Abstract

Reversible Peptide-Protein Interactions Inside Cells: Enabling a New Approach for Achieving Super-Resolution Imaging in Live Cells

Curran Oi

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Cells are the basic unit of life and, within cells, thousands of unique proteins work in concert to perform a vast array of tasks. Visualizing and tracking proteins inside live cells is therefore critical to understanding the behavior of these proteins *in vivo*. The invention of fluorescence microscopy has enabled proteins to be tagged and tracked using fluorescent molecules. More recently, the development of super-resolution microscopy has enabled very high resolution images of proteins in cells to be collected, both *in vitro* and *in vivo*.

Currently, one major challenge in super-resolution microscopy is the fact that many proteins are not amenable to tagging and imaging using existing methods. For example, many proteins mislocalize or misfunction when fused to another protein as large as a fluorescent protein. Similarly, proteins with short half-lives are difficult to image, because they are degraded before a fused fluorescent protein has time to mature and become fluorescent.

In this dissertation I present a new super-resolution imaging method called Live cell Imaging using reVersible intEractions - Point Accumulation In Nanoscale Topography (LIVE-PAINT). In this technique, reversible peptide-protein interaction pairs are used to transiently associate a fluorescent protein with a protein of interest. To implement LIVE-PAINT, I fused one half of a peptide-protein interaction pair to a protein I want to image at its genomic locus, thus labeling all copies of the protein in the cell with a peptide tag. Then, I separately fused the other half of the peptide-protein interaction pair to a fluorescent protein and integrated the construct into the genome, under control of the galactose inducible promoter. When both constructs are expressed concurrently, binding events between the protein of interest and fluorescent protein are mediated by the peptide-protein interaction pair.

I have demonstrated that LIVE-PAINT can be performed using coiled coil interaction pairs and peptide-tetratricopeptide interaction pairs with a range of binding affinities between approximately 1 and 300 nM. I have also shown that LIVE-PAINT can be performed using many different color fluorescent proteins, demonstrating the flexibility of the method.

LIVE-PAINT has many strengths which make it a useful new super-resolution tool. One example of this is given by proteins which do not tolerate direct fusions to fluorescent protein. I have tagged several putative plasma membrane proteins which localize to the vacuole when directly fused to fluorescent proteins and shown

they localize to the plasma membrane as expected when tagged using peptide-protein interaction pairs and imaged with LIVE-PAINT. This putative localization to the plasma membrane is also confirmed by immunostaining data in one case. I have also demonstrated that LIVE-PAINT enables signal replenishment. In my work, the peptide-protein interactions used to tag the protein of interest are reversible and I restrict the illumination volume during imaging. This means that after a fluorescent protein unbinds from a protein of interest, another one can diffuse in from a part of the cell outside the illumination volume and bind in its place.

Because the fluorescent protein is expressed separately from the protein of interest, much larger constructs can be reversibly associated to a protein of interest without increasing the size of the fusion to the protein of interest. To show this, I expressed a tandem array of three identical fluorescent proteins and demonstrated that this construct could be used for LIVE-PAINT imaging without any noticeable effect on the proper localization or function of the protein of interest. An additional benefit of the fact that the fluorescent protein is expressed separately from the protein of interest is that the expression level of the fluorescent protein is therefore not directly tied to the expression level of the protein of interest. This property of LIVE-PAINT makes it a good tool for imaging very low and very high abundance proteins, which suffer from too little or too much fluorescent signal in traditional fluorescence microscopy approaches.

Thus, I have shown that LIVE-PAINT is a useful new super-resolution imaging technique and there are a number of applications for which it is uniquely well suited. LIVE-PAINT is particularly useful for studying proteins which are not amenable to direct fusion to fluorescent proteins, proteins which are short-lived, and proteins which are expressed at a very low or very high level.

Reversible Peptide-Protein Interactions Inside Cells: Enabling a New Approach for Achieving Super-Resolution Imaging in Live Cells

A Dissertation

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By

Curran Oi

Dissertation Director: Lynne Regan, Ph.D.

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Glossary of Terms

FP	Fluorescent Protein
GFP	Green Fluorescent Protein
mNG	mNeonGreen (a bright green fluorescent protein)
mEoS	A green to red photoconvertible fluorescent protein
K_d	Dissociation constant
TRAP	Tetratricopeptide repeat affinity protein
TPR	Tetratricopeptide repeat
SYNZIP	Synthetic heterodimeric coiled coil
TIRF	Total internal reflection fluorescence
FWHM	Full width at half maximum
SMLM	Single Molecule Localization Microscopy
PAINT	Point accumulation in nanoscale topography
LIVE-PAINT	Live cell Imaging using reVersible intEractions PAINT
PALM	PhotoActivated Localization Microscopy
STORM	STochastic Optical Reconstruction Microscopy

STED	STimulated Emission Depletion

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1 Introduction

[Some text and figures adapted from Oi, C. *et al.* (2020). PAINT using proteins: A new brush for super-resolution artists. *Protein Science*. **5** (11)[1]]

Proteins are macromolecules ubiquitous in life. They coordinate a vast array of cellular functions, including cell division, DNA repair, and endocytosis, among many others. Because proteins are critical for proper cellular function, and because thousands of different proteins are made in every cell, elucidating protein function and regulation is a grand challenge of biology. One important characteristic of protein function is localization: where a protein is inside a cell, at a given time, or in the presence of a given stimulus. Observing protein localization and its colocalization with other molecules in the cell is critical to understanding protein function, including protein behavior in diseases.

Many approaches to studying protein localization have been developed, with ever-improving methods continually being developed to visualize proteins with better resolution, or to tag them in less perturbative ways. A number of different protein tagging techniques have been developed. Below I discuss some of the major techniques, including their benefits and drawbacks. I then turn my focus to the development and advancement of super-resolution techniques, which have improved resolution of fluorescence microscopy to localize proteins to sub-20 nm. I end with a summary of the work presented in this thesis.

1.1 Immunohistochemistry

A common method for studying proteins inside cells is immunohistochemistry. This method, which dates back to 1941, uses an antibody to bind to a protein of interest inside a cell which has been fixed and permeabilized[2] (Figure 1-1). The antibody can be either conjugated to an enzyme, such as peroxidase, or to a fluorescent dye[3]. When conjugated to an enzyme, the enzyme can produce a colored or fluorescent product, which makes it possible to see where the antibody has bound. The primary antibody can also be unlabeled. In this case, a secondary antibody conjugated to an enzyme or fluorescent dye will be used to bind to the primary antibody and produce a signal.

Despite being an old method, it is still very commonly employed and has a number of appealing benefits. One of the greatest strengths of this method is that no modification to the protein of interest is needed. This is important, as modifications to the sequence of the protein of interest can change its function. Many proteins are very sensitive to even very small genetic fusions, so the fact that immunohistochemistry does not require any modification to the target protein is a tremendous benefit. Additionally, antibodies tend to bind proteins with high specificity and affinity (nanomolar to picomolar)[4, 5], resulting in protein labeling with high signal to background. Antibodies which bind a large number of protein targets already exist and are commercially available, which makes them quite easy to acquire and use.

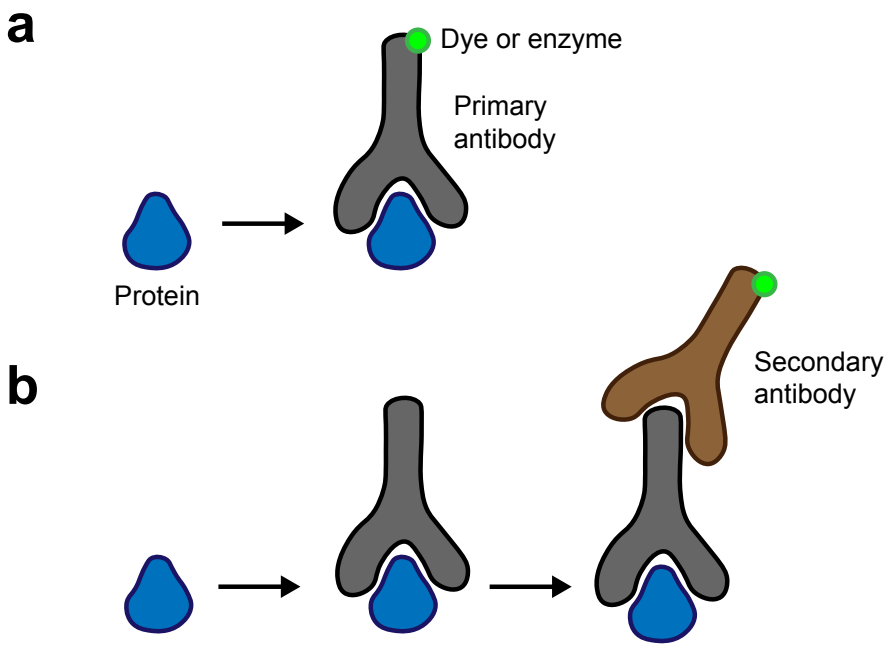

Figure 1-1. Immunohistochemistry uses antibodies to determine protein localization. (a) A protein of interest (blue) is bound specifically by a primary antibody (gray) that targets an epitope on the protein of interest. A dye or enzyme (green) conjugated to the primary antibody produces a spectroscopic readout for the protein's localization. (b) Same process as in (a), except the primary antibody is not functionalized. Instead, a secondary antibody (brown) that binds to the primary antibody is functionalized with a dye or enzyme (green) to produce a spectroscopic readout.

Some drawbacks to methods using antibodies include the need to fix and permeabilize cells, the large size of antibodies, that some antibodies have issues with nonspecific binding, and that it can be expensive and time consuming to generate new antibodies.

Among these, perhaps the biggest drawback to immunohistochemistry is the need to fix and permeabilize cells. This requirement means this method cannot be used to study protein behavior inside live cells. When studying protein behavior, it is often desirable to study how these proteins behave in live cells, because it is difficult to know how the fixation and permeabilization process affects cells. Additionally, cells are very dynamic environments and proteins are continually binding, diffusing, catalyzing reactions, and being translated and degraded. Studying the dynamic processes of proteins is best done in living cells; it is equivalent to the increased information content contained in a video, relative to a few photos.

The large size of antibodies, which are on the order of 10 nm in length and 150 kDa in weight[6], also presents a limitation of immunohistochemistry. For advanced imaging methods, such as super-resolution imaging (discussed later), individual proteins can be localized to within 10-20 nm of their true position. This would be very difficult to do using an antibody, because the large size of the antibody would increase the uncertainty in the true location of the protein of interest. For this

reason, methods using full-sized antibodies are not ideal for imaging proteins to very high resolution.

Recently, however, single domain antibodies have begun to be used to perform super-resolution imaging[7]. Single domain antibodies are approximately 15 kDa in size[8]. This reduces the distance between the protein of interest and the fluorescent probe attached to the single domain antibody compared to full-sized antibodies. This small size makes single domain antibodies good tools for super-resolution imaging. Using single domain antibodies is an appealing approach for super-resolution imaging, but only when a single domain antibody already exists for the protein.

It can take substantial time and resources to generate antibodies capable of binding new proteins of interest, due to the need to inoculate live animals. Despite the drawbacks I have discussed, imaging using antibodies is still quite useful when live cell work and high image resolution are not critical, such as pathology, where an established set of antibodies are used very regularly for diagnosing diseases[9-14].

1.2 Bioconjugation

Another approach used to covalently tag biological molecules with another molecule is called bioconjugation. There are multiple ways to do this. A common

approach is to use dyes which can react with the side chain of one particular amino acid (Figure 1-2). For example, N-Hydroxysuccinimidyl (NHS) esters will conjugate to primary amines (-NH2)[15], which are present on lysine side chains and the N-terminus of a protein sequence. Using this approach, an organic dye can be conjugated to all the exposed lysine side chains (as well as the N-terminus of the protein) in a purified protein sample. Approaches to conjugate molecules to other reactive groups of proteins, including cysteine[16], tyrosine[17], and N- and C- termini also exist[18, 19].

Bioconjugation is a robust method and enables the conjugation of a wide variety of molecules to a protein. For the purpose of protein imaging, this means that many different organic fluorescent dyes can be used, so long as they are properly functionalized to react specifically to primary amines, or another reactive group. Organic dyes can be quite bright and since a single protein can have more than one reactive group accessible to the reaction (e.g. multiple available lysine residues), it is possible to conjugate many copies of a fluorescent dye to a single protein, making the fluorescent signal from the protein very bright, which is advantageous for imaging with high signal to background.

Despite the benefits of this style of bioconjugation, it suffers from some major drawbacks. The most prominent is that this labeling cannot be done inside living cells. This is because all the lysines in the cells, for example, would be labeled with the fluorescent probe, and you would not obtain labeling specific to the protein you

would like to study. Additionally, many fluorescent probes are not cell permeable[20], which limits the set of probes which can be used. Finally, the labeled proteins must be re-introduced into living cells after labeling *in vitro*, if it is desirable to study them in their native environment. This is difficult and has the added drawback that the existing copies of the protein of interest in the cell would be unlabeled, so you cannot achieve 100% labeling of the protein of interest using this method. As such, this bioconjugation approach is better suited to *in vitro* study of proteins.

More recently, other bioconjugation approaches have emerged which circumvent some of the shortcomings associated with classical bioconjugation approaches. These methods use proteins expressed inside the cell that are capable of covalently conjugating to exogenously supplied organic dyes (Figure 1-3). Three commonly used orthogonal chemistries exist for this approach and the associated proteins are called SNAP-tag[21, 22], CLIP-tag[23], and HaloTag[24]. These protein tags are moderately sized: ~20 kDa for SNAP-tag and CLIP-tag and ~33 kDa for HaloTag. SNAP-tag and CLIP-tag are smaller than most fluorescent proteins (FPs), which are ~27 kDa in size, but they are much larger than organic fluorophores themselves, which are typically less than 1 kDa in size.

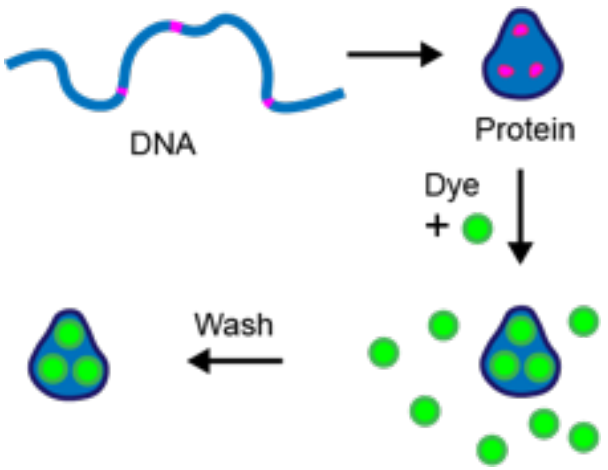

Figure 1-2. Bioconjugation by labeling all of a specific residue in a protein. DNA (blue strand) codes for a protein (blue enclosed shape) which contains some residues (pink) capable of reacting to an organic dye (green). After the protein is expressed, the residues available for conjugation to the dye are reacted with the dye. By then washing the sample, unbound dye molecules can be removed.

Figure 1-3. Bioconjugation using protein tags. A protein of interest (blue) is genetically fused to a protein tag such as SNAP-tag (purple) that can form a covalent bond with an organic dye (green). After the dye is added and it reacts to form a covalent bond to the protein tag, unconjugated dye can be removed using washing steps.

The use of protein tags capable of covalent conjugation to organic dyes is a major advancement of the bioconjugation approach, because a dye can be added to cells and labeling will be specific to the protein of interest, which is not possible with bioconjugation approaches which tag all accessible lysines, for example. There remains the challenge of adding the organic dye to the cells in such a way that it is transduced into the cells and high labeling efficiency is achieved, without high background fluorescence from unconjugated fluorophores. This can involve many washing steps in the procedure or the use of strategies aimed to label only a small fraction of proteins with the reactive protein tag.

SNAP-tag, CLIP-tag, and HaloTag move the bioconjugation approach forward by enabling specific tagging and the ability to genetically encode everything in the system except the organic dye. The primary drawbacks of the approach are the need to get the dye into cells, the removal of excess unconjugated dye by washing steps, and the moderate size of the dye-conjugating protein tags. The first two issues are not a significant weakness of the approach, as cell permeable dyes are available and washing steps are not particularly difficult. The size of the dye conjugating protein tags, however, suggests that the approach will not enable tagging of proteins of interest that are sensitive to direct fusion to FPs, since the dye conjugating protein tags are similar in size to FPs.

1.3 Fluorescent labeling using unnatural amino acids

In a method similar to bioconjugation approaches, fluorescent probes can be specifically incorporated into proteins using unnatural amino acids. This approach uses organisms that have been recoded to remove one of the codons from the coding sequence of the genome[25]. Typically, the amber stop codon (TAG) is used for this purpose. This codon can then be used for a new amino acid, which can be synthetic and introduced to the organism by addition to the growth media.

The new amino acid can itself be fluorescent[26], or it can be conjugated to a fluorescent dye in a second step[27]. Fluorescent amino acids that have been developed to date are quite dimmer than commonly used organic fluorophores and intrinsically FPs, by approximately an order of magnitude[28]. They will also be fluorescent whether or not they have been incorporated into the protein of interest. This means that there will be unconjugated fluorescent amino acids in the media that increase the background fluorescence. These can be removed but require washing steps. Conjugating the fluorescent dye in a second step to a reactive unnatural amino acid enables the use of brighter fluorescent molecules, but this second molecule must be added to the media as well and unreacted molecules must also be removed by repeated washing to reduce the background signal from unconjugated fluorophores.

Using unnatural amino acids has the benefit that precise locations in a protein can be labeled, since the unnatural amino acid can be placed anywhere in the coding

sequence that does not disrupt the folding, function, or localization of the protein of interest. This makes it a powerful tool for labeling very specific parts of a protein, which is difficult to achieve with other methods.

One of the challenges of using unnatural amino acids to label proteins is that a new tRNA synthetase must be evolved to bind to both the tRNA which recognizes the repurposed codon and the new amino acid[29]. This must be done for each different synthetic amino acid used. Additionally, due to the fact that only one codon is recoded in unnatural amino acid approaches, it is not possible to readily use two unnatural amino acids at once in a controlled and sequence specific manner.

Recently, a method was developed using unnatural amino acids to image two proteins at once, using different color fluorophores[30]. However, this method requires the two proteins to be expressed using two different amino acids, in different sets of cells. The cells are then merged to enable imaging of both proteins concurrently. This example illustrates how difficult it is to extend unnatural amino acid tagging approaches to labeling multiple proteins concurrently. This drawback limits the potential for labeling multiple proteins or multiple sites with different labels at once with this strategy.

1.4 Intrinsically fluorescent proteins

In the early 1990's, FPs, which had been known to exist since the 1960's and 1970's, were developed into incredibly powerful biological tools for imaging proteins. First, Douglas Prasher cloned and sequenced the gene for green fluorescent protein (GFP)[31] and then Martin Chalfie expressed the protein in *Escherichia coli*[32]. GFP is a medium-sized protein, with a molecular weight of about 27 kDa. This is a bit larger than SNAP-tag and CLIP-tag, which are 20 kDa, but smaller than the 33 kDa HaloTag. Because it can be genetically fused to a protein of interest, it has the advantage that specific fluorescent labeling of a protein of interest can be obtained without the need to add any exogenous organic dyes to the media or to wash the cells.

Conversely, the size of FPs can pose a burden to a protein of interest, when directly fused. Many proteins do not tolerate a direct fusion to an FP, with the direct fusion causing either misfunction or mislocalization of the protein of interest. Proteome-wide studies comparing immunofluorescence data to fluorescence data collected using GFP fusions to proteins of interest have estimated the percentage of proteins whose localization is disrupted by direct fusion to an FP to be about 20-25%[33, 34]. Membrane proteins have been shown to be particularly sensitive to direct fusions to FPs, with only 46 of 139 putative transporter proteins in *Saccharomyces cerevisiae* showing localization to the plasma membrane when directly fused to GFP[34, 35].

After the initial successes of cloning GFP and using it to tag a protein for fluorescence imaging *in vivo*, FPs have quickly become ubiquitous tools for imaging proteins *in vivo*, along with a plethora of other uses I will not discuss further. Soon, scientists like Roger Tsien began mutating GFP to generate brighter and more stable versions of the protein[36]. Mutations to the chromophore and surrounding residues gave rise to a family of related proteins with different photophysical properties[37-39]. In addition to GFP, FPs with different excitation and emission wavelengths have been developed as imaging tools: everything from ultraviolet, blue, green, and yellow FPs to orange, red, and infrared FPs have been engineered. Many of these FPs have been derived from random mutation and screening of GFP, though other FPs have been cloned and engineered from other organisms as well[40, 41]. Most notably, a yellow to deep red family of FPs has been engineered from a tetrameric red FP from the sea anemone *Discosoma sp*.[40]. The resulting set of engineered FPs has made it possible to tag multiple proteins for imaging concurrently, by using spectrally distinct FPs.

Despite the power of intrinsically fluorescent proteins for imaging, they have some drawbacks. Although they can be quite bright (not quite as bright as the brightest organic dyes), the chromophore in the center of the FPs needs to mature in order for the protein to be fluorescent. In some FPs, this maturation process has been made quite rapid, on the order of tens of minutes, but for others it can take several hours for the chromophore to mature[42]. This is a downside compared with approaches that use organic dyes, because not all of the FPs will be fluorescent

at any given time. Additionally, some fraction of FPs never develop a mature chromophore, resulting in some of the proteins tagged with an FP never developing a fluorescent signal[42]. This is a drawback for imaging approaches in which an FP is directly fused to a protein of interest, because that protein of interest will never be imaged. This issue is less determinantal when imaging using reversible interaction pairs, because a non-fluorescent FP bound to a protein of interest will quickly unbind and a fluorescent one will be free to bind.

The desire to be able to control the conditions under which fluorescence "turns on" led to the development of split FPs. Split FPs have been very useful for probing protein-protein interactions. Several different groups have used engineering approaches to split FPs into two halves[43-45]. Both halves have little to no fluorescence on their own and only generate fluorescence when they bind to one another and the FP chromophore is able to mature (Figure 1-4). Typically, each half of the split FP is fused to a different protein. If these proteins interact, they drive binding of the split FP halves and generate a fluorescent signal. While useful for probing protein-protein interactions, this method is limited due to the fact that the interaction is not readily reversible[43, 45]. It is also therefore not a good method for measuring binding kinetics, or for quantifying equilibrium binding values.

Recently, advances in split fluorescent technology have been made which use FPs which bind small molecule chromophores that give rise to a fluorescent signal[46, 47]. These interactions are reversible and have the potential to be used for quantitative

binding assays. However, they are much less bright than FPs conventionally used for imaging purposes and are therefore not yet ideal for live cell applications.

In all, FPs are versatile and useful tools for imaging proteins inside cells. It is trivial to tag multiple proteins simultaneously inside cells, with no background signal from unbound or unconjugated fluorescent molecules, unlike other labeling methods I have previously discussed. This tagging specificity, along with no need for any exogenously added dyes or washing steps, makes work with FPs very simple. Additionally, the availability of many different FPs for imaging purposes makes it easy to select spectrally distinct FPs so the tagged proteins are easily distinguishable.

Mutagenesis of intrinsically fluorescent proteins has given rise to FPs with varied spectral and physical properties. In the 2000's, the discovery of mutations and novel proteins that cause an FP to be photoconvertible revolutionized imaging methods in biology[48-51]. Photoconvertible proteins, which can be toggled between two spectral states, were used to develop the super-resolution imaging method called PhotActivated Localization Microscopy (PALM)[52]. Super-resolution imaging methods, which dramatically improve the resolution with which molecules can be imaged, both *in vivo* and *in vitro*, have been a critical advance to molecular imaging.

1.5 Super-resolution microscopy

In traditional, diffraction-limited, fluorescence imaging, the maximum resolution achievable is on the order of 200-250 nm, due to the wavelength of visible light. This limit was discovered by Ernst Abbe, who found that the minimum resolvable distance *d* between two points is $d = \frac{\lambda}{2NA}$, where λ is the wavelength of light used for imaging and NA is the numerical aperture. NA is dependent on both *n* the index of refraction of the medium the light passes through and θ, the maximal half angle of the cone of light that can enter or exit the microscope lens, with the dependence $NA = n \sin \theta$. Some media have NA larger than 1, like immersion oil, which has a NA of about 1.5; immersion oil is commonly used in microscopy.

Since the resolution achieved depends on the wavelength of the photons used, UV and X-ray microscopes have been developed, which use UV light and x-rays, both of which have shorter wavelengths than visible light. While these microscopes have higher resolution, they suffer from poor contrast and the high energy photons used damage biological samples. Super-resolution microscopy techniques that use fluorescent molecules have the benefit that the fluorescent molecules emit fluorescence that is typically much higher than the natural autofluorescence of the biological samples being studied, giving the technique high contrast.

Proteins are only a few nanometers in diameter, much smaller than the 200-250 nm diffraction limit of light. This means that precise imaging of proteins is not

possible with diffraction-limited imaging and methods with superior resolution must be used to obtain more detailed information of protein location in the cell[52-56].

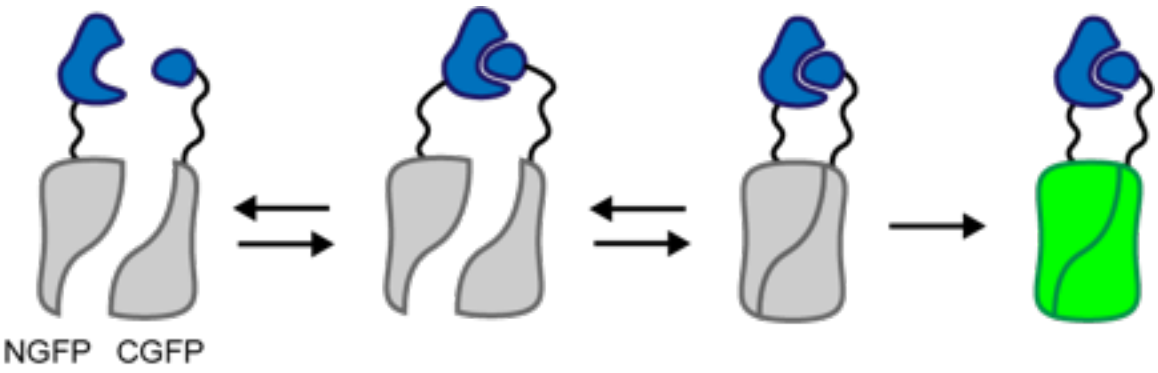

Figure 1-4. Split FPs only fluoresce upon binding. A FP is genetically split into N-terminal (NGFP) and C-terminal (CGFP) halves (gray). When two proteins (blue) fused to the split FP halves bind to one another, they induce binding of NGFP and CGFP. Upon binding, the chromophore of the FP is able to mature and product fluorescence (fused green protein). The first two steps are readily reversible, as represented by the reversible arrows. Once the chromophore has matured, the interaction is no longer readily reversible, as indicated by the unidirectional arrow.

In diffraction-limited fluorescence imaging, all fluorescent molecules in the cell are excited and imaged at once. In contrast, in PALM, imaging occurs in two steps: first one laser is used to photoconvert a subset of the fluorescent molecules in the sample to their alternate state (e.g. convert from green to red state); then, the converted molecules are excited using a second laser and imaged[52] (Figure 1-5). In this way, only a subset of the fluorescent molecules in the sample are imaged at once. These molecules can be precisely localized, because they are separate enough from one another to have distinct point spread functions, which look like distinct spots. The more photons collected for each of these localization events, the better precision with which the center of each localization event is known. This photoconversion and imaging process is repeated iteratively, to obtain a super-resolution image.

At the same time PALM was being developed, other super-resolution methods such as STochastic Optical Reconstruction Microscopy (STORM)[56] and STimulated Emission Depletion (STED)[53] microscopy were also being invented. Since then, many additional super-resolution microscopy techniques have emerged to expand the imaging toolkit and improve on earlier methods[54, 57-63].

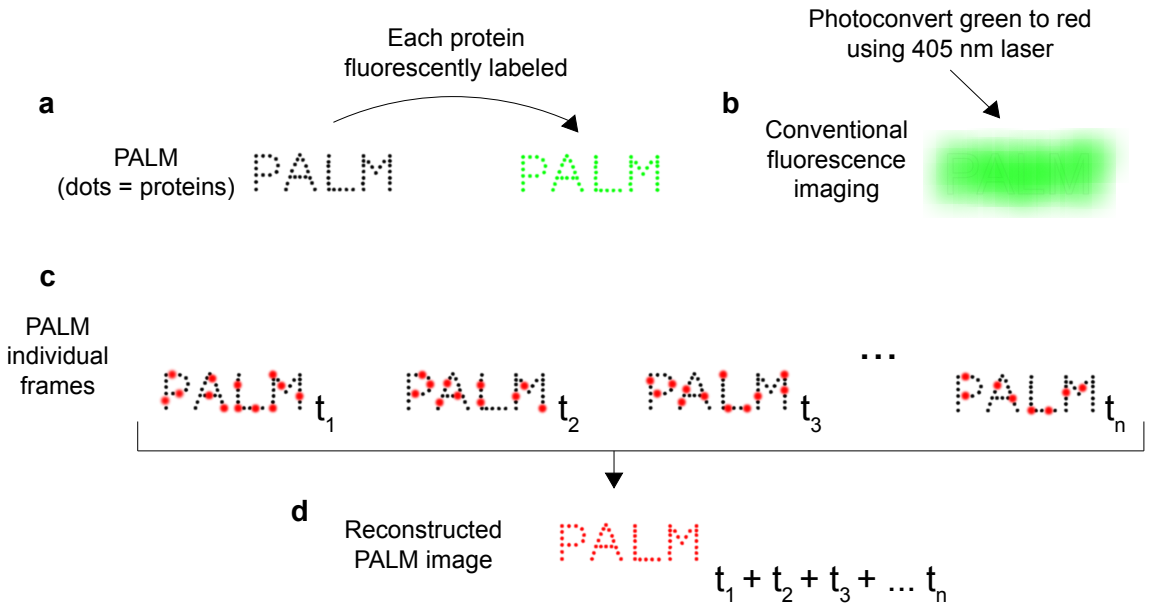

Figure 1-5. Cartoon illustration of the principle of the PALM method. PALM achieves super-resolution by summing sparse, temporally-separated localization events. (a) A biomolecular structure 'PALM' (dimensions of the order of 500 x 2000 nm) composed of multiple proteins. Individual proteins are shown as black dots. If each protein is directly fused to a fluorescent molecule (green dots) (b) conventional fluorescence imaging cannot resolve individual fluorophores, so the PALM structure is fluorescent, but individual proteins cannot be visualized - because the proteins are too close together to be resolved by diffraction-limited microscopy. The fluorophores must be photoconverted from green-emitting to red-emitting by exposure to a short laser pulse from a blue laser. (c) Single fluorescent localizations by PALM. The proteins in the biomolecular structure are directly fused to a photoconvertible fluorescent molecule. A small subset of these molecules are photoconverted to red-emitting using a short blue laser pulse and imaged using a red emission filter. This is repeated and such data are collected iteratively over time (t_1, t_2, t_3, ….t_n). At each timepoint, a different subset of the proteins is photoconverted to red-emitting. (d) The localization events collected at each timepoint (t_1, t_2, t_3, ….t_n) in (c) are summed to generate a final super-resolution image, in which the location of each protein can now be resolved.

Super-resolution imaging methods have improved the possible achievable resolution down to approximately 20 nm for imaging proteins in live cells. An unfortunate reality of working with fluorescent molecules, however, is that they eventually photobleach after being exposed to lasers. Without photobleaching, PALM imaging could in principle be continued forever, continually exciting and re-exciting the same fluorescent molecules. In this way, many localization events could be obtained, increasing the precision with which the location of the proteins would be known in the cell. Because fluorescent molecules, including both FPs and organic fluorescent dyes photobleach, this data collection cannot continue indefinitely. This constraint of modern super-resolution imaging constitutes a "photobleaching limit".

1.6 Circumventing the photobleaching limit

In the 2000's, a super-resolution imaging technique called point accumulation in nanoscale topography (PAINT) [63] was developed (Figure 1-6), which aimed to circumvent the photobleaching limit of super-resolution imaging. In the original iteration of PAINT, a small molecule dye, which reversibly binds to a biomolecule, is used for imaging. This dye will only fluoresce when bound to its target molecule. For example, when the dye Nile red binds to a lipid membrane it will fluoresce red and generate a localization event. It will then unbind and be non-fluorescent. Repeated binding and unbinding events will give rise to a large number of

localization events which can be distinguished from one another, because the dye concentration will be tuned so that only a few molecules are bound to the lipid membrane at once.

A key advantage of PAINT-based methods, compared to other SMLM approaches, is their ability to circumvent the issue of fluorescent probes photobleaching over time, which is an inevitable consequence of irradiation by the excitation laser. In approaches employing covalently-bound fluorescent probes, the number of emitting fluorophores decreases as the experiment proceeds, thus progressively fewer localization events are recorded as time continues. Eventually all the fluorophores are bleached. The duration of data acquisition and the number of localizations is thus strictly limited. By contrast, in PAINT-based approaches, because the interaction between the biomolecule and the fluorescent probe is transient, bound but bleached fluorescent molecules will be continually replaced by exchanging with unbleached, unbound molecules. Data acquisition can thus continue beyond the time scale for bleaching, enabling extended data accumulation, consequently generating higher resolution images. This also allows dim bursts that are localized with a low accuracy to be removed during analysis, since there are many more localizations of higher precision. An additional advantage of PAINT methods is that they do not require a photoconvertible fluorophore. There are thus many more small molecule fluorophores or fluorescent proteins, with a greater range of emission wavelengths, to choose from.

Recently, a PAINT imaging approach called protein-PAINT has been developed for imaging proteins inside live cells[58]. This approach makes use of a 12 kDa protein tag called Y-FAST, which reversibly binds to fluorogenic rhodamine derivatives and undergoes a spectral shift upon binding[58]. Y-FAST is fused to the protein of interest and the dye that it reversibly binds is added to the growth or imaging media. Binding events are recorded as localization events and iterative binding and unbinding gives rise to a super-resolution image.

This work uses a relatively small protein tag for imaging proteins (12 kDa). This decreased size, relative to FPs (27 kDa) and dye-conjugating tags such as SNAP-tag (20 kDa), helps move the field towards smaller and smaller tags. However, the lack of additional protein tags with similar behavior to Y-FAST makes it nontrivial to extend the method to concurrent imaging of multiple protein targets. Additionally, there does not exist a set of spectrally distinct dyes usable with this system; at the moment, only one protein tag and one dye is usable with this system, limiting the method's generalizability.

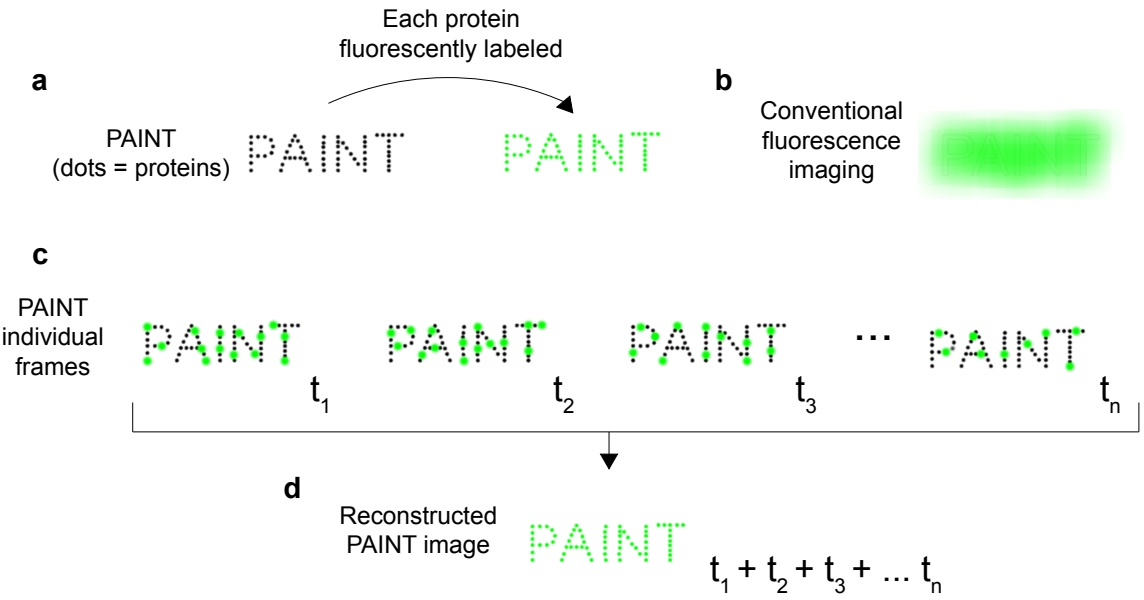

Figure 1-6. Cartoon illustration of the principle of the PAINT method. PAINT achieves super-resolution by summing sparse, temporally-separated localization events. (a) A biomolecular structure 'PAINT' (dimensions of the order of 500 x 2000 nm) composed of multiple proteins. Individual proteins are shown as black dots. If each protein is directly fused to a fluorescent molecule (green dots) (b) conventional fluorescence imaging cannot resolve individual fluorophores, so the PAINT structure is fluorescent, but individual proteins cannot be visualized - because the proteins are too close together to be resolved by diffraction-limited microscopy. (c) Single localizations by PAINT. The proteins in the biomolecular structure are not directly fused to a fluorescent molecule. They are only visible when a fluorescent molecule transiently binds to any of them, resulting in intense bursts of fluorescence (green spots). Such data are collected iteratively over time (t_1, t_2, t_3, ….t_n). At each timepoint, a different subset of the proteins is bound to the fluorescent molecule. (d) The localization events collected at each timepoint (t_1, t_2, t_3, ….t_n) in (c) are summed to generate a final super-resolution image, in which the location of each protein can now be resolved.

1.7 Encoding specificity using DNA-PAINT

A limitation of PAINT using small molecules is the lack of specificity in their interaction. The development of DNA-PAINT provided a strategy for PAINT-type single molecule localization microscopy (SMLM) visualization, but via a highly specific interaction. DNA-PAINT is an elegant technique which uses two short complementary oligonucleotides, one attached to a biomolecule of interest and one labeled with a fluorescent dye[64] (Figure 1-7b). These oligonucleotides interact transiently, resulting in bursts of fluorescence, just as in the original small-molecule PAINT experiments. In this implementation of the method, however, the transient interaction is highly specific, dictated by the sequence of the two complementary DNA strands. DNA-PAINT has been widely used to image DNA origami type structures *in vitro*[64-75].

The enormous advantage of DNA-PAINT is that it is relatively straightforward to manipulate the specificity and affinity of the two interacting ssDNA strands. In more elaborate implementations, involving a ssDNA attached to a nanobody or aptamer for example, DNA-PAINT has been used to image proteins within fixed, permeabilized cells[65, 70-73, 76, 77]. These examples, however, make clear one of the main limitations of DNA-PAINT: It cannot be used inside live cells.

In its original implementation, DNA-PAINT was constrained by the intrinsically slow binding rate of complementary ssDNA oligonucleotides, leading to long image acquisition times at the solution concentrations needed to avoid significant

background from unbound labelled oligonucleotide. It could take hours to obtain high resolution images[74]. Recently, however Strauss and Jungmann have shown that on-rates can be increased about a hundred-fold by using multiple concatenated repeats of a short DNA sequence[74]. This modification of the method enables sufficient data for a 20 nm resolution image to be acquired in minutes. Chung and colleagues have also recently reported a new approach to DNA-PAINT, in which a DNA-PAINT imaging strand self quenches, so it is dark when unbound and only fluoresces when it binds to the docking DNA strand[78]. This enables imaging to be performed at a high concentration of fluorescent probes and image localization events to be collected at 100 frames per second. This resulted in 1,000 blinking events per micron of microtubule to be collected in ~4 mins instead of ~1.4 hrs as was the case using traditional DNA-PAINT probes.

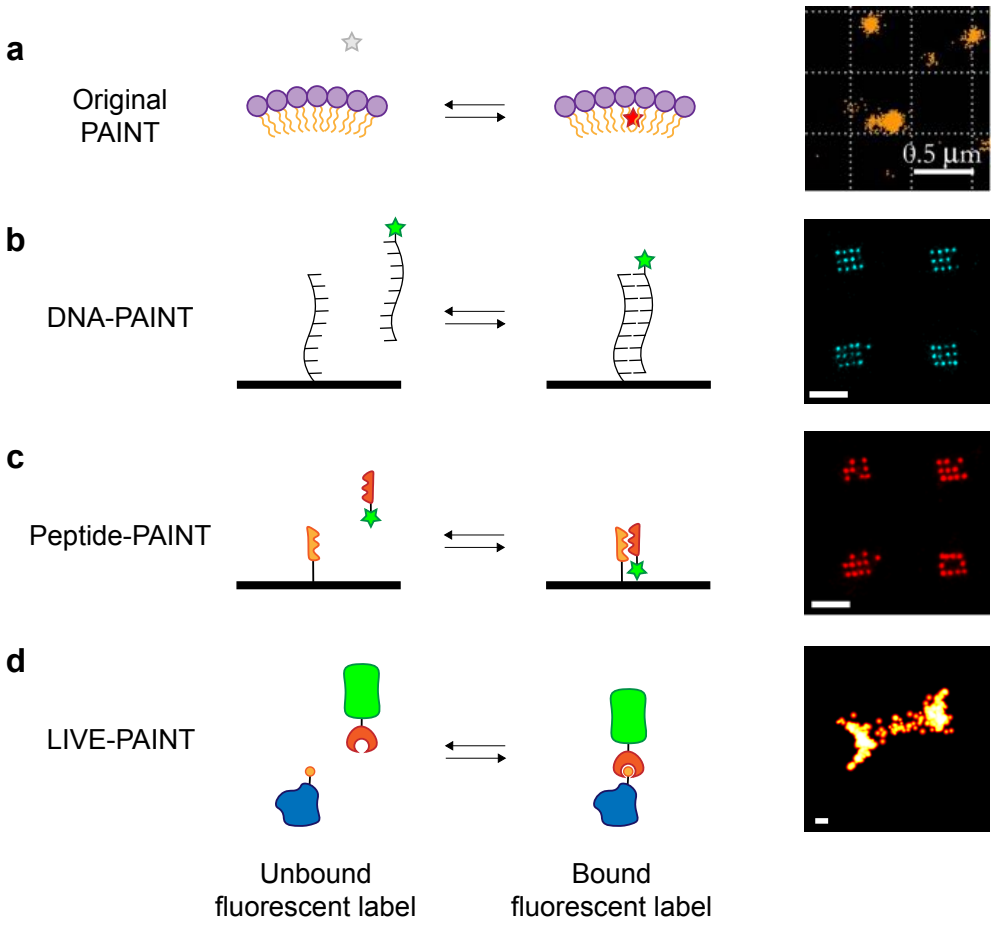

Figure 1-7. Cartoon illustration of different PAINT methods. (a) 'Original PAINT'. Left: a cartoon representation of a small molecule dye which is nonfluorescent in aqueous solution (grey star) but which fluoresces (red star) when it transiently interacts with the hydrophobic lipid membrane of the LUV (purple circles represent polar headgroups, yellow tails represent the aliphatic tails). Right: image of LUVs imaged using Nile red modified from Sharonov et al.[63] (copyright (2006) National Academy of Sciences). (b) DNA-PAINT. Left: cartoon representation in which the short ssDNA oligonucleotide to be imaged (for example part of a DNA origami surface array) is shown as a black strand. The complementary ssDNA oligonucleotide is shown as a black strand attached to a fluorescent dye (green star). Right: DNA origami nanostructures imaged using DNA-PAINT, reproduced from Eklund et al.[79] (copyright Creative Commons Attribution 4.0 International license: https://creativecommons.org/licenses/by/4.0/). (c) Peptide-PAINT. Left: cartoon representation in which the protein to be imaged is fused to a peptide (orange saw- tooth). The protein is visualized by the interaction of that peptide with a protein that binds it (red sawtooth) fused to a fluorescent dye (green star). Right: DNA origami nanostructures imaged using Peptide-PAINT, reproduced from

Eklund et al.[79] (copyright Creative Commons Attribution 4.0 International license: https://creativecommons.org/licenses/by/4.0/). (d) LIVE-PAINT. Left: cartoon illustration in which the protein to be imaged (blue) is fused, at the gene level, to a peptide (orange circle). The protein is visualized by the interaction of that peptide with a protein that binds to it (red crescent), fused to a fluorescent protein (green barrel). Any fluorescent protein can be used. This method can also be used with coiled coils, as are used in (c). Oi et al. used the bright mNG. Right: image of the septum in live Saccharomyces cerevisiae, obtained by labeling Cdc12p and imaged using LIVE-PAINT. Scalebars are 500 nm (a) and 100 nm (b-d). All images shown in a-d were acquired using TIRF.

1.8 Development of protein-based PAINT

Recent papers describe the successful use of transient peptide-protein interactions to perform super-resolution microscopy[61, 79, 80], in particular as a new way to implement the PAINT method[63] (Figure 1-6).

Protein-based PAINT methods (Figure 1-7c-d) therefore have the potential to provide a straightforward route to increased on-rates. Indeed, in a method they named Peptide-PAINT (Figure 1-7c), Eklund et al. showed that using peptide-peptide interactions can increase imaging speeds of a DNA origami array two-fold, relative to imaging the same array using ssDNA-ssDNA interactions, in DNA-PAINT[79].

Eklund et al. started with the E_3/K_3 coiled coil pair of 21 amino acid (aa) peptides, where each E unit is a negatively charged 7 aa 'heptad repeat' and each K unit is a positively charged 7 aa 'heptad repeat' peptide[81-83]. Keeping the length of the negatively charged peptide constant, Eklund et al. explored the effect of decreasing the length of the K peptide on coiled-coil stability. They chose to work with K peptides of 18 or 19 aa, which interact with the E peptide with dissociation constants of 1.7 μM, and 81 nM respectively. These dissociation constants are similar to the dissociation constants of DNA duplexes that have previously been effective in DNA-PAINT.

The majority of the testing of Peptide-PAINT was *in vitro*, in the context of a DNA origami array, which allowed a direct comparison between the behavior of the

peptide pair with that of a DNA duplex. It proved possible to image the DNA origami surface using Peptide-PAINT in a similar fashion to using DNA-PAINT. An advantage of Peptide-PAINT is faster 'on-rates' than for conventional DNA-PAINT. With Peptide-PAINT, the mean dark time between fluorescent bursts for a given binding site is approximately 30 s, compared with approximately 70 s for DNA-PAINT.

1.9 Imaging proteins in cells using protein-based PAINT

Eklund et al. showed that the Peptide-PAINT method has the potential to be used in fixed permeabilized cells. In addition to requiring that a cell is fixed and permeabilized, in its current implementation, Peptide-PAINT also requires an antibody or (antibody equivalent) against any protein of interest and, chemical coupling of a peptide to a secondary antibody (Figure 1-8a). The stoichiometry between the antibody and the coiled coil strand is variable, because the conjugation attaches the coiled coil peptide to any accessible primary amine on the antibody[79]. The complementary strand of the coiled coil duplex is conjugated to a fluorophore and added exogenously to the fixed and permeabilized cells. This strategy has the disadvantage of increasing the distance between the protein of interest and the fluorophore, thus decreasing the precision of localization of the protein of interest. It has been previously shown that conjugating a fluorophore to a primary antibody increases the distance between the target and fluorophore by

~12.5 nm[84]. Using both primary and secondary antibodies will increase this distance between target molecule and fluorophore even more, likely to more than 20 nm.

Other key developments in using peptide-peptide or peptide-protein interactions for PAINT-type super-resolution imaging have focused on the important advantage that they can be genetically encoded and thus work inside live cells.

The idea of fluorescently labelling a protein of interest via a non-covalent interaction with a fluorescent molecule, has previously been described for traditional fluorescence imaging. For example, Pratt et al. fused a 5 aa peptide to the protein of interest via an 8 aa linker sequence, which was then visualized in live *E. coli*, by its interaction with a 120 aa tetratricopeptide repeat (TPR) domain fused to a fluorescent protein[85, 86]. Related work by Hinrichsen et al. showed that a similar method could be used to fluorescently label a membrane protein post-translationally in live yeast[87], thus avoiding the perturbation of function associated with direct fusion of a fluorescent protein to a membrane protein.

Work performed by Doh et al. demonstrated that a tight binding coiled coil interaction pair could be used to associate various chemical reporters for use with fluorescence microscopy and electron microscopy[88]. In this work, the protein of interest is tagged using a 5.2 kDa peptide, which binds a 7.5 kDa peptide to form a coiled coil with a binding affinity of 13 pM. This tight affinity helps enable high signal to background, by minimizing the fraction of chemical reports which are not

bound to the protein of interest. This work is a good demonstration of the use of peptide-protein interaction pairs for labeling and imaging proteins in live cells, though the tight binding affinity of the peptide-protein pair is not compatible with PAINT style imaging, which requires frequent unbinding of the peptide-protein pair.

Perfilov et al. showed that different versions of the E_3/K_3 peptides (containing point mutations) could be used to perform super-resolution imaging in live cells[61]. In this example, they used a peptide attached to a photo-convertible fluorescent protein, and used photoactivated localization microscopy (PALM) to obtain data for a super-resolution image. Although a peptide-protein interaction is used in this work, it differs from the work of Eklund et al.[79] and Oi et al.[80], in not employing a PAINT approach to data acquisition. The work is analogous to the peptide-protein pair mediated fluorescence labeling of Pratt et al. but with PALM super-resolution imaging rather than diffraction limited imaging. It also provides evidence complementary to the data shown in this thesis that super-resolution imaging can be performed using peptide-protein interactions.

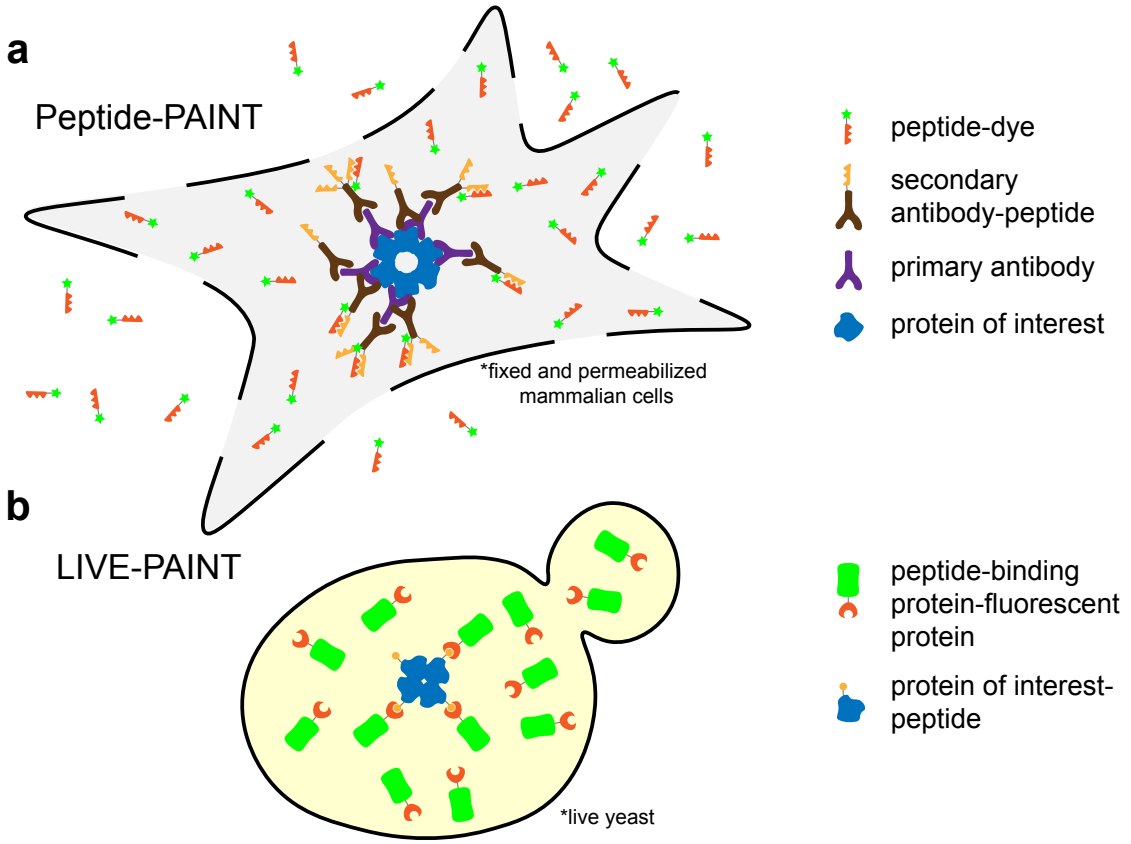

Figure 1-8. Cartoon representations of using Peptide-PAINT in fixed, permeabilized mammalian cells and LIVE-PAINT inside live yeast. (a) In Peptide-PAINT, he protein of interest (blue) is bound by a primary antibody (purple). A secondary antibody (brown), which binds to the primary antibody, is attached to one or more peptides (orange sawtooth). A peptide (red sawtooth) that interacts with the antibody-linked peptide is synthesized with a fluorescent dye (green star) attached. Cells are fixed and permeabilized and the peptide-dye fusion (red sawtooth-green star) is added exogenously and can diffuse in and out of the cell. Excess antibodies and fluorescently labelled peptide can be washed out prior to imaging in TIRF, which further decrease the background. (b) In LIVE-PAINT, the protein of interest (blue) is fused to a peptide (orange circle), at the gene level, and integrated into the chromosome. A peptide-binding protein, comprising the recognition element for the peptide (red crescent) is fused to a fluorescent protein (green barrel), at the gene level, and integrated into the chromosome. Labeling is performed inside live cells, with the expression level of the labelling protein controlled. Background from unbound labeling protein is reduced by data acquisition in TIRF.

1.10 Aims of this work

While PAINT-like methods can achieve high resolution by using reversible interactions, the method suffers from the fact that it is not easy to tag proteins in cells and it is not currently possible to tag proteins for PAINT imaging inside live cells. The exception is the protein-PAINT method, which is quite limited and requires the addition of an organic dye to the media, as I've described in section 1.6. In order to develop a PAINT imaging strategy for studying proteins inside live cells, I have proposed to use peptide-protein interactions to reversibly associate an FP to a protein of interest, using a fully genetically encodable system. Using reversible interaction pairs to tag proteins for imaging using PALM is possible and similarly has the benefit of using only small peptide fusions to a protein of interest. The primary benefits of PAINT over PALM are that PAINT can use any bright FP, while PALM requires a photoconvertible FP, and the fact that PAINT can naturally extend to highly multiplexed multicolor imaging, which I discuss in detail in Section 3.4.

I investigated the use of peptide-protein interactions to perform super-resolution imaging inside live yeast cells, naming this method Live cell Imaging using reVersible intEractions PAINT (LIVE-PAINT) [80] (Figure 1-7d and Figure 1-8b). All the imaging was performed on live cells, in which the chromosome was engineered to express the desired proteins. In this work, the protein of interest was fused to a peptide (either a 5 aa peptide for the TPR interaction, or a 42 aa peptide for the

coiled coil interaction) via an 8 aa linker sequence. I use a heterodimeric antiparallel coiled coil[89], or a peptide-TPR pairs[90], having observed that that fusion of highly charged peptides[91] to my protein of interest (Cdc12p) resulted in aberrant cell morphology and growth. While not any peptide fusion will be an improvement over direct fusion to an FP, using modular recruitment tags has the benefit that many different interaction pairs can be tested to find a minimally perturbative interaction pair for tagging the protein of interest. I explore how the labeling efficacy changes with the dissociation constant of the peptide-protein pair and the amount of the labeling protein expressed. The key difference between LIVE-PAINT and DNA-PAINT and Peptide-PAINT is that in LIVE-PAINT all the components are genetically encoded and expressed within the cell.

This approach uses the same principles of reversible binding utilized in the original PAINT method, as well as the DNA-PAINT method and its associated advancements. This method is very flexible due to the number of well-characterized reversible peptide-protein interaction pairs and the number of bright FPs in the literature. In Chapter 2, I present the evidence that it is possible to use reversible peptide-protein interactions to perform LIVE-PAINT imaging in live *S. cerevisiae*. I show that this imaging can be performed using several different peptide-protein interaction pairs, different FPs, and can be used to image different proteins of interest.

LIVE-PAINT has an added benefit over other super-resolution imaging methods such as PALM: the genetic modification to the protein of interest in LIVE-PAINT can be much smaller than what is used in PALM (less than 5 kDa for LIVE-PAINT, compared with ~27 kDa when using PALM). This is also a smaller modification to the protein of interest than that used in the protein-PAINT method (12 kDa). The small tag used in LIVE-PAINT makes it possible to tag and image proteins inside live cells that do not tolerate direct fusion to a protein as large as an FP. I also show that LIVE-PAINT can be used to image cofilin, which does not tolerate a direct fusion to an FP[92].

Because true positive controls for live cell super-resolution imaging are rare, when imaging proteins it makes sense to use a tagging approach which has been shown to be minimally perturbing to other proteins. This is important, because large fusions to proteins of interest can result in mislocalization or stabilization of the protein of interest, which can result in collecting imaging data for artifactual behavior. Therefore, I believe that the least perturbative approach to super-resolution imaging is likely to be the best one. Thus, it would be beneficial to use LIVE-PAINT instead of directly fusing proteins of interest to an FP, even when there is not an obvious defect in protein localization when the protein of interest is directly fused to an FP. This is especially important when tagging and imaging a protein for which the function and localization is not very well understood, so the results best reflect the true behavior of the protein of interest.

2 Super-resolution microscopy using reversible peptide-protein interactions

[Text and Figures adapted from Oi, C. *et al.* (2020). LIVE-PAINT allows super-resolution microscopy inside living cells using reversible peptide-protein interactions. *Communications Biology*. **5** (458)[80] and Oi, C. *et al.* (2020). PAINT using proteins: A new brush for super-resolution artists. *Protein Science*. **5** (11)[1]]

2.1 Introduction

Here, I describe a PAINT-based method that has all the advantages of DNA-PAINT, but with the enormous benefit that it can be used for imaging inside live cells. I refer to this approach as LIVE-PAINT.

In LIVE-PAINT, reversible peptide-protein interactions, rather than zipping/unzipping of a DNA oligonucleotide duplex, are responsible for the transient localizations required for SMLM. The protein to be imaged is genetically fused to a short peptide and expressed from the protein's endogenous promoter. Additionally, integrated at a suitable place in the genome, a peptide-binding protein is genetically fused to an FP and expressed from an inducible promoter, allowing its expression level to be controlled and optimized. The small size of the peptide tags fused to the protein of interest is another important strength of the method. It enables post-translational fluorescent labeling of target proteins that do not tolerate a direct fusion to an FP. To illustrate this point, we show that LIVE-PAINT can be used to perform *in vivo* super-resolution imaging of proteins, such as actin and

cofilin, which are notoriously refractory to direct fusions[92, 93]. Furthermore, in a collaboration with Mathew Horrocks at the University of Edinburgh, we show that LIVE-PAINT can be used to perform diffraction-limited tracking of individual biomolecules for extended periods of time.

2.2 Important requirements for a good protein-peptide or peptide-peptide pair for imaging using PAINT methods

When considering which peptide-peptide or peptide-protein interactions are suitable for PAINT imaging, there are several important considerations. The protein-peptide pair must be specific. The 1:1 heterodimer should be overwhelmingly favored over all other states, such as higher order oligomers. There should be minimal or no homodimer formation by either component, and neither component should interact significantly with any cellular protein. The peptide fused to the protein of interest should be small, to avoid perturbing that protein's function. The peptide-protein interaction should be of a suitable strength to function as desired. It should not be too tight, because the PAINT approach relies on transient interactions and exchange of the bound state with the unbound pool. Operationally, a dissociation constant of about 1 μM is desirable[61, 64, 79], although the on-rate and off-rate of binding is more important than the K_d.

It is desirable for the peptide-protein interaction to have a relatively fast off-rate. Most DNA-PAINT experiments use interactions with an off-rate of approximately 1

s^{-1} [64]. In the original DNA-PAINT experiments, that the off-rate of the DNA strands is not highly dependent on the length of the DNA strands used, suggesting that there is little scope for modulating the off-rate for interactions between two short DNA strands [64]. In principle, the desirable off-rate for PAINT experiments is one which allows sufficient photons to be observed during a localization event to achieve 'good' resolution. In DNA-PAINT experiments, exposure times of 100 ms are typically used[64, 74] and in LIVE-PAINT I have used 50 ms[80]. With exposure times of 50-100 ms, PAINT methods using currently available fluorophores, would benefit from using interactions with even higher off-rates, up to 10-20 s^{-1}. On-rates ideal for PAINT experiments are those that will enable rapid re-binding of fluorescent probes to molecules of interest. The ideal value will be dictated by the off-rate of the interaction, the desired K_d of the interaction (which will depend on the circumstances of the imaging) and the concentration of the fluorescently labeled construct the user desires to use.

Although K_d has been reported for many peptide -protein interactions, far fewer on- and off-rates for peptide-protein interactions have been measured.

2.3 Peptide-protein pairs can be used to achieve super-resolution

The essence of LIVE-PAINT is to visualize individual fluorescent molecules transiently attached to a cellular structure of interest. The individual fluorophores are thus identified by temporal, rather than spatial, separation. LIVE-PAINT

achieves sparse labeling by using reversible peptide-protein interactions. The protein of interest is directly fused to a peptide and an FP is fused to the cognate protein (Figure 2-1a and Figure 2-2). The peptide-protein interactions are chosen so that solution exchange occurs on a timescale shorter than or comparable to the bleaching lifetime, allowing many sequential images to be obtained. In each image, a different peptide-tagged protein of interest is bound to a different protein-FP, allowing individual proteins to be precisely localized (Figure 2-1a-d). These localization events are then summed to generate a super-resolution image (Figure 2-1E).

As a test case with which to optimize this approach, I visualized the cell division septin protein Cdc12, a component of the readily-identifiable septum that is formed during *Saccharomyces cerevisiae* budding. I tested LIVE-PAINT using two different peptide-protein interactions with very different dissociation constants and molecular structures: TRAP4-MEEVF (a TPR-peptide pair with a dissociation constant (K_D) of 300 nM) and SYNZIP17-SYNZIP18 (an antiparallel coiled coil pair with a K_D of 1 nM)[89, 90, 94, 95]. In both cases, the peptide (MEEVF or SYNZIP18) is fused to Cdc12 and the cognate protein or peptide (TRAP4 or SYNZIP17, respectively) is fused to the bright green FP mNeonGreen (mNG) (11). Although mNG is known to blink intrinsically[96], I chose to use it in the experiments because it is very bright and therefore can produce very precise localization events. mNG

has a brightness of 93[41], while other FPs I use in this work, mKO and mOrange, have brightness of 31[97] and 49[40], respectively. Most importantly, I show that mKO and mOrange, which are not known to blink intrinsically, are also compatible with LIVE-PAINT (Figure 2-3). TRAP-peptide pairs have been shown previously to be less perturbative for cellular imaging than direct fusion to an FP[87]. Both TRAP4-MEEVF and SYNZIP17-SYNZIP18, were well-tolerated by the cell, and both can be used for either diffraction limited or super-resolution imaging of the septum in live yeast (Figure 2-1e). I observed no distorted cell morphology or changes in growth rate in liquid media when using the TRAP4-MEEVF and SYNZIP17-SYNZIP18 interaction pairs. In previous work, I observed distorted cell morphology for ~5% of yeast expressing a direct fusion of Cdc12 to an FP[87]. As with other super-resolution imaging methods, resolution improves as more localizations are acquired. With LIVE-PAINT, I was able to obtain approximately 20 nm resolution in about 5 s when imaging Cdc12p-SYNZIP18 using SYNZIP17-mNG (Figure 2-4). I also provide evidence that LIVE-PAINT can be performed with additional peptide-protein interaction pairs (Figure 2-5), that the localizations events observed are specific to the protein being labeled (Figure 2-6), and that two orthogonal interaction pairs can be used with two FPs to tag two different proteins specifically and concurrently (Figure 2-7).

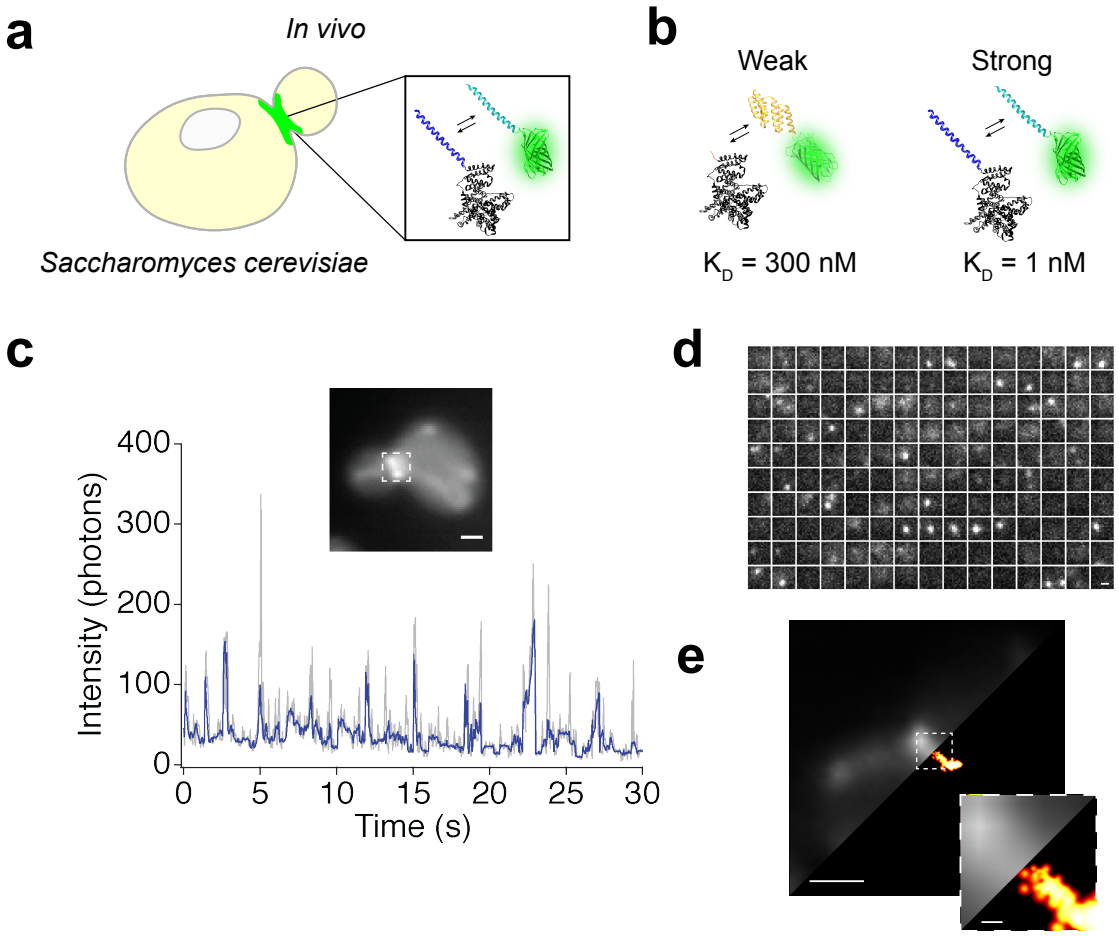

Figure 2-1. LIVE-PAINT achieves sparse labeling using reversible peptide-protein interactions. (a) Details of the LIVE-PAINT imaging method, as applied to Cdc12p. A peptide tag (dark blue) is fused to the target protein that will be imaged, Cdc12p (black). A peptide-binding protein (light blue) is fused to a fluorescent protein (bright green). The peptide tag and peptide-binding protein reversibly associate, as indicated by the double arrows. (b) Molecular details of the peptide-protein pairs used: TRAP4 (yellow) binds to the peptide MEEVF (red) and SYNZIP18 (dark blue) binds to SYNZIP17 (light blue) with the dissociation constants shown. Proteins are shown with a ribbon representation of their structures, and are approximately to scale. Ribbon structure diagrams were generated using PDB files for interaction pairs similar to those used in this work: TRAP4-MEEVF is represented using the structure for a tetratricopeptide repeat protein in complex with the MEEVF peptide (PDB ID: 3FWV) and SYNZIP17-SYNZIP18 is represented using the structure for the antiparallel coiled coil Kif21A (PDB ID: 5NFD). (c) Binding and unbinding of the peptide-binding module-fluorescent protein to the peptide tag generate blinking events. Plot of the fluorescence intensity (photons) at a particular location (in a

square shown as a dotted box around the septum in the florescence image) in the septum versus time. We interpret peaks in the signal as indicating that mNG is bound to Cdc12p and troughs indicating mNG is dissociated from Cdc12p. (d) Montage of frames from a fluorescence microscopy video collected of the area of the septum boxed in part (c). Each frame in the montage is separated by 0.2 s and the bright blinking events correspond to fluorescence peaks in (c). (e) Diffraction limited (left) and super-resolution (right) images of Cdc12p imaged using Cdc12p-SYNZIP18 and SYNZIP17-mNG. The image was generated from a video with 6,000 frames, with an exposure time of 50 ms per frame and a laser power density of 3.1 W/cm^2. Number of super-resolution localization events: 448. Scale bars are 1 μm, except for the inset to (e), which has a 100 nm scale bar.

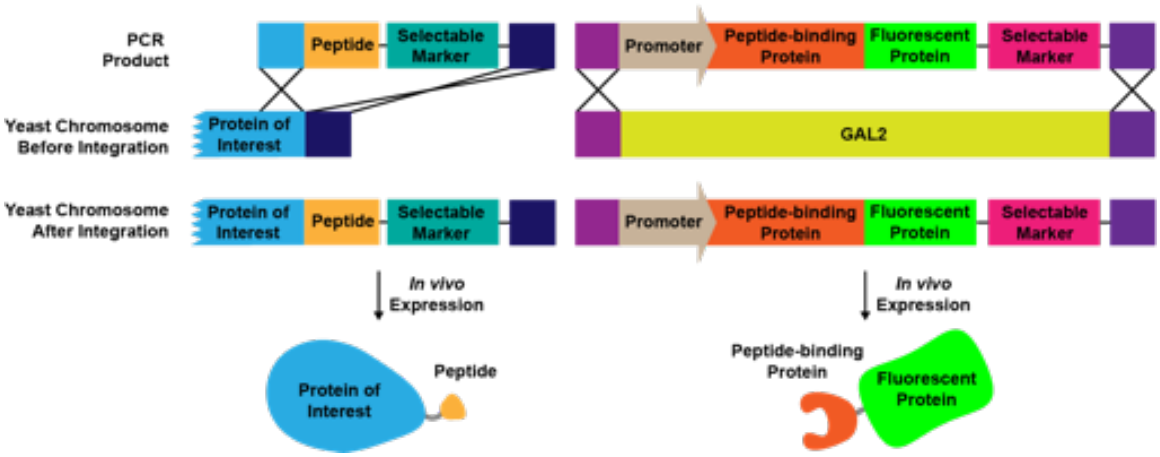

Figure 2-2. Strategy for genomic modifications in yeast. (Left) Strategy for fusing a peptide to the protein of interest. A peptide is added to the protein of interest, by integrating a PCR product that encodes the C-terminus of the protein of interest fused to the peptide, a selectable marker, and a section of the chromosome beyond the gene into the genome. The PCR product and the chromosome before and after integration are labeled. The final protein products are shown at the bottom of the cartoon. The components are shown as: C-terminus of the coding region of the protein of interest (pale blue rectangle); a region of the chromosome just beyond the end of the coding region of the protein of interest (dark blue rectangle); DNA encoding the peptide to be fused to the protein of interest (pale orange rectangle); DNA encoding a selectable marker (turquoise rectangle). (Right) Strategy for making the promoter-peptide-binding-protein-FP chromosomal integrations. A PCR product containing the promoter, peptide-binding protein- FP fusion and a selectable marker is integrated at the GAL2 locus. The components are shown as: regions of identity adjacent to the GAL2 locus, for integration into the chromosome by homologous recombination (light and dark purple rectangles); promoter (beige arrow); DNA encoding peptide-binding-protein (dark orange rectangle); DNA encoding FP (green rectangle); DNA encoding a selectable marker (magenta rectangle).

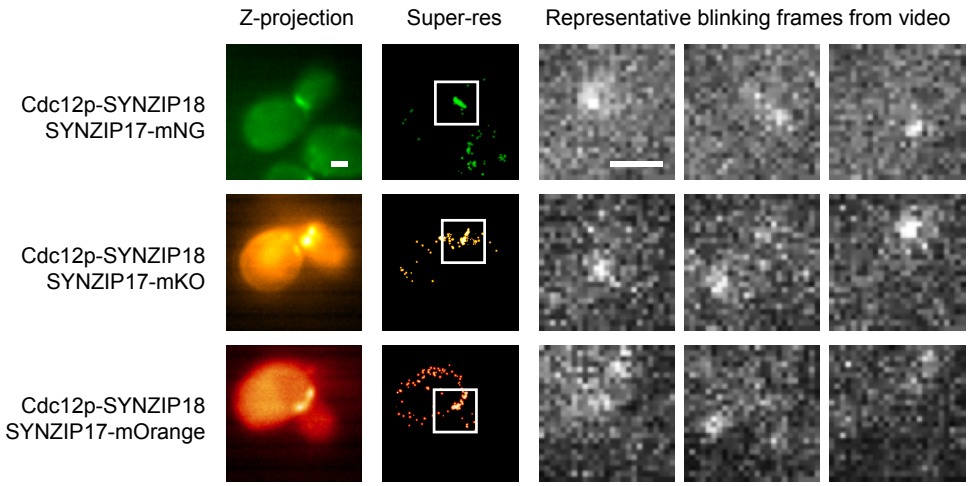

Figure 2-3. LIVE-PAINT can be performed with different FPs. LIVE-PAINT was performed using the SYNZIP17-18 interaction pair to tag Cdc12p and three different fluorescent proteins: mNG, mKO, and mOrange, as indicated. (Left) Z-projections showing the average fluorescence signal for each video, calculated by integrating the average intensity over the entire video. (Middle) Super-resolution images for each video. The white box corresponds to the cropped region shown in the "representative blinking frames from video" section at right. (Right) Representative frames from the video, showing bright "blinks" in different locations. All images were constructed from videos collected for 1,000 frames, with a 50 ms exposure per frame. Number of localization events: mNG: 531; mKO: 280; mOrange: 154. Scale bars are 1 μm. The mNG images were obtained with a 488 nm laser, using a power of 3 W/cm^2, while the mKO and mOrange images were obtained with a 561 nm laser, using a power density of 50 W/cm^2 (see methods).

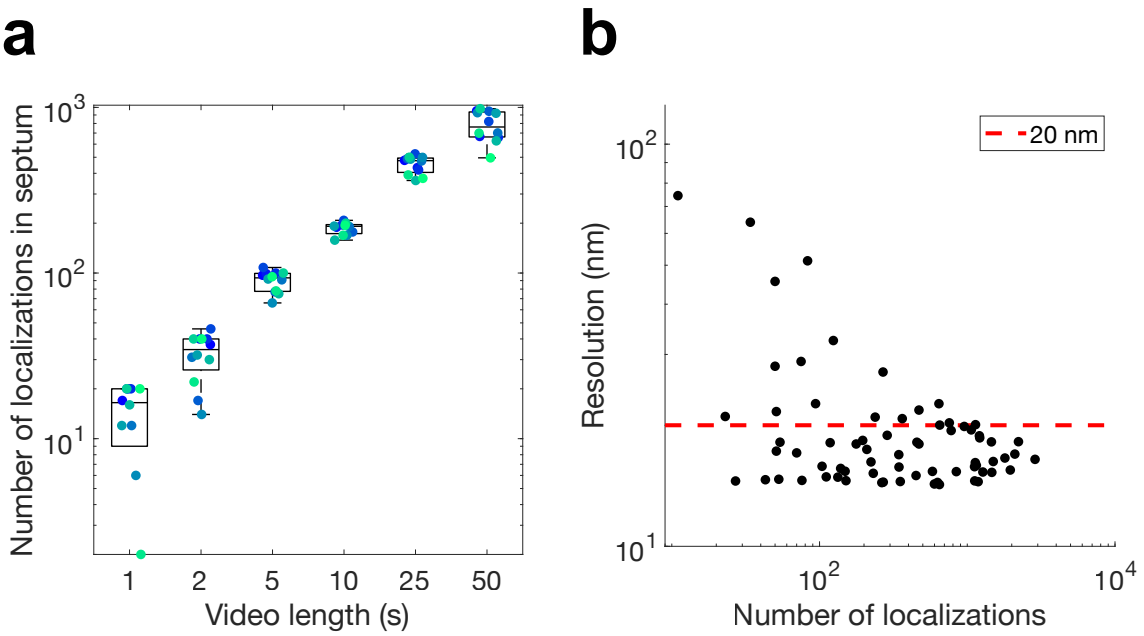

Figure 2-4. Image resolution can reach 20 nm within five seconds while imaging Cdc12p-SYNZIP18 + SYNZIP17-mNG. 12 individual cells were imaged for 50 s (1,000 frames with 50 ms exposures). (a) Videos were truncated after the given number of seconds as shown. For each video the number of localizations in the septum is shown. Each cell is indicated by a different color dot, which can be tracked across the different video lengths. In the box, the middle line represents the median, the bottom line represents the 25th percentile, and the bottom line represents the 75th percentile. The whiskers reach to the furthest points in the data which are not outliers. (b) Resolution was calculated for each of the points in (A) and shown as a function of the number of localizations in the septum (as described in Methods). Cells were grown in media containing 0.005% galactose.

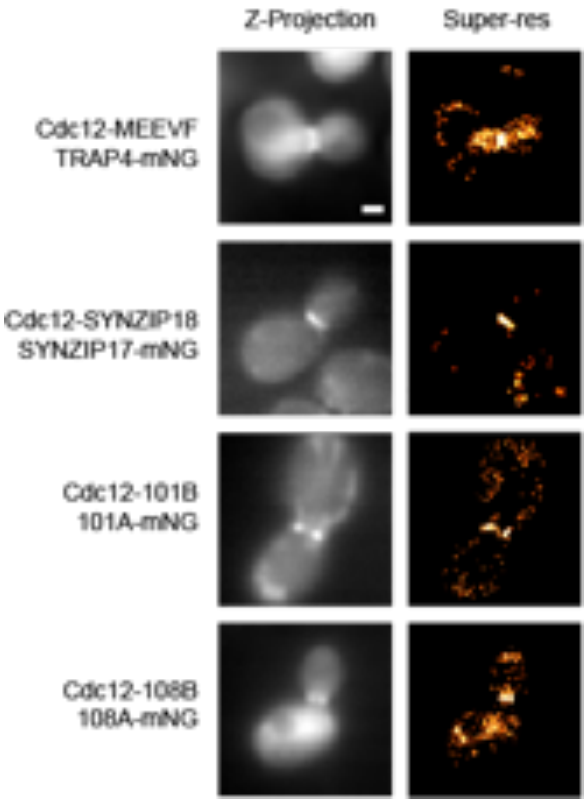

Figure 2-5. LIVE-PAINT can be performed with different peptide-protein interaction pairs. From top to bottom, the interaction pairs tested were TRAP4-MEEVF, SYNZIP17-SYNZIP18, 101A-101B, and 108A-108B, as indicated. (Left) Z-projections showing the average fluorescence signal for each video, calculated by integrating the average intensity over the entire video. (Right) Super-resolution images for each video. Number of localization events: TRAP4-MEEVF: 429; SYNZIP17-SYNZIP18: 398; 101A-101B: 582; 108A-108B: 803. Scale bars are 1 μm.

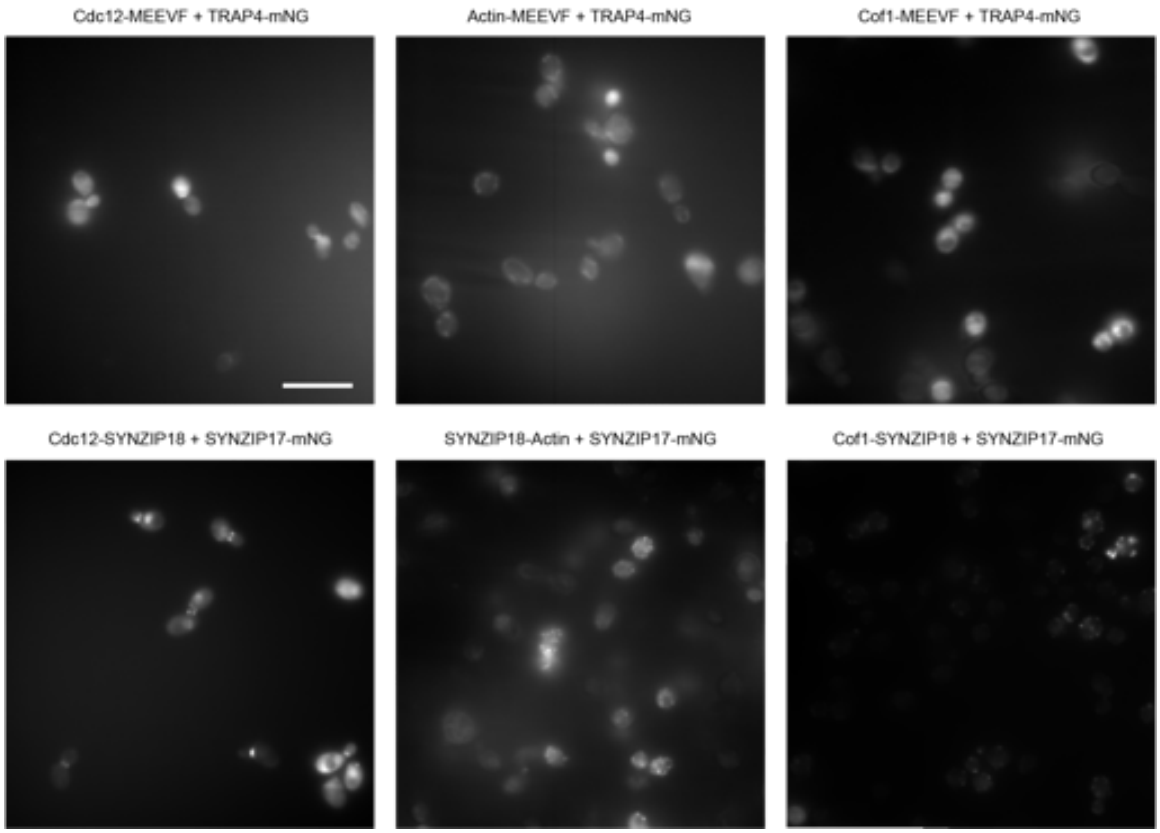

Figure 2-6. TRAP4-MEEVF and SYNZIP17-SYNZIP18 interactions are specific inside the cell. Maximum projection images of Cdc12p, Act1p, and Cof1p, which were tagged by either the TRAP4-MEEVF (top row) or SYNZIP17-SYNZIP18 (bottom row) interaction pair. Structures localized to the septum are seen when tagging Cdc12 and puncta around the edge of the cell are observed when tagging actin of Cof1. All images were constructed from videos collected for 1,000 frames, with a 50 ms exposure per frame and a laser power density of 3.1 W/cm^2. Scale bar is 10 μm.

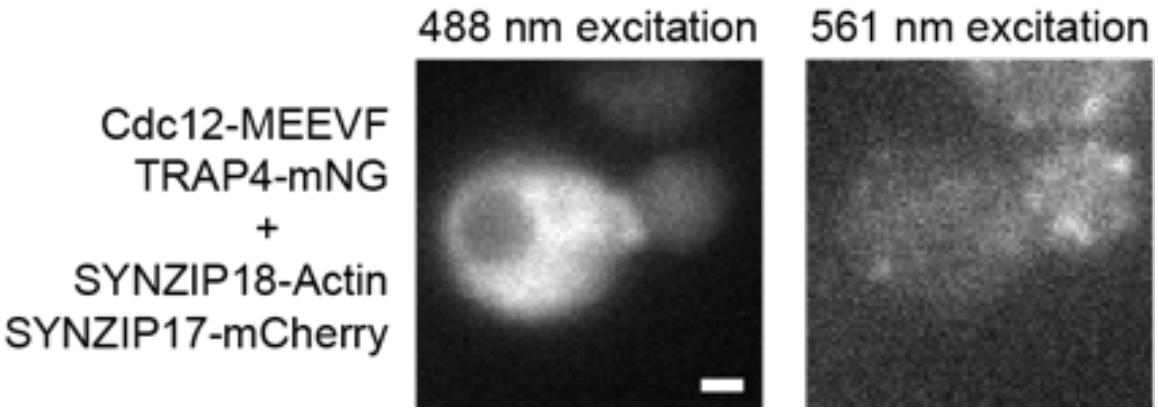

Figure 2-7. TRAP4-MEEVF and SYNZIP17-SYNZIP18 interaction pairs are orthogonal to one another and can be used with two different FPs for concurrent imaging. Fluorescence images of a cell expressing both Cdc12p-MEEVF+TRAP4-mNG and SYNZIP18-Act1p+SYNZIP17-mCherry. (Left) Cell imaged using a 488 nm excitation laser and green emission filter. Structure at yeast septum, corresponding to the location of Cdc12p, is clearly visible. mNG fluorescence would be detected using these excitation and emission settings. (Right) Cell imaged using a 561 nm excitation laser, using a power density of 50 W/cm^2. Distinctive structures around the edge of the cell, corresponding to the location of Act1p, are clearly visible. mCherry fluorescence would be detected using these excitation and emission settings. Images were collected using a 1 s exposure time. Scale bars are 1 μm.

2.4 Signal to background dictated by amount of labelling protein

In LIVE-PAINT, the peptide-binding proteins fused to mNG (TRAP4-mNG and SYNZIP17-mNG), are expressed from an inducible promoter[98]. See Figure 2-8 for fluorescence induction profile. Using an inducible promoter for expressing the fluorescent construct is useful, because it makes it possible to test many different expression levels of the fluorescent construct using only one strain. This simplifies the process for determining the best expression level of the fluorescent construct to use for imaging.

By varying the expression level of either TRAP4-mNG or SYNZIP17-mNG, for the TRAP4-MEEVF and SYNZIP17-SYNZIP18 interaction pairs respectively, I can determine which conditions generate the highest percentage of localizations at the septum relative to non-specific localizations (Figure 2-9). For very low expression levels, for example for 0% galactose with 'leaky' expression, not enough mNG is expressed and not enough localization events are achieved to generate a super-resolution image. Conversely, for example for 0.1% galactose, expression levels are too high and very few individual localization events can be visualized, because the density of mNG is too high to achieve sparse labeling. At intermediate expression levels, for example with 0.005% or 0.02% galactose, there are sufficient FPs that enough localization events can be recorded to resolve a super-resolution image, but the FP expression level is not so high that single localization events cannot be recorded.

I performed cluster analysis using the DBSCAN function (see methods) to quantify the number of localization events in the septum versus in the rest of the cell. I was thus able to identify the conditions that produced the most specific super-resolution images. In an analogous fashion to DNA-PAINT, the FP mNG does not give rise to a localization event until it binds and is immobilized. Some non-specific localization or blinking events are recorded, these are randomly distributed within the cell and can be removed from further analysis by using DBSCAN. The number of these non-specific localization events increases with galactose concentration, because by increasing galactose, the number of free mNG which are not bound to a Cdc12 protein increases. For this reason, I choose not to work with very high galactose concentrations for most of my experiments. I observed that the highest percentage of localization events in the septum for the 0.005% galactose condition when imaging both the TRAP4-MEEVF interaction pair (45% of localization events in the septum) and the SYNZIP17-SYNZIP18 interaction pair (98% of localization events in the septum). The septum was identified by cluster analysis (see methods).

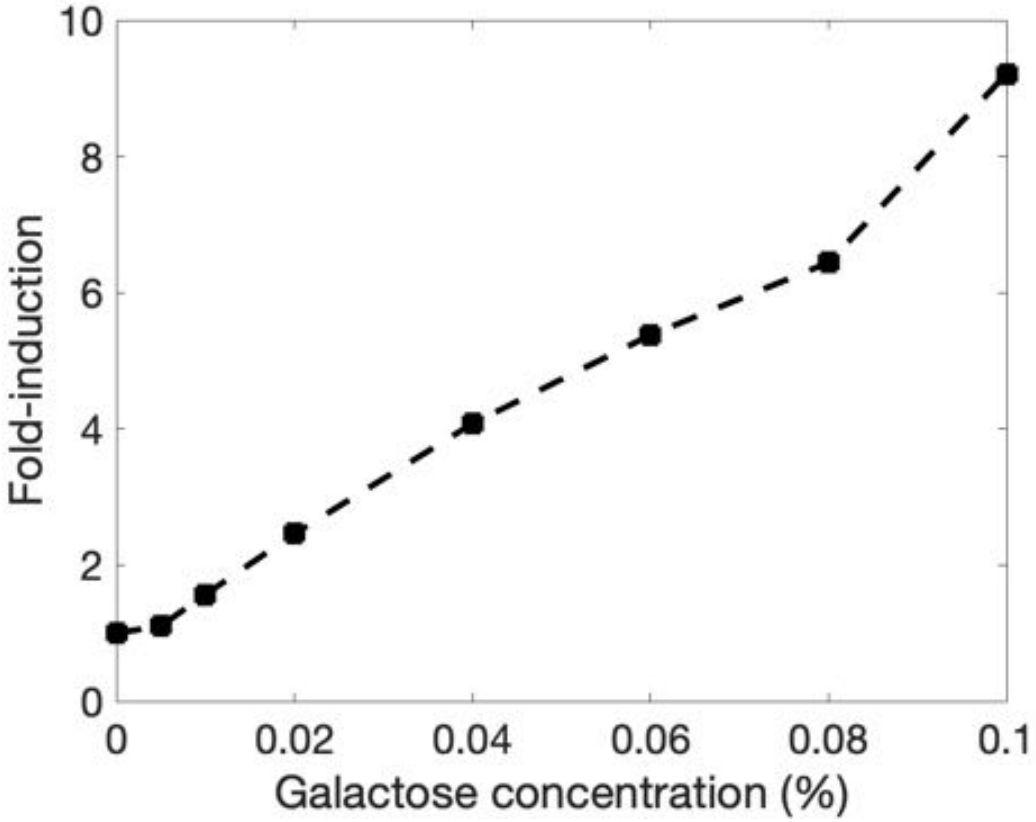

Figure 2-8. Expression of mNG under pGAL1 is linear with galactose concentration in gal2Δ background. SYNZIP17-mNG was expressed under pGAL1 and grown overnight in synthetic complete media supplemented with 1% raffinose and a variable amount of galactose. No glucose was added to the media, as glucose represses the *GAL1* promoter. The expression of mNG was normalized first to the OD_{600} of the culture, which was between 0.12 and 0.16. This fluorescence value was then normalized to the expression level at 0% galactose. At higher galactose concentrations (2%) we have seen fold-induction values of ~30.

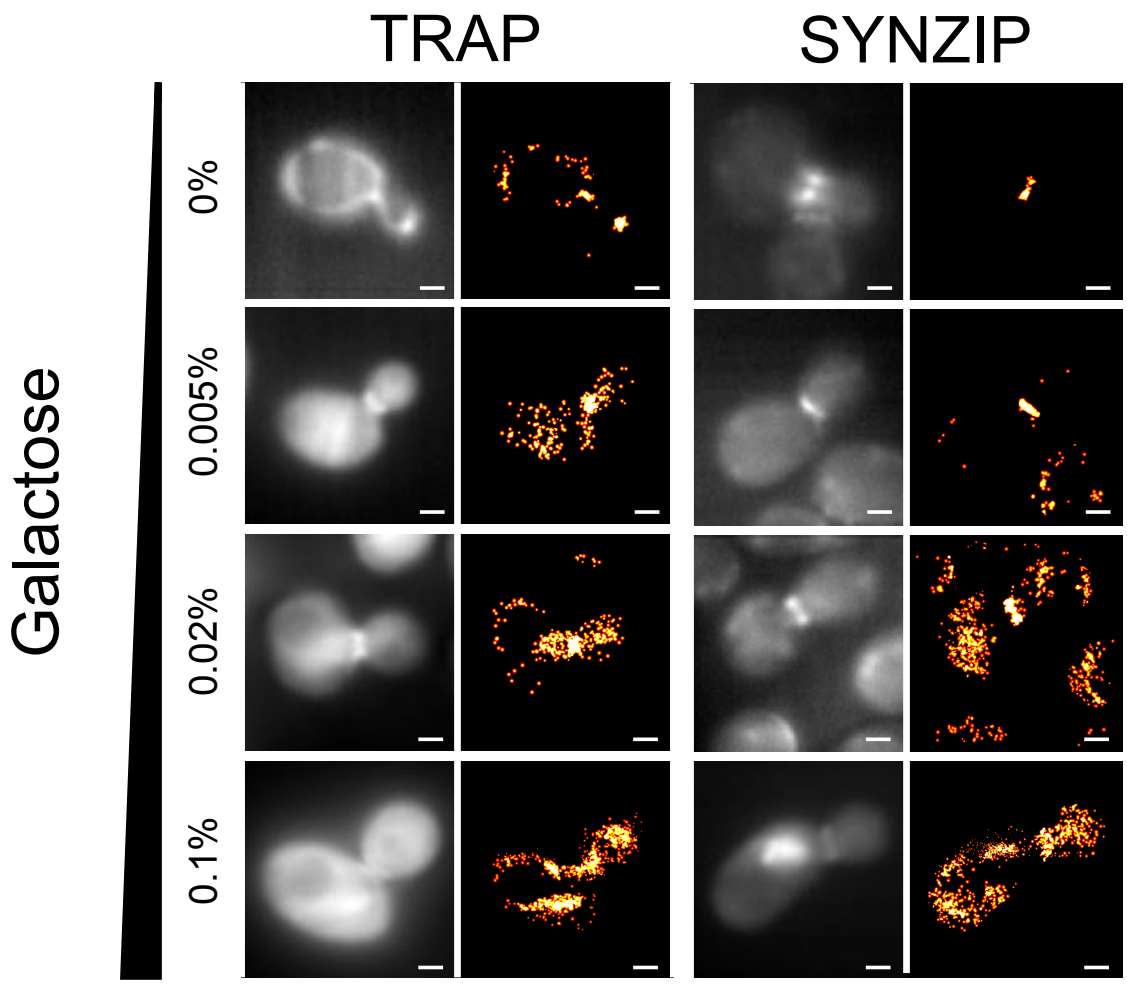

Figure 2-9. Varying FP expression level and interaction pairs affects specificity of localization events. Pairs of diffraction-limited and super-resolution images are shown for Cdc12p-MEEVF + TRAP4-mNG (left) and Cdc12p-SYNZIP18 + SYNZIP17-mNG (right), at different concentrations of galactose (as indicated on the left). All images were generated from 1,000 frame videos, with each frame having an exposure time of 50 ms and a laser power density of 3.1 W/cm^2. Percent of localizations in septum, at different concentrations of galactose, for Cdc12p-MEEVF + TRAP4-mNG: 0% galactose - 15%; 0.005% galactose - 45%; 0.02% galactose - 38%; 0.1% galactose - 23%. Percent of localizations in septum, at different concentrations of galactose, for Cdc12p-SYNZIP18 + SYNZIP17-mNG: 0% galactose - 94%; 0.005% galactose - 98%; 0.02% galactose - 43%; 0.1% galactose -19%. See Supplementary Table 1 for total number of localizations per image. Scale bars are 1 μm.

2.5 Septum width increases as daughter to mother ratio increases

To demonstrate the potential of LIVE-PAINT, I show an example of how it can be used to study a biological structure in live cells. By analyzing SMLM data for Cdc12 in individual cells, obtained using LIVE-PAINT with the SYNZIP17-SYNZIP18 interaction pair, I am able to describe various features of the yeast budding process. For example, I find that for small daughter cell sizes (daughter:mother diameter ratio less than approximately 0.85), the septum width is of the order 200 nm. As the daughter cell gets larger (daughter:mother diameter ratio approximately 0.85 to 1.0), the septum is clearly visible as two separate rings, with a septum width of approximately 400-800 nm. See Figure 2-10. This example demonstrates that LIVE-PAINT can be used to study a biological structure in live cells on the single cell level.

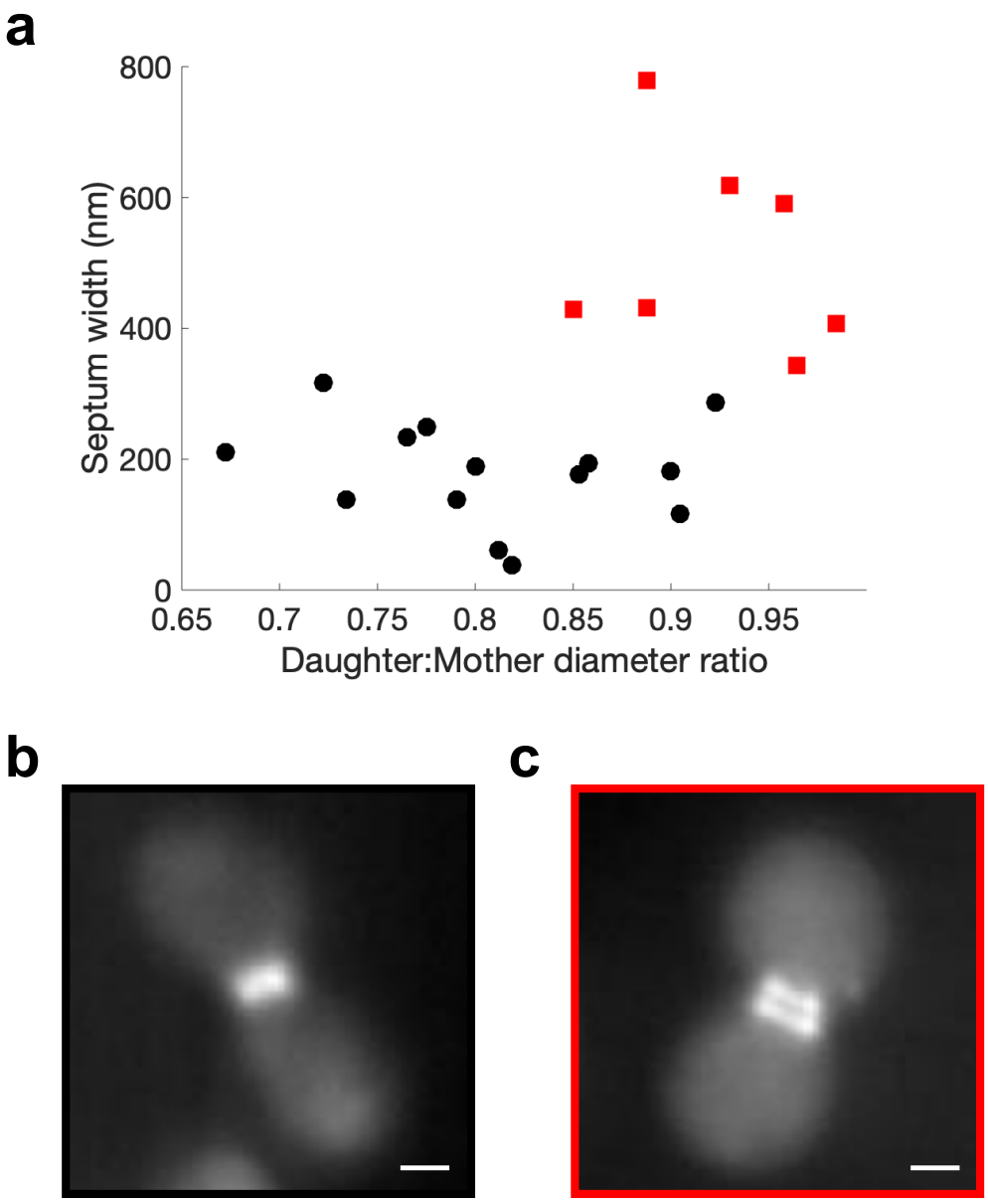

Figure 2-10. Yeast septum width increases with daughter:mother diameter ratio. (a) septum width plotted as a function of yeast daughter:mother diameter ratio, with single-ring septa plotted with black dots and double-ring septa plotted with red squares. See methods for how I determined septum width. Single-ring and double-ring septa were readily identifiable from fluorescence images of single cells. (b) and (c) show representative fluorescence images for single-ring and double-ring septa, respectively. Scale bars are 1 μm.

2.6 Multiple tandem mNG improves localization precision

In current super-resolution imaging techniques used inside live cells, such as PALM, the target protein is directly fused to an FP. This fusion adds a large, 25 kDa, modification to the target protein. Trying to enhance the PALM signal by fusing three FPs to the same target protein, would increase the size of the overall protein by about 75 kDa. Many proteins are unable to fold and correctly mature to their functional state when fused to a single FP, therefore a larger modification to a target protein, on the order of 75 kDa would likely be even more detrimental.

With the LIVE-PAINT method, however, the protein of interest is labeled post-translationally and reversibly. Thus, labeling with multiple tandem FPs should be more feasible. I performed LIVE-PAINT on Cdc12-SYNZIP18 using the SYNZIP17 fused to one or three tandem copies of mNG and compared the super-resolution data obtained for both conditions (Figure 2-11 and Figure 2-12). Cdc12 not only tolerates such post-translational labeling with the three tandem mNG, but labeling with this construct results in better localization precision. I note, however, that the larger size of the three tandem mNG construct creates additional distance between the protein of interest which would result in increased uncertainty about its actual position.

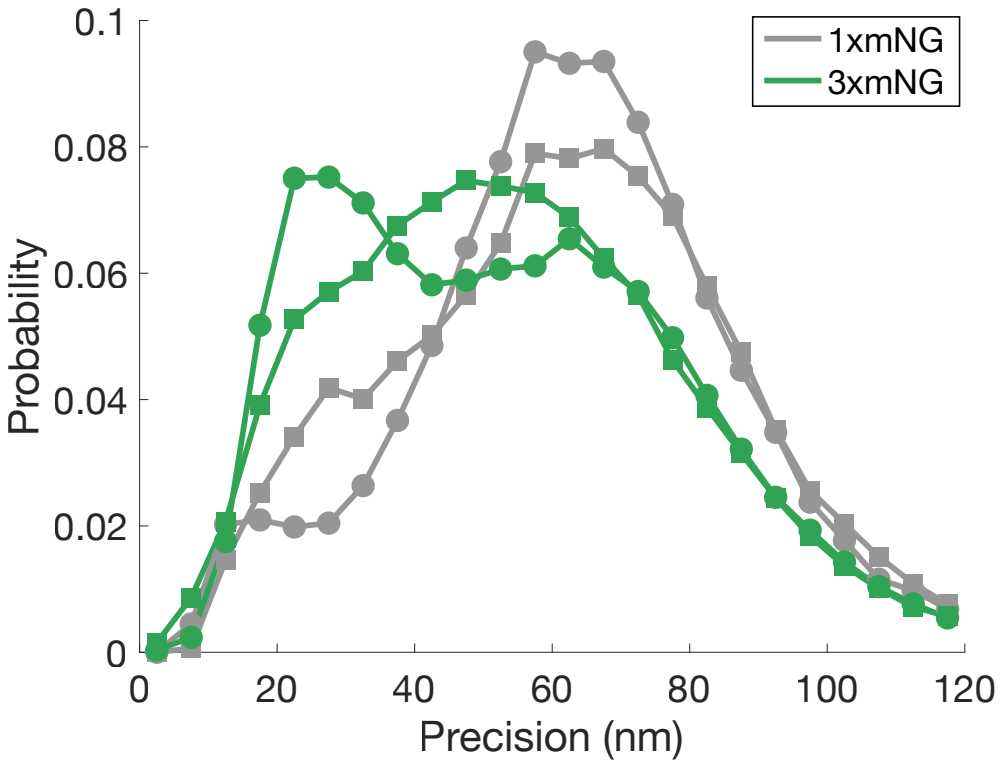

Figure 2-11. SYNZIP17-3xmNG shows improved localization precision compared with SYNZIP17-1xmNG. Full distribution of localization precision shown for Cdc12p-SYNZIP18 + SYNZIP17-3xmNG (green line; circles and squares indicate two replicates) and Cdc12-SYNZIP18 + SYNZIP17-1xmNG (gray line; circles and squares indicate two replicates). Both experiments were performed by expressing the fluorescent protein construct using 0.005% galactose in the yeast growth media. The curves show the average of replicates for both the 3xmNG and 1xmNG constructs (n = 2 biological replicates), while the data points for both replicates are given as spots. Microscopy videos used to generate this data was collected for 4,000 frames, with a 50 ms exposure per frame and a laser power density of 3.1 W/cm^2. The mean precision values of the 1xmNG replicates (63.4 nm and 63.9 nm) is significantly different from the mean precision of the 3xmNG replicates (52.2 nm and 53.1 nm), a with p-value of 0.05 as determined by a one-tailed t-test (see methods). When comparing the medians between 1xmNG replicates (63.1 nm and 63.5 nm) and 3xmNG replicates (52.2 nm and 53.1 nm), we obtained a similar result, with a p-value of 0.038. The number of localizations events for each curve is: 1xmNG: 31503 (gray circles) and 28053 (gray squares); 3xmNG: 56269 (green circles) and 69565 (green squares). Precision determined using GDSC SMLM Fiji plugin as described in section 4.10.

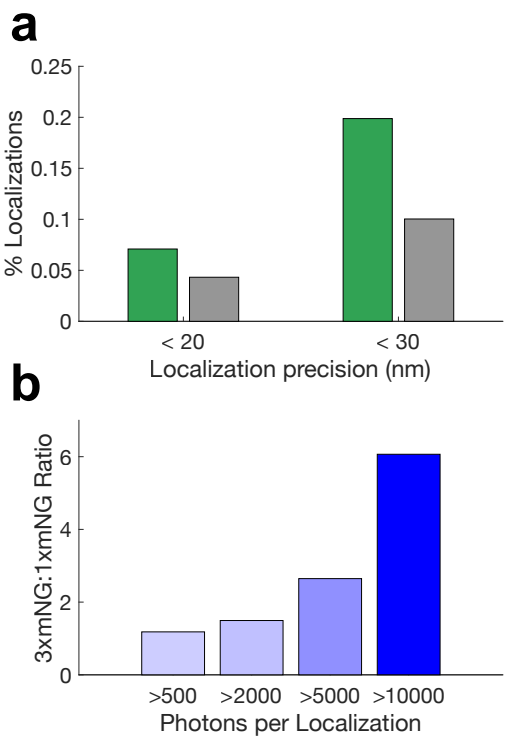

Figure 2-12. Three tandem copies of mNeonGreen (3xmNG) shows improved localization precision compared with a single copy of mNG fused to the cognate peptide binding protein (a) Percentage of localizations with precision values < 20 nm or < 30 nm. Green bars represent data for 3xmNG, and the gray bars represents data for 1xmNG. (b) LIVE-PAINT with 3xmNG gives higher numbers of localizations with a large number of photons than with mNG. The 3xmNG:mNG ratio of number of localizations is plotted for each 'photons per localization' bin. The darker the blue bar, the greater the enrichment in probability for 3xmNG compared with mNG in that bin. Data was collected over a field of view including multiple cells for both the 1xmNG and 3xmNG data, and two technical replicates were collected. Microscopy videos used to generate this data was collected for 4,000 frames, with a 50 ms exposure per frame and a laser power density of 3.1 W/cm^2. This data was combined for this analysis. Number of localizations events: 1xmNG: 59556; 3xmNG: 125834.

2.7 LIVE-PAINT enables longer data acquisition times

An additional advantageous feature of the LIVE-PAINT method is that it allows bleached fluorescent labels to exchange with unbleached fluorescent labels, *in vivo*. In the case of STORM and PALM imaging methods, photobleaching of the probe adds a limitation to the number of emitters that can be localized. This photobleaching reduces the resolution of the image because it limits the density of emitters that can be measured. Thus, researchers have to resort to using localization events with lower signal to noise than is optimal. In many cases, control of the emission is difficult to achieve, and much of the fluorescent probe is bleached early in the acquisition when individual emitters cannot be discerned due to their density being too high, further limiting the density of localizations measured. Here I demonstrate the ability to image for longer periods of time with LIVE-PAINT, using the SYNZIP labeling pair.

When imaging using a conventional direct fusion of Cdc12 to mNG, I observe that after I deliberately photobleach by irradiating with high laser power for two minutes, very few localization events are subsequently observed. In contrast, when using SYNZIP17-SYNZIP18 to localize mNG to Cdc12, after I deliberately photobleach by irradiating with high laser power for two minutes, I subsequently observe many more new localization events. For the direct fusion of Cdc12 to mNG, I measured an average of 1,802 localizations per cell before bleaching and 181 after bleaching; when using the SYNZIP17-SYNZIP18 interaction pair to perform the same

imaging, I measured an average of 1,536 localizations before bleaching and 289 after bleaching. This indicates that the bleached SYNZIP17-mNGs can unbind and be replaced by unbleached SYNZIP17-mNGs from the cytoplasm. This result shows that the LIVE-PAINT imaging strategy allows one to obtain more total localization events during an imaging session, because they allow for longer imaging times (Figure 2-13). The individual cells imaged using LIVE-PAINT for the data in Figure 2-13 were measured to have a resolution of ~20 nm (see Figure 2-14 for maximum projection images of the individual cells analyzed in Figure 2-13).

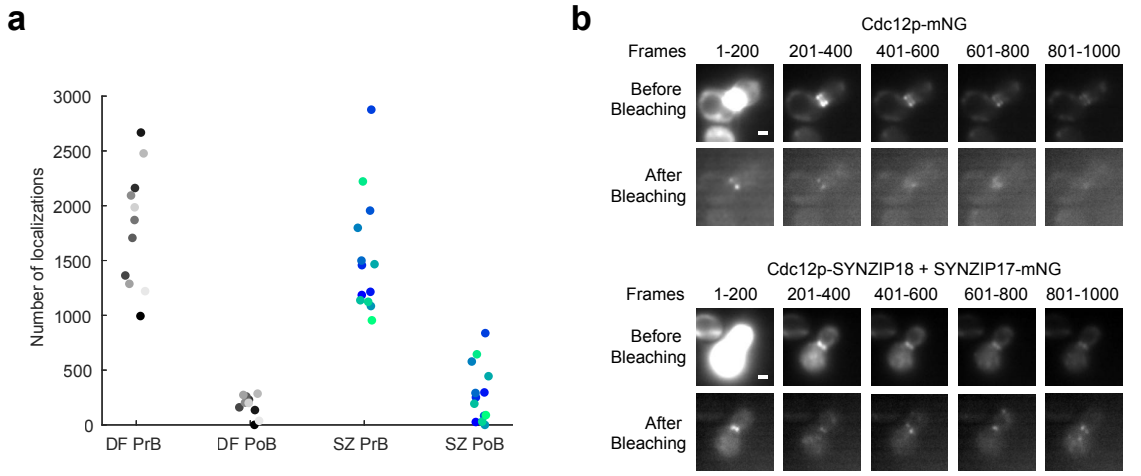

Figure 2-13. LIVE-PAINT shows recovery of signal after bleaching. (a) LIVE-PAINT interaction pairs show more recovery in number of localization events than a direct fusion to a fluorescent protein. In this experiment, fluorescence images were collected for 1,000 frames (50 s) at standard imaging power (3.1 W/cm^2), then the sample was photobleached using high laser power (26.6 W/cm^2), and then the sample was again imaged for 1,000 frames (50 s) at standard imaging power. Cdc12p-SYNZIP18 + SYNZIP17-mNG (blue/green circles, each representing a single cell) retain many more localization events than Cdc12p-mNG (gray circles, each representing a single cell) after two minutes of photobleaching. Each shade of gray or blue/green represents a single cell, which can be color-matched between pre-photobleaching (PrB) and post-photobleaching (PoB) conditions. DF = Cdc12p-mNG (Direct Fusion); SZ = Cdc12p-SYNZIP18 + SYNZIP17-mNG (SYNZIP pair). (b) Maximum projections for different frame ranges in both “before bleaching” and “after bleaching” videos demonstrate that signal obtained after bleaching continues to localize to the yeast septum. (Top) Maximum projections are shown for 200 frame ranges for a representative cell expressing Cdc12p-mNG. (Bottom) Maximum projections are shown for a representative cell expressing Cdc12p-SYNZIP18 + SYNZIP17-mNG. All “before bleaching” images are normalized to one another and, similarly, all “after bleaching” images are normalized to one another. Scale bar is 1 μm.

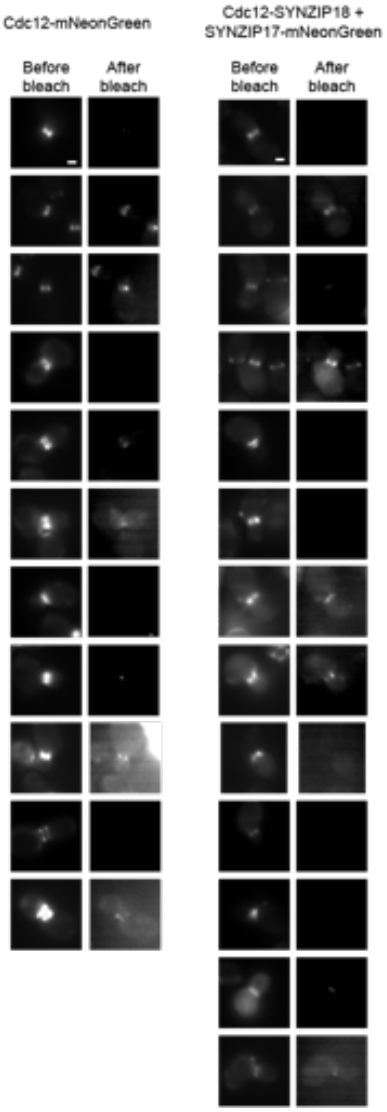

Figure 2-14. Maximum projection images generated from videos collected before and after bleaching, for all cells analyzed for the data shown in Figure 2-13. Left) Maximum projection images are shown for cells expressing Cdc12p-mNG. (Right) Maximum projection images are shown for cells expressing Cdc12p-SYNZIP18 + SYNZIP17-mNG. All images were constructed from videos collected for 1,000 frames, with a 50 ms exposure per frame and a laser power density of 3.1 W/cm^2. Scale bar is 1 μm.

2.8 Increasing exchangeable label extends data acquisition times

The data in Figure 2-13 shows that reversible interaction pairs can unbind from the target protein and signal can be replenished by free protein-mNG binding to the target protein.

Building on this result, I compared how long data collection can be continued, when there is a high versus low level of peptide-binding protein-mNG in the cytoplasm. Figure 2-15 shows the results of such experiments for both the SYNZIP17-SYNZIP18 and TRAP4-MEEVF interaction pairs. For 0% galactose, where the expression level of peptide-binding-module-FP is low, almost all the binding-module-FP will be initially bound to Cdc12, thus all FPs will be illuminated and bleached rapidly, because there is not a cytoplasmic pool of peptide-binding-protein-FP for them to exchange with. By contrast, for 0.1% galactose, where the expression level of the peptide-binding protein-FP is high, there is a sizeable cytoplasmic pool available to exchange with molecules bound to peptide-Cdc12, but which have been bleached. In Figure 2-15b, for example, I observe that when imaging Cdc12-SYNZIP18 + SYNZIP17-mNG using 0.1% galactose, even after 200 s of imaging, localizations are still being recorded at about 30-40% of the initial rate.

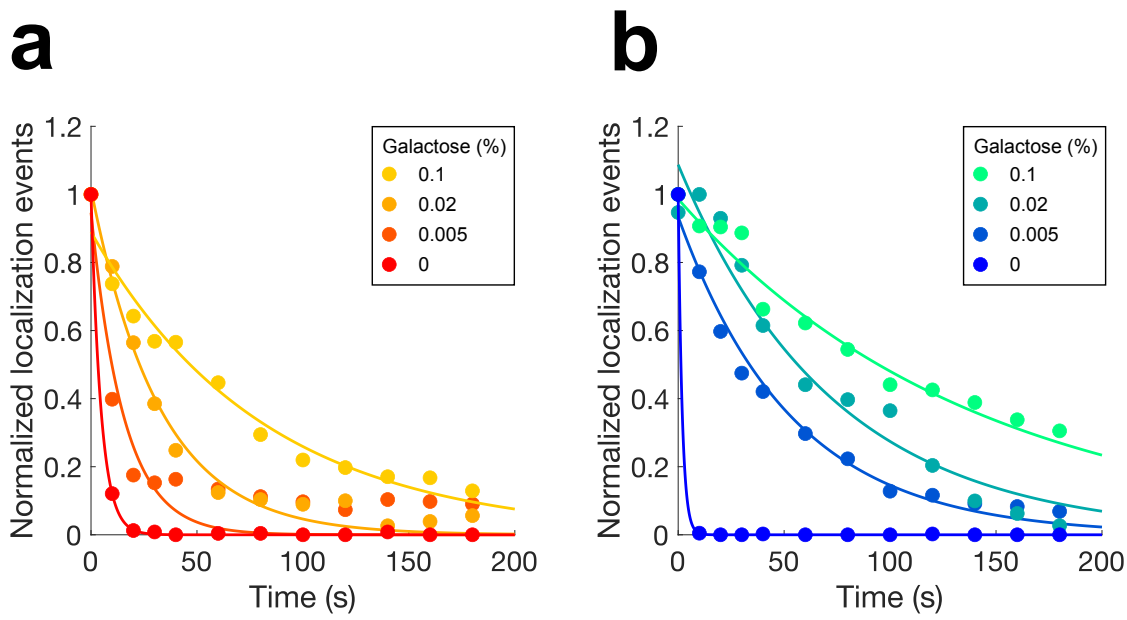

Figure 2-15. Localization rate decays more slowly with increased FP expression. Localization rate as a function of imaging time for (a) Cdc12p-MEEVF + TRAP4-mNG and (b) Cdc12p-SYNZIP18 + SYNZIP17-mNG, each at four different concentrations of galactose. Data for the MEEVF-TRAP4 interaction pair is for 0% galactose (red), 0.005% galactose (dark orange), 0.02% galactose (light orange) and 0.1% galactose (yellow). Data for the SYNZIP18-SYNZIP17 interaction pair is for 0% galactose (bright blue), 0.005% galactose (blue), 0.02% galactose (teal) and 0.1% galactose (mint). All images were generated from 4,000 frame videos, with each frame having an exposure time of 50 ms and a laser power density of 3.1 W/cm^2. The data for each concentration of galactose were fit to a single exponential (shown as a solid line with matching color). For the MEEVF-TRAP4 interaction pair (a), the exponential time constant (τ) for the different concentrations of galactose is 0%: 4.7 s; 0.005%: 15 s; 0.02%: 32 s; 0.1%: 81 s. For the SYNZIP18-SYNZIP17 interaction pair (b), the exponential time constant (τ) for the different concentrations of galactose is: 0%: 1.9 s; 0.005%: 54 s; 0.02%: 73 s; 0.1%: 139 s.

2.9 Difficult to tag proteins can be labeled using LIVE-PAINT

Actin (Act1p), an important cytoskeletal protein, is notoriously difficult to tag and image. A number of different methods have been developed to circumvent this problem, but they are not without issues, including changing the stability, dynamics, and lifetime of Act1p structures[93, 99, 100]. Direct fusion of Act1p to the photoconvertible fluorescent protein mEos, expressed alongside unmodified Act1p, has been used to image Act1p using PALM[101, 102]. The mEos protein is a rather large addition to Act1p, and undoubtedly results in some perturbation of function (as evidenced by cells expressing only Act1p-mEos, in the absence of any unmodified Act1p, being unviable).

It is therefore desirable to use a tagging method which requires a smaller fusion to Act1p than the full mEos protein. LifeAct, a peptide that binds to the polymerized form of Act1p, and not the unpolymerized form, has been used to image actin. Because LifeAct binds wildtype actin, imaging can be performed without making any genetic modifications to actin. However, using LifeAct to image actin results in a perturbation to the equilibrium distribution of Act1p forms in the cell[99]. Nevertheless, the binding and unbinding of LifeAct has been used to image Act1p filaments in live cells using a PAINT-like approach[103]. I note and reference this result, however polymerized Act1p is the only protein that can be imaged using LifeAct, LIVE-PAINT can be applied to any protein, including Act1p, and I present its application to Act1p to provide another possible tool for actin researchers.

Wild-type Act1p was chromosomally expressed from its endogenous promoter. We expressed SYNZIP18-Act1p from a low copy number plasmid, using a copper-inducible promoter. SYNZIP17-mNG was expressed, as previously, from the galactose inducible promoter, chromosomally integrated at the *GAL2* locus (Figure 2-16).

Using LIVE-PAINT, I was able to readily visualize actin patches, which assemble at the cell membrane, at sites of endocytosis[104] (Figure 2-16a). Because actin structures are quite dynamic, I investigated how quickly I could obtain super-resolution images (compared to the acquisition time of 200 s for the data shown in Figure 2-16c). Actin rings, or actin cables that span the cell, are likely not observed because I am imaging in Total Internal Reflection Fluorescence (TIRF), which illuminates only about 200 nm into the cell (a typical yeast cell is 1-3 μm thick). Alternatively, or additionally, it could be that the stringent structural requirements for actin in these assemblies means that even actin with very small ~2 kDa tags may be excluded from ring and cable structures[105].

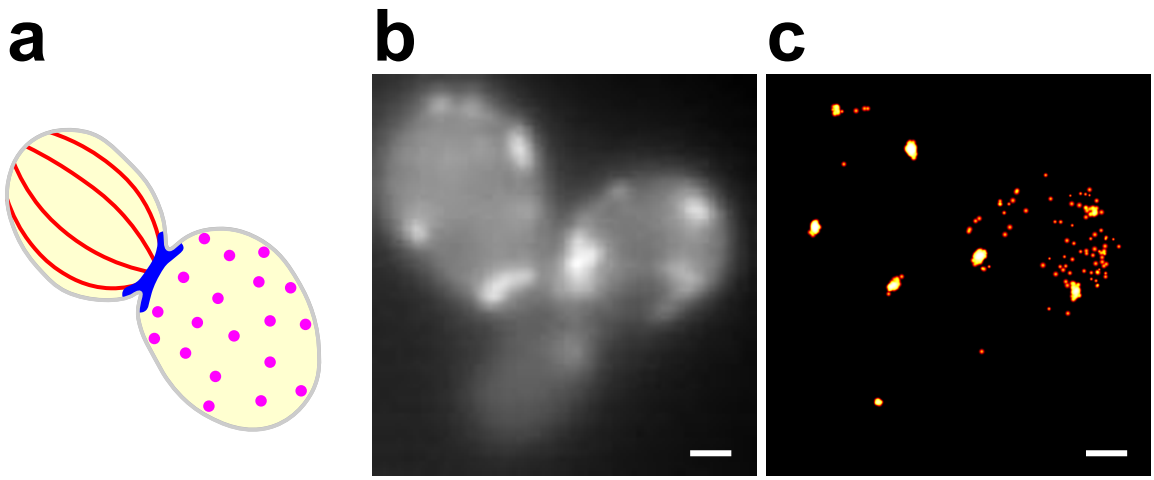

Figure 2-16. Actin patches can be imaged using LIVE-PAINT in live yeast. a) Cartoon showing the three distinctive actin structures that have been observed in fixed and immunostained *S cerevisiae*: actin cables (red), actin rings (blue), and actin patches (magenta). (b) Diffraction limited image of SYNZIP18-Act1p + SYNZIP17-mNG. (c) LIVE-PAINT super-resolution image constructed from 200 s video imaging SYNZIP18-Act1p + SYNZIP17-mNG (50 ms exposure per frame and a laser power density of 3.1 W/cm^2). Number of localization events obtained: 778. Only localization events with precision < 30 nm were used to construct the super-resolution image. Scale bars are 1 μm.

2.10 LIVE-PAINT enables long tracking times *in vivo*

In the data presented so far, I have used LIVE-PAINT to generate super-resolution images of proteins which do not move significantly during the period of data acquisition. In some cases, the protein-of-interest may move on the timescale of imaging, and whilst an increase in the imaging frame rate could resolve this to some extent, it may not always be possible if the proteins move too quickly. The extended imaging lifetime enabled by LIVE-PAINT, however, offers the opportunity to detect and track the motion of diffusing molecules within live cells. Cofilin (Cof1p) is an important protein that binds to actin filaments promoting severing[92]. It has so far, however, proven difficult to image due to its function being affected by either N- or C-terminal direct fusion of a fluorescent protein[92]. I therefore C-terminally tagged Cof1p with SYNZIP18, in the same method I used previously to tag and image Cdc12p, and tracked it using the LIVE-PAINT strategy (diffraction-limited, not super-resolution). In collaboration with Mathew Horrocks, I was able to observe the diffusion of Cof1p during the 100 s of imaging (Figure 2-17). We observed a wide range of behaviors (Figure 2-17).

Cof1p was tolerated by the cell as observed by no reduction in growth rate and it localized to distinct spots near the periphery of the cell as expected due to its known association eisosomes. The success of the LIVE-PAINT tagging approach in these examples demonstrates the value of the method for visualizing proteins

that are refractory to direct fusion to an FP[92], and also its potential to be developed to track moving proteins.

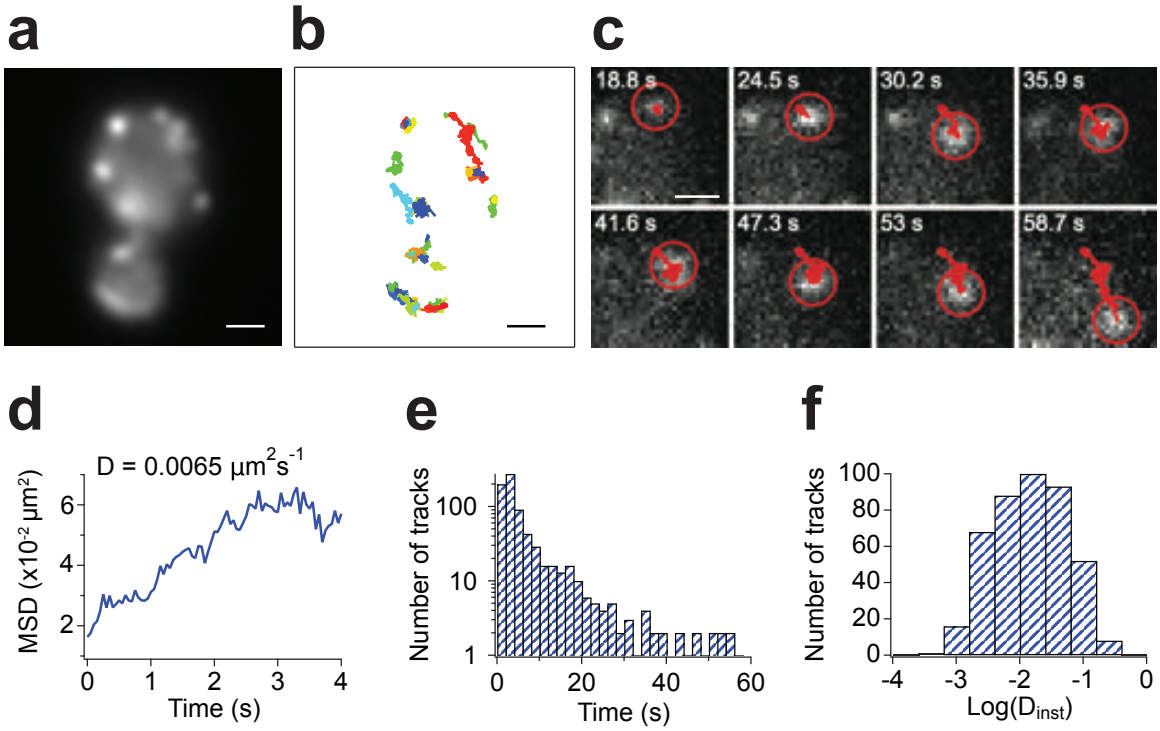

Figure 2-17. Clusters of cofilin can be tracked using LIVE-PAINT. (a) Diffraction-limited image of example live yeast in which Cof1p was tagged and tracked. Larger field of view shown in Figure 2-18. (b) Individual tracks of Cof1p from the cells shown in (a). The yeast cells were imaged for 100 s (50 ms per frame) and a laser power density of 3.1 W/cm^2, and each colored track corresponds to an individual diffusing cluster. (c) Example montage of one of the diffusing Cof1p clusters. (d) The mean squared displacement of the tracked Cof1p cluster shown in (c). (e) Histogram of track lengths from all clusters detected in the cells in Figure 2-18. The tracks last for 6.59 ± 9.83 s (mean ± S.D., n = 426). (f) Histogram of the diffusion coefficients of the tracked Cof1p clusters. D = 0.031 ± 0.043 μm^{2}s^{-1} (mean ± S.D., n = 426). Scale bars are 1 μm.

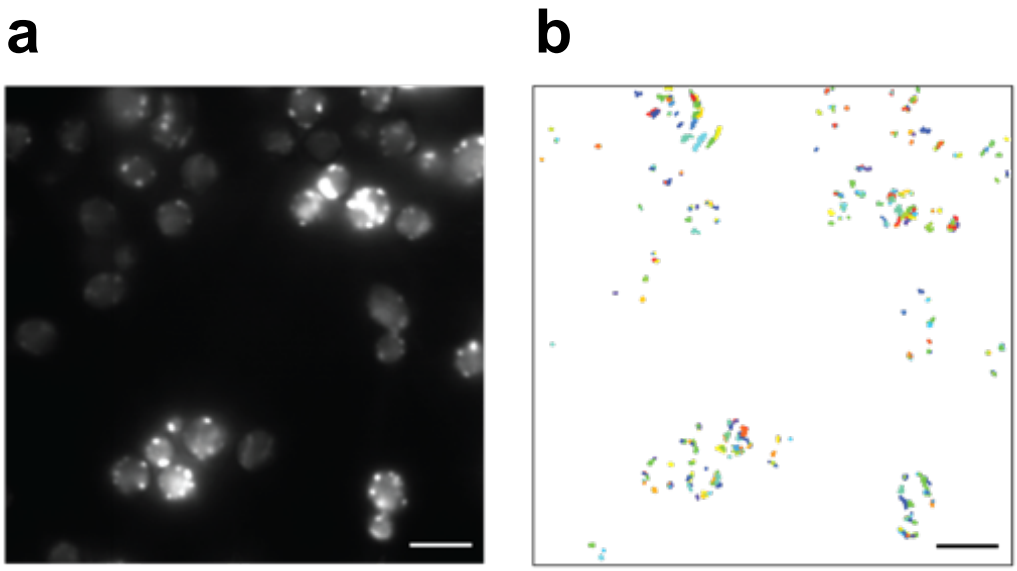

Figure 2-18. Tracking of cofilin in yeast cells. (a) Diffraction-limited image of the Cof1p in the yeast cells. (b) Tracks from individual Cof1p clusters. The image was constructed from a video collected for 4,000 frames, with a 50 ms exposure per frame and a laser power density of 3.1 W/cm^2. Scale bar is 5 μm.

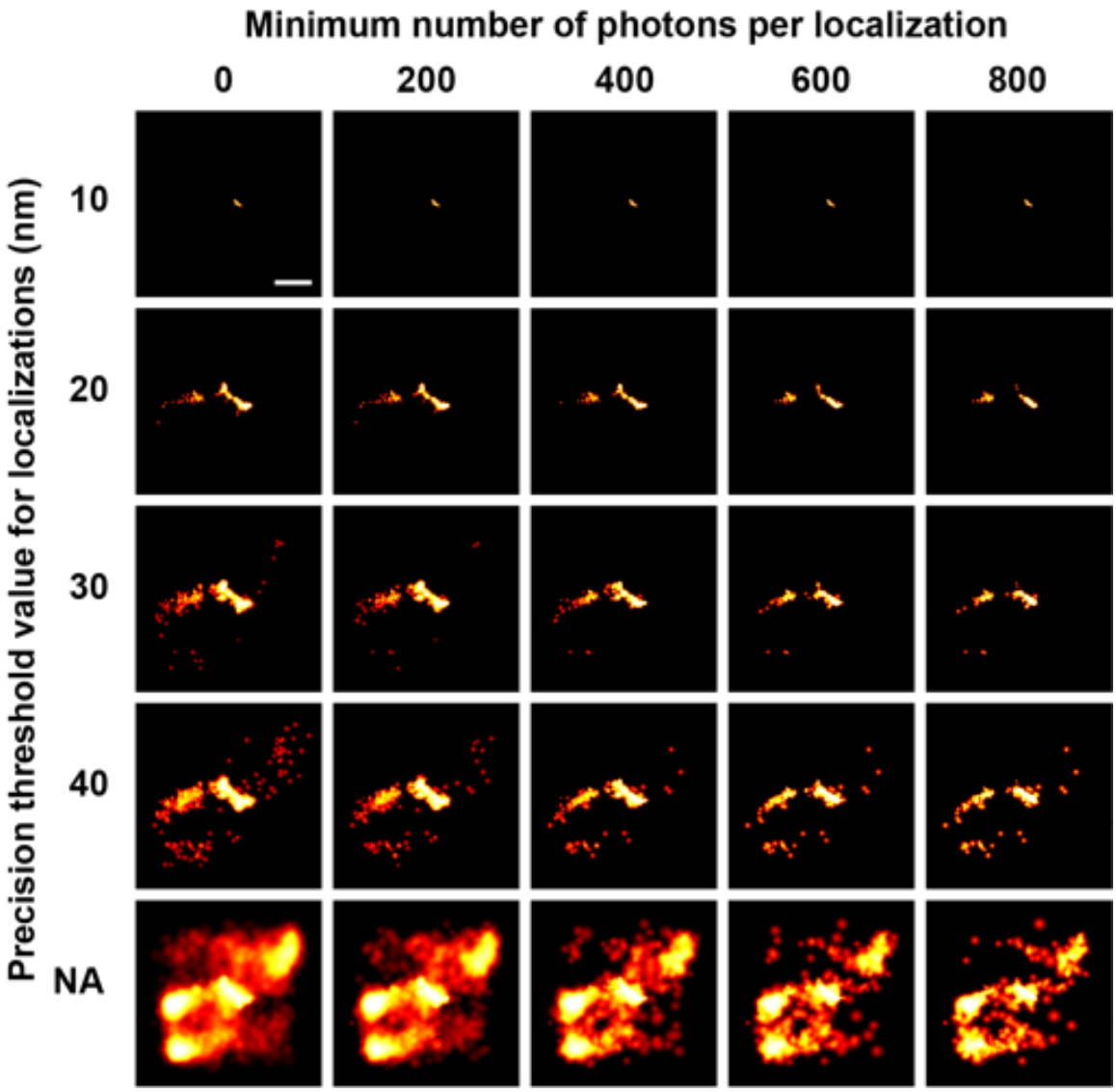

Figure 2-19. One stack of images analyzed using different thresholds for localization precision and minimum number of photons per localization. NA indicates no precision value specified. The video used to construct the images collected for 6,000 frames, with a 50 ms exposure per frame and a laser power density of 3.1 W/cm^2. Number of localizations for each precision value, from left to right in the image: 10 nm: 46, 46, 46, 46, 46; 20 nm: 633, 617, 497, 328, 247; 30 nm: 1386, 1169, 746, 438, 330; 40 nm: 2013, 1547, 887, 526, 392; NA: 6248, 3764, 1855, 1089, 724. Scale bar is 1 μm.

2.11 Discussion

I have developed an imaging strategy, LIVE-PAINT, which enables a new approach to super-resolution imaging inside live cells. I have demonstrated the effectiveness of LIVE-PAINT, which makes use of reversible protein-peptide interactions to obtain SMLM in live *S. cerevisiae*. The data I obtained for Cdc12p enabled me, for example, to quantitatively track the width of the septum at the bud neck of budding yeast as a function of daughter:mother cell diameter ratio and showed that septum width does not change significantly until the daughter diameter reaches approximately 0.85 of the mother cell diameter, at which point the septum divides into two distinct rings.

LIVE-PAINT has a number of advantages over existing super-resolution imaging methods. The main advantage over DNA-PAINT is that LIVE-PAINT works inside living cells; all the components that I describe are chosen to function in that *milieu*. Also, LIVE-PAINT is extendible to concurrent tagging of multiple protein targets (Figure 2-3, Figure 2-5, Figure 2-6, Figure 2-7). Recently, interacting charged coiled coil pairs have been used to label proteins to perform PALM imaging in live mammalian cells[61]. This work provides a valuable independent validation of my approach.

LIVE-PAINT requires neither photo-conversion of fluorophores (as PALM does) nor selective deactivation of fluorophores as stimulated emission depletion (STED) does. Not only do these methods require special instrumentation, but the high laser

power that is typically required can often cause cell damage during live cell imaging experiments, in addition to bleaching of fluorophores[106]. LIVE-PAINT is performed inside living cells, typically in minimal growth medium, with no potentially toxic additions, such as oxygen scavengers, required. LIVE-PAINT requires only that the protein of interest is directly fused to a small peptide tag, a strategy with a number of advantages. Labeling is post-translational, and therefore the method is suitable for labeling proteins for which direct fusion to a larger FP abrogates function[87]. Other approaches to performing PAINT in live cells, such as protein-PAINT, require the addition of organic dyes, cannot be used to image multiple targets simultaneously, and require a larger fusion to the target protein[58].

The intensity of the signal from each localization event can be increased, by using a tandem array of FPs attached to the peptide-binding protein. It is also straightforward to change the identity of the FP, without needing to change the peptide fusion to the protein to be imaged. Because LIVE-PAINT does not rely on the use of photoactivatable proteins, any FP can be used. This flexibility in choice of FP means that the method could be extended to concurrent super-resolution imaging of multiple targets. One of the limitations of existing live cell super-resolution methods is the reliance on FPs that are all very spectrally similar to one another, which prevents accurate imaging of multiple target proteins concurrently. Even though recent methods have been extended to image two targets in live cells, they require harsh oxygen scavengers and are limited to only two colors by the lack of additional orthogonal chemistries for attachment of dyes to protein tags

such as SNAP-tag[57]. LIVE-PAINT does not require oxygen scavengers and is limited only by the number of orthogonal peptide-protein interaction pairs and the number of available spectrally distinct FPs, both of which are abundant.

Importantly, the protein of interest is expressed from its endogenous promoter and the conditions for detection of fluorescence localizations are optimized by adjusting the intracellular concentration of the peptide-binding protein-FP that is used for labeling. This means that for a very abundant protein of interest, for example, in LIVE-PAINT the number of fluorescent proteins can be reduced by reducing the expression level of the peptide-binding protein-fluorescent protein, instead of having to photobleach some of the fluorescent proteins to reduce the number so that individual fluorescent proteins can be localized. Similarly, for a low abundance protein of interest directly fused to a fluorescent protein, photobleaching is especially problematic, because the starting number of molecules is very low. In LIVE-PAINT, more localization events can be observed by imaging for longer, during which time any bleached peptide-binding protein-fluorescent proteins can be refreshed by exchange with an unbleached pool.

Finally, LIVE-PAINT, especially in a TIRFM (or light sheet fluorescence microscopy) format, enables data to be acquired for much longer than other current methods (such as PALM) that can be used inside live cells. In such methods, the fluorescent protein is directly fused to the protein of interest, so once a fluorophore is bleached, it is not replaced and from then onwards is dark. By contrast, in LIVE-

PAINT, the non-covalently bound fluorescent protein can be exchanged after bleaching, with a non-bleached fluorescent protein from the cytoplasm. Acquiring data for longer results in more localizations being detected and consequently higher resolution images being obtained. Unbound fluorescent proteins in LIVE-PAINT result in background fluorescence, but this effect can be mitigated by reducing the illumination volume in the cell, as we have done using TIRFM in this work, or by using other strategies, such as light-sheet fluorescence microscopy.

I have demonstrated the power of LIVE-PAINT in *S. cerevisiae* by using it to image Cdc12 and hence to study septum formation. Furthermore, I have used it to image Act1p and Cof1p, two important proteins that are intractable to direct fusion. Finally, I showed that this approach is fundamentally compatible with tracking the movement of individual proteins inside live cells.

I expect that it will be straightforward to extend LIVE-PAINT to other organisms and cell types. In this work, I found that four of the five peptide-pairs that I tested were suitable for LIVE-PAINT. Many more potentially compatible interaction pairs exist, and may be better suited for particular applications. In future work, I will investigate how the optimal labeling requirements differ for different cellular proteins and how best to label and image multiple proteins simultaneously.

3 Applications for LIVE-PAINT

3.1 Tagging membrane proteins

Many super-resolution methods require direct fusion of a fluorescent protein, which is on the order of 25 kDa in size, to a protein of interest[107]. This is a large fusion and many proteins do not tolerate such a large fusion well[87, 108].

One example of such a class of proteins is membrane proteins in *S. cerevisiae*. It is estimated that only 46 out of 139 plasma membrane proteins in *S. cerevisiae* correctly localize to the plasma membrane when directly fused to GFP at their C-terminus[35, 108]. Among those which do not correctly localize to the plasma membrane, many mislocalize to the vacuole and some are not visualized at all[108]. Thus, it would be advantageous to develop an imaging approach which would enable tagging and imaging of difficult-to-tag proteins such as membrane proteins. It would be doubly advantageous if this approach also enabled live cell super-resolution imaging of these membrane proteins.

This mislocalization of membrane proteins to the vacuole in *S. cerevisiae* can be remedied, in at least some cases, by fusing only a short peptide tag to the protein of interest. Recently, our lab has used the peptide-protein pair SpyTag-SpyCatcher to post-translationally tag the plasma membrane-associated protein Pma1p with an FP[87] in live *S. cerevisiae*. Pma1p typically shows mislocalization to the vacuole in yeast when directly fused to an FP. To post-translationally label Pma1p, first Pma1p was fused to SpyTag and an FP to SpyCatcher. When SpyTag associates

with SpyCatcher, it forms a spontaneous covalent bond[109]. Using this approach, Pma1p was covalently labeled with an FP and no longer mislocalized to the vacuole. Additionally, Pma1p-FP shows a severe growth defect and this was eliminated using the SpyTag-SpyCatcher tagging approach. This work demonstrates the potential for using peptide-protein tags to tag proteins in a less perturbative manner.

In other work, it has been demonstrated that plasma membrane proteins can be tagged using coiled coils and diffraction limited imaging can be performed[110], though this was not demonstrated for proteins which suffer mislocalization upon direct fusion to GFP and this approach was not used to perform super-resolution imaging.

Due to the small size of the peptides used in LIVE-PAINT, I rationalized that the LIVE-PAINT tagging method is likely to be less perturbative to plasma membrane protein localization than direct fusion to GFP. Additionally, because LIVE-PAINT uses transient peptide-protein interactions to achieve super-resolution imaging, this approach to tagging proteins of interest enables super-resolution imaging of these membrane proteins in live cells.

I chose three difficult-to-tag membrane proteins in yeast to tag using the LIVE-PAINT approach, using the 101A/101B coiled coil interaction pair. When tagged using the 101A/101B interaction pair, with 101A fused to mNG and 101B fused to the C-terminus of the membrane protein, the proteins correctly localize to the

plasma membrane (Figure 3-1b), instead of localizing to the vacuole as is observed when these same membrane proteins are directly fused to GFP.

In order to demonstrate that the improved localization to the plasma membrane is due to using the smaller, less perturbative peptide tag, rather than a fusion to GFP, I performed the following controls.

First, for two of the membrane proteins I tagged, I compared the effect of direct fusion to GFP to both the reversible tagging approach using 101A/101B and to a direct fusion to mNG. For one of the two target proteins, only the 101A/101B tagging approach was successful in achieving high specificity in plasma membrane labeling. In the other, both the direct fusion to mNG and the 101A/101B tagging approach achieved high specificity in plasma membrane labeling. This suggests that in some cases a direct fusion to mNG may be less perturbative to protein localization than direct fusion to GFP. However, the 101A/101B tagging approach was successful for both proteins, suggesting that it is superior to direct fusion of either GFP or mNG.

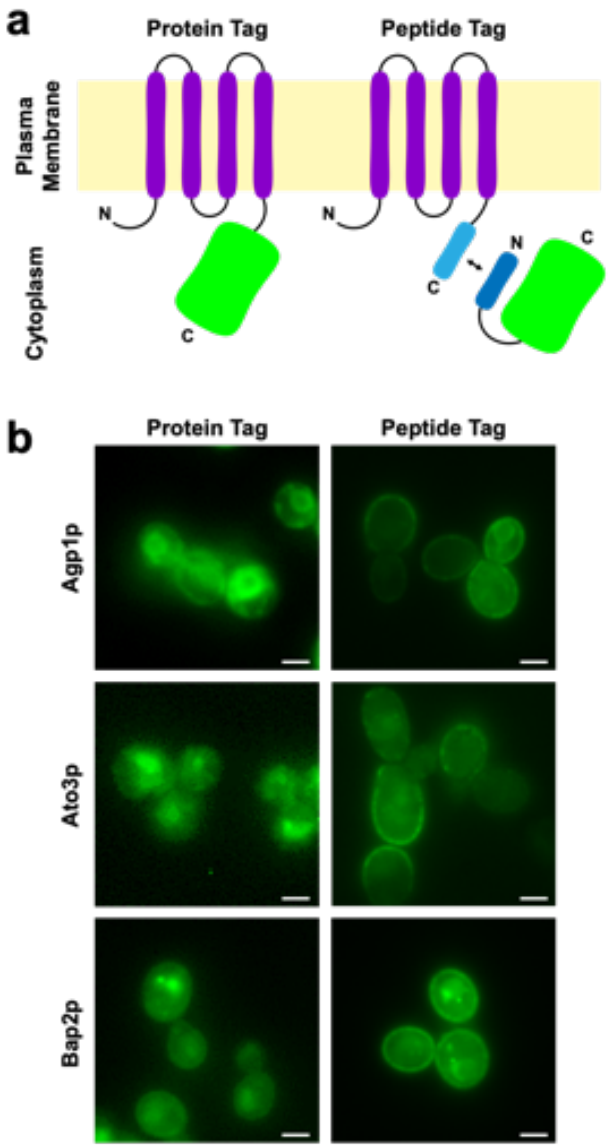

Figure 3-1. Approaches for tagging membrane proteins. (a) Membrane proteins (purple) can be directly fused to a fluorescent protein (green), using a protein tag approach (left). Alternatively, the membrane protein can be fused to one half of a coiled coil (light blue) and the other half of the coiled coil (dark blue) can be fused to a fluorescent protein and expressed separately. We call this the peptide tag approach. The membrane protein is then labeled by the reversible binding of the coiled coil pair. “N” and “C” indicate the N- and C-termini of the proteins. (b) Diffraction limited images of membrane proteins tagged using the protein tag approach (left), in which GFP is directly fused to the protein’s C-terminus. On the right, the peptide tag approach is shown, in which half of a coiled coil pair,

101A/101B is fused to the C-terminus of the membrane protein and the other half is fused to mNG and expressed in vivo. This is shown for three different membrane proteins. These cells were grown in 0.005% w/v galactose. Protein tag data is from the Yeast GFP Fusion Localization Database[108]. Scalebar is 2 μm.

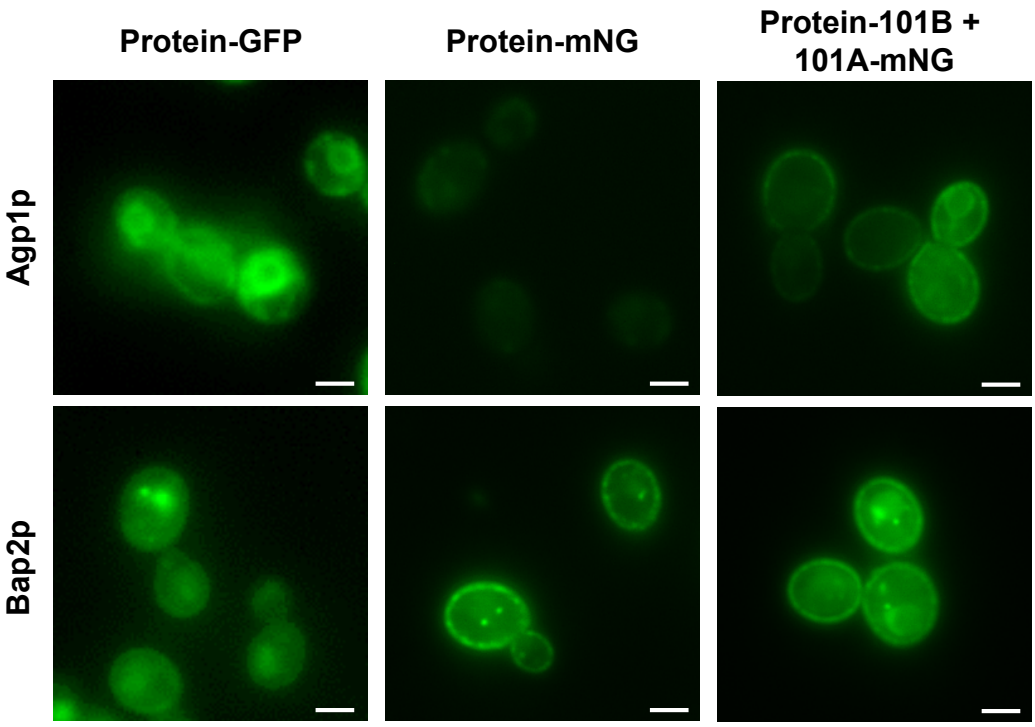

Figure 3-2. Direct fusion versus coiled coil tagging approaches. Fluorescent images of Agp1p (top) or Bap2p (bottom) proteins tagged with GFP (left), mNG (middle), or the coiled coil pair 101A/101B using mNG (right). Scalebars are 2 μm.

Next, I sought to demonstrate that correct localization to the plasma membrane was not due to an intrinsic propensity for the 101B coiled coil half to localize to the plasma membrane. As evidence of this, when I tagged other proteins of interest which are known to localize to other loci in the cell, they correctly localized to their expected loci (Figure 3-3).

Finally, because the 101A-mNG half of the imaging construct is expressed under control of the galactose inducible *GAL1* promoter, I used different concentrations of galactose in the media to induce varied expression levels of 101A-mNG. For the two membrane proteins I imaged for a range of galactose concentrations, a galactose concentration of 0.005% provided the best signal to background of the tested galactose concentrations (Figure 3-4). At higher galactose concentrations, the extra 101A-mNG appeared to begin collecting in the nucleus and at lower concentrations there was not significant plasma membrane signal relative to background fluorescence. This optimal concentration of galactose was also the same concentration of galactose that proved optimal for imaging during my original LIVE-PAINT experiments.

Together, this data demonstrates that tagging membrane proteins with reversible peptide-protein interaction pairs can enable them to be imaged in live cells when they do not tolerate direct fusion to GFP or other fluorescent proteins. This is highly beneficial, because so many membrane proteins mislocalize to the vacuole upon direct fusion to GFP.

This data also suggests that other proteins which do not correctly localize when fused to GFP would likely benefit from being tagged using reversible peptide-protein interactions.

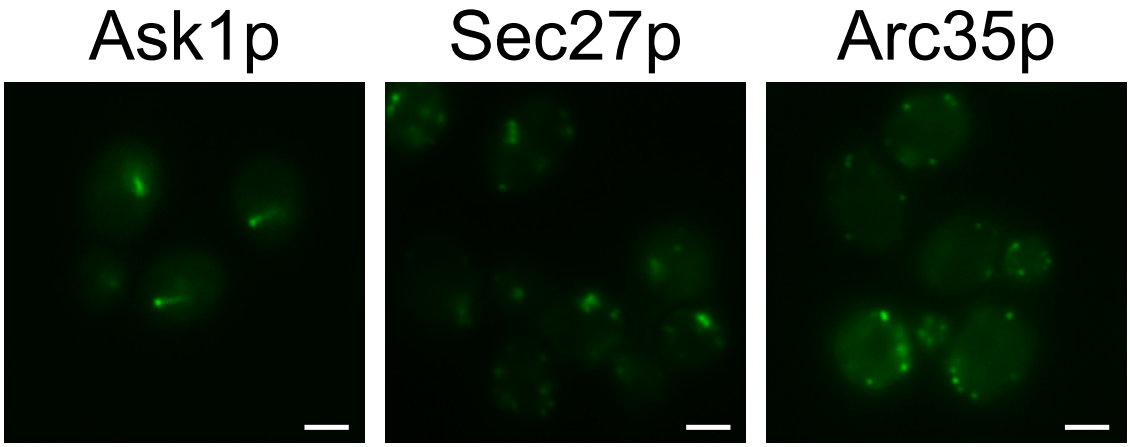

Figure 3-3. Proteins labeled and imaged using 101A/101B coiled coil pair associate to expected loci.

Proteins are fused to 101B at their C-terminus and 101A-mNG is co-expressed in the cell. Binding of 101A to 101B drives association of mNG to the tagged protein. Ask1p, part of the DASH complex, binds to microtubules and kinetochores and is present in cells in 1-2 distinct puncta[111]; Sec27p is involved in ER to golgi transport and localizes to ER and golgi membranes[112]; Arc35p is part of the Arp2/3 complex and localizes to site of actin branching[113]. The observed localization pattern matches that observed when these same proteins were directly fused to GFP and imaged[108]. Scalebars are 2 μm.

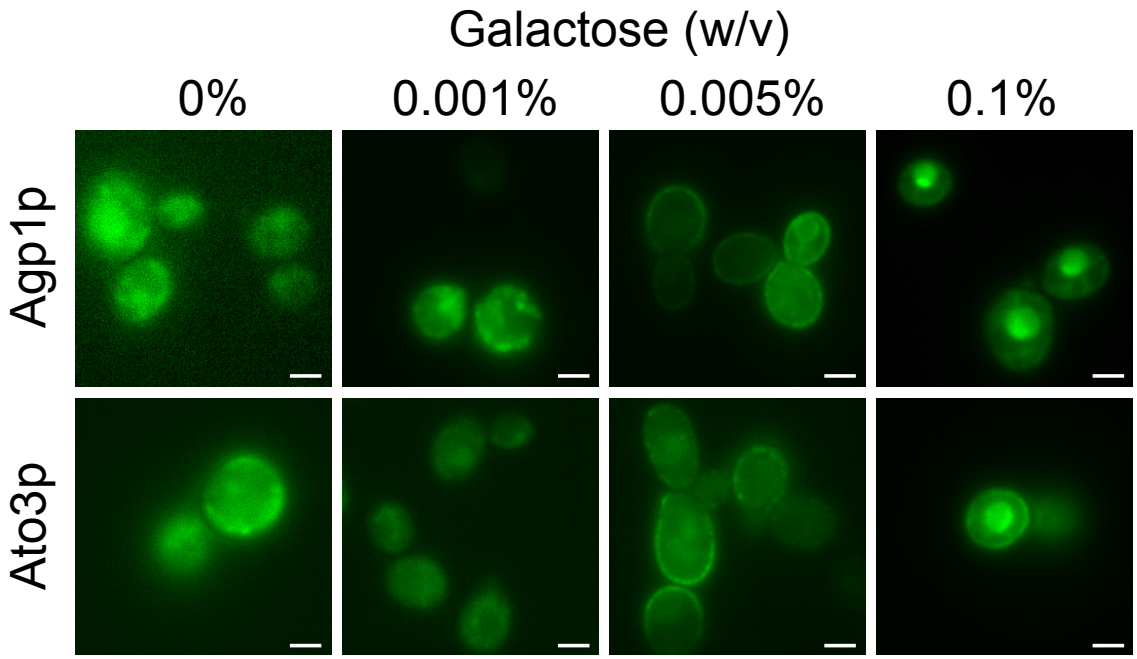

Figure 3-4. Images of membrane proteins using different expression levels of the labelling fluorescent protein. Cells were grown in synthetic complete media containing 0% to 0.1% w/v galactose. The labelling strand 101A-mNeonGreen was under control of the galactose inducible promoter, resulting in higher fluorescence levels when cells are grown in media with higher concentration of galactose. Diffraction limited fluorescence images of Agp1p and Ato3p, where each protein is fused to 101B, which interacts transiently with 101A-mNeonGreen. Scalebar is 2 μm.

3.2 Tagging very high and very low abundance proteins

Just like membrane proteins, very high and very low abundance proteins of interest are challenging to image by most super-resolution microscopy methods. This is because most other methods directly fuse or bind a fluorescent molecule to a protein of interest. Because of this, the number of fluorescent molecules is approximately equal to the number of proteins of interest in the cell. This means that when imaging very low abundance proteins of interest there are very few fluorescent molecules (Figure 3-5a). The end result is that the fluorescent molecules photobleach very quickly and very little total fluorescent signal can be collected. Conversely, for very high abundance proteins, the signal is too high due to the overabundance of fluorescent molecules. The end result is that the density of fluorescent molecules in the cell is so high that many of these molecules must first be photobleached in order to begin obtaining single localization events. There exist tricks to circumvent this, such as expressing a fluorescently labeled copy of the protein of interest ectopically, leaving most of the copies of the protein unlabeled.

Often, however, this approach is undesirable as the majority of the proteins of interest will never be imaged, so only sparse data for the protein will be collected. In LIVE-PAINT, this issue can be circumvented, because the protein of interest is not directly fused to the FP construct; it is expressed separately, under control of a different promoter (cartoon of anticipated data in Figure 3-5b). For low

abundance proteins of interest, this means that the FP construct can be present at higher levels than the protein of interest, acting as a reservoir of unbleached FPs which are capable of binding transiently and producing localization events. In this way, the total number of available fluorescent molecules can be much higher than the total abundance of the protein of interest. It would be desirable, however, to not make this number too high, as then background localization events from unbound FPs would come to dominate the super-resolution image produced.

Similarly, for high abundance proteins, the FP construct can be expressed at a lower level than the protein of interest. This is preferable to expressing a small amount of the protein of interest directly fused to an FP, because the FPs expressed in LIVE-PAINT are capable of binding to any of the tagged protein of interest, meaning that the resulting super-resolution image would be produced by binding and localization of FPs to a much larger fraction of the total expressed proteins of interest.

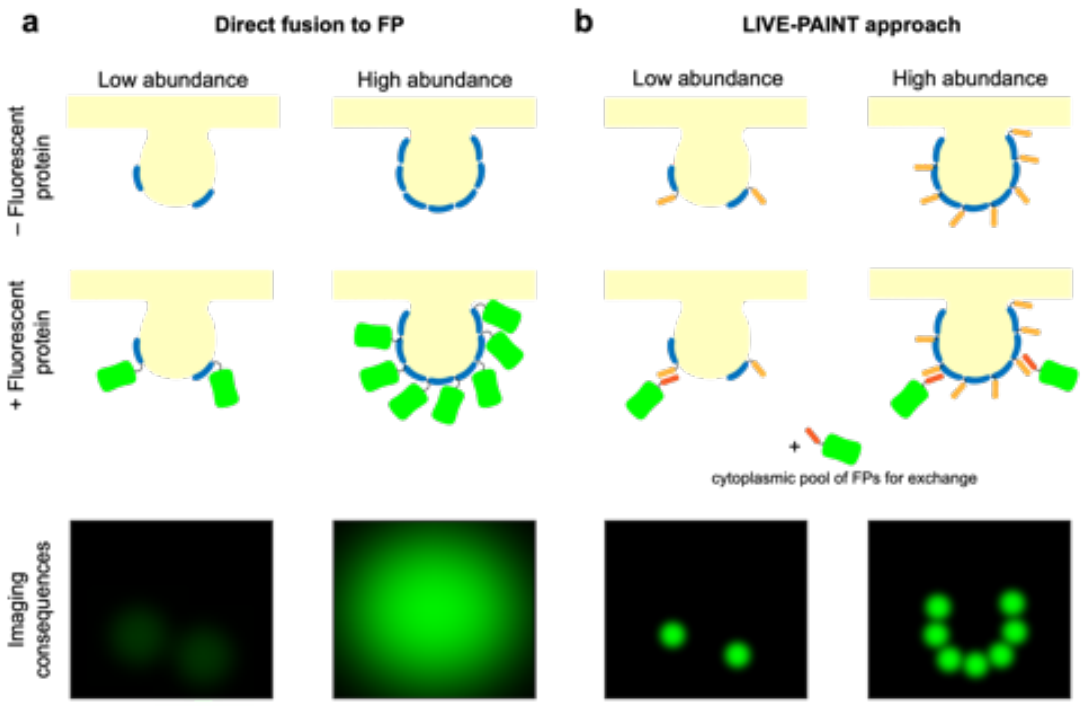

Figure 3-5. Direct fusion versus LIVE-PAINT for low and high abundance proteins. The protein of interest (blue) schematized here is Pil1p, which localizes to the eisosome during endocytosis. Pil1p localizes to the invaginated cell membrane (yellow). (a) A protein of interest (blue) is directly fused to an FP (green). In low abundance cases, the imaging consequences are that there is very little fluorescence signal generated. In high abundance cases, too much signal is generated to discern the location of individual FPs. (b) A protein of interest is directly fused to a peptide (light orange). A peptide-binding protein (red) is directly fused to an FP (green) and expressed separately. The abundance of FP is therefore controlled separately from the abundance of the protein of interest. Therefore, for both high and low abundance proteins of interest the imaging consequences are that the level of expressed FP is high enough to achieve good signal and low enough to be able to resolve individual FPs.

3.3 Tagging proteins which are rapidly turned over in the cell

When expressing FPs *in vivo*, the FP is first translated and folded. At this point, the FP is still not fluorescent. It requires a spontaneous backbone cyclization to occur to make the chromophore mature. The amount of time it takes for chromophore maturation depends on the FP used, with GFP maturing in approximately 25 minutes, and fast maturing FPs such as mNG more rapidly, in approximately 10 minutes[41]. While this may seem like a short amount of time, many proteins have half-lives much shorter than 25, or even 10, minutes[114]. Thus, if an FP is directly fused to a protein of interest that has a very short half-life, it will be degraded before the FP has time to mature (Figure 3-6). In this case, traditional methods for imaging proteins by directly fusing them to FPs will not work.

In LIVE-PAINT, the FP construct is expressed separately from the protein of interest. This enables fully mature, fluorescent FPs to bind to proteins which have only just been translated. Because the FP associates with the protein of interest transiently and reversibly, this approach can be used to label and image proteins which have very short half-lives. Short-lived proteins are a good target for LIVE-PAINT methods, because it is desirable to study them in live cells, in which the proteins are most likely to be turning over and behaving most naturally. Transient binding of non-fluorescent FPs is not expected to be a major issue for this approach, because the fast off-rate of the interaction pairs used ensure that immature or photobleached FPs will quickly unbind from the protein of interest.

Additionally, due to the requirement for an FP's chromophore to mature before it is fluorescent, short-lived proteins which are directly fused to FPs will be degraded before the FP's chromophore has time to mature.

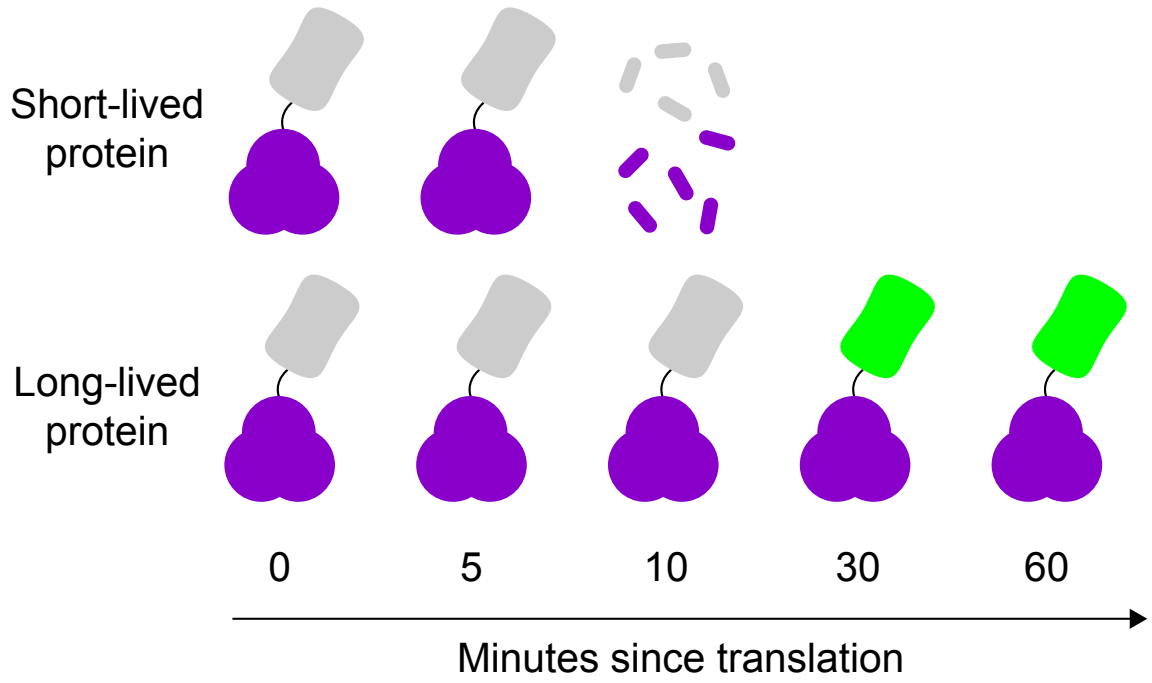

Figure 3-6. Short-lived proteins are degraded before FPs can mature.

(Top) A short-lived protein (purple) is fused to an FP (gray). By 10 minutes post translation, the protein has been degraded. (Bottom) A long-lived protein (purple) is fused to an FP (gray). By 30 minutes post translation, the FP chromophore has matured, thus turning green. The long-lived protein has not been degraded within the 60 minutes shown.

3.4 Tagging many proteins concurrently

The ability to perform super-resolution imaging of multiple proteins simultaneously in live cells is highly desirable. This is because many cellular processes are carried out by a large number of proteins, the details of which are difficult to tease apart. In endocytosis, for example, dozens of unique proteins assembly at a membrane bound structure called an eisosome. This structure is approximately 300 nm in diameter, which is of the order of the diffraction limit of light. In order to determine the relative spatial localization of the proteins in an eisosome and track them over time a super-resolution technique is needed which can be used in live cells and is capable of tagging and imaging multiple proteins concurrently. This is only one example. Many processes inside the cell are carried out by the concerted action of many different proteins.

Imaging many proteins concurrently is difficult to do using traditional live cell super-resolution techniques. For example, in PALM, two color super-resolution imaging can be performed using two different photoconvertible FPs. However, this approach is not extendible to tagging and imaging many more proteins of interest concurrently, due to the limited variety in available photoconvertible FPs. Because LIVE-PAINT can use any bright FP, it can be used to tag many different proteins of interest concurrently for live cell super-resolution imaging. I propose that LIVE-PAINT can be used to simultaneous super-resolution imaging of up to eight proteins concurrently in live cells. This method will give researchers a tool for better

understanding cellular processes and how proteins coordinate their behavior in live cells.

Currently, it is not possible to perform super-resolution imaging in live cells on more than two targets simultaneously. This is due to the fact that many live cell super-resolution methods require the use of photoconvertible fluorescent proteins, for which there are not many spectrally distinct options. Those methods which do not use photoconvertible fluorescent proteins cannot be used in live cells, because they use organic fluorophores, which cannot be genetically encoded and expressed inside cells. To be used inside cells, the cells must first be fixed and permeabilized, a process which kills the cells. Thus, a new and different super-resolution imaging approach is required in order to accomplish highly multiplexed multi-color super-resolution imaging in live cells. Recently, Zhang and colleagues developed an approach to perform super-resolution imaging of three targets concurrently in fixed mammalian cells by collecting "salvaged fluorescence"[115]. They use this salvaged fluorescence to assign the identity of different fluorescent dyes by calculating the ratio of the salvaged fluorescence to the conventional collected fluorescence. The approach they use is similar to the one I present here, though their work has only been performed in fixed cells to date and has not yet aimed to perform more than three color imaging.

Compared with other super-resolution imaging methods for studying proteins, such as PALM, which uses photoconvertible fluorescent proteins, LIVE-PAINT can be

used with any fluorescent protein. Brighter fluorescent proteins will produce more precise super-resolution localizations, due to the fact that more photons can be collected from the fluorescent proteins in a shorter amount of time, compared with a dimmer fluorescent protein. LIVE-PAINT can therefore be performed on as many simultaneous protein targets as we have orthogonal peptide-protein interactions and spectrally distinct fluorescent proteins at our disposal. In the initial implementation of LIVE-PAINT, I have preliminary data showing that it can be used with many different, spectrally distinct fluorescent proteins (Figure 2-3), and many mutually orthogonal peptide-protein interaction pairs (Figure 2-5).

LIVE-PAINT is perfectly poised to extend the number of simultaneous super-resolution imaging targets that can be studied simultaneously in live cells. This will be a powerful advancement for studying the interplay between proteins in complex processes. Because this will enable highly multiplexed multi-color super-resolution imaging in live cells for the first time, many proteins inside single cells can be studied over time, tracking the dynamic interplay of a set of proteins. Being able to image many proteins simultaneously in this way will be a major advancement over what is currently possible, which is simultaneous live cell super-resolution imaging of two proteins.

I propose a simple approach for separating fluorescent emission from multiple fluorescent proteins simultaneously with LIVE-PAINT, using only a dichroic mirror and standard fluorescence emission filters. These fluorescent proteins, which

range from green to red (peak emission wavelengths from 517 nm to 610 nm), can be excited by two lasers.

Here I use a simple approach, which takes advantage of the broad emission spectra of fluorescent proteins, which are much broader than those for organic dyes. Because of this broad emission spectrum, fluorescent proteins which have a peak emission wavelength between green and red will emit a mixture, with the ratio of red to green fluorescence being distinct for each fluorescent protein (Figure 3-7). These emission spectra can thus be split into “green” and “red” halves, with different fluorescent proteins emitting a different ratio of green to red wavelengths. Three example proteins are given in Figure 3-7: mNeonGreen (mostly green emission), mCherry (mostly red emission), and mKO (a mixture of red and green emission).

This approach makes use of a single dichroic mirror to separate red and green wavelengths from each other (Figure 3-8a). In this setup, a very green fluorescent protein, such as mNG, will be expected to have upwards of 95% of its emission filtered onto the “green half” of the camera by the dichroic mirror. Similarly, a very red fluorescent protein, like mCherry, will have the majority of its fluorescent emission deflected to the “red half” of the camera. Fluorescent proteins such as mKO and mOrange have emission spectra that contain some red and some green emission.

In order to predict how many photons will need to be collected for each super-resolution localization event in order to distinguish four fluorescent protein colors from one another, I first looked at the emission spectra for different fluorescent proteins. I then split the emission into a green half (emission wavelengths less than 590 nm) and a red half (emission wavelengths greater than 590 nm). In the simulation, these halves were then passed through standard fluorescence emission bandpass filters that were fairly permissive. For the green filter, a peak wavelength 525 nm and FWHM of 50 nm was chosen and for the red filter, a peak wavelength of 607 nm and FWHM of 42 nm was chosen. The FWHM of the filters was chosen to maximize the ability to discriminate mKO from mOrange emission; these filters result in a large relative difference in the amount of emission that will be incident on the red and green halves on the camera.

By simulating photons emitted from different FPs, I show that by collecting a minimum of approximately 100 photons per localization event, it is expected to be possible to discriminate the four fluorescent proteins from one another using our proposed dichroic mirror setup (Figure 3-8b). When performing LIVE-PAINT with mNG, high precision localization events (precision value of 20 nm or better) are all generated from at least 200 photons, and approximately two thirds of these events generated from over 500 photons. Based on our simulations, it will be possible to distinguish the four fluorescent proteins in Figure 3-8b using this approach.

A consideration I am aware of is that not all proteins are as bright as mNG. The other fluorescent proteins I propose to use are less bright, by approximately 2-4 fold. This means that fewer photons will be collected for each localization event. However, I note that based on the brightness of the fluorescent proteins and the number of photons per localization I have collected using mNG, it is very reasonable to assume a large number of localization events with at least 200 photons will be collected for these fluorescent proteins. In the case that the majority of localization events I collect are not generated from at least 100-200 photons, I will simply disregard the localization events which do not contain enough photons to assign a fluorescent protein to a localization event.

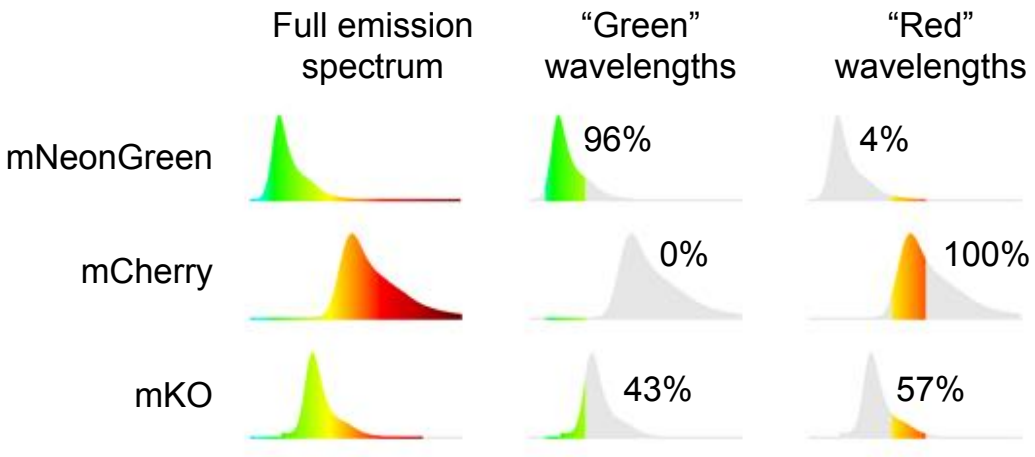

Figure 3-7. Fluorescent proteins have broad emission spectra. This emission spectra can be split into “green” wavelengths and “red” wavelengths, using a dichroic mirror and appropriate fluorescence emission filters. Some proteins, like mNeonGreen, emit almost entirely green wavelengths; some, like mCherry, emit almost entirely red wavelengths; some, like mKO, emit a mixture of red and green wavelengths.

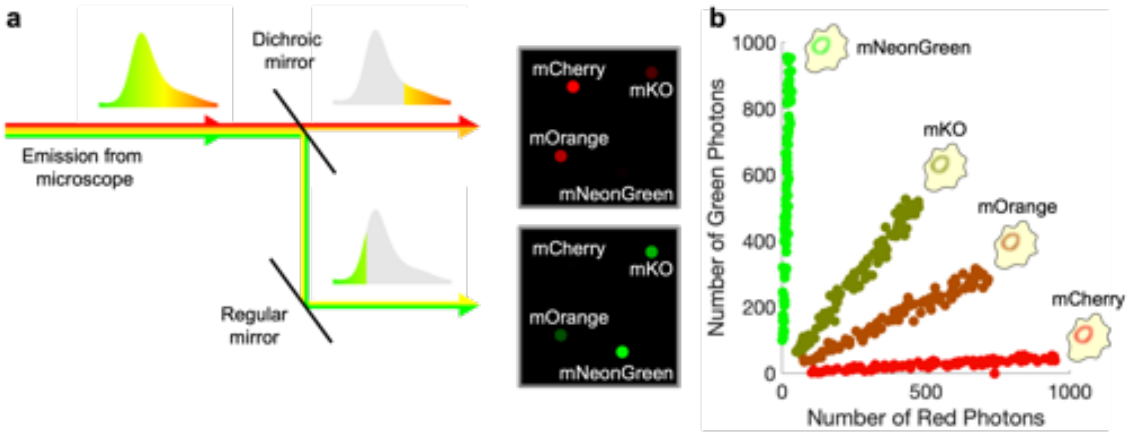

Figure 3-8. A dichroic mirror can be used to distinguish four fluorescent proteins.

(a) The fluorescent emission from the microscope first encounters a dichroic mirror. Longer wavelength photons pass through the dichroic mirror, while shorter wavelength photons are reflected and directed to the second half of a camera. Four fluorescent proteins and their approximate amount of relative red and green emission are shown on the top “red” camera half and the bottom “green” camera half. (b) Number of photons incident on the red half (x-axis) or green half (y-axis) of the camera for a given localization event, with each individual point drawn from a binomial distribution as described in the text. The color of the points matches the color of the fluorescent protein used in the simulation. Cells with an example cellular structure highlighted are shown next to the points in the plot, for each fluorescent protein color.

In order to test the ability for LIVE-PAINT to perform LIVE-PAINT on four or more protein targets simultaneously, I need to first identify a set of proteins which will be ideal to image concurrently. These proteins are ideally part of fixed structures in the cell, so they will not diffuse rapidly through the cell, and are spatially separated from one another. In the original implementation of LIVE-PAINT, I obtained super-resolution images for Cdc12p and Act1p. In addition to these two targets, I have also labeled and performed diffraction-limited imaging of three other proteins: Ask1p, a spindle pole body protein, which forms 1-2 bright spots in each cell; Sec27p, which localizes to the golgi apparatus; and Arc35p, which localizes to actin branch sites (Figure 3-3). This is in addition to the membrane proteins I have tagged and imaged using the 101A/101B interaction pair (Figure 3-1). Other potential targets include Nup120p, a protein that forms part of the nuclear pore complex, and Pil1p, a protein which localizes to the eisosome during endocytosis. This illustrates that there are many different protein targets which appear amenable to tagging using reversible peptide-protein interactions and would thus be suitable for multicolor LIVE-PAINT imaging.

Because LIVE-PAINT uses reversible peptide-protein interactions to transiently associate a fluorescent protein with a protein of interest, four-color LIVE-PAINT imaging requires using a set of four mutually orthogonal peptide-protein interactions. A selection of peptide-protein interactions that can be used for LIVE-PAINT is given in

Table 3-1. I have so far used two of these four coiled coil pairs, in addition to two other peptide-protein interactions I have also used, to perform LIVE-PAINT (Figure 2-5).

While concurrent super-resolution imaging of four target proteins concurrently is a very exciting prospect, I believe that the LIVE-PAINT approach is actually amenable to even higher multiplexing. In order to expand LIVE-PAINT to concurrent imaging of eight target proteins simultaneously, eight mutually orthogonal peptide-proteins interaction pairs which also do not interact with other proteins inside the cell would be required. The interaction pairs listed in Table 3-1 represent a set of eight mutually orthogonal interaction pairs suitable for LIVE-PAINT imaging we can use to perform highly multiplexed LIVE-PAINT. The first four interaction pairs, TRAP1-4 and their cognate 5-residue peptides, are a set of engineered tetratricopeptide repeat proteins engineered and characterized by our lab[90]. The last four interaction pairs, 101A-101B to 108A-108B, are a set of synthetic coiled coils engineered by the Keating and Dueber labs[95], which were engineered for use in *S. cerevisiae* and for mutual orthogonality. Because the TRAP interaction pairs and the coiled coil interaction pairs have different binding modes and the TRAP proteins do not bind other proteins inside *S. cerevisiae*[90], the TRAP interaction pairs are very likely to be orthogonal to the synthetic coiled coil pairs. There exist other orthogonal peptide-protein interaction pairs which can be used as well, including PDZ-peptide interactions and other coiled coil interactions.

Table 3-1. Orthogonal peptide-protein interaction pairs for use with LIVE-PAINT. This set of eight peptide-protein interactions pairs have been shown to be orthogonal, bind reversibly, and can be used inside cells[90, 95].

Number	1	2	3	4	5	6	7	8
Protein	TRAP1	TRAP2	TRAP3	TRAP4	101A	102A	107A	108A
Peptide	MEEVV	MERVW	MRRVW	MEEVF	101B	102B	107B	108B

One difference between the eight-color imaging and the four-color imaging approach is that proteins would need to be integrated into the yeast chromosome using CRISPR/Cas9, because there are not enough selectable markers available in yeast to make all genomic integrations using only selectable markers. Therefore, these additional genomic integrations should be made using CRISPR/Cas9 gene editing technology to integrate the peptide fusions to the proteins of interest and the fluorescent proteins, fused to the peptide-binding proteins, into the yeast chromosome.

The approach for eight-color imaging differs from the four-color imaging approach in an additional way: it requires a second dichroic mirror to be used to split fluorescence emitted from the microscope and a third emission filter to filter the fluorescence (Figure 3-9a). In this way, the emitted fluorescence is divided in three parts: red, green, and blue. A very red fluorescent protein such as mCherry would have almost all its emission on the red detector; an orange fluorescent protein such as mKO would have some fluorescence incident on the red and some on the green detector; a blue fluorescent protein such as mBlueberry2 would have most of its fluorescence incident on the blue detector and only a little on the green detector. The different fluorescent proteins would have distinct spectral fingerprints in this way.

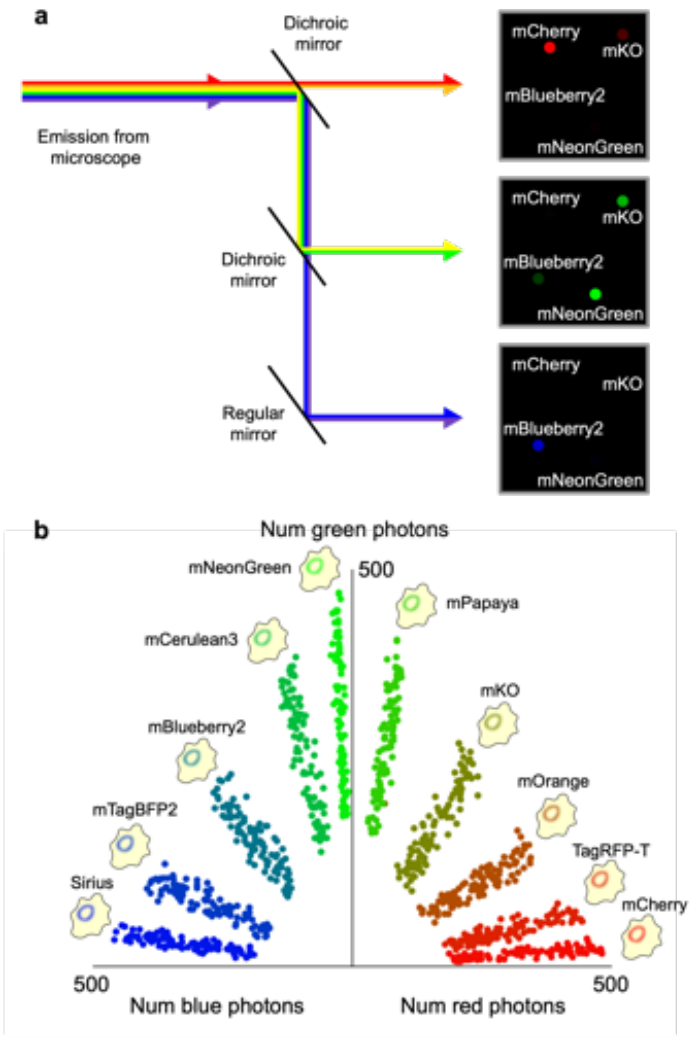

Figure 3-9. Two dichroic mirrors can be used to distinguish more than eight fluorescent proteins. (a) The fluorescent emission from the microscope first encounters a dichroic mirror. Longer wavelength photons pass through the dichroic mirror, while shorter wavelength photons are reflected and directed to the second half of a camera. Four fluorescent proteins and their approximate amount of relative red and green emission are shown on the top “red” camera half and the bottom “green” camera half. (b) Number of blue or red photons incident on the camera (x-axis) or green photons incident on the camera (y-axis) for a given localization event, with each individual point drawn from a binomial distribution as described in the text. The color of the points matches the color of the fluorescent protein used in the simulation. Cells with an example cellular structure highlighted are shown next to the points in the plot, for each fluorescent protein color.

Figure 3-9b shows the number of green, red, and blue photons that would be collected for individual localization events, for simulated data. The data was simulated by calculating the probability of an emitted photon being red, green, or blue for a given fluorescent protein. This information was then used to simulate different localization events by randomly drawing events from a binomial distribution built using the calculated probabilities for red, green, and blue photons.

3.5 Super-resolution imaging of GFP tagged proteins

GFP has become such a powerful tool in biology that there is now an incredible abundance of strains and constructs in which proteins have been tagged with GFP. There are even strain libraries for *S. cerevisiae*[108] and *E. coli* in which all open reading frames have been attempted to be tagged with GFP. Direct fusions of proteins to GFP has proven to be an immensely powerful tool for diffraction limited imaging of many proteins inside live cells. However, a direct fusion of a protein of interest to GFP alone cannot be used to perform super-resolution imaging. It would be very beneficial to develop a method for live cell super-resolution imaging of GFP-tagged proteins, due to the abundance of GFP-tagged protein constructs which exist.

Previously, a group has used nanobodies which bind to GFP with high affinity to perform super-resolution imaging of GFP tagged proteins in fixed cells[116]. This technique uses nanobodies conjugated to bright photoconvertible organic dyes,

which enables PALM imaging. The drawbacks of this approach are that the system is not fully genetically encodable, meaning that the nanobody-dye conjugate must be introduced to the cells exogenously, and the organic dyes require specific, non-native buffer conditions in order to achieve robust blinking.

Using the LIVE-PAINT approach, I propose that this can be done using a weak-binding GFP nanobody, fused to any bright red fluorescent protein (Figure 3-10). This system is fully genetically encodable, imaging can be performed in live cells, and no special buffer conditions are required for proper function of the fluorescent proteins and the subsequent super-resolution imaging.

Imaging of this system could be done in one of two ways. In the first approach, the red fluorescent protein can be directly excited and imaged (Figure 3-10b). This is most analogous to how I have performed LIVE-PAINT to date. The drawbacks to this approach are that imaging would need to be performed in TIRF so as to excite only a subset of the FPs at once and that there would likely be background signal from unbound nanobody-red FPs.

The other approach is to take advantage of the fused GFPs to perform FRET (Figure 3-10c). In this approach, the GFP would be directly excited by a green laser, the GFP would FRET to a bound red FP, and then the red fluorescence emission would be collected and recorded. This approach would have the benefit that a red FP would need to be in close proximity to a GFP (presumably most would be bound) and therefore would need to be very near the protein of interest

in order to emit red fluorescence. Additionally, because unbound red FPs would not be excited, the imaging would not need to be performed in TIRF and thus more cellular structures would be easy to image, as they would not need to be near the cell surface in order to be observed.

One potential drawback to this FRET approach is that the GFP donor molecules may photobleach before many localizations can be recorded. If that is the case, this issue could perhaps be mitigated by making GFP the acceptor fluorophore and instead express a blue fluorescent protein donor fused to the GFP nanobody. In this case, the donor can be expressed at a high level and the photobleaching will proceed more slowly.

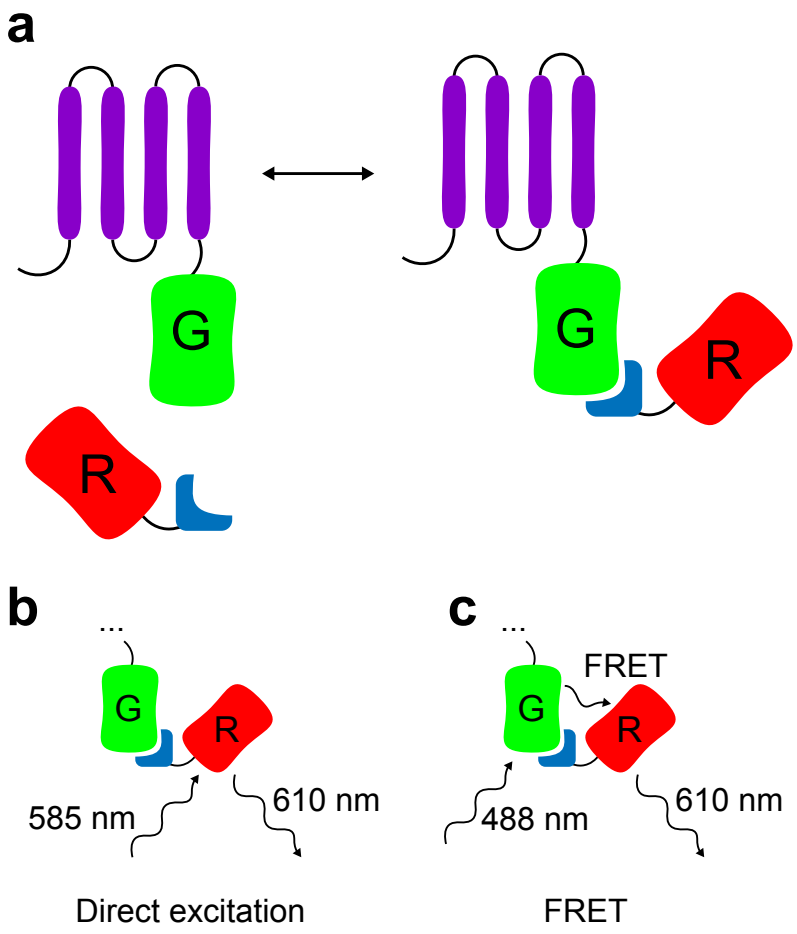

Figure 3-10. LIVE-PAINT of GFP-tagged proteins using nanobodies. (a) A protein of interest (purple) is genetically fused to GFP (green, labeled “G”). A weak binding GFP nanobody (blue) is fused to a bright red fluorescent protein (red, labeled “R”) and reversibly binds to GFP. (b) Imaging can be performed by directly exciting and imaging the red fluorescent protein. (c) Alternatively, imaging can be performed by FRET, by exciting the green fluorescent protein and following red emission.

One important requirement for LIVE-PAINT imaging using GFP nanobodies to be possible is that a GFP nanobody with relatively weak affinity be used. Nanobodies are typically selected for their high affinity, because tight binding and slow unbinding are often desired properties. In this case, however, unbinding of the GFP nanobody is critical to this approach, because without repeated binding and unbinding LIVE-PAINT would not work. Thus, it is important to identify a GFP nanobody which binds with tight enough affinity to achieve good signal to background, but a fast enough off-rate to ensure that frequent unbinding will occur. In the literature, I have identified a GFP nanobody which appears to have appropriate kinetics, with an approximate off-rate of 1.5/s and a binding affinity of 600 nM [117]. This is comparable to the properties of the TRAP4-MEEVF interaction pair I have used for LIVE-PAINT imaging.

Another important consideration when working with nanobodies is the fact that they contain a conserved disulfide linkage, which may not form when expressed *in vivo*. Nevertheless, nanobodies have been successfully expressed in cells and used for *in vivo* diffraction-limited imaging in *S. cerevisiae*[118]. This suggests that at least some nanobodies can properly fold and identify their targets *in vivo* and that this approach has potential.

3.6 A use for non-monomeric FPs

Fluorescence imaging is typically performed using monomeric FPs. This is because FPs are typically genetically fused to a protein of interest. Therefore, if the FP is not monomeric, the natural oligomeric state of the protein of interest will not be perturbed by bringing together multiple copies of the protein[119]. Weak dimerization of FPs can affect protein function. This has been observed with several fluorescent proteins, which have a weak tendency to form oligomers[120]. Most naturally occurring FPs, however, are tetrameric. Much protein engineering work has been done to make monomeric FP variants from their naturally occurring dimers and tetramers[39-41, 121].

The lack of uses for non-monomeric FPs is something of a missed opportunity, however, because they tend to be very bright naturally[41] and engineering a tetramer into a monomer typically results in a loss of brightness (per monomer) [40]. Thus, it would be valuable to be able to use these bright dimeric and tetrameric FPs for cellular imaging.

In LIVE-PAINT, the protein of interest is not directly fused to the labeling FP. In fact, the affinities used to associate the protein of interest to the FP are often quite weak. I propose that dimeric and tetrameric FPs might be possible to use with LIVE-PAINT. The protein of interest would still be fused to a short peptide and the peptide-binding protein would still be fused to a fluorescent protein. The only difference is that the fluorescent protein would form a homo-oligomer post-

translationally. I expect that this approach would not work well when using tight binding peptide-protein interaction pairs, as the fluorescent protein will likely often bind multiple proteins simultaneously and could perturb localization and function in this way. However, I think that this approach could be successful in enhancing the effective on-rate of the FP construct. Thus, this approach could enable peptide-protein pairs to be used with LIVE-PAINT which would otherwise be too weak to attain good signal to background during imaging.

This approach would additionally provide a method for modulating the effective affinity for a single peptide-protein interaction pair and would increase the pool of available FPs that could be used for super-resolution imaging.

3.7 Discussion

I have demonstrated that LIVE-PAINT is an attractive method for tagging and imaging proteins which do not tolerate direct fusion to an FP. To show this, I tagged and imaged membrane proteins which mislocalize to the vacuole when directly fused to GFP. When fused to only a short peptide and imaged using the LIVE-PAINT approach, these membrane proteins correctly localized to the plasma membrane. This is good evidence that proteins sensitive to fusions to FPs are likely to better tolerate the smaller peptide fusions used in LIVE-PAINT. This is an important benefit and the evidence I have presented suggests that this peptide-protein tagging strategy is likely less perturbative to protein function in general.

A set of proteins which have been challenging to image to date are proteins which have very low or very high abundance. I have proposed that these proteins will be easier to image using LIVE-PAINT, because for low abundance proteins the FP construct can be overexpressed, while for high abundance proteins the FP construct can be expressed at a level lower than the protein of interest. The fact that the FP is expressed separately from the protein of interest will enable many FP expression levels to be tested for various proteins of interest. It would be beneficial to develop a set of “rules” for choosing FP expression levels based on the abundance of the protein of interest and the affinity of the reversible peptide-protein interaction pair used. This would enable easier super-resolution imaging of low and high abundance proteins.

Proteins with short half-lives present an exciting application for LIVE-PAINT imaging, because they will be degraded before the chromophore in a fused FP has time to mature and become fluorescent. This is a straightforward but powerful application of LIVE-PAINT and will enable another class of difficult-to-image proteins to be imaged in super-resolution, in live cells.

Over the course of my work developing LIVE-PAINT, I have demonstrated that the LIVE-PAINT approach works for tagging and imaging a wide array of proteins. I have shown this, in particular, for proteins such as Cdc12p, Ask1p, Sec27p, and Arc35p, which localize to varied distinct structures within the cell. There exist many well characterized orthogonal peptide-protein interactions and spectrally distinct

FPs which can be used along with these proteins of interest to perform highly multiplexed concurrent super-resolution imaging with LIVE-PAINT. I have presented a simple ratiometric approach for splitting fluorescence emitted from the sample in real time to distinguish different FPs. This simple approach can, in principle, be used to tag and image upwards of eight different proteins concurrently in live cells. This highlights the potential for LIVE-PAINT to be used to study complex biological processes in live cells, which are often caried out by dozens of proteins working in concert. Being able to tag and image several proteins simultaneously, rather than only one or two as is possible with existing techniques, will be very beneficial for elucidating protein behavior in complex biological processes.

In the past, many proteins' functions have been determined by fusing them to GFP and imaging them within cells. This approach has become ubiquitous in biology and has resulted in a vast number of biological discoveries. Because of GFP's popularity, there exist many strains and plasmids in which proteins are tagged with GFP. While this is incredibly useful, GFP alone, when fused to a protein of interest, cannot be used to perform super-resolution imaging. I present an approach which will enable these constructs to be used for live cell super-resolution imaging. This is a simple extension of the LIVE-PAINT approach, in which a GFP nanobody is fused to a red FP and the transient binding events of the GFP nanobody to GFP are collected. This will likely be a very useful tool for researchers, who will now be

able to use their GFP-tagged strains to perform super-resolution imaging in live cells.

Finally, I present a use for non-monomeric FPs in super-resolution imaging. In existing super-resolution methods, only monomeric FPs are desired, because they are directly fused to the protein of interest. The work I present suggests that LIVE-PAINT is a potentially useful application for non-monomeric FPs. The increased oligomeric state of the FPs can enhance the effective on-rate of the peptide-binding protein-FP fusion, thus making weak interaction pairs effectively tighter. It also expands the set of available FPs for use in applications such as multicolor imaging.

Together, I present data and propose applications for LIVE-PAINT which will advance the super-resolution toolkit for biologists, in particular for proteins which mislocalize upon direct fusion to an FP, proteins which are expressed at very low or very high levels, and proteins with short half-lives.

4 Methods

4.1 Media, buffers, and antibiotics

Table 4-1. Commonly used media and buffers

Media/Buffer	Purpose	Ingredients	Storage
RT	Liquid growth media for *E. coli*	For 1 L: 10 g Tryptone 5 g Yeast Extract 5 g NaCl Autoclave	RT
LB Agar	Solid growth media for *E. coli*	For 1L: 10 g Tryptone 5 g Yeast Extract 5 g NaCl 15 g Agar Autoclave	RT
TSS	Preparing chemically competent *E. coli*	For 50 mL: 5 g PEG 3350 1.5 mL 1M $MgCl_2$ 2.5 mL DMSO Add LB to 50 mL Filter sterilize	4°C
SOC	Transforming chemically competent *E. coli*	For 1 L: 5 g Yeast extract 20 g Tryptone 0.584 g NaCl 0.186 g KCl 2.4 g $MgSO_4$ Autoclave	RT
YPD	Liquid growth media for *S. cerevisiae*	For 1 L: 20 g Peptone 10 g Yeast Extract 20 g Dextrose (glucose) Autoclave	RT
YPD Agar	Agar growth media for *S. cerevisiae*	For 1 L: 20 g Peptone 10 g Yeast Extract 20 g Dextrose (glucose)	RT

		15 g Agar Autoclave	
5x YNB + AA	Yeast nitrogenous base plus amino acids for yeast synthetic media	For 200 mL: 6.7 g YNB with ammonium sulfate 0.342 g Edinburgh AA mix (lacking Met, Ura, Trp, Leu, His, Ade) Filter sterilize	RT
Yeast Synthetic Agar (Dropout) (lacking Met, Ura, Trp, Leu, His, Ade)	Synthetic dropout solid growth media for yeast	For 1L: 6.7 g YNB with ammonium sulfate 0.342 g Edinburgh AA mix (lacking Met, Ura, Trp, Leu, His, Ade) 20 g Dextrose (glucose) 15 g Agar Filter sterilize 10 mL each 100x amino acid stocks as necessary	
Yeast Synthetic Media (Glucose)	Liquid growth media for *S. cerevisiae* for imaging or maintaining selection	For 100 mL: 20 mL 5x YNB+AA 5 mL 40% Glucose 1 mL each 100x amino acid stocks as necessary	Make fresh before use
Yeast Synthetic Media (Galactose)	Liquid growth media for *S. cerevisiae* for imaging or maintaining selection	For 100 mL: 20 mL 5x YNB+AA 10 mL 20% Galactose 10 mL 10% Raffinose 1 mL each 100x amino acid stocks as necessary	Make fresh before use
50x TAE	For casting and running agarose gels	For 1L: 242 g Tris-base 57.1 mL 100% acetic acid	RT

		100 mL 0.5 M EDTA pH 8.0	
TE	For storing DNA in solution	For 100 mL: 1 mL Tris pH 8.0 200 µL 0.5 M EDTA pH 8.0 Filter sterilize	RT

Table 4-2. Stock solution recipes. All solutions are filter sterilized using a 0.22 µm filter.

Stock	Solvent	Stock	Working	Storage
Antibiotics				
Ampicillin	ddH_2O	100 mg/mL	100 µg/mL	-20°C
Chloramphenicol	Ethanol	25 mg/mL	25 µg/mL	-20°C
Amino Acids and Nucleobases				
Arginine	ddH_2O	2 g/L	20 mg/L	4°C
Histidine	ddH_2O	1 g/L	10 mg/L	4°C
Leucine	ddH_2O	6 g/L	60 mg/L	RT
Lysine	ddH_2O	4 g/L	40 mg/L	4°C
Methionine	ddH_2O	1 g/L	10 mg/L	4°C
Tryptophan	ddH_2O	4 g/L	40 mg/L	4°C
Adenine	0.1M NaOH	2 g/L	20 mg/L	4°C
Uracil	0.1M NaOH	2 g/L	20 mg/L	4°C
Carbon Sources				
Glucose	ddH_2O	400 g/L	20 g/L	RT
Galactose	ddH_2O	200 g/L	20 g/L	RT
Raffinose	ddH_2O	100 g/L	10 g/L	RT
Yeast Glycerol Stocks				
Glycerol	ddH_2O	300 g/L	150 g/L	4°C

4.2 *E. coli* overnight growth

Individual colonies were picked from LB agar plates and grown in 3 mL LB for 16 hrs overnight at 37°C with shaking at 180 rpm. When growing cells containing a plasmid, the appropriate antibiotic was also added to the media.

4.3 *E. coli* chemically competent cell preparation

E. coli competent cells (TOP10) were prepared by starting an overnight culture at 37°C with shaking at 180 rpm in 5 mL LB by picking a single colony from an LB agar plate. Cells were diluted 1:200 in 200 mL first thing the next morning and grown at 37°C with shaking at 180 rpm. After reaching an OD_{600} of 0.5-0.7, cells were quickly removed from the incubator and chilled in a bucket of ice for 10 minutes. In all following steps, cells were kept on ice. Cells were centrifuged at 4°C for 10 minutes at 10,000 rpm. Supernatant was discarded. Cells were resuspended in 50 mL of ddH_2O chilled to 0-4°C and again centrifuged at 4°C for 10 minutes at 10,000 rpm. After three washes with water, the cells were resuspended in chilled 2 mL TSS buffer (see Table 4-1 for recipe). Approximately 80 1.5 mL tubes (Eppendorf) were labeled and set open on dry ice to pre-chill. 25 µL of cells resuspended in TSS buffer were quickly pipetted into each open tube and closed. Tubes were then placed in a freezer box pre-chilled to -70-80°C for long term storage.

4.4 Transformation of chemically competent *E. coli*

A 25 µL aliguot of chemically competent cells in a 1.5 mL tube (Eppendorf) was thawed on ice. Plasmid DNA (typically 10-50 ng) is then mixed with the cells by pipetting up and down. This mixture is then left to incubate on ice for 30-60 minutes. The cells are then heat shocked at 42°C for 40s on a heat block. Following this, the cells are chilled on ice again for 2 minutes and resuspended in 1 mL SOC

buffer (see Table 4-1 for recipe). If using an antibiotic other than ampicillin, first grow this 1 mL culture in an incubator at 37°C with shaking at 180 rpm for 1 hr. Turn on a flame and work near the flame from this point on. Plate 200 µL on an LB agar plate containing the appropriate antibiotic, which has been pre-warmed to 37°C. After plating the cells, spread them evenly using either a spreader or glass beads. After the cells have been spread and the plate has mostly dried, place the lid on the plate, invert it so the agar is near the top, and leave to grow overnight in the 37°C plate incubator.

4.5 General cloning protocol

All cloning was performed in *Escherichia coli* strain TOP10. Unless otherwise noted, cloning was performed by amplifying a destination vector and an insert containing 25 bp homology with the destination vector on either end, and joining them together using NEBuilder® HiFi DNA Assembly Master Mix (New England Biolabs). After PCR amplifying the vector and insert, both products were cleaned up using a QIAquick PCR purification kit (QIAGEN) and eluted in 30 uL of the EB buffer provided in the kit. Sometimes a gBlock (Integrated DNA Technologies) was used in place of a PCR amplified insert. A DeNovix DS-11+ spectrophotometer was used to measure the DNA concentration. The instrument was first blanked using 1.5 uL EB buffer and then the concentration of the PCR products was measured using 1.5 uL of the eluted product. 100 ng of the PCR amplified backbone was mixed with 2-3x molar excess of the insert. 5 uL of 2x NEBuilder®

HiFi DNA Assembly Master Mix (New England Biolabs) was added to this mixture and water was added to a total volume of 10 uL. This reaction was incubated at 50°C for one hour. Following this, 8.5 uL of the reaction, 1 uL of CutSmart buffer (New England Biolabs), and 0.5 uL DpnI (New England Biolabs) were mixed together and incubated overnight at 37°C. Following this, the reaction was heated to 80°C for 20 minutes to inactivate the DpnI. 5uL of this reaction was mixed with 25 uL of chemically competent TOP10 cells and transformed as in “Transformation of chemically competent *E. coli*”.

4.6 Yeast strain construction

Except where otherwise noted, standard methods for genetically modifying yeast and preparing growth media were used. Yeast strains constructed in this work are all derived from the parent strain BY4741. PCR products containing an appropriate selection marker for integration into the yeast genome were amplified from yeast integration vectors. The amplification primers also included 45 bp homology arms, which matched the final 45 bp preceding the stop codon in the protein to be tagged and 45 bp downstream of the stop codon.

When using the kanR marker, transformants were selected by plating first on YPD plates and then replica plating to yeast agar plates including 600 mg/L geneticin (Gibco) and incubating for a further 48 hours. When using a nutritional deficiency marker such as His3, Ura3, Leu2, or Met15, cells were simply plated on synthetic complete yeast agar plates lacking the appropriate amino acid.

FP fusions were inserted into the yeast genome at the GAL2 locus by amplifying the desired protein's sequence from a plasmid. The amplification primers also included 45 bp homology arms that match sequences upstream and downstream of the GAL2 gene, and the HIS3 gene. Strain construction was verified by PCR amplification of the modified locus.

4.7 Yeast genomic DNA extraction

First, genomic DNA (gDNA) was isolated from colonies obtained from yeast transformations. This was done by resuspending a single yeast colony in 100 µL of 0.2 M LiAc + 1% SDS. This mixture was incubated at 75°C in a heat block for 5 minutes. Afterwards, 300 µL 100% ethanol was added to the mixture and centrifuged at 15,000 xg for 3 minutes. The resulting pellet was washed with 100 µL 70% ethanol, the ethanol was removed, and the pellet allowed to dry for 10 minutes. The dried pellet was then dissolved in 20 µL TE buffer (see Table 4-1 for recipe) and centrifuged at 15,000 xg for 15 seconds. The supernatant containing the gDNA was then transferred to a fresh tube.

This gDNA was then used as a template for a PCR reaction to confirm the presence of the inserted DNA at the genomic locus being checked. The PCR reactions were carried out using Phusion High-Fidelity DNA Polymerase and GC buffer (New England Biolabs) in a ProFlex PCR system (Thermo Fisher Scientific). Unless otherwise noted, PCR reactions to validate the insertion of DNA at genomic loci ran for 35 cycles, had an annealing temperature of 55°C, and used an

extension time of 1 minute, with a reaction volume of 10 uL. For the following genomic targets, the annealing temperature was varied CDC12 (59°C), GAL2 (64°C), and COF1 (61°C) and the extension time used was 2 minutes. The primers used for checking genomic loci in modified yeast are given in Table A5-2.

PCR products were run on a 1% agarose gel in TAE buffer (see Table 4-1 for recipe) for 30 minutes at 120 V. Full lists of primers used are given in Appendix section “Primers, gBlocks, and strains

Table A5-1 provides a full list of the yeast strains used in this work.

4.8 Yeast glycerol stocks

A single yeast colony was picked from an agar plate and used to inoculate a 3 mL YPD liquid culture. When appropriate, synthetic media was instead used to maintain selection of plasmids. The culture was grown overnight at 30°C for 16 hrs while shaking at 180 rpm. After growth, 1 mL culture was mixed with 1 mL 30% glycerol (see Table 4-2 for recipe) and stored in a 2 mL screw cap tube at -80°C.

4.9 Super-resolution imaging of yeast strains

For imaging experiments, yeast cells were grown overnight in 500 µL of synthetic complete media. Constructs using the galactose inducible promoter, pGAL1, were all grown with 1% w/v raffinose plus the concentration of galactose desired for a particular experiment. The concentration of galactose used varied between 0% and 2% w/v.

One colony was picked into a 500 µL overnight culture to ensure that the OD_{600} of the cells was between 0.1 and 0.5 by the time of imaging. Two dilutions of the overnight culture, 1:1 and 1:5, were prepared to ensure that one would fall in this OD_{600} range.

22x22 mm glass coverslips with thickness no. 1 (VWR) were cleaned by a 20 minute exposure in a 2.6 L Zepto plasma laboratory unit (Diener Electronic). Frame-Seal slide chambers (9 × 9 mm^2, Biorad, Hercules, CA) were then secured to a coverslip. The surface was prepared for the attachment of yeast cells by coating the surface with 2 mg/mL concanavalin A (Sigma-Aldrich), which was dissolved in PBS pH 7.4, using approximately 100 µL per well. After leaving the concanavalin A on the surface of the slide for 30 seconds, it was removed using a pipette tip and by tilting the slide to ensure all liquid was removed. Then, 150 µL of prepared yeast culture was pipetted onto the slide. The yeast culture was left to sit on the slide for approximately 5 minutes. The cells were then aspirated from the slide, the surface washed with milliQ water three times, and then 150 µL fresh milliQ water was then added to the slide before imaging.

Single-molecule imaging was performed using a custom-built TIRF microscope, which restricts the illumination to within 200 nm of the sample slide. The fluorophores were excited with 488 nm illumination. Collimated laser light at a wavelength of 488 nm (Cobolt MLD 488-200 Diode Laser System, Cobalt, Sweden) was aligned and directed parallel to the optical axis at the edge of a 1.49

NA TIRF objective (CFI Apochromat TIRF 60XC Oil, Nikon, Japan), mounted on an inverted Nikon TI2 microscope (Nikon, Japan). The microscope was fitted with a perfect focus system to autocorrect the *z*-stage drift during imaging. Fluorescence collected by the same objective was separated from the returning TIR beam by a dichroic mirror (Di01-R405/488/561/635 (Semrock, Rochester, NY, USA), and was passed through appropriate filters (BLP01-488R, FF01-520/44 (Semrock, NY, USA)). The fluorescence was then passed through a 2.5× beam expander and recorded on an EMCCD camera (Delta Evolve 512, Photometrics, Tucson, AZ, USA) operating in frame transfer mode (EMGain = 11.5 e^{-}/ADU and 250 ADU/photon). Each pixel was 103 nm in length. Images were recorded with an exposure time of 50 ms with a laser power density of 3.1 W/cm^2. The lasers were first attenuated with neutral density filters to reduce the excitation power. The power at the back aperture of the objective lens was measured, and the excitation area determined using tetraspeck beads immobilized on a glass coverslip. The microscope was automated using the open source microscopy platform Micromanager.

For photobleach-and-recovery experiments I first imaged the samples at very high laser power density (26.6 W/cm^2). After 1,000 frames (50 s) of imaging, this power density was dropped to 3.1 W/cm^2. The sample was then imaged for another 1,000 frames (50 s).

4.10 Microscope settings and imaging parameters

Images were analyzed using Fiji and single localizations were processed using the Peak Fit function of the Fiji GDSC SMLM plugin, using a signal strength threshold of 30, a minimum photon threshold of 100, and a precision threshold of 20 nm. The precision threshold was sometimes changed to 30 nm, 40 nm, or 1000 nm, in order to obtain the distribution of precision values for all obtained localization events. Figure 2-19 shows a matrix of precision and minimum photons per localization thresholds applied to one stack of images, which helped select the cutoffs used.

4.11 Single-molecule tracking analysis

The LIVE-PAINT images were recorded at a frame rate of 50 ms for 2000 frames. The images were first analyzed using Trackpy[122]. Individual puncta corresponding to cofilin clusters were selected by applying a mask size of 7 and a minimum mass of 2000. The puncta were linked into tracks by applying a maximum displacement of 3 pixels/frame, and a memory of 3 frames (i.e. if the puncta were absent in more than 3 frames, then they were no longer linked to those in previous or subsequent frames). Tracks shorter than 20 frames were discarded, and the mean squared displacement (MSD) plot for the remaining tracks were calculated.

Custom-written code in Igor Pro (Wavemetrics) was used to calculate the initial diffusion co-efficient for each track by fitting the first 250 ms of the MSD to a straight line and determining the gradient. The log of the diffusion coefficients determined

from fits with an $r^2 > 0.5$ were then used to populate the diffusion coefficient histogram.

4.12 Image resolution calculation

Image resolution was calculated by first performing cluster analysis using DBSCAN[123] in Python 2.7 to identify localizations in the yeast bud neck. Then, resolution was measured using the equation $R_{eff} = \sqrt{(\bar{r}_{nn})^2 + (\bar{\sigma})^2}$, where R_{eff} is the effective image resolution, $\bar{r}_{nn}$ is the mean nearest neighbor distance between localizations in the septum, and $\bar{\sigma}$ is the average localization precision[107].

4.13 Cluster analysis for identifying yeast septum

For the images shown in Figure 2-9, septum localizations were identified from total cellular localization events using DBSCAN[123] in Python 2.7 and the percent of total cellular localizations in the septum was determined. In order to prevent misidentification of septa in background localizations, DBSCAN was applied to localizations within a 1 μm radius of the center of the cell. DBSCAN parameters were maintained for images of cells the same galactose concentration: 0% galactose - ε = 2, N = 25; 0.005% galactose - ε = 2, N = 50; 0.02% galactose - ε = 1.75, N = 50; 0.1% galactose - ε = 2.8, N = 75.

4.14 Quantifying yeast septum width

Budding yeast with septa were identified from z-projections and following thresholding, ImageJ's Analyze Particles tool was used to determine: the

maximum Feret's diameter of the cell, the starting coordinates of the Feret's diameter, the angle between the Feret's diameter and the x-axis, and the coordinates of the cellular center of mass. The end coordinates of the Feret's diameter were calculated from the Feret's diameter data. In the same cells, septum localizations were identified from total cellular localization events as described in above cluster analysis within a radius of the Feret's diameter/5 from the cell's center of mass and using parameters of $\varepsilon = 2$, $N = 100$.

The distance between the center of the septum points and the coordinates of both the start and end of the Feret's diameter was determined and the larger of the two was taken to be the mother cell diameter and the smaller, the daughter cell diameter.

To find the septum width, the mean absolute perpendicular distance between all the septum localizations and the line bisecting the angle between the center of the septum, and the mother and daughter diameters was doubled.

4.15 Measuring yeast fluorescence using plate reader

Plate reader measurements were carried out on a POLARstar Omega microplate reader (BMG LABTECH). To observe the galactose dependent induction of mNG under the pGAL1 promoter in a Δgal2 background, budding yeast cells were grown overnight in 500 µL of synthetic complete media plus 1% w/v raffinose and galactose concentrations ranging from 0 to 0.1% w/v.

The next morning, 200 µL of this culture was added to individual wells in a 96 well clear bottom plate (Greiner bio-one, item 655096). Cellular fluorescence was excited using the 485 nm excitation filter and measured using the 520 nm emission filter.

The optical density of the cells was measured using the absorbance setting at 600 nm. The fluorescence readings were then normalized to the number of cells by dividing the measured cellular fluorescence by the optical density.

5 Appendix

5.1 Primers, gBlocks, and strains

Table A5-1. Yeast strains used in this study.

Strain	Parent	Genotype
BY4741	-	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0
CDC12-MEEVF	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 CDC12-MEEVF::KANMX6
CDC12-SYNZIP18	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 CDC12-SYNZIP18::KANMX6
CDC12-CCBN3,5	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 CDC12-CCBN3,5::KANMX6
pGAL1 TRAP4-mEOS	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 TRAP4-mEOS
pGAL1 TRAP4-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 TRAP4-mNeonGreen
pGAL1 SYNZIP17-mEOS	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-mEOS
pGAL1 SYNZIP17-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-mNeonGreen
CDC12-MEEVF + pGAL1 TRAP4-mEOS	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 TRAP4-mEOS CDC12-MEEVF::KANMX6
CDC12-MEEVF + pGAL1 TRAP4-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 TRAP4-mNeonGreen CDC12-MEEVF::KANMX6
CDC12-SYNZIP18 + pGAL1 SYNZIP17-mEOS	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-mEOS CDC12-SYNZIP18::KANMX6
CDC12-SYNZIP18 + pGAL1 SYNZIP17-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-mNeonGreen CDC12-SYNZIP18::KANMX6
CDC12-SYNZIP18 + pGAL1 SYNZIP17-3xmNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-3xmNeonGreen CDC12-SYNZIP18::KANMX6

CDC12-SYNZIP18 + pGAL1 SYNZIP17-GRvT	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-GRvT CDC12-SYNZIP18::KANMX6
CDC12-CCBN3,5 + pGAL1 CCAN3,5-mEOS	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 CCAN3,5-mEOS CDC12-CCBN3,5::KANMX6
COF1-MEEVF + pGAL1 TRAP4-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 TRAP4-mNeonGreen COF1-MEEVF::KANMX6
COF1-SYNZIP18 + pGAL1 SYNZIP17-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-mNeonGreen COF1-SYNZIP18::KANMX6
pGAL1 LifeAct-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 LifeAct-mNeonGreen
Cdc12-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Cdc12-mNeonGreen::KANMX6
Cdc12-SYNZIP18 + pGAL1 SYNZIP17-EYFP	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-EYFP CDC12-SYNZIP18::KANMX6
Cdc12-SYNZIP18 + pGAL1 SYNZIP17-mKO	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-mKO CDC12-SYNZIP18::KANMX6
Cdc12-SYNZIP18 + pGAL1 SYNZIP17-mOrange	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-mOrange CDC12-SYNZIP18::KANMX6
Cdc12-SYNZIP18 + pGAL1 SYNZIP17-mCherry	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-mCherry CDC12-SYNZIP18::KANMX6
pGAL1 101A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 101A-mNeonGreen
pGAL1 102A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 102A-mNeonGreen
pGAL1 107A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 107A-mNeonGreen
pGAL1 108A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 108A-mNeonGreen
Cdc12-MEEVF + pGAL1 TRAP4-SNAP-tag	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 TRAP4-SNAP-tag CDC12-MEEVF::KANMX6
Cdc12-SYNZIP18 + pGAL1 SYNZIP17-SNAP-tag	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 SYNZIP17-SNAP-tag CDC12-SYNZIP18::KANMX6

Cdc12-MEEVF + pGAL1 TRAP4-mNG + pGAL1 SYNZIP17-mCherry	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 TRAP4-mNeonGreen CDC12-MEEVF::KANMX6 pGAL1 SYNZIP17-mCherry::URA3
Cdc12-101B + pGAL1 101A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 101A-mNeonGreen CDC12-101B::KANMX6
Cdc12-102B + pGAL1 102A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 102A-mNeonGreen CDC12-102B::KANMX6
Cdc12-107B + pGAL1 107A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 107A-mNeonGreen CDC12-107B::KANMX6
Cdc12-108B + pGAL1 108A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 108A-mNeonGreen CDC12-108B::KANMX6
Cdc12-101B + pGAL1 101A-mNeonGreen + pGAL1 108A-mCherry	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 101A-mNeonGreen CDC12-101B::KANMX6 pGAL1 108A-mCherry::URA3
PIL1-101B + pGAL1 101A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 101A-mNeonGreen PIL1-101B::KANMX6
ASK1-101B + pGAL1 101A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 101A-mNeonGreen ASK1-101B::KANMX6
SEC27-101B + pGAL1 101A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 101A-mNeonGreen SEC27-101B::KANMX6
ARC35-101B + pGAL1 101A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 101A-mNeonGreen ARC35-101B::KANMX6
AGP1-101B + pGAL1 101A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 101A-mNeonGreen AGP1-101B::KANMX6
ATO3-101B + pGAL1 101A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 101A-mNeonGreen ATO3-101B::KANMX6
BAP2-101B + pGAL1 101A-mNeonGreen	BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gal2Δ::HIS3MX6 pGAL1 101A-mNeonGreen BAP2-101B::KANMX6

Table A5-2. Sequencing primers.

Label	Name	Sequence	Purpose
S1	CDC12_CT_F	GAGGGTCACGAGAACACC	Check C-terminus of CDC12
S2	CDC12_CT_R	CAGTTACTTCTGCTGGTTCC	Check C-terminus of CDC12
S3	GAL2_Seq_F	CTAATCCAAGGAGGTTTAC	Check GAL2 locus
S4	GAL2_Seq_R	TAAGAGAGATGATGGAGC	Check GAL2 locus
S5	SP6_Seq_F	ATTTAGGTGACACTATAG	Sequence pFA6a-HIS3MX6 and pFA6a-KANMX6 plasmids
S6	T7_Seq_F	TAATACGACTCACTATAGGG	Sequence pFA6a-HIS3MX6 and pFA6a-KANMX6 plasmids
S7	HIS_SEQ_F	CGTTAGAACGCGGCTAC	Sequence pFA6a-HIS3MX6 plasmids
S8	GAL2_SEQ_F2	GCTGCAGAAGGCACATCTA	Check GAL2 locus
S9	GAL2_SEQ_R2	CCCAGAGATAAGTCTGGTGATG	Check GAL2 locus
S10	pCUP1_seq_F	CATATAGAAGTCATCGACTAGT	Check pCu415CUP1 plasmid
S11	pCUP1_seq_R	GACGGTATCGATAAGCTT	Check pCu415CUP1 plasmid
S12	COF1_seq_F	CCTTAAACGGTGTCTCTACC	Check C-terminus of COF1
S13	COF1_seq_R	GGTGTACGGGACCTTAAATC	Check C-terminus of COF1
S14	p6k_seq	ATATTGTCGTTAGAACGC	Check ADY2 locus, with MEM_SEQ_R (S37)
S15	URA3_seq_F	AGGCATATTTATGGTGAAGGA	Check AGP1 locus, with MEM_SEQ_R (S37)
S16	URA3_seq_R	CAGTCCTGTCTTATTGTTCTT	Check ATO3 locus, with MEM_SEQ_R (S37)
S17	ADY2_SEQ_F	GGTTAGCTCCTGCTCCAGTG	Check AZR1 locus, with MEM_SEQ_R (S37)
S18	AGP1_SEQ_F	TGTCCATGGCTAACTCCTCC	Check BAP2 locus, with MEM_SEQ_R (S37)
S19	ATO3_SEQ_F	GATGGTAAATGGGCGCTAAG	Check BAP3 locus, with MEM_SEQ_R (S37)

S20	AZR1_SEQ_F	GCTGTACACCTGGTTGCTTG	Check BOR1 locus, with MEM_SEQ_R (S37)
S21	BAP2_SEQ_F	TGTTGCAGCTTCATCAAAGG	Check CAN1 locus, with MEM_SEQ_R (S37)
S22	BAP3_SEQ_F	GGCCTTGGTAGTGTGCTCTC	Check CCH1 locus, with MEM_SEQ_R (S37)
S23	BOR1_SEQ_F	AAGCACCATTGCACACAGAG	Check DIP5 locus, with MEM_SEQ_R (S37)
S24	CAN1_SEQ_F	ACAACCATTATTTCTGCCGC	Check FAT1 locus, with MEM_SEQ_R (S37)
S25	CCH1_SEQ_F	TCGGACTGAACAAAGAGCTG	Check FCY2 locus, with MEM_SEQ_R (S37)
S26	DIP5_SEQ_F	ATATGCGTTGGCAATTGATG	Check FEN2 locus, with MEM_SEQ_R (S37)
S27	FAT1_SEQ_F	TGGCGCATGTAGGAACTATG	Check GAP1 locus, with MEM_SEQ_R (S37)
S28	FCY2_SEQ_F	TAATGACCCAACCTGGAAGG	Check MUP1 locus, with MEM_SEQ_R (S37)
S29	FEN2_SEQ_F	TAGGCATTGGCATGTTTCTG	Check MUP3 locus, with MEM_SEQ_R (S37)
S30	GAP1_SEQ_F	AACAATGGTTGCCCTAGCTG	Check PMA1 locus, with MEM_SEQ_R (S37)
S31	MUP1_SEQ_F	CCCAATGGTTGGTTTGTTTC	Check PTR2 locus, with MEM_SEQ_R (S37)
S32	MUP3_SEQ_F	CCAGAGTTGGTGGCAAATTC	Check SIT1 locus, with MEM_SEQ_R (S37)
S33	PMA1_SEQ_F	TCTCCAAAGCCCGTTAAATG	Check STE6 locus, with MEM_SEQ_R (S37)
S34	PTR2_SEQ_F	TGCGTTGATCATTTTCATCC	Check membrane protein tag incorporations with one of S17-S36, S38-42
S35	SIT1_SEQ_F	TTAGGTTTCGGTGCAGGTTC	Check PIL1 locus, with MEM_SEQ_R (S37)
S36	STE6_SEQ_F	CATTGGTGAATCAGGCACAG	Check ASK1 locus, with MEM_SEQ_R (S37)
S37	MEM_SEQ_R	CTGTCAAGGAGGGTATTCTG	Check SEC27 locus, with MEM_SEQ_R (S37)
S38	PIL1_SEQ_F	AAGATCACCGACAAAATCGC	Check ARC35 locus, with MEM_SEQ_R (S37)
S39	ASK1_SEQ_F	GCCTAACGAGGCAGATTCAG	Check PIL1 (exogenous copy) at URA3 locus, with MEM_SEQ_R (S37)

S40	SEC27_SEQ_F	AATGAAATGGAGAGCCTTGG	Check LEU2 tag incorporations with one of S44-47
S41	ARC35_SEQ_F	AATTACTGGCGGTGAGGTTG	Check NUP53 locus, with LEU2_SEQ_R (S45)
S42	PIL1_EXOG_SEQ_F	CTTGAGCTGCTTGATGATTC	Check NUP84 locus, with LEU2_SEQ_R (S45)
S43	LEU2_SEQ_R	ATACCTGAGTATTCCCACAG	Check NUP85 locus, with LEU2_SEQ_R (S45)
S44	NUP53_SEQ_F	GGGTCAAGTTGACTTACAAATC	Check NUP120 locus, with LEU2_SEQ_R (S45)
S45	NUP84_SEQ_F	AGCCGATAACATATCAAAGG	Check ADY2 locus, with MEM_SEQ_R (S37)
S46	NUP85_SEQ_F	GGTAAAGTCATATTCGTGGC	Check AGP1 locus, with MEM_SEQ_R (S37)
S47	NUP120_SEQ_F	TTGAGATTGGGTCACGAGTG	Check ATO3 locus, with MEM_SEQ_R (S37)

Table A5-3. Cloning primers used in this study.

Label	Name	Sequence	Purpose
C1	p6h_ath1_F2	CAGCGTACGAAGCTTCAG	Amplify pFA6a-HIS3MX6 around the horn to replace GAL1 with another promoter
C2	p6h_ath1_R2	AGAAAAAACCCCGGATTC	Amplify pFA6a-HIS3MX6 around the horn to replace GAL1 with another promoter
C3	p6k_ath1_F	TTGCAAACCAGAGCCTG	Amplify pFA6a-KANMX6 around the horn to replace MEEVF with another peptide
C4	p6k_ath1_R	TGATGAGTCATGTAATTAGTTATGT	Amplify pFA6a-KANMX6 around the horn to replace MEEVF with another peptide
C5	p6h_ath2_F	GAATCCGGGGTTTTTTCT	Amplify pFA6a-HIS3MX6 around the horn to replace TRAP4 with another protein
C6	p6h_ath2_R	CTGCAGATGAGTGCGATTA	Amplify pFA6a-HIS3MX6 around the horn to replace TRAP4 with another protein
C7	p6h_int_F	CTAATCCAAGGAGGTTTACGGACCAGGGGAACTTTCCAGATTCAGAAGCTTCGTACGCTGCA	Amplify the entire cassette from pFA6a-HIS3MX6 (e.g. TRAP4-mEOS under GAL1, plus HIS3 marker) for transformation into yeast
C8	p6h_int_R	CATGAAAAATTAAGAGAGATGATGGAGCGTCTCACTTCAAACGCAGGCGTTAGTATCGAATCG	Amplify the entire cassette from pFA6a-HIS3MX6 (e.g. TRAP4-mEOS under GAL1, plus HIS3 marker) for transformation into yeast
C9	p6k_int_F	GAAGAGCAGGTCAAAAGCTTGCAAGTAAAAAAATCCCATTTAAAAGGTGGATCAGGCTCTGG	Amplify the entire cassette from pFA6a-KANMX6 (e.g. GS-MEEVF, plus KANMX6 marker) for transformation into yeast
C10	p6k_int_R	AGGCGTTGAAATTGACGAGACAAAGAGGAAGACATTAATTAATCATTAGAAAAACTCATCGAGCATC	Amplify the entire cassette from pFA6a-KANMX6 (e.g. GS-MEEVF, plus KANMX6 marker) for transformation into yeast
C11	cPRO_rep_F2	CGCGGCCGCCAGCTGAAGCTTCGTACG	Amplify constitutive promoter sequence from pYTK0XX plasmids for replacing

		CTGCGTAGTCGGTC TCAAACG	GAL1 using C1 and C2 primers that amplify pFA6a-HIS3MX6 around the horn
C12	cPRO_re p_R2	GAATCCGGGGTTTT TTCTTTTATTGGTCT GGTCTCACATA	Amplify constitutive promoter sequence from pYTK0XX plasmids for replacing GAL1 using C1 and C2 primers that amplify pFA6a-HIS3MX6 around the horn
C13	p6h_ath 3_t4_F	TTGCTCCTTCAGGA TTTTCT	Amplify pFA6a-HIS3MX6 around the horn to replace TRAP4 mEOS with another FP (e.g. mNeonGreen). TRAP4 is left to make a TRAP4-FP fusion.
C14	p6h_ath 3_sz_F	CTTGTAAGCTTCAAT TTCCTTTCTCAAGT	Amplify pFA6a-HIS3MX6 around the horn to replace SYNZIP17 mEOS with another FP (e.g. mNeonGreen). SYNZIP17 is left to make a SYNZIP17-FP fusion.
C15	p6h_ath 3_R	TGATAAGTCATGTAA TTAGTTATGTC	Amplify pFA6a-HIS3MX6 around the horn to replace TRAP4 or SYNZIP mEOS with another FP (e.g. mNeonGreen). TRAP4 or SYNZIP17 is left to make a TRAP4/SYNZIP17-FP fusion.
C16	pCUP1_ ATH_F	AAGCTTATCGATAC CGTC	Amplify pCu415CUP1 around the horn to replace product under expression on pCUP1
C17	pCUP1_ ATH_R	ACTAGTCGATGACT TCTATATG	Amplify pCu415CUP1 around the horn to replace product under expression on pCUP1
C18	p6kcof1i ntF	TACGATTCTGTTTTG GAAAGAGTCAGCAG AGGCGCTGGTTCTC ATGGTGGATCAGGC TCTGG	Amplify the entire cassette from pFA6a-KANMX6 (e.g. GS-MEEVF, plus KANMX6 marker) for transformation into yeast at Cof1 locus
C19	p6kcof1i ntR	TTTCATTTTTCTTGA AGATTGTTGTCATTT GTGAAATCATTTACC ATTAGAAAAACTCAT CGAGCATC	Amplify the entire cassette from pFA6a-KANMX6 (e.g. GS-MEEVF, plus KANMX6 marker) for transformation into yeast at Cof1 locus
C20	p6h_ath 3_mNG_ R	ATAACATGGCCTCT CTCC	Amplify pFA6a-HIS3MX6 around the horn to replace TRAP4-mNG with LifeAct-mNG (use with C5)

C21	SZ17mCher_EYFP _F	ACTTGAGAAAGGAAATTGAAGCTTACAAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAGGGCG	Amplify mCherry to insert into pFA6a-HIS3MX6 in place of mNeonGreen in SZ17-mNeonGreen
C22	SZ17mCher_EYFP _R	GACATAACTAATTACATGACTTATCACTTGTACAGCTCGTCCATG	Amplify mCherry to insert into pFA6a-HIS3MX6 in place of mNeonGreen in SZ17-mNeonGreen
C23	SZ17EYFP _F	ACTTGAGAAAGGAAATTGAAGCTTACAAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGTAAAGGAGAAGAACTTTTCAC	Amplify EYFP to insert into pFA6a-HIS3MX6 in place of mNeonGreen in SZ17-mNeonGreen
C24	SZ17EYFP _R	GACATAACTAATTACATGACTTATCATTTGTATAGTTCATCCATGCC	Amplify EYFP to insert into pFA6a-HIS3MX6 in place of mNeonGreen in SZ17-mNeonGreen
C25	kanmx_gs_mng_F	TGGTGGATCAGGCTCTGGTTTGCAAATGGTGAGCAAGGGCG	Amplify mNeonGreen to insert into pFA6a-KANMX6 in place of SYNZIP18
C26	kanmx_gs_mng_R	ACATAACTAATTACATGACTCATCACTTGTACAGCTCGTCCATG	Amplify mNeonGreen to insert into pFA6a-KANMX6 in place of SYNZIP18
C27	GSmNG_insA_F	AGGCTCTGGTTTGCAAATGGTGAGCAAGGGC	Fix deletion of A in GS-mNG construct to be used for mNG direct fusion
C28	GSmNG_insA_R	GCCCTTGCTCACCATTTGCAAACCAGAGCCT	Fix deletion of A in GS-mNG construct to be used for mNG direct fusion
C29	p6k_ath2_F	AGCGTACGAAGCTTCAG	Amplify pFA6a-KANMX6 around the horn to replace GS-MEEVF with GS-mNG using the gBlock
C30	p6h_ath4_F	GAATCCGGGGTTTTTTCTCC	Amplify pFA6a-HIS3MX6 around the horn to replace SZ17-mNG with 101/2/7/8A-mNG (use with C31)
C31	p6h_ath4_R	ATGGTGAGCAAGGGCG	Amplify pFA6a-HIS3MX6 around the horn to replace SZ17-mNG with 101/2/7/8A-mNG (use with C30)

C32	URA3_rep_HIS3_F	GCCAGATCTGTTTAGCTTGCCTCGTTTCAATTCAATTCATCATTTTTTTTT	Amplify URA3 gene from pRS306 plasmid to Gibson into pFA6aHIS3MX6 (PCR with C33)
C33	URA3_rep_HIS3_R	GAATCTTTTTATTGTCAGTACTTCAGTTTTGCTGGCCGCATC	Amplify URA3 gene from pRS306 plasmid to Gibson into pFA6aHIS3MX6 (PCR with C32)
C34	HIS3_rep_ath_F	ACGAGGCAAGCTAAACAG	Amplify pFA6aHIS3MX6 around the horn to replace with URA3 (URA3 pcr'd from pRS306 using C32 and C33). PCR with C35.
C35	HIS3_rep_ath_R	TGAAGTACTGACAATAAAAAGAT	Amplify pFA6aHIS3MX6 around the horn to replace with URA3 (URA3 pcr'd from pRS306 using C32 and C33). PCR with C34.
C36	URA3_int_F	GAGGCATATTTATGGTGAAGGATAAGTTTTGACCATCAAAGAAGGTAAGCTTCGTACGCTGCA	Seq not correct? Use C51 instead.PCR to amplify product from pFA6aHIS3MX6 (which now has URA3 marker instead) to insert into URA3 locus. PR with C37.
C37	URA3_int_R	GTCTTATTGTTCTTGATTTGTGCCCCGTAAAATACTGTTACTTGGGGCGTTAGTATCGAATCG	PCR to amplify product from pFA6aHIS3MX6 (which now has URA3 marker instead) to insert into URA3 locus. PR with C36.
C38	LEU2_F	GGCGCGCCAGATCTGTTTAGCTTGCAACTGTGGGAATACTCAG	PCR to amplify LEU2 from pRS305 to swap in place of KanMX6 in pFA6a-KanMX6 plasmids
C39	LEU2_R	AGAATCTTTTTATTGTCAGTACTGATTAAGCAAGGATTTTCTTAACT	PCR to amplify LEU2 from pRS305 to swap in place of KanMX6 in pFA6a-KanMX6 plasmids
C40	p6k_sel_amp_F	GCAAGCTAAACAGATCTG	PCR to amplify pFA6a-KanMX6 plasmids to swap selection marker with another, e.g. LEU2 using C38/C39
C41	p6k_sel_amp_R	TCAGTACTGACAATAAAAAGA	PCR to amplify pFA6a-KanMX6 plasmids to swap selection marker with another, e.g. LEU2 using C38/C39
C42	108A_R	CATGTTATCCTCCTCGCCCTTGCTCACCATTTGCAATCCACTACCACTTCCACCCTCT	For use with primer C2 to amplify 108A to swap with SZ17 in SZ17-mCherry (URA3 plasmid)

		AATTTGTCTTTCTCTTTCT	
C43	Nup53_F	TGGTTAAATAGATTGAATAATTGGTTATTTGGATGGAATGATTTGGGTGGATCAGGCTCTGG	To amplify pFA6a-LeuMX6 plasmids to tag Nup53 (e.g. tag with 108B)
C44	Nup53_R	GCTGGAATCGCACCAAAGCACTACATTTGGGGGTAAGGTTTTTCATTAAGCAAGGATTTTCTTAACTTCT	To amplify pFA6a-LeuMX6 plasmids to tag Nup53 (e.g. tag with 108B)
C45	Nup84_F	AAAGAGTATCTGGATCTCGTTGCTCGCACAGCAACCCTTTCGAACGGTGGATCAGGCTCTGG	To amplify pFA6a-LeuMX6 plasmids to tag Nup53 (e.g. tag with 108B)
C46	Nup84_R	GCTGTTTACTTAAAATATAAACTTATTCTGCAATACATTAATTGATTAAGCAAGGATTTTCTTAACTTCT	To amplify pFA6a-LeuMX6 plasmids to tag Nup53 (e.g. tag with 108B)
C47	Nup85_F	AATTTAAGAAAGAAACTAAATTTCAAATTATGTCAAGCGTTCATGGGTGGATCAGGCTCTGG	To amplify pFA6a-LeuMX6 plasmids to tag Nup53 (e.g. tag with 108B)
C48	Nup85_R	GTCATATATCCTAATATACCATCCGAAGGACATTACTATACTGATTTAAGCAAGGATTTTCTTAACTTCT	To amplify pFA6a-LeuMX6 plasmids to tag Nup53 (e.g. tag with 108B)
C49	Nup120_F	AAAGTGGTTACTTTAACTGATTTAAGAGATGAGTTACGAGGTCTAGGTGGATCAGGCTCTGG	To amplify pFA6a-LeuMX6 plasmids to tag Nup53 (e.g. tag with 108B)
C50	Nup120_R	GATCAATTTTTTAAATGAAGTATTAATTTACAGTTTATATATTCATTAAGCAAGGATTTTCTTAACTTCT	To amplify pFA6a-LeuMX6 plasmids to tag Nup53 (e.g. tag with 108B)

C51	URA3_int_F2	GAGGCATATTTATGGTGAAGGATAAGTTTTGACCATCAAAGAAGGTCTGAAGCTTCGTACGCTG	PCR to amplify product from pFA6aHIS3MX6 (which now has URA3 marker instead) to insert into URA3 locus. PCR with C37.
C52	GSmEOS_F	TGGTGGATCAGGCTCTGGTTTGCAAATGAGTGCGATTAAGCCA	To make GS-mEOS in pFa6a-KanMX6. Amplify GS-MEEVF vector using C3 and C4, amplify mEOS using below (anneal 60C), then Gibson together
C53	GSmEOS_R	ACATAACTAATTACATGACTCATCATCGTCTGGCATTGTCAG	To make GS-mEOS in pFa6a-KanMX6. Amplify GS-MEEVF vector using C3 and C4, amplify mEOS using below (anneal 60C), then Gibson together
C54	URA3_p6k_int_F	GAGGCATATTTATGGTGAAGGATAAGTTTTGACCATCAAAGAAGGTCTAGTTAGAAAGACATTTTTGCTG	To insert pCUP1 PIL1-GS-101B or PIL1-GS-mEOS at URA3 locus (yeast integration amplification pcr)
C55	URA3_p6k_int_R	GTCTTATTGTTCTTGATTTGTGCCCCGTAAAATACTGTTACTTGGATTAGAAAAACTCATCGAGCATC	To insert pCUP1 PIL1-GS-101B or PIL1-GS-mEOS at URA3 locus (yeast integration amplification pcr)
C56	PIL1_F	ACACCAGCAAAGTGAGTCTCTTCCCCAACAAACAACAGCTGGTGGATCAGGCTCTGG	To amplify pFA6a-HIS3MX6 plasmids to tag PIL1 (along with primer C57) for yeast integrations
C57	PIL1_R	TTTTTTTTTTTGTTTCTAATAGATTGTTGATTTATTTTGAATTAGAAAAACTCATCGAGCATC	To amplify pFA6a-HIS3MX6 plasmids to tag PIL1 (along with primer C56) for yeast integrations
C58	ASK1_F	ACCAAAGCCTGGGACCATCATTCATTTTTCTACGAATAGAGGTGGATCAGGCTCTGG	To amplify pFA6a-HIS3MX6 plasmids to tag ASK1 (along with primer C59) for yeast integrations
C59	ASK1_R	GATATTCATCACTAGTAAAAATTGTATGTACTTATTTATTATTAGAAAAACTCATCGAGCATC	To amplify pFA6a-HIS3MX6 plasmids to tag ASK1 (along with primer C58) for yeast integrations

C60	SEC27_F	GCAAGGAGAGGCAGTGCCGGAGCCTGTGGAAGAAGAGAGTGGTGGATCAGGCTCTGG	To amplify pFA6a-HIS3MX6 plasmids to tag SEC27 (along with primer C61) for yeast integrations
C61	SEC27_R	ATTCGTTGATTAGTTCTTTATTTGTCTTTCTATGAGGTGGATTAGAAAAACTCATCGAGCATC	To amplify pFA6a-HIS3MX6 plasmids to tag SEC27 (along with primer C60) for yeast integrations
C62	ARC35_F	ACAGGCAAGAAGAACCTTCACCGGTAGAAAGATTGTCTACGGTGGATCAGGCTCTGG	To amplify pFA6a-HIS3MX6 plasmids to tag ARC35 (along with primer C63) for yeast integrations
C63	ARC35_R	TAACCCTTTTTACGGATTCTTACGTACTTATTTAATCTTTATTAGAAAAACTCATCGAGCATC	To amplify pFA6a-HIS3MX6 plasmids to tag SEC27 (along with primer C62) for yeast integrations

Table A5-4. gBlocks used in this study. Sequence is listed below each construct.

Label	Name	Purpose
G1	CCAN35_GB2	gBlock for inserting CCAN3,5 coil into pFA6a-HIS3MX6 in place of TRAP4, using plasmid amplified around the horn using primers C5 and C6
TTAACGTCAAGGAGAAAAAACCCCGGATTCATGGGTCTCGAACAAGAAATTGCAGCTTTGGAGAAGGAAAACGCTGCTTTGGAATGGGAAATTGCTGCATTGGAACAAGGTGGAAGTGGTAGTGGAAGTGGCCTGCAGATGAGTGCGATTAAGCCAGACATG		
G2	CCBN35_GB2	gBlock for inserting CCBN3,5 coil into pFA6a-KANMX6 in place of MEEVF, using plasmid amplified around the horn using primers C1 and C2
TTAACGTCAAGGAGAAAAAACCCCGGATTCATGGGTCTCGAACAAGAAATTGCAGCTTTGGAGAAGGAAAACGCTGCTTTGGAATGGGAAATTGCTGCATTGGAACAAGGTGGAAGTGGTAGTGGAAGTGGCCTGCAGATGAGTGCGATTAAGCCAGACATG		
G3	SYNZIP17_GB2	gBlock for inserting SYNZIP17 into pFA6a-HIS3MX6 in place of TRAP4, using plasmid amplified around the horn using primers C5 and C6
TTAACGTCAAGGAGAAAAAACCCCGGATTCATGAACGAAAAGGAAGAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGAAACAAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAATTGAAGCTTACAAGAGTGGTAGTGGAAGTGGCCTGCAGATGAGTGCGATTAAGCCAGACATG		
G4	SYNZIP18_GB2	gBlock for inserting SYNZIP18 into pFA6a-KANMX6 in place of MEEVF, using plasmid amplified around the horn using primers C1 and C2
TTAACGTCAAGGAGAAAAAACCCCGGATTCATGAACGAAAAGGAAGAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGAAACAAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAATTGAAGCTTACAAGAGTGGTAGTGGAAGTGGCCTGCAGATGAGTGCGATTAAGCCAGACATG		
G5	mNG_for_T4	gBlock for inserting mNeonGreen into pFA6a-HIS3MX6 in place of mEOS in TRAP4-mEOS using plasmid amplified around the horn using primers P13 and P15
AGAAAATCCTGAAGGAGCAAGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAGGGCGAGGAGGATAACATGGCCTCTCTCCCAGCGACACATGAGTTACACATCTTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGCACCGGCAATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTC		

CACCAAGGGTGACCTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATC GGGTATGGCTTCCATCAGTACCTGCCCTACCCTGACGGGATGTCGCCTT TCCAGGCCGCCATGGTAGATGGCTCCGGCTACCAAGTCCATCGCACAAT GCAGTTTGAAGATGGTGCCTCCCTTACTGTTAACTACCGCTACACCTACG AGGGAAGCCACATCAAAGGAGAGGCCCAGGTGAAGGGGACTGGTTTCC CTGCTGACGGTCCTGTGATGACCAACTCGCTGACCGCTGCGGACTGGTG CAGGTCGAAGAAGACTTACCCCAACGACAAAACCATCATCAGTACCTTTA AGTGGAGTTACACCACTGGAAATGGCAAGCGCTACCGGAGCACTGCGC GGACCACCTACACCTTTGCCAAGCCAATGGCGGCTAACTATCTGAAGAA CCAGCCGATGTACGTGTTCCGTAAGACGGAGCTCAAGCACTCCAAGACC GAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTTACCGATGTGATGGGCA TGGACGAGCTGTACAAGTGATAAGTCATGTAATTAGTTATGTC		
G6	mNG_for_SZ17	gBlock for inserting mNeonGreen into pFA6a-HIS3MX6 in place of mEOS in SYNZIP17-mEOS using plasmid amplified around the horn using primers P14 and P15
ACTTGAGAAAGGAAATTGAAGCTTACAAGGGCGGCAGCGGCAGCGGCC TGCAGATGGTGAGCAAGGGCGAGGAGGATAACATGGCCTCTCTCCCAG CGACACATGAGTTACACATCTTTGGCTCCATCAACGGTGTGGACTTTGAC ATGGTGGGTCAGGGCACCGGCAATCCAAATGATGGTTATGAGGAGTTAA ACCTGAAGTCCACCAAGGGTGACCTCCAGTTCTCCCCCTGGATTCTGGT CCCTCATATCGGGTATGGCTTCCATCAGTACCTGCCCTACCCTGACGGG ATGTCGCCTTTCCAGGCCGCCATGGTAGATGGCTCCGGCTACCAAGTCC ATCGCACAATGCAGTTTGAAGATGGTGCCTCCCTTACTGTTAACTACCGC TACACCTACGAGGGAAGCCACATCAAAGGAGAGGCCCAGGTGAAGGGG ACTGGTTTCCCTGCTGACGGTCCTGTGATGACCAACTCGCTGACCGCTG CGGACTGGTGCAGGTCGAAGAAGACTTACCCCAACGACAAAACCATCAT CAGTACCTTTAAGTGGAGTTACACCACTGGAAATGGCAAGCGCTACCGG AGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAATGGCGGCTAACT ATCTGAAGAACCAGCCGATGTACGTGTTCCGTAAGACGGAGCTCAAGCA CTCCAAGACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTTACCGAT GTGATGGGCATGGACGAGCTGTACAAGTGATAAGTCATGTAATTAGTTAT GTC		
G7	SZ18-Actin	gBlock for inserting SZ18-Actin into pCu415CUP1 using plasmid amplified around the horn using primers C16 and C17
TGCAATATCATATAGAAGTCATCGACTAGTATGTCTATTGCTGCTACTTTG GAAAACGACTTGGCTAGACTTGAAAACGAAAACGCTAGATTGGAAAAGG ACATTGCTAACTTGGAAAGAGACTTGGCTAAGTTGGAAAGAGAAGAAGCT TACTTCGGTGGATCAGGCTCTGGTATGGATTCTGAGGTTGCTGCTTTGGT TATTGATAACGGTTCTGGTATGTGTAAAGCCGGTTTTGCCGGTGACGACG CTCCTCGTGCTGTCTTCCCATCTATCGTCGGTAGACCAAGACACCAAGGT ATCATGGTCGGTATGGGTCAAAAAGACTCCTACGTTGGTGATGAAGCTCA ATCCAAGAGAGGTATCTTGACTTTACGTTACCCAATTGAACACGGTATTG		

TCACCAACTGGGACGATATGGAAAAGATCTGGCATCATACCTTCTACAAC GAATTGAGAGTTGCCCCAGAAGAACACCCTGTTCTTTTGACTGAAGCTCC AATGAACCCTAAATCAAACAGAGAAAAGATGACTCAAATTATGTTTGAAAC TTTCAACGTTCCAGCCTTCTACGTTTCCATCCAAGCCGTTTTGTCCTTGTA CTCTTCCGGTAGAACTACTGGTATTGTTTTGGATTCCGGTGATGGTGTTA CTCACGTCGTTCCAATTTACGCTGGTTTCTCTCTACCTCACGCCATTTTGA GAATCGATTTGGCCGGTAGAGATTTGACTGACTACTTGATGAAGATCTTG AGTGAACGTGGTTACTCTTTCTCCACCACTGCTGAAAGAGAAATTGTCCG TGACATCAAGGAAAAACTATGTTACGTCGCCTTGGACTTCGAACAAGAAA TGCAAACCGCTGCTCAATCTTCTTCAATTGAAAAATCCTACGAACTTCCA GATGGTCAAGTCATCACTATTGGTAACGAAAGATTCAGAGCCCCAGAAG CTTTGTTCCATCCTTCTGTTTTGGGTTTGGAATCTGCCGGTATTGACCAAA CTACTTACAACTCCATCATGAAGTGTGATGTCGATGTCCGTAAGGAATTA TACGGTAACATCGTTATGTCCGGTGGTACCACCATGTTCCCAGGTATTGC CGAAAGAATGCAAAAGGAAATCACCGCTTTGGCTCCATCTTCCATGAAGG TCAAGATCATTGCTCCTCCAGAAAGAAAGTACTCCGTCTGGATTGGTGGT TCTATCTTGGCTTCTTTGACTACCTTCCAACAAATGTGGATCTCAAAACAA GAATACGACGAAAGTGGTCCATCTATCGTTCACCACAAGTGTTTCTAATG AAAGCTTATCGATACCGTCGACCTCGAGTCA		
G8	Actin-SZ18	gBlock for inserting Actin-SZ18 into pCu415CUP1 using plasmid amplified around the horn using primers C16 and C17
TGCAATATCATATAGAAGTCATCGACTAGTATGGATTCTGAGGTTGCTGC TTTGGTTATTGATAACGGTTCTGGTATGTGTAAAGCCGGTTTTGCCGGTG ACGACGCTCCTCGTGCTGTCTTCCCATCTATCGTCGGTAGACCAAGACA CCAAGGTATCATGGTCGGTATGGGTCAAAAAGACTCCTACGTTGGTGAT GAAGCTCAATCCAAGAGAGGTATCTTGACTTTACGTTACCCAATTGAACA CGGTATTGTCACCAACTGGGACGATATGGAAAAGATCTGGCATCATACCT TCTACAACGAATTGAGAGTTGCCCCAGAAGAACACCCTGTTCTTTTGACT GAAGCTCCAATGAACCCTAAATCAAACAGAGAAAAGATGACTCAAATTAT GTTTGAAACTTTCAACGTTCCAGCCTTCTACGTTTCCATCCAAGCCGTTTT GTCCTTGTACTCTTCCGGTAGAACTACTGGTATTGTTTTGGATTCCGGTG ATGGTGTTACTCACGTCGTTCCAATTTACGCTGGTTTCTCTCTACCTCAC GCCATTTTGAGAATCGATTTGGCCGGTAGAGATTTGACTGACTACTTGAT GAAGATCTTGAGTGAACGTGGTTACTCTTTCTCCACCACTGCTGAAAGAG AAATTGTCCGTGACATCAAGGAAAAACTATGTTACGTCGCCTTGGACTTC GAACAAGAAATGCAAACCGCTGCTCAATCTTCTTCAATTGAAAAATCCTA CGAACTTCCAGATGGTCAAGTCATCACTATTGGTAACGAAAGATTCAGAG CCCCAGAAGCTTTGTTCCATCCTTCTGTTTTGGGTTTGGAATCTGCCGGT ATTGACCAAACTACTTACAACTCCATCATGAAGTGTGATGTCGATGTCCG TAAGGAATTATACGGTAACATCGTTATGTCCGGTGGTACCACCATGTTCC CAGGTATTGCCGAAAGAATGCAAAAGGAAATCACCGCTTTGGCTCCATCT TCCATGAAGGTCAAGATCATTGCTCCTCCAGAAAGAAAGTACTCCGTCTG		

GATTGGTGGTTCTATCTTGGCTTCTTTGACTACCTTCCAACAAATGTGGAT CTCAAAACAAGAATACGACGAAAGTGGTCCATCTATCGTTCACCACAAGT GTTTCGGTGGATCAGGCTCTGGTTCTATTGCTGCTACTTTGGAAAACGAC TTGGCTAGACTTGAAAACGAAAACGCTAGATTGGAAAAGGACATTGCTAA CTTGGAAAGAGACTTGGCTAAGTTGGAAAGAGAAGAAGCTTACTTCTAAT GAAAGCTTATCGATACCGTCGACCTCGAGTCA		
G9	Actin-MEEVF	gBlock for inserting Actin-MEEVF into pCu415CUP1 using plasmid amplified around the horn using primers C16 and C17
TGCAATATCATATAGAAGTCATCGACTAGTATGGATTCTGAGGTTGCTGC TTTGGTTATTGATAACGGTTCTGGTATGTGTAAAGCCGGTTTTGCCGGTG ACGACGCTCCTCGTGCTGTCTTCCCATCTATCGTCGGTAGACCAAGACA CCAAGGTATCATGGTCGGTATGGGTCAAAAAGACTCCTACGTTGGTGAT GAAGCTCAATCCAAGAGAGGTATCTTGACTTTACGTTACCCAATTGAACA CGGTATTGTCACCAACTGGGACGATATGGAAAAGATCTGGCATCATACCT TCTACAACGAATTGAGAGTTGCCCCAGAAGAACACCCTGTTCTTTTGACT GAAGCTCCAATGAACCCTAAATCAAACAGAGAAAAGATGACTCAAATTAT GTTTGAAACTTTCAACGTTCCAGCCTTCTACGTTTCCATCCAAGCCGTTTT GTCCTTGTACTCTTCCGGTAGAACTACTGGTATTGTTTTGGATTCCGGTG ATGGTGTTACTCACGTCGTTCCAATTTACGCTGGTTTCTCTCTACCTCAC GCCATTTTGAGAATCGATTTGGCCGGTAGAGATTTGACTGACTACTTGAT GAAGATCTTGAGTGAACGTGGTTACTCTTTCTCCACCACTGCTGAAAGAG AAATTGTCCGTGACATCAAGGAAAAACTATGTTACGTCGCCTTGGACTTC GAACAAGAAATGCAAACCGCTGCTCAATCTTCTTCAATTGAAAAATCCTA CGAACTTCCAGATGGTCAAGTCATCACTATTGGTAACGAAAGATTCAGAG CCCCAGAAGCTTTGTTCCATCCTTCTGTTTTGGGTTTGGAATCTGCCGGT ATTGACCAAACTACTTACAACTCCATCATGAAGTGTGATGTCGATGTCCG TAAGGAATTATACGGTAACATCGTTATGTCCGGTGGTACCACCATGTTCC CAGGTATTGCCGAAAGAATGCAAAAGGAAATCACCGCTTTGGCTCCATCT TCCATGAAGGTCAAGATCATTGCTCCTCCAGAAAGAAAGTACTCCGTCTG GATTGGTGGTTCTATCTTGGCTTCTTTGACTACCTTCCAACAAATGTGGAT CTCAAAACAAGAATACGACGAAAGTGGTCCATCTATCGTTCACCACAAGT GTTTCGGTGGATCAGGCTCTGGTATGGAAGAGGTTTTTTAATGAAAGCTT ATCGATACCGTCGACCTCGAGTCA		
G10	Actin-GS	gBlock for inserting Actin-GS into pCu415CUP1 using plasmid amplified around the horn using primers C16 and C17
TGCAATATCATATAGAAGTCATCGACTAGTATGGATTCTGAGGTTGCTGC TTTGGTTATTGATAACGGTTCTGGTATGTGTAAAGCCGGTTTTGCCGGTG ACGACGCTCCTCGTGCTGTCTTCCCATCTATCGTCGGTAGACCAAGACA CCAAGGTATCATGGTCGGTATGGGTCAAAAAGACTCCTACGTTGGTGAT GAAGCTCAATCCAAGAGAGGTATCTTGACTTTACGTTACCCAATTGAACA CGGTATTGTCACCAACTGGGACGATATGGAAAAGATCTGGCATCATACCT		

TCTACAACGAATTGAGAGTTGCCCCAGAAGAACACCCTGTTCTTTTGACT GAAGCTCCAATGAACCCTAAATCAAACAGAGAAAAGATGACTCAAATTAT GTTTGAAACTTTCAACGTTCCAGCCTTCTACGTTTCCATCCAAGCCGTTTT GTCCTTGTACTCTTCCGGTAGAACTACTGGTATTGTTTTGGATTCCGGTG ATGGTGTTACTCACGTCGTTCCAATTTACGCTGGTTTCTCTCTACCTCAC GCCATTTTGAGAATCGATTTGGCCGGTAGAGATTTGACTGACTACTTGAT GAAGATCTTGAGTGAACGTGGTTACTCTTTCTCCACCACTGCTGAAAGAG AAATTGTCCGTGACATCAAGGAAAAACTATGTTACGTCGCCTTGGACTTC GAACAAGAAATGCAAACCGCTGCTCAATCTTCTTCAATTGAAAAATCCTA CGAACTTCCAGATGGTCAAGTCATCACTATTGGTAACGAAAGATTCAGAG CCCCAGAAGCTTTGTTCCATCCTTCTGTTTTGGGTTTGGAATCTGCCGGT ATTGACCAAACTACTTACAACTCCATCATGAAGTGTGATGTCGATGTCCG TAAGGAATTATACGGTAACATCGTTATGTCCGGTGGTACCACCATGTTCC CAGGTATTGCCGAAAGAATGCAAAAGGAAATCACCGCTTTGGCTCCATCT TCCATGAAGGTCAAGATCATTGCTCCTCCAGAAAGAAAGTACTCCGTCTG GATTGGTGGTTCTATCTTGGCTTCTTTGACTACCTTCCAACAAATGTGGAT CTCAAAACAAGAATACGACGAAAGTGGTCCATCTATCGTTCACCACAAGT GTTTCGGTGGATCAGGCTCTGGTTAATGAAAGCTTATCGATACCGTCGAC CTCGAGTCA		
G11	LifeAct-mNG	gBlock for inserting LifeAct-mNG into pFA6a-HIS3MX6 TRAP4-mNG in place of TRAP4 using plasmid amplified around the horn using primers C5 and C20
TTAACGTCAAGGAGAAAAAACCCCGGATTCATGGGCGTGGCCGACTTGA TCAAGAAGTTCGAGTCCATCTCCAAGGAGGAGCGCATTCCGGGCCTGAT TAACATGGTGAGCAAGGGCGAGGAGGATAACATGGCCTCTCTCCCAGCG ACACATG		
G12	SZ17-GRvT	gBlock for inserting GRvT into pFA6aHIS3MX6 to replace mNeonGreen in SZ17-mNeonGreen
ACTTGAGAAAGGAAATTGAAGCTTACAAGGGCGGATCAGGCTCCGGATT GCAGATGGTCAGTAAGGGGGAGGAGGTCATAAAGGAATTCATGCGTTTC AAGGTGAGAATGGAAGGGTCAATGAATGGACATGAATTCGAAATTGAAG GGGAAGGCGAGGGGAGACCGTATGAAGGGACTCAAACGGCCAAGTTAA AGGTAACAAAGGGTGGGCCGCTTCCCTTCGCTTGGGACATTCTTTCTCC ACTATTGATGTATGGGTCTAAAATGTACGTTAAACACCCGGCGGACATCC CGGACTATAAGAAACTTTCTTTTCCGGAAGGTTTTAAATGGGTCAGAGTT ATGAATTTTGAGGATGGTGGCTTGGTCACAGCCACACAGGACAGCTCCC TACAGGACGGCACTCTTATCTATGAAGTCAAGATGAGGGGGACAAACTTT CCCCCCGACGGACCAGTGATGCAGAAGAAAACAATGGGTTGGGAAGCTA GTACGGAGCGTCTTTACCCCAGGGATGGAGTATTAAAGGGCGAGATTCA CCAAGCTCTAAAACTGAAAGACGGTGGGCATTACTTAGTAGAATTTAAAA CGATCTACATGGCTAAGAAGCCAGTTCAACTTCCAGGTTACTATTATGTC GACACGAAGCTGGACATAACCAGCCACAACGAGGACTATACGAGAGTCG AGCAGTACGAGAGGTCAGAGGGAAGGCATCACCTATTCCTGTACGACGG		

AAGCACCGGAAGTGGCTCATCAGGTACCATGGTATCAAAAGGGGAAGAA
GTGATTAAAGAGTTCATGAGGTTTAAAGTCAGAATGGAAGGGAGTATGAA
CGGACATGAATTCGAGATAGAAGGAGAGGGTGAGGGGAGACCATATGA
GGGTACTCAGACCGCTAAGTTAAAGGTGACAAAAGGTGGCCCACTTCCG
TTCGCCTGGGACATTCTATCTCCGCAGTTTATGTACGGGTCCAAAGCATA
CGTTAAGCACCCAGCTGATATCCCGGATTATAAAAAACTAAGCTTTCCTG
AGGGTTTCAAATGGGAAAGGGTGATGAACTTCGAGGATGGTGGATTGGT
AACTGTCACTCAGGACTCCTCTATTCAGGACGGAACGCTAATTTATAAGG
TCAAAATGAGGGGTACAAACTTTCCGCCTGATGGCCCAGTCATGCAGAA
AAAGACAATGGGATGGGAAGCATCCACTGAGAGGTTATATCCACGTGAT
GGGGGTTTTGAAGGGCGAGATCCATCAGGCGCTAAAACTAAAGGATGGGG
GCCACTACCTGGTGGAGTTCAAGACTATCTACATGGCCAAAAAGCCAGT
CCAACTACCTGGTTATTATTATGTAGATACAAAGCTTGATATTACATCACA
TAACGAGGATTACACCATTGTCGAACAGTACGAGAGAAGCGAGGGTAGA
CACCATCTATTTCTGTACGGTATGGATGAGTTGTACAAGTGATAAGTCAT
GTAATTAGTTATGTC

G13	SZ17-3xmNG	gBlock for inserting 3xmNeonGreen into pFA6aHIS3MX6 to replace mNeonGreen in SZ17-mNeonGreen

ACTTGAGAAAGGAAATTGAAGCTTACAAGGGCGGCAGCGGCAGCGGCC
TGCAGATGGTGTCCAAAGGAGAAGAAGATAATATGGCAAGCCTTCCCGC
CACTCACGAGTTACACATATTCGGGTCTATCAATGGGGTGGACTTTGACA
TGGTAGGGCAGGGTACTGGTAACCCAAATGACGGCTACGAAGAATTAAA
TTTGAAATCAACGAAGGGGGATCTGCAGTTTTCCCCATGGATACTAGTAC
CTCACATAGGCTACGGATTCCACCAGTATCTTCCGTATCCTGATGGAATG
AGCCCGTTCCAAGCCGCGATGGTGGATGGTAGCGGCTATCAGGTACATC
GTACTATGCAATTCGAAGACGGAGCCAGCCTTACTGTCAACTACAGGTAC
ACATATGAGGGGTCCCACATTAAGGGGGAGGCACAGGTGAAAGGTACTG
GGTTTCCAGCGGATGGACCTGTCATGACCAACTCACTAACCGCAGCAGA
TTGGTGTAGGAGCAAAAAAACATATCCTAACGACAAAACAATAATATCCA
CGTTTAAATGGAGTTACACGACTGGAAATGGGAAACGTTATAGGTCTACG
GCCAGAACAACCTATACCTTCGCGAAGCCGATGGCAGCTAATTATTTAAA
GAACCAACCGATGTACGTTTTTAGGAAGACCGAGTTGAAACATTCAAAGA
CAGAGTTAAATTTCAAGGAGTGGCAAAAAGCCTTTACCGATGTCATGGGG
ATGGATGAATTATACAAGGGTAGCATGGTAAGCAAAGGGGAAGAGGACA
ACATGGCAAGTTTGCCGGCGACCCATGAGCTTCATATATTCGGATCCATC
AATGGAGTGGATTTTGATATGGTTGGTCAAGGAACCGGCAACCCAAATG
ATGGGTATGAAGAACTTAATCTTAAGTCTACTAAGGGCGACCTTCAATTC
TCACCCTGGATCCTAGTTCCTCATATAGGGTACGGCTTCCACCAATACCT
ACCGTACCCGGATGGCATGTCACCTTTTCAAGCAGCTATGGTAGACGGT
TCTGGTTACCAAGTCCACAGGACTATGCAGTTCGAAGATGGCGCATCTTT
AACTGTAAACTATAGATACACTTATGAAGGTAGTCATATTAAGGGAGAGG
CACAGGTAAAAGGAACTGGTTTTCCGGCGGACGGCCCGGTTATGACTAA
TAGCTTAACGGCTGCGGACTGGTGCAGAAGCAAGAAGACGTATCCGAAT

GACAAGACCATCATTTCTACTTTCAAATGGTCATACACAACAGGTAATGG CAAAAGGTATAGGAGTACCGCTAGAACTACTTATACTTTTGCGAAACCAA TGGCTGCCAATTATCTGAAGAATCAACCCATGTACGTATTTCGTAAGACC GAACTTAAGCATAGCAAGACGGAGCTAAACTTCAAGGAATGGCAAAAGG CGTTTACTGACGTTATGGGTATGGATGAATTGTATAAAGGCAGTATGGTC AGTAAAGGTGAGGAGGATAATATGGCCAGCCTGCCGGCCACGCACGAG TTGCACATTTTCGGTTCCATTAACGGAGTCGACTTCGATATGGTTGGACA GGGAACCGGGAATCCAAATGACGGGTACGAAGAATTAAACCTAAAAAGT ACAAAAGGCGATCTACAATTTTCTCCCTGGATACTAGTACCACATATCGG CTATGGTTTTCACCAGTACCTTCCTTACCCTGATGGCATGTCCCCCTTTC AAGCTGCTATGGTGGATGGTTCCGGGTACCAAGTGCATAGAACCATGCA GTTCGAGGATGGAGCTAGTCTAACTGTTAACTACAGATATACATACGAGG GATCCCATATTAAAGGCGAGGCTCAGGTGAAAGGGACGGGGTTCCCGG CGGATGGCCCGGTCATGACTAATTCCTTAACGGCGGCAGATTGGTGTAG GTCAAAAAAGACATACCCAAACGATAAAACGATTATAAGTACATTTAAGTG GTCTTATACCACCGGTAATGGAAAGCGTTATCGTAGCACGGCTAGGACT ACATACACGTTCGCCAAGCCGATGGCGGCTAATTACCTTAAGAACCAGC CGATGTATGTGTTCCGTAAGACCGAATTAAAACATTCTAAAACAGAGCTA AATTTTAAGGAGTGGCAAAAAGCATTCACGGATGTCATGGGAATGGATGA ACTGTATAAGTGATAAGTCATGTAATTAGTTATGTC		
G14	SZ17-mOrange	gBlock for inserting mOrange into pFA6aHIS3MX6 to replace mNeonGreen in SZ17-mNeonGreen
ACTTGAGAAAGGAAATTGAAGCTTACAAGGGCGGCAGCGGCAGCGGCC TGCAGATGGTGAGCAAGGGCGAGGAGAATAACATGGCCATCATCAAGGA GTTCATGCGCTTCAAGGTGCGCATGGAGGGCTCCGTGAACGGCCACGA GTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCTTTCA GACCGCTAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTG GGACATCCTGTCCCCTCAGTTCACCTACGGCTCCAAGGCCTACGTGAAG CACCCCGCCGACATCCCCGACTACTTCAAGCTGTCCTTCCCCGAGGGCT TCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCG TGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAA GCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAA GACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGG CGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAGGACGGCGG CCACTACACCTCCGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTG CAGCTGCCCGGCGCCTACATCGTCGGCATCAAGTTGGACATCACCTCCC ACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCC GCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTGATAAGTCATGTA ATTAGTTATGTC		
G15	SZ17-mKO	gBlock for inserting mKO into pFA6aHIS3MX6 to replace mNeonGreen in SZ17-mNeonGreen
ACTTGAGAAAGGAAATTGAAGCTTACAAGGGCGGCAGCGGCAGCGGCC TGCAGATGGTGAGTGTGATTAAACCAGAGATGAAGATGAGGTACTACAT		

GGACGGCTCCGTCAATGGGCATGAGTTCACAATTGAAGGTGAAGGCACA GGCAGACCTTACGAGGGACATCAAGAGATGACACTACGCGTCACAATGG CCAAGGGCGGGCCAATGCCTTTCGCGTTTGACTTAGTGTCACACGTGTT CTGTTACGGCCACAGACCTTTTACTAAATATCCAGAAGAGATACCAGACT ATTTCAAACAAGCATTTCCTGAAGGCCTGTCATGGGAAAGGTCGTTGGAG TTCGAAGATGGTGGGTCCGCTTCAGTCAGTGCGCATATAAGCCTTAGAG GAAACACCTTCTACCACAAATCCAAATTTACTGGGGTTAACTTTCCTGCC GATGGTCCTATCATGCAAAACCAAAGTGTTGATTGGGAGCCATCAACCGA GAAAATTACTGCCAGCGACGGAGTTCTGAAGGGTGATGTTACGATGTAC CTAAAACTTGAAGGAGGCGGCAATCACAAATGCCAATTCAAGACTACTTA CAAGGCGGCAAAAAAGATTCTTAAAATGCCAGGAAGCCATTACATCAGCC ATCGCCTCGTCAGGAAAACCGAAGGCAACATTACTGAGCTGGTAGAAGA TGCAGTAGCTCATTCCTGATAAGTCATGTAATTAGTTATGTC		
G16	GS_mNG_for_DF	gBlock for inserting GS-mNG into pFA6aKANMX6 to replace GS-MEEVF
GCCGCCAGCTGAAGCTTCGTACGCTGGTGGATCAGGCTCTGGTTTGCAA ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCTCTCTCCCAGCGACA CATGAGTTACACATCTTTGGCTCCATCAACGGTGTGGACTTTGACATGGT GGGTCAGGGCACCGGCAATCCAAATGATGGTTATGAGGAGTTAAACCTG AAGTCCACCAAGGGTGACCTCCAGTTCTCCCCCTGGATTCTGGTCCCTC ATATCGGGTATGGCTTCCATCAGTACCTGCCCTACCCTGACGGGATGTC GCCTTTCCAGGCCGCCATGGTAGATGGCTCCGGCTACCAAGTCCATCGC ACAATGCAGTTTGAAGATGGTGCCTCCCTTACTGTTAACTACCGCTACAC CTACGAGGGAAGCCACATCAAAGGAGAGGCCCAGGTGAAGGGGACTGG TTTCCCTGCTGACGGTCCTGTGATGACCAACTCGCTGACCGCTGCGGAC TGGTGCAGGTCGAAGAAGACTTACCCCAACGACAAAACCATCATCAGTA CCTTTAAGTGGAGTTACACCACTGGAAATGGCAAGCGCTACCGGAGCAC TGCGCGGACCACCTACACCTTTGCCAAGCCAATGGCGGCTAACTATCTG AAGAACCAGCCGATGTACGTGTTCCGTAAGACGGAGCTCAAGCACTCCA AGACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTTACCGATGTGAT GGGCATGGACGAGCTGTACAAGTAATGAGTCATGTAATTAGTTATGT		
G17	SZ17-SNAP-tag	gBlock for inserting SNAP-tag into pFA6a-HIS3MX6 in place of mEOS in SYNZIP17-mEOS using plasmid amplified around the horn using primers P14 and P15
ACTTGAGAAAGGAAATTGAAGCTTACAAGGGCGGCAGCGGCAGCGGCC TGCAGATGGACAAAGACTGCGAAATGAAGCGCACCACCCTGGATAGCCC TCTGGGCAAGCTGGAACTGTCTGGGTGCGAACAGGGCCTGCACCGTAT CATCTTCCTGGGCAAAGGAACATCTGCCGCCGACGCCGTGGAAGTGCCT GCCCCAGCCGCCGTGCTGGGCGGACCAGAGCCACTGATGCAGGCCACC GCCTGGCTCAACGCCTACTTTCACCAGCCTGAGGCCATCGAGGAGTTCC CTGTGCCAGCCCTGCACCACCCAGTGTTCCAGCAGGAGAGCTTTACCCG CCAGGTGCTGTGGAAACTGCTGAAAGTGGTGAAGTTCGGAGAGGTCATC AGCTACAGCCACCTGGCCGCCCTGGCCGGCAATCCCGCCGCCACCGCC		

GCCGTGAAAACCGCCCTGAGCGGAAATCCCGTGCCCATTCTGATCCCCT GCCACCGGGTCGTTCAGGGCGACCTGGACGTGGGGGGCTACGAGGGC GGGCTCGCCGTGAAAGAGTGGCTGCTGGCCCACGAGGGCCACAGACTG GGCAAGCCTGGGCTGGGTTGATAAGTCATGTAATTAGTTATGTC		
G18	TRAP4-SNAP-tag	gBlock for inserting SNAP-tag into pFA6a-HIS3MX6 in place of mEOS in TRAP4-mEOS using plasmid amplified around the horn using primers P13 and P15
AGAAAATCCTGAAGGAGCAAGGCGGCAGCGGCAGCGGCCTGCAGATGG ACAAAGACTGCGAAATGAAGCGCACCACCCTGGATAGCCCTCTGGGCAA GCTGGAACTGTCTGGGTGCGAACAGGGCCTGCACCGTATCATCTTCCTG GGCAAAGGAACATCTGCCGCCGACGCCGTGGAAGTGCCTGCCCCAGCC GCCGTGCTGGGCGGACCAGAGCCACTGATGCAGGCCACCGCCTGGCTC AACGCCTACTTTCACCAGCCTGAGGCCATCGAGGAGTTCCCTGTGCCAG CCCTGCACCACCCAGTGTTCCAGCAGGAGAGCTTTACCCGCCAGGTGCT GTGGAAACTGCTGAAAGTGGTGAAGTTCGGAGAGGTCATCAGCTACAGC CACCTGGCCGCCCTGGCCGGCAATCCCGCCGCCACCGCCGCCGTGAAA ACCGCCCTGAGCGGAAATCCCGTGCCCATTCTGATCCCCTGCCACCGG GTCGTTCAGGGCGACCTGGACGTGGGGGGCTACGAGGGCGGGCTCGC CGTGAAAGAGTGGCTGCTGGCCCACGAGGGCCACAGACTGGGCAAGCC TGGGCTGGGTTGATAAGTCATGTAATTAGTTATGTC		
G19	101A_FOR_MNG	gBlock for inserting 101A into pFA6a-HIS3MX6 in place of SYNZIP17, using plasmid amplified around the horn using primers C30 and C31
GTCAAGGAGAAAAAACCCCGGATTCATGAATAGAGTAAAGGACTTGAAG AACAGAGTTAAAGATTTGAAGAAAGAATTGGCTAGGTTGGAAAAGGAAAA TGAATCTTTGAAGAGAGAAATAGCTTATATGGAAAAGGAATTAGCTAGATT AGAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAGGGCGAGG AGGATA		
G20	102A_FOR_MNG	gBlock for inserting 102A into pFA6a-HIS3MX6 in place of SYNZIP17, using plasmid amplified around the horn using primers C30 and C31
GTCAAGGAGAAAAAACCCCGGATTCATGAATAGGTTAGCTGCCTTGAGA AATGAAAACAACATTTTGAAGAATGAGAATAATATTTTGAAGTACGAGTTG GCAGCAATTGAGAACAGATTAGCTGCATTGAGGTACGAGTTGGCCGCTA TTGAAGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAGGGCGAG GAGGATA		
G21	107A_FOR_MNG	gBlock for inserting 107A into pFA6a-HIS3MX6 in place of SYNZIP17, using plasmid amplified around the horn using primers C30 and C31
GTCAAGGAGAAAAAACCCCGGATTCATGGAAAGGATTAACAGATTGAGA AGAGAAGCAACTGAGTTAGAAGACAGGAACCAGAGATTGAAAGACAGAA ACCAGAGATTAAAAGATAGGAACCAAAGGTTAAAACAAGAATTAGCCGCA		

CATAGGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAGGGCGAGGAGGATA		
G22	108A_FOR_MNG	gBlock for inserting 108A into pFA6a-HIS3MX6 in place of SYNZIP17, using plasmid amplified around the horn using primers C30 and C31
GTCAAGGAGAAAAAACCCCGGATTCATGAATAGAGTAGCCCATTTGAAGTCAGAAAACGAACAATTGAAAGAGAGGAATGCCGAATTGAAAGAAAGAAATGCTGAATTAAAAGCAGAATTGGATTCTTTGGAGAAAGAGAAAGACAAATTAGAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAGGGCGAGGAGGATA		
G23	GS-101B	gBlock for inserting 101B into pFA6a-KANMX6 in place of MEEVF, using plasmid amplified around the horn using primers C3 and C4
GCTTCGTACGCTGGTGGATCAGGCTCTGGTTTGCAAAGAGAAATAGCTTACATGGAGAAAGAATTGGCCAGATTAGAAAACAGAGTTAAGGATTTGAAGGAGGAAAATGCTACATTGAAAGATAAGGTTAAGACATTGAAGAATAGGGTAAAGGACTTAAAGTGATGAGTCATGTAATTAGTTATGTCA		
G24	GS-102B	gBlock for inserting 102B into pFA6a-KANMX6 in place of MEEVF, using plasmid amplified around the horn using primers C3 and C4
GCTTCGTACGCTGGTGGATCAGGCTCTGGTTTGCAATATGAATTAGCCGCAATTGAAAATGAAAACAACATATTGAAGAATGAGAATAATATATTAAAGAATAGATTGGCAGCATTGAGATATGAATTGGCAGCAATCGAAAATAGATTGGCTGCTTTGAGATGATGAGTCATGTAATTAGTTATGTCA		
G25	GS-107B	gBlock for inserting 107B into pFA6a-KANMX6 in place of MEEVF, using plasmid amplified around the horn using primers C3 and C4
GCTTCGTACGCTGGTGGATCAGGCTCTGGTTTGCAAAGAGAGGCAACTGAATTGGAAGAAAGGATCAACAGGTTGAGGAACGACAACGCCACATTGGAAAATGATAATGCAACCTTGGAAAACGACAATGCTACCTTGGAACAGGAATTAGCAGCACATAGGTGATGAGTCATGTAATTAGTTATGTCA		
G26	GS-108B	gBlock for inserting 108B into pFA6a-KANMX6 in place of MEEVF, using plasmid amplified around the horn using primers C3 and C4
GCTTCGTACGCTGGTGGATCAGGCTCTGGTTTGCAAAAGGAAAAGGATAAATTAGAGTCAGAAAACGAACAATTAAAGAAAGACAATGCAAATTTAGAAAAGGATAACGCCAACTTAGAAAATAGAGTCGCACATTTGAAGAATAGAGTTGCACATTTAAAATGATGAGTCATGTAATTAGTTATGTCA		
G27	pCUP1_PIL1_GS_101B	gBlock for inserting pCUP1 PIL1-GS-101B into pFA6a-KanMX6
GCCGCCAGCTGAAGCTTCGTACGCTCTAGTTAGAAAGACATTTTTGCTGTCAGTCACTGTCAAGAGATTCTTTTGCTGGCATTTCTTCTAGAAGCAAAAAGAGCGATGCGTCTTTTCCGCTGAACCGTTCCAGCAAAAAAGACTACCAA		

CGCAATATGGATTGTCAGAATCATATAAAAGAGAAGCAAATAACTCCTTG
TCTTGTATCAATTGCATTATAATATCTTCTTGTTAGTGCAATATCATATAGA
AGTCATCGACTAGTATGCATCGTACCTATTCCCTGCGTAACTCACGTGCT
CCCACAGCATCCCAGCTGCAAAATCCTCCTCCTCCTCCTTCCACTACAAA
GGGCCGTTTTTTCGGTAAAGGCGGCCTGGCCTATAGTTTCCGTCGTAGC
GCCGCCGGGGCCTTCGGACCTGAGCTGTCACGTAAACTTTCCCAGCTTG
TCAAAATCGAGAAAAACGTCCTTCGTTCAATGGAGCTTACTGCAAATGAG
CGTAGAGATGCCGCCAAACAGCTTTCCATCTGGGGTCTTGAGAATGACG
ATGACGTCTCAGATATAACCGACAAGCTGGGCGTACTTATATACGAGGTC
AGCGAGCTGGATGATCAGTTCATAGACAGATACGATCAGTATCGTCTTAC
CCTGAAATCAATACGTGACATAGAGGGCTCCGTCCAGCCTTCCCGTGAT
AGAAAAGATAAAATAACAGATAAGATAGCATATCTTAAGTATAAGGACCC
CCAGTCGCCCAAAATCGAAGTACTTGAGCAGGAGCTTGTTAGAGCCGAA
GCCGAGTCCCTTGTAGCCGAGGCCCAGCTGTCCAACATAACTCGTTCGA
AACTTCGTGCCGCCTTTAATTATCAGTTCGATTCAATAATAGAGCACTCG
GAAAAGATCGCCCTGATAGCCGGCTATGGCAAAGCCCTACTTGAGCTGC
TTGATGATTCCCCCGTAACCCCTGGCGAGACACGTCCCGCCTATGACGG
TTACGAGGCATCCAAGCAGATAATCATCGACGCCGAGAGTGCTTTAAAC
GAGTGGACTCTGGATTCCGCACAGGTAAAACCCACCCTGAGCTTTAAAC
AAGACTATGAGGATTTTGAGCCCGAGGAGGGAGAGGAGGAAGAGGAAG
AGGATGGCCAGGGACGTTGGTCAGAGGATGAGCAGGAGGACGGGCAGA
TCGAGGAGCCCGAGCAGGAGGAGGAGGGCGCCGTCGAGGAGCACGAG
CAGGTAGGGCATCAACAGAGCGAATCCTTGCCACAGCAGACTACTGCCG
GTGGATCAGGCTCTGGTTTGCAAAGAGAAATAGCTTACATGGAGAAAGA
ATTGGCCAGATTAGAAAACAGAGTTAAGGATTTGAAGGAGGAAAATGCTA
CATTGAAAGATAAGGTTAAGACATTGAAGAATAGGGTAAAGGACTTAAAG
TGATGAGTCATGTAATTAGTTATGT

G28	pCUP1_PIL1_GS_mEOS	gBlock for inserting pCUP1 PIL1-GS-mEOS into pFA6a-KanMX6

GCCGCCAGCTGAAGCTTCGTACGCTCTAGTTAGAAAGACATTTTTGCTGT
CAGTCACTGTCAAGAGATTCTTTTGCTGGCATTTCTTCTAGAAGCAAAAA
GAGCGATGCGTCTTTTCCGCTGAACCGTTCCAGCAAAAAAGACTACCAA
CGCAATATGGATTGTCAGAATCATATAAAAGAGAAGCAAATAACTCCTTG
TCTTGTATCAATTGCATTATAATATCTTCTTGTTAGTGCAATATCATATAGA
AGTCATCGACTAGTATGCATCGTACCTATTCCCTGCGTAACTCACGTGCT
CCCACAGCATCCCAGCTGCAAAATCCTCCTCCTCCTCCTTCCACTACAAA
GGGCCGTTTTTTCGGTAAAGGCGGCCTGGCCTATAGTTTCCGTCGTAGC
GCCGCCGGGGCCTTCGGACCTGAGCTGTCACGTAAACTTTCCCAGCTTG
TCAAAATCGAGAAAAACGTCCTTCGTTCAATGGAGCTTACTGCAAATGAG
CGTAGAGATGCCGCCAAACAGCTTTCCATCTGGGGTCTTGAGAATGACG
ATGACGTCTCAGATATAACCGACAAGCTGGGCGTACTTATATACGAGGTC
AGCGAGCTGGATGATCAGTTCATAGACAGATACGATCAGTATCGTCTTAC
CCTGAAATCAATACGTGACATAGAGGGCTCCGTCCAGCCTTCCCGTGAT

AGAAAAGATAAAATAACAGATAAGATAGCATATCTTAAGTATAAGGACCC
CCAGTCGCCCAAAATCGAAGTACTTGAGCAGGAGCTTGTTAGAGCCGAA
GCCGAGTCCCTTGTAGCCGAGGCCCAGCTGTCCAACATAACTCGTTCGA
AACTTCGTGCCGCCTTTAATTATCAGTTCGATTCAATAATAGAGCACTCG
GAAAAGATCGCCCTGATAGCCGGCTATGGCAAAGCCCTACTTGAGCTGC
TTGATGATTCCCCCGTAACCCCTGGCGAGACACGTCCCGCCTATGACGG
TTACGAGGCATCCAAGCAGATAATCATCGACGCCGAGAGTGCTTTAAAC
GAGTGGACTCTGGATTCCGCACAGGTAAAACCCACCCTGAGCTTTAAAC
AAGACTATGAGGATTTTGAGCCCGAGGAGGGAGAGGAGGAAGAGGAAG
AGGATGGCCAGGGACGTTGGTCAGAGGATGAGCAGGAGGACGGGCAGA
TCGAGGAGCCCGAGCAGGAGGAGGAGGGCGCCGTCGAGGAGCACGAG
CAGGTAGGGCATCAACAGAGCGAATCCTTGCCACAGCAGACTACTGCCG
GTGGATCAGGCTCTGGTTTGCAAATGAGTGCGATTAAGCCAGACATGAA
GATCAAACTCCGTATGGAAGGCAACGTAAACGGGCACCACTTTGTGATC
GACGGAGATGGTACAGGCAAGCCTTTTGAGGGAAAACAGAGTATGGATC
TTGAAGTCAAAGAGGGCGGACCTCTGCCTTTTGCCTTTGATATCCTGACC
ACTGCATTCCATTACGGCAACAGGGTATTCGCCAAATATCCAGACAACAT
ACAAGACTATTTTAAGCAGTCGTTTCCTAAGGGGTATTCGTGGGAACGAA
GCTTGACTTTCGAAGACGGGGGCATTTGCAACGCCAGAAACGACATAAC
AATGGAAGGGGACACTTTCTATAATAAAGTTCGATTTTATGGTACCAACTT
TCCCGCCAATGGTCCAGTTATGCAGAAGAAGACGCTGAAATGGGAGCCC
TCCACTGAGAAAATGTATGTGCGTGATGGAGTGCTGACGGGTGATATTG
AGATGGCTTTGTTGCTTGAAGGAAATGCCCATTACCGATGTGACTTCAGA
ACTACTTACAAAGCTAAGGAGAAGGGTGTCAAGTTACCAGGCGCCCACT
TTGTGGACCACTGCATTGAGATTTTAAGCCATGACAAAGATTACAACAAG
GTTAAGCTGTATGAGCATGCTGTTGCTCATTCTGGATTGCCTGACAATGC
CAGACGATGATGAGTCATGTAATTAGTTATGT

5.2 Plasmid Sequences

Table A5-5 contains a list of plasmids used in the study. The pages following this table have more detailed sequence info for each plasmid.

Table A5-5. Plasmids used in this study.

#	Name	Parent Vector
1	pYTK023	pYTK001
2	pYTK024	pYTK001
3	pYTK025	pYTK001
4	pYTK026	pYTK001
5	pYTK027	pYTK001
6	pGAL1 TRAP4-mEOS	pFA6a-His3MX6
7	pRNR2 TRAP4-mEOS	pFA6a-His3MX6
8	pPOP6 TRAP4-mEOS	pFA6a-His3MX6
9	pRAD27 TRAP4-mEOS	pFA6a-His3MX6
10	pPSP2 TRAP4-mEOS	pFA6a-His3MX6
11	pREV1 TRAP4-mEOS	pFA6a-His3MX6
12	GS-MEEVF	pFA6a-KanMX6
13	GS-CCBN3,5	pFA6a-KanMX6
14	GS-SYNZIP18	pFA6a-KanMX6
15	pGAL1 SYNZIP17-mEOS	pFA6a-His3MX6
16	pGAL1 CCAN3,5-mEOS	pFA6a-His3MX6
17	pGAL1 TRAP4-mNeonGreen	pFA6a-His3MX6
18	pGAL1 SYNZIP17-mNeonGreen	pFA6a-His3MX6
19	VFP-MEEVF	pCu415CUP1
20	pCUP1 SZ18-Actin	pCu415CUP1
21	pCUP1 Actin-SZ18	pCu415CUP1
22	pCUP1 Actin-MEEVF	pCu415CUP1
23	pCUP1 Actin-GS	pCu415CUP1
24	pGAL1 LifeAct-mNeonGreen	pFA6a-His3MX6
25	pGAL1 SYNZIP17-EYFP	pFA6a-His3MX6
26	pGAL1 SYNZIP17-mKO	pFA6a-His3MX6
27	pGAL1 SYNZIP17-mOrange	pFA6a-His3MX6
28	pGAL1 SYNZIP17-mCherry	pFA6a-His3MX6
29	pGAL1 101A-mNeonGreen	pFA6a-His3MX6
30	pGAL1 102A-mNeonGreen	pFA6a-His3MX6
31	pGAL1 107A-mNeonGreen	pFA6a-His3MX6
32	pGAL1 108A-mNeonGreen	pFA6a-His3MX6

33	GS-101B	pFA6a-KanMX6
34	GS-102B	pFA6a-KanMX6
35	GS-107B	pFA6a-KanMX6
36	GS-108B	pFA6a-KanMX6
37	pGAL1 TRAP4-SNAP-tag	pFA6a-His3MX6
38	pGAL1 SYNZIP17-SNAP-tag	pFA6a-His3MX6
39	pGAL1 SYNZIP17-mCherry	pFA6a-His3MX6
40	GS-108B	pFA6a-Leu2MX6
41	pGAL1 108A-mCherry	pFA6a-Ura3MX6
42	GS-mNeonGreen	pFA6a-KanMX6
43	GS-mEOS	pFA6a-KanMX6
44	pGAL1 SYNZIP17-GRvT	pFA6a-His3MX6
45	pGAL1 SYNZIP17-3xmNeonGreen	pFA6a-His3MX6
46	pCUP1 PIL1-GS-101B	pFA6a-KanMX6
47	pCUP1 PIL1-GS-mEOS	pFA6a-KanMX6

Plasmid	pYTK023		
Freezer Box	CO Plasmids	#	1
Parent Vector	pYTK001	Source	Addgene
Bacterial Selection	Cam	Yeast Selection	NA
Full Sequence	benchling.com/s/seq-cMrOpwgltbG3B9QnNGST		

Description
Encodes pRNR2 as a Type 2 part to be used in the Dueber YTK system

Shading Key
Plasmid backbone
pRNR2

DNA
GGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTT
CCTGCGTTATCCCCTGATTCTGTGGATAACCGTAGTCGGTCTCAAACGAGTC
GAACAAGAAGCAGGCAAAGTTTAGAGCACTGCCCCTCCGCACTCAAAAAAG
AAAAAACTAGGAGGAAAATAAAATTCTCAACCACACAAACACATAAACACAT
ACAAATACAAATACAAGCTTATTTACTTGACATCGCGCGATCTTCCACTATTC
AGCGCCGTCCGCCCTCTCTCGTGTTTTTTGTTTACGCGACAACTATGCGAAA
TCCGGAGCAACGGGCAACCGTTTGGGGAAAGACCACACCCACGCGCGATC
GCCATGGCAACGAGGTCGCACACGCCCCACACCCAGACCTCCCTGCGAGC
GGGCATGGGTACAATGTCCCCGTTGCCACAGACACCACTTCGTAGCACAGC
GCAGAGCGTAGCGTGTTGTTGCTGCTGACAAAAGAAAATTTTTCTTAGCAAA
GCAAAGGAGGGGAAGCACGGGCAGATAGCACCGTACCATACCCTTGGAAA
CTCGAAATGAACGAAGCAGGAAATGAGAGAATGAGAGTTTTGTAGGTATATA
TAGCGGTAGTGTTTGCGCGTTACCATCATCTTCTGGATCTATCTATTGTTCTT
TTCCTCATCACTTTCCCCTTTTTCGCTCTTCTTCTTGTCTTTTATTTCTTTCTTT
TTTTTAATTGTTCCCTCGATTGGCTATCTACCAAAGAATCCAAACTTAATACA
CGTATTTATTTGTCCAATTACCAGATCTATGTGAGACCAGACCAATAAAAAAC
GCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGT
CATTACTGGATCTATCAACAGGA

Expression Product Sequence
NA

Plasmid	pYTK024		
Freezer Box	CO Plasmids	#	2
Parent Vector	pYTK001	Source	Addgene
Bacterial Selection	Cam	Yeast Selection	NA
Full Sequence	https://benchling.com/s/seq-T01j9KANvj0x5Df1MF6f		

Description
Encodes pPOP6 as a Type 2 part to be used in the Dueber YTK system

Shading Key
Plasmid backbone
pPOP6

DNA
GGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTT
CCTGCGTTATCCCCTGATTCTGTGGATAACCGTAGTCGGTCTCAAACGTTCG
TGCTTTGTGATAAAGTGTTTCACGTCATCCGACATGACTTCGTAGTTATGGA
CTGAACTGTGTGGTGAGGTTCCATGATTTCTTAGGTCCAGCAGATACATGTC
TCTTCCCAATTTCTTGTTAAGGTTACGGCCAATGCTTCGGTTGTTGAGCTTG
TTACCGAATAAGCCGTGAAGTATGATAATAGGTGGTCTTGGCTTCCCTTCAT
CCCCAGTTTTTACTGCATCTCTCTTGATTATGTCATATGAAAGGTCCAGTGG
GACTTGCTTTTGTTGCAGCACCTTTGCTAATGAATGAAAGGCACATAGTGAC
TGCTTAAAAATGCAGGAACTTAAATTATTCCGAATGGTATTTTGTCTCACATA
TATTGTCCCATACTGTGCCAAGATCCCGGCTTTACCCAGTATCATCATTGTA
CCGTTACCAATTCTCCTCGTATATCACGGTTAGTTTTTAAACCTCGGGGTGA
CGTTTACTATTGGCGTACTAATATATTCTTATTTTCTTTTCTTTTTTGTTGGCA
GTTTCAAGCAACACATGTACTGGATAACCAACCCCCGCACGCTCTTGGAAA
AAATTGAGAAGGCATCGGACACTTGCTGATGAGTATTTCGAAAAATTCCATG
AAGATGAGGCCAAGATTGTTTGGAAGAGATTGAAAAGAAGAAGAAGAAAAA
AAGATAAAAGCAAATCAAAAGATCTATGTGAGACCAGACCAATAAAAAACGC
CCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCAT
TACTGGATCTATCAACAGGA

Expression Product Sequence
NA

Plasmid	pYTK025		
Freezer Box	CO Plasmids	#	3
Parent Vector	pYTK001	Source	Addgene
Bacterial Selection	Cam	Yeast Selection	NA
Full Sequence	https://benchling.com/s/seq-U6kfhKXzkj6ncwPng2rd		

Description
Encodes pRAD27 as a Type 2 part to be used in the Dueber YTK system

Shading Key
Plasmid backbone
pRAD27

DNA
GGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTT
CCTGCGTTATCCCCTGATTCTGTGGATAACCGTAGTCGGTCTCAAACGCCTT
GTGAAATTGCAAATATGGTGATTTGAAACGTTTCCTAGTGCAGCAGGATCAC
AGATAACGTGTAAAGGGCTTAGCAGTTGATAATCCTCTCTAGTTAAGACCTA
AACAAAATGCTGTCACTAACCGTAGTATTAAATGACACACTTTGGTGACTTTC
GTTAATGGGGATGTGGTAGTGGCCATTGCCAATAAACAAAAAGAACAGGGA
AAGAAGTAGAAAGTGATATAAGTTTGCTTGCCACTTTTCGTTTTTCACGAAAA
AAACAGGCGAAAAAAAATGCTAGACAAGTACCCGGCTGAATCACACCTCGT
TAACAGTGACTTTCGGTGACAGATACCCGATTGGGCACCCGGCTGGTAAGT
TATGATAGAAAGCCAACGCTGTACTATTGGCTTAGCTATGGCAATATTTTGA
TTATCAGCTAGTTTTATTAACGTTATAATTAGTGTAACCAGTTTTTCATCTATT
TCATTTATTTCATTTATTTACTTTAATTGCAGATCCCCCTAACGCGTTTAAAGC
TTTTATTCACTAGCTTATGTATTTTTTATAGGAAACGCGACGCGTAACATCGC
GCAAATGAAGGTTTTGATGTATTATAATGAGGTATTCTTCCTTATATACATCG
ATGAAAAGCGTTGACAGCATACATTGGAAAGAAATAGGAAACGGACACCGG
AAGAAAAAATAGATCTATGTGAGACCAGACCAATAAAAAACGCCCGGCGGC
AACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGAT
CTATCAACAGGA

Expression Product Sequence
NA

Plasmid	pYTK026		
Freezer Box	CO Plasmids	#	4
Parent Vector	pYTK001	Source	Addgene
Bacterial Selection	Cam	Yeast Selection	NA
Full Sequence	https://benchling.com/s/seq-t8yR0zpCM4ml62o8YfJJ		

Description
Encodes pPSP2 as a Type 2 part to be used in the Dueber YTK system

Shading Key
Plasmid backbone
pPSP2

DNA
GGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTT
CCTGCGTTATCCCCTGATTCTGTGGATAACCGTAGTCGGTCTCAAACGTGAC
CCAACATCAGATGACCCAAGGTCCACCTCTTATTAAAGGACGTTTGATCCTT
CGACACCATGGCTCTGTTGAACTTTTATCTGAGAGAGGAAAAAAAGGAAGG
AAAAAAAAGAAGAAACTTCCTTTATTTATTTGTCTTAACCACAACACACAATG
CAATAAGATGCAATATAATATCAAAGCCAATATCTTATGTTGCTGATCCTGAG
AAGGAATATATACAATTTATGTAGTAAAATACCTTTTCTTCTGCGAGTTGCAA
GAAATAGAAAAGACTCCGATTGCGCATCGCCAGAATAAAATTTCACAACCAC
ACTTTTTGGCTGAACTTTTTATTACCTGATTAAACAGAGAGAGAAAAGGTAGA
GGTCAAAATTTTTTAAGCAAAACTAAAAAAGATGCAAAATCACGTGCTGAAAA
TCTAACATAAGGGTTAAGATTAGAGTTTTATAGGACTTGTTTTGTAATATTTC
AAATACGAGCTAACCCTACTGATTTCAATTAGGTCTAATTTAGGGTTGAGCT
GCACTGAAATTTCGGAAATTTTGGGTTATTTTAAATGAGACAGAAGAACTAC
AGAGATACGTTCTTCAGACTTTAAAGCTTATCTCCACAAAGAATTGGTCAAG
AAATCATCCTAGAAAAACACGTTTGCTCACTCGATCTTAATCACATAGAGTG
CTGGAACGGGAAGAAAGATCTATGTGAGACCAGACCAATAAAAAACGCCCG
GCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTAC
TGGATCTATCAACAGGA

Expression Product Sequence
NA

Plasmid	pYTK027		
Freezer Box	CO Plasmids	#	5
Parent Vector	pYTK001	Source	Addgene
Bacterial Selection	Cam	Yeast Selection	NA
Full Sequence	https://benchling.com/s/seq-QyrkE0Rcx1hU8WB5BkM4		

Description
Encodes pREV1 as a Type 2 part to be used in the Dueber YTK system

Shading Key
Plasmid backbone
pREV1

DNA
GGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTT
CCTGCGTTATCCCCTGATTCTGTGGATAACCGTAGTCGGTCTCAAACGGTGT
TGTTATCCGATACAACCGGATATTTTTCTTTTAATGAGTCTAAACCGTGATAG
CTTCAGGTTAATACAATCAAAAAAAGCTCAAATATTCTTTTAATGCCGCGTTC
ACAGATTCCAATTGAATACAACTAGGTAGTTCATTATATGAAGCCTTTGCTAC
TATTTTTCACTATAGTCTGCCTTCACCTTAATGCAGACATCCACATATTTTAAT
CACTTTAAAATAAAAAGGAAGATATATTAGAAGCTATGATCCAATCTGTAAGC
CAGATTAAAATTCACGAACTCTTCTTTCATTTGAATTGAATGCTTTGAGTTGG
GGTAGATTATCGCAAATTACTCATCACATTTATTGACTACGAACTTGCTGATG
TCCTTTTTTTATTTATATTTTTCTTCAGTGAAGCGATTTTTTTTTTACACAGAC
CAAGACGGAAAAAAGTAGCTAAGGAAGAAAACAAAATCATGAAAAAAATGTG
AAGTGATCATGCACATCGCATCAACTTAAACATTGGCTTAGAGATATATAGA
GTTAGAGTTTACGGCAACCTTTAAGCACCAATACCTTTTGGCATAGTCTAAA
GACCTGGTTCTTAATTTTAAACAAATTTAACTAAAGATTTCCCTATCAAAGAA
GTAACGAGTTGACAGATTTTCTCAAAATAAATCGATACTGCATTTCTAGGCAT
ATCCAGCGAGATCTATGTGAGACCAGACCAATAAAAAACGCCCGGCGGCAA
CCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTA
TCAACAGGA

Expression Product Sequence
NA

Plasmid	pGAL1 TRAP4-mEOS		
Freezer Box	CO Plasmids	#	6
Parent Vector	pFA6a-His3MX6	Source	Michael Hinrichsen
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-gQibzK4OWh81ywqt6XPX		

Description
pGAL1 driven expression. TRAP4-mEOS in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
TRAP4
Linker
mEOS
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAAGCAGGCA
CTGAAAGAAAAAGAGCTGGGGAACGATGCCTACAAGAAGAAAGACTTTGAC
ACAGCCTTGAAGCATTACGACAAAGCCAAGGAGCTGGACCCCACTAACATG
ACTTACATTATCAATCAAGCAGCGGTATACTTTGAAAAGGGCGACTACAATA
AGTGCCGGGAGCTTTGTGAGAAGGCCATTGAAGTGGGGAGAGAAAACCGA
GAGGACTATCGATGGATTGCCATTGCATATGCTCGAATTGGCAACTCCTACT
TCAAAGAAGAAAAGTACAAGGATGCCATCCATTTCTATAACAAGTCTCTGGC
AGAGCACCGAACCCCAAAGGTGCTAAAAAAGTGCCAACAGGCGGAGAAAAT
CCTGAAGGAGCAAGGCGGCAGCGGCAGCGGCCTGCAGATGAGTGCGATTA
AGCCAGACATGAAGATCAAACTCCGTATGGAAGGCAACGTAAACGGGCACC
ACTTTGTGATCGACGGAGATGGTACAGGCAAGCCTTTTGAGGGAAAACAGA
GTATGGATCTTGAAGTCAAAGAGGGCGGACCTCTGCCTTTTGCCTTTGATAT

CCTGACCACTGCATTCCATTACGGCAACAGGGTATTCGCCAAATATCCAGA
CAACATACAAGACTATTTTAAGCAGTCGTTTCCTAAGGGGTATTCGTGGGAA
CGAAGCTTGACTTTCGAAGACGGGGGCATTTGCAACGCCAGAAACGACATA
ACAATGGAAGGGGACACTTTCTATAATAAAGTTCGATTTTATGGTACCAACTT
TCCCGCCAATGGTCCAGTTATGCAGAAGAAGACGCTGAAATGGGAGCCCTC
CACTGAGAAAATGTATGTGCGTGATGGAGTGCTGACGGGTGATATTGAGAT
GGCTTTGTTGCTTGAAGGAAATGCCCATTACCGATGTGACTTCAGAACTACT
TACAAAGCTAAGGAGAAGGGTGTCAAGTTACCAGGCGCCCACTTTGTGGAC
CACTGCATTGAGATTTTAAGCCATGACAAAGATTACAACAAGGTTAAGCTGT
ATGAGCATGCTGTTGCTCATTCTGGATTGCCTGACAATGCCAGACGATGATA
AGTCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCC
GCTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTT
ATTTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCT
TTTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGC
TTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCG
CGCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAA
TTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MKQALKEKELGNDAYKKKDFDTALKHYDKAKELDPTNMTYIINQAAVYFEKGDY
NKCRELCEKAIEVGRENREDYRWIAIAYARIGNSYFKEEKYKDAIHFYNKSLAEH
RTPKVLKKCQQAEKILKEQGGSGSGLQMSAIKPDMKIKLRMEGNVNGHHFVID
GDGTGKPFEGKQSMDLEVKEGGPLPFAFDILTTAFHYGNRVFAKYPDNIQDYF
KQSFPKGYSWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTNFPANGPV
MQKKTLKWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDFRTTYKAKEKGV
KLPGAHFVDHCIEILSHDKDYNKVKLYEHAVAHSGLPDNARR

Plasmid	pRNR2 TRAP4-mEOS		
Freezer Box	CO Plasmids	#	7
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-DhzKTw554axgw7Ft1Hiu		

Description
pRNR2 driven expression. TRAP4-mEOS in yeast integration vector with HIS3 marker.

Shading Key
pRNR2
TRAP4
Linker
mEOS
Stop
CYC1 terminator

DNA
TAATACATAACCTTATGTATCATACACATACGATTTAGGTGACACTATAGAAC
GCGGCCGCCAGCTGAAGCTTCGTACGCTGCGTAGTCGGTCTCAAACGAGT
CGAACAAGAAGCAGACAAAGTTTAGAGCACTGCCCCTCCGCACTCAAAAAA
GAAAAAACTAGGAGGAAAATAAAATTCTCAACCACACAAACACATAAACACA
TACAAATACAAATACAAGCTTATTTACTTGACATCGCGCGATCTTCCACTATT
CAGCGCCGTCCGCCCTCTCTCGTGTTTTTTGTTTACGCGACAACTATGCGAA
ATCCGGAGCAACGGGCAACCGTTTGGGGAAAGACCACACCCACGCGCGAT
CGCCATGGCAACGAGGTCGCACACGCCCCACACCCAGACCTCCCTGCGAG
CGGGCATGGGTACAATGTCCCCGTTGCCACAGACACCACTTCGTAGCACAG
CGCAGAGCGTAGCGTGTTGTTGCTGCTGACAAAAGAAAATTTTTCTTAGCAA
AGCAAAGGAGGGGAAGCACGGGCAGATAGCACCGTACCATACCCTTGGAA
ACTCGAAATGAACGAAGCAGGAAATGAGAGAATGAGAGTTTTGTAGGTATAT
ATAGCGGTAGTGTTTGCGCGTTACCATCATCTTCTGGATCTATCTATTGTTCT
TTTCCTCATCACTTTCCCCTTTTTCGCTCTTCTTCTTGTCTTTTATTTCTTTCTT
TTTTTTAATTGTTCCCTCGATTGGCTATCTACCAAAGAATCCAAACTTAATAC
ACGTATTTATTTGTCCAATTACCAGATCTATGTGAGACCAGACCAATAAAATG
AAGCAGGCACTGAAAGAAAAAGAGCTGGGGAACGATGCCTACAAGAAGAAA
GACTTTGACACAGCCTTGAAGCATTACGACAAAGCCAAGGAGCTGGACCCC
ACTAACATGACTTACATTATCAATCAAGCAGCGGTATACTTTGAAAAGGGCG
ACTACAATAAGTGCCGGGAGCTTTGTGAGAAGGCCATTGAAGTGGGGAGAG
AAAACCGAGAGGACTATCGATGGATTGCCATTGCATATGCTCGAATTGGCA
ACTCCTACTTCAAAGAAGAAAAGTACAAGGATGCCATCCATTTCTATAACAA
GTCTCTGGCAGAGCACCGAACCCCAAAGGTGCTAAAAAAGTGCCAACAGGC

GGAGAAAATCCTGAAGGAGCAAGGCGGCAGCGGCAGCGGCCTGCAGATGA
GTGCGATTAAGCCAGACATGAAGATCAAACTCCGTATGGAAGGCAACGTAA
ACGGGCACCACTTTGTGATCGACGGAGATGGTACAGGCAAGCCTTTTGAGG
GAAAACAGAGTATGGATCTTGAAGTCAAAGAGGGCGGACCTCTGCCTTTTG
CCTTTGATATCCTGACCACTGCATTCCATTACGGCAACAGGGTATTCGCCAA
ATATCCAGACAACATACAAGACTATTTTAAGCAGTCGTTTCCTAAGGGGTAT
TCGTGGGAACGAAGCTTGACTTTCGAAGACGGGGGCATTTGCAACGCCAGA
AACGACATAACAATGGAAGGGGACACTTTCTATAATAAAGTTCGATTTTATG
GTACCAACTTTCCCGCCAATGGTCCAGTTATGCAGAAGAAGACGCTGAAAT
GGGAGCCCTCCACTGAGAAAATGTATGTGCGTGATGGAGTGCTGACGGGT
GATATTGAGATGGCTTTGTTGCTTGAAGGAAATGCCCATTACCGATGTGACT
TCAGAACTACTTACAAAGCTAAGGAGAAGGGTGTCAAGTTACCAGGCGCCC
ACTTTGTGGACCACTGCATTGAGATTTTAAGCCATGACAAAGATTACAACAA
GGTTAAGCTGTATGAGCATGCTGTTGCTCATTCTGGATTGCCTGACAATGCC
AGACGATGATAAGTCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTC
CCCCCACATCCGCTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCT
AGGTCCCTATTTATTTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATAT
TTCAAATTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATAC
TGAAAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGG
TTAATTAAGGCGCGCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCC
GCAGAATCTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MKQALKEKELGNDAYKKKDFDTALKHYDKAKELDPTNMTYIINQAAVYFEKGDY
NKCRELCEKAIEVGRENREDYRWIAIAYARIGNSYFKEEKYKDAIHFYNKSLAEH
RTPKVLKKCQQAEKILKEQGGSGSGLQMSAIKPDMKIKLRMEGNVNGHHFVID
GDGTGKPFEGKQSMDLEVKEGGPLPFAFDILTTAFHYGNRVFAKYPDNIQDYF
KQSFPKGYSWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTNFPANGPV
MQKKTLKWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDFRTTYKAKEKGV
KLPGAHFVDHCIEILSHDKDYNKVKLYEHAVAHSGLPDNARR

Plasmid	pPOP6 TRAP4-mEOS		
Freezer Box	CO Plasmids	#	8
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-2aO7cR1EvgArD6NXWJLv		

Description
pPOP6 driven expression. TRAP4-mEOS in yeast integration vector with HIS3 marker.

Shading Key
pPOP6
TRAP4
Linker
mEOS
Stop
CYC1 terminator

DNA
TAATACATAACCTTATGTATCATACACATACGATTTAGGTGACACTATAGAAC
GCGGCCGCCAGCTGAAGCTTCGTACGCTGCGTAGTCGGTCTCAAACGTTC
GTGCTTTGTGATAAAGTGTTTCACGTCATCCGACATGACTTCGTAGTTATGG
ACTGAACTGTGTGGTGAGGTTCCATGATTTCTTAGGTCCAGCAGATACATGT
CTCTTCCCAATTTCTTGTTAAGGTTACGGCCAATGCTTCGGTTGTTGAGCTT
GTTACCGAATAAGCCGTGAAGTATGATAATAGGTGGTCTTGGCTTCCCTTCA
TCCCCAGTTTTTACTGCATCTCTCTTGATTATGTCATATGAAAGGTCCAGTGG
GACTTGCTTTTGTTGCAGCACCTTTGCTAATGAATGAAAGGCACATAGTGAC
TGCTTAAAAATGCAGGAACTTAAATTATTCCGAATGGTATTTTGTCTCACATA
TATTGTCCCATACTGTGCCAAGATCCCGGCTTTACCCAGTATCATCATTGTA
CCGTTACCAATTCTCCTCGTATATCACGGTTAGTTTTTAAACCTCGGGGTGA
CGTTTACTATTGGCGTACTAATATATTCTTATTTTCTTTTCTTTTTTGTTGGCA
GTTTCAAGCAACACATGTACTGGATAACCAACCCCCGCACGCTCTTGGAAA
AAATTGAGAAGGCATCGGACACTTGCTGATGAGTATTTCGAAAAATTCCATG
AAGATGAGGCCAAGATTGTTTGGAAGAGATTGAAAAGAAGAAGAAGAAAAA
AAGATAAAAGCAAATCAAAAGATCTATGTGAGACCAGACCAATAAAATGAAG
CAGGCACTGAAAGAAAAAGAGCTGGGGAACGATGCCTACAAGAAGAAAGAC
TTTGACACAGCCTTGAAGCATTACGACAAAGCCAAGGAGCTGGACCCCACT
AACATGACTTACATTATCAATCAAGCAGCGGTATACTTTGAAAAGGGCGACT
ACAATAAGTGCCGGGAGCTTTGTGAGAAGGCCATTGAAGTGGGGAGAGAAA
ACCGAGAGGACTATCGATGGATTGCCATTGCATATGCTCGAATTGGCAACT
CCTACTTCAAAGAAGAAAAGTACAAGGATGCCATCCATTTCTATAACAAGTC
TCTGGCAGAGCACCGAACCCCAAAGGTGCTAAAAAAGTGCCAACAGGCGG

AGAAAATCCTGAAGGAGCAAGGCGGCAGCGGCAGCGGCCTGCAGATGAGT
GCGATTAAGCCAGACATGAAGATCAAACTCCGTATGGAAGGCAACGTAAAC
GGGCACCACTTTGTGATCGACGGAGATGGTACAGGCAAGCCTTTTGAGGGA
AAACAGAGTATGGATCTTGAAGTCAAAGAGGGCGGACCTCTGCCTTTTGCC
TTTGATATCCTGACCACTGCATTCCATTACGGCAACAGGGTATTCGCCAAAT
ATCCAGACAACATACAAGACTATTTTAAGCAGTCGTTTCCTAAGGGGTATTC
GTGGGAACGAAGCTTGACTTTCGAAGACGGGGGCATTTGCAACGCCAGAAA
CGACATAACAATGGAAGGGGACACTTTCTATAATAAAGTTCGATTTTATGGT
ACCAACTTTCCCGCCAATGGTCCAGTTATGCAGAAGAAGACGCTGAAATGG
GAGCCCTCCACTGAGAAAATGTATGTGCGTGATGGAGTGCTGACGGGTGAT
ATTGAGATGGCTTTGTTGCTTGAAGGAAATGCCCATTACCGATGTGACTTCA
GAACTACTTACAAAGCTAAGGAGAAGGGTGTCAAGTTACCAGGCGCCCACT
TTGTGGACCACTGCATTGAGATTTTAAGCCATGACAAAGATTACAACAAGGT
TAAGCTGTATGAGCATGCTGTTGCTCATTCTGGATTGCCTGACAATGCCAGA
CGATGATAAGTCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCC
CCACATCCGCTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGG
TCCCTATTTATTTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCA
AATTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGA
AAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTA
ATTAAGGCGCGCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGCA
GAATCTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MKQALKEKELGNDAYKKKDFDTALKHYDKAKELDPTNMTYIINQAAVYFEKGDY
NKCRELCEKAIEVGRENREDYRWIAIAYARIGNSYFKEEKYKDAIHFYNKSLAEH
RTPKVLKKCQQAEKILKEQGGSGSGLQMSAIKPDMKIKLRMEGNVNGHHFVID
GDGTGKPFEGKQSMDLEVKEGGPLPFAFDILTTAFHYGNRVFAKYPDNIQDYF
KQSFPKGYSWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTNFPANGPV
MQKKTLKWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDFRTTYKAKEKGV
KLPGAHFVDHCIEILSHDKDYNKVKLYEHAVAHSGLPDNARR

Plasmid	pRAD27 TRAP4-mEOS		
Freezer Box	CO Plasmids	#	9
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-H1Dxd2Tm67cOlDJsMd6C		

Description
pRAD27 driven expression. TRAP4-mEOS in yeast integration vector with HIS3 marker.

Shading Key
pRAD27
TRAP4
Linker
mEOS
Stop
CYC1 terminator

DNA
TAATACATAACCTTATGTATCATACACATACGATTTAGGTGACACTATAGAAC
GCGGCCGCCAGCTGAAGCTTCGTACGCTGCGTAGTCGGTCTCAAACGCCTT
GTGAAATTGCAAATATGGTGATTTGAAACGTTTCCTAGTGCAGCAGGATCAC
AGATAACGTGTAAAGGGCTTAGCAGTTGATAATCCTCTCTAGTTAAGACCTA
AACAAAATGCTGTCACTAACCGTAGTATTAAATGACACACTTTGGTGACTTTC
GTTAATGGGGATGTGGTAGTGGCCATTGCCAATAAACAAAAAGAACAGGGA
AAGAAGTAGAAAGTGATATAAGTTTGCTTGCCACTTTTCGTTTTTCACGAAAA
AAACAGGCGAAAAAAAATGCTAGACAAGTACCCGGCTGAATCACACCTCGT
TAACAGTGACTTTCGGTGACAGATACCCGATTGGGCACCCGGCTGGTAAGT
TATGATAGAAAGCCAACGCTGTACTATTGGCTTAGCTATGGCAATATTTTGA
TTATCAGCTAGTTTTATTAACGTTATAATTAGTGTAACCAGTTTTTCATCTATT
TCATTTATTTCATTTATTTACTTTAATTGCAGATCCCCCTAACGCGTTTAAAGC
TTTTATTCACTAGCTTATGTATTTTTTATAGGAAACGCGACGCGTAACATCGC
GCAAATGAAGGTTTTGATGTATTATAATGAGGTATTCTTCCTTATATACATCG
ATGAAAAGCGTTGACAGCATACATTGGAAAGAAATAGGAAACGGACACCGG
AAGAAAAAATAGATCTATGTGAGACCAGACCAATAAAATGAAGCAGGCACTG
AAAGAAAAAGAGCTGGGGAACGATGCCTACAAGAAGAAAGACTTTGACACA
GCCTTGAAGCATTACGACAAAGCCAAGGAGCTGGACCCCACTAACATGACT
TACATTATCAATCAAGCAGCGGTATACTTTGAAAAGGGCGACTACAATAAGT
GCCGGGAGCTTTGTGAGAAGGCCATTGAAGTGGGGAGAGAAAACCGAGAG
GACTATCGATGGATTGCCATTGCATATGCTCGAATTGGCAACTCCTACTTCA
AAGAAGAAAAGTACAAGGATGCCATCCATTTCTATAACAAGTCTCTGGCAGA
GCACCGAACCCCAAAGGTGCTAAAAAAGTGCCAACAGGCGGAGAAAATCCT

GAAGGAGCAAGGCGGCAGCGGCAGCGGCCTGCAGATGAGTGCGATTAAG
CCAGACATGAAGATCAAACTCCGTATGGAAGGCAACGTAAACGGGCACCAC
TTTGTGATCGACGGAGATGGTACAGGCAAGCCTTTTGAGGGAAAACAGAGT
ATGGATCTTGAAGTCAAAGAGGGCGGACCTCTGCCTTTTGCCTTTGATATCC
TGACCACTGCATTCCATTACGGCAACAGGGTATTCGCCAAATATCCAGACAA
CATACAAGACTATTTTAAGCAGTCGTTTCCTAAGGGGTATTCGTGGGAACGA
AGCTTGACTTTCGAAGACGGGGGCATTTGCAACGCCAGAAACGACATAACA
ATGGAAGGGGACACTTTCTATAATAAAGTTCGATTTTATGGTACCAACTTTCC
CGCCAATGGTCCAGTTATGCAGAAGAAGACGCTGAAATGGGAGCCCTCCAC
TGAGAAAATGTATGTGCGTGATGGAGTGCTGACGGGTGATATTGAGATGGC
TTTGTTGCTTGAAGGAAATGCCCATTACCGATGTGACTTCAGAACTACTTAC
AAAGCTAAGGAGAAGGGTGTCAAGTTACCAGGCGCCCACTTTGTGGACCAC
TGCATTGAGATTTTAAGCCATGACAAAGATTACAACAAGGTTAAGCTGTATG
AGCATGCTGTTGCTCATTCTGGATTGCCTGACAATGCCAGACGATGATAAGT
CATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCT
CTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATT
TTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTT
TTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTT
GAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGC
GCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAAT
TAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MKQALKEKELGNDAYKKKDFDTALKHYDKAKELDPTNMTYIINQAAVYFEKGDY
NKCRELCEKAIEVGRENREDYRWIAIAYARIGNSYFKEEKYKDAIHFYNKSLAEH
RTPKVLKKCQQAEKILKEQGGSGSGLQMSAIKPDMKIKLRMEGNVNGHHFVID
GDGTGKPFEGKQSMDLEVKEGGPLPFAFDILTTAFHYGNRVFAKYPDNIQDYF
KQSFPKGYSWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTNFPANGPV
MQKKTLKWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDFRTTYKAKEKGV
KLPGAHFVDHCIEILSHDKDYNKVKLYEHAVAHSGLPDNARR

Plasmid	pPSP2 TRAP4-mEOS		
Freezer Box	CO Plasmids	#	10
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-5thjST949EkHteqQ1aJD		

Description
pPSP2 driven expression. TRAP4-mEOS in yeast integration vector with HIS3 marker.

Shading Key
pPSP2
TRAP4
Linker
mEOS
Stop
CYC1 terminator

DNA
TAATACATAACCTTATGTATCATACACATACGATTTAGGTGACACTATAGAAC
GCGGCCGCCAGCTGAAGCTTCGTACGCTGCGTAGTCGGTCTCAAACGTGA
CCCAACATCAGATGACCCAAGGTCCACCTCTTATTAAAGGACGTTTGATCCT
TCGACACCATGGCTCTGTTGAACTTTTATCTGAGAGAGGAAAAAAAGGAAG
GAAAAAAAAGAAGAAACTTCCTTTATTTATTTGTCTTAACCACAACACACAAT
GCAATAAGATGCAATATAATATCAAAGCCAATATCTTATGTTGCTGATCCTGA
GAAGGAATATATACAATTTATGTAGTAAAATACCTTTTCTTCTGCGAGTTGCA
AGAAATAGAAAAGACTCCGATTGCGCATCGCCAGAATAAAATTTCACAACCA
CACTTTTTGGCTGAACTTTTTATTACCTGATTAAACAGAGAGAGAAAAGGTA
GAGGTCAAAATTTTTTAAGCAAAACTAAAAAAGATGCAAAATCACGTGCTGA
AAATCTAACATAAGGGTTAAGATTAGAGTTTTATAGGACTTGTTTTGTAATAT
TTCAAATACGAGCTAACCCTACTGATTTCAATTAGGTCTAATTTAGGGTTGAG
CTGCACTGAAATTTCGGAAATTTTGGGTTATTTTAAATGAGACAGAAGAACTA
CAGAGATACGTTCTTCAGACTTTAAAGCTTATCTCCACAAAGAATTGGTCAA
GAAATCATCCTAGAAAAACACGTTTGCTCACTCGATCTTAATCACATAGAGT
GCTGGAACGGGAAGAAAGATCTATGTGAGACCAGACCAATAAAATGAAGCA
GGCACTGAAAGAAAAAGAGCTGGGGAACGATGCCTACAAGAAGAAAGACTT
TGACACAGCCTTGAAGCATTACGACAAAGCCAAGGAGCTGGACCCCACTAA
CATGACTTACATTATCAATCAAGCAGCGGTATACTTTGAAAAGGGCGACTAC
AATAAGTGCCGGGAGCTTTGTGAGAAGGCCATTGAAGTGGGGAGAGAAAAC
CGAGAGGACTATCGATGGATTGCCATTGCATATGCTCGAATTGGCAACTCC
TACTTCAAAGAAGAAAAGTACAAGGATGCCATCCATTTCTATAACAAGTCTCT
GGCAGAGCACCGAACCCCAAAGGTGCTAAAAAAGTGCCAACAGGCGGAGA

AAATCCTGAAGGAGCAAGGCGGCAGCGGCAGCGGCCTGCAGATGAGTGCG
ATTAAGCCAGACATGAAGATCAAACTCCGTATGGAAGGCAACGTAAACGGG
CACCACTTTGTGATCGACGGAGATGGTACAGGCAAGCCTTTTGAGGGAAAA
CAGAGTATGGATCTTGAAGTCAAAGAGGGCGGACCTCTGCCTTTTGCCTTT
GATATCCTGACCACTGCATTCCATTACGGCAACAGGGTATTCGCCAAATATC
CAGACAACATACAAGACTATTTTAAGCAGTCGTTTCCTAAGGGGTATTCGTG
GGAACGAAGCTTGACTTTCGAAGACGGGGGCATTTGCAACGCCAGAAACGA
CATAACAATGGAAGGGGACACTTTCTATAATAAAGTTCGATTTTATGGTACC
AACTTTCCCGCCAATGGTCCAGTTATGCAGAAGAAGACGCTGAAATGGGAG
CCCTCCACTGAGAAAATGTATGTGCGTGATGGAGTGCTGACGGGTGATATT
GAGATGGCTTTGTTGCTTGAAGGAAATGCCCATTACCGATGTGACTTCAGAA
CTACTTACAAAGCTAAGGAGAAGGGTGTCAAGTTACCAGGCGCCCACTTTG
TGGACCACTGCATTGAGATTTTAAGCCATGACAAAGATTACAACAAGGTTAA
GCTGTATGAGCATGCTGTTGCTCATTCTGGATTGCCTGACAATGCCAGACG
ATGATAAGTCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCC
ACATCCGCTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTC
CCTATTTATTTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAA
TTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAA
ACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAAT
TAAGGCGCGCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGA
ATCTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MKQALKEKELGNDAYKKKDFDTALKHYDKAKELDPTNMTYIINQAAVYFEKGDY
NKCRELCEKAIEVGRENREDYRWIAIAYARIGNSYFKEEKYKDAIHFYNKSLAEH
RTPKVLKKCQQAEKILKEQGGSGSGLQMSAIKPDMKIKLRMEGNVNGHHFVID
GDGTGKPFEGKQSMDLEVKEGGPLPFAFDILTTAFHYGNRVFAKYPDNIQDYF
KQSFPKGYSWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTNFPANGPV
MQKKTLKWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDFRTTYKAKEKGV
KLPGAHFVDHCIEILSHDKDYNKVKLYEHAVAHSGLPDNARR

Plasmid	pREV1 TRAP4-mEOS		
Freezer Box	CO Plasmids	#	11
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-80CXdefuWi6xfuoivBMy		

Description
pREV1 driven expression. TRAP4-mEOS in yeast integration vector with HIS3 marker.

Shading Key
pREV1
TRAP4
Linker
mEOS
Stop
CYC1 terminator

DNA
TAATACATAACCTTATGTATCATACACATACGATTTAGGTGACACTATAGAAC
GCGGCCGCCAGCTGAAGCTTCGTACGCTGCGTAGTCGGTCTCAAACGGTG
TTGTTATCCGATACAACCGGATATTTTTCTTTTAATGAGTCTAAACCGTGATA
GCTTCAGGTTAATACAATCAAAAAAAGCTCAAATATTCTTTTAATGCCGCGTT
CACAGATTCCAATTGAATACAACTAGGTAGTTCATTATATGAAGCCTTTGCTA
CTATTTTTCACTATAGTCTGCCTTCACCTTAATGCAGACATCCACATATTTTA
ATCACTTTAAAATAAAAAGGAAGATATATTAGAAGCTATGATCCAATCTGTAA
GCCAGATTAAAATTCACGAACTCTTCTTTCATTTGAATTGAATGCTTTGAGTT
GGGGTAGATTATCGCAAATTACTCATCACATTTATTGACTACGAACTTGCTG
ATGTCCTTTTTTTATTTATATTTTTCTTCAGTGAAGCGATTTTTTTTTTTACACAG
ACCAAGACGGAAAAAAGTAGCTAAGGAAGAAAACAAAATCATGAAAAAAATG
TGAAGTGATCATGCACATCGCATCAACTTAAACATTGGCTTAGAGATATATA
GAGTTAGAGTTTACGGCAACCTTTAAGCACCAATACCTTTTGGCATAGTCTA
AAGACCTGGTTCTTAATTTTAAACAAATTTAACTAAAGATTTCCCTATCAAAG
AAGTAACGAGTTGACAGATTTTCTCAAAATAAATCGATACTGCATTTCTAGGC
ATATCCAGCGAGATCTATGTGAGACCAGACCAATAAAATGAAGCAGGCACT
GAAAGAAAAAGAGCTGGGGAACGATGCCTACAAGAAGAAAGACTTTGACAC
AGCCTTGAAGCATTACGACAAAGCCAAGGAGCTGGACCCCACTAACATGAC
TTACATTATCAATCAAGCAGCGGTATACTTTGAAAAGGGCGACTACAATAAG
TGCCGGGAGCTTTGTGAGAAGGCCATTGAAGTGGGGAGAGAAAACCGAGA
GGACTATCGATGGATTGCCATTGCATATGCTCGAATTGGCAACTCCTACTTC
AAAGAAGAAAAGTACAAGGATGCCATCCATTTCTATAACAAGTCTCTGGCAG
AGCACCGAACCCCAAAGGTGCTAAAAAAGTGCCAACAGGCGGAGAAAATCC

TGAAGGAGCAAGGCGGCAGCGGCAGCGGCCTGCAGATGAGTGCGATTAAG
CCAGACATGAAGATCAAACTCCGTATGGAAGGCAACGTAAACGGGCACCAC
TTTGTGATCGACGGAGATGGTACAGGCAAGCCTTTTGAGGGAAAACAGAGT
ATGGATCTTGAAGTCAAAGAGGGCGGACCTCTGCCTTTTGCCTTTGATATCC
TGACCACTGCATTCCATTACGGCAACAGGGTATTCGCCAAATATCCAGACAA
CATACAAGACTATTTTAAGCAGTCGTTTCCTAAGGGGTATTCGTGGGAACGA
AGCTTGACTTTCGAAGACGGGGGCATTTGCAACGCCAGAAACGACATAACA
ATGGAAGGGGACACTTTCTATAATAAAGTTCGATTTTATGGTACCAACTTTCC
CGCCAATGGTCCAGTTATGCAGAAGAAGACGCTGAAATGGGAGCCCTCCAC
TGAGAAAATGTATGTGCGTGATGGAGTGCTGACGGGTGATATTGAGATGGC
TTTGTTGCTTGAAGGAAATGCCCATTACCGATGTGACTTCAGAACTACTTAC
AAAGCTAAGGAGAAGGGTGTCAAGTTACCAGGCGCCCACTTTGTGGACCAC
TGCATTGAGATTTTAAGCCATGACAAAGATTACAACAAGGTTAAGCTGTATG
AGCATGCTGTTGCTCATTCTGGATTGCCTGACAATGCCAGACGATGATAAGT
CATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCT
CTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATT
TTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTT
TTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTT
GAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGC
GCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAAT
TAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MKQALKEKELGNDAYKKKDFDTALKHYDKAKELDPTNMTYIINQAAVYFEKGDY
NKCRELCEKAIEVGRENREDYRWIAIAYARIGNSYFKEEKYKDAIHFYNKSLAEH
RTPKVLKKCQQAEKILKEQGGSGSGLQMSAIKPDMKIKLRMEGNVNGHHFVID
GDGTGKPFEGKQSMDLEVKEGGPLPFAFDILTTAFHYGNRVFAKYPDNIQDYF
KQSFPKGYSWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTNFPANGPV
MQKKTLKWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDFRTTYKAKEKGV
KLPGAHFVDHCIEILSHDKDYNKVKLYEHAVAHSGLPDNARR

Plasmid	GS-MEEVF		
Freezer Box	CO Plasmids	#	12
Parent Vector	pFA6a-KanMX6	Source	Michael Hinrichsen
Bacterial Selection	Amp	Yeast Selection	G418
Full Sequence	https://benchling.com/s/seq-cH8vtb33COM2sBsHTH2L		

Description
MEEVF peptide in yeast integration vector with KanR marker. Meant for as a template for tagging genomic target proteins

Shading Key
Linker
MEEVF
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGGTG
GATCAGGCTCTGGTTTGCAAATGGAAGAGGTTTTTTAATGAGTCATGTAATT
AGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAA
AAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGT
TATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTAC
AGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTT
TGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTG
TTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCC
CAGAATACCCTCCTTGACAGTCTTG

Expression Product Sequence
GGSGSGLQMEEVF

Plasmid	GS-CCBN3,5		
Freezer Box	CO Plasmids	#	13
Parent Vector	pFA6a-KanMX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	G418
Full Sequence	https://benchling.com/s/seq-gbZBrJqbqs3F2GWXHLvj		

Description
CCBN3,5 coil in yeast integration vector with KanR marker. Meant for as a template for tagging genomic target proteins

Shading Key
Linker
CCBN3,5
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGGTG
GATCAGGCTCTGGTTTGCAAGGTTTGAAGCAAAAGATTGCAGCTTTGGAATA
CAAGAACGCTGCTTTGAAGAAGAAGATTGCTGCTTTGAAGCAAGGTGGTTG
ATGAGTCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACA
TCCGCTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCT
ATTTATTTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTT
TTCTTTTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACC
TTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAA
GGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCC
AGCGACATGGAGGCCCAGAATACCCTCCTTGACAGTCTTG

Expression Product Sequence
GGSGSGLQGLKQKIAALEYKNAALKKKIAALKQGG

Plasmid	GS-SYNZIP18		
Freezer Box	CO Plasmids	#	14
Parent Vector	pFA6a-KanMX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	G418
Full Sequence	https://benchling.com/s/seq-vj9MUSkhiQdyIJarTaXT		

Description
SYNZIP18 coil in yeast integration vector with KanR marker. Meant for as a template for tagging genomic target proteins

Shading Key
Linker
SYNZIP18
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGGTG
GATCAGGCTCTGGTTTGCAATCTATTGCTGCTACTTTGGAAAACGACTTGGC
TAGACTTGAAAACGAAAACGCTAGATTGGAAAAGGACATTGCTAACTTGGAA
AGAGACTTGGCTAAGTTGGAAAGAGAAGAAGCTTACTTCTGATGAGTCATGT
AATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAAC
CGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTT
ATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTC
TGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAA
GGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAG
ATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGA
GGCCCAGAATACCCTCCTTGACAGTCTTG

Expression Product Sequence
GGSGSGLQSIAATLENDLARLENENARLEKDIANLERDLAKLEREEAYF

Plasmid	pGAL1 SYNZIP17-mEOS		
Freezer Box	CO Plasmids	#	15
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-I7p5eAGtiRTHp4shi7I1		

Description
pGAL1 driven expression. SYNZIP17-mEOS in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
SYNZIP17
Linker
mEOS
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCAACGAAAAGGAA
GAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGAAAC
AAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAATTGA
AGCTTACAAGAGTGGTAGTGGAAGTGGCCTGCAGATGAGTGCGATTAAGCC
AGACATGAAGATCAAACTCCGTATGGAAGGCAACGTAAACGGGCACCACTT
TGTGATCGACGGAGATGGTACAGGCAAGCCTTTTGAGGGAAAACAGAGTAT
GGATCTTGAAGTCAAAGAGGGCGGACCTCTGCCTTTTGCCTTTGATATCCTG
ACCACTGCATTCCATTACGGCAACAGGGTATTCGCCAAATATCCAGACAACA
TACAAGACTATTTTAAGCAGTCGTTTCCTAAGGGGTATTCGTGGGAACGAAG
CTTGACTTTCGAAGACGGGGGCATTTGCAACGCCAGAAACGACATAACAAT
GGAAGGGGACACTTTCTATAATAAAGTTCGATTTTATGGTACCAACTTTCCC
GCCAATGGTCCAGTTATGCAGAAGAAGACGCTGAAATGGGAGCCCTCCACT
GAGAAAATGTATGTGCGTGATGGAGTGCTGACGGGTGATATTGAGATGGCT

TTGTTGCTTGAAGGAAATGCCCATTACCGATGTGACTTCAGAACTACTTACA
AAGCTAAGGAGAAGGGTGTCAAGTTACCAGGCGCCCACTTTGTGGACCACT
GCATTGAGATTTTAAGCCATGACAAAGATTACAACAAGGTTAAGCTGTATGA
GCATGCTGTTGCTCATTCTGGATTGCCTGACAATGCCAGACGATGATAAGTC
ATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTC
TAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTT
TTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTT
TTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTG
AGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCG
CCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAATTA
AGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
NEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYKGGSGSGLQMSAI
KPDMKIKLRMEGNVNGHHFVIDGDGTGKPFEGKQSMDLEVKEGGPLPFAFDIL
TTAFHYGNRVFAKYPDNIQDYFKQSFPKGYSWERSLTFEDGGICNARNDITME
GDTFYNKVRFYGTNFPANGPVMQKKTLKWEPSTEKMYVRDGVLTGDIEMALLL
EGNAHYRCDFRTTYKAKEKGVKLPGAHFVDHCIEILSHDKDYNKVKLYEHAVAH
SGLPDNARR

Plasmid	pGAL1 CCAN3,5-mEOS		
Freezer Box	CO Plasmids	#	16
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-AZ2PbROjt7z6oAWa1GnZ		

Description
pGAL1 driven expression. CCAN3,5-mEOS in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
CCAN3,5
Linker
mEOS
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCGGTCTCGAACAA
GAAATTGCAGCTTTGGAGAAGGAAAACGCTGCTTTGGAATGGGAAATTGCT
GCATTGGAACAAGGTGGAAGTGGTAGTGGAAGTGGCCTGCAGATGAGTGC
GATTAAGCCAGACATGAAGATCAAACTCCGTATGGAAGGCAACGTAAACGG
GCACCACTTTGTGATCGACGGAGATGGTACAGGCAAGCCTTTTGAGGGAAA
ACAGAGTATGGATCTTGAAGTCAAAGAGGGCGGACCTCTGCCTTTTGCCTTT
GATATCCTGACCACTGCATTCCATTACGGCAACAGGGTATTCGCCAAATATC
CAGACAACATACAAGACTATTTTAAGCAGTCGTTTCCTAAGGGGTATTCGTG
GGAACGAAGCTTGACTTTCGAAGACGGGGGCATTTGCAACGCCAGAAACGA
CATAACAATGGAAGGGGACACTTTCTATAATAAAGTTCGATTTTATGGTACC
AACTTTCCCGCCAATGGTCCAGTTATGCAGAAGAAGACGCTGAAATGGGAG
CCCTCCACTGAGAAAATGTATGTGCGTGATGGAGTGCTGACGGGTGATATT
GAGATGGCTTTGTTGCTTGAAGGAAATGCCCATTACCGATGTGACTTCAGAA

CTACTTACAAAGCTAAGGAGAAGGGTGTCAAGTTACCAGGCGCCCACTTTG
TGGACCACTGCATTGAGATTTTAAGCCATGACAAAGATTACAACAAGGTTAA
GCTGTATGAGCATGCTGTTGCTCATTCTGGATTGCCTGACAATGCCAGACG
ATGATAAGTCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCC
ACATCCGCTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTC
CCTATTTATTTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAA
TTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAA
ACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAAT
TAAGGCGCGCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGA
ATCTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
GLEQEIAALEKENAALEWEIAALEQGGGGSGSGLQMSAIKPDMKIKLRMEGNV
NGHHFVIDGDGTGKPFEGKQSMDLEVKEGGPLPFAFDILTTAFHYGNRVFAKY
PDNIQDYFKQSFPKGYSWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTN
FPANGPVMQKKTLKWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDFRTTY
KAKEKGVKLPGAHFVDHCIEILSHDKDYNKVKLYEHAVAHSGLPDNARR

Plasmid	pGAL1 TRAP4-mNeonGreen		
Freezer Box	CO Plasmids	#	17
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-RLzDwsLjYwYYipR8nS7v		

Description
pGAL1 driven expression. TRAP4-mNeonGreen in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
TRAP4
Linker
mNeonGreen
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAAGCAGGCA
CTGAAAGAAAAAGAGCTGGGGAACGATGCCTACAAGAAGAAAGACTTTGAC
ACAGCCTTGAAGCATTACGACAAAGCCAAGGAGCTGGACCCCACTAACATG
ACTTACATTATCAATCAAGCAGCGGTATACTTTGAAAAGGGCGACTACAATA
AGTGCCGGGAGCTTTGTGAGAAGGCCATTGAAGTGGGGAGAGAAAACCGA
GAGGACTATCGATGGATTGCCATTGCATATGCTCGAATTGGCAACTCCTACT
TCAAAGAAGAAAAGTACAAGGATGCCATCCATTTCTATAACAAGTCTCTGGC
AGAGCACCGAACCCCAAAGGTGCTAAAAAAGTGCCAACAGGCGGAGAAAAT
CCTGAAGGAGCAAGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAG
GGCGAGGAGGATAACATGGCCTCTCTCCCAGCGACACATGAGTTACACATC
TTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGCACCGGC
AATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGAC
CTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCATC

AGTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCGCCATGGTAG
ATGGCTCCGGCTACCAAGTCCATCGCACAATGCAGTTTGAAGATGGTGCCT
CCCTTACTGTTAACTACCGCTACACCTACGAGGGAAGCCACATCAAAGGAG
AGGCCCAGGTGAAGGGGACTGGTTTCCCTGCTGACGGTCCTGTGATGACC
AACTCGCTGACCGCTGCGGACTGGTGCAGGTCGAAGAAGACTTACCCCAA
CGACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGGAAATGGC
AAGCGCTACCGGAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAAT
GGCGGCTAACTATCTGAAGAACCAGCCGATGTACGTGTTCCGTAAGACGGA
GCTCAAGCACTCCAAGACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTT
TACCGATGTGATGGGCATGGACGAGCTGTACAAGTGATAAGTCATGTAATTA
GTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAA
AGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTT
ATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACA
GACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTT
GGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTGT
TTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAATTAAGGCGCGCC
AGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence

MKQALKEKELGNDAYKKKDFDTALKHYDKAKELDPTNMTYIINQAAVYFEKGDY
NKCRELCEKAIEVGRENREDYRWIAIAYARIGNSYFKEEKYKDAIHFYNKSLAEH
RTPKVLKKCQQAEKILKEQGGSGSGLQMVSKGEEDNMASLPATHELHIFGSIN
GVDFDMVGQGTGNPNDGYEELNLKSTKGDLQFSPWILVPHIGYGFHQYLPYPD
GMSPFQAAMVDGSGYQVHRTMQFEDGASLTVNYRYTYEGSHIKGEAQVKGT
GFPADGPVMTNSLTAADWCRSKKTYPNDKTIISTFKWSYTTGNGKRYRSTART
TYTFAKPMAANYLKNQPMYVFRKTELKHSKTELNFKEWQKAFTDVMGMDELY
K

Plasmid	pGAL1 SYNZIP17-mNeonGreen		
Freezer Box	CO Plasmids	#	18
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-FlqbO1w4YUpxuglnvG0p		

Description
pGAL1 driven expression. SYNZIP17-mNeonGreen in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
SYNZIP17
Linker
mNeonGreen
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAACGAAAAG
GAAGAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGA
AACAAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAAT
TGAAGCTTACAAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAGG
GCGAGGAGGATAACATGGCCTCTCTCCCAGCGACACATGAGTTACACATCT
TTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGCACCGGCA
ATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGACC
TCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCATCA
GTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCGCCATGGTAGA
TGGCTCCGGCTACCAAGTCCATCGCACAATGCAGTTTGAAGATGGTGCCTC
CCTTACTGTTAACTACCGCTACACCTACGAGGGAAGCCACATCAAAGGAGA
GGCCCAGGTGAAGGGGACTGGTTTCCCTGCTGACGGTCCTGTGATGACCA
ACTCGCTGACCGCTGCGGACTGGTGCAGGTCGAAGAAGACTTACCCCAAC

GACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGGAAATGGCA
AGCGCTACCGGAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAATG
GCGGCTAACTATCTGAAGAACCAGCCGATGTACGTGTTCCGTAAGACGGAG
CTCAAGCACTCCAAGACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTT
ACCGATGTGATGGGCATGGACGAGCTGTACAAGTGATAAGTCATGTAATTA
GTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAA
AGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTT
ATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACA
GACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTT
GGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTGT
TTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAATTAAGGCGCGCC
AGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MNEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYKGGSGSGLQMVS
KGEEDNMASLPATHELHIFGSINGVDFDMVGQGTGNPNDGYEELNLKSTKGDL
QFSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVHRTMQFEDGASL
TVNYRYTYEGSHIKGEAQVKGTGFPADGPVMTNSLTAADWCRSKKTYPNDKTII
STFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYVFRKTELKHSKTE
LNFKEWQKAFTDVMGMDELYK

Plasmid	VFP-MEEVF		
Freezer Box	CO Plasmids	#	19
Parent Vector	pCu415CUP1	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	Leu2
Full Sequence	https://benchling.com/s/seq-K1pnvrso0irHeeAzu6hq		

Description
For inserting actin constructs for yeast imaging of actin

Shading Key
pCUP1
VFP
MEEVF
Stop

DNA
GACTGGACTCTAGTTAGAAAGACATTTTTGCTGTCAGTCACTGTCAAGAGAT
TCTTTTGCTGGCATTTCTTCTAGAAGCAAAAAGAGCGATGCGTCTTTTCCGC
TGAACCGTTCCAGCAAAAAAGACTACCAACGCAATATGGATTGTCAGAATCA
TATAAAAGAGAAGCAAATAACTCCTTGTCTTGTATCAATTGCATTATAATATC
TTCTTGTTAGTGCAATATCATATAGAAGTCATCGACTAGTATGAATGTGATTA
AGCCAGACATGAGGATCAAGCTGCGTATGGAAGGTGCTGTAAACGGGCAC
AAGTTCGTTATTTTAGGAGACGGAAATGGCAAGCCTTACGAGGGAACGCAG
ACCATAGACGTTACAGTCAAAGAAGGTGGACCTCTGCCTTTTGCTTACGACA
TCTTAACATCAGCATTCCAGTACGGCAACAGGGTGTTTACCAAATATCCAGA
TGATATTGCAGATTATTTCAAGCAATCTTTTCCTGTGGGGTATTCCTGGGAA
CGAAGCATGACTTACGAAGACGGAGGCATTTGTACTGTTTCAAGTGACATAA
AAATGGAAGGCAACTCCTTTATCTATGAAATTCGATTTCATGGCTTGAACTTT
CCCTCCGATGGTCCAGTTATGCAGAAGAAGACCGTGAAATGGGAGCCATCC
ACTGAGAAAATGTATGTGCGTGATGGAGTGCTGAAGGGTGATGTTAACATG
CGTCTGTTGCTGGAAGGAGGTGGTCATTACCGATGTGACTTCAAAAGTACTT
ACAAAGCCAAGAGGGCTGTCCAGTTGCCAGACTATCACTATATTGACCACC
GCATTGAGATCTTAAGCCATGACAAGGATTACAACAAAGTTAAGCTGTGTGA
GAACGCCGCAGCTCGCTGTTCTATGCTGCCGAGTCAGGCCAAGCTCGAGC
TGGTTCCGCGTGGATCCCCGAATTCCGAAAACCTGTATTTTCAGGGCTCCA
GTGCTGCTGTAACTGAAGAAATGCCACCCCTAGAAGGAGATGACGACACAT
CACGCATGGAAGAAGTATTCTAAAAGCTTATCGATACCGTCGACCTCGAGTC
ATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTC
TAACCGAAAAGGAAGGAGT

Expression Product Sequence

MNVIKPDMRIKLRMEGAVNGHKFVILGDGNGKPYEGTQTIDVTVKEGGPLPFAY
DILTSAFQYGNRVFTKYPDDIADYFKQSFPVGYSWERSMTYEDGGICTVSSDIK
MEGNSFIYEIRFHGLNFPSDGPVMQKKTVKWEPSTEKMYVRDGVLKGDVNMR
LLLEGGGHYRCDFKSTYKAKRAVQLPDYHYIDHRIEILSHDKDYNKVKLCENAA
ARCSMLPSQAKLELVPRGSPNSENLYFQGSSAAVTEEMPPLEGDDDTSRMEE
VF

Plasmid	pCUP1 SZ18-Actin		
Freezer Box	CO Plasmids	#	20
Parent Vector	pCu415CUP1	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	Leu2
Full Sequence	https://benchling.com/s/seq-4o5hkdTI5RzFHhMnCmHQ		

Description
CUP1 driven expression. SYNZIP18-Actin in yeast shuttle vector with LEU2 marker.

Shading Key
pCUP1
SYNZIP18
Linker
Actin
Stop

DNA
GACTGGACTCTAGTTAGAAAGACATTTTTGCTGTCAGTCACTGTCAAGAGAT
TCTTTTGCTGGCATTTCTTCTAGAAGCAAAAAGAGCGATGCGTCTTTTCCGC
TGAACCGTTCCAGCAAAAAAGACTACCAACGCAATATGGATTGTCAGAATCA
TATAAAAGAGAAGCAAATAACTCCTTGTCTTGTATCAATTGCATTATAATATC
TTCTTGTTAGTGCAATATCATATAGAAGTCATCGACTAGTATGTCTATTGCTG
CTACTTTGGAAAACGACTTGGCTAGACTTGAAAACGAAAACGCTAGATTGGA
AAAGGACATTGCTAACTTGGAAAGAGACTTGGCTAAGTTGGAAAGAGAAGA
AGCTTACTTCGGTGGATCAGGCTCTGGTATGGATTCTGAGGTTGCTGCTTTG
GTTATTGATAACGGTTCTGGTATGTGTAAAGCCGGTTTTGCCGGTGACGAC
GCTCCTCGTGCTGTCTTCCCATCTATCGTCGGTAGACCAAGACACCAAGGT
ATCATGGTCGGTATGGGTCAAAAAGACTCCTACGTTGGTGATGAAGCTCAAT
CCAAGAGAGGTATCTTGACTTTACGTTACCCAATTGAACACGGTATTGTCAC
CAACTGGGACGATATGGAAAAGATCTGGCATCATACCTTCTACAACGAATTG
AGAGTTGCCCCAGAAGAACACCCTGTTCTTTTGACTGAAGCTCCAATGAACC
CTAAATCAAACAGAGAAAAGATGACTCAAATTATGTTTGAAACTTTCAACGTT
CCAGCCTTCTACGTTTCCATCCAAGCCGTTTTGTCCTTGTACTCTTCCGGTA
GAACTACTGGTATTGTTTTGGATTCCGGTGATGGTGTTACTCACGTCGTTCC
AATTTACGCTGGTTTCTCTCTACCTCACGCCATTTTGAGAATCGATTTGGCC
GGTAGAGATTTGACTGACTACTTGATGAAGATCTTGAGTGAACGTGGTTACT
CTTTCTCCACCACTGCTGAAAGAGAAATTGTCCGTGACATCAAGGAAAAACT
ATGTTACGTCGCCTTGGACTTCGAACAAGAAATGCAAACCGCTGCTCAATCT
TCTTCAATTGAAAAATCCTACGAACTTCCAGATGGTCAAGTCATCACTATTG
GTAACGAAAGATTCAGAGCCCCAGAAGCTTTGTTCCATCCTTCTGTTTTGGG
TTTGGAATCTGCCGGTATTGACCAAACTACTTACAACTCCATCATGAAGTGT

GATGTCGATGTCCGTAAGGAATTATACGGTAACATCGTTATGTCCGGTGGTA
CCACCATGTTCCCAGGTATTGCCGAAAGAATGCAAAAGGAAATCACCGCTTT
GGCTCCATCTTCCATGAAGGTCAAGATCATTGCTCCTCCAGAAAGAAAGTAC
TCCGTCTGGATTGGTGGTTCTATCTTGGCTTCTTTGACTACCTTCCAACAAAT
GTGGATCTCAAAACAAGAATACGACGAAAGTGGTCCATCTATCGTTCACCAC
AAGTGTTTCTAATGATAAAAGCTTATCGATACCGTCGACCTCGAGTCATGTA
ATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACC
GAAAAGGAAGGAGT

Expression Product Sequence
MSIAATLENDLARLENENARLEKDIANLERDLAKLEREEAYFGGSGSGMDSEVA
ALVIDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGIMVGMGQKDSYVGDEA
QSKRGILTLRYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPMNP
KSNREKMTQIMFETFNVPAFYVSIQAVLSLYSSGRTTGIVLDSGDGVTHVVPIYA
GFSLPHAILRIDLAGRDLTDYLMKILSERGYSFSTTAEREIVRDIKEKLCYVALDF
EQEMQTAAQSSSIEKSYELPDGQVITIGNERFRAPEALFHPSVLGLESAGIDQTT
YNSIMKCDVDVRKELYGNIVMSGGTTMFPGIAERMQKEITALAPSSMKVKIIAPP
ERKYSVWIGGSILASLTTFQQMWISKQEYDESGPSIVHHKCF

Plasmid	pCUP1 Actin-SZ18		
Freezer Box	CO Plasmids	#	21
Parent Vector	pCu415CUP1	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	Leu2
Full Sequence	https://benchling.com/s/seq-VHya07dCQ0PeIv8zEPhD		

Description
CUP1 driven expression. Actin-SYNZIP18 in yeast shuttle vector with LEU2 marker.

Shading Key
pCUP1
Actin
Linker
SYNZIP18
Stop

DNA
GACTGGACTCTAGTTAGAAAGACATTTTTGCTGTCAGTCACTGTCAAGAGAT
TCTTTTGCTGGCATTTCTTCTAGAAGCAAAAAGAGCGATGCGTCTTTTCCGC
TGAACCGTTCCAGCAAAAAAGACTACCAACGCAATATGGATTGTCAGAATCA
TATAAAAGAGAAGCAAATAACTCCTTGTCTTGTATCAATTGCATTATAATATC
TTCTTGTTAGTGCAATATCATATAGAAGTCATCGACTAGTATGGATTCTGAGG
TTGCTGCTTTGGTTATTGATAACGGTTCTGGTATGTGTAAAGCCGGTTTTGC
CGGTGACGACGCTCCTCGTGCTGTCTTCCCATCTATCGTCGGTAGACCAAG
ACACCAAGGTATCATGGTCGGTATGGGTCAAAAAGACTCCTACGTTGGTGA
TGAAGCTCAATCCAAGAGAGGTATCTTGACTTTACGTTACCCAATTGAACAC
GGTATTGTCACCAACTGGGACGATATGGAAAAGATCTGGCATCATACCTTCT
ACAACGAATTGAGAGTTGCCCCAGAAGAACACCCTGTTCTTTTGACTGAAGC
TCCAATGAACCCTAAATCAAACAGAGAAAAGATGACTCAAATTATGTTTGAAA
CTTTCAACGTTCCAGCCTTCTACGTTTCCATCCAAGCCGTTTTGTCCTTGTAC
TCTTCCGGTAGAACTACTGGTATTGTTTTGGATTCCGGTGATGGTGTTACTC
ACGTCGTTCCAATTTACGCTGGTTTCTCTCTACCTCACGCCATTTTGAGAAT
CGATTTGGCCGGTAGAGATTTGACTGACTACTTGATGAAGATCTTGAGTGAA
CGTGGTTACTCTTTCTCCACCACTGCTGAAAGAGAAATTGTCCGTGACATCA
AGGAAAAACTATGTTACGTCGCCTTGGACTTCGAACAAGAAATGCAAACCG
CTGCTCAATCTTCTTCAATTGAAAAATCCTACGAACTTCCAGATGGTCAAGT
CATCACTATTGGTAACGAAAGATTCAGAGCCCCAGAAGCTTTGTTCCATCCT
TCTGTTTTGGGTTTGGAATCTGCCGGTATTGACCAAACTACTTACAACTCCA
TCATGAAGTGTGATGTCGATGTCCGTAAGGAATTATACGGTAACATCGTTAT
GTCCGGTGGTACCACCATGTTCCCAGGTATTGCCGAAAGAATGCAAAAGGA
AATCACCGCTTTGGCTCCATCTTCCATGAAGGTCAAGATCATTGCTCCTCCA

GAAAGAAAGTACTCCGTCTGGATTGGTGGTTCTATCTTGGCTTCTTTGACTA
CCTTCCAACAAATGTGGATCTCAAAACAAGAATACGACGAAAGTGGTCCATC
TATCGTTCACCACAAGTGTTTCGGTGGATCAGGCTCTGGTTCTATTGCTGCT
ACTTTGGAAAACGACTTGGCTAGACTTGAAAACGAAAACGCTAGATTGGAAA
AGGACATTGCTAACTTGGAAAGAGACTTGGCTAAGTTGGAAAGAGAAGAAG
CTTACTTCTAATGAAAGCTTATCGATACCGTCGACCTCGAGTCATGTAATTA
GTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAA
AGGAAGGAGT

Expression Product Sequence
MDSEVAALVIDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGIMVGMGQKDS
YVGDEAQSKRGILTLRYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLT
EAPMNPKSNREKMTQIMFETFNVPAFYVSIQAVLSLYSSGRTTGIVLDSGDGVT
HVVPIYAGFSLPHAILRIDLAGRDLTDYLMKILSERGYSFSTTAEREIVRDIKEKLC
YVALDFEQEMQTAAQSSSIEKSYELPDGQVITIGNERFRAPEALFHPSVLGLESA
GIDQTTYNSIMKCDVDVRKELYGNIVMSGGTTMFPGIAERMQKEITALAPSSMK
VKIIAPPERKYSVWIGGSILASLTTFQQMWISKQEYDESGPSIVHHKCFGGSGS
GSIAATLENDLARLENENARLEKDIANLERDLAKLEREEAYF

Plasmid	pCUP1 Actin-MEEVF		
Freezer Box	CO Plasmids	#	22
Parent Vector	pCu415CUP1	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	Leu2
Full Sequence	https://benchling.com/s/seq-wzwyaeVWicue9tQCsxTv		

Description
CUP1 driven expression. Actin-MEEVF in yeast shuttle vector with LEU2 marker.

Shading Key
pCUP1
Actin
Linker
MEEVF
Stop

DNA
GACTGGACTCTAGTTAGAAAGACATTTTTGCTGTCAGTCACTGTCAAGAGAT
TCTTTTGCTGGCATTTCTTCTAGAAGCAAAAAGAGCGATGCGTCTTTTCCGC
TGAACCGTTCCAGCAAAAAAGACTACCAACGCAATATGGATTGTCAGAATCA
TATAAAAGAGAAGCAAATAACTCCTTGTCTTGTATCAATTGCATTATAATATC
TTCTTGTTAGTGCAATATCATATAGAAGTCATCGACTAGTATGGATTCTGAGG
TTGCTGCTTTGGTTATTGATAACGGTTCTGGTATGTGTAAAGCCGGTTTTGC
CGGTGACGACGCTCCTCGTGCTGTCTTCCCATCTATCGTCGGTAGACCAAG
ACACCAAGGTATCATGGTCGGTATGGGTCAAAAAGACTCCTACGTTGGTGA
TGAAGCTCAATCCAAGAGAGGTATCTTGACTTTACGTTACCCAATTGAACAC
GGTATTGTCACCAACTGGGACGATATGGAAAAGATCTGGCATCATACCTTCT
ACAACGAATTGAGAGTTGCCCCAGAAGAACACCCTGTTCTTTTGACTGAAGC
TCCAATGAACCCTAAATCAAACAGAGAAAAGATGACTCAAATTATGTTTGAAA
CTTTCAACGTTCCAGCCTTCTACGTTTCCATCCAAGCCGTTTTGTCCTTGTAC
TCTTCCGGTAGAACTACTGGTATTGTTTTGGATTCCGGTGATGGTGTTACTC
ACGTCGTTCCAATTTACGCTGGTTTCTCTCTACCTCACGCCATTTTGAGAAT
CGATTTGGCCGGTAGAGATTTGACTGACTACTTGATGAAGATCTTGAGTGAA
CGTGGTTACTCTTTCTCCACCACTGCTGAAAGAGAAATTGTCCGTGACATCA
AGGAAAAACTATGTTACGTCGCCTTGGACTTCGAACAAGAAATGCAAACCG
CTGCTCAATCTTCTTCAATTGAAAAATCCTACGAACTTCCAGATGGTCAAGT
CATCACTATTGGTAACGAAAGATTCAGAGCCCCAGAAGCTTTGTTCCATCCT
TCTGTTTTGGGTTTGGAATCTGCCGGTATTGACCAAACTACTTACAACTCCA
TCATGAAGTGTGATGTCGATGTCCGTAAGGAATTATACGGTAACATCGTTAT
GTCCGGTGGTACCACCATGTTCCCAGGTATTGCCGAAAGAATGCAAAAGGA
AATCACCGCTTTGGCTCCATCTTCCATGAAGGTCAAGATCATTGCTCCTCCA
GAAAGAAAGTACTCCGTCTGGATTGGTGGTTCTATCTTGGCTTCTTTGACTA

CCTTCCAACAAATGTGGATCTCAAAACAAGAATACGACGAAAGTGGTCCATC
TATCGTTCACCACAAGTGTTTCGGTGGATCAGGCTCTGGTATGGAAGAGGT
TTTTTAATGAAAGCTTATCGATACCGTCGACCTCGAGTCATGTAATTAGTTAT
GTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAAAGGA
AGGAGT

Expression Product Sequence
MDSEVAALVIDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGIMVGMGQKDS
YVGDEAQSKRGILTLRYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLT
EAPMNPKSNREKMTQIMFETFNVPAFYVSIQAVLSLYSSGRTTGIVLDSGDGVT
HVVPIYAGFSLPHAILRIDLAGRDLTDYLMKILSERGYSFSTTAEREIVRDIKEKLC
YVALDFEQEMQTAAQSSSIEKSYELPDGQVITIGNERFRAPEALFHPSVLGLESA
GIDQTTYNSIMKCDVDVRKELYGNIVMSGGTTMFPGIAERMQKEITALAPSSMK
VKIIAPPERKYSVWIGGSILASLTTFQQMWISKQEYDESGPSIVHHKCFGGSGS
GMEEVF

Plasmid	pCUP1 Actin-GS		
Freezer Box	CO Plasmids	#	23
Parent Vector	pCu415CUP1	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	Leu2
Full Sequence	https://benchling.com/s/seq-GhQC0FDjm6ZqKh9gPIsA		

Description
CUP1 driven expression. Actin-GS in yeast shuttle vector with LEU2 marker.

Shading Key
pCUP1
Actin
Linker
Stop

DNA
GACTGGACTCTAGTTAGAAAGACATTTTTGCTGTCAGTCACTGTCAAGAGAT
TCTTTTGCTGGCATTTCTTCTAGAAGCAAAAAGAGCGATGCGTCTTTTCCGC
TGAACCGTTCCAGCAAAAAAGACTACCAACGCAATATGGATTGTCAGAATCA
TATAAAAGAGAAGCAAATAACTCCTTGTCTTGTATCAATTGCATTATAATATC
TTCTTGTTAGTGCAATATCATATAGAAGTCATCGACTAGTATGGATTCTGAGG
TTGCTGCTTTGGTTATTGATAACGGTTCTGGTATGTGTAAAGCCGGTTTTGC
CGGTGACGACGCTCCTCGTGCTGTCTTCCCATCTATCGTCGGTAGACCAAG
ACACCAAGGTATCATGGTCGGTATGGGTCAAAAAGACTCCTACGTTGGTGA
TGAAGCTCAATCCAAGAGAGGTATCTTGACTTTACGTTACCCAATTGAACAC
GGTATTGTCACCAACTGGGACGATATGGAAAAGATCTGGCATCATACCTTCT
ACAACGAATTGAGAGTTGCCCCAGAAGAACACCCTGTTCTTTTGACTGAAGC
TCCAATGAACCCTAAATCAAACAGAGAAAAGATGACTCAAATTATGTTTGAAA
CTTTCAACGTTCCAGCCTTCTACGTTTCCATCCAAGCCGTTTTGTCCTTGTAC
TCTTCCGGTAGAACTACTGGTATTGTTTTGGATTCCGGTGATGGTGTTACTC
ACGTCGTTCCAATTTACGCTGGTTTCTCTCTACCTCACGCCATTTTGAGAAT
CGATTTGGCCGGTAGAGATTTGACTGACTACTTGATGAAGATCTTGAGTGAA
CGTGGTTACTCTTTCTCCACCACTGCTGAAAGAGAAATTGTCCGTGACATCA
AGGAAAAACTATGTTACGTCGCCTTGGACTTCGAACAAGAAATGCAAACCG
CTGCTCAATCTTCTTCAATTGAAAAATCCTACGAACTTCCAGATGGTCAAGT
CATCACTATTGGTAACGAAAGATTCAGAGCCCCAGAAGCTTTGTTCCATCCT
TCTGTTTTGGGTTTGGAATCTGCCGGTATTGACCAAACTACTTACAACTCCA
TCATGAAGTGTGATGTCGATGTCCGTAAGGAATTATACGGTAACATCGTTAT
GTCCGGTGGTACCACCATGTTCCCAGGTATTGCCGAAAGAATGCAAAAGGA
AATCACCGCTTTGGCTCCATCTTCCATGAAGGTCAAGATCATTGCTCCTCCA
GAAAGAAAGTACTCCGTCTGGATTGGTGGTTCTATCTTGGCTTCTTTGACTA
CCTTCCAACAAATGTGGATCTCAAAACAAGAATACGACGAAAGTGGTCCATC

TATCGTTCACCACAAGTGTTTCGGTGGATCAGGCTCTGGTTAATGAAAGCTT
ATCGATACCGTCGACCTCGAGTCATGTAATTAGTTATGTCACGCTTACATTC
ACGCCCTCCCCCCACATCCGCTCTAACCGAAAAGGAAGGAGT

Expression Product Sequence
MDSEVAALVIDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGIMVGMGQKDS
YVGDEAQSKRGILTLRYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLT
EAPMNPKSNREKMTQIMFETFNVPAFYVSIQAVLSLYSSGRTTGIVLDSGDGVT
HVVPIYAGFSLPHAILRIDLAGRDLTDYLMKILSERGYSFSTTAEREIVRDIKEKLC
YVALDFEQEMQTAAQSSSIEKSYELPDGQVITIGNERFRAPEALFHPSVLGLESA
GIDQTTYNSIMKCDVDVRKELYGNIVMSGGTTMFPGIAERMQKEITALAPSSMK
VKIIAPPERKYSVWIGGSILASLTTFQQMWISKQEYDESGPSIVHHKCFGGSGS
G

Plasmid	pGAL1 LifeAct-mNeonGreen		
Freezer Box	CO Plasmids	#	24
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-fNs4lNEttwOLPVZYhu8e		

Description
pGAL1 driven expression. LifeAct-mNeonGreen in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
LifeAct
Linker
mNeonGreen
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGGGCGTGGC
CGACTTGATCAAGAAGTTCGAGTCCATCTCCAAGGAGGAGCGCATTCCGGG
CCTGATTAACATGGTGAGCAAGGGCGAGGAGGATAACATGGCCTCTCTCCC
AGCGACACATGAGTTACACATCTTTGGCTCCATCAACGGTGTGGACTTTGAC
ATGGTGGGTCAGGGCACCGGCAATCCAAATGATGGTTATGAGGAGTTAAAC
CTGAAGTCCACCAAGGGTGACCTCCAGTTCTCCCCCTGGATTCTGGTCCCT
CATATCGGGTATGGCTTCCATCAGTACCTGCCCTACCCTGACGGGATGTCG
CCTTTCCAGGCCGCCATGGTAGATGGCTCCGGCTACCAAGTCCATCGCACA
ATGCAGTTTGAAGATGGTGCCTCCCTTACTGTTAACTACCGCTACACCTACG
AGGGAAGCCACATCAAAGGAGAGGCCCAGGTGAAGGGGACTGGTTTCCCT
GCTGACGGTCCTGTGATGACCAACTCGCTGACCGCTGCGGACTGGTGCAG
GTCGAAGAAGACTTACCCCAACGACAAAACCATCATCAGTACCTTTAAGTGG
AGTTACACCACTGGAAATGGCAAGCGCTACCGGAGCACTGCGCGGACCAC

CTACACCTTTGCCAAGCCAATGGCGGCTAACTATCTGAAGAACCAGCCGAT
GTACGTGTTCCGTAAGACGGAGCTCAAGCACTCCAAGACCGAGCTCAACTT
CAAGGAGTGGCAAAAGGCCTTTACCGATGTGATGGGCATGGACGAGCTGTA
CAAGTGATAAGTCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCC
CCCACATCCGCTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAG
GTCCCTATTTATTTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTC
AAATTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTG
AAAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTT
AATTAAGGCGCGCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGC
AGAATCTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MGVADLIKKFESISKEERIPGLINMVSKGEEDNMASLPATHELHIFGSINGVDFD
MVGQGTGNPNDGYEELNLKSTKGDLQFSPWILVPHIGYGFHQYLPYPDGMSP
FQAAMVDGSGYQVHRTMQFEDGASLTVNYRYTYEGSHIKGEAQVKGTGFPAD
GPVMTNSLTAADWCRSKKTYPNDKTIISTFKWSYTTGNGKRYRSTARTTYTFA
KPMAANYLKNQPMYVFRKTELKHSKTELNFKEWQKAFTDVMGMDELYK

Plasmid	pGAL1 SYNZIP17-EYFP		
Freezer Box	CO Plasmids	#	25
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-1RubyQQTmv7HBadKgQEp		

Description
pGAL1 driven expression. SYNZIP17-EYFP in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
SYNZIP17
Linker
EYFP
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAACGAAAAG
GAAGAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGA
AACAAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAAT
TGAAGCTTACAAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGTAAAG
GAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGA
TGTTAATGGGCACAAATTTTCTGTCAGTGGAGACGGTGAAGGTGATGCAAC
ATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTC
CATGGCCAACACTTGTCACTACTTTCGGTTATGGTCTAAAATGCTTTGCTAG
ATACCCAGATCATATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAA
GGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGA
CACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTT
AAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAAT
ACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGG

AATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAA
CTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTT
TACCAGACAACCATTACCTGTCCTATCAATCTAAGCTTTCGAAAGATCCCAA
CGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGAT
TACACATGGCATGGATGAACTATACAAATGATAAGTCATGTAATTAGTTATGT
CACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAAAGGAAG
GAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTTATGTTA
GTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACAGACGC
GTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTTGGGAC
GCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTGTTTAGCT
GCCTCTCCCCGCCGGTCACCCGCAGAATCTTAATTAAGGCGCGCCAGATCT
GTTTAGCTTGCCTCGTC

Expression Product Sequence
MNEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYKGGSGSGLQMVS
KGEELFTGVVPILVELDGDVNGHKFSVSGDGEGDATYGKLTLKFICTTGKLPVP
WPTLVTTFGYGLKCFARYPDHMKRHDFFKSAMPEGYVQERTIFFKDDGNYKTR
AEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVN
FKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSYQSKLSKDPNEKRDH
MVLLEFVTAAGITHGMDELYK

Plasmid	pGAL1 SYNZIP17-mKO		
Freezer Box	CO Plasmids	#	26
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-8qlWOnGNcHHq09c5wY17		

Description
pGAL1 driven expression. SYNZIP17-mKO in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
SYNZIP17
Linker
mKO
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAACGAAAAG
GAAGAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGA
AACAAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAAT
TGAAGCTTACAAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGTGTGA
TTAAACCAGAGATGAAGATGAGGTACTACATGGACGGCTCCGTCAATGGGC
ATGAGTTCACAATTGAAGGTGAAGGCACAGGCAGACCTTACGAGGGACATC
AAGAGATGACACTACGCGTCACAATGGCCAAGGGCGGGCCAATGCCTTTC
GCGTTTGACTTAGTGTCACACGTGTTCTGTTACGGCCACAGACCTTTTACTA
AATATCCAGAAGAGATACCAGACTATTTCAAACAAGCATTTCCTGAAGGCCT
GTCATGGGAAAGGTCGTTGGAGTTCGAAGATGGTGGGTCCGCTTCAGTCAG
TGCGCATATAAGCCTTAGAGGAAACACCTTCTACCACAAATCCAAATTTACT
GGGGTTAACTTTCCTGCCGATGGTCCTATCATGCAAAACCAAAGTGTTGATT
GGGAGCCATCAACCGAGAAAATTACTGCCAGCGACGGAGTTCTGAAGGGT

GATGTTACGATGTACCTAAAACTTGAAGGAGGCGGCAATCACAAATGCCAAT
TCAAGACTACTTACAAGGCGGCAAAAAAGATTCTTAAAATGCCAGGAAGCCA
TTACATCAGCCATCGCCTCGTCAGGAAAACCGAAGGCAACATTACTGAGCT
GGTAGAAGATGCAGTAGCTCATTCCTGATAAGTCATGTAATTAGTTATGTCA
CGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAAAGGAAGGA
GTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTTATGTTAGT
ATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACAGACGCGT
GTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTTGGGACGC
TCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTGC
CTCTCCCCGCCGGTCACCCGCAGAATCTTAATTAAGGCGCGCCAGATCTGT
TTAGCTTGCCTCGTC

Expression Product Sequence
MNEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYKGGSGSGLQMVS
VIKPEMKMRYYMDGSVNGHEFTIEGEGTGRPYEGHQEMTLRVTMAKGGPMPF
AFDLVSHVFCYGHRPFTKYPEEIPDYFKQAFPEGLSWERSLEFEDGGSASVSA
HISLRGNTFYHKSKFTGVNFPADGPIMQNQSVDWEPSTEKITASDGVLKGDVT
MYLKLEGGGNHKCQFKTTYKAAKKILKMPGSHYISHRLVRKTEGNITELVEDAV
AHS

Plasmid	pGAL1 SYNZIP17-mOrange		
Freezer Box	CO Plasmids	#	27
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-0f3wc6Mvbd1nhvOX2Luk		

Description
pGAL1 driven expression. SYNZIP17-mOrange in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
SYNZIP17
Linker
mOrange
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAACGAAAAG
GAAGAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGA
AACAAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAAT
TGAAGCTTACAAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAGG
GCGAGGAGAATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGC
GCATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGG
CGAGGGCCGCCCCTACGAGGGCTTTCAGACCGCTAAGCTGAAGGTGACCA
AGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCACCT
ACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTCA
AGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAG
GACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCG
AGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCC
CCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATG

TACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCT
GAAGGACGGCGGCCACTACACCTCCGAGGTCAAGACCACCTACAAGGCCA
AGAAGCCCGTGCAGCTGCCCGGCGCCTACATCGTCGGCATCAAGTTGGAC
ATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCC
GAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTGATAAGT
CATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCT
CTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATT
TTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTT
TTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTT
GAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGC
GCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAAT
TAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MNEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYKGGSGSGLQMVS
KGEENNMAIIKEFMRFKVRMEGSVNGHEFEIEGEGEGRPYEGFQTAKLKVTKG
GPLPFAWDILSPQFTYGSKAYVKHPADIPDYFKLSFPEGFKWERVMNFEDGGV
VTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGA
LKGEIKMRLKLKDGGHYTSEVKTTYKAKKPVQLPGAYIVGIKLDITSHNEDYTIVE
QYERAEGRHSTGGMDELYK

Plasmid	pGAL1 SYNZIP17-mCherry		
Freezer Box	CO Plasmids	#	28
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-BVpO2ML3irlpuquPNFfv		

Description
pGAL1 driven expression. SYNZIP17-mCherry in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
SYNZIP17
Linker
mCherry
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAACGAAAAG
GAAGAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGA
AACAAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAAT
TGAAGCTTACAAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAGG
GCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGC
ACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGG
CGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACC
AAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATG
TACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTG
AAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGA
GGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGC
GAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGC
CCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGAT

GTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAG
CTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGC
CAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGG
ACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCG
CCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTGATAA
GTCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCG
CTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTA
TTTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTT
TTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCT
TGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCG
CGCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAA
TTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MNEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYKGGSGSGLQMVS
KGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKG
GPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGV
VTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGA
LKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIV
EQYERAEGRHSTGGMDELYK

Plasmid	pGAL1 101A-mNeonGreen		
Freezer Box	CO Plasmids	#	29
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-44iVh8fAL0PtH14VmlBL		

Description
pGAL1 driven expression. 101A-mNeonGreen in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
101A
Linker
mNeonGreen
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAATAGAGTA
AAGGACTTGAAGAACAGAGTTAAAGATTTGAAGAAAGAATTGGCTAGGTTGG
AAAAGGAAAATGAATCTTTGAAGAGAGAAATAGCTTATATGGAAAAGGAATT
AGCTAGATTAGAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAG
GGCGAGGAGGATAACATGGCCTCTCTCCCAGCGACACATGAGTTACACATC
TTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGCACCGGC
AATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGAC
CTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCATC
AGTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCGCCATGGTAG
ATGGCTCCGGCTACCAAGTCCATCGCACAATGCAGTTTGAAGATGGTGCCT
CCCTTACTGTTAACTACCGCTACACCTACGAGGGAAGCCACATCAAAGGAG
AGGCCCAGGTGAAGGGGACTGGTTTCCCTGCTGACGGTCCTGTGATGACC
AACTCGCTGACCGCTGCGGACTGGTGCAGGTCGAAGAAGACTTACCCCAA

CGACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGGAAATGGC
AAGCGCTACCGGAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAAT
GGCGGCTAACTATCTGAAGAACCAGCCGATGTACGTGTTCCGTAAGACGGA
GCTCAAGCACTCCAAGACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTT
TACCGATGTGATGGGCATGGACGAGCTGTACAAGTGATAAGTCATGTAATTA
GTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAA
AGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTT
ATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACA
GACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTT
GGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTGT
TTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAATTAAGGCGCGCC
AGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MNRVKDLKNRVKDLKKELARLEKENESLKREIAYMEKELARLEGGSGSGLQMV
SKGEEDNMASLPATHELHIFGSINGVDFDMVGQGTGNPNDGYEELNLKSTKGD
LQFSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVHRTMQFEDGAS
LTVNYRYTYEGSHIKGEAQVKGTGFPADGPVMTNSLTAADWCRSKKTYPNDKT
IISTFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYVFRKTELKHSKT
ELNFKEWQKAFTDVMGMDELYK

Plasmid	pGAL1 102A-mNeonGreen		
Freezer Box	CO Plasmids	#	30
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-N26lnTD9oysF1KPtvwlz		

Description
pGAL1 driven expression. 102A-mNeonGreen in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
102A
Linker
mNeonGreen
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAATAGGTTA
GCTGCCTTGAGAAATGAAAACAACATTTTGAAGAATGAGAATAATATTTTGAA
GTACGAGTTGGCAGCAATTGAGAACAGATTAGCTGCATTGAGGTACGAGTT
GGCCGCTATTGAAGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAG
GGCGAGGAGGATAACATGGCCTCTCTCCCAGCGACACATGAGTTACACATC
TTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGCACCGGC
AATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGAC
CTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCATC
AGTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCGCCATGGTAG
ATGGCTCCGGCTACCAAGTCCATCGCACAATGCAGTTTGAAGATGGTGCCT
CCCTTACTGTTAACTACCGCTACACCTACGAGGGAAGCCACATCAAAGGAG
AGGCCCAGGTGAAGGGGACTGGTTTCCCTGCTGACGGTCCTGTGATGACC
AACTCGCTGACCGCTGCGGACTGGTGCAGGTCGAAGAAGACTTACCCCAA

CGACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGGAAATGGC
AAGCGCTACCGGAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAAT
GGCGGCTAACTATCTGAAGAACCAGCCGATGTACGTGTTCCGTAAGACGGA
GCTCAAGCACTCCAAGACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTT
TACCGATGTGATGGGCATGGACGAGCTGTACAAGTGATAAGTCATGTAATTA
GTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAA
AGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTT
ATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACA
GACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTT
GGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTGT
TTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAATTAAGGCGCGCC
AGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MNRLAALRNENNILKNENNILKYELAAIENRLAALRYELAAIEGGSGSGLQMVSK
GEEDNMASLPATHELHIFGSINGVDFDMVGQGTGNPNDGYEELNLKSTKGDLQ
FSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVHRTMQFEDGASLT
VNYRYTYEGSHIKGEAQVKGTGFPADGPVMTNSLTAADWCRSKKTYPNDKTII
STFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYVFRKTELKHSKTE
LNFKEWQKAFTDVMGMDELYK

Plasmid	pGAL1 107A-mNeonGreen		
Freezer Box	CO Plasmids	#	31
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-86v5ECb3TFErUB4bgVEM		

Description
pGAL1 driven expression. 107A-mNeonGreen in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
107A
Linker
mNeonGreen
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGGAAAGGATT
AACAGATTGAGAAGAGAAGCAACTGAGTTAGAAGACAGGAACCAGAGATTG
AAAGACAGAAACCAGAGATTAAAAGATAGGAACCAAAGGTTAAAACAAGAAT
TAGCCGCACATAGGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAA
GGGCGAGGAGGATAACATGGCCTCTCTCCCAGCGACACATGAGTTACACAT
CTTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGCACCGG
CAATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGA
CCTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCAT
CAGTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCGCCATGGTA
GATGGCTCCGGCTACCAAGTCCATCGCACAATGCAGTTTGAAGATGGTGCC
TCCCTTACTGTTAACTACCGCTACACCTACGAGGGAAGCCACATCAAAGGA
GAGGCCCAGGTGAAGGGGACTGGTTTCCCTGCTGACGGTCCTGTGATGAC
CAACTCGCTGACCGCTGCGGACTGGTGCAGGTCGAAGAAGACTTACCCCA

ACGACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGGAAATGG
CAAGCGCTACCGGAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAAT
GGCGGCTAACTATCTGAAGAACCAGCCGATGTACGTGTTCCGTAAGACGGA
GCTCAAGCACTCCAAGACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTT
TACCGATGTGATGGGCATGGACGAGCTGTACAAGTGATAAGTCATGTAATTA
GTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAA
AGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTT
ATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACA
GACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTT
GGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTGT
TTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAATTAAGGCGCGCC
AGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MERINRLRREATELEDRNQRLKDRNQRLKDRNQRLKQELAAHRGGSGSGLQM
VSKGEEDNMASLPATHELHIFGSINGVDFDMVGQGTGNPNDGYEELNLKSTKG
DLQFSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVHRTMQFEDGA
SLTVNYRYTYEGSHIKGEAQVKGTGFPADGPVMTNSLTAADWCRSKKTYPND
KTIISTFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYVFRKTELKHS
KTELNFKEWQKAFTDVMGMDELYK

Plasmid	pGAL1 108A-mNeonGreen		
Freezer Box	CO Plasmids	#	32
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-VnsKVOHuzAbeBRozBN1c		

Description
pGAL1 driven expression. 108A-mNeonGreen in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
108A
Linker
mNeonGreen
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAATAGAGTA
GCCCATTTGAAGTCAGAAAACGAACAATTGAAAGAGAGGAATGCCGAATTG
AAAGAAAGAAATGCTGAATTAAAAGCAGAATTGGATTCTTTGGAGAAAGAGA
AAGACAAATTAGAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAG
GGCGAGGAGGATAACATGGCCTCTCTCCCAGCGACACATGAGTTACACATC
TTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGCACCGGC
AATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGAC
CTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCATC
AGTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCGCCATGGTAG
ATGGCTCCGGCTACCAAGTCCATCGCACAATGCAGTTTGAAGATGGTGCCT
CCCTTACTGTTAACTACCGCTACACCTACGAGGGAAGCCACATCAAAGGAG
AGGCCCAGGTGAAGGGGACTGGTTTCCCTGCTGACGGTCCTGTGATGACC
AACTCGCTGACCGCTGCGGACTGGTGCAGGTCGAAGAAGACTTACCCCAA

CGACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGGAAATGGC
AAGCGCTACCGGAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAAT
GGCGGCTAACTATCTGAAGAACCAGCCGATGTACGTGTTCCGTAAGACGGA
GCTCAAGCACTCCAAGACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTT
TACCGATGTGATGGGCATGGACGAGCTGTACAAGTGATAAGTCATGTAATTA
GTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAA
AGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTT
ATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACA
GACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTT
GGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTGT
TTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAATTAAGGCGCGCC
AGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MNRVAHLKSENEQLKERNAELKERNAELKAELDSLEKEKDKLEGGSGSGLQM
VSKGEEDNMASLPATHELHIFGSINGVDFDMVGQGTGNPNDGYEELNLKSTKG
DLQFSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVHRTMQFEDGA
SLTVNYRYTYEGSHIKGEAQVKGTGFPADGPVMTNSLTAADWCRSKKTYPND
KTIISTFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYVFRKTELKHS
KTELNFKEWQKAFTDVMGMDELYK

Plasmid	GS-101B		
Freezer Box	CO Plasmids	#	33
Parent Vector	pFA6a-KanMX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	G418
Full Sequence	https://benchling.com/s/seq-7RHn97DOeBHP2hkNGq05		

Description
For endogenous tagging of a protein of interest with GS-101B.

Shading Key
Linker
101B
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGGTG
GATCAGGCTCTGGTTTGCAAAGAGAAATAGCTTACATGGAGAAAGAATTGG
CCAGATTAGAAAACAGAGTTAAGGATTTGAAGGAGGAAAATGCTACATTGAA
AGATAAGGTTAAGACATTGAAGAATAGGGTAAAGGACTTAAAGTGATGAGTC
ATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTC
TAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTT
TTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTT
TTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTG
AGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCG
CCAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGAC
ATGGAGGCCCAGAATACCCTCCTTGACAGTCTTG

Expression Product Sequence
GGSGSGLQREIAYMEKELARLENRVKDLKEENATLKDKVKTLKNRVKDLK

Plasmid	GS-102B		
Freezer Box	CO Plasmids	#	34
Parent Vector	pFA6a-KanMX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	G418
Full Sequence	https://benchling.com/s/seq-hojVUiOqSOVA90xqnZF0		

Description
For endogenous tagging of a protein of interest with GS-102B.

Shading Key
Linker
102B
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGGTG
GATCAGGCTCTGGTTTGCAATATGAATTAGCCGCAATTGAAAATGAAAACAA
CATATTGAAGAATGAGAATAATATATTAAAGAATAGATTGGCAGCATTGAGAT
ATGAATTGGCAGCAATCGAAAATAGATTGGCTGCTTTGAGATGATGAGTCAT
GTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTA
ACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTT
TTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTT
TCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAG
AAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCC
AGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATG
GAGGCCCAGAATACCCTCCTTGACAGTCTTG

Expression Product Sequence
GGSGSGLQYELAAIENENNILKNENNILKNRLAALRYELAAIENRLAALR

Plasmid	GS-107B		
Freezer Box	CO Plasmids	#	35
Parent Vector	pFA6a-KanMX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	G418
Full Sequence	https://benchling.com/s/seq-2p75CzKgqNyJFtbJtSzi		

Description
For endogenous tagging of a protein of interest with GS-107B.

Shading Key
Linker
107B
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGGTG
GATCAGGCTCTGGTTTGCAAAGAGAGGCAACTGAATTGGAAGAAAGGATCA
ACAGGTTGAGGAACGACAACGCCACATTGGAAAATGATAATGCAACCTTGG
AAAACGACAATGCTACCTTGGAACAGGAATTAGCAGCACATAGGTGATGAG
TCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGC
TCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTAT
TTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTT
TTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTT
GAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGC
GCCAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGA
CATGGAGGCCCAGAATACCCTCCTTGACAGTCTTG

Expression Product Sequence
GGSGSGLQREATELEERINRLRNDNATLENDNATLENDNATLEQELAAHR

Plasmid	GS-108B		
Freezer Box	CO Plasmids	#	36
Parent Vector	pFA6a-KanMX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	G418
Full Sequence	https://benchling.com/s/seq-XuGrep5o1E7LCjvA51Qx		

Description
For endogenous tagging of a protein of interest with GS-108B.

Shading Key
Linker
108B
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGGTG
GATCAGGCTCTGGTTTGCAAAAGGAAAAGGATAAATTAGAGTCAGAAAACG
AACAATTAAAGAAAGACAATGCAAATTTAGAAAAGGATAACGCCAACTTAGA
AAATAGAGTCGCACATTTGAAGAATAGAGTTGCACATTTAAAATGATGAGTC
ATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTC
TAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTT
TTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTT
TTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTG
AGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCG
CCAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGAC
ATGGAGGCCCAGAATACCCTCCTTGACAGTCTTG

Expression Product Sequence
GGSGSGLQKEKDKLESENEQLKKDNANLEKDNANLENRVAHLKNRVAHLK

Plasmid	pGAL1 TRAP4-SNAP-tag		
Freezer Box	CO Plasmids	#	37
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-rXBiGD3qAUlKMuXeMfxE		

Description
pGAL1 driven expression. TRAP4-SNAP-tag in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
TRAP4
Linker
SNAP-tag
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAAGCAGGCA
CTGAAAGAAAAAGAGCTGGGGAACGATGCCTACAAGAAGAAAGACTTTGAC
ACAGCCTTGAAGCATTACGACAAAGCCAAGGAGCTGGACCCCACTAACATG
ACTTACATTATCAATCAAGCAGCGGTATACTTTGAAAAGGGCGACTACAATA
AGTGCCGGGAGCTTTGTGAGAAGGCCATTGAAGTGGGGAGAGAAAACCGA
GAGGACTATCGATGGATTGCCATTGCATATGCTCGAATTGGCAACTCCTACT
TCAAAGAAGAAAAGTACAAGGATGCCATCCATTTCTATAACAAGTCTCTGGC
AGAGCACCGAACCCCAAAGGTGCTAAAAAAGTGCCAACAGGCGGAGAAAAT
CCTGAAGGAGCAAGGCGGCAGCGGCAGCGGCCTGCAGATGGACAAAGACT
GCGAAATGAAGCGCACCACCCTGGATAGCCCTCTGGGCAAGCTGGAACTG
TCTGGGTGCGAACAGGGCCTGCACCGTATCATCTTCCTGGGCAAAGGAACA
TCTGCCGCCGACGCCGTGGAAGTGCCTGCCCCAGCCGCCGTGCTGGGCG
GACCAGAGCCACTGATGCAGGCCACCGCCTGGCTCAACGCCTACTTTCACC

AGCCTGAGGCCATCGAGGAGTTCCCTGTGCCAGCCCTGCACCACCCAGTG
TTCCAGCAGGAGAGCTTTACCCGCCAGGTGCTGTGGAAACTGCTGAAAGTG
GTGAAGTTCGGAGAGGTCATCAGCTACAGCCACCTGGCCGCCCTGGCCGG
CAATCCCGCCGCCACCGCCGCCGTGAAAACCGCCCTGAGCGGAAATCCCG
TGCCCATTCTGATCCCCTGCCACCGGGTCGTTCAGGGCGACCTGGACGTG
GGGGGCTACGAGGGCGGGCTCGCCGTGAAAGAGTGGCTGCTGGCCCACG
AGGGCCACAGACTGGGCAAGCCTGGGCTGGGTTGATGATAAGTCATGTAAT
TAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGA
AAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAG
TTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTA
CAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTT
TTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCT
GTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAATTAAGGCGCG
CCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MKQALKEKELGNDAYKKKDFDTALKHYDKAKELDPTNMTYIINQAAVYFEKGDY
NKCRELCEKAIEVGRENREDYRWIAIAYARIGNSYFKEEKYKDAIHFYNKSLAEH
RTPKVLKKCQQAEKILKEQGGSGSGLQMDKDCEMKRTTLDSPLGKLELSGCE
QGLHRIIFLGKGTSAADAVEVPAPAAVLGGPEPLMQATAWLNAYFHQPEAIEEF
PVPALHHPVFQQESFTRQVLWKLLKVVKFGEVISYSHLAALAGNPAATAAVKTA
LSGNPVPILIPCHRVVQGDLDVGGYEGGLAVKEWLLAHEGHRLGKPGLG

Plasmid	pGAL1 SYNZIP17-SNAP-tag		
Freezer Box	CO Plasmids	#	38
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-za4EHciKGFZcDQeYezyc		

Description
pGAL1 driven expression. SYNZIP17-SNAP-tag in yeast integration vector with URA3 marker.

Shading Key
pGAL1
SYNZIP17
Linker
SNAP-tag
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAACGAAAAG
GAAGAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGA
AACAAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAAT
TGAAGCTTACAAGAGTGGTAGTGGAAGTGGCCTGCAGATGGACAAAGACTG
CGAAATGAAGCGCACCACCCTGGATAGCCCTCTGGGCAAGCTGGAACTGT
CTGGGTGCGAACAGGGCCTGCACCGTATCATCTTCCTGGGCAAAGGAACAT
CTGCCGCCGACGCCGTGGAAGTGCCTGCCCCAGCCGCCGTGCTGGGCGG
ACCAGAGCCACTGATGCAGGCCACCGCCTGGCTCAACGCCTACTTTCACCA
GCCTGAGGCCATCGAGGAGTTCCCTGTGCCAGCCCTGCACCACCCAGTGT
TCCAGCAGGAGAGCTTTACCCGCCAGGTGCTGTGGAAACTGCTGAAAGTG
GTGAAGTTCGGAGAGGTCATCAGCTACAGCCACCTGGCCGCCCTGGCCGG
CAATCCCGCCGCCACCGCCGCCGTGAAAACCGCCCTGAGCGGAAATCCCG
TGCCCATTCTGATCCCCTGCCACCGGGTCGTTCAGGGCGACCTGGACGTG

GGGGGCTACGAGGGCGGGCTCGCCGTGAAAGAGTGGCTGCTGGCCCACG
AGGGCCACAGACTGGGCAAGCCTGGGCTGGGTTGATGATAAGTCATGTAAT
TAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGA
AAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAG
TTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTA
CAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTT
TTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCT
GTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAATTAAGGCGCG
CCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MNEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYKSGSGSGLQMDK
DCEMKRTTLDSPLGKLELSGCEQGLHRIIFLGKGTSAADAVEVPAPAAVLGGPE
PLMQATAWLNAYFHQPEAIEEFPVPALHHPVFQQESFTRQVLWKLLKVVKFGE
VISYSHLAALAGNPAATAAVKTALSGNPVPILIPCHRVVQGDLDVGGYEGGLAV
KEWLLAHEGHRLGKPGLG

Plasmid	pGAL1 SYNZIP17-mCherry (Ura3)		
Freezer Box	CO Plasmids	#	39
Parent Vector	pFA6a-Ura3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	Ura3
Full Sequence	https://benchling.com/s/seq-uNT8eWjLy2rcpfTex8Vh		

Description
pGAL1 driven expression. SYNZIP17-mCherry in yeast integration vector with URA3 marker.

Shading Key
pGAL1
SYNZIP17
Linker
mCherry
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAACGAAAAG
GAAGAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGA
AACAAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAAT
TGAAGCTTACAAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGAGCAAGG
GCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGC
ACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGG
CGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACC
AAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATG
TACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTG
AAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGA
GGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGC
GAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGC
CCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGAT

GTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAG
CTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGC
CAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGG
ACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCG
CCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTGATAA
GTCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCG
CTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTA
TTTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTT
TTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCT
TGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCG
CGCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAA
TTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MNEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYKGGSGSGLQMVS
KGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKG
GPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGV
VTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGA
LKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIV
EQYERAEGRHSTGGMDELYK

Plasmid	GS-108B (Leu2)		
Freezer Box	CO Plasmids	#	40
Parent Vector	pFA6a-Leu2MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	Leu2
Full Sequence	https://benchling.com/s/seq-o5z8uxqEOZUbiygLuWR6		

Description
For tagging Nup proteins (or others) and then will separately express 108A-mCherry separately using plasmid No 41

Shading Key
Linker
108B
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGGTG
GATCAGGCTCTGGTTTGCAAAAGGAAAAGGATAAATTAGAGTCAGAAAACG
AACAATTAAAGAAAGACAATGCAAATTTAGAAAAGGATAACGCCAACTTAGA
AAATAGAGTCGCACATTTGAAGAATAGAGTTGCACATTTAAAATGATGAGTC
ATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTC
TAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTT
TTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTT
TTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTG
AGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCG
CCAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGAC
ATGGAGGCCCAGAATACCCTCCTTGACAGTCTTG

Expression Product Sequence
GGSGSGLQKEKDKLESENEQLKKDNANLEKDNANLENRVAHLKNRVAHLK

Plasmid	pGAL1 108A-mCherry (Ura3)		
Freezer Box	CO Plasmids	#	41
Parent Vector	pFA6a-Ura3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	Ura3
Full Sequence	https://benchling.com/s/seq-8R4Ywd9HUveV50xa9qcn		

Description
For tagging Nup proteins (or others) and then will separately express GS-108B separately using plasmid No 40

Shading Key
pGAL1
108A
Linker
mCherry
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAATAGAGTA
GCCCATTTGAAGTCAGAAAACGAACAATTGAAAGAGAGGAATGCCGAATTG
AAAGAAAGAAATGCTGAATTAAAAGCAGAATTGGATTCTTTGGAGAAAGAGA
AAGACAAATTAGAGGGTGGAAGTGGTAGTGGATTGCAAATGGTGAGCAAGG
GCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGC
ACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGG
CGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACC
AAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATG
TACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTG
AAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGA
GGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGC
GAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGC
CCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGAT

GTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAG
CTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGC
CAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGG
ACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCG
CCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTGATAA
GTCATGTAATTAGTTATGTCACGCTTACATTCACGCCCTCCCCCCACATCCG
CTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTA
TTTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTT
TTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCT
TGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCG
CGCCAGATCTGTTTAGCTGCCTCTCCCCGCCGGTCACCCGCAGAATCTTAA
TTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTC

Expression Product Sequence
MNRVAHLKSENEQLKERNAELKERNAELKAELDSLEKEKDKLEGGSGSGLQM
VSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVT
KGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDG
GVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPED
GALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDY
TIVEQYERAEGRHSTGGMDELYK

Plasmid	GS-mNeonGreen		
Freezer Box	CO Plasmids	#	42
Parent Vector	pFA6a-KanMX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	G418
Full Sequence	https://benchling.com/s/seq-EHmomOrb1DNLgIYp9fhs		

Description
GS-mNeonGreen in yeast integration vector with KanR marker for direct fusion to FP. Meant for as a template for tagging genomic target proteins

Shading Key
Linker
mNeonGreen
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGGTG
GATCAGGCTCTGGTTTGCAAATGGTGAGCAAGGGCGAGGAGGATAACATG
GCCTCTCTCCCAGCGACACATGAGTTACACATCTTTGGCTCCATCAACGGT
GTGGACTTTGACATGGTGGGTCAGGGCACCGGCAATCCAAATGATGGTTAT
GAGGAGTTAAACCTGAAGTCCACCAAGGGTGACCTCCAGTTCTCCCCCTGG
ATTCTGGTCCCTCATATCGGGTATGGCTTCCATCAGTACCTGCCCTACCCTG
ACGGGATGTCGCCTTTCCAGGCCGCCATGGTAGATGGCTCCGGCTACCAA
GTCCATCGCACAATGCAGTTTGAAGATGGTGCCTCCCTTACTGTTAACTACC
GCTACACCTACGAGGGAAGCCACATCAAAGGAGAGGCCCAGGTGAAGGGG
ACTGGTTTCCCTGCTGACGGTCCTGTGATGACCAACTCGCTGACCGCTGCG
GACTGGTGCAGGTCGAAGAAGACTTACCCCAACGACAAAACCATCATCAGT
ACCTTTAAGTGGAGTTACACCACTGGAAATGGCAAGCGCTACCGGAGCACT
GCGCGGACCACCTACACCTTTGCCAAGCCAATGGCGGCTAACTATCTGAAG
AACCAGCCGATGTACGTGTTCCGTAAGACGGAGCTCAAGCACTCCAAGACC
GAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTTACCGATGTGATGGGCATG
GACGAGCTGTACAAGTGATGAGTCATGTAATTAGTTATGTCACGCTTACATT
CACGCCCTCCCCCCACATCCGCTCTAACCGAAAAGGAAGGAGTTAGACAAC
CTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTTATGTTAGTATTAAGAACGT
TATTTATATTTCAAATTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCATGTA
ACATTATACTGAAAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTT
AATTTGCGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCG
CCGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGACAG
TCTTG

Expression Product Sequence
GGSGSGLQMVSKGEEDNMASLPATHELHIFGSINGVDFDMVGQGTGNPNDGY
EELNLKSTKGDLQFSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVH
RTMQFEDGASLTVNYRYTYEGSHIKGEAQVKGTGFPADGPVMTNSLTAADWC
RSKKTYPNDKTIISTFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYV
FRKTELKHSKTELNFKEWQKAFTDVMGMDELYK

Plasmid	GS-mEOS		
Freezer Box	CO Plasmids	#	43
Parent Vector	pFA6a-KanMX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	G418
Full Sequence	https://benchling.com/s/seq-QhXy9YnUtLdwba5qLYRN		

Description
GS-mEOS in yeast integration vector with KanR marker for direct fusion to FP. Meant for as a template for tagging genomic target proteins

Shading Key
Linker
mEOS
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGGTG
GATCAGGCTCTGGTTTGCAAATGAGTGCGATTAAGCCAGACATGAAGATCA
AACTCCGTATGGAAGGCAACGTAAACGGGCACCACTTTGTGATCGACGGAG
ATGGTACAGGCAAGCCTTTTGAGGGAAAACAGAGTATGGATCTTGAAGTCA
AAGAGGGCGGACCTCTGCCTTTTGCCTTTGATATCCTGACCACTGCATTCCA
TTACGGCAACAGGGTATTCGCCAAATATCCAGACAACATACAAGACTATTTT
AAGCAGTCGTTTCCTAAGGGGTATTCGTGGGAACGAAGCTTGACTTTCGAA
GACGGGGGCATTTGCAACGCCAGAAACGACATAACAATGGAAGGGGACAC
TTTCTATAATAAAGTTCGATTTTATGGTACCAACTTTCCCGCCAATGGTCCAG
TTATGCAGAAGAAGACGCTGAAATGGGAGCCCTCCACTGAGAAAATGTATG
TGCGTGATGGAGTGCTGACGGGTGATATTGAGATGGCTTTGTTGCTTGAAG
GAAATGCCCATTACCGATGTGACTTCAGAACTACTTACAAAGCTAAGGAGAA
GGGTGTCAAGTTACCAGGCGCCCACTTTGTGGACCACTGCATTGAGATTTT
AAGCCATGACAAAGATTACAACAAGGTTAAGCTGTATGAGCATGCTGTTGCT
CATTCTGGATTGCCTGACAATGCCAGACGATGATGAGTCATGTAATTAGTTA
TGTCACGCTTACATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAAAGG
AAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTTATG
TTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACAGAC
GCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTTGGG
ACGCTCGAAGGCTTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTGTTTAG
CTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGAA
TACCCTCCTTGACAGTCTTG

Expression Product Sequence

GGSGSGLQMSAIKPDMKIKLRMEGNVNGHHFVIDGDGTGKPFEGKQSMDLEV
KEGGPLPFAFDILTTAFHYGNRVFAKYPDNIQDYFKQSFPKGYSWERSLTFEDG
GICNARNDITMEGDTFYNKVRFYGTNFPANGPVMQKKTLKWEPSTEKMYVRD
GVLTGDIEMALLLEGNAHYRCDFRTTYKAKEKGVKLPGAHFVDHCIEILSHDKD
YNKVKLYEHAVAHSGLPDNARR

Plasmid	pGAL1 SYNZIP17-GRvT		
Freezer Box	CO Plasmids	#	44
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-ExyAMgbI9mGBpBZUbbwO		

Description
pGAL1 driven expression. SYNZIP17-GRvT in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
SYNZIP17
Linker
GRvT
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAACGAAAAG
GAAGAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGA
AACAAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAAT
TGAAGCTTACAAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTCAGTAAGG
GGGAGGAGGTCATAAAGGAATTCATGCGTTTCAAGGTGAGAATGGAAGGGT
CAATGAATGGACATGAATTCGAAATTGAAGGGGAAGGCGAGGGGAGACCG
TATGAAGGGACTCAAACGGCCAAGTTAAAGGTAACAAAGGGTGGGCCGCTT
CCCTTCGCTTGGGACATTCTTTCTCCACTATTGATGTATGGGTCTAAAATGTA
CGTTAAACACCCGGCGGACATCCCGGACTATAAGAAACTTTCTTTTCCGGAA
GGTTTTAAATGGGTCAGAGTTATGAATTTTGAGGATGGTGGCTTGGTCACAG
CCACACAGGACAGCTCCCTACAGGACGGCACTCTTATCTATGAAGTCAAGA
TGAGGGGGACAAACTTTCCCCCCGACGGACCAGTGATGCAGAAGAAAACAA
TGGGTTGGGAAGCTAGTACGGAGCGTCTTTACCCCAGGGATGGAGTATTAA

AGGGCGAGATTCACCAAGCTCTAAAACTGAAAGACGGTGGGCATTACTTAG
TAGAATTTAAAACGATCTACATGGCTAAGAAGCCAGTTCAACTTCCAGGTTA
CTATTATGTCGACACGAAGCTGGACATAACCAGCCACAACGAGGACTATAC
GAGAGTCGAGCAGTACGAGAGGTCAGAGGGAAGGCATCACCTATTCCTGTA
CGACGGAAGCACCGGAAGTGGCTCATCAGGTACCATGGTATCAAAAGGGG
AAGAAGTGATTAAAGAGTTCATGAGGTTTAAAGTCAGAATGGAAGGGAGTAT
GAACGGACATGAATTCGAGATAGAAGGAGAGGGTGAGGGGAGACCATATG
AGGGTACTCAGACCGCTAAGTTAAAGGTGACAAAAGGTGGCCCACTTCCGT
TCGCCTGGGACATTCTATCTCCGCAGTTTATGTACGGGTCCAAAGCATACGT
TAAGCACCCAGCTGATATCCCGGATTATAAAAAACTAAGCTTTCCTGAGGGT
TTCAAATGGGAAAGGGTGATGAACTTCGAGGATGGTGGATTGGTAACTGTC
ACTCAGGACTCCTCTATTCAGGACGGAACGCTAATTTATAAGGTCAAAATGA
GGGGTACAAACTTTCCGCCTGATGGCCCAGTCATGCAGAAAAAGACAATGG
GATGGGAAGCATCCACTGAGAGGTTATATCCACGTGATGGGGTTTTGAAGG
GCGAGATCCATCAGGCGCTAAAACTAAAGGATGGGGGCCACTACCTGGTG
GAGTTCAAGACTATCTACATGGCCAAAAAGCCAGTCCAACTACCTGGTTATT
ATTATGTAGATACAAAGCTTGATATTACATCACATAACGAGGATTACACCATT
GTCGAACAGTACGAGAGAAGCGAGGGTAGACACCATCTATTTCTGTACGGT
ATGGATGAGTTGTACAAGTGATAAGTCATGTAATTAGTTATGTCACGCTTAC
ATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAAAGGAAGGAGTTAGAC
AACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTTATGTTAGTATTAAGAA
CGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCAT
GTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGC
TTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTGCCTCTCCCC
GCCGGTCACCCGCAGAATCTTAATTAAGGCGCGCCAGATCTGTTTAGCTTG
CCTCGTC

Expression Product Sequence
MNEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYKGGSGSGLQMVS
KGEEVIKEFMRFKVRMEGSMNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLP
FAWDILSPQFMYGSKAYVKHPADIPDYKKLSFPEGFKWERVMNFEDGGLVTVT
QDSSIQDGTLIYKVKMRGTNFPPDGPVMQKKTMGWEASTERLYPRDGVLKGEI
HQALKLKDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNEDYTIVEQYE
RSEGRHHLFLYGMDELYK

Plasmid	pGAL1 SYNZIP17-3xmNeonGreen		
Freezer Box	CO Plasmids	#	45
Parent Vector	pFA6a-His3MX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	His3
Full Sequence	https://benchling.com/s/seq-ecDsSyNFm2CQuTR2paGa		

Description
pGAL1 driven expression. SYNZIP17-3xmNeonGreen in yeast integration vector with HIS3 marker.

Shading Key
pGAL1
SYNZIP17
Linker
mNeonGreen
Stop
CYC1 terminator

DNA
GCCAGCTGAAGCTTCGTACGCTGTTGGCATATACACATACGATTTAGGTGA
CACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTGAGCTCTAGTAC
GGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAGGAAGACTCTCCT
CCGTGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTC
GCGCCGCACTGCTCCGAACAATAAAGATTCTACAATACTAGCTTTTATGGTT
ATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAAC
GAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATT
TCTGGGGTAATTAATCAGCGAAGCAATGATTTTTGATCTATTAACAGATATAT
AAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTT
GTATTACTTCTTATTCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATA
CCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATTCATGAACGAAAAG
GAAGAATTGAAGTCTAAGAAGGCTGAATTGAGAAACAGAATTGAACAATTGA
AACAAAAGAGAGAACAATTGAAGCAAAAGATTGCTAACTTGAGAAAGGAAAT
TGAAGCTTACAAGGGCGGCAGCGGCAGCGGCCTGCAGATGGTGTCCAAAG
GAGAAGAAGATAATATGGCAAGCCTTCCCGCCACTCACGAGTTACACATATT
CGGGTCTATCAATGGGGTGGACTTTGACATGGTAGGGCAGGGTACTGGTAA
CCCAAATGACGGCTACGAAGAATTAAATTTGAAATCAACGAAGGGGGATCT
GCAGTTTTCCCCATGGATACTAGTACCTCACATAGGCTACGGATTCCACCAG
TATCTTCCGTATCCTGATGGAATGAGCCCGTTCCAAGCCGCGATGGTGGAT
GGTAGCGGCTATCAGGTACATCGTACTATGCAATTCGAAGACGGAGCCAGC
CTTACTGTCAACTACAGGTACACATATGAGGGGTCCCACATTAAGGGGGAG
GCACAGGTGAAAGGTACTGGGTTTCCAGCGGATGGACCTGTCATGACCAAC
TCACTAACCGCAGCAGATTGGTGTAGGAGCAAAAAAACATATCCTAACGACA

AAACAATAATATCCACGTTTAAATGGAGTTACACGACTGGAAATGGGAAACG
TTATAGGTCTACGGCCAGAACAACCTATACCTTCGCGAAGCCGATGGCAGC
TAATTATTTAAAGAACCAACCGATGTACGTTTTTAGGAAGACCGAGTTGAAA
CATTCAAAGACAGAGTTAAATTTCAAGGAGTGGCAAAAAGCCTTTACCGATG
TCATGGGGATGGATGAATTATACAAGGGTAGCATGGTAAGCAAAGGGGAAG
AGGACAACATGGCAAGTTTGCCGGCGACCCATGAGCTTCATATATTCGGAT
CCATCAATGGAGTGGATTTTGATATGGTTGGTCAAGGAACCGGCAACCCAA
ATGATGGGTATGAAGAACTTAATCTTAAGTCTACTAAGGGCGACCTTCAATT
CTCACCCTGGATCCTAGTTCCTCATATAGGGTACGGCTTCCACCAATACCTA
CCGTACCCGGATGGCATGTCACCTTTTCAAGCAGCTATGGTAGACGGTTCT
GGTTACCAAGTCCACAGGACTATGCAGTTCGAAGATGGCGCATCTTTAACT
GTAAACTATAGATACACTTATGAAGGTAGTCATATTAAGGGAGAGGCACAGG
TAAAAGGAACTGGTTTTCCGGCGGACGGCCCGGTTATGACTAATAGCTTAA
CGGCTGCGGACTGGTGCAGAAGCAAGAAGACGTATCCGAATGACAAGACC
ATCATTTCTACTTTCAAATGGTCATACACAACAGGTAATGGCAAAAGGTATA
GGAGTACCGCTAGAACTACTTATACTTTTGCGAAACCAATGGCTGCCAATTA
TCTGAAGAATCAACCCATGTACGTATTTCGTAAGACCGAACTTAAGCATAGC
AAGACGGAGCTAAACTTCAAGGAATGGCAAAAGGCGTTTACTGACGTTATG
GGTATGGATGAATTGTATAAAGGCAGTATGGTCAGTAAAGGTGAGGAGGAT
AATATGGCCAGCCTGCCGGCCACGCACGAGTTGCACATTTTCGGTTCCATT
AACGGAGTCGACTTCGATATGGTTGGACAGGGAACCGGGAATCCAAATGAC
GGGTACGAAGAATTAAACCTAAAAAGTACAAAAGGCGATCTACAATTTTCTC
CCTGGATACTAGTACCACATATCGGCTATGGTTTTCACCAGTACCTTCCTTA
CCCTGATGGCATGTCCCCCTTTCAAGCTGCTATGGTGGATGGTTCCGGGTA
CCAAGTGCATAGAACCATGCAGTTCGAGGATGGAGCTAGTCTAACTGTTAA
CTACAGATATACATACGAGGGATCCCATATTAAAGGCGAGGCTCAGGTGAA
AGGGACGGGGTTCCCGGCGGATGGCCCGGTCATGACTAATTCCTTAACGG
CGGCAGATTGGTGTAGGTCAAAAAAGACATACCCAAACGATAAAACGATTAT
AAGTACATTTAAGTGGTCTTATACCACCGGTAATGGAAAGCGTTATCGTAGC
ACGGCTAGGACTACATACACGTTCGCCAAGCCGATGGCGGCTAATTACCTT
AAGAACCAGCCGATGTATGTGTTCCGTAAGACCGAATTAAAACATTCTAAAA
CAGAGCTAAATTTTAAGGAGTGGCAAAAAGCATTCACGGATGTCATGGGAAT
GGATGAACTGTATAAGTGATAAGTCATGTAATTAGTTATGTCACGCTTACATT
CACGCCCTCCCCCCCACATCCGCTCTAACCGAAAAGGAAGGAGTTAGACAAC
CTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTTATGTTAGTATTAAGAACGT
TATTTATATTTCAAATTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCATGTA
ACATTATACTGAAAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTT
AATTTGCGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTGCCTCTCCCCGC
CGGTCACCCGCAGAATCTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCC
TCGTC

Expression Product Sequence
MNEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYKGGSGSGLQMVS
KGEEDNMASLPATHELHIFGSINGVDFDMVGQGTGNPNDGYEELNLKSTKGDL

QFSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVHRTMQFEDGASL
TVNYRYTYEGSHIKGEAQVKGTGFPADGPVMTNSLTAADWCRSKKTYPNDKTII
STFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYVFRKTELKHSKTE
LNFKEWQKAFTDVMGMDELYKGSMVSKGEEDNMASLPATHELHIFGSINGVDF
DMVGQGTGNPNDGYEELNLKSTKGDLQFSPWILVPHIGYGFHQYLPYPDGMS
PFQAAMVDGSGYQVHRTMQFEDGASLTVNYRYTYEGSHIKGEAQVKGTGFPA
DGPVMTNSLTAADWCRSKKTYPNDKTIISTFKWSYTTGNGKRYRSTARTTYTF
AKPMAANYLKNQPMYVFRKTELKHSKTELNFKEWQKAFTDVMGMDELYKGSM
VSKGEEDNMASLPATHELHIFGSINGVDFDMVGQGTGNPNDGYEELNLKSTKG
DLQFSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVHRTMQFEDGA
SLTVNYRYTYEGSHIKGEAQVKGTGFPADGPVMTNSLTAADWCRSKKTYPND
KTIISTFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYVFRKTELKHS
KTELNFKEWQKAFTDVMGMDELYK

Plasmid	pCUP1 PIL1-GS-101B		
Freezer Box	CO Plasmids	#	46
Parent Vector	pFA6a-KanMX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	G418
Full Sequence	https://benchling.com/s/seq-P1h7DbfTww5w4XPVeV9z		

Description
pCUP1 driven expression. PIL1-GS-101B in yeast integration vector with KANMX6 marker.

Shading Key
pCUP1
PIL1
Linker
101B
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTCTAG
TTAGAAAGACATTTTTGCTGTCAGTCACTGTCAAGAGATTCTTTTGCTGGCAT
TTCTTCTAGAAGCAAAAAGAGCGATGCGTCTTTTCCGCTGAACCGTTCCAGC
AAAAAAGACTACCAACGCAATATGGATTGTCAGAATCATATAAAAGAGAAGC
AAATAACTCCTTGTCTTGTATCAATTGCATTATAATATCTTCTTGTTAGTGCAA
TATCATATAGAAGTCATCGACTAGTATGCATCGTACCTATTCCCTGCGTAACT
CACGTGCTCCCACAGCATCCCAGCTGCAAAATCCTCCTCCTCCTCCTTCCA
CTACAAAGGGCCGTTTTTTCGGTAAAGGCGGCCTGGCCTATAGTTTCCGTC
GTAGCGCCGCCGGGGCCTTCGGACCTGAGCTGTCACGTAAACTTTCCCAG
CTTGTCAAAATCGAGAAAAACGTCCTTCGTTCAATGGAGCTTACTGCAAATG
AGCGTAGAGATGCCGCCAAACAGCTTTCCATCTGGGGTCTTGAGAATGACG
ATGACGTCTCAGATATAACCGACAAGCTGGGCGTACTTATATACGAGGTCA
GCGAGCTGGATGATCAGTTCATAGACAGATACGATCAGTATCGTCTTACCCT
GAAATCAATACGTGACATAGAGGGCTCCGTCCAGCCTTCCCGTGATAGAAA
AGATAAAATAACAGATAAGATAGCATATCTTAAGTATAAGGACCCCCAGTCG
CCCAAAATCGAAGTACTTGAGCAGGAGCTTGTTAGAGCCGAAGCCGAGTCC
CTTGTAGCCGAGGCCCAGCTGTCCAACATAACTCGTTCGAAACTTCGTGCC
GCCTTTAATTATCAGTTCGATTCAATAATAGAGCACTCGGAAAAGATCGCCC
TGATAGCCGGCTATGGCAAAGCCCTACTTGAGCTGCTTGATGATTCCCCCG
TAACCCCTGGCGAGACACGTCCCGCCTATGACGGTTACGAGGCATCCAAG
CAGATAATCATCGACGCCGAGAGTGCTTTAAACGAGTGGACTCTGGATTCC
GCACAGGTAAAACCCACCCTGAGCTTTAAACAAGACTATGAGGATTTTGAGC

CCGAGGAGGGAGAGGAGGAAGAGGAAGAGGATGGCCAGGGACGTTGGTC
AGAGGATGAGCAGGAGGACGGGCAGATCGAGGAGCCCGAGCAGGAGGAG
GAGGGCGCCGTCGAGGAGCACGAGCAGGTAGGGCATCAACAGAGCGAAT
CCTTGCCACAGCAGACTACTGCCGGTGGATCAGGCTCTGGTTTGCAAAGAG
AAATAGCTTACATGGAGAAAGAATTGGCCAGATTAGAAAACAGAGTTAAGGA
TTTGAAGGAGGAAAATGCTACATTGAAAGATAAGGTTAAGACATTGAAGAAT
AGGGTAAAGGACTTAAAGTGATGAGTCATGTAATTAGTTATGTCACGCTTAC
ATTCACGCCCTCCCCCCACATCCGCTCTAACCGAAAAGGAAGGAGTTAGAC
AACCTGAAGTCTAGGTCCCTATTTATTTTTTTATAGTTATGTTAGTATTAAGAA
CGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCAT
GTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGC
TTTAATTTGCGGTTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCC
CCGCCGGGTCACCCGGCCAGCGACATGGAGGCCCAGAATACCCTCCTTGA
CAGTCTTG

Expression Product Sequence
MHRTYSLRNSRAPTASQLQNPPPPPSTTKGRFFGKGGLAYSFRRSAAGAFGP
ELSRKLSQLVKIEKNVLRSMELTANERRDAAKQLSIWGLENDDDVSDITDKLGV
LIYEVSELDDQFIDRYDQYRLTLKSIRDIEGSVQPSRDRKDKITDKIAYLKYKDPQ
SPKIEVLEQELVRAEAESLVAEAQLSNITRSKLRAAFNYQFDSIIEHSEKIALIAGY
GKALLELLDDSPVTPGETRPAYDGYEASKQIIIDAESALNEWTLDSAQVKPTLSF
KQDYEDFEPEEGEEEEEEDGQGRWSEDEQEDGQIEEPEQEEEGAVEEHEQV
GHQQSESLPQQTTAGGSGSGLQREIAYMEKELARLENRVKDLKEENATLKDKV
KTLKNRVKDLK

Plasmid	pCUP1 PIL1-GS-mEOS		
Freezer Box	CO Plasmids	#	47
Parent Vector	pFA6a-KanMX6	Source	Curran Oi
Bacterial Selection	Amp	Yeast Selection	G418
Full Sequence	https://benchling.com/s/seq-1Qz6Bkc9qYL4Pi5gYt1H		

Description

Shading Key
pCUP1
PIL1
Linker
mEOS
Stop
CYC1 terminator

DNA
GTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATACGAT
TTAGGTGACACTATAGAACGCGGCCGCCAGCTGAAGCTTCGTACGCTCTAG
TTAGAAAGACATTTTTGCTGTCAGTCACTGTCAAGAGATTCTTTTGCTGGCAT
TTCTTCTAGAAGCAAAAAGAGCGATGCGTCTTTTCCGCTGAACCGTTCCAGC
AAAAAAGACTACCAACGCAATATGGATTGTCAGAATCATATAAAAGAGAAGC
AAATAACTCCTTGTCTTGTATCAATTGCATTATAATATCTTCTTGTTAGTGCAA
TATCATATAGAAGTCATCGACTAGTATGCATCGTACCTATTCCCTGCGTAACT
CACGTGCTCCCACAGCATCCCAGCTGCAAAATCCTCCTCCTCCTCCTTCCA
CTACAAAGGGCCGTTTTTTCGGTAAAGGCGGCCTGGCCTATAGTTTCCGTC
GTAGCGCCGCCGGGGCCTTCGGACCTGAGCTGTCACGTAAACTTTCCCAG
CTTGTCAAAATCGAGAAAAACGTCCTTCGTTCAATGGAGCTTACTGCAAATG
AGCGTAGAGATGCCGCCAAACAGCTTTCCATCTGGGGTCTTGAGAATGACG
ATGACGTCTCAGATATAACCGACAAGCTGGGCGTACTTATATACGAGGTCA
GCGAGCTGGATGATCAGTTCATAGACAGATACGATCAGTATCGTCTTACCCT
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CCGAGGAGGGAGAGGAGGAAGAGGAAGAGGATGGCCAGGGACGTTGGTC
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GAGGGCGCCGTCGAGGAGCACGAGCAGGTAGGGCATCAACAGAGCGAAT
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TTCAAATTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATAC
TGAAAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGG
TTAATTAAGGCGCGCCAGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCA
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Expression Product Sequence
MHRTYSLRNSRAPTASQLQNPPPPPSTTKGRFFGKGGLAYSFRRSAAGAFGP
ELSRKLSQLVKIEKNVLRSMELTANERRDAAKQLSIWGLENDDDVSDITDKLGV
LIYEVSELDDQFIDRYDQYRLTLKSIRDIEGSVQPSRDRKDKITDKIAYLKYKDPQ
SPKIEVLEQELVRAEAESLVAEAQLSNITRSKLRAAFNYQFDSIIEHSEKIALIAGY
GKALLELLDDSPVTPGETRPAYDGYEASKQIIIDAESALNEWTLDSAQVKPTLSF
KQDYEDFEPEEGEEEEEEDGQGRWSEDEQEDGQIEEPEQEEEGAVEEHEQV
GHQQSESLPQQTTAGGSGSGLQMSAIKPDMKIKLRMEGNVNGHHFVIDGDGT
GKPFEGKQSMDLEVKEGGPLPFAFDILTTAFHYGNRVFAKYPDNIQDYFKQSFP
KGYSWERSLTFEDGGICNARNDITMEGDTFYNKVRFYGTNFPANGPVMQKKTL
KWEPSTEKMYVRDGVLTGDIEMALLLEGNAHYRCDFRTTYKAKEKGVKLPGAH
FVDHCIEILSHDKDYNKVKLYEHAVAHSGLPDNARR

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