

# Yeast library creation using the SWAT method

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## Introduction

The SWAp-Tag (SWAT) method removes the major hurdles of yeast strain collection (library) building – cost, time and labor, making creativity the only barrier to designing and building new libraries. The methodology is based on a template (*acceptor*) library, SWAT-GFP, which can be “swapped” into any other library of choice (See Appendix, Figure 1). Using the “template library” of strains, in which an *acceptor* module with a GFP was integrated into a different gene in each strain (at the N' terminus), one can easily create any other yeast collection with their desired genomic modification or tag inserted into each ORF at the N'. The method relies on the Synthetic Genetic Array (SGA) procedures to cross the SWAT library with a *donor* strain. The *donor* strain harbors the desired module to be inserted on a *donor* plasmid, and an inducible I-SceI restriction enzyme that facilitates high efficiency of swapping (See Appendix, Figure 2). The SWAT methodology is easy and fast, highly efficient and accurate. It enables any lab to create their own “tailor made” strain collection in a matter of a few weeks.

## Materials

To create a new yeast library one must have in hand the three basic features of the method:

Feature needed	Material provided	Actions needed
Donor module plasmid	Template plasmids	Cloning of desired modification and validation of swapping
SWAT donor strain	Template Strain	Introduction of the cloned donor plasmid
SWAT acceptor library	SWAT acceptor library	None

## Procedure for creation of libraries using SWAT

### Summary

First, the desired genomic modification must be cloned into a *donor* plasmid. This plasmid should then be transformed into a SWAT donor strain, which already contains the SGA procedure genetic traits, and is compatible with the SWAT method as it harbors a galactose induced I-SceI. Once introduced with the donor plasmid the donor strain is crossed with the SWAT library, and the following steps of the SGA process are performed: mating, diploid selection, sporulation, haploid selection and double-mutant selection. At this point, swapping of the desired module into the genome is induced by replicating the double mutant plates onto galactose containing media. Finally, selection for positively swapped yeast is performed by 5-FOA (negative selection for the SWAT *acceptor* module), and/or by a selection marker introduced with the donor module.

## **Protocol**

### **1. Donor module plasmid cloning**

The donor plasmid is a yeast shuttle vector and must contain the following features:

(Note: All of the below instructions are for N'-tag swapping with the available SWAT-GFP library)

- A. Bacterial origin of replication and selection marker, for maintenance and plasmid production.
- B. Yeast centromere and autonomously replicating sequence (CEN-ARS).
  - This feature ensures a low-copy number of the plasmid per cell, helping avoid a possible problem of non-cut plasmid after SclI induction, and recombination between copies of donor plasmids.
- C. Yeast selection marker.
  - Possible common markers that can be used are: KAN, NAT, MET, and Bleomycin. (Hygromycin may be used if not used as the swap selection).
  - Markers that **cannot** be used, as they are utilized as SGA markers are: URA (the SWAT library marker), HIS, LEU, LYS, and ARG, as well as the CAN1 and LYP1 transporters.
- D. Two SclI sites, directly up- and downstream of the swap module.
  - The I-SclI site sequence is (restriction site alone underlined) :  
5' agttacgctagggataacagggtaatatag 3'.
- E. Two recombination sequences, homologous to regions in the SWAT module flanking the SWAT module SclI site. These can be any combination of the following:

Swap type	5' recombination sequence	3' recombination sequence
Seamless tagging	L1	L2
Selection reconstitution and tag swap	HYGΔC	L2
Selection and tag swap	L1	L2

The sequences of these features can be found in the appendix, and are annotated in the plasmid sequence files.

- F. Optional – selection marker.

Swap type	Selection marker sequence	Selection introduced
Seamless tagging	None	None
Selection reconstitution and tag swap	HYGΔC (TEFpr-HYG <sub>1-576</sub> )	HYG
Selection and tag swap	Full marker sequence	Depending of marker selected

- G. The desired genomic modification. This may be either a promoter, a promoter and a tag, or any other regulatory element (protein coding or other).
- One can also introduce a tag at the 5' end, to be driven by each gene's native promoter, and another promoter + tag at the 3' end, to be fused to each protein.
  - Proteins containing regulatory features at their 5' end were tagged downstream to these features with a SWAT module which has a generic feature in exchange (such as signal peptides). Therefore, non-seamless swapping donor plasmids should contain another version of these features as well (such as a different signal peptide), and introduced separately to assemble a donor strain to be crossed with this subset of strains of the SWAT library.

To ease the cloning of donor plasmids, several template plasmids were prepared by us and are freely available. These plasmids contain all the features above, and should only be modified by insertion of the desired genomic modification to be introduced instead of the SWAT tag in the library.

**Please refer to the appendix Table 1 and Table 2 for a complete list of template plasmids with information of swapping types, selection markers, and cloning sequences.**

The plasmids do not contain multiple cloning sites and therefore we highly recommend using either restriction free (RF) cloning (Unger et al., 2010) or Gibson assembly (Gibson et al., 2009) to perform plasmid cloning steps, and to sequence the entirety of the swap module (sequence between and including both SclI sites) before testing the cloned constructs.

## 2. SWAT donor strain preparation

Once prepared, the donor plasmid should now be transformed into a SWAT donor template strain. As detailed in the table below, the template strain (yMS2085) has the following traits: *his3 $\Delta$ 1 leu2 $\Delta$ 0 met15 $\Delta$ 0 ura3 $\Delta$ 0 LYS2+ can1 $\Delta$ ::GAL1pr-Scel::STE2pr-SpHIS5 lyp1 $\Delta$ ::STE3pr-LEU2*.

If you wish to screen for additional genetic traits across the entire library (an additional fluorescent reporter, gene deletion, etc.), they may be incorporated into the genome of the donor strain at this stage. For example, one may wish to incorporate a reporter for glycosylation into each protein represented in the library, and also test this reporter on the background of a deletion in one of the genes participating in this process. Therefore this gene should be deleted in the donor strain prior to the beginning of its SGA with the SWAT library.

### SWAT donor template strain traits

Class	Trait function	Genotype
Swapping	Inducible expression of Scel enzyme	<i>can1<math>\Delta</math>::GAL1pr-Scel::STE2pr-SpHIS5</i>
SGA procedure	Deletions for Insertion of markers	<i>his3<math>\Delta</math>1 leu2<math>\Delta</math>0 met15<math>\Delta</math>0 ura3<math>\Delta</math>0</i>
	Haploid selection and a mating type selection	<i>can1<math>\Delta</math>::GAL1pr-Scel::STE2pr-SpHIS5</i>
	Haploid selection and alpha mating type selection	<i>lyp1<math>\Delta</math>::STE3pr-LEU2</i>
Functional screening	Screening reporter / genetic modification	According to the desired screen objective

### 3. Donor plasmid verification

Before continuing to perform high-throughput experiments, it is advisable to test swapping capability of the cloned donor plasmid on a few strains. The test strains have the same traits as the SWAT donor template strain (see above), with the addition of a SWAT *acceptor* module introduced into the genome, tagging a different protein in each strain. These testing strains are provided with the library, or can be made by tagging of specific proteins with the SWAT module (plasmid pST-N2). The genotype for these strains is (HMG1 as an example): *SWAT-GFP-HMG1 ; can1Δ::GAL1pr-Scel::STE2pr-SpHIS5 ; lyp1Δ::STE3pr-LEU2 ; his3Δ1 ; leu2Δ0 met15Δ0 ; ura3Δ0 ; LYS2+*.

Follow these steps to assure the donor plasmid functionality:

1. Transform the donor plasmid into the test donor strains, using the selection found in the donor plasmid (see 1.C). Incubate at 30°C for 2-3 days, according to the transformation protocol.
2. Pick a few single colonies from each transformation, and patch them onto a fresh selection plate. Incubate at 30°C for 1-2 days.
3. Either pick and re-patch or replica plate (using a velvet cloth) the yeast onto plates containing 2% galactose media (YPGal). Incubate at 30°C for 1-2 days. Repeat this induction step another time.
  - Save the plates from the previous step for comparison later on.
4. Either pick and re-patch or replica plate the yeast onto plates containing one of the following selection media:

Swap type	Selection
Seamless tagging	5-FOA
Selection reconstitution and tag swap	Hygromycin B
Selection and tag swap	Depending of marker selected

Incubate at 30°C for 1-2 days.

Repeat this selection step another time.

5. Repeat step 4, for another selection round. Refresh yeast from step 2.
6. Perform an experiment that enables testing the swapping of the donor module, comparing yeast before (step 2) and after (step 5) swapping. This may include a Validation by PCR, visualization of fluorescent tags, etc.

#### 4. SGA of SWAT library with SWAT donor and tag swap

Once the SWAT donor strain is ready it should be crossed with the SWAT library, creating haploid, double-mutant yeast (See Appendix, Figure 2). We highly recommend using the [Singer RotorHD](#) colony arrayer to preform high-throughput library plate manipulation (transferring of yeast from one selection media to another, manual pinners can also be used ([V&P scientific](#))).

A complete description of the SGA method is published in numerous manuscripts (Tong et al., 2001), and a full protocol was previously published by us (Cohen and Schuldiner, 2011).

Step ID	Step	Media	Time	Temp.
1	Donor strain liquid starter	Liquid media with the specific donor plasmid selection, e.g. YEPD+NAT (Neuruseothricin, 200µg/mL).	Over night	30°C
2a	Donor strain arraying	Agar plates with the specific donor selection (e.g. YEPD+NAT).	1 day	30°C
2b	SWAT library arraying	Agar plates with SD-Ura.	1 day	30°C
3	Mating	YEPD plates.	1 day	RT
4	Diploid selection	Plates that contain the specific donor plasmid selection, and lacks uracil (e.g. SD-Ura+NAT).	1-2 days	30°C
4b	2 <sup>nd</sup> Diploid selection	Plates that contain the specific donor plasmid selection, and lacks uracil (e.g. SD-Ura+NAT).	1-2 days	30°C
5	Sporulation	Nitrogen starvation plates. Note: Do not wrap plates, oxygen is essential for this step	5-7 days	22°C/ RT
10*	Haploid Selection	SD-Ura-His-Arg-Lys + canavanine + thialysine plates to select for <u>MATa</u> haploids, or SD-Ura-Leu-Arg-Lys + canavanine + thialysine plates to select for <u>MATα</u> haploids.	2 days	30°C
12*	Double mutant selection	SD -His/-Leu and -Ura-Arg-Lys + canavanine + thialysine + specific donor selection (e.g. NAT).	1 day	30°C
13	I-SceI induction	YEPGalactose (2%)	1 day	30°C
13b	2 <sup>nd</sup> I-SceI induction	YEPGalactose (2%)	1 day	30°C
14*	Swap selection	SD -His/-Leu and -Arg-Lys + canavanine + thialysine + a. Seamless tagging - 5-FOA (1g/L). b. Selection reconstitution and tag swap - HYG (HygromycinB, 200µg/mL).	1 day	30°C

		c. Selection and tag swap - Depending of marker selected (e.g NAT).		
<b>15**</b>	Second swap selection	Same as #14.	1 day	30°C
<p>* - These steps may be carried out twice to ensure even more full elimination of undesired yeast from the previous steps.</p> <p>** - The second swap selection step should be made prior to the planned screening.</p>				

At each step, an extra plate with the same media should be used to test growth of strains from previous steps as a control for the selection used. For example:

- The diploid selection media (#4) should be tested with the original donor strain and several colonies from the library array (before mating).
- At Scel induction (#13), the original donor strain and strains from the library should be plated, and then transferred to the Swap selection (#14) to verify that they do not grow (as swapping doesn't occur in the library, and the donor plasmid should be cut in the donor).

### 5. New library swap verification

Validation of correct swapping events can be performed in various methods, depending on the type of swap and type of tag introduced.

- A. The SWAT libraries contain 3 control strains, each placed in multiple repeats per each 384 well plate. In addition, there are blank locations without any colonies.
  - a. Check that the blank locations did not get contaminated during plate replication at the various stages.

Control strain	Genotype	Description
his3Δ1-SWAT / his3Δ1-SWAT-SP	<i>his3Δ1::SWAT-GFP-STOP / his3Δ1::SWAT-SP-GFP-STOP ; leu2Δ0 met15Δ0 ura3Δ0</i>	The SWAT-GFP / SWAT-SP-GFP module was inserted into the his3 deletion locus in BY4741, with a STOP codon following the L2-linker. This strain is to be used to measure either GFP levels (before swap) or the donor tag phenotype, w/o any protein attached to the tag.
his3Δ1-GFPΔC	<i>his3Δ1::sfGPFΔC-Scel-URA-sfGFPΔC; leu2Δ0 met15Δ0 ura3Δ0</i>	BY4741 was inserted with a fragment containing the following: L1-sfGPFΔC-Scel-CYC1ter-URA3-NOP1pr-Scel-sfGFPΔC-Stop. Strain is good for auto-fluorescence measurements. This strain is meant to be able to grow on -URA (with the SWAT-GFP library), and also be able to lose its URA selection upon Scel induction via recombination of the sfGPFΔC fragments. It is therefore compatible with all SGA procedures, regardless of the donor plasmid used, until after the 5-FOA selection.
URA <sup>+</sup>	<i>ΔYDL121C::URA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	BY4741 was transformed with a URA marker inserted deleting the ORF YDL121C. This control strain expresses URA3, and should be used to



		verify 5-FOA selection, as it cannot lose it due to I-SceI induction like the library strains.
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- B. PCR analysis can be performed, using swapped and original colonies. Primers should be planned to test both the presence of the new tag in the yeast genome, and to make sure no non-swapped yeast remain. We recommend using the following PCR reactions after swap of the N' SWAT-GFP libraries:

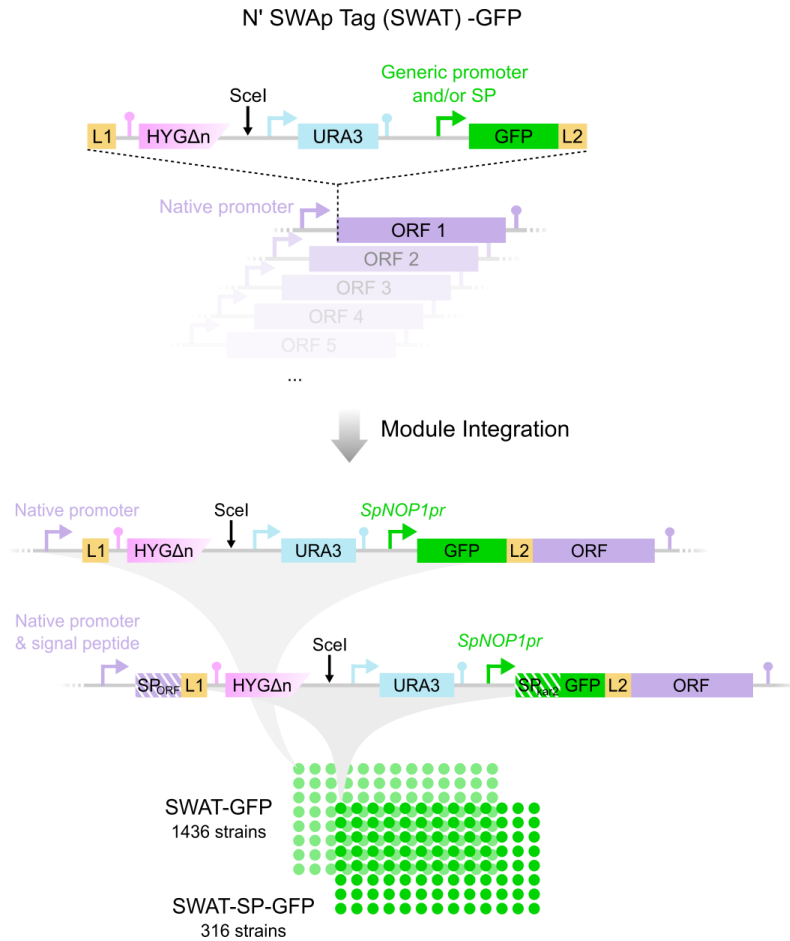
Swap type	Validation	F primer binding site	R primer binding site	Amplicon size [bp]
Seamless tagging	Swapped	L1	From introduced tag	*
	Swapped	From introduced tag	L2	*
	Original	NOP1pr	GFP tag	494
Seamless GFP tagging	Swapped	L1	GFP tag	390
	Original	NOP1pr	GFP tag	494
Selection reconstitution and tag swap	Swapped	From introduced tag	Gene specific	*
	Original	NOP1pr	GFP tag	494
Selection and tag swap	Swapped	From introduced tag	Gene specific	*
	Original	NOP1pr	GFP tag	494

\* - Depending on the tag used.

Primer name	Sequence 5' – 3'
L1 CHK F	cgtacgctgcaggctcgac
L1 CHK R	catcgatgaattctctgtcg
sfGFP CHK R	ttcgaatttcacttctgctc
NOP1pr CHK F	agccaacaagtttccttac

- C. Functional analysis should be performed on positive and negative control gene colonies, based on the introduced module. This may also be performed after streaking of the swapped colonies to single colonies, to assess percent of swapped vs. non-swapped clones.

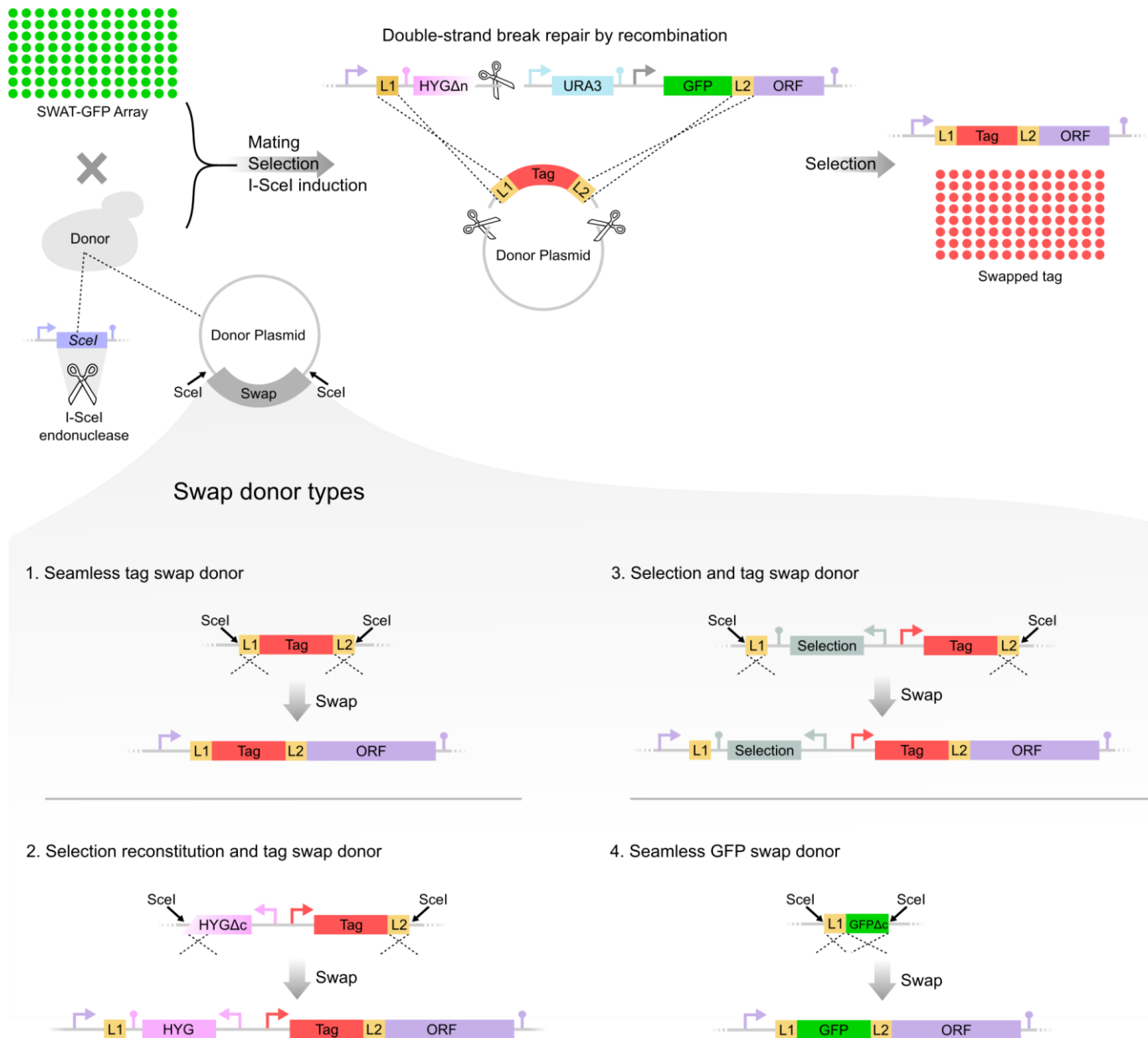
## Appendix



**Figure 1. The SWAT-GFP library assembly.**

SWAT-GFP acceptor modules were used to tag proteins at their N- terminus. The modules contain the restriction site for the I-SceI endonuclease (SceI), a URA3 selection marker (URA3), a truncated Hygromycin B selection marker (HYGΔn), the *SpNOP1* promoter (“Generic”) and/or a signal peptide (see below), and are flanked by two generic sequences for homologous recombination that also serve as linkers for protein fusion (L1, L2). To target specific genes in transformations, the SWAT modules are amplified with primers which include a 40bp gene-specific homology sequence (Methods). Promoters are designated in the illustration by a line-arrow combination (where the arrow reflects directionality), and terminators are designated by a line-circle combination.

Two strain collections were assembled, in which proteins were tagged at their N’ with an acceptor SWAT module that contains the *SpNOP1* constitutive promotor and a GFP. Proteins predicted to harbor a signal peptide (SP) were tagged with a similar acceptor SWAT module that contains a SP upstream to the GFP (SP of *ScKAR2*).



**Figure 2. Generating new libraries from the SWAT-GFP library.**

The SWAT-GFP library can be crossed with a donor strain to create a new library of your choice. The donor strain encodes a galactose induced I-SceI and harbors a donor plasmid with the desired tag flanked by two generic sequences (L1, L2) and two SclI restriction sites for systematic insertion (top left). An SGA procedure is used to create the new library and includes mating of the SWAT –GFP arrays with the donor strain, followed by a diploid selection step. Diploids are induced to sporulate, and thereafter haploids are selected for using the unique SGA markers. Next, selection is made for double mutants that contain both the acceptor tagged locus and the donor plasmid and I-SceI locus. At this point, I-SceI expression is induced by a growth step on galactose, leading to double strand breaks in the tagged gene locus and the donor plasmid. The genomic break is mended by homologous recombination of the donor tag

(top middle). Correct swapping is selected for and a library featuring the new tag is created (top right).

Several types of tag swaps can be carried out by using various designs of donor plasmid features, which result in several types of tagged-gene libraries (indicated by the Swap arrow). The possible swap donor types include: (1) Seamless tag swap in which proteins are fused to a new tag, restoring the native regulatory features (promoter and/or signal peptide), (2) Selection reconstitution tag swap in which a new tag is introduced with a common regulatory features (e.g. a specific promoter), and the Hygromycin selection marker is reconstituted, (3) Selection and tag swap in which both a new tag as well as a new selection marker are introduced, and (4) Seamless GFP swap in which proteins are still fused to the GFP tag, while restoring their native regulatory features (promoter and/or signal peptide).

Table 1. Available donor plasmids

Name	Description	N' / C' swapping	Cloning Info	5' – 3' recombination sequences	Swap tag	Swap selection	Swap promoter (N') / terminator (C')	HYG $\Delta$ c	Plasmid selection
pSD-N1	Donor template	N	A	L1 – L2	-	-	-	-	kanMX4
pSD-N2	Donor template	N	A	L1 – L2	-	-	-	-	<i>S.c MET15</i>
pSD-N3	Donor template	N	A	L1 – L2	-	-	-	-	natPP <sup>3</sup>
pSD-N4	HYG $\Delta$ c Donor template	N	B	HYG $\Delta$ c – L2	-	hphNT1	-	+	kanMX4
pSD-N5	HYG $\Delta$ c Donor template	N	B	HYG $\Delta$ c – L2	-	hphNT1	-	+	<i>S.c MET15</i>
pSD-N6	HYG $\Delta$ c Donor template	N	B	HYG $\Delta$ c – L2	-	hphNT1	-	+	natPP <sup>3</sup>
pSD-N7	HYG $\Delta$ c:: <i>TEF2</i> pr template	N	C	HYG $\Delta$ c – L2	-	hphNT1	<i>S.c TEF2</i>	+	<i>S.c MET15</i>
pSD-N8	HYG $\Delta$ c:: <i>TEF2</i> pr- SP <sub>Kar2</sub> template	N	D	HYG $\Delta$ c – L2	SP <sub>Kar2</sub> <sup>1</sup>	hphNT1	<i>S.c TEF2</i>	-	napPP <sup>3</sup>
pSD-N9	Seamless GFP	N	-	L1 – sfGFP $\Delta$ c	-	-	-	-	kanMX4
pSD-N10	Seamless GFP	N	-	L1 – sfGFP $\Delta$ c	-	-	-	-	<i>S.c MET15</i>
pSD-N11	Seamless GFP	N	-	L1 – sfGFP $\Delta$ c	-	-	-	-	natPP <sup>3</sup>
pSD-N12	Seamless mCherry	N	-	L1 – L2	mCherry	-	-	-	kanMX4
pSD-N13	Seamless mCherry	N	-	L1 – L2	mCherry	-	-	-	<i>S.c MET15</i>
pSD-N14	Seamless mCherry	N	-	L1 – L2	mCherry	-	-	-	natPP <sup>3</sup>
pSD-N15	NAT:: <i>TEF2</i> pr-mCherry	N	E	L1 – L2	mCherry	natMX6	<i>S.c TEF2</i>	-	<i>S.c MET15</i>
pSD-N16	NAT:: <i>TEF2</i> pr- SP <sub>Kar2</sub> -mCherry	N	F	L1 – L2	SP <sub>Kar2</sub> -mCherry <sup>1</sup>	natMX6	<i>S.c TEF2</i>	-	<i>S.c MET15</i>
pSD-N17	HYG $\Delta$ c:: <i>TEF2</i> pr-mCherry	N	-	HYG $\Delta$ c – L2	mCherry	hphNT1	<i>S.c TEF2</i>	+	natPP <sup>3</sup>
pSD-N18	HYG $\Delta$ c:: <i>CYC1</i> pr-BFP	N	-	HYG $\Delta$ c – L2	BFP	hphNT1	<i>S.c CYC1</i>	+	natPP <sup>3</sup>
pSD-N19	HYG $\Delta$ c:: <i>Z4</i> pr	N	-	HYG $\Delta$ c – L2	-	hphNT1	<i>Z4</i> EV inducible <sup>2</sup>	+	<i>S.c MET15</i>
pSD-N20	HYG $\Delta$ c:: <i>Z4</i> pr	N	-	HYG $\Delta$ c – L2	-	hphNT1	<i>Z4</i> EV inducible <sup>2</sup>	+	natPP <sup>3</sup>

<sup>1</sup> SP<sub>Kar2</sub> – a codon changed version of *S.c* Kar2 signal peptide. See full sequence in experimental procedures.

<sup>2</sup> *Z4*EV inducible – A modified GAL4 promoter engineered to be activated by an estradiol binding transcription factor (*Z4*EV), see Mclsaac & Noyes et al. 2013.

<sup>3</sup> natPP – A Nourseothricin (NAT) selection marker was created by combining the coding sequence from natMX6 with *S.c* PGK1 promoter and PGK1 terminator (PP)

**Table 2. Donor plasmids cloning information**

The table below describes which features should be cloned into each donor template plasmid in order to construct SWAT donor plasmids.  
The features should be introduced directly between the indicated “cloning upstream sequence” and “cloning downstream sequence”.

Cloning Info ‡	Plasmids	5' – 3' recombination sequences	<i>Donor type:</i> Features to clone into plasmid for swapping	Cloning upstream sequence	Cloning downstream sequence
<b>A*</b>	pSD-N1/2/3	L1 – L2	<b><i>Seamless tag donor:</i></b> Tag coding sequence. <b><i>Selection and tag donor:</i></b> Selection marker, promoter and tag.	L1 linker: cgtacgctgcaggtcgacggtggcggttctggcggtggcggatcc	L2 linker: ggcggttcctctggtggtggtggtgcgacagagaattcatcgatg
<b>B*</b>	pSD-N4/5/6	HYGΔc – L2	<b><i>Selection reconstitution and tag donor:</i></b> Promoter and tag.	HYGΔc (TEF promoter 5' end): cctccatgtcgtggcgggtgacccggcggggacgaggcaagct	L2 linker: ggcggttcctctggtggtggtggtgcgacagagaattcatcgatg
<b>C</b>	pSD-N7	HYGΔc – L2	<b><i>Selection reconstitution and tag donor:</i></b> Tag (TEF2pr driven expression).	TEF2 promoter 3' end: tagaatatacgggtcaacgaactataattaactaaacactagtacc	L2 linker: ggcggttcctctggtggtggtggtgcgacagagaattcatcgatg
<b>D</b>	pSD-N8	HYGΔc – L2	<b><i>Selection reconstitution and tag donor:</i></b> Tag (TEF2pr driven expression with Kar2 signal peptide).	SP <sub>Kar2</sub> 3' end: caaaactcctttcactcttctaataatgtcctggtcagaggcgagac	L2 linker: ggcggttcctctggtggtggtggtgcgacagagaattcatcgatg
<b>E</b>	pSD-N15	L1 – L2	<b><i>Selection and tag donor:</i></b> 1. Promoter and tag (replacing TEF2pr-mCherry). 2. Tag (replacing mCherry).	1. NAT selection (TEF terminator 3' end): gtaatatcatgcgtcaatcgatatgtgaatgctggtcgctatactg  2. TEF2 promoter 3' end: tagaatatacgggtcaacgaactataattaactaaacactagtacc	L2 linker: ggcggttcctctggtggtggtggtgcgacagagaattcatcgatg
<b>F</b>	pSD-N16	L1 – L2	<b><i>Selection and tag donor:</i></b> Tag (replacing mCherry, TEF2pr driven expression with Kar2 signal peptide).	SP <sub>Kar2</sub> 3' end: caaaactcctttcactcttctaataatgtcctggtcagaggcgagac	L2 linker: ggcggttcctctggtggtggtggtgcgacagagaattcatcgatg

‡ - Refers to annotations in the “Available donor plasmids” table.

\* - Cloning can also be performed using the restriction sites BamHI and SpeI.