

Using the yeast gene deletion collection to customize gene expression

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The pioneering sequencing of the *Saccharomyces cerevisiae* genome (1) formed the basis for genome-wide, large-scale approaches to studying gene function. One such project was the *S. cerevisiae* Genome Deletion Project that systematically replaced each yeast open reading frame (ORF) with a kanamycin resistance module (KanMX), yielding precise start-to-stop codon deletions for more than 95% of the genes (2,3). This replacement is widely used for phenotypic analysis of gene function. We have taken advantage of the thousands of KanMX-promoter fusions generated by the yeast deletion project to create insertion modules for replacing a normal promoter with any one of thousands of different possible yeast promoters.

This method takes advantage of the DNA templates produced by the gene knockout project, each possessing a specific ORF that has been replaced by a KanMX module (Figure 1). Since the flanking regions for the deleted ORFs are left intact, what remains produces a set of strains carrying unique DNA templates in which almost any intergenic region is flanked by a KanMX marker. For almost any yeast promoter, there exists a strain in which a selectable marker has been inserted immediately 5' of that promoter. This KanMX marker-intergenic region can be PCR-amplified as a cassette, and flanking sequences can be designed to target the product for insertion at the desired site by homologous recombination (4).

The ORF immediately 5' to the promoter of interest is identified so that the knockout strain for which this ORF has been replaced by KanMX can be used as a source of template DNA. To target the PCR product for insertion, the amplification primers are designed with 5' ends that are homologous to the

intended insertion sites (40–60 bp). This can be accomplished with a single PCR step. In the results shown herein, we used genomic DNA as the template, but we have also used the colony PCR method (5), which could potentially allow high-throughput workflows.

Our schematic shows the KanMX-promoter cassette inserted between the normal promoter and the ORF, but the upstream primer can be designed to cause replacement of the normal promoter sequence. We have successfully used both methods. This concept could also be used to design insertion fragments to replace 3' untranslated sequences with those from another gene, as well as to make in-frame fusions between practically any two coding regions.

PCR fragments containing the KanMX-promoter cassette were amplified using genomic DNA from the chosen deletion strain (Table 1) purchased from Research Genetics (Huntsville, AL, USA).

For all reactions, primers are shown in Table 2. Bolded and underlined nucleotides indicate the portion that anneals to sequences flanking the KanMX-promoter fragment, while plain text identifies the portion of the primer required for targeting insertion. Our typical PCR conditions used 0.1 µg purified genomic DNA as the template, 2 mM MgCl₂, 1× *Taq* DNA polymerase buffer, 5 U *Taq* DNA polymerase (Promega, Madison, WI, USA), 0.2 mM dNTPs, and 1 µM each primer in a total volume of 50 µL, using a GeneAmp® 9700 (Applied Biosystems, Foster City, CA, USA) thermal cycler with start at 94°C, followed by 35 cycles of 94°C for 30 s, 60°C for 45 s, 72°C for 120 s, followed by 72°C for 7 min. PCR products were purified using QIAquick® PCR columns (Qiagen, Valencia, CA, USA), and 0.5 µg amplified DNA was used for each transformation using a Frozen-EZ Yeast Transformation II™ kit (Zymo Research, Orange, CA, USA). Yeast transformants were allowed to express the resistance marker by plating onto nonselective medium and then replica plated to G418 (0.25 mg/mL) for integrant selection. Proper insertion was confirmed via PCR and sequencing. The rate of successful transformation into the appropriate position was comparable to the integration of commonly used plasmid-generated fragments.

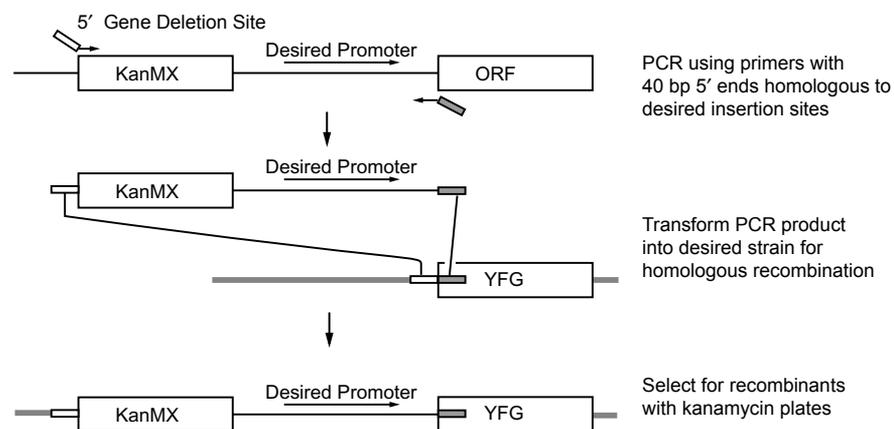


Figure 1. Amplification of a desired promoter region for insertion upstream of your favorite gene (YFG). Primers are selected to amplify the promoter of interest along with the KanMX marker that has been inserted immediately 5' to the promoter in a specific strain of the gene deletion set. Amplification primers have regions at the 5' ends that are homologous to the target site (shown as small rectangles). Amplified products are transformed into the strain of interest, and the PCR products are inserted by homologous recombination via the targeting sequences. ORF, open reading frame.

As an example of this technique, we used the *CCR4* primer set and template DNA from strain 10388 (carrying a deletion in *yal020c*) to produce a KanMX-*CCR4* promoter fragment that was targeted to replace the normal promoter sequence upstream of the *CLN3* ORF. We chose the *CCR4* promoter as a potential constitutive promoter because *CCR4* messenger RNA (mRNA) levels, encoding a general transcriptional regulator (6), were relatively constant in published microarray experiments (7). Replacement of the *CLN3* promoter with that from *CCR4* reduced *CLN3* mRNA levels and blunted *CLN3* induction by nutrients (8) (Figure 2A). Thus, this method was effective in manipulating expression of the target gene.

We also used the *LSR1* promoter, which normally drives transcription of the U2 small nuclear RNA (snRNA) (9), as a test promoter. We chose U2 RNA as a loading control because it is constant and abundant. The KanMX-*LSR1* promoter cassette was amplified with a DNA template from strain 13371 (carrying a deletion in *ybr231c*) using the *LSR1* primers for insertion immediately 5' of the *RPB4* ORF. That produced overexpression of *RPB4* mRNA, which encodes a subunit of RNA polymerase II (10) (Figure 2B).

To make a conditional promoter fusion, we used the *SMF3* promoter. *SMF3* encodes an iron transporter that is induced in low iron environments (11,12). An *SMF3* promoter fusion driving *CLN3* was constructed with prior amplification of the KanMX-*SMF3* promoter fragment using genomic DNA from strain 16433 as a template (strain 16433 carries KanMX inserted in place of *ylr035c*). *CLN3* mRNA levels were determined in mid-log cultures grown in either the absence or presence of an iron chelator, bathophenanthroline-disulfonic acid (BPS). As expected, BPS increased *CLN3* mRNA in the altered strain (Figure 2C). The *SMF3* promoter is just one example out of the hundreds of regulated promoters that could be selected for use.

Since expression can also be regulated through translational regulatory elements in the mRNA,

Table 1. Yeast Strains

Strain	Description	Reference
BY4742	MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Research Genetics
10388	BY4742 carrying <i>yal020c::KanMX</i>	Research Genetics
13371	BY4742 carrying <i>ybr231c::KanMX</i>	Research Genetics
16433	BY4742 carrying <i>ylr035c::KanMX</i>	Research Genetics
11597	BY4742 carrying <i>yor301w::KanMX</i>	Research Genetics
DP2	BY4742 carrying <i>CCR4-CLN3::KanMX</i>	This study
DLY2	BY4742 carrying <i>LSR1-RPB4::KanMX</i>	This study
DLY4	BY4742 carrying <i>SMF3-CLN3::KanMX</i>	This study
DLY5	BY4742 carrying <i>CPA1-CLN3::KanMX</i>	This study

we moved the 5' noncoding region of *CPA1* to regulate production of *CLN3*. Translation of Cpa1 is inhibited by arginine through regulation of ribosomal scanning of an upstream ORF (13,14). The *CPA1* promoter driving *CLN3* fusion was constructed as above, amplifying the KanMX-*CPA1* promoter fragment out of strain 11597 (carrying a deletion in *yor301w*). The addition of arginine (40 mM) to the medium decreased cell size in the transformants, which was the expected phenotype for decreased Cln3 levels (not shown).

It is commonly useful to drive expression of a gene of interest with a heterologous promoter. While vector-based chromosomal insertion tools for yeast, such as the STIK system (15), have been available for many years, our method takes advantage of the previously accomplished generation of thousands of KanMX-promoter fusions, providing ready-made templates for generating a targeted insert with a single PCR. The availability of these templates obviates the need for the intermediate step of creating a recombinant clone carrying the promoter fused to a marker. The collection of *S. cerevisiae* deletion strains provides a set of readily amplified KanMX markers lying immediately 5' to intragenic regions that include approximately 95% of the promoters in *S. cerevisiae*. This provides a useful set of ordered templates that can be used to substitute almost any yeast promoter into a site driving almost any yeast gene. This method involves only a single PCR amplification and a standard yeast transformation. Furthermore, the growing body of microarray data

allows predictions to be made regarding the strength of a given promoter under different circumstances, which should enable custom expression patterns to be readily designed.

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

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Table 2. PCR Primers

Constructs	Upstream Primer	Downstream Primer
<i>SMF3</i> promoter - <i>CLN3</i>	5'-ACTCTCGTTCAAGACACTGATTTGATACGCTTTC TGAC <u>CGGTCCATATCCCTCATATACATG</u> -3'	5'-CATTAGCGTATCTAATTATGGTATCCTTCAATAT GGCCAT <u>TTTTCTCTTGTGATGCTACTACTG</u> -3'
<i>CPA1</i> promoter - <i>CLN3</i>	5'-ACTCTCGTTCAAGACACTGATTTGATACGCTTTC TGAC <u>CGGGAAATTCATTGTGCACAA</u> -3'	5'-CATTAGCGTATCTAATTATGGTATCTTTCAATAT GGCCAT <u>TGAAATATTTTTAGGAGTGGT</u> -3'
<i>LSR1</i> promoter - <i>RPB4</i>	5'-TTCATCCGTATTGAAATATATATAAGAATATAG AAA <u>GTTTCAAGAAAGAAAACCCAC</u> -3'	5'-TTCTCCGTCTTGTGTTGAAAGGTTGATGTAGAA ACATTCAT <u>ATTCGTGGGGATTAAAAAA</u> -3'
<i>CCR4</i> promoter - <i>CLN3</i>	5'-AATTATATCAGTTATTACCCTCGACCCGGGTACC AGATCTCTATTCCGTCGCACACGATC-3'	5'-TGCCTGGCCATCCACGCTATATACACGCCTG GCGGATCTAAAACTTTTCGTTTCAGTTG-3'

Bolded and underlined nucleotides indicate the portion that anneals to sequences flanking the KanMx-promoter fragment. Plain text identifies the portion of the primer required for targeting insertion.

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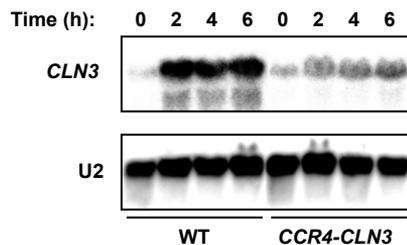
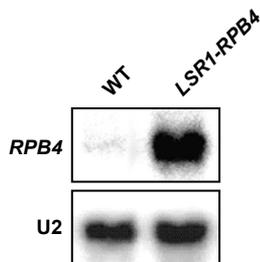


Figure 2. Examples demonstrating altered gene expression after heterologous promoter insertion. (A) Northern blot detection of *CLN3* expression levels in wild-type and *CCR4-CLN3* strains. Wild-type (BY4742) and the isogenic *CCR4-CLN3* promoter fusion (DP2) strains were grown for 48 h to post-log phase ($6 A_{660}$) in yeast extract peptone dextrose (YEPD) medium and transferred to fresh YEPD at an A_{660} of 0.4. Samples were collected at the indicated time points for RNA preparation and Northern blot analysis. For Northern blot analysis, RNA was isolated (16) and probed with a 1.8-kb *Bam*HI fragment from *CLN3*. Blots were probed with a radiolabeled 0.6-kb *Sac*I fragment from *U2* to confirm uniform loading. (B) Northern blot detection of *RPB4* expression levels in wild-type and *LSR1-RPB4* strains. Wild-type (BY4742) and the isogenic *LSR1-RPB4* promoter fusion (DLY2) strains were grown to mid-log phase ($1 A_{660}$) in YEPD medium. Samples were collected for RNA preparation and Northern blot analysis with a 558-bp fragment from *RPB4* (*68-626). (C) Northern blot detection of induction of *SMF3-CLN3* by iron chelation. Wild-type (BY4742) and the isogenic *SMF3-CLN3* promoter fusion (DLY4) strains were grown to mid-log phase ($1 A_{660}$) in Synthetic dropout (SD) medium with or without the 1.6 μ M iron chelator bathophenanthroline-disulfonic acid (BPS) as indicated. Samples were collected for RNA preparation and Northern blot analysis with a *CLN3* probe. A *U2* probe was used as a loading and transfer control.

B



C

