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## Cloning the *Schizosaccharomyces pombe lys2<sup>+</sup>* gene and construction of new molecular genetic tools

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### Abstract

Molecular genetic analyses in *Schizosaccharomyces pombe* rely on selectable markers that are used in cloning vectors or to mark targeted gene deletions and other integrated constructs. In this study, we used genetic mapping data and genomic sequence information to predict the identity of the *S. pombe lys2<sup>+</sup>* gene, which is homologous to *Saccharomyces cerevisiae LYS4<sup>+</sup>*. We confirmed this prediction, showing that the cloned SPAC343.16 gene can complement a *lys2-97* mutant allele, and constructed the *lys2<sup>+</sup>*-based cloning vector pRH3. In addition, we deleted the *S. pombe his7<sup>+</sup>* gene with a *lys2<sup>+</sup>*-marked polymerase chain reaction (PCR) product and the *S. pombe lys2<sup>+</sup>* gene with a *his7<sup>+</sup>*-marked PCR product. Strains carrying these deletions of *lys2<sup>+</sup>* or *his7<sup>+</sup>* serve as relatively efficient hosts for the deletion of the *ade6<sup>+</sup>* gene by *lys2<sup>+</sup>*- or *his7<sup>+</sup>*-marked PCR products when compared with hosts carrying *lys2* or *his7* point mutations. Therefore, these studies provide plasmids and strains allowing the use of *lys2<sup>+</sup>* as a selectable marker, along with improved strains for the use of *his7<sup>+</sup>* to mark gene deletions.

### Keywords

*Schizosaccharomyces pombe*; Fission yeast; *his7<sup>+</sup>*; *lys2<sup>+</sup>*; Selectable marker; Deletion

### Introduction

Selectable markers are essential to molecular genetic studies in the fission yeast *Schizosaccharomyces pombe*. They are incorporated into autonomously replicating plasmids and used to mark DNA fragments inserted into chromosomes by homologous recombination in order to delete or alter the sequence of a gene at its endogenous locus. As more molecular genetic tools are developed and strains carrying gene deletions or epitope tags at multiple loci are constructed, the number of usable selectable markers can become limiting.

The first plasmid markers used in *S. pombe* were *Saccharomyces cerevisiae URA3<sup>+</sup>*, which complements an *S. pombe ura4<sup>-</sup>* mutation, and *Saccharomyces cerevisiae LEU2<sup>+</sup>*, which

complements an *S. pombe leu1<sup>-</sup>* mutation (Beach and Nurse 1981; Losson and Lacroute 1983). These markers function well on multicopy plasmids; however, single copy *URA3<sup>+</sup>* fails to complement a *ura4<sup>-</sup>* mutant allele, while single copy *LEU2<sup>+</sup>* (expressed from its own promoter) only weakly complements a *leu1<sup>-</sup>* mutant allele. Therefore, these markers are generally insufficient for marking a gene deletion. Subsequently, the *S. pombe ura4<sup>+</sup>* (Bach 1987; Grimm et al. 1988) and *leu1<sup>+</sup>* (Kikuchi et al. 1988) genes were cloned, although while *ura4<sup>+</sup>* is commonly used, the *Saccharomyces cerevisiae LEU2<sup>+</sup>* gene remains the preferred marker to *S. pombe leu1<sup>+</sup>*. In addition, the *S. pombe his7<sup>+</sup>* (Apolinario et al. 1993), *his3<sup>+</sup>* (Burke and Gould 1994) and *arg3<sup>+</sup>* (Waddell and Jenkins 1995) genes have been cloned and shown to fully restore His<sup>+</sup> and Arg<sup>+</sup> growth in single copy. Very recently, a series of cloning vectors has been constructed carrying the *arg12<sup>+</sup>*, *tyr1<sup>+</sup>*, *ade7<sup>+</sup>*, and *his2<sup>+</sup>* genes, and a *vps34<sup>+</sup>* gene disruption was constructed using each marker (Fujita et al. 2005).

In addition to biosynthetic pathway genes, drug-resistance markers are used in *S. pombe*. The *kanMX6* marker, which confers G418<sup>R</sup> growth, is extensively used in gene deletions and epitope-tagging of chromosomal genes (Bähler et al. 1998), while the bacterial *hph* and *nat* genes confer resistance to hygromycin B and nourseothricin, respectively (Burland et al. 1991; Sato et al. 2005). The advantage of drug resistance markers is that, except for strains already carrying these markers, all *S. pombe* strains are suitable hosts for transformation with these markers due to their natural sensitivity to the drugs to which these markers confer resistance. In contrast, biosynthetic pathway genes can only be introduced into a host strain that is defective in that step of the pathway.

To facilitate their use in targeted gene deletions, unmarked chromosomal deletions of *ura4<sup>+</sup>* (Grimm et al. 1988), *his3<sup>+</sup>* (Burke and Gould 1994) and *arg3<sup>+</sup>* (Waddell and Jenkins 1995) were constructed. In addition, using a Cre-*loxP* system, *arg12:loxP*, *tyr1:loxP*, *ade7:loxP* and *his2:loxP* deletion alleles have also been constructed (Fujita et al. 2005). These deletion alleles do not possess any sequences homologous to the selectable marker, thus increasing the frequency with which transformants that regain the relevant prototrophy carry the gene deletion. We have also used the SV40 promoter-driven *LEU2<sup>+</sup>* selectable marker present in the pNMT-TOPO vectors (Invitrogen) for gene deletions, as there is insufficient homology between *S. pombe leu1<sup>+</sup>* and *Saccharomyces cerevisiae LEU2<sup>+</sup>* to promote targeting of *LEU2<sup>+</sup>* to the *leu1<sup>+</sup>* locus, while the SV40 promoter allows sufficient expression of Leu2 to confer Leu<sup>+</sup> growth in single copy (Wang et al. 2004).

In this study, we cloned the *S. pombe lys2<sup>+</sup>* gene and incorporated it into a versatile cloning vector to create plasmid pRH3. In addition, we carried out polymerase chain reaction (PCR) based gene deletions of *lys2<sup>+</sup>* and *his7<sup>+</sup>*, marking each deletion with the other selectable marker. This work allowed us to identify PCR primers capable of amplifying each marker to produce a product that functions well in single copy. In addition, the resulting *lys2 :his7<sup>+</sup>* deletion allele lacks the entire *lys2<sup>+</sup>* sequence that is amplified to mark the *his7 :lys2<sup>+</sup>* deletion, while the *his7 :lys2<sup>+</sup>* deletion allele lacks the entire *his7<sup>+</sup>* sequence that is amplified to mark the *lys2 :his7<sup>+</sup>* deletion. As expected, these strains are superior to *lys2-97* and *his7-366* strains for the construction of *lys2<sup>-</sup>* or *his7<sup>-</sup>*-marked gene deletions when using PCR products, as demonstrated by comparing the efficiency of deleting the *ade6<sup>+</sup>* gene. Thus, these studies make the *his7<sup>+</sup>* marker easier to use in future gene

disruptions, and provide new reagents for the use of *lys2*<sup>+</sup> as a selectable marker in *S. pombe*.

## Materials and methods

### Yeast strains and growth media

Yeast strains used in this study are listed in Table 1. Only relevant genotypes are given in the text and figures. Standard *S. pombe* media YEA, PM and EMM (Watanabe et al. 1988; Alfa et al. 1993) were used for culturing cells. Crosses were carried out on MEA medium (Gutz et al. 1974) and tetrads were dissected on YEA medium.

### Cloning of *lys2*<sup>+</sup> gene

SPAC343.16 (*lys2*<sup>+</sup>) was PCR-amplified from strain 972 genomic DNA using oligonucleotides *lys2*-for (5'-CGTAAAAAAGAAAGAGCTGAGCG-3') and *lys2*-rev (5'-GCAGTACGCAAAGATCCTAAGCT-3'). The PCR product was cloned into vector pNMT41 (Invitrogen) and used to transform *Escherichia coli* according to the manufacturer's instructions. Plasmids from *E. coli* transformants were purified and screened by a *Hind*III digest to identify pRH1 and pRH2, which carry the insert PCR product in the two orientations relative to the *nmt41* promoter.

### Construction of the pRH3 *lys2*<sup>+</sup>-marked cloning vector

The *lys2*<sup>+</sup> gene was PCR-amplified using oligonucleotides URA3*lys2*-for (5'-GATTCGTAATCTCCGAACAGAAGGAAGAACGAAGGAAGGAGCACAGACTTAGTCTGAATAATCATGCTTGAAACC-3') and URA3*lys2*-rev (5'-CCTGATGCGGTATTTTCCTTACGCATCTGTGCGGTATTTACACCCGCATATTTAGCGATGTTATTCAGCTTTAC-3') with plasmid pRH1 as template. The 3' ends of the oligonucleotides (underlined) are identical to sequences flanking *lys2*<sup>+</sup>, while the 5' ends are identical to sequences flanking the *URA3*<sup>+</sup> selectable marker in plasmid pSP2 (Cottarel et al. 1993). This PCR product was used to replace the *URA3*<sup>+</sup> marker in plasmid pSP2 by co-transformation of strain CHP871 (*lys2-97*) with plasmid pSP2 (digested with *Nsi*I and *Stu*I) and the PCR product, following the marker-swap protocol of Kelly and Hoffman (Kelly and Hoffman 2002), to create plasmid pRH3.

### Construction of *lys2* ::*his7*<sup>+</sup> and *his7* ::*lys2*<sup>+</sup> deletion alleles

The *lys2* ::*his7*<sup>+</sup> and *his7* ::*lys2*<sup>+</sup> deletion alleles were constructed by a PCR-based strategy. A *lys2* ::*his7*<sup>+</sup> deletion construct was created by PCR amplification of *his7*<sup>+</sup> from plasmid pMN1 (Apolinario et al. 1993), using oligonucleotides *lys2* ::*his7*for (5'-ATTTATCCAGATTTAGAGCGTAACTTGTAAGCTAATAGTGAAATTTATTATTATATCCAAGCAAGGATTTGCTATGCC-3') and *lys2* ::*his7*rev (5'-AAAATAAAGTTGAAAAAATGAAAATCAACGTTGAAAAGAATGTCAACATATTAATGACCGACTGCTATCATCTGCG-3'). Sequences complementary to *his7*<sup>+</sup> are underlined, while the oligonucleotide 5' ends contain sequences flanking *lys2*<sup>+</sup>. A *his7D* ::*lys2*<sup>+</sup> deletion construct was created by PCR amplification of *lys2*<sup>+</sup> from plasmid pRH1, using oligonucleotides *his7* ::*lys2*for (5'-

TGATTTTATTTCAACTAAATTAAGAGTATTATAAATGCCACCAAAGACTGTTTC  
 CTAACGCATGCTACGAACTAGTCTG-3') and *his7*:*lys2*rev (5'-  
 AATAATTACACAAAGTAAAAATTTCCGCATTAGCAAGGTTTTAGTAGACTACAC  
 CTTAGTGGGAAGATAGATTGATGGAC-3'). Sequences complementary to *lys2*<sup>+</sup> are  
 underlined, while the oligonucleotide 5' ends contain sequences flanking *his7*<sup>+</sup>. Cells were  
 transformed to His<sup>+</sup> or Lys<sup>+</sup> (Bähler et al. 1998) and homologous recombination events were  
 confirmed by PCR analysis using *his7*testfor (5'-GACTCGCATGATTCCATCCATC-3')  
 and *his7*testrev (5'-CAGAAGGTATACACAGCGACA-3') or *lys2*testfor (5'-  
 CGTAAAAAAGAAAGAGCTGAGCG-3') and *lys2*testrev (5'-  
 GCAGTACGCAAAGATCCTAAGCT-3').

### Construction of *ade6* ::*his7*<sup>+</sup> and *ade6* ::*lys2*<sup>+</sup> deletion alleles

The *ade6*<sup>+</sup> gene was deleted by a one-step PCR-based method. Oligonucleotides *ade6*-  
*his7*for (5'-  
 CTATGCAGAATAATTTTTCCAACCAACTTTTTCTAATTTATCAGCCTTTTCTAAA  
 ACCAAGCAAGGATTTTCGCTATGCC-3') and *ade6*-*his7*rev (5'-  
 ATGAGCGAAAAACAGGTTGTAGGGATCCTTGGAGGTGGTCAATTGGGCCGAAT  
 GATGGACCGACTGCTATCATCTGCG-3') were used to PCR-amplify *his7*<sup>+</sup> from  
 plasmid pMN1, while oligonucleotides *ade6*-*lys2*for (5'-  
 CTATGCAGAATAATTTTTCCAACCAACTTTTTCTAATTTATCAGCCTTTTCTAAA  
 AACGCATGCTACGAACTAGTCTG-3') and *ade6*-*lys2*rev (5'-  
 ATGAGCGAAAAACAGGTTGTAGGGATCCTTGGAGGTGGTCAATTGGGCCGAAT  
 GATGGTGGGAAGATAGATTGATGGAC-3') were used to PCR-amplify *lys2*<sup>+</sup> from  
 plasmid pRH1. Strains CHP1029 (*lys2* : *his7*<sup>+</sup>), CHP1030 (*his7* : *lys2*<sup>+</sup>), and CHP1031  
 (*lys2*-97 *his7*-366) were transformed to Lys<sup>+</sup> and His<sup>+</sup>, as appropriate (Bähler et al. 1998).  
 The percent of Ade<sup>-</sup> transformants was scored by replica plating to EMM-*ade* and to YEA-  
*ade*.

## Results and discussion

### *lys2*<sup>+</sup> Gene prediction and cloning

The initial goal of this study was to clone and develop a new selectable marker for *S. pombe*,  
 preferably in a biosynthetic pathway for which other markers are not available. While the *S.*  
*pombe lys1*<sup>+</sup> gene had been cloned (Beach et al. 1982; Ford et al. 1993), it is too large to be  
 used as a selectable marker. We chose to pursue *lys2*<sup>+</sup>, due to mapping data on the website  
<http://www.izb.unibe.ch/res/kohli/Chromo1.html> placing it 4.3 cM from *rad9*<sup>+</sup> (164 PD:1  
 NPD:9 TT). As *rad9*<sup>+</sup> has been cloned (Murray et al. 1991), its physical location on the  
 chromosome is known. Other chromosome 1 cross data (Table 2) indicate that 1 cM is  
 equivalent to approximately 7–12 kb in physical map distance. Examination of the *S. pombe*  
 GeneDB (<http://www.genedb.org/genedb/pombe/index.jsp>) for a *Saccharomyces cerevisiae*  
*LYS* gene homolog within 30.5–52.5 kb of *rad9*<sup>+</sup> reveals that SPAC343.16 is approximately  
 38 kb from *rad9*<sup>+</sup>, and is predicted to encode a homoaconitate hydratase that is 62%  
 identical to *Saccharomyces cerevisiae* Lys4. Based on these data, we set out to test whether  
 SPAC343.16 is *lys2*<sup>+</sup>.

To determine if SPAC343.16 is *lys2*<sup>+</sup>, we PCR-amplified this gene from strain 972 (*lys2*<sup>+</sup>) and cloned it into a vector that can transform *S. pombe leu1-32* strains to Leu<sup>+</sup>. The PCR product includes the SPAC343.16 promoter, so that the orientation of insertion into this *nmt*-driven expression vector will not affect function. Plasmids pRH1 and pRH2, carrying the insert in either orientation, complement *lys2-97* in strain CHP880, while failing to complement *lys1-131* in strain FWP42 (Fig. 1). Conversely, a clone of the *lys1*<sup>+</sup> gene, *plys1*<sup>+</sup>, which we previously obtained from a pWH5-based genomic library (Feilotter et al. 1991), transforms FWP42, but not CHP880, to Lys<sup>+</sup>. Thus, SPAC343.16 is *lys2*<sup>+</sup>.

### Construction of a *lys2*<sup>+</sup>-based cloning vector

Using a marker-swap protocol (Kelly and Hoffman 2002), we replaced *Saccharomyces cerevisiae URA3*<sup>+</sup> in plasmid pSP2 (Cottarel et al. 1993) with *lys2*<sup>+</sup> to create plasmid pRH3 (Fig. 2). This plasmid possesses the *S. pombe ars1* to facilitate high-efficiency transformation, along with a polylinker with unique restriction sites for *XhoI*, *EcoRI*, *SmaI*, *XmaI*, *BamHI*, *SpeI*, *NotI*, *EagI*, *SacII*, and *BstXI*. In addition, pRH3 can be used in a blue/white screen in *E. coli* to identify plasmids containing insert DNA. Plasmid pRH3 can transform CHP871 (*lys2-97*) cells to Lys<sup>+</sup> and produces blue colonies when introduced into *E. coli* strain TOP10 (data not shown). Thus, pRH3 should be a useful cloning vector for transforming *S. pombe* strains that carry markers in the more commonly used leucine, uracil and histidine biosynthetic pathways due to the presence of other plasmids or marked chromosomal insertions. Using the *lys2*<sup>+</sup>-specific sequences employed in this construction (see Materials and methods), it would be a simple matter to replace the selectable marker in other *S. pombe* vectors with *lys2*<sup>+</sup>.

### Construction of *lys2* ::*his7*<sup>+</sup> and *his7* ::*lys2*<sup>+</sup> deletion alleles using PCR products

Polymerase chain reaction-based gene deletions generally utilize long oligonucleotides whose 3' ends prime amplification of a selectable marker and whose 5' ends provide targeting sequences to target insertion into the genome by homologous recombination. We designed two sets of oligonucleotides; one to target *his7*<sup>+</sup> to the *lys2*<sup>+</sup> locus (*lys2* ::*his7*<sup>+</sup>) and a second to target *lys2*<sup>+</sup> to the *his7*<sup>+</sup> locus (*his7* ::*lys2*<sup>+</sup>). PCR amplification from plasmids pMN1 (*his7*<sup>+</sup>, Apolinario et al. 1993) and pRH1 (*lys2*<sup>+</sup>) resulted in the expected 1.7 and 2.8 kb products (data not shown), which were used to transform strain FWP58 (*his7-366*) to His<sup>+</sup> and strain CHP871 (*lys2-97*) to Lys<sup>+</sup>. Less than one percent of transformants in each transformation acquired the corresponding auxotrophy due to homologous recombination. This low frequency is presumably due to the ability of the transforming DNA to repair *his7-366* and *lys2-97* at their respective loci. PCR analysis confirmed the homologous recombination events to create the *lys2* ::*his7*<sup>+</sup> and *his7* ::*lys2*<sup>+</sup> alleles (Fig. 3a) in strains CHP1029 and CHP1030, respectively. As expected, the 3.2 kb product for the *lys2*<sup>+</sup> locus is reduced to 2.1 kb due to its replacement with the smaller *his7*<sup>+</sup> gene to create the *lys2* ::*his7*<sup>+</sup> allele. Conversely, the 2.2 kb product for the *his7*<sup>+</sup> locus is increased to 3.4 kb due to its replacement with the larger *lys2*<sup>+</sup> to create the *his7* ::*lys2*<sup>+</sup> allele (an additional 1.9 kb nonspecific band is also visible in the *his7* diagnostic lanes).

This was our first attempt to use either *lys2*<sup>+</sup> or *his7*<sup>+</sup> in a PCR-based deletion protocol. We therefore needed to verify that these amplified products confer histidine and lysine

prototrophy in single copy, so that future deletion protocols can use the same priming sequences. The PCR analysis in Fig. 3a uses oligonucleotides that flank the targeting sites for each deletion, thus the size of the PCR product demonstrates that each deletion contains a single insertion of the selectable marker, as opposed to a tandem insertion. To determine whether the deletion strains CHP1029 (*his7-366 lys2 ::his7<sup>+</sup>*) and CHP1030 (*his7 ::lys2<sup>+</sup> lys2-97*) carry insertions of the PCR products at additional genetic loci, we crossed each strain with CHP1031 (*his7-366 lys2-97*) and examined the segregation pattern for the His<sup>+</sup> and Lys<sup>+</sup> phenotypes in the progeny. A single integration should produce a 2:2 pattern, while integrations at multiple loci would result in other patterns depending on whether a single copy of the marker in question is sufficient to confer a His<sup>+</sup> or Lys<sup>+</sup> phenotype. As shown in Fig. 3b, all ten tetrads from each cross display a 2:2 pattern indicating the presence of a single copy of the respective markers. More than 30 additional tetrads from each cross also showed a 2:2 segregation pattern (data not shown). Therefore, single copy integrations of these *his7<sup>+</sup>*- and *lys2<sup>+</sup>*-marked PCR products confer His<sup>+</sup> and Lys<sup>+</sup> prototrophy, respectively.

### Increased efficiency of homologous gene deletion in *lys2 ::his7<sup>+</sup>* and *his7 ::lys2<sup>+</sup>* strains

As mentioned above, we assumed that the low homologous recombination frequency observed while constructing the *lys2 ::his7<sup>+</sup>* and *his7 ::lys2<sup>+</sup>* strains was due to relatively high frequency repair of the *his7-366* and *lys2-97* alleles. To determine whether strains bearing the *lys2 ::his7<sup>+</sup>* and *his7 ::lys2<sup>+</sup>* deletions could serve as improved hosts for PCR-based gene deletions, we compared the efficiency with which we could delete *ade6<sup>+</sup>* from CHP1029 (*his7-366 lys2 ::his7<sup>+</sup>*), CHP1030 (*his7 ::lys2<sup>+</sup> lys2-97*), and CHP1031 (*his7-366 lys2-97*). When using CHP1031 cells, >0.3% of the Lys<sup>+</sup> transformants (0/350) were Ade<sup>-</sup>, and 0.2% of the His<sup>+</sup> transformants (1/550) were Ade<sup>-</sup>. In contrast, 14.3% of the Lys<sup>+</sup> CHP1029 transformants (1/7) were Ade<sup>-</sup>, and 13.7% of the His<sup>+</sup> CHP1030 transformants (19/139) were Ade<sup>-</sup>. Therefore, strains bearing these deletion alleles are significantly better for use in PCR-based gene deletion protocols. In addition, we have carried out subsequent crosses to produce sets of strains appropriate for the construction of diploid strains that are homozygous for *lys2 ::his7<sup>+</sup>* or *his7 ::lys2<sup>+</sup>* to be used in gene knockout experiments (see Table 1, strains CHP1050-1057).

## Conclusions

In this study, we used genetic and physical mapping information to identify the *S. pombe lys2<sup>+</sup>* gene, demonstrating that three different plasmid constructs carrying this gene complement *lys2-97*, but not *lys1-131* (Fig. 1; data not shown). One of these plasmids, pRH3 (Fig. 2), should be a useful cloning vector as it contains an *S. pombe ars*, conferring high efficiency transformation, and a large polylinker to accommodate a variety of cloning strategies. In addition, one can apply a blue/white screen to identify pRH3 derivatives that carry insert DNA.

We have also demonstrated the ability to use both *lys2<sup>+</sup>* and *his7<sup>+</sup>* in PCR-based gene disruption constructs. In doing this, we have identified sequences (see Materials and methods) capable of PCR-amplifying these two markers such that they are functional in

single copy. By deleting the *his7<sup>+</sup>* gene with *lys2<sup>+</sup>* and deleting the *lys2<sup>+</sup>* gene with *his7<sup>+</sup>*, we have created strains that are useful hosts for subsequent gene deletions. Previous efforts to use *his7<sup>+</sup>* as a selectable marker for gene deletions were hampered by the fact that *his7-366* can be directly repaired by the transforming DNA (Apolinario et al. 1993; Jin et al. 1995; Landry et al. 2000). As shown here, this problem is greatly enhanced when using a PCR-based strategy, since the deletion cassette possesses little sequence homology to the gene to be deleted relative to the homology between the selectable marker and the spontaneous mutant allele in the host. Since the PCR-amplified markers used in this study do not share homology with the deletion alleles, repair at the site of the deletion allele is not possible. As expected, the PCR products used to delete *ade6<sup>+</sup>* produced more than tenfold fewer total transformants in strains CHP1029 (*his7-366 lys2<sup>+</sup> :his7<sup>+</sup>*) and CHP1030 (*his7<sup>+</sup> :lys2<sup>+</sup> lys2-97*), than in strain CHP1031 (*his7-366 lys2-97*) using comparable amounts of transforming DNA. The total number of Lys<sup>+</sup> CHP1029 transformants was especially low, possibly indicating suboptimal expression of Lys2 in this particular context, however we were readily able to identify an Ade<sup>-</sup> transformant among the seven Lys<sup>+</sup> colonies.

*lys2<sup>+</sup>* is one of many genes whose identities could be predicted using genetic mapping data and genomic sequence information. In fact, a list of gene predictions can be found at [http://www.sanger.ac.uk/Projects/S\\_pombe/genetic\\_map.shtml](http://www.sanger.ac.uk/Projects/S_pombe/genetic_map.shtml). While most genes of the methionine biosynthetic pathway have not been cloned, the fact that methionine added to the growth medium derepresses conjugation and other glucose-regulated behaviors suggests that the use of a marker in this pathway could be problematic (Schweingruber et al. 1998). The list of predicted, but not cloned, genes includes all four tryptophan biosynthesis genes. Thus, while many biosynthetic pathway genes remain to be cloned, either *trp3<sup>+</sup>* or *trp4<sup>+</sup>* may be particularly useful due to their small size and the fact that no other marker from this pathway is currently in use.

## Acknowledgments

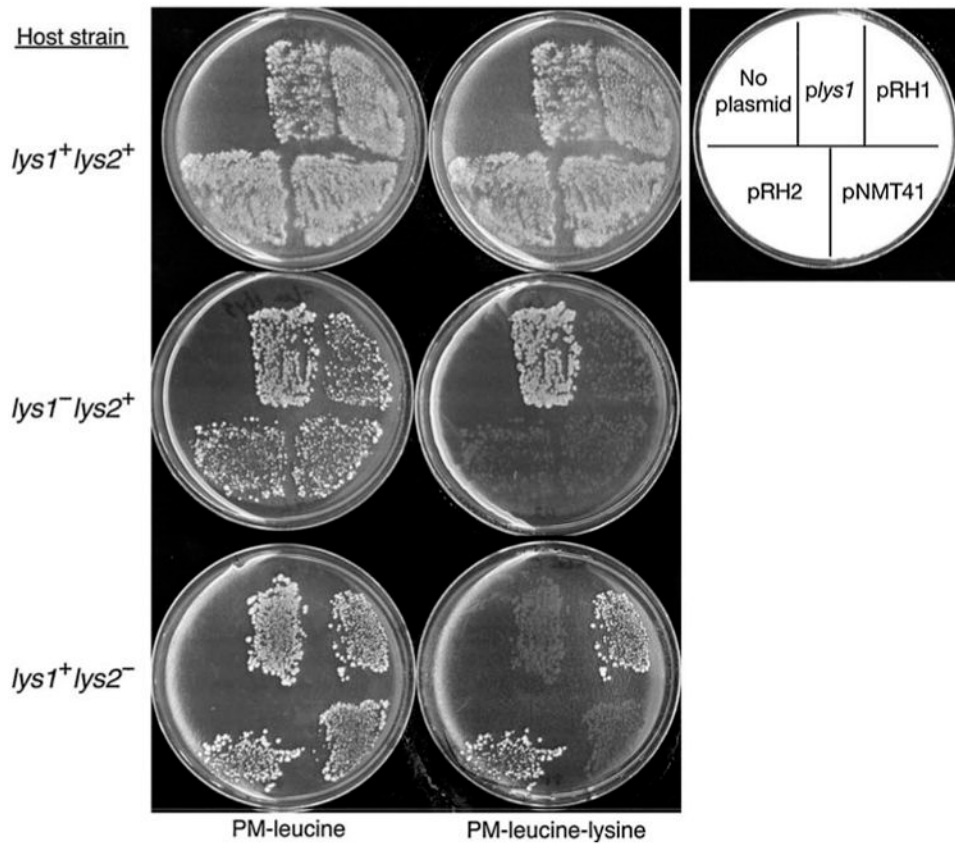
We thank Val Wood for pointing out other efforts to predict the identities of *S. pombe* genes based on genetic and map distance information. We thank Doug Ivey for critical reading of the manuscript. This work was supported by National Institutes of Health grant GM46226 to C.S.H. and a Boston College Undergraduate Research Fellowship to R.L.H.

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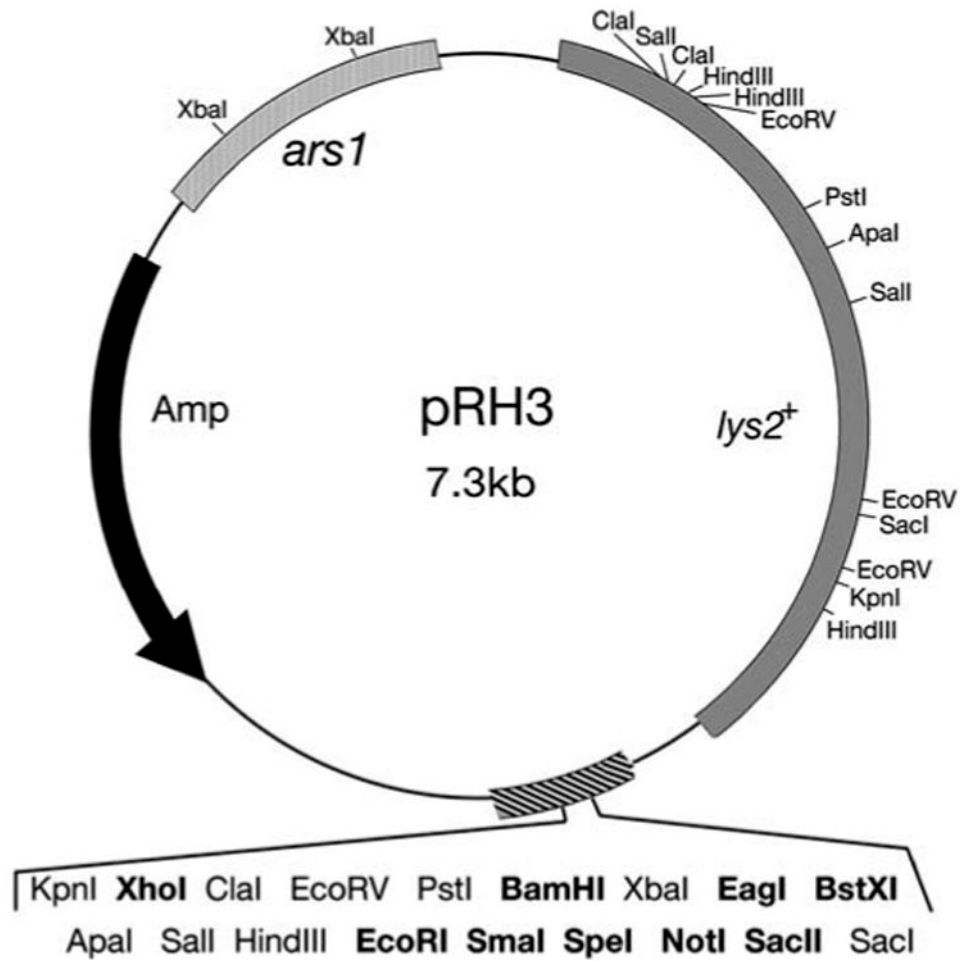
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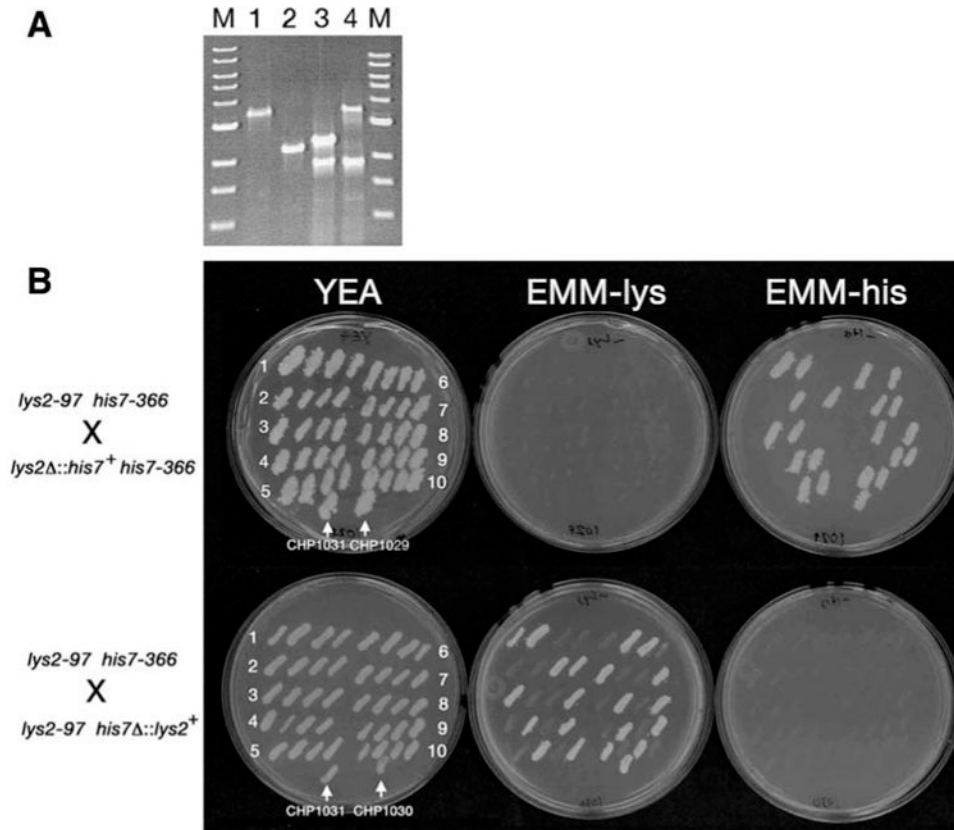




**Fig. 1.** Plasmid complementation of *lys1-131* and *lys2-97* mutant alleles. Strains FWP45 (*lys1<sup>+</sup>lys2<sup>+</sup>*), FWP42 (*lys1-131*), and CHP880 (*lys2-97*) were transformed to Leu<sup>+</sup> with plasmids *plys1<sup>+</sup>* (pWH5 vector carrying *lys1<sup>+</sup>* genomic DNA), pRH1 (pNMT41 carrying *lys2<sup>+</sup>* PCR product), pRH2 (pNMT41 carrying *lys2<sup>+</sup>* PCR product inserted in the opposite orientation from that of pRH1), or pNMT41 (empty vector). A mock transformation (no plasmid) was carried out in the absence of plasmid DNA. Leu<sup>+</sup> transformants were replica plated to PM medium lacking leucine and lysine. Plasmid *plys1<sup>+</sup>* is shown to complement the *lys1-131* mutant allele, while plasmids pRH1 and pRH2 complement the *lys2-97* allele



**Fig. 2.** Schematic of the pRH3 cloning vector. Plasmid pRH3 was constructed by marker swap transformation, replacing the *URA3<sup>+</sup>* marker of plasmid pSP2 (Cottarel et al. 1993) with the *lys2<sup>+</sup>* gene. Unique restriction sites in the polylinker are shown in *bold*. The relative locations of polylinker sites that are present elsewhere in the plasmid are also shown



**Fig. 3.** PCR-based gene disruptions using *lys2*<sup>+</sup> and *his7*<sup>+</sup> markers. **a** PCR analysis of the *lys2*<sup>+</sup> (Lanes 1 and 2) and *his7*<sup>+</sup> (Lanes 3 and 4) loci from strains FWP58 (*lys2*<sup>+</sup>; Lane 1), CHP1029 (*lys2* :*his7*<sup>+</sup>; Lane 2), CHP1028 (*his7*<sup>+</sup>; Lane 3), and CHP1030 (*his7* :*lys2*<sup>+</sup>; Lane 4). Lanes marked M contain a 1 kb ladder size marker. **b** Tetrad analysis of strains CHP1029 (*lys2* :*his7*<sup>+</sup>) and CHP1030 (*his7* :*lys2*<sup>+</sup>) crossed with CHP1031. Ten tetrads from each cross were patched onto a YEA plate and then replica plated to EMM-lys, EMM-his, and YEA. The 2:2 segregation patterns for the growth phenotype conferred by the respective selectable markers demonstrates that CHP1029 and CHP1030 each carry integration at a single genetic locus. The PCR product size in **a** demonstrates that a single copy of the integration cassette is present at each locus

Table 1

## Strain list

Strain	Genotype
972	<i>h</i> <sup>-</sup>
FWP42	<i>h</i> <sup>-</sup> <i>leu1-32 lys1-131</i>
FWP45	<i>h</i> <sup>+</sup> <i>leu1-32</i>
FWP58	<i>h</i> <sup>+</sup> <i>his7-366</i>
CHP871	<i>h</i> <sup>-</sup> <i>his6-365 lys2-97</i>
CHP880	<i>h</i> <sup>-</sup> <i>ura4::jbp1-lacZ leu1-32 his6-365 lys2-97</i>
CHP1028	<i>h</i> <sup>+</sup> <i>lys2-97</i>
CHP1029	<i>h</i> <sup>+</sup> <i>his7-366 lys2::his7</i> <sup>+</sup>
CHP1030	<i>h</i> <sup>+</sup> <i>lys2-97 his7::lys2</i> <sup>+</sup>
CHP1031	<i>h</i> <sup>-</sup> <i>ura4-D18 leu1-32 his7-366 lys2-97</i>
CHP1050	<i>h</i> <sup>+</sup> <i>ura4-D18 leu1-32 ade6-M210 his7::lys2</i> <sup>+</sup>
CHP1051	<i>h</i> <sup>-</sup> <i>ura4-D18 leu1-32 ade6-M210 his7::lys2</i> <sup>+</sup>
CHP1052	<i>h</i> <sup>+</sup> <i>ura4-D18 leu1-32 ade6-M216 his7::lys2</i> <sup>+</sup>
CHP1053	<i>h</i> <sup>-</sup> <i>ura4-D18 leu1-32 ade6-M216 his7::lys2</i> <sup>+</sup>
CHP1054	<i>h</i> <sup>+</sup> <i>ura4-D18 leu1-32 ade6-M210 lys2::his7</i> <sup>+</sup>
CHP1055	<i>h</i> <sup>-</sup> <i>ura4-D18 leu1-32 ade6-M210 lys2::his7</i> <sup>+</sup>
CHP1056	<i>h</i> <sup>+</sup> <i>ura4-D18 leu1-32 ade6-M216 lys2::his7</i> <sup>+</sup>
CHP1057	<i>h</i> <sup>-</sup> <i>ura4-D18 leu1-32 ade6-M216 lys2::his7</i> <sup>+</sup>

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**Table 2**  
**Genetic and physical map distances for Chromosome 1 gene pairs**

Gene pair	Map distance (cM)	Physical distance	kb/cM
<i>cdc3-cdc16</i>	23.7	168 kb	7.1
<i>cdc12-cdc25</i>	26.7	195 kb	7.3
<i>arg3-spo3</i>	18.0	220 kb	12.2
<i>rad9-lys2</i>	4.3	30.5–52.5 <sup>a</sup>	

Values obtained from <http://www.izb.unibe.ch/res/kohli/Chromo1.html>

<sup>a</sup>Distance range is an estimate based on the ratio of physical to genetic map distance from the other three gene pairs

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