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# *S. pombe* genome deletion project
## An update

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The fission yeast *Schizosaccharomyces pombe* is a model organism used widely to study various aspects of eukaryotic biology. A collection of heterozygous diploid strains containing individual deletions in nearly all *S. pombe* genes has been created using a PCR based strategy. However, deletion of some genes has not been possible using this methodology. Here we use an efficient knockout strategy based on plasmids that contain large regions homologous to the target gene to delete an additional 29 genes. The collection of deletion mutants now covers 99% of the fission yeast open reading frames.

## Introduction



Although forward genetic screens have provided many important insights into various aspects of cell biology, they are limited by the large numbers of mutants that must be analyzed and inherent biases in mutagenesis techniques. Systematic reverse genetic screens using genome-wide gene deletion collections or RNAi libraries provide a powerful alternative to forward genetic screens (reviewed in refs. 1–5). Studies with the budding yeast *Saccharomyces cerevisiae* have shown the usefulness of such gene deletion collection for numerous studies including drug discovery (reviewed in refs. 6–9). The fission yeast *S. pombe* is an important model organism sharing many features with cells of higher eukaryotes. The availability of the *S. pombe* genome sequence[10] and PCR-based gene deletion technology[11] allowed researchers involved in the *S. pombe* genome deletion project (KRIBB-Bioneer-CRUK consortium) to generate a set of 4.836 heterozygous diploid deletion mutants covering 98.4% of the fission yeast open reading frames (Kim et al., in press). However, for some genes a deletion could either not be constructed or the analysis of mutant phenotypes gave ambiguous results. Here we use an efficient knock-out strategy to delete 29 of these genes.

## Results

We speculated that some of the genes which could not be deleted by the KRIBB-Bioneer-CRUK consortium may require longer regions of homology for efficient gene targeting[12,13] and may be amenable to knockout by our recently developed technique.[14] This protocol is based on knockout constructs that contain large regions homologous to the target gene cloned into vectors. The cloning vectors pCloneNat1 and pCloneHyg1 contain dominant drug resistance markers conferring resistance to nourseothricin (clonat) and hygromycin B, respectively.[15] Here we introduce two new cloning vectors pCloneKan1 and pCloneBle1 which contain dominant drug resistance markers conferring resistance to geneticin and phleomycin, respectively (**Fig. 1**). Both pCloneKan1 and pCloneBle1 are compatible with our knockout protocol as described in Gregan et al.[15] and mendel.imp.ac.at/Pombe_deletion.

To demonstrate the usefulness of the pCloneBle1 vector, we used it to create knockout construct for deletion of the *hhp1* gene encoding the casein kinase 1.[16] After transformation of a linearized pCloneBle1-*hhp1* plasmid into fission yeast we were able to recover phleomycin-resistant colonies. Colony PCR showed that we had successfully deleted the *hhp1* gene in 7 out of 10 phleomycin-resistant transformants. The pCloneBle1 vector conferred resistance to both phleomycin and zeocin, which is a commercially produced antibiotic containing phleomycin (**Fig. 2**).

We used the pCloneKan1 to create knockout constructs of 64 genes for which deletion could either not be constructed, the analysis of mutant phenotypes gave ambiguous results such as abnormal segregation of the selection marker after tetrad analysis or the phenotype was different from published data (Kwang-Lae Hoe, unpublished data). These constructs contained regions of homology, which were larger than those used by the

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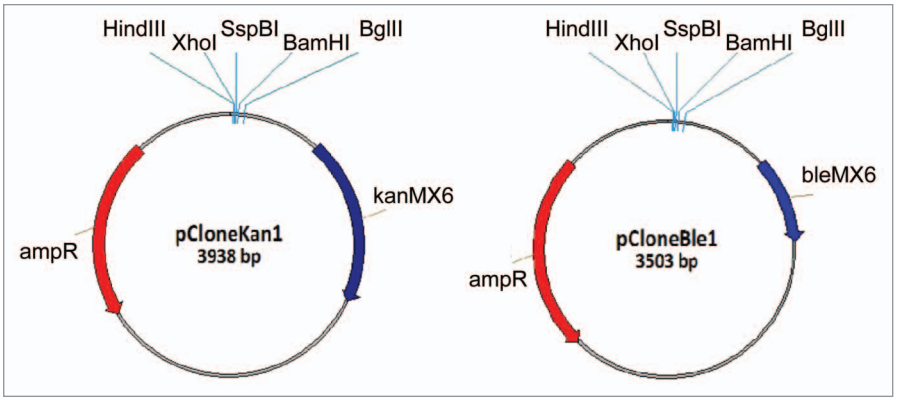


**Figure 1.** Maps of cloning vectors pCloneKan1 and pCloneBle1. Restriction sites used for cloning of the homology regions are indicated. Nucleotide sequence of pCloneKan1 (GQ354684) and pCloneBle1 (GQ354685) can be found in GenBank.

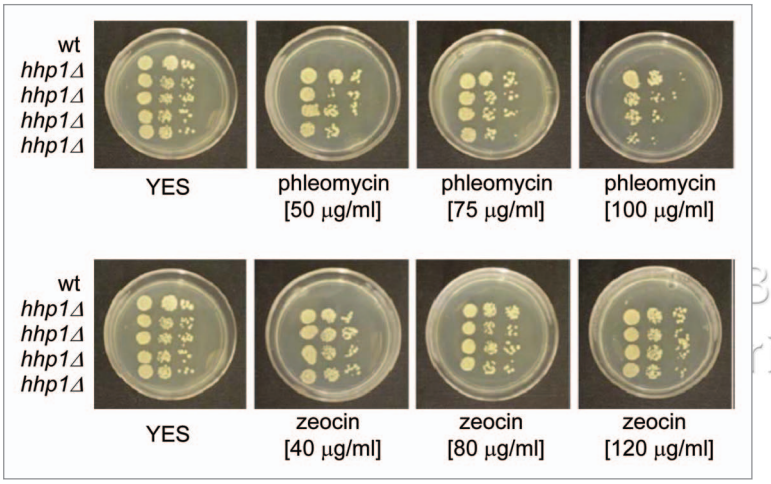


**Figure 2.** pCloneBle1 confers resistance to phleomycin and zeocin. To test the usefulness of the pCloneBle1, we used this plasmid to delete *hhp1* gene. We first constructed *hhp1* deletion plasmid (pCloneBle1-*hhp1*) according to protocol described in Gregan et al.[15] After linearization of the pCloneBle1-*hhp1* plasmid with the restriction enzyme XbaI, we transformed it into a wild-type *S. pombe* strain and selected for phleomycin-resistant colonies. Colony-PCR showed that we successfully deleted *hhp1* in 7 out of 10 tested colonies. Transformants were resistant to both phleomycin and zeocin, which is a commercially produced antibiotic containing phleomycin. A 10-fold dilution series of wild-type (wt) (JG11318) and four transformants where *hhp1* has been deleted with pCloneBle1-*hhp1* construct (JG15352) were spotted onto YES plates containing the indicated concentrations [μg/ml] of either phleomycin or zeocin. Plates were incubated at 32°C for 3 days.

KRIBB-Bioneer-CRUK consortium (350–800 bp compared to 80–350 bp) (Kim et al. in press). We found that indeed, after transformation of knockout plasmids into a diploid *S. pombe* strain we were able to create heterozygous knockout mutants for 49 genes. Out of the 49 deleted genes, 29 strains were missing from the *S. pombe* genome deletion collection and the remaining 21 strains were used to confirm ambiguous data (Kwang-Lae Hoe, unpublished data). To identify genes essential for vegetative growth, we sporulated the heterozygous diploid strains and dissected asci on rich medium. 13 of the 49 genes analyzed were essential for vegetative growth (**Table 1, Fig. S1**). Five of these 13 essential genes have been previously described.[17-21]

We next tried to find common characteristics that could explain why these genes were difficult to delete. However, gene ontology (GO) analysis of the deleted genes showed no enrichment for any particular GO category. The genes were randomly distributed along all three *S. pombe* chromosomes and showed a similar percentage of essential genes (26%) to that calculated from the analysis of 4.836 *S. pombe* genes (26.1%) (Kim et al., in press). We conclude that for these genes the length of homology for recombination during construction of the deletion is likely to be important.

## Discussion

In summary, we describe two new plasmids for gene deletions in the fission yeast *S. pombe*. The plasmids pCloneKan1 and pCloneBle1 can be used for single gene deletions, but they are particularly suitable for high-throughput knockout screens and for deletion of genes which require large regions of homology. Both pCloneKan1 and pCloneBle1 are compatible with the knockout protocol described in Gregan et al.[14,15] Detailed instructions how to use these plasmids to prepare knockout constructs for all predicted fission yeast genes is available in a form of searchable database//mendel.imp.ac.at/Pombe_deletion. The added advantage of this strategy is that a library of knockout plasmids is created which can be used to knockout genes in strains with various genetic backgrounds. Importantly, we used the pCloneKan1 plasmid to make 29 deletion strains, which were missing from the *S. pombe* genome deletion collection (Kim et al. in press). Together with the previously constructed 4.836 heterozygous deletions, this covers 99% of the fission yeast open reading frames. Given the fact that fission yeast is an important model organism, we expect that this genome-wide deletion collection will become an important tool for studying molecular aspects of eukaryotic biology and will accelerate the use of *S. pombe* for various comparative studies of eukaryotic cell processes.

## Materials and Methods

*S. pombe* media and growth conditions were as described in.[22,23] G418 (Gibco), Zeocin (InvivoGen) and Phleomycin (Sigma) solutions were added to media after autoclaving.

To create plasmid pCloneKan1, we first mutated HindIII restriction site in *kanMX6* gene of the plasmid pFA6aKanMX6 using site-directed mutagenesis kit (Stratagene) and the following mutagenic oligonucleotides: TCT GGA AAG AAA TGC ATA AAC TTT TGC CAT TCT CAC CGG and CCG GTG

AGA ATG GCA AAA GTT TAT GCA TTT CTT TCC AGA. Then we replaced the EcoRI/PstI fragment of the pCloneNat1 plasmid containing *natMX4* by the EcoRI/PstI fragment of the mutated plasmid pFA6aKanMX6.

To create plasmid pCloneBle1, we replaced the BglII/SpeI fragment of the pCloneNat1 plasmid containing *natMX4* by the BglII/SpeI fragment of the plasmid pFA6BleMX6 containing *bleMX6* conferring resistance to phleomycin.[24,25]

To create plasmid pCloneBle1-*hhp1*, we cut out *hhp1* homology regions from the plasmid pCloneNat1-*hhp1*,[26] using restriction enzymes HindIII and BamHI and inserted into HindIII/BamHI sites of the plasmid pCloneBle1.

All the pCloneKan1 and pCloneBle1 deletion constructs used in this study were made according to protocol described in Gregan et al.[15] and mendel.imp.ac.at/Pombe_deletion. In addition, dwin and upin oligonucleotides used for constructing pCloneKan1 deletion constructs contained tag sequences (barcodes) of 20 bp unique to each deletion mutant (**Table S1**). Large numbers of deletion strains can be pooled and analyzed in parallel in competitive growth assays. The barcodes allow identification of individual mutants in the pool of mutants.[1] The ability to assess deletion strains in parallel will significantly decrease the amount of labor and materials needed for fitness screens.

The pCloneKan1 deletion constructs were transformed into *S. pombe* strain SP286 (*ade6-M210/ade6-M216, leu1-32/leu1-32, ura4-D18/ura4-D18 h⁺/h⁺*) using a lithium acetate method. Transformants were selected on YES agar plates containing 100 μg/ml G418.

Essentiality was determined by a microscopic observation of colony-forming ability of spores on YES (rich medium) at 32°C. The spores were derived from corresponding heterozygous diploid deletion strains transformed with the pON177 plasmid containing the mat1-M sequence (a gift from O. Nielson).

Gene Ontology analysis was performed according to go.princeton.edu/cgi-bin/GOTermFinder.[27]

**Table 1.** Deletion phenotype

| Deleted gene | Mutant phenotype | Deleted gene | Mutant phenotype |
|---|---|---|---|
| SPAC1250.07* | essential | SPAC15E1.03* | viable |
| SPAC23C4.04c* | viable | SPCC1682.05c* | essential |
| SPAC27E2.12* | viable | SPBC119.10* | viable |
| SPAC56F8.07* | essential | SPAC4D7.08c* | viable |
| SPAPB1A10.16* | viable | SPAC1786.02 | viable |
| SPCC1393.14* | viable | | |
| SPAC1F3.04c* | viable | SPAC30D11.09 | viable |
| SPAC23D3.14c* | viable | SPAC3A11.05c | viable |
| SPAC2F7.16c* | viable | SPBC543.04 | essential |
| SPAC9.09* | viable | SPCC191.02c | viable |
| SPAPB15E9.01c* | viable | SPAC1952.13 | essential |
| SPAPB18E9.02c* | viable | SPAC1B1.03c | essential |
| SPBC16G5.19* | viable | SPAC1F7.04 | essential |
| SPBC17G9.06c* | viable | SPAC2C4.11c | essential |
| SPBC215.15* | viable | SPAC3H8.10 | viable |
| SPBC21D10.06c* | viable | SPAC56F8.10 | essential |
| SPBC23G7.09* | viable | SPAPB1E7.02c | viable |
| SPBC365.05c* | essential | SPBC146.13c | viable |
| SPBC685.08* | viable | SPBC16C6.09 | viable |
| SPBC6B1.12c* | viable | SPBC428.19c | essential |
| SPBC800.14c* | viable | SPCC18.04 | essential |
| SPBP23A10.11c* | viable | SPCC645.05c | essential |
| SPBPB8B6.03* | viable | SPAC589.12 | viable |
| SPBPB8B6.06c* | viable | SPBC1778.01c | viable |
| SPCC290.04* | viable | SPBC32F12.01c | viable |

*genes which were missing from the *S. pombe* genome deletion collection.




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### Note

Supplementary materials can be found at:
www.landesbioscience.com/supplement/SpirekCC9-12-Sup.pdf

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