A recombinatorial method useful for cloning dominant alleles in *Saccharomyces cerevisiae*

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A major inconvenience in cloning a dominant mutation in *Saccharomyces cerevisiae* is the requirement for the construction of a genomic library from the mutant strain (1). To alleviate the need for library construction, we present an alternative that is based on the ability of yeast cells to carry out precise and efficient homologous recombination (2).

In principle, a dominant mutation may be cloned by co-transforming (3) a genomic library of a wild-type strain with genomic DNA derived from the mutant strain and selecting for transformants with the mutant phenotype. These should represent the following events: (i) Recombination between the genomic DNA fragment from the mutant strain, carrying the mutant gene, and the respective locus in the genome of the wild-type recipient strain. (ii) Recombination between the same DNA and a library plasmid that contains the gene of interest, if they coexisted within the same cell. (iii) A spontaneous mutation resulting in the mutant phenotype. A fourth possibility where a few additional copies of a wild-type gene would result in the same mutant phenotype, is considered unlikely and would be a caveat even for the classical approach. Co-segregation of the mutant phenotype with the plasmid should facilitate the selection of the second class events that eventually result in the isolation of the mutant gene of interest.

We initially exploited the possibility of correcting a defect in a known gene carried on a centromeric plasmid, by applying the above outlined scheme of recombination between a plasmid and a genomic DNA fragment. A gcn2Δ, ura3-52 strain (4), sensitive to 10 mM of the drug 3-aminotriazole (3-AT), was co-transformed with 20 µg of a URA3 marked plasmid (YCp50) harbouring a defective GCN2 gene, along with 50 µg of genomic DNA (5) from the wild-type strain S288C. The GCN2 gene on the plasmid had been inactivated by digesting, filling in and religating the unique *Xho*I site within the GCN2 ORF (Fig. 1). Out of 20 000 primary Ura[+] transformants, seven were resistant to 3-AT after replica-plating on minimal plates containing 10 mM 3-AT. In six cases this resistance was shown to be plasmid borne following eviction of the plasmid by treating the transformed cells with 1.0 mg/ml 5-Fluoroorotic acid (5-FOA; ref. 2). The plasmids from these six transformants were recovered (6) and checked for the fidelity of the correction, i.e. restoration of the *Xho*I restriction site. The results are shown in Figure 1. In this experiment, a correction could not have resulted from exchange of information between the plasmid,
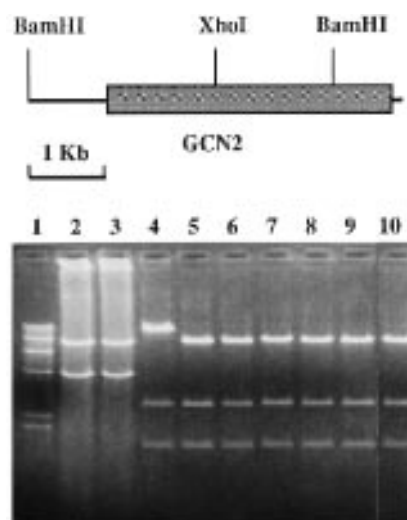
Figure 1. Repair of a gcn2 allele following co-transformation with genomic DNA fragments. (Top) Diagrammatic representation of the GCN2 gene locus showing the positions of the unique *Xho*I and the flanking *Bam*HI sites. (Bottom) Restriction analysis of GCN2 bearing plasmids. Lane 1, *Hin*dIII digest of phage λ DNA, used as size standard. Lane 2, the YCp50 plasmid carrying the gnc2 mutant gene generated by destroying the *Xho*I site, digested with *Bam*HI and *Xho*I. Lane 3, *Bam*HI/*Xho*I double digest of a plasmid recovered from a 3-AT sensitive transformant. No restoration of the *Xho*I is evident. Lanes 4–9, *Bam*HI/*Xho*I double digests of the plasmids recovered from the 3-AT resistant transformants. In all cases the *Xho*I site has been restored. Lane 10, *Bam*HI/*Xho*I control digest of YCp50 carrying the wild-type GCN2 gene.

carried by all transformed cells and the genome, since the strain transformed was deleted for the GCN2 gene.

In order to simulate conditions for cloning dominant alleles, the following approach was undertaken. A ura3-52, leu2-1 strain (RH1168) was transformed with 50 µg of a genomic DNA library derived from the same strain, on a URA3 marked plasmid (pRS316), along with 100 µg of genomic DNA prepared from the wild-type strain S288C. The complexity of the library was ~8000 independent plasmid clones with an average insert size of 7 kb. The size of the genomic DNA used was in the range of 50 kb. Following transformation, cells were allowed to recover for 6 h in

liquid minimal media containing 2.0 mM leucine, before plating on minimal plates for direct selection of uracil and leucine proto-trophs. About 100 000 primary Ura$^+$ transformants were plated, as measured by plating a fraction (1/1000) of the cells on a minimal plate containing 2.0 mM leucine. Out of those, 68 were also leucine prototrophs. These cells were cured of the library plasmid by replica plating on minimal, 0.3 mM uracil, 2.0 mM leucine, 1.0 mg/ml 5-FOA plates and in five instances the leucine prototrophy co-segregated with the plasmid (Table 1). The plasmids from these transformants were isolated and shown to complement both uracil and leucine auxotrophies when reintroduced to a ura3-52, leu2-1 strain. In addition these five plasmids carried DNA sequences encompassing the *LEU2* gene, as confirmed by restriction analysis (not shown). Finally, in a control experiment when the genomic DNA from the strain S288C was replaced with DNA prepared from the RH1168 strain, no leucine prototroph transformants were recovered (Table 1).

Table 1.

Transformants	RH1168 genomic library	
	+S288C genomic DNA	+RH1168 genomic DNA
Ura^{+a}	100 000	100 000
Ura$^+$, Leu^{+a}	68	0
5-FOA^r, Leu^{+b}	63	–
5-FOA^r, Leu^{-b}	5	–

A genomic library derived from RH1168 yeast cells was introduced to the RH1168 strain, either along with genomic DNA derived from the strain S288C (wild-type), or from the RH1168 strain (ura3-52, leu2-1).
[a]The numbers of prototroph transformants for the indicated metabolites, is shown.
[b]The segregation of the leucine prototrophy was examined following counter-selection for the plasmid borne URA3 marker gene.

The overall frequency of recovering useful recombination events among the transformants was an estimated 0.5×10^{-4}. This is much higher than predicted by simply multiplying the probability of coexistence within a cell, of a certain plasmid clone and its cognate piece of genomic DNA, with the probability of a productive recombination event between the two. Apparently more than one library plasmids and genomic DNA fragments enter a single cell during the transformation.

The described method may also be applied as an alternative to the Gap-Repair method (7) for retrieving mutant alleles of known genes from the genome of mutant strains, when there are not suitable restriction sites available to introduce a convenient gap in the gene. According to this alternative, genomic DNA from the mutant strain can be co-transformed with a plasmid bearing the wild-type gene of interest into mutant or wild-type cells depending on whether the allele is recessive or dominant. Plasmids that confer the mutant phenotype can then be isolated and analysed.

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