Problems with construction of haploid yeast with DNA polymerase alleles reducing fitness.

In previous classic studies, pol mutants were created either by a plasmid shuffling method (ARAKI *et al.* 1992) or by integration-excision into the genome of a vector carrying the allele of interest (MORRISON *et al.* 1991). The low stability of replicative plasmids is the first method's limitation, but it is popular because of simplicity (ARAKI *et al.* 1992; KESTI *et al.* 1999; DEVBHANDARI AND REMUS 2020). Integration is the most popular method (JIN *et al.* 2001; SIEBLER *et al.* 2014; BARBARI *et al.* 2018), but it is not well-suited for examining the phenotypes of lethal mutations or mutations severely reducing fitness. In this method, the mutant copy of a truncated or full-length *pol* allele of interest is integrated into the genome at its original location by transformation by a linearized plasmid. The procedure creates a duplication of the original and plasmid-borne alleles with a selective marker between them. It is essential to use a marker that can be counter-selected, like *URA3*. The single-copy mutant allele is obtained by selecting pop-out with the excision of one gene copy and the *URA3* marker on FOA medium, selective for *ura3* mutants. The integration-excision method works well for haploids and mutator pol alleles (MORRISON *et al.* 1991; MORRISON AND SUGINO 1992; BARBARI *et al.* 2018). If the mutation leads to lethality or weak growth, the pop-outs will possess almost always only a wild-type allele reconstituted by recombination. Problematic *pol* alleles could be created in diploids in a heterozygous state, but duplication of a polymerase gene and *URA3* will still be present inside the repeat (PAVLOV *et al.* 2001). Selection for pop-outs

in such diploids is difficult (though successes are reported (GARBACZ *et al.* 2018; TER BEEK *et al.* 2019)) because the rate of mitotic recombination between the gene and centromere is typically much higher than the rate of intra-chromosomal recombination and thus, most Ura⁻ clones will have a wild-type pol allele. If heterozygous diploids with *polX-URA3-POLX* sporulate, haploid segregants will possess mutant and wild-type alleles, and selection on FOA will again tend to give only clones bearing the wild-type allele. Because of these complications with the integration-excision approach, alternative methods are desirable.

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