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Short communication

A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance

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Summary. Mutations at the *URA3* locus of *Saccharomyces cerevisiae* can be obtained by a positive selection. Wild-type strains of yeast (or *ura3* mutant strains containing a plasmid-borne *URA3*$^+$ gene) are unable to grow on medium containing the pyrimidine analog 5-fluoro-orotic acid, whereas *ura3*$^-$ mutants grow normally. This selection, based on the loss of orotidine-5′-phosphate decarboxylase activity seems applicable to a variety of eucaryotic and procaryotic cells.

A 1.1 kb DNA fragment bearing the *URA3* gene (Bach et al. 1979) is commonly used as a selectable marker on many yeast/*E. coli* shuttle plasmids. Episomal plasmids (2 µm, YEp24; *ars*, YRp10; or centromere, YCp19, YCp50) and integrating plasmids (YIp5) are used extensively (Botstein et al. 1979) in transformation experiments. Often it is desirable to be able to select for the loss of a recombinant plasmid; when the plasmid is lost at low frequency, as in the case of an integrated plasmid, a positive selection for loss of the plasmid is almost a necessity. Such a positive selection based on increased resistance of *ura1*, *ura3* and *ura5* mutants to ureidosuccinate has been described (Bach and LaCroute 1972). However, this technique is very sensitive to physiological conditions and hence is not routinely used. Therefore, a new method for selection of *ura3*$^-$ cells using 5-fluoro-orotic acid has been developed. A recipe for the medium is given in Table 1. At the concentrations described, 5-FOA prevents the growth of wild-type yeast cells and permits the growth of cells with a mutation in the *URA3* gene. Strains containing the commonly used allele *ura3-52* grow well on 5-FOA. Hence, it is possible to select *ura3*$^-$ cells from among a large population of *URA*$^+$ cells. *ura3*$^-$ cells have an efficiency of plating of 1 on 5-FOA medium, whereas *URA*$^+$ cells will form colonies on the medium at a frequency of approximately 10^{-7}. When a relatively high frequency event ($10^{-4}-10^{-6}$) such as loss of an episomal or integrative plasmid, or homozygosis of a *ura3* mutation in a diploid is selected by 5-FOA, most or all of the 5-FOA^r colonies will be of the desired class. For example, of several hundred 5-FOA^r derivatives of integrated *URA3* plasmids, only one was *URA*$^+$. In experiments where low frequency events are selected, 5-FOA resistant colonies may result from mutations in genes other than *ura3*. In experiments where spontaneous or UV-induced 5-FOA resistant mutants were selected, only 5–10% of the mutants were *ura3*$^-$. We do not know how many different classes of mutants can give rise to a 5-FOA resistant phenotype; however, *ura1, 2* and *4* mutants are sensitive to the drug whereas *ura3* and *ura5* mutants are resistant (*ura5* mutants are only partially resistant) (see also Jund and LaCroute 1979).

5-FOA medium can be used to simplify gene replacement by transformation. In this procedure, mutations are made in a cloned yeast gene inserted into the integrating *URA3* plasmid Yip5 and then transferred to the yeast genome. The object of this experiment is to replace the resident wild-type gene with the mutationally altered plasmid borne gene. If *ura3-52* is used as a recipient the plasmid will usually integrate at the locus of the gene of interest (Winston et al. 1983). Integration of a circular plasmid by homologous recombination results in a tandem repeat of the gene carried on the plasmid (one mutant, one wild-type copy) flanking the plasmid sequences and the *URA3* gene. The final step of the procedure is to identify the *ura3*$^-$ cells in the population which have "popped out" the plasmid (and the *URA3* gene) by homologous recombination between the repeated segment leaving behind either the mutant or wild-type allele of the plasmid above. This event occurs at a frequency of 10^{-4} or less, so it is a tedious process to identify popouts by replica plating. The last step

Table 1. 5-FOA medium

1)	2× concentrate (filter sterilize):	
	Yeast nitrogen base	7 g
	5-fluoro-orotic acid[a]	1 g
	Uracil	50 mg
	Glucose	20 g
	H_2O	500 ml
2)	Add to 500 ml 4% agar (autoclaved):	

The concentration of 5-FOA may be cut in half for many applications, however, the background growth of *URA*$^+$ cells will be heavier

[a] Available from SCM Specialty Chemicals, P.O. Box 1466, Gainesville, FL, 32602, USA

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is greatly simplified by the use of 5-FOA medium. Cells containing the integrated plasmid are simply patched directly onto the 5-FOA plates. In this way, hundreds of independent popouts can be isolated with ease, since only strains lacking a *URA3*$^+$ region can grow. We have used this procedure to produce popouts at several different loci in yeast. The direct substitution method of Orr-Weaver et al. (1983) could be used to introduce in vitro constructed alleles into any gene. A recipient strain carrying the *URA3*$^+$ gene inserted within the gene to be mutagenized would simply be transformed with linear, mutated DNA spanning the point at which *URA3* is inserted, followed by 5-FOA selection. It should be noted that in this case one may have to apply the 5-FOA selection after a delay sufficient to allow expression of the *ura3*$^-$ phenotype.

5-FOA can also be used in the fission yeast, *Schizosaccharomyces pombe*. Wild-type *URA*$^+$ cells are sensitive to 5-FOA medium, but the *S. pombe ura4-294* mutant (analogous to a *S. cerevisiae ura3*$^-$ mutant) grows normally on 5-FOA medium. Episomal plasmids bearing the *S. cerevisiae URA3* gene can be selected against in strains carrying the *ura4-294* mutation.

There are many other uses for 5-FOA medium. For example, Winston et al. (1984) have shown that yeast transposons (Ty elements) marked with the *URA3* gene can be selected against with 5-FOA medium. 5-FOA resistant derivatives include "popouts" (delta-delta recombinants) of the Ty element and gene convertants of the Ty element (both classes lose the *URA3* gene). 5-FOA makes curing episomal plasmids for cosegration studies an easy task since hundreds of independent segregants can be obtained on a few plates. Another potential use for 5-FOA is the construction of isogenic strains by transformation with cloned *ura3-52* DNA (Rose and Winston 1984).

5-FOA may be useful in isolating *ura3* mutants in industrial or brewing yeasts where no useful auxotrophic markers for transformation exist. A protocol that would work even with diploid cells would be to mutagenize yeast cells and look for colonies which upon replica plating to 5-FOA medium would papillate 5-FOA resistant derivatives. If the mutagenesis produced *URA3/ura3* heterozygotes, these could papillate to *ura3/ura3* as they do in *S. cerevisiae URA3/ura3* heterozygotes.

5-FOA appears to have a broad spectrum. We have found that it inhibits the growth of *Saccharomyces, Schizosaccharomyces* and *Candida*. Also, *E. coli* HB101 is sensitive, while its *pyr*F derivative, DB6507, is not (*pyr*F encodes the same enzymatic step as *URA3*), suggesting a similar mode of action against yeast and bacteria.

The means by which 5-FOA kills yeast cells is not certain. It seems likely, however, in view of the resistance of OMP pyrophosphorylase-minus mutants (*ura5*) and OMP decarboxylase mutants (*ura3*) that this toxicity occurs through the conversion to 5-fluoro-UMP. Whether the lethality is ultimately caused by a fluorinated ribonucleotide or deoxyribonucleotide is uncertain. We found that of 40 *URA*$^+$ cells plated on 5-FOA medium and incubated for three days at 30° C, 15 recovered when transferred to YPD medium by micromanipulation, indicating that growth inhibition by 5-FOA is at least partially reversible. Interestingly, all of these survivors were petite. Petites are also found occasionally among the *ura3*$^-$ colonies selected on 5-FOA medium.

5-FOA is one member of a small chemical repertoire that permit yeast geneticists to select mutations in specific nutritional genes. α-amino-adipate selects mutations at the *LYS2* and *LYS5* loci (Chattoo et al. 1979) and methyl mercury selects for *met2* and *met15* mutations (Singh and Sherman 1974, 1975). However, 5-FOA promises to be the most versatile of these due to the wide variety of cloning vectors already available with the conveniently small *URA3* gene, which has been fully sequenced (Rose et al. 1984).

References

Bach M, LaCroute F (1972) Direct selective techniques for the isolation of pyrimidine auxotrophs in yeast. Mol Gen Genet 115:126–130

Bach M, LaCroute F, Botstein D (1979) Evidence for transcriptional regulation of orotidine-5′-phosphate decarboxylase in yeast by hybridization of mRNA to the yeast structural gene cloned in *Escherichia coli*. Proc Natl Acad Sci USA 76:386–391

Botstein D, Falco SC, Stewart SE, Brennan M, Scherer S, Stinchomb D, Struhl K, Davis RW (1979) Sterile host yeasts (SHY): A eukaryotic system of biological containment for recombinant DNA materials. Gene 8:17–24

Chattoo BB, Sherman F, Azubalis DA, Fjellstedt TA, Mehvert D, Ogur M (1979) Selection of *lys2* mutants of the yeast *Saccharomyces cerevisiae* by the utilization of α-amino-adipate. Genetics 93:51–65

Jund R, LaCroute F (1972) Regulation of orotidylic acid pyrophosphorylase in *Saccharomyces cerevisiae*. J Bacteriol 109:196–202

Orr-Weaver TL, Szostak JW, Rothstein RJ (1983) Genetic applications of yeast transformation with linear and gapped plasmids. In: Wu R, Grossman L, Moldave K (eds) Methods in enzymology: Recombinant DNA, Part B. Academic Press, New York, p 228

Rose M, Winston F (1984) Identification of a Ty insertion within the coding sequence of the *Saccharomyces cerevisiae URA3* gene. Mol Gen Genet 193:557–560

Rose M, Grisafi P, Botstein D (1984) Structure and function of the yeast *URA3* gene: Expression in *E. coli*. Gene, in press

Singh A, Sherman F (1974) Characteristics and relationships of mercury-resistant mutants and methionine auxotrophy of yeast. J Bacteriol 118:911–918

Singh A, Sherman F (1975) Genetic and physiological characterization of *met15* mutants of *Saccharomyces cerevisiae:* A selective system for forward and reverse mutations. Genetics 81:75–97

Winston F, Chumley FG, Fink GR (1983) Eviction and transplacement of mutant genes in yeast. In: Wu R, Grossman L, Moldave D (eds) Methods in enzymology: Recombinant DNA, Part B. Academic Press, New York, p 211

Winston F, Chaleff DT, Valent B, Fink GR (1984) Mutations affecting Ty-mediated expression of the *HIS4* gene of *Saccharomyces cerevisiae*. Genetics 107:179–197

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