

Yeast Idiosyncrasies

From Cora Styles (Fink Lab)

ade1* and *ade2 cultures turn red. The intensity depends on the amount of adenine in the medium. YPD is not rich in adenine, so cultures turn red in 2-3 days. Petites don't turn red.

asp5 requires asparagine added to YPD ascospore dissection plate. Extra asp needed only for germination; cultures grow normally on YPD. *asp5* turns red in aged cultures; not as fast or intense as *ade2* or *ade1*.

gcn4 has an arginine requirement (partial?) *canr* (arginine permease) makes *gcn4* grow very slowly.

trp slows growth, especially at lower temperatures. Extra *trp* in medium helps, but does not restore wildtype growth. Different mutants may show a range of phenotypes.

Petites may be rho minus or rho zero. Colonies are smaller and white, and their size can vary over a wide range in one culture.

Dissections Usually done on YPD. Some slow-growing mutants (*sit*, eg.) show increased germination frequency on GNA; other mutants germinate worse (report Botstein lab) on GNA.

Drop in The amount of amino acid stock solutions recommended for supplementing minimal medium: YNB (SD in the literature) is based on empirical and practical factors, and allows mutants with a single auxotrophy to grow like wild-type. Minimal medium with one to several additions is called drop-in.

Drop-out This is complete drop-in minus one. It is the same as the SC (synthetic complete) formula in the Cold Spring Harbor Yeast Manual. One or more amino acids is intentionally omitted (dropped out).

Minimal Media *E. coli* minimal vs. yeast minimal: wild-type *E. coli* grow equally well on complete (LB) and minimal (M9). Wild-type yeast grows slower on minimal (YNB = SD) than on complete (YPD). Wild-type yeast seems to grow equally well on complete (YPD) and on synthetic complete (SC), however.

Doubling time *S. cerevisiae* haploid in YPD liquid is 90 min. at 30°C. Stationary phase has a cell density of 2×10^8 per ml. In YNB (SD) it is less, about 5×10^7 per ml.

Psi Factor Zygote viability and possibly sporulation and spore viability may fail due to Ψ (psi), a cytoplasmic factor, that can arise and be lost spontaneously. Ψ enhances the effect of suppressors which can act as dominant (semi-) lethals in diploids when introduced from one parent into the Ψ^+ cytoplasm brought in by the other parent. See Protocol: Curing cytoplasm of Ψ^+ , C. Styles 1981 (Blue Protocols Notebook).

Sporulation Quality and efficiency is sensitive to many factors (genotypes and conditions). Sometimes, simply repeating the procedure (GNA 2 days at 30°C, SPO 1 day at 23°C, then 1-4 days at 30°C) improves results. A second sporulation medium (NGS) occasionally gives better results. Sometimes the opposite is true.

- Tetrad storage** Storage on sporulation medium should be at 4°C after one week of incubation. They remain reasonably viable for a couple of months. Longer incubation above 4°C toughens the diploid wall, requiring increased digestion for dissection. Asci of wild strains are harder to digest than those of laboratory strains.
- Homozygous diploids** Haploids form homozygous (a/a , α/α) diploids spontaneously at low frequency in cultures. Their proportions increase in continuously sub-cultured strains and can take over the culture (a common problem of "old" lab strains). Spheroplast transformation sometimes produces a high proportion of homozygous diploids. These are not easily distinguished from haploids. They mate the same as haploids and the resulting triploids form 4-spored tetrads. The differences in cell sizes is too slight to be a clear indicator of ploidy.
- Tetrads from triploids** When dissected, give extremely poor viability, in the range of 0 to 3 colonies from 10 tetrads. Yeast can't tolerate genomic imbalances of the disomic mixes resulting from meiosis. This is the key to detecting diploidy in a parent strain. The observation can be confirmed and the homozygous diploid recovered as a haploid:
- A) Cross the putative diploid by a known homozygous diploid of opposite MAT. Dissection shows restored ascospore viability, and both mating (a/a α/α) and non mating (a/α) offspring. The latter can be sporulated and will produce haploids.
 - B) Sporulate zygotes en mass on NGS from a 4-hour 30C mix with a *kar1* parent. The zygote "ascus" will contain a tetrad and a dyad. Dissection will produce haploids. Frequently the dyad spores do not germinate (conveniently).
- Inositol** Inositol is present in trace amounts in Difco Yeast Nitrogen Base. The medium supports partial growth of *ino* mutants, making it difficult to distinguish the mutant phenotype from wild type. We regularly supplement YNB preparations with inositol to allow *ino* mutants to grow like wild type. Medium lacking inositol is made "from scratch," using stock solutions stored over chloroform in a cold room according to the Cold Spring Harbor Manual formula. Difco Bacto agar should be used to make solid medium.
- Agar** Agar is a crudely refined natural product and varies greatly from one processor to another. More highly refined agar is more expensive, has fewer impurities, and usually makes weaker gels. We are using Difco Bacto agar for routine purposes. Less refined agar should be tested carefully. Some agars support *ura* mutants and some have a water-soluble substance toxic to wild type. Agar can be washed to avoid these problems if necessary.
- ade3** Requires both adenine and histidine.
- hom3** Requires methionine and threonine.
- TRP** *TRP* cultures leak tryptophan into the medium. After 2-3 days, the edges of *trp* patches in prints to *trp*-plates start to grow if they are adjacent to *TRP* patches.

- URA** Does the same as *TRP* cultures, but the cross-feeding is not seen until after 7-9 days.
- ade2** (and *ade 1*?) ("red ade's") grow slightly slower than wild type. Spontaneous mutations to Ade- which affect genes earlier in the pathway prevent intermediate products from reaching the point where the red pigment accumulates (which seems to inhibit growth). Such double mutants are white and have a competitive growth advantage. When doing sectoring assays, white sectors overgrow red sectors, and may blot them out over time.
- sup11** *Sup11* (and possibly other tRNA suppressors) elevate the rate of chromosome loss (J. Haber, recent Genetics paper). Experiments designed to use a *sup11-ade2* system for the purpose of detecting plasmid loss or chromosome segregation may be internally flawed. An *ade2-ade3* system may be safer (Fred Dietrich).
- Winston's S288C-isogenic** This series of strains, which are all GAL2, may all contain a chromosomal mutation which causes an elevated frequency of petites formation. When single colonies are plated on GNA medium, 1/4 or more are petites, and the rest are sectoring. The colonies after 5 days on GNA (and after a week on YPD) have a ragged circumference. This is not seen in Jim Thomas' S288C-derived *trp 1Δ1* series. When the series are crossed, the phenotype segregates 2:2. Fred is checking out the series at key points and will give us an update.
- trp1Δ1** inhibits growth of GAL strains (the deletion extends into the adjacent GAL3 gene). *trp1Δ63* does not inhibit GAL growth (deletion stops short of GAL3).
- ts-mutations** Ts mutations on plasmids covering a lethal null mutation may turn out to actually be nonsense mutations. After much time spent in characterizing their "ts" strains, this discovery has been made independently in our lab alone by Mark Rose (KAR1), Meg McLaughlin (GCN4) and Laura Davis (NUP1). Sequence to be sure you're not dealing with a nonsense mutation. Suppression may be being enhanced by PSI+. These false ts won't make colonies on YPD with guanidine hydrochloride at permissive temperature, i.e. curing PSI+ is lethal.
- Canavanine resistance** Is a spontaneous mutation easy to isolate. It is always a mutation in a single gene; the arginine permease. Wild type strains (CAN1) cease making arginine in the presence of the analog canavanine, take it up through their normal permease, and build it into their proteins - with fatal consequences. The addition of arginine to canavanine-containing medium restores viability. Hence media used for detection/selection of *can1* must be arginine free.
- Leucine** Leucine (L+) contains trace amounts of methionine as an impurity virtually impossible to entirely eliminate in its manufacture. Biochemically, the leucine is "essentially methionine-free," but in a growth test, methionine auxotrophs appear to be "leaky" or "disappear" when grown on medium containing leucine, especially in larger quantities as in drop-out. If your having difficulty tracking a methionine auxotroph, try to use strains which don't require leucine and omit it from the medium, or do a complementation test using Leu+ testers.