How to do recombination assays.
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Background

Recombination assays are a useful way of assessing the effect of mutations or natural polymorphism on recombination at a particular locus, either allelic recombination, which is a measure of gene conversion, or between two genes in a particular chromosomal interval, which is a measure of crossing over. Allelic assays require a cross that is heteroallelic, for example, between two strains each with a different *his-3* mutation. Non-allelic assays are between strains that have mutations in two distinct genes, each requiring a different supplement, which are in repulsion. The two assays differ only in the composition of selective plates. Prototrophy in a single gene is the outcome of allelic recombination so selective plates lack the corresponding supplement, while in the non-allelic assay, selective plates lack two supplements, which selects for progeny with a wild type copy of both genes on the same chromosome. Neither assay yields an absolute recombination frequency but both are useful for comparative studies..

It is quite difficult to obtain repeatable recombination data unless the crosses and subsequent assays are performed in a controlled way. For a meaningful comparison all crosses should be inoculated into the same batch of medium at the same time and all strains should be relatively fresh. They can be stored at -20ºC, but desiccated cultures are undesirable. Crosses should be incubated at 25ºC – this is critical, as variations in temperature during incubation can reduce fertility and alter recombination frequency. In warmer locations a refrigerated incubator is necessary to obtain repeatable data. This need not be a large expense, as we use a small wine chiller with Peltier cooling that we bought from a department stores for less than $200 AUD ($100 or so USD), and which was modified by the addition of a small heater and thermostat. For less tha $500AUD we have an incubator that maintains the temperature nicely .

It is important that for each assay all spores have as close to the same heat-shock time as possible, or germination rates and viability will vary. If I am doing more than four assays at the same time, I allow a 15 minute gap between each set of four to minimise …. Dilutions must be done with care, and as spores settle quickly, even in agar, pipetting should be carried out immediately after vortex mixing. When using a mechanical pipette, do not use the same tip more than once, as heating the tip changes the volume it will draw up. Test pipettes (just by weight, using distilled water) before a set of assays. We use 10 ml disposable pipettes for plating the spore suspension, as each holds three 3 ml aliquots at one time. These pipettes need not be discarded after each pipetting operation since they are graduated. One 10 ml pipette can be used for each assay, providing that plating begins with the highest dilution of spores onto the non-selective plates.

In order to count a large number of colonies, necessary for accurate data, it is helpful if the strains in the cross all have the colonial temperature-sensitive mutation *cot-1*. After germination at 20-25ºC for two hours to overnight, the plates can be moved to 34ºC to trigger the colonial phenotype, yielding compact colonies. Little time at the lower temperature yields highly compact colonies making it possible (though not much fun!) to count over 500 colonies on a single 8.5 cm petri dish. Good recombination data can be obtained from Cot^+ cultures but the spore density must be lower and plates must contain sorbose. *cot-1* progeny can be plated on sucrose, but the plates should be moved to 34ºC after no more than two hours, and incubated for at least three days at 34C before counting.

When adding spores to layer agar, the number to be added depends on the expected recombination frequency. *his-3* allelic recombination frequency varies from less than 0.01% to about 1%, depending on the alleles of *cog* and *rec-2* in the cross. Add the amount of spores that, after your chosen dilution, will result in 30-60 spores per ml in the layer agar to be plated on the non-selective medium, thus giving you 90-180 colonies to count on each plate. If you have no idea of the allelic recombination frequency, try a 1

in 20, followed by a 1 in 40 dilution, and plate 3 x 3 ml aliquots both of the two higher spore concentrations on selective medium.

It also assists counting if the plates are poured and the layer agar added on a level surface. It is very annoying if all your colonies end up on one half of the plate!

Procedure

Ascospores are harvested from a single crossing tube in sterile distilled water. After estimation of the number of spores by haemocytometer, an appropriate volume (in the above suggestion, this volume would include ~5 x 10^5 spores) is added to 20 ml layer agar (0.8% Difco agar, 2% sucrose, 2% Vogel's N medium), in an appropriate container (we use disposable 30 ml sterile plastic universal tubes, 24mm x 90mm), kept in a 60ºC waterbath or incubator. For a 1 in 800 dilution, vortex vigorously and transfer 1 ml to 19 ml molten layer agar; vortex this vigorously and transfer 0.5 ml to 19.5 ml layer agar. Following incubation at 60ºC for 45 - 70 minutes, 3 ml aliquots of the highest and lowest dilutions are plated on selective and non-selective medium respectively. Incubation can take as little as overnight (for Cot^+ cultures incubated on sorbose medium at 34ºC) to three days (2 hours at 20ºC, followed by three days at 34ºC on sucrose medium for *cot-1* strains).