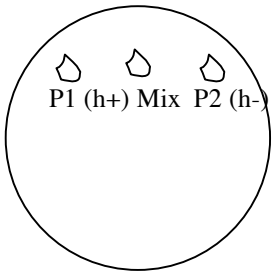
Rough guide to pombe genetics

Waking up strains

1. Use a sterile toothpick to transfer cells from frozen vial (-70°C) to plate. Do not thaw vial, put it back into the freezer as soon as possible, and do not remove whole strain box, only relevant vial. Use YES (no phloxin) plates if strain is not thiamine sensitive and you are not needing to select for unstable plasmid.
2. Check that h+ strains are haploid by streaking on phloxin YES (pink) plates – diploids are red, haploids are pink. It is a good idea to colony purify h+ strains by streaking from a single colony.

Setting up pombe crosses

3. Strains to use should be growing freshly (use YES unless there is some good reason to keep the strains on EMM).
4. Set up cross on EMMG3S or ME4S plate as follows (P1 parent 1, P2 parent 2).



To set the mix up a few microlitres of sterile water and mix with a toothpick.

Note that the plates must contain all supplements needed for both parents.

5. Incubate at 28-30°C for 2-3 days (25°C if one parent is ts). Examine mix for asci – if positive check that they are absent from h+ parent (h90 reversion can occur).
6. Proceed to derive haploid strains by random spore analysis or tetrads.

Random spore analysis.

Using a three day old cross check for the presence of asci under the light microscope. Random spore analysis allows many more spores to be examined than in tetrad analysis and in this way recombination mapping and strain construction can be carried out. However, it is important that all the classes of spores are viable when studying recombination frequencies, otherwise tetrad analysis becomes necessary
1 ml of sterile distilled water is inoculated with a loopful of the cross, 5 ul of Helicase (Helix pomatia juice) is added and the mixture incubated overnight at 25-29°C or for at least 6 hours at 29°C. Helicase is a crude snail gut enzyme that breaks down the ascus wall and kills vegetative cells.

Spin down spores and resuspend in 1 ml of 30% ethanol (this kills any remaining vegetative cells). Sonicate on setting 6 for 30 seconds to break up spore clumps

The spore number/ml is counted using a haemocytometer. Between 200-1000 spores/plate can be plated out on YES Agar or selective medium. The plates are then incubated until colonies form.
NB: if the cross works reasonably well 100uL of a 1:1000 dilution will give approx. 200 colonies per plate if you inoculate the 1ml culture with a generous loopful of cross. Easier than counting, especially if you are doing large numbers of crosses.

Tetrads

Suspend mating mix in sterile water and spread on thin plate (YES unless thiamine sensitive). Allow plate to sit for a few hours at 32°C so that ascus wall breaks down, then dissect. Spores from asci can usually be recognized from characteristic appearance: