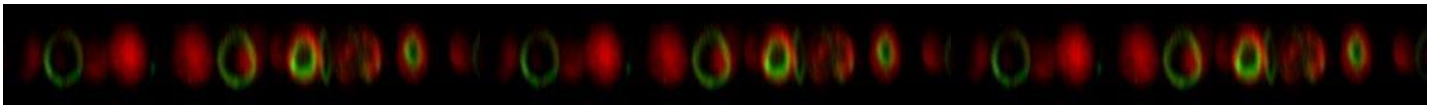

STRAIN DEVELOPMENT

1. Before setting up a cross grow the strains for 2-3 days on YE media (or selection media if necessary).
2. Check the strains all the markers and phenotypes. This will ensure that you do not use the wrong strain.
3. Patch the strains on a fresh YE plate the afternoon before you set up the cross. This will ensure that the cells are actively growing. Old and sick cells do not mate very well.
4. For a typical h+ and h- cross, mix approximately equal amounts of cells on the plate. You can make several copies of the cross on the same ME plate.
5. For a cross with an h90 strain mix 20 times less h90 strain with the other strain. This will force a cross between the two different strains.
6. You can use 5 ul of sterile water to mix the two strains and make it more homogenous.
7. Spores should after 2 days of incubation at 25ºC.
8. For random spore analysis you can incubate for an extra day.
9. For tetrad analysis, you will have to proceed as soon as spores appear to avoid premature rupture of the ascus walls.
10. DO NOT USE THE tetrad dissection microscope till you have been fully trained. Dr. Das loves to pull tetrads and will be happy to give you a demonstration of how the scope is used.
11. For random spore analysis, after spore digestion, wash then twice with sterile water, resuspend in sterile water, determine the spore concentration, label the tube well and store the remaining spore solution at 4ºC.
12. ALWAYS store strains after confirming the genotype. Then start working on the experiments. Don’t just store them and forget about them. The strains were made for a reason. At the very least inform Dr. Das that the strain is ready.

Calculating spore concentration using a haemocytometer.

1. Do a 1000X dilution of your spore sample. Load 10ul on the haemocytometer slide as follows.
2. To load the sample first place a coverslip in the haemocytometer slide. Then gently release the sample from the pipette tip into the wedge on the slide, making sure the coverslip is on top. Wait a few seconds for the sample to spread out properly.
3. Count the spores in each of the 4 (16 squared) corners of the slide.
4. From this calculate the average number of spores in a 16 squared corner.
5. The volume of the 16 squared corner is 1mm X 1mm X 0.1mm. This is 0.1 c.mm or 0.1ul. Thus the spore concentration is average number of spores calculated above/0.1ul. Compute the actual spore concentration by taking into account the initial dilution you made before counting.
6. Now calculate the volume of spore solution needed to get 100 spores. Load that amount on a plate and spread well with sterile glass beads. Note: the minimum volume that you can spread well is 50ul. Avoid volumes higher than 200ul as they make the plates too wet. If you are forced to use high volumes, dry the plate before transferring to the incubator.

PLEASE NOTE: DO not use the excel calculator for spore calculations. Do it manually. If you don't know how ASK Dr. DAS!!!