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

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

how ASK Dr. DAS!!!