Spot testing (eg for comparing strain viabilities)

This procedure assumes that strains contain pREP series plasmids and that the nmt1 promoter must be induced for the comparison

1. Grow up strains in EMM-leu+thiamine (5 ug/ml) - inoculate liquid cultures, grow for 12-24 hrs.

2. When strains are in log phase (od 0.2-1.0), measure od, spin down, and wash x2 in sterile deionized water (sdw) (it is important to get rid of last trace of thiamine).

3. Resuspend strains in sterile deionized water so that OD of each culture is 0.5 (ie cell densities must be about the same).

4. Make serial dilution as follows in sdw

1 undiluted
2 100 ul of 1 into 900ul water(ie 1:10 dilution)
3 100ul of 2 into 900ul water ie 1:100 dilultion
4 etc 1:1000
5 etc 1:10000

5. Spot out 10 ul aliquots (start with dilution 5 and move to more concentrated solutions obvioiusly) on :

EMM-leu-thiamine

EMM-leu+thiamine

plates (use a grid pattern to get a neat array)

6. Incubate plates at 30°C (unless ts strain is being used) and photograph after 2-5 days (will depend on strain).