

Rapid screening of strains by fluorescence microscopy

1. Replica plate strains to be screened onto YES. Grow o/n at 32°C (I grew for 16 h but probably not critical).
2. Use multispot slides for the next step: put 3 ul or so water on each spot and using a toothpick spread cells from the overnight plate over the entire area of each spot. Allow slide to dry (can put on 37°C block if in a hurry).
3. Dip slide in 100% ethanol* (could also use methanol but ethanol works fine for Cdt1 and is not toxic). Leave for 5 mins.
4. Dip slide in acetone* for 1 min. Remove slide and allow to dry. Can store at this stage at -20°C for a few hours, maybe a few days.
5. To mount, warm slide to 37°C on block. Add a few ul of agarose mountant to each spot and cover with large coverslip (ie one large enough for entire slide). Push gently on coverslip to spread mountant to thin layer.
6. Put slide in fridge for 5 mins or so to set mountant.
7. When viewing it is not necessary to raise objective when moving from spot to spot (just put a small amount of immersion oil on each spot).

**Keep solvent bottles at -20°C and do slide incubations on ice (I don't know if this is important)*

*RapidFluorScreen
24.2.09*