

Coverslip preparation (Observations & Info from April Horton, 9/13/04)

Tends to do 24 at a time (with a couple extra in case of breakage). 3 (100 mm x 20 mm Falcon) dishes x 8 coverslips each. Parafilm in the bottom of each dish.

18 mm coverslips (Carolina Biological Supply -- Assistant 1001/18) stored in 100% ethanol (Nalgene screw-top jar). This is the only cleaning she does right now -- no acid wash.

Plops a few in each dish and rearranges with forceps to a single layer.

Doesn't prop them up on the edges of the dish like some people do.

Didn't flame forceps before starting because they are dipped in ethanol to grab coverslips.

April reuses plates (petri dishes), so they're not sterile, but the UV must do the job because contamination hasn't been a problem lately.

UV exposure in clean hood for ~20 minutes.

PDL (poly-D-lysine) is stored in -80° C freezer (bottom R drawer in top freezer). Red PDL label ~5 ml/week, so one tube lasts about 2 weeks

Keep track of batch (date) since bad PDL is often a culprit when problems arise.

Gloves up to handle coverslips after UV treatment and sprays PDL bottle & gloves.

Flame forceps.

Opens sterile 10 or 5 ml pipette (more sterile than little pipette tips like she used to use) -- open end of package, put tip on pipettor and then take off rest of wrapping. Placing pipettor upside down reduces chance of touching other objects with tip or shaft, but sideways is better overall so that liquid from the end of the tip doesn't drip down into the pipettor.

To conserve PDL stock, put 200-500 μ l on half of the coverslips, then sandwich rest on top of the first batch.

Put trays of coverslips in incubator overnight. Keep extra trays and parafilm sterile because they will be used the next day when coverslips are un-sandwiched and rinsed.

After coating, suck PDL off, bead water up, then transfer to 12 well plates and rinse 2x with 1-2 ml of water in wells.

Label trays with name and date.

Take water off, then plates can be wrapped in foil and can be kept in the fridge for even a couple weeks if necessary before plating cells.

[Tom has a bulk coverslip prep if it is useful sometime, which works okay for him, but April had trouble with getting cells to grow correctly the one time she tried it.]

Stack up plates in the Left hood on Tuesdays for plating with cells.

Plating day with shift to Friday soon.