

VR's protocol, adapted from Somero lab protocol, as adapted from DeRisi lab protocol

Preparing poly-lysine slides for printing microarrays:

Goal: To coat glass microscope slides with poly-lysine ("PLL"), so they are "sticky". Then when the arraying robot prints DNA spots onto them (making a microarray) the DNA sticks.

Materials:

Gold Seal Micro Slides, Cat. No. 3010, 3" x 1", 1mm thick, Fisher # 12-518-100A	1 secondary containment tray
NaOH pellets, e.g. Sigma # S8045	3L glass beakers (2+)
95% EtOH	1 1L plastic beaker
lots of Milli-Q H ₂ O	plastic boxes for PLL-coating of slides (pipette-tip boxes work fine)
1X or 10X PBS	metal slide racks
Poly-L-lysine solution, 0.1% (w/v), Sigma # P8920	plastic washing container
slide boxes, Fisherbrand, foam-lined not cork-lined, Fisher #03-448-4	plastic wrap or tinfoil
	rubber band

Notes:

- *rinse all containers with RO before using, to remove dust, etc.. Keep these containers separate (at Gin's bench).
- * throughout this process, make sure the slides in the racks stay well separated, by running a gloved finger across the top edge of the slides, etc - during rinsing, etc. - at *all* steps
- * to keep dust from sticking to the slides, once the protocol is begun try to keep the slides submerged in solution at all times, &/or covered.
- * do NOT use powdered gloves during this protocol.
- * slides **MUST be stored at least two weeks before spotting DNA** (DeRisi lab says 2 weeks, Schoolnik lab says 3 weeks, Somero lab says 1 week). **Don't use slides that are > 4 mos. old for printing**, sometimes the poly-lysine degrades (DeRisi says 4 mos., Schoolnik labs says 3 mos.).

1. Wash slides: Although the slides come "clean" in a box, there is still a fair bit of dust and dirt on them and they need to be extremely clean before they are coated, because any dirt will cause irregularities in the coating which will be weak spots, and may peel off.

make up wash solution:

need about 600mls of solution to cover a metal slide rack in a 3L beaker
in a glass beaker (2L or bigger), mix:

For 60 slides: 1200mls

120 g NaOH pellets
720 mls 95% EtOH
480 mls Milli-Q H₂O

For 90 slides: 1800mls

180 g NaOH pellets
1080 mls 95% EtOH
720 mls Milli-Q H₂O

For 120 slides: 2400mls

240 g NaOH pellets
1440 mls 95% EtOH
960 mls Milli-Q H₂O

(final conc. for this solution is 57% EtOH, 10% w/v NaOH)

VR's protocol, adapted from Somero lab protocol, as adapted from DeRisi lab protocol
mix with stir-bar on stir-plate (no heat) until fully dissolved (takes ~15min)
Thoroughly rinse 1 glass 3L beaker for each 30 slides.

Place slides into metal racks (our current racks take 30 slides each), use air canister to spray off slides.

Put racks into beakers, and beakers into one large plastic tub (for secondary containment on shaking table - this solution is highly basic and we don't want it to spill). Put secondary containment tray on shaker, into hood.

pour 600mls wash solution into each 3L beaker. Cover the beakers, with plastic wrap or foil, to prevent dust from getting in.

shake gently on table to wash, about 2hrs

2. Rinse slides: The wash solution must be fully rinsed from the slides or it will interfere with the coating process.

wash slides 5x vigorously with clean Milli-Q water - e.g., put two racks at a time in long narrow plastic tubs, place a rubber band around the slides - **as close to the two ends as possible!!!** - to hold them in the rack securely, run Milli-Q water over racks and then swoosh racks up and down *and* back and forth in water vigorously, repeatedly, for maybe 30 seconds. Dump water and repeat process 4 more times.

let the slides sit in clean water while you prepare the poly-lysine solution

3. Coat slides:

only use PLASTIC with poly-lysine!

So, to coat the slides use the empty plastic pipette-tip boxes labeled "for poly-lysine". To make up poly-lysine solution, use a plastic beaker or dish.

For coating 2 racks of slides at once:

For 750mls poly-lysine solution:

(This solution's final concentrations are ~0.0169% w/v PLL, and 0.0984X PBS.)

550mls Milli-Q H₂O

73.8mls 1X PBS (kept in fridge once opened, post-autoclaving)

126.72mls poly-lysine solution, 0.1% w/v in H₂O

mix ingredients in order listed, in plastic, with stir-bar on stir-plate

dump excess water from slides

place each of two slide racks into its own poly-lysine pipette-tip box

immediately pour 375mls poly-lysine solution over each rack

VR's protocol, adapted from Somero lab protocol, as adapted from DeRisi lab protocol

close each box

put the two boxes into a plastic tub for secondary containment

let slides shake on shaker table in poly-lysine solution for 30 minutes

(Note: each box of poly-lysine solution can be re-used the same day, to coat one more rack of slides. Also, if a lot of slides will be prepared on several consecutive days, the solution can be filtered after the first day and stored in plastic in the fridge. When ready to use again, add an additional 3-5 mls of poly-lysine. This can be repeated a maximum of six times.)

4. Rinse slides: All the excess poly-lysine solution should be removed, so that the coating is as even as possible.

lift the metal slide racks out of the poly-lysine solution, and place the two racks into the long narrow plastic tub for washing. Wash slides 5x vigorously with clean Milli-Q water – following the same protocol as first rinse step.

(Meanwhile, if needed, start next racks of slides coating.)

Keep the rinsed slides in water until they are spun – they can wait a *few* minutes if someone else is using the centrifuge, for example.

Drain the excess water from racks, and put them into the centrifuge, on top of several folded up large kimwipes.

Position the racks with the slides in a consistent orientation so you know which way is “facing” the direction of motion. (Imagine riding on a very fast merry-go-round coating in maple syrup. As you move, the syrup would dry off the front side of you first, and also flow around your edges a little to build up along the sides of your back. We see this with the slides – on their back faces, the edges show signs of PLL wrapping around during the spin... No problem on the backside, but not so great for the side we want to print on.)

Make sure the racks are balanced in weight (same number of slides) *and position*. Check the separation of the slides in the racks (finger run across top edge).

spin ~150xg (in Ed's plate-spinner centrifuge, a Sorvall Legend RT, with the plate-spinner rotor in it) for 5-10 min., *at room temperature*, until dry. Before putting the slides in the centrifuge, spray out the rotor, plate holders, etc., with an air canister, to prevent dust.

remove the slides from the racks and place into a slide storage box, (having sprayed the box dust-free with an air-can), all slides in the same orientation (e.g. all with the slide faces that were at the “front” – in the direction of spinning motion – pointing the same direction in the box. Label the outside of the box with tape saying “PLL-coated slides” and **the date**, and your name, and the directionality of the slides in the box (based on direction of spin, and see below).

Place storage box into dessicator cabinet.

5. To QC the slides: It's a good idea to see how the slides look, to make sure nothing obvious has gone wrong. This can be done the same day or in the next several days.

- breathe on them, look for surface irregularities.
- check their background by scanning on the array scanner
 - edge effects not that uncommon, shouldn't interfere with where array goes
 - slide sidedness where one face of slide is more speckly than the other also not uncommon. If this occurs, **orient slides** methodically in the case so that the better side always faces the same direction, and note this on the outside of the storage box - this will be the face used for spotting the DNA onto.
 - it's probably good to save a few of the slide scans, and print out on a single page, to keep a record of that batch's general properties post-coating. Just doing Preview Scans is fine. Save the file, and also export it using both our standard brightness/contrast and the auto-brightness/contrast.

Our standard settings for scanning PLL slides:

635nm laser (red): 100%, PMT Gain: 600

532nm laser (green): 100%, PMT Gain: 600

brightness at 95, contrast at 93

but, also look at and save a few images using the auto-brightness/contrast button

our file naming convention:

Year_Month_Day_description_slidenummer_front_or_back_autosettings

e.g. 2005_01_19_newPLLslides_1f or 2004_12_20_newPLLslides_3b_auto

VR's protocol, adapted from Somero lab protocol, as adapted from DeRisi lab protocol

Protocol Summary:

1. Wash slides

make up wash solution:

in a glass beaker, mix:

For 60 slides: 1200mls

120 g NaOH pellets

720 mls 95% EtOH

480 mls Milli-Q H₂O

For 90 slides: 1800mls

180 g NaOH pellets

1080 mls 95% EtOH

720 mls Milli-Q H₂O

For 120 slides: 2400mls

240 g NaOH pellets

1440 mls 95% EtOH

960 mls Milli-Q H₂O

pour 600mls wash solution into each *clean* 3L beaker, containing rack of slides.

Cover the beakers.

shake gently on table to wash, about 2hrs

2. Rinse slides: wash slides 5x vigorously with clean Milli-Q water

let the slides sit in clean water while you prepare the poly-lysine solution

3. Coat slides:

only use PLASTIC with poly-lysine!

for 750mls poly-lysine solution:

550mls Milli-Q H₂O

73.8mls 1X PBS (kept in fridge once opened, post-autoclaving)

126.72mls poly-lysine solution, 0.1% w/v in H₂O

mix ingredients in order listed, in plastic, with stir-bar on stir-plate

dump excess water from slides

place each of two slide racks into its own poly-lysine pipette-tip box

immediately pour 375mls poly-lysine solution over each rack

close boxes & put into a plastic tub for secondary containment

let slides shake on shaker table in poly-lysine solution for 30 minutes

any additional racks of slides can sit in Mill-Q water while waiting their turn for the poly-lysine

4. Rinse slides:

Wash slides 5x vigorously with clean Milli-Q water

(Meanwhile, if needed, start next racks of slides coating.)

Drain the excess water from racks, and put them into the centrifuge, on top of several folded up large kimwipes. Balance them, and note direction of motion of slides (which faces point "forward").

spin 150g for 5 min., or until dry.

remove the slides from the racks and place into a slide storage box, all in the same orientation. Label the outside of the box. Place storage box into dessicator cabinet.

5. QC the slides

VR's protocol, adapted from Somero lab protocol, as adapted from DeRisi lab protocol

Form for Preparing Poly-Lysine coated slides, for microarrays:

Date: _____

Preparer: _____

Number of slides being prepped: _____

slide lot #: _____

NaOH lot #: _____

EtOH lot#: _____

NaOH slide wash:

time in washing: _____ time out: _____ total time: _____

PLL slide coating:

1st batch, time in coating: _____ time out: _____ total time: _____

2nd batch, time in coating: _____ time out: _____ total time: _____

Notes about any changes, observations, accidents, etc:

Q/C of slides:

1. Visual inspection when breathed upon:

2. General appearance using scanner:

3. A few representative scans, using both standard settings (brightness 95, contrast 93) and auto-settings: