

Southern Protocol

1) Perform clean tail prep with barrier tips using existing protocol.

2) Read ODs on all samples using spectrophotometer with a 1:200 dilution. To calculate concentration, use this formula:
 $A_{260} \times 10 = \mu\text{g}/\text{ul}$. Use Microsoft Excel to make a table of the various amounts of DNA (typically 5 μg is used), enzyme (ex. HIND III or Eco R1), water, compatible buffer, and if necessary, BSA, you will need for your digest. The digest total for each sample is 30 μl . Here is an ex.:

ID #	A260	Conc.	5 μg	Water	BSA	Buffer B	HIND III
2F-6	0.2609	2.609	1.916443	23.78356	0.3	3	1

Explanation of example listed:

- To calculate amt. of DNA needed: $=5/\text{conc}$.
- To calculate amt. of water needed: $=30-4.3-\text{DNA}(\mu\text{l})$
- Amt. of BSA is constant at 0.3 μl
- To calculate the amt. of buffer needed: 10% of total volume
- Amt. of enzyme is constant at 1 μl

3) To set up your digest: thaw buffer completely, add water first, then all other components and lastly, add enzyme (keep enzyme in freezer until ready to add). Put at 37° for 1 hr. and then spike again with 1 μl of enzyme and return to 37° overnight. In the morning, spin samples down to get rid of condensation and then add 1 μl of enzyme and return to 37°. *Pour the gel while you are waiting for the 37° incubation for 1 hr.* After 1 hr., you are ready to add blue loading dye to the samples (10% of total vol., so 3 μl for 30 μl digest) and load samples on gel.

4) To make gel:

- Use 10xTBE for the stock running buffer but it needs to be diluted to 1xTBE (for 1L use 900ml of MilliQ H₂O + 100ml of 10xTBE)
- Typically, a .8% or 1% agarose gel is used. Remember, 1%=1g/100ml, so add .8g/100 ml to make a 0.8% gel or 1g/100 ml to make a 1% gel.
- So, grab a 250ml Erlenmeyer flask and add 100 ml 1xTBE and then weigh out .8g of agarose and add to flask and put in microwave until it boils (~1.5 min.) Make sure there are no granules after you shake flask to mix, if there are, you need to put back in microwave. Take out with paper towels and let cool for a few minutes. Then add Ethidium bromide (10% of total volume added, so add 10 μl for a 100 ml gel) (Be careful with Ethidium bromide, it's a known carcinogen so always use gloves). Flask should still be warm to the touch when pouring it. Push bubbles to the side and place comb on side opposite the knob and twist in. Let solidify 15-20 min.

- 5) Once solidified, place gel in gel rig with the wells closest to the black electrode so DNA can run towards pos., red electrode. After samples are loaded, add 5-10ul DNA ladder. Put top on and set desired voltage (ex. 85volts) and hit run, should see tiny bubbles at bottom of gel rig if it's working.

- 6) When gel is done running, you need to make a mask of it and take a picture. Grab a transparency and marker and if DNA is still in gel it will fluoresce light back due to the Ethidium bromide staining. Place gel on UV machine being careful to use the shield and cover arms with lab coat as the UV light will burn exposed skin. Also, do not leave gel on too long as the UV light can nick the DNA if on for extended period of time. Cut a notch in the top, left corner near your first lane and lay transparency on top of gel and trace the perimeter of gel and the lanes and the DNA ladder marks.

- 7) Move gel into the other machine to take a picture. Make sure camera on top is on the right side with the setting at 1. Flip UV switch on, click camera icon, adjust exposure time ~1 sec (do not want to see a lot of orange fluoresce), click manipulate to sharpen image, click brightness to adjust to ~10, and click contrast to adjust to ~60 and click print. Put gel back on tray and grab the scraps of gel and put in Ethidium bromide waste.

- 8) Washes for the gel (To prepare DNA to be transferred to a gene screen):
 - Order of washes: a) .25N HCl (15 min.)
 - b) .4M NaOH/.6M NaCl (30 min.)
 - c) 1.5M NaCl /0.6M tris pH 7.5 (30 min.)
 - d) 10X SSC (15 min.)
 - Grab a Pyrex dish and slide gel into it with .25N HCl (enough to cover whole gel) and put on red rotor for 15 min.
 - Rinse gel with deionized water 3x, and pour in second wash, .4M NaOH/.6M NaCl for 30 min.
 - Wash gel with dH2O again and add third wash, 1.5M NaCl/0.6M tris pH 7.5 for 30 min.
 - Wash with dH2O again and add final wash 10X SSC for 15 min. from stock 20X SSC. (To make 1 L, add 500ml MilliQ H2O + 500ml 20X SSC= 10X SSC)

- 9) Ready for the transfer:
 - Get gene screen and cut out a membrane to fit your gel and place it in water for 30 sec. in pyrex dish and dump out and then pour in 10X SSC for 5-10 min. and put on red rotor again.
 - Grab a baking dish and put glass on top and and pour 10X SSC in bottom about halfway to the top. Cut out a long sheet of Whatman paper that's wider than gel but smaller than pyrex and cut out a smaller square that's bigger than your gel. Use 10 ml pipette to smooth out Whatman paper after you wrap it around the glass and saturate it.
 - After the membrane wash is completed, place gel on top of glass wrapped with Whatman paper with the corner on the upper right (if you notched it on the left). Put membrane on top of gel and cut 4 strips of parafilm to go around gel and 1 corner piece for the gel and place around gel on Whatman #3. Soak Whatman square in 10X SSC and place on top of membrane and roll out bubbles with pipette. Take half a pack of paper

towels and place on top of Whatman square and put small square glass plate on top with a little weight (ex. small H₂O bottle) on top of that and leave overnight.

10) Take down the membrane:

- Take off paper towels and Whatman square and throw away. Cut notch in membrane to match the gel notch. Label membrane (# and initials) on side that touched wide piece of Whatman paper on glass.
- Grab pyrex dish and add .4N NaOH for 30-60 sec. and dump out and add .2M tris/2X SSC and put on red rotor for 10 min.
- Air dry for 10 min. and then place membrane on paper towel in UV stratolinker and hit auto crosslink and start, then flip over and do other side.

11) Ready to hybridize (hyb):

- Warm up hyb solution: Grab Quick Hyb bottle (Stratagene) and invert to mix (very viscous). Add 10ml quick hyb (or 14ml if it's a larger blot) to glass tube with 10 ml pipette. Put glass tube in hyb oven at 58° for 5 min.
- Pre-hyb: Thaw Salmon sperm tube completely and probe and add 30ul Salmon sperm and calculated amt. of probe (Look below for calculations) into an eppi tube. Boil for 10 min. in 100° block with a lead lid over it to prevent tube from bursting off (dangerous since it's radioactive) At the same time, place dried membrane in MilliQ H₂O and dab off with paper towel and place in pre-hybed bottle for 10 min. in hyb oven.
- After 10 min., cool probe + SS tube on ice for 1-2 min. and do quick spin. Add the whole tube to the hyb bottle and place back in hyb oven for 3 hrs.

12) Ready for hyb washes:

- Order of hyb washes:
 - a) 2X SSC (room temp. for 10 min.)
 - b) 2X SSC/1% SDS (room temp. for 10 min.)
 - c) 1X SSC/1% SDS (58° for 10 min. typically but with PGC-1 KO screen and HIND III frag as probe do it for 40 min.)
- Dump out remaining quick hyb solution from bottle in radioactive waste. Add 2X SSC to bottle and shake around to get quick hyb solution off membrane blot and dump in radioactive waste. Then add 2X SSC again and place bottle back in hyb oven for 10 min. for a room temp. wash so open door of hyb oven and cool hyb oven down to ~25-33°. (Can dump the first 2 washes in radioactive waste and the last wash down the sink in the same room *only this sink can be used for radioactive waste*) Must sign sheet for how much you put down sink.
- Pull blot out with clippers and blot dry with paper towels. Wrap blot in saran wrap. Tape down in metal cassette and put film over it with film notch in upper left corner and place in -80° overnight and develop next morning.

13) To label a probe:

- Need to know conc. of the desired plasmid in order to figure out how much you need to use to label a probe. Add x amt. DNA to an eppi tube + x amt. of H₂O to equal 8ul (25ng). (ex. 2ul of plasmid + 6ul H₂O = 8ul)
- Boil for 3 min. and chill on ice for 1-2 min. and add 3ul AGT mix, 4ul rxn mix #1 (High Prime Kit-Roche), and 5ul a³²P-dCTP (50uCi) to the same tube.

(Make sure to keep AGT and rxn mix on ice while using and sign out for radioactivity). (20ul total)

-- Put in 37° block for 30 min.

-- Grab the tube and do a quick spin. Add 60ul 1X STE to this tube to stop the rxn.

-- Next, will need to grab tube apparatus, syringe, and push column. Take off blue tips of column and remove big tube and put column in smaller tube, small tip first. Put an empty eppi tube in bottom of tube apparatus. Pre-wet column with 80ul 1X STE and push down with large outer tube (should see small amt. 1X STE in bottom of eppi tube or droplets on bottom of column). Throw that eppi tube away.

--Next, grab a new eppi tube and place in the bottom of tube apparatus. Add entire probe tube down the column (~80ul) and collect new tube. Add another 80ul 1X STE down the column and label tube. Take 2ul probe in order to count in scintillation counter and place in plastic vial. (leave tip in plastic vial) Use #1 card in blue slot and put plastic vial in #1 position and hit automatic counting and start. Tear off computer count.

14) To calculate amt. probe needed for hyb:

-- Remember *500,000 counts/ml quick hyb*

-- So, take your computer readout # / 2 (2ul place in plastic vial) = x.

-- 500,000 counts x 10 (10 ml quick hyb used) = 5,000,000.

-- 5,000,000 / x = amt. needed to label probe.

--For larger blots, use 14 ml quick hyb.

-- So, take computer readout # / 2 = x.

-- 500,000 counts x 14ml = 7,000,000

-- 7,000,000 / x = amt. needed to label probe.