

Southern Blot

Heat FBI Buffer to 55-65C

Place membrane in hyb tube

Boil Salmon Sperm DNA //denature and used as a block, 5mg/ml

Add 20ml FBI

400ul Salmon sperm DNA to hyb tube //final [sperm] = 100ug/ml

Prehyb for ~1 hr @ 65C

While Prehyb, make probe

Calculate how much template/ water needed for rxns

Prepare 37C waterbath

100C waterbath //add water to holes in 100C heat block

Defrost P32-dCTP

Make sure Geiger counter is on

Open trash containers before beginning

Double glove

When pipetting, after releasing liquid, don't let plunger back up, put over trash can, then release, eject tip

On bench

add dH2O and template to tube according to instructions //total vol = 42ul

__ ul dH2O

__ ul Probe (~50ng)

Check if dCTP is thawed

Yes?

Heat probe/water tubes @100C for 5 min //denatures dsDNA

In hood

Set up

-open trash

-turn on counter

-pipett tips ready? Make sure filter tips

-bring pipetters

-napkins

-2 x 15ml conical vials

-Quickspin column

-hot box

-something to hold small test tube

add 5ul P32 dCTP/ tube

Add 3ul Magenta DNA pol/ tube

Mix gently

Incubate 37C 10 min in bacteria incubator //cover with plastic shielding
Stop rxn by adding 2ul Stop Mix
Remove unincorporated nts using Quick Spin column

Quick Spin

Tweezers
2x 15 ml conical vials

Invert column to resuspend
Remove top, then bottom
Drain by centrifuging 1100x G for a few minutes
Discard waste, centrifuge again
Discard old tube, place in new tube
Keeping column upright, add DNA sample to CENTER
Place column in 15ml conical
Cap off and centrifuge 1100x G 4 min
SAVE ELUATE
Discard old column in radioactive waste
Lock/ Seal tubes // hold up to counter to check radioactivity
Heat 95-100C for 5 min //denature probes
Add to center of hyb tubes
Heat on rollers 65C O/N

CHECK TO MAKE SURE NOTHING IS HOT

Washes

Open trash lids
Have paper towels ready

*Open hyb tube, pour straight into radioactive waste, DON'T TILT BACK UP, or radioactive waste will drip down side. Take paper towel, wipe of neck/ mouth, place in radioactive waste, now can pour fresh wash buffer into hyb tube

Washes are 20 min each @ 55C

Washes

| | |
|---------------------------|-------------------------------------|
| #1 20ml, 1X SSPE, 1%SDS | 1ml SDS, 1ml 20X SSPE, 18ml ddH2O |
| #2 “ “ “ | “ “ “ |
| #3 100 ml, 1X SSPE, 1%SDS | 5ml SDS, 5ml 20X SSPE, 90ml ddH2O |
| #4 100ml, .5X SSPE, 1%SDS | 5ml SDS, 2.5ml 20X SSPE 93ml ddH2O |
| #5 100ml, .1X SSPE, 1%SDS | 5ml SDS, 500ul 20X SSPE, 95ml ddH2O |

Seran wrap to keep wet
Mount membrane on filter paper //use tape
Put in film cassette with film, make sure to mark a corner
If using an intensifying screen, store at -80C O/N