

## Southern blot

### Day 1 Genomic DNA preparation

Refer to the protocol of “*Drosophila* genomic DNA preparation”

### Day 2 Digestion of genomic DNA

Digest 10ug of genomic DNA with one or more restriction enzyme at appropriate temperature overnight.

### Day 3 Run gel

Load digest on 0.7% agarose gel and run gel O/N at 25V. Make a picture of gel with a fluorescent ruler along.

### Day 4 Transfer and crosslink genomic DNA to membrane

1. Transfer DNA from gel to membrane using invitrogen iBlot DNA gel transfer device and kit.
2. Denature DNA on the membrane immediately after transfer with 0.5N NaOH/1.5M NaCl (denature buffer) at RT for 10min with shaking.
3. Wash the membrane with 2×SSC at RT 2×5 minutes with gentle shaking.
4. Dry the membrane at RT for 10min.
5. Crosslink DNA to membrane with UV.
6. Dry the membrane at RT O/N.

\*You can keep the membrane at 4°C for at least 2 weeks if you don't want to do hybridization immediately.

### Day 5 Probe labeling and membrane hybridization

1. Label probe following the instrument of GE AlkPhos direct labeling and detection system (GE, RPN3680/3681).
2. Pre-hybridize the membrane with hybridization buffer (GE, RPN3680/3681) containing 50ug/ml ssDNA at 55°C for 2~4 hrs.
3. Pour off pre-hybridization buffer. Hybridize the membrane with hybridization buffer containing labeled probe (5-10ng probe/ml buffer) and 25ug/ml ssDNA at 55°C O/N.

Day 6 Signal generation and detection

1. Wash the membrane with 2×SSC/0.1%SDS at RT 2×10min each with gentle shaking.
2. Wash the membrane with 0.1×SSC/0.1%SDS at RT 2×10min each with gentle shaking.
3. Wash the membrane with secondary wash buffer at RT 2×10min each with gentle shaking.
4. Pipette detection reagent (GE, RPN3680/3681) onto the blot and incubate at RT for 2~5min.  
Wrap the blot in the film cassette.

5. Place a sheet of autoradiography film on the top of the blot and expose the film at RT. start with 1 hour.
6. Take the film out of the cassette and develop it.

\* If necessary, you can stripe and reprobe the blot at least twice (following the instrument of GE AlkPhos direct labeling and detection system).