

Dot Blot

Solutions:

TE1X

SSC20X

Procedure:

1. Take eppendorf tubes from ChIP containing 30 μl of DNA in 1XTE, and add 111 μl TE 0.2X.
2. Add 9 μl NaOH 5M to denature DNA.
3. Incubate at 50°C for 1h.
4. Add 150 μl ammonium acetate 2M
5. In dot blot apparatus, put 2 filter papers Bio Dot SF (BioRad) and 1 filter of Nylon Hybond N+, previously wet in SSC 6X.
6. With closed vacuum, add twice 300 μl SSC 6X to wash the wells of the apparatus. Then, open vacuum until all liquid disappears from wells.
7. With closed vacuum, add the samples from the ChIP (300 μl), and fill empty wells with 300 μl SSC 6X. Then, open vacuum until all liquid disappears from wells. Some wells with sample may take a while to empty (just be patient!...).
8. With closed vacuum, add twice 300 μl SSC 6X.
9. Take membrane out of apparatus, and wash briefly in SSC 2X.
10. Dry filter and fix DNA to membrane with UV light at 0.30 j/cm^2
11. Membrane can be stored at 4°C wrapped in plastic until the hybridization reaction.