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Protocol for Northern Blotting

Northern blotting was performed using DIG Wash and Block Buffer Set (Sigma-Aldrich; code no. 11585762001). For more information, please contact Sigma-Aldrich Co, LLC.

Day 1

Electrophoresis and Transfer

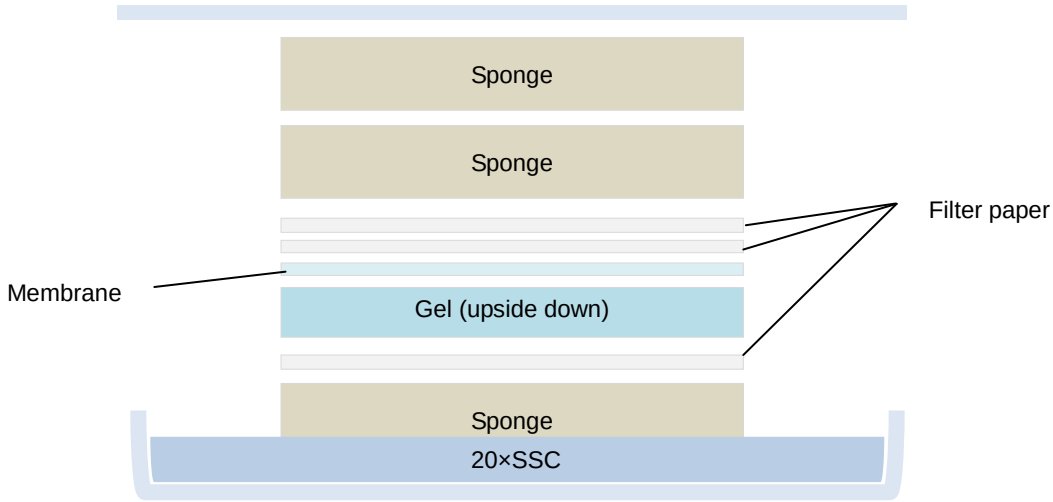
↓ Dilute total RNA samples with 2×Loading Buffer (50% formamide, 6.14% formaldehyde, 1×MOPS, 10% Glycerol, 0.05% Bromophenol Blue).

↓ Heat total RNA samples at 65°C for 10 min., then quench at 4°C for 5 min.

↓ Load the samples in a 1% denaturing agarose gel (1% Agarose S, 1×MOPS, 2% formaldehyde), and conduct electrophoresis in 1×MOPS at 50 V for 2 hr.

↓ Rinse the gel in 20×SSC for 10 min, put the following things in order ; one dried sponge, one filter paper, an agarose gel (upside down), one membrane, two filter papers, two dried sponges.

↓ Soak the lower sponge in 20×SSC, incubate them at room temperature overnight. (Please see below.)



Day 2

UV cross-link and Hybridization

↓ Soak the membrane in 2×SSC and put it on the filter paper soaked with 2×SSC.

↓ Make UV cross-link (120 mJ/cm^2) using FUNA-UV-LINKER FS-800 (Funakoshi).

↓ Put the membrane and the prewarmed PerfectHyb (TOYOBO; code no. HYB-101) in a hybridization bag at 68°C for at least 30 min.

↓ Denature the RNA probe at 98°C for 5 min, then quench at 4°C for 5 min.

↓ Mix the probe solution with 68°C prewarmed PerfectHyb.

↓ Discard the solution and put the probe solution in the hybridization bag, incubate the membrane at 68°C for 16 hr. (The condition of the hybridization reaction depends on the probe being used.)

Day 3

Wash step after hybridization

↓ Wash the membrane twice with Low stringency buffer (2×SSC, 0.1% SDS) at room temperature for 5 min. each.

↓ Wash the membrane twice with High stringency buffer (2×SSC, 0.1% SDS) at 68°C for 15 min. each.

↓ Rinse the membrane with Wash Buffer for 2 min.

Detection

↓ To reduce nonspecific binding, soak the membrane in Blocking Buffer at room temperature for 30 min.

↓ Incubate the membrane with Anti-Digoxigenin (DIG) mAb (MBL; code no. M227-3) diluted with Blocking Buffer at room temperature for 1 hr.

↓ Wash the membrane twice with Wash Buffer for 15 min. each.

↓ Incubate the membrane with 1: 5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with Blocking Buffer at room temperature for 1 hr.

↓ Wash the membrane twice with Wash Buffer for 15 min. each.

↓ Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.

↓ Expose for 1 min. with ImageQuant LAS 4000 imaging system (Fujifilm). The condition for exposure and development may vary.