* + 1. Subsequently submit blotting gel to following washes:
       1. Depurination: Add 1 L 0.25 M HCl in ddH2O per gel, incubate for 20 min at RT on a rocking platform at 40 rpm, remove the solution and rinse once with ddH2O.
       2. Denaturation: Add 1 L 0.5 M NaOH + 1.5 M NaCl in ddH2O per gel, incubate for 30 min at RT on a rocking platform at 40 rpm, remove the solution and wash once with ddH2O.
       3. Neutralization: Add 1 L 0.5 M Tris pH 7.4 + 1.5 M NaCl in ddH2O per gel, incubate for 30 min at RT on a rocking platform at 40 rpm, remove the solution and wash once with ddH2O.
    2. Blot the gel o/n at RT onto a positively charged nylon membrane through capillary transfer (see manufacturer's recommendations) using 20x saline-sodium citrate (SSC) buffer (0.3 M sodium citrate, 3 M NaCl).
    3. After blotting, dry membrane for 30 min at 80 °C and crosslink using an UV crosslinker. At this stage, membranes can be stored at RT for several days.

Note: The following steps involving radioactive probe generation, hybridization and post-hybridization washes should be carried out in an appropriate facility for work with radioactive materials.

* + 1. Prepare Church hybridization buffer. For this, first prepare pH buffer (720 mL 1 M Na2HPO4 + 280 mL 1 M NaH2PO4). To prepare Church buffer supplement 500 mL Ph buffer with 10 g BSA, 2 mL 0.5 M EDTA, 70 g SDS and fill up to 1 L with ddH2O.
    2. Transfer blot membrane into glass hybridization tube and add 15 mL Church buffer. Pre-hybridize for 1 h at 65 °C rotating. Use different hybridization tubes for each probe.
    3. Prepare radioactively labelled probes.

6.1. Denature 25 ng of probe DNA prepared in step 3.4.2 in 20 µL ddH2O for 10 min at 95 °C in screw cap tubes and transfer onto ice.

6.2. Add 4 µL of 5x concentrated random primer mix containing 1 U/mL Klenow polymerase, 0.125 mM dATP, dGTP and dTTP each in 50% (v/v) glycerol (commercial kit for random-primed labeling of DNA) and mix thoroughly by pipetting. Add 2 µL 10 mCi/mL [α-32P]dCTP, mix and incubate for 10 min at 37 °C. Stop the reaction by adding 2 µL 0.2 M EDTA (pH 8).

6.3. Remove non-incorporated [α-32P]dCTP using commercial chromatograpy spin-colums for purification of labeled probes according to the manufacturer’s recommendations.

6.4. Denature radioactive labelled probe by incubation for 10 min at 95 °C and transfer immediately onto ice.

7. Add denatured probe to the 15 mL Church buffer of the pre-hybridized membranes. Hybridize o/n at 65 °C rotating.

8. After hybridization, discard hybridization buffer and rinse membranes once in prewarmed low stringency buffer (2x SSC + 0.1% SDS). Subsequently wash membranes in 20 mL prewarmed low stringency buffer at 65 °C rotating for 20 min.

9. Discard first wash buffer and incubate in 20 mL prewarmed high stringency buffer (0.5x SSC + 0.1% SDS) for 10 to 20 min at 65 °C rotating. Discard second wash and rinse membranes once in high stringency buffer.

10. Drain excess liquid from washed membranes using filter paper, wrap membranes in clear plastic foil and expose to autoradiography film in appropriate cassette o/n at -80 °C.

11. Develop autoradiograph film. Compare obtained banding pattern with expected pattern according to Southern strategy (for example results see **Figure 4b**).