Synthesis of digoxigenin-labeled RNA probe

1. Mix these reagents in the following order at room temperature:

Sterile distilled water	11µ1
10X transcription buffer (Roche)	2µl
0.1 M DTT (Roche; freshly diluted from 1M stock stored at -80°C)	2µl
RNA DIG labelling mix (Roche)	2µl
Linearised plasmid (2ug)	1µl
Placental ribonuclease inhibitor (100 U/µl; Roche)	1µl
SP6, T7 or T3 RNA polymerase (10 U/µl; Roche)	1µl
	20µl total volume

2. Incubate at 37°C for 2 h.
3. Add 2µl DNase I (ribonuclease-free, Roche).
4. Incubate at 37°C, 15 min.
5. Remove a 1µl aliquot and electophorese on a 1% agarose TAE gel containing 0.2 µg/ml ethidium bromide.
6. Bring sample volume to 40µl with ddH_2O.
7. Purify samples using BioRad Micro Bio-Spin 30 columns (cat. #732-6250) as follows:
    1. Invert the column sharply several times to resuspend the settled gel. Tap the column to remove all air bubbles. Snap off the tip and place the column in a 2.0 ml microcentrifuge tube (supplied). Remove the cap. If buffer does not begin to flow from the column, push the cap back on the column and remove it again to start the flow. Allow the excess packing buffer to drain by gravity to the top of the gel bed (about 2 min). Discard the drained buffer and place the column back into the 2.0 ml tube.

    2. Centrifuge for 2 min in a microcentrifuge at 1,000 x g (4000rpm) to remove the remaining packing buffer. Note: The speed is important to ensure proper performance of the columns. Discard the tube.

    3. Place the column in a clean 1.5 ml microcentrifuge tube (supplied with 25 and 100 packs). Carefully apply the sample (10–75 µl) directly onto the top center of the gel bed. Do not disturb the gel bed. Application of more or less than the recommended sample volume may decrease column performance.

    4. After loading the sample, centrifuge the column for 4 min at 1,000 x g (4000rpm). The purified sample is now in 10 mM Tris buffer. Properly dispose of the used column.

8. Alternatively, to purify samples by precipitation:
    1. Add 100µ1 TE (50 mM TrisHCI, 1 mM EDTA, pH 8.0) or water, 10µl 4M LiCl, 300ul EtOH, mix and precipitate on dry ice, for 30 min.
    2. Spin in a microcentrifuge at 4°C for 30 min, wash pellet twice with 80% EtOH and air dry.
    3. Redissolve in TE or water at ~0.1µg/µl, run aliquot out on gel to ensure integrity.

8. Quantify RNA by measuring absorbance at 260/280 using UV spectrophotometer.
9. Dilute RNA probes to 20ng/µl (20X) in Hybridization buffer.
10. Store probes at -80°C.