Cut ~5ug of plasmid DNA with a linearizing restriction enzyme in a 100L volume overnight. Check 10L on a 1% gel. The band should be a single band. Phenol-chloroform extract and EtOH precipitate probe template and resuspend in 20L of RNAse -free water. Store at -20°C indefinitely.

All reactions are done with Filter tips, using RNAse free plastic ware, and wearing gloves.

Thaw and vortex transcription buffer very well, this reagent precipitates easily on ice. Keep at the reaction at RT as you build the reaction, NOT on ice!

To make 20L of DIG-labeled probe:

* ~1ug of probe template DNA ------------------------ X L
* RNF water ------------------------ X L
* Transcrition buffer (10X) ------------------------- 2 L
* 0.1 M DTT ------------------------- 1 L
* DIG labeled ddNTPs ------------------------- 2 L
* RNasin or RNAout ------------------------ 1 L
* Polymerase (SP6, T7, or T3) ------------------------- 2 L

Volume total- 20L

After 2 hrs. at 37°C the reaction should be complete. It may be possble to continue the reaction overnight but I have never tried this.

Take 1 L of your reaction and mix with gel loading dye (blue dye), heat to 65 for ~ 5mins. Then run on a 2-2.5% gel (20mins at 120 V). You shoul dsee a RNA band and a band from your probe template. w

If so, proceed to DNAse treatment by adding 1 uL of Turbo DNAse or RNAse-free DNAse. Incubate at 37°C for 15 mins. At this point you must pruify the RNA or place at -80°C to avoid degreading the RNA.

Use a column to purify. Nanodrop and store at -80C in the probe box.