Preparation of Probe for *In Situ* Hybridization

(Rivera lab)

1. Prepare reaction mix

50 ng/µl	1.0 µl	1 µg/µl DNA template (Linear plasmid, PCR fragment)
1X	2.0 µl	10X Transcription buffer
1X	2.0 µl	10X DIG RNA labeling mix (Roche Cat # 1277073)
0.01 M	2.5 µl	0.1M DTT
1 U/µl	0.5 µl	40 U/µl RNAse inhibitor (RNAsin, Promega N2611)
2 U/µl	2.0 µl	T3, T7 or SP6 RNA Polymerase (20 U/µl)
	10.0 µl	depc ddH2O

2. Incubate at 37 ºC for 2 hours.
3. Stop the reaction by adding 1 µl of 0.5 M EDTA.
4. Add 2.5 µl of 4 M LiCl and 75 µl of cold 100% ethanol, to precipitate.
5. Chill at -20 ºC for 2 hours (or 30 minutes at -60 ºC or below)
6. Centrifuge at 13,000g for 5 minutes.
7. Wash the pellet with 70% ethanol and let dry.
8. Resuspend in 200 µl of depc TE and add 1µl of RNAse inhibitor.
9. Check the RNA probe by running 1 µl on an agarose gel. The signal from the RNA should be 10X stronger than that of the DNA template.
10. Prepare aliquots of 20 µl and store at -20 ºC. It can last for 1 year.
    Use ~ 20 µl (0.1 - 1 µg) per wholemount in situ hybridization assay.