

Making an *in situ* probe

1. Linearize Qiagen-purified plasmid with appropriate blunt or 5' overhang enzyme.
2. Add 2 μl of Proteinase K (10mg/ml), 37°C 1 hr
3. Phenol/Chloroform extract digest and precipitate with 100% EtOH
4. Resuspend pellet in DEPC-treated or RNase-free H₂O (to a concentration of 1-2 $\mu\text{g}/\mu\text{l}$)
5. Synthesis of probe:

DNA	5 μl
RNase-free H ₂ O	8 μl
10X buffer	2 μl
Dig NTP mix (Roche kit)	2 μl
T7, T3, or SP6 (Roche)	1.5 μl
RNase Inhibitor (Roche)	<u>0.5 μl</u>
	20 μl

6. Run above 20 μl reaction 3 hrs at 37°C
7. DNase treatment: Add 2 μl to reaction, return to 37°C for 20 min
8. Stop reaction: Add 2 μl to reaction
9. Precipitation of probe:

To 24 μl reaction, add:	
-20°C cold 4M LiCl	2.5 μl
-20°C cold 100% EtOH	75 μl
Place reaction in -80°C	40 min
Spin max speed, 4°C	15 min
Pour off supernatant	
Add -20°C cold 70% EtOH	300 μl
Spin max speed, 4°C	15 min
Pour off supernatant	

10. Remove excess EtOH with pipette (do not vacuum dry) resuspend RNA in 20 μl DEPC-treated or RNase-free H₂O; gently mix
11. Add 0.5 μl of RNase Inhibitor to RNA. Proceed to run gel and spot test probe.
12. Store at -20°C