Prepare ribro-probe for RNA in situ hybridization

Plasmid linearization

1. In 100 μl of reaction, add 20 μg of plasmid DNA and 50 U of designed restriction enzyme.
2. 37 °C for 2 hr.
3. Gel check for complete digestion.
4. Inactivate enzyme at 65 °C for 20 min (optional).

DNA purification

1. Add 100 μl of H_2O to the above reaction.
2. Add 200 μl of Phenol:Chloroform (1:1) and mix.
3. Spin for 10 min.
4. Transfer the supernatant to a new tube and mix with 200 μl of Chloroform.
5. Spin for 5 min.
6. Transfer the supernatant and add 20 μl of 3 M NaOAc and mix. Then add 400 μl of EtOH.
7. Sit on ice for 15 min and spin for 10 min.
8. Wash with 1 ml of 70% EtOH.
9. Air dry and resuspend the pellet with 20 μl of H_2O.
10. Gel check (Optional).

RNA probe synthesis

1. Prepare the following reaction mixture:

	1X (μl)
H_2O	23.0
10X Buffer	4.0
0.1 M DTT	4.0
Nucleotide mix	4.0
RNA inhibitor (100 U/l)	1.0
RNA polymerase	2.0

2. Add 2 μl of linearized DNA template.
3. Incubate at 37 °C for 2 hr.
4. Take 0.5 μl for gel check.
5. Add 2 μl of DNase (RNA free).
6. 37 °C for 15 min.
7. Add 100 μl TE, 4 μl LiCl, and 300 μl EtOH.
8. Sit on –20 °C for 30 min.
9. Spin at 4 °C for 10 min.
10. Wash pellet twice with 1 ml of 70% EtOH.
11. Resuspend with 40-60 μl H_2O. Take 0.5 μl for gel check.
12. Store at –20 °C.