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 H₂O 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 H₂O.
10. Gel check (Optional).

**RNA probe synthesis**
1. Prepare the following reaction mixture:
   - H₂O: 23.0 µl
   - 10X Buffer: 4.0 µl
   - 0.1 M DTT: 4.0 µl
   - Nucleotide mix: 4.0 µl
   - RNA inhibitor (100 U/l): 1.0 µl
   - RNA polymerase: 2.0 µl
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 H₂O. Take 0.5 µl for gel check.
12. Store at –20 °C.