Simple protocol for Dig-RNA probes for in situ hybridization

(Wim Damen & Martin Klingler 12/99)

- Prepare plasmid (JETSTAR columns)
- Linearize plasmid (use enzyme that generates blunt end or 5' overhang)
- Purify by phenol/chloroform extraction and EtOH precipitation
- *In vitro* transcription:

  1µl DNA (500 ng)
  5µl H_2O
  1µl 10x reaction buffer
  1µl rNTP mix **DIG** labeling (or rNTP mix **fluorescein** labeling)
  1µl RNase inhibitor (20 units)
  1 µl RNA polymerase (T3/T7/Sp6)

  10 µl

- Incubate 2 h at 37°C
- Add 90 µl H_2O, mix
- Run 5 µl on a mini-gel (0.8% gel, 60 V, 15 min), place 25 ng template DNA in neighboring lane. RNA band should be 10 times stronger than template DNA.

- Add 1 µl tRNA (20 µg/µl)
- Add 16 µl 3.2M LiCl, mix, and add 400 µl EtOH
- Precipitate 2 h at -20°C
- Centrifuge 20 min at 15,000 rpm; pellet should be well visible
- Wash pellet with 500 µl 70% EtOH (centrifuge again if pellet gets loose!)
- Dry in speedvac (about 5 min)
- Dissolve in 100 µl H_20 (DEPC-treated)

- Store aliquots at -80°C
- Test optimal probe concentration (try 1, 2, 4 ul)