**RNA probe synthesis with Roche SP6 or T7 RT enzyme (30-5-08)**

Set up a 20 µl reaction with template: 2µl for linearized plasmid, 800ng for PCR product. Keep buffer and reaction at RT during pipetting the reaction.

| total volume | 20 µl |
|---|---|
| $H_2O$ (MilliQ is fine) | variable |
| DNA template (max. 12µl) | variable |
| Buffer (Roche [Sp6]: 10 X. Roche [T7]: 10 X) | 2 |
| NTP labeling mix (either DIG, FITC, BIOTIN) | 2 |
| RNase Inhibitor (Roche 03 335 399 001, 40 units/ul) | 2 |
| RNA polymerase (T7 or SP6 RT Roche) | 2 |

2 hrs @ 37 °C.

Prepare hyb buffer, RNA gel

**DNase treatment**
Treat with 1 µl DNase (Megascript) for 15min at 37ºC

**RNA precipitation**
Add 70 µl $H_2O$ (RNase free)
Add 10 µl Na-Acetate 3M pH 5.5 (final 0.3M)
Add 300 µl of ice-cold EtOH (100 %) (3 volumes)

Precipitate for 30' @ -70 °C, spin 30' @ 4°C, 15000 rpm.
Wash pellet with EtOH (70 %, DEPC $H_2O$), remove residual liquid and air dry (5').
Dissolve pellet in 20 µl DEPC $H_2O$

**Measure concentration**
Take out 0.5 µl of the sample and add 3.5µl RNase free $H_2O$ (dilution 1:8)
Spec on nanodrop and run 300ng on gel
(The average total yield of the 20 µl reaction is between 30-40 µg)

Store 10µl of RNA probe at -80 ºC
Fill up 9µl with x µl Hyb buffer (depending on concentration, final conc. 100ng/µl) and store at -20 °C.