RNA probe synthesis with Ambion Megascript SP6 or T7 Kit (30-5-08)

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

total volume	20 µl
Megascript buffer (10x)	2
ATP (different for SP6 and T7)	2
CTP (different for SP6 and T7)	2
GTP (different for SP6 and T7)	2
UTP (different for SP6 and T7)	1.5
DIG-11-UTP (10nmol/µl Boehringer)	4
H_2O (MilliQ is fine)	variable
DNA template (max. 4.5µl)	variable
RNA polymerase (T7 or SP6 Megascript)	2

6 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 150 µl Hyb buffer (depending on concentration, final conc. 50-100ng/µl) and store at -20 °C.

Protocol by Franz Kainz