In Vitro Transcription

1. Wear gloves all the time, work on a cleaned work bench and keep all samples in closed tubes whenever possible to avoid RNase contaminations. Use very fresh MilliQ H_2O.
2. Thaw samples on ice.
3. Prepare *in vitro* transcription mixture by pipetting the following components in the given order while keeping the in vitro transcription tube at room temperature (minimizes chance of NTP precipitation):

	V, ml	final Conc.	V, ml (15mL tube)
MilliQ H_2O	50 ml		1000 ml
5x TraB (transcription buffer)	10 ml	1x	200 ml
100 mM DTT	5 ml	10 mM	100 ml
25 mM NTPs	6 ml	3 mM	120 ml
100 mM GMP	2.5 ml	5 mM	50 ml
0.5 U/ml iPPase	1 ml	0.01U/ml	20 ml
T7-RNA-Polymerase	4 ml	0.3 mM	80 ml
40 U/ml RNase Inhibitor	0.15ml	0.12 U/ml	3ml
Template DNA	5 ml	10% (v/v)	100 ml

1. Incubate *in vitro* transcription at 37°C.
2. Remove two 15 mL samples within a few hours (1-4 h) and continue incubating the remaining sample.
3. Stop reaction in this sample by adding 3 ml of 20% KOAc, pH 5.0 (1/5 Vol.). Store the removed sample at -20°C.
4. Remove a 15 mL sample on the next day after having incubated the reaction mixture overnight.
5. Stop reaction in this sample by adding 3 ml of 20% KOAc, pH 5.0.
6. Add 4 mL 6x RNA-loading dye to your sample.
7. Boil the sample for 3 min (100°C).

8. Analyze the samples on a 15% RNA urea gel.

For Preparative in vitro transcriptions:

12. Add 2U (2 mL) of DNaseI (1U/mL, Fermentas) to 1mL in vitro transcription reaction to digest DNA and incubate for 1h at 37 °C

5x TraB (transcription buffer):

	For 50mL	Final conc (5x)	Final conc (1x)
1M Tris-HCL, pH 7.5	10 mL	200 mM	40 mM
1M $MgCl_2$	3.75 mL	75 mM	15 mM
100mM spermidine	5 mL	10 mM	2 mM
1M NaCl	2.5 mL	50 mM	10 mM

Add MilliQ H_2O to 50mL
Check pH with pH paper to be approximately 7.5
Filter the buffer using a syringe and a syringe filter
Aliquot the buffer in 1mL samples and store it at -20C

20%KOAc pH 5.0 (potassium acetate): 50 mL
Dissolve 10g potassium acetate in approximately 25 mL Milliq H_2O
Add glacial acetic acid until pH of 5.0 is reached
Add MilliQ H_2O to 50mL