Protocol 1: Standard In Vitro T7 RNA Polymerase Transcription reaction

Step1: Reaction mix for 500 μl transcription.

Water	xxx μl
10X Trx Buffer (New)	50 μl
50 mM DTT	50 μl
25 mM AUGC (NTP mix)	80 μl (4 mM Each Final Conc.)
T7 RNA Polymerase (JF prep)	5 - 10 μl (Depending on activity)
Digested Plasmid	25 μl (40 μgs)
[α-^{32}P] UTP (Optional)	2 μl
	500 μl

1. Incubate for 6 hours at 37 °C.

2. Add 10 μl of RQI DNaseI (Promega), incubate for 37 °C for 30 minutes.

3. Add 50 μl of 0.5M EDTA, pH8.0 and 50 μl of 3M Sodium acetate pH 5.2.

4. Add 600 μl of chloroform, extract by vortexing rapidly for 30 sec. Spin in microfuge for 1 min at max speed.

5. Transfer upper aqueous phase to new tube. Add chloroform and repeat step 4.

6. To aqueous phase add 1.2 mls of 100% Ethanol. Precipate RNA at –80 °C for at least 30 min or –20 °C for 12 hours.

7. Spin tubes at 4 °C for 30 minutes.

8. Carefully remove and discard ethanol without disturbing the RNA pellet.

9. Add 500 μl of 70% ethanol, spin at max for 30 seconds. Carefully remove all the ethanol. Dry pellet for 5 minutes in the SpeedVac without heat.

10. Resuspend in 100 μl of 2XSLB loading dye.

11. Purify the RNA on denaturing polyacrylamide gel. The percentage of the gel depends on the size of RNA you are purifying. Refer to chart for determining the best %.

12. Stop gel electrophoresis. Place gel between Saran Wrap. Visualize the RNA by placing the gel on TLC paper and shining short wave UV light (hand held lamp).

13. Mark the band with a marker pen.

14. Excise the band using clean razor blades. Place the band in eppendorf tube.

15. Add 600 µl of RNA elution buffer.

16. Vortex for 12-14 hours at 4 °C using eppendorf platform shaker.

17. Add 300 µl of chloroform. Vortex for 10 minutes. Spin at max for 2 minutes.

18. Carefully transfer all the liquid to new tube, do not transfer gel pieces.

19. Add 600 µl of chloroform. Repeat step 4 and 5. (total of 3 chloroform extractions).

20. Repeat step 6-9 (ethanol precipitation).

21. Resuspend in 20 µl of TE. Determine the concentration of RNA using a Spectrophotometer. Usually 1:150 dilution of the RNA is used for estimation. Calculate the molar concentration.

$$\text{Molar Conc.} = \frac{\text{Conc. in } \mu g/\mu l}{330 \times \text{length of RNA (nt)}} = \mu moles/\mu l$$

10X Trx Buffer (New)

10X Conc.	1X Conc.	Stock Sol.	Volume
400 mM Tris pH 8.0	40 mM	2 M	200 µl
200 mM $MgCl_2$	20 mM	1 M	200 µl
20 mM Spermidine	2 mM	1 M	20 µl
1% Triton X100	0.1%	100%	10 µl
		Water	570 µl
			1000 µl

Store at -20 C.