Protocol 2: In vitro Transcription of tRNA

Step1: Digest the plasmid encoding tRNA with BstNI

Water	xx µl	
10X NEB2 buffer	10 µl	
1 µg/µl BSA	10 µl	(not 100X)
BstNI (NEB)	10 µl	
Plasmid	xx µl	(80 µg)
	100 µl	

1. Incubate at 60 °C for 2 hours.

2. Add 10 µl of 0.5 M EDTA, pH8.0 and 10 µl of 3M Sodium acetate pH 5.2.

3. Add 120 µl of phenol-chloroform-isoamylalcohol mix, extract by vortexing rapidly for 30 sec. Spin in microfuge for 1 min at max speed.

4. Transfer upper aqueous phase to new tube. Add chloroform and repeat step 3.

6. To aqueous phase add 350 µl of 100% Ethanol. Precipate DNA at –80 °C for atleast 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 DNA 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 50 µl of TE.

Step2: Reaction mix for 500 μl transcription.

Water	223 μl	
10X Trx Buffer (New)	50 μl	
50 mM GMP	100 μl	(10 mM Final Conc.)
50 mM DTT	50 μl	
25 mM AUGC (NTP mix)	40 μl	(2 mM Each Final Conc.)
T7 RNA Polymerase (JF prep)	10 μl	
Digested Plasmid	25 μl	(40 μgs)
[α-^{32}P] UTP (Optional)	2 μl	
	500 μl	

Follow Protocol 1: Steps 1-21.