Protocol 3: In vitro Transcription of ASL4-33

Step1: Anneal 1000 picomoles of 18T7T (T7 Top Strand) and 1000 picomoles of ASL template by heating to 90 °C for 2 minutes and cool slowly to room temperature in metal tray containing hot water. (Annealed Template = 20 µl Final Volume)

Step 2: Reaction mix for 500 µl transcription.

Water	116 µl	
500 mg/ml PEG8000	100 µl	
50 mM GMP	100 µl	(10 mM Final Conc.)
1M Tris pH 8.1	20 µl	
50 mM Spermine	20 µl	
125 mM DTT	20 µl	
0.1% TritonX 100	50 µl	
25 mM AUGC (NTP mix)	40 µl	(2 mM Each Final Conc.)
1M $MgCl_2$	10 µl	
T7 RNA Polymerase (SJ prep)	2 µl	
Annealed Template	20 µl	
[α-^{32}P] UTP (Optional)	2 µl	
	500 µl	

Incubate at 37 C for 3 to 6 hours.

Then for RNA purification follow the detailed protocol for T7 RNA polymerase transcription: Steps 1 to 21