In vitro mRNA synthesis

This protocol is transcribing capped mRNA from linearized plasmid DNA. For example, for making transposase mRNA, linearize plasmid #123 (pCS2FA CO Tol2 TPase) with NotI-HF then synthesize mRNA with SP6.

Linearize plasmid

1. For a 50 ul reaction:

10 x NEB buffer	5 ul	1x
Plasmid DNA (5 ug)	x ul	
Restriction enzyme	2 ul	
Water	make up to 50 ul	
Final	**50 ul**	

2. Incubate at appropriate temperature for 2-3 h
3. Run 2 ul on an agarose gel alongside an equivalent amount of unlinearized plasmid
4. If linearized, proceed to purification. If not, incubate in enzyme for longer

Purify linearized plasmid

For this, we typically use the Zymo DNA Clean & Concentrator-5 kit:

1. Add two volumes of DNA binding buffer for one volume of sample (i.e. add 96 ul of DNA binding buffer to 48 ul of linearized plasmid)
2. Transfer mixture to a spin column in a collection tube and centrifuge at 13,000 rpm in desktop centrifuge for 30 s.
3. Discard flow through. Add 200 ul DNA wash buffer and centrifuge for 30 s
4. Discard flow through. Add 200 ul DNA wash buffer and centrifuge for 30 s
5. Transfer column to fresh RNAse-free tube and pipette 6 ul of nuclease-free water onto membrane. Centrifuge for 30 s to elute purified linearized plasmid
6. Quantify with Nanodrop

mRNA synthesis

Before starting, clean the lab bench and pipettes with RNA Zap. Maintain RNase-free conditions throughout.

For mRNA synthesis, we use Ambion mMessage mMachine kits:

1. Thaw everything except enzyme at RT. Vortex 2X NTP/CAP buffer until in solution and then let sit on ice during set-up. Vortex 10X Reaction Buffer until in solution and then let sit at RT during set-up. Microfuge all reagents briefly to get solutions out of the lids.

2. Assemble the following reaction IN ORDER at RT (not on ice):

Nuclease-free water	to 20 ul
2X NTP/CAP	10 ul
10X Reaction Buffer	2 ul
Linearized plasmid (1 ug)	x ul
Enzyme Mix	2 ul

3. Pipette the mixture up and down (gently, without causing bubbles to form) and briefly microfuge to collect everything in the bottom of the tube.
4. Incubate at 37°C for 2 h (but no longer)
5. Add 1 ul of TURBO DNase, mix and incubate at 37°C for 15 min

mRNA purification

We often use the MEGAClear Kit for mRNA purification:

1. Add 80 ul Elution Solution. Mix gently by pipetting.
2. Add 350 ul Binging Solution Concentrate. Mix gently by pipetting.
3. Add 250 ul of 100% EtOH. Mix gently by pipetting.
4. Add sample to the filter cartridge. Centrifuge for 1 min at 13,000 rpm.
5. Discard flow through. Add 500 ul Wash Solution.
6. Discard flow through. Add 500 ul Wash Solution.
7. Discard flow through. Centrifuge again to remove traces of Wash Solution.
8. Place cartridge into elution tube. Add 50 ul Elution Solution to the center of the filter. Close lid and incubate in heatblock at 70°C for 10 min.
9. Centrifuge for 1 min at 13,000 rpm.
10. Check yield with Nanodrop and assess size of RNA band on agarose gel.

mRNA concentration

It may be useful to concentrate the mRNA sample using the following procedure:

1. Add 1:10 vol 5 M ammonium acetate (e.g. 5 ul in 50 ul euluted RNA)
2. Add 2.5 vol 100% EtOH. Mix and incubate at -20°C for 30 min
3. Centrifuge at 13,000 rpm for 15 min (it is okay to do this at RT)
4. Carefully remove supernatant, leaving pellet behind
5. Wash the pellet with 500 ul 70% ice-cold EtOH by dripping EtOH down the side of the tube. Centrifuge at 13,000 rpm for 15 min

6. Remove supernatant. Perform a quick spin and remove any remaining EtOH with a very fine tipped pipette or a syringe

7. Air dry pellet then resuspend with desired volume of nuclease-free water