

Preparation of mRNA for transfection

Overview: mMessage mMachine (Ambion)

1. Transcribe mRNA
2. Digest DNA with DNase I
3. Precipitate with LiCl
4. Resuspend pellet

Notes on RNA handling:

1. Clean bench with RNase away and put down new bench pad
2. Put tips and tubes out (RNA only drawer)
3. Use 0.6 mL siliconized tubes for all reactions
4. RNA stored @ -20°C
5. Use clean gloves
6. Microfuge reagents prior to use

Materials:

1. mMessage Machine T7 kit (ambion, -20°C)
2. Linearized DNA template
3. Ethanol (-20°C)
4. Yeast Poly adenylase (USB, -20°C-yellow stratacooler)
5. 10 mM ATP (-20°C-yellow stratacooler)

Procedures:

RNA- Transcription Reaction Assembly: Procedure modified from mMessage mMachine (p. 7-8), mMessage mMachine kit stored @ -20C

1. Thaw frozen reagents
 - a. Vortex 10X Reaction Buffer & 2X NTP/CAP until completely in solution.
 - b. Place 2X NTP/CAP on ice.
 - c. Keep 10X Reaction Buffer at RT (or might coprecipitate template).
 - d. RNA polymerase enzyme mix- keep in StrataCooler (stored in glycerol)
 - e. Microfuge reagents before opening to prevent loss and contamination.
2. Assemble transcription reaction at RT in siliconized tubes.
 - a. Add in following order

<u>Amount</u>	<u>Component</u>
To 20uL	Nuclease-free H ₂ O
10 uL	2X NTP/CAP
2 uL	10X Reaction Buffer
1 ug	linear template DNA
2 uL	Enzyme Mix

Note: this can be done on a 3 ug scale

- b. Flick tube.
- c. Microfuge briefly.
- d. Incubate in 37°C incubator for 2 hr (1hr minimum, 2 hr for maximum yield).

Digestion of DNA Template

1. Add 1 uL of DNaseI (per ug of template),
2. Mix well by flicking
3. Microfuge briefly
4. Incubate @ 37°C for 15 min.

LiCl Precipitation of RNA

1. Add 15 uL LiCl (per 3 ug linear template; can be cold; LiCL from Kit)
2. Flick tube
3. Microfuge briefly
4. Incubate @ -20°C for at least 30 min.
5. Pellet RNA in cold room in microfuge
6. Wash pellet w/ 70% EtOH for RNA
7. Spin
8. Remove supernatant
9. Air dry pellet on RNA bench
10. Resuspend pellet in 30 µL H₂O for RNA
11. Refreeze
12. Thaw
13. Quantitate RNA

Polyadenylation of RNA

Reaction:

	<u>1x</u>	<u>4x (do)</u>
RNA	7 μ L	28 μ L
5x buffer	2 μ L	8 μ L
10 mM ATP	0.2 μ L	0.8 μ L
yeast polyA polymerase	1 μ L	4 μ L

1. Leave RNA in the tube it's in
2. Add 5x buffer to each template (do NOT pipet up and down)
3. Add polymerase
4. Flick tubes until well mixed
5. Microfuge briefly
6. Incubate 20 min @ **30°C** – (can use heating block but turn on early)
7. LiCl precipitate RNA
 - a. Add 5 μ L (20 μ L for 4x) LiCl (from Ambion Kit)
 - b. Incubate at -20°C for at least 30 min
 - c. Pellet RNA in cold room in microfuge
 - d. Wash pellet w/ 70% EtOH for RNA
 - e. Spin
 - f. Remove supernatant
 - g. Air dry pellet on RNA bench
 - h. Resuspend pellet in 30 μ L H₂O for RNA
 - i. Refreeze
 - j. Thaw
 - k. Quantitate RNA

Quantitation:

Make 1:10 to 1:50 dilution for Abs

Storage of mRNA:

-20°C