POLY A TAILING

Introduction

The mRNA is mixed with poly-A polymerase, ATP, buffer and salts to create a poly(A)-tailing reaction. After a poly(A)-tailing reaction, the RNA must be precipitated to remove everything that is not RNA in the solution. The protocol is derived from New England Biolabs' protocols regarding their Poly(A) Polymerase product.

Materials

- Poly(A) polymerase 2U/ml
- mRNA sample from previous steps
- 25mM MnCl2
- 10mM ATP
- 5x Poly(A) buffer
- RNase-free water
- Sodium acetate
- Glyco-blue glycogen
- Ethanol 99%
- Ethanol 70%

Procedure

Poly(A)-tailing reaction

1. Prepare the working solution in a new tube. **For this to work, you need to have a set total volume**
2. Poly(A)polymerase: 2**U**
3. mRNA: **max 40**µg (in reality about 4 µg)
4. MnCl2: **2,5mM**
5. ATP: **1mM**
6. PAP 5X buffer: **diluted to 1x**
7. RNase-free water: **fill up to remaining volume**
8. **AFTER PUTTING THE WORKING SOLUTION TOGETHER, incubate at 37 celsius for 1h**

Precipitation

9. To start the precipitation, add 1/10 of the samples total volume of **Sodium acetate,** 1/50 of the samples total volume of **GlycoBlue,** and 3x the samples total volume of **cold 99% ethanol.**

10. **EXAMPLE REACTION FOR TOTAL VOLUME 100µl:** 10**µl** of sodium acetate, 2**µl** of GlycoBlue and 336**µl** of 99.7% of ice cold ethanol

11. Put the sample in the -80 celsius freezer for at least 1h

12. Take out the sample and centrifuge it for 30min at 13.000rpm in the cold room

13. Carefully remove the supernatant and **dont touch the pellet**

14. Add 750**µl** of cold 70% ethanol, vortex briefly and centrifuge for 10 mins at 13.000rpm in the cold room

15. Remove supernatant, add another 750**µl** of 70% ethanol, vortex and centrifuge for 10 mins at 13.000rpm again

16. Remove the supernatant (**try to get rid of all ethanol)**, and keep the tube **slightly open with your thumb on the lid for 5mins**

17. Resuspend the pellet in 15µl RNase-free water, depending on your RNA inout requirement (cDNA has a minimal input conc. of 66ng/µl). Make sure to scratch the wall of where the pellet was supposed to be, RNA can form an invisible film on a large surface along the wall of the tube.

18. Let the pellet rehydrate for 15min in room temperature. If needed, vortex the tube to get the pellet to resuspend in the water.

19. Check concentration in Qubit (HS RNA) and take out 1,5 ul of sample into Bioanalyzer Analysis.