Protocol 5: Deprotection and Purification of Synthetic RNA

CAUTION: *Wear gloves, use RNAase free materials.*

1. Carefully open the column and transfer CPG beads into a 1.5 ml screw-cap tube.

2. Add 1 ml of 40% Methylamine solution (Aldrich#42646-6). Close the tubes tightly and seal with parafilm.

3. Incubate at 65 °C for 10 minutes. Cool to –20 °C for 10 minutes. Spin at max for 1 min and transfer supernatant to 2 sets of new 1.5 ml screw-cap tubes (500 µl each tube). Wash beads with 1 ml of Ethanol : Acetonitrile : Water :: 3 : 1 : 1. Transfer 500 µl each to above 2 sets of tubes respectively.

4. Dry above mix to a white powder in Speed Vac with No Heat. Takes 10 to 12 hours.

5. Resuspend dried pellet in 250 µl TEA.HF/NMP solution. (Make this solution by combining 1.5 ml N-Methylpyrrolidinone, 750 µl Triethylamine and 1 ml Triethylamine trihydrofluoride; order of addition is important to make this solution).

Important Note: *I add 125 µl TEA.HF/NMP solution to each of my 2 sets of dried pellet, throughly vortex and combine the 2 sets into one tube.*

6. Parafilm the tubes and incubate at 65 °C for 1 hour 30 minutes. Cool tubes, place on ice for 30 minutes.

7. Add 25 µl of 3M NaoAc pH 5.2 and 1 ml of 1-butanol. Vortex.

8. Incubate at –70 °C for atleast 1 hour (can leave it overnight).

9. Spin in microfuge at max speed for 30 minutes at 4° C.

10. Carefully remove butanol. Wash RNA pellet with 70% ethanol. Dry pellet for 5 min in Speed Vac with no heat.

11. Resuspend in 100 µl of TE, pH 7.5.

12. Further purify the RNA on a denaturing polyacrylamide gel. Load about 30 µl RNA + 30 µl 2XSLB mix/lane. Follow standard procedures for RNA identification by UV-shadowing and passive elution from gel slice.

REAGENTS:

40% Methylamine, Aldrich#42646-6
Acetonitrile, Aldrich#27071-7
Triethylamine trihydrofluoride, Aldrich#34464-8
Triethylamine, Aldrich#47128-3
1-butanol, Aldrich#27067-9
1-Methyl-2-Pyrrolidinone, Aldrich#32863-4

Reference: Wincott et al., NAR (1995), Vol23, No 14, pp2677-2684.