Labeling of mRNA with Pyrene

Deprotection of Synthetic RNA (standard DHARMACON protocol):

- Centrifuge tube with dried RNA briefly
- Add 400µL of 2'-Deprotection buffer to each tube of RNA (2'-Deprotection buffer = 100mM acetic acid adjusted to pH 3.8 with TEMED).
- Completely dissolve RNA pellet by pipetting up and down.
- Vortex for 10 seconds and centrifuge for 10 seconds
- Incubate at 60°C for 30min.
- SpeedVac to dryness before use.

Extraction of Deprotected RNA:

- Resuspend dried RNA pellet in water to concentration of ~1mM (150µL is fine for a 0.4 µmol synthesis)
- Extract three times with chloroform
    - Add chloroform 1:1 to dissolved RNA volume
    - Vortex for 30sec.
    - Centrifuge for 1min.
    - Remove aqueous layer (top)
    - Repeat twice.
- Ethanol precipitate by adding 0.1vol of 3M NaoAc and 2.5 vol of ice cold 100% ethanol. Leave at -20°C for 1hour.
- Pellet by centrifuging 30min 4°C at max.
- Dry pellet in speedvac and resuspend in water to a final concentration of 1000 µM.
- Store at -20°C

Labeling Reaction:

- Reagents:
    - 0.1M Sodium Tetraborate pH 8.5 (Borax)
        - Dissolve 1.9g of Borax in 40mL of milliQ water. Adjust pH to 8.5 using concentrated HCl. Raise volume to 50mL final.
        - Store at -80°C in 1mL aliquots.
        - ***Note:*** Borax absorbs CO_2 from the air, resulting in a change in pH. Use each aliquot once then discard.
    - Pyrene Succinimide solution
        - Dissolve 10mg of Pyrene Succinimide in 560µL DMSO.
        - Use only once and discard.

- Reaction:

1000 µM mRNA	7.0 µL
Pyrene Succinimide	14.0 µL
0.1M Borax pH 8.5	75.0 µL
MilliQ Water	4.0 µL
	100.0 µL

Note: The mixture will get cloudy upon addition of pyrene succinimide in DMSO

- Incubate at room temp. ***in the dark*** on an eppendorf shaker for at least 6 hours (overnight is ok)
- Ethanol precipitate by adding 10 µL of 3M NaoAc and 300 µL of ice cold 100% ethanol. Incubate -20^{o}C for 1 hour.
- Centrifuge 4^{o}C for 30min at max
- Dry pellet, resuspend in 50 µL water and 50 µL 2X Sequencing Loading Buffer (SLB)
- Purify labeled mRNA on 15% Acrylamide-Urea gel
- Visualize band by UV shadowing. Mark and cut out bands with a clean razor blade.
- Elute overnight on eppendorf shaker at 4^{o}C overnight in 600 µL Elution Buffer.
- Extract twice with chloroform (same as above)
- Precipitate by adding 1mL ice cold 100% ethanol, leave at -20^{o}C for 1hour.
- Centrifuge 4^{o}C at max for 30min.
- Dry pellet.
- Resuspend in 10 µL water per labeling reaction.
- Quantitate and raise volume to final concentration of 100 µM.