Pre-stock (Gear, Track)

Prestock

1. Spin plates down at 2000 rpm for 1 min.
2. Pipette 20 uL of oligos into basin in accordance with master prestock sheet using the multichannel pipette.
3. Transfer into respective prestock plate & respective well in accordance with the master PS/WS sheet on drive. (i.e. In the sheet, for workstock A1 it need oligos from prestock A1-A5. Pippete 10 uL for each prestock oligo A1-A5 in to worstock A1.)

Working stock (Gear, Track)

Working Stock

1. Using master WS sheet on drive, scale up amounts of oligos to transfer into a new falcon tube such that the end result will be 1 mL of WS of a specific structure. (i. e. If a sheet calls for 170 uL from A1 and 30 uL of ddH20, add 850 uL from A1 and 150 uL of ddH20 to get 1 mL of WS)
2. Invert PCR tube to mix WS.

Folding Reaction

Components

1. 8064 scaffold @100nM 10 uL.
2. Working stock 20uL.
3. 10x FOBXM (50nM TRIS, 10mM EDTA) 5uL.
4. ddH2O 10uL.
5. 10x $MgCl_2$ Screen Strip Mastermix 5uL.

Agarose gel electrophoresis (gel purification)

Gel viewing

Gel Protocol

1. Place 250 mL beaker on scale and add 1.25 g agarose. Then fill to 62,5g with .5x TBE buffer and heat in microwave for 1.5 min. Refill to 62.5 g with ddH2O and 0.5mL 1.375M $MgCl_2$.
2. Carefully add 2 uL of ethidium bromide and mix contents of beaker thoroughly before pouring out into a gel rig. Place comb into gel rig and allow mixture to cool 15-20 mins before removing comb.
3. Add 300 mL of .5 x TBE buffer to gel rig & load wells.
4. To prep for loading wells, take an 8-strip tube and add 3 uL of loading dye (varies by experiment), and load bottom to top so that the first well is 1 kb ladder, and the second well is control (MF or scaffold).
5. Secure the cathode and anode to the rig and power source, and run for 2 hours. Place ice around rig so that overheating does not occur.
6. Remove gel from rig and place on the UV table.
7. Place the camera over the gel and turn the camera on.
8. Open the imaging software on the computer. Center camera over the gel and take the image.

PEG purification

PEG protocol

- 1. In 1.5 mL epi tube, add desired volume of structure.
- 2. Add equivalent volume of 15% PEG8000, and pipette up and down to mix.

- 1. Spin in centrifuge at 16,000 G for 30 min.
- 2. Resuspend in 1x tris and EDTA based dye w/ 20 mM MgCl2, with volume equal to initial volume of structure.

Nanodrop concentration measurement

1. Open the NanoDrop program on the thermocycler computer.
2. Select nucleic acids.
3. Leave the arm down for NanoDrop to calibrate.
4. Once the calibration is done, add 1-2 μL of the buffer to the NanoDrop and click "BLANK".
5. Name the sample in the bar on the top right.
6. Clean the buffer on the platform and the arm using a KimWipe.
7. Add 1-2 μL of sample, close the arm, and click "Run".
8. Save with a descriptive name.
9. Repeat steps 6-8 at least three times and ensure close values. Take the average of those values.
10. Clean with 2 μL of ddH2O. Add the drop and close the device to clean both the arm and the platform.
11. Calculate molarity using the following equation.

$$\frac{A\ [ng/\mu L]}{B*660} * 1,000,000 = C\ nM$$

Where:
A: the concentration, in $ng/\mu L$, obtained from the NanoDrop.
B: the number of bases of the scaffold being used. (8064 in this project)
C: the concentration, in nM.

- For single-stranded DNA, use 330 instead of 660.

TEM

1. Before grid preparation, prepare Uranyl formate (UFo) staining solution.

a. Thaw out premade stock of UFo, and place 1 mL droplet of 5M NaOH on the inner wall of the tube.

b. Vortex on high setting for 2 minutes, then centrifuge at high setting for 3 mins.
2. Place TEM grids on a slide using tweezers, and clean and prep surface using the plasma etcher.
3. Hold grid using tweezers, and add 3 uL of sample onto the carbon coated side of the grid. Allow sample to adsorb onto grid surface for 4 minutes.
4. With approximately 1 min left, place one 10 uL drop and one 20 uL drop of UFo on a parafilm surface.
5. When time has elapsed, wick off excess solution on the grid using a filter paper. Invert grid onto the 10 mL UFo droplet such that the carbon coated side is in contact with UFo. Immediately wick off excess UFo using the filter paper.
6. Invert grid onto the 20 uL UFo droplet. Maintain contact with UFo for 40 sec. After 40 sec has elapsed, wick off excess UFo using the filter paper.
7. Store prepared grid in a TEM grid box.