Nucleosome assembly: modified from Wang Lab nucleosome assembly protocol

1. Buffers:

High salt: 400 mL

4mL 1M Tris pH 7.5

0.8 mL 0.5 M EDTA

160 mL 5M NaCl

230.8 mL MQ H2O

400 uL 1M DTT

Low salt: 1400 mL

14mL 1M Tris pH 7.5

2.8 mL 0.5 M EDTA

70 mL 5M NaCl

1297.8 mL MQ H2O

1.4 mL 1M DTT

Zero salt: 400 mL

4mL 1M Tris pH 7.5

0.8 mL 0.5 M EDTA

394.8 mL MQ H2O

400 uL 1M DTT (add fresh)

1. Make assembly reactions: 50 uL final volume

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ratio octamer: 601 |  |  |  |  | Final conc. |
| 20x assembly buffer | 2.5 uL |  |  |  | 1 X |
| 5 M NaCl | 20 uL |  |  |  | 2 M |
| 601-DNA |  |  |  |  | 100 nM |
| octamer |  |  |  |  | Specified molar ratio |
| diH2O |  |  |  |  | To 50 uL total volume |

1. Dialysis
   1. Soak membrane in diH2O ~10 min. Cut and unfold membrane such that it is a single layer
   2. Cut PCR tubes as described in lambda chromatin assembly
   3. Add assembly to tube caps, making sure to avoid/ remove any bubbles
   4. Overlay dialysis membrane and seal buttons with the lower part of the PCR tube
   5. Float in high salt buffer, making sure buttons are minimally perturbed by stir bar and that there are no bubbles on the outer surface of the dialysis membrane.
   6. Dialyze overnight at ~1.2 mL/ min with low salt buffer
   7. Check low salt buffer volume in the morning, can increase flow rate to 2.4 mL/ min if desired
   8. Transfer buttons to zero salt buffer when low salt buffer is gone (2-4 hours at 2.4 mL/min) and dialyze for at least 4 hours
2. Remove reactions from buttons by piercing the membrane with a pipette tip and extracting the solution. Spin down in mini-centrifuge to remove any bubbles and store at 4°C for up to a month
3. Run 10 uL of assembly on a 4% native PAGE gel
   1. 120V, 4 deg, 1X TBE
   2. run bromophenol blue just off the gel
   3. post-stain with EtBR

4% native PAGE gel

2.5 mL 40% acrylamide (19:1)

1 mL 10x TBE

H20 to 20 mL

200 uL 10% APS

20 uL TEMED