Chromatin assemblies on lambda

High salt buffer (500 mL) low salt buffer (1 L) TCS-20 (2 L)

5 mL 1 M Tris 10 mL 20 mL

400 mL 2.5 M KCl 100 mL 8 mL

1 mL 0.5 M EDTA 2 mL 4 mL

0.5 mL 1 M DTT (added fresh) 1 mL 2 mL

Assembly reactions: 50 uL, 0.2 uM “601 sites”

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ratio octamer: DNA\* |  |  |  |  | Final conc |
| 20X assembly buffer | 2.5 uL |  |  |  | 1X |
| 5 M NaCl | 20 uL |  |  |  | 2 M |
| Lambda DNA |  |  |  |  | 0.2 uM 601 sites |
| Histone octamer |  |  |  |  | Specified ratio |
| water |  |  |  |  | To 50 uL final |

\* This ratio is determined from the ratio at which a mono-601 substrate (167bp) is fully assembled. The number of 601-sites on lambda DNA is assumed to be ~200; calculated by assuming a 200 bp nucleosome repeat length.

20X assembly buffer

100 mM Tris

10 mM EDTA

Dialysis button assembly

1. Cut PCR tube ~ 1/3 of the way from the lid, trying not to deform it
   1. This with snap to the lid, holding the dialysis membrane in place
2. Cut off lid and save
   1. This is where the assembly volume will go
3. Soak dialysis membrane (snake-skin, 8000 KDa MWCO) in MQ-water on a hot plate
   1. Boiling is okay, I usually set it and forget it as I pipette my assembly reactions
4. Cut membrane length-wise to create a single sheet (as opposed to a tube) and place in the high salt buffer until use
5. Pipette assembly reactions into the tube-top, being careful to avoid and remove any bubbles
6. Overlay the membrane taking care not to wick away any of the reaction
7. Place the lower half of the tube over the top and firmly snap into place using the flat cap of a 1.5 mL eppendorf tube
8. Pipette some high salt buffer over top of the membrane to ensure there isn’t an air bubble at the membrane interface
9. Float in the high salt buffer
10. Pump low salt buffer at ~ 1 mL/ min into high salt buffer, removing high salt buffer at the same rate
    1. Slower is fine, faster is not so great (at least for transitioning from high to low)
11. Once low salt buffer is gone, pump at least 1 L TCS-20 at the same rate
    1. Sometimes I forget and let it pump more than 1L, sometimes the whole 2 L
12. Dip reactions into remaining 1 L of TCS-20 for at least 2 hr
13. Pierce membrane to remove assemblies
14. Assess quality via MNase digestion (see protocol)
15. Store at 4deg for up to a month