Nucleosome Spacing Assay

1. Reagents

- a) Extraction buffer 5/50 (ExB 5/50) containing 0.1 µg / µl BSA (Boehringer).
- b) McNAP
- c) Core histones
- d) Hi buffer (2M NaCl, 10 mM Tris 7.5, 1 mM EDTA, 0.05% NP40)
- e) Purified NAP1 (TS19-2, ~1.5 µg / µl)
- f) Buffer H 0.3
- g) Your favorite chromatin remodeling complex
- h) Template DNA. Can be circular or linear. I normally use lambda DNA.
- i) MNase stop solution and glycogen (Boehringer). See chromatin assembly protocol.

2. Reaction

Add the following reagents IN ORDER. Mix gently immediately after adding components.

- a) ExB 5/50 +BSA (to final 30 µl).
- b) 3 µl of Mc NAP.
- c) 0.5 µl of yeast NAP1.
- d) 0.5 µl of core histones (~0.5 µg / µl).
- e) ATP-dependent chromatin remodeling complex (0.5 µl for YB95 complex from TB790-5 #19). For no factor control, use buffer H 0.3.
- f) DNA. I use 0.5 µl of 0.5 µg / µl lambda DNA.
- g) Incubate @ 30 ¡C for 4 hrs.
- h) Add 0.6 µl of 0.1 M CaCl2 per tube.
- i) Digest with MNase. Depending on the freshness of the enzyme, I use 1 µl of X10 ~ X30 dilution (original: 50 u / µl).
- j) Take out 15 µl ea at 3' and 15' of digestion, and add to stop solution (4 µl of X5 MNase Stop plus 1 µl of glycogen per tube).
- k) Add 18 µl of water, 40 µl of X2 Stop buffer, and 2 µl of 10 mg / ml proteinase K per tube. Incubate at 37 ¡C overnight.
- l) Add 20 µl of 10 M Ammonium acetate and 250 µl of EtOH.
- m) Do EtOH ppt, dissolve ppt in 4 µl of X1 Orange G loading buffer, and run on 1.3 % agarose in 0.5 X TBE.
- n) Take photo.
- o) Have a good day.