

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 $^{\circ}\text{C}$ for 4 hrs.
- h) Add 0.6 μl of 0.1 M CaCl_2 per tube.
- i) Digest with MNase. Depending on the freshness of the enzyme, I use 1 μl of X10 $\sim$ 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 $^{\circ}\text{C}$ 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.