

# Nucleosome Spacing Assay

## 1. Reagents

- a) Extraction buffer 5/50 (ExB 5/50) containing 0.1  $\mu\text{g} / \mu\text{l}$  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,  $\sim 1.5 \mu\text{g} / \mu\text{l}$ )
- 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  $\mu\text{l}$ ).
- b) 3  $\mu\text{l}$  of Mc NAP.
- c) 0.5  $\mu\text{l}$  of yeast NAP1.
- d) 0.5  $\mu\text{l}$  of core histones ( $\sim 0.5 \mu\text{g} / \mu\text{l}$ ).
- e) ATP-dependent chromatin remodeling complex (0.5  $\mu\text{l}$  for YB95 complex from TB790-5 #19). For no factor control, use buffer H 0.3.
- f) DNA. I use 0.5  $\mu\text{l}$  of 0.5  $\mu\text{g} / \mu\text{l}$  lambda DNA.
- g) Incubate @ 30  $^{\circ}\text{C}$  for 4 hrs.
- h) Add 0.6  $\mu\text{l}$  of 0.1 M  $\text{CaCl}_2$  per tube.
- i) Digest with MNase. Depending on the freshness of the enzyme, I use 1  $\mu\text{l}$  of X10 ~ X30 dilution (original: 50 u /  $\mu\text{l}$ ).
- j) Take out 15  $\mu\text{l}$  ea at 3' and 15' of digestion, and add to stop solution (4  $\mu\text{l}$  of X5 MNase Stop plus 1  $\mu\text{l}$  of glycogen per tube).
- k) Add 18  $\mu\text{l}$  of water, 40  $\mu\text{l}$  of X2 Stop buffer, and 2  $\mu\text{l}$  of 10 mg / ml proteinase K per tube. Incubate at 37  $^{\circ}\text{C}$  overnight.
- l) Add 20  $\mu\text{l}$  of 10 M Ammonium acetate and 250  $\mu\text{l}$  of EtOH.
- m) Do EtOH ppt, dissolve ppt in 4  $\mu\text{l}$  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.