Judd Rice Laboratory
at the USC/Norris Comprehensive Cancer Center
www.histonecode.com

Isolation of Oligonucleosomes by MNase Digestion

Revised by J Sims from O'Neill and Turner, 2003
March 2006

Procedure

Nuclear Extraction

1. Trypsinize cells off of 15 cm plates (I use no less than 3 confluent 15 cm plates), pellet the cells, wash in PBS.

2. Resuspend the cell pellet in 20 mL cold NIB buffer and spin down 600 x g for 7 minutes at 40C.

3. Wash nuclear pellet 2X PBS/1mM PMSF
   Nuclei can be stored at -800C if needed.

MNase Digestion of Nuclei

1. Resuspend nuclei in MNase Digestion Buffer to a final concentration of 0.5mg/mL as determined by UV Spectroscopy.

2. Add 50U MNase/0.5mg chromatin and digest 7 minutes at 370C.

3. Stop the reaction by adding 10mM EDTA and leave overnight at 40C.

4. Spin down 5 minutes 11,600 x g at 40C. The supernatant should contain oligonucleosomes.
   To determine the final concentration of oligonucleosomes, re-read the samples at A260.

5. The oligonucleosomes should be stable at -80°C up to 4 weeks.
   Be sure to add additional protease inhibitors before storage and avoid multiple freeze/thaw cycles.

Solutions

NIB Buffer

150mM NaCl
10mM HEPES, pH 7.4
1.5mM MgCl2
10mM KCl
0.5% NP-40
0.5mM DTT
protease inhibitors

MNase Digestion Buffer

0.32M sucrose
50mM Tris, pH 7.4
4mM MgCl2
1mM CaCl2
1mM PMSF