Reconstitution of Recombinant Nucleosome Core Particle

Adapted from: Dyer, PN *et al* (2004) Reconstitution of nucleosome core particles from recombinant histones and DNA. *Meth. Enzymol.* **375**: 23–44.

1) Combine equimolar ratios of 601 DNA (see ref 1) and recombinant octamer. Final concentrations of 0.5-2 µM DNA are used routinely with success.

2) Transfer DNA/octamer mixture to a dialysis apparatus (6-8 kDa MWCO)

3) Dialyze DNA/octamer against a step-wise dilution series as follows:
   i) 2 M NaCl, 10 mM Tris pH 8.0, 1 mM EDTA, 1 mM DTT (O/N)
   ii) 0.85 M NaCl, 10 mM Tris pH 8.0, 1 mM EDTA, 1 mM DTT (≥ 2 hours)
   iii) 0.65 M NaCl, 10 mM Tris pH 8.0, 1 mM EDTA, 1 mM DTT (≥ 2 hours)
   iv) 0.2 M NaCl, 10 mM Tris pH 8.0, 1 mM EDTA, 1 mM DTT (O/N)

4) Analyze nucleosome reconstitution on a 5% 0.2xTBE nondenaturing PAGE (37.5:1 acrylamide:bis-acrylamide) gel using a 100 bp DNA ladder as a standard. Nucleosomes can be visualized by ethidium bromide and Coomassie Brilliant Blue staining. A 186 bp DNA fragment (based on the 601 sequence) wrapped around octamer into functional nucleosome core particle will shift from ~200 bp to 500-600 bp.

5) Reconstituted nucleosomes can be used or purified by preparative nondenaturing PAGE.

References:

1. Lowary PT and J Widom (1988). New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J. Mol. Biol.* **276**: 19–42.