Gozani Lab
9-14-05

Monomucleosome binding assay

2x Binding buffer: 40 mM HEPES7.9, 160 mM KCl, 0.2 mM ZnCl2, 0.2% EDTA, 20% glycerol, 0.2% NP40, 1mM DTT, 1 mM PMSF.

Total vol: 20 µl.
Mononucleosome: ~ 1-1.5 µg. (4-5 µl of 0.3 mg/ml) per binding.
Proteins: varient. Depend on binding affinity to nucleosome.

Core mix for 10 rx: 40 µl mononucleosome + 40 µl 2x binding buffer, aliquot 10 µl each.
Add proteins: GST-ING2PHD: 0, 5, 10, 20, 50 µg (1 ul, 2ul, 4 ul and 10 ul of 5 mg/ml).
GST control 0, 20, 50 µg (5µl and 12.5 µl of 4 mg/ml). (diluted with binding buffer).

Binding condition: leave at 30oC 30 min.

Add 5 µl 6x DNA sample buffer, load onto 5% acrymide/TBE gel, ran with **1xTBE buffer** under 120 V 60-70 min.

Stained in EB/TBE (5 µl EB in 50 ml TBE) for 1 h.