Chromatin Beads

Thio-dCTP (100 mM), 1 μmol (N-8002-1 from TriLink BioTechnologies)
Thio-dGTP (100 mM), 1 μmol (N-8003-1 from TriLink BioTechnologies)
Biotin-14-dATP (0.5mM), 50 nmol (19524-016 from Invitrogen)
Biotin-21-dUTP, 0.5 mM (635701 from BD Biosciences)

DNA Polymerase I, Large (Klenow) Fragment, 5 Units/μl (M0210S from NEB)

Dynabeads M-280 Streptavidin 10mg/ml (6-7 x 10^8 beads/ml)[1]
Dynal® kilobaseBINDER™ Kit (SKU# 601-01, Invitrogen)

Linearize plasmid

With BamHI and NotI, ideal ~ 4kb

DNA biotinylation

1. Fill-in reaction

DNA	30 μg
Klenow buffer 10x	7 μl
0.4 mM Biotin-14-dATP	8.7 μl
0.5 mM Biotin-21-dUTP	7 μl
5 mM Thio-dCTP	1 μl
5 mM Thio-dGTP	1 μl
Klenow (5μ/μl)	4 μl
ddH_2O	add 70 μl

1h @ 37°C

2. Separate unincorporated nucleotides

Precipitate DNA with EtOH
Check DNA concentration by measuring OD_{260}

Coupling DNA to beads

using the Dynabeads kilobaseBINDER™ Kit
(>182 μg of a 4 kb DNA fragment/mg beads)

1. Resuspend the Dynabeads M-280 Streptavidin by shaking the vial to obtain a homogenous suspension.
2. Transfer 60 μl (3 μl beads/ μg DNA) resuspended Dynabeads to an Eppi, place the tube in a Dynal MPC for 1 min until the Dynabeads have settled on the wall.

3. Remove supernatant. Avoid touching the Dynabeads pellet!
4. Add 240 µl binding solution[2] and gently resuspend the beads.
5. Place the tube in the Dynal MPC and remove supernatant.
6. Resuspend the pellet in 134 µl binding solution.
7. Add 134 µl (20 µg DNA) biotinylated DNA to the beads, mix carefully to avoid foaming!
8. Incubate at RT (18°C) o/n on a roller (about 1 turn / 10 s).
9. Place the tube in the Dynal MPC and remove supernatant (measure OD_{260}).
10. Wash twice with binding solution.
11. Repeat a long (>4h) incubation at 4°C with 40% of the initial amount of DNA in "fresh" incubation mix. Measure OD_{260} of the supernatant.
12. Wash the Dynabeads/DNA-complex twice in washing solution[3] and once in CSF-XB.
13. Calculate binding of DNA.
14. Resuspend the Dynabeads/DNA-complex in CSF-XB to get final conc. of 1µg DNA/ 3 µl beads.

Making chromatin beads

1. Take 230 µl CSF-extract containing 1 µM Nocodazole.
2. Resuspend 10 µl of DNA-beads (3µl beads /µg DNA) in 150 µl of CSF-extract containing 1 µM Nocodazole. Incubate for 5 – 10 min at 20°C (rather 16°C).
3. Add Ca^{2+} to final conc. of 0.6 mM.
4. Incubate 90 min at 20°C, rotating.
5. Re-arrest in M-phase by adding 80 µl CSF-extract.
6. Aliquot IMMEDIATELY and freeze in LN.

20 µl of chromatin beads should contain enough beads for resuspension in 60 µl new CSF!

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1) in PBS pH 7.4, containing 0.1% BSA, 0.02 NaN_3
2)
3) 10 mM Tris-Hcl (pH 7.5), 1 mM EDTA, 2.0 M NaCl