

Isolation of Metaphase Chromosomes

The described protocol is a modification of a method developed by Gasser and Laemmli (Exp Cell Res. 1987, 173(1):85-98). At a density of $2 \cdot 10^5$ cells/ml, HeLa S3 cells grown in suspension (0.5 liter culture) were synchronized by a thymidine-nocodazole-protocol (Fang et al., 1998, Mol. Cell 2, 163). The metaphase-arrested cells could not be stored and were immediately swollen for 5 minutes in 50 ml 1xPME (5 mM Pipes/NaOH pH 7.2, 5 mM NaCl, 5 mM MgCl₂, 1 mM EGTA). This step was repeated once. Cells were pelleted at 1000g for 5 minutes and the supernatant was removed. All following steps were carried out at 4°C. The cells were resuspended in 25 ml lysis buffer 4 (1xPME, 1% thiodiethylene glycol, complete protease inhibitor cocktail minus EDTA (Roche), 2.5 μ M microcystin-LR, 1 μ M ocadaic acid, 1 mM ATP, 10 μ g/ml cytochalasinB, 0.2% digitonin) and immediately lysed by 12 gentle strokes with the B pestle of a Dounce homogenizer. The lysate was then divided and put on top of six 30 ml sucrose step gradients each consisting of 2 ml HSS (1xPME, 1% thiodiethylene glycol, complete protease inhibitor cocktail without EDTA (Roche), 1 μ M microcystin-LR, 1 mM ATP, 1.8 M sucrose) at the bottom and 28 ml LSS (1xPME, 1% thiodiethylene glycol, complete protease inhibitor cocktail without EDTA (Roche), 1 μ M microcystin-LR, 1 mM ATP, 0.9 M sucrose, 0.02% digitonin) at the top. After centrifugation in a SW28 rotor (Beckman) for 30 minutes at 4000 rpm, the cytosol and the LSS layer were aspirated off for all but 2 ml. Chromosomes were resuspended in the remaining liquid and combined with wash solution (1xPME, 0.25% thiodiethylene glycol, complete protease inhibitor cocktail without EDTA (Roche), 1 μ M microcystin-LR, 1 mM ATP), 1.6 ml 0.2 M spermidine, and 0.8 ml 0.2 M spermine (in this order) to give a total volume of 49 ml. After incubation for 5 minutes, 31 ml percoll (Amersham) were added. The mixture was carefully dounced 8 times and then centrifuged in a 70 Ti rotor (Beckman) for 30 minutes at 21,000 rpm. Individual chromosomes were recovered from a diffuse band 1 cm above the bottom of the tubes. They were passed through a 70 μ m cell strainer (Falcon) and mixed with 35 ml wash solution in a 50 ml conical tube. After putting 0.3 ml storage solution (1xPME, 70% glycerol) to the bottom of the tube, the tube was spun in a RC3C centrifuge (Sorvall) for 30 minutes at 3300 rpm. This washing step was repeated once more but in a 15 ml tube and with centrifugation at only 2200 rpm. The chromosomes were resuspended in 0.3 ml storage solution and stored in aliquots at -80°C. To determine the DNA concentration the preparation was diluted 1:100 in 5 M urea, 2M NaCl and heated for 15 minutes at 80°C. The absorption at 260 nm was then measured.

Note: It is crucial to adjust the pH of the buffers containing spermine or spermidine to neutral (pH=7.0-7.5).