Metaphase Spreading for DT40

Fukagawa 020712

Grow cells at density of 1-5 X 10^6/ 10 mls flask.

Add Colcemid (10 μg/ml, Gibco) for 90mins: 30 – 100 μl per 10 mls medium.

Centrifuge cells at 1000rpm for 5min. Remove supernatant almost completely. Re-suspend pellet in residual supernatant by gentle, but persistent tapping until all cell clumps are broken up.

Add 10mls pre-warmed hypotonic solution (40mM KCl, 0.5mM EDTA, 20mM HEPES, PH7.4), gently mixing and incubate at 37°C for 10mins.

Centrifuge cells at 1000rpm for 5min. Remove supernatant almost completely. Re-suspend pellet in residual supernatant by gentle, but persistent tapping until all cell clumps are broken up.

Add pre-cooled fixative (methanol:acetic acid 3:1, 4°C) initially dropwise and keep tapping gently until all cell clumps are re-suspended. (Do not use vortex mixer or other rough shaking which breaks up individual mitosis.) Then fill up tube completely with fixative. Store over night at -20°C.