Sister Chromatid Exchange Assay

1. Grow cells in 10 µM BrdU for two cell divisions (40 hrs for 293 cells). Cover plates with aluminum foil (BrdU is light sensitive).
2. Add Colcemid to a final concentration of: 150 ng/mL (13,333X of 2 mg/mL stock, ie 0.75 µL per 10 mL) for the final 30 min to enrich mitotic cells.
3. Transfer medium (floating cells) and trypsinized cells to a new tube. Spin cells at 180 xg for 10 min.
4. Aspirate supernatant leaving 0.2 mL of media and resuspend cells by gently tapping bottom of tube.
5. Add 6 mL of prewarmed 75 mM KCl dropwise for first 1mL while *gently* vortexing. Incubate 16 min at 37ºC (incubator).
6. Add four drops of fixative, gently invert, and spin down cells at 180 xg for 10 min.
7. Aspirate supernatant leaving 0.2 mL of hypotonic solution. Resuspend pellet by *gently* tapping and add 5 mL of freshly prepared fixative (3:1 solution of methanol:glacial acetic acid), dropwise for the first 1 mL while *gently* vortexing. Incubate 20 min at 4ºC. Spin down cells at 180 xg for 10 min.
8. Repeat step 6 two or three times until the cell pellet is colorless.
9. Resuspend the pellet in a small volume of fixative (<1 mL), until the cell suspension looks slightly cloudy.
10. Slides should be prechilled in freezer before used.
11. Draw cells into a plastic transfer pipet. Hold tip 6 in above the slide and place 2 drops onto the slide with enough room for the drops to not touch. Stand slides on slant to dry.
12. Let slides air dry in dark for 2-3 days.
13. Stain slides with 0.1 mg/mL acridine orange (Molecular Probes; alternatively Giemsa Stain can be used) in dH_2O for 5 min at room temperature.
14. Wash slides extensively for 2 min under running dH_2O tap water.
15. Incubate slides for 1 min in Sorenson Buffer, pH 6.8 (0.1M Na2HPO4, 0.1M NaH2PO4) and then mount in Sorenson Buffer, pH 6.8.
16. View immediately under FITC filter and count reciprocal exchange events.

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