Preparation of metaphase spreads

1. Grow cells to ~40% confluence.
2. Add 0.1 µg/ml demecolcin (colcemid) for 1~ 2 hrs in growth medium.
3. Harvest cells by trypsinization, suspend in media, and spin them down.
4. Remove supernatant completely, and resuspend in 5 ml of 0.075 M KCl (pre-warmed to 37°C), be gentle to prevent lysis.
5. Incubate at 37°C for 30 min with occasional gentle mixing.
6. Spin the cells down for 5 min at 500 rpm.
7. Decant the buffer, and resuspend the cells in left over solution by tapping.
8. Add 500 µl of fixative (3:1 MeOH and glacial acetic acid, prepared fresh) drop by drop while the cells are slowly and gently mixed on a vortex (<1000 rpm).
9. Add another 500 µl of fixative.
10. Fill to 10 ml with the fixative and store at 4°C overnight or longer.
11. When ready to drop, spin the cells down (500 rpm).
12. Remove 9 ml of fixative and resuspend cells in the 1 ml (or smaller if fewer cells are left over).
13. Place a few slides in cold water. Place wet paper towels on top of a heating block set to 70°C.
14. Drop the resuspended cells to the wet slide.
15. Wash the cells with fixative gently using Pasteur pipette.
15. Place slides for 1 min on the humidified 70-80°C heating block (place wet paper towels on the hot plate and place the slides on top of the wet paper towels).
16. Check the slides under a regular light microscope for spreading efficiency.
17. Let the slides dry overnight in a fume hood.