Metaphase Chromosome Spreads-Human Cells

Pretreatment of Superfrost Plus slides (Fisher Scientific cat#1 2-550-15)

1. Treat slides with 40% MeOH. Slides can be stored in 40% MeOH at 4°C.
2. Replace the MeOH with 100% EtOH for 1 hour. Air dry slides at RT.

Harvest cells:

1. Grow cells according to specific cell culturing conditions until the cells have reached logarithmic phase (80-90% confluency). A minimum of 2 x 10^6 cells is recommended. Change media 2-3 hours prior harvest.
2. Add 10 µl/ml of Colcemid (10 µg/ml) to the 175 cm^2 cell culture flask.
3. Incubate cells in a 5% CO_2 incubator at 37 °C for 3 hours. Using a sterile pipette, transfer media from cells into a 15 ml conical tube. Set aside.
4. Wash the cells by adding 10 ml of Dulbecco's phosphate-buffered saline (DPBS) into the flask, gently swirl the buffer, use a pipet to remove the wash buffer and discard.
5. Add 5 ml of TrypLE Express (ThermoFisher Scientific) ensuring that it covers the entire surface of the flask. Treat the cells for about 5 minutes at 37 °C and monitor the progress of cell detachment. After the majority of the cells have detached, pipette the media saved from step 3 back into the flask. This is your cell suspension.
6. Transfer the cell suspension in 10 ml aliquots into 15 ml conical tubes. Centrifuge at 200 x g for 10 min. Remove supernatant and resuspend the pellet with the small amount of KCl buffer remaining in the tube.
7. Add 10 ml of 37 °C prewarmed 0.075 M KCl to the pellet in the conical tube. Vortex tube at medium speed to resuspend the cells.
8. Incubate cells at 37 °C for 10 min. Centrifuge at 200 x g for 5 min at 25°C. Remove supernatant (until about 0.5 ml remains) and resuspend the pellet in 5 ml of fresh Carnoy's Fixative (3:1 ratio of methanol:glacial acetic acid). Add additional 5 ml of fixative without mixing for a total of 10 ml.
9. Centrifuge at 200 x g for 5 min. Remove supernatant and resuspend cells in 5 ml of fixative. Repeat for a total of 3 times.
10. The resuspended cells can be stored in the fixative at 4°C for up to one year.

Spread chromosomes:

1) Spin down cells at 200 g for 5 mins at 25°C.
2) Replace the Carnoy's Fixative with 5 ml of fresh Carnoy's fixative and resuspend the cells
3) Take 20 µl the of cell suspension and release the drop on to a pretreated slide tipped at a 45° angle.
4) Set the slide, chromosome side up, over a beaker that has 70°C -80°C distilled water for 30 seconds.
5) Air dry then incubate the slides at 37°C overnight prior to using for DNA-FISH experiments.

Sources:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3073051/

Moralli, Daniela et al. “An improved technique for chromosomal analysis of human ES and iPS cells.” *Stem cell reviews and reports* vol. 7,2 (2011): 471-7. doi:10.1007/s12015-010-9224-4

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4091199/

Howe, Bradley et al. “Chromosome preparation from cultured cells.” *Journal of visualized experiments : JoVE* ,83 e50203. 28 Jan. 2014, doi:10.3791/50203

https://www.komp.org/pdf/Chromosome_Spreads_and_Counting_KOMP_ES_cell_clones_Protocol.pdf