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| National Repository of Fish Cell Lines |
| Standard Operating Procedure  |
| Title: Chromosome preparation from cultured cells |
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**Materials:**

1. 15-ml conical tube.
2. 0.05 % Cochicine in MiliQ/double distilled water (DDW).
3. Hypotonic solution- 0.56 % KCl.
4. Carnoy's fixative- 3:1 (methanol: glacial acetic acid).
5. Giemsa stain (stock solution)
6. Gurr Buffer:
7. Prepare separate solutions: 0.54g/l conc. of KH2PO4 and 0.57g/l conc. ofNa2HPO4 (anhyd.).
8. Mix 2 vols. of KH2PO4 and ~1 vol. of Na2HPO4 until one get buffer pH 6.8. OR
9. Weigh 0.469g of NaH2PO4 and 0.937g of Na2HPO4 and dissolve in 1000-ml of DDW.

**Procedure:**

**Chromosome preparation:**

1. Grow desired cells/ cell line according to the specific culture conditions in the incubator. When the cells attain logarithmic phase, i.e. confluence level up to 70-80%, add 10 µl of 0.05% colchicine in the culture flask (25 cm2). Keep flask in the incubator for 60-70 minutes. Decant the culture media using pipette without disturbing monolayer of the cells.
2. Add 1 ml of 0.25% trypsin, ensuring that it covers the entire surface of the flask, to detach cells from the surface of the flask. Leave the cells in trypsin for about 2 min. When majority of the cells detach, add 6 ml of culture media enriched with 20% FBS and mix gently.
3. Transfer the cell suspension into 15-ml conical tubes. Centrifuge the tube at 1,000 rpm for 5 min and pipette out the supernatant without disturbing the pellet.
4. Add 6-8 ml of prewarmed (37°C) 0.56% (0.075M) KCl to the pellet in the conical tube and mix cells gently. Incubate cells at 37°C for 15 min.
5. Centrifuge the tube at 1,000 rpm for 5-6 minutes at room temperature (RT). Remove supernatant, leaving 0.5 ml supernatant at the bottom and re-suspend the pellet.
6. Carefully add 5 ml of freshly prepared chilled Carnoy's fixative to the cells, mix gently and keep the tube for 30 minutes.
7. Repeat steps 5 and 6 for 3-4 times for cell fixation.
8. The slides can be prepared from the fixed cells using standard slash drop technique. The cells can further be stored at 4°C.

**Slide preparation for metaphase spreads:**

1. Centrifuge the cells at 1,000 rpm for 5 minutes. Remove the supernatant until only 0.3-0.5 ml of fixative remains.
2. After gently re-suspending the pellet in the remaining fixative, pipette the cell suspension and drop onto a tilted slide (~45°angle) from a distance of about 1.0-1.5 feet height and allow the suspension to roll across the slide.
3. Wipe the back of the slide on a tissue paper and then allow the slide to air-dry for at least 10 minutes. The slide should be dried completely before Giemsa staining.
4. Observe the slide under microscope for the presence of good quality chromosomes and proceed for Giemsa staining.
5. Prepare fresh 5% Giemsa Staining solution in PBS/Gurr Buffer (pH 6.8) in 50 ml Coplin jar. Place slides into Coplin jar for 10-15 min. Rinse the slide with distilled water and allow it to air-dry for at least one day.
6. Add 2-3 drops of DPX Mountant and cover the slide with cover slip ensuring that there is no air bubble under the coverslip. The excess mountant can be removed. Allow the slide to dry on hot-plate at 60°C overnight.
7. Analyze cells under light microscope with 10X and 100X magnification. Take photograph of good quality metaphase spreads and prepare the karyotype.