Karyotyping ES Cells

Materials & Reagents required:

- Depex Mounting Medium
- ES Cell Medium:
  - DMEM (Cat. No. SLM-220-B)
  - 15-20% Fetal Bovine Serum (Cat. No. ES-009-B or ES-011-B)
  - 1% Nucleosides,100x (Cat. No. ES-008-D)
  - 1% Penicillin-Streptomycin, 100x (Cat. No. TMS-AB2-C)
  - 1% Non-Essential Amino Acids, 100x (Cat. No. TMS-001-C)
  - 1% L-Glutamine Solution, 100x (Cat. No. TMS-002-C)
  - 1% 2-Mercaptoethanol, 100x (Cat. No. ES-007-E)
  - 1000 units/mL ESGRO mLIF Supplement (Cat. No. ESG1106 or ESG1107)
- Fixative (MeOH:Glacial Acetic Acid, 3:1)
- Hypotonic KCl Solution
- Leishman's Stain (0.2% w/v solution in methanol)
- Microscope Slides
- Pipette
- 0.05% Trypsin-0.53mM EDTA (Cat. No. SM-2002-C)
- Xylene

Procedure:

This method is recommended for use with actively growing cultures of ES cells (i.e. 1–2 day cultures).

1. One day prior to karyotyping, passage a 70% confluent ES cell plate at a 1:2 ratio.
2. At least 3 hours prior to karyotyping, transfer the ES cells into fresh medium.
3. Trypsinize the ES cells and transfer the cell suspension to a conical tube. Centrifuge the cells at 300 xg for 5 minutes, and then aspirate the medium. Avoid allowing the pellets to dry out.
4. Resuspend each cell pellet in 8 mL of hypotonic KCl solution, gently flicking the tube to avoid clumping and ensure an even suspension.

5. Incubate the tube at 37 °C for 10 minutes (this may vary for each type of cell line used).
6. Add 2 mL of freshly made fixative (MeOH:Glacial Acetic Acid, 3:1) and mix by gentle inversion.
7. Centrifuge cells (300 xg, 5 minutes) and aspirate the supernatant.
8. Using a Pasteur pipette, carefully add 2 mL of fixative solution drop wise, with gentle mixing to avoid clumping. Add an additional 6 mL of fixative and mix by gentle inversion of the tube.
9. Centrifuge cells (300 xg, 5 minutes) and aspirate supernatant.
10. Repeat steps 8 & 9 three times.
11. Resuspend the pellet in 1 mL of fixative (this volume may need to be adjusted slightly according to pellet size).
12. To make cell spreads, first humidify the surface of a dried cold slide by exhaling on the slide surface while holding the slide at a 45° angle. Using a Pasteur pipette, carefully dispense one drop of the suspended cells onto the top surface of the slide and allow to air dry.

Staining:

1. Stain slides with freshly made Leishman's stain for 8 minutes.
2. Rinse in running water for 1 minute and air dry.
3. Clear slides in 2 changes of xylene and mount cover slip using Depex mounting medium.

Notes:

- Colcemid is not used in this method, as the mitotic index of actively growing ES cells is generally high enough to obtain an ideal chromosome spread.
- High quality slides are recommended. Slides should be soaked in 100% ethanol overnight and dried with lint-free tissue before use. As it is important to have slides cold before use, slides can be stored in the refrigerator or freezer in an ethanol bath prior to making cell spreads.
- Most labs use 0.56% KCl and some labs use 0.2% KCl + 0.2% Na Citrate as an alternative. This depends entirely on the cell types being analyzed. The time in KCl is crucial — too short and the chromosomes will be too tightly packed; too long and they will not remain in their appropriate group.