

## Karyotyping a cell line

optimized by Noranne Enzer at MIT, Nov 2005

### Prep work:

Check that all solutions are in stock and slides are prepped.

Prepare glass slides (5 per cell line) by soaking overnight in EtOH:HCl 1:1.

Rinse w/ dH<sub>2</sub>O and soak in 200 proof EtOH until use.

### Solutions

50 mg/mL colchicin in EtOH

0.56% KCl

MeOH:Acetic Acid (3:1), ice cold

EtOH:HCl (1:1)

200 Proof EtOH

Giemsa stain

(Invitrogen/Gibco #10092-013)

Gurr buffer

(Invitrogen/Gibco #10582-013)

Glycerol/PBS (1:1)

### Supplies

coplin jar dedicated to Giemsa Staining

glass slides

hotplate

pyrex dish (8x8 or 9x13 is fine)

microscope with 63x-100x objective

attached to computer to save images

22mm x 22mm coverslips

### Preparing cells:

- 1) Feed exp. growing cells (50-75% confluent in one well of a 6-well plate or in a 6cm dish) with media containing 10 µg/ml colchicin and grow cells for 6-12 hours for ES cells, 12-18 hours for other cells. (stock is 50 mg/mL in EtOH, protect from light, store at -20°C) 50mg/ml is 5,000x, so 2µL stock for 10ml media.
- 2) Rinse with PBS, trypsinize for 5 min. (make sure the cells are in single-cell suspension by pipetting 10 times and checking under the scope. This is critical for getting good spreads) and spin through media for 2 min at 2K rpm.
- 3) Resuspend in 1 ml PBS. Divide evenly between 2 eppendorf tubes and spin for 2 min at 2K rpm (Biofuge Pico).
- 4) Pull off PBS and resuspend each pellet in 500 µl of 0.56% KCl. Sit at RT 15 min.
- 5) Spin for 2 min at 2K rpm.
- 6) Remove KCl and resuspend pellets gently in cold MeOH:Acetic Acid 3:1 and let sit on ice for 10 min.
- 7) Pellet at 3K rpm, 2 min (\*Note different conditions – the cells are now hard to pellet, but if you spin too hard, they will break open in the tube)
- 8) Carefully remove the supernatant and resuspend in 200µl fresh, cold MeOH:Acetic Acid 3:1.(Can leave cells at 4°C overnight.)

## **Karyotyping**

### **Dropping Cells**

Prepare a steam bath by placing a pyrex dish (8x8 or 9x13) with 1-2 inches of dH<sub>2</sub>O onto a hotplate, adjusting the temp to get simmering and a lot of steam, but not boiling.

Dry prepared slides carefully with kimwipe before dropping cells.

Resuspend cells with gentle vortexing. Drop 10 µl of suspended cells w/ a pipetman approximately 8 inches onto a slide that is held at a 60 degree angle in steam. Tilt the slide to spread. Let the slide sit in steam while the MeOH evaporates off.

Let air dry. (Here you can quickly check under 10x scope to see if it worked).

Giemsa stain for 2 hours, overnight is fine.

Rinse in dH<sub>2</sub>O. Mount in 50% glycerol/50% PBS and secure coverslip with fingernail polish. Make sure the polish dries COMPLETELY before taking it near an objective.

Take pictures at 63x. Print out each karyotype and count each chromosome by writing a number on each sister chromatid pair. This helps to ensure correct counting and provides a record for your notebook.

### **Solutions**

Giemsa stain (Invitrogen/Gibco 10092-013): 4% in Gurr Buffer. Note- assume bottle from Gibco is 100%- Put 2 ml Giemsa in 48 mL Gurr, pour into coplin jar.

\*Note: Giemsa stain is collected as hazardous waste.

Gurr buffer: One tablet in 1 liter dH<sub>2</sub>O