Karyotyping
1. Split cells that need to be karyotyped onto matrigel at 1/6 or 1/12 dilutions off from MEFs.

2. Cells should be in a stage of active division (not confluent) when initiating the karyotyping procedure.

3. Wear gloves. Add 23 ul of Vinblastine Sulfate (Vb) working solution (25ul/25ml ddH2O) to each well of a 6-well plate (23ul/3ml or 15ul/2ml Vb). Working conc of VB is 2 ug / ml.

4. Incubate cells 2 – 3 hours in 37°C incubator.

5. Meanwhile, put one bottle of sterile ddH20 and one 50ml tube filled with 70% Ethanol in fridge (4C). Also, put one box of slides at –20C.

6. 45 minutes prior to hood work, put hypotonic solution, 1x PBS, and wash media (DMEM + 5% FBS) in 37C water bath. 20 minutes prior to hood work, put trypsin in 37°C water bath.

7. After 2-3 hour incubation, prepare 3 15ml tubes with 8ml of wash media in each. Label as 15, 30, and 40.

8. Get cells from incubator and aspire media from wells, add 1ml 1x PBS/well, swirl and aspire. Repeat.

9. Add 1ml/well of warm trypsin and incubate for 1-2 min in 37°C incubator.

10. Pipette cells up/down with P1000 and transfer 1/3 (2 wells) of cells to each 15ml tube.

11. Spin tubes 5 minutes @ 800rpm.

12. Remove sup by either aspiring it or pouring it out and resuspend pellet by flicking tube.

13. SLOWLY (i.e. drop by drop) add 2.3ml hypotonic solution per tube. Put tubes in 37C water bath for 15, 30, and 40 minutes.

14. When incubation period is over, take tube and spin 5 minutes @ 800rpm.

15. Meanwhile, make 50ml of Carnoy’s fixative. It must be made FRESH 1:3 Glacial acetic acid: Methanol, 12.5ml Glacial acetic acid ,37.5ml Methanol
14. Remove most of sup, and resuspend pellet in remaining media.

15. Using a dropper, slowly add 10 drops of Carnoy's fixative, flicking tube in between drops to prevent clumping. Bring volume to 2ml with fixative.

16. Spin 5 minutes.

17. Remove supernatant and slowly add 4ml of fixative, flick tube, spin and discard sup. Repeat, but don't remove all of sup. the second time around. Leave about 1ml of sup. and flick.

18. When you are ready to drop the cells, fill plastic box with ice and pour enough of the chilled ddH2O and Ethanol into two separate beakers to dip and cover the cold slides. Leave the beakers on ice. (Use slide holder for ethanol and large square beaker for ddH2O).

19. Dip slides in chilled 70% ethanol and then rinse well with in the chilled ddH2O. Leave slides in the chilled water until you are ready to use them – slides must remain COLD in order for karyotype to work!!!. Using a glass pipette, aspire some of the cells and drop them onto the clean, wet and chilled slides either at arms length away or at varying heights from the floor. Dry slides on hot plate at LOW setting.

20. Stain with filtered Giemsa for 4 minutes.

21. Wash with 1x PBS for 5 minutes.

22. Wash with ddH2O.

23. Count at least 15 cells. Significant problem if more than 4 cells have more or less than 40 chromosomes (mouse) [or 46 for human].

**Solutions for chromosome preparation**

Vinblastine sulfate salt. Sigma, cat# V1377
Add 0.5 ml sterile water to original vial containing 1mg of crystal vinblastine sulfate for a 2mg/ml stock solution. Aliquot and dilute to working solution of 25ul/25mlsterile water. Keep stock and working solution covered with foil at 4C.

Hypotonic solution
1.5g sodium citrate
1.12 g potassium chloride
q.s to .5L