

# Metaphase Chromosome Spread Preparation Protocol

Revised April, 2015

Chromosome counting is performed at the Mouse Biology Program to determine the percentage of euploid metaphase chromosomes in the targeted ES cell clones. A normal diploid mouse chromosome count is 40. We count at least 20 spreads. If 50% or more of the spreads counted contain 40 chromosomes each, the clone is considered to be more likely to contribute to both somatic cell chimerism and to germline transmission.

Chromosome counting is strongly encouraged prior to injection of targeted ES cells into blastocysts.

## **Reagents and Supplies**

Item	Vendor	Catalog Number
Karyomax (Colcemid) 10 ug/ml	Gibco	15210-040
PBS (1X without CA or Mg)	Gibco	14190-144
ES Cell Medium (see specific ES cell culture protocols for various KOMP ES cells)		
Trypsin (see specific ES cell culture protocols for various KOMP ES cells)		
KCl 10X Stock, 5.6% KCl: 5.6 g KCl in 100 ml Sterile Distilled Water. Mix until completely dissolved. Store at room temperature for about 2 months.	Fisher	P217
Methanol	Fisher	A412SK-4
Glacial Acetic Acid (GAA)	Fisher	UN2789
Vectashield Mounting Medium with DAPI	Vector Labs	H1200

## **Preparation of Metaphase Spreads**

1. Thaw or passage ES cells onto one well of gelatinized 6 well dish or 35 mm dish approximately 2 to 3 days prior to preparation of chromosome spreads.
2. Feed cells with 3 ml of ES cell media 30 to 60 minutes prior to adding Colcemid.
3. Add 300 ul of Colcemid to a final concentration of 1 ug/ml, incubate at 37°C for 1 hour.

4. Aspirate medium and rinse with PBS. Trypsinize cells as recommended for each particular cell line and resuspend in media.
5. Centrifuge at 1000 rpm for 4 minutes.
6. Aspirate supernatant, leaving about 200 ul in the tube. Gently tap bottom of tube to break up any clumps.
7. Add 5 ml of ice cold 0.56% KCl solution (see following chart for preparation from stock) and invert once. Stand at room temperature (RT) for 6 minutes. Spin at 1000 rpm for 4 minutes.
8. Aspirate supernatant, resuspend pellet in remaining drops of liquid by gently tapping the bottom of tube and add 5 ml of methanol: glacial acetic acid (3:1) fixative solution. (See following chart for preparation directions.) This is the most critical stage of the preparation. It is very important to disrupt the pellet before addition of the fixative and to add the fixative only one or two drops at a time for the first ~4 ml, tapping the bottom of the tube to mix cells continuously or a Vortex mixer on low speed may be used. The remaining ~1 ml can be added all at once. Spin at 1000 rpm for 4 minutes.
9. Aspirate supernatant. Resuspend pellet in small volume of fixative (~1ml) or less if the pellet is small. Cell suspension will be slightly milky.
10. Take a small quantity of cell suspension (~20 ul) in a 20 ul pipetter. Release 1-3 drops in a row onto an alcohol cleaned slide, a single drop at a time. Having the slide over a beaker or slide jar of water may be helpful in controlling humidity. Allow the slides to air dry thoroughly for minimum of 1 hour. Prepare at least 2 slides for each cell line.

### **Photographing and Counting Chromosomes**

1. Add 1-2 drops of mounting medium with DAPI to each slide, over the dried cell prep. Carefully add a coverslip and seal around sides with clear nail polish (currently we're using Sally Hanson Diamond Shine, for example.)
2. Examine under oil immersion at 100X magnification, using a UV light source. Scan each slide and photograph the first 25 good spreads you find for each clone. (The "good" spreads are those in which the chromosome spread is clearly from one cell only. Sometimes the chromosomes from 2 cells overlap. Also, odd small numbers of chromosomes can sometimes be seen, that are clearly less than a complete representation of one cell. Don't count those.)
3. Count the number of chromosomes in each spread. Typically we count 20 or more spreads. Chromosomes can be accurately counted by identifying the centromeres,

which light up a little more than the rest of the chromosome. Determine the percentage of euploid cells (those with 40 chromosomes) from the total number counted. Most commonly we find cells containing 38 to 42 chromosomes. We blast inject the clones which are identified as being at least 50% euploid as we've found this gives a good chance of successfully achieving germline transmission.

### **Solutions Guide**

#### **5.6% KCl (10X Stock)**

5.6 g KCl in 100 ml Sterile Distilled Water. Mix until completely dissolved. Store at room temperature for up to 2 months.

#### **0.56% KCl (Working Solution – 5 ml per sample)**

Chill on ice before using.

Number of Samples	Volume of 5.6% Stock (mls)	Volume of D. H <sub>2</sub> O (mls)
1	0.5	4.5
2	1.0	9.0
3	1.5	13.5
4	2.0	18.0
5	2.5	22.5
6	3.0	27.0
7	3.5	31.5
8	4.0	36.0
12	6.0	54.0

**Fixative (3 parts Methanol + 1 part GAA)**

Prepare at least 10 ml per sample. Chill on ice before using.

Number of Samples	Volume of Methanol (mls)	Volume of Glacial Acetic Acid (mls)
1	6	2
2	9	3
3	15	5
4	18	6
5	21	7
6	27	9
7	30	10
8	33	11
12	2 x 27	2 x 9