Cell Growth Protocol for GM12878-XiPat Cell Lines

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GM12878 human lymphoblastoid cells were cloned and tested to determine whether the maternal or paternal X chromosome was inactive. Two clones, #34 and #40, with paternally inactive X chromosomes were isolated. These two clones are treated as biological replicates. Their clone number is designed in the metadata by the labVersion as "34" and "40".

Cell Culture and Single cell cloning

GM12878 cells were diluted in 50% conditioned media, seeded in 96-well plates and grown in a humidified incubator at 37°C and 5% CO_2 until expansion. To make conditioned media, cells were grown in fresh media over night and then removed by filtration. The filtered conditioned media was diluted 1:1 with fresh media. Upon expansion, cells were grown according to the ENCODE GM12878 cell culture protocol described below.

Testing of X-inactivation skewing

DNA and RNA from each clone were isolated from fresh cells and stored at -20°C and -80°C respectively. A quantitative Q-SNaPshot[1], a modification of the commercial SNaPshot assay (Applied Biosystems), was employed to detect relative allelic expression levels of selected monoallelically expressed genes at heterozygous SNPs. Genes with established expression patterns (XIST[2] and EBP[1]) were tested to screen isolates for complete nonrandom inactivation. Only those clones that had the correct DNA genotype and properly phased monoallelic expression (XIST was expressed from one X chromosome and subject genes from the other) were selected for further analysis. Complete nonrandom inactivation was verified in these isolates at ATRX, TBL1X, MCART6, and GPC4, all of which were monoallelically expressed from the active X.

Gene	SNP	Paternal	Maternal	Expression
XIST	rs1620574	C	T	Xi
EBP	rs3048	T	G	Xa
TBL1X	rs16985675	A	G	Xa
MCART6	rs5916825	G	A	Xa
ATRX	rs3088074	G	C	Xa
GPC4	rs1048369	A	G	Xa

1. Carrel, L. and H.F. Willard, *X-inactivation profile reveals extensive variability in X-linked gene expression in females.* Nature, 2005. **434**(7031): p. 400-4.

2. Brown, C.J., et al., *A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome.* Nature, 1991. **349**(6304): p. 38-44.

GM12878 cell culture

NOTES:

A. Upon receipt, cells should be expanded for a few days (see #3 below) and then frozen in aliquots (see #6 below).

B. Cell density is critical for lymphoblastoid cells. If they ever grow to more than 1,000,000 cells/ml, they start slowing down. If they are diluted to less than 200,000, they can start dying off. Cells need to clump to some degree to stay healthy.
Here are guidelines from Coriell for growing lymphoblastoid cells. The media and conditions are identical to those being used by HapMap.

1. **How are lymphoblast cultures established?**
Lymphoblastoid cell lines obtained from the Coriell Cell Repositories were established by Epstein-Barr Virus transformation of peripheral blood mononuclear cells using phytohemagluttinin as a mitogen. All cells lines are free of bacterial, fungal or mycoplasma contamination.

2. **What are the basic culture conditions for lymphoblasts?**
Recommended Medium: RPMI 1640 2mM L-glutamine
15% fetal bovine serum Culture Conditions: T25 tissue culture flask with 10-20 ml medium
upright position 37°C under 5% carbon dioxide

NOTE: Groups should also include 1x penicillin/streptomycin (Invitrogen)

3. **What should I do when I first receive lymphoblast cultures?**
Lymphoblast cultures are shipped in T25 tissue culture flasks that have been filled to capacity with carbon dioxide-equilibrated medium to provide sufficient nutrients for extended transport times. Upon receipt, cell culture flasks should be incubated unopened overnight at 37°C. Lymphoblast cultures should be counted the next day and either split if sufficient growth has occurred or the medium volume decreased to yield a cell density of 200,000 - 500,000 viable cells/ml. Flasks should be incubated in an upright position with vented or loose caps.4. **How do lymphoblasts grow in culture?**
Lymphoblastoid cell lines grow in suspension culture with cells clumped in loose aggregates. These aggregates can be dissociated by gently agitating the culture or by gentle trituration with a pipet.
Cultures should be seeded at a concentration of no less than 200,000 viable cells/ml. In three to four days the culture is either refed with fresh medium or split again depending upon how fast the particular line grows and the desired number of cells. The plateau level

for most cultures is about one million viable cell/ml and is reached three to five days after subculturing. The pH of cultures will be quite acidic at this point, appearing distinctly yellow if phenol red is used as an indicator. Cultures left in plateau phase tend to exhibit a decrease in viability accompanied by a lengthening of the doubling time.
The volume of medium in the flask can effect the growth of cells as the surface to air ratio is important in maintaining the proper pH of the medium. No more than 20 ml of medium should be used in a T25 flask.

5. **What are the common problems in growing lymphoblasts?**
Factors that can affect the growth characteristics of a cell line include: temperature, pH, the particular lot of medium or serum, depletion of L-glutamine, mycoplasma contamination and the length of time in continuous culture.
To control these factors, medium is prewarmed to 37°C before addition to the cells, new lots of serum and medium are prequalified on control cell lines, and medium (with glutamine added just before use) is replaced at regular intervals depending on rate of growth.
Cell cultures are not usually maintained in continuous culture, but frozen to create "seed stocks" for future use.
The percentage of fetal bovine serum used can depend on the individual cell line and can be varied if growth seems slow. The usual range is 5 to 15 percent. Most lymphoblast cell lines grow well in FBS that has NOT been heat-inactivated (**un**inactivated). Poorly growing lines can be tried in heat-inactivated FBS (30 min. at 56°C).
Poorly growing lines should be cultured at somewhat higher cell concentrations (300,000 to 500,000 viable cells/ml). Slowly growing lines can be "half-fed" instead of subcultured (allow cells to settle to bottom of flask; remove approximately half volume of spent medium; replace with equal volume of pre-warmed fresh medium.

6. **How should lymphoblasts be frozen?**
Pool sufficient flasks for freezing a seed stock. Dissociate the cell clumps by trituration and count the viable cells. Calculate the total number of viable cells. Centrifuge the culture for 10 minutes at 100 +/- 20 X g at 4-10°C. Resuspend the cell pellet in the appropriate volume of cold (4-10°C) freeze medium [RPMI 1640 with 20% FBS and 6% DMSO] to yield approximately five million viable cells/ml. Dispense the cell suspension in 1-ml aliquots into plastic or glass cryovials. Freeze at -1°C/min to -80°C (either in microprocessor controlled freezer or passively in an isopropanol bath placed in a -80°C freezer overnight). Store in liquid nitrogen (vapor or liquid phase as appropriate).
Recover cell lines by thawing a vial rapidly in a 37°C water bath. Resuspend the entire contents of the vial in fresh culture medium. The volume to be used will depend on the number of viable cells in the vial and should be adjusted to give a cell density of not less than 200,000 cells/ml.