Transformation of Animal Cells

Animal Cell Culture and Media:

Animal cell culture and maintenance require expertise and experience. These cell lines cannot be maintained for a long time, in some cases fresh batch of cell cultures have to be initiated and some has to be transferred to fresh media for some period of time (8to 10 passage can be OK for some cell lines). Some of the cell lines are genetically transformed. One has to use all his ingenuity to keep them intact and functional.

Mouse connective tissue,
M.fibroblast,
M.embryonic stem cells,
M.monocyte,
M.macrophages,
M.spleen cells,
Mouse 3T3 NIH cell lines,
Rat fibroblasts,
Rat hepatomas,
Human lymphomas,
Human keratinocytes,
H. small cell lung cancer cells,
Lymphocytes EBV transformed,
H embryonic kidney cell HEK293 cell lines,
Chinese hamster ovary cell lines (CHO),
Cat kidney cell lines,
African green monkey kidney cell lines,
SV 40 transformed African monkey kidney cell lines (COS),
Dog's primary hapatocytes,
Chick embryonic fibroblast cell lines,
Hela cells (Henrietta Lock),
Myeloma cell lines,
Bovine fetal heart cells,

Cells cultured including Egg cells for Transgenic and animal cloning experiments:

Human egg cells,
Mouse eggs,
Xenopus eggs,
Cow eggs,
Pig eggs,
Sheep eggs,
Sheep udder epithelial cells,
Sheep embryonic epidermal cells,
Mouse blastocysts,
Many stem cells from variety sources have been cultured and used.

A list of cell lines commercially available:

3T3: Mouse, 2n=40, endothelial fibroblast, show heteroploidy cannot grow in suspension media.

L: Mouse, 2n=40, connective tissue –fibroblasts, grow in suspension media, show heteroploidy.

CHO: Chinese hamster ovarian cell, epithelia, grows in suspension cultures and show pseudo diploidy.

BHK-1: Syrian hamsters, kidney cell-fibroblasts, grow in suspension and show diploidy.

BSC: Monkey, 2n=42, kidney epithelial cells, don't grow in suspensions, show diploidy.

MPC: Mouse, 2n=40, bone marrow, Myeloma-lymphoid, grow in suspension, show heteroploidy.

RHP: Frog, 2n=26, egg-epithelial, don't grow in suspensions, show haploids.

HeLa: Human, 2n=46, cervical tumor-epithelial, show growth in suspension and show heteroploidy.

KB: Human, 2n=46, Nasopharyngeal tumor-epithelial, grow in suspension culture, and heteroploidy.

Today perhaps hundreds of such lines are available.

Cell Type	Animal	Tissue origin	Type o tissue	Growth in suspension	Chromosoma number
3T3	Mouse (2n=	Connective	Fibrobl	No	Heteroploidy
L	Mouse, 2n=40	Connective	Fibrobl	Yes	Heteroploidy
CHO	Chinese Hamster	Ovary	Epithel	Yes	Pseudodiploic
BHK-	Syrian ham kidney	Kidney	Fibrobl	Yes	Diploid
BSC Monk	Kidney	Epithelial	No	Diploid	
MPC	Mouse	Bone marrow Meyloma	Lymph	Yes	Heteroploidy
RHP	Frog 2n=26	Egg	Epithel	No	Haploid
HeLa	Human fen	Cervical tumor	Epithel	Yes	Heteroploids
KB	Human 2n=46	Nasopharynge tumor	Epithel	Heteroploids	

Some promoters used for expression in animals:

Promoter	Response Element	Gene cloned	Animal system Used
Metallotheonein		HGH	Mice, Rat

BPV		Bovine growth Hormone	Bovine
Avian leucosis viral Promoter			Chick
MMtr	DRE, GRE	HGH, b-Gal	Fish
MMtr MuMLV		HGH, porcine Growth hormon Rat growth Hormone, Bovin GH, Thymidine Kinase,	Pig, Sheep
Ovine-blactoglobin Promoter,		Human factor-X, Human antitryp	Ovine
Prolactin promoter		Tissue type plasr nogen activating factor	
SV40 early Promoter	SV40 enhance		
Beta Actin			
DJHFR			Increases copy Numbers with Methotrexate

Mouse: Promoter Metallotheonein gene:
Genes cloned- Human growth hormone, rat GH, bovine GH.
Chick: Promoter- ALV (Avian leucosis viral promoter.
BPV- Bovine Papilloma viral promoter.
Fish- MMtr- cloned genes are HGH, b-Gal.
Pig- MMtr promoter- genes cloned are- HGH, BGH, porcine GH, rat GH.
MuMLV: promoter-gene cloned is rat GH.
Rabbit: MMtr promoter- genes cloned are HGH,

hMT promoter- genes cloned are –HGH.
RbEu promoter- gene-rbc-myc.
Sheep: MMtr- genes cloned are –HGH, TK, b-GH, hGRF (growth releasing factor), oBLG- human Factor-X, oBLG- human XI antitrypsin gene, Factor IZX.
Goat- La promoter- genes cloned are Prolactin, tissue specific plasminogen activating factor.
GH = growth hormone,
Hu = human,
MuMLV = Murine leukemia virus,
B = Bovine,
O = Ovine,
TK = Thymidine kinase,

General procedure for isolation of animal cells:

Dissected out tissues are Trypsinized (0.05% Trypsin plus 0.53mM EDTA in buffered saline).

Monitor the cell shape. When cells become spherical they are filtered through 4-layered cheeses cloth.

Then the cells are washed with serum containing growth medium.

Plate them in growth medium and allow them to grow as single layers or what is called monolayer.

The most common medium used is GIBCo-BRL's Dulbecco's modified Eagle medium (DMEM). To this Glutamine is added as additive to 2mM concentration.

In many cases fetal bovine serum (heat killed and filtered) is also added to 10 %(V/V). This supports growth of cells.

Antibiotics such as Ampicillin or penicillin 100ug per ml are added to prevent bacterial contamination.

The pH of the culture medium at 7.2 is maintained by adding bicarbonates (2.0 to 3.7gm per liter).

During Transfection the cells have to be prepared in serum free state.

When cells are added onto plates they adhere to the surface and divide and redivide and grow to density called confluence at which time cell-to-cell contact is maximal. This contact inhibits them further growth. In cancer cells contact inhibition is lost so cells pile up one upon another. The number of passages is limited to 8-10 times, and then the cells have to be extracted from fresh tissues and cultured. The term cell passage refers to how many times the extracted cells can be for replating. During Transfection cells should be in 70-80% confluence. Such cells are repeatedly sub cultured once in every 4-5 days. Cells at the density of 1-4 x 10^4 cells/cm^2 before the cells are used for Transfection. Viable cell concentration can be accounted by treating a sample of cells with Trypan blue stain. Stained cells are considered as dead cells and unstained are living cells. The cells used for the said purpose should be competent, whose efficiency can be estimated by using control experiments.

Embryonic Stem cells:

- Cells are obtained from embryonic blastula stage.
- Blastocysts can be cultured in Petri plate with suitable culture media providing primary embryonic cells from fibroblast as feeder layer.
- When blastula embryos are grown on feeder layer of cells, the ectoderm spreads out and inner embryonic stem cells come out and now they are exposed to feeder layer of cells.

- Such stem cells can be expanded and maintained for a number of generations by reculturing. Precaution should be taken about the change in chromosomal number.
- Such cells can be used for developmental studies for they have potentiality to develop into different types of tissues, which depends upon the kind of stimulants you provide.
- The feeder layer prevents stem cells from differentiating. Addition of Leukemia inhibitor factor (LIF) also prevents stem cells from differentiating.
- The number of passages for keeping stem cells in active state is possibly 12-14.

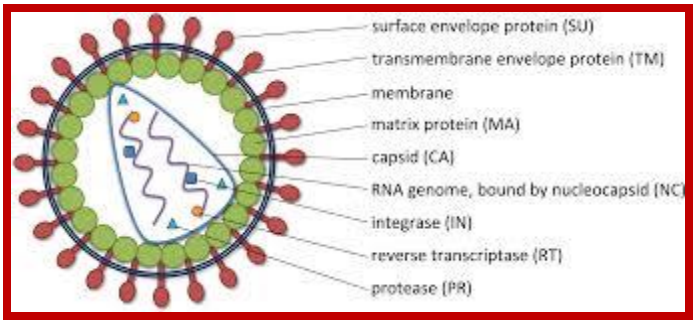
Transfection Protocol:

Transfection by Calcium Phosphate method:

- Take the desired recombinant DNA and linearize them.

Then take about 10-20ug in 225 ul of H2O, then Add this to 25ul of 2.5M Ca Cl2 drop wise and mix. Then add 250ul of 2xHEPES buffered with saline.

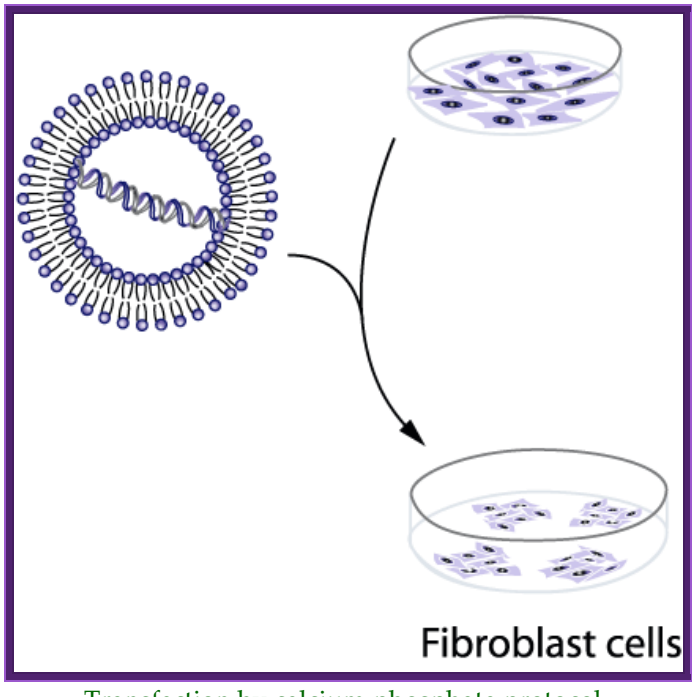
- A fine ppt. develops. Add the ppt. to ES cells drop wise, incubates for 4hrs. Remove the liquid.
- Give glycerol shock by adding glycerol to DMEM 15%.
- Incubate for 4 minutes and remove glycerol.
- Add DMEM and incubate for some time and remove DMEM.
- Then add DMEM incubate overnight.
- Select the cell colonies on specific antibiotics after 12 -24 hrs of culture.



Nucleic Acid Delivery: Lentiviral and Retroviral Vectors

www.Labome.com

Simple and Complex Retrovirus Virion Structure; The viral particle contains two copies of reverse transcriptase (RT)-associated positive-stranded RNA within the internal core. Also located here are the nucleocapsid (NC), capsid (CA), integrase (IN), and protease (PR). The inner core is surrounded by an outer Matrix (MA) layer which is in turn encompassed by the glycoprotein (ENV)-studded, host cell membrane-derived envelope.



Transfection by calcium phosphate protocol;

Tips for Eukaryotic Cell Transfection:

January 29, 2009 by Shoba in Tech Tips .

I find that the simplest, fastest and cheapest transfection method for eukaryotic cells is calcium phosphate mediated transfection. Its main advantage is that, since Ca2+ is a small ion and part of the culture medium, cell viability is not a problem.

The exact mechanism of calcium phosphate mediated transfection is not known, but what we do know is that calcium, being poorly soluble in culture medium, forms micro precipitates in the presence of phosphate ions. These micro precipitates are believed to have a positive effect on transfection efficiency. DNA/Calcium reaction time: No more than 1 min. Don't leave it for more time.

Concentration of components in the precipitation mixture:

DNA: 25ug/mL; Calcium: 125mM and Phosphate: 0.77mM.

The reason for this is that when DNA is mixed with calcium phosphate micro precipitates, co-precipitates of DNA-calcium mixtures are formed. These strongly bind to the surface of the cell monolayer and enhance uptake of DNA by the cells possibly by endocytosis.

The key to reproducible transfection efficiencies is to have a high concentration of calcium phosphate-DNA micro precipitates.

Liposome Mediated Transfection:

Cationic liposomes are available in many companies, which provide molecular materials for research activities.

- Transfectum from Promega are called DOGs.
- Boehringer Manhiem provides them as DOTAP.
- GIBCI-BRL provides lipofectine as DOTMA.
- GIBCO-BRL also provides another lipofectamine as DOSPA- (cationic lipids).

Non-chemical methods (Wikipedia);

- Electroporation is a popular method, creates micro-sized holes transiently in the plasma membrane of cells under an electric discharge.

  Similarly, transfection applying sonic forces to cells, referred as Sono-poration.

- Optical transfection is a method where a tiny (~1 μm diameter) hole is transiently generated in the plasma membrane of a cell using a highly focused laser. one cell at a time can be used.
- Gene electrotransfer is a technique that enables transfer of genetic material into prokaryotic or eukaryotic cells. It is based on a physical method named electroporation, where transient increase in the permeability of cell membrane is achieved when submitted to short and intense electric pulses.

- Impalefection is a method of introducing DNA bound to a surface of a nanofiber that is inserted into a cell. This approach can also be implemented with arrays of nanofibers that are introduced into large numbers of cells and intact tissue.

- Hydrodynamic delivery .In mice and rats, but to a lesser extent in larger animals, DNA most often in plasmids, including transposons, can be delivered to the liver using hydrodynamic injection that involves infusion of a relatively large volume in the blood in less than 10 seconds; nearly all of the DNA is expressed in the liver by this procedure.

Particle-based methods

- A direct approach to transfection is the **gene gun**, where the DNA is coupled to a nanoparticle of an inert solid (commonly gold) which is then "shot" directly into the target cell's nucleus.

- Magnetofection, or Magnet assisted transfection is a transfection method, which uses magnetic force to deliver DNA into target cells. Nucleic acids are first associated with magnetic nanoparticles. Then, application of magnetic force drives the nucleic acid particle complexes towards and into the target cells, where the cargo is released.[12][13][14]

- Impalefection is carried out by impaling cells by elongated nanostructures and arrays of such nanostructures such as carbon

nanofibers or silicon nanowires which have been functionalized with plasmid DNA.

Viral methods

DNA can also be introduced into cells using viruses as a carrier. In such cases, the technique is called viral transduction, and the cells are said to be transduced. This can be done using insect cells.

General Protocol:

- The above said lipid contain quaternary amino group. Such cationic lipids are blended with neutral but natural lipids.
- When such materials are mixed with water they form a monolayer of vesicles where hydrophilic part faces water and hydrophobic part faces inside of the vesicle.

Such mixture of lipofectamine is mixed with recombinant linear DNA in 10:1 ratio. The phosphate backbones spontaneously react with cationic head of lipids and form a complex and remain as the complex.

1. Dilute DNA to 100ul in 150mM NaCl, 20mM HEPES pH 7.5.
    - Dilute cationic lipofectamine to 100ul in NaCl/HEPES buffer. Then add serum free medium and mix.
    - Prepare cells with washing in serum free culture medium, then lipofectamine-DNA complexes are added to competent animal cell culture.
    - The positive charged liposomal components react with negatively charged lipid membrane and they fuse with the cell membranes and deliver the DNA into the cell.
    - Incubate cells 1-24 hrs at 37^oC. Remove the medium and add growth medium.

- Grow them and allow for transient expression of the selection marker gene. Then plate on selection medium.

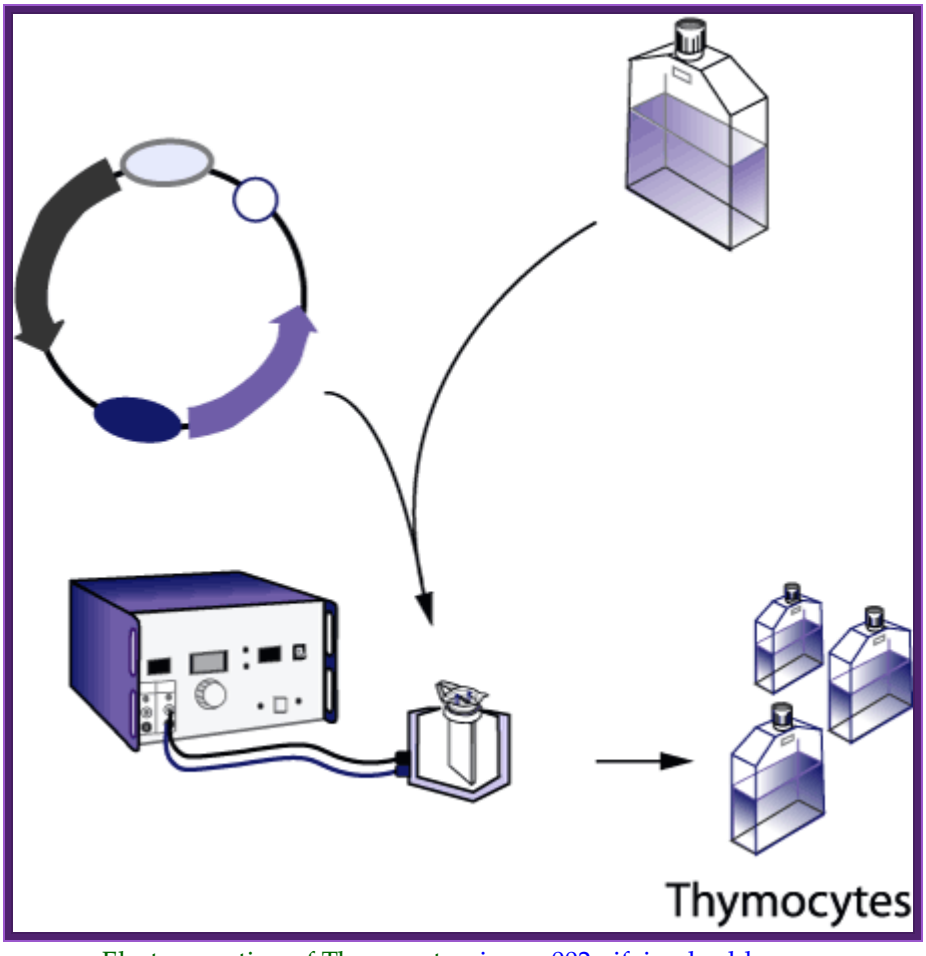
For ES cells:

- Add 10ug of linearized vector DNA to 2ml of OPTI-MEM serum reduced medium.
- Then add 100ug of cationic liposome. Mix and set at 30^oC.
- Add this final preparation to cells suspended in OPTE-MEM-1 medium. Incubate for 4 hrs.
- Plate them on feeder layers.
- Allow cells to recover and express the selection marker gene.
- Remove the medium and plate the cells on regular growth medium containing proper antibiotic or the drug.

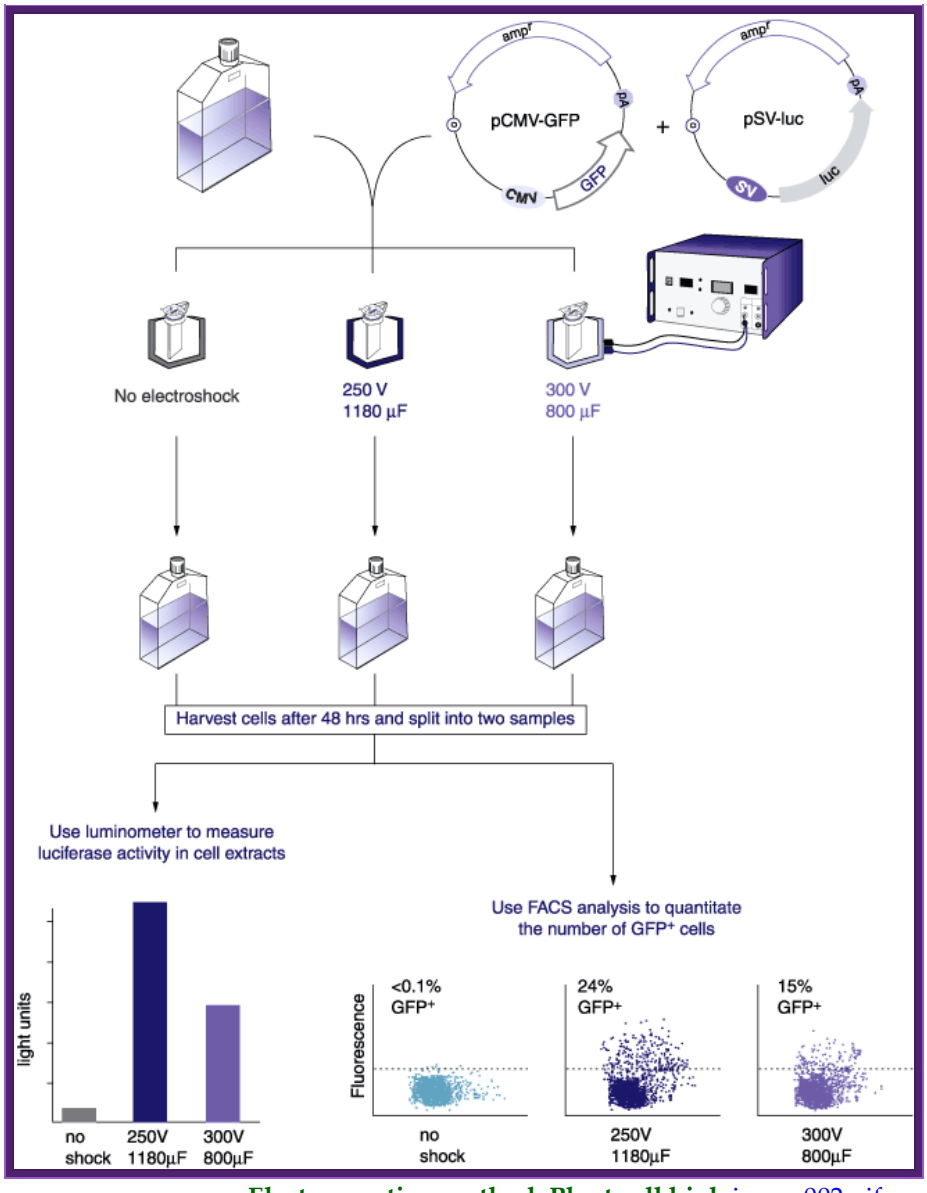
Electroporation Protocol:

- Prepare the cells with culturing and reculturing and finally grow the cells free from serum.
- Take cells in PBS pH 7.5, swirl for 1-2 minutes decant.
- Then add Trypsin dilution, incubate for 1-3 minutes at 37^oC.
- When cells detach from the surface of the glass container add DMEM-H medium, mix gently to create single cell suspension.
- Pellet the cells and remove the supernatant.
- Then suspend cells in 200ul of the same medium.
- Add purified Recombinant DNA to 3nM concentration.
- Mix the contents and transfer cells into the cuvette.
- Pulse at 270 volts, 50 u Faraday and 360-ohm resistance for 50 to 100 milliseconds.
- Remove the cells and decant and add DMEM-H media and plate cells on a feeder layer and allow cells to recover and express a marker gene.

- Then plate the cells on a growth medium containing proper drug for selection.



Electroporation of Thymocytes; image002.gif; imgbuddy.com



Electroporation method; Plant cell biol; image002.gif; imgbuddy.com