Mammalian Cell Expression Systems

Mammalian Cell Expression Systems

- Important for producing proteins with correct post-translational modifications.
- Proteins are expressed in a stable functional form since the machinery to facilitate proper protein folding and assembly are conserved in higher eukaryotic cells.
- DNA cloned from higher eukaryotic cells (both cDNAs and genomic clones) is readily expressed since the signals for transcription, mRNA processing, and translation are conserved in higher eukaryotic systems.
- Many proteins are readily secreted from mammalian cells providing the ability to isolate the protein from conditioned medium that contains low amounts of protein when cells are propagated under serum-free conditions.
- Mammalian cells have the capacity to express large and complex recombinant proteins.
- Optimization for each production

Application of Mammalian Protein Production

Mammalian cells are used as a host for gene expression in order to:

- Confirm that cloned genes can direct the synthesis of desired proteins;
- Study protein structure-function relationships;
- Isolate genes by direct screening or selecting transfected cells that express a desired protein;
- Produce proteins that are available in limited quantity
- Evaluate the physiologic consequences of expression of specific proteins in mammalian cells in order to study biological regulatory control mechanisms.

Transiently producing cell lines

Short-term (transient) production of protein.

The foreign DNA is not integrated into genome. The efficiency of expression after transient transfection of plasmid DNA is dependent upon the number of cells that incorporate DNA, the gene copy number, and the expression level per gene.

The amount of DNA, RNA, and protein per mammalian cell is roughly 100–1000 times greater for mammalian cells than for *Escherichia coli.*

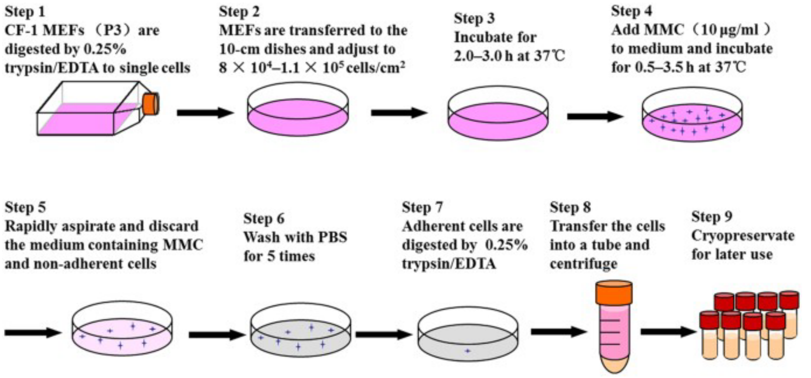
Adherent versus suspension cell cultures

Adherent cell cultures:

Require periodic passaging, but allow easy visual inspection under inverted microscope
Cells are dissociated enzymatically (e.g., Gibco™ TrypLE™ Express, trypsin) or mechanically

Growth is limited by surface area, which may limit product yields

Used for cytology, harvesting products continuously, and many research applications



mouse embryonic fibroblast (MEF)
SCiENTifiC RepoRts | 7: 12266 | DOI:10.1038/s41598-017-10428-5

Suspension cell cultures

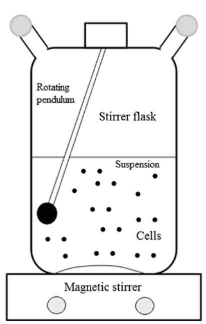
Appropriate for cells adapted to suspension culture and a few other cell lines that are nonadhesive (e.g., hematopoietic)

Easier to passage, but require daily cell counts and viability determination to follow growth patterns; culture can be diluted to stimulate growth

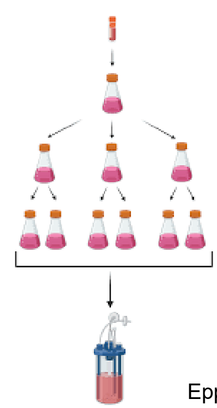
Does not require enzymatic or mechanical dissociation

Growth is limited by concentration of cells in the medium, which allows easy scale-up

Used for bulk protein production, batch harvesting, and many research applications



doi:10.1088/2053-2563/aafac0ch1



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Mammalian cell lines

Most commonly used cell lines, are responsive to majority of expression vectors:

CHO cells (Chinese hamster ovary cells)

HEK 293 – derived from human embryonic kidney 293 cells transformed with fragments of adenovirus type 5 DNA

HEK 293T encode the SV40 large T antigen: episomal replication of plasmids with SV40 origin of replication

Other cell lines:

NSO and Sp2/0 cell lines mouse myeloma cells

PER.C6: from human retina

Cell lines are quite easily transfected, doubling time about 24 h in suspension culture, grow up to high densities, can produce grams of protein per liter

Composition of media can influence the yield.

Media for transient expression must contain nutrients supporting the growth

Mammalian cells for production of pharmaceutics

CHO, NS0, and Sp2/0 are the main cell lines used for the expression of recombinant biopharmaceuticals.

CHO cells are involved in production of over 70% of therapeutic proteins

The **CHO cell-based systems contribute 84% of approved antibody productions**. The remaining antibodies are expressed in either NS0 cells or Sp2/0 cells

Mammalian cells for SARS Cov-2 study

Vero and Vero E6 cells are one of the most extensively used kidneys based epithelial cell lines for the culture of SARS-CoV-2 due to the presence of high expression of ACE-2 receptors.

The Vero cell line was established by Japanese scientists Yasumura and Kawakita in 1962 from Chlorocebus sp. of African green monkeys.

Vero E6 or Vero 1008 cell line are the clone of Vero 76 cells and are better option than Vero cells as they show some contact inhibition and imitates properties more like primary cells.

Other applications of Vero cells

Vero and Vero E6 cell lines are used for the antibodies production,

vaccine production, Type I IFNs as a target of antiviral therapy,

Vero cells were found to be the best screening models for new drugs as well as for already available anti-viral drugs for their repositioning

What we can optimize

Selection of cell lines

Culture media: complex media (fetal bovine serum

Serum free media: complete basal medium supplemented with hormones, growth factors, binding proteins, etc. Composition of media can influence glycosylation (glucose starvation reduces glycosylation at Asn.

Transient expression: nutrients supporting the growth of high density cultures

Stable expression: media must support cell growth for a longer time (glucose, amino acids, lipid addition), buffering chemicals, e.g. HEPES to control pH. Additives must not change osmolarity of medium).

Methods for Transfer of Genetic Material into Mammalian Cells

Transient transfection

Chemical methods using $CaCl_2$ and DEAE-dextran

cationic lipids: transfectin, Lipofectamine, FectoPro

polyplexes like polyethelyimine (PEI)

Physical methods: electroporation, laser-based transfection

Pulsed electric field to introduce DNA into the cells

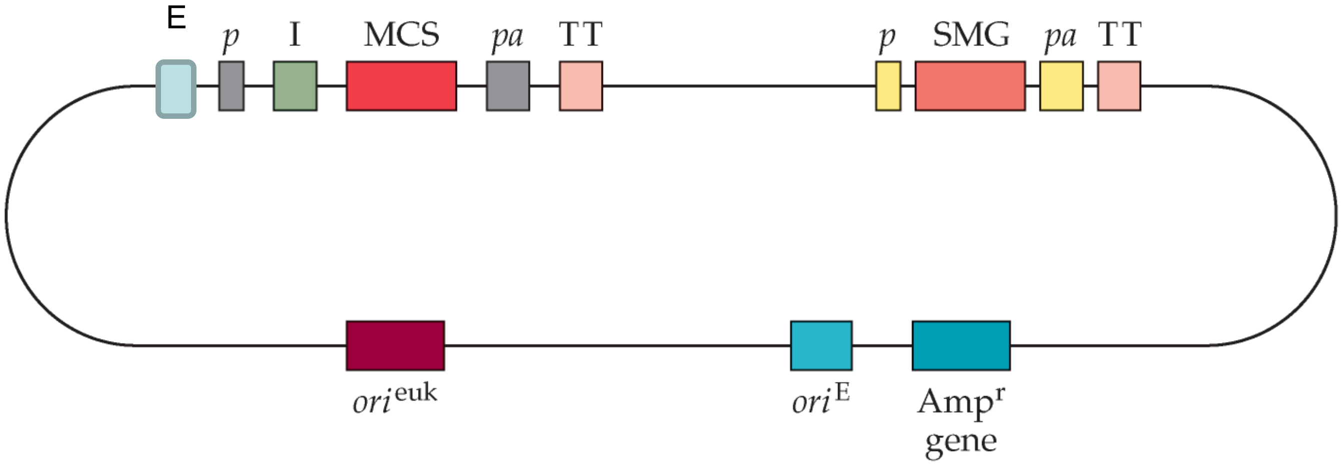
Viral methods: defective virus encapsulates the gene of interest for transfer: lentiviral vectors, bacMam system with baculoviruses, cytomegalovirus vectors, adenovirus vectors

Mammalian expression Vector

- Generalized mammalian expression vector.
- The MCS (multiple clonning sites) and SMG (selectable marker gene) are under the control of eukaryotic promoter (p), polyadenylation (pa), and termination transcription sequences (TT). E, enhancers, promote the rate o transcription, bind to activator proteins
- An intron (I) enhances the production of heterologous protein.
- The Amp^r gene is used for selecting transformed *E. coli.*

E: CMV
EF1α
SV40

P: CMV
EF1α
GAPDH



Mol. Biotechnol., Glick and Pasternak

Promoters and vectors

SV40 early promoter

Rous sarcoma virus promoter

Adenovirus major late promoter

Human cytomegalovirus immediate early promoter

Vector used: pED, pTBL_KN, pCEP4, and others.

Efficient Protein Production

- The foreign gene must be associated with transcriptional and translation control elements appropriate for the cell type.

- Selection of suitable promoter, requirement of polyadenylation site, transcriptional termination signal, and variety of translation control element.

- Genes containing introns are expressed at a higher level than the equivalent cDNA copy of the gene (coupling of transcription, splicing and mRNA processing in higher eukaryotic cells).

Adenovirus vector

Adenovirus: non-enveloped icosahedral virus with ds linear DNA genome
In vectors, early region E1 and E3 of the viral genome are deleted, virus cannot replicate
Instead is present an expression cassette, in which is a host gene under control of promoter, eg. cytomegalovirus promoter

Virus can be propagated in suspensions
Ad vector can infect a wide range of mammalian cell lines

Large scale production involves vector propagation in packaging cell lines in Bioreactors

Large scale purification: affinity, anion-exchange chromatography, size-exclusion chromatography

Applications using AD vectors

Application for influenza vaccines
Development of vaccines against SARS Cov2

Oxford University/AstraZeneca life science vaccine ChAdOx1 nCov19(AZD1222)

Russian vaccine	Gam-COVID vac
China CanSino Biologics vaccine	Ad5-nCoV2
Janssen	Ad26.COV2-S

Stably Transfected Mammalian Cells

- The foreign DNA becomes integrated into an apparently random location within the host genome.
- Ones integrated, the foreign DNA is replicated together with the host chromosome.
- The foreign gene is controlled by a suitable transcriptional control element.

- Problems with efficient integration process during preparation of the stably transfected cell line.