Swayam Course - Analytical Techniques

Week 13, Tutorial 34 - Expression Techniques of Recombinant proteins by insect and mammalians cells

Content Writer - Dr. Ruby Dhar, DBT-Biocare Fellow, Department of Biochemistry, All India Institute of Medical Sciences, New Delhi

Introductions

Recent advances in genomics, proteomics, and bioinformatics have facilitated the use of recombinant DNA technology in order to evaluate any protein of interest, without prior knowledge of the protein's cellular location or function. The parallel use of affinity tags with recombinant DNA techniques, allows the facile modification of proteins of interest leading to efficient identification, production, and isolation from the host system. However, protein insolubility, conformation, stability, and structural flexibility, as well as low purification yields and host cell toxicity are challenges and the host system should be carefully selected.

In one of the previous module, I have discussed regarding the expression of recombinant proteins in bacteria and yeast.

In this module I would be discussing regarding the recombinant expression using insects and mammalian cells.

This module covers the following objectives:

OBJECTIVES

1. INTRODUCTION TO RECOMBINANT PROTEIN EXPRESSION IN INSECT CELLS
2. RECOMBINANT PROTEINS USING INSECT CELLS

   INTRODUCTION TO BACULOVIRUS

   EXPRESSION VECTORS FOR INSECT CELLS

   STEPS TOWARDS EXPRESSION OF RECOMBINANT PROTEIN USING INSECT CELLS

3. INTRODUCTION TO RECOMBINANT PROTEIN EXPRESSION IN MAMMALIAN CELLS
4. KEY FEATURES OF PROTEIN EXPRESSED IN MAMMALIAN CELLS

   CHARACTERISTICS OF MAMMALIAN EXPRESSION VECTOR

   SELECTION MARKERS FOR MAMMALIAN CELLS

   MODE OF TRANSFECTION OF DNA INTO MAMMALIAN CELLS

   TRANSIENT AND STABLE EXPRESSION IN MAMMALIAN CELLS

5. CELL -FREE PROTEIN EXPRESSION SYSTEM
6. COMPARISON OF VARIOUS PROTEIN EXPRESSION METHODS

EUKARYOTIC PROTEIN EXPRESSION SYSTEM:

Eukaryotic expression systems are frequently employed for the production of recombinant proteins as therapeutics as well as research tools.

Most commonly used expression systems are based on stably transfected adherent CHO cells or nonadherent lymphoid cell lines.

An efficient alternative is the infection of insect cells by recombinant baculoviruses.

The choice of a suitable expression system depends largely on the biochemical and biological properties of the protein of interest, as well as on the nature of the planned experiments and the amount of recombinant protein required.

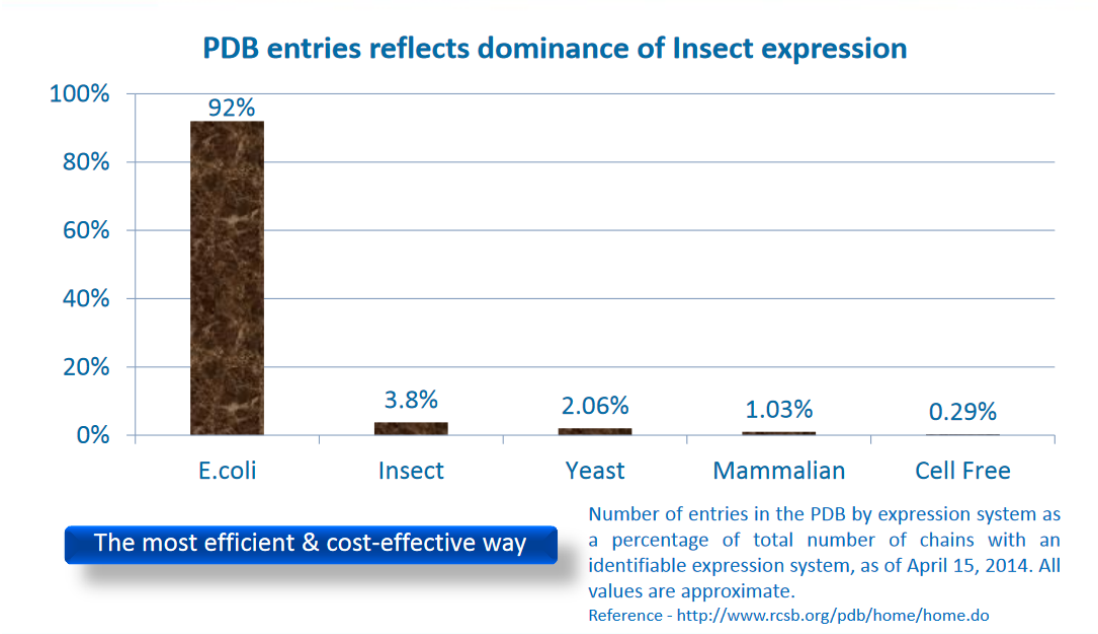
1. INTRODUCTION OF RECOMBINANT PROTEINS USING INSECT CELLS:

1. Expression using baculoviruses: Insect cells are infected with recombiunant baculovirus bearing a host gene.

2. Stable transformation of insect cell: Using selective marker long term protein expression is achieved . Mostly used for expression of receptors, membrane and glycoproteins.

3. Transient expression without any selectable marker is mostly used for immunodetection and functional studies.

4.Since the first insect cell line was isolated in 1963, more than 500 different cell lines have become available. The most common expression systems are based on cell lines derived from *Spodoptera frugiperda* (Sf-9 and Sf-21), *Trichoplusia ni* (BTI-TN-5B1-4, marketed as High Five™) and *Drosophila melanogaster* (S2)



2. LIST OF RECOMBINANT PROTEIN PRODUCED USING INSECT CELLS FOR HUMAN AND VETERINARY USE

Application	Product name	Company	Stage
For human use			
Cervical cancer	CERVARIX®	GSK	Approved
Prostate cancer	PROVENGE®	Dendreon	Approved
Influenza	FluBlok®	Protein Sciences	Approved
Influenza	A/H5N1 Virus-like particle	Novavax	Phase I (NCT01596725)
For veterinary use			
Procrine circovirus 2 (PCV_2)	Porcilis® PCV	Merck	Approved
PCV_2	CircoFLEX®	Boehringer Ingelheim	Approved
Swine fever	Porcilis Pesti®	Merck	Approved

BACULOVIRUS

- In 1983, Smith and Summers established the baculovirus-based expression system.

Two of the most common isolates used in foreign gene expression are Autographa californica multiple nuclear polyhedrosis virus (AcMNPV) and Bombyx mori(silkworm)nuclear polyhedrosis virus (BmNPV)

- Baculoviruses are large, complex deoxyribonucleic acid (DNA) viruses that infect arthropods.
- Baculoviruses are rod-shaped, enveloped viruses of 30–60 nm in diameter and 250–300 nm in length.
- Baculoviruses use actin filaments for intracellular transport.
- Baculoviruses use host RNA polymerase for synthesis of early genes and encode a viral RNA polymerase for transcription of late genes.
- Baculoviruses have been genetically engineered to be highly efficient eukaryotic expression systems. is widely used as a eukaryotic expression system for the production of vaccines and medical products of medicinal and biologicalimportance.

Generally, the process of viral replication is divided into three phases, throughout which wild-type baculovirus exhibits both lytic and occluded life cycles.

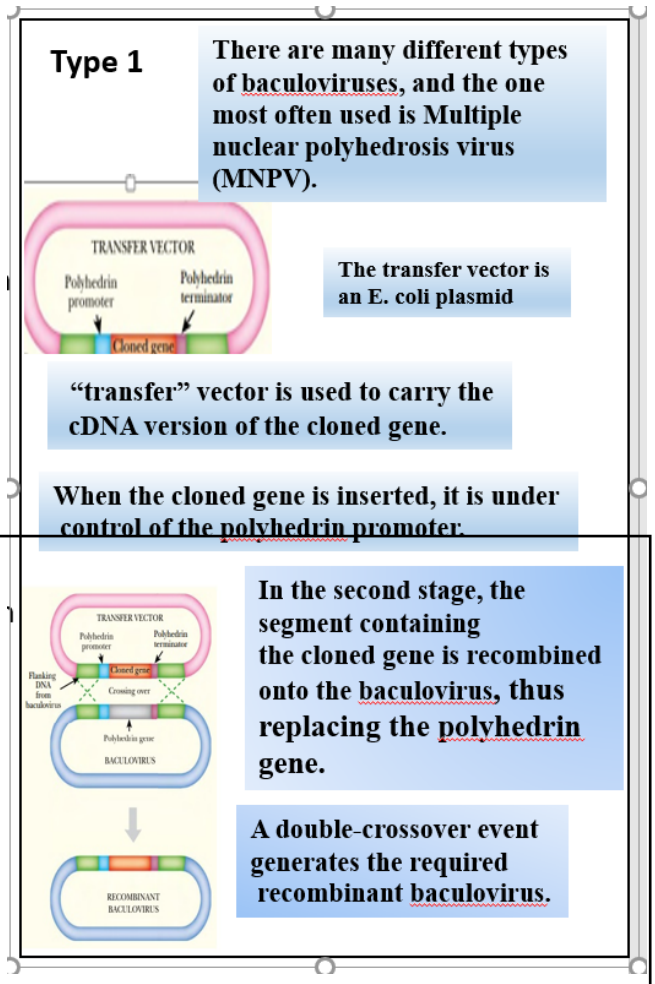
The three phases are characterized as follows ：

- Early Phase: In this phase, the virus infects the insect cell by attachment, penetration and uncoating. In this phase, the infected cells are prepared for viral DNA replication. Normally, initial viral synthesis occurs 0.5-6h post-infection, along with the shutting down of host gene expression.
- Late Phase: Genes that code for replication of viral DNA and assembly of virus are expressed during this time. Cells begin to produce extra-cellular virus that contains the plasma membrane envelope and glycoprotein during the time range of 6-12h post-infection. Both are necessary elements for viral infection through the process of endocytosis. The virions are then assembled and budded. Recombinant virions are released 18-36h post-infection.
- Very Late Phase: Occlusion derived virus particles are produced and cell lysis occurs in this phase.

There are two main types of Vectors to transfer gene of interest into the Baculovirus genome

TYPE 1 VECTOR :Multiple nuclear polyhedrosis virus (MNPV)

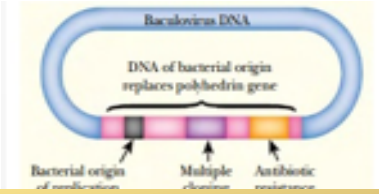
- ✓ The BEVS patented by Max Summers and Gale Smith in 1983, used homologous recombination to integrate the GOI into a polyhedrin locus of the baculovirus genome.
- ✓ Polyhedrin is not essential for virus replication in cultured cells because its function is to form the occlusion bodies that protect the virus against UV light and high temperatures during the natural infection cycle.
- ✓ Usually it requires crossovers to integrate the GOI, which occurred at a low frequency (~0.1%). This drawback was addressed by inserting three BsuI36 restriction sites and a *lac*Z cassette into the baculovirus genome .
- ✓ Digestion with Bsu36I linearized the virus DNA (called BacPAK6) and co-transfection of the linearized virus DNA and transfer plasmid was followed by homologous recombination to restore the circularity of the virus DNA, leading to the replication of recombinant viruses.
- ✓ BacPAK6 was optimized by deleting the chitinase gene (*chi*A) that inhibits the secretory pathway in insects, resulting in higher protein concentrations when the product is membrane targeted or secreted.



Type 2 Vector **Bacmids**

TYPE 2 VECTOR: Bacmids

- ✓ To enable the replication of baculovirus DNA in bacteria, a bacterial artificial chromosome (BAC) was integrated into BacPAK6 to produce a bacmid vector.
- ✓ Recombinant baculoviruses can also be generated using transposon activity which is marketed as the Bac-to-Bac® system.



- ✓ The baculovirus DNA contains an integrated BAC, an antibiotic resistance gene, a *lac*Z cassette and an attachment site for the bacterial transposon Tn*7*.
- ✓ The cloning and amplification of recombinant viral DNA is therefore carried out in *Escherichia coli*. The corresponding transfer plasmid consists of the GOI flanked by two mini- mini-transposon sites (Tn*7R* and Tn*7L*).

Shuttle vector that replicates as a plasmid in E.Coli and as a virus in insect cells

Such baculovirus-plasmid hybrids are referred to as bacmids

- ➢ The cloned gene is inserted into the MCS giving a recombinant bacmid

- ✓ The *E. coli* strain carries a helper plasmid providing all necessary transposon system elements. Following the transformation of the DH10Bac strain, clones carrying recombinant bacmid can be identified by blue/white screening and PCR analysis

Mode of transfection :

A. Calcium phosphate–DNA co-precipitation

- ✓ One of the oldest transfection methods is calcium phosphate-DNA co-precipitation which was adapted for insect cell lines in the 1980s. Mixing calcium chloride with a phosphate-buffered DNA-containing solution results in the formation of a fine calcium phosphate/DNA co-precipitate that binds to the cell surface and penetrates the cells by endocytosis.
- ✓ **B.Lipid-mediated and polymer-mediated transfection**
- ✓ In the late 1980s, a transfection method was developed based on the synthetic positively charged lipid N-[1-(2, 3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA). The cationic head groups of DOTMA interact with the anionic phosphate backbone of DNA to form a complex can bind to the cell membrane and probably taken up by endocytosis .
- ✓ Many different lipid formulations are available to achieve highly efficient transduction, including BaculoPORTER (Biocat), Cellfectin, Cellfectin II and Lipofectin (all Thermo Fisher Scientific).
- ✓ Various non-lipid transfection reagents can also form complexes with DNA, including baculoFECTIN II and flashFECTIN (Oxford Expression Technologies), FuGENE 6 (Promega), GeneJuice® (Merck Millipore) and *Trans*IT®-Insect (Mirus Bioscience).
- ✓ **C. Electroporation**

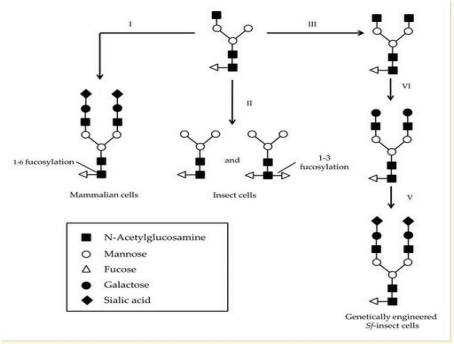
✓ Electroporation is a convenient and efficient transfection method, but specialized equipment is required. It is based on a short electrical pulse at an optimal voltage (specific for each cell line) to from transient pores in the plasma membrane. This in turn facilitates the intake of small molecules such as DNA, RNA or proteins .

Insect cells form shorter and less complex N-glycan structures than mammalian cells

Many therapeutic proteins require a

specific glycosylation pattern, hence insect cells are genetically engineered

Cell lines have been modified to express recombinant glucosaminyltransferases, galactosyltransferases and sialyltransferases. In the presence of the sialic acid precursor acetyl-D-mannosamine, human-like glycans can be synthesized in these cells.



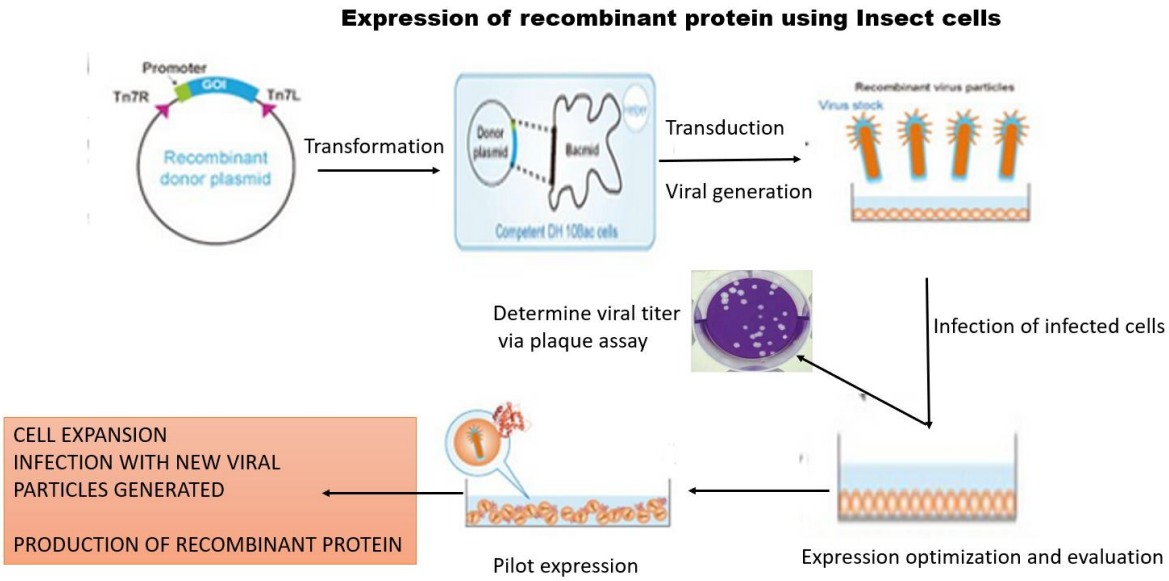
The GlcNAcase was suppressed and the simultaneous expression of a galactosyltransferase (GalT) resulted in more complex but still unsialylated and heterogeneous glycans.

STEPS INVOLVED IN RECOMBINANT PROTEIN EXPRESSION IN INSECT CELLS:

- In the first step, the gene of interest (GOI) must be integrated into a transfer vector The most important differences among these vectors are the promoter system, the protein tag and the secretion signal.
- When the transfer vector is ready, the desired parts need to be integrated into the baculovirus genome.
- The recombinant bacmid DNA is then transfected into insect cells (Sf9, Sf21 or HighFive™) for virus production. Following the generation of the P1 virus stock, the virus needs to be amplified to increase the titre and culture volume. The titre of the P2 stock is typically $\geq 10^8$ pfu/mL.

  Insect cells are cultivated in shaker flasks with a culture volume of 50 mL. For industrial applications, virus stocks with an even higher volume are necessary, so P3 stocks are generated using bioreactors.
- The titre of infectious virus particles must be determined before the viruses can be used for protein expression using plaque assay and End point dilution assay .
- The protein expression step can be divided into two stages: cell expansion and infection. In the first step, the bioreactor is inoculated with a low cell concentration, and at the desired time of infection (TOI), the baculovirus stock is added to the cell suspension.

Industrial baculovirus-based processes use a volume of 10–100 $m^{3.}$ A multicipility of Infection()MOI > 1 statistically ensures the infection of all insect cells immediately after virus addition. These cells start to produce the recombinant protein and also produce new virus



particles which infect other neighboring cells too.

3. INTRODUCTION TO RECOMBINANT PROTEIN EXPRESSION USING MAMMALIAN CELLS

rHuman protein must be identical to the natural protein.

The first recombinant therapeutic protein produced inmammalian cells, **tissue plasminogen activator** (r-tPA,Activase) synthesized using CHO cells, was approved for clinical use in 1987

The table below show the list of recombinant proteins generated by using mammalian cells

Table. Selected list of approved biologics produced in Chinese Hamster Ovary cell lines.

Product	Type	Therapeutic use	Manufacturer	Year of approval (FDA)
Vectibix	Anti-EGFR mAb	Metastatic colorectal cancer	Amgen	2006
Myozyme	α-glucosidase	Pompe disease	Genzyme	2006
Aldurazyme	Laronidase	Mucopolysaccharidosis I	Genzyme	2006
Orencia	Ig-CTLA4 fusion	Rheumatoid arthritis	Bristol-Myers Squibb	2005
Naglazyme	N-acetylgalactosamine-4-sulfatase	Mucopolysaccharidosis VI	BioMarin Pharmaceutical	2005
Luveris	Luteinizing hormone	Infertility	Serono	2004
Avastin	Anti-VEGF mAb	Metastatic colorectal cancer & lung cancer	Genentech	2004
Advate	Factor VIII (engineered)	Hemophilia A	Baxter	2003
Xolair	Anti-IgE mAb	Moderate/severe asthma	Genentech	2003
Raptiva	Anti-CD11a mAb	Chronic psoriasis	Genentech	2003
Fabrazyme	α-galactosidase	Fabry disease	Genzyme	2003
Rebif	Interferon-β	Relapsing multiple sclerosis	Serono	2002
Humira	Anti-TNFα mAb	Rheumatoid arthritis	Abbott	2002
Aranesp	Erythropoietin (engineered)	Anemia	Amgen	2001
Campath	Anti-CD52 mAb	Chronic lymphocytic leukemia	Genzyme, Bayer	2001
ReFacto	Factor VIII	Hemophilia A	Wyeth	2000
Tenecteplase	Tissue plasminogen activator (engineered)	Myocardial infraction	Genentech	2000
Herceptin	Anti-HER2 mAb	Metastatic breast cancer	Genentech	1998
Enbrel	TNFα receptor fusion	Rheumatoid arthritis	Amgen, Wyeth	1998
Benefix	Factor IX	Hemophilia B	Wyeth	1997
Follistim/Gonal-F	Follicle stimulating hormone	Infertility	Serono/NV Organon	1997
Rituxan	Anti-CD20 mAb	Non-Hodgkin's lymphoma	Genentech, Biogen Idec	1997
Avonex	Interferon-β	Relapsing multiple sclerosis	Biogen Idec	1996
Cerezyme	β-glucocerebrosidase	Gaucher's disease	Genzyme	1994
Pulmozyme	Deoxyribonuclease I	Cystic fibrosis	Genentech	1993
Epogen/Procrit	Erythropoietin	Anemia	Amgen/Ortho Biotech	1989
Activase	Tissue plasminogen activator	Acute myocardial infraction	Genentech	1987

4.Key features of recombinant proteins expressed in mammalian cells

Prokaryotic yeast and insect expression systems are generally unable to produce authentic mammalian proteins due to the absence of appropriate mechanisms for carrying out the necessary post-translational modifications to the protein

Correct disulphide bond formation Reaction mediated by the enzyme disulphide isomerase. An improperly

folded protein is unstable and lacks activity

Proteolytic cleavage of a precursor form Selected segments of amino acid sequences are removed to yield a

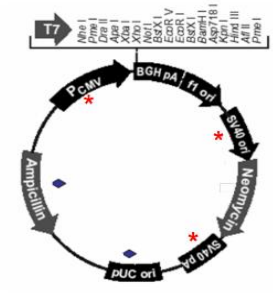
functional protein

Glycosylation Gives a protein with stability and, in some instances, its distinctive properties. The most common protein glycosylations occur by the addition of specific sugar residues to serine or threonine (O-linked) or to

asparagine (N-linked)

Addition of amino acids within proteins Modification of this type includes phosphorylation, acetylation, sulfation

Characteristics of Mammalian expression vector



- ✓ Contain an efficient promoter elements for high level transcriptional initiation
- ✓ mRNA processing signals
- ✓ selectable markers
- ✓ Plasmid sequences for propagation in bacterial hosts
- ✓ a eukaryotic origin of replication from an animal virus, e.g. Simian virus 40 (SV40)
- ✓ Origin of replication from E. coli
- ✓ promoter sequences that drive both the cloned gene(s) and the selectable marker gene(s)
- ✓ transcription termination sequences -adenylation signals from animal virus
- ✓ e.g. SV40
- ✓ Matrix-attachment regions
- ✓ Chromatin insulators, locus control region

Eukaryotic promoters commonly used in mammalian expression vectors

- ✓ **Animal viruses:**
- ✓ Cytomegalovirus (CMV) promoter
- ✓ SV40 promoter/enhancer
- ✓ Vaccinia virus promoter
- ✓ Viral LTRs (MMTV, RSV, HIV)
- ✓ **Mammalian genes:**
- ✓ Promoters of constitutively expressed genes (actin, GAPDH),
- ✓ Promoters of genes expressed in a tissue-specific manner (albumin, NSE)

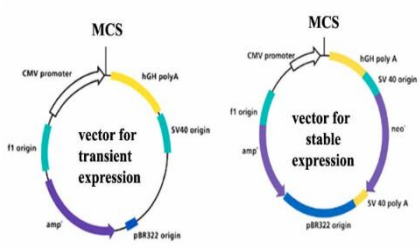
  Promoters of inducible genes (Metallothionin, steroid hormones)

What is the difference between Transient and Stable transfection?

- ✓ **Transient transfection**
- ✓ - standard non-selectable transformations of mammalian cells that replicate the
- ✓ plasmid DNA, but do not faithfully segregate the plasmid to progeny; in time
- ✓ the plasmid will be lost from the cultured cells.
- ✓ **Stable transfection**
- ✓ - a selectable marker is included in the plasmid vector so that after the cells have been transformed,the rare cell that incorporates the plasmid DNA into its genome (through DNA

repair and recombination enzymes) can be isolated and cloned (because progeny of the transformed cell will inherit the plasmid DNA including the selectable marker gene, e.g. antibiotic resistance gene)

VECTOR DESIGN FOR TRANSIENT AND STABLE FUNCTION



The strong human cytomegalovirus (CMV) promoter regulatory region drives constitutive protein expression levels.
The presence of the SV40 replication origin will result in high levels of DNA replication in SV40 replication permissive COS cells.
These vectors contain the pMB1 (derivative of pBR322)origin for replication in bacterial cells, the b-lactamase gene for ampicillin resistance selection in bacteria, hGH polyA, and the f1 origin.
Stable expression vectors carry the aminoglycoside phosphotransferase II gene (neomycin resistance gene or neor) that confers resistance to aminoglycosides such as G418 sulfate, allowing selection of stable transfectants.

G418 is used for the selection and maintenance of eukaryotic cells G418 is an aminoglycoside antibiotic produced by Micromonospora rhodorangea.

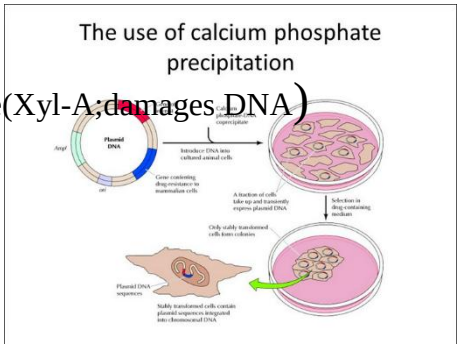
G418 blocks polypeptide synthesis in eukaryotic cells by binding irreversibly to 80S ribosomes and therefore disrupting their proofreading capability.

RESISTANCE TO G418

Resistance to G418 is conferred by the neo gene from transposon Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH 3' II3. This protein inactivates G418 by covalently modifying its amino or hydroxyl functions therefore inhibiting the antibiotic-ribosome interaction

Enzyme	Drug
Aminiglycoside phosphotransferase(APH)	=G418(Inhibits protein synthesis)
Dihydrofolate reductase(DHFR)	= Methotrexate (Inhibits DHFR)
Hygromycin B phosphotransferase(HPH)	=Hygromycin B (inhibits protein systhesis
Thymidine Kinase(TK)	= Aminopterin (Inhibits de novo purine and thymidylate synthesis)
Xanthine-guanine Phosphoribosyltransferase (XGPRT)	= Mycophenolic acid(Inhibits de novo GMP synthesis
Adenosine deaminase(ADA)	=9-b-D-xylofuranosyl adenine(Xyl-A;damages DNA)

Transfection of DNA into mammalian cells :



- ***• Calcium phosphate***

– Mix cells with DNA in a phosphate buffer

– Then solution of calcium salt added to form a precipitate

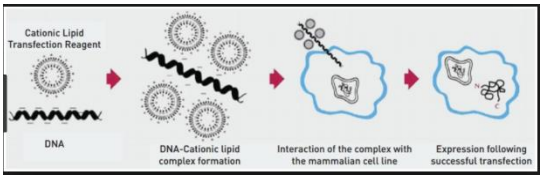
– Cells take up the calcium phosphate crystals which include some DNA

➢ Liposomes

– DNA mixed with lipid to form liposome,

small vesicles with some of the DNA inside

– DNA-bearing liposomes fuse with cell membrane carrying DNA inside the cell



➢ <u>Non-liposomal methods of transfection</u>

- ***Non-liposomal transfection agents (lipids and polymers)***

Alternatives to liposomes include non-liposomal lipids and polymers capable of forming complexes with nucleic acid to form micelles, or tiny encapsulating droplets. The transfection is usually performed under aqueous conditions, which enables the lipophilic portion of the amphiphilic compound, or the part of the droplet that displays affinity for fatty-acid compounds such as the cell membrane, to form the micelle capsule that encases the exogenous nucleic acids.

- ***Dendrimer-based transfection***

Dendrimers are highly branched, globular macromolecules that are capable of interacting with DNA to form small complexes. Dendrimers are stable in biological liquids and are not sensitive to temperature. These properties make dendrimers highly efficient tools for tissue culture transfections. The downside of dendrimers is that they are non-biodegradable and therefore may be toxic to cells with prolonged exposure.

- ***Microinjection***

In certain cases, permanent expression of a probe or gene of interest is desirable in your experimental system. This requires targeting specific cells within a population for gene delivery by microinjecting specific DNA sequences into the nuclei of target cells. The limitation of this method is that the number of cells that can effectively be transfected by this method is limited by the skill of the person performing the microinjection.

- ***Virus-mediated gene delivery (transduction)***

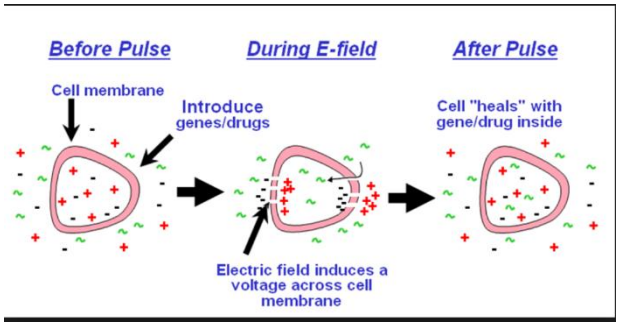
Exogenous genes or probes can be introduced into cultured mammalian cells by viral transduction techniques that use viruses as carriers. Viral delivery is useful for transfecting primary cell cultures, and there are numerous *in vivo* gene delivery approaches that allow the delivered genetic material to be integrated into the target cell genome using the viral machinery. Often, a selection marker (either antibiotic-based or GFP-based) is used to select for cells that have been successfully transduced with the virus.

- **<u>Electroporation</u>**

Electroporation is a powerful transfection tool useful for studying gene function.

Electroporation permeabilizes the membranes of cells when an electrical current is applied. The long dsRNA is usually delivered in a high ionic strength medium. Once these pores have resealed, normal cell functions can continue.

Process of Electroporation



Images of Electrophotometer

CULTURING OF MAMMALIAN CELLS:

Mammalian cells are adherent or non-adherent

Cultured in plates or flasks

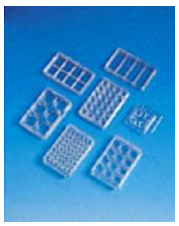
Grow in monolayer or suspension culture on specially treated surfaces or flask.

Medium supplemented with 5-10% Fetal Calf Serum

Laminar flow cabinet is used to maintain sterility and perform all cell culture work inside it.

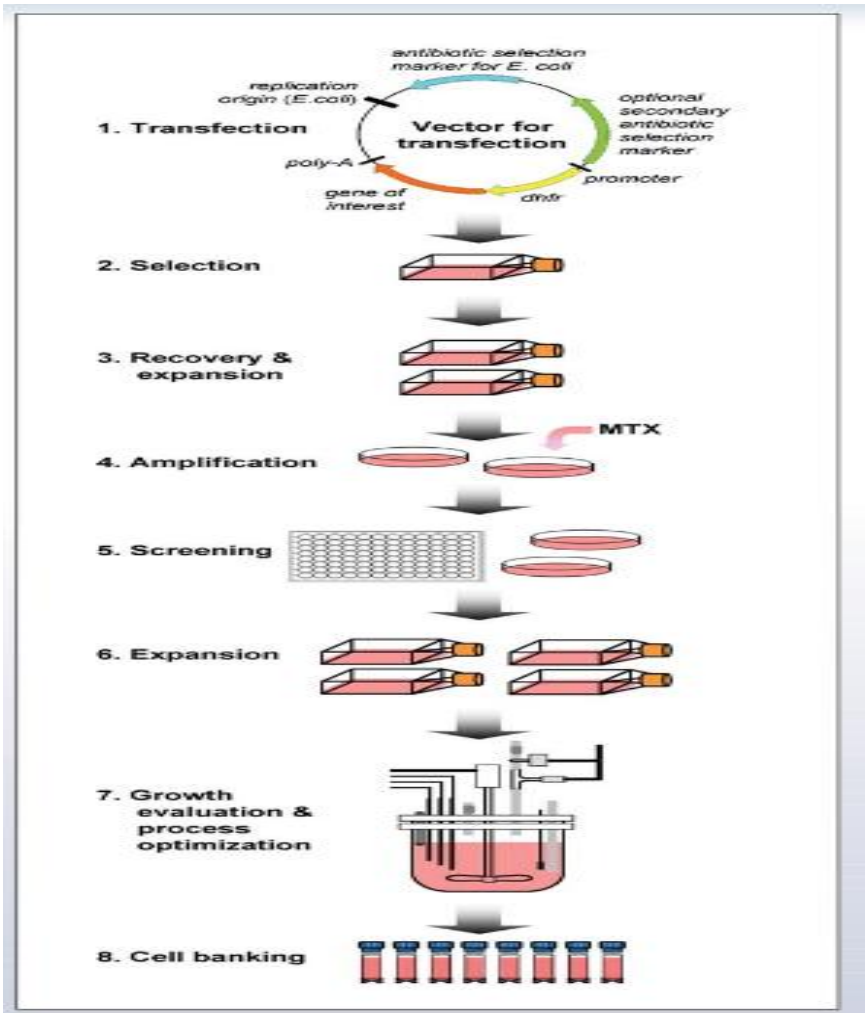
Cells are grown in CO_2 incubator at 37 degree celcius

PICSTURES OF COMMONLY USED THINGS FOR MAMMALIAN CULTURE:



Schematic steps involved in cell line development in production of recombinant proteins using mammalian cell line

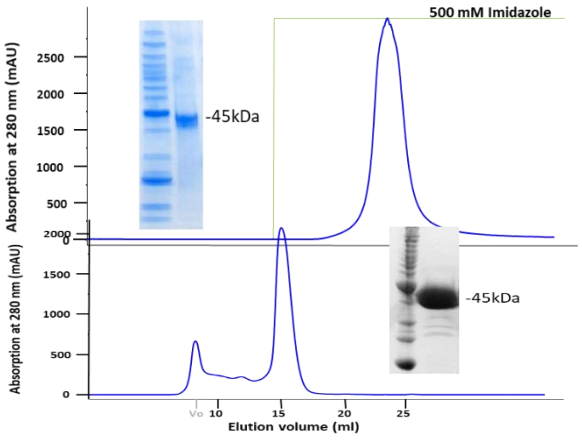
Ref: CHO Consortium



HOW THE PROTEINS ARE FINALLY PURIFIED?

Depending on the charge and tag on the expressed protein, two commonly used methods to purify the recombinant proteins are :

Affinity chromatography



Gel Filteration:

These techniques have been described in details in previous module , hence are not talked about much here

.5. CELL-FREE PROTEIN EXPRESSION STUDIES

Cell-free protein expression is performed without the use of living cells. Instead, all components needed to transcribe DNA to RNA and translate the RNA to protein (e.g. ribosomes, tRNAs, enzymes, cofactors, amino acids) are provided in solution for use *in vitro*.

Generally, such solutions are obtained through making a cell lysate from a desired cell type.

Cell-free mixtures have been made from both bacterial and eukaryotic cells.

Cell-free systems are generally not practical for large-scale protein expression.

Use of Cell-free protein expression system:

- ✓ Rapid generation of a smaller amount of recombinant protein .
- ✓ Proteins required for high-throughput screening of truncated proteins for structural or functional studies.
- ✓ Making proteins that are toxic to expression hosts *in vivo*
- ✓ Expression of proteins with modified amino acids,
- ✓ Incorporation of post-translational modifications, or studies on protein folding

Limitations of Cell-free protein system:

- ✓ Lack of post translational modification
- ✓ Cumbersome, as need to extract all the translational machinery from cells first

Predominantly used cell free systems:

- ❑ E.coli 30S extract
- ❑ Rabbit reticulocyte lysate
- ❑ Wheat germ extract

E.coli cell free system:

- Crude extract (30S)
- Endogenous mRNA removed by run-off translation and
- subsequent degradation
- Simple translation apparatus
- Comparatively simple control of initiation

This is one of the cheap method.

Rabbit reticulocyte lysate:

- Efficient in vitro eukaryotic protein synthesis system

Reticulocytes: Immature red blood cells specialized for hemoglobin

synthesis (Hb is 90% of protein content) lacking nuclei but complete translation machinery

- Endogenous globin mRNA removed by treatment with Ca2+-dependent

micrococcal nuclease, which is then inactivated by EGTA-chelation of Ca2+

- Exogenous proteins are synthesized at a rate close to that observed in intact Reticulocytes
- Both capped (eukaryotic) and uncapped (viral) RNA is translated

Kozak consensus and polyA signal have to be provided on RNA

Synthesizes mainly full-length product

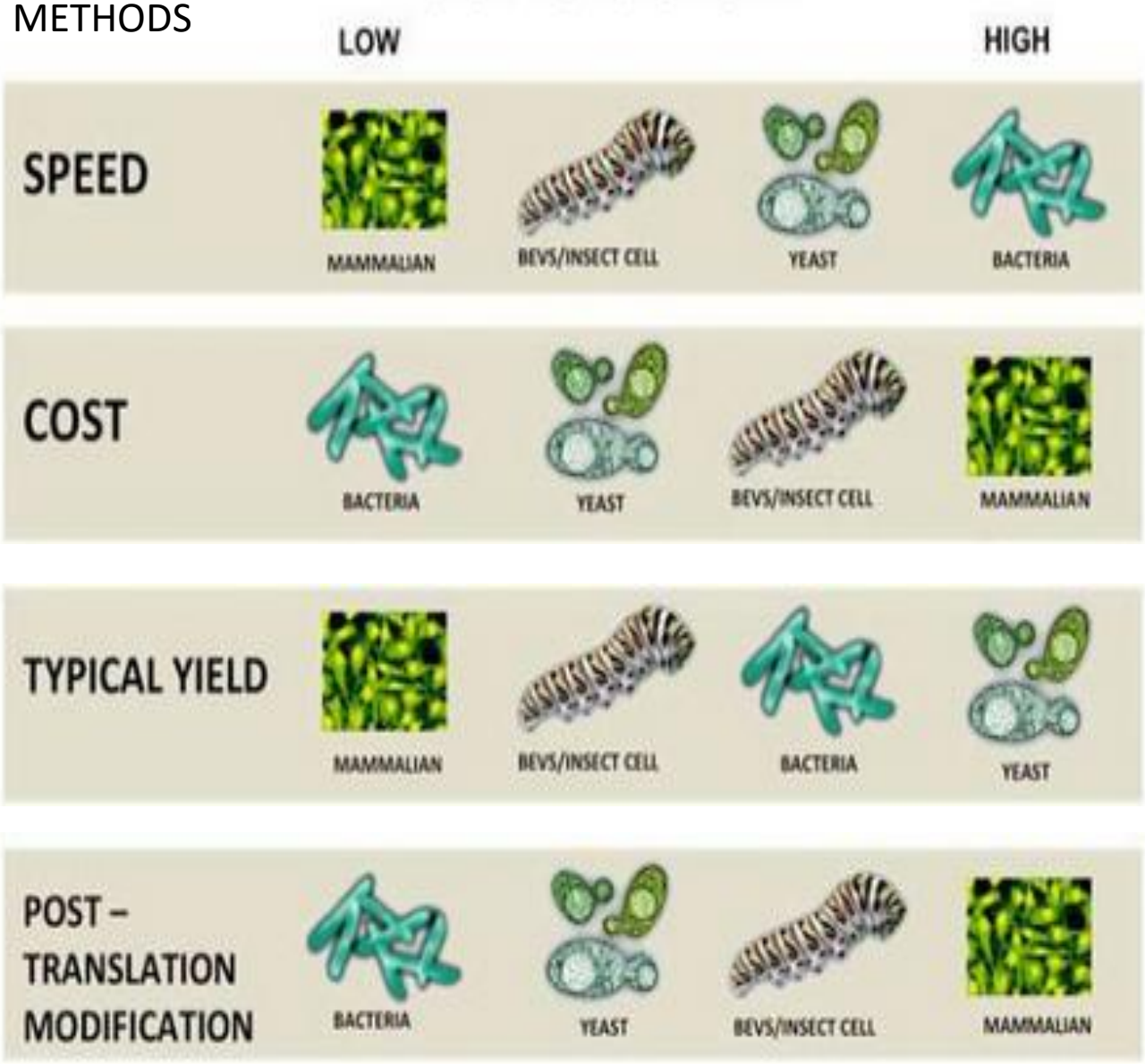
Wheat germ extract:

- Convenient alternative to rabbit reticulocyte lysate
- Low levels of endogenous mRNA: low background
- No micrococcal treatment necessary
- Exogenous proteins (mammalian, viral, plant) synthesized at high levels

What are the advantages of using cell free extract system?

- Express proteins toxic to cells
- Express insoluble proteins
- Mutational analysis
- Protein labeling: Radiolabeling or fluor
- escent incorporate unusual amino acids
- Producing proteins for structural studies, inc. NMR, crystallography, mass spec
- Eliminates the time and cost required with cell-based systems:
- Cell line maintenance
- Expression optimization
- Transformation or transfection

6. COMPARISON OF VARIOUS PROTEIN EXPRESSION METHODS



SUMMARY:

1. Eukaryotic expression systems are frequently employed for the production of recombinant proteins as therapeutics as well as research tools.

2. After *E.Coli* , insect cells are widely in terms of recombinant protein expression . In insect cells the most common expression systems are based on cell lines derived from *Spodoptera frugiperda* (Sf-9 and Sf-21), *Trichoplusia ni* (BTI-TN-5B1-4, marketed as High Five™) and *Drosophila melanogaster* (S2).

3. Insect cells have been successfully used to produce many recombinant proteins used as therapeutics for human treatment like CERVERIX for cervical cancer , PROVENGE for prostate cancer, FluBLOK Influenza control.

4. In 1983, Smith and Summers established the baculovirus-based expression system.

Two of the most common isolates used in foreign gene expression are Autographa californica multiple nuclear polyhedrosis virus (AcMNPV) and Bombyx mori(silkworm)nuclear polyhedrosis virus (BmNPV).

5. Baculoviruses have been genetically engineered to be highly efficient eukaryotic expression systems. is widely used as a eukaryotic expression system for the production of vaccines and medical products of medicinal and biological importance.

6. Two main expression vectors used for insect cells are BEVS(based on Multiple nuclear polyhedrosis virus (MNPV) and the Bacmid virus

7.For recombinant protein expression in insects cells first the GOI is put into the transfer vector which in turn need to be integrated into the baculovirus genome and infected to insect cells for viral production and further protein expression.

8. Prokaryotic yeast and insect expression systems are generally unable to produce authentic mammalian proteins due to the absence of appropriate mechanisms for carrying out the necessary post-translational modifications to the protein , hence if required we need to use mammalian cells for protein expression.

9. Correct disulphide bonding, glycolsylation and amino acid modification like phosphorylation, acetylation and sulfation of protein is required for proper folding and activity of proteins.