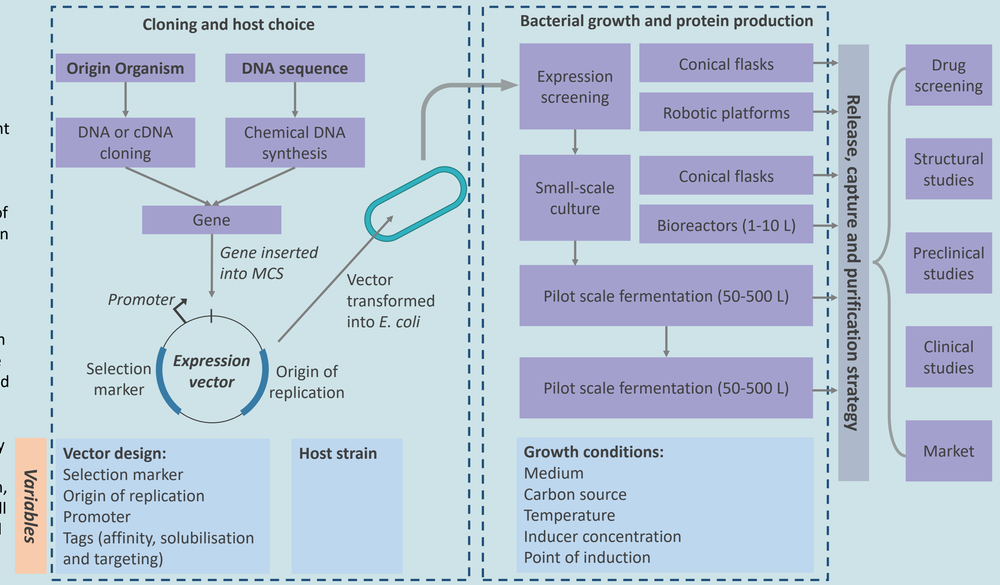
Recombinant Protein Expression

Recombinant proteins include various receptors, cytokines, growth factors, transcription factors, hormones, enzymes, toxic proteins, viral antigens, etc., which are widely used in scientific research, medical and industrial fields. Expression systems for the production of these recombinant proteins include mammalian cells, bacteria, yeast, insect cells, and plants, etc.

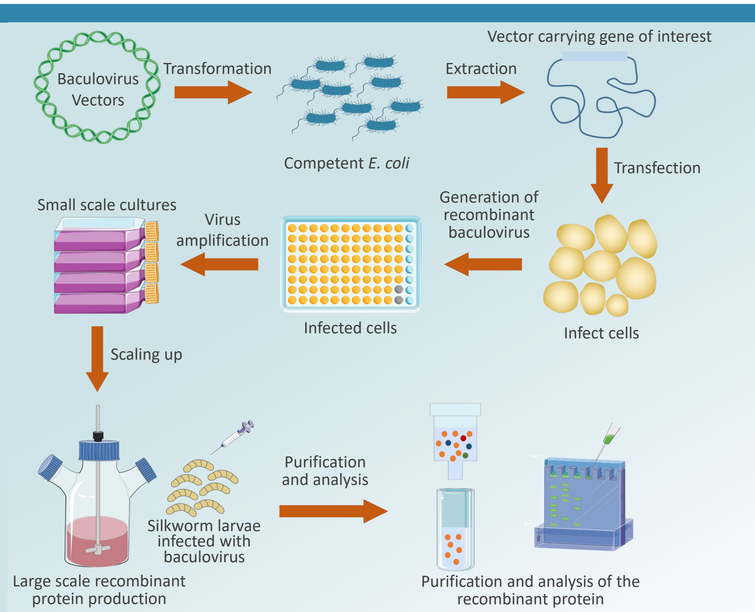
Escherichia coli Expression System

E. coli is the most preferred system for the production of recombinant proteins in bacteria and the most popular method of producing recombinant proteins. Expression of recombinant proteins requires multiple steps. First, the gene encoding the desired protein is cloned into the MCS of the expression vector under the control of a promoter that will regulate the expression of the gene. The promoter is a key component of an expression system because of its role in controlling the initiation of transcription of the associated gene. The plasmid vector is then transformed into an *E. coli* strain capable of producing the recombinant protein and grown in liquid medium. At a specific stage of growth, the production of the recombinant protein is induced by the addition of a chemical inducer that will activate the promoter on the expression vector. The recombinant protein of interest is formed by the expression of recombinant genes and the folding of recombinant polypeptide chains. Then, the recombinant protein is released from the cell and captured and purified. The scale of bacterial growth depends on the application of different recombinant proteins.



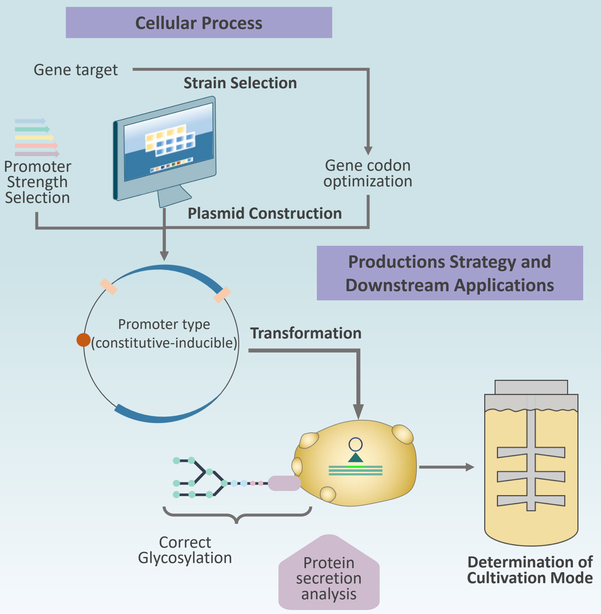
Baculovirus–insect Cell Systems

The baculovirus-insect cell expression system is a binary system consisting of two basic components, including a baculovirus expression vector that delivers a foreign gene encoding a protein of interest to the host and its host, an insect cell line, usually a lepidopteran insect cell line. The important steps of the baculovirus-insect cell expression system include baculovirus production, insect cell infection and purification. First, rBV carrying foreign genes is produced by recombination or transposition methods. The rBV is then amplified by several rounds of passage to generate a high-titer rBV stock. Insect cells with an optimized MOI are infected with rBV stock to produce the protein of interest. Finally, collect and purify the cells and/or supernatant to obtain the protein of interest. The baculovirus-insect cell expression system has the advantages of ease of manipulation, low cost, accommodating large DNA inserts, relatively high production levels, and necessary eukaryotic protein modifications similar to mammalian cells, and has been used for the expression of various proteins such as enzymes, glycoproteins, recombinant viruses and vaccines.



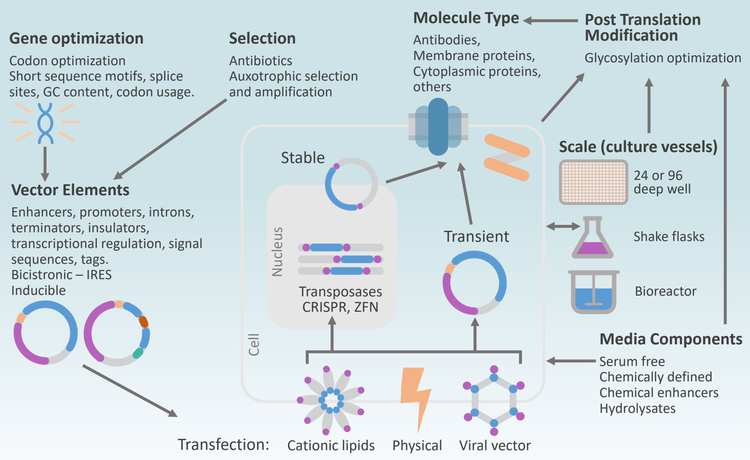
Yeast Expression System

Yeast expression system is an important tool for the production of functional recombinant proteins for industrial or medical applications, with the ability to complete proper post-translational modifications, rapid growth, simple genetic manipulation, scalable fermentation, high biomass concentration, and safe pathogen-free production. *Saccharomyces cerevisiae*, *Pichia pastoris*, *Hansenula polymorpha*, *Yarrowia lipolytica*, *Arxula adeninivorans*, *Kluyveromyces lactis*, and *Schizosaccharomyces pombe* are all common yeast expression hosts, among which *S. cerevisiae* and *P. pastoris* are especially popular. The yeast expression system can be divided into methylotroph and non-methylotroph. *P. pastoris* and *S. cerevisiae* belong to methylotrophic and nonmethylotrophic yeast, respectively. Variations in culture conditions during protein production can affect protein yield, such as pH, oxygen density, temperature, aeration, and induction techniques.



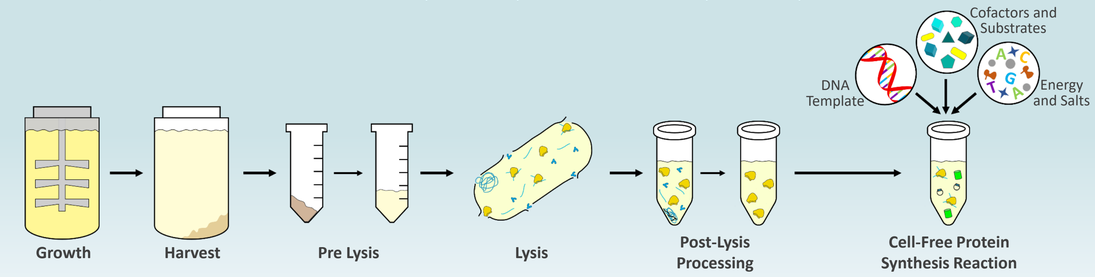
Mammalian Cell Expression System

Mammalian cell expression system is one of the routine methods for protein production. Protein production by mammalian cell lines enables the generation of a near-wild-type transcriptional and translational environment, coupled with relevant chaperone, secretory, and redox environments, as well as post-translational modifications that lead to functionally relevant and active proteins. Mammalian cell lines commonly used for protein production are CHO, cultivated from an ovarian biopsy of Chinese hamsters, and HEK-293, generated when human embryonic kidney cells were transformed with fragments of adenovirus type 5 DNA. Both cell lines can produce and post-translationally modify eukaryotic proteins in a high-level functional form, and can also be readily transfected or virally transduced to introduce foreign DNA encoding the target protein. The mammalian cell expression system introduces the vector DNA encoding the recombinant protein into the host cell line, and utilizes the synthetic ability of the cell to express and secrete the encoded protein into the cell culture medium.



Preparation of Cell-Free Extract and Set Up of CFPS Reactions

The basic steps of cell-free platforms based on different organisms are similar. First the cell line of interest needs to be cultured from which transcription and translation machinery are to be extracted. Next, cells are lysed while maintaining ribosomal activity in the lysate. The lysate is clarified by various methods to prepare cell extracts, and then utilize the prepared cell extract in CFPS reactions to synthesize the protein of interest. The CFPS reaction consists of the cell extract, a reaction mixture, and a mixture of DNA and inducers encoding the genetic instructions for the reaction. The extract contains ribosomes, RNA polymerase, other transcription and translation accessory proteins derived from a source strain, and metabolic enzymes for energy and cofactor regeneration. The reaction mixture of supplemental cofactors for protein synthesis including amino acids, nucleotides, salts, polyamines, an energy source (sugars or polysaccharides, glycolytic intermediates or creatine phosphate), molecular crowding agents, metabolic cofactors, buffer, transfer RNAs, and other metabolic additives. The DNA template can be plasmid DNA or a linear expression template obtained from PCR.



Creative BioMart

Starting from a small supplier of proteins and enzymes for academic institutes and biotech companies, Creative BioMart has always been focusing on developing high quality protein products and efficient protein manufacturing techniques. Over the past decade, our products and services are proved to have served our customers well and our brand has become one of the most trustworthy in the market.

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Protein Expression Systems Provided by Creative BioMart:

- Bacterial Expression Systems (*E. coli* / *Bacillus*)
- Baculovirus-Insect Cell Expression Systems (SF9, SF21, S2, T.ni / HI-5)
- Cell-Free *In Vitro* Protein Expression
- Mammalian Expression Systems (CHO / HEK293)
- Yeast Expression Systems (*P. pastoris* / *S. cerevisiae*)
- *Nicotiana tabacum* Specific Expression Platform
- Rice Endosperm Specific Expression Platform
- Other More

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Abbreviation:

CFPS, Cell-free protein synthesis; CHO, Chinese hamster ovary cells; HEK-293, human embryonic kidney 293 cells; MCS, multiple cloning site; MOI, multiplicity of infection; rBV, recombinant baculovirus.