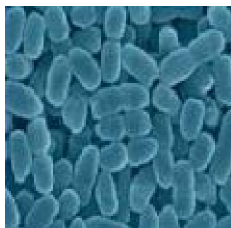
NRC·CNRC CNRC·NRC
Biotechnology Research Institute
Institut de recherche en biotechnologie

Protein expression technologies

The Biotechnology Research Institute of the National Research Council Canada (NRC-BRI) developed innovative technologies that can enhance research in protein expression or commercial protein production in bacterial, viral, or eukaryotic expression systems. The following unique vectors, regulatory elements, cells, and processes invented at the NRC-BRI are available for research and commercial licensing.

Protein Expression Technologies Available

Bacterial

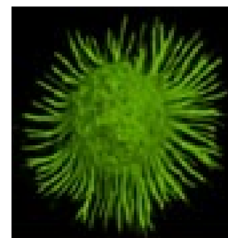


1. Alternative prokaryotic recombinant protein production: Expression using methylotrophic bacteria
2. Novel carrier proteins for enhanced peptide production and purification

Viral

3. Method for the large-scale production of E1 deficient adenovirus
4. Generation of adenovirus-based libraries by positive selection of recombinants

Eukaryotic



5. A HEK293 cell line for suspension culture and serum-free production of recombinant proteins and viral vectors
6. Recombinant proteins production by large-scale transfection of suspension-growing mammalian cells
7. Cumate-inducible expression system in eukaryotic cells
8. Yeast extracts for serum-free insect cell growth cultures
9. A coumarin-regulated on/off switch for gene expression in mammalian cells

Bacterial Expression Technologies

1. Alternative prokaryotic recombinant protein production: Expression using methylotrophic bacteria (L-11404 & L-11327).

The bacterium *Escherichia coli* is the best known and the most used prokaryotic expression system. The *E. coli* system, however, has drawbacks that include inclusion body formation, high acetate production, which tends to inhibit growth, and product formation, and a requirement for relatively expensive carbon sources such as glucose. Methylotrophic bacteria are a group of prokaryotic microorganisms that can utilize one-carbon compounds as a sole source of carbon and energy. This technology allows the production of recombinant peptides and proteins using a genetically modified methylotrophic bacterium (*Methylobacterium extorquens*). The bacteria are grown in minimal salts medium lacking organic sugars and containing methanol. The technology encompasses the needed expression vector containing a regulated promoter, methods of transfection, and methods of protein production.

Applications

- Alternative to *E. coli* and other prokaryotic expression systems.
- High-yield production of recombinant peptide/proteins (e.g. enzymes, bioactive peptides, bio-insecticides).

2. Novel carrier proteins for enhanced peptide production and purification (L-11391).

The synthesis of peptides larger than 40 amino acids relies mainly on chemical methods that generally suffer from cost disadvantages and low yield. This technology overcomes these drawbacks by providing novel carrier proteins, derived from *Staphylococcus* nuclease, for the production of small or large peptides as recombinant fusion proteins. These Fusion Carriers confer peptide stability and solubility and allow highly efficient and low cost production of small or large recombinant peptides (2-100 amino acids) using any suitable bacterial expression systems. This high efficiency is achieved through the use of a small carrier protein that allows C- or N-terminal fusions, the formation of inclusion bodies to protect the recombinant peptide from cellular proteases, and the improvement of downstream purification processes.

Applications

- Stable, high-yield and low-cost peptide production of peptides regardless of their size.
- Production of isotopically labeled peptides for NMR spectroscopy.
- Alternative for the production of pharmaceutical peptides, markers for medical imaging and diagnostics.

Viral Expression Technologies

3. Method for the large-scale production of E1 deficient adenovirus (L-10624).

Adenoviral vector systems are one of the most powerful platforms in biotechnology for gene transfer and protein expression. E1 gene products are essential for adenovirus replication. Deletion of this gene sequence produces a virus that is incapable of replication. Therefore, E1-deleted adenoviral vectors can only be generated in a cell line that constitutively expresses E1 proteins. This technology provides an adenovirus E1-complementing cell line that is particularly useful for simple, efficient and scalable production of infectious but replication-defective viral particles. In addition, a recombinant vector allows the creation of E1-complementing cell lines from different cell lineages.

Applications

- Large-scale production of infectious but replication-defective adenovirus viral particles that may be used for therapeutic and research applications requiring efficient gene transfer.
- Establishment of E1-complementing cells from any pharmaceutically acceptable cell line in order to significantly reduce the contamination of those cell cultures by replication-competent adenoviruses.

4. Generation of adenovirus-based libraries by positive selection of recombinants (L-10890).

Adenoviruses (Ad) are among the preferred viral vectors used in gene therapy, vaccination, and recombinant-protein production. This technology improves the safety and general utility of this important class of vectors by offering a novel Ad mutant (Ad2-deleted) for efficient library generation. In addition, novel cell lines derivatives of HEK293 cells expressing the Ad2 protease gene under tight regulation have been produced to simplify preparation of the defective virus particle. Adenovirus-based libraries are generated by positive selection of E1-deleted and viral protease-deleted (Ad2) recombinant vectors in the propagation-competent HEK293 cells. The technology allows the production of high purity, high diversity and high titer (> 10^6 clones) adenovirus-based expression libraries.

Applications

- Generation of safe adenoviral recombinants for applications where vector delivery to mammalian cells is desired such as gene therapy and vaccination, transfer of sensitizing genes into cancer cells, and recombinant protein expression.
- Use of Ad2 protease-complementing cell lines for safe and easy propagation of any therapeutic protease-defective adenoviral vector or for amplification of a protease-defective adenoviral library.
- Screening of highly diversified adenoviral expression libraries in mammalian cells.

Eukaryotic Expression Technologies

5. A HEK293 cell line for suspension culture and serum-free production of recombinant proteins and viral vectors (L-10894).

Mammalian cell culture systems are attractive for high yield production of viral vectors and recombinant proteins requiring complex posttranslational modifications. This technology provides a broadly applicable stepwise approach for the selection of animal cells capable of growth in serum-free conditions; such cells simplify downstream processing and allow the production of biologicals in the quantities and purities required for human use. This approach was successfully applied for the selection of a serum-free adapted clone (293SF-3F6) from a suspension-growing derivative of the HEK293 cell line. Furthermore, it allowed for the selection and improvement of a chemically defined formulation that most efficiently sustained serum-free suspension culture as well as high yield production of recombinant proteins and adenoviral vectors in this cell line.

Applications

- Selection of animal cell lines viable in serum-free medium for the expression of recombinant proteins and/or viral vectors.
- Screening and optimization of serum-free medium.
- Large scale transient or stable production of recombinant proteins and/or viral vectors in suspension and under serum-free conditions.
- Production of biopharmaceuticals using a cGMP master cell bank of a suspension-growing serum-free adapted HEK293 cell line.

6. Recombinant proteins production by large-scale transfection of suspension-growing mammalian cells (L-11266).

The production of active recombinant proteins with mammalian expression systems is often long, tedious, or of limited scalability. Hence, transient gene expression is often preferable to the establishment of stable transfectant as this latter approach is time consuming and requires that the expressed protein not adversely affect the growth of the cells. This technology provides a robust large-scale transient transfection process for the production of recombinant proteins in suspension-growing, serum-free adapted HEK293 mammalian cells. This validated process, carried out in a single step, achieves high expression levels in a very short period of time by combining optimized parameters in four key aspects: the cell line, the expression vector, the transfection vehicle and the serum-free culture medium. It is easily scalable and amenable to high throughput production.

Applications

- High yield and low cost protein production in a few days to a couple of weeks (mg or g of protein).
- Production of proteins, including antibodies, for high-throughput screening assays, antibody generation, structure-activity analyses, surface plasmon resonance, NMR or crystallography.

7. Cumate-inducible expression system in eukaryotic cells (L-11648 & L-11225).

In some circumstances, it is helpful to be able to control the timing, duration or level of production of a recombinant protein. Inducible expression systems are designed to provide this kind of control. However, these systems are generally of limited use in mammalian cells because of the side effects associated with the inducers, the low level of expression achieved and their inability to tightly regulate the expression. This technology provides a gene-switch that allows turning on quickly the expression of a recombinant protein in mammalian cells, in response to the presence (repressor configuration) or absence (transactivator configuration) of a specific and non-toxic regulator, cumate. Such a system is highly valuable for the *in vitro* expression of recombinant proteins that would slow down or even prevent the growth of the very cells that are used to produce them. In addition, it can also be adapted for *in vivo* applications where turning the protein production on or off at a specific time may be needed.

Applications

- Production of therapeutic proteins and viral vectors in tissue culture.
- Cell-based assays.
- Potential applications for gene therapy.

8. Yeast extracts for serum-free insect cell growth cultures (L-11389).

Yeast extracts from *Saccharomyces cerevisiae* have been traditionally used in animal cell cultures as substitutes for fetal bovine serum. Yeastolate ultrafiltrate is a key component in formulating serum-free medium for insect cell cultures. It is a product derived from natural sources and as such yeastolate preparations often show significant variability in their composition. This technology offers a robust method to prepare and characterize the active fraction from yeastolate ultrafiltrate that promotes cell growth and protein production in insect cells.

Applications

- Substitute for yeast extract for all cells.
- Rapid method to fingerprint the active fraction in each lot of yeastolate ultrafiltrate.
- Efficient characterization of the active fraction by analytical methods such as HPLC and NMR.

9. A coumarin-regulated on/off switch for gene expression in mammalian cells (L-11444).

Regulated expression of transgenes in mammalian cells is a valuable tool for both functional genomic studies and clinical applications. Inducible mammalian expression systems that feature low levels of basal activity and rapid induction of gene expression in response to safe and reliable inducers are needed to control protein expression. This technology features a rapid and sensitive gene-switch that relies on modulation by coumarins to provide regulated gene expression in mammalian cells. The system uses a coumarin-responsive chimeric transactivator together with a positive regulatory feedback loop to achieve efficient and tight control of expression. It can be turned on by coumermycin or promptly switched off by addition of novobiocin. This highly inducible and dynamic system is well suited for regulated mammalian transgene expression *in vitro* and *in vivo*.

Applications

- Tightly controlled regulation of gene expression in mammalian cells for functional analysis of cellular proteins and drug discovery applications.
- Transient production of highly toxic proteins or proteins interfering with host cell functions.
- Rapid and precise regulation of gene expression in vivo using low antibiotics concentration for gene therapy applications.

CONTACTS:

Yves Quenneville
Tel.: (514) 496-8507
Business Development Officer
E-mail: yves.quenneville@cnrc-nrc.gc.ca

Daniel Desmarteaux
Tel.: (514) 496-5300
Business Development Officer
E-mail: daniel.desmarteaux@cnrc-nrc.gc.ca