

Enhanced system for baculovirus-mediated transient transgene expression

SUMMARY

The current invention represents a further improvement on Bac-Mam technology and combines the versatility of the baculovirus vector with advanced molecular switch technology and powerful viral promoters for a tightly regulated gene expression system capable of delivering high yields of high quality protein in serum-free CHO cells. This technology allows precise inducible control of gene expression, which can be timed to limit cell growth inhibition, or to coincide with a specific growth phase or co-expression event. Combined with the large baculovirus insert capacity and CHO cell expression, this invention is discovery and pre-clinical production ready. Further development using this technology could facilitate an innovative expression system for precise control of large scale GMP biologic product manufacturing.

APPLICATIONS

- Non-mammalian viral vector delivery of genes to mammalian cell targets.
- Production of high quality biologics, including antibodies and other large macromolecules that require mammalian post-translational modifications.
- Inducible expression and high yields.

CONCEPT

Baculovirus was initially developed as technology to be used in gene therapy, due to its non-mammalian non-cytological properties. Subsequently, due to their ability to rapidly produce biologically active proteins and the relative ease of culturing insect cells, baculovirus has become a common tool for gene expression and protein production as part of the discovery research process. With the discovery that baculovirus could transform a range of mammalian cell types, an opportunity to merge the advantages of the baculovirus platform with industry standard mammalian cell models arose, leading to the invention of the Bac-Mam system. The novel technology

described here involves the use of a tailored DNA shuttle vector that allows for the convenient regulation of transgene expression and is based upon the gene regulatory mechanism of the bacterial operon p-cym of *Pseudomonas putida*. Inducible gene expression provides a “switch” mechanism to regulate both the level and the duration of expression, which is suitable for producing proteins whose constitutive expression may not be well tolerated by the cell. Therefore, the use of cumate gene-switch-modified baculoviruses to transfer therapeutic genes in CHO cells utilizing the Bac-Mam platform represents a cost-efficient, fast and high-yield recombinant bio-manufacturing method. In fact, this improved method generates 80-90 times more protein as compared to other commercially available systems. A NRC-BRI proprietary promoter CR5 constructed under the regulation of the rcTA transactivator has been tested using this platform and demonstrated a high level of activity with an additional inducible feature in stable cell lines 227#-13 and 10#-35 adapted for growth in suspension serum-free media.

FEATURES AND BENEFITS

Timing selectable induction

Tight regulation of gene expression prevents protein production until desired. Induction with cumate allows fast and reliable control for the onset of gene expression and allows optimization of cell culture yields and synchronized expression of gene combinations.

Time and cost savings

The system provides the means to reduce production time and cost for large scale production of therapeutic or industrial proteins and biologics. Cost savings could be particularly significant for molecules that would inhibit cell growth. Regulated gene expression, high transduction efficiency, and strong viral promoter expression provide high yields, reducing purification and concentration requirements.

Vector and cell line versatility

Baculoviral vectors are fast and easy to use for cloning large gene inserts. Viral vectors are capable of transducing and supporting expression of genes of interest in a range of mammalian cell types including CHO and 293 cells. Baculovirus’s vectors may also be useful for *in vivo* gene delivery.

Harmless inducer

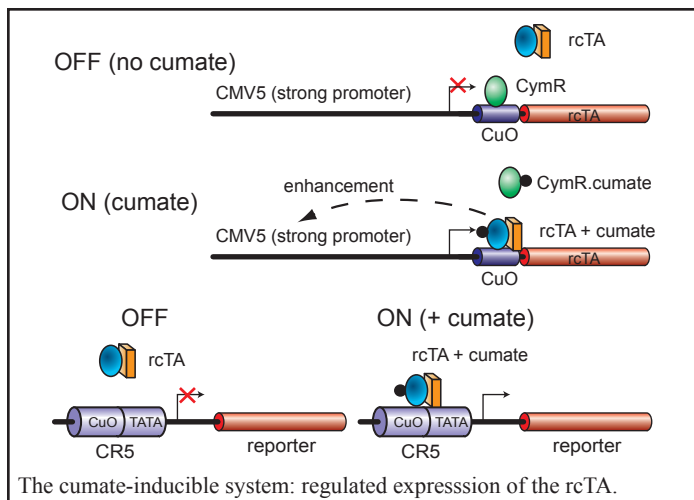
The technology uses an inducer – cumate, which is non-toxic in mammalian cells.

Intellectual property suite

Technology available as part of an intellectual property platform that includes several variations on the gene switch, use in eukaryotic cells, and several stable cell lines, which can be licensed individually or together as a platform.

PROTECTION STATUS

A novel system for enhanced baculovirus-mediated transient transgene expression in CHO cells (NRC no. 11770, 11648); A system for inducible expression in eukaryotic cells (NRC no. 11225).



CONTACTS

Daniel Desmarteaux

Tel.: (514) 496-5300

Business Development Officer

E-mail: daniel.desmarteaux@cnrc-nrc.gc.ca

Yves Quenneville

Tel.: (514) 496-8507

Business Development Officer

E-mail: yves.quenneville@cnrc-nrc.gc.ca

Dr. Bernard Massie

Tel.: (514) 496-6131

Group Leader, Genomics & Gene Therapy Vectors

E-mail: bernard.massie@cnrc-nrc.gc.ca