L-11947

Novel gene expression system for *Escherichia coli*

SUMMARY

Biomanufacturing is a burgeoning industry, expected to surpass $2.8 billion dollars by 2011. A variety of host types, including *Escherichia coli,* can be used for the production of proteins, enzymes and DNA that are needed in biopharmaceutical industries (e.g., insulin, growth hormones) and other industries (e.g., amylase). A novel gene expression system was developed for *E. coli.* This system could replace the IPTG-based systems currently available as it achieves higher product yields, uses p-cumate, a non-toxic, cost-competitive inducer, and is suitable for use with common laboratory strains of *E. coli*.

APPLICATIONS

- Production of biopharmaceutical proteins.
- Production of industrial enzymes.
- Basic and applied use in universities and research institutes.

CONCEPT

Inducible-expression systems are valuable assets for the study and scalable production of proteins including those that are toxic to the host. A cumate gene-switch that allows tight regulation of protein expression in mammalian cells was recently developed and commercialized. This system uses regulatory elements of the *Pseudomonas putida* F1 *cym* and *cmt* operons in order to control target gene expression at the transcriptional level, by using cumate as an inducer. A variation of the cumate-inducible switch was developed to provide a novel *E. coli* gene expression system. This system involves a new expression vector designed to express the *P. putida* repressor gene (*cymR*) constitutively in the host strain. Induction of recombinant gene transcription relies on the addition of an exogenous inducer, p-cumate, which is non-toxic, cost-competitive and easy to handle. The system provides high induction of transcription, extremely low basal expression and a degree of control far superior to other currently available *E. coli* expression systems. Further, results indicate that the system is compatible with a wide range of *E. coli* strains (e.g., BL-21[DE3]) and can potentially allow the expression of any gene produced using IPTG-inducible systems.

FEATURES AND BENEFITS

Applicable to commonly used *E. coli* strains

The expression vector can be used with common laboratory strains such as BL-21 (DE3), enabling those currently using IPTG-based systems to adopt the novel *E. coli* gene expression system.

High product yield

The *E. coli* gene expression system is capable of high induction of transcription (and low basal expression) surpassing the yields of IPTG-based gene expression systems.

Alternative to IPTG-induced *E. coli* gene expression systems

IPTG is a widely used inducer for *E. coli* recombinant protein production, but IPTG-based systems do not express some genes well, leading to insoluble or incorrectly folded proteins. The novel *E. coli* gene expression system may be an avenue to the production of these types of genes.

Novel, non-toxic, cost-competitive inducer

The *E. coli* gene expression system utilizes the non-toxic inducer p-cumate. This inducer is also cost-competitive with IPTG.

PROTECTION STATUS

Development of an inducible/regulated gene expression system in *Escherichia coli* based on regulatory elements of the *Pseudomonas putida* F1 *cym* operon (NRC no. 11947).

Induction time (h)	Inducer (100µM)			
	Specific GFP (mg/g DW)		[GFP] (mg/L)	
	Cumate	IPTG	Cumate	IPTG
1	30	28	602	554
2	69	60	1644	1300
3	104	74	3002	1778
4	141	82	4719	2486
5	153	72	5443	2035
6	146	50	6194	2015
7	170	58	6977	1948
8	196	58	7838	2090

Final Cell Dry Weight (g/L): Cumate 37.6
IPTG 35.9

Partial Cell Lysis- Foaming

GFP expression levels for T7 (IPTG) and cumate expression systems in *E. coli* BL21(DE3)pLysS

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. Carlos B. Miguez
Tel.: (514) 496-6280
Microbial & Enzymatic Technology Group
E-mail: carlos.miguez@cnrc-nrc.gc.ca

IPSO 11947 09/04/08