

## Cumate-inducible expression system in eukaryotic cells

### SUMMARY

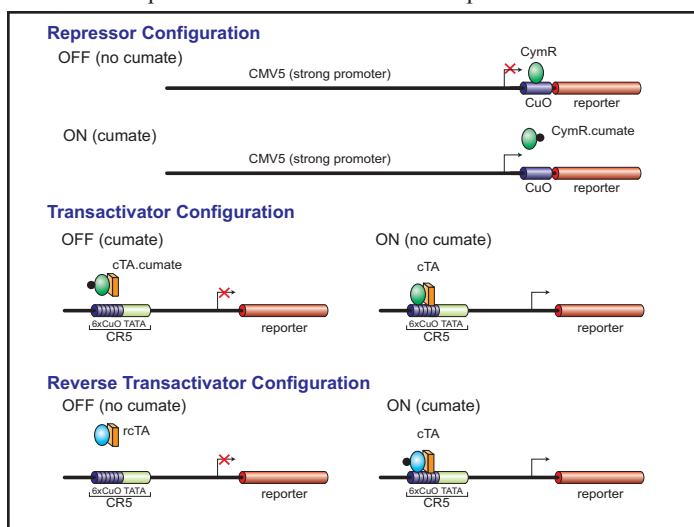
The cumate-inducible expression system allows turning on the expression of a given protein only when needed. This system is especially important for proteins that would slow down or even prevent the growth of the very cells that are used to produce the protein. Additional uses include human gene therapy where turning the protein production on or off at a specific time may be needed.

### APPLICATIONS

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

### CONCEPT

The cumate-inducible expression system has three configurations: the Repressor, the Transactivator and the Reverse Transactivator. In the first configuration, the repressor molecule, CymR, is used to repress transcription from a mammalian promoter (CMV5) by binding the CymR binding element, the cumate operator (CuO) placed downstream of the initiation site. Addition of the inducer (cumate), at concentrations that are not toxic to mammalian cells, causes a change in the configuration of CymR such that it can no longer bind the DNA sequence of the operator thus relieving repression and allowing production of the desired protein (see the Repressor Configuration diagram). In the second configuration, a chimeric transactivator (cTA) was generated by the fusion of CymR, as a DNA binding domain, with the activation domain of VP16. A chimeric promoter, CR5, was also created by inserting 6 copies of the CuO upstream of the CMV minimal promoter. This scheme



allows activation of the CR5 promoter only in the absence of cumate (expression ON in the Transactivator Configuration diagram). Finally, a reverse CymR molecule was generated by mutagenesis, such that it binds CuO in the presence rather than in the absence of cumate. The transactivator formed by the fusion of the reverse CymR mutant with VP16, gives rise to the reverse transactivator, which activates expression from the CR5 promoter in the presence of cumate. The ability to regulate target gene expression by any one of these three configurations makes the system more versatile and thus suitable for a large range of applications.

### FEATURES AND BENEFITS

#### Timing selectable induction

This novel system can remain off until there are enough cells to produce a batch of protein that would otherwise prevent the bioreactor cells from growing.

#### Rapid response after induction

In its Repressor and Reverse Transactivator Configuration, this system can be turned on quickly by the addition of cumate.

#### Time and cost savings

The system provides the means to reduce production time and cost for large scale protein production. Cost savings is particularly noteworthy in this system where the protein is inhibitory to cell growth. It allows all carbon sources and energy to be focused on expanding the bioreactor cell count prior to induction.

#### Compatible with other inducible systems

The system can work in concert with other inducible systems to allow the sequential expression of multiple proteins.

#### Control of gene expression in mammalian cells

This system allows inducible production of proteins in commercially important mammalian cell lines, such as CHO and 293. It can also be adapted to control gene expression *in vivo* in animal studies.

#### Harmless effector

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

### PROTECTION STATUS

Expression system, components thereof and methods of use (NRC no. 11648); A system for inducible expression in eukaryotic cells (NRC no. 11225).

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