

## DEREPRESSOR SYSTEMS: P<sub>ON</sub> and E<sub>ON</sub>

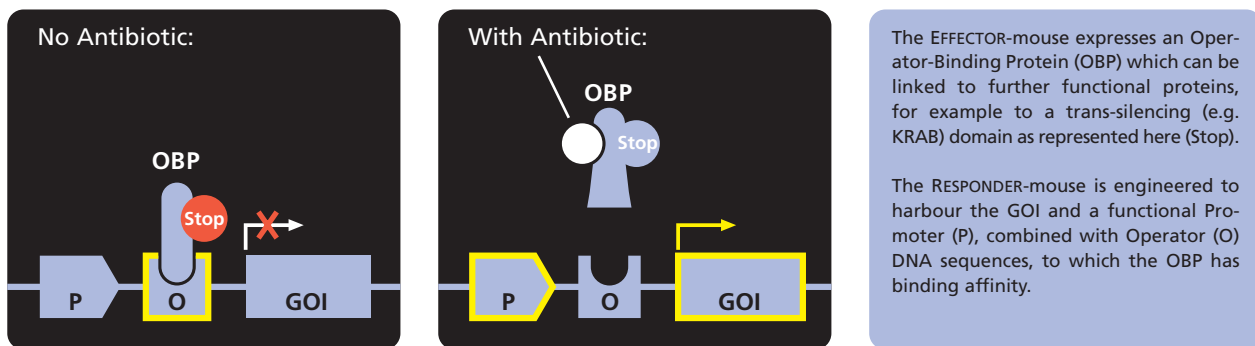
### Transgene Activation with Antibiotics

Two novel systems for independent modulation of transgene expression in mice, i.e. Pristinamycin-inducible (P<sub>ON</sub> or Pip<sub>ON</sub>)<sup>1</sup> and Erythromycin-inducible (E<sub>ON</sub>)<sup>2</sup> set-ups, are now available at PolyGene.

#### Mode of Function

These unique systems, developed at the Swiss Federal Institute of Technology in Zurich, are based on the capacity of antibiotics to bind repressor molecules and thus their capacity to modulate gene expression.

Crossing of two complementary (EFFECTOR and RESPONDER) transgenic mouse lines yields offspring, in which expression of the Gene Of Interest (GOI) is actively inhibited.



Transcription is **de-repressed** by administration of the respective antibiotic, which dissociates the fused-Operator Binding Protein (OBP) from the Operator. GOI expression is thus unleashed and free to be driven by a definable (e.g. tissue specific) Promoter.

#### Dose-Response

While binding of OBP alone strongly reduces GOI expression, it is literally abolished when KRAB-OBP fusion protein is bound to the operator sequences<sup>1</sup>.

Expression, as shown in cell culture experiments, is gradually increased up to roughly 100-fold at antibiotic concentrations ranging from about 0.1 to 2 µg/ml<sup>1,2</sup>.

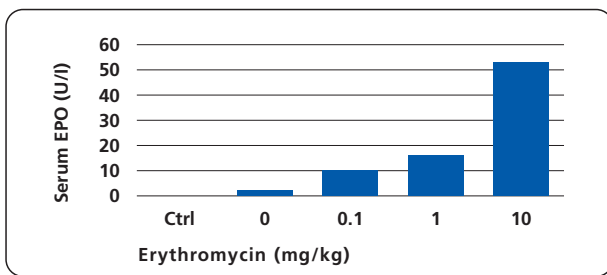
As evidenced *in vitro*, protein production follows linear kinetics over a period of approx. 70 hours<sup>2</sup>.

#### Bioavailability

Streptogramins (e.g. Pristinamycin) and Macrolides (e.g. Erythromycin), many of which have had a proven track record as antibiotic agents for over half a century, are reputed for excellent bio-availability and optimal pharmacokinetics. The low concentrations required for adjusting responsive elements are reached in all tissues following as well intravenous as oral administration.

## System Compatibility

Tested on mice with implanted HT-1080 cell derivatives, the E<sub>ON</sub> system was also proven to respond *in vivo*, in a dose-dependent manner<sup>2</sup>.



Microencapsulated cells producing Erythropoietin (EPO) under Erythromycin control were implanted in the peritoneal cavity of receptor mice. EPO synthesis was monitored 72 hrs after injection of Erythromycin at indicated doses.

Non-transplanted control mice (Ctrl), irrespective of the quantities of antibiotic administered, showed endogenous EPO production levels at the detection limit.

## Independent Control

As evidenced in antibiotic interference studies<sup>2</sup>, the different systems are fully compatible, thus paving the way for independent regulation of different transgenes in a single cell or combinatorial transcription control<sup>3</sup>.

## Positioning

PolyGene offers free promoter choice in RESPONDER constructs, thus ideally positioning both (P<sub>ON</sub> and E<sub>ON</sub>) DEREPRESSOR systems for **tissue specific** applications.

Note that the initial RESPONDER mouse, exhibiting promoter-driven GOI expression, can serve as constitutive EXPRESSOR. Caution is however warranted for transgenes affecting embryogenesis. Hence, the DEREPRESSOR systems are best suited for transgene expression modulation in **adult mice**.

Streptogramins as well as Macrolides are known for their low interference with host metabolism. Apart from possible concerns in the field of immunology (with P<sub>ON</sub> as preferred option), or in that of neurology (for which E<sub>ON</sub> may seem safer), both the P<sub>ON</sub> and the E<sub>ON</sub> systems are indicated for research applications in a **broad range of therapeutic areas**.

### References:

1. Fussenegger, M. *et al.*, Streptogramin-based gene regulation systems for mammalian cells. *Nat. Biotechnol.* **18**, 1203-1208 (2000)
2. Weber, W. *et al.*, Macrolide-based transgene control in mammalian cells and mice. *Nat. Biotechnol.* **20**, 901-906 (2002)
3. Kramer, B. *et al.*, BioLogic Gates Enable Logical Transcription Control in Mammalian Cells. *Biotechnol. Bioeng.* **87**, 478-484 (2002)

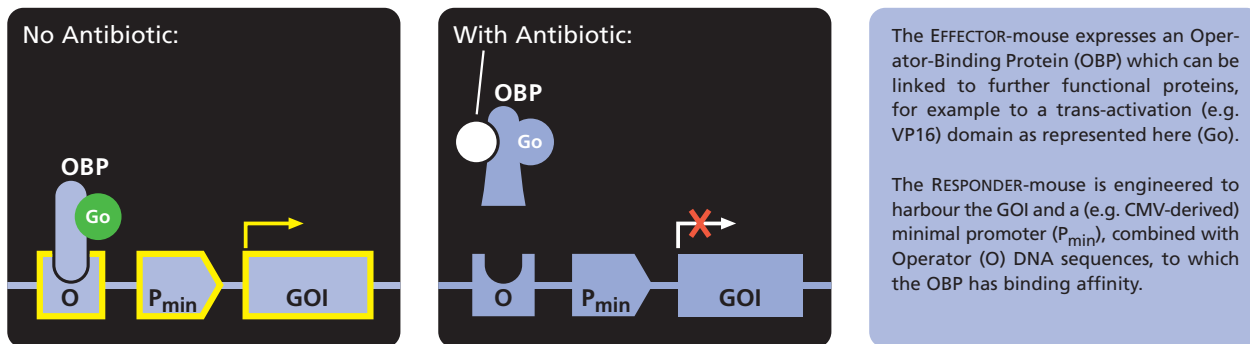
**DEACTIVATOR SYSTEMS: P<sub>OFF</sub> and E<sub>OFF</sub>**

**Transgene Suppression with Antibiotics**

PolyGene's Pristinamycin- and Erythromycin-inducible expression systems are also available as DEACTIVATORS, i.e. P<sub>OFF</sub> and E<sub>OFF</sub>, respectively<sup>1,2</sup>.

**Mode of Function**

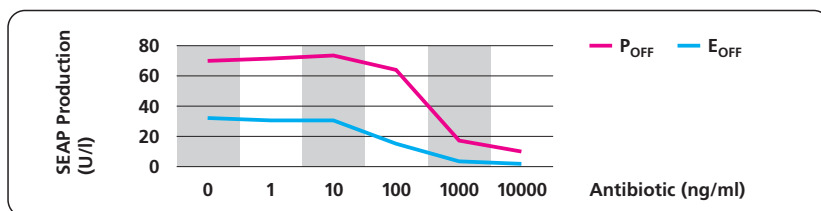
In their P<sub>OFF</sub> and E<sub>OFF</sub> versions, the co-expression of two distinct (EFFECTOR and RESPONDER) transgenes gives the possibility to modulate the expression of a given Gene Of Interest (GOI).



Transcription is **de-activated** by administration of the respective antibiotic, dissociating OBP from Operator and thus depriving the GOI of a functional activator.

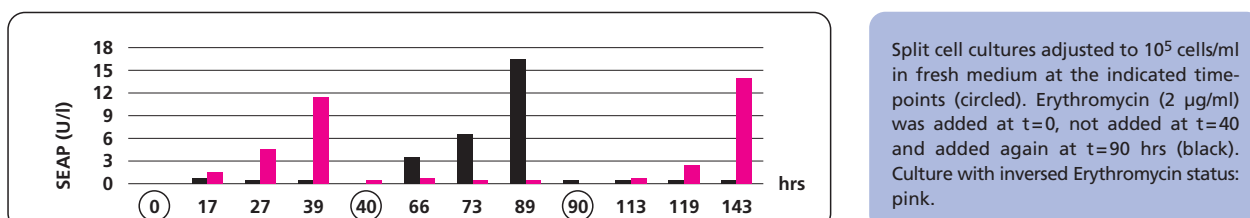
**Dose-Response**

As demonstrated in CHO cell culture experiments, GOI expression is reduced by approximately 100-fold over respective antibiotic concentrations ranging from roughly 0.01 to 2 µg/ml<sup>1,2</sup>.



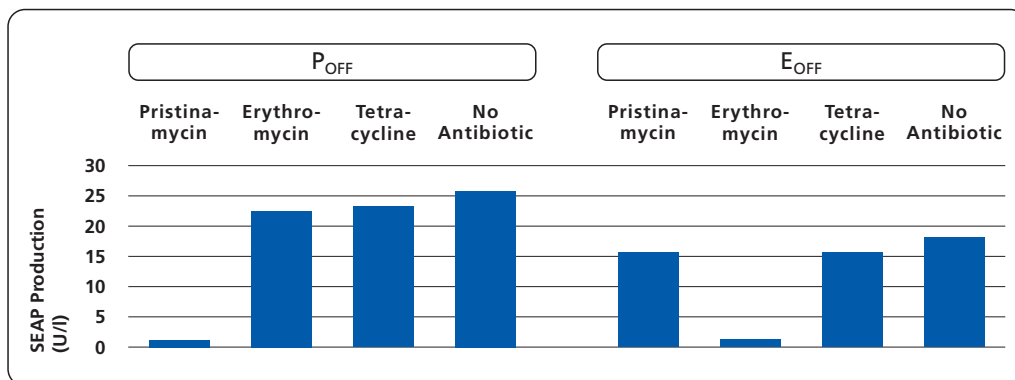
**Reversibility**

The responsiveness of SEAP producing E<sub>OFF</sub> cells to recurrent addition and withdrawal of Erythromycin reveals a swift and efficient on/off state transition<sup>2</sup>.



## Independent Control

As demonstrated by interference studies on CHO cells co-transfected with pairs of EFFECTOR and RESPONDER elements, gene expression modulation is achieved with the system-compatible antibiotic, only<sup>2</sup>.



## System Compatibility *in vivo*

In mice, the E<sub>OFF</sub> system was proven to respond in a dose-dependent fashion<sup>2</sup>. Moreover, the availability of different systems for independent control of transgene expression opens perspectives in terms of multi-gene and multi-regulated interventions<sup>3, 4</sup>.

## Positioning

To be provided with custom-made RESPONDER mice, PolyGene will maintain various EFFECTOR mouse lines constitutively expressing the fused OBP at determined levels. Thus, mating of RESPONDER mice with different EFFECTORS (each of a given trans-activation potential) allows studying GOI expression beyond a 2-log range.

Since the initial (OFF-type) RESPONDER mouse does not express the GOI, DEACTIVATOR systems are also suited for investigating **embryonic lethal genes**.

The reputed excellence of both, Streptogramins and Macrolides, in terms of bioavailability and of low interference with host metabolism, distinguishes the P<sub>OFF</sub> and E<sub>OFF</sub> systems for a **variety of applications**, including for **pregnancy-related studies**.

### References:

1. Fussenegger, M. *et al.*, Streptogramin-based gene regulation systems for mammalian cells. *Nat. Biotechnol.* **18**, 1203-1208 (2000)
2. Weber, W. *et al.*, Macrolide-based transgene control in mammalian cells and mice. *Nat. Biotechnol.* **20**, 901-906 (2002)
3. Fux, C. *et al.*, Streptogramin- and tetracycline-responsive dual regulated expression of p27Kip1 sense and antisense enables positive and negative growth control of Chinese hamster ovary cells. *Nucleic Acids Res.* **29**, e19 (2001)
4. Kramer, B. *et al.*, An engineered epigenetic switch in mammalian cells. *Nat. Biotechnol.* **22**, 867-870 (2004)