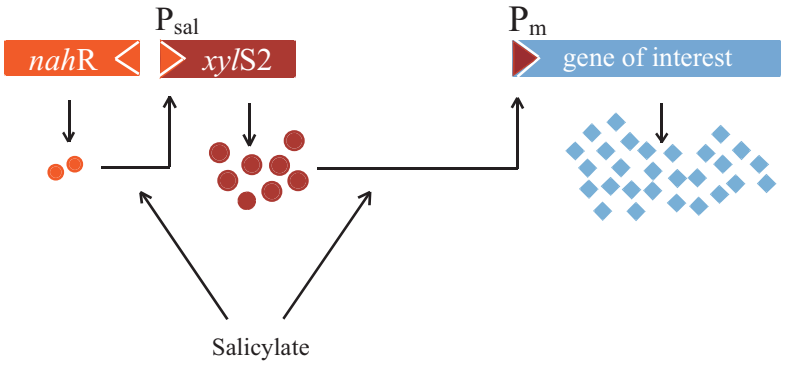
CASCADE™

BACTERIAL PROTEIN EXPRESSION SYSTEM

CASCADE™ (*) is a bacterial protein expression system that provides tightly regulated, high-level expression. The system makes use of linked regulatory circuits to amplify gene expression levels when induced, maintaining low basal expression levels under non-inducing conditions.



Applications:

- Laboratory and large scale recombinant protein expression/purification:optimization of host, expression systems, and downstream processing with the use of our genetic technology.
- Whole cell biocatalysis: stable assemblage of metabolic pathways.
- Programming therapeutic bacteria expression: antigen expression and display in attenuated pathogenic bacteria.
- Expression of heteromultimeric proteins.

Advantages:

- Tight control of gene expression with the highest level compared to other systems.
- Broad range of expression levels using different inducer concentrations.
- Modular structure compatible with mini-Tn5 delivery vectors.
- Stable phenotypes by using mini-Tn5 mediated integration.
- Active at low temperature (16°C) and low sensitivity to media formulation.
- Availability of vectors with protein fusion tags for purification using low cost affinity supports.
- Cost effective and scalable bioprocess engineering.
- Higher rate of success in obtaining soluble proteins.

TECHNOLOGY OFFER

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BACTERIAL PROTEIN EXPRESSION SYSTEM

CASCADE™ expression system is a transcriptional regulatory circuit to amplify the cell response to a given signal. In the current configuration, CASCADE™ is composed by the salicylate-responsive activators of *Pseudomonas putida* *Nah*R of the naphthalene degradation plasmid NAH7 and *Xyl*S2, a mutant regulator of the TOL plasmid for catabolism of m-xylene and their respective cognate promoters P*sal* and P*m*: Control of the expression of *xylS2* with the *nahR*/P*sal* system permitted either their selective activation with specific effectors for each protein or the simultaneous activation of both of them with salicylate. When cells face the common effector of the two regulators, both the increase in *Xyl*S2 concentration and the stimulation of its activity act synergistically on the P*m*: promoter, amplifying the gene expression capacity by at least one order of magnitude with respect to the individual systems.

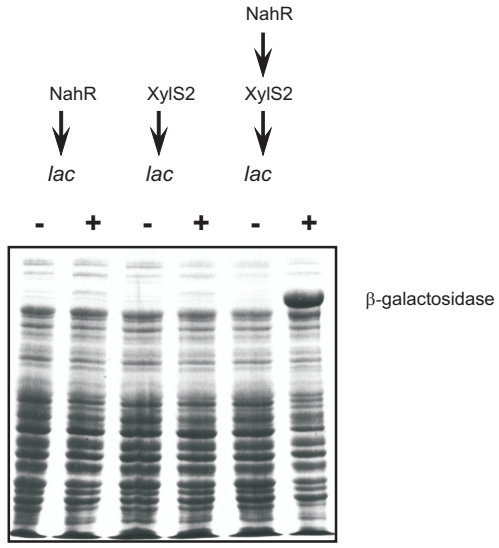

Figure 1. Compared overexpression of β-galactosidase in single copy using single expression systems and the CASCADE™

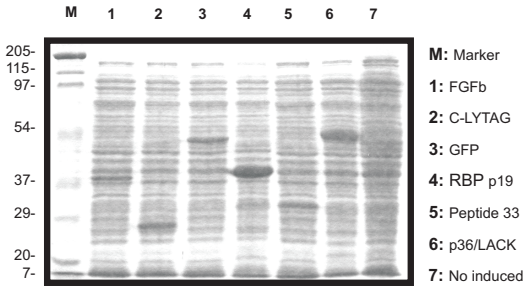

Figure 2. Cell extracts of *E.coli* cultures overexpressing different C-LytA tagged proteins with CASCADE™

The system has a modular design with a regulatory module (*nahR*-P*sal*::*xylS2*) and an expression module (with the terminal promoter P*m* and the gene/s of interest). Both modules can be stably inserted in the chromosome of gram negative bacteria by the use of mini-Tn5 delivery vectors (figure 1). The system has been used with dozens of proteins (see for instance figure 2), with an improved yield of active protein compared to any other competitor bacterial system.

PRODUCTS	DESCRIPTION	Cat. No.
E. coli REG-1 Strain	*E. coli* REG-1 strain contains the regulatory element *nahR/Psal::xylS2* integrated into the chromosome. The strain genotype is mini-Tn5 (kan^R *nahR/Psal::xylS2*) *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 (ara-leu)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G. The strain is shipped at room temperature. Upon arrival, stored at 4°C.	BS-3262
pALEX Vectors	pALEX vectors carry the 3´moiety of the *Streptococcus pneumoniae lytA* gene (*C-LytA*) between the CASCADE™ P*m* terminal promoter and the multiple cloning site for efficient protein expression and purification. These vectors contain a MCS that has seven unique restriction sites: *Bam*HI, *Xho*I, *Sac*I, *Bgl*II, *Kpn*I, *Bst*BI, and *Hin*DIII (refer to vector map for further details). These are available in all three reading frames (pALEXa, pALEXb, pALEXc), to facilitate cloning. These vectors also carry an enterokinase recognition sequence that enables removal of the C-LYTAG moiety from the fusion protein.	EV-3413
pCCD5 Vector	pCCD5 is a 20-40 copy number, 3.4 Kb, ColE1-derived vector usable for cloning and expression of protein genes in *E. coli* with the CASCADE ™ system. pCCD5 contains the *Pm* promoter preceded by a transcripcional terminator, and located upstream of a multi-cloning site that includes an efficient translation initiation region.	EV-3408
pCNB4-S2 Vector	pCNB4-S2 is a mini-Tn5 Km transposon delivery plasmid designed for stable integration of the CASCADE™ regulatory module in the chromosome of a wide variety of Gram-negative bacteria. pCNB4-S2 delivers a mobile element expressing *xylS2* under the control of the *Psal* promoter and its cognate salicylate-responsive regulator NahR. Transposition of the CASCADE™ regulatory module from this plasmid depends on the expression of a transposase gene in pCNB4-S2 external to the mobile element, allowing stability of the transposed module by simply curing of pCNB4-S2 from the recipient bacterial strain.	EV-3407

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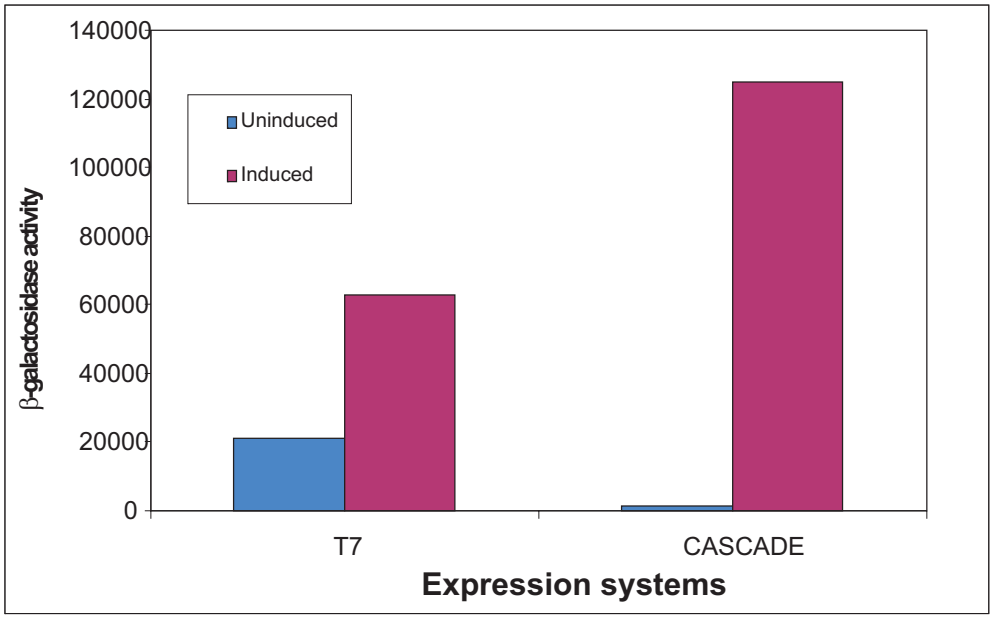
CASCADE™: SCALABLE PROTEIN EXPRESSION SYSTEM

CASCADE™ is especially convenient for scaling the production of recombinant proteins. The industrial production of recombinant enzymes, antigens, biopharmaceuticals or industrial proteins is highly recommended using CASCADE™ expression system because of its high protein production yield, combined with the fact that the protein expression is also tightly regulated, and can be controlled adding different inducer concentrations so the solubility of the protein is facilitated, reducing the risk of inclusion bodies.



Another fact that will surprise you about CASCADE™ Protein Expression System is the extremely low cost of the inducer (salicylate) compared with competitors systems (i.e. IPTG). CASCADE™ is the only bacterial protein expression system which allows you to save costs and scale your protein production, and also is able to express difficult proteins as hidrophobic, toxic, etc.

INDUCER PRICE COMPARATIVE		
INDUCER	€/g	€/cultive litre 1 mM
IPTG	**35.80 €**	**8.34 €**
SALICYLATE	**0.13 €**	**0.02 €**

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