pALEX1a, b & c

(Cat. No. EV-3439; EV-3440; EV-3441)

For research use only.

1. About BIOMEDAL CASCADE™ System.

The *CASCADE™* expression system uses two salicylate-responsive transcriptional activators, NahR and XylS2. In the presence of salicylate, the NahR protein induces expression of XylS2 from the P_{sal} promoter. Salicylate also activates XylS2, and hence its cognate promoter *Pm*, inducing high-level expression of the gene or genes of interest. The synergistic effect of combining two transcriptional regulators in a sequential cascade amplifies expression levels nearly 20-fold compared to expression from either promoter individually.

2. Description.

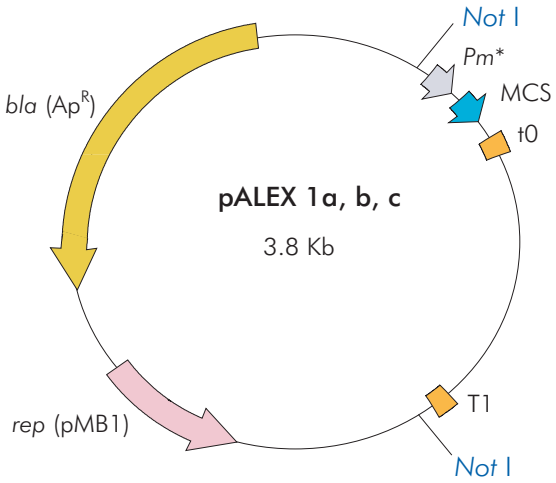
PRODUCT	CAT.No.	FORMAT
pALEX 1a	EV-3439	8 µg
pALEX 1b	EV-3440	8 µg
pALEX 1c	EV-3441	8 µg

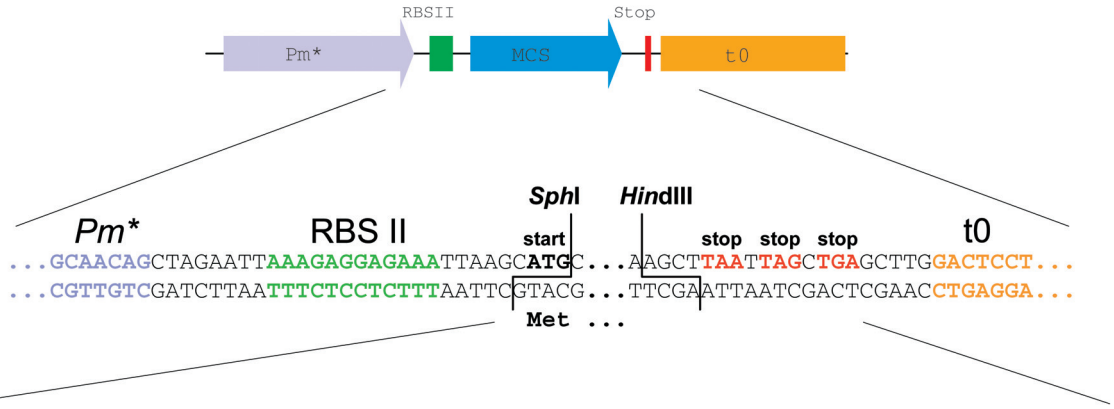
3. pALEX1 vectors.

3.1. General information.

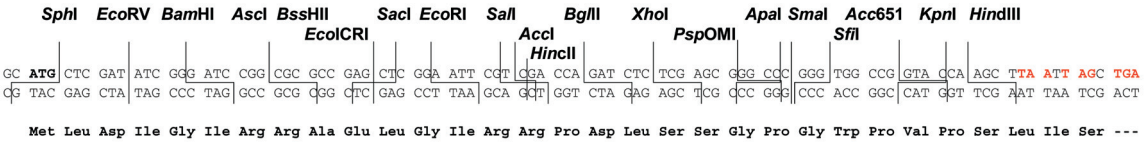
pALEX1a, b and c are highly improved *CASCADE™* expression vectors to be used in *E. coli* hosts strains bearing the salicylate-inducible *nahR/Psal::xylS2* regulatory module, such as REG1 or MEDAL-GOLD (Cat. No. BS-3262 and BS-3432). These new vectors are high-copy number, ampicilin-selectable plasmids that incorporate a mutant *Pm* promoter with even lower basal transcriptional activity, for tightly regulated and high capacity protein expression. Among other features, pALEX1a, b and c have an improved multiple cloning site (MCS), for easy and flexible cloning in-frame with an ATG start codon located at an optimal distance from a highly efficient ribosome binding site (RBSII), as well as stop codons in all three forward reading frames, followed by strong transcriptional terminator sequences (t0 and T1). In addition, the expression cassette in pALEX1a, b and c is flanked by "rare cutter" *NotI* sites, for convenient subcloning into mini Tn5 delivery vectors and construction of genetically stable expression strains.

3.2. pALEX1a, 1b and 1c maps.

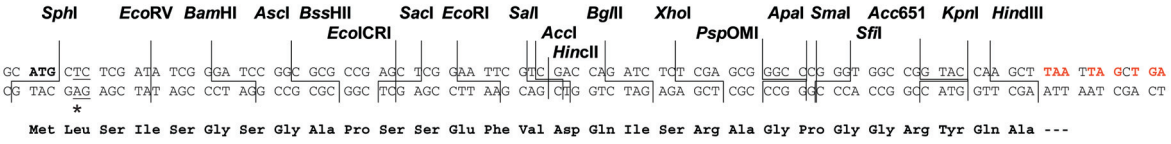




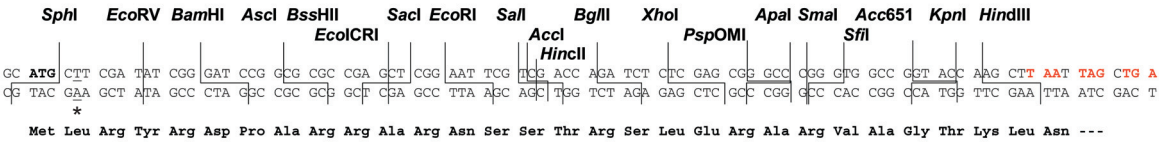
pALEX1a



pALEX1b



pALEX1c



*Variable region in pALEX1b and pALEX1c vectors

4. Protocol.

4.1. Cloning into pALEX1 vectors and host strain transformation.

To facilitate cloning strategies, the MCSs of pALEX1a, b and c contain unique recognition sites for a wide variety of restriction enzymes. Assembly of a construct leading to expression of the desired protein will require using the appropriate restriction enzymes, to insert the coding sequence in-frame with the ATG start codon in the MCS. The pALEX1a, b and c vectors differ in one, or two base-pair insertions that introduce frame shifts in the MCS region located between the *Sph*I and *Eco*RV sites, simplifying in-frame cloning by choosing the appropriate pALEX1 vector. Expression of the resulting protein can be carried out in one of the *CASCADE*^{TM} bacterial hosts (*E. coli* REG-1, Cat. No. BS-3262 or *E. coli* MEDAL GOLD, Cat. No. BS-3432). Biomedal recommends TSS solution (Cat. No. RS-3215/16), as a fast and simple method to prepare transformation-competent bacteria (for more information, visit our website at www.biomedal.com)

4.2. Protein expression.

Optimal expression conditions should be determined for each particular recombinant protein with small scale cultures before attempting large scale expression procedures. Expression can be optimized by varying inducer concentration (0.01 to 2 mM), temperature (22 – 37°C) and time of induction (4 h – overnight). The following can be a start point expression protocol:

1. Inoculate 5 ml of liquid culture medium, containing 100 µg/ml ampicillin, with a single, freshly transformed colony. Incubate this pre-culture overnight at 37 °C with shaking (200 r.p.m.).

2. Dilute pre-culture 1:100 in fresh, ampicillin-containing medium and continue incubation in the same conditions until OD_{600} reaches 0.7 – 1.0.

3. Add the inducer (salicylate) to a final concentration of 2 mM. Incubate 5 h at 30°C with shaking (200 r.p.m.).

4. Harvest cells by centrifugation at 4000 xg for 15 min at 4°C. Disrupt cells for protein extraction and analysis, or store pellet at -20°C until use.

5. Storage Conditions.

Store at 4°C. For long periods, it is recommended store at -20°C.

For more information,
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