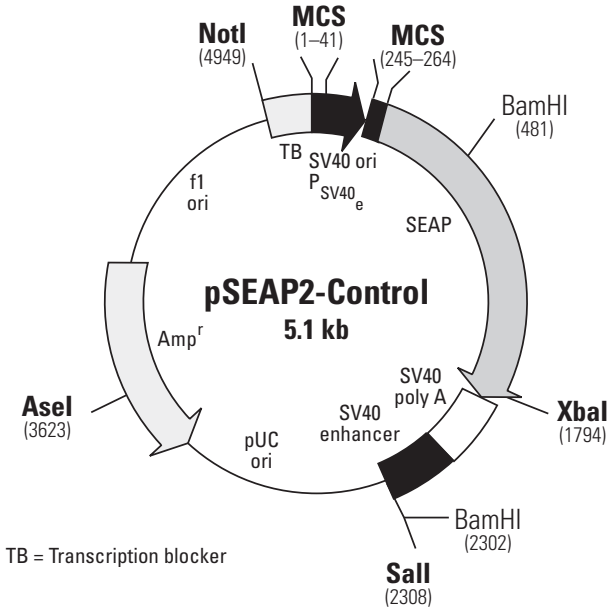
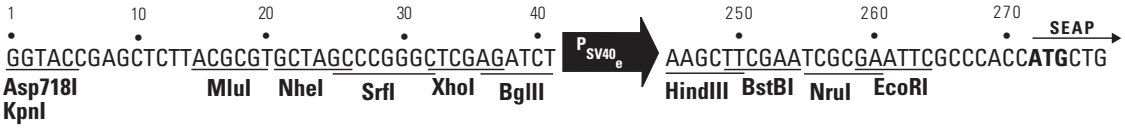
Vector Information

pSEAP2-Control Vector Information PT3074-5

GenBank Accession No: U89938 Cat. Nos. 631717 & 631735





Restriction Map and Multiple Cloning Site (MCS) of pSEAP2-Control. Unique restriction sites are in bold.

Description

pSEAP2-Control is a positive control vector expressing secreted alkaline phosphatase (SEAP) under the control of the SV40 early promoter and the SV40 enhancer. The SEAP coding sequence is followed by the SV40 late polyadenylation signal to ensure proper, efficient processing of the SEAP transcript in eukaryotic cells. A synthetic transcription blocker (TB), composed of adjacent polyadenylation and transcription pause sites, located upstream of the MCS reduces background transcription (1). The vector backbone also contains an f1 origin for single-stranded DNA production, a pUC origin of replication, and an ampicillin resistance gene for propagation and selection in *E. coli*. The SEAP2 Vectors incorporate a number of features that improve the sensitivity of SEAP by increasing the efficiency of SEAP expression or that enhance the utility of the vectors. These include: an improved Kozak consensus translation initiation site (2); the removal of the SV40 small-t intron, which can cause cryptic splicing and reduced expression in some genes and/or cell types (3, 4); switching from the early to late polyadenylation signal of SV40, which typically causes a five-fold increase in mRNA levels (5); an expanded multiple cloning site (MCS); compact plasmid size; and removal of extraneous sequences from the 3' untranslated region of the SEAP mRNA.

Use

The integrated set of Great EscAPe™ SEAP2 Vectors has been designed to provide maximal flexibility in studying regulatory sequences from the gene of interest. pSEAP2-Control expresses SEAP in most cell types and provides an important control. The secreted SEAP enzyme is assayed directly from the culture medium and permits time-course studies not possible with assays dependent on cell lysates. Furthermore, the cells can be used for further investigations such as RNA or protein studies. The SEAP2 Vectors can be transfected into mammalian cells by any standard method.

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Location of features

- Multiple Cloning Sites: 1–41 & 245–264
- SV40 early promoter:
  - Promoter insert: 37–245
  - 21 bp repeats: 75–95, 96–116 & 118–138
  - Early promoter element: 151–157
  - Major transcription start points: 147, 185, 191 & 196
- SV40 origin of replication: 95–230
- Secreted alkaline phosphatase (SEAP) gene:
  - Kozak consensus translation initiation signal: 267–274
  - SEAP coding sequences:
    - start codon (ATG): 272–274; stop codon: 1829–1831
    - signal peptide: 272–322
    - mature protein: 323–1828
    - C-terminal extension to SEAP: 1790–1828
- SV40 late mRNA polyadenylation signal: 1942–1947
  - mRNA 3' end: 1961
- SV40 enhancer:
  - Enhancer insert: 2056–2301
  - 72-bp tandem repeats: 2134–2205 & 2206–2277
  - 21-bp repeats: 2067–2087, 2089–2109 & 2110–2130
- pUC plasmid replication origin: 2586–3229
- Ampicillin resistance gene:
  - Promoter: –35 region: 4307–4302; –10 region: 4284–4279
  - Transcription start point: 4272
  - Ribosome binding site: 4249–4245
  - β-lactamase coding sequences:
    - start codon (ATG): 4237–4235; stop codon: 3379–3377
  - β-lactamase signal peptide: 4237–4169
  - β-lactamase mature protein: 4168–3380
- f1 single-strand DNA origin (packages the coding strand of SEAP): 4369–4824
- Transcription blocker (TB): 4955–5108
  - Synthetic polyadenylation site (6): 4955–5003
  - Transcription pause site from human α2 globin gene (7): 5017–5108

Recommended sequencing primers

5′ of MCS: 5057–5076 (5'-CTAGCAAAATAGGCTGTCCC-3')

3′ of MCS: 377–357 (5'-CCTCGGCTGCCTCGCGGTTCC-3')

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains. Single-stranded DNA production requires a host containing an F' episome such as JM109.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtai ned by Clontech. This vector has not been completely sequenced.

References

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6. Levitt, N. *et al*. (1989) *Genes Dev.* **3**:1019–1025.
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