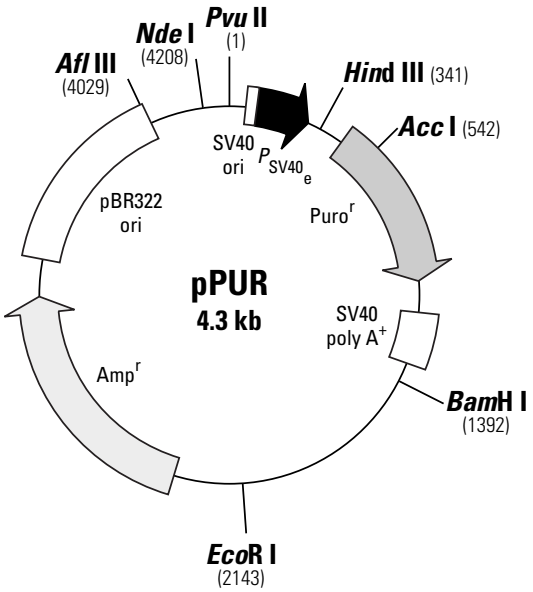
Vector Information

pPur Vector Information

PT2031-5

Cat. No. 631601



Restriction Map of pPur Vector. All restriction sites shown are unique.

Description:

pPUR is a selection vector that confers puromycin resistance to eukaryotic cells. The puromycin resistance gene of pPUR can be used as a dominant selectable marker to select for stably transformed mammalian cell lines in a manner analogous to that using neomycin (G418) or hygromycin resistance genes (1). In pPUR (referred to as pBSpacΔP in reference 2), the *Streptomyces alboniger* puromycin-N-acetyl-transferase (*pac*) gene, or Puro^r, is cloned between the SV40 early promoter and polyadenylation signals to create a cassette that will be expressed in mammalian cells. Cells expressing *pac* are resistant to the antibiotic puromycin (Cat. No. 631305). pPUR has the pBR322 origin of replication and ampicillin resistance gene for propagation and selection in *E. coli.*

Use:

pPUR, or the puromycin resistance gene from pPUR, can be used in a variety of ways:

- pPUR can be cotransfected with another vector expressing your gene of interest to select for integration of both DNA molecules.
- An expression cassette, or other DNA, can be inserted into pPUR using the *Bam*H I, *Eco*R I, *Nde* I, or *Pvu* II restriction sites. Successful transfectants containing the modified vector can be selected for puromycin resistance.
- The puromycin resistance cassette can be excised, using the *Nde* I and *Bam*H I or the *Pvu* II and *Bam*H I sites, and inserted into another vector. The resulting construct can then be used to confer puromycin resistance in transformed cells.
- The *pac* gene can be used as a reporter gene using an assay analogous to the CAT assay (1).

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Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Location of features:
- SV40 origin of replication: 190–325
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 23–94 & 95–166
  - 21-bp repeats: 170–190, 191–211 & 213–233
  - Early promoter element: 246–252
  - Major transcription start points: 242, 280, 286 & 291
- Puromycin resistance gene
  - Puromycin-N-acetyl-transferase (*pac*) coding sequences:
  - Start codon (ATG): 432–434; stop codon: 1029–1031
- Polyadenylation signal
  - SV40 early mRNA polyadenylation signals: 1292–1297 & 1321–1326
  - mRNA 3' ends: 1330 & 1342
- Ampicillin resistance (β-lactamase) gene
  - Promoter: –35 region: 2283–2288; –10 region: 2306–2311
  - Transcription start point: 2318
  - Ribosome binding site: 2341–2345
  - β-lactamase coding sequences: start codon (ATG): 2353–2355; stop codon: 3211–3213
  - β-lactamase signal peptide: 2353–2421
  - β-lactamase mature protein: 2422–3210
- pBR322 plasmid replication origin: 3361–4004

Propagation in *E. coli:*
- Suitable host strains: DH5α, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pBR322 (rop^-)
- Copy number: ~100–200
- Plasmid incompatibility group: pMB1/Col E1

Selection marker in mammalian hosts:

Plasmid confers resistance to puromycin (2.5–10 µg/ml) to mammalian host cells.

References:

1. de la Luna, S. & Ortin, J. (1992) *Methods Enzymol.* **216**:376–385.
2. de la Luna, S., *et al.* (1988) *Gene* **62**:121–126.

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