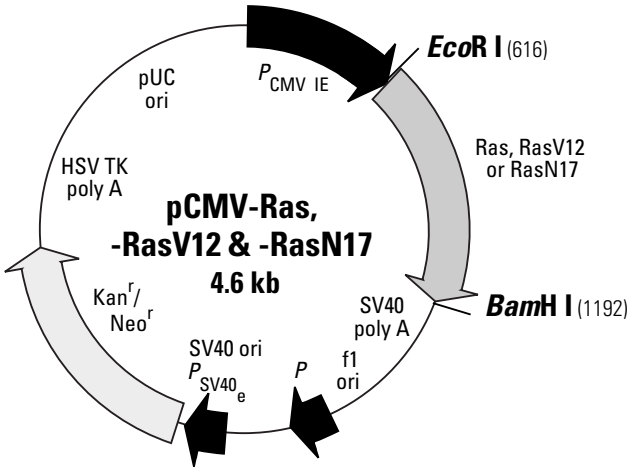
Vector Information

Ras Dominant-Negative Vector Set Information PT3456-5

Cat. No. 631924



Combined map of pCMV-Ras, pCMV-RasV12 & pCMV-RasN17 Vectors. All restriction sites shown are unique. The Ras Dominant-Negative Vector Set includes three vectors; each vector contains a different Ras coding sequence, as shown

Description

The Ha-ras (p21) oncogene regulates multiple signal transduction pathways including MAPK, the most widely studied Ras-dependent pathway. Ras transduces extracellular signals by binding to effector proteins, like Raf-1, MEKK1, and PKCζ (1). However, the direct effect of Ras binding to various effectors is not well understood. The Ras Dominant-Negative Vector Set provides convenient tools for studying Ras-related signal transduction pathways. The set consists of three vectors:

- **pCMV-Ras Vector**—constitutively expresses the wild-type (wt) Ha-ras protein.
- **pCMV-RasV12 Vector**—expresses a constitutively active form of the Ras protein that contains a glycine-to-valine mutation at residue 12.
- **pCMV-RasN17 Vector**—expresses a dominant-negative form of the Ras protein that contains a serine-to-asparagine mutation at residue 17. Expression of this mutant Ras variant will "knock out" endogenous Ras expression in mammalian cells.

These proteins are expressed at high levels from the constitutive CMV promoter. The SV40 polyadenylation sequence directs proper processing of the 3' end of the mRNAs. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neo^r)—consisting of the SV40 early promoter, the Tn5 neomycin/kanamycin resistance gene, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene—allows kanamycin selection in *E. coli* and neomycin selection in eukaryotic cells. The vector backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

These vectors can be transfected into mammalian cells using any standard method. Stable transformants can be selected using G418 (2).

The Ras Dominant-Negative Vector Set can be used with our Mercury™ *cis*-acting reporter vectors, such as pSRE-SEAP (Cat. No. 631901). This combination allows you to set up a complete assay system to measure differences in activation of the Ras pathway. For more information about our Mercury™ Pathway Profiling Vectors, visit our web site at **www.clontech.com**.

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Note: The following list of features is based on the pCMV-Ras Vector. Complete sequence and restriction digest information for all of these vectors are available at **vectors.clontech.com.**

Location of Features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
  Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- Ras variant (each vector contains one of the following):
  wild-type Ras only: Start codon (ATG): 621–623; stop codon: 1188–1190
  RasV12 only: Start codon (ATG): 621–623; stop codon: 1188–1190
  G→T (gly-to-val) mutation: 655
  RasN17 only: Start codon (ATG): 621–623; stop codon: 1188–1190
  G→A (ser-to-asn) mutation: 670
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1351–1355 & 1380–1384; mRNA 3' ends: 1389 & 1401
- f1 single-strand DNA origin: 1448–1903 (Packages the noncoding strand of Ras.)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 1965–1970; –10 region: 1988–1993
  Transcription start point: 2000
- SV40 origin of replication: 2244–2321
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2077–2148 & 2149–2222
  21-bp repeats: 2224–2244, 2245–2265 & 2267–2287
  Early promoter element: 2300–2306
  Major transcription start points: 2296, 2334, 2340 & 2345
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences: start codon (ATG): 2428–2430; stop codon: 3220–3222
  G→A mutation to remove *Pst* I site: 2610
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 2956
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3458–3463 & 3471–3476
- pUC plasmid replication origin: 3812–4450

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Katz, M. & McCormic, F. (1997) *Curr. Opin. Genet. Devel.* **7**:75–79.
2. Gorman, C. (1985) In *DNA cloning: A practical approach, Vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, UK) pp. 143–190.

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.

Related Products

- Mercury Pathway Profiling SEAP System (Cat. No. 631910)
- Mercury Pathway Profiling Luciferase System (Cat. No. 631911)
- pSRE-SEAP (Cat. No. 631901)