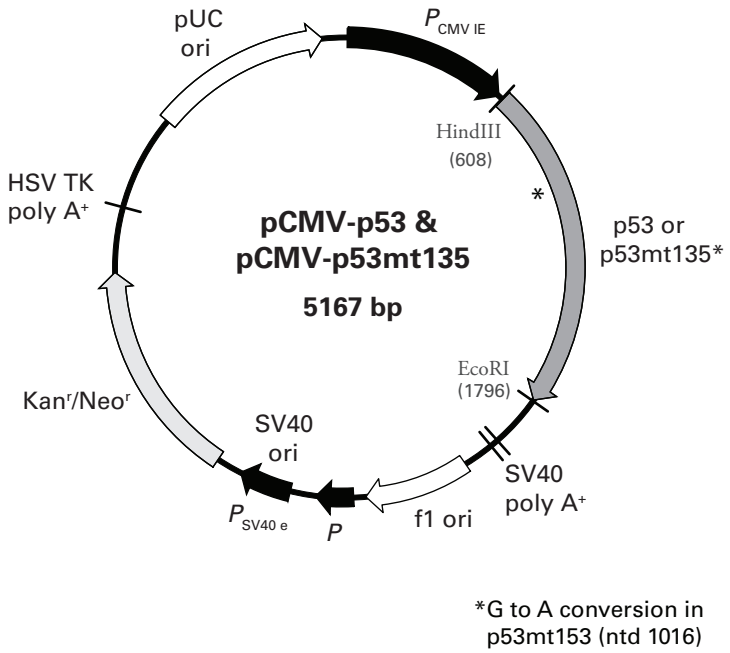
Vector Information

**p53 Dominant-Negative Vector Set Information** PT3348-5

Catalog No. 631922




*G to A conversion in p53mt153 (ntd 1016)

**Combined map of pCMV-p53 and pCMV-p53mt135.** All restriction sites shown are unique. The p53 Dominant-Negative Vector Set includes two vectors; each vector contains a different p53 coding sequence, as indicated above.

## Description

The p53 Dominant-Negative Vector Set is a convenient tool for monitoring p53-mediated signal transduction pathways. This vector set consists of two vectors, pCMV-p53 and pCMV-p53mt135. pCMV-p53 expresses the wild-type p53 tumor suppression protein and pCMV-p53mt135 expresses a dominant-negative mutant. Both proteins are expressed at high levels from the constitutive CMV promoter. The p53 gene and p53mt135 gene differ by a G to A conversion at nucleotide 1017.

The SV40 polyadenylation sequence directs proper processing of the 3' end of the p53 or p53mt135 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

pCMV-p53 is used to examine the physical interactions between p53 and other proteins, to induce p53-mediated cell cycle arrest, or to study p53-induced gene expression. pCMV-p53mt135 expresses the p53mt135 mutant, which because of a conformational change, can no longer interact with p53-binding sites. When p53mt135 and p53 are co-expressed, they form a mixed tetramer that is unable to interact with p53-binding sites; therefore, the downstream effects of p53 are blocked (1, 2).

Both vectors can be transfected into mammalian cells using any standard method. Stable transformants can be selected using G418 (3).

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**Note:** The following list of features is based on the pCMV-p53 Vector. Complete sequence and restriction digest information for all of these vectors are available at **http://www.clontech.com/support/vectors.asp.**

## Location of Features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
  Transcription start point: 583
  C to G mutation to remove *Sac* I site: 569
- p53 or p53mt135 gene: 613–1791
  **p53mt135 only:** G to A mutation: 1016
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1986–1991 & 2016–2020; mRNA 3' ends: 2024 & 2036
- f1 single-strand DNA origin: 2083–2538 (Packages the noncoding strand of p53 & p53mt135.)
- Bacterial promoter for expression of $Kan^r$ gene
  –35 region: 2600–2605; –10 region: 2623–2628
  Transcription start point: 2635
- SV40 origin of replication: 2879–3014
- SV40 early promoter
  Enhancer (72 bp tandem repeats): 2712–2783 & 2784–2855
  21 bp repeats: 2859–2879, 2880–2900 & 2902–2922
  Early promoter element: 2935–2941
  Major transcription start points: 2931, 2969, 2975 & 2980
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences: start codon (ATG): 3063–3065; stop codon: 3855–3857
  G to A mutation to remove *Pst* I site: 3245
  C to A (Arg to Ser) mutation to remove *Bss*H II site: 3591
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 4093–4098 & 4106–4111
- pUC plasmid replication origin: 4442–5085

## 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) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

## References

1. Scheffner, M., *et al.*, (1992) *J. Virol.* **66**(8):5100–5104.
2. Vogelstein, B. & Kinzler, K. W. (1992) *Cell* **70**(4):523–526.
3. Gorman, C. (1985) In *DNA cloning: A practical approach, Vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, UK) pp. 143–190.

## Note

The vector sequence was 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.