Technical Manual

# pCAT®3 Reporter Vectors

INSTRUCTIONS FOR USE OF PRODUCTS E1851, E1861, E1871 AND E1881.

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# pCAT®3 Reporter Vectors

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## 1. Description

The pCAT®3 Reporter Vectors provide a basis for the quantitative analysis of factors that potentially regulate mammalian gene expression. These factors may be *cis*-acting, such as promoters and enhancers, or *trans*-acting, such as various DNA-binding factors. The backbone of the pCAT®3 Reporter Vectors (1) is similar to the pGL3 Luciferase Vectors, with the exception of an intron located 5´ of the chloramphenicol acetyltransferase (CAT) gene. The vector backbones were designed to increase expression of the reporter gene for easier monitoring of transcriptional activity in transfected eukaryotic cells. In addition, the pCAT®3 Reporter Vectors contain numerous features that aid in the structural characterization of putative regulatory sequences.

For peer-reviewed articles that cite use of the pCAT®3 Vectors, visit:
**www.promega.com/citations/**

## 2. Product Components and Storage Conditions

| Product | Size | Cat.# |
|---|---|---|
| pCAT®3-Control Vector | 20µg | E1851 |
| pCAT®3-Promoter Vector | 20µg | E1861 |
| pCAT®3-Basic Vector | 20µg | E1871 |
| pCAT®3-Enhancer Vector | 20µg | E1881 |

Information on related products, including the CAT Assay System, is provided in Section 11.E.

**Storage Conditions:** Store the Vector DNA at –20°C.

## 3. General Considerations

### 3.A. Common Structural Elements of the pCAT®3 Reporter Vectors

Except for the inclusion of promoters and enhancers, the four pCAT®3 Reporter Vectors are structurally identical. The distinguishing features of each vector are summarized in Section 3.C. The pCAT®3 Vectors each contain a high-copy-number replicon for maintenance in *E. coli*, an ampicillin-resistance gene for selection, and a filamentous phage origin of replication (f1 ori) for single-stranded DNA (ssDNA) production. Restriction sites for insertion of DNA fragments are located upstream and downstream of the CAT gene.

### 3.B. Advantages of the pCAT®3 Vectors

The pCAT®3 Reporter Vector family provides significant advances over the first generation of pCAT® Reporter Vectors (Table 1). The pCAT®3 Reporter Vectors contain a redesigned vector backbone, which increases CAT expression, improves in vivo vector stability, provides greater flexibility in performing genetic manipulations and facilitates transfer of cloned fragments between the pCAT®3 Vectors and pGL3 Luciferase Reporter Vectors. The modifications in the reporter vectors result in CAT expression levels higher than those obtained with the first generation of pCAT® Reporter Vectors, while maintaining relatively low background CAT expression.

The increase in the CAT expression observed with the pCAT®3 Vectors provides greater sensitivity. It may now be possible to obtain measurable CAT expression in cell types that are difficult to transfect or when studying weak promoter elements. However, relative expression profiles may vary between cell types (1). Therefore, it is important to include the appropriate control vectors in all experiments.

**Table 1. Characteristics of the pCAT®3 Vectors.**

| Feature | Purpose of Feature |
|---|---|
| Poly(A) signal derived from the late SV40 poly(A) signal. | Late SV40 poly(A) signal is more efficient than the early SV40 poly(A) (2). |
| Synthetic poly(A) transcriptional pause site located 5′ of the multiple cloning site. | Reduces background CAT expression while avoiding possible recombination between two SV40 poly(A) sequences in the same plasmid (3,4). |
| Chimeric intron located 5′ of the CAT gene.* | The SV40 small-t antigen intron in the first generation of pCAT® Vectors reduced expression when placed 3′ of the CAT gene due to cryptic splicing (5–7). |
| Kozak consensus sequence located at the 5′ end of the CAT gene. | Provides for optimal translation efficiency (8). |
| Multiple cloning region. | Increases convenience and provides compatibility with the pGL3 Vectors. |
| f1 ori site in the vector backbone. | Provides the ability to generate ssDNA for sequencing or mutagenesis. |
| Unique NcoI site in the Kozak sequence. | Facilitates subcloning. |
| Unique XbaI site downstream of the CAT gene. | Facilitates subcloning. |
| An EcoRI site has been removed from the CAT gene, resulting in the amino acid change Phe73→Leu73. | Facilitates subcloning. |

***Note:** The chimeric intron is composed of the donor site from the first intron of the human β-globin gene and the branch and acceptor site from the intron of an immunoglobulin gene (9). The sequences of the donor and acceptor sites, along with the branchpoint site, have been optimized to match the consensus sequence (10).

While the chimeric intron placed 5′ of the CAT gene increases CAT expression, its location 5′ of the gene may also result in spurious transcription in some cell lines. We have removed some of the known potential regulatory sequences from the intron; however, any unidentified regulatory sequences still present within the vector can lead to increased background CAT expression. The user should recognize this possibility and use the proper experimental controls. If the intron is not required for increased expression, it is conveniently flanked by HindIII sites for easy removal from the pCAT®3 Vectors.

## 3.C. Distinguishing Features of the pCAT®3 Reporter Vectors

Maps of the pCAT®3-Basic, Enhancer, Promoter and Control Vectors are shown in Figures 1–4. Sequence accession numbers and information on restriction enzyme sites present in these vectors are provided in Section 11.

### pCAT®3-Basic Vector

The pCAT®3-Basic Vector lacks eukaryotic promoter and enhancer sequences, allowing maximum flexibility in cloning putative regulatory sequences. Expression of CAT activity in cells transfected with this plasmid depends on insertion and proper orientation of a functional promoter upstream from the intron and the CAT gene. Potential enhancer elements can also be inserted upstream of the promoter or in the BamHI or SalI sites downstream of the CAT transcription unit.

### pCAT®3-Enhancer Vector

The pCAT®3-Enhancer Vector contains an SV40 enhancer located downstream of the CAT gene and the poly(A) signal. This aids in the verification of functional promoter elements because the presence of an enhancer will often result in transcription of the CAT gene at higher levels.

### pCAT®3-Promoter Vector

The pCAT®3-Promoter Vector contains an SV40 promoter upstream of the intron and the CAT gene. DNA fragments containing putative enhancer elements can be inserted either upstream or downstream of the promoter-CAT transcriptional unit.

### pCAT®3-Control Vector

The pCAT®3-Control Vector contains SV40 promoter and enhancer sequences, resulting in strong expression of CAT in many mammalian cell types. The pCAT®3-Control Vector is useful for monitoring transfection efficiency, and provides a convenient standard for comparing promoter and enhancer activities expressed by pCAT®3 recombinants.

**Note:** The specific transcriptional characteristics of the pCAT®3 Vectors will vary for different cell types. This may be particularly true for COS cells, which contain the SV40 large-T antigen. The SV40 large-T antigen promotes replication from the SV40 origin, which is found in the promoter of the pCAT®3-Promoter and pCAT®3-Control Vectors. The combination of large-T antigen and SV40 origin will result in a higher copy number of these vectors in COS cells, which in turn may result in increased expression of the reporter gene compared to other cell and vector combinations.

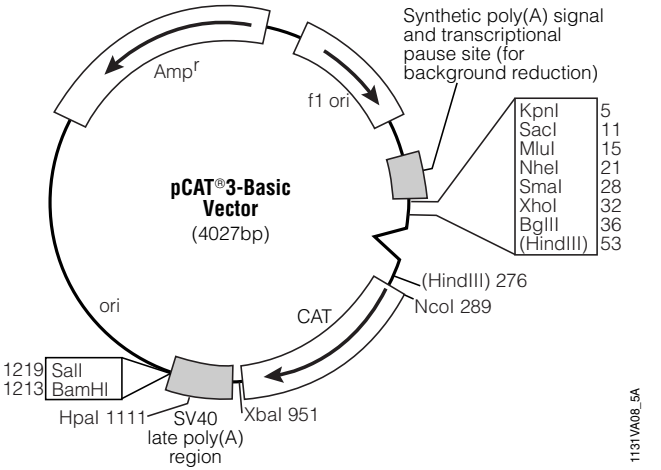


**Figure 1. The pCAT®3-Basic Vector circle map.** –^–, position of intron; CAT, cDNA encoding the chloramphenicol acetyltransferase gene; Amp$^r$, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli*. Arrows within CAT and the Amp$^r$ genes indicate the direction of transcription; the arrow in f1 ori indicates the direction of ssDNA strand synthesis. Restriction sites shown in parentheses are not unique sites.

**Sequence reference points:**

| | |
|---|---|
| SV40 Promoter | (none) |
| SV40 Promoter-directed transcriptional start sites | (none) |
| SV40 Enhancer | (none) |
| SV40 late poly(A) region | 981–1202 |
| CAT gene | 291–947 |
| chimeric intron | 103–235 |
| upstream poly(A) region | 3867–4020 |
| multiple cloning region | 1–58 |
| RVprimer3 binding site | 3969–3988 |
| RVprimer4 binding site | 1289–1270 |
| β-lactamase gene (Amp$^r$) | 3149–2292 |
| f1 origin | 3282–3736 |
| *Col*E1-derived plasmid replication origin | 1527 |

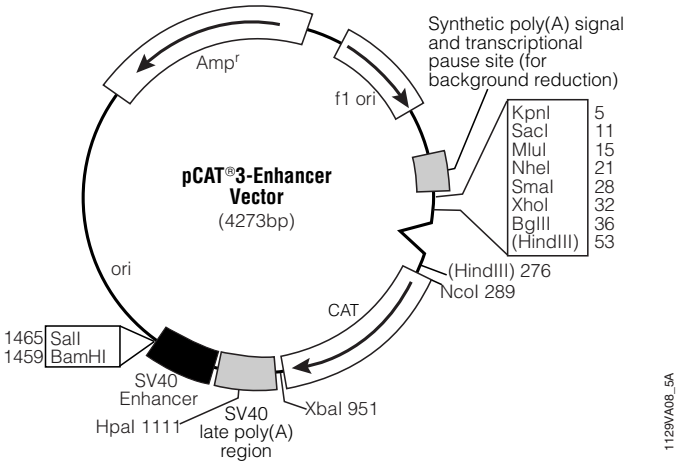


**Figure 2. The pCAT®3-Enhancer Vector circle map.** –^–, position of intron; CAT, cDNA encoding the chloramphenicol acetyltransferase gene; Amp$^r$, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli*. Arrows within CAT and the Amp$^r$ genes indicate the direction of transcription; the arrow in f1 ori indicates the direction of ssDNA strand synthesis. Restriction sites shown in parentheses are not unique sites.

**Sequence reference points:**

| | |
|---|---|
| SV40 Promoter | (none) |
| SV40 Promoter-directed transcriptional start sites | (none) |
| SV40 Enhancer | 1222–1458 |
| SV40 late poly(A) region | 981–1202 |
| CAT gene | 291–947 |
| chimeric intron | 102–234 |
| upstream poly(A) region | 4113–4266 |
| multiple cloning region | 1–58 |
| RVprimer3 binding site | 4215–4234 |
| RVprimer4 binding site | 1535–1516 |
| β-lactamase gene (Amp$^r$) | 3395–2538 |
| f1 origin | 3528–3982 |
| *Col*E1-derived plasmid replication origin | 1773 |

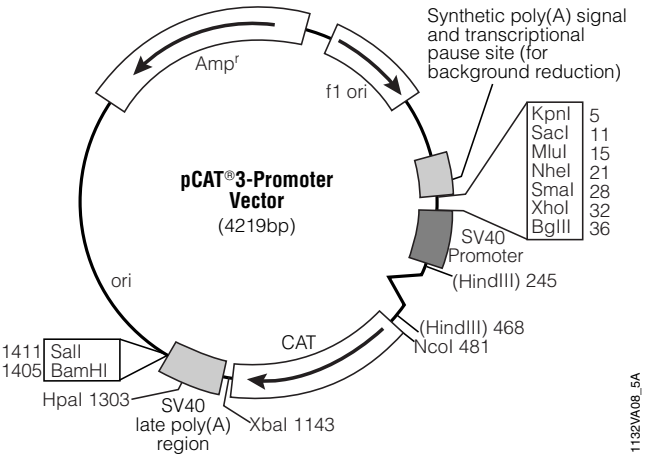


**Figure 3. The pCAT®3-Promoter Vector circle map.** –^–, position of intron; CAT, cDNA encoding the chloramphenicol acetyltransferase gene; Amp$^r$, gene conferring ampicillin resistance in *E. coli;* f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli.* Arrows within CAT and the Amp$^r$ genes indicate the direction of transcription; the arrow in f1 ori indicates the direction of ssDNA strand synthesis. Restriction sites shown in parentheses are not unique sites.

**Sequence reference points:**

| | |
|---|---|
| SV40 Promoter | 48–250 |
| SV40 Promoter-directed transcriptional start sites | 185, 191, 196 |
| SV40 Enhancer | (none) |
| SV40 late poly(A) region | 1173–1394 |
| CAT gene | 483–1139 |
| chimeric intron | 295–427 |
| upstream poly(A) region | 4059–4212 |
| multiple cloning region | 1–41 |
| RVprimer3 binding site | 4161–4180 |
| RVprimer4 binding site | 1481–1462 |
| β-lactamase gene (Amp$^r$) | 3341–2484 |
| f1 origin | 3474–3928 |
| *Col*E1-derived plasmid replication origin | 1719 |

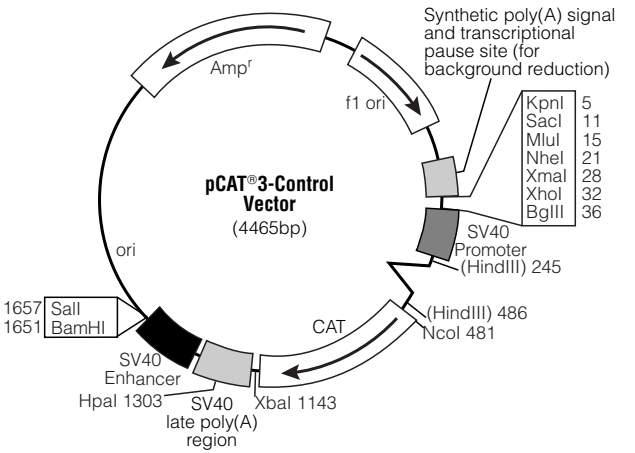


**Figure 4. The pCAT®3-Control Vector circle map.** –^–, position of intron; CAT, cDNA encoding the chloramphenicol acetyltransferase gene; Amp$^r$, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli*. Arrows within CAT and the Amp$^r$ genes indicate the direction of transcription; the arrow in f1 ori indicates the direction of ssDNA strand synthesis. Restriction sites shown in parentheses are not unique sites.

**Sequence reference points:**

| | |
|---|---|
| SV40 Promoter | 48–250 |
| SV40 Promoter-directed transcriptional start sites | 185, 191, 196 |
| SV40 Enhancer | 1414–1650 |
| SV40 late poly(A) region | 1173–1394 |
| CAT gene | 483–1139 |
| chimeric intron | 295–427 |
| upstream poly(A) region | 4305–4458 |
| multiple cloning region | 1–41 |
| RVprimer3 binding site | 4407–4426 |
| RVprimer4 binding site | 1727–1708 |
| β-lactamase gene (Amp$^r$) | 3587–2730 |
| f1 origin | 3720–4174 |
| *Col*E1-derived plasmid replication origin | 1965 |

### 3.D. CAT Assay

Chloramphenicol acetyltransferase (CAT), encoded by a bacterial drug-resistance gene, inactivates chloramphenicol by acetylating the drug at one or both of its two hydroxyl groups (11). This gene is not found in eukaryotes, and therefore eukaryotic cells contain no background CAT activity. This characteristic, along with assay sensitivity, has made the CAT gene a good reporter for studies of mammalian gene expression (12,13).

### 3.E. Mapping Genetic Elements Located Within DNA Fragments

The locations of functional elements within a DNA fragment are often determined by making a set of unidirectional nested deletions following the method of Henikoff (14) and then assaying for changes in biological activity. This method takes advantage of the unique properties of Exonuclease III (ExoIII), which will digest 5´ overhangs but not 3´ overhangs or α-phosphorothioate nucleotide filled-in overhangs. Nested deletions of an insert DNA can be made directly in the pCAT®3 family of reporter vectors using this method, eliminating the need for subcloning steps. The multiple cloning site of the pCAT®3 Vectors contains upstream KpnI and SacI restriction sites, which can be used to generate the 3´ overhangs resistant to ExoIII (Figures 1–5). After treatment with ExoIII, S1 Nuclease is added to remove the resulting ssDNA overhangs, Klenow Fragment is added to flush the ends, and the ends are ligated to circularize the vectors. Deletion clones can be screened by gel electrophoresis of miniprep DNA, and the precise deletion endpoints within the promoter region can be determined by DNA sequencing (see Section 9).

### 3.F. Site-Specific Mutagenesis of the DNA Fragments

Once a DNA fragment's biological activity has been identified, site-specific mutagenesis may be used to further define the sequences associated with the activity. To provide the ssDNA template necessary for some mutagenesis reactions, the pCAT®3 Reporter Vectors contain an origin of replication derived from filamentous phage. This allows single-stranded plasmid DNA to be produced and secreted in phage-like particles from *E. coli* infected with the appropriate helper phage.

Mutagenesis is performed using oligonucleotides that are complementary to the ssDNA but contain the desired changes to the nucleic acid sequence (15). The oligonucleotides are hybridized to the template DNA, and double-stranded DNA is synthesized using a DNA polymerase. After amplification of the DNA in *E. coli*, the mutations may be verified by DNA sequencing.

**Figure 5. pCAT®3 Vector multiple cloning regions.** Upstream and downstream cloning sites and the locations of sequencing primer (RVprimer3 and RVprimer4) binding sites are shown. The large primer arrows indicate the direction of sequencing. The positions of the promoter (in the pCAT®3-Promoter and pCAT®3-Control Vectors) and the enhancer (in the pCAT®3-Enhancer and pCAT®3-Control Vectors) are shown as insertions into the sequence of the pCAT®3-Basic Vector. (Note that the promoter replaces four bases [AAGT] of the pCAT®3-Basic Vector.) The sequence shown is of the DNA strand generated from the f1 ori.

## 4. Cloning Methods

### 4.A. Cloning Strategies

The restriction sites for XhoI and SalI have compatible ends, as do BglII and BamHI. Therefore, cloning into the XhoI or BglII sites upstream of CAT or the downstream SalI or BamHI sites allows easy positioning of DNA inserts either upstream or downstream of the CAT reporter gene. Thus, positional effects of a putative genetic element may be readily tested. Cloning fragments into a single site generally will yield both possible orientations relative to the reporter gene, making these effects also readily testable. It should be noted that the area encompassing the XhoI restriction site in the multiple cloning region exhibits considerable secondary structure when the plasmid is in a supercoiled configuration and, as a result, is resistant to digestion. We therefore recommend gel purifying the vector after digestion with XhoI to avoid an excessive number of background colonies.

The other upstream restriction sites may be used for cloning. However, note that some of the sites are required for generation of nested deletions (see Section 7). Specifically, the KpnI or SacI site is needed to generate a 3´ overhang upstream of the insert.

### 4.B. Preparation of pCAT®3 Vectors and Insert DNA for Cloning

The fragment and vector DNA should be digested with restriction enzymes that generate compatible ends for cloning. In most cases, the ends of the DNA fragment may require modification, either by using synthetic linkers, by using PCR primers containing recognition sites for the desired restriction enzymes, or by filling in restriction site overhangs. It may be advantageous to treat the vector DNA with Calf Intestinal Alkaline Phosphatase (CIAP) to remove 5´-phosphate groups, thus preventing recircularization of vector without insert. Sufficient DNA should be prepared to perform control reactions for digestion, ligation and transformation steps.

To ensure capture of the correct insert DNA, the desired restriction fragment can be purified by electrophoresis on an agarose gel and then recovered from the gel using the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281), or an equivalent method. Alternatively, unfractionated restriction fragments can be cloned into the target plasmid, and the desired recombinant identified by gel electrophoresis of plasmid DNA.

### 4.C. Transformation Protocols for pCAT®3 Vectors

Because the CAT Reporter Vectors are supplied as modified DNA, *E. coli* hosts may be either restriction+ or restriction-. Use of a *rec*A host such as JM109 is preferred to prevent undesirable recombination between the insert and the host chromosomal DNA. A strain that has an F´ episome is required for ssDNA production.

Grow JM109 on minimal plates (M-9) supplemented with thiamine HCl prior to preparation of competent cells and transformation. This selects for the F´ episome.

### 4.D. Isolation of Plasmid DNA

The Wizard® *Plus* SV Minipreps DNA Purification System (Cat.# A1340, A1470) may be used for small-scale preparation of plasmid DNA for screening clones. DNA suitable for transfection may be purified using the PureYield™ Plasmid Midipreps System (Cat.# A2492, A2495).

## 5. Transfection of Mammalian Cells

Transfection of DNA into eukaryotic cells may be mediated by cationic liposomes (16), calcium phosphate (13,17), DEAE-dextran (13,18), or electroporation (17). Transfection systems based on cationic lipids (TransFast™ Transfection Reagent, Transfectam® Reagent and Tfx™ Reagents) and calcium phosphate (Profection® Mammalian Transfection Systems) are available from Promega. For more information on these transfection reagents, please request the *TransFast™ Transfection Reagent Technical Bulletin* (#TB260), the *Transfectam® Reagent Technical Bulletin* (#TB116), the *Tfx™-Reagents Technical Bulletin* (#TB216) or the *ProFection® Mammalian Transfection Systems Technical Manual* (#TM012). All of these documents are available on our web site at: **www.promega.com/tbs/**

## 6. Assay of CAT Activity

For CAT transient expression assays, cell extracts are typically prepared 48–72 hours post-transfection. CAT activity may then be monitored by two alternative methods using the CAT Enzyme Assay System with Reporter Lysis Buffer (Cat.# E1000). The most rapid, sensitive, and convenient of these is based on liquid scintillation counting (LSC) of CAT reaction products. Cell extracts are incubated in a reaction mix containing $^{14}C$- or $^{3}H$-labeled chloramphenicol and n-Butyryl Coenzyme A. CAT transfers the n-butyryl moiety of the cofactor to chloramphenicol. For the LSC assay, the reaction products are extracted with a small volume of xylene. The n-butyryl chloramphenicol partitions mainly into the xylene phase, while unmodified chloramphenicol remains predominantly in the aqueous phase (19). The xylene phase is mixed with scintillant and counted in a scintillation counter. This assay can be completed in as little as 2–3 hours, is linear for nearly three orders of magnitude and can detect as little as $3 \times 10^{-4}$ units of CAT. CAT activity can also be analyzed using thin layer chromatography (TLC). This method is more time-consuming than LSC but allows visual confirmation of the data. For more information, see the *CAT Enzyme Assay System with Reporter Lysis Buffer Technical Bulletin* #TB084 (available at **www.promega.com/tbs/**).

## 7. Generation of Nested Deletions

Unidirectional deletions of any inserted DNA can be made using a procedure developed by Henikoff (14) in which Exonuclease III (ExoIII) is used to specifically digest insert DNA from a 5´-protruding or blunt-end restriction site. In the pCAT®3 Reporter Vectors, these 5´ overhangs or blunt ends are supplied by digesting the plasmid with BglII, MluI, NheI, XhoI or XmaI. When the plasmids are cut with KpnI or SacI, which yield 3´ overhangs, the ExoIII will be unable to digest in the other direction.

The uniform rate of enzyme digestion allows deletions of various lengths to be made simply by removing timed aliquots from the reaction. Given that small deletions (less than 500 bases) are probably desired, we recommend performing the reaction at a lower temperature (between 4–16°C). Samples from the ExoIII reaction are removed at timed intervals to tubes containing S1 nuclease, which removes the remaining single-stranded tails. The low pH and the presence of zinc cations in the S1 buffer effectively inhibit further digestion by ExoIII. After neutralization and heat inactivation of the S1 nuclease, Klenow is added to flush the ends, and the ends are ligated to circularize the vectors. The ligation mixtures are used directly to transform competent cells. Each successive timepoint yields a collection of subclones containing clustered deletions extending further into the original insert. For a more detailed protocol, please see the *Erase-a-Base® System Technical Manual* (#TM006; **www.promega.com/tbs/**).

## 8. Generation of Single-Stranded DNA and Site-Specific Mutations

### 8.A. Production of Single-Stranded DNA

To generate single-stranded DNA (ssDNA) from the pCAT®3 Vectors, bacterial cells containing pCAT®3 Vectors are infected with an appropriate helper phage. The plasmid then enters the f1 replication mode, and the resulting ssDNA is exported from the cell as an encapsulated phage-like particle. The single-stranded plasmid DNA is purified from the supernatant by simple precipitation and extraction procedures (20–22).

### 8.B. Generation of Site-Specific Mutations

Site-specific mutagenesis, as developed by Hutchinson *et al.* (15), is accomplished by hybridizing a synthetic oligonucleotide that is complementary to the single-stranded template except for a region of mismatch near the center. This region contains the desired nucleotide change or changes. Following hybridization to the single-stranded target DNA, the oligonucleotide is extended with DNA polymerase to create a double-stranded structure. The nick is then sealed with DNA ligase, and the duplex structure is transformed into an *E. coli* host. Theoretically, the yield of mutants using the Hutchinson procedure should be 50% (due to semi-conservative replication). In practice, however, the mutant yield may be much lower, often only a few percent or less. This is presumably due to factors such as incomplete in vitro polymerization, primer displacement by the DNA polymerase used in the fill-in reaction, and in vivo host-directed mismatch repair mechanisms, which favor repair of the unmethylated newly synthesized DNA strand. Because of the low mutant yield, methods have been developed to increase the mutation frequency (23).

## 9. Sequencing of pCAT®3 Reporter Vectors

It may be desirable to sequence the DNA inserted into the CAT® 3 Reporter Vectors. Two examples of such applications are to determine the exact position of generated deletions (Section 7) and to confirm production of a site-specific mutation (Section 8). Two primers are available for sequencing the pCAT®3 Vectors: RVprimer3 (Reporter Vector Primer 3) for sequencing clockwise across the upstream cloning sites and RVprimer4 for sequencing counterclockwise across the BamHI and SalI cloning sites downstream of CAT.

| | |
|---|---|
| RVprimer3 | 5´-CTAGCAAAATAGGCTGTCCC-3´ |
| RVprimer4 | 5´-GACGATAGTCATGCCCCGCG-3´ |

RVprimer3 is especially useful for identifying positions of nested deletions.

**Note:** Both primers can be used for dsDNA sequencing, but only the RVprimer4 may be used for ssDNA sequencing.

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## 11. Appendix

### 11.A. pCAT®3-Basic Vector Restriction Sites

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3´-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. The pCAT®3-Basic Vector sequence is available in the GenBank® database (GenBank®/EMBL Accession Number **U57024**) and at **www.promega.com/vectors/**

**Table 2. Restriction Enzymes That Cut the pCAT®3-Basic Vector 1–5 Times.**

| Enzyme | # of Sites | Location |
|---|---|---|
| **AccB7I** | 1 | 736 |
| **AccI** | 1 | 1220 |
| **AccIII** | 1 | 500 |
| **Acc65I** | 1 | 1 |
| AcyI | 1 | 2899 |
| AflII | 2 | 66, 263 |
| AflIII | 2 | 15, 1469 |
| **Alw26I** | 5 | 128, 153, 728, 2423, 3199 |
| **Alw44I** | 2 | 1783, 3029 |
| AlwNI | 1 | 1885 |
| AspHI | 4 | 11, 1787, 2948, 3033 |
| **AvaI** | 2 | 26, 32 |
| **AvaII** | 2 | 2500, 2722 |
| **BalI** | 1 | 771 |
| **BamHI** | 1 | 1213 |
| **BanII** | 3 | 11, 33, 3440 |
| BbsI | 2 | 174, 1298 |
| **BglI** | 2 | 2482, 3750 |
| **BglII** | 1 | 36 |
| BsaI | 2 | 128, 2423 |
| BsaAI | 1 | 3511 |
| BsaBI | 1 | 1212 |
| BsaHI | 1 | 2899 |
| **BsaMI** | 4 | 497, 904, 1032, 1125 |
| BsmI | 4 | 497, 904, 1032, 1125 |
| BspHI | 2 | 2189, 3197 |
| BspMI | 2 | 90, 3990 |
| BssSI | 2 | 1642, 3026 |
| **Bst98I** | 2 | 66, 263 |
| **BstZI** | 5 | 60, 269, 964, 968, 3860 |
| Cfr10I | 4 | 966, 1337, 2442, 3406 |
| **ClaI** | 3 | 1206, 3918, 4022 |
| DraIII | 1 | 3514 |
| DrdI | 2 | 1577, 3558 |
| DsaI | 1 | 289 |
| EagI | 5 | 60, 269, 964, 968, 3860 |
| EarI | 3 | 1353, 3157, 3795 |
| EclHKI | 1 | 2362 |
| **Eco47III** | 1 | 1345 |
| Eco52I | 5 | 60, 269, 964, 968, 3860 |
| **EcoICRI** | 1 | 9 |
| **FokI** | 5 | 196, 484, 2328, 2509, 2796 |
| FseI | 1 | 970 |
| FspI | 2 | 2584, 3757 |
| **HaeII** | 4 | 1347, 1717, 3356, 3364 |
| HgaI | 4 | 1580, 2158, 2888, 3289 |
| **HincII** | 2 | 1111, 1221 |
| HindII | 2 | 1111, 1221 |
| **HindIII** | 2 | 53, 276 |
| **HpaI** | 1 | 1111 |
| **Hsp92I** | 1 | 2899 |
| **KpnI** | 1 | 5 |
| **MluI** | 1 | 15 |
| **MspA1I** | 5 | 404, 848, 1811, 2056, 2997 |
| **NaeI** | 3 | 968, 1339, 3408 |
| **NciI** | 5 | 27, 28, 1849, 2545, 2896 |
| **NcoI** | 1 | 289 |
| **NgoMIV** | 3 | 966, 1337, 3406 |
| **NheI** | 1 | 21 |

**Note:** The enzymes listed in boldface type are available from Promega.

**Table 2. Restriction Enzymes That Cut the pCAT®3-Basic Vector 1–5 Times (continued).**

| Enzyme | # of Sites | Location | Enzyme | # of Sites | Location |
|---|---|---|---|---|---|
| **NotI** | 3 | 60, 269, 3860 | **SmaI** | 1 | 28 |
| NspI | 1 | 1473 | SrfI | 1 | 28 |
| PflMI | 1 | 736 | **SspI** | 4 | 816, 3166, 3719, 3834 |
| PshAI | 1 | 1284 | | | |
| PspAI | 1 | 26 | **StyI** | 1 | 289 |
| **PstI** | 1 | 76 | TfiI | 3 | 854, 1444, 3915 |
| **PvuI** | 2 | 2732, 3778 | **VspI** | 1 | 2534 |
| **PvuII** | 1 | 404 | **XbaI** | 1 | 951 |
| **SacI** | 1 | 11 | **XhoI** | 1 | 32 |
| **SalI** | 1 | 1219 | **XmaI** | 1 | 26 |
| **ScaI** | 3 | 921, 2842, 3925 | **XmnI** | 1 | 2961 |
| **SinI** | 2 | 2500, 2722 | | | |

**Table 3. Restriction Enzymes That Do Not Cut the pCAT®3-Basic Vector.**

| | | | | | |
|---|---|---|---|---|---|
| ***Aat*II** | Bpu1102I | DraII | **NdeI** | RsrII | **StuI** |
| **AgeI** | Bsp120I | Eco72I | **NruI** | **SacII** | SwaI |
| **ApaI** | BsrGI | Eco81I | **NsiI** | **SfiI** | **Tth111I** |
| AscI | **BssHII** | EcoNI | PacI | **SgfI** | XcmI |
| AvrII | Bst1107I | **EcoRI** | PinAI | SgrAI | |
| BbeI | **BstEII** | **EcoRV** | PmeI | **SnaBI** | |
| BbrPI | **BstXI** | EheI | PmlI | **SpeI** | |
| **BbuI** | **Bsu36I** | **I-PpoI** | Ppu10I | **SphI** | |
| **BclI** | **CspI** | KasI | PpuMI | SplI | |
| BlpI | **Csp45I** | **NarI** | Psp5II | Sse8387I | |

**Table 4. Restriction Enzymes That Cut the pCAT®3-Basic Vector 6 or More Times.**

| | | | | | |
|---|---|---|---|---|---|
| AciI | **BsrSI** | **DraI** | **Hsp92II** | **MspI** | ScrFI |
| **AluI** | Bst71I | EaeI | MaeI | **NdeII** | SfaNI |
| **BanI** | **BstOI** | Fnu4HI | MaeII | NlaIII | **TaqI** |
| BbvI | BstUI | **HaeIII** | MaeIII | NlaIV | **Tru9I** |
| BsaOI | **CfoI** | **HhaI** | **MboI** | PleI | **XhoII** |
| BsaJI | **DdeI** | **HinfI** | **MboII** | **RsaI** | |
| **Bsp1286I** | **DpnI** | **HpaII** | MnlI | **Sau3AI** | |
| BsrI | DpnII | HphI | MseI | Sau96I | |

**Note:** The enzymes listed in boldface type are available from Promega.

### 11.B. pCAT®3-Enhancer Vector Restriction Sites

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3´-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. The pCAT®3-Enhancer Vector sequence is available in the GenBank® database (GenBank®/EMBL Accession Number **U57026**) and at **www.promega.com/vectors/**

**Table 5. Restriction Enzymes That Cut the pCAT®3-Enhancer Vector 1–5 Times.**

| Enzyme | # of Sites | Location |
|---|---|---|
| **AccB7I** | 1 | 736 |
| **AccI** | 1 | 1466 |
| **AccIII** | 1 | 500 |
| **Acc65I** | 1 | 1 |
| AcyI | 1 | 3145 |
| AflII | 1 | 263 |
| AflIII | 2 | 15, 1715 |
| **Alw26I** | 5 | 127, 152, 728, 2669, 3445 |
| **Alw44I** | 2 | 2029, 3275 |
| AlwNI | 1 | 2131 |
| AspHI | 4 | 11, 2033, 3194, 3279 |
| **AvaI** | 2 | 26, 32 |
| **AvaII** | 2 | 2746, 2968 |
| **BalI** | 1 | 771 |
| **BamHI** | 1 | 1459 |
| **BanII** | 3 | 11, 33, 3686 |
| BbsI | 2 | 173, 1544 |
| **BbuI** | 2 | 1317, 1389 |
| **BglI** | 2 | 2728, 3996 |
| **BglII** | 1 | 36 |
| BsaI | 2 | 127, 2669 |
| BsaAI | 1 | 3757 |
| BsaBI | 1 | 1212 |
| BsaHI | 1 | 3145 |
| **BsaMI** | 4 | 497, 904, 1032, 1125 |
| BsmI | 4 | 497, 904, 1032, 1125 |
| BspHI | 2 | 2435, 3443 |
| BspMI | 2 | 89, 4236 |
| BssSI | 2 | 1888, 3272 |
| **Bst98I** | 1 | 263 |
| **BstZI** | 5 | 60, 269, 964, 968, 4106 |
| Cfr10I | 4 | 966, 1583, 2688, 3652 |
| **ClaI** | 3 | 1206, 4164, 4268 |
| DraIII | 1 | 3760 |
| DrdI | 2 | 1823, 3804 |
| DsaI | 1 | 289 |
| EagI | 5 | 60, 269, 964, 968, 4106 |
| EarI | 3 | 1599, 3403, 4041 |
| EclHKI | 1 | 2608 |
| **Eco47III** | 1 | 1591 |
| Eco52I | 5 | 60, 269, 964, 968, 4106 |
| **EcoICRI** | 1 | 9 |
| FseI | 1 | 970 |
| FspI | 2 | 2830, 4003 |
| **HaeII** | 4 | 1593, 1963, 3602, 3610 |
| HgaI | 4 | 1826, 2404, 3134, 3535 |
| **HincII** | 2 | 1111, 1467 |
| HindII | 2 | 1111, 1467 |
| **HindIII** | 2 | 53, 276 |
| **HpaI** | 1 | 1111 |
| **Hsp92I** | 1 | 3145 |
| **KpnI** | 1 | 5 |
| **MluI** | 1 | 15 |
| **NaeI** | 3 | 968, 1585, 3654 |
| **NciI** | 5 | 27, 28, 2095, 2791, 3142 |
| **NcoI** | 1 | 289 |
| **NgoMIV** | 3 | 966, 1583, 3652 |
| **NheI** | 1 | 21 |
| **NotI** | 3 | 60, 269, 4106 |
| **NsiI** | 2 | 1315, 1387 |
| NspI | 3 | 1317, 1389, 1719 |

**Note:** The enzymes listed in boldface type are available from Promega.

**Table 5. Restriction Enzymes That Cut the pCAT®3-Enhancer Vector 1–5 Times (continued).**

| Enzyme | # of Sites | Location | Enzyme | # of Sites | Location |
|---|---|---|---|---|---|
| PflMI | 1 | 736 | **SphI** | 2 | 1317, 1389 |
| Ppu10I | 2 | 1311, 1383 | SrfI | 1 | 28 |
| PshAI | 1 | 1530 | **SspI** | 4 | 816, 3412, 3965, 4080 |
| PspAI | 1 | 26 | | | |
| **PstI** | 1 | 75 | **StyI** | 1 | 289 |
| **PvuI** | 2 | 2978, 4024 | TfiI | 3 | 854, 1690, 4161 |
| **PvuII** | 1 | 404 | **VspI** | 1 | 2780 |
| **SacI** | 1 | 11 | **XbaI** | 1 | 951 |
| **SalI** | 1 | 1465 | **XhoI** | 1 | 32 |
| **ScaI** | 3 | 921, 3088, 4171 | **XmaI** | 1 | 26 |
| **SinI** | 2 | 2746, 2968 | **XmnI** | 1 | 3207 |
| **SmaI** | 1 | 28 | | | |

**Table 6. Restriction Enzymes That Do Not Cut the pCAT®3-Enhancer Vector.**

| | | | | | |
|---|---|---|---|---|---|
| **AatII** | Bpu1102I | **Csp45I** | KasI | Psp5II | Sse8387I |
| **AgeI** | Bsp120I | DraII | **NarI** | RsrII | **StuI** |
| **ApaI** | BsrGI | Eco72I | **NdeI** | **SacII** | SwaI |
| AscI | **BssHII** | Eco81I | **NruI** | **SfiI** | **Tth111I** |
| AvrII | Bst1107I | EcoNI | PacI | **SgfI** | XcmI |
| BbeI | **BstEII** | **EcoRI** | PinAI | SgrAI | |
| BbrPI | **BstXI** | **EcoRV** | PmeI | **SnaBI** | |
| **BclI** | **Bsu36I** | EheI | PmlI | **SpeI** | |
| BlpI | **CspI** | **I-PpoI** | PpuMI | SplI | |

**Table 7. Restriction Enzymes That Cut the pCAT®3-Enhancer Vector 6 or More Times.**

| | | | | | |
|---|---|---|---|---|---|
| AciI | Bst71I | Fnu4HI | MaeII | NlaIII | **Tru9I** |
| **AluI** | **BstOI** | **FokI** | MaeIII | NlaIV | **XhoII** |
| **BanI** | BstUI | **HaeIII** | **MboI** | PleI | |
| BbvI | **CfoI** | **HhaI** | **MboII** | **RsaI** | |
| BsaOI | **DdeI** | **HinfI** | MnlI | **Sau3AI** | |
| BsaJI | **DpnI** | **HpaII** | MseI | Sau96I | |
| **Bsp1286I** | DpnII | HphI | **MspI** | ScrFI | |
| BsrI | **DraI** | **Hsp92II** | **MspA1I** | SfaNI | |
| **BsrSI** | EaeI | MaeI | **NdeII** | **TaqI** | |

**Note:** The enzymes listed in boldface type are available from Promega.

## 11.C. pCAT®3-Promoter Vector Restriction Sites

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3´-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. The pCAT®3-Promoter Vector sequence is available in the GenBank® database (GenBank®/EMBL Accession Number **U57027**) and at **www.promega.com/vectors/**

**Table 8. Restriction Enzymes That Cut the pCAT®3-Promoter Vector 1–5 Times.**

| Enzyme | # of Sites | Location | Enzyme | # of Sites | Location |
|---|---|---|---|---|---|
| **AccB7I** | 1 | 928 | Cfr10I | 4 | 1158, 1529, 2634, 3598 |
| **AccI** | 1 | 1412 | | | |
| **AccIII** | 1 | 692 | **ClaI** | 3 | 1398, 4110, 4214 |
| **Acc65I** | 1 | 1 | DraIII | 1 | 3706 |
| AcyI | 1 | 3091 | DrdI | 2 | 1769, 3750 |
| AflII | 2 | 258, 455 | DsaI | 1 | 481 |
| AflIII | 2 | 15, 1661 | EagI | 5 | 252, 461, 1156, 1160, 4052 |
| **Alw26I** | 5 | 320, 345, 920, 2615, 3391 | EarI | 3 | 1545, 3349, 3987 |
| **Alw44I** | 2 | 1975, 3221 | EclHKI | 1 | 2554 |
| AlwNI | 1 | 2077 | **Eco47III** | 1 | 1537 |
| AspHI | 4 | 11, 1979, 3140, 3225 | Eco52I | 5 | 252, 461, 1156, 1160, 4052 |
| **AvaI** | 2 | 26, 32 | | | |
| **AvaII** | 2 | 2692, 2914 | **EcoICRI** | 1 | 9 |
| AvrII | 1 | 229 | FseI | 1 | 1162 |
| **BalI** | 1 | 963 | FspI | 2 | 2776, 3949 |
| **BamHI** | 1 | 1405 | **HaeII** | 4 | 1539, 1909, 3548, 3556 |
| **BanII** | 3 | 11, 33, 3632 | | | |
| BbsI | 2 | 366, 1490 | HgaI | 4 | 1772, 2350, 3080, 3481 |
| **BglI** | 3 | 182, 2674, 3942 | | | |
| **BglII** | 1 | 36 | **HincII** | 2 | 1303, 1413 |
| BsaI | 2 | 320, 2615 | HindII | 2 | 1303, 1413 |
| BsaAI | 1 | 3703 | **HindIII** | 2 | 245, 468 |
| BsaBI | 2 | 48, 1404 | **HpaI** | 1 | 1303 |
| BsaHI | 1 | 3091 | **Hsp92I** | 1 | 3091 |
| **BsaMI** | 4 | 689, 1096, 1224 , 1317 | **KpnI** | 1 | 5 |
| | | | **MluI** | 1 | 15 |
| BsmI | 4 | 689, 1096, 1224, 1317 | **MspA1I** | 5 | 596, 1040, 2003, 2248, 3189 |
| BspHI | 2 | 2381, 3389 | **NaeI** | 3 | 1160, 1531, 3600 |
| BspMI | 2 | 282, 4182 | **NciI** | 5 | 27, 28, 2041, 2737, 3088 |
| BssSI | 2 | 1834, 3218 | | | |
| **Bst98I** | 2 | 258, 455 | **NcoI** | 1 | 481 |
| **BstZI** | 5 | 252, 461, 1156, 1160, 4052 | **NgoMIV** | 3 | 1158, 1529, 3598 |
| | | | **NheI** | 1 | 21 |

**Note:** The enzymes listed in boldface type are available from Promega.

**Table 8. Restriction Enzymes That Cut the pCAT®3-Promoter Vector 1–5 Times (continued).**

| Enzyme | # of Sites | Location | Enzyme | # of Sites | Location |
|---|---|---|---|---|---|
| **NotI** | 3 | 252, 461, 4052 | **SmaI** | 1 | 28 |
| NspI | 1 | 1665 | SrfI | 1 | 28 |
| Pfl MI | 1 | 928 | **SspI** | 4 | 1008, 3358, 3911, 4026 |
| PshAI | 1 | 1476 | | | |
| PspAI | 1 | 26 | **StuI** | 1 | 228 |
| **PstI** | 1 | 268 | **StyI** | 2 | 229, 481 |
| **PvuI** | 2 | 2924, 3970 | TfiI | 3 | 1046, 1636, 4107 |
| **PvuII** | 1 | 596 | **VspI** | 1 | 2726 |
| **SacI** | 1 | 11 | **XbaI** | 1 | 1143 |
| **SalI** | 1 | 1411 | **XhoI** | 1 | 32 |
| **ScaI** | 3 | 1113, 3034, 4117 | **XmaI** | 1 | 26 |
| **SfiI** | 1 | 182 | **XmnI** | 1 | 3153 |
| **SinI** | 2 | 2692, 2914 | | | |

**Table 9. Restriction Enzymes That Do Not Cut the pCAT®3-Promoter Vector.**

| | | | | | |
|---|---|---|---|---|---|
| **AatII** | Bpu1102I | **Csp45I** | KasI | PpuMI | SplI |
| **AgeI** | Bsp120I | DraII | **NarI** | Psp5II | Sse8387I |
| **ApaI** | BsrGI | Eco72I | **NdeI** | RsrII | SwaI |
| AscI | **BssHII** | Eco81I | **NruI** | **SacII** | **Tth111I** |
| BbeI | Bst1107I | EcoNI | **NsiI** | **SgfI** | XcmI |
| BbrPI | **BstEII** | **EcoRI** | PacI | SgrAI | |
| **BbuI** | **BstXI** | **EcoRV** | PinAI | **SnaBI** | |
| **BclI** | **Bsu36I** | EheI | PmeI | **SpeI** | |
| BlpI | **CspI** | **I-PpoI** | PmlI | **SphI** | |

**Table 10. Restriction Enzymes That Cut the pCAT®3-Promoter Vector 6 or More Times.**

| | | | | | |
|---|---|---|---|---|---|
| AciI | **BsrSI** | **DraI** | HphI | MseI | Sau96I |
| **AluI** | Bst71I | EaeI | **Hsp92II** | **MspI** | ScrFI |
| **BanI** | **BstOI** | Fnu4HI | MaeI | **NdeII** | SfaNI |
| BbvI | BstUI | **FokI** | MaeII | NlaIII | **TaqI** |
| BsaOI | **CfoI** | **HaeIII** | MaeIII | NlaIV | **Tru9I** |
| BsaJI | **DdeI** | **HhaI** | **MboI** | PleI | **XhoII** |
| **Bsp1286I** | **DpnI** | **HinfI** | **MboII** | **RsaI** | |
| BsrI | DpnII | **HpaII** | MnlI | **Sau3AI** | |

**Note:** The enzymes listed in boldface type are available from Promega.

### 11.D. pCAT®3-Control Vector Restriction Sites

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3´-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. The pCAT®3-Control Vector sequence is available in the GenBank® database (GenBank®/EMBL Accession Number **U57025**) and at **www.promega.com/vectors/**

**Table 11. Restriction Enzymes That Cut the pCAT®3-Control Vector 1–5 Times.**

| Enzyme | # of Sites | Location |
|---|---|---|
| **AccB7I** | 1 | 928 |
| **AccI** | 1 | 1658 |
| **AccIII** | 1 | 692 |
| **Acc65I** | 1 | 1 |
| AcyI | 1 | 3337 |
| AflII | 2 | 258, 455 |
| AflIII | 2 | 15, 1907 |
| **Alw26I** | 5 | 320, 345, 920, 2861, 3637 |
| **Alw44I** | 2 | 2221, 3467 |
| AlwNI | 1 | 2323 |
| AspHI | 4 | 11, 2225, 3386, 3471 |
| **AvaI** | 2 | 26, 32 |
| **AvaII** | 2 | 2938, 3160 |
| AvrII | 1 | 229 |
| **BalI** | 1 | 963 |
| **BamHI** | 1 | 1651 |
| **BanII** | 3 | 11, 33, 3878 |
| BbsI | 2 | 366, 1736 |
| **BbuI** | 2 | 1509, 1581 |
| **BglI** | 3 | 182, 2920, 4188 |
| **BglII** | 1 | 36 |
| BsaI | 2 | 320, 2861 |
| BsaAI | 1 | 3949 |
| BsaBI | 2 | 48, 1404 |
| BsaHI | 1 | 3337 |
| **BsaMI** | 4 | 689, 1096, 1224, 1317 |
| BsmI | 4 | 689, 1096, 1224, 1317 |
| BspHI | 2 | 2627, 3635 |
| BspMI | 2 | 282, 4428 |
| BssSI | 2 | 2080, 3464 |
| **Bst98I** | 2 | 258, 455 |
| **BstZI** | 5 | 252, 461, 1156 1160, 4298 |
| Cfr10I | 4 | 1158, 1775, 2880, 3844 |
| **ClaI** | 3 | 1398, 4356, 4460 |
| DraIII | 1 | 3952 |
| DrdI | 2 | 2015, 3996 |
| DsaI | 1 | 481 |
| EagI | 5 | 252, 461, 1156 1160, 4298 |
| EarI | 3 | 1791, 3595, 4233 |
| EclHKI | 1 | 2800 |
| **Eco47III** | 1 | 1783 |
| Eco52I | 5 | 252, 461, 1156, 1160, 4298 |
| **EcoICRI** | 1 | 9 |
| FseI | 1 | 1162 |
| FspI | 2 | 3022, 4195 |
| **HaeII** | 4 | 1785, 2155, 3794, 3802 |
| HgaI | 4 | 2018, 2596, 3326, 3727 |
| **HincII** | 2 | 1303, 1659 |
| HindII | 2 | 1303, 1659 |
| **HindIII** | 2 | 245, 468 |
| **HpaI** | 1 | 1303 |
| **Hsp92I** | 1 | 3337 |
| **KpnI** | 1 | 5 |
| **MluI** | 1 | 15 |
| **NaeI** | 3 | 1160, 1777, 3846 |
| **NciI** | 5 | 27, 28, 2287, 2983, 3334 |
| **NcoI** | 1 | 481 |
| **NgoMIV** | 3 | 1158, 1775, 3844 |
| **NheI** | 1 | 21 |
| **NotI** | 3 | 252, 461, 4298 |
| **NsiI** | 2 | 1507, 1579 |
| NspI | 3 | 1509, 1581, 1911 |
| PflMI | 1 | 928 |

**Note:** The enzymes listed in boldface type are available from Promega.

**Table 11. Restriction Enzymes That Cut the pCAT®3-Control Vector 1–5 Times (continued).**

| Enzyme | # of Sites | Location | Enzyme | # of Sites | Location |
|---|---|---|---|---|---|
| Ppu10I | 2 | 1503, 1575 | **SphI** | 2 | 1509, 1581 |
| PshAI | 1 | 1722 | SrfI | 1 | 28 |
| PspAI | 1 | 26 | **SspI** | 4 | 1008, 3604, 4157, 4272 |
| **PstI** | 1 | 268 | | | |
| **PvuI** | 2 | 3170, 4216 | **StuI** | 1 | 228 |
| **PvuII** | 1 | 596 | **StyI** | 2 | 229, 481 |
| **SacI** | 1 | 11 | TfiI | 3 | 1046, 1882, 4353 |
| **SalI** | 1 | 1657 | **VspI** | 1 | 2972 |
| **ScaI** | 3 | 1113, 3280, 4363 | **XbaI** | 1 | 1143 |
| **SfiI** | 1 | 182 | **XhoI** | 1 | 32 |
| **SinI** | 2 | 2938, 3160 | **XmaI** | 1 | 26 |
| **SmaI** | 1 | 28 | **XmnI** | 1 | 3399 |

**Table 12. Restriction Enzymes That Do Not Cut the pCAT®3-Control Vector.**

| | | | | | |
|---|---|---|---|---|---|
| **AatII** | Bpu1102I | **CspI** | EheI | PmeI | **SnaBI** |
| **AgeI** | Bsp120I | **Csp45I** | **I-PpoI** | PmlI | **SpeI** |
| **ApaI** | BsrGI | DraII | KasI | PpuMI | SplI |
| AscI | **BssHII** | Eco72I | **NarI** | Psp5II | Sse8387I |
| BbeI | Bst1107I | Eco81I | **NdeI** | RsrII | SwaI |
| BbrPI | **BstEII** | EcoNI | **NruI** | **SacII** | **Tth111I** |
| **BclI** | **BstXI** | **EcoRI** | PacI | **SgfI** | XcmI |
| BlpI | **Bsu36I** | **EcoRV** | PinAI | SgrAI | |

**Table 13. Restriction Enzymes That Cut the pCAT®3-Control Vector 6 or More Times.**

| | | | | | |
|---|---|---|---|---|---|
| AciI | **BsrSI** | **DraI** | HphI | MseI | **Sau3AI** |
| **AluI** | Bst71I | EaeI | **Hsp92II** | **MspI** | Sau96I |
| **BanI** | **BstOI** | Fnu4HI | MaeI | **MspA1I** | ScrFI |
| BbvI | BstUI | **FokI** | MaeII | **NdeII** | SfaNI |
| BsaOI | **CfoI** | **HaeIII** | MaeIII | NlaIII | **TaqI** |
| BsaJI | **DdeI** | **HhaI** | **MboI** | NlaIV | **Tru9I** |
| **Bsp1286I** | **DpnI** | **HinfI** | **MboII** | PleI | **XhoII** |
| BsrI | DpnII | **HpaII** | MnlI | **RsaI** | |

**Note:** The enzymes listed in boldface type are available from Promega.

## 11.E. Related Products

### CAT Assay Reagents

| Product | Size | Cat.# |
|---|---|---|
| CAT Enzyme Assay System With Reporter Lysis Buffer | 50 reactions | E1000 |
| Reporter Lysis Buffer, 5X | 30ml | E3971 |
| Chloramphenicol Acetyltransferase (CAT) | 100u | E1051 |

### Reporter Vector Sequencing Primers

| Product | Size | Cat.# |
|---|---|---|
| RVprimer3 (clockwise) | 2µg | E4481 |
| RVprimer4 (counterclockwise) | 2µg | E4491 |

### Mammalian Transfection Systems

| Product | Size | Cat.# |
|---|---|---|
| TransFast™ Transfection Reagent | 1.2mg | E2431 |
| Tfx™-50 Reagent | 2.1mg | E1811 |
| Tfx™-20 Reagent | 4.8mg | E2391 |
| ProFection® Mammalian Transfection System Calcium Phosphate | 40 reactions | E1200 |

### Competent Cells

| Product | Size | Cat.# |
|---|---|---|
| JM109 Competent Cells, >$10^8$cfu/µg* | 1ml | L2001 |
| JM109 Competent Cells, >$10^7$cfu/µg | 1ml | L1001 |

*For Laboratory Use. Each 1ml order is provided as 5 × 200µl aliquots. Also included with each order is pGEM®-3Z Competent Cell Control DNA.

### pGL3 Luciferase Reporter Vectors

Please visit **www.promega.com** to see a complete listing of our reporter vectors.

| Product | Size | Cat.# |
|---|---|---|
| pGL3-Control Vector | 20µg | E1741 |
| pGL3-Enhancer Vector | 20µg | E1771 |
| pGL3-Promoter Vector | 20µg | E1761 |
| pGL3-Basic Vector | 20µg | E1751 |

**pGL4 Luciferase Reporter Vectors**

| Vector | Multiple Cloning Region | Reporter Gene | Protein Degradation Sequence | Reporter Gene Promoter/ Response Element | Mammalian Selectable Marker | Cat.# |
|---|---|---|---|---|---|---|
| pGL4.10[*luc2*] | Yes | *luc2*[A] | No | No | No | E6651 |
| pGL4.11[*luc2*P] | Yes | " | hPEST | No | No | E6661 |
| pGL4.12[*luc2*CP] | Yes | " | hCL1-hPEST | No | No | E6671 |
| pGL4.13[*luc2*/SV40] | No | " | No | SV40 | No | E6681 |
| pGL4.14[*luc2*/Hygro] | Yes | " | No | No | Hygro | E6691 |
| pGL4.15[*luc2*P/Hygro] | Yes | " | hPEST | No | Hygro | E6701 |
| pGL4.16[*luc2*CP/Hygro] | Yes | " | hCL1-hPEST | No | Hygro | E6711 |
| pGL4.17[*luc2*/Neo] | Yes | " | No | No | Neo | E6721 |
| pGL4.18[*luc2P*/Neo] | Yes | " | hPEST | No | Neo | E6731 |
| pGL4.19[*luc2CP*/Neo] | Yes | " | hCL1-hPEST | No | Neo | E6741 |
| pGL4.20[*luc2*/Puro] | Yes | " | No | No | Puro | E6751 |
| pGL4.21[*luc2P*/Puro] | Yes | " | hPEST | No | Puro | E6761 |
| pGL4.22[*luc2CP*/Puro] | Yes | " | hCL1-hPEST | No | Puro | E6771 |
| pGL4.23[*luc2*/minP] | Yes | " | No | minP | No | E6691 |
| pGL4.24[*luc2*P/minP] | Yes | " | hPEST | " | No | E6701 |
| pGL4.25[*luc2*CP/minP] | Yes | " | hCL1-hPEST | " | No | E6711 |
| pGL4.26[*luc2*/minP/Hygro] | Yes | " | No | " | Hygro | E6721 |
| pGL4.27[*luc2P*/minP/Hygro] | Yes | " | hPEST | " | Hygro | E6731 |
| pGL4.28[*luc2CP*/minP/Hygro] | Yes | " | hCL1-hPEST | " | Hygro | E6741 |
| pGL4.29[*luc2P*/CRE/Hygro] | No | " | hPEST | CRE | Hygro | E6751 |
| pGL4.30[*luc2P*/NFAT-RE/Hygro] | No | " | hPEST | NFAT-RE | Hygro | E6761 |
| pGL4.31[*luc2P*/*GAL4*UAS/Hygro] | No | " | hPEST | *GAL4*UAS | Hygro | E6771 |
| pGL4.70[hR*luc*] | Yes | h*Rluc*[B] | No | No | No | E6881 |
| pGL4.71[hR*luc*P] | Yes | " | hPEST | No | No | E6891 |
| pGL4.72[hR*luc*CP] | Yes | " | hCL1-hPEST | No | No | E6901 |
| pGL4.73[hR*luc*/SV40] | No | " | No | SV40 | No | E6911 |
| pGL4.74[hR*luc*/TK] | No | " | No | HSV-TK | No | E6921 |
| pGL4.75[hR*luc*/CMV] | No | " | No | CMV | No | E6931 |
| pGL4.76[hR*luc*/Hygro] | Yes | " | No | No | Hygro | E6941 |
| pGL4.77[hR*luc*P/Hygro] | Yes | " | hPEST | No | Hygro | E6951 |
| pGL4.78[hR*luc*CP/Hygro] | Yes | " | hCL1-hPEST | No | Hygro | E6961 |
| pGL4.79[*hRluc*/Neo] | Yes | " | No | No | Neo | E6971 |
| pGL4.80[*hRlucP*/Neo] | Yes | " | hPEST | No | Neo | E6981 |
| pGL4.81[*hRlucCP*/Neo] | Yes | " | hCL1-hPEST | No | Neo | E6991 |
| pGL4.82[*hRluc*/Puro] | Yes | " | No | No | Puro | E7501 |
| pGL4.83[*hRlucP*/Puro] | Yes | " | hPEST | No | Puro | E7511 |
| pGL4.84[*hRlucCP*/Puro] | Yes | " | hCL1-hPEST | No | Puro | E7521 |

[A]*luc2* = synthetic firefly luciferase gene. [B]h*Rluc* = synthetic *Renilla* luciferase gene.

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## DNA Purification Products

| Product | Size | Cat.# |
|---|---|---|
| Wizard® SV Gel and PCR Clean-Up System* | 50 preps | A9281 |
| | 250 preps | A9282 |
| Wizard® *Plus* SV Minipreps DNA Purification System* | 50 preps | A1330 |
| | 250 preps | A1460 |
| PureYield™ Plasmid Midipreps System | 50 preps | A2492 |
| | 100 preps | A2495 |

*For Laboratory Use