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.

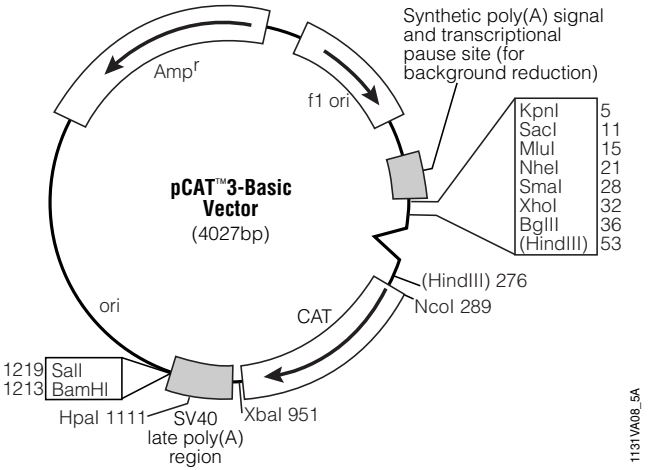

Figure 1. The pCAT™3-Basic Vector circle map. –^–, position of intron; CAT, cDNA encoding the chloramphenicol acetyltransferase gene; Amp^r, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli*. 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

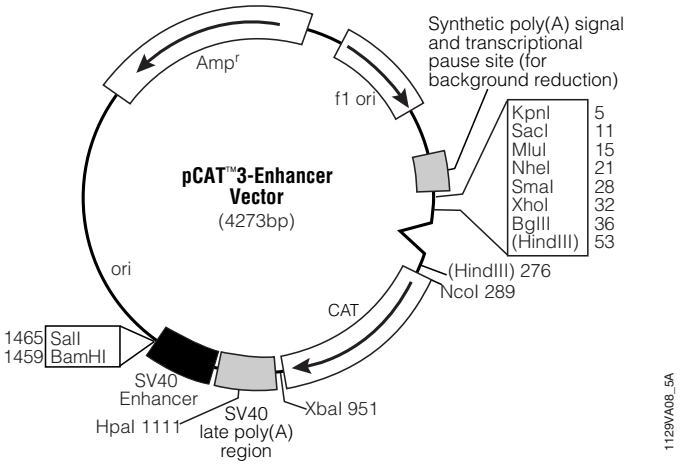

Figure 2. The pCAT™3-Enhancer Vector circle map. –^–, position of intron; CAT, cDNA encoding the chloramphenicol acetyltransferase gene; Amp^r, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli*. 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

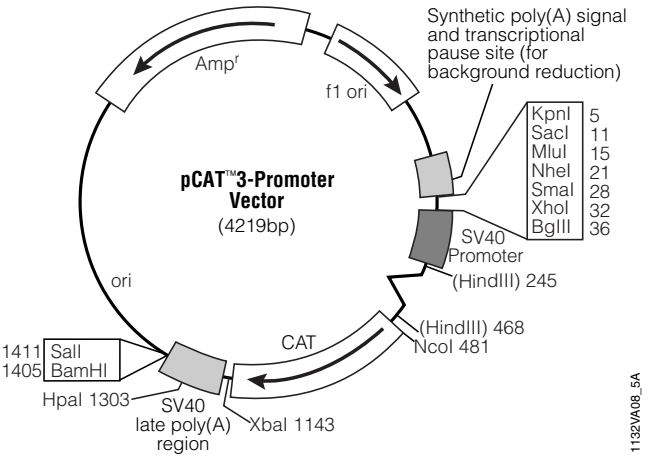

Figure 3. The pCAT™3-Promoter Vector circle map. –^–, position of intron; CAT, cDNA encoding the chloramphenicol acetyltransferase gene; Amp^r, gene conferring ampicillin resistance in *E. coli;* f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli.* 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

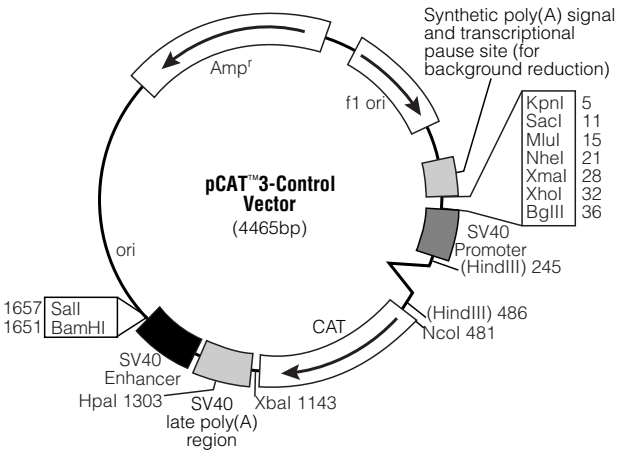

Figure 4. The pCAT™3-Control Vector circle map. –^–, position of intron; CAT, cDNA encoding the chloramphenicol acetyltransferase gene; Amp^r, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli*. 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) 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) or the *ProFection® Mammalian Transfection Systems Technical Manual* (#TM012). All of these documents are available on our web site at: **www.promega.com/protocols/**

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 3×10^{-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.

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.

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.

10. References

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

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

Product	Size	Cat.#
Reporter Lysis Buffer, 5X	30ml	E3971

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
ProFection® Mammalian Transfection System Calcium Phosphate	40 reactions	E1200

Competent Cells

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

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]hR*luc* = synthetic *Renilla* luciferase gene.

Promega

DNA Purification Products

Product	Size	Cat.#
Wizard® SV Gel and PCR Clean-Up System	50 preps	A9281
	250 preps	A9282
Wizard® *Plus* SV Minipreps	50 preps	A1330
DNA Purification System	250 preps	A1460
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495