Technical Manual

Chroma-Luc™ Reporter Vectors

INSTRUCTIONS FOR USE OF PRODUCTS E1411, E1421, E1431, E1441, E1451 AND E1461.

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Chroma-Luc™ Reporter Vectors

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1. Description

Normalizing the expression of an experimental reporter to the expression of a control reporter can help to differentiate specific from nonspecific effects of cell-treatment protocols. This normalization is helpful for both transiently and stably transfected cell lines. Theoretically, the more similar the experimental and control reporters are, the more relevant the normalization. Based on this principle, we have developed a luciferase-based technology, the Chroma-Luc™ Reporter Vectors[a–d]. The Chroma-Luc™ Vectors include a vector encoding one red-emitting luciferase (CBR*luc*) and two vectors encoding green-emitting luciferases (CBG68*luc* and CBG99*luc*; Figure 1).

The design of the synthetic Chroma-Luc™ genes is based on a native Yellow-Green luciferase gene originally cloned from *Pyrophorus plagiophthalamus*, a large click beetle indigenous to the Caribbean. To ensure reliability and high levels of expression, the Chroma-Luc™ genes have been codon optimized for mammalian expression and cleared of most known consensus transcription

factor binding sites. In addition, the predicted peroxisome targeting sequences have been removed. Upon addition of the luciferase substrates, the engineered CBRluc luciferase emits red light (613 nm), which has optimal color separation from the corresponding 537 nm green-emitting Chroma-Luc™ luciferases (i.e., CBG68luc and CBG99luc).

Because of different substrate requirements between the Chroma-Luc™ luciferases and other beetle luciferases, Promega has developed Chroma-Glo™ Reagent[a,c,d,e] (Cat.# E4910, E4920 and E4950) as a companion reagent to the Chroma-Luc™ Vectors. The Chroma-Glo™ Reagent has been designed and optimized to elicit maximal luminescence from all the Chroma-Luc™ enzymes. Color-separating filters allow the user to measure the red and green luminescent signals independently.

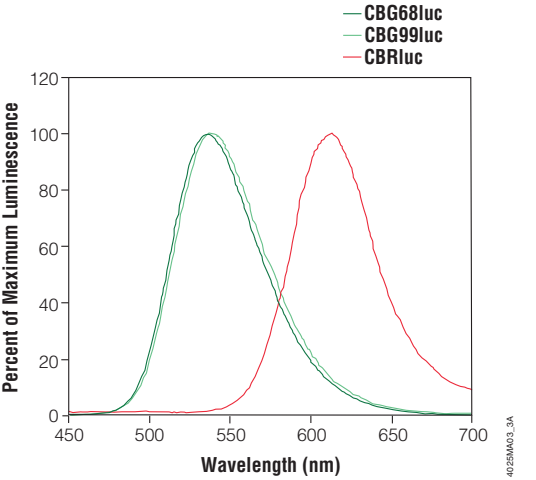

Figure 1. Emission spectra of the Chroma-Luc™ enzymes. Independent populations of CHO cells were transfected with either pCBR-Control, pCBG68-Control, or pCBG99-Control. Twenty-four hours after transfection, the cells were lysed by adding Glo Lysis Buffer (Cat.# E2661). Equal volumes of cell lysate and Chroma-Glo™ Reagent were mixed and incubated for 5 minutes at room temperature. The emission spectra were generated using the Spex Fluorolog®-2 spectrofluorometer with the excitation source turned off. Emission data were collected from 450–700 nm. Maximum emission for CBG68luc and CBG99luc is 537 nm, while CBRluc has a maximum emission of 613 nm.

2. Product Components and Storage Conditions

Product	Size	Cat.#
pCBR-Basic Vector	20 µg	E1411
pCBR-Control Vector	20 µg	E1421
pCBG68-Basic Vector	20 µg	E1431
pCBG68-Control Vector	20 µg	E1441
pCBG99-Basic Vector	20 µg	E1451
pCBG99-Control Vector	20 µg	E1461

Storage Conditions: Store the vectors at –20°C.

3. General Considerations

In cell biology research and pharmaceutical discovery, testing a wide variety of experimental conditions or a large number of chemical compounds for their effects on cellular physiology is a common practice. This testing is typically achieved through transcriptional assays using genetic reporters coupled to physiologically regulated genetic elements.

Monitoring upregulation of these genetic elements is relatively simple using luciferase assays because they are extremely sensitive, rapid and easy to perform. However, monitoring the downregulation of these genetic elements is more difficult because the generalized effects of cell death can be interpreted easily as a specific decrease in luciferase production. Normalizing the expression of an experimental reporter to the expression of a control reporter can help to distinguish specific from nonspecific effects. This normalization can also reduce the effect of variability of the transfection step. The Chroma-Glo™ Luciferase Assay System and the Chroma-Luc™ luciferases provide an ideal dual-reporter system, because the two reporter enzymes are highly similar (>98% amino acid identity) and because both signals are generated by a single-reagent addition.

Pyrophorus plagiophthalamus has two sets of light-emitting organs: a pair located on the dorsal surface of the prothorax, and a single organ located in the ventral cleft of the abdomen. The dorsal anterior organs emit a yellow to green luminescence, while the ventral cleft emits a higher-emission orange luminescence. Four luciferase-expressing genes were cloned from the ventral cleft. The emission for these four luciferases ranges from green to orange (544–593 nm). Based on their emission color, the four genes were named Green, Yellow-Green, Yellow and Orange. These four genes express luciferases that are highly similar; the proteins share between 95–99% amino acid identity. Sequence alignment of the four proteins revealed that key amino acids were responsible for the differences in emission colors.

The experimental information gathered on how to manipulate emission color was used to design both a synthetic red-emitting and green-emitting luciferase. The wildtype Yellow-Green luciferase demonstrated the brightest signal, and this gene was selected as the template for designing the red- and green-emitting Chroma-Luc™ luciferases. In addition to changes to generate different emission colors, several other sequence modifications were made to generate the most favorable synthetic genetic reporters.

To improve the expression, low-usage mammalian codons were replaced in the synthetic Chroma-Luc™ genes (Table 1).

Table 1. Comparison of Codon Usage Among the Wildtype Yellow-Green Luciferase and the Synthetic CBR*luc*, CBG99*luc* and CBG68*luc* Luciferase Genes.

Note: In order to remove the predicted peroxisome targeting sequence found in the wildtype Yellow-Green luciferase, we have removed the C-terminal amino acid.

Amino Acid/ Codon	Number of Codons in Yellow-Green Luciferase	Number of Codons in CBR*luc* (Red)	Number of Codons in CBG99*luc* (Green)	Number of Codons in CBG68*luc* (Green)	Percent Use in Human Cells
Ala/ GCA	15	4	4	5	20.0
GCC	4	12	12	14	41.6
GCG	3	1	0	0	10.3
GCT	15	20	21	18	28.0
Arg/ AGA	7	0	0	0	18.8
AGG	7	0	0	0	21.0
CGA	6	0	0	2	10.2
CGC	1	11	11	11	21.4
CGG	0	0	0	0	19.7
CGT	4	14	14	13	8.9
Asn/ AAC	7	12	13	13	57.7
AAT	16	9	9	9	42.3
Asp/ GAC	6	12	12	14	57.2
GAT	20	14	14	12	42.8
Cys/ TGC	4	4	4	3	59.4
TGT	9	7	7	8	40.6
Gln/ CAA	8	7	7	11	24.8
CAG	6	8	7	3	75.2
Glu/ GAA	26	18	18	19	39.3
GAG	12	19	20	19	60.7
Gly/ GGA	17	3	3	1	24.1
GGC	2	21	21	21	35.8
GGG	3	2	2	1	24.4
GGT	16	14	14	16	15.8

Amino Acid/ Codon	Number of Codons in Yellow-Green Luciferase	Number of Codons in CBR*luc* (Red)	Number of Codons in CBG99*luc* (Green)	Number of Codons in CBG68*luc* (Green)	Percent Use in Human Cells
His/ CAC	6	4	5	7	60.4
CAT	7	9	8	6	39.6
Ile/ ATA	13	0	0	0	12.9
ATC	7	21	20	23	54.0
ATT	19	18	18	15	33.1
Leu/ CTA	5	0	0	0	6.5
CTC	4	11	11	12	20.8
CTG	4	18	18	19	44.4
CTT	13	1	1	1	11.2
TTA	18	0	0	0	5.5
TTG	13	25	25	23	11.5
Lys/ AAA	25	12	13	19	38.9
AAG	12	22	22	16	61.1
Met/ ATG	11	10	10	10	100.0
Phe/ TTC	9	12	12	15	58.9
TTT	15	13	13	10	41.4
Pro/ CCA	9	12	12	9	25.7
CCC	8	1	1	2	35.3
CCG	2	1	1	0	11.6
CCT	9	14	14	17	27.3
Ser/ AGC	2	11	13	14	25.8
AGT	8	3	2	1	13.0
TCA	5	2	2	1	12.8
TCC	2	2	2	4	24.4
TCG	7	0	0	0	5.8
TCT	8	11	12	11	18.2
Thr/ ACA	9	2	1	0	25.4
ACC	1	11	11	8	40.5
ACG	1	0	0	0	11.8
ACT	8	11	10	14	22.4
Trp/ TGG	2	2	2	2	100.0
Tyr/ TAC	9	13	12	12	60.1
TAT	11	7	7	7	39.9
Val/ GTA	13	2	1	1	9.3
GTC	4	25	25	21	25.7
GTG	11	18	19	26	48.7
GTT	19	6	6	3	16.4
Total Number of Codons	**543**	**542**	**542**	**542**	

To reduce the potential for anomalous expression, virtually all of the known consensus transcription factor binding sites were removed. The wildtype Yellow-Green luciferase gene possessed 152 known consensus transcription factor binding sites; the resulting synthetic genes, CBR*luc* and CBG68*luc*, have two known consensus transcription factor sites (a 99% reduction), and CBG99*luc* has four (a 97% reduction; Figure 2). Finally, the synthetic genes express a luciferase enzyme without a peroxisome targeting sequence, and all synthetic genes have a Kozak consensus sequence.

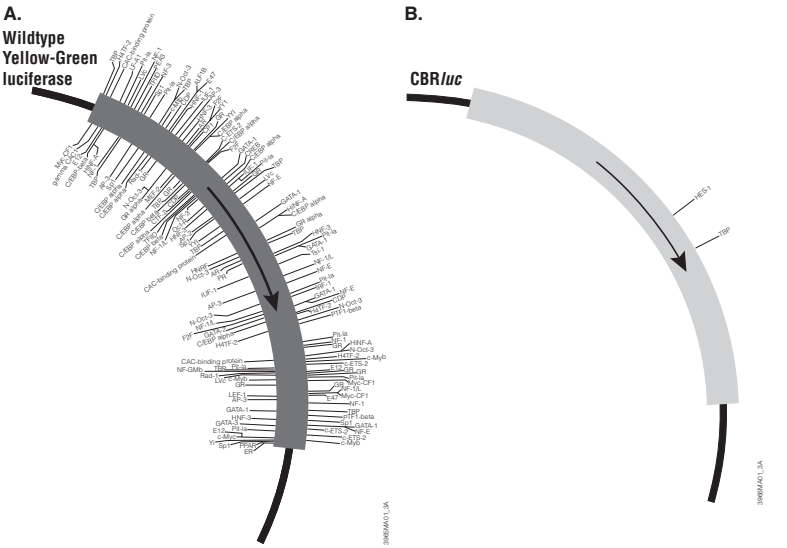

Figure 2 (continued on next page). Known transcription factor binding sites in the wildtype Yellow-Green luciferase gene (Panel A), red luciferase (CBR*luc*; Panel B), green luciferase (CBG99*luc*; Panel C, next page), and green luciferase (CBG68*luc*; Panel D, next page). Most (>97%) of the known transcription factor binding sites from the wildtype Yellow-Green gene have been removed to create the synthetic Chroma-Luc™ genes. The wildtype Yellow-Green luciferase gene (Panel A) has 152 known transcription factor binding sites.

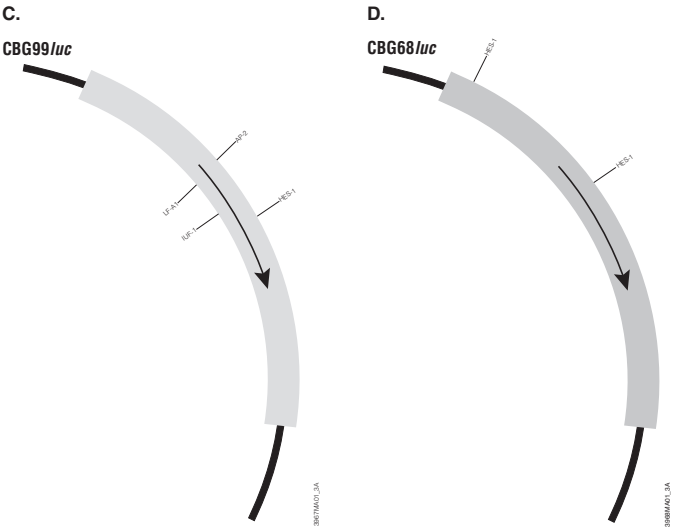

Figure 2 (continued). Known transcription factor binding sites in the wildtype Yellow-Green luciferase gene (Panel A), red luciferase (CBR*luc*; Panel B), green luciferase (CBG99*luc*; Panel C), and green luciferase (CBG68*luc*; Panel D).

The data in Figure 3 demonstrate that all the synthetic genes exhibit a significant increase in expression over the native gene. Figure 4 demonstrates that the synthetic Chroma-Luc™ genes have reduced anomalous expression.

The data in Figures 3 and 4 are not corrected for photomultiplier (PMT) efficiency. To obtain a significant amount of the red-emitted light from the expression of CBR*luc*, the luminometer (more specifically, the PMT) must be selected with consideration. Generally a PMT is more sensitive in the blue to green wavelengths and least sensitive in the red wavelengths. A charged coupled device (CCD) instrument works well for detecting the Chroma-Luc™ luciferases because it possesses a detection range that is more sensitive in the red spectrum.

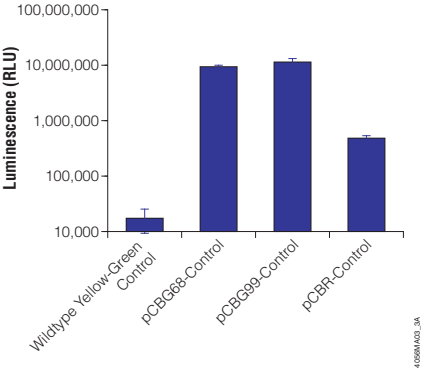

Figure 3. Increase in expression from the synthetic Chroma-Luc™ genes. The three synthetic Chroma-Luc™ genes and the wildtype Yellow-Green luciferase gene were independently cloned into the pGL3-Control Vector, replacing the *luc*+ present in this vector. At twenty-four hours post-transfection of CHO cells an equal amount of Chroma-Glo™ Reagent was added to the media and the cells. The relative light units were detected using a Berthold Centro LB 960 Luminometer. When compared to the wildtype Yellow-Green luciferases, the synthetic Chroma-Luc™ luciferases, CBG68 and CBG99, display 546- and 660-fold increases in relative light units, respectively. The synthetic CBR luciferase displays a 28-fold increase in relative light units compared to the wildtype Yellow-Green luciferase. All values have been corrected for transfection efficiency but not for photomultiplier efficiency.

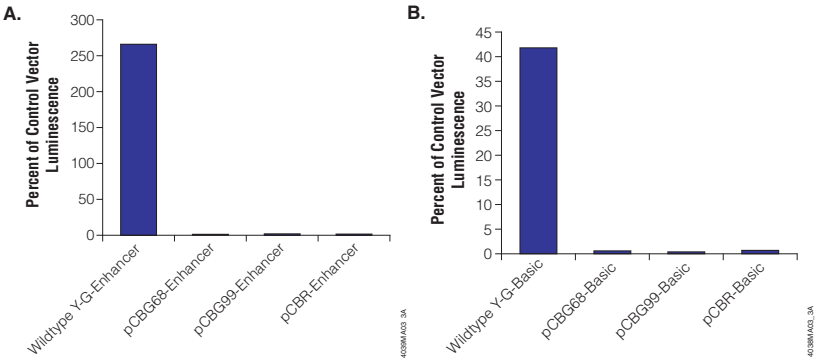

Figure 4. Reduction in anomalous expression for the synthetic Chroma-Luc™ genes. The synthetic CBR*luc*, CBG68*luc*, and CBG99*luc* genes and the wildtype Yellow-Green luciferase gene were cloned into a pGL3-Control Vector (contains SV40 promoter and enhancer), a pGL3-Enhancer Vector (contains only SV40 enhancer) and pGL3-Basic Vector (no promoter or enhancer added). The vectors were transfected separately into CHO cells. At 24 hours post-transfection, an equal amount of Chroma-Glo™ Reagent was added to the media and cells. The relative light units were collected using a Berthold Centro LB 960. Expression levels are shown as percentages of the corresponding Control vectors. The data show greatly reduced relative expression of the synthetic reporter in the absence of promoter. **Panel A.** The luciferase expression from the pGL3-Enhancer Vector with the synthetic Chroma-Luc™ genes was reduced to an average of 1.8% of control. **Panel B.** The basal level of luciferase transcription from the pGL3-Basic Vector with the synthetic Chroma-Luc™ genes was reduced to an average of 0.6% of control. These experiments were repeated with similar results.

Apart from the different colors of luminescence, the significant differences among the three Chroma-Luc™ genes are at the level of DNA. CBG99*luc* and CBR*luc* have 99% DNA identity, while CBG68*luc* and CBR*luc* have 68.9% DNA identity. The two green-emitting luciferases are expressed from CBG68*luc* and CBG99*luc* and are 69.2% identical. The Chroma-Luc™ genes CBR*luc* and CBG68*luc* are most dissimilar genetically while retaining a high degree of protein homology. These two genes express proteins that are 98.3% identical. The CBR*luc* and CBG99*luc* genes possess a high degree of protein similarity, approximately 98.5%. Finally the proteins expressed by CBG68*luc* and CBG99*luc* genes are 99.6% homologous; there is one amino acid difference.

4. Chroma-Luc™ Reporter Vector Backbones

The Chroma-Luc™ Basic Vector series includes pCBR-Basic (Cat.# E1411), pCBG68-Basic (Cat.# E1431) and pCBG99-Basic (Cat.# E1451). No promoter or enhancer sequence has been added to the Basic series. The vector backbone is from the pGL3 series and contains a multiple cloning region for cloning regulator elements of interest.

The Chroma-Luc™ Control Vector series includes pCBR-Control (Cat.# E1421), pCBG68-Control (Cat.# E1441) and pCBG99-Control (Cat.# E1461). These vectors contain an SV40 promoter and SV40 enhancer and have the same vector backbone as the Basic series.

SV40 Promoter and Enhancer

The Control Vectors possess an SV40 Early Promoter and an Enhancer, which have been shown to provide strong, constitutive expression in a variety of cell types. The Basic Vectors do not possess these elements. The Control Vectors contain the SV40 origin of replication within the SV40 promoter. The SV40 origin of replication results in transient, episomal replication in cells expressing the SV40 large T antigen such as COS-1 and COS-7 cells (1).

SV40 poly(A)

Polyadenylation signals cause the termination of transcription by RNA polymerase II and signal the addition of approximately 200–250 adenosine residues to the 3´ end of the RNA transcription (2). Polyadenylation has been shown to enhance RNA stability and translation efficiency (3,4).

f1 Origin of Replication

To provide the ssDNA template (i.e., for use in mutagenesis) the Chroma-Luc™ 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. **Note:** Only the sequence of the f1 origin has been verified; no quality control assays were performed to test the functionality of this region.

5. Chroma-Luc™ Reporter Vector Maps and Sequence Information

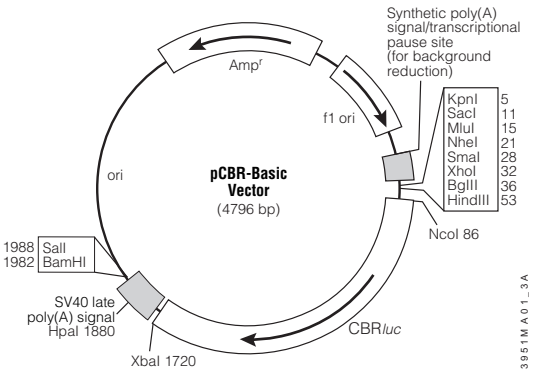

Figure 5. The pCBR-Basic Vector circle map. This vector contains: CBR*luc*, synthetic DNA sequence encoding the red luciferase enzyme; Amp^r, gene conferring ampicillin resistance in *E. coli*; ori, origin of plasmid replication in *E. coli*. Arrows within the CBR*luc* and Amp^r genes indicate the direction of functionality.

pCBR-Basic Vector Sequence Reference Points:

Multiple cloning region	1–58
CBR*luc* reporter gene	88–1716
SV40 late poly(A) region	1750–1971
Reporter Vector Primer 4 (RV Primer 4)	2039–2058
β-lactamase (Amp^r) coding region	3058–3918
f1 origin of replication	4050–4505
Synthetic poly(A) signal	4636–4789
Reporter Vector Primer 3 (RV Primer 3)	4738–4757

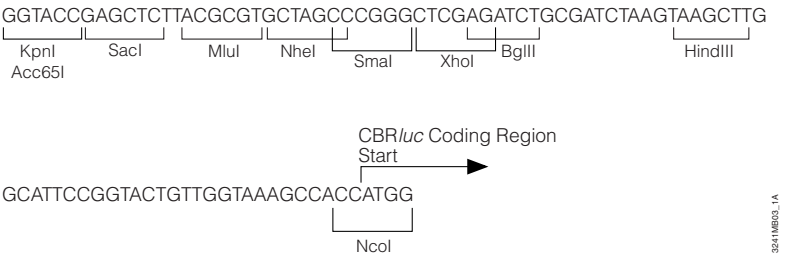

Figure 6. Multiple cloning region of the pCBR-Basic Vector.

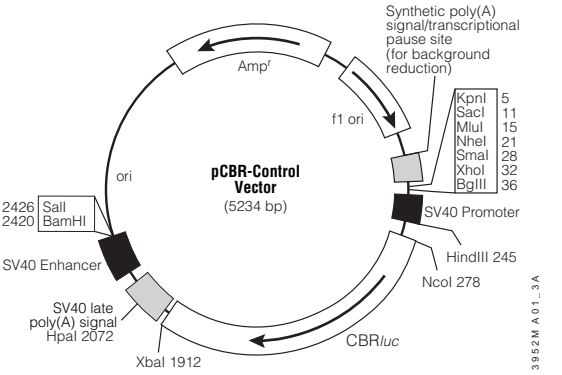

Figure 7. The pCBR-Control Vector circle map. This vector contains: CBR*luc*, synthetic cDNA sequence encoding the red luciferase enzyme; Amp^r, gene conferring ampicillin resistance in *E. coli*; ori, origin of plasmid replication in *E. coli*. Arrows within the CBR*luc* and Amp^r genes indicate the direction of functionality.

pCBR-Control Vector Sequence Reference Points:

SV40 promoter	48–250
CBR*luc* reporter gene	280–1908
SV40 late poly(A) region	1942–2163
SV40 Enhancer	2183–2419
Reporter Vector Primer 4 (RV Primer 4)	2477–2496
β-lactamase (Amp^r) coding region	3496–4356
f1 origin of replication	4488–4943
Synthetic poly(A) signal	5074–5227
Reporter Vector Primer 3 (RV Primer 3)	5176–5195

Figure 8. Multiple cloning region of the pCBR-Control Vector.

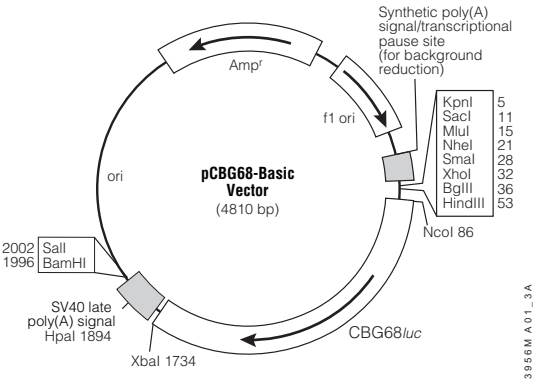

Figure 9. The pCBG68-Basic Vector circle map. This vector contains: CBG68*luc*, synthetic cDNA sequence encoding the green 68 luciferase enzyme; Amp^r, gene conferring ampicillin resistance in *E. coli*; ori, origin of plasmid replication in *E. coli*. Arrows within the CBG68*luc* and Amp^r genes indicate the direction of functionality.

pCBG68-Basic Vector Sequence Reference Points:

Multiple cloning region	1–58
CBG68*luc* Reporter gene	88–1716
SV40 late poly(A) region	1764–1985
Reporter Vector Primer 3 (RV Primer 3)	2053–2072
β-lactamase (Amp^r) coding region	3072–3932
f1 origin of replication	4064–4519
Synthetic poly(A) signal	4650–4803
Reporter Vector Primer 4 (RV Primer 4)	4752–4771

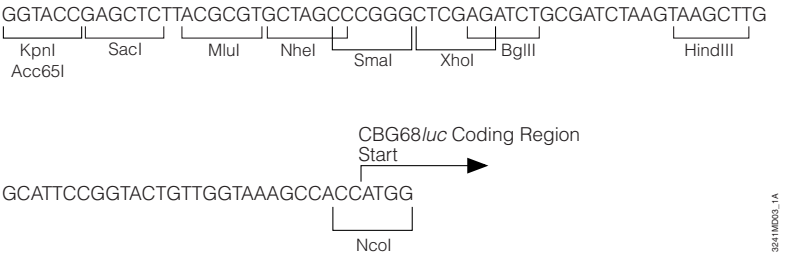

Figure 10. Multiple cloning region of the pCBG68-Basic Vector.

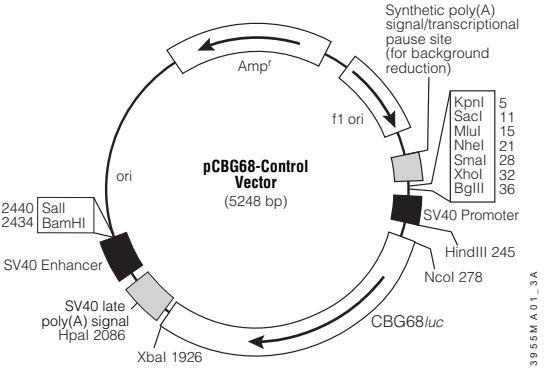

Figure 11. The pCBG68-Control Vector circle map. This vector contains: CBG68*luc*, synthetic DNA sequence encoding the green 68 luciferase enzyme; Amp^r, gene conferring amplicillin resistance in *E. coli*; ori, origin of plasmid replication in *E. coli*. Arrows within the CBG68*luc* and Amp^r genes indicate the direction of functionality.

pCBG68-Control Vector Sequence Reference Points:

SV40 promoter	48–250
CBG68*luc* Reporter gene	280–1908
SV40 late poly(A) region	1956–2177
SV40 Enhancer	2197–2433
Reporter Vector Primer 3 (RV Primer 3)	2491–2510
β-lactamase (Amp^r) coding region	3510–4370
f1 origin of replication	4502–4957
Synthetic poly(A) signal	5088–5241
Reporter Vector Primer 4 (RV Primer 4)	5190–5209

Figure 12. Multiple cloning region of the pCBG68-Control Vector.

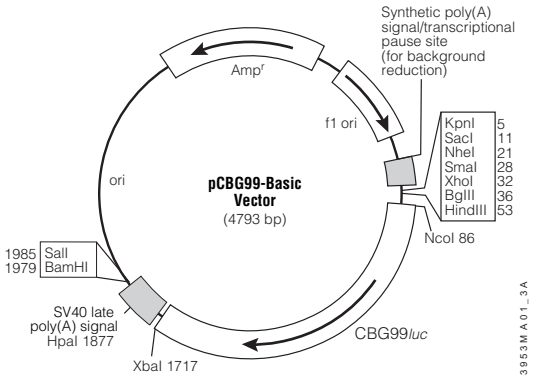

Figure 13. The pCBG99-Basic Vector circle map. This vector contains: CBG99*luc*, synthetic DNA sequence encoding the green 99 luciferase enzyme; Amp^r, gene conferring ampicillin resistance in *E. coli*; ori, origin of plasmid replication in *E. coli*. Arrows within the CBG99*luc* and Amp^r genes indicate the direction of functionality.

pCBG99-Basic Vector Sequence Reference Points:

Multiple cloning region	1–58
CBG99*luc* reporter gene	88–1716
SV40 late poly(A) region	1747–1968
Reporter Vector Primer 4 (RV Primer 4)	2036–2055
β-lactamase (Amp^r) coding region	3055–3915
f1 origin of replication	4047–4502
Synthetic poly(A) signal	4633–4786
Reporter Vector Primer 3 (RV Primer 3)	4735–4754

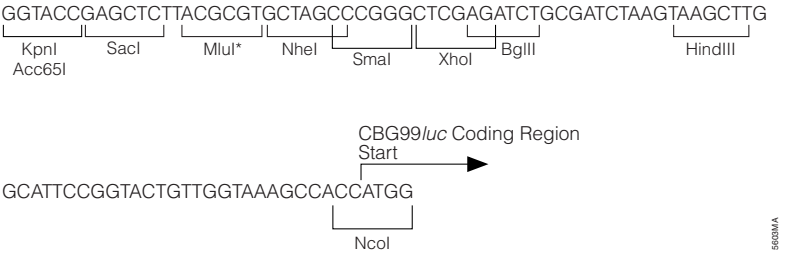

Figure 14. Multiple cloning region of the pCBG99-Basic Vector.
*The MluI site is also present in the CBG99*luc* gene and should not be used for cloning into the pCBG99-Basic Vector.

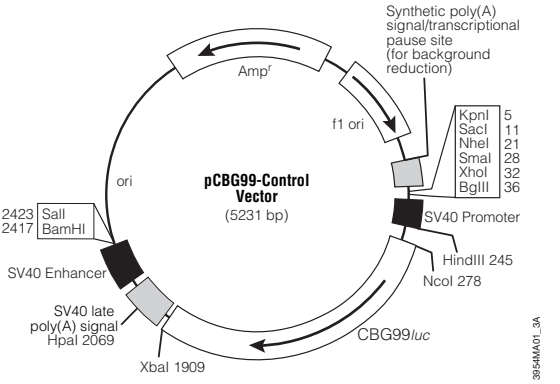

Figure 15. The pCBG99-Control Vector circle map. This vector contains: CBG99*luc*, synthetic DNA sequence encoding the green 99 luciferase enzyme; Amp^r, gene conferring amplicillin resistance in *E. coli*; ori, origin of plasmid replication in *E. coli*. Arrows within the CBG99*luc* and Amp^r genes indicate the direction of transcription.

pCBG99-Control Vector Sequence Reference Points:

SV40 promoter	48–250
CBG99*luc* Reporter gene	280–1908
SV40 late poly(A) region	1939–2160
SV40 Enhancer	2180–2416
Reporter Vector Primer 3 (RV Primer 3)	2474–2493
β-lactamase (Amp^r) coding region	3493–4353
f1 origin of replication	4485–4940
Synthetic poly(A) signal	5071–5224
Reporter Vector Primer 4 (RV Primer 4)	5173–5192

Figure 16. Multiple cloning region of the pCBG99-Control Vector.
*The MluI site is also present in the CBG99*luc* gene and should not be used for cloning into the pCBG99-Control Vector.

6. Emission Color Separation

In order to measure the different luminescent signals, filters must be used to separate the red and green Chroma-Luc™ signals.

6.A. Filter Choices

Promega has used 510/60 nm and 610 nm long-pass filters from Chroma Corporation (Chroma Part# D510/60 and E610LP, respectively) to successfully separate the red and green luminescent signals generated by the Chroma-Luc™ luciferases. These filters permit transmittance in the spectral region shown in Figure 17.

The 510/60 nm and 610 nm long-pass filter pair provide a balance between sensitivity and separation. This filter pair was chosen so that the two colored signals have the least amount of overlap, while permitting transmittance of the largest possible portion of each luminescent spectrum. Other filter pairs can be used if extreme separation or extreme sensitivity is critical. If extreme separation of signal is required, filter pairs may be chosen so that no green spectrum tailing is captured in the red signal. A 640 nm long-pass filter, for example, might be chosen for this application and combined with a 510/60 nm filter.

If extreme sensitivity is critical, filter pairs may be chosen so that more of each signal spectrum is transmitted. This means that more of the red luminescent signal will be transmitted through the red filter. Regardless of the filter set chosen, the luminescent signal transmittance through each filter must be calculated. Section 6.B describes the mathematical formulae that are required to separate the red and the green luminescent signals. These calculations must be performed regardless of filter choice and must be performed for each combination of filter pair and luminometer.

6.B. Data Analysis

Color Separation

Filter sets provide the ability to measure a consistent portion of the red or green luminescence generated by a sample. If the same filters are used for all measurements in an experiment, then it is not necessary to calculate the total luminescence generated by each luciferase. The relative proportion that is measured therefore can be used to calculate induction ratios and other experimentally relevant information.

Figure 17 shows the overlap of the spectra generated by the Chroma-Luc™ luciferase enzymes in the transmittance window of two filters. Filter corrections can be made to factor out the tail of the green luminescent signal from a red luminescent measurement. A single calculation of the filter corrections is adequate for all measurements using a single luminometer or CCD camera with a particular filter set. However, measurements using a different instrument or filter set require calculation of different correction factors.

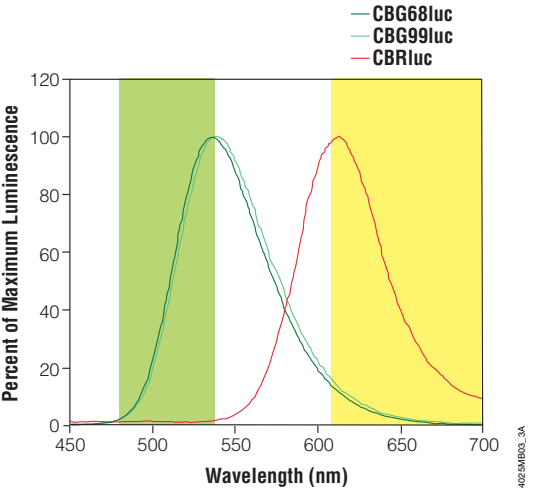

Figure 17. Emission spectra for Chroma-Luc™ luciferases. Separate populations of CHO cells were transiently transfected with the Chroma-Luc™ Vectors. CBR*luc* is the DNA coding for the red-emitting luciferase, and CBG68*luc* and CBG99*luc* are the two different DNA sequences coding for the green-emitting luciferase. At 24 hours post-transfection the cells were removed by trypsin treatment, lysed by the addition of Glo Lysis Buffer (Cat.# E2661) and an equal amount of Chroma-Glo™ Reagent. The spectra data were captured using a Spex Fluorolog®-2 spectrofluorometer with the excitation source off. Emission data were collected from 450–700 nm. Maximum emission for CBG68*luc* and CBG99*luc* is 537 nm, while CBR*luc* has a maximum emission of 613 nm. The light block indicates the maximal transmittance range of a 610 nm long-pass (greater than 610 nm) filter. The dark block indicates the maximal transmittance range of a 510/60 (510±30 nm) filter.

Calculating the filter corrections (calibration constants) requires a one-time measurement of two samples of cells, one expressing only a red-emitting luciferase and the other expressing only a green-emitting luciferase. A total of 6 one-time measurements (Table 2) must be made to determine calibration constants used to normalize filter and detector efficiencies. The calibration constants should not change over time; however, these must be recalculated upon substituting filter sets, luminometers or detectors. After these calibration constants are known, the corrected red and green luminescence values (R´ and G´) can be determined using two experimental luminescence values (Lrf and Lgf, see Table 3) using the equations provided below.

$$R' = \frac{Lrf - [Lgf \times (Grf/Ggf)]}{(Rrf/R) - (Rgf/R) \times (Grf/Ggf)}$$

The corrected amount of red luminescence in a mixed sample, R´, is calculated by subtracting the portion of the green luminescence measured using the red filter [Lgf × (Grf/Ggf)] from the amount of total luminescence measured with the red filter (Lrf) that is normalized for filter efficiencies [(Rrf/R) – (Rgf/R) × (Grf/Ggf)].

$$G' = \frac{Lgf - [R' \times (Rgf/R)]}{(Ggf/G)}$$

The corrected amount of green luminescence from the same sample, G´, is calculated as the amount of total luminescence measured with the green filter (Lgf) minus the amount of corrected red luminescence (R´) measured with the green filter [R´ × (Rgf/R)] that has been normalized for filter efficiencies (Ggf/G).

Measurements for the calibration constants Rrf/R, Rgf/R, Grf/Ggf and Ggf/G must be made every time filter sets and/or detection equipment changes.

To assist you in the determination of corrected luminescence values, Promega has prepared the Chroma-Luc™ Calculator, a PC-based spreadsheet. This tool is available for download at: **www.promega.com/techserv/tools/**

Table 2. Descriptions of Initial Measurements Required for Calibration Constants Used to Determine Corrected Experimental Luminescence Values from Overlapping Chroma-Luc™ Spectra.

Name	Symbol	Measurement
Total Red Luminescence, no filter	R	No filter used. Total luminescence measured from the red-emitting luciferase.
Total Green Luminescence, no filter	G	No filter used. Total luminescence measured from the green-emitting luciferase.
Red Luminescence, using red filter	Rrf	Luminescence measured from the red-emitting luciferase. Red filter used.
Green Luminescence, using red filter	Grf	Luminescence measured from the green-emitting luciferase. Red filter used.
Red Luminescence, using green filter	Rgf	Luminescence measured from the red-emitting luciferase. Green filter used.
Green Luminescence, using green filter	Ggf	Luminescence measured from the green-emitting luciferase. Green filter used.

Table 3. Experimental Luminescence Values Required to Determine Corrected Luminescence Values, R´ and G´.

Name	Symbol	Measurement
Experimental luminescence, red filter	Lrf	Experimental luminescence measured with the red filter.
Experimental luminescence, green filter	Lgf	Experimental luminescence measured with the green filter.

7. Appendix

7.A. pCBR-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. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY258591) and online at: **www.promega.com/vectors/**

Table 4. Restriction Enzymes That Cut the pCBR-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	314
AccB7I	1	138
AccI	2	794, 1989
Acc65I	1	1
AcyI	2	311, 3668
AflIII	3	5, 1590, 2238
Alw26I	3	859, 3192, 3968
Alw44I	2	2552, 3798
AlwNI	1	2654
ApaI	1	1228
AspHI	5	11, 177, 2556, 3717, 3802
AvaI	3	26, 32, 1135
AvaII	2	3269, 3491
BamHI	1	1982
BanII	5	11, 33, 973, 1228, 4209
BbsI	3	453, 989, 2067
BclI	2	882, 1400
BglI	2	3251, 4519
BglII	1	36
BsaI	2	859, 3192
BsaAI	1	4280
BsaBI	1	1981
BsaHI	2	311, 3668
Bsp120I	1	1224
BspHI	2	2958, 3966
BspMI	1	4759
BsrBRI	1	1981
BssSI	3	976, 2411, 3795
BstXI	1	292
BstZI	3	1733, 1737, 4629
ClaI	3	1975, 4687, 4791
DraI	5	621, 1941, 2997, 3016, 3708
DraII	1	1258
DraIII	2	1006, 4283
DrdI	2	2346, 4327
DsaI	1	86
EaeI	5	1055, 1733, 1737, 3519, 4629
EagI	3	1733, 1737, 4629
EarI	3	2122, 3926, 4564
EclHKI	1	3131
Eco47III	1	2114
Eco52I	3	1733, 1737, 4629
EcoICRI	1	9
FseI	1	1739
FspI	2	3353, 4526
HaeII	4	2116, 2486, 4125, 4133
HgaI	5	718, 2349, 2927, 3657, 4058
HincII	4	399, 1635, 1880, 1990
HindII	4	399, 1635, 1880, 1990
HindIII	1	53
HpaI	1	1880
Hsp92I	2	311, 3668
KpnI	1	5
MluI	1	15
NciI	5	27, 28, 2618, 3314, 3665

Table 4. Restriction Enzymes That Cut the pCBR-Basic Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NcoI	1	86	**SalI**	1	1988
NheI	1	21	**ScaI**	3	1609, 3611, 4694
NotI	1	4629	**SinI**	2	3269, 3491
NruI	2	1205, 1472	**SmaI**	1	28
NsiI	1	1468	SrfI	1	28
NspI	1	2242	**SspI**	5	477, 555, 3935, 4488, 4603
PaeR7I	1	32			
PflMI	1	138	**StyI**	3	86, 206, 1301
Ppu10I	1	1464	**VspI**	1	3303
PshAI	1	2053	**XbaI**	1	1720
PspAI	1	26	**XhoI**	1	32
PvuI	2	3501, 4547	**XmaI**	1	26
PvuII	2	768, 1432	**XmnI**	1	3730
SacI	1	11			

Table 5. Restriction Enzymes That Do Not Cut the pCBR-Basic Vector.

AccIII	BsrGI	**EcoRI**	PpuMI	SplI
AflII	**BssHII**	**EcoRV**	Psp5II	Sse8387I
AgeI	Bst1107I	EheI	**PstI**	**StuI**
AscI	**Bst98I**	**I-PpoI**	RsrII	SwaI
AvrII	**BstEII**	KasI	**SacII**	**Tth111I**
BalI	**Bsu36I**	**NarI**	**SfiI**	XcmI
BbeI	**CspI**	**NdeI**	**SgfI**	
BbrPI	**Csp45I**	PacI	SgrAI	
BbuI	Eco72I	PinAI	**SnaBI**	
BlpI	Eco81I	PmeI	**SpeI**	
Bpu1102I	EcoNI	PmlI	**SphI**	

Table 6. Restriction Enzymes That Cut the pCBR-Basic Vector 6 or More Times.

AciI	**BstOI**	**HinfI**	**MspI**	ScrFI
AluI	BstUI	**HpaII**	**MspA1I**	SfaNI
BanI	**CfoI**	HphI	**NaeI**	**TaqI**
BbvI	Cfr10I	**Hsp92II**	**NdeII**	TfiI
BsaJI	**DdeI**	MaeI	**NgoMIV**	**Tru9I**
BsaOI	**DpnI**	MaeII	NlaIII	**XhoII**
BsaMI	DpnII	MaeIII	NlaIV	
Bsp1286I	**FokI**	**MboI**	PleI	
BsrI	Fnu4HI	**MboII**	**RsaI**	
BsrSI	**HaeIII**	MnlI	**Sau3AI**	
Bst71I	**HhaI**	MseI	Sau96I	

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

7.B. pCBR-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. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY258592) and online at: **www.promega.com/vectors/**

Table 7. Restriction Enzymes That Cut the pCBR-Control Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	506
AccB7I	1	330
AccI	2	986, 2427
Acc65I	1	1
AcyI	2	503, 4106
AflIII	3	15, 1782, 2676
Alw26I	3	1051, 3630, 4406
Alw44I	2	2990, 4236
AlwNI	1	3092
ApaI	1	1420
AspHI	5	11, 369, 2994, 4155, 4240
AvaI	3	26, 32, 1327
AvaII	2	3707, 3929
AvrII	1	229
BamHI	1	2420
BanII	5	11, 33, 1165 1420, 4647
BbsI	3	645, 1181, 2505
BbuI	2	2278, 2350
BclI	2	1074, 1592
BglI	3	182, 3689, 4957
BglII	1	36
BsaI	2	1051, 3630
BsaAI	1	4718
BsaBI	2	48, 2173
BsaHI	2	503, 4106
Bsp120I	1	1416
BspHI	2	3396 , 4404
BspMI	1	5197
BsrBRI	2	48, 2173
BssSI	3	168, 2849, 4233
BstXI	1	484
BstZI	3	1925, 1929, 5067
ClaI	3	2167, 5125, 5229
DraI	5	813, 2133, 3435, 3454, 4146
DraII	1	1450
DraIII	2	1198, 4721
DrdI	2	2784, 4765
DsaI	1	278
EaeI	5	1247, 1925, 1929, 3957, 5067
EagI	3	1925, 1929, 5067
EarI	3	2560, 4364, 5002
EclHKI	1	3569
Eco47III	1	2552
Eco52I	3	1925, 1929, 5067
EcoICRI	1	9
FseI	1	1931
FspI	2	3791, 4964
HaeII	4	2554, 2924, 4563, 4571
HgaI	5	910, 2787, 3365, 4095, 4496
HincII	4	591, 1827, 2072, 2428
HindII	4	591, 1827, 2072, 2428
HindIII	1	245
HpaI	1	2072
Hsp92I	2	503, 4106
KpnI	1	5
MluI	1	15
NciI	5	27, 28, 3056, 3752, 4103

Table 7. Restriction Enzymes That Cut the pCBR-Control Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NcoI	1	278	**SfiI**	1	182
NheI	1	21	**SinI**	2	3707, 3929
NotI	1	5067	**SmaI**	1	28
NruI	2	1397, 1664	**SphI**	2	2278, 2350
NsiI	3	1660, 2276, 2348,	SrfI	1	28
NspI	3	2278, 2350, 2680	**SspI**	5	669, 747, 4373,
PaeR7I	1	32			4926, 5041
PflMI	1	330	**StuI**	1	228
Ppu10I	3	1656, 2272, 2344	**StyI**	4	229, 278, 398,
PshAI	1	2491			1493
PspAI	1	26	**VspI**	1	3741
PvuI	2	3939, 4985	**XbaI**	1	1912
PvuII	2	960, 1624	**XhoI**	1	32
SacI	1	11	**XmaI**	1	26
SalI	1	2426	**XmnI**	1	4168
ScaI	3	1801, 4049, 5132			

Table 8. Restriction Enzymes That Do Not Cut the pCBR-Control Vector.

AccIII	BsrGI	EcoNI	PmeI	**SpeI**
AflII	**BssHII**	**EcoRI**	PmlI	SplI
AgeI	Bst1107I	**EcoRV**	PpuMI	Sse8647I
AscI	**Bst98I**	EheI	Psp5II	SwaI
BalI	**BstEII**	**I-PpoI**	**PstI**	**Tth111I**
BbeI	**Bsu36I**	KasI	RsrII	XcmI
BbrPI	**CspI**	**NarI**	**SacII**	
BlpI	**Csp45I**	**NdeI**	**SgfI**	
Bpu1102I	Eco72I	PacI	SgrAI	
Bpu1268I	Eco81I	PinAI	**SnaBI**	

Table 9. Restriction Enzymes That Cut the pCBR-Control Vector 6 or More Times.

AciI	Bst71I	**HhaI**	MseI	Sau96I
AluI	**BstOI**	**HinfI**	**MspI**	ScrFI
BanI	BstUI	**HpaII**	**MspA1I**	SfaNI
BbvI	**CfoI**	HphI	**NaeI**	**TaqI**
BsaOI	Cfr10I	**Hsp92II**	**NdeII**	TfiI
BsaJI	**DdeI**	MaeI	**NgoMIV**	**Tru9I**
BsaMI	**DpnI**	MaeII	NlaIII	**XhoII**
BsmI	DpnII	MaeIII	NlaIV	
Bsp1286I	Fnu4HI	**MboI**	PleI	
BsrI	**FokI**	**MboII**	**RsaI**	
BsrSI	**HaeIII**	MnlI	**Sau3AI**	

Note: The enzymes listed in bold face type are available from Promega Corporation.

7.C. pCBG68-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. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY258593) and online at: **www.promega.com/vectors/**

Table 10. Restriction Enzymes That Cut the pCBG68-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	218
AccI	2	1571, 2003
Acc65I	1	1
AcyI	3	215, 1622, 3682
AflIII	2	15, 2252
AgeI	1	1657
Alw44I	2	2566, 3812
AlwNI	1	2668
ApaI	2	1228, 1261
AvaI	2	26, 32
AvaII	2	3283, 3505
BalI	1	1036
BamHI	1	1996
BanII	5	11, 33, 1228, 1261, 4223
BbsI	3	149, 1714, 2081
BclI	1	932
BglI	2	3265, 4533
BglII	1	36
BlpI	1	1061
Bpu1102I	1	1061
BsaI	3	1201, 1272, 3206
BsaAI	2	1281, 4294
BsaBI	1	1995
BsaHI	3	215, 1622, 3682
BsaMI	3	60, 1815, 1908
BsmI	3	60, 1815, 1908
Bsp120I	2	1224, 1257
BspHI	3	1725, 2972, 3980
BspMI	1	4773
BsrBRI	1	1995
BssSI	4	208, 752, 2425, 3809

Enzyme	# of Sites	Location
BstXI	2	292, 1044
BstZI	3	1747, 1751, 4643
ClaI	3	1989, 4701, 4805
Csp45I	1	254
DraI	4	1955, 3011, 3030, 3722
DraII	2	1257, 1258
DraIII	1	4297
DrdI	3	1480, 2360, 4341
DsaI	2	86, 558
EagI	3	1747, 1751, 4643
EarI	4	172, 2136, 3940, 4578
EclHKI	1	3145
Eco47III	1	2128
Eco52I	3	1747, 1751, 4643
EcoICRI	1	9
FseI	1	1753
FspI	2	3367, 4540
HincII	3	981, 1894, 2004
HindII	3	981, 1894, 2004
HindIII	1	53
HpaI	1	1894
Hsp92I	3	215, 1622, 3682
KpnI	1	5
MluI	1	15
MspA1I	5	154, 1088, 2594, 2839, 3780
NaeI	3	1751, 2122, 4191
NcoI	1	86
NgoMIV	3	1749, 2120, 4189
NheI	1	21
NotI	1	4643

Table 10. Restriction Enzymes That Cut the pCBG68-Basic Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NspI	1	2256	SrfI	1	28
PaeR7I	1	32	**SspI**	3	3949, 4502, 4617
PinAI	1	1657	**StyI**	1	86
PshAI	1	2067	TfiI	4	403, 1636, 2227, 4698
PspAI	1	26			
PvuI	3	1386, 3515, 4561	**VspI**	1	3317
SacI	1	11	**XbaI**	1	1734
SalI	1	2002	**XhoI**	1	32
ScaI	2	3625, 4708	**XmaI**	1	26
SinI	2	3283, 3505	**XmnI**	1	3744
SmaI	1	28			

Table 11. Restriction Enzymes That Do Not Cut the pCBG68-Basic Vector.

AccB7I	**Bst98I**	KasI	Psp5II	SplI
AccIII	**BstEII**	**NarI**	**PstI**	Sse8387I
AflII	**Bsu36I**	**NdeI**	**PvuII**	**StuI**
AscI	**CspI**	**NruI**	RsrII	SwaI
AvrII	Eco72I	**NsiI**	**SacII**	**Tth111I**
BbeI	Eco81I	PacI	**SfiI**	XcmI
BbrPI	EcoNI	PflMI	**SgfI**	
BbuI	**EcoRI**	PmeI	SgrAI	
BsrGI	**EcoRV**	PmlI	**SnaBI**	
BssHII	EheI	Ppu10I	**SpeI**	
Bst1107I	**I-PpoI**	PpuMI	**SphI**	

Table 12. Restriction Enzymes That Cut the pCBG68-Basic Vector 6 or More Times.

AciI	Bst71I	**HaeII**	**MboI**	**Sau3AI**
AluI	**BstOI**	**HaeIII**	**MboII**	Sau96I
Alw26I	BstUI	HgaI	MnlI	ScrFI
AspHI	**CfoI**	**HhaI**	MseI	SfaNI
BanI	Cfr10I	**HinfI**	**MspI**	**TaqI**
BbvI	**DdeI**	**HpaII**	**NciI**	**Tru9I**
BsaOI	**DpnI**	HphI	**NdeII**	**XhoII**
BsaJI	DpnII	**Hsp92II**	NlaIII	
Bsp1286I	EaeI	MaeI	NlaIV	
BsrI	Fnu4HI	MaeII	PleI	
BsrSI	**FokI**	MaeIII	**RsaI**	

Note: The enzymes listed in bold face type are available from Promega Corporation.

7.D. pCBG68-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. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY258594) and online at: **www.promega.com/vectors/**

Table 13. Restriction Enzymes That Cut the pCBG68-Control Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	410
AccI	2	1763, 2441
Acc65I	1	1
AcyI	3	407, 1814, 4120
AflIII	2	15, 2690
AgeI	1	1849
Alw44I	2	3004, 4250
AlwNI	1	3106
ApaI	2	1420, 1453
AvaI	2	26, 32
AvaII	2	3721, 3943
AvrII	1	229
BalI	1	1228
BamHI	1	2434
BanII	5	11, 33, 1420, 1453, 4661
BbsI	3	341, 1906, 2519
BbuI	2	2292, 2364
BclI	1	1124
BglI	3	182, 3703, 4971
BglII	1	36
BlpI	1	1253
Bpu1102I	1	1253
BsaI	3	1393, 1464, 3644
BsaAI	2	1473, 4732
BsaBI	2	48, 2187
BsaHI	3	407, 1814, 4120
BsaMI	3	252, 2007, 2100
BsmI	3	252, 2007, 2100
Bsp120I	2	1416, 1449
BspHI	3	1917, 3410, 4418
BspMI	1	5211
BsrBRI	2	48, 2187

Enzyme	# of Sites	Location
BssSI	4	400, 944, 2863, 4247
BstXI	2	484, 1236
BstZI	3	1939, 1943, 5081
ClaI	3	2181, 5139, 5243
Csp45I	1	446
DraI	4	2147, 3449, 3468, 4160
DraII	2	1449, 1450
DraIII	1	4735
DrdI	3	1672, 2798, 4779
DsaI	2	278, 750
EagI	3	1939, 1943, 5081
EarI	4	364, 2574, 4378, 5016
EclHKI	1	3583
Eco47III	1	2566
Eco52I	3	1939, 1943, 5081
EcoICRI	1	9
FseI	1	1945
FspI	2	3805, 4978
HincII	3	1173, 2086, 2442
HindII	3	1173, 2086, 2442
HindIII	1	245
HpaI	1	2086
Hsp92I	3	407, 1814, 4120
KpnI	1	5
MluI	1	15
NaeI	3	1943, 2560, 4629
NcoI	1	278
NgoMIV	3	1941, 2558, 4627
NheI	1	21
NotI	1	5081

Table 13. Restriction Enzymes That Cut the pCBG68-Control Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NsiI	2	2290, 2362	**SmaI**	1	28
NspI	3	2292, 2364, 2694	**SphI**	2	2292, 2364
PaeR7I	1	32	SrfI	1	28
PinAI	1	1849	**SspI**	3	4387, 4940, 5055
Ppu10I	2	2286, 2358	**StuI**	1	228
PshAI	1	2505	**StyI**	2	229, 278
PspAI	1	26	TfiI	4	595, 1828, 2665, 5136
PvuI	3	1578, 3953, 4999			
SacI	1	11	**VspI**	1	3755
SalI	1	2440	**XbaI**	1	1926
ScaI	2	4063, 5146	**XhoI**	1	32
SfiI	1	182	**XmaI**	1	26
SinI	2	3721, 3943	**XmnI**	1	4182

Table 14. Restriction Enzymes That Do Not Cut the pCBG68-Control Vector.

AccB7I	**Bst98I**	EheI	PmlI	**SnaBI**
AccIII	**BstEII**	**I-PpoI**	PpuMI	**SpeI**
AflII	**Bsu36I**	KasI	Psp5II	SplI
AscI	**CspI**	**NarI**	**PstI**	Sse8387I
BbeI	Eco72I	**NdeI**	**PvuII**	SwaI
BbrPI	Eco81I	**NruI**	RsrII	**Tth111I**
BsrGI	EcoNI	PacI	**SacII**	XcmI
BssHII	**EcoRI**	PflMI	**SgfI**	
Bst1107I	**EcoRV**	PmeI	SgrAI	

Table 15. Restriction Enzymes That Cut the pCBG68-Control Vector 6 or More Times.

AciI	Bst71I	**HaeII**	**MboI**	**RsaI**
AluI	**BstOI**	**HaeIII**	**MboII**	**Sau3AI**
Alw26I	BstUI	HgaI	MnlI	Sau96I
AspHI	**CfoI**	**HhaI**	MseI	ScrFI
BanI	Cfr10I	**HinfI**	**MspI**	SfaNI
BbvI	**DdeI**	**HpaII**	**MspA1I**	**TaqI**
BsaOI	**DpnI**	HphI	**NciI**	**Tru9I**
BsaJI	DpnII	**Hsp92II**	**NdeII**	**XhoII**
Bsp1286I	EaeI	MaeI	NlaIII	
BsrI	Fnu4HI	MaeII	NlaIV	
BsrSI	**FokI**	MaeIII	PleI	

Note: The enzymes listed in bold face type are available from Promega Corporation.

7.E. pCBG99-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. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY258595) and online at: **www.promega.com/vectors/**

Table 16. Restriction Enzymes That Cut the pCBG99-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	314
AccB7I	1	138
AccI	2	794, 1986
Acc65I	1	1
AcyI	2	311, 3665
AflIII	4	15, 753, 1590, 2235
Alw26I	3	859, 3189, 3965
Alw44I	2	2549, 3795
AlwNI	1	2651
ApaI	1	1228
AspHI	5	11, 177, 2553, 3714, 3799
AvaI	3	26, 32, 1135
AvaII	2	3266, 3488
BamHI	1	1979
BanII	5	11, 33, 973, 1228, 4206
BbsI	3	453, 989, 2064
BclI	2	882, 1400
BglI	2	3248, 4516
BglII	1	36
BsaI	2	859, 3189
BsaAI	1	4277
BsaBI	1	1978
BsaHI	2	311, 3665
Bsp120I	1	1224
BspHI	2	2955, 3963
BspMI	1	4756
BsrBRI	1	1978
BssSI	3	976, 2408, 3792
BstXI	1	292
BstZI	3	1730, 1734, 4626

Enzyme	# of Sites	Location
ClaI	3	1972, 4684, 4788
DraI	5	621, 1938, 2994, 3013, 3705
DraII	1	1258
DraIII	2	1006, 4280
DrdI	2	2343, 4324
DsaI	1	86
EaeI	5	1055, 1730, 1734, 3516, 4626
EagI	3	1730, 1734, 4626
EarI	3	2119, 3923, 4561
EclHKI	1	3128
Eco47III	2	1119, 2111
Eco52I	3	1730, 1734, 4626
EcoICRI	1	9
FseI	1	1736
FspI	2	3350, 4523
HaeII	5	1121, 2113, 2483, 4122, 4130
HgaI	5	718, 2346, 2924, 3654, 4055
HincII	4	399, 1635, 1877, 1987
HindII	4	399, 1635, 1877, 1987
HindIII	1	53
HpaI	1	1877
Hsp92I	2	311, 3665
KpnI	1	5
MluI	2	15, 753
NciI	5	27, 28, 2615, 3311, 3662
NcoI	1	86

Table 16. Restriction Enzymes That Cut the pCBG99-Basic Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NheI	1	21	**SalI**	1	1985
NotI	1	4626	**ScaI**	3	1609, 3608, 4691
NruI	2	1205, 1472	**SinI**	2	3266, 3488
NsiI	1	1468	**SmaI**	1	28
NspI	1	2239	SrfI	1	28
PaeR7I	1	32	**SspI**	5	477, 555, 3932, 4485, 4600
PflMI	1	138			
Ppu10I	1	1464	**StyI**	3	86, 206, 1301
PshAI	1	2050	**VspI**	1	3300
PspAI	1	26	**XbaI**	1	1717
PvuI	2	3498, 4544	**XhoI**	1	32
PvuII	2	768, 1432	**XmaI**	1	26
SacI	1	11	**XmnI**	1	3727

Table 17. Restriction Enzymes That Do Not Cut the pCBG99-Basic Vector.

AccIII	Bpu1102I	Eco81I	PinAI	SgrAI
AflII	BsrGI	EcoNI	PmeI	**SnaBI**
AgeI	**BssHII**	**EcoRI**	PmlI	**SpeI**
AscI	Bst1107I	**EcoRV**	PpuMI	**SphI**
AvrII	**Bst98I**	EheI	Psp5II	SplI
BalI	**BstEII**	**I-PpoI**	**PstI**	Sse8387I
BbeI	**Bsu36I**	KasI	RsrII	**StuI**
BbrPI	**CspI**	**NarI**	**SacII**	SwaI
BbuI	**Csp45I**	**NdeI**	**SfiI**	**Tth111I**
BlpI	Eco72I	PacI	**SgfI**	XcmI

Table 18. Restriction Enzymes That Cut the pCBG99-Basic Vector 6 or More Times.

AciI	Bst71I	**HhaI**	MseI	Sau96I
AluI	**BstOI**	**HinfI**	**MspI**	ScrFI
BanI	BstUI	**HpaII**	**MspA1I**	SfaNI
BbvI	**CfoI**	HphI	**NaeI**	**TaqI**
BsaOI	Cfr10I	**Hsp92II**	**NdeII**	TfiI
BsaJI	**DdeI**	MaeI	**NgoMIV**	**Tru9I**
BsaMI	**DpnI**	MaeII	NlaIII	**XhoII**
BsmI	DpnII	MaeIII	NlaIV	
Bsp1286I	Fnu4HI	**MboI**	PleI	
BsrI	**FokI**	**MboII**	**RsaI**	
BsrSI	**HaeIII**	MnlI	**Sau3AI**	

Note: The enzymes listed in bold face type are available from Promega Corporation.

7.F. pCBG99-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. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY258596) and online at: **www.promega.com/vectors/**

Table 19. Restriction Enzymes That Cut the pCBG99-Control Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	506
AccB7I	1	330
AccI	2	986, 2424
Acc65I	1	1
AcyI	2	503, 4103
AflIII	4	15, 945, 1782, 2673
Alw26I	3	1051, 3627, 4403
Alw44I	2	2987, 4233
AlwNI	1	3089
ApaI	1	1420
AspHI	5	11, 369, 2991, 4152, 4237
AvaI	3	26, 32, 1327
AvaII	2	3704, 3926
AvrII	1	229
BamHI	1	2417
BanII	5	11, 33, 1165, 1420, 4644
BbsI	3	645, 1181, 2502
BbuI	2	2275, 2347
BclI	2	1074, 1592
BglI	3	182, 3686, 4954
BglII	1	36
BsaI	2	1051, 3627
BsaAI	1	4715
BsaBI	2	48, 2170
BsaHI	2	503, 4103
Bsp120I	1	1416
BspHI	2	3393, 4401
BspMI	1	5194
BsrBRI	2	48, 2170
BssSI	3	1168, 2846, 4230
BstXI	1	484
BstZI	3	1922, 1926, 5064
ClaI	3	2164, 5122, 5226
DraI	5	813, 2130, 3432, 3451, 4143
DraII	1	1450
DraIII	2	1198, 4718
DrdI	2	2781, 4762
DsaI	1	278
EaeI	5	1247, 1922, 1926, 3954, 5064
EagI	3	1922, 1926, 5064
EarI	3	2557, 4361, 4999
EclHKI	1	3566
Eco47III	2	1311, 2549
Eco52I	3	1922, 1926, 5064
EcoICRI	1	9
FseI	1	1928
FspI	2	3788, 4961
HaeII	5	1313, 2551, 2921, 4560, 4568
HgaI	5	910, 2784, 3362, 4092, 4493
HincII	4	591, 1827, 2069, 2425
HindII	4	591, 1827, 2069, 2425
HindIII	1	245
HpaI	1	2069
Hsp92I	2	503, 4103
KpnI	1	5
MluI	2	15, 945
NciI	5	27, 28, 3053, 3749, 4100
NcoI	1	278

Table 19. Restriction Enzymes That Cut the pCBG99-Control Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NheI	1	21	**SfiI**	1	182
NotI	1	5064	**SinI**	2	3704, 3926
NruI	2	1397, 1664	**SmaI**	1	28
NsiI	3	1660, 2273, 2345	**SphI**	2	2275, 2347
NspI	3	2275, 2347, 2677	SrfI	1	28
PaeR7I	1	32	**SspI**	5	669, 747, 4370,
PflMI	1	330			4923, 5038
Ppu10I	3	1656, 2269, 2341	**StuI**	1	228
PshAI	1	2488	**StyI**	4	229, 278, 398,
PspAI	1	26			1493
PvuI	2	3936, 4982	**VspI**	1	3738
PvuII	2	960, 1624	**XbaI**	1	1909
SacI	1	11	**XhoI**	1	32
SalI	1	2423	**XmaI**	1	26
ScaI	3	1801, 4046, 5129	**XmnI**	1	4165

Table 20. Restriction Enzymes That Do Not Cut the pCBG99-Control Vector.

AccIII	BsrGI	Eco81I	PacI	**SgfI**
AflII	**BssHII**	EcoNI	PinAI	SgrAI
AgeI	Bst1107I	**EcoRI**	PmeI	**SnaBI**
AscI	**Bst98I**	**EcoRV**	PmlI	**SpeI**
BalI	**BstEII**	EheI	PpuMI	SplI
BbeI	**Bsu36I**	**I-PpoI**	Psp5II	Sse8387I
BbrPI	**CspI**	KasI	**PstI**	SwaI
BlpI	**Csp45I**	**NarI**	RsrII	**Tth111I**
Bpu1102I	Eco72I	**NdeI**	**SacII**	XcmI

Table 21. Restriction Enzymes That Cut the pCBG99-Control Vector 6 or More Times.

AciI	Bst71I	**HhaI**	MseI	Sau96I
AluI	**BstOI**	**HinfI**	**MspI**	ScrFI
BanI	BstUI	**HpaII**	**MspA1I**	SfaNI
BbvI	**CfoI**	HphI	**NaeI**	**TaqI**
BsaOI	Cfr10I	**Hsp92II**	**NdeII**	TfiI
BsaJI	**DdeI**	MaeI	**NgoMIV**	**Tru9I**
BsaMI	**DpnI**	MaeII	NlaIII	**XhoII**
BsmI	DpnII	MaeIII	NlaIV	
Bsp1286I	Fnu4HI	**MboI**	PleI	
BsrI	**FokI**	**MboII**	**RsaI**	
BsrSI	**HaeIII**	MnlI	**Sau3AI**	

Note: The enzymes listed in bold face type are available from Promega Corporation.

7.G. References

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	100 ml	E4920
	10 × 100 ml	E4950

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Product	Size	Cat.#
Glo Lysis Buffer, 1X	100 ml	E2661

Plasmid DNA Purification System

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495

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