Promega

Technical Bulletin

siCHECK™ Vectors

INSTRUCTIONS FOR USE OF PRODUCTS C8011 AND C8021.

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siCHECK™ Vectors

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1. Description

The psiCHECK™-1 Vector[a–d] (Cat.# C8011) and psiCHECK™-2 Vector[a–f] (Cat.# C8021) are designed to provide a quantitative and rapid approach for optimization of RNA interference (RNAi). The vectors enable the monitoring of changes in expression of a target gene fused to the reporter gene. In both vectors, *Renilla* luciferase is used as a primary reporter gene, and the gene of interest can be cloned into the multiple cloning region located downstream of the *Renilla* luciferase translational stop codon. Initiation of the RNAi process toward a gene of interest results in cleavage and subsequent degradation of fusion mRNA. Measurement of decreased *Renilla* luciferase activity is a convenient indicator of RNAi effect (1).

RNAi is a phenomenon by which double-stranded RNA complementary to a target mRNA can specifically inactivate gene function by stimulating the degradation of the target mRNA (2–4). Because of the ability to inactivate genes, RNAi has emerged as a powerful tool for analyzing gene function.

In mammalian systems, including cultured mammalian cells, chemically synthesized double-stranded short interfering RNA molecules (<30 nucleotides; siRNA) or endogenously expressed short hairpin RNA molecules (shRNA) result in dsRNA duplexes <30 base pairs in length that induce RNAi (5–10). RNAi duplexes >30bp induce the interferon response and nonspecific degradation of mRNA and cannot be used as tools for specific gene silencing (11,12).

Interestingly, a significant percentage of the siRNA or shRNA designed for a specific gene are not effective (5,13–16). On average only 1 in 5 of the siRNA/shRNAs selected for targeting a specific region show efficient gene silencing (16,17). Possible causes for the failure of a particular siRNA/shRNA may be instability of an siRNA probe in vivo, inability to interact with components of the RNAi machinery or the inaccessibility of the target mRNA due to local secondary structural constraints. Analysis of nucleotide sequences, melting temperatures and secondary structures have not revealed any obvious difference between effective and ineffective siRNA/shRNA (18).

At present, one of the most serious limitations for the RNAi technology is the lack of a rapid, reliable, quantitative target-site screening method. Various algorithm programs exist that aid in the design of potential siRNA targets. However, an experimental method is needed to screen these siRNAs. Current screening technologies include such semi-quantitative, time-consuming methods as fluorescence change for GFP-target fusions, Western blot analysis, monitoring phenotypic changes or RT-PCR. In addition, the current screening technologies are not easily modified for the rapid, simultaneous screening of multiple siRNA/shRNA.

2. Product Components and Storage Conditions

Product	Size	Cat.#
psiCHECK™-1 Vector	20µg	C8011
psiCHECK™-2 Vector	20µg	C8021

Storage Conditions: Store the psiCHECK™-1 and psiCHECK™-2 Vectors at –20°C.

3. General Considerations

3.A. siCHECK™ Vector Features

Current methods to monitor changes in gene expression as the result of RNAi are either semi-quantitative, time-consuming or not applicable to high-throughput screening. The siCHECK™ Vectors are easier to use than currently available methods, allow optimal quantitative target site selection and can be adapted for use in high-throughput methodologies.

There are two siCHECK™ Vectors, the psiCHECK™-1 Vector and the psiCHECK™-2 Vector. Both vectors contain as the primary reporter gene the synthetic version of *Renilla* luciferase, *hRluc*, which is used to monitor changes in expression as the result of RNAi induction. This synthetic gene is engineered for more efficient expression in mammalian cells and for reduced anomalous transcription.

To aid in fusion of the target gene to the synthetic *Renilla* luciferase reporter gene, a region of restriction sites (i.e., the multiple cloning region) has been added 3´ to the *Renilla* translational stop. The restriction sites present in the multiple cloning region can be used to create genetic fusions between the gene of interest and the *Renilla* reporter gene. Because no fusion protein is expressed, there is no need to be concerned about whether you have cloned into a proper translational reading frame.

The multiple cloning region of the psiCHECK™-1 Vector contains unique restriction sites SgfI, XhoI, SmaI, EcoRI, PmeI and NotI. Due to the presence of the firefly expression cassette, the psiCHECK™-2 Vector contains fewer unique restriction sites. The restriction sites in the psiCHECK™-2 Vector multiple cloning region are SgfI, XhoI, PmeI and NotI.

The promoter used for *Renilla* luciferase expression in the siCHECK™ Vectors is the SV40 promoter. Experimental results (data not shown) demonstrate that the SV40 promoter results in the best balance between *Renilla* luciferase expression and the detection of RNAi activity when used with siRNA or vectors expressing shRNA.

The difference between the two siCHECK™ Vectors is that the psiCHECK™-2 Vector possesses a secondary firefly reporter expression cassette. The firefly expression cassette consists of an HSV-TK promoter, a synthetic firefly luciferase gene and an SV40 late poly(A) signal. To reduce the potential for recombination events, the *Renilla* luciferase reporter gene in the psiCHECK™-2 Vector uses a synthetic poly(A). This firefly reporter cassette has been specifically designed to be an intraplasmid transfection normalization reporter; thus when using the psiCHECK™-2 Vector, the *Renilla* luciferase signal can be normalized to the firefly luciferase signal.

If no transfection normalization is required or one would prefer to have the transfection normalization reporter on a second plasmid, the psiCHECK™-1 Vector is the vector of choice.

3.A. siCHECK™ Vector Features (continued)

The psiCHECK™-1 Vector is recommended for use in monitoring RNAi effects in live cells. The changes in *Renilla* luciferase activity are measured with EnduRen™ Live Cell Substrate (Cat.# E6481), which allows continuous monitoring of intracellular *Renilla* luminescence (19; Figure 2). EnduRen™ Live Cell Substrate is for use only with *Renilla* luciferase.

Promega offers several reagents that can be used in conjunction with the siCHECK™ Vectors to monitor *Renilla* and/or firefly luciferase signals. For the psiCHECK™-1 Vector, which only contains the *Renilla* luciferase reporter gene, the *Renilla* Luciferase Assay System (Cat.# E2810, E2820) can be used. The psiCHECK™-2 Vector, which contains *Renilla* and firefly luciferase reporter genes, requires the use of either the Dual-Luciferase® Reporter Assay System (Cat.# E1910) or the Dual-Glo™ Luciferase Assay System (Cat.# E2920) to generate the firefly and *Renilla* luciferase signals.

3.B. How the siCHECK™ Vectors Work

Figure 1 provides a basic description of how the siCHECK™ Vectors work. Using the unique restriction sites, the gene of interest is cloned into the multiple cloning region located 3′ to the synthetic *Renilla* luciferase gene and its translational stop codon. After cloning, the vector is transfected into the mammalian cell line of choice, and a fusion of the *Renilla* luciferase gene and the gene of interest is transcribed. Vectors expressing potential shRNA or siRNA can be cotransfected simultaneously or sequentially, depending on your experimental design. If a specific shRNA/siRNA binds to the target mRNA and initiates the RNAi process, the fused *Renilla* luciferase:gene of interest mRNA will be cleaved and subsequently degraded, decreasing the *Renilla* luciferase signal.

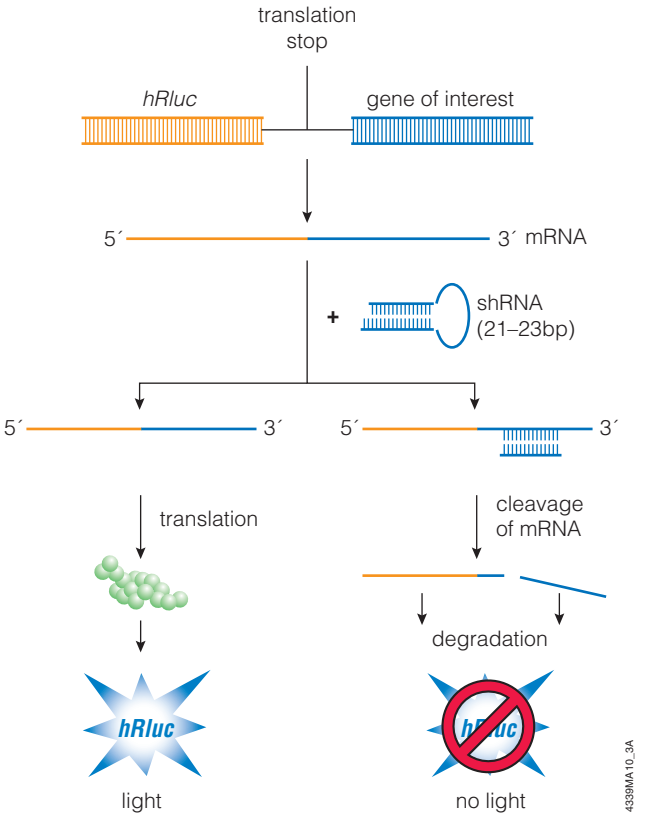

Figure 1. Mechanism of action of the siCHECK™ Vectors.

3.C. Sample Experiments Using the siCHECK™ Vectors

To demonstrate the utility of the siCHECK™ Vectors, two experiments are detailed in this Technical Bulletin. In the first experiment, human p53 cDNA was subcloned into the psiCHECK™-1 and the psiCHECK™-2 Vectors using the SgfI and NotI restriction sites located in the multiple cloning region of both vectors. Note the SgfI and NotI restriction sites are located 3´ to the *Renilla* luciferase translational stop codon. As shown in Figure 2, the psiCHECK™-1 Vector containing the human p53 cDNA was cotransfected into HEK-293T cells with the psiLentGene™ Basic Vector expressing either a *Renilla* luciferase (*hRluc*) or p53 shRNA. The negative control was the psiLentGene™ Basic Vector with a nonspecific 19bp insert. (A BLAST search using this 19bp sequence and a threshold >90% revealed no homology to any known mammalian gene or to the synthetic *Renilla* luciferase gene.) This nonspecific sequence was used for all RNAi experiments in this Technical Bulletin.

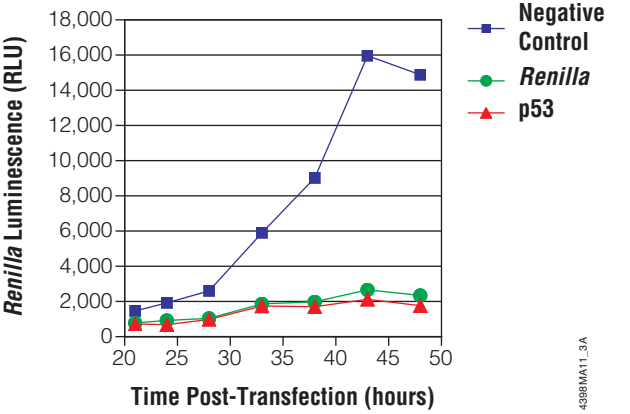

Figure 2. Inhibition of *Renilla* luciferase expression by targeting either the *Renilla* luciferase or p53 gene. The human p53 cDNA was subcloned into the psiCHECK™-1 Vector using the SgfI and NotI restriction sites located 3´ to the *Renilla* luciferase translational stop codon. To begin the transfection assay, HEK-293T cells were plated in a 96-well plate at 3,000 cells/well. After an overnight incubation, the cells were treated with a transfection mixture consisting of 35µl of serum-free medium, 0.3µl of TransFast™ Transfection Reagent (Cat.# E2431), 0.02µg of psiCHECK™-1:p53 vector and 0.08µg of psiLentGene™ Basic Vector per well. For this experiment, the psiLentGene™ Vector expressed shRNAs directed against human p53, *Renilla* luciferase or the nonspecific 19bp sequence, which serves as a negative control, (Section 3.C). After a one-hour incubation, 100µl of serum-containing medium was added to the wells. At 21 hours post-transfection, EnduRen™ Live Cell Substrate (Cat.# E6481) was added to a final concentration of 60µM, and *Renilla* luciferase activity was monitored. *Renilla* luciferase activities were normalized to the number of viable cells using the CellTiter-Glo® Luminescent Cell Viability Assay (Cat.# G7573; 20).

At 21 hours post-transfection, nonlytic EnduRen™ Live Cell Substrate was added to the wells; luminescence was monitored for the next 27 hours until 48 hours post-transfection. The data in Figure 2 show that the psiLentGene™ Basic Vector expressing either *Renilla* luciferase or p53 shRNA inhibits the expression of the *Renilla* luciferase reporter gene from the psiCHECK™-1:p53 vector. Interestingly, using either *Renilla* luciferase or p53 shRNA results in virtually identical inhibition of *Renilla* luciferase expression.

In a second experiment, the human p53 cDNA used in Figure 2 was subcloned into the psiCHECK™-2 Vector using the SgfI and NotI restriction sites. Five potential p53 shRNAs designed to bind to five different target sites were cloned into the psiLentGene™ Basic Vector; the resulting vectors were named Site 1 through Site 5. The control is a psiLentGene™ Vector containing the nonspecific 19bp sequence. The psiCHECK™-2 Vector containing the p53 cDNA was cotransfected with the psiLentGene™ Vector expressing either a p53 shRNA (Figure 3, Sites 1–5) or the nonspecific shRNA into HEK-293T cells as described in Figure 3. Forty-eight hours after transfection, the medium was removed and cells were lysed in Passive Lysis Buffer (Cat.# E1941). The firefly and *Renilla* luciferase signals were generated using the Dual-Luciferase® Reporter 1000 Assay System (21).

Figure 3, Panel A, displays the *Renilla* luciferase signal, while Figure 3, Panel B, shows the *Renilla* luciferase signal normalized (corrected for transfection efficiency to the firefly luciferase signal). The data in Figure 3, Panel A, is difficult to interpret due to transfection variations. The *Renilla* luciferase positive control, which should demonstrate inhibition of reporter expression, is not statistically different (i.e., overlapping error bars) from the negative control (no effect on reporter expression was detected). The inability to distinguish between the positive and negative controls renders any conclusion regarding the effectiveness of potential shRNAs suspect.

However, when the *Renilla* luciferase signals are normalized (see Figure 3, Panel B) to the internal firefly luciferase transfection control, the data interpretation is different, as the *Renilla* luciferase positive control is statistically different from the negative control. In addition, the normalized data allow the ability to distinguish the effectiveness of the various target site shRNAs.

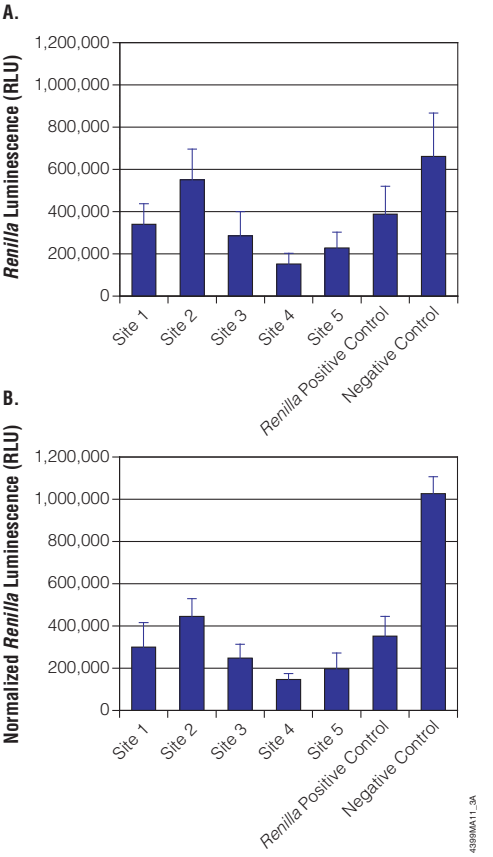

Figure 3. Target site selection using the psiCHECK™-2 Vector. HEK-293T cells were seeded into a 96-well plate at a density of 3,000 cells/well. Human p53 cDNA was subcloned into the psiCHECK™-2 Vector using the SgfI and NotI restriction sites. After an overnight incubation, the cells were treated with a transfection mixture consisting of 35µl of serum-free medium, 0.3µl of TransFast™ Transfection Reagent (Cat.# E2431), 0.02µg of psiCHECK™-2 Vector:p53 and 0.08µg of psiLentGene™ Basic Vector per well. The psiLentGene™ Basic Vector expressed one of five different shRNAs directed against human p53, *Renilla* luciferase or a nonspecific 19bp sequence (Section 3.C) as a negative control. After a one-hour incubation, 100µl of serum-containing medium was added to the wells. Forty-eight hours post-transfection *Renilla* and firefly luciferase activities were measured using the Dual-Luciferase® Reporter 1000 Assay System (Cat.# E1980; 21). **Panel A** displays the raw *Renilla* luciferase data, while in **Panel B**, the *Renilla* luciferase data has been normalized to firefly luciferase data. The data represent the mean of 12 wells plus or minus the standard deviation. Note that in other experiments the ability of different shRNAs to inhibit gene expression might vary more dramatically.

4. siCHECK™ Vector Maps

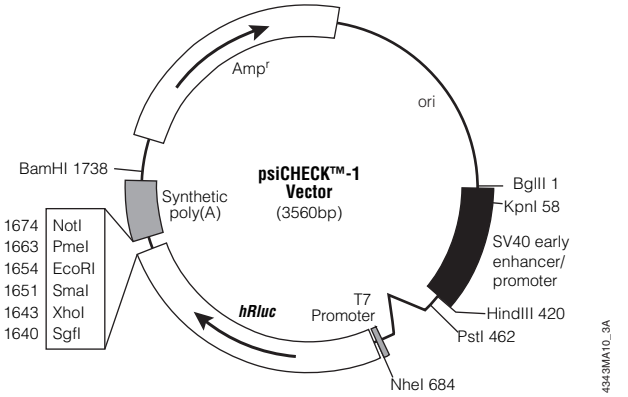

Figure 4. psiCHECK™-1 Vector map. –^– denotes the intron.

psiCHECK™-1 Vector sequence reference points:

SV40 early enhancer/promoter	7–425
Chimeric intron	489–621
T7 RNA polymerase promoter	666–684
Synthetic *Renilla* luciferase gene (*hRluc*)	694–1629
Multiple cloning region	1636–1680
Synthetic poly(A)	1688–1736
β-lactamase (Amp^r) coding region	1874–2734

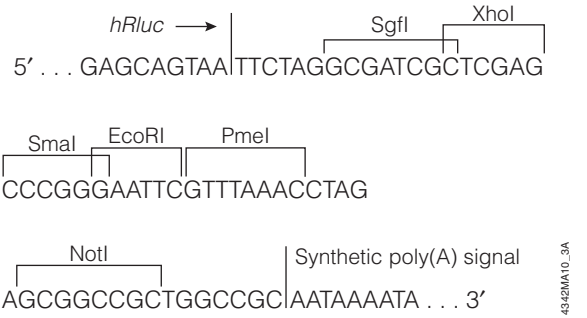

Figure 5. psiCHECK™-1 Vector multiple cloning region.

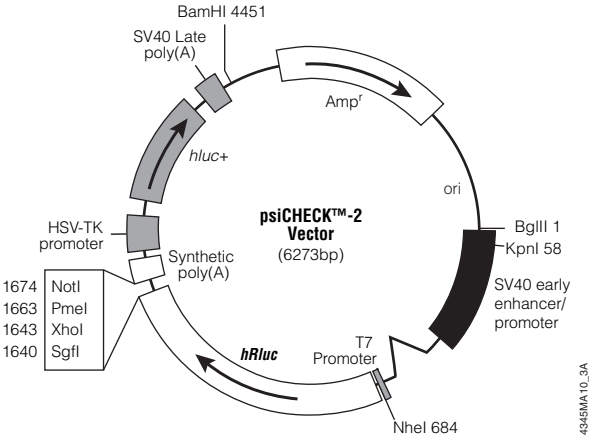

Figure 6. psiCHECK™-2 Vector map. –^– denotes the intron.

psiCHECK™-2 Vector sequence reference points:

SV40 early enhancer/promoter	7–425
Chimeric intron	489–621
T7 RNA polymerase promoter	666–684
Synthetic *Renilla* luciferase gene (*hRluc*)	694–1629
Multiple cloning region	1636–1680
Synthetic poly(A)	1688–1736
HSV-TK promoter	1744–2496
Synthetic firefly luciferase gene (*hluc*+)	2532–4184
SV40 late poly(A)	4219–4440
β-lactamase (Amp^r) coding region	4587–5447

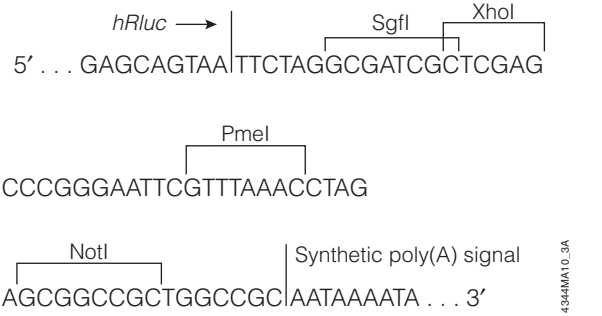

Figure 7. psiCHECK™-2 Vector multiple cloning region.

5. siCHECK™ Vector Restriction Enzyme Tables

5.A. Restriction Enzyme Sites for the psiCHECK™-1 Vector

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 to report 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 available from the GenBank® database (GenBank®/EMBL accession number **AY535006**) and online at: **www.promega.com/vectors/**

Table 1. Restriction Enzymes That Cut the psiCHECK™-1 Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	1391
Acc65I	1	54
AcyI	2	1388, 2121
AflII	2	452, 649
Alw44I	2	1989, 3235
AlwNI	1	3140
AspHI	4	1091, 1993, 2078, 3239
AvaI	3	715, 1643, 1649
AvaII	2	2297, 2519
AvrII	1	404
BamHI	1	1738
BanI	3	54, 575, 2708
BanII	3	759, 899, 1650
BbsI	1	560
BbuI	2	152, 224
BclI	2	734, 1187
BglI	3	357, 694, 2543
BglII	1	1
BsaI	3	514, 1234, 2595
BsaOI	5	1640, 1677, 2143, 2292, 3215
BsaBI	1	1453
BsaHI	2	1388, 2121
BspHI	2	1821, 2829
BspMI	1	476
BssSI	2	1992, 3376
Bst98I	2	452, 649
BstZI	1	1280
Cfr10I	1	2576

Enzyme	# of Sites	Location
DraI	4	1663, 2083, 2775, 2794
DraII	1	1539
DraIII	1	882
DrdI	2	441, 3447
DsaI	4	15, 311, 692, 899
EaeI	3	1674, 1681, 2268
EagI	1	1674
EarI	2	1193, 1862
EclHKI	1	2661
Eco52I	1	1674
Eco81I	1	1280
EcoRI	1	1654
EcoRV	1	1179
FspI	2	8, 2438
HaeII	1	3309
HgaI	4	1570, 2129, 2859, 3437
HindIII	1	420
Hsp92I	2	1388, 2121
KpnI	1	58
MspA1I	5	80, 1679, 2025, 2966, 3211
NciI	5	1650, 1651, 2125, 2476, 3172
NcoI	3	15, 311, 692
NheI	1	684
NotI	1	1674
NruI	1	1355
NsiI	3	154, 226, 913

5.A. Restriction Enzyme Sites for the psiCHECK™-1 Vector (continued)

Table 1. Restriction Enzymes That Cut the psiCHECK™-1 Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NspI	2	152, 224	**SmaI**	1	1651
PaeR7I	1	1643	**SphI**	2	152, 224
PmeI	1	1663	**SspI**	1	1856
Ppu10I	3	150, 222, 909	**StuI**	1	403
PspAI	1	1649	**StyI**	5	15, 311, 404, 692, 701
PstI	1	462			
PvuI	2	1640, 2292	TfiI	2	426, 805
PvuII	1	80	**Tth111I**	1	1390
ScaI	2	662, 2180	**VspI**	1	2486
SfiI	1	357	**XhoI**	1	1643
SgfI	1	1640	**XmaI**	1	1649
SinI	2	2297, 2519	**XmnI**	2	1228, 2061

Table 2. Restriction Enzymes That Do Not Cut the psiCHECK™-1 Vector.

***Acc*B7I**	BsaAI	**Eco47III**	**NaeI**	**SacI**
AccI	**BsaMI**	Eco72I	**NarI**	**SacII**
AccIII	BsmI	**EcoICRI**	**NdeI**	**SalI**
AflIII	Bsp120I	EcoNI	**NgoMIV**	SgrAI
AgeI	BsrGI	EheI	PacI	**SnaBI**
ApaI	**BssHII**	FseI	PflMI	**SpeI**
AscI	Bst1107I	**HincII**	PinAI	SplI
BalI	**BstEII**	HindII	PmlI	SrfI
BbeI	**BstXI**	**HpaI**	PpuMI	Sse8387I
BbrPI	**ClaI**	**I-PpoI**	PshAI	SwaI
BlpI	**CspI**	KasI	Psp5II	**XbaI**
Bpu1102I	**Csp45I**	**MluI**	RsrII	XcmI

Table 3. Restriction Enzymes That Cut the psiCHECK™-1 Vector 6 or More Times.

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

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

5.B. Restriction Enzyme Sites for the psiCHECK™-2 Vector

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 to report 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 available from the GenBank® database (GenBank®/EMBL accession number **AY535007**) and online at: **www.promega.com/vectors/**

Table 4. Restriction Enzymes That Cut the psiCHECK™-2 Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	1391
AccI	2	2079, 3132
Acc65I	1	54
AflII	4	452, 649 , 1773, 1897
AflIII	1	2450
Alw44I	2	4702, 5948
AlwNI	2	2094, 5853
ApaI	1	2562
AvrII	2	404, 2059
BalI	3	1865, 3513, 4038
BamHI	1	4451
BanII	5	759, 899, 1650, 2050, 2562
BbeI	4	2030, 2815, 3481, 3613
BbsI	2	560, 1743
BbuI	2	152, 224
BclI	5	734, 1187, 3112, 3853, 4147
BglII	1	1
BsaI	4	514, 1234, 2123, 5308
BsaAI	2	2083, 3734
BsaBI	4	1453, 2979, 4146, 4450
BsaMI	3	2504, 4270, 4363
BsmI	3	2504, 4270, 4363
Bsp120I	1	2558
BspHI	3	3115, 4534, 5542
BspMI	2	476, 3463
BsrGI	1	3022
BssHII	1	1978
BssSI	3	3459, 4705, 6089
Bst1107I	1	2080
Bst98I	4	452, 649, 1773, 1897
BstXI	1	3650
BstZI	3	1674, 4202, 4206
Bsu36I	3	1280, 3145, 3745
ClaI	1	4444
Csp45I	1	2390
DraI	5	1663, 4410, 4796, 5488, 5507
DraIII	1	882
DrdI	2	441, 6160
EagI	3	1674, 4202, 4206
EarI	5	1193, 1874, 2616, 2727, 4575
EclHKI	1	5374
Eco47III	1	3519
Eco52I	3	1674, 4202, 4206
Eco81I	3	1280, 3145, 3745
EcoNI	3	2721, 3144, 4149
EcoRI	2	1654, 2386
EcoRV	1	1179
EheI	4	2028, 2813, 3479, 3611
FseI	2	3943, 4208
FspI	3	8, 3354, 5151
HincII	1	4349
HindII	1	4349

5.B. Restriction Enzyme Sites for the psiCHECK™-2 Vector (continued)

Table 4. Restriction Enzymes That Cut the psiCHECK™-2 Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
HindIII	2	420, 2497	PpuMI	1	2056
HpaI	1	4349	Psp5II	1	2056
KasI	4	2026, 2811, 3477, 3609	PspAI	2	1649, 2019
			PvuI	2	1640, 5005
KpnI	1	58	**PvuII**	3	80, 2268, 2606
MluI	1	2450	**SacII**	1	2036
NaeI	3	3941, 3962, 4206	**ScaI**	3	662, 2697 ,4893
NarI	4	2027, 2812, 3478, 3610	**SfiI**	1	357
			SgfI	1	1640
NcoI	5	15, 311, 692, 2067, 2530	**SmaI**	2	1651, 2021
			SphI	2	152, 224
NgoMIV	3	3939, 3960, 4204	**SspI**	1	4569
NheI	1	684	**StuI**	1	403
NotI	1	1674	TfiI	2	426, 805
NruI	1	1355	**Tth111I**	1	1390
NsiI	3	154, 226, 913	**VspI**	1	5199
NspI	5	152, 224, 2336, 3023, 3278	**XbaI**	1	4189
			XhoI	1	1643
PaeR7I	1	1643	**XmaI**	2	1649, 2019
PmeI	1	1663	**XmnI**	2	1228, 4774
Ppu10I	3	150, 222, 909			

Table 5. Restriction Enzymes That Do Not Cut the psiCHECK™-2 Vector.

AccB7I	Bpu1102I	**NdeI**	RsrII	SplI
AccIII	**BstEII**	PacI	**SacI**	SrfI
AgeI	**CspI**	PflMI	**SalI**	Sse8387I
AscI	Eco72I	PinAI	SgrAI	SwaI
BbrPI	**EcoICRI**	PmlI	**SnaBI**	XcmI
BlpI	**I-PpoI**	PshAI	**SpeI**	

Table 6. Restriction Enzymes That Cut the psiCHECK™-2 Vector 6 or More Times.

AciI	**Bsp1286I**	EaeI	MaeII	**PstI**
AcyI	BsrI	Fnu4HI	MaeIII	**RsaI**
AluI	**BsrSI**	**FokI**	**MboI**	**Sau3AI**
Alw26I	Bst71I	**HaeII**	**MboII**	Sau96I
AspHI	**BstOI**	**HaeIII**	MnlI	ScrFI
AvaI	BstUI	HgaI	MseI	SfaNI
AvaII	**CfoI**	**HhaI**	**MspI**	**SinI**
BanI	Cfr10I	**HinfI**	**MspA1I**	**StyI**
BbvI	**DdeI**	**HpaII**	**NciI**	**TaqI**
BglI	**DpnI**	HphI	**NdeII**	**Tru9I**
BsaOI	DpnII	**Hsp92I**	NlaIII	**XhoII**
BsaHI	DraII	**Hsp92II**	NlaIV	
BsaJI	DsaI	MaeI	PleI	

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

6. siCHECK™ Vector Backbones and Components

The vector backbones of the psiCHECK™-1 and psiCHECK™-2 Vectors are based on the phRL-SV40 Vector (Cat.# E6261). Both the psiCHECK™-1 Vector and psiCHECK™-2 Vector contain the synthetic *Renilla* luciferase reporter gene. The psiCHECK™-2 Vector also contains a synthetic firefly luciferase gene. These synthetic luciferase genes have been codon optimized for more efficient mammalian expression and have been designed with a greatly reduced number of consensus transcription factor binding sites for reduced risk of anomalous transcriptional behavior.

SV40 Early Enhancer/Promoter

The psiCHECK™-1 Vector and psiCHECK™-2 Vector contain the SV40 early enhancer/promoter region, which provides strong, constitutive expression of *Renilla* luciferase in a variety of cell types.

Chimeric Intron

Downstream of the SV40 enhancer/promoter region is a chimeric intron composed of the 5′-donor site from the first intron of the human β-globin and the branch and 3′-acceptor site from the intron that is between the leader and the body of an immunoglobin gene heavy chain variable region (22). The sequences of the donor and acceptor sites, along with the branch point site, have been changed to match the consensus sequence for splicing (23). Transfection studies have demonstrated that the presence of an intron flanking the cDNA insert frequently increases the level of gene expression (24–27).

T7 Promoter

A T7 RNA polymerase promoter is located downstream of the chimeric intron and immediately precedes the synthetic *Renilla* luciferase reporter gene. This promoter can be used to synthesize RNA transcripts in vitro using T7 RNA Polymerase (Cat.# P2075). Note that the T7 promoter has been verified by sequence only; there has been no functional testing of the T7 promoter.

Polyadenylation Signals (SV40 Late and Synthetic)

Polyadenylation signals are coupled to 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 transcript (28). Polyadenylation has been shown to enhance RNA stability and translation (29,30). The late SV40 polyadenylation signal is extremely efficient and has been shown to increase the steady-state level of RNA to approximately fivefold more than that of the early SV40 polyadenylation signal (31). The synthetic poly(A) was cloned from our pCI-neo Vector (Cat.# E1841). The synthetic poly(A) signal is based on the highly efficient polyadenylation signal of the rabbit β-globin gene (32).

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8. Related Products

RNA Interference Products

Product	Size	Cat.#
GeneClip™ U1 Hairpin Cloning System—Basic	1 system	C8750
GeneClip™ U1 Hairpin Cloning System—Puromycin	1 system	C8760
GeneClip™ U1 Hairpin Cloning System—Hygromycin	1 system	C8770
GeneClip™ U1 Hairpin Cloning System—Neomycin	1 system	C8780
GeneClip™ U1 Hairpin Cloning System—hMGFP	1 system	C8790

Firefly and *Renilla* Luciferase Reagents

Product	Size	Cat.#
EnduRen™ Live Cell Substrate	0.34mg	E6481
	3.4mg	E6482
	34mg	E6485
Passive Lysis 5X Buffer	30ml	E1941
Glo Lysis Buffer, 1X	100ml	E2661

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