Instruction Manual

pSV40β Mammalian *lacZ*nls12co Expression Vector

Version 1.01
March 29, 2004

A plasmid expression vector for cloning and expression of proteins into mammalian cells with detection using the *lacZ*nls12co -Galactosidase marker gene.

Catalog no. M1018

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Important Information:

MSDS Sheets and product safety information are available by request from Marker Gene Technologies, Inc. and by accessing our web site at www.markergene.com.

Shipping and Storage

The pSV40β*lacZ*nls12co vector is shipped at room temperature. Store at –20°C once resuspended. Products are guaranteed for six months from date of shipment when stored properly.

Contents

Item	Concentration
pSV40β*lacZ*nls12co Vector, lyophilized in TE buffer, pH 8.0	20 μg

Quality Control

The pSV40β*lacZ*nls12co vector has been qualified by restriction endonuclease digestion. pSV40β*lacZ*nls12co is further qualified by transformation using an appropriate *E. coli* and mammalian cell strain in culture.

Accessory Products
Additional products that may be used with the pSV40β*lacZ*nls12co vector are now available from Marker Gene.

Ordering information is provided below.

Product	Unit Size	Catalog no.
pCMVβ Mammalian *lacZ* Expression Vector	20μg	M0951
pSV40β Mammalian *lacZ* Expression Vector	20μg	M0952
Expression of your recombinant fusion protein can be detected using:		
Fluorescein di-β-D-Galactopyranoside (FDG)	5 mg	M0250
Methylumbelliferyl-β-D-Galactopyranoside (MUG)	1g	M0241
Resorufin-β-D-Galactopyranoside (Res-Gal)	10 mg	M0203
Trifluoromethylumbelliferyl-β-D-Galactopyranoside	100mg	M0252
Carboxyumbelliferyl-β-D-Galactopyranoside (CUG)	5mg	M0257
FACS Fluorescent Blue *lacZ* β-Galactosidase Detection Kit	1 kit	M0255
β-Galactosidase Sample Kit	1 kit	M0276
in vivo lacZ β-Galactosidase Detection Kit	1 kit	M0259
Chemiluminescent *lacZ* β-Galactosidase Detection Kit	1 kit	M0855

Methods

Overview

Description

- This common eukaryotic expression vector, pSV40β expresses the full-length codon-optimized β-galactosidase gene (*lacZ*nls12co) under the control of simian virus 40 (SV40) early promoter. When expressed in mammalian cells, the codon-optimized gene results in expression levels of β-galactosidase 15-fold higher than those resulting from an analogous construct containing the native *E. coli* gene sequence. Enhanced transcript stability and increased translational efficiency provide for increased β-galactosidase expression, as suggested by RNA analysis. In addition, codon-optimization results in the elimination of several cryptic splice acceptor sites that are present in the native *E. coli* gene sequence and increases the amounts of un-spliced, full-length genomic RNA when used in a lentiviral vector containing a 5' splice donor. The nls12 variant results from the addition of a twelve amino acid sequence, ProLysLysLysArgLysValGluAspProLysAsp (from the SV40 T antigen nuclear localization signal) after the methionine initiation residue.

- This vector is very useful for transfection of mammalian cells in culture and for use in other species. The β-galactosidase enzyme expression is enhanced by the SV40 late polyadenylation signal.

- pSV40β*lacZ*nls12co expression vector also contains the β-lactamase gene, which acts as a selection marker (100μg/mL ampicillin resistance) in *E. coli* host.

- The β-galactosidase gene can be excised using the 5' *XhoI* and 3' *NotI* sites to allow the insertion of other genes to be expressed under the same regulatory elements in mammalian cells.

- For a map of pSV40β, see page 10.

The pSV40β*lacZ*nls12co Vector System

The pSV40β*lacZ*nls12co vector is a cloning vector that takes advantage of the site-specific recombination properties of bacteriophage lambda (Landy, 1989) to provide a rapid and highly efficient way to move your gene of interest into multiple vector systems. To express your gene of interest simply:
1. Clone your gene of interest into an entry vector to create an entry clone.
2. Perform an LR recombination reaction between the entry clone and a destination vector *(e.g.* pSV40β) to generate the expression vector.
3. Transfect your expression clone into the cell line of choice for stable expression of your gene of interest.

Using pSV40β*lacZ*nls12co

The pSV40β*lacZ*nls12co vector is supplied as a supercoiled plasmid. Although Marker Gene has previously recommended using a linearized destination vector for more efficient recombination, further testing has found that linearization of this vector is NOT required to obtain optimal results for a downstream application.

Propagating pSV40β:
If you wish to propagate and maintain pSV40β*lacZ*nls12co, we recommend using JM109 *E. coli* Competent Cells.

Entry Clone:
To recombine your gene into pSV40β*lacZ*nls12co, you should have an entry clone containing your gene of interest.

Points to consider before recombining*:*
Your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). Other sequences are also possible, but the G or A at position –3 and the G at position +4 are the most critical for functional expression. If you wish to include the V5 epitope and 6xHis tag, your gene in the entry clone should not contain a stop codon. The gene should also be designed to be in frame with the C-terminal epitope tag after recombination. If you do NOT wish to include the V5 epitope and 6xHis tag, please be sure that your gene contains a stop codon in the entry clone.

Transfection

Introduction: This section provides general information for transfecting your expression clone into the mammalian cell line of choice. We recommend that you include a positive control vector and a mock transfection (negative control) in your experiments to evaluate your results.

Plasmid Preparation:
Once you have generated your expression clone, you must isolate plasmid DNA for transfection. Plasmid DNA must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating the plasmid using a mini/midi prep kit such as the one available from Qiagen (Plasmid Midi Kit, Cat# 12143).

Methods of Transfection:
For established cell lines *(e.g.* HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. We recommend that you follow exactly the protocol for your particular cell line. Please pay particular attention to cell medium requirements, confluency and when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology* (Ausubel *et al*., 1994). Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler *et al*., 1977), lipid-mediated (Felgner *et al*., 1989; Felgner and Ringold, 1989) and electroporation (Chu *et al*., 1987; Shigekawa and Dower, 1988). If you wish to use a lipid-based reagent for transfection, we recommend using Lipofectamine Reagent available from GIBCO-BRL. For more information contact our Technical Assistance Staff (www.markergene.com or techservice@markergene.com).

Positive Control:
We recommend the use of a positive control vector for mammalian cell transfection and expression which may be used to optimize recombinant protein expression levels in your particular cell line. A vector that allows expression of a C-terminally tagged β-galactosidase fusion protein that may be detected by Western blot or functional assay provides the easiest way to measure protein expression levels. Consult our technical assistance for more information about C-terminal fusion protein expression systems.

To propagate and maintain the plasmid:
1. Resuspend the vector in 20 μl sterile water to prepare a 1μg/μL stock solution and store at -20°C. Use the stock solution to transform a *rec*A, *end*A *E. coli* strain like TOP10, JM109, or equivalent.
2. Select transformants on LB agar plates containing 50-100 μg/ml ampicillin.
3. Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

Expression and Analysis

Expression of your gene of interest from the expression clone can be performed in transiently transfected cells or stable cell lines.

Preparation of Cell Lysates:
To lyse cells:
1. Wash cell monolayers (~5 x 10^5 to 1 x 10^6 cells) once with phosphate-buffered saline (PBS, available from Gibco, Catalog no. 10010-023 or see page 9 for a recipe).
2. Scrape cells into 1 ml PBS and pellet the cells at 1500 x g for 5 minutes.
3. Resuspend in 50 µl Cell Lysis Buffer (Marker Gene Product # M0626-003 or see page 9 for a recipe). Other cell lysis buffers are also suitable. Vortex mix.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells. Note: You may prefer to lyse the cells at room temperature or on ice if degradation of your protein is a potential problem.
5. Centrifuge the cell lysate at 10,000 x g for 10 minutes at 4°C to pellet nuclei and transfer the supernatant to a fresh tube. Assay the lysate for protein concentration. Do not use protein assays utilizing Coomassie Blue or other dyes. NP-40 may interfere with the binding of the dye with the protein.
6. Add SDS-PAGE sample buffer (see page 12 for a recipe) to a final concentration of 1X and boil the sample for 5 minutes.
7. Load 20 µg of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your fusion protein.

Detecting Recombinant Fusion Proteins:
To detect expression of your recombinant fusion protein by Western blot analysis, you may use the Anti-V5 antibodies or the Anti-His(C-term) antibodies available from Invitrogen or Amersham Biosciences or an antibody to your protein of interest.

Assay for β-galactosidase:
If you use a positive control vector, you may assay for β-galactosidase expression by Western blot analysis or activity assay using cell-free lysates (Miller, 1972). X-Gal staining, or fluorescence detection are common methods of analysis. Marker Gene offers a FACS Fluorescent Blue *lacZ* β-Galactosidase Detection Kit (Product M0255), and the *in vivo lacZ* β-Galactosidase Detection Kit (Product M0259) for fast and easy detection of β-galactosidase expression.

Purification of Recombinant Fusion Proteins:
The presence of the C-terminal polyhistidine (6xHis) tag in your recombinant fusion protein allows for purification using a metal-chelating resin (available from Life Technologies). Note: Other purification methods are suitable.

Creating Stable Cell Lines:
The neomycin resistance gene can be cloned into the pSV40β*lacZ*nls12co vector to allow selection of stable cell lines. If you wish to create stable cell lines, transfect your construct into the mammalian cell line of choice and select for foci using Geneticin®. General guidelines are provided below.

To obtain stable transfectants, we recommend that you linearize your pSV40β*lacZ*nls12co construct before transfection. While linearizing the vector may not improve the efficiency of transfection, it increases the chances that the vector does not integrate in a way that disrupts elements necessary for expression in mammalian cells. To linearize your construct, cut at a unique site that is neither located within a critical element nor within your gene of interest.

Geneticin® (G418) blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression in mammalian cells of the bacterial aminoglycoside phosphotransferase gene (APH), derived from T*n*5, results in detoxification of Geneticin® (Southern and Berg, 1982).

Geneticin® Selection Guidelines:
Geneticin ® is available from GIBCO (Catalog no. 11811-023). Use as follows:
1. Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
2. Use 100 to 1000 μg/ml of Geneticin® in complete medium.
3. Calculate concentration based on the amount of active drug.
4. Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to Geneticin®. Cells will divide once or twice in the presence of lethal doses of Geneticin® Selective Antibiotic, so the effects of the drug takes several days to become apparent. Complete selection for positive clones of cells can take up to 2 to 3 weeks of growth in selection medium.

Appendix

Recipes

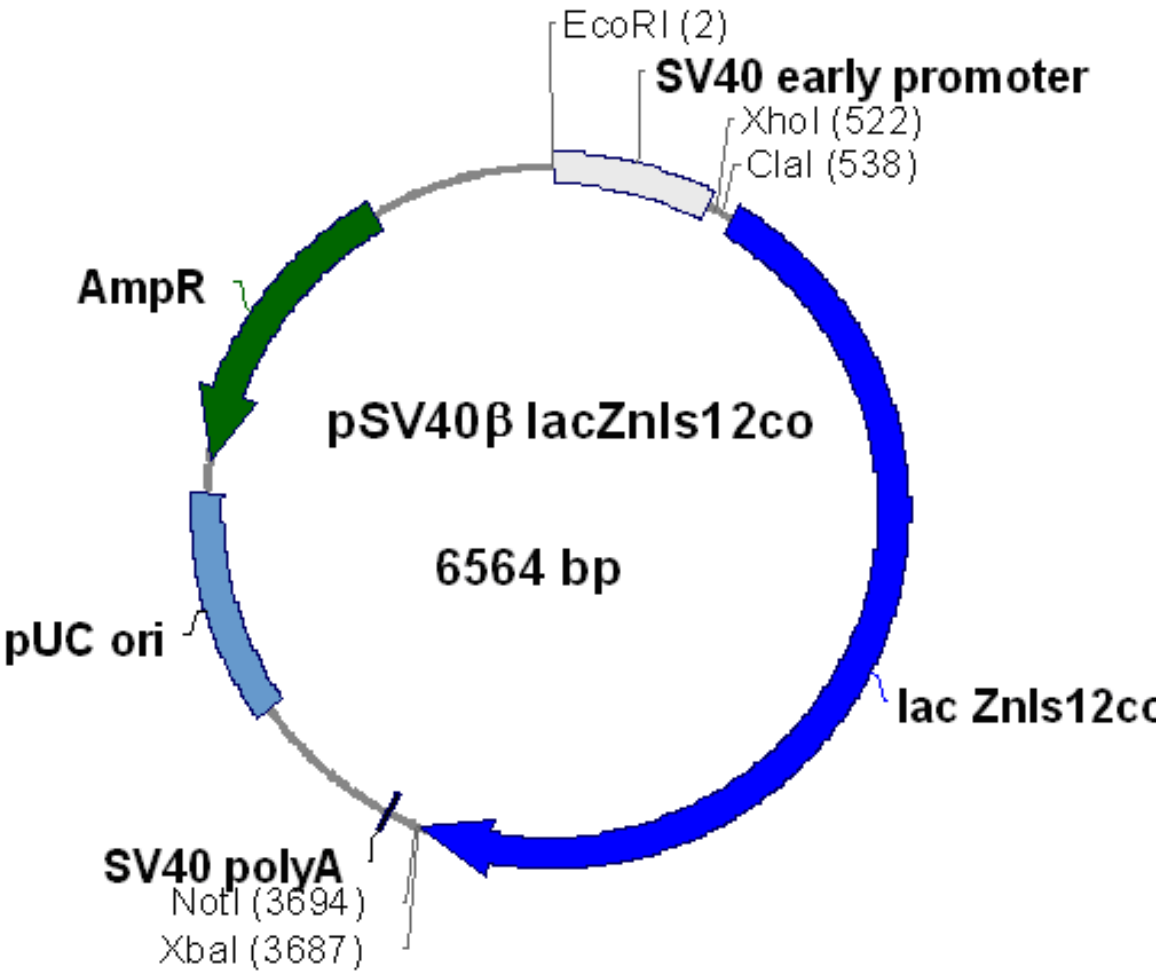
LB (Luria-Bertani) Medium and Plates Composition:
1.0% Tryptone
0.5% Yeast Extract
1.0% NaCl
pH 7.0
1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 ml deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow solution to cool to 55°C and add antibiotic if needed. Store at room temperature or at +4°C.

LB agar plates:
1. Prepare LB medium as above, but add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle for 20 minutes at 15 psi.
3. After autoclaving, cool to ~55°C, add antibiotic if needed, and pour into 10 cm plates.
4. Let harden, then invert and store at +4°C.

Cell Lysis Buffer:
25 mM Tris-phosphate (pH 7.8) containing 10% glycerol, 1% Triton X-100, 1 mg/ml BSA, 2 mM EGTA and 2 mM DTT 50 mM Tris, pH 7.8.

4X SDS-PAGE Sample Buffer:
1. Combine the following reagents:
0.5 M Tris-HCl, pH 6.8, 5 ml
Glycerol (100%), 4 ml
β-mercaptoethanol, 0.8 ml
Bromophenol Blue, 0.04 g
SDS, 0.8 g
2. Bring the volume to 10 ml with sterile water.
3. Aliquot and freeze at -20°C until needed.

Map and Features of pSV40β*lacZ*nls12co:
The map below shows the elements of pSV40β*lacZ*nls12co. The β-galactosidase gene can be excised using the 5' *XhoI* and 3' *NotI* sites to allow the insertion of other genes to be expressed under the same regulatory elements in mammalian cells.



Full Length Sequence Data of pSV40β*lacZ*nls12co:

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   1 GAATTCTAGT TGTGGTTTGT CCAAACTCAT CAATGTATCT TATCATGTCT
  51 GGATCCGCTG TGGAATGTGT GTCAGTTAGG GTGTGGAAAG TCCCCAGGCT
 101 CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA GTCAGCAACC
 151 AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT
 201 GCATCTCAAT TAGTCAGCAA CCATAGTCCC GCCCCTAACT CCGCCCATCC
 251 CGCCCCTAAC TCCGCCCAGT TCCGCCCATT CTCCGCCCCA TGGCTGACTA
 301 ATTTTTTTTA TTTATGCAGA GGCCGAGGCC GCCTCGGCCT CTGAGCTATT
 351 CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG CCTAGGCTTT TGCAAAAAGC
 401 TTGGACACAA GACAGGCTTG CGAGATATGT TTGAGAATAC CACTTTATCC
 451 CGCGTCAGGG AGAGGCAGTG CGTAAAAAGA CGCGGACTCA TGTGAAATAC
 501 TGGTTTTTAG TGCGCCAGAT CTCGAGGTCG ACGGTATCGA TAAGCTTAAC
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 551 CATCAGCAAG CAGGTCATTG TGCCACCACC ATGCCCAAGA AGAAGAGGAA
 601 GGTGGAGGAC CCCAAGGACA TCACCGACTC CCTGGCCGTG GTGCTGCAGC
 651 GCCGCGACTG GGAGAACCCC GGCGTGACCC AGCTGAACCG CCTGGCCGCC
 701 CACCCCCCCT TCGCCTCCTG GCGCAACTCC GAGGAGGCCC GCACCGACCG
 751 CCCCTCCCAG CAGCTGCGCT CCCTGAACGG CGAGTGGCGC TTCGCCTGGT
 801 TCCCCGCCCC CGAGGCCGTG CCCGAGTCCT GGCTGGAGTG CGACCTGCCC
 851 GAGGCCGACA CCGTGGTGGT GCCCTCCAAC TGGCAGATGC ACGGCTACGA
 901 CGCCCCCATC TACACCAACG TGACCTACCC CATCACCGTG AACCCCCCCT
 951 TCGTGCCCAC CGAGAACCCC ACCGGCTGCT ACTCCCTGAC CTTCAACGTG
1001 GACGAGTCCT GGCTGCAGGA GGGCCAGACC CGCATCATCT TCGACGGCGT
1051 GAACTCCGCC TTCCACCTGT GGTGCAACGG CCGCTGGGTG GGCTACGGCC
1101 AGGACTCCCG CCTGCCCTCC GAGTTCGACC TGTCCGCCTT CCTGCGCGCC
1151 GGCGAGAACC GCCTGGCCGT GATGGTGCTG CGCTGGTCCG ACGGCTCCTA
1201 CCTGGAGGAC CAGGACATGT GGCGCATGTC CGGCATCTTC CGCGACGTGT
1251 CCCTGCTGCA CAAGCCCACC ACCCAGATCT CCGACTTCCA CGTGGCCACC
1301 CGCTTCAACG ACGACTTCTC CCGCGCCGTG CTGGAGGCCG AGGTGCAGAT
1351 GTGCGGCGAG CTGCGCGACT ACCTGCGCGT GACCGTGTCC CTGTGGCAGG
1401 GCGAGACCCA GGTGGCCTCC GGCACCGCCC CCTTCGGCGG CGAGATCATC
1451 GACGAGCGCG GCGGCTACGC CGACCGCGTG ACCCTGCGCC TGAACGTGGA
1501 GAACCCCAAG CTGTGGTCCG CCGAGATCCC CAACCTGTAC CGCGCCGTGG
1551 TGGAGCTGCA CACCGCCGAC GGCACCCTGA TCGAGGCCGA GGCCTGCGAC
1601 GTGGGCTTCC GCGAGGTGCG CATCGAGAAC GGCCTGCTGC TGCTGAACGG
1651 CAAGCCCCTG CTGATCCGCG GCGTGAACCG CCACGAGCAC CACCCCCTGC
1701 ACGGCCAGGT GATGGACGAG CAGACCATGG TGCAGGACAT CCTGCTGATG
1751 AAGCAGAACA ACTTCAACGC CGTGCGCTGC TCCCACTACC CCAACCACCC
1801 CCTGTGGTAC ACCCTGTGCG ACCGCTACGG CCTGTACGTG GTGGACGAGG
1851 CCAACATCGA GACCCACGGC ATGGTGCCCA TGAACCGCCT GACCGACGAC
1901 CCCCGCTGGC TGCCCGCCAT GTCCGAGCGC GTGACCCGCA TGGTGCAGCG
1951 CGACCGCAAC CACCCCTCCG TGATCATCTG GTCCCTGGGC AACGAGTCCG
2001 GCCACGGCGC CAACCACGAC GCCCTGTACC GCTGGATCAA GTCCGTGGAC
2051 CCCTCCCGCC CCGTGCAGTA CGAGGGCGGC GGCGCCGACA CCACCGCCAC
2101 CGACATCATC TGCCCCATGT ACGCCCGCGT GGACGAGGAC CAGCCCTTCC
2151 CCGCCGTGCC CAAGTGGTCC ATCAAGAAGT GGCTGTCCCT GCCCGGCGAG
2201 ACCCGCCCCC TGATCCTGTG CGAGTACGCC CACGCCATGG GCAACTCCCT
2251 GGGCGGCTTC GCCAAGTACT GGCAGGCCTT CCGCCAGTAC CCCCGCCTGC
2301 AGGGCGGCTT CGTGTGGGAC TGGGTGGACC AGTCCCTGAT CAAGTACGAC
2351 GAGAACGGCA ACCCCTGGTC CGCCTACGGC GGCGACTTCG GCGACACCCC
2401 CAACGACCGC CAGTTCTGCA TGAACGGCCT GGTGTTCGCC GACCGCACCC
2451 CCCACCCCGC CCTGACCGAG GCCAAGCACC AGCAGCAGTT CTTCCAGTTC
2501 CGCCTGTCCG GCCAGACCAT CGAGGTGACC TCCGAGTACC TGTTCCGCCA
2551 CTCCGACAAC GAGCTGCTGC ACTGGATGGT GGCCCTGGAC GGCAAGCCCC
2601 TGGCCTCCGG CGAGGTGCCC CTGGACGTGG CCCCCCAGGG CAAGCAGCTG
2651 ATCGAGCTGC CCGAGCTGCC CCAGCCCGAG TCCGCCGGCC AGCTGTGGCT
2701 GACCGTGCGC GTGGTGCAGC CCAACGCCAC CGCCTGGTCC GAGGCCGGCC
2751 ACATCTCCGC CTGGCAGCAG TGGCGCCTGG CCGAGAACCT GTCCGTGACC
2801 CTGCCCGCCG CCTCCCACGC CATCCCCCAC CTGACCACCT CCGAGATGGA
2851 CTTCTGCATC GAGCTGGGCA ACAAGCGCTG GCAGTTCAAC CGCCAGTCCG
2901 GCTTCCTGTC CCAGATGTGG ATCGGCGACA AGAAGCAGCT GCTGACCCCC
2951 CTGCGCGACC AGTTCACCCG CGCCCCCCTG GACAACGACA TCGGCGTGTC
3001 CGAGGCCACC CGCATCGACC CCAACGCCTG GGTGGAGCGC TGGAAGGCCG
3051 CCGGCCACTA CCAGGCCGAG GCCGCCCTGC TGCAGTGCAC CGCCGACACC
3101 CTGGCCGACG CCGTGCTGAT CACCACCGCC CACGCCTGGC AGCACCAGGG
3151 CAAGACCCTG TTCATCTCCC GCAAGACCTA CCGCATCGAC GGCTCCGGCC
3201 AGATGGCCAT CACCGTGGAC GTGGAGGTGG CCTCCGACAC CCCCCACCCC
3251 GCCCGCATCG GCCTGAACTG CCAGCTGGCC CAGGTGGCCG AGCGCGTGAA
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3301 CTGGCTGGGC CTGGGCCCCC AGGAGAACTA CCCCGACCGC CTGACCGCCG
3351 CCTGCTTCGA CCGCTGGGAC CTGCCCCTGT CCGACATGTA CACCCCCTAC
3401 GTGTTCCCCT CCGAGAACGG CCTGCGCTGC GGCACCCGCG AGCTGAACTA
3451 CGGCCCCCAC CAGTGGCGCG GCGACTTCCA GTTCAACATC TCCCGCTACT
3501 CCCAGCAGCA GCTGATGGAG ACCTCCCACC GCCACCTGCT GCACGCCGAG
3551 GAGGGCACCT GGCTGAACAT CGACGGCTTC CACATGGGCA TCGGCGGCGA
3601 CGACTCCTGG TCCCCCTCCG TGTCCGCCGA GTTCCAGCTG TCCGCCGGCC
3651 GCTACCACTA CCAGCTGGTG TGGTGCCAGA AGTAGTCTAG AGCGGCCGCG
3701 GGGATCCAGA CATGATAAGA TACATTGATG AGTTTGGACA AACCACAACT
3751 AGAATGCAGT GAAAAAAATG CTTTATTTGT GAAATTTGTG ATGCTATTGC
3801 TTTATTTGTA ACCATTATAA GCTGCAATAA ACAAGTTAAC AACAACAATT
3851 GCATTCATTT TATGTTTCAG GTTCAGGGGG AGGTGTGGGA GGTTTTTTCG
3901 GATCCTCTAG AGTCGACCTG CAGGCATGCA AGCTTGGCGT AATCATGGTC
3951 ATAGCTGTTT CCTGTGTGAA ATTGTTATCC GCTCACAATT CCACACAACA
4001 TACGAGCCGG AAGCATAAAG TGTAAAGCCT GGGGTGCCTA ATGAGTGAGC
4051 TAACTCACAT TAATTGCGTT GCGCTCACTG CCCGCTTTCC AGTCGGGAAA
4101 CCTGTCGTGC CAGCTGCATT AATGAATCGG CCAACGCGCG GGGAGAGGCG
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4251 ACGGTTATCC ACAGAATCAG GGGATAACGC AGGAAAGAAC ATGTGAGCAA
4301 AAGGCCAGCA AAAGGCCAGG AACCGTAAAA AGGCCGCGTT GCTGGCGTTT
4351 TTCCATAGGC TCCGCCCCCC TGACGAGCAT CACAAAAATC GACGCTCAAG
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4551 ACGCTGTAGG TATCTCAGTT CGGTGTAGGT CGTTCGCTCC AAGCTGGGCT
4601 GTGTGCACGA ACCCCCCGTT CAGCCCGACC GCTGCGCCTT ATCCGGTAAC
4651 TATCGTCTTG AGTCCAACCC GGTAAGACAC GACTTATCGC CACTGGCAGC
4701 AGCCACTGGT AACAGGATTA GCAGAGCGAG GTATGTAGGC GGTGCTACAG
4751 AGTTCTTGAA GTGGTGGCCT AACTACGGCT ACACTAGAAG GACAGTATTT
4801 GGTATCTGCG CTCTGCTGAA GCCAGTTACC TTCGGAAAAA GAGTTGGTAG
4851 CTCTTGATCC GGCAAACAAA CCACCGCTGG TAGCGGTGGT TTTTTTGTTT
4901 GCAAGCAGCA GATTACGCGC AGAAAAAAAG GATCTCAAGA AGATCCTTTG
4951 ATCTTTTCTA CGGGGTCTGA CGCTCAGTGG AACGAAAACT CACGTTAAGG
5001 GATTTTGGTC ATGAGATTAT CAAAAAGGAT CTTCACCTAG ATCCTTTTAA
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5801 TCTCAAGGAT CTTACCGCTG TTGAGATCCA GTTCGATGTA ACCCACTCGT
5851 GCACCCAACT GATCTTCAGC ATCTTTTACT TTCACCAGCG TTTCTGGGTG
5901 AGCAAAAACA GGAAGGCAAA ATGCCGCAAA AAAGGGAATA AGGGCGACAC
5951 GGAAATGTTG AATACTCATA CTCTTCCTTT TTCAATATTA TTGAAGCATT
6001 TATCAGGGTT ATTGTCTCAT GAGCGGATAC ATATTTGAAT GTATTTAGAA

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6051 AAATAAACAA ATAGGGGTTC CGCGCACATT TCCCCGAAAA GTGCCACCTG
6101 ACGTCTAAGA AACCATTATT ATCATGACAT TAACCTATAA AAATAGGCGT
6151 ATCACGAGGC CCTTTCGTCT CGCGCGTTTC GGTGATGACG GTGAAAACCT
6201 CTGACACATG CAGCTCCCGG AGACGGTCAC AGCTTGTCTG TAAGCGGATG
6251 CCGGGAGCAG ACAAGCCCGT CAGGGCGCGT CAGCGGGTGT TGGCGGGTGT
6301 CGGGGCTGGC TTAACTATGC GGCATCAGAG CAGATTGTAC TGAGAGTGCA
6351 CCATATGCGG TGTGAAATAC CGCACAGATG CGTAAGGAGA AAATACCGCA
6401 TCAGGCGCCA TTCGCCATTC AGGCTGCGCA ACTGTTGGGA AGGGCGATCG
6451 GTGCGGGCCT CTTCGCTATT ACGCCAGCTG GCGAAAGGGG GATGTGCTGC
6501 AAGGCGATTA AGTTGGGTAA CGCCAGGGTT TTCCCAGTCA CGACGTTGTA
6551 AAACGACGGC CAGT
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Restriction Sites:

AarI (CACCTGCnnnn'nnnn_)
Cuts 1 time.
Cuts at position 3543.

AatII (G_ACGT'C) [ZraI]
Cuts 1 time.
Cuts at position 6104.

AccI (GT'mk_AC) [FblI,XmiI]
Cuts 2 times.

AclI (AA'CG_TT) [Psp1406I]
Cuts 2 times.

AcuI (CTGAAGnnnnnnnnnnnnnn_nn') [Eco57I]
Cuts 2 times.

AfeI (AGC'GCT) [Eco47III,Aor51HI,FunI]
Cuts 2 times.

AhdI (GACnn_n'nnGTC) [Eam1105I,AspEI,DriI,EclHKI]
Cuts 2 times.

AleI (CACnn'nnGTG) [OliI]
Cuts 2 times.

ApaI (G_GGCC'C) [Bsp120I,PspOMI]
[dcm methylated]
Cuts 1 time.
Cuts at position [3317].

ApoI (r'AATT_y) [AcsI,XapI]
Cuts 2 times.

***Avr*II** (C'CTAG_G) [AspA2I,BlnI,XmaJI]
Cuts 1 time.
Cuts at position 381.

***Ban*II** (G_rGCy'C) [Eco24I,EcoT38I,FriOI]
Cuts 1 time.
Cuts at position 3317.

***Bci*VI** (GTATCCnnnnn_n') [BfuI]
Cuts 2 times.

***Bfr*BI** (ATG'CAT) [EcoT22I,Mph1103I,NsiI,Zsp2I]
Cuts 2 times.

***Bgl*II** (A'GATC_T)
Cuts 2 times.

***Bsa*BI** (GATnn'nnATC) [Bse8I,BseJI,MamI]
[dam methylated]
Cuts 2 times.

***Bsm*I** (GAATG_Cn') [BsaMI,Mva1269I,PctI]
Cuts 2 times.

***Bsm*BI** (CGTCTCn'nnnn_) [Esp3I]
Cuts 2 times.

***Bsr*DI** (GCAATG_nn') [Bse3DI,BseMI]
Cuts 2 times.

***Bsr*GI** (T'GTAC_A) [Bsp1407I,BstAUI,SspBI]
Cuts 1 time.
Cuts at position 3387.

***Bss*HII** (G'CGCG_C) [BsePI,PauI]
Cuts 1 time.
Cuts at position 1144.

***Bst*EII** (G'GTnAC_C) [BstPI,Eco91I,EcoO65I,PspEI]
Cuts 1 time.
Cuts at position 2524.

***Bst*XI** (CCAn_nnnn'nTGG)
Cuts 1 time.
Cuts at position 3214.

ClaI **(AT'CG_AT)**
[BanIII,Bsa29I,BseCI,Bsp106I,BspDI,BspXI,Bsu15I,BsuTUI,ZhoI]
[dam methylated]
Cuts 1 time.
Cuts at position 537.

DraIII **(CAC_nnn'GTG) [AdeI]**
Cuts 1 time.
Cuts at position 1815.

DrdI **(GACnn_nn'nnGTC) [AasI,DseDI]**
Cuts 2 times.

EcoRI **(G'AATT_C) [FunII]**
Cuts 1 time.
Cuts at position 1.

FalI **(AAGnnnnnCTTnnnnnnnn_nnnnn')**
Cuts 1 time.
Cuts at position 432.

FseI **(GG_CCGG'CC)**
Cuts 1 time.
Cuts at position 2748.

FspAI **(rTGC'GCAy)**
Cuts 1 time.
Cuts at position 1619.

HpaI **(GTT'AAC) [KspAI]**
Cuts 1 time.
Cuts at position 3837.

MfeI **(C'AATT_G) [MunI]**
Cuts 1 time.
Cuts at position 3846.

MscI **(TGG'CCA) [BalI,MlsI,MluNI,Msp20I]**
[dcm methylated]
Cuts 2 times.

NdeI **(CA'TA_TG) [FauNDI]**
Cuts 1 time.
Cuts at position 6353.

NotI **(GC'GGCC_GC) [CciNI]**
Cuts 1 time.
Cuts at position 3693.

***Nsi*I** (A_TGCA'T) [BfrBI,EcoT22I,Mph1103I,Zsp2I]
Cuts 2 times.

***Pfo*I** (T'CCnGG_A)
Cuts 1 time.
Cuts at position 6215.

***Pml*I** (CAC'GTG) [PmaCI,AcvI,BbrPI,Eco72I,PspCI]
Cuts 1 time.
Cuts at position 1291.

***Ppu*MI** (rG'GwC_Cy) [PpuXI,Psp5II,PspPPI]
[dcm methylated]
Cuts 2 times.

***Psi*I** (TTA'TAA)
Cuts 1 time.
Cuts at position 3817.

***Psp*OMI** (G'GGCC_C) [ApaI,Bsp120I]
Cuts 1 time.
Cuts at position 3313.

***Pvu*I** (CG_AT'CG) [BspCI,Ple19I]
Cuts 2 times.

***Sac*II** (CC_GC'GG) [Cfr42I,KspI,Sfr303I,SgrBI]
Cuts 2 times.

***Sal*I** (G'TCGA_C)
Cuts 2 times.

***Sap*I** (GCTCTTCn'nnn_)
Cuts 1 time.
Cuts at position 4173.

***Sbf*I** (CC_TGCA'GG) [Sse8387I,SdaI]
Cuts 2 times.

***Sca*I** (AGT'ACT) [ZrmI]
Cuts 2 times.

***Sex*AI** (A'CCwGG_T) [MabI]
[dcm methylated]
Cuts 1 time.
Cuts at position [148].

***Sfi*I** (GGCCn_nnn'nGGCC)
[dcm methylated]
Cuts 2 times.

***Sgr*AI** (Cr'CCGG_yG)
Cuts 1 time.
Cuts at position 1148.

***Ssp*I** (AAT'ATT)
Cuts 1 time.
Cuts at position 5986.

***Tfi*I** (G'AwT_C) [PfeI]
Cuts 2 times.

***Tth*111I** (GACn'n_nGTC) [AspI,PflFI,PsyI,TelI]
Cuts 1 time.
Cuts at position 1384.

***Xba*I** (T'CTAG_A)
[dam methylated]
Cuts 2 times.

***Xcm*I** (CCAnnnn_n'nnnnTGG)
Cuts 2 times.

***Xho*I** (C'TCGA_G) [BssHI,PaeR7I,Sfr274I,SlaI,TliI]
Cuts 1 time.
Cuts at position 521.

***Xmn*I** (GAAnn'nnTTC) [Asp700I,MroXI,PdmI]
Cuts 2 times.

***Zra*I** (GAC'GTC) [AatII]
Cuts 1 time.
Cuts at position 6102.

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University of Oregon Riverfront Research Park
1850 Millrace Drive
Eugene, Oregon 97403
1-888-218-4062
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