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ab275768 pDC57 Mammalian Luciferase Expression Vector

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For cloning and expression of luciferase into mammalian cells

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1. Overview

This high copy number eukaryotic vector, pDC57 expresses a mutant luciferase gene under the control of the cytomegalovirus immediate early gene (CMV IE) promoter. This vector encodes a mutant enzyme that catalyzes the production of long wavelength (red) light (Em: 605) from D-Luciferin, product M0237. This mutant luciferase is optimized for expression in mammalian cells, resulting in drastically improved light production, allowing detection of expression at extremely low levels. The mutant luciferase also has been shown to remain stable at 37°C over long time periods, a characteristic not seen in wild type luciferases. This vector is very useful for transfection of mammalian cells in culture and may also be used in other species. The mutant luciferase enzyme expression is enhanced by the Kozak's consensus sequence and SV40 late polyadenylation signal.

pDC57 expression vector also contains the β-lactamase gene, which acts as a selection marker (100 µg/mL ampicillin resistance) in *E.coli* host.

The mutant luciferase 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 sequence and enzyme cleavage site information for pDC57, see Appendix 9.2 and 9.3.

The pDC57 vector is a cloning vector that takes advantage of the site-specific recombination properties of bacteriophage lambda 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. pCMVβ) to generate the expression vector.

3. Transfect your expression clone into the cell line of choice for stable expression of your gene of interest.

2. Storage and Stability

Store all samples of the vector at –20°C or below, once resuspended.

3. Quality Control

The pDC57 mammalian luciferase expression vector has been qualified by restriction endonuclease digestion. pDC57 is further qualified by transformation using an appropriate *E.coli* and mammalian cell strain in culture.

4. Using pDC57 Mammalian Luciferase Expression Vector

4.1 Propagating pDC57:

If you wish to propagate and maintain pDC57, we recommend using JM109 *E.coli* Competent Cells for transformation.

4.2 Entry Clone:

To recombine your gene into pDC57, you should have an entry clone containing your gene of interest.

4.3 Points to consider before recombining:

Your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation. 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.

5. Transfection

5.1 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.

5.2 Plasmid Preparation:

Once you have generated your expression clone, you must isolate plasmid DNA for transfection. Plasmid DNA for 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.

5.3 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.

5.4 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.

5.5 To propagate and maintain the plasmid:

5.5.1 Resuspend the vector in 20 µL sterile water to prepare a 1 µg/µL stock solution and store at -20°C. Use the stock solution to transform a *recA*, *endA E.coli* strain like TOP10, JM109, or equivalent.

5.5.2 Select transformants on LB agar plates containing 50-100 µg/ml ampicillin.

5.5.3 Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

6. Expression and Analysis

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

6.1 Preparation of Cell Lysates:

To lyse cells:

6.1.1 Wash cell monolayers (~5 x 10^5 to 1 x 10^6 cells) once with phosphate-buffered saline (PBS).

6.1.2 Scrape cells into 1 mL PBS and pellet the cells at 1500 x *g* for 5 minutes.

6.1.3 Resuspend in 50 µL Cell Lysis Buffer (Live Cell Luciferase Assay Kit (ab253393) or see Appendix 9.1 for a recipe). Other cell lysis buffers are also suitable. Vortex mix.

6.1.4 Incubate cell suspension at 37°C for 10 minutes to lyse the cells.

Δ Note: Other You may prefer to lyse the cells at room temperature or on ice if degradation of your protein is a potential problem.

6.1.5 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.

6.1.6 Add SDS-PAGE sample buffer (see Appendix for a recipe) to a final concentration of 1X and boil the sample for 5 minutes.

6.1.7 Load 20 µg of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your fusion protein.

6.2 Luciferase Assay using Cell Lysates:

Cell lysate from above step can be assay for luciferase activity using the Live Cell Luciferase Assay Kit (ab253393).

6.3 Detecting Recombinant Fusion Proteins:

To detect expression of your recombinant fusion protein by Western blot analysis, you may use Anti-V5 antibodies or the Anti-His(C-term) antibodies.

6.4 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. X-Gal staining, or fluorescence detection are common methods of analysis. Abcam offers a FACS Blue LacZ beta Galactosidase detection kit (ab189815), and the LacZ beta Galactosidase Intracellular Detection Kit (ab189816) for fast and easy detection of β-galactosidase expression.

6.5 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.

Δ Note: Other purification methods may also be suitable.

6.6 Creating Stable Cell Lines:

The neomycin resistance gene can be cloned into the pDC57 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®.

To obtain stable transfectants, we recommend that you linearize your pDC57 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 Tn5, results in detoxification of Geneticin®.

6.7 Geneticin® Selection Guidelines:

Use Geneticin®as follows:

6.7.1 Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
6.7.2 Use 100 to 1000 µg/ml of Geneticin® in complete medium.
6.7.3 Calculate concentration based on the amount of active drug.

6.7.4 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.

7. Appendix

7.1 Recipes

LB (Luria-Bertani) Medium and Plates Composition:

1.0% Tryptone
0.5% Yeast Extract
1.0% NaCl
pH 7.0

1. For 1 litre, 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 litre.
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.

7.2 Full Length Sequence Data of pDC57

1 GAATTCGAGC TTGCATGCCT GCAGGTCGTT ACATAACTTA CGGTAAATGG
51 CCCGCCTGGC TGACCGCCCA ACGACCCCCG CCCATTGACG TCAATAATGA
101 CGTATGTTCC CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG
151 GTGGAGTATT TACGGTAAAC TGCCCACTTG GCAGTACATC AAGTGTATCA
201 TATGCCAAGT ACGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCCGCCT
251 GGCATTATGC CCAGTACATG ACCTTATGGG ACTTTCCTAC TTGGCAGTAC
301 ATCTACGTAT TAGTCATCGC TATTACCATG GTGATGCGGT TTTGGCAGTA
351 CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC
401 ACCCCATTGA CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC
451 TTTCCAAAAT GTCGTAACAA CTCCGCCCCA TTGACGCAAA TGGGCGGTAG
501 GCGTGTACGG TGGGAGGTCT ATATAAGCAG AGCTCGTTTA GTGAACCGTC
551 AGATCGCCTG GAGACGCCAT CCACGCTGTT TTGACCTCCA TAGAAGACAC
601 CGGGACCGAT CCAGCCTCCG GACTCTAGAG GATCCGGTAC TCGAGGCCAC
651 CATGGAAAAT ATGGAAAACG ACGAGAACAT CGTGGTGGGC CCCAAGCCCT
701 TCTACCCCAT CGAGGAAGGC AGCGCCGGCA CCCAGCTGCG GAAGTACATG
751 GAAAGATACG CCAAGCTGGG CGCCATTGCC TTCACCAACG CCGTGACCGG
801 CGTGGACTAC AGCTACGCCG AGTACCTGGA AAAGAGCTGC TGCCTGGGCA
851 AGGCTCTGCA GAACTACGGC CTGGTGGTGG ACGGCCGGAT CGCCCTGTGC
901 AGCGAGAACT GCGAGGAATT CTTCATCCCC GTGATCGCCG GCCTGTTCAT
951 CGGCGTGGGC GTGGCTCCCA CCAACGAGAT CTACACCCTG CGGGAGCTGG
1001 TGCACAGCCT GGGCATCAGC AAGCCCACCA TCGTGTTCAG CAGCAAGAAG
1051 GGCCTGGACA AAGTCATCAC CGTGCAGAAA ACCGTGACCA CCATCAAGAC
1101 CATCGTGATC CTGGACAGCA AGGTGGACTA CCGGGGCTAC CAGTGCCTGG
1151 ACACCTTCAT CAAGCGGAAC ACCCCCCCTG GCTTCCAGGC CAGCAGCTTC
1201 AAGACCGTGG AGGTGGACCG GAAAGAACAG GTGGCCCTGA TCATGAACAG
1251 CAGCGGCAGC ACCGGCCTGC CCAAGGGCGT GCAGCTGACC CACGAGAACA
1301 CCGTGACCCG GTTCAGCCAC GCCAGGGACC CCATCTACGG CAACCAGGTG
1351 TCCCCCGGCA CCGCCGTGCT GACCGTGGTG CCCTTCCACC ACGGCTTCGG
1401 CATGTTCACC ACCCTGGGCT ACCTGATCTG CGGCTTCCGG GTGGTGATGC
1451 TGACCAAGTT CGACGAGGAA ACCTTCCTGA AAACCCTGCA GGACTACAAG
1501 TGCACCTACG TGATTCTGGT GCCCACCCTG TTCGCCATCC TGAACAAGAG
1551 CGAGCTGCTG AACAAGTACG ACCTGAGCAA CCTGGTGGAG ATCGCCAGCG
1601 GCGGAGCCCC CCTGAGCAAA GAAGTGGGAG AGGCCGTCGC CAGGCGGTTC
1651 AATCTGCCCG GCGTGCGGCA GGGCTACGGC CTGACCGAGA CAACCAGCGC
1701 CATCATCATC ACCCCCGAGG GCGACGACAA GCCTGGAGCC AGCGGCAAGG
1751 TGGTGCCCCT GTTCAAGGCC AAAGTGATCG ACCTGGACAC CAAGAAGAGC
1801 CTGGGCCCCA ACAGACGGGG CGAAGTGTGC GTGAAGGGCC CCATGCTGAT
1851 GAAGGGCTAC GTGAACAACC CCGAGGCCAC CAAAGAGCTG ATCGACGAAG
1901 AGGGCTGGCT GCACACCGGC GACATCGGCT ACTACGACGA AGAGAAGCAC
1951 TTCTTCATCG TGGACCGGCT GAAGAGCCTG ATCAAGTACA AGGGCTATCA
2001 GGTGCCCCCT GCCGAGCTGG AAAGCGTCCT GCTGCAGCAC CCCAGCATCT
2051 TCGACGCCGG CGTGGCCGGG GTGCCAGATC CTGTGGCCGG CGAGCTGCCT
2101 GGCGCCGTGG TGGTGCTGGA ATCCGGCAAG AACATGACCG AGAAAGAAGT
2151 GATGGACTAC GTCGCCAGCC AGGTGTCCAA CGCCAAGCGG CTGAGAGGCG
2201 GCGTGAGATT CGTGGACGAA GTGCCAAAGG GCCTGACCGG CAAGATCGAC
2251 GGCAGGGCCA TCCGGGAGAT CCTGAAGAAA CCCGTGGCCA AGATGTGATG
2301 AGCGGCCGCG GGGATCCAGA CATGATAAGA TACATTGATG AGTTTGGACA
2351 AACCACAACT AGAATGCAGT GAAAAAAATG CTTTATTTGT GAAATTTGTG
2401 ATGCTATTGC TTTATTTGTA ACCATTATAA GCTGCAATAA ACAAGTTAAC
2451 AACAACAATT GCATTCATTT TATGTTTCAG GTTCAGGGGG AGGTGTGGGA
2501 GGTTTTTTCG GATCCTCTAG AGTCGACCTG CAGGCATGCA AGCTTGGCGT
2551 AATCATGGTC ATAGCTGTTT CCTGTGTGAA ATTGTTATCC GCTCACAATT
2601 CCACACAACA TACGAGCCGG AAGCATAAAG TGTAAAGCCT GGGGTGCCTA
2651 ATGAGTGAGC TAACTCACAT TAATTGCGTT GCGCTCACTG CCCGCTTTCC
2701 AGTCGGGAAA CCTGTCGTGC CAGCTGCATT AATGAATCGG CCAACGCGCG

2751 GGGAGAGGCG GTTTGCGTAT TGGGCGCTCT TCCGCTTCCT CGCTCACTGA
2801 CTCGCTGCGC TCGGTCGTTC GGCTGCGGCG AGCGGTATCA GCTCACTCAA
2851 AGGCGGTAAT ACGGTTATCC ACAGAATCAG GGGATAACGC AGGAAAGAAC
2901 ATGTGAGCAA AAGGCCAGCA AAAGGCCAGG AACCGTAAAA AGGCCGCGTT
2951 GCTGGCGTTT TTCCATAGGC TCCGCCCCCC TGACGAGCAT CACAAAAATC
3001 GACGCTCAAG TCAGAGGTGG CGAAACCCGA CAGGACTATA AAGATACCAG
3051 GCGTTTCCCC CTGGAAGCTC CCTCGTGCGC TCTCCTGTTC CGACCCTGCC
3101 GCTTACCGGA TACCTGTCCG CCTTTCTCCC TTCGGGAAGC GTGGCGCTTT
3151 CTCATAGCTC ACGCTGTAGG TATCTCAGTT CGGTGTAGGT CGTTCGCTCC
3201 AAGCTGGGCT GTGTGCACGA ACCCCCCGTT CAGCCCGACC GCTGCGCCTT
3251 ATCCGGTAAC TATCGTCTTG AGTCCAACCC GGTAAGACAC GACTTATCGC
3301 CACTGGCAGC AGCCACTGGT AACAGGATTA GCAGAGCGAG GTATGTAGGC
3351 GGTGCTACAG AGTTCTTGAA GTGGTGGCCT AACTACGGCT ACACTAGAAG
3401 GACAGTATTT GGTATCTGCG CTCTGCTGAA GCCAGTTACC TTCGGAAAAA
3451 GAGTTGGTAG CTCTTGATCC GGCAAACAAA CCACCGCTGG TAGCGGTGGT
3501 TTTTTTGTTT GCAAGCAGCA GATTACGCGC AGAAAAAAAG GATCTCAAGA
3551 AGATCCTTTG ATCTTTTCTA CGGGGTCTGA CGCTCAGTGG AACGAAAACT
3601 CACGTTAAGG GATTTTGGTC ATGAGATTAT CAAAAAGGAT CTTCACCTAG
3651 ATCCTTTTAA ATTAAAAATG AAGTTTTAAA TCAATCTAAA GTATATATGA
3701 GTAAACTTGG TCTGACAGTT ACCAATGCTT AATCAGTGAG GCACCTATCT
3751 CAGCGATCTG TCTATTTCGT TCATCCATAG TTGCCTGACT CCCCGTCGTG
3801 TAGATAACTA CGATACGGGA GGGCTTACCA TCTGGCCCCA GTGCTGCAAT
3851 GATACCGCGA GACCCACGCT CACCGGCTCC AGATTTATCA GCAATAAACC
3901 AGCCAGCCGG AAGGGCCGAG CGCAGAAGTG GTCCTGCAAC TTTATCCGCC
3951 TCCATCCAGT CTATTAATTG TTGCCGGGAA GCTAGAGTAA GTAGTTCGCC
4001 AGTTAATAGT TTGCGCAACG TTGTTGCCAT TGCTACAGGC ATCGTGGTGT
4051 CACGCTCGTC GTTTGGTATG GCTTCATTCA GCTCCGGTTC CCAACGATCA
4101 AGGCGAGTTA CATGATCCCC CATGTTGTGC AAAAAAGCGG TTAGCTCCTT
4151 CGGTCCTCCG ATCGTTGTCA GAAGTAAGTT GGCCGCAGTG TTATCACTCA
4201 TGGTTATGGC AGCACTGCAT AATTCTCTTA CTGTCATGCC ATCCGTAAGA
4251 TGCTTTTCTG TGACTGGTGA GTACTCAACC AAGTCATTCT GAGAATAGTG
4301 TATGCGGCGA CCGAGTTGCT CTTGCCCGGC GTCAATACGG GATAATACCG
4351 CGCCACATAG CAGAACTTTA AAAGTGCTCA TCATTGGAAA ACGTTCTTCG
4401 GGGCGAAAAC TCTCAAGGAT CTTACCGCTG TTGAGATCCA GTTCGATGTA
4451 ACCCACTCGT GCACCCAACT GATCTTCAGC ATCTTTTACT TTCACCAGCG
4501 TTTCTGGGTG AGCAAAAACA GGAAGGCAAA ATGCCGCAAA AAAGGGAATA
4551 AGGGCGACAC GGAAATGTTG AATACTCATA CTCTTCCTTT TTCAATATTA
4601 TTGAAGCATT TATCAGGGTT ATTGTCTCAT GAGCGGATAC ATATTTGAAT
4651 GTATTTAGAA AAATAAACAA ATAGGGGTTC CGCGCACATT TCCCCGAAAA
4701 GTGCCACCTG ACGTCTAAGA AACCATTATT ATCATGACAT TAACCTATAA
4751 AAATAGGCGT ATCACGAGGC CCTTTCGTCT CGCGCGTTTC GGTGATGACG
4801 GTGAAAACCT CTGACACATG CAGCTCCCGG AGACGGTCAC AGCTTGTCTG
4851 TAAGCGGATG CCGGGAGCAG ACAAGCCCGT CAGGGCGCGT CAGCGGGTGT
4901 TGGCGGGTGT CGGGGCTGGC TTAACTATGC GGCATCAGAG CAGATTGTAC
4951 TGAGAGTGCA CCATATGCGG TGTGAAATAC CGCACAGATG CGTAAGGAGA
5001 AAATACCGCA TCAGGCGCCA TTCGCCATTC AGGCTGCGCA ACTGTTGGGA
5051 AGGGCGATCG GTGCGGGCCT CTTCGCTATT ACGCCAGCTG GCGAAAGGGG
5101 GATGTGCTGC AAGGCGATTA AGTTGGGTAA CGCCAGGGTT TTCCCAGTCA
5151 CGACGTTGTA AAACGACGGC CAGT

7.3 Enzyme cleavage sites

Restriction Enzyme	Site 1	Site 2
Acc36I	12	2535
AccI	2523	
AccIII	617	
AclI	4018	4391
AflIII	2899	
BalI	2287	
BcgI	4329	
BclI	1238	1979
BfuI	3102	4629
BglII	977	
Bpu10I	1573	1612
BsaXI	1614	2739
BsmI	2368	2461
Bso31I	3853	
BstAPI	4964	
BstV2I	600	
BtgZI	300	
CspCI	376	
DrdI	3007	4876
Eam1105I	3792	
Eco52I	882	2303
EcoICRI	532	
EcoRI	1	916
FalI	1963	
FspI	4014	5037
HincII	2447	2524
HindIII	2540	
HpaI	2447	
MunI	2456	
NcoI	326	650
NdeI	200	4963
NotI	2303	

PasI	1413	
PciI	2899	
PpiI	3608	4420
PpuMI	1326	
PsiI	2427	
PspXI	640	
PvuI	4162	5058
SacI	534	
SacII	2309	
SalI	2522	
SanDI	1326	
ScaI	4272	
SexAI	1343	1580
SgrAI	1915	2056
SnaBI	306	
SphI	17	2538
SspI	4596	
TsoI	2435	4191
TspGWI	4232	4574
TstI	1477	
Tth111I	1060	2158
Van91I	1146	
XbaI	624	2516
XhoI	640	
XmnI	1948	4391

8. Notes

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