

A Comparison of pCI-neo Vector and pcDNA4/HisMax Vector



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The pCI-neo Mammalian Expression Vector contains a cytomegalovirus (CMV) immediate-early enhancer/promoter, an optimized chimeric intron and the simian virus 40 (SV40) late polyadenylation signal. These three elements combine to yield strong, constitutive expression of the cloned genes in mammalian cells. The vector also contains a neomycin phosphotransferase gene for selection of stably transfected clones, a T7 promoter, an f1 origin of replication, unique restriction enzyme sites flanking each of the elements of the vector, a high-copy plasmid replicon, and a versatile multiple cloning region. In this article, we compare the expression of three transgenes cloned into Promega's pCI-neo Vector and the pcDNA4/HisMax Vector from Invitrogen.

INTRODUCTION

Increasing eukaryotic expression by optimization of promoters and/or vectors is a major driving force in the field of mammalian gene expression. In the past, eukaryotic viral promoter units (i.e., CMV, SV40 and RSV) were included in vectors, and these promoters alone were sufficient to provide protein expression in mammalian cells. Currently, the CMV promoter is accepted as the standard of mammalian promoter strength due to its demonstrated high level of transient transgene expression in a majority of mammalian cells (1). In pursuit of increased transgene expression, additional features have been included in mammalian vectors. One such feature is the inclusion of an intron, which increases the expression of all cDNA inserts (2,3). A chimeric intron was designed using the 5'-donor site from the first intron of the human β -globin and the branch and 3'-acceptor site from the intron located between the leader and body of an immunoglobulin gene heavy chain variable region (4). This chimeric intron, which has been demonstrated to significantly increase transgene expression (5), is included in a variety of the mammalian expression vectors offered by Promega.

Recently, a mammalian expression vector has been designed that includes a 163bp DNA sequence isolated from the 5'-untranslated region of the mouse vascular endothelial growth factor (VEGF) gene, which acts as a translational enhancer. According to the technical literature on this product, when a transgene is subcloned in-frame with this translational enhancer and transfected into a variety of mammalian cell lines, the expression level of the transgene increases.

To determine the differences in transgene expression between a vector with a chimeric intron (Promega) or a vector with a translational enhancer (Invitrogen), three reporter genes (i.e., *luc* [luciferase], *lacZ*, and the green fluorescent protein gene [GFP]) were subcloned into the pCI-neo Mammalian Expression Vector^(a,b) (Promega Cat.# E1841) and the pcDNA4/HisMax (Invitrogen) eukaryotic expression vector. After subcloning, the vectors containing the reporter genes were transfected into various mammalian cell lines. Protein expression levels were determined for each of the reporter genes 48 hours post-transfection.

LUCIFERASE EXPRESSION

Firefly luciferase cDNA (*luc*) was subcloned into the pCI-neo and pcDNA4/HisMax Vectors, resulting in the constructs pCI-neo+*luc* and pcDNA4/HisMax+*luc*, respectively. The vector constructs were transfected into NIH3T3, CHO, COS-1, COS-7, HeLa and 293 cells using Promega's TransFast™ Transfection Reagent^(c) (Cat.# E2431). Cells were harvested 48 hours post-transfection, and levels of luciferase expression were determined using the Luciferase Assay System^(d) (Cat.# E1500; see Figure 1).

The data demonstrate that NIH3T3, COS-1 and COS-7 cells transfected with pCI-neo+*luc* express greater amounts of luciferase than the same cell lines transfected with pcDNA4/HisMax+*luc*. Equal levels of luciferase expression are displayed by CHO, HeLa and 293 cells transfected with either pCI-neo+*luc* or pcDNA4/HisMax+*luc*.

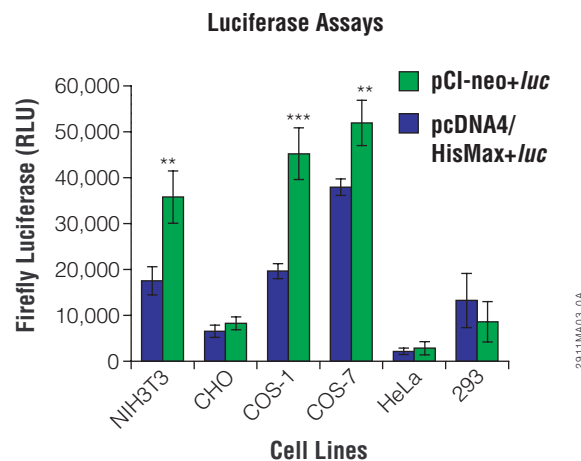


Figure 1. Relative levels of luciferase expression for pCI-neo+*luc* and pcDNA4/HisMax+*luc* in different cell lines. The luciferase cDNA was subcloned into the pCI-neo and pcDNA4/HisMax vectors, and the resulting constructs were transfected into NIH3T3, CHO, COS-1, COS-7, HeLa or 293 cells using the TransFast™ Transfection Reagent. Forty-eight hours post-transfection, cells were harvested using Luciferase Cell Culture Lysis Reagent^(c) (Cat.# E1531), and luciferase expression was determined using the Luciferase Assay System. The data are represented as the mean and standard deviation relative light units (RLU) for each cell line and construct. The experiment was repeated with identical results. ** $p < 0.01$; *** $p < 0.001$.

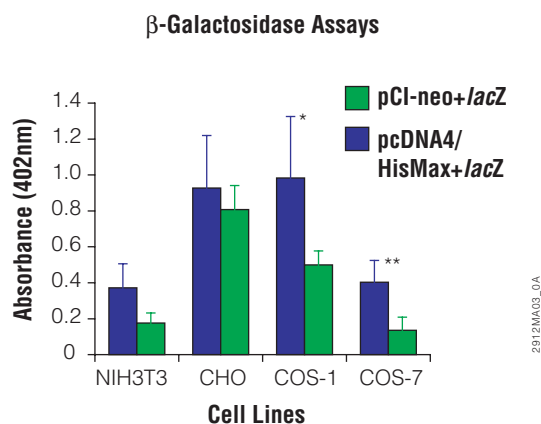


Figure 2. Levels of β-galactosidase expression for pCI-neo+lacZ and pcDNA4/HisMax+lacZ in different cell lines. The *lacZ* gene was subcloned into the pCI-neo and pcDNA4/HisMax vectors, and the resulting constructs were transfected into NIH3T3, CHO, COS-1 or COS-7 cells using the TransFast™ Transfection Reagent. Forty-eight hours post-transfection, the cells were harvested with Reporter Lysis Buffer (Cat.# E3971) and expression levels of β-galactosidase were determined using the β-Galactosidase Enzyme Assay System (Cat.# E2000). The data are represented as the mean and standard deviation for each cell line and construct. Transfection controls (no DNA) were used to normalize the data. The experiment was repeated an additional three times with identical results. *p<0.05; **p<0.01.

β-GALACTOSIDASE EXPRESSION

pCI-neo+lacZ (resulting from the subcloning of *lacZ* into the pCI-neo Vector) and pcDNA4/HisMax+lacZ (supplied as a control by Invitrogen) constructs were transfected into NIH3T3, CHO, COS-1 and COS-7 cells. Cells were harvested 48 hours post-transfection, and expression levels of β-galactosidase (β-gal) were determined using the β-Galactosidase Enzyme Assay System^(c) (Cat.# E2000).

The data shown in Figure 2 demonstrate that β-gal expression levels for NIH3T3 and CHO cells transfected with either *lacZ*-containing construct are similar. However, β-gal expression levels from COS-1 and COS-7 cells transfected with pcDNA4/HisMax+lacZ are higher than the same cells transfected with pCI-neo+lacZ. The COS-1 data are statistically significant at p<0.05, while the COS-7 data are statistically significant at p<0.01.

GFP Fluorescence Assays

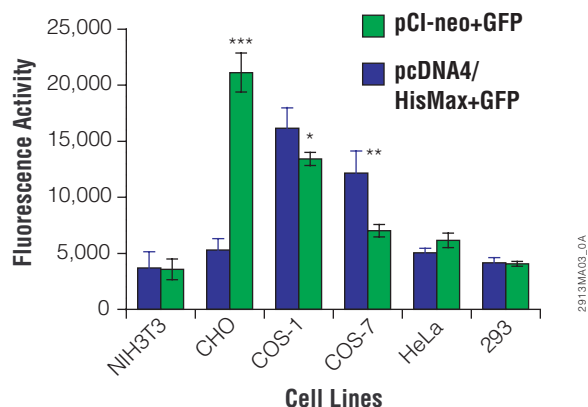


Figure 3. The levels of GFP expression for pCI-neo+GFP and pcDNA4/HisMax+GFP in different cell lines. The green fluorescent protein cDNA was subcloned into the pCI-neo and pcDNA4/HisMax vectors and transfected into NIH3T3, CHO, COS-1, COS-7, HeLa or 293 cells using the TransFast™ Transfection Reagent. Forty-eight hours post-transfection, the cells were trypsinized, and GFP expression was measured using a fluorometer. The data are represented as the mean and standard deviation for each cell line and construct. No DNA transfection controls were used to normalize the data. The experiment was repeated an additional three times with identical results. *p<0.05; **p<0.01; ***p<0.001.

GREEN FLUORESCENT PROTEIN EXPRESSION

cDNA encoding the green fluorescence protein (6,7) was subcloned into the pCI-neo and the pcDNA4/HisMax vectors, producing pCI-neo+GFP and pcDNA4/HisMax+GFP constructs, respectively. NIH3T3, CHO, COS-1, COS-7, HeLa and 293 cells were transfected with pCI-neo+GFP or pcDNA4/HisMax+GFP using Promega's TransFast™ Transfection Reagent. Cells were subsequently harvested 48 hours post-transfection and analyzed for GFP expression with a fluorometer (see Figure 3).

CHO cells transfected with pCI-neo+GFP express 4-fold more GFP than CHO cells transfected with pcDNA4/HisMax+GFP (data statistically significant at p<0.001). Conversely, COS-7 cells transfected with pcDNA4/HisMax+GFP express more GFP than the same cell line transfected with pCI-neo+GFP (data statistically significant at p<0.01). COS-1 cells transfected with pcDNA4/HisMax+GFP express higher GFP levels than cells transfected with pCI-neo+GFP (data statistically significant at p<0.05). Upon transfection with either GFP-expressing vector, the remaining cells (i.e., NIH3T3, HeLa and 293) express equivalent levels of GFP.



SUMMARY

The data suggest that no fundamental difference in transgene expression exists between the pCI-neo Mammalian Expression Vector from Promega and the pcDNA4/HisMax vector (Invitrogen). In this work, the level of transgene expression varied depending on both transgene and cell line. In some instances, transgene expression levels resulting from subcloning into the pCI-neo Mammalian Expression Vector and subsequent transfection were significantly greater. In the majority of instances, transgene expression levels were equivalent.

Importantly, Promega's pCI-neo Mammalian Expression Vector possesses noteworthy advantages over the pcDNA4/HisMax vector. First, subcloning into pCI-neo Vector is much more straightforward than subcloning into pcDNA4/HisMax. The pcDNA4/HisMax vector system contains three different vectors, referred to as pcDNA4/HisMax A, B and C, and each vector's multiple cloning site possesses a different translational reading frame. Thus, when subcloning into the pcDNA4/HisMax vector, an out-of-frame subcloning will result in no expression of your protein of interest. In addition, to preserve original protein function or structure, the transgene should be subcloned with little or no 5'-untranslated region present.

A second advantage of Promega's pCI-neo Vector is that expression from this vector does not generate an unnecessary N-terminal fusion peptide. The additional N-terminal fusion peptide generated by subcloning into the existing pcDNA4/HisMax vector reading frame may adversely affect folding or function of a protein of interest.

REFERENCES

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Ordering Information

Product	Size	Cat. #
pCI-neo Mammalian Expression Vector	20µg	E1841
β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer		E2000
TransFast™ Transfection Reagent	1.2mg	E2431
Bright-Glo™ Luciferase Assay System ^(d)	10ml	E2620
Luciferase Assay System	100 assays	E1500
Luciferase 1000 Assay System ^(d)	1,000 assays	E4550
Luciferase Cell Culture Lysis Reagent, 5X	30ml	E1531

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^(a)U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.

^(b)The CMV promoter and its use are covered under U.S. Pat. Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation, Iowa City, Iowa, and licensed FOR RESEARCH USE ONLY. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.

^(c)The cationic lipid component of the TransFast™ Transfection Reagent is covered by U.S. Pat. Nos. 5,824,812, 5,869,715 and pending foreign patents.

^(d)U.S. Pat. No. 5,283,179, Australian Pat. No. 649289 and other patents. Certain applications of this product may require licenses from others.

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