

## Certificate of Analysis

### pNLCol3[*luc2*-P2A-*NlucP*/CMV/Hygro] Vector:

<b>Part No.</b>	<b>Size</b>
N148A	20µg

**Description:** The pNLCol3[*luc2*-P2A-*NlucP*/CMV/Hygro] Vector<sup>(a,b)</sup> is a second generation coincidence reporter vector (Col; 1) that allows expression of both firefly luciferase (Fluc, *luc2* gene) from *Photinus pyralis* and NanoLuc<sup>®</sup> Luciferase fused to a PEST destabilization domain (NanoLuc<sup>®</sup>-PEST, *NlucP* gene) from the same mRNA transcript. The stoichiometric expression of both luciferases is achieved by use of the P2A sequence from porcine teschovirus-1, which promotes a ribosomal skip and expression of the two unfused enzymes with different compound interaction profiles. When used in high-throughput screening, true hits will show a similar response from both reporters, helping to reduce the number of false hits and the workload associated with follow-up screens.

The *luc2* and *NlucP* genes are codon optimized for mammalian expression, and all pNL vectors have minimal consensus transcription factor-binding sites to reduce anomalous expression. The pNLCol3[*luc2*-P2A-*NlucP*/CMV/Hygro] Vector contains a CMV promoter for high-level expression of both reporters, allowing its use as a constitutive expression control.

The pNLCol3[*luc2*-P2A-*NlucP*/CMV/Hygro] Vector contains the following key features:

- Firefly Luciferase reporter gene (*luc2*).
- P2A peptide sequence that promotes a ribosomal skip, resulting in the stoichiometric expression of two unfused reporter proteins from the same mRNA transcript.
- NanoLuc<sup>®</sup> Luciferase reporter gene fused to the PEST destabilization domain (*NlucP*). PEST-mediated turnover by the proteasome provides a reporter with a very short intracellular protein half-life, providing a reporter protein that responds more quickly and typically with greater signal-to-background compared to other reporters. NanoLuc<sup>®</sup>-PEST is also substantially brighter than *Renilla* luciferase, providing a dramatic increase in sensitivity for the second generation coincidence reporter system.
- Ampicillin resistance gene for selection in bacteria.
- Hygromycin resistance gene for selection in mammalian cells.
- A CMV promoter for high-level expression in mammalian cells.

**Concentration:** 1µg/µl.

**GenBank<sup>®</sup> Accession Number:** KM359773.

**Storage Buffer:** The pNLCol3[*luc2*-P2A-*NlucP*/CMV/Hygro] Vector is supplied in 10mM Tris-HCl (pH 7.4), 1mM EDTA.

**Storage Conditions:** See the product information label for storage recommendations and expiration date.

## Quality Control Assays

### Contaminant Assays

**Contaminating Nucleic Acids:** RNA, single-stranded DNA and chromosomal DNA are not evident in specified quantities of the vector as determined by agarose gel electrophoresis.

**Endotoxin Concentration:** Endotoxin Units (EU) are obtained using *Limulus amoebocyte* lysate testing. The specification is <100EU/mg of plasmid DNA.

**Nuclease Assay:** Following incubation of 1µg of the vector in restriction enzyme buffer at 37°C for 16–24 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

**Physical Purity:**  $A_{260}/A_{280} \geq 1.80$ ,  $A_{260}/A_{250} \geq 1.05$  at pH 7.4.

### Functional Assays

**Identity Assay:** The vector was sequenced completely and has 100% identity with the published sequence available at:

[www.promega.com/vectors](http://www.promega.com/vectors)

**Restriction Digestion:** The functional purity of vector DNA is verified by successful digestion with restriction enzymes at the optimal temperature for one hour. Samples are examined by agarose gel electrophoresis to compare cut and uncut vector DNA with marker DNA.

## Reference

1. Cheng, K.C. and Inglesse, J. (2012) A coincidence reporter-gene system for high-throughput screening. *Nat. Methods*, **9**, 937.

Signed by:

R. Wheeler, Quality Assurance

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**Promega**

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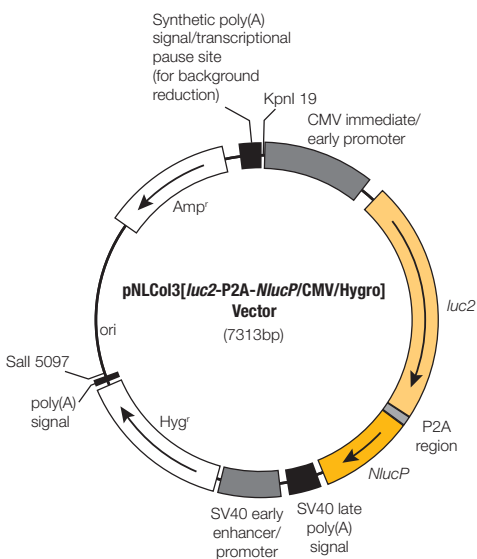
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## pNLCol3[*luc2*-P2A-*NlucP*/CMV/Hygro] Vector Features and Map

The following features are present in the vector based on nucleotide sequence.

CMV immediate early enhancer/promoter	20–761
<i>luc2</i> reporter gene	865–2514
P2A region	2515–2580
<i>NlucP</i> reporter gene	2581–3219
SV40 late poly(A) region	3259–3480
SV40 early enhancer/promoter	3528–3946
Hygromycin resistance coding region	3971–5008
Synthetic poly(A) sequence	5032–5080
Reporter Vector primer 4 (RVprimer4) binding region	5147–5166
<i>Col/E1</i> -derived plasmid replication origin	5404
Synthetic $\beta$ -lactamase ( <i>Amp<sup>r</sup></i> ) coding region	6195–7055
Synthetic poly(A) signal/transcriptional pause region	7160–7313
Reporter Vector primer 3 (RVprimer3) binding region	7262–7281



Sequence information and vector maps are available online at: [www.promega.com/vectors](http://www.promega.com/vectors)

For information on the Nano-Glo® Dual-Luciferase® Assay System, see Technical Manual #TM426, available online at: [www.promega.com/protocols](http://www.promega.com/protocols)

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