Promega

Technical Bulletin

pSP-*luc*+NF Fusion Vector

INSTRUCTIONS FOR USE OF PRODUCT E4471.

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1. Description

The pSP-*luc*+NF Fusion Vector[a,b,c] is a luciferase cassette vector containing the engineered firefly luciferase gene, *luc*+NF. The *luc*+NF gene is related to the *luc*+ gene found in the pGL3 family of eukaryotic reporter vectors (1) but has been further modified for maximum flexibility in constructing N-terminal fusions (NF) with luciferase. The pSP-*luc*+NF Fusion Vector is not itself intended for the expression of luciferase in eukaryotic cells because it does not contain eukaryotic promoters, enhancers or polyadenylation signals.

The *luc*+NF gene is positioned downstream of an SP6 promoter and ribosome binding site. An opposing T7 promoter is located immediately downstream of *luc*+NF. Thus, the pSP-*luc*+NF Fusion Vector provides a convenient template for the in vitro synthesis of both sense and antisense luciferase transcripts for studies involving in situ hybridization, RNA processing, RNA transfection or coupled in vitro transcription/translation and protein folding. Multiple cloning regions containing recognition sequences for commonly used restriction enzymes are positioned at the 5´ and 3´ ends of *luc*+NF to provide maximum flexibility in cloning. Subcloning *luc*+NF into expression vectors provides a useful genetic reporter with exceptional sensitivity. Luciferase enzymatic activity can be assayed most efficiently using the Luciferase Assay System.

Luciferase is a 61kDa monomeric protein that does not require post-translational modifications for enzymatic activity. Thus, it can function as a genetic reporter immediately upon translation (2–4). Luciferase synthesized by in vitro translation can be labeled with ^{35}S, as the protein contains 4 cysteine and 14 methionine residues. To ensure full enzymatic activity of luciferase, no more than 5 codons can be deleted from either the 5´- or 3´-end of the coding region.

Properties of the *luc*+NF Gene

The numerous modifications that distinguish *luc*+NF from the native luciferase gene are detailed in Table 1. Briefly, the *luc*+NF gene (and the related *luc*+ gene) contains four general classes of modifications: 1) The C-terminal tripeptide has been mutated to eliminate peroxisome targeting of the expressed protein; 2) codon usage was improved for expression in plant and animal cells; 3) two potential sites of N-glycosylation were removed; and 4) several DNA sequence changes were made to disrupt extended palindromes, remove internal restriction sites and eliminate consensus sequences recognized by genetic regulatory binding proteins (5), helping to ensure that the reporter gene itself is unaffected by spurious host transcriptional signals.

In addition, the *luc*+NF gene contains a unique BstEII restriction site inserted immediately downstream of the luciferase ATG translation start codon. The BstEII site allows a cloned insert to be positioned immediately downstream of the *luc*+NF initiation codon. If the cloned insert already contains an initiation codon, the *luc*+NF initiation sequences can be removed using BstEII and another restriction enzyme that cuts in the upstream multiple cloning region. This eliminates the possibility of expressing low levels of functional luciferase through internal initiation in the luciferase portion of the gene fusion.

As a result of the insertion of the BstEII site, the glutamic acid at position 2 of the native protein has been replaced with a valine-threonine at positions 2 and 3 of the *luc*+NF protein. Because of the altered N-terminal amino acid sequence, luciferase encoded by *luc*+NF may exhibit slightly lower enzymatic activity than that encoded by *luc*+.

Note: The numbering scheme for amino acids is relative to the first amino acid, Met #1, of luciferase (*luc*). The numbering of nucleotides is relative to the first base, "A", of the luciferase open reading frame. All numberings for Table 1 refer to the amino acid or nucleic acid positions for the native luciferase gene. Because of the addition of the BstEII site at position 4 of the native nucleic acid sequence to create *luc*+NF, all of the nucleic acid numbering will be shifted by 3 nucleotides and all amino acid numbering will be shifted by 1 residue for the *luc*+NF gene.

Table 1. Summary of Modifications in the *luc*+NF Gene.

Purpose of Modification	Sequence Modification in *luc*+NF
Introduce NcoI and BstEII sites for the construction of N-terminal fusions with *luc*+NF.	Met$_1$ Glu$_2$ Asp$_3$ *luc*: AAA ATG GAA GAC Met$_1$ Val$_2$ Thr$_3$ Asp$_4$ *luc*+NF: TCC ATG GTC ACC GAC NcoI BstEII
Remove internal XbaI site; disrupt extended palindrome.	#50 XbaI *luc*: CTCTAGAGG *luc*+NF: CGCTGGAAG
Eliminate potential glycosylation and ATF sites.	Asn$_{51}$ Ile$_{52}$ Thr$_{53}$ *luc*: AAC ATC ACG TACGCGGAA Asp$_{51}$ Ile$_{52}$ Thr$_{53}$ *luc*+NF: GAC ATC ACT TACGCTGAG
Eliminate potential glycosylation sites.	Asn$_{120}$ Ile$_{121}$ Ser$_{122}$ *luc*: AAC ATT TCG Gly$_{120}$ Ile$_{121}$ Ser$_{122}$ *luc*+NF: GGC ATT TCG
Remove potential TGT-3 site; improve codon usage.	#376 *luc*: GTAGTGTTTGTT *luc*+NF: GTGGTGTTCGTT
Improve codon usage.	#429 *luc*: ATTACCAATAATCCAG *luc*+NF: GCTCCCAATCATCCAA
Improve codon usage.	#549 *luc*: ACCAGAGTCCTTTGATCGTGACAAA *luc*+NF: GCCAGAGTCCTTCGATAGGGACAAG
Remove internal EcoRI site, improve codon usage.	#586 EcoRI *luc*: ATAATGAATTCC *luc*+NF: ATCATGAACTCC
Remove internal BstEII site and potential AP-2 and LF-A1 sites; improve codon usage.	#611 BstEII *luc*: GGTTACCTAAGGGTGTGGCCCTTCCG *luc*+NF: GTCTGCCTAAAGGTGTCGCTCTGCCT
Remove potential AP-1 site.	#649 *luc*: TGCGTCAG *luc*+NF: TGCGTGAG

Table 1. Summary of Modifications in the *luc*+NF Gene (continued).

Purpose of Modification	Sequence Modification in *luc*+NF
Improve codon usage.	#823 *luc*: TTACGATCCCTTCAGGATTACAAA *luc*+NF: CTGAGGAGCCTTCAGGATTACAAG
Improve codon usage.	#859 *luc*: TTGCTAGTACCAACCCTATTTTCA *luc*+NF: CTGCTGGTGCCAACCCTATTCTCC
Eliminate internal palindrome; improve codon usage.	#948 *luc*: GGGCGCACCTCTTTCGAAA *luc*+NF: TGGCGCTCCCCTCTCTAAG
Improve codon usage.	#987 *luc*: AAAACGCTTCCATCTTCCAGGGATACGA *luc*+NF: CAAGAGGTTCCATCTGCCAGGTATCAGG
Eliminate potential AP-1 site; improve codon usage.	#1161 *luc*: GAGAGGCGAATTATGTGTCAGAGGA *luc*+NF: AAGAGGCGAACTGTGTGTGAGAGGT
Eliminate palindrome structure; improve codon usage.	#1305 *luc*: AGTTGACCGCTTGAAGTCTTTAATTAAATAC *luc*+NF: CGTTGACCGCCTGAAGTCTCTGATTAAGTAC
Remove internal EcoRV site; improve codon usage.	#1336 EcoRV *luc*: AAAGGATATCAGGTGGCC *luc*+NF: AAAGGCTATCAGGTGGCT
Remove internal ClaI site; improve codon usage.	#1368 ClaI *luc*: ATCGATATTGTTA *luc*+NF: ATCCATCTTGCTC
Remove potential SP-1 site and AP-2 sites.	#1403 *luc*: CGGGCGTGGC *luc*+NF: CAGGTGTCGC
Remove peroxisome targeting sequence.	Gly_{547} Lys_{548} Ser_{549} Lys_{550} Leu_{551} stop *luc*: GGA AAG TCC AAA TTG TAA Gly_{547} Lys_{548} Ile_{549} Ala_{550} Val_{551} stop *luc*+NF: GGA AAG ATC GCC GTG TAA

2. Product Components and Storage Conditions

Product	Size	Cat.#
pSP-*luc*+NF Fusion Vector	20µg	E4471

Storage Conditions: Store the pSP-*luc*+NF Fusion Vector at –20°C.

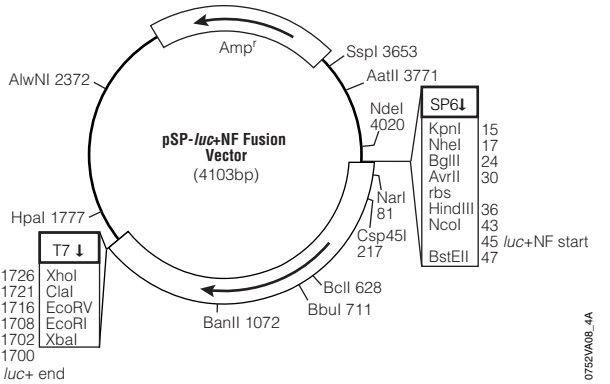

Figure 1. pSP-*luc*+NF Fusion Vector circle map and sequence reference points.
(rbs = ribosome binding site)

Sequence reference points:

SP6 RNA polymerase initiation site	1
upstream multiple cloning region	11–53
luciferase coding region	45–1700
downstream multiple cloning region	1702–1731
T7 RNA polymerase promoter (–17 to +3)	1740–1759
T7 RNA polymerase initiation site	1742
ColE1-derived replication origin	2014
β-lactamase (Amp^r) coding region	3636–2776
SP6 RNA polymerase promoter (–17 to +3)	4087–3

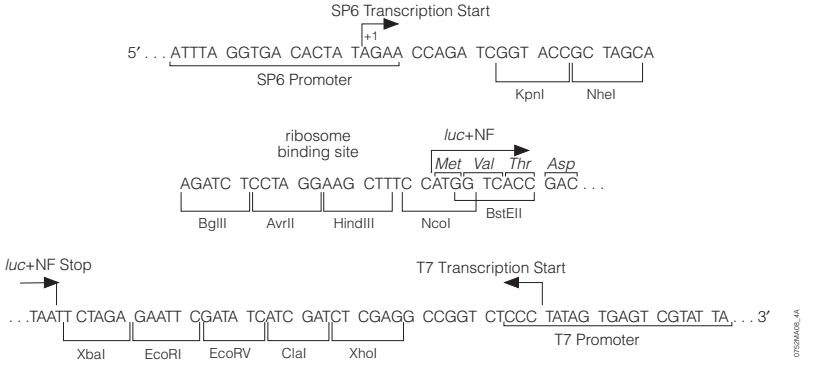

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Figure 2. pSP-*luc*+NF Fusion Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by SP6 RNA polymerase and corresponds to the sequence of *luc*+NF mRNA. Note that cleavage at the ClaI site is blocked when the pSP-*luc*+NF Fusion Vector is propagated in host strains containing *dam* methylase activity.

3. Related Products

Product	Size	Cat.#
Luciferase Assay System (with Cell Culture Lysis Reagent)	100 assays	E1500
Luciferase Assay System with Reporter Lysis Buffer	100 assays	E4030
Luciferase Assay Reagent 10-Pack	1,000 assays	E1501
Luciferase Assay Reagent (bulk liquid reagent)	100ml	E1483
pGL3-Control Vector	20µg	E1741
pGL3-Enhancer Vector	20µg	E1771
pGL3-Promoter Vector	20µg	E1761
pGL3-Basic Vector	20µg	E1751

4. References

1. Sherf, B. and Wood, K.V. (1994) Increased expression and convenience with the new pGL3 Luciferase Reporter Vectors. *Promega Notes* **49**, 14–21.

2. Ow, D. *et al.* (1986) Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. *Science* **234**, 856–9.

3. deWet, J.R. *et al.* (1987) Firefly luciferase gene: Structure and expression in mammalian cells. *Mol. Cell Biol.* **7**, 725–37.

4. Wood, K.V. (1990) Firefly luciferase: A new tool for molecular biologists. *Promega Notes* **28**, 1–3.

5. Faisst, S. and Meyer, S. (1992) Compilation of vertebrate-encoded transcription factors. *Nucleic Acids Res.* **20**, 3–26.

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[b]U.S. Pat. No. 5,670,356.

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