

Vector pEi-ck-myc-Luc for Gene Expression in *Entamoeba invadens*

Catalog No. NR-42013

Product Description: pEi-ck-myc-Luc is an expression vector for use in *Entamoeba invadens* (*E. invadens*). The pBluescript SK- vector was modified to contain the luciferase gene from *Photinus pyralis* flanked by promoter sequences from the CKII gene to drive gene expression. A Myc tag and *NheI* and *AvrII* sites have been introduced for inserting the gene of interest. The vector also contains the gene for neomycin resistance flanked by 5' and 3' regions of *enolase* for selection. The resulting plasmid, NR-42013, may be used as a vector for both transient and stable transfection of *E. invadens*. The plasmid was produced in One Shot[®] TOP10 chemically competent *Escherichia coli* (Invitrogen[™]) and extracted using a QIAGEN[®] EndoFree[®] Plasmid Maxi Kit.

Lot¹: 61329982

Manufacturing Date: 22MAR2013

TEST	SPECIFICATIONS	RESULTS
Confirmation of Insert by PCR ²	~ 1670 base pair amplicon	~ 1670 base pair amplicon
Confirmation of Insert by Sequencing (Figure 1)	Consistent with <i>Photinus pyralis</i> luciferase gene	Consistent with <i>Photinus pyralis</i> luciferase gene ^{3,4}
DNA Content by PicoGreen [®] Measurement	0.7 µg to 1.5 µg per vial	0.8 µg per vial
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 2.1	1.8
Bacterial Transformability (Post-Vial) ⁵	≥ 100 colonies	≥ 100 colonies

¹Produced in One Shot[®] TOP10 chemically competent *Escherichia coli* (Invitrogen[™]) and extracted using a QIAGEN[®] EndoFree[®] Plasmid Maxi Kit.

²PCR was performed with primers designed to amplify the luciferase gene. PCR primers used for amplification were Luc Forward- 5' GGGAATTCATGGAAGACGCCAAAAAC 3' and Luc Reverse- 5' CCAAGCTTTTACAATTTGGACTTTCC 3'. Conditions used for PCR are available upon request.

³GenBank: X84846 bases 115 to 1767

⁴Also consistent with other luciferase reporter vectors

⁵One Shot[®] TOP10 *Escherichia coli* were transformed with 23 ng of plasmid DNA.

Figure 1. Vector pEi-ck-myc-Luc Insert Sequence

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1      GGGAAATTCATGGAAGACGCCAAAAACATAAAGAAAGGCCCGCGCCATTCTATCCTCTAG 60
61     AGGATGGAACCGCTGGAGAGCAACTGCATAAGGCTATGAAGAGATACGCCCTGGTTCCCTG 120
121    GAACAATTGCTTTTACAGATGCACATATCGAGGTGAACATCACGTACGCGGAATACTTCG 180
181    AAATGTCCGTTTCGGTTGGCAGAAGCTATGAAACGATATGGGCTGAATACAAATCACAGAA 240
241    TCGTCGTATGCAGTGAAAACCTCTCTCAATTCTTTATGCCGGTGTGGGGCGCGTTATTTA 300
301    TCGGAGTTGCAGTTGCGCCCGCGAACGACATTTATAATGAACGTGAATTGCTCAACAGTA 360
361    TGAACATTTTCGCAGCTACCGTAGTGTGTTGTTTCCAAAAAGGGGTTGCAAAAAATTTTGA 420
421    ACGTGCAAAAAAATTACCAATAATCCAGAAAATTATTATCATGGATTCTAAAACGGATT 480
481    ACCAGGGATTTTCAGTCGATGTACACGTTTCGTACACATCTCATCTACCTCCCGGTTTTAATG 540
541    AATACGATTTTGTACCAGAGTCCTTTGATCGTGACAAAACAATTGCACTGATAATGAATT 600
601    CCTCTGGATCTACTGGGTTACCTAAGGGTGTGGCCCTTCCGCATAGAAGTGCCTGCGTCA 660
    
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661 GATTCTCGCATGCCAGAGATCCTATTTTTGGCAATCAAATCATTCCGGATACTGCGATTT 720
721 TAAGTGTGTTCCATTCCATCACGGTTTTGGAATGTTTACTACACTCGGATATTTGATAT 780
781 GTGGATTCGAGTCGTCTTAATGTATAGATTTGAAGAAGAGCTGTTTTTACGATCCCTTC 840
841 AGGATTACAAAATTCAAAGTGC GTTGTCTAGTACCAACCCTATTTTCATTCTTCGCCAAAA 900
901 GCACTCTGATTGACAAAATACGATTTATCTAATTTACACGAAATTGCTTCTGGGGGCGCAC 960
961 CTCTTTCGAAAGAAGTCGGGGAAGCGGTTGCAAAACGCTTCCATCTTCCAGGGATACGAC 1020
1021 AAGGATATGGGCTCACTGAGACTACATCAGCTATTCTGATTACACCCGAGGGGGATGATA 1080
1081 AACCGGGCGCGGTTCGTAAGTTGTTCCATTTTTTGAAGCGAAGGTTGTGGATCTGGATA 1140
1141 CCGGAAAACGCTGGGCGTTAATCAGAGAGGCGAATTATGTGTCAGAGGACCTATGATTA 1200
1201 TGTCCGTTATGTAAACAATCCGGAAGCGACCAACGCCTTGATTGACAAGGATGGATGGC 1260
1261 TACATTCTGGAGACATAGCTTACTGGGACGAAGACGAACACTTCTTCATAGTTGACCGCT 1320
1321 TGAAGTCTTTAATTAAATACAAAGGATATCAGGTGGCCCCCGCTGAATTGGAATCGATAT 1380
1381 TGTTACAACACCCCAACATCTTCGACGCGGGCGTGGCAGGTCTTCCCGACGATGACGCCG 1440
1441 GTGAACTTCCCGCCGCGGTTGTTGTTTTGGAGCACGGAAAGACGATGACGGAAAAAGAGA 1500
1501 TCGTGGATTACGTCGCCAGTCAAGTAACAACCGCGAAAAAGTTGCGCGGAGGAGTTGTGT 1560
1561 TTGTGGACGAAGTACCGAAAGGTCTTACCGGAAAACCTCGACGCAAGAAAAATCAGAGAGA 1620
1621 TCCTCATAAAGGCCAAGAAGGGCGAAAGTCCAAATTGTAAAAGCTTGG 1670
    
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Date: 20 MAY 2014

Signature: 

Title: Technical Manager, BEI Authentication or designee

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