

Plasmid Containing 18S Ribosomal RNA Gene Fragment from *Babesia bigemina*

Catalog No. NR-28918

Product Description: A 582 base pair fragment of the 18S ribosomal RNA gene from *Babesia bigemina* (*B. bigemina*) was amplified by PCR and cloned into vector pPCR-Script Amp SK(+) (Agilent Technologies). The resulting plasmid, NR-28918, may be used in PCR assays for the detection of *B. bigemina* in cattle ticks or cattle blood. The plasmid was produced in One Shot[®] TOP10 chemically competent *Escherichia coli* (Invitrogen[™]) and extracted using a QIAGEN[®] EndoFree[®] Plasmid Maxi Kit.

Lot¹: 61329983

Manufacturing Date: 22MAR2013

TEST	SPECIFICATIONS	RESULTS
Confirmation of Insert by PCR ²	~ 580 base pair amplicon	~ 580 base pair amplicon
Confirmation of Insert by Sequencing (Figure 1)	Consistent with <i>B. bigemina</i> 18S ribosomal RNA gene	Consistent with <i>B. bigemina</i> 18S ribosomal RNA gene ³
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	1.7
Bacterial Transformability (Post-Vial) ⁴	≥ 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 16 bases upstream and 17 bases downstream of the 18S ribosomal RNA region. PCR primers used for amplification were KB-16 forward primer 5' CATCAGCTTGACGGTAGGG 3' and KB-17 reverse primer 5' GTCCTTGGCAAATGCTTTC 3'. Conditions used for PCR are available upon request.

³GenBank: FJ426361

⁴One Shot[®] TOP10 *Escherichia coli* were transformed with 19 ng of plasmid DNA.

Figure 1. Plasmid 18S Ribosomal RNA Insert Sequence

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1      TCATCAGCTTGACGGTAGGGTATTGGCCTACCGAGGCAGCAACGGGTAACGGGGAATTAG 60
61     GGTTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGC 120
121    GCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAGAAATAACAATACAGGGCTT 180
181    TCCCGGGACAGAAACAAGCCCTTTAAGTTTATGCTAGGCAAGCAGGAGGTGATCCGAGGC 240
241    AGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAACCTTGTTGCA 300
301    GTTAAAAAGCTCGTAGTTGTATTTAGCCTCGCGTTTTTCCCTKYTKTTGGGTCTTTTC 360
361    GCTGGCTTTTTTTTTACTTTGAGAAAATTAGAGTGTTC AAGCAGACTTTTGTCTTGAAT 420
421    ACTTCAGCATGGAATAATAGAGTAGGACCTTGTTCTATTTGTTGGTTTTGAGCCTTG 480
481    TAATGGTTAATAGGAACGGTTGGGGCATTTCGTATTTAACTGTCAGAGGTGAAATCTTA 540
541    GATTTGTTAAAGACGAACCACTGCGAAAGCATTGCCAAGG 581
    
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Date: 19 MAY 2014

Signature:



Title: Technical Manager, BEI Authentication or designee

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