

# **Certificate of Analysis for NR-28919**

### Plasmid Containing 18S Ribosomal RNA Gene Fragment from Babesia bovis

### Catalog No. NR-28919

**Product Description:** A 565 base pair fragment of the small subunit ribosomal RNA gene from *Babesia bovis* (*B. bovis*) was amplified by PCR and cloned into vector pPCR-Script Amp SK(+) (Agilent Technologies). The resulting plasmid, NR-28919, may be used in PCR assays for the detection of *B. bovis* in cattle ticks or cattle blood. The plasmid was produced in One Shot<sup>®</sup> TOP10 chemically competent *Escherichia coli* (Invitrogen<sup>™</sup>) and extracted using a QIAGEN <sup>®</sup> EndoFree<sup>®</sup> Plasmid Maxi Kit.

Lot<sup>1</sup>: 61330033 Manufacturing Date: 22MAR2013

TEST	SPECIFICATIONS	RESULTS
Confirmation of Insert by PCR <sup>2</sup>	~ 570 base pair amplicon	~ 570 base pair amplicon
Confirmation of Insert by Sequencing (Figure 1)	Consistent with <i>B. bovis</i> 18S ribosomal RNA gene	Consistent with <i>B. bovis</i> 18S ribosomal RNA gene <sup>3</sup>
DNA Content by PicoGreen® Measurement	0.7 μg to 1.5 μg per vial	0.8 μg per vial
OD <sub>260</sub> /OD <sub>280</sub> Ratio	1.7 to 2.1	1.8
Bacterial Transformability (Post-Vial) <sup>4</sup>	≥ 100 colonies	≥ 100 colonies

<sup>&</sup>lt;sup>1</sup>Produced in One Shot® TOP10 chemically competent *Escherichia coli* (Invitrogen™) and extracted using a QIAGEN® EndoFree® Plasmid Maxi Kit.

<sup>2</sup>PCR 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.

#### Figure 1. Plasmid 18S rRNA Insert Sequence

1	TCATCAGCTTGACGGTAGGGTATTGGCCTCCCGAGGCATCGACGGGTAACGGGGAATTAG	60
61	GGTTCGATTCCGGAGAGGGAGCCTGCGAGACGGCTACCACATCTAAGGAAGG	120
121	GCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAGAAATACCAATACGGGGCTA	180
181	$\tt CTGCTCTGTAATTGGCATGGGGGGCGACCTTCACCCTCGCCCGAGTACCCATTGGAGGGCA$	240
241	AGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAACTTGTTGCA	300
301	GTTAAAAAGCTCGTAGTTGAATCTCACGTCCGCCCTAGTGTTTTCCACTACGCGGACGCC	360
361	TCGTTACTTTGAGAAAATTAGAGTGTTTCAAGCAGGTTTCGCCTGTATAATTGAGCATGG	420
421	AATAACCTTGTATGACCCTGTCGTACCGTTGGTTGACTTTGGGTAATGGTTAATAGGAAC	480
481	GGTTGGGGGCATTCGTACTCGACTGTCAGAGGTGAAATTCTTAGATTTGTCGATGACGCA	540
541	CGACTGCGAAAGCATTTGCCAAGGACA 567	

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<sup>&</sup>lt;sup>3</sup>GenBank: HQ264111

<sup>&</sup>lt;sup>4</sup>One Shot<sup>®</sup> TOP10 Escherichia coli were transformed with 13 ng of plasmid DNA.



# **Certificate of Analysis for NR-28919**

**Date:** 19 MAY 2014 **Signature:** 

Title: Technical Manager, BEI Authentication or designee

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