

Certificate of Analysis for NR-4708

Genomic DNA from Yersinia pestis, Strain KIM Derivative 22 (D22)

Catalog No. NR-4708

Product Description: Genomic DNA was isolated from a preparation of Yersinia pestis (Y. pestis), strain KIM Derivative 22 (D22). Y. pestis, strain KIM(D22) is an avirulent derivative that contains the pMT1 plasmid as well as the unstable chromosomal pgm locus, but lacks the pCD1 and pPCP1 plasmids that are essential for virulence.

Lot1: 58268397 Manufacturing Date: 15AUG2008

TEST	SPECIFICATIONS	RESULTS
Sequencing of 16S Ribosomal RNA Gene (~ 1460 bp)	Identical to BEI Resources NR-4684 Identical to GenBank: AE009952 Consistent with Y. pestis	Identical to BEI Resources NR-4684 Identical to GenBank: AE009952 Consistent with Y. pestis ²
Presence of Plasmids Confirmed by PCR Amplification pMT1 (pFra; 100 kb plasmid) pCD1 (pYV; 70 kb plasmid) pPCP1 (pPla; 9.5 kb plasmid)	Positive Negative Negative	Positive Negative Negative
Agarose Gel Electrophoresis	High molecular weight chromosomal DNA	High molecular weight chromosomal DNA (Figure 1)
Concentration by PicoGreen® Measurement	4 to 6 μg in 25 to 100 μL per vial	5.3 μg in 49 μL per vial (109 μg/mL)
Functional Activity by PCR Amplification Y. pestis specific sequence (YPO0396)³ 16S ribosomal RNA gene Virulence-associated plasmids pMT1 (pFra; 100 kb plasmid) pCD1 (pYV; 70 kb plasmid) pPCP1 (pPla; 9.5 kb plasmid)	~ 800 bp amplicon ~ 1500 bp amplicon ~ 1200 bp amplicon None detected None detected	~ 800 bp amplicon ~ 1500 bp amplicon ~ 1200 bp amplicon None detected None detected
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 1.9	1.9
Bacterial Inactivation 10% of total yield plated on Tryptic Soy Agar ^{4,5}	No viable bacteria detected	No viable bacteria detected

Y. pestis, strain KIM(D22) was deposited by Professor Robert R. Brubaker of the Department of Microbiology and Molecular Genetics at Michigan State University, East Lansing, Michigan. The bacterial preparation used for extraction of genomic DNA was produced by broth (Tryptic Soy Broth; BD 211768) culture of the deposited material. After incubation for 48 hours at 28°C and aerobic atmosphere, genomic DNA was extracted using proprietary technology.

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²Also consistent with other Yersinia species.

³Sequence locus tag YPO0396 codes for an uncharacterized protein that is highly conserved in Y. pestis

⁴7 days at 37°C in an aerobic atmosphere.

⁵An extraction procedure was used that has been shown to consistently inactivate 100% of Gram-negative bacteria.



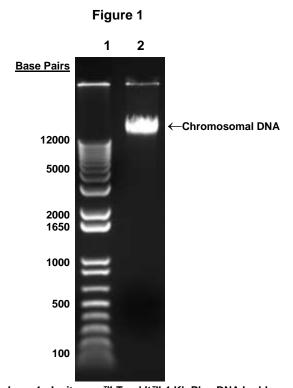
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Date: 05 NOV 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder

Lane 2: 200 ng of NR-4708

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