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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for HM-277D

Genomic DNA from Microbial Mock Community B (Staggered, High Concentration), v5.2H, for Whole Genome Shotgun Sequencing

Catalog No. HM-277D

Product Description: A mixture of genomic DNA from 20 bacterial strains containing staggered ribosomal RNA operon counts (10,000 – 10,000,000 operons per organism per µL). Note: The label for HM-277D is incorrect. HM-277D contains genomic DNA from microbial mock community B and not microbial mock community A.

Lot[1,2]: 60257284 **Manufacturing Date: 31AUG2011**

TEST	SPECIFICATIONS	RESULTS
DNA Sequencing of Individual 16S Ribosomal RNA Genes from Mock Community B (~ 1500 base pairs)	Consistent with *Acinetobacter baumannii* Consistent with *Actinomyces odontolyticus* Consistent with *Bacillus cereus* Consistent with *Bacteroides vulgatus* Consistent with *Clostridium beijerinckii* Consistent with *Deinococcus radiodurans* Consistent with *Enterococcus faecalis* Consistent with *Escherichia coli* Consistent with *Helicobacter pylori* Consistent with *Lactobacillus gasseri* Consistent with *Listeria monocytogenes* Consistent with *Neisseria meningitidis* Consistent with *Propionibacterium acnes* Consistent with *Pseudomonas aeruginosa* Consistent with *Rhodobacter sphaeroides* Consistent with *Staphylococcus aureus* Consistent with *Staphylococcus epidermidis* Consistent with *Streptococcus agalactiae* Consistent with *Streptococcus mutans* Consistent with *Streptococcus pneumoniae*	Consistent with *Acinetobacter baumannii*[§] Consistent with *Actinomyces odontolyticus*[§] Consistent with *Bacillus cereus*[§] Consistent with *Bacteroides vulgatus*[§] Consistent with *Clostridium beijerinckii*[‡] Consistent with *Deinococcus radiodurans*[§] Consistent with *Enterococcus faecalis*[§] Consistent with *Escherichia coli*[£] Consistent with *Helicobacter pylori*[†] Consistent with *Lactobacillus gasseri*[‡] Consistent with *Listeria monocytogenes*[§] Consistent with *Neisseria meningitides*[†] Consistent with *Propionibacterium acnes*[§,3] Consistent with *Pseudomonas aeruginosa*[£] Consistent with *Rhodobacter sphaeroides*[£] Consistent with *Staphylococcus aureus*[§,4] Consistent with *Staphylococcus epidermidis*[§,4] Consistent with *Streptococcus agalactiae*[§] Consistent with *Streptococcus mutans*[§] Consistent with *Streptococcus pneumoniae*[§]
Agarose Gel Electrophoresis	High molecular weight chromosomal DNA	High molecular weight chromosomal DNA (Figure 1)

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TEST	SPECIFICATIONS	RESULTS
Theoretical DNA Concentration for Individual Members of Mock Community B [based on number of ribosomal RNA (rRNA) operons input DNA] *Acinetobacter baumannii* -100,000 operons *Actinomyces odontolyticus* -10,000 operons *Bacillus cereus* - 1,000,000 operons *Bacteroides vulgatus* - 10,000 operons *Clostridium beijerinckii* - 1,000,000 operons *Deinococcus radiodurans* - 10,000 operons *Enterococcus faecalis* - 10,000 operons *Escherichia coli* - 10,000,000 operons *Helicobacter pylori* - 100,000 operons *Lactobacillus gasseri* - 100,000 operons *Listeria monocytogenes* - 100,000 operons *Neisseria meningitidis* - 100,000 operons *Propionibacterium acnes* - 100,000 operons *Pseudomonas aeruginosa* - 1,000,000 operons *Rhodobacter sphaeroides* - 10,000,000 operons *Staphylococcus aureus* - 1,000,000 operons *Staphylococcus epidermidis* - 10,000,000 operons *Streptococcus agalactiae* - 1,000,000 operons *Streptococcus mutans* - 10,000,000 operons *Streptococcus pneumoniae* - 10,000 operons	Report results	82 pg/µL *Acinetobacter baumannii*[§] 10 pg/µL *Actinomyces odontolyticus*[§] 450 pg/µL *Bacillus cereus*[§] 7.6 pg/µL *Bacteroides vulgatus*[§] 440 pg/µL *Clostridium beijerinckii*[‡] 10 pg/µL *Deinococcus radiodurans*[§] 7.0 pg/µL *Enterococcus faecalis*[§] 6.8 ng/µL *Escherichia coli*[£] 86 pg/µL *Helicobacter pylori*[†] 32 pg/µL *Lactobacillus gasseri*[‡] 50 pg/µL *Listeria monocytogenes*[§] 58 pg/µL *Neisseria meningitidis*[†] 88 pg/µL *Propionibacterium acnes*[§] 1.6 ng/µL *Pseudomonas aeruginosa*[£] 14 ng/µL *Rhodobacter sphaeroides*[£] 590 pg/µL *Staphylococcus aureus*[§] 5.1 ng/µL *Staphylococcus epidermidis*[§] 32 pg/µL *Streptococcus agalactiae*[§] 4.1 ng/µL *Streptococcus mutans*[§] 5.5 pg/µL *Streptococcus pneumoniae*[§]
Total Amount of DNA per vial	≥ 50 ng per µL	56 ng per µL
Functional Activity by PCR Amplification 16S ribosomal RNA gene	~ 1500 base pair amplicon	~ 1500 base pair amplicon (Figure 1)
OD_{260}/OD_{280} Ratio	Report results	1.9
Bacterial Inactivation 10% of total yield plated on Tryptic Soy agar with 5% sheep blood[5]	No viable bacteria detected	No viable bacteria detected

[1]Preparation and QC testing (with the exception of Bacterial Inactivation) were performed by Baylor College of Medicine in Houston, Texas.
[2]Genomic DNA was extracted using the following methods: [§]SDS Lysis, CsCl, [‡]Modified SDS Lysis, CsCl, [£]Triton Lysis, CsCl and [†]Omega E.Z.N.A.® Bacterial DNA Kit.
[3]Also consistent with other *Propionibacterium* species
[4]Also consistent with other *Staphylococcus* species
[5]7 days at 37°C under both anaerobic atmosphere (80% N_2:10% CO_2:10% H_2) and aerobic atmospheric conditions

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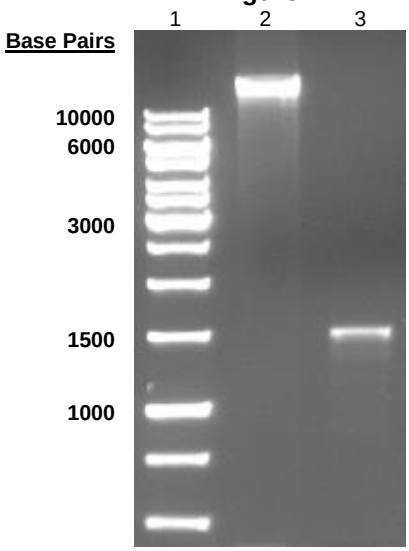
Figure 1



Lane 1: 1 Kb DNA Ladder (Fermentas, Cat. No. SM0311)
Lane 2: 200 ng of gDNA HM-277D
Lane 3: PCR of 16S ribosomal RNA gene from HM-277D

Date: 07 OCT 2014 **Signature:**

Title: Technical Manager, BEI Authentication or designee

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