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Technical Data Sheet:

3 Strain Tagged Whole Cell Even Mix

ATCC® Number	MSA-2014™
Components	33.3% *Escherichia coli* with Tag 1 33.3% *Clostridium perfringens* with Tag 2 33.3% *Staphylococcus aureus* with Tag 3
Product Description	We have engineered three bacterial genomes (*Escherichia coli, Staphylococcus aureus,* and *Clostridium perfringens*) to contain a unique synthetic DNA tag that can be detected via 16S rRNA profiling and whole genome sequencing assays. The unique tag comprises four artificial variable regions (corresponding to the V1 through V4 regions in the 16S rRNA gene) flanked by conserved regions for PCR amplification, thereby enabling the identification of spike-in reads during the analysis of next-generation sequencing data. Using these recombinant bacterial strains, we prepared an even mixture of three tagged strains for use as a spike-in control in microbiome research.

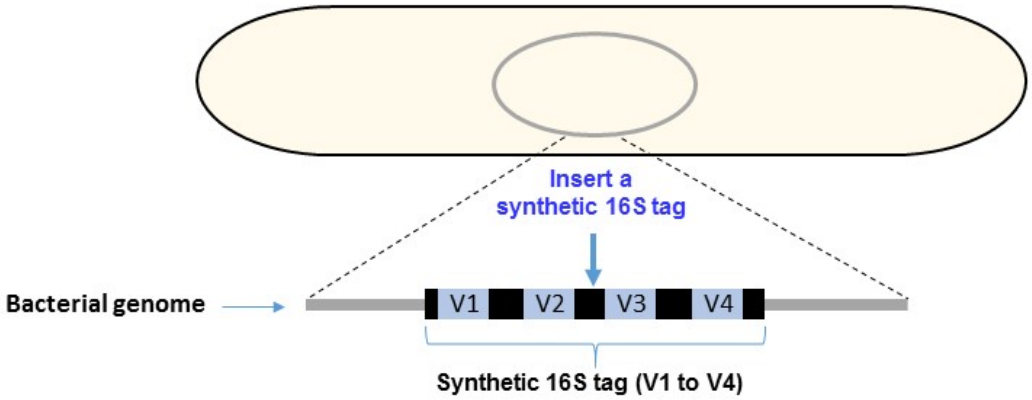


Figure 1. Production of tagged strains. ATCC created 3 unique synthetic 16S tag sequences mimicking the native 16S rRNA gene from 3 bacterial strains (*E. coli*, *C. perfringens*, and *S. aureus*). Each tag consists of 4 artificial variable regions (corresponding to V1 through V4 of the 16S rRNA gene) flanked by conserved regions for PCR amplification. Each tag sequence was integrated into the genome of their cognate strains to create three tagged strains. The tag sequences are provided below.

Escherichia coli 16S Tag 1

AAATTGAAGAGTTTGATCATGGCTCAGATTACATGCGGATTCGAGGGCCACAGGAGGCATCACTGACATGCCCTATCGTGAT
AGGGGCTAGCTACAGCAGAGTGGCGGACGGGTGAGTAATGTGAGTAGCGAAAAGTCATGGCTAAAGGTACTGTTTCGTCAT
CCGATAAGATTGACGGAAATTGATTCTCACACGTCCCGATGTGGGAGCCGCGACCGTCACAGGTGAAGAATCTCTCTCAAAA
GATTTATGGCCATAGTAGATTTCACTCACAAATCCAGACACACGGGTAGTTCGCTGCGACTCGATTTCTAATATCTTATGGAT
CCTAATCTAGACTCCTACGGGAGGCAGCAGTGGTCTACTGCATGATCAACCAAAGGTGTTCCGGCTTACGTTCAATTTGAGA
ACGGCGGTCTGGAGCATGAAAGGACGAGACGGCATTAGGACTTGCCAGGCGATGTATGCTGATCGGGAAGTAGGGAAACAT
GAGAGGCCGCTCTAAATCCTCTTCCGTGCCAGCAGCCGCGGTAATACGACGTGTGATCATGGTAGACGTCCACTTTTACCGT
GTGTGGGCGATGAGGGATGCAAGAGGATCATTGGTTAGCGTATTTGTGACTCTGAAACTAAGGCAGGAGACCAGGTTAGGT
AAACGGTGCGTAGATAATCCGCAGACCGCCGGTCGCGTAGCTTAAGGAAAGGGTATGCCCGACGTGGCTTGAAACAGATAC
CTAAACCAGGTGACGCCTATAGAGAACGACGAAGCTAATCTATCCGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA
AACGATGTCGAC

Clostridium perfringens 16S Tag 2

AAATTGAGAGTTTGATCCTGGCTCAGGATGTACGACGAGGATTTAGGTGGGGAGGGACTGGCACGAGTAGTATACGGTTTT
AAAAAGTATTGGAGCGGCGGACGGGTGAGTAACACCTTAAGGCGGGTTGGGGCGTCCGAAACATACGATCCCGCTGGCAAA
GGTGCCAGTGGCAGACCTGGCGGGGAGTACCGGAGCATAAAGGATTCGCAAGCACGTTCAGCGGTTAGGGAGCCTGGGCT
GCAAGCGCGAAGGCCAGCGCTTTACCGTGCATGGTTAGCAAATGAGTCCCTGACCGACCACCACATAATCGTACGTCCGTAT
CCTCTCTACAGACTCCTACGGGAGGCAGCAGTGGTAGGCTCCATAATGCTAGTCGACCTCGTGCTTGGTCGCTGCTTCAACC
GTTCACAAGAACTCTCCTGCCAACGTTAATCGGCGTAGCGCGTAATCGATCACCGAGTGTAGTACGTATCTATCCCTATACGT
GCCAGCAGCCGCGGTAATACGTGAAGGTTTGAACTGAAATCAAGAAAGTTAATCAAGGGTTGCGTGCGCGGAATCGGCGTG
AAAACAAATTGAGCGGGTGGGAACAAAACGAAGATGGTAGTTCTATAGGTTGCAGATAACTCCCGTAACTTTAGCTGCGGTA
AGGAAGTGTGCGCTTACGGGATAGCGATATGTACTGGCCTAAGAGCTCCGGATTTCTAAGCTGCTGGGTCGAATGTAAGCAC
GACACATCTCTAATCCGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATAC

Staphylococcus aureus 16S Tag 3

TTTATGGAGAGTTTGATCCTGGCTCAGGATCATTACCTCGATTGAGATAAGCAAACAAGTCTCGCCTAGTGAAGGCACGTCT
GATCGTCACACCTGGAAGCGGCGGACGGGTGAGTAACACCTTACAGTATGGTATCCTGGATATTCACTTAGTATTCACTGGT
CCTCCATAGTAACGGCGTGCTCGATAAGCGGTCCCAGGATTAGATAAATCGACTGGCCTATGAATGCCTAGGCACACGAGTG
GGATACGATCAAGAGTGACACAGAGCGAACCCCGTCAAATGTCCGGAAATATGGGTGTATATGACGTACCCAGAGTGGTATA
GAAAATCCTTGTGAAGACTCCTACGGGAGGCAGCAGTAGAGGTGGTGCGAGGCACAGTTAGGTTCATGATCATGGCGACAG
AGAAGCTGGGCACAGCGAAGGTCAAACCAACAACAGTAGCAGTCACTACTAAGCTGGGAGGTGATGGTTCTGCGCATAGGG
TATTAAACACACTGGTAATTGATCCTGATACGTGCCAGCAGCCGCGGTAATACCGAAGTCTATTATCTCGGCATGCTCGTGG
AGCTCAGACCGCTGAGGTGAAGTATAAAGTGTTCGCAGGATCGAGATATAACGGCTCATATATGTGATGGGACCAGTTTAAA
ATACGCGGATATGCAGTGCACGGACCAGGAGGGACGGAGAGGGACCTCTTACTTGCAATCGTTCAATGGAGGTCAGTACCG
CAGAGAGTAGGTAATACTGTGAGACGAAGAGAAAGAGATTTGTGAATCCTCAAACAGGATTAGATACCCTGGTAGTCCACGC
CGTAAACGATGAGTGC

Table 1. Composition of the 3 Strain Tagged Whole Cell Even Mix (ATCC® MSA-2014™)

Species	Gram Stain	Genome size (Mb)	Tag size (bp)	G/C Content (%)	Number of 16S Copies	Number of Tag Copies	Cells per vial*
Escherichia coli Tag1	Negative	4.59	829	50.8	7	1	2×10^7
Clostridium perfringens Tag2	Positive	3.25	799	29.0	10	1	2×10^7
Staphylococcus aureus Tag3	Positive	2.70	833	32.8	6	1	2×10^7

*Specification Range: Total of 6.0×10^7 cells/vial ± 1 log. Whole cell concentrations indicate ATCC® manufacturing specifications and are provided as a reference only.

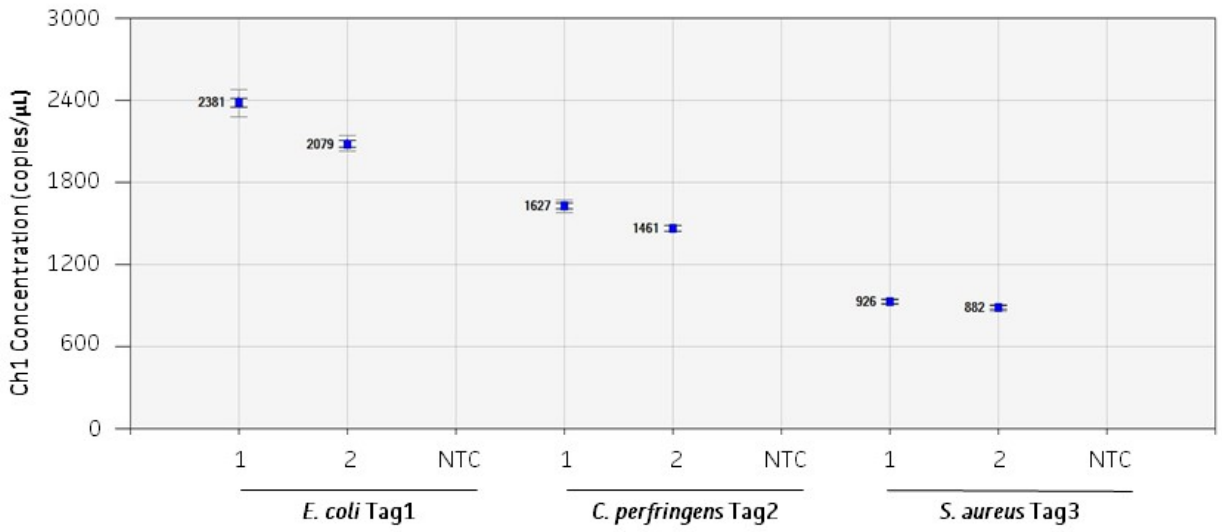
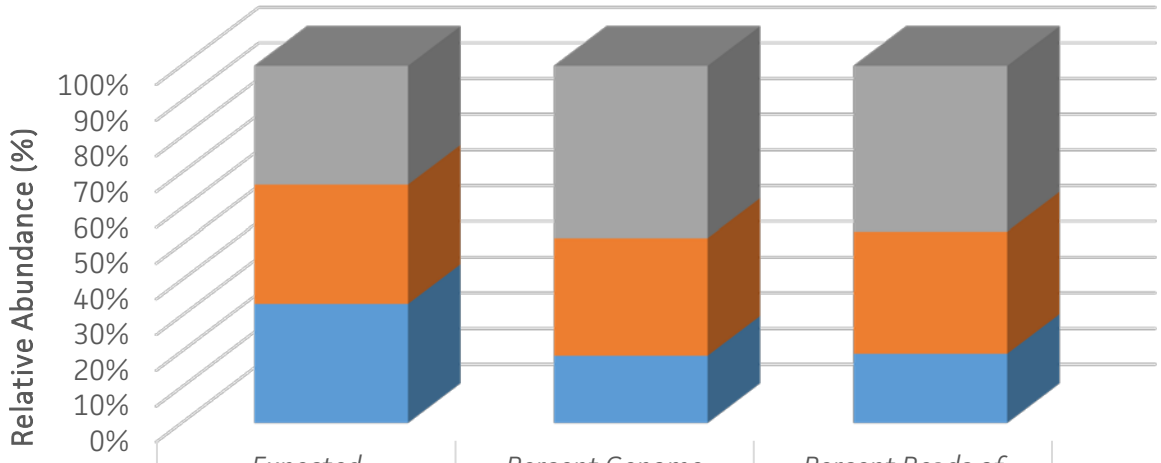


Figure 2. Genome copy number of individual bacteria determined by Droplet Digital™ PCR (ddPCR™; Bio-Rad) assay after extraction of DNA from ATCC® MSA-2014™. Total DNA was extracted from two vials of ATCC® MSA-2014™ by using a DNeasy® Powelyzer® Microbial Kit (QIAGEN) and eluted in a final volume of 50 µL according to the manufacturer's instruction. The concentration of extracted DNA was assayed by PicoGreen® dsDNA quantitation assay (Thermo Fisher Scientific); Vial 1 (1) = 9.7 ng/µL and Vial 2 (2) = 8.6 ng/µL. Both vials were diluted 100-fold and assayed in triplicate (1, 2) along with a no template control (NTC). The primer and probe set used for the three tag amplicons were the following: *E. coli* Tag1 (Forward: 5'-GAACGGGTGAGTAATGTGAGTAG -3'; Reverse: 5'-CGGGACGTGTGAGAATCAAT -3'; Probe: 5'-TCGGATGACGAAACAGTACCTTTAGCC -3'), *C. perfringens* Tag2: (Forward: 5'- TAACCATGCACGGTAAAGCG -3'; Reverse: 5'-AAGGATTCGCAAGCACGTTC -3'; Probe: 5'-TTCGCGCTTGCAGCCCAGGCTCCCT -3'), and *S. aureus* Tag3: (Forward: 5'- AATACGCGGATATGCAGTGC 3'; Reverse: 5'-CTGACCTCCATTGAACGATTGC -3'; Probe: 5'-AGAGGTCCCTCTCCGTCCCTCCTGGTCCGT -3'). The concentration (genome copies/µL) represents the average of three wells in triplicate assays. The total genome copy number per organism per vial was calculated based on the average of triplicate reactions per specific ddPCR™ assay (Table 2).

Table 2. Genome copy number of individual bacteria in the 3 Strain Tagged Whole Cell Even Mix

Species	Genome copy number per vial	
	Vial 1	Vial 2
Escherichia coli Tag1	$4.75 \pm 0.18 \times 10^7$	$4.16 \pm 0.10 \times 10^7$
Clostridium perfringens Tag2	$3.25 \pm 0.08 \times 10^7$	$2.92 \pm 0.04 \times 10^7$
Staphylococcus aureus Tag3	$1.85 \pm 0.03 \times 10^7$	$1.76 \pm 0.04 \times 10^7$



	Expected	*Percent Genome copies by ddPCR™*	*Percent Reads of Tags by Shotgun Sequencing*
E. coli Tag1	*33.3%*	*48.2%*	*46.4%*
C. perfringens Tag 2	*33.3%*	*32.9%*	*34.2%*
S. aureus Tag3	*33.3%*	*18.8%*	*19.4%*

Figure 3. Relative abundance of three genomes extracted from ATCC® MSA-2014™. Shotgun metagenomic sequencing was performed on the Illumina® platform (Nextera XT DNA Library Preparation Kit and MiniSeq 2x150). Total shotgun reads (7.27 million) were mapped to unique tag sequences (total = 1856 reads), and the percent of reads mapped to individual tags were calculated. Reads mapping to tag sequences was performed via Bowtie2 tool in Geneious 11.1.4 Software. Note: The discrepancy between the expected and observed ratios may be attributed to cell counting and extraction efficiency, which is related to the diverse physical properties of the bacterial species.