¹US Food and Drug Administration, Office of Regulatory Science, Pacific Northwest Laboratory, Applied Technology Branch. 22201 23rd Dr. SE, Bothell, WA 98021. Jason.Neal-Mckinney@fda.hhs.gov

Introduction

Whole genome sequencing (WGS) techniques allow FDA scientists to identify food determine relationships between strains, and predict the virulence and antimicrobial r bacterial isolates. The ultimate goal of any WGS effort is to have a complete and sequence, where the entire genetic code of the organism is known. However, due to each sequencing technology, most genome assemblies are fragmented into many o assembly errors that can affect gene characterization. In this study, we improved completeness of our genome assemblies by combining data from two complement platforms, the Illumina MiSeq and the Oxford Nanopore MinION. Raw sequence instrument was uploaded to the FDA GalaxyTrakr environment, which hosts a bioinformatics programs. QUAST and Nanostat were used to determine basic read me while SPAdes and Canu were used to assemble MiSeq and MinION data, respectively assembly was performed using Unicycler to combine both data types. We found that data is highly accurate and useful for single nucleotide polymorphism analysis, it resu genome assemblies that did not include all genes. In contrast, the MinION data resu genomes that contained many errors affecting gene characterization. By combining b were able to generate a highly accurate and complete genome assembly that allows for analysis of every gene in the organism.

FDA U.S. FOOD & DRUG

ADMINISTRATION

OFFICE OF REGULATORY AFFAIRS

Genomic DNA was extracted from 7 *Listeria monocytogenes* isolates using the Qiagen DNEASY kit. For MiSeq, libraries were prepared using the Illumina DNA prep kit and sequenced using paired-end 2 x 250bp reads. For MinION, libraries were prepared using the Oxford Nanopore Technologies Rapid Barcocding kit and sequenced on a single 106D Flow cell. Phylogenetic relatedness was obtained from the NCBI pathogen Detection Portal. Basic read metrics were determined using FastQC and NanoStat for Miseq and MinION data, respectively. *De novo* assemblies were generated using SPAdes for MiSeq data and Unicycler to combine both MiSeq and MinION data. Assembly metrics were determined using QUAST and the assembly graphs were visualized using Bandage. Whole genome alignment was performed using the progressiveMauve algorithm. All of the programs used were hosted in the Galaxytrakr.gov virtual environment

Figure 1. SNP Tree of 7 *L. monocytogenes* isolates

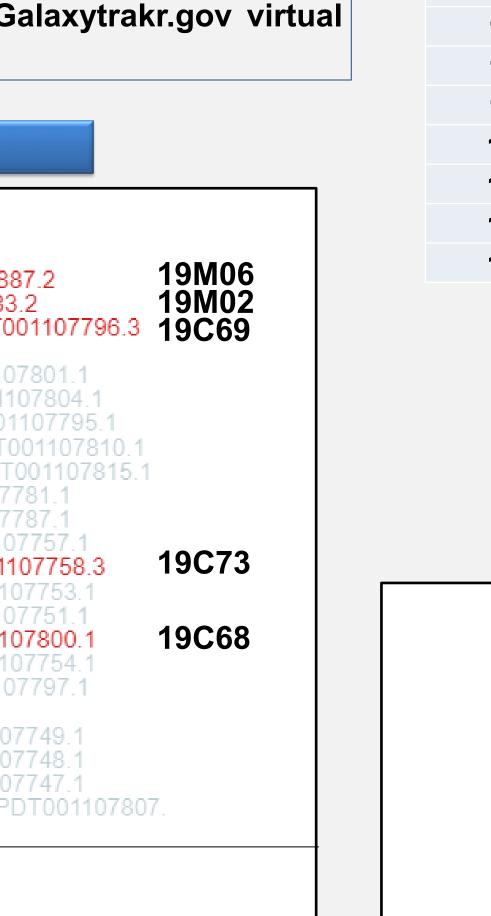
Methods

				<u>(</u>	<u>Cluster</u>	<u>1</u>					
				JSA:WA, en							
				SA:WA, envii .:WA, enviror							
environm	ental/other	2018-09-07	7, USA:WA,	environment	al swab, I	FDA102	7238-0	35-006	, PDT0	0037	2785.1
🔁 environn	nental/other	; 2021-08-1	1, USA:WA	environmen	ital swab	sponge,	FDA1	166408-	S047-0	004, F	PDT00
				A, environme		The second se					
				VA, environm A:WA, enviro							
en	vironmental	/other, 202	1-08-11, US	A:WÁ, enviro	onmental	swab sp	onge,	FDA116	6408-5	S080-	-003, P
				environmenta							
				environmenta							
				environmen A, environme							
				, environme							
environn	nental/other	; 2021-08-1	1, USA:WA	environmen	ital swab	sponge,	FDA1	166408-	-S057-0	003, [′] F	PDT00
				, environme							
				, environmen environmen		and the second se					
				, FDA11330						001,1	
o environm	ental/other,	2021-08-1	1, ÚSA:WA,	environmen	tal swab s	sponge,	FDA11	66408-	S099-0)01, F	PDT00
				environmen							
			1 I I C A NAIA								
environm	ental/other,	2021-08-1 tal/other 20	021-08-11 l	ISA:WA env	tal swab s vironment	sponge, tal swab	Spond	e FDA'	SU59-0 116640	101, F 18-S08	1D I OU 89-004
• environm	ental/other, environmen	2021-08-1 ital/other, 20	021-08-11, l	JSA:WA, env	tal swab s vironment	sponge, tal swab	spong	e, FDA	5059-0 116640	8-S08	20100 89-004
• environm	ental/other, environmen	2021-08-1 ntal/other, 20	021-08-11, l	JSA:WA, env	tal swab s vironment	sponge, tal swab	spong	e, FDA	5059-0 116640	101, F 8-S08	2D100 89-004
• environm	ental/other, environmen	tal/other, 2	021-08-11, l	JSA:WA, env	vironment	tal swab	spong	e, FDA	5059-0 116640	8-S08	20100 89-004
environm 1 2 3	ental/other, environmen	2021-08-1 ntal/other, 20	021-08-11, l	JSA:WA, env	tal swab s vironment	tal swab	spong	e, FDA	5059-0 116640	001, F 8-S08	20100 89-004
	environmen	tal/other, 2	021-08-11, l	JSA:WA, env	vironment	tál swab <u>2</u>	spong	e, FDA	116640	8-S08	89-004
	environmen	ntal/other, 20	021-08-11, l	JSA:WA, env <u>C</u> 21, USA:WA	vironment Iuster	tál swab <u>2</u> imental	spong	e, FDA	116640 32175-(8-S08	89-004 96, PE
	environmen environme environme	ental/other, 20 ental/other ental/other	, 2016-03-2 , 2016-11-1 , 2016-03-2	JSA:WA, env <u>C</u> 1, USA:WA 6, USA, sal 1, USA:WA	vironment Iuster , environ mon, WA	al swab	spong swab, S-A00 swab,	e, FDA FDA93 045, PI FDA93	116640 2175-0 DT000 32175-3	61-09 1610 52-05	96, PE 87.2 56, PE
	environmen environme environme environme	ental/other, 20 ental/other ental/other ental/other	021-08-11, l 2016-03-2 2016-11-1 2016-03-2 2016-03-2	JSA:WA, env <u>C</u> 1, USA:WA 6, USA, sal 1, USA:WA 1, USA:WA	vironment luster , environ mon, WA , environ	al swab	spong swab, S-A00 swab, swab,	e, FDA FDA93 045, PI FDA93 FDA93	116640 2175-0 0T000 2175-0 2175-0	61-09 1610 52-05 87-21	96, PE 87.2 56, PE 11, PE
	environmen environme environme environme environme	ental/other, 20 ental/other ental/other ental/other ental/other	021-08-11, l 2016-03-2 2016-11-1 2016-03-2 2016-03-2 2016-03-2	USA:WA, env <u>C</u> 1, USA:WA 6, USA, sal 1, USA:WA 1, USA:WA 1, USA:WA	vironment luster , environ mon, WA , environ , environ	al swab	spong swab, S-A00 swab, swab, swab,	e, FDA FDA93 045, PI FDA93 FDA93 FDA93	116640 2175- 0T000 2175- 32175- 32175-	8-S08 61-09 1610 52-05 87-21 86-19	89-004 96, PE 87.2 56, PE 11, PE 97, PE
	environmen environme environme environme environme	ental/other, 2 ental/other ental/other ental/other ental/other ental/other	021-08-11, l 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2	USA:WA, env <u>C</u> 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA	vironment luster , environ mon, WA , environ , environ , environ	al swab	spong Swab, S-A00 swab, swab, swab, swab,	e, FDA FDA93 045, PI FDA93 FDA93 FDA93 FDA93	116640 2175- 0T000 2175- 2175- 2175- 2175-	8-S08 61-09 52-05 87-21 86-19 81-16	89-004 86, PE 87.2 56, PE 11, PE 97, PE 52, PE
	environmen environme environme environme environme environme	ental/other, 2 ental/other ental/other ental/other ental/other ental/other ental/other	021-08-11, l 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2	USA:WA, env <u>C</u> 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA	ironment luster , environ , environ , environ , environ	al swab	spong Swab, S-A00 swab, swab, swab, swab, swab,	e, FDA FDA93 045, PI FDA93 FDA93 FDA93 FDA93 FDA93	116640 2175- 2175- 2175- 2175- 2175- 2175- 2175-	8-S08 61-09 1610 52-05 87-21 86-19 81-16 45-02	89-004 96, PE 87.2 56, PE 97, PE 92, PE 26, PE
	environmen environme environme environme environme environme environme environme	ental/other, 20 ental/other ental/other ental/other ental/other ental/other ental/other ental/other	021-08-11, 1 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2	JSA:WA, env 21, USA:WA 6, USA, sal 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA	ironment	al swab	spong Swab, S-A00 swab, swab, swab, swab, swab, swab,	e, FDA FDA93 045, PI FDA93 FDA93 FDA93 FDA93 FDA93	116640 2175- 2175- 2175- 2175- 2175- 2175- 2175- 32175-	61-09 1610 52-05 87-21 86-19 81-16 45-02 72-12	99-004 96, PC 87.2 56, PC 11, PD 26, PC 26, PC 22, PC
	environmen environme environme environme environme environme environme environme	ental/other, 20 ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other	021-08-11, 1 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2	USA:WA, env <u>C</u> 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA	ironment	al swab	spong swab, S-A00 swab, swab, swab, swab, swab, swab, swab,	e, FDA FDA93 045, PI FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93	116640 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175-	8-S08 61-09 1610 52-05 87-21 86-19 81-16 81-16 45-02 72-12 73-13	89-004 89-004 86, PC 87.2 56, PC 26, PC 26, PC 26, PC 27, PC
	environmen environme environme environme environme environme environme environme environme environme	ental/other, 20 ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other	021-08-11, 1 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2	USA:WA, env 21, USA:WA 6, USA, sal 21, USA:WA 21, USA:WA 21, USA:WA 21, USA:WA 21, USA:WA 21, USA:WA 21, USA:WA 21, USA:WA 21, USA:WA	ironment luster , environ mon, WA , environ , environ , environ , environ , environ , environ	2 mental mental mental mental mental mental mental mental mental mental	spong swab, S-A00 swab, swab, swab, swab, swab, swab, swab, swab,	e, FDA FDA93 045, PI FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93	116640 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175-	8-S08 61-09 1610 52-05 87-21 86-19 81-16 81-16 81-16 81-16 81-16 81-16 81-16 81-16 81-16 81-16 81-16 81-16 81-16 81-16 81-16 81-16	89-004 6, PC 87.2 56, PC 11, PD 27, PC 26, PC 26, PC 27, PC 37, PC
	environmen environme environme environme environme environme environme environme environme environme environme environme	ental/other, 20 ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other	021-08-11, l 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2	USA:WA, env 21, USA:WA 6, USA, sal 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA	ironment luster , environ mon, WA , environ , environ , environ , environ , environ , environ , environ	2 mental mental mental mental mental mental mental mental mental mental mental mental	spong swab, S-A00 swab, swab, swab, swab, swab, swab, swab, swab, swab, swab,	e, FDA FDA93 045, PI FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93	116640 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175-	8-S08 61-09 1610 52-05 87-21 86-19 86-19 81-16 81-16 81-16 81-16 80-15	89-004 89-004 87.2 56, PC 56, PC 52, PC 26, PC 26, PC 27, PC 27, PC 27, PC 37, PC 37, PC 37, PC 37, PC
	environmen environme environme environme environme environme environme environme environme environme environme environme environme	ental/other, 20 ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other	021-08-11, l 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2	USA:WA, env 21, USA:WA 6, USA:WA 1, USA:WA	ironment luster , environ , environ , environ , environ , environ , environ , environ , environ , environ , environ	2 mental mental mental mental mental mental mental mental mental mental mental mental mental mental mental	spong swab, S-A00 swab, swab, swab, swab, swab, swab, swab, swab, swab, swab, swab, swab, swab,	e, FDA FDA93 045, PI FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93	116640 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175-	8-S08 61-09 1610 52-05 87-21 86-19 86-19 86-19 86-19 86-19 86-11 80-15 80-15	89-004 96, PC 87.2 56, PC 11, PD 52, PC 26, PC 26, PC 26, PC 27, PC 37, PC 37, PC 37, PC 37, PC
	environmen environme environme environme environme environme environme environme environme environme environme environme environme environme	ental/other, 20 ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other ental/other	021-08-11, 0 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2 2016-03-2	USA:WA, env 21, USA:WA 6, USA, sal 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA 1, USA:WA	ironment	2 mental mental mental mental mental mental mental mental mental mental mental mental mental mental mental mental mental mental mental	spong swab, S-A00 swab, swab, swab, swab, swab, swab, swab, swab, swab, swab, swab, swab, swab, swab,	e, FDA FDA93 045, PI FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93 FDA93	116640 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175- 2175-	8-S08 61-09 1610 52-05 87-21 86-19 86-19 81-16 45-02 72-12 73-13 82-17 80-15 80-15 80-15 80-22	89-004 96, PC 87, 2 56, PC 56, PC 52, PC

Two are Better Than One: Hybrid Genome Assembly using MiSeq and MinION

Jason Neal-McKinney

		Tab	le 1. MiSeq and MinION	Read Metrics	
dborne pathogens,	Strain	Sequencer	Mean Read Length	Mean Read Quality	Number of Reads
resistance traits of	19C68	MiSeq	236	36	727299
accurate genomic	19C69	MiSeq	236.5	36.3	1813006
o the limitations of	19C73	MiSeq	198.9	36.2	2396644
contigs or contain	19M02	MiSeq	185.2	35.9	816386
ed the quality and	19M04	MiSeq	173.7	34.9	1118504
nentary sequencing	19M06	MiSeq	174.4	35.2	1155587
e data from each	19M08	MiSeq	181.2	36	1199700
a wide variety of	19C68	MinION	9314.5	11.9	4138
metrics and quality,	19C69	MinION	9704.2	11.8	48784
ely. Hybrid genome	19C73	MinION	7858.4	11.7	133865
nat while the MiSeq	19M02	MinION	8973.9	11.7	119543
ulted in incomplete	19M04	MinION	10386	11.7	131541
esulted in complete	19M06	MinION	10650.9	11.9	10038
both datatypes, we	19M08	MinION	9066.1	11.7	126983
or analysis of every					

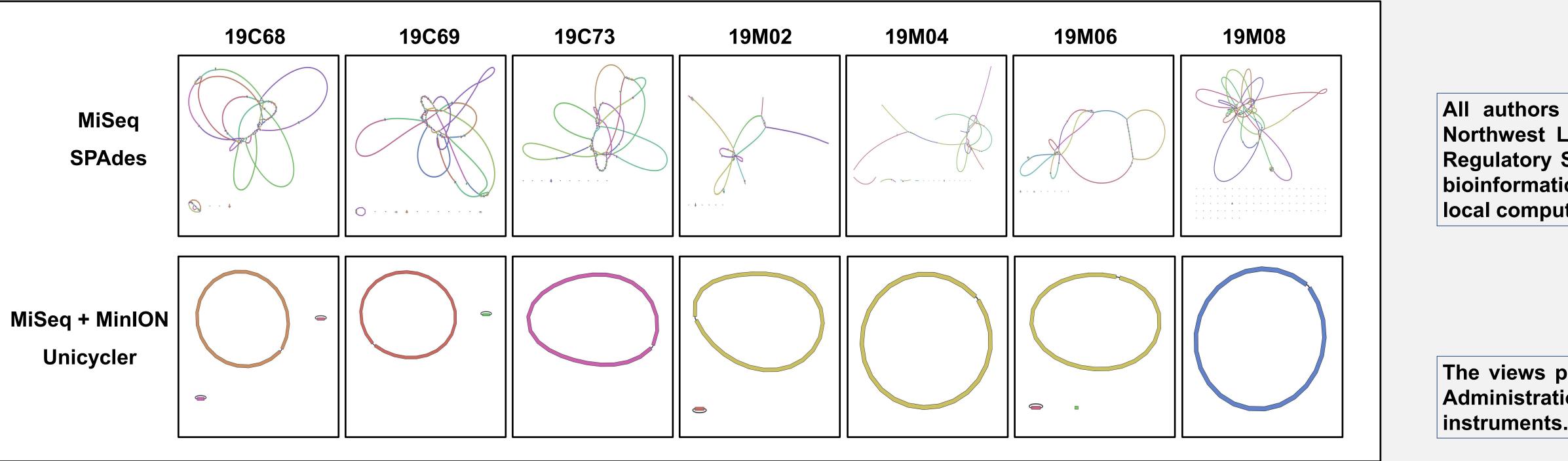


00119886.2 00119884.2 00119896.2 00119895.2 00119892.2 00119882.2 00119889.2 **19M08** 00119890.: 00119893.2 00119888.2 00119891.2 00119894.2 100119885.2 **19M04**

Table 2. De novo Assembly Metrics

Strain	Sequencer	Assembler	Contigs	Total Length	Largest Contig	Circular Contigs
19C68	MiSeq	SPAdes	29	3,228,591	823,173	0
19C69	MiSeq	SPAdes	22	3,147,021	823,173	0
19C73	MiSeq	SPAdes	23	3,066,348	823,173	0
19M02	MiSeq	SPAdes	22	3,117,063	791,224	0
19M04	MiSeq	SPAdes	19	2,940,821	693,838	0
19M06	MiSeq	SPAdes	31	3,117,184	418,169	0
19M08	MiSeq	SPAdes	24	3,021,530	585,188	0
19C68	MiSeq/MinION	Unicycler	3	3,267,493	3,101,927	3
19C69	MiSeq/MinION	Unicycler	2	3,184,668	3,101,936	2
19C73	MiSeq/MinION	Unicycler	1	3,101,947	3,101,947	1
19M02	MiSeq/MinION	Unicycler	2	3,152,721	3,069,989	2
19M04	MiSeq/MinION	Unicycler	1	2,968,820	2,968,820	1
19M06	MiSeq/MinION	Unicycler	3	3,154,255	3,069,829	2
19M08	MiSeq/MinION	Unicycler	1	3,054,986	3,054,986	1

Figure 2. Assembly Graph Images



Interest-Model Interes
etaset_44499 44099 440000 50000 60000 70000 100000 100000 2000000 2000
10000 20000 30000 40000 50000 2000000 2000000 2000000
Image: 140000 200000 300000 100000 2000000 <td< th=""></td<>
19C69 10000 20000 30000 60000 50000 60000 70000 80000 100000 1100000 120000 130000 160000 150000 160000 190000 200000 210000 220000 230000 260000 270000 280000 280000 30000 0000 3101 19C73 dstateM0899.dst (to annotations loaded) 10000 20000 30000 60000 50000 60000 80000 100000 1100000 1200000 130000 1400000 150000 180000 190000 200000 2100000 2200000 2300000 240000 280000 280000 300000 30000 1000000
dataset_3408988.dat (ino annotations loaded) 100000 300000 600000 200000 800000 200000 100000 2200000
10000 20000 30000 60000 200000 200000 2200000 2200000 2200000 </th
Instance 1 2408989.dat (no annotations loaded) Indogo 200000 300000 400000 500000 800000 800000 1000000 1100000 1200000 1600000 1600000 1800000 2000000 2100000 2200000 2200000 2200000 2200000 2200000 2200000 2200000 2200000 2200000 2200000 2200000 2200000 2200000 2200000 2200000 200000 200000 300000 300000 300000 300000 300000 300000 300000 300000 300000 300000 300000 300000 1000000 1200000 1500000 1600000 1500000 1600000 1800000 1200000 1200000 1200000 1200000 1200000 1200000 1200000 200000 2100000 2200
19C73 staset_3409989.dat (no annotations loaded) 100000 200000 500000 600000 700000 1000000 1200000 1200000 1200000 1200000 200000 200000 200000 200000 200000 200000 2000000 200000
dataset_3408989.dat (no annotations loaded) 100000 200000 300000 400000 500000 600000 200000 300000 200000 <t< th=""></t<>
100000 200000 300000 400000 500000 500000 200000 200000 1000000 1100000 1200000 1300000 1400000 1500000 1500000 1500000 2000000 2100000 2200000 250000
Image: 100000 100000 500000 600000 700000 800000 1000000 1500000 1600000 1500000 22000000 2200000 2200000
IPMO2 dataset_3408990.dat (no annotations loaded) 100000 200000 300000 400000 500000 20000
dataset_3408990.dat (no annotations loaded) 100000 200000 300000 600000 700000 800000 1000000 1200000 1800000 1800000 2200000 2300000 2600000
100000 200000 300000 400000 500000 600000 700000 800000 900000 1100000 1200000 1300000 1600000 1600000 100000 2000000 2100000 2200000 2300000 2600000 2700000 2800000 2900000 100000 100000 100000 100000 100000 100000 1100000 100000 1100000 100000 100000 100000 100000 2000000 2100000 2500000 2600000 2700000 2800000 2900000 100000 100000 100000 100000 100000 100000 1100000 100000 1100000 100000 100000 100000 100000 2000000 200000 200000 2500000 2600000 2700000 280000 2900000 100000 100000 100000 100000 100000 100000 100000 100000 100000 100000 100000 100000 100000 20000 200000 20000 20000 20000 20000 20000 20000 20000 20000 20000 20000 20000 20000 20000 20000 20000 20000 20000 200000 200000 200000 20000 20000 20000 20000 20000 20000 20000 20000 20000 2000 20000 20000 20000 20000 20000 2000 20000 20000 20000 20000 2000 20000 20000 2000000
▲ 19M04 dataset_3413345.dat (no annotations loaded)
P 19M04 dataset_3413345.dat (no annotations loaded)
dataset_3413345.dat (no annotations loaded)
100000 200000 300000 400000 500000 600000 700000 800000 900000 1000000 1200000 1300000 1500000 1600000 1900000 2000000 2100000 2200000 2300000 2600000 2600000 2700000 2800000 2900000 3000000 3100000
19M06
dataset_3408992.dat (no annotations loaded) 100000 200000 300000 400000 500000 600000 700000 800000 900000 100000 1200000 1300000 1500000 1600000 1700000 1800000 200000 2100000 2200000 2300000 2600000 2600000 2600000 2900000 3000000
R 19M08
dataset_3408993.dat (no annotations loaded)

- plasmids in the hybrid assemblies are circularized and separate.
- that show more detail between closely related strains than the SNP analysis.

- complete genome sequences useful for distinguishing closely related isolates.



Conclusions

 Single Nucleotide Polymorphism (SNP) analysis of the MiSeq data revealed the seven Listeria monocytogenes strains belong to two distinct clusters: Cluster 1 (19C68, 19C69, 19C73, 19M02, and 19M06) and Cluster 2 (19M04 and 19M08).

 De novo assembly of short-read data from the MiSeq using SPAdes results in fragmented genome assemblies, while the combination of MiSeq and long-read MinION data using Unicycler results in more complete genome assemblies.

The assembly graphs of MiSeq data do not clearly show what contigs are extrachromosomal, while the chromosome and

The completed genome sequences from hybrid assembly using Unicycler enable direct alignments of the whole genome

Whole genome alignment reveals that isolates from Cluster 1 have distinct insertions and deletions relative to Cluster 2.

Isolate 19C69 has a genomic inversion relative to other isolates in its cluster that is not apparent from SNP typing.

The combination of long-read MinION data with the existing MiSeq data available in GenomeTrakr can be used to generate

Acknowledgements

All authors are FDA full-time employees. We would like to thank the FDA Pacific Northwest Laboratory for scientific assistance and support as well as the Office of Regulatory Science for review. Special thanks to the GalaxyTrakr team for hosting the bioinformatics programs used for analysis, as well as Federico Grau and Brad Tenge for local computing support.

Disclaimer

The views presented here do not necessarily reflect those of the U.S. Food and Drug Administration, nor do the authors specifically endorse the listed reagents and