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Hybrid Assembly from 75 *E. coli* Genomes Isolated from French Bovine Food Products between 1995 and 2016

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ABSTRACT Here, we report the complete (or near-complete) genome sequences of 75 *Escherichia coli* isolates, including 71 *stx*-positive *E. coli* isolates, isolated in France between 1995 and 2016 from food of bovine origin. Genomes were assembled using a combination of long- and short-read sequencing.

Shiga toxin-producing *Escherichia coli* (STEC) food contamination can lead to severe human symptoms (1). Here, we assembled 75 *E. coli* genomes, including 31 *eae*-positive STEC and 39 *eae*-negative STEC genomes, isolated from food of bovine origin within a 21-year period in France.

E. coli strains were originally isolated on Trypticase soy broth with yeast extract (TSYE) plates overnight at 37°C. Isolates were revived from 20 to 30% glycerol stock on TSYE plates and incubated overnight at 37°C. One colony was cultured overnight at 37°C in brain heart infusion broth (BHI) with rotation. Genomic DNA was extracted from BHI culture (1 mL) using different extraction methods and sequenced using both MiSeq (Illumina, Inc., San Diego, CA, USA) and MinION (Nanopore, Oxford Science Park, Oxford, UK) technologies (2). Default parameters were used for all software unless otherwise specified. Illumina libraries were constructed using Nextera XT kit and sequenced on v2 micro or standard flow cells (2 × 150 bp or 2 × 250 bp). MinION libraries were constructed from unsheared and non-size-selected DNA using the SQK-LSK109 ligation kit and EXP-NBD104 or EXP-NBD114 barcoding kit and sequenced on FLO-MIN106 flow cells using an Mk1B device.

Illumina reads were trimmed and quality checked using the CLC Genomics Workbench v21 (Qiagen). We generated 299,212 to 3,972,904 reads (mean, 1,368,471 ± 720,963.42) with an average read length of 198.08 (±37.99) bases per sample, representing 12 to 174× (mean, 51 ± 22.65) (Table 1). MinION FAST5 files were basecalled and demultiplexed using guppy_basecaller and guppy_barcode v3.4.5+fb1fbfb or v5.0.11+2b6dbff using “–compress-fastq” and “–trim-barcodes” parameters (3). Statistics on raw reads were performed using NanoPlot v1.29.0 (4). Between 14,450 and 1,992,878 reads (mean, 239,919 ± 341,570.565) were generated with N_{50} values ranging from 2,565 bp to 29,351 bp (mean, 16,330 ± 7,056.38). Assemblies were generated using Unicycler v0.4.8 (–min_fasta_length 1,000), Flye v2.6 or v2.8.1-b1676 (–genome-size 5m), and Raven v1.2.2 (5–7). Assembly quality and contiguity were assessed using QUAST v5.0.2 (8) and manual inspection of the assemblies (*stx*-phage and large plasmids integrity). Either the Unicycler-generated assembly was conserved or the Flye and/or Raven assemblies were further polished with short reads using racon v1.4.13, medaka v0.12.1 (optional), and pilon v1.24 polishing tools (3 to 5 rounds) (9, 10; <https://github.com/nanoporetech/medaka>). If none of these strategies produced satisfying assemblies, Canu v1.8 or v2.1.1 (genomeSize 5m) or Unicycler based on Flye or Raven assembly (–existing_long-read_assembly) was generated (11). Because assemblers have different performances regarding plasmid or phage reconstruction, several assemblies

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TABLE 1 Assembly metrics of *Escherichia coli* draft genomes and NCBI accession numbers for each isolate sequenced

NCBI accession no. (ONT, Illumina)	DNA extraction method(s)/	Strain	Serotype ^a	stx subtype(s)	ee	Hybrid assembly	MinION read length (N ₅₀ [bp])	No. of contigs	Genome size (bp)	Assembly N ₅₀ (bp)	GC content (%)	Genome coverage (x)	GenBank accession no.
SRR18191646, SRR18191606	Bead based	1429	O76:H19	1c	—	Unicycler	10,334	3	5,316,065	5,183,674	50.64	107	JACQM1000000000
SRR18191539, SRR18191528	Salting out	2206	O79:H48	1a, 2a	—	Canu ^e Unicycler	14,002	5 16	5,528,843 5,258,581	5,270,995 1,387,201	50.64 50.73	107 184	JACWPQ0000000000 JACQMHH0000000000
SRR18191517, SRR18191506	Salting out	06QMA126.1	O153/O178:H19	1a, 2a, 2d	—	Unicycler	17,384	6	5,257,865	5,088,236	50.71	184	JACWPFF0000000000
SRR18191495, SRR18191580	Salting out	06QMA140.2	O149:H1	1d	—	Flye ^d Unicycler	4	7 10	5,368,292 5,348,285	4,589,384 5,086,995	50.59 50.67	179 179	JACQMG0000000000 JACWPE0000000000
SRR18191569, SRR18191644	Bead based, salting out	06QMA181.1a	O91:H14	1a, 2b, 2b	—	Unicycler	28,527	13	5,618,742	5,174,383	50.74	242	JACQME0000000000
SRR18191633, SRR18191566	Salting out	06QMA227.3	O116:H48	2a	—	Raven ^d Unicycler	13,538	5	5,602,836 4,987,186	5,378,985 4,797,877	50.76 50.62	242 230	JAOWN100000000000 JACQMD0000000000
SRR18191555, SRR18191544	Salting out	07HMPA386	O46/O134:H38	1a, 2a	—	Unicycler	18,809	5	4,943,051	4,790,646	50.57	150	JACQMC0000000000
SRR18191613, SRR18191610	Salting out, bead based	07HMPA966	O91:H10	2a	—	Canu ^a Flye ^b Unicycler	7,440 25,581 22,124	10 3 6	4,965,900 5,144,288	4,764,076 4,966,076	50.63 50.73	150 238	JACWPDP0000000000 JACQMB0000000000
SRR18191609, SRR18191608	Salting out	07HMPA299.3B	O175:H16	2a	—	Unicycler	25,581	7	5,331,927	5,45,461	50.76	706	JACWPPO0000000000
SRR18191607, SRR18191605	Salting out	09QMA303.1	O88:H19	2g	—	Raven ^b Unicycler	4,203,6	4	5,088,106 5,132,730	4,812,392 4,862,173	50.73 50.73	207 207	JACQMA0000000000 JAOWN100000000000
SRR18191604, SRR18191603	Salting out	09QMA311.2.3	O175:H16	2d	—	Unicycler	23,479	4	5,179,346	5,125,959	50.77	168	JACQLZ000000000000
SRR18191602, SRR18191601	Salting out	09QMA33.1	O24:H27	2a	—	Unicycler	20,366	11	5,535,100	5,226,378	50.61	149	JACQLY000000000000
SRR18191600, SRR18191599	Salting out	09QMA47.1	O6:H10	1c	—	Flye ^d Unicycler	17,841	2	5,382,235	5,226,869	50.66	223	JACWPB0000000000
SRR18191542, SRR18191541	Salting out	18904	O26:H11	1a	+	Unicycler	22,209	4	5,283,406	4,794,423	50.53	215	JACQLW0000000000
SRR18191540, SRR18191538	Salting out	2011-026	O26:H11	1a	+	Raven ^d Unicycler	17,173	3	5,801,088	4,709,082	50.54	215	JACWPAG0000000000
SRR18191537, SRR18191536	Salting out	2026-0145	O145:H28	2c	+	Unicycler	23,580	11	5,834,018	5,743,918	50.64	272	JACQLV000000000000
SRR18191535, SRR18191534	Salting out	2039-O26	O26:H11	1a	+	Canu ^e Raven ^d Unicycler	9,087	3	5,891,761	2,948,002	50.45	272	JAOWNH000000000000
SRR18191533, SRR18191532	Salting out	2048-O145	O145:H28	2c	+	Flye ^d Raven ^d Unicycler	18,920	15	5,906,758	5,674,337	50.51	262	JACQLU000000000000
SRR18191531, SRR18191530	Salting out	2142-O103	O103:H25	— ^m	+	Raven	22,773	10	5,509,245	4,442,030	50.60	295	JACQLOZ000000000000
SRR18191529, SRR18191527	Salting out	2149-O103	O103:H11	1a	+	Unicycler	14,099	27	5,844,869	5,319,265	50.69	153	JACQLR000000000000
SRR18191526, SRR18191525	Salting out					Unicycler		12	5,758,941	5,122,111	50.69	153	JACQNW000000000000

(Continued on next page)

TABLE 1 (Continued)

NCBI accession no. (ONT, Illumina)	DNA extraction method/s/ method/s/	Strain	Serotype ^k	stx subtype(s)	eae	Hybrid assembly	MinION read length (N _{so} [bp])	No. of contigs	Genome size [bp]	Assembly N _{so} [bp]	GC content (%)	Genome coverage (X)	GenBank accession no.	
SRR18191524, SRR18191523,	Salting out	2236-3b	O26:H11	1a	+	Fly ^e	16,957	23	5,997,576	5,003,748	50.63	154	JAOWOX0000000000	
SRR18191522, Bead based	2918-0145	0145:H28	2a	+	Fly	Raven ^b	6,716	30	6,044,444	5,011,133	50.61	154	JAOWNW0000000000	
SRR18191521, SRR18191520,	Salting out	3273-0103	0103:H2	1a	+	Unicycler	Fly ^e	20,998	28	5,535,909	5,335,429	50.63	72	JAOWND0000000000
SRR18191519, SRR1721513,	Bead based, salting out	3313-0111	0111:H8	1a	+	Fly ^e	4,229	7	5,492,229	1,347,297	50.54	72	JAOQLP0000000000	
SRR18191516, SRR1721512,	Bead based, salting out	3313-0111	0111:H8	1a	+	Unicycler	10,277	17	5,769,060	5,373,151	50.72	238	JAOOWO0000000000	
SRR18191516, SRR18191518,	Salting out	3313-0111	0111:H8	1a	+	Unicycler	21,079	19	5,488,508	1,232,028	50.55	90	JAOQL0000000000	
SRR18191515, SRR18191514,	Salting out	3382-0103	0103:H2	1a	+	Fly ^e	13,920	18	5,719,857	4,395,063	50.65	128	JACWOU0000000000	
SRR18191513, Bead based	3383-0103	0103:H2	1a	+	Unicycler	Fly ^e	5,882	3	5,540,746	5,463,884	50.67	128	JAOWNL0000000000	
SRR18191512, SRR18191511,	Bead based	3383-026b	O26:H11	1a	+	Fly ^e	6,945	18	5,719,857	4,395,063	50.65	671	JACWOT0000000000	
SRR18191510, SRR18191509,	Bead based	3430-0103	0103:H2	1a	+	Fly ^e	8,683	9	5,542,730	5,454,423	50.65	671	JAOWNTO0000000000	
SRR18191508, SRR18191507,	Bead based, salting out	429-026	O26:H11	1a	+	Unicycler	12,758	6	5,838,831	5,709,619	50.59	778	JAOWOS0000000000	
SRR21721511, SRR18191507,	Salting out	429-026	O26:H11	1a	+	Fly ^e	12,758	12	5,822,451	5,697,780	50.62	778	JAOWOPR0000000000	
SRR18191505, SRR18191504,	Salting out	4712-026	O26:H11	1a	+	Unicycler	Fly ^e	25,662	4	5,558,675	4,743,383	50.73	259	JAOWNNS0000000000
SRR18191503, SRR18191502,	Salting out	4747-026	O26:H11	1a	+	Unicycler	Canu ^a	39	5,664,587	2,049,284	50.56	148	JACQLM0000000000	
SRR18191501, SRR18191500,	Salting out	6423-026	O26:H11	1a	—	Unicycler	Canu ^e	6	5,856,627	5,621,430	50.64	148	JACQPN0000000000	
SRR18191499, SRR18191498,	Bead based	97HMP449	O3:H12	1a	—	Unicycler	Fly ^e	6	5,714,135	5,609,626	50.62	148	JAOWP0000000000	
SRR18191497, SRR18191496,	Salting out	97HMP473	O3:H12	1a	—	Unicycler	Canu ^e	7	5,992,428	5,821,752	50.66	264	JAOWPML0000000000	
SRR18191590, SRR18191589,	Bead based, salting out	97HMP650	O110:H9	1c	—	Unicycler	Raven ^b	28,759	4	5,371,299	5,104,752	50.51	334	JAOQLL0000000000
SRR18191588, SRR18191587,	Bead based, salting out	97HMP652	O110:H9	1c	—	Unicycler	Unicycler	22,595	3	5,472,012	3,671,848	50.47	334	JAOQPL0000000000
SRR18191586, SRR21721510,	Bead based, salting out	97HMP657	O136:H2	1a	—	Unicycler	21,241	9	5,439,851	5,167,313	50.67	175	JAOWNQ0000000000	
SRR18191585, SRR18191584,	Salting out	97HMP657	O136:H2	1a	—	Fly ^e	21,241	44	5,655,428	5,156,955	50.68	125	JAOWON0000000000	
SRR18191583, SRR18191582	Bead based	97HMP915	O112ac:H19	2c	—	Unicycler	8,149	2	5,247,946	5,142,912	50.78	805	JACQLH0000000000	

(Continued On next page)

TABLE 1 (Continued)

NCBI accession no. (ONT, Illumina)	DNA extraction method/s/ method/s/ method/s/	Strain	Serotype ^k	stx subtype(s)	eae	Hybrid assembly	MinION read length (N ₅₀ [bp])	No. of contigs	Genome size [bp]	Assembly N ₅₀ [bp]	GC content (%)	Genome coverage (X)	GenBank accession no.
SRR18191581, SRR18191579	Bead based	98HMPL324	O174:H21	2c	—	Raven ^d	5,263	4	5,214,333	5,042,330	50.76	908	JAOWNBN000000000
SRR18191578, SRR18191577	Salting out	98HMPL325	O17/044/O77:H18	2d	—	Canu	8	5,317,997	5,045,734	50.79	908	JAOWPK000000000	
SRR18191576, SRR18191575	Bead based	98HMPL475	O110:H9	1c	—	Unicycler	14,916	6	5,373,540	5,090,743	50.71	199	JAOQLG000000000
SRR18191574, SRR18191573	Salting out	98HMPL479	O91:H14	1a, 2b, 2b	—	Unicycler	8,894	13	5,831,137	5,558,854	50.68	152	JAOQLE000000000
SRR18191572, SRR18191571	Salting out	98HMPL487	O136:H12	1a	—	Unicycler	20,281	2	5,430,616	5,174,002	50.58	191	JACQLD000000000
SRR18191570, SRR18191568	Salting out	Crl154393	O157:H7	2a, 2c	+	Unicycler	21,282	11	5,744,891	5,126,924	50.47	134	JACQLC000000000
SRR18191567, SRR18191598	Salting out	Crl154395	O157:H7	2c	+	Flye ^b	5,807,355	3	5,666,998	5,047	134	JAOWM000000000	
SRR18191597, SRR18191596	Salting out	Crl154397	O157:H7	2c, 2c	+	Raven ^d	5,273,188	3	5,789,274	5,273,188	50.49	134	JACWNA000000000
SRR18191595, SRR18191594	Salting out	Crl169922	O82:H8	—	Unicycler	21,865	17	5,720,557	4,991,949	50.39	129	JAOQLB000000000	
SRR18191593, SRR18191592	Salting out	Crl169937	O51:H41	—	Unicycler	20,389	19	5,644,625	5,555,517	50.48	129	JAOWOL000000000	
SRR18191591, SRR18191643	Salting out	CrlO103	O103:H2	—	Unicycler	20,244	6	5,749,327	5,423,006	50.49	240	JACQLA000000000	
SRR18191642, SRR18191641	Salting out	CrlO157	O157:H7	2c	+	Flye ^b	4,868,876	9	5,476,119	4,761,119	50.80	225	JAOQKZ000000000
SRR18191640, SRR18191639	Bead based, salting out	E. coli 12-1	O157:H7	2a, 2c	+	Canu ^a	5,642,849	10	5,232,969	4,232,969	50.43	278	JACQKWW000000000
SRR18191638, SRR18191637	Salting out	EC14	O174:H21	2c	—	Flye ^b	25,609	3	5,909,043	5,474,499	50.44	284	JACWOJ000000000
SRR18191636, SRR18191635	Salting out, Bead based	ECA135	O22:H16	2b, 2c, 2d	—	Unicycler	20,105	4	5,807,203	5,689,286	50.50	284	JAOWOI000000000
SRR18191634, SRR18191632	Salting out, Bead based, salting out	ECA15	O79:H48	1a, 2a	—	Flye ^b	29,351	5	5,082,783	4,945,157	50.83	219	JACQKY000000000
SRR18191631, SRR18191630	Salting out	ECA193	O84:H21	1a, 2c	—	Raven ^d	19,793	5	5,155,830	4,911,711	50.83	219	JAOWPWH000000000
SRR18191629, SRR18191628	Bead based	ECA194	O179:H8	1a, 2a	—	Unicycler	20,554	9	5,386,935	5,386,935	50.63	276	JAOWOG000000000
SRR18191627, SRR18191626	Bead based	ECA279	O174:H2	1a, 2d	—	Unicycler	15,826	3	5,596,707	5,158,603	50.69	222	JACQKU000000000
SRR18191625, SRR18191624	Bead based, salting out	ECA329	O91:H21	2a, 2d	—	Flye ^b	2,565	7	5,094,287	4,913,760	50.72	154	JACQKT000000000
SRR18191623, SRR18191565	Salting out	ECA329	O91:H21	2a, 2d	—	Unicycler	15,119	5	5,126,030	2,145,451	50.72	199	JACQKS000000000
SRR18191564	Salting out	ECA34	O5:H9	1a	+	Flye ^b	6,208	14	5,167,400	5,037,956	50.76	199	JAOWF000000000
									5,167,518	5,016,862	50.71	235	JAOWNPM000000000
									5,342,559	4,901,208	50.76	167	JAOWOE000000000
									5,341,159	4,796,583	50.64	86	JAOWOD000000000

(Continued On next page)

TABLE 1 (Continued)

NCBI accession no. (ONT, Illumina)	DNA extraction method(s) ^j	Strain	Serotype ^k	stx subtype(s)	eae	Hybrid assembly	MinION read length (N ₅₀ [bp])	No. of contigs	Genome size (bp)	Assembly N ₅₀ (bp)	GC content (%)	Genome coverage (X)	GenBank accession no.
SRR18191623, SRR18191564	Salting out	ECA34	O5:H9	1a	+	Raven ^d	24,947	4	5,414,456	4,637,316	50.68	115	JACOMWY0000000000
SRR18191563, SRR18191562	Bead based, salting out	ECA36	O5:H9	1a, 1a	+	Unicycler	22,228	14	5,418,653	4,566,021	50.66	100	JAOWNNN0000000000
SRR18191561, SRR18191560	Bead based, salting out	ECA37	O5:H9	1a, 1a	+	Flye ^e	24,413	11	5,403,523	5,306,342	50.69	112	JAOWOC0000000000
SRR18191559, SRR18191558	Bead based	ECA399	O174:H21	2c	-	Unicycler	3,390	5	5,141,946	3,448,145	50.78	479	JAOQKR0000000000
SRR18191557, SRR18191556	Salting out	ECA400	O6:H10	2d	-	Canu ^a	24,727	2	5,061,078	5,053,258	50.68	200	JAOWPFG0000000000
SRR18191554, SRR18191553	Bead based	ECA447	O22:H16	1a, 2b, 2d	-	Unicycler	6,419	5	5,351,044	5,298,558	50.63	121	JACQKQ0000000000
SRR18191552, SRR18191551	Salting out	ECA89	OgN-PRK2:H16	1c	-	Raven ^d	18,224	52	5,345,303	3,229,988	50.64	121	JACWMX0000000000
SRR18191550, SRR18191549	Salting out	ECA97	O113:H4	2d	-	Unicycler	17,597	4	5,163,781	4,943,802	50.72	180	JACWNY0000000000
SRR18191548, SRR18191547	Salting out	HSV03	O157:H7	2c	+	Raven ^d	22,885	40	5,262,159	5,257,847	50.76	180	JAOWNK0000000000
SRR18191546, SRR18191545	Salting out	HSV04	O157:H7	2c	+	Flye ^e	22,926	11	5,843,094	5,515,994	50.55	149	JAOQKP0000000000
SRR18191543, SRR18191622	Bead based	NC672mucus	Ox13:H2	-	Flye ^e	6,632	3	5,323,626	5,187,741	50.49	67	JAOWMWW0000000000	
SRR18191621, SRR18191620	Salting out	NC809	O41:H7	-	Unicycler	19,581	8	5,227,833	4,891,608	50.51	230	JACQKQ0000000000	
SRR18191619, SRR18191618	Bead based	NV34	O168:H8	2d	-	Unicycler	6,450	6	5,269,996	4,249,978	50.77	402	JACQKN0000000000
SRR18191617, SRR18191616	Bead based	NV36	O75:H8	1c, 2b	-	Raven ^d	3	5,314,568	5,184,508	50.81	402	JACWMV0000000000	
SRR18191615, SRR18191614	Salting out	Slk8430761	O177:H25	2c, 2d	+	Unicycler ^f	8	5,575,909	5,306,095	50.65	468	JACQKM0000000000	
SRR18191612, SRR18191611	Salting out	Slk8430767	O177:H25	2c, 2d	+	Flye ^e	20,113	11	5,577,269	5,301,141	50.65	113	JAOWNM0000000000
						Raven ^d	28	30	5,639,340	5,231,455	50.58	180	JAOWNZ0000000000
								3	5,658,335	5,248,496	50.58	180	JAOQKL0000000000
								3	5,420,630	5,017,089	50.54	150	JAOWMU0000000000
								3	5,458,939	5,276,849	50.57	150	JAOWMU0000000000

^aLong-read assembly polished with pilon.^bLong-read assembly polished with racon and medaka.^cLong-read assembly polished with racon (2 rounds) and medaka.^dLong-read assembly polished with racon, medaka, and pilon.^eLong-read assembly polished with racon, medaka, and pilon, and contigs <1 kb were removed.^fLong-read assembly polished with racon (3 rounds), medaka, and pilon, and contigs <1 kb were removed.^gLong-read assembly polished with racon (4 rounds), medaka, and pilon, and contigs <1 kb were removed.^hLong-read assembly polished with racon (3 rounds) and pilon.ⁱLong-read assembly polished with racon (3 rounds), pilon, and contigs <1 kb were removed.^jIf two methods were used for DNA extraction, the first one corresponds to the DNA extraction method used for sequencing using Illumina and the second for MinION sequencing.^kSerotypes were determined using *in silico* analysis.^l50 × subsampling.^m - are used to describe bash arguments/options.

were retained for some of the samples. Whenever possible, Unicycler performed the circularization of the replicons (5).

Assemblies from 1 to 52 contigs (mean, 10.33 ± 9.73) with a total assembly length between 4,849,707 and 6,118,277 bp (mean, $5,468,530.42 \pm 292,599.77$ bp) and mean GC content of 50.64% (± 0.13) were generated. N_{50} values ranged from 1,082,226 to 5,821,752 bp (mean, $4,759,454.32 \pm 1,018,105.98$ bp). The main sample characteristics, including serotype, *stx* and *eae* gene presence (determined using abricate [-min-cov 60 –min-id 80]), genome coverage, and accession numbers are reported in Table 1. All genomes were annotated using the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) v6.3 at http://www.ncbi.nlm.nih.gov/genome/annotation_prok (12).

Data availability. Short and long raw reads were deposited in the NCBI SRA database under BioProject accession number [PRJNA808207](#). Additionally, assembled genomes were deposited in GenBank under accession numbers as referred to in Table 1 within BioProject accession numbers [PRJNA808207](#), [PRJNA885284](#), [PRJNA884285](#), [PRJNA884276](#), and [PRJNA883638](#).

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