Supplementary Material

Rapid Detection of Genetic Engineering, Structural Variation, and Antimicrobial Resistance Markers in Bacterial Biothreat Pathogens by Nanopore Sequencing

Amy S. Gargis[1*], Blake Cherney[1], Andrew B. Conley[2], Heather P. McLaughlin[1], David Sue[1]

[1] Division of Preparedness and Emerging Infections, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

[2] IHRC-Georgia Tech Applied Bioinformatics Laboratory, Atlanta, Georgia, USA.

[*]Correspondence and requests for materials should be addressed to A.S.G. (email: AGargis@cdc.gov). Current Address: Division of Healthcare Quality Promotion, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Supplemental Table 1. *Y. pestis* and *B. anthracis* DNA extraction quality and quantity.

Strain	**Extraction**	**A 260/280nm***	**A 260/230nm***	**Estimated DNA Concentration (ng/µl)**
Y. pestis A1122	SP	1.94	1.78	69.7
Y. pestis S1037	SM	1.97	2.11	81.5
	SP-1	1.95	2.06	67
	SP-2	1.91	2.15	78
B. anthracis Sterne/pUTE29	SM	1.96	2.16	103
B. anthracis 411A2	SM	1.87	1.53	60.9
B. anthracis UT308	SM	1.96	1.56	81.8

(A) absorbance, (SM) silica-membrane extraction, (SP) salt-precipitation extraction

*Oxford Nanopore Technologies recommends optical density absorbancy ratios of A260/280nm: ~1.8 and A260/230nm: 2.0-2.2.

Supplemental Table 2. Details of *Y. pestis* and *B. anthracis* nanopore sequencing runs included in this study.

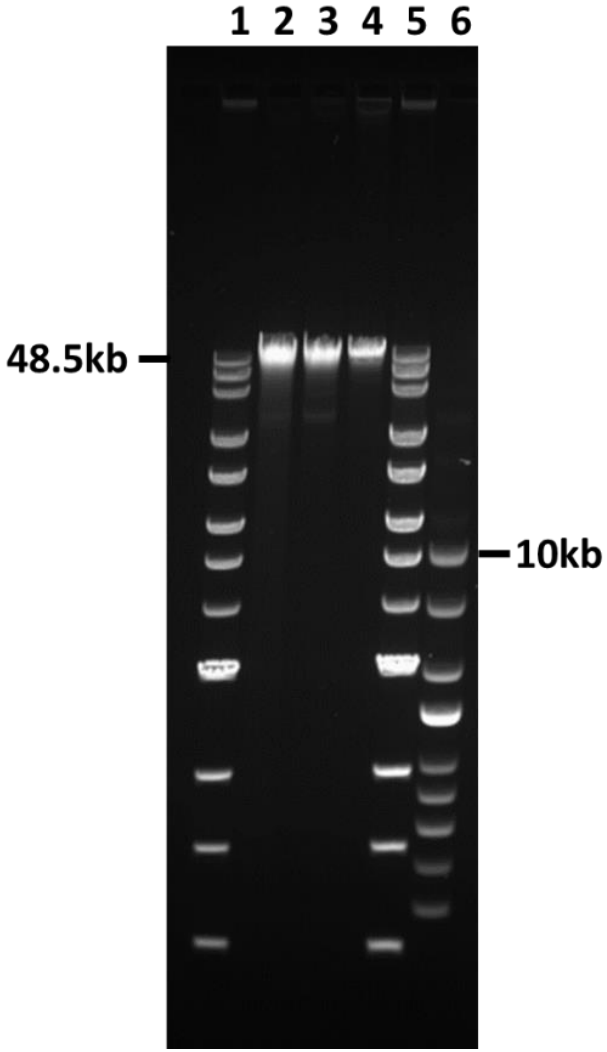
Y. pestis **Strain (Extraction)**	**Library Preparation**	**Run Date**	**MinKNOW Sequencing Script**	**Total Run Time**	**Time to 100,000 passed fast5 reads**	**Strand to Pore Ratio (~10 run time)**	**fast5 Accession Number**	**FASTQ Accession Number**
S1037 (SM)	Rapid 1	2/14/2018	SQK-RAD004	17 hr 54 min	1 hr 2 min	263/326 (80%)	SRR8608868	SRR8608876
S1037 (SM)	Rapid 2	2/15/2018	SQK-RAD004	17 hr 1min	57 min	239/317 (75%)	SRR8608869	SRR8608877
S1037 (SM)	Rapid 3	2/16/2018	SQK-RAD004	24 hr 9 min	1 hr 38 min	256/312 (82%)	SRR8608870	SRR8608878
S1037 (SM)	Field	03/14/2018	SQK-RAD003	28 hr 27 min	1hr 35 min	298/340 (87%)	SRR8608871	SRR8608879
S1037 (SP-1)	Rapid 1	2/28/2018	SQK-RAD004	20 hr 29 min	1 hr 37 min	153/252 (60%)	SRR8608853	SRR8608872
S1037 (SP-1)	Rapid 2	3/1/2018	SQK-RAD004	23 hr 29 min	2 hr 41 min	159/251 (63%)	SRR8608852	SRR8608873
S1037 (SP-1)	Rapid 3	3/2/2018	SQK-RAD004	46 hr 24 min	3 hr 18 min	157/244 (64%)	SRR8608855	SRR8608874
S1037 (SP-1)	Field	3/5/2018	SQK-RAD003	24 hr	1 hr 57min	257/344 (74%)	SRR8608854	SRR8608875
S1037 (SP-2)	Rapid	09/18/2018	SQK-RAD004	43 hr 47min	8 hr 12 min	84/218 (38%)	SRR8608849	SRR8608880
A1122 (SP)	Rapid	09/19/2018	SQK-RAD004	49 hr 16 min	1 hr 47min	260/347 (74%)	SRR8608851	SRR8608862
B. anthracis **Strain (Extraction)**	**Library Preparation**	**Run Date**	**MinKNOW Sequencing Script**	**Total Run Time**	**Time to 100,000 passed fast5 reads**	**Strand to Pore Ratio (~10 run time)**	**fast5 Accession Number**	**FASTQ Accession Number**
Sterne/pUTE29 (SM)	Rapid	4/12/2018	SQK-RAD004	24 hr	3 hr 55 min	107/201 (53%)	SRR8608850	SRR8608863
Sterne/pUTE29 (SM)	Field	09/13/2018	SQK-RAD003	40 hr 3 min	1 hr 43 min	208/330 (63%)	SRR8608847	SRR8608864
411A2 (SM)	Rapid	8/3/2018	SQK-RAD004	48 hr	1 hr 36 min	Not recorded	SRR8608846	SRR8608865
411A2 (SM)	Field	6/28/2018	SQK-RAD003	Not recorded	4 hr 53 min	Not recorded	SRR8608860	SRR8608866
UT308 (SM)	Rapid	6/27/2018	SQK-RAD004	22 hr 47 min	2 hr 15min	87/237 (37%)	SRR8608861	SRR8608867

All runs were sequenced using the Rapid Sequencing Kit (SQK-RAD004), or the Field Sequencing Kit (SQK-LRK001), R9.4.1 flow cells, and MinKNOW (Version 18.01.6). (SM) silica-membrane extraction, (SP) salt-precipitation extraction

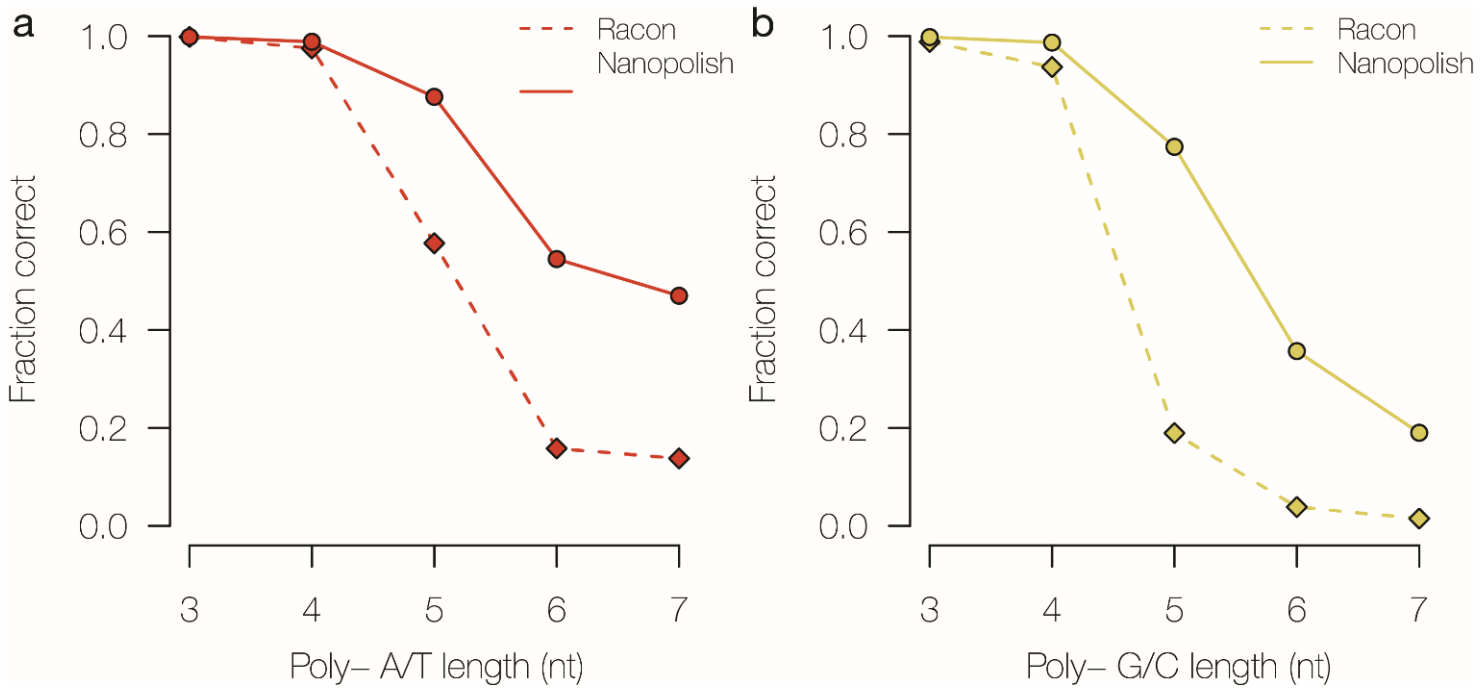
Supplemental Table 3. Metrics of 16 hour nanopore sequencing for *Y. pestis* strain S1037.

***Y. pestis* S1037 Sequencing Run**	**Active Pores**	**Passed Reads**	**Failed Reads**	**≥Q7 Reads (Albacore)**	**# Reads Lost After Basecalling**	**% Reads Lost After Basecalling**	**Avg. Read Length (nt)**	**Longest Read (nt)**	**Data Generated (Gb)**	**Avg. Quality Score**
SP Rapid (1)	1,061	676,497	54,793	639,598	36,899	5.45	4,769	144,371	3.1	13.1
SP Rapid (2)	888	410,515	46,382	388,435	22,080	5.38	4,690	144,371	1.8	13.2
SP Rapid (3)	759	329,875	85,554	310,906	18,969	5.75	8,239	126,776	2.6	13.1
SM Rapid (1)	1,358	1,190,946	75,040	305,824	885,122	74.32	4,377	101,096	1.3	12.6
SM Rapid (2)	1,276	1,294,029	144,023	1,172,803	121,226	9.37	3,927	149,903	4.6	12.5
SM Rapid (3)	968	650,090	180,273	477,864	172,226	26.49	5,533	93,179	2.6	12.8
SM Field (1)	1,326	621,837	52,943	594,145	27,692	4.45	5,030	86,368	3.0	13.9
SP Field (1)	1,502	579,770	60,298	545,442	34,328	5.92	6,284	116,662	3.4	13.5

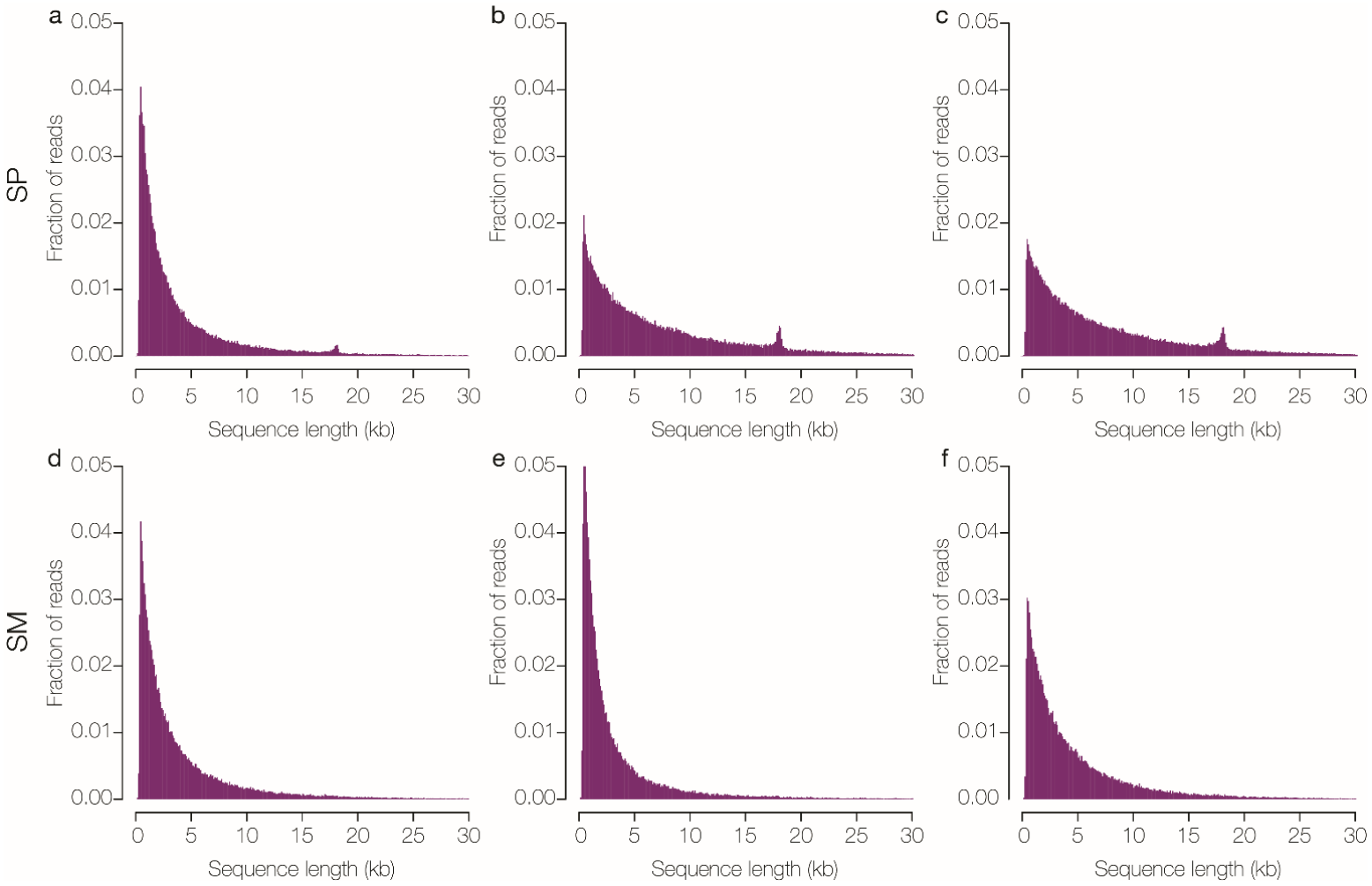
Supplementary Figure 1. Agarose gel electrophoresis of *Y. pestis* S1037 DNA. gDNA (100 ng/lane) extracted using the silica-membrane (lane 2) and salt-precipitation (lane 3) methods was visualized. DNA Ladders (NEB 1 kb Extend, lanes 1 and 5; and NEB Supercoiled DNA Ladder, lane 6) and lambda phage gDNA (ONT, lane 4) were also included.



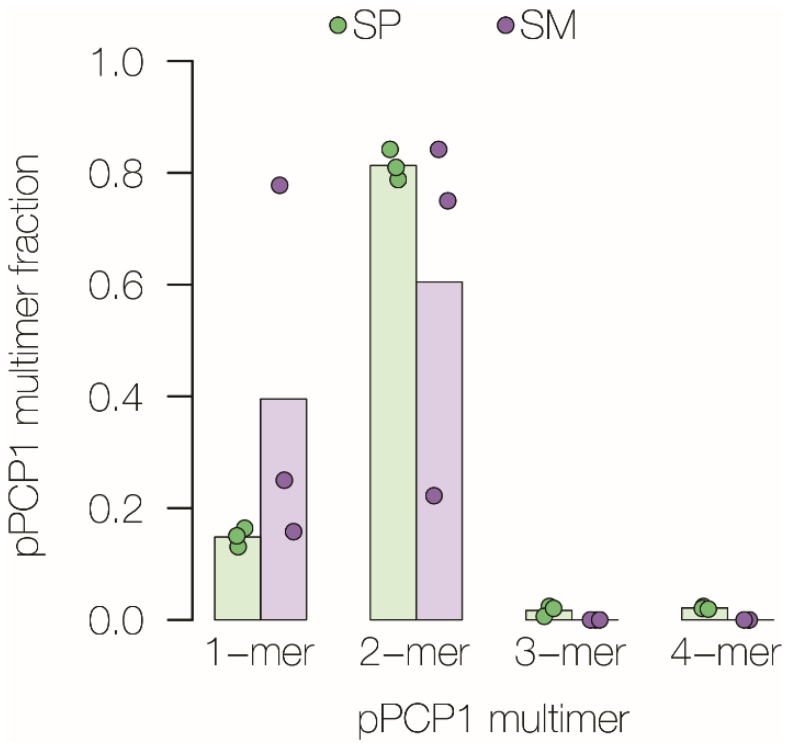
Supplementary Figure 2. Poly-N length and fraction of poly-N's correct in *Y. pestis* S1037 assemblies using 100,000 fast5 read data sets. **(a)** The average fraction of poly-A/T's or **(b)** poly-G/C's, with sequence lengths of 3 to 7 nucleotides, called correctly in *Y. pestis* S1037 nanopore assemblies using sequence polishing tools RACON (dotted line) and Nanopolish (solid line). The fraction correct was calculated as an average using all salt-precipitation and silica-membrane replicate data sets (n=6).



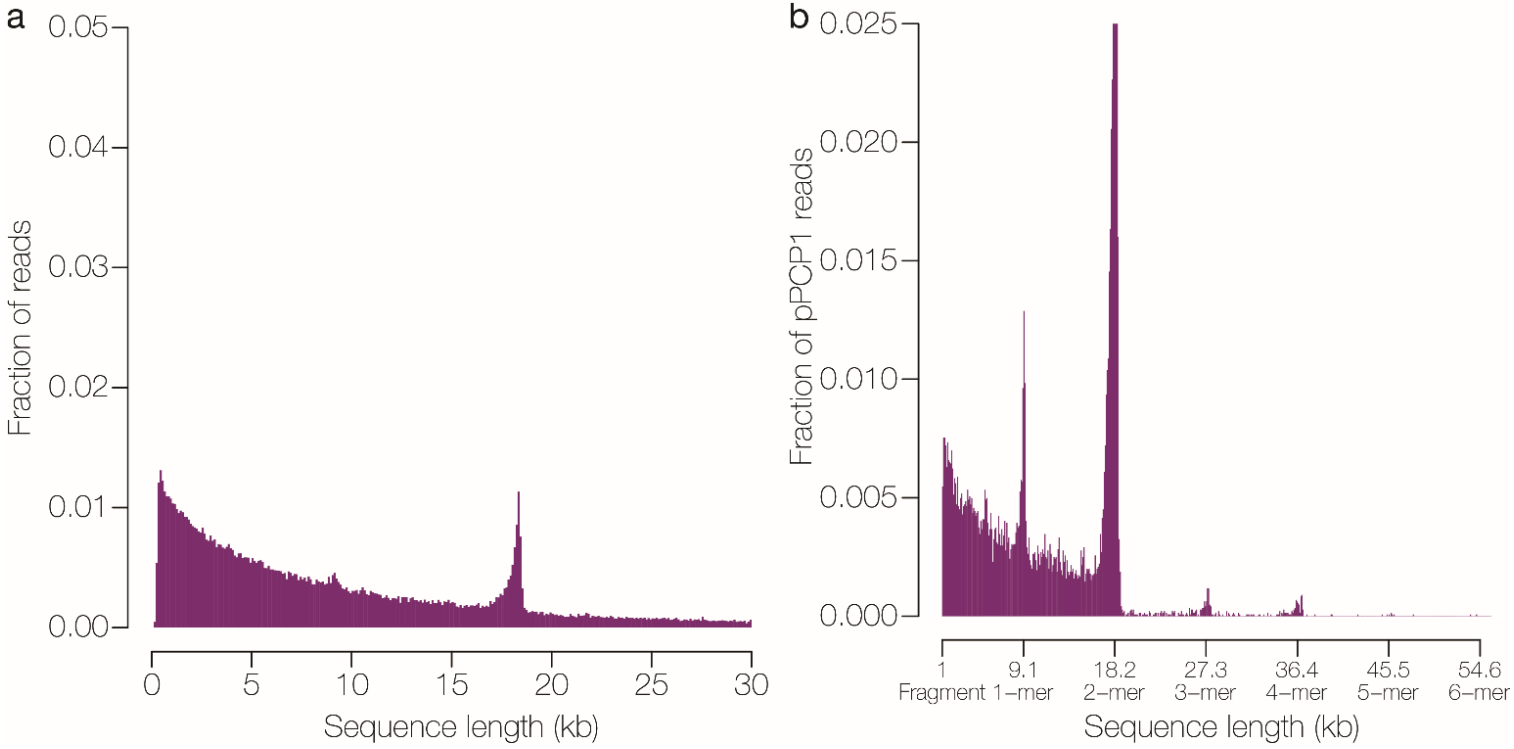
Supplementary Figure 3. Distribution of nanopore sequencing reads by length using the first 100,000 fast5 nanopore reads analyzed for *Y. pestis* S1037 data sets. (**a-c)** Overall read length distributions for the three salt-precipitation (SP) replicates. (**d-f)** Overall read length distributions for the three silica-membrane (SM) replicates. The peak in graphs a-c corresponds to the increased fraction of ~18.2 kb reads in the SP data sets that represent the pPCP1 dimer.



Supplementary Figure 4. Fraction of the pPCP1 multimers found in *Y. pestis* S1037 nanopore data sets of 100,000 fast5 reads. Based on length, reads were assigned to a multimeric form of pPCP1 (x-axis) and the fraction of each multimer is displayed (y-axis). Salt-precipitation (SP) data sets are in green and silica-membrane (SM) data sets are in purple. Each dot represents the multimer fraction of a rapid technical replicate and bars depict the average multimer fraction for all three technical replicates. The 3-mer and 4-mer forms were not detected in the SM data sets.

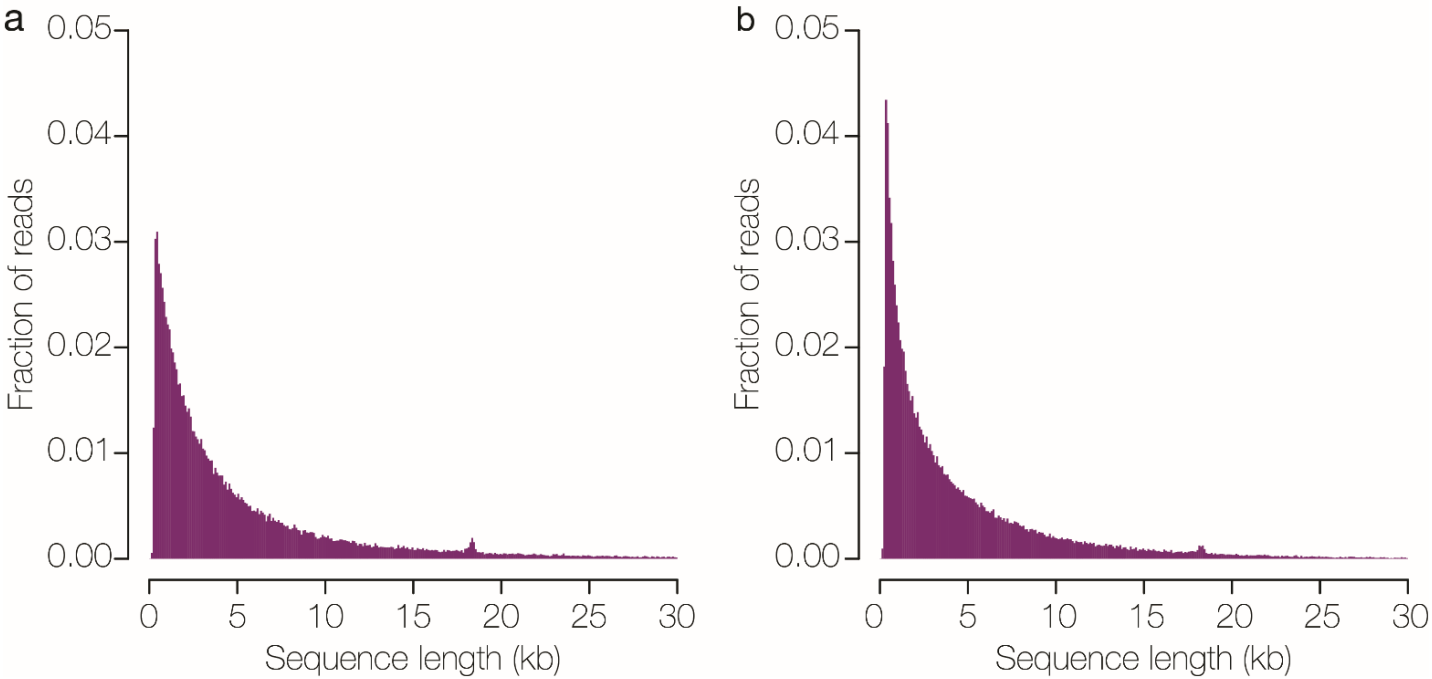


Supplementary Figure 5. Distribution and fraction of reads in *Y. pestis* S1037 SP-2 100,000 read assembly. (a) Distribution of nanopore sequencing reads by length using the first 100,000 fast5 reads analyzed for the *Y. pestis* S1037 SP-2 data set in which additional care was taken to minimalize shearing during extraction. The increased fraction of ~18.2 kb reads in the SP data sets corresponds to the pPCP1 dimer. **(b)** Fraction of nanopore sequencing reads with homology to pPCP1 using the first 100,000 fast5 reads analyzed for the *Y. pestis* S1037 SP-2.

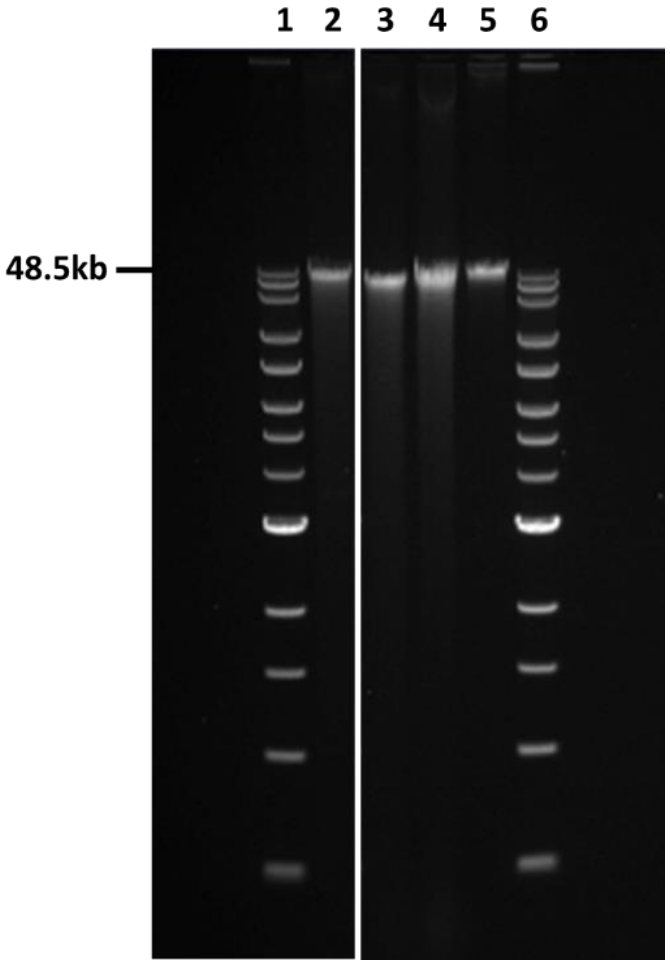


Supplementary Figure 6. Distribution of nanopore sequencing reads by length using the first 100,000 fast5 reads analyzed for *Y. pestis* A1122. (**a)** salt-precipitation (SP) data set, and (**b)** silica-membrane (SM) data set

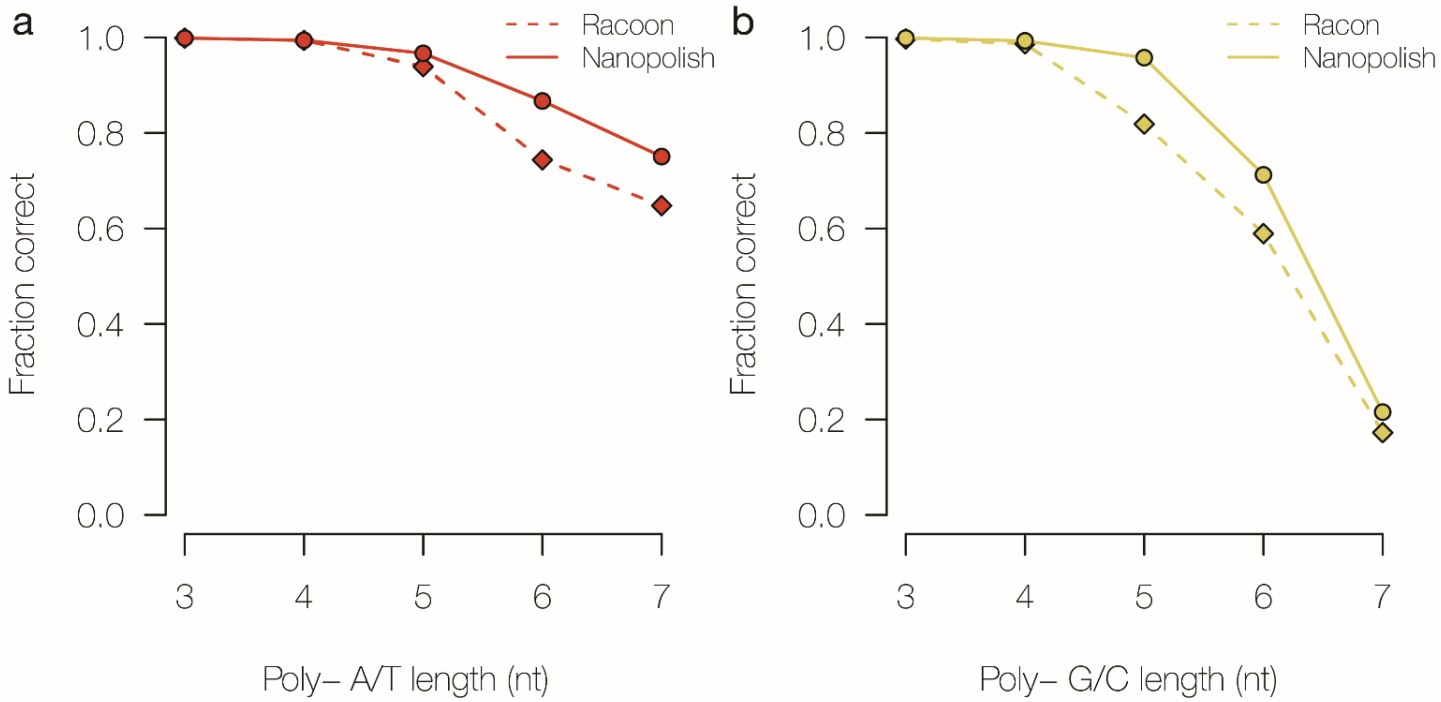
.



Supplementary Figure 7. Agarose gel electrophoresis of *B. anthracis* DNA extracted using the silica-membrane method. gDNA (100 ng/lane) was visualized for *B. anthracis* Sterne/pUTE29 (lane 2), 411A2 (lane 3), and UT308 (lane 4). DNA Ladder (NEB 1 kb Extend, lanes 1 and 6) and lambda phage gDNA (ONT, lane 5) were also included. The white line between lanes 2 and 3 indicates where the gel was cropped to remove lanes not relevant to this study.

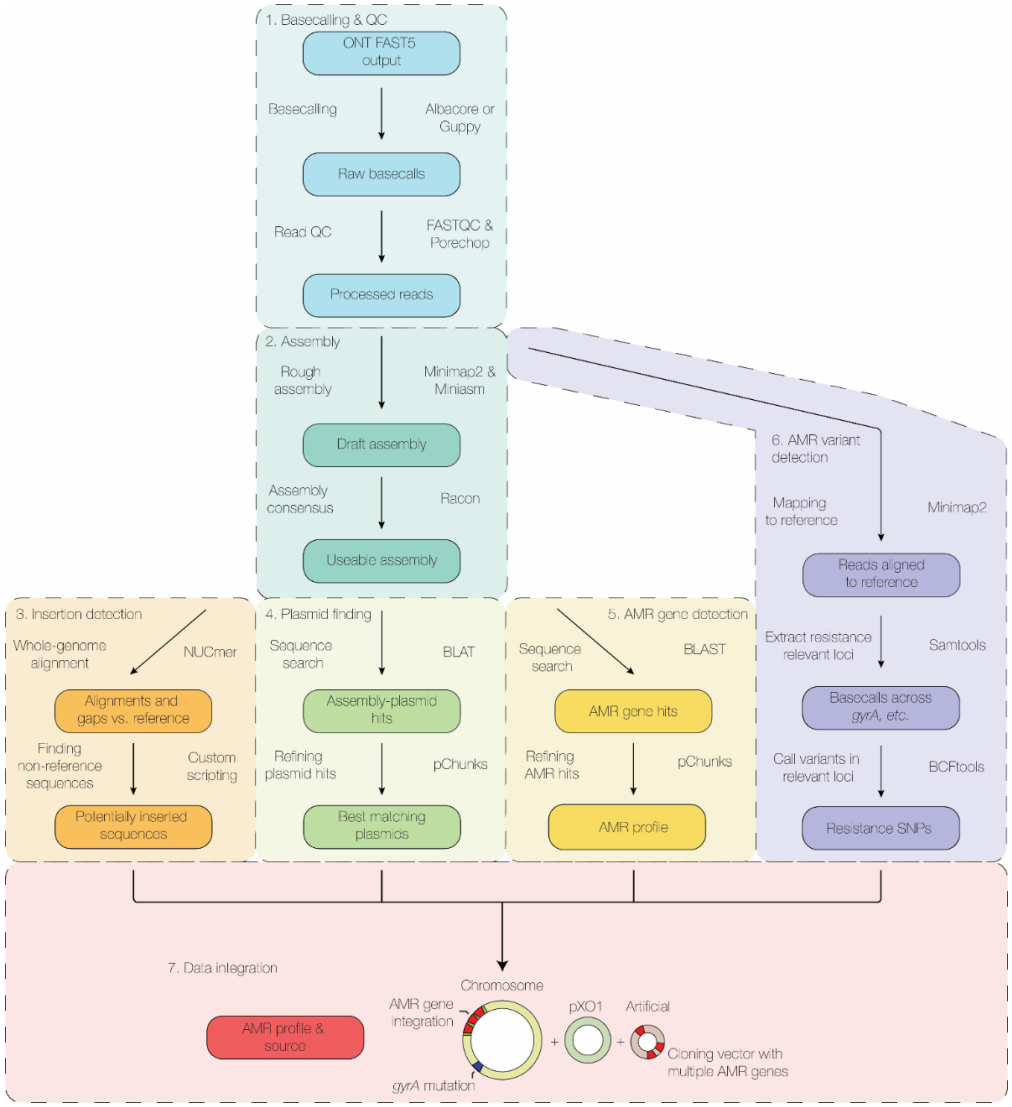


Supplementary Figure 8. **Poly-N length and fraction of poly-N's correct in *B. anthracis* Sterne/pUTE29 100,000 read assemblies.** (**a)** The average fraction of poly A/Ts or **(b)** poly G/C's, with sequence lengths of 3-7 nucleotides, called correctly in *B. anthracis* nanopore assemblies using sequence polishing tools RACON (dotted line) and Nanopolish (solid line). The 100,000 fast5 read data sets were used for this analysis and the fraction correct was calculated as an average using the *B. anthracis* Sterne/pUTE29 (Rapid and Field), 411A2 (Rapid and Field), and UT308 (Rapid) assemblies (n=5).

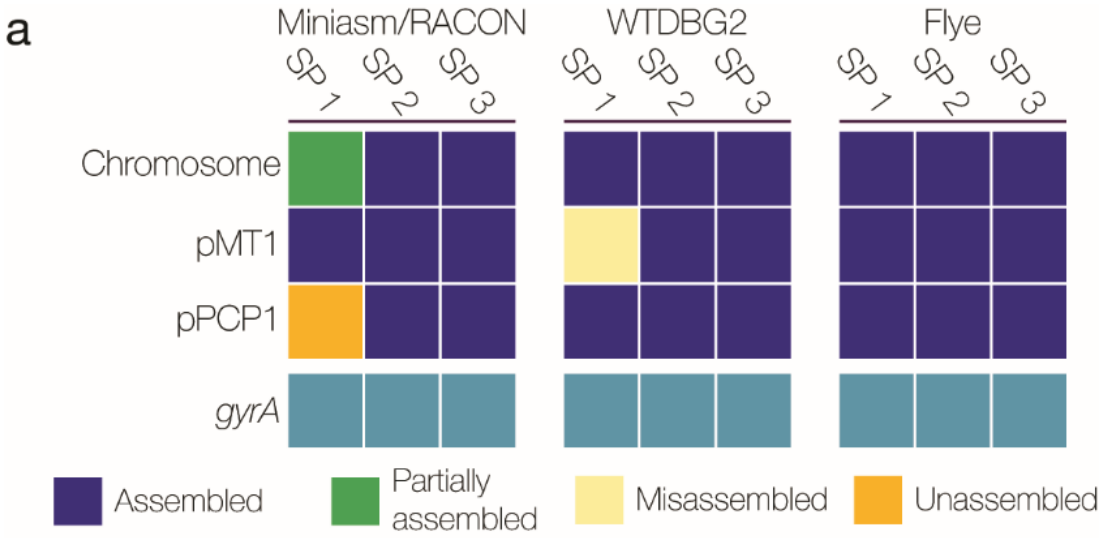


Supplementary Figure 9. Bioinformatics pipeline for analysis of *Y. pestis* and *B. anthracis* whole genome sequencing nanopore data.

A pipeline was developed to perform rapid *de novo* genome and plasmid assembly, to detect known markers for antimicrobial resistance, and to identify variants (SNPs, insertions and deletions).



Supplementary Figure 10. Performance assessment of different software to improve *Y. pestis de novo* assembly. Three different algorithms were used to assemble the genome sequences from the three salt precipitation (SP) replicates: Miniasm, WTDBG2, and Flye. The Miniasm assembly was post-processed with RACON. **(a)** Completeness of each assembly was determined by comparing the assembled genome to the A1122 *Y. pestis* reference sequence. **(b)** Assemblies were compared to the A1122 *Y. pestis* reference sequence using the QUAST utility in order to quantify the number of mismatches and indels in each assembly.



b

	Miniasm/RACON			WTDBG2			Flye		
	SP 1	SP 2	SP 3	SP 1	SP 2	SP 3	SP 1	SP 2	SP 3
Mismatches (k)	24.2	22.6	27.0	3.0	2.9	3.3	0.8	0.7	0.7
Indels (k)	58.4	54.4	60.4	53.8	50.2	51.8	9.9	9.0	9.7