Supplementary Material

Accurate bacterial outbreak tracing with Oxford Nanopore sequencing and reduction of methylation-induced errors

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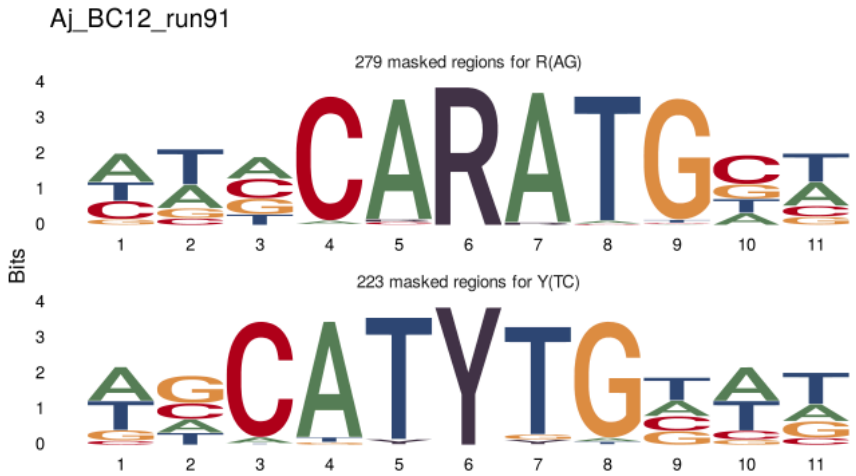
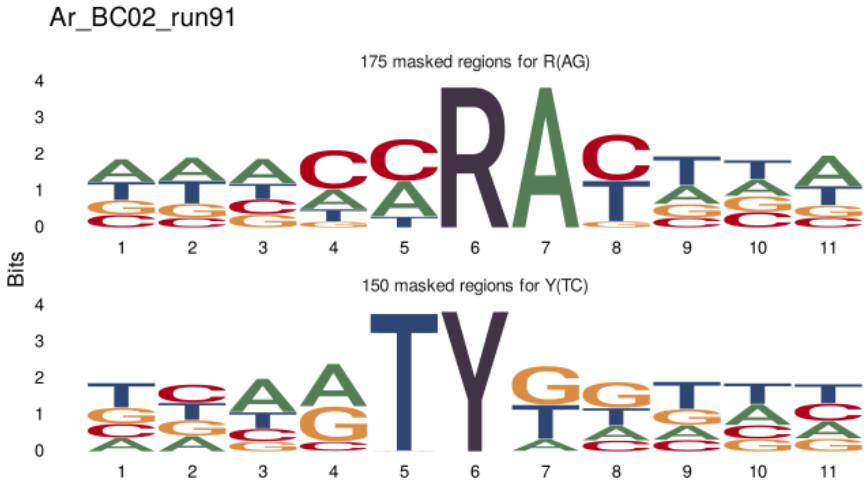
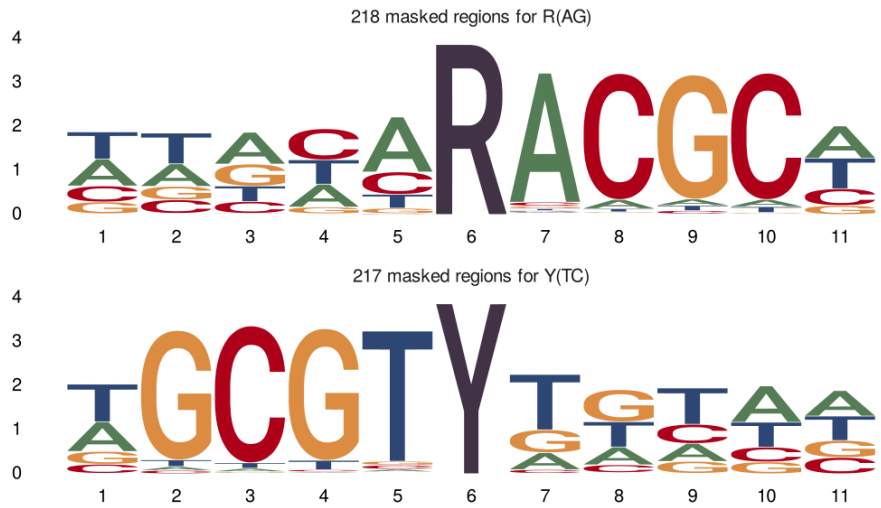
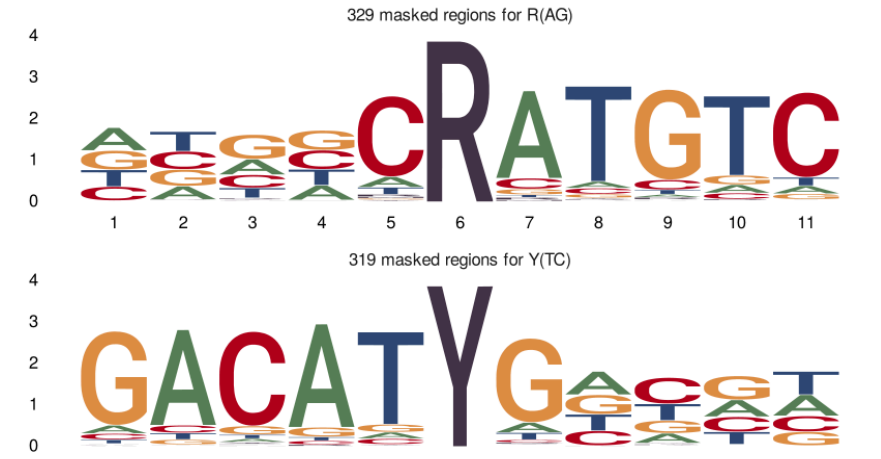
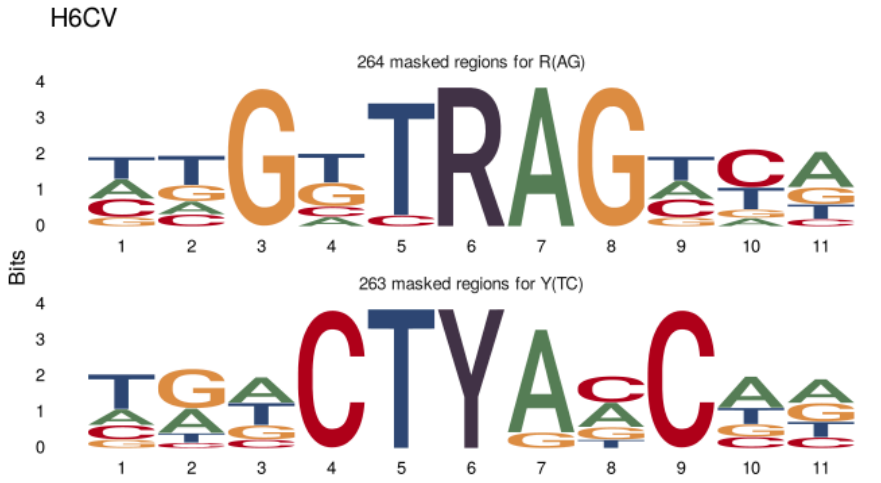
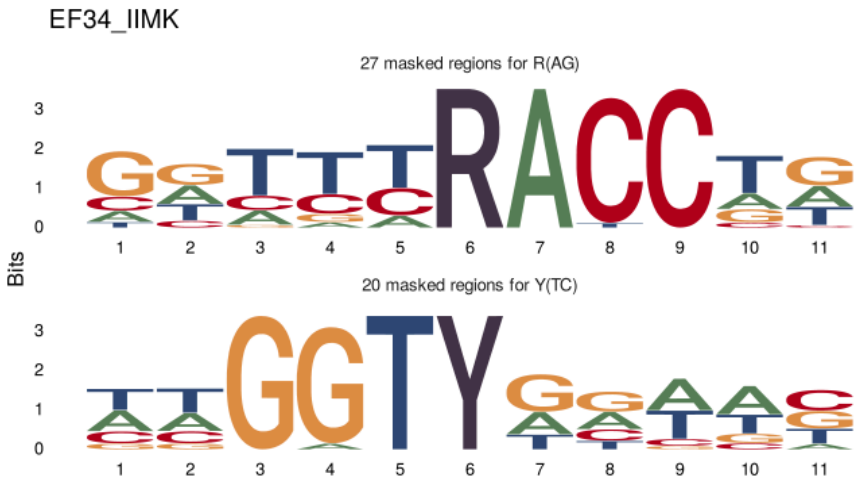
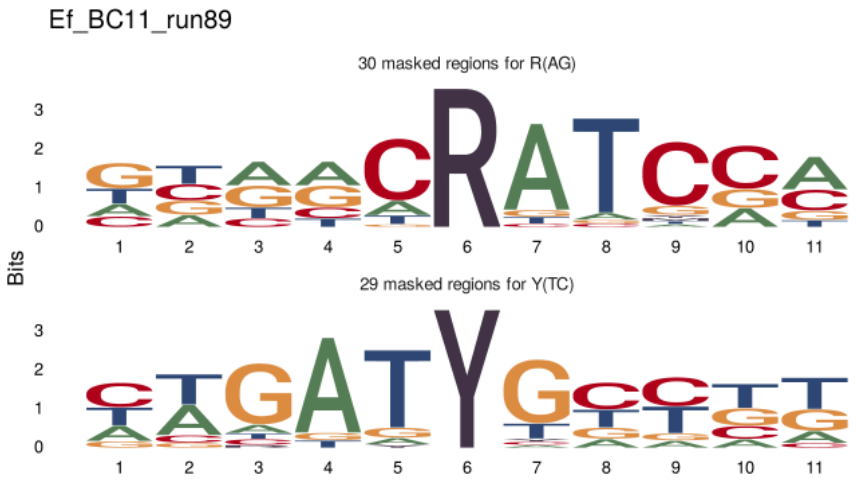
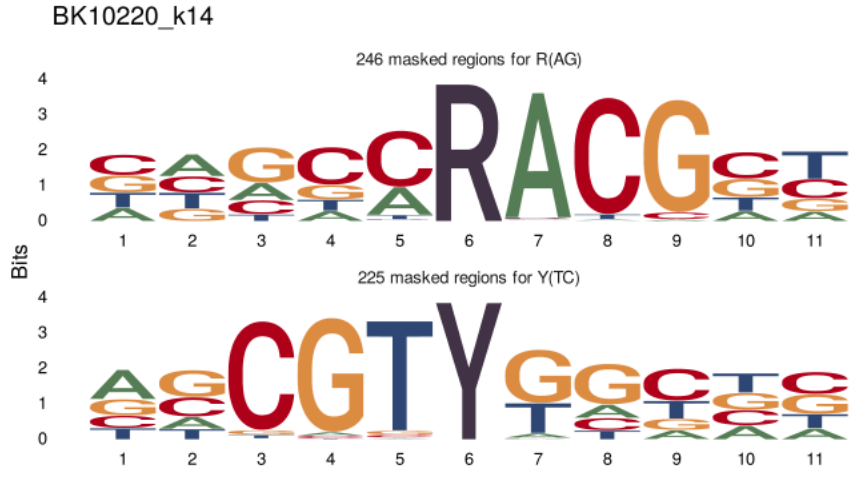
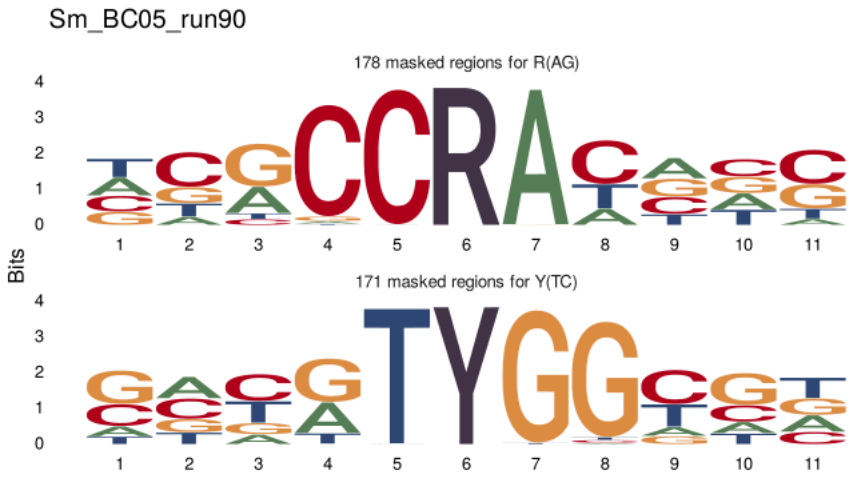
Supplementary Tables

Supplementary Table 1: Sequence logos of observed sequence pattern around the ambiguous bases R and Y on the chromosomal contig for different species based on one sample.

Species	Motif type
Acinetobacter junii	
Acinetobacter radioresistens	

Chryseobacterium gleum	
Citrobacter freundii	
Enterococcus faecalis	

Enterococcus faecium	
Escherichia flexneri	
Klebsiella pneumoniae	

Serratia marcescens	

Supplementary Table 2: Evaluation of errors in reads for different species. Native reads (SQK-NBD114.24) and PCR reads (SQK-RPB114.24) were compared against the same native genome. Only the most problematic observed ambiguous positions, R and Y, within the chromosome, are included in this table. The evaluation was done using MPOA workflow.

	native reads (SQK-NBD114.24)		PCR reads (SQK-RPB114.24)	
	R (A or G)	Y (T or C)	R (A or G)	Y (T or C)
Klebsiella pneumoniae (UR2602)	260	241	0	0
*Klebsiella pneumoniae (*VA13414)	257	234	1	0
Klebsiella pneumoniae (BK12739)	244	243	0	0
Klebsiella pneumoniae (VA18342)	247	230	0	0
Klebsiella pneumoniae (VA23130)	111	99	3	3
Klebsiella pneumoniae (BK13728)	53	55	0	1
Klebsiella pneumoniae (UR14350)	14	5	0	0
Klebsiella pneumoniae (D411554)	11	12	3	3
Klebsiella pneumoniae (VA13324)	13	9	0	1
Klebsiella pneumoniae (TP3419)	14	5	12	17
Klebsiella aerogenes (BC05_run91)	22	15	1	1
Shewanella algae (BC11_run90)	40	34	0	0
Acinetobacter soli (BC11_run91)	288	239	0	0
Acinetobacter junii (BC12_run91)	42	44	1	2
Enterobacter hormaechei (BC21_run89)	345	333	79	69
Citrobacter freundii (BC22_run90)	22	29	57	64

Supplementary Table 3: Evaluation of errors in reads for different species, prepared with predecessor sequencing kit SQK-LSK109. Only the ambiguous positions on the chromosomes are considered in this table. The evaluation was done using MPOA workflow.

Species	N(ATCG)	W(AT)	S(CG)	M(AC)	K(TG)	R(AG)	Y(TC)
Achromobacter bronchisepticus	408	0	0	0	0	4	4
Acinetobacter baumannii	92	1	2	1	1	63	50
Acinetobacter baumannii	601	1	0	0	0	42	53
Acinetobacter baumannii	23	3	0	0	1	36	27
Acinetobacter baumannii	10381	0	0	0	1	3	4
Acinetobacter baumannii	1615	0	0	0	0	39	36
Acinetobacter baumannii	1936	0	0	0	0	1	1
Acinetobacter baumannii	132	1	0	0	0	3	2
Acinetobacter baumannii	3569	0	0	0	0	0	2
Chryseobacterium bernardetii_A	18413	19	6	8	13	27	26
Chryseobacterium indologenes	14	2	0	0	1	20	16
Elizabethkingia anophelis	29	2	0	3	2	7	1
Elizabethkingia anophelis	136	5	0	0	0	4	5
Enterobacter asburiae	10	0	0	0	0	4	1
Enterobacter cloacae	5	0	0	0	1	83	55
Enterobacter ludwigii	2	0	0	0	0	0	3
Escherichia coli	45	0	0	0	0	2	3
Escherichia coli	67	15	33	17	15	50	47
Escherichia coli	19	0	0	0	0	27	34
Escherichia coli	24736	0	0	0	0	0	1
Escherichia flexneri	10	1	0	0	0	20	32
Klebsiella pneumoniae	0	0	0	0	0	2	1
Klebsiella pneumoniae	13	1	0	0	0	27	20
Klebsiella pneumoniae	4	1	0	0	0	7	4
Klebsiella pneumoniae	6974	0	0	0	1	7	5
Klebsiella quasipneumoniae	1210	0	0	0	2	1	3
Pseudomonas aeruginosa	18871	0	0	0	0	28	18
Pseudomonas aeruginosa	958	1	51	2	11	35	30
Pseudomonas aeruginosa	41	0	10	0	5	12	12
Pseudomonas aeruginosa	21	1	8	4	0	34	38

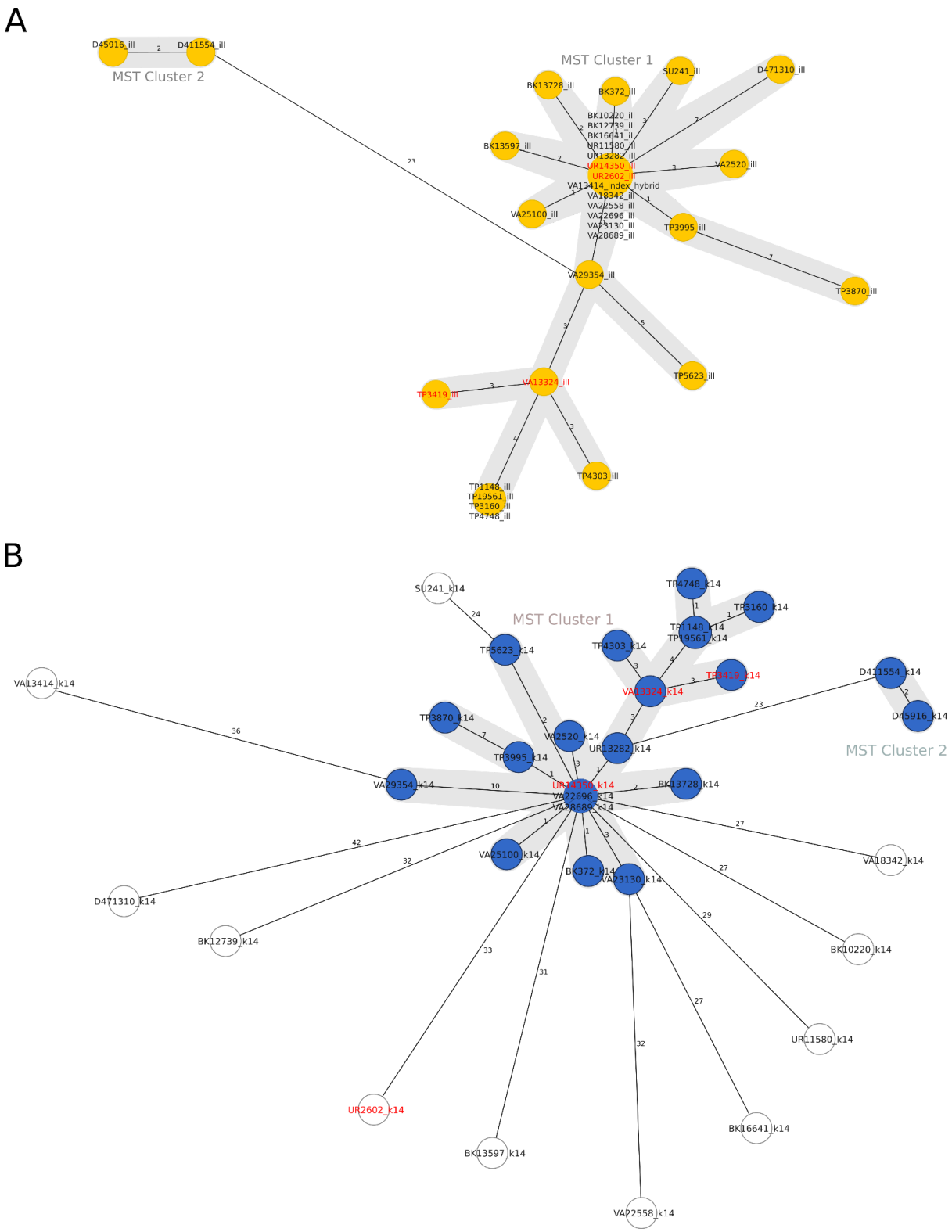
Species	N(ATCG)	W(AT)	S(CG)	M(AC)	K(TG)	R(AG)	Y(TC)
Pseudomonas aeruginosa	16022	0	1	0	0	19	34
Pseudomonas aeruginosa	15763	0	0	0	2	14	5
Pseudomonas aeruginosa	28	0	0	0	0	2	2
Pseudomonas aeruginosa	7612	0	0	1	6	12	13
Pseudomonas aeruginosa	10	0	0	1	0	11	13
Pseudomonas aeruginosa	129	0	0	1	0	10	6
Salmonella enterica	312	0	0	0	0	2	1
Serratia marcescens	31	0	0	0	0	15	17
Serratia marcescens	596	0	0	0	0	5	4
Serratia marcescens	43	1	0	0	1	7	8
Serratia marcescens	3	0	0	0	0	1	9

Supplementary Table 4: Comparison of errors in reads for 11 different species when basecalled with different models and basecaller. Only the most problematic observed ambiguous positions, R and Y, within the chromosome, are included in this table. The evaluation was done using MPOA workflow.

	Guppy (*dna_r10.4.1_e8.2_260bps_sup.cfg*)		Dorado (*res_dna_r10.4.1_e8.2_400bps_sup.cfg*)	
Species	R(AG)	Y(TC)	R(AG)	Y(TC)
Pseudomonas aeruginosa	49	54	15	14
Pseudomonas aeruginosa	1091	1189	896	791
Pseudomonas aeruginosa	1	1	1	0
Pseudomonas aeruginosa	138	100	37	38
Pseudomonas aeruginosa	0	1	0	0
Pseudomonas aeruginosa	138	136	61	51
Pseudomonas aeruginosa	0	0	0	0
Pseudomonas aeruginosa	33	40	9	14
Pseudomonas aeruginosa	0	0	0	0
Pseudomonas aeruginosa	111	99	52	48
Pseudomonas aeruginosa	33	40	9	14
Klebsiella pneumoniae	47	42	17	22

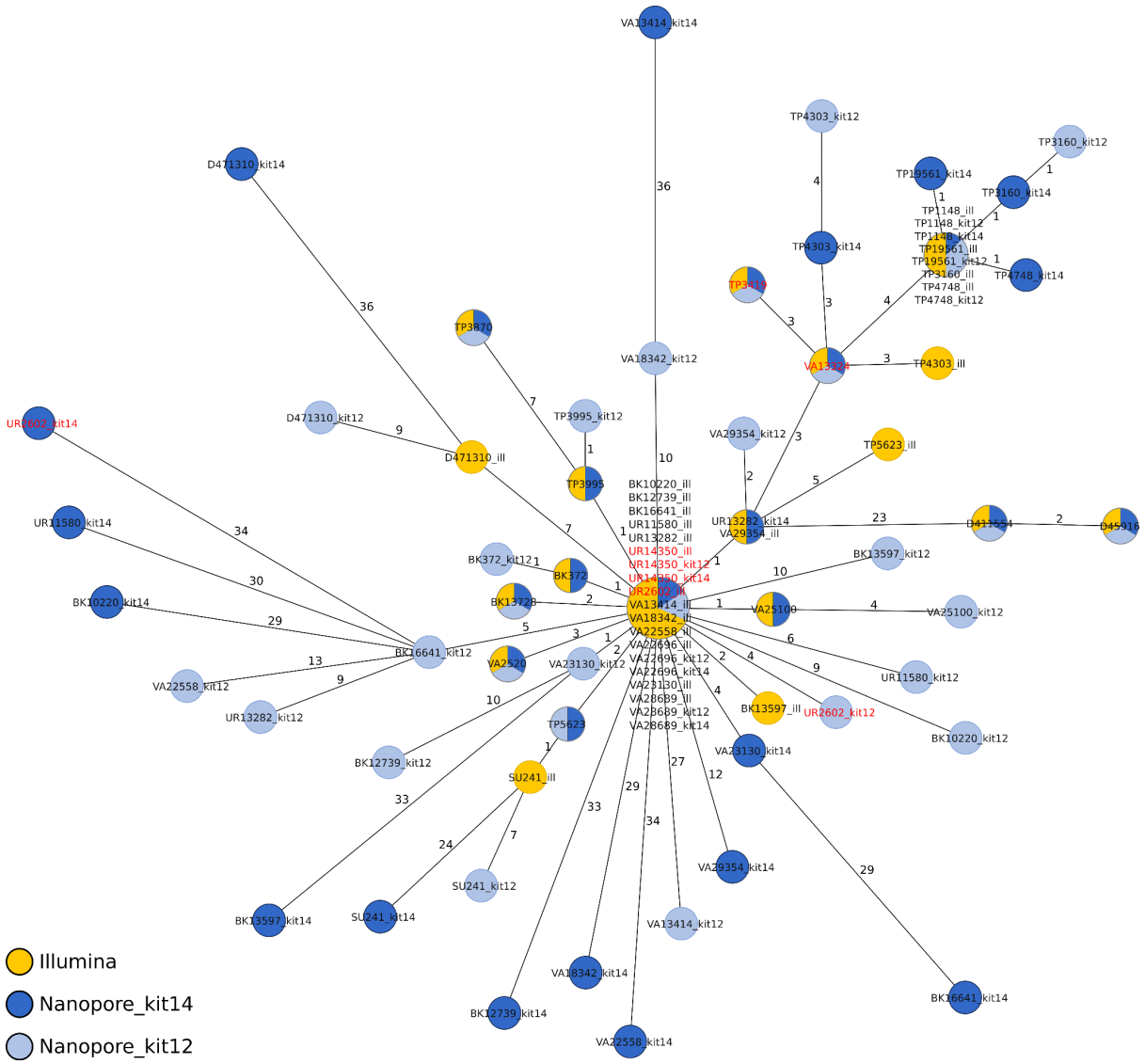
Klebsiella pneumoniae	54	40	22	12
Acinetobacter baumannii	1	0	0	0
Acinetobacter baumannii	10	9	2	1
Achromobacter xylosoxidans	6	4	1	0
Citrobacter freundii	48	38	5	10
Enterobacter hormaechei	4	6	3	1
Escherichia flexneri	85	86	32	29
Klebsiella_A oxytoca	5	9	1	1
Klebsiella_A michiganensis	22	29	18	19
Salmonella enterica	82	85	23	19
Stenotrophomonas maltophilia	228	167	198	170

Supplementary Figures

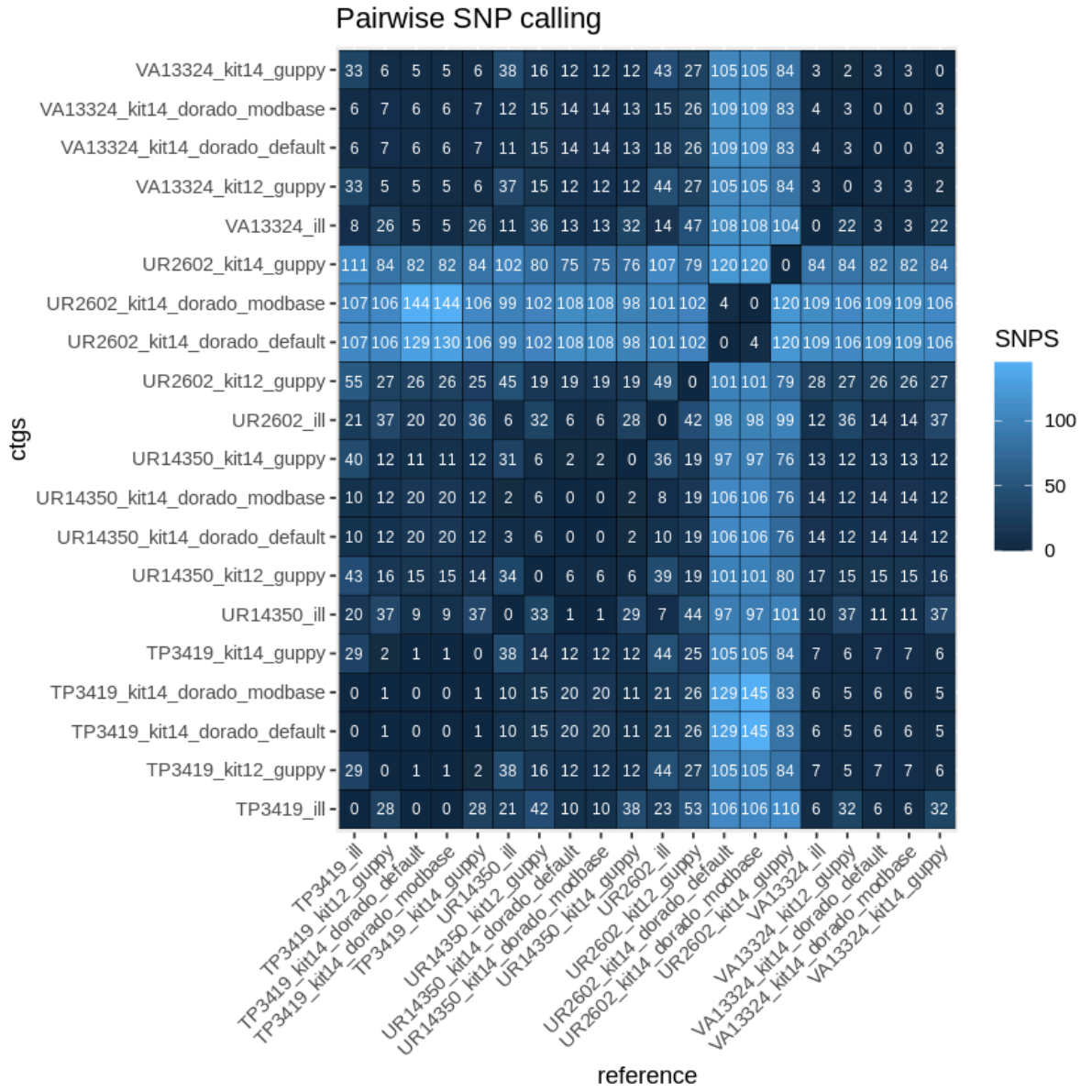


Supplementary Figure 1: Both Minimal spanning trees (pairwise ignore missing values showing) based on 2358 genes of each 33 *K. pneumoniae* outbreak samples. Samples used in the manuscript for Figure 1 are highlighted in red. Samples with allelic differences ≤15 are

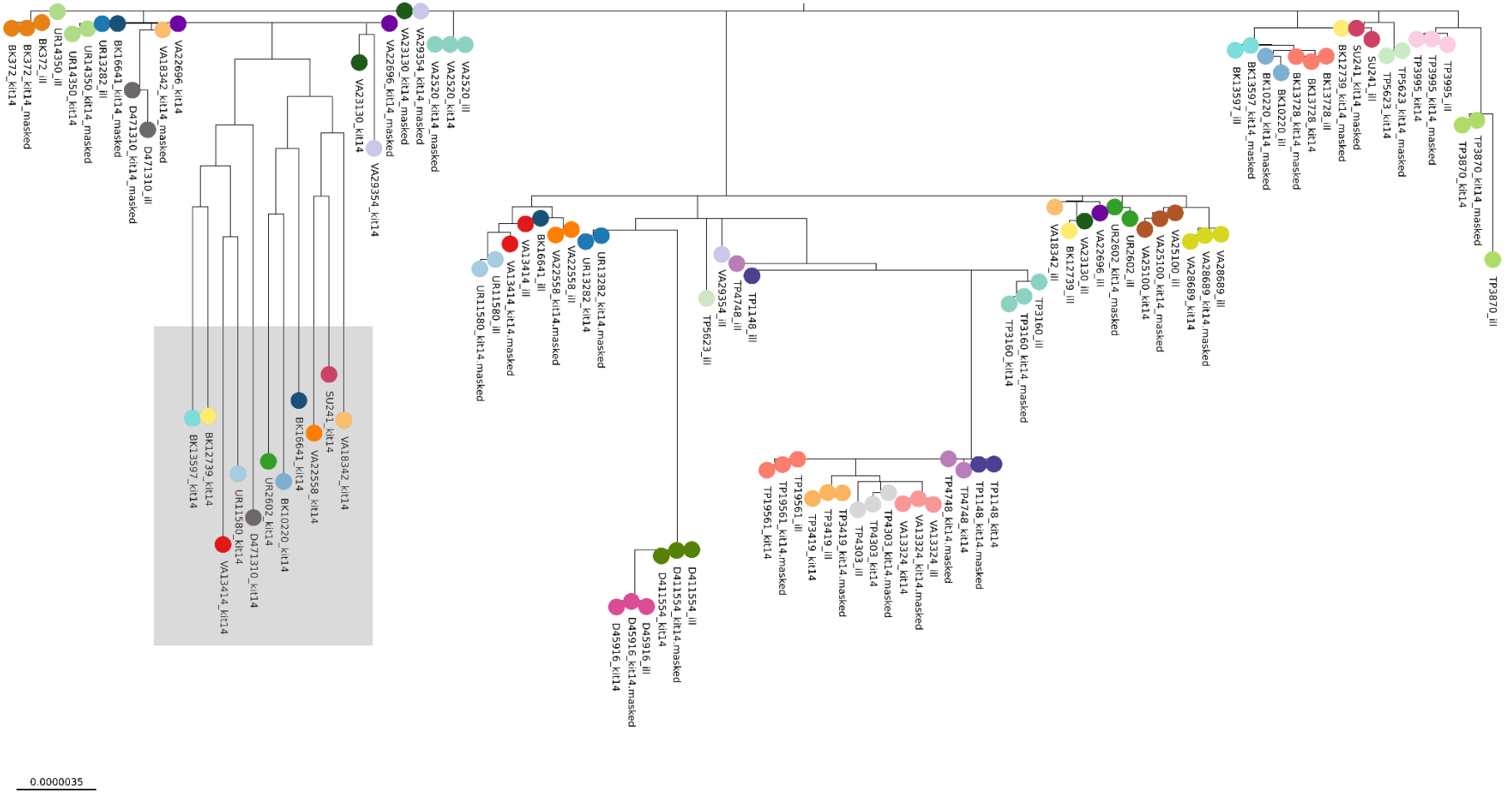
considered as part of the cluster. **A:** Assemblies built from Illumina sequencing data (yellow). **B:** Assemblies built from Nanopore Kit14 sequencing data (blue). Outlier samples not in the outbreak cluster and showing significant differences from the Illumina data are shown in white. Nodes (samples) are connected by lines depicting the distance by numbers of allelic differences. Loci are considered different if one or more bases change between the samples. Loci without allelic differences are described as being the same.



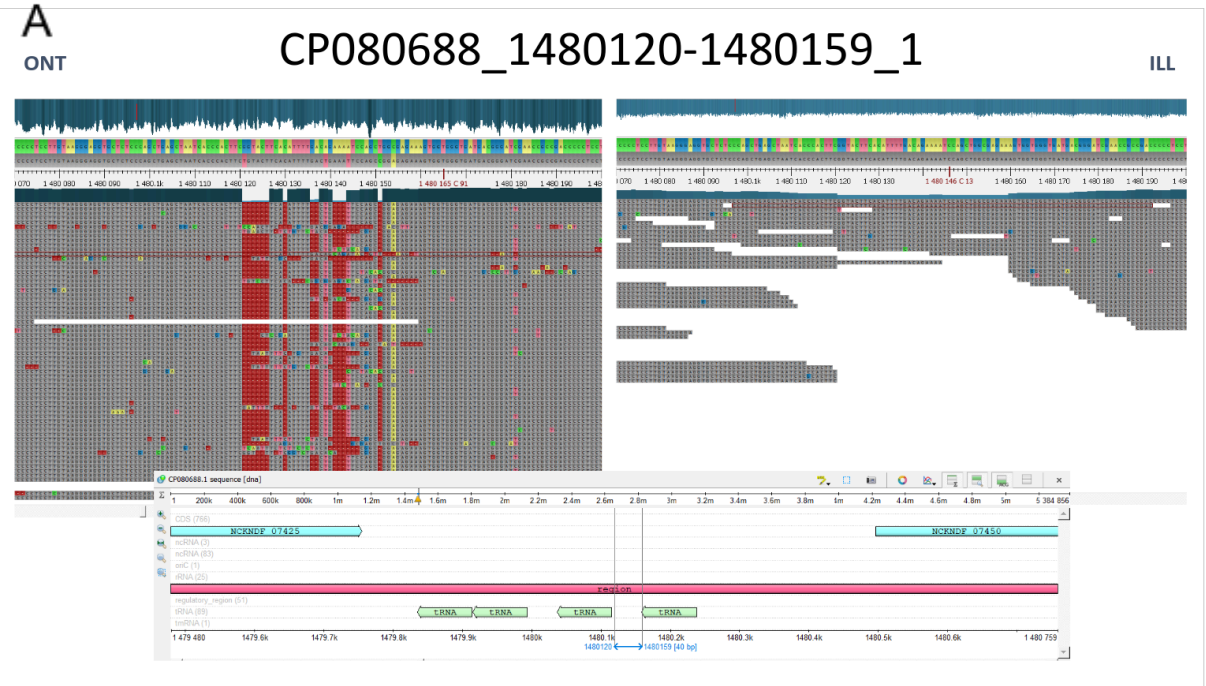
Supplementary Figure 2: Minimum spanning trees (pairwise ignore missing values showing) of each 33 *K. pneumoniae* outbreak samples based on 2358 genes to compare the allelic variations between Illumina genomes, Nanopore SQK-NBD114.24 (kit14) and SQK-NBD114.24 (kit12). Samples used in the manuscript for Figure 1 are highlighted in red. in Nodes (samples) are connected by lines depicting the distance by numbers of allelic differences. Loci are considered different if one or more bases change between the samples. Loci without allelic differences are described as being the same. Nodes are colored according to the sequencing technology used. Samples with allelic differences ≤15 are considered as part of the cluster.

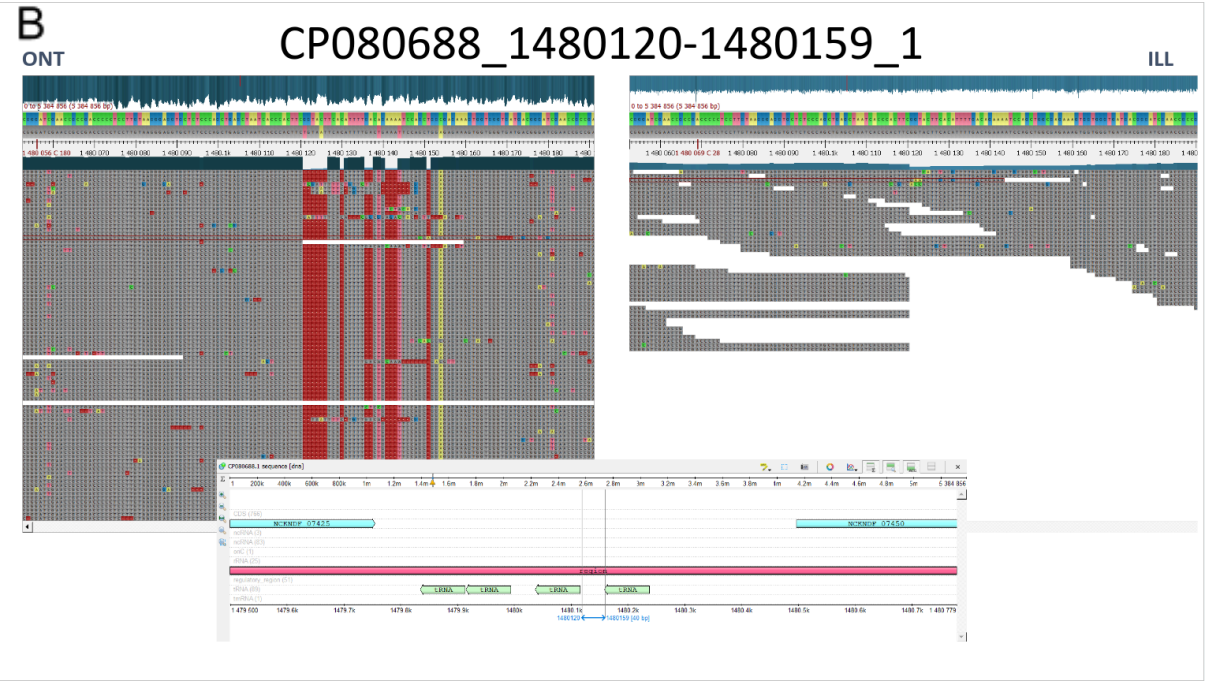


Supplementary Figure 3: Heatmap of pairwise SNP comparison of four genomes based on Snippy analysis. The four isolates used were each prepared with Illumina (gold standard and reference), Kit 14, and Kit 12 and basecalled with each respective Guppy “super accurate” basecalling model (see methods “Basecalling and Assembly”). All Kit 14-prepared isolates were additionally basecalled with Dorado using the default and a modification-aware model.

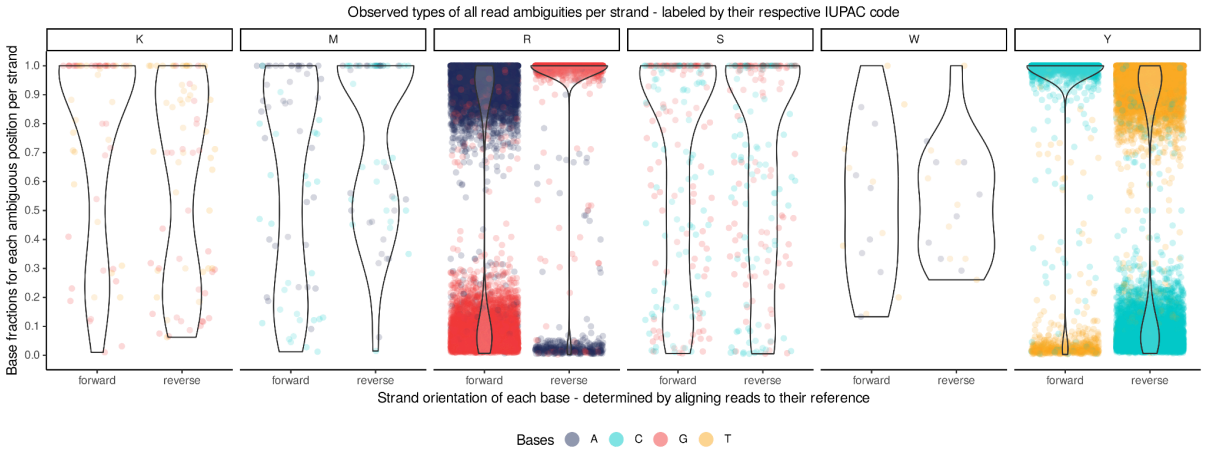


Supplementary Figure 4: Phylogenetic tree based on core genome SNP alignment to figure the genetic distances between 33 *K. pneumoniae* outbreak samples (colored nodes), sequenced using Illumina (ill) and Nanopore SQK-NBD114.24 (kit14) compared to the masked Kit 14 assemblies (masked). Detected outliers are highlighted in grey.

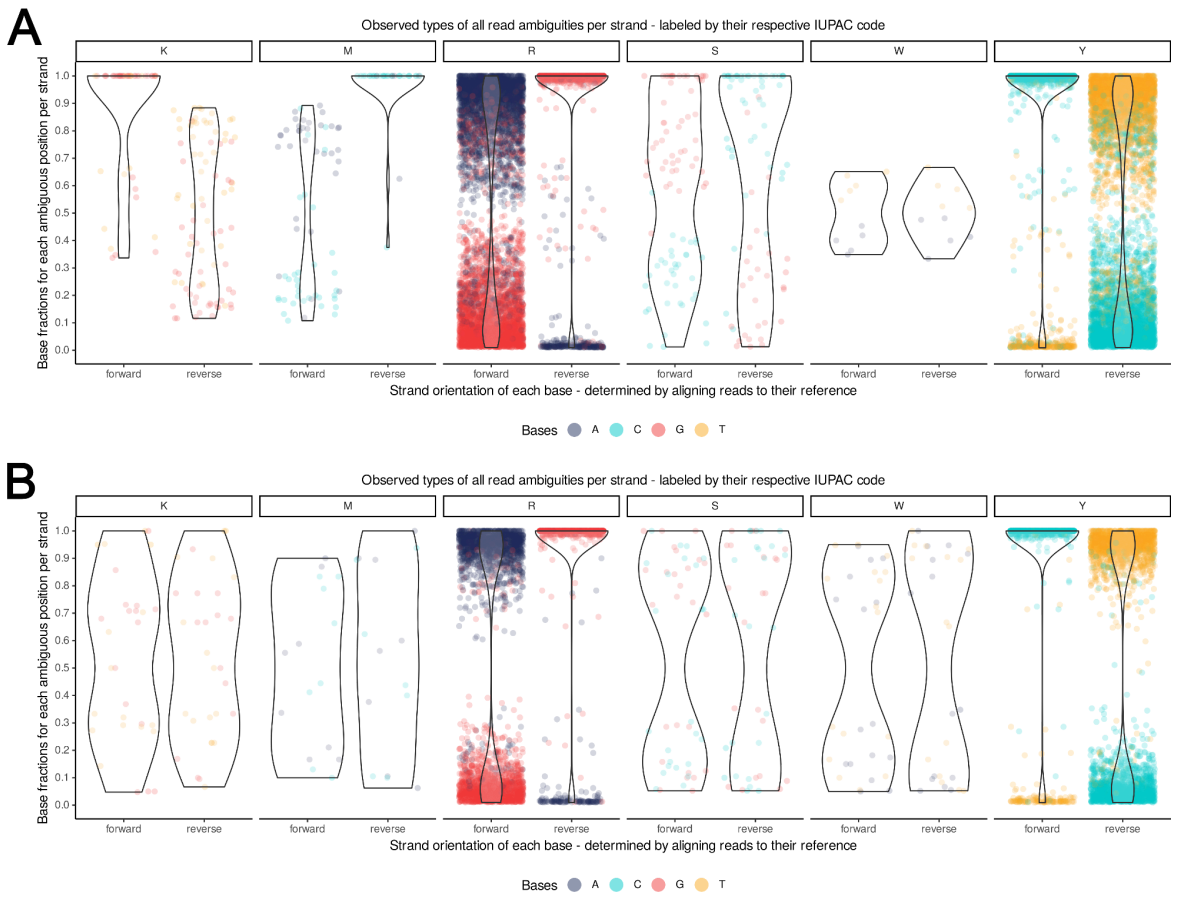




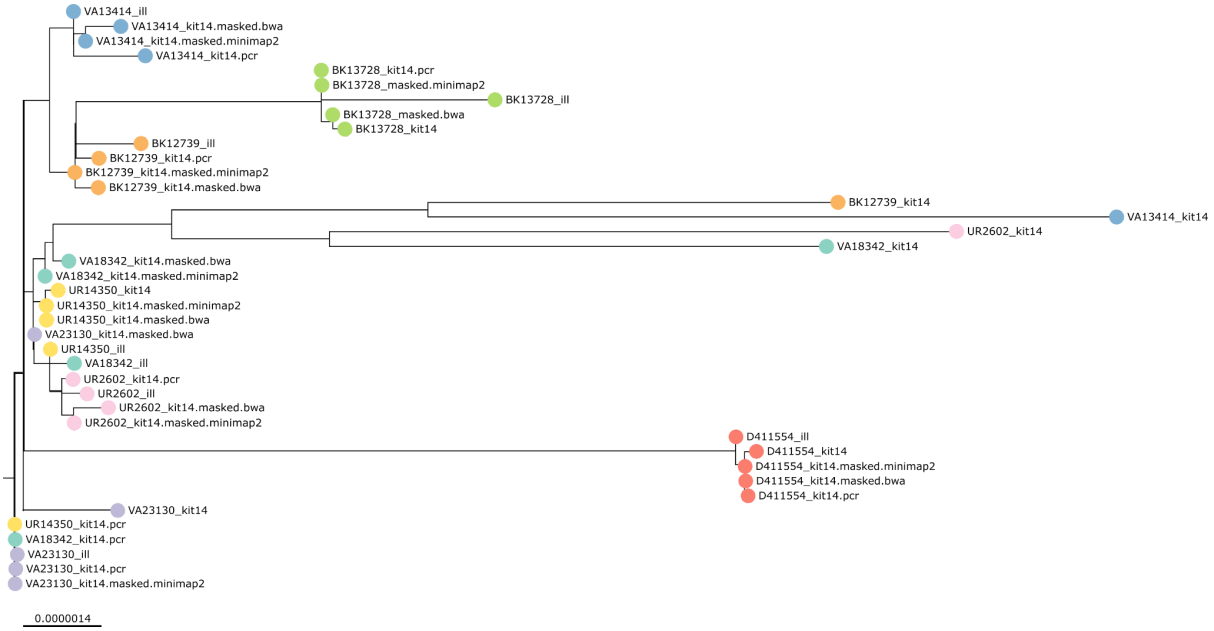
Supplementary Figure 5: Identified errors in Illumina Assemblies by mapped reads of the same sample and sequence region Nanopore (left) and Illumina (right) against Illumina Hybrid Assembly of Index patients in the position 1480120-1480159 on the chromosome. **A:** VA28689 **B**: BK16641.



Supplementary Figure 6: Violin chart showing the ratio between two bases within the mapped read data distinguished by strand orientation for 19 *Pseudomonas aeruginosa* samples.



Supplementary Figure 7: Mapping strategies - minimap2 vs bwa Frequency plot over minimap2 (A) and bwa (B) mapped reads on polished assembly.



Supplementary Figure 8: Mapping strategies - minimap2 vs BWA Phylogenetic tree based on core genome SNP alignment to figure the genetic distances between eight *K. pneumoniae* outbreak samples (colored nodes), prepared with Illumina (ill), Nanopore SQK-NBD114.24 (kit14) and SQK-RPB114.24 (pcr) compared to masked Kit 14 assemblies based on minimap2 mapping (masked.minimap2) and masked Kit 14 assemblies based on BWA mapping (masked.bwa).

Supplementary Code

Supplementary Code 1: Used Code for Assembly, Polishing and phylogenetic Analysis

```
flye --plasmids --meta -t ${task.cpus} --nano-hq ${read} -o
assembly
minimap2 -x map-ont -t ${task.cpus} ${assembly} ${read} >
${name}.paf
minimap2 -ax map-ont ${assembly} ${read} | samtools view -bS - |
samtools sort -@ ${task.cpus} - > ${name}_${technology}_sorted.bam
racon -t ${task.cpus} ${read} ${mapping} ${assembly} >
${name}_consensus.fasta
medaka_consensus -i ${read} -d ${consensus} -o polished -t
${task.cpus} -m \${POLISHMODEL}
FastTree -gtr -nt ${clean_core_alignment} > clean.core.tree.nwk
for fasta in genomes/*
      do
                FILENAME=\$(basename \${fasta%.*})
                snippy --cpus ${task.cpus} --ram ${maxmemory}
--outdir ${cluster_ID}/\${FILENAME}/ --ref reference/* --ctgs
\${fasta}
          done
snippy-core --ref reference/* --prefix results ${cluster_ID}/*/
```