Genomic DNA Extraction

JANUS Chemagic Workstation & Chemagic Viral DNA/RNA Kit (PerkinElmer)
1-6

GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich)
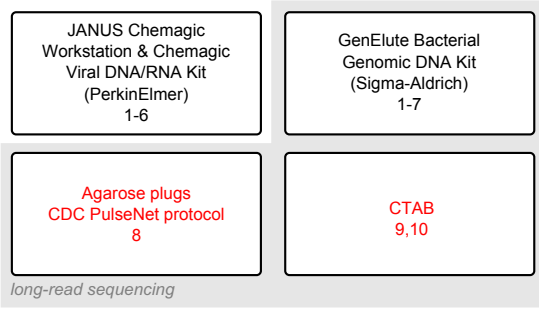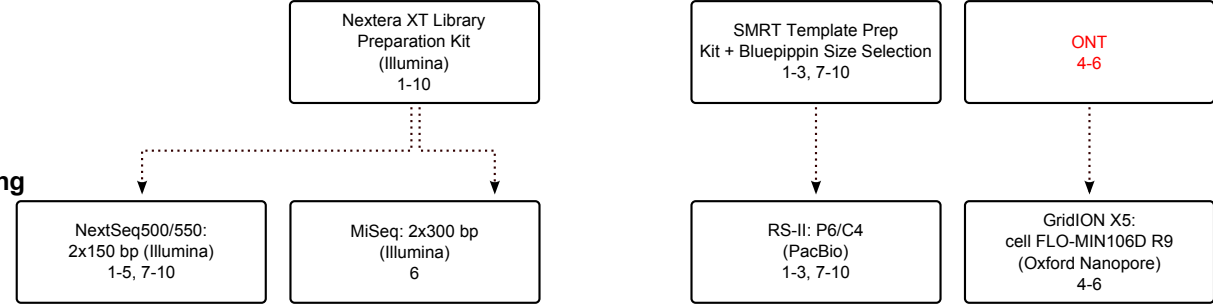1-7

Agarose plugs CDC PulseNet protocol
8

CTAB
9,10

long-read sequencing

Assembly Groups
1: *L. monocytogenes* (*224, *235, *7774); N. gonorrhoeae (*10541); N. meningitidis (*5726); S. enterica subsp. enterica Birkenhead (*10532), Enteritidis (*10527, *10528), Typhimurium (*10530), Virchow (*10533); S. pyogenes (*10539)
2. E. coli (*2545); S. enterica subsp. enterica Typhimurium (*10529), S. pneumoniae (*10538)
3. S. enterica subsp. enterica Hvittingfoss (*5056)
4. E. faecium (*4167); E.coli (*14361); S. flexneri (*10535)
5. S. enterica subsp. enterica Saintpaul (*10531), Typhimurium (*8979)
6. K. pneumoniae (*8079)
7. N. meningitidis (*10537)
8. L. pneumophila (*10536);
9. M.chimaera (*7395)
10. M. tuberculosis (*18547)

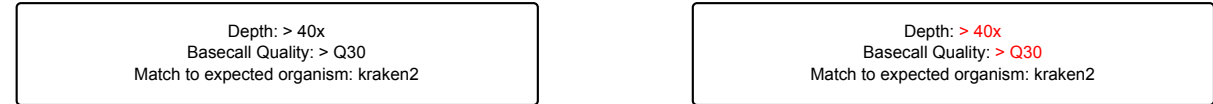## Library Construction

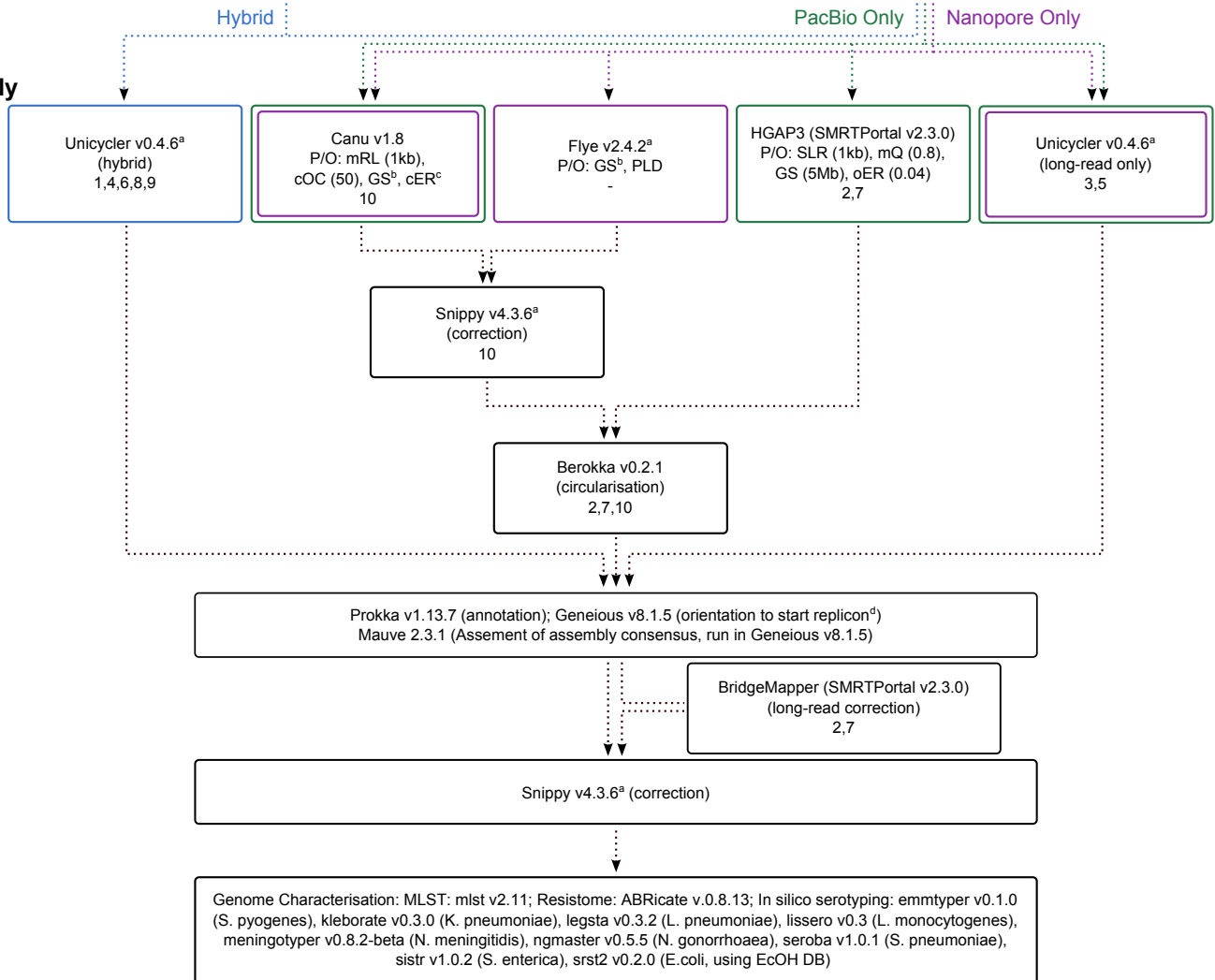Nextera XT Library Preparation Kit (Illumina)
1-10

SMRT Template Prep Kit + Bluepippin Size Selection
1-3, 7-10

ONT
4-6

Sequencing

NextSeq500/550: 2x150 bp (Illumina)
1-5, 7-10

MiSeq: 2x300 bp (Illumina)
6

RS-II: P6/C4 (PacBio)
1-3, 7-10

GridION X5: cell FLO-MIN106D R9 (Oxford Nanopore)
4-6

Quality Control

Depth: > 40x
Basecall Quality: > Q30
Match to expected organism: kraken2

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Hybrid   PacBio Only   Nanopore Only

Assembly

Unicycler v0.4.6[a] (hybrid)
1,4,6,8,9

Canu v1.8
P/O: mRL (1kb), cOC (50), GS[b], cER[c]
10

Flye v2.4.2[a]
P/O: GS[b], PLD
-

HGAP3 (SMRTPortal v2.3.0)
P/O: SLR (1kb), mQ (0.8), GS (5Mb), oER (0.04)
2,7

Unicycler v0.4.6[a] (long-read only)
3,5

Snippy v4.3.6[a] (correction)
10

Berokka v0.2.1 (circularisation)
2,7,10

Prokka v1.13.7 (annotation); Geneious v8.1.5 (orientation to start replicon[d])
Mauve 2.3.1 (Assement of assembly consensus, run in Geneious v8.1.5)

BridgeMapper (SMRTPortal v2.3.0) (long-read correction)
2,7

Snippy v4.3.6[a] (correction)

Genome Characterisation: MLST: mlst v2.11; Resistome: ABRicate v.0.8.13; In silico serotyping: emmtyper v0.1.0 (S. pyogenes), kleborate v0.3.0 (K. pneumoniae), legsta v0.3.2 (L. pneumoniae), lissero v0.3 (L. monocytogenes), meningotyper v0.8.2-beta (N. meningitidis), ngmaster v0.5.5 (N. gonorrhoaea), seroba v1.0.1 (S. pneumoniae), sistr v1.0.2 (S. enterica), srst2 v0.2.0 (E.coli, using EcOH DB)

Abbreviations and Footnotes: [a] multiple versions utilised (Unicycle: v0.4.6, v0.4.8-beta; Flye v2.4.2, v2.6-release; Snippy: v4.3.6, v4.3.7, v4.4.0, v4.4.5) P/O = parameters/options (that differ from default); mRL = minimum read length; cOR = corOutCoverage (Canu); GS = genome size ([b] set as Mb closest to species average); cER = correctedErrorRate (Canu, [c] set as 0.144 for Nanopore or 0.045 for PacBio); PLD = plasmid (Flye); SLR = seed read length; mQ = minimum read quality; oER = overlapper error rate; [d] start replicon was *dnaA* for chromosome and *rep* for plasmid based on prokka annotation.