PATRIC Bioinformatics Resource Center

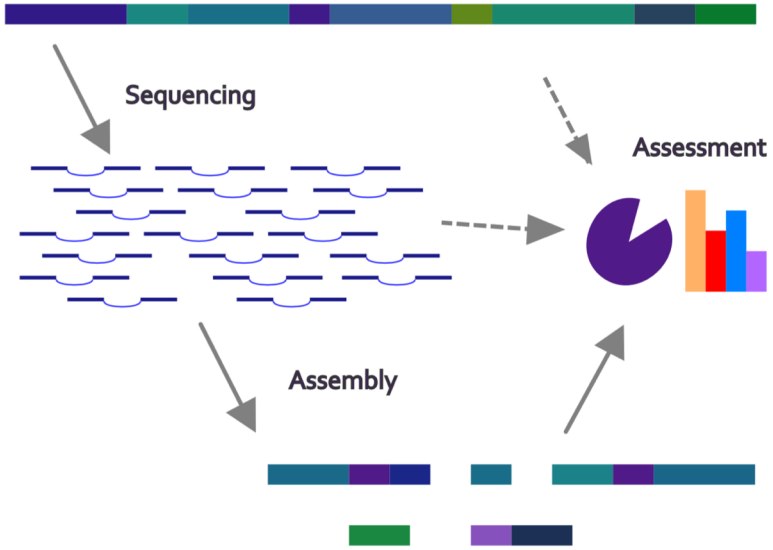
Genome Assembly in PATRIC

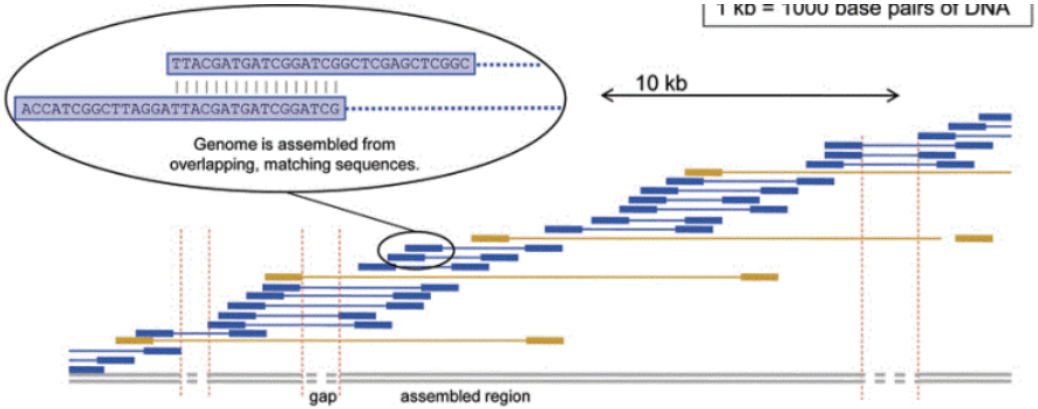
Presented by Neal Conrad

Slides originally by Fangfang Xia

The Sequence Assembly problem

- Reconstructing contiguous DNA regions (contigs) from a set of short sequences (reads)





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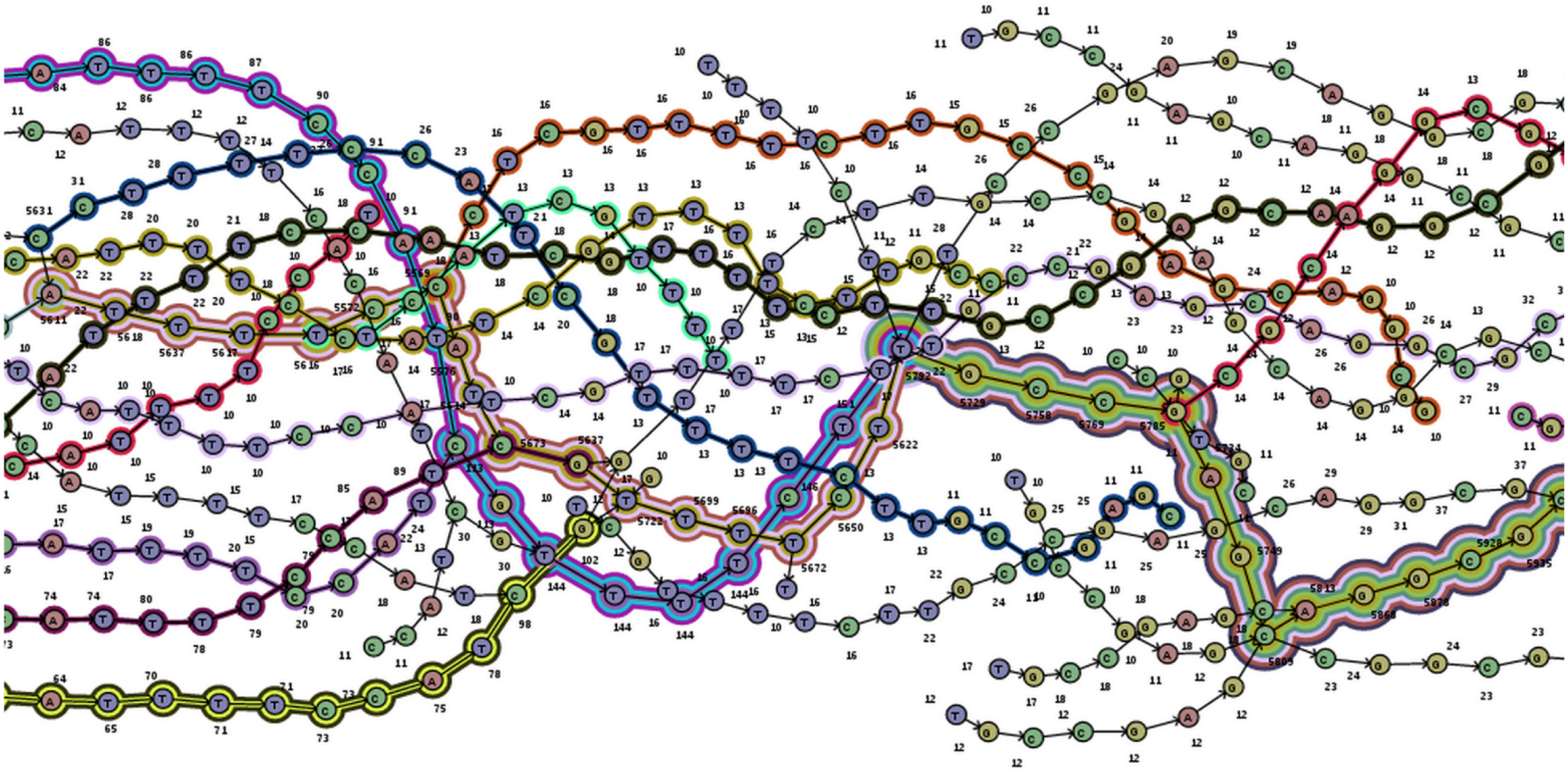
An incomplete list of assemblers

Abyss, AllPaths, AllPaths-LG, AMOS, Arapan-M, Arapan-S, Celera, CLC, Clustgun, Cortex, Discovar, DNA Baser, Dragon, Edena, Euler, Euler-sr, FERMI, Forge, Geneious, Graph Constructor, HGAP, IDBA, IDBA-UD, Kiki, Meta-velvet, Minia, MIRA, NextGENe, Newbler, PADENA, PASHA, Phrap, Ray, Ray-meta, REAPR, Sequencher, SeqMan, SGA, SHARCGS, SOPRA, SSAKE, SOAPdenovo, SPAdes, Staden, Taipan, TIGR, VCAKE, Phusion, QSRA, Velvet, YAGA

Assembly graph



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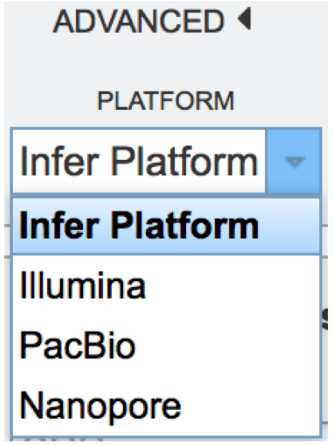
"Assemblers should take in your data and automatically do the best possible job with it."

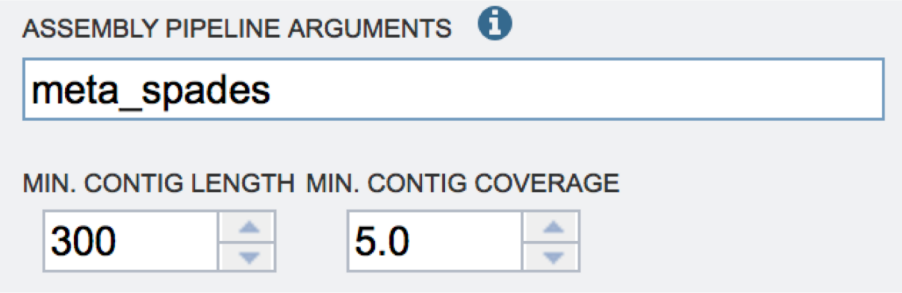
– A reviewer for Assemblathon

Common Assembly Scenarios

- *Short read assembly* — Illumina sequencers
- *Long read assembly* — PacBio, Oxford Nanopore
- *Hybrid assembly* — Short reads + long reads
  - Short for assembly + long for scaffolding (spades)
  - Long for assembly + short for finetuning (auto; planned)



- *Whole genome assembly*
- *Plasmid assembly*
- *Metagenome assembly*



- *De novo assemby vs reference-guided assembly*
  - lib.consensus.fa output in variation analysis

ASSEMBLY SERVICE

COMPUTE CAPABILITIES

PREPROCESSING

- SolexaQA
- Length Filtering
- SGA: Q-trim / Q-filter
- TagDust: Adapter Removal
- BayesHammer EC
- SGA: Error Correction
- ...

ASSEMBLY

- Kiki
- SPAdes
- SGA
- MaSuRCA
- Discovar
- IDBA-UD
- Velvet
- A5
- ...

POST-ASSEMBLY

- SSPACE:Scaffold
- REAPR: Break
- GAM-NGS Merge
- ...

ALIGNMENT

- BWA
- Bowtie2
- ...

EVALUATION

- ALE
- REAPR
- QUAST

VISUALIZATION

- Nx Plots
- Contig Length
- ALE Comparison
- Benchmarks

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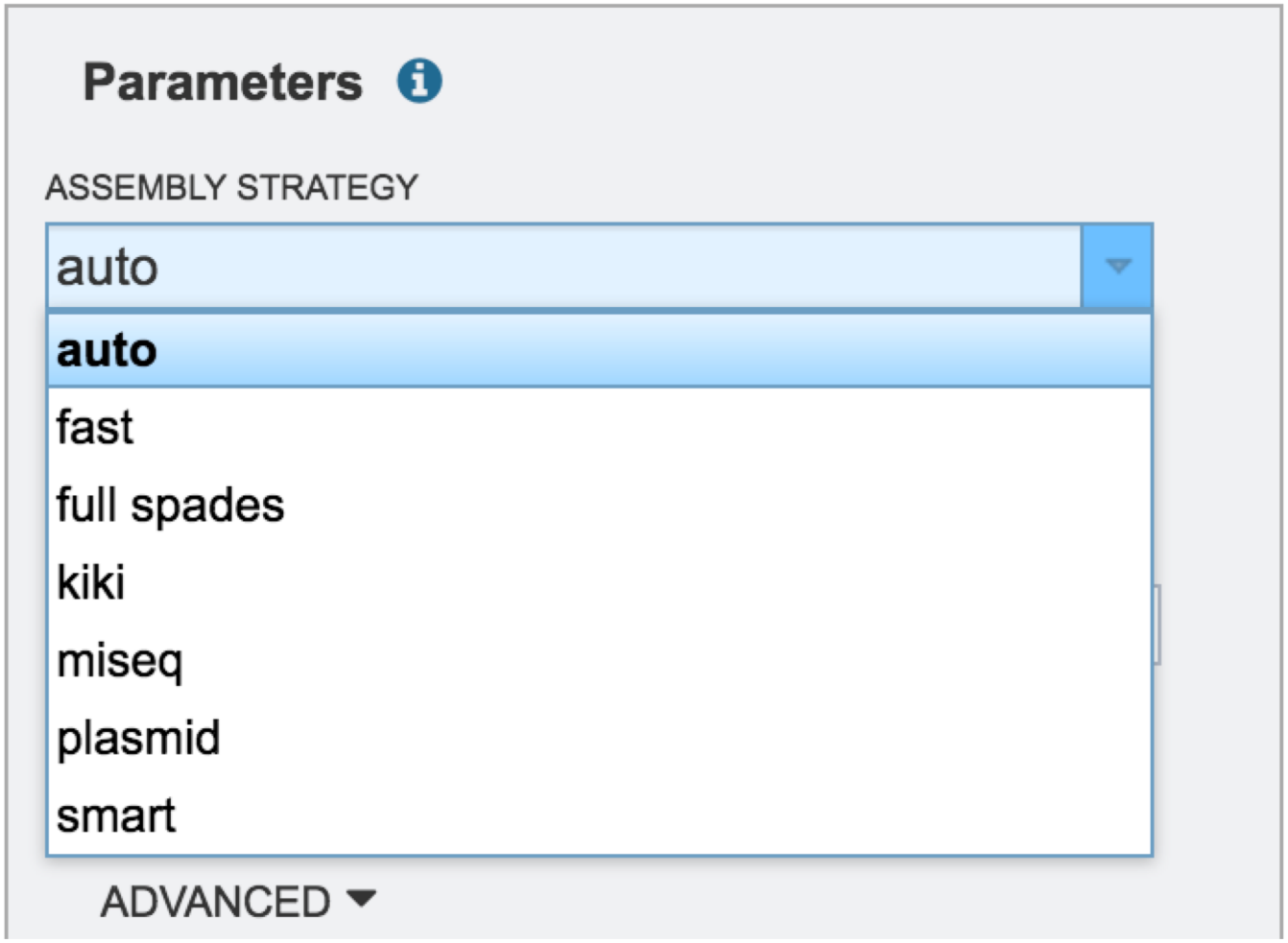
Curated assembly strategies



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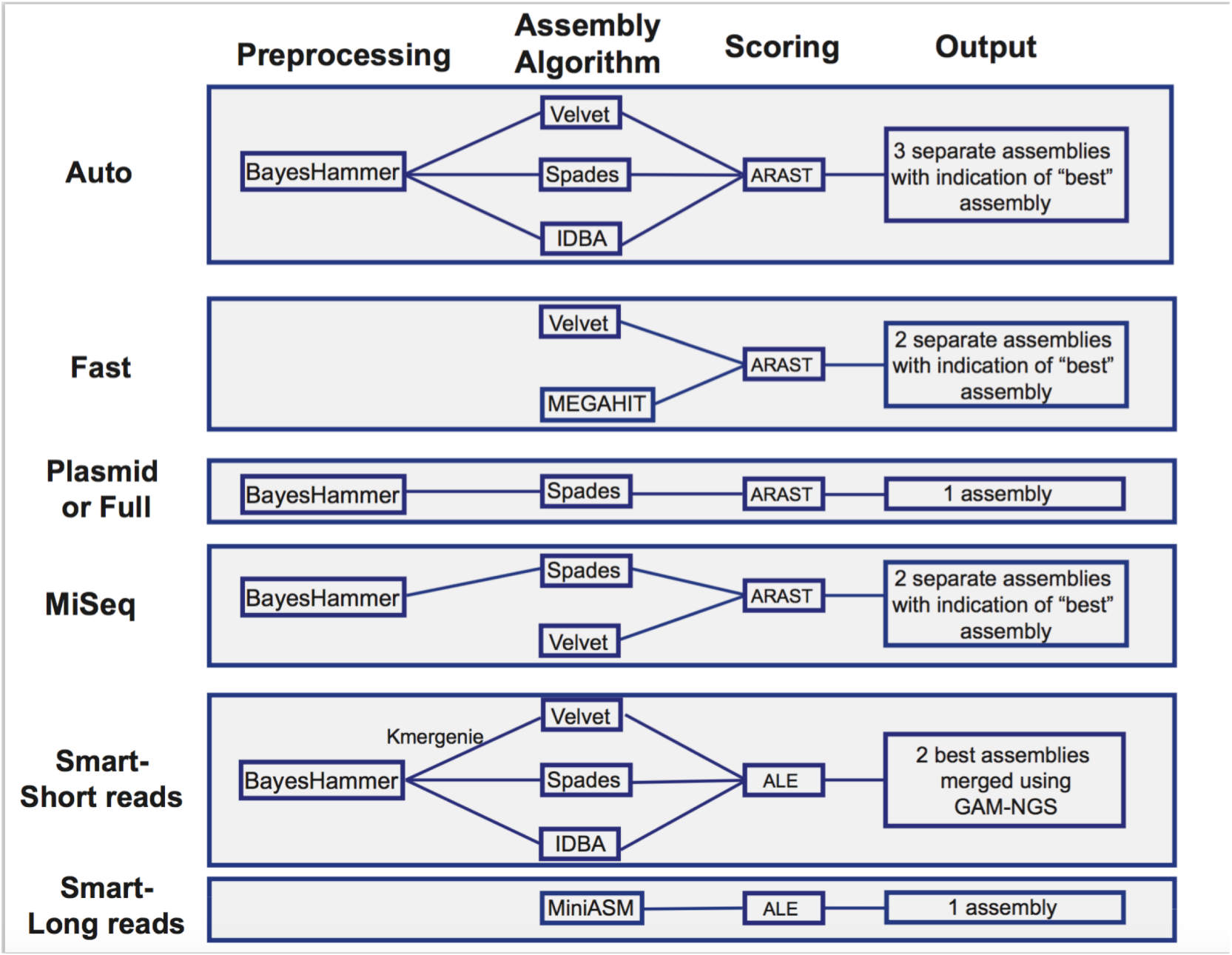
Preprocessing
Assembly Algorithm
Scoring
Output
Auto
BayesHammer
Velvet
Spades
IDBA
ARAST
3 separate assemblies with indication of "best" assembly
Fast
Velvet
MEGAHIT
ARAST
2 separate assemblies with indication of "best" assembly
Plasmid or Full
BayesHammer
Spades
ARAST
1 assembly
MiSeq
BayesHammer
Spades
Velvet
ARAST
2 separate assemblies with indication of "best" assembly
Smart-Short reads
Kmergenie
BayesHammer
Velvet
Spades
IDBA
ALE
2 best assemblies merged using GAM-NGS
Smart-Long reads
MiniASM
ALE
1 assembly

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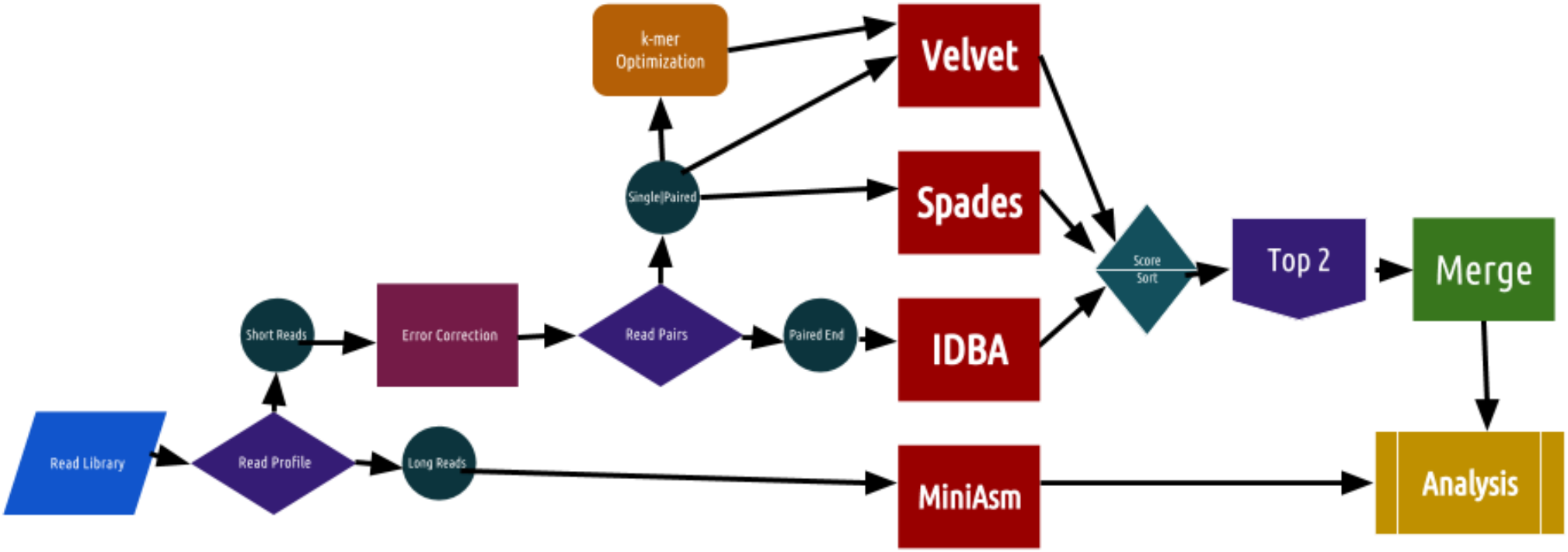
The “smart” assembly recipe



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Which assembly recipe to use

- ***auto*** — the evolving default strategy recommended for most data
- ***full spades*** – runs the full SPAdes pipeline, one of the best assemblers for microbial genomes
- ***fast*** — ~2X faster than *auto*; suited for large genomes or simple microbial communities (velvet + megahit)
- ***kiki*** — very fast but does not use paired end information; good for metagenome assembly
- ***miseq*** — good for Illumina MiSeq reads that are 250-350 bp long (Spades with more k-mer iterations)
- ***smart*** — the slowest and sometimes the most accurate
- ***plasmid*** —plasmid assembly (plasmidSPAdes)

Typical execution times

for a typical microbial genome

Recipe	Hours
smart	3 ~ 100
auto / miseq	2 ~ 80
fast	1 ~ 12
kiki	1 ~ 6

Depends on read depth, sequencing errors, genome size, repeat structure, etc.

Evaluate assembled contigs

QUAST report

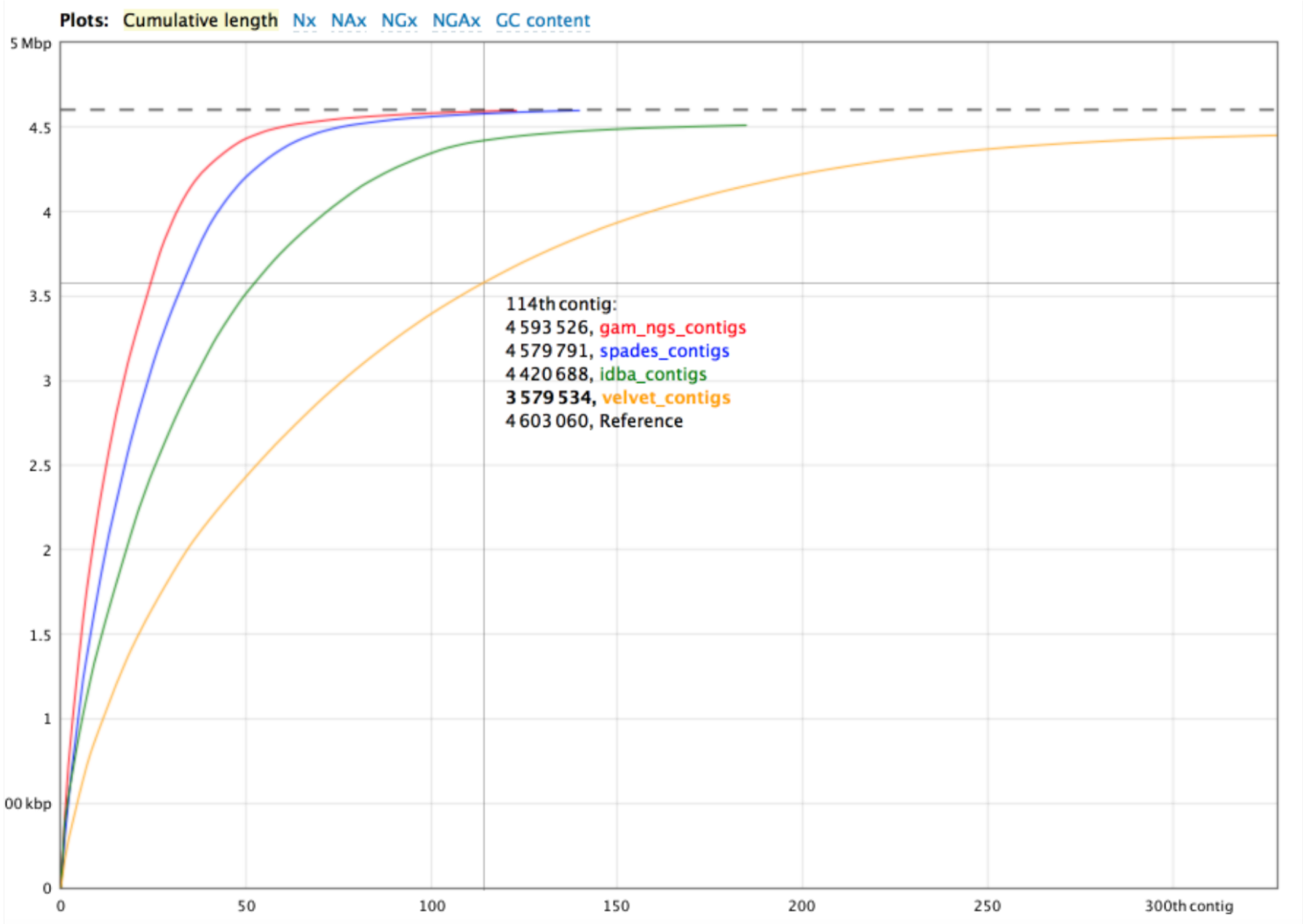
19 June 2014, Thursday, 08:19:51

All statistics are based on contigs of size >= 500 bp, unless otherwise noted (e.g., "# contigs (>= 0 bp)" and "Total length (>= 0 bp)" include all contigs.)

Extended report worst.....best

Genome: 4 603 060 bp, G+C content: 68.79%

Statistics without reference	gam_ngs_contigs	spades_contigs	idba_contigs	velvet_contigs
# contigs	123	140	185	328
Largest contig	399 330	292 708	378 933	160 339
Total length	4 598 516	4 598 552	4 511 207	4 451 511
N50	130 248	95 827	61 227	26 869
Misassemblies				
# misassemblies	6	6	1	29
Misassembled contigs length	153 182	150 167	24 718	876 583
Mismatches				
# mismatches per 100 kbp	16.080	16.52	6.08	12.69
# indels per 100 kbp	4.020	3.91	3.5	10.210
# N's per 100 kbp	0	0	0	373.58
Genome statistics				
Genome fraction (%)	98.898	98.895	97.953	96.39
Duplication ratio	1.01	1.01	1.001	1.003
NGA50	130 247	95 827	61 162	24 307
Predicted genes				
# predicted genes (unique)	4480	4501	4464	4695
# predicted genes (>= 0 bp)	4519	4540	4464	4695
# predicted genes (>= 300 bp)	3970	3984	3919	3989
# predicted genes (>= 1500 bp)	550	545	537	480
# predicted genes (>= 3000 bp)	44	44	38	34


Plots: Cumulative length Nx NAx NGx NGAx GC content
5 Mbp
4.5
4
3.5
3
2.5
2
1.5
1
00 kbp
0
0
50
100
150
200
250
300th contig
114th contig:
4 593 526, gam_ngs_contigs
4 579 791, spades_contigs
4 420 688, idba_contigs
3 579 534, velvet_contigs
4 603 060, Reference

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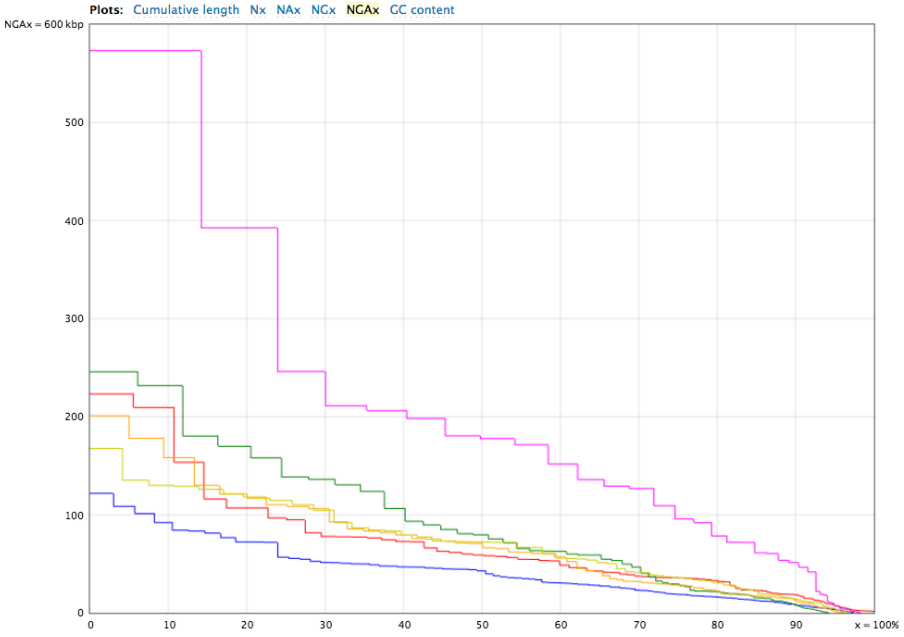
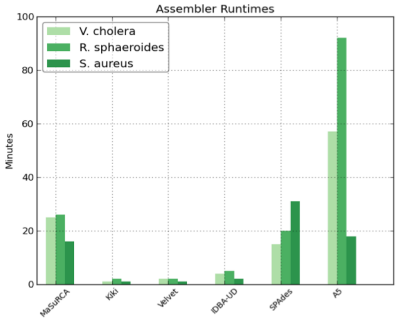
AssemblyRAST: Assembler Comparison

```
for LIB in $(ls)
do
  ar_run -f $LIB/rd*.fq -a masurca kiki velvet spades idba a5
done
```



Assembly	MaSurca	Kiki	Velvet	IDBA-UD	SPAdes
# contigs (>= 0 bp)	135	1228	733	301	316
# contigs (= 1000 bp)	125	1228	520	140	133
Total length (= 0 bp)	4454671	4211242	4604732	4550407	4661794
Total length (= 1000 bp)	4447475	4211242	4523850	4490529	4604439
# contigs	133	1228	587	173	161
Largest contig	159677	33373	56029	378946	230007
Total length	4453816	4211242	4572094	4512010	4625338
Reference length	4603060	4603060	4603060	4603060	4603060
GC (%)	68.86	68.55	68.68	68.81	68.82
Reference GC (%)	68.79	68.79	68.79	68.79	68.79
N50	74831	4406	14135	73097	71177
NG50	66418	3936	13982	72396	73118
N75	34138	2548	7489	42086	47310
NG75	31030	2088	7240	41189	47310
# misassemblies	7	32	14	3	9
# local misassemblies	12	5	1322	4	7
Unaligned contigs length	0	2760	117	0	55
Genome fraction (%)	95.315	90.776	95.681	97.712	99.048
Duplication ratio	1.016	1.006	1.036	1.005	1.017
# N's per 100 kbp	0.00	0.00	3249.32	0.00	8.76
# mismatches per 100 kbp	31.34	32.95	8.54	4.16	12.11
# indels per 100 kbp	5.54	6.99	23.91	3.58	5.75
Largest alignment	159677	33373	53699	378908	230007
NA50	74744	4285	12944	73097	67626
NGA50	66418	3893	12905	72357	71175
NA75	34138	2483	6437	41275	42056
NGA75	31030	2048	6263	37563	42056

Organism	MaSuRCA	Kiki	Velvet	IDBA	SPAdes	A5
B. cereus HiSeq*	52644	59995	42763	31347	**78420**	45935
S. aureus	22603	1854	11540	34957	**50888**	8188
V. cholera HiSeq	59028	42804	47191	70796	**177768**	72282
V. cholera MiSeq	50207	70738	19767	44178	198488	57376
R. sphaeriodes HiSeq	66418	3893	33342	**72357**	71175	20356*
R. sphaeriodes MiSeq	-	33589	62923	60228	**126502**	83693



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Interactive Demo

Acknowledgements

- PATRIC Team:
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    - Rick Stevens
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  - Stevens Group
    - Chris Bun
    - Sebastien Boisvert

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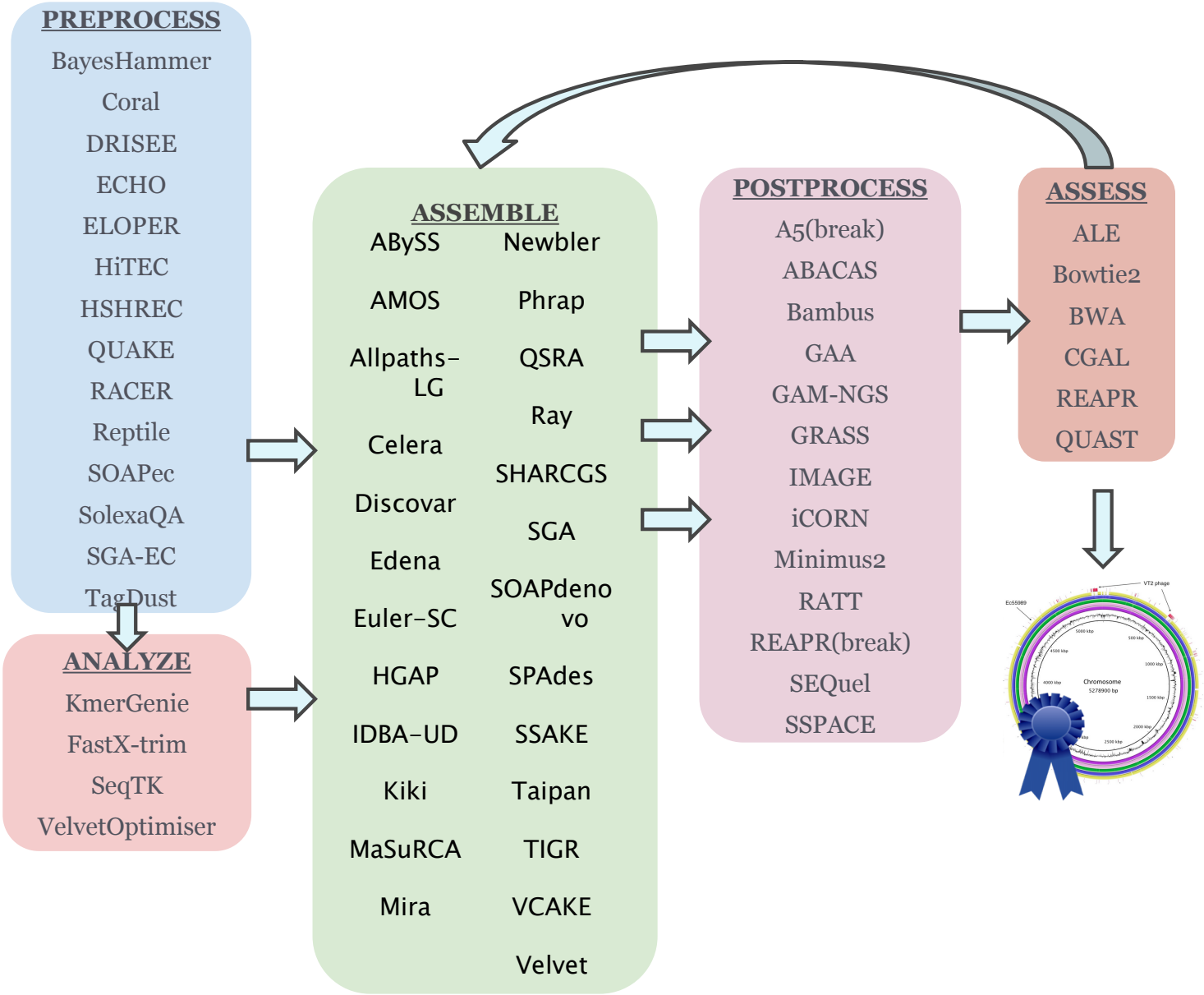
Advanced pipelines



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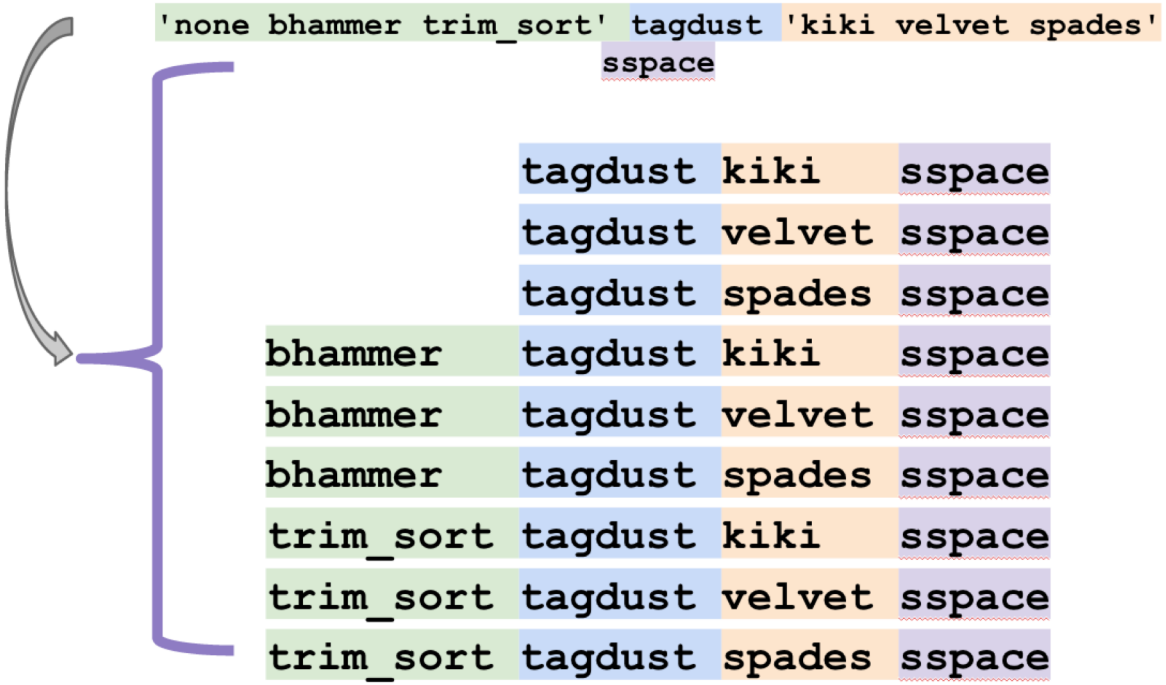
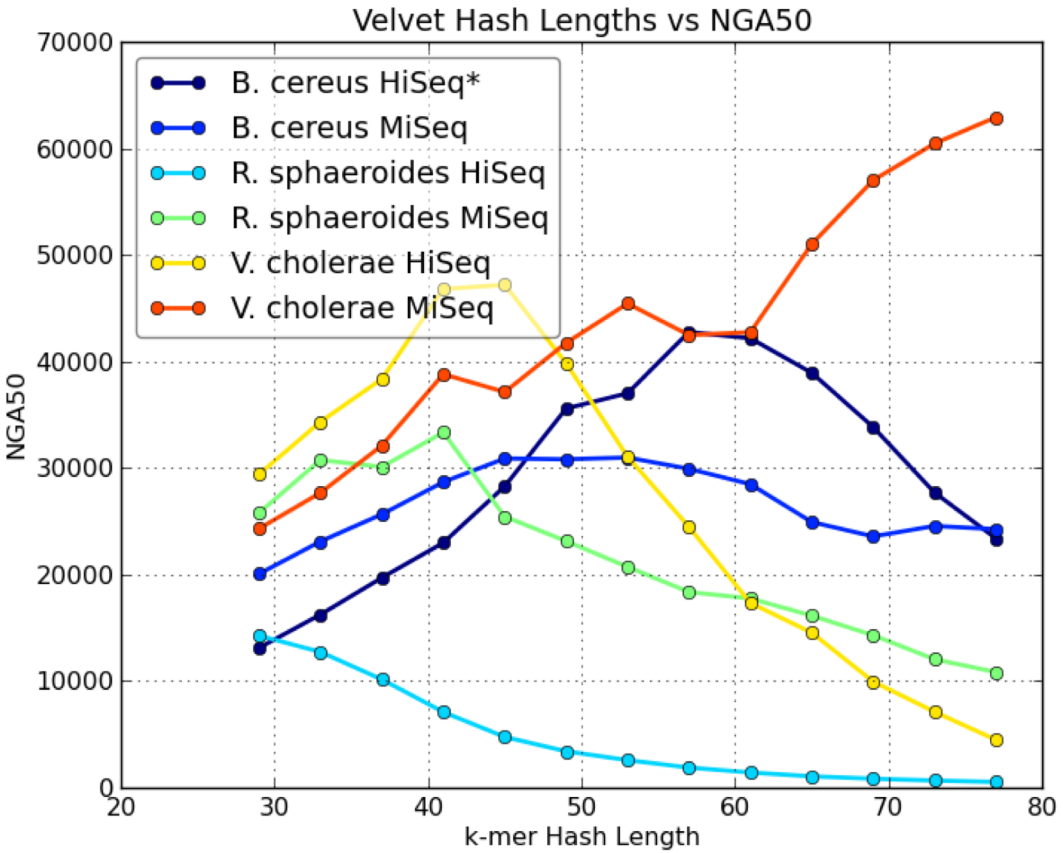
Pipeline design



Parameter scan: k-mer Optimization

```
velvet ?hash_length=29-77:4
```



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Upcoming improvements

- Improved error detection and classification using supervised learning
- Workflows for new sequencing technology (MinION), Hybrid assembly