Overcoming uncollapsed haplotypes in long-read assemblies of non-model organisms

Nadège Guiglielmoni, Antoine Houtain, Alessandro Derzelle, Karine Van Doninck, Jean-François Flot

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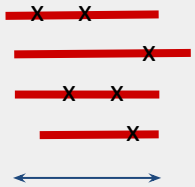
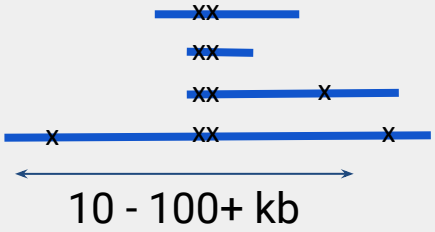
Long-reads and genome assembly

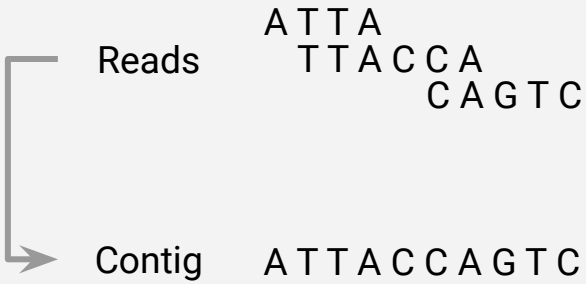
PacBio reads



Nanopore reads



Assembly process



Long-read assemblers

Canu	Ra	Shasta
Flye	Raven	wtdbg2
NextDeNovo		

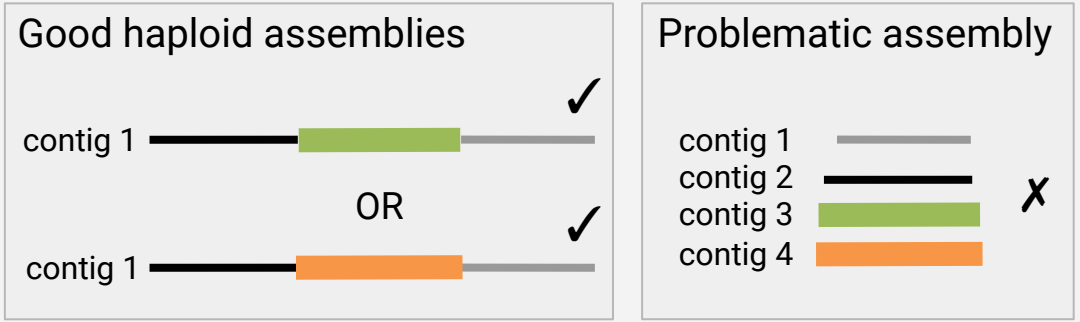
The problem of highly heterozygous regions

Haplotype 1 ATTACCAGTCTCAA**TGGATGGCTACTC**TTTGACGATAGCT

Haplotype 2 ATTACCAGTCTCAA**AGGCTGCTAGTG**TTTGACGATAGCT

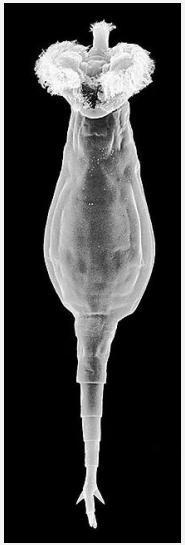
Assembly process

Assembly output

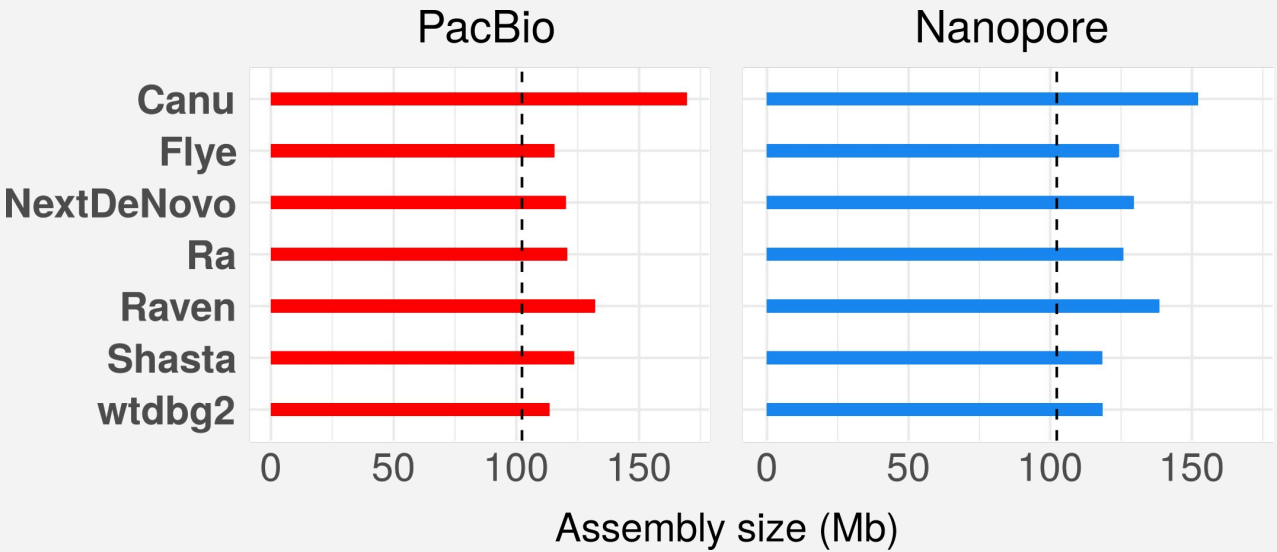


Symptoms of uncollapsed haplotypes

Assemblies of bdelloid rotifer *Adineta vaga* → expected haploid size 102.3 Mb



Who Needs Sex (or Males) Anyway?
Liza Gross, PloS Biology, 2007



Strategies to reduce uncollapsed haplotypes

- ➔ Strategy 1: choose a better assembler
- ➔ Strategy 2: removing uncollapsed haplotypes

  Tool: Purge Haplotigs

  Purge Haplotigs: allelic contig reassignment for third-gen diploid genome assemblies, Roach et *al.*, BMC Bioinformatics, 2018
- ➔ Strategy 3: select longest reads for assembly

2 long-read datasets for *Adineta vaga*:	**PacBio**	23.5 Gb, 230X
	Nanopore	17.5 Gb, 171X

Evaluation criteria

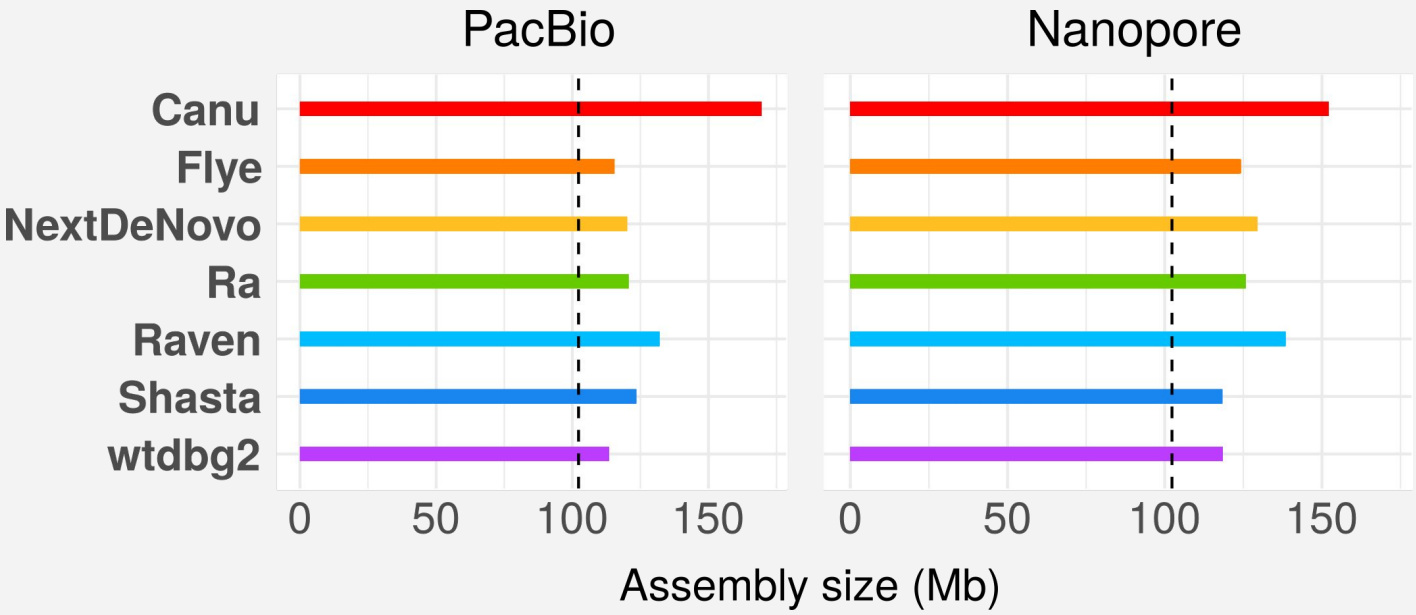
- **Assembly size**: sum of the lengths of all contigs, compared to the **estimated size of 102.3 Mb**

- **BUSCO score**: **number of orthologs** from a specific lineage (Metazoa) retrieved in the assembly, **either in a single-copy or duplicated**

- **Coverage**: number of reads covering a given position in a contig

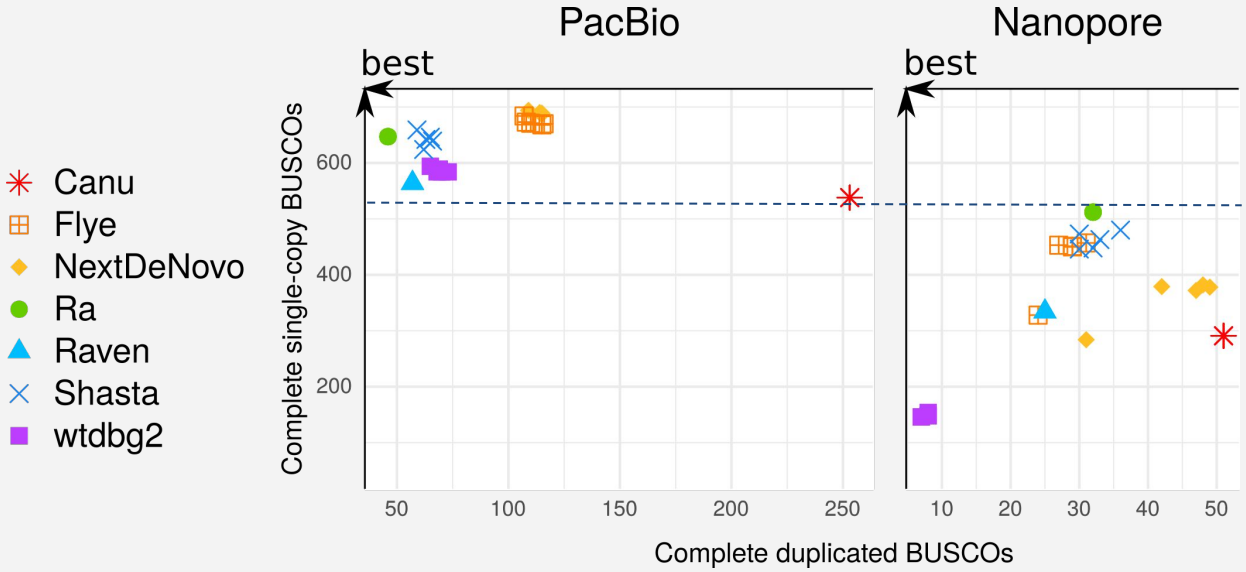
Evaluation criteria: assemblies of all reads

Assembly size: sum of the lengths of all contigs, compared to the estimated size of 102.3 Mb



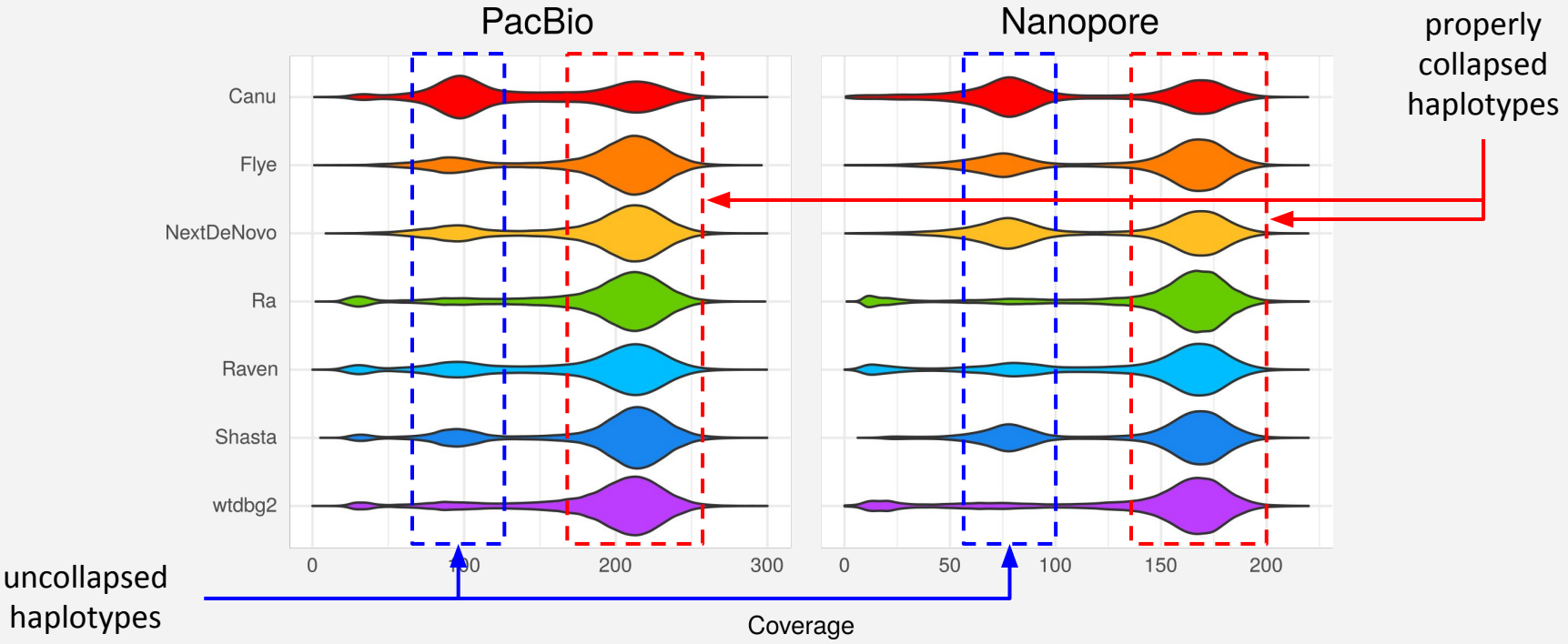
Evaluation criteria: assemblies of all reads

BUSCO score: number of orthologs from a specific lineage (Metazoa, 954 features) retrieved in the assembly, either in a single-copy or duplicated



Evaluation criteria: assemblies of all reads

Coverage: number of reads covering a given position in a contig, based on long reads mapping



Collapsing haplotypes: PacBio assemblies

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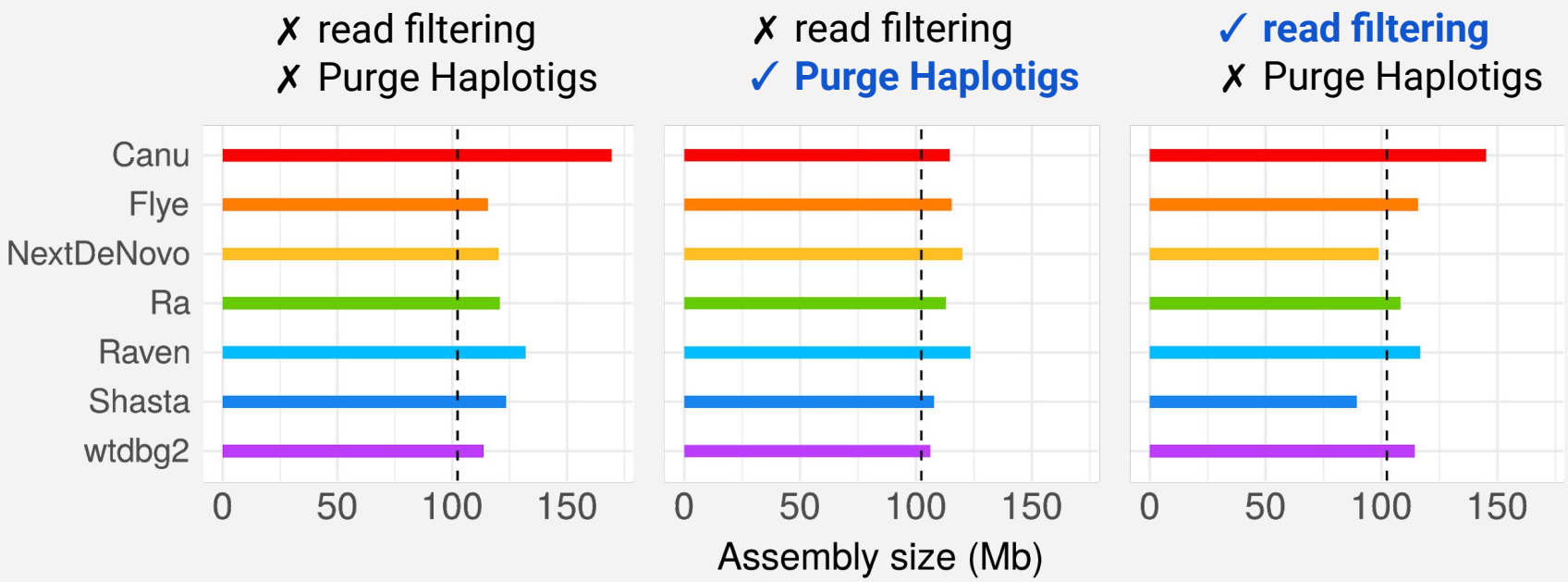

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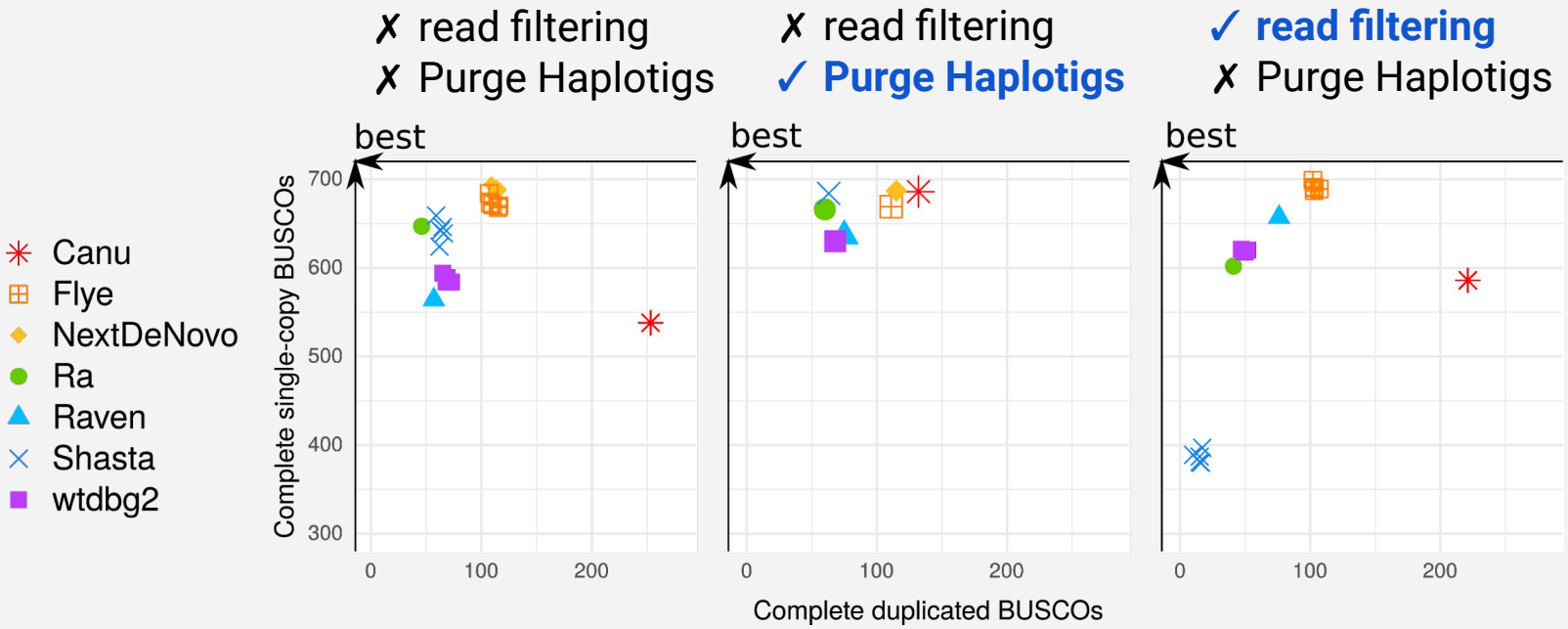

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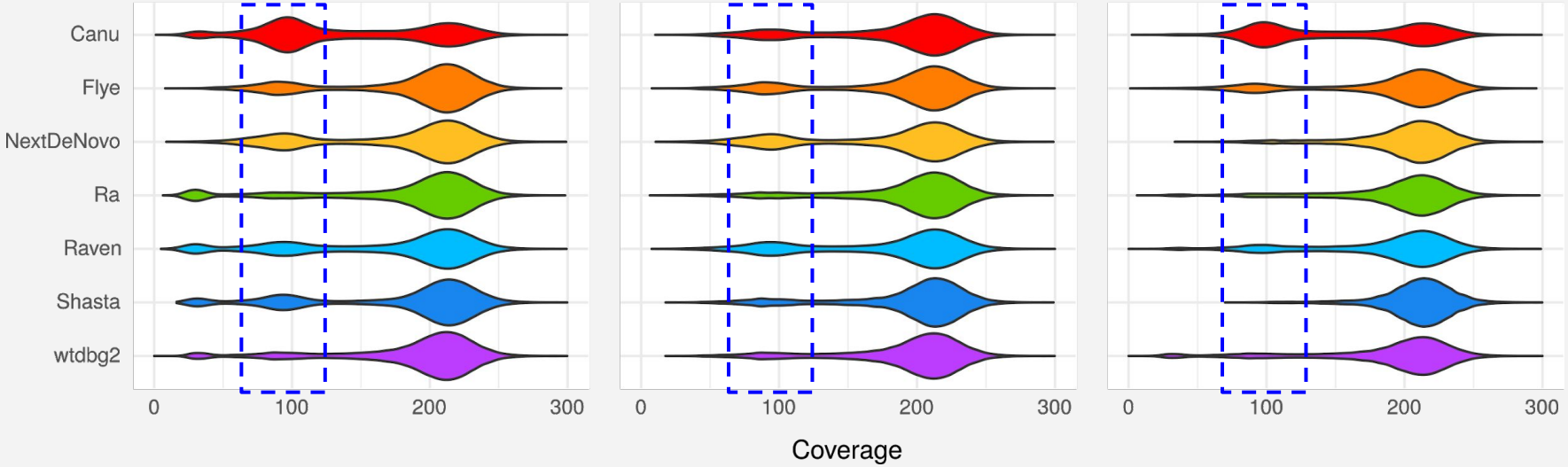

Collapsing haplotypes: Nanopore assemblies

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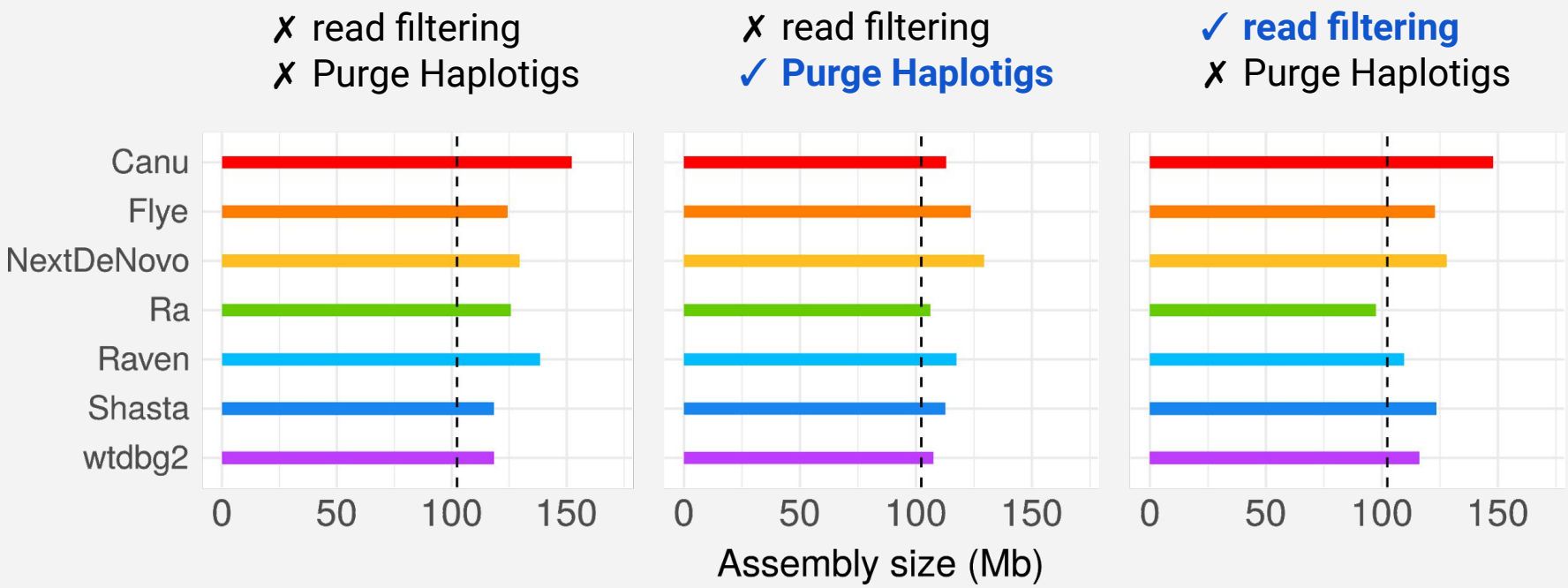

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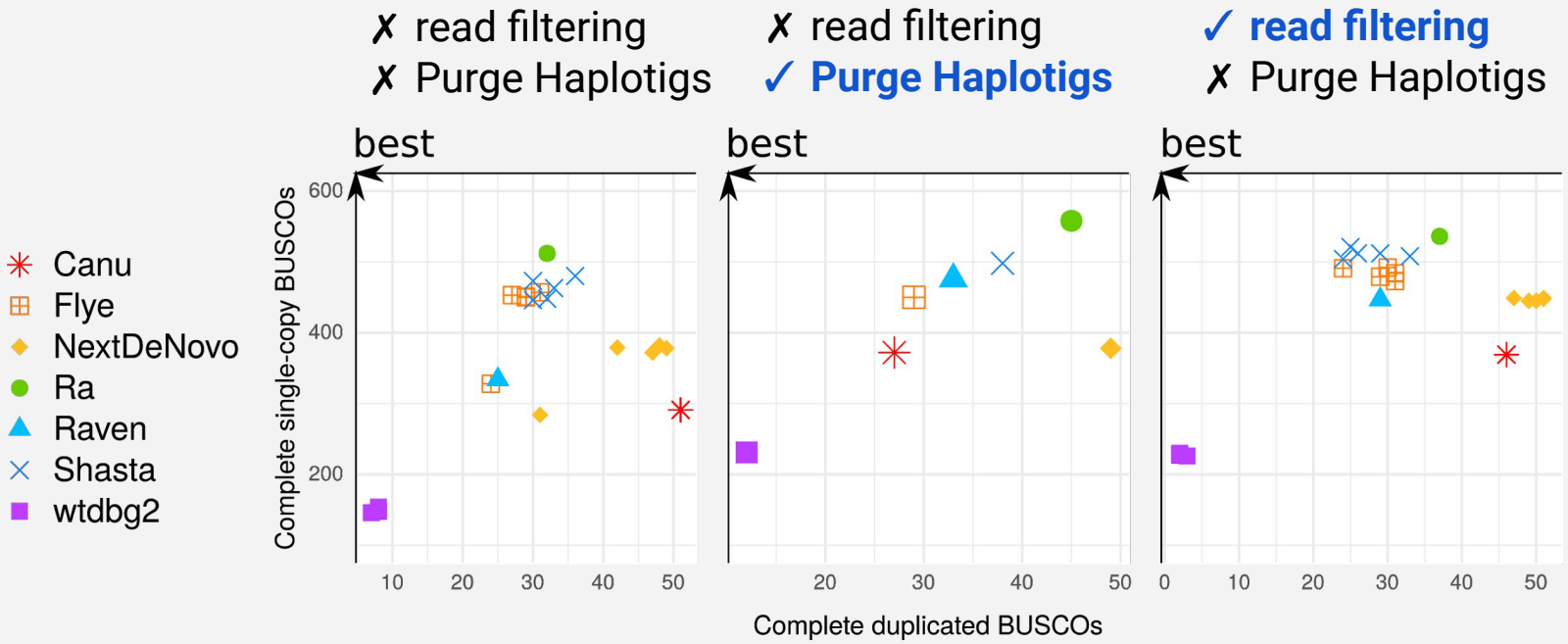

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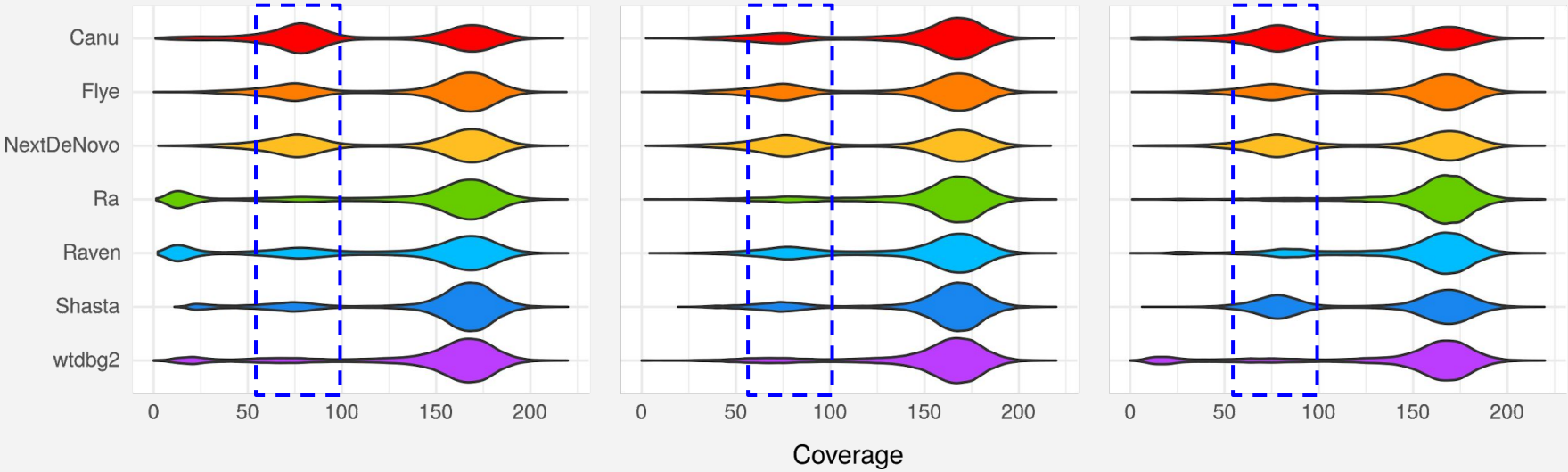

Take-home message

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- ➔ Strategy 2: removing uncollapsed haplotypes works better on some assemblies than others
- ➔ Strategy 3: Read filtering improves structure and does not decrease quality
- ➔ Need for better assessment of assemblies ≠ contiguity
- ➔ There is not one measure to pick the best assembly

Acknowledgements

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Alessandro Derzelle
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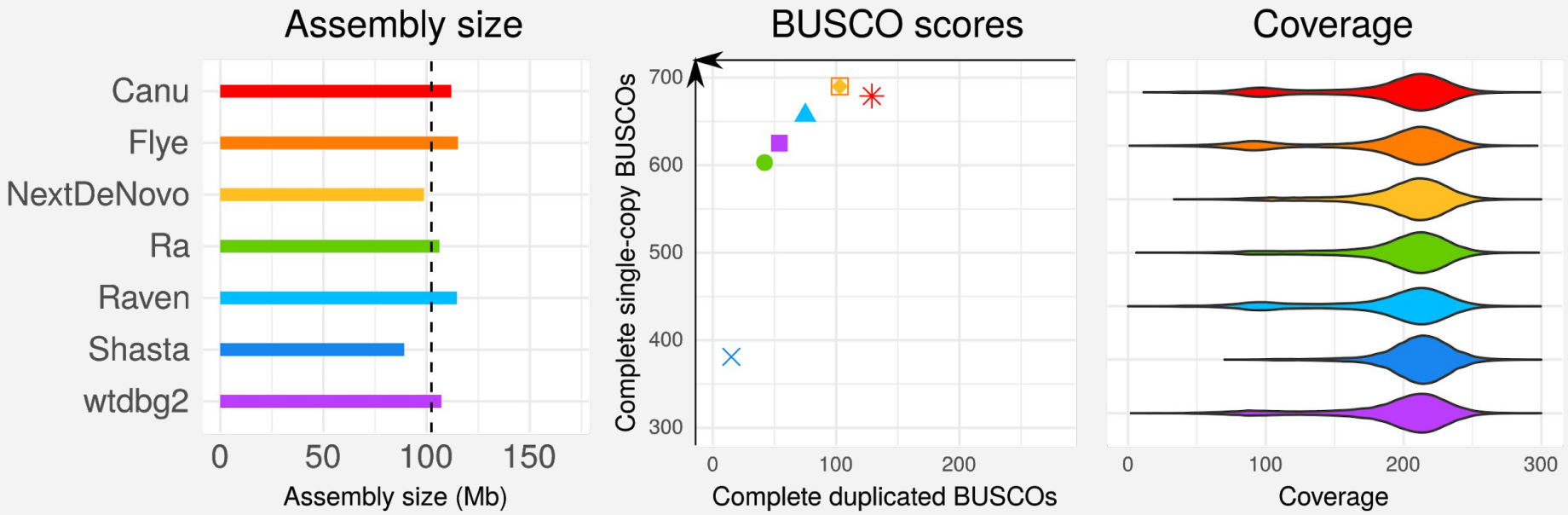
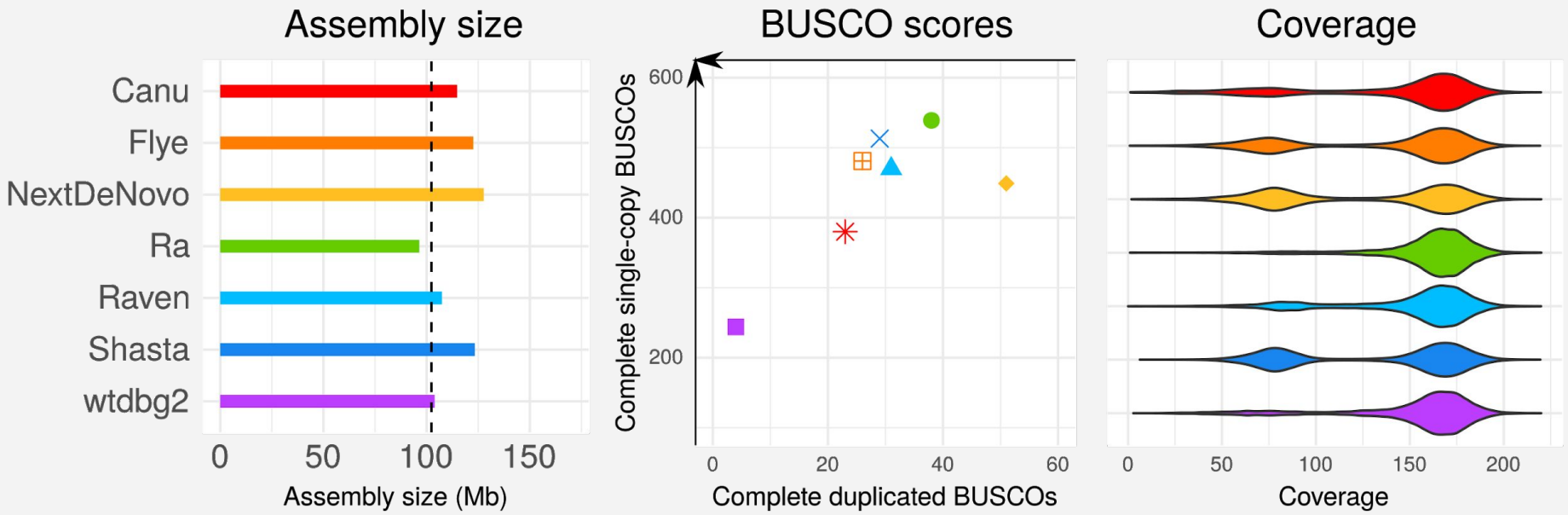
Thank you for your attention!
Questions?

PacBio: Purge Haplotigs + read filtering



Nanopore: Purge Haplotigs + read filtering



Performance