Future impact of ALPACA

Research prospects by
Luca Denti

Open problems on pangenomes

(currently under investigation)

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Luca Denti

1) Pangenomic analysis in transcriptomics *(aka pantranscriptomics)*

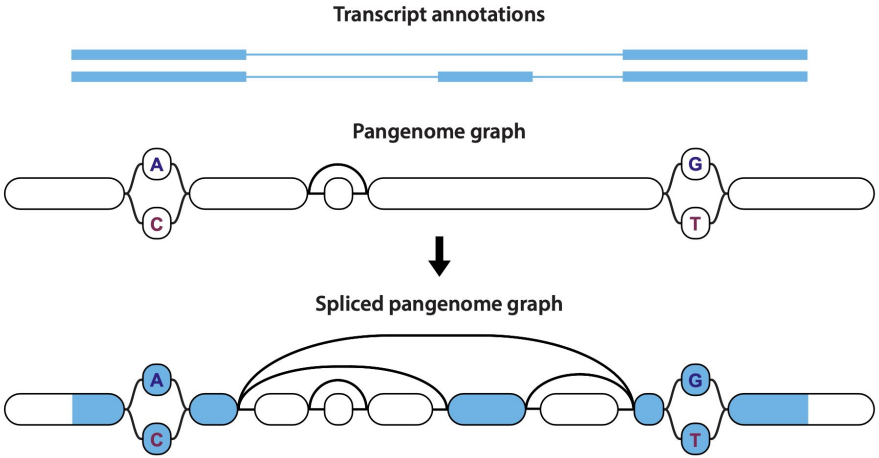
RSEM is to RPVG* as rMATS is to pantas

PLOS COMPUTATIONAL BIOLOGY

Differential quantification of alternative splicing events on spliced pangenome graphs

Simone Ciccolella, Davide Cozzi, Gianluca Della Vedova, Stephen Njuguna Kuria, Paola Bonizzoni, Luca Denti

doi: https://doi.org/10.1101/2023.11.06.565751



*Sibbesen et al. ***Haplotype-aware pantranscriptome analyses using spliced pangenome graphs.*** Nature Methods (2023)

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Based on **spliced alignment** to spliced pangenomes with `vg mpmap` (`GCSA2`-based)

- efficiency
  - *graph needs to be **heavily pruned** (haplotype-aware transcripts from dros. n.o. genes, >256GB RAM)*
  - *one replicate required more than **3 days** to align*
- backward extensions limited to 256bp (short reads) - *longer patterns can return **false positives***
- inaccuracies when aligning over **novel splicing sites**

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↳ *`ropebwt3`?*

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2) Long reads and pangenomes

Common short reads practice (e.g., `Giraffe-DV`):

1. Align to graph → 2. Surject to reference → 3. Reference-based approach

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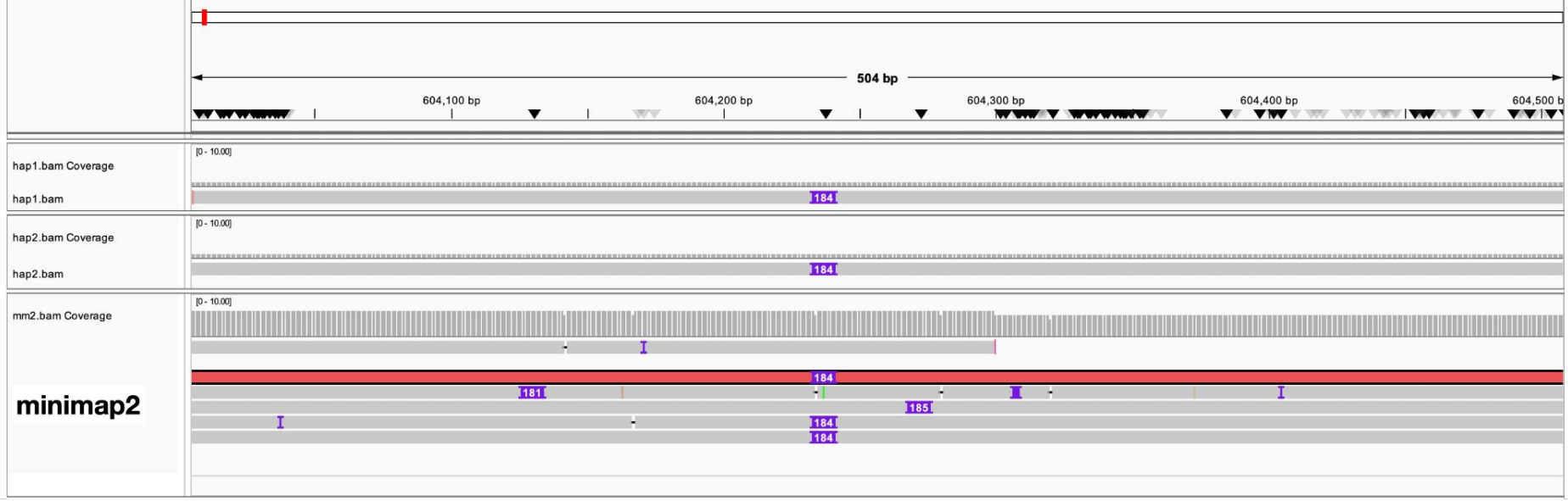
This is **not applicable to long reads** *(for now and at least for SV calling)*:

- ➢ improve long read alignments (1)
- ➢ design new pangenomic approaches for downstream analyses (2,3 / 3)
- ➢ design alignment-free methodologies (1,2,3)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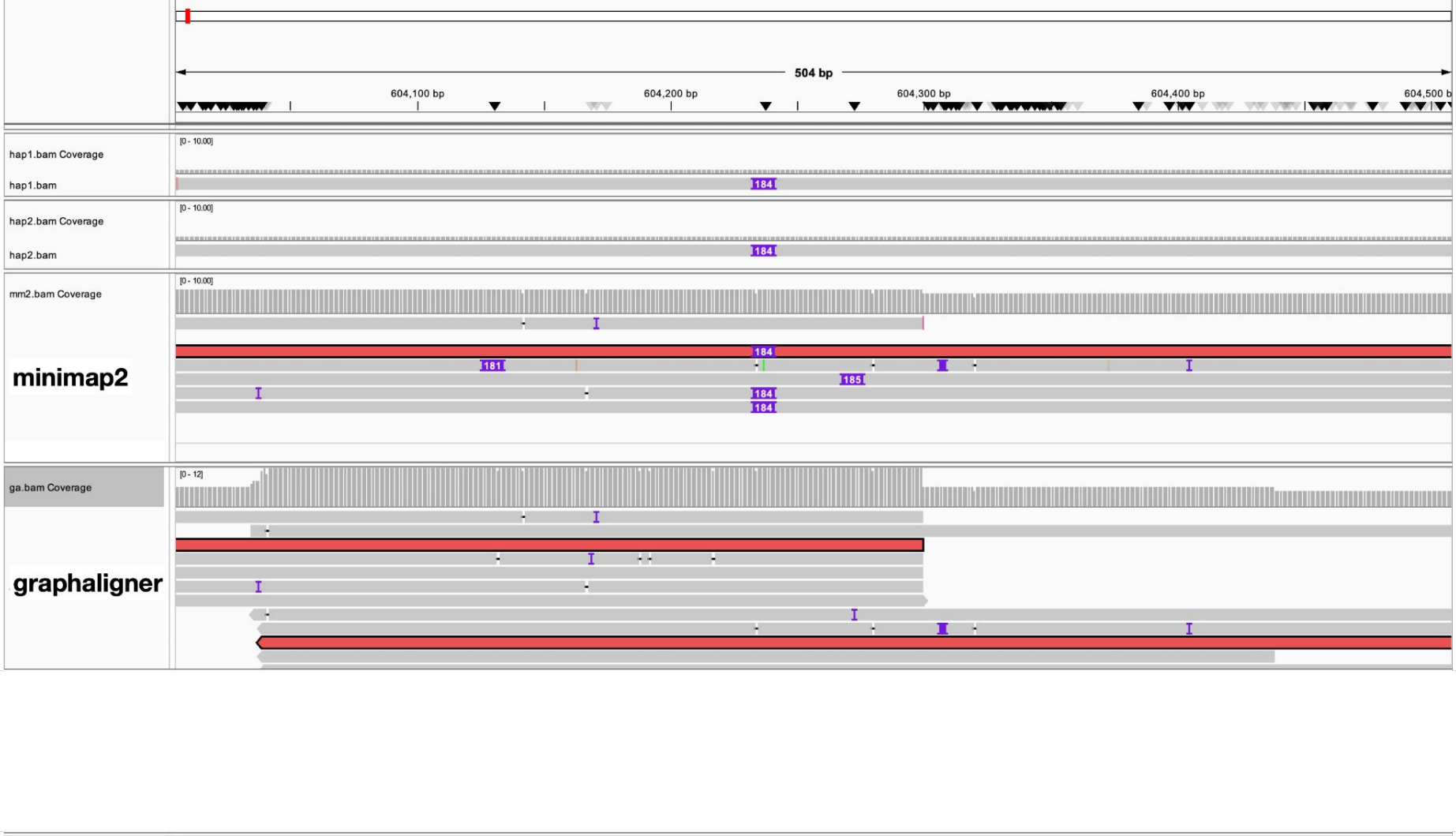
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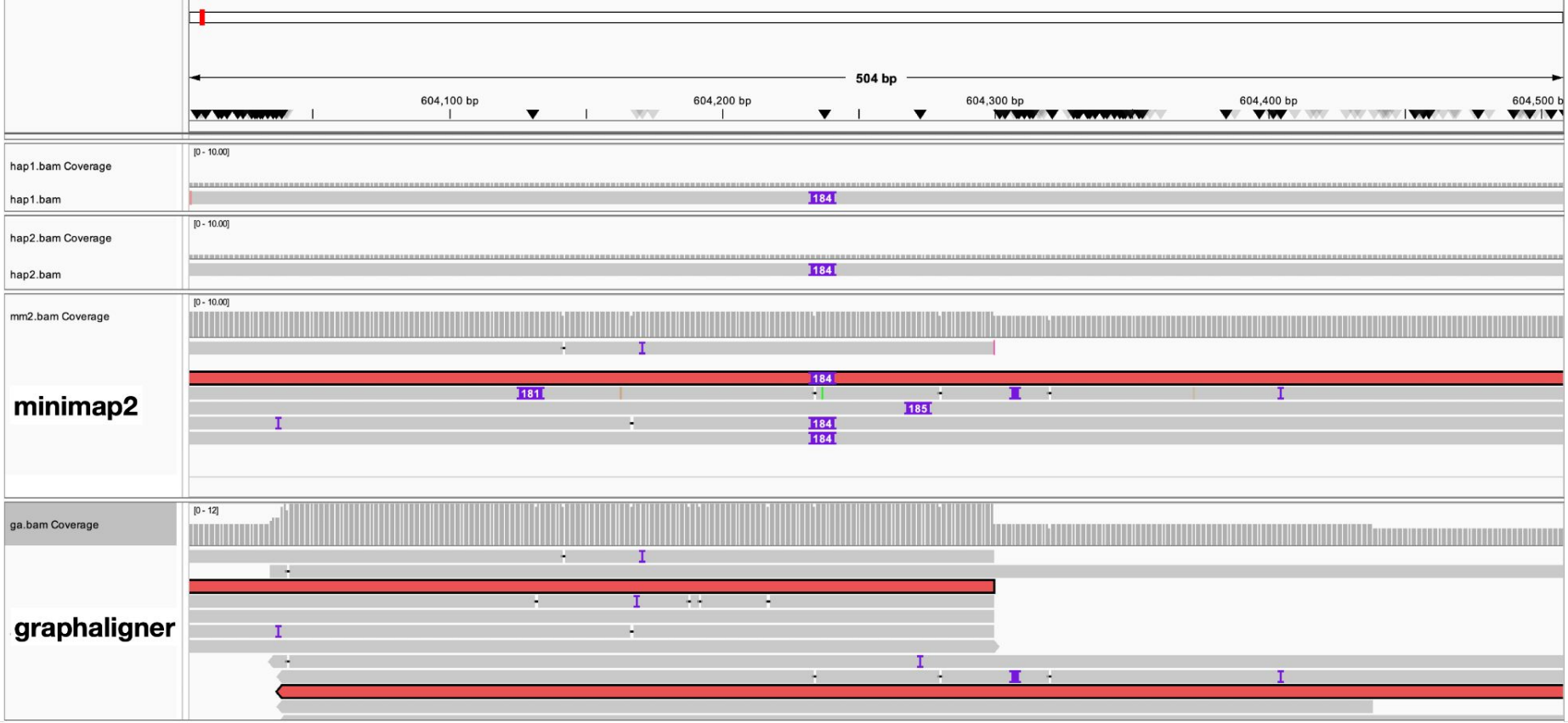

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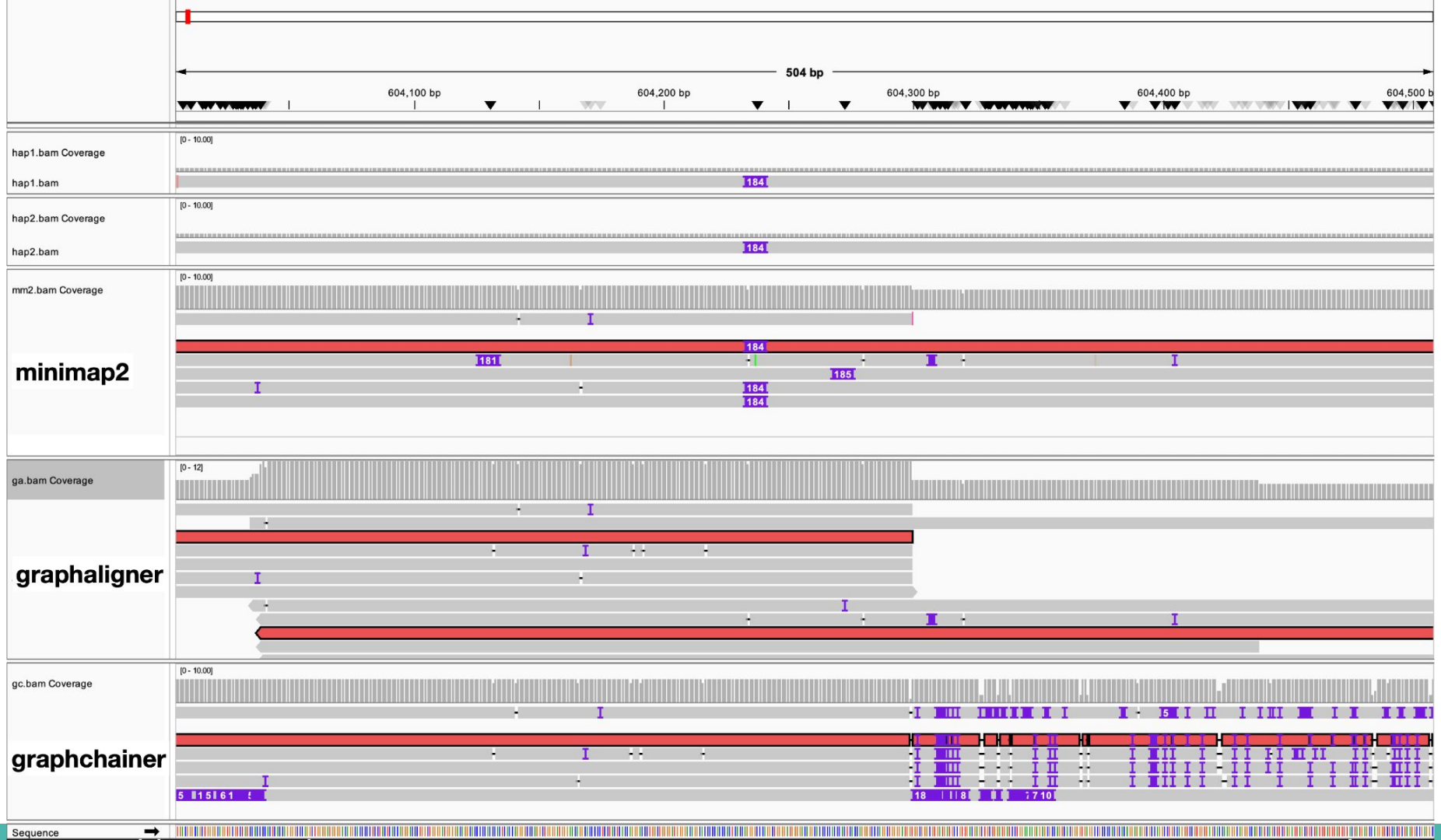
3304M 1I 4624M 34I 1M7I1M2I894M1I 2654M 1I248M1I874M1I **842M : 590M**1I90M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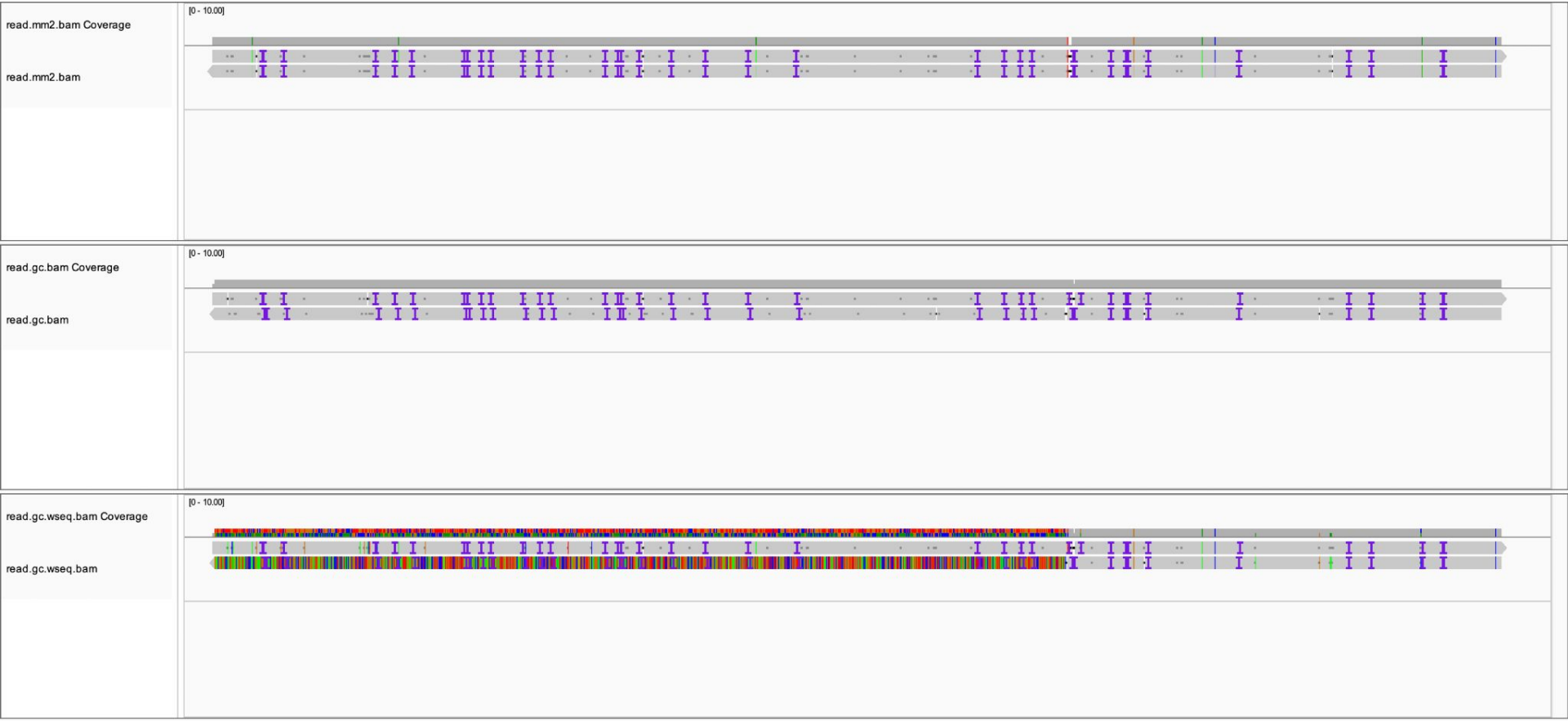
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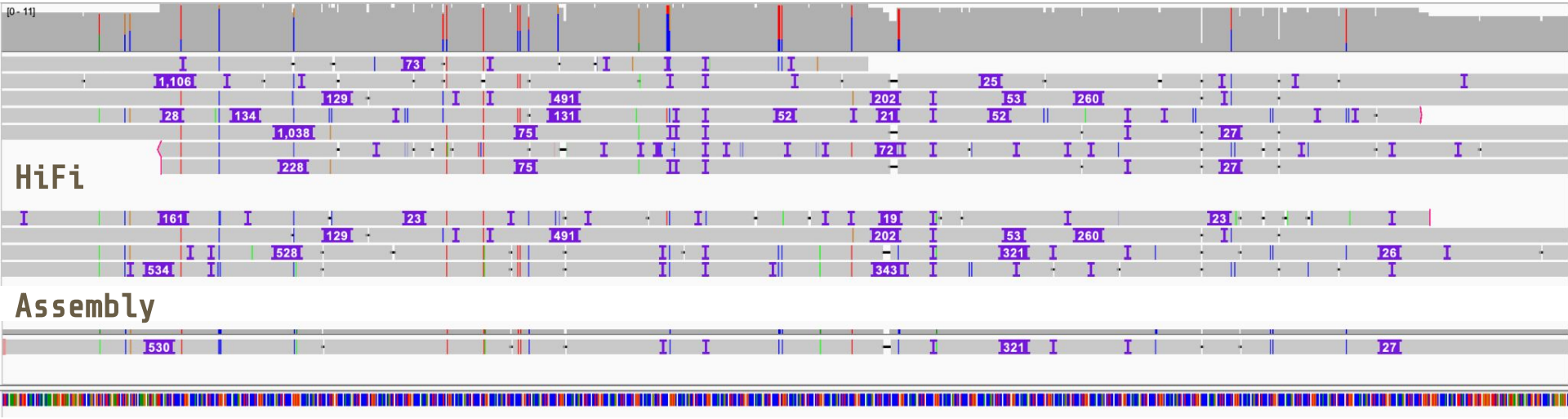
3) Reference-bias vs Alignment-bias

*"No single genome can represent the **diversity** in the human population. Using a single reference genome creates **reference biases**, adversely affecting variant discovery, gene–disease association studies and the accuracy of genetic analyses."*

https://humanpangenome.org/

*"Any downstream analysis is affected by the **choices** made by the aligner"*

3) Reference-bias vs Alignment-bias (CGR)



(mainly due to scores/penalties and query lengths)

- What should we “fix”?
- Is alignment to pangenomes going to improve/facilitate this?
- Is this just a problem of “representation”?

4) How to represent SVs *(or any variant)* wrt a pangenome?

- One VCF against each path *(genome)* of the pangenome?
- FASTA with *svtigs** ?
- Can we find a more compact way?
- What about a GAF-like file format for variations?

*Soylev, ..., Marschall. ***SVarp: pangenome-based structural variant discovery.*** *bioRxiv (2024)*