Current options to index, represent, and visualize annotations in a pangenome with the vg toolkit

MIGGS workshop

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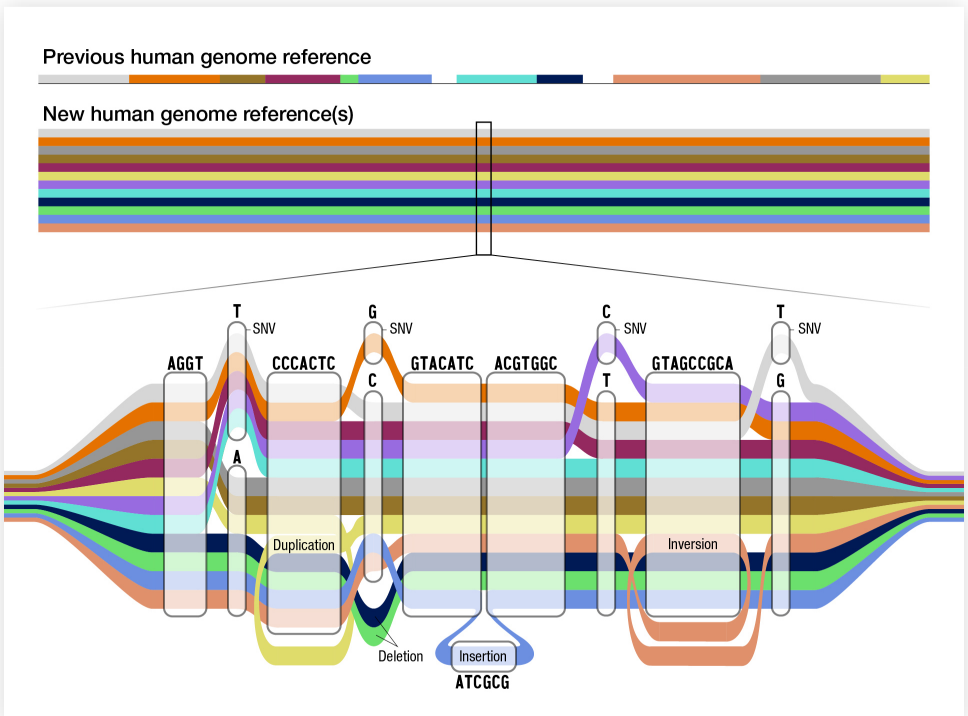
Pangenome graph from assemblies

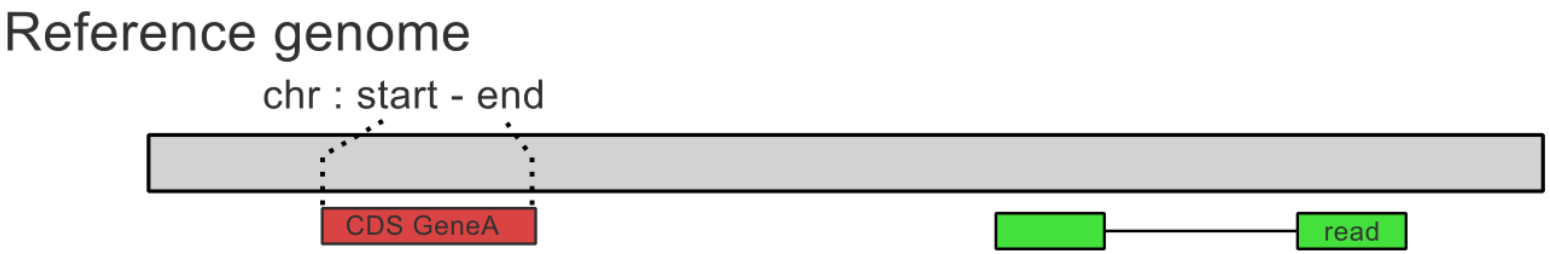
Built by aligning high-quality genomes, saved as paths through the pangenome.

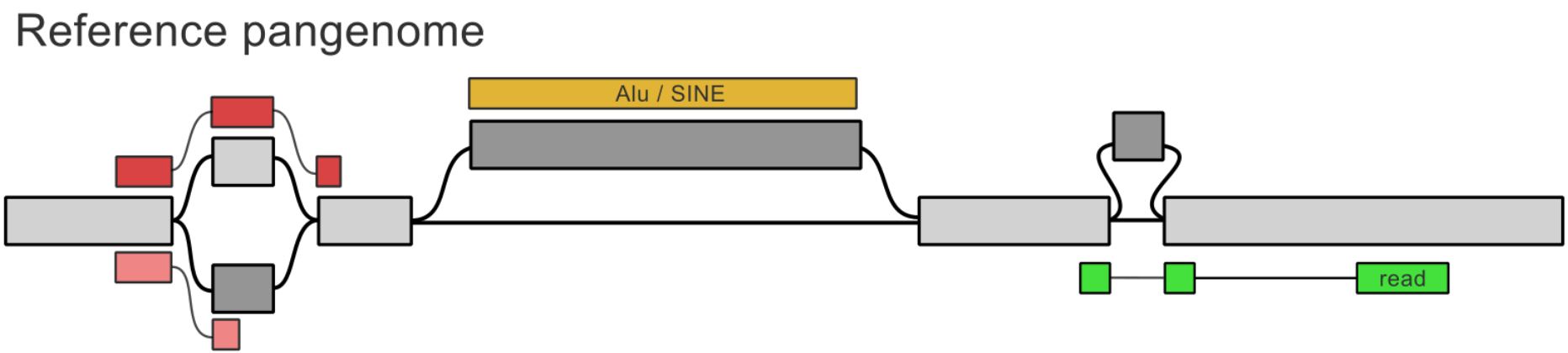


Human Pangenome Reference Consortium (HPRC)

Liao, Asri, Ebler, et al. Nature 2023

Annotations could be paths through the pangenome





How to **represent** those paths and **index** them efficiently?

(doing a minimum amount of work)

GAM format

- Reads aligned to a pangenome graph.
- Used by `vg`, `GraphAligner`, …
- Binary/protobuf format, indexable but unwieldy.

```
{
    "mapping_quality": 60,
    "name": ">44878920>44878957",
    "path": {
        "mapping": [
            {"edit": [{"from_length": 1, "to_length": 1}],
             "position": {"node_id": "44878920"}, "rank": "1"},
            {"edit": [{"from_length": 1, "to_length": 1}],
             "position": {"node_id": "44878957"}, "rank": "2"}
        ]
    },
    "score": 2,
    "sequence": "AC"
}
```

**json* representation

Graph Alignment Format (GAF)

- **Text** format describes mapping between sequences and a graph.

```
read_name_6 100 0   100 +   <394<393<392<391    128 25  124 100 100 60 cs:Z::100
```

```
!-----query info----------!-----target info----------------!--------------------!
  name size range strand         path size range                 align + opt. tags
```

  - First, **query** information: name, size, aligned range.
  - Then, **target** information: **path**, size, aligned range.
  - Finally, additional information: MAPQ, CIGAR, …

Indexing genomic files with HTSlib

https://github.com/samtools/htslib

- Index text format on the genomic coordinates, e.g. `chr:start-end`.
  - For example: VCF, BED, SAM/BAM, GFF
- Sorted by `chr`, then `start`, then `end`.
- Compressed with BGZIP (~block gzip).
- Fast extraction of slices.

Indexing using minimum/maximum node IDs

Assumes node IDs are **sorted** integers.

```
read_name_9 100 0   100 +   <3222<3221<3220<3219    128 18  117 100 1
```

- "genomic range": **3219-3222**.
- Same as current strategy for GAM files.
- Modified HTSlib (`bgzip`/`tabix`) to **index based on min/max ID**.
- Modified `vg gamsort` to **sort GAFs based on min/max ID**.

New commands available

Sorting a GAF file

```
vg gamsort -G reads.gaf.gz | bgzip > reads.sorted.gaf.gz
```

Indexing sorted bgzipped GAF

```
tabix -p gaf reads.sorted.gaf.gz
```

Extracting a node interval

```
tabix reads.sorted.gaf.gz {node}:20034-20158
```

Sorting short sequencing reads

Illumina 30x short reads for HG002, aligned to the HPRC pangenome with `vg giraffe`.

Format	Time (H:M:S)	Max. memory used (Kb)	File size (Gb)
GAM	11:46:58	6,236.60	108
GAF	6:50:28	1,904.83	52

Faster, less memory, and **smaller files** using GAF.

Projecting annotation to the pangenome

Input annotation **relative to one haplotype** (BED or GFF), present in the pangenome (path)

```
vg annotate -x pg.gbz -f genes.hapH.gff -F > genes.hapH.gaf
```

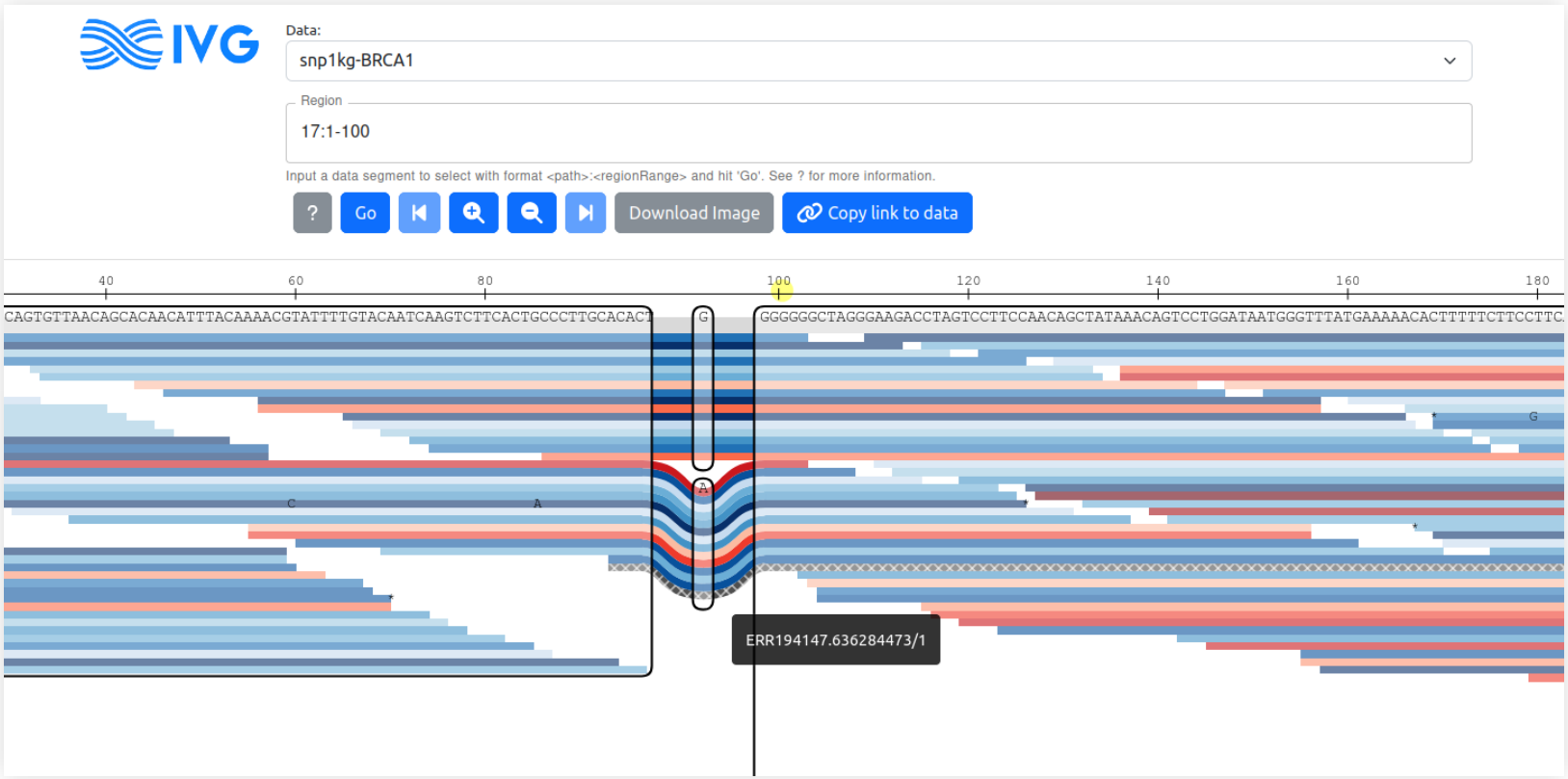
Application

Projected the HPRC v1 assemblies' annotations:

- ~4M **gene annotations** in ~16 mins
- ~5.5M **repeats** from RepeatMasker in ~9 mins

Visualization with the sequenteTubemap

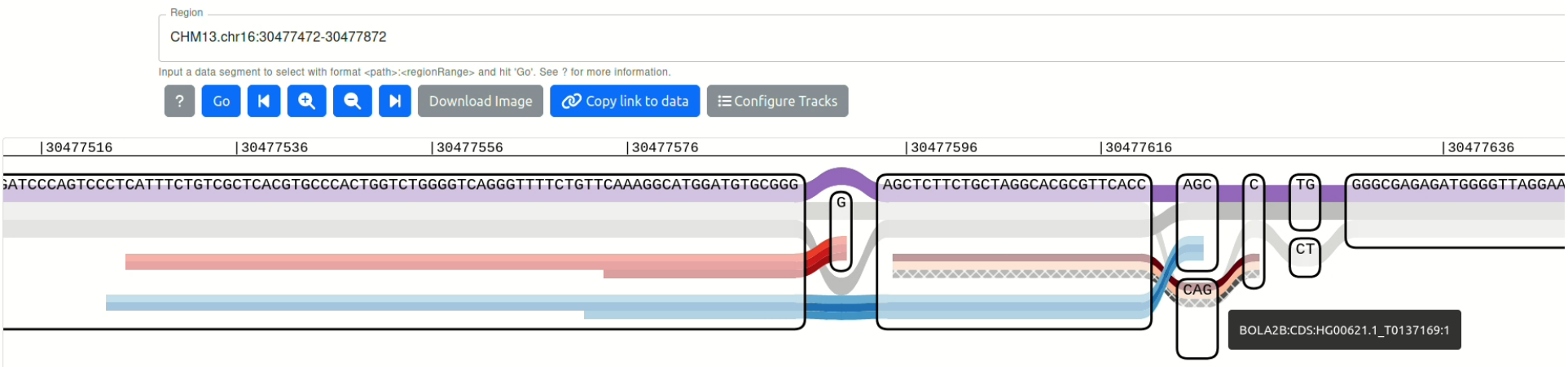
Interactively query a subgraph and aligned reads.



Recently modified to accept indexed GAF files.

https://github.com/vgteam/sequenceTubemap

Coding sequence (CDS) for two haplotypes in the HPRC pangenome.



Haplotypes: CHM13 (***purple***), HG00621 (***greys***). Annotated CDS for HG00621 hap 1 (***reds***) and 2 (***blues***).

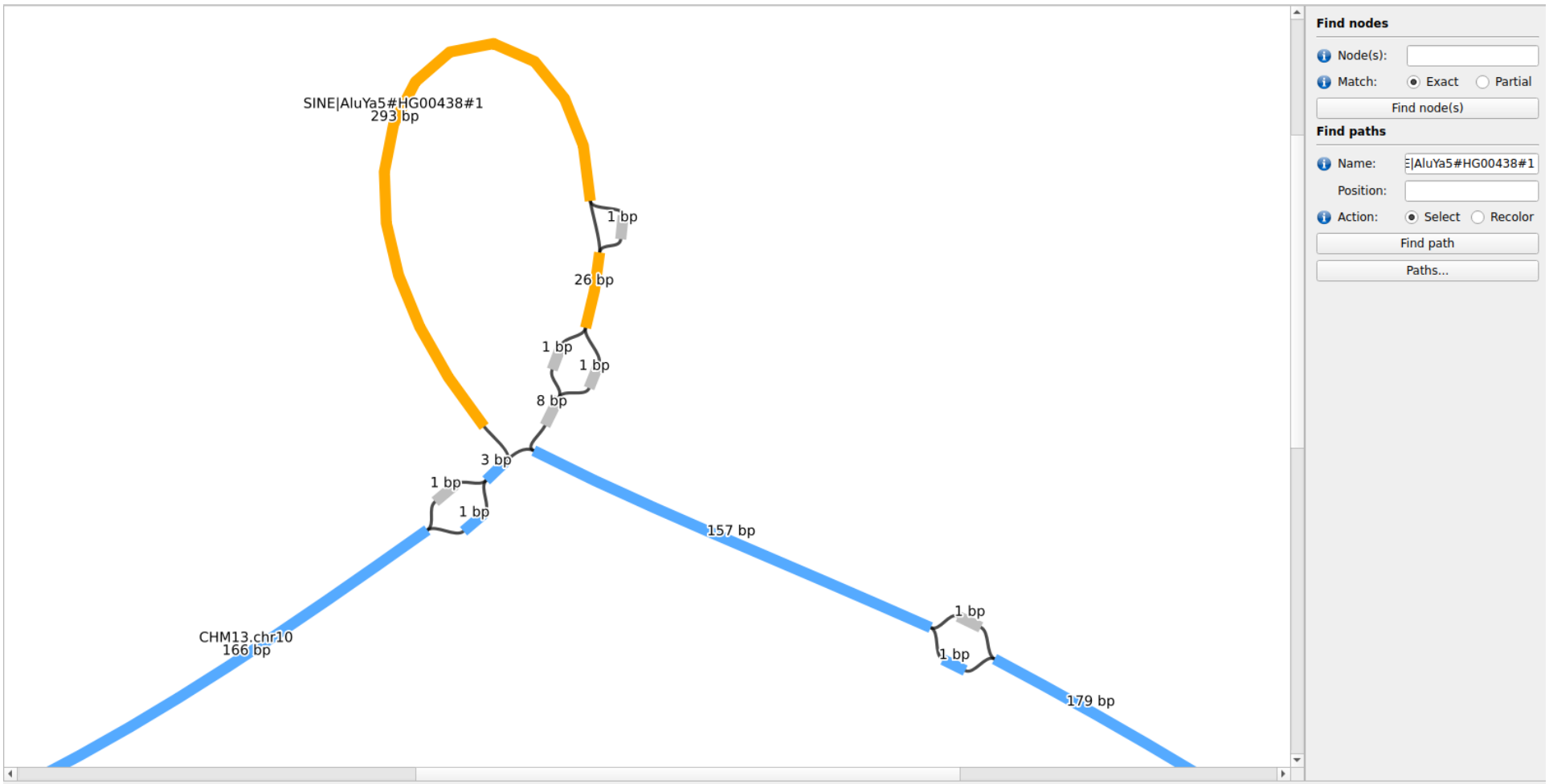
Visualization with Bandage?

BandageNG can color nodes by paths, **present in the input graph** (GFA).

https://github.com/asl/BandageNG

1. Optional: **extract subgraph** for the region of interest.
   - `vg chunk` or `odgi extract`
2. Add the external annotation as **paths in the graph file**.
   - `vg augment -BF subgraph.pg annot.gaf.gz`
   - `vg convert -f augmented.pg > augmented.gfa`
3. Open with BandageNG, search path by name, **color nodes**.

Mobile element insertion



Node color: ***blue*** for the reference path, ***orange*** for the AluYa5 transposon.

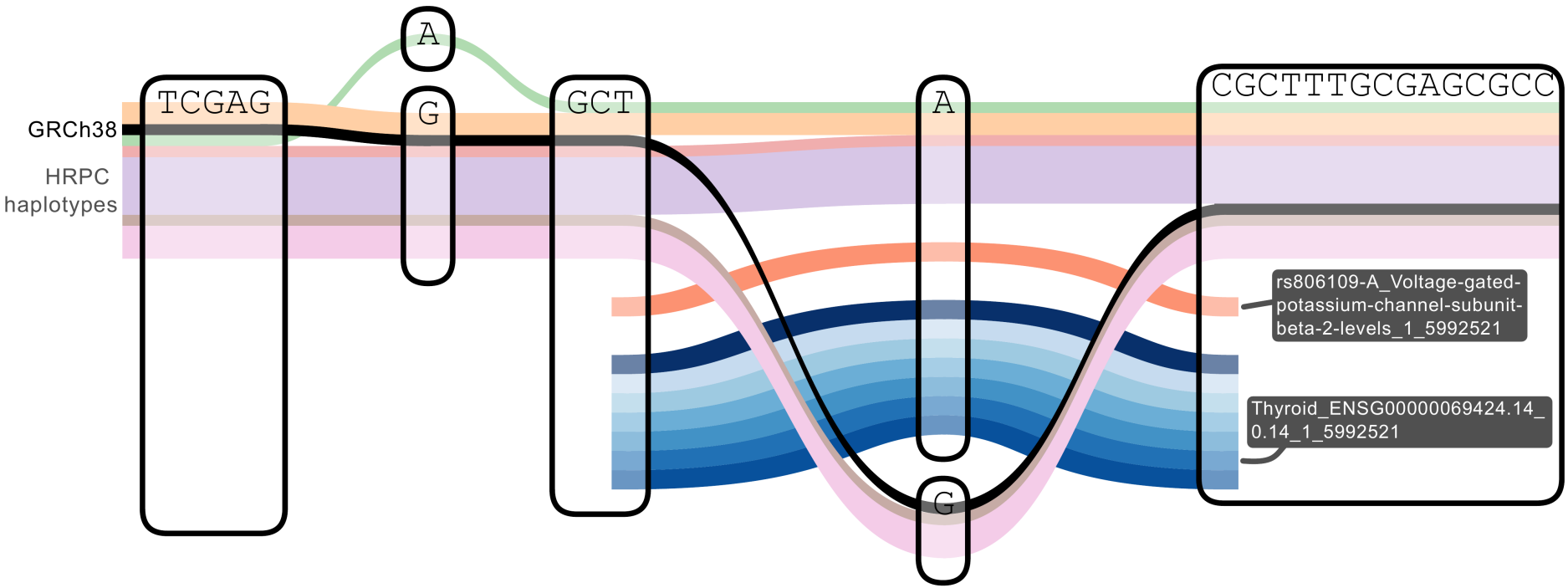
Projecting variants to the pangenome

Custom scripts to (try to) **find and annotate known variants** in the pangenome.

Application

- **GWAS** hits and **eQTLs** from GTEx
  - ~500Mb bgzipped GAF file for ~75M variants
- **Genotype** calls from short reads.

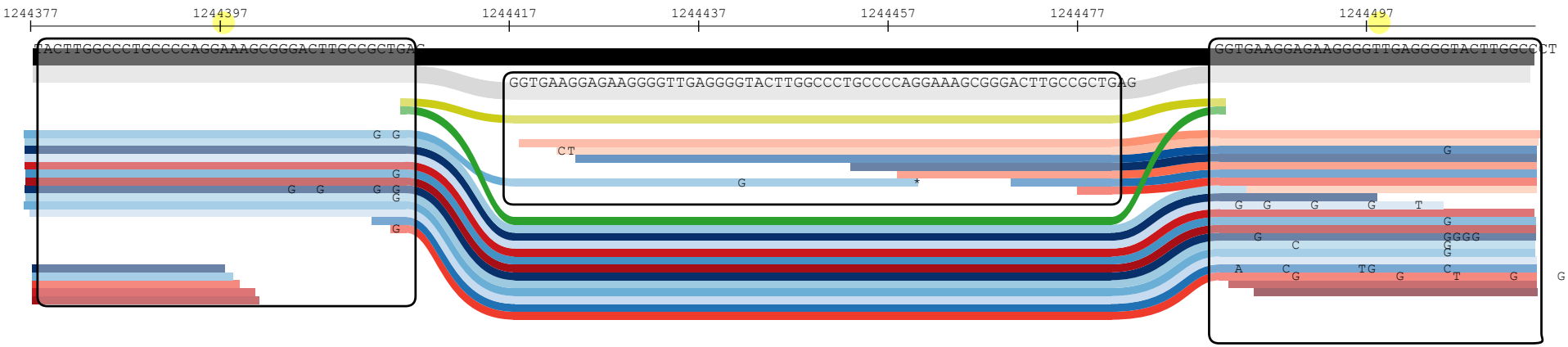
Annotating the GWAS catalog and eQTLs



black: Reference path (GRCh38), ***pale colors***: other human haplotypes, ***reds***: GWAS catalog, ***blues***: eQTLs across tissues (GTEx)

Annotating genotype calls

E.g. to investigate supporting reads.

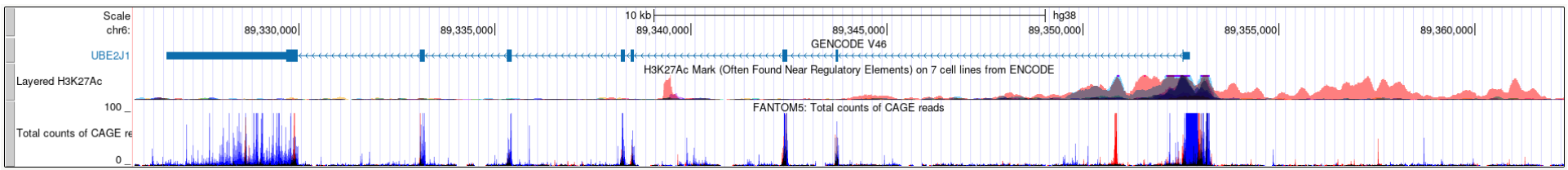


yellow/***green***: annotation paths from `vg call` genotypes. ***reds***/***blues***: short sequencing reads

Summarizing read coverage

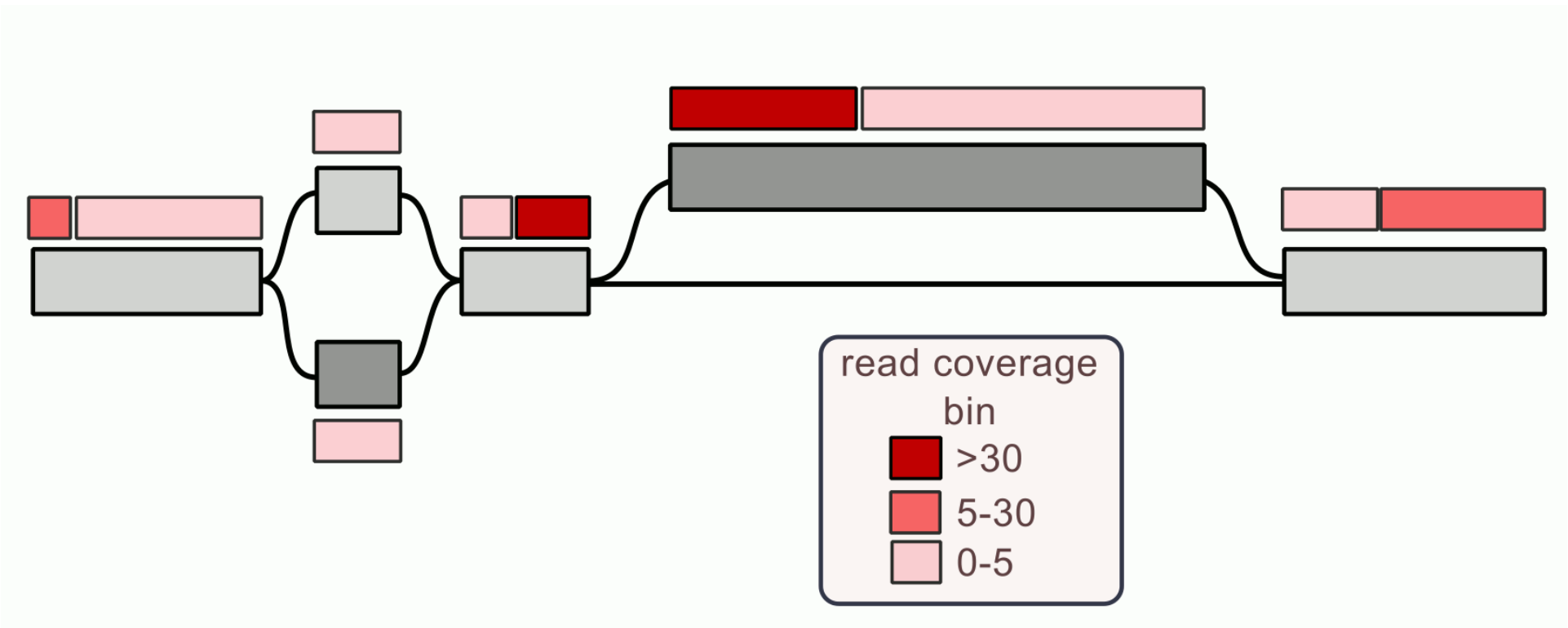
E.g. for epigenomics tracks.

On the linear genome:



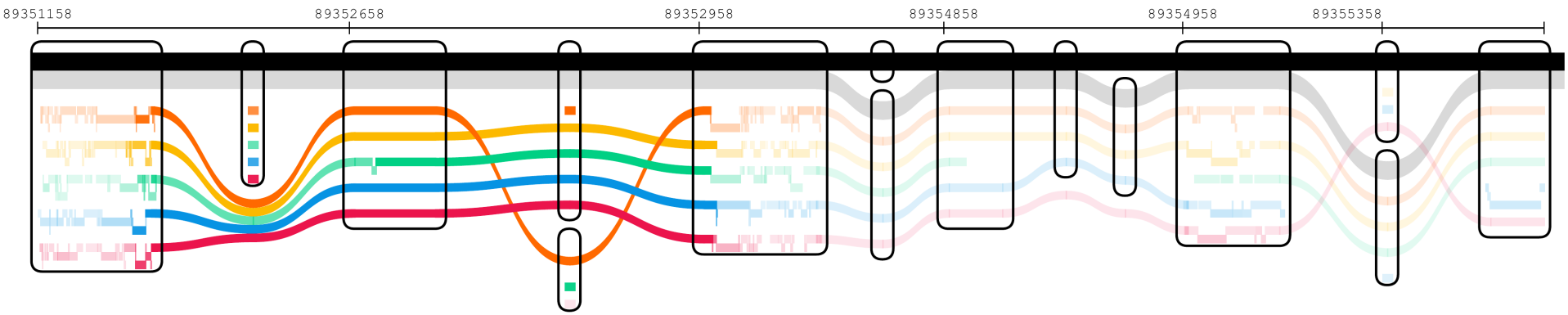
Summarizing read coverage

- Re-analyzing ENCODE ATAC-seq data across a few tissues.
- Implemented a naive approach to **bin read coverage**.
  1. Split the coverage in bins, e.g. (0, 5, 30, +)
  2. Extend them to create paths of contiguous bins.



Coverage of ATAC-seq data from ENCODE

Reads from 7 cell types aligned with `vg giraffe` to the HPRC pangenome.



Compressed view (no sequence shown, node size not to scale).

Reference paths CHM13/GRCh38 (***black***/***grey***) and ATAC-seq coverage track for different tissues (***colors***). Opacity represents coverage level.

Conclusion

GAF files can now be sorted/indexed, and then **queried fast**.

Limitations

- Indexing relies on **integer compact node IDs**
  - Output of *Minigraph-Cactus*/*PGGB*, otherwise convert with *vg*/*odgi*
- Designed for **short paths**
- Ugly **metadata handling**: all in a *name*
- Requires **annotations of the input genomes**
- In general, we need **more efficient** visualization tools

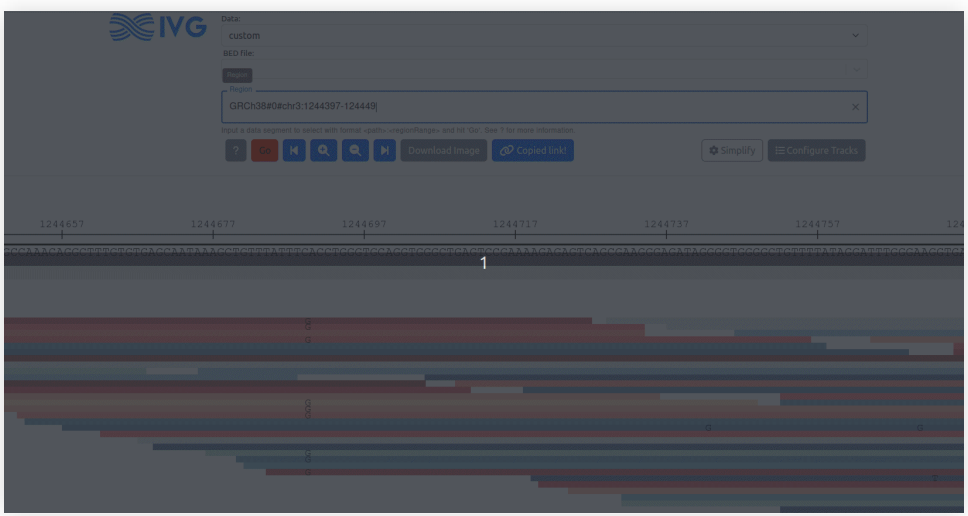
Acknowledgments

Adam M. Novak, Dickson Chung, Glenn Hickey, Sarah Djebali, Toshiyuki T. Yokoyama, Erik Garrison, Benedict Paten.

Manuscript in preparation + analysis + talk:

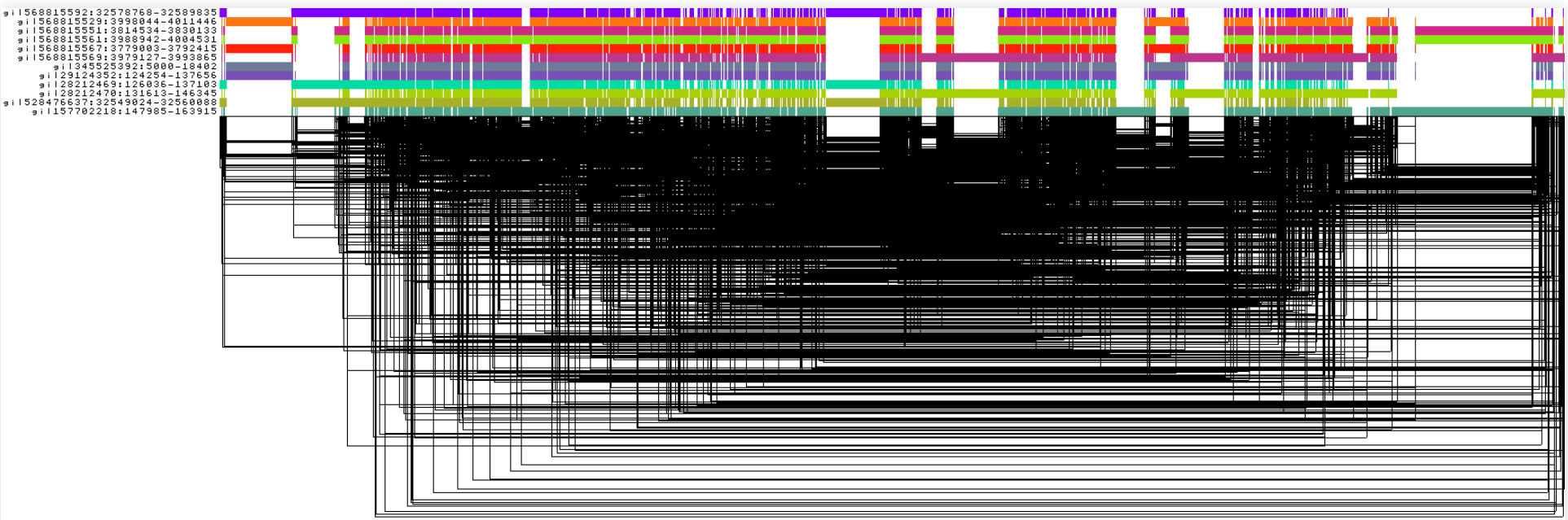
https://github.com/jmonlong/manu-vggafannot

Questions?



Compact sorted node IDs through graph sorting

Graph sorting aims to find the best node order for a 1D and 2D layout.



https://odgi.readthedocs.io/en/latest/rst/tutorials/sort_layout.html