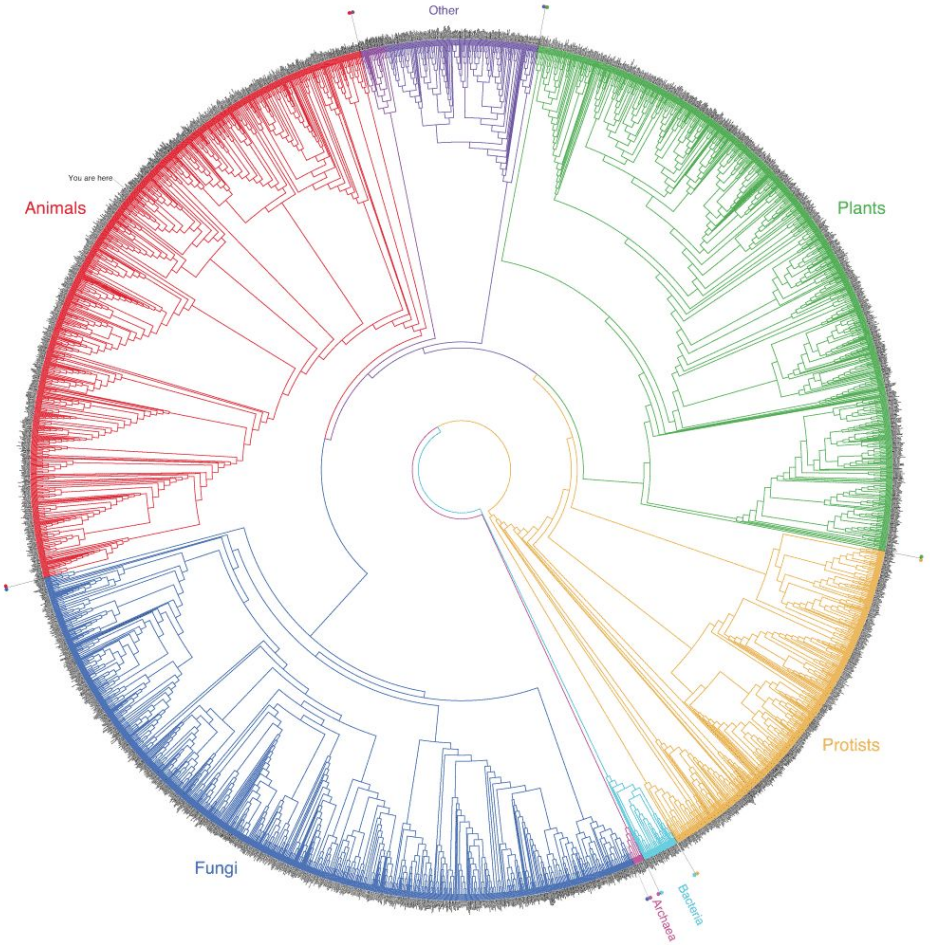
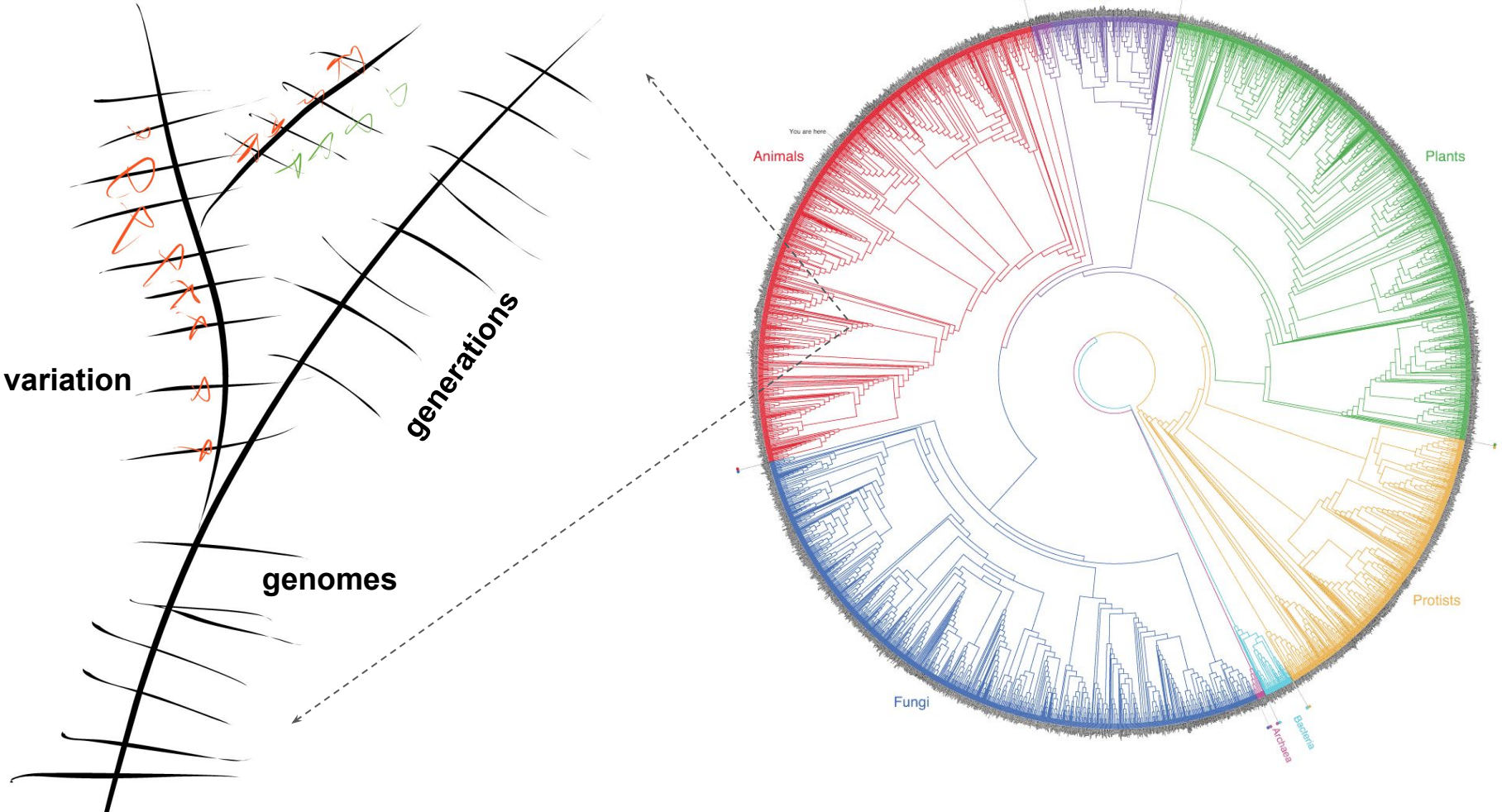
Computational Pangenomics #CPANG19

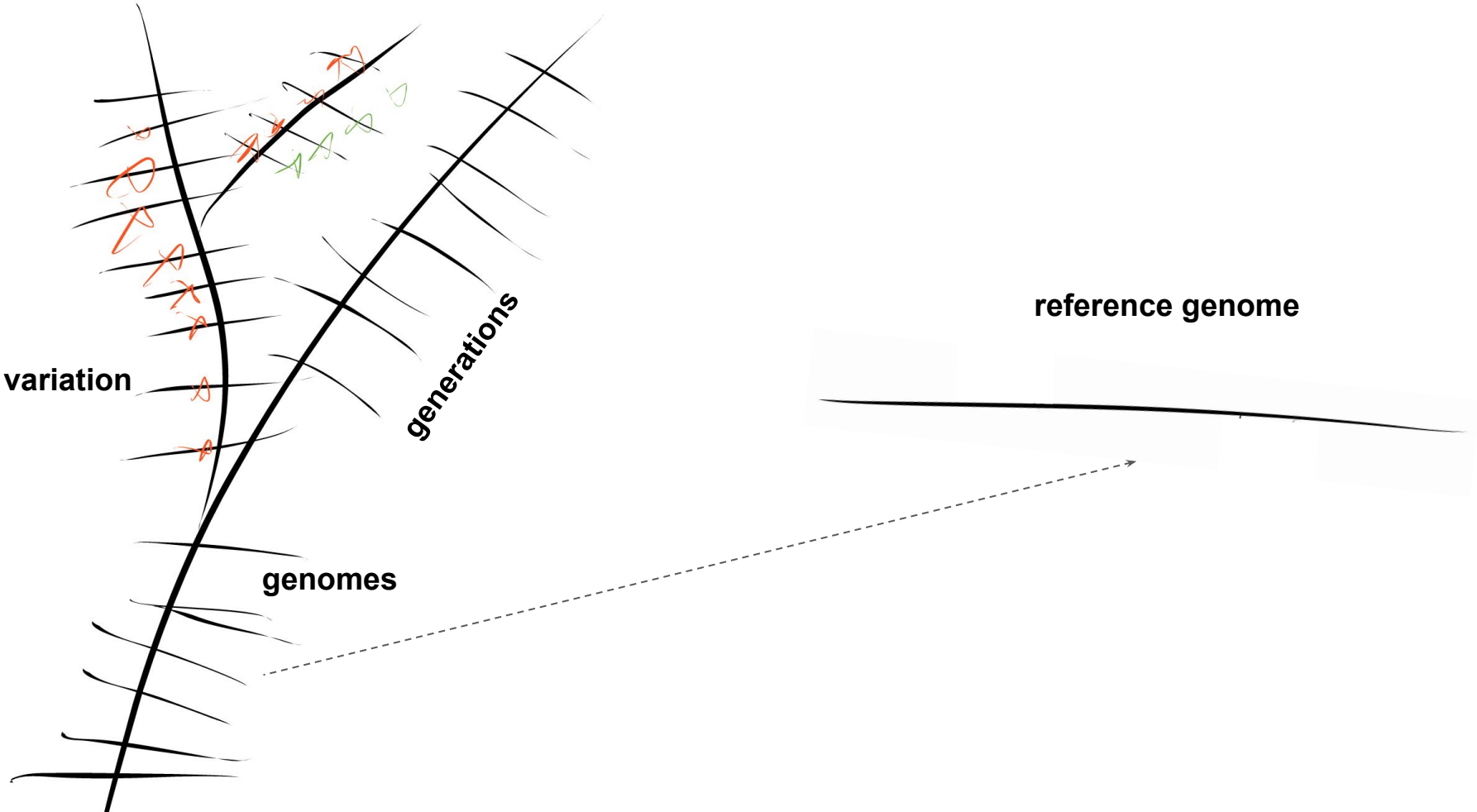
Day 1 (September 9, 2018)

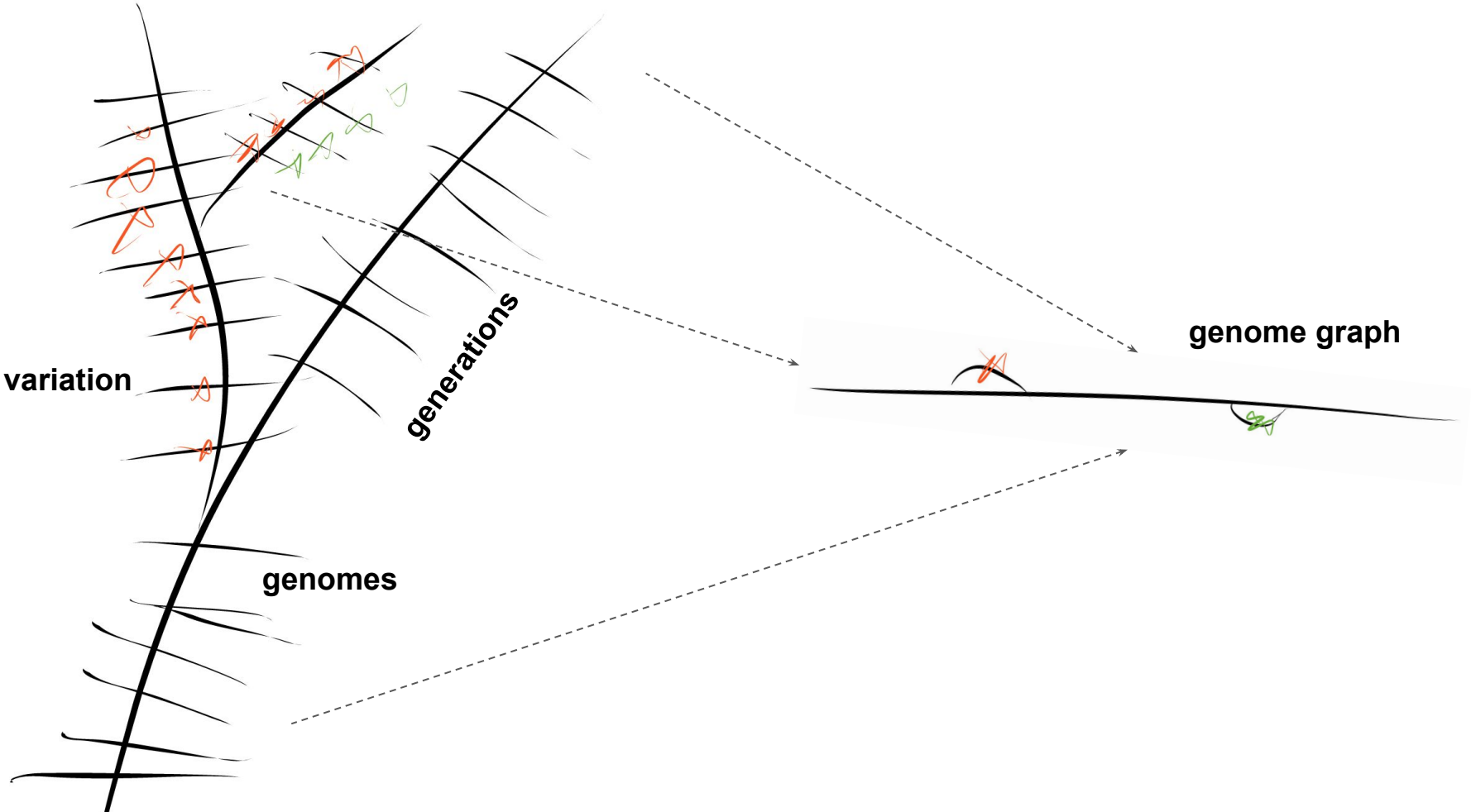
Erik Garrison and Mikko Rautiainen

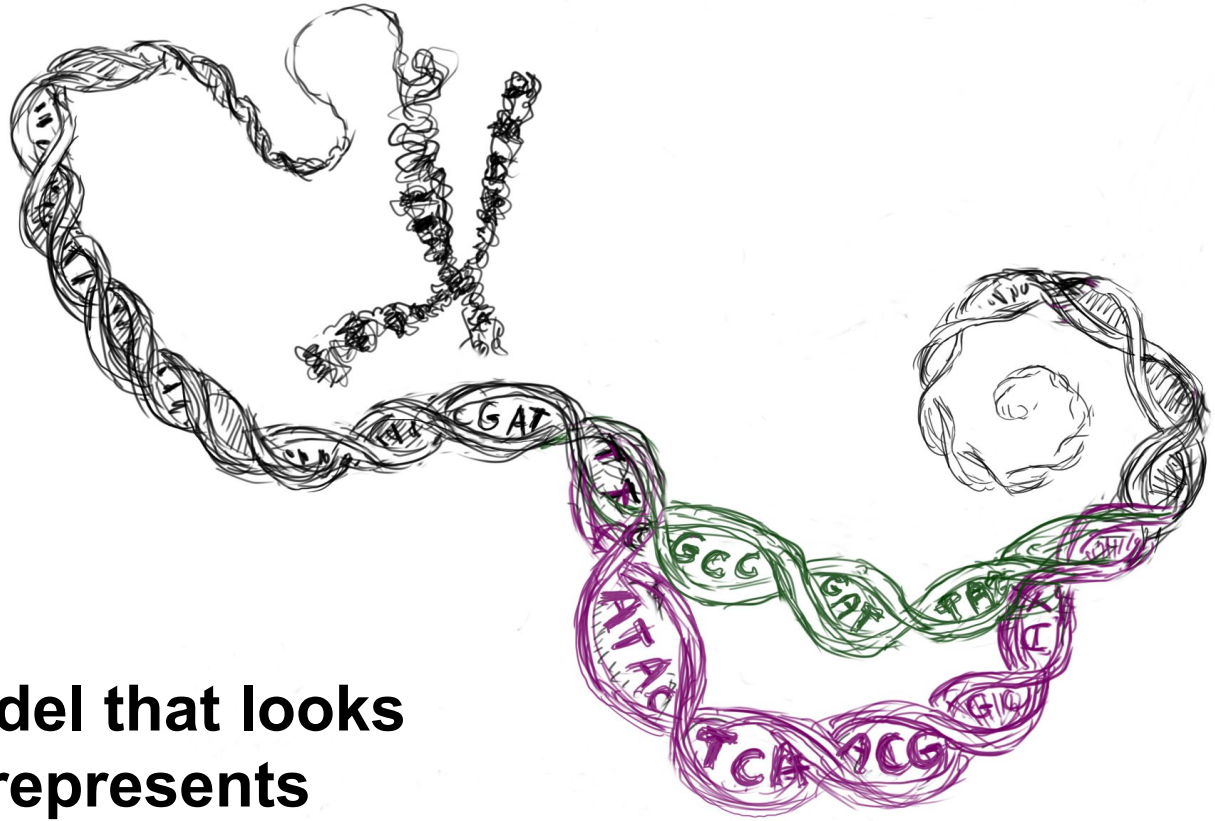
Genome variation graphs


Other
You are here
Animals
Plants
Protists
Fungi
Bacteria
Archaea


variation
generations
genomes
Other
You are here
Animals
Plants
Protists
Fungi
Archaea
Bacteria

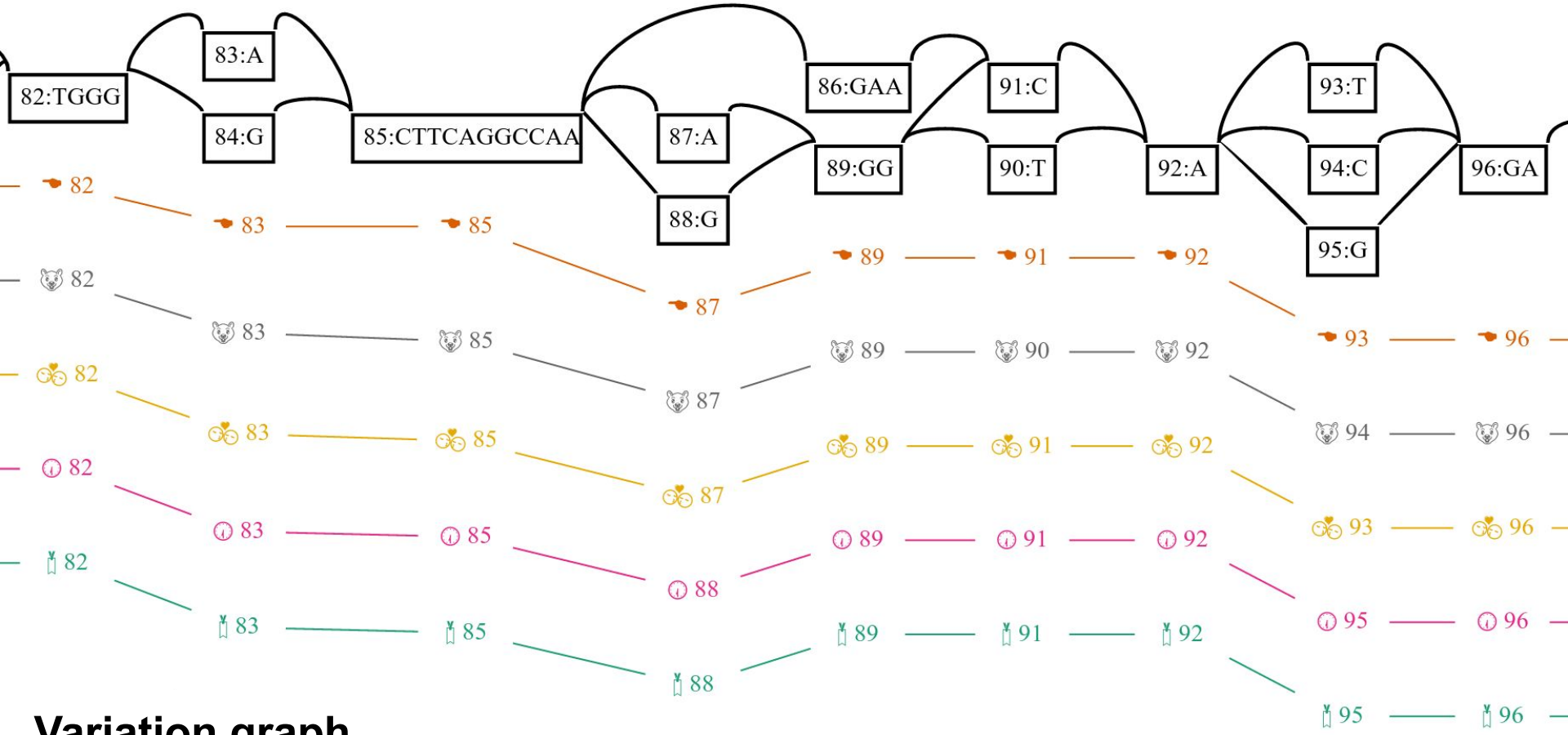

variation
generations
genomes
reference genome


variation
generations
genomes
genome graph

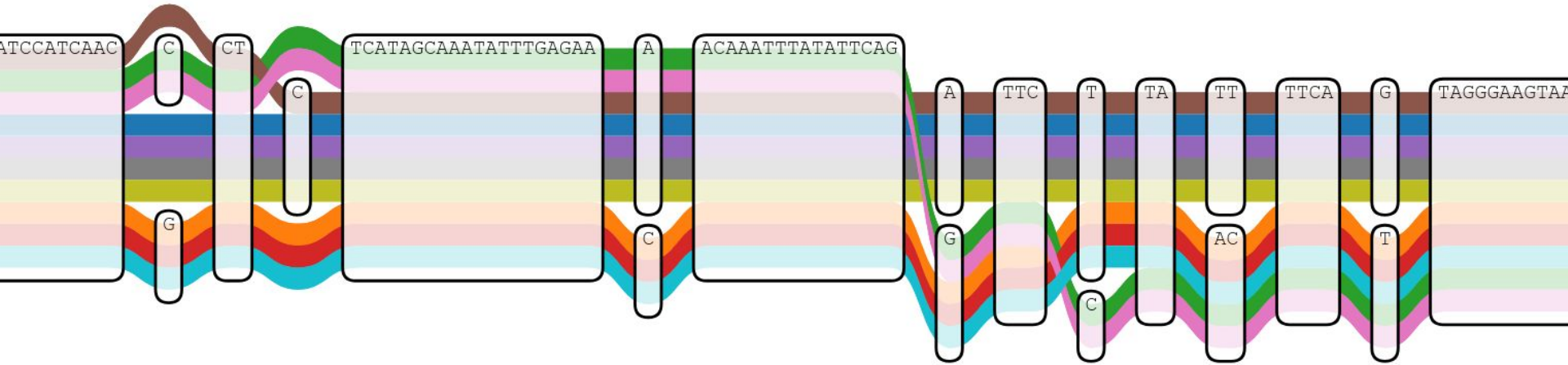


We want a model that looks like DNA, but represents many genomes at the same time.

Maciej Smuga-Otto
http://www.smuga-otto.com/mso/



Variation graph



Variation graph

https://vgteam.github.io/sequenceTubeMap/

Multiple sequence alignments ~ variation graphs

traditional MSA

(a)

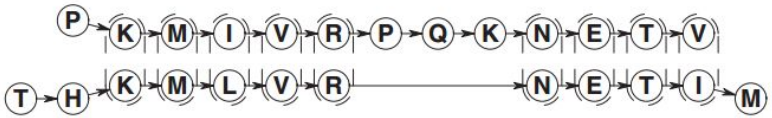
. . P K M I V R P Q K N E T V .
T H . K M L V R . . . N E T I M

consensus sequence

(b)

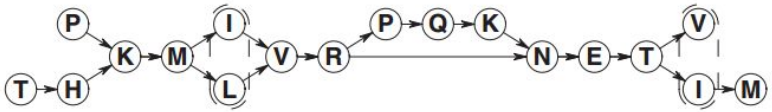
positionally-matching regions aligned

(c)



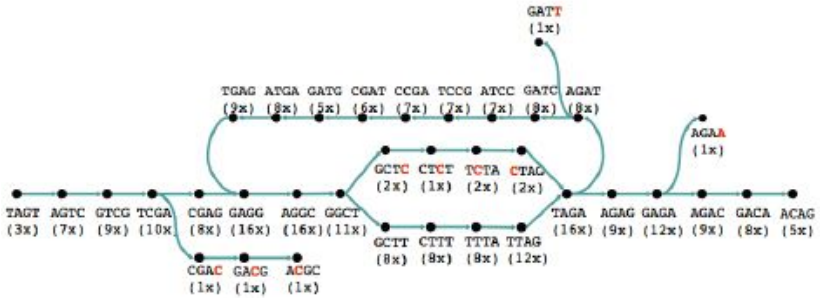
multiple sequence alignment

(d)

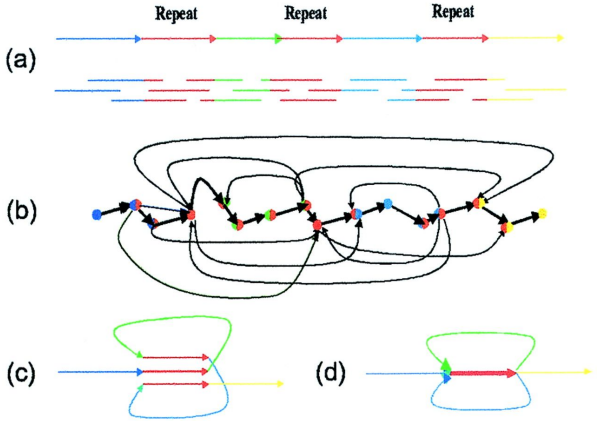


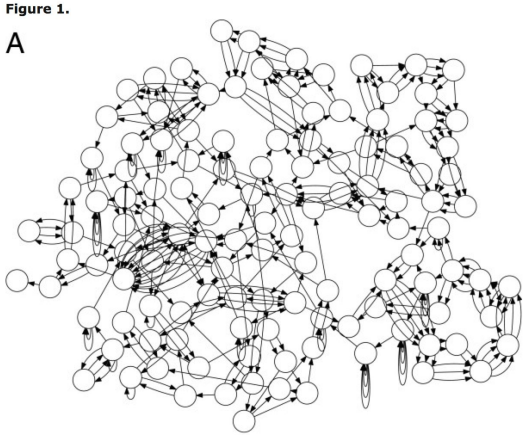
Christopher Lee, Catherine Grasso, Mark F. Sharlow. **Multiple sequence alignment using partial order graphs.** *Bioinformatics*, 2002.

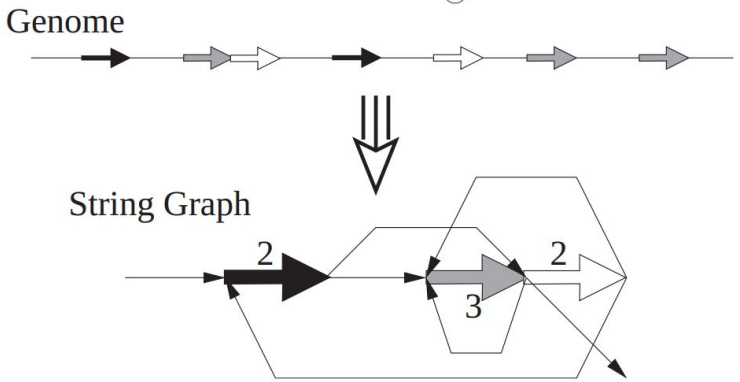
Assembly graphs ~ variation graphs



http://plus.maths.org/content/os/issue55/features/sequencing/index, credit Daniel Zerbino

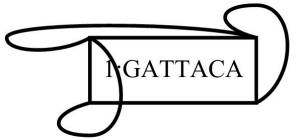






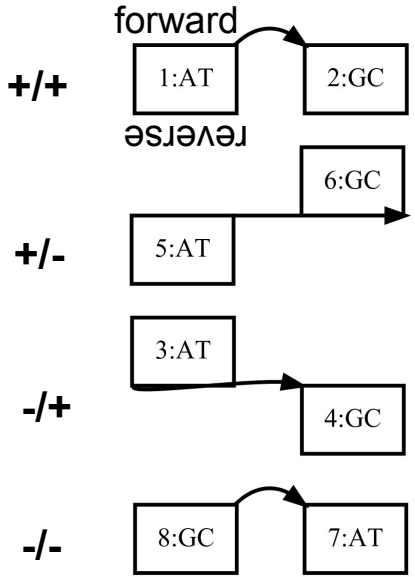
Eugene Myers. **The fragment assembly string graph**. *Bioinformatics*, 2005.

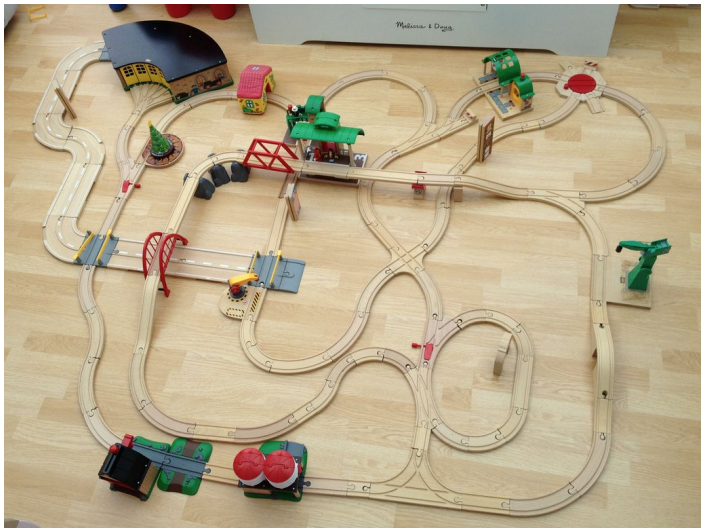
Train track graphs

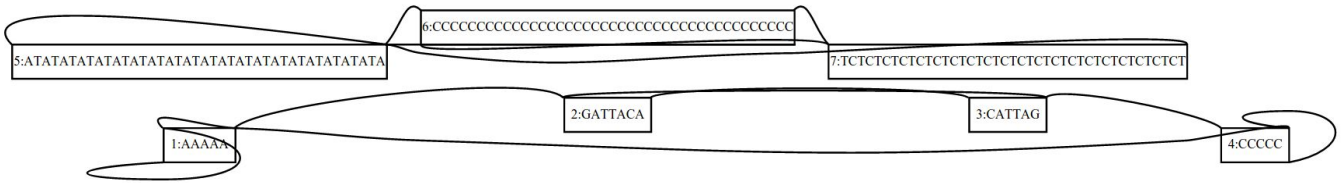


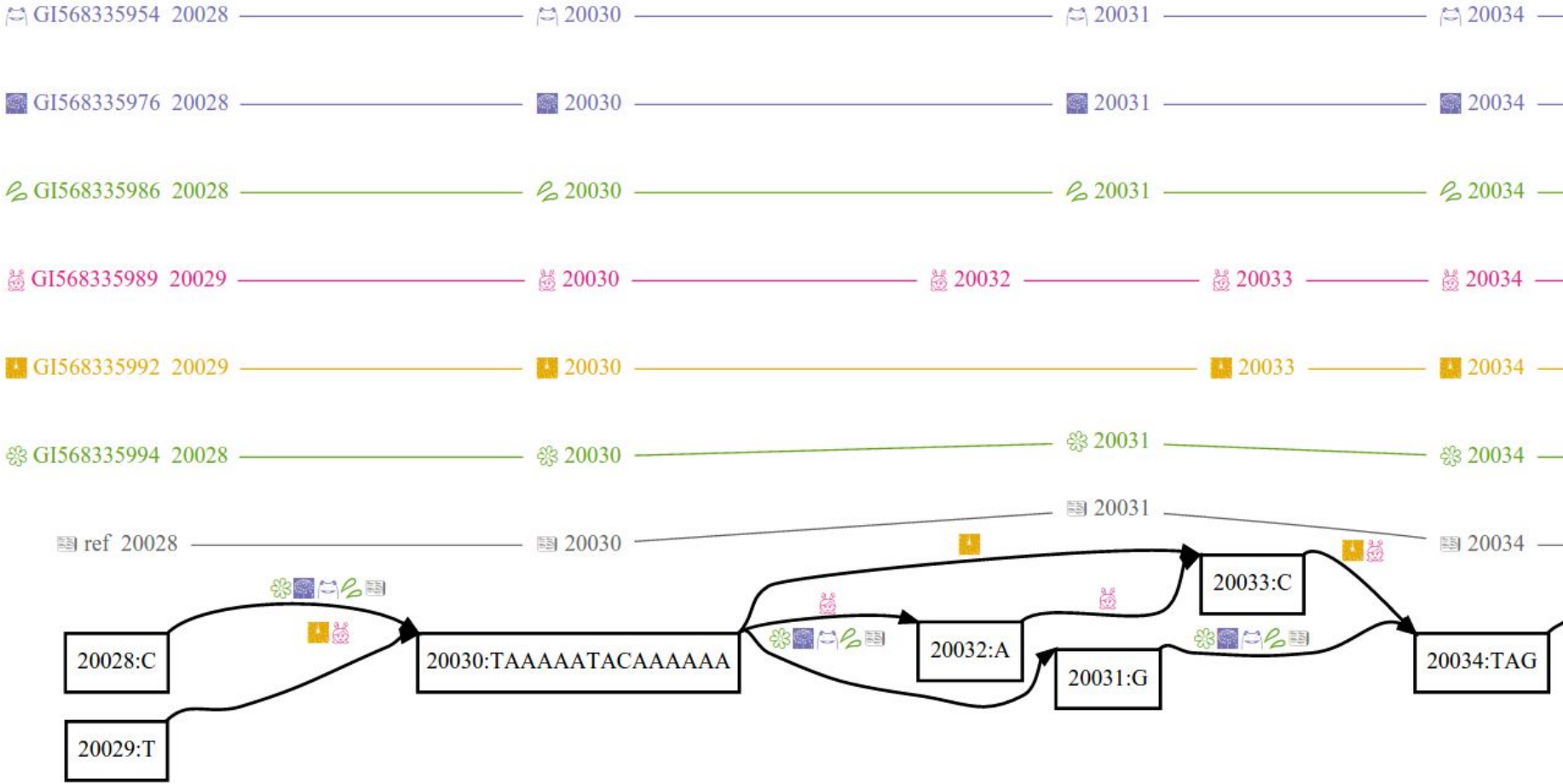
The graph is implicitly bidirectional, encoding both the forward and reverse complement.

Edges switching from the forward (+) to reverse (-) represent inversions.

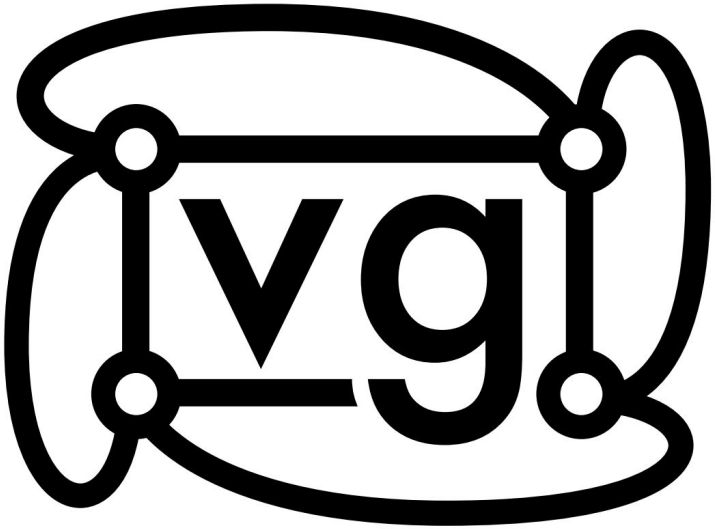




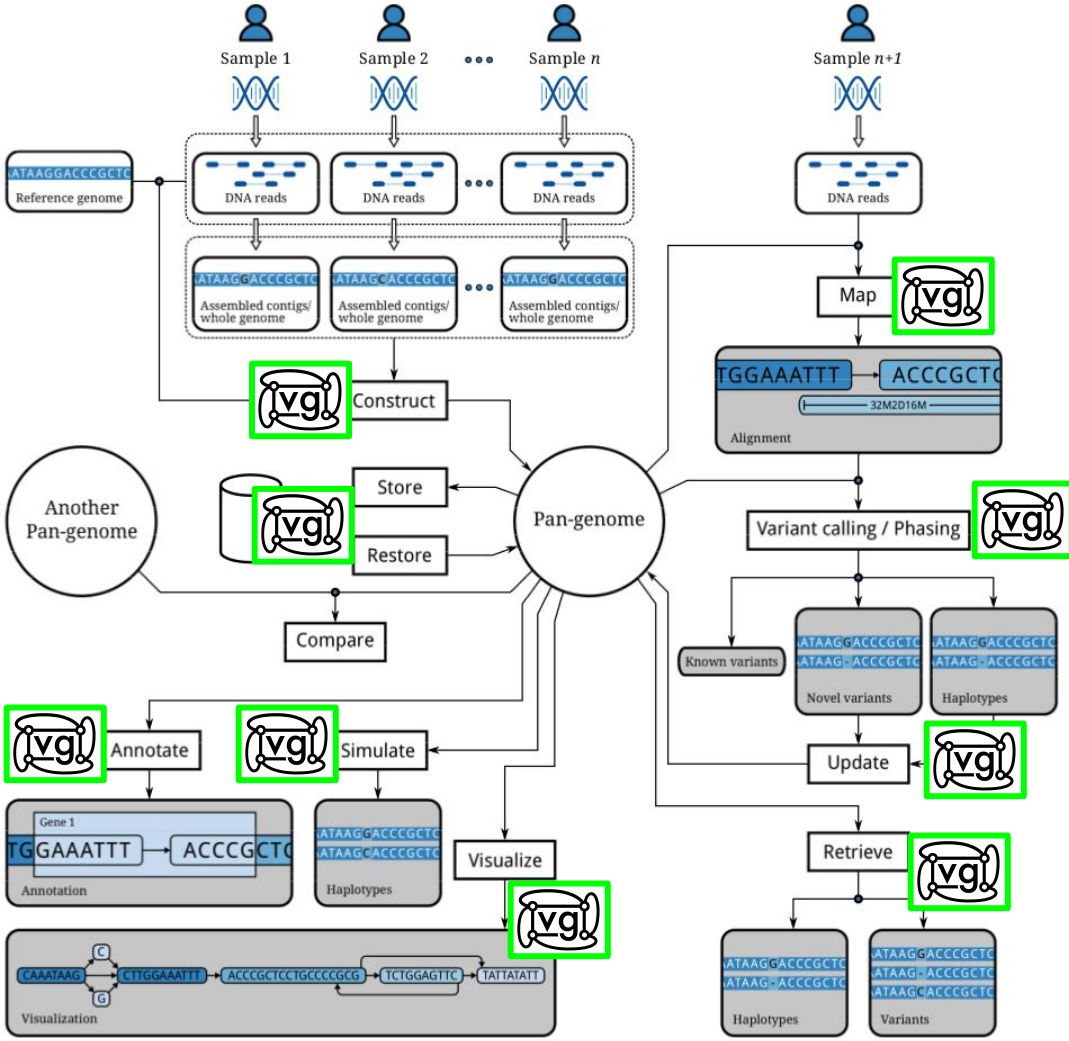




*a fragment of the MHC



github.com/vgteam/vg



Construction (from VCF)

```
POS  ID   REF  ALT
...
```

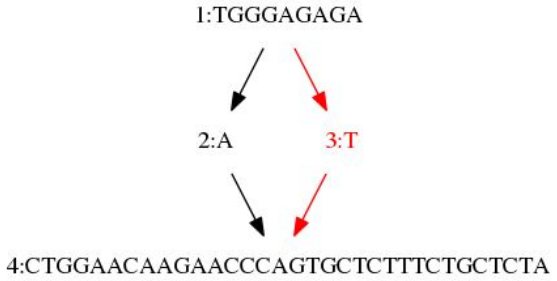
1:TGGGAGAGAACTGGAACAAGAACCCAGTGCTCTTTCTGCTCTA

For each variant

1. cut the reference path around the variant
2. add the novel (ALT) sequence to the graph

Construction (from VCF)

```
POS  ID   REF  ALT
10   .    A    T
...
```

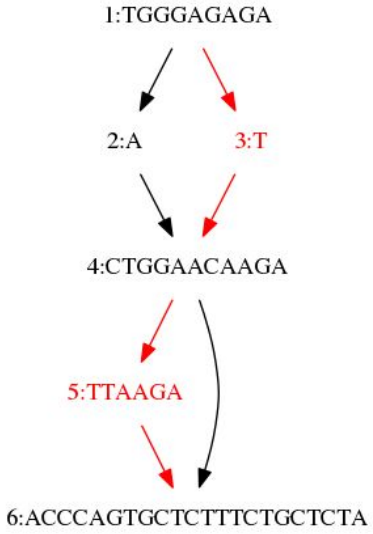


For each variant

1. cut the reference path around the variant
2. add the novel (ALT) sequence to the graph

Construction (from VCF)

```
POS  ID   REF  ALT
10   .    A    T
21   .    A    ATTAAGA
...
```



For each variant

1. cut the reference path around the variant
2. add the novel (ALT) sequence to the graph

Construction (from VCF)

For each variant

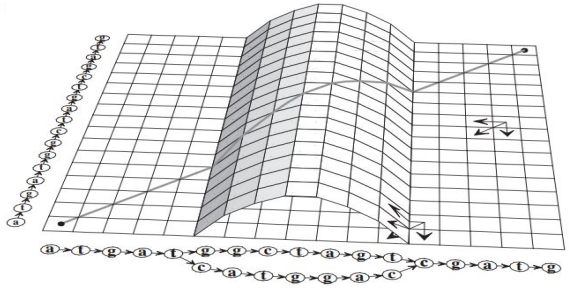
1. cut the reference path around the variant
2. add the novel (ALT) sequence to the graph

POS	ID	REF	ALT
10	.	A	T
21	.	A	ATTAAGA
31	.	TCTTT	T

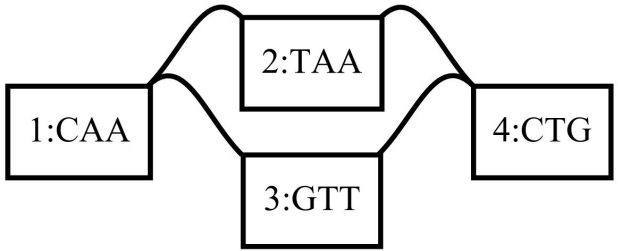
Local alignment to the graph

query:
CAAATTCT

	T	A	A
C	0	0	0
A	0	2	2
A	3	2	4
A	4	5	4
T	6	3	3
T	4	4	1
C	2	2	2
T	2	0	0



	C	A	A
C	**2**	0	0
A	0	**4**	2
A	0	2	**6**
A	0	2	4
T	0	0	2
T	0	0	1
C	2	0	0
T	0	0	0



	C	T	G
C	2	0	0
A	0	0	0
A	1	0	0
A	2	0	0
T	2	4	1
T	5	4	3
C	**10**	7	6
T	7	**12**	9

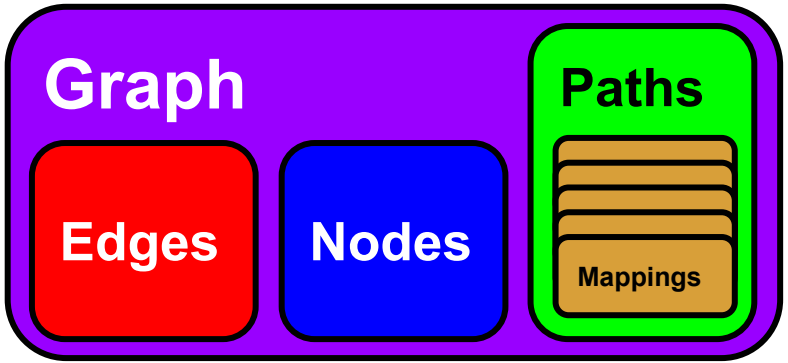
	G	T	T
C	0	0	0
A	0	0	0
A	3	2	1
A	**4**	1	0
T	2	**6**	3
T	0	4	**8**
C	0	2	5
T	0	2	4

1. fill the score matrixes
2. find the maximum score
3. trace back for alignment

scores:
match = 2
mismatch = 2
gap_open = 3
gap_extension = 1

Data model

Basic entity is a *Graph*:



Implemented in vg using protobuf, JSON, RDF, and GFA

Graph

```
// *Graphs* are collections of nodes and edges.
// They can represent subgraphs of larger graphs
// or be wholly-self-sufficient.
// Protobuf memory limits of 67108864 bytes mean we typically keep the size
// of them small generating graphs as collections of smaller subgraphs.
//
message Graph {
    repeated Node node = 1; // The `Node`s that make up the graph.
    repeated Edge edge = 2; // The `Edge`s that connect the `Node`s in the graph.
    repeated Path path = 3; // A set of named `Path`s that visit sequences of oriented `Node`s.
}
```

https://github.com/ekg/vg/blob/master/src/vg.proto

Node

```
// *Nodes* store sequence data.
message Node {
    string sequence = 1;   // Sequence of DNA bases represented by the Node.
    string name = 2;  // A name provides an identifier.
    int64 id = 3;     // Each Node has a unique positive nonzero ID within its Graph.
}
```

Edge

```
// *Edges* describe linkages between nodes. They are bidirected, connecting the
// end (default) or start of the "from" node to the start (default) or end of
// the "to" node.
//
message Edge {
    int64 from = 1; // ID of upstream node.
    int64 to = 2;   // ID of downstream node.
    bool from_start = 3; // If the edge leaves from the 5' (start) of a node.
    bool to_end = 4; // If the edge goes to the 3' (end) of a node.
    int32 overlap = 5; // Length of overlap between the connected `Node`s.
}
```

Path

```
// Paths are walks through nodes defined by a series of `Edit`s.
// They can be used to represent:
//    - haplotypes
//    - mappings of reads, or alignments, by including edits
//    - relationships between nodes
//    - annotations from other data sources, such as:
//          genes, exons, motifs, transcripts, peaks
//
message Path {
    string name = 1; // The name of the path.
    repeated Mapping mapping = 2; // describe the order and orientation in which the Path visits `Node`s.
    bool is_circular = 3; // Set to true if the path is circular.
    int64 length = 4; // Optional length annotation for the Path.
}
```

Mapping

```
// A Mapping defines the relationship between a node in system and another entity.
// An empty edit list implies complete match, however it is preferred to specify the full edit structure.
// as it is more complex to handle special cases.
//
message Mapping {
    Position position = 1; // The position at which the first Edit, if any, in the Mapping starts. Inclusive.
    repeated Edit edit = 2; // The series of `Edit`s to transform to region in read/alt.
    int64 rank = 5; // The 1-based rank of the mapping in its containing path.
}
```

Position

```
// A position in the graph is a node, direction, and offset.
// The node is stored by ID, and the offset is 0-based and
// counts from the start of the node in the specified orientation.
// The direction specifies which orientation of the node we are
// considering, the forward (as stored) or reverse complement.

message Position {
    int64 node_id = 1; // The Node on which the Position is.
    int64 offset = 2; // The offset into that node's sequence at which the Position occurs.
    bool is_reverse = 4; // True if we obtain the original sequence of the path by reverse complementing
    string name = 5; // If the position is used to represent a position against a reference path
}
```

Position

```
// Example:
//
//     seq+         G A T T A C A
//     offset+  → 0 1 2 3 4 5 6 7
//
//     seq-         C T A A T G T
//     offset-  → 0 1 2 3 4 5 6 7
//
// Or both at once:
//
//     offset-    7 6 5 4 3 2 1 0 ←
//     seq+         G A T T A C A
//     offset+  → 0 1 2 3 4 5 6 7
```

Edit

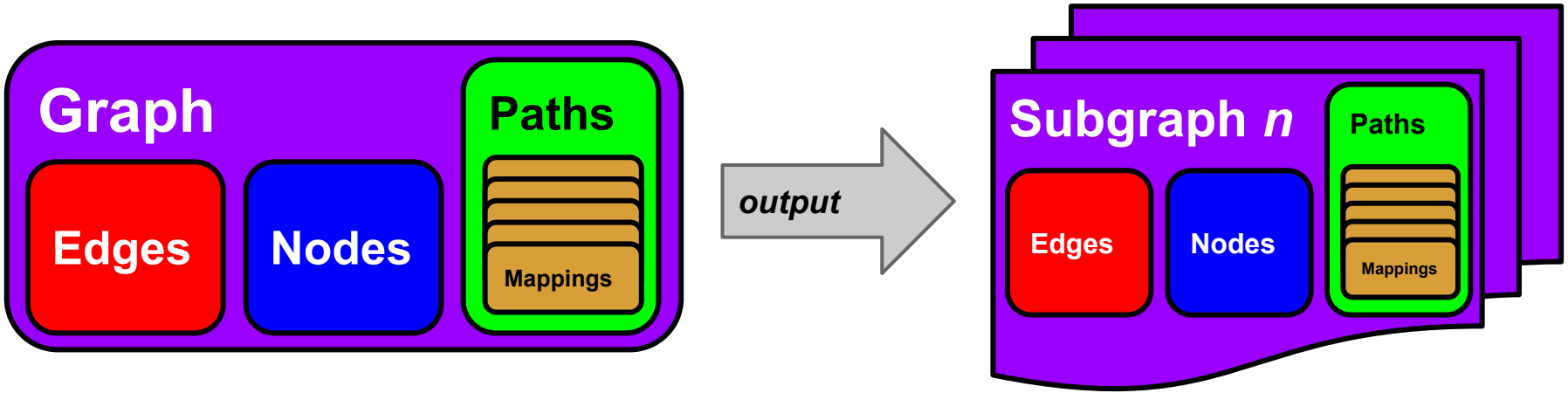
```
// Edits describe how to generate a new string from elements
// in the graph. To determine the new string, just walk the series of edits,
// stepping from_length distance in the basis node, and to_length in the
// novel element, replacing from_length in the basis node with the sequence.
//
//
// There are several types of Edit:
// - *matches*: from_length == to_length; sequence is empty
// - *snps*: from_length == to_length; sequence = alt
// - *deletions*: to_length == 0 && from_length > to_length; sequence is empty
// - *insertions*: from_length < to_length; sequence = alt
//
message Edit {
    int32 from_length = 1; // Length in the target/ref sequence that is removed.
    int32 to_length = 2; // Length in read/alt of the sequence it is replaced with.
    string sequence = 3; // The replacement sequence, if different from the original sequence.
}
```

Alignment

```
// Alignments link query strings, such as other genomes or reads, to Paths.
//
message Alignment {
    string sequence = 1; // The sequence that has been aligned.
    Path path = 2; // The Path that the sequence follows in the graph it has been aligned to
    string name = 3; // The name of the sequence that has been aligned. Similar to read name in BAM.
    bytes quality = 4; // The quality scores for the sequence, as values on a 0-255 scale.
    int32 mapping_quality = 5; // The mapping quality score for the alignment, in Phreds.
    int32 score = 6; // The score for the alignment, in points.
    ….
```

Serialization

To serialize the graph, we generate a stream of sub-graphs that can be reassembled into the whole.



How to resequence using vg

Import a graph: ***vg construct / vg view***

Index it: ***vg index***

Query it: ***vg find***

Sample it: ***vg sim***

Map to it: ***vg map***

Call variants: ***vg call***

Build a graph: ***vg msga***

Components of the vg toolchain

- Data model
  - https://github.com/vgteam/vg/blob/master/src/vg.proto
- VG C++ API
  - https://github.com/vgteam/vg/blob/master/src/vg.hpp
- XG (graph index)
  - https://github.com/vgteam/vg/blob/master/src/xg.hpp
- GCSA2 (sequence path index)
  - https://github.com/jltsiren/gcsa2

vg construct

tiny.fa

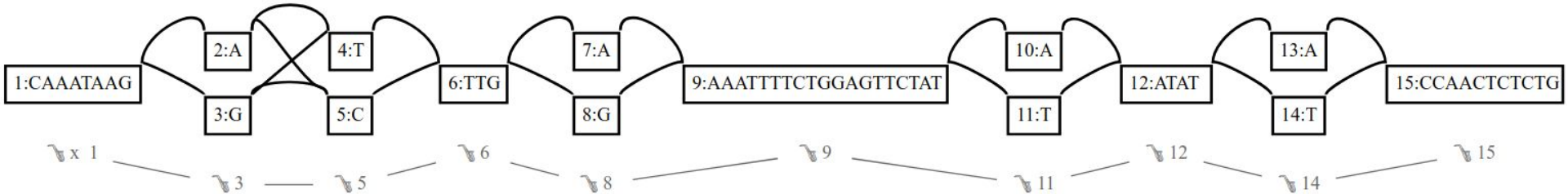
```
1:CAAATAAGGCTTGGAAATTTTCTGGAGTTCTATTATATTCCAACTCTCTG
```

tiny.vcf.gz

#CHROM	POS	REF	ALT
x	9	G	A
x	10	C	T
x	14	G	A
x	34	T	A
x	39	T	A

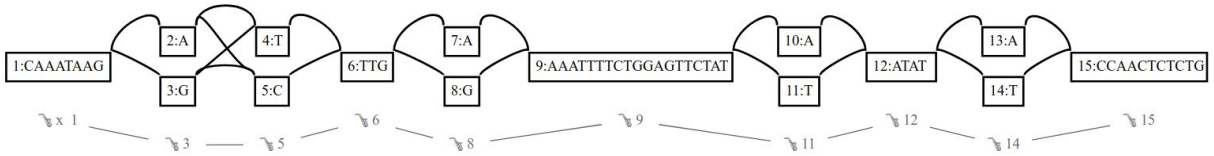
```
vg construct \
    -v tiny/tiny.vcf.gz \
    -r tiny/tiny.fa >tiny.vg
```

tiny.vg



vg index

tiny.vg



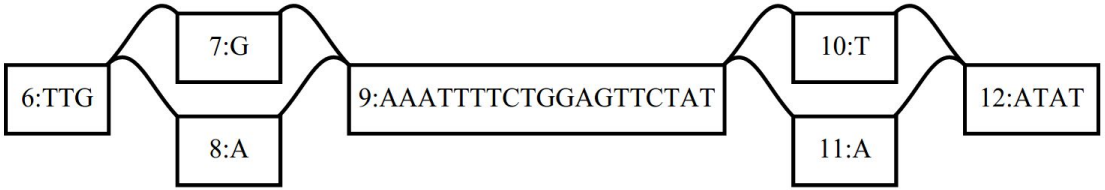

```
vg index tiny.vg \
    -x tiny.xg \
    -g tiny.gcsa -k 16
```

```
vg kmers -gk 16 tiny.vg | head -50
ATTTGGAAATTTTCTG        2:0     G       G       9:10
GTTTGGAAATTTTCTG        3:0     G       G       9:10
CAAATAAGATTTGAAA        1:0     #       A       9:2
GTTTGAAAATTTTCTG        3:0     G       G       9:10
ATTTGAAAATTTTCTG        2:0     G       G       9:10
GCTTGAAAATTTTCTG        3:0     G       G       9:10
TAAGATTTGAAAATTT        1:4     A       T       9:6
GCTTGGAAATTTTCTG        3:0     G       G       9:10
AATAAGATTTGAAAAT        1:2     A       T       9:4
CCTTATTTG$$$$$$$        3:-0    A,G     $       17:7
ACTTGAAAATTTTCTG        2:0     G       G       9:10
CTTGAAAATTTTCTGG        5:0     A,G     A       9:11
CAAATAAGATTTGGAA        1:0     #       A       9:2
CTTGGAAATTTTCTGG        5:0     A,G     A       9:11
AAATAAGATTTGAAAA        1:1     C       T       9:3
```

vg find

```
vg find -x tiny.xg \
    -p x:20-25 -c 1 \
   | vg view -d -
```

Query the nodes around x:20-25 in the reference path “x”.



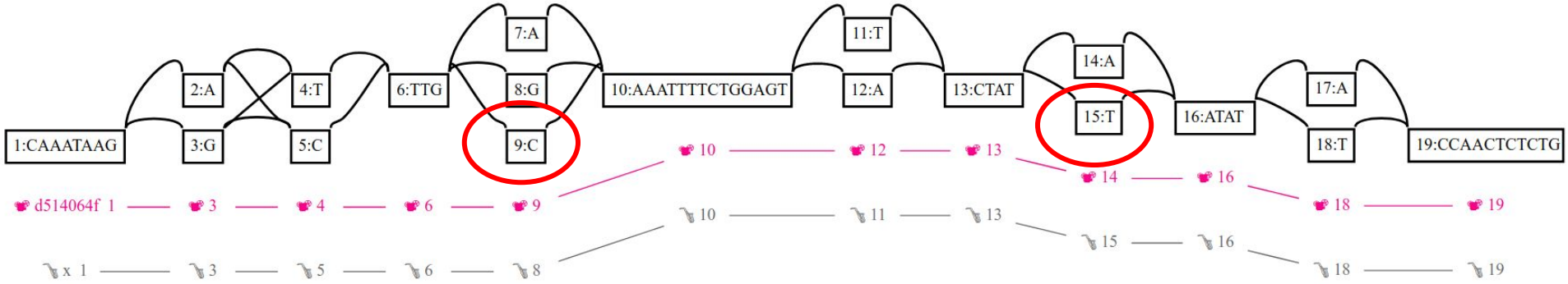

```
vg find -g tiny.gcsa \
    -S TCCAGAAAATTTTCAA
→ 9:-7
```

Query the position of a particular sequence in the GCSA2 index.

vg sim

Use a haplotype representing some variants relative to the tiny.vg to build a new graph:

```
vg msga -g tiny.vg -Nz \
    -s CAAATAAGGTTTGCAAATTTTCTGGAGTACTATAATATTCCAACTCTCTG \
    >truth.vg
```



We can then use it as a generative model and sample reads from it:

```
vg sim -l 50 -n 10 -s 1337 -x truth.xg >truth.reads
```

vg map

```
vg map -x tiny.xg -g tiny.gcsa -T truth.reads >aln.gam
```

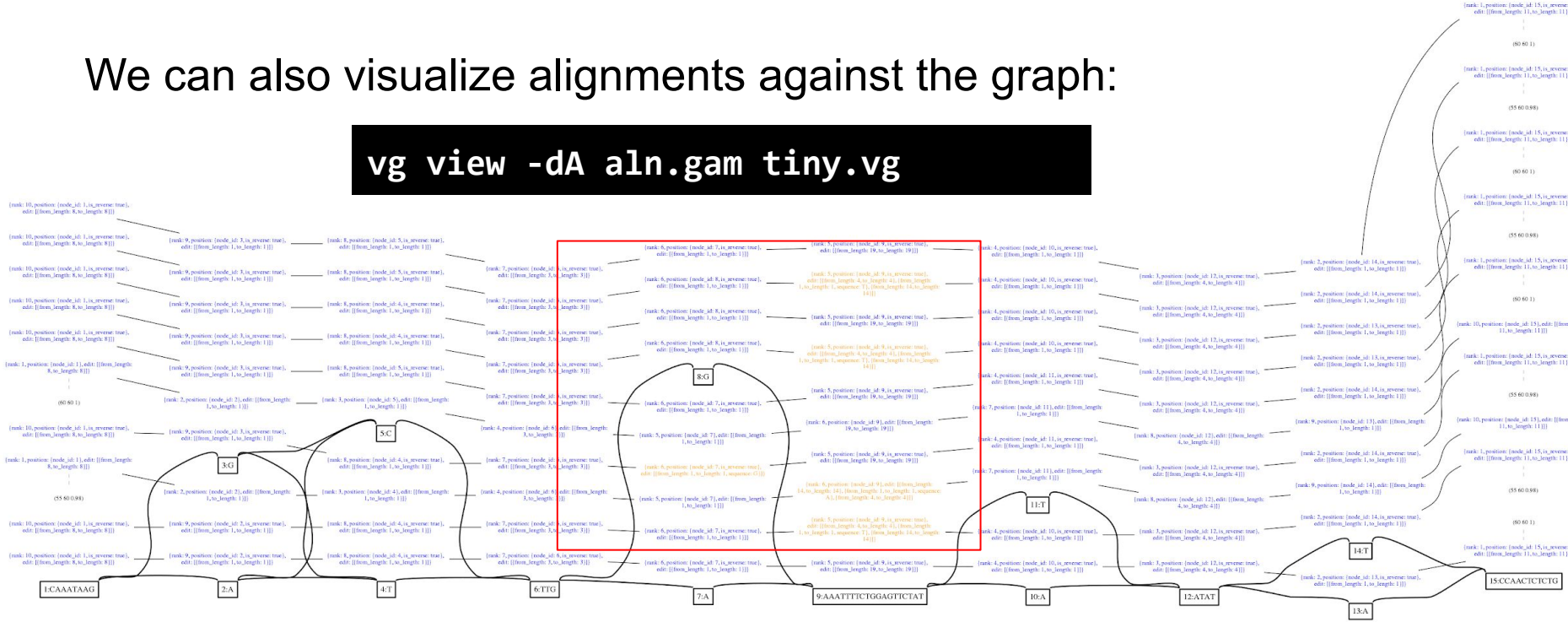

```
{
  "sequence": "CAGAGAGTTGGTATATTATAGAACTCCAGAAAATTTCCAAACCTTATTTG",
  "identity": 1,
  "path": {
    "mapping": [
      {
        "position": {
          "node_id": 15,
          "is_reverse": true
        },
        "edit": [
          {
            "from_length": 11,
            "to_length": 11
          }
        ],
        "rank": 1
      },
      {
        "position": {
          "node_id": 13,
          "is_reverse": true
        },
        "edit": [
          {
            "from_length": 1,
            "to_length": 1
          }
      .....
```


```
vg view -a aln.gam
```

alignment viz

We can also visualize alignments against the graph:

```
vg view -dA aln.gam tiny.vg
```



Blue represents perfect match.
Yellow represents a mismatch.

Day 2

Introduction to viral quasispecies

Subcommands

Introduce subcommands: surject, vectorize, msga, prune

Interleave: explain subcommands and have participants try them on toy examples from previous day

Practical: five virus mix

Day 3

Introduction to drug resistance in bacteria

Practical 1: Build a single-gene graph

- build a gene-model for gyrA and map reads to it for a handful of samples.
- Infer the sequence of this gene in each sample (vg call/mod)

Practical 2: Gene presence via assembly

- Assembly (minia3) of each sample, thread mcr-1/2/3 gene sequences through it to determine presence/absence of this colistin-resistance gene

Day 4

vg overview and practical

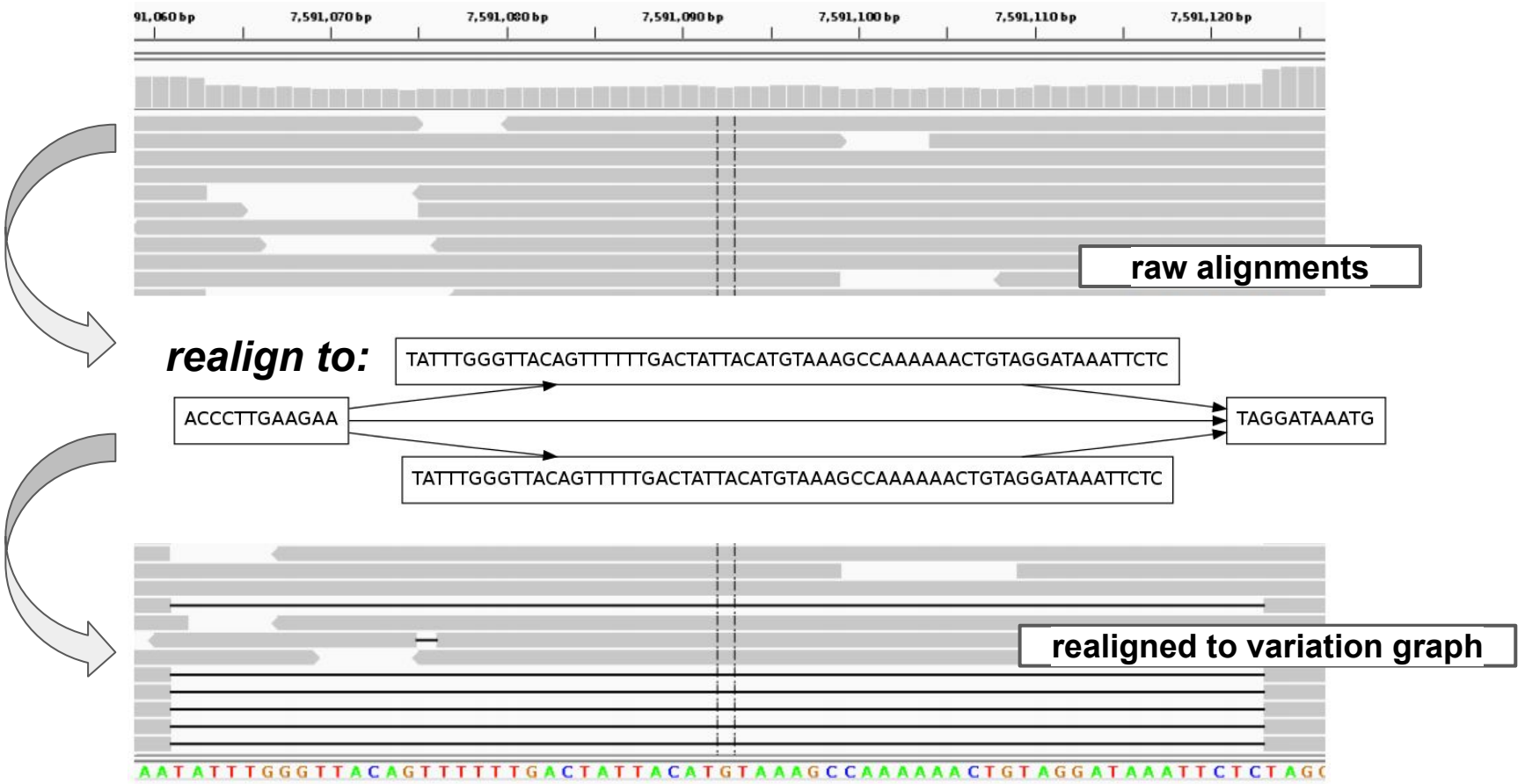
One slide per interesting subcommand, describing general idea:

Construct, view, index, find, sim, map, mpmap, **surject, msga, mod, prune, call**, **augment**, **vectorize, pack, chunk**, deconstruct, snarls, **explode**, concat, simplify, translate

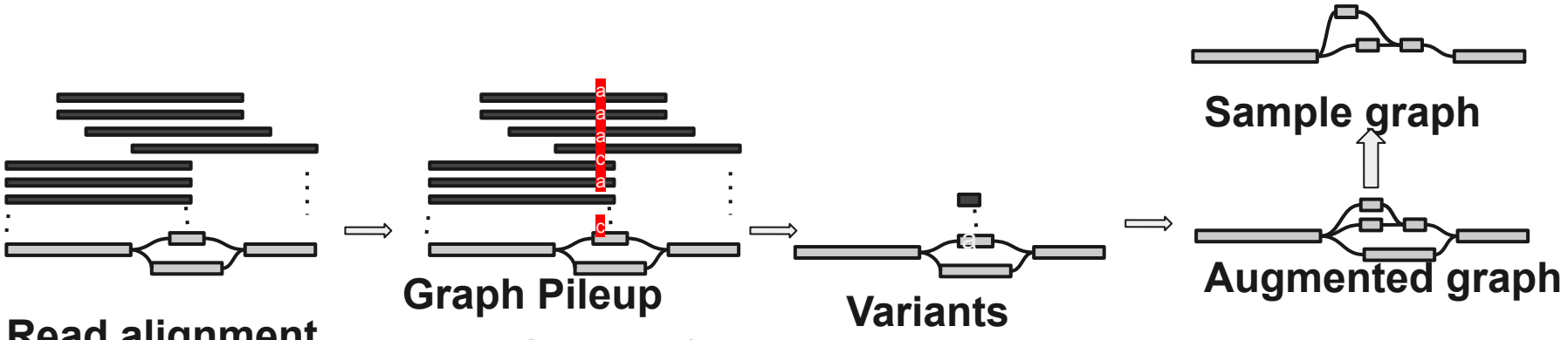
One slide showing an example use case.

Day 1: construct, view, index, find, sim, map
Day 2: surject, vectorize, msga, prune
Day 3: call, mod, pack, augment, chunk, explode
Day 4: snarls, mpmap, simplify, translate

When does a linear reference fail?



Variant calling on the graph



Read alignment

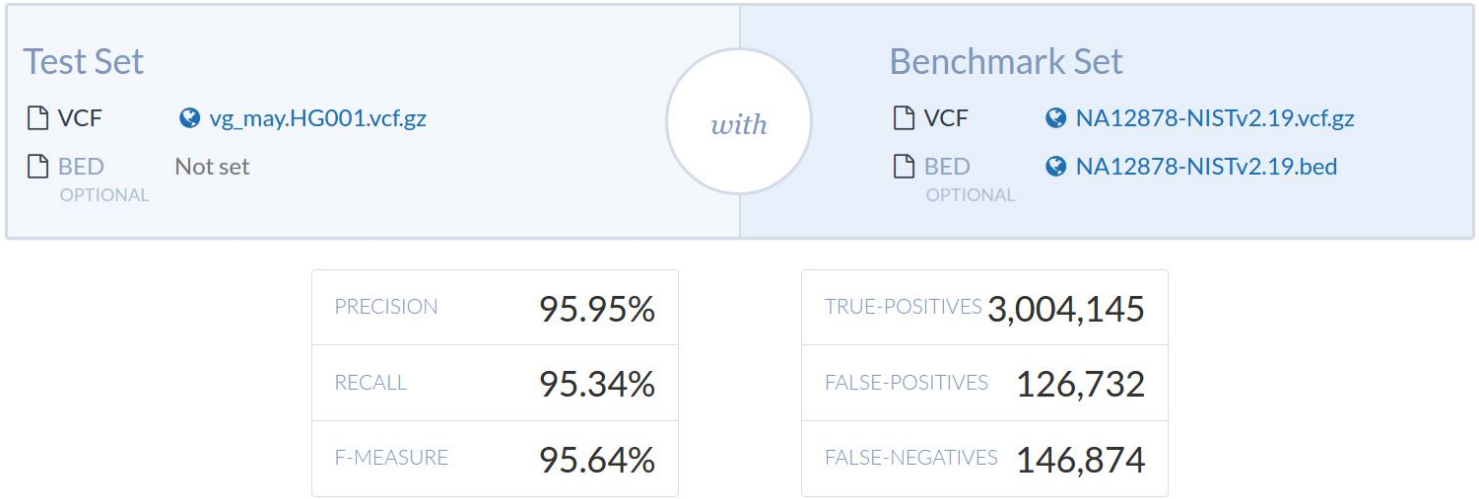
- GAM format
- from `vg map`

- VG pileup format
- from `vg pileup`

- VG format
- from `vg call`

Glenn Hickey, Adam Novak, Benedict Paten, Mike Lin

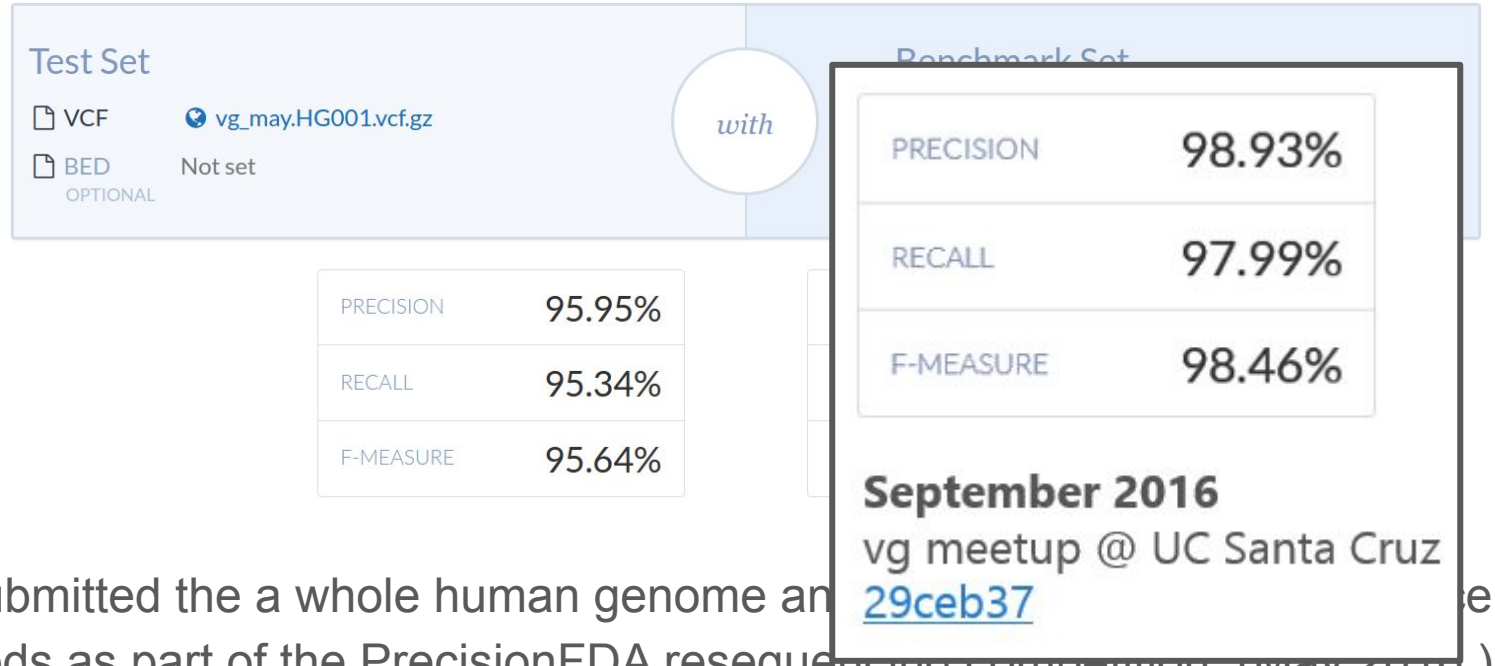
Whole human genome analysis pipeline



We submitted the a whole human genome analysis using variation reference methods as part of the PrecisionFDA resequencing competition. (May 2016.)

We did not win... but we did get a star for: ★ HEROIC-EFFORT

Whole human genome analysis pipeline



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A community evalution of reference graphs (in MHC)