VG cheat sheet 2017

How to construct, modify and view a variation graph with vg?

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Best viewed on Marp

Create a graph

From a multi FASTA sequences file:

```
% vg msga -f multi.fa > graph.vg
```

From a reference sequence FASTA file + VCF file:

```
% vg construct -r ref.fa -v var.vcf > graph.vg
```

If `--alt-paths` (`-a`) option is given, all variants in VCF will be treated as (very short) paths (one base length path for each SNP).

```
% vg construct -a -r ref.fa -v var.vcf > graph.vg
```

Note that, `vg` can consume FASTA, VCF and GFA1 format files only.

Modify a graph

Add variations in a VCF file to a graph by `vg add` with `-v` option:

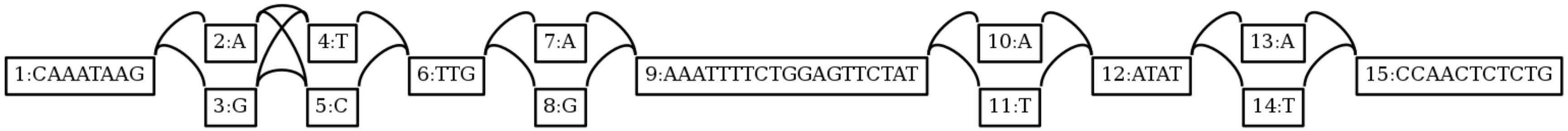
```
% vg msga -f multi.fasta > old.vg
% vg add -v add.vcf old.vg > new.vg
```

Path names are teken from FASTA identifiers by default. So, if you want to have multiple paths in a graph, you need to feed multiple sequences to `vg msga`.

View a graph (as an image)

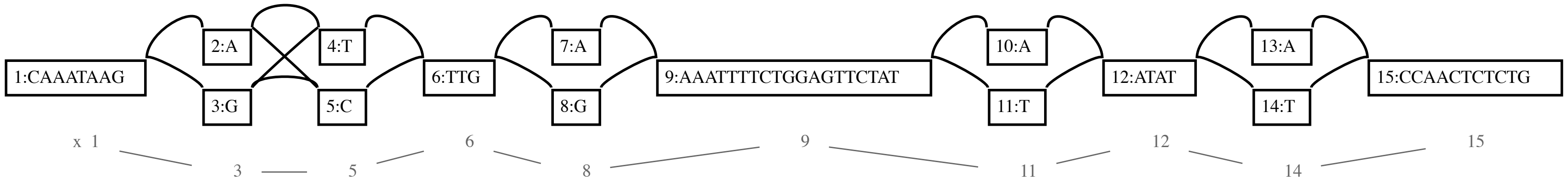
Output as PNG file (via a Graphviz `dot` command):

```
% vg view -d graph.vg | dot -Tpng -o graph.png
```



Output as SVG file (via a Graphviz `dot`) with paths (`-p` option):

```
% vg view -dp graph.vg | dot -Tsvg -o graph.svg
```



View a graph (for apps)

As a JSON format (use `jq` to make the output human friendly):

```
% vg view -j graph.vg
{"node": [{"sequence": "CAAATAAG", "id": 1}, {"sequence": "A",
```

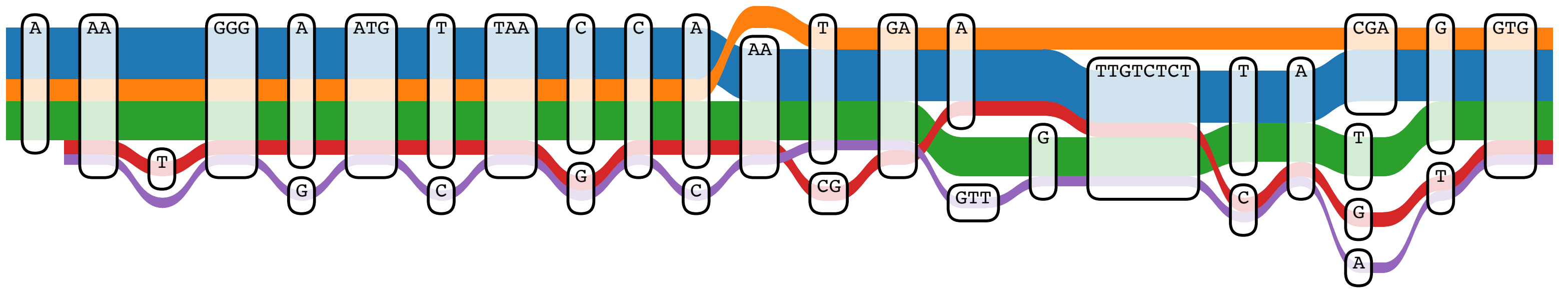
```
% vg view -j graph.vg | jq
{ "path": [
    { "name": "x",
      "mapping": [
        { "position": { "node_id": 1 },
          "edit": [
            { "from_length": 8,
              "to_length": 8
            }
          ],
          "rank": 1
        },
  :
```

View a graph (in web app)

View on the Tube Maps web application (via JSON output)

```
% vg view -j graph.vg > graph.json
```

Just upload JSON to https://wolfib.github.io/sequenceTubeMap/ at the Use Custom Data tab.



View a graph (default)

As a GFA (Graphical Fragment Assembly) format:

```
% vg view graph.vg
H       VN:Z:1.0
P       x       1+,3+,5+,6+,8+,9+,11+,12+,14+,15+       8M,1M,1
S       1       CAAATAAG
L       1       +       2       +       0M
L       1       +       3       +       0M
S       2       A
L       2       +       4       +       0M
L       2       +       5       +       0M
S       3       G
L       3       +       4       +       0M
L       3       +       5       +       0M
S       4       T
L       4       +       6       +       0M
S       5       C
  :
```

Application of vg in analysis pipeline

- vg construct - create a reference graph
- vg find - extract regions of interest
- vg sim - sample reads from simulation
- vg annotate - ??
- vg map - read alignment
- vg pileup - read depth
- vg call - variant calling
- vg mod - update reference
- vg view - visualization

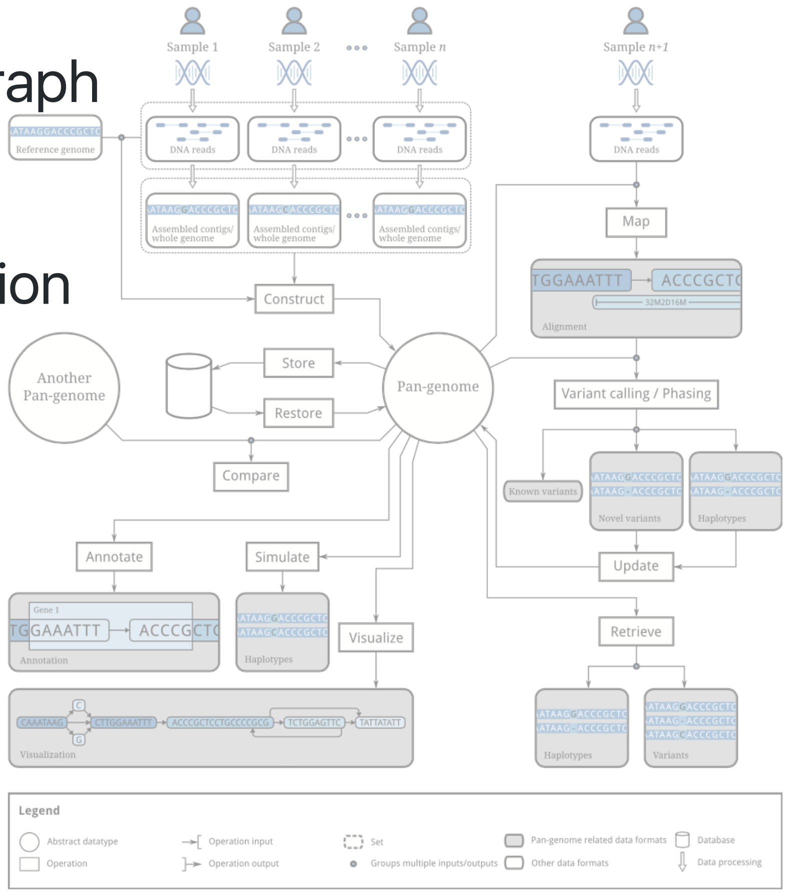


Figure 2. Illustration of operations to be supported by a pan-genome data structure.

Figure: Briefings in Bioinformatics 2016, doi:10.1093/bib/bbw089

Sub graph extraction

`xg` index created by `vg index` with `-x` option is used to find nodes and edges on a path (chr1) at a 0-based base position (123) or a region from (123) - to (456) bases.

```
% vg index -x graph.xg graph.vg
% vg find -x graph.xg -p chr1:123 | vg view -
{"node": [{"sequence": "CAAATAAG", "id": 1}], "edge": [{"from":
```

```
% vg find -x graph.xg -p chr1:123-456 | vg view -j - | jq
{ "node": [ { "sequence": "CCAACTCTCTG", "id": 15 } ],
  "edge": [ { "from": 13, "to": 15 }, { "from": 14, "to": 15 }
  "path": [
    { "name": "chr1",
      "mapping": [
        { "position": { "node_id": 15 },
          "rank": 10
        }
      ]
  :
```

Sequence matching

`GCSA2` index created by `vg index` with `-g` option is used to find nodes and positions which contains a given sub-sequence (need to specify the order of the De Bruijn graph with `-k` option).

```
% vg index -k 16 -g graph.gcsa graph.vg
% vg find -g graph.gcsa -S GAGT
9:10
15:-4
```

This means, sequences starting from

- the 11th base (10+1) on the node 9 and
- the 5th base (4+1) of the reverse complement strand on the node 15

match with the query (note that base count is 0-based).

Haplotype

`vg construct` uses VCF to make all possible edges (REF/ALT), then `vg index` uses VCF again to take samples' information. `-x` creates GPBWT (stable) index and `-G` creates GBWT (under dev) index.

```
#CHROM  POS  D  REF  ALT  ... FORMAT  1     2
x        9   .   G    A   ...     GT  1|0   1|0
x       10   .   C    T   ...     GT  1|1   0|1
x       14   .   G    A   ...     GT  1|0   0|1
x       34   .   T    A   ...     GT  1|1   1|1
x       39   .   T    A   ...     GT  1|0   0|1
```

Save as tiny2.vcf and apply bgzip/tabix (see installation). Samples can be phased or unphased but there was a bug when mixed.

```
% vg construct -v tiny2.vcf.gz -r tiny.fa > tiny2.vg
% vg index -v tiny2.vcf -x tiny2.xg -G tiny2.gbwt -p tiny2.vg
```

Haplotype (continued)

`vg find` with `-t` returns all threads (paths) of haplotypes

```
% (vg find -x tiny2.xg -t; cat tiny2.vg) | vg view -
  :
P    _thread_1_x_0_0    1+,2+,4+,6+,7+,9+,10+,12+,13+,15+    8M
P    _thread_1_x_1_0    1+,3+,4+,6+,8+,9+,10+,12+,14+,15+    8M
P    _thread_2_x_0_0    1+,2+,5+,6+,8+,9+,10+,12+,14+,15+    8M
P    _thread_2_x_1_0    1+,3+,4+,6+,7+,9+,10+,12+,13+,15+    8M
  :
```

`vg find` with `-p` to specify a specific haplotype with the prefix

```
% (vg find -x tiny2.xg -q _thread_2; cat tiny2.vg) | vg view -
  :
P    _thread_2_x_0_0    1+,2+,5+,6+,8+,9+,10+,12+,14+,15+    8M
P    _thread_2_x_1_0    1+,3+,4+,6+,7+,9+,10+,12+,13+,15+    8M
  :
```

TODO: check why cat is needed; how to use `.gbwt` instead of `.xg`.

Read mapping

First, generate some simulated reads with length 30 for test.

```
% vg sim -n 2 -l 30 -x graph.xg graph.vg
TGGAATATTATAGAACTCCAGAAAATTTTC
TATAGAACTCCAGAAAATTTCCAAGCCTTA
```

Then map one of those reads and store the results in a GAM format file (GAM is vg's BAM equivalent).

```
% vg map -g graph.gcsa -x graph.xg -s TGGAATATTATAGAACTCCAGAAAA
% vg view -a graph.gam
{"mapping_quality": 60, "sequence": "TGGAATATTATAGAACTCCAGAAAAT
```

Note that, the `-d` option is provided as a shortcut to specify a prefix for `.xg` and `.gcsa` index files.

```
% vg map -d graph -s TGGAATATTATAGAACTCCAGAAAATTTTC -j
```

Multiple read mapping

Generates more simulated reads.

```
% vg sim -l 30 -n 100 -x graph.xg graph.vg > graph.reads
```

Then map those reads to a graph.

```
% vg map -d graph -T graph.reads > graph.gam
```

And make a pileup.

```
% vg pileup graph.vg graph.gam > graph.pileup
```

Then call and store the result into a VCF file.

```
% vg call graph.vg graph.pileup > call.vcf
```

RDF/Turtle dump of a graph (SemWeb)

```
% vg view -t graph.vg > graph.ttl
```

Schema of RDF for VG consists of three classes and predicates.

- `:Node` node:1 (http://example.org/vg/node/1)
  - `rdf:value` "ATGC" (DNA sequence fragment)
  - `:linksForwardToForward`, `:linksForwardToReverse`, `:linksReverseToForward`, `:linksReverseToReverse` node:2
- `:Step` step:x-1 (http://example.org/vg/step/x-1)
  - `:path` path:x (path identifier; arbitrary)
  - `:node` node:1 (node identifier; 1-based)
  - `:rank` 1 (order of the nodes on the path; 1-based)
  - `:position` 0 (sequence position on the path; 0-based)
- `:Path` path:x (http://example.org/vg/path/x)

SPARQL for RDF graph

RDF model contains all the variation graph structure and sequences of nodes, which can be concatenated in the order of rank of steps along with the path to reconstruct the original sequence.

```
PREFIX vg: <http://example.org/vg/>
PREFIX rdf: <http://www.w3.org/1999/02/22-rdf-syntax-ns#>
SELECT ?path (GROUP_CONCAT(?subseq; separator='') AS ?sequence)
WHERE {
  ?step vg:path ?path ;
        vg:node ?node ;
        vg:rank ?rank .
  ?node rdf:value ?subseq .
}
GROUP BY ?path
ORDER BY ?rank
```

Installation

Codebase: https://github.com/vgteam/vg/

```
% git clone https://github.com/vgteam/vg.git
```

VG docker: https://github.com/vgteam/vg_docker

It is recommended to use Docker for resolving dependencies (need to give >3~4GB memory to docker; 2GB was not enough to build).

```
% git clone https://github.com/vgteam/vg_docker.git
% cd vg_docker
% docker build -t vg -f Dockerfile.build .
```

Installation (optional)

You may want to add following line to Dockerfile for handling VCF files and PNG/SVG images:

```
RUN apt-get install -y tabix graphviz
```

You need to use bgzip in htslib for making a tabix indexed VCF file compressed. On Mac with homebrew:

```
% brew install htslib
% bgzip test.vcf
% tabix -p vcf test.vcf.gz
```

Running vg on docker

Suppose if you tagged (named) the image as `vg` and mount the `~/vg` folder in your home directory to `/opt` inside the docker:

```
% docker run --rm -t -i -v ~/vg:/opt vg /bin/bash
```

You can put your data files in the `/opt` directory (`~/vg` on the host) to make them persistent.

```
root@e7de87ec79ea:/vg# vg
vg: variation graph tool, version v1.5.0-272-g187be7c

usage: vg <command> [options]

commands:
  -- add            add variants from a VCF to a graph
  -- annotate       annotate alignments with graphs and graphs w
  :
```

Other options

```
usage: vg <command> [options]

commands:
  -- add            add variants from a VCF to a graph
  -- annotate       annotate alignments with graphs and graphs w
  -- call           call variants on a graph from a pileup
  -- chunk          split graph or alignment into chunks
  -- construct      graph construction
  -- explode        split graph into connected components
  -- genotype       Genotype (or type) graphs, GAMS, and VCFs.
  -- homogenize     homogenize augmented graphs
  -- index          index graphs or alignments for random access
  -- mod            filter, transform, and edit the graph
  -- sift           Filter Alignments by various metrics relatec
  -- simplify       graph simplification
  -- snarls         compute snarls and their traversals
  -- deconstruct    convert a graph into VCF relative to a refer
  -- view           format conversions for graphs and alignments
  -- vectorize      transform alignments to simple ML-compatible
  -- find           use an index to find nodes, edges, kmers, or
  -- paths          traverse paths in the graph
  -- align          local alignment
  -- map            global alignment
  -- stats          metrics describing graph properties
  -- join           combine graphs via a new head
  -- ids            manipulate node ids
  -- concat         concatenate graphs tail-to-head
  -- kmers          enumerate kmers of the graph
  -- sim            simulate reads from the graph
  -- mod            filter, transform, and edit the graph
  -- homogenize     homogenize long variants in the graph to imp
  -- surject        map alignments onto specific paths
  -- msga           multiple sequence graph alignment
  -- pileup         build a pileup from a set of alignments
  -- genotype       compute genotypes from aligned reads
  -- compare        compare the kmer space of two graphs
  -- circularize    circularize a path within a graph.
  -- translate      project alignments and paths through a graph
  -- validate       validate the semantics of a graph
  -- sort           sort variant graph using max flow algorithm
  -- test           run unit tests
  -- version        version information
```

Advanced options for `vg construct`

`-t, --threads N` use N threads to construct graph (defaults to numCPUs)

If you have multiple cores on your computer, use `--threads` (`-t`) to specify number of threads.

```
% vg construct -t 10 -r ref.fa -v var.vcf > graph.vg
```

`-n, --rename V=F` rename contig V in the VCFs to contig F in the FASTAs (may repeat)

If the sequence names in the reference FASTA and VCF differs, convert them with `--rename` (`-n`) option.

```
% vg construct -n 1:hg19_chr1 -r ref.fa -v var.vcf > graph.vg
```

Other options for `vg construct`

```
usage: vg construct [options] >new.vg

    -R, --region REGION   specify a particular chromosome or 1-
    -C, --region-is-chrom don't attempt to parse the region (us
                          sequence name could be inadvertently
    -z, --region-size N   variants per region to parallelize
    -m, --node-max N      limit the maximum allowable node sequ
                          nodes greater than this threshold wil
                          Note: nodes larger than ~1024 bp can'
    -p, --progress        show progress
    -S, --handle-sv       include SVs in construction of graph.
    -I, --insertions FILE a FASTA file containing insertion seq
                           (referred to in VCF) to add to graph
    -f, --flat-alts N     don't chop up alternate alleles from
```

Advanced options for `vg xxx`

To be written

```
% vg xxx
```

Other options for `vg xxx`

To be written

```
% vg xxx
```

Misc notes

`vg align` is for testing local alignment functions.

```
% vg align -s TGGAATATTATAGAACTCCAGAAAATTTTC graph.vg > graph.g
% vg view -a graph.gam
```

To be added