Gen: a version control system for biology

Abstract

. . . (write me in assets/pandoc_metadata.yaml)

Introduction

The conventional digital representation of a genetic sequence as a string of characters assumes that a species is sufficiently defined by a single reference genome. As whole genome sequencing has become more accessible, limitations of this model have become increasingly apparent. The field of *pangenomics* has emerged in response, bringing software tools and data models that represent genetic diversity from thousands of individuals at once in the form of a graph structure [@eizengaPangenomeGraphs2020]. Thus far, these tools have been predominantly applied to fundamental biology, but a similar problem exists in engineering biology. Developing a microbial strain, plant cultivar, cell line, or engineered protein also involves hundreds to thousands genetic variants obtained from environmental samples, breeding, mutagenesis, or genetic engineering. An additional challenge for genetic engineering is the distinction between *observed* and *intended* sequence variants. Digital tools tend to emphasize one type over the other: a synthetic biology workflow may involve DNA sequencing to confirm that an intended genome edit took place, but discard any additonal observed mutations. Likewise, a variant calling pipeline may identify all observed mutations, but lose the engineering context.

Sequence management in engineering biology is further complicated by the iterative nature of the development process. Organisms or biological parts are commonly subject to multiple, sequential rounds of engineering to arrive at a desired state. Pangenomes are an underutilized but highly useful tool for modeling iterative engineering. A graph structure can also represent sequence edits made over time, in addition to the variants that are studied in one experiment. However, the existing data models are not optimal for representing this type of iterative engineering. Thus, we set out on a data model which could be a common, growable database of both reference and sample-specific sequences.

While not directly related to pangenomes, we saw another opportunity to couple graph models with another common need in synthetic biology – tracking the

engineering process itself. The current state of sharing the evolution of a strain to its final state is ad-hoc. We saw that the addition of variants to a graph can be captured as a set of changes. And if we can annotate these sets of changes, we are able to devise a version control system for genetic sequences analogous to *git* in software engineering. Such a system enables workflows common in software engineering, such as continuous integration and code review. Additionally, this naturally links the process of genetic engineering with tracking the work that was done. This provides an easier path for follow-up work such as patent applications and regulatory compliance.

For these two purposes, Gen was created. Gen offers a growable database of tracking changes to a sequence. Changes are recorded and can be gathered into a patch, allowing for sharing of work, code review, and testing. Other git-like functions are also included, such as the ability to create new branches and merge work across branches. Thus, teams can work in parallel and incorporate changes across branches when necessary. Gen is written in rust and binaries are available for Linux and OSX platforms.

Methods

Graph Representation

Fundamentally, Gen's data model is similar to most graph models where sequences are encoded as vertices with edges connecting sequences in various orientations. Gen expands on this common model by defining an edge as a connection between positions and strands within nodes. This enables Gen to model changes in an append-only mode, where changes to the graph require only addition of data. This is a significant advantage, as the traditional model requires splitting nodes into sub-nodes when changes are made, introducing computational overhead for many operations (fig. 1).

On importing data, Gen creates a Sequence object, which is a unique database entry. Nodes are then created which reference a slice of the stored Sequence. This separation has several uses. One is data compression where the same sequence or subsequence may be referenced by multiple nodes. The other is to resolve cases where an identical sequence is referenced multiple times within the same graph. For instance, if multiple copies of the same sequence are repeated, without the node intermediate, Gen would create an unintentional loop by creating an edge between the same sequence. Instead, Gen creates a node for each repeat, with a unique edge between each. In this way gen can faithfully represent duplicate segments.

To help illustrate this model, an insertion and deletion are shown in fig. 2. An insertion or replacement of a sequence is represented by creating a new sequence, a corresponding node, and two edges. The first edge has its source as the start of the region being replaced (the coordinate of the insertion start) on the source node, and its target as the start of the created node. The second edge has its

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b
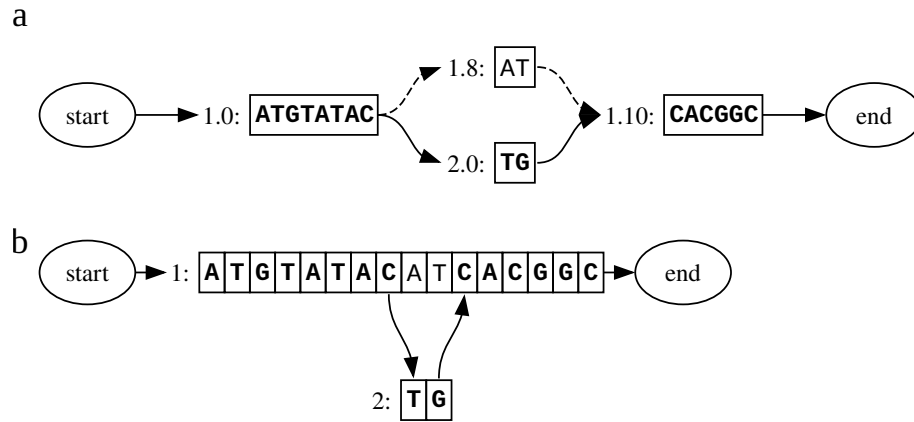
Fig. 1: **Overview of Graph data model**
a. Traditional graph where sequences are encoded as nodes and edges connect two nodes together. **b.** Gen data model where sequences are encoded as nodes and edges connect positions within nodes together.

source as the end of the created node, and its target as the end of the region being replaced. Deletions can be encoded as just one new edge, from the start coordinate of the region being deleted to the end coordinate of that region.

For modeling real world data, such as chromosomes, Gen represents a contig using one directed graph of nodes and edges. This grouping is termed a Block Group and a join table between Block Groups and Edges is used to record all the edges in a block group. Because of the additive nature of insertions, replacements, and deletions, block groups can only grow over time. The block group represents all possible sequences that can be generated from the graph.

Graph Traversal

Several conventions and models are used to facilitate graph traversal. Problems that are common in biological sequence graphs are: having a defined beginning and end point, predefined paths, and indexing into the graph.

Without a defined beginning and end point, traversing a graph requires enumerating all nodes and edges and identifying which have no incoming edges (start nodes) and which have no outgoing edges (end nodes). To facilitate this, Gen defines a virtual start node and a virtual end node to avoid enumerating all nodes to identify starts and ends. On imports and updates, edges are added to these virtual nodes as needed.

Next, several paths are commonly used in a graph, such as the sequence and coordinates corresponding to the reference genome. Paths specify an ordered list of edges to traverse, from a start node to an edge node. Paths have a unique

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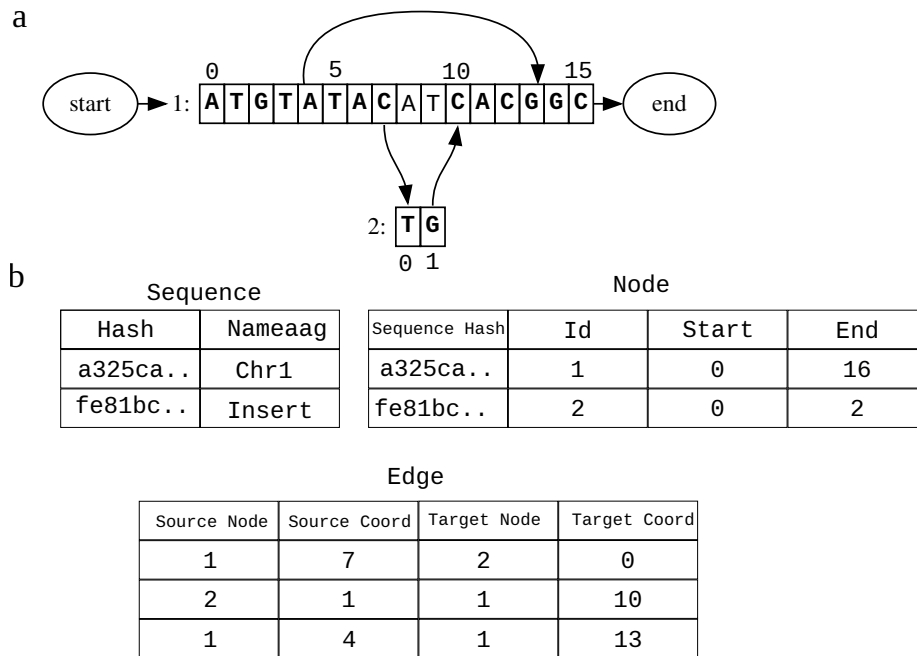
Fig. 2: **Graph updates**
   a. Additions of a sequence and deletion to the graph. Inserts and swaps
   of sequences are handled by the same logic. **b.** Database representation
   of additions and deletions. Models are simplified to illustrate the essential
   parts. The first 2 rows of edges correspond to the addition of the TG
   sequence and the 3rd edge corresponds to the deletion. Coordinate is
   abbreviated Coord in column headers.

Sequence

Hash	Nameaag
a325ca..	Chr1
fe81bc..	Insert

Node

Sequence Hash	Id	Start	End
a325ca..	1	0	16
fe81bc..	2	0	2

Edge

Source Node	Source Coord	Target Node	Target Coord
1	7	2	0
2	1	1	10
1	4	1	13

name per collection and sample, meaning the chr1 path can exist only once within a sample.

While paths are able to provide paths from the beginning to the end of a graph, it is just as useful to index within a graph. Accessions provide a system for relative coordinates within a graph. An accession is a named list of ordered edges within a graph. Accessions are almost identical to paths but do not have to run from a start node to end node. Common use of accessions are to identify useful regions in a graph, such as landing pads for genetic modification or genes of interest.

Phasing, Ploidyness, and Haplotypes

While we continually add new edges, we need to indicate which edges belong together. Phasing is supported and changes on the same chromatid will be exported together. Phasing is stored as the chromosome_index field, xx, and yyy.

A similar need for phasing is within combinatorial assembly. This is where a set of parts are engineered in series, leading to a massive diversity of end products. However, in many cases users want a desired set of paths through these parts. This is another form of phasing, where a set of parts are linked together much like a haplotype.

To support both of these cases, the concept of a phase layer is utilized. A phase layer groups together variants representing both haplotypes and linked parts.

Data Organization

Data is stored in on-disk sqlite database files that act as compartmentalized repositories. Within each database file, Gen has 3 mechanisms for faceting data – a graph name, a sample, and a collection. The name of a graph refers to its underlying biological identity as polynucleotide or polypeptide; the sample identifies a specific variation or instance of the graph; and a collection groups a set of graphs together. Practically this means that "chromosome I in sample S1" is a different object than "chromosome I in sample S2", and one graph can be modified without forcing the change to propagate to the other. But because both graphs share a common universe of node and edge identifiers, variants and annotations can still be easily compared or propagated between samples if desired.

The interpretation of the sample facet is flexible by design. Depending on the application domain or needs of the user a sample could refer to a physical specimen or individual, for example, but also an environmental population or pool of experimental samples. Samples do not have to have a counterpart in the physical world, they can be purely virtual and refer to a digitally designed sequence or model-derived screening library. Multiple graphs can be associated to the same sample (e.g. all chromosomes in a genome), but not every graph has

to be associated with a sample. If it is not, that graph is said to be part of the *null* sample.

Updating the Graph

Updating a graph has varying levels of difficulty. Linear sequences can be indexed in an intuitive way by coordinates whereas graphs are indexed by nodes. Sequence graphs are difficult to refer to specific positions within the graph because positions are dependent upon the edges traversed to each position. Thus, the trivial graph with only a single path can be treated like a linear sequence, but graphs full of heterozygous changes require far more effort as changes of unequal length create ambiguity into positions conditionally on which edges were traversed. For this, various approaches are possible to address the increasing levels of complexity. Where possible, existing standards were used or expanded upon.

A vcf file of alternative alleles can be used to incorporate changes with multiple samples supported. Accessions may also be encoded into the VCF file via the INFO tag, where the GAN and GAA tag can be used to provide the accession name and the allele index for the named accession (fig. 3 a). If an accession has been made, it can be used in subsequent changes to refer to a relative region within the graph (fig. 3 b).

A graph alignment format (GAF) can be used to insert aligned sequences into the graph (fig. 4). Here, a csv file can be used to provide anchoring sequences to identify a position for a change to be inserted between. The transform command will take this csv, and produce a fasta file that can be aligned via available graph alignment tools such as minigraph or vg. Then, the resulting GAF and csv file can be provided to the update command to apply these changes to the graph. Additionally, the GAF file can be manually edited to fine tune the precise update location as graph aligners may not fully match what users want.

A graphical fragment assembly (GFA) can be used to augment a graph. Here, we add any new nodes and paths in the supplied GFA and identify which nodes and paths overlap between the existing database and GFA. Changes to the overlapping nodes and paths are identified and the database is updated to reflect the additional information.

For simple graphs such as a plasmid or haploid organisms where linear indexing is possible, a fasta file may be provided to insert its contents ito a given position. This syntax can also be utilized for complex examples if a linear reference path is established through the graph. For example, a human genome may have many heterozygous changes, but chr1 can still correspond to the reference genome's path and be used as an index into the graph.

A GenBank file can be provided with changes annotated. Currently, we only support changes encoded in the Geneious format. Thus, GenBanks can be modified in Geneious and exported to Gen. A similar GenBank export exists

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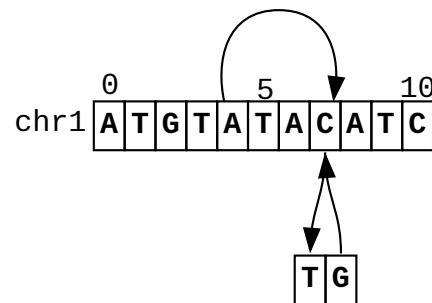

```
 #CHROM POS ID REF ALT QUAL FILTER INFO FORMAT foo
 chr1 5 . ATA A . . GAN=del1;GAA=1;TYPE=del GT 0/1
 chr1 8 . C CTG . . GAN=ins1;GAA=1;TYPE=ins GT 1/1
```

b

```
 #CHROM POS ID REF ALT QUAL FILTER INFO FORMAT bar
 ins1 2. T A . . TYPE=snp GT 0/1
```

Fig. 3: **Updating a graph with a VCF**
　　　a. A VCF file with the corresponding graph. An insertion and a deletion
　　　are encoded into a VCF file to apply to chr1. Each change is provided
　　　an accession (del1 and ins1, respectively) which enables future updates
　　　to refer to the these regions. A new sample, foo, is created with these
　　　changes. **b.** Example VCF using the previously defined accession. A
　　　new sample, bar, is created from the previously defined foo sample. The
　　　accession, ins1, is further modified in this new child sample.
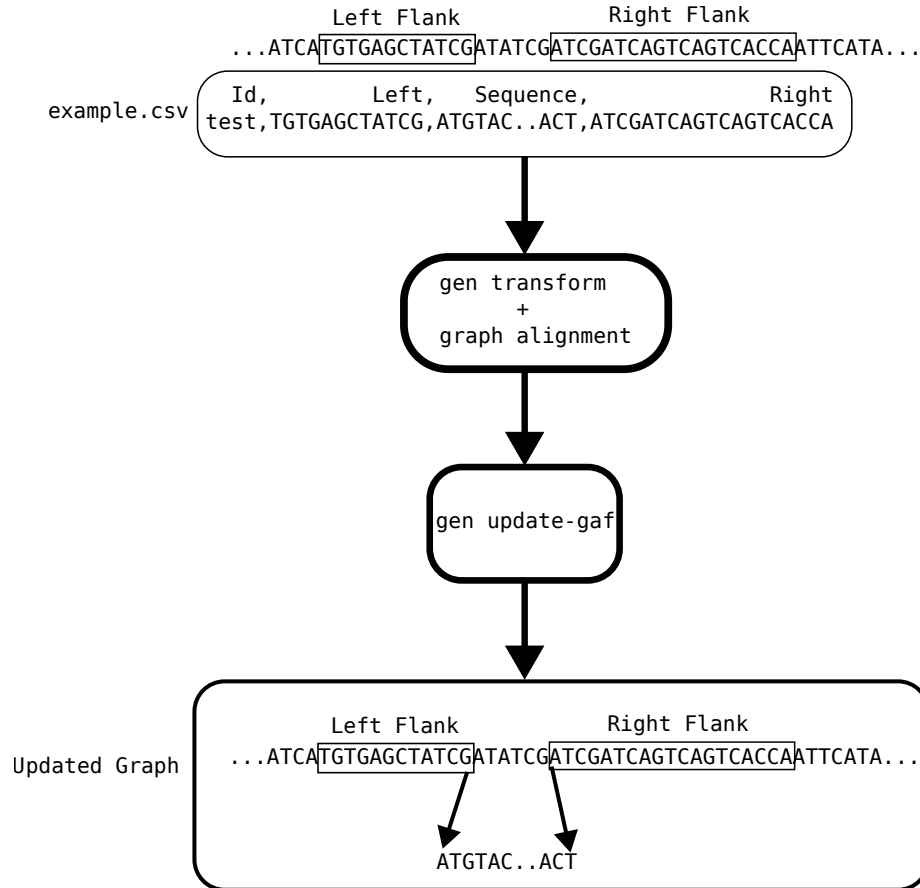
Fig. 4: **Updating a graph with a GAF**
A csv file can be used to modifed a region of the graph. Anchoring sequences are transformed into a fasta file by gen transform, which can then be fed into a graph aligner to identify graph nodes corresponding to the flanking sequences. The specified sequence is then added to the graph between the aligned flanks. Deletions can be modeled by a blank sequence to insert, and left or right flanks can be omitted to model edits at the extremes of a sequence.

where changes are encoded in a Geneious compatible format so bidirectional information transfer is possible. This allows users to edit their sequences using a GUI and leverage Gen for tracking of work and graph based analyses.

Sample Lineage

Gen represents lineage across updates and other operations using samples. For instance, importing a fasta file into a new collection creates the null sample with block groups for each contig. If we then update that collection with a vcf file, gen creates new samples specified in the vcf, and any new sample in the vcf is created as a child of the null sample. Gen copies any graph data created by the fasta import in the null sample over to each child sample before applying the updates specified for each sample.

Using samples seems to be the best fit for tracking lineage, instead of say updating block groups with new information within an existing sample. A sample can be used to capture the two main use cases for gen operations: Intended edits, and observed variations. An intended edit can be represented by updating an existing sample using say a fasta file plus a region and coordinates. That operation would capture the integration of a cassette, and create a new sample with a path that represents the updated sequence. That new sample would be "virtual" in the sense that it doesn't exist in the real world, but is treated by gen as separate from any real world samples, which seems appropriate. Observed variations can be represented by updating an existing sample using say a vcf file. That operation will create new samples, each of which represents variations observed during sequencing. In this way, gen spans both intended and unintended genetic changes, and can be used to track changes across repeated cycles of development and testing.

Pooling

Add details about how gen represents pooling.

Operations

There are various methods to change a graph, which we term an operation. Each command that alters the data model such as update or import is recorded as an operation. Operations are analogous to a commit in git and many git-like functionalities are present in Gen.

Branches allow work to be carried out in parallel. By default, Gen creates a main branch operations are recorded against. Operations form a simple tree structure, with each operation having a single parent operation at this time. Branches may be created off any operation, and nested branches are supported. Branches can be merged into one another via the merge command. This workflow enables parallel work by teams and easy experimentation. Operations may be reverted

and individual operations can be applied across branches, similar to the git cherry-pick command (fig. 5).

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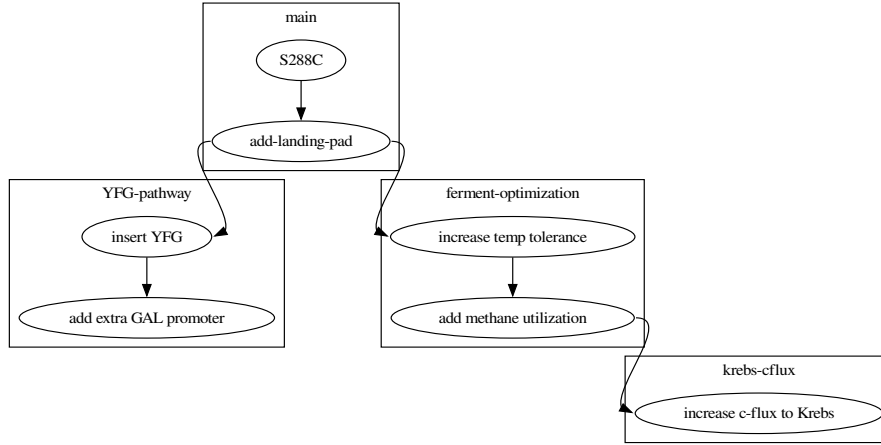
b

Operation	Git Command	Gen Command
Create Branch	git checkout -b	gen checkout -b
Merge Branch	git merge name	gen merge name
Checkout	git checkout name	gen checkout name
Reset	git reset hash	gen reset hash
Create Patch	git diff	gen patch-create
Apply Patch	git apply x.diff	gen patch-apply file
View a diff	NA	gen patch-view file

Fig. 5: **Operations**
    a. Tree structure formed by a series of operations in different branches.
    b. Summary of commands related to operations and their similar git equivalents.

A set of operations can be collected into a patch, which is analogous to the git patch which represents a diff of how the codebase is changed (fig. 6). However, due to the purely additive data model of Gen, diffs are much simpler to create as there are no rewrites. Patches are stored as a gzip file and can be shared to distribute changes. Viewing of patches is possible via the patch-view command, which will render a dot graph of changes within the patch. By commiting these patches and changes into git, this workflow enables many features common to

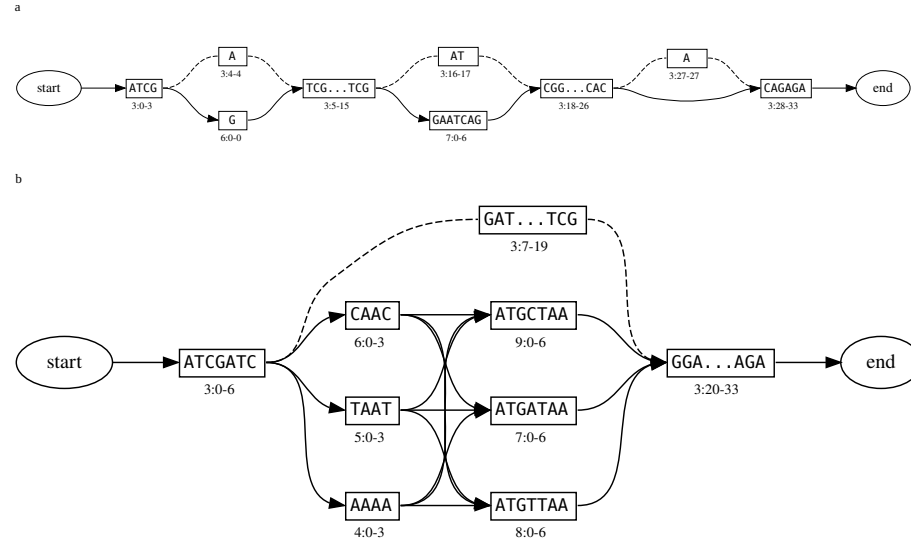software development such as code review and continuous integration testing.

Fig. 6: **Changes to a sample created by patch-view and viewed in GraphViz.**
a. A simple basepair change, a sequence swap, and a deletion are shown. A dashed line indicates the path along the reference sequence whereas a solid line indicates paths adding sequences from other non-reference sources. **b.** Viewing a combinatorial assembly of 6 parts across two segments.

Translating coordinate schemes

Annotations can be propagated through the graph structure. Thus, annotations on the reference genome can be translated into the coordinates of new samples. Coordinates are translated with the following rules:

- If the start and end of the annotation range on the reference map to valid coordinates on the sample sequence, gen translates the entire annotation to the later sample. Gen does this even if there is a gap in the middle of the later sequence that doesn't correspond to anything in the reference sequence, say if a subsequence were replaced.
- If say the start of the range translates to a valid coordinate on the later sample, but the end does not (say because a replacement wiped out the stretch of the reference sequence that contained the end), gen truncates the translated annotation range to only include the stretch of sequence that the reference shares with the later sample.

Exports

A design goal of Gen was to not replace existing formats, but to complement them. Gen can export its data into a variety of formats for subsequent use by 3rd party tools. Because graphs can vary significantly in their complexity, several export options are available to accommodate the varying degrees.

Graphs can be exported into the Graphical Fragment Assembly (GFA) format. Nodes in the Gen graph are translated into GFA segment ids and paths are exported as well. Gen nodes are converted into segments by concatenating the Gen node id with the sequence coordinates the node maps to. For example, node 5 which references positions 9-20 of a sequence will be exported as 5.9.20. The lineage of a sample can also be encoded as a GFA file, where changes from a parent sample to a descendent will be exported. This is incredibly useful for representing and tracking iterative strain engineering.

Simple graphs can be exported into a GenBank format. Due to the limited information encoded in GenBanks, only simple graphs such as plasmid or haploid samples will produce a meaningful output. The data export for GenBank currently uses the Geneious conventions for encoding changes to a sequence, so data can be imported and exported into Geneious for sequence editing.

Paths within a graph may be exported to a fasta format. This is commonly used to generate a fasta file of a reference genome or simple graphs where changes are unambiguous (for example, changes to a haploid organism).

Database

Gen uses a sqlite database. Sqlite was chosen because it is extensively used across all major platforms. It is additionally a local database, enabling users to use gen without having to set up more complicated databases or require an internet connection to a cloud service. Sqlite also allows extensibility of the data model via schema migrations and users can extend the Gen model with new tables to add custom functionality if desired. Updates to gen will be possible with schema migrations, which help mitigate the version issues plaguing many bioinformatic data formats.

Another consideration for sqlite is it can be accessed via web assembly (WASM), meaning databases stored in places such an Amazon s3 bucket can be accessed without any dedicated servers. Thus, applications can be developed and ran entirely on the client side.

Results

Performance Benchmarks

While extensive benchmarking and performance tuning has not been carried out, owing to the use of Rust, Gen is fairly performant without much attention to

optimizations. Benchmarks were carried out to evaluate the time and storage needs for importing the reference human genome and updating it with variant data from the 1000 genomes project. (See table tbl. 1)

Tab. 1: my caption

Task	Time	Storage
Importing GrCh38 (shallow)	7s	496kb
Importing GrCh38 (full)	49s	3.0 Gb
Adding variants from chr22 of 1000 genomes project (HG00096 sample)	7.7s	21.8 Mb
Adding variants from chr22 of 1000 genomes project (HG00097 sample)	7.2s	21.7 Mb
Adding variants from chr22 of 1000 genomes project (HG00096 + HG00097 sample)	14.9s	32.6 Mb
Translating xxx from A to B (gff coordinate example)	xxxs	yyy Mb
Export of GFA stuff	xxxs	yyy Mb

Shallow imports of data record only minimal information and do not store the genome sequence. This allows the database to work strictly off coordinates and fetch the sequence data from disk when needed. Notably, it is extremely useful for collaborating on large files as it enables a shallow working copy to be used and shared.

For updating with variant data, the size of each vcf file was approximately 17.1 Mb. With addition of many samples within the same graph can lead to improved data compression as variants shared between samples require less data to be created.

translating annotations

data export time

Discussion

- The lack of a growable database. Minor changes required regenerating the entire graph and re-exporting it. Graph genomes grow at a rate faster than a linear alignment. For example, a single copy of hg38 suffices for analyses of many samples. Whereas with sample specific graphs, each sample requires its own graph as an additional tracked asset. This also made it difficult to compare across analyses, as nodes which may be in common between two graph samples would have different labels. This could be mitigated by creating a graph encompassing all samples, but

this approach simply delays the inevitable addition of a new sample, a derivative lineage, or an updated set of variant calls.

Removed sections

Inroduction

- observation about in many genetic analyses, the genome doesn't change among samples, the same reference genome is used for each. It's good enough for many applications, but syn bio has genomes that change often. The lack of an updateable graph format is a major hindrance here, where regenerating a GFA is time consuming and makes it difficult to compare across analyses. So we worked on a format where the reference genome could be uodated In many conexs whee he efeence genome is used, he pae of updaes is infequen, where the genome is unchanged or slowly changing. This is why there are so many examples where hg18 and hg19 are still utilized. Conversely, in scenarios where the genome is changing often, such as in synthetic biology where multiple generations of a strain is generated, the reference genome is updating. Thus, the previous approaches of a largely static genome are unsuitable. This makes it difficult to compare analyses across generations

Updating Graph section

- Removed `For pooled updates, a custom library format may be provided.`. Should put pooled stuff in its own section.

Coordinates and indexing

Coordinates in gen are 0-based, and ranges are closed/open. For instance, (0, 5) represents the first five base pairs of a sequence, and (10, 11) represents the eleventh base pair.

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References

Eizenga, Jordan M., Adam M. Novak, Jonas A. Sibbesen, Simon Heumos, Ali Ghaffaari, Glenn Hickey, Xian Chang, et al. 2020. "Pangenome Graphs." *Annual Review of Genomics and Human Genetics* 21 (1):139–62. https://doi.org/10.1146/annurev-genom-120219-080406.