Sequence Graph format (SQG) specification version 0.1

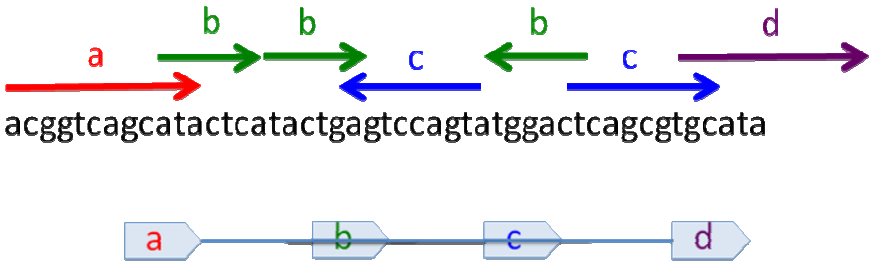
This is a straw man proposal for a standard exchange format for graphs made of connected segments of DNA. A basic implementation in C may emerge soon, depending on feedback.

SQG is designed to provide a standard for the graphs used within assembly software (assembly graphs including de Bruijn graphs and string graphs), and also to represent repeat structure in a large sequence such as a genome (repeat graphs), variation in a population, and transcript structures. The basic model is that there are two primary types of object: segments with sequence attached, and connections (adjacencies or overlaps or gaps) between the segments.

SQG supports attachment of arbitrary information to segments and connections to allow for extendability and a wide variety of uses. This is done by allowing the declaration of named properties, which are strongly typed.

SQG supports representing the consensus sequence of segments, but is not designed primarily to represent the relationship between primary sequencing reads and the segments in an assembly. We recommend use of BAM for representing the detailed alignments (for further discussion see further below).

1. Example and discussion of representation




```
SQG  0 1
HISTORY  2011/03/14_19:04:00  made by hand
PROPERTY  CopyNumber  int  number of times the node is traversed
PROPERTY  Comment  string  arbitrary text
PROPERTY  Chromosome  flag  the true chromosomal sequence
SEGMENT  a 10 CopyNumber=1 acggtcagca
SEGMENT  b  6 CopyNumber=3 catact
SEGMENT  c  8 CopyNumber=2 ggactcta
SEGMENT  d 10 CopyNumber=1 agcgtgcata
CONNECTION a  >>  b  -2
```

```
CONNECTION  b  >>  b  0  Comment="tandem repeat"
CONNECTION  b  ><  c  -2
CONNECTION  c  <<  b  0
CONNECTION  b  <>  c  -1
CONNECTION  c  >>  d  -2
WALK 1>a>>b>>b><c<<b<>c>>d>10 Chromosome
```

In the picture for this example we have shown sequence segments as oriented blocks, and connections as non-directed edges. In terms of graph theory this is completely equivalent to two standard representations:

- The breakpoint graph, originally introduced in the context of representing the relationship between synteny blocks in chromosomes of related species. These are graphs with two types of edge: labelled segment edges, and connection edges. Any walk must alternate between segment edges and connection edges. SQG format corresponds to attaching sequence to the segments, with the nodes in the graph implicitly defined at the ends of segments. The '>' and '<' symbols in the connection lines indicate which ends of the segment edges are connected, e.g. **a>>b** means the end of **a** is connected to the start of **b** and **b><c** means that the end of **b** in connected to the end of **c**.
- Representations in which sequence is attached to nodes of a graph and there are bi-oriented edges, with rules about consecutive edges being traversed having to be "compatible". For this, SQG notation **>>** is the type of the edge, compatibility means that adjacent edges in a sequence walk need to end and start with the same symbol (so **>>** and **><** are compatible, but **>>** and **<<** are not), and sequence is read from notes in the forward direction if they are flanked by **>>** and in the reverse complement direction if they are flanked by **<<**.

Which of these (or any other equivalent) is used internally by software implementing SQG is up to the software developer.

Comment: "Bidirected" might be a more common term for this in the literature

There is however an alternative natural representation of a sequence assembly as a directed graph where sequence is attached to the directed edges, and a sequence walk is just any walk following edges in the correct orientation. We believe we can map between this representation and the SQG representation, but can not guarantee to get back to the same actual version. An open question is whether we can define a canonical version that is stable.

2. Record types

SQG is a record based format, designed to also support a binary implementation. Records are tab delimited. The first field comes from a fixed vocabulary and determines the record type.

SQG <major> <minor>

This record must be present, must be the first record, and must be unique. <major> and <minor> are integers denoting the major and minor versions.

HISTORY <date_time> <command>

These are optional records, to record the sequence of commands that created the file.

- <date_time> is date and time string with format yyyy/mm/dd_hh:mm:ss.
- <command> is an arbitrary string – typically the literal command line obtained with ARGV[0] or the equivalent for the language being used.

Good practice is to copy any existing lines and add one more each time the file is transformed. This self-documentation feature has proved useful in BAM and VCF files.

PROPERTY <id> <type> <description>

Properties are used to attach extra information to segments, . Property declaration lines must preceed the first use of the property.

- <id> is a legitimate C identifier, satisfying regexp [**a**-**zA**-**Z**][**a**-**zA**-**Z0**-**9**_]+. Ids starting with a capital letter are reserved for definition in this or a future version of the specification – see below for reserved property names.
- <type> is currently one of {**int**, **float**, **string**, **flag**}.
    - int values are signed integers maximum 2^{32}-1, minimum -2^{32}.
    - float values are parsed using a scanf family function into a 4 byte float.
    - string values are an arbitrary length sequence of printable ascii characters enclosed in double quotes, allowing the space character, newline as \n, tab as \t, double quotes as \" and backslash as \\.
    - dna_string values are an arbitrary length sequence of the characters a, c, g, t, n.

  It would be attractive from the user perspective to extend the type system to support more complex constructed types, in particular fixed and variable size arrays, and ideally compound types/structures. And/or we could define special types such as "annot" for annotations with start and end coordinates in the sequence and some attached information (what type?), or "depth" for pairs of a string and a non-negative floating point depth. The advantage of such special types is that they could in principle automatically transform correctly with operations on the graph.
- <description> is a free text description of the property. There must be some description.

We allow the same property to be used for different object types, e.g. segments, connections or walks, but the attached data will be the same.

Comment: Having this as a type might be useful for the purposes of compression later

SEGMENT <id> <length>[<properties>[<sequence>]]

These define the sequence units. The sequence is optional, so as to allow a more compact representation with sequence stored elsewhere. Properties are optional because there might not be any.

- <id> is a standard C type identifier. Identifiers must be unique in a graph; there can not be more than one SEGMENT record with the same id.

Comment: Since this is optional, why not make it into a property? This could lead to a cleaner API interface.

- <length> is a positive integer denoting the length of the sequence. In some cases where the length is uncertain, this is presumed to be a plausible "best-guess" value.
- <properties> is a string of the form <prop_id>[=<value>][;<prop_id>[=<value>]]*, where is a property id, and value is a legal value. The equals sign and value must be present unless the property has type flag, in which case they can not be present. If there are no properties, but the sequence is present, then the reserved property None of type flag must be used (i.e. write None in this field).
- <sequence> is the sequence of the segment in the alphabet [acgtn]. It is annoying to support n's, but experience from other formats is that it is necessary. An application can check if n's are absent to implement more efficient bitwise compression.

CONNECTION <seg1-id> <join> <seg2-id> <separation>[<properties>]

These define the connections between segments.

- <seg1-id> and <seg2-id> are segment ids. They need to have been declared already in SEGMENT records.
- <join> is one of
  - **>>** if the end of seg1 joins the start of seg2
  - **><** if the end of seg1 joins the end of seg2
  - **<<** if the start of seg1 joins the end of seg2
  - **<>** if the start of seg1 joins the start of seg2
- <separation> is an integer. If it has value 0 then the segments abut. If it is negative then they overlap by minus the number of bases specified. If it is positive then there is a gap between the segments of the number of bases specified. As with segment lengths, the connection separation must be present and should be set to a working "best guess" if uncertain.
- <properties> is a property string as for segments. In this case if there are no properties None does not need to be used since this is the last field.

There can be at most one connection with each join type between each pair of segment. Note that there can be multiple connections between segments if they have different join types (e.g. the graph of a diploid sequence with an inversion b between segments a and c would have connections a>>b, b>>c, a><b and b<>c). It is legal for there to be a connection between a segment and itself (for example this is used for tandem repeats, or exact palindromes).

WALK <specification> <properties>

Walks define sequences that can be obtained by traversing arbitrary sections of the graph, starting and ending at any base position within segments.

- <specification> is a string with the following syntax <int><directed_segment>+<int>,

where <directed_segment> is **>**node_id**>** or **<**node_id**<**, where the greater than and less than symbols in these are literals indicating whether the segment is traversed in the forwards or reverse direction. So **17>a>>b>5** starts at position 17 in segment a, moves forwards along a to its end, moves into the start of node b

Comment: It might be useful to allow this to be a negative number, which would represent an offset from the end of an edge. For example, if we have an edge a and an edge b of length 19, then a walk going through both edges would be 1<a<<b<-1 instead of 1<a<<b<17. This would be easier to read. Also, it might make sense if the length of a segment is not known exactly.

Comment: Is this notation redundant? We would have the weirdness that 3>a>14 would be the same as 3<a<14. Could we drop the first and last inequality sign? So we would have 3a14, or 17a>>b5 for the other example.

(correctly handling any gap or overlap caused by the separation of the nodes a and b, which must have an edge a>>b) and moves forwards to base 5 of b. The positions for the starts and ends are 1-based and inclusive. Segments of a single node can be specified with e.g. **27>a>192**.

Note that the reverse complement of a walk is specified by reversing the elements, so for example the reverse complement of **17>a>>b>5** is **5<b<<a<17**. Also note that walks have an implicit sequence and length. We support circular sequences such as mitochondrial genomes by a flag property Circular.

We could allow the length and sequence to be given in the format. Although redundant, this would have the advantage of having a standard format for extracting sequences of walks.

Comment: I think this would be a good idea, and I don't think it would be necessarily redundant. For example, edges might represent inexact overlaps for some assemblers, so there may not be a systematic way to recover the sequence of the walk from the sequences of the segments. If the sequence of the walk could be specified, then the assembler could transfer this information using the format.

Walks are a key concept. Basically, any sequence in the true genome(s) represented by the graph will correspond to a walk. In particular, full chromosomes will correspond to walks, as will assembly contigs (which might be single segments, but might also be bigger than segments, e.g. when choosing arbitrarily a route through polymorphisms). By creating new connections with gaps as necessary, we could also represent scaffolds as walks. Note that scaffolds and chromosomes are long and may contain thousands of elements. Should we allow recursive definition of walks in terms of previous walks?

Comment: I would say yes because of scaffolds. A scaffold could be very large (millions of bp), and it would be inefficient to repeat the sequence of each individual contig.

How different is a walk from a recursively defined segment?

Comment: We could require for walks to also have ids. This id space would be shared with the segments. Then, allowing recursively defined walks becomes very easy, with segments becoming base cases of the recursion.

Furthermore, any specific match of another sequence to a graph will match a walk, so a walk replaces the standard concept of a triple (<sequence>,<start>,<end>).

A set of walks induces an arbitrary subgraph, so in particular the set of matches into a graph will generate a subgraph. I am not sure how useful that concept is.

3. Reserved properties

Comment: I think the different properties get into exactly what the format is used for. Some properties only make sense if the graph is used as an exchange between assemblers, and others make sense only if the graph is used as a "population graph." Maybe its worthwhile to point this out and to split this section into (at least) two parts.

Segment

- **ReadStartCount** *int* How many reads start in this segment
- **CopyNumber** *int* How many times this segment is traversed in a sequence set
  - Typically the sequence set in question would be the standard chromosome set for the organism.
- **CopyNumberEstimate** *float* Estimated copy number
  - It would be nice to give a distribution with probabilities, actually likelihoods. For this we could have an array of floats, with values from 0 up to *k* copies, and a final value indicating *>k*.
- **Comment** *string*

Connection

- **CopyNumber** *int* How many

Walk

- **Circular** *flag* Denotes that the described sequence is circular
  - Require that end point is start point minus 1
- **Chromosome** *string* Claim this is a full chromosome sequence
  - *string* is name of chromosome
- **Reference** *string* Corresponds to a publicly known reference sequence
  - *string* is identifier for reference
- **Annotation** *string* This walk corresponds to an annotation
- **Contig** *string* This walk is an assembly contig – the string is the ID
- **Scaffold** *string* This walk is an assembly scaffold – the string is the ID
- **ReadAlignment** *string* This represents an alignment of the read. The ID is the name of the read, or * if it is not known.

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4. Potential operations

We can think of a number of operations.

- Format validation.
- Nx determination, e.g. N50.
- Finding sequences of walks.
- Tip removal, subject to criteria such as maximum length.
- Bubble identification and collapsing.
- Standardising overlap length. There are various reasons to standardise overlap length. Removing overlaps, so that all separations are 0 (or positive if there were any positive overlaps in the original sequence) gives a non-redundant representation of all the sequence, and can in principle simplify bubbles and other types of variation. On the other hand, increasing overlaps to a standard value k will ensure that all subsequences of the full sequence (whatever it is) are represented in the node sequences, which can be useful for matching operations.
- Transformation into graphviz dot format for visualisation and some standard graph operations. And back again if sufficient information is preserved.
- Matching an arbitrary sequence. This is probably the killer app.

5. Recording read placement

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It is also possible to maintain information about where reads align to the graph. In the context of a graph, an alignment is represented as a WALK. We describe two ways of storing such alignments: with a SAM/BAM file, and by using the WALK record type in the SQG file.

First, the alignments may be stored in SAM/BAM format. The following alignment fields have special meaning when recording alignments to a graph:

- RNAME is the segment where the walk begins. It should cross-reference a valid SEGMENT id.
- POS is the position within RNAME where the walk begins. It should be between 1 and the length of the SEGMENT.
- An optional field with the WK tag may be used in the case that the alignment walk contains more than a single segment. This string should be formatted in the exact same way as the specification string format in a WALK record.

Additionally, in the header section of the SAM/BAM file, each SEGMENT id which appears in an RNAME field must have an entry in the reference sequence dictionary as an @SQ line. The SN property must cross-reference a SEGMENT id, and the LN must correspond to that SEGMENT's length.

Alternatively, an alignment can be stored as a WALK record in the SQG file. Such an alignment must have the ReadAlignment property set. This is the name of the read, or * if the name is not known.

Comment: There may be another way to store the full alignment in SAM. SAM allows to chain together alignments of multiple reads within a template, such as matepairs or strobe-reads. This functionality could be overloaded by thinking of each read as a "template", and the segments of an alignment walk as a chain of "read alignments."

This could work, but the downsides are: 1) there would be no way to store real matepair information, and 2) it seems unnatural, from a user-perspective.

Comment: I'm not sure if this is really necessary, but it is required according to the SAM/BAM specifications.

5. Open issues/options

5.1 How to handle typed properties?

5.2 Should we have some sort of hierarchical substructure?

5.3 Compression, binary version of format?

5.4 Sorting, indexing

5.5 Include/import macro

Might be good for walks in particular, and in context of hierarchical structure

5.6 Recording information for arbitrary segment pairs

This is important for scaffolding. Different from connections because the segments are not necessarily consecutive. Can have distance and/or orientation (e.g. transcript exon matches give orientation not distance), and a type. Perhaps a new record type?

5.7 Add read placement – Celera people want this

Full alignments are walks. Really these are better held in BAM. But could record start points, or start point density along segments.

6. Comparators

- Amos (U Maryland, TIGR, Celera and others)
  - Open sources assemblers, visualisers etc.
  - http://sourceforge.net/apps/mediawiki/amos/index.php?title=AMOS
- Ace files (including CAF)
  - Used by Phrap, CAP, other assemblers and viewers (e.g. consed, xgap)

    - Older, capillary-based. Focus more on read alignments and consensus, not on graph structure.
- More general fomats, like GraphML, GraphViz dot

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