SOLiD™ GFF File Format

1 Introduction

The GFF file is a text based repository and contains data and analysis results; colorspace calls, quality values (QV) and variant annotations.

The inputs to the GFF file generation are the matching files (xxx.ma), the reference sequence, and the QV files xxxx.qv.qual .

The generation process is as follows

- *Unique mapping* is defined for the mapping parameters used when the mapping to the genome was performed and when MaToGff or MatesToGff program was run to generate the gff file. For example, take a mate pair run that allowed two mismatches and for which the MatesToGff parameter clear was set to 1; in this case, the possibility of a second mapping with three mismatches would not be assessed.

- Using the location information from the matching file the read is compared to the reference (in colorspace). The changes relative to the reference are annotated.

- xxx.gff file is produced.

2 Specification

GFF (General Feature Format) is a record based file format, where each line describes a single *feature* (in this case, a *read*) with a list of tab-delimited *fields* in a fixed order specified by the GFF specification. See Appendix A for a general definition of the format, and Section 3.2 for a specific definition of the fields for SOLiD™ files.

If a '#' character appears anywhere on a line, then the rest of that line is a *comment*. Ordinarily, an application reading the file will ignore comments and *comment lines* (lines starting with '#' after white space). Comment lines that begin with '##' are called *meta-data*, which are data that apply to all of the features in the file. These meta-data, defined in Section 3.1, need not be ignored by all applications.

2.1 Meta-data

The GFF format allows for the use of meta-data in the form of '##' comment lines; including the following standard GFF meta-data tags:

2.1.1 ##solid-gff-version

This is currently "`##solid-gff-version 0.2`".

2.1.2 ##gff-version

This is "`##gff-version 2`".

2.1.3 ##source-version <source> <version text>

A comment describing the version of the program that produced this file. The source and the version text should be free of white space. For an acceptable example:

```
##source-version MaToGff.java v0.2
```

2.1.4 ##date <date>

The date that the file was generated, in astronomical (yyyy-mm-dd) format. This example designates 1 October 2007:

```
##date 2007-10-01
```

2.1.5 ##Type <type>[<reference name>]

The type will be set to **solid_read**. The reference name is the name of the reference to which *all* reads in this file were aligned. For example

```
##Type solid_read chr1
```

2.1.6 ##history <command line>

These comment lines allows us to record source information in addition to the program name, which is recorded by the ##source-version tag, and to record how previous processing steps lead to the data in the current SOLiD™ GFF v0.2 file. There is one history line for each processing step, with earlier steps appearing above later ones. We recognize that it may not always be feasible to record all aspects of a command line, such as its file indirections, for example.

```
##history map Sample1_F3.csfasta myReference.fasta
##history MaToGff.java --convert=unique -sort Sample1.ma
##history AnnotateChanges.java Sample1.gff myReference.fasta
##history filter_fasta.pl --noduplicates --output=qvs.qual
##history AddQvs.java Sample1.gff qvs.qual
```

2.1.7 ##time <current time>

The local time, in 24 hour format, when the generating software wrote this line. For example,

```
##time 18:02:36
```

2.1.8 ##color-code <code string>

This line specifies the color code used to generate *all* color reads in this file. The code-string is a comma-separated string of <motif>=<code> pairs. For example, the following entry specifies our current two-base encoding:

```
##color-code
AA=0,AC=1,AG=2,AT=3,CA=1,CC=0,CG=3,CT=2,GA=2,GC=3,GG=0,GT=1,TA=3,TC=2,TG=1,TT=0
```

2.1.9 ##primer-base <code string>

A comma separated string of <primer set>=<base> pairs, each of which specifies the last base for each primer set in this file. For example,

##primer-base F3=T,R3=G

2.1.10 ##max-num-mismatches <count>

The largest number of mismatches (in colorspace) for any reported hit, and therefore for any read, in the file. E.g.,

##max-num-mismatches 6

2.1.11 ##max-read-length <count>

The largest number of positions (colors and the leading base) for any read in the file. E.g.,

##max-read-length 20

2.1.12 ##line-order <order>

<order> has one of three values: "fragment", "mates", or "none".

The GFF standard allows lines in any order ("line order is not relevant"). The SOLiD™ GFF v0.2 files, however, should have meta-data come before feature lines, and feature lines should be ordered by increasing sequence index, then start position, panel number, *x* coordinate, *y* coordinate, and finally primer set id of the reads (see Attributes below). This ordering is said to be *fragment-ordered* and is indicated by:

##line-order fragment

2.1.12.1 Mate-paired data

Mate-paired data can also be ordered in this manner, one line for the "forward" read and a second for the "reverse", again indicated by <order> = fragment. Some applications, however, may prefer an alternative, called *mates-ordered*, where the "forward" reads are fragment-ordered as before, but each "reverse" read appears immediately after its corresponding "forward" read. This ordering is indicated by:

##line-order mates

If the data are not known to be either fragment ordered or mates ordered, they are said to be unordered, which is indicated by

##line-order unordered

2.2 Fields

The feature lines specify one read each, with the following fields, as specified in the GFF standard (see Appendix A).

2.2.1 seqname

The name is the bead identifier (panel_x_y) plus a suffix indicating the primer set id (either "_F3" or "_R3" currently). For example:

```
8_1727_1389_F3
```

Because each run was analyzed separately, it is probable that users will want to use data from multiple files. Because each bead is identified by its panel number and X, Y coordinates it is possible that the same bead identifier may be used in multiple files before concatenating or merging files. The user may want to add an identifier to the seqname to avoid issues; it is suggested that an identifier is appended to the end of seqname (as often files may be sorted by seqname).

2.2.2 source

This name is always **solid**.

2.2.3 feature

This name is always **read**.

2.2.4 start

The inclusive start point of the aligned read on the 1-based base-space reference sequence, indexed from 5´ to 3´. See ‘end’ for details.

2.2.5 end

The inclusive end point of the aligned read on the 1-based base-space reference sequence, indexed from 5´ to 3´. If this read maps to the reference at multiple locations, start and end will correspond to an alignment with the least number of mismatches. Ties will be broken either by increasing start or randomly, depending on the application that generated the file.

By the GFF specification (see Appendix A), start must be less than or equal to end. For example, if the read aligns to the forward strand of the reference (see strand field below), as in:

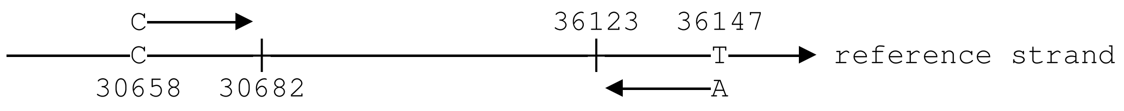
```
start  end    strand  [attributes]
30658  30682    +     g=C0103112003130213233311032
```

then the initial ‘C’ aligns with position 30658 of the reference strand, which should be a matching ‘C’; the base following ‘0’, which is another ‘C’ (by the color code in §3.1.7), aligns with position 30659; and the base corresponding to the last ‘2’ aligns with position 30682. See the diagram below.

If, on the other hand, the read aligns to the reverse strand of the reference, as in:

start	end	strand	[attributes]
36123	36147	-	g=A322121003310310232103022

then the A aligns with position 36147 of the reference strand, which should be a complementing T; the base following the first ‘3’ after the initial ‘A’, which corresponds to a ‘T’ by the color code, aligns with position 36146 of the (forward) reference strand, and the base following the last ‘2’ aligns with position 36123.



2.2.6 score

A summary quality score for the read, recorded to one decimal place:

$$score = -10\log_{10} P$$

where P is the average probability of error at any read position:

$$P = \frac{1}{n}\sum_{i=1}^{n} 10^{-QV_i/10}$$

and QV_i is the quality value of the color at read position i (see Attribute q). Note that high scores correspond to high quality reads.

2.2.7 strand

Either '+', if the read aligns to the "forward", "nominal", or "given" strand (i.e., the sequence provided as the reference) or '-' if the read aligns to the other strand.

2.2.8 frame

The SOLiD™ system does not use the frame and therefore always sets it to ".".

2.2.9 Attributes

This is a semi-colon separated list of key-value pairs of the form "key=value"..

2.2.9.1 b

The corrected base-space representation of the read. Construct it in three steps:

1. Identify the isolated and invalid mismatches (see the definitions in the *s* attribute) in the read, and replace them with the aligned reference color-calls.

2. Convert the result to a DNA sequence using the color code (see 2.1.8).

[see Appendix B for more details]

3. Set the bases that differ from the reference in lower case and those that do not differ in upper case.

2.2.9.2 c

The *category* of the mate-pair for this read. It is defined and present only for mate pair runs. *Category* is written as a three letter code that describes the relative mapping of the two reads that make up a mate pair. In the definitions below, an acceptable insert size is defined in the mate pair analysis settings.

- AAA: same reference, same strand, correct ordering, acceptable insert size
- AAB: same reference, same strand, correct ordering, small insert size
- AAC: same reference, same strand, correct ordering, large insert size
- BAA: same reference, different strands, acceptable insert size
- BAB: same reference, different strands, small insert size
- BAC: same reference, different strands, large insert size
- ABA: same reference, same strand, incorrect ordering, acceptable insert size
- ABB: same reference, same strand, incorrect ordering, small insert size
- ABC: same reference, same strand, incorrect ordering, large insert size
- C**: different references

2.2.9.3 g

The color-space string for this read, written from 5´ (the bead end) to 3´. In addition, prepended to the string is the first (5´ most) nucleotide of the DNA sequence of the read. This, together with the coding algorithm specified by the color-code meta-data tag, allows any application to reconstruct the DNA sequence for the read. By referring to the 'strand' meta-data tag, the application can reconstruct the color-call substring and the corresponding DNA subsequence for either strand of the reference.

It is important to note that FASTA files generated by the instrument store their data in a different format. There, the color-string stores the last base of the primer, presumably the phase 5 primer, as its first base. Fortunately, given the color-code tag, it is easy for any application to convert FASTA notation to GFF notation, simply by reconstructing the first base of the read. For example, it would convert the FASTA sequence T210033221 to the GFF sequence C10033221 by converting T2 to TC and dropping the primer base T. Note that this means that the first color reported by 'g' is really the second color in the read from the instrument.

2.2.9.4 i

The 1-based *index* of the reference sequence. For example, i=3 says that this read is aligned to the third reference sequence in the reference name (see the ##type meta-data tag). If this value is not specified it defaults to 1.

2.2.9.5 p

This *mappability* measure is intended to give a count of the "effective number of hits" for this read onto the reference. A small value close to 1 indicates that our read is effectively unique in the reference. A higher value indicates that our read effectively matches at multiple locations. More formally, we need these definitions:

L	Length of the read
k	Number of mismatches in an alignment. Note $k \leq L$.
m	Least number of mismatches over all alignments for this read. By construction, this is the number for that alignment reported by 'start' and 'end'. See the 'end' field for more details.
N_k	Number of reference positions where the read aligns with exactly k mismatches. Note that $N_k = 0$ for all $k < m$, because m is the *least* number of mismatches for this read. We will also assume that $N_k = 0$ for all k greater than the largest number of mismatches reported by the 'u' attribute. For example, if u=0,0,3,1, then $N_0 = 0$, $N_1 = 0$, $N_2 = 3$, $N_4 = 1$, and $N_k = 0$ for all $k > 4$.
X_{Lk}	The expected number of alignments with exactly k mismatches as a multiple of the expected number of alignments with 0 mismatches.

Define $X_{Lk} = 3^k \binom{L}{k}$ and $mappability = X_{Lm} \sum_{k=m}^{L} \left(\frac{1}{X_{Lk}} N_k \right)$.

In the following examples, let L=25: For a read with exactly one alignment with no mismatches, m=0, and $N_m = 1$. Then $X_{L,m} = X_{25,0} = 1$ and *mappability* = 1, indicating an ideal unique match.

Even if our unique alignment has (say two) mismatches, then L=25, m=2, $N_m = 1$, and again *mappability* = 1 (the X_{Lm}'s cancel out).

If the read has two perfect alignments, then L=25, m=0, and N_m = 2. Again, $X_{L,m} = X_{25,0} = 1$. But now *mappability* = 2, indicating an ambiguous match. In general, if our read maps perfectly to the reference in *n* places, i.e. $N_0 = n$, then the mappability measure will also be *n*.

A final example shows how more complicated values arise: if $N_0 = 0$, $N_1 = 3$, and $N_2 = 25$, then *mappability* = 3.694. The three alignments with one mismatch contribute 3 and the 25 alignments with two mismatches contribute the remaining 0.694 to the measure.

2.2.9.6 q

A comma-separated list of quality values, one per color-call. Each quality value is an integer between 0 and 100, exclusive. In addition, the value -1 indicates a missing quality value for the corresponding color-call.

2.2.9.7 r

Reference call at mismatch. A comma-separated list of {position}_{ref_color} for all of the color-calls in the read sequence that differ from the reference sequence. Position is 1-based relative to the sequence specified in the "g" attribute (again, the prepended base has position 1 and the first color in 'g' has position 2.). This is different from the basechange format in that only the reference sequence call is provided and the positions are 1-based and positive. For example,

```
r=18_3,21_1
```

means that the reference has color 3 at position 18, and color 1 at position 21.

2.2.9.8 s

This is a comma-separated string representing annotations on the sequence. The format is '{char}{position}' where {char} is a character representing the type of annotation (typically a formatting request to visualization software) and {position} is the position of this annotation in the read. The position is 1-based on the string recorded in the 'g' attribute. That is, the prepended base has position 1, and the first color has position 2. For example, "a5,g7,g8" means "format the color call at position 5 gr*a*y, format call 7 *g*reen, and format call 8 *g*reen". The SOLiD™ system follows this convention:

a	(grAy) is an *isolated mismatch*; it is a mismatch and neither the color-call on its left nor the call on its right is a mismatch,
g	(Green) is a *valid adjacent mismatch*; it is a mismatch and it, together with the adjacent mismatch on its left or right, could correspond to an isolated SNP,
y	(Yellow) is a color call that is consistent with an isolated *two*-base change. In general, these will be mismatches. But a conserved color between two mismatches is also a possibility.
r	(Red) is a color call that is consistent with an isolated *three*-base change.
b	(Blue) is an *invalid adjacent mismatch*; it is any other mismatch.

2.2.9.9 u

Mismatch count. This is a comma separated list of non-negative integers. The *i* th number specifies the number of positions in the reference to which this read aligns with exactly $i - 1$ mismatches. For example, u=0,3,15 says that this read does *not* align to the reference anywhere with exactly 0 mismatches, but that it does align with one mismatch at 3 reference positions, and with exactly two mismatches at 15 reference positions. All unspecified mismatch counts are undefined. For example, this example gives no information about the number of reference positions where the read aligns with exactly four mismatches.

3 Example

```
##gff-version 2
##source-version AnnotateChanges.java v0.2
##date 2007-08-28
##time 09:30:18
##Type solid_read DH10B_WithDup_FinalEdit
##color-code AA=0,AC=1,AG=2,AT=3,CA=1,CC=0,CG=3,CT=2,GA=2,GC=3,GG=0,GT=1,TA=3,TC=2,TG=1,TT=0
##generated-by "AnnotateChanges.java test-etc/modules/temp.gff test-
etc/modules/DH10B_WithDup_FinalEdit_validated.fasta"
##hdr seqname    source   feature start    end      score    strand   frame    [attributes]      [comments]
389_495_172_F3   Solid   read   906843   906867   -1   +    .    g=T2121033030302201123013333;r=18_0,19_1,24
                                                                ;s=y18,y19,r24;u=0,0,0,1
389_595_202_F3   Solid   read   912290   912314   -1   -    .    g=T3113211132100321303001020;u=1
```

This is an example of single-primer data which conforms to the specification described above. The reads have been annotated with reference sequence discrepancies, style annotations, and uniqueness counts. The example also shows a convenience "header" line (##hdr), which illustrates that not all comments, not even meta-data, need come from this specification.

A GFF specification

The SOLiD systems GFF format v2 has been designed to adhere to the GFF specification available for download at http://www.sanger.ac.uk/Software/formats/GFF/GFF_Spec.shtml

B Corrected base space representation

Stepwise process used in the generation of corrected base space representation

Step 1: Convert Base space (BS) reference to colorspace (CS) reference

```
    Reference BS      T T G A C T G A G T A C T
    Reference CS       0 1 2 1 2 1 2 2 1 3 1 2
```

Step 2: Using the known alignment (from the xxx.ma file) to the reference, align the read to the reference sequence in colorspace. Identify colorspace mismatches.

```
    Reference CS       0 1 2 1 2 1 2 2 1 3 1 2
    Read CS           T0 1 2 1 2 2 2 2 1 3 1 2
```

Step 3: Determine whether the mismatches are valid or invalid using two base encoding rules (see SOLiD system White paper Theoretical Understanding of Two-Base Color Codes and its Application to Annotation, Error Detection, and Error Correction. The code used is based on getCompatibility code listed in the white paper).

Example (i) Invalid
```
    Reference CS       0 1 2 1 2 1 2 2 1 3 1 2
    Read CS           T0 1 2 1 2 2 2 2 1 3 1 2
```

Example (ii) Valid
```
    Reference CS       0 1 2 1 2 1 2 2 1 3 1 2
    Read CS           T0 1 2 1 2 2 1 2 1 3 1 2
```

Step 4: If a color change is not valid, substitute the reference color to give the corrected color read:

Example (i) Invalid
```
    Reference CS       0 1 2 1 2 1 2 2 1 3 1 2
    Read CS           T0 1 2 1 2 2 2 2 1 3 1 2
    Corrected Read CS T0 1 2 1 2 1 2 2 1 3 1 2
```

Example (ii) Valid
```
    Reference CS       0 1 2 1 2 1 2 2 1 3 1 2
    Read CS           T0 1 2 1 2 2 1 2 1 3 1 2
    Corrected Read CS T0 1 2 1 2 2 1 2 1 3 1 2
```

Step 5: Convert to base space.

Example (i) Invalid

```
Corrected Read CS T0 1 2 1 2 1 2 2 1 3 1 2
Read in BS        T T G A C T G A G T A C T
```

Example (ii) Valid

```
Corrected Read CS T0 1 2 1 2 2 1 2 1 3 1 2
Read in BS        T T G A C T C A G T A C T
```

Step 6: Write bases that differ from the reference in lowercase.

Example (i) Invalid

```
Reference BS      T T G A C T G A G T A C T
Corrected Read BS T T G A C T G A G T A C T
```

Example (ii) Valid

```
Reference BS      T T G A C T G A G T A C T
Corrected Read BS T T G A C T c A G T A C T
```

Note change to base relative to reference

Step 7: Write corrected base read into GFF file (see 2.2.9.1)

Example (i) Invalid

b=TTGACTGAGTACT

Example (ii) Valid

…….. b=TTGACTcAGTACT