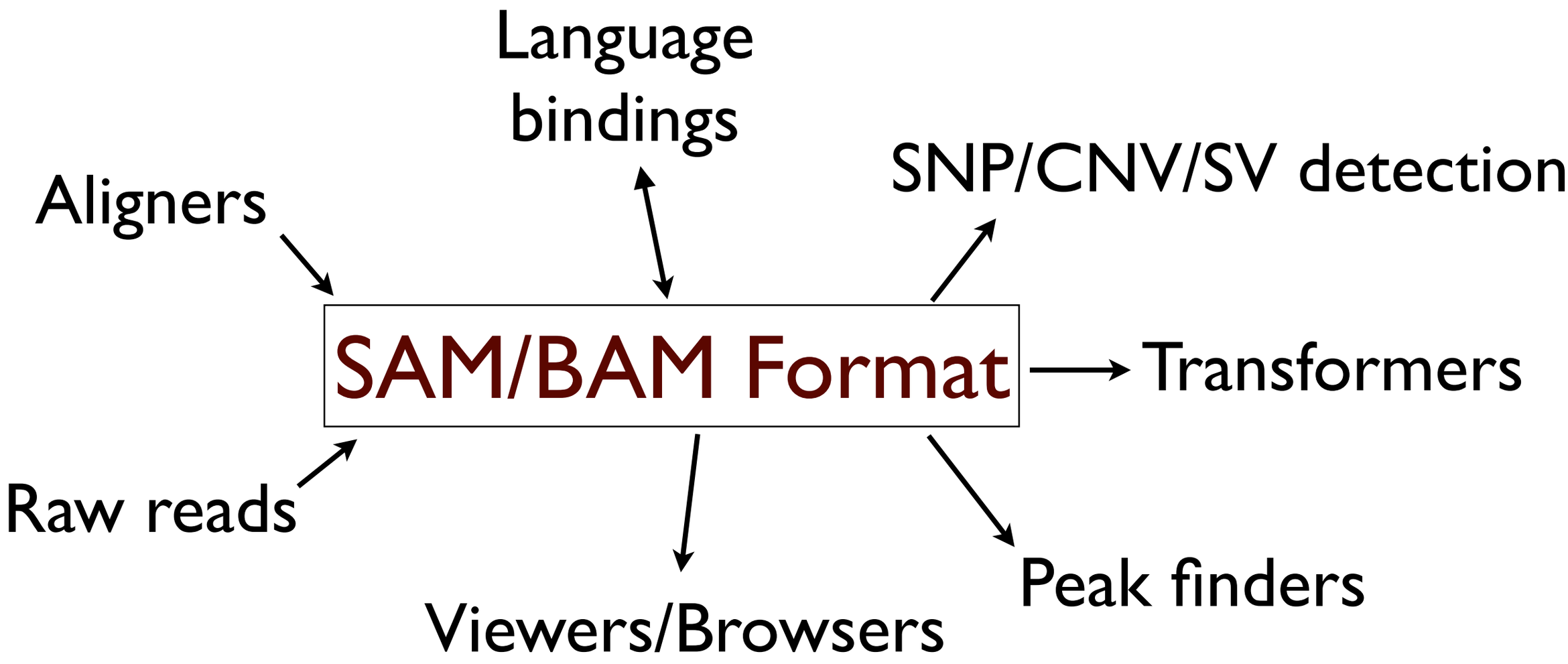
Samtools & Gbrowse2 for NGS

10/19/10

What is Samtools?

- A supported format for alignments
  - SAM - human-readable, scriptable
    - BAM - compact, indexed alignments
- A software toolset
- An API

Samtools and Friends



http://samtools.sourceforge.net/swlist.shtml

Samtools and Friends

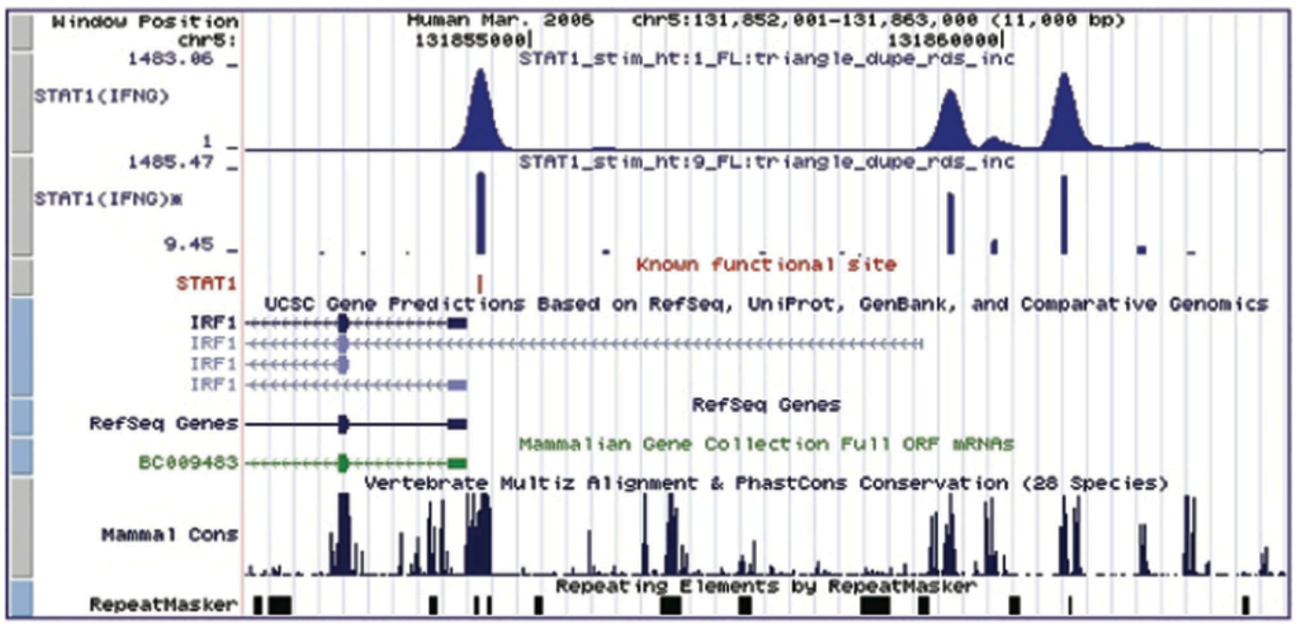
Aligners

BFAST
Bowtie & TopHat
BWA
Mosaik
Novoalign
SSAHA2

http://samtools.sourceforge.net/swlist.shtml

Samtools and Friends

FindPeaks (Vancouver)
MACS
QuEST(Stanford)



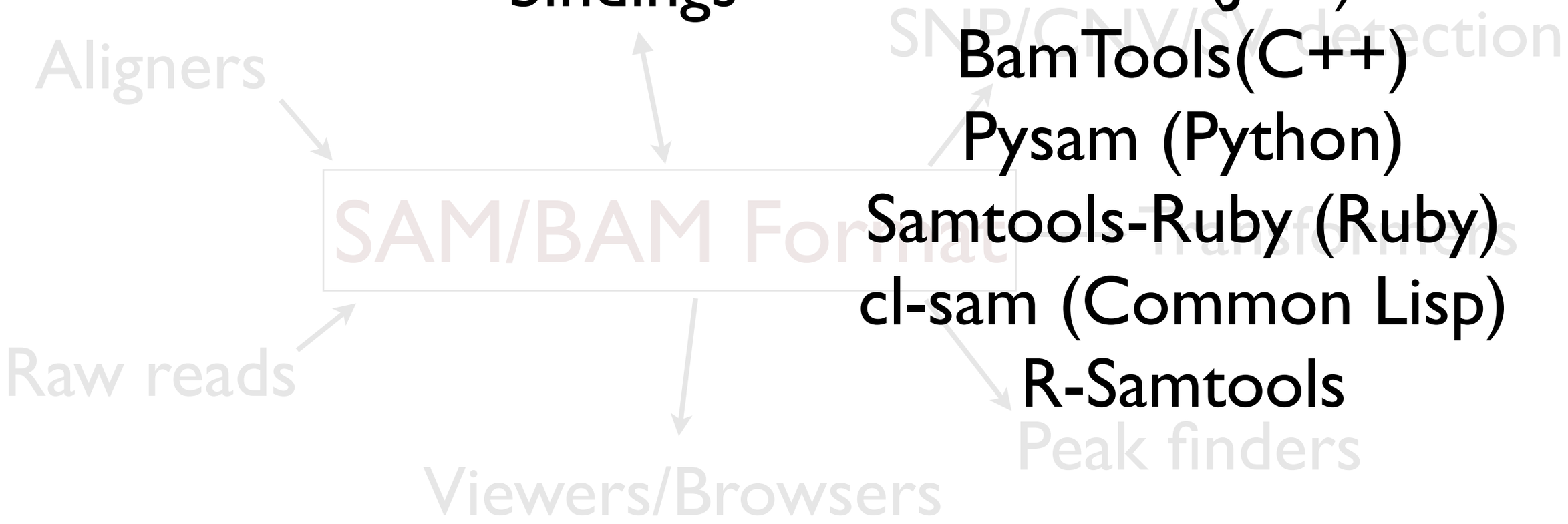
Peak finders

http://samtools.sourceforge.net/swlist.shtml

Samtools and Friends

Language bindings

Bio-SamTools (Perl)
Picard (Java)
BamTools(C++)
Pysam (Python)
Samtools-Ruby (Ruby)
cl-sam (Common Lisp)
R-Samtools



http://samtools.sourceforge.net/swlist.shtml

Samtools and Friends

SV Detection

Breakdancer (WashU)

SNP/CNV/SV detection

SNP pipelines:

Genome Analysis Toolkit (Broad)

CNV Detection

CNVer (Toronto)

http://samtools.sourceforge.net/swlist.shtml

Simple Pileup

```
seq1 272 T 24  ,.$.....,.,.,...,,,,.^+. <<<+;<<<<<<<<<<=<;<;7<&
seq1 273 T 23  ,.....,.,.,...,,,,,,,A <<<;<<<<<<<<3<=<<<;<<+
seq1 274 T 23  ,.$.....,.,.,...,,,,,,    7<7;<;<<<<<<<<<=<;<;<<6
seq1 275 A 23  ,$.....,.,.,...,,,,,.^l.  <+;9*<<<<<<<<<=<<:;<<<<
seq1 276 G 22  ...T,,.,.,...,,,,,,,,  33;+<<7=7<<7<&<<1;<<6<
seq1 277 T 22  ....,,.,.,.C.,,,,,,.G.  +7<;<<<<<<<&<=<<:;<<&<
seq1 278 G 23  .....,.,.,...,,,,,,,.^k.   %38*<<;<7<<7<=<<<;<<<<<
seq1 279 C 23  A..T,,.,.,...,,,,,,,,, ;75&<<<<<<<<<=<<<9<<:<<
```

chr

pos

refbase

depth

read bases

qualities

samtools pileup -cf ex1.fa ex1.bam

Verbose Pileup

samtools-0.1.8/samtools pileup -v -c -f yeasties.fa SRR003681.sorted.bam > SRR003681.pileup

I do not understand the columns in the pileup output.

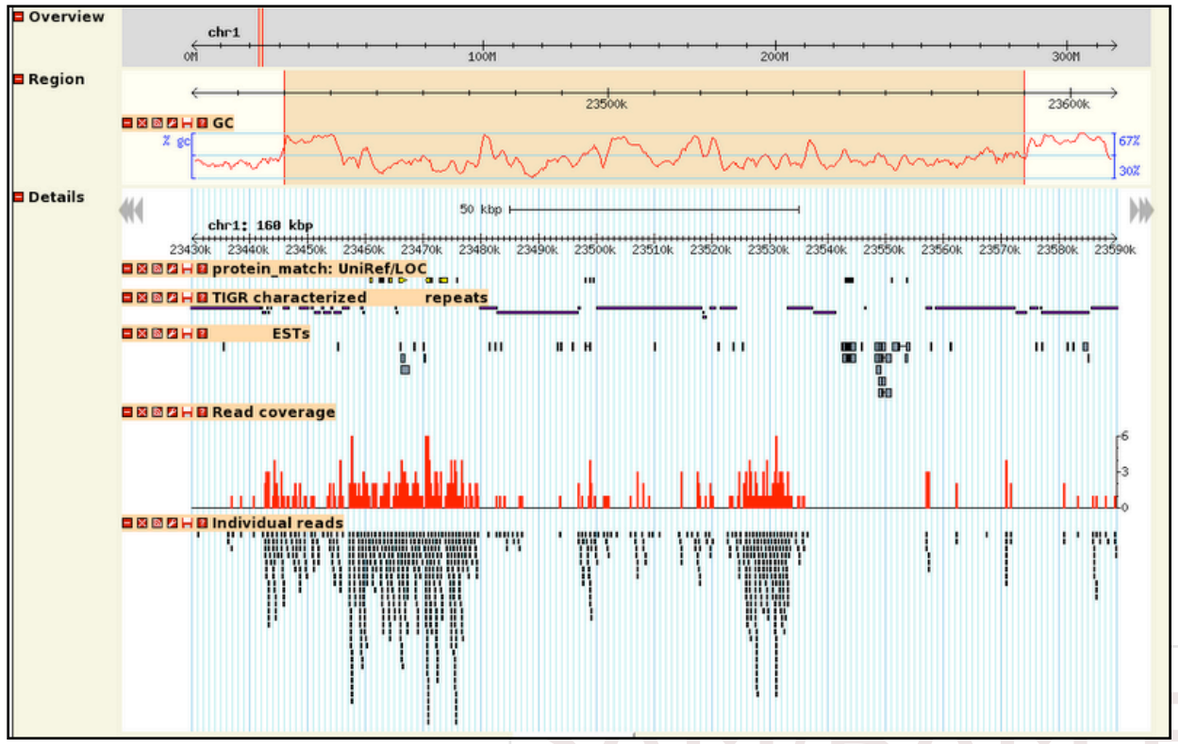
This is explained in the manual page. Or briefly (when you invoke pileup with the `-c` option):

1. reference sequence name
2. reference coordinate
3. reference base, or `*' for an indel line
4. genotype where heterozygotes are encoded in the IUB code: `M=A/C, R=A/G, W=A/T, S=C/G, Y=C/T` and `K=G/T`; indels are indicated by, for example, `*/+A, -A/*` or `+CC/-C`. There is no difference between `*/+A` or `+A/*`.
5. Phred-scaled likelihood that the genotype is wrong, which is also called `consensus quality'.
6. Phred-scaled likelihood that the genotype is identical to the reference, which is also called `SNP quality'. Suppose the reference base is `A` and in alignment we see 17 `G` and 3 `A`. We will get a low consensus quality because it is difficult to distinguish an `A/G` heterozygote from a `G/G` homozygote. We will get a high SNP quality, though, because the evidence of a SNP is very strong.
7. root mean square (RMS) mapping quality
8. # reads covering the position
9. read bases at a SNP line (check the manual page for more information); the 1st indel allele otherwise
10. base quality at a SNP line; the 2nd indel allele otherwise
11. indel line only: # reads directly supporting the 1st indel allele
12. indel line only: # reads directly supporting the 2nd indel allele
13. indel line only: # reads supporting a third indel allele

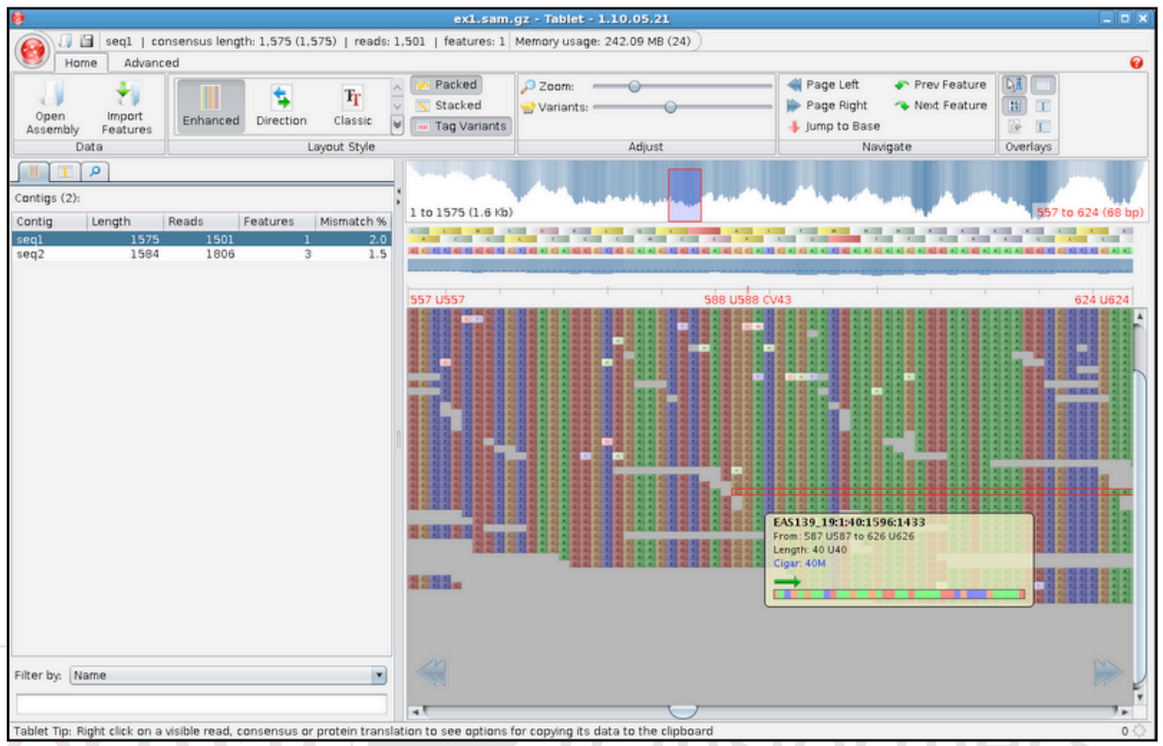
If pileup is invoked without `-c', indel lines and columns between 3 and 7 inclusive will not be outputted.

http://sourceforge.net/apps/mediawiki/samtools/index.php?title=SAM_FAQ

Samtools and Friends



gbrowse2



Tablet

Raw reads

Peak finders

Viewers/Browsers

http://jermdemo.blogspot.com/2010/08/ngs-viewers-reviewed.html

http://samtools.sourceforge.net/swlist.shtml

Sam Output

SRR003681.6593 16 chrII 159978 255 36M * 0 0 AAAACCCTTTAGTTTTTTTACTGTGTGTCCGGCCGC .4:$-.'"("&2&+$1.-)/C'1%)&.)I@IIIIII XA:i:2 MD:Z:3G6T8T16 NM:i:3

QNAME = name of read
FLAG = Bitwise FLAG (2^{16}-1)
RNAME = Reference sequence name
POS = Position (1-based)
MAPQ = Mapping Quality (Phred-based)
CIGAR = CIGAR STRING
MRNM = Mate Reference Sequence
MPOS = 1-based Mate Position of the other seq
ISIZE = Inferred Insert Size
SEQ = Sequence reported on the + strand
QUAL = Quality scores (ASCII-33 = Phred)
TAG = TAG

Tag	Meaning
NM	Edit distance
MD	Mismatching positions/bases
AS	Alignment score
X0	Number of best hits
X1	Number of suboptimal hits found by BWA
XN	Number of ambiguous bases in the referenece
XM	Number of mismatches in the alignment
XO	Number of gap opens
XG	Number of gap extentions
XT	Type: Unique/Repeat/N/Mate-sw
XA	Alternative hits; format: (chr,pos,CIGAR,NM;)*
XS	Suboptimal alignment score
XF	Support from forward/reverse alignment
XE	Number of supporting seeds

High-level API

```
use Bio::DB::Sam;

# high level API
my $sam = Bio::DB::Sam->new(-bam  =>"data/ex1.bam",
                            -fasta=>"data/ex1.fa",
                            );

my @targets    = $sam->seq_ids;
my @alignments = $sam->get_features_by_location(-seq_id => 'seq2',
                                                -start  => 500,
                                                -end    => 800);
for my $a (@alignments) {

   # where does the alignment start in the reference sequence
   my $seqid  = $a->seq_id;
   my $start  = $a->start;
   my $end    = $a->end;
   my $strand = $a->strand;
   my $cigar  = $a->cigar_str;
   my $paired = $a->get_tag_values('PAIRED');

   # where does the alignment start in the query sequence
   my $query_start = $a->query->start;
   my $query_end   = $a->query->end;

   my $ref_dna    = $a->dna;        # reference sequence bases
   my $query_dna = $a->query->dna; # query sequence bases

   my @scores    = $a->qscore;     # per-base quality scores
   my $match_qual= $a->qual;       # quality of the match
}

my @pairs = $sam->get_features_by_location(-type   => 'read_pair',
                                           -seq_id => 'seq2',
                                           -start  => 500,
                                           -end    => 800);

for my $pair (@pairs) {
   my $length                    = $pair->length;   # insert length
   my ($first_mate,$second_mate) = $pair->get_SeqFeatures;
   my $f_start = $first_mate->start;
   my $s_start = $second_mate->start;
}
```

Low-level API

```
# low level API
my $bam          = Bio::DB::Bam->open('/path/to/bamfile');
my $header       = $bam->header;
my $target_count = $header->n_targets;
my $target_names = $header->target_name;
while (my $align = $bam->read1) {
   my $seqid     = $target_names->[$align->tid];
   my $start     = $align->pos+1;
   my $end       = $align->calend;
   my $cigar     = $align->cigar_str;
}

my $index = Bio::DB::Bam->index_open('/path/to/bamfile');
my $index = Bio::DB::Bam->index_open_in_safewd('/path/to/bamfile');

my $callback = sub {
    my $alignment = shift;
    my $start       = $alignment->start;
    my $end         = $alignment->end;
    my $seqid       = $target_names->[$alignment->tid];
    print $alignment->qname," aligns to $seqid:$start..$end\n";
}
my $header = $index->header;
$index->fetch($bam,$header->parse_region('seq2'),$callback);
```

http://search.cpan.org/~lds/Bio-SamTools/lib/Bio/DB/Sam.pm

Problem Set

Get Bowtie for Mac (unzip, make). You may use another SAM-compliant aligner if you are adventurous.

http://bowtie-bio.sourceforge.net/

Download the reference sequences that came with gbrowse2

http://localhost/gbrowse2/databases/yeast_scaffolds/

Concatenate them:
cat chr1.fa chr2.fa > yeasties.fa

Build the indices for those two reference sequences:
bowtie-build yeasties.fa yeasties

Download this short read archive:
http://infoserver.cshl.edu/LectureNotes/ngs2/SRR003681.subset.fq

Study the section on FASTQ encoding: http://en.wikipedia.org/wiki/FASTQ_format#Encoding

Type 'tail SRR003681.subset.fq' to see some less than perfect sequences at the end of the file. Can you tell what encoding was used for these sequences?

bowtie-0.12.7/bowtie --sam yeasties SRR003681.subset.fq > SRR003681.sam

Can you explain to my grandmother what Bowtie's default alignment settings are?
http://bowtie-bio.sourceforge.net/manual.shtml#the--n-alignment-mode

Problem Set

Copy Samtools to your current directory
cp /Network/Servers/infoserver.cshl.edu/Users/Shared/samtools-0.1.8 .

These three incantations are always necessary to get a sorted, index BAM from a SAM file:

samtools-0.1.8/samtools view -b -S -T yeasties.fa SRR003681.sam > SRR003681.bam

samtools-0.1.8/samtools sort SRR003681.bam SRR003681.sorted

samtools-0.1.8/samtools index SRR003681.sorted.bam

The script calculates coverage depth at each position of chrI.
Use your perl skills to calculate the maximum, minimum, and average depth.
The script is located at: http://infoserver.cshl.edu/LectureNotes/ngs2/getCoverage.pl

```
#!/usr/bin/perl
use strict;
use warnings;
use Bio::DB::Sam;

 # high level API
my $sam = Bio::DB::Sam->new(-bam  =>"SRR003681.sorted.bam",
                            -fasta=>"yeasties.fa",
);

my $segment = $sam->segment(-seq_id=>'chrI');
my ($coverage)=$segment->features('coverage');
my @data_points=$coverage->coverage;
foreach my $depth(@data_points){
    print $depth."\n";
}
```

Problem Set

Copy the following four files into a new directory called *bamfiles*:

SRR003681.sorted.bam
SRR003681.sorted.bam.bai
yeasties.fa
yeasties.fa.fai

Move this directory: (do you have admin privileges on your computer yet?)
sudo mv bamstuff /Library/WebServer/Documents/gbrowse2/databases/

Add this to the database definitions section of /etc/gbrowse2/yeast_chr1+2.conf

```
[SRR003681:database]
db_adaptor     = Bio::DB::Sam
db_args        = -fasta /Library/Webserver/Documents/gbrowse2/databases/bamfiles/yeast_chromosomes.fa
                 -bam /Library/Webserver/Documents/gbrowse2/databases/bamfiles/SRR003681.sorted.bam
search options = default
```

Add these to the tracks section of /etc/gbrowse2/yeast_chr1+2.conf

```
[CoverageXyplot]
feature        = coverage
glyph          = wiggle_xyplot
database       = SRR003681
height         = 50
fgcolor        = black
bicolor_pivot  = 20
pos_color      = blue
neg_color      = red
key            = Coverage (xyplot)
category       = NGS
label          = 0
```

```
[Reads]
feature        = match
glyph          = segments
draw_target    = 1
show_mismatch  = 1
mismatch_color = red
database       = SRR003681
bgcolor        = blue
fgcolor        = white
height         = 5
label density  = 50
bump           = fast
key            = Reads
category       = NGS
```

Go to http://localhost/cgi-bin/gb2/gbrowse/yeast_advanced/
Turn on the NGS tracks.
NOTE this is the advanced yeast gbrowse instance not the basic one

Having problems?
tail /var/log/apache2/error_log