Challenges and Solutions in the Analysis of Next Generation Sequence Data

Heng Li

Broad Institute

2nd CHOP/PENN NGS Symposium

About me

- One of the major contributors to the SAM specification
- Key developer of several popular software packages:
  - Short-read alignment: MAQ and BWA
  - Long-read alignment: BWA-SW
  - Variant calling and data processing: SAMtools and Tabix
- Involved in the early development of the 1000 Genomes Project
- Google (US/UK) "heng li" for the slides.

Outline

1. Overview of the next-generation sequencing
    - Messages from the 1000 Genomes Project
    - Sequencing machines vs. computers

2. Quest for standards

3. The SAM-centric data processing
    - Making a choice: alignment
    - Making a choice: visualization
    - Making a choice: SNP/INDEL discovery
    - SAM-centric data processing

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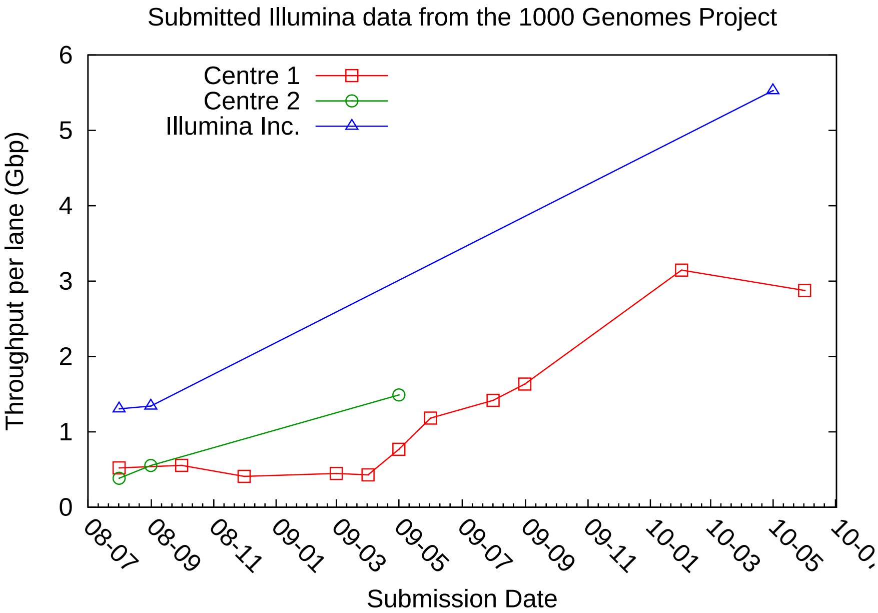
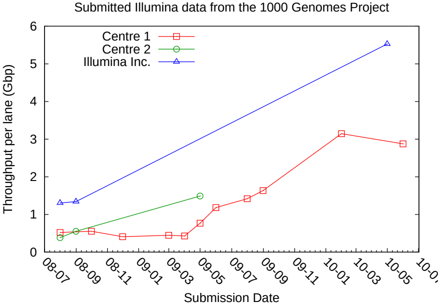
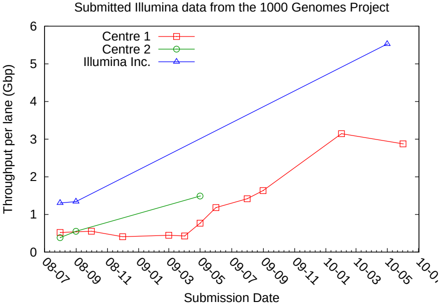

Submitted Illumina data from the 1000 Genomes Project
Centre 1
Centre 2
Illumina Inc.
Throughput per lane (Gbp)
0
1
2
3
4
5
6
08-07
08-09
08-11
09-01
09-03
09-05
09-07
09-09
09-11
10-01
10-03
10-05
10-0
Submission Date



Illumina sequencing

- 5X increased throughput in $<$2 years
- 4–5Gbp raw sequences per machine day at present



Illumina sequencing

- 5X increased throughput in <2 years
- 4–5Gbp raw sequences per machine day at present
- HiSeq: 14Gbp per lane (not formally submitted yet); ∼30X mappable data to human per half run

Current sequencing technologies

	GA IIx	HiSeq	SOLiD4	454FLX
Read length (bp/color)	2x100	2x100	2x50	400
Run time (days)	9.5	8	14	0.4
Mappable per run (Gbp)	40	160	90	0.5
Throughput (Gbp/day)	4.2	20	6.4	1.1

- Machine yield obtained from vendors' website.
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- HiSeq and SOLiD4 sequence two flow cells/slides per run.

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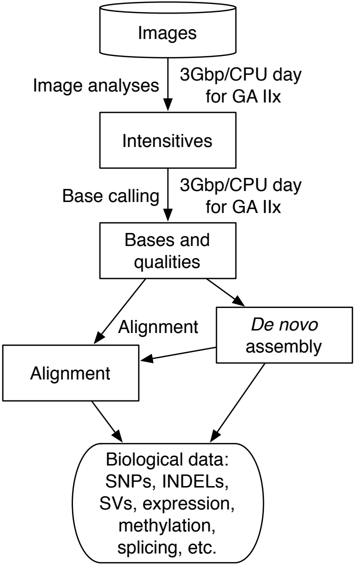
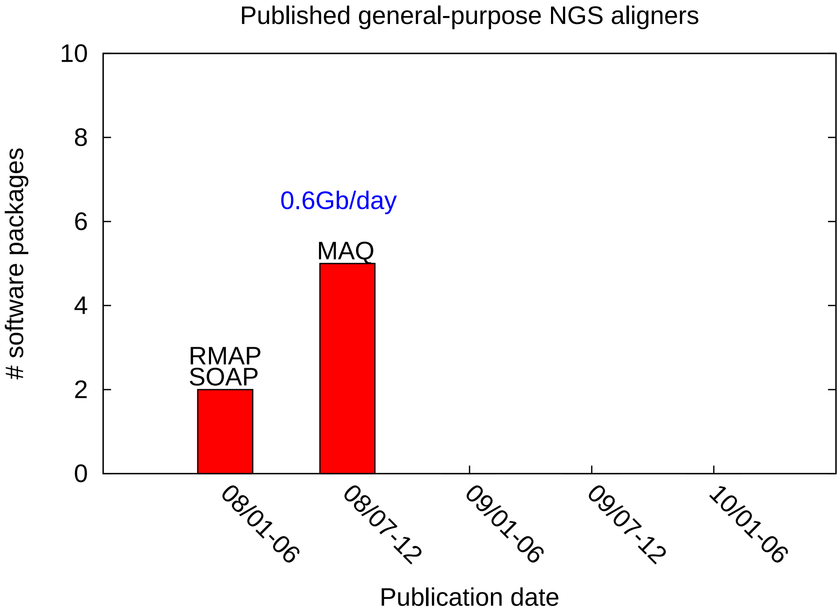
Do computers match sequencing machines in throughput?

Typical NGS workflow



- Image analyses and base calling software are provided by vendors.
- Image are usually analyzed in real time.
- 454 alignment and assembly can be done on intesities.

For image analyses and base calling, an 8-core computer matches HiSeq in throughput.


Published general-purpose NGS aligners
# software packages
0
2
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6
8
10
0.6Gb/day
RMAP
SOAP
MAQ
08/01-06
08/07-12
09/01-06
09/07-12
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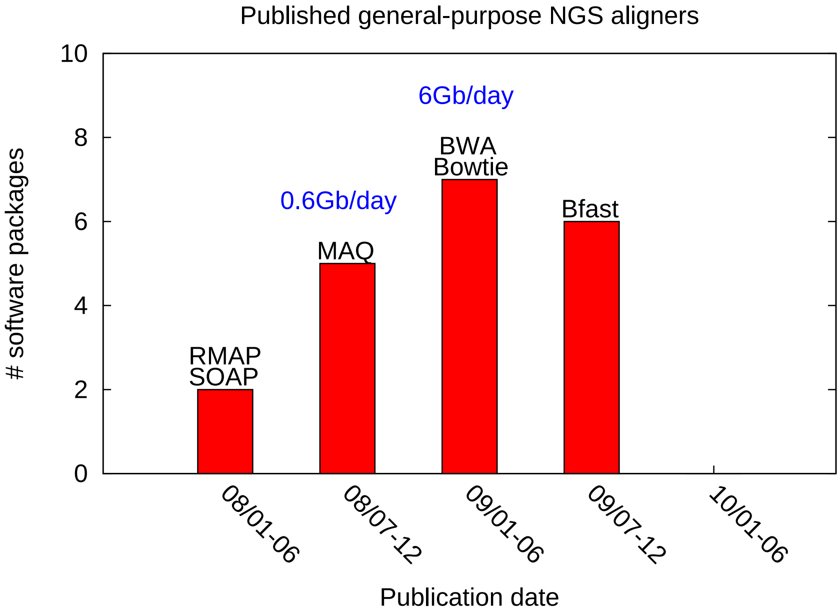
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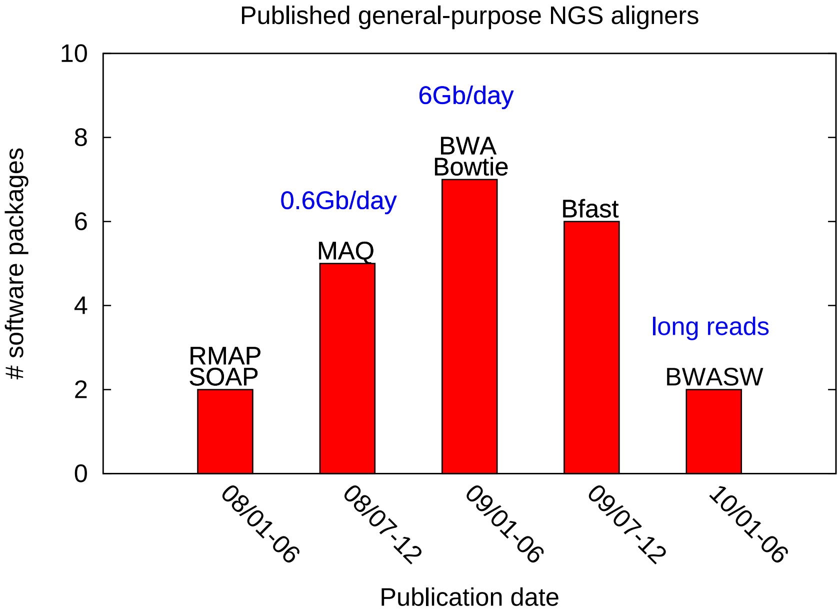
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Image analysis (Gbp/CPU day)	3	3		
Base calling (Gbp/CPU day)	3	3		
Aln. to human (Gbp/CPU day)	6	6		3

- Machine yield obtained from vendors' website.
- Assuming 90% of raw seqences mappable for GA IIx and HiSeq.
- HiSeq and SOLiD4 sequence two flow cells/slides per run.
- Image analyses done in real-time (on sequencing machines).
- Illumina alignment done by Bowtie/BWA/SOAP2; 454 by BWASW.

A word about Pacific Biosciences (PacBio)

- Polymerase for sequencing
- Single-molecule sequencing
  - less amplification bias
  - DNA methylation (no bisulfite treatment)
- Long reads (In Sequence, 02/10/2009; Eid *et al.*, 2009)
  - $\sim$1000bp in average
  - exponentially distributed: a few very long, many short (Sanger and 454: normal distributed)
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- Potential for a variety of new applications
- Not an immediate replacement of current technologies

Sequencing machines vs. computers

- *At present*, a tie.
- *In future*, sequencing machines may pull ahead.
- Alignment used to be the bottleneck, but base calling is the slowest step now.
  - Base calling: platform dependent and mostly by vendors. (closed)
  - Alignment: community efforts. (open)

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The dilemma of openness

A year ago:

- 14 published aligners, 14 (primitive) alignment formats.
- No generic alignment viewers, no generic variant callers.
- Everyone writes their own pipeline from scratch.

Now (a year after the publication of SAM):

- Most popular aligners generate a single format: SAM.
- 10 SAM supported alignment viewers, 3 generic SNP/indel callers.
- Build pipeline upon high-performance tools as well as upon libraries in C, C++, Java, Perl, Python and Ruby.

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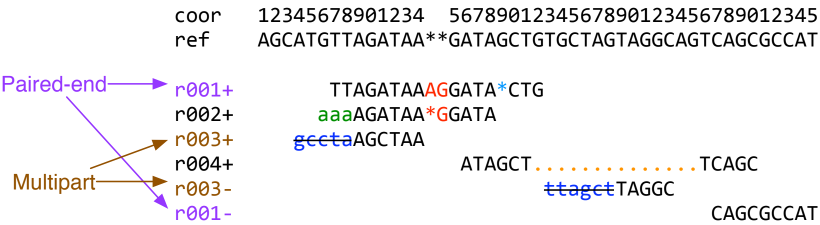

```
coor   12345678901234  5678901234567890123456789012345
ref    AGCATGTTAGATAA**GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT

r001+        TTAGATAAAGGATA*CTG
r002+       aaaAGATAA*GGATA
r003+     ~~gccta~~AGCTAA
r004+                   ATAGCT..............TCAGC
r003-                          ~~ttagct~~TAGGC
r001-                                        CAGCGCCAT
```

Ins & padding
Soft clipping

Splicing
Hard clipping

```
@SQ SN:ref LN:45
r001 163 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTA *
r002   0 ref  9 30 3S6M1P1I4M * 0    0 AAAAGATAAGGATA    *
r003   0 ref  9 30 5H6M       * 0    0 AGCTAA   *   NM:i:1
r004   0 ref 16 30 6M14N5M    * 0    0 ATAGCTTCAGC       *
r003  16 ref 29 30 6H5M       * 0    0 TAGGC    *   NM:i:0
r001  83 ref 37 30 9M         = 7  -39 CAGCGCCAT         *
```

```
ref  7 T 1 .    |ref 12 T 3 ...         |ref 17 T 3 ...
ref  8 T 1 .    |ref 13 A 3 ...         |ref 18 A 3 .-1G..
ref  9 A 3 ...  |ref 14 A 2 .+2AG.+1G.  |ref 19 G 2 *.
ref 10 G 3 ...  |ref 15 G 2 ..          |ref 20 C 2 ..
ref 11 A 3 ..C  |ref 16 A 3 ...         |...
```

Features

- Flexibility:
  - Variable read lengths, from short reads to BACs.
  - Indels, splicing, clipping and multi-part alignment.
  - User defined or aligner specific information.
- Efficiency:
  - Compact in file size (one byte per raw base).
  - Minimal memory requirement.
  - Random access.
- Open alignment files over FTP/HTTP.
  - Interested in a few genes, but cannot afford 40TB alignments.
- Matured and stable.

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The SAM/BAM format is the standard.

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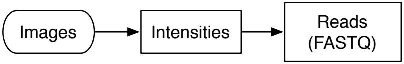
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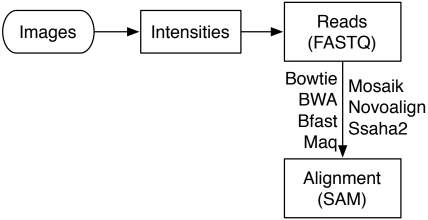
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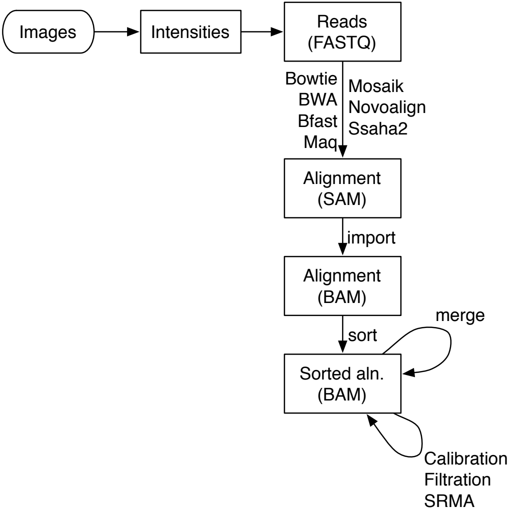
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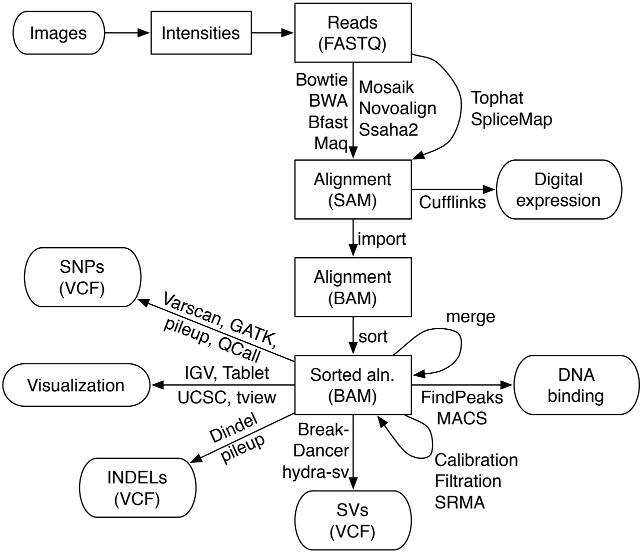
SRMA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Alignment (SAM)
Cufflinks
Digital expression
import
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Varscan, GATK, pileup, QCall
SNPs (VCF)
IGV, Tablet
UCSC, tview
Visualization
Dindel
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INDELs (VCF)
Break-Dancer
hydra-sv
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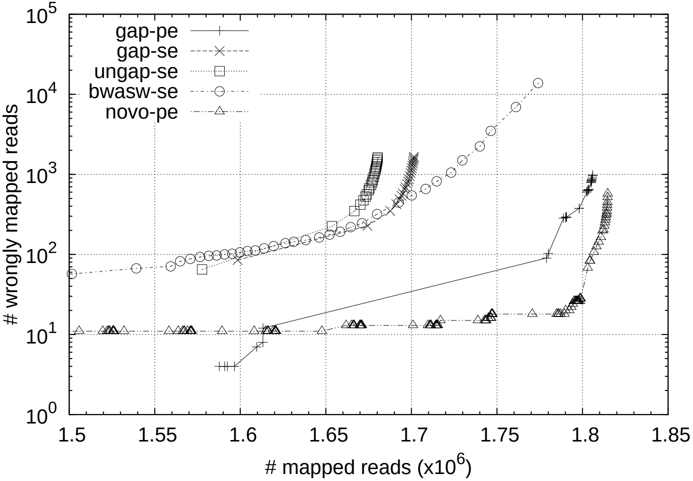

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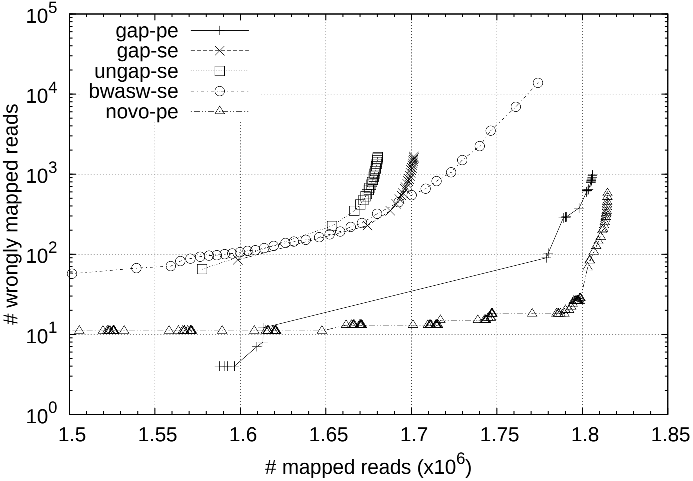
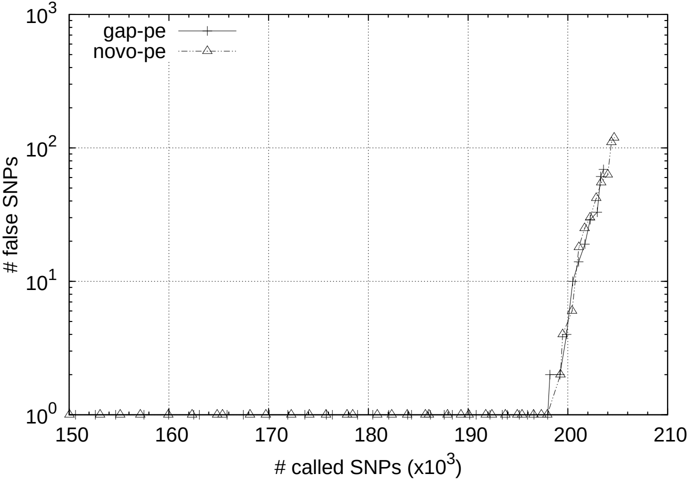


Systematic errors are more dangerous.



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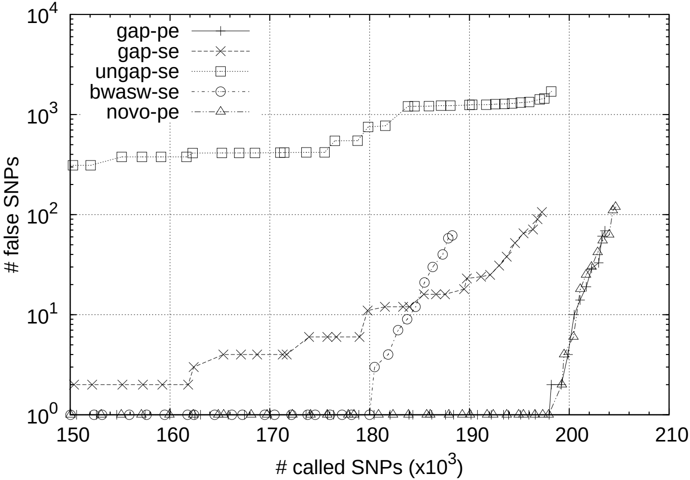

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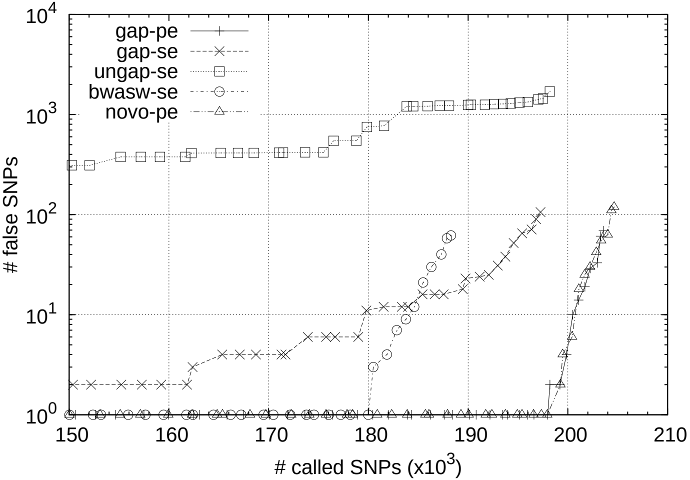

Choose an alignment algorithm

Sources of alignment errors:

- Repeats and *known* segmental duplications
- Approximations and heuristics
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The baseline:

Don't let the alignment errors be dominant.

Choose alignment algorithms based on needs

Depth based CNV discovery and study of highly expressed genes:

- Other sources of errors/variation dominate
- Use a fast aligner (e.g. Bowtie/SOAP2)

SNP/INDEL discovery and study of weakly expressed genes:

- 10–20% of short variants in human are indels.
- Use a gapped and accurate aligner (e.g. bwa/novoalign).

SV discovery; somatic mutations

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- Combine distinct algorithms (e.g. bwa+mosaik/bwasw)

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Visualization

- Base-pair resolution:
  - IGV: highly tuned for SNP/SV discovery
  - SAMtools tview: fast over slow network
  - Tablet: Support many alignment/assembly formats; elegant interface
- Low resolution:
  - SeqMonk: highly tuned for ChIP/RNA-seq
- Web based:
  - UCSC: BAM support plus much more
  - GBrowse: BAM support

Types of SNP calling

- Single sample: samtools, GATK, QCall and VarScan
- Pooled sample: Syzygy
- Multi-sample, without LD: GATK and QCall
- Multi-sample, with LD: GATK+Beagle and QCall
- Contrast samples (e.g. tumor vs. normal): ?

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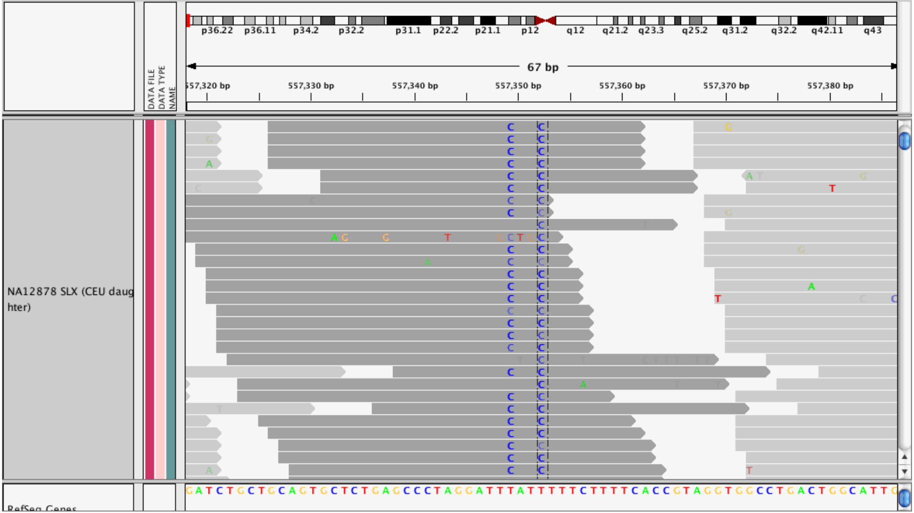
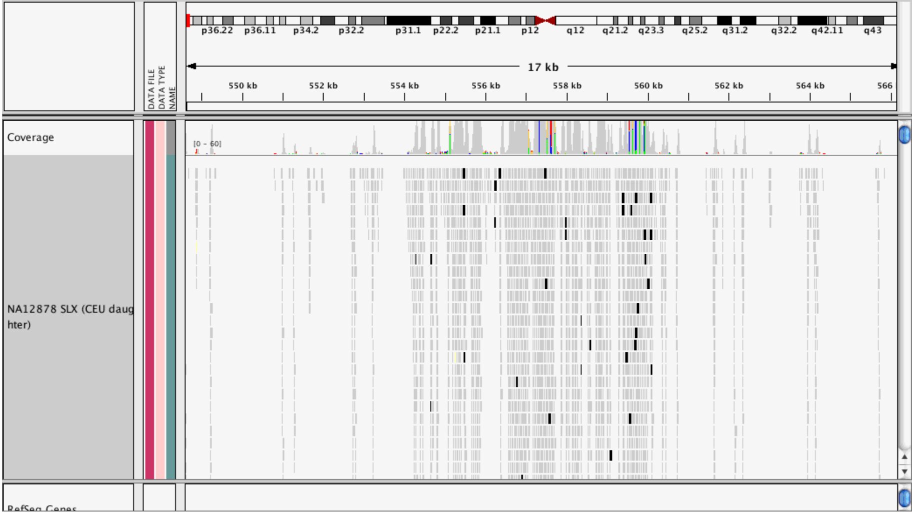


from the GATK website

Artefacts: excessive coverage



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Other factors considered in filtering

- Allele balance: #ref-alleles/#snp-alleles
- Mapping quality: the accessibility of the region
- Clustered SNPs: sign of paralogous mapping
- Length of the longest homopolymer run
- Break of diploidity: paralogous mapping

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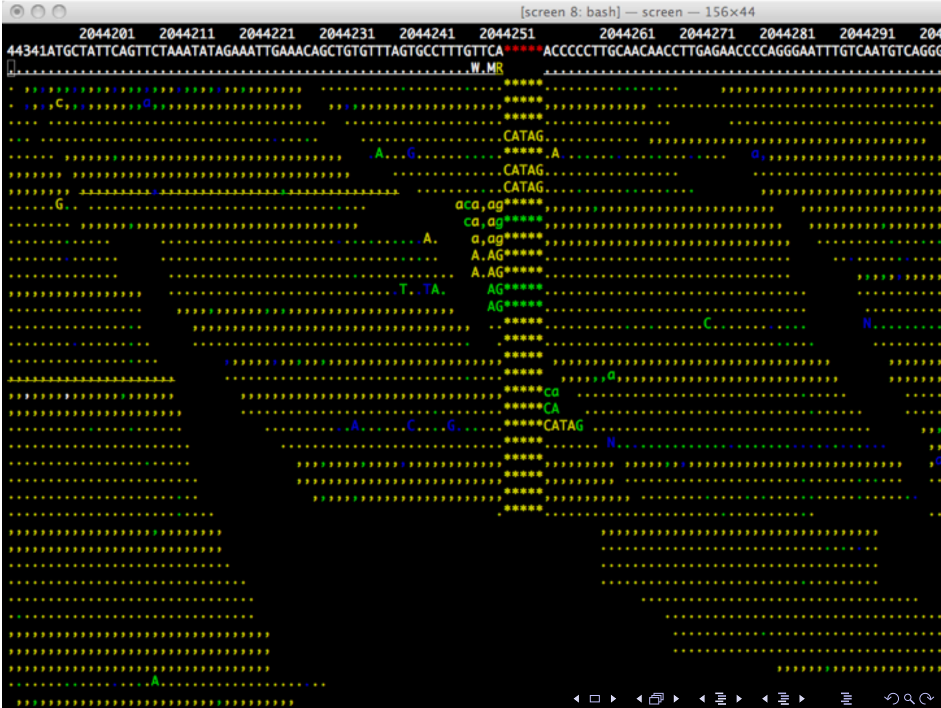
Towards high accuracy SNP calling:

- Necessary for somatic mutations.
- A good alignment viewer (e.g. IGV and tview) is essential – SAM only!



[screen 8: bash] — screen — 156×44

```
         2044201   2044211   2044221   2044231   2044241   2044251       2044261   2044271   2044281   2044291   204
44341ATGCTATTCAGTTCTAAATATAGAAATTGAAACAGCTGTGTTTAGTGCCTTTGTTCA*****ACCCCCTTGCAACAACCTTGAGAACCCCAGGGAATTTGTCAATGTCAGGG
.............................................................W.MR     ..................................................
```

INDEL calling

Problem:

- Reads are indenpendent in alignment, but reads mapped to the same locus are correlated.
- Naive indel callers would not work well.

Solution:

- Sophisticated indel callers all do realignment implicitly (e.g. Dindel and samtools).
- Explicit realignment (e.g. SRMA and GATK).

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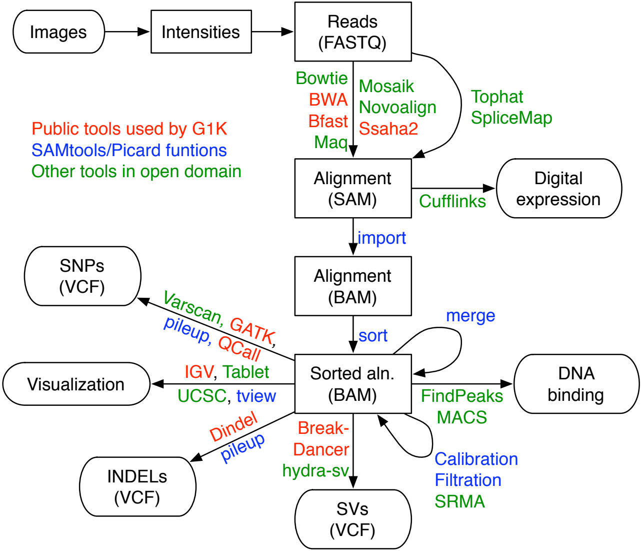

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- Efficiency: processing 40X human resequencing data in a week (∼30 CPU days) with 500GB disk space.

- Following the best practices, small labs can afford analyzing deep human resequencing data.
- Analyzing data of smaller scale is even easier.

Summary

- A sequencing machine can produce up to 20G base pairs per day.
- Data processing is keeping the pace with the increasing throughput of sequence machines.
- The SAM/BAM format is the pivot of data processing. Adopted by most major sequencing centers.
- Following the best practices, small labs can handle data from the latest sequencing machines.

Acknowledgements

- 1000 Genomes Project analyses group
- Bob Handsaker and Richard Durbin
- Tim Fennel, Mark Depristo and the GSA group at Broad
- SAMtools/Picard users
- Altshuler/Daly lab and Reich lab
- Xiaowu Gai

Thank You