Low level Processing and Visualization of Genome Sequencing Data

GSND 5340Q, BMDA

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Section 1

Motivating Example: X-linked Disease

Rare X-linked Disease



Rare X-linked Disease

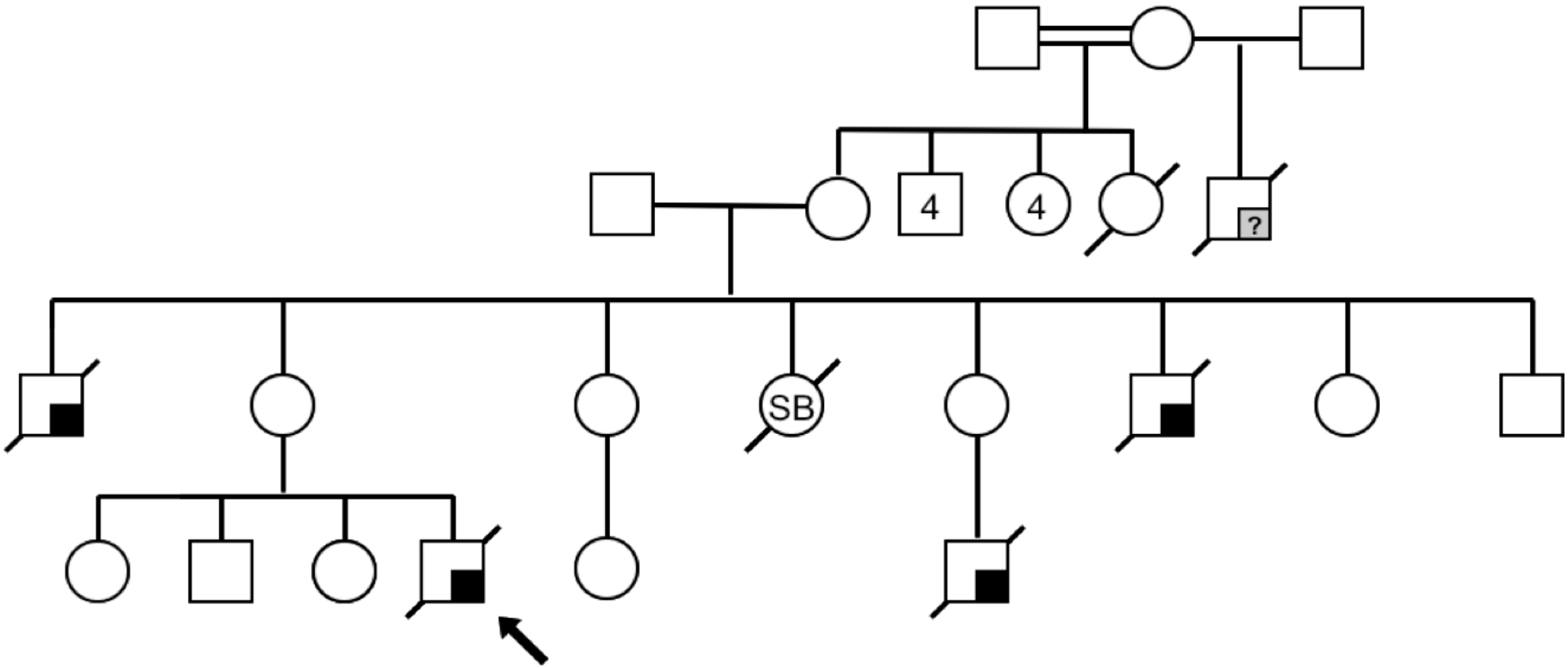
Uncle #1

Uncle #2

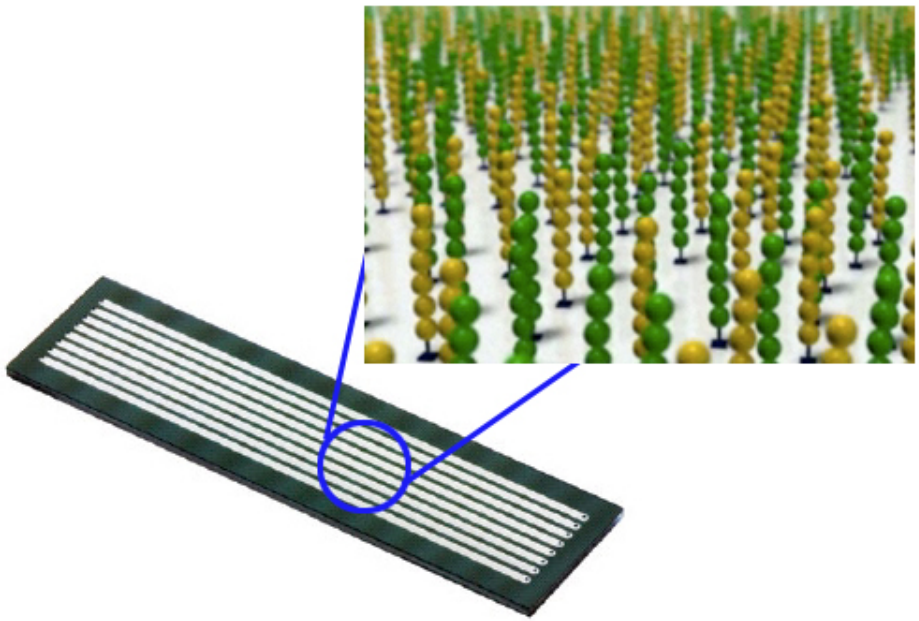
cousin

proband

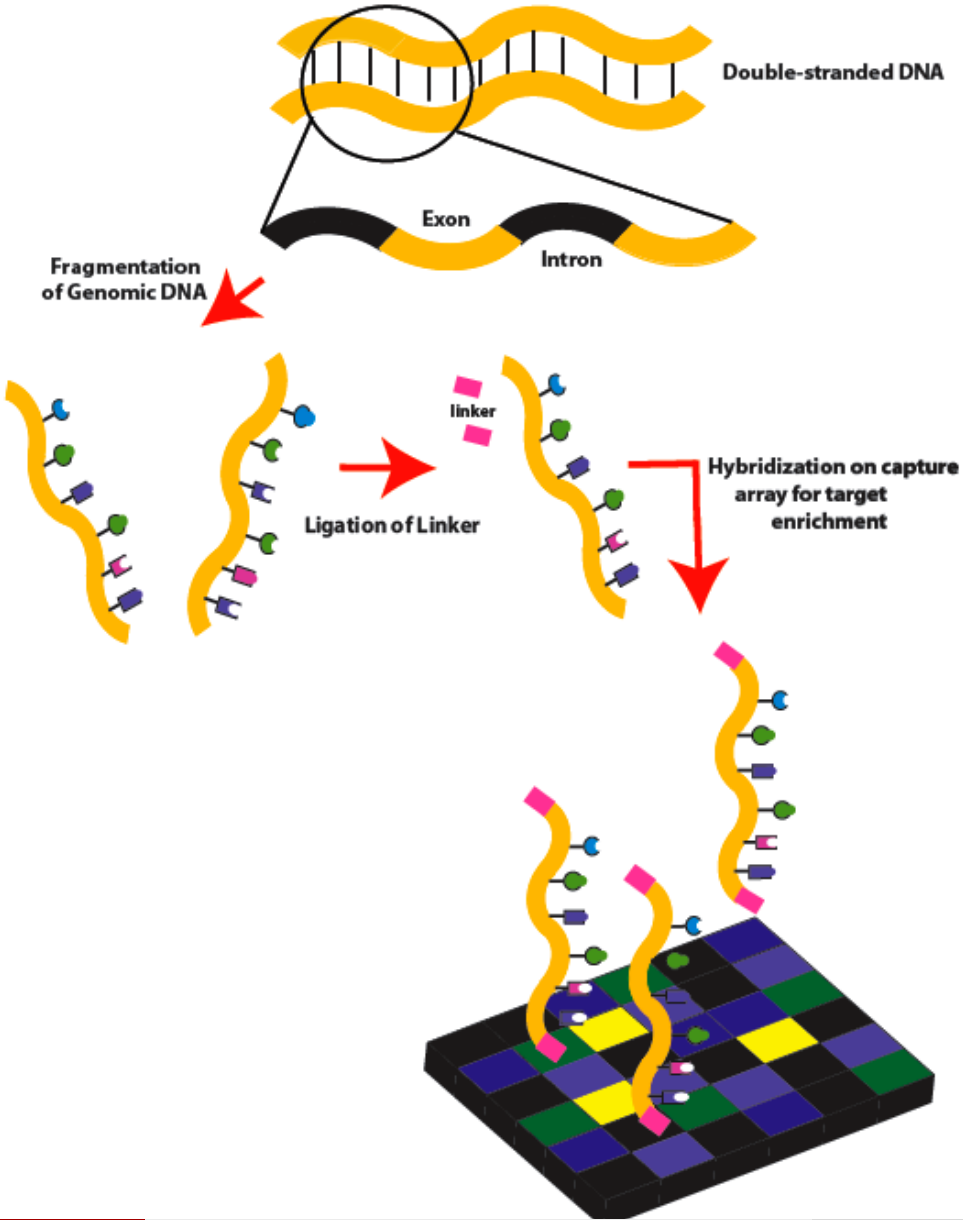
Rare X-linked Disease



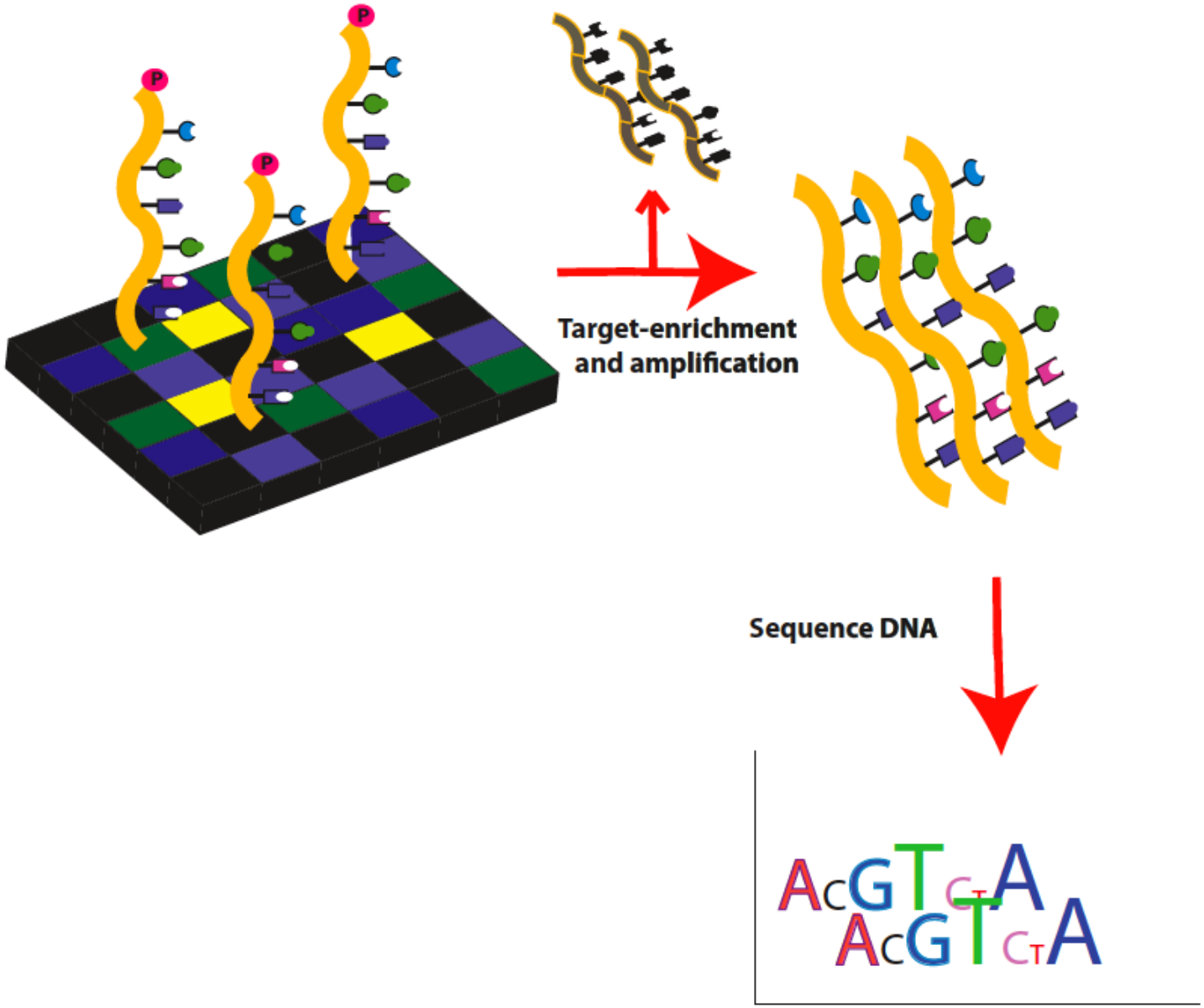
Exon Capture Sequencing



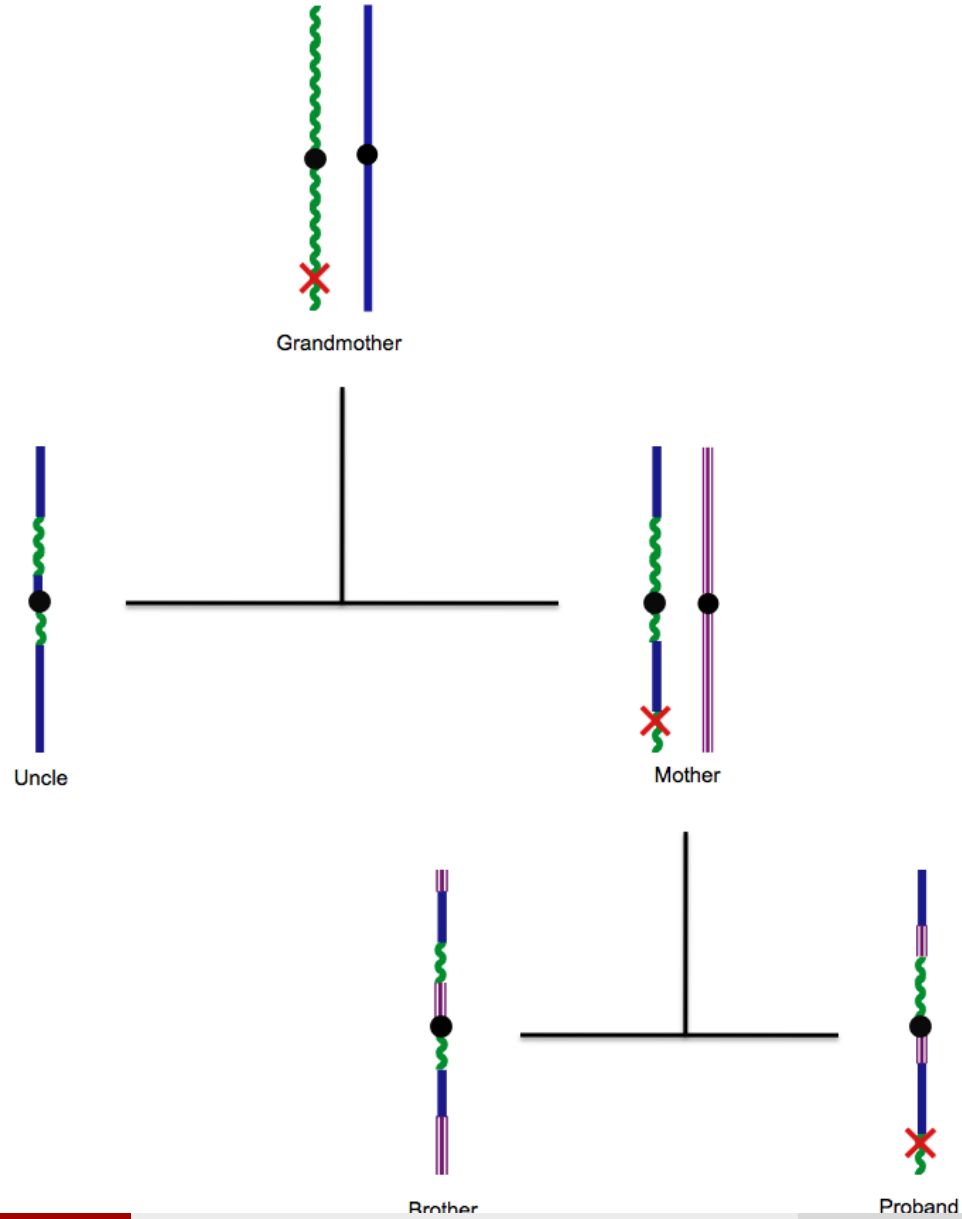
Exome Capture



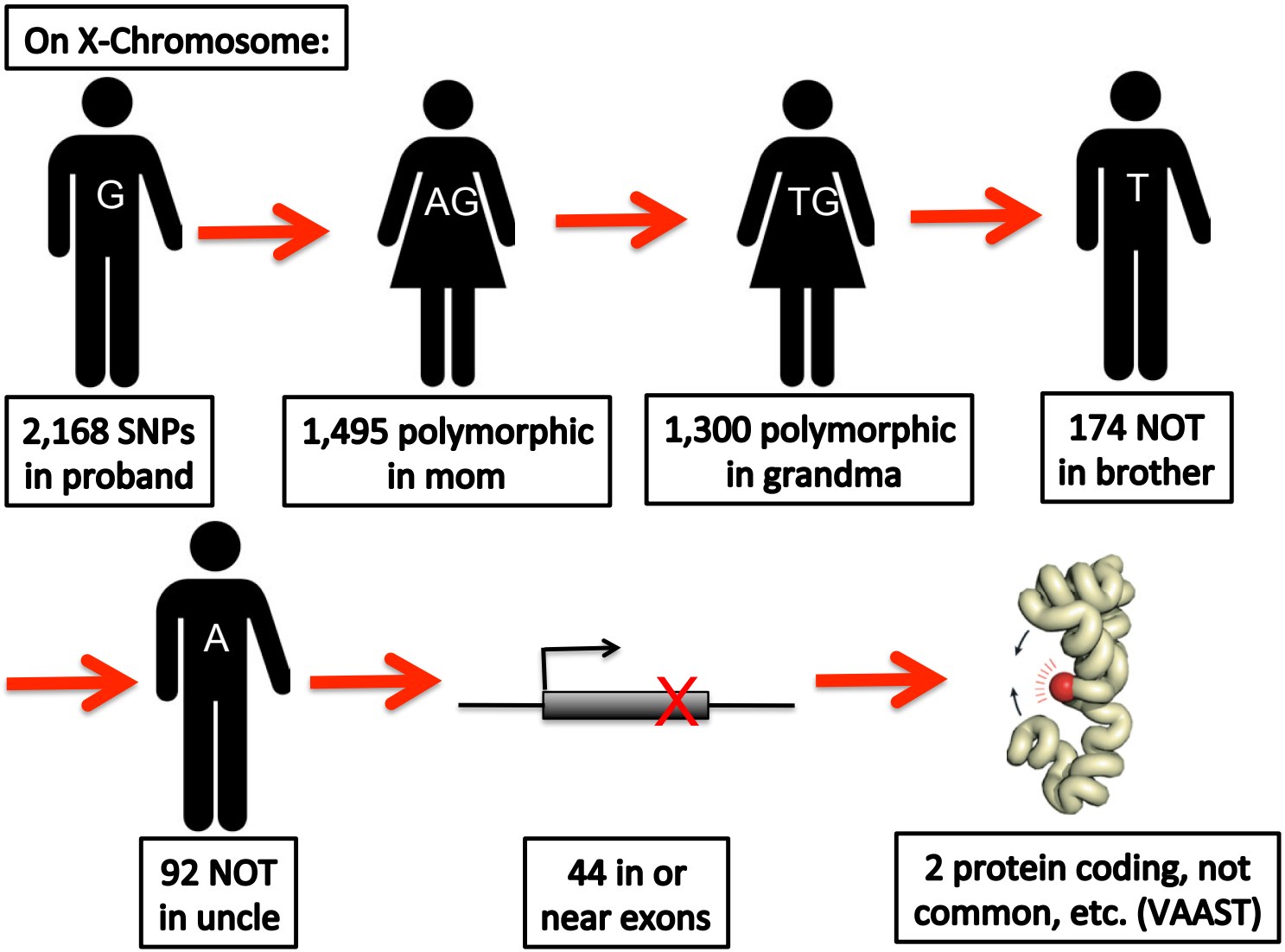
Exome Capture



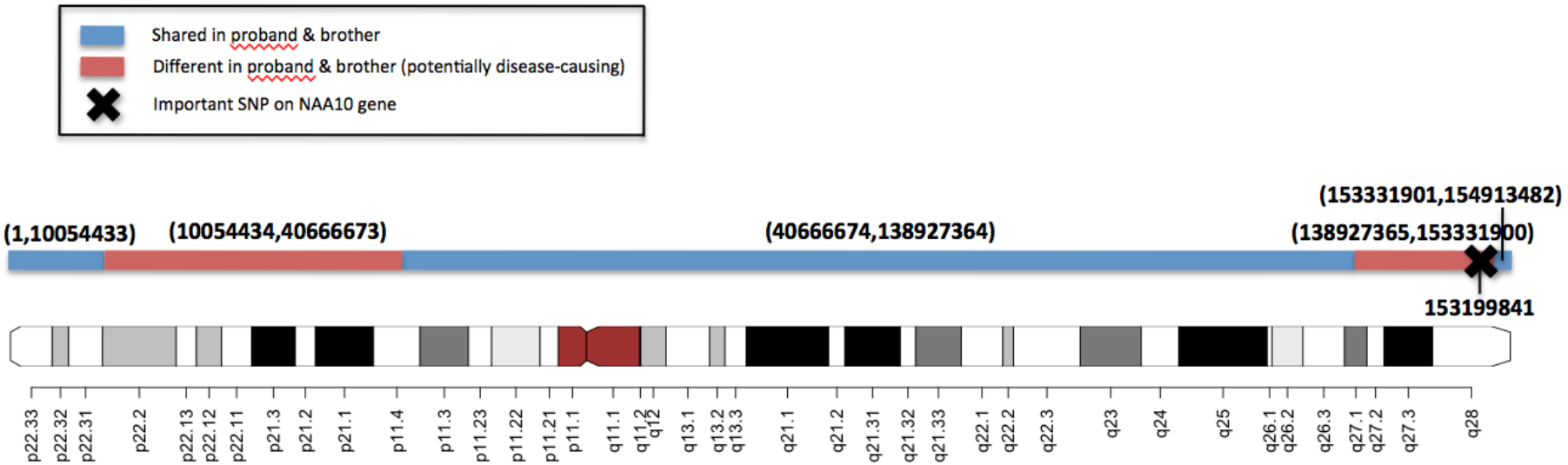
Rare X-linked Disease



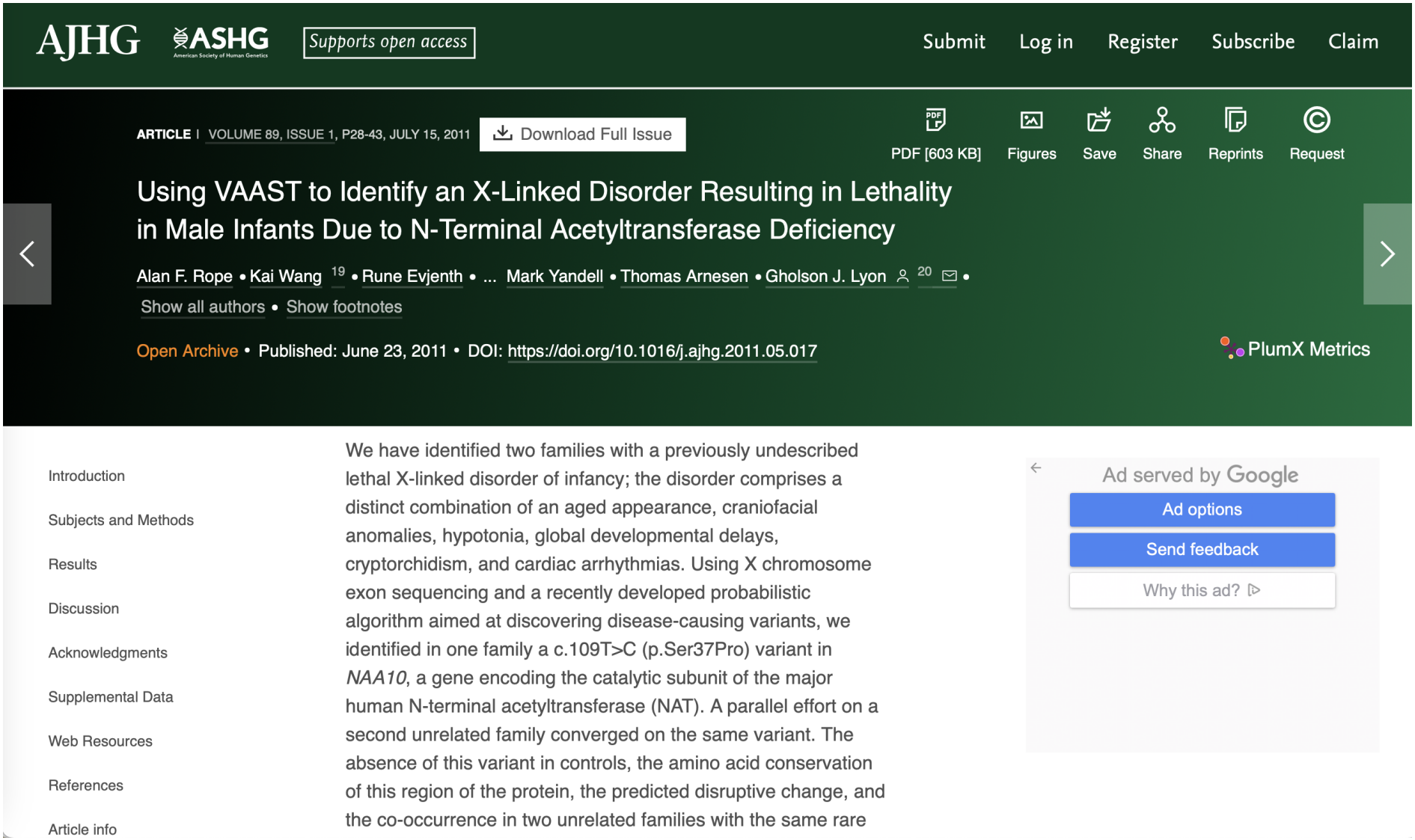
Rare X-linked Disease



Rare X-linked Disease



Rare X-linked Disease



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Using VAAST to Identify an X-Linked Disorder Resulting in Lethality in Male Infants Due to N-Terminal Acetyltransferase Deficiency

Alan F. Rope • Kai Wang [19] • Rune Evjenth • ... Mark Yandell • Thomas Arnesen • Gholson J. Lyon [20] •

Show all authors • Show footnotes

Open Archive • Published: June 23, 2011 • DOI: https://doi.org/10.1016/j.ajhg.2011.05.017

PlumX Metrics

Introduction

Subjects and Methods

Results

Discussion

Acknowledgments

Supplemental Data

Web Resources

References

Article info

We have identified two families with a previously undescribed lethal X-linked disorder of infancy; the disorder comprises a distinct combination of an aged appearance, craniofacial anomalies, hypotonia, global developmental delays, cryptorchidism, and cardiac arrhythmias. Using X chromosome exon sequencing and a recently developed probabilistic algorithm aimed at discovering disease-causing variants, we identified in one family a c.109T>C (p.Ser37Pro) variant in *NAA10*, a gene encoding the catalytic subunit of the major human N-terminal acetyltransferase (NAT). A parallel effort on a second unrelated family converged on the same variant. The absence of this variant in controls, the amino acid conservation of this region of the protein, the predicted disruptive change, and the co-occurrence in two unrelated families with the same rare

N-terminal acetyltransferase (NAA10)

N-terminal acetyltransferase (NAT):

- Common modification (~80-90% of human proteins)
- Depletion from cancer cells linked to cell cycle arrest and apoptosis (Starheim, *BMC Proc* 2009)
- NAT genes directly implicated as cause of genetic disease
- Mutation demonstrated a significantly impaired biochemical activity *in vitro*
- NAA10 lethal if knocked out of Drosophila

Section 2

Generating Sequencing Data

Next-Generation Sequencing

- Expensive to purchase (hundreds of thousands $USD)
- Expensive to operate (e.g. reagents, flow cells)
- You can sequence your genome at 30X depth for <$1000 USD.

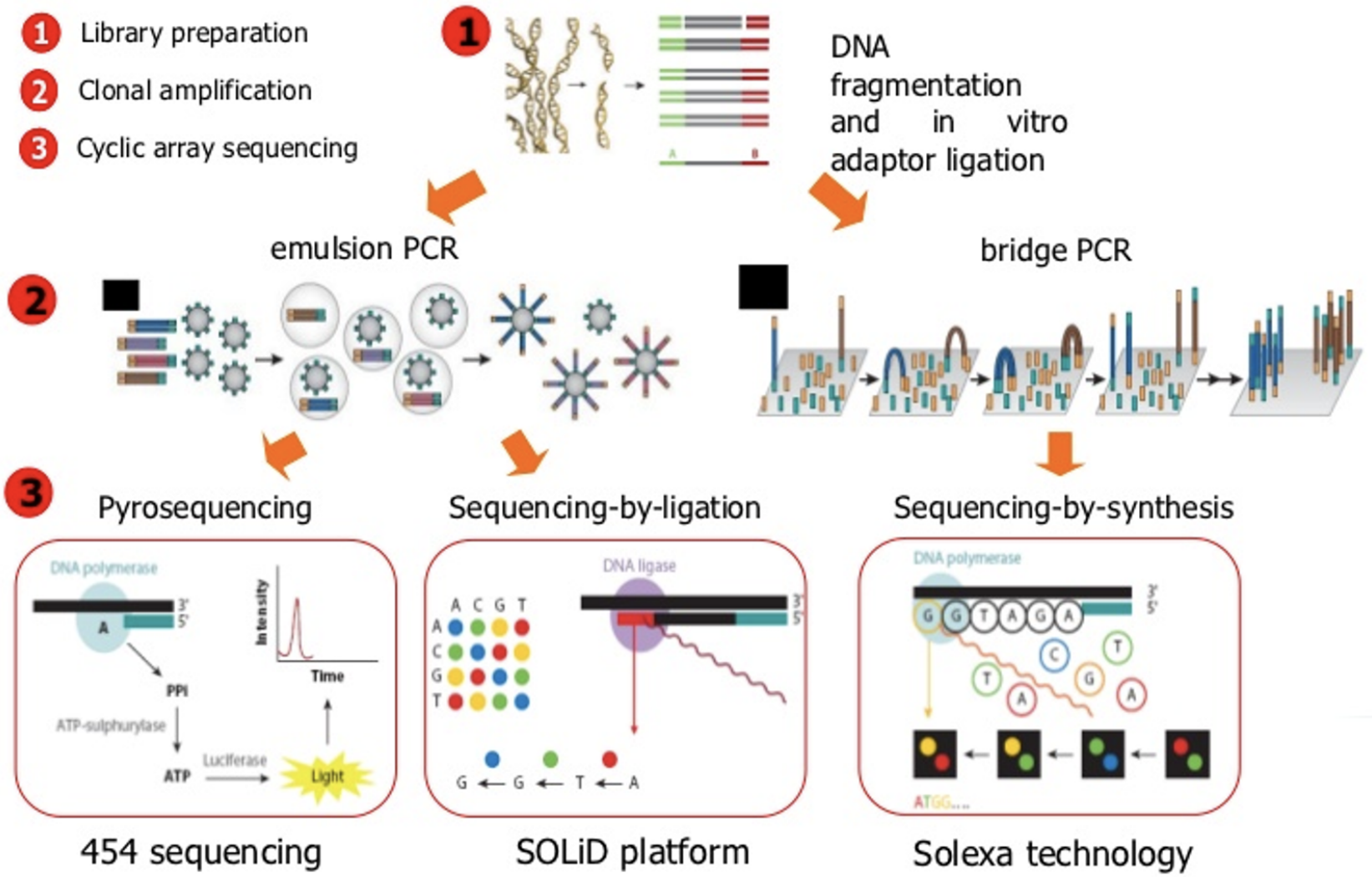
Roche 454

Ion Torrent

Illumina HiSeq 2500

Illumina NovaSeq 6000

Next-Generation Sequencing



Alex Sanchez, Statistics and Bioinformatics Research Group, Statistics Department, Universitat de Barcelona

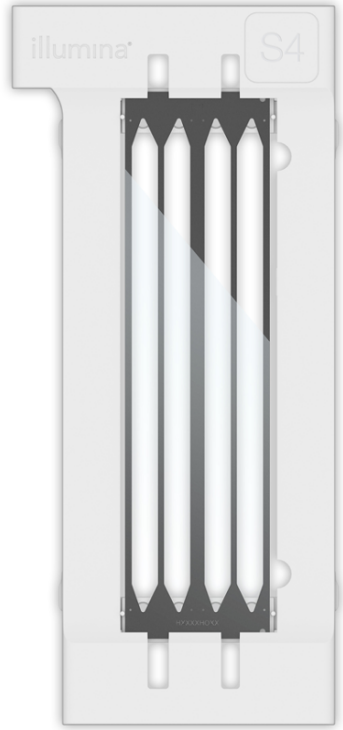
Illumina Sequencing

- Most common sequencing technology today
- Sequences any DNA
- Sequencing by synthesis method
- Sequences (reads) are short (<300bp)
- 2 gigabases - 6 terabases per run
- Hours to days to complete one run

For more information, you can watch the following:
https://www.youtube.com/watch?v=fCd6B5HRaZ8

Illumina Sequencing

- Sequencing occurs on a **flow cell**
- Each flow cell has 1 to 8 **lanes**
- Number of reads for overall flow cell varies
- Length of reads is fixed (e.g. 250 bp)
- Read format:
  - **Single end** - one read per molecule
  - **Paired end** - two reads per molecule
- **Multiplexing**: sequence many samples at once using molecular barcodes



NovaSeq Flowcell
Courtesy of Illumina, Inc.

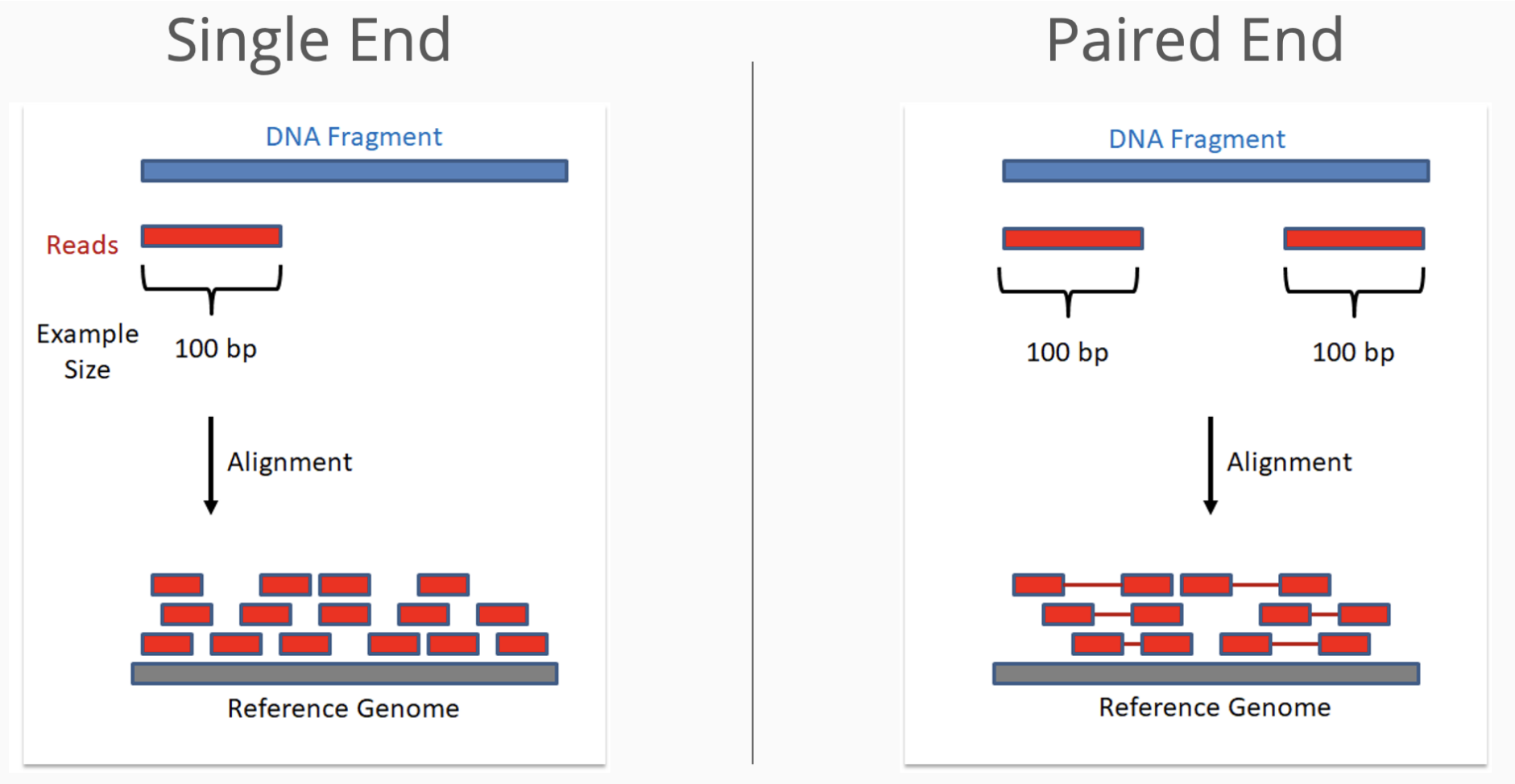
Sequencing Library Generation Workflow

Sequencing Library: DNA prepared for sequencing

Workflow:

- Extract RNA/DNA from sample
  - If RNA, reverse transcribe to cDNA
- Size select using gel cut or random shearing
- PCR amplify DNA if concentration is low
- Add sequencing adapters
  - If multiplexing, use barcoded adapters
- Pool samples, load across flow lanes for sequencing
- Typically only perform 1, sequencing cores

Design Choice: Single End vs Paired End



Design Choice: Number of Reads

Table 1: Coverage and Read Recommendations by Application

Category	Detection or Application	Recommended Coverage (x) or Reads (millions)	References
Whole genome sequencing	Homozygous SNVs	15x	Bentley et al., 2008
	Heterozygous SNVs	33x	Bentley et al., 2008
	INDELs	60x	Feng et al., 2014
	Genotype calls	35x	Ajay et al., 2011
	CNV	1-8x	Xie et al., 2009; Medvedev at al., 2010
Whole exome sequencing	Homozygous SNVs	100x (3x local depth)	Clark et al., 2011; Meynert et al., 2013
	Heterozygous SNVs	100x (13x local depth)	Clark et al., 2011; Meynert et al., 2013
	INDELs	not recommended	Feng et al., 2014
Transcriptome Sequencing	Differential expression profiling	10-25M	Liu Y. et al., 2014; ENCODE 2011 RNA-Seq
	Alternative splicing	50-100M	Liu Y. et al., 2013; ENCODE 2011 RNA-Seq
	Allele specific expression	50-100M	Liu Y. et al., 2013; ENCODE 2011 RNA-Seq
	De novo assembly	>100M	Liu Y. et al., 2013; ENCODE 2011 RNA-Seq
DNA Target-Based Sequencing	ChIP-Seq	10-14M (sharp peaks); 20-40M (broad marks)	Rozowsky et al., 2009; ENCODE 2011 Genome; Landt et al., 2012

https://genohub.com/recommended-sequencing-coverage-by-application/

Design Choice: Sequencing Depth

Whole Exome

- Less expensive
- Nearly complete ascertainment of variation in the coding ~1% of the genome (i.e. exons)
- Will miss functional variants outside of the coding region

Low Coverage Whole Genome

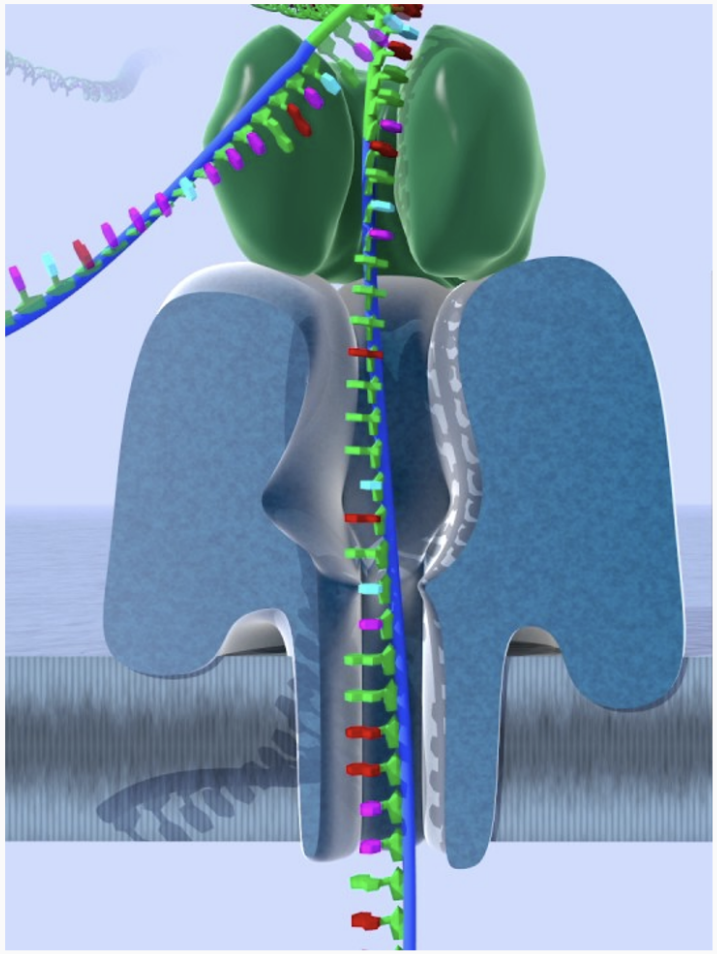
- Less expensive
- Reasonably good ascertainment of shared variation, but not unique variation

Deep Whole Genome

- More expensive
- Capture most of the genetic information
- Sequence the entire genome of each subject

3rd Gen Sequencing: Oxford Nanopore

- "Real time single molecule"



https://www.youtube.com/watch?v=GUb1TZvMWsw

Section 3

Sequencing Data Formats

Biological Data Formats are standardized

- Many different types of data
- Standard formats exist for many of them
- Always use/extend standard formats
- Don't save data in non standard formats when standards are available - No .xslx!
- For complete list of formats see: https://genome.ucsc.edu/FAQ/FAQformat.html

Sequencing Data Formats

format	data	tool(s)
FASTA	sequence of nucleotides	Samtools, Biopython
FASTQ	sequenced reads	FASTQC, Biopython
SAM/BAM/CRAM	aligned reads	Samtools, Deeptools, PySam
VCF	variant calls	vcftools bedtools
BED / BED-PE	genomic regions	bedtools
GFF	general features	Biopython, Manual Parsing
GTF	gene features	Biopython, Manual Parsing

Sequencing Data Formats

format	data	tool(s)
FASTA	sequence of nucleotides	samtools faidx
FASTQ	sequenced reads	-
SAM/BAM/CRAM	aligned reads	samtools
VCF	variant calls	vcftools bedtools
BED / BED-PE		bedtools
GFF		-
GTF	gene feature	-

Contains sequences of nucleotides / AA / bases

Sequencing Data Formats

format	data	
FASTA	s	faidx
FASTQ	sequ	
SAM/BAM/CRAM	aligned reads	samtools
VCF	variant calls	vcftools bedtools
BED / BED-PE	genomic regions	bedtools
GFF	general features	-
GTF	gene features	-

Contains positions: chromosome, start and end

Data Formats Example

EXAMPLE

Align reads to the reference, sort and index, call SNPs, extract the SNPs on chromosome Y overlapping with all the Alu elements.

Data Formats Example

EXAMPLE

Align reads to the reference, sort and index, call SNPs on chromosome Y overlapping with all the Alu elements.

FASTQ format

2

Data Formats Example

EXAMPLE

Align reads to the reference, sort and index, call SNPs on chromosome Y overlapping with all the Alu elements.

FASTA format

3

Data Formats Example

EXAMPLE

Align reads to the reference, sort and index, call SNPs, extract the SNPs on chromosor with all the Alu elements.

save in BAM format

4

Data Formats Example

EXAMPLE

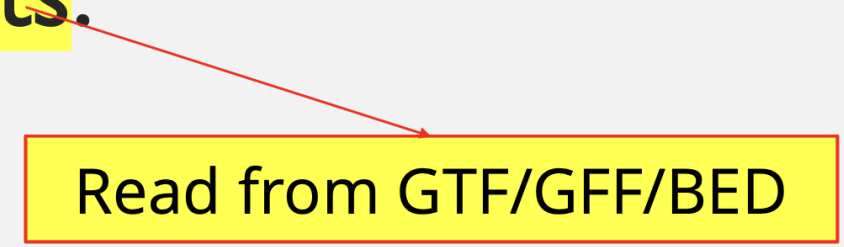
Align reads to the reference, sort and index, call SNPs, extract the SNPs on chromosom ith all the Alu elements.

5

Data Formats Example

EXAMPLE

Align reads to the reference, sort and index, call SNPs, extract the SNPs on chromosome Y overlapping with all the Alu elements.



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Data Formats Example

EXAMPLE

Align reads to the reference, sort and index, call SNPs, extract the SNPs on chromosome Y overlapping with all the Alu elements.

BWA/BOWTIE2

7

Data Formats Example

EXAMPLE

Align reads to the reference, sort and index, call SNPs, extract the SNPs on chromos[illegible] with all the Alu elements.

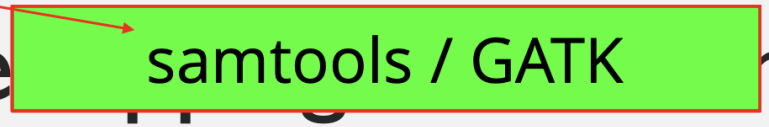
samtools

8

Data Formats Example

EXAMPLE

Align reads to the reference, sort and index, call SNPs, extract the SNPs on chromosome Y ove[illegible]he Alu elements.



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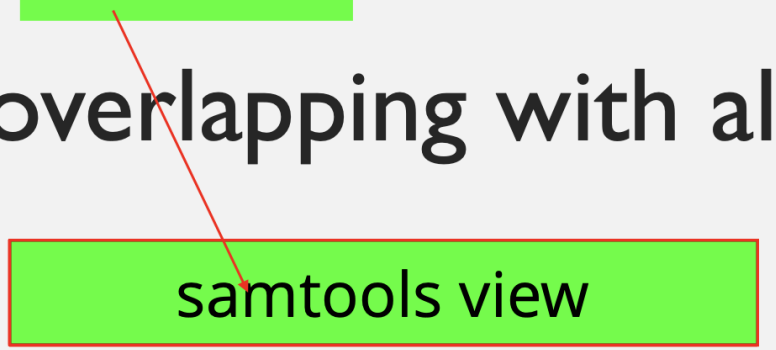
Data Formats Example

EXAMPLE

Align reads to the reference, sort and index, call SNPs, extract the SNPs on chromosome Y overlapping with all the Alu elements.



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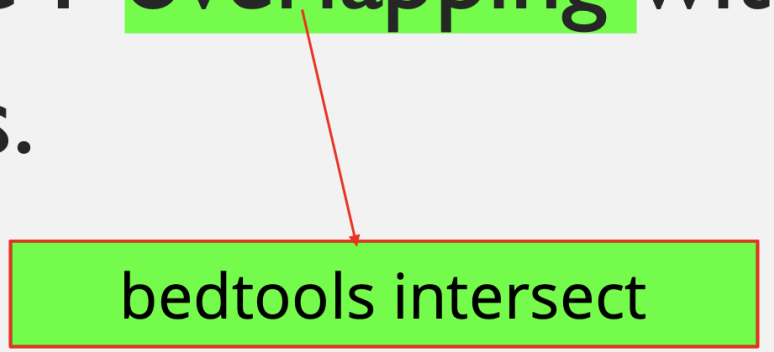
Data Formats Example

EXAMPLE

Align reads to the reference, sort and index, call SNPs, extract the SNPs on chromosome Y overlapping with all the Alu elements.



Section 4

Raw Sequencing Data and QC

History and Evolution of Illumina Data Output

Illumina sequencers have given output in many different formats:

- Illumina .PRB and .INT files
  - Better access to raw data.
  - Base calling algorithms (Bravo and Irizarry, *Biometrics*, 2010)
  - Mapping algorithms (GNUMAP, NOVO)
  - Confusing formats; Large data files
- Illumina .FASTQ files
- Sanger .FASTQ files

Illumina .INT and .PRB

```
1    1    125 771 1651.8 2189.6 228.1 549.9   219.0 202.5  48.4 3016.8    127.8   6.1 204
1    1    478 16   1050.0 969.9 149.5 311.7    0.0   0.0  39.3 134.1    0.0   0.0   0.0   0.
1    1    780 553   639.6 980.8 555.2 6412.8   1040.1 4408.7 750.3 638.1   946.2 4351.1 7
1    1    123 685 -116.5 341.5 -14.0 -985.9   231.5 1090.0  88.3 -102.2   240.2 513.6
1    1    61   934   40.7  87.3  38.5  21.3   16.4  31.7 100.9  68.8      41.4  29.4  40.4  3
1    1    866 972 2820.5 4698.9 435.8 8502.2 4740.3 4890.2 1491.5 1241.7 2137.5 2505.6 6
```

```
 40 -40 -40 -40  -40 -40 -40  40  -40 -40  40 -40  -40 -40  40 -40  -4
 28 -28 -40 -40   -5  -5  -5  -5    -5  -5  -5  -5    -5  -5  -5  -5    -5
-17 -16 -30  13  -40  26 -26 -40  -21  17 -20 -27   21 -21 -40 -40  -4
-40  40 -40 -40  -40  40 -40 -40   -1   1 -40 -40    -0 -40  -0 -14   26
 -1 -40  -0 -10  -20 -18  16 -35   -1  -8  -2 -13   12 -34 -13 -26   5
-27 -40 -40  27   40 -40 -40 -40  -40 -40  40 -40  -40 -40  40 -40  -4
```

.fasta or .fa

```
>chrX
ttgaactcctgacctcaggtgatccgcccgccttgacctcccaaagcgct
cctgcctcagcctcccgagtagctgggactacaggtgcctgccaccatgc
....
caggctaatttttgtatttttagtagagacggggtttcaccatgttagc
caggatggtctcaatctcctgacctcatgatccgcctgcctcggcctccc
>chrY
tgtacactttaaatgggtgaattttatggaatgtgaattataCGTGTTGG
CTTGTAAAAAAAAATGATGGAGATGGAGACGTGACTCTAGCGTGAAGGGG
...
GTGGGGAGAGTAGATCTAGAGTGGAGACACCACTTTTAGGAGGTATGATC
cctgccaccatgcctggctaatttttgtatttttagtagagacagggttt
```

.fastq or .fq (or .fq.gz)

```
@HWI-EAS240_0001:2:1:1142:17571#0/1
CTCTCTTTTCTCCCCANGTCTCCTCATGACCATATCCNTGTTGTCCATTGTGTANGGNNNCTT
+HWI-EAS240_0001:2:1:1142:17571#0/1
bababb`abbbbabbYB^[^^`_`_bbbbbbbbbb`_B`_``^`^bbbba^`^^BOOBBBNOO
@HWI-EAS240_0001:2:1:1142:6453#0/1
CAGGACGTGCACTATGNCATCCACGATGCAAGTCTTANCATTATTCAGGATACANATANNGGC
+HWI-EAS240_0001:2:1:1142:6453#0/1
_U][aa_aa^^```^[B][][]]]S```Y``a`aaZ]B]]^^^[^```[OVY]XBNNNBB]]]
@HWI-EAS240_0001:2:1:1142:19443#0/1
TCAGAAAACAGAAAGGTCATTTTCTTACTTCTTTGCANGATGCCACCCTCCAGANCAGNNAAT
+HWI-EAS240_0001:2:1:1142:19443#0/1
bbbabb^^bbb^bbabb]bb]^]`]]]bbbbbbbbYYBYXOOOOOBb`bbVY^HBOOOBBOJO
```

.FASTQ Paired End

fastq_SRR1997469_1.fastq

```
@SRR1997469.1 1 length=36
CAGTCTTCTTAGAAATATCCACTTCGGAATAAAAGA
+SRR1997469.1 1 length=36
BBBBBFFFFF<FFFFFFFFFFFFBFFFFFFFFFFF
@SRR1997469.2 2 length=36
ACAGTTGAACGATCCTTTACAGANAGNAGNCTNGTA
+SRR1997469.2 2 length=36
<BBBBFFFBBF<BFF/<FFFFFF############
...
```

fastq_SRR1997469_2.fastq

```
@SRR1997469.1 1 length=36
AGATAAGATGGTAATCTTTGATGGAGAACATTAAGA
+SRR1997469.1 1 length=36
BBBBBFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
@SRR1997469.2 2 length=36
ACTGGGAANCCTTCTGTCTAGCCTTATATGAAAAAA
+SRR1997469.2 2 length=36
BBB/BFFB#BB/FFFF/FFFFFFBBFFFFFFFFFBF
...
```

.FASTQ Comparison

```
 SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
 ..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....................
 ..............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII.....................
 ................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.....................
 LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................................
 !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
  |                         |    |        |                              |                     |
33                        59   64       73                            104                   126

S - Sanger        Phred+33,  raw reads typically (0, 40)
X - Solexa        Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64,  raw reads typically (3, 40)
   with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
   (Note: See discussion above).
L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
```

https://en.wikipedia.org/wiki/Phred_quality_score

Sequence Quality Score (PHRED)

Phred quality scores are logarithmically linked to error probabilities

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

https://en.wikipedia.org/wiki/Phred_quality_score

Sequence Quality Score (PHRED)

Quality scores (Phred)

- Sanger Phred: Range=(0,40), $P = 1 - 10^{-(ASCII-33)/10}$
- Solexa: Range= (-5,40), $P = \frac{10^{(ASCII-64)/10)}}{1+10^{(ASCII-64)/10}}$
- Illumina 1.3: (0,40), $P = \frac{10^{(ASCII-64)/10)}}{1+10^{(ASCII-64)/10}}$
- Illumina 1.5: Range=(2,40), $P = 1 - 10^{-(ASCII-64)/10}$
- Illumina 1.8: Same as Sanger except Range=(0,41)

.FASTQ Comparison

> Sanger

	ASCII	Quality
!	33	0.0000
"	34	0.2057
#	35	0.3690
$	36	0.4988
%	37	0.6019
&	38	0.6838
'	39	0.7488
(	40	0.8005
)	41	0.8415
*	42	0.8741
+	43	0.9000
,	44	0.9206
-	45	0.9369
.	46	0.9499
/	47	0.9602
0	48	0.9684
1	49	0.9749
2	50	0.9800

> Solexa

	ASCII	Quality
;	59	0.2403
<	60	0.2847
=	61	0.3339
>	62	0.3869
?	63	0.4427
@	64	0.5000
A	65	0.5573
B	66	0.6131
C	67	0.6661
D	68	0.7153
E	69	0.7597
F	70	0.7992
G	71	0.8337
H	72	0.8632
I	73	0.8882
J	74	0.9091
K	75	0.9264
L	76	0.9406
M	77	0.9523
N	78	0.9617

> Illumina1.3

	ASCII	Quality
@	64	0.5000
A	65	0.5573
B	66	0.6131
C	67	0.6661
D	68	0.7153
E	69	0.7597
F	70	0.7992
G	71	0.8337
H	72	0.8632
I	73	0.8882
J	74	0.9091
K	75	0.9264
L	76	0.9406
M	77	0.9523
N	78	0.9617
O	79	0.9693
P	80	0.9755
Q	81	0.9804
R	82	0.9844
S	83	0.9876

> Illumina1.5

	ASCII	Quality
B	66	0.3690
C	67	0.4988
D	68	0.6019
E	69	0.6838
F	70	0.7488
G	71	0.8005
H	72	0.8415
I	73	0.8741
J	74	0.9000
K	75	0.9206
L	76	0.9369
M	77	0.9499
N	78	0.9602
O	79	0.9684
P	80	0.9749
Q	81	0.9800
R	82	0.9842
S	83	0.9874
T	84	0.9900
U	85	0.9921

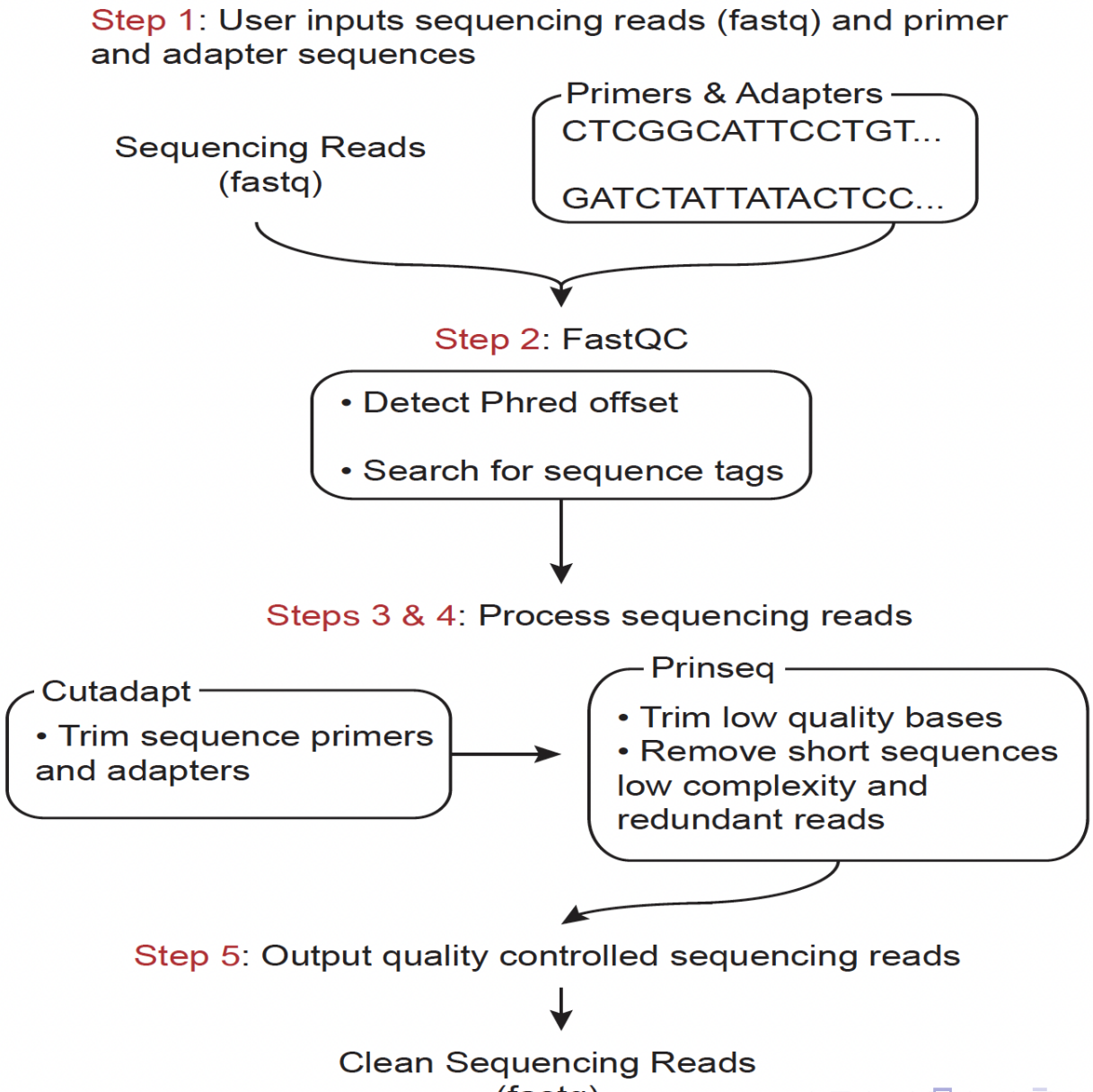
Quality Control

Need to preprocess the reads:

- Check for quality (FASTQC)
- Trim adapter and (Cutadapt, others)
- Remove duplicate reads, trim low complexity/quality bases/reads (Prinseq)
- Complete pipelines: NCBI Toolkit, QC-Chain, PathoQC (pathoscope.sourceforge.net), others

Note: Not comprehensive or updated!

Read Quality Checks

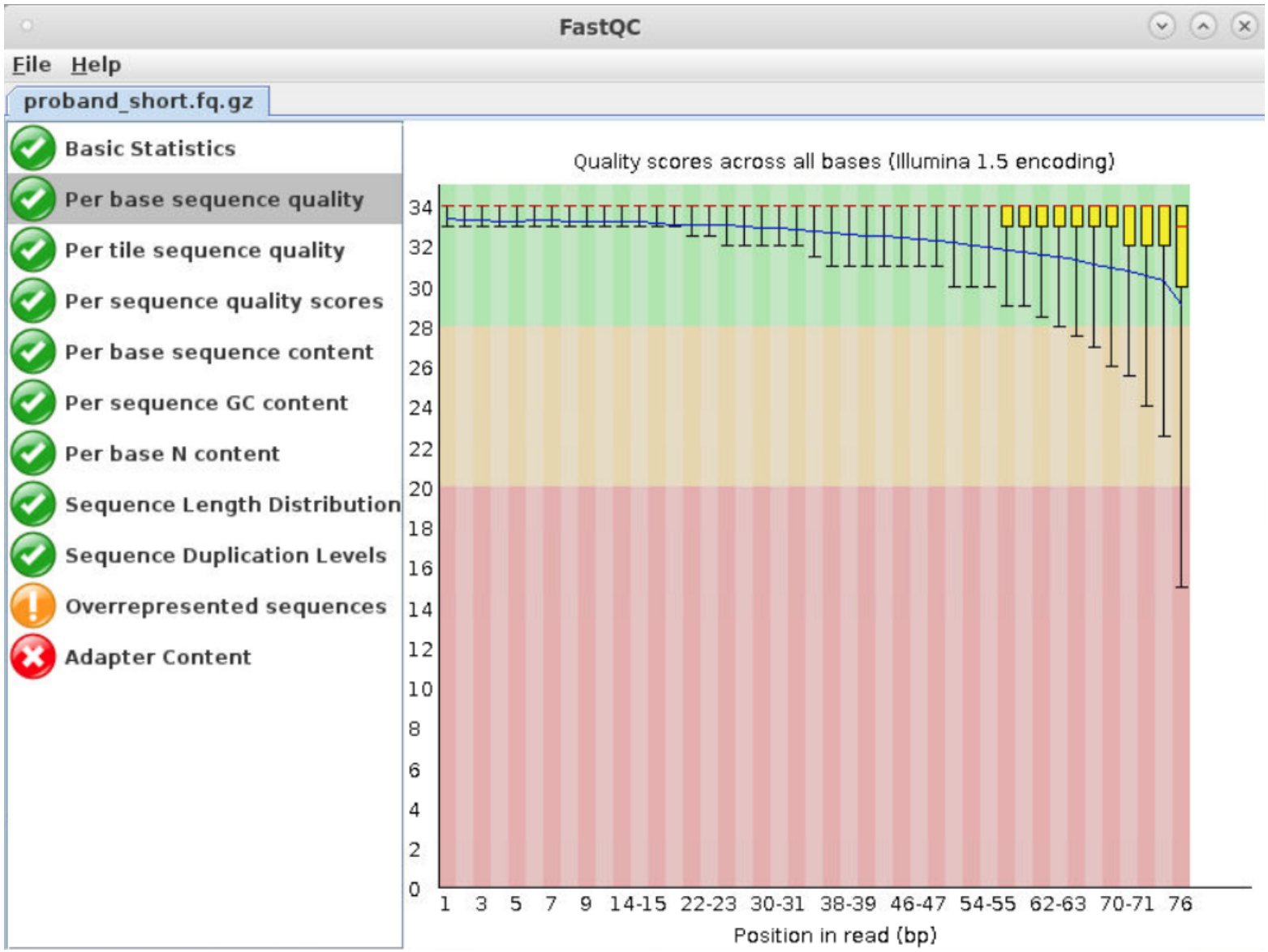


Read Quality Checks (Outdated!)

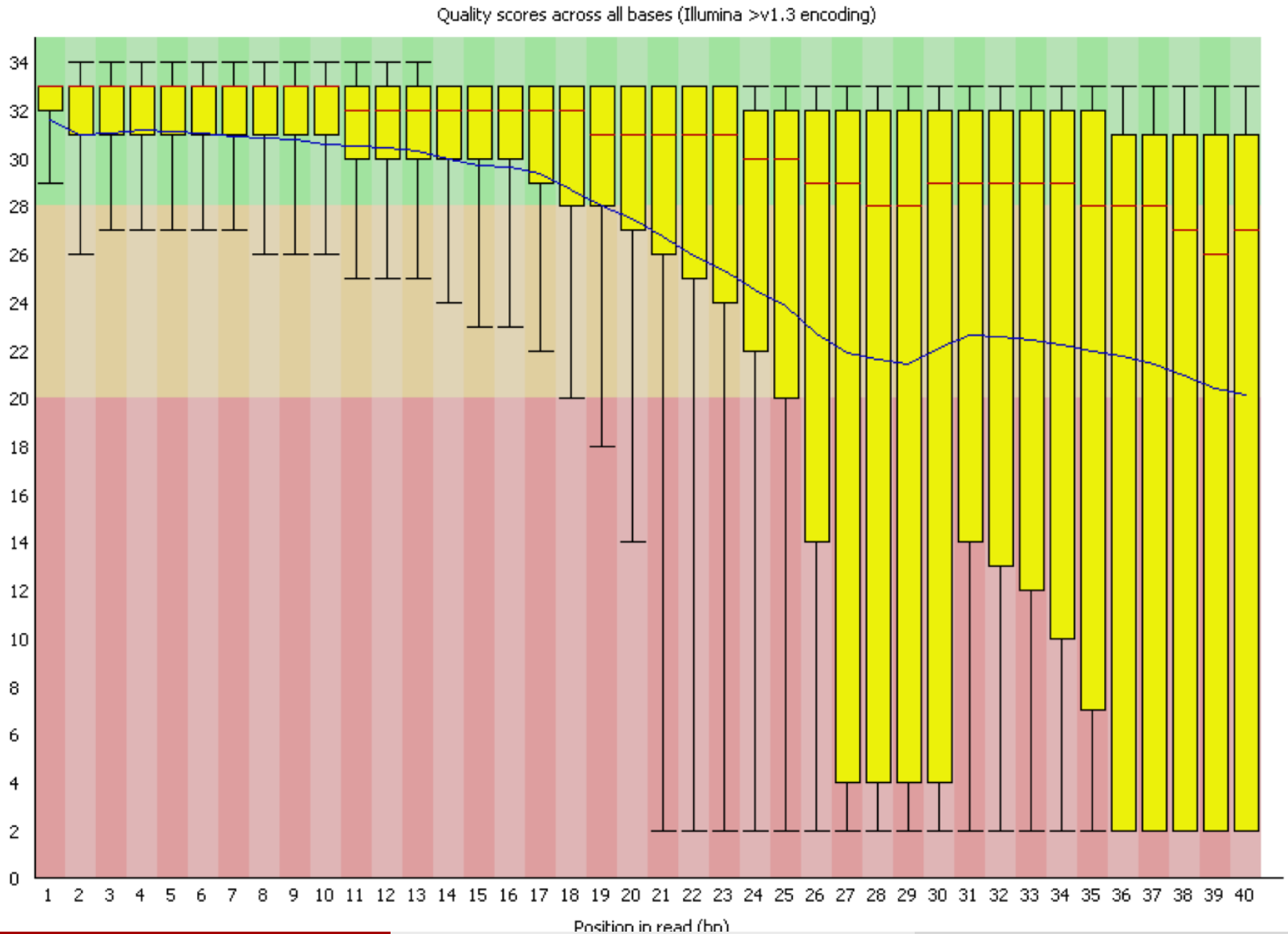
Comparison of some of the more popular QC pipelines:

QC Features	QCToolkit	QC-Chain	Cutadapt	Prinseq
Parallel computation	X	X		
Phred offset detection	X	X		
Tag sequence removal	X	X	X	
Poly-A/T tail trimming			X	X
PCR duplication filtering		X		X
Low complexity filtering				X
Homopolymer removal	X			X
GC content filtering		X		X
N/X content filtering				X

FASTQC


FastQC
File Help
proband_short.fq.gz
Basic Statistics
Per base sequence quality
Per tile sequence quality
Per sequence quality scores
Per base sequence content
Per sequence GC content
Per base N content
Sequence Length Distribution
Sequence Duplication Levels
Overrepresented sequences
Adapter Content
Quality scores across all bases (Illumina 1.5 encoding)
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1 3 5 7 9 14-15 22-23 30-31 38-39 46-47 54-55 62-63 70-71 76
Position in read (bp)

FASTQC


Quality scores across all bases (Illumina >v1.3 encoding)
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Position in read (bp)

FastQC Example

Interactive GUI from Amarel Desktop:

```
module spider fastqc
#module avail fastqc
module load FastQC
fastqc
```

Running FastQC from command-line (single file):

```
fastqc myfastqfile.fq.gz --outdir=output/
```

Running FastQC from command-line (multiple files):

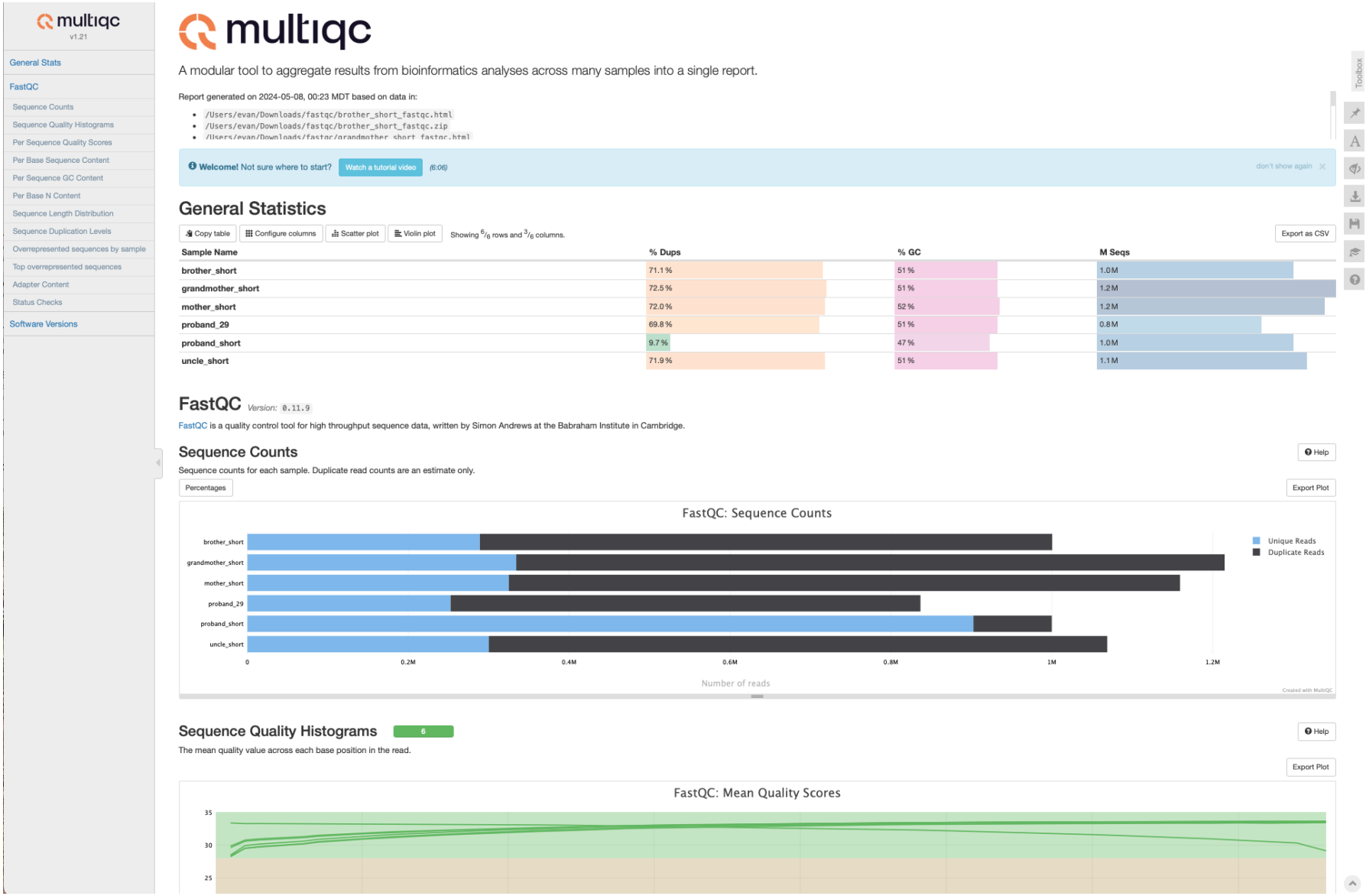
```
fastqc *.fq.gz --outdir=output/
```

MultiQC Example

You can use `multiqc` to combine FastQC results.

```
pip install multiqc
cd my_fastqc_dir
multiqc .
```

MultiQC Example



Section 5

Mapping Reads

Sequencing data alignment

Next-Generation Sequencing (NGS) Data present new challenges:

- Map 'reads' to genome
- Call SNPs and variants from the reads
- Other Applications: RNA-seq, miRNAs, alternative splicing, ChIP-seq, BS-seq, RNA editing

Obstacles:

- Massive data size
- Repeat regions, rare variants

Goal: Develop an approach to put the puzzle together!

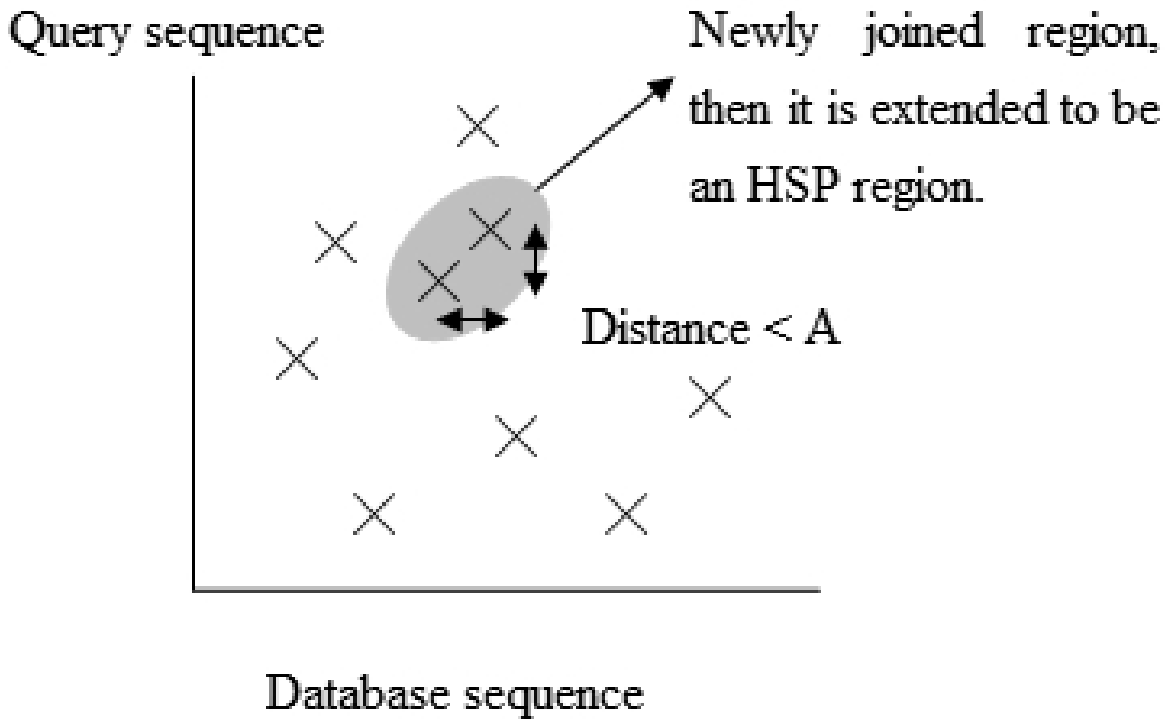
Mapping Algorithms

Here are a few examples of alignment algorithms (not comprehensive – some of Dr. Johnson's favorites!)

- BLAST
- BLAT
- SOAP2
- Bowtie2
- BWA
- STAR
- Subread (Rsubread)
- GNUMAP
- NovoAlign
- Many others!!

Basic Local Alignment Search Tool (BLAST)

BLAST is a "heuristic" method that identifies and combined *High-scoring Segment Pairs (HSPs)* between two sequences. BLAST searches for high scoring sequence alignments between the query sequence and the existing sequences in the database using an approach that approximates the Smith-Waterman algorithm (described later).



Basic Local Alignment Search Tool (BLAST)

BLAST can be found at: https://blast.ncbi.nlm.nih.gov/Blast.cgiBLAST. Try "BLASTING" the following sequences:

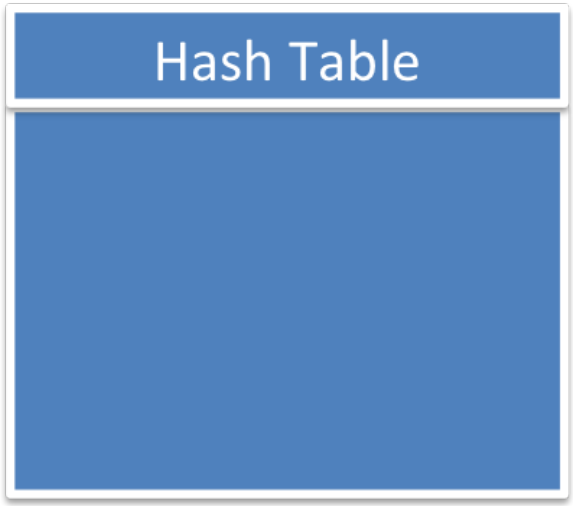
```
>NC_045512.2 Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1
ATTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCTGTTCTCTAAACGAACTTTAA

>NC_001802.1 Human immunodeficiency virus 1
GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGC

>NR_102810.2 Mycobacterium tuberculosis H37Rv 16S ribosomal RNA
TGTTTGGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGA
AAGGTCTCTTCGGAGATACTCGAGTGGCGAACGGGTGAGTAACACGTGGGTGATCTGCCCTGCACTTCGG

>NR_041248.1 Bacillus anthracis strain ATCC 14578 16S ribosomal RNA
AGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGG
```

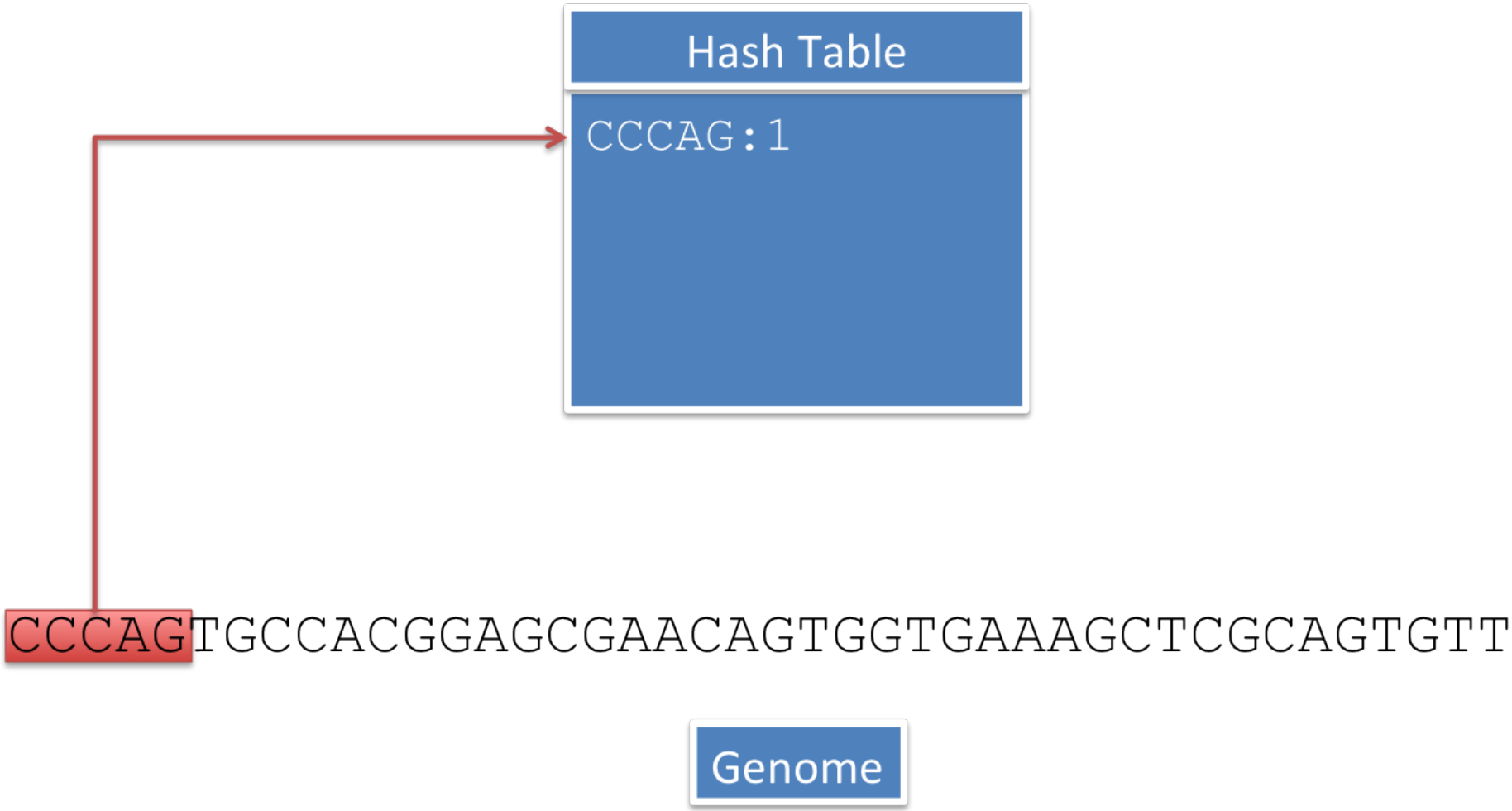
Creating a Hash Index Table



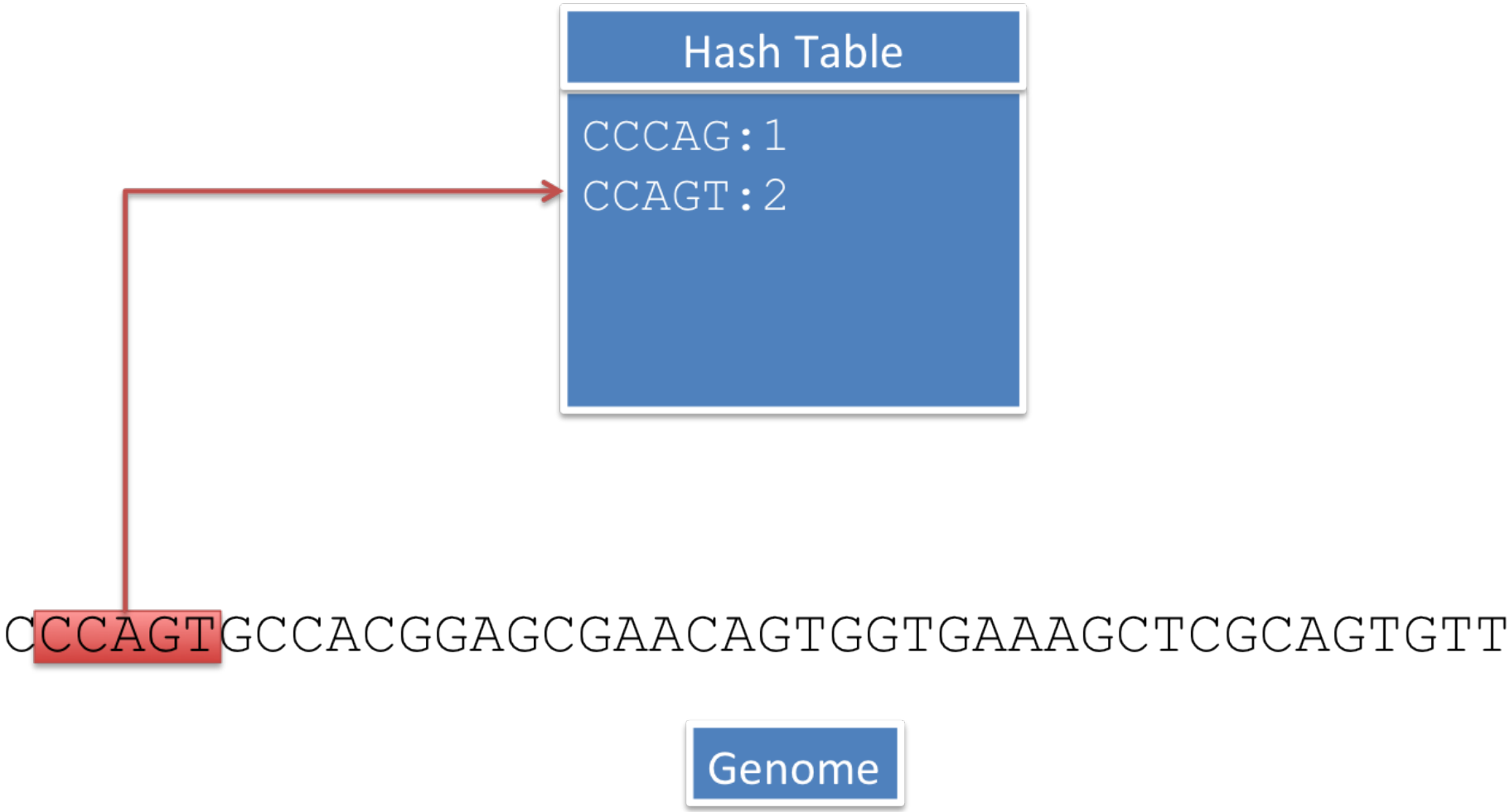
CCCAGTGCCACGGAGCGAACAGTGGTGAAAGCTCGCAGTGTT

Genome

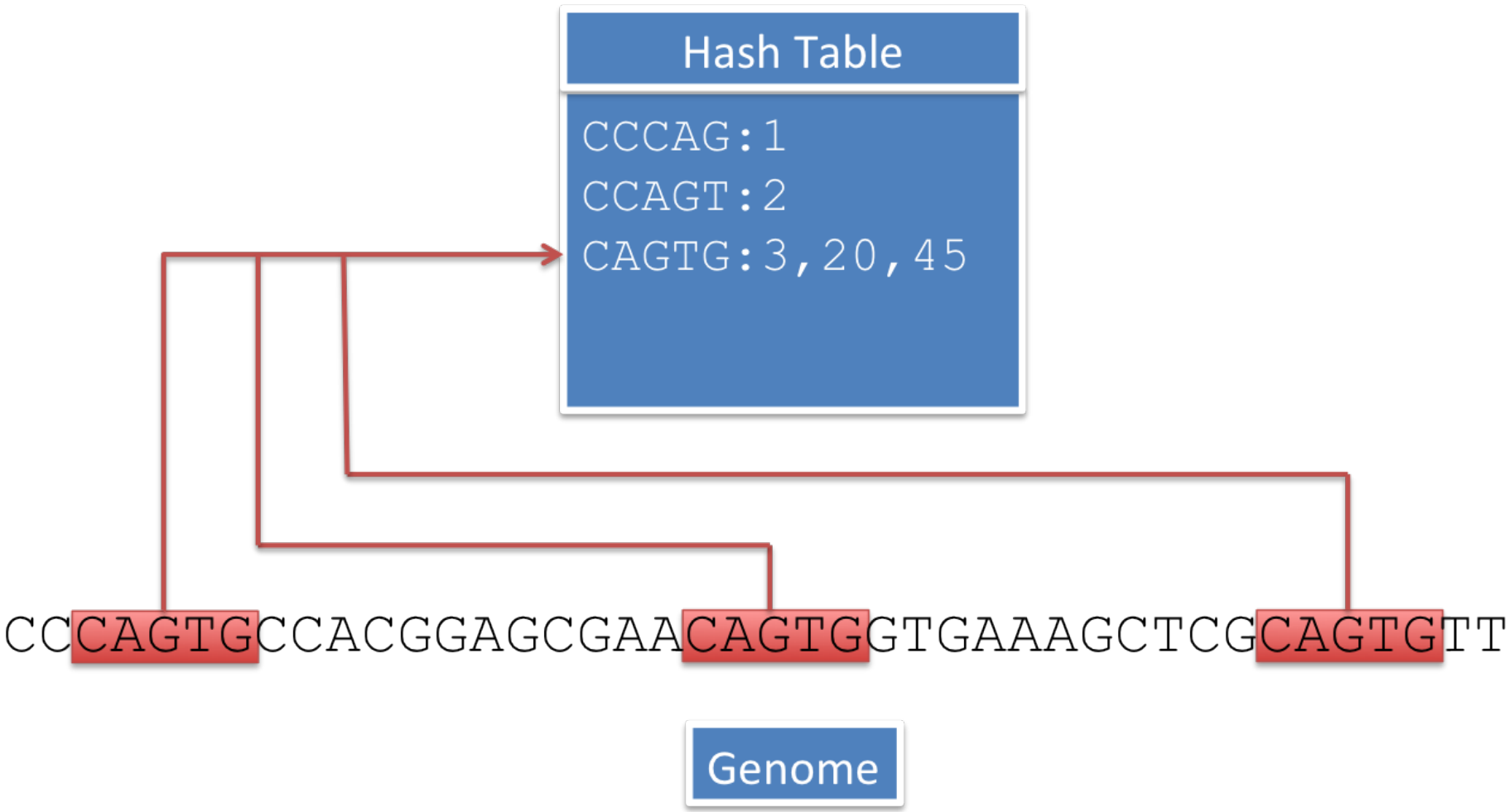
Creating a Hash Index Table



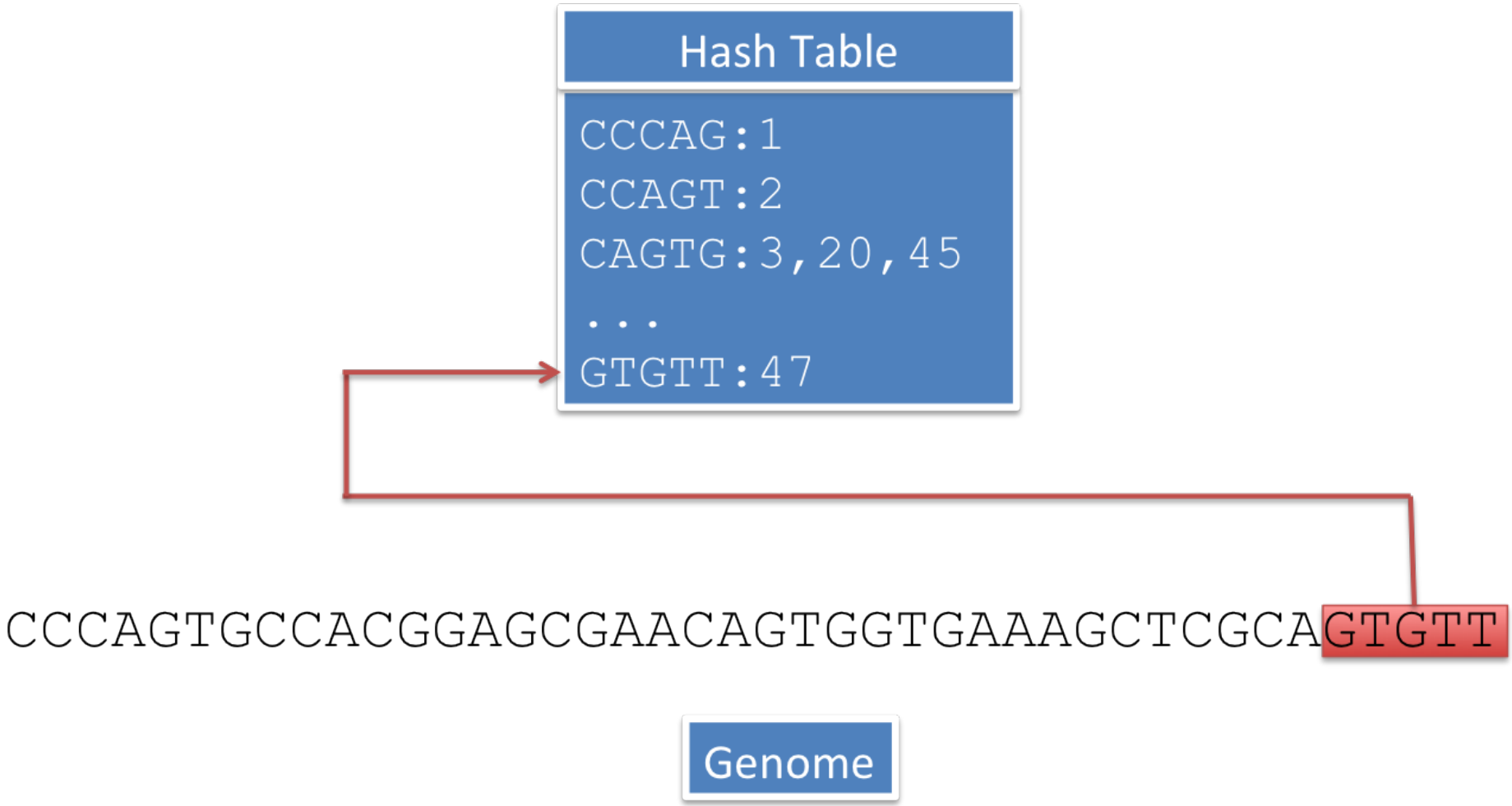
Creating a Hash Index Table



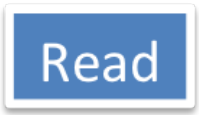
Creating a Hash Index Table



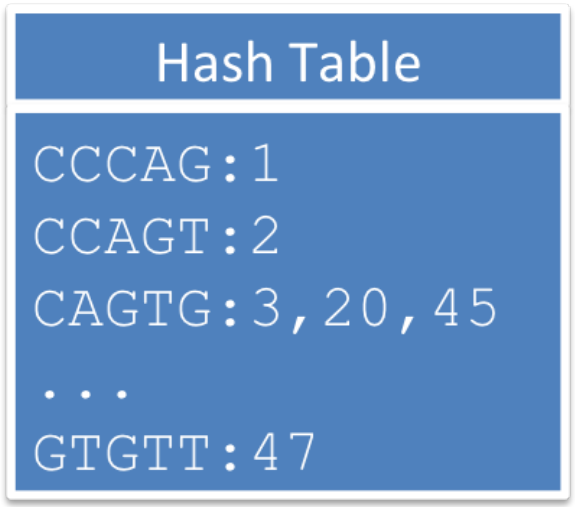
Creating a Hash Index Table



Read Lookup

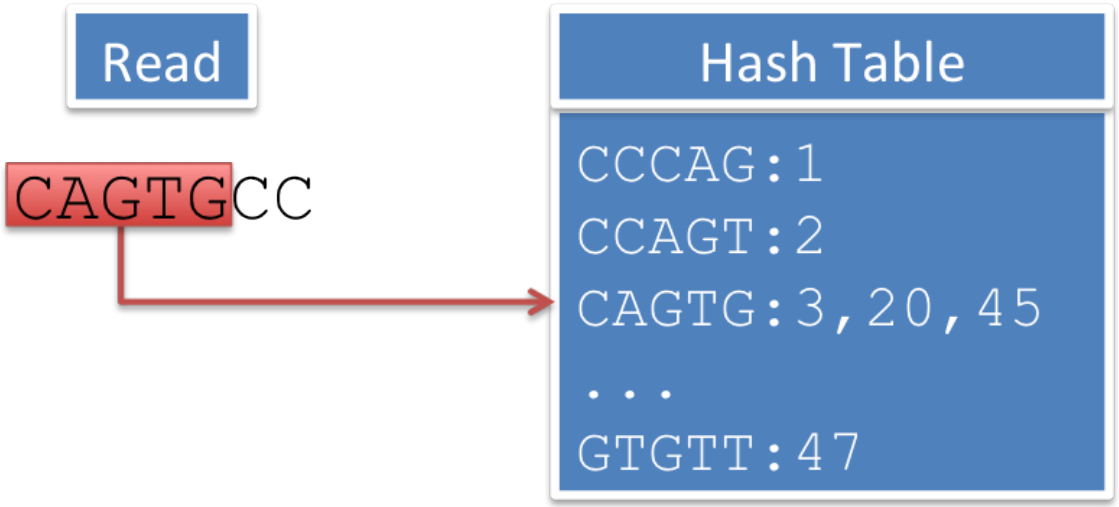


CAGTGCC



CCCAGTGCCACGGAGCGAACAGTGCTGAAAGCTCGCAGTGTT

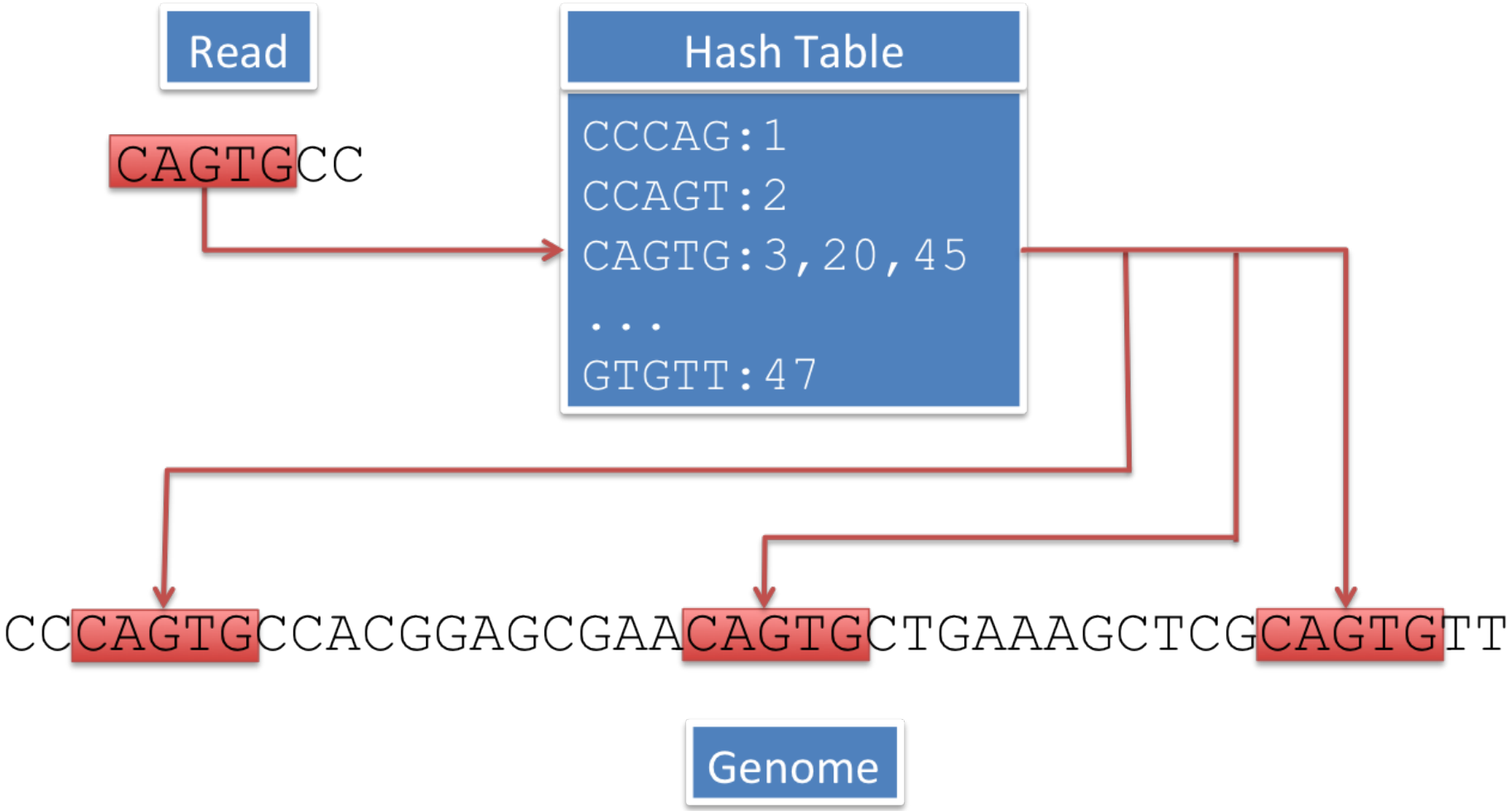
Read Lookup



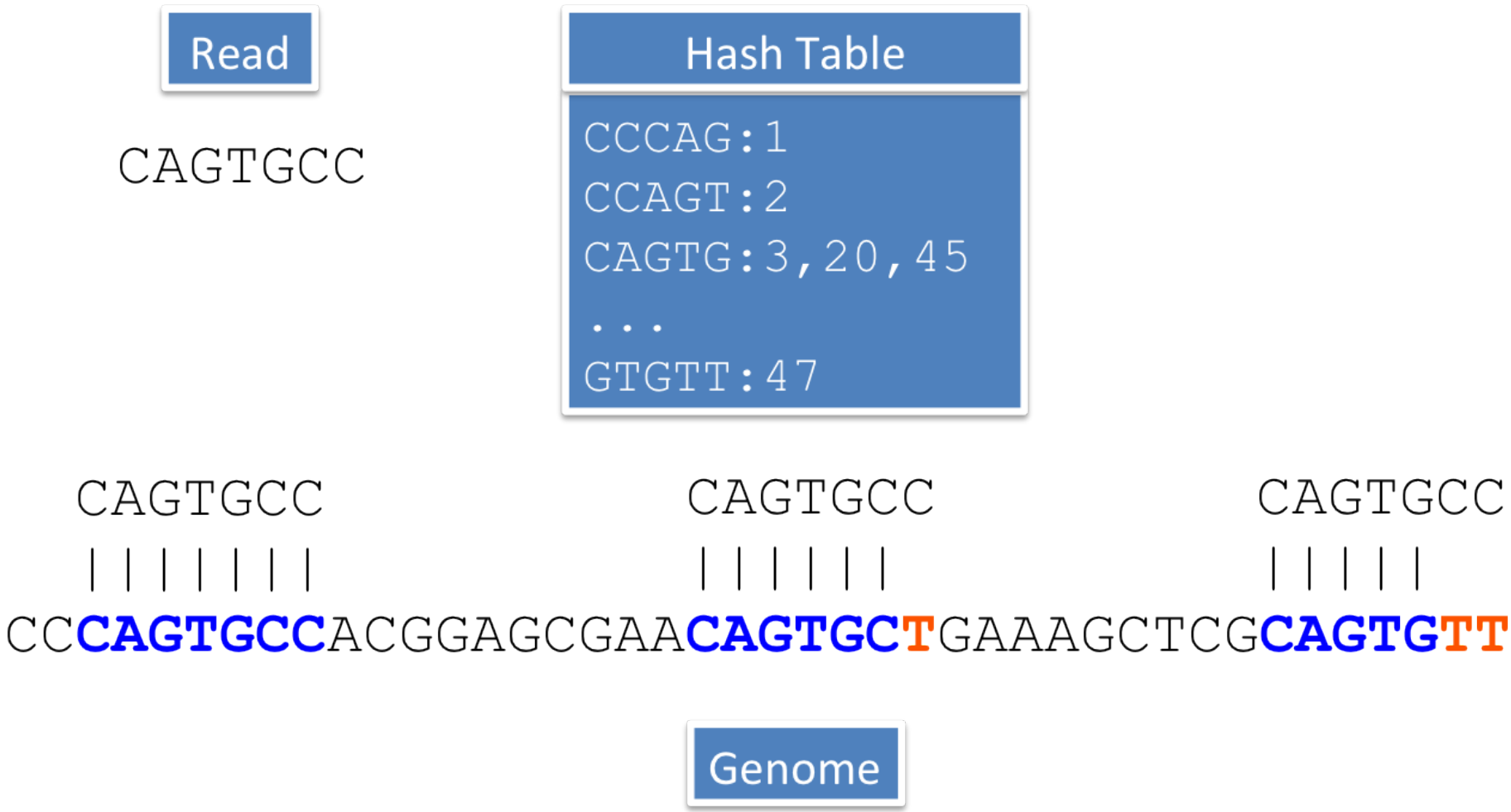
CCCAGTGCCACGGAGCGAACAGTGCTGAAAGCTCGCAGTGTT

Genome

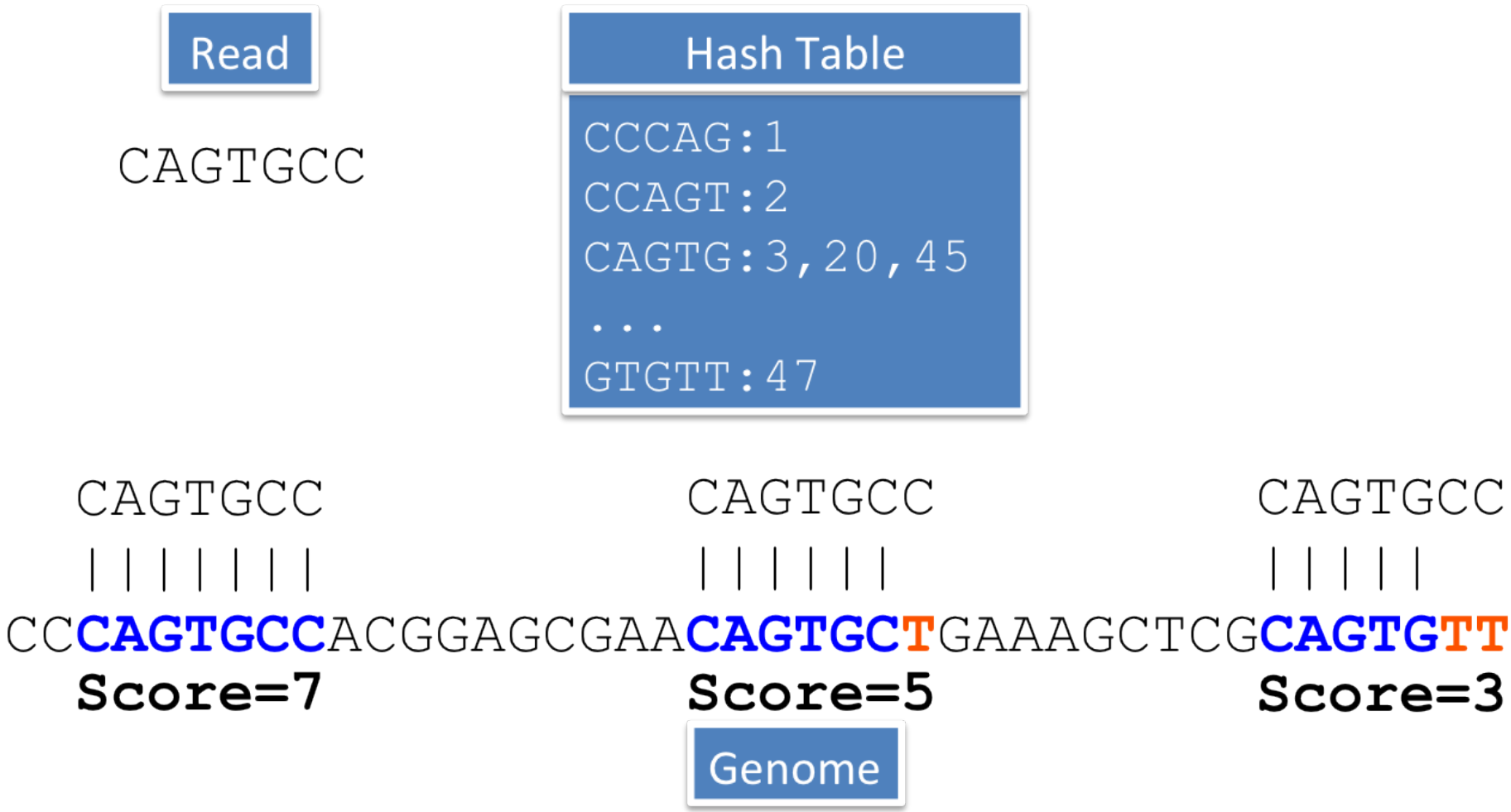
Read Lookup


Read
CAGTGCC
Hash Table
CCCAG:1
CCAGT:2
CAGTG:3,20,45
...
GTGTT:47
CCCAGTGCCACGGAGCGAACAGTGCTGAAAGCTCGCAGTGTT
Genome

Read Lookup


Read
CAGTGCC
Hash Table
CCCAG:1
CCAGT:2
CAGTG:3,20,45
...
GTGTT:47
CAGTGCC
CAGTGCC
CAGTGCC
CCCAGTGCCACGGAGCGAACAGTGCTGAAAGCTCGCAGTGTT
Genome

Read Lookup


Read
CAGTGCC
Hash Table
CCCAG:1
CCAGT:2
CAGTG:3,20,45
...
GTGTT:47
CAGTGCC
CAGTGCC
CAGTGCC
CCCAGTGCCACGGAGCGAACAGTGCTGAAAGCTCGCAGTGTT
Score=7
Score=5
Score=3
Genome

Needleman-Wunsch

T A C A T C

T T C A - C

		t	a	c	a	t	c
t							
t							
c							
a							
c							

- Allows for mismatches and gaps
- Scoring function: +1 match; -1 for mismatch; -2 for gap
- Produces maximum likelihood alignment

Needleman-Wunsch

T A C A T C

T T C A - C

		t	a	c	a	t	c
	0	-2	-4	-6	-8	-10	-12
t	-2						
t	-4						
c	-6						
a	-8						
c	-10						

- Allows for mismatches and gaps
- Scoring function: +1 match; -1 for mismatch; -2 for gap
- Produces maximum likelihood alignment

Needleman-Wunsch

```
T A C A T C
|
T T C A - C
```

		t	a	c	a	t	c
	0	-2	-4	-6	-8	-10	-12
t	-2	1					
t	-4						
c	-6						
a	-8						
c	-10						

- Allows for mismatches and gaps
- Scoring function: +1 match; -1 for mismatch; -2 for gap
- Produces maximum likelihood alignment

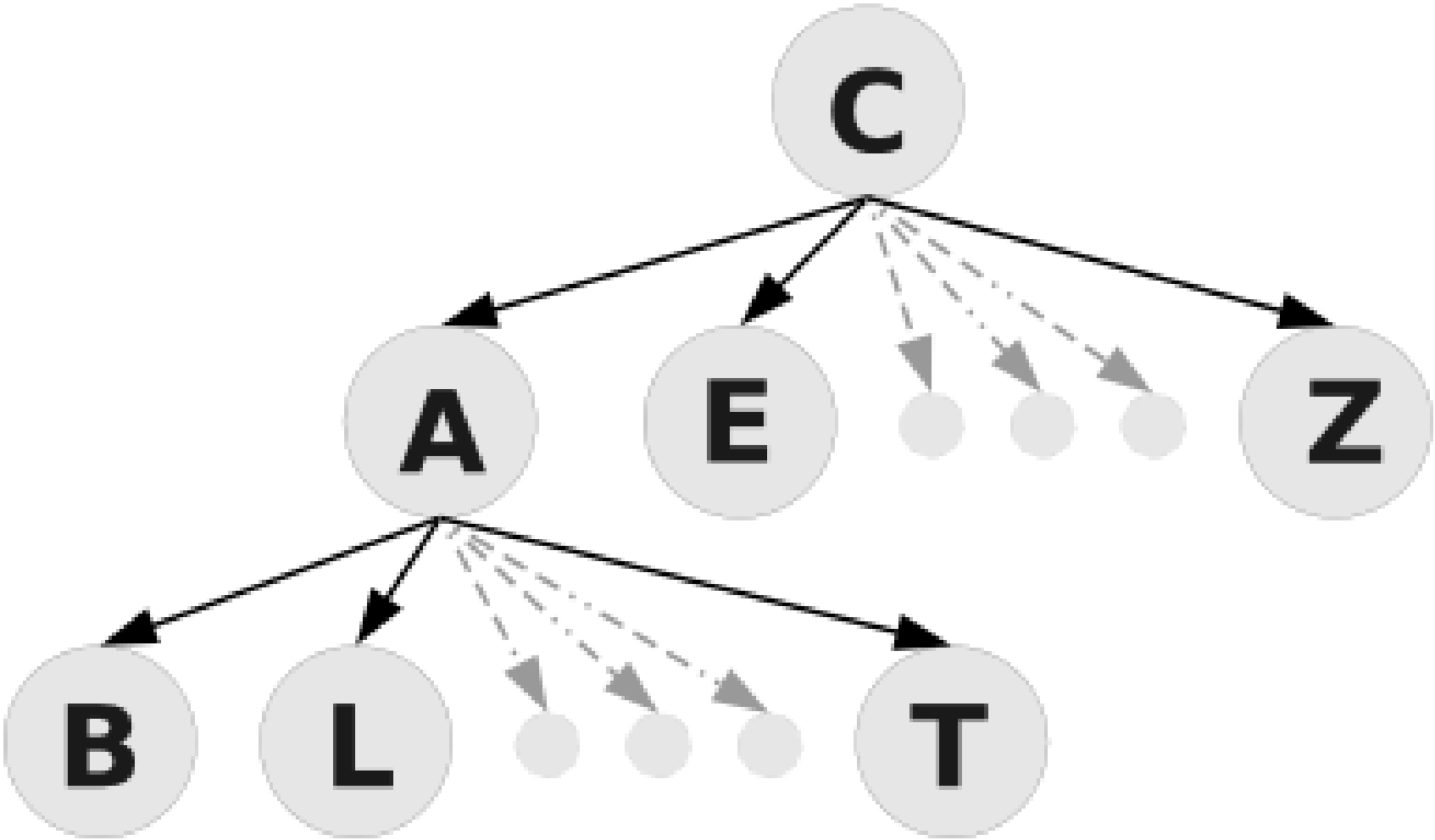
Needleman-Wunsch

```
T A C A T C
|   | |   |
T T C A - C
```

		t	a	c	a	t	c
	0	-2	-4	-6	-8	-10	-12
t	-2	**1**	-1	-3	-5	-7	-9
t	-4	-1	**0**	-2	-4	-4	-6
c	-6	-3	-2	**1**	-1	-3	-3
a	-8	-5	-2	-1	**2**	**0**	-2
c	-10	-7	-4	-1	0	1	**1**

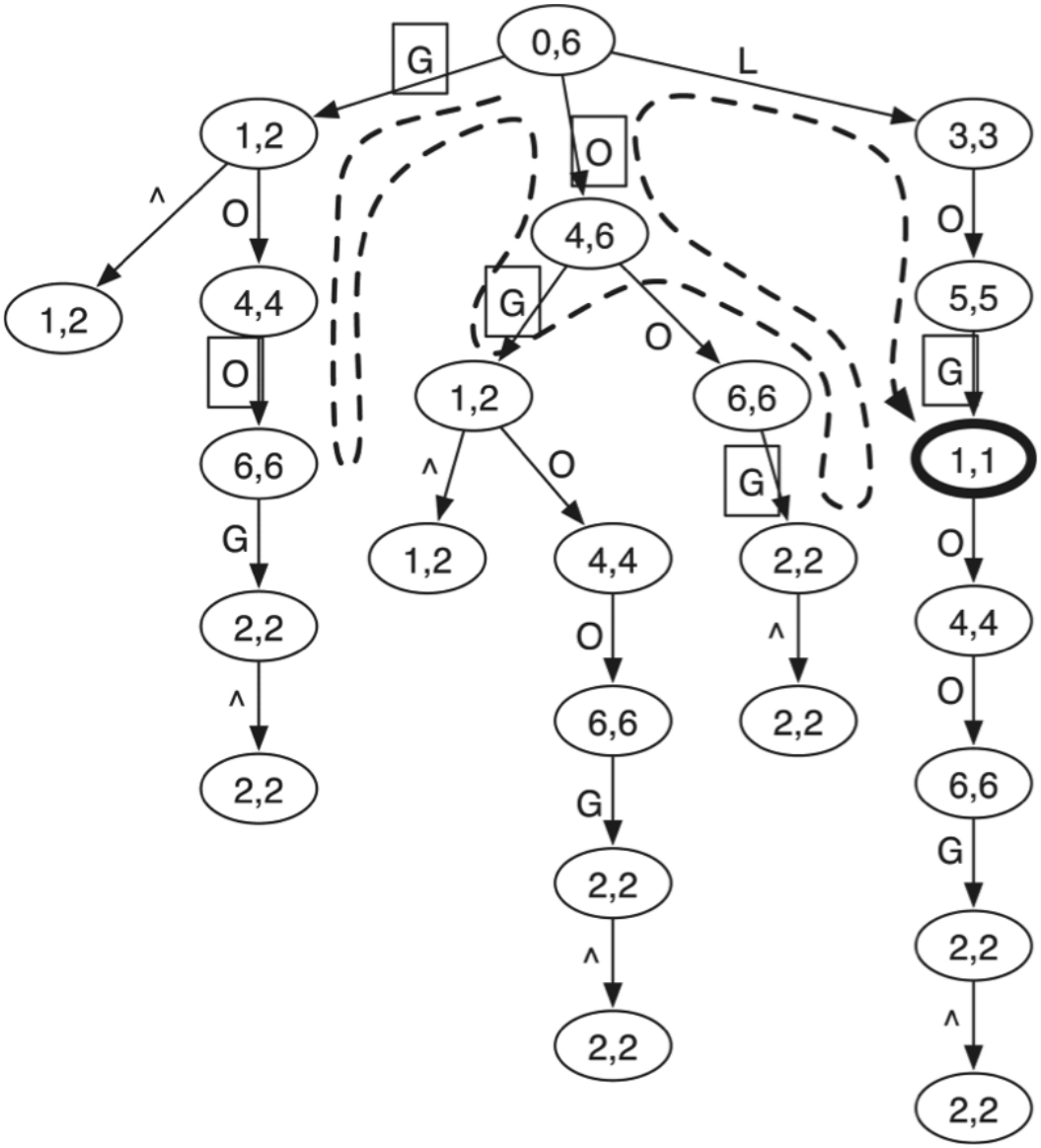
- Allows for mismatches and gaps
- Scoring function: +1 match; -1 for mismatch; -2 for gap
- Produces maximum likelihood alignment

Prefix Trees



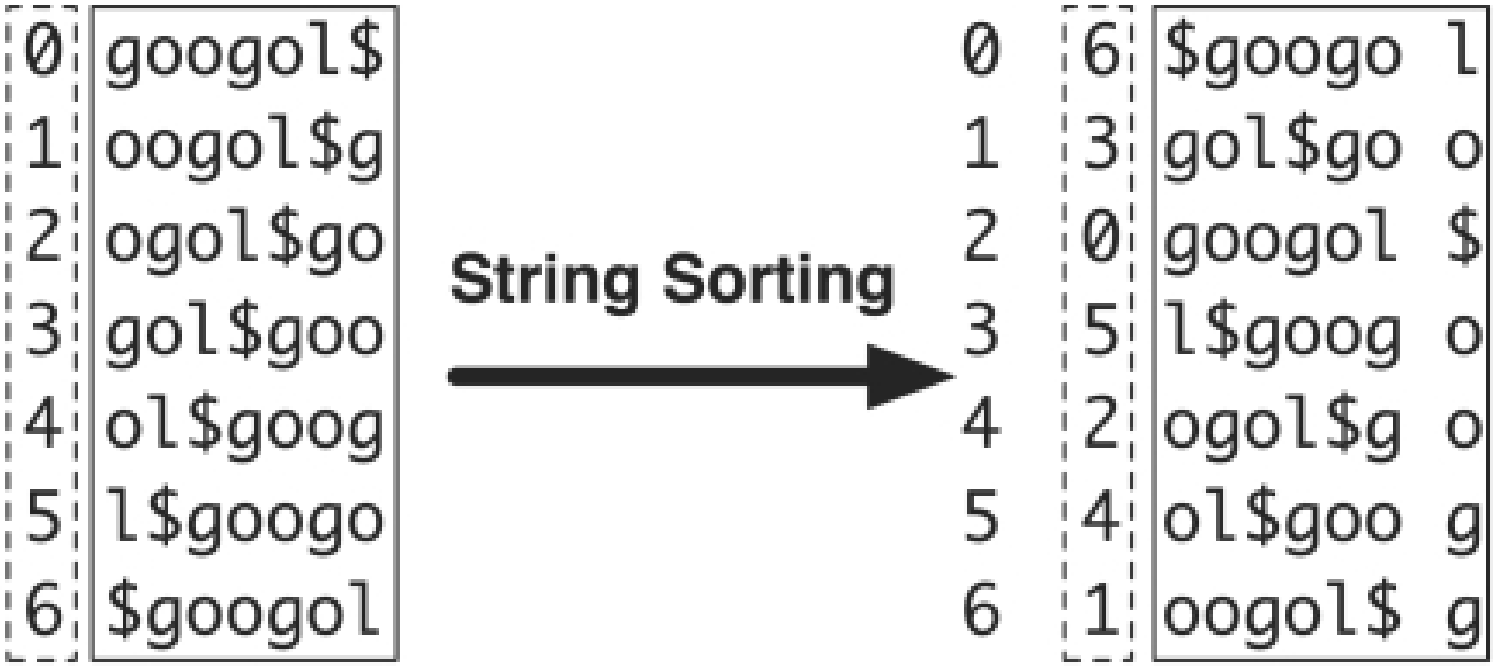
{hei

Prefix Trees



Li H , and Durbin R Bioinformatics 2009;25:1754-1760

Burrows-Wheeler Transformation (BWA, Bowtie)



Pos

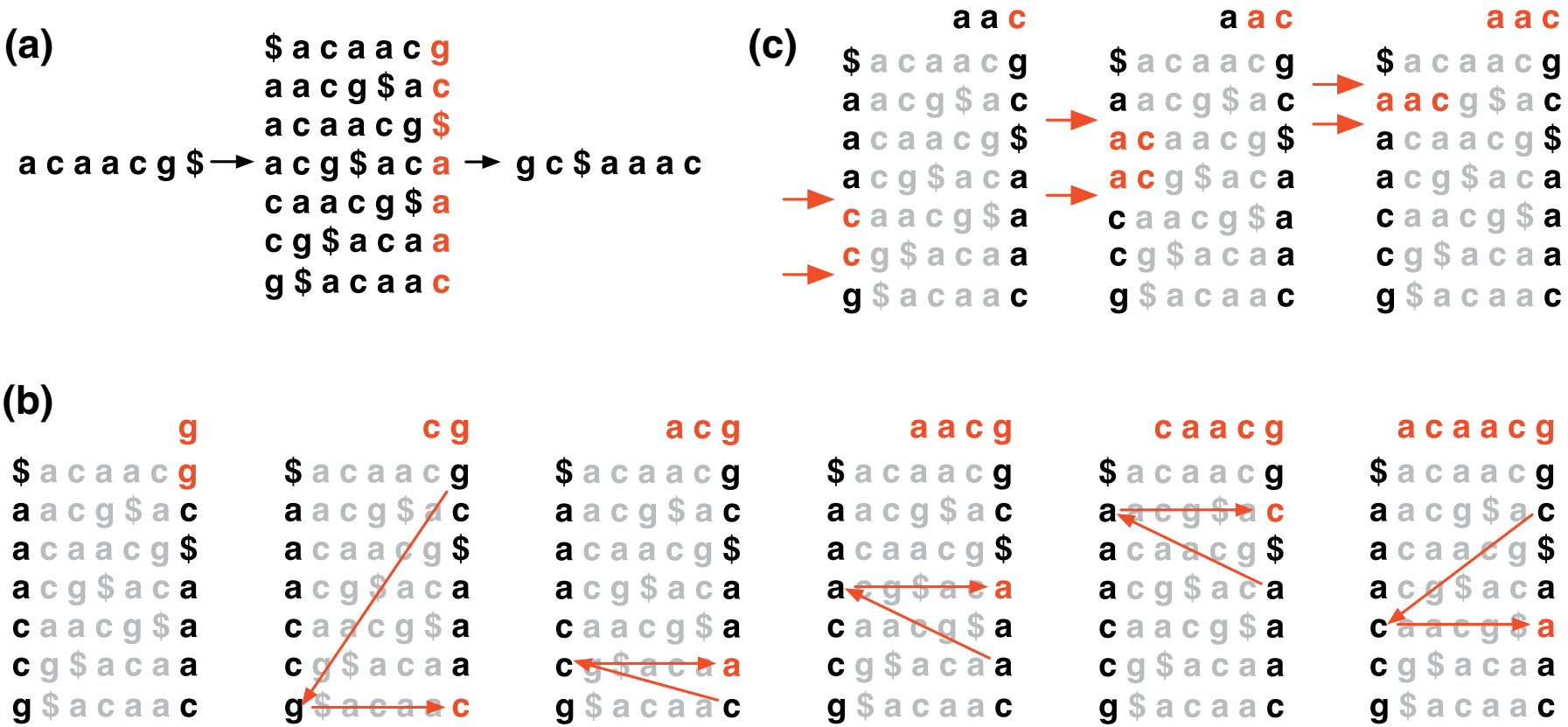
X = googol$

i S(i) B[i]

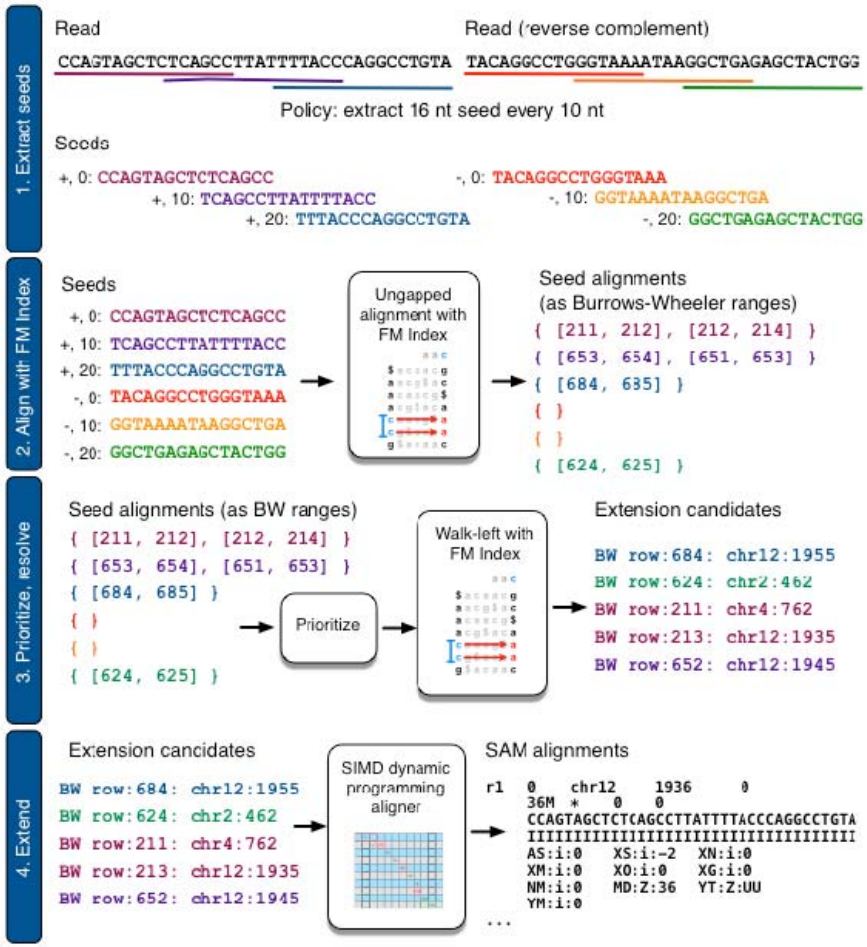
lo$oogg

(6,3,0,5,2,4,1)

Burrows-Wheeler Transformation (BWA, Bowtie)



Hybrid Methods (BWA-SW, Bowtie2)



Read alignments are stored in SAM/BAM/CRAM

- BAM and CRAM are compressed SAM files
- One line per alignment
- Use `samtools` to view, sort, merge, concatenate, index, get statistics, etc, on alignment files.

SAM/BAM/CRAM Format – Header

The *header* lines start with @:

- @HD = header definition
- @SQ = a sequence in the reference file you used, followed by how long it was and it's comment (from the reference file)
- @RG = read groups you assigned while mapping the reads
- @PG = programs used to obtain this bam, in order

SAM/BAM/CRAM Format – Body

Each alignment has 11 mandatory fields:

Col	Field	Type	Regexp/Range	Brief description
1	QNAME	String	`[!-?A-~]{1,255}`	Query template NAME
2	FLAG	Int	$[0,2^{16}-1]$	bitwise FLAG
3	RNAME	String	`\*|[!-()+-<>-~][!-~]*`	Reference sequence NAME
4	POS	Int	$[0,2^{29}-1]$	1-based leftmost mapping POSition
5	MAPQ	Int	$[0,2^{8}-1]$	MAPping Quality
6	CIGAR	String	`\*|([0-9]+[MIDNSHPX=])+`	CIGAR string
7	RNEXT	String	`\*|=|[!-()+-<>-~][!-~]*`	Ref. name of the mate/next segment
8	PNEXT	Int	$[0,2^{29}-1]$	Position of the mate/next segment
9	TLEN	Int	$[-2^{29}+1,2^{29}-1]$	observed Template LENgth
10	SEQ	String	`\*|[A-Za-z=.]+`	segment SEQuence
11	QUAL	String	`[!-~]+`	ASCII of Phred-scaled base QUALity+33

SAM/BAM/CRAM Format – Flag

The flag is the summation of the following binary attributes:

Binary (Decimal)	Hex	Description
00000000001 (1)	0x1	Is the read paired?
00000000010 (2)	0x2	Are both reads in a pair mapped "properly" (i.e., in the correct orientation with respect to one another)?
00000000100 (4)	0x4	Is the read itself unmapped?
00000001000 (8)	0x8	Is the mate read unmapped?
00000010000 (16)	0x10	Has the read been mapped to the reverse strand?
00000100000 (32)	0x20	Has the mate read been mapped to the reverse strand?
00001000000 (64)	0x40	Is the read the first read in a pair?
00010000000 (128)	0x80	Is the read the second read in a pair?
00100000000 (256)	0x100	Is the alignment not primary? (A read with split matches may have multiple primary alignment records.)
01000000000 (512)	0x200	Does the read fail platform/vendor quality checks?
10000000000 (1024)	0x400	Is the read a PCR or optical duplicate?

Example flags:

Single end read mapped to + strand: 0 (no flags apply)

Single end read mapped to - strand: 16

Unmapped, paired end first mate read with unmapped mate: 69 = 1 + 4 + 8 + 64

SAM/BAM/CRAM Format – Example

```
@HD     VN:1.0  SO:coordinate
@SQ     SN:chr20        LN:64444167
@PG     ID:TopHat       VN:2.0.14       CL:/srv/dna_tools/tophat/tophat -N 3 --read-edit-dist 5 --read-rea
lign-edit-dist 2 -i 50 -I 5000 --max-coverage-intron 5000 -M -o out /data/user446/mapping_tophat/index/chr
20 /data/user446/mapping_tophat/L6_18_GTGAAA_L007_R1_001.fastq
HWI-ST1145:74:C101DACXX:7:1102:4284:73714        16      chr20   190930  3       100M    *       0       0
       CCGTGTTTAAAGGTGGATGCGGTCACCTTCCCAGCTAGGCTTAGGGATTCTTAGTTGGCCTAGGAAATCCAGCTAGTCCTGTCTCTCAGTCCCCCCTCT
C    BBDCCDDCCDDDDCDDDDDDCDCCCDBC?DDDDDDDDDDDDDDDCCDCDDDDDDDDDDCCCCEDDDC?DDDDDDDDDDDDDDDDDDDDDBDHFFFFDC@@
    AS:i:-15        XM:i:3  XO:i:0  XG:i:0  MD:Z:55C20C13A9 NM:i:3  NH:i:2  CC:Z:=  CP:i:55352714   HI:i:0
HWI-ST1145:74:C101DACXX:7:1114:2759:41961        16      chr20   193953  50      100M    *       0       0
       TGCTGGATCATCTGGTTAGTGGCTTCTGACTCAGAGGACCTTCGTCCCCTGGGGCAGTGGACCTTCCAGTGATTCCCCTGACATAAGGGGCATGGACGA
G    DCDDDDEDDDDDDCDDDDDDDCCCDDDCDDDDDEEC>DFFFEJJJJJIGJJJJIHGBHHGJIJJJJJJGJJJIJJJJJIHJJJJJJHHHHHFFFFFCCC
    AS:i:-16        XM:i:3  XO:i:0  XG:i:0  MD:Z:60G16T18T3 NM:i:3  NH:i:1
HWI-ST1145:74:C101DACXX:7:1204:14760:4030        16      chr20   270877  50      100M    *       0       0
       GGCTTTATTGGTAAAAAAGGAATAGCAGATTTAATCAGAAATTCCCACCTGGCCCAGCAGCACCAACCAGAAAGAAGGGAAGAAGACAGGAAAAAACCA
C    DDDDDDDDDCCDDDDDDDDDDEEEEEEEFFFEFFEGHHHHFGDJJIHJJIJIJJJIIIIGGFJJIHIIIIJJJJJJIGHHFAHGFHJHFGGHFFFDD@BB
    AS:i:-11        XM:i:2  XO:i:0  XG:i:0  MD:Z:0A85G13    NM:i:2  NH:i:1
HWI-ST1145:74:C101DACXX:7:1210:11167:8699        0       chr20   271218  50      50M4700N50M     *       0
       0       GTGGCTCTTCCACAGGAATGTTGAGGATGACATCCATGTCTGGGGTGCACTTGGGTCTCCGAAGCAGAACATCCTCAAATATGACCTCTCG
```

SAM/BAM/CRAM Format

- Use `samtools` view to see the content of a SAM/BAM/CRAM file.
- Always sort and index them
- Use `samtools view -f` (include) and `-F` (exclude) to filter by flags
- Use `samtools view -q` to filter by quality.
- More on `samtools` later!!

BWA Example (OnDemand Amarel Desktop)

```
# load bwa
module load bwa

# Index the genome:
bwa index genomefile.fa

# Align the reads:
bwa mem genomefile.fa myfastqfile.fq > bwaalign.sam

# Note: for use with gatk (will learn later) bwa mem
# should include a `read group' header as well, e.g.:
-R "@RG\tID:group1\tSM:sam1\tPL:illumina\tLB:lib1\tPU:unit1"
```

Bowtie2 Example (OnDemand Amarel Desktop)

```
# Load Bowtie2
module load bowtie2

# Index the genome:
bowtie2-build genomefile.fa output/genomefile

# Align the reads:
bowtie2 -x output/genomefile -U myfastqfile.fq \
    -S bowtiealign.sam
```

RSubread Example (OnDemand RStudio)

```
# Install the Bioconductor installer
install.packages("BiocManager")

# Install Rsubread
BiocManager::install("Rsubread")

# Index the genome:
buildindex("genomefile", "genomefile.fa")

# Align the reads:
align("genomefile", "myfastqfile.fq",
      output_file="myfastq.bam", nthreads = 4)
```

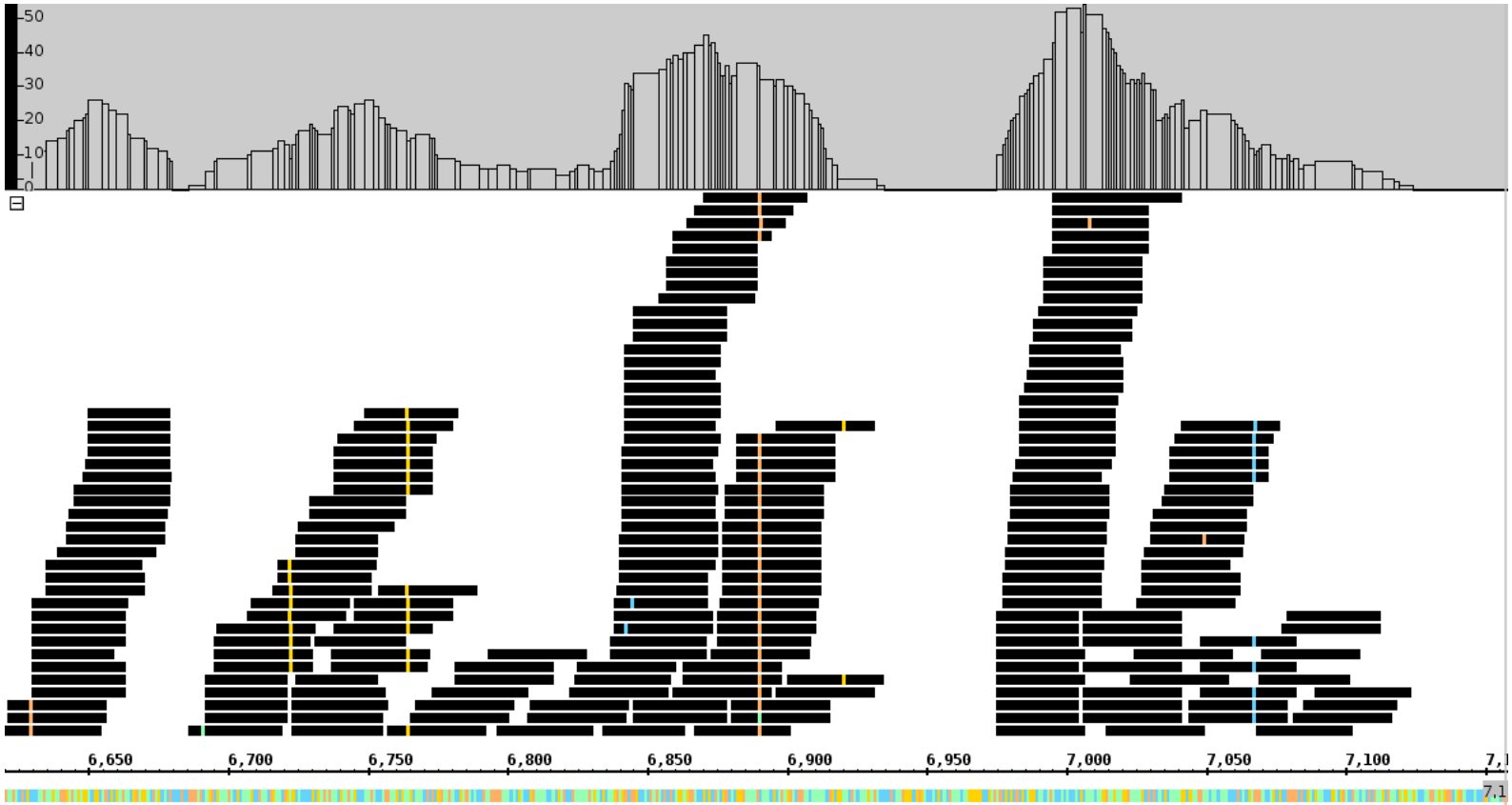
Section 6

Visualization of Aligned Sequencing Data

Options for data visualization

- UCSC Genome Browser
- IGB (http://bioviz.org/igb/)
- IGV (http://www.broadinstitute.org/igv/)
- SAMtools

IGB Example


50
40
30
20
10
0
6,650
6,700
6,750
6,800
6,850
6,900
6,950
7,000
7,050
7,100

IGV Example

DePristo et al. *Nature Genetics 2011; 43: 491-8.*

SAMtools


152853001 152853011 152853021 152853031 152853041 152853051 152853061
TGCCCAAATTCAGAAGCTGCCCACCTGGGGCCAGGAAAGGCCATGGTAGAAGTAGTATTTCATCTGGTAGTTCTCGGGC
tgcc aaattcagaagctgcccacctggggccagggaaggccatggtagaagtagtatttcatctggtagttctcgggc
tgcccaaattcagaagctgcccacc GGGGCCAGGGAAGGCCATGGTAGAAGTAGTATTTCATCTGGTAGTTCTCGGGC
tgcccaaattcagaagctgcccacc GGGGCCAGGGAAGGCCATGGTAGAAGTAGTATTTCATCTGGTAGTTCTCGGGC
tgcccaaattcagaagctgcccacct GGGCCAGGGAAGGCCATGGTAGAAGTAGTATTTCATCTGGTAGTTCTCGGGC
tgcccaaattcagaagctgcccacct GGGCCAGGGAAGGCCATGGTAGAAGTAGTATTTCATCTGGTAGTTCTCGGGC
tgcccaaattcagaagctgcccacct GGGCCAGGGAAGGCCATGGTAGAAGTAGTATTTCATCTGGTAGTTCTCGGGC
tgcccaaattcagaagctgcccacct GGGCCAGGGAAGGCCATGGTAGAAGTAGTATTTCATCTGGTAGTTCTCGGGC
tgcccaaattcagaagctgcccacct GGCCAGGGAAGGCCATGGTAGAAGTAGTATTTCATCTGGTAGTTCTCGGGC
tgcccaaattcagaagctgcccacctggggccagggaa gccatggtagaagtagtatttcatctggtagttctcgggc
tgcccaaattcagaagctgcccacctggggccagggaa gccatggtagaagtagtatttcatctggtagttctcgggc
tgcccaaattcagaagctgcccacctggggccagggaagg CATGGTAGAAGTAGTATTTCATCTGGTAGTTCTCGGGC
tgcccaaattcagaagctgcccacctggggccagggaaggccatg TAGAAGTGGTATTTCATCTGGTAGTTCTCGGGC
tgcccaaattcagaagctgcccacctggggccagggaaggccatgga GAAGTAGTATTTCATCTGGTAGTTCTCGGGC
tgcccaaattcagaagctgcccacctggggccagggaaggccatgga gaagtagtatttcatctggtagttctcgggc
TGCCCAAATTCAGAAGCTGCCCACCTGGGGCCAGGGAAGGCCATGGTAGAA tagtatttcatctggtagttctcgggc
TGCCCAAATTCAGAAGCTGCCCACCTGGGGCCAGGGAAGGCCATGGTAGAA agtatttcatctggtagttctcgggc
tgcccaaattcagaagctgcccacctggggccagggaaggccatggtagaagtagtattt ATCTGGTAGTTCTCGGGC
tgcccaaattcagaagctgcccacctggggccagggaaggccatggtagaagtagtattt tctggtagttctcgggc
TGCCCAAATTCAGAAGCTGCCCACCTGGGGCCAGGGAAGGCCATGGTAGAAGTAGTATTTC TCTGGTAGTTCTCGNNN
tgcccaaattcagaagctgcccacctggggccagggaaggccatggtagaagtagtatttc tctggtagttctcgggc
tgcccaaattcagaagctgcccacctggggccagggaaggccatggtagaagtagtatttcatc GGTAGTTCTCGGGC

SAMtools Example (Amarel Desktop)

```
# Load SAMtools
module load samtools

# Convert SAM file to BAM format:
samtools view -bS myalignments.sam > myalignments.bam

# Sort the BAM file:
samtools sort myalignments.bam -o myalignments.sorted.bam

# Index the BAM file:
samtools index myalignments.sorted.bam

# View BAM file in SAMtools:
samtools tview myalignments.sorted.bam genomefile.fa
```

SAMtools Example

SAMtools commands:

- The one command to remember: '?'
- g: go to a specific location (i.e. chrX:152852988 or chrX_149913753:2939235)
- m,b: mapping quality, base quality
- n: color by nucleotide
- '.': dot/base view
- r: read name
- q: quit SAMtools

SAM/BAM/CRAM Format – Conversions

```
# Convert SAM to BAM
samtools view -bS in.sam > out.bam

# Convert BAM to SAM
samtools view -ho out.sam in.bam

# Convert BAM to fastq
samtools bam2fq in.bam > out.fastq
```

RSamtools

`Rsamtools` is a very useful (although somewhat limited) version of Samtools available in R:

```
# Install Rsamtools
BiocManager::install("Rsamtools")

# Convert SAM to BAM
asBam(in.sam, out.bam)
```

You can also index and sort .bam files, as well as extract alignments from a .bam file (very useful!).

Section 7

Other Data Formats

Standard format for keeping tables

field1	field2	field3	...
...	...	...	...

Fields (columns) separated by a character on each line:

- Comma (or Character) Separated Vector (CSV)
- Tab Separated Vector (TSV)
- Some interpreters take any space (space or tab) as a separator (such as `awk`, `cut`).
- Some have column name as first row (header), some don't

Genomic regions

- A region is defined by three required fields:
  - sequence name (e.g. chromosome)
  - start coordinate
  - end coordinate
- Define regions of interest: introns, exons, genes, etc.
- Additional information saved as fields after the first three.
- Three standard tab-separated formats: BED, GFF, GTF No headers

BED Format

Mandatory fields:

1. chrom - Name of the chromosome/scaffold/reference sequence
2. chromStart - 0-based starting position of the feature on chrom
3. chromEnd - Ending position of the feature in the chromosome or scaffold.

The chromEnd base is not included in the display of the feature.

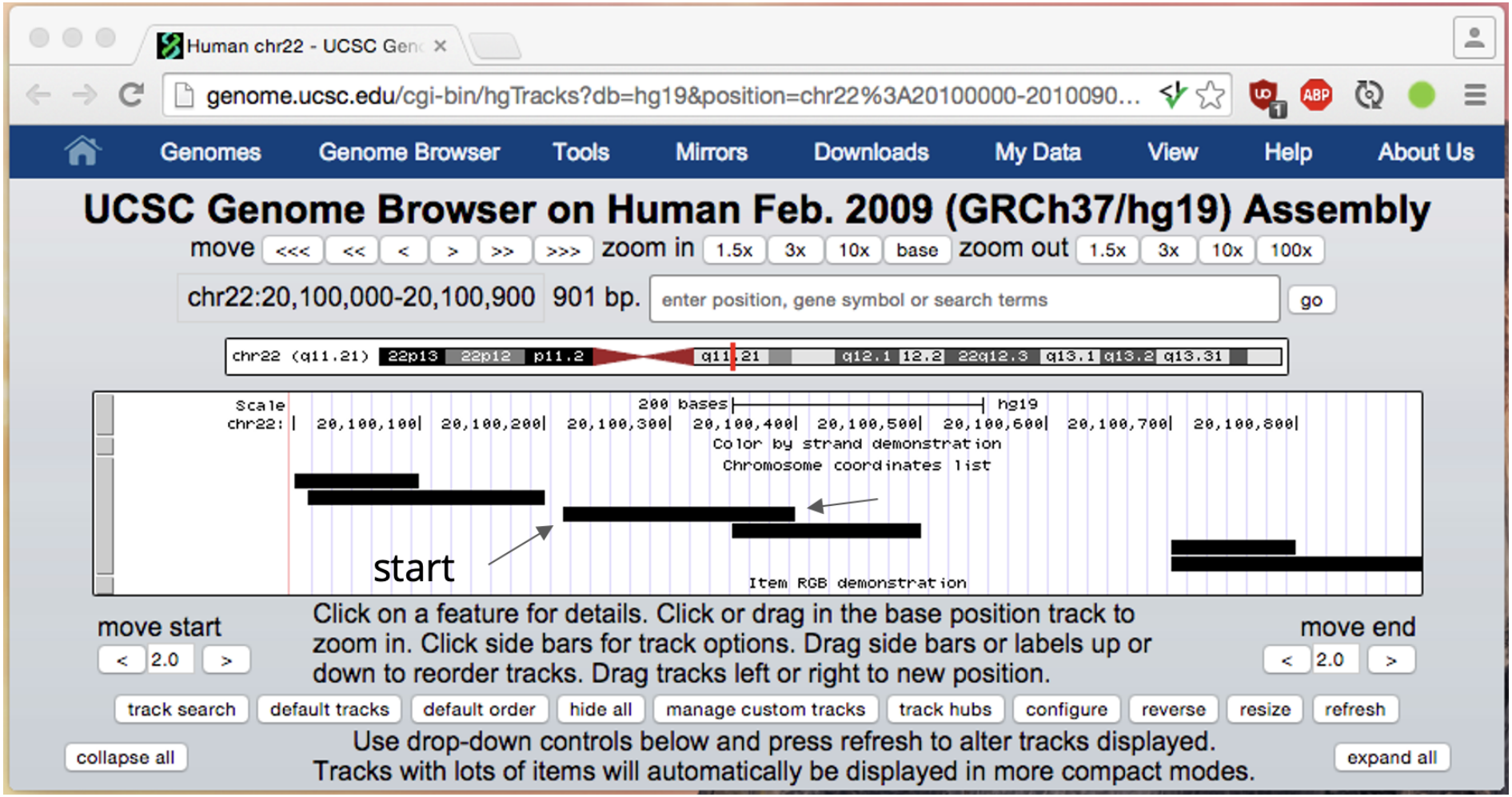
For example, the first 100 bases of a chromosome are defined as:

- chromStart=0
- chromEnd=100
- span the bases numbered 0-99

BED Format

chr1	11873	14409	uc001aaa.3	0	+	11873	11873
chr1	11873	14409	uc010nxr.1	0	+	11873	11873
chr1	11873	14409	uc010nxq.1	0	+	12189	13639
chr1	14361	16765	uc009vis.3	0	-	14361	14361
chr1	14361	19759	uc009vit.3	0	-	14361	14361
chr1	14361	19759	uc009viu.3	0	-	14361	14361
chr1	14361	19759	uc001aae.4	0	-	14361	14361
chr1	14361	29370	uc001aah.4	0	-	14361	14361
chr1	14361	29370	uc009vir.3	0	-	14361	14361
chr1	14361	29370	uc009viq.3	0	-	14361	14361
chr1	14361	29370	uc001aac.4	0	-	14361	14361
chr1	14406	29370	uc009viv.2	0	-	14406	14406
chr1	14406	29370	uc009viw.2	0	-	14406	14406
chr1	15602	29370	uc009vix.2	0	-	15602	15602
chr1	15795	18061	uc009vjd.2	0	-	15795	15795
chr1	16606	29370	uc009viy.2	0	-	16606	16606
chr1	16606	29370	uc009viz.2	0	-	16606	16606
chr1	16857	17751	uc009vjc.1	0	-	16857	16857
chr1	16857	19759	uc001aai.1	0	-	16857	16857

BED Format


Human chr22 - UCSC Gen
genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&position=chr22%3A20100000-2010090...
Genomes
Genome Browser
Tools
Mirrors
Downloads
My Data
View
Help
About Us
UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly
move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x
chr22:20,100,000-20,100,900 901 bp.
enter position, gene symbol or search terms
go
chr22 (q11.21) 22p13 22p12 p11.2 q11.21 q12.1 12.2 22q12.3 q13.1 q13.2 q13.31
Scale
chr22:
200 bases
hg19
20,100,100| 20,100,200| 20,100,300| 20,100,400| 20,100,500| 20,100,600| 20,100,700| 20,100,800|
Color by strand demonstration
Chromosome coordinates list
start
Item RGB demonstration
move start
< 2.0 >
Click on a feature for details. Click or drag in the base position track to zoom in. Click side bars for track options. Drag side bars or labels up or down to reorder tracks. Drag tracks left or right to new position.
move end
< 2.0 >
track search
default tracks
default order
hide all
manage custom tracks
track hubs
configure
reverse
resize
refresh
collapse all
Use drop-down controls below and press refresh to alter tracks displayed.
Tracks with lots of items will automatically be displayed in more compact modes.
expand all

BED Format

Optional fields:

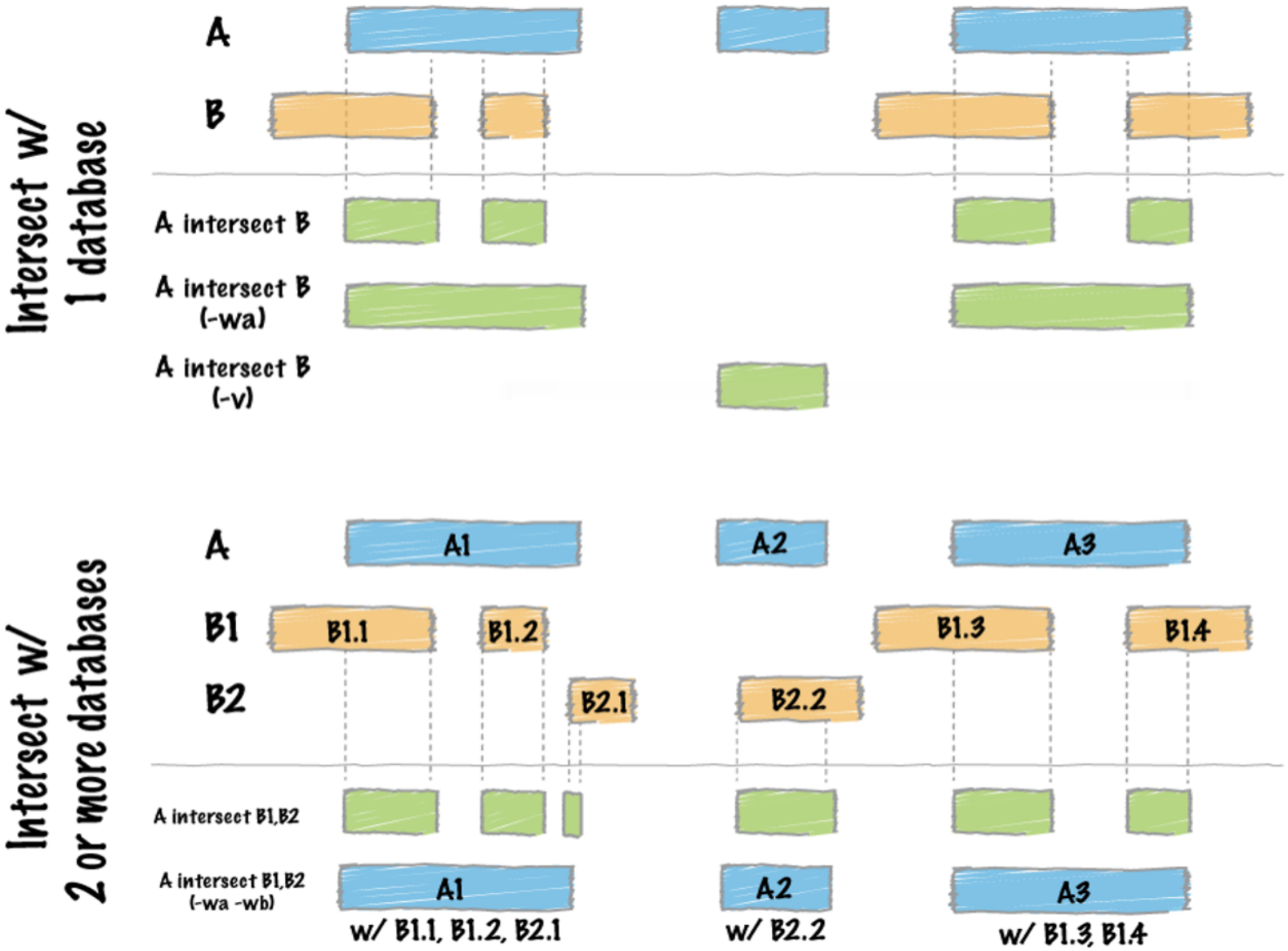
4. Name
5. Score
6. Strand
7. 7-12. Display options (thick starts and end, color, blocks. . .) to control the view on the genome browser

bedtools

http://bedtools.readthedocs.io/

- sort (sort bed files)
- Intersect (get intersections of bed files)
- merge
- coverage
- overlap
- substract
- ...

bedtools intersect



bedtools merge

GFF - General features

9 mandatory fields, tab separated:

1. seqname - The name of the sequence. Must be a chromosome or scaffold.
2. source - The program that generated this feature.
3. feature - The name of this type of feature (e.g. gene, exon, etc).
4. start - The starting position of the feature in the sequence (1-based)
5. end - The ending position of the feature (inclusive).
6. score - A score between 0 and 1000.
7. strand - Valid entries include "+", "-", or "." (for don't know/don't care).
8. frame - If the feature is a coding exon, frame should be a number between 0-2 that represents the reading frame of the first base. If the feature is not a coding exon, the value should be ".".
9. group - All lines with the same group are linked together into a single item

Gene Information

GTF (Gene Transfer Format, GTF2.2)

- Extension to GFF2, backwards compatible
- First eight GTF fields are the same as GFF
- *feature field* is the same as GFF, has controlled vocabulary:
  - *gene, transcript, exon, CDS, 5UTR, 3UTR, inter, inter_CNS, and intron_CNS, etc*
- group field expanded into a list of attributes (i.e. key/value pairs)

The attribute list must begin with the one mandatory attribute: - gene_id value - A globally unique identifier for the genomic source of the sequence

GTF format

```
##description: evidence-based annotation of the human genome (GRCh38), version 27 (Ensembl 90)
##provider: GENCODE
##contact: gencode-help@sanger.ac.uk
##format: gtf
##date: 2017-08-01
chr1    HAVANA  gene    923928  944581  .       +       .       gene_id "ENSG00000187634.11"; gene_type
"protein_coding"; gene_name "SAMD11"; level 2; havana_gene "OTTHUMG00000040719.10";
```

- seqname: chr1
- source: HAVANA
- feature: gene
- start: 923928
- end: 944581
- score: . (no score)
- strand: +
- frame: . (not coding feature)
- attributes:
  - gene_id: ENSG00000187634.11
  - gene_type: protein_coding
  - gene_name: SAMD11
  - level: 2
  - havana_gene: OTTHUMG00000040719.10

GFF/GTF encodes relationships

- Features are hierarchical, e.g.:
  - A gene has 1 or more transcripts
  - A transcript has 1 or more exons

Section 8

SNP and Variant Calling

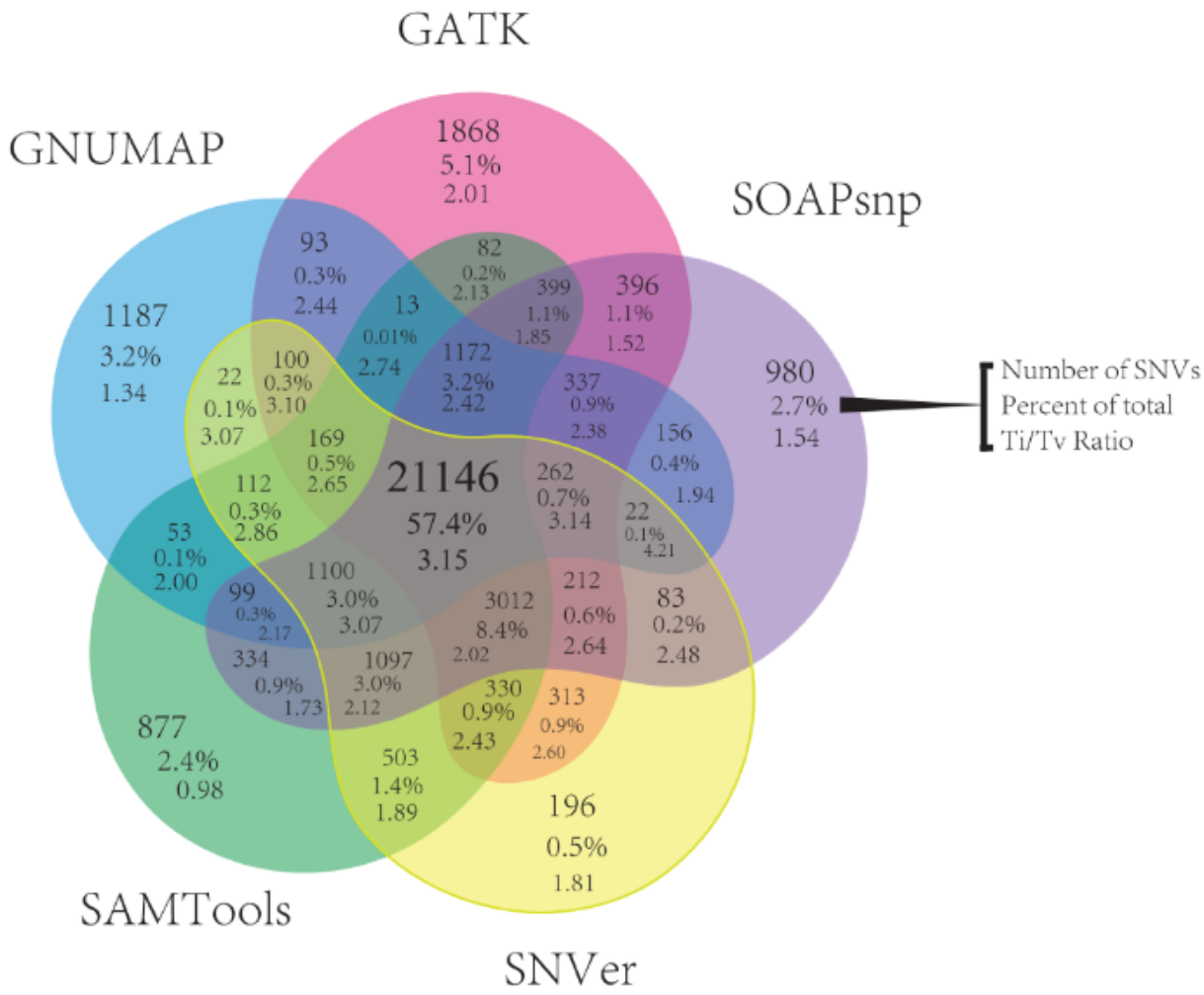
Methods for SNP Calling

Methods for SNP calling:

- Mapper/callers: MAQ, SOAPsnp, GNUMAP, Crossbow (Bowtie)
- Callers: SAMtools (mpileup), GATK (HaplotypeCaller, Mutect2), FreeBayes, others

Inconsistencies Among Aligners

Low concordance of variant-calling pipelines (O'Rawe, *Genome Med*, 2013)



SAMtools Example

Multiple sample SNP calling:

```
samtools mpileup -f genomefile.fa \
  myalignments.sorted.bam > myalignments.vcf
```

VCF files

8 fixed columns: #CHROM, POS, ID, REF, ALT, QUAL, FILTER, INFO, FORMAT
Additional columns, one for each sample, with sample ID

(a) **VCF example**

Header

```
##fileformat=VCFv4.1
##fileDate=20110413
##source=VCFtools
##reference=file:///refs/human_NCBI36.fasta
##contig=<ID=1,length=249250621,md5=1b22b98cdeb4a9304cb5d48026a85128,species="Homo Sapiens">
##contig=<ID=X,length=155270560,md5=7e0e2e580297b7764e31dbc80c2540dd,species="Homo Sapiens">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##ALT=<ID=DEL,Description="Deletion">
##INFO=<ID=SVTYPE,Number=1,Type=String,Description="Type of structural variant">
##INFO=<ID=END,Number=1,Type=Integer,Description="End position of the variant">
```

Body

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	SAMPLE1	SAMPLE2
1	1	.	ACG	A,AT	40	PASS	.	GT:DP	1/1:13	2/2:29
1	2	.	C	T,CT	.	PASS	H2;AA=T	GT	0|1	2/2
1	5	rs12	A	G	67	PASS	.	GT:DP	1|0:16	2/2:20
X	100	.	T	<DEL>	.	PASS	SVTYPE=DEL;END=299	GT:GQ:DP	1:12:.	0/0:20:36

Danecek et al. Bioinformatics 2011

Complete Variant Calling Pipeline (Outdated!)}

Our analysis pipeline consisted of the following:

- Align the FASTQ files to genome
- Convert SAM file to BAM, add read group info
- Filter the reads based on quality (BAMTools)
- Samtools to sort and index, and use Picard to mark duplicates
- GATK calibration, realignment, variant calling (HaplotypeCaller, Mutect2)
- Filter the called variants (GATK filtersnps and filterindels).
- Annotation of SNPs (snpEff, condel)
- Filter by frequency (thousand genomes, TCGA, etc.)
- Downstream analysis (rare variants, pedigree, pathway level, etc)

Downstream Annotation and Analysis (Outdated!)

Downstream Annotation Tools (old list):

- snpEff (http://snpeff.sourceforge.net/)
- Condel (http://bg.upf.edu/condel/home)
- SIFT http://sift.jcvi.org/
- Polyphen 2 http://genetics.bwh.harvard.edu/pph2/
- http://mutationassessor.org/
- Ensembl variant effect predictor (http://www.ensembl.org/info/docs/variation/vep/index.html)
- Thousand Genomes variant frequency (e.g. 1% threshold) and Exome Sequence Project variant frequency (e.g. 1%).

Session info

```
sessionInfo()
```

```
## R version 4.4.0 (2024-04-24)
## Platform: aarch64-apple-darwin20
## Running under: macOS Sonoma 14.2.1
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRlapack.dylib;  LAPACK version 3
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## time zone: America/Denver
## tzcode source: internal
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## loaded via a namespace (and not attached):
##  [1] digest_0.6.35     fastmap_1.2.0     xfun_0.44         Matrix_1.7-0
##  [5] lattice_0.22-6    reticulate_1.37.0 knitr_1.46        htmltools_0.5.8.1
##  [9] png_0.1-8         rmarkdown_2.27    cli_3.6.2         grid_4.4.0
## [13] compiler_4.4.0    rstudioapi_0.16.0 tools_4.4.0       evaluate_0.23
## [17] Rcpp_1.0.12       yaml_2.3.8        jsonlite_1.8.8    rlang_1.1.3
```