Genomic data file formats and tools

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Talk typesetting

- Commands/options are in `typewriter font`
- URLs are highlighted in blue

Unix: recap

- Lecture from SIB
  https://edu.sib.swiss/pluginfile.php/2878/mod_resource/content/4/couselab-html/content.html

Exercises

- Run the exercises till number 4

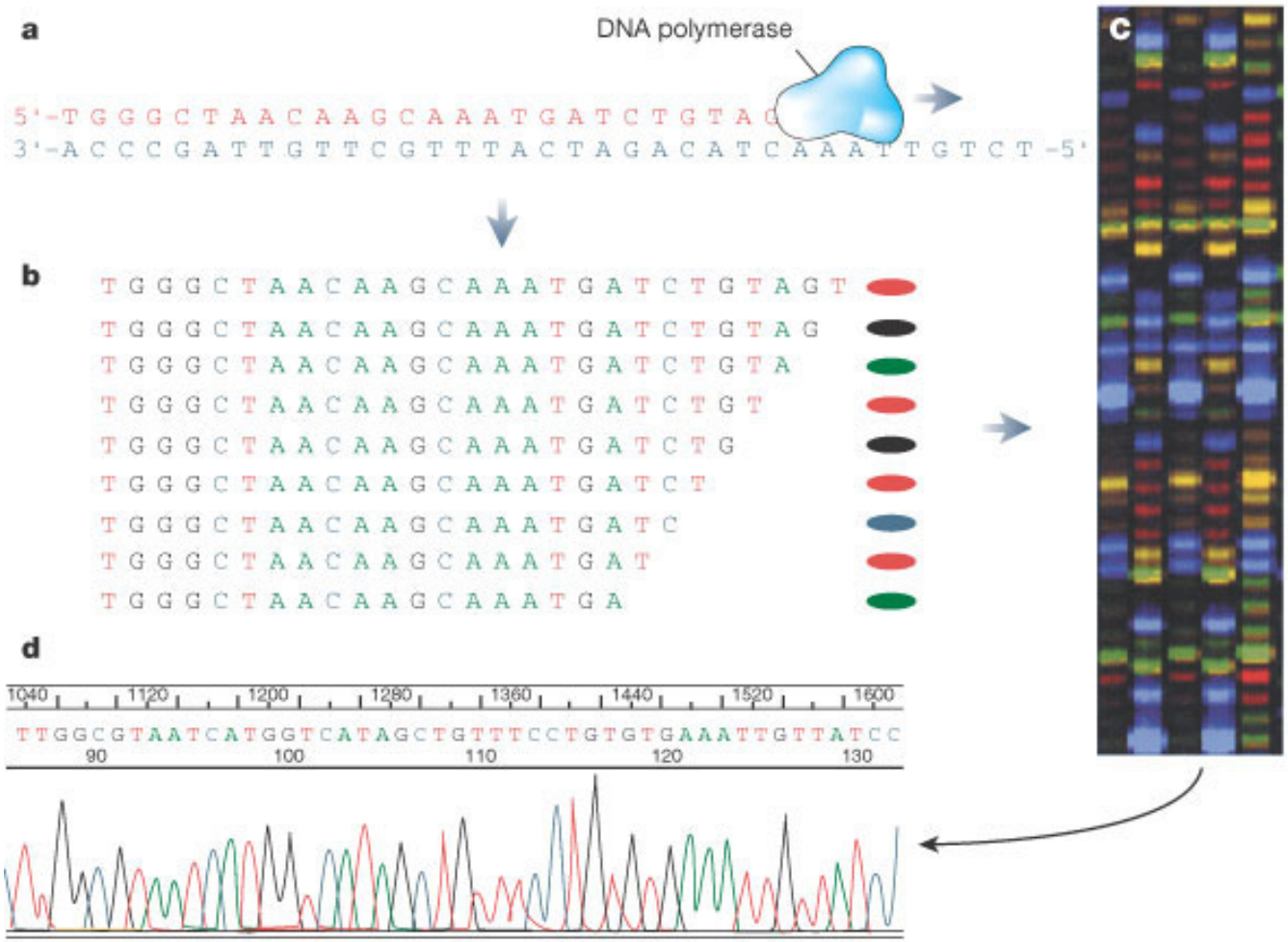
Commonly used formats

- Reference genomes
- Fasta and FastQ (Unaligned sequences)
- SAM/BAM (Alignments)
- BED (Genomic ranges)
- GFF/GTF (Gene annotation)
- BEDgraphs (Genomic ranges)
- Wiggle files, BEDgraphs and BigWigs (Genomic scores).
- Indexed BEDgraphs/Wiggles
- VCFs (variants)

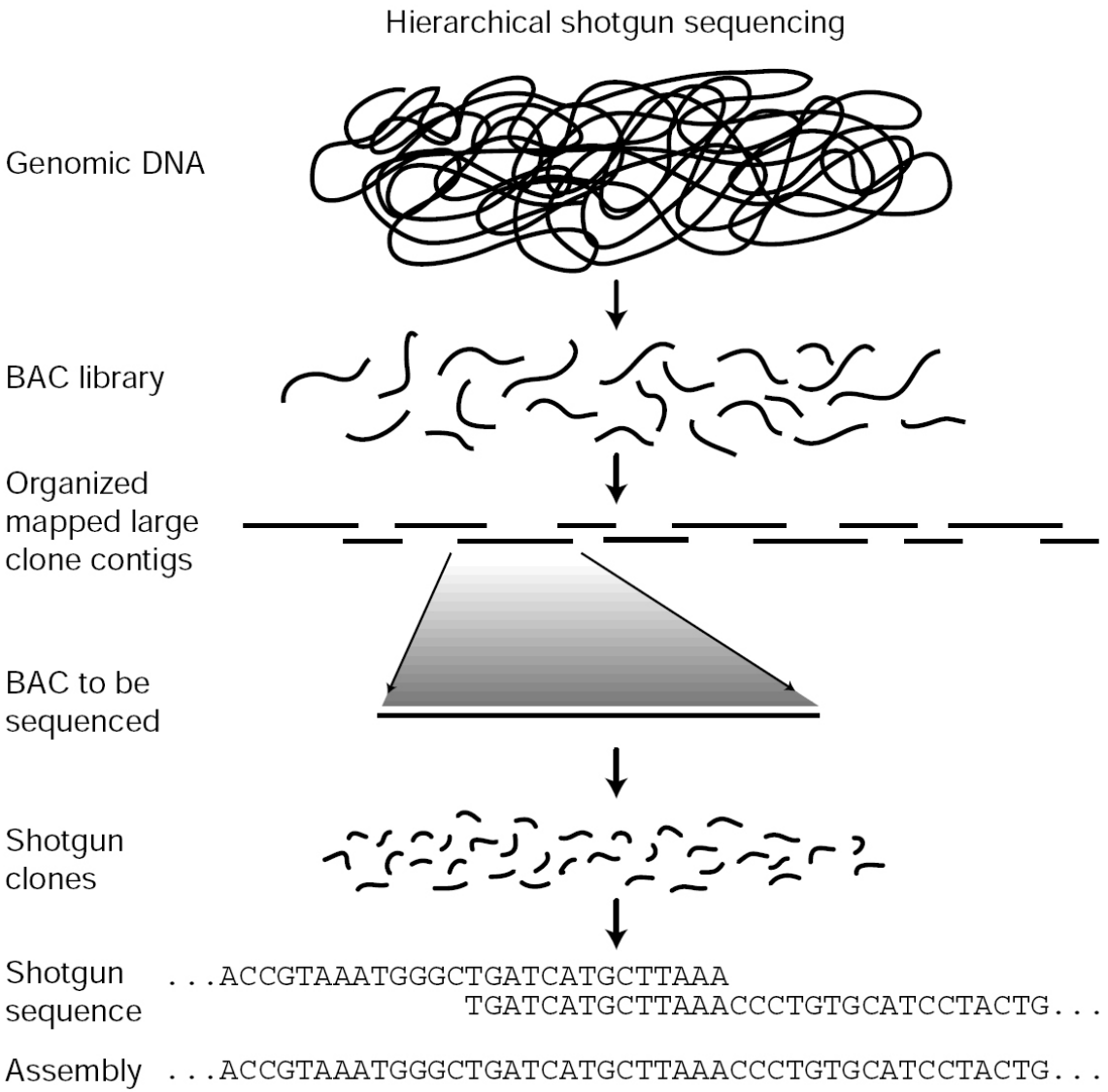
Reference genomes

- Reference genomes describe the 'consensus' DNA sequence
- (Who is the human consensus for DNA sequencing?)
- Aside of human variation, multiple assemblies have been released

Sanger sequencing Nature 409, 863 (2001)



Hierarchical shotgun Nature 409, 863 (2001)



Reference genomes

GRCh stands for 'Genome Reference Consortium'

- Human GRCh37 (hg19)
- Human GRCh38
- Mouse mm10
- Mouse GRCm38
- Zebrafish, chicken and others: https://www.ncbi.nlm.nih.gov/grcThe Genome Reference consortium

Reference genomes: FASTA

- A reference genome is a collection of contigs/scaffolds
- A contig is a stretch of DNA sequence encoded as A,G,C,T,N.
- Typically comes in FASTA format.
- ">" line contains the scaffold name
- Following lines contain the sequence (single line, 80 nt-column sized...)

Reference genomes: FASTA

```
>NC_009902.1 Babesia bovis T2Bo mitochondrion, complete genome
TTTAAAAAAGTGTTAAAAACTTTATACATTAAAAAATTTAAACAAGTGATCATGTATAAAGTACACTTGT
TACTGTGTAAATATCAAAAACAATTTAATTTCAAAATTTTTGAAATATGTTTTTTGTGTTGTGTTATAAA
GTTTTTTTTCAAAATTATATATGTTTGCATTTGCTGGATATAGTTCGGTCTCTGCAAACCATAAAGTCAT
CGGTATATCCTACATATGGCTTTCATATTGGTTTGGAGTTATTGGATTTTATATGAGTATTTTGATAAGA
ACAGAATTGAGTATGAGTGGTTTAAAGATTATGACAATGGATACTCTTGAGATATACAATATGATGTTTT
```

Patches, alternate loci and primary assembly

- Primary assembly: the best known assembly of a haploid genome
  - Chromosome assembly
  - Unlocalized sequence (associated to a chromosome but whose order/orientation is unknown)
  - Unplaced sequence (not linked to any chromosome)
- Alternate loci: An alternate representation of a locus (usually highly polimorphic regions, such as the MHC region)
- Patches: A contig sequence that is released outside of the full assembly release
  - Fix: error correction
  - Novel: new sequences that will be included into the next full assemblty release

Browsing genomic patches

- ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/001/405/GCA_000001405.27_GRCh38.p12/README_patch_release.txt

Retrieving fasta sequences manually (UCSC)

- Try to retrieve the `DIEXF` gene promoter
- (What is a promoter in terms of sequence?)
- Go to an assembly https://genome-euro.ucsc.edu/cgi-bin/hgTracks?db=hg38
- Query gene symbol (i.e. `DIEXF`)
- Click into the gene (gencode track)
- Click into the `sequence and links` item
- Specify your promoter definition

Manually downloading the DIEXF promoter

https://genome-euro.ucsc.edu/cgi-bin/hgGene?hgg_gene=uc001hhr.3&hgc

Genomes | Genome Browser | Tools | Mirrors | Downloads | My Data | He

Human Gene DIEXF (ENST00000491415.6) Description and Page Index

Description: Homo sapiens digestive organ expansion factor homolog (zebrafish) (DIEXF), mRN
Gencode Transcript: ENST00000491415.6
Gencode Gene: ENSG00000117597.17
Transcript (Including UTRs)
Position: hg38 chr1:209,828,007-209,857,565 **Size:** 29,559 **Total Exon Count:** 12 **Strand:** +
Coding Region
Position: hg38 chr1:209,828,064-209,851,447 **Size:** 23,384 **Coding Exon Count:** 12

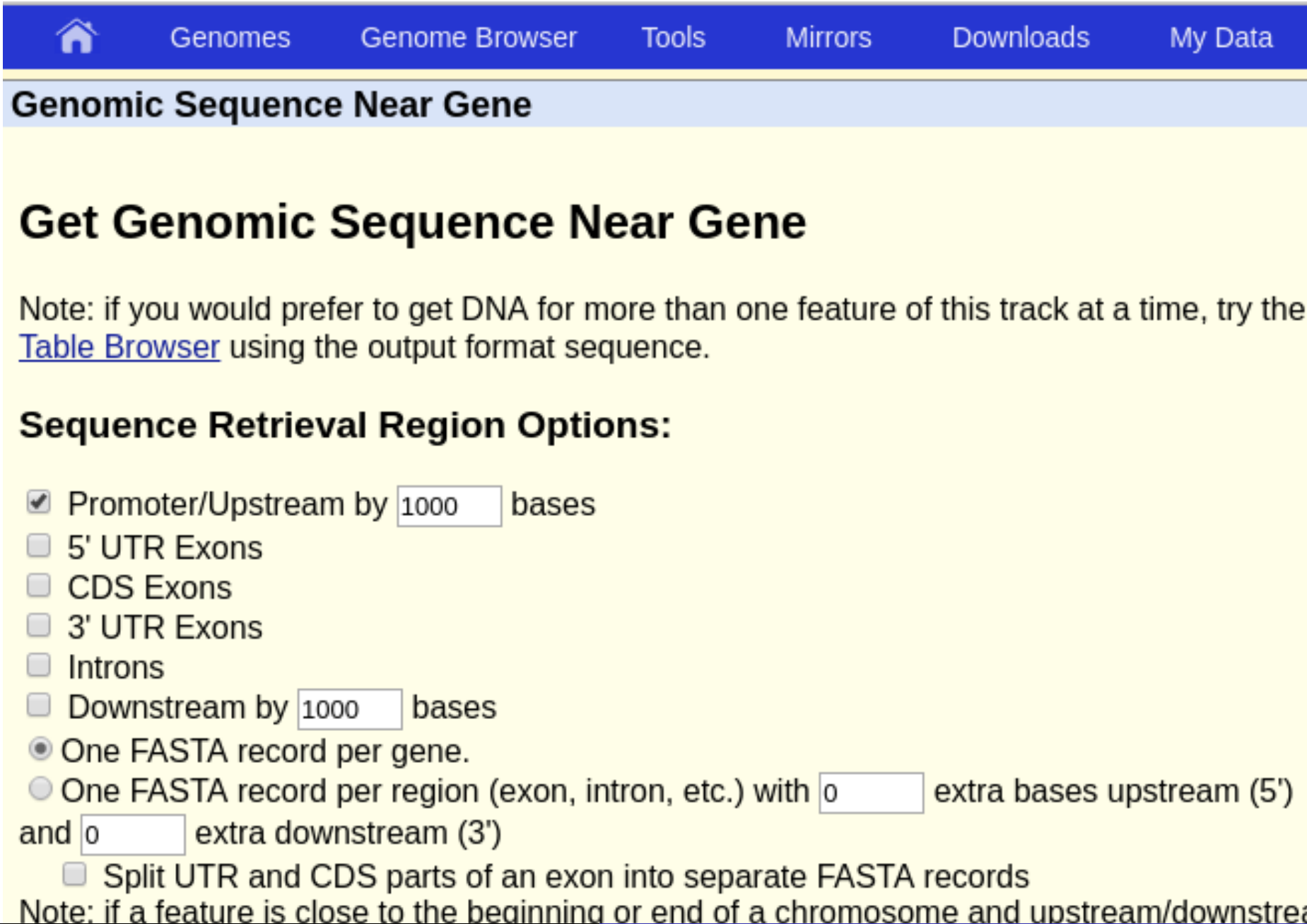
Page Index	Sequence and Links	UniProtKB Comments	CTD	RNA-Seq Express
RNA Structure	Protein Structure	Other Species	GO Annotations	mRNA Description
Other Names	Methods			

Data last updated: 2016-03-28

− Sequence and Links to Tools and Databases

Genomic Sequence (chr1:209,828,007-209,857,565)			mRNA (may differ from genome)		Protein
Gene Sorter	Genome Browser	Other Species FASTA	Gene interactions	Table Schema	BioGP
CGAP	Ensembl	Entrez Gene	ExonPrimer	GeneCards	Gepis
HGNC	HPRD	Lynx	MGI	MOPED	neXtP

Manually downloading the DIEXF promoter



Manually downloading the DIEXF promoter

```
>hg38_knownGene_uc001hhr.3 range=chr1:209827007-209
gtttctgctgttgttaaatggggaatgctggaacagatttgtttgcgggg
actcttccaatactttcagaaaatgcgagaatagggtgagggtgggaatc
tcagacttgtgggccccatgattgatataacacacacaggcggcagaccc
taatgggtaaaagcatgtggttgcatcagttaaggtttttctctcttctc
ttgctagcgtgttatctttttctttttcttttcttttttttttttttcg
agatggagtctagcttttgtcgcccaggctggagtaggctggagtgcagt
ggagtgatctcggctcattgcaacctccacctcccgggttccagcgattc
tcctgcctcacctcctgagtagctgggattacaggcgcccgctaccacgc
ccggctgatttttgtacttttagtagagacggggtttcaccatgtttggc
catgctggtctcgaactcctgacctcaggtgatccgcccatctcggcctc
ccaaagtgttgagattacaggcgtgagccaccgcgcccggccgctagcgt
gttatcttttctaagcatcagtttccttatctgcaacaccaggcttatta
acaagacctatctgtacactgttgtggtgatgaagtgagatgttcaggca
cccttaaatgttggttgatattttattgcagtatactgtaaagtcactg
cattcgactatctccgctactacacatttacgcagactgatttccataac
caaaacacaagcacaaagctcatgcccccgactcacgcaacccgggaagc
ccagctgcccacgttctagggctctgagaacactagtgaacgaactcccg
tgctttcaaagagctgcggtaggggcagaaccgggaaccggatgttcta
agcctgtcgtacgagcgcgacgtaaagcggatctgctttatggcacctg
```

FASTQ: Short read sequencing

- Next step to FASTA: including quality data
- Standard de facto for high-throughput sequencing instruments such (i.e. Illumina)

phred scores

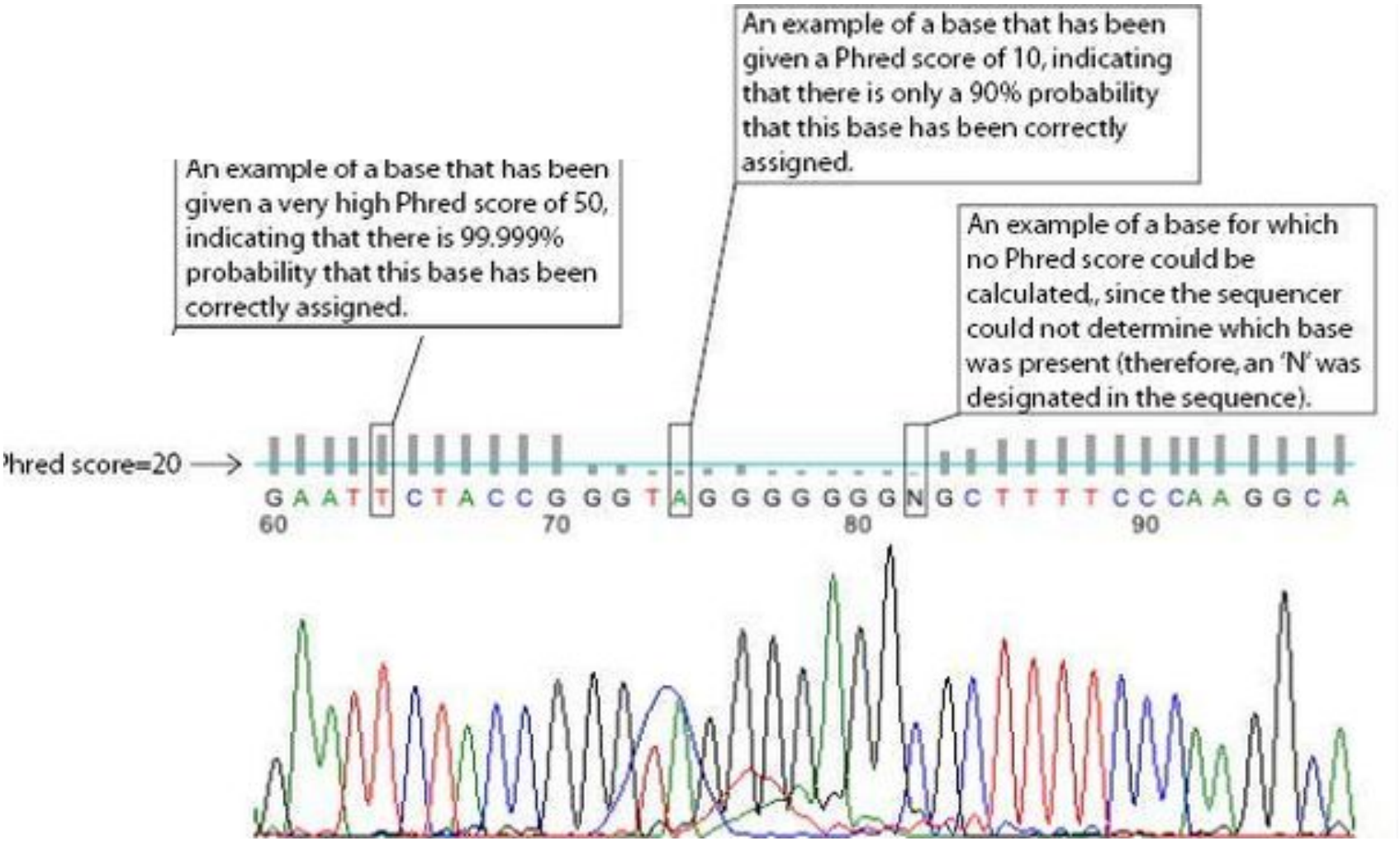
- Sequence quality is represented using Phred scores
- The sequencing quality score of a given base Q is defined by as
- $Q = -10 \ \log_{10} P$

phred scores

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%

phred scores



Unaligned sequences (from sequencers): FASTQ

- FASTQs stands for FASTA with Qualities
- Plain text files with chunks of four lines:
  - @ identifier line
  - Sequence
  - "+"
  - Quality scores (different encodings exist)

Example FASTQ entry

```
@SRR001666.1 071112_SLXA-EAS1_s_7:5:1:817:345 length=36
GGGTGATGGCCGCTGCCGATGGCGTCAAATCCCACC
+SRR001666.1 071112_SLXA-EAS1_s_7:5:1:817:345 length=36
IIIIIIIIIIIIIIIIIIIIIIIIIIIIII9IG9IC
```

Phred scores encoding

- https://wiki.bits.vib.be/index.php/Identify_the_Phred_scale_of_quality_scores_used_in_fastQ

Working with fastq files

```
## retrieving an example fasta file
curl  https://molb7621.github.io/workshop/_downloads/SP1.fq
 > file.fastq

## counting number of reads
awk 'END{print NR/4}' file.fastq

## transforming into fasta
awk 'NR%4==1{a=substr($0,2);} NR%4==2 {print ">"a"\n"$0}' \
  file.fastq
```

Using awk

- https://en.wikipedia.org/wiki/AWK
- http://bioinformatics.cvr.ac.uk/blog/essential-awk-commands-for-next-generation-sequence-ana
- Dissect why the previous awk commands worked

awk: Counting the number of items in a fastq

- `awk 'ENDprint NR/4' file.fastq`
- NR gives the number of records (line numbers)
- FASTQ are chunks of 4 lines for each sequence
- NR/4 at the END of the file indicates the number of sequences

Working with fastq files

```
## retrieving an example fasta file
curl  https://molb7621.github.io/workshop/_downloads/SP1.fq
 > file.fastq

## counting number of reads
awk 'END{print NR/4}' file.fastq

## transforming into fasta
awk 'NR%4==1{a=substr($0,2);} NR%4==2 {print ">"a"\n"$0}' \
  file.fastq
```

awk: fastq to fasta

```
awk 'NR%4==1{a=substr($0,2);} NR%4==2 {print ">"a"\n"$0}'
file.fastq
```

- % is a modulo operator
- NR%4==1 will retrieve the first line of a fastq chunk (header)
- NR%4==2 will retrieve the second line (the sequence)
- the id line will be preprended with the > and reduced to a substring (chopped)
- This will be applied to all the lines!

In class exercises for fastq

- Activity: FASTQ/A exercises (exercise 5 on)

Alignment file format

- SAM - Sequence Alignment Map.
- Standard format for sequence data
- Recognised by majority of software and browsers: standard

What is an alignment?

- Sequence alignment: arrange a set of sequences to identify regions of similarity/identity
- Mapping short reads against a reference genome: aligning large amounts short reads to a reference genome

Local alignments vs global alignment

(a) Global alignment

(b) Local alignment

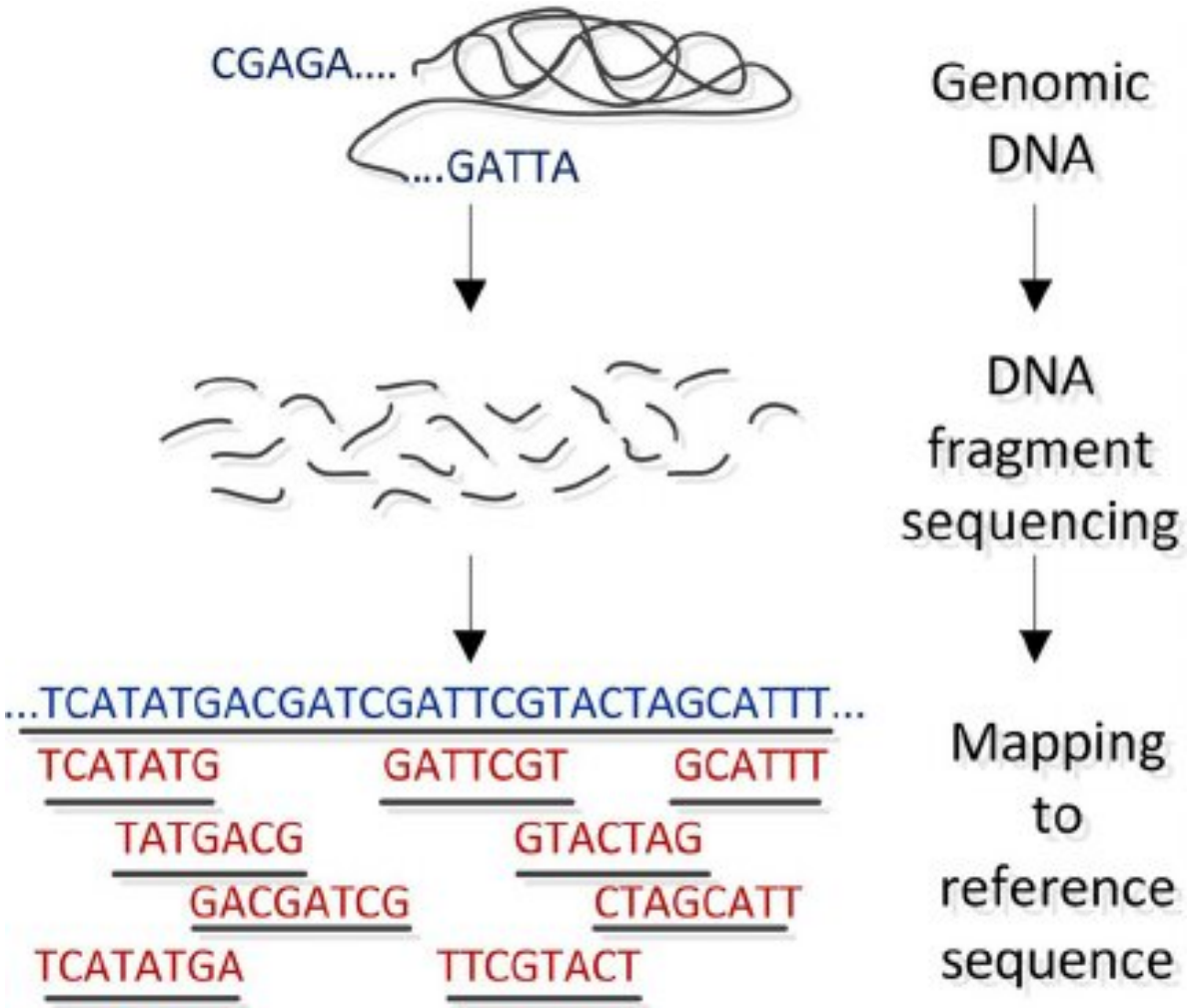
(c) Semi-global alignment

Alachiotis et al, 2013

SAM format

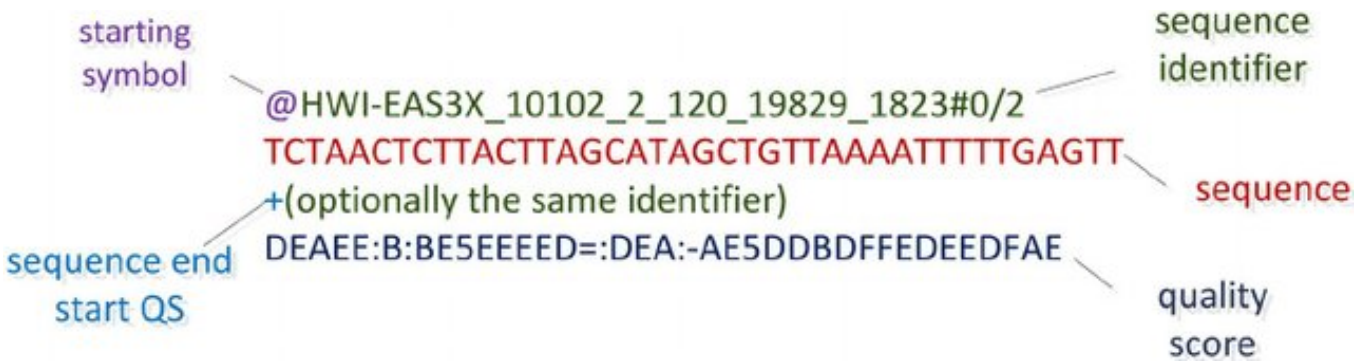
- Chromosome
- Locus (coordinate)
- CIGAR string, i.e.
- 30M1D2M - 30 bases continuously match, 1 deletion from reference, 2 base match
- Flags (https://broadinstitute.github.io/picard/explain-flags.html)

Next generation sequencing to genomic variation



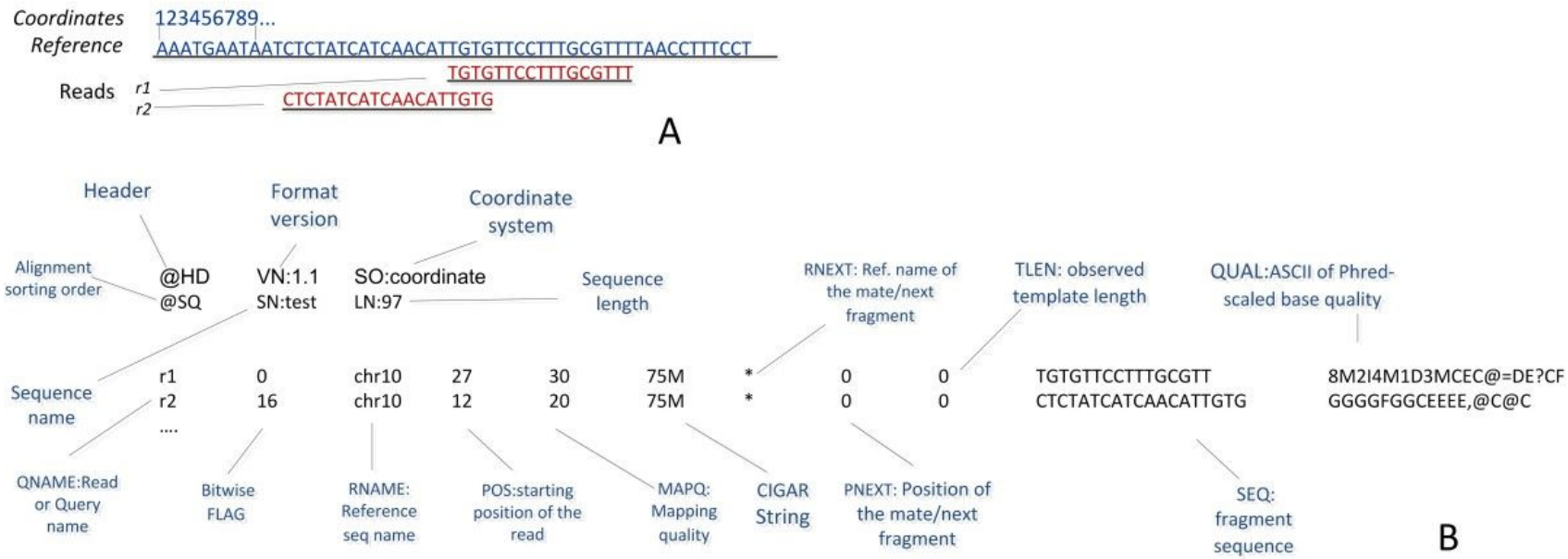
Pavlopoulos et al 2013

Next generation sequencing to genomic variation



Pavlopoulos et al 2013

Next generation sequencing to genomic variation



Pavlopoulos et al 2013

SAM format

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2723002/

Parsing SAM files

```
curl -L curl https://github.com/samtools/samtools/raw/develop/ex
  > ex1.sam.gz

gunzip ex1.sam.gz
head ex1.sam
```

From SAM to BED: counts

- Read https://bedtools.readthedocs.io/en/latest/content/tools/genomecov.html

Keep it simple: count and transform into BED files

- BED (Browser Extensible Data)
- BED3: 3 tab separated columns, chromosome (scaffold), start, end
- BED6: BED3 plus name, score, strand

BED3

```
chr22 1000 5000
chr22 2000 6000
```

BED6

```
chr22 1000 5000 cloneA 960 +
chr22 2000 6000 cloneB 900 -
```

BED12

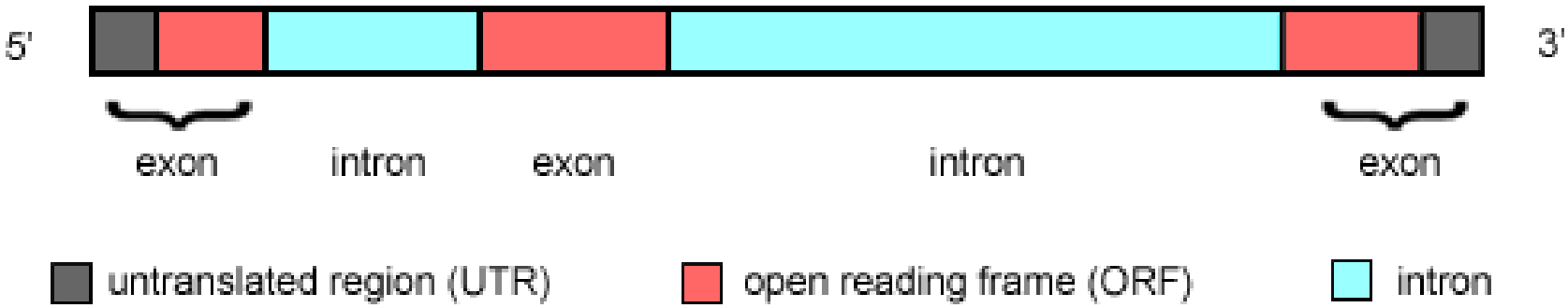
```
chr22 1000 5000 cloneA 960 + 1000 5000 0 2 567,488, 0,3512
chr22 2000 6000 cloneB 900 - 2000 6000 0 2 433,399, 0,3601
```

BEDtools

- Which fraction of the human genome is covered by exons?
- http://pedagogix-tagc.univ-mrs.fr/courses/jgb53d-bd-prog/practicals/03_bedtools/

What is genomic annotation?

- Adding layers to genomic coordinates



Henrik Lantz, BILS/SciLifeLab

What is genomic annotation?

Seqid	source	type	start	end	score	strand	phase	attributes
Chr1	Snap	gene	234	3657	.	+	.	ID=gene1; Name=Snap1;
Chr1	Snap	mRNA	234	3657	.	+	.	ID=gene1.m1; Parent=gene1;
Chr1	Snap	exon	234	1543	.	+	.	ID=gene1.m1.exon1; Parent=gene1.m1;
Chr1	Snap	CDS	577	1543	.	+	0	ID=gene1.m1.CDS; Parent=gene1.m1;
Chr1	Snap	exon	1822	2674	.	+	.	ID=gene1.m1.exon2; Parent=gene1.m1;
Chr1	Snap	CDS	1822	2674	.	+	2	ID=gene1.m1.CDS; Parent=gene1.m1;
		start_codon						Alias, note, ontology_term ...
		stop_codon						

Henrik Lantz, BILS/SciLifeLab

What is genomic annotation?

Seqid	source	type	start	end	score	strand	phase	attributes
Chr1	Snap	exon	234	1543	.	+	.	gene_id "gene1"; transcript_id "transcript1";
Chr1	Snap	CDS	577	1543	.	+	0	gene_id "gene1"; transcript_id "transcript1";
Chr1	Snap	exon	1822	2674	.	+	.	gene_id "gene1"; transcript_id "transcript1";
Chr1	Snap	CDS	1822	2674	.	+	2	gene_id "gene1"; transcript_id "transcript1";
		start_codon						
		stop_codon						

Henrik Lantz, BILS/SciLifeLab

Open reading frames

Steven M. Carr

GTF

- http://mblab.wustl.edu/GTF22.html

BEDgraph

```
chromA   chromStartA   chromEndA   dataValueA
chromB   chromStartB   chromEndB   dataValueB

chr19 49303800 49304100 0.50
chr19 49304100 49304400 0.75
chr19 49304400 49304700 1.00
```

BEDgraphs

- To display continuous-valued data in track format.
- Uuseful for probability scores

Which are the differences with BED?

- BED, BED, BED12?
- Advantages: the coordinates are specified, so sparsity is allowed

Wig files

- To display continuos-value data
- GC percent, probability scores, and transcriptome data.
- Data is not sparse! Wiggle data elements must be equally sized (step)

```
variableStep chrom=chr2
300701 12.5
300702 12.5
300703 12.5
300704 12.5
300705 12.5
```

Wig with added span

```
variableStep chrom=chr2 span=5
300701 12.5
```

Wig with fixedStep and span

```
fixedStep chrom=chr3 start=400601 step=100 span=5
11
22
33
```

VCF

- Standard file format for storing variation data
- Unambiguous, scalable and flexible
- 8 columns:
  1. CHROM
  2. POS
  3. ID
  4. REF
  5. ALT
  6. QUAL
  7. FILTER
  8. INFO

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	NA19909
11	5248232	rs334	T	A	100	PASS	AA=T|||;AC=1;AF=0.0273562;AFR_AF=0.0998;AMR_AF=0.0072;AN=2;DP=22876;EAS_AF=0;EUR_AF=0;EX_TARGET;NS=2504;SAS_AF=0;VT=SNP	GT	0|1

EMBL/EBI training

Quality values: which one?

- Phred-scaled quality score for the assertion made in ALT. i.e. $Q = -10 \log_{10} P$ being P(call in ALT is wrong)
- Read quality
- Mapping quality
- Variant calling quality

Variant calling

- Lecture by Michael Lawrence (VariantExplore package)
- https://www.bioconductor.org/help/course-materials/2014/CSAMA2014/3_Wednesday/lectures/VariantCallingLecture.pdf