

NGS - quality control, alignment, visualisation

File types

File types

fasta	sequences
fastq	reads
sam/bam	alignments
bed	regions
gff	annotations
vcf	variants

fasta

- Plain sequence: *.fasta or *.fa
- Nucleotides or amino acids (proteins)
- Useful command:

```
grep -c "^>" sequence.fasta
```

```
sequence.fasta
```

```
>sequence title1
```

```
ATCGTATCT
```

```
>sequence title2
```

```
ATGATGACGT
```

fastq

reads.fastq

```
@D00283R:66:CC611ANXX:4:2311:2596:2330 1:N:0:TCCGGAG  
ACTCTACGCTCAATAAAGATTTCTGATACGGCTCCTGAAATGCAGAATGAGT  
+  
B/<<<B<FFFFFFFFFBBFFFBFFFBFFFF/FFFFFFFF/BFFFBFFF
```

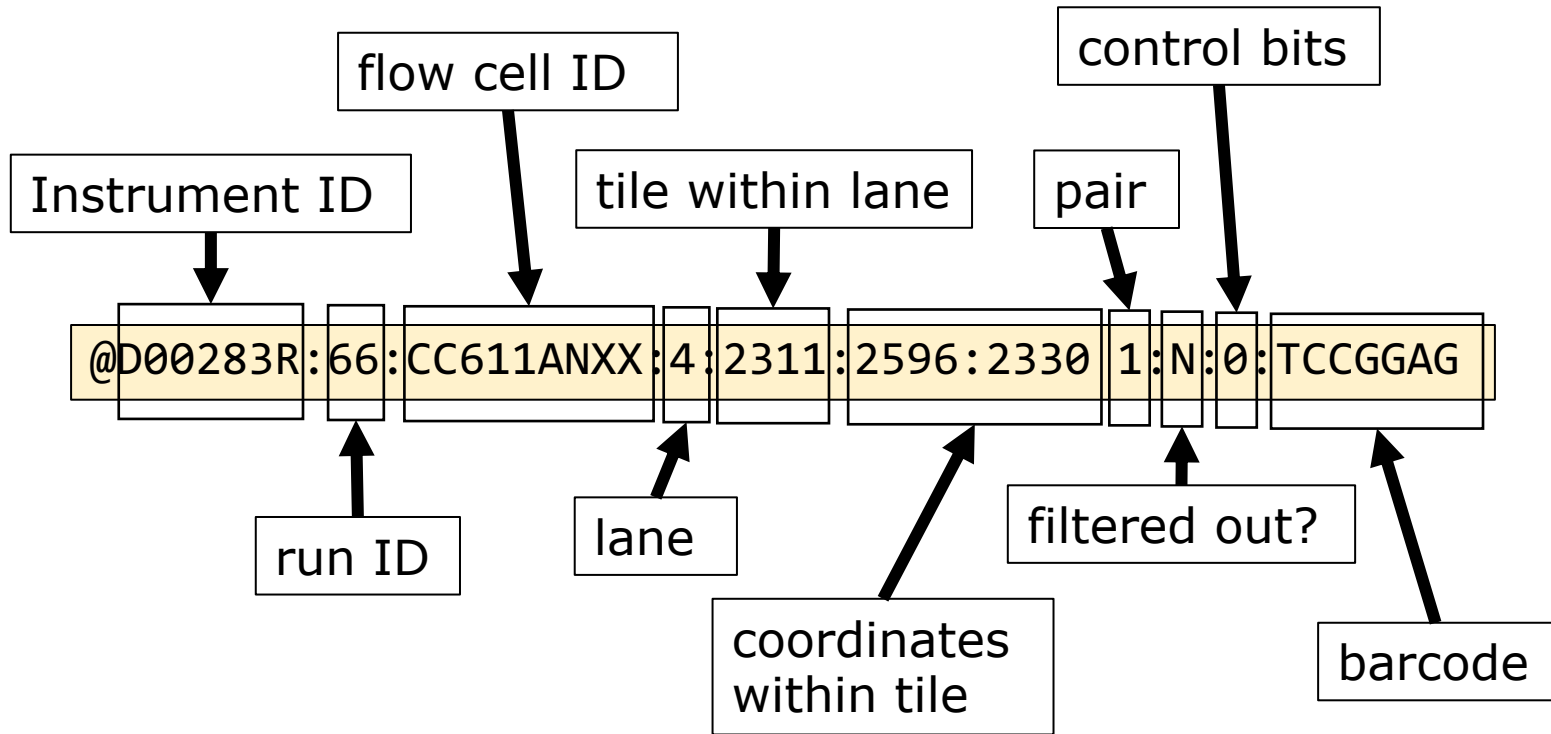
title, starts with @

nucleotide sequence

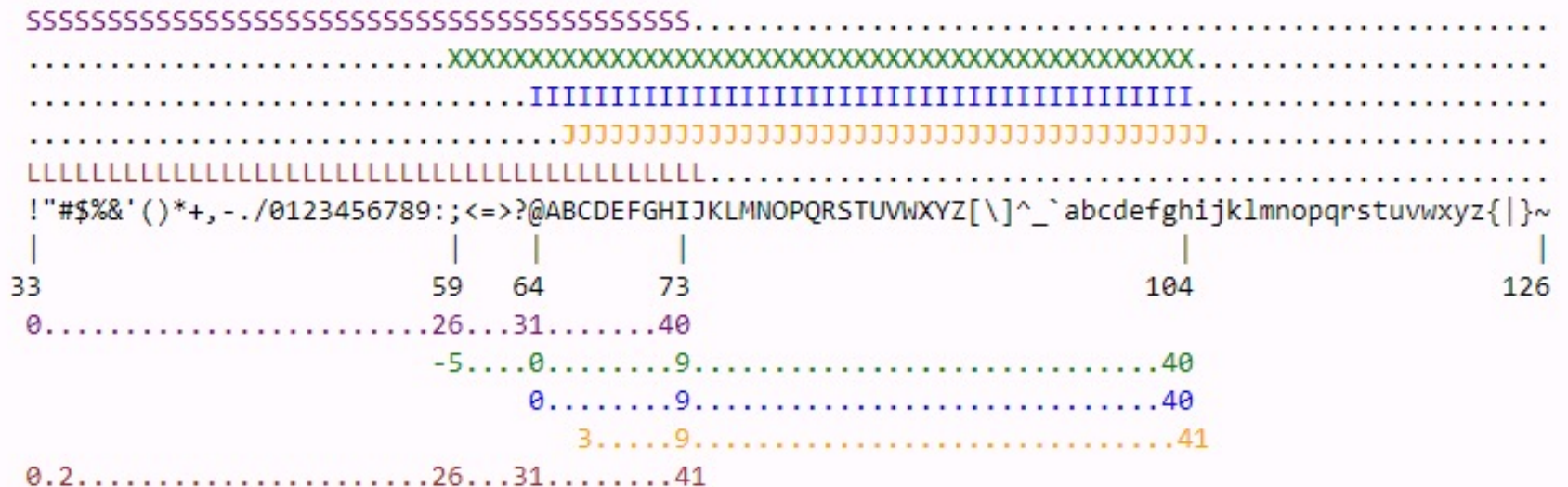
optional description

base quality

fastq header (Illumina)



Base quality (phred)



- 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, 41)
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)

Quiz Question 5

Sam

sequence alignment format

Aim: alignments

sam header

```
@HD      VN:1.0  SO:coordinate
@SQ      SN:U00096.3      LN:4641652
@PG      ID:bowtie2      PN:bowtie2      VN:2.4.1
CL: "/opt/miniconda3/envs/ngs/bin/bowtie2-align-s \
--wrapper basic-0 \
-x /home/ubuntu/ecoli/ref_genome//ecoli-strK12-MG1655.fasta \
-1 /home/ubuntu/ecoli/trimmed_data/paired_trimmed_SRR519926_1.fastq \
-2 / home/ubuntu/ecoli/trimmed_data/paired_trimmed_SRR519926_2.fastq"
```

SAM column	example
read name	SRR519926.5
flag	89
reference	U00096.3
start position	61
mapping quality	42
CIGAR string	214M
reference name mate is mapped	=
start position mate	476
fragment length	515
sequence	CATCACCATTCCCAC
base quality	@>4:4C@89+&9CC@
optional	AS:i:-2
optional	XN:i:0

Quiz Question 6

sam flags

Bit	Description
1	0x1 template having multiple segments in sequencing
2	0x2 each segment properly aligned according to the aligner
4	0x4 segment unmapped
8	0x8 next segment in the template unmapped
16	0x10 SEQ being reverse complemented
32	0x20 SEQ of the next segment in the template being reverse complemented
64	0x40 the first segment in the template
128	0x80 the last segment in the template
256	0x100 secondary alignment
512	0x200 not passing filters, such as platform/vendor quality controls
1024	0x400 PCR or optical duplicate
2048	0x800 supplementary alignment

	read paired?	properly aligned?	unmapped?	next unmapped?	flag
read1	0	1	0	0	2
read2	1	0	1	1	13
read3	1	1	0	1	11

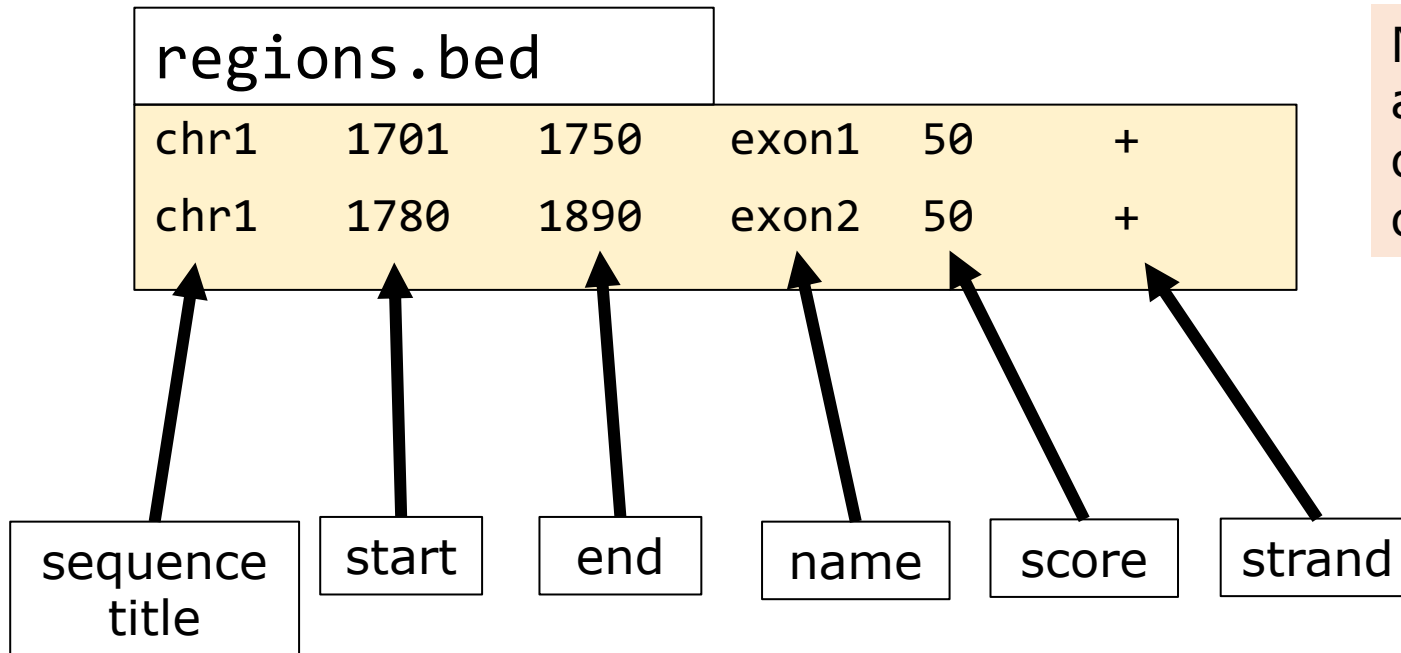
1	2	4	8
---	---	---	---

Quiz Question 7

bed

Browser Extensible Data

Aim: specify regions



Numbering starts at 0!
chr1:1702-1750
chr1:1781-1890

gff

General Feature Format

Aim: annotation

seq name	source	feature	start	end	score	strand	frame	attributes
1	ensembl	mRNA	339070	346959	.	-	.	ID=...;
1	ensembl	exon	339070	339312	.	-	.	Parent=; ...
1	ensembl	CDS	339070	339312	.	-	0	ID=;...

vcf

Variant Call Format

Aim: variants

```
##fileformat=VCFv4.2
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##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">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT NA00001 NA00002 NA00003
20 14370 rs6054257 G A 29 PASS NS=3;DP=14;AF=0.5;DB;H2 GT:GQ:DP:HQ 0|0:48:1:51,51 1|0:48:8:51,51 1/1:43:5:.,.
20 17330 . T A 3 q10 NS=3;DP=11;AF=0.017 GT:GQ:DP:HQ 0|0:49:3:58,50 0|1:3:5:65,3 0/0:41:3
20 1110696 rs6040355 A G,T 67 PASS NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27 2|1:2:0:18,2 2/2:35:4
20 1230237 . T . 47 PASS NS=3;DP=13;AA=T GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 0/0:61:2
20 1234567 microsat1 GTC G,GTCT 50 PASS NS=3;DP=9;AA=G GT:GQ:DP 0/1:35:4 0/2:17:2 1/1:40:3
```


vcf

```
##fileformat=VCFv4.3
##fileDate=20090805
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##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
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##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
```

```
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```

```
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##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
```

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	NA00001	NA00002
20	14370	rs6054257	G	A	29	PASS	NS=3;DP=14;AF=0.5;DB;H2	GT:GQ:DP:HQ	0 0:48:1:51,51	1 0:48:8:51,51
20	17330	.	T	A	3	q10	NS=3;DP=11;AF=0.017	GT:GQ:DP:HQ	0 0:49:3:58,50	0 1:3:5:65,3
20	1110696	rs6040355	A	G,T	67	PASS	NS=2;DP=10;AF=0.333,0.667;AA=T;DB	GT:GQ:DP:HQ	1 2:21:6:23,27	2 1:2:0:18,2
20	1230237	.	T	.	47	PASS	NS=3;DP=13;AA=T	GT:GQ:DP:HQ	0 0:54:7:56,60	0 0:48:4:51,51
20	1234567	microsat1	GTC	G,GTCT	50	PASS	NS=3;DP=9;AA=G	GT:GQ:DP	0/1:35:4	0/2:17:2

samples