Genome assembly and annotation

Day 2: Read trimming

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Slides modified from Igor Pessi, with permission

Aims for this part of MMB-114

Day 1: Basics of UNIX and working with the command line

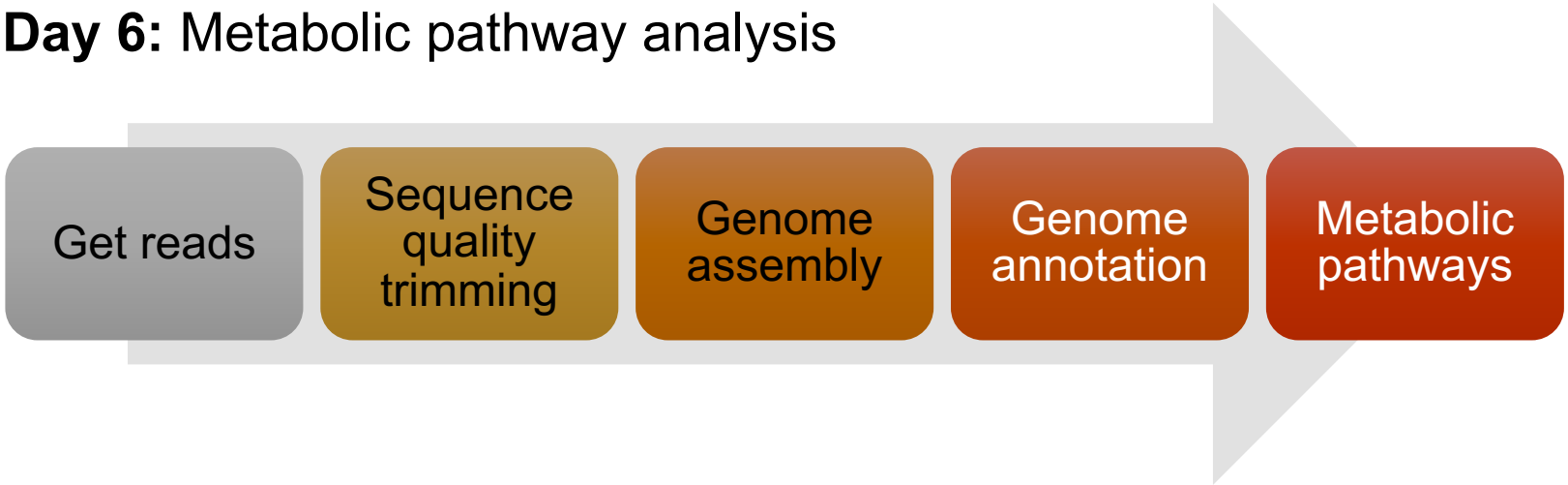
Day 2: Handling of Illumina data

Day 3: Genome assembly

Day 4: Check-up and report

Day 5: Genome annotation

Day 6: Metabolic pathway analysis



Before we start…

Let’s go through the exercise from last week together

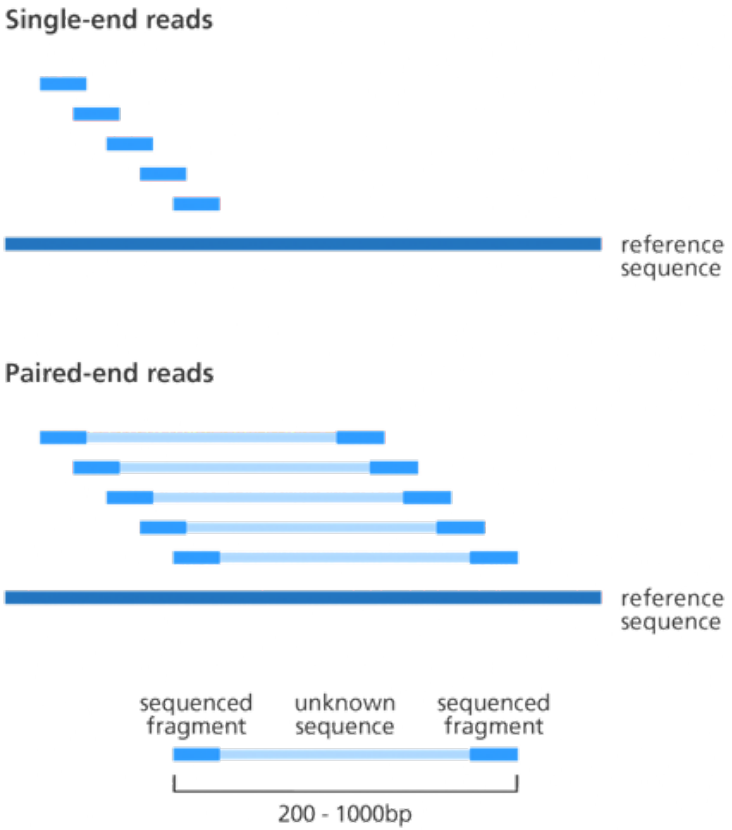
https://github.com/karkman/MMB-114_Genomics

(**Day 1:** UNIX and CSC)

Raw data

We have received paired-end data for one strain:

What does paired-end data mean?



FASTQ's anatomy

```
@M02764:119:000000000-C5R9K:1:1101:15139:1363 1:N:0:24
GCTAACCCCATTGCAACGTGGTAACTTGTTAGACCGTTTTTAAAAGTCGCTGAAGCAGCCACGATAAACGACATCCCGATTGAACCCCCAGGACCATGTTG
ACCACGCCCGAAGCTGCACCGGCATCTGCAGGTAACGTATTGGCAACACCGGCGACCGTTAATGGACTGAGTGTTAAACCTTGTCCAATCTCCATCAGGAT
CATGGGAACCTCAATACCTAGCGCATAGCCCACTTGTCCCGAGAAAAAGCCTAAGGCACCCACCCCAATTGCGGTGGTTGCAATCCCCACGATTAGTAATT
TCTGTTTCCAAAAGCGCTTTGTT
+
CCCCCEFGGGGGFCGGGGGGGGGGGFCCDFGGFFCCCFCC8EFF@FFCCECC@F<FGFGGGGGG7B7<C7:FFEE@EGGDGCEFFFCBFG7D,CFC,EEEC
GG<FG>@<F=EG=7<<EB8:+8:@@FDDFGGG8FDECF8FFFEFGGCEGDD:7++@FGDEE>EG9AFBCC<>>FCCFB,:8?FG;;B,?8,6B;BC@C6E6
823B9C?+8C**0><++++1+6***=C:E+*:7:?7+*395CD)92*9<FFFCFFFC<FD;))1>55)*/29@)<))00)7*1*)09))7))54-
=8*9.9/5*-)-*).))..3:(((43).(
```

ASCII_BASE-33 Illumina, Ion Torrent, PacBio and Sanger

Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII
0	1.00000	33 !	11	0.07943	44 ,	22	0.00631	55 7	33	0.00050	66 B
1	0.79433	34 "	12	0.06310	45 -	23	0.00501	56 8	34	0.00040	67 C
2	0.63096	35 #	13	0.05012	46 .	24	0.00398	57 9	35	0.00032	68 D
3	0.50119	36 $	14	0.03981	47 /	25	0.00316	58 :	36	0.00025	69 E
4	0.39811	37 %	15	0.03162	48 0	26	0.00251	59 ;	37	0.00020	70 F
5	0.31623	38 &	16	0.02512	49 1	27	0.00200	60 <	38	0.00016	71 G
6	0.25119	39 '	17	0.01995	50 2	28	0.00158	61 =	39	0.00013	72 H
7	0.19953	40 (	18	0.01585	51 3	29	0.00126	62 >	40	0.00010	73 I
8	0.15849	41)	19	0.01259	52 4	30	0.00100	63 ?	41	0.00008	74 J
9	0.12589	42 *	20	0.01000	53 5	31	0.00079	64 @	42	0.00006	75 K
10	0.10000	43 +	21	0.00794	54 6	32	0.00063	65 A			

Phred quality score (Q score)

Indicates the probability that a given base is called correctly by the sequencer

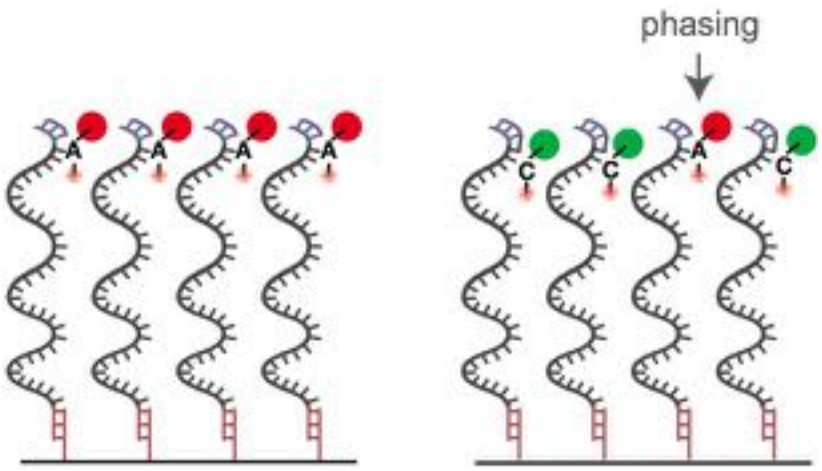
Defined logarithmically to the base calling error probability

- $Q = -10 \log_{10} p$

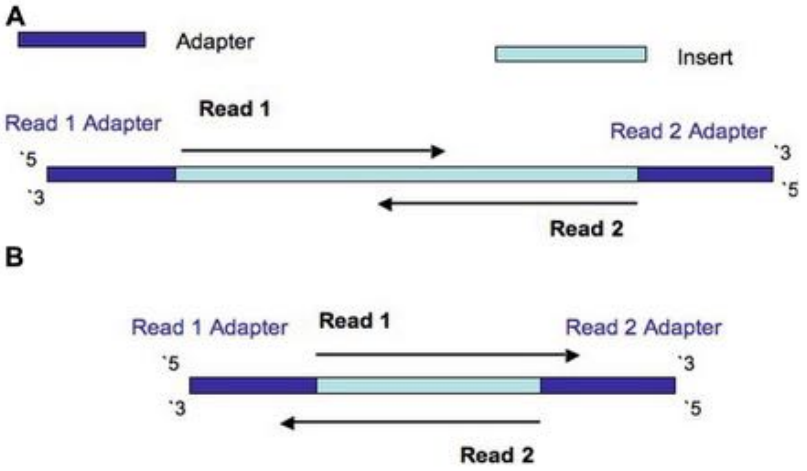
Phred Quality Score	Probability of incorrect base call	Base call accuracy	ASCII
10	1 in 10	90%	+
20	1 in 100	99%	5
30	1 in 1,000	99.9%	?
40	1 in 10,000	99.99%	I

Quality filtering and adapter removal

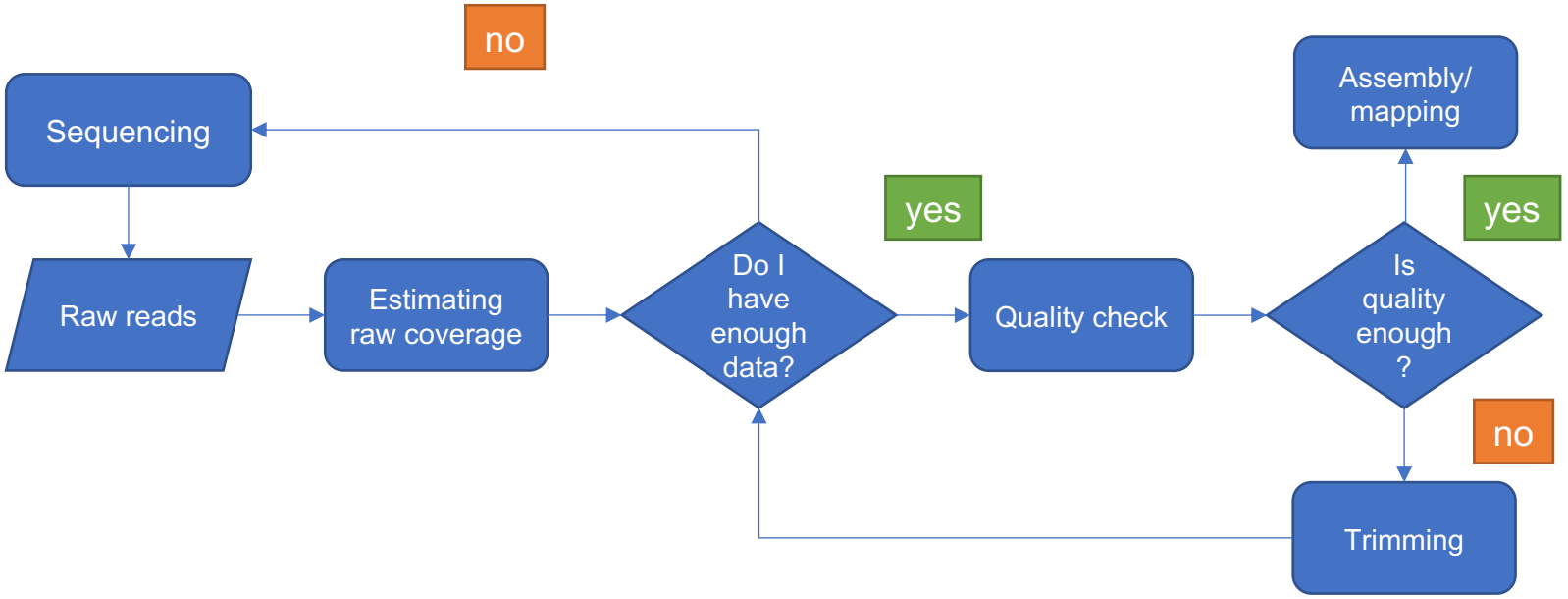
Phasing



Adapter read-through



Read quality assessment



Additional basic UNIX commands

Compressing/decompressing

tar

gunzip

Visualization

head

tail

less

Operations

Piping (|)

Redirection (>)

Remember:

Commands have to be typed in a single line, one at a time

- Backslash (\ )

Whenever possible, type everything, don't copy and paste

- Tabulator!

Additional notes about Puhti

Interactive partition

`sinteractive`

Max. rrunning time: 7 days (default 24 h)

Computing capacity up to 8 cores (default 1)

Memory capacity of up to 76 GB (default 2 GB)

The module system

`module load biokit`

Convenient way to manage applications

FASTQC

http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Quality assessment of FASTQ files

The output of FASTQC is one ZIP and one HTML file

We will look at the HTML file in a web browser.

Need to download it to your own computer

CUTADAPT

https://cutadapt.readthedocs.io/en/stable/

Removal of low quality regions and adapters

- *“Rubbish in = Rubbish out”*

In addition to generating new files with the quality-filtered reads, CUTADAPT prints a log of the operation in the screen

- We want to store this info in a file so that we can check later if needed
- For that we will use “>” to redirect the output to a file

Time to take a look at our genome data

https://github.com/karkman/MMB-114_Genomics

(**Day 2:** Read trimming)