

Genomics

An introduction to genome sequencing, data processing, and analysis

#DatAlumni course series @ ZMT, July 6/7, 2021

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```
martin — haex1482@hpc1004:~/data/shared/Serranus — ssh haex1482@carl.hpc.uni-oldenburg.de — 1...
[~/data/shared/Serranus]> zcat < G09452-L1_S5_L001_R1_001.fastq.gz | head -n 4
@J00124:43:HM5WNBBXX:1:1101:22932:1173 1:N:0:GNTTCG
ACCCANGGGAGAACCTGCAAACCTCCATACAGAAGTGTCCGAGCNGGATTGAACTCACGACCCAGGNTCNGAACACNCNTGCNGTAAGGCATGAGTGCTAACAC
TNCGCCACNTGGAGGCCCTCAAACNGAACAGTTAGCANAAN
+
AAFFF#JJFJJJJJJJJJJJJAJAJJFFFJJF<A7FJJFJ<JJ#FFJJ7AF<FJJJ<JJFJJJJFJJ#FJ#JFFJJF#J#FFF#FJF7FJJAJJJJ-FJJJ<JFFJ
F#JJJFJJJ#JJJFFJJJ<FFFFJJ#JFFAJFJJFFJFJA#FJ#
[~/data/shared/Serranus]>
```

Workshop plan

Time	Block	Topics
Jul 06, 14:00–14:30	1. Background and introduction	<ul style="list-style-type: none">– Sequencing techniques and applications– Workflow overview– Planning a genome project
Jul 06, 14:30–16:00	2. Data pre-processing and QA/QC	<ul style="list-style-type: none">– Introduction to bioinformatics (exercise)– Raw sequencing data, trimming & filtering– Quality assurance / control
Jul 07, 14:00–15:00	3. Assembly and annotation	<ul style="list-style-type: none">– Genome assembly (exercise)– Genome re-sequencing– Genome annotation
Jul 06, 15:00–16:00	4. Data management	<ul style="list-style-type: none">– Documentation– Data organization– Publishing / sharing genome data

Objective: Convey basic knowledge of the steps required to sequence a genome, from project planning to data publication

Exercises

Required software

- git
- bash

Windows

- install git from here: <https://git-scm.com/download> (use all default settings)
follow instructions at: <https://www.computerhope.com/issues/ch001927.htm>
- bash comes with git, type “git bash” in Start menu to launch

macOS

- both should come preinstalled
- open Terminal (/Applications/Utilities/Terminal.app), type “git --version” to check
- If you receive an error message, install from here: <https://sourceforge.net/projects/git-osx-installer/>

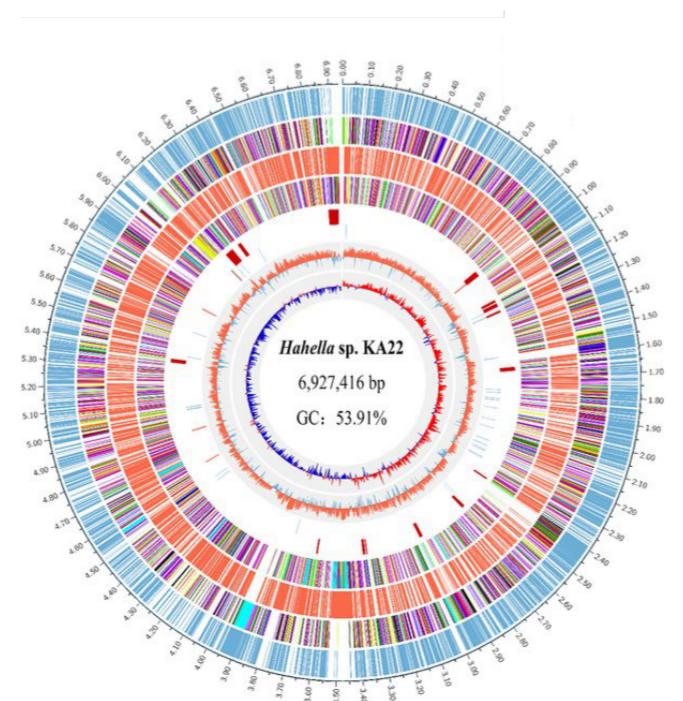
1. Background and introduction

- What is genomics?
- Sequencing technologies and applications
- Basic workflow
- Project planning

What is genomics and what can we do with it?

Understanding genomes

- Structure
- Function (e.g. gene prediction and interactions)
- Evolution (> comparative genomics)

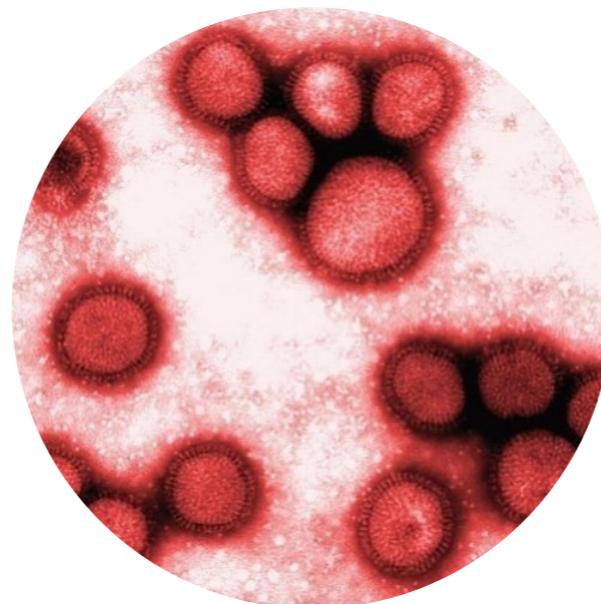


adapted from Feng et al. 2019, Marine Genomics

What is genomics and what can we do with it?

Studying phylogenetic relationships

- Reconstruct evolutionary history of organisms or genes
- Identify unknown organisms (e.g. new pathogens, metagenomics)
- How do species evolve?



What is genomics and what can we do with it?

Understanding the genetic basis of phenotypic traits

(e.g. GWAS, Genome-wide association studies)

- Diseases and risk factors
- Yield and resistance in domestic plants and animals



What is genomics and what can we do with it?

Population and conservation genomics

- Estimate population history and size
- Assess genetic diversity / identify adaptive loci
- Detect hybrids / effects of hybridization



Genome sequencing basics

Whole genome sequencing (WGS) strategies

- *de novo*
- re-sequencing (mapping)

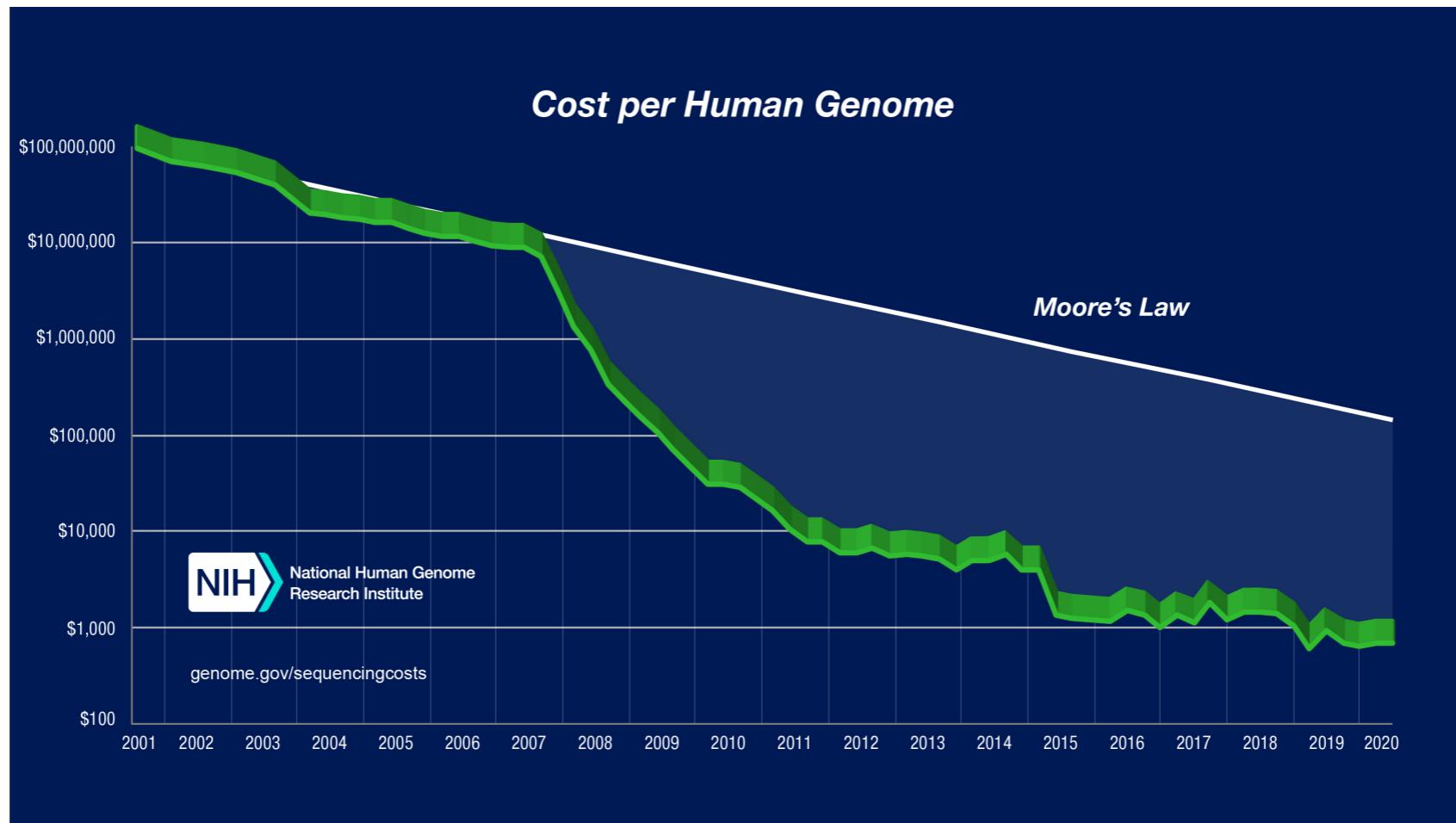
Shotgun principle /
Massive parallel sequencing



Many competing high-throughput methods

- short read sequencing: Illumina (next-gen)
- long read sequencing: PacBio, Nanopore (third gen)
- linked read sequencing: e.g. 10x Genomics

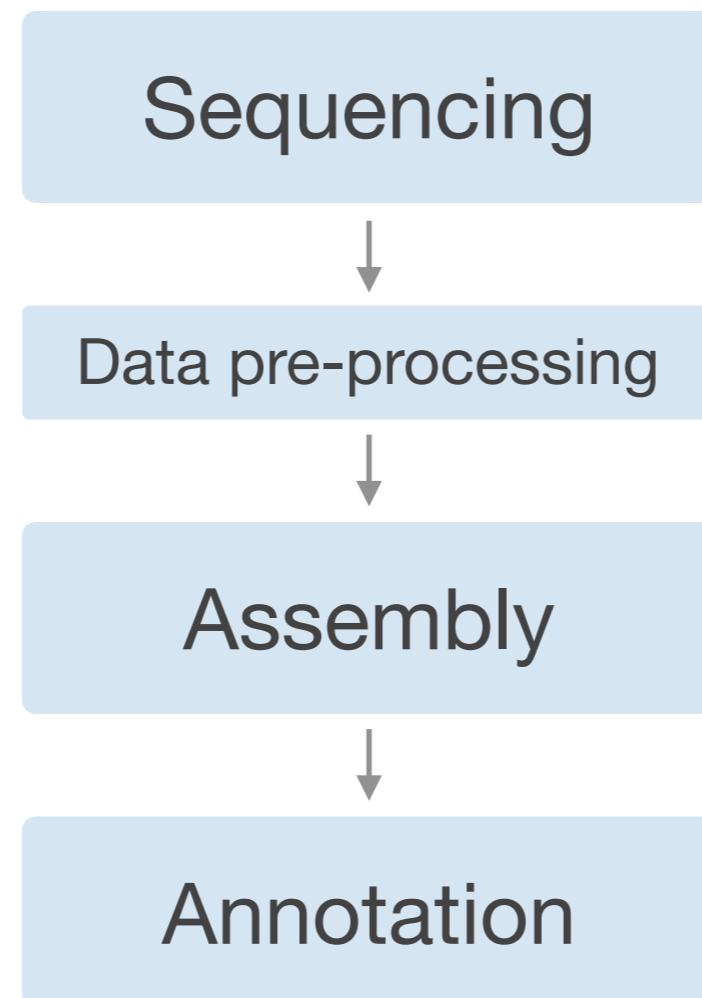
Genome sequencing costs



The bottleneck is not cost anymore, but knowing how to process the data

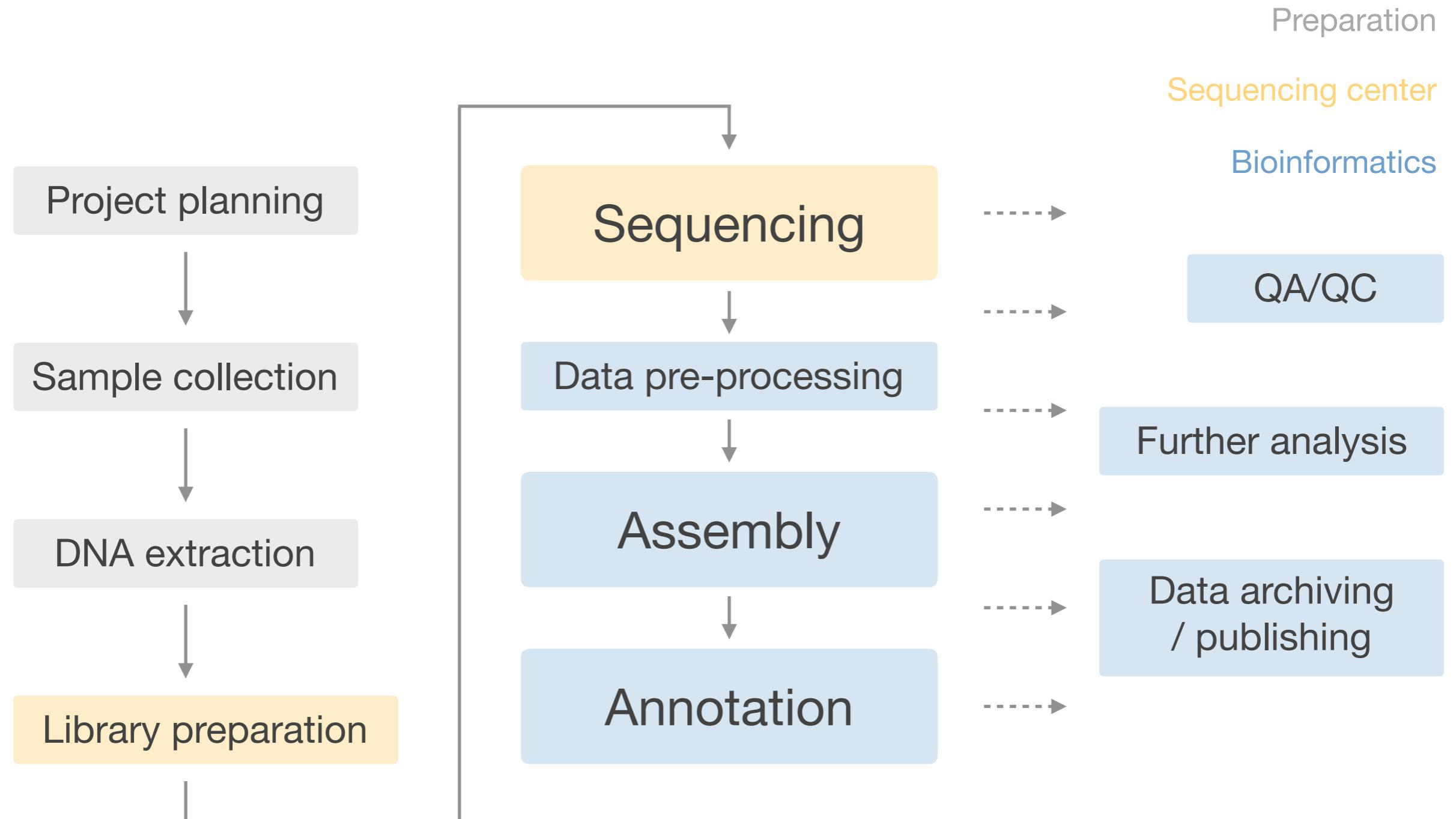
www.genome.gov/sequencingcostsdata, accessed Jul 5 2021

De novo genome sequencing workflow



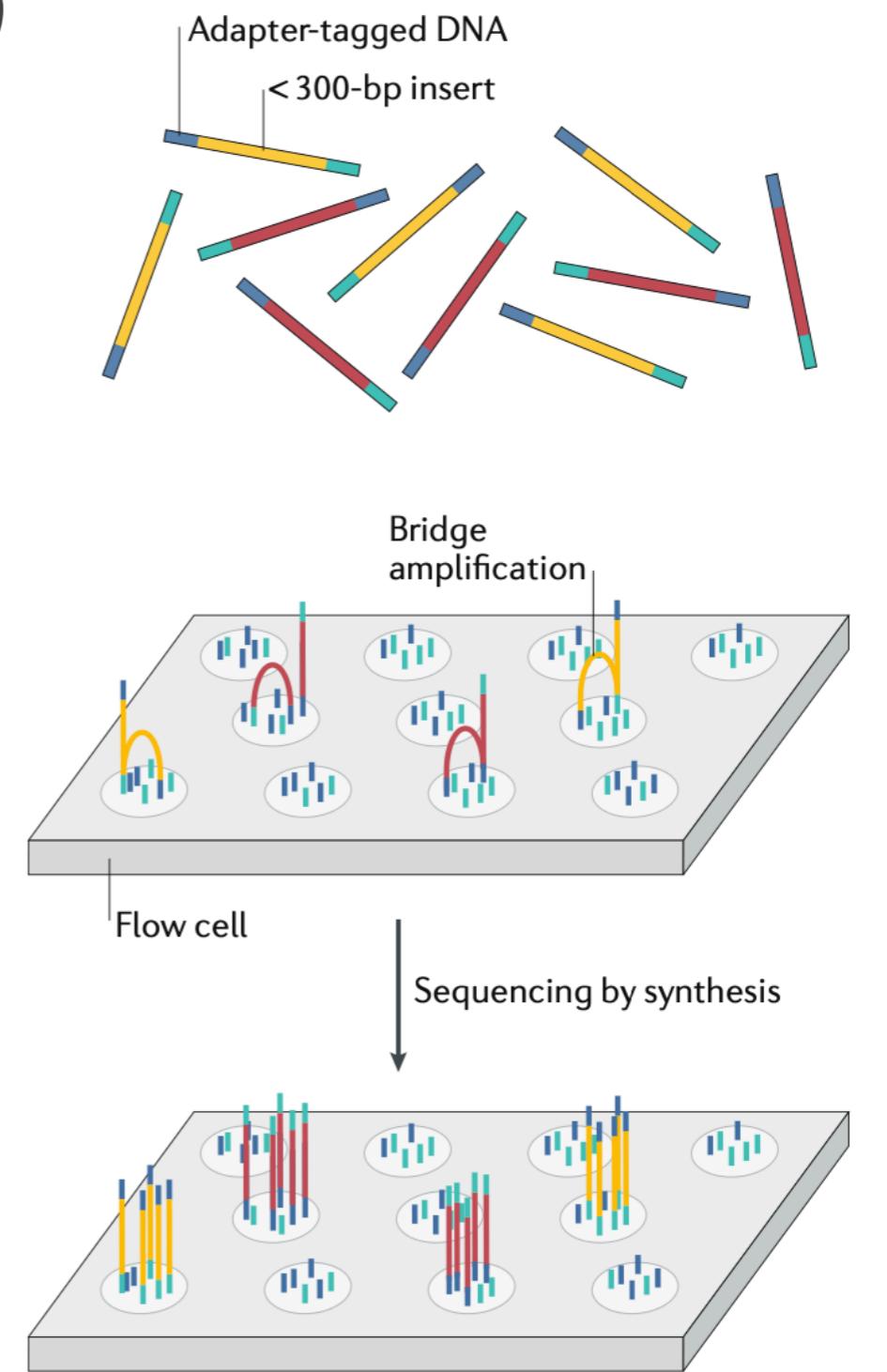
De novo genome sequencing workflow

Legend



Illumina sequencing (short reads)

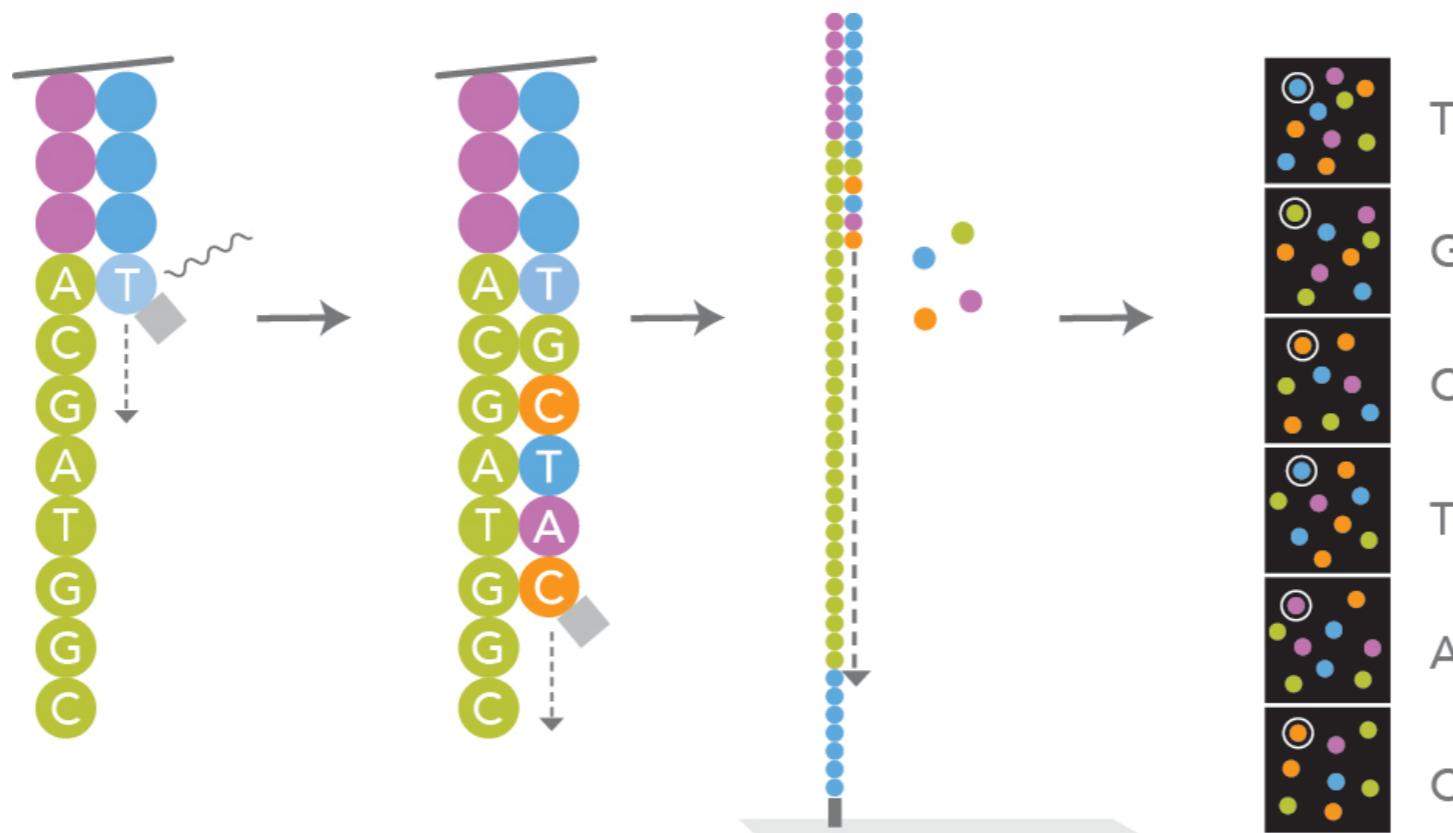
1. DNA fragmentation
2. Adapter ligation
3. Attachment to flow cell
4. Cluster generation (bridge PCR)
5. Sequencing by synthesis



Logsdon et al. 2020, Nature Reviews

Illumina sequencing (short reads)

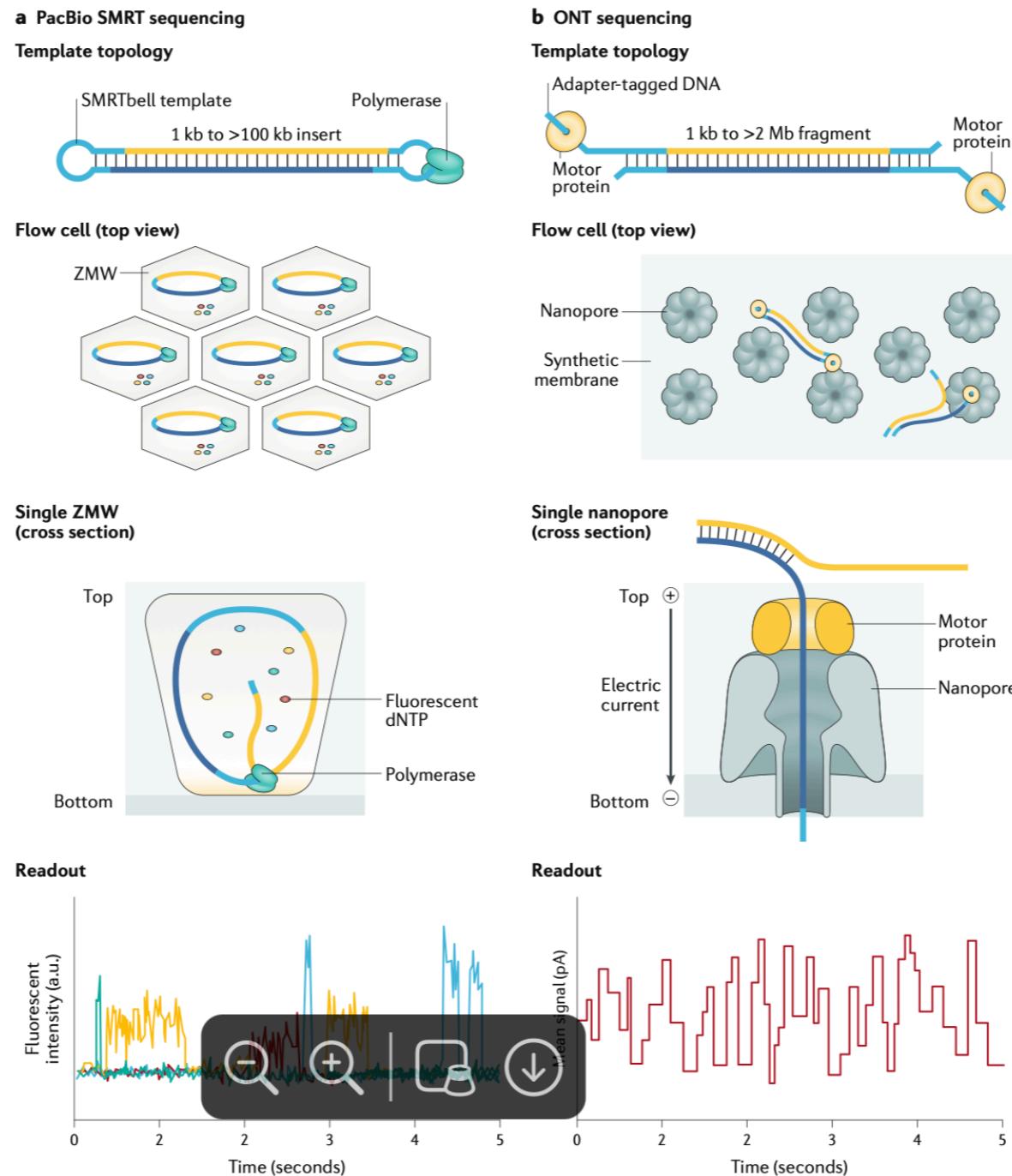
Sequencing by synthesis



Illumina, Inc.

Long read sequencing

PacBio
SMRT



Oxford
Nanopore
Technologies

Logsdon et al. 2020, Nature Reviews

Sequencing technologies compared

Technology	Typical read length	Accuracy	Gb per run	Cost per Gb	Devices
Illumina	50–250 bp	>99.9 %	15–3000	\$10–60	HiSeq, MiSeq, NovaSeq
PacBio	10–60 kb	87–99%	5–100	\$20–200	Sequel, Sequel II
Nanopore	10–200 kb	87–98%	1–100	\$20–2000	PromethION, MinION

Legend: bp = base pairs, kb = kilo bases (1000 bp), Mb = Mega bases (mill. bp), Gb = Giga bases (bill. bp)

Logsdon et al. 2020, Nature Reviews

Sequencing technologies compared



Illumina HiSeq 4000



Nanopore MinION

Illumina, Inc.

Oxford Nanopore Technologies

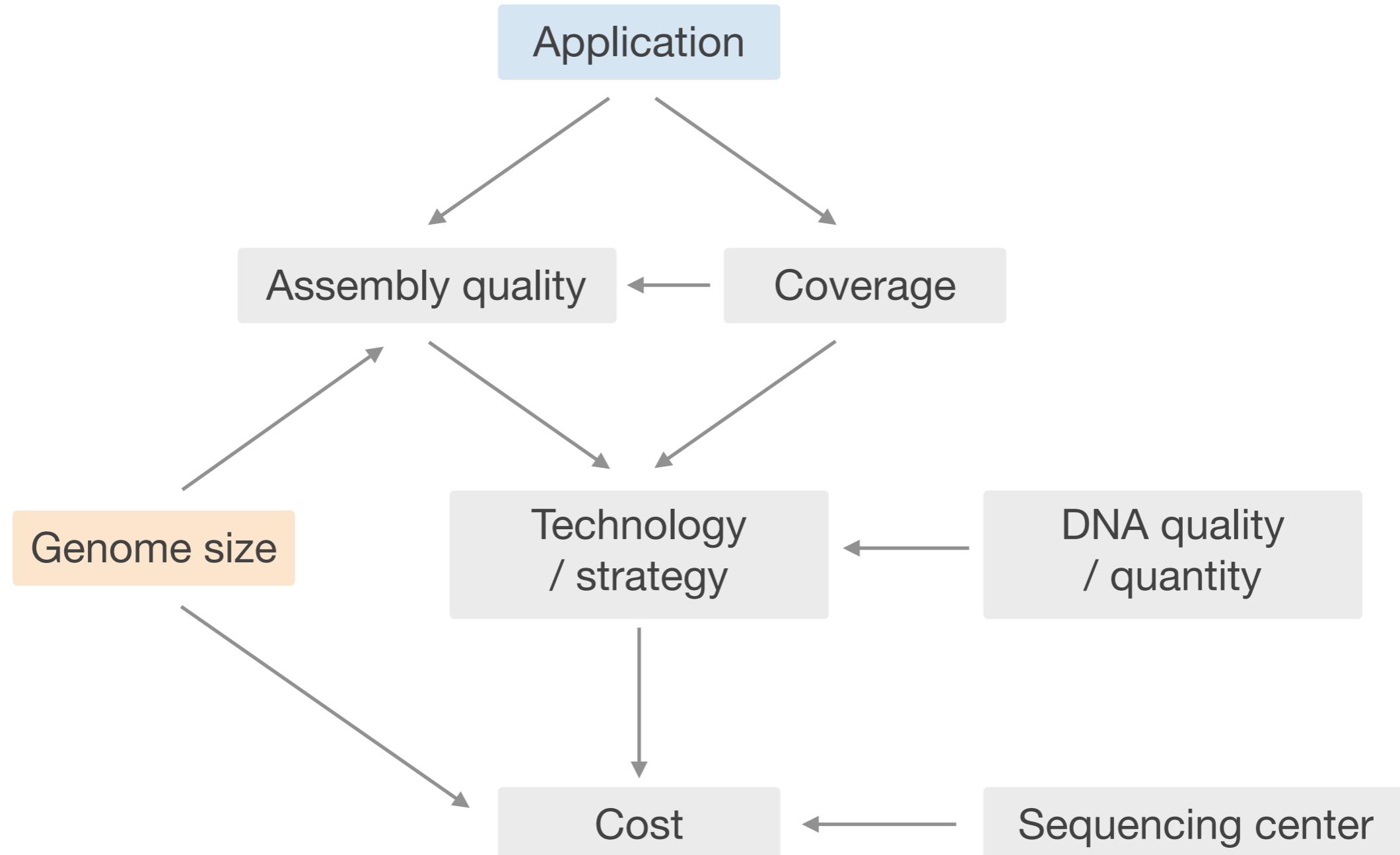
Coverage

- Average number of reads representing each position in the genome
- coverage or depth = **read count × read length / genome size**
- high coverage facilitates assembly, detection of sequencing errors
- Typical coverage: **50–100×** (or more) for *de novo* genome sequencing

10–30× for re-sequencing

```
Genome: CGTAATGGCATATGCCTAGATTGAAACG
Read 1: TAATGGCATATGCCTAGAT
Read 2: CATATGCCTAGATTGAAA
Read 3: TATGCCTAGATTGAAACG
Depth: 00111112233333333322222211
```

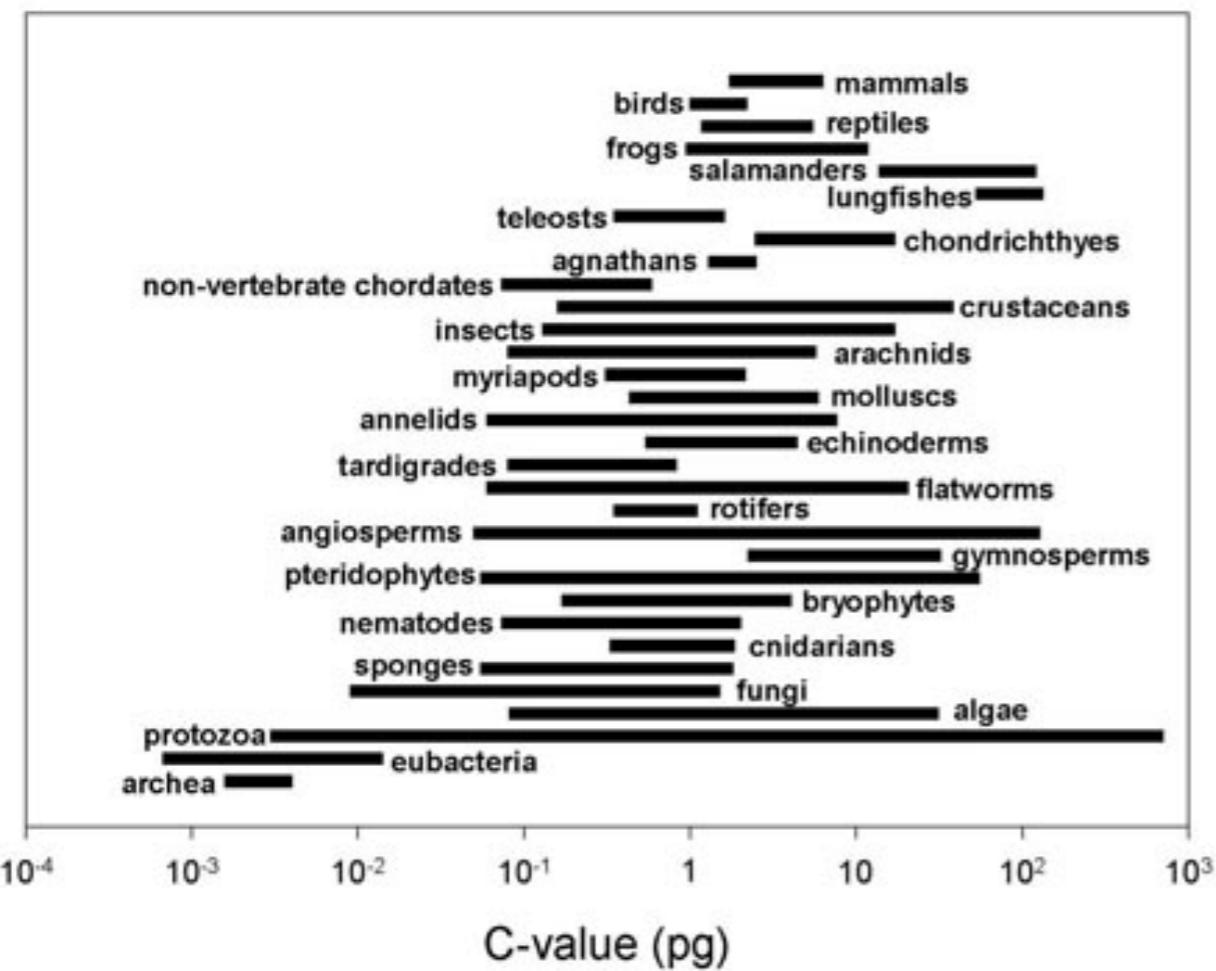
Planning your genome project



Genome size

How large is your genome?

Search Animal Genome Size Database: www.genomesize.com



1 pg ~ 1 Gb

Genome size is often correlated with repetitive DNA, which is difficult to sequence and assemble

Gregory 2021, Animal Genome Size Database

Sample collection

- Permits required?
- Suitable tissue / cell types vary by organism
(e.g. whole body, coral cores, blood, gills, fin clips)
- Store in **100% ethanol** or stabilizing solution (e.g. DNA Shield), ideally cold
- Do not freeze and thaw repeatedly
- Collect **metadata**

Metadata

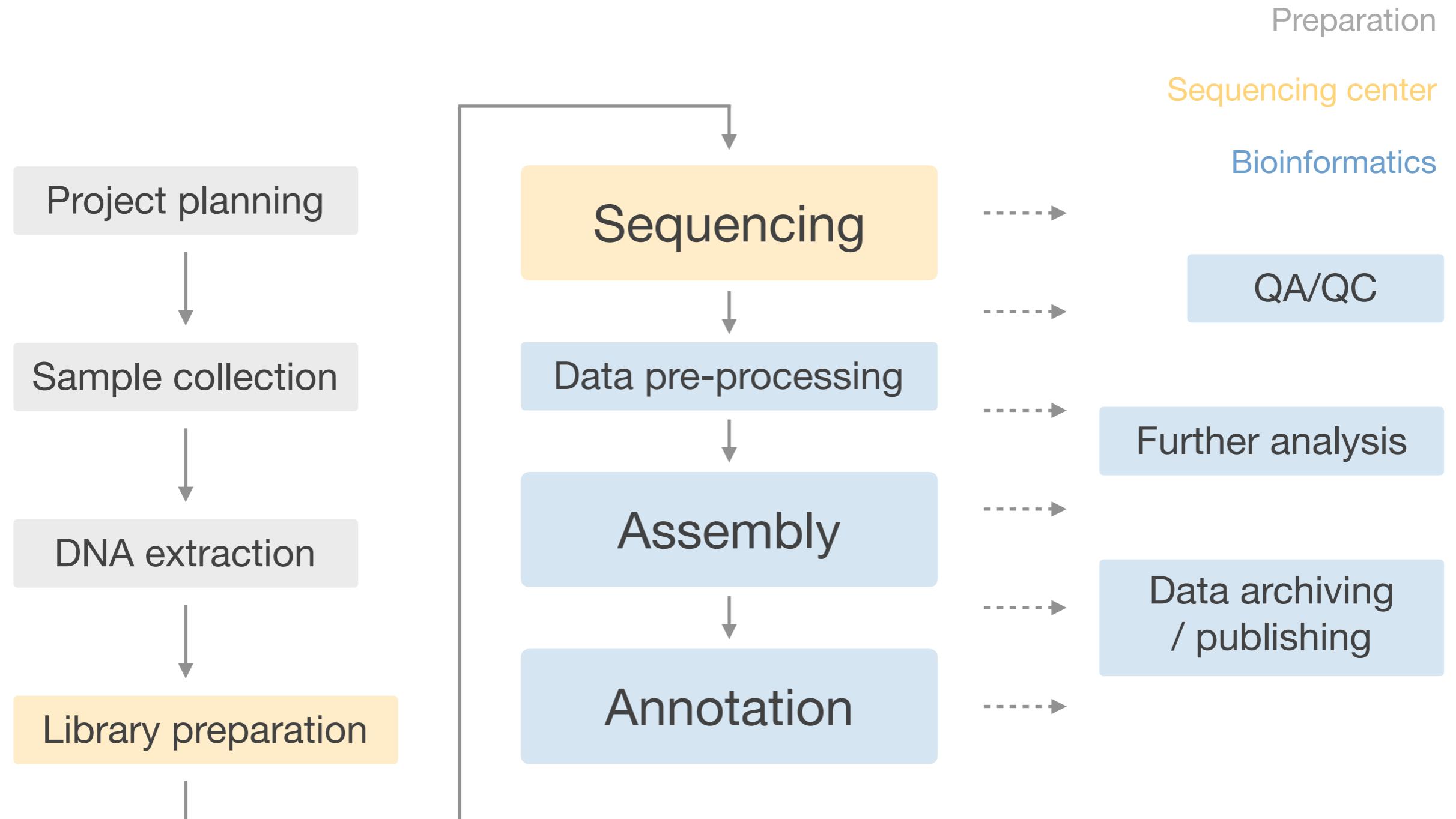
- Species / population
- Date and time of day
- Geographic coordinates (possibly depth, site name)
- Tissue type (possibly developmental stage)
- Person collecting
- Possibly ecological context (e.g. coral reef)
- Sampling method (e.g. snorkeling, core sample)
- Assign unique sample IDs if collecting population samples

DNA extraction

- Protocol depends on tissue and desired quality (perform test runs)
- Common methods include commercial kits and phenol:chloroform extraction
- Document DNA quality and concentration
- Store and ship DNA cold if possible
- Do not freeze and thaw repeatedly

De novo genome sequencing workflow

Legend



Library preparation and sequencing

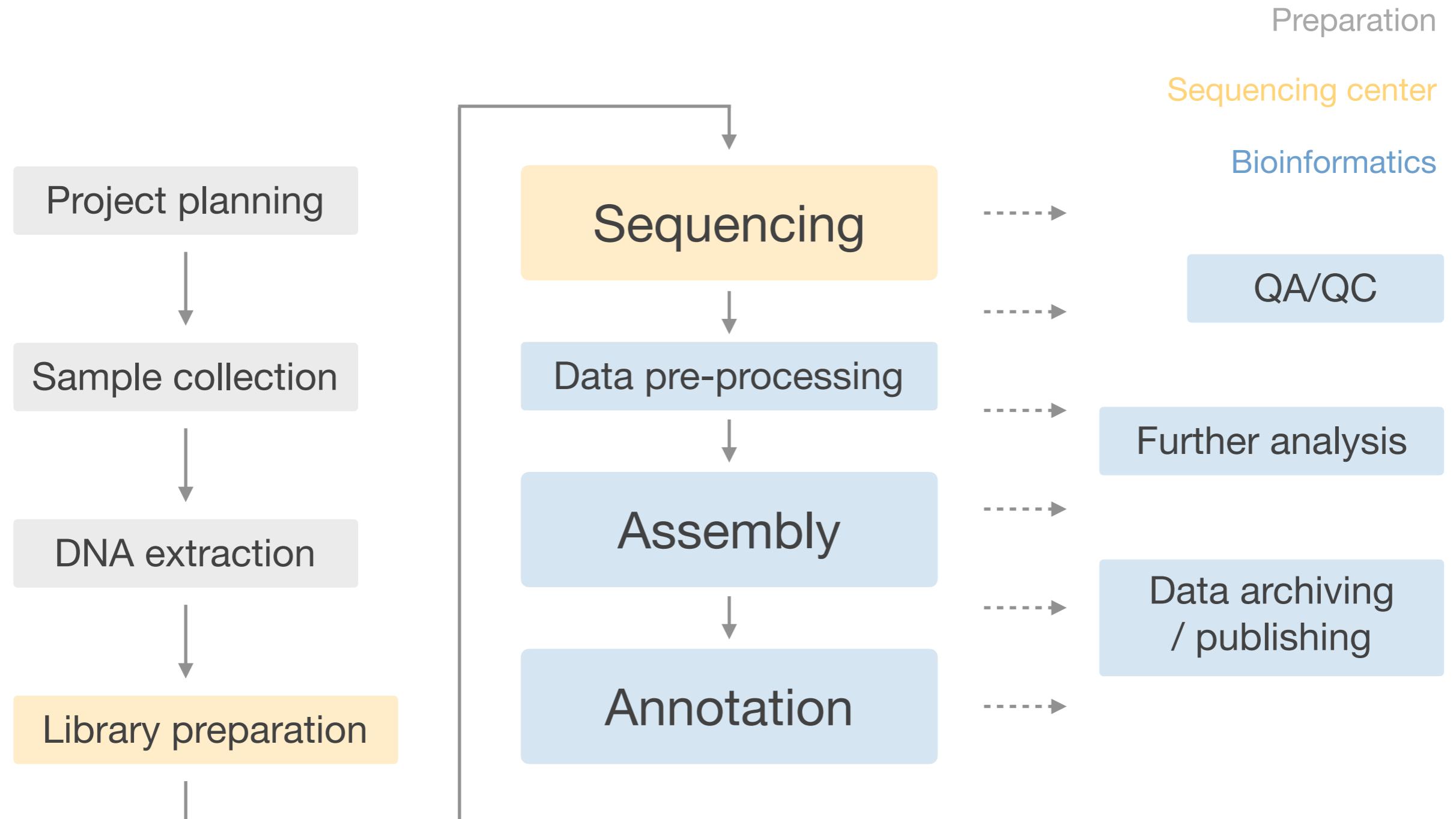
- Typically outsourced to academic or commercial sequencing center
- Academic providers usually offer discounts to affiliates
- Services, DNA requirements often found on website, but not prices
(request quotes, possibly try www.scienceexchange.com)
- Contact center well in advance for scheduling, shipping and labeling preferences

2. Data pre-processing and QA/QC

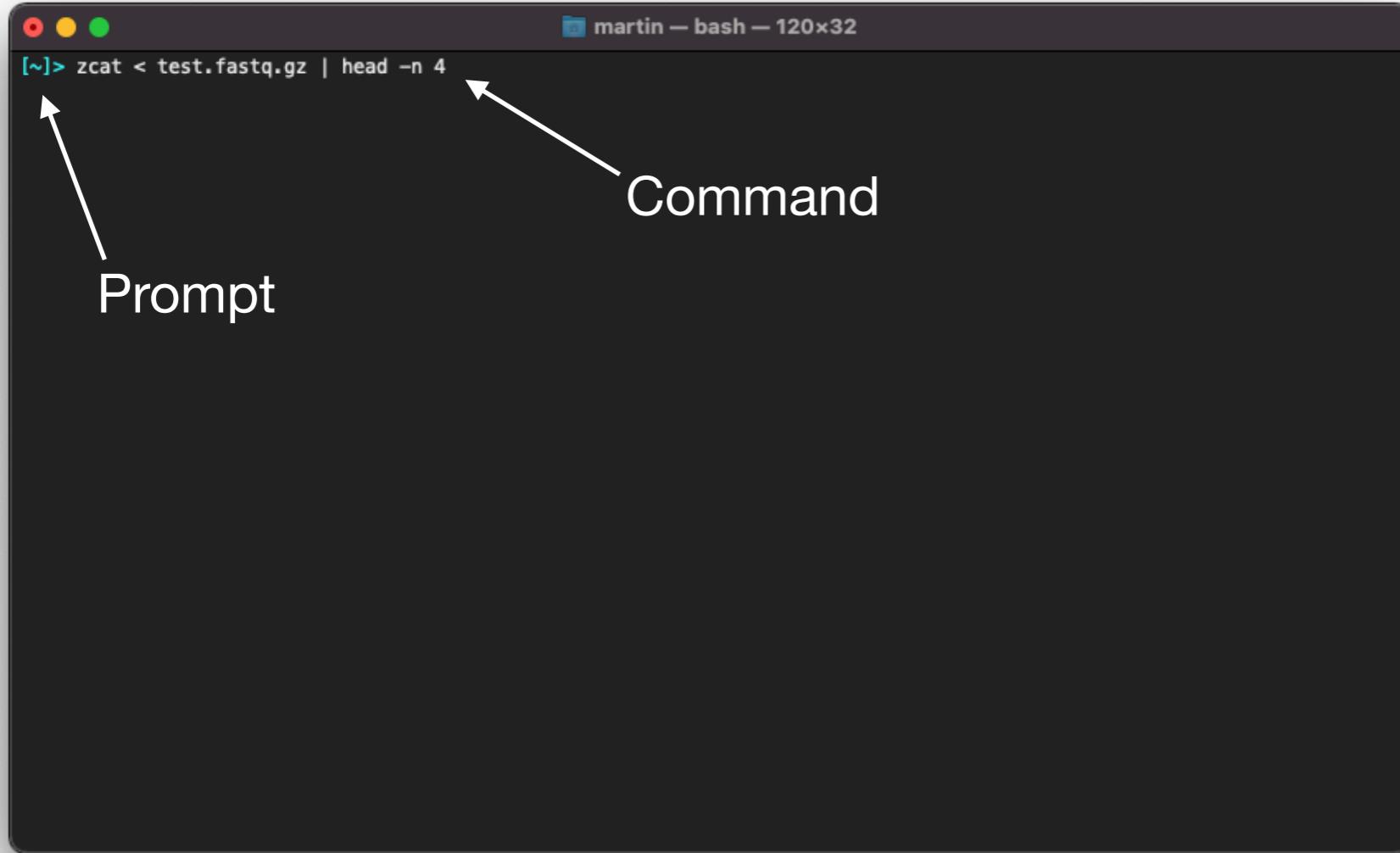
- Introduction to bioinformatics and the command line
- Raw sequencing data, trimming & filtering
- Read quality assurance / control

De novo genome sequencing workflow

Legend



The command line



Shell window
(terminal / console)

Command line interface + command language = **shell**

Most common shell: **bash**

The command line

Advantages

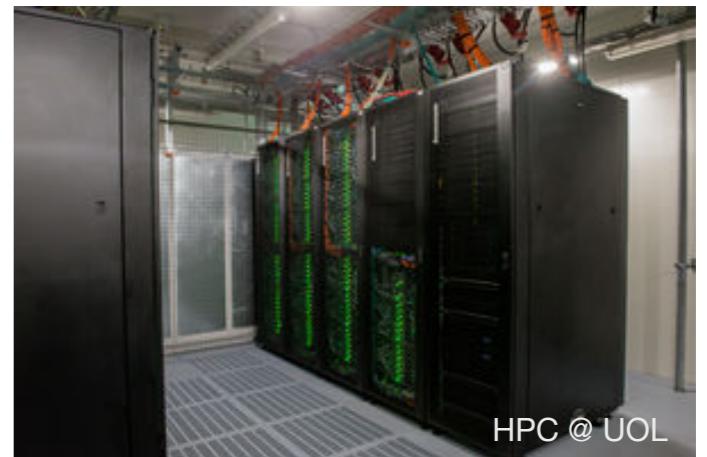
- High **flexibility**
- **Automation / pipelines > complex workflows**
- High **reproducibility / ease of documentation**
- Use of **computing clusters**

Many tools are only available on the command line

A note on high performance computing

HPC cluster advantages

- Parallelization
- Memory capacity (storage and RAM)
- Rich, actively managed software environment
(Linux based)
- Job submission system



Possible alternative: cloud computing (e.g. Amazon Web Services)

How to access and use bash

macOS

- open Terminal: /Applications/Utilities/Terminal.app
- (type “bash”)

Windows

- shortcut for this workshop: install git, type “git bash” from Start menu
- for serious use, run Linux subsystem on Windows
- Alternatively, install Cygwin

Text editors

Large text-based files are ubiquitous in genomics and programming

Recommended for all OS: Atom (<https://atom.io>)  ATOM

Built-in:

- TextEdit (macOS)
- Notepad (Windows)

Exercise instructions

Command (type this and press enter to execute) # comments will be ignored
 # replace < > with appropriate content

Output (this should appear as a result to your commands)

Additional information and tips

Exercise instructions

Download example data using git

```
git clone https://github.com/mhelmkampf/workshop_zmt.git      # download example data
```

```
Cloning into 'workshop_zmt' ...
```

```
...
```

```
...
```

```
...
```

```
Resolving deltas: 100% (7/7), done.
```

Bash basics

Typical usage (syntax)

`command [-options] [file]`

Navigating the file system

```
ls                                     # list directory contents  
ls -l                                 # list in long format  
  
cd <dir>                               # change directory  
cd ..                                  # move back one level (to parent directory)  
  
pwd                                    # display path of current directory  
  
man <command>                          # help manual (not available in git bash)
```

Bash basics

Path: location of file or directory in system's file structure

workshop_zmt/project/1_rawdata

Path and keyboard shortcuts

..	# = parent directory
.	# = current directory
tab	# autocomplete file and directory names
up arrow	# display previous command

Bash basics

Organizing files

```
mkdir <dir>                                # create new directory

mv <file / dir>                            # move or rename file or directory
cp <file> <new path / name>                # make copy of file under new name
rm <file>                                    # delete file
rm -r <dir>                                  # delete directory

wget <URL>                                   # download file
gzip <file>                                 # compress file
gzip -d <file>                              # uncompress file
```

Bash basics

Displaying and manipulating files

```
cat <file>                      # display text file  
head <file>                     # display the first 10 lines of file  
tail <file>                     # display the last 10 lines of file  
  
nano <file>                      # edit file using the Nano text editor  
  
grep 'pattern' <file>           # search for 'pattern' in file  
wc -l <file>                     # count number of lines in file
```

Bash basics

Operators

```
<command> > <file>                                # write output to file  
<command1> | <command2>                            # execute second command on output of first (pipe)
```

Looping over multiple files

```
for FILE in <list>                                # list: e.g. *.fastq  
do  
    <command> ${FILE}  
    ...  
done
```

Raw data

Receiving data: FTP server link, wget command

```
cd workshop_zmt/projects/1_rawdata  
gzip -d Hpue_raw300_F.fastq.gz          # decompress file  
ls -l                                     # check if successful
```

```
total 68936  
-rw-r--r--  1 martin  staff   26M May 21 13:13 Hpue_raw300_F.fastq  
-rw-r--r--  1 martin  staff  7.7M May 21 13:16 Hpue_raw300_R.fastq.gz  
drwxr-xr-x  4 martin  staff  128B Jun 28 20:42 md5
```

Raw data

The FASTQ format

```
head -n 4 Hpue_raw300_F.fastq # display first 4 lines of file
```

Raw data

The FASTQ format

```
head -n 4 Hpue_raw300_F.fastq # display first 4 lines of file
```

1. @ followed by sequence id and optional info (e.g. instrument/run id, multiplex barcode)
 2. DNA sequence
 3. +, sometimes followed by sequence id
 4. per-base quality score (same length as sequence)

Raw data

The FASTQ format

Phred **quality score**:

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

Common benchmark:

% bases with $Q \geq 30$

Quality score	P 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 10000	99.99%

FASTQ encoding (Illumina 1.8+):

ASCII Symbol:	!"#\$%&'()*)+,.-./0123456789:;=>?@ABCDEFGHIJ
Quality Score:	0.2.....10.....20.....30.....41

Raw read quality check

Using the FastQC software

```
cd ../../apps          # change into apps folder  
unzip fastqc_v0.11.9.zip # decompress software  
FastQC/fastqc -h       # confirm software works, show help  
  
cd ../project/1_rawdata # change into raw data folder  
../../apps/FastQC/fastqc Hpue_raw300_F.fastq # run FastQC on file
```

```
Started analysis of Hpue_raw300_F.fastq  
Approx 5% complete for Hpue_raw300_F.fastq  
Approx 10% complete for Hpue_raw300_F.fastq  
...  
Analysis complete for Hpue_raw300_F.fastq
```

Results can also be found in workshop_zmt/project/2_qc

Raw read quality check

Using the FastQC software



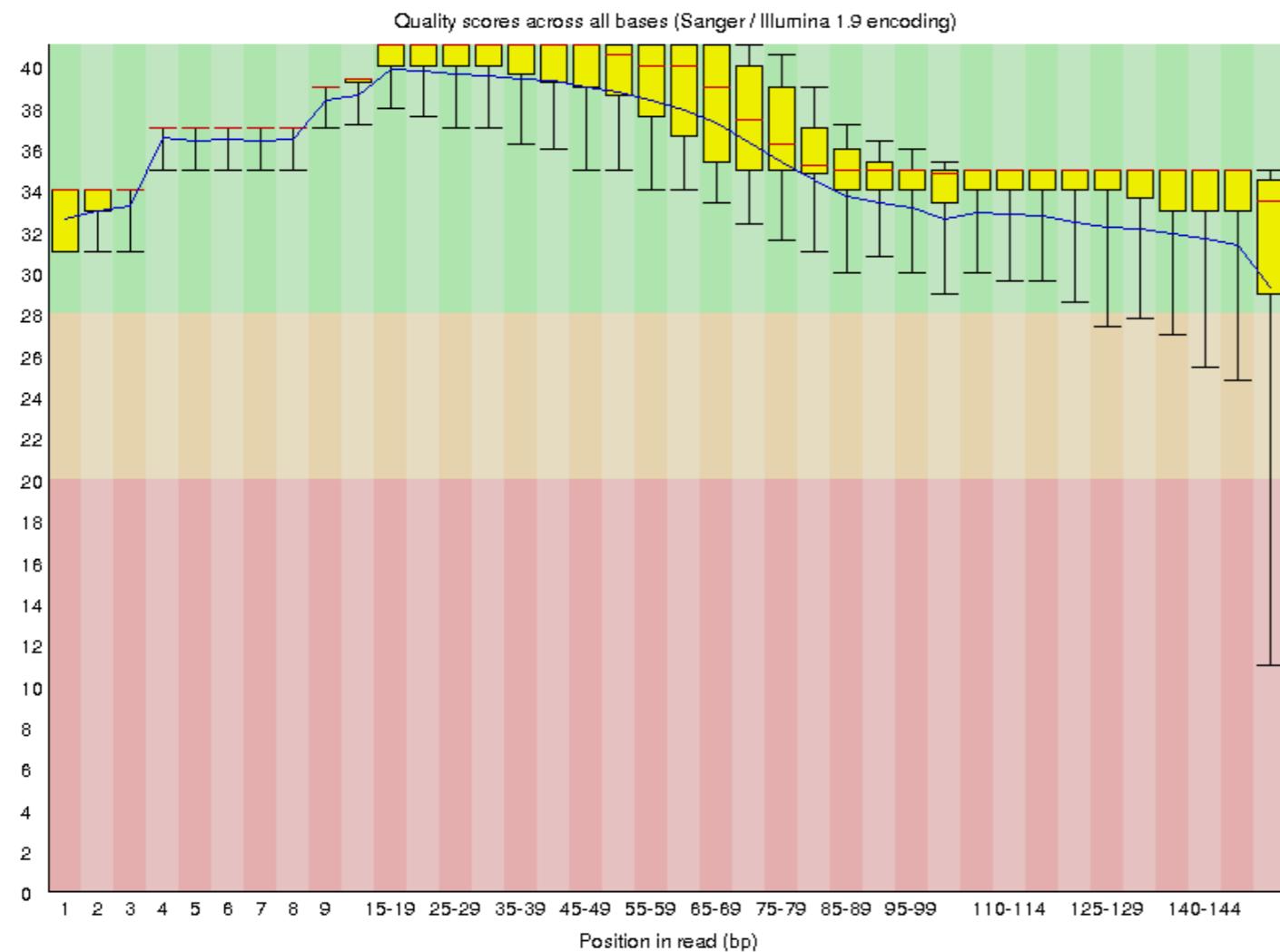
Basic Statistics

Measure	Value
Filename	Hpue_raw300_F.fastq
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	75000
Sequences flagged as poor quality	0
Sequence length	151
%GC	40

Raw read quality check

Using the FastQC software

Per base sequence quality



Raw read quality check

Using the FastQC software

⚠ Overrepresented sequences

Sequence	Count	Percentage	Possible Source
GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGC	640	0.8533333333333334	TruSeq Adapter, Index 2 (100% over 50bp)

Example for bad Illumina data:

https://www.bioinformatics.babraham.ac.uk/projects/fastqc/bad_sequence_fastqc.html

Read trimming & filtering

Using cutadapt for adapter removal and quality trimming

```
.../..../apps/cutadapt-3.4.exe -help          # confirm app works, show help (from 1_rawdata)

.../..../apps/cutadapt-3.4.exe -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCA -q 10 -o
Hpue_raw300_F_trim.fastq Hpue_raw300_F.fastq
# run FastQC (-q 10: quality-filter last 10 bases, -a: trim Illumina adapter)
```

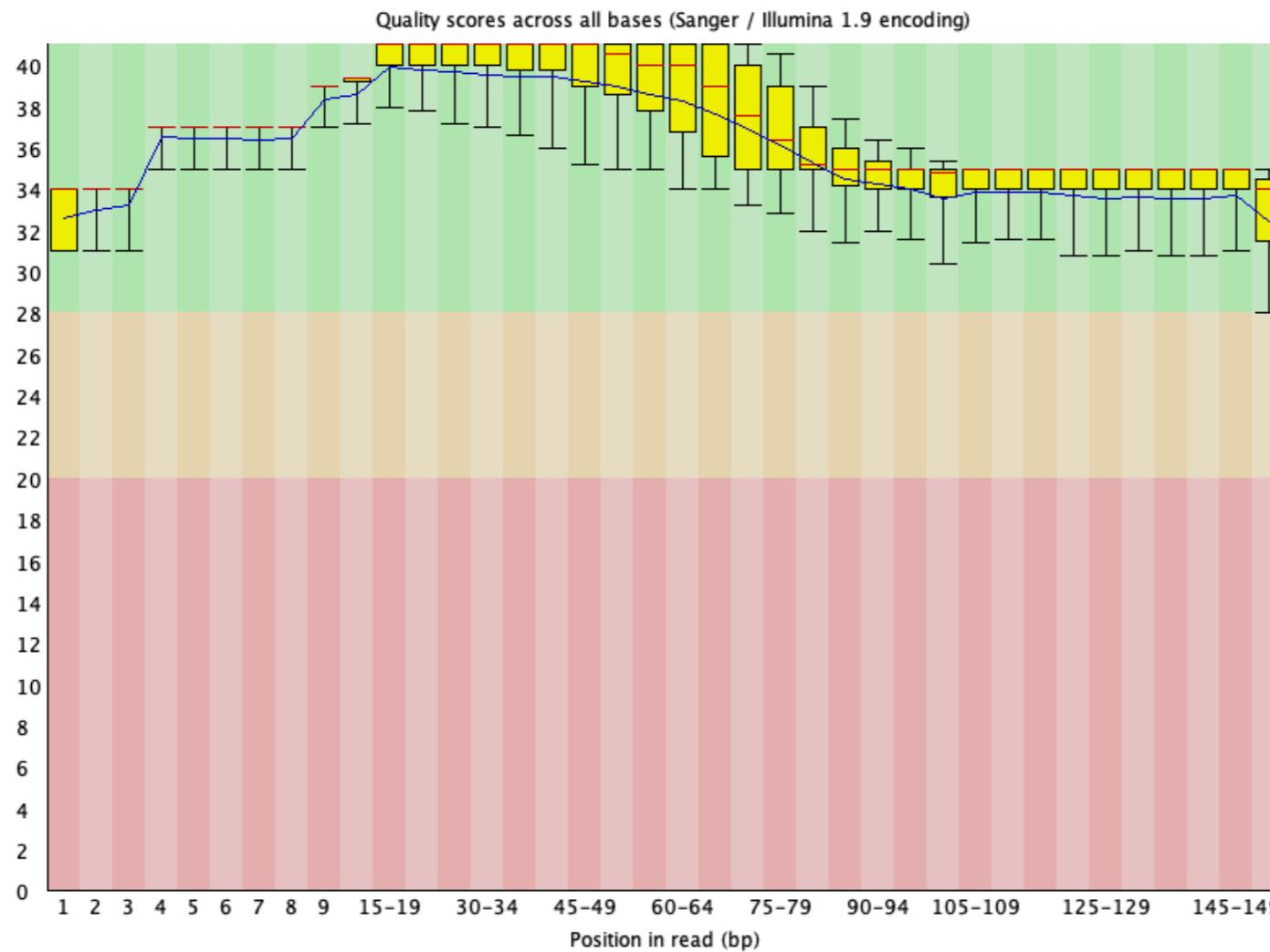
This is cutadapt 3.4 with Python 3.9.2
Command line parameters: ...
Processing reads ...

Rerun FastQC

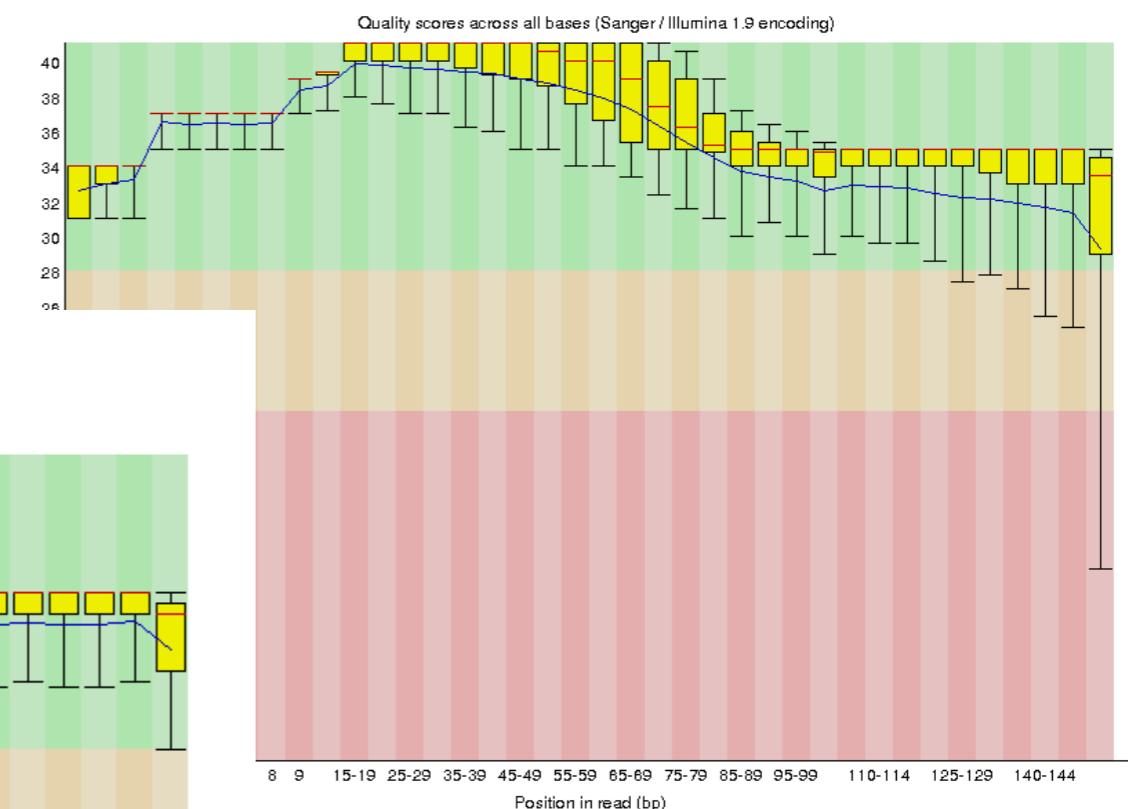
Filtered read quality check

Using the FastQC software

Per base sequence quality



Per base sequence quality



Raw reads

Filtered reads

Data pre-processing summary

- Raw read quality assessment
- Adapter removal
- Quality filtering (trim low-quality bases / reads)
- Check for contamination (reads of viral, bacterial, human origin)
- Filtered read quality assessment

De novo genome sequencing workflow

Legend

