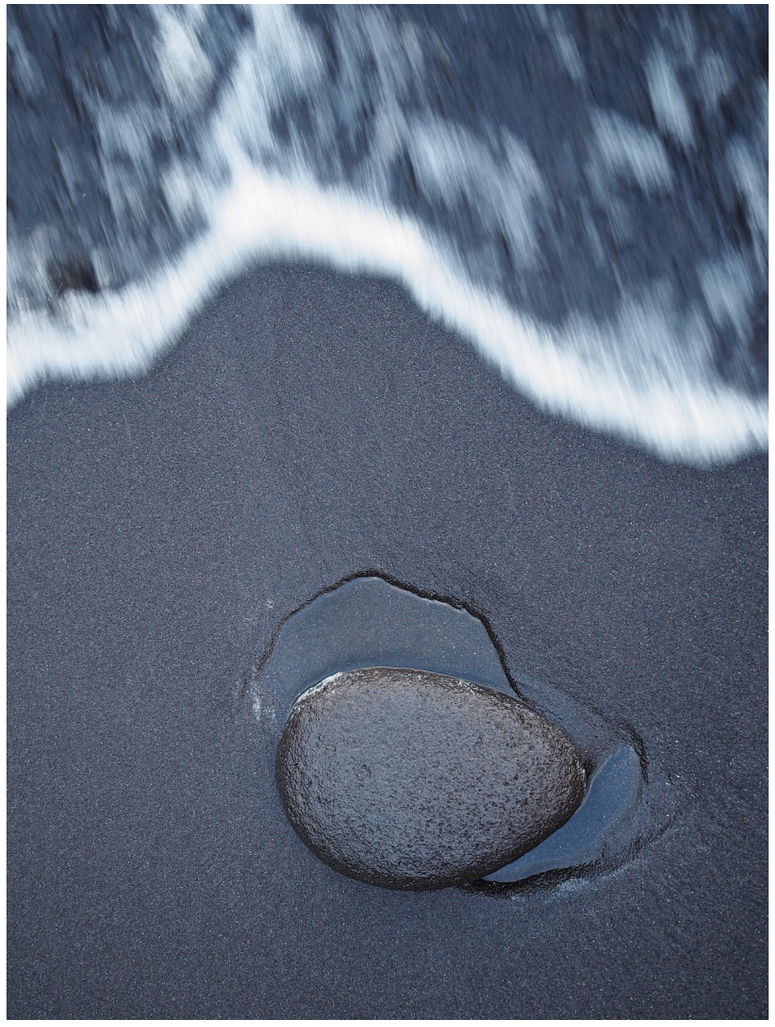
Exercises in Marine Ecological Genetics

06. Genome sequencing and assembly



- Become familiar with short and long sequencing reads
- Trim reads and assess read quality
- Calculate genome assembly metrics
- Learn to use a high performance computing cluster

Martin Helmkampf

Download course materials using git

Go to project directory

```
cd dir          # e.g. Documents/meg23_exercises
ls -l    # view directory contents, long format
```

```
meg23_exercises
├── local
└── meg23_repo
    ├── code
    ├── data
    ├── other
    └── slides
```

Update course repository

```
cd meg23_repo
git pull
```

In case of an error message

```
cd ..                                                  # go back to project directory
rm -rf meg23_repo                                      # delete old repository
git clone https://github.com/mhelmkampf/meg23_repo.git
```

Avoiding version conflict

Please do not save over files in the course repository. Instead, save your own scripts to the local subdirectory (including copies of course scripts you would like to edit), e.g with

```
cp code/06_genseq.sh ../local/06_genseq_lc.sh        # cp [source] [destination]
```

High performance computing (HPC) at UOL


TOP 500 The List. CERTIFICATE

CARL - Lenovo NeXtScale nx360M5, Xeon E5-2650v4 12C 2.2GHz, Infiniband FDR

Carl von Ossietzky University of Oldenburg, Germany

is ranked

No. 363

among the World's TOP500 Supercomputers

with 457.23 Tflop/s Linpack Performance

in the 48th TOP500 List published at SC16, Salt Lake City, UT on Novem

Congratulations from the TOP500 Editors

Erich Strohmaier
NERSC/Berkeley Lab

University of Tennessee

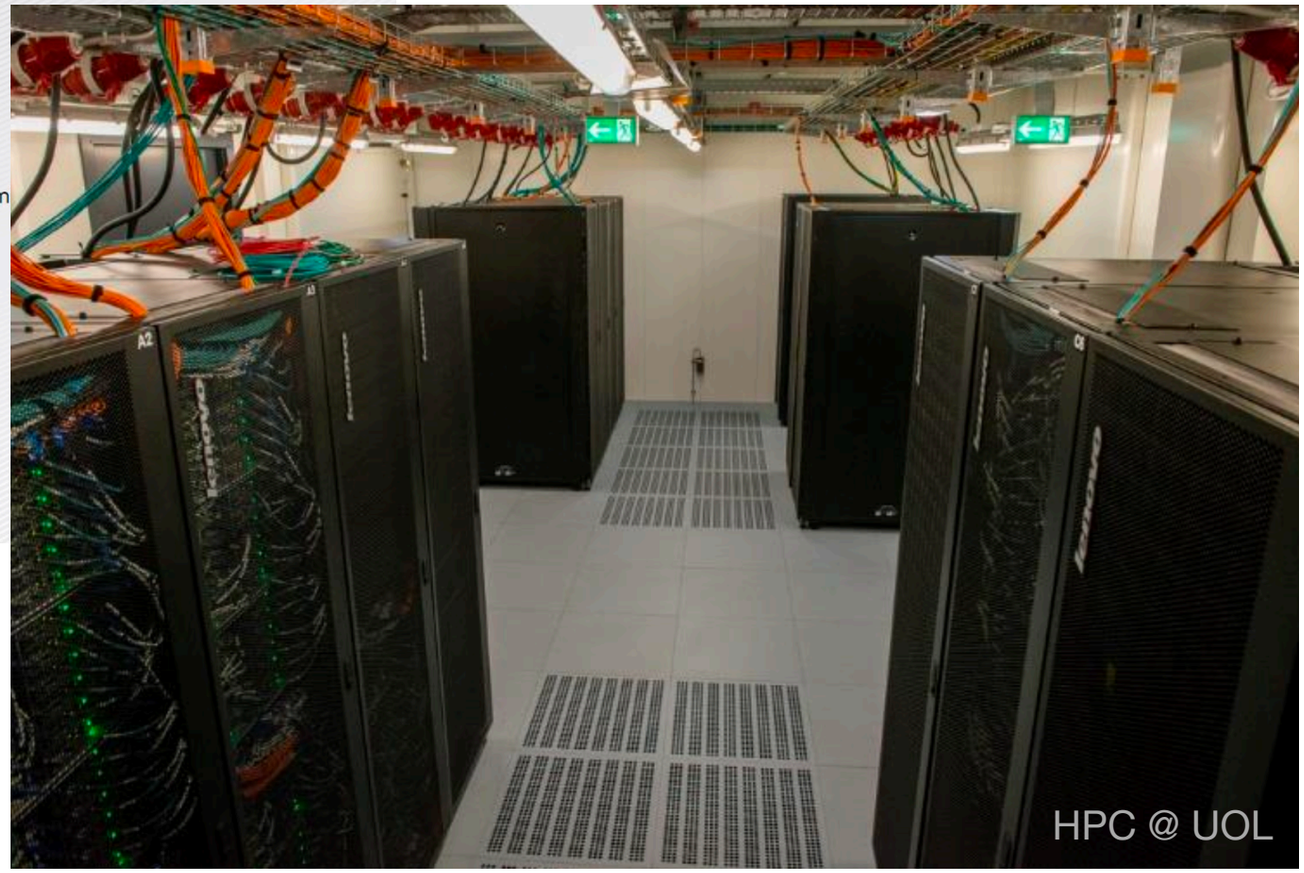
Horst Simon
NERSC/Berkeley Lab

327 compute nodes

7640 cores (CPUs)

77 TB RAM total

271 TFlop/s


HPC @ UOL

Get set up on the HPC cluster

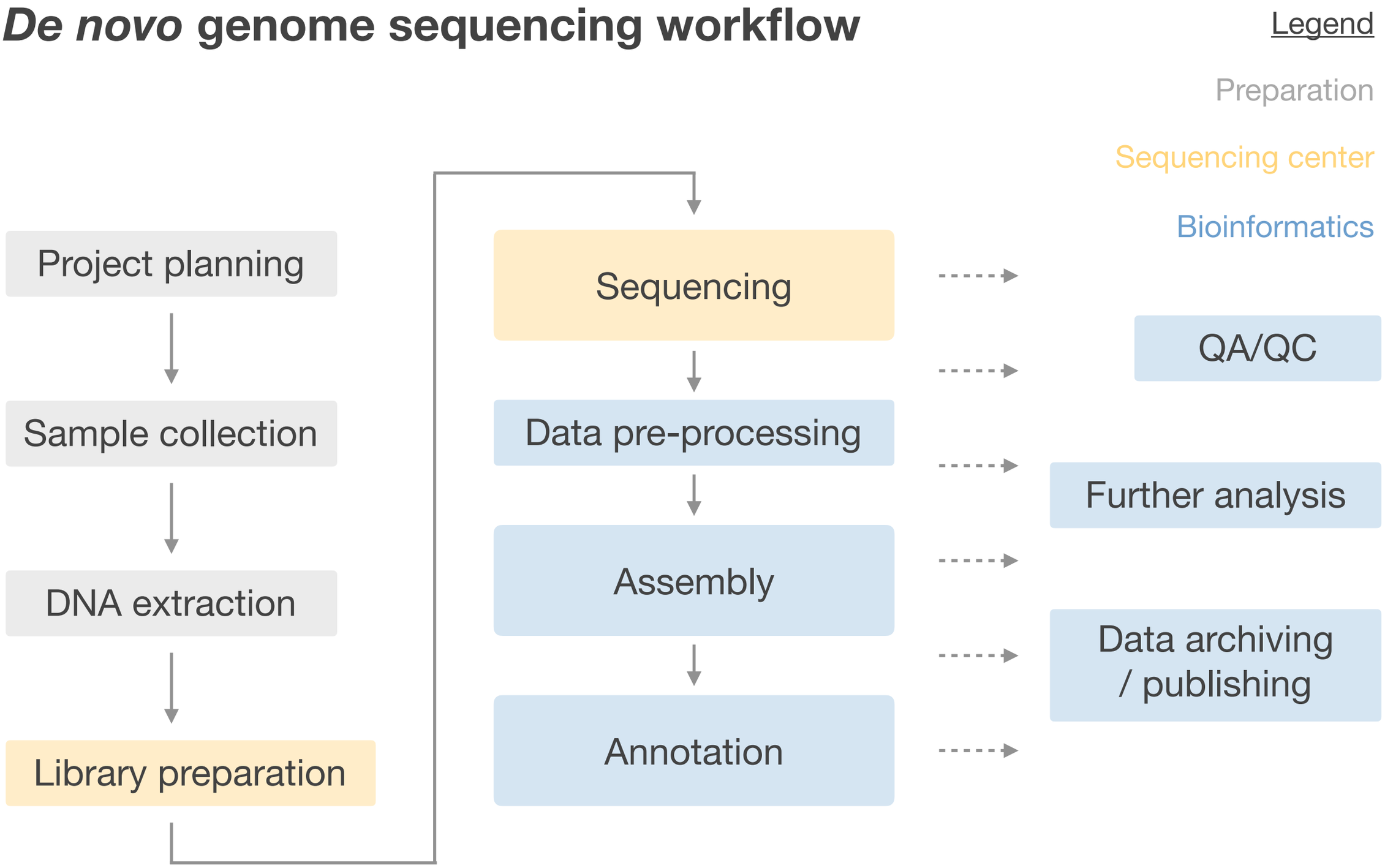
Connect to login node

```
ssh <account>@carl.hpc.uni-oldenburg.de
# Account ids and passwords can be found on StudIP in Files | course_accounts.csv
```

Download course materials to cluster account using git

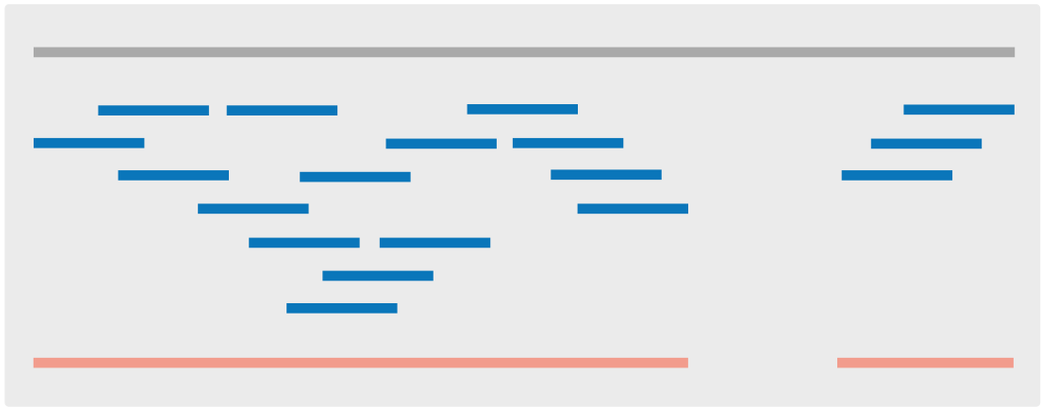
```
git clone https://github.com/mhelmkampf/meg23_repo.git
```

De novo genome sequencing workflow



Genome assembly

- Reconstructing long, continuous sequence from millions of overlapping reads
- Reads can be very short (e.g. Illumina) or long (e.g. PacBio)
- Segments of assembled sequence are called contigs,
  which may be combined into scaffolds
- Scaffolds or PacBio contigs can be up to chromosome-length



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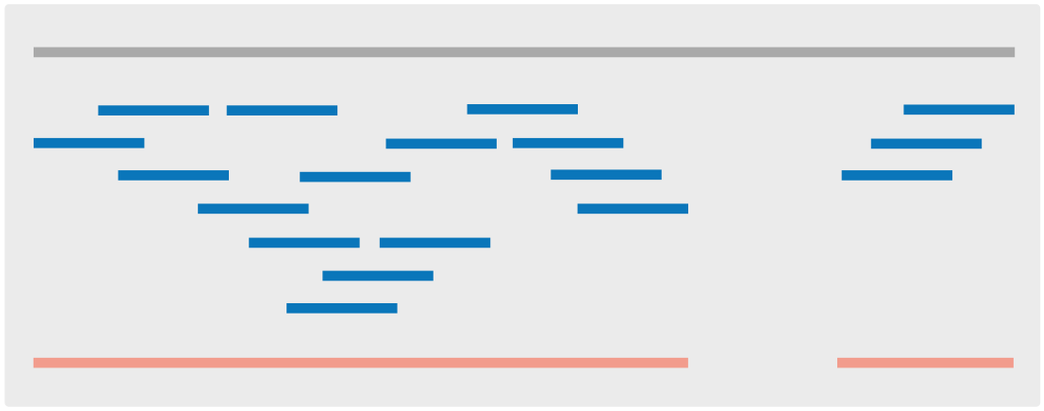
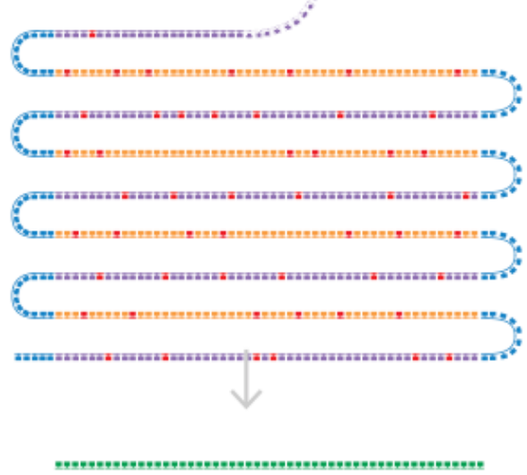




Sequencing reads in FASTQ format

```
head -n 4 HypPue1_illumina_raw_F.fastq          # display first 4 lines of file
```

```
@HWI-ST1293:199:HA9JHADXX:1:1101:1044:1603 1:N:0:CGATGT
NCCCTGTTAAAGGATCATCTCTGACCTATCATTGTGGTGTAATCACATTTAACACAATCACGATGTGCTTTACCTGCAGC
ATCTTTACAGCAGGGCTGGGAGATATGACCAAAAACAGTTATGATAATATGTTTATTTCTATTGAAAATCA
+
#1=DDFFFHHHHHHJJJJJJJJJJJJJJJJJJJJJJGHIJJJJJIIIJJJJHJJJJJJJJJJJJIJIJJJJJJJHHHHH
FFFFFFEEEEEEDDDDDDDDD@DDDEDEDDDBDDDDDDCDCEDDEEDDEEEDEECDDEDEDEEDDDDDDDC
```

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

Base quality

Phred quality score:

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

Common benchmark:

% bases with Q ≥ 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
```

Assessing assembly quality

- Sequencing depth / coverage
- Assembly metrics: size distribution of contigs / scaffolds
- Average base accuracy (Q score)
- Percentage of assembly assigned to chromosomes
- Gene completeness
- Phasing information

Challenges

- Contamination
- Misassembled regions
- Presence of false duplications

Sequencing depth / 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: CGTAATGGCATATCGCCTAGATTCGAAACG
Read 1:   TAATGGCATATCGCCTAGAT
Read 2:         CATATCGCCTAGATTCGAAA
Read 3:           TATCGCCTAGATTCGAAACG
Depth:  00111111223333333333322222211
```

Assembly metrics

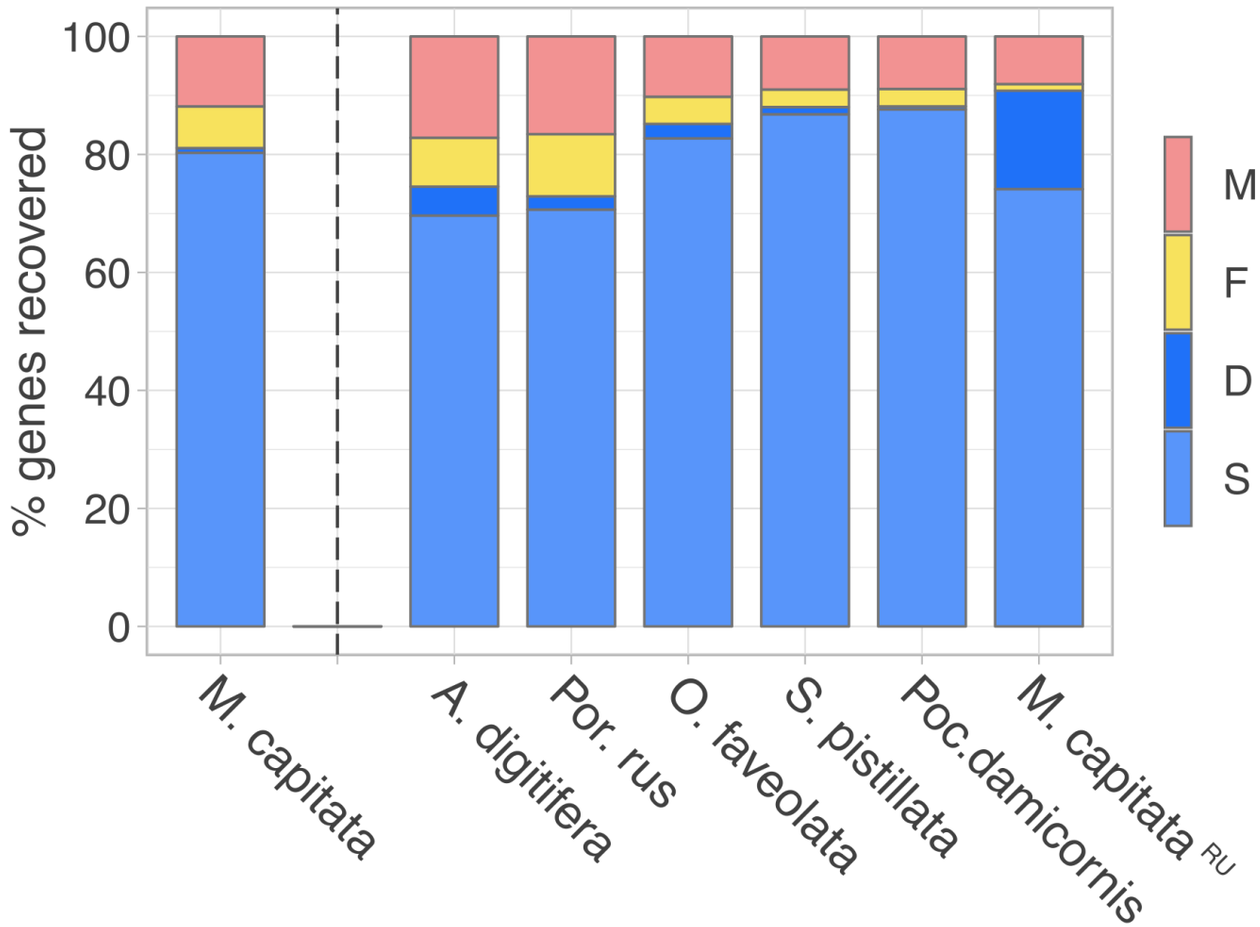
- Total size (compare to expected genome size)
- Number of contigs / scaffolds
- Largest scaffold
- N50: contig / scaffold size where 50% of assembly is found on contigs / scaffolds of equal or larger size (measure for sequence continuity)

```
Scaffolds: 530, 760, 1050, 610, 450, 800, 220, and 1200 kb
Reorder: 1200, 1050, 800, 760, 610, 530, 450, 220 kb
Sum/2: 5620/2 = 2810
Add up until sum/2 is reached: 1200 + 1050 + 800 > 2810
N50 = 800 kb
```

Gene completeness with BUSCO

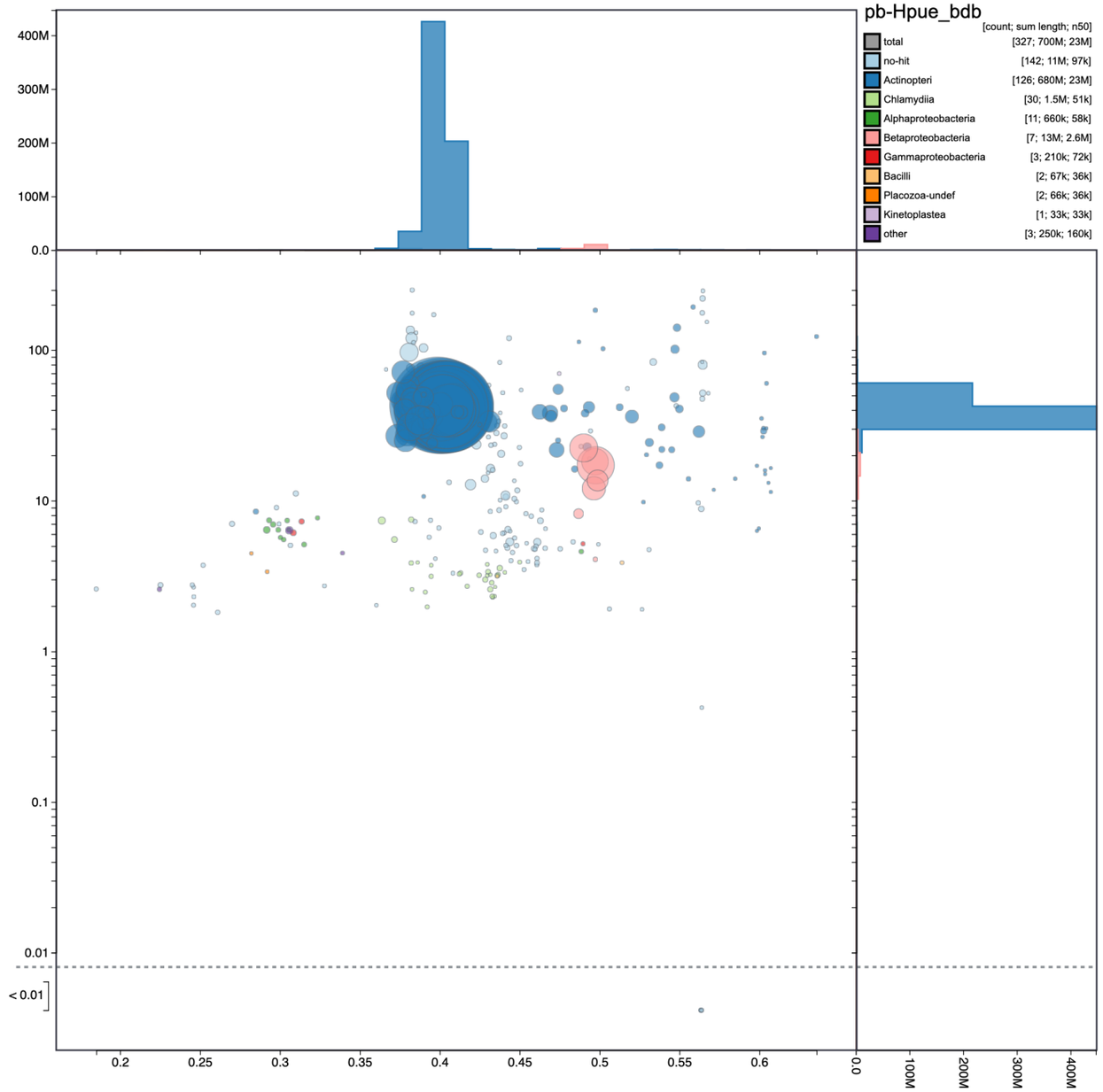
https://busco.ezlab.org

Quantifies assembly completeness based on presence of universal, highly conserved, single-copy genes (e.g. housekeeping genes)



Helmkampf et al. 2019 (Genome Biology and Evolution)

Contamination QC with BlobTools



Color:

Most similar

known taxon

Coverage

% GC

HypPue2.1_pacbio_pctg.fas

Course outline

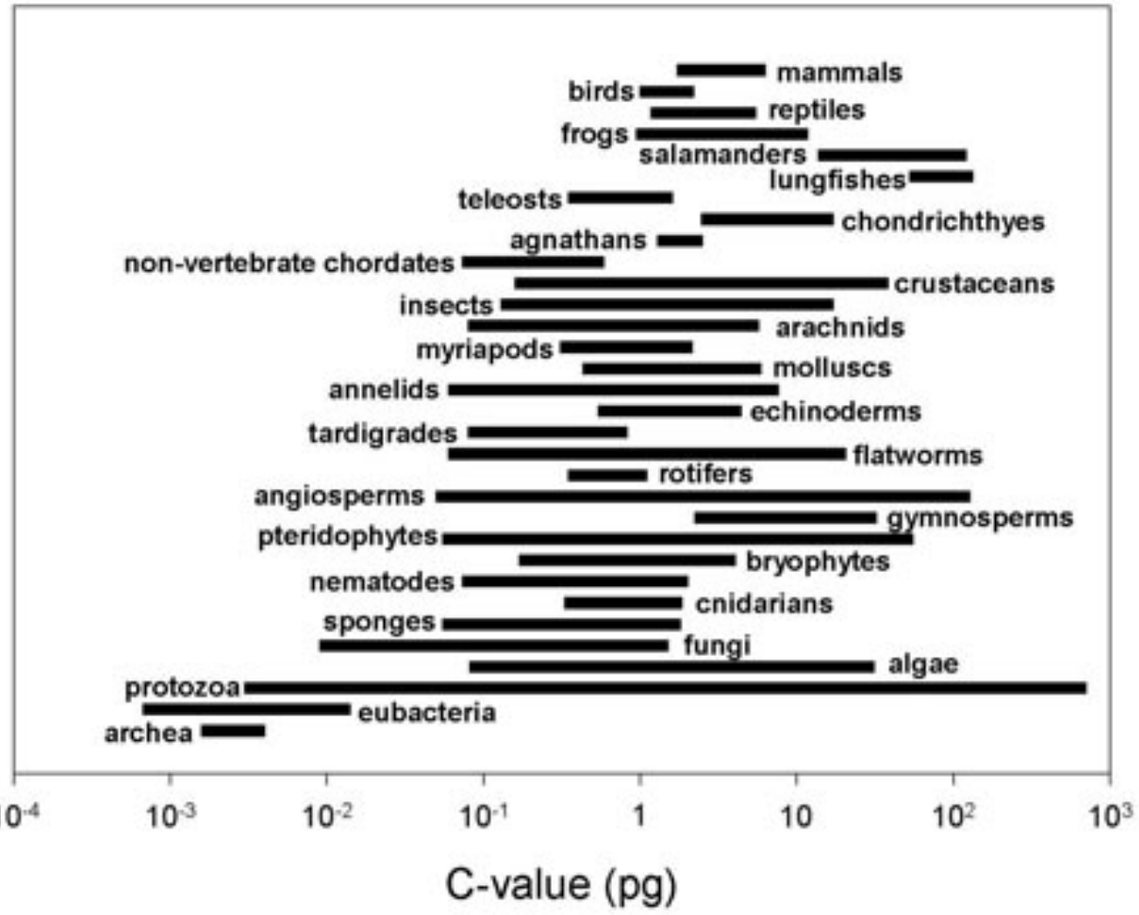
May be subject to change

Class	Date	Topics	Script
01	Apr 14	Introduction, software installation	01_intro.R
02	Apr 21	Hardy-Weinberg equilibrium	02_hwe.R
03	Apr 28	Genetic drift and effective population size	03_drift.R
04	May 05	Population structure and gene flow	04_structure.R
05	May 12	Isolation by distance (lecture online, exercises in person)	05_ibd.R
–	May 19	Himmelfahrt break	–
06	May 26	Genome sequencing and assembly	06_genseq.sh
07	Jun 02	Genotyping, SNPs and population genomics	07_snps.R
08	Jun 09	Recombination and linkage disequilibrium	08_linkage.R
–	Jun 16	Student presentations	–
09	Jun 23	Selection and mutation	09_selection.R
10	Jun 30	DNA barcoding	10_barcode.sh
11	Jul 07	Metabarcoding	11_meta.sh
–	Jul 14	To be determined	–

Genome size

How large is the 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