# Exercises in Marine Ecological Genetics

## 07. Genotyping, SNPs and population genomics

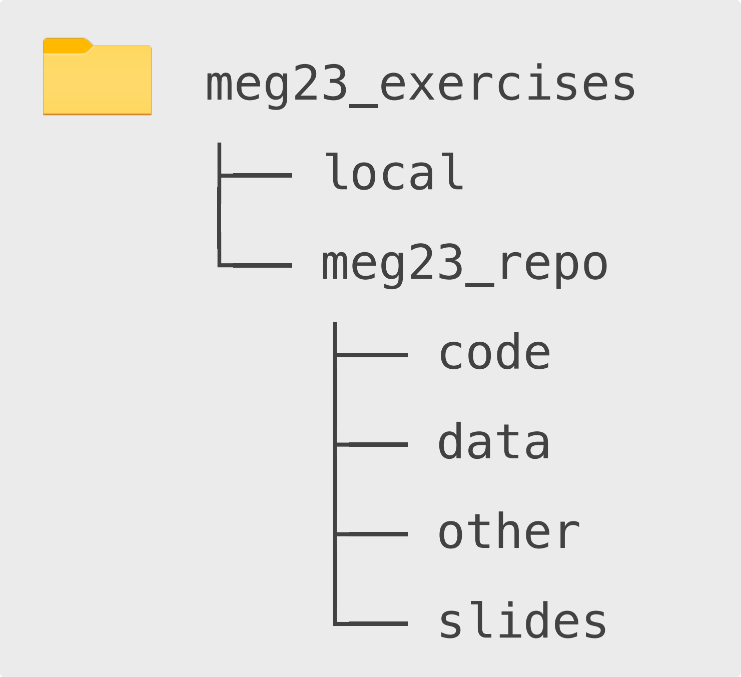
- Get overview of whole-genome genotyping
- View and filter VCF files storing SNP data
- Run population genetic calculations on SNP data
- Work on a high performance computing cluster

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/07_snps.sh ../local/07_snps_lc.sh          # cp [source] [destination]
```

# 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
```

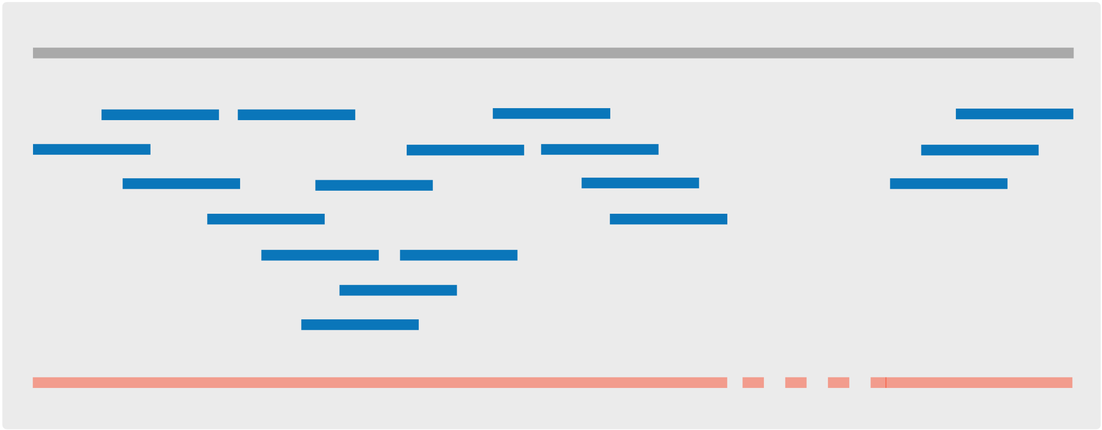
Download course materials to cluster account using git

```
cd meg23_repo
git pull
# first time: git clone https://github.com/mhelmkampf/meg23_repo.git
```

# Genome assembly recap

- 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



Genome

Reads

Contigs / scaffolds

# Genome assembly recap

- Reconstructing long, continuous sequence from millions of overlapping reads
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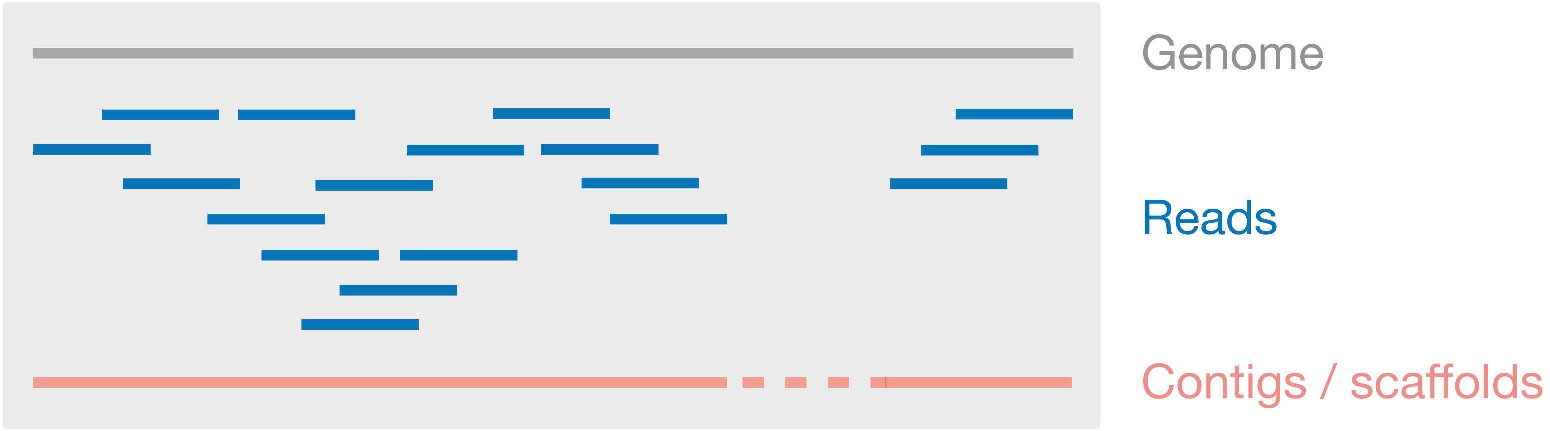
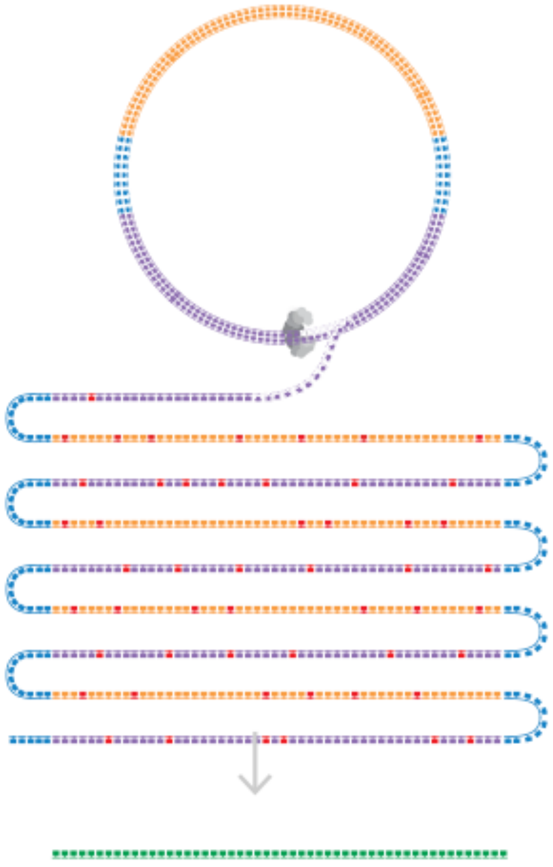
- Scaffolds or PacBio contigs can be up to chromosome-length

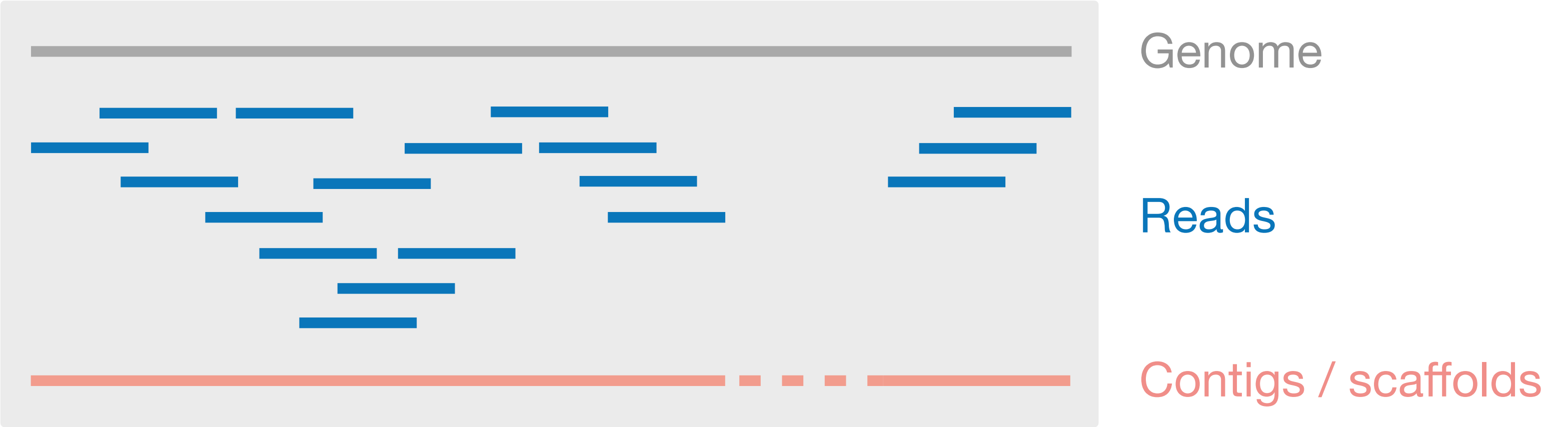




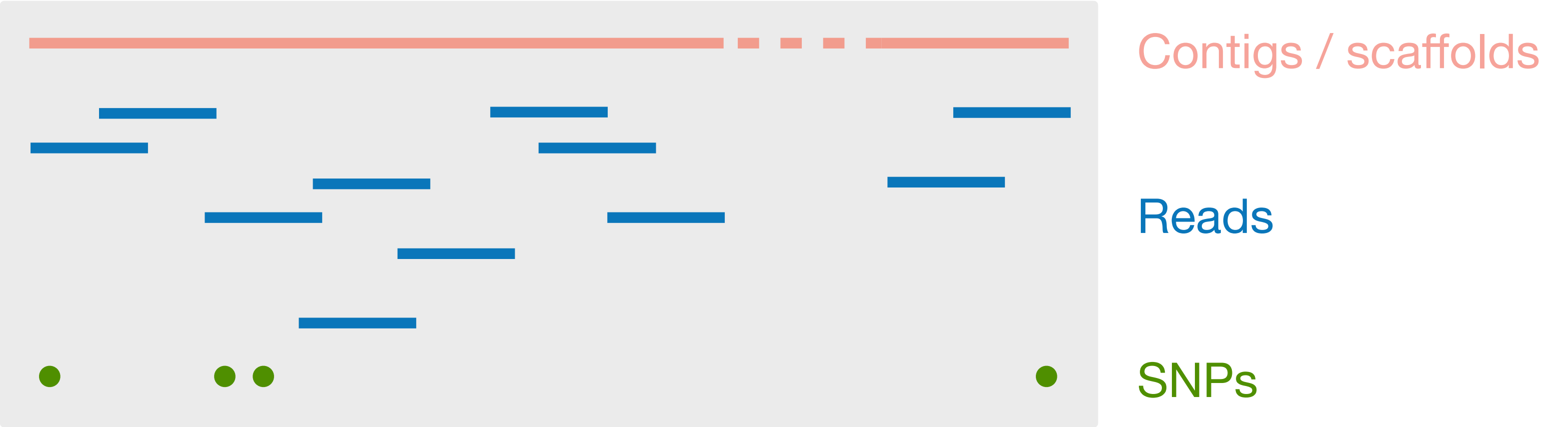
PacBio HiFi read

# Genome sequencing strategies

*De novo*



Re-sequencing



~ Reduced representation sequencing, e.g. RADseq

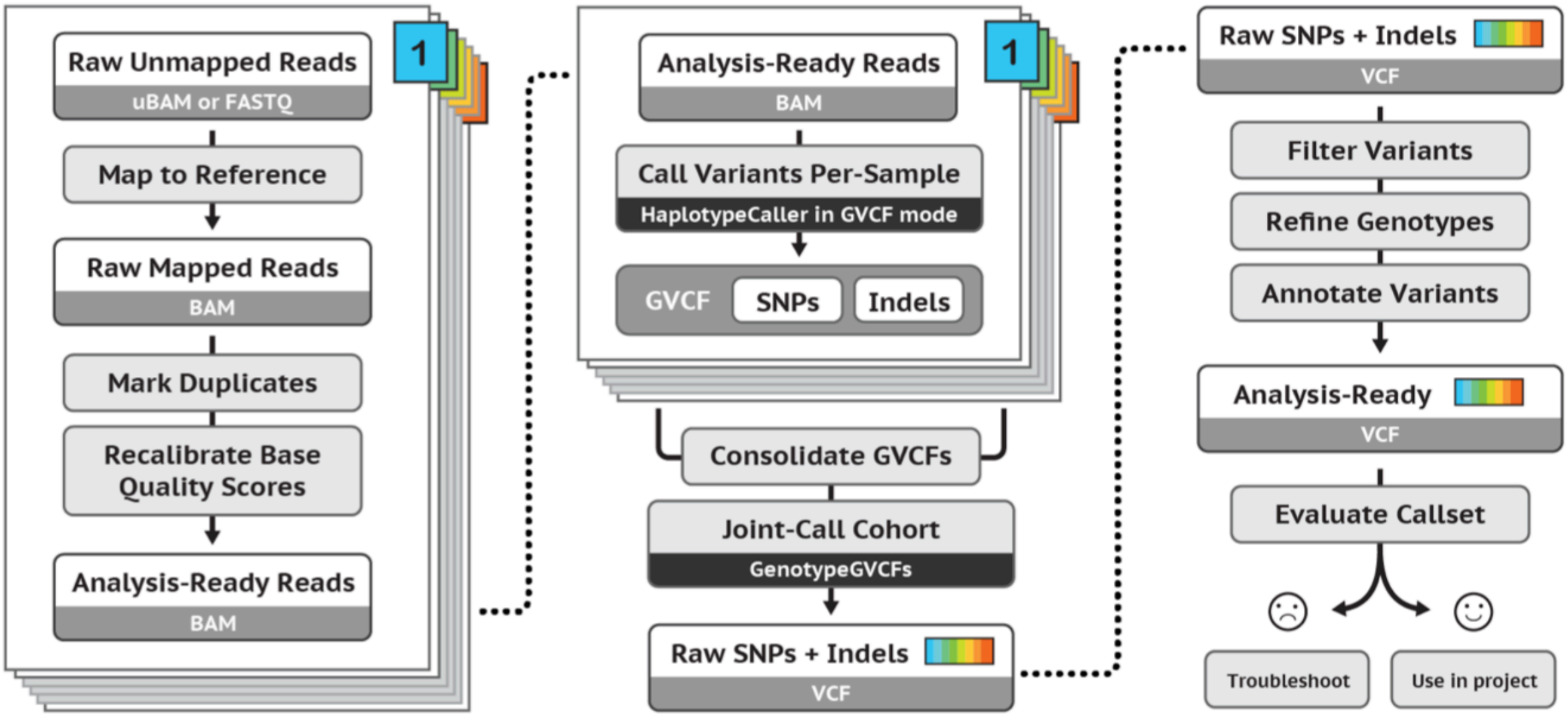
# Sequencing reads in FASTQ format

```
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=DDFFFHHHHHHJJJJJJJJJJJJJJJJJJJJJJJGHIJJJJJIIIJJJJHJJJJJJJJJJJJIJIJJJJJJJHHHHH
FFFFFFEEEEEEDDDDDDDDD@DDDEDEDDDBDDDDDDCDCEDDEEDDEEEDEECDDEDEDEEDDDDDDDC
```

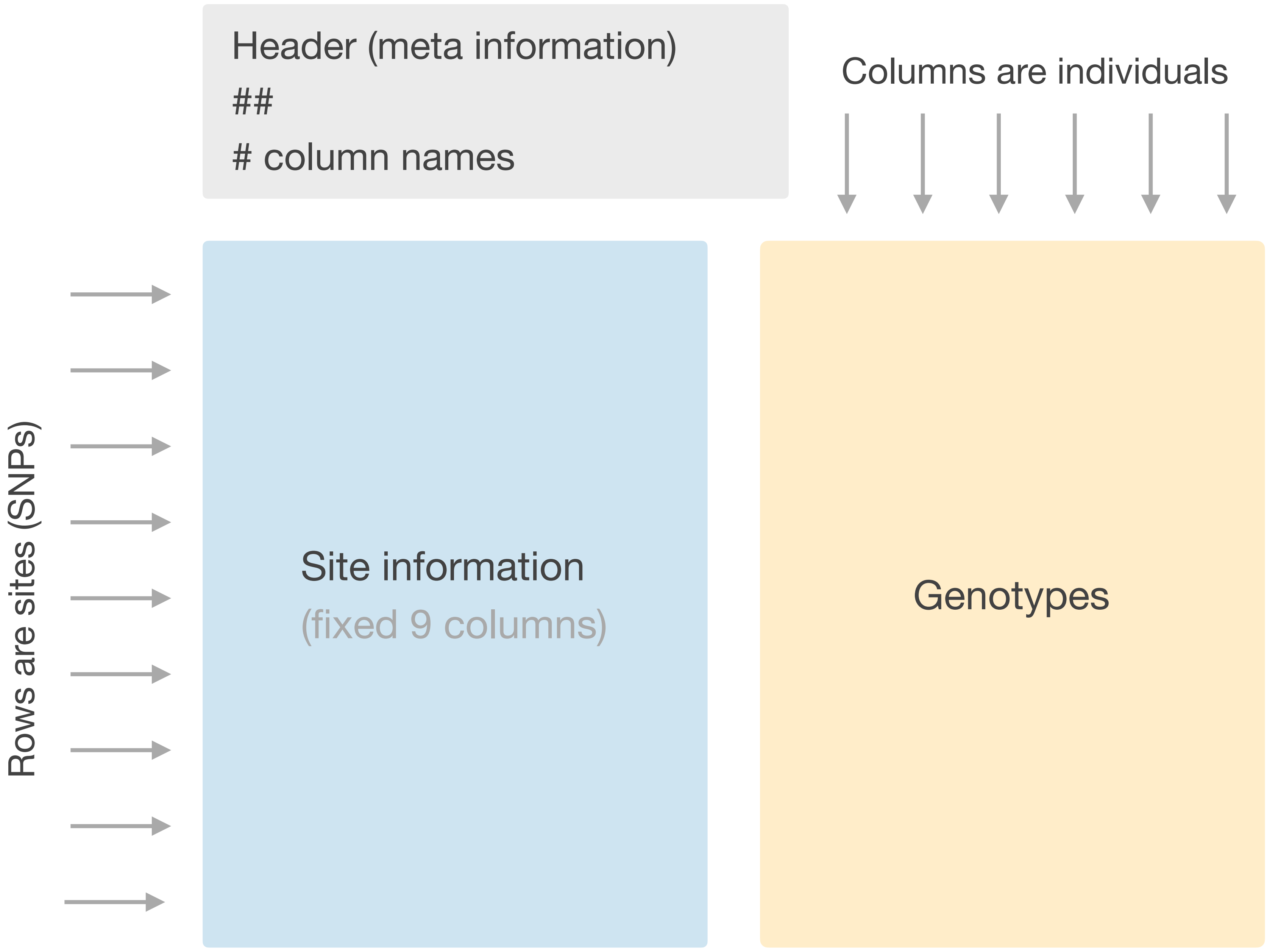
```
1. @ followed by sequence id and optional info (e.g. instrument/run id, barcode)
2. DNA sequence
3. +, sometimes followed by sequence id
4. base quality score (same length as sequence)
```

# Whole-genome genotyping workflow with GATK



gatk.broadinstitute.org

# Variant call format (VCF)



Header (meta information)

##

# column names

Columns are individuals

Rows are sites (SNPs)

Site information

(fixed 9 columns)

Genotypes

Efficiently stores

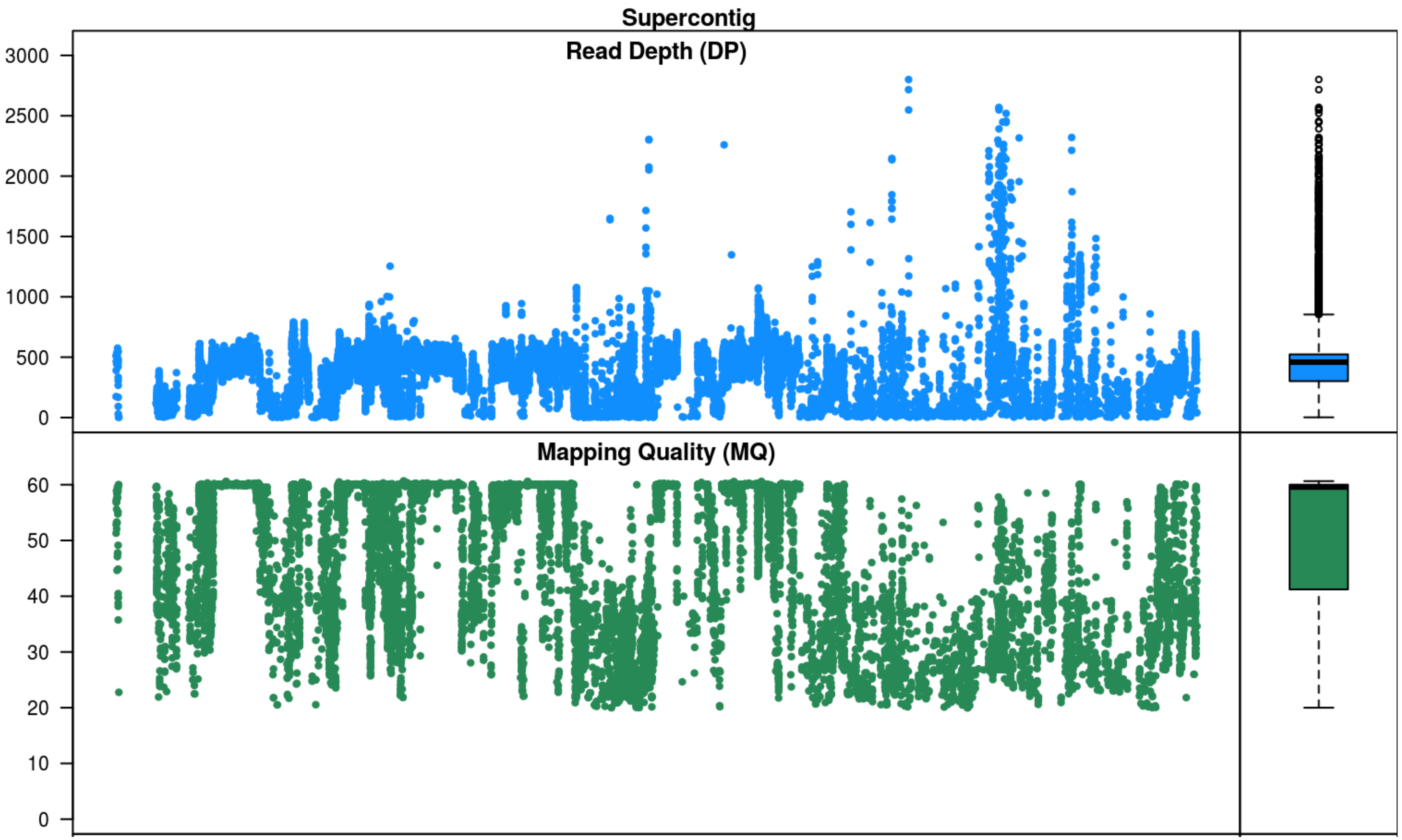
genetic variation data

Optional data e.g:

- allele frequencies
- no. samples with data
- read depth
- genotype likelihood

# Read depth and mapping quality

Exercise 3



vcfR documentation

# Example dataset

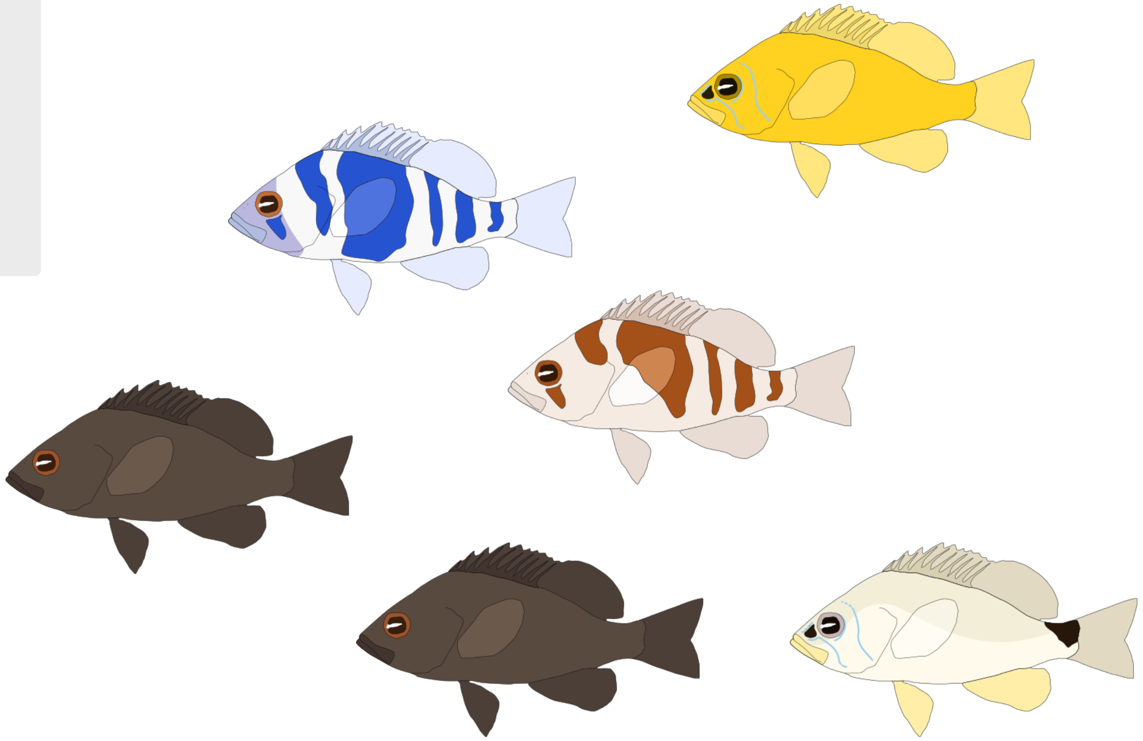
Exercise 1–4

- 8 species of hamlet (genus *Hypoplectrus*)
- 3 Caribbean sites: Belize, Honduras, Panama
- 167 hamlet samples total
- Illumina short-read resequencing (mean depth 17×)
- Genotyping with GATK
- High-quality reference genome of *H. puella*

↓

**snps_hamlets_lg12.vcf.gz**

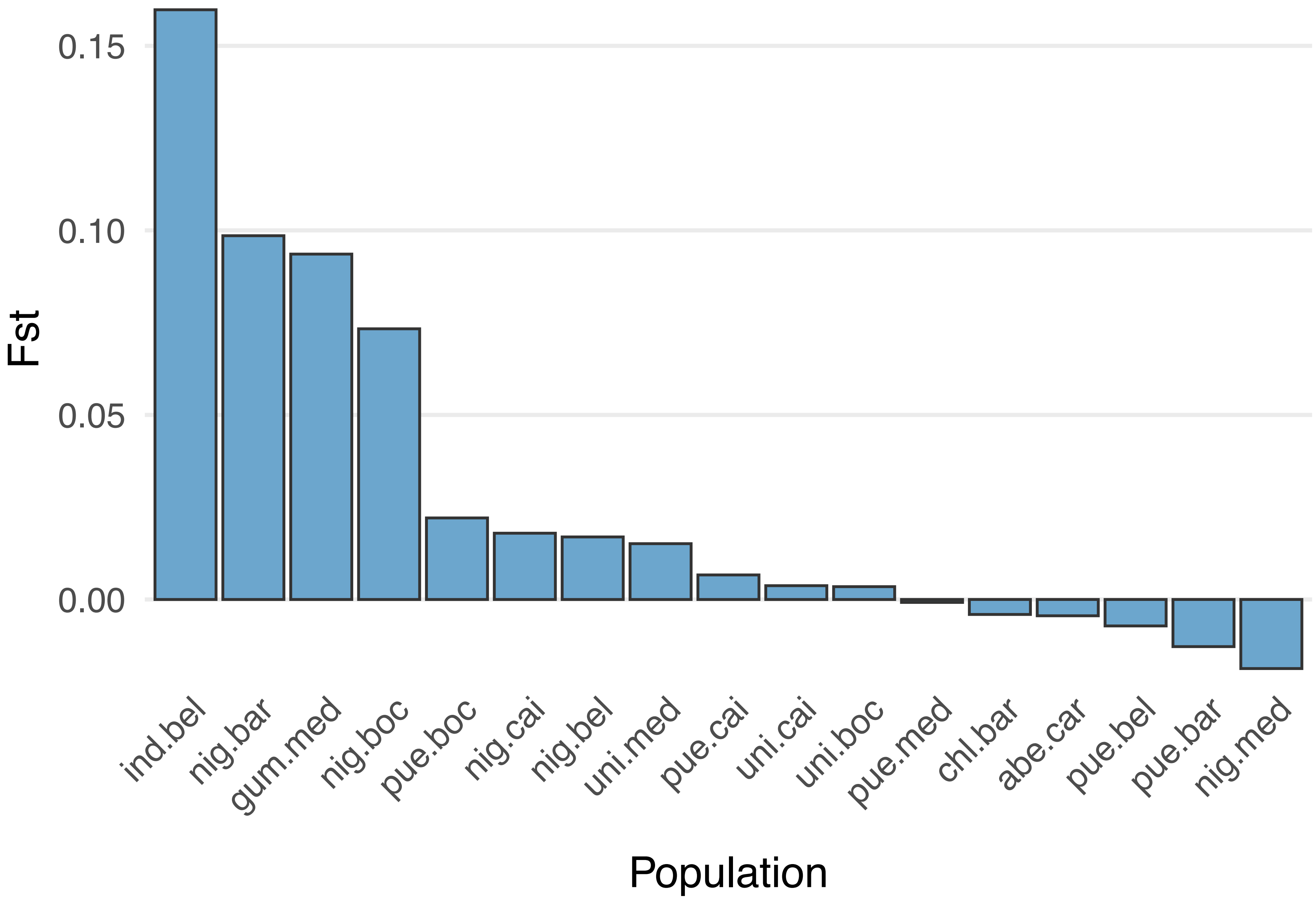
- Chromosome 12 only
- Subset to 36 samples from 6 populations



Illustrations by Kosmas Hench

# Compare PCA with population-specific $F_{ST}$

Exercise 3



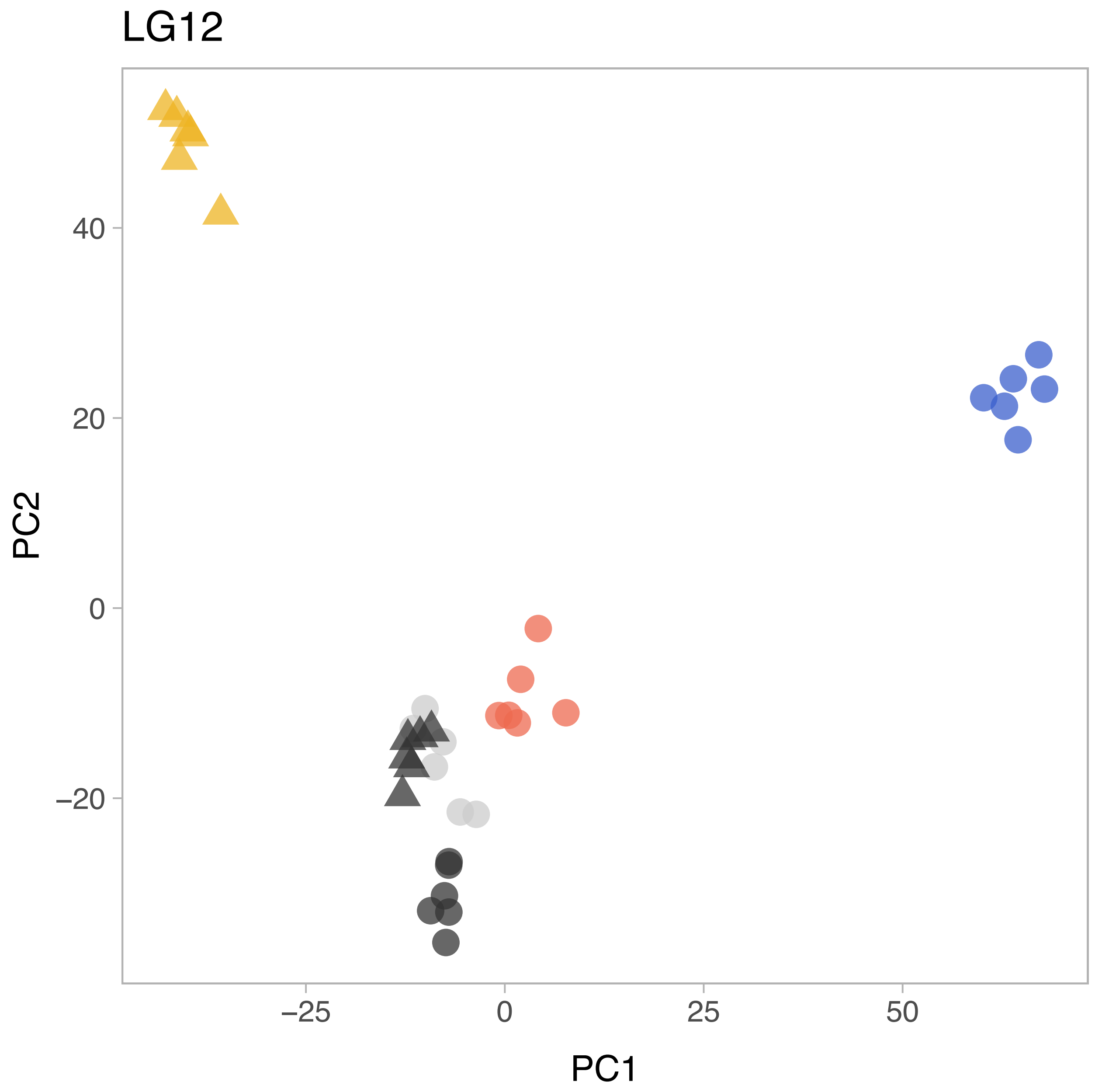
In PCA:

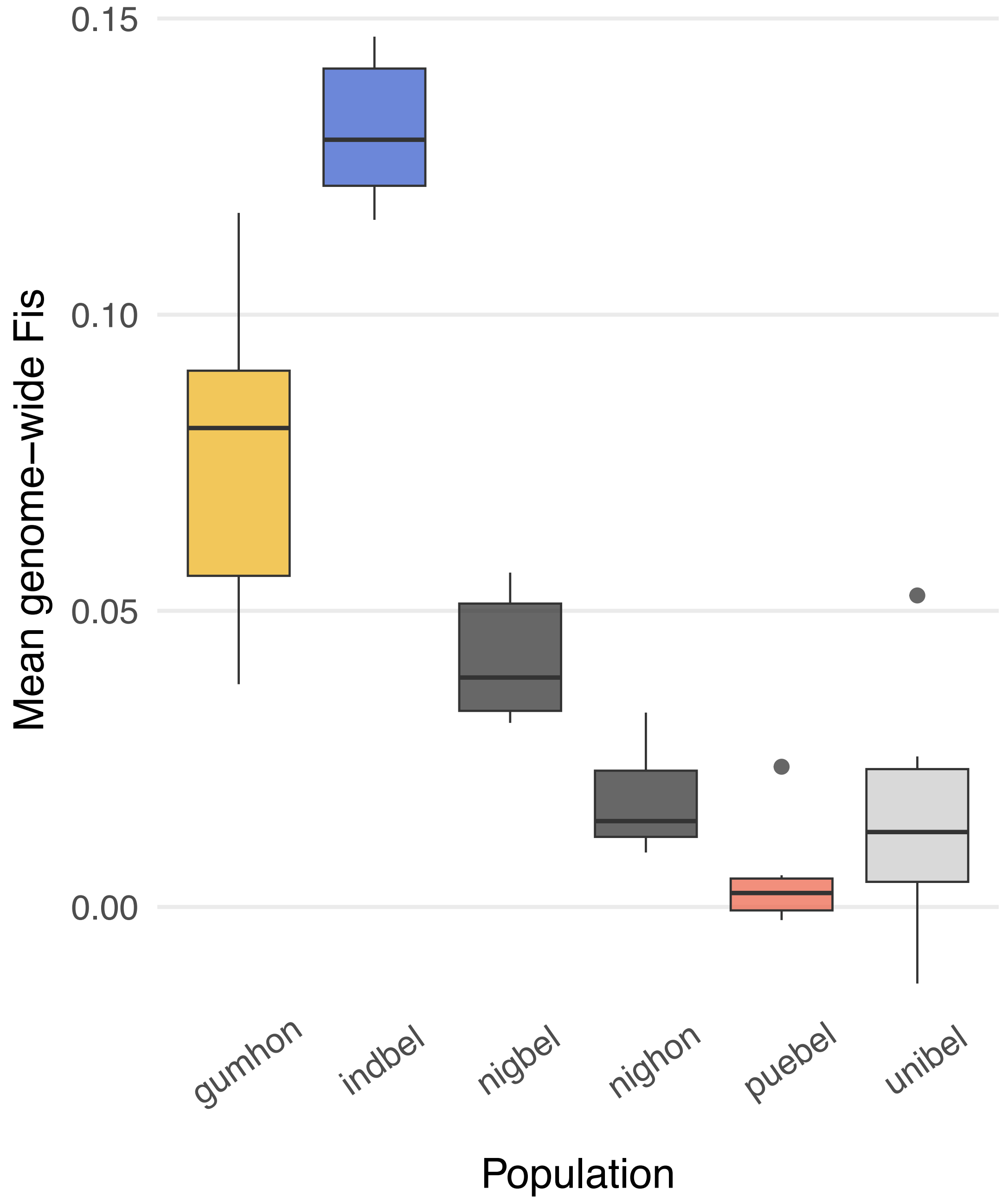
boc = pan

med ~ hon

# Compare PCA with population-specific $F_{ST}$

Exercise 3/4





# 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 | – |