Exercises in Marine Ecological Genetics

10. DNA barcoding

- Extract DNA barcodes from Sanger reads
- Identify samples with the Barcode of Life Data System
- Evaluate genetic distances and id quality

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/10_barcode.sh ../local/10_barcode_lc.sh      # cp [source] [destination]
```

#fischdetektive

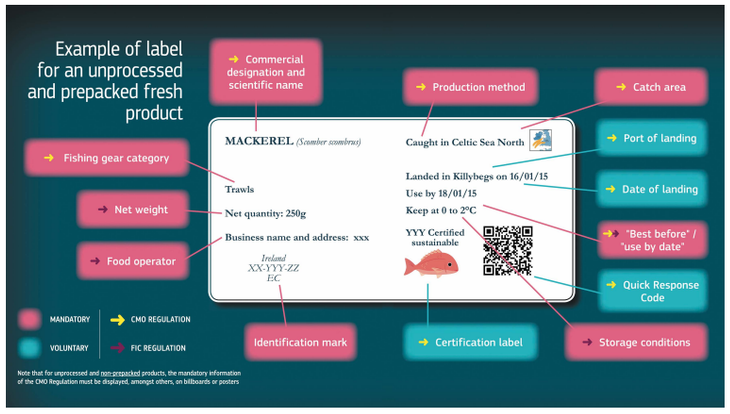
Citizen science project

at GEOMAR (2017) with over

700 participants (10–14 years)

Where does our seafood come

from, and is it labeled correctly?



Thorsten Reusch, GEOMAR

Sample collection

↓

DNA extraction

↓

PCR (COI)

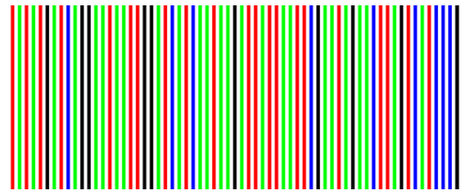
↓

Sequencing

←

ID

COI barcode



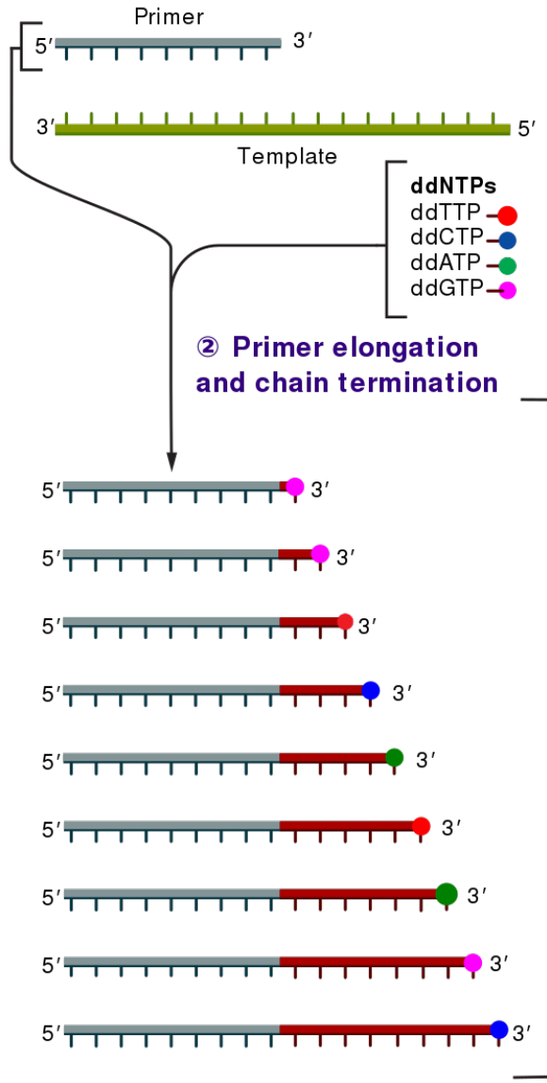
Approx. 650 bp in 5’ region of cytochrome c oxidase subunit I (COI)

```
>MN604318.1 Oncorhynchus keta cytochrome c oxidase subunit I gene, complete cds; mitochondrial
GTGGCAATCACACGATGATTCTTCTCAACCAACCACAAAGACATTGGCACCCTCTATTTAGTATTTGGTGCCTGAGCCGGGATAGTAGGCACCGCCCTG
AGCCTACTAATTCGGGCAGAACTAAGCCAGCCAGGCGCTCTTCTAGGGGATGACCAGATCTACAATGTAATCGTTACAGCCCATGCCTTCGTTATAATT
TTCTTTATAGTCATACCAATTATAATCGGAGGCTTTGGAAACTGATTAATCCCCCTAATGATCGGGGCACCAGATATAGCATTCCCACGAATAAATAAC
ATAAGCTTCTGACTCCTACCTCCGTCCTTCCTCCTCCTCCTTTCTTCATCTGGAGTTGAAGCCGGCGCTGGTACCGGGTGGACAGTTTATCCCCCTCTA
GCCGGAAACCTTGCCCACGCAGGAGCATCTGTCGACTTAACCATCTTCTCCCTCCATTTAGCTGGAATCTCCTCAATTTTGGGGGCCATTAATTTTATT
ACGACCATTATCAACATAAAACCCCCAGCTATTTCTCAGTACCAAACCCCGCTTTTTGTCTGAGCTGTACTAATCACTGCTGTACTTCTACTATTATCA
CTCCCCGTTCTGGCAGCAGGTATTACTATGTTGCTCACAGATCGAAATTTAAACACCACTTTCTTTGACCCGGCGGGTGGCGGAGATCCAATTTTATAC
CAACACCTCTTTTGATTCTTCGGTCACCCAGAGGTCTATATTCTGATCCTCCCAGGCTTTGGTATAATTTCACATATCGTTGCATATTACTCTGGTAAG
AAAGAACCTTTCGGGTACATAGGAATAGTGTGAGCTATAATAGCCATCGGCTTGTTAGGATTTATCGTTTGAGCCCACCACATATTTACTGTCGGGATG
GACGTGGACACTCGTGCCTACTTTACATCTGCCACCATAATTATCGCTATCCCCACAGGAGTAAAAGTATTTAGCTGACTAGCTACACTGCACGGAGGC
TCGATCAAATGAGAGACACCACTTCTCTGAGCCCTAGGATTTATCTTCCTATTTACAGTGGGCGGATTAACGGGCATCGTCCTTGCTAACTCCTCATTA
GACATTGTTTTACATGACACTTATTACGTAGTCGCCCATTTCCACTACGTACTCTCAATAGGAGCTGTATTTGCCATTATGGGCGCTTTCGTACACTGA
TTCCCCCTATTCACAGGGTACACCCTTCACAGCACATGAACCAAAATCCATTTTGGAATTATATTTATCGGTGTAAATTTAACCTTTTTCCCACAGCAT
TTCCTAGGCCTCGCAGGGATACCACGACGGTACTCTGACTACCCGGACGCCTACACGCTATGAAACACTGTATCCTCAATCGGATCCCTTGTCTCCTTA
GTAGCTGTAATTATGTTCCTATTTATTCTTTGAGAGGCTTTTGCTGCCAAACGAGAAGTAGCATCAATCGAAATAACTTCAACAAACGTAGAATGACTA
CACGGATGCCCCCCACCCTACCACACATTCGAGGAACCAGCATTTGTCCAAGTACGAACGTACTAA
```

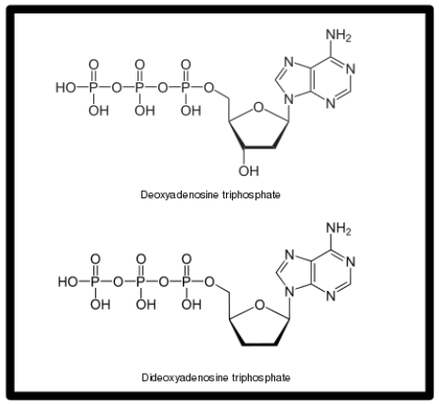
Sanger sequencing

① Reaction mixture

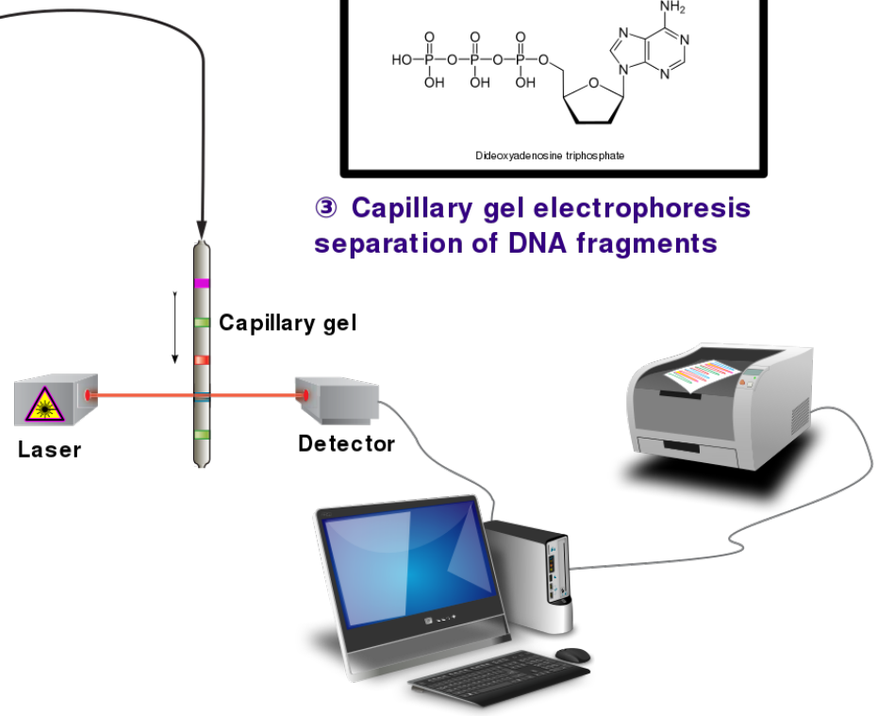
- **Primer and DNA template**
- **ddNTPs with flourochromes**
- **DNA polymerase**
- **dNTPs (dATP, dCTP, dGTP, and dTTP)**

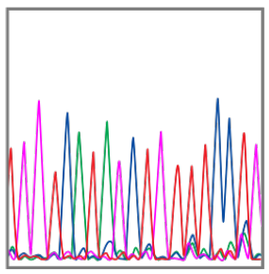


② Primer elongation and chain termination



③ Capillary gel electrophoresis separation of DNA fragments

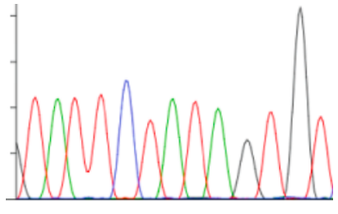




④ Laser detection of flourochromes and computational sequence analysis

Sanger read processing

Exercise 1



Trace file (F + R)

Basecalling

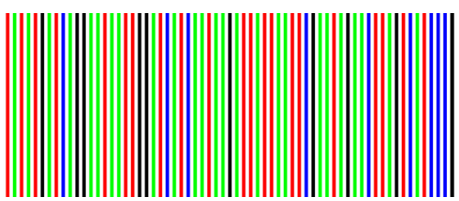
Fasta file (F+R)

Trimming

Reverse complement R

Align F and R

Alignment



Consensus

Create consensus sequence

Sequence alignment

Exercise 1

```
G A T G T T C G A A
G A T C – – – G A A
G A C C – T C G – T
```

Arranges nucleotide or amino acid sequences

so that the number of mismatches and gaps are minimized

- Multiple sequence alignments can be constructed progressively from pairwise alignments
- Computationally complex, often requires heuristic solutions
- Key to identify evolutionary relationships between sequences (e.g. homology)

Matching sequences to database with BLAST

Exercise 2

Algorithm overview

- Split query into very short segments (*k*-mers or words)
- Find exact matches between words and sequences in database (seeds)
- Extend matches to local alignments (HSP; stops once too many mismatches occur)
- Evaluate statistical significance of each HSP (e-value)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Oncorhynchus keta mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: OK_M08F	Oncorhynchus keta	1029	1029	100%	0.0	99.64%	772	LC094471.1
☑	Oncorhynchus keta mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds, isolate: OK_M01F	Oncorhynchus keta	1029	1029	100%	0.0	99.64%	772	LC094464.1
☑	Oncorhynchus keta isolate 10_Narva cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Oncorhynchus keta	1029	1029	100%	0.0	99.64%	655	KR778851.1

...

Genetic distances

Exercise 2

```
AAGCCAGCCAGGCGCTCTTCTAGGGGATGACCAGATCTACAATGTAATCG   # 50 positions total
AAGTCAACCTGGTGCACTTCTTGGTGATGATCAAATTTATAATGTGATCG
***.**.** **.** ***** ** ** **.**.**.**.*****.****   # 13 differences
```

Uncorrected distance

p = 13 / 50 = 0.26

K2P distance (Kimura 1980)

$$K = -\frac{1}{2}\ln((1-2p-q)\sqrt{1-2q})$$

K = 0.33

p: proportion of transitions (A<>G, C<>T)

8 / 50 = 0.16

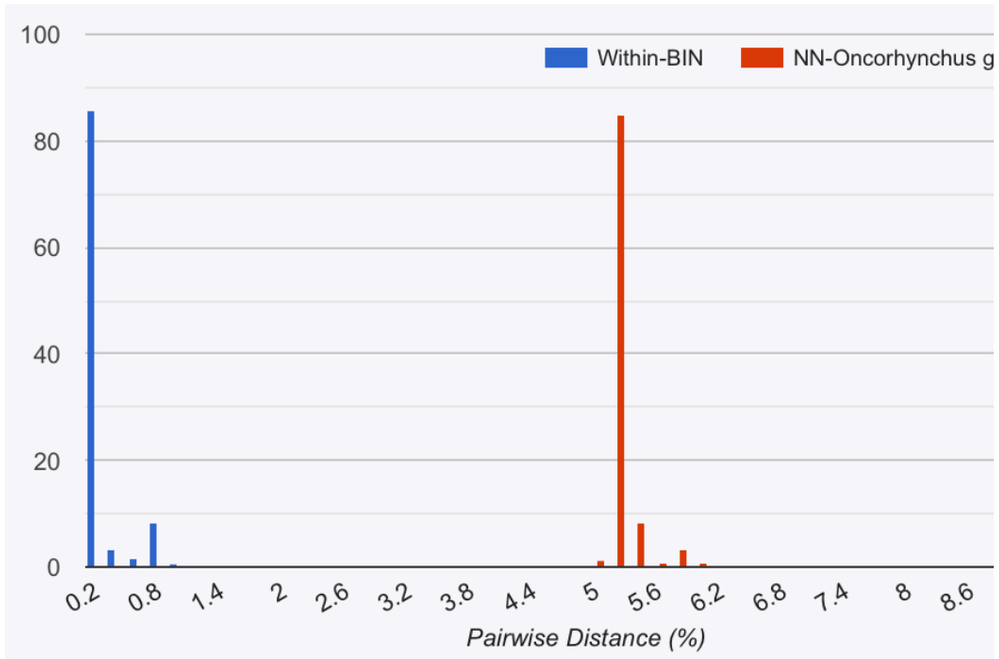
q: proportion of transversions

5 / 50 = 0.1

Barcode gap

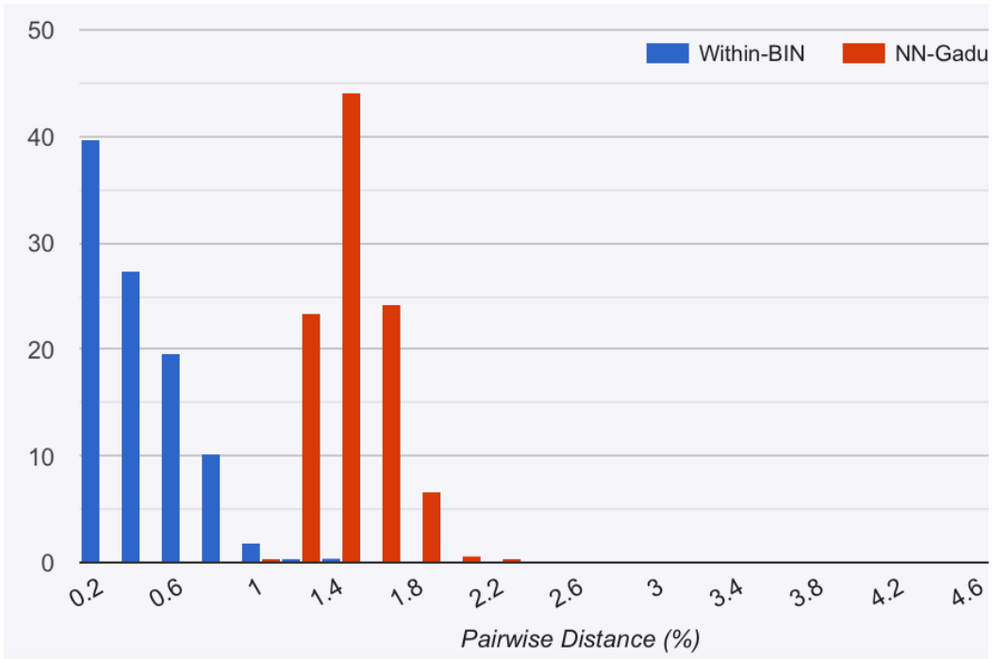
Exercise 2

Comparing genetic distances within BIN and to nearest neighbor (NN) BIN



Large barcode gap

Oncorhynchus keta

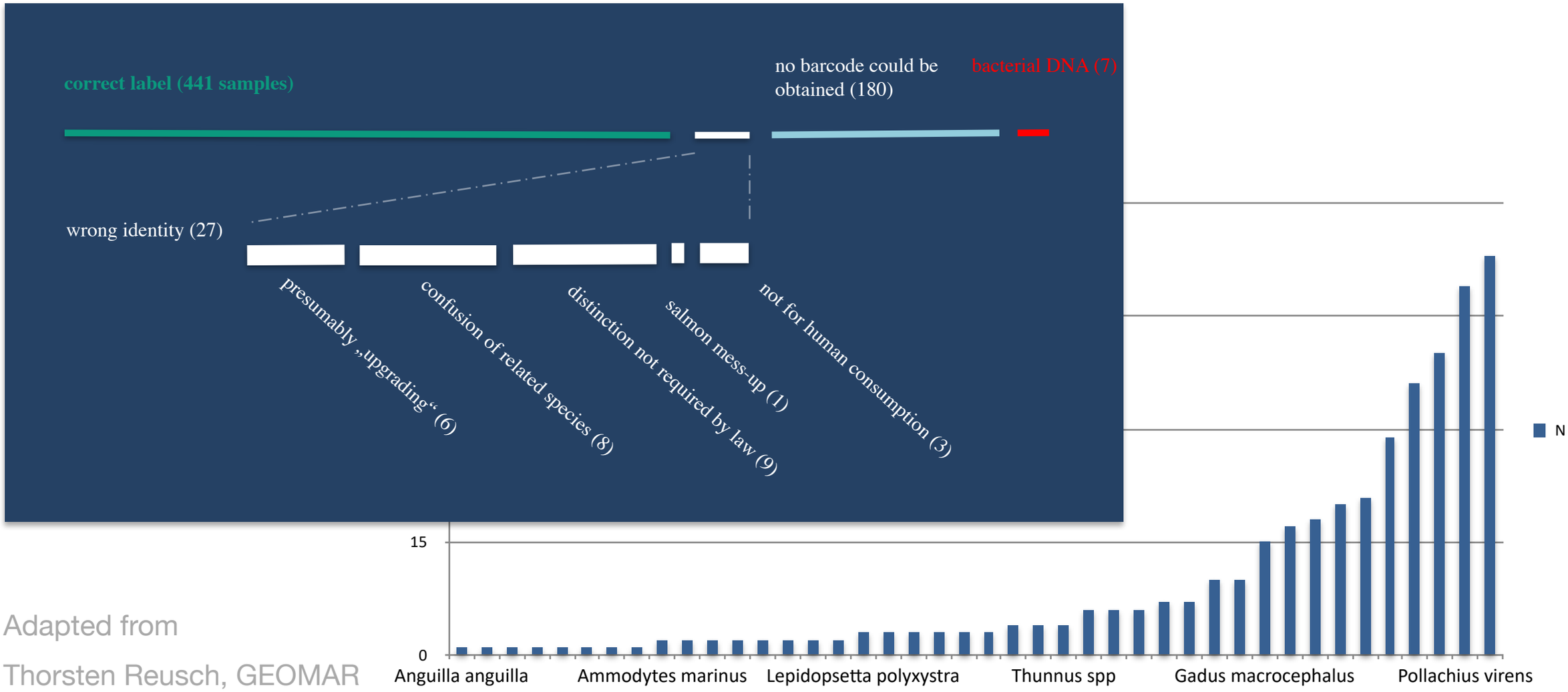


Small barcode gap

Gadus chalcogrammus

#fischdetektive results

Mislabeling seems to be only a moderate problem in Germany in frozen and fresh fish (but may be higher in Sushi-grade fish and processed fish products)

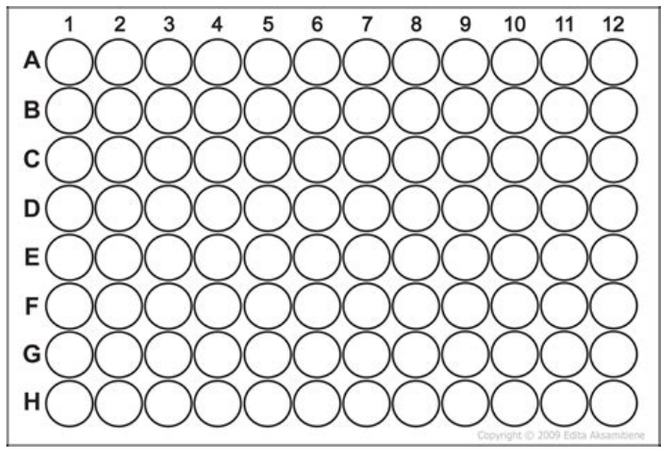


Adapted from

Thorsten Reusch, GEOMAR

Portable 3rd gen sequencing

Oxford Nanotechnologies MinION



96-well plate

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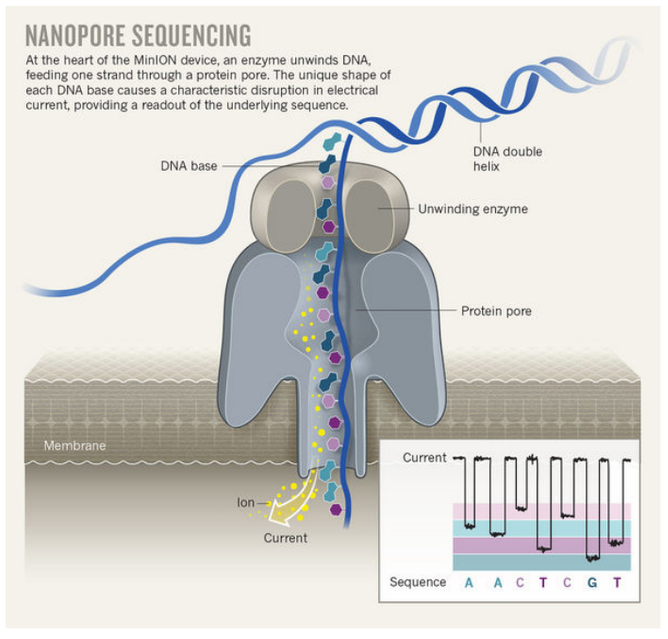
Indexing during PCR
Library preparation

whatech.com



blogs.nature

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.sh
08	Jun 09	Recombination and linkage disequilibrium	08_recomb.R
–	Jun 16	Student presentations	–
09	Jun 23	Selection and mutation	09_sel.R
10	Jun 30	DNA barcoding	10_barcode.sh
11	Jul 07	Metabarcoding I	
12	Jul 14	Metabarcoding II	