DNA barcoding
with Oxford Nanopore

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ARISE (*Authoritative and Rapid Identification System for Essential biodiversity information*)

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Naturalis Biodiversity Center

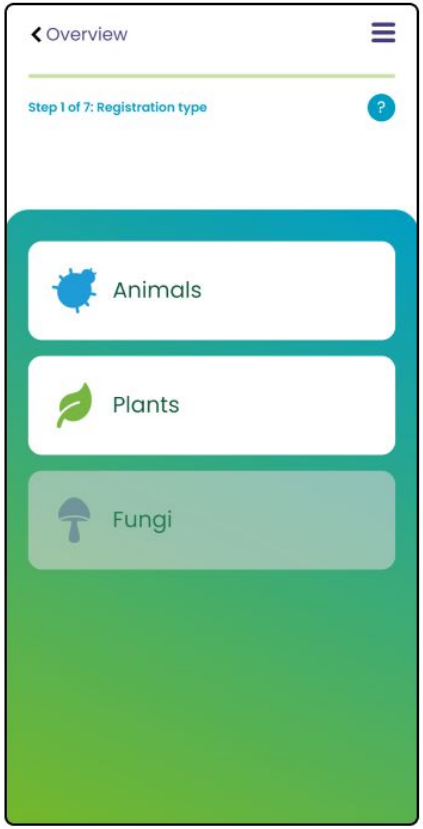
WESTERDIJK FUNGAL BIO DIVERSITY INSTITUTE

Upscaling

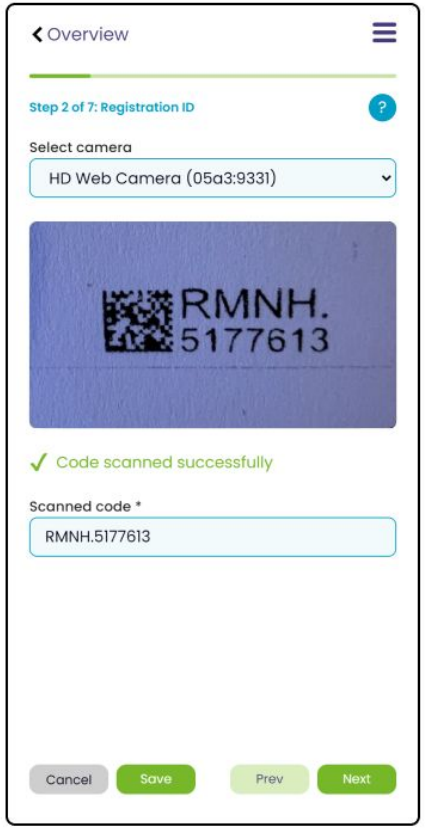
- Upscale DNA barcoding to **>100.000** samples/year
- Lower costs for barcoding to **<€2.00** per sample
- **Automation** (lab + bio-info)
- But do not compromise on **quality** of DNA extract!

Collecting

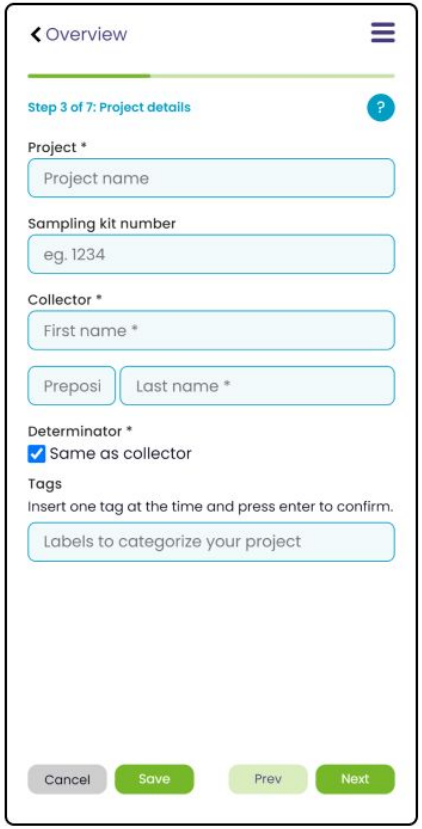
- Complete sampling kit with instructions and registration numbers for specimens
- Insects / plants / fungi
- Registration via mobile app for easy data collection
- Online target list



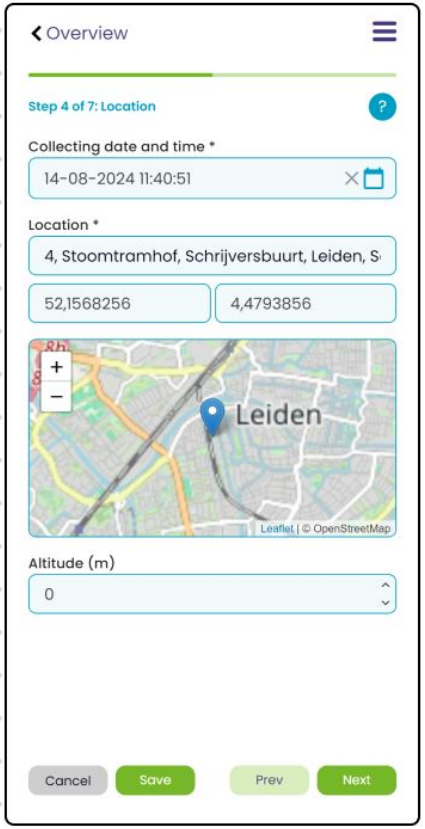
Select registration type



Scan or type ID code



Project details



Location

charts

Filter by: species | Search: Search 4458 result | Status: All | Occurrence status: All | Locality: Search countries | 4458 results found

Export

Order	Family	Genus	Species	Species status	Species with barcode	All	ARISE	Non-ARISE	Occ. status	Collected	Locality
Coleoptera	Cerambycidae	Chlorophorus	Chlorophorus diadema	Wanted	1	3	0	3	2d	0	Russia;South Korea
Coleoptera	Cerambycidae	Chlorophorus	Chlorophorus annularis	Wanted	1	3	0	3	2d	0	India;Japan
Coleoptera	Cerambycidae	Chlorophorus	Chlorophorus varius	Wanted	1	3	0	3	1a	0	Austria;France;Slovakia
Coleoptera	Cerambycidae	Pterolophia	Pterolophia angusta	Wanted	0	0	0	0	2d	0	
Coleoptera	Cerambycidae	Phoracantha	Phoracantha semipunctata	Wanted	1	15	0	15	2d	0	Australia;France;Italy;Portugal
Coleoptera	Cerambycidae	Apriona	Apriona germari	Wanted	1	16	0	16	2d	0	India;South Korea
Coleoptera	Cerambycidae	Apriona	Apriona rugicollis	Wanted	1	1	0	1	2d	0	Taiwan
Coleoptera	Cerambycidae	Xylotrechus	Xylotrechus arvicola	Wanted	1	5	0	5	1a	0	Austria;France;Germany
Coleoptera	Cerambycidae	Xylotrechus	Xylotrechus antilope	Wanted	1	13	0	13	1a	0	Austria;Belgium;France;Germany;Hungary;Russia;Slovakia
Coleoptera	Cerambycidae	Xylotrechus	Xylotrechus rusticus	Wanted	1	30	0	30	0a	0	Austria;Belgium;Bulgaria;Finland;France;Germany;Russia
Coleoptera	Cerambycidae	Xylotrechus	Xylotrechus rufilius	Wanted	1	17	0	17	2d	0	
Coleoptera	Cerambycidae	Batocera	Batocera lineolata	Wanted	1	26	0	26	2d	0	
Coleoptera	Cerambycidae	Batocera	Batocera rufomaculata	Wanted	1	7	0	7	2d	0	
Coleoptera	Cerambycidae	Acanthocinus	Acanthocinus griseus	Wanted	1	13	0	13	2d	0	
Coleoptera	Cerambycidae	Acanthocinus	Acanthocinus aedilis	Wanted	1	25	0	25	1a	0	
Coleoptera	Cerambycidae	Phytoecia	Phytoecia nigricornis	Wanted	1	16	1	15	1	0	
Coleoptera	Cerambycidae	Phytoecia	Phytoecia cylindrica	Wanted	1	11	0	11	1a	0	
Coleoptera	Cerambycidae	Psacothea	Psacothea hilaris	Wanted	1	54	0	54	2d	0	
Coleoptera	Cerambycidae	Ozodes	Ozodes multituberculatus	Wanted	1	11	0	11	2d	0	
Coleoptera	Cerambycidae	Calamobius	Calamobius filum	Wanted	1	9	0	9	0a	0	

Go to page: 1

1 of 223

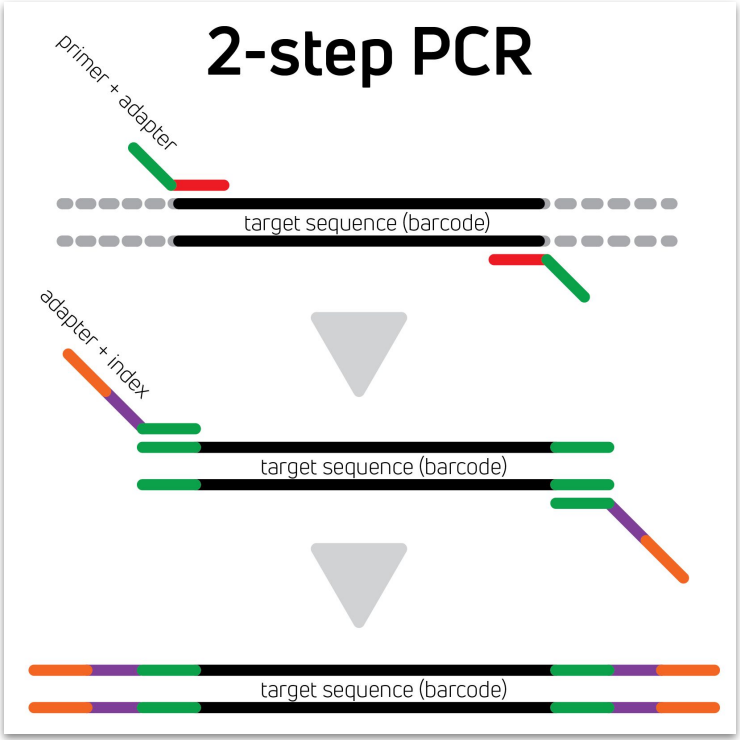
Extraction

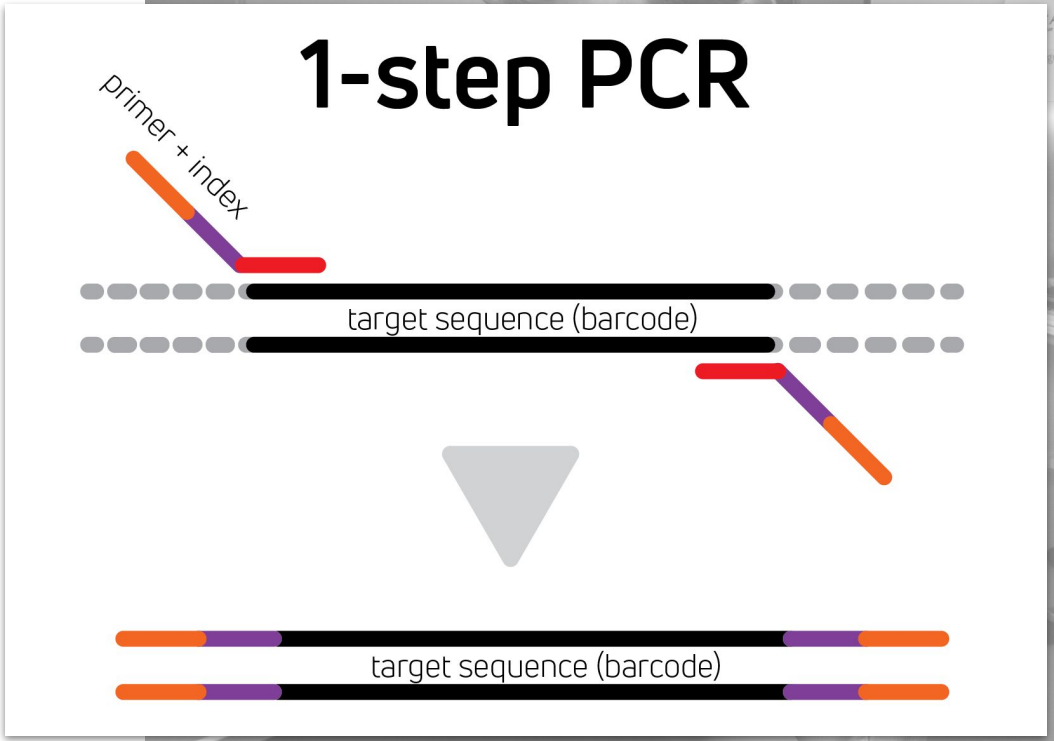
- Combination of the Mu-DNA and Bio-On-Magnetic-Beads (BOMB) protocols
- Serasil beads (commercial)
- Automated on Janus
- **€0.72** (vs. €4.00)
  50% of costs is plastics!

Sequencing

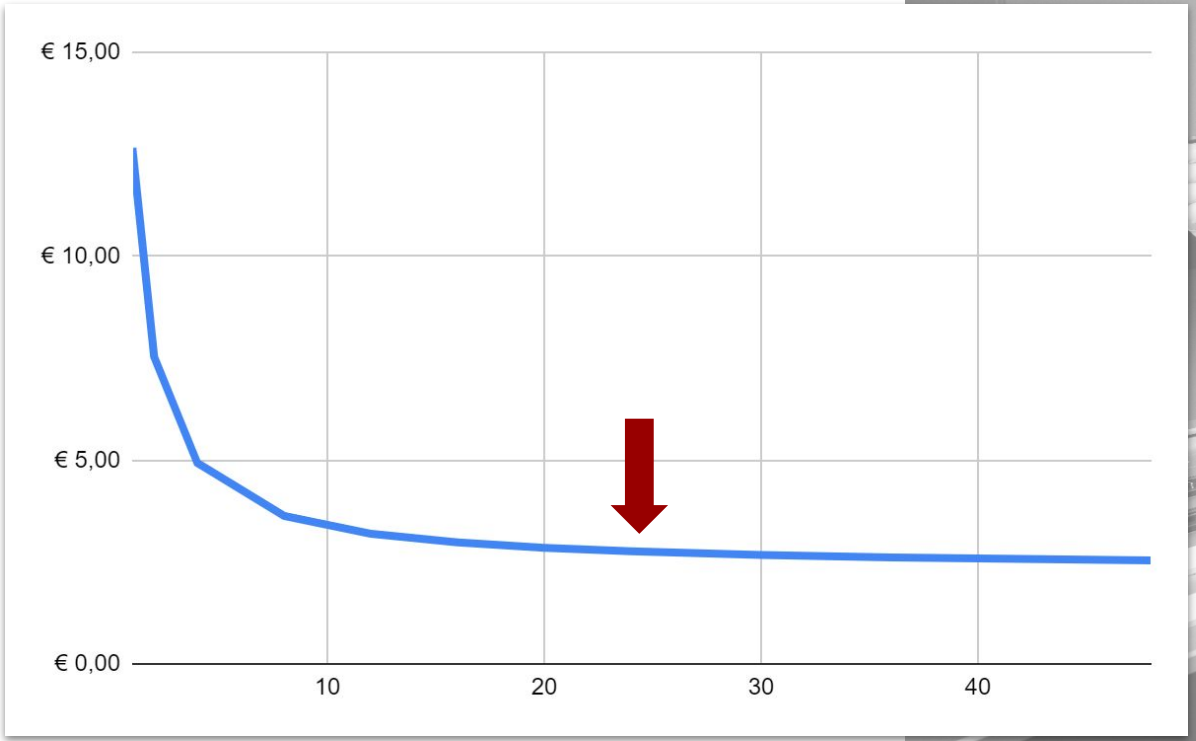
- 1-step PCR with (96) indexed primers
- ligation of (24) native barcodes to each plate pool
- 2265 samples per flowcell
- **€1.44** (vs. €5.00) amplification + sequencing

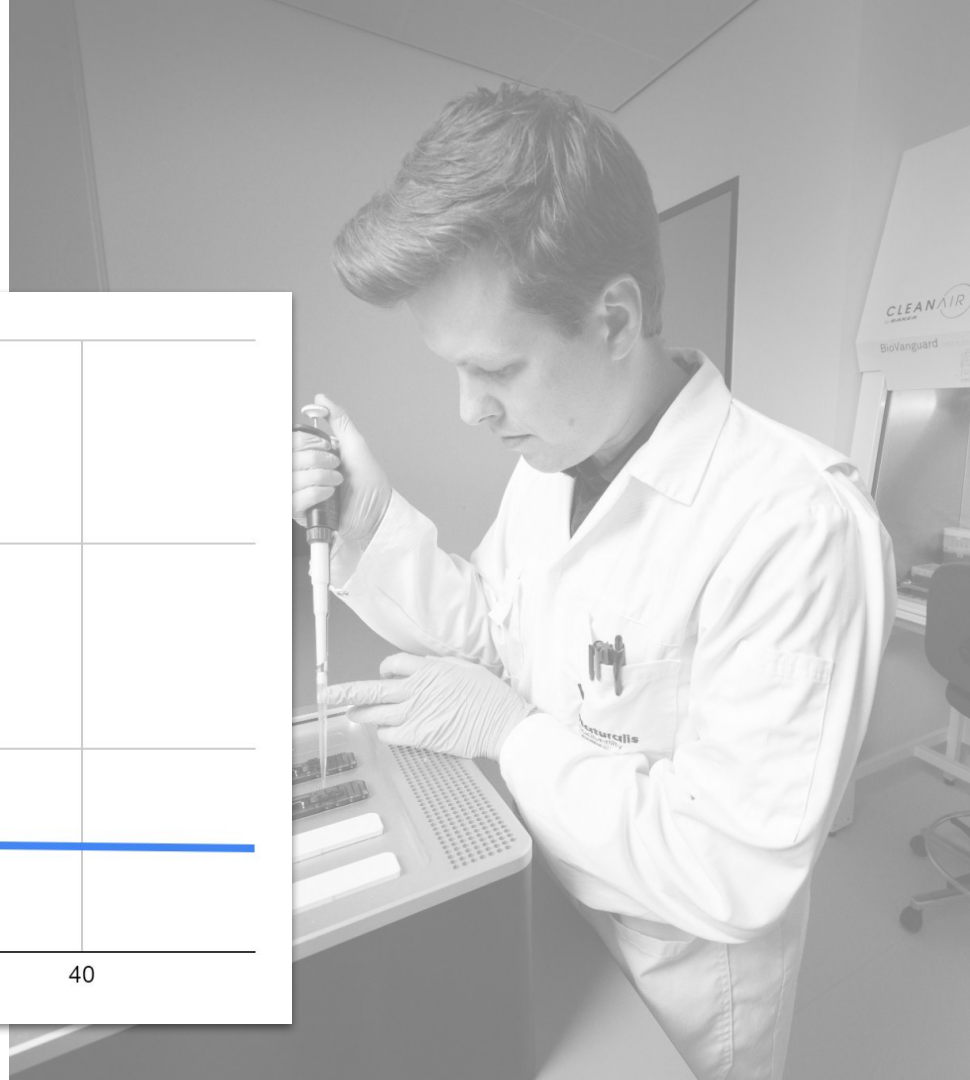
Sequencing

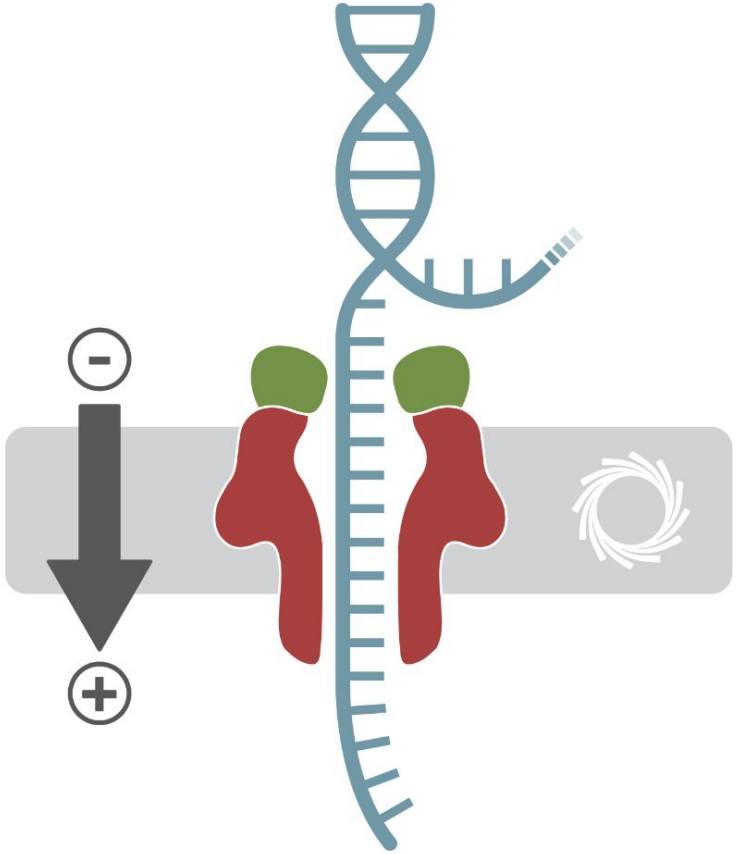

2-step PCR
primer + adapter
target sequence (barcode)
adapter + index
target sequence (barcode)
target sequence (barcode)

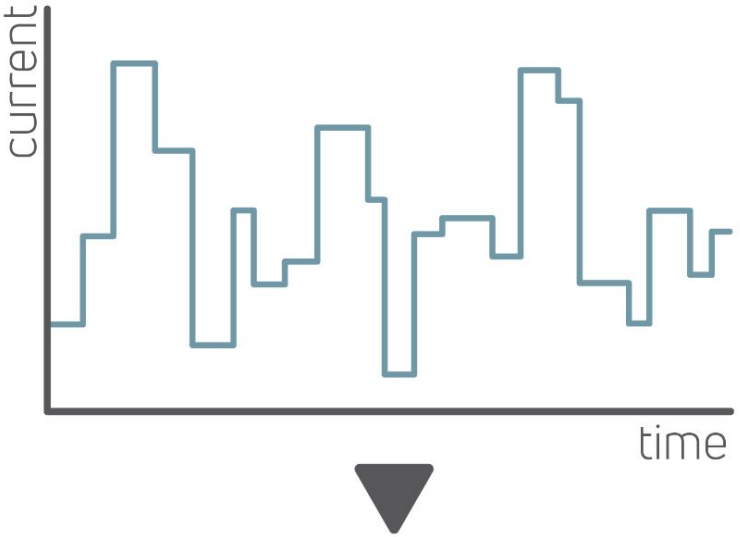

1-step PCR
primer + index
target sequence (barcode)
target sequence (barcode)

Sequencing







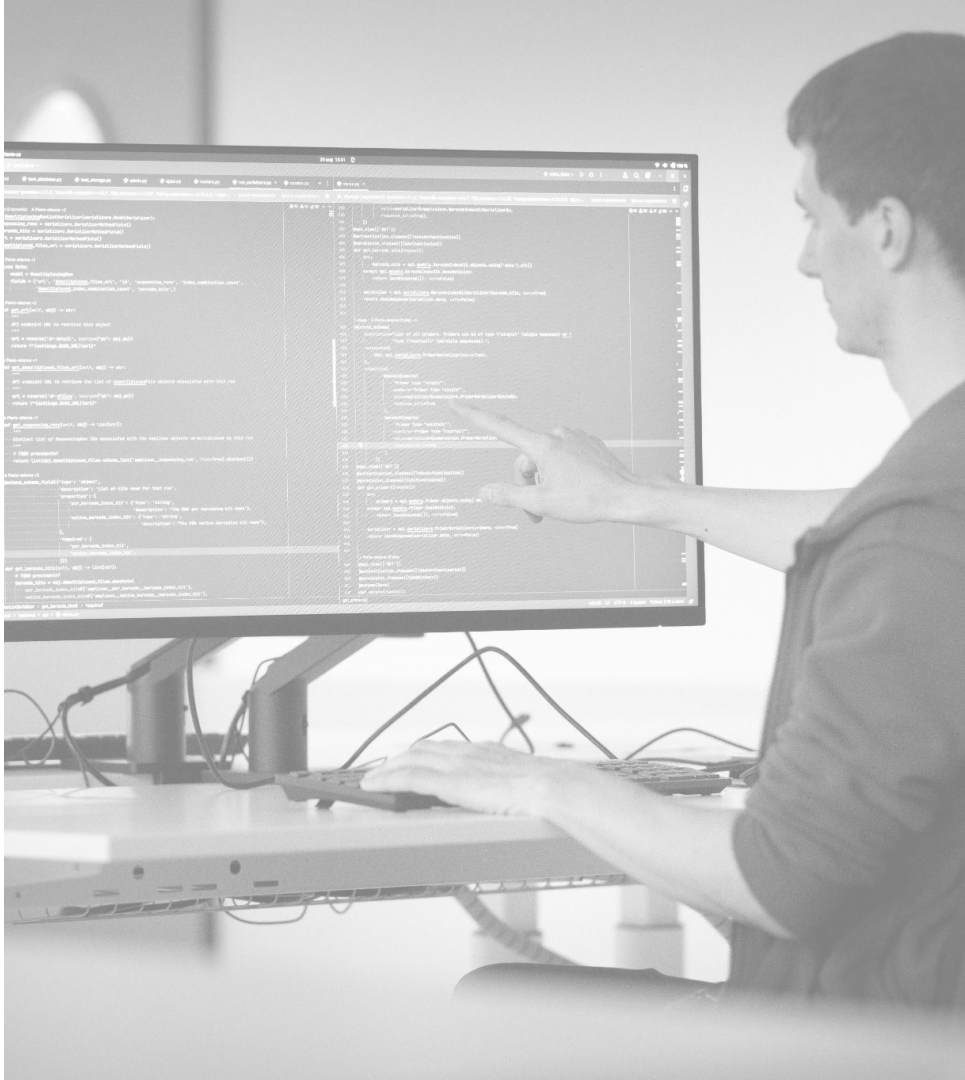


AACCTTGTATTTTATTTTTGG
GGCATGAGCAGGGATAGTAGA
ACATCATTAAGAATATTAATT
CGAGCAGAATTAGGAAATCCA
GGATCTCTTATTGGGGAT . . .

Oxford NANOPORE Technologies

Analysis

- Basecalling live GridION
- Custom demultiplexing pipeline using Guppy
- NGSpeciesID / Medaka to create consensus
- Data sent to Core Sequence Cloud via Django API



Details for Amplicon: RMNH.5142473~NI175~e1100037242

General Information:

Field	Value
Name	RMNH.5142473
Specimen Taxon	Insect
Sequencing Run	a0193cc2-3b67-48f4-b0ed-3c544730d292
Project ID	23009-5004000157
Label	[A]:RMNH.5142473 | NI175 | e1100037242 | COI-5P | ...
Is Control	false
Marker	COI-5P
Marker Min Length	658
Marker Max Length	658
Translation Table	5. Invertebrate Mitochondrial
Sample Plate ID	ARISE_SEQ_0135
Stock Plate ID	NCBN001674
PCR Plate Position	H06
Extract ID	e1100037242
PCR ID	NI175

Primer Information:

Field	Value
Forward Primer ID	ONT_LepF1+ONT_LCO1490
Forward Primer Type/Direction	cocktail / F
Forward Primer Sequences	ATTCAACCAATCATAAAGATATTGG, GGTCAACAAATCATAAAGATATTGG
Forward Primer Full Sequences	TTTCTGTTGGTGCTGATATTGCATTCAACCAATCATAAAGATATTGG, TTTCT...
Reverse Primer ID	ONT_LepR1+ONT_HCO2198
Forward Primer Type/Direction	cocktail / R
Reverse Primer Sequences	TAAACTTCTGGATGTCCAAAAAATCA, TAAACTTCAGGGTGACCAAAAAAT...
Reverse Primer Full Sequences	ACTTGCCTGTCGCTCTATCTTCTAAACTTCTGGATGTCCAAAAAATCA, ACT...

Barcode Information:

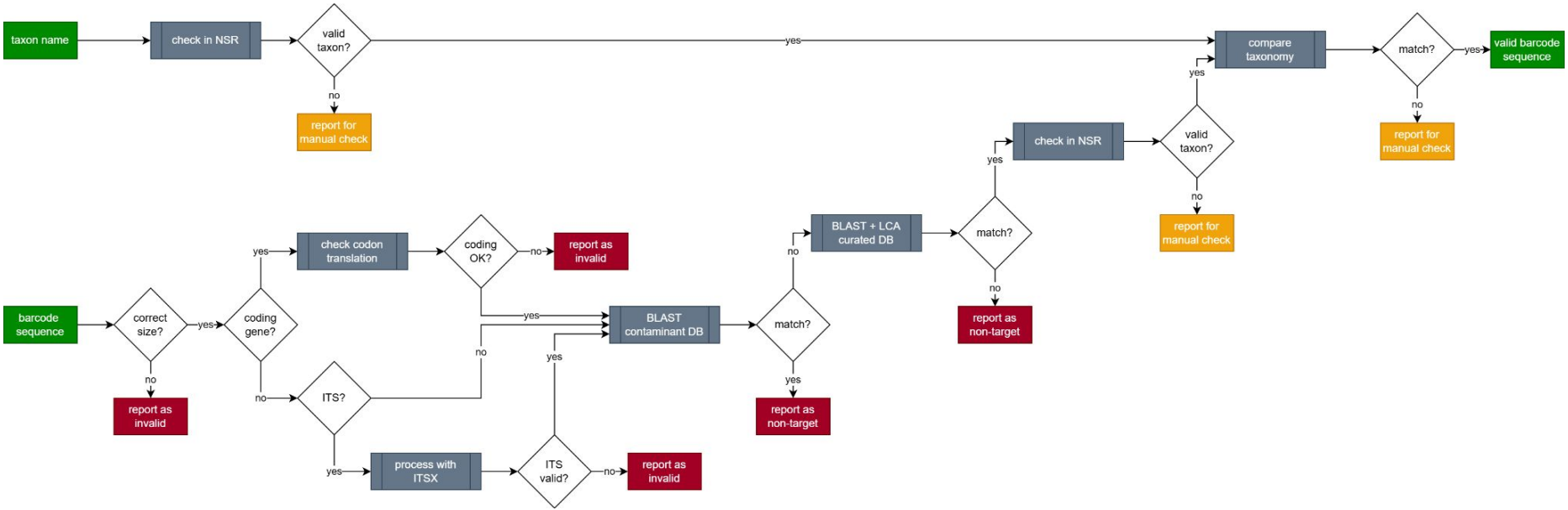
Field	Value
PCR Barcode ID	BC48
PCR Barcode Sequence	CATCTGGAACGTGGTACACCTGTA
PCR Barcode Kit	EXP-PBC096
Native Barcode ID	NB07

Statistics

- 21.123 samples
- 29.022 barcodes

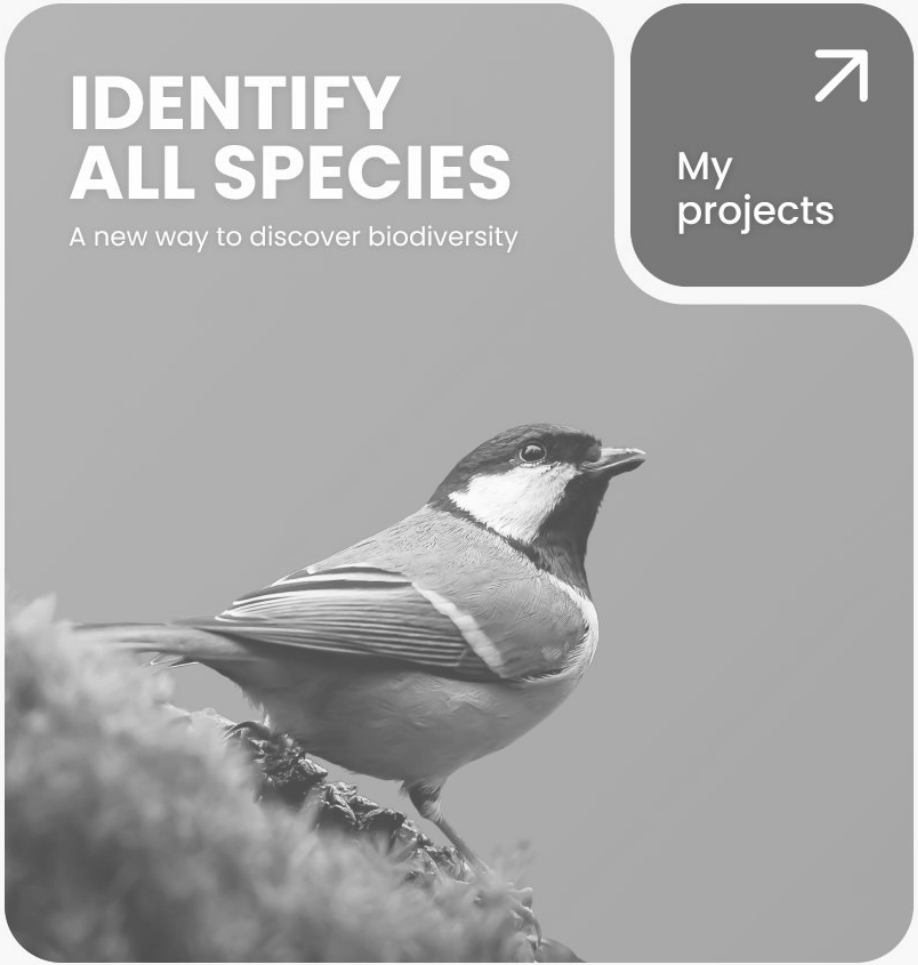
15.243 with 1 barcode (72.2%)
3.136 with 2 barcodes (14.8%)
2.744 with >3 barcodes (13.0%)
maximum **27** barcodes

Validation / Identification



Publication

- All sequence (meta)data and sample metadata collected in data lakehouse: **Core Sequence Cloud**
- “My Sample” dashboard for the collectors



Now you!

- ONT barcoding is relatively cheap to start with, and fits in any lab

MinION Mk1B

- Pocket-sized, portable device for biological analysis
- Simple 10-min sample prep available
- Real-time analysis for rapid, efficient workflows
- Adaptable to direct DNA or RNA sequencing

Packages from: **€1,900.00**

- Many online protocols and scripts, and an active user community

Now you!

- Training opportunities though the Biodiversity Genomics Europe program

Now you!

- Online seminars via the European iBOL node

iBOL EUROPE

publication about E2E workflow in preparation

thank you!

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