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An AI-Powered Online Platform for Food Species Authentication Using Oxford Nanopore Metabarcoding

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Try the platform
nu3gen.eu/metabarcoding/

01 Overview

- **Purpose:** one-step species authentication from raw ONT metabarcoding data
- **Input:** demultiplexed FASTQ files uploaded via web interface
- **Pipeline:** QC & length filtering → taxonomic assignment → per-read confidence classes
- **AI layer:** dominant vs minor taxa, mixed samples, uncertain assignments
- **Test run:** 16 samples, 39 426 reads; 86–93 % assigned (verified samples)

02 Introduction

- ONT: rapid, portable sequencing for food authentication [1–3]
- Metabarcoding: many species in one assay — meat, seafood, complex foods [2,5–8]
- Barrier: bioinformatics skills, command-line tools, expert interpretation [2,4]
- **Objective:** raw ONT reads → interpreted species report, no bioinformatics needed

03 Methods — end-to-end automated workflow

1 Upload — Demultiplexed raw FASTQ via web browser

2 Filter — Read quality & length filtering, sequence processing

3 Assign — Taxonomic assignment against reference sequence databases

4 Classify — Each read placed in one of six confidence categories

5 AI interpretation — Integrates assignments, read abundance & quality parameters [9–11]

6 Report — Simplified species report; detail kept for experts

Per-read categories: Assigned · Probable · Low-confidence · Ambiguous · Unassigned · Insufficient length

Test data & sequencing: run AMP23 · 16 barcoded samples with animal-derived DNA (MBC1–MBC16, barcodes 29–44) · marker: mitochondrial 16S rRNA minibarcode · Native Barcoding Kit 96 V14 (SQK-NBD114-96) · Flongle Flow Cell FLO-FLG114 · MinION Mk1C · R10.4.1 pore, E8.2 chemistry, 400 bases s^{-1} (MIN114_DNA_e8_2_400K)

04 Results

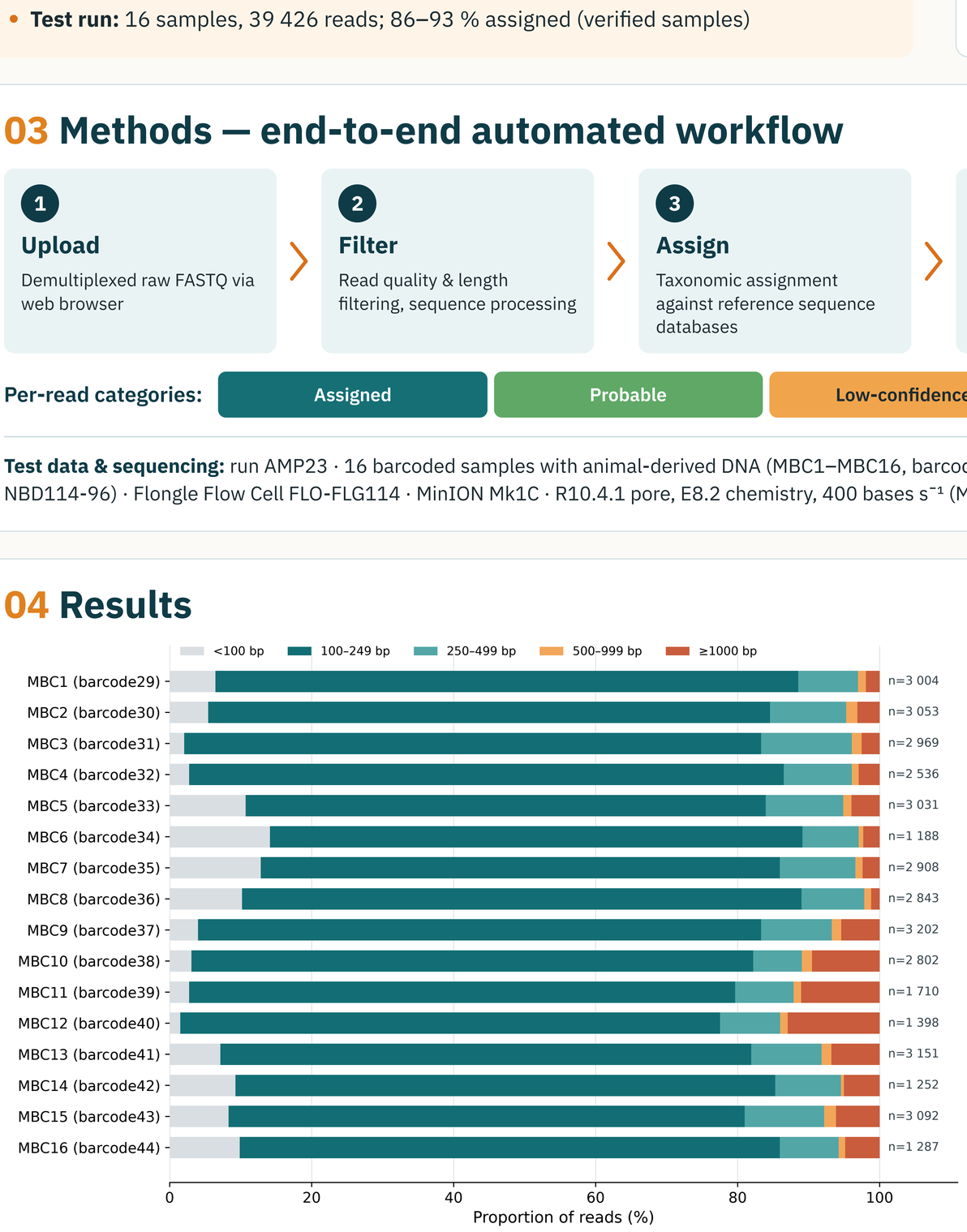


Figure 1. Read-length distribution, run AMP23 (n = demultiplexed reads); 100–249 bp reads dominate in every sample.

AUTOMATED SAMPLE REPORT — MBC1 · barcode29

Dominant species: ***Capra hircus*** GOAT

Total reads	Assigned	Low confidence
3 004	**2 611 (86.9 %)**	**145 (4.8 %)**
Unassigned	Insufficient length	Mixed sample
55 (1.8 %)	**193 (6.4 %)**	**No evidence***

Summary: Goat DNA was identified as the dominant component of the sample.

Dominant consensus sequence: `MBC1_barcode29_dominant_consensus`

DOWNLOAD CONSENSUS FASTA · COPY SEQUENCE · OPEN IN NCBI BLAST

* Minor-taxon composition is required for definitive mixed-sample reporting.

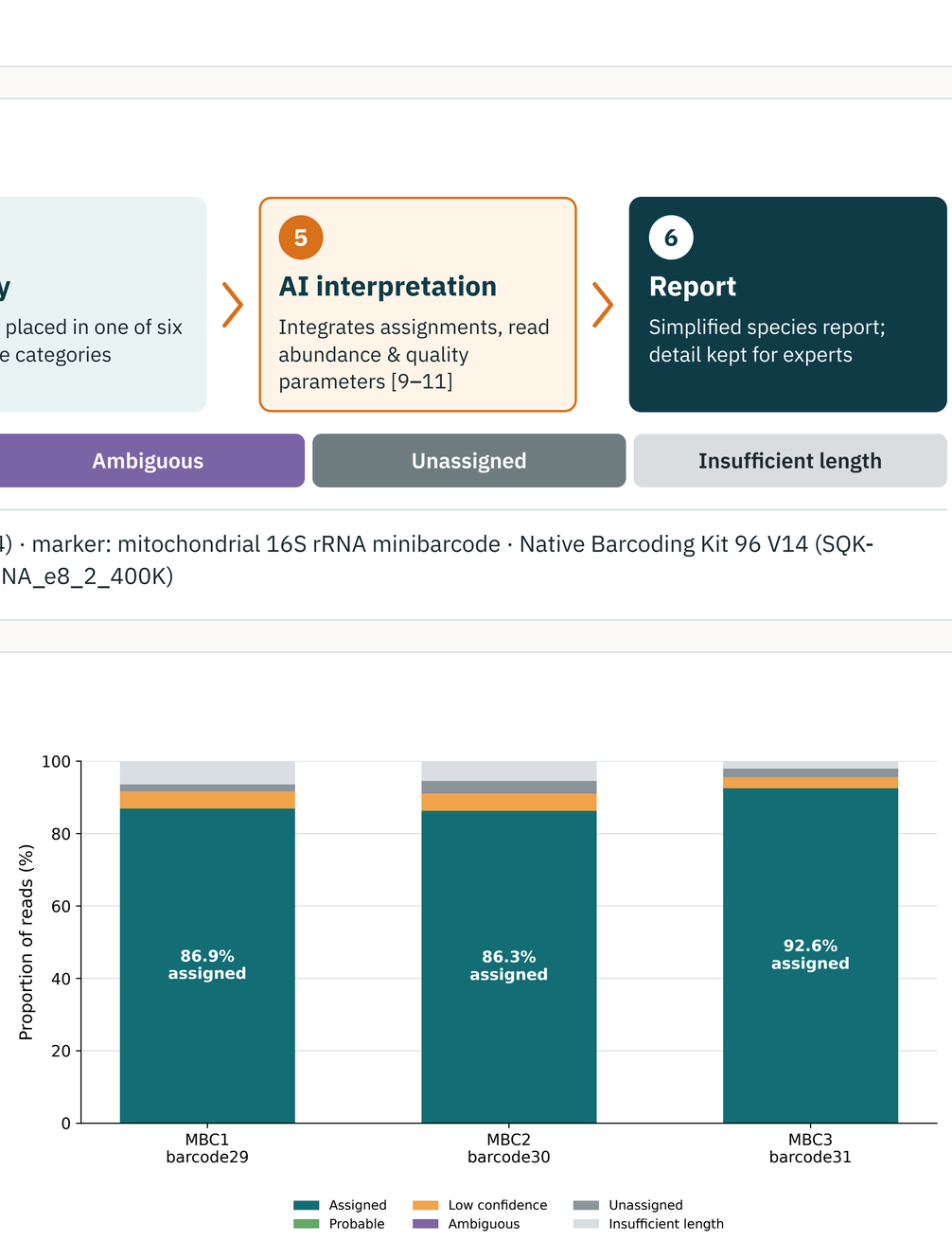


Figure 2. Classification outcome for samples with complete verified category counts (MBC1–MBC3).

Sample	Total reads	Assigned	Dominant species
MBC1 · bc29	3 004	86.9 %	*Capra hircus* (goat)
MBC2 · bc30	3 053	86.3 %	*Capra hircus* (goat)
MBC3 · bc31	2 969	92.6 %	*Bos taurus* (cattle)

Key findings

- 16 samples processed end-to-end from raw FASTQ: 39 426 reads (1 188–3 202 per sample)
- 86.3–92.6 % of reads confidently assigned in verified samples (MBC1–MBC3)
- Dominant species identified: goat (MBC1, MBC2), cattle (MBC3)
- Platform output translated automatically into a plain-language report (example: MBC1)
- Dominant consensus sequence exported as FASTA or sent to NCBI BLAST for independent verification

05 Conclusions

- Demanding metabarcoding workflow reduced to **upload → automated analysis → interpretation**
- Less dependence on local bioinformatics infrastructure & command-line expertise
- Use cases: authenticity control, undeclared animal species, rapid screening of complex foods [1–4]
- **Limitations:** prototype; full category counts verified for 3 of 16 samples; minor-taxon proportions not yet reported, so mixed-sample calls remain provisional
- Web service: **nu3gen.eu/metabarcoding/**

References

1. Voorhuijzen-Harink, M.M., et al. Food Chemistry: X (2019) 2: 100035.
2. Giusti, A., et al. Comprehensive Reviews in Food Science and Food Safety (2024) 23(1): e13256.
3. Sharma, A., et al. Annual Review of Food Science and Technology (2025) 16: 219–244.
4. Hakimzadeh, A., et al. Molecular Ecology Resources (2024) 24(5): e13847.
5. Fernandes, T.J.R., et al. Critical Reviews in Food Science and Nutrition (2021) 61(22): 3904–3935.
6. Böhme, K., et al. Journal of Agricultural and Food Chemistry (2019) 67(14): 3854–3864.
7. Staats, M., et al. Analytical and Bioanalytical Chemistry (2016) 408(17): 4615–4630.
8. Lo, Y.T., Shaw, P.C. Food Chemistry (2018) 240: 767–774.
9. Magdas, D.A., et al. Foods (2025) 14(10): 1808.
10. Osemwowa, E.U. Food Additives & Contaminants: Part A (2026) 43(6): 795–805.
11. Jiménez-Carvelo, A.M., et al. Food Research International (2019) 122: 25–39.
12. Nehal, N., et al. Critical Reviews in Biotechnology (2021) 41(5): 767–791.

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