

# Rapid, Portable Species-Identification with full-length mtDNA Sequencing to Combat the Illegal Wildlife Trade

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The illegal wildlife trade (IWT) is estimated to be worth £17 billion annually. Tackling IWT as a serious organized crime has been highlighted by the UK government as a priority [1]. In line with this, we have been developing a **rapid, portable, universal DNA test** to identify the species-of-origin of suspected IWT products (Figure 1). The test is designed to generate full-length mtDNA reads with no prior knowledge of the species being investigated and to be performed with minimal equipment. The use of this test by enforcement officials at docksides or airports enables the identification of illegally trafficked goods in circumstances where visual inspection is inconclusive. We have been applying this test to 'Europe's own Ivory trade', the smuggling of critically endangered and CITES appendix II listed European eel (*Anguilla anguilla*) out of the EU and into Asian markets, fetching up to £20,000 per kilogram of juvenile eel.



- (A) **DNA extraction, 10 min** – automated with a PDQeX device
- Enzymatic nDNA depletion, 5 min** – linear DNA is digested preserving supercoiled circular mtDNA
- Library preparation, 10 min** – RAD004 (or Field Kit)
- MinION sequencing, 60 min** – Flongle flow cells reduce per sample cost
- Species ID, 5 min** – *De novo* mtDNA consensus building and BLAST database search

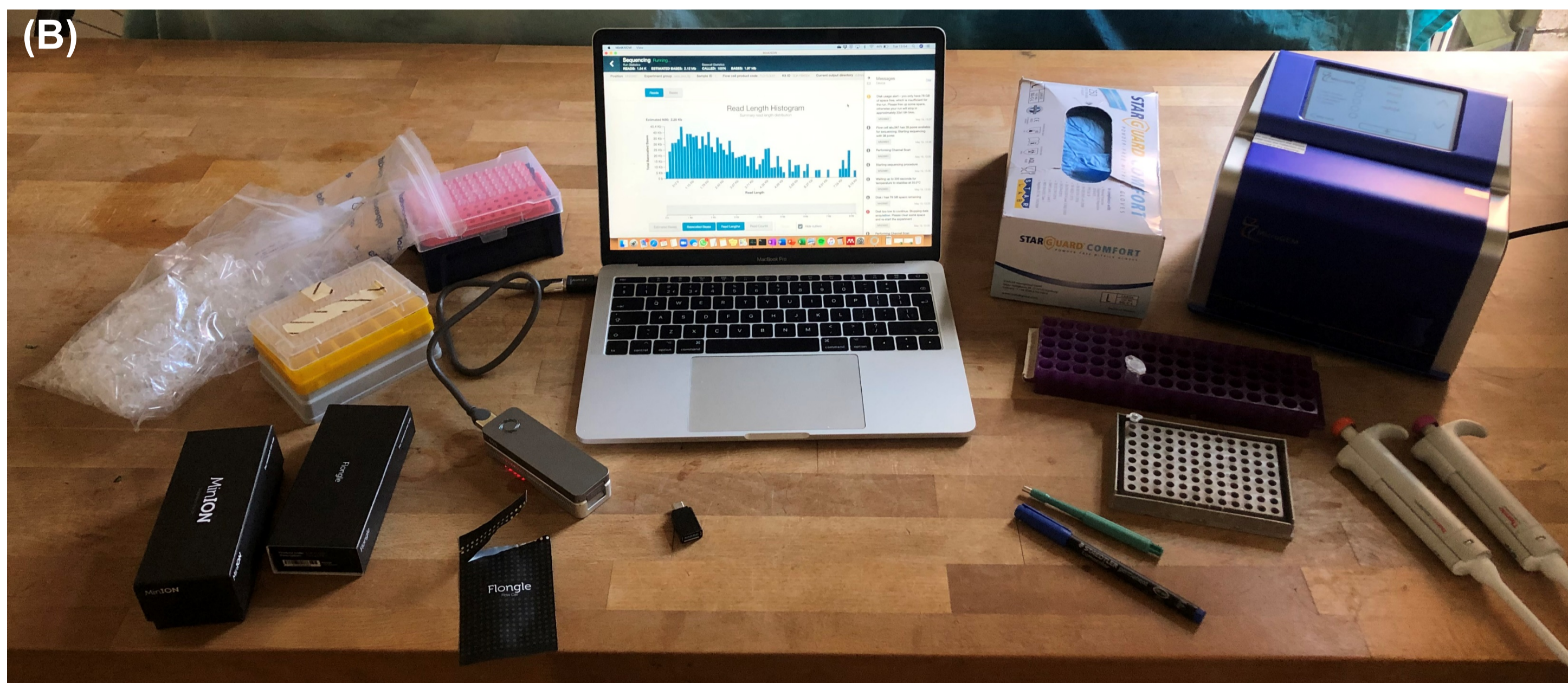


Figure 1: Overview of the species ID method (A) and all equipment required to carry out extraction and sequencing (B).

Validation of mtDNA enrichment by nDNA depletion was performed on human DNA and evaluated by gel electrophoresis (Figure 2) and qPCR (Figure 3), revealing a 64-fold increase in mtDNA relative to nDNA. nDNA depletion increased coverage of the complete human mtDNA sequence by more than double compared to an unenriched flowcell (Table 1). Compared against the slower and non-generic nCATS approach [2] nDNA depletion was able to generate 6-fold more coverage of the European eel mitogenome, enabling accurate identification of the sample in less than 90 minutes (Table 1).

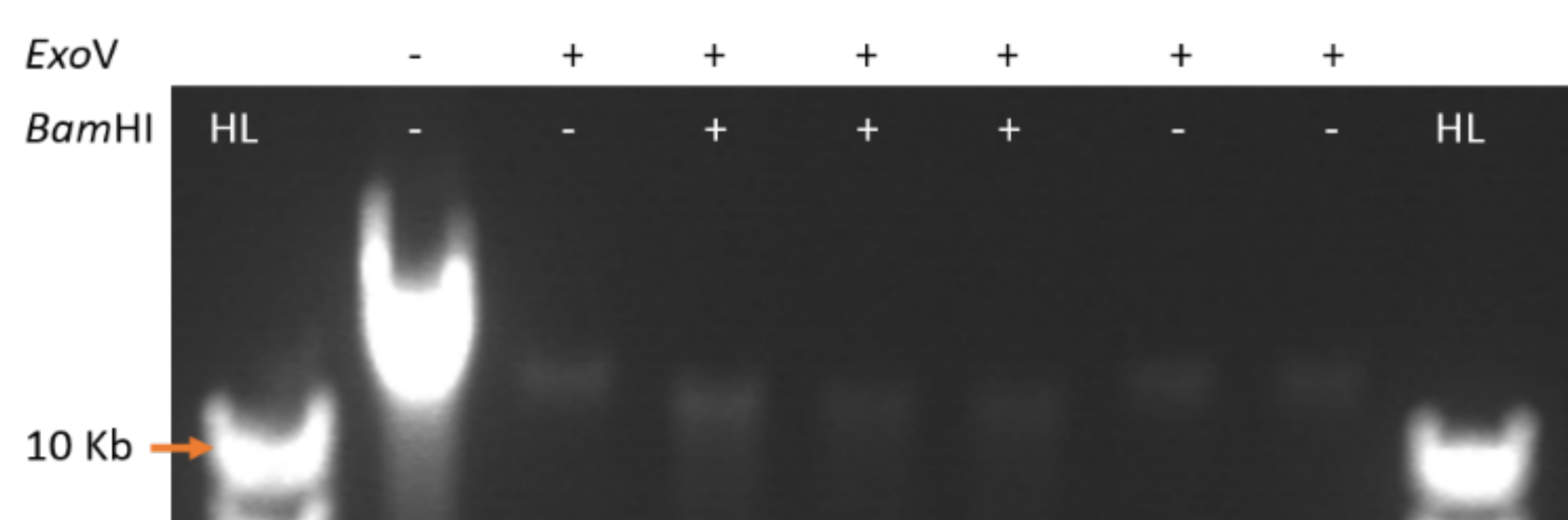


Figure 2: nDNA depletion of a human gDNA sample. Untreated gDNA (-/-) in comparison to ExoV treated (+/-) and ExoV/BamHI (+/+) treated gDNA. The mtDNA sample used is subsequently linearised by BamHI.

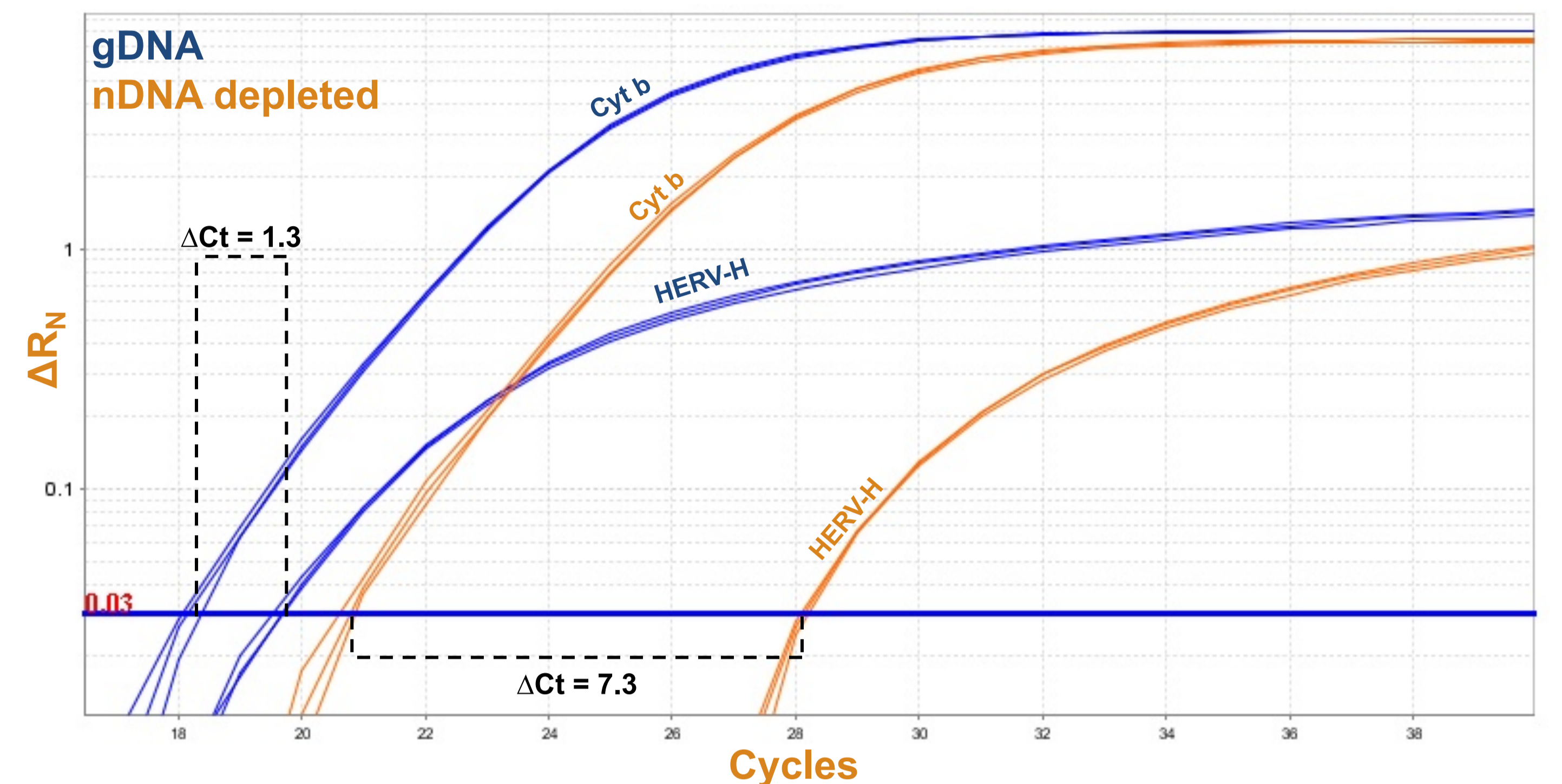


Figure 3: qPCR amplification of mitochondrial and nuclear DNA in genomic (blue) and n-depleted (orange) human DNA. TaqMan probes designed against the mitochondrial Cyt b gene and multicopy nuclear HERV-H genes were used as proxies for mitochondrial and nuclear DNA.

Sample	mtDNA reads (%)	Coverage of reference	Identity to reference (%)
Human control (unenriched)	0.24	18.3	98.86
nDNA depleted human	1.18	42.7	99.48
nDNA depleted <i>Anguilla</i>	0.45	33.2	99.21
nCATS <i>Anguilla</i>	0.35	4.7	97.91

Table 1: Comparison of mtDNA enrichment by nDNA depletion and published nCATS method.

Sequencing full length mtDNA enables greater resolution of closely related species such as European and Japanese eel (*Anguilla japonica*) by utilizing all of the species defining mtDNA variation (Figure 4). Through nDNA depletion we have successfully identified European eel in less than 90 mins generating a consensus sequence concordant with European eel at 841/845 species defining loci (Table 2); with the 650 bp cytochrome oxidase I (COI) barcode region alone this drops to 16/17 loci.

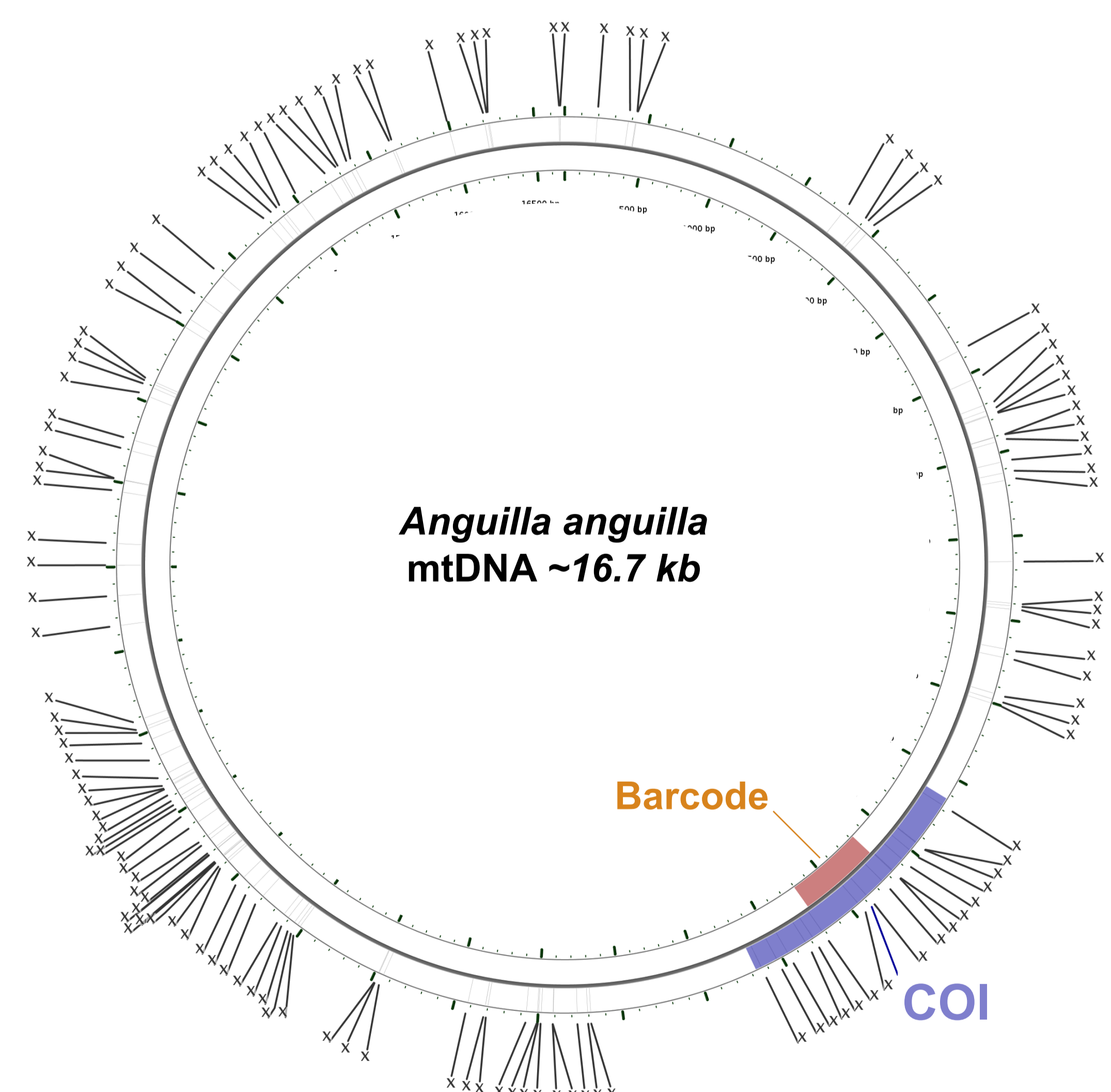


Figure 4: Ideogram of European eel specific mitochondrial DNA variation. The COI gene is highlighted in purple and the barcode region typically used for species identification in orange.

Eel species	Species-defining DNA variants			
	Full length mtDNA	European eel NS consensus	COI barcode	European eel NS COI barcode consensus
European (n = 55)	131	130	4	3
Japanese (n = 4)	714	3	13	0
American (n = 50)	98	0	3	0

Table 2: mtDNA variation in published eel mitogenomes. Live European glass eels/elvers which are not morphologically distinct from other species are typically trafficked under falsified permits as less protected species (e.g. the Japanese eel, *Anguilla japonica*).

## References

- [1] London Conference on the Illegal Wildlife Trade (October 2018): Declaration – [www.gov.uk/government/publications/](http://www.gov.uk/government/publications/)  
 [2] Gilpatrick et al. Targeted nanopore sequencing with Cas9-guided adapter ligation. *Nat Biotechnol* 38, 433-438 (2020)