

Workshop Title: Interpreting Non-Human Molecular Trace evidence

Venue:

i3S-Institute for Research and Innovation in Health, University of Porto,
Rua Alfredo Allen, 208
4200-135 Porto, Portugal

Day 1, 31 August, 09h – 17h:

- **“Non-Human Forensic Genetics: Methodologies, Applications, Exceptions, and Case Studies”**, by Nádia Pinto, i3S-Institute for Research and Innovation in Health, University of Porto, Portugal, and Henk Braig, National University of San Juan (UNSJ), San Juan, Argentina
 - Foundations and scope
 - ✓ Basic concepts in non-human forensic genetics.
 - ✓ Why non-human genetic material matters in forensic contexts.
 - ✓ When the field behaves like human forensic genetics and when it does not.
 - Methodologies
 - ✓ Sampling, extraction, quantification, and validation.
 - ✓ Marker types
 - ✓ Software and laboratory workflow, including sequence curation and quality control.
 - Interpretation
 - ✓ Database-based identification and its limits.
 - ✓ Statistical evaluation of evidence.
 - ✓ Phylogenetic and evolutionary inference, including recombination and model choice.
 - Applications
 - ✓ Zoology, botany, microbiology, and food forensics.
 - ✓ Species identification, individualization, kinship, population assignment, outbreak tracing, and traceability.
 - Exceptions and pitfalls
 - ✓ Nonstandard or difficult cases.
 - ✓ Low-quality databases, poor population structure, degraded samples, mixtures, and validation limits.
 - ✓ Situations where the usual forensic assumptions do not hold.
 - Case studies

Day 2, 01 September, 09h – 17h:

- **“Molecular Identification of Non-Human Biological Evidence: A Comprehensive Workflow”** (hands-on) by Filipe Pereira, Department of Genetics and Biotechnology (DGB), University of Trás-os-Montes and Alto Douro, Portugal
 - Basic concepts related to biological identifications using DNA
 - Overview of DNA sequencing in the laboratory

- Introduction to software for DNA sequence analyses
- Installation and use of the Geneious software for DNA sequence management
- Sequence curation and quality control (electropherograms, formats)
- Brief overview of alignment of DNA sequences and phylogenetic inferences
- Database-based identifications (BLAST, BOLD): strengths and pitfalls
- Examples of real cases of using DNA for biological identifications
- Data interpretation and reporting

Day 3, 02 September, 09h – 17h:

- **“Introduction to Biological Sequence Databases”** by Pedro G. Ferreira, Computer Sciences Department (hands-on), Faculty of Sciences of the University of Porto, Portugal
 - Sequence data formats and metadata.
 - Types of biological sequence databases (primary/non-curated and secondary/curated).
 - How sequences are organized, annotated, and linked.
 - Retrieving sequences efficiently.
 - Database quality assessment and common pitfalls.
 - Using sequence databases in non-human forensic genetics and metagenomics.

- **“Introduction to Metagenomic Data Analysis: From Raw Sequencing Reads to Microbial Profiles”** (hands-on), by Eran Elhaik, Department of Biology, Lund University, Sweden
 - Introduction to Metagenomics and Microbiome Research
 - ✓ What metagenomics is and why it is used
 - ✓ Difference between shotgun sequencing and amplicon sequencing
 - ✓ Applications in ecology, health, and forensic science
 - Understanding Sequencing Data
 - ✓ What sequencing reads are
 - ✓ Structure of FASTQ files
 - ✓ Paired-end sequencing and quality scores
 - Quality Control and Data Cleaning
 - ✓ Why sequencing data must be cleaned
 - ✓ Identifying sequencing errors and contaminants
 - ✓ Read trimming and filtering
 - ✓ Example tools: FastQC, MultiQC, fastp, Trimmomatic

Day 4, 03 September, 09h – 17h:

- **“Introduction to Metagenomic Data Analysis: From Raw Sequencing Reads to Microbial Profiles” - continuation**, by Eran Elhaik, Department of Biology, Lund University, Sweden

- Preparing Reads for Analysis
 - ✓ Handling paired-end reads
 - ✓ Removing host DNA contamination
 - ✓ Mapping reads to reference genomes
 - ✓ Example tools: repair.sh, Bowtie2, Samtools
- Microbial Identification and Classification
 - ✓ Assigning sequencing reads to microbial taxa
 - ✓ How reference databases are used
 - ✓ Generating microbial abundance tables
 - ✓ Example tools: Kraken2 and Bracken
- Amplicon Analysis and Microbial Community Profiles
 - ✓ Processing amplicon sequencing data
 - ✓ Merging reads and removing chimeras
 - ✓ Clustering sequences and generating OTU tables
 - ✓ Example tools: PANDAseq and VSEARCH

➤ **Workshop's challenge**