

Applied Genomics Project: Compliance Verification

Confirmation of Alignment with AG Course Requirements

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The project “**Hybrid Genome Assembly and Integrative Multi-Omics of Compost-Derived Fungal Isolates**” is fully aligned with the theoretical and practical modules of the *Applied Genomics* course. It integrates environmental sampling, hybrid sequencing, genome assembly, functional annotation, transcriptomics, comparative genomics, and translational outcomes relevant to biotechnology and EU bioeconomy policy.

1. Sampling and Organism Choice

- **Project:** Compost sampling (Bologna, Italy) with 20 fungal isolates; preliminary screening with PDA + PLA + Rhodamine B; 5 isolates expected to show strong degradative activity.
- **Course Topics:** Environmental genomics, targeted organism selection, biotechnology applications.
- **Justification:** PLA/PHA-rich compost represents a hotspot for fungi with unique hydrolytic capacities.

2. DNA/RNA Extraction and Quality Control

- **Project:** CTAB-based genomic DNA extraction; QC with Nanodrop, Qubit, gel (>20 kb). RNA-Seq libraries (12: Control, PLA, PHA × 4 replicates) validated with Bioanalyzer.
- **Course Topics:** NGS-grade nucleic acid preparation and QC.
- **Justification:** High integrity nucleic acids are prerequisites for long-read sequencing and reliable transcriptomics.

3. Hybrid Sequencing Strategy

- **Project:** Illumina NovaSeq PE150 (100× coverage) + Oxford Nanopore GridION (30–40× coverage).
- **Course Topics:** Sequencing platforms, trade-offs, hybrid strategies.

- **Justification:** Combines short-read accuracy with long-read contiguity for reference-quality assemblies.

4. Genome Assembly and QC

- **Project:** Flye (Nanopore-first assembly) + Pilon (Illumina polishing). Quality assessed with QUAST and BUSCO (*fungi_odb10*); completeness expected >90%.
- **Course Topics:** Assembly metrics (N50, L50, GC%), completeness benchmarking.
- **Justification:** Ensures reliable representation of gene space for downstream functional studies.

5. Functional Annotation

- **Project:** MAKER3 with Augustus + GeneMark-ES; CAZyme annotation via db-CAN3; manual curation with Geneious. Expected to reveal cutinases, esterases, lipases, PHA depolymerase-like proteins.
- **Course Topics:** Gene prediction, domain-based functional annotation, manual model validation.
- **Justification:** Multi-layer annotation links sequence evidence with functional enzyme repertoires.

6. Transcriptomics

- **Project:** RNA-Seq quantified with Salmon, summarized by tximport, DEG analysis with DESeq2 (FDR <0.05, $|\log_2 FC| \geq 2$). Expected: PLA-induced esterases, lipases, cutinases.
- **Course Topics:** RNA-Seq design, quantification, DEG analysis.
- **Justification:** Links genome predictions with inducible enzymatic responses under PLA/PHA.

7. Comparative Genomics and Phylogenomics

- **Project:** OrthoFinder for orthogroups; MCSanX + MAUVE for synteny; phylogeny from single-copy orthologs (MAFFT, Gblocks, AMAS, RAxML, MrBayes). Comparative focus: degraders vs. non-degraders.
- **Course Topics:** Orthology inference, genome rearrangements, phylogenetic reconstruction.
- **Justification:** Provides evolutionary context for plastic-degrading capacity.

8. Expected Outcomes and Bioeconomy Relevance

- **Project:** Predict high-contiguity assemblies, rich CAZyme repertoires, inducible hydrolase genes, and comparative signatures of degraders.
- **Course Topics:** Translational genomics, environmental applications, EU Circular Economy goals.
- **Justification:** Builds a molecular resource for enzyme discovery and sustainable composting strategies.

Integrated Checklist

- Targeted sampling and isolate screening
- DNA/RNA extraction with stringent QC
- Hybrid sequencing (Illumina + Nanopore)
- Genome assembly + BUSCO/QUAST metrics
- Functional annotation (MAKER3, dbCAN3, manual curation)
- Transcriptomics (Salmon + DESeq2)
- Comparative genomics + phylogenomics
- Application to EU bioeconomy and compost valorization