INCT Fungi of Brazil

SOP 1: DNA Extraction, Quality Control, and Shipping

> **Disclaimer:** *This document was translated from brazilian portuguese with the assistance of generative AI (Gemini).*

User Manual

Objective:

To guide INCT Fungi of Brazil researchers on extraction protocols, purification procedures, and minimum quality/quantity specifications for submitting genomic DNA intended for Illumina PCR-free library sequencing.

1. Culture Purity Verification

> **CAUTION:** This is a critical prerequisite prior to DNA extraction. Whole-genome sequencing is highly sensitive and will detect any contaminating organism. Contamination by bacteria, yeasts, or foreign fungi compromises genome assembly and analysis, leading to wasted resources and required resequencing.

1.1. Monosporic Cultures (Strongly Recommended)

To ensure genetic homogeneity, DNA extraction should be performed from monosporic cultures (derived from a single spore). This ensures all material originates from a single individual and eliminates intra-strain genetic polymorphism. Cultures derived from mycelial fragments or multi-spore isolates may contain genomic variants that impair de novo assembly.

1.2. Visual and Microbiological Inspection

Prior to extraction, thoroughly inspect the culture:

Culture Type	What to Check	Signs of Contamination (DO NOT extract)
Solid Media (Plate)	Uniform colony morphology, color, and texture. Absence of satellite colonies or atypical zones.	Bacterial colonies (shiny, mucoid spots); distinct fungal morphotypes; irregular colored borders.
Liquid Media (Broth)	Homogeneous mycelial pellets or mat. Clear broth between mycelial masses.	Turbid medium (classic bacterial contamination); surface biofilm; discoloration or off-odors; atypical structures.

1.3. Remediation for Suspected Contamination

If contamination is suspected, abort DNA extraction and proceed with:

- Subculturing onto fresh media using hyphal tip isolation from a clean zone.
- Re-isolating a single spore for a new monosporic culture.
- Performing serial dilution of hyphal fragments and replating if the fungus does not sporulate.
- Allowing complete regrowth and repeating visual verification prior to extraction.

1.4. Optional Microscopic Confirmation

When feasible, inspect culture mounts under optical microscopy (400x to 1000x) to confirm the absence of bacterial cells (rods or cocci) among hyphae.

2. DNA Extraction Guidelines

Isolating high-molecular-weight, intact DNA is essential for sequencing success. Rigorous extraction protocols must be maintained.

- **Extraction Method:** Specific commercial kits are not mandated, but the exact protocol used must be declared in the submission spreadsheet (e.g., CTAB, Qiagen DNeasy Plant Kit, Zymo Research Kit, Phenol-Chloroform, etc.) to aid troubleshooting if sequencing issues arise.

General Recommendations:

Aspect	Recommendation
Starting Material	Use young (3–7 days for most filamentous fungi), healthy cultures to minimize secondary metabolites and polysaccharides.
Cell Lysis	Avoid prolonged vortexing during lysis to prevent DNA shearing. Prefer liquid nitrogen grinding or short-pulse bead beating.
Melanized Fungi	For melanized or polysaccharide-rich species, include additional clean-up steps (e.g., CTAB/NaCl precipitation or column purification).
RNase Treatment	Treat with RNase A to remove RNA, which interferes with accurate quantification and library prep.
Post-Extraction Storage	Store at -20°C (short-term) or -80°C (long-term). Avoid repeated freeze-thaw cycles.

3. Minimum DNA Quality and Quantity Standards

Parameter	Minimum Requirement	Notes
Total DNA Amount	≥ 500 ng	Required for PCR-free library preparation.
Minimum Concentration	≥ 10 ng/µL	Higher concentrations preferred when possible.
Sample Volume	20 µL to 50 µL	—
Suspension Buffer	Ultrapure water or EDTA-FREE buffer	Standard TE buffer is prohibited; EDTA inhibits downstream enzymatic steps.
Purity (A260/A280)	1.6 to 2.0	Measured via NanoDrop or equivalent spectrophotometer.
Purity (A260/A230)	≥ 1.8	Measured via NanoDrop. Lower values indicate carbohydrate, salt, or phenol carryover.
Integrity (Size)	> 50% of fragments > 2 kb	Verified by 0.8%–1% Agarose Gel or Bioanalyzer/TapeStation. Smeared/degraded DNA is unacceptable.
Official Quantification	Fluorometric Assay (e.g., Qubit)	Final reported concentration must be fluorometric. NanoDrop overestimates DNA concentration.

4. Local Packaging and Handling

Store samples in sterile, DNase/RNase-free 1.5 mL or 2.0 mL microcentrifuge tubes. Clearly label tube caps and sides with the **Sample ID** (matching the submission spreadsheet) using an alcohol- and freeze-resistant permanent marker. Maintain at -20°C or -80°C until dispatch to the Focal Point/Regional Hub.

5. Labs Without Qubit or NanoDrop Access

The INCT Fungi of Brazil operates as a collaborative network. If your laboratory lacks fluorometric (Qubit) or spectrophotometric (NanoDrop) equipment, follow the National Logistics Plan: extract DNA, perform preliminary agarose gel validation, and ship samples to your designated Regional Support Hub for full quality validation before submission to the National Focal Point.

6. Required Documentation

Every submission must include a completed **Spreadsheet 1: DNA Sample Submission**, delivered digitally to the Committee with a hard copy accompanying the physical shipment.