



INCT Fungi of Brazil

National Logistics Plan

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

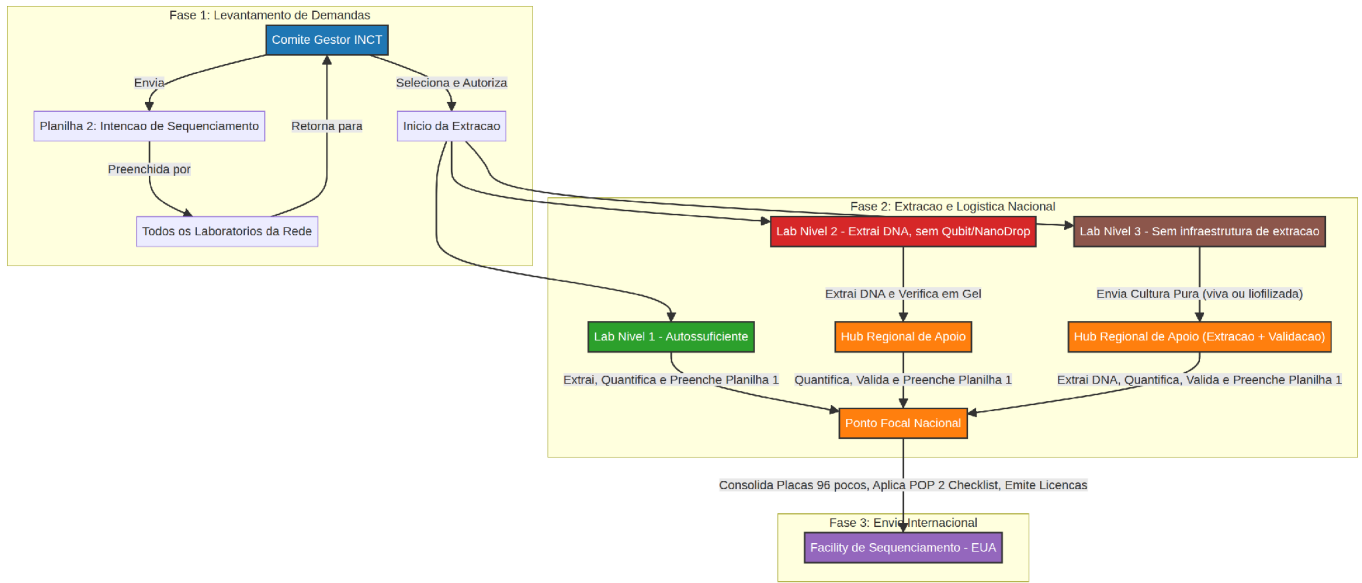
DNA Extraction, Validation, and Shipping Workflow for Sequencing

Objective:

To establish an efficient, collaborative network ensuring that all INCT Fungi of Brazil laboratories —regardless of local infrastructure—can extract and ship high-quality genomic DNA to the sequencing facility in the USA.

1. Logistics Workflow Chart

The workflow is designed to optimize resources and guarantee the rigorous quality control required by the facility (*PCR-free library prep*).



2. Network Laboratory Classification

For logistical purposes, network laboratories are classified into three categories based on available infrastructure. This classification allows the Committee to assess the required support and realistically plan the sample flow.

Level	Designation	Available Infrastructure	Role in the Workflow
Level 1	Self-sufficient	DNA extraction, Qubit (fluorometry), NanoDrop (spectrophotometry), agarose gel	Extracts DNA, conducts all quality and purity analyses (SOP 1), fills out Spreadsheet 1, and ships validated samples directly to the National Focal Point .
Level 2	Partially Dependent	DNA extraction and agarose gel, but without Qubit and/or NanoDrop	Extracts DNA following SOP 1, checks preliminary integrity on gel, and ships (frozen) samples to the Regional Support Hub for final quantification and validation.
Level 3	Fully Dependent	Lacks infrastructure for both DNA extraction and quantification	Maintains and cultures strains of interest, ensures culture purity (monosporic cultures), and ships live cultures (or freeze-dried/frozen mycelium) to the Regional Support Hub or a Level 1/2 partner lab to perform extraction and quantification.

Level 3 Laboratory Details:

These laboratories play a fundamental role in the network by housing strains of taxonomic, ecological, or biotechnological interest, even without the necessary equipment for DNA preparation. Their contribution is enabled by the collaborative framework of the INCT. The workflow for these laboratories is as follows:

1. The Level 3 Laboratory researcher fills out **Spreadsheet 2 (Sequencing Request)** as usual, indicating in the infrastructure column that full support is required.
2. Following Committee approval, the researcher prepares a pure culture (monosporic, visually verified per SOP 1) and ships it to the Regional Hub or designated partner laboratory.

3. The Regional Hub/partner carries out DNA extraction, quantification (Qubit), purity assessment (NanoDrop), and integrity check (gel), fills out Spreadsheet 1, and forwards everything to the National Focal Point.
4. The originating researcher remains the scientific lead for the sample and must provide all associated metadata (genus, species, strain, genome size).

3. Support and Consolidation Structure

To enable this workflow, the INCT relies on strategic support centers:

Regional Support Hubs:

- **Definition:** Strategically located anchor laboratories (e.g., one in the Southeast, one in the Northeast, one in the South/Center-West) equipped with robust infrastructure and trained personnel.
- **Role:** Receive samples from Level 2 Laboratories within their geographical region. The Hub performs official quantification via Qubit and purity analysis via NanoDrop. If approved, the Hub completes "Spreadsheet 1" and forwards the sample to the National Focal Point. If rejected, the Hub guides the originating lab through a new extraction.

National Focal Point:

- **Definition:** The central laboratory responsible for international interface.
- **Role:** Receive all approved samples (from Level 1 Labs and Regional Hubs). Apply **SOP 2 (Committee Checklist)** for document verification. Consolidate samples into 96-well PCR plates (optimizing batch sizes). Manage export permits (SisGen, Ibama, Federal Revenue) and handle physical shipment on dry ice to the US facility.

4. Macro Execution Schedule (Per Batch)

- **Month 1:** Committee distributes Spreadsheet 2 (Sequencing Request).
- **Month 2:** Committee evaluates requests, obtains facility quotes, and authorizes the start of extractions.
- **Month 3:** Laboratories extract DNA. Level 2 Labs send samples to Regional Hubs. Hubs validate samples.
- **Month 4:** Level 1 Labs and Hubs ship final samples to the National Focal Point.
- **Month 5:** National Focal Point consolidates plates, issues permits, and dispatches shipments to the USA.



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