INCT Fungi of Brazil

SOP 2: Sample Reception and Quality Checklist

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

Steering Committee Manual

Objective:

To establish standard procedures for Committee reviewers and Focal Points when receiving, checking, and validating DNA samples from network researchers, ensuring compliance before shipment to the US sequencing facility.

1. Physical Reception and Document Verification

Upon receiving a sample batch from a network laboratory, the Focal Point manager must immediately begin verification.

Step 1: Package Integrity Check

- Did the package arrive frozen (dry ice/ice packs still frozen)?
- Are tubes or plates intact, without cracks or leaks?

Step 2: Document Check

- Was "Spreadsheet 1: DNA Sample Submission" sent digitally?
- Does the physical tube count match the spreadsheet exactly?

- Do tube labels correspond exactly to the spreadsheet codes?

2. DNA Quality Checklist (Technical Validation)

The Committee must evaluate user-submitted data in the spreadsheet for each sample. If a sample fails any parameter below, it must be flagged as **"REJECTED"** or **"PENDING RE-EXTRACTION"**.

Evaluation Criterion	Acceptable Parameter	Action in Case of Non-Compliance
Extraction Method Reported?	Yes	Request information from researcher.
Suspension Buffer	Ultrapure water or EDTA-free buffer	Reject. Request re-extraction (precipitation and resuspension carries high risk of quality loss).
Total Volume	20 µL to 50 µL	If < 20 µL, evaluate total mass. If > 50 µL, request concentration (if feasible) or re-extraction.
Total DNA Amount	≥ 500 ng	Reject. Facility strictly requires this minimum for PCR-free prep.
Quantification Method	Qubit (or equivalent fluorometer)	If measured solely by NanoDrop, Focal Point/Hub must re-quantify via Qubit.
260/280 Ratio	1.6 to 2.0	Reject. Request clean-up or re-extraction.
260/230 Ratio	≥ 1.8	Reject. High inhibitor risk. Request clean-up.
DNA Integrity (Gel)	Intact DNA on 0.8% gel	Reject severely degraded samples.

3. Consolidation into 96-Well Plates

Following validation, approved samples must be transferred into 96-well PCR plates for international shipment.

- **Mapping:** Complete the plate map (A1 to H12) linking original sample IDs to well positions.

- **Pipetting:** Ensure the sample is thoroughly homogenized prior to plate loading.
- **Sealing:** Seal plates tightly using foil seals.
- **Protection:** Secure a rigid plastic cover over the foil seal or cushion with paper towels secured by rubber bands.
- **Batching:** Group samples in batches of 50 to 96 per plate. Plates containing fewer than 50 samples are not optimized for robotic processing at the facility.

4. International Shipping Preparation

- Export the finalized plate map spreadsheet.
- Ensure active export authorizations (such as SisGen) are documented in the submission form.
- Pack plates in insulated styrofoam coolers with sufficient dry ice for international transit.
- Address to: Facility – [US Address].
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