



Fungal cultures and DNA for whole genome sequencing

How to keep fungal cultures pure and extract clean, high-molecular-weight DNA for fungal whole genome sequencing, with the DNA quality targets.

A contaminated culture gives a contaminated genome assembly. Keep the culture pure from start to finish.

1. Keep the culture pure

1. Start from a single, well-isolated colony, a single spore or a hyphal-tip culture.
2. Use strict aseptic technique, sterile tools, tips and fresh media; minimise exposure to the air.
3. Check purity by colony appearance and microscopy; sub-culture if in doubt and discard plates with mixed growth.
4. Use actively growing, healthy cultures for DNA extraction.
5. Keep verified isolates as glycerol stocks or other validated stocks.
6. Clean surfaces with 70% ethanol before and after work; wear gloves, mask and lab coat.

2. Extract the DNA

- Grind mycelium in liquid nitrogen or use bead-beating; CTAB-based or kit methods work well for fungi.
- Remove RNA (RNase A), proteins, polysaccharides and phenolic compounds.
- Pipette gently; do not vortex, to keep the DNA long.

3. DNA quality we need

Parameter	Requirement
Concentration	At least 50 ng/ μ L
Total DNA	At least 1 μ g
A260/280	1.8–2.0
A260/230	2.0 or higher
Integrity	High molecular weight, one clear band on a gel
Contaminants	Free from RNA, protein, polysaccharides and phenolics

You can also send a pure culture or mycelium and we will extract the DNA. Sequencing: Illumina NovaSeq 6000, PE150, over 300 $\times$ coverage for genomes of about 20–40 Mb.