# From specimen to sequence: Huang MEGA Lab Nanopore workflow

amplicon (two-step or one-pot PCR) or whole genome · scan a QR code to open the protocol · LIMS stages 1 → 2 → 3a → 3b → 4a

**How to read**

- Head seq: universal tail on the gene-region primers
- Barcode: sample-specific, on the barcode primers
- Gene region: target amplicon (colour = sample)

QR code: scan it with a phone to open that step's protocol PDF
Left bands = LIMS stages; /lims-register ends every stage; dashed = QC / info
Strain phenotyping (MIC + biofilm): Stage 2 side branch → Culture record

Route C uses native barcodes (NBD114.96) in the library: no PCR, no Stage 3b
/nanoamp, /nanogenome: Claude Code analysis skills for the run data

**Stage 1** — Specimen# / Culture#

**Specimen**
soil / environmental / insect | fungal strain in culture

**/lims-register**
Stage 1 → Specimen# (soil, environmental) or Culture# (strain)

**isolate fungi (strains, Route B / C)**

**Stage 2** — isolate + culture + phenotype · Culture#

**Microbe isolation**
environmental sample → vial

**Culture media**
MEA, PDA and other recipes

**Isolation and culturing**
primary plate on MEA (Stage 2a) → subculture one colony / hyphal tip on PDA
one Stage-2b row per isolate · preserve in vials
sequence from a pure culture only

**/lims-register**
Stage 2a plate → Stage 2b isolate → Culture# per strain

**strain with a Culture#:**
phenotype (optional, any time after Stage 2b)

**Strain phenotyping: MIC + biofilm**
antifungal MIC: RPMI-MOPS broth microdilution
8 drugs · 0.5 McFarland inoculum · 35 °C, 48 h
biofilm on the same inoculum: 72 h, then
crystal violet (biomass) and MTT (metabolic activity)

**MIC + biofilm**
microdilution · CV / MTT

**/lims-register**
Stage 2c → MIC per drug, CV, MTT on the Culture record

soil / environmental sample,
or an existing pure culture:
extract directly (skip isolation)

**DNA extraction**

**Stage 3a** — extraction + Qubit · MolWork#

**Soil DNA extraction**
SuperSoil · Zixpress 32

**Fungal DNA extraction**
modified BOMB v9.0

**DNA extraction**
soil: SuperSoil, Zixpress 32 | fungal / HMW gDNA: BOMB v9
genome route: keep the DNA high-molecular-weight

**quantify**

**Qubit Flex**
concentration of the extract (dsDNA HS)



**/lims-register**
Stage 3a → MolWork# (minted only after quantification)

choose a route

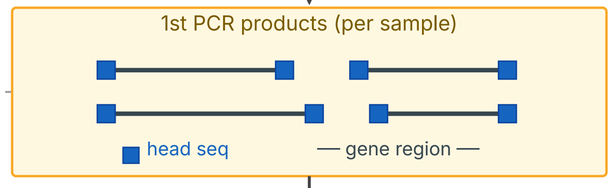
**Route A · amplicon, two-step PCR**
environmental samples (soil, water) and mixed templates

**1st PCR (gene regions)**

**Stage 3b** — PCR + gel + Qubit · pcr_reaction

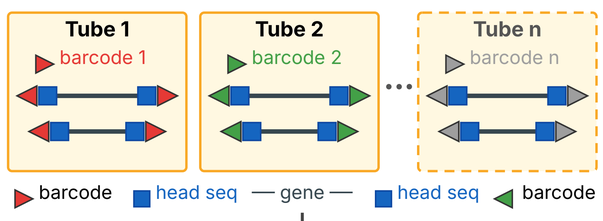
**2-step PCR protocol**
mixtures + cycling

1st PCR products (per sample)




**2nd PCR (barcoding)**
gel check #1: good amplicons only

2nd PCR products: one tube per sample, one barcode per tube




**Route B · amplicon, one-pot PCR**
strains: single- or multi-locus

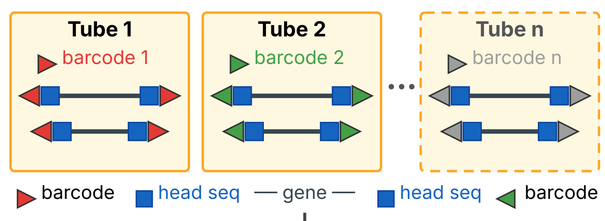
**Set up one-pot PCR**

One reaction tube (per strain × locus)
**gene-region primers** (head-tailed, 0.1 µM each) + **barcode primers** (barcode + head seq, 0.3 µM each)
50 µL: KAPA HiFi 2X + both primer sets + 3 µL template

**One-pot PCR**
phase 1: 25 cycles, 60–66 °C
phase 2: 15 cycles, 65 °C

**One-pot PCR protocol**
50 µL mix + 25/15 cycles

One-pot PCR products: one tube per sample, one barcode per tube




same barcoded molecule from either route

**Gel check → Qubit Flex (dsDNA BR)**
confirm the barcoded product

**/lims-register**
Stage 3b → pcr_reaction: method, conc., 2nd_pcr_ok, barcode

**/lims-register**
Stage 4a → Nanopore#: experiment + flow cell, add samples (PCR barcode) → pooling volumes

**Pooling (equal molar)**

**Pool all barcoded samples**
equal molar of every sample

**Purification (bead clean-up)**
PCR sample : bead = 1 : 1

**Size selection 1X**
bead purification protocol

**Library construction**

**Stage 4a** — Nanopore experiment · Nanopore#

**KAPA EvoPrep library**
PCR-barcoded pool, ~200 fmol
end-prep → adapter ligation → clean-up
PromethION

**EvoPrep library prep**
KAPA EvoPrep NBD114.96

**Load the flow cell and sequence**
PromethION R10.4.1 (FLO-PRO114M)

run data (POD5) + NanoAmp sample sheet (export from LIMS Stage 4a)

**/nanoamp**
**amplicon runs (Routes A and B)**
basecall → demultiplex → trim → cluster
→ BLAST classification
run it on the run folder with the LIMS sample sheet

**/nanogenome**
**genome runs (Route C)**
basecall → demultiplex → assemble → polish
→ annotate → compare
run it on the project directory

**after the run (bench)**

**Flow cell wash and reuse**
EXP-WSH004

**Flow cell wash**
EXP-WSH004 PromethION

**/lims-register**
Stage 4a → flow cell: pores after wash, date of use

**Route C ↓**

**Route C · genome, no PCR (Stage 3a → 4a, no 3b)**

**QC the gDNA**

**High-molecular-weight gDNA**
integrity: gel / TapeStation
input per ONT table
(10 kb avg → 200 fmol ≈ 1.3 µg)

**Library construction**

**KAPA EvoPrep + NBD114.96**
end-prep → native barcode
ligation (NB01–96) → pool
→ adapter ligation
~140 min

**EvoPrep library prep**
KAPA EvoPrep NBD114.96

**/lims-register**
Stage 4a → genome samples: MolWork# + NB barcode