From specimen to sequence: Huang MEGA Lab Nanopore workflow

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

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)

MIC + biffilm
microdilution · CV / MTT

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)

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

DNA extraction

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

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

Stage 3b PCR + gel + QubIt · pcr_reaction

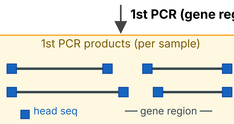
Route C ↓

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

1st PCR (gene regions)

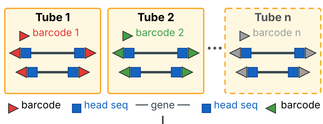
2-step PCR protocol
mixtures + cycling

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)

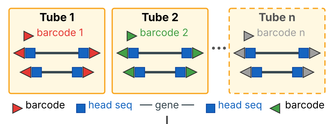
barcode primers
(barcode + head seq, 0.3 µM each)

50 µL: KAPA HiFi 2X + both primer sets + 3 µL template

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

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

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



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

QC the gDNA

same barcoded molecule from either route



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



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

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

Library construction

Stage 4a Nanopore# · experiment · flow cell · Nanopore#

/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

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

EvoPrep library prep
KAPA EvoPrep NBD114.96

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

Size selection 1X
bead purification protocol

Library construction

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

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
EXP-WSH004 PromethION

Flow cell wash and reuse
EXP-WSH004

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

Legend

- 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, or click on the website, 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