



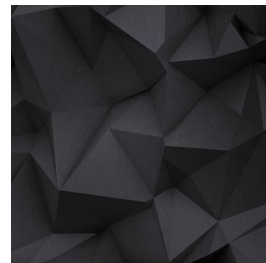
Mar 18, 2023

Version 3

🌐 ONT DNA Barcoding Fungal Amplicons w/ MinION & Flongle V.3

DOI

<https://dx.doi.org/10.17504/protocols.io.36wgq7qyk5/v3>



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Protocol status: Working

We use this collection and it's working

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Keywords: dna, sequencing, MinION, nanopore, oxford nanopore technologies, ONT, extraction, amplification, PCR, master mix, primers, NGSspeciesID, guppy, minibar, linux, PopOS, flongle, 10.4.1, adapter ligation, dA tailing, basecalling, demultiplex, methodology to dna barcode fungal specimen, dna barcode fungal specimen, fungal samples from specimen, fungal sample, dna extraction, software setup through dna extraction, dna template, alternative extraction protocol, genbank accessions with this protocol, extraction protocol, genbank accession, specimen, v14 ligation chemistry, dna,

Abstract

This collection of protocols outline a working methodology to DNA barcode fungal specimens. This process will work for dried tissue, fresh tissue, or with DNA template that has already gone through an alternative extraction protocol. It takes you from computer/software setup through DNA extraction, amplification, library preparation, and sequencing. It also includes primary and secondary data analysis. Take your fungal samples from specimens to GenBank accessions with this protocol!

This particular workflow is designed for use with **Flongle 10.4.1 flowcells** and **V14 Ligation** chemistry.

Materials

Total equipment needs and cost:

PCR tube rack x10 ([Amazon](#)) : \$33.78 (would be good to have at least 10)
 Tweezers (flat, non-serrated tips - Ebay): \$10.00
 Teasing needle ([Amazon](#)): \$12.35
 Mini centrifuge ([Ebay](#)): \$70.00
 PCR tube rack 1.5mL ([Amazon](#)): \$26.42
 0.5 -10uL multichannel pipette ([Amazon](#)): \$155.51 (may be able to find used cheaper on Ebay)
 50 - 300uL multichannel pipette ([Amazon](#)): \$172.89 (may be able to find used cheaper on Ebay)
 Summit Professional Freezer -20C (Facebook Marketplace / [1](#) / [2](#)): \$150 (gotta get lucky)
 Magnetic bead separator for 1.5mL eppi tubes ([Ebay](#)): \$59.00
 Tip disposal bucket
 Gel electrophoresis system ([miniPCR](#)): \$300
 Heat block ([Amazon](#)): \$179.99
 Hula mixer (Ebay): \$200.00 (optional)
 Flongle Starter Pack: \$1,460.00
 MinION Mk1B Starter Pack: \$1000.00
 Thermocycler: \$100 - \$1600

Total upfront equipment cost: ~\$3,200 - \$3,700, excluding thermocycler and computer
 Total upfront equipment cost: ~\$3,200 - \$3,700, excluding thermocycler and computer- \$3,700, excluding thermocycler and computer

Eppendorf Mastercycler (used) - \$1600 (can find less expensive models used, reasonable units in the \$200-\$400 range)

System 76 laptop - \$4,000 (can probably find something that works for much cheaper...depending on your use case)

Consumables:

0.2mL PCR tubes ([Amazon](#)): \$12.83
 0.2 non-skirted 96-well PCR plates ([USA Scientific/Amazon](#)): \$22.00/10 ×3 = \$66.00
 8-strip PCR caps ([USA Scientific](#)): \$11.00/125 strips x3 = \$33.00
 PCR Sealing Film ([Amazon](#)): \$36.74
 Eppendorf DNA LoBind 1.5mL tubes ([USA Scientific](#)): \$31.75 per 250 tubes
 15mL tubes ([Amazon](#)): \$17.99
 10ul-100uL Pipette ([Amazon](#)): \$32.39
 1000uL pipette ([Amazon](#)): \$32.39
 10uL filtered pipette tips ([Amazon](#)): \$63.99
 200uL filtered pipette tips ([Amazon](#)): \$57.77
 1000uL pipette tips ([Amazon](#)): \$13.28
 Alcohol swabs ([Amazon](#)): \$5.49
 Kimwipes ([Amazon](#)): \$24.30
 Fine-tip Sharpies ([Amazon](#)): \$1.84

Eliminase (Ebay): \$60.00

Total upfront consumable purchases: \$400 - \$500

Primers

ONT-tagged Forward Primers \$87.50

ONT-tagged Reverse Primers \$396.16

Total upfront primer cost: ~\$500

Reagents:

X-Amp DNA Reagent (IBI Scientific): 50mL kit is \$128.47. Cost per sample between \$0.039 (15uL) - \$0.064 (25uL)

PCR Master Mix (Empirical Bioscience): \$206.90 shipped (\$10.34 per plate; \$0.108 per reaction/sample)

Ethanol: \$56.18 per 1L

 NEBNext Ultra II End Repair/dA-Tailing Module - 24 rxns **New England Biolabs Catalog #E7546S** \$283.00
per 24 reactions

 Molecular Water **IBI Scientific Catalog #IB42130**

 HighPrep™ PCR Clean-up System **MagBio Genomics Inc. Catalog #AC-60005** : \$117.88 per 50 mL. \$0.047
per rxn.

 Ligation Sequencing Kit V14 **Oxford Nanopore Technologies Catalog #SQK-LSK114** : \$694.43 per 6
reactions (MinION) or 12 reactions (Flongle)

 NEBNext Quick Ligation Module **New England Biolabs Catalog #E6056S** : \$361.00 per 20 reactions

Total upfront reagent costs: ~\$1,600

Extraction ongoing costs:

Total per sample: \$0.071

Total per 96 samples: \$6.88

Total per Flongle run (480 samples) : \$34.40

Total per Flongle run (672 samples) : \$48.16

Total per Flongle run (960 samples) : \$68.88

PCR ongoing costs:

Total per sample: ~\$0.10 (\$796.54 / 10,000 rxns from Empirical Biosciences)

Total per Flongle run (480 samples) : ~\$48.00

Total per Flongle run (672 samples) : ~\$67.20

Total per Flongle run (960 samples) : ~\$96.00

dA-tailing ongoing costs:

Total per Flongle run (1/2 rxns): \$5.95



Total per MinION run: \$11.85

Total per sample (Flongle: 480 samples): \$0.012

Total per sample (Flongle: 672 samples): \$0.0089

Total per sample (Flongle: 960 samples): \$0.0061

Ligation ongoing costs:

Total per Flongle run (1/2 rxns): \$66.95

Total per MinION run: \$133.84

Total per sample (Flongle: 480 samples): \$0.139

Total per sample (Flongle: 960 samples): \$0.07

Sequencing ongoing costs:

Flongle cells: \$810 per 12 cells + \$50 per shipment (likely want 3-4 shipments) = ~\$100 per cell

Total upfront costs:

~\$5700 - \$6200 + thermocycler + compute

Total ongoing costs:

Per Flongle run: \$255 per 480 samples = \$0.53 per specimen

Per Flongle run: \$288 per 672 samples = \$0.43 per specimen

Per Flongle run: \$338 per 960 samples = \$0.35 per specimen

Files

 SEARCH

Protocol



NAME

ONT Sequencing IT/Compute Pop!_OS 22.04 Setup

VERSION 2

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NAME

Preparing ONT-tagged Primers and Master Mix for Fungal DNA Barcoding

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Quick DNA Extraction for Fungal Barcoding (X-Amp)

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Modified Promega Wizard Extraction for Barcoding Macrofungi

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ONT Post-PCR Pooling & Purification for Fungal Barcoding

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ONT dA-tailing for Fungal Barcoding

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ONT V14 Nanopore Adapter Ligation for Fungal DNA Barcoding

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ONT Flongle Flowcell Loading with Q20+ (V14) Chemistry

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Primary Data Analysis - Basecalling, Demultiplexing, and Consensus Building for ONT Fungal Barcodes

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Secondary Data Analysis - Creating a MycoMap Project from ONT Amplicon/Barcode Data

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