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Version 2

ONT DNA Barcoding Fungal Amplicons w/ MinION & Flongle V.2

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Protocol status: Working

We use this collection and it's working

Created: September 11, 2022

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Keywords: dna barcode fungal specimen, methodology to dna barcode fungal specimen, dna extraction, dna template, software setup through dna extraction, use with flongle, alternative extraction protocol, extraction, dna, specimen, ligation, v12 ligation chemistry, flongle

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, sequencing, and primary data analysis.

This particular workflow is designed for use with **Flongle 9.4.1 flowcells** and **V12 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

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

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 (Q20) **Oxford Nanopore Technologies Catalog #SQK-LSK112** : \$694.43 per 6 reactions

⊗ 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: \$0.093 - \$0.118

Total per 96 samples: \$8.93 - \$11.33

Total per Flongle run (480 samples) : \$44.65 - \$56.65

Total per Flongle run (672 samples) : \$62.50 - \$79.30

PCR ongoing costs:

Total per sample: ~\$0.225

Total per 96 samples: ~\$22.50

Total per Flongle run (480 samples) : ~\$110.00

Total per Flongle run (672 samples) : ~\$150.00

dA-tailing ongoing costs:

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

Total per MinION run: \$11.85

Total per 96 samples: \$0.061

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

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

**Ligation ongoing costs:**

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

Total per MinION run: \$133.89

Total per 96 samples: \$13.38

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

Total per sample (Flongle: 672 samples): \$0.0996

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: \$333 per 480 samples = \$0.69 per specimen

Per Flongle run: \$402 per 672 samples = \$0.59 per specimen

Troubleshooting

Files

 SEARCH

Protocol

NAME

ONT Sequencing IT/Compute Pop!_OS 22.04 Setup

VERSION 1

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Protocol

NAME

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

VERSION 2

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Quick DNA Extraction for Fungal Barcoding (X-Amp)

VERSION 3

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ONT Post-PCR Pooling & Purification for Fungal Barcoding

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ONT dA-tailing for Fungal Barcoding

VERSION 2

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ONT Q20+ (V12) Adapter Ligation for Fungal DNA Barcoding

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ONT Flongle Flowcell Loading with Q20+ (V12) Chemistry

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ONT Basecalling, Demultiplexing, and Analysis for Fungal Barcodes

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

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