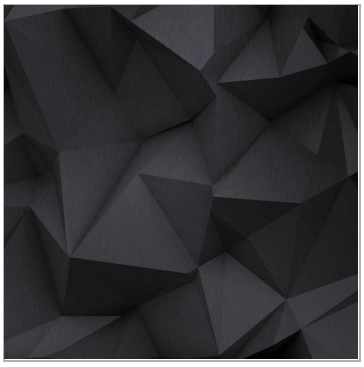

VERSION 3

MAR 17, 2023

OPEN ACCESS

Collection Citation: Stephen Douglas Russell 2023. ONT DNA Barcoding Fungal Amplicons w/ MinION & Flongle. **protocols.io** https://protocols.io/view/ont-dna-barcoding-fungal-amplicons-w-minion-amp-fl-cq2avyaeVersion created by Stephen Douglas Russell

Protocol status: Working
We use this collection and it's working

Created: Mar 12, 2023

Last Modified: Mar 17, 2023

COLLECTION integer ID: 78626

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

Stephen Douglas Russell[1]

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The Hoosier Mushroom Society

Stephen Douglas Russell

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

Keywords: dna, sequencing, MinION, nanopore, oxford nanopore technologies, ONT, extraction, amplification, PCR, master mix, primers, NGSpeciesID, guppy, minibar, linux, PopOS, flongle, 10.4.1, adapter ligation, dA tailing, basecalling, demultiplex

Total upfront equipment cost: ~$3,200 - $3,700, excluding thermocycler and computerTotal upfront equipment cost: ~$3,200 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 x3 = $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

Protocol

NAME
ONT Sequencing IT/Compute Pop!_OS 22.04 Setup

VERSION 2

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Protocol

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

VERSION 3

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Protocol

NAME
Quick DNA Extraction for Fungal Barcoding (X-Amp)

VERSION 5

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NAME
Modified Promega Wizard Extraction for Barcoding Macrofungi

VERSION 2

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NAME

ONT Post-PCR Pooling & Purification for Fungal Barcoding

VERSION 3

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NAME

ONT dA-tailing for Fungal Barcoding

VERSION 3

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Protocol

NAME

ONT V14 Nanopore Adapter Ligation for Fungal DNA Barcoding

VERSION 5

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NAME

ONT Flongle Flowcell Loading with Q20+ (V14) Chemistry

VERSION 4

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Protocol

NAME

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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