NOV 21, 2022

WORKS FOR ME 1

Modified EMP ITS Illumina Amplicon Protocol

Forked from EMP ITS Illumina Amplicon Protocol

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.6qpvrdmeogmk/v1

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ABSTRACT

The ITS protocol detailed here is designed to amplify fungal microbial eukaryotic lineages using paired-end community sequencing on the Illumina platform with primers ITS1f-ITS2 (EMP.ITSkabir).

Note: This a modified version used in biocrust project at University of California Riverside.

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EXTERNAL LINK

http://www.earthmicrobiome.org/protocols-and-standards/its/

PROTOCOL CITATION

Dylan P. Smith, Kabir G. Peay, Gail Ackermann, Amy Apprill, Markus Bauer, Donna Berg-Lyons, Jason Betley, T. D. Bruns, J. Greg Caporaso, Noah Fierer, Louise Fraser, Jed A. Fuhrman, M. Gardes, Jack A. Gilbert, Niall Gormley, Greg Humphrey, James Huntley, Janet K. Jansson, Rob Knight, Chris L. Lauber, S Lee, Sarah M. Owens, Alma E. Parada, Geoff Smith, J. Taylor, Luke Thompson, Willam A. Walters, T. J. White 2022. Modified EMP ITS Illumina Amplicon Protocol . **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvrdmeogmk/v1

FORK NOTE

FORK FROM

Forked from EMP ITS Illumina Amplicon Protocol, Luke Thompson

CREATED

Mar 02, 2021

LAST MODIFIED

Nov 21, 2022

PROTOCOL INTEGER ID

47834

GUIDELINES

Ordering primers

The primer sequences in this protocol are always listed in the 5' -> 3' orientation. This is the orientation that should be used for ordering. See the page Primer Ordering and Resuspension for more information. Primer constructs were designed by Dylan Smith and Kabir Peay.

- Illumina ITS Primer Constructs (ITS1f-ITS2)

Note: Unlike the 16S and 18S sequencing primers, the ITS sequencing primers have additional 3' bases beyond the PCR primers, in order to match the melting temperature of the Illumina adapters. The forward sequencing primer has 19 and the reverse sequencing primer has 15 additional 3' bases; therefore the amplicon sequences will begin 19 bp (forward read) and 15 bp (reverse read) after the PCR primers.

EMP.ITSkabir forward primer (ITS1f)

Field descriptions (space-delimited):

1. 5' Illumina adapter
2. Forward primer linker
3. Forward primer (ITS1f; Note: This is 38 bp upstream of ITS1 from White et al., 1990.)

```
AATGATACGGCGACCACCGAGATCTACAC GG CTTGGTCATTTAGAGGAAGTAA
```


Citation: Dylan P. Smith, Kabir G. Peay, Gail Ackermann, Amy Apprill, Markus Bauer, Donna Berg-Lyons, Jason Betley, T. D. Bruns, J. Greg Caporaso, Noah Fierer, Louise Fraser, Jed A. Fuhrman, M. Gardes, Jack A. Gilbert, Niall Gormley, Greg Humphrey, James Huntley, Janet K. Jansson, Rob Knight, Chris L. Lauber, S. Lee, Sarah M. Owens, Alma E. Parada, Geoff Smith, J. Taylor, Luke Thompson, Willam A. Walters, T. J. White Modified EMP ITS Illumina Amplicon ProtocolÃÂ https://dx.doi.org/10.17504/protocols.io.6qpvrdmeogmk/v1

EMP.ITSkabir reverse primer (ITS2), barcoded

Field descriptions (space-delimited):

1. Reverse complement of 3' Illumina adapter
2. Golay barcode
3. Reverse primer linker
4. Reverse primer (ITS2; Note: This is identical to ITS2 from White et al., 1990.)

```
CAAGCAGAAGACGGCATACGAGAT NNNNNNNNNN CG GCTGCGTTCTTCATCGATGC
```


Citation: Dylan P. Smith, Kabir G. Peay, Gail Ackermann, Amy Apprill, Markus Bauer, Donna Berg-Lyons, Jason Betley, T. D. Bruns, J. Greg Caporaso, Noah Fierer, Louise Fraser, Jed A. Fuhrman, M. Gardes, Jack A. Gilbert, Niall Gormley, Greg Humphrey, James Huntley, Janet K. Jansson, Rob Knight, Chris L. Lauber, S. Lee, Sarah M. Owens, Alma E. Parada, Geoff Smith, J. Taylor, Luke Thompson, Willam A. Walters, T. J. White Modified EMP ITS Illumina Amplicon ProtocolÃÂ https://dx.doi.org/10.17504/protocols.io.6qpvrdmeogmk/v1

PCR reaction mixtures

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Reagent	Volume
PCR-grade water	13.0 µL
PCR master mix (2x)	10.0 µL
Forward primer (10 µM)	0.5 µL
Reverse primer (10 µM)	0.5 µL
Template DNA	1.0 µL
Total reaction volume	25.0 µL

Notes:

- PCR-grade water from Sigma (cat. no. W3500) or MoBio (cat. no. 17000-11)
- Platinum Hot Start PCR Master Mix (2x) from ThermoFisher (cat. no. 13000014)
- Final master mix concentration in 1x reaction: 0.8x
- Final primer concentration in 1x reaction: 0.2 µM

Thermocycler conditions

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ITS amplification

- Primers: ITS1f-ITS2
- Amplicon size: ~230 bp

Temperature	Time	Repeat
94 °C	1 min	
94 °C	30 s	x35
52 °C	30 s	x35
68 °C	30 s	x35
68 °C	7 min	
4 °C	hold	

References

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6, 1621–1624. http://doi.org/10.1038/ismej.2012.8

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Smith, D. P., & Peay, K. G. (2014). Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. PLoS ONE, 9(2), e90234–e90234. http://doi.org/10.1371/journal.pone.0090234
Walters, W., Hyde, E. R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J. A., Jansson, J. K., Caporaso, J. G., Fuhrman, J. A., Apprill, A., & Knight, R. (2016). Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. mSystems, 1(1), e00009–15. http://doi.org/10.1128/mSystems.00009-15
White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR protocols: a guide to methods and applications (pp. 315–322). New York: Academic Press.

MATERIALS TEXT

MATERIALS

- PCR-Grade Water **Sigma Aldrich Catalog #W3500**
- Platinum Hot Start PCR Master Mix (2x) **Thermo Fisher Scientific Catalog #13000014**
- Quant-iT™ PicoGreen™ dsDNA Assay Kit **Invitrogen - Thermo Fisher Catalog #P11496**
- UltraClean PCR Clean Up Kit **Mobio Catalog #12500-250-1**

STEP MATERIALS

- Quant-it™ PicoGreen® dsDNA Assay Kit **Life Technologies Catalog #P7589**
- UltraClean PCR Clean Up Kit **Mobio Catalog #12500-250-1**
- Quant-it™ PicoGreen® dsDNA Assay Kit **Life Technologies Catalog #P7589**
- UltraClean PCR Clean Up Kit **Mobio Catalog #12500-250-1**

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for safety and hazard information.

BEFORE STARTING

For running these libraries on the MiSeq and HiSeq, please make sure you read the supplementary methods of Caporaso et al. (2012). You will need to make your sample more complex by adding 5-10% PhiX to your run.

Amplification Protocol

1 Amplify samples in triplicate.

Note

Each sample will be amplified in 3 replicate 25-µL PCR reactions.

2 Pool triplicate PCR reactions for each sample into a single volume (75 µL). Do not combine amplicons from different samples at this point.

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3 Run amplicons from each sample on an agarose gel.

Note

Low-biomass samples may yield faint or no visible bands; alternative methods such as a Bioanalyzer could be used to verify presence of PCR product.

Expected result

Expected band size for ITS1f-ITS2 is ~230 bp.

4 Quantify amplicons (DNA concentration) with NanoDrop spectrophotometer.

5 Clean up the amplicons using magnetic bead clean up. Please contact Matthew Collin matthew.collin@ucr.edu for the bead clean up protocol.

6 Combine an equal amount of amplicon from each sample (240 ng) into a single, sterile tube. Higher amounts can be used if the final pool will be gel-isolated or when working with low-biomass samples.

Note

When working with multiple plates of samples, it is typical to produce a single tube of amplicons for each plate of samples.

7 Measure concentration and A260/A280 ratio of final pool that has been cleaned.

Expected result

For best results the A260/A280 ratio should be between 1.8-2.0.

8 Send an aliquot for sequencing along with sequencing primers listed below.

ITS sequencing primers

Read 1 sequencing primer

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Field descriptions (space-delimited):

1. Forward primer segment
2. Extended region into amplicon

```
TTGGTCATTTAGAGGAAGTAA AAGTCGTAACAAGGTTTCC
```

Read 2 sequencing primer

Field descriptions (space-delimited):

1. Reverse primer segment
2. Extended region into amplicon

```
CGTTCTTCATCGATGC VAGARCCAAGAGATC
```

Index sequencing primer

1. Reverse complement of extended amplicon region
2. Reverse complement of reverse primer
3. Reverse complement of linker

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```
TCTC GCATCGATGAAGAACGCAGC CG
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