1st BASE
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Fungal DNA Barcoding PCR Kit (KIT-1210-50)

Fungal DNA Barcoding PCR Kit

Product No: KIT-1210-50

This kit contains PCR reagents to amplify the ITS gene for Sanger sequencing. The optimized PCR protocol generate end products for Sequencing+ PLUS Services from 1st BASE sequencing services. Customer can align and BLAST the obtained sequencing results to their choice of database for barcoding purposes. If customer choose to send their PCR products for other sequencing provider, the PCR products need to be purified before sequencing. It is an ideal kit to perform DNA barcoding of various fungal samples readily.

For Research Use Only. Not for use in Diagnostic Procedures.

Kit Contents

No	Product	KIT-1210-50	Storage
1	ITS Primer Mix	1 mL	-20 °C
2	ITS Enzyme	15 µL	
3	ITS PCR Buffer	300 µL	
4	Fungal Plasmid Positive Control	25 µL	
5	Sequencing Forward Primer, SPF (10 µM)	150 µL	
6	Sequencing Reverse Primer, SPR (10 µM)	150 µL	

Product Specification

	KIT-1210-50
Sample	Purified Genomic DNA from fungus
Duration	PCR amplification ~ 45 minutes
Storage	-20 °C

Materials Supplied by Users

- ✓ Thermocycler
- ✓ Electrophoresis reagents and system
- ✓ Sterile nuclease-free 0.2 mL PCR tubes or 96-well plate
- ✓ Sterile nuclease-free pipette and pipette tips

Precautions for Users

- ✓ Always wear a lab coat, disposable gloves and apply proper aseptic techniques to conduct molecular biology experiments.

Protocol

PCR	1. Prepare the PCR Mix according to Table 1. 2. Add 2 µL of purified genomic DNA (gDNA) at the concentration of 15 – 25 ng/µL as DNA Template with each 23 µL of **PCR Mix** into 0.2mL tube or 96-well plate. 3. Run the PCR Cycle Protocol on Thermocycler according to Table 2. 4. After the PCR cycle is completed, check the present of ~700 bp PCR products on 1% agarose gel according to Figure 1.
Sequencing	5. For the unpurified PCR products that shows single band at ~700 bp on agarose gel electrophoresis, they are ready to send for 1st BASE Sequencing+ PLUS Services using the provided SPF and SPR sequencing primers. *Tips:* ✓ *If there is no amplification or no PCR products generated from your fungal sample, please refer Troubleshooting Guidelines.* ✓ *1st BASE Sequencing+ PLUS Services has included PCR clean-up before sequencing. The turnaround time is ~3 – 4 working days from the day of the unpurified PCR products received by 1st BASE.*
Barcoding	6. After the sequencing results are ready, trim off the reads with Quality Value (QV) < 20, align the Forward and Reverse sequencing results. 7. BLAST the aligned sequence against your preferred database, e.g. NCBI, Greengenes or others. *8.* The identification of the fungus is reliable up to genus level and it typically appears within the top-10 of nucleotide BLAST results.

Table 1: Preparation of PCR Mix

Number of Reactions	ITS Primer Mix (µL)	ITS Enzyme (µL)	ITS PCR Buffer (µL)	Total PCR Mix (µL)
4	71.0	1.0	20	92
6	106.5	1.5	30	138
8	142.0	2.0	40	184
10	177.5	2.5	50	230
12	213.0	3.0	60	276
14	248.5	3.5	70	322
16	284.0	4.0	80	368
18	319.5	4.5	90	414
20	355.0	5.0	100	460

Note 1:

- ✓ The PCR Mix must be freshly prepared.
- ✓ The recommended DNA template amount in each PCR is 30 – 50 ng.
- ✓ Each PCR consists of 23 µL of PCR Mix and 2 µL of diluted DNA Template.
- ✓ Both NTC (No Template Control) and positive control reactions are recommended to be included into each round of PCR preparation.
- ✓ For each positive control reaction, use 1 µL of the provided plasmid positive control (5 ng/µL) as DNA Template.
- ✓ For each NTC reaction, use 1 µL of the provided TE Buffer as DNA Template.

Table 2: PCR Cycle Protocol

Step	PCR Process	Time	Temp. (°C)	Number of Cycle
1	Initial Denaturation	2 min	98	1
2	Denaturation	15 sec	98	25 cycles
3	Annealing	30 sec	60	
4	Extension	30 sec	72	
5	Final Extension	10 min	72	1

Note 2:

- ✓ Always check the present of PCR end products, which is ~700 bp of size on agarose gel electrophoresis before send for 1st BASE Sequencing+ PLUS Services.
- ✓ If you have alternative sequencing service provider, please purify the PCR products (PCR purification reagents not provided with this kit) before sequencing.

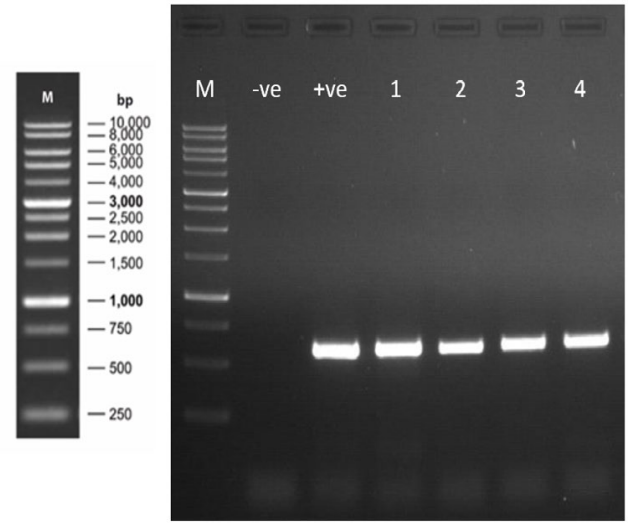

Figure 1. 1uL of un-purified PCR products on 1% TAE Agarose Gel. PCR was performed in 25uL according to Table 2.

-ve: NTC (No Template Control)
+ve: Fungal Plasmid Positive Control, 1uL per reaction
1 to 4: Fungal Nucleic Acid, 30 to 50ng per reaction

Troubleshooting Guidelines

Problems	Reasons
No PCR amplification	▪ Presence of PCR inhibitors: Perform 5x – 20x dilution in Step 10 and repeat PCR using the diluted DNA Template.
NTC shown amplification	▪ PCR mix in Table 1 must be prepared freshly. Do not use the premix that was prepared overnight.
Sanger sequencing results shown mixture of signal	▪ More than 1 different copy of ITS gene were detected due to presence of more than 1 type of fungus. a) Re-isolate single colony from the fungal culture. Repeat the process of extraction and sequencing using the new PCR products amplified from pure culture; or b) If the mixture of fungus is less than 5, by using the remaining PCR products, you may order cloning prior sequencing services using product code: **MBS-3006**. If the mixture of fungus is a lot more complex, you may consider our next-generation sequencing services using product code: **NGS-7008**. New gDNA is required.

Product Ordering Information

Product Name	*Packaging Size*	*Product No.*
Fungal DNA Barcoding PCR Kit	50 preps	KIT-1210-50
Fungal DNA Barcoding Kit, including DNA Extraction	50 preps	KIT-1200-50
1st BASE Sequencing+ PLUS Services	2 sequencing reactions for each sample	SS1201
PCR Product Cloning Service PLUS (up to 1.5kb) + Cloning of unpurified PCR product into pJET1.2/ Blunt vector + Colony PCR screening and pick 5 positive colony PCR products for bi-directional sequencing.	1 sample	MBS-3006
Amplicon Sequencing Lite, partial gene (ITS v2) with Basic Bioinformatics Analysis.	1 sample	NGS-7008
Fungal DNA Barcoding Services from pure isolates, ITS gene + Extraction of gDNA + PCR amplification and Purification + Bidirectional PCR product sequencing + Data analysis (BLAST to show the top 10 matches from database)	1 sample	MBS-5004
Fungal DNA Barcoding Services from mixed isolates, ITS gene + Extraction of gDNA, PCR Amplification and Purification + Cloning and 8 positive clones of plasmid DNA to send for Forward Single Pass Sequencing + Data analysis (Alignment & BLAST to show the top 10 matches from database)	1 sample	MBS-5104
<Optional> Custom DNA Barcoding Services	Minimum 5 samples	MBS-5007

Customization of DNA Barcoding kit for your choice of organism is available. Please contact us at http://www.base-asia.com/find-us for more information.

Note

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