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Bacterial DNA Barcoding Kit (KIT-1100-50)

Bacterial DNA Barcoding Kit

Product No: KIT-1100-50

This kit allows crude nucleic acid extraction from pure isolate of bacterial sample from agar culture or cell pellet from liquid culture, followed by PCR amplification of the 16s rRNA full length gene for Sanger sequencing. It is a solution-based extraction method that utilizes high salt to extract nucleic acid for PCR applications. The kit includes PCR primers, PCR reagents and optimized PCR protocol to 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 bacterial samples readily.

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

Kit Contents

No	Product	KIT-1100-50	Storage
1	Bacterial Lysis Buffer	50 mL	Room temperature (21 °C – 25 °C)
2	TE Buffer	11 mL	
3	Proteinase K Solution	180 µL	-20 °C
4	16S Primer Mix	600 µL	
5	16S Enzyme	30 µL	
6	16S PCR Buffer	750 µL	
7	Bacterial Plasmid Positive Control	25 µL	
8	Sequencing Forward Primer, 785F (10 µM)	150 µL	
9	Sequencing Reverse Primer, 907R (10 µM)	150 µL	

Product Specification

	KIT-1100-50
Sample	0.5 cm x 0.5 cm cut agar **or** Cell pellet from ≤ 2 mL fresh liquid culture
Elution	50 – 100 µL
Duration	a) Nucleic acid extraction: ≤ 2 hours or overnight b) PCR amplification: ~ 45 min
Storage	- 20 °C and Room Temperature (21 °C – 25 °C)

Materials Supplied by Users

- ✓ Thermo block to set at 56 °C
- ✓ Micro-centrifuge (non-refrigerated) with minimum speed of 14,000 × *g*
- ✓ Thermocycler
- ✓ Electrophoresis reagents and system
- ✓ Spectrophotometer
- ✓ Sterile nuclease-free 1.5 mL micro-centrifuge tubes (2x units per sample)
- ✓ Sterile nuclease-free 0.2 mL PCR tubes or 96-well plate
- ✓ Sterile nuclease-free pipette and pipette tips
- ✓ Isopropanol
- ✓ 70% ethanol

Precautions for Users

- ✓ Always wear a lab coat, disposable gloves, anti-fog protective goggles and surgical mask.
- ✓ For bacterial sample that is potentially contagious, suitable protective PPE lab coats and face shield must be equipped. The extraction works should be conducted in BSL2 or higher grade, which is according to interim guidelines of laboratories.

Protocol

Lysis	1. Add 500 µL of **Bacterial Lysis Buffer** into 1.5 mL micro-centrifuge tube that contains the recommended sample size of bacteria. *Tips:* ✓ *1 mL filter pipette tip is recommended to be used to add Lysis Buffer.* ✓ *Ensure the entire sample size is 100% submerged into Lysis Buffer.* ✓ *Handle each sample size one by one. Do not open ≥ two tubes together within the same time to avoid cross contamination.* 2. Add 3 µL of **Proteinase K solution**. Vortex to mix and spin down briefly. 3. Incubate at 56 °C for 30 minutes. Overnight incubation is optional. 4. Centrifuge the lysate at 14,000 – 16,000 × *g* for 10 minutes.
Washing	5. Transfer ~500 µL of supernatant to a new 1.5 mL micro-centrifuge tube, which contains 500 µL of **isopropanol**. Invert the tube several times to mix gently. 6. Centrifuge at 14,000 – 16,000 × *g* for 10 min and discard the supernatant. 7. Add 1 mL of **70% ethanol**. Centrifuge again at 14,000 – 16,000 × *g* for 5 minutes and discard the supernatant. *Tip:* 70% ethanol should be prepared freshly or less than a week. 8. Air dry the pellet for 3 minutes.
Elution	9. Re-suspend the dried DNA pellet with 50 µL **TE Buffer** and incubate at 56 °C for < 1 hr. *Tip: If necessary, increase the elution volume using* not more than 100 µL TE Buffer *to dissolve the DNA pellet completely.* 10. Measure the Optical Density (OD) reading using spectrophotometer. Dilute the nucleic acid to 15 – 25 ng/µL. Use 2 µL of this diluted nucleic acid as DNA Template for PCR.

PCR	11. Prepare the PCR Mix according to Table 1. 12. Add 2 µL of DNA Template from Step 10 with each 23 µL of **PCR Mix** into 0.2mL tube or 96-well plate. 13. Run the PCR Cycle Protocol on Thermocycler according to Table 2. 14. After the PCR cycle is completed, check the presence of ~1.5 kb PCR products on 1% agarose gel according to Figure 1.
Sequencing	15. For the unpurified PCR products that shows single band at ~1.5 kb on agarose gel electrophoresis, they are ready to send for 1st BASE Sequencing+ PLUS Services using the provided 785F and 907R sequencing primers. *Tips:* ✓ *If there is no amplification or no PCR products generated from your bacteria 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	16. After the sequencing results are ready, trim off the reads with Quality Value (QV) < 20, align the Forward and Reverse sequencing results. 17. BLAST the aligned sequence against your preferred database, e.g. NCBI, Greengenes or others. 18. The identification of the bacteria 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	16S Primer Mix (µL)	16S Enzyme (µL)	16S PCR Buffer (µL)	Total PCR Mix (µL)
2	20	1.0	25.0	46
3	30	1.5	37.5	69
4	40	2.0	50.0	92
5	50	2.5	62.5	115
6	60	3.0	75.0	138
7	70	3.5	87.5	161
8	80	4.0	100.0	184
9	90	4.5	112.5	207
10	100	5.0	125.0	230

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	94	1
2	Denaturation	10 sec	98	25 cycles
3	Annealing	30 sec	53	
4	Extension	60 sec	68	

Note 2:

- ✓ Always check the presence of PCR end products, which is ~1.5 kb 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.

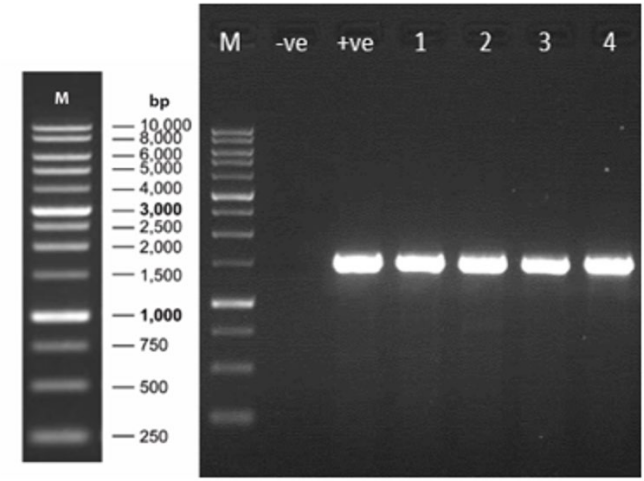

Figure 1. 1uL of un-purified PCR products on 1% TAE Agarose Gel. PCR was performed in 25uL according to Table 2.

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

Troubleshooting Guidelines

Problems	Reasons
Low yield of nucleic acid	▪ The culture agar exceeds the recommendation cut size of 0.5cm x 0.5cm. The sample size not submerged entirely in Bacterial Lysis Buffer.
No PCR amplification	▪ Presence of PCR inhibitors: a) Check whether dilution was performed correctly in Step 10; or b) 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 16s gene were detected due to presence of more than 1 type of bacteria. a) Re-isolate single colony from the bacteria culture. Repeat the process of extraction and sequencing using the new PCR products amplified from pure culture; or b) If the mixture of bacteria 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 bacteria is a lot more complex, you may consider our next-generation sequencing services using product code: **NGS-7008**. New gDNA is required.

The kit has been tested with wide range of bacteria fresh culture as following:

Gram-negative Bacteria		Gram-positive Bacteria
Acinetobacter	*Comamonas*	*Bacillus*
Alcaligenes	*Enterobacter*	*Cellulomonas*
Azotobacter	*Klebsiella*	*Enterococcus*
Brucella	*Pseudomonas*	*Lactobacillus*
Burkholderia	*Salmonella*	*Micrococcus*
Chryseobacterium	*Stenotrophomonas*	*Oceanobacillus*
Citrobacter	*Xanthomonas*	*Staphylococcus*

More information can contact us at mbs@apicalscientific.com.

Product Ordering Information

Product Name	***Packaging Size***	***Product No.***
Bacterial DNA Barcoding Kit	50 preps	KIT-1100-50
Bacterial DNA Barcoding PCR Kit, without DNA Extraction	50 preps	KIT-1110-50
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 (16s v3-v4 or 16s v4) with Basic Bioinformatics Analysis.	1 sample	NGS-7008
Bacterial DNA Barcoding Services from pure isolates, full gene of 16s rRNA + 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-5002
Bacterial DNA Barcoding Services from mixed isolates, full gene of 16s rRNA + Extraction of gDNA, PCR Amplification and Purification + Cloning and 8 positive clones of plasmid DNA to send for Bidirectional Sequencing + Data analysis (Alignment & BLAST to show the top 10 matches from database)	1 sample	MBS-5102
<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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