Quick-Start Protocol April 2019

QIAseq® 16S/ITS Region Panel

Further information

- *QIAseq 16S/ITS Panel Handbook*: **www.qiagen.com/HB-2547**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Important points before starting

- Use high-quality DNA.
- If DNA concentration is >1 ng/µl, then dilute to 1 ng/µl. If DNA concentration is <1 ng/µl, then use 1 µl of sample per PCR reaction.
- If using samples that have low bacterial content or low biomass (body swabs, environmental swabs, etc.), then simply add 1 µl of DNA per PCR reaction.
- Ensure reactions are thoroughly mixed, as well as prepared and incubated at the recommended temperatures.
- Prepare fresh 80% ethanol using nuclease-free water. Mix thoroughly by vortexing.
- QIAseq Beads need to be homogenous. Thoroughly resuspend beads immediately before use, and process the beads quickly. If a delay in the protocol occurs, vortex the beads again.

Preparation of QIAseq 16S/ITS Region Panel PCR Reaction

If more than one 16S region is to be sequenced, refer to *QIAseq 16S/ITS Handbook*.

1. On ice, prepare PCR reactions per gDNA sample by following Table 1. Mix well by pipetting up and down 10 times or by vortexing, and then spin down.
2. Incubate the reactions in a thermal cycler by following Table 2.

Table 1. Preparation of QIAseq 16S/ITS Region Panel PCR

	Per sample		
Component	**Panel pool 1**	**Panel pool 2**	**Panel pool 3**
Microbial DNA sample	1 µl	1 µl	1 µl
UCP Master Mix	2.5 µl	2.5 µl	2.5 µl
Region panel 1	1 µl	1 µl	1 µl
Region panel 2	optional (1 µl)	optional (1 µl)	optional (1 µl)
Region panel 3	optional (1 µl)	optional (1 µl)	optional (1 µl)
UCP PCR water	varies	varies	varies
Total volume	**10 µl**	**10 µl**	**10 µl**

Table 2. Setup of QIAseq 16S/ITS Region PCR Reaction

Step	**Time**	**Temperature**
Hold	2 min	95°C
3-step cycling		
Denaturation	30 s	95°C
Annealing	30 s	50°C
Extension	2 min	72°C
12 cycles*		
Final extension (1 cycle)	7 min	72°C
Hold	∞	4°C

* If samples are low biomass or contain low abundance of bacteria, use 20 cycles of 16S PCR.

3. If the sample required multiple PCR reactions, add 20 µl UCP PCR water and pool into single tube/well; if only one PCR reaction per sample, add 40 µl UCP PCR water.
4. According to the number of PCR reactions per samples, add QIAseq Beads (for 1 reaction, 55 µl per tube/well; for 2 reactions, 66 µl; for 3 reactions, 99 µl).
5. Mix well by pipetting up and down 12 times.
6. Incubate for 5 min at room temperature (15–25°C).
7. Place the tubes/plate on a magnetic rack for 5 min and discard the supernatant.
8. Centrifuge and carefully remove residual liquid. Add 55 µl nuclease-free water. Pipet up and down 12 times until beads are fully resuspended. Incubate for 2–5 min at room temperature.
9. Place the tubes/plate on a magnetic rack for 5 min. Carefully transfer 50 µl of the supernatant that contains the 16S/ITS PCR product to new tubes or plate.
10. Add 55 µl of QIAseq Beads to each sample. Mix well by pipetting up and down.
11. Incubate for 5 min at room temperature.

12. Place the tube/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant.
13. Wash with 200 µl of 80% ethanol. Carefully remove and discard the wash.
14. Repeat the ethanol wash and ensure that all traces of ethanol have been removed.
15. With the tubes/plate still on the magnet, air-dry at room temperature for 5 min.
16. Elute the DNA from the beads by adding 35 µl UCP PCR water.
17. Return the tubes/plate to the magnetic rack until the solution has cleared.
18. Transfer 32.5 µl supernatant to clean tubes/plate, then proceed to the protocol below.

Preparation of QIAseq 16S/ITS Region Panel Sample Index PCR Reaction

1. To tubes/plate containing the QIAseq 16S/ITS PCR product, add the components according to Table 3. Mix well by pipetting up and down 12 times or by vortexing, then spin down.

Table 3. Preparation of QIAseq 16S/ITS Region Panel Sample Index PCR Reaction

Step	Per sample	HT array
16S/ITS Region Panel PCR Product	32.5 µl	32.5 µl
UCP Master Mix	12.5 µl	12.5 µl
p5-RS2-ID# (4 µM)*	2.5 µl	–
p7-FS2-ID# (4 µM)*	2.5 µl	–
UCP water		5 µl
Total volume	**50 µl**	**50 µl**

* Use a unique p5-RS2-ID# + p7-FS2-ID# combination for each microbial DNA sample.

2. Incubate the reactions in a thermal cycler according to Table 4.

Table 4. Setup of QIAseq 16S/ITS Region Panel Sample Index PCR Reaction

Step	Time	Temperature
Hold	2 min	95°C
3-step cycling		
Denaturation	30 s	95°C
Annealing	30 s	60°C
Extension	2 min	72°C
19 cycles		
Final extension (1 cycle)	7 min	72°C
Hold	∞	4°C

3. Remove the tubes/plate from the thermal cycler and briefly centrifuge.
4. Add 45 µl QIAseq Beads to each and mix well by pipetting up and down 12 times.
5. Incubate for 5 min at room temperature.
6. Place the tubes/plate on a magnetic rack for 5 min and discard the supernatant.
7. Wash with 200 µl of 80% ethanol. Carefully remove and discard the wash.
8. Repeat the ethanol wash and ensure that all traces of ethanol have been removed.
9. With the tubes/plate still on the magnet, air-dry at room temperature for 10 min.
10. Elute the DNA from the beads by adding 30 µl nuclease-free water. Mix well.
11. Return the tubes/plate to the magnetic rack until the solution has cleared.
12. Transfer 25 µl of the supernatant to clean tubes/plate.
13. Proceed to "Protocol: Library QC and Quantification" in the *QIAseq 16S/ITS Panel Handbook*. Alternatively, the completed QIAseq 16S/ITS Region Panel Sequencing Library can be stored at –20°C in a constant-temperature freezer.

Revision History

Document	Changes	Date
HB-2564-003	Updated QR code to lead to correct handbook. Modified steps 3, 4, 8, 9 and 10, in page 2. Updated information on some incubation and air-drying times. Changed "Preparation of QIAseq 16S/ITS Region Panel PCR Reaction" in page 3 to "Preparation of QIAseq 16S/ITS Region Panel **Sample Index** PCR Reaction".	April 2019

Scan QR code for handbook.

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