Quick-Start Protocol April 2019

QIAseq® 16S/ITS Screening 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.
- 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 Screening Panel PCR Reaction

1. On ice, prepare 3 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.

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 Multiplex Master Mix	2.5 µl	2.5 µl	2.5 µl
Panel pool 1	1 µl	–	–
Panel pool 2	–	1 µl	–
Panel pool 3	–	–	1 µl
UCP PCR water	5.5 µl	5.5 µl	5.5 µl
Total volume	**10 µl**	**10 µl**	**10 µl**

2. Incubate the reactions in a thermal cycler as described in Table 2.

Table 2. Setup of QIAseq 16S/ITS 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. After PCR is finished, add 20 µl of UCP water to each of the PCR reactions.
4. Pool the PCR reactions into a single LoBind® tube or the well of a 96-well PCR plate.
5. Add 99 µl of QIAseq Beads and 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. Centrifuge and carefully remove any residual liquid.
8. Add 55 µl of nuclease-free water. Mix by pipetting up and down 12 times until the 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 tubes/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. Mix well. Incubate for 2–5 min at room temperature.

17. Return the tubes/plate to the magnetic rack until the solution has cleared.
18. Transfer 32.5 µl of the supernatant to clean tubes/plate.
19. Proceed to "Preparation of QIAseq 16S/ITS Screening Panel Sample Index PCR Reaction" below.

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

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

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

Step	Time	Temperature
Hold	2 mins	95°C
3-step cycling Denaturation Annealing Extension 14 cycles	 30 secs 30 secs 2 mins	 95°C 60°C 72°C
Final extension (1 cycle)	7 mins	72°C
Hold	∞	4°C

3. Remove the tubes/plate from the thermal cycler and briefly centrifuge.

4. Add 45 µl of QIAseq Beads 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 5 min.
10. Elute the DNA from the beads by adding 30 µl nuclease-free water. Mix well. Incubate for 2–5 min at room temperature.
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 Screening Panel Sequencing Library can be stored at –20°C in a constant-temperature freezer.

Revision History

Document	Changes	Date
HB-2565-003	Corrected QR code to lead to HB-2547 instead of HB-2541. Modified volumes of nuclease-free water and QIAseq Beads in steps 8, 9, and 10, page 2. Updated information on some incubation and air-drying times.	April 2019

Scan QR code for handbook.

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