Mycobiome_ITS1_ITS2_NGS_Kit

Manual

CELEMICS

Kit includes

Package Name	Product Name	1 rxn (µl)	96 rxn (µl)	Expiry date
Celemics ITS1 NGS Kit (kit storage condition: -20℃)	PCR Master Mix	37.5	1,900 X 2	1 year
	ITS_1 PCR I Forward Primer	1	100	
	ITS_2 PCR I Reverse Primer	1	100	
	Dual Index Forward Primer (YELLOW CAP)	1	56 (each)	
	Dual Index Reverse Primer (GREEN CAP)	1	84 (each)	

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Step 1. 1ST PCR Amplification

[Preparation]

- PCR Master Mix
- ITS PCR I Primer Set
- High Quality Genomic DNA

[Procedures]

1. Calculate the required volumes of each component based on the **Table 1**.

Table 1. Components for 1st PCR Amplification

Reagent	Volume for 1 rxn (μL)
ITS_1 PCR I Forward Primer	1
ITS_2 PCR I Reverse Primer	1
PCR Master Mix	12.5
High-quality genomic DNA	X
Nuclease-free water	10.5 - X
Total Volume	25 μL

2. Transfer the appropriate volumes of PCR Master Mix, high-quality genomic DNA and ITS PCR primer set to individual 0.2 mL PCR tubes or wells of a PCR plates.
   * Important – Combine all reagents on ice.

3. Cap or seal individual reactions, mix and centrifuge briefly.

4. Run the PCR program shown in **Table 2** using a thermal cycler.

Table 2. PCR program for 1st PCR Amplification

Step	Temperature	Time
1	95 ℃	5 minute
2	95 ℃	30 seconds
3	55 ℃	30 seconds
4	72 ℃	30 seconds
5	-	Repeat step 2 to step 4 for 25 cycle
6	72 ℃	5 minutes
7	4 ℃	Hold

Step 2. Purification of the sample using Celemics DNA purification Beads

[Preparation]

- Celemics DNA purification beads.
  Put in room temperature for at least 30 minutes before using.
  Store the Celemics DNA purification beads at 4 ℃ after use.
- 80% Ethanol solution
- Nuclease-free water

[Procedures]

1. Mix the Celemics DNA purification Beads well in order to achieve a homogenous state.

2. Add the Celemics DNA purification beads (20 µL) to a new 1.5 ml Lobind Tube and transfer PCR product (25 µL) to the tube. Mix well on a vortex mixer and pulse-spin down the tube. Incubate at room temperature for 5 minutes. (Don't spin down the Celemics DNA purification Beads too strongly to prevent it from precipitating)

Table 3. Volumes of Celemics DNA purification Beads

Regent	Volume for 1 sample
Celemics DNA purification Beads	Beads : DNA sample = 0.8 : 1 (Example) Beads : DNA sample = 50 µL : 63 µL

3. Put the tube in the magnetic separator for 3~5 minutes until the solution is clear.

4. Keep the tube in the magnetic separator and discard the supernatant. (*Be careful not to touch the beads while you remove the supernatant to keep the beads maximized*)

5. Continue to keep the tube in magnetic separator and put 500 µL (180 µL for PCR tube or 96 well plate) fresh 80% ethanol in each sample.

6. Incubate for 30 seconds at room temperature and then discard the ethanol.
   (Be careful not to touch the beads while you remove the supernatant to keep the beads maximized)

7. Repeat the 80% ethanol wash. (step 5~6)

8. Spin down the tube once again and discard residual ethanol.

Step 2. Purification of the sample using Celemics DNA purification Beads

9. Dry the sample at room temperature for 2 minutes or until residual ethanol has completely evaporated. (*Do not dry the beads until pellet appears cracked due to decreased elution efficiency which occurs when the bead pellets are excessively dried*).

10. Add 22 µL of Nuclease-free water to the sample, mix well on a vortex mixer or tap the tube. Pulse-spin down and incubate the sample at room temperature for 2 minutes.

11. Put the tube in the magnetic separator for 2 minutes until solution is clear.

12. Transfer the Supernatant (**20 µL**) to a new 1.5 mL Lobind Tube. (Retain the supernatant in this step)

Stop point

If you do not continue to the next step, You can store the sample at -20℃

Step 3. 2nd PCR Amplification

[Preparation]

- PCR Master Mix
- Dual index Forward Primer (YELLOW CAP)
- Dual index Reverse Primer (GREEN CAP)
- Purified PCR products from **Step 2**

[Procedures]

1. Calculate the required volumes of each component based on the **Table 4**.

Table 4. Components for 1st PCR Amplification

Reagent	Volume for 1 rxn (μL)
Dual index Forward Primer (YELLOW CAP)	1
Dual index Forward Primer (GREEN CAP)	1
PCR Master Mix	25
Purified PCR products from **Step 2**	5
Nuclease-free water	18
Total Volume	50 μL

2. Transfer the appropriate volumes of PCR Master Mix, purified PCR products and dual index PCR primers to individual 0.2 mL PCR tubes or wells of a PCR plates.
   * Important – Combine all reagents on ice.
   * Important – Check the appropriate of dual index combination in **Table 6**.

3. Cap or seal individual reactions, mix and centrifuge briefly.

4. Run the PCR program shown in **Table 5** using a thermal cycler.

Table 5. PCR program for 2nd PCR Amplification

Step	Temperature	Time
1	95 ℃	3 minute
2	95 ℃	30 seconds
3	60 ℃	30 seconds
4	72 ℃	30 seconds
5	-	Repeat step 2 to step 4 for 10 cycle
6	72 ℃	5 minutes
7	4 ℃	Hold

Step 3. 2nd PCR Amplification

Table 6. Dual Index sequences

Index	Sequence	Index	Sequence
Dual Index 501 Primer	TATAGCCT	Dual Index 701 Primer	ATTACTCG
Dual Index 502 Primer	ATAGAGGC	Dual Index 702 Primer	TCCGGAGA
Dual Index 503 Primer	CCTATCCT	Dual Index 703 Primer	CGCTCATT
Dual Index 504 Primer	GGCTCTGA	Dual Index 704 Primer	GAGATTCC
Dual Index 505 Primer	AGGCGAAG	Dual Index 705 Primer	ATTCAGAA
Dual Index 506 Primer	TAATCTTA	Dual Index 706 Primer	GAATTCGT
Dual Index 507 Primer	CAGGACGT	Dual Index 707 Primer	CTGAAGCT
Dual Index 508 Primer	GTACTGAC	Dual Index 708 Primer	TAATGCGC
Dual Index 509 Primer	GACCTGTA	Dual Index 709 Primer	CGGCTATG
Dual Index 510 Primer	CGGTGGTA	Dual Index 710 Primer	TCCGCGAA
Dual Index 511 Primer	GTTGGACT	Dual Index 711 Primer	TCTCGCGC
Dual Index 512 Primer	CTAAGATC	Dual Index 712 Primer	AGCGATAG
Dual Index 513 Primer	AATGGTTC	Dual Index 713 Primer	GCATAGTG
		Dual Index 714 Primer	CATTCCAG
		Dual Index 715 Primer	GGCAGGAT
		Dual Index 716 Primer	CAACAACA

Step 4. Purification of the sample using Celemics DNA purification Beads

[Preparation]

- Celemics DNA purification beads.
  Put in room temperature for at least 30 minutes before using.
  Store the Celemics DNA purification beads at 4 ℃ after use.
- 80% Ethanol solution
- Nuclease-free water

[Procedures]

1. Mix the Celemics DNA purification Beads well in order to achieve a homogenous state.

2. Add the Celemics DNA purification beads (90 µL) to a new 1.5 ml Lobind Tube and transfer PCR product (50 µL) to the tube. Mix well on a vortex mixer and pulse-spin down the tube. Incubate at room temperature for 5 minutes. (Don't spin down the Celemics DNA purification Beads too strongly to prevent it from precipitating)

Table 7. Volumes of Celemics DNA purification Beads

Regent	Volume for 1 sample
Celemics DNA purification Beads	Beads : DNA sample = 1.8 : 1 (Example) Beads : DNA sample = 90 µL : 50 µL

3. Put the tube in the magnetic separator for 3~5 minutes until the solution is clear.

4. Keep the tube in the magnetic separator and discard the supernatant. (*Be careful not to touch the beads while you remove the supernatant to keep the beads maximized*)

5. Continue to keep the tube in magnetic separator and put 500 µL (180 µL for PCR tube or 96 well plate) fresh 80% ethanol in each sample.

6. Incubate for 30 seconds at room temperature and then discard the ethanol.
   (Be careful not to touch the beads while you remove the supernatant to keep the beads maximized)

7. Repeat the 80% ethanol wash. (step 5~6)

8. Spin down the tube once again and discard residual ethanol.

Step 4. Purification of the sample using Celemics DNA purification Beads

9. Dry the sample at room temperature for 2 minutes or until residual ethanol has completely evaporated. (*Do not dry the beads until pellet appears cracked due to decreased elution efficiency which occurs when the bead pellets are excessively dried*).

10. Add 32 µL of Nuclease-free water to the sample, mix well on a vortex mixer or tap the tube. Pulse-spin down and incubate the sample at room temperature for 2 minutes.

11. Put the tube in the magnetic separator for 2 minutes until solution is clear.

12. Transfer the Supernatant (**30 µL**) to a new 1.5 mL Lobind Tube. (Retain the supernatant in this step)

Stop point

If you do not continue to the next step, You can store the sample at -20℃