16S Microbial ID Kit

96 Samples – Illumina MiSeq Compatible

Kit Preparation Protocol

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Contents

Introduction

The 16S Microbial ID Kit in conjunction with the RIDI™ cloud analysis software allows for the rapid preparation and analysis of microbial-specific amplicons suitable for taxonomically relevant identification. This kit supports the preparation and analysis of up to 96 individual samples in a single run or the preparation and analysis of up to 48 samples across two runs on the Illumina MiSeq. Kit highlights and features include:

Library Preparation by Amplification

Library preparation and indexing by amplification reduces hands-on time and simplifies library preparation.

Robust Sample Indexing

Simple, robust, and unique sample indexing method allows for efficient demultiplexing of up to 96 samples in a single run.

One 96-Sample Run or Two 48-Sample Runs

The kit may be used for one run of up to 96-samples or split into two runs of up to 48-samples. Flexibility to meet your project's needs.

Integrated and Useful Data Analysis

Use the RIDI™ cloud software to seamlessly analyze, identify, explore, and report the microbes detected within your sample pool.

16S Microbial ID Kit Contents

The 16S Microbial ID Kit contains several reagents with different storage conditions. Please open and distribute the reagents to the appropriate storage conditions after receipt. **Do not mix reagents from different lots. A unique Kit Access Code is found inside of the box lid. Protect this code as it is required to register your kit and submit your data for analysis.**

Kit Content	Storage Temp	Location
Amplification Plate 1 (AP1)	-20°C	Preamp
Amplification Plate 2 (AP2)	-20°C	Preamp
Master Mix (MM)	-20°C	Preamp
Normalization Beads (NB)	Room Temp	Postamp
Custom Primer 1 (CP1)	-20°C	Postamp
Custom Primer 2 (CP2)	-20°C	Postamp

Preamp: Pre-amplification, Postamp: Post-amplification, Library Preparation, and Sequencing

If components are missing, contact us at **bioidgenomics.com/contact/** for assistance. Visit the **MSDS Forms** link at **bioidgenomics.com/16s/** for a kit MSDS.

RIDI™ Cloud Analysis Software Requirements

The resulting FASTQ data for both Read 1 and Read 2 must be uploaded to the RIDI™ cloud for analysis. RIDI™ cloud supports most modern internet browsers. A minimum internet speed of 10 Mbps is suggested for reliable data upload and analysis performance. For best performance we recommend Google Chrome. Visit the **Bioinformatics** link at **bioidgenomics.com/16S/** for more information.

User-Supplied Consumables and Reagents

User-Supplied Item	Storage Temp	Location
Assorted Filter Pipette Tips	Room Temp	Preamp, Postamp
Molecular-Grade H_2O	Room Temp	Preamp, Postamp
Reagent Troughs	Room Temp	Preamp, Postamp
PCR Plate Seals	Room Temp	Preamp
Molecular-Grade EtOH	Room Temp	Postamp
200 mM Tris-HCl (pH 7.0)	Room Temp	Postamp
1.0 N NaOH	Room Temp	Postamp
Library Quantification	Various	Postamp
1.5 mL LoBind Eppendorf Tubes	Room Temp	Postamp

Preamp: Pre-amplification, Postamp: Post-amplification, Library Prep, and Sequencing

Required Equipment

Required Equipment	Location	Suggested Manufacturer	Part #
1000 µL Pipette	Var	Rainin	17014382
200 µL Pipette	Var	Rainin	17014384
10 µL Pipette	Var	Rainin	17014388
200 µL Multi-channel Pipette	Var	Rainin	17013810
Plate Centrifuge	Var	Optional	Optional
Dual Block Thermal Cycler	Amp	ThermoFisher	4484076
Magnetic Rack	Postamp	Alpaqua	009225

Var: Various Locations, Amp: Amplification, Postamp: Post-amplification, Library Prep, and Sequencing

Compatible DNA Extraction Kits

Generally, DNA prepared by standard extraction kits are compatible with DNA sequencing. The QIAamp DSP Blood Kit (Cat# 61104, Qiagen) has performed suitably for blood samples. The QIAamp PowerFecal DNA Kit (Cat#12830, Qiagen) and the MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (Cat# A36570, Thermo Scientific) have both performed suitably for stool or complex environmental samples, respectively. Samples or extraction methods that introduce PCR inhibitors would not be expected to perform properly and will not generate adequate results. A non-exhaustive list of extraction methods that have been found to work with this kit may be accessed by the **Compatible Extraction** link at **bioidgenomics.com/16s/**.

Amplification Plate Preparation

In this step, the user will prepare the Amplification Plate(s) prior to PCR. Good molecular biology techniques are critical to avoid reagent contamination and limit sample cross-contamination. Once this step is complete, the extracted DNA samples can be added to the prepared Amplification Plate(s).

Total Estimated Time

5 – 10 minutes

Materials, Reagents, and Consumables

Item	Amount	Supplier	Storage Temperature
Amplification Plate(s) (AP1/AP2)	1 or 2 Plates	BioID Genomics	-20°C
Master Mix (MM)	1 Tube	BioID Genomics	-20°C
Molecular-Grade H_2O	2 mL	User	Room Temp
Filter Pipette Tip	As required	User	Room Temp
Reagent Troughs	As required	User	Room Temp

Protocol

1. If more than 48 samples will be tested, remove Amplification Plate 1 (AP1) and Amplification Plate 2 (AP2) from the -20°C storage. If 48 or less samples will be tested, remove either Amplification Plate 1 (AP1) or Amplification Plate 2 (AP2) from -20°C storage.
2. Allow the Amplification Plate(s) to come to room temperature.
3. Thaw the Master Mix (MM) tube on ice or in a 4°C block.
4. Thaw all extracted DNA samples that will be tested on ice or in a 4°C block. It may be helpful to keep these samples separate from the Master Mix (MM) to reduce the risk of contamination.

> ***Note: An amplification positive control and an extraction control are suggested to ensure run-to-run performance. Positive controls may consist of a known microbial template DNA, while an extraction control may consist of microbes spiked into a negative sample matrix and is extracted in parallel with unknown samples.***

5. Set a thermal cycler to hold at 65°C.
6. Once the Amplification Plate(s) have reached room temperature, gently remove the Amplification Plate(s) from the sealed foil pouch. Centrifuge briefly or gently tap plate(s) to collect any contents that may have shifted during shipment or storage in the bottom of the wells.
7. Carefully remove the plate seal(s) from the Amplification Plate(s).
8. Add 8 µL of molecular-grade H_2O into each well using a multi-channel pipette. Briefly centrifuge or gently tap plate(s) to collect the solution in the bottom of the wells, take care to not cross-contaminate the wells.

> ***Note: If the extracted DNA sample(s) consist of a low number of genomic equivalents, the amount of molecular-grade H_2O may be reduced, and a corresponding volume of the extracted DNA sample(s) can be added. Caution is warranted as overloading the Amplification Plate with excessive extracted DNA can result in inconsistent or skewed amplification of the target microbial targets. Optimization may be required if rare targets are suspected. The total volume of molecular-grade H_2O and extracted DNA***

sample should always reach a combined volume of 10 µL. Decreasing the molecular-grade H_2O volume below 5 µL can evaporate quickly in the subsequent steps; therefore, care is warranted.

9. Place the Amplification Plate(s) on the thermal cycler that was preheated to 65°C for 2 minutes.
10. Briefly centrifuge or gently tap the Amplification Plate(s) to collect the primer and water solution in the bottom of the wells, again taking care to not cross-contaminate the wells.
11. Add 10 µL of the Master Mix (MM) into each well of the Amplification Plate(s) using a multi-channel pipette.
12. If the kit has a remaining unused Amplification Plate, return the remaining Master Mix (MM) to the -20°C storage location with the remaining Amplification Plate.
13. Proceed to **Sample Loading and Amplification**.

Sample Loading and Amplification

In this step, the user will load the samples to be sequenced into the Amplification Plate(s). Take care when loading the extracted DNA sample(s) in the appropriate positions as it is critical for successful sample indexing and DNA sequencing. Once complete, the resulting amplicons will be ready for library normalization and pooling.

Total Estimated Time

90-120 minutes (depending on sample number and thermal cycler)

Materials, Reagents, and Consumables

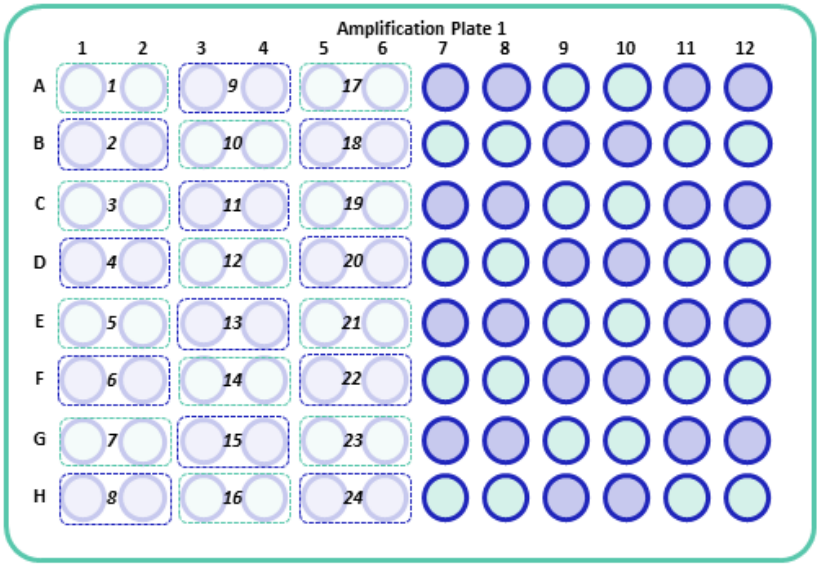
Item	Amount	Supplier	Storage Temperature
Prepared Amplification Plate(s) (AP1/AP2)	1 or 2 Plates	BioID Genomics	Prepared at Room Temp
Extracted DNA Sample(s)	Various	User	Thawed at 4°C
PCR Plate Seals	1 or 2 Seals	User	Room Temp
Filter Pipette Tip	Various	User	Room Temp

Protocol

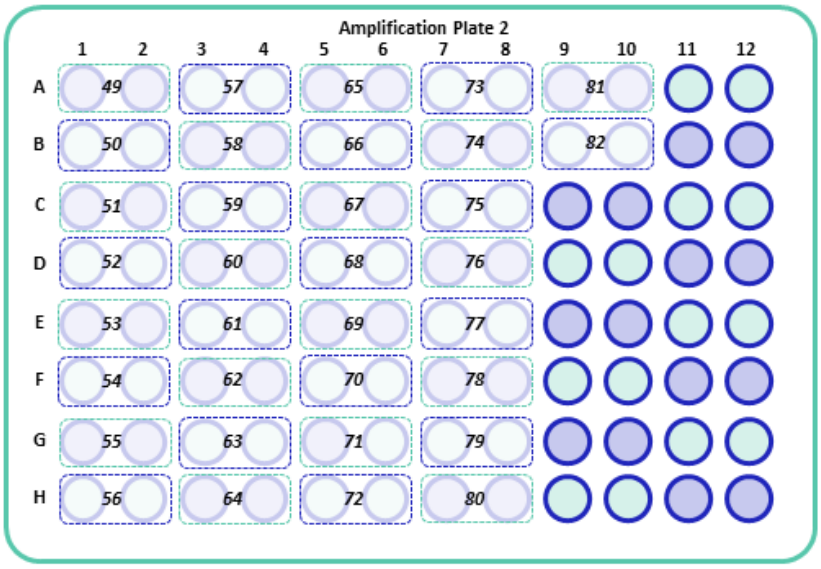
1. Add 2 µL of the extracted DNA sample(s) to each pair of wells to be used for each sample. For example, 2 µL of the first extracted DNA sample should be added to well A1 and another 2 µL of the first extracted DNA sample should be added to well A2. *See Sample Index Key on page 18 – 20.*

   Note: The extracted DNA sample(s) should be added sequentially in each column, from top to bottom, before moving on to another column, left to right. Using the wells out of order may result in nonoptimal sequencing performance. If Plate 1 is used, start with Indexed Sample 1 in wells A1 and A2. If Plate 2 is used start with Indexed Sample 49 in wells A1 and A2.

   Example A – A total of 24 extracted DNA samples are tested on Plate 1. The first sample, Sample 1, is loaded into wells A1 and A2. The last sample, Sample 24, is loaded into wells H5 and H6.



Example B – A total of 34 extracted DNA samples are tested on Plate 2. The first sample, Sample 49, is loaded into wells A1 and A2. The last sample, Sample 82, is loaded into wells B9 and B10.



2. After all necessary wells are loaded with the extracted DNA sample(s), seal the Amplification Plate(s) with the user-supplied PCR plate seal(s) and briefly centrifuge or gently tap the loaded Amplification Plate(s) to collect the solution in the bottom of the wells. Exercise caution to reduce well cross-contamination.

   Note: It is recommended that a dual block thermal cycler is used so both Amplification Plates, if two are used, are amplified simultaneously. Alternatively, but not recommended, a single block thermal cycler may be used with the amplifications performed one-after-another. In this case, store the sealed Amplification Plate at 4°C when not in the thermal cycler.

3. Place the loaded Amplification Plate(s) on a dual block thermal cycler and amplify using the following PCR conditions:
   - 96°C for 1 minute
   - 35 cycles of:
     - 96°C for 20 seconds
     - 48°C for 30 seconds
     - 72°C for 30 seconds
   - 72°C for 7 minutes
   - Hold at 4°C
4. Remove the Amplification Plate(s) and briefly centrifuge or gently tap the plate(s) to collect the solution containing the amplicons in the bottom of the wells.
5. Proceed to **Library Normalization and Pooling**.

Library Normalization and Pooling

In this step, the user will use Normalization Beads to rapidly produce a sequencing library with a uniform DNA fragment representation. Normalization is achieved by the limited binding capacity of the magnetic Normalization Beads. When this step is complete, the library pool will consist of amplicons in approximate equimolar ratios.

Total Estimated Time

30 – 40 minutes

Materials, Reagents, and Consumables

Item	Amount	Supplier	Storage Temperature
Cycled Amplification Plate(s) (AP1/AP2)	1 or 2 Plates	BioID Genomics	Prepared at Room Temp
Normalization Beads (NB)	Various	BioID Genomics	Room Temp
Molecular-Grade H_2O	2 mL	User	Room Temp
85% Molecular-Grade EtOH	Various	User	Room Temp
1.5 mL LoBind Eppendorf Tube	1 Tube	User	Room Temp
Reagent Troughs	Various	User	Room Temp

Protocol

1. Fully resuspend by mixing the Normalization Beads (NB) until homogenous using a vortex. Avoid introducing bubbles or air into the Normalization Beads (NB). Keep the resuspended beads away from magnetic sources until use.
2. Carefully remove the seal from the cycled Amplification Plate(s).
3. Add 80 µL of molecular-grade H_2O to each well.

> ***Note: Reagents only need to be added to wells that had extracted DNA sample(s) added and could contain amplicons resulting from the PCR reaction. This will save time and reagents as it is unnecessary to normalize and pool unused wells on the Amplification Plate(s).***

4. Add 90 µL of magnetic Normalization Beads (NB) to each well of the Amplification Plate(s).
5. Incubate the Amplification Plate(s) containing the Normalization Beads (NB) for 1 minute at room temperature to allow for bead / amplicon binding.
6. Incubate the Amplification Plate(s) containing the Normalization Beads (NB) for 5-10 minutes on a magnetic rack to collect the magnetic Normalization Beads (NB).
7. Prepare at least 800 µL per sample of 85% molecular-grade EtOH (400 µL for each well).

> ***Note: If 96 samples are to be tested, approximately 80 mL of 85% EtOH will be required. If 48 samples are to be tested, approximately 40 mL of 85% EtOH will be required.***

8. Remove and discard only 120 µL of the supernatant from each well of the Amplification Plate(se) without disturbing the magnetic Normalization Beads (NB).
9. Remove the Amplification Plate(s) containing the Normalization Beads (NB) from the magnetic rack, add 200 µL of 85% molecular-grade EtOH, and resuspend the Normalization Beads (NB) by gently pipetting up and down.

10. Incubate the Amplification Plate(s) containing the Normalization Beads (NB) for 5 minutes on a magnetic rack to collect the magnetic Normalization Beads (NB).
11. Remove and discard 200 µL of the supernatant from each well of the Amplification Plate(s) without disturbing the magnetic Normalization Beads (NB).
12. Keep the Amplification Plate(s) containing the Normalization Beads (NB) on the magnetic rack and add 200 µL of 85% molecular-grade EtOH to further wash the magnetic Normalization Beads (NB).
13. Incubate the Amplification Plate(s) containing the Normalization Beads (NB) for 1 minute on the magnetic rack to collect the magnetic Normalization Beads (NB).
14. Slowly remove all the supernatant from each well of the Amplification Plate(s) without disturbing the magnetic Normalization Beads (NB).

    Note: Gentle tapping of the magnet or Amplification Plate may help to collect any residual supernatant in the bottom of the wells.

15. Keep the Amplification Plate(s) containing the Normalization Beads (NB) on the magnetic rack and air-dry the Normalization Beads (NB) for 5 – 10 minutes.
16. Remove the Amplification Plate(s) containing the Normalization Beads (NB) from the magnetic rack and resuspend the Normalization Beads (NB) in 40 µL of molecular-grade H_2O.
17. Incubate the Amplification Plate(s) containing the Normalization Beads (NB) for 1 – 2 minutes at room temperature to fully elute the sequencing-ready amplicon DNA from the Normalization Beads (NB).
18. Incubate the Amplification Plate(s) containing the Normalization Beads (NB) for 5 minutes on a magnetic rack to collect the Normalization Beads (NB).
19. Remove 5 µL from each well of the Amplification Plate(s) and pool together in a 1.5 mL LoBind Eppendorf tube. Keep the pooled library on ice or in a 4°C block until denaturation. This is your normalized and Pooled Library.

    Note: Rapid pooling may be achieved by using a user supplied 8-well strip tube and a multi-channel pipette, rather than pooling 5 µL individually. By this method, 5 µL from each column of the plate will be removed and placed into the 8-well strip tube. When 5 µL from all columns of the plate have been removed, each well of the 8-well strip tube can be rapidly combined into the 1.5 mL LoBind Eppendorf tube.

    Note: Consider resealing and saving the Amplification Plate(s) at -20°C as a backup amplicon source. Library pools can be accidentally spilled or lost. Also, the sequencer may suffer from a chemistry or hardware failure, requiring a new library to be prepared. It may be useful to keep these plates until the sequencing run is complete and the data is analyzed.

20. To quantify your Pooled Library with the method of your choice, proceed to **Library Quantification**.

    Note: This is a potential stopping point, whereby the pooled library may be stored short-term at -20°C. Long-term storage should be at -70°C to reduce DNA degradation. Degradation of the prepared amplicons will significantly reduce the quality of the sequence data.

Library Quantification

It is recommended that users quantify the concentration of the Pooled Library prior to loading, especially for the first few times using the kit. Quantification can be skipped if the Pooled Library concentration is reliably predictable for the types of extracted DNA samples that are tested. A loading density between 400 – 700 (K/mm^2) is recommended. Libraries consisting of extracted DNA samples that contain significant off-target genomic DNA may benefit from lower loading densities (< 500 K/mm^2). It is expected that 50% or less of the identified clusters will pass the filter at these loading densities. The expected average fragment size for the Pooled LIbrary prepared by this kit is 425bp. Additionally, a Pooled Library concentration between 0.5 – 10.0 nM is typical and depends on the input extracted DNA samples.

Suitable quantification methods include, but are not limited to:

Method	Kit Name	Part #	Manufacturer
qPCR	Library Quantification Kit	0960140001	Roche / KAPA Biosystems
Fluorometric	dsDNA HS Assay	Q32854	ThermoFisher Scientific

Total Estimated Time

5 – 60 minutes (varies depending on method)

Protocol

1. Follow the quantification protocol of the method of your choice and calculate the total nM concentration for the Pooled Library.
2. Proceed to **Library Denaturation and Dilution**.

Library Denaturation and Dilution

In this step, the user will prepare the Pooled Library for sequencing by the proper denaturation and dilution.

Total Estimated Time

5 minutes

Materials, Reagents, and Consumables

Item	Amount	Supplier	Storage Temperature
Pooled Library	Various	User	4°C or Ice
HT1 Buffer	Various	Illumina	4°C or Ice
1.0 N NaOH	200 µL	User	Room Temp
Molecular-Grade H_2O	800 µL	User	Room Temp
200 mM Tris-HCl (pH 7.0)	Various	User	Room Temp
1.5 mL LoBind Eppendorf Tube	2 Tubes	User	Room Temp

Protocol

1. If frozen, thaw the Pooled Library and place on ice or in a 4°C block until use.
2. Thaw HT1 buffer at Room Temp and place on ice or in a 4°C block until use.
3. Prepare a fresh 1 mL aliquot of 0.2 N NaOH by mixing 800 µL molecular-grade H_2O and 200 µL of 1.0 N NaOH in a 1.5 mL LoBind Eppendorf tube (discard aliquot after use).
4. Determine the volume of the Pooled Library required to obtain 20 nM. Add this volume of library to a new 1.5 mL LoBind Eppendorf tube. This volume is referred to as the "*equal volume*" for the following steps.

Note: See the Example after step 9 for an example of an "equal volume" of 2.5 µL, for clarity.

5. Add an *equal volume* of the freshly prepared 0.2 N NaOH to the library. Briefly vortex or mix the solution and centrifuge to collect the solution in the bottom of the tube.
6. Incubate the tube at room temperature for 5 minutes.
7. Add an *equal volume* of 200 mM Tris-HCl (pH 7.0) to the tube. Briefly vortex or mix the solution and centrifuge to collect the solution at the bottom of the tube.
8. Place the library mixture on ice or in a 4°C block to stop denaturation.

Note: Rapid cooling of the sample ensures the sample will remain denatured. The use of wet ice (water to ice 1:1) or metal freezer beads may promote rapid cooling.

9. Add ice cold (or 4°C) HT1 buffer to bring the volume of the Denatured Library to 1000 µL.

Example – If the *equal volume* was 2.5 µL then the following reagents will be present in the 1.5 mL LoBind Eppendorf tube.

Reagent	Amount
Pooled Library	2.5 µL
0.2 N NaOH Solution	2.5 µL
200 mM Tris	2.5 µL
HT1	992.5 µL
Denatured Library	1000 µL

10. Place the Denatured Library on ice or in a 4°C block.

Note: Rapid cooling of the library mixture ensures the sample will remain denatured. The use of wet ice (water to ice 1:1) or metal freezer beads may assist in rapid cooling.

11. To prepare the Final Library, add 150 µL of the Denatured Library (20 pM) to a new 1.5 mL LoBind Eppendorf tube.
12. To this tube add 450 µL of ice cold (or 4°C) HT1 for a Final Library concentration of 5 pM with a total volume of 600 µL. When loaded, this Final Library should result in a cluster density between 400 – 700 K/mm^2; however, optimization may be required for the specific library and sample types.

Note: Consider saving the original Pooled Library from step 1 at -20°C as a backup library source. The Final Library can be accidentally spilled or lost. Also, the sequencer may suffer from a chemistry or hardware failure, requiring a new library to be prepared. It may be useful to keep the original Pooled Library until the sequencing run is complete and the data is analyzed.

Load Library, Load Primers, and Sequence

In this step, the user will dilute the Custom Primer 1 (CP1) and Custom Primer 2 (CP2) used for sequencing. The user will also load the diluted Custom Primers and the Final Library into the appropriate reservoirs in the MiSeq reagent cartridge. A full sequencing run of 2 x 250 for a total of 500 cycles is recommended for initial sequencing requirements. With optimization, it is possible to reduce the total cycles to further reduce the on-sequencer time.

Total Estimated Time

5 – 10 minutes for dilution / loading

Up to 39 hours (the maximum recoverable data is achieved after a full paired end read of 2 x 250 – or 500 bp total).

Materials, Reagents, and Consumables

Item	Amount	Supplier	Storage Temperature
Final Library	600 µL	BioID Genomics	4°C or Ice
Custom Primer 1 (CP1)	1 Tube	BioID Genomics	-20°C
Custom Primer 2 (CP2)	1 Tube	BioID Genomics	-20°C
HT1 Buffer	~ 1.5 mL	Illumina	4°C or Ice
Sequencing Cartridge	1	Illumina	-20°C
1.5 mL LoBind Eppendorf Tube	2 Tubes	User	Room Temp

Protocol

1. Thaw both Custom Primer 1 (CP1) and Custom Primer 2 (CP2) tubes and place on ice or in a 4°C block.
2. Prepare the reagent cartridge according to the *MiSeq System User Guide* for a 2 x 250 bp Paired End Version 2 run.
3. Add 60 µL of the Custom Primer 1 (CP1) and 540 µL of ice cold (or 4°C) HT1 to a new labeled 1.5 mL LoBind Eppendorf tube. This is the Diluted Custom Primer 1.
4. Add 60 µL of the Custom Primer 2 (CP2) and 540 µL of ice cold (or 4°C) HT1 to a new labeled 1.5 mL LoBind Eppendorf tube. This is the Diluted Custom Primer 2.

> ***Note: Some Illumina protocols refer to custom primers as C1, C2, and C3. This should not be confused with the CP1 (Custom Primer 1) and CP2 (Custom Primer 2) provided by in this kit. Specifically, CP1 is placed into Reservoir 18 and CP2 is placed into Reservoir 20. Do not accidentally place CP2 into Reservoir 19.***

5. Remove any residual liquid or condensation from the foil seals for Reservoir 17, 18, and 20.
6. Pierce each of the foil seals for Reservoir 17, 18, and 20 with a fresh 1 mL pipette tip, changing the tip between each reservoir. When complete dispose of all tips used for piercing the foil seals.
7. Add all 600 µL of the Final Library into Reservoir 17 with a new pipette tip and without touching the foil seal or introducing bubbles into the reservoir.
8. Add all 600 µL of the Diluted Custom Primer 1 into Reservoir 18 with a new pipette tip and without touching the foil seal or introducing bubbles into the reservoir.
9. Add all 600 µL of the Diluted Custom Primer 2 into Reservoir 20 with a new pipette tip and without touching the foil seal or introducing bubbles into the reservoir.

Note: Reservoir 19 is not used as Illumina Indexing is not used. Ensure that you do not add sequencing Read 2 primer into Reservoir 19.

10. Set up the sequencing run according to the *MiSeq System User Guide* for a 2 x 250 bp Paired End Version 2 Run.
11. Ensure that the Sample Sheet is appropriately configured to use custom R1 and R2 sequencing primers. The run Settings fields in the Sample Sheet should appear as follows:

```
[Settings]
CustomRead1PrimerMix   C1
CustomRead2PrimerMix   C3
```

12. Proceed with sequencing according to the *MiSeq System User Guide* for a 2 x 250 bp Paired End Version 2 Run.

Upload, Demultiplex, and Analyze Data

In this step, the user will upload the resulting FASTQ data from the sequence run to the RIDI™ cloud analysis software. With paired end reads, two files are generated and both files need to be uploaded. A one-time setup of a RIDI™ cloud account is required to register kits, upload data, and generate results. Once an account is created, sequencing kits must be registered prior to data submission.

Total Estimated Time

30 – 240 minutes for upload (depending on internet speed)

Variable analysis time (depending on submission queue and data complexity)

Protocol

1. Visit the **Bioinformatics** link at **bioidgenomics.com/16S/**.
2. Register the kit that was used by entering the Kit Access Code.
3. When the sequencing run is complete, submit the paired end FASTQ files in "*fastq.gz*" format.
4. After the analysis is complete, explore or download your data.

Sample Index Key

Up to 96 samples can be added to 96 pairs of wells split across two Amplification Plates. Each pair of wells on each Amplification Plate has its own individual sample index from 1 to 96. If a single Amplification Plate is used, it is important that sample indices are used sequentially from lowest to highest to ensure the highest quality sequencing results. Always use the lowest available sample index and increase by 1 for each additional sample.

Index Key (Amplification Plate 1)

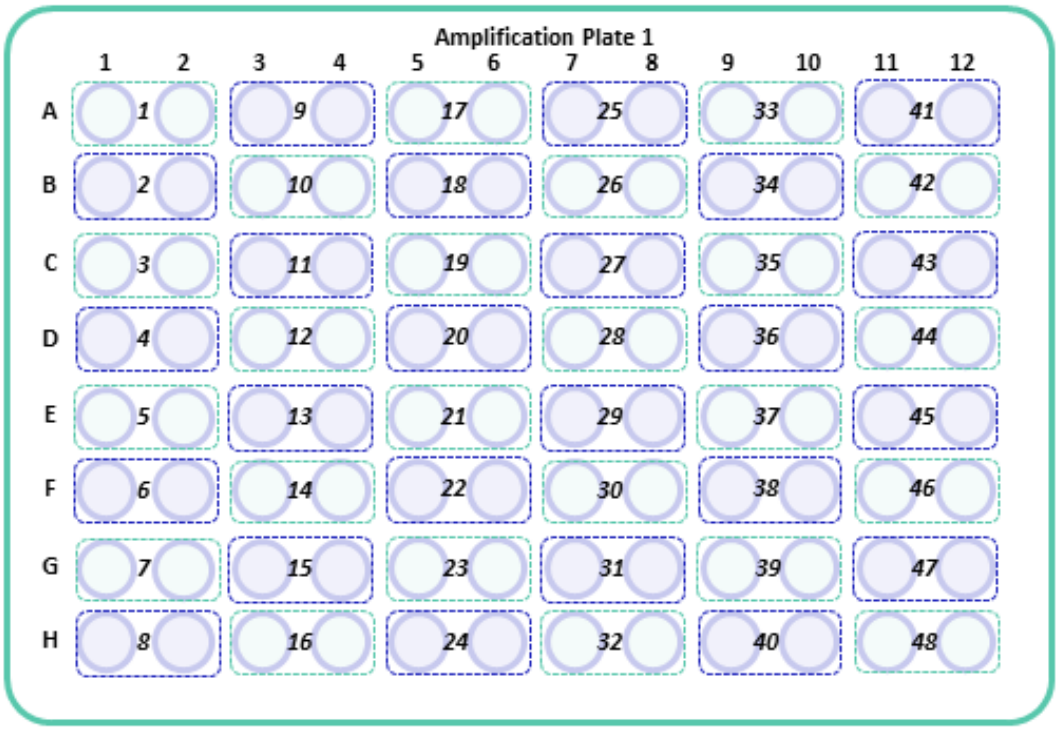
Sample Index	Plate	Well 1 Position	Well 2 Position	Sample Index	Plate	Well 1 Position	Well 2 Position
1	1	A1	A2	25	1	A7	A8
2	1	B1	B2	26	1	B7	B8
3	1	C1	C2	27	1	C7	C8
4	1	D1	D2	28	1	D7	D8
5	1	E1	E2	29	1	E7	E8
6	1	F1	F2	30	1	F7	F8
7	1	G1	G2	31	1	G7	G8
8	1	H1	H2	32	1	H7	H8
9	1	A3	A4	33	1	A9	A10
10	1	B3	B4	34	1	B9	B10
11	1	C3	C4	35	1	C9	C10
12	1	D3	D4	36	1	D9	D10
13	1	E3	E4	37	1	E9	E10
14	1	F3	F4	38	1	F9	F10
15	1	G3	G4	39	1	G9	G10
16	1	H3	H4	40	1	H9	H10
17	1	A5	A6	41	1	A11	A12
18	1	B5	B6	42	1	B11	B12
19	1	C5	C6	43	1	C11	C12
20	1	D5	D6	44	1	D11	D12
21	1	E5	E6	45	1	E11	E12
22	1	F5	F6	46	1	F11	F12
23	1	G5	G6	47	1	G11	G12
24	1	H5	H6	48	1	H11	H12

Index Key (Amplification Plate 2)

Sample Index	Plate	Well 1 Position	Well 2 Position
49	2	A1	A2
50	2	B1	B2
51	2	C1	C2
52	2	D1	D2
53	2	E1	E2
54	2	F1	F2
55	2	G1	G2
56	2	H1	H2
57	2	A3	A4
58	2	B3	B4
59	2	C3	C4
60	2	D3	D4
61	2	E3	E4
62	2	F3	F4
63	2	G3	G4
64	2	H3	H4
65	2	A5	A6
66	2	B5	B6
67	2	C5	C6
68	2	D5	D6
69	2	E5	E6
70	2	F5	F6
71	2	G5	G6
72	2	H5	H6

Sample Index	Plate	Well 1 Position	Well 2 Position
73	2	A7	A8
74	2	B7	B8
75	2	C7	C8
76	2	D7	D8
77	2	E7	E8
78	2	F7	F8
79	2	G7	G8
80	2	H7	H8
81	2	A9	A10
82	2	B9	B10
83	2	C9	C10
84	2	D9	D10
85	2	E9	E10
86	2	F9	F10
87	2	G9	G10
88	2	H9	H10
89	2	A11	A12
90	2	B11	B12
91	2	C11	C12
92	2	D11	D12
93	2	E11	E12
94	2	F11	F12
95	2	G11	G12
96	2	H11	H12

Index Schematic (Amplification Plate 1)



Index Schematic (Amplification Plate 2)