NEXTFLEX® Metagenomics Application for Sciclone NGS Workstation

(Compatible with NEXTFLEX® 16S V1-V3 Amplicon-Seq Kit, NEXTFLEX® 16S V4 Amplicon-Seq Kit 2.0, NEXTFLEX® 16S V5-V6 Amplicon-Seq Kit and NEXTFLEX® 18S ITS Amplicon-Seq Kit)

 V17.01

This product is for research use only.
Not for use in diagnostic procedures.

NEXTflex™ Metagenomics Amplicon-Seq Automation Guide

Product Overview

The NEXTflex™ Metagenomics Amplicon-Seq Application is designed to prepare multiplexed amplicon libraries. The NEXTflex 16S Amplicon-Seq Kits enable analysis of the entire microbial community within a sample, without the bias introduced by the culturing required for other, non-NGS based 16S rRNA analysis methods. The NEXTflex™ 18S ITS Amplicon-Seq Kit is designed to prepare multiplexed amplicon libraries that span the hypervariable Internal Transcribed Spacer (ITS) region of eukaryotic 18S ribosomal RNA (rRNA) genes. These libraries are compatible with paired-end sequencing on the Illumina® MiSeq platform.

There are two main steps involved in NEXTflex amplicon processing: an initial PCR amplification using customized PCR primers that target the desired domain, and a subsequent PCR amplification that integrates relevant flow cell binding domains and unique 12 base pair sample indices. A limited number of cleanup steps ensures maximum recovery of amplicons for downstream sequencing. Using this kit, library preparation may be automated using the Maestro-based NEXTflex Metagenomics Amplicon-Seq Application on the PerkinElmer Sciclone NGS Workstation.

The NEXTflex Metagenomics Amplicon-Seq Workflow on the Sciclone NGS Workstation allows for preparation of up to 96 libraries at a time from between 1 ng and 50 ng high quality genomic DNA, using up to 384 unique barcoded primers. The user provides the number of samples to process (1 to 12 columns of 8 samples each) and selects either to use pre-arrayed barcoded primers in a hard shell PCR plate, or have the workstation array up to 32 barcoded primers at the start of each run. The workflow typically takes less than 3 hours to complete.

Application Name	NEXTflex Metagenomics Amplicon-Seq Steps
NEXTflex Metagenomics Amplicon-Seq	PCR 1 setup
	PCR 1 cleanup
	PCR 2 setup
	PCR 2 cleanup

Required Hardware

Part	Vendor / Part Number
Sciclone NGS Workstation	Perkin Elmer
Inheco 384-well plate adapter	NGS Sciclone accessory CLS 100853
Inheco 96-well adapters (2)	NGS Sciclone accessory CLS 128372
Inheco 96-well adapter/shaker	NGS Sciclone accessory CLS 100852
Magnet	Agencourt 96 ring magnet CLS128316

Required Software

- Maestro 6.0 software or later
- Maestro-based NEXTflex Metagenonics Amplicon-Seq Application

Required Consumables

Reagents

- NEXTflex™ reagents:
  - ◊ 16S V5-V6 Amplicon-Seq Kit (4205-01--07) or
  - ◊ 16S V4 Amplicon-Seq Kit 2.0 (4203-01--07) or
  - ◊ 16S V1-V3 Amplicon-Seq Kit (4202-01--07) or
  - ◊ 18S ITS Amplicon-Seq Kit (4210-01--07)
  - ◊ See kit manual for components and storage conditions.
- Ethanol 80% (room temperature)
- Agencourt® AMPure® XP beads (Beckman Coulter Genomics, Cat # A63881)

Plates and Reservoirs

PerkinElmer Part No.	Vendor / Part Number	Part	Quantity Needed
CLS127737	BioRad HSP-9631	96 Well PCR Plate, Bio-Rad Hardshell, Full Skirt	6-7 dependent on options selected
CLS111426	PerkinElmer	Pipette Tip, 150 µL, Art, Box, 10-96 Sterile Racks	2-12 dependent on number of samples processed and options selected
CLS133355	Seahorse Bioscience 201379-100	Deepwell-96 POS, Square 2.0 mL well, Polypropylene, Seahorse	1
CLS128469	Seahorse Bioscience 201244-100	Reservoir-Deepwell, 252 mL, Seahorse	1
CLS112785	Seahorse Bioscience 200856-100	946 Lid-Universal, Robotic friendly, Polystyrene	7
6008890	Corning 3672	Microplate-384 well, Round bottom, Polypropylene, pack of 10	1

Miscellaneous Equipment

- Adhesive PCR Plate Seal (BioRad, Cat # MSB1001)
- Thermal cycler
- 2, 10, 20, 200 and 1000 µL pipettes / multichannel pipettes
- Nuclease-free barrier pipette tips
- Microcentrifuge
- 1.5 mL nuclease-free microcentrifuge tubes
- Agilent 2100 Bioanalyzer
- Applied Biosystems 7900HT Fast Real-Time PCR System (or equivalent)

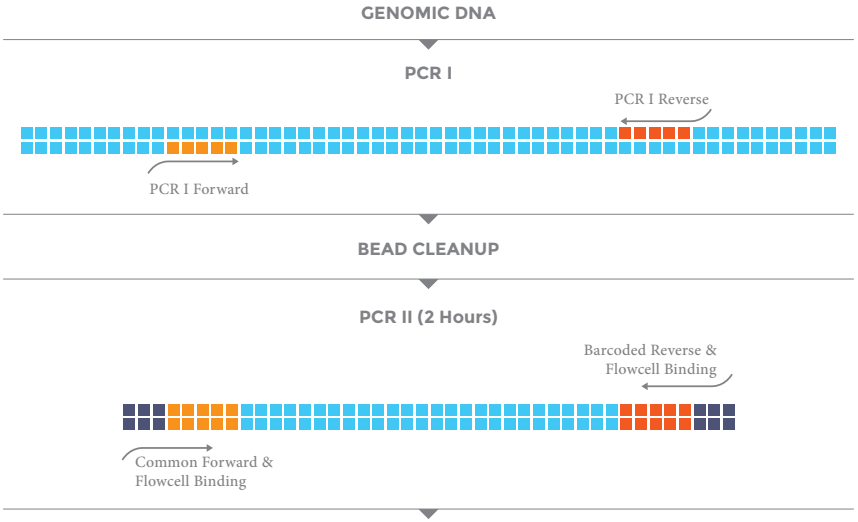
Warnings and Precautions

Bioo Scientific strongly recommends that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor or Bioo Scientific at nextgen@biooscientific.com.

- Follow all safety precautions as recommended by PerkinElmer for the Sciclone NGS Workstation.
- Do not use the kit past the expiration date.
- Ensure pipettes are properly calibrated, as library preparations are highly sensitive to pipetting error.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- Genomic DNA sample quality may vary between preparations. It is the user's responsibility to utilize high quality genomic DNA. Genomic DNA that is heavily nicked or damaged may cause library preparation failure. Absorbance measurements at 260 nm are commonly used to quantify DNA, and 260 nm / 280 nm ratios of 1.8 - 2.0 usually indicate relatively pure DNA. Other quantification methods using fluorescent dyes may also be used. The user should be aware that contaminating RNA, nucleotides, and single-stranded DNA may affect the amount of usable DNA in a sample preparation.
- Allow AMPure XP beads to come to room temperature for at least 30 minutes prior to use

Metagenomics Amplicon-Seq Workflow on the Sciclone NGS Workstation

Figure 1: Sample flow chart with approximate times necessary for each step.



Starting Material

Up to 96 samples of 1 ng - 50 ng high quality DNA in 33 µL nuclease-free water.

NEXTflex Metagenomics Amplicon-Seq Library Preparation

This application begins with the 33 µL DNA samples in a hard-shell, 96-well PCR plate and proceeds with PCR I setup and cleanup, followed by PCR II setup and cleanup. PCR is performed off the deck.

Materials for Amplicon-Seq Library Preparation

Bioo Scientific Supplied

NEXTflex Metagenomics Amplicon-Seq Components
GREEN CAP - NEXTflex™ PCR Master Mix
ORANGE CAP - NEXTflex™ PCR I Primer Mix*
YELLOW CAP or PCR PLATE - NEXTflex™ PCR II Barcoded Primer Mix
CLEAR CAP BOTTLE - Resuspension Buffer
* Use the appropriate Primer 1 mix for your application

User Supplied

Ethanol, 80%, freshly prepared, 100 mL
Agencourt AMPure XP Magnetic Beads

NEXTflex Metagenomics Amplicon-Seq Library Preparation Method

The library preparation method allows for NEXTflex Barcode Primer Mixes that are pre-aliquoted in a hard-shell PCR plate (where sample in sample well A1 would receive the barcoded primer mix from well A1 of the barcode plate, etc…), or for up to 32 NEXTflex Barcoded Primer Mixes to be arrayed by the machine, according to the Barcode Indexing tab in the NEXTflex Metagenomics Amplicon-Seq Workbook.

1. Power on the Sciclone NGS Workstation and the Inheco unit, and then start the PC controller.

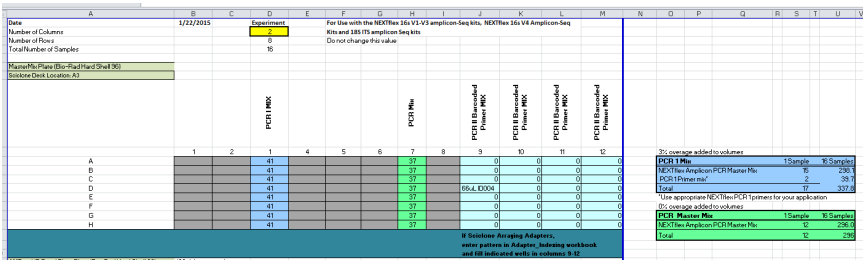


Figure 2: Setup guide for the NEXTflex Metagenomics Amplicon-Seq. Enter the number of columns to process (1-12) in cell 'D2'

2. Specify the number of samples and, if necessary, the appropriate NEXTflex Barcoded Primer Mixes, in the Maestro-based NEXTflex Metagenomics Amplicon-Seq Application Workbook:

   - The path of the workbook is: C:\ProgramData\Caliperls\Maestro\Workbooks\NEXTflex Metagenomics Amplicon-Seq Workbook.xls
   - Only columns of 8 samples can be processed at a time.
   - Enter the number of columns to process in cell 'D2' of the library preparation tab.
   - If Barcoded Primer Mixes are to be arrayed by the Sciclone, respective barcoded adapters are set by well ID in the Indexing tab of the workbook. Up to 32 NEXTflex Barcoded Primer Mixes for sample multiplexing can be used in one run. Alternatively, the NEXTflex Barcoded Primer Mixes can be placed in a hard-shell, 96-well PCR plate, allowing for up to 96 samples to be multiplexed.

Enter Barcoded Primer Index Well # below

Sample Well	Index Well	Index Name
A1	1	ID001
B1	2	ID002
C1	3	ID003
D1	4	ID004
E1	5	ID005
F1	6	ID006
G1	7	ID007
H1	8	ID008
A2	9	ID009
B2	10	ID010
C2	11	ID011
D2	12	ID012
E2	1	ID001
F2	2	ID002
G2	3	ID003
H2	4	ID004
A3	4	ID004
B3	5	ID005
C3	6	ID006
D3	7	ID007
E3	8	ID008
F3	9	ID009
G3	10	ID010
H3	11	ID011
A4	12	ID012
B4	13	ID013
C4	14	ID014
D4	15	ID015
E4	16	ID016
F4	17	ID017
G4		#N/A
H4		#N/A
A5		#N/A

Enter Name of barcoded primer Mix in the Index Name column

Index Well	Index Name	# Samples	Vol Needed
1	ID001	2	10
2	ID002	2	10
3	ID003	2	10
4	ID004	3	14
5	ID005	2	10
6	ID006	2	10
7	ID007	2	10
8	ID008	2	10
9	ID009	2	10
10	ID010	2	10
11	ID011	2	10
12	ID012	2	10
13	ID013	1	6
14	ID014	1	6
15	ID015	1	6
16	ID016	1	6
17	ID017	1	6
18	ID018	0	0
19	ID019	0	0
20	ID020	0	0
21	ID021	0	0
22	ID022	0	0
23	ID023	0	0
24	ID024	0	0
25	ID025	0	0
26	ID026	0	0
27	ID027	0	0
28	ID028	0	0
29	ID029	0	0
30	ID030	0	0
31	ID031	0	0
32	ID032	0	0

Figure 3: The Adapter Indexing worksheet in the NEXTflex Metagenomics Amplicon-Seq Workbook. The Sciclone can array up to 32 NEXTflex Barcoded Primer Mixes according to the user input in the adapter indexing worksheet. Alternatively, the NEXTflex barcoded primers can be pre-arrayed in a hard shell PCR plate.

   - Save the worksheet after all edits have been made. **DO NOT change the name or path of the workbook.**

3. The NEXTflex Metagenomics Amplicon-Seq Workbook contains reagent and plate setup maps for processing the number of columns specified in step 2 above. Refer to the Library Prep tab for the library preparation method.

   - Master mix calculations, where necessary, are found in the workbook to the right of the plate setup maps

     This application requires one master mix to be prepared by the user. The master mix must be combined prior to starting the Maestro-based NEXTflex Metagenomics Amplicon-Seq Library Preparation application.

The PCR I Master Mix is composed of the following components*:

15 µL	NEXTflex™ PCR Master Mix
2 µL	NEXTflex™ PCR I Primer Mix
17 µL	TOTAL VOLUME

*See workbook for preparation of master mix with appropriate overages for automation.

- Aliquot the prepared reaction mixes into hard-shell PCR plates as determined by the spreadsheet.
- Keep sample plate and master mix plate on ice until ready to run the application.
- Ensure no wells used have trapped air bubbles.
- Prepare the remaining plates as depicted in the workbook.
- Into a Seahorse deep well reservoir, add 100 mL of freshly prepared 80% EtOH solution. Cover the reservoir with a lid and store at room temperature.
- In addition to the reagents and plates listed in the workbook, 1-2 clean hard-shell PCR plates are needed depending on the options selected for the run. Also, a Seahorse deep well plate is needed for waste collection.

4. Run the Maestro software. Open the NEXTflex Metagenomics Amplicon-Seq Library Preparation application.

5. Start the run by pressing the play button. If operating under the Editor Mode, make sure to start the Main Method.

   At the start of the run, the user will be prompted with a pop-up window. The user must select the NEXTflex Barcoded Primer Mix format. NEXTflex Barcoded Primer Mixes can be pre-arrayed in a Hard Shell PCR plate or placed in the Master Mix plate (arranged according to the NEXTflex Metagenomics Amplicon-Seq Workbook) and arrayed by the workstation. Select the correct format for your application.

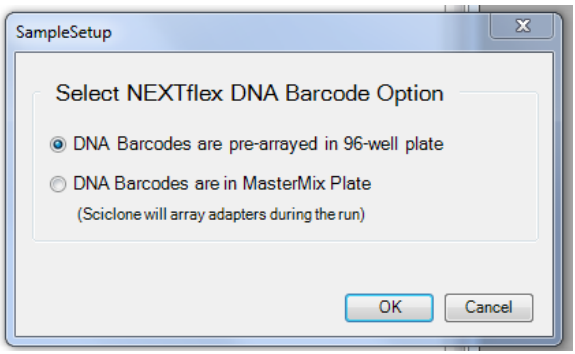


Figure 4: Barcoded Primer Mix options

6. The user will be prompted to verify the number of sample columns to be processed. Ensure the software shows the correct number of sample columns being processed. If not, the Excel workbook from Step 2 will need to be corrected, and the application restarted from the Main Method.

7. The NEXTflex Metagenomics Amplicon-Seq Library Preparation application requires the user to verify that the following Inheco adapters and magnets are on the deck:

Position A3:	96-well
Position A4:	384-well
Position D2:	96-well
Position D4:	96-well shaker
Position B4:	Magnet

8. Set up the Sciclone NGS Workstation deck as indicated by the application. This involves placement of reagent and sample plates, as well as consumables such as pipette tip boxes.

9. The Setup window will show a graphic of the completed deck setup. Ensure the positioning of all plates and boxes is correct, and then click "Finished" to begin the automated library preparation method. A blinking green light on top of the machine indicates the Maestro-based NEXTflex Metagenomics Amplicon-Seq Application is running.

10. The Sciclone NGS Workstation will proceed through the automated reaction steps and their respective cleanups: PCR I setup, PCR I cleanup, PCR II setup, and PCR II cleanup.

11. Once PCR setup is completed, the application will pause. Seal the PCR plate and place it on a thermocycler. Use the appropriate cycling conditions found in the kit manual.

    Once the final cleanup is completed, the final library plate can be safely stored at -20°C or used for immediate validation (qPCR and/or Bioanalyzer).

RELATED PRODUCTS

Illumina Compatible RNA NGS Kits and Adapters

- NEXTflex™ Rapid Directional RNA-Seq Kit
- NEXTflex™ RNA-Seq Barcodes
- NEXTflex-96™ RNA-Seq Barcodes

- NEXTflex™ Rapid Directional qRNA-Seq™ Kit

- NEXTflex™ Small RNA Sequencing Kit v2
- NEXTlfex™ Small RNA Barcode Primers

- NEXTflex™ Poly(A) Beads

Illumina Compatible DNA NGS Kits and Adapters

- NEXTflex™ 16S V4 Amplicon-Seq Kit
- NEXTflex™ 16S V4 Amplicon-Seq Kit 2.0
- NEXTflex™ 16S V1-V3 Amplicon-Seq Kit
- NEXTflex™ 18S ITS Amplicon-Seq Kit

- NEXTflex™ Rapid DNA-Seq Kit
- NEXTflex™ Cell Free DNA-Seq Kit
- NEXTflex™ DNA Barcodes
- NEXTflex-96™ DNA Barcodes
- NEXTflex-HT™ Barcodes
- NEXTflex™ Dual-Indexed DNA Barcodes

- NEXTflex™ Bisulfite-Seq Kit
- NEXTflex™ Bisulfite-Seq Barcodes
- NEXTflex™ Methyl-Seq 1 Kit
- NEXTflex™ Msp 1

- NEXTflex™ ChIP-Seq Kit
- NEXTflex™ ChIP-Seq Barcodes
- NEXTflex-96™ ChIP-Seq Barcodes

- NEXTflex™ Pre-Capture Combo Kit
- NEXTflex™ Rapid Pre-Capture Combo Kit
- NEXTflex™ DNA Barcode Blockers

- NEXTflex™ PCR-Free DNA Sequencing Kit
- NEXTflex™ PCR-Free Barcodes

WE WANT TO HEAR FROM YOU!

Your feedback is important to us. Tell us what you think of our kits by scanning the QR code or visiting our website at www.biooscientific.com/NGSfeedback. We can't wait to hear from you!

Bioo Scientific

a PerkinElmer company

THE NGS EXPERTS™

Bioo Scientific Corporation · 7050 Burleson Road, Austin, Texas 78744 · BiooScientific.com
P: 1.888.208.2246 · F: 512.707.8122 · Bioo Research Products Group · nextgen@biooscientific.com
Made in the USA