SPECIFICATIONS

NEXTFLEX® Combo-Seq™ mRNA/miRNA Automation Kit

NOVA-5139-53

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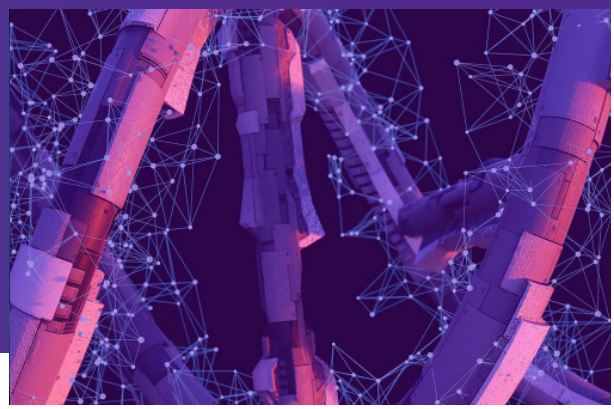


Product Overview

The NEXTFLEX® Combo-Seq™ mRNA/miRNA Automation Kit enables the user to generate up to 96 combined mRNA and microRNA libraries from 5 ng – 100 ng of total RNA, without the need for rRNA depletion or poly(A) selection on the PerkinElmer® Sciclone® G3 NGSx workstation. The workflow relies on a novel technique in which poly(A)-tailed RNA species are selectively reverse transcribed then sheared by RNase H into fragments of a useful length. The mRNA fragments are subsequently processed along with the small RNAs present in the sample. Libraries prepared with this kit are directional (strand-specific) and compatible with Illumina® sequencers.

NEXTFLEX® Combo-Seq™ mRNA/miRNA sequencing libraries contain Unique Dual Indices (UDIs), which are designed to specifically address the index-hopping phenomenon associated with Illumina® platforms utilizing a patterned flow cell. These UDIs prevent mis-assigned reads from appearing in final datasets, allowing for the highest assurance of data integrity.

PerkinElmer®
For the Better

Materials and Reagents

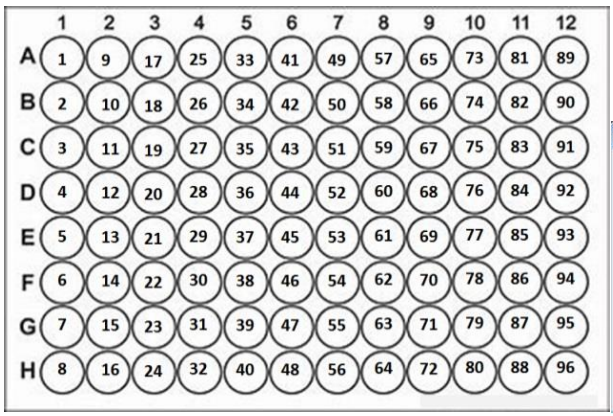
Provided Reagents

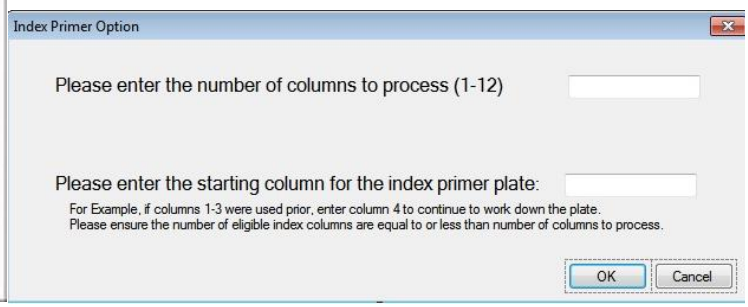
Contents, Storage and Shelf Life

The NEXTFLEX® Combo-Seq™ mRNA/miRNA Automation Kit contains enough material to prepare 96 prepare 96 libraries using the PerkinElmer® Sciclone® G3 NGSx workstation for Illumina® sequencing. The volumes provided support a single, 96-samples run or two, 48-sample runs. The shelf life of all reagents is at least 6 months when stored properly. All components can safely be stored at -20°C, except: NEXTFLEX® Adapter Depletion Solution, Resuspension Buffer, and Nuclease-free Water, which can be stored at room temperature, and NEXTFLEX® Cleanup Beads, which should be stored at 4°C.

Kit Contents	Cap Color	Amount	Storage Temp.
NEXTFLEX® Anchored Oligo(dT) Primer	PINK CAP	227 µL	-20°C
RT/RNase H Annealing Buffer	PINK CAP	227 µL	-20°C
RT/RNase H Reaction Buffer	PINK CAP	578 µL	-20°C
NEXTFLEX® Combo-Seq™ Reverse Transcriptase	PINK CAP	378 µL	-20°C
NEXTFLEX® Thermostable RNase H	PINK CAP	608 µL	-20°C
NEXTFLEX® PAP Buffer	RED CAP	578 µL	-20°C
NEXTFLEX® Poly(A)	RED CAP	145 µL	-20°C
NEXTFLEX® tRNA/YRNA Blocker	RED CAP	131 µL	-20°C
Adapter Depletion Solution	WHITE CAP	5 mL	Room Temp.
NEXTFLEX® Combo-Seq™ 5′ Ligation Buffer	LIGHT PURPLE CAP	854 µL	-20°C
NEXTFLEX® Combo-Seq™ 5′ 4N Adapter	LIGHT PURPLE CAP	197 µL	-20°C
NEXTFLEX® 5′ Ligation Enzyme Mix	LIGHT PURPLE CAP	263 µL	-20°C
NEXTFLEX® Combo-Seq™ RT Buffer	BLUE CAP	(2) 757 µL	-20°C
NEXTFLEX® UDI Barcoded Primer Mix*	GREEN CAP	6 µL each*	-20°C
NEXTFLEX® PCR Master Mix	GREEN CAP	832 µL	-20°C
Resuspension Buffer	YELLOW CAP	(2) 1.5 mL	Room Temp.
Nuclease-free Water	WHITE CAP	10 mL	Room Temp.
NEXTFLEX® Cleanup Beads	WHITE CAP	16 mL	4°C

*** Pre-plated, see figure below**





Pre-plated NEXTFLEX® UDI Barcoded PCR Primer Plate

(left) Barcode ID for the provided plate. Sequences can be found at the bottom of this document. Barcodes will be added column-wise to the PCR reaction. Any unused columns can be used in a subsequent run by selecting the starting column from an interface (right) in the Maestro software.

Sciclone® NGS Hardware, Software, Applications and Consumables

Required Hardware

Part	Vendor/Part Number
Sciclone® NGS Workstation	PerkinElmer®
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
Agencourt® 96 ring magnet	Agencourt® CLS128316
Spacer Assembly for Agencourt® 96 ring magnet	Agencourt® CLS128316

Required Software

Maestro 6.3 software or later

Required Consumables

Part No.	Vendor / Part Number	Part	Quantity Needed for 96 samples
6008870	PerkinElmer®	Bio-Rad® Hard-Shell® 96 Well PCR Plate, Full Skirt	15
111426	PerkinElmer®	Pipette Tip, 150 µL, Art, Box, 10-96 Sterile Racks	33
6008880	PerkinElmer®	Deepwell-96 POS, Square 2.0 mL well, Polypropylene, Seahorse	2
6008700	PerkinElmer®	Reservoir-Deepwell, 12 column, 21mL	3
6000030	PerkinElmer®	946 Lid-Universal, Robotic friendly, Polystyrene	9
6008890	PerkinElmer®	Microplate-384 well, Round bottom, Polypropylene, pack of 10	1

Required Reagents Not Provided

- **Vapor-Lock – Qiagen; Cat No./ID: 981611**
- 5 ng – 100 ng total RNA in up to 11 µL Nuclease-free Water
- Human Universal RNA (Biochain # R4234565) or other total RNA, as a control (optional)
- Isopropanol
- 80% Ethanol, freshly prepared
- Adhesive PCR Plate Seal (Bio-Rad, Cat # MSB1001)
- Thermal cycler compatible with Bio-Rad® Hard Shell Plates (see Bio-Rad website for recommendations)
- Vortex

Provided Maestro Applications

Application Name	NEXTFLEX® Combo-Seq Steps	Approximate Run Time (Including offline Incubations and PCR)
Library Prep 1	Step A – Reverse Transcription and RNase H Digestion	1 hour, 40 minutes
	Step B - Polyadenylation	1 hour, 45 minutes
	Step C – 5′ 4N Adapter Ligation	1 hour
	Step D – First Strand Synthesis	1 hour, 30 minutes
Library Prep 2	Step E – Barcoded PCR Setup	20-45 minutes (depending on # of cycles)
Post-PCR Size Selection Cleanup	Step F – Size Selection & Cleanup	1 hour

Warnings and Precautions

- Ensure that all pipette tips, microcentrifuge tubes, and other consumables are RNase-free.
- Take extreme care to keep an RNase-free workspace. It is recommended that an RNase decontamination solution such as RNase-Zap® (SigmaAldrich) be used on gloves and workspace surfaces before making mastermixes.
- DTT in buffers may precipitate after freezing. If precipitate is seen, vortex buffer for 1 - 2 minutes or until the precipitate is in solution. The performance of the buffer is not affected once the precipitate is in solution.
- Vortex and centrifuge each component prior to use, in order to ensure material has not lodged in the cap or side of the tube.
- Do not remove enzymes from -20°C until immediately before use; return to -20°C immediately after use.
- Do not freeze NEXTFLEX® Cleanup Beads. NEXTFLEX® Cleanup Beads should be stored at 4°C and brought to room temperature before use. Vortex and ensure homogenous appearance before use.
- Except where specified, thermal cycling should be performed with a heated lid.

Starting Material

The NEXTFLEX® Combo-Seq™ mRNA/miRNA Automation Kit is designed for use with 5 ng – 100 ng of total RNA. Upstream depletion of ribosomal RNA is neither required nor recommended. RNA should be free of DNA, divalent cations, salts such as guanidinium salts, organics such as phenol, reducing agents such as DTT, and chelators such as EDTA. Best results are obtained using high-quality starting material with a RIN ≥ 8. The use of degraded RNA will result in a low proportion of mRNA-mapping reads in the sequencing library.

Some total RNA extraction and purification methods may not efficiently isolate small RNAs. Users should verify that their extraction and purification method isolates total RNA including small RNAs such as miRNA.

The NEXTFLEX® Combo-Seq™ mRNA/miRNA Kit includes an optional reagent designed to deplete tRNA fragments and Y RNA fragments which are abundant in many types of input RNA. Users who wish to deplete these RNA fragments should add 1 µL of NEXTFLEX® tRNA/YRNA Blocker prior to 5′ ligation, as indicated in Steps B-11 and B-14.

If the user is performing the procedure for the first time, we recommend including a positive control such as 20 ng of total RNA (Biochain R4234565 or similar).

Using Qiagen® Vapor-Lock™ barrier

Qiagen® Vapor-Lock™ barrier must be used during the Library Prep 1 application. Qiagen® Vapor-Lock™ barrier should be manually applied directly to the top of the samples by pipetting prior to beginning the run. Qiagen® Vapor-Lock™ barrier will be removed during the bead cleanup at the end of Step A and will not inhibit any downstream applications.

Offline Steps

Two steps are done offline in a thermal cycler: Step B – Polyadenylation and Step E – Library Amplification.

Step ID	Condition
Step B - Polyadenlyation	15 minutes – 37°C 20 minutes – 90°C < 5 minutes – 4°C
Step E – PCR	30 sec – 98°C 10 sec – 98°C 20 sec – 65°C 15 sec – 72°C } cycle as per chart below 2 min – 72°C Hold – 4°C

Input RNA Amount	# of PCR Cycles
100 ng	13
20 ng	16
10 ng	17
5 ng	18

Appendix-PCR Primer Sequences

NEXTFLEX®	Sequence
NEXTFLEX® Anchored Oligo(dT) Primer	TTTTTTTTTTTTTTTTTTTVN
NEXTFLEX® Combo-Seq™ 5′ 4N Adapter	3′ddNVTTTTTTTTTT5′5′rGrUrUrCrArGrArGrUrUrCrUrArCrArGrUrCrCrGrArCrGrArUrCrNrNrNrN3′
RT primer (included in NEXTFLEX® Combo-Seq™ RT Buffer)	TCCTTGGCACCCGAGAATTCCATTTTTTTTTTTTTTTTTTTVN
UDI primer 1 – P7 (included in NEXTFLEX® UDI Barcoded Primer Mix)	CAAGCAGAAGACGGCATACGAGATXXXXXXXX[1]GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
UDI primer 2 – P5 (included in NEXTFLEX® UDI Barcoded Primer Mix)	AATGATACGGCGACCACCGAGATCTACACXXXXXXXX[2]ACACGTTCAGAGTTCTACAGTCCGA

XXXXXXXX[1] denotes the P7 index region of the primer. The index sequences that are added to each barcoded library via PCR are listed below. Note: UDI primer 1 contains the reverse complement of the sequence listed under P7 Index. The final library, however, will contain the P7 Index listed below (see also Fig. 2), and the sequencer will read the index as listed below.

XXXXXXXX[2] denotes the P5 index region of the primer. The index sequences that are added to each barcoded library via PCR are listed below.

NEXTFLEX® Combo-seq™ mRNA/miRNA UDI primers

NEXTFLEX®	i7 sequences	i5 sequences
UDI 1	AAGATCAT	AATAATAG
UDI 2	TGCTATTC	TTAGTAGC
UDI 3	GACGTGTC	TGCGTGGC
UDI 4	CGGTAGTC	ACCAATTG
UDI 5	GACAGCAG	CTACTGGT
UDI 6	CTTGTACA	AGCAGAGT
UDI 7	GACAAGTG	CATTATCG
UDI 8	CTCGCCTT	GTGCAGTC
UDI 9	GAGCGTCA	GTTCACAC
UDI 10	CGCGCTCG	CCGCATAC
UDI 11	GAGCTCTA	GTGGCGAA
UDI 12	CTATAGGA	GTGGATAC
UDI 13	AAGAGAGC	AATATAAC
UDI 14	TTATAGCG	TTCTAGGT
UDI 15	GAGCTAAG	AGATTGTG
UDI 16	CGAAGCCA	CAATCCGT
UDI 17	TTGTGGCT	TTACTTAC
UDI 18	CGCGAGAC	TTGTCGAC
UDI 19	GAGATCGG	AAGGAGCG
UDI 20	CGCACTTA	CTCGAAGC
UDI 21	GATGTCAG	GTTAGAAC
UDI 22	CTACTTCG	CGAACTGT
UDI 23	GATTACTC	CATGTCTC
UDI 24	ACTCAGAC	CGACTATA

NEXTFLEX®	i7 sequences	i5 sequences
UDI 25	AAGAGTTG	AATATTGA
UDI 26	TTATACAA	TTCTCAAT
UDI 27	GATTAGGA	GTGGAACG
UDI 28	CTCTGGCG	AGTTACGG
UDI 29	GATACCTA	GCGAGATC
UDI 30	CGCGTATC	AGCGTACG
UDI 31	GATACGAT	CTTGGTAC
UDI 32	CTGGAGCT	CGCAGCTG
UDI 33	GAACGATA	ACGGCACA
UDI 34	CGGTCCAT	GCGGTGTG
UDI 35	GAACTGGC	CGATACTA
UDI 36	CGGTGAGA	CAGCTACA
UDI 37	AAGACATA	AATATGCT
UDI 38	TTATGTAT	TTCTCCGC
UDI 39	GAACAGAT	CTATATTG
UDI 40	CTTGGCCT	CAGGAAGG
UDI 41	GAACATTC	GCCGTGCA
UDI 42	CTGATATA	GAGTTGCG
UDI 43	GAAGCGAG	AACCTCAC
UDI 44	CGGCCTCT	AACCATGG
UDI 45	GAAGTCGA	TCACTGTT
UDI 46	CGGCCATG	GACTGAGC
UDI 47	GAAGTTAC	GTCCAAGT
UDI 48	CTTAGAGA	CCGATGCG

NEXTFLEX®	i7 sequences	i5 sequences
UDI 49	AAGACTGT	AATATCTG
UDI 50	TTCGGAAC	TTAGCTAA
UDI 51	GAAGATCG	GCTTCTTG
UDI 52	CGGAGGCT	CGTTATAT
UDI 53	GAAGAAGC	TGCGCGAT
UDI 54	CTGCGAAG	CGACATGG
UDI 55	GAATCTGA	GAAGCCTC
UDI 56	CTGCGGTA	GATTGGAG
UDI 57	GTCTTGCT	GTCTATCG
UDI 58	CTGACAGT	AATACAGG
UDI 59	GAATTGAA	TACTCTGT
UDI 60	CGTCGTCA	TGGTGGTA
UDI 61	AAGTAAGT	AATAGATT
UDI 62	TGACGGAA	TTCTCTCA
UDI 63	GAATACGG	GCACACAA
UDI 64	CTTATCAG	TGTGAGTC
UDI 65	TCCGCGTC	GTCGCAGG
UDI 66	CTGAGTGC	GCATCACA
UDI 67	TCCTAACG	TCCAACTA
UDI 68	CGAGATAA	TGGTCACA
UDI 69	TCGCGATT	CCAAGCAC
UDI 70	CTAGTTAT	CCTTGATG
UDI 71	TCGCACTC	GATGGTAA
UDI 72	CTATTATC	CCTCCGAG

NEXTFLEX®	i7 sequences	i5 sequences
UDI 73	AAGTACAG	AATAGCAA
UDI 74	TGAATGGA	TTCGCTTG
UDI 75	TCGAAGCT	GCCACGCG
UDI 76	CTCATAAT	GCGCTGAC
UDI 77	TCTTAGGC	TACACAAC
UDI 78	CTCTACAC	GTATGGTC
UDI 79	TCTAGGTT	AGTCACAA
UDI 80	CGTTCTGC	CCGCTAAT
UDI 81	TCACTACG	CCTAGTGG
UDI 82	CTTCACGA	GCTAGAGT
UDI 83	TCAGTGAG	CGTGCAGG
UDI 84	CTTCTTAA	CGTAGTTC
UDI 85	AAGTTATC	AATTAATG
UDI 86	TGACCGCG	TTAATTCA
UDI 87	TCAGACTT	TGTGCAAC
UDI 88	CTGCATCA	CGATCGTT
UDI 89	TCATGCGT	CCGACGAC
UDI 90	CTTGCGGA	CTCCGCGC
UDI 91	TCAATCCT	GCCACCTC
UDI 92	CCGGCTGA	CCTCTGGA
UDI 93	TGCCGCCG	GACACAGA
UDI 94	CAGAGCTC	GCAATTGC
UDI 95	TGCGATAG	CACACATG
UDI 96	CCATTGAC	CAGATTCT

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