NEXTFLEX® Rapid Directional RNA-Seq Automation Kit 2.0

NOVA-5198-53

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This product is for research use only.

Not for use in diagnostic procedures.

GENERAL INFORMATION

Product Overview

Automation of NGS library preparation on the Revvity Sciclone® G3 NGSx workstation reduces sample tracking errors and sample-to-sample variability while dramatically increasing throughput. Samples are processed in 96-well PCR plates, and the number of samples to be processed (1 to 12 columns of 8 samples each) is selected at the start of each run. Pre-set tip-tracking utilities written into the Maestro applications guide the instrument to pick up appropriate numbers of tips and refill/replace tip boxes as needed. Inheco temperature blocks installed on the Sciclone® deck allow for appropriate 4°C storage of reagents and controlled incubation temperatures for enzymatic steps. Reaction mixes are pre-arrayed prior to sample addition to eliminate incubation time variability between samples. The easy-to-follow user interfaces guide the reagent and deck setup process and prompt the user for any necessary interventions.

The NEXTFLEX® Rapid Directional RNA-Seq Automation kit 2.0 is designed to prepare directional, strand-specific RNA libraries from total RNA if used in conjunction with the NEXTFLEX® Poly(A) Beads 2.0 (10 ng - 5 µg) or NEXTFLEX® RiboNaut™ rRNA depletion kit (human, mouse, rat) (5 ng - 1µg) for sequencing using Illumina® sequencers. The workflow is broken into three separate applications and can be completed in approximately 9-10 hours.

Provided Reagents

Contents, Storage, and Shelf Life

The NEXTFLEX® Rapid Directional RNA-Seq Automation kit 2.0 contains enough material to prepare 96 RNA samples for sequencing on an Illumina® instrument if used in conjunction with the NEXTFLEX® Poly(A) Beads 2.0 or RiboNaut™ rRNA depletion kit from 10 ng - 5 µg to enrich for mRNA or total RNA (excluding rRNA) from RNA samples of 5 ng – 1 µg total RNA input and NEXTFLEX® adapters. The shelf life of all reagents is at least 12 months when stored properly. The Nuclease-free Water and Resuspension Buffer can be stored at room temperature. The NEXTFLEX® Cleanup Beads XP should be stored at 4°C, and all other components should be stored at -20°C.

Kit Contents	Amount
BROWN CAP	
NEXTFLEX® RNA-Seq Fragmentation Buffer Mix 2.0	760 µL
RED CAP	
NEXTFLEX® RNA-Seq Directional First Strand Synthesis Buffer Mix 2.0	558 µL
NEXTFLEX® RNA-Seq Rapid Reverse Transcriptase 2.0	140 µL
BLUE CAP	
NEXTFLEX® RNA-Seq Directional Second Strand Synthesis Mix 2.0	(2) 1300 µL
CLEAR CAP	
NEXTFLEX® RNA-Seq End Repair & Adenylation Buffer Mix 2.0	(2) 883 µL
NEXTFLEX® RNA-Seq End Repair & Adenylation Enzyme 2.0	353 µL
PURPLE CAP	
NEXTFLEX® RNA-Seq Ligase Buffer Mix 2.0	(4) 1236 µL
NEXTFLEX® RNA-Seq Ligase Enzyme 2.0	334 µL
GREEN CAP	
NEXTFLEX® RNA-Seq PCR Master Mix 2.0	(2) 1314 µL
NEXTFLEX® RNA-Seq Primer Mix 2.0 (50 µM)	211 µL
WHITE CAP BOTTLE	
Nuclease-free Water	25 mL
Resuspension Buffer	20 mL
NEXTFLEX® Cleanup Beads XP	35 mL

Optional Reagents

NEXTFLEX Poly(A) Beads 2.0 Automation

Kit Contents	Amount
YELLOW CAP	
NEXTFLEX® Poly (A) Beads 2.0	(2) 1 mL
WHITE CAP BOTTLE	
NEXTFLEX® Poly (A) Washing Buffer 2.0	60 mL
NEXTFLEX® Poly (A) Elution Buffer 2.0	9 mL
NEXTFLEX® Poly (A) Binding Buffer 2.0	21 mL

NEXTFLEX® RiboNaut™ rRNA Depletion Kit (H/M/R)

Kit Contents	Amount
PINK CAP	
NEXTFLEX® Bait Hybridization Buffer	411 µL
NEXTFLEX® RiboNaut™ Bait Mix	514 µL
BROWN CAP	
NEXTFLEX® Cleanup Beads XP	(2) 1.5 mL
WHITE CAP	
Resuspension Buffer (used as elution buffer)	(2) 1.5 mL
WHITE CAP BOTTLE	
NEXTFLEX® RiboNaut™ Beads	24 mL
NEXTFLEX® RiboNaut™ Bead Wash Buffer	72 mL
NEXTFLEX® Cleanup Buffer	12 mL

NEXTFLEX® Barcoded Adapter Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Representative plate layout of NEXTFLEX® RNA-Seq 2.0 UDI Barcodes 1 – 96 (6.25 µM stock plate) for automation. The NEXTFLEX® NGS barcode index is contained within the adapter sequence. For more information on format and customization options for automation, contact NGS@revvity.com

Sciclone® NGS Hardware, Software, Applications and Consumables

Required Hardware

Part	Vendor/Part Number
Sciclone® NGS Workstation	Revvity
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

Provided Maestro Applications

Application Name	NEXTFLEX® Rapid Directional RNA-Seq 2.0 Steps	Approximate Run Time (Including offline Incubations and PCR)
cDNA Synthesis	Optional Poly(A) Enrichment/rRNA Depletion	70 minutes
	RNA Fragmentation	25 minutes
	1st Strand Synthesis	70 minutes
	2nd Strand Synthesis	65 minutes
	Bead Cleanup	45 minutes
Library Prep	3′ Adenylation	45 minutes
	Adapter Ligation	20 minutes
	Bead Cleanups	45 minutes
	PCR Setup and Amplification	30-60 minutes (depending on # of cycles)
Post PCR Cleanup	NEXTFLEX® Bead Cleanups	45-75 minutes (depending on # of cleanups selected)

Required Consumables

Part No.	Vendor	Part	Quantity Needed for 96 samples (including Poly-A enrichment)	Quantity Needed for 96 samples (including RiboNaut™ rRNA Depletion)
6008870	Revvity	Bio-Rad Hard-Shell® 96 Well PCR Plate, Full Skirt	18	16
111426	Revvity	Pipette Tip, 150 µL, Art, Box, 10-96 Sterile Racks	31	33
6008880	Revvity	Deepwell-96 POS, Square 2.0 mL well, Polypropylene, Seahorse	3	3
6008700	Revvity	Reservoir-Deepwell, 12 column, 21mL	4	4
6000030	Revvity	946 Lid-Universal, Robotic friendly, Polystyrene	13	12
6008890	Revvity	Microplate-384 well, Round bottom, Polypropylene, pack of 10	1	1
6008290	Revvity	StorPlate-96V, PP, 96 well, V-bottom, (V), 450µL, 50/box	1	3

Required Materials Not Provided

- Ethanol 100%
- Adhesive PCR Plate Seal (Bio-Rad, Cat # MSB1001)
- Thermal cycler compatible with Bio-Rad Hard-Shell®96 Well Full Skirted PCR Plate
- 2, 10, 20, 200 and 1000 µL pipettes / multichannel pipettes
- Nuclease-free barrier pipette tips
- Microcentrifuge
- 1.5 mL nuclease-free microcentrifuge tubes
- Revvity LabChip® GX/GXII Touch™ instrument or equivalent
- High Sensitivity DNA chips and reagents

Warnings and Precautions

We strongly recommend 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 NGS@revvity.com

- Do not use the kit past the expiration date.
- The NEXTFLEX® Directional First Strand Synthesis Buffer Mix 2.0 may appear yellow in color.
- The NEXTFLEX® RNA-Seq 2.0 UDI barcodes (NOVA-51292X series) are intended to be used with the NEXTFLEX® Rapid Directional RNA-Seq 2.0 Kit and are at a 6.25 µM starting concentration. The NEXTFLEX® RNA-Seq Barcodes (NOVA-51291X series) are not compatible with the the NEXTFLEX® Rapid Directional RNA-Seq 2.0 Kit as they are at a concentration of 0.6 µM starting concentration. Most NEXTFLEX adapter barcodes are typically provided at 25 µM, and would require different dilution recommendations if using with the NEXTFLEX® (R) Rapid Directional RNA-Seq 2.0 Kit. Inquire for more details at NGS@revvity.com
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- Ensure that all pipette tips, microcentrifuge tubes, and other consumables are RNase-free.
- 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 precipitate is in solution.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Vortex and micro-centrifuge each component prior to use, to ensure material has not lodged in the cap or the 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 XP.
- Thermal cycling should be performed with a heated lid except where specified.
- Do not heat the NEXTFLEX® adapter barcodes above room temperature.
- For multiplexing options, please use the appropriate NEXTFLEX® adapter barcodes during STEP E: Adapter Ligation. Inquire at NGS@revvity.com
- RNA sample quality may vary between preparations. High quality RNA should have either an RNA Quality Score (RQS) greater than or equal to 7 or a 28S band that is twice as intense as the 18S band of ribosomal RNA.
- Vortex beads until they are a uniform suspension.
- Allow beads to come to room temperature for 30 minutes prior to use.

Starting Material

The NEXTFLEX® Rapid Directional RNA-Seq Kit 2.0 has been optimized and validated using poly(A) enriched or rRNA depleted RNA (~1 ng – 100 ng). Only 10 ng - 5 µg of total RNA is required if NEXTFLEX Poly(A) beads are used to enrich for mRNA*. The NEXTFLEX® Poly(A) beads have been optimized and validated using 10 ng - 5 µg of total RNA for RNA-seq applications. Only 5 ng - 1 µg of total RNA is required for the NEXTFLEX® RiboNaut™ rRNA Depletion Kit (H/M/R).

We recommend examining total RNA integrity using a LabChip® GX/GXII Touch™ instrument or equivalent. High quality total RNA preparations should have an RNA Quality Score (RQS) greater than or equal to 7. Alternatively, total RNA may be run on a 1 - 2% agarose gel and integrity examined by staining with ethidium bromide. High quality RNA should have a 28S band that is twice as intense as the 18S band of ribosomal RNA. Lower amounts of starting material result in higher duplication rates and other changes in sequencing data quality.

*Low RNA inputs may reduce library complexity. To request additional information, email NGS@revvity.com

The NEXTFLEX® RNA-Seq 2.0 UDI Barcodes are recommended for use with this kit. These barcodes are at a starting stock concentration of 6.25 µM. If using other NEXTFLEX adapter barcodes, please inquire at NGS@revvity.com for additional guidance.

Previously Poly(A) enriched RNA, Previously rRNA depleted RNA, Total RNA, or FFPE Total RNA	Desired Adapter Concentration	Adapter Dilution Required	PCR Cycles
1 ng	0.3125 µM	1/20	13 – 14
10 ng	1.56 µM	1/4	10 – 11
50 ng	3.125 µM	1/2	8 – 9
100 ng	6.25 µM	None	7 – 8
Input RNA: Total RNA enriched using NEXTFLEX® Poly(A) Beads 2.0	**Desired Adapter Concentration**	**Adapter Dilution Required**	**PCR Cycles**
10 ng	0.104 µM	1/60	16 – 17
100 ng	0.3125 µM	1/20	13 – 14
1000 ng	1.56 µM	1/4	9 – 10
5000 ng	6.25 µM	None	7 – 8
Input RNA: Total RNA depleted using NEXTFLEX® RiboNaut™ rRNA Depletion Kit (H/M/R)	**Desired Adapter Concentration**	**Adapter Dilution Required**	**PCR Cycles**
5 ng	0.104 µM	1/60	16 – 17
100 ng	0.3125 µM	1/20	12 – 13
1000 ng	3.125 µM	1/2	8 – 9
Input RNA: FFPE Total RNA depleted using NEXTFLEX® RiboNaut™ rRNA Depletion Kit (H/M/R)	**Desired Adapter Concentration**	**Adapter Dilution Required**	**PCR Cycles**

5 ng	0.104 µM	1/60	17 – 20
50 ng	0.104 µM	1/60	14 – 17

APPENDIX

Oligonucleotide Sequences

NEXTFLEX®	Sequence (5′ → 3′)
PCR Primer 1	AATGATACGGCGACCACCGAGATCTACAC
PCR Primer 2	CAAGCAGAAGACGGCATACGAGAT
NEXTFLEX® UDI Barcode	AATGATACGGCGACCACCGAGATCTACACXXXXXXXX[1]AC ACTCTTTCCCTACACGACGCTCTTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXX X[2]ATCTCGTATGCCGTCTTCTGCTTG

XXXXXXXX[1] denotes the P5 index region of adapter. The index sequences contained in each adapter are listed below.

XXXXXXXX[2] denotes the P7 index region of the adapter. The index sequences contained in each adapter are listed below.

When entering index sequences for the Illumina® MiniSeq®, NextSeq®, HiSeq® 3000 or HiSeq® 4000 platforms, enter the P5 Index Reverse Complement. For all other Illumina® platforms, enter the P5 Index in the first column.

	P5 Index	P5 Index Reverse Complement	P7 Index
UDI 1	AATAACGT	ACGTTATT	AATCGTTA
UDI 2	TTCTTGAA	TTCAAGAA	GTCTACAT
UDI 3	GGCAGATC	GATCTGCC	CGCTGCTC
UDI 4	CTATGTTA	TAACATAG	GATCAACA
UDI 5	GTTGACGC	GCGTCAAC	CGAAGGAC
UDI 6	ATCTACGA	TCGTAGAT	GATGCCGG
UDI 7	CTCGACAG	CTGTCGAG	CTACGAAG
UDI 8	GAGGCTGC	GCAGCCTC	GATGCGTC
UDI 9	CCTCGTAG	CTACGAGG	CTACGGCA
UDI 10	CATAGGCA	TGCCTATG	GATTCCTT
UDI 11	AGATGAAC	GTTCATCT	CTACTCGA
UDI 12	CCGAGTAT	ATACTCGG	GATTCGAG
UDI 13	AATATTGA	TCAATATT	AATCGGCG
UDI 14	GTATACCG	CGGTATAC	TTCGCCGA
UDI 15	GATCCAAC	GTTGGATC	CTGGCCTC
UDI 16	AGATACGC	GCGTATCT	GAACTTAT
UDI 17	GGTATCTT	AAGATACC	CGTATTGG
UDI 18	CCTCTGGC	GCCAGAGG	GAAGCACA

UDI 19	CCATTGTG	CACAATGG	CTTAATAC
UDI 20	ACTACGGT	ACCGTAGT	GAAGTCTT
UDI 21	AAGTGCTA	TAGCACTT	GAAGAGGC
UDI 22	GCCGAACG	CGTTCGGC	CGGATAAC
UDI 23	TGTCCACG	CGTGGACA	GAATCTGG
UDI 24	GACACACT	AGTGTGTC	CTGATTGA
UDI 25	AATATGCT	AGCATATT	AATCCGTT
UDI 26	TTCTCATA	TATGAGAA	TGCGTACA
UDI 27	TCTGTGAT	ATCACAGA	GAATCAAT
UDI 28	CCGAACTT	AAGTTCGG	TGAGTCAG
UDI 29	GTCTAACA	TGTTAGAC	GAATGCTC
UDI 30	GACGCCAT	ATGGCGTC	GAATATCC
UDI 31	GCCAATGT	ACATTGGC	CTTATGAA
UDI 32	CCAACGTC	GACGTTGG	TCGGCACC
UDI 33	GTAGATAA	TTATCTAC	AAGAAGCG
UDI 34	CTTACGGC	GCCGTAAG	CTCACGAT
UDI 35	CCAAGTGC	GCACTTGG	TCGGTCGA
UDI 36	CTAACTCA	TGAGTTAG	TCGGTAAG
UDI 37	AATATCTG	CAGATATT	AAGATACA
UDI 38	TTATATCA	TGATATAA	GTCGCTGT
UDI 39	CTGCGGAT	ATCCGCAG	TCGGATGT
UDI 40	GCGGCTTG	CAAGCCGC	CGAGCCGG
UDI 41	GAGTTGAT	ATCAACTC	CGATTATC
UDI 42	GCACTGAG	CTCAGTGC	TCGAAGCT
UDI 43	GACCACCT	AGGTGGTC	CTATCATT
UDI 44	TGGCTAGG	CCTAGCCA	CGCGCCAA
UDI 45	CCTACCGG	CCGGTAGG	CGAACGGA
UDI 46	GGAGGATG	CATCCTCC	CTACTGAC
UDI 47	CGCTGAAT	ATTCAGCG	TCTTAAGT
UDI 48	TGTGACGA	TCGTCACA	TTAGAGTC
UDI 49	AATAGATT	AATCTATT	AAGACGAA
UDI 50	TTAGCGCA	TGCGCTAA	TTATTATG
UDI 51	GCGGCCGT	ACGGCCGC	CGCTATTA
UDI 52	CAGTAACC	GGTTACTG	TCTATCAG
UDI 53	GCCTAGTA	TACTAGGC	CGGTGGTA
UDI 54	CACGGCGC	GCGCCGTG	TCACCAAT

UDI 55	GGTGCAGA	TCTGCACC	CTGGAAGC
UDI 56	TCGCTGAC	GTCAGCGA	CGTAAGAG
UDI 57	CAGCCAGT	ACTGGCTG	AAGAGAGC
UDI 58	CGTCAACC	GGTTGACG	TCAACGAG
UDI 59	GCCGGCGA	TCGCCGGC	TGCGAGAC
UDI 60	GCCTCCGG	CCGGAGGC	CCTGGTGT
UDI 61	AATAGTCC	GGACTATT	AAGTAAGT
UDI 62	TTAGACGT	ACGTCTAA	TGACTGAA
UDI 63	GTGGACTA	TAGTCCAC	AAGACTGT
UDI 64	CACGGACG	CGTCCGTG	CAATGATG
UDI 65	CACTAGAG	CTCTAGTG	CACAGTAA
UDI 66	GCAGATGG	CCATCTGC	TGGTCATT
UDI 67	CTCTCACG	CGTGAGAG	CAACCGTG
UDI 68	GGAATCAC	GTGATTCC	TGGTGCAC
UDI 69	CGTTGACG	CGTCAACG	CCACAATG
UDI 70	CATCAGGT	ACCTGATG	TGTGTGCC
UDI 71	CGTTGTAA	TTACAACG	CACCACGG
UDI 72	GGCACGGT	ACCGTGCC	TGTGTTAA
UDI 73	AATAGCAA	TTGCTATT	AAGTTATC
UDI 74	TGATCGGT	ACCGATCA	GTACAGCT
UDI 75	AGTAGTAT	ATACTACT	CAACTGCT
UDI 76	GTTAGAGG	CCTCTAAC	CATGATGA
UDI 77	CCTTACAG	CTGTAAGG	TGACTACT
UDI 78	GTACATTG	CAATGTAC	CAGAAGAT
UDI 79	GGAGACCA	TGGTCTCC	TGAGGCGC
UDI 80	CGAACACC	GGTGTTCG	CAGGTTCC
UDI 81	GAGAACAA	TTGTTCTC	TGAACAGG
UDI 82	TGTGAATC	GATTCACA	CAGTGTGG
UDI 83	GGTTAAGG	CCTTAACC	TTCCACCA
UDI 84	AGACCGCA	TGCGGTCT	CCGCTGTT
UDI 85	AATACAGG	CCTGTATT	AAGTTGGA
UDI 86	TGATGGCC	GGCCATCA	GGACAACG
UDI 87	TGTCACCT	AGGTGACA	TTCGAACC
UDI 88	GCTTCGGC	GCCGAAGC	CAGACCAC
UDI 89	CCAGTGGT	ACCACTGG	TTCTGGTG
UDI 90	GCACACGC	GCGTGTGC	CAATCGAA

UDI 91	GTCACGTC	GACGTGAC	AAGTACAG
UDI 92	GCAGCTCC	GGAGCTGC	CCGTGCCA
UDI 93	CATGCAGC	GCTGCATG	CATTGCAC
UDI 94	ACGATTGC	GCAATCGT	TTACCTGG
UDI 95	GACATTCG	CGAATGTC	CTGCAACG
UDI 96	GCGAATAC	GTATTCGC	TACTGTTA
UDI 97	AATTAATG	CATTAATT	AAGTGTAT
UDI 98	TTAATTCC	GGAATTAA	GTCCACTC
UDI 99	GAAGGAAC	GTTCCTTC	CTTGCTAT
UDI 100	CAGGCATA	TATGCCTG	TACATAGA
UDI 101	CCAATACT	AGTATTGG	TAGCCGAT
UDI 102	TCGCGCAT	ATGCGCGA	CGATCCAC
UDI 103	GTGTGAAC	GTTCACAC	TAGCGTTG
UDI 104	GGTGGCAC	GTGCCACC	CTCATCAC
UDI 105	TAGGTGCT	AGCACCTA	TATGCGGT
UDI 106	GAGCGGTT	AACCGCTC	TAACTCGC
UDI 107	AGGATTAG	CTAATCCT	CGTACGTT
UDI 108	GTGAGCCA	TGGCTCAC	TAAGTACC
UDI 109	AATTATGC	GCATAATT	AAGTCGTG
UDI 110	TTACGCCG	CGGCGTAA	TTCAGAAC
UDI 111	GTAGTGAT	ATCACTAC	GTTATATA
UDI 112	TGGTTGCA	TGCAACCA	ACCGCTAT
UDI 113	GTCCGACC	GGTCGGAC	ACCGTCCT
UDI 114	GCCAGCCG	CGGCTGGC	TGTCTAAC
UDI 115	CTCGTGTC	GACACGAG	ACCGAGGT
UDI 116	GCCGTGGC	GCCACGGC	ACCGATTA
UDI 117	GTAGCCAC	GTGGCTAC	GTTCTACT
UDI 118	GACTTATA	TATAAGTC	ACCTGACT
UDI 119	GAACGTCG	CGACGTTC	GGTAATCG
UDI 120	ATGCGTAG	CTACGCAT	ACCTTAGA
UDI 121	AATTAGAT	ATCTAATT	AAGGAGTT
UDI 122	TTACGATA	TATCGTAA	TGAAGCCA
UDI 123	CTGTACAA	TTGTACAG	GTGTTGTA
UDI 124	CGTGGTTG	CAACCACG	ACCACACG
UDI 125	TGTAGCGG	CCGCTACA	ACCAGGAC
UDI 126	GTGTCATG	CATGACAC	TTGGCAGG

UDI 127	GAACCGGT	ACCGGTTC	ACCATTAA
UDI 128	CACTCTGA	TCAGAGTG	TTGTAGAT
UDI 129	TACACGTT	AACGTGTA	ACCATATC
UDI 130	GAGTGCCT	AGGCACTC	TTGGTGGC
UDI 131	GACGGTCC	GGACCGTC	ACCAACAT
UDI 132	CTGATGCT	AGCATCAG	GTCTGTGC
UDI 133	AATTACCA	TGGTAATT	AAGGCAAT
UDI 134	GTCCTGTT	AACAGGAC	TGCATTGC
UDI 135	GTTAACAG	CTGTTAAC	ACGCCACT
UDI 136	AGCCTGTA	TACAGGCT	AAGTCTCC
UDI 137	ACGTTGCT	AGCAACGT	GGATCTCT
UDI 138	GGTGTTGC	GCAACACC	ACGCGATC
UDI 139	TGGTACTA	TAGTACCA	GGATAATA
UDI 140	CATCATTG	CAATGATG	ACGCTTAT
UDI 141	GACGATTG	CAATCGTC	TTAGGTTG
UDI 142	CGGCCGAA	TTCGGCCG	ACGCACAA
UDI 143	CTGCCGCC	GGCGGCAG	TGAATATA
UDI 144	GCATTAGC	GCTAATGC	GGCAGGAG
UDI 145	AATTGAGA	TCTCAATT	AAGCAATA
UDI 146	TTCAAGAG	CTCTTGAA	TTATGTAT
UDI 147	GTCCGCAA	TTGCGGAC	ACGGCAGA
UDI 148	CAAGGTAG	CTACCTTG	GGACTCTG
UDI 149	AGCGACTC	GAGTCGCT	GTACGTAC
UDI 150	GTAGTATC	GATACTAC	GGAAGGTA
UDI 151	GTGGCGAA	TTCGCCAC	ACGTCCAT
UDI 152	ACGTGCGC	GCGCACGT	ACGTGTTG
UDI 153	GAAGACAG	CTGTCTTC	TGAAGAAT
UDI 154	GCGCAGAT	ATCTGCGC	ACGTAGTC
UDI 155	AGAGACAT	ATGTCTCT	AAGGATAA
UDI 156	CACTTGTT	AACAAGTG	GGCGAGGA
UDI 157	AATTGGTC	GACCAATT	AACAAGGC
UDI 158	TTAACACT	AGTGTTAA	TTGGTCCG
UDI 159	GCTCAGCG	CGCTGAGC	ACGAGCCT
UDI 160	GTTCAGAC	GTCTGAAC	GGAGATTC
UDI 161	CAGTTGCG	CGCAACTG	TGCGCGCT
UDI 162	GCAATATG	CATATTGC	ACGATCTA

UDI 163	CGCAACGT	ACGTTGCG	TGCTGAGG
UDI 164	GGTGAGAT	ATCTCACC	ACTCCTCC
UDI 165	CCAGCTGA	TCAGCTGG	ACTCTACG
UDI 166	GTTGAGCA	TGCTCAAC	TTCGTTCT
UDI 167	CTGTAGTG	CACTACAG	TGCAGTCG
UDI 168	GTGCATCC	GGATGCAC	TGCCGTAA
UDI 169	AATTCACT	AGTGAATT	AACAGTTG
UDI 170	TTCAGTGA	TCACTGAA	TGTTAACA
UDI 171	AGTCGCGA	TCGCGACT	ACTTCCTG
UDI 172	CCATATCG	CGATATGG	TGAATGCG
UDI 173	AAGCATGG	CCATGCTT	ACTTGTAC
UDI 174	CGTGTCTA	TAGACACG	ACTACTTA
UDI 175	TCAGTTGC	GCAACTGA	TGAGATCG
UDI 176	CGAGAGTG	CACTCTCG	ACTAGCTC
UDI 177	CGAGTACG	CGTACTCG	TTATCAAC
UDI 178	GTGTCTCA	TGAGACAC	TGCTTGTC
UDI 179	CCATCGCT	AGCGATGG	AAGGTAGG
UDI 180	CTTCGCTC	GAGCGAAG	TGGTATGG
UDI 181	AATTCTAA	TTAGAATT	AACACATA
UDI 182	TTCATCTG	CAGATGAA	TGGTGTCT
UDI 183	ACAGTGAA	TTCACTGT	ACACTAAC
UDI 184	AGATGTGA	TCACATCT	TTGTGTTC
UDI 185	GGCAACTG	CAGTTGCC	ACACACCT
UDI 186	CAGCGTCT	AGACGCTG	GGTGTCCG
UDI 187	CTGTCGGT	ACCGACAG	ACACATTC
UDI 188	TCGGCGTT	AACGCCGA	TTGCTTAA
UDI 189	CACACGCG	CGCGTGTG	ACAGCCTT
UDI 190	AGCCAACA	TGTTGGCT	TTGCAGTA
UDI 191	AGCAAGTT	AACTTGCT	ACAGGCAG
UDI 192	ACGCGTCA	TGACGCGT	TTGCCATC
UDI 193	AATGAACC	GGTTCATT	ATAATGTA
UDI 194	GGAAGTGG	CCACTTCC	TCGTACCG
UDI 195	CGCTAAGC	GCTTAGCG	GTTATTAT
UDI 196	GTCATAGC	GCTATGAC	ACAGAAGC
UDI 197	GTGACCAT	ATGGTCAC	TTGATAAT
UDI 198	GAAGTTGA	TCAACTTC	ACATCGGA

UDI 199	CGCTACCG	CGGTAGCG	ACATCTAG
UDI 200	CGCCTCTT	AAGAGGCG	GTGAGTGT
UDI 201	GTTCTGGA	TCCAGAAC	ACATCACC
UDI 202	GAGATGCC	GGCATCTC	TTGAACGC
UDI 203	GGTTAGCC	GGCTAACC	ACATGGAT
UDI 204	GGCTCGAC	GTCGAGCC	TGGCAGAG
UDI 205	AATGTGGC	GCCACATT	ATAAGGCT
UDI 206	GTCACAAG	CTTGTGAC	TCTTACTC
UDI 207	CGTAGATA	TATCTACG	ACATGATA
UDI 208	TGCGGCTA	TAGCCGCA	TTGCCTCT
UDI 209	ACAACTCT	AGAGTTGT	ACATTCTC
UDI 210	GATCGCAG	CTGCGATC	ACATATGT
UDI 211	TGGAACAT	ATGTTCCA	GTTGGTAG
UDI 212	CACATACC	GGTATGTG	ACAACCGC
UDI 213	GCTAAGAA	TTCTTAGC	AAGCAGAC
UDI 214	CTTAACCA	TGGTTAAG	ACAAGGTG
UDI 215	GCCTTCAA	TTGAAGGC	ACAAGAGT
UDI 216	AACTGGAA	TTCCAGTT	TTGGAATT
UDI 217	AATGGAAG	CTTCCATT	ATAACAGA
UDI 218	TTCAATTC	GAATTGAA	TATTGTCG
UDI 219	CGTCCTTA	TAAGGACG	ACAATGCC
UDI 220	CACCGGTA	TACCGGTG	TATGGTTC
UDI 221	AGGAACCA	TGGTTCCT	AGCCACAG
UDI 222	GATCTTCC	GGAAGATC	GCTATCGA
UDI 223	CGGCTTGT	ACAAGCCG	AGCGCTCG
UDI 224	GCCGACAC	GTGTCGGC	TGCGCTTC
UDI 225	CGGCGTAC	GTACGCCG	TCTACGCC
UDI 226	GCCTCATT	AATGAGGC	TATCACAA
UDI 227	CGGCACGA	TCGTGCCG	AGCTTGGT
UDI 228	GCCTGTAG	CTACAGGC	AGCTTATG
UDI 229	AATGGCCT	AGGCCATT	ATAACGAC
UDI 230	TTCCAGGC	GCCTGGAA	GATGGAGT
UDI 231	TTAACCTC	GAGGTTAA	TGATCTAA
UDI 232	CGTTAGGA	TCCTAACG	AGCTATAT
UDI 233	GCCATAAT	ATTATGGC	AGCAGAGA
UDI 233	GCCATAAT	ATTATGGC	AGCAGAGA

UDI 234	GCCAAGCC	GGCTTGGC	GACGTAAG
UDI 235	CTCGGTCA	TGACCGAG	AGGCCTGA
UDI 236	CGCAGGCT	AGCCTGCG	TAAGATTA
UDI 237	GCGTCCTC	GAGGACGC	AGGCGAAT
UDI 238	CTACAAGT	ACTTGTAG	TTGTTCTT
UDI 239	GCGTGTCC	GGACACGC	TACTCCTG
UDI 240	GCGACTGA	TCAGTCGC	GACTGGCG
UDI 241	AATGCTTG	CAAGCATT	ATATATAC
UDI 242	TTAATCAA	TTGATTAA	TAGATCGG
UDI 243	CTCTGGAC	GTCCAGAG	AGGCAAGC
UDI 244	GCTCCAGT	ACTGGAGC	TCCAGGTA
UDI 245	CGCGTTAT	ATAACGCG	AGGTCAAG
UDI 246	GCTCAATC	GATTGAGC	TACACTGG
UDI 247	CGCATGTG	CACATGCG	TCCTTGCG
UDI 248	GCTGCCAA	TTGGCAGC	AGGACCTC
UDI 249	CGAACTAT	ATAGTTCG	TAAGCATT
UDI 250	GCTGTCTC	GAGACAGC	AGGATATT
UDI 251	CGACGGTT	AACCGTCG	TCATTCGG
UDI 252	GCTTCACA	TGTGAAGC	AGTCCGAC
UDI 253	AATGCGCA	TGCGCATT	ATATTACA
UDI 254	TGAATCGT	ACGATTCA	TCTCCGTG
UDI 255	CTAACCAG	CTGGTTAG	TAATAGGA
UDI 256	GCTTGTGA	TCACAAGC	TTGTAAGA
UDI 257	CTGGCACT	AGTGCCAG	GAATGTAA
UDI 258	GCACGGTC	GACCGTGC	AGTCAATT
UDI 259	CTTGGATT	AATCCAAG	GAACAATT
UDI 260	GCACATCA	TGATGTGC	AGTGGTCA
UDI 261	CTTCAATA	TATTGAAG	TACAATAT
UDI 262	GCAGGTAT	ATACCTGC	AGTGTGAG
UDI 263	CTGAGTCC	GGACTCAG	GAACCTTA
UDI 264	GCATACTT	AAGTATGC	AGTGTACT
UDI 265	AATGCCAC	GTGGCATT	TATACCAT
UDI 266	GGCCGTTG	CAACGGCC	ATCGTGGA
UDI 267	GCATAGAC	GTCTATGC	GCAAGACA
UDI 268	CATTCGCC	GGCGAATG	TACAGGCC
UDI 269	GGCCGCGT	ACGCGGCC	AGTTCCGT

UDI 270	CCTGAAGG	CCTTCAGG	TCAATTAT
UDI 271	GGCCTTCA	TGAAGGCC	AGTTCATA
UDI 272	GGCTTACT	AGTAAGCC	TCACCGGC
UDI 273	CCGGTCTG	CAGACCGG	TACAACTA
UDI 274	GGCACTCC	GGAGTGCC	AGTTGGCT
UDI 275	CATGTCGT	ACGACATG	TACGTCTC
UDI 276	GGCAATAA	TTATTGCC	AGTACGCG
UDI 277	AATCTGAA	TTCAGATT	TATACATC
UDI 278	TGCGAAGT	ACTTCGCA	ATCGTTAG
UDI 279	CAAGGAGA	TCTCCTTG	GAATTACG
UDI 280	GGTCTTAG	CTAAGACC	AGTACTGC
UDI 281	CAAGTAAT	ATTACTTG	TACTAACG
UDI 282	GGTCAGTA	TACTGACC	AGTATGTC
UDI 283	CCAGTCAC	GTGACTGG	TAAGTGTG
UDI 284	GGTCATGT	ACATGACC	ATATAAGG
UDI 285	CAACGACC	GGTCGTTG	ATAGAATA
UDI 286	GGTTCTTG	CAAGAACC	TCTAGAGA
UDI 287	CAACATAC	GTATGTTG	ATAGGCCA
UDI 288	GGTTGATT	AATCAACC	ATAGCGGT
UDI 289	AATAATAG	CTATTATT	ATATCCTA
UDI 290	GTCGGATA	TATCCGAC	GATAGGAT
UDI 291	CACATTGT	ACAATGTG	ATACTGCG
UDI 292	GGTTACAA	TTGTAACC	TCTTCCGA
UDI 293	CACGAATT	AATTCGTG	ATACCTAT
UDI 294	GGTATGCA	TGCATACC	TCGTTATA
UDI 295	CAGGACCT	AGGTCCTG	TAATTAGT
UDI 296	GGACTGGC	GCCAGTCC	ATTATTCG
UDI 297	CAGAGCGA	TCGCTCTG	TAATAATC
UDI 298	GGAGCTAG	CTAGCTCC	ATTATCGC
UDI 299	CAGCTTAG	CTAAGCTG	TAATATAG
UDI 300	CCGCCAAC	GTTGGCGG	ATTAGACA
UDI 301	CCTATGCG	CGCATAGG	ATAGATCT
UDI 302	GGATCCGA	TCGGATCC	TAGAGCTC
UDI 303	GGATTGTT	AACAATCC	ATTACAAT
UDI 304	CAGGTGAA	TTCACCTG	ATTGAAGT
UDI 305	GGAACATT	AATGTTCC	ATTGATTC

UDI 306	CAAGACTC	GAGTCTTG	TAACTAAG
UDI 252	GCTTCACA	TGTGAAGC	AGTCCGAC
UDI 253	AATGCGCA	TGCGCATT	ATATTACA
UDI 254	TGAATCGT	ACGATTCA	TCTCCGTG
UDI 255	CTAACCAG	CTGGTTAG	TAATAGGA
UDI 256	GCTTGTGA	TCACAAGC	TTGTAAGA
UDI 257	CTGGCACT	AGTGCCAG	GAATGTAA
UDI 258	GCACGGTC	GACCGTGC	AGTCAATT
UDI 259	CTTGGATT	AATCCAAG	GAACAATT
UDI 260	GCACATCA	TGATGTGC	AGTGGTCA
UDI 261	CTTCAATA	TATTGAAG	TACAATAT
UDI 262	GCAGGTAT	ATACCTGC	AGTGTGAG
UDI 263	CTGAGTCC	GGACTCAG	GAACCTTA
UDI 264	GCATACTT	AAGTATGC	AGTGTACT
UDI 265	AATGCCAC	GTGGCATT	TATACCAT
UDI 266	GGCCGTTG	CAACGGCC	ATCGTGGA
UDI 267	GCATAGAC	GTCTATGC	GCAAGACA
UDI 268	CATTCGCC	GGCGAATG	TACAGGCC
UDI 269	GGCCGCGT	ACGCGGCC	AGTTCCGT
UDI 270	CCTGAAGG	CCTTCAGG	TCAATTAT
UDI 271	GGCCTTCA	TGAAGGCC	AGTTCATA
UDI 272	GGCTTACT	AGTAAGCC	TCACCGGC
UDI 273	CCGGTCTG	CAGACCGG	TACAACTA
UDI 274	GGCACTCC	GGAGTGCC	AGTTGGCT
UDI 275	CATGTCGT	ACGACATG	TACGTCTC
UDI 276	GGCAATAA	TTATTGCC	AGTACGCG
UDI 277	AATCTGAA	TTCAGATT	TATACATC
UDI 278	TGCGAAGT	ACTTCGCA	ATCGTTAG
UDI 279	CAAGGAGA	TCTCCTTG	GAATTACG
UDI 280	GGTCTTAG	CTAAGACC	AGTACTGC
UDI 281	CAAGTAAT	ATTACTTG	TACTAACG
UDI 282	GGTCAGTA	TACTGACC	AGTATGTC
UDI 283	CCAGTCAC	GTGACTGG	TAAGTGTG
UDI 284	GGTCATGT	ACATGACC	ATATAAGG
UDI 285	CAACGACC	GGTCGTTG	ATAGAATA
UDI 286	GGTTCTTG	CAAGAACC	TCTAGAGA

UDI 287	CAACATAC	GTATGTTG	ATAGGCCA
UDI 288	GGTTGATT	AATCAACC	ATAGCGGT
UDI 289	AATAATAG	CTATTATT	ATATCCTA
UDI 290	GTCGGATA	TATCCGAC	GATAGGAT
UDI 291	CACATTGT	ACAATGTG	ATACTGCG
UDI 292	GGTTACAA	TTGTAACC	TCTTCCGA
UDI 293	CACGAATT	AATTCGTG	ATACCTAT
UDI 294	GGTATGCA	TGCATACC	TCGTTATA
UDI 295	CAGGACCT	AGGTCCTG	TAATTAGT
UDI 296	GGACTGGC	GCCAGTCC	ATTATTCG
UDI 297	CAGAGCGA	TCGCTCTG	TAATAATC
UDI 298	GGAGCTAG	CTAGCTCC	ATTATCGC
UDI 299	CAGCTTAG	CTAAGCTG	TAATATAG
UDI 300	CCGCCAAC	GTTGGCGG	ATTAGACA
UDI 301	CCTATGCG	CGCATAGG	ATAGATCT
UDI 302	GGATCCGA	TCGGATCC	TAGAGCTC
UDI 303	GGATTGTT	AACAATCC	ATTACAAT
UDI 304	CAGGTGAA	TTCACCTG	ATTGAAGT
UDI 305	GGAACATT	AATGTTCC	ATTGATTC
UDI 306	CAAGACTC	GAGTCTTG	TAACTAAG
UDI 307	GTGCGGCG	CGCCGCAC	ATTGACAA
UDI 308	CAATCTTC	GAAGATTG	ATTGTGTT
UDI 309	GTGCTCAG	CTGAGCAC	ATTGCTGA
UDI 310	CAACGCAT	ATGCGTTG	TACATCCT
UDI 311	GTGTAGGC	GCCTACAC	ATTCACGG
UDI 312	CAACTATA	TATAGTTG	TAATTGAC
UDI 313	GCATTGCA	TGCAATGC	ATAGTCTG
UDI 314	CTTACATG	CATGTAAG	TATAAGAC
UDI 315	GTGTATAG	CTATACAC	ATTCTTAC
UDI 316	AATCACTC	GAGTGATT	TAAGAAGG
UDI 317	CACTATCT	AGATAGTG	ATTCTCTA
UDI 318	GTGATCTC	GAGATCAC	ATTCGATG
UDI 319	CAATCCGT	ACGGATTG	TAAGCTAC
UDI 320	GTTCGTCA	TGACGAAC	ATTCGTGT
UDI 321	CCACTTAA	TTAAGTGG	ATGAATAT
UDI 322	GTTGAATG	CATTCAAC	ATGAAGGA

UDI 323	CCAGCAAG	CTTGCTGG	ATGAACTG
UDI 324	GTTAGTTC	GAACTAAC	ATGAGCAC
UDI 325	CCAGGCTA	TAGCCTGG	ATAGGAAT
UDI 326	GGTCCGCT	AGCGGACC	TAGACGGC
UDI 327	CACTTCCA	TGGAAGTG	GAATAGTG
UDI 328	GTTAAGTT	AACTTAAC	ATGACACC
UDI 329	AATCTAGT	ACTAGATT	ATGACGTT
UDI 330	GTTAATGA	TCATTAAC	ATGTATTA
UDI 331	CATACCTA	TAGGTATG	ATGTACCT
UDI 332	GTATGAGT	ACTCATAC	TCAATGGA
UDI 333	CTTGCGTA	TACGCAAG	ATGTTGAG
UDI 334	GACCGAAT	ATTCGGTC	TAACCAGA
UDI 335	CGTTACTT	AAGTAACG	ATGTGATT
UDI 336	GACCTGAC	GTCAGGTC	TAACAGCC
UDI 337	TTCGGCAT	ATGCCGAA	TATCTGTC
UDI 338	CCGAAGGC	GCCTTCGG	AGATAACT
UDI 339	GACCATGA	TCATGGTC	ATGTGGCA
UDI 340	GACGTGCA	TGCACGTC	TAACACTG
UDI 341	CGGCGACA	TGTCGCCG	ATGTCTGG
UDI 342	GACTAGGT	ACCTAGTC	TCAAGCAC
UDI 343	CGGTAGAC	GTCTACCG	ATGGTAAC
UDI 344	GACAGCTT	AAGCTGTC	ATGGTTCA
UDI 345	CGCTCGCA	TGCGAGCG	TCCAACGG
UDI 346	GAGATAGT	ACTATCTC	GAACAGAA
UDI 347	AACACTAC	GTAGTGTT	ATGGCCAG
UDI 348	GTGTGCGG	CCGCACAC	TCACGTGA
UDI 349	ACCGTGTG	CACACGGT	ATACAACC
UDI 350	TGGACACA	TGTGTCCA	TATGTTGG
UDI 351	AACTACTT	AAGTAGTT	ATGCTAGA
UDI 352	TTGAGGAA	TTCCTCAA	ATGCTGTC
UDI 353	AACTTAAT	ATTAAGTT	GATTGACC
UDI 354	TTGACTTA	TAAGTCAA	ATCATACT
UDI 355	AACTTGCC	GGCAAGTT	ATCAGCTA
UDI 356	GTGCACGT	ACGTGCAC	ATCACGCA
UDI 357	AACTTCGG	CCGAAGTT	GAGTTCTG
UDI 358	TGTACGAA	TTCGTACA	ATCACCGT

UDI 359	AACTCAGC	GCTGAGTT	TCTTGGAA
UDI 360	TGTATTCT	AGAATACA	ATCTAATC
UDI 361	GTCGTCGG	CCGACGAC	ATACATGA
UDI 362	CCGACGCA	TGCGTCGG	TATTGAAT
UDI 363	AACGAAGA	TCTTCGTT	GCTAGTCT
UDI 364	TGTCGGAT	ATCCGACA	ATCTAGCG
UDI 365	AACGACCG	CGGTCGTT	TCTCGCTA
UDI 366	TGTCGTGC	GCACGACA	ATCTGAAG
UDI 367	AACGTCAA	TTGACGTT	TCTAATGC
UDI 368	TGTAAGGT	ACCTTACA	GATAGCGC
UDI 369	AACGGATC	GATCCGTT	TATAGTGT
UDI 370	TTGAATCG	CGATTCAA	TATCAAGC
UDI 371	AACGGTGT	ACACCGTT	ATCGCATT
UDI 372	AACCAGTG	CACTGGTT	ATCCAGAA
UDI 373	AATATAAC	GTTATATT	ATACTATT
UDI 374	GGCGCGTG	CACGCGCC	GATGATAC
UDI 375	TTGTTACC	GGTAACAA	GCGGTATT
UDI 376	AACCTTGC	GCAAGGTT	ATCCGTTC
UDI 377	TGTGCAAT	ATTGCACA	AGAATTCA
UDI 378	AACCGAGG	CCTCGGTT	TATGACTT
UDI 379	TTGGATAC	GTATCCAA	GAGTCAGA
UDI 380	AACCGCAC	GTGCGGTT	AGAAGACG
UDI 381	TGTTAGTG	CACTAACA	AGAAGCTT
UDI 382	TATTCGTA	TACGAATA	GAGTAGCA
UDI 383	ATAATTGT	ACAATTAT	AGAACCAA
UDI 384	TAGTCCAA	TTGGACTA	GCTTGGTG

Low Level Multiplexing Guidelines

Barcodes 1 and 2, 13 and 14, 25 and 26, 37 and 38, 49 and 50, 61 and 62, 73 and 74, and 85 and 86 are fully color balanced and are suitable to be used in a pool of two samples. When designing low-plexy index pools, always include two libraries barcoded with a set of two unique and fully color balanced barcodes to avoid laser color complexity issues during de-multiplexing. Additional libraries may be safely multiplexed with one set of fully color balanced barcodes in a pool.

www.revvity.com

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com