SEQuoia Dual Indexed Primers

Catalog #	Description
12011928	**SEQuoia Dual Indexed Primers Set**, 12 vials of unique dual indexes, 96 reactions
12011930	**SEQuoia Dual Indexed Primers Plate**, 96-well plate of unique dual indexes, 96 reactions

For research purposes only.

Introduction

SEQuoia Dual Indexed Primers are designed for use with SEQuoia Complete Stranded RNA Library Prep Kits to construct libraries for multiplex sequencing using Illumina® platforms. The nucleotide sequence of each primer set has been balanced to achieve optimal multiplexed sequencing results. SEQuoia Dual Indexed Primers are attached to the sample insert during PCR amplification.

The SEQuoia Dual Indexed Primers Set contains 12 unique dual indexed adapters in 12 separate vials with sufficient volume for eight reactions per vial. The SEQuoia Dual Indexed Primers Plate contains 96 unique dual indexed adapters plated along the columns in a 96-well PCR plate. The first 12 sets of indexes (A1–H1, A2–D2) are identical to the indexes supplied in the SEQuoia Dual Indexed Primers Set. See Table 1 for kit contents.

Storage and Stability

SEQuoia Dual Indexed Primers are shipped on dry ice or ice packs, depending on the destination country. Upon receipt, store the product at –20°C in a constant temperature freezer. Indexes must not be exposed to temperatures above room temperature. SEQuoia Dual Indexed Primers are guaranteed for 12 months after the shipping date if stored properly. For best performance, avoid repeated freeze-thaw cycles.

Table 1. Kit contents.

Component	Catalog #	Volume	Storage Temperature
SEQuoia Dual Indexed Primers Set	12011928	20 µl/vial	–20°C
SEQuoia Dual Indexed Primers Plate*	12011930	10 µl/well	

* This plate is only for single use. Excess volume is provided to enable easy and accurate transfer of contents to PCR reaction plate. To avoid contamination, do not reseal and reuse excess volume.

Important Considerations — Please Read Before Starting

- Before using, thaw contents completely. Spin vials or plate for 1 min to ensure all liquid settles to the bottom of the vessel
- The plate seal is intended to be pierced. Do not peel the plate seal from the plate as doing so can easily lead to cross-contamination
- The SEQuoia Dual Indexes contain an excess volume to ensure accurate dispensing
- Before use, carefully mix the primers in the plate by pipetting up and down several times with a multichannel pipet equipped with barrier tips or by vortexing the vials. Do not vortex plates
- Primer concentrations are optimized for a wide range of input RNA. Do not adjust concentrations

Quality Control

SEQuoia Dual Indexed Primers are subject to stringent quality control. See Table 2 for sequences of dual indexes.

Table 2. Sequences of SEQuoia Dual Indexed Primers.

UDI #	Well	i7 Bases in Adapter	i7 Bases for Sample Sheet	i5 Bases for Sample Sheet (NovaSeq®, MiSeq®, HiSeq® 2000/2500)	i5 Bases for Sample Sheet (iSeq®, MiniSeq®, NextSeq®, HiSeq 3000/4000/X)
DI_1*	A1	AACCGCGG	CCGCGGTT	AGCGCTAG	CTAGCGCT
DI_2*	B1	GGTTATAA	TTATAACC	GATATCGA	TCGATATC
DI_3*	C1	CCAAGTCC	GGACTTGG	CGCAGACG	CGTCTGCG
DI_4*	D1	TTGGACTT	AAGTCCAA	TATGAGTA	TACTCATA
DI_5*	E1	CAGTGGAT	ATCCACTG	AGGTGCGT	ACGCACCT
DI_6*	F1	TGACAAGC	GCTTGTCA	GAACATAC	GTATGTTC
DI_7*	G1	CTAGCTTG	CAAGCTAG	ACATAGCG	CGCTATGT
DI_8*	H1	TCGATCCA	TGGATCGA	GTGCGATA	TATCGCAC
DI_9*	A2	CCTGAACT	AGTTCAGG	CCAACAGA	TCTGTTGG
DI_10*	B2	TTCAGGTC	GACCTGAA	TTGGTGAG	CTCACCAA
DI_11*	C2	AGTAGAGA	TCTCTACT	CGCGGTTC	GAACCGCG
DI_12*	D2	GACGAGAG	CTCTCGTC	TATAACCT	AGGTTATA
DI_13	E2	AGACTTGG	CCAAGTCT	AAGGATGA	TCATCCTT
DI_14	F2	GAGTCCAA	TTGGACTC	GGAAGCAG	CTGCTTCC
DI_15	G2	CTTAAGCC	GGCTTAAG	TCGTGACC	GGTCACGA
DI_16	H2	TCCGGATT	AATCCGGA	CTACAGTT	AACTGTAG
DI_17	A3	CTGTATTA	TAATACAG	ATATTCAC	GTGAATAT
DI_18	B3	TCACGCCG	CGGCGTGA	GCGCCTGT	ACAGGCGC
DI_19	C3	ACTTACAT	ATGTAAGT	ACTCTATG	CATAGAGT
DI_20	D3	GTCCGTGC	GCACGGAC	GTCTCGCA	TGCGAGAC
DI_21	E3	AAGGTACC	GGTACCTT	AAGACGTC	GACGTCTT
DI_22	F3	GGAACGTT	AACGTTCC	GGAGTACT	AGTACTCC
DI_23	G3	AATTCTGC	GCAGAATT	ACCGGCCA	TGGCCGGT
DI_24	H3	GGCCTCAT	ATGAGGCC	GTTAATTG	CAATTAAC
DI_25	A4	ATCTTAGT	ACTAAGAT	AACCGCGG	CCGCGGTT
DI_26	B4	GCTCCGAC	GTCGGAGC	GGTTATAA	TTATAACC
DI_27	C4	ATACCAAG	CTTGGTAT	CCAAGTCC	GGACTTGG
DI_28	D4	GCGTTGGA	TCCAACGC	TTGGACTT	AAGTCCAA
DI_29	E4	CTTCACGG	CCGTGAAG	CAGTGGAT	ATCCACTG
DI_30	F4	TCCTGTAA	TTACAGGA	TGACAAGC	GCTTGTCA
DI_31	G4	AGAATGCC	GGCATTCT	CTAGCTTG	CAAGCTAG
DI_32	H4	GAGGCATT	AATGCCTC	TCGATCCA	TGGATCGA
DI_33	A5	CCTCGGTA	TACCGAGG	CCTGAACT	AGTTCAGG
DI_34	B5	TTCTAACG	CGTTAGAA	TTCAGGTC	GACCTGAA
DI_35	C5	ATGAGGCT	AGCCTCAT	AGTAGAGA	TCTCTACT
DI_36	D5	GCAGAATC	GATTCTGC	GACGAGAG	CTCTCGTC
DI_37	E5	CACTACGA	TCGTAGTG	AGACTTGG	CCAAGTCT
DI_38	F5	TGTCGTAG	CTACGACA	GAGTCCAA	TTGGACTC
DI_39	G5	ACCACTTA	TAAGTGGT	CTTAAGCC	GGCTTAAG
DI_40	H5	GTTGTCCG	CGGACAAC	TCCGGATT	AATCCGGA
DI_41	A6	ATCCATAT	ATATGGAT	CTGTATTA	TAATACAG
DI_42	B6	GCTTGCGC	GCGCAAGC	TCACGCCG	CGGCGTGA
DI_43	C6	AGTATCTT	AAGATACT	ACTTACAT	ATGTAAGT
DI_44	D6	GACGCTCC	GGAGCGTC	GTCCGTGC	GCACGGAC
DI_45	E6	CATGCCAT	ATGGCATG	AAGGTACC	GGTACCTT
DI_46	F6	TGCATTGC	GCAATGCA	GGAACGTT	AACGTTCC

* Indexes provided in SEQuoia Dual Indexed Primers Set.

continues

UDI #	Well	i7 Bases in Adapter	i7 Bases for Sample Sheet	i5 Bases for Sample Sheet (NovaSeq, MiSeq, HiSeq 2000/2500)	i5 Bases for Sample Sheet (iSeq, MiniSeq, NextSeq, HiSeq 3000/4000/X)
DI_47	G6	ATTGGAAC	GTTCCAAT	AATTCTGC	GCAGAATT
DI_48	H6	GCCAAGGT	ACCTTGGC	GGCCTCAT	ATGAGGCC
DI_49	A7	CGAGATAT	ATATCTCG	ATCTTAGT	ACTAAGAT
DI_50	B7	TAGAGCGC	GCGCTCTA	GCTCCGAC	GTCGGAGC
DI_51	C7	AACCTGTT	AACAGGTT	ATACCAAG	CTTGGTAT
DI_52	D7	GGTTCACC	GGTGAACC	GCGTTGGA	TCCAACGC
DI_53	E7	CATTGTTG	CAACAATG	CTTCACGG	CCGTGAAG
DI_54	F7	TGCCACCA	TGGTGGCA	TCCTGTAA	TTACAGGA
DI_55	G7	CTCTGCCT	AGGCAGAG	AGAATGCC	GGCATTCT
DI_56	H7	TCTCATTC	GAATGAGA	GAGGCATT	AATGCCTC
DI_57	A8	ACGCCGCA	TGCGGCGT	CCTCGGTA	TACCGAGG
DI_58	B8	GTATTATG	CATAATAC	TTCTAACG	CGTTAGAA
DI_59	C8	GATAGATC	GATCTATC	ATGAGGCT	AGCCTCAT
DI_60	D8	AGCGAGCT	AGCTCGCT	GCAGAATC	GATTCTGC
DI_61	E8	CAGTTCCG	CGGAACTG	CACTACGA	TCGTAGTG
DI_62	F8	TGACCTTA	TAAGGTCA	TGTCGTAG	CTACGACA
DI_63	G8	CTAGGCAA	TTGCCTAG	ACCACTTA	TAAGTGGT
DI_64	H8	TCGAATGG	CCATTCGA	GTTGTCCG	CGGACAAC
DI_65	A9	CTTAGTGT	ACACTAAG	ATCCATAT	ATATGGAT
DI_66	B9	TCCGACAC	GTGTCGGA	GCTTGCGC	GCGCAAGC
DI_67	C9	AACAGGAA	TTCCTGTT	AGTATCTT	AAGATACT
DI_68	D9	GGTGAAGG	CCTTCACC	GACGCTCC	GGAGCGTC
DI_69	E9	CCTGTGGC	GCCACAGG	CATGCCAT	ATGGCATG
DI_70	F9	TTCACAAT	ATTGTGAA	TGCATTGC	GCAATGCA
DI_71	G9	ACACGAGT	ACTCGTGT	ATTGGAAC	GTTCCAAT
DI_72	H9	GTGTAGAC	GTCTACAC	GCCAAGGT	ACCTTGGC
DI_73	A10	GTTAATTG	CAATTAAC	CGAGATAT	ATATCTCG
DI_74	B10	ACCGGCCA	TGGCCGGT	TAGAGCGC	GCGCTCTA
DI_75	C10	GGAGTACT	AGTACTCC	AACCTGTT	AACAGGTT
DI_76	D10	AAGACGTC	GACGTCTT	GGTTCACC	GGTGAACC
DI_77	E10	GTCTCGCA	TGCGAGAC	CATTGTTG	CAACAATG
DI_78	F10	ACTCTATG	CATAGAGT	TGCCACCA	TGGTGGCA
DI_79	G10	GCGCCTGT	ACAGGCGC	CTCTGCCT	AGGCAGAG
DI_80	H10	ATATTCAC	GTGAATAT	TCTCATTC	GAATGAGA
DI_81	A11	CTACAGTT	AACTGTAG	ACGCCGCA	TGCGGCGT
DI_82	B11	TCGTGACC	GGTCACGA	GTATTATG	CATAATAC
DI_83	C11	GGAAGCAG	CTGCTTCC	GATAGATC	GATCTATC
DI_84	D11	AAGGATGA	TCATCCTT	AGCGAGCT	AGCTCGCT
DI_85	E11	TATAACCT	AGGTTATA	CAGTTCCG	CGGAACTG
DI_86	F11	CGCGGTTC	GAACCGCG	TGACCTTA	TAAGGTCA
DI_87	G11	TTGGTGAG	CTCACCAA	CTAGGCAA	TTGCCTAG
DI_88	H11	CCAACAGA	TCTGTTGG	TCGAATGG	CCATTCGA
DI_89	A12	GTGCGATA	TATCGCAC	CTTAGTGT	ACACTAAG
DI_90	B12	ACATAGCG	CGCTATGT	TCCGACAC	GTGTCGGA
DI_91	C12	GAACATAC	GTATGTTC	AACAGGAA	TTCCTGTT
DI_92	D12	AGGTGCGT	ACGCACCT	GGTGAAGG	CCTTCACC
DI_93	E12	TATGAGTA	TACTCATA	CCTGTGGC	GCCACAGG
DI_94	F12	CGCAGACG	CGTCTGCG	TTCACAAT	ATTGTGAA
DI_95	G12	GATATCGA	TCGATATC	ACACGAGT	ACTCGTGT
DI_96	H12	AGCGCTAG	CTAGCGCT	GTGTAGAC	GTCTACAC

Alignment and Analysis Guidelines

SEQuoia Complete Stranded RNA Library Prep Kit captures both long and short RNA. As such, secondary analysis requires a novel solution to simultaneously process both short and long RNA reads.

There are three main steps to processing and analyzing SEQuoia Complete Stranded RNA data:

FASTQ Preprocessing

- Trimming of SEQuoia Complete specific poly(A) tails
- Optional quality trimming

Alignment

- Optional unique molecular identifier–based PCR deduplication
- Single-pass alignment of all reads, both short and long RNA, to a conjoined annotation set of the transcriptome and known microRNAs, using the STAR aligner

Feature Counting

- Assignment of aligned reads to exons
- Single output for all mapped reads with raw and normalized counts (TPM, RPKM, FPKM)

Bio-Rad offers two streamlined bioinformatic workflow options that ensure consistent, high-quality data analysis. The parameters and settings of both options are identical.

Option 1: Web-Based Platform

SeqSense.bio-rad.com

This secure, web-based environment provides an integrated preconfigured data analysis workflow that allows users to perform complex analyses with only a few mouse clicks and to visualize and further interrogate their results.

Option 2: Docker Container

https:// hub.docker.com/r/bioraddbg/sequoia_analysis_toolkit

A command line application packaged with required accessories, such as libraries and other dependencies, is available to run on your infrastructure (Cloud or on-premises).

Trimming Guidelines

A read trimming step is recommended before proceeding with any quality metric calculations or downstream analysis. The SEQuoia Complete Kit adapters contain a single cytosine nucleotide base that appears immediately upstream of the insert and a poly(A) sequence immediately following the insert. To trim the cytosine base and the poly(A) tail, use cutadapt, which runs on Linux, macOS, and Windows (cutadapt.readthedocs.io/en/stable/guide.html).

Work with the reads in a FASTQ file (compressed or uncompressed). The reads can be trimmed running cutadapt from the command line: cutadapt -u 1 -a A{10} -m 15 -o output_file.fastq.gz input_file.fastq.gz (see Table 3 for description of command line).

Table 3. Command line.

Component	Description
-u 1	Directs cutadapt to trim the first base (5') of the read
-a A{10}	Directs cutadapt to trim any poly(A) track and all following bases in the read. The poly(A) track must be at least 10 bases long (unless it appears truncated at the end of the read) and contain no more than 1 error (that is, a nonadenine base). Poly(A) removal is important
-m 15	Removes reads from the FASTQ file that are shorter than 15 bases after trimming

Related Products

Catalog #	Description
17005726	**SEQuoia Complete Stranded RNA Library Prep Kit**, 24 reactions
17005710	**SEQuoia Complete Stranded RNA Library Prep Kit**, 96 reactions
1863040	**ddPCR Library Quantification Kit for Illumina TruSeq®**

Visit **bio-rad.com/SEQuoiaComplete** for more information.

Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Drive, Hercules, CA 94547
510-741-1000

10000119322 Ver B 20-0403 0620