Technical Bulletin

Ordering Additional Indexes for the BD Rhapsody™ Library Reagent Kits

Introduction

Index primers are used to uniquely label individual libraries so that multiple libraries can be sequenced in a single Illumina® sequencing run. This technical bulletin provides quick guides and instructions for ordering additional indexing primers for BD Rhapsody™ Sequencing Libraries.

BD Rhapsody Kits Index Primers

The BD Rhapsody Library Preparation Kits include four unique 3' index primers and one common 5' index primer. Table 1 lists the index primers included with the BD Rhapsody Kit. To increase the number of libraries that can be multiplexed in a single sequencing run, additional indexes are required: see the Ordering Additional Indexes section below.

Note: The sequences of the BD Rhapsody Reverse Index *Primers* are reverse complements of the Index 1 (i7) Adapter sequences in the Illumina *Adapter Sequences* document (see References). Any additional index primers ordered for use with BD Rhapsody Libraries must follow this reverse complement sequence convention for all Reverse Index Primers.

Name	Part number	Reference	Sequence (5' – 3')
BD Rhapsody™ Library Forward Primer	91-1085	TruSeq D501	AATGATACGGCGACCACCGAGATCTACAC**TATAGCCT**ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
BD Rhapsody™ Library Reverse Primer 1	650000080	Nextera N709	CAAGCAGAAGACGGCATACGAGAT**AGCGTAGC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD Rhapsody™ Library Reverse Primer 2	650000091	Nextera N710	CAAGCAGAAGACGGCATACGAGAT**CAGCCTCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD Rhapsody™ Library Reverse Primer 3	650000092	Nextera N711	CAAGCAGAAGACGGCATACGAGAT**TGCCTCTT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD Rhapsody™ Library Reverse Primer 4	650000093	Nextera N712	CAAGCAGAAGACGGCATACGAGAT**TCCTCTAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T

Table 1. List of indexes included in the BD Rhapsody Library Amplification Kits

Highlighted in bold sequence are the unique bases of the i5 or i7 indexes that are read during index read cycles during sequencing. The asterisk (*) represents 3' Phosphorothioate bond(s).

Ordering additional index primers

Table 2 lists the manufacturing grade, purification and resuspension specifications for custom ordering additional indexing primers from Integrated DNA Technologies (IDT) to increase the number of libraries that can be multiplexed per run. To ensure equivalent performance with the indexes provided with each BD Rhapsody Kit (Table 1), indexes should be ordered from IDT (**idtdna.com**) according to the specifications in Table 2.

Specifications

Order additional indexes from Integrated DNA Technologies (IDT), according to the following specifications.

IDT services	Resuspension buffer	Purification	Concentration
TruGrade	IDTE pH 8.0	HPLC	10 µM

Table 2. Index manufacturing specifications

Tables 3 and 4 list the sequences of the indexing primers that may be ordered from Integrated DNA Technologies (IDT) to increase the number of BD Rhapsody Libraries that can be multiplexed per run.

TruSeq combinatorial dual (CD) index primers

The index primers listed below can be used as Combinatorial Index Adapters with 96 different combinations possible. Thus, 96 different samples could be multiplexed together with this limited set of 20 indexes.

Note: This set of combinatorial dual indexes limits the user to 8 *unique* dual index pairs. See below for how to order additional unique dual index primers.

Table 3

Forward index name	Sequence (5' – 3')	i5 bases for sample sheet NovaSeq, MiSeq, HiSeq 2000/ 2500	i5 bases for sample sheet iSeq, MiniSeq, NexSeq, HiSeq 3000/4000
TruSeq D501*	AATGATACGGCGACCACCGAGATCTACAC**TATAGCCT**ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T	TATAGCCT	AGGCTATA
TruSeq D502	AATGATACGGCGACCACCGAGATCTACAC**ATAGAGGC**ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T	ATAGAGGC	GCCTCTAT
TruSeq D503	AATGATACGGCGACCACCGAGATCTACAC**CCTATCCT**ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T	CCTATCCT	AGGATAGG
TruSeq D504	AATGATACGGCGACCACCGAGATCTACAC**GGCTCTGA**ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T	GGCTCTGA	TCAGAGCC
TruSeq D505	AATGATACGGCGACCACCGAGATCTACAC**AGGCGAAG**ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T	AGGCGAAG	CTTCGCCT
TruSeq D506	AATGATACGGCGACCACCGAGATCTACAC**TAATCTTA**ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T	TAATCTTA	TAAGATTA
TruSeq D507	AATGATACGGCGACCACCGAGATCTACAC**CAGGACGT**ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T	CAGGACGT	ACGTCCTG
TruSeq D508	AATGATACGGCGACCACCGAGATCTACAC**GTACTGAC**ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T	GTACTGAC	GTCAGTAC

Table 4

Reverse index name	Sequence (5' – 3')	i7 bases for sample sheet
TruSeq D701	CAAGCAGAAGACGGCATACGAGAT**CGAGTAAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	ATTACTCG
TruSeq D702	CAAGCAGAAGACGGCATACGAGAT**TCTCCGGA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TCCGGAGA
TruSeq D703	CAAGCAGAAGACGGCATACGAGAT**AATGAGCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CGCTCATT
TruSeq D704	CAAGCAGAAGACGGCATACGAGAT**GGAATCTC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GAGATTCC
TruSeq D705	CAAGCAGAAGACGGCATACGAGAT**TTCTGAAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	ATTCAGAA
TruSeq D706	CAAGCAGAAGACGGCATACGAGAT**ACGAATTC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	GAATTCGT
TruSeq D707	CAAGCAGAAGACGGCATACGAGAT**AGCTTCAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CTGAAGCT
TruSeq D708	CAAGCAGAAGACGGCATACGAGAT**GCGCATTA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TAATGCGC
TruSeq D709	CAAGCAGAAGACGGCATACGAGAT**CATAGCCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	CGGCTATG
TruSeq D710	CAAGCAGAAGACGGCATACGAGAT**TTCGCGGA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TCCGCGAA
TruSeq D711	CAAGCAGAAGACGGCATACGAGAT**GCGCGAGA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	TCTCGCGC
TruSeq D712	CAAGCAGAAGACGGCATACGAGAT**CTATCGCT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	AGCGATAG

Tables 3 and 4. Additional index oligonucleotides that can be used with the BD Rhapsody Library Amplification Kits
Highlighted in bold sequence are the unique bases of the i5 or i7 indexes that are read during index read cycles during sequencing. The asterisk (*) represents 3' Phosphorothioate bond(s).

Ordering unique dual indexing primers

Unique dual index (UDI) sequences can also be ordered from Integrated DNA Technologies (IDT) to increase the number of BD Rhapsody Libraries that can be multiplexed per run, with a unique dual index combination per primer pair (n=96) for the resolution of reads produced by index hopping on Illumina sequencers. See the Illumina Adapter Sequences document (References) for more detailed information about UDIs.

UDI primers should be ordered from IDT such that:

1) Each Forward Index Primer has the sequence:
AATGATACGGCGACCACCGAGATCTACAC**[i5]**ACACTCTTTCCCTACACGACGCTCTTCCGAT*C*T
2) Each Reverse Index Primer has the sequence:
CAAGCAGAAGACGGCATACGAGAT**[i7]**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T

The eight (8) nucleotide i5 and i7 sequences to order in the primer are the reverse orientation of the index sequence. The asterisk (*) represents 3' Phosphorothioate bond(s).

Summary

Following the instructions provided in this technical bulletin, BD Rhapsody System users should be able to order additional indexing primers for BD Rhapsody Sequencing Libraries and thereby sequence multiple libraries in a single Illumina sequencing run. For additional questions or queries please contact **scomix@bdscomix.bd.com**

References

1 mRNA Targeted Library Preparation with the BD Rhapsody™ and BD Rhapsody™ Express Single-Cell Analysis Systems Doc ID: 210968 Rev. 3.0
2 Illumina Adapter Sequences; Illumina Document#1000000002694 v11
3 Illumina White Paper 770-2017-004-D: Effects of Index Misassignment on multiplexing and downstream analysis

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