

Technical Bulletin

Sequences of Oligonucleotides in BD Rhapsody™ Library Reagent Kits

Introduction

BD Rhapsody™ Kits and BD® Single-Cell Multiplexing Kits include several primers used in BD Rhapsody Library preparation. This technical bulletin consolidates the sequences into tables which can be used to identify relevant sequences for specific applications.

Oligonucleotides in BD Rhapsody Targeted mRNA and AbSeq Kit

Table 1 lists the sequences of all the oligonucleotides that are included in the BD Rhapsody Targeted mRNA and AbSeq Amplification Kit (Cat No. 633774). To order additional indexing primers for BD Rhapsody Sequencing Libraries, see the Ordering Additional Indexes for the BD Rhapsody Library Reagent Kits technical bulletin.

Oligonucleotide	Use	Part Number	Sequence (5' – 3')
BD Rhapsody™ Universal Oligo	Forward primer for Targeted PCR1 and 2, Sample Tag PCR1 and 2 and AbSeq PCR1	650000074	ACACGACGCTCTTCCGATCT
BD Rhapsody™ BD® AbSeq Primer	Reverse primer for AbSeq PCR1	91-1086	CAGACGTGTGCTCTTCCGATCT
BD Rhapsody™ Sample Tag PCR1 Primer	Reverse primer for Sample Tag PCR1	91-1088	GTTGTCAAGATGCTACCGTT
BD Rhapsody™ Sample Tag PCR2 Primer	Reverse primer for Sample Tag PCR2	91-1089	CAGACGTGTGCTCTTCCGATCTGTTGTCAAGATGCTACCGTT
BD Rhapsody™ Library Forward Primer	Forward primer in mRNA Targeted, Sample Tag and AbSeq Index PCR	91-1085	AATGATACGGCGACCACCGAGATCTACACT TATAGCCT ACACTCTTCCCTACACGACGCTCTTCCGATC*T
BD Rhapsody™ Library Reverse Primer 1	mRNA Targeted, Sample Tag and AbSeq Index PCR	650000080	CAAGCAGAAGACGGCATAACGAGAT AGCGTAGCGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD Rhapsody™ Library Reverse Primer 2		650000091	CAAGCAGAAGACGGCATAACGAGAT CAGCCTCGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD Rhapsody™ Library Reverse Primer 3		650000092	CAAGCAGAAGACGGCATAACGAGAT TGCCTCTTGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC*T
BD Rhapsody™ Library Reverse Primer 4		650000093	CAAGCAGAAGACGGCATAACGAGAT TCCTCTACGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC*T

Table 1. Oligonucleotides included in the BD Rhapsody Targeted mRNA and AbSeq Amplification Kit

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).



Oligonucleotides in BD Rhapsody Whole Transcriptome Analysis Amplification Kit

Table 2 lists the sequences of all the oligonucleotides that are included in the BD Rhapsody Whole Transcriptome Analysis Amplification Kit (Cat No. 633801). Note that the BD AbSeq Primer and BD Rhapsody WTA Amplification Primer have the same sequence. To order additional indexing primers for BD Rhapsody sequencing libraries, see the Ordering Additional Indexes for the BD Rhapsody Library Reagent Kits technical bulletin.

Oligonucleotide	Use	Part Number	Sequence (5' – 3')
BD Rhapsody™ Universal Oligo	Forward primer for WTA RPE PCR, Sample Tag PCR1 and 2 and AbSeq PCR1	650000074	ACACGACGCTCTCCGATCT
BD Rhapsody™ AbSeq Primer	Reverse primer for AbSeq PCR1	91-1086	CAGACGTGTGCTCTCCGATCT
BD Rhapsody™ Sample Tag PCR1 Primer	Reverse primer for Sample Tag PCR1	91-1088	GTTGTCAAGATGCTACCGTT
BD Rhapsody™ Sample Tag PCR2 Primer	Reverse primer for Sample Tag PCR2	91-1089	CAGACGTGTGCTCTCCGATCTGTTGTCAAGATGCTACCGTT
BD Rhapsody™ WTA Extension Primers	Random primers ¹ for WTA RPE	91-1115	TCAGACGTGTGCTCTCCGATCTNNNNNNNNN
BD Rhapsody™ WTA Amplification Primer	Reverse primer for WTA RPE PCR	91-1116	CAGACGTGTGCTCTCCGATCT
BD Rhapsody™ Library Forward Primer	Forward primer for WTA, Sample Tag and AbSeq Index PCR	91-1085	AATGATACGGCGACCACCGAGATCTACACT TATAGCCT TACTCTTTCCCTA CACGACGCTCTCCGAT* C*T
BD Rhapsody™ Library Reverse Primer 1		650000080	CAAGCAGAAGACGGCATAACGAGAT AGCGTAG CGTGACTGGAGTTCAGAC GTGTGCTCTCCGATC*T
BD Rhapsody™ Library Reverse Primer 2		650000091	CAAGCAGAAGACGGCATAACGAGAT CAGCCTCGG TGACTGGAGTTCAGAC GTGTGCTCTCCGATC*T
BD Rhapsody™ Library Reverse Primer 3	WTA, Sample Tag and AbSeq Index PCR	650000092	CAAGCAGAAGACGGCATAACGAGAT TGCCTCTT TGACTGGAGTTCAGAC GTGTGCTCTCCGATC*T
BD Rhapsody™ Library Reverse Primer 4		650000093	CAAGCAGAAGACGGCATAACGAGAT TCCTCTACG TGACTGGAGTTCAGAC GTGTGCTCTCCGATC*T

¹The WTA extension primers are random primers that share a common 5' end but have a degenerate 3' end (9 Ns), for which mixed bases are achieved by hand mixing of 25% per nucleotide content.

Table 2. Oligonucleotides included in the BD Rhapsody Whole Transcriptome Analysis Amplification Kit

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).

BD Single-Cell Multiplexing Sample Tags

Table 3 lists all the Sample Tag sequences that are included in the BD Human Single-Cell Multiplexing Kit (Cat No. 633781). Each Sample Tag is a human universal antibody conjugated with a unique oligonucleotide sequence to allow for sample identification. Each Sample Tag has common 5' and 3' ends and the Sample Tag sequence (5' – 3'):

GTTGTCAAGATGCTACCGTTCAGAG[**Sample Tag sequence**]AAAAAAAAAAAAAAAAAAAAAAAAAAAA

Sample Tag	Part Number	Sample Tag sequence (5' – 3')
Sample Tag 1—Human	51-9014988	ATTCAAGGGCAGCCGCGTCACGATTGGATACGACTGTTGGACCGG
Sample Tag 2—Human	51-9014995	TGGATGGGATAAGTGCCTGATGGACCGAAGGGACCTCGTGGCCGG
Sample Tag 3—Human	51-9014996	CGGCTCGTGCTGCGTCGTCTCAAGTCCAGAACTCCGTGTATCCT
Sample Tag 4—Human	51-9014997	ATTGGGAGGCTTTCGTACCGCTGCCGCCACCAGGTGATACCCGCT
Sample Tag 5—Human	51-9014998	CTCCCTGGTGTTC AATACCCGATGTGGTGGGCAGAATGTGGCTGG
Sample Tag 6—Human	51-9014999	TTACCCGCAGGAAGACGTATACCCCTCGTGCCAGGCGACCAATGC
Sample Tag 7—Human	51-9015000	TGTCTACGTCGGACCGCAAGAAGTGAGTCAGAGGCTGCACGCTGT
Sample Tag 8—Human	51-9015001	CCCCACCAGGTTGCTTTGTTCGGACGAGCCCGCACAGCGCTAGGAT
Sample Tag 9—Human	51-9015002	GTGATCCGCGCAGGCACACATACCGACTCAGATGGGTTGTCCAGG
Sample Tag 10—Human	51-9015003	GCAGCCGGCGTCGTACGAGGCACAGCGGAGACTAGATGAGGCCCC
Sample Tag 11—Human	51-9015004	CGCGTCCAATTTCCGAAGCCCCGCCCTAGGAGTCCCCTGCGTGC
Sample Tag 12—Human	51-9015005	GCCCATTCATTGCACCCGCCAGTGATCGACCCTAGTGGAGCTAAG

Table 3. Sample Tags included in the BD Human Single-Cell Multiplexing Kit

Table 4 lists all the Sample Tag sequences that are included in the BD Mouse Immune Single-Cell Multiplexing Kit (Cat No. 633793). Each Sample Tag is an anti-mouse CD45, clone 30-F11 antibody conjugated with a unique oligonucleotide sequence to allow for sample identification. Each Sample Tag has common 5' and 3' ends and the Sample Tag sequence (5' – 3'):

Sample Tag	Part Number	Sample Tag sequence (5' – 3')
Sample Tag 1—Mouse Immune	51-9016750	AAGAGTCGACTGCCATGTCCCCTCCGCGGGTCCGTGCCCCCAAG
Sample Tag 2—Mouse Immune	51-9016751	ACCGATTAGGTGCGAGGCGCTATAGTCGTACGTCGTTGCCGTGCC
Sample Tag 3—Mouse Immune	51-9016752	AGGAGGCCCCGCGTGAGAGTGATCAATCCAGGATACATCCCCTGC
Sample Tag 4—Mouse Immune	51-9016753	TTAACCGAGGCGTGAGTTTGGAGCGTACCGGCTTTGCGCAGGGCT
Sample Tag 5—Mouse Immune	51-9016754	GGCAAGGTGTCACATTGGGCTACCGCGGGAGGTGACCAGATCCT
Sample Tag 6—Mouse Immune	51-9016755	GCGGGCACAGCGGCTAGGGTGTTCGGGTGGACCATGGTTACGGC
Sample Tag 7—Mouse Immune	51-9016756	ACCGGAGGCGTGTGTACGTGCGTTTCGAATTCCTGTAAGCCCACC
Sample Tag 8—Mouse Immune	51-9016757	TCGCTGCCGTGCTTCATTGTGCGCGTTCTAACCTCCGATGTCTCG
Sample Tag 9—Mouse Immune	51-9016758	GCCTACCCGCTATGCTCGTCGGCTGGTTAGAGTTTACTGCACGCC
Sample Tag 10—Mouse Immune	51-9016759	TCCCATTCGAATCACGAGGCCGGGTGCGTTCTCCTATGCAATCCC
Sample Tag 11—Mouse Immune	51-9016760	GGTTGGCTCAGAGGCCCCAGGCTGCGGACGTGTCGGACTCGCGT
Sample Tag 12—Mouse Immune	51-9016761	CTGGGTGCCTGGTTCGGGTTACGTGCGCCCTCGGGTTCGGAAGGTC

Table 4. Sample Tags included in the BD Mouse Immune Single-Cell Multiplexing Kit

BD AbSeq Ab-Oligo common motifs

The antibody in each BD AbSeq Ab-Oligo is conjugated to an oligonucleotide that contains an antibody clone-specific barcode (ABC) flanked by a poly-A tail on the 3' end and a common PCR handle (PCR primer binding site) followed by a unique molecular identifier on the 5' end and the AbSeq Ab-Oligo sequence (5' – 3'):

CAGACGTGTGCTCTTCCGATCTVNNVNNNNVNNV[Barcode sequence (ABC)]AAAAAAAAAAAAAAAAAAAAAAAAAAAA

The poly-A tail of the oligonucleotide allows the ABC to be captured by the BD Rhapsody System or other oligo-dT-based capture systems. The 5' PCR handle allows for efficient sequencing library generation for Illumina® sequencing platforms. The ABC (barcode sequence) for each antibody can be found in the Product Details section of the respective Ab-Oligo product page online:

bdbiosciences.com/bd-abseq-assay

Summary

The provided tables will enable users to easily identify sequences of oligonucleotides that are included in BD Rhapsody Library Preparation Kits and BD Single-Cell Multiplexing Kits and sequences of the common motifs of BD AbSeq Ab-Oligos. For additional queries, please contact scomix@bdscomix.bd.com

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23-22746-00

BD Life Sciences, San Jose, CA, 95131, USA

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