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ARTseq™ Index PCR Primers (1-12)

Cat. No. SMIP2124

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1. Introduction

The ARTseq Index PCR Primers (1-12) are used to add an Illumina® Index (barcode) to an ARTseq Ribosome Profiling Kit library. The ARTseq Index PCR Primers should not be used with any Ilumina® sample preparation kit.

Each Index PCR Primer is supplied in a yellow-cap tube in 48 µl of nuclease-free water.

2. Product Specifications

Storage: Store the ARTseq Index PCR Primers at –20°C.

Quality Control: The ScriptMiner Index PCR Primers are function-tested by preparing Indexed libraries using the ARTseq Ribosome Profiling Kit. Incorporation of an Index is confirmed by PCR.

3. Related Products

The following products are also available:

– ScriptSeq™ v2 RNA-Seq Library Preparation Kit

4. Sequences of the ARTseq Index PCR Primers (1-12)

The sequence of the 6-base Index is underlined.* The actual Index sequence read during sequencing will be the reverse complement of the underlined sequence in each Primer (see Table 1).

Index 1 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**CGTGAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Index 2 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**ACATCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Index 3 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**GCCTAA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Index 4 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**TGGTCA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Index 5 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**CACTGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Index 6 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**ATTGGC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Index 7 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**GATCTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Index 8 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**TCAAGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Index 9 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**CTGATC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Index 10 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**AAGCTA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Index 11 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**GTAGCC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Index 12 PCR Primer

5′ CAAGCAGAAGACGGCATACGAGAT**TACAAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3′

Table 1. The Index sequence of the respective ARTseq Index PCR Primers (column 2) and the Index sequence read during sequencing (column 3).

	ARTseq Index Primer	Index sequence read during sequencing
Index 1 PCR Primer	5′-CGTGAT-3′	5′-ATCACG-3′
Index 2 PCR Primer	5′-ACATCG-3′	5′-CGATGT-3′
Index 3 PCR Primer	5′-GCCTAA-3′	5′-TTAGGC-3′
Index 4 PCR Primer	5′-TGGTCA-3′	5′-TGACCA-3′
Index 5 PCR Primer	5′-CACTGT-3′	5′-ACAGTG-3′
Index 6 PCR Primer	5′-ATTGGC-3′	5′-GCCAAT-3′
Index 7 PCR Primer	5′-GATCTG-3′	5′-CAGATC-3′
Index 8 PCR Primer	5′-TCAAGT-3′	5′-ACTTGA-3′
Index 9 PCR Primer	5′-CTGATC-3′	5′-GATCAG-3′
Index 10 PCR Primer	5′-AAGCTA-3′	5′-TAGCTT-3′
Index 11 PCR Primer	5′-GTAGCC-3′	5′-GGCTAC-3′
Index 12 PCR Primer	5′-TACAAG-3′	5′-CTTGTA-3′

ARTseq Index PCR Primers Usage

An ARTseq Index PCR Primer should be used in place of the Reverse PCR Primer in the ARTseq Kit procedure

Pooling Multiplexed ARTseq Libraries

Illumina sequencers use a green laser to read G/T nucleotides and a red laser to read A/C nucleotides. With each sequencing cycle at least one of the two nucleotides for each color channel must be read to ensure proper registration. Since the Index sequence of pooled, multiplexed ARTseq libraries will be read simultaneously, it is important to maintain color balance for each base of the Index sequences in the pooled library. Otherwise, Index sequencing will fail due to registration failure.

Example 1. Improper pooling of multiplexed ARTseq libraries. Pooling and sequencing ARTseq libraries containing Index reads 1,2,3,4 or Index reads 7,8,9,10 for example will result in a registration failure when sequencing base 2 and 3 of the Indexes.

√=signal in both color channels x=signal missing in one color channel

	Index sequence read		Index sequence read
Index 1	ATCACG	Index 7	CAGATC
Index 2	CGATGT	Index 8	ACTTGA
Index 3	TTAGGC	Index 9	GATCAG
Index 4	TGACCA	Index 10	TAGCTT
	√XX√√√		√XX√√√

Example 2. Proper pooling of multiplexed ARTseq libraries. Pooling and sequencing ARTseq libraries containing Index reads 4,5,6,7 or Index reads 9,10,11,12 for example will result in successful registration when sequencing all six bases of the Indexes.

√=signal in both color channels x=signal missing in one color channel

	Index sequence read		Index sequence read
Index 4	TGACCA	Index 9	GATCAG
Index 5	ACAGTG	Index 10	TAGCTT
Index 6	GCCAAT	Index 11	GGCTAC
Index 7	CAGATC	Index 12	CTTGTA
	√√√√√√		√√√√√√

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