

# ScriptMiner™ Index PCR Primers (1-12)

Cat. No. SMIP2124

**Important!** *The ScriptMiner Small RNA-Seq Library Preparation Kit and the ScriptMiner Index PCR Primers will be discontinued on December 31, 2013 or when current inventory is sold.*

*The ScriptMiner Index PCR Primers will continue to be provided with Epicentre's ARTseq™ Ribosome Profiling Kit.*

*Please consider the Illumina® TruSeq™ Small RNA Sample Preparation Kit as an alternative.*

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## 1. Introduction

The ScriptMiner™ Index PCR Primers (1-12) are used to add an Illumina® Index (barcode) to an RNA-Seq library prepared using Epicentre's ScriptMiner™ v2 RNA-Seq Library Preparation Kit. The ScriptMiner Index PCR Primers should not be used with any Illumina® TruSeq® RNA sample preparation kit.

Each Index PCR Primer is supplied in a yellow-cap tube in 48 µl of nuclease-free water. Each tube contains sufficient primer for preparing 24 ScriptMiner v2 RNA-Seq libraries.

## 2. Product Specifications

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

**Quality Control:** The ScriptMiner Index PCR Primers are function-tested by preparing barcoded RNA-Seq libraries using the ScriptMiner Small RNA-Seq Library Preparation 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
- Ribo-Zero™ rRNA Removal Kit (Human/Mouse/Rat)
- Ribo-Zero™ rRNA Removal Kit (Human/Mouse/Rat)–Low Input
- Ribo-Zero™ Gold Kit (Human/Mouse/Rat)
- Ribo-Zero™ rRNA Removal Kit (Gram-Negative Bacteria)
- Ribo-Zero™ rRNA Removal Kit (Gram-Positive Bacteria)
- Ribo-Zero™ rRNA Removal Kit (Meta-Bacteria)
- Ribo-Zero™ rRNA Removal Kit (Plant Leaf)
- Ribo-Zero™ rRNA Removal Kit (Plant Seed/Root)

## 4. Sequences of the ScriptMiner 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' CAAGCAGAAGACGGCATAACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'

### Index 2 PCR Primer

5' CAAGCAGAAGACGGCATAACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'

### Index 3 PCR Primer

5' CAAGCAGAAGACGGCATAACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'

### Index 4 PCR Primer

5' CAAGCAGAAGACGGCATAACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'

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**Index 5 PCR Primer**5' CAAGCAGAAGACGGCATAACGAGAT**CACTGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'**Index 6 PCR Primer**5' CAAGCAGAAGACGGCATAACGAGAT**ATTGGC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'**Index 7 PCR Primer**5' CAAGCAGAAGACGGCATAACGAGAT**GATCTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'**Index 8 PCR Primer**5' CAAGCAGAAGACGGCATAACGAGAT**TCAAGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'**Index 9 PCR Primer**5' CAAGCAGAAGACGGCATAACGAGAT**CTGATC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'**Index 10 PCR Primer**5' CAAGCAGAAGACGGCATAACGAGAT**AAGCTA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'**Index 11 PCR Primer**5' CAAGCAGAAGACGGCATAACGAGAT**GTAGCC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'**Index 12 PCR Primer**5' CAAGCAGAAGACGGCATAACGAGAT**TACAAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3'**Table 1.** The Index sequence of the respective ScriptMiner™ Index PCR Primers (column 2) and the Index sequence read during sequencing (column 3).

	<b>ScriptMiner Index Primer</b>	<b>Index sequence read during sequencing</b>
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'

### ScriptMiner Index PCR Primers Usage

A ScriptMiner Index PCR Primer should be used in place of the Reverse PCR Primer in a ScriptMiner Small RNA-Seq Library Preparation Kit procedure. Use 1 µl of the desired ScriptMiner Index PCR Primer in place of the Reverse PCR Primer in the ScriptMiner Small Kit procedure.

### Pooling Multiplexed ScriptMiner 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 ScriptMiner 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 ScriptMiner libraries.** Pooling and sequencing ScriptMiner 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 <b>ATCACG</b>	Index 7 <b>CAGATC</b>
Index 2 <b>CGATGT</b>	Index 8 <b>ACTTGA</b>
Index 3 <b>TTAGGC</b>	Index 9 <b>GATCAG</b>
Index 4 <b>TGACCA</b>	Index 10 <b>TAGCTT</b>
√XX√√√	√XX√√√

**Example 2. Proper pooling of multiplexed ScriptMiner libraries.** Pooling and sequencing ScriptMiner 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 <b>TGACCA</b>	Index 9 <b>GATCAG</b>
Index 5 <b>ACAGTG</b>	Index 10 <b>TAGCTT</b>
Index 6 <b>GCCAAT</b>	Index 11 <b>GGCTAC</b>
Index 7 <b>CAGATC</b>	Index 12 <b>CTTGTA</b>
√√√√√√	√√√√√√

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