

ScriptSeq™ Index PCR Primers

Set 1

Cat. No. RSBC10948

Set 2

Cat. No. SSIP1202

Set 3

Cat. No. SSIP1203

Set 4

Cat. No. SSIP1204

1 Each of Set 1, Set 2, Set 3, Set 4

Cat. No. SSIP1234

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1. Introduction

ScriptSeq™ Index PCR Primers are used to add an Illumina® Index (barcode) to an RNA-Seq library prepared using ScriptSeq™ v2 RNA-Seq Library Preparation Kit or a ScriptSeq™ Complete Kit. The ScriptSeq Index PCR Primers are provided in sets of 12 Primers each. The Primer composition of each Set was selected in order to provide the user the greatest flexibility in preparing multiplexed ScriptSeq libraries.

Each ScriptSeq Index PCR Primer is supplied in a yellow-cap tube at 10 µM in 48 µl of nuclease-free water. Each tube contains sufficient primer for preparing 48 Indexed ScriptSeq v2 RNA-Seq libraries.

2. Product Specifications

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

Quality Control: Each Primer is function-tested by preparing a ScriptSeq v2 RNA-Seq library. The incorporation of an Index read is confirmed by Bioanalyzer (Agilent) profile and by PCR.

3. ScriptSeq Index PCR Primers Usage

A ScriptSeq Index PCR Primer should be used in place of the Reverse PCR Primer in a ScriptSeq v2 RNA-Seq Library Preparation Kit procedure. Use 1 µl of the desired ScriptSeq Index PCR Primer in place of the Reverse PCR Primer that is included in the ScriptSeq v2 RNA-Seq Library Preparation Kit.

The ScriptSeq Index PCR Primers should not be used with any Illumina® TruSeq™ RNA preparation kit. Even though the 6-base Index sequences are identical, the flanking sequences of the ScriptSeq and TruSeq Primers are different.

4. Pooling Indexed ScriptSeq RNA-Seq Libraries

Pooling multiplexed ScriptSeq v2 RNA-Seq 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 nucleotide for each color channel must be read to ensure proper registration. The following examples demonstrate proper and improper pooling strategies.

Example 1. Proper pooling of multiplexed ScriptSeq v2 libraries. Pooling and sequencing ScriptSeq v2 RNA-Seq libraries containing Indexes 4, 5, 6, 7 or Indexes 9, 10, 11, 12 will result in successful registration due to the presence of both red and green signals when sequencing each of the six nucleotides of the Indexes.

$\sqrt{\text{ }}$ =signal in both red and green color channels x =signal missing in one color channel

Index Sequence

Index 4 T G A C C A

Index 5 A C A G T G

Index 6 G C C A A T

Index 7 C A G A T C

$\sqrt{\text{ }}$ $\sqrt{\text{ }}$ $\sqrt{\text{ }}$ $\sqrt{\text{ }}$ $\sqrt{\text{ }}$ $\sqrt{\text{ }}$

Index Sequence

Index 9 G A T C A G

Index 10 T A G C T T

Index 11 G G C T A C

Index 12 C T T G T A

$\sqrt{\text{ }}$ $\sqrt{\text{ }}$ $\sqrt{\text{ }}$ $\sqrt{\text{ }}$ $\sqrt{\text{ }}$ $\sqrt{\text{ }}$

Example 2. Improper pooling of multiplexed ScriptSeq v2 libraries. Pooling and sequencing ScriptSeq v2 RNA-Seq libraries containing Indexes 1, 2, 3, 4 or Indexes 7, 8, 9, 10 may result in a registration failure due to lack of both **green** and **red** color signals when sequencing nucleotides 2 and 3 of the Indexes.

$\sqrt{\text{ }}$ =signal in both red and green color channels x =signal missing in one color channel

Index Sequence

Index 1 A T C A C G

Index 2 C G A T G T

Index 3 T T A G G C

Index 4 T G A C C A

$\sqrt{\text{ }}$ x x $\sqrt{\text{ }}$ $\sqrt{\text{ }}$ $\sqrt{\text{ }}$

Index Sequence

Index 7 C A G A T C

Index 8 A C T T G A

Index 9 G A T C A G

Index 10 T A G C T T

$\sqrt{\text{ }}$ x x $\sqrt{\text{ }}$ $\sqrt{\text{ }}$ $\sqrt{\text{ }}$

The sequence of each ScriptSeq Index PCR Primer is:

5’ CAAGCAGAAGACGGCATACGAGATNNNNNNGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT 3’

Where **NNNNNN** is the **reverse complement** of the Index sequence generated during sequencing.

ScriptSeq™ Index PCR Primers (Set 1), Catalog Number RSBC10948

Contents	Index sequence generated during sequencing	I7 Index ID for entry into the IEM Sample Sheet-Wizard
Index 1 PCR Primer	5’-ATCACG-3’	A001
Index 2 PCR Primer	5’-CGATGT-3’	A002
Index 3 PCR Primer	5’-TTAGGC-3’	A003
Index 4 PCR Primer	5’-TGACCA-3’	A004
Index 5 PCR Primer	5’-ACAGTG-3’	A005
Index 6 PCR Primer	5’-GCCAAT-3’	A006
Index 7 PCR Primer	5’-CAGATC-3’	A007
Index 8 PCR Primer	5’-ACTTGA-3’	A008
Index 9 PCR Primer	5’-GATCAG-3’	A009
Index 10 PCR Primer	5’-TAGCTT-3’	A010
Index 11 PCR Primer	5’-GGCTAC-3’	A011
Index 12 PCR Primer	5’-CTTGTA-3’	A012

Suggested options for low-plexity pooling of Indexed ScriptSeq libraries using ScriptSeq Index PCR Primers (Set 1):

Index 6 **G C C A A T**
Index 12 **C T T G T A**

Index 1 **A T C A C G**
Index 4 **T G A C C A**
Index 8 **A C T T G A**

Index 5 **A C A G T G**
Index 10 **T A G C T T**
Index 11 **G G C T A C**

Additional Indexes can be added to any of the above Index combinations in order to make high-complexity pools of ScriptSeq libraries.

ScriptSeq™ Index PCR Primers (Set 2), Catalog Number SSIP1202

Contents	Index sequence read during sequencing	I7 Index ID for entry into the IEM Sample Sheet-Wizard
Index 13 PCR Primer	5'-AGTCAA-3'	A013
Index 14 PCR Primer	5'-AGTTCC-3'	A014
Index 15 PCR Primer	5'-ATGTCA-3'	A015
Index 16 PCR Primer	5'-CCGTCC-3'	A016
Index 17 PCR Primer	5'-GTAGAG-3'	****
Index 19 PCR Primer	5'-GTGAAA-3'	A019
Index 20 PCR Primer	5'-GTGGCC-3'	A020
Index 22 PCR Primer	5'-CGTACG-3'	A022
Index 23 PCR Primer	5'-GAGTGG-3'	A023
Index 30 PCR Primer	5'-CACCGG-3'	A030
Index 31 PCR Primer	5'-CACGAT-3'	A031
Index 46 PCR Primer	5'-TCCCGA-3'	A046

**** Index 17 must be added manually into the IEM Sample Sheet-Wizard. To use Index 17 select A011 and finish the Sample Sheet. Then, manually correct the finished Sample Sheet by changing A011 to A017 in the column labeled "I7_Index_ID" and by changing **GGCTAC** to **GTAGAG** in the column labeled "Index". Index 11 (**GGCTAC**) will provide correct color balance information at all but the base position 6 in Index 17 (**GTAGAG**).

Suggested options for low-plexity pooling of Indexed ScriptSeq libraries using ScriptSeq Index PCR Primers (Set 2):

Index 13 **AGTCAA**
 Index 23 **GAGTGG**
 Index 30 **CACCGG**

Index 14 **AGTTCC**
 Index 23 **GAGTGG**
 Index 46 **TCCCGA**

Index 16 **CCGTCC**
 Index 17 **GTAGAG**
 Index 19 **GTGAAA**

Additional Indexes can be added to any of the above Index combinations in order to make high-complexity pools of ScriptSeq libraries.

ScriptSeq™ Index PCR Primers (Set 3), Catalog Number SSIP1203

Contents	Index sequence read during sequencing	I7 Index ID for entry into the IEM Sample Sheet-Wizard
Index 18 PCR Primer	5'-GTCCGC-3'	A018
Index 25 PCR Primer	5'-ACTGAT-3'	A025
Index 27 PCR Primer	5'-ATTCCT-3'	A027
Index 29 PCR Primer	5'-CAACTA-3'	A029
Index 33 PCR Primer	5'-CAGGCG-3'	A033
Index 35 PCR Primer	5'-CATTTT-3'	A035
Index 37 PCR Primer	5'-CGGAAT-3'	A037
Index 39 PCR Primer	5'-CTATAC-3'	A039
Index 41 PCR Primer	5'-GACGAC-3'	A041
Index 43 PCR Primer	5'-TACAGC-3'	A043
Index 45 PCR Primer	5'-TCATTTC-3'	A045
Index 47 PCR Primer	5'-TCGAAG-3'	A047

Suggested options for low-plexity pooling of Indexed ScriptSeq libraries using ScriptSeq Index PCR Primers (Set 3):Index 18 **GTCCGC**Index 25 **ACTGAT**Index 27 **ATTCCT**Index 29 **CAACTA**Index 41 **GACGAC**Index 33 **CAGGCG**Index 37 **CGGAAT**Index 45 **TCATTTC**

Additional Indexes can be added to any of the above Index combinations in order to make high-complexity pools of ScriptSeq libraries.

ScriptSeq™ Index PCR Primers (Set 4), Catalog Number SSIP1204

Contents	Index sequence read during sequencing	I7 Index ID for entry into the IEM Sample Sheet-Wizard
Index 21 PCR Primer	5'-GTTTCG-3'	A021
Index 24 PCR Primer	5'-GGTAGC-3'	****
Index 26 PCR Primer	5'-ATGAGC-3'	****
Index 28 PCR Primer	5'-CAAAAG-3'	A028
Index 32 PCR Primer	5'-CACTCA-3'	A032
Index 34 PCR Primer	5'-CATGGC-3'	A034
Index 36 PCR Primer	5'-CCAACA-3'	A036
Index 38 PCR Primer	5'-CTAGCT-3'	A038
Index 40 PCR Primer	5'-CTCAGA-3'	A040
Index 42 PCR Primer	5'-TAATCG-3'	A042
Index 44 PCR Primer	5'-TATAAT-3'	A044
Index 48 PCR Primer	5'-TCGGCA-3'	A048

**** Index 24 and Index 26 must be added manually into the IEM Sample Sheet-Wizard.

*Index 24: To use Index 24 select A019 and finish the Sample Sheet. Then, manually correct the finished Sample Sheet by changing A019 to A024 in the column labeled "I7_Index_ID" and by changing **GTGAAA** to **GGTAGC** in the column labeled "Index". Index 19 (**GTGAAA**) will provide correct color balance information at all but the base position 5 of Index 24 (**GGTAGC**).*

*Index 26: To use Index 26, select A013 and finish the Sample Sheet. Then, manually correct the finished Sample Sheet by changing A013 to A026 in the column labeled "I7_Index_ID" and by changing **AGTCAA** to **ATGAGC** in the column labeled "Index". Index 13 (**AGTCAA**) will provide correct color balance information at all but the base position 5 of Index 26 (**ATGAGC**).*

Suggested options for low-plexity ScriptSeq libraries using ScriptSeq Index PCR Primers (Set 4):

Index 26 **ATGAGC**

Index 42 **TAATCG**

Index 21 **GTTTCG**

Index 28 **CAAAAG**

Index 40 **CTCAGA**

Index 24 **GGTAGC**

Index 36 **CCAACA**

Index 38 **CTAGCT**

Additional Indexes can be added to any of the above Index combinations in order to make high-complexity pools of ScriptSeq libraries.

Sequencing Indexed ScriptSeq v2 RNA-Seq libraries

Please see Appendix 2 of the ScriptSeq v2 RNA-Seq Library Preparation Kit protocol or Appendix 2 of a ScriptSeq Complete Kit protocol for details.

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