

If more than 8 samples need to be multiplexed on *one lane on an Illumina sequencer*, the following **additional 8 index pairs** may be purchased from IDT and used for Library PCR.

<b>i5 index Primer</b>	<b>Sequence</b>	<b>Index Sequence</b>
i5_Unique_9	AATGATACGGCGACCACCGAGATCTACAC AGAACGAG TCGTCGGCAGCGTC	AGAACGAG
i5_Unique_10	AATGATACGGCGACCACCGAGATCTACAC TGCTTCCA TCGTCGGCAGCGTC	TGCTTCCA
i5_Unique_11	AATGATACGGCGACCACCGAGATCTACAC CTTCGACT TCGTCGGCAGCGTC	CTTCGACT
i5_Unique_12	AATGATACGGCGACCACCGAGATCTACAC CACCTGTT TCGTCGGCAGCGTC	CACCTGTT
i5_Unique_13	AATGATACGGCGACCACCGAGATCTACAC ATCACACG TCGTCGGCAGCGTC	ATCACACG
i5_Unique_14	AATGATACGGCGACCACCGAGATCTACAC CCGTAAGA TCGTCGGCAGCGTC	CCGTAAGA
i5_Unique_15	AATGATACGGCGACCACCGAGATCTACAC TACGCCTT TCGTCGGCAGCGTC	TACGCCTT
i5_Unique_16	AATGATACGGCGACCACCGAGATCTACAC CGACGTTA TCGTCGGCAGCGTC	CGACGTTA

<b>i7 index Primer</b>	<b>Sequence</b>	<b>Index Sequence</b>
i7_Unique_9	CAAGCAGAAGACGGCATAACGAGATATTAGCCGGTCTCGTGGGCTCGG	CGGCTAAT
i7_Unique_10	CAAGCAGAAGACGGCATAACGAGATCGATCGATGTCTCGTGGGCTCGG	ATCGATCG
i7_Unique_11	CAAGCAGAAGACGGCATAACGAGATGATCTTGCCTCTCGTGGGCTCGG	GCAAGATC
i7_Unique_12	CAAGCAGAAGACGGCATAACGAGATAGGATAGCGTCTCGTGGGCTCGG	GCTATCCT
i7_Unique_13	CAAGCAGAAGACGGCATAACGAGATGTAGCGTAGTCTCGTGGGCTCGG	TACGCTAC
i7_Unique_14	CAAGCAGAAGACGGCATAACGAGATAGAGTCCAGTCTCGTGGGCTCGG	TGGACTCT
i7_Unique_15	CAAGCAGAAGACGGCATAACGAGATGCTACTCTGTCTCGTGGGCTCGG	AGAGTAGC
i7_Unique_16	CAAGCAGAAGACGGCATAACGAGATCTGGATGTCTCGTGGGCTCGG	ATCCAGAG

## ORDER

1. Order oligos (single-stranded DNA) on IDT:  
<https://www.idtdna.com/pages/support/how-to-order>
2. Purification method: **standard desalting, 25nmole**

## LIBRARY PCR SETUP

3. Dilute each oligo to 4  $\mu$ M with nuclease-free water (typically from 100  $\mu$ M stock)
4. For each sample combine **5  $\mu$ l of i5 index primer** and **5  $\mu$ l of i7 index primer** (see Table 1 on the next page) → The combined primers represent the “V2 Index Primer”
5. Proceed as outlined in the User Guide

V2 Index Primer Name	i5 Index Primer	i7 Index Primer
Index Pair 9	i5_Unique_9	i7_Unique_9
Index Pair 10	i5_Unique_10	i7_Unique_10
Index Pair 11	i5_Unique_11	i7_Unique_11
Index Pair 12	i5_Unique_12	i7_Unique_12
Index Pair 13	i5_Unique_13	i7_Unique_13
Index Pair 14	i5_Unique_14	i7_Unique_14
Index Pair 15	i5_Unique_15	i7_Unique_15
Index Pair 16	i5_Unique_16	i7_Unique_16
Index Pair 17	i5_Unique_9	i7_Unique_10
Index Pair 18	i5_Unique_9	i7_Unique_11
Index Pair 19	i5_Unique_10	i7_Unique_11
Index Pair 20	i5_Unique_10	i7_Unique_12
Index Pair 21	i5_Unique_11	i7_Unique_12
Index Pair 22	i5_Unique_11	i7_Unique_13
Index Pair 23	i5_Unique_12	i7_Unique_13
Index Pair 24	i5_Unique_12	i7_Unique_14
Index Pair 25	i5_Unique_13	i7_Unique_14
Index Pair 26	i5_Unique_13	i7_Unique_15
Index Pair 27	i5_Unique_14	i7_Unique_15
Index Pair 28	i5_Unique_14	i7_Unique_16

Table 1. Index i5/i7 combinations for 20 additional samples