

V2/V3 DNA Additional Index Guide

If more than 8 samples need to be multiplexed on *one lane on an Illumina sequencer*, the following **additional i5 and i7 indexes** may be purchased from IDT and used for Library PCR by making unique pairs following the instructions below.

i5 index Primer	Sequence	Index Sequence
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

i7 index Primer	Sequence	Index Sequence
i7_Unique_9	CAAGCAGAAGACGGCATAACGAGATATTAGCCGGTCTCGTGGGCTCGG	CGGCTAAT
i7_Unique_10	CAAGCAGAAGACGGCATAACGAGATCGATCGATGTCTCGTGGGCTCGG	ATCGATCG
i7_Unique_11	CAAGCAGAAGACGGCATAACGAGATGATCTTGCGTCTCGTGGGCTCGG	GCAAGATC
i7_Unique_12	CAAGCAGAAGACGGCATAACGAGATAGGATAGCGTCTCGTGGGCTCGG	GCTATCCT
i7_Unique_13	CAAGCAGAAGACGGCATAACGAGATGTAGCGTAGTCTCGTGGGCTCGG	TACGCTAC
i7_Unique_14	CAAGCAGAAGACGGCATAACGAGATAGAGTCCAGTCTCGTGGGCTCGG	TGGACTCT
i7_Unique_15	CAAGCAGAAGACGGCATAACGAGATGCTACTCTGTCTCGTGGGCTCGG	AGAGTAGC
i7_Unique_16	CAAGCAGAAGACGGCATAACGAGATCTCTGGATGTCTCGTGGGCTCGG	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 μ M with nuclease-free water (typically from 100 μ M stock)
4. For each sample combine **5 μ l of i5 index primer** and **5 μ l of i7 index primer** -
The combined primers represent the "V2/V3 Index Primer"
5. Proceed as outlined in the User Guide