

Single Indexing Truseq "Y" Adapter (Index #1/I7 = ATCACG):

AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GTTCTGTCTTCTGCGGTATGCTCTAGCACTACACTGACCTCAAGTCTGCACACGAGAAGGCTAG-P

Double-stranded Standard TruSeq Library Molecule post PCR:

AATGATACGGCGACCACCGAGA TCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNN-AGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG
TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNN-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTAGTGC TAGAGCATACGGCAGAAGACGAAC

Flow cell linkages:

P5 F/C (Index 2)---> Read 1 primer ----> Index read 1 primer ---->
AATGATACGGCGACCACCGAGA ACAC TCTTTCCC TACACGACGCTCTTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG
3' TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNN-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG TAGTGCTAGAGCATACGGCAGAAGACGAAC--| f/c

f/c |--AATGATACGGCGACCACCGAGA TCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNN-AGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG 3'
3' TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
<---- Read 2 primer

When this molecule is sequenced with standard "Nextera"/Amplicon protocol as below...

1x151 [read1]
1x8 [index1] (cycles 152 – 159)
1x7 DARK, 1x8 seq [index2, primed off flowcell before Paired end turnaround] (cycles 160 – 167)
1x151 [read2] (cycled 168 – 318)

...the red bases highlighted above are the observed sequence from index2 (see below). The green bases are skipped by the 7 dark cycles.

Index	Lane	Tile	Cycle	Time	%A	%C	%G	%T
160	1	1	160	05/07/201...	0.1	0.2	0.1	99.6
161	1	1	161	05/07/201...	0.1	98.9	0.3	0.6
162	1	1	162	05/07/201...	0.1	0.2	0.0	99.7
163	1	1	163	05/07/201...	0.1	0.1	0.1	99.8
164	1	1	164	05/07/201...	0.1	0.4	0.2	99.4
165	1	1	165	05/07/201...	0.1	99.3	0.0	0.6
166	1	1	166	05/07/201...	0.1	99.4	0.0	0.4
167	1	1	167	05/07/201...	0.2	98.7	0.3	0.8