Illumina - all flavors (USE with Caution, this is outdated but can be useful for a basic understanding of the adapters, the GSAF primarily only uses UDI's for all projects)

If this page isn't formatted well on your screen, try shrinking the left side bar.

Here is a helpful document from Illumina discussing the compatibility between various library primers and various sequencing platforms and kits.

Canonical ILLUMINA library design as of June 2012 (all 5'-3'), "TruSeq V3": NOTE all sequences shown are TOP STRAND 5' to 3'

<P5 primer/capture site>
<IndexRead2>
<Read1 primer site>

<template - gDNA, RNA, amplicon, whatever>
<Read2 primer site>
<IndexRead1>
<P7 primer/capture site>

If you'd like a different description, this one from the Tufts core facility is quite good.

NOTE THAT THE SHADED PORTIONS SHOULD NOT BE CHANGED if you are designing your own primers!! The only flexibility one has is in the "template" section and in the two "index read" sections. Every other nucleotide shown matters as-is.

Single index adaptor design on a standard Illumina HiSeq or MiSeq run

1. P5 PCR primer/flowcell capture site:

   AATGATACGGCGACCACCGAGA
2. IndexRead2:

   NONE - as in **do NOT** put an index here. If you want to add an index here, use one of the "Dual-index" designs below.
3. Read1 primer site:

   Either the small RNA sequencing primer site: (NEB: TCTACACGTTCAGAGTTCTACAGTCCGACGATCA [Illumina lists this but it is UNPROVEN: CAGGTTCAGAGTTCTACAGTCCGACGATCA]) OR the standard TruSeq Read 1 primer site: TCTACACTCTTTCCCTACACGACGCTCTTCCGATCT. Which to choose? The TruSeq Read 1 primer site is complementary to the Read 2 primer site, so if you are designing amplicons do NOT use the TruSeq Read 1 primer site, use the small RNA sequencing primer site.
4. The insert to be sequenced
5. Read2 primer site:

   Then the Index read primer site: AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC (**NOTE:** the initial A is from the dA tailing of the insert and is not included in the index primer or adaptor sequences; note also the reverse-complement of this is the Read 2 sequencing primer, but the Read 2 sequencing primer includes the T corresponding to the dA insert tail so sequencing starts with the insert)
6. IndexRead1:

   The index sequence (usually 6 bp) - see many examples below in the **Barcodes** section. Within a lane, image analysis works best with as much base diversity as possible.
7. P7 PCR primer/flowcell capture site:

   ATCTCGTATGCCGTCTTCTGCTTG

Here is an example of a read-pair from an RNA-seq library generated from the NEB small RNA kit with an insert size of 62 nt:

```
Read 1 sequence (note adaptor sequence starting with "AGATCGGAA...")

AAGGGATCATAGACGGTATTTCTATGTAAACGAACAGTCGGGCGAGTCTCAGTGGGAGTTTCAGATCGGAAGAGCACACGTCTGAACTCCAGNCACCGATG
ACCGAGATCTACACGTTCAGAGTTCTACAGTCCGACGATCAGGGATCATAGACGGTATTTCTATGTAAACGAACAGTCGGGCGAGTCTCAGTGGGAGTTTC
5' SR adaptor:GUUCAGAGUUCUACAGUCCGACGAUC
Read 2 sequence, reverse complemented (note adaptor sequence RC ending with "...CCGACGATCA")
```

Dual-index TruSeq (NOT Nextera) adaptor design on a standard Illumina PE HiSeq or MiSeq run

1. P5 PCR primer/flowcell capture site:

   AATGATACGGCGACCACCGAGATCTACAC
2. IndexRead2:

   Optional. Example: TAGATCGC. This is called "IndexRead2" because it is read after index read 1. The GSAF does not normally sequence this barcode - please request if you need it read. We have little guidance to offer on designs other than to re-use the same sequences as in the Index Read 1 site - base diversity is your friend.
3. Read1 primer site:

   The standard TruSeq Read 1 primer site: ACACTCTTTCCCTACACGACGCTCTTCCGATCT. We are not sure at this point whether the small RNA primer site is compatible with dual-indexes or not.
4. The remaining template elements are identical to the Single-index adaptor design above.
5. Note that an artifact of this design is that a SINGLE index (I7 index) TS library will read the sequence "TCTTTC..." as the I5 index if it is run in dual-index mode.

Dual-index Nextera adaptor design (we believe these are compatible with Illumina V3 PE HiSeq or V2/V3 MiSeq run)

```
Nextera® DNA Sample Preparation Kit (Illumina) 1,2
Nextera® transposase sequences (FC-121-1031, FC-121-1030)
5’ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
                      (a) Read 1 -->
5’ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG
                       (d) Read 2 -->

Nextera® Index Kit - PCR primers (FC-121-1012, FC-121-1011)
5’ AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC
           (c) i5 Index read -->
5’ CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG
                               <-- i7 Index read (b)
```

Nextera codes for entry on sample sheet:

i5 bases in adapter	Nextera DNA i5 index name	Nextera XT i5 index name	Nextera Enrichment i5 index name	HiSeq 2500 and MiSeq i5 bases for entry on sample sheet	NextSeq and HiSeq 4000 i5 bases for entry on sample sheet
TAGATCGC	N501	S501	E501	TAGATCGC	GCGATCTA
CTCTCTAT	N502	S502	E502	CTCTCTAT	ATAGAGAG
TATCCTCT	N503	S503	E503	TATCCTCT	AGAGGATA
AGAGTAGA	N504	S504	E504	AGAGTAGA	TCTACTCT
GTAAGGAG	N505	S505	E505	GTAAGGAG	CTCCTTAC
ACTGCATA	N506	S506	E506	ACTGCATA	TATGCAGT
AAGGAGTA	N507	S507	E507	AAGGAGTA	TACTCCTT
CTAAGCCT	N508	S508	E508	CTAAGCCT	AGGCTTAG
i7 bases in adapter	**Nextera DNA i7 index name**	**Nextera XT i7 index name**	**Nextera Enrichment i7 index name**	**i7 bases for entry on sample sheet (HiSeq, MiSeq, or NextSeq)**	
TCGCCTTA	N701	N701	N701	TAAGGCGA	
CTAGTACG	N702	N702	N702	CGTACTAG	
TTCTGCCT	N703	N703	N703	AGGCAGAA	
GCTCAGGA	N704	N704	N704	TCCTGAGC	

AGGAGTCC	N705	N705	N705	GGACTCCT
CATGCCTA	N706	N706	N706	TAGGCATG
GTAGAGAG	N707	N707	N707	CTCTCTAC
CCTCTCTG	N708	N708	N708	CAGAGAGG
AGCGTAGC	N709	N709	N709	GCTACGCT
CAGCCTCG	N710	N710	N710	CGAGGCTG
TGCCTCTT	N711	N711	N711	AAGAGGCA
TCCTCTAC	N712	N712	N712	GTAGAGGA

Barcodes (also known as Indexes)

NOTE: Illumina barcodes (indexes) have varied significantly over time NOT ONLY in their sequence but also in WHERE they are placed in the sequencing construct.

The GSAF expects indexes to be in the 3' end of the final sequencing construct, between the Index read sequencing primer site and the P7 PCR primer site. If you are using dual-indexed samples with an additional barcode between the P5 bridge PCR primer site and the Read 1 sequencing primer site, we can easily accommodate that on a run but do not normally do so - you need to tell us.

The GSAF uses the following names for the following barcodes. Note that these sequences are shown 5'-3' when the P5 sequence is on the left. In other words, here is the first barcode shown in the context of the full 3'-end adaptor construct:
GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG

Sequence	TruSeq name	NEXTFlex number
ATCACG	TSBC01	NFBC07
CGATGT	TSBC02	NFBC01
TTAGGC	TSBC03	NFBC08
TGACCA	TSBC04	NFBC02
ACAGTG	TSBC05	NFBC03
GCCAAT	TSBC06	NFBC04
CAGATC	TSBC07	NFBC05
ACTTGA	TSBC08	NFBC09
GATCAG	TSBC09	NFBC10
TAGCTT	TSBC10	NFBC11
GGCTAC	TSBC11	NFBC12
CTTGTA	TSBC12	NFBC06
AGTCAA	TSBC13	NFBC13
AGTTCC	TSBC14	NFBC14
ATGTCA	TSBC15	NFBC15
CCGTCC	TSBC16	NFBC16
GTAGAG	TSBC17	NFBC17
GTCCGC	TSBC18	NFBC18
GTGAAA	TSBC19	NFBC19
GTGGCC	TSBC20	NFBC20
GTTTCG	TSBC21	NFBC21
CGTACG	TSBC22	NFBC22
GAGTGG	TSBC23	NFBC23
GGTAGC	TSBC24	NFBC24
ACTGAT	TSBC25	NFBC25

ATGAGC	TSBC26	NFBC26
ATTCCT	TSBC27	NFBC27
CAAAAG	TSBC28	NFBC28
CAACTA	TSBC29	NFBC29
CACCGG	TSBC30	NFBC30
CACGAT	TSBC31	NFBC31
CACTCA	TSBC32	NFBC32
CAGGCG	TSBC33	NFBC33
CATGGC	TSBC34	NFBC34
CATTTT	TSBC35	NFBC35
CCAACA	TSBC36	NFBC36
CGGAAT	TSBC37	NFBC37
CTAGCT	TSBC38	NFBC38
CTATAC	TSBC39	NFBC39
CTCAGA	TSBC40	NFBC40
GTGATC	NEB40	
GACGAC	TSBC41	N/A
GCGCTA	N/A	NFBC41
TAATCG	TSBC42	NFBC42
TACAGC	TSBC43	NFBC43
TATAAT	TSBC44	NFBC44
TCATTC	TSBC45	NFBC45
TCCCGA	TSBC46	NFBC46
TCGAAG	TSBC47	NFBC47
TCGGCA	TSBC48	NFBC48

NOTE that TSBC41 is hamming distance 2 away from both TSBC31 and TSBC11; all others are hamming distance >=3.

Some additional 5 bp barcodes can be found here:http://comailab.genomecenter.ucdavis.edu/index.php/Barcodes

After exhaustive searching of all 4096 6-mers, the following table is all remaining 6 bp barcodes that have hamming distance of at least 3 from each other and the table above of 49 barcodes (NOTE: these have NOT been tested on the sequencer as of 2/7/12):

Sequence	GSAF name	
AAACAC	UTBC50	
TGAAGG	UTBC51	
AACATA	UTBC52	
CGCGTC	UTBC53	
GATACA	UTBC54	
GGTGTG	UTBC55	
TAAGAA	UTBC56	
AGCGAG	UTBC57	
CGGTTA	UTBC58	
AGCTTT	UTBC59	

TGGTCT	UTBC60	
TATCCC	UTBC61	
TGTCGT	UTBC62	
CCCCAC	UTBC63	
ATACGA	UTBC64	
CCCTTG	UTBC65	
ACCGGC	UTBC66	
TTACTG	UTBC67	
GGAACT	UTBC68	
GTTATT	UTBC69	
AAAAGT	UTBC70	
AAGGGA	UTBC71	
AAGTAT	UTBC72	
ACATCT	UTBC73	
ACGATT	UTBC74	
ACGCCG	UTBC75	
ACTCTC	UTBC76	
AGAATC	UTBC77	
ATTGGG	UTBC78	
CCGCGT	UTBC79	
CGCCCT	UTBC80	
CTGCAG	UTBC81	
GAAGTT	UTBC82	
GCACCC	UTBC83	
GCAGGA	UTBC84	
GCCGCG	UTBC85	
GGCGGT	UTBC86	
GTATTA	UTBC87	
TACGTG	UTBC88	
TCACAT	UTBC89	
TCTATA	UTBC90	
TGCAAA	UTBC91	
TGGCAC	UTBC92	
TGTTAG	UTBC93	
TTCTAT	UTBC94	
GGTACG	UTBC95	

Excruciating details - USE WITH CAUTION - RNA PCR primers are NOT current as of Dec. 2011

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Oligonucleotide sequences for TruSeqTM RNA and DNA Sample Prep Kits1

TruSeq Universal Adapter
     5’ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
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TruSeq Adapters
barcode:                            ATCACG
TruSeq Adapter, Index 1
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG
TruSeq Adapter, Index 2
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGCCGTCTTCTGCTTG
TruSeq Adapter, Index 3
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACTTAGGCATCTCGTATGCCGTCTTCTGCTTG
TruSeq Adapter, Index 4
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACTGACCAATCTCGTATGCCGTCTTCTGCTTG
TruSeq Adapter, Index 5
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACACAGTGATCTCGTATGCCGTCTTCTGCTTG
TruSeq Adapter, Index 6
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGCCAATATCTCGTATGCCGTCTTCTGCTTG
TruSeq Adapter, Index 7
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCAGATCATCTCGTATGCCGTCTTCTGCTTG
TruSeq Adapter, Index 8
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACACTTGAATCTCGTATGCCGTCTTCTGCTTG
TruSeq Adapter, Index 9
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGATCAGATCTCGTATGCCGTCTTCTGCTTG
TruSeq Adapter, Index 10
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACTAGCTTATCTCGTATGCCGTCTTCTGCTTG
TruSeq Adapter, Index 11
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGGCTACATCTCGTATGCCGTCTTCTGCTTG
TruSeq Adapter, Index 12
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTTGTAATCTCGTATGCCGTCTTCTGCTTG
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCAC Mux index read seq primer
  AGATCGGAAGAGCACACGTCTG - my 3' adaptor
my RT primer:
5' TCAGACGTGTGCTCTTCCGATCT 3'
  TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG Mux read 2 seq primer (reversed)
                       CTTGAGGTCAGTGGAACATTAGAGCATACGGCAGAAGACGAAC Index 12 PCR primer (reversed)
So order RC of TruSeq Adaptor Indexes as PCR primers

Oligonucleotide sequences for TruSeq Small RNA Sample Prep Kits
*****************************
Example (all Illumina sequences unless noted):
RNA PCR Primer (RP1),  part # 15005505
5’ AATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCGA
   AATGATACGGCGACCACCGA      CAGGTTCAGAGTTCTACAGTCCGA - this is the NEB SR Primer R1 - pads added by SPHS
                 5' RNA adaptor GUUCAGAGUUCUACAGUCCGACGAUC  (NEB kit calls this the SR Adaptor 1)

RNA PCR Primer, Index 1 (RPI1) code ATCACG in DNA, RC (CGTGAT)
5' CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA 3' - RNA pcr primer
   CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC (RC of DNA Index 1 for comparison)
                         3' RNA adaptor   3'  CCTTGGCACCCGAGAATTCCA - 5'
*****************************
Differences with NEB Small RNA kit (RP1/SR Primer R1/SR Adaptor 1/5' RNA adaptor are all the same):
5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTTGTAATCTCGTATGCCGTCTTCTGCTTG  (TruSeq Adaptor Ind 12)
 NEB 3' adaptor (3' SR Adaptor 1)       5'  ATCGTATGCCGTCTTCTGCTTG 3'
                                       3'    AGCATACGGCAGAAGACGAAC 5' (reverse of NEB SR Primer F1)
3' CTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGCCGATGTAGAGCATACGGCAGAAGACGAAC 5' (rev. of Ind. 12 adaptor)
CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTC (TruSeq Adaptor Ind 12 PCR primer)
5' CAAGCAGAAGACGGCATACGA 3' (NEB SR Primer F1)
5' CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT   (Illumina genomic PCR primer)
3' CAAGCAGAAGACGGCATACGAT 5' - NEB 3' SR Adaptor 1 (RC)
5' CAAGCAGAAGACGGCATACGA 3' - NEB RT Primer 1
For reference:
>TruSeqAdapterIndex11_RC
CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
>TruSeqAdapterIndex12_RC
CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
*****************************

RNA 5’ Adapter (RA5),  part # 15013205
5’ GUUCAGAGUUCUACAGUCCGACGAUC

RNA 3’ Adapter (RA3),  part # 15013207
5’ TGGAATTCTCGGGTGCCAAGG
3' ACCTTAAGAGCCCACGGTTCCG 5' (RT primer, reversed)
```

RNA RT Primer (RTP), part # 15013981
5’ GCCTTGGCACCCGAGAATTCCA

Multiplexing Index Read Sequencing Primer
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
Multiplexing Read 2 Sequencing Primer
5' GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
RNA PCR Primer (RP1), part # 15005505
5’ AATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCGA
RNA PCR Primer, Index 1 (RPI1)
CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 2 (RPI2)
CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 3 (RPI3)
CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 4 (RPI4)
CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 5 (RPI5)
CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 6 (RPI6)
CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 7 (RPI7)
CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 8 (RPI8)
CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 9 (RPI9)
CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 10 (RPI10)
CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 11 (RPI11)
CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 12 (RPI12)
CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 13 (RPI13)
CAAGCAGAAGACGGCATACGAGATTTGACTGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 14 (RPI14)
CAAGCAGAAGACGGCATACGAGATGGAACTGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 15 (RPI15)
CAAGCAGAAGACGGCATACGAGATTGACATGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 16 (RPI16)
CAAGCAGAAGACGGCATACGAGATGGACGGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 17 (RPI17)
CAAGCAGAAGACGGCATACGAGATCTCTACGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 18 (RPI18)
CAAGCAGAAGACGGCATACGAGATGCGGACGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 19 (RPI19)
CAAGCAGAAGACGGCATACGAGATTTTCACGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 20 (RPI20)
CAAGCAGAAGACGGCATACGAGATGGCCACGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 21 (RPI21)
CAAGCAGAAGACGGCATACGAGATCGAAACGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 22 (RPI22)
CAAGCAGAAGACGGCATACGAGATCGTACGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 23 (RPI23)
CAAGCAGAAGACGGCATACGAGATCCACTCGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 24 (RPI24)
CAAGCAGAAGACGGCATACGAGATGCTACCGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 25 (RPI25)
CAAGCAGAAGACGGCATACGAGATATCAGTGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 26 (RPI26)
CAAGCAGAAGACGGCATACGAGATGCTCATGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 27 (RPI27)
CAAGCAGAAGACGGCATACGAGATAGGAATGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 28 (RPI28)
CAAGCAGAAGACGGCATACGAGATCTTTTGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 29 (RPI29)
CAAGCAGAAGACGGCATACGAGATTAGTTGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 30 (RPI30)
CAAGCAGAAGACGGCATACGAGATCCGGTGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 31 (RPI31)

CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 32 (RPI32)
CAAGCAGAAGACGGCATACGAGATTGAGTGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 33 (RPI33)
CAAGCAGAAGACGGCATACGAGATCGCCTGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 34 (RPI34)
CAAGCAGAAGACGGCATACGAGATGCCATGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 35 (RPI35)
CAAGCAGAAGACGGCATACGAGATAAAATGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 36 (RPI36)
CAAGCAGAAGACGGCATACGAGATTGTTGGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 37 (RPI37)
CAAGCAGAAGACGGCATACGAGATATTCCGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 38 (RPI38)
CAAGCAGAAGACGGCATACGAGATAGCTAGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 39 (RPI39)
CAAGCAGAAGACGGCATACGAGATGTATAGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 40 (RPI40)
CAAGCAGAAGACGGCATACGAGATTCTGAGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 41 (RPI41)
CAAGCAGAAGACGGCATACGAGATGTCGTCGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 42 (RPI42)
CAAGCAGAAGACGGCATACGAGATCGATTAGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 43 (RPI43)
CAAGCAGAAGACGGCATACGAGATGCTGTAGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 44 (RPI44)
CAAGCAGAAGACGGCATACGAGATATTATAGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 45 (RPI45)
CAAGCAGAAGACGGCATACGAGATGAATGAGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 46 (RPI46)
CAAGCAGAAGACGGCATACGAGATTCGGGAGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 47 (RPI47)
CAAGCAGAAGACGGCATACGAGATCTTCGAGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
RNA PCR Primer, Index 48 (RPI48)
CAAGCAGAAGACGGCATACGAGATTGCCGAGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA

Oligonucleotide sequences for Genomic DNA

Adapters
5' P-GATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG
5' ACACTCTTTCCCTACACGACGCTCTTCCGATCT
PCR Primers
5' AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
5' CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT

Genomic DNA Sequencing Primer
5' ACACTCTTTCCCTACACGACGCTCTTCCGATCT

Paired End DNA oligonucleotide sequences

PE Adapters
5' P-GATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
5' ACACTCTTTCCCTACACGACGCTCTTCCGATCT
PE PCR Primer 1.0
5' AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
PE PCR Primer 2.0
5' CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT

PE Read 1 Sequencing Primer
5' ACACTCTTTCCCTACACGACGCTCTTCCGATCT
PE Read 2 Sequencing Primer
5' CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT

Oligonucleotide sequences for the Multiplexing Sample Prep Oligo Only Kit

Multiplexing Adapters
5' P-GATCGGAAGAGCACACGTCT
5' ACACTCTTTCCCTACACGACGCTCTTCCGATCT

```
Multiplexing PCR Primer 1.0
5' AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Multiplexing PCR Primer 2.0

Multiplexing Read 1 Sequencing Primer
5' ACACTCTTTCCCTACACGACGCTCTTCCGATCT
Multiplexing Index Read Sequencing Primer
5' GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
Multiplexing Read 2 Sequencing Primer
5' GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

PCR Primer, Index 1
CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTC
PCR Primer, Index 2
CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTC
PCR Primer, Index 3
CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTC
PCR Primer, Index 4
CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTC
PCR Primer, Index 5
CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTC
PCR Primer, Index 6
CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTC
PCR Primer, Index 7
CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTC
PCR Primer, Index 8
CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTC
PCR Primer, Index 9
CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTC
PCR Primer, Index 10
CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTC
PCR Primer, Index 11
CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTC
PCR Primer, Index 12
CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTC

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instruments and products only. All other uses are strictly prohibited.
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Nextera adaptor style:

 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGT-3'
                  |||||||||||||||||||
3'-CAGAGCACCCGAGCCTCTACACATATTCTCTGTC-5'

TruSeq truncated adaptor:
  5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'
                         ||||||||||||||
 3'-CACTGACCTCAAGTCTGCACACGAGAAGGCTAGA-5'

Nextera adaptor style, with primers overlaid:
First cycle:
                                                                                                            3'-
GGCTCGGGTGCTCTG CAAAGC TAGAGCATACGGCAGAAGACGAAC-5'
                                  5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGT-3'   <UNKNOWN>   5'-
CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-3'
                                                   |||||||||||||||||||                      |||||||||||||||||||
                                 3'-CAGAGCACCCGAGCCTCTACACATATTCTCTGTC-5'    <UNKNOWN>  3'-
TGACAGAGAATATGTGTAGACTGCGACGGCTGCT-5'


Second and subsequent cycles:
AATGATACGGCGACCACCGAGATCTACAC ATCACG TCGTCGGCAGCGTC
                                  3'-AGCAGCCGTCGCAGTCTACACATATTCTCTGTCA   <UNKNOWN>  3'-
TGACAGAGAATATGTGTAGACTGCGACGGCTGCT-5'
```

Cautions, common mistakes, and lessons learned from failure

1. Assembling the P7 side adaptor or primer wrong - the key thing to note is that the "cannonical designs" are shown 5' to 3' across the entire finished sequencing construct. So if you're designing a reverse primer for the P7 side you have to use the reverse complement of ALL 3 DESIGN ELEMENTS (flow cell binding site, barcode, and sequencing primer site) and make sure they're in the right order.
2. Incorrect P5 dual-index design - the "ACAC" motif in the single index design MUST be repeated on both sides of an index within P5 - see the "dual index" designs specifically.
3. Reverse complement barcode sequences in either P5 or P7 side indexes, especially from amplicons - the fact that the Illumina sequencers read i5 differently is a pain - pay attention to that when submitting barcode sequences that will wind up in a sample sheet. And remember that the i7 index is read "forward, top strand" of the canonical design, which is reverse complement of the sequence that appears in a reverse primer used when creating a library.