Illumina - all flavors

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:
   - AGATATCGGAGGACACGTCTGAACTCCAGTCAC
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: TCTACAGTTCCAGTTCTACAGTCCCGAGATCA [Illumina lists this but it is UNPROVEN: CAGTTCCAGTTCTACAGTCCCGAGATCA]) OR the standard TruSeq Read 1 primer site: TCTACACTCTTTCCCTACAGGCTCTTCCGATCT. 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:
   - ATCTCGTATGGCTCTTCTCTTG

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 "AGATCGGA...")
<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>AAGGGATCATAGACGTTATTCTATGTAAACGAACATCGGGCAGTCTGTGGGAGGTTCAGATCGGAAGAGCACCGTCTAATCGGAGCTCTCAGGCAGTCTTCTACATCGTCCAGCAGATCAAGATCGGGAGGGTTCTATGTAAACGAACAGTCGGGCGAGTCTCAGTGGGAGTTTCAGATCGGAAGAGCACCGTCTAATCGGAGCTCTCAGGCAGTCTTCTACATCG</td>
</tr>
<tr>
<td>5' SR adaptor: GUUCAGAGUUCUACAGGUUCAGGAGCATCA</td>
</tr>
</tbody>
</table>
| Read 2 sequence, reverse complemented (note adaptor sequence RC ending with "...CCGACGATCA")
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ACACGTCTGGAACCTCCAGGCAGTCTTCTACATCGTCCAGCAGATCAAGATCGGGAGGGTTCTATGTAAACGAACAGTCGGGCGAGTCTCAGTGGGAGTTTCAGATCGGAAGAGCACCGTCTAATCGGAGCTCTCAGGCAGTCTTCTACATCG</td>
</tr>
</tbody>
</table>
Dual-index TruSeq (NOT Nextera) adaptor design on a standard Illumina PE HiSeq or MiSeq run

1. P5 PCR primer/flowcell capture site:
   AATGATACGGCAGACACGTCTACAC

2. 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: ACACCTTTTCCTACAGCGCCGCTTTCGATCT. 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’ AATGATACGGCAGACACGTCTACAC[i5]TCGTCGGCAGCGT
    (c) i5 Index read -->
5’ CAAGCAGAAGACGCTACGAGATGTATAAGAGACAG
    <-- i7 Index read (b)

Nextera codes for entry on sample sheet:

<table>
<thead>
<tr>
<th>i5 bases in adapter</th>
<th>Nextera DNA i5 index name</th>
<th>Nextera XT i5 index name</th>
<th>Nextera Enrichment i5 index name</th>
<th>HiSeq 2500 and MiSeq i5 bases for entry on sample sheet</th>
<th>NextSeq and HiSeq 4000 i5 bases for entry on sample sheet</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAGATCGC</td>
<td>N501</td>
<td>S501</td>
<td>E501</td>
<td>TAGATCGC</td>
<td>GCGATCTA</td>
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<td>S502</td>
<td>E502</td>
<td>CTCTCTAT</td>
<td>ATAGAGAG</td>
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<tr>
<td>TATCCTCT</td>
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<td>S503</td>
<td>E503</td>
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<td>AGAGGATA</td>
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<td>AGAGTAGA</td>
<td>N504</td>
<td>S504</td>
<td>E504</td>
<td>AGAGTAGA</td>
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<td>S506</td>
<td>E506</td>
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</table>

<table>
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<tr>
<th>i7 bases in adapter</th>
<th>Nextera DNA i7 index name</th>
<th>Nextera XT i7 index name</th>
<th>Nextera Enrichment i7 index name</th>
<th>i7 bases for entry on sample sheet (HiSeq, MiSeq, or NextSeq)</th>
</tr>
</thead>
<tbody>
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<td>GCTCAGGA</td>
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</table>
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

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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](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):

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

Oligonucleotide sequences for TruSeqTM RNA and DNA Sample Prep Kits

TruSeq Universal Adapter
5’ AATGATACGGGCAGGCACACCGAGATCTACACTCTTTCCCTACACGAGCTCTTCCGATCT

TruSeq Adapters
barcode: ATCACG
TruSeq Adapter, Index 1
5’ GATCGGAAGAGCACACGTCTGCTGAATCCAAGTCACATCGATATTCTATCGAGATCT
TruSeq Adapter, Index 2
5’ GATCGGAAGAGCACACGTCTGCTGAATCCAAGTCACATCGATATTCTATCGAGATCT
TruSeq Adapter, Index 3
5’ GATCGGAAGAGCACACGTCTGCTGAATCCAAGTCACATCGATATTCTATCGAGATCT
TruSeq Adapter, Index 4
5’ GATCGGAAGAGCACACGTCTGCTGAATCCAAGTCACATCGATATTCTATCGAGATCT
TruSeq Adapter, Index 5
5’ GATCGGAAGAGCACACGTCTGCTGAATCCAAGTCACATCGATATTCTATCGAGATCT
TruSeq Adapter, Index 6
5’ GATCGGAAGAGCACACGTCTGCTGAATCCAAGTCACATCGATATTCTATCGAGATCT
TruSeq Adapter, Index 7
5’ GATCGGAAGAGCACACGTCTGCTGAATCCAAGTCACATCGATATTCTATCGAGATCT
TruSeq Adapter, Index 8
5’ GATCGGAAGAGCACACGTCTGCTGAATCCAAGTCACATCGATATTCTATCGAGATCT
TruSeq Adapter, Index 9
5’ GATCGGAAGAGCACACGTCTGCTGAATCCAAGTCACATCGATATTCTATCGAGATCT
TruSeq Adapter, Index 10
5’ GATCGGAAGAGCACACGTCTGCTGAATCCAAGTCACATCGATATTCTATCGAGATCT
TruSeq Adapter, Index 11
5’ GATCGGAAGAGCACACGTCTGCTGAATCCAAGTCACATCGATATTCTATCGAGATCT
TruSeq Adapter, Index 12

my RT primer:
5’ TCAGACGTGTTGCTCTTTCCGATCT 3’
TCTAGCCTTTCTCGTGTGCAGACTTGAGGTCAGTG 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’ AATGATACGGGACACCACTATACAGTTCAGTCTACAGTCCA
AATGATACGGGACACCACTATACAGTTCAGTCTACAGTCCA - 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' CAAGCAGAAGAGCGCATACGAGATCGTGATGAGTTGACTGGACCTTTGGAAGGACCCGAAATCCA 3' - RNA PCR primer
   CAAGCAGAAGAGCGCATACGAGATCGTGATGACTGGACCTTTGGAAGGACCCGAAATCCA (RC of DNA Index 1 for comparison)
3' RNA adaptor 3' CCTTGGCAACCCGAAATCCA - 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' CTAGCCCTCTTCGTGCGAGACTTTGAGCTGACGTCCAGATCACTCGTATGCCGTCTTCTGCTTG  (Illumina genomic PCR primer)
   3' CTAGCCCTCTTCGTGCGAGACTTTGAGCTGACGTCCAGATCACTCGTATGCCGTCTTCTGCTTG 5' (rev. of Ind. 12 adaptor)
   CAAGCAGAAGAGCGCATACGAGATCGTGATGACTGGACCTTTGGAAGGACCCGAAATCCA (TruSeq Adaptor Ind 12 PCR primer)
   5' CAAGCAGAAGAGCGCATACGAGATCGTGATGACTGGACCTTTGGAAGGACCCGAAATCCA 3' (NEB SR Primer F1)
   5' CAAGCAGAAGAGCGCATACGAGATCGTGATGACTGGACCTTTGGAAGGACCCGAAATCCA (Illumina genomic PCR primer)
3' CAAGCAGAAGAGCGCATACGAGATCGTGATGACTGGACCTTTGGAAGGACCCGAAATCCA 5' - NEB 3' SR Adaptor 1 (RC)
   5' CAAGCAGAAGAGCGCATACGAGATCGTGATGACTGGACCTTTGGAAGGACCCGAAATCCA 3' - NEB RT Primer 1
For reference:
>TruSeqAdapterIndex11_RC
   CAAGCAGAAGAGCGCATACGAGATCGTGATGACTGGACCTTTGGAAGGACCCGAAATCCA
>TruSeqAdapterIndex12_RC
   CAAGCAGAAGAGCGCATACGAGATCGTGATGACTGGACCTTTGGAAGGACCCGAAATCCA
**********************************************************************

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

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

RNA RT Primer (RTP), part # 15013981
5' GCCTTGGCAACCCGAGAATCCA

Multiplexing Index Read Sequencing Primer
5' GATCGGAAGAGGCCACGATCGTGGCCTCCAGTCAC
Multiplexing Read 2 Sequencing Primer
5' GTGACTGGAGTTCACTACGAGTTTCTCGGATCT
RNA PCR Primer (RP1), part # 15005505
5' AATGATACGGGCGAGATGTGACAGTGCTAGGTGATGACTGGAGTTTCTCGGATCT
RNA PCR Primer, Index 1 (RPI1)
   CAAGCAGAAGAGCGCATACGAGATCGTGATGACTGGAGTTTCTCGGATCT
RNA PCR Primer, Index 2 (RPI2)
   CAAGCAGAAGAGCGCATACGAGATCGTGATGACTGGAGTTTCTCGGATCT
RNA PCR Primer, Index 3 (RPI3)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 4 (RPI4)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 5 (RPI5)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 6 (RPI6)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 7 (RPI7)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 8 (RPI8)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 9 (RPI9)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 10 (RPI10)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 11 (RPI11)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 12 (RPI12)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 13 (RPI13)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 14 (RPI14)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 15 (RPI15)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 16 (RPI16)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 17 (RPI17)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 18 (RPI18)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 19 (RPI19)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 20 (RPI20)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 21 (RPI21)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 22 (RPI22)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 23 (RPI23)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 24 (RPI24)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 25 (RPI25)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 26 (RPI26)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 27 (RPI27)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 28 (RPI28)
CAAGCAGAAGACGGCATACGAGATCTGCTTGGCAACCCGAGAATTCCA
RNA PCR Primer, Index 29 (RPI29)
CAAGCAGAAGACGGCATACGAGAATTAGTGGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 30 (RPI30)
CAAGCAGAAGACGGCATACGAGATCCGGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 31 (RPI31)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 32 (RPI32)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 33 (RPI33)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 34 (RPI34)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 35 (RPI35)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 36 (RPI36)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 37 (RPI37)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 38 (RPI38)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 39 (RPI39)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 40 (RPI40)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 41 (RPI41)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 42 (RPI42)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 43 (RPI43)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 44 (RPI44)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 45 (RPI45)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 46 (RPI46)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 47 (RPI47)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA
RNA PCR Primer, Index 48 (RPI48)
CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCCTTGGCACCAGAGAATTCCA

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' ACACCTTTTTCCCTACACGACGCTCTTCCGATCT
PE PCR Primer 1.0
5' AATGATACGGCACACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
PE PCR Primer 2.0
5' CAAGCAGAAGACGCTACGATCGCTCTTCCGATCT

PE Read 1 Sequencing Primer
5' ACACCTTTTTCCCTACACGACGCTCTTCCGATCT
PE Read 2 Sequencing Primer
5' CGGTCTCGGCAATTCCGCTCGAACCCTCTTTCCGATCT

Oligonucleotide sequences for the Multiplexing Sample Prep Oligo Only Kit

Multiplexing Adapters
5' P-GATCGGAAGAGCGACACGTCT
5' ACACCTTTTTCCCTACACGACGCTCTTCCGATCT
Multiplexing PCR Primer 1.0
5' AATGATACGGCACACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Multiplexing PCR Primer 2.0

Multiplexing Read 1 Sequencing Primer
5' ACACCTTTTTCCCTACACGACGCTCTTCCGATCT
Multiplexing Index Read Sequencing Primer
5' GATCGGAAGACGCTACGATCGCTCTTCCGATCT
Multiplexing Read 2 Sequencing Primer
5' GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

PCR Primer, Index 1
CAAGCAGAAGACGCTACGATCTGATGTGACTGGAGTTC
PCR Primer, Index 2
CAAGCAGAAGACGCTACGATCTGATGTGACTGGAGTTC
PCR Primer, Index 3
CAAGCAGAAGACGCTACGATCTGATGTGACTGGAGTTC
PCR Primer, Index 4
CAAGCAGAAGACGCTACGATCTGATGTGACTGGAGTTC
PCR Primer, Index 5
CAAGCAGAAGACGCTACGATCTGATGTGACTGGAGTTC
PCR Primer, Index 6
CAAGCAGAAGACGCTACGATCTGATGTGACTGGAGTTC
PCR Primer, Index 7
CAAGCAGAAGACGCTACGATCTGATGTGACTGGAGTTC
PCR Primer, Index 8
CAAGCAGAAGACGCTACGATCTGATGTGACTGGAGTTC
PCR Primer, Index 9
CAAGCAGAAGACGCTACGATCTGATGTGACTGGAGTTC
PCR Primer, Index 10
CAAGCAGAAGACGCTACGATCTGATGTGACTGGAGTTC
PCR Primer, Index 11
CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTC
PCR Primer, Index 12
CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTC

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Nextera adaptor style:

5'-TCGTCGCGCAGCTCACTGCTGCTAAGAGACAGT-3'
3'-CAGAGCACCAGGAGCCCTCTACTACATATTTCTCTGTC-5'

TruSeq truncated adaptor:

5'-ACACCTCTTCCTACACGACGCTCTTCCGATCT-3'
3'-CACTGACCTCAAGTCTGCACACGAGAAGGCTAGA-5'

Nextera adaptor style, with primers overlaid:
First cycle:

3'-GGCTCGGGTGCCTCG CAAAGC TAGAGCATAACGCAGCAGACGAAC-5'
5'-TCGTCGCGCAGCTCACTGCTGCTAAGAGACAGT-3'
3'-CAGAGCACCAGGAGCCCTCTACTACATATTTCTCTGTC-5'

Second and subsequent cycles:
AATGATACGGCGACCACCGAGATCTACACTCACG TCGTCGCGCAGCGTCACTGCTGCTGCT-5'
3'-AGCAGCCGTCGAGCTACATATTTCTCTGTCA-3'
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.