2012-06-27, E.Olivares

There are different P5 primers for PE and single read flowcells, attached at their 5' ends to the flowcell.

P5 PairedEnd: 5'-TTTTTTTTTT**AATGATACGGCGACCACCGAGA**UCTACAC-3'
P5 SingleRead: 5'-TTTTTTTTTT-(diol)3-**AATGATACGGCGACCACCGA**-3´

TruSeq Review:

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

```
      AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GTTCGTCTTCTGCCGTATGCTCTAGCACTACACTGACCTCAAGTCTGCACACGAGAAGGCTAG-P
```

Double-stranded Standard TruSeq Library Molecule post PCR:

```
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNN-AGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG
TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNN-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTAGTGCTAGAGCATACGGCAGAAGACGAAC
```

Denatured ss-Library molecules with read primers:

```
                        Read 1 primer ---->                   Index read 1 primer ---->
                        ACACTCTTTCCCTACACGACGCTCTTCCGATCT      GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNN-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGTAGTGCTAGAGCATACGGCAGAAGACGAAC--| f/c
```

[PE turnaround]

```
|--AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNN-AGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG 3'
                                                             TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
                                                                       <----  Read 2 primer
```

Double-indexed adapter design suitable for sequencing with "Nextera" protocol on MiSeq with a PE flowcell (as of 6/2012)

Annealed adapter example

```
      5'-AATGATACGGCGACCACCGAGATCTACACTAGATCGCACACTCTTTCCCTACACGACGCTCTTCCGATC*T N501_truseq
         3'-GTTCGTCTTCTGCCGTATGCTCTAAGCGGAATCACTGACCTCAAGTCTGCACACGAGAAGGCTAG-P          N701_truseq
```

Double-stranded Library Molecule post PCR (as an example this is a 701-501 combination)

```
AATGATACGGCGACCACCGAGATCTACACTAGATCGCACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNN-AGATCGGAAGAGCACACGTCTGAACTCCAGTCACTAAGGCGAATCTCGTATGCCGTCTTCTGCTTG
TTACTATGCCGCTGGTGGCTCTAGATGTGATCTAGCGTGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNN-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGATTCCGCTTAGAGCATACGGCAGAAGACGAAC
```

Denatured ss-Library molecules with read primers:

--Note that index2 is primed from the flow cell primer, POST CLEAVAGE of the 7bp leader sequence. This is why the Nextera recipe requires 7-dark cycles to skip the constant region before reading index2.

```
   AATGATACGGCGACCACCGAGA (index2primer)
   P5 Flowcell Cluster primer-->        Read 1 primer ---->                   Index read 1 primer ---->
   AATGATACGGCGACCACCGAGAUCTACAC        ACACTCTTTCCCTACACGACGCTCTTCCGATCT     GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
3' TTACTATGCCGCTGGTGGCTCTAGATGTGATCTAGCGTGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNN-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGATTCCGCTTAGAGCATACGGCAGAAGACGAAC--| f/c
```

[PE turnaround]

```
|-AATGATACGGCGACCACCGAGATCTACACTAGATCGCACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNN-AGATCGGAAGAGCACACGTCTGAACTCCAGTCACTAAGGCGAATCTCGTATGCCGTCTTCTGCTTG 3'
                                                          <----  3' TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG
                                                                          Read 2 primer
```

Example sample sheet:

```
[Header]
IEMFileVersion,3
Investigator Name,ECO
Project Name,DOUBLEINDEX
Experiment Name,EXPT
Date,2012 06/05/12 21:28:11
Workflow,Resequencing
Assay,Nextera
Description
Chemistry,Amplicon
[Reads]
151
151
[Settings]
OnlyGenerateFASTQ,1
[Data]
Sample_ID,Sample_Name,Sample_Plate,Sample_Well,Sample_Project,index,I7_Index_ID,index2,I5_index_ID,Description,GenomeFolder
1,LS594,RE1018,A01,Exp135,TAAGGCGA,N701,TAGATCGC,N501,,C:\Illumina\MiSeq Reporter\Genomes\PhiX\Illumina\RTA\Sequence\Chromosomes
2,LS594,RE1018,A01,Exp135,CGTACTAG,N702,TAGATCGC,N501,,C:\Illumina\MiSeq Reporter\Genomes\PhiX\Illumina\RTA\Sequence\Chromosomes
3,LS594,RE1018,A01,Exp135,AGGCAGAA,N703,TAGATCGC,N501,,C:\Illumina\MiSeq Reporter\Genomes\PhiX\Illumina\RTA\Sequence\Chromosomes
```