Diagram of Illumina protocol using single read adapter sequences

Sequences from Bentley et al., Nature 2008

Genomic Y adapter, single read sequences
* = phosphorothioate bond
-p phosphate group

```
5'-ACACTCTTTCCCTACACGAC
                        \
                         CTCTTCCGATC*T-3'
3'-GTTCGTCTTCTGCCGTATGCTCGAGAAGGCTAG-p-5'
```

DNA fragment, after overhang attached, phosphorylated

```
5'-p.......A-3'
3'-A.......p-3'
```

Ligate Genomic Y adapter to DNA fragment with overhang

```
5'-ACACTCTTTCCCTACACGAC
                        \
                         GCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'

3'-GTTCGTCTTCTGCCGTATGCTCGAGAAGGCTAGA..........TCTAGCCTTCTCG
                                                \
                                                 CAGCACATCCCTTTCTCACA-5'
```

Gel purify ligation product

Amplify with PCR primers

Short primer sequence 5'-CAAGCAGAAGACGGCATACGAGCTCTTCCGATC*T-3'
Long primer sequence 5’- AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3'

Round 1 of PCR amplification

Denaturation of ligation product, primer annealling and product synthesis. Arrow goes in direction of synthesis. Long primer does not anneal in this step because it is identical to the maroon adapter, and requires the complementary sequence to anneal.

```
5'-ACACTCTTTCCCTACACGAC
                        \
                         GCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
                                                TCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'
```

```
3'-GTTCGTCTTCTGCCGTATGCTCGAGAAGGCTAGA..........TCTAGCCTTCTCG
                                                \
                                                 CAGCACATCCCTTTCTCACA
```

5'-CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT

Resulting products from Round 1 synthesis. Pink indicates newly synthesized strand

Product 1:

5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
3'-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........TCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'

Product 2, same as Product 1, rotated 180 degrees

3'-GTTCGTCTTCTGCCGTATGCTCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACA-5'
5'-CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'

Round 2 synthesis.
Denaturation and primer annealling

Long primer anneals to Product 1, synthesis occurs. Short primer cannot anneal to this fragment

5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
                          3'-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........CTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'

Resulting product after Round 2 of PCR

5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
                          3'-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........TCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'

Round 3 of PCR:

Denaturation of this asymmetric product

5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'

                          3'-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........ACTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'

Annealling of long primer and extension. Long primer will only anneal to this fragment. Results again in an asymmetric product

 5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
                           3'-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........ACTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'

Annealling of short primer and extension. Short primer will only anneal to this fragment

5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
                                                    TCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'

Resulting PCR product from short primer annealling is uniform

```
5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........TCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'
```

Annealling and synthesis now proceeds as normal with both long and short primers from this product. Presumably the asymmetric product continues to be generated linearly compared with the full length product, which is generated exponentially.

```
5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
                          <---------------------------------------------TCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'

3'-TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........TCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'
5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT ---------------------------------------------->
```

Products are denatured and added to flow cell in cluster processing station

Flow cell oligos
oligo 'A': 5 ´-PS-TTTTTTTTTT-(diol)3-AATGATACGGCGACCACCGA-3
oligo 'B': 5 ´-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGA-3 ´

Denaturation of PCR products, single strands bind to fixed flow cell oligos.

Bound to oligo B:

```
5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
                                                                        3'-AGCATACGGCAGAAGACGAACTTTTTTTTTT-5'
```

Bound to oligo A:

```
                    3'-TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........TCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'
5 ´-PS-TTTTTTTTTT-(diol)3-AATGATACGGCGACCACCGA-3'
```

These are extended with Taq

Product bound to Oligo B, now has a strand complementary to oligo A after synthesis. Presumably this binds to an oligo A substrate

```
                    5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
                       TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........TCTAGCCTTCTCGAGCATACGGCAGAAGACGAACTTTTTTTTTT-PS-5'
5 ´-PS-TTTTTTTTTT-(diol)3-AATGATACGGCGACCACCGA-3
```

Denaturation with formamide removes the seed template leaving the bridge structure (double stranded at A end)

```
                       TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........TCTAGCCTTCTCGAGCATACGGCAGAAGACGAACTTTTTTTTTT-PS-5'
5 ´-PS-TTTTTTTTTT-(diol)3-AATGATACGGCGACCACCGA-3
```

Product bound to Oligo A, now has a strand complementary to oligo B after synthesis. Presumably this binds to an oligo B substrate

```
                        3'-TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........TCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'
5 -PS-TTTTTTTTTT-(diol)3-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG
                                                                                                AGCATACGGCAGAAGACGAACTTTTTTTTTT-PS-5'
```

Denaturation removes the seed template leaving this bridge structure (double stranded at B end)

```
5 -PS-TTTTTTTTTT-(diol)3-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG
                                                                                                AGCATACGGCAGAAGACGAACTTTTTTTTTT-PS-5'
```

Bridge structures are amplified with BST polymerase, then denatured. 35 cycles. Unclear on how the strand “finds” the anchored oligo after denaturation, but amplification has to be exponential.

Synthesis of the bridge structure double stranded at B end (same process will occur for structure double stranded at A end)

```
5 -PS-TTTTTTTTTT-(diol)3-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG
                        TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........TCTAGCCTTCTCGAGCATACGGCAGAAGACGAACTTTTTTTTTT-PS-5'
```

Denaturation and reannealling:

Binds to oligo B:

```
                                                                                                AGCATACGGCAGAAGACGAACTTTTTTTTTT-PS-5'
5 -PS-TTTTTTTTTT-(diol)3-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG
```

Binds to oligo A:

```
5 -PS-TTTTTTTTTT-(diol)3-AATGATACGGCGACCACCGA
                        TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........TCTAGCCTTCTCGAGCATACGGCAGAAGACGAACTTTTTTTTTT-PS-5'
```

- Double stranded bridge structures are linearized at oligo A using periodate, which cleaves the diol

We now have dsDNA immobilized by binding to oligo B

```
   TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA..........TCTAGCCTTCTCGAGCATACGGCAGAAGACGAACTTTTTTTTTT-PS-5'
5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT..........AGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
```

- Clusters are blocked from further extension with terminal transferase and ddNTP
- Linearized dsDNA is denatured with NaOH
- Sequencing primer is hybridized to one strand

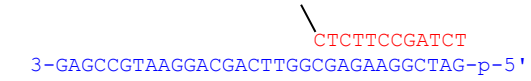
Genomic DNA sequencing primer: 5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT Exactly matches end of long read PCR primer

Flow cell is removed from cluster station and transferred to sequencer

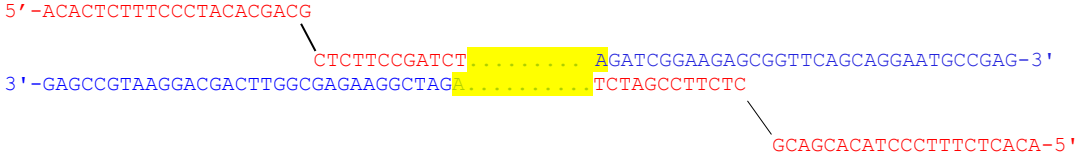
Using Paired End adapter sequences

5’ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
5’-p-GATCGGAAGAGCGGTTCAGCAGGAATGCCGAG

Formation of Y structure:



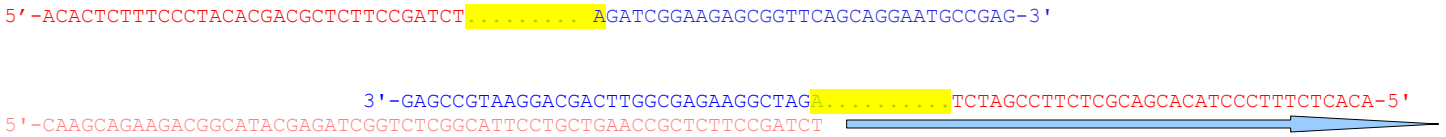
Ligate Genomic Y adapter to DNA fragment with overhang



Amplification with paired end PCR primers

PE1 5’-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
PE2 5’-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT

Denaturation of adapter complex, binding of primer PE1 to one strand, synthesis in round 1 PCR



Round 2 PCR

Asymmetric substrate (black represents newly synthesized strand)

```
                            3'-GAGCCGTAAGGACGACTTGGCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACA-5'
5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
```

Denaturation of asymmetric substrate, binding of primer PE2 to one strand, and extension:

```
                            3'-GAGCCGTAAGGACGACTTGGCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACA-5'


5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3
  ═══════════════════════════════════════════════════════════════════════ TCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCTAGAGCCACCAGCGGCATAGTAA-5'
```

Round 3 PCR

Asymmetric product from round 2:

```
5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3
   GTTCGTCTTCTGCCGTATGCTCTAGCCAGAGCCGTAAGGACGACTTGGCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCTAGAGCCACCAGCGGCATAGTAA-5'
```

Denaturation of asymmetric product from round 2, followed by binding of primer PE1 to newly synthesized strand. Primer PE2 will bind to shorter strand, resulting in a repeat of round 2 amplification:

```
5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3
  ═══════════════════════════════════════════════════════════════════════ TCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCTAGAGCCACCAGCGGCATAGTAA-5


3'-GTTCGTCTTCTGCCGTATGCTCTAGCCAGAGCCGTAAGGACGACTTGGCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCTAGAGCCACCAGCGGCATAGTAA-5'
5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT ═══════════════════════════════════════════════════════════════════════
```

Resulting in a symmetric product:

```
3'-GTTCGTCTTCTGCCGTATGCTCTAGCCAGAGCCGTAAGGACGACTTGGCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCTAGAGCCACCAGCGGCATAGTAA-5'
5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT-3'
```

Annealling of PCR products to flow cells
Immobilised oligos on paired end flowcell surface:

oligo 'C': 5'-PS-TTTTTTTTTTAATGATACGGCGACCACCGAGAUCTACAC-3'
(U = 2-deoxyuridine)
oligo 'D': 5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGoxoAT-3',
(Goxo = 8-oxoguanine) immobilised on the surface in a ratio C:D = 1:1.

```
             3'-GTTCGTCTTCTGCCGTATGCTCTAGCCAGAGCCGTAAGGACGACTTGGCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCTAGAGCCACCAGCGGCATAGTAA-5'
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGoxoAT-3
```

```
5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT-3'
                                                                                                                                3'-CACATCUAGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
```

Formation of double stranded bridge structures and isothermal amplification is the same as for single-read:

```
                GTTCGTCTTCTGCCGTATGCTCTAGCCAGAGCCGTAAGGACGACTTGGCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCUAGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
```

Linearization of oligo C with "USER" enzyme retains strand 1, attached to "D" oligo. USER generates gap at location of the uracil in dsDNA only (so it doesn't cleave the single stranded clusters):

```
                GTTCGTCTTCTGCCGTATGCTCTAGCCAGAGCCGTAAGGACGACTTGGCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACATC AGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
```

Blocking with terminal transferase and ddNTPs
Denaturation with NaOH
Hybridization of read 1 sequencing primer
Transfer of flow cell to sequencer
First round of sequencing:

```
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
                                                                                        <-- TCTAGCCTTCTCGCAGCACATCCCTTTCTCACA-5'
```

Denaturation to remove read 1 product leaves single strand again, attached to oligo 'D':

```
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT-3'
```

This strand forms a bridge structure again with oligo 'C':

```
                                                                                                                CACATCUAGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
```

3' Dephosphorylation of strand, followed by isothermal synthesis of new strand produces bridged cluster:

```
                GTTCGTCTTCTGCCGTATGCTCTAGCCAGAGCCGTAAGGACGACTTGGCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCUAGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
```

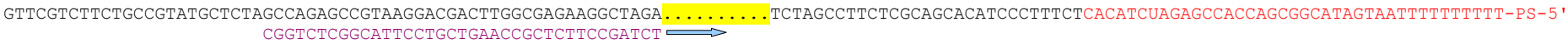
Second strand is cleaved at the 8-oxoguanine in oligo 'D' using Fpg enzyme:

```
                GTTCGTCTTCTGCCGTATGCTCTAGCCAGAGCCGTAAGGACGACTTGGCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCUAGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAG
                                    \ATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
```

Now strands are attached to oligo 'C':

```
                GTTCGTCTTCTGCCGTATGCTCTAGCCAGAGCCGTAAGGACGACTTGGCGAGAAGGCTAGA..........TCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCUAGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
                          ATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT..........AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT
```

Blocking with TT, denaturation with NaOH, hybridization of read 2 sequencing primer:

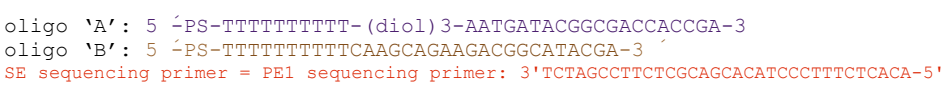


Transfer to sequencer to get second read. Note immobilized oligos are permanently altered by the cleavage process.

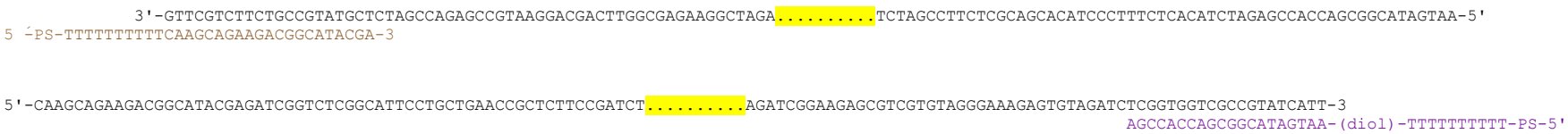
Could paired end sequences anneal to a single read flow cell? Yes, flow cell oligos are compatible with paired end primers/adapters, and sequencing primer will hybridize correctly:

Paired end product:

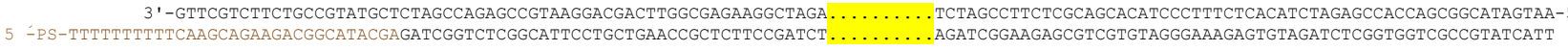
Flow cell oligos for single read:



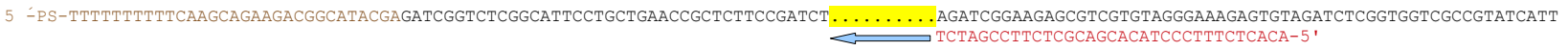
Denature product, annealling:



Isothermal synthesis, cleavage of diol to form dsDNA attached to oligo B:



Denaturation of product, annealling of sequencing primer, sequencing:



Comparison of SE and PE adapter sequences with identical portions highlighted. PE sequences are 1 base longer than SE sequences

SE1 5'-ACACTCTTTCCCTACACGACC-TCTTCCGATC*T-3'
PE1 5'-ACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3'

SE2 3'-GTTCGTCTTCTGCCGTATGCTCGAGAAGGCTAG-p-5'
PE2 3'- GAGCCGTAAGGACGACTTGGCGAGAAGGCTAG-p-5'

Comparison of SE and PE PCR primer sequences with identical portions highlighted. PE1 primer is longer than SE1 primer, SE2 and PE2 primers are identical.

SE1 5'-CAAGCAGAAGACGGCATACGAGCTC----------TTCCGATC*T-3'
PE1 5’-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T

SE2 5’-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3'
PE2 5’-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T

Immobilised oligos on paired end flowcell surface:

oligo ‘C’: 5’-PS-TTTTTTTTTTAATGATACGGCGACCACCGAGAUCTACAC-3’
(U = 2-deoxyuridine)
oligo ‘D’: 5’-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGoxoAT-3’,
(Goxo = 8-oxoguanine) immobilised on the surface in a ratio C:D = 1:1.

Comparison of SE (A,B)and PE (C,D) immobilised oligo sequences:

oligo ‘C’: 5’-PS-TTTTTTTTTT---------AATGATACGGCGACCACCGAGAUCTACAC-3’
oligo ‘A’: 5 ´-PS-TTTTTTTTTT-(diol)3-AATGATACGGCGACCACCGA-3

oligo ‘D’: 5’-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGoxoAT-3’,
oligo ‘B’: 5 ´-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGA-3

Oligo B same as oligo D except for the GoxoAT end on D
Oligo A has stretch of same sequences as oligo C

Comparison of sequencing primers. SE and PE1 primers are identical, therefore SE primer could be used on a PE adapter sequence:

Single read: 5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'
PE1: 5’-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3’

PE2: 5’-CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT -3