Diagram of Illumina protocol using single read adapter sequences

Sequences of flow cell oligos taken from Bentley et al., Nature 2008
Attempt to diagram how direct sequencing of short amplicons would work, as described by Quail et al. 2008:

"Direct sequencing of short amplicons. To avoid unnecessary PCR amplification steps, which would potentially exacerbate biases, we can perform extremely deep sequencing of short amplicons using locus-specific primers that possess tails that can hybridize to the oligos tethered to the flowcell surface. The tailless forward and reverse oligos are then used as primers in the sequencing steps.
Supplementary Protocol 10:
This is achieved by attaching a tail on the amplicon-specific primers that will allow amplicons to hybridise directly to the flowcell after PCR. Sequencing is performed using the tailless specific primers at a concentration of 500nM.
[AATGATACGGCGACCACCGAGATCTACACT] [specific primer 1]
[CAAGCAGAAGACGGCATACGAGAT] [specific primer 2]"

This would have to be done on a paired end module.

Let's look at how these match up with existing Illumina sequences.
Paired end PCR primer 1 CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
Quail specific primer 2 CAAGCAGAAGACGGCATACGAG

Single read/Paired read PCR primer 2 AATGATACGGCGACCACCGAGATCTACACT
Quail specific primer 1 AATGATACGGCGACCACCGAGATCTACACT

Standard PE sequencing primer 1 ACACTCTTTCCCTACACGACGCTCTTCCGATCT
Standard PE sequencing primer 2 CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT

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, shown as G

Here's what we should have after 1 round of PCR with our extended amplicon specific primers:

```
3'-GTTCGTCTTCTGCCGTATGCTCTA[locus primer].........[locus primer]TCACATCUAGAGCCACCAGCGGCATAGTAA-5'
5'-CAAGCAGAAGACGGCATACGAGAT[locus primer].........[locus primer]AGTGTAGATCTCGGTGGTCGCCGTATCATT-3'
```

Some steps are skipped below. I'm showing the denatured product hybridizing to flow cell oligos:

Oligo D:

```
            3'-GTTCGTCTTCTGCCGTATGCTCTA[locus primer].........[locus primer]CACATCUAGAGCCACCAGCGGCATAGTAA-5'
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGA-AT-3'
```

Oligo C:

```
5'-CAAGCAGAAGACGGCATACGAGAT[locus primer].........[locus primer]AGTGTAGATCTCGGTGGTCGCCGTATCATT-3'
                                                    3'CACATCUAGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
```

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

```
              GTTCGTCTTCTGCCGTATGCTCTA[locus primer].........[locus primer]TCACATCUAGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGAT[locus primer].........[locus primer]AGTGTAGATCTCGGTGGTCGCCGTATCATT
```

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):

```
              GTTCGTCTTCTGCCGTATGCTCTA[locus primer].........[locus primer]TCACATC*AGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGAT[locus primer].........[locus primer]AGTGTAGATCTCGGTGGTCGCCGTATCATT
```

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 on strand attached to oligo D:

```
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGA-AT[locus primer].......[locus primer]AGTGTAGATCTCGGTGGTCGCCGTATCATT
                                                  <---------- TCACATCTAGAGCCACCAGCGGCATAGTAA
```

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

```
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGA-AT[locus primer].........[locus primer]AGTGTAGATCTCGGTGGTCGCCGTATCATT-3'
```

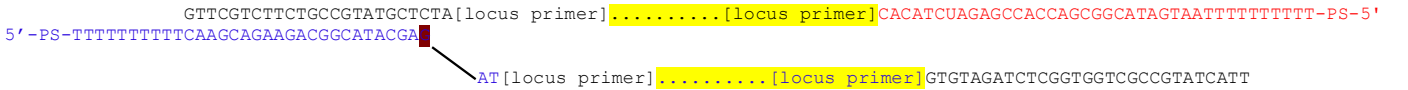
This strand forms a bridge structure with oligo 'C':

```
                                                                  CACATCUAGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGAT[locus primer].........[locus primer]AGTGTAGATCTCGGTGGTCGCCGTATCATT
```

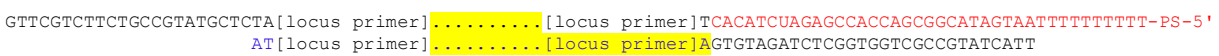
3' Dephosphorylation of strand, followed by isothermal synthesis of new strand produces bridged cluster:

```
              GTTCGTCTTCTGCCGTATGCTCTA[locus primer].........[locus primer]TCACATCUAGAGCCACCAGCGGCATAGTAATTTTTTTTTT-PS-5'
5'-PS-TTTTTTTTTTCAAGCAGAAGACGGCATACGAGAT[locus primer].........[locus primer]AGTGTAGATCTCGGTGGTCGCCGTATCATT
```

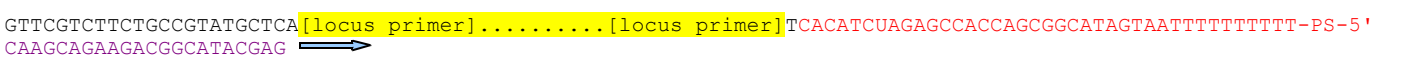
Second strand is cleaved at the 8-oxoguanine in oligo 'D' using Fpg enzyme, resulting in disassociation from oligo D:



Now strands are attached to oligo 'C':



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.