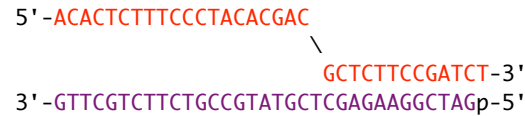


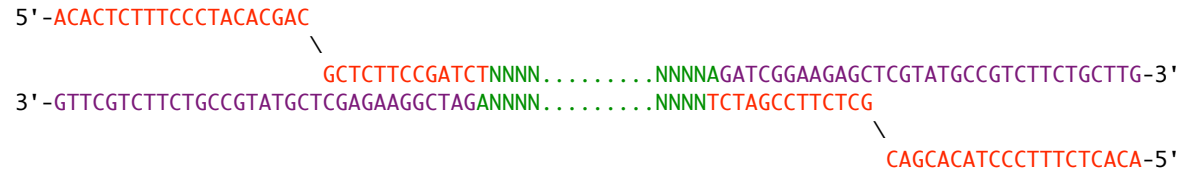
Fragmented, end polished, phosphorylated, dA ligated DNA sample (fragment size is <800nt)



Genomic "Y" Adapter



Ligate

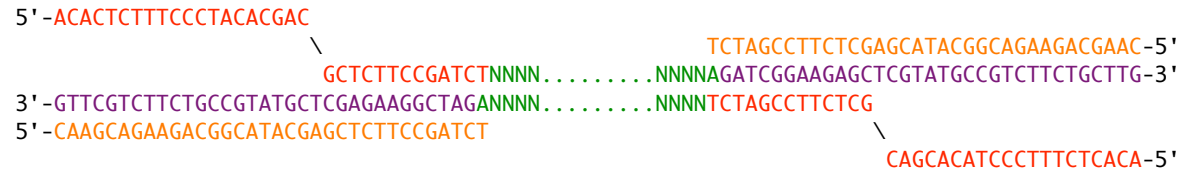


[Ligation product is gel purified, selecting only those products in the 150-200nt range]

PCR Primers

In the first round of PCR the short PCR primer will fully anneal to both 3' ends; the long primer would not be used. The product of the first round is then asymmetric. In subsequent rounds the long primer would anneal to one end and the short primer to the other.

Round 1



Product of first round (showing only one of the two possible asymmetric products)



Rounds 2-18

```
5'-ACACTCTTTCCTACACGACGCTCTCCGATCTNNNN.....NNNNAGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
3'-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGANNN.....NNNNTCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'
5'-AATGATACGGCGACCACCGAGATCTACACTCTTCCCTACACGACGCTCTCCGATCT
```

Product of PCR amplification

```
5'-AATGATACGGCGACCACCGAGATCTACACTCTTCCCTACACGACGCTCTCCGATCTNNNN.....NNNNAGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGANNN.....NNNNTCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'
```

[Anneal to flow cell. Perform cluster generation]

Genomic DNA Sequencing Primer

```
5'-AATGATACGGCGACCACCGAGATCTACACTCTTCCCTACACGACGCTCTCCGATCTNNNN.....NNNNAGATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGANNN.....NNNNTCTAGCCTTCTCGAGCATACGGCAGAAGACGAAC-5'
5'-ACACTCTTTCCTACACGACGCTCTCCGATCT
```