

Fragmented, end polished, phosphorylated, dA ligated DNA or cDNA sample prepared using the TruSeq DNA or RNA sample prep kit. Size is should be less than 800nt.

5'-pNNNN.....NNNA-3'
3'-ANNN.....NNNNp-5'

TruSeq “Y” adapter for DNA or RNA. The top strand is the TruSeq Universal Adapter, the bottom is one of twelve TruSeq Adapter sequences which include a 6nt MID sequence (barcode), represented by the blue “N’s”.

5'-AATGATACGGCGACCACCGA GATCTACACTCTTTCCTACACGAC
3'-GTTTCGTCTTCTGCCGTATGCTCTANNNNNNCACTGACCTCAAGCTGCACACGAGAAGGCTAGp-5'
GCTCTTCCGATCT-3'

Ligate

5'-AATGATACGGCGACCACCGA GATCTACACTCTTTCCTACACGAC
3'-GTTTCGTCTTCTGCCGTATGCTCTANNNNNNCACTGACCTCAAGCTGCACACGAGAAGGCTAGANNNN.....NNNNAGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNATCTCGTATGCCGTCTTCTGCTTG-3'
GCTCTTCCGATCTNNNN.....NNNNAGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNATCTCGTATGCCGTCTTCTGCTTG-3'
CAGCACATCCCTTTCTCACATCTAGAGCCACCAGCGGCATAGTAA-5'

PCR Amplify, size select in range of 350-450nt. (Exact PCR primer sequences not known, but P5 & P7 should work.)

5'-CAAGCAGAAGACGGCATACGAGATNNNNNGTGACTGGAGTTCAAGCTGTGCTCTTCCGATCTNNNN.....NNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT-3'
3'-GTTTCGTCTTCTGCCGTATGCTCTANNNNNNCACTGACCTCAAGCTGCACACGAGAAGGCTAGANNNN.....NNNNTCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCTAGAGCCACCAGCGGCATAGTAA-5'

Flow cell generation (Anneal, bridged amplification, linearization). The clusters are ssDNA of the top strand, anchored to the flow cell at the 5' end.

Sequencing reads

Index (i7) Read Sequencing Primer
<-CACTGACCTCAAGTCTGCACACGAGAAGGCTAG-5'
Read 1 Sequencing Primer
<-TCTAGCCTTCTCGCAGCACATCCCTTTCTCACA-5'
5'-CAAGCAGAAGACGGCATACGAGATNNNNNGTGACTGGAGTTCAAGCTGTGCTCTTCCGATCTNNNN.....NNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT-3'

After reamplification the ssDNA clusters are composed of the bottom strand, again anchored to the flow cell at its 5' end.

3'-GTTTCGTCTTCTGCCGTATGCTCTANNNNNNCACTGACCTCAAGTCTGCACACGAGAAGGCTAGANNNN.....NNNNTCTAGCCTTCTCGCAGCACATCCCTTTCTCACATCTAGAGCCACCAGCGGCATAGTAA-5'
5'-GTGACTGGAGTTCAAGCTGTGCTCTTCCGATCT->
Read 2 Sequencing Primer

p5: 5'-AATGATACGGCGACCACCGA
p5rc: 3'-TTACTATGCCGCTGGTGGCT

p7: 5'-CAAGCAGAAGACGGCATACGA
p7rc: 3'-GTTTCGTCTTCTGCCGTATGCT