Step 1 Digestion with ApeKI

CWG-insert-CWG

Step 2 Ligation of barcoded and common adapters

Barcoded adapter (x96):
5’-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-inlinebarcode-CWG

5’-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-inlinebarcode-CWG-insert-CWG-AGATCGGAAGAGCGGTTCAGCAGGAATGCCGAG-3’
3’-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-inlinebarcode-CWG-insert-CWG-TCTAGCCTTCTCGCCAAGTCGTCCTTACGGCTC-5’

3’-CWG-TCTAGCCTTCTCGCCAAGTCGTCCTTACGGCTC-5’
Common adapter (x1):

Step 3 Amplification with P5-P7 PCR primers

PCR primer A with P5 and Truseq
5’AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT

Correct P7 and Truseq
AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC---ATCTCGTATGCCGTCTTCTGCTTG

AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-inlinebarcode-CWG-insert-CWG-AGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCTCGTATGCCGTCTTCTGCTTG
TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-inlinebarcode-CWG-insert-CWG-TCTAGCCTTCTCGCCAAGTCGTCCTTACGGCTCTGGCTAGAGCATACGGCAGAAGACGAAC

TCTAGCCTTCTCGCCAAGTCGTCCTTACGGCTCTGGCTAGAGCATACGGCAGAAGACGAAC-5’
PCR primer B with WRONG P7 and WRONG TruSeq

NEW PROPOSED INDEXED LIBRARY STRUCTURE

NEW PCR primer A with P5 and Truseq and i5
5’AATGATACGGCGACCACCGAGATCTACACACGATCAGACACTCTTTCCCTACACGACGCTCTTCCGATCT

Barcoded adapter (x96):
5’-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-BC-CWG

5'AATGATACGGCGACCACCGAGATCTACACACGATCAGACACTCTTTCCCTACACGACGCTCTTCCGATCT-BC-CWG-ins-CWG-AGATCGGAAGAGCACACGTCTGAACTCCAGTCACGCGCATATATCTCGTATGCCGTCTTCTGCTTG3’
3’TTACTATGCCGCTGGTGGCTCTAGATGTGTGCTAGTCTGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-BC-CWG-ins-CWG-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGCGCGTATATAGAGCATACGGCAGAAGACGAAC5’

3’-CWG-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-5’
NEW common adapter (x1):

3’TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGCGCGTATATAGAGCATACGGCAGAAGACGAAC5’
NEW PCR PRIMER B with correct P7 and Truseq and i7

Reference: TruSeq Dual Index Library https://teichlab.github.io/scg_lib_structs/methods_html/Illumina.html

ALL ADAPTERS 5’->3’:
Barcoded: ACACTCTTTCCCTACACGACGCTCTTCCGATCT-BC-CWG
NEW Common: GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-CWG
NEW PCR A: AATGATACGGCGACCACCGAGATCTACACACGATCAGACACTCTTTCCCTACACGACGCTCTTCCGATCT
NEW PCR B: CAAGCAGAAGACGGCATACGAGATATATGCGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT