Forward primer with index

CAAGCAGAAGACGGCATACGAGATCNNNNNNtttcttgggtagtttgcagtttt

Reverse primer

AATGATACGGCGACCACCGAGATCTACACCGACTCGGTGCCACTTTT

NNNNNN denotes a user-specified sample barcode sequence

* Using ExTaq from Takara:

Library: up to 2.5ug

Buffer: 5uL

dNTP: 4uL

primers mix (10uM): 1uL

enzyme: 0.25uL

H2O to 50uL

* Amplify reactions in a thermocycler using the following program.

1 cycle 94°C 2 minutes

30 cycles 94°C 20 seconds

63°C 30 seconds

72°C 45 seconds

1 cycle 72°C 1 minute1

1 cycle 4°C HOLD

* PCR purify
* Send to NextSeq75SR (asking for only 20 cycles) with index: NNNNNN
* Please note that indexing primer will bind to the reverse strand; therefore, index will be read as reverse complement

Illumina primer 1 (with index): TTTCAAGTTACGGTAAGCATATGATAGTCCATTTTAAAACATAATTTTAAAACTGCAAACTACCCAAGAAA

Illumina primer 2 (to sequence):

CGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC