sgRNA barcode PCR primers

Forward: AATGATACGGCGACCACCGAGATCTACACcccactgacgggcaccgga

Reverse: *CAAGCAGAAGACGGCATACGAGATCnnnnnnTTTCTTGGGTAGTTTGCAGTTTT*

*nnnnnn* denotes a user-specified sample barcode sequence

Sequencing primers for Illumina HiSeq

Read 1 primer: *CGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC*

Indexing primer: *TTTCAAGTTACGGTAAGCATATGATAGTCCATTTTAAAACATAATTTTAAAACTGCAAACTACCCAAGAAA*

Use the following per-sample recipe to assemble the 8-10X of the total reaction mixture and dispense into 8-10 PCR strip tubes in 50-µL aliquots on ice.

6 µg Genomic DNA

2 µL forward sgRNA PCR primer (10µM)

2 µL sample-specific barcoded reverse sgRNA PCR primer (10µM)

25 µL Phusion PCR Master Mix

Up to 50 µL H2O

Amplify reactions in a thermocycler using the following program.

1 cycle 98°C 2 minutes

30 cycles 98°C 10 seconds

 60°C 15 seconds

 72°C 45 seconds

1 cycle 72°C 5 minutes

1 cycle 4°C HOLD