



double stranded PCR product,
gel purify band/smear at (300-400bp)
(insert at 200-300bp)

1: Arbitrary PCR

using phusion PCR enzyme, so that no A's added at 3' end

1_transp_deep_seq_F1: TCTATCGCCTTCTTGACGAG

1_transp_deep_seq_R2: GTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT NNNNNNNNNggtgc
 1_transp_deep_seq_R3: GTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT NNNNNNNNNgatata
 1_transp_deep_seq_R4: GTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT NNNNNNNNNnagtac

2: Nested PCR (addition of P7 and P5)

using phusion PCR enzyme, so that no A's added at 3' end

2_transp_deep_seq_FP5: AATGATACGGCGACCACCGAGATCT actggtcggcg CATTAGGGGATTCATCAG

Rev_P7 6_02
 CAAGCAGAAGACGGCATAACGAGAT agtctt GTGACTGGAGTTCAGACGTGT
 CAAGCAGAAGACGGCATAACGAGAT gtatct GTGACTGGAGTTCAGACGTGT
 CAAGCAGAAGACGGCATAACGAGAT tcatgg GTGACTGGAGTTCAGACGTGT
 CAAGCAGAAGACGGCATAACGAGAT cgcgac GTGACTGGAGTTCAGACGTGT
 CAAGCAGAAGACGGCATAACGAGAT acgata GTGACTGGAGTTCAGACGTGT
 CAAGCAGAAGACGGCATAACGAGAT tataga GTGACTGGAGTTCAGACGTGT

3: Sequencing on Illumina chip

transp_deep_seq_Custom seq 1: actggtcggcgCATTAGGGGATTCATCAG

4: Barcode is read on Illumina chip

transp_deep_seq_Index seq: GATCGGAAGAGCACACGTCTGAACTCCAGTCAC