

A

P1

INDEX1

5' ACACTCTTTCCCTACACGACGCTCTTCCGATCT *ACGTAGCT*\*T (sense)

5'P-*AGCTACGT*AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG\*T (antisense)

P2

5' P-GATCGGAAGAGCGGTTTCAGCAGGAATGCCGA\*G (sense)

5' CTCGGCATTCTGCTGAACCGCTCTTCCGATC\*T (antisense)

B



**Figure S3** Design of custom adapters used for multiplexing. A) Oligo sequences for the indexing adapters. The index is added to the 3' end of the Illumina P1 adapter. An example index sequence (in italics) is shown. See Table S1 for all 96 index sequences. "P" is used to indicate a 5' phosphate addition. The \* shows the position of a phosphorothioate bond. Oligo sequences for the universal Illumina P2 adapter are also shown. B) Schematic diagram showing adapters ligated to DNA fragments. The arrow indicates the direction of sequencing using the Illumina primer for the first read. The first 8 bases of sequence for read 1 are the index sequence.