A

Original: 5’ - NNN12345NNN - 3’

Frame 1: 5’ - N12 345 TGC GGC CGC A12 345 NNN - 3’
X X Cys Gly Arg X^1 X X

Frame 2: 5’ - NN1 234 5TG CGG CCG CA1 234 5NN - 3’
X X Leu Arg Pro His X X
Met Gln
Val

Frame 3: 5’ - NNN 123 45T GCG GCC GCA 123 45N - 3’
X X X^2 Ala Ala Ala X X

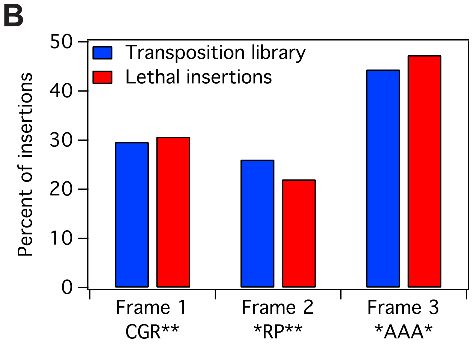
Example insertion:

Original: 5’ - CAAGTGGTTAT - 3’

Frame 1: 5’ - AGT GGT TGC GGC CGC AGT GGT TAT - 3’
Ser Gly Cys Gly Arg Ser Gly Tyr

Frame 2: 5’ - AAG TGG TTG CGG CCG CAG TGG TTA - 3’
Lys Trp Leu Arg Pro Gln Trp Leu

Frame 3: 5’ - CAA GTG GTT GCG GCC GCA GTG GTT - 3’
Gln Val Val Ala Ala Ala Val Val



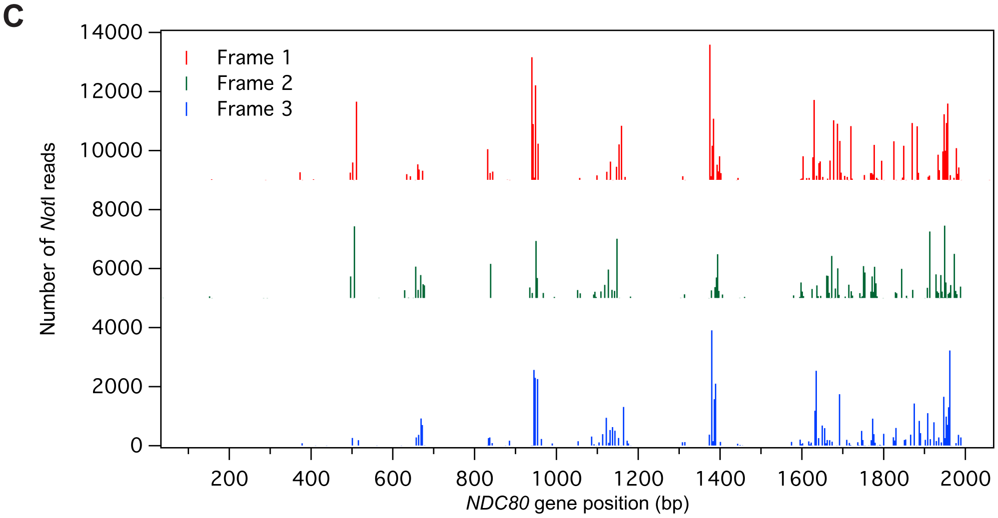


Figure S2 The reading frame targeted by transposition dictates the residues inserted. (A) The translation of the insertion depends on both the frame of insertion and the target sequence (adapted from Finnzymes Manual F-701). In the transposition library, each 15-bp insertion includes 5 bp of duplicated target sequence (red) and 10 additional nucleotides (blue). X is any amino acid; X^1 is Ile, Met, Thr, Asn, Lys, Ser, or Arg; X^2 is any amino acid except Gln, Glu, Lys, Met, and Trp. An example insertion is also shown. (B) The proportion of lethal insertions in each frame (red) is similar to that of the initial transposition library (blue). (C) The positions of lethal insertions (from Figure S1) are separated based on the frame of insertion. Traces are offset vertically for visual clarity.