File S2

Calculating fold coverage of Tn-mutagenized pools.

For entry pools: Fold coverage was calculated by dividing the number of Tmp^RKan^R transformants by the length of plasmid DNA "available" for mutagenesis. The target plasmid, *DCW1* entry vector, is 3608bp long. When considering the portions of the entry vector into which we could expect insertions, we excluded the origin (683 bp) and Kan^R gene (807bp), because plasmids with insertions in these regions would not be expected to propagate. Thus, 4236bp (2118 bp x 2 strands of DNA) of sequence in *DCW1* entry vector was available for mutagenesis. We observed 2.01×10^5 Tmp^RKan^R colonies / 4236bp = 47-fold coverage.

For expression pools: There are 1373 bp between Gateway recombination sites in a wild type *DCW1* expression vector. In our mutagenized expression pools, all Tn7 insertions will be present in this area, since that is the only region mobilized from the mutagenized *DCW1* entry pool by Gateway recombination. Because the Tn7 can insert in either direction, we consider the "available" DNA for mutagenesis in these constructs to be 2746 bp long. Fold coverage is calculated using the number of Tmp^RCar^R colonies in the expression pools, or Car^R colonies in the final pool. See Supplementary Note 3 for a discussion of how the number of Tmp^RCar^R colonies was calculated in the xpC pool. We observed 91x fold coverage in the initial expression pool (xpC), and recovered sufficiently numbers of transformants in subsequent steps to maintain this coverage. As the initial mutagenesis of the *DCW1* entry vector had 47x coverage, these expression pools are sufficiently large to maintain the complexity present in the initial mutagenized entry pool.