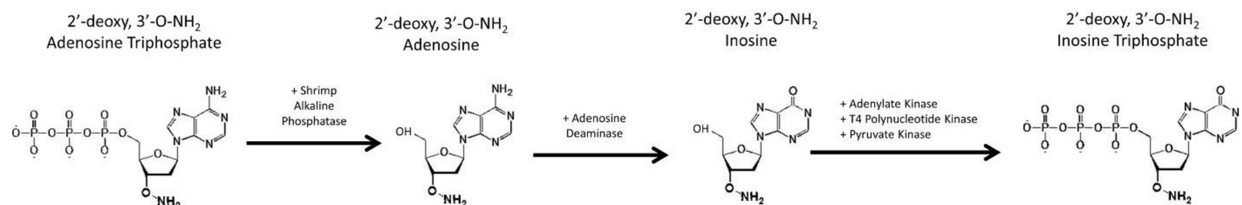
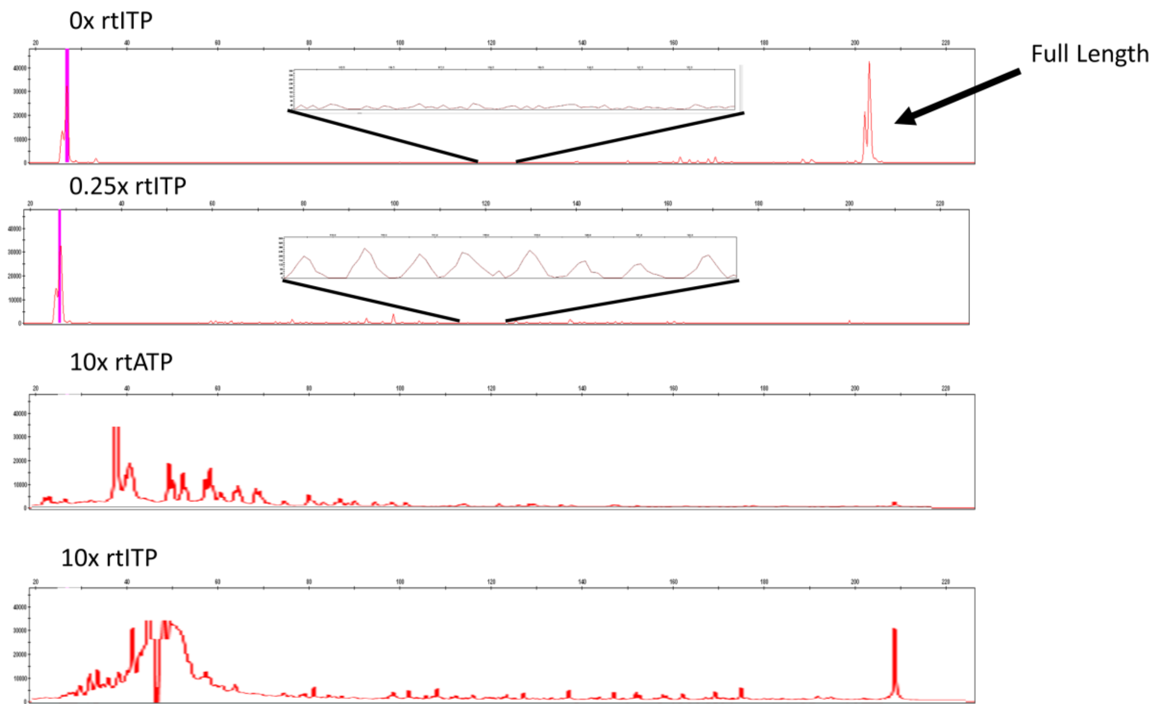


Supplementary Figure 1

Schematic of enzymatic creation of reversibly terminated 2'-deoxy, 3'-O-NH2 inosine triphosphate

Due to the inability of adenosine deaminase to convert adenosine triphosphate into inosine triphosphate, we dephosphorylated reversibly-terminated deoxyadenosine triphosphate before deamination. We subsequently re-phosphorylated the newly formed reversibly-terminated deoxyinosine using a mixture of three nucleotide/nucleoside kinases to form a deoxyinosine triphosphate capable of being incorporated into a PCR product.

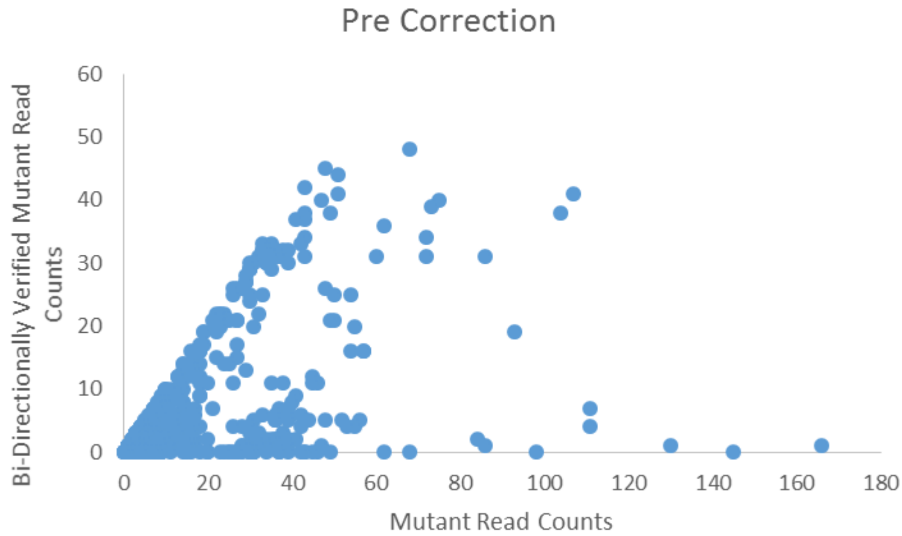


Supplementary Figure 2

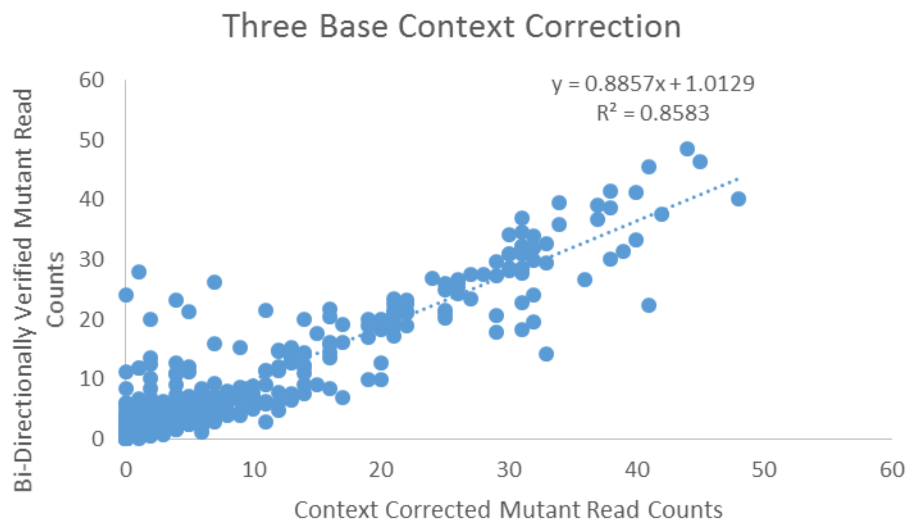
Demonstration of incorporation of reversibly-terminated deoxyinosine triphosphate (rtITP)

Linear amplification of a 212bp PCR product template with a fluorescently-labeled primer (ROX) was performed. Products were cleaned using ethanol precipitation and run on a 3730 Applied Biosystems capillary sequencer. Peak on left hand side is primer. Peak on right hand side is full length product. Peaks at every possible nucleotide as estimated with size markers were detected.

A



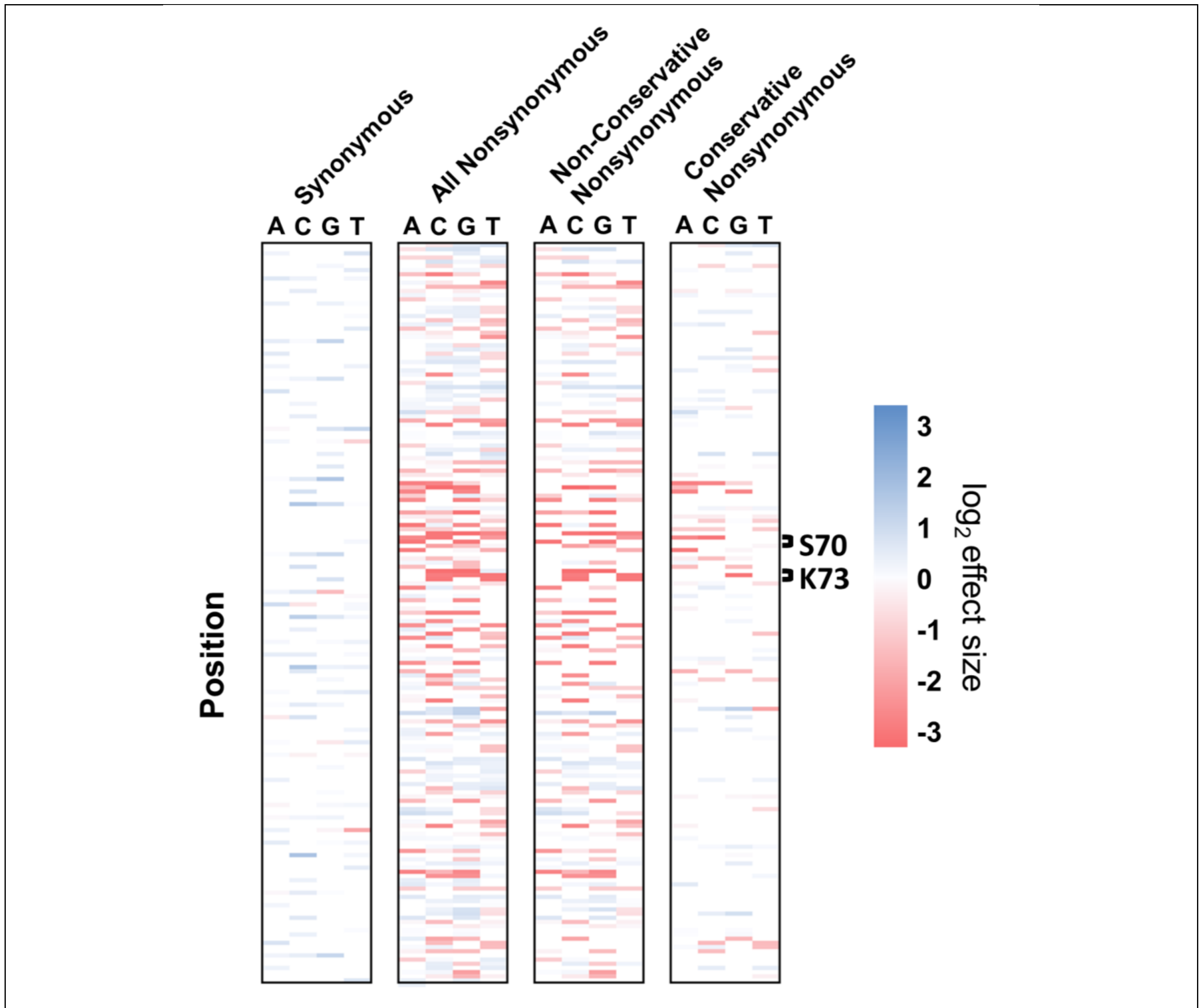
B



Supplementary Figure 3

Context corrected mutant read counts

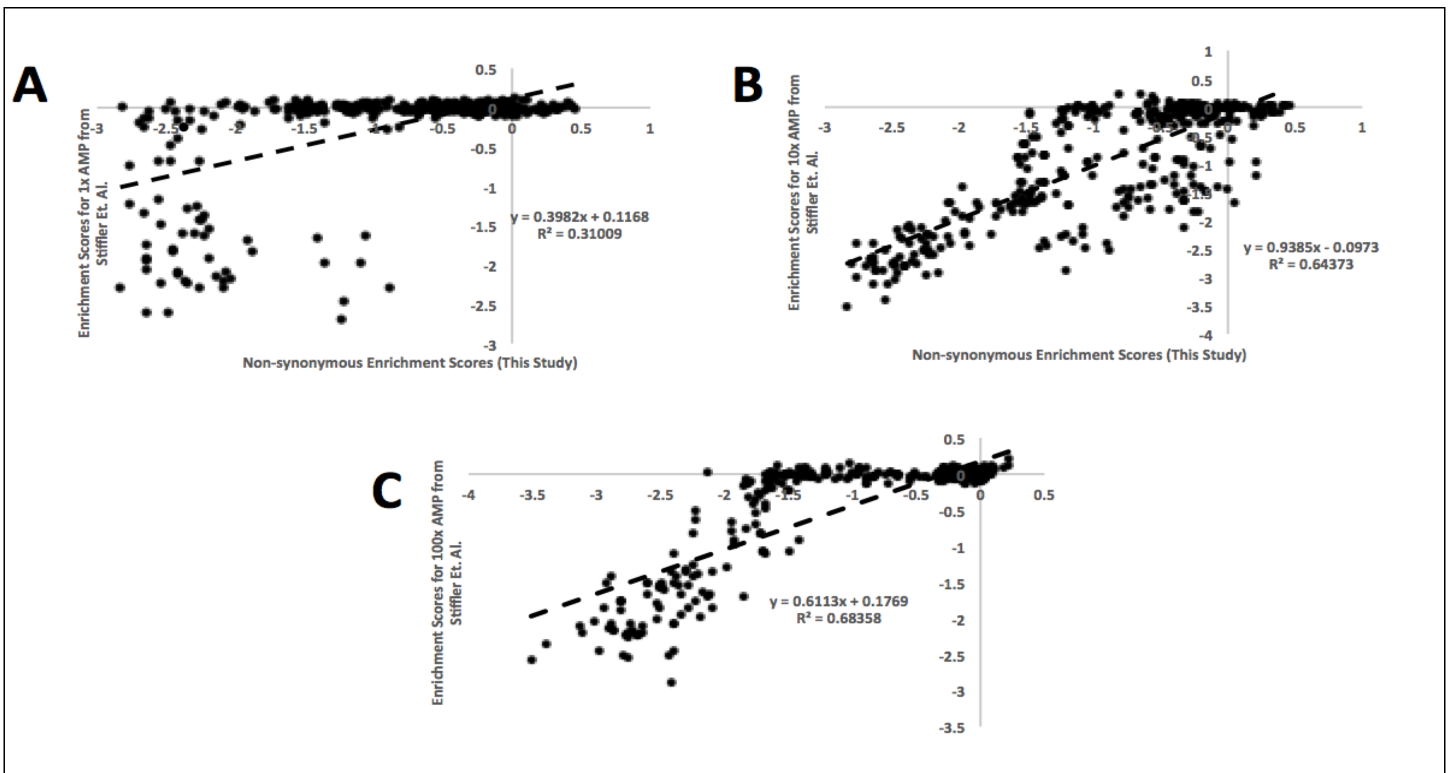
Comparisons between (A) observed mutation containing read counts (each dot is the number of reads containing a specific alternative nucleotide at a given position i.e. there were 80 C to T variant containing reads at position 100) compared to mutations verified to be true by virtue of being sequenced on both paired-end reads via Illumina sequencing or context or (B) 3bp context read-count-normalized mutation read counts.



Supplementary Figure 4

Functional map of mutation effect sizes for all possible single nucleotide changes.

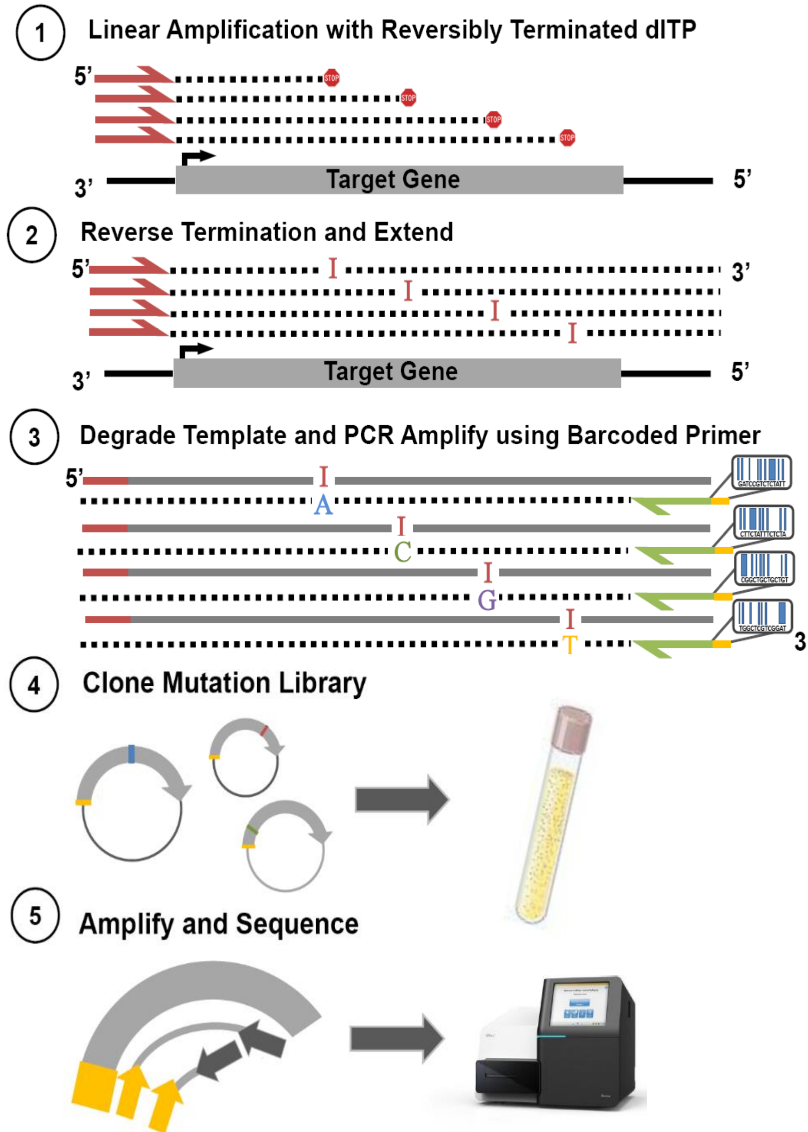
Y-axis indicates nucleotide 141 of the TEM-1 β-lactamase gene (top, AA 47) to nucleotide 315 (bottom, AA 105). Non-conservative amino acid changes are those that alter the amino acid character, i.e. hydrophobic to nonpolar. Conservative changes are non-synonymous changes that do not alter the amino acid type. Sites S70 and K73 within the active site are labeled.



Supplementary Figure 5

Correlation between effect-sizes for ampicillin mutations observed in this study and those reported previously

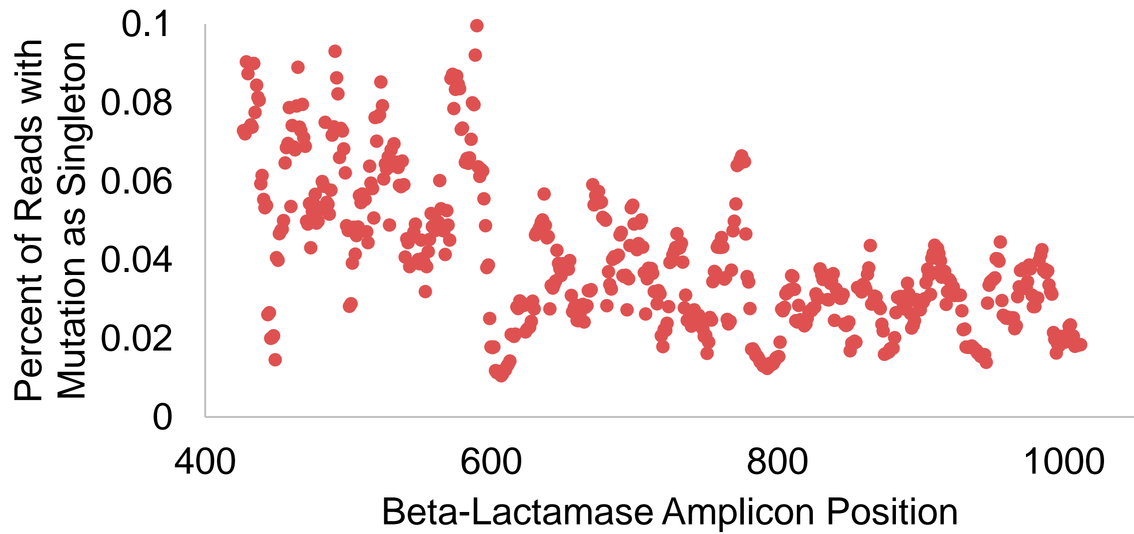
As observed in the previous study (1)of TEM-1 Beta-lactamase, mutations generally fell into a bi-modal distribution of either "damaging" or "not damaging". Comparisons are for (A) 1x ampicillin (B) 10x ampicillin and (C) 100x ampicillin.



Supplementary Figure 6

Extended schematic and library characteristics of SAS mutagenesis

Schematic of SAS mutagenesis 1) Linear amplification of a target genomic region is performed using a mixture of dNTPs and reversibly-terminated dITP and a biotinylated primer 2) The termination of products from step 1 is reversed and products are extended using the original template. 3) Reverse primer is added for one cycle, products are hybridized with streptavidin coated magnetic beads and washed to remove template. PCR is then performed to introduce single point mutations complementary to each inosine on the forward strand and to add a 20-mer unique molecular identifier (UMI) for the purpose of validating library creation. 4) Products are then cloned to reduce library complexity. 5) Minimal cycles of PCR are then used to amplify overlapping sections, each containing the UMI to perform subassembly of the SAS library.



Supplementary Figure 7

Distribution of mutations in long SAS mutation library

Plotted is the total number of reads harboring a mutation as a singleton (i.e. a molecule with only one mutation) at each position along the long SAS mutation library created using the TEM-1 Beta-lactamase gene. PCR of the distal three sections totaling 607bp was performed. Paired-end reads, one harboring the UMI barcode and the other harboring a segment of the SAS library was sequenced on an Illumina MiSeq lane.