Charge-Seq Analysis:

Fastq files for the pooled samples obtained from genewiz were demultiplexed according to the five-nucleotide sample barcode contained within the ligated adapter using a custom python script. Eighty six percent of all read pairs were found to contain a sample barcode. Pairs without an identifiable barcode could not be matched to a sample and were thus discarded. Illumina Sequencing adapters and ligated sample barcode adapters were trimmed using cutadapt in paired read mode and reads with less than twenty nucleotides remaining after trimming, corresponding to empty adapters, were removed. A custom reference file for human tRNA was constructed from the gtRNAdb GRCh38 high confidence tRNA gene set by removing duplicate sequences and appending ‘CCA’ to the 3’ end of each unique tRNA gene sequence. Trimmed reads were then aligned to the custom tRNA reference file using bowtie2 short read aligner with options “--dovetail -D 20 -R 3 -N 1 -L 20 -i S,1,0.50”. The charging status of the aligned tRNA was determined from the 3’ end of the aligned portion of each read pair, where reads ending in ‘CCA’ were determined to be charged, and reads ending in ‘CC’ were determined to be uncharged. This method was able to determine the charging status of approximately 85.5 percent of all mapped reads. Counts of charged and uncharged tRNA for each tRNA gene in each sample were then imported to R for further analysis. Differences in read counts between samples were accounted for by multiplying the read counts in each sample by a normalization coefficient equal to the average number of reads across all samples divided by the read count in that sample. The fraction charged for each tRNA isodecoder within each sample was then determined summing the normalized counts of charged and uncharged reads for each gene corresponding to that isodecoder, and the mean was then averaged for each of the four replicates of each treatment. The mean fraction charged for each isodecoder was then visualized as a heatmap and barplot +/- S.D. of the mean using ggplot2. Statistical significance in difference of the mean fraction charged was determined using a Welch’s T-test with Benjamini-Hochberg FDR correction to account for repeated measures.