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Supplemental Information

Genome-wide Analysis of *Salmonella enterica* serovar Typhi in Humanized Mice Reveals Key Virulence Features

Joyce E. Karlinsey, Taylor A. Stepien, Matthew Mayho, Larissa A. Singletary, Lacey K. Bingham-Ramos, Michael A. Brehm, Dale L. Greiner, Leonard D. Shultz, Larry A. Gallagher, Matt Bawn, Robert A. Kingsley, Stephen J. Libby, and Ferric C. Fang

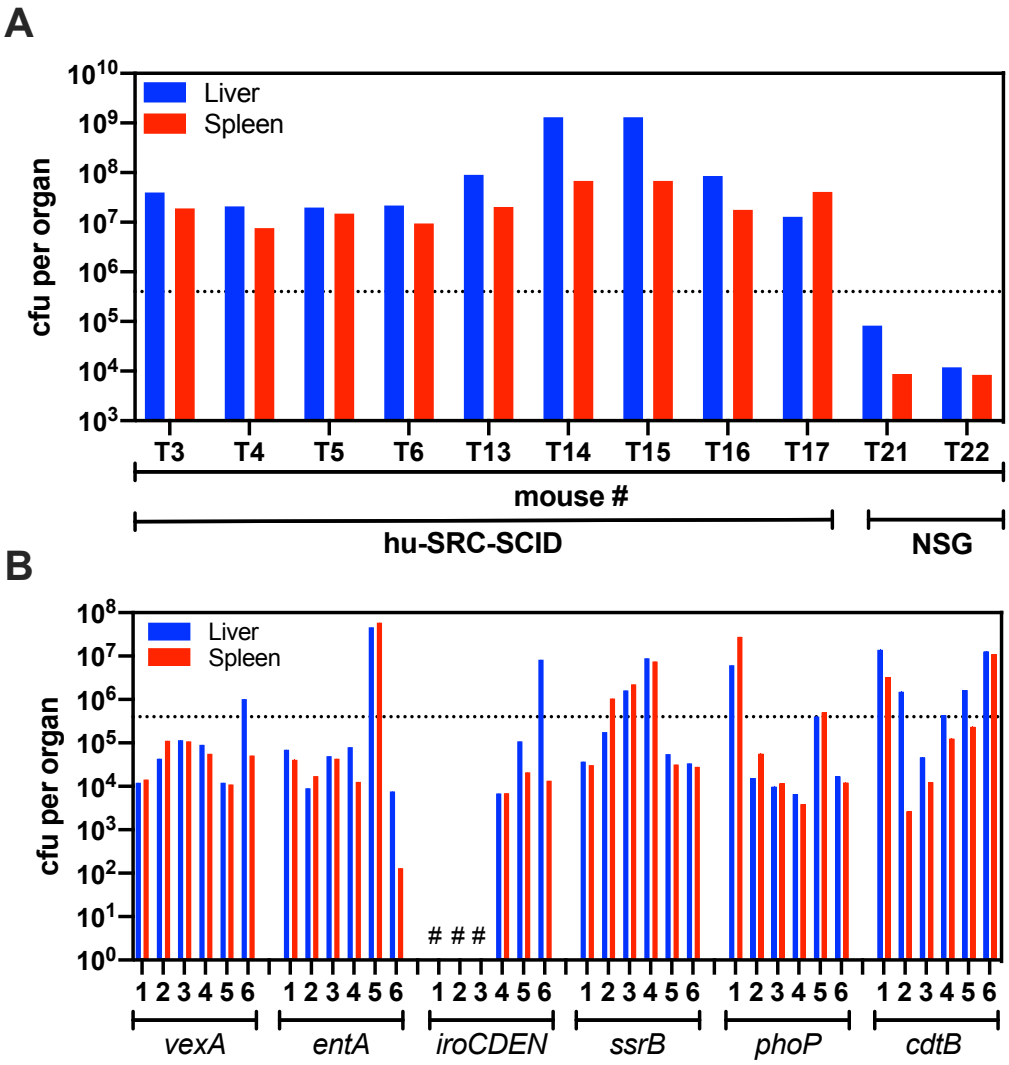

Figure S1 Related to Figure 2A. Bacterial Burdens in Liver and Spleen of hu-SRC-SCID and Non-Engrafted NOD-*scid IL2rγ*^{null} (NSG) Mice Infected with *S.* Typhi. (**A**) Hu-SRC-SCID mice (T2, T3, T5, T6, T13, T14, T15, T16 and T17) and non-engrafted NSG mice (T21 and T22) were infected i.p. with ~4 x 10^5 cfu *S.* Typhi transposon library (y-axis dotted line). Twenty-four hours p.i., the organs were harvested, homogenized and a sample plated for cfu determination in livers (blue bars) and spleens (red bars). The remaining homogenates were processed for Illumina-based TraDIS as outlined in Supplemental Figure S4, STAR Methods and the results presented in Supplemental Tables S2 and S3. (**B**) The competitive indexes (CI) of *S.* Typhi mutants *vexA*, *entA*, *iroCDEN*, *ssrB*, *phoP* and *cdtB* compared to wild-type were

determined by mixed infections in hu-SRC-SCID mice (see Figure 2A). The organism burdens in livers (blue bars) and spleens (red bars) from hu-SRC-SCID mice infected i.p. with $2x10^5$ cfu (y-axis dotted line) with an equal mixture of wild-type and mutant strain. The animals were monitored daily and sacrificed before moribund. Mixed infections of wild-type and *iroCDEN*, *ssrB*, *phoP*, *cdtB* were sacrificed at 24 h p.i. and *vexA*, *entA* at 72 h p.i. Six mice were infected in each group, the # indicates samples where no colonies were isolated in the organs for subsequent CI determination.

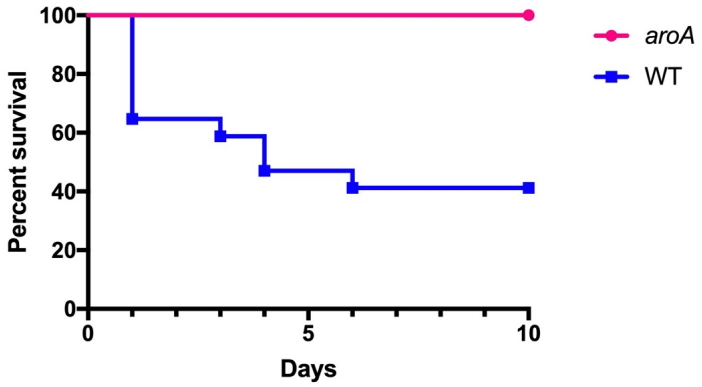

Figure S2 Related to Figure 2A. *S.* Typhi Carrying an *aroA* Mutation is Attenuated for Virulence in Humanized Mice. Humanized hu-SRC-SCID mice were challenged with i.p. inoculation of wild-type *S.* Typhi Ty2 (1-$3.5x10^5$ cfu) or an isogenic *aroA* mutant strain ($3x10^5$ to $2.5x10^6$ cfu) (n=17 per group). Differences in survival were highly significant ($p = 0.0002$ by Mantel-Cox test).

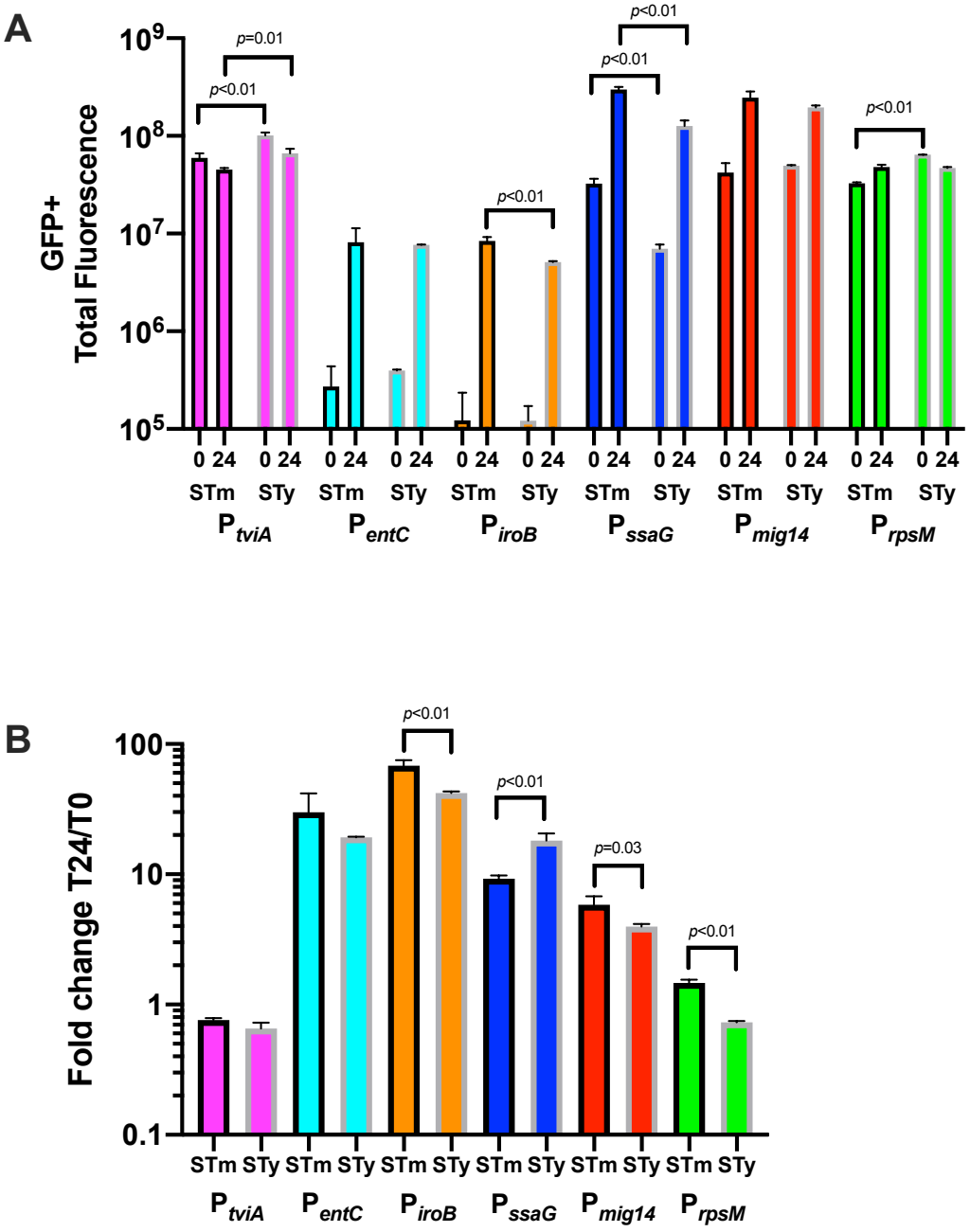

Figure S3 Related to Figure 2B and 2C. Infection of *Salmonella* Strains in Human THP-1 Macrophages. (**A**) Comparison of gene expression between *S.* Typhi (STy) and *S.* Typhimurium (STm) of representative promoters involved

in *Salmonella* pathogenesis. *Salmonella* strains with GFP fusions to representative promoters for Vi antigen (*tviA*), Iron acquisition (*entC* and *iroB*), SPI-2 (*ssaG*), PhoP regulon (*mig14*) and a constitutive promoter (*rpsM*) were assayed for GFP expression by FACS analysis. All reporter-carrying *Salmonella* strains also carried a plasmid constitutively expressing mCherry to identify viable intracellular bacteria. THP-1 macrophages infected with S*almonella* strains (STm black bordered bars, STy grey bordered bars) with GFP promoter fusions to *tviA* (magenta bars), *entC* (cyan bars), *iroB* (orange bars), *ssaG* (blue bars), *mig14* (red bars) or *rpsM* (green bars) at a MOI of ~15:1 for 24 h. The bar graph shows total fluorescence intensity of GFP+ cells at pre-infection (0) and 24 h p.i. (24) where the error bars are the means from 3 biological replicates. Statistical significance *p* between STm and STy reporter plasmids was determined by unpaired two-tailed Student t test. (**B**) Fold change T24/T0 of total fluorescence intensity of GFP+ cells from data in S3A.

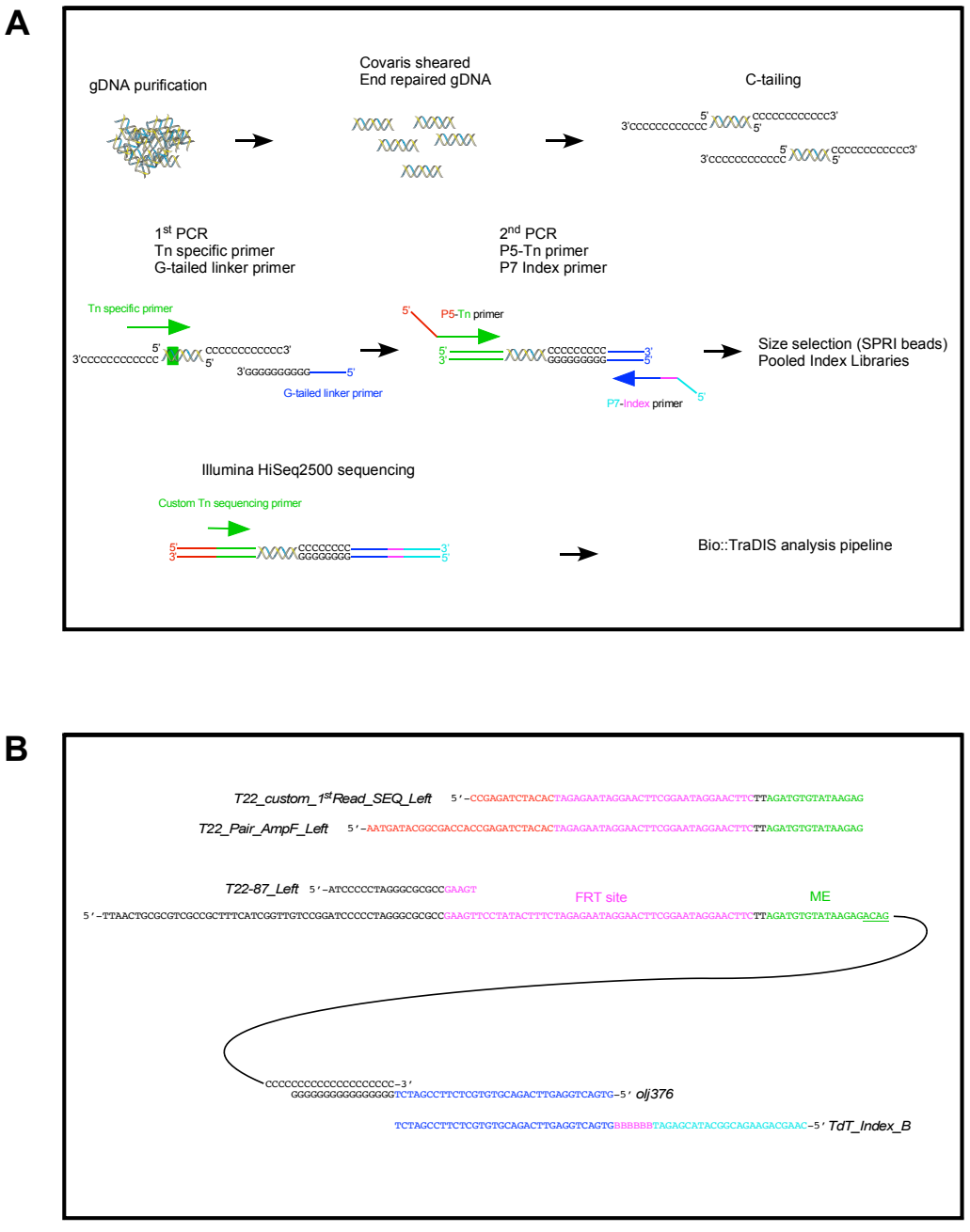

Figure S4 Related to STAR Methods. Overview of Illumina-Based Transposon-Directed Insertion Site Sequencing (TraDIS). (**A**) Construction of transposon specific libraries using the TdT method for Illumina sequencing. Genomic DNA was isolated from the "input" and "output" cultures and Covaris sheared to ~300bp. The sheared DNA ends were end-repaired, and the 3'-ends were C-tailed using terminal deoxynucleotidyl transferase (TdT). The transposon specific fragments were PCR amplified using a transposon specific primer and a G-tailed linker primer. A second

PCR reaction was performed to add the P5 and P7-indexed sequences. The libraries were size selected using SPRI beads and the indexed transposon specific libraries were pooled and sequenced using a custom transposon specific sequencing primer on an Illumina HiSeq2500 platform. The sequences were processed using Bio::TraDIS analysis pipeline (Barquist et al., 2016). (**B**) Primer design for Illumina-based transposon-directed insertion site sequencing for the left end of transposable element T22 (STAR Methods).

Table S4 related to STAR Methods. Primers Used in This Study.

Primer	Sequence 5'-3'	Purpose
TYP5	GTACTGGTTGTAGAGGATAATGCATTATTACGCCACCACCGTGTAGGCTGGAGCTGCTTC	Deletion of *phoP* in STy and STm
TYP6	TCAAAAAGATATCCTTGTCCGCGTACGGTGGTAATGACATCATATGAATATCCTCCTTAG	Deletion of *phoP* in STy and STm
TYP9	CTGACGTTACAACCCATCGCGCGGGTCGATGGCGCCATTAGTGTAGGCTGGAGCTGCTTC	Deletion of *aroA* in STy
TYP10	CGTACTCATCCGCGCCAGTTGTTCGAAATAATCAGGGAACCATATGAATATCCTCCTTAG	Deletion of *aroA* in STy
TYP45	ATCATCATATTACTAACGACATTTTTCCTGCTTTCGGGATGTGTAGGCTGGAGCTGCTTC	Deletion of *vexA* in STy
TYP46	TTAGTGCCGCGGGTCAAAAAGCTATCGAATGCGGCTTTCACATATGAATATCCTCCTTAG	Deletion of *vexA* in STy
TYP13	TATAAGATCTTATTAGTAGACGATCATGAAATCATCATTAGTGTAGGCTGGAGCTGCTTC	Deletion of *ssrB* in STy
TYP14	ATTAACCTCATTCTTCGGGCGCAGTTAAGTAACTCTGTCACATATGAATATCCTCCTTAG	Deletion of *ssrB* in STy
TYP17	TCTCTACTTAACAGTGCTCGTTTACGACCTGAATTACTGAGTGTAGGCTGGAGCTGCTTC	Deletion of *invA* in STy
TYP18	TTTATAACATTCACTGACTTGCTATCTGCTATCTCACCGACATATGAATATCCTCCTTAG	Deletion of *invA* in STy
JKP696	CGCGAGGGCAGCAAAATGAAAGAATATAAGATCTTATTAGGTGTAGGCTGGAGCTGCTTC	Deletion of *ssrB* in STm
JKP697	AGTTAAGTAACTCTGTCACTTTATGAACCTGTAGCTTTCTC	Deletion of *ssrB* in STm
TSP303	GCTTTGATTTTTCAGACAAAACGGTATGGGTGACCGGGGCGTGTAGGCTGGAGCTGCTTC	Deletion of *entA* in STy and STm
TSP304	TCAGGCTCCCAATGTTGAACCGCCGTCCACCACGATATCCCATATGAATATCCTCCTTAG	Deletion of *entA* in STy and STm
JKP899	ATGCCCGCGACTCATTCCCCCATGCCCGCTCGTGCCTGGAGTGTAGGCTGGAGCTGCTTC	Deletion of *iroCDEN* in STy and STm
JKP912	ATGAGAGTTAAGAAGTTCATCTGGTTAATAACCGTGGTTTCATATGATTATCCTCCTTAG	Deletion of *iroCDEN* in STy and STm
JKP684	TGTTTTTTTCCTTCTGACCATGATCATCTGCAGCTATATGTGTAGGCTGGAGCTGCTTC	Deletion of *cdtB* in STy
JKP685	TAATGCTTCAACCCTTTGTGAATAAGGTGCTCGATCGACACATATGAATATCCTCCTTAG	Deletion of *cdtB*

		in STy
JKP965	TGGATCCCCCGGGCTGCAGGTGGGAGTTTGGGACTACAG	Construction of pJK741
JKP966	GGCATGCAAGCTTGATATCGATAACCGTTAGCGCTGGTAAC	Construction of pJK741
JKP969	TGGATCCCCCGGGCTGCAGGAACTATTTCATACGCGGATGTG	Construction of pJK744 and pJK745
JKP970	GGCATGCAAGCTTGATATCGTATTGATACTACCGCCGTATTGC	Construction of pJK744 and pJK745
JKP971	TGGATCCCCCGGGCTGCAGGAGTCTCACAATAGCGTCCTG	Construction of pJK746 and pJK747
JKP972	GGCATGCAAGCTTGATATCGAAGCGATCGGGAGCAAGC	Construction of pJK746 and pJK747
JKP973	TGGATCCCCCGGGCTGCAGGTCCACGGGCGTCTGGTATG	Construction of pJK748 and pJK749
JKP974	GGCATGCAAGCTTGATATCGGACAGCACAGGGTATAGCAGTC	Construction of pJK748 and pJK749
JKP975	TGGATCCCCCGGGCTGCAGGCCAGTATGACGTTCTGACG	Construction of pJK750
JKP976	GGCATGCAAGCTTGATATCGTAATGCCAGCAGCTCCAAC	Construction of pJK750
JKP979	GGCCGCTCTAGAACTAGTGTTCGAGCTCGGTACCCGG	Construction of pJK753
JKP980	GAATTCCTGCAGCCCGGGGTTACTTGTACAGCTCGTCCATGC	Construction of pJK753
JKP981	TGGATCCCCCGGGCTGCAGGTTCGAGCTCGGTACCCGG	Construction of pJK754
JKP982	GGCATGCAAGCTTGATATCGGATCTTAACATTTTCAGCGATACCCG	Construction of pJK754
T22—87_Left	ATCCCCCTAGGGCGCGCCGAAGT	TraDIS
olj376	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGGGGGGGGGGGGG	TraDIS
T22_PAIR_AmpF_LEFT	AATGATACGGCGACCACCGAGATCTACACTAGAGAATAGGAACTTCGGAATAGGAACTTCTTAGATGTGTATAAGAG	TraDIS
TdT_Index_01_ATCACG	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_02_CGATGT	CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_03_TTAGGC	CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_04_TGACCA	CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_05_ACAGTG	CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_06_GCCAAT	CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_07_CAGATC	CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_08_ACTTGA	CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_09_GATCAG	CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_10_TAGCTT	CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_11_GGCTAC	CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_12_CTTGTA	CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_13_AGTCAA	CAAGCAGAAGACGGCATACGAGATTTGACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_14_AGTTCC	CAAGCAGAAGACGGCATACGAGATGGAACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_15_ATGTCA	CAAGCAGAAGACGGCATACGAGATTGACATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS

TdT_Index_16_CCGTCC	CAAGCAGAAGACGGCATACGAGATGGACGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_18_GTCCGC	CAAGCAGAAGACGGCATACGAGATGCGGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_19_GTGAAA	CAAGCAGAAGACGGCATACGAGATTTTCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_20_GTGGCC	CAAGCAGAAGACGGCATACGAGATGGCCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_21_GTTTCG	CAAGCAGAAGACGGCATACGAGATCGAAACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_22_CGTACG	CAAGCAGAAGACGGCATACGAGATCGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_23_GAGTGG	CAAGCAGAAGACGGCATACGAGATCCACTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_25_ACTGAT	CAAGCAGAAGACGGCATACGAGATATCAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
TdT_Index_27_ATTCCT	CAAGCAGAAGACGGCATACGAGATAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	TraDIS
T22_custom_1stRead_SEQ_Left	CCGAGATCTACACTAGAGAATAGGAACTTCGGAATAGGAACTTCTTAGATGTGTATAAGAG	TraDIS