Supplementary Information

Substrate specificities of Escherichia coli ItaT that acetylates aminoacyl-tRNAs

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Figure S1

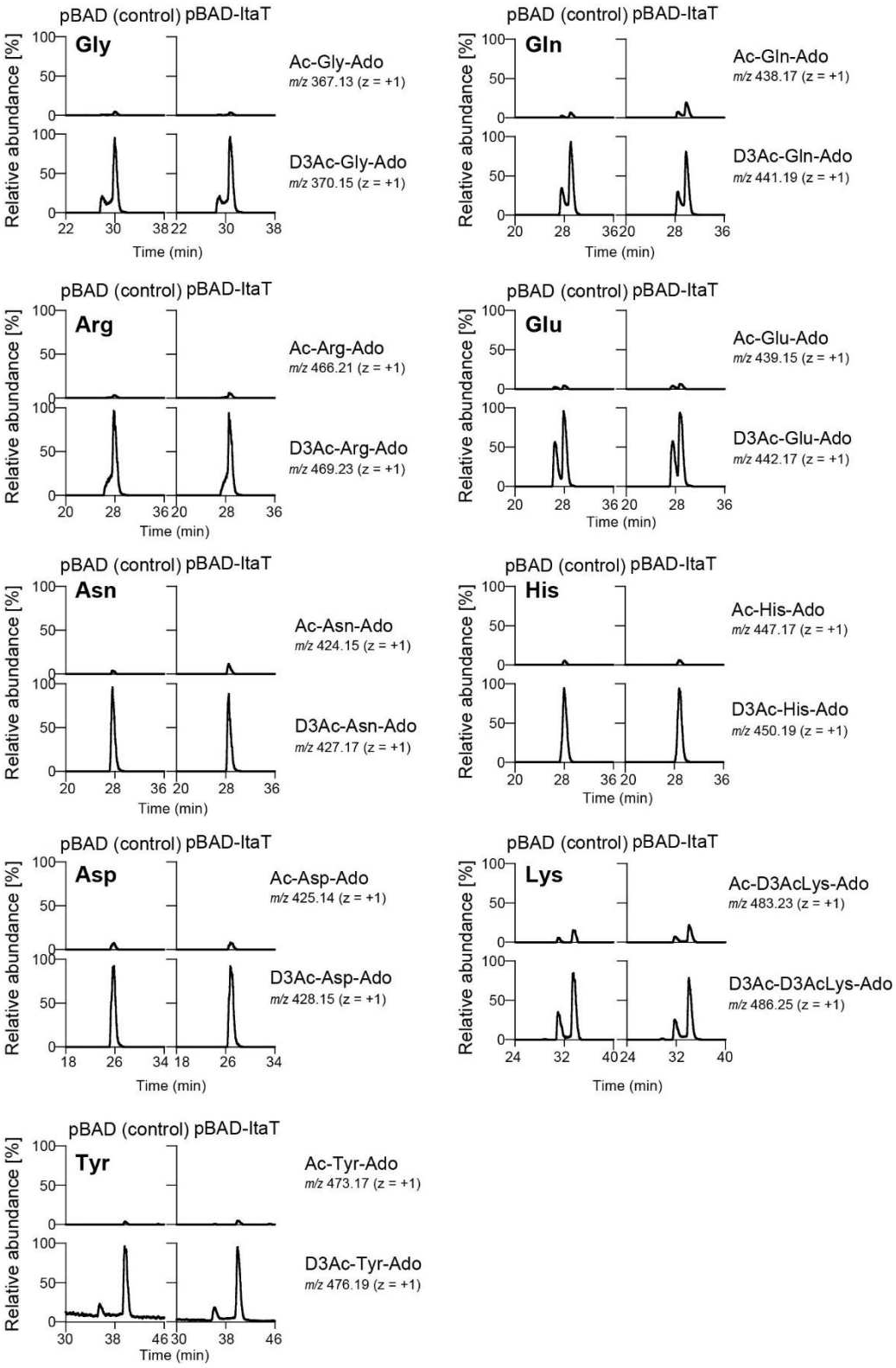

Figure S1: LC/MS analyses of RNase I-digests of RNA prepared from *E. coli* with ItaT induction. Asterisks indicate nonspecific peaks with the same m/z value.

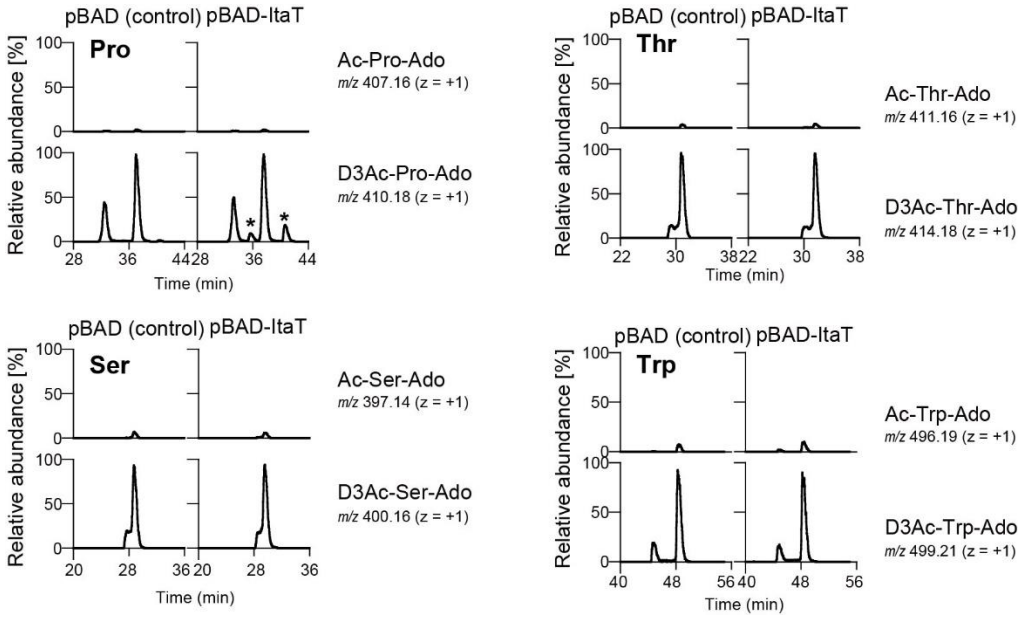

Figure S1: Continued.

Figure S2

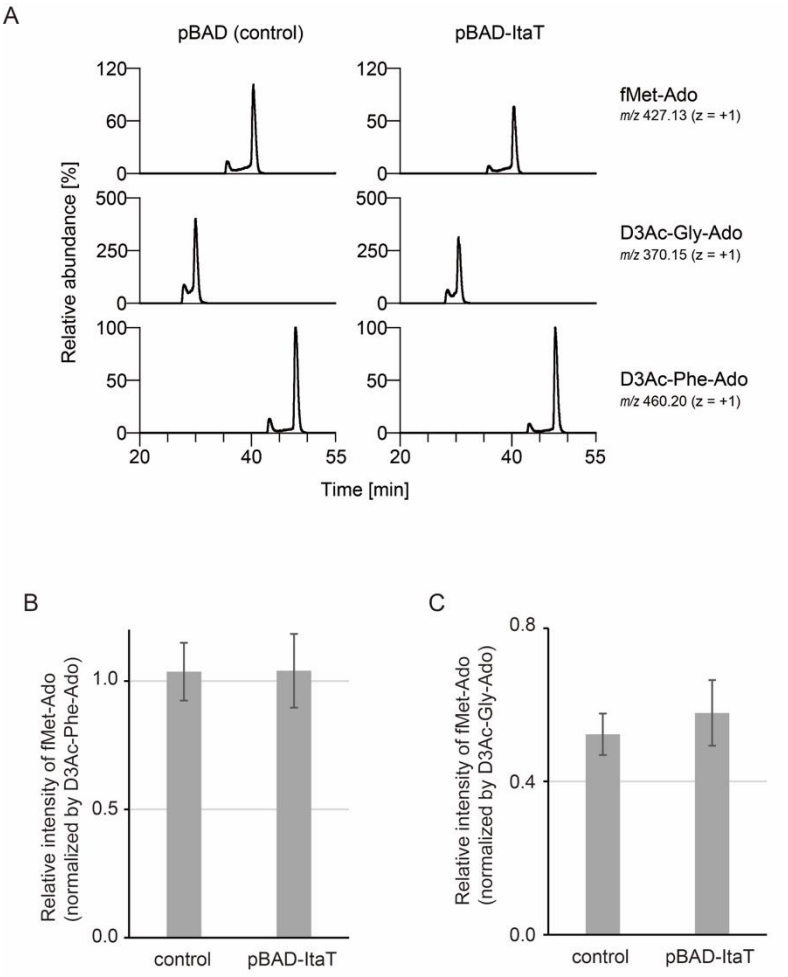

Figure S2: Identification of *N*-formyl-Met-tRNAf^{Met}.

(**A**) Identification of the molecular mass corresponding to f-Met-A76 (N-formyl methionyl-adenosine, m/z = 427.13) in RNA preparations from *E. coli* with or without ItaT induction. The intensity of f-Met-A76 or D_3Ac-Gly-A76 (control) in each sample is expressed relative to the intensity of D_3Ac-Phe-A76. (**B**) (**C**) Quantification of the relative intensities of f-Met-A76. Intensity of f-Met-A76 in RNA preparations from *E. coli* with or without ItaT induction was normalized by the intensity of D_3Ac-Phe-A76 (**B**) or D_3Ac-Gly-A76 (**C**). The bars in the graphs are SD of duplicate for control or triplicate for ItaT from independent experiments. The amounts of f-Met-tRNAf^{Met} in the cells are not significantly altered by the ItaT induction *in vivo*.

Figure S3

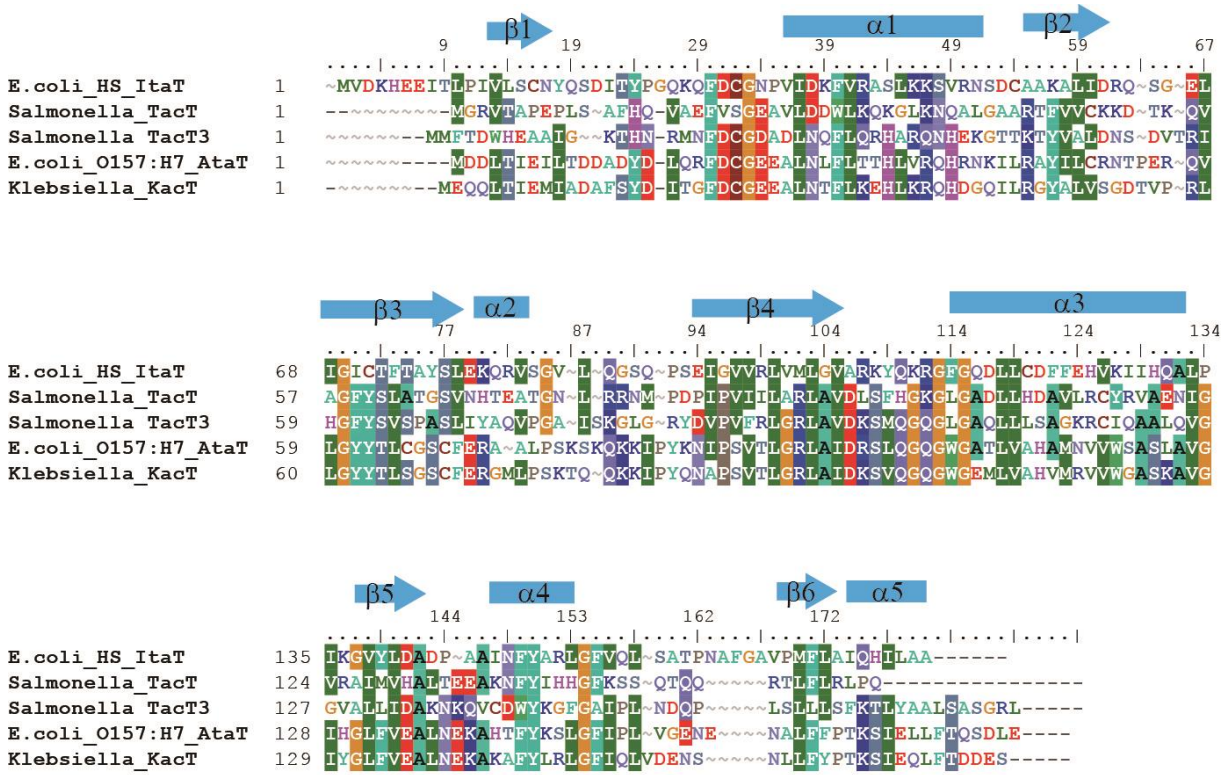

Figure S3: Sequence alignment of type II GNAT toxins targeting aminoacyl-tRNAs.
Amino acid sequences of *S. enterica* Typhimurium TacT, *E. coli* AtaT, *K. pneumoniae* KacT and *E. coli* ItaT are aligned. The secondary structure elements (α1-α5 and β1-β6) of ItaT are depicted above the alignment.

Figure S4

A

tRNA	Acc-stem		D-stem	D-loop	D-stem		Ac-stem	Ac-loop	Ac-stem	V-region	T-stem	T-loop	T-stem	Acc-stem		
	1	8	10	14	22	26	27	32	39	44	49	53	61	66	73	74
Ile	GGCCCCT	TA	GCTC	AGT--GGTT-A	GAGC	A	GGCGA	CTCATAA	TCGCT	TG----------GTC	GCTGG	TTCAAGT	CCAGC	AGGGGCC	A	CCA
Ile	AGGCTTG	TA	GCTC	AGGT-GGTT-A	GAGC	G	CACCC	CTGATAA	GGGTG	AG----------GTC	GGTGG	TTCAAGT	CCACT	CAGGCCT	A	CCA
Metf	CGCGGGG	TG	GAGC	AGCCTGGT--A	GCTC	G	TCGGG	CTCATAA	CCCGA	AG----------GTC	GTCGG	TTCAAAT	CCGGC	CCCCGCA	A	CCA
Metm	GGCCCCT	TA	GCTC	AGT--GGTT-A	GAGC	A	GGCGA	CTCATAA	TCGCT	TG----------GTC	GCTGG	TTCAAGT	CCAGC	AGGGGCC	A	CCA
Val	GCGTTCA	TA	GCTC	AGTT-GGTT-A	GAGC	A	CCACC	TTGACAT	GGTGG	GG----------GTC	GTTGG	TTCGAGT	CCAAT	TGAACGC	A	CCA
Val	GGGTGAT	TA	GCTC	AGCT-GGG--A	GAGC	A	CCTCC	CTTACAA	GGAGG	GG----------GTC	GGCGG	TTCGATC	CCGTC	ATCACCC	A	CCA

B

tRNA	Acc-stem		D-stem	D-loop	D-stem		Ac-stem	Ac-loop	Ac-stem	V-region	T-stem	T-loop	T-stem	Acc-stem		
	1	8	10	14	22	26	27	32	39	44	49	53	61	66	73	74
Leu	GCCGAAG	TG	GCGA	AATC-GGTA-G	ACGC	A	GTTGA	TTCAAAA	TCAAC	CGTA-GAAA-TACGT	GCCGG	TTCGAGT	CCGGC	CTTCGGC	A	CCA
Leu	GCGAAGG	TG	GCGG	AATT-GGTA-G	ACGC	G	CTAGC	TTCAGGT	GTTAG	TGTTCTTACGGACGT	GGGGG	TTCAAGT	CCCCC	CCCTCGC	A	CCA
Leu	GCGAAGG	TG	GCGG	AATT-GGTA-G	ACGC	G	CTAGC	TTCAGGT	GTTAG	TGTCCTTACGGACGT	GGGGG	TTCAAGT	CCCCC	CCCTCGC	A	CCA
Leu	GCCGAGG	TG	GTGG	AATT-GGTA-G	ACAC	G	CTACC	TTGAGGT	GGTAG	TGCCCAATAGGGCTT	ACGGG	TTCAAGT	CCCGT	CCTCGGT	A	CCA
Leu	GCCCGGA	TG	GTGG	AATC-GGTA-G	ACAC	A	AGGGA	TTTAAAA	TCCCT	CGGCGTTCGCGCTGT	GCGGG	TTCAAGT	CCCGC	TCCGGGT	A	CCA
Leu	GCGGGAG	TG	GCGA	AATT-GGTA-G	ACGC	A	CCAGA	TTTAGGT	TCTGG	CGCC-GCAA-GGTGT	GCGAG	TTCAAGT	CTCGC	CTCCCGC	A	CCA
Phe	GCCCGGA	TA	GCTC	AGTC-GGT--A	GAGC	A	GGGGA	TTGAAAA	TCCCC	GT----------GTC	CTTGG	TTCGATT	CCGAG	TCCGGGC	A	CCA
Ala	GGGGCTA	TA	GCTC	AGCT-GGG--A	GAGC	G	CTTGC	ATGGCAT	GCAAG	AG----------GTC	AGCGG	TTCGATC	CCGCT	TAGCTCC	A	CCA
Ala	GGGGCTA	TA	GCTC	AGCT-GGG--A	GAGC	G	CCTGC	TTTGCAC	GCAGG	AG----------GTC	TGCGG	TTCGATC	CCGCA	TAGCTCC	A	CCA

Figure S4: Sequence alignment of tRNA isoacceptor genes.
(**A**) $tRNA^{Ile}$, $tRNA^{Val}$ and $tRNA^{Met}$. Metf and Metm are initiator $tRNAf^{Met}$ and elongator $tRNAm^{Met}$, respectively. (**B**) $tRNA^{Leu}$, $tRNA^{Phe}$ and $tRNA^{Ala}$.

Table S1: Sequences of synthetic genes

ItaR-ItaT gene: catATGCCTGGAAAAACAGCCACTCTACCCGATGTCGATAAAACGCTGAAAAATGCGC GGGTCGAACTCAAAACTAGTCCCGACGCCAAAAACAAACTTCGTGAAGCTGCGCAAG CCGTTGGGGTAGATTTAAGCGCTTTTATTTTGAGCGCTGCTATGGAACGCGCCGAAAG TGTGCTTGATAACCAACGTCGCCGTGAGCTTTCGAATCAAAGCTGGGAACTGATGAAC CAACTCATCGCTGAACCTGCTCAACCGACGCTCGCCCTCAAGGCGTTAATGAAAAGG AAAAACAGCGATGGTCGACAAGCATGAAGAGATTACTCTGCCCATAGTCCTCTCCTGT AATTATCAGTCTGATATTACTTATCCTGGGCAAAAACAGTTTGATTGCGGTAACCCTG TTATCGATAAATTTGTACGCGCATCGCTAAAGAAAAGTGTGCGTAATAGCGACTGTGC GGCTAAAGCACTTATTGACAGACAAAGTGGTGAACTGATCGGCATCTGTACTTTTACG GCATATTCGCTGGAAAAACAACGCGTTTCTGGCGTCCTTCAGGGTTCACAACCTTCAG AAATTGGTGTTGTCAGATTAGTCATGTTGGGGGTAGCACGGAAGTATCAAAAGCGGG GCTTTGGTCAGGACCTACTATGTGATTTTTTTGAACATGTAAAAATAATTCACCAGGC ATTACCAATTAAAGGGGTTTATCTTGATGCTGACCCTGCCGCCATTAATTTTTATGCTC GTCTCGGCTTTGTTCAGCTTTCAGCGACACCAAATGCTTTTGGTGCTGTACCTATGTTT TTGGCGATTCAGCATATTCTCGCGGCTctcgag
tRNA ^{Phe} gene: GCCCGGATAGCTCAGTCGGTAGAGCAGGGGATTGAAAATCCCCGTGTCCTTGGTTCGA TTCCGAGTCCGGGCACCA
tRNA^{Ala} gene: GGGGCTATAGCTCAGCTGGGAGAGCGCTTGCATGGCATGCAAGAGGtCAGCGGTTCGA TCCCGCTTAGCTCCACCA
tRNA^{Leu} gene: GCCCGGATGGTGGAATCGGTAGACACAAGGGATTTAAAATCCCTCGGCGTTCGCGCT GTGCGGGTTCAAGTCCCGCTCCGGGTACCA

Table S2: Sequences of oligonucleotides

isoS_fw for IRS	AGCTAGCTCATATGAGTGACTATAAATCAACCC
isoS_rv for IRS	AGCTCTCGAGGGCAAACTTACGTTTTTCACC
ItaT_fw_NdeI for pET/pBAD	ATACATATGGTCGACAAGCATGAAGAGATTAC
ItaT_rv_XhoI for pET	CCGCTCGAGAGCCGCGAGAATATGCTG
ItaT_rv_HindIII for pBAD	CCCAAGCTTTCAAGCCGCGAGAATATGCTG
ItaR_fw_NdeI for pET	AGCTGACATATGCCTGGAAAAACAGC
ItaT_R115D _fw	GATCAGGACCTACTATGTGATTTTTTTGAAC
ItaT_R115D _rv	AAAGCCCCGCTTTTGATACTTCC
ItaT_N34A_FW	GCG CCTGTTATCGATAAATTTGTACGCGC
ItaT_N34A_RV	ACCGCAATCAAACTGTTTTTGCCC
ItaT_V36A/I37A_FW	GCGGCG GATAAATTTGTACGCGCATCGCTAAAG
ItaT_V36G/I37G_FW	GGCGGC GATAAATTTGTACGCGCATCGCTAAAG
ItaT_V36D/I37D_FW	GATGAT GATAAATTTGTACGCGCATCGCTAAAG
ItaT_V36D/I37D _RV	AGGGTTACCGCAATCAAACTGTTTTTG
ItaT_K39A_FW	GCG TTTGTACGCGCATCGCTAAAGAAAAGTGTG
ItaT_K39A/F40A_FW	GCGGCG GTACGCGCATCGCTAAAGAAAAGTGTG
ItaT_K39A/F40A_RV	ATCGATAACAGGGTTACCGCAATC
ItaT_R42A_FW	GCG GCATCGCTAAAGAAAAGTGTGCG
ItaT_R42A_RV	TACAAATTTATCGATAACAGGGTTACC
ItaT_K46A/K47A_FW	GCGGCTAGTGTGCGTAATAGCGACTGTGCGGC
ItaT_K46A/K47A_RV	TAGCGATGCGCGTACAAATTTATCG
ItaT_R50A_FW	GCG AATAGCGACTGTGCGGCTAAAGCAC
ItaT_R50A_RV	CACACTTTTCTTTGACGATGCGCG
ItaT_K80A_FW	GCG CAACGCGTTTCTGGCGTCCTTCAGGG
ItaT_K80A_RV	AAGATAAACCCCTTTAATTGGTAATG
ItaT_R82A_FW	GCG GTTTCTGGCGTCCTTCAGGGTTCAC
ItaT_R82A_RV	TTGTTTTTCCAGCGAATATGCCG
ItaT_M102A_FW	GCG TTGGGGGTAGCACGGAAGTATCAAAAG
ItaT_M102R_FW	CGC TTGGGGGTAGCACGGAAGTATCAAAAG
ItaT_M102A_RV	GACTAATCTGACAACACCAATTTCTG
ItaT_Y150F_FW	TTT GCTCGTCTCGGCTTTGTTCAGCTTTC
ItaT_Y150F_RV	AAAATTAATGGCGGCAGGGTCAGC
ItaT_F40A_FW	GCG GTACGCGCATCGCTAAAGAAAAGTGTG
ItaT_F40A_RV	TTTATCGATAACAGGGTTACCGCAATC
ItaT_L133E_FW	GAACCAATTAAAGGGGTTTATCTTGATGCTGA
ItaT_L133E_RV	TGCCTGGTGAATTATTTTTACATGTTC