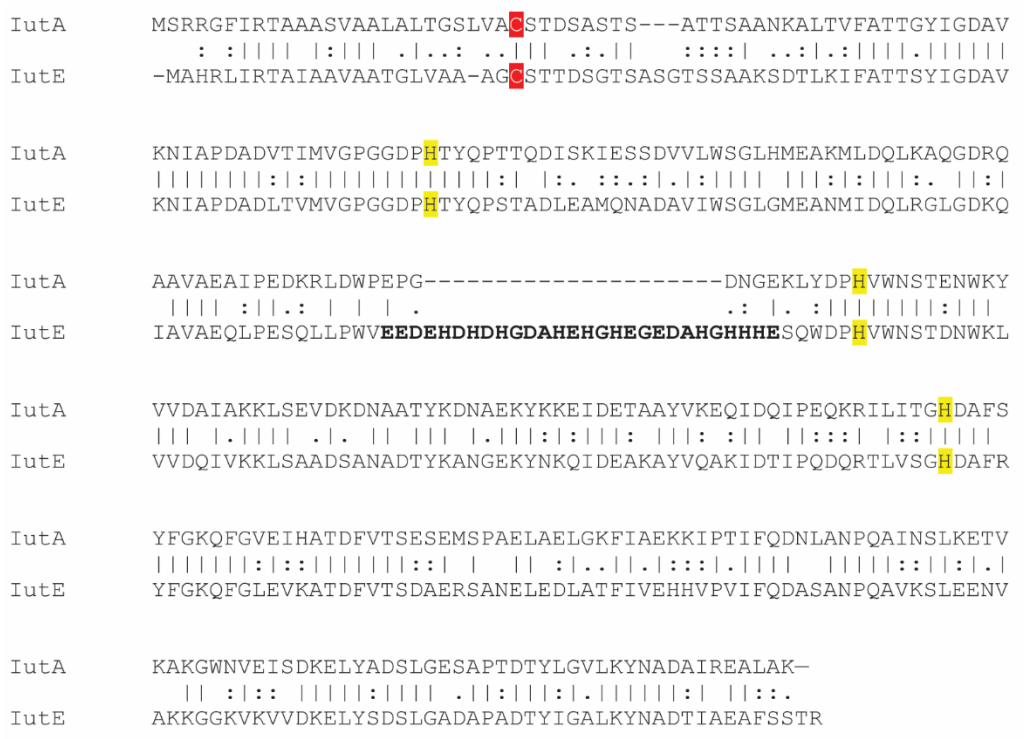

Figure S1 Alignment of IutA and IutE. Amino acid sequence alignment of IutA and IutE using ClustalO (1). The indicated Cys residue (red) is the predicted lipoprotein anchor and indicated His residues (yellow) are associated with metal ion coordination in crystalized homologues. Residues in **bold** indicate a region rich in acidic and His residues (His-rich region) that is associated with Zn-binding proteins of the cluster 9 family.

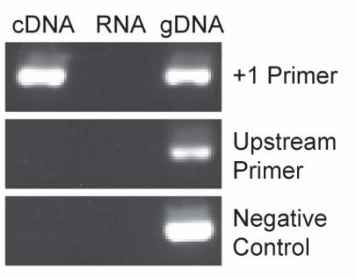

Figure S2 PCR validation of *iutE* RACE. Primers annealing at the +1 site (+1 Primer), -20 site (Upstream Primer), and further upstream (Negative Control) were paired with a common reverse primer to PCR amplify targets from total cDNA, total RNA, and genomic DNA (gDNA).

Table S1. Primers used in this study for cloning.

Plasmid	Primer sequence [a]	RE	Vector
pKΔiut	GTATTGGGTGTGCATGCGTCCGC [b]	SphI	pK18mobsacB
	TTACTTAGCCAAAGCCTCGCGTCTAGACATGCGAGAAGTTTCCTTAG [b]	XbaI	
	GTGGCGGTCATGGCACATCTAGAGGAGGCTTTCTCCTCGACCC [c]	XbaI	
	GCGCCAATGGGAATTCCGCGTCAGC [c]	EcoRI	
pKΔripA.	GATCGGATCCCATAGCCCAATCGGCAGAG [d]	BamHI	pK19mobsacB
	AAGACTCACGAGCCGAAACC [d]		
	GGTTTCGGCTCGTGAGTCTTTTGGTTCCTTCTGTGTAAGTTGAT [e]		
	GATCGTCGACCGGAGTACAGACCTAGGTTG [e]	SalI	
pIutAHis.	CCGCTACCACCATGGCAGCTAACAAAGC	NcoI	pET30a
	CAGATGTGCGACAGGATCCGCGCTAC	BamHI	
pIutEHis.	CTTCGAGCGCCGTCATGAGCGACACTC	BspHI	pET30a
	CTCTTCGTGAGCGGCCGCAGCCCACC	NotI	
pSPZ-iutA	TGAAGCCGGATCCTGACATGCG	BamHI	pSPZ
	TATCTGAAGTCGACAGCGGCAT	SalI	
pSPZ-iutE(FL)	GATCGGATCCACAAGACCTGTGGCAGCCA	BamHI	pSPZ
	GCTCAGATCTGCTGTTGCGTTGATGATTGTGC [f]		
pSPZ-iutE	CGGCTGGATCCACAAGACCTGTG	BamHI	pSPZ
	AGACCTGTGGGATCCACAGCTGC	SalI	
pSPZ-iutE5'	CGTCGGATCCTCATGTGCACTAGGAAGCGC	BamHI	pSPZ
	GCTCAGATCTGCTGTTGCGTTGATGATTGTGC [f]		
pET30zur	GGCCATGGCCATGAATCGCACCATTG	NcoI	pET30a
	GCGTTTGGATCCGCGCTCGGAT	BamHI	
pET30ripA	GCCCAGATCTAAGTCTTCCATCAATTAAGACGACC	BglII	pET30a
	ATTCGGATCCTTACACAGAAGGAACCAACACC	BamHI	

[a] Primer sequences are oriented 5' to 3'. Restriction enzyme (RE) sites used are underlined.

[b-e] Indicated primers were paired for PCR amplification.

[f] No restriction site is indicated. A native SalI site in the *Corynebacterium diphtheriae* genomic sequence was used for cloning.

Table S2. Primers used in this study for qPCR.

Primer	Gene	Primer sequence (5'-3')	Amplicon size (bp)
RTiutA1	*iutA*	ACGAGACTGCTGCGTATGTG	222
RTiutA2		TGTCCTGGAAAATCGTAGGG	
RTiutD1	*iutD*	TCGTCGATTTCTGAGCACAC	182
RTiutD2		ACACCATCGTCATCACCAGA	
RTiutE1.1	*iutE*	ACCACGTCCCAGTGATCTTC	171
RTiutE2.1		ACTTCAGGGCACCGATGTAG	
RTgyrB1	*dip0005*	GGTCTGACCATTACGCTGGT	166
RTgyrB2		TCTTCTCGCGTTTCTTTGGT	
RTDIP0168_1	*dip0168*	AAAGTTGAGTCCGGAGCGTA	179
RTDIP0168_2		AACTCATCGACCCCAACAAC	
RTripA1	*dip0922*	GGAAGGTTGCTCAGAAGCTG	174
RTripA2		ATAAATCAGCCGCCACAGAC	
RTDIP1283_1	*acn*	TCCTACTCCAGAGGGCAAGA	190
RTDIP1283_2		ACCAGGTTTGATGGACGAAG	

Table S3. Primers for 5'RACE and PCR validation.

Target Gene	Purpose	Primer Sequence (5'-3')	Notes
RACE			
iutA	GSP1	CATTATCGCCTGGCTCTGG	
	GSP2	CATCTTGGCCTCCATATGCAG	
iutE	GSP1	GGAGAGCTTCTTAACGATCTG	
	GSP2	AACCCATGGCAACAGCTGG	
PCR validation			
iutE	+1	ATGGCACATCGACTAATCCGTA	[a]
	Upstream	GTTGCCTATAGTGGCGGTC	[a]
	Negative control	GGCTGCGGTGTTAAAAACTGC	[a]
	Reverse primer	GAGGATCCCACTGCGACTC	

[a] Indicated forward primers were paired with the reverse primer for PCR.

Table S4. Primers used in this study for EMSA.

Region name	Amplicon size [a]	Primer sequence (5'-3') [b]	Notes
p*tox*	232	CCCCCCCTCATTGAGGAGTAGGTCCC	
		CCCCCCCATGGGCTGAAGGTGGGG	
p*iutA*	187	CCCCCCAGCGGCATGGTTCTTATTGTG	
		CCCCCCTGACATGCGAGAAGTTTCCTTAG	
p*iutE*-FL	206	CCCCCCGTCGACGGCCTCATCTTTC	
		CCCCCCACAAGACCTGTGGCAGCCA	[c]
p*iutE*	153	CCCCCCGGCTGCGGTGTTAAAAAC	[d]
		CCCCCCACAAGACCTGTGGCAGCCA	[c]
p*iutE* A	94	CCCCCCGGCTGCGGTGTTAAAAAC	[d]
		CCCCCCGCCACTATAGGCAACGATTGT	
p*iutE* B	91	CCCCCCAGTGCACATGACAATCGTTGC	
		CCCCCCACAAGACCTGTGGCAGCCA	[c]
p*iutE* C	63	CCCCCCGGCTGCGGTGTTAAAAAC	[d]
		CCCCCCAGGAAGCGCACACTTAGATAC	
p*acn*	400	CCCCCCCTTGGCCAATCACCATTCCATA	
		CCCCCCAGTGAGCTCCACTTCGTTTTG	

[a] Sizes include what is amplified from genomic template, a string of six C residues are added to the 5' end of each primer to assist in stability of the double-stranded product.

[b] All primers are biotinylated on the 5' end.

[c] Primers used to amplify these constructs are shared among indicated constructs.

[d] Primers used to amplify these constructs are shared among indicated constructs.

Supplemental References

1. **Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG.** 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol **7:**539.