

Supporting information

**Effects on IS1 transposition frequency of a mutation in the *ygjD* gene
involved in an essential tRNA Modification in *Escherichia coli***

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Figure legends

Fig. S1. Cloning of the junction regions of the insertion / deletion in the *yjgD_{sup}* mutant genome. (a) The original *yjgD_{sup}* mutant has disruptions of the *recA* ($\Delta recA:Tc$) and the *yjgD* genes ($\Delta yjgD:Sm$) and the plasmid (mini-F-*yjgD^{ts1}*(Ap)). (b) To obtain the strains, in which the plasmids pSG76A2-A or -B were integrated into the *yjgD_{sup}* chromosome by homologous recombination, two plasmids, pACYC184-*yjgD⁺* and mini-F-*recA⁺* (Km), were introduced into the *yjgD_{sup}* mutant. (c) Then the plasmids pSG76A2-A or -B and a helper plasmid, pPIR-K, were introduced into the resultant strain. (d) And the strains with the integrated plasmids were obtained at 42°C. The chromosomal DNA was prepared, digested with the restriction enzymes, ligated, and introduced into DH1 together with pPIR-K. The plasmids carrying the junction regions were obtained and sequenced. The arrows on the cloned plasmids indicate the primers for sequencing.

Fig. S2. The PFGE patterns predicted from the genome sequences. Because Tn10 has an *XbaI* site, a 206,624 base pair fragment was split to ca. 171 KB and ca. 45 kb

bands. In the presence of Dam methylase, two *XbaI* sites (3,861,916 and 4,308,586) are not digested by *XbaI*.

Fig. S3. Growth of the *ygjD*_{sup} mutant with the *dinG*, *rep*, and *rnhA* multi-copy plasmids. The *ygjD*_{sup} mutant and the isogenic *ygjD*⁺ strain were transformed with pACYC184 and its derivatives carrying the *dinG*, *rep*, and *rnhA* genes. The transformants were incubated on Antibiotic Medium 3 containing Cm and streptomycin at 30, 37, or 42°C.

Fig. S4. Junction sequences of the insertion / deletion in the *ygjD*_{sup} mutant genome. Sequences of the three junctions are shown: *insB* - *yjgJ* (4,517,262 - 4,472,838); *yjhG* - *insA* (4,520,435 - *insA*); *insB* - *ubiD* (*insB* - 4,023,119). The putative junctions are indicated by arrows.

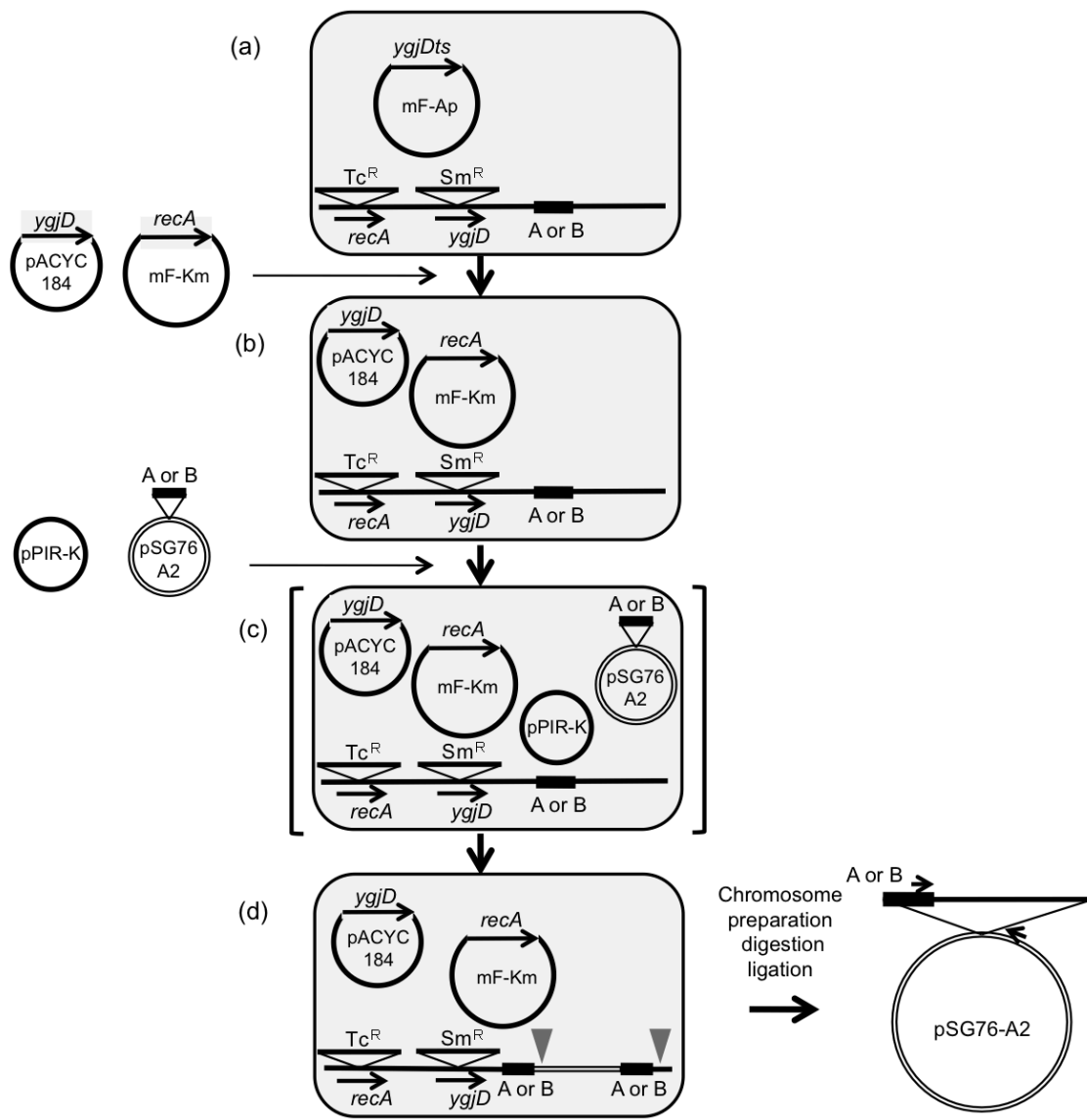


Fig. S1. Hashimoto *et al.*

(1) <i>NotI</i>		(2) <i>XbaI</i> (-Dam methylation)		(3) <i>XbaI</i> (+Dam methylation)	
< MG1655 >	< <i>ygjD_{sup}</i> >	< MG1655 >	< <i>ygjD_{sup}</i> >	< MG1655 >	< <i>ygjD_{sup}</i> >
1003721 base	1003721 base	440059 base	440059 base	440059 base	440059 base
	377505 base	335008 base	335008 base	335008 base	335008 base
361369 base	361369 base	323965 base	323965 base	323965 base	323965 base
358533 base		269545 base	269545 base	297926 base	297926 base
281227 base	281227 base	262133 base	262133 base		276555 base
273618 base	273618 base	254644 base	254644 base	269545 base	269545 base
261674 base	261674 base	248418 base	248418 base	262133 base	262133 base
250536 base	250536 base	224937 base	224937 base	254644 base	254644 base
250163 base	250163 base	214863 base	214863 base	248418 base	248418 base
248520 base	248520 base	206624 base	206624 base	224937 base	224937 base
	248520 base	197148 base	197148 base	214863 base	214863 base
214239 base	214239 base		195472 base	206624 base	(206624 base)
207784 base	207784 base		175777 base		195472 base
193356 base	193356 base	155297 base	155297 base	155297 base	155297 base
	174719 base	149210 base	149210 base	149210 base	149210 base
156067 base	156067 base	117291 base	117291 base	137243 base	137243 base
132769 base	132769 base	106065 base	106065 base	117291 base	117291 base
108684 base	108684 base	105763 base	105763 base	106065 base	106065 base
98648 base	98648 base	105747 base	105747 base	105763 base	105763 base
92214 base	92214 base	100778 base	100778 base	105747 base	105747 base
40597 base	40597 base		100778 base	94058 base	94058 base
36074 base	36074 base	94058 base	94058 base	86685 base	86685 base
35142 base	35142 base	86685 base	86685 base	72559 base	72559 base
15443 base	15443 base	79553 base	79553 base	66883 base	66883 base
14962 base	14962 base	72559 base	72559 base	48824 base	48824 base
	4335 base	66883 base		48417 base	48417 base
4335 base	4335 base	57690 base	57690 base	41402 base	41402 base
		48824 base	48824 base		41402 base
		48417 base	48417 base	39700 base	39700 base
		41402 base	41402 base	33538 base	33538 base
				28987 base	28987 base
		39700 base	39700 base	28538 base	28538 base
		33538 base	33538 base	26503 base	26503 base
		28987 base	28987 base	21775 base	21775 base
		28538 base	28538 base	14081 base	14081 base
		26503 base	26503 base	12523 base	12523 base
		21775 base	21775 base	12223 base	12223 base
		14081 base	14081 base	6055 base	6055 base
		12523 base	12523 base	2067 base	2067 base
		12223 base	12223 base	119 base	119 base
		6055 base	6055 base		
		2067 base	2067 base		
		119 base	119 base		

Fig. S2. Hashimoto *et al.*

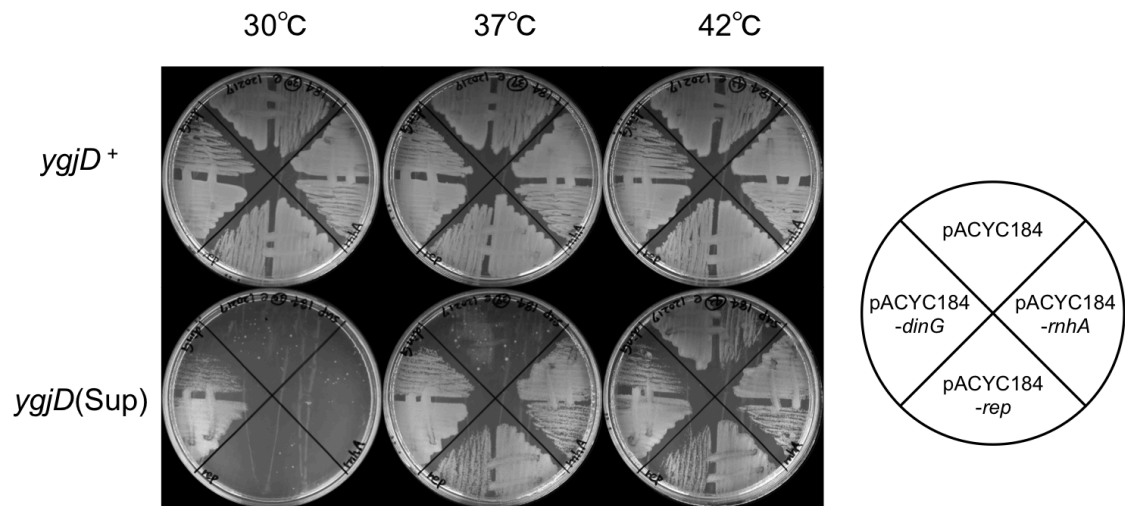


Fig. S3 Hashimoto *et al.*

insB-yjgJ

insB CACTATCAATAAGTTGGAGTCATTACCGACCATGTTTATTTTCATACATT
genome CACTATCAGTAAGTTGGAGTCATTACCCGAAAAGATAATCAATTCCGCT
yjgJ AAACAACGTTTCATTTCTATCGTTATACGAAAAGATAATCAATTCCGCT
↑

yjhG-insA

yjhG CCGGAGGCCCCATCGGTAAATTACGCACCGGGGATTTAATTGAAATTAA
genome CCGGAGGCCCCATCGGTAAATTAGGTGATGCTGCCAACTTACTGATTTA
insA CTGCCCTAAAGGATGGGGATTTTCGGTAATGCTGCCAACTTACTGATTTA
↑

ubiD-insB

ubiD CGGCACGCAAAGTGCGGTCAGCAATTTTCAGTGATTTCCAGATGCGGATC
genome CGGCACGCAAAGTGCGGTCAGCAATTTTCGGTAATGACTCCAACCTATTG
insB ACCCACAATGTATGAAATAAACATGGTTCGGTAATGACTCCAACCTATTG
↑↑↑

Fig. S4. Hashimoto *et al.*

Table S1 Primer sequences

Primer name	Sequence
182-34	5'-CCGGATCCTTTCCCGTCAACCTTGATG-3'
182-35	5'-CCGGATCCTGCGATAGTCCATTACGAC-3'
454-59	5'-CCGGATCCACTGACTTGTGTACCAGGTC-3'
454-60	5'-CCGGATCCTTGCTCAGGAAAGCCAGTGG-3'
495-1	5'-CACCGAGGATGAGAAGTGC-3'
500-9	5'-CATTTCCCGGTTAATACAGGTATACAAAAGTGGATAGACTGGTGTCCCTGTTGATACC-3'
500-10	5'-CCATATCTACATGGGGCAGTTGTTTATTCTTTTAGTGTGGCACTTATTCAGGCGTAGCAC-3'
500-11	5'-ATGGGTCGCTACCCTGTTATGAGAGGACGTTATGCCACTACTGGTGTCCCTGTTGATACC-3'
500-12	5'-ACAACAGGTTGGCTCACTGGCAAGAACCCGATTATAAAACCCTTATTCAGGCGTAGCAC-3'
500-13	5'-CAGGGCATTACCAGTCTGAGGAGAACTCATGTCTGTTCTGGTGTCCCTGTTGATACC-3'
500-14	5'-TTCAGGTGTCTGGATGTTTTTATTTATGCTTTCAGTTTTTCACTTATTCAGGCGTAGCAC-3'
500-29	5'-CCCTGCAGCGCAAGCTGGTTGCCGAGTTTTTC-3'
500-30	5'-CCGAGCTCAATCAGTAAGTTGGCAGCATTACCG-3'
500-32	5'-CCGCATGCATCGCATTCTCTATCGCTTTATCGG-3'
500-34	5'-CCGAATTCGCTTACCACCGATTCTTGAAGCATC-3'
500-35	5'-AAAGGTGTGGCGAAGGTTTATCTGTCCGAG-3'
500-36	5'-TAACCTCGCGCATACAACCGGGCAGTGATG-3'
tatD-CmN	5'-ACAGTGTGAAGAATACCGAGTTCGCAAACTCTAAAACGCCTGGTGTCCCTGTTGATACC-3'
tatD-CmC	5'-CCGTCAGGGCGGTTGTCATATGGAGTACAGGATGTTTGATCACTTATTCAGGCGTAGCAC-3'
fadA-CmN	5'-CGCATCCGGCAAGTGGTTAAACCCGCTCAAACACCGTCGCCTGGTGTCCCTGTTGATACC-3'
fadA-CmC	5'-GAAAACGGCTTAAGGAGTCACAATGGAACAGGTTGTCATTCCTTATTCAGGCGTAGCAC-3'
ppc-CmN	5'-GGTTTGAGAAGAGGAAGATTAGCCGGTATTACGCATACCCTGGTGTCCCTGTTGATACC-3'
ppc-CmC	5'-TCAAACGATAAGATGGGGTGTCTGGGGTAAATGAACGAACACTTATTCAGGCGTAGCAC-3'
malK-CmN	5'-AGGCTTTGTGTGTTTTGTGGGGTGTCTAAACGCCCGGCTCCTGGTGTCCCTGTTGATACC-3'
malK-CmC	5'-CGATGACAGGTTGTTACAAAGGGAGAAGGGCATGGCGAGCCACTTATTCAGGCGTAGCAC-3'
mgtA-CmN	5'-ATCGTGCCAGTTTATTCTTTATTGCCAGCCGTAACGACGCTGGTGTCCCTGTTGATACC-3'
mgtA-CmC	5'-GGGACTCCTTATGTTTAAAGAAATTTTACCCGGCTCATTCACTTATTCAGGCGTAGCAC-3'
idnT-CmN	5'-CCCCGAAACTACATCACAATATTTTATTCTTTCAGTGCAACTGGTGTCCCTGTTGATACC-3'
idnT-CmC	5'-GCACTAATAAGAGATAAAGACTATGCCATTAATCATTATTCCTTATTCAGGCGTAGCAC-3'
groES-CmN	5'-AGATACGGACTTTCTCAAAGGAGAGTTATCAATGAATATT cTggTgTcccTgTgATAcc-3'
groEL-CmC	5'-GGTTTGTTTATTTCTGCGAGGTGCAGGGCAATTACATCAT cAcTTATTcAggcgTAgcAc-3'
lacI-CmN	5'-GAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAACGTTA cTggTgTcccTgTgATAcc-3'
lacA-CmC	5'-gcccagcgtatcaggcaattttataattaaactgacgacAcTTATTcAggcgTAgcAc-3'

Table S2 Transcription of collision-related genes in the *ygjD*^{ts} mutant.

Gene	<i>ygjD</i> (Ts) signal intensity	Wild-type signal intensity	Ratio
<i>dinG</i>	326.0	284.9	1.1443
	453.4	515.6	0.8794
<i>rep</i>	909.1	1272.0	0.7147
	652.9	463.9	1.2074
<i>uvrD</i>	1664.8	1649.3	1.0094
	1457.2	1352.6	1.0773
<i>mfd</i>	1217.4	1045.9	1.1640
	1054.5	707.3	1.4909
<i>rnhA</i>	1823.9	1327.2	1.3742
	1659.4	1881.8	0.8818

The data are derived from the microarray experiments (Hashimoto *et al.*, 2011).

Upper, cells grown at 37°C; lower, cells grown at 30°C