Supporting Information

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In Vivo Roles of Conjugation with Glutathione and O^6-Alkylguanine DNA-Alkyltransferase in the Mutagenicity of the *bis*-Electrophiles 1,2-Dibromoethane and 1,2,3,4-Diepoxybutane in Mice

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Figure S1. GSH levels (A) and AGT activities (B) in livers of B6C3F1 male mice 6 h after treatment with vehicle, 1,2-dibromooethane (DBE, 30 mg/kg, ip), O^6-BzGua (80 mg/kg, ip), or BSO (8 mg/kg, ip). n = 5

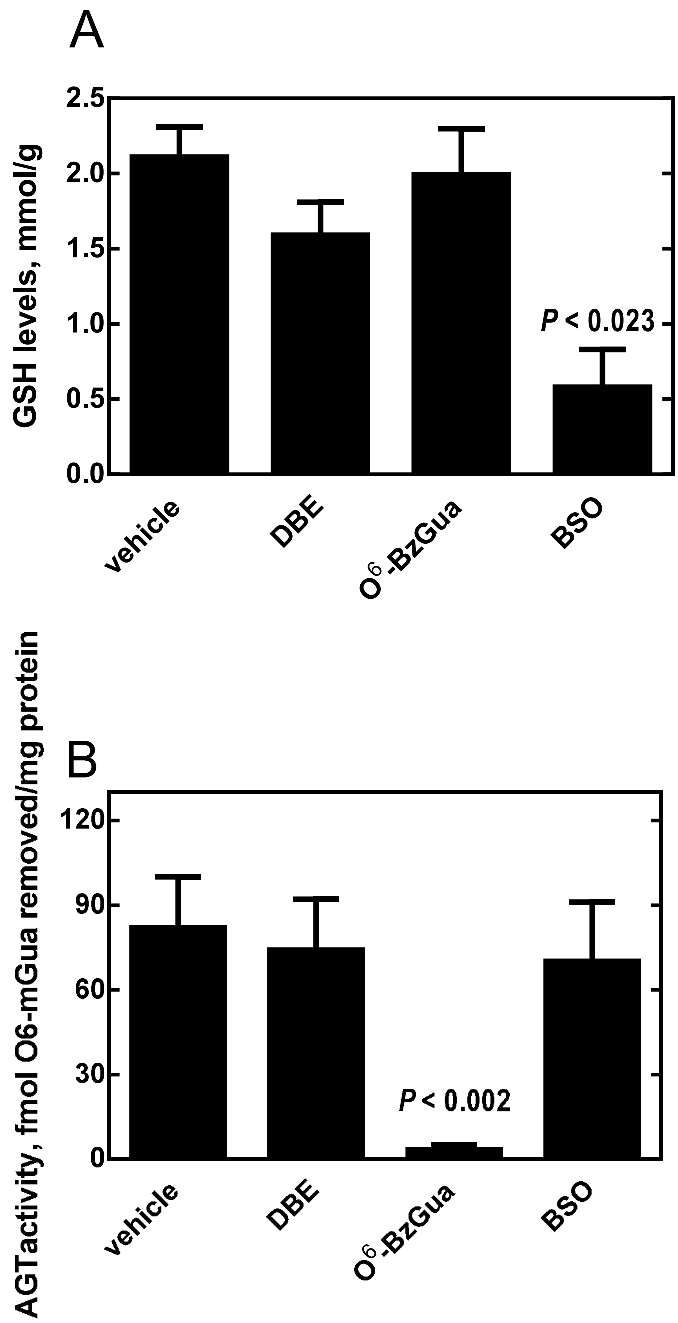
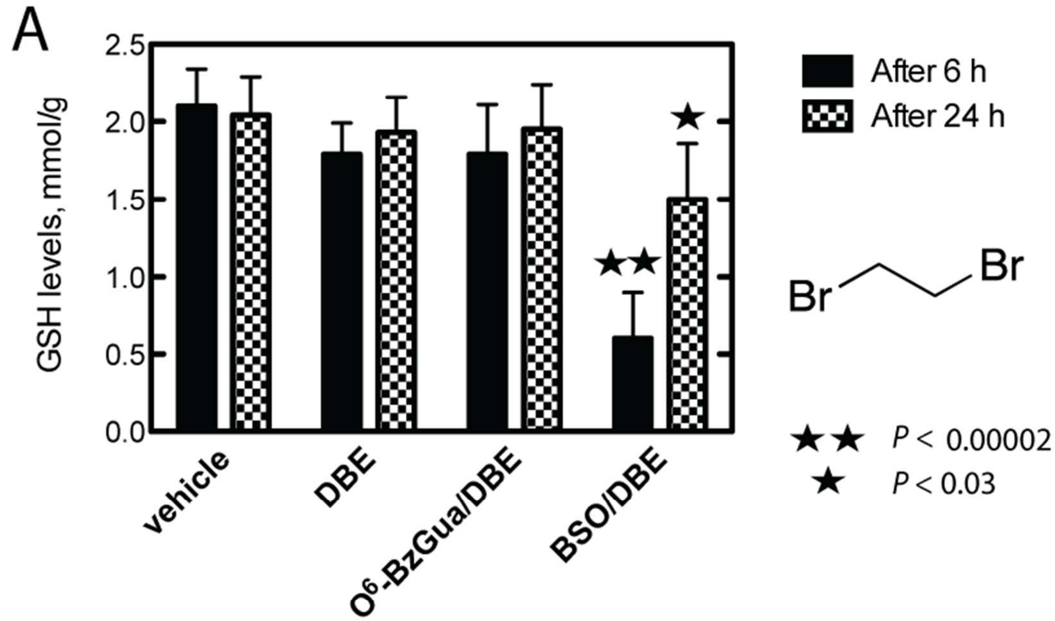


Figure S2. GSH levels in liver of Big Blue® transgenic mice 6 and 24 h after treatment with (A) vehicle, 1,2-dibromooethane (DBE), *O*[6]-BzGua/DBE, or BSO/DBE and (B) vehicle, DEB, *O*[6]-BzGua/DEB, or BSO/DEB. *O*[6]-BzGua (80 mg/kg, ip) was administered 1 h prior to treatment with DBE (30 mg/kg, ip) or DEB (25 mg/kg, ip), and BSO (8 mg/kg, ip) was administered 2 h prior to treatment with DBE (30 mg/kg, ip) or DEB (25 mg/kg, ip).



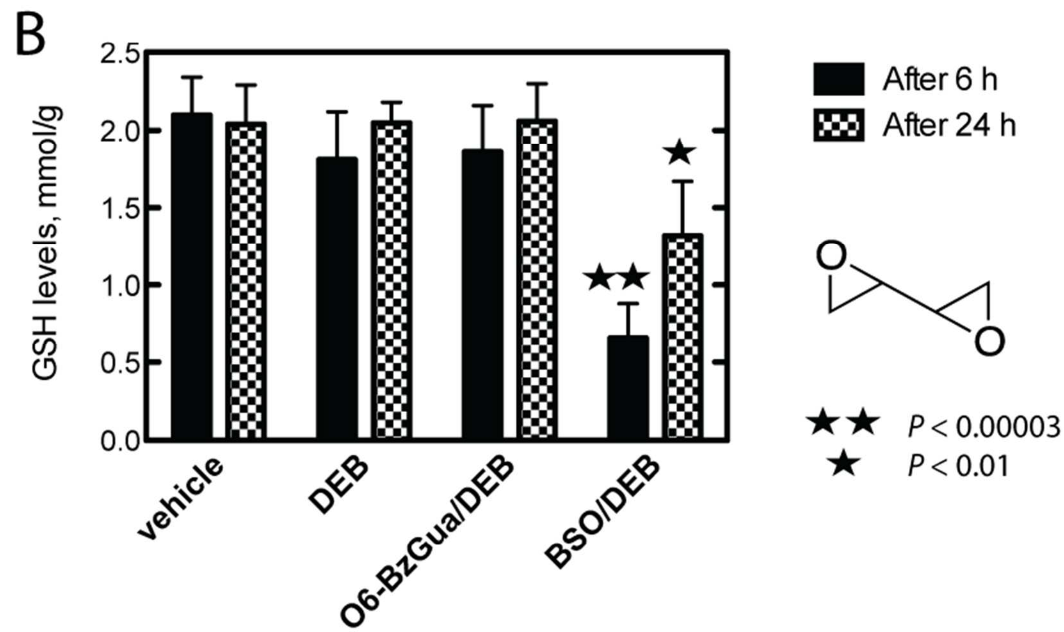


Figure S3. Gel electrophoresis imaging measurements of AGT activity in liver extracts of Big Blue® mice treated with vehicle, dibromoethane (DBE), BSO, or *O*^{6}-BzGua. The substrate (*upper bands*) and product (*lower bands*) were separated by denaturing gel electrophoresis after uracil DNA glycosylase/piperidine hydrolysis (3'-CGGAGCTCGGTCGGCGTCTGCG**U**C**X**CTCCTGCGGCT-^{32}P 5' (X: *O*^{6}-MeGua)/5'-GCCTCGAGCCAGCCGCAGACGCAGCGAGGA-3').

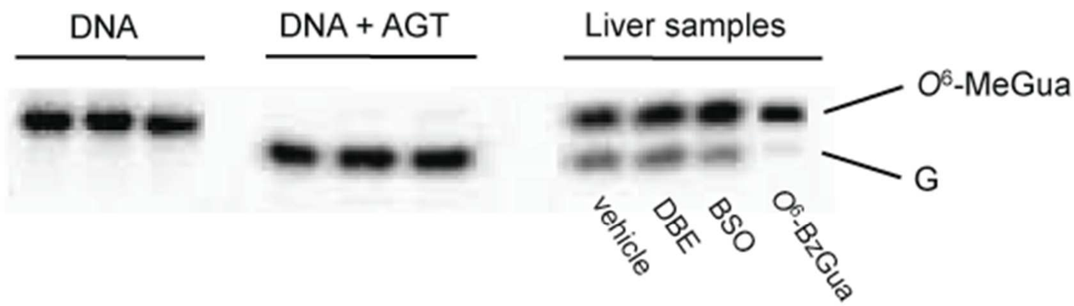


Figure S4. AGT activities in liver of Big Blue® transgenic mice at 6 and 24 h after treatment with (A) vehicle, DBE, O^6-BzGua/DBE, or BSO/DBE and (B) vehicle, DEB, O^6-BzGua/DEB, or BSO/DEB. O^6-BzGua (80 mg/kg, ip) was administered 1 h prior to treatment with DBE (30 mg/kg, ip) or DEB (25 mg/kg, ip), and BSO (8 mg/kg, ip) was administered 2 h prior to treatment with DBE (30 mg/kg, ip) or DEB (25 mg/kg, ip).

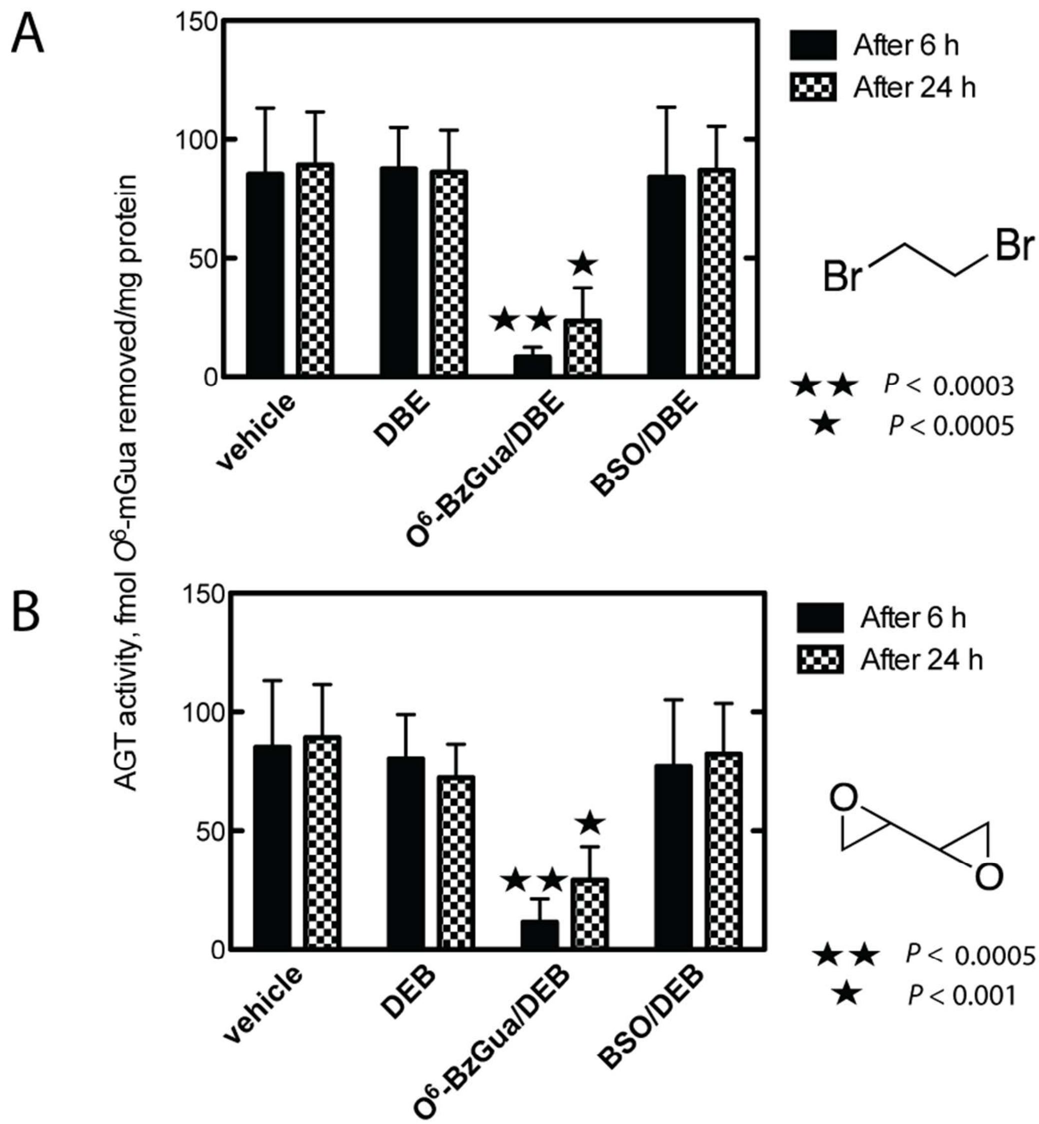


Table S1. Quantitative Analysis of DNA Adducts in Livers of Big Blue® Transgenic Mice 6 and 24 h after Treatment with Vehicle, Dibromoethane (, O^6-BzGua/ Dibromoethane, or BSO/ Dibromoethane

time	adducts per 10^5 bases	control[a] (n = 5)	dibromoethane (n = 5)	BSO/ dibromoethane (n = 5)	O^6BzGua/ dibromoethane (n = 5)
after 6 h	N^7G-ethyl-GSH	ND[b]	4.8 ± 0.8	2.2 ± 0.3	5.2 ± 0.8
	N^6dA-ethyl-GSH	ND	ND	ND	ND
	N^1A-ethyl-GSH	ND	ND	ND	ND
after 24 h	N^7G-ethyl-GSH	ND	3.5 ± 0.8	1.8 ± 0.6	3.4 ± 0.9
	N^6dA-ethyl-GSH	ND	ND	ND	ND
	N^1A-ethyl-GSH	ND	ND	ND	ND

[a]Control: not treated with DBE.
[b]ND, not detected (limit of detection 5 adducts/10^8 bases)

Table S2. Quantitative Analysis of DNA Adducts in Livers of Big Blue® Transgenic Mice 6 and 24 h after Treatment with Vehicle, DEB, O^6-BzGua/DEB, or BSO/DEB

time	adducts per 10^7 bases	control[a] (n = 5)	DEB (n = 5)	BSO/DEB (n = 5)	O^6BzGua/DEB (n = 5)
after 6 h	N^7G-$(OH)_2$butyl-GSH	ND[b]	0.24 ± 0.07	0.13 ± 0.03	0.22 ± 0.02
	N^6dA-$(OH)_2$butyl-GSH	ND	0.22 ± 0.06	0.12 ± 0.02	0.23 ± 0.04
	N^3A-$(OH)_2$butyl-GSH	ND	ND	ND	ND
	N^1dG-$(OH)_2$butyl-GSH	ND	ND	ND	ND
	N^3dT-$(OH)_2$butyl-GSH	ND	ND	ND	ND
	N^4dC-$(OH)_2$butyl-GSH	ND	ND	ND	ND
after 24 h	N^7G-$(OH)_2$butyl-GSH	ND	0.19 ± 0.06	0.07 ± 0.02	0.17 ± 0.04
	N^6dA-$(OH)_2$butyl-GSH	ND	0.18 ± 0.03	0.08 ± 0.04	0.17 ± 0.04
	N^3A-$(OH)_2$butyl-GSH	ND	ND	ND	ND
	N^1dG-$(OH)_2$butyl-GSH	ND	ND	ND	ND
	N^3dT-$(OH)_2$butyl-GSH	ND	ND	ND	ND
	N^4dC-$(OH)_2$butyl-GSH	ND	ND	ND	ND

[a]Control: not treated with DBE.
[b]ND, not detected (limit of detection 0.03 adducts/10^7 bases)

Table S3. Independent Mutations in the Liver *cII* Gene of Big Blue® Transgenic Mice Treated with Vehicle, Diibromoethane (DBE), BSO/ Diibromoethane, or O^6-BzGua/ Dibromoethane

position[a]	mutation[b]	amino acid change	sequence context 5' → 3'[c]	number of independent mutations			
				vehicle	DBE	BSO/DBE	O^6-BzGua/DBE
1	A → G	Met → Val	catATGgtt		1		
	A → T	Met → Leu	catATGgtt			1	
2	T → A	Met → Lys	catATGgtt			1	
	T → C	Met → Thr	catATGgtt		1		
3	G → A	Met → Ile	catATGgtt		1	1	2
	G → C	Met → Ile	catATGgtt			1	
	G → T	Met → Ile	catATGgtt		1	1	1
15	C → A	Asn → Lys	gcaAACaaa	1		1	
16	A → T	Lys → Stop	aacAAAcgc			1	
19	C → T	Arg → Cys	aaaCGCaac		1	1	
25	G → A	Glu → Lys	aacGAGgct	2	1	1	1
	G → T	Glu → Stop	aacGAGgct	1	1		
26	A → G	Glu → Gly	aacGAGgct			1	
31	C → A	Leu → Ile	gctCTAcga		1		1
34	C → T	Arg → Stop	ctaCGAatc	3	2	2	1
35	G → A	Arg → Glu	ctaCGAatc	1	1	1	
	G → T	Arg → Leu	ctaCGAatc				1
39	C → G	Ile → Met	cgaATCgag			1	
40	G → A	Glu → Lys	atcGAGagt		1		
39-40	CG → AT	Glu → Stop	cgaATCGAGagt			1	
41	A → T	Glu → Val	atcGAGagt		1		
	A → G	Glu → Gly	atcGAGagt				1
50	T → C	Leu → Ser	gcgTTGctt		1		
51	G → T	Leu → Phe	gcgTTGctt			1	1
55	A → C	Asn → His	cttAACaaa		1		1
64	G → A	Ala → Thr	atcGCAatg		1	1	
74	G → A	Gly → Glu	cttGGAact		1	1	1
	G → T	Gly → Val	cttGGAact	1		1	
89	C → A	Ala → Glu	acaGCGgaa				1
	C → T	Ala → Val	acaGCGgaa	1	1	2	1
101	G → T	Gly → Val	gtgGGCgtt		1		
103	G → A	Val → Ile	ggcGTTgat	3	1	2	1
	G → C	Val → Leu	ggcGTTgat	1			1
	G → T	Val → Phe	ggcGTTgat	1		1	
108	T → C	Asp → Asp	gatGATaag				1
113	C → T	Ser → Leu	aagTCGcag	1	1	1	
115	C → A	Gln → Lys	tcgCAGatc		1		1
	C → T	Gln → Stop	tcgCAGatc		1	1	
118	A → T	Ile → Phe	cagATCagc	1			
124	A → G	Arg → Gly	agcAGGtgg				1

125	G → T	Arg → Met	agcAGGtgg				1
141	G → A	Trp → Stop	gacTGGatt		1	1	1
	G → T	Trp → Cys	gacTGGatt	1		1	
145	C → A	Pro → Thr	attCCAaag		1		1
160	C → A	Leu → Met	atgCTGctt		1		
	C → G	Leu → Val	atgCTGctt				1
164	T → A	Leu → His	ctgCTTgct				1
175	G → T	Glu → Stop	cttGAAtgg		1		
178/185	+G	Frameshift	tGGGGGGt	3	1	3	2
179	G → A	Trp → Stop	gaaTGGggg		1		1
	G → T	Trp → Leu	gaaTGGggg		1		1
179-184	−G	Frameshift	tGGGGGGt	1	1	1	1
182	G → T	Gly → Val	tggGGGgtc		1		
185	T → G	Val → Gly	gggGTCgtt	1	1	1	1
190	G → C	Asp → Gln	gttGACgac	1			
193	G → T	Asp → Tyr	gacGACgac		1		1
196	G → A	Asp → Asn	gacGACatg	4	1	3	1
	G → T	Asp → Tyr	gacGACatg		1		
200	T → A	Met → Lys	gacATGgct				1
206	G → T	Arg → Leu	gctCGAttg				1
	G → A	Arg → Gln	gctCGAttg	2	1	2	1
211	G → C	Ala → Pro	ttgGCGcga	1			
	G → A	Ala → Thr	ttgGCGcga			1	
212	C → T	Ala → Val	ttgGCGcga	2	1	1	1
214	C → T	Arg → Stop	gcgCGAcaa	2	1	1	1
220	G → T	Val → Phe	caaGTTgct	1		1	
224-225	GT → TG	Ala → Val	gttGCTgcg				1
274	C → A	Gln → Lys	gaaCAAatc		1		1
287	A → T	Glu → Val	atgGAGttc	1			
Total				37	40	42	38

[a]Position 1 is the first base of the start codon in the *cII* coding sequuence.

[b]Presented in term of sequence change on nontranscribed DNA strand.

[c]Uppercase indicates target codon and target basees are underlined.

Table S4. Independent Mutations in the Liver *cII* Gene of Big Blue® Transgenic Mice Treated with Vehicle, DEB, BSO/DEB, or O^6-BzGua/DEB

		amino acid	sequence context	number of independent mutations			
position[a]	mutation[b]	change	5' → 3'[C]	vehicle	DEB	BSO/DEB	O^6-BzGua/DEB
1	A → G	Met → Val	catATGgtt		2	1	
	A → T	Met → Leu	catATGgtt		1		
2	T → A	Met → Lys	catATGgtt				1
	T → C	Met → Thr	catATGgtt				1
3	G → A	Met → Ile	catATGgtt			1	1
	G → T	Met → Ile	catATGgtt		1		
15	C → A	Asn → Lys	gcaAACaaa	1			
16	A → T	Lys → Stop	aacAAAcgc				1
19	C → T	Arg → Cys	aaaCGCaac		1	1	1
25	G → A	Glu → Lys	aacGAGgct	2	1	1	2
	G → T	Glu → Stop	aacGAGgct	1		1	1
26	A → G	Glu → Gly	aacGAGgct		1		
31	C → A	Leu → Ile	gctCTAcga		1		1
32	T → C	Leu → Pro	gctCTAcga		1		1
34	C → T	Arg → Stop	ctaCGAatc	3	2	1	2
35	G → A	Arg → Glu	ctaCGAatc	1			1
	G → T	Arg → Leu	ctaCGAatc		1	1	1
40	G → A	Glu → Lys	atcGAGagt		1		1
41	A → T	Glu → Val	atcGAGagt			1	
	A → G	Glu → Gly	atcGAGagt		1	1	
50	T → C	Leu → Ser	gcgTTGctt		1	1	
51	G → T	Leu → Phe	gcgTTGctt		1	1	
55	A → C	Asn → His	cttAACaaa		2	1	1
58	A → G	Lys → Glu	aacAAAatc		1	1	
64	G → A	Ala → Thr	atcGCAatg			1	1
73-74	GG → CT	Glu → Ile	cttGGAact			1	
74	G → T	Gly → Val	cttGGAact	1		1	
82	A → G	Lys → Glu	gagAAGaca		1		1
89	C → A	Ala → Glu	acaGCGgaa		1		1
	C → T	Ala → Val	acaGCGgaa	1		1	1
100	G → T	Gly → Cys	gtgGGCgtt		1	1	
101	G → T	Gly → Val	gtgGGCgtt			1	
103	G → A	Val → Ile	ggcGTTgat	3		2	1
	G → C	Val → Leu	ggcGTTgat	1		1	
	G → T	Val → Phe	ggcGTTgat	1			1
108	T → C	Asp → Asp	gatGATaag				1
109	A → G	Lys → Glu	gatAAGtcg				1
113	C → T	Ser → Leu	aagTCGcag	1	1	1	1
115	C → T	Gln → Stop	tcgCAGatc		1	1	1
118	A → T	Ile → Phe	cagATCagc	1			
124	A → G	Arg → Gly	agcAGGtgg		1		1
125	G → T	Arg → Met	agcAGGtgg				1
141	G → A	Trp → Stop	gacTGGatt		1	1	

	G → T	Trp → Cys	gacTGGatt	1		1	
145	C → A	Pro → Thr	attCCAaag				1
154-155	TC → AG	Ser → Arg	ttcTCAatg		1		
160	C → G	Leu → Val	atgCTGctt				1
175	G → C	Glu → Gln	cttGAAtgg				1
	G → T	Glu → Stop	cttGAAtgg				1
178/185	+G	Frameshift	tGGGGGGt	3	2	2	2
179	G → A	Trp → Stop	gaaTGGggg		1	1	1
179-184	−G	Frameshift	tGGGGGGt	1	3		1
185	T → G	Val → Gly	gggGTCgtt	1			
190	G → C	Asp → Gln	gttGACgac	1	1		
196	G → A	Asp → Asn	gacGACatg	4	1	2	1
	G → T	Asp → Tyr	gacGACatg			1	
200	T → C	Met → Thr	gacATGgct		1		
206	G → A	Arg → Gln	gctCGAttg	2	1	2	1
211	G → C	Ala → Pro	ttgGCGcga	1			
212	C → A	Ala → Glu	ttgGCGcga		2		
	C → T	Ala → Val	ttgGCGcga	2		1	1
214	C → T	Arg → Stop	gcgCGAcaa	2	1	2	1
215	G → T	Arg → Leu	gcgCGAcaa			1	
220	G → T	Val → Phe	caaGTTgct	1			
274	C → A	Gln → Lys	gaaCAAatc			1	
287	A → T	Glu → Val	atgGAGttc	1			
Total				37	40	39	40

[a]Position 1 is the first base of the start codon in the *cII* coding sequence.

[b]Presented in term of sequence change on nontranscribed DNA strand.

[c]Uppercase indicates target codon and target bases are underlined.