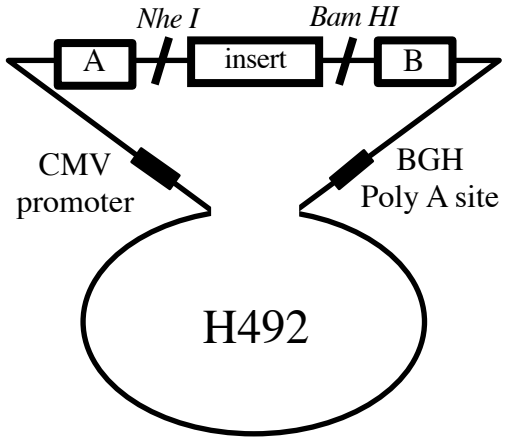
Supplementary Table S1. Primer list in this study.

Construction	Direction	Primer Sequence
YH303	forward	5′-GGTACCACAGCTGGATTACTCGCTC-3′
YH304	reverse	5′-GTGAGAGACTTAACTGGCTGCTCGAG -3′
YH307	forward	5′-ATTACTCGCTCAGAAGCTGTGTTGC-3′
YH308	reverse	5′-CTGCCAGTTGCTAAGTGAGAGACTT-3′
Linearized primer (1)	forward	5′-TAGCGTTAACATCGATATCCGGATCCTGG-3′
Linearized primer (1)	reverse	5′-GCTAACAAAGCACGGAGTTTACAAGCAGC-3′
MG-COL4A5 exn15-16	forward	5′-CCGTGCTTTGTTAGCACACAGTATCCCATGAACCATTG-3′
MG-COL4A5 exn15-16	reverse	5′-TCGATGTTAACGCTAATGCTTGAGGGGATGAATACC-3′
MG-COL4A5 exon19	forward	5′-CCGTGCTTTGTTAGCTCAGGCTTAGGAGATTGGAATGA-3′
MG-COL4A5 exon19	reverse	5′-TCGATGTTAACGCTAAAGAGTTGGGAAGTATCTGTCAA-3′
MG-COL4A5 exon21	forward	5′-CCGTGCTTTGTTAGCTTCTGCTTTTTGGGTTTTGG-3′
MG-COL4A5 exon21	reverse	5′-TCGATGTTAACGCTAGGGTTGGAGTATTGCTGGAG-3′
MG-COL4A5 exon25	forward	5′-CCGTGCTTTGTTAGCTCTGTTGTATCACCAGCACTTGA-3′
MG-COL4A5 exon25	reverse	5′-TCGATGTTAACGCTATAAACGGCCAGGGAAACACA-3′
MG-COL4A5 exon28	forward	5′-CCGTGCTTTGTTAGCAAGGAATGTAAGATGATTGAGAGGA-3′
MG-COL4A5 exon28	reverse	5′-TCGATGTTAACGCTATTGCTCCAAATGTGACCTTG-3′
MG-COL4A5 exon30	forward	5′-CCGTGCTTTGTTAGCCCCAAGGACTAGTGACTCAGG-3′
MG-COL4A5 exon30	reverse	5′-TCGATGTTAACGCTAGCCTCTGCTTCCAAAACCATG-3′
MG-COL4A5 exon31	forward	5′-CCGTGCTTTGTTAGCGCACTGTCTTAGAGCAATCTGC-3′
MG-COL4A5 exon31	reverse	5′-TCGATGTTAACGCTATGAACCAGTCAAGTCATCCCTTA-3′
MG-COL4A5 exon 32	forward	5′-CCGTGCTTTGTTAGCCCCTAGTTCCAGGTGAGGG-3′
MG-COL4A5 exon 32	reverse	5′-TCGATGTTAACGCTATCCAAACTAAGCACGTAACCA-3′
MG-COL4A5 exon35	forward	5′-CCGTGCTTTGTTAGCGGGAAAGAAACAAGGTGTTCTGG-3′
MG-COL4A5 exon35	reverse	5′-TCGATGTTAACGCTAAGGGGGTATAGCTGGAGAAA-3′
MG-COL4A5 exon36	reverse	5′-TCGATGTTAACGCTAGACTGGCCACAAACCCTAAAAC-3′
MG-COL4A5 exon37	forward	5′-CCGTGCTTTGTTAGCGCACTTGGCTTCTCAAGATTC-3′
MG-COL4A5 exon37	reverse	5′-TCGATGTTAACGCTAGGGCAAAGAAAAGGTAAAGAGA-3′
MG-COL4A5 exon41	forward	5′-CCGTGCTTTGTTAGCTGCTCTGTCTTAACACAAAGGA-3′
MG-COL4A5 exon41	reverse	5′-TCGATGTTAACGCTAAGAACCATGGAAATAGATGGAGT-3′
MG-COL4A5 exon42	forward	5′-CCGTGCTTTGTTAGCGCCACACATTGTTAAGCCATGA-3′
MG-COL4A5 exon42	reverse	5′-TCGATGTTAACGCTAACCAAAGACTCCAGGTGAAGTA-3′
MG-COL4A5 exon43	forward	5′-CCGTGCTTTGTTAGCCTTTGCGCACCTCAGTTAGC-3′
MG-COL4A5 exon43	reverse	5′-TCGATGTTAACGCTAATGGCAAAGCAACAGGG-3′
MG-COL4A5 exon44	forward	5′-CCGTGCTTTGTTAGCTCCTCTGGGACAAGATAGCCA-3′

MG-COL4A5 exon44	reverse	5′-TCGATGTTAACGCTAGGAAGCATTTTTGTTGATCCTG-3′
MG-COL4A5 exon45	reverse	5′-TCGATGTTAACGCTAATGAGAACACATGGACACAGGG-3′
MG-COL4A5 exon46	forward	5′-CCGTGCTTTGTTAGCCCTTGTTGGCTTCTTTGGTC-3′
MG-COL4A5 exon46	reverse	5′-TCGATGTTAACGCTATGAGTTTTCCACTGCTCTAAACA-3′
MG-COL4A5 exon47	reverse	5′-TCGATGTTAACGCTAGCACTTTGGCCAAGGCTACTC-3′
MG-COL4A5 exon48	forward	5′-CCGTGCTTTGTTAGCGGGAATATATAATGAGCTACCACAA-3′
MG-COL4A5 exon48	reverse	5′-TCGATGTTAACGCTACAAAAGCAGACCCCCAATAA-3′
MG-COL4A5 exon50	forward	5′-CCGTGCTTTGTTAGCTACCTCTGGGCCTGTTCCTTC-3′
MG-COL4A5 exon50	reverse	5′-TCGATGTTAACGCTAAGTCTGTTTTTCTGACCTGAGTC-3′
Mutagenesis c.1165G>A	forward	5′-ACCTCCAAGTAAATGAGATTGCATTTA-3′
Mutagenesis c.1165G>A	reverse	5′-CATTTACTTGGAGGTCCAGGAAGGCC-3′
Mutagenesis c.1423G>A	forward	5′-AGTGAAAAGTTTGATCTCCAAACATA-3′
Mutagenesis c.1423G>A	reverse	5′-ATCAAACTTTTCACTCCTTGTTCTCC-3′
Mutagenesis c.1948G>A	forward	5′-AATACCAAGTAAGTTTACTGTGTTTTG-3′
Mutagenesis c.1948G>A	reverse	5′-AACTTACTTGGTATTCCTGGCTGGCC-3′
Mutagenesis c.2244G>T	forward	5′-GACCAAATGTCTGGGACATTTTTC-3′
Mutagenesis c.2244G>T	reverse	5′-CCCAGACATTTGGTCCTGGAAAGCCG-3′
Mutagenesis c.2509G>A	forward	5′-TCAACCTAGTAAGATTAGAGTAAATG-3′
Mutagenesis c.2509G>A	reverse	5′-ATCTTACTAGGTTGACCTATTGGCCC-3′
Mutagenesis c.2677G>C	forward	5′-ATTTCCACGTAATTTGTTTAAAGTTTTC-3′
Mutagenesis c.2677G>C	reverse	5′-AAATTACGTGGAAATCCAGAGGCACC-3′
Mutagenesis c.2677G>A	forward	5′-ATTTCCAAGTAATTTGTTTAAAGTTTTC-3′
Mutagenesis c.2677G>A	reverse	5′-AAATTACTTGGAAATCCAGAGGCACC-3′
Mutagenesis c.3373G>A	forward	5′-ATTCCCAAGTAAAATTTCTTCTCTTA-3′
Mutagenesis c.3373G>A	reverse	5′-ATTTTACTTGGGAATCCAGGAAGGCC-3′
Mutagenesis c.3924G>C	forward	5′-GACTCCACGTAGGAAATGGAAGTAGA-3′
Mutagenesis c.3924G>C	reverse	5′-TTCCTACGTGGAGTCCTGGTGGTCCTTG-3′
Mutagenesis c.3997G>A	forward	5′-ATTCCCAAGTATTTGAAGGGATTTTTG-3′
Mutagenesis c.3997G>A	reverse	5′-CAAATACTTGGGAATCCTGGAACACC-3′
Mutagenesis c.4069G>A	forward	5′-TCCTCCAAGTAAGACTTATTCCTGAA-3′
Mutagenesis c.4069G>A	reverse	5′-GTCTTACTTGGAGGACCAATAAGTCC-3′
Mutagenesis c.4688G>A	forward	5′-ATTAGTCAGTAAGGCATTGATTTAGC-3′
Mutagenesis c.4688G>A	reverse	5′-GCCTTACTGACTAATGAATGGCTGGA-3′
Mutagenesis c.4976G>A	forward	5′-ATGTTCAAGTAAAGTGCTTATAGCTTTA-3′
Mutagenesis c.4976G>A	reverse	5′-ACTTTACTTGAACATGTCTGACACATC-3′
cDNA-COL4A5-exon25-1st	forward	5′-TTTCCTGGAGAAAGGGGTCAG-3′
cDNA-COL4A5-exon25-1st	reverse	5′-ACCTGCTTTTCCTGGAAGCCCTG-3′

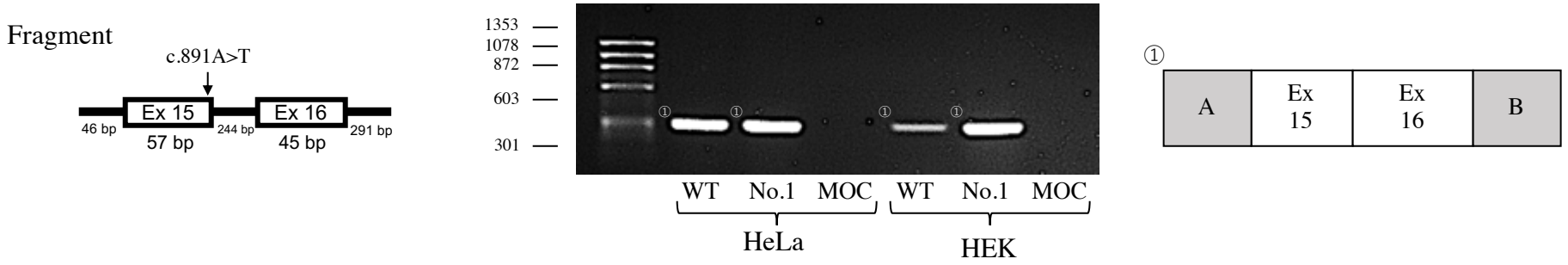
cDNA-COL4A5-exon25-2nd	forward	5′-CTCCAGGATCTCTTGGTTT-3′
cDNA-COL4A5-exon25-2nd	reverse	5′-GATCCCTGGACTGCCTCTTT-3′
cDNA-COL4A5-exon41-1st	forward	5′-CTGGTATCAAAGGTTCTGTG-3′
cDNA-COL4A5-exon41-1st	reverse	5′-ATCAAAGCCAGGGAGTCCAT-3′
cDNA-COL4A5-exon41-2nd	forward	5′-TACCAGGAACCCCTGGAGCA-3′
cDNA-COL4A5-exon41-2nd	reverse	5′-AGGACCTTCTGGACCTGGTAG3′
cDNA-COL4A5-exon48-1st	forward	5′-GTCCTTCAGGACAGAGTATC-3′
cDNA-COL4A5-exon48-1st	reverse	5′-TGATCATATATAGTAGCACCA-3′
cDNA-COL4A5-exon48-2nd	forward	5′-ATGCTGGTCCTCCAGGAATC-3′
cDNA-COL4A5-exon48-2nd	reverse	5′-CAATGAGACACTGCATCCTAG-3′

Supplementary Figure S1.

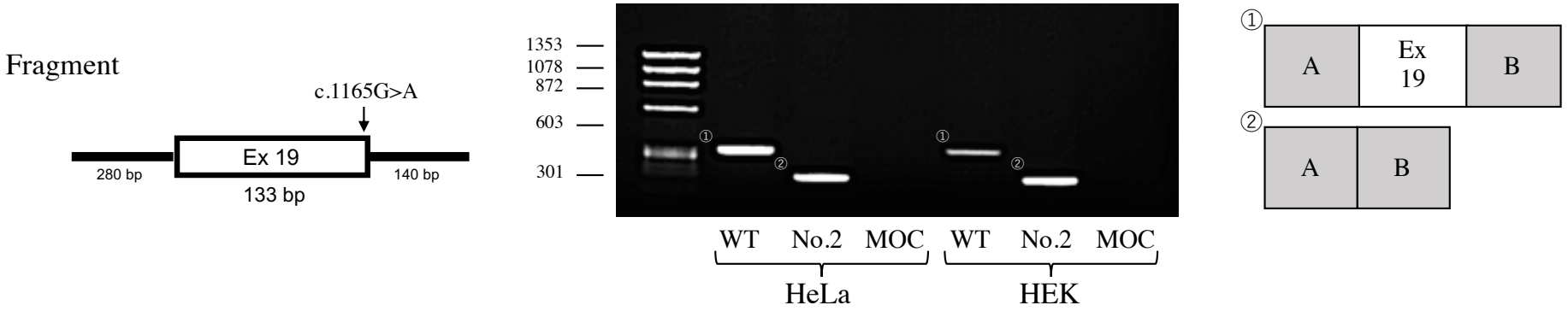


Supplementary Figure S2.

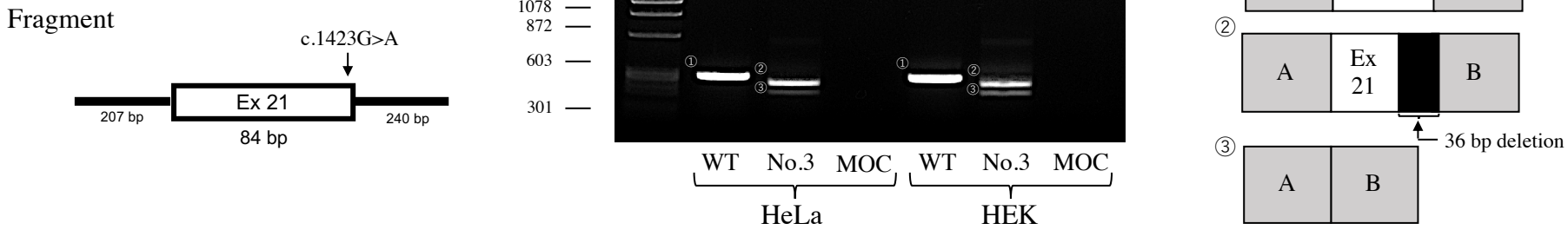
(a) No.1: c.891A>T, p.(Arg297Ser)



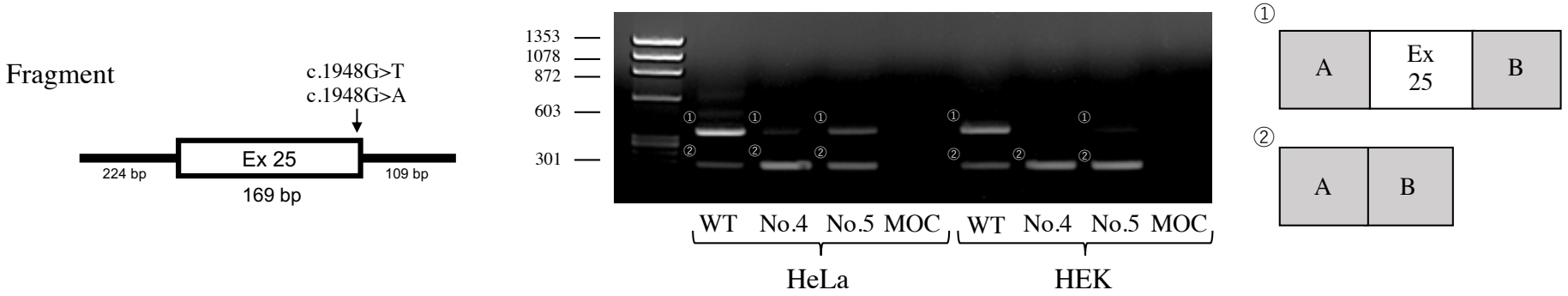
(b) No.2: c.1165G>A, p.(Gly389Arg)



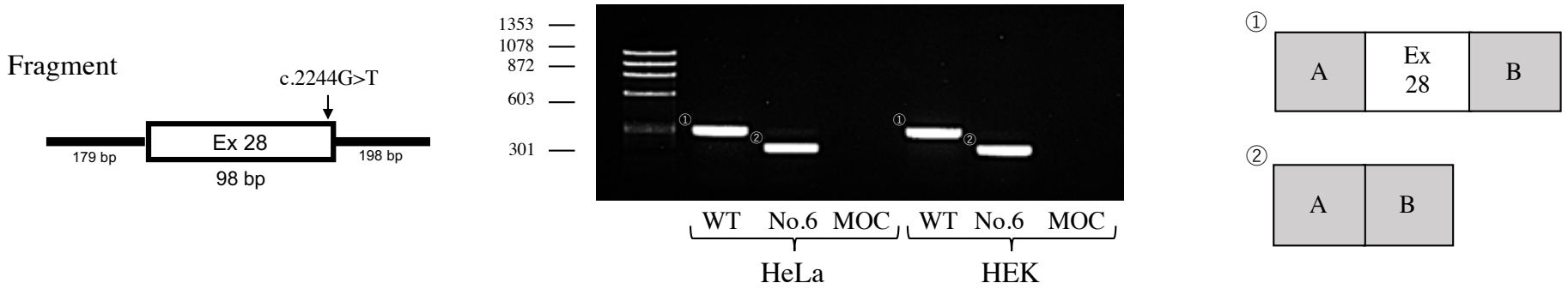
(c) No.3: c.1423G>A, p.(Gly457Arg)



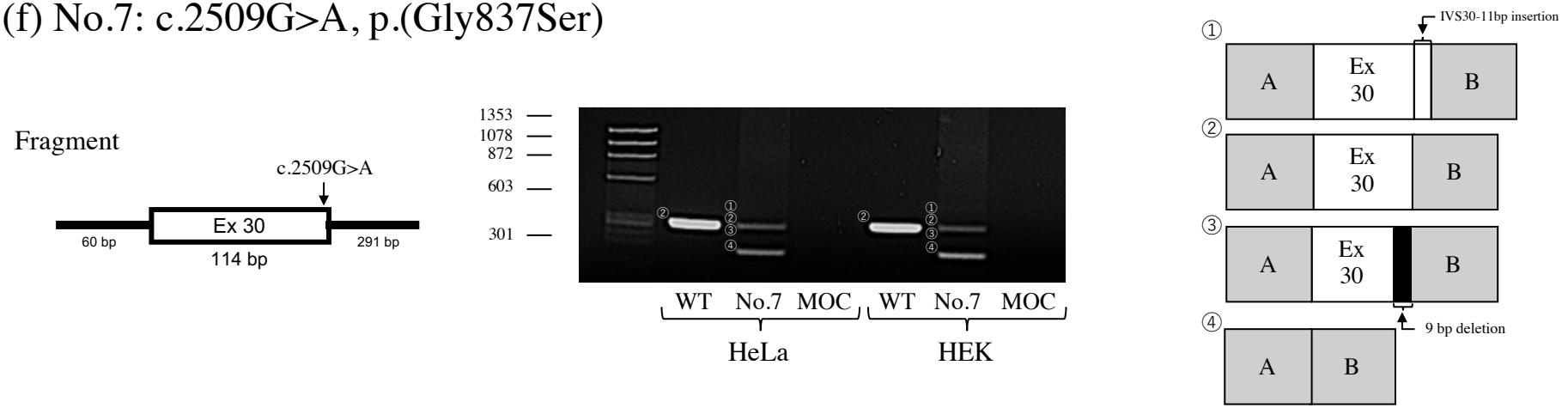
(d) No.4: c.1948G>T, p.(Gly650Cys)
No.5: c.1948G>A, p.(Gly650Ser)



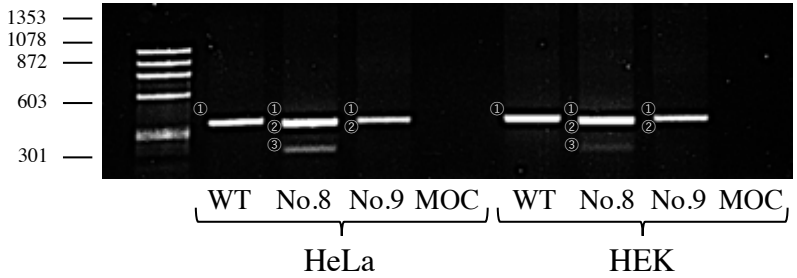
(e) No.6: c.2244G>T, p.(Lyn748Asn)

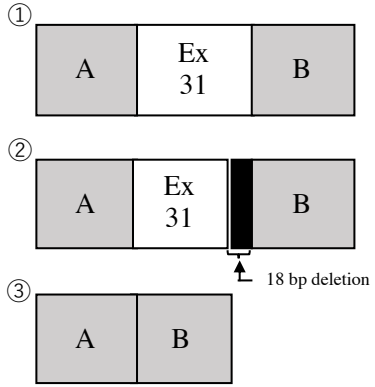


(f) No.7: c.2509G>A, p.(Gly837Ser)

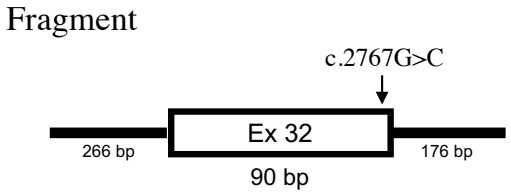


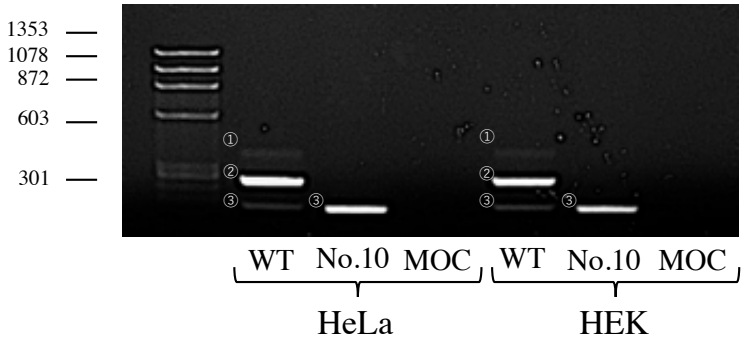
(g) No.8: c.2677G>C, p.(Gly893Arg)
No.9: c.2677G>A, p.(Gly893Ser)

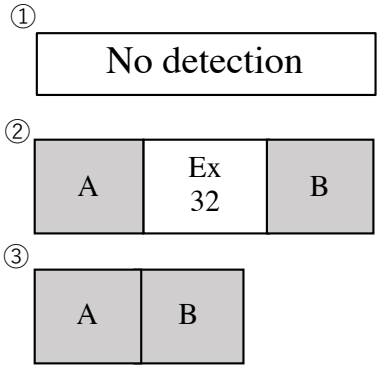




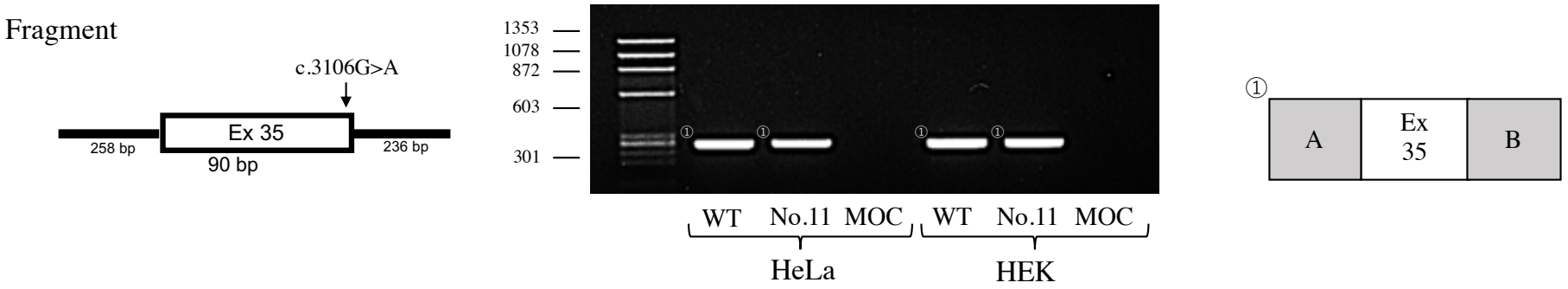
(h) No.10: c.2767G>C, p.(Gly923Arg)



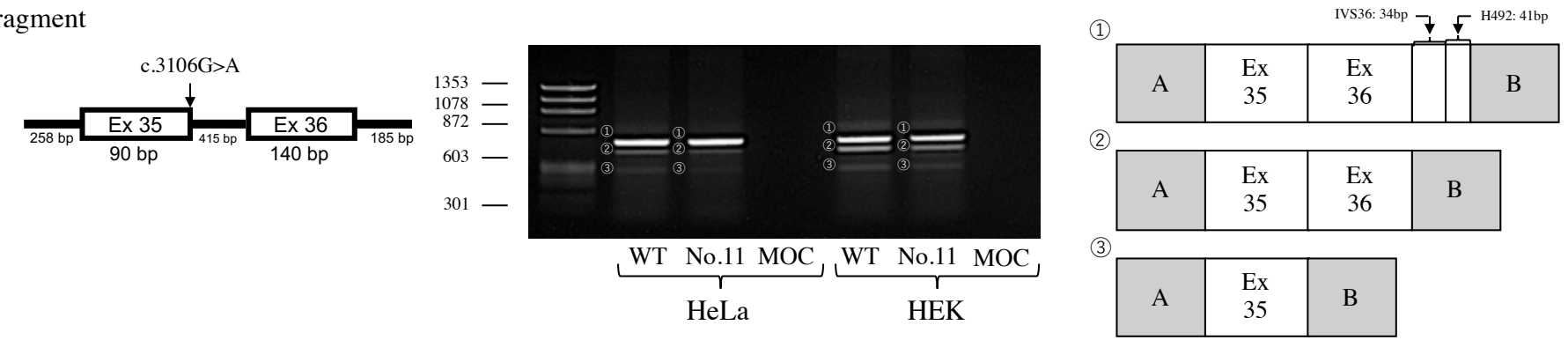




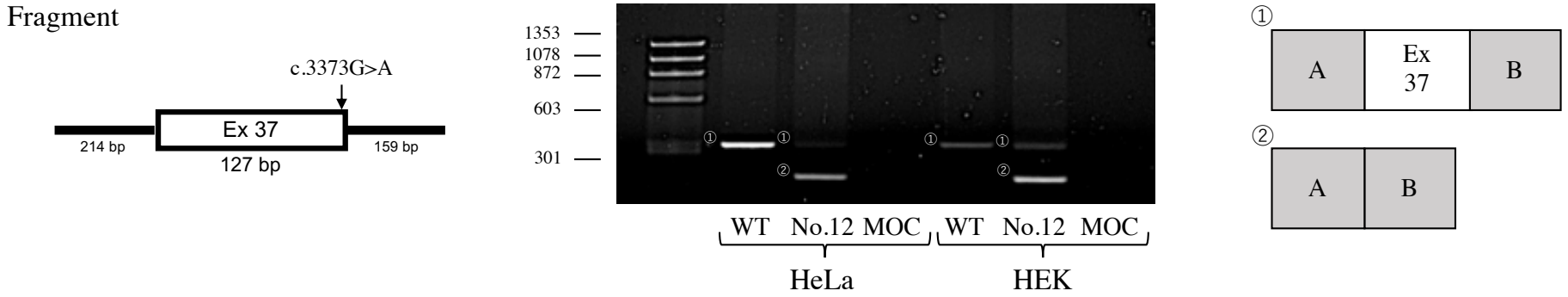
(i-1) No.11: c.3106G>A, p.(Gly1036Arg)



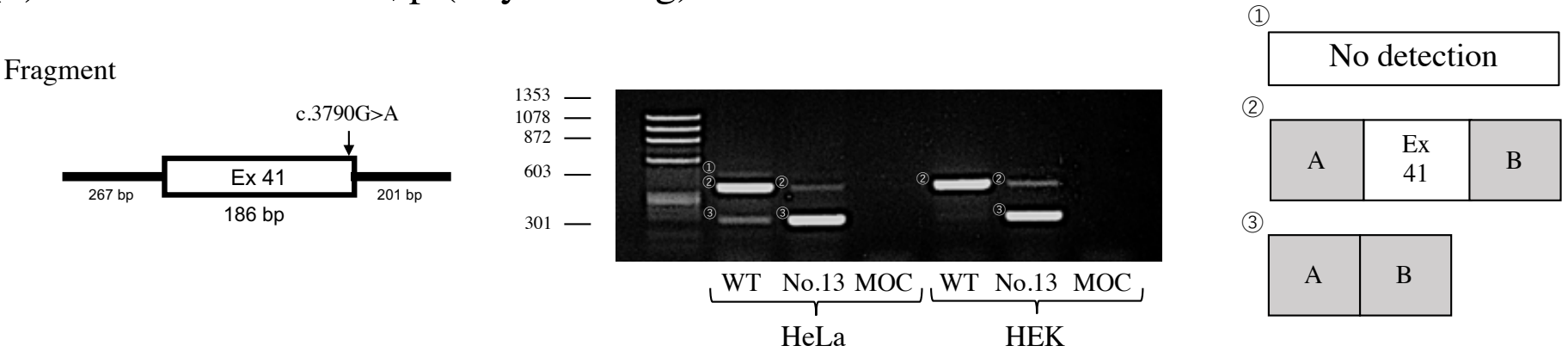
(i-2) No.11: c.3106G>A, p.(Gly1036Arg)



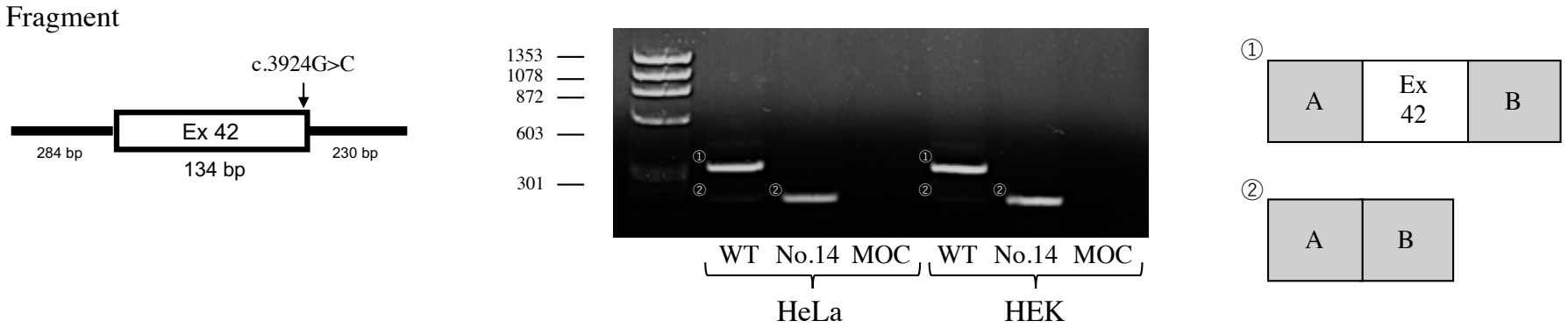
(j) No.12: c.3373G>A, p.(Gly1125Arg)



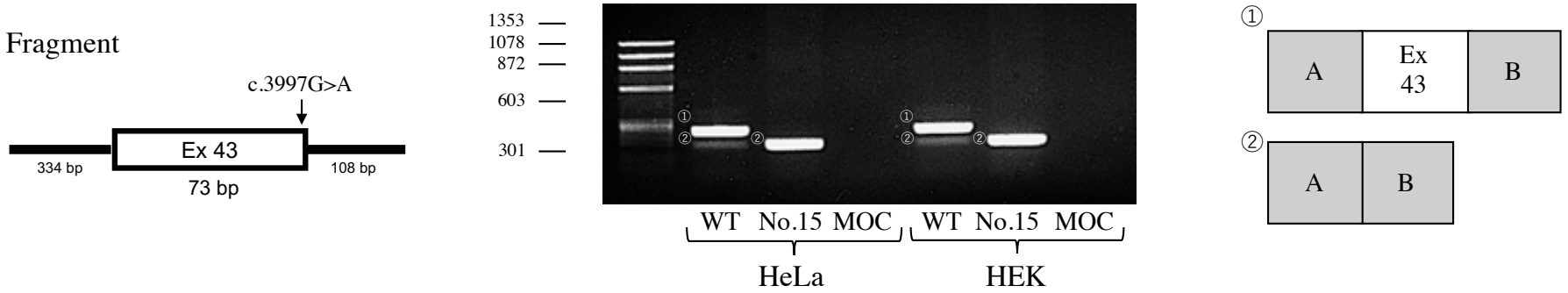
(k) No.13: c.3790G>A, p.(Gly1264Arg)



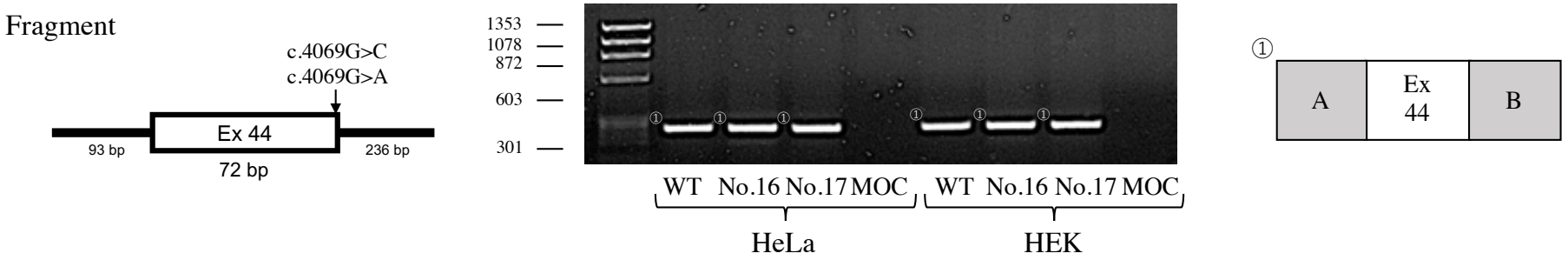
(l) No.14: c.3924G>C, p.(Gln1308His)



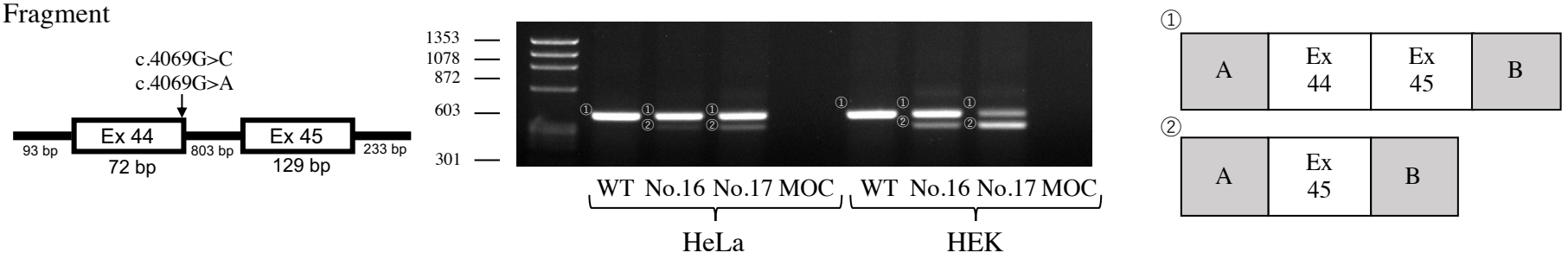
(m) No.15: c.3997G>A, p.(Gly1333Ser)



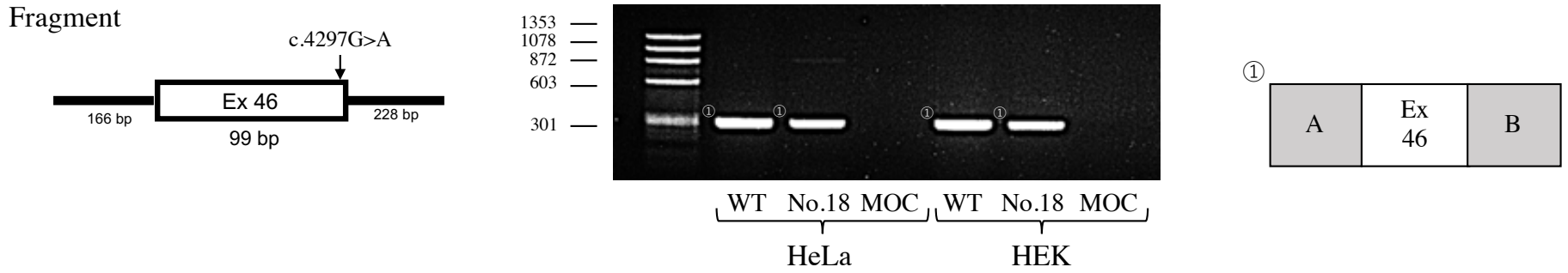
(n-1) No.16: c.4069G>C, p.(Gly1357Arg)
No.17: c.4069G>A, p.(Gly1357Ser)



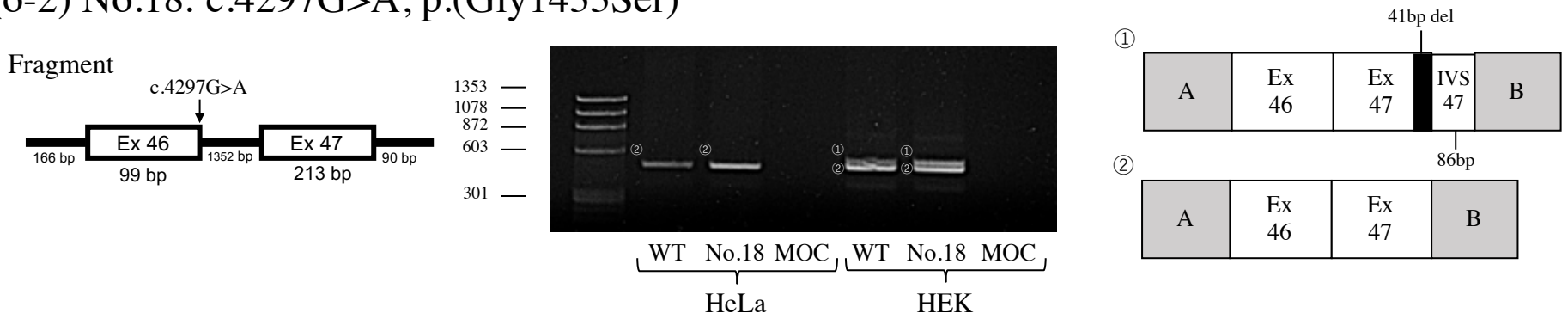
(n-2) No.16: c.4069G>C, p.(Gly1357Arg)
No.17: c.4069G>A, p.(Gly1357Ser)



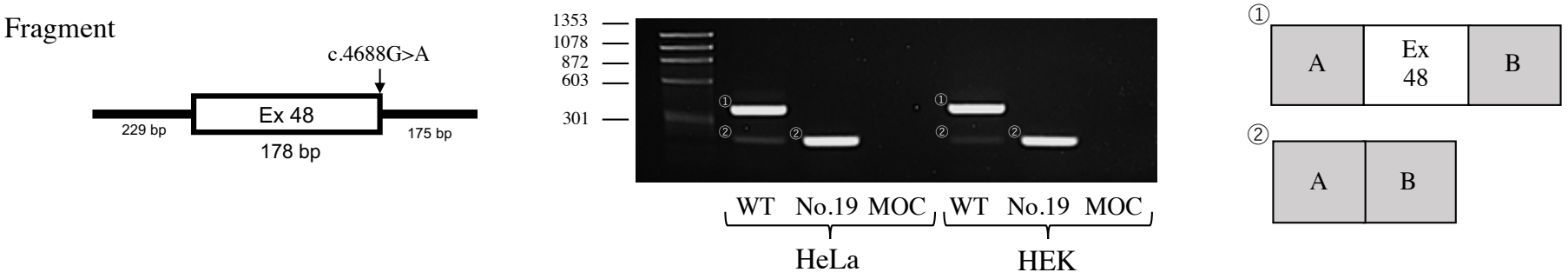
(o-1) No.18: c.4297G>A, p.(Gly1433Ser)



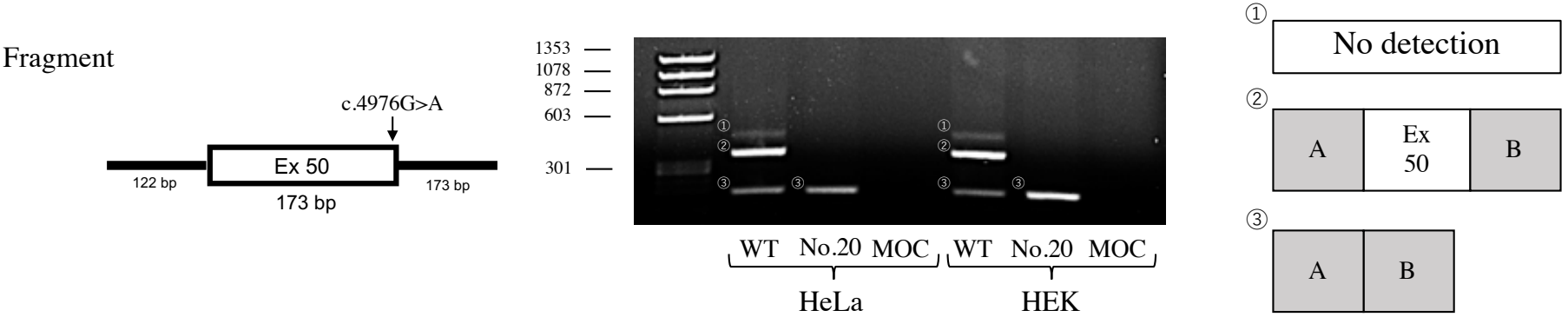
(o-2) No.18: c.4297G>A, p.(Gly1433Ser)



(p) No.19: c.4688G>A, p.(Arg1563Glu)

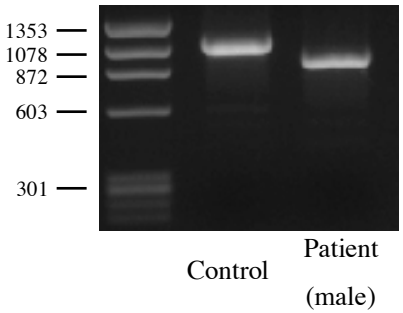


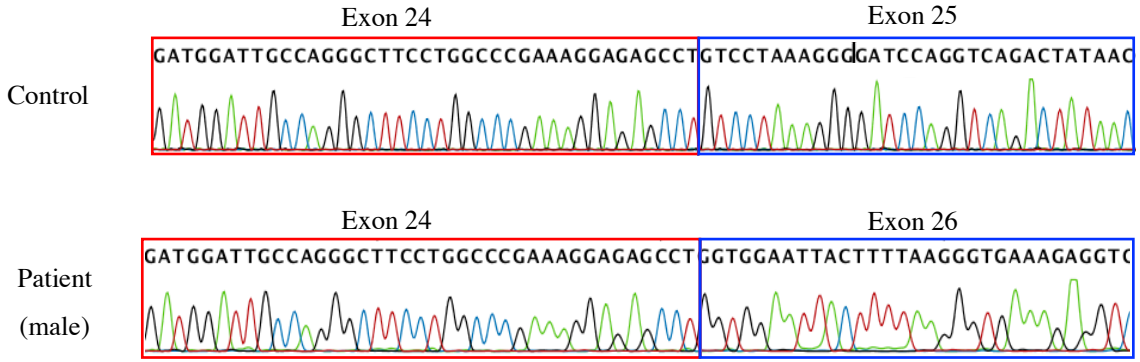
(q) No.20: c.4976G>A, p.(Ser1659Asn)



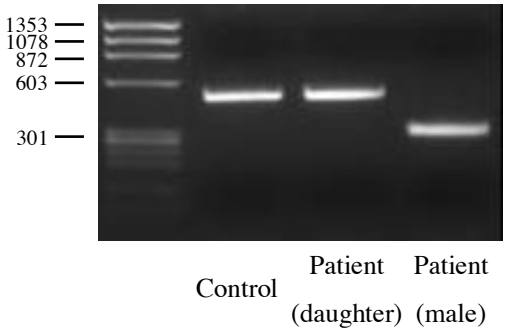
Supplemental Figure S3.

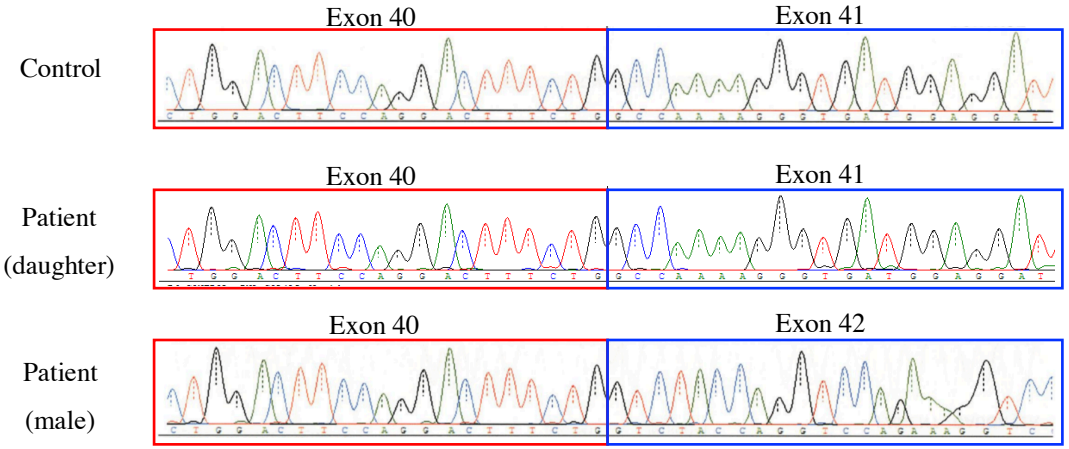
(a) No.4: c.1498G>T,



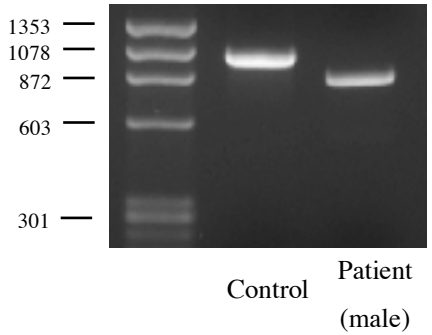


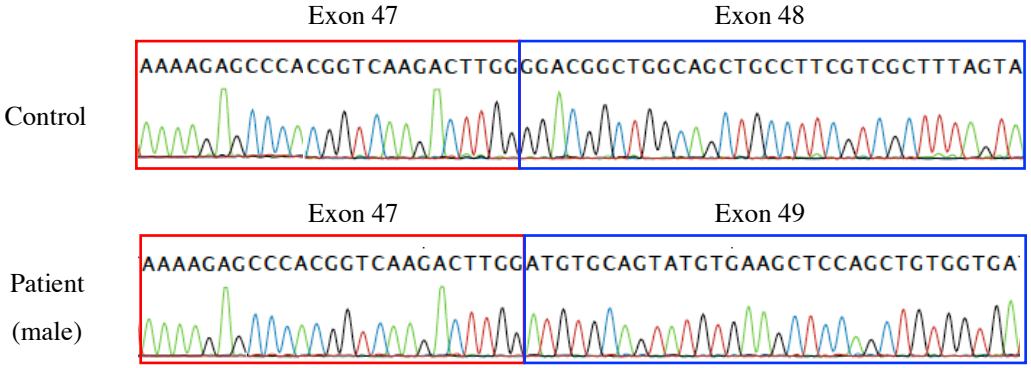
(b) No.13: c.3790G>A, p.(Gly1264Arg)





(c) No.19: c.4688G>A, p.(Arg1563Glu)





Supplementary Figure Legends

Supplementary Figure S1.

Schematic for the hybrid minigene. The H492 vector contains two cassette exons, A and B, with a multiple cloning site that includes *NheI* and *BamHI* restriction sites. In addition, the vector contains a cytomegalovirus (CMV) enhancer-promotor and a bovine growth hormone gene (BGH) polyadenylation site.

Supplementary Figure S2.

Fragmentary component of the minigene, the electrophoresis results, and schematic for the transcript analysis from the minigene assay. (a) Both WT and the No. 1 variant (c.891A>T) showed only canonical transcripts. (b) WT showed only a canonical transcript, whereas the No. 2 variant (c.1165G>A) showed only a transcript with exon 19 skipping. (c) WT showed only a canonical transcript, whereas the No. 3 variant (c.1423G>A) showed transcripts with a 36-bp deletion or exon 21 skipping. (d) WT and Nos. 4 and 5 variants (c.1948G>T, c.1948G>A) showed transcripts that were canonical or had exon 25 skipping, and there was a higher proportion of exon skipping in these variants than in the WT. (e) WT showed only a canonical transcript, whereas the No. 6 variant (c.2244G>T) showed only a transcript with exon 28 skipping. (f) WT showed only a canonical transcript, whereas the No. 7 variant (c.2509G>A) showed transcripts that were canonical or had an 11-bp insertion, 9-bp deletion, or exon 30 skipping. (g) WT showed only a canonical transcript, whereas the No. 8 variant (c.2677G>C) showed transcripts that were canonical or had an 18-bp deletion or exon 31 skipping. The No. 9 variant (c.2677G>A) showed transcripts that were canonical or had an 18-bp deletion. (h) WT showed transcripts that were canonical or had exon 32 skipping; there was also a thin band that could not be sequenced. The No. 10 variant (c.2767G>C) showed only a transcript with exon 32 skipping. (i-1) When the fragment comprised exon 35 and a flanking intron, both WT and the No. 11 variant (c.3106G>A) showed only canonical transcripts. (i-2) When

the fragment included exons 35–36 and a flanking intron, both WT and the No. 11 variant showed transcripts that were canonical or had a 75-bp insertion or exon 36 skipping. (j) WT showed only a canonical transcript, whereas the No. 12 variant (c.2373G>A) showed transcripts that were canonical or had exon 37 skipping. (k) WT and the No. 13 variant (c.3790G>A) showed transcripts that were canonical or had exon 41 skipping; there was a higher proportion of exon skipping in the variants than in the WT, and only WT from the HeLa cells showed a thin band that could not be sequenced. (l) WT showed transcripts that were canonical or had exon 42 skipping, whereas the No. 14 variant showed only a transcript with exon 42 skipping. (m) WT showed transcripts that were canonical or had exon 43 skipping, whereas the No. 15 variant (c.3997G>A) showed only a transcript with exon 43 skipping. (n-1) When the fragment comprised exon 44 and a flanking intron, WT and Nos. 16 and 17 variants (c.4069G>C, c.4069G>A) showed only canonical transcripts. (n-2) However, when the fragment comprised exons 44–45 and a flanking intron, WT showed only a canonical transcript, whereas Nos. 16 and 17 variants showed transcripts that were canonical or had exon 44 skipping. (o-1) When the fragment comprised exon 46 and a flanking intron, both WT and the No. 18 variant (c.4297G>A) showed only canonical transcripts. (o-2) When the fragment comprised exons 46–47 and a flanking intron, both WT and the No. 18 variant showed the same transcripts. cDNA from HeLa cells showed only a canonical transcript and cDNA from HEK cells showed transcripts that were canonical or had an exon 46 deletion plus an intron 46 insertion. (p) WT showed transcripts that were canonical or had exon 48 skipping, whereas the No. 19 variant (c.4688G>A) showed only a transcript with exon 48 skipping. (q) WT showed transcripts that were canonical or had exon 50 skipping; there was also a thin band that could not be sequenced. The No. 20 variant (c.4976G>A) showed only a transcript with exon 50 skipping. WT, wild-type

Supplementary Figure S3.

Results from the electrophoresis and direct sequencing of the reverse transcription-polymerase chain

reaction with patients' blood samples. (a) Male patient with the No. 4 variant (c.1948G>T) expressed only a transcript with exon 25 skipping. (b) Male patient hemizygous for the No. 13 variant (c.3790G>A) expressed only a transcript with exon 41 skipping; however, the daughter heterozygous for the No. 13 variant expressed only a canonical transcript. (c) Male patient with the No. 19 variant (c.4688G>A) expressed only a transcript with exon 48 skipping.