Supplemental Appendix

Contents

sTable 1. Prediction by the Human Splicing Finder.

Family number	Result	Mutation	Transcription	CV of original site (WT)	CV of original site (Mutant)	HSF prediction	CV of cryptic site (WT)	CV of cryptic site (Mutant)	Patient's site	CV of patient's site (WT)	CV of patient's site (Mutant)
A7	-	c.610-2A>T	19bp deletion	88.39	59.44	-	-	-	19bp down stream from the original site	87.63	87.63
A8	Compatible	c.3247-2A>C	127bp deletion (Exon 37 skipping)	90.2	61.26	-	-	-	-	-	
A17	Compatible	c.3455-1G>A	1bp deletion	81.72	52.77	1bp downstream from the original site	46.06	75.11	1bp downstream from the original site	46.06	75.11
A19	Compatible	c.1948+894C>G	106bp insersion with stop codon	-	-	c.1948+893 (donor)	60.6	89.55	c.1948+893 (donor)	60.6	89.55
A21	Incompatible	c.3790G>A	186bp deletion (Exon 41 skipping)	91.2	80.62	-	-	-	-	-	-
A27	Compatible	c. 991-1G>A	1bp deletion	83.19	54.24	1bp down stream from the original site	42.41	71.36	1bp down stream from the original site	42.41	71.36
A28	-	c. 2147-2A>G	18bp deletion	89.64	60.7	18bp downstream from the original site	85.97	85.97	18bp downstream from the original site	85.97	85.97
A30	Compatible	c. 990+1G>T	54bp deletion (Exon 17 skipping)	96.71	69.88	-	-	-	-	-	-
A43	-	c.1948+1G>A	169bp delesion (Exon 25 skipping)	100	73.16	-	-	-	-	-	-
A48	Compatible	c.4511-345A>G	74bp insersion with stop codon	-	-	c.4511-345 (donor)	64.1	90.93	c.4511-345 (donor)	64.1	90.93
A91	Compatible	c.646-2A>G	42bp deletion (Exon 12 skipping)	98.28	69.33	-	-	-	-	-	-
A97	-	c.1166-1G>A	174bp deletion (Exon 20 skipping)	81.24	52.29	1bp down stream from the original site	51.09	80.03	-	-	-
A116	Compatible	c.646-1G>C	42bp deletion (Exon 12 skipping)	98.28	69.33	-	-	-	-	-	-
A121	-	c.546+2_3 insT	81bp deletion (Exon 9 skipping)	89.42	70.3	1bp down stream from the original site	22.38	72.21	-	-	-
A126	Compatible	c.609+875G>T	123bp insersion with stop codon	-	-	c.609+874 (donor)	64.21	91.05	c.609+874 (donor)	64.21	91.05
A128	Incompatible	c.2042-18A>G	105bp deletion (Exon 27 skipping)	87.29	87.29	15bp up stream from the original site	51.52	80.46	-	-	-
A141	Compatible	c.3247-2A>G	127bp deletion (Exon 37 skipping)	90.2	61.26	-	-	-	-	-	-
A158	-	c.2245-8T>A	6bp insertion	79.9	77.91	6bp upstream from the original site	42.25	71.19	6bp upstream from the original site	42.25	71.19
A178	-	c.876A>T	35bp deletion	83.61	83.61	17bp up stream from the original site	68.88	95.71	C. 857	95.55	95.55
A196	Compatible	c.1588-1G>A	1bp deletion	90.89	61.95	1bp down stream from the original site	50.76	79.71	1bp down stream from the original site	50.76	79.71
A217	Compatible	c.4510+1754T>G	84bp insertion with stop codon	-	-	c.4510+1750 (donor)	76.8	89.1	c.4510+1750 (donor)	76.8	89.1
A231	-	c.3107-4A>G	3bp insertion	90.34	90.27	3bp up stream from the original site	53.4	82.34	3bp up stream from the original site	53.4	82.34
A244	Compatible	c.4069+1G>A	72bp deletion (Exon 44 skipping)	89.32	62.49	-	-	-	-	-	-
A247	-	c.687+5G>A	42bp deletion (Exon 12 skipping)	87.31	75.14	-	-	-	-	-	-
A258	-	c.3107-4A>G	3bp insertion	90.34	90.27	3bp up stream from the original site	53.4	82.34	3bp up stream from the original site	53.4	82.34
A276	Compatible	c.892-2A>G	45bp deletion (Exon 16 skipping)	83.68	54.73	-	-	-	-	-	-
A298	-	c.3107-4A>G	3bp insertion	90.34	90.27	3bp up stream from the original site	53.4	82.34	3bp up stream from the original site	53.4	82.34
A299	-	c.2395+3A>G	151bp delesion (Exon 29 skipping)	88.31	87.15	-	-	-	-	-	-
A302	-	c.1948+1G>A	169bp delesion (Exon 25 skipping)	100	73.16	-	-	-	-	-	-
A323	-	c.3605-9C>G	8bp insertion	83.96	82.05	8bp upstream from the original site	48.66	77.61	8bp upstream from the original site	48.66	77.61
A326	-	c.2678-1G>A	90bp delesion (Exon 32 skipping)	84.18	55.23	1bp down stream from the original site	46.92	75.87	-	-	-
A329	Compatible	c.1424-367C>T	93bp insersion	-	-	c.1424-368 (donor)	81.7	84.5	c.1424-368 (donor)	81.7	84.5
A333	Incompatible	c.4803+1G>A	345bp insersion	91.34	64.5	-	-	-	-	-	-
A365	Compatible	c.3604+1G>A	51bp delesion (Exon 40 skipping)	80.91	54.08	-	-	-	-	-	-
A371	Compatible	c.1032+3_6 del AA	42bp delesion (Exon 18 skipping)	84.38	58.24	-	-	-	-	-	-
A375	-	c.1948G>T	169bp delesion (Exon 25 skipping)	100	89.13	-	-	-	-	-	-
A384	-	c.4511-11A>G	10bp insertion	80.19	80.44	10bp upstream from the original site	67.51	96.45	10bp upstream from the original site	67.51	96.45
A402	Compatible	c.2146+4delT	105bp delesion (Exon 27 skipping)	71.45	47.18	-	-	-	-	-	-
A422	Incompatible	c.548dupG	63bp delesion (Exon 10 skipping)	86.25	86.25	-	-	-	-	-	-
A424	Compatible	c.385-1G>A	1bp deletion	83.09	54.15	1bp down stream from the original site	51.89	80.83	1bp down stream from the original site	51.89	80.83
A452	Incompatible	c.2395+5G>A	151bp deletion (Exon 29 skipping)	76.46	76.46	-	-	-	-	-	-

HSF: human splicing finder, CV: consensus value, WT: wild-type

sTable 2. Clinical and genetic findings of patients analyzed by genotype-phenotype correlation.

Family number	Effect	ESRD Age (yr)	Mutation	Intron/Exon	Transcription
Splice consensus sequence mutations					
A7*	Truncating	9	c.610-2A>T	Intron 10	19bp deletion
A8*	Truncating	16	c.3247-2A>C	Intron 36	127bp deletion
A8-1	Truncating	14	c.3247-2A>C	Intron 36	127bp deletion
A27*	Truncating	no (5yo)	c.991-1G>A	Intron 17	1bp deletion
A30-1	Non-truncating	34	c.990+1G>T	Intron 17	54bp deletion
A43*	Truncating	no (2yo)	c.1948+1G>A	Intron 25	169bp deletion
A196*	Truncating	no (7yo)	c.1588-1G>A	Intron 23	1bp deletion
A244-1	Non-truncating	17	c.4069+1G>A	Intron 44	72bp deletion
A244-2	Non-truncating	25	c.4069+1G>A	Intron 44	72bp deletion
A276*	Non-truncating	29	c.892-2A>G	Intron 15	45bp deletion
A276-1	Non-truncating	36	c.892-2A>G	Intron 15	45bp deletion
A302-1	Truncating	20	c.1948+1G>A	Intron 25	169bp deletion
A302-2	Truncating	23	c.1948+1G>A	Intron 25	169bp deletion
A326*	Non-truncating	no (12yo)	c.2678-1G>A	Intron 31	90bp deletion
A365*	Non-truncating	no (3yo)	c.3604+1G>A	Intron 40	51bp deletion
A424*	Truncating	no (11yo)	c.385-1G>A	Intron 6	1bp deletion
A424-1	Truncating	15	c.385-1G>A	Intron 6	1bp deletion
A424-2	Truncating	no (8yo)	c.385-1G>A	Intron 6	1bp deletion
A424-3	Truncating	30	c.385-1G>A	Intron 6	1bp deletion
Suspected splice site mutations					
A121*	Non-truncating	no (25yo)	c.546+2_3insT	Intron 9	81bp deletion
A128*	Non-truncating	no (46yo)	c.2042-18A>G	Intron 26	105bp deletion
A128-1	Non-truncating	35	c.2042-18A>G	Intron 26	105bp deletion
A231-1	Non-truncating	no (3yo)	c.3107-4A>G	Intron 35	3bp insertion

A231-2	Non-truncating	42	c.3107-4A>G	Intron 35	3bp insertion
A231-3	Non-truncating	42	c.3107-4A>G	Intron 35	3bp insertion
A258*	Non-truncating	no (3yo)	c.3107-4A>G	Intron 35	3bp insertion
A298*	Non-truncating	25	c.3107-4A>G	Intron 35	3bp insertion
A298-1	Non-truncating	25	c.3107-4A>G	Intron 35	3bp insertion
A298-2	Non-truncating	no (35yo)	c.3107-4A>G	Intron 35	3bp insertion
A299-1	Truncating	21	c.2395+3A>G	Intron 29	151bp deletion
A371*	Non-truncating	no (12yo)	c.1032+3_6 delAAGT	Intron 18	42bp deletion
A371-1	Non-truncating	40	c.1032+3_6 delAAGT	Intron 18	42bp deletion
A402*	Non-truncating	no (7yo)	c.2146+4delT	Intron 27	105bp deletion
Deep intronic mutations creating cryptic exon					
A19*	Truncating	no (22yo)	c.1948+894C>G	Intron 25	106bp insertion with stop codon
A48*	Truncating	no (6yo)	c.4511-345A>G	Intron 47	74bp insertion with stop codon
A126*	Truncating	no (12yo)	c.609+875G>T	Intron 10	123bp insertion with stop codon
A217*	Truncating	no (3yo)	c.4510+1754T>G	Intron 47	84bp insertion with stop codon
A217-1	Truncating	no (1yo)	c.4510+1754T>G	Intron 47	84bp insertion with stop codon
A329*	Non-truncating	22	c.1424-367C>T	Intron 21	93bp insertion
Exonic mutations					
A21*	Non-truncating	24	c.3790G>A	Exon 41	186bp deletion
A21-1	Non-truncating	20	c.3790G>A	Exon 41	186bp deletion
A21-2	Non-truncating	no (10yo)	c.3790G>A	Exon 41	186bp deletion
A178*	Truncating	18	c.876A>T	Exon 15	35bp deletion
A178-1	Truncating	no (21yo)	c.876A>T	Exon 15	35bp deletion
A375*	Truncating	11	c.1948G>T	Exon 25	169bp deletion
A422*	Non-truncating	29	c.548dupG	Exon 10	63bp deletion

* shows proband, ǂ shows the specifically reported case

ESRD: end stage renal disease, yo: years old

sTable3: Immunohistochemical analyses

Family number	Gender	Effect	α5(IV)
Splice consensus sequence mutations			
A7	Male	Truncating	Negative
A8	Male	Truncating	N/A
A17	Female	Truncating	Positive
A27	Male	Truncating	Negative
A28	Female	Non-truncating	Positive
A30	Female	Non-truncating	Positive
A43	Male	Truncating	Negative
A91	Female	Non-truncating	Positive
A97	Female	Non-truncating	Positive
A116	Female	Non-truncating	Positive
A141	Female	Truncating	Positive
A196	Male	Truncating	Negative
A244	Female	Non-truncating	Positive
A276	Male	Non-truncating	N/A
A302	Female	Truncating	N/A
A326	Male	Non-truncating	Negative
A333	Male	Truncating	N/A
A365	Male	Non-truncating	Negative
A424	Male	Truncating	Negative
Suspected splice site mutations			
A121	Male	Non-truncating	Positive
A128	Male	Non-truncating	Negative (skin)
A158	Female	Non-truncating	N/A
A231	Female	Non-truncating	Positive
A247	Female	Non-truncating	Positive
A258	Male	Non-truncating	N/A

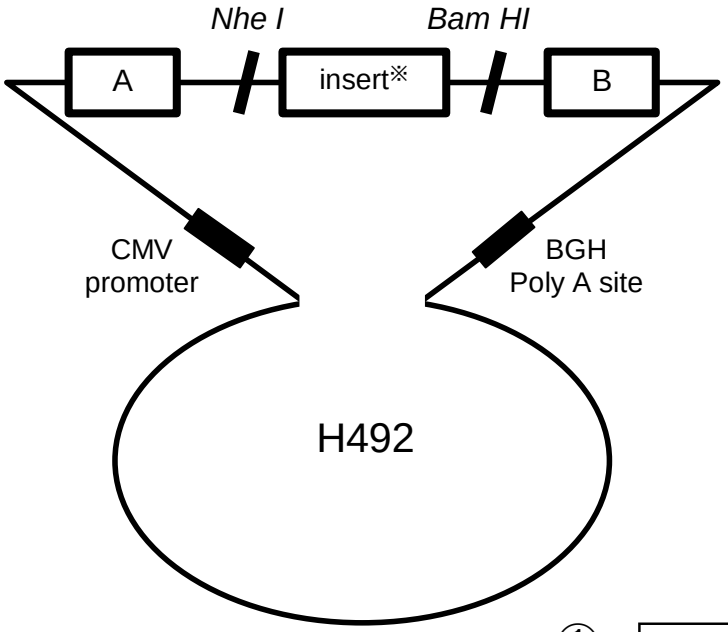
A298	Male	Non-truncating	Positive
A299	Female	Truncating	Positive
A323	Female	Non-truncating	Positive
A371	Male	Non-truncating	N/A
A384	Female	Truncating	Positive
A402	Male	Non-truncating	N/A
A452	Female	Truncating	Positive
Deep intronic mutations creating cryptic exon			
A19	Male	Truncating	Negative
A48	Male	Truncating	Negative (skin)
A126	Male	Truncating	Negative
A217	Male	Truncating	Negative
A329	Male	Non-truncating	Negative
Exonic mutations			
A21	Male	Non-truncating	N/A
A178	Male	Truncating	Negative
A375	Male	Truncating	N/A
A422	Male	Non-truncating	Positive

N/A: not available, α5(IV): α5 chain of type IV collagen

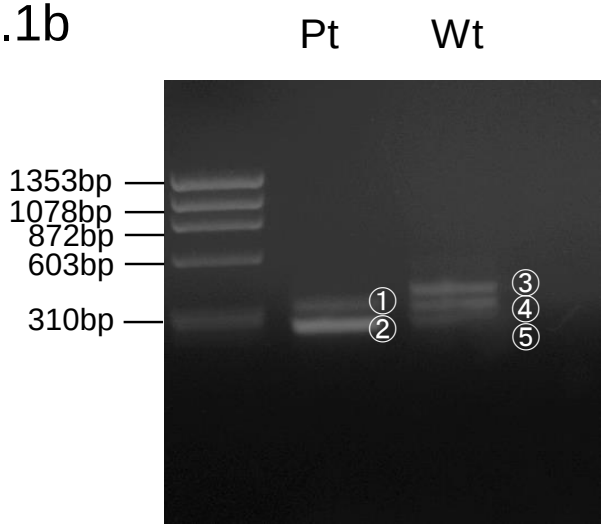
sTable4:α5 chain of Type IV collagen expression in male cases

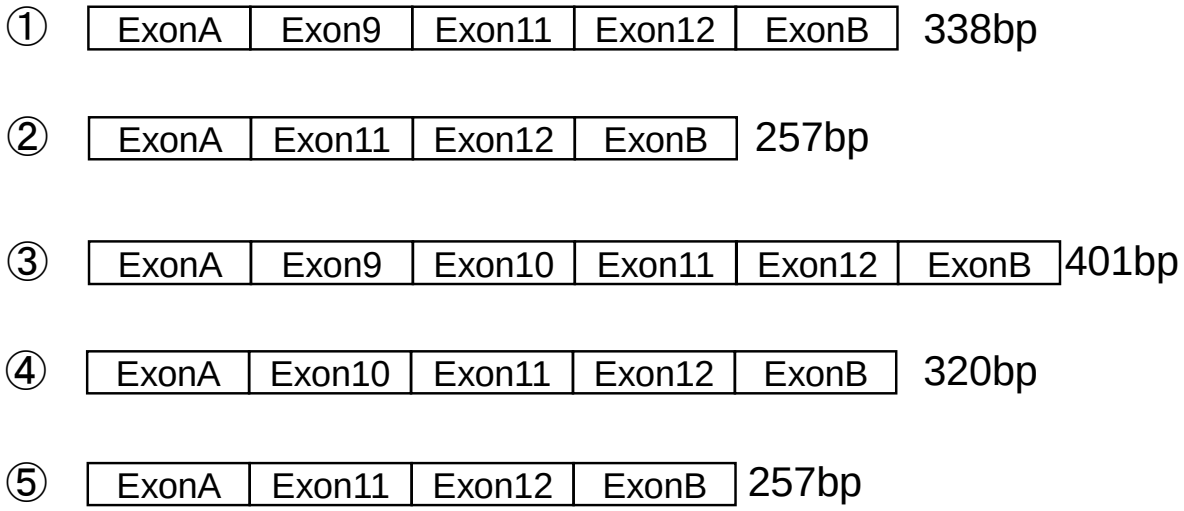
	Positive	Negative
Truncating	0	10
Non-truncating	3	4

sFig.1a

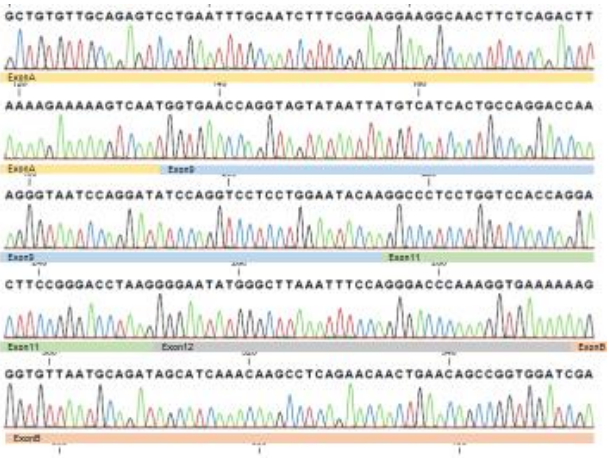


sFig.1b





sFig.1c

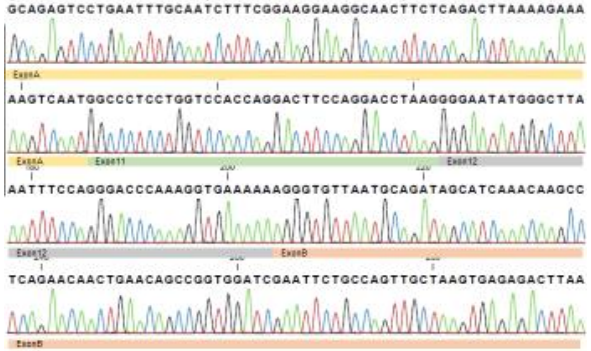


Pt①: Exon10 skipping

Pt②: Exon9+Exon10 skipping

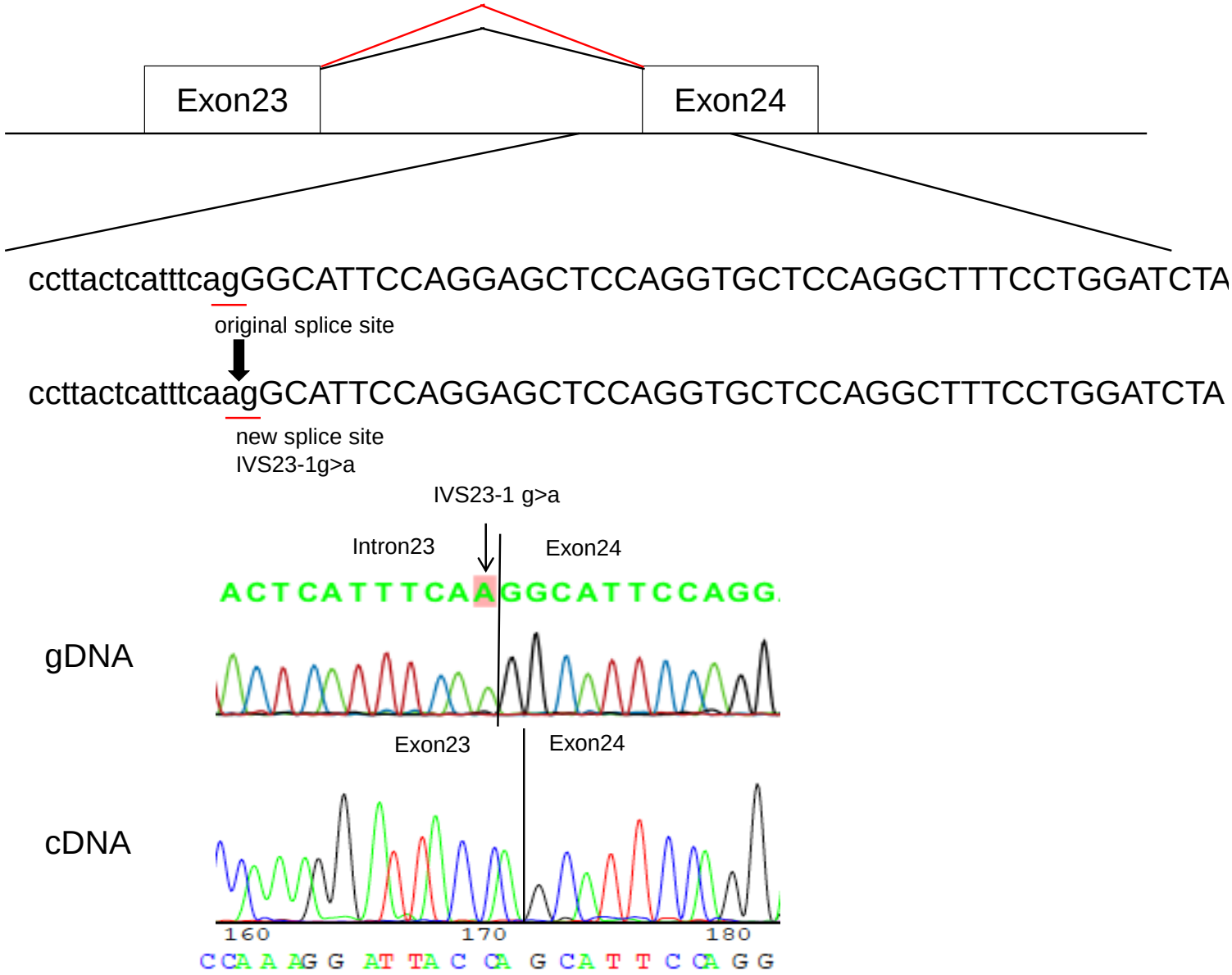
Wt③: full

Wt④: Exon9 skipping



Wt⑤: Exon9+Exon10 skipping

A


Exon23
Exon24
Normal:
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original splice site
Patient:
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new splice site
IVS23-1g>a
IVS23-1 g>a
Intron23
Exon24
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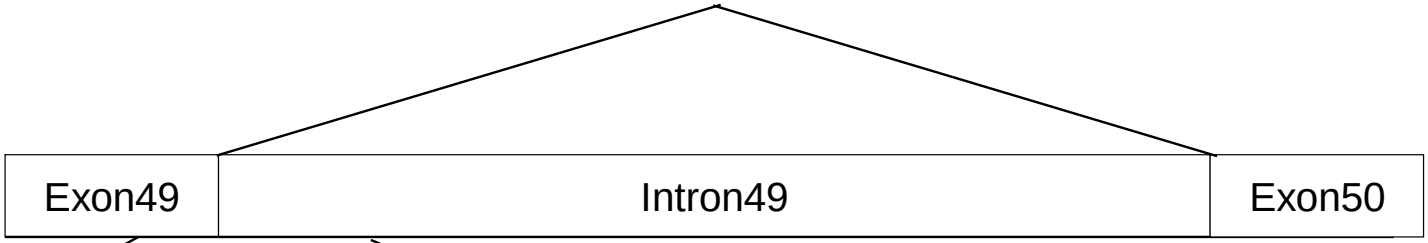
gDNA
Exon23
Exon24
cDNA
160
170
180
CCAAAGG ATTACCAGCATTCCAGG

B



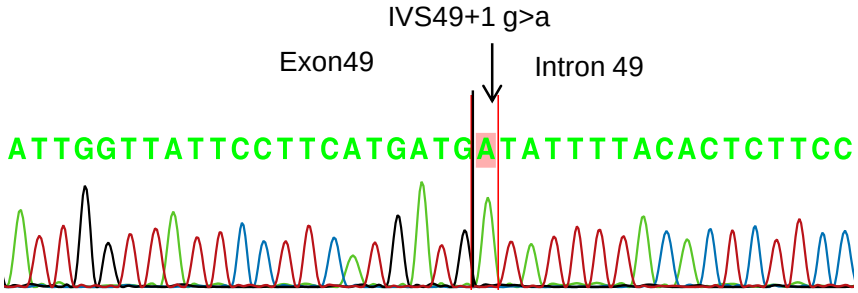
Normal: GTTATTCCTTCATGATGg t a t t t t a c a c t c t t

original splice site

Patient: GTTATTCCTTCATGATGATATTTTACACTCTT

IVS49+1 g>a

gDNA



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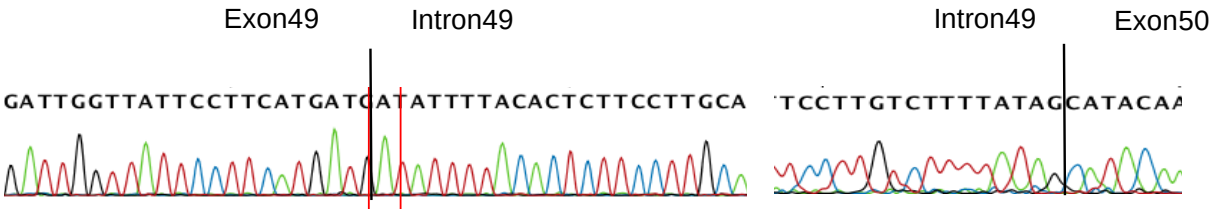

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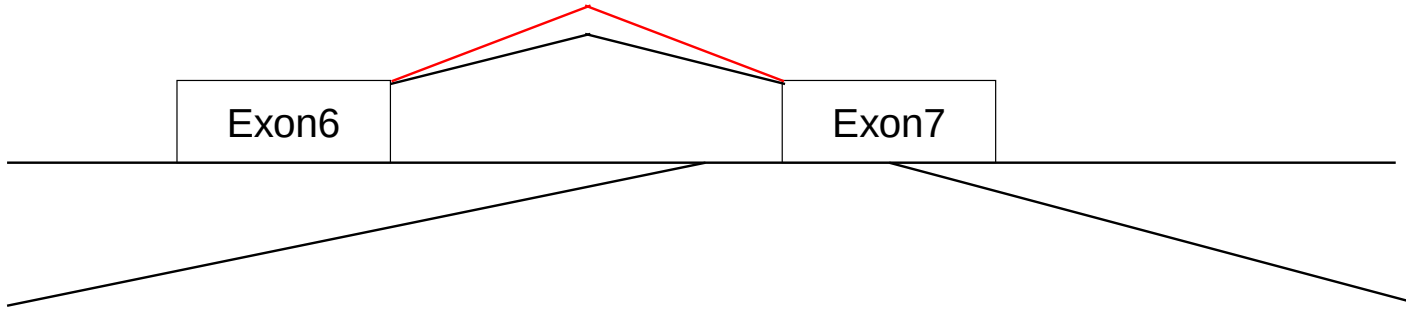
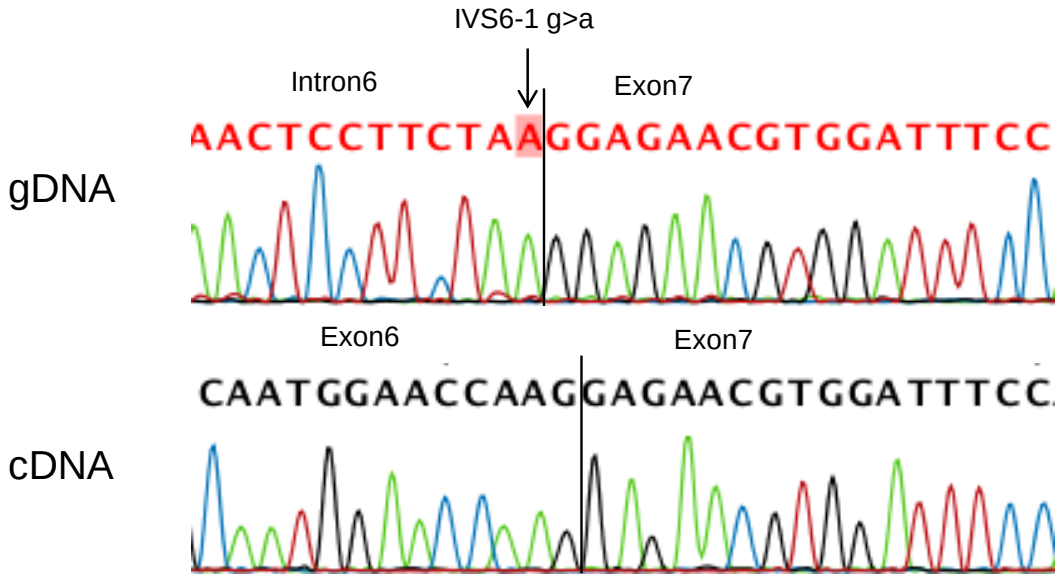


Normal: tttaactccttctagGGAGAACGTGGATTTCCAGGCAGTCCCGGTTTTCCTGGTTTACAG

original splice site

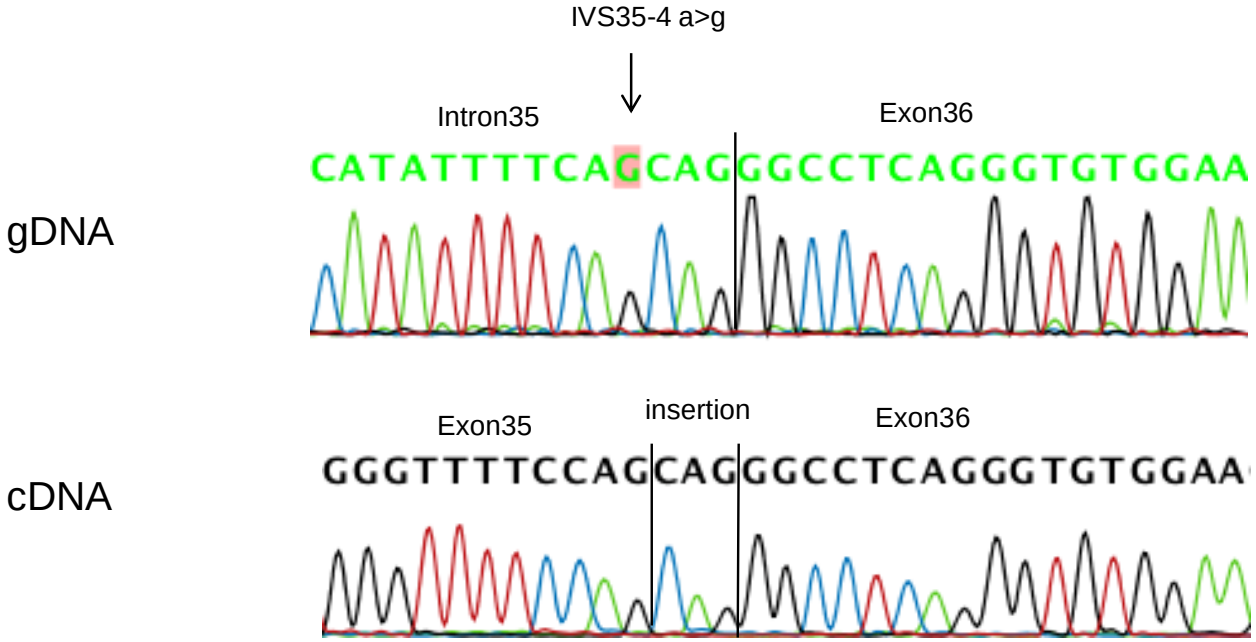
Patient: tttaactccttctaagGAGAACGTGGATTTCCAGGCAGTCCCGGTTTTCCTGGTTTACAG

new splice site
IVS6-1 g>a



D


Exon35
Exon36
Normal:
atatcacatattttcaa c a g GGCCTCAGGG
original splice site
Patient:
atatcacatattttcagCAGGGCCTCAGGG
new splice site
IVS35-4 a>g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Intron35
Exon36
gDNA
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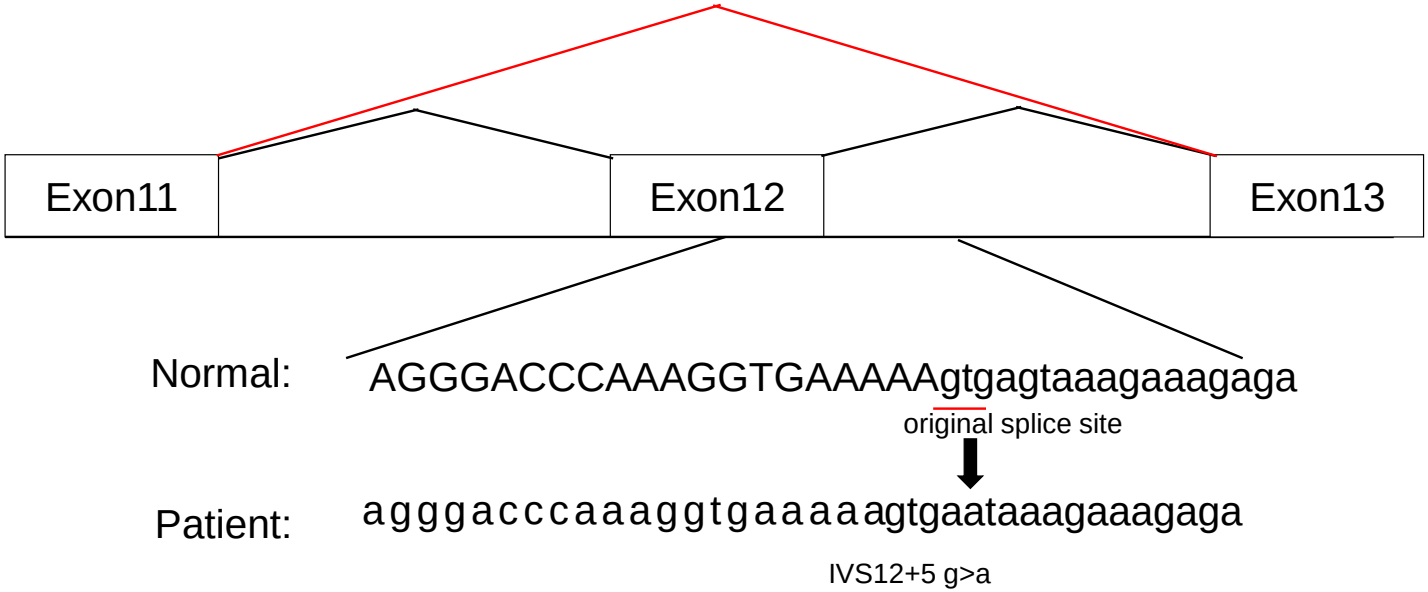
Exon35
insertion
Exon36
cDNA
GGGTTTTCCAGCAGGGCCTCAGGGTGTGGAA

E


Exon11
Exon12
Exon13
Normal:
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original splice site
Patient:
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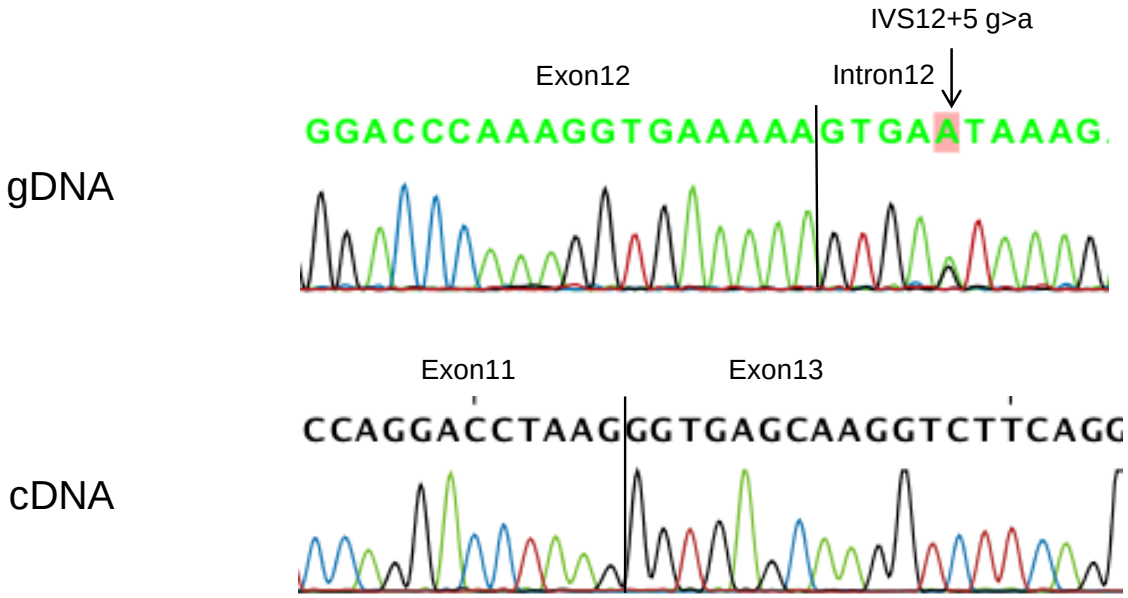
IVS12+5 g>a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Exon12
Intron12
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gDNA
Exon11
Exon13
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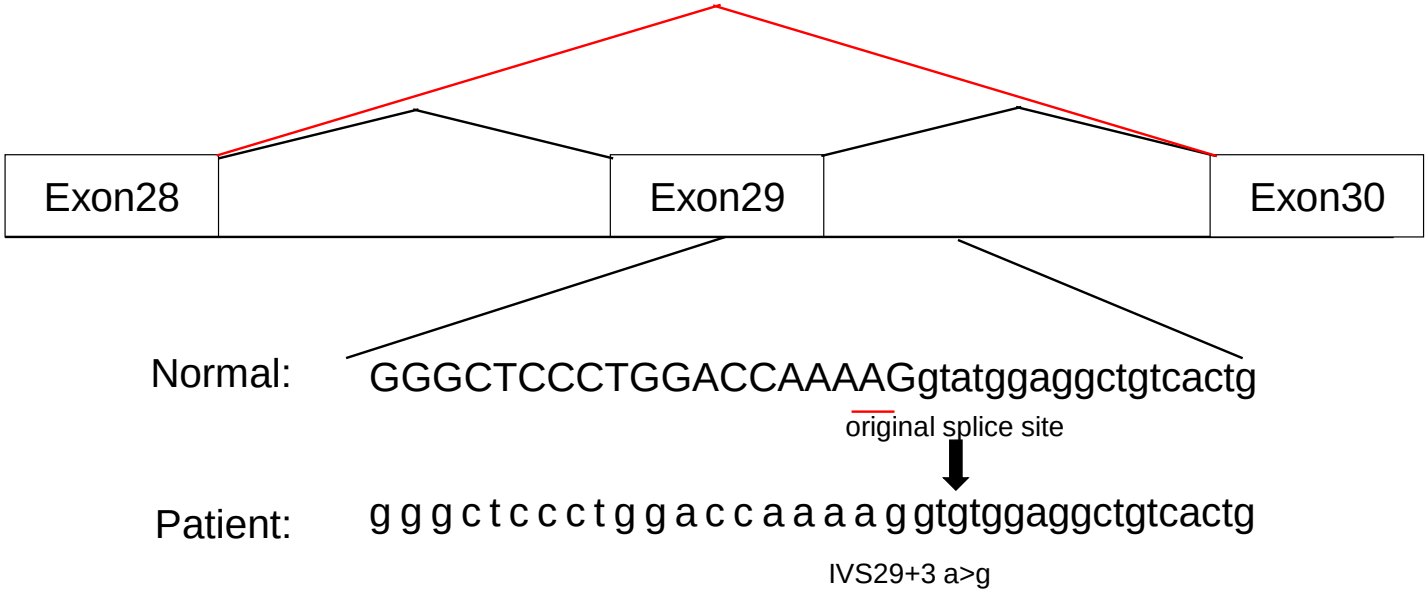
cDNA

F


Exon28
Exon29
Exon30
Normal:
GGGCTCCCTGGACCAAAAGgtatggaggctgtcactg
original splice site
Patient:
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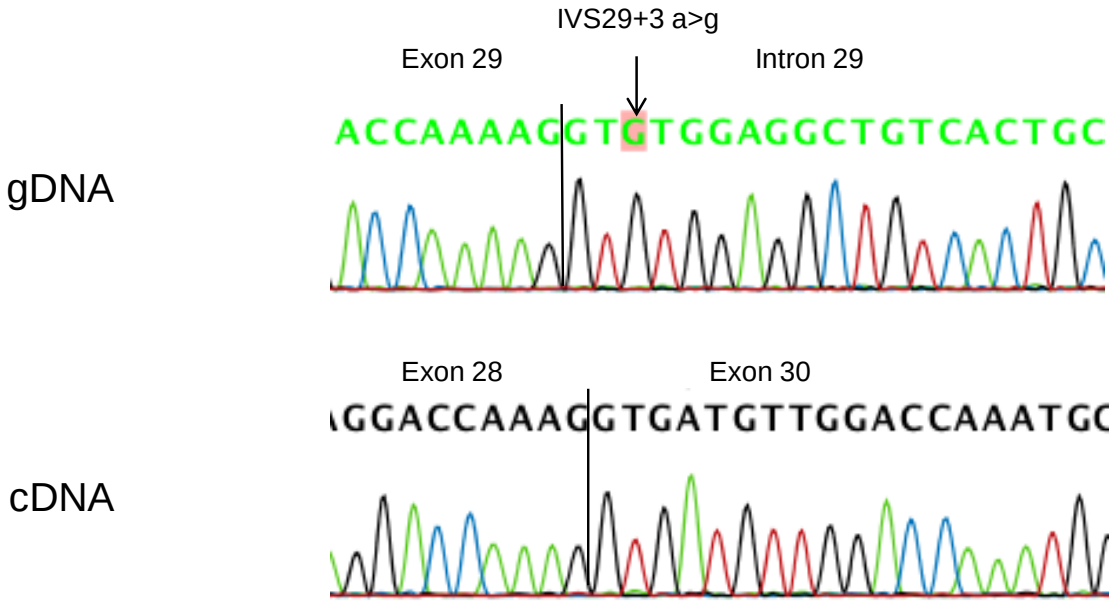
IVS29+3 a>g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Exon 29
Intron 29
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gDNA
Exon 28
Exon 30
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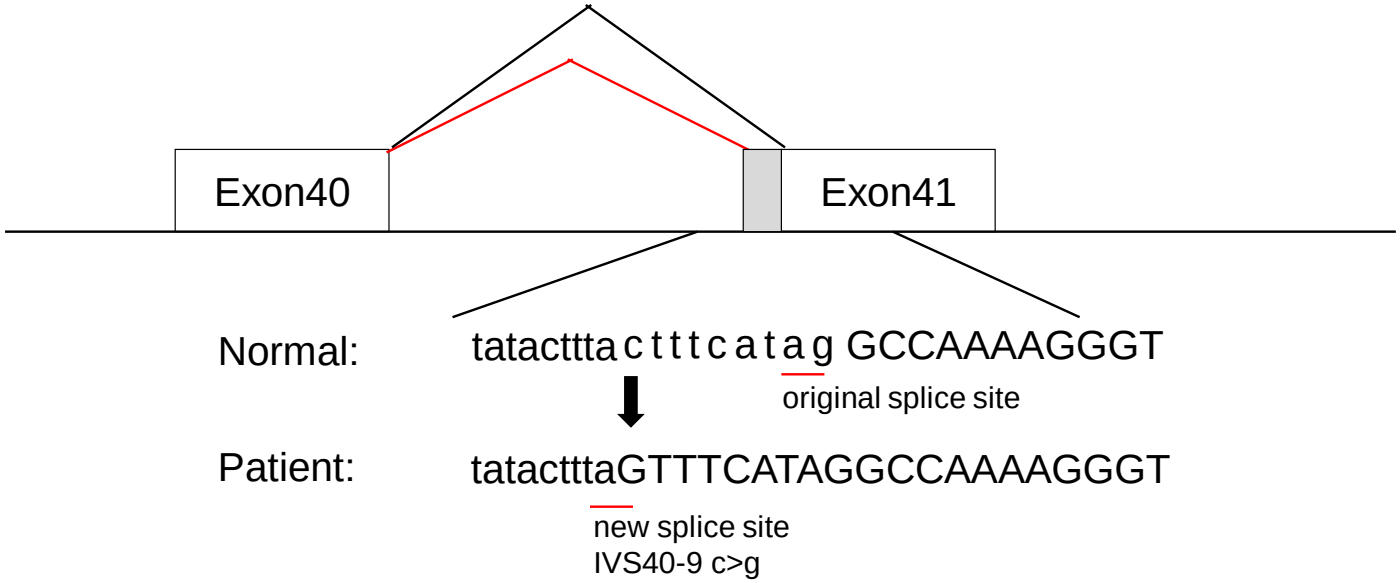
cDNA

G


Exon40
Exon41
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original splice site
Patient:
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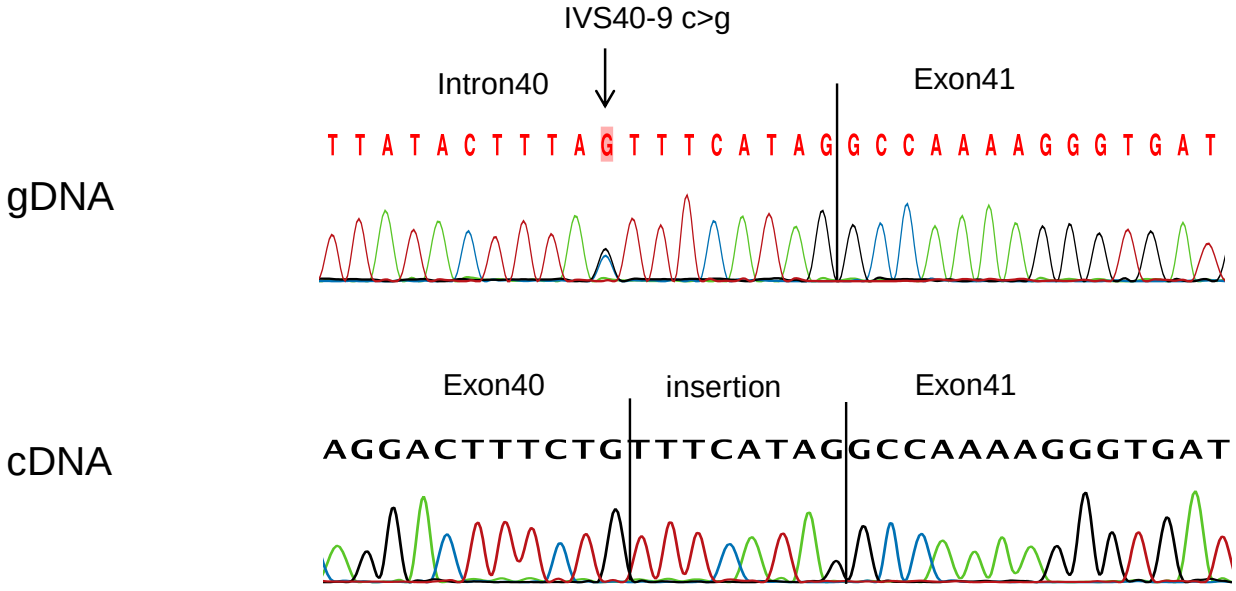
new splice site
IVS40-9 c>g


IVS40-9 c>g
Intron40
Exon41
gDNA
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Exon40
insertion
Exon41
cDNA
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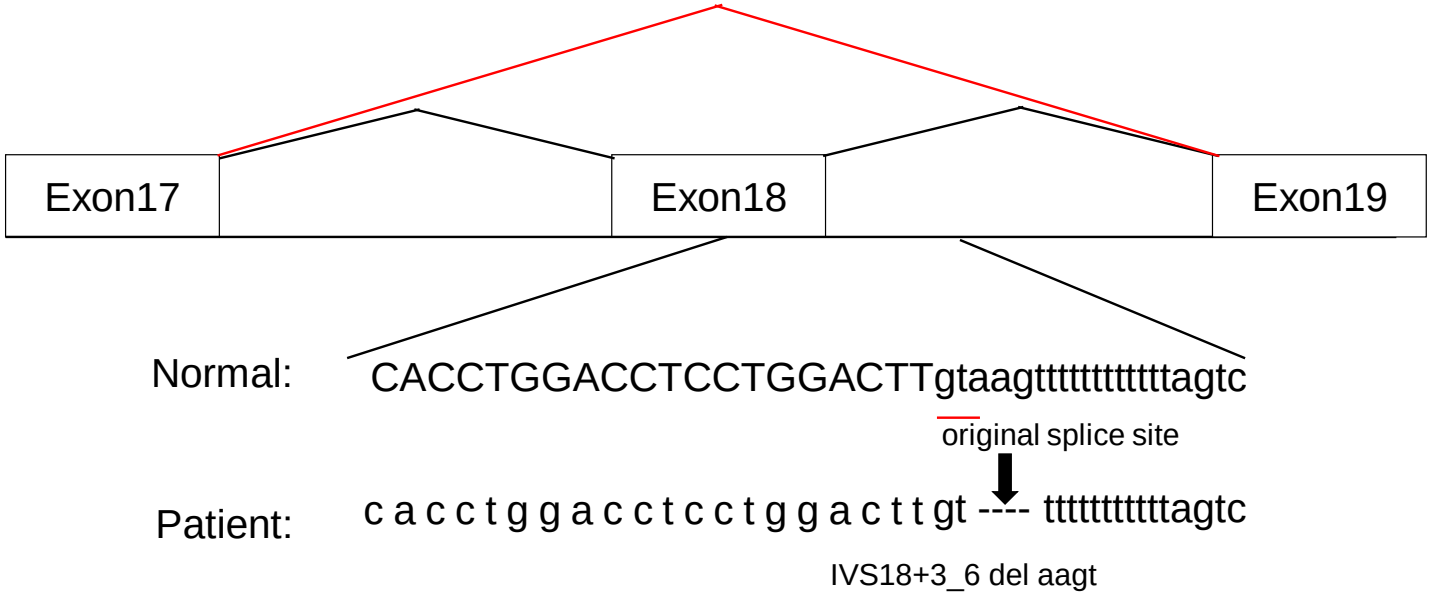
H


Exon17
Exon18
Exon19
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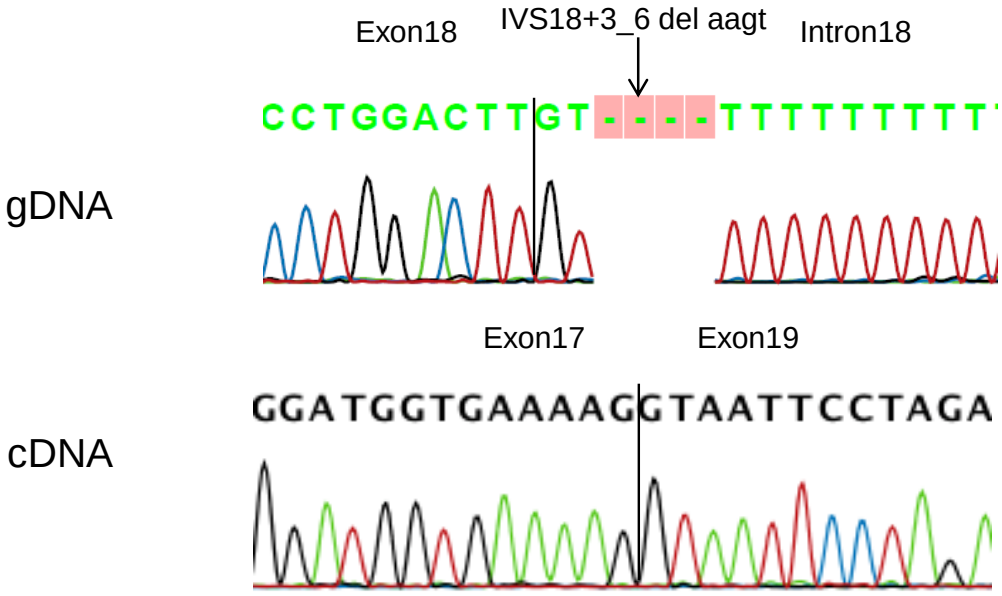
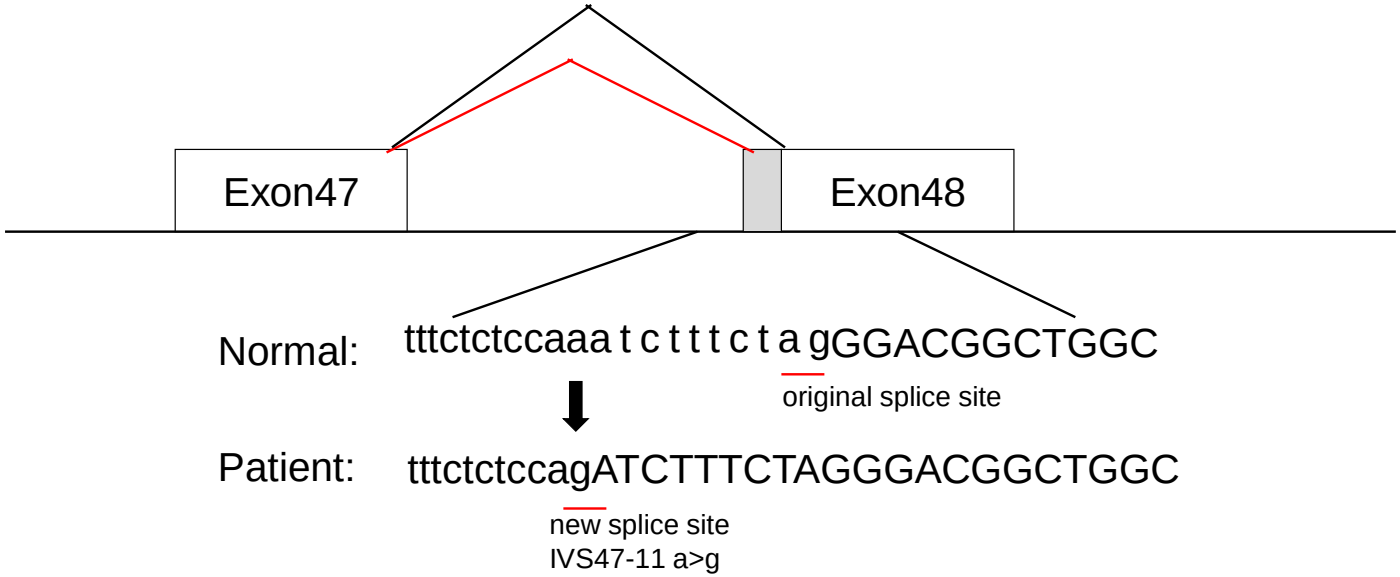
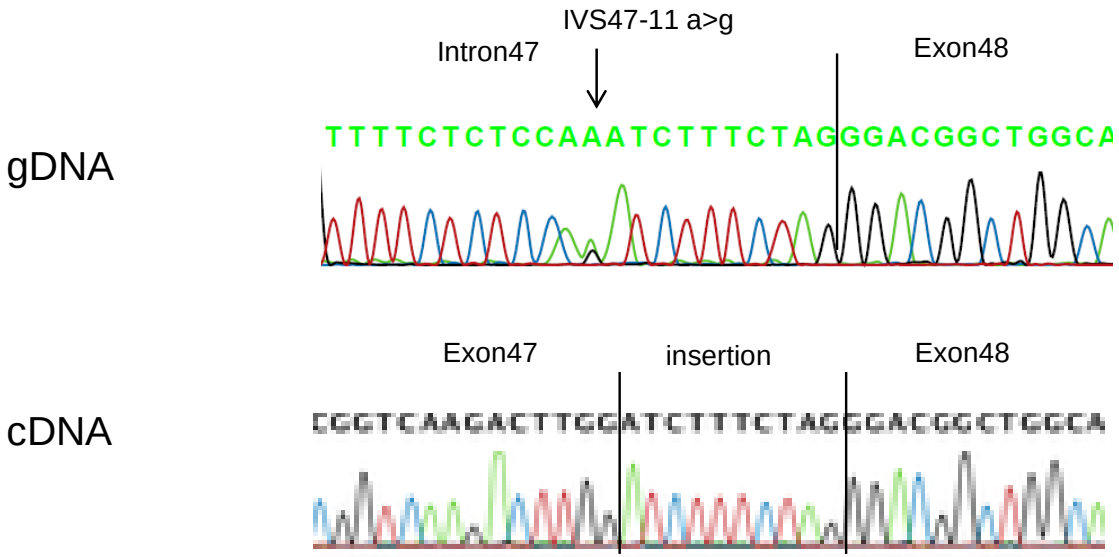
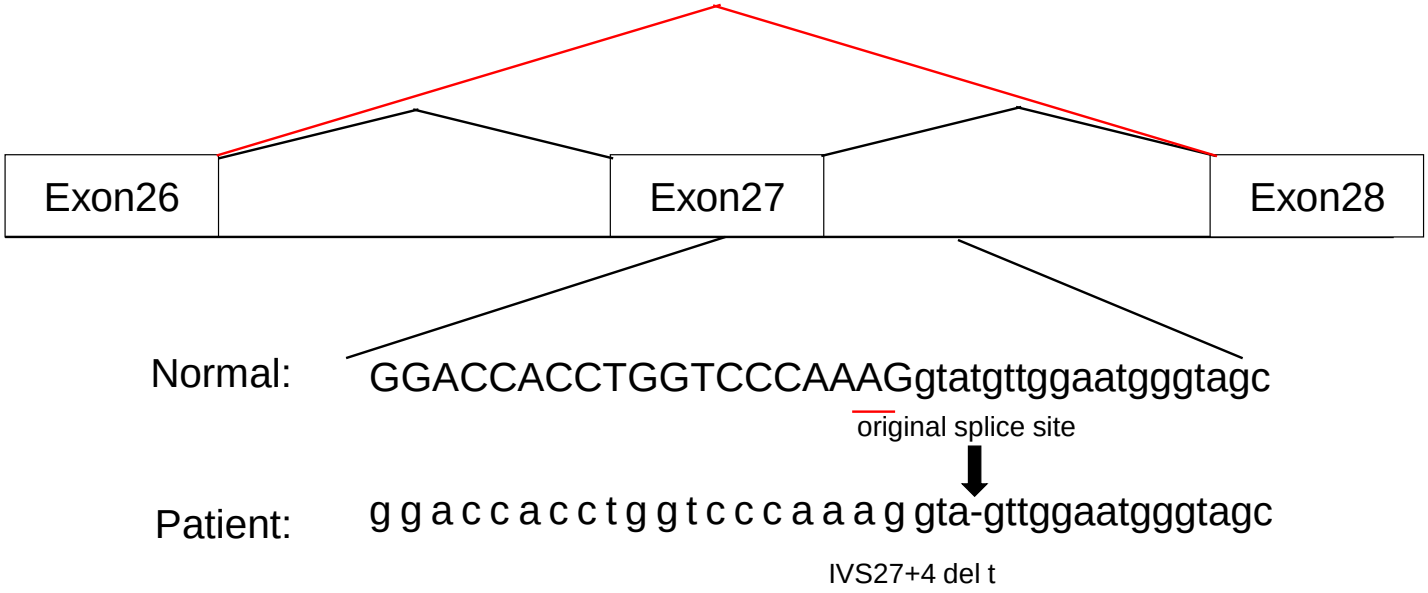
original splice site
Patient:
c a c c t g g a c c t c c t g g a c t t gt ---- ttttttttttagtc
IVS18+3_6 del aagt

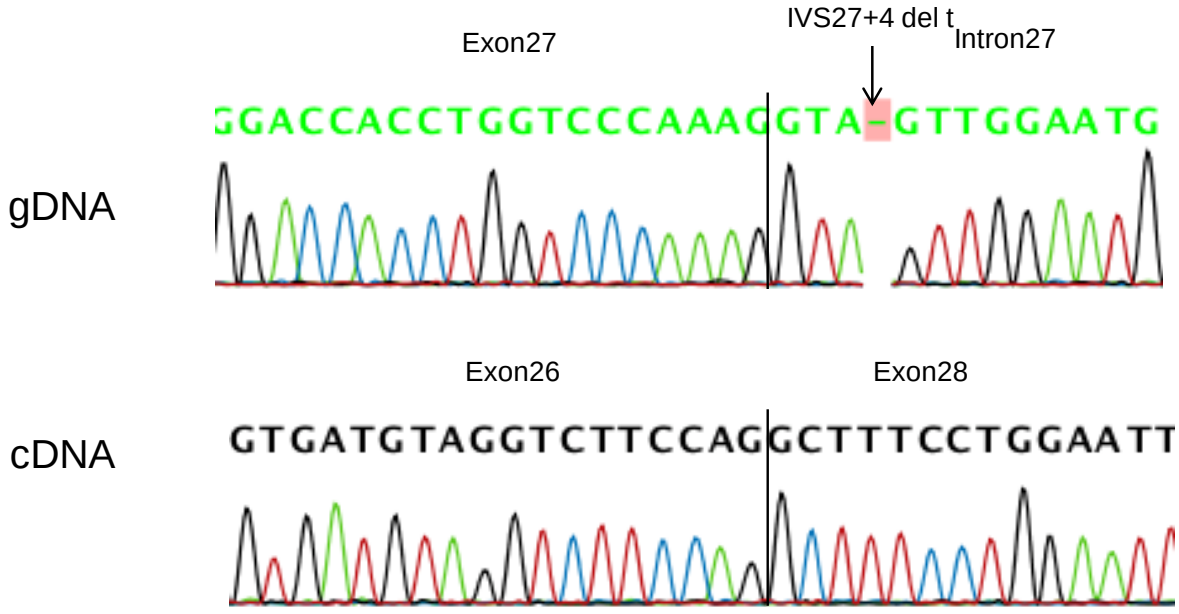

Exon18
IVS18+3_6 del aagt
Intron18
CCTGGACTTGT----TTTTTTTTTT
gDNA
Exon17
Exon19
GGATGGTGAAAAGGTAATTCCTAGA
cDNA


Exon47
Exon48
Normal: tttctctccaaa t c t t t c t a gGGACGGCTGGC
original splice site
Patient: tttctctccagATCTTTCTAGGGACGGCTGGC
new splice site
IVS47-11 a>g

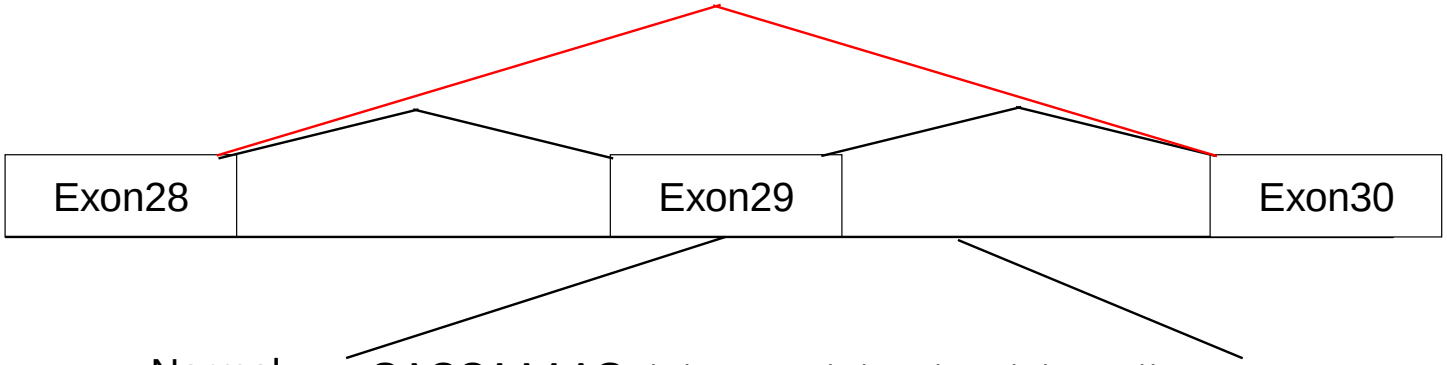

gDNA
IVS47-11 a>g
Intron47
Exon48
TTTTCTCTCCAAATCTTTCTAGGGACGGCTGGCA
cDNA
Exon47
insertion
Exon48
CGGTCAAGACTTGGATCTTTCTAGGGACGGCTGGCA

J


Exon26
Exon27
Exon28
Normal:
GGACCACCTGGTCCCAAAGgtatgttggaatgggtagc
original splice site
Patient:
g g a c c a c c t g g t c c c a a a g gta-gttggaatgggtagc
IVS27+4 del t


IVS27+4 del t
Exon27
Intron27
gDNA
GGACCACCTGGTCCCAAAGGTA-GTTGGAATG
Exon26
Exon28
cDNA
GTGATGTAGGTCTTCCAGGCTTTCCTGGAATT

K

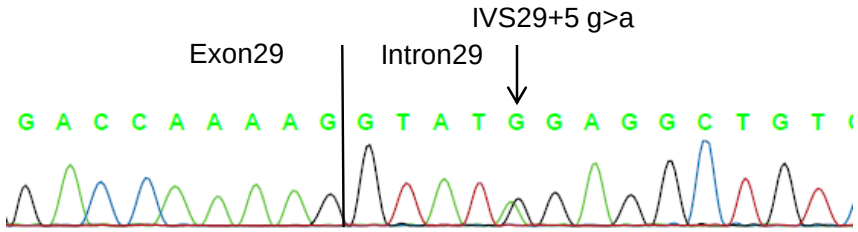


Normal: GACCAAAAGgtatggaggctgtcactgcatctcaactt

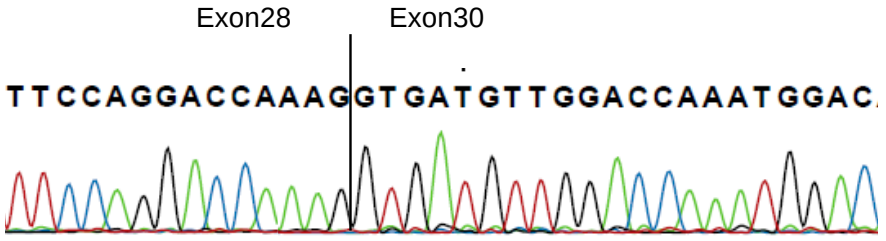
original splice site

IVS12+5 g>a

Patient: g a c c a a a a g gtatagaggctgtcactgcatctcaactt

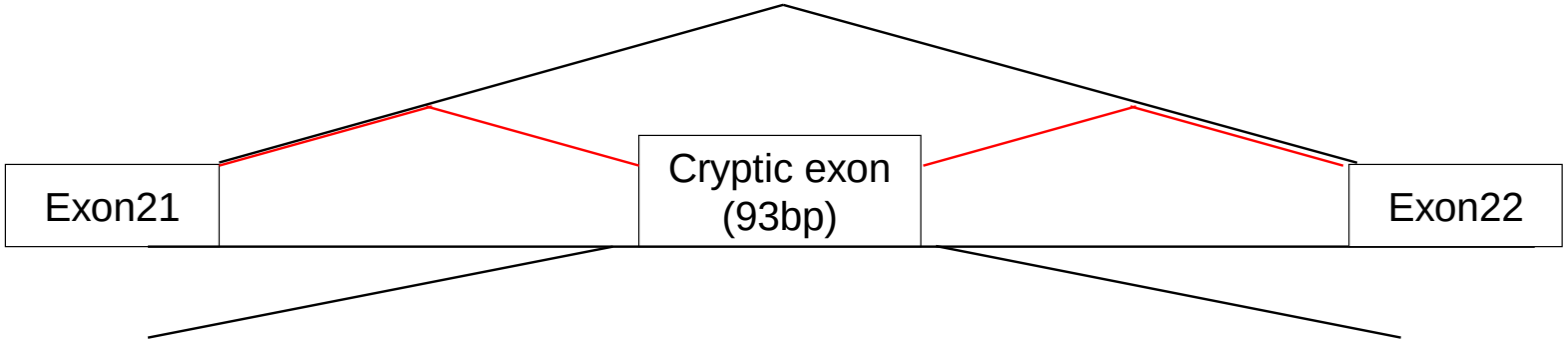


gDNA



cDNA

L

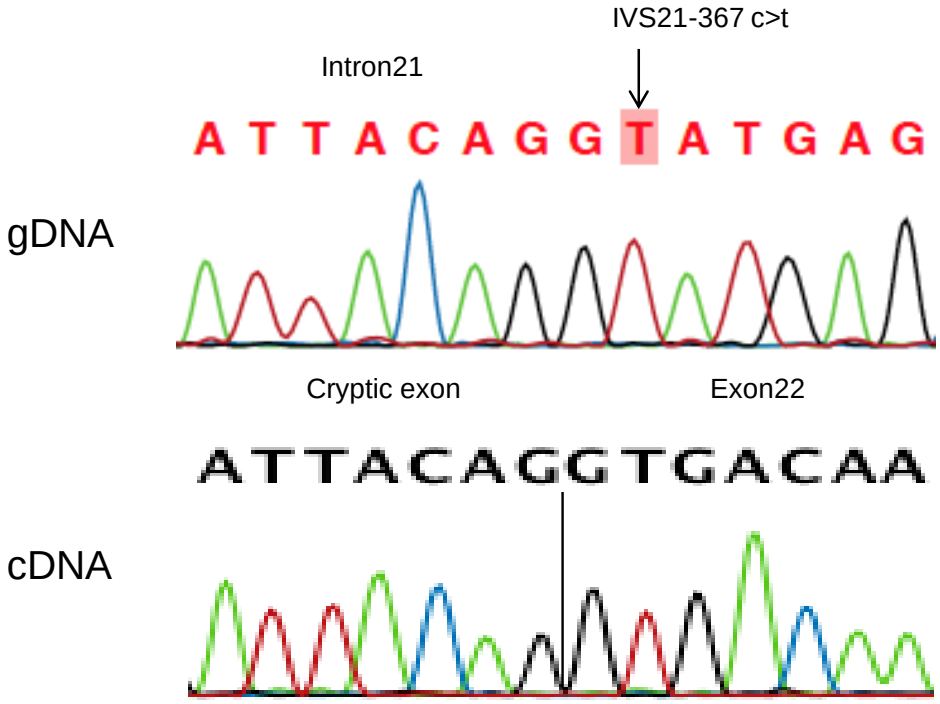


Normal: ttttctgtag a g a t g g g g t t t c g a g t g c t g g g a t t a c a g gcatgagctactg

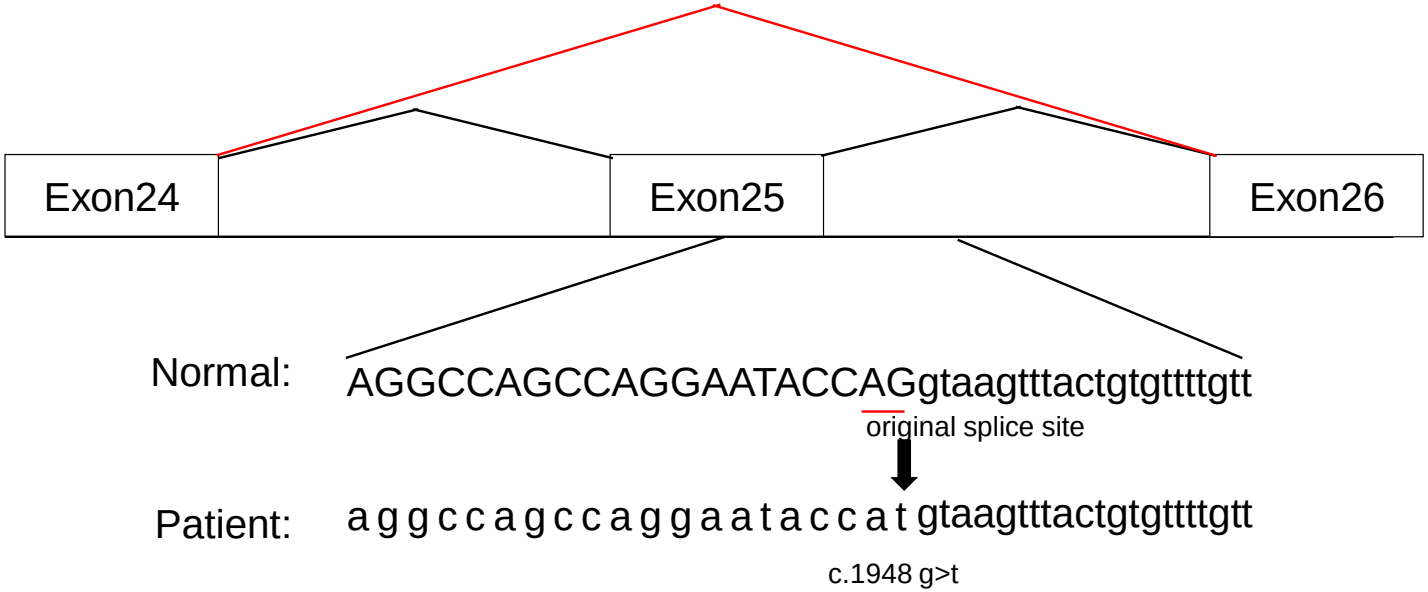
Patient: ttttctgtagAGATGGGGTTTCG AGTGCTGGGATTACAGgtatgagctactg

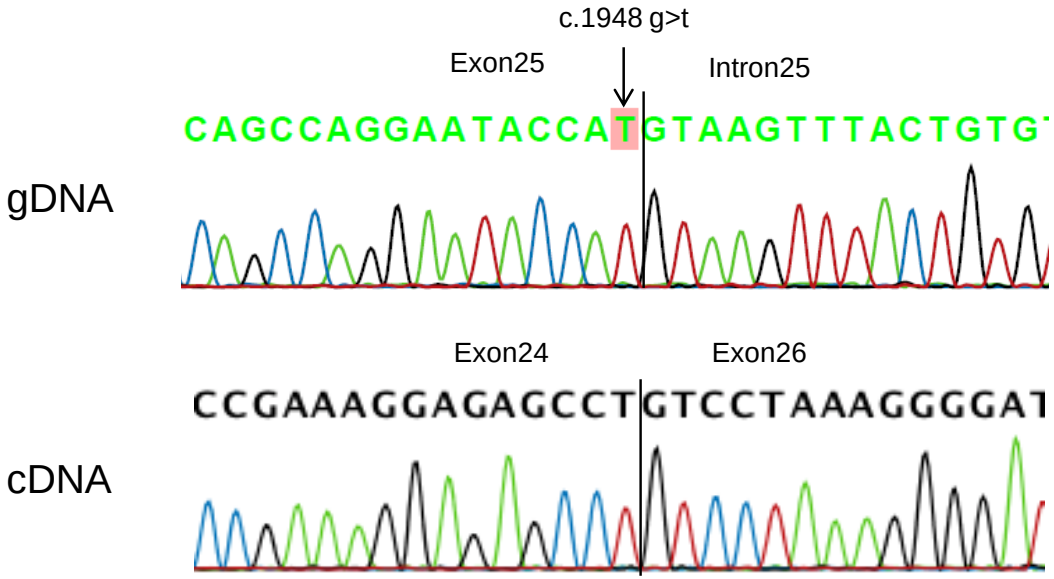
new splice site

new splice site
IVS21-367 c>t

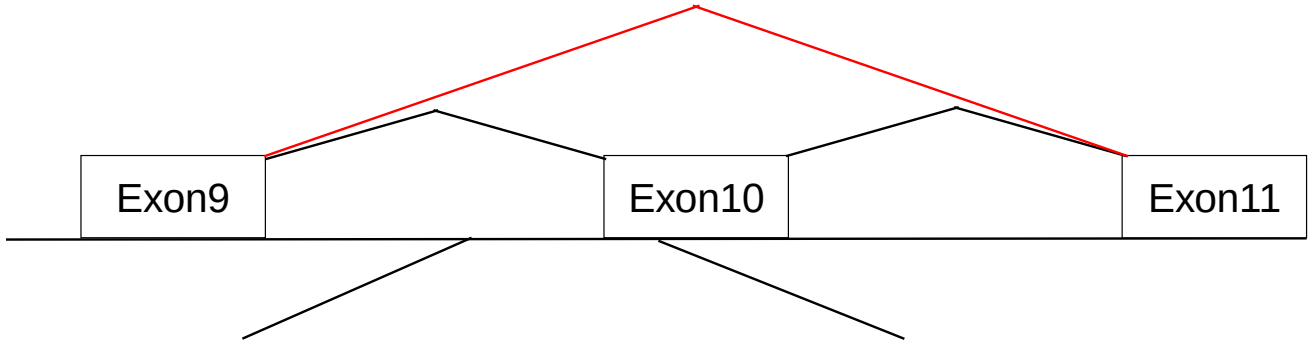


M


Exon24
Exon25
Exon26
Normal:
AGGCCAGCCAGGAATACCAGgtaagtttactgtgttttgtt
original splice site
Patient:
a g g c c a g c c a g g a a t a c c a t gtaagtttactgtgttttgtt
c.1948 g>t


c.1948 g>t
Exon25
Intron25
CAGCCAGGAATACCATGTAAGTTTACTGTG
gDNA
Exon24
Exon26
CCGAAAGGAGAGCCTGTCCTAAAGGGGAT
cDNA

N



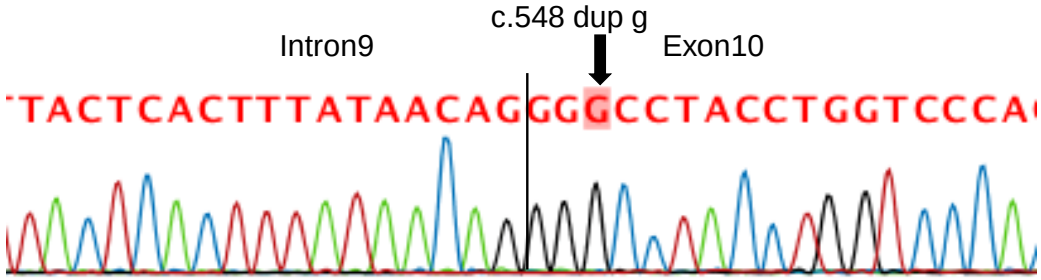
Normal: ctcactttataacagGGCCTACCTGGTCC

original splice site

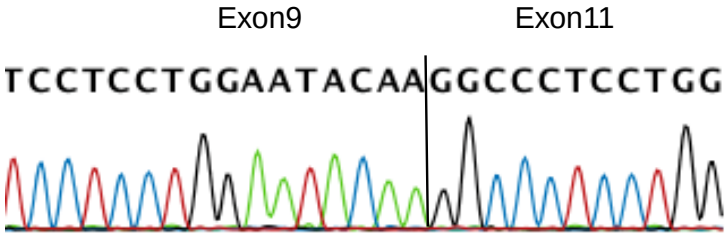
Patient: ctcactttataacagg g g c c t a c c t g g t c c

c.548 dup g

gDNA



cDNA



Supplementary Figure legends

sFigure. 1

a: Schematic for the hybrid minigene. The H492 vector contains two cassette exons, A and B, with a multiple cloning site including *NheI* and *BamHI* restriction sites. The H492 vector also contains a cytomegalovirus (CMV) enhancer-promotor and a bovine growth hormone gene (BGH) polyadenylation site. b: Electrophoresis and schematic for transcript analysis from the minigene constructs. Patients exhibited double bands (exon 10 skipping and exon 9+10 skipping) and wild-type exhibited triple bands. (full and exon 9 skipping and exon 9+10 skipping). Pt: patient; Wt: wild-type.

c: Direct sequencing for transcript analysis from the minigene constructs. Pt: patient; Wt: wild-type.

sFigure. 2

Upper panels show schemas of aberrant splicing (red lines). Normal splicing is indicated by black lines. The original and new splice sites and flanking sequences are shown below. Patients' flanking genomic DNA and cDNA sequences are shown in the lower panels. (A) Patient ID A196. IVS23-1 G>A eliminated the splice acceptor site of intron 23 to activate a new splice site, one nucleotide downstream. (B) Patient ID A333. IVS49+1 G>A disrupted the splicing donor site of intron 49, resulting in an intron 49 insertion, which creates a transcript with a 345-bp insertion. (C) Patient ID A424. IVS 6-1 G>A altered the splice acceptor site of intron 6 one nucleotide downstream, which creates a transcript with a 1-bp deletion. (D) Patient ID A231, A258, A298. IVS35-4 A>G altered the splice acceptor site of intron 35 three nucleotides upstream, which creates a transcript with a 3-bp insertion. (E) Patient ID A247. IVS 12+5 G>A disrupted the splice donor site

of intron 12, resulting in exon 12 skipping, which creates a transcript with a 42-bp deletion. (F) Patient ID A299. IVS29+3 A>G disrupted the splice donor site of intron 29, resulting in exon 29 skipping, which creates a transcript with a 151-bp deletion. (G) Patient ID A323. IVS40-9 C>G altered the splice acceptor site of intron 40 nine nucleotides upstream, which creates a transcript with a 9-bp insertion. (H) Patient ID A371. IVS 18+3_6 del AAGT disrupted the splice donor site of intron 18, resulting in exon 18 skipping, which creates a transcript with a 42-bp deletion. (I) Patient ID A384. IVS48-11A>G altered the splice acceptor site of intron 48 ten nucleotides upstream, which creates a transcript with a 10-bp insertion. (J) Patient ID A402. IVS27+4 del T disrupted the splice donor site of intron 27, resulting in exon 27 skipping, which creates a transcript with a 105-bp deletion. (K) Patients ID A452. IVS29+5 G>A disrupted the splice donor site of intron 29, resulting in exon 29 skipping, which creates a transcript with a 151-bp deletion. (L) Patient ID A329. IVS21-367 C>T produced a new splice donor site, resulting in a cryptic exon activation between exons 21 and 22 and creating a transcript with a 93-bp insertion. (M) Patient ID A375. Mutation in last nucleotide of exon 25, C1948 G>T, disrupted the splice donor site of intron 25, resulting in exon 25 skipping, which creates a transcript with a 169-bp deletion. (N) Patient ID A422. Mutation of the second nucleotide of exon 10, C548 dup G, disrupted the splicing acceptor site of intron 9, resulting in exon 10 skipping, which creates a transcript with a 63-bp deletion.