Supp. Table S1. Oligos used for direct sequencing and confirmation of the detected variants.

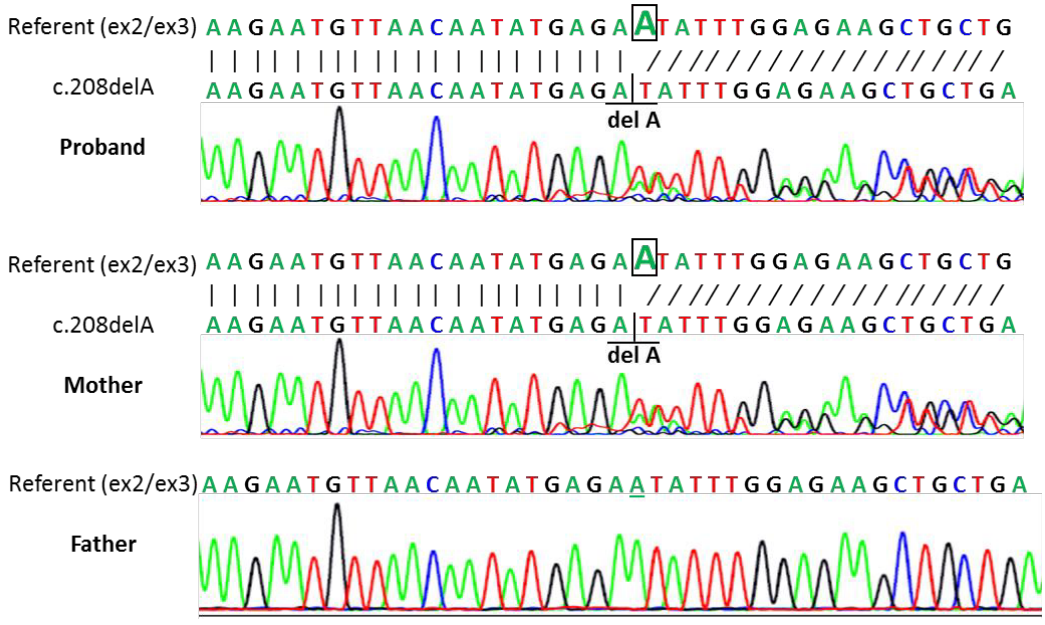
Oligos used for direct DNA sequencing (in house design)			
Target	**Oligo name**	**Oligo sequence**	**Patient ID**
Exon 5	NF1 ex5_F	CAGAATTTAGGGATACCCGAAGAC	#3
	NF1 ex5_R	TTGGTGTTCTAGTTCAGCACAAT	
Exon 9	NF1_F9_F	GAC CTC TCA TTT TGC CAA GAG AT	#4
	NF1_F9_R	TAC AGT GCC TCA GTG ATG CC	
Exon 10	NF1_ex10_F	TCT TCT GGC AGC TGG ATT TT	#5, #6
	NF1_ex10_R	TGA AGC CAA AAA GAA CAG CA	
Exon 11	NF1_ex11_F	TTG ATG TTC GTT TCA AGA CCT	#7
	NF1_ex11_R	TAT GGT CCC TTC GGT CAA GA	
Exon 13	NF1_ex13_F	GTGCAAAAACGATTTTCATTGT	#8
	NF1_ex13_R	TGTTAGGAAATGATAAGGGCAAT	
Exon 19 and 20	NF1 ex19/20_F	TTGGCTTTATCATTTGAAGCA	#10
	NF1 ex19/20_R	GCAGATCAGTTAACAGACAAAAGTC	
Exon 21	NF1_ex21_F	GAA ATT TGA CAC TCG GCT GAT	#11, #12, #28
	NF1_ex21_R	AGA GGC AAG CTG ACC CCT AC	
Exon 28 and 29	NF1_ex28/29_F	GCA AGT GGT TGT CAA CTT TGG	#29, #14, #15
	NF1_ex28/29_R	TCC CTG GAT CTA AGG CAA AT	
Exon 34	NF1_ex34_F	CCG GGT ATC AGA AAT GGA AA	#30
	NF1_ex34_R	CAA GAA GAT GCA AAG TAA AAA GCA	
Exon 35	NF1_ex35_F	TGG TCC TGA GGT CTT TTT GG	#16
	NF1_ex35_R	TGT TGT CTT CAC TCC CTG GT	
Exon 38	NF1_ex38_F	CAATGGTGGGAACTCTTCCT	#18, #33
	NF1_ex38_R	CCCCACAACTTGATGAGGTC	
Exon 46	NF1 ex46_F	AGCTACCAAGATCACCATAGCA	#20
	NF1 ex46_R	AGCGCTTGAGAACATACTATCCA	
Exon 51	NF1_ex51_F	CCACTTGGAAGGAGCAAACG	#23
	NF1_ex51_R	GTCTCAGAGTCCCATTCCCT	
Oligos used for RNA sequencing (from Vatero et al.)			
Target	**Oligo name**	**Oligo sequence**	**Patient ID**
Exon 3	#F1_F	GAGGACATGGCCGCGCACA	#1
	#F1_R	GAAAATAAAACCCCAGAGGCAGAA	
Exon 18	#F6_F	CTACGTACTCCTGGAGCCTCT	#9
	#F6_R	TTGGCTTTTGGATAGTTAAGGAT	
Exon 29	#F11_F	TCTCGGCATTTACTCTACCAAC	#13
	#F11_R	TGGTATAAACAGTGGCACACAC	
Exon 35	#F13_F	TTGTGAAAAGCAACTTTGATG	#17
	#F13_R	ATTGATTTGACCAGTTTTGAA	
Exon 40	#F16_F	CTGGGACACTGCTCAATATCG	#19
	#F16_R	AGGCTTCCCCATATTTTTGCTT	
Exon 47	#F19_F	ATGGGCAGATAAAGCAGATAAT	#21
	#F19_R	CCACGCTCTGTGTATTCACTT	
Exon 49	#F20_F	ATCCTTCACCTGCTATTGTTG	#22
	#F20_R	TTAGGAGCCTTTGTGTCTGATA	
Confirmed with MLPA analysis with P081D1 0418 NFmix1			
Target	**Probe mix**	**Result**	**Patient ID**
NF1_ex4	P081D1	seen as ex4 deletion	#2

Supp. Table S2. ACMG criteria used for classification of the novel variants.

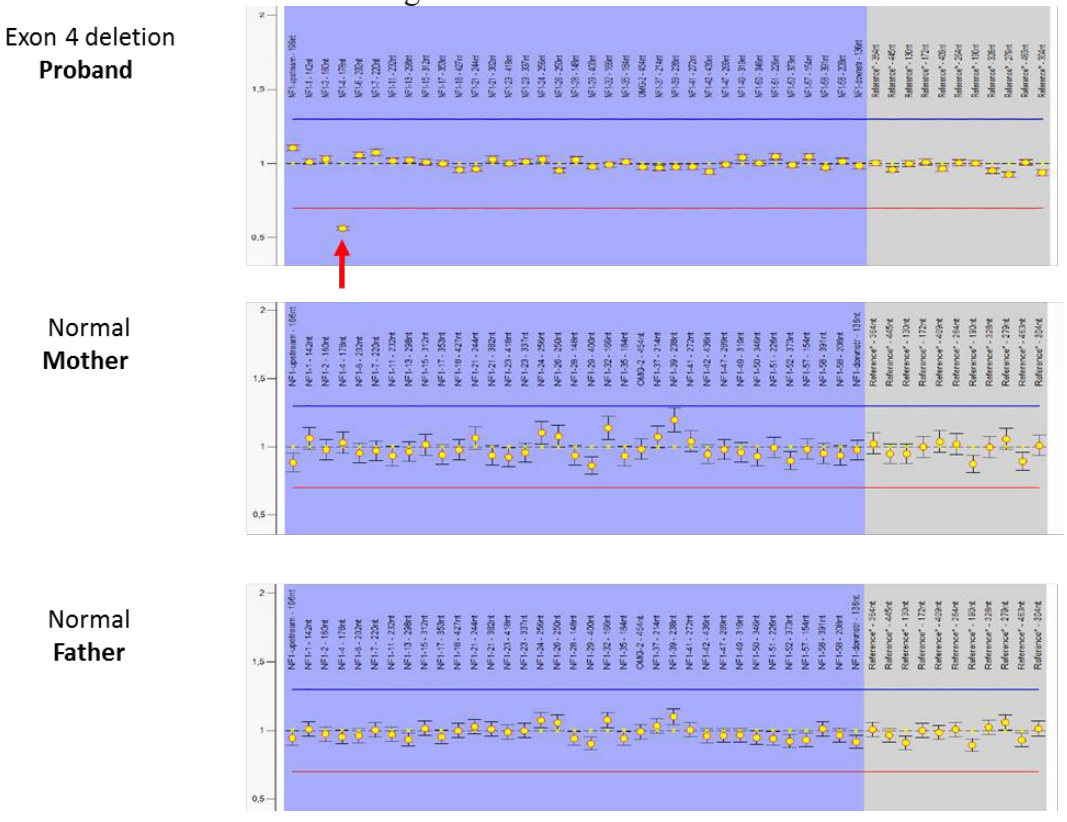
Case ID	Position chr17 (hg19)	Detected variant NM_001042492.3; NP_001035957.1	Exon / Intron	Type of variant	ACMG criteria	ACMG classification
#1	29486029	c.208delA; p.(Ile70TyrfsTer15)	3	frameshift	PVS1, PM2, PP1	Pathogenic
#2	29490256-29490279	c.341_364del; p.(Leu114_Leu121del)	4	in frame	PM4, PM2, PS2	Likely Pathogenic
#8	29541555	c.1480_1481delTT; p.(Leu494ValfsTer16)	13	frameshift	PVS1, PM2, PP1	Pathogenic
#10	29554310	c.2325+1G>C	19i	splice	PVS1, PM2, PP1	Pathogenic
#11	29556127	c.2495_2496dupAC; p.(Ser833ThrfsTer9)	21	frameshift	PVS1, PM2, PS2	Pathogenic
#19	29661887	c.5844C>G; p.(Tyr1948Ter)	40	nonsense	PVS1, PS1, PM2, PP1	Pathogenic
#21	29667571	c.6971delA; p.(Gln2324ArgfsTer22)	47	frameshift	PVS1, PM2, PS2	Pathogenic
#23	29679423	c.7605_7606delinsAT; p.(Lys2536Ter)	51	nonsense	PVS1, PM2, PS2	Pathogenic
#28	29556166_29556174	c.2533_2541del; p.(Cys845_Leu847del)	21	in frame	PM1, PM4, PM2, PS2	Pathogenic
#30	29587472	c.4517delC; p.(Ala1506ValfsTer68)	34	frameshift	PVS1, PM2, PP1	Pathogenic

Supp. Figure S1. Direct sequencing and MLPA results for confirmation of the detected novel variants.

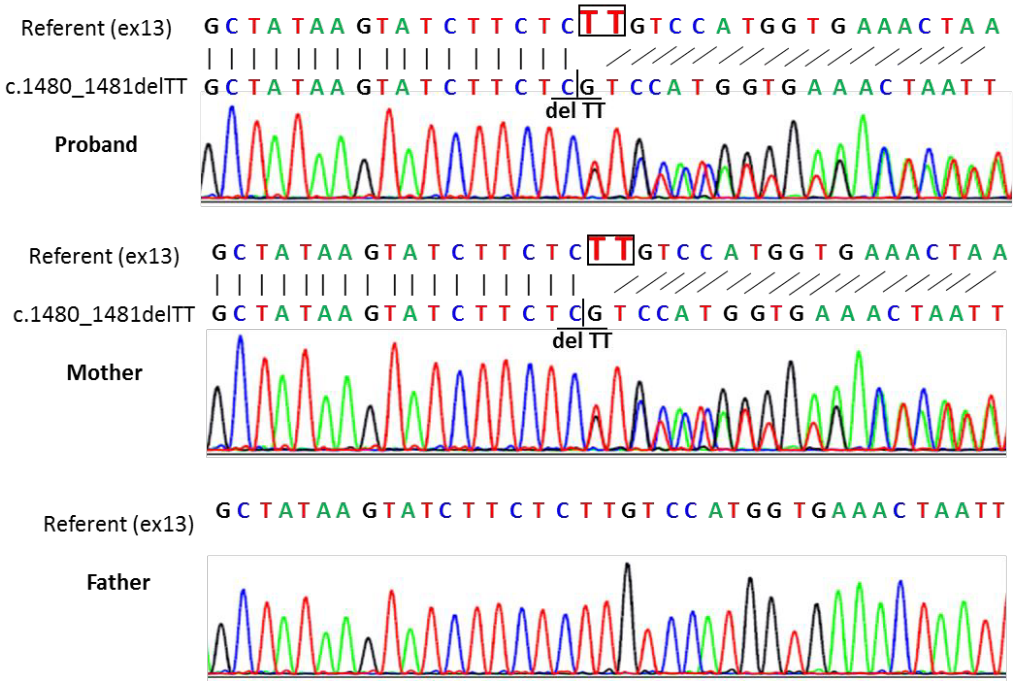
Case #1



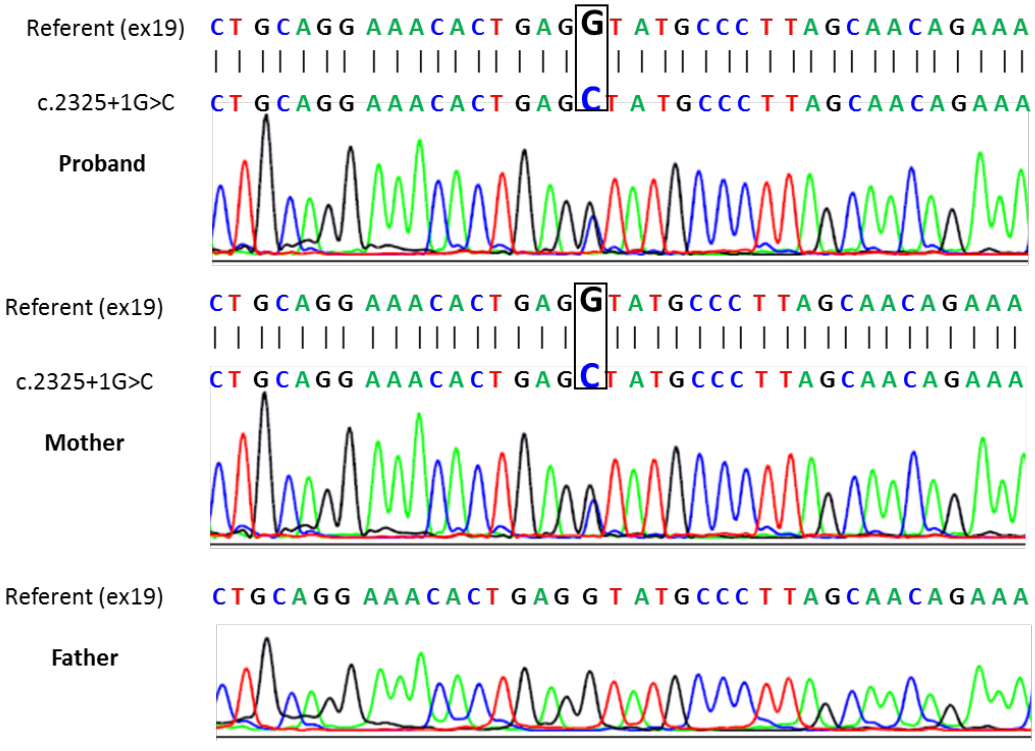
Case #2 MLPA results - variant c.341_364del; p.(Leu114_Leu121del) seen as deletion of exon 4 in patient. Parents normal elektroforetogram.



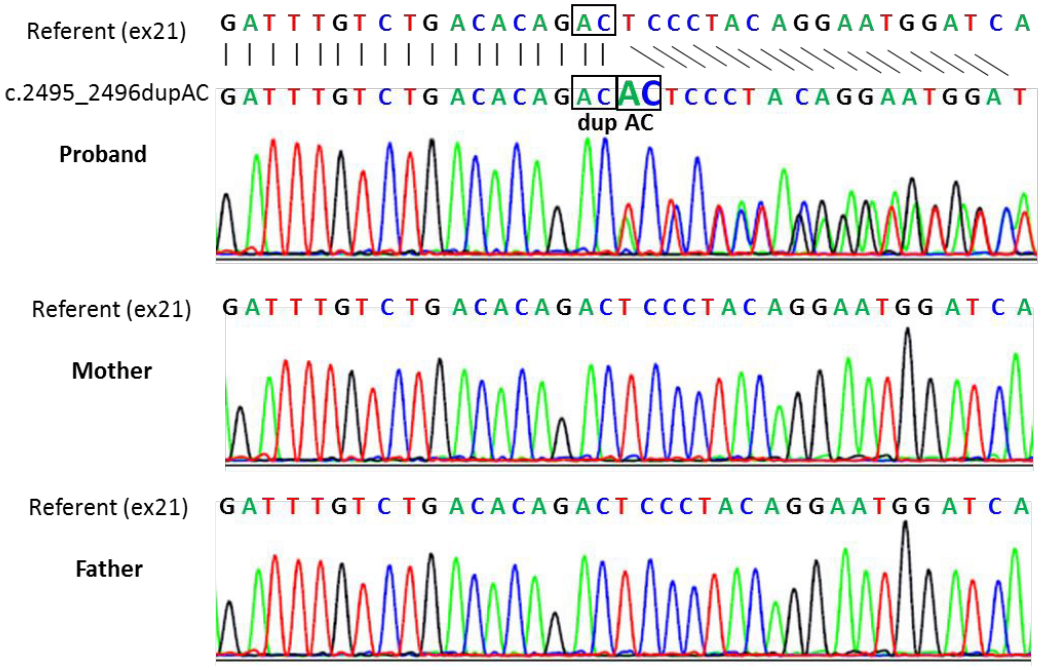
Case #8



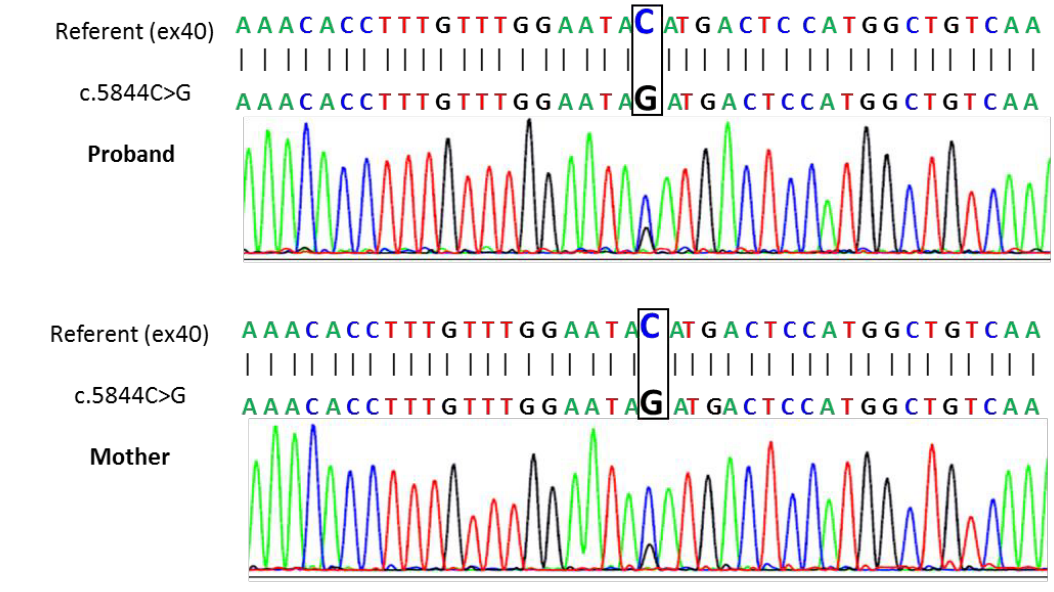
Case #10



Case #11



Case #19



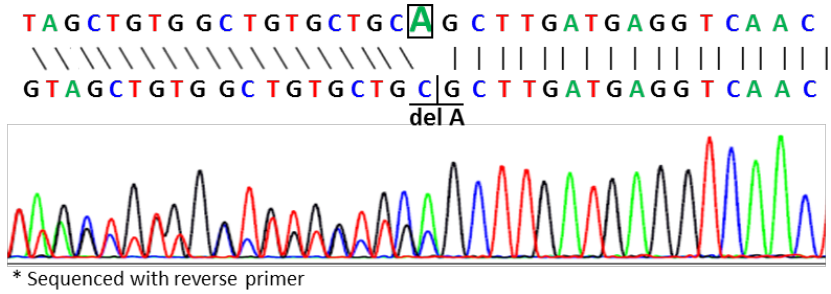
Father – Unavailable

Case #21

Referent (ex47)

c.6971delA

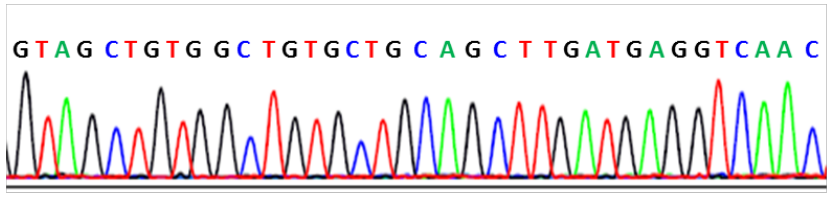
Proband



* Sequenced with reverse primer

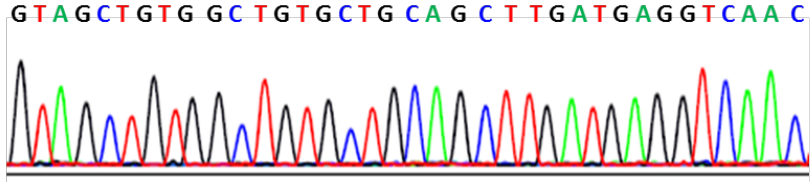
Referent (ex47)

Mother



Referent (ex47)

Father

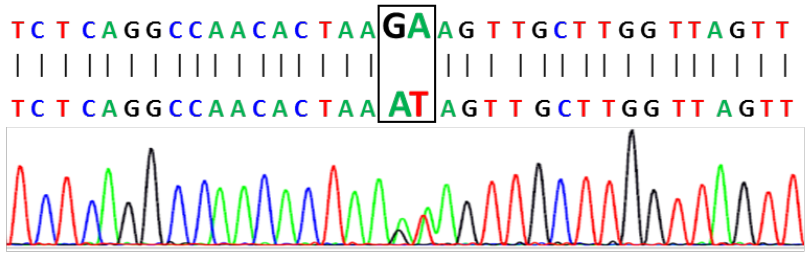


Case #23

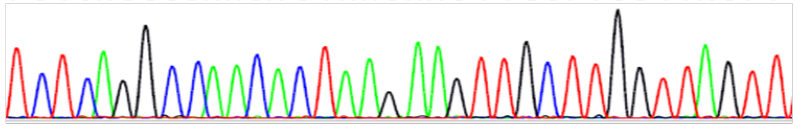
Referent (ex51)

c.7605_7606delinsAT

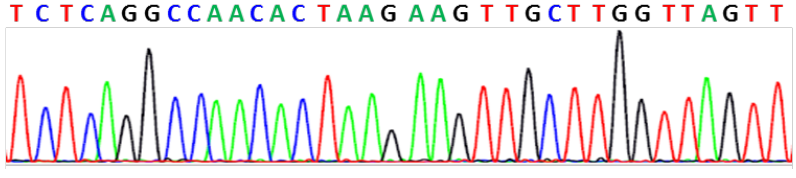
Proband



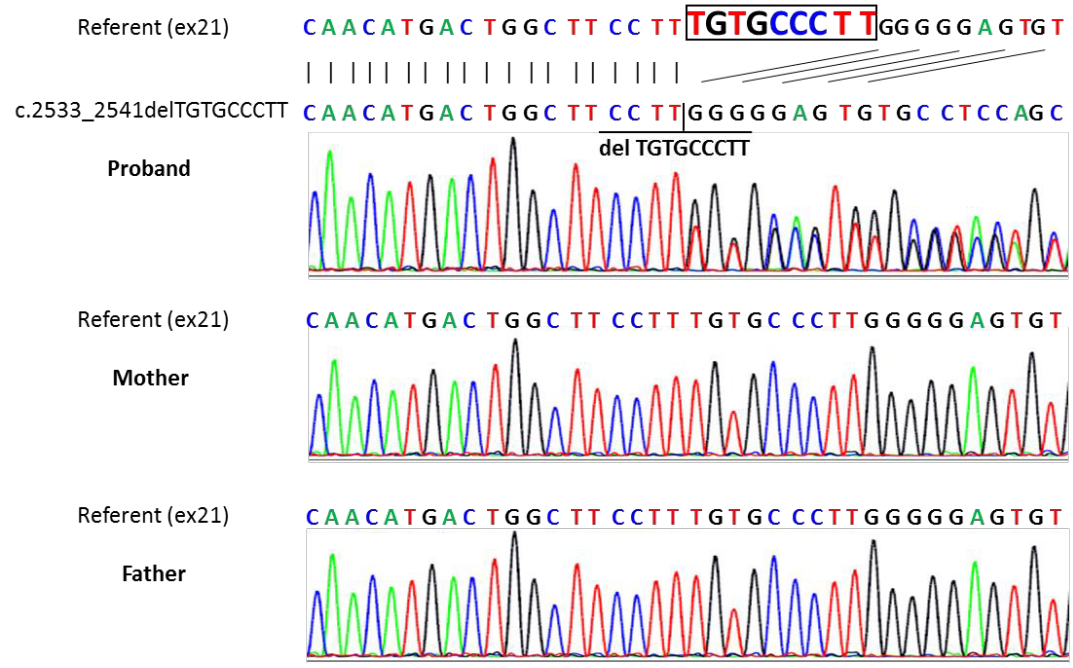
Referent (ex51)
Mother



Referent (ex51)
Father



Case #28



Case #30