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Supplemental Information

Genome Editing in Patient iPSCs Corrects the Most Prevalent *USH2A* Mutations and Reveals Intriguing Mutant mRNA Expression Profiles

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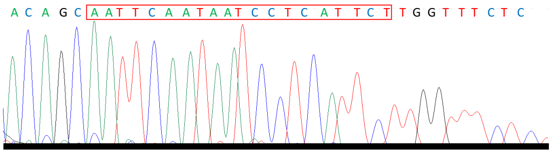
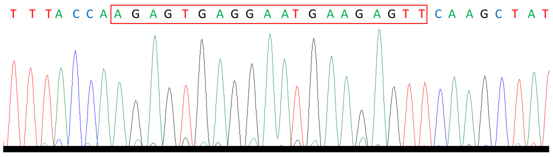
OF1B *ARHGAP18* (intron) Chr6 129617889 (+)

AATTCTGCAATCCTCACTCT	GGG	sgRNA-2
AATTCaatAATCCTCAtTCT	TGG	OF1



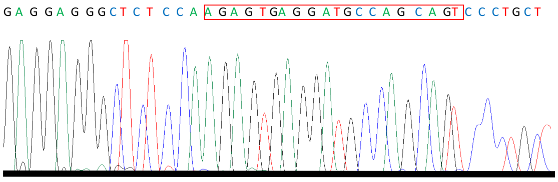
OF2B *GABRB1* (intron) Chr4 47400277 (-)

AATTCTGCAATCCTCACTCT	GGG	sgRNA-2
AAcTCTtCAtTCCTCACTCT	TGG	OF2
CCA AGAGTGAGGAaTGaAGAgTT		OF2 (-)



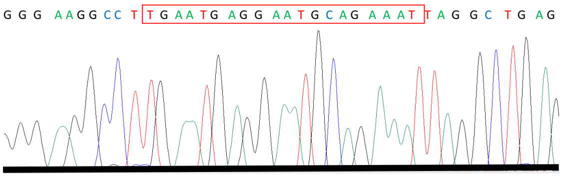
OF3B Intergenic Chr1 5386235 (-)

AATTCTGCAATCCTCACTCT	GGG	sgRNA-2
AcTgCTGgcATCCTCACTCT	TGG	OF3
CCA AGAGTGAGGATgcCAGcAgT		OF3 (-)



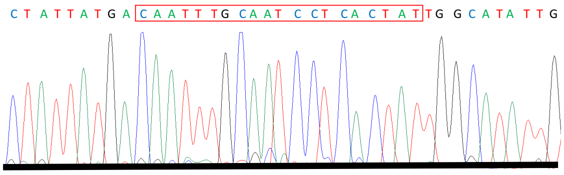
OF4B Intergenic Chr14 31209345 (-)

AATTCTGCAATCCTCACTCT	GGG	sgRNA-2
AtTTCTGCAtTCCTCAtTCa	AGG	OF4
CCT tGAaTGAGGAaTGCAGAAaT		OF4 (-)



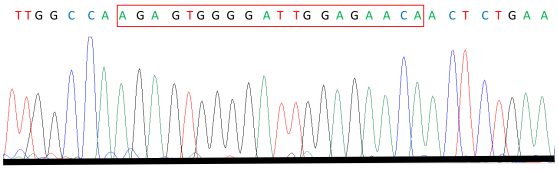
OF5B Intergenic Chr3 87701684 (+)

AATTCTGCAATCCTCACTCT	GGG	sgRNA-2
cAaTtTGCAATCCTCACTaT	TGG	OF5



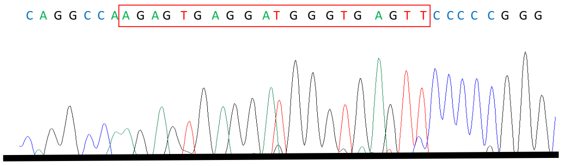
OF6B Intergenic Chr2 71792972 (-)

AATTCTGCAATCCTCACTCT	GGG	sgRNA-2
tgTTCTcCAATCCcCACTCT	TGG	OF6
CCA AGAGTGgGGATTGgAGAAca		OF6 (-)



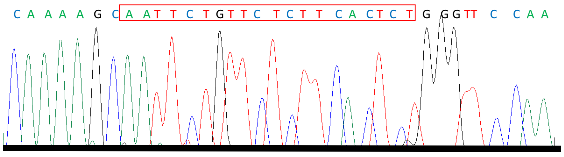
OF7B Intergenic Chr18 75074856 (-)

AATTCTGCAATCCTCACTCT	GGG	sgRNA-2
AAcTCacCcATCCTCACTCT	TGG	OF7
CCA AGAGTGAGGATgGgtGAgTT		OF7 (-)



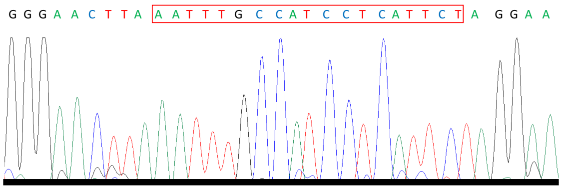
OF8B *DIP2C* (intron) Chr10 616144 (+)

AATTCTGCAATCCTCACTCT	GGG	sgRNA-2
AATTCTGttcTCtTCACTCT	GGG	OF8



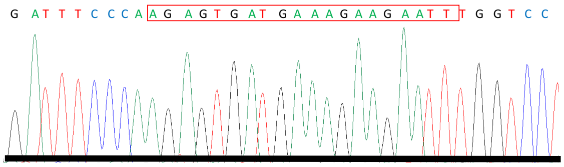
OF9B *NCOA2* (intron) Chr8 70355855 (+)

AATTCTGCAATCCTCACTCT	GGG	sgRNA-2
AAaTtTGCcATCCTCAtTCT	AGG	OF9



OF10B *GAP43* (intron) Chr3 115644091 (-)

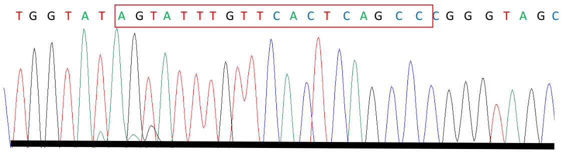
AATTCTGCAATCCTCACTCT	GGG	sgRNA-2
AATTCTtCttTCaTCACTCT	TGG	OF10
CCA AGAGTGAtGAaaGaAGAATT		OF10 (-)



OF1M

LINC00879 (intron) Chr3 94971838 (+)

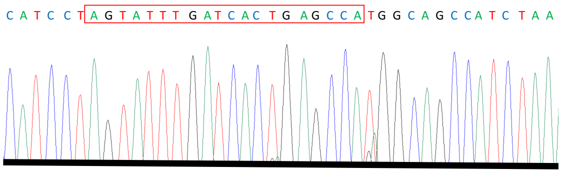
AGAATTTGTTCACTGAGCCG TGG sgRNA-1S
AGtATTTGTTCACTcAGCCc GGG OF1



OF2M

Intergenic Chr3 2012988 (+)

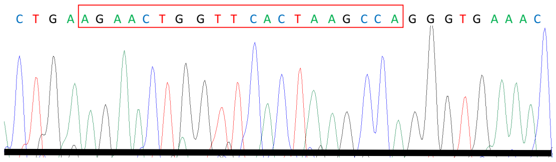
AGAATTTGTTCACTGAGCCG TGG sgRNA-1S
AGtATTTGaTCACTGAGCCa TGG OF2



OF3M

LRRK1 (intron) Chr15 100962092 (+)

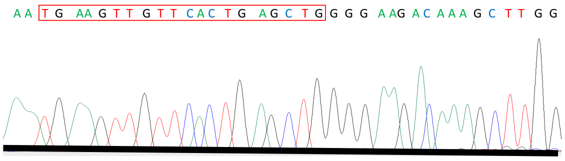
AGAATTTGTTCACTGAGCCG TGG sgRNA-1S
AGAAcTgGTTCACTaAGCCa GGG OF3



OF4M

Intergenic Chr5 2894427 (+)

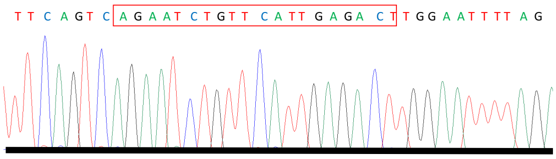
AGAATTTGTTCACTGAGCCG TGG sgRNA-1S
tGAAgTTGTTCACTGAGCtG GGG OF4



OF5M

Intergenic Chr2 210765079 (+)

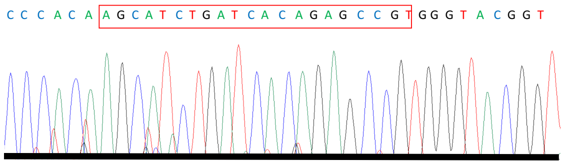
AGAATTTGTTCACTGAGCCG TGG sgRNA-1S
AGAATcTGTTCAtTGAGaCt TGG OF5



OF6M

Intergenic Chr1 30855755 (+)

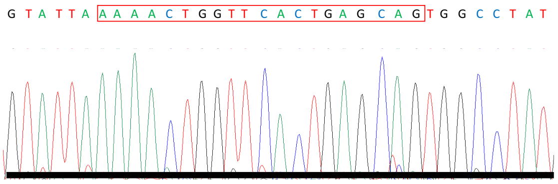
AGAATTTGTTCACTGAGCCG TGG sgRNA-1S
AGcATcTGaTCACaGAGCCG TGG OF6



OF7M

LOC646241 (intron) Chr5 17830676 (+)

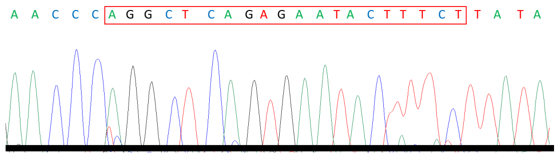
AGAATTTGTTCACTGAGCCG TGG sgRNA-1S
AaAAcTgGTTCACTGAGCaG TGG OF7



OF8M

MED13L (intron) Chr12 116261613 (-)

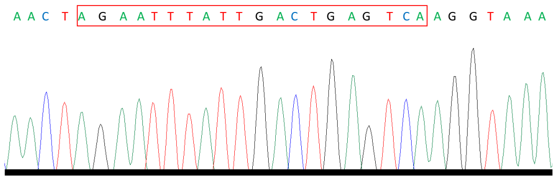
AGAATTTGTTCACTGAGCCG TGG sgRNA-1S
AGAAagTaTTCACTGAGCCt GGG OF8
CCC aGGCTCAGTGAAtActTTCT OF8 (-)



OF9M

Intergenic Chr5 35529692 (+)

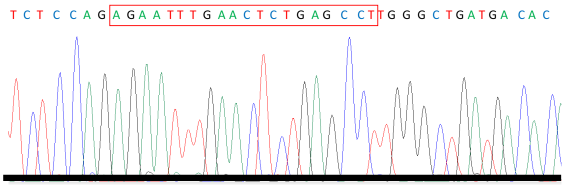
AGAATTTGTTCACTGAGCCG TGG sgRNA-1S
AGAATTTaTTgACTGAGtCa AGG OF9



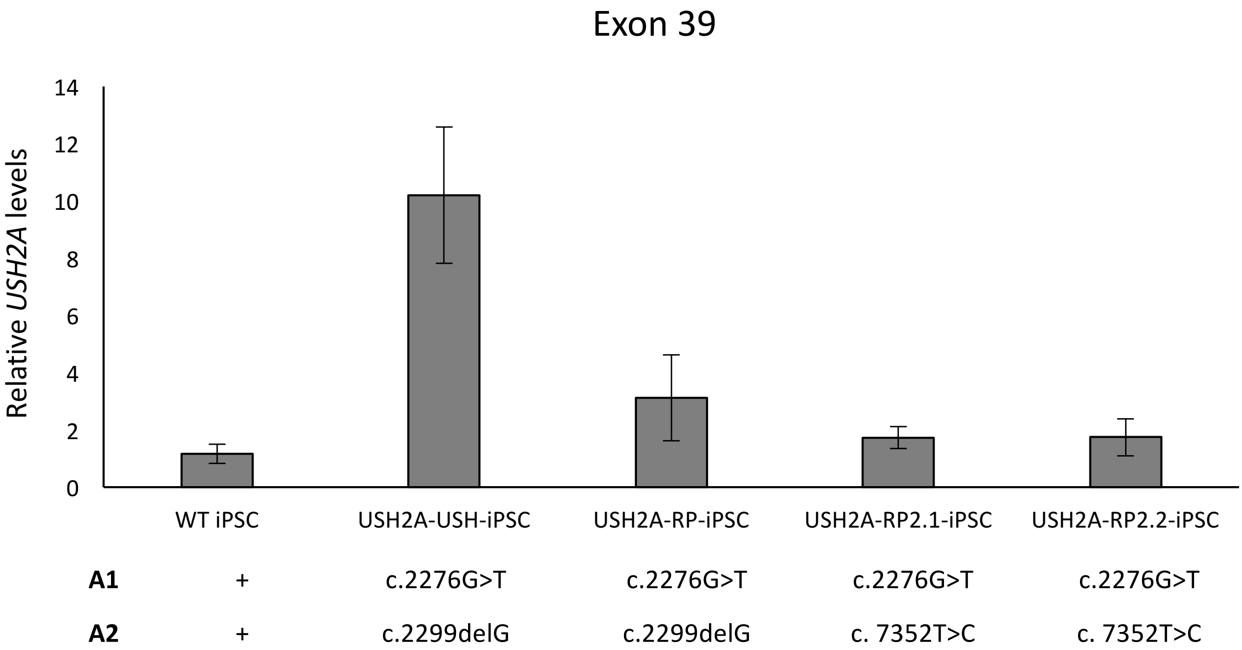
OF10M

C2orf48 (intron) Chr2 10203876 (+)

AGAATTTGTTCACTGAGCCG TGG sgRNA-1S
AGAATTTGaaCtCTGAGCCt TGG OF10



A


Exon 39
Relative USH2A levels
0
2
4
6
8
10
12
14
WT iPSC
USH2A-USH-iPSC
USH2A-RP-iPSC
USH2A-RP2.1-iPSC
USH2A-RP2.2-iPSC
A1
+
c.2276G>T
c.2276G>T
c.2276G>T
c.2276G>T
A2
+
c.2299delG
c.2299delG
c. 7352T>C
c. 7352T>C

B

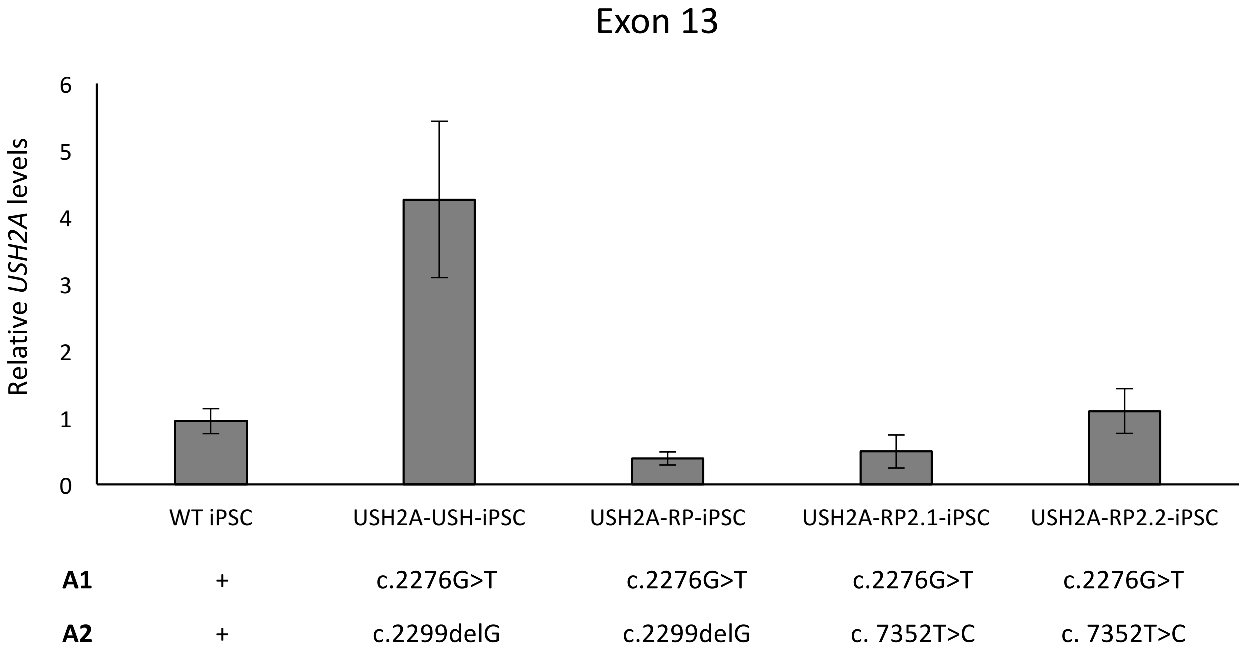

Exon 13
Relative USH2A levels
0
1
2
3
4
5
6
WT iPSC
USH2A-USH-iPSC
USH2A-RP-iPSC
USH2A-RP2.1-iPSC
USH2A-RP2.2-iPSC
A1
+
c.2276G>T
c.2276G>T
c.2276G>T
c.2276G>T
A2
+
c.2299delG
c.2299delG
c. 7352T>C
c. 7352T>C

Figure S1: Non-exonic off-target analysis for *USH2A*-USH-iPSC clone B3B1. For each off-target (OF) site, the gene, the chromosome, and the position is shown. In brackets, the positive (+) or negative (-) strand is indicated. The electropherograms show the Sanger sequencing results of the predicted off-target regions and the off-target sequence is boxed in red. The sgRNA-2 sequence and its PAM sequence are shown above the off-target sequence. Lowercase letters represent the mismatched nucleotides between sgRNA-2 and the off-target. When the off-target site is on the negative strand (-) the reverse complement sequence is indicated.

Figure S2: Non-exonic off-targets analysis for *USH2A*-RP-iPSC clone MS3F7. For each off-target (OF) site, the gene, the chromosome, and the position is shown. In brackets, the positive (+) or negative (-) strand is represented. The electropherograms show the Sanger sequencing results of the predicted off-target regions and the off-target sequence is boxed in red. The sgRNA-1S sequence and its PAM sequence are shown above the off-target sequence. Lowercase letters represent the mismatched nucleotides between sgRNA-1S and the off-target. When the off-target site is found in the negative strand (-) the reverse complement sequence is represented.

Figure S3: *USH2A* mRNA expression levels in a second RP iPSC line. *USH2A* mRNA expression levels in exon 39 (**A**) and exon 13 (**B**) for the *USH2A*-USH-iPSC and *USH2A*-RP-iPSC lines, and for two iPSC lines, *USH2A*-RP2.1-iPSC and *USH2A*-RP2.2-iPSC, from another patient with arRP and compound heterozygous for c.2276G>T and c.7352T>C. A WT iPSC line was used as a control and its relative expression was set at 1. The allelic (A1 and A2) mutations carried by each cell line are indicated below the graphs. A plus sign indicates the absence of a mutation. Results were normalized to *GAPDH* expression. Data is expressed as mean ± SEM, *n*=3 technical replicates.

Table S1: sgRNA, ssODN and primer sequences

	Name	**Sequence**
sgRNAs	sgRNA-1	5' AGA ATT TGT TCA CTG AGC CA (**TGG**) 3'
	sgRNA-2	5' AAT TCT GCA ATC CTC ACT CT (**GGG**) 3'
	sgRNA-3	5' CCC TGC CAG TGT AAC CTC CA (**TGG**) 3'
	sgRNA-4	5' CTG AGC CAT GGA GGT TAC AC (**TGG**) 3'
	sgRNA-1S	5' AGA ATT TGT TCA CTG AGC CG (**TGG**) 3'
ssODNs	ssODN-1	5' GGC TTA GGT GTG ATC ATT GCA ATT TTG GAT TTA AAT TTC TCC GAA GCT TTA ATG ATG TTG GAT GTG AGC CCT GCC AGT GTA ACC TAC ATG GCT CAG TGA ACA AAT TCT GCA ATC CTC ACT CTG GG CA 3'
	ssODN-2	3' TAC AAT TGG TGA CAT CTA ACC CAT AAA AGT TTT CTC TGC AGG TGT CAC ACT GAA GTC CTT TGG CTT CTT TTT TGC ACT CAC ACT GGC CAG AGT GAG GAT TGC AGA ATT TGT TCA CTG AGC CAT GGA G 5'
***USH2A* DNA**	exon 13	F: 5' GGC ATT GCT TGT GAG AAA ACA C 3' R: 5' AGG AAT CAC ACT CAC ACA TCT G 3'
CNV analysis	*USH2A*	F: 5' CAA TCC TCA CTC TGG GCA GT 3' R: 5' CAC AGG CCT TAC AAT TGG TG 3'
	TRMT10C	F: 5' TGG TGC CAT TTA CCC TTC AT 3' R: 5' AGC TCT TCA GAA GGG GGT GT 3'
	TERT	F: 5' GCG TTT GGT GGA TGA TTT CT 3' R: 5' AAC TGC AAA GCC CAC AGG 3'
***USH2A* mRNA analysis**	exon 39	F: 5' CAG CGG AGA AGA GAC AAA CC 3' R: 5' TCA AGC TGC CTT GGC TAT TT 3'
	exon 13	F: 5' CCT CCC TGG GAC TGT CTG TA 3' R: 5' CAG TTG CAA GGC AGA CAG AG 3'
	GAPDH	F: 5' AAC CAT GAG AAG TAT GAC AAC 3' R: 5' CTT CCA CGA TAC CAA AGT T 3'
Off-target analysis B3B1	OF1B	F: 5' TGT CTT TGT GAA TGT TAC CAC AGC 3' R: 5' TCT CTG TTG ACA AGG CTT TAC CT 3'
	OF2B	F: 5' CCT CAT CAG TGG CCA TCT TC 3' R: 5' CCC AGT AGC CCT AGA GAA GGA 3'
	OF3B	F: 5' TCC ACT CTC TAC CTG GCA CA 3' R: 5' TTG TCA ACA CTG CGT CAG GAC 3'
	OF4B	F: 5' CCC CAG CAA AGA ACC AAC AG 3' R: 5' AGT GTT GTA GTC CAG AAC TTG CTA 3'
	OF5B	F: 5' TCT TCA CAT ATT GGA GTC ATG ACT 3' R: 5' AGC ATC AAC ATC TGT GGC CAT 3'
	OF6B	F: 5' TCT TGT GGG AGG AGG GGA TG 3' R: 5' GGT GGG TAG CAG AAT GGC TTT 3'
	OF7B	F: 5' GAC AAG ACT CAC AGC TGG GG 3' R: 5' TCC ACC ACC CTC ACT CCT CC 3'
	OF8B	F: 5' TTC ACC CAG GTC ATC CCT CA 3' R: 5' CGG TCC AGA TTA CAA ACG GCT 3'
	OF9B	F: 5' AGC TAA AGA CAG AAT GGG AAC T 3' R: 5' CCC ATT CAG AAT CAT GAG AGA GAT 3'
	OF10B	F: 5' TCC ACT TCC TTC CTG ATT GC 3' R: 5' ACC ACA GCA GGA CAA TCC TC 3'

	Name	Sequence
Off-target analysis MS3F7	OF1M	F: 5' CCA TTC CTT ACT TTA GAA GCC ATT 3' R: 5' AGC AAA GGT TGA CTG GAG GA 3'
	OF2M	F: 5' TTG CCT TAT CAC CAC CAT CA 3' R: 5' TTG CCT AGA TGC TTT GCT GA 3'
	OF3M	F: 5' AGC AAC TGA GTC AAG ATT CGG T 3' R: 5' TGT CCA GCA AAA TGT TAA CTG TGT T 3'
	OF4M	F: 5' TGG GGC TGT CAG AAA TGA GG 3' R: 5' CTC TTC TGA GGG CTG ATG GAG C 3'
	OF5M	F: 5' CAG CCT TTA AAA ATG CGC TAG T 3' R: 5' GCA ACA GGA AAA AGC CAG AG 3'
	OF6M	F: 5' GAC CTG TGG GGA CAA GAG AC 3' R: 5' GCC ATC TGC ATC ACA ATG TC 3'
	OF7M	F: 5' GTG GAA GTG GGG AGT GAA AA 3' R: 5' CAA AGG ATG GGC GCT TAC TA 3'
	OF8M	F: 5' TGA TTT CTG TAG GGT TTA AAA GCA A 3' R: 5' ATG CAA AAC GGG GAG GAA CAG 3'
	OF9M	F: 5' TGC TTT TGC CTT GCT TCT TT 3' R: 5' AGT GTT GAA GGT AGG CAA CGA 3'
	OF10M	F: 5' GGG GCT CAC GGT TAA GTC AA 3' R: 5' GGA AGC ACC AGC AGT CTC TGG 3'