# Supplementary Information File

## Functional assays of non-canonical splice-site variants in inherited retinal dystrophies genes.

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**Supplementary Table 1**. Detailed information about the clinical and genetic diagnosis from patients carrying putative splicing-affecting variants.

| Patient ID | Phenotype | Gene:transcript | Coding DNA | Protein | State | Classification* |
|---|---|---|---|---|---|---|
| RPN-410# | STGD | ***ABCA4*:NM_000350** | **c.G2972T** | **p.G991V** | **het** | **LP** |
| | | *ABCA4*:NM_000350 | c.4253+4C>T | p.? | het | P |
| RPN-536 | RP | ***ABCA4*:NM_000350** | **c.G6148C** | **p.V2050L** | **het** | **US** |
| | | *CNGA1*:NM_001142564 | c.C1892T | p.T631M | het | LP |
| | | *CNGB1*:NM_001297 | c.3150delG | p.(Phe1051Leufs*12) | het | P |
| RPN-106# | BMD | *BEST1*:NM_004183 | c.T602C | p.I201T | het | LP |
| | | ***BEST1*:NM_004183** | **c.637-2_637del** | **p.Glu213del** | **het** | **LP** |
| RP-677 | STGD | ***CNNM4*:NM_020184** | **c.C2039T** | **p.A680V** | **het** | **US** |
| RPN-304# | MD STGD-like | ***FSCN2*:NM_012418** | **c.G1105A** | **p.G369S** | **het** | **US** |
| | | *PRPH2*:NM_000322 | c.G499A | p.G167S | het | P |
| RPN-299 | RP | *C21orf2*:NM_004928 | c.G172T | p.V58L | het | US |
| | | ***IDH3B*:NM_001258384** | **c.A532G** | **p.S178G** | **het** | **US** |
| RPN-326 | BMD | *ABCA4*:NM_000350 | c.C5642T | p.A1881V | het | LP |
| | | *BEST1*:NM_004183 | c.C698A | p.P233Q | het | LP |
| | | ***MAK*:NM_005906** | **c.A755G** | **p.N252S** | **het** | **US** |
| RPN-442# | RP | *MERTK*:NM_006343 | MERTK del (chr2:111371701-113132395) | | het | P |
| | | ***MERTK*:NM_006343** | **c.G1450A** | **p.G484S** | **het** | **LP** |
| RPN-451# | RP | *PRCD*:NM_001077620 | c.102_111dup | p.Ser38X | het | P |
| | | ***PRCD*:NM_001077620** | **c.74+5G>C** | **p.?** | **het** | **LP** |
| RPN-461# | RP | ***PRPF8*:NM_006445** | **c.434+3G>A** | **p.?** | **het** | **US** |
| | | *ROM1*:NM_000327 | ROM1 dup | p.? | het | LP |
| RPN-284 | STGD | ***PRPF31*:NM_015629** | **c.C182G** | **p.A61G** | **het** | **US** |
| RPN-253# | RP | ***RHO*:NM_000539** | **c.G316A** | **p.G106R** | **het** | **P** |
| RPN-104# | LCA | *AIPL1*:NM_014336 | c.97_104dup | p.(Phe35Leufs*2) | hom | P |
| | | *IQCB1*:NM_001023570 | c.1518_1519del | p.(His506Glnfs*13) | het | P |
| | | ***RIMS1*:NM_014989** | **c.2544+4A>G** | **p.?** | **het** | **US** |
| RP-632 | RP | *EYS*:NM_001142800 | ex. 32-33 del | p.? | het | P |
| | | ***RPGRIP1*:NM_020366** | **c.930+3A>G** | **p.?** | **het** | **US** |
| RPN-424 | RD | ***CACNA2D4: NM_172364.5*** | **c.2153-12_2155del** | **p.?** | **het** | **US** |

In bold are marked the variants analyzed by minigene assay. # Cases with segregation analysis carried out. *Classification prior to the minigenes analysis. Abbreviations: STGD: Stargardt disease; RP: retinitis pigmentosa; BMD: Best macular dystrophy; MD STGD-like: autosomal dominant macular dystrophy STGD disease-like; LCA: Leber congenital amaurosis; RD: Retinal dystrophy; P: Pathogenic. LP: Likely pathogenic. US: Uncertain significance.

**Supplementary Table 2.** Primers used to amplify the specific insert.

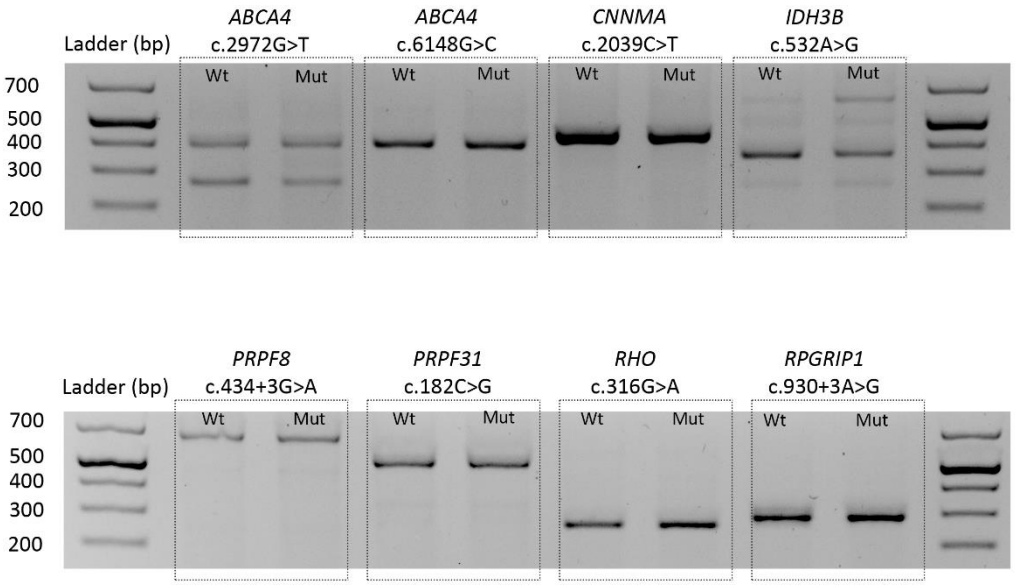
| Primer | Sequence (5'-3') | Size (bp) | Flanking Intronic Region (Upstream / Downstream bp) |
|---|---|---|---|
| ABCA4(20D)_XhoI | **AAGAAT***CTCGAG*GGGAGCACCATGAAACAAGT | 593 | 225 / 218 |
| ABCA4(20R)_NheI | **AAGAAT***GCTAGC*GGAGGAGCCCTCAGCTCT | | |
| ABCA4(45D)_XhoI | **AAGAAT***CTCGAG*CGAGACCCACTGTCTTGCTC | 676 | 290 / 248 |
| ABCA4(45R)_NheI | **AAGAAT***GCTAGC*ACAGGAGACATCCTGGGAAC | | |
| BEST(6D)_XhoI | **AAGAAT***CTCGAG*CTGAGGGTCTTCCGAGAGC | 654 | 296 / 261 |
| BEST(6R)_NheI | **AAGAAT***GCTAGC*TCAGCTTCCCAAAGTGCTG | | |
| CACNA2D4 (23D)_XhoI | **AAGAAT***CTCGAG*TGAGGTCACTGAGGGTCTCC | 566 | 268 / 204 |
| CACNA2D4 (23R)_NheI | **AAGAAT***GCTAGC*CAACCCAGAGAAAGGAGCTG | | |
| CNNM4(6 D)_XhoI | **AAGAAT***CTCGAG*TGTTTGGTTGATTTGTTTTGG | 688 | 251 / 255 |
| CNNM4(6 R)_NheI | **AAGAAT***GCTAGC*AGCCACATGCACACCTGTAG | | |
| FSCN2 (3D)_XhoI | **AAGAAT***CTCGAG*CATCACCTGCACCATCACTC | 557 | 233 / 202 |
| FSCN2 (3R)_NheI | **AAGAAT***GCTAGC*TCCCAGCAGCGGAGATAAG | | |
| IDH3B(7D)_XhoI | **AAGAAT***CTCGAG*ATATGCGGCTGAGGTAGGTG | 800 | 333 / 335 |
| IDH3B(7R)_NheI | **AAGAAT***GCTAGC*TCCCATAGAGATTGGGCATC | | |
| MAK(8D)_XhoI | **AAGAAT***CTCGAG*CGCATTGTTTCCTAACTCTCAG | 576 | 216 / 192 |
| MAK(8R)_NheI | **AAGAAT***GCTAGC*TTTCTGCATTTCTCCCAATG | | |
| MERTK(9D)_NheI | **AAGAAT***GCTAGC*GGGTCTCACTCTGTTACCTAGC | 889 | 249 / 271 |
| MERTK(9D)_XhoI | **AAGAAT***CTCGAG*GTGTGACTGGCGTATTGTGC | | |
| PRCD (1D)_ XhoI | **AAGAAT***CTCGAG*TTAGAGTGGCAGCTCCTTGC | 979 | 203 / 599 |
| PRCD (1R)_ NheI | **AAGAAT***GCTAGC*TGGAGATGAGAGGCACAGC | | |
| PRPF8(4D)_ XhoI | **AAGAAT***CTCGAG*TGGCCTGACAGACATGAGAC | 950 | 363 / 477 |
| PRPF8(4R)_ NheI | **AAGAAT***GCTAGC*AAGGCCCACCTCAAGTAAGC | | |
| PRPF31(3 D)_XhoI | **AAGAAT***CTCGAG*TAGCAAGGTGGCGGTCATAG | 835 | 221 / 429 |
| PRPF31(3 R)_NheI | **AAGAAT***GCTAGC*GTCTGGGAAACCTCAAGCTG | | |
| RHO(1D)_XhoI | **AAGAAT***CTCGAG*GGACAGACAAGTCATGCAG | 945 | 249 / 240 |
| RHO(1R)_NheI | **AAGAAT***GCTAGC*GACAAGCGCATATTGCTCCA | | |
| RIMS1 (14D) _XhoI | **AAGAAT***CTCGAG*GCGACACATCATTTAGAATGTACC | 647 | 224 / 251 |
| RIMS1 (14R) _NheI | **AAGAAT***GCTAGC*TCCATGAATATGTCGCCTTG | | |
| RPGRIP1(7D)_XhoI | **AAGAAT***CTCGAG*GAAATGGTGTGAATGATTGCAG | 497 | 205 / 268 |
| RPGRIP1(7R)_NheI | **AAGAAT***GCTAGC*AATTTGCTCCAGCAATAGGC | | |

Tails added at the beginning of the primer are indicated in bold. Enzyme restriction sites used in this study are indicated in italics.

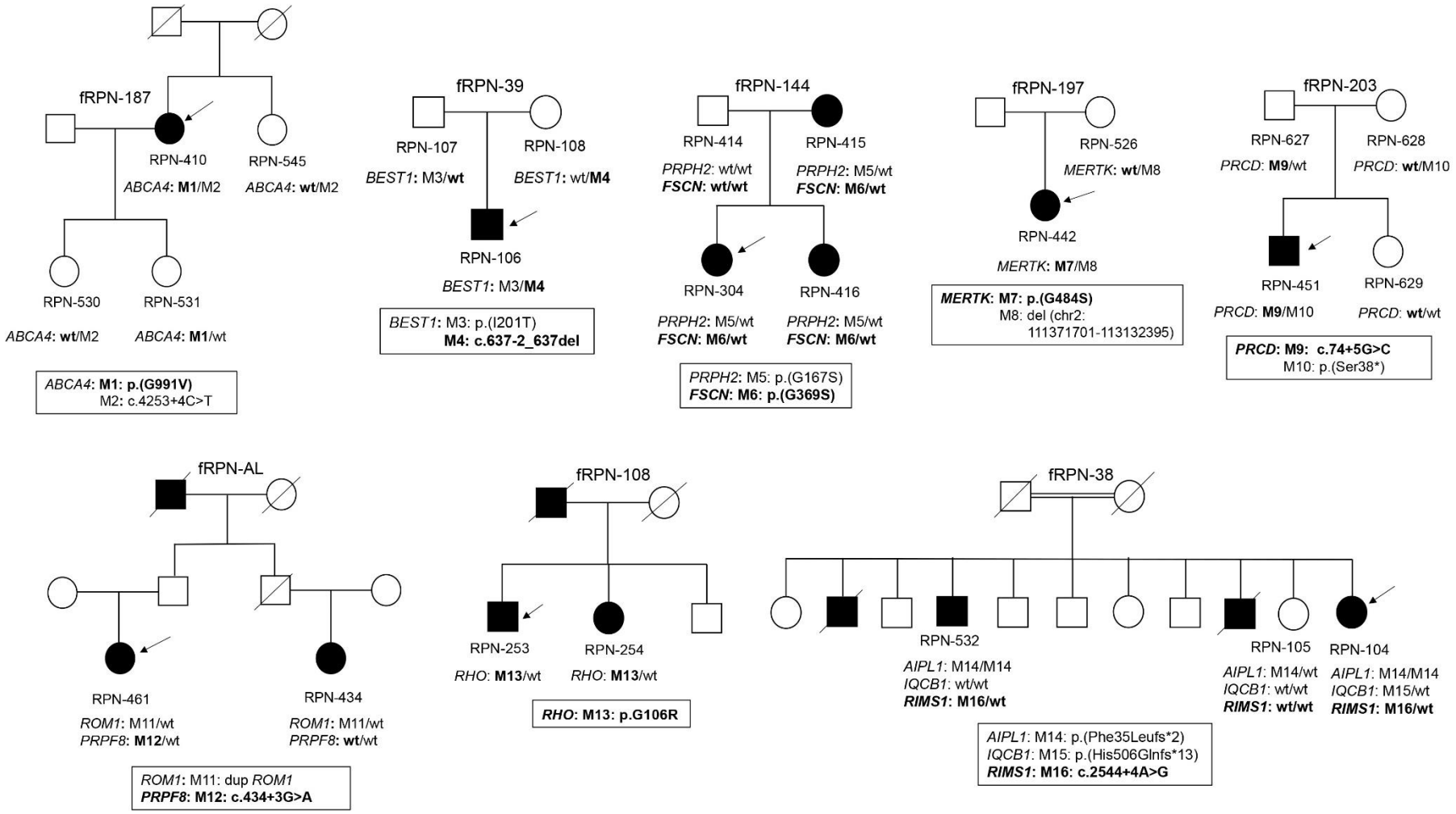
**Supplementary Table 3.** Primers used for the site-directed mutagenesis.

| Primer | Sequence (5'-3') |
|---|---|
| PRCD(1D) | GCATGGCCAGGG**A**CCTG**T**G**A**AGGAAAAGGGTGGTGCACATGGC |
| PRCD(1R) | GCCATGTGCACCACCCTTTTCCT**T**C**A**CAG**GT**CCCTGGCCATGC |

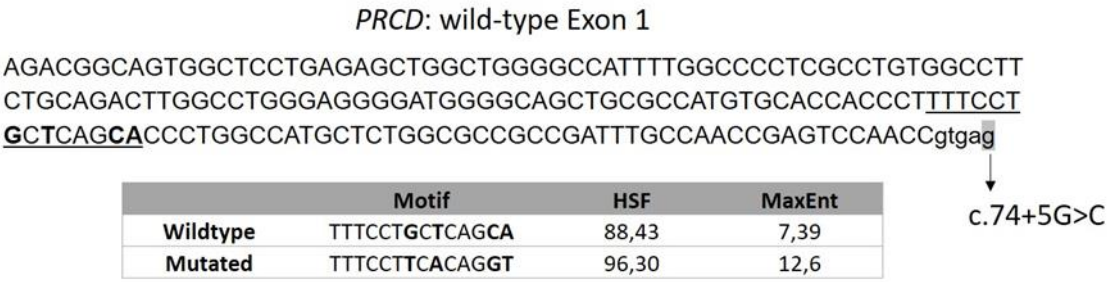
The nucleotides that have been modified are indicated in bold.




**Supplementary Figure 1**. Representation of the amplified product of the variants that did not affect the splicing.

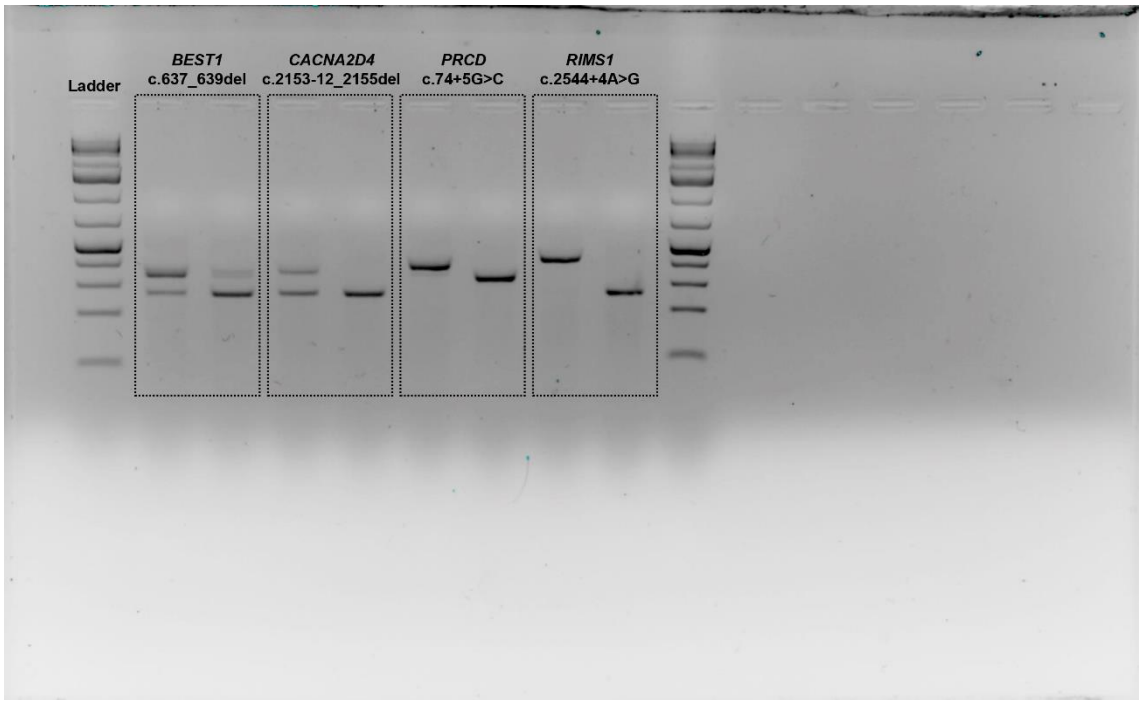



**Supplementary Figure 2.** Cosegregation analysis for the variants analysed in present study. The arrow indicates the index cases in each family. WT indicates “wild-type allele”. M indicates the mutation analysed in each family. Individuals from whom DNA sample were available are indicated with identification numbers.

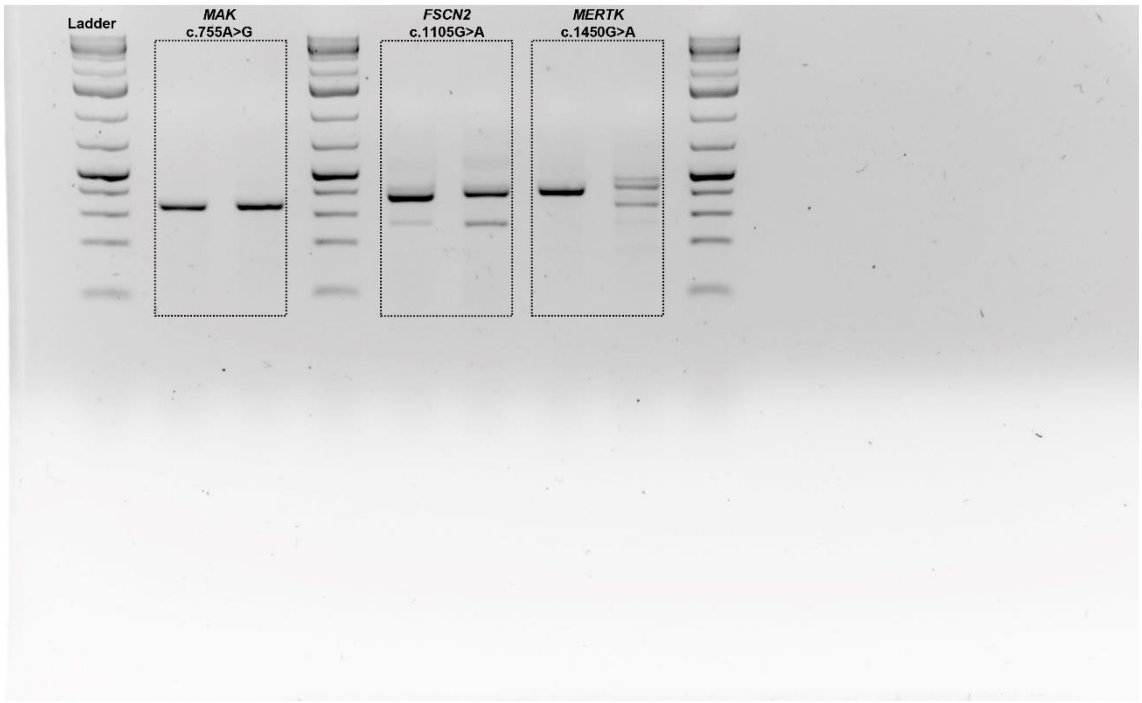



| | Motif | HSF | MaxEnt |
|---|---|---|---|
| **Wildtype** | TTTCCT**GCTCAGCA** | 88,43 | 7,39 |
| **Mutated** | TTTCCT**TCACAGGT** | 96,30 | 12,6 |

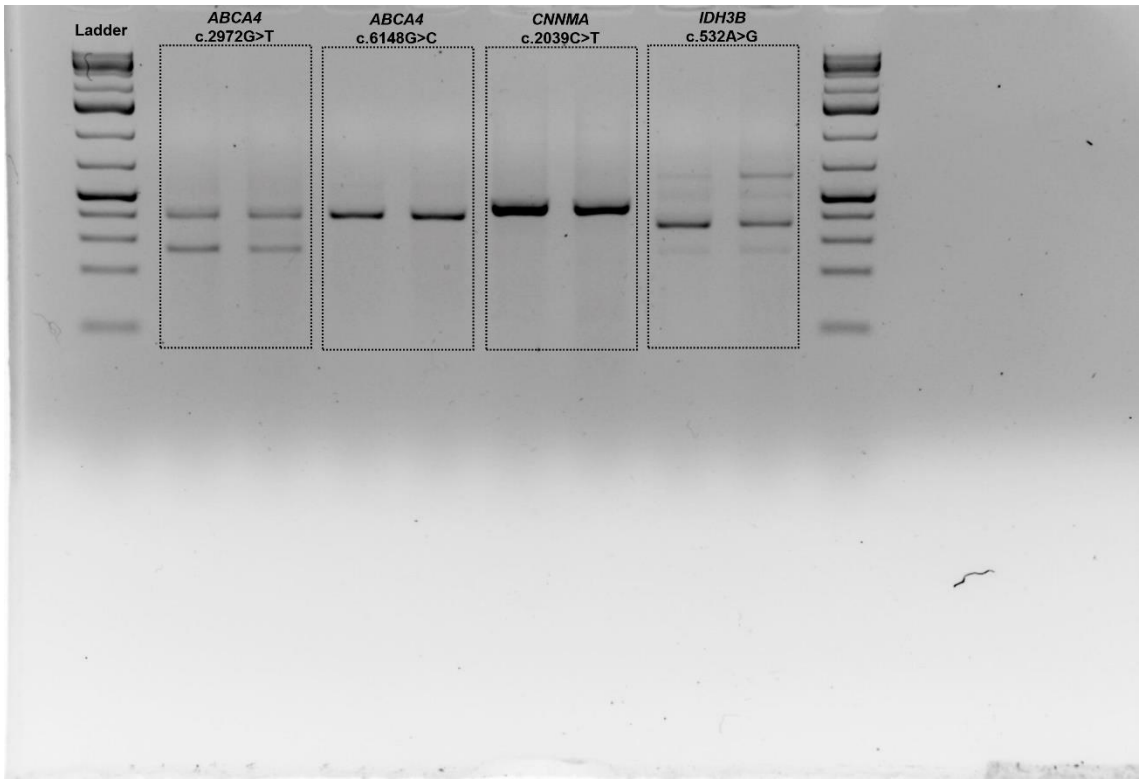
**Supplementary Figure 3**. Modifications generated in the *PRCD* exon 1 sequence by site-directed mutagenesis to promote the recognition of the first exon.



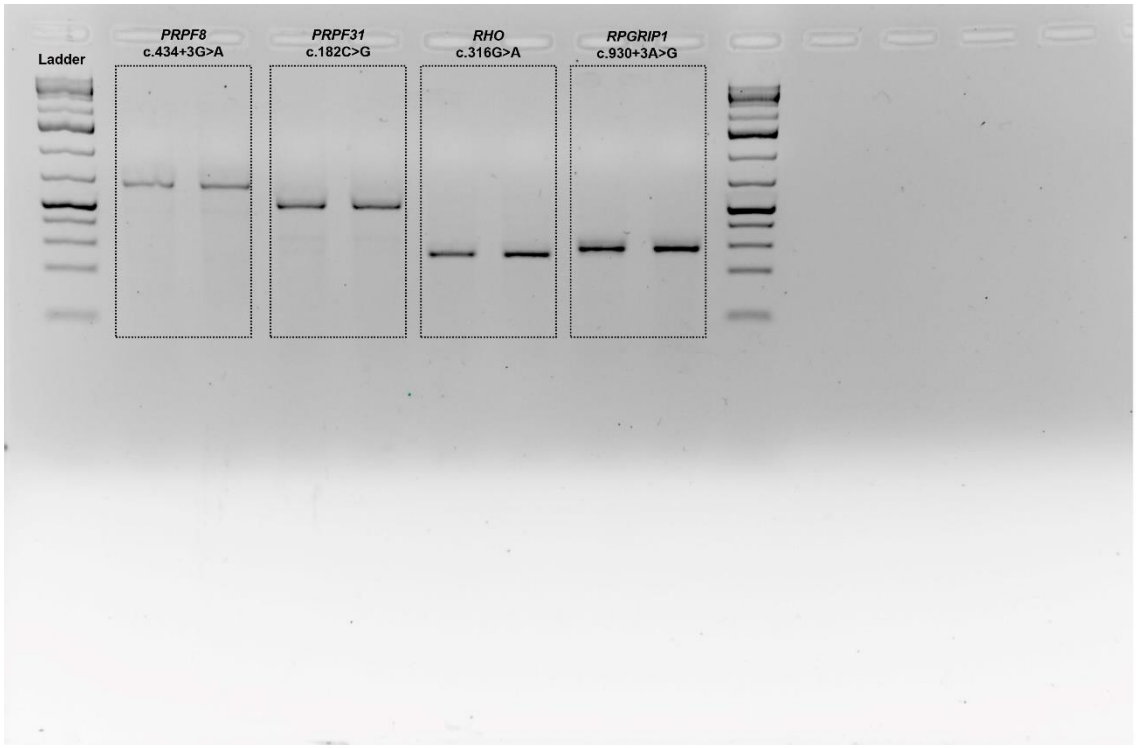

Raw Figure 1 (unprocessed version).




Raw Figure 1 (unprocessed version).




Raw Supplementary Figure 1 (unprocessed version).




Raw Supplementary Figure 1 (unprocessed version).