

Supplementary table 1. Primers and PCR conditions for characterization of *BRCA1/2* deep intronic variants at RNA level.

A. Primers for short (up to 1.5kb) and long (1.5-6kb) RT-PCR assays for splicing characterization in variant carriers and controls.

GENE	Deep intronic variant (VAR)	Location	Forward Primer	Reverse Primer	FL size (bp)	PCR kit	TA (°C)	Cycles
BRCA1	c.80+909T>C	intron 2	1F-ACAGGCTGTGGGTTTCTC	7R-TTGGCACGGTTTCTGTAGC	495	EcoTaq	58	x35
	c.81-1360delA	intron 2	1F-ACAGGCTGTGGGTTTCTC	7R-TTGGCACGGTTTCTGTAGC	495	EcoTaq	58	x35
	c.134+382C>T	intron 3	1F-ACAGGCTGTGGGTTTCTC	7R-TTGGCACGGTTTCTGTAGC	495	EcoTaq	58	x35
	c.134+835A>G	intron 3	2F-TATCTGCTCTCCGGTTGAA	7R-TTGGCACGGTTTCTGTAGC	397	EcoTaq	58	x35
	c.135-633C>T	intron 3	2F-TATCTGCTCTCCGGTTGAA	7R-TTGGCACGGTTTCTGTAGC	397	EcoTaq	58	x35
	c.212+253G>A	intron 5	2F-TATCTGCTCTCCGGTTGAA	7R-TTGGCACGGTTTCTGTAGC	397	EcoTaq	58	x35
	c.441+294G>A	intron 7	5F-TCAACCAGAAGAAGGGCC	10R-TTATCCCTGGTTCCTTGAG	491	EcoTaq	58	x35
	c.671-260C>T	intron 10	1F-ACAGGCTGTGGGTTTCTC	11R-CTCTCAGCTGCACGCTTCT	1062	EcoTaq	57	x35
	c.4185+194C>G	intron 12	11F-CAAGAAGAGCAAAGCATGGA	13R-TGTTCTGGATTTCGCAGGTC	275	EcoTaq	56	x35
	c.4185+1684A>C	intron 12	11F-CAAGAAGAGCAAAGCATGGA	13R-TGTTCTGGATTTCGCAGGTC	275	EcoTaq	56	x35
	c.4185+2967G>A	intron 12	11F-CAAGAAGAGCAAAGCATGGA	13R-TGTTCTGGATTTCGCAGGTC	275	EcoTaq	56	x35
	c.4185+4105C>T	intron 12	11F-CAAGAAGAGCAAAGCATGGA*	13R-TGTTCTGGATTTCGCAGGTC	275	EcoTaq	56	x35
	c.4185+4151A>G	intron 12	11F-CAAGAAGAGCAAAGCATGGA	13R-TGTTCTGGATTTCGCAGGTC	275	EcoTaq	56	x35
	c.4186-3530A>G	intron 12	11F-CAAGAAGAGCAAAGCATGGA	13R-TGTTCTGGATTTCGCAGGTC	275	EcoTaq	56	x35
	c.4186-2448_4186-2447insGGA	intron 12	11F-CAAGAAGAGCAAAGCATGGA	13R-TGTTCTGGATTTCGCAGGTC	275	EcoTaq	56	x35
	c.4358-2859G>A	intron 13	12F-AAGACTGCTCAGGGCTATCCTCTC	15R-GACTCCCAGAGCAACTGTGC	411	EcoTaq	58	x35
	c.4484+643C>G	intron 14	12F-AAGACTGCTCAGGGCTATCCTCTC	15R-GACTCCCAGAGCAACTGTGC	411	EcoTaq	58	x35
	c.4676-284_4676-283insAGTGAACATT	intron 15	14F-GGCCTTTCTGTGACAAGTT	16R-TGACTCTGGGCTCTGTCTT	351	EcoTaq	58	x35
	c.4676-113C>G	intron 15	14F-GGCCTTTCTGTGACAAGTT	16R-TGACTCTGGGCTCTGTCTT	351	EcoTaq	58	x35
	c.4987-635A>G	intron 16	16F-GGGAGAAGCCAGAATTGACA	18R-TCCGTTACACACAAAACCTCAG	198	EcoTaq	58	x35
	c.5075-1544G>C	intron 17	16F-GGGAGAAGCCAGAATTGACA	18R-TCCGTTACACACAAAACCTCAG	198	EcoTaq	58	x35
	c.5075-1445G>C	intron 17	16F-GGGAGAAGCCAGAATTGACA	18R-TCCGTTACACACAAAACCTCAG	198	EcoTaq	58	x35
	c.5075-1061T>A	intron 17	16F-GGGAGAAGCCAGAATTGACA	18R-TCCGTTACACACAAAACCTCAG	198	EcoTaq	58	x35
	c.5278-2042C>T	intron 20	20F1-CAAGGTCCAAGCGAGCAAG	22R-GGGTGAATGATGAAAGCTCCT	160	EcoTaq	58	x35
	c.5278-1708_5278-1697del	intron 20	20F1-CAAGGTCCAAGCGAGCAAG	22R-GGGTGAATGATGAAAGCTCCT	160	EcoTaq	58	x35
c.5278-1238C>T	intron 20	20F1-CAAGGTCCAAGCGAGCAAG	22R-GGGTGAATGATGAAAGCTCCT	160	EcoTaq	58	x35	
c.5278-801T>G	intron 20	20F1-CAAGGTCCAAGCGAGCAAG	22R-GGGTGAATGATGAAAGCTCCT	160	EcoTaq	58	x35	
BRCA2	c.-39-137_-39-135del	intron 1	1F5-AAGCGTGAGGGGACAGATTT	6R-TGAAACAAACTCCCACATACCA	617	EcoTaq	58	x35
	c.316+368A>G	intron 3	1F5-AAGCGTGAGGGGACAGATTT	6R-TGAAACAAACTCCCACATACCA	617	EcoTaq	58	x35
	c.316+1053C>T	intron 3	1F5-AAGCGTGAGGGGACAGATTT	6R-TGAAACAAACTCCCACATACCA	617	EcoTaq	58	x35
	c.316+1669A>G	intron 3	1F5-AAGCGTGAGGGGACAGATTT	6R-TGAAACAAACTCCCACATACCA	617	EcoTaq	58	x35
	c.316+2196A>G	intron 3	1F5-AAGCGTGAGGGGACAGATTT	6R-TGAAACAAACTCCCACATACCA	617	EcoTaq	58	x35
	c.317-339C>T	intron 3	1F5-AAGCGTGAGGGGACAGATTT	6R-TGAAACAAACTCCCACATACCA	617	EcoTaq	58	x35
	c.425+33A>G	intron 4	1F5-AAGCGTGAGGGGACAGATTT	6R-TGAAACAAACTCCCACATACCA	617	EcoTaq	58	x35
	c.632-1101G>A	intron 7	4F-AGCAGATGATGTTTCTGTCC	10R2-ACCATTACACAGGCCAAAGAC	809	EcoTaq	58	x35
	c.632-1136dup	intron 7	4F-AGCAGATGATGTTTCTGTCC	10R2-ACCATTACACAGGCCAAAGAC	809	EcoTaq	58	x35
	c.632-943dup	intron 7	4F-AGCAGATGATGTTTCTGTCC	10R2-ACCATTACACAGGCCAAAGAC	809	EcoTaq	58	x35
	c.6841+1018A>G	intron 11	10F2-TATGTCCAATAATTAATGATAT	14R2-TTGGTCTGCCTGTAGTAATC	5570	Expand™ Long Range dNTPack	58	x10; x30
	c.6937+302C>T	intron 12	11F2-CTGCCAAGTCATGCCACAC	14R2-TTGGTCTGCCTGTAGTAATC	475	EcoTaq	58	x35
	c.7007+2200C>T	intron 13	11F2-CTGCCAAGTCATGCCACAC	14R2-TTGGTCTGCCTGTAGTAATC	475	EcoTaq	58	x35
	c.7008-1374G>T	intron 13	11F2-CTGCCAAGTCATGCCACAC	14R2-TTGGTCTGCCTGTAGTAATC	475	EcoTaq	58	x35
	c.7008-1289A>C	intron 13	11F2-CTGCCAAGTCATGCCACAC	14R2-TTGGTCTGCCTGTAGTAATC	475	EcoTaq	58	x35
	c.7436-182G>T	intron 15	11F2-CTGCCAAGTCATGCCACAC	17R-AAGCTTTGGATCCACACCTG	1110	EcoTaq	57	x35
	c.8331+1543C>T	intron 18	16F-TACAGTTGGCTGATGGTGGGA	20R-TTGTTGGGCTCCACATATT	842	EcoTaq	56	x35
	c.8331+2157A>T	intron 18	16F-TACAGTTGGCTGATGGTGGGA	20R-TTGTTGGGCTCCACATATT	842	EcoTaq	56	x35
	c.8332-2898A>G	intron 18	16F-TACAGTTGGCTGATGGTGGGA	20R-TTGTTGGGCTCCACATATT	842	EcoTaq	56	x35
	c.8332-2638C>T	intron 18	16F-TACAGTTGGCTGATGGTGGGA	20R-TTGTTGGGCTCCACATATT	842	EcoTaq	56	x35
	c.8633-1008G>A	intron 20	19F-CATCGCTTTTCAGTGATGGA	25R-CACTGGAGTTGCTTGCGAG	981	EcoTaq	58	x35
	c.8754+759A>G	intron 21	19F-CATCGCTTTTCAGTGATGGA	25R-CACTGGAGTTGCTTGCGAG	981	EcoTaq	58	x35
	c.9256+256A>G	intron 24	19F-CATCGCTTTTCAGTGATGGA	25R-CACTGGAGTTGCTTGCGAG	981	EcoTaq	58	x35
	c.9257-4843C>T	intron 24	19F-CATCGCTTTTCAGTGATGGA	25R-CACTGGAGTTGCTTGCGAG	981	EcoTaq	58	x35
	c.9257-3591G>T	intron 24	19F-CATCGCTTTTCAGTGATGGA	25R-CACTGGAGTTGCTTGCGAG	981	EcoTaq	58	x35
c.9257-882T>C	intron 24	19F-CATCGCTTTTCAGTGATGGA	25R-CACTGGAGTTGCTTGCGAG	981	EcoTaq	58	x35	
c.9501+599T>C	intron 25	24F-AGATTTACCAGCCACGGGAG	27R2-CCGATACACAAACCTGAGG	1265	EcoTaq	57	x35	

* A 5' 6-Carboxyfluorescein (6-FAM) labeled primer was also used

B. Primers for long RT-PCRs and internal sequencing primers to determine allelic imbalance at informative heterozygous loci.

GENE	Long PCR primers (5' to 3')	TA (°C)	FL size (bp)	PCR kit	Cycles	informative exonic variants
BRCA1	11F-CAAGAAGAGCAAAGCATGGA	57	1882	EcoTaq	x35	c.4308T>C; c.4837A>G
	24R-ACTCTGTGCTTCCAGCCCTA					
BRCA2	1F5-AAGCGTGAGGGGACAGATTT	56	2275	Expand™ Long Range dNTPack	x10; x30	c.-52A>G; c.-26G>A; c.865A>C; c.1114A>C
	11R2-GCAGGCATGACAGAGAATCA					
	15F-CCACTCTGCCTCGAATCTC	56	2858	Expand™ Long Range dNTPack	x10; x30	c.7242A>G; c.9976A>T
	27R2-CCGATACACAAACGCTGAGG					

GENE	internal sequencing primers (5' to 3')
BRCA1	11F-CAAGAAGAGCAAAGCATGGA
	14F-GGCCTTTCTGCTGACAAGTT
	16R-TGACTCTGGGGCTCTGTCTT
	18R-TCCGTTACACACAAACTCAG
BRCA2	6R-TGAAACAAACTCCCACATACCA
	9F-TCCAATCATGATGAAAGTCTGA
	16R-TCCACCATCAGCCAAGTGA
	27R2-CCGATACACAAACGCTGAGG

Supplementary Table 2. *BRCA1/2* deep intronic variants identified in >1 patient from our cohort that are predicted to alter splicing.

GENE	HGVS nomenclature*	Location	dbSNP	Population variant frequencies (gnomAD)	Frequency of occurrence in our cohort	Local Splicing Effect (Alamut software)	ClinVar review status
<i>BRCA1</i>	c.80+1312A>G	intron 2	-	-	1,04%	New Acceptor Site	not reported
	c.134+224dup	intron 3	-	ALL:4.46% - AFR:13.85% - AMR:1.17% - EAS:0.074% - NFE:0.17% - FIN:1.19% - OTH:1.14%	2,60%	Cryptic Acceptor Weakly Activated	not reported
	c.442-1887C>A	intron 7	rs191341657	ALL:0.18% - AFR:0.023% - AMR:0.36% - NFE:0.27% - FIN:0.26% - OTH:0.10%	2,08%	Cryptic Acceptor Strongly Activated	not reported
	c.594-447A>G	intron 9	-	-	2,08%	Cryptic Acceptor Strongly Activated	not reported
	c.4358-2509C>A	intron 13	rs562207926	ALL:0.097% - AFR:0.034% - ASJ:0.33% - NFE:0.17% - FIN:0.029%	1,04%	New Acceptor Site	not reported
	c.5193+1234del	intron 19	-	ALL:0.57% - AFR:0.23% - AMR:3.07% - ASJ:0.83% - NFE:0.34% - FIN:3.69% - OTH:0.91%	28,13%	New Donor Site	not reported (c.5193+1232_5193+1234del is classified as benign by expert panel)
<i>BRCA2</i>	c.6841+625T>C	intron 11	-	ALL:0.014% - AFR:0.012% - NFE:0.021%	1,04%	Cryptic Acceptor Strongly Activated	not reported
	c.6841+686del	intron 11	-	ALL:0.84% - AFR:0.34% - AMR:4.14% - NFE:0.49% - FIN:9.59% - OTH:2.11%	47,92%	New Donor Site	not reported
	c.6841+686dup	intron 11	-	ALL:2.11% - AFR:3.46% - AMR:5.73% - ASJ:0.75% - EAS:0.37% - NFE:0.58% - FIN:12.03% - OTH:3.16%	9,90%	New Donor Site	not reported
	c.6937+239A>T	intron 12	rs190434310	ALL:0.27% - AFR:0.11% - AMR:0.12% - ASJ:0.33% - NFE:0.41% - FIN:0.086% - OTH:0.61%	1,04%	Cryptic Acceptor Strongly Activated	not reported
	c.6937+594T>G **	intron 12	rs191253965	ALL:0.036% - AMR:0.95% - ASJ:0.33% - NFE:0.0067% - OTH:0.10%	1,04%	Cryptic Donor Strongly Activated	benign (reviewed by expert panel)
	c.7007+811C>T	intron 13	rs552519251	ALL:0.12% - AFR:0.046% - AMR:0.12% - NFE:0.21%	2,08%	New Donor Site	not reported
	c.8331+3112A>G	intron 18	-	-	1,56%	Cryptic Donor Strongly Activated	not reported
	c.8332-1343C>T	intron 18	rs564842026	ALL:0.013% - AMR:0.12% - FIN:0.087%	1,04%	Cryptic Acceptor Strongly Activated	not reported
	c.8332-2706G>A	intron 18	rs190505464	ALL:0.074% - AFR:0.023% - AMR: 0.24% - NFE:0.13%	1,56%	Cryptic Donor Strongly Activated	not reported
c.9256+2960_9256+2991 dup	intron 24	rs140864865	-	1,04%	New Donor Site	not reported	

* *BRCA1* and *BRCA2* variants are annotated according to NM_007294.3 and NM_000059.3 transcripts. HGVS nomenclature guidelines were used for variant annotation (<http://varnomen.hgvs.org/>)

** First reported in Anczukow et al., 2012