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Based on the information obtained from the CGH array, primers were designed spanning the putative breakpoints for each case and used in long-range PCR

Mutation	From	To	5´--3´ primer sequence	maximum amplicon size
Exon 7 deletion	MSH2-47507393	MSH2-47515906	Intr6-MSH2-S- GGCTCTTCAACTCTGTTGATTAGTT Intr8-MSH2-A- AGTGAAATGGGAGCCAAAA	**860bp**
Exon 4_8 deletion	MSH2-47492237	MSH2-47527926	Intr3-MSH2-S- TCGAGTGGAACTTTAGCCTGTT Intr8-MSH2- A- GGGCAAGTATTAACTCAAACCACAA	**1979bp**

PCR conditions

Buffer Kappa Taq 1X	2,5 **µl**
Mix dNTPs 0,2 mM/cada uno	1,5 **µl**
Primers 12 pmol/ each	1-1 **µl**
Kappa Taq polimerase	0,5 **µl**
300 ng DNA	2 **µl**
H2O	
final volume	**25 µl**

°C	Time		Cicles
94	3	min	
94	30	sec	
60	30	sec	X40
72	1	min/kb*	cicles
72	5	min	
15	∞		

Fluorescently labeled primers were used to amplify the microsatellite polymorphic regions

multiplex	Microsatellite	oligonucleotide	5`-3`sequence	Amplicon size	Td (ºC)
A	*D2S119*	**MSH2-D2S119-S**	CTTGGGGAACAGAGGTCATT	**214-232bp**	**61**
		MSH2-D2S119-A-FAM	GAGAATCCCTCAATTTCTTTGGA		
B	*Clen27*	**MSH2-Clen27-S-FAM**	AACACTGCCATAGTCACAACTGCCA	**241bp**	**60**
		MSH2-Clen27-A	ACCAATTACATGCACTTAACGTGAT		
	D2S391	**MSH2-D2S391-S-HEX**	GTAATGGAGCCAGTAGGTTACA	**149 bp**	
		MSH2-D2S391-A	AGAGGGTATGATGGAAAAGC		
	D2S2227	**MSH2-D2S2227-S**	CACGCTGTCCATCTCTGAAT	**179-221bp**	
		MSH2-D2S2227-A-HEX	GCAGTTTCTCGGAATAACCA		
C	*Clen30*	**MSH2-Clen30-S**	ATTTGTGACTGTTCAGCTGCTCTCT	**268 bp**	**58**
		MSH2-Clen30-A-HEX	ACCAGCACCATACAGTCAGCTCCTA		
	D2S1248	**MSH2-D2S1248-S-FAM**	GCTCCCATACTCTCACTTG	**380bp**	
		MSH2-D2S1248-A	TTAATACCATCCTCAGTAACC		
D	*D2S1247*	**MSH2-D2S1247-S**	TTTTCCTGCTTCCCACCTG	**250 bp**	**61**
		MSH2-D2S1247-A-FAM	GCAAAAAGGGGCTGTCTTC		
	D2S123	**MSH2-D2S123-S**	TCAACATTGCTGGAAGTTCT	**196bp**	
		MSH2-D2S123-A-HEX	GACTTTCCACCTATGGGACT		

PCR conditions

Buffer Kappa Taq 1X	2,5 **µl**
Mix dNTPs 0.2 mM/each	1 **µl**
oligonucleotides pmoles/each: 1 (F, R) 2 (F, R) 3 (F, R)	12 1-1 **µl /each**
Kappa Taq polimerasa	0,2 **µl**
300 ng DNA	2 **µl**
H2O	
Final volume	**25 µl**

°C	Time		Cicles
94	3	min	
94	30	sec	X40 cicles
X*	30	sec	
72	30	sec	
72	5	min	
15	∞		

The two intragenic single base substitutions located within intron 1 and intron 9 of MSH2 (rs2162123 and rs3771278) were screened through High Resolution Meelting (HRM) thechnology [LightCycler® 480 Instrument (Roche)]

marker	change	position	**Primer sequence**	**TºC**	**size**
rs2162122	T/C	Intron 1	**MSH2-In1-S**- GTCCCTCCCACAATACATGG	**60**	**191pb**
			MSH2-In1-A- ACAGGCTCATATGCGGAAAG		
rs3771278	C/T	Intron 9	**MSH2-In9-S**- GAATGGGTCATTGGAGGTTG	**60**	**256pb**
			MSH2-In9-A- ATCATACAAGGGCCTGTTGG		

PCR conditions

LightCycler®480 High Resolution Meeting X2	5 **µl**
Oligonucleotides 0,2 **µM**/each (S,A)	0,25-0,25 **µl**
Cl2Mg(25mM)	1 **µl**
30 ng ADN	2 **µl**
H2O	
Final volume	**10 µl**

Programs

Program Name	Cycles	Analysis Mode
DESNATURALIZACION	1	None
PCR	40	Quantification
MELTING	1	Melting Curves
HOLD	1	None

DESNATURALIZACION Temperature Targets

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:10:01	4,4		0	0	0

PCR Temperature Targets

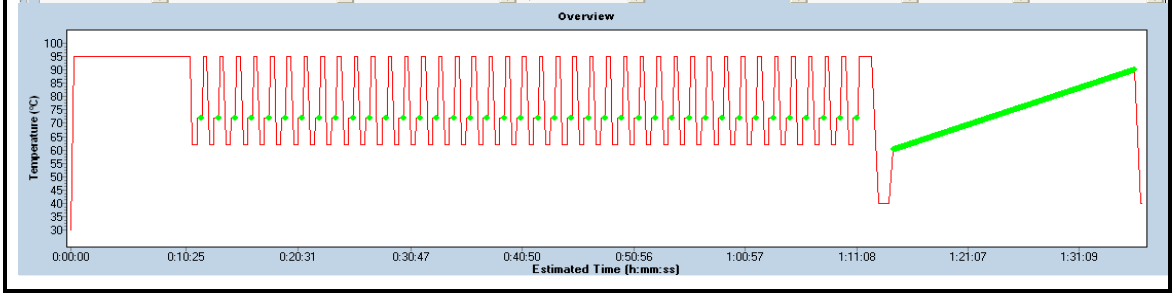
Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:00:10	4,4		0	0	0
62	None	00:00:13	2,2		0	0	0
72	Single	00:00:14	4,4		0	0	0

MELTING Temperature Targets

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	None	00:01:00	4,4				
40	None	00:01:00	1,5				
60	None	00:00:01	1				
90	Continuous		0,02	25			

HOLD Temperature Targets

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisitions (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
40	None	00:00:10	1,5		0	0	0



We designed a PCR test to screen these deletions in first degree relatives. A routine PCR procedure was optimized. We used three primer sequences: one forward and two reverse

EXONS 4-8 MSH2 primer sequence	Amplicon size
MSH2-In3S: TCGAGTGGAACTTTAGCCTGTT **MSH2-In8A:** GGGCAAGTATTAACTCAAACCACAA **MSH2-In3´A:** CCGGCCACAATAACAACC	**WT:** 1175pb **Del:** 751pb
EXON 7 MSH2 primer sequence	**Amplicon size**
MSH2-In6S: GGCTCTTCAACTCTGTTGATTAGTT **MSH2-In8A:** CGTAATTAGCTGGGCATGGT **MSH2-In6´A:** AGTGAAATGGGAGCCAAAAA	**WT:** 586pb **Del:** 861pb

PCR conditions

MegaMix-double (microzone)	15ul
MSH2-InXS (8 µM) S:	2ul
MSH2-InXA (8 µM) S´:	2ul
MSH2-InX´A (8 µM) A:	0.5ul
DNA(150ng)	2ul
H20	
Final Volume	**25 µl**

T°C	time		Cicles
94	9	min	
94	1	min	X35
60	1	min	
72	2	min	
72	10	min	
15	∞