Loss of Heterozygosity Analysis at selected Single Nucleotide Polymorphism Sites in the Intronic Regions of *PAX7* via *In Silico* Biology & Microsatelite Analysis.

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Abstract

Motivation. In this study, the eight intronic regions of *PAX7* were scanned for Single Nucleotide Polymorphisms (SNPs), loss of heterozygosity via allele quantification, and tandem repeats that exist immediately before or after the genetic variations.

Method. Culmination of results based on the output of gel electrophoresis and Microsatelite analysis.

Results. The use of less invasive methods, *In Silico* Biology & Microsatellite analysis to identify Rhabdomyosarcoma offering reduced discomfort to cancer patients.

Conclusions. Results of microsatellite analysis show a new method for detecting ERMS/ARMS in patients

Keywords. Single Nucleotide Polymorphisms; PAX7; Rhabdomyosarcoma; Loss of heterozygosity; ERMS, ARMS.

Abbreviations and notations

MSI, Microsatellite Instability	chr., chromosome
ARMS, alveolar rhabdomyosarcoma	5', five prime end of DNA strand
3', three prime end of DNA strand	electropherogram, picture file
ERMS, embryonal rhabdomyosarcoma	RD, Rhabdomyosarcoma
CNP, Copy Number Polymorphism	SNP, Single Nucleotide Polymorphism
AQ, Allele Quantification	TSS, transcription start site
LOH, Loss of heterozygosity	SSLP, Simple sequence length polymorphisms
bp, base pair	nt, nucleotide

INTRODUCTION

Testing for the loss of heterozygosity and mutation detection is performed routinely at hospitals across the United States for confirmation of a cancer diagnosis and identification of the molecular lesion [Maris et al., 2005; Kelloff et al.,2006]. Researchers and clinicians have bridged the gap between the discovery and experimentation of new methods to diagnose patients sooner at a molecular level enabling them to create/modify new therapeutics. Much research has been performed on Rhabdomyosarcomas and the chimeric translocation between *PAX7* on chromosome 1 and the Forkhead gene on chromosome 13. In studies performed recently [Hirsch et al., 2001], alveolar rhabdomyosarcoma (ARMS) and embryonal rhabdomyosarcoma (ERMS) have been used to test for single nucleotide polymorphisms (SNPs) which may lead to the location of acquired homozygosity. Thus far, researchers have looked towards chimeric translocations of *PAX7* with other chromosomes (chr.3 & chr.11), (Davis and Barr 1997; Steenman et al., 2000) besides the well known translocation with chromosome 13 (Gattenlöhner et al., 1998). Loss of heterozygosity (LOH) at multiple chromosomal loci is strongly associated with clinical phenotype including patient outcome [Loh et al., 2002].

In this study, the eight intronic regions of *PAX7* were scanned for SNPs that exhibit loss of heterozygosity and were quantified by allele quantification, and tandem/microsatellite repeats that exist immediately before or after the genetic variations. Based on the genotype data stored in the NCBI databank and in house genomic DNA controls, a mutation was detected in one of the seven (14.28%) of the samples tested.

MATERIALS AND METHODS

Tumor Samples & Control Samples

Five ARMS samples were analyzed; all five have PAX7-FKHR translocations and were obtained from The five ARMS samples were delivered as isolated DNA cores in paraffin, from Biomaxx , Inc.(http://www.biomax.us/). All five samples were excised from patient tumour samples. ERMS samples from established cell line ATCC#: CCL 136 and five normal DNA samples isolated from buccal swabs submitted by volunteers were also examined.

LOH Analysis. Primary normal/tumor pairs were investigated using fluorescently labeled microsatellites. Primer sequences were obtained from the Primer3 Database (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Amplifications of each microsatellite were done in 7.5-µl volumes with 10 ng of each respective genomic DNA, 8 pmol of each primer (5' primer, fluorescently labeled), 100 µM each dNTP, 0.6 unit of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA), 10 mM Tris·HCl (pH 8.3), 50 mM KCl, and 2 mM $MgCl_2$.

DNA Isolation

EPICENTRE® Biotechnologies (http://www.epibio.com) BuccalAmp™ DNA Extraction Kit, QuickExtract™ DNA Extraction Solution, and Catch-All™ Sample Collection Swabs were used for the DNA Isolation of the five normal samples submitted by volunteers. The QuickExtract™ DNA Extraction Solution was also used for the ERMS sample from the cell line ATCC#: CCL 136. The five ARMS samples were delivered as isolated DNA cores formalin fixed, paraffin embedded histology tissue sections. The tissues were fixed by formalin no longer than 48 hours, and then processed to be ready for sectioning. Two serial tissue sections with 5 µm thickness are mounted on a SuperFrost Plus glass slides.

***In Silico* Investigation**

The introns of *PAX7* were scrutinized with four (3 online, 1 offline) unique programs which store databases of SNPs submitted to the Human Genome Project. The names of the four programs are:

1) dbSNP (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=Pager&DB=snp) - Search SNPs with heterozygosity between 0 and 90 percent.
2) SNP500Cancer (http://snp500cancer.nci.nih.gov/snplist.cfm) - The goal of the SNP500Cancer project is to resequence 102 reference samples to find known or newly discovered single nucleotide polymorphisms (SNPs) which are of immediate importance to molecular epidemiology studies in cancer. SNP500Cancer provides a central resource for sequence verification of SNPs.
3) Mreps: (http://bioweb.pasteur.fr/seqanal/interfaces/mreps.html) - Mreps is a flexible and efficient software program for identifying serial repeats (usually called *tandem repeats*) in DNA sequences.
4) GeneMarker® (http://www.softgenetics.com/gm/gm_fraganal.htm) for fragment analysis is a genotyping tool with an integrated pedigree function.

The eight introns of *PAX7* were scanned for genetic variations consisting of single nucleotide polymorphisms and microsatellites using the databases stated above. This practice of searching for mutations has been previously utilised elsewhere (Ceccherini, I. et al, 1994; Carrasquillo et al., 2002)

Primer & Assay Design

The primer design for microsatellite analysis was performed utilizing the Biotage AB Assay Design Software version 1.0.6. Thirty to sixty base pairs before and after the target polymorphisms were loaded into the Assay Design program to provide the best options of forward, reverse, and sequencing primers.

Location of the primers used in the investigation of the microsatellites within intron 5 and intron 8 of the *PAX7* gene.

Primer 5: rs12740463 [*Homo sapiens*] Intron 8; chromosome position: chr1:18783428+18783596

Primer	Sequence	Genotype Detail
Forward PCR Primer	CTGGGGAAGGACGAGCTGA	C: 1.000
Reverse PCR Primer	GTACCAACCGCACTGGTCATG	

Primer 16: rs2236830 [*Homo sapiens*] Intron 5; chromosome position: chr1:18726993-18727052

Primer	Sequence	Genotype Detail
Forward PCR Primer	TTTTTCCAAGGCGATCGG	T: 0.955
Reverse PCR Primer	CTGTGGCTGCCTTACCTGA	C: 0.045

P 2) rs742077 [*Homo sapiens*] Intron 8; chromosome position: chr1:18802224-18802395

Primer	Sequence	Genotype Detail
Forward PCR Primer	CCTGCAGCCTTGACCTGATTC	G: 0.080
Reverse PCR Primer	GGGAGTGTGGCTGCAATTAGAA	A: 0.920

***Figure 1**. Above are the primers used for the analyses of the seven samples. Given for each are: 1) the primer name, the reference SNP cluster report ID; 3) species; 4) Intron #; 5) chromosome position; 6)Genotype detail; 7) sequence of the primers.*

The three sets of primers were selected based on previous research which indicated several novel cis-acting elements present in the *immediate* vicinity of SNPs detected via *In Silico* biology (Mitchell and Ziman 2006). The results of this research generated enough investigational interest to test the theories with bench work. These primers where also selected due to their positioning on the two (2) transcript variants for ARMS (NM_013945 and NM_002584). The primers were also generated on the basis of the genotype detail (see above) for loss of heterozygosity. Loss of heterozygosity has been studied in ARMS previously (White *et al.* 1995; Staehelin, F. *et al*., 2000) where chromosome 1 and chromosome 11 had exhibited chimeric translocations. In this study, we used the SNP data from previous findings (Mitchell and Ziman 2006) to test ARMS patient DNA, ERMS Cell line DNA and control genomic DNA from healthy individuals for the alleles reported in the NCBI database.

For microsatellite analysis, fluorescent dye labels (6-FAM (blue), HEX (green), and NED (yellow)) were used for each primer. Size standards (Rox (red)) are used for all samples. There is no clean-up for PCR products used in this process. One to four microliters of PCR product was used for testing. A mixture of 7.5 ul/ sample (7 ul of Hi-Di-Formamide and 0.5 uls of Rox standard) was added and then denatured at 98°C for 4 minutes and then cooled for an additional 4 minutes. Analysis of samples was performed using the 16-array 3100 Genetic Analyzer (Applied Biosystems).The analysis of the processed samples was performed with GeneMapper Software version 3.0 (Applied Biosystems) and SoftGenetics GeneMarker® version 1.51. The analysis software uses the size standard to create a standard curve for each lane and then determines the length of each dye-labeled fragment by comparing it with the standard curve for that specific lane. Accuracy of less than one base pair difference is seen between sample replicates.

***In Silico* PCR Amplification**

In-Silico PCR amplification provides the user a systematic approach to prescreen the custom-designed primers for the given template before purchase. Programs (http://insilico.ehu.es/) & (http://genome.ucsc.edu/cgi-bin/hgPcr) were used and the output of these programs allow for quick and easy changes to primer design or template choice. The two websites cited compare the user's template and primers.

PCR Analysis / Gel Electrophoresis

DNA dilution–based PCR assay was used for allele quantification and SNP detection. The following DNA concentrations (serial dilutions) were examined for the five cases; 2, 1, 0.5, 0.25, 0.125 ng/ul. One ul of each dilution was used as the DNA template for the PCR reactions. PCR was performed in a volume of 25 ul with the presence of 100 uM of each dNTP, 10 mM Tris-HCl (pH 8.3), 2 mM magnesium chloride, 50 mM potassium chloride, 0.75 unit of Taq DNA polymerase, and 15 pmoles of each custom primer. The following reaction conditions were employed: denaturation at 96°C for 5 min, followed by 60 cycles each of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, with a final extension of 5 min at 72°C. Sixty-five cycles were used due to the small size of the fragment and the low concentration of the isolated patient DNA. Subsequently, aliquots of the PCR reaction were resolved on 2% / 4% Invitrogen™ commercial pre-cast agarose E-gel, and the products were visualized *on Bio-Rad UV Gel Doc 2000*.

Microsatellite Analysis Instrument: ABI PRISM ® 3100 Genetic Analyzer

The ABI PRISM®3100 Genetic Analyzers are automated capillary electrophoresis systems that can separate, detect, and analyze fluorescent-labeled DNA fragments in one run.

RESULTS

Standard PCR Analysis/ Gel electrophoresis

Standard PCR assays performed on all seven samples revealed amplification of the selected SNPs found in the intronic regions of the *PAX7* gene (***Fig.2***). The gene-specific fragment was visualized at all dilution levels for these cases.

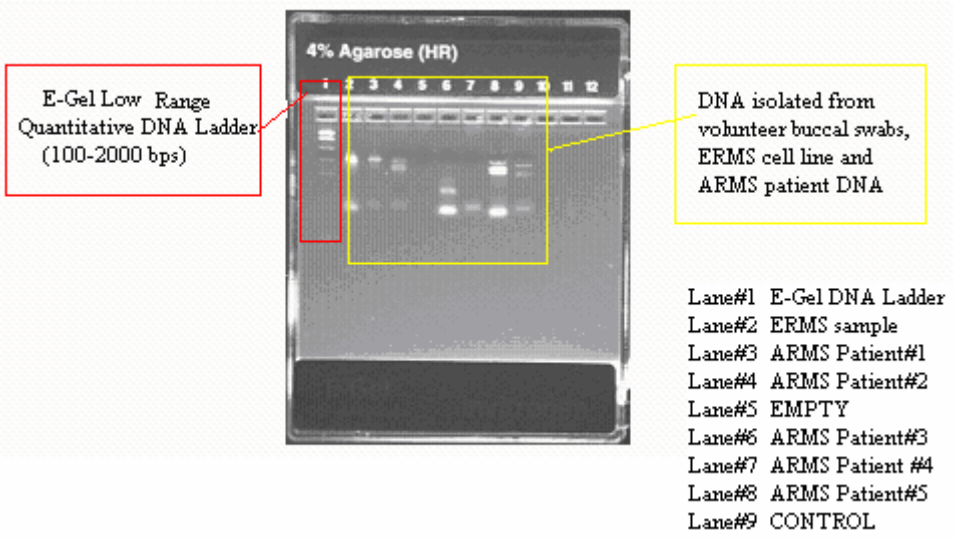
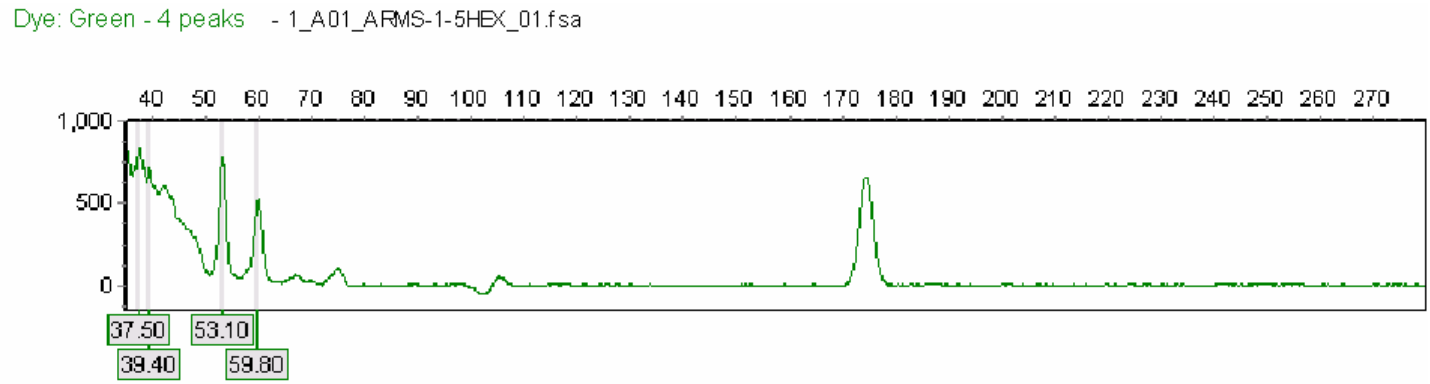


Figure 2

Figure 2. *Invitrogen™ 4% precast Agarose Gel electrophoresis showing serial dilution for DNA isolated from volunteer buccal swabs, ERMS cell line and ARMS patient DNA. Sybr-Safe staining resolved on Bio-Rad UV Gel Doc 2000. All samples were exposed to the three primer sets; Primer 5 (C/T), Primer 16 (C/T), and Primer 37(G/A).*

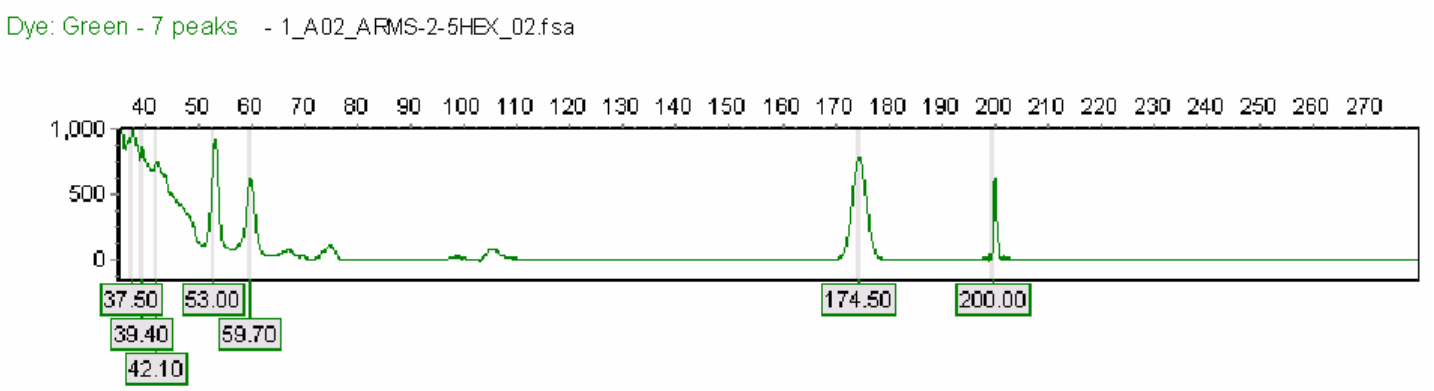
Microsatellite Analysis Results

Microsatellite Analysis provides for a visual and qualitative approach to gene copy number modifications that occur from the loss of tumor suppressor genes. In past studies, the loss of heterozygosity was detected by restriction fragment length polymorphism (RFLP) and microsatellite markers. More recently, the HuSNP GeneChip© (Affymetrix) has made the completion of large projects more attainable through allelotyping whole genomes by screening for more than 1500 SNPs in one process cycle [Wong et al., 2004].



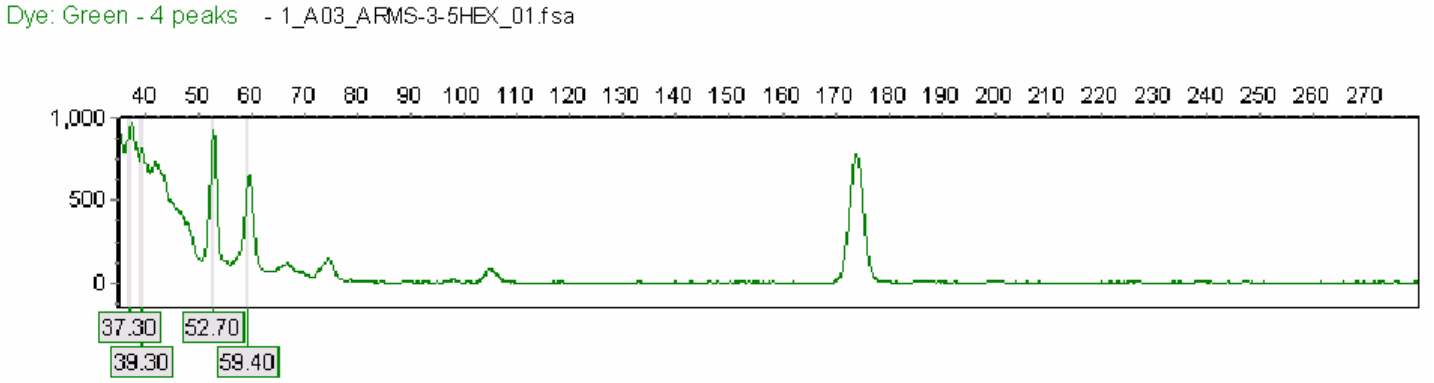
No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	37.50	843	9440			1.0	Unknown	4.4	
2	39.40	710	6010			1.0	Unknown	4.6	
3	53.10	775	11531			1.0	Unknown	9.8	
4	59.80	524	8639			1.0	Unknown	3.3	

Figure 3. *Fragment analysis results for ARMS Patient #1, Primer 5 with electropherogram and peak table.*



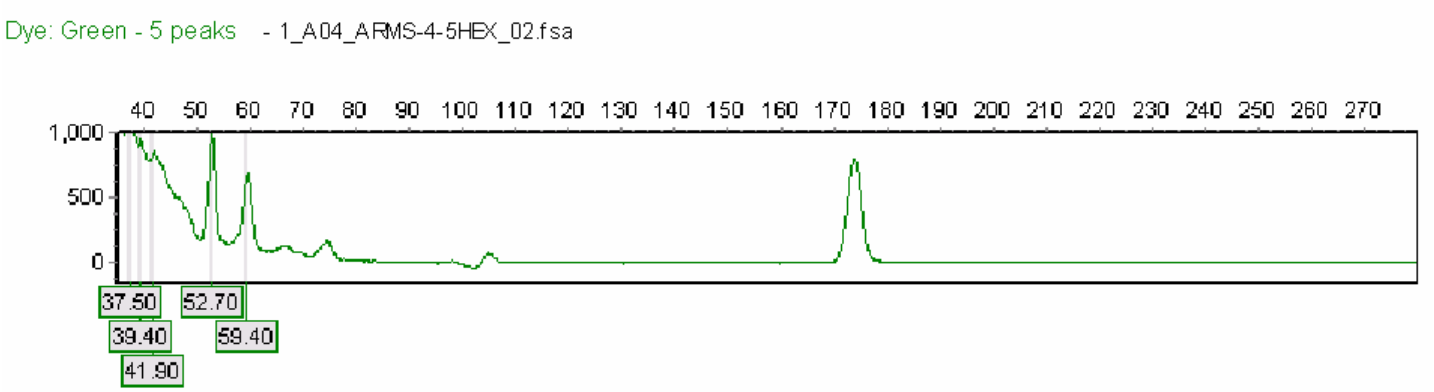
No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	37.50	1015	10575			1.0	Unknown	4.4	
2	39.40	858	6540			1.0	Unknown	5.0	
3	42.10	750	9386			1.0	Unknown	1.1	
4	53.00	917	13789			1.0	Unknown	13.6	
5	59.70	625	10861			1.0	Unknown	3.9	
6	174.50	784	23081			1.0	Unknown	1.3	
7	200.00	620	3542			1.0	Unknown	78.7	

Figure 4. *Fragment analysis results for ARMS Patient #2, Primer 5 with electropherogram and peak table. The presence of the mutant allele in this sample appears as an extra peak labeled with HEX (Patient#2, sample A02.205HEX) at 200 nt. The highest peak was chosen as the reference. False alleles were called when they were >5% of the most prominent allele, but were not present in the genotype of the buccal swab control DNA samples.*



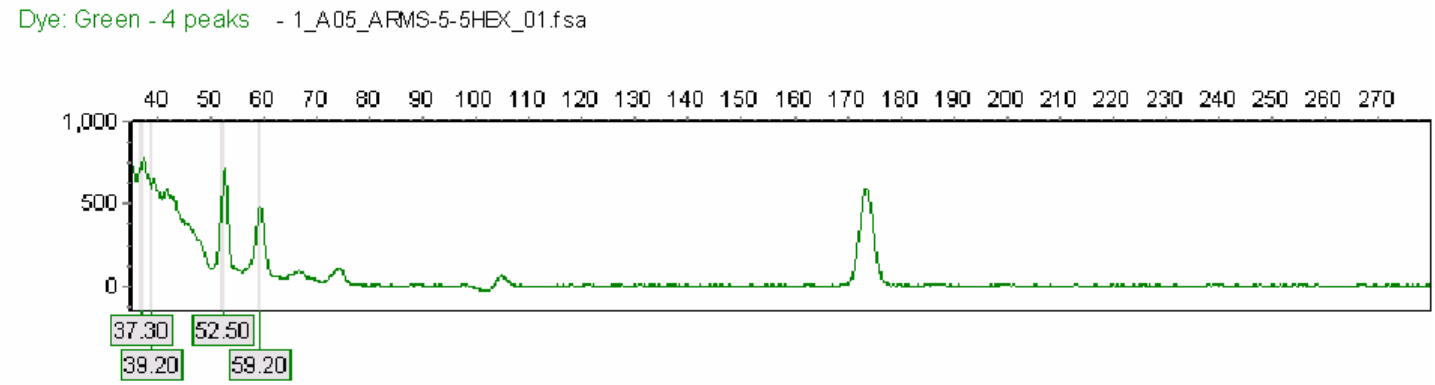
No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	37.30	970	10967			1.0	Unknown	3.7	
2	39.30	819	7035			1.0	Unknown	3.6	
3	52.70	922	13713			1.0	Unknown	13.3	
4	59.40	650	11235			1.0	Unknown	4.7	

Figure 5. *Fragment analysis results for ARMS Patient 31, Primer 5 with electropherogram and peak table.*



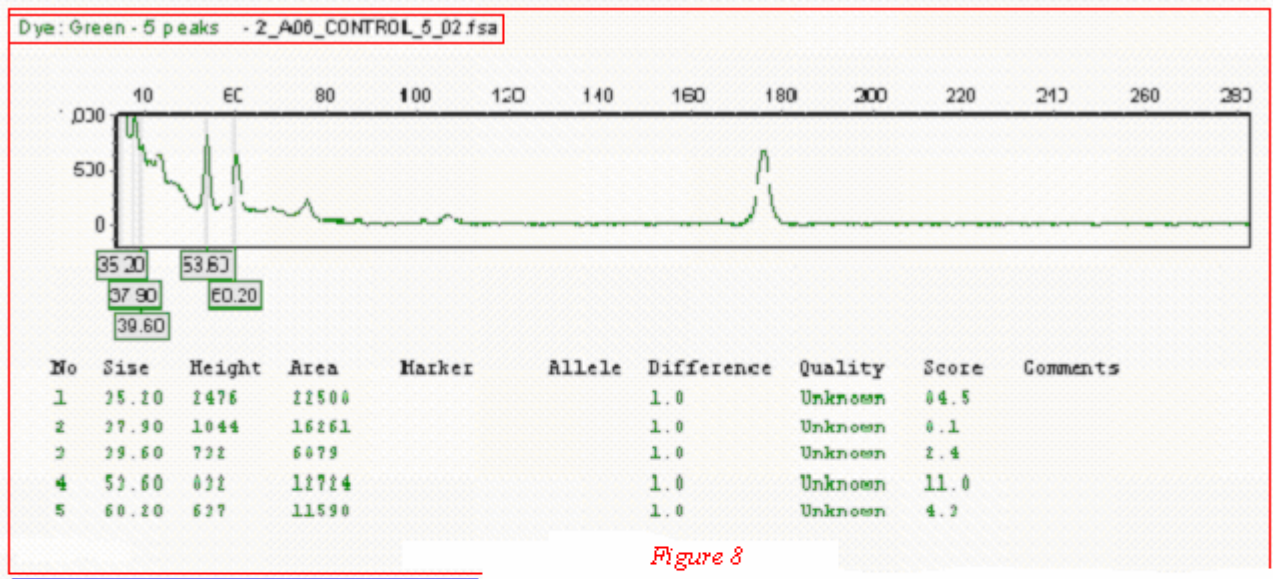
No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	37.50	1145	19182			1.0	Unknown	6.0	
2	39.40	956	8254			1.0	Unknown	4.0	
3	41.90	855	10711			1.0	Unknown	1.2	
4	52.70	994	14912			1.0	Unknown	15.6	
5	59.40	684	11771			1.0	Unknown	4.6	

Figure 6. *Fragment analysis results for ARMS Patient #4, Primer 5 with electropherogram and peak table.*



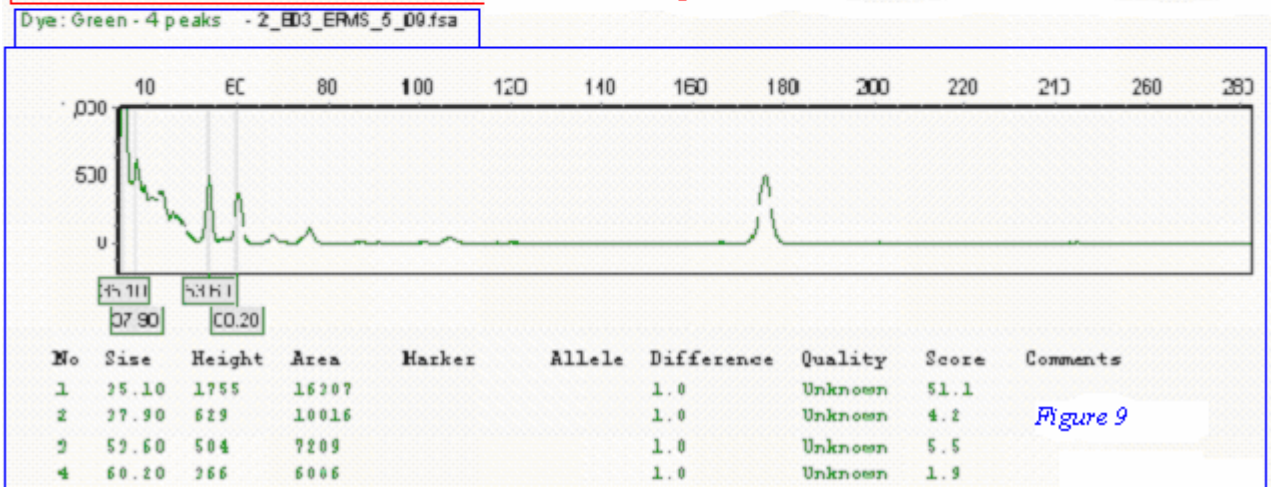
No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	37.30	772	12917			1.0	Unknown	2.1	
2	39.20	654	5604			1.0	Unknown	2.4	
3	52.50	711	10521			1.0	Unknown	9.1	
4	59.20	483	8093			1.0	Unknown	1.9	

Figure 7*. Fragment analysis results for ARMS Patient #5, Primer 5 with electropherogram and peak table.*



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	35.20	1476	22500			1.0	Unknown	84.5	
2	37.90	1044	16261			1.0	Unknown	6.1	
3	39.60	722	6079			1.0	Unknown	2.4	
4	53.60	832	12724			1.0	Unknown	11.0	
5	60.20	627	11590			1.0	Unknown	4.2	

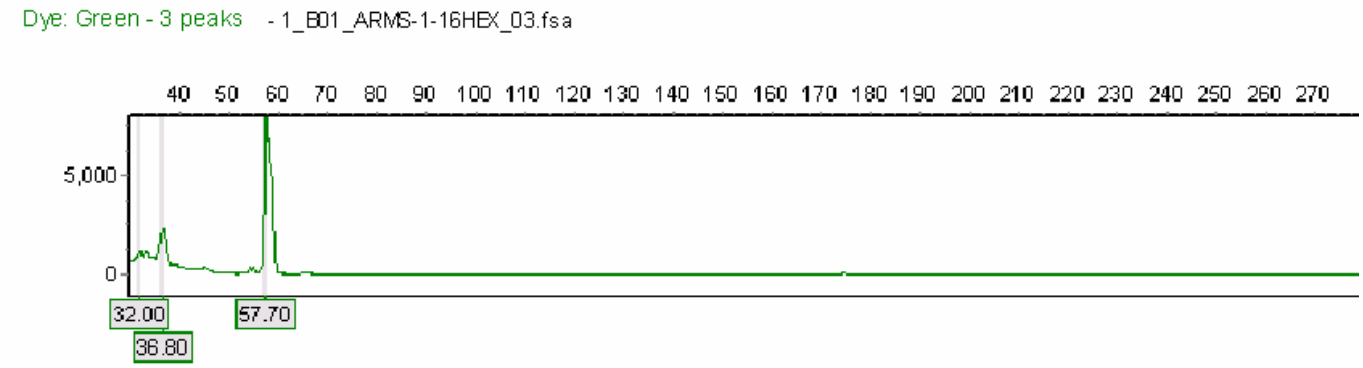
Figure 8



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	35.10	1755	16207			1.0	Unknown	51.1	
2	37.90	629	10016			1.0	Unknown	4.2	
3	53.60	504	7209			1.0	Unknown	5.5	
4	60.20	266	6006			1.0	Unknown	1.9	

Figure 9

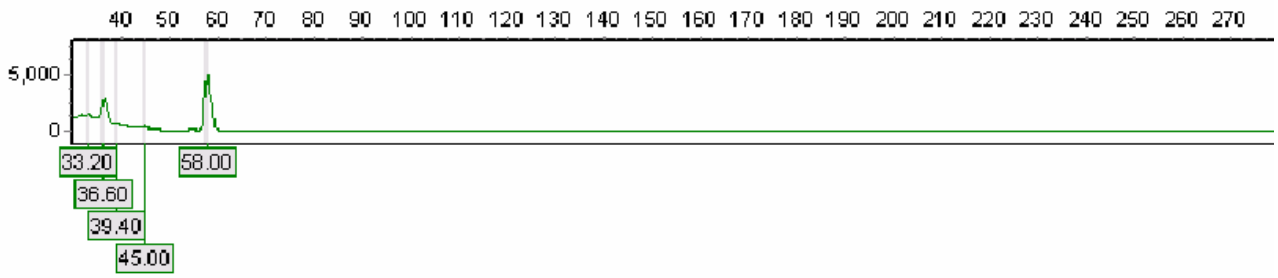
Figure 8 **&** ***Figure 9***: *Fragment analysis results for ERMS cell line(*ATCC#: CCL 136) *and control sample, with Primer 5 showing electropherograms and peak tables.*



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	32.00	1196	11614			1.0	Unknown	25.7	
2	36.80	2368	34146			1.0	Unknown	59.3	
3	57.70	8181	115835			1.0	Unknown	500.0	[<SAT (Repaired)>]

Figure 10: *Fragment analysis results for ARMS Patient #1, Primer 16 with electropherogram and peak table.*

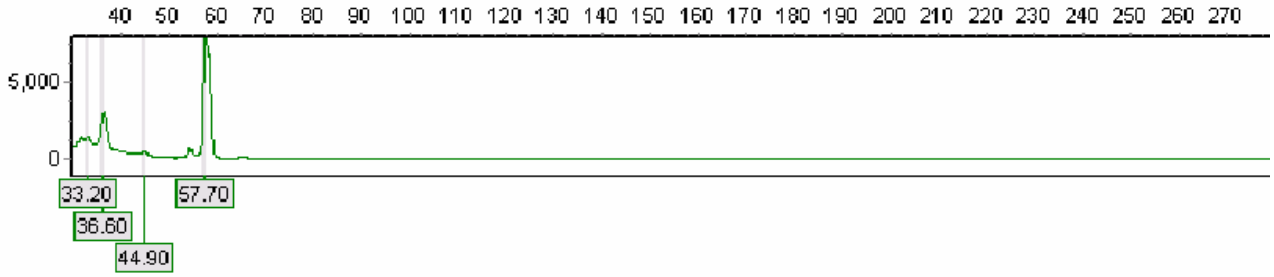
Dye: Green - 5 peaks - 1_B02_ARMS-2-16HEX_04.fsa



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	33.20	1542	21502			1.0	Unknown	5.8	
2	36.60	2841	43188			1.0	Unknown	65.8	
3	39.40	734	6238			1.0	Unknown	2.6	
4	45.00	492	6136			1.0	Unknown	1.4	
5	58.00	5014	73882			1.0	Unknown	328.9	

Figure 11. *Fragment analysis results for ARMS Patient #2, Primer 16 with electropherogram and peak table.*

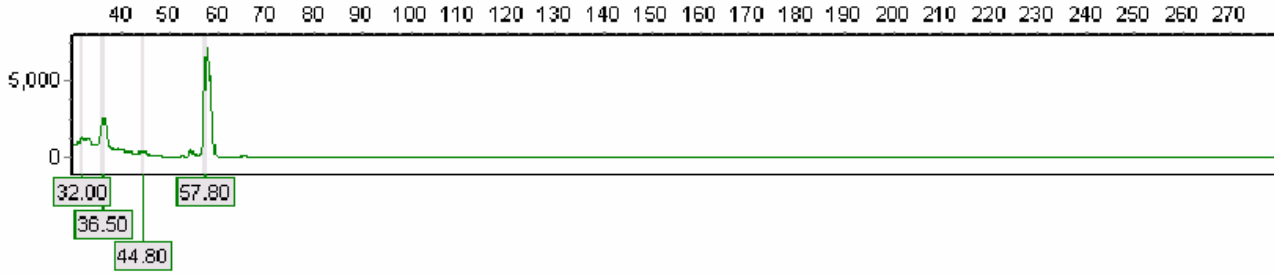
Dye: Green - 4 peaks - 1_B03_ARMS-3-16HEX_03.fsa



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	33.20	1529	18978			1.0	Unknown	19.2	
2	36.60	3132	44102			1.0	Unknown	103.2	
3	44.90	507	6826			1.0	Unknown	2.7	
4	57.70	9131	130623			1.0	Unknown	500.0	[<SAT (Repaired)>]

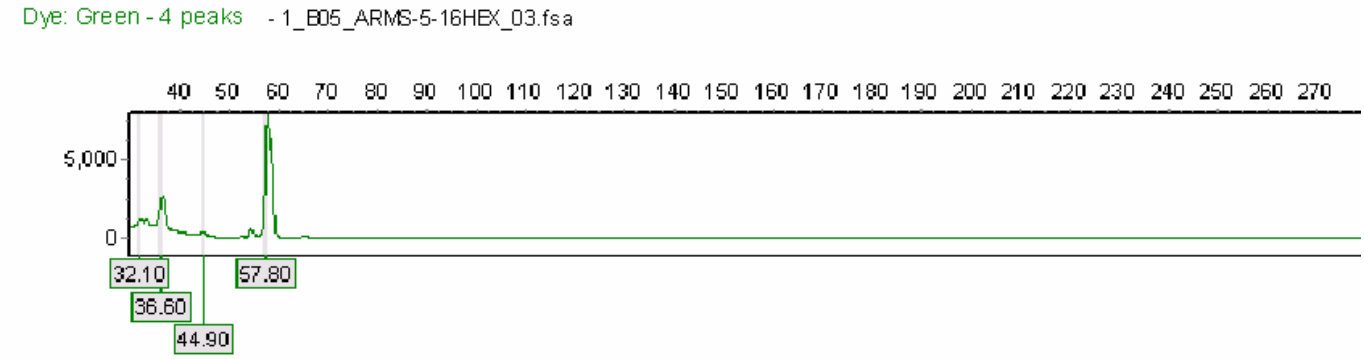
Figure 12. *Fragment analysis results for ARMS Patient #3, Primer 16 with electropherogram and peak table.*

Dye: Green - 4 peaks - 1_B04_ARMS-4-16HEX_04.fsa



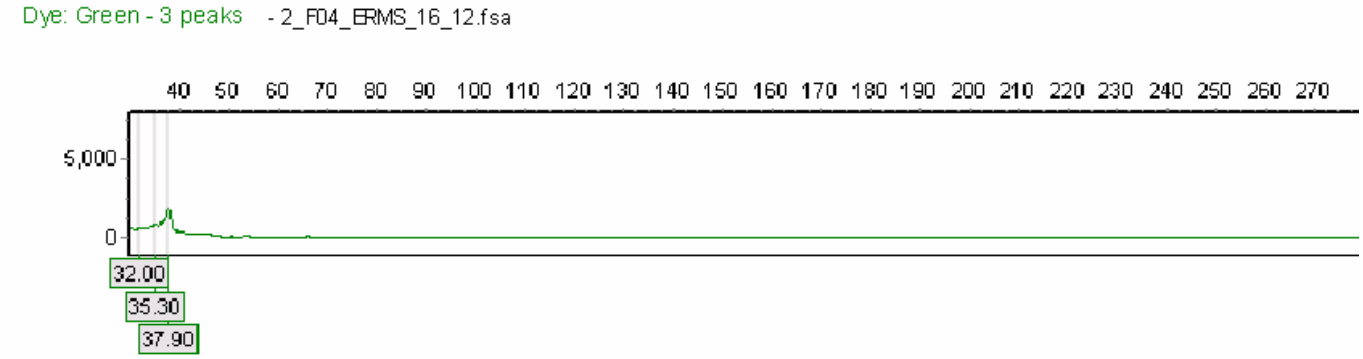
No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	32.00	1363	12516			1.0	Unknown	20.4	
2	36.50	2625	36989			1.0	Unknown	81.7	
3	44.80	400	7855			1.0	Unknown	1.3	
4	57.80	7110	102485			1.0	Unknown	500.0	

Figure 13. *Fragment analysis results for ARMS Patient #4, Primer 16 with electropherogram and peak table.*



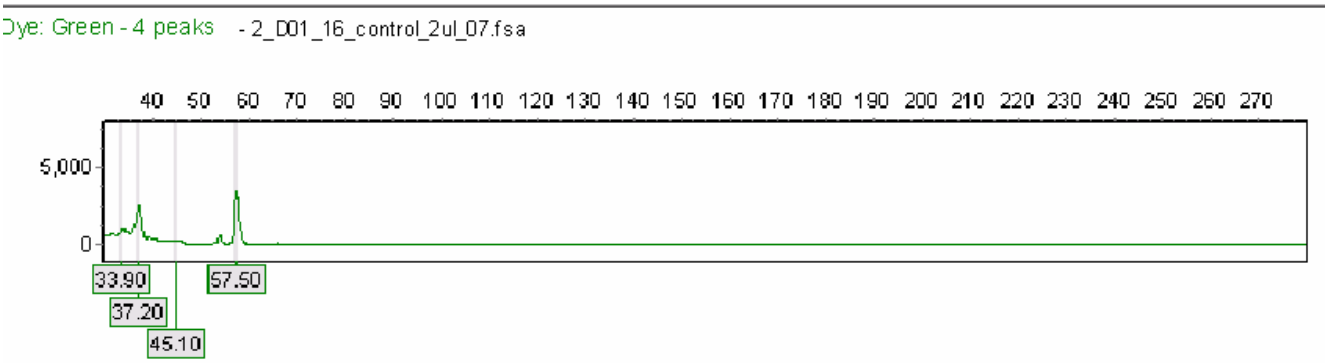
No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	32.10	1297	11714			1.0	Unknown	24.6	
2	36.60	2708	36169			1.0	Unknown	94.9	
3	44.90	399	5426			1.0	Unknown	1.9	
4	57.80	7961	111354			1.0	Unknown	500.0	[<SAT (Repaired)>]

Figure 14. *Fragment analysis results for ARMS Patient #5, Primer 16 with electropherogram and peak table.*



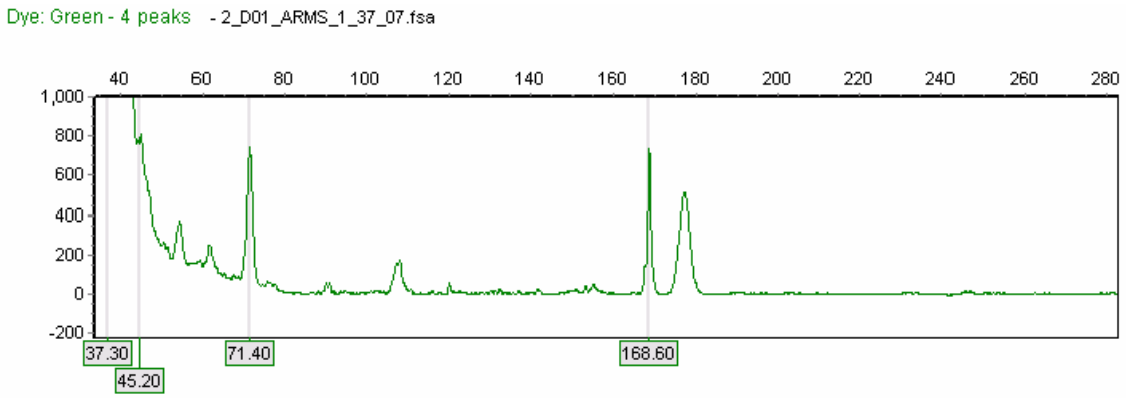
No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	32.00	701	7803			1.0	Unknown	3.0	
2	35.30	905	16647			1.0	Unknown	3.8	
3	37.90	1921	23720			1.0	Unknown	61.5	

Figure 15. *Fragment analysis results for ERMS Cell line (*ATCC#: CCL 136*), Primer 16 with electropherogram and peak table.*
Note: Peak 57.50 is absent from this sample.



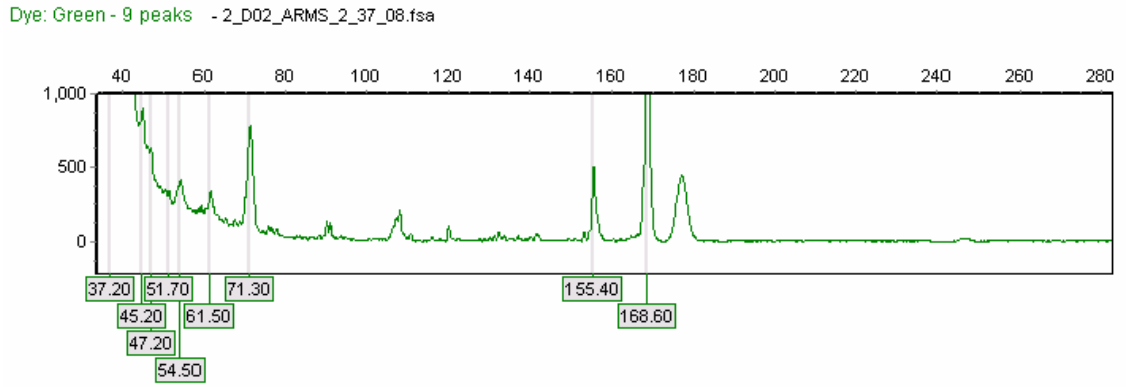
No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	33.90	991	19417			1.0	Unknown	3.7	
2	37.20	2584	35117			1.0	Unknown	107.5	
3	45.10	331	5111			1.0	Unknown	1.4	
4	57.50	3474	30708			1.0	Unknown	392.3	

Figure 16. *Fragment analysis results for CONTROL sample (genomic DNA) with Primer 16 with electropherogram and peak table.*



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	37.30	7511	70078			1.0	Unknown	500.0	
2	45.20	808	8852			1.0	Unknown	2.8	
3	71.40	745	11662			1.0	Unknown	10.5	
4	168.60	735	5569			1.0	Unknown	57.1	

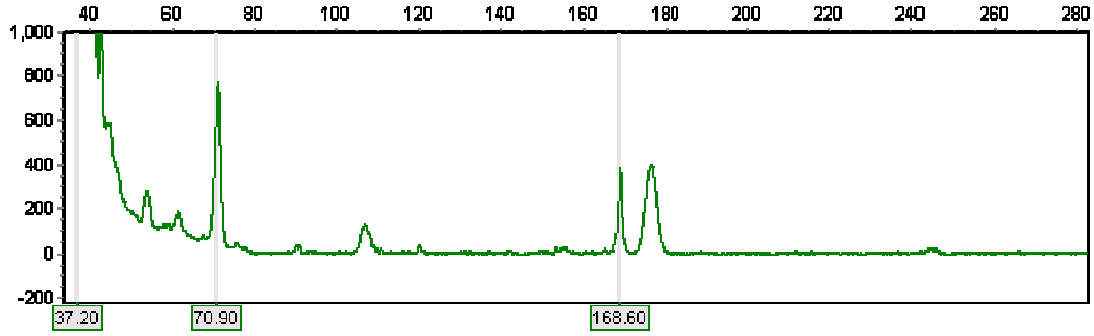
Figure 17*. Fragment analysis results for ARMS Patient #1, Primer 37 with electropherogram and peak table.*



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	37.20	6578	62174			1.0	Unknown	500.0	
2	45.20	898	9104			1.0	Unknown	7.7	
3	47.20	628	5760			1.0	Unknown	4.2	
4	51.70	335	3057			1.0	Unknown	1.7	
5	54.50	408	8215			1.0	Unknown	1.5	
6	61.50	333	3150			1.0	Unknown	3.6	
7	71.30	784	12024			1.0	Unknown	11.1	
8	155.40	502	4223			1.0	Unknown	24.8	
9	168.60	2390	19105			1.0	Unknown	317.9	

Figure 18. *Fragment analysis results for ARMS Patient #2, Primer 37 with electropherogram and peak table.*

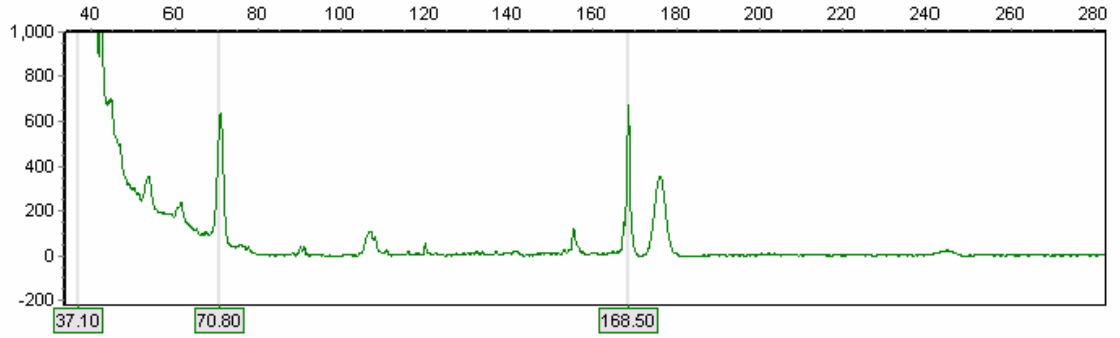
Dye: Green - 3 peaks - 2_D03_ARMS_3_37_07.fsa



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	37.20	5702	50832			1.0	Unknown	500.0	
2	70.90	772	11871			1.0	Unknown	8.0	
3	168.60	382	3056			1.0	Unknown	18.8	

Figure 19. *Fragment analysis results for ARMS Patient #3, Primer 37 with electropherogram and peak table.*

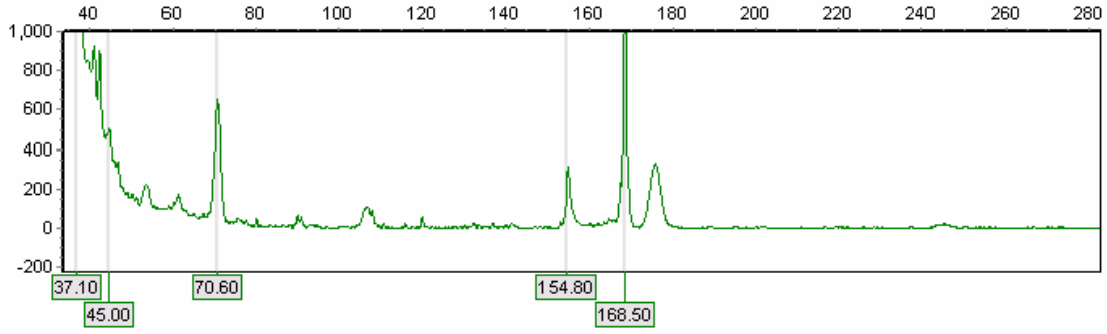
Dye: Green - 3 peaks - 2_D04_ARMS_4_37_08.fsa



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	37.10	5347	47566			1.0	Unknown	500.0	
2	70.80	632	10747			1.0	Unknown	4.7	
3	168.50	674	5296			1.0	Unknown	49.2	

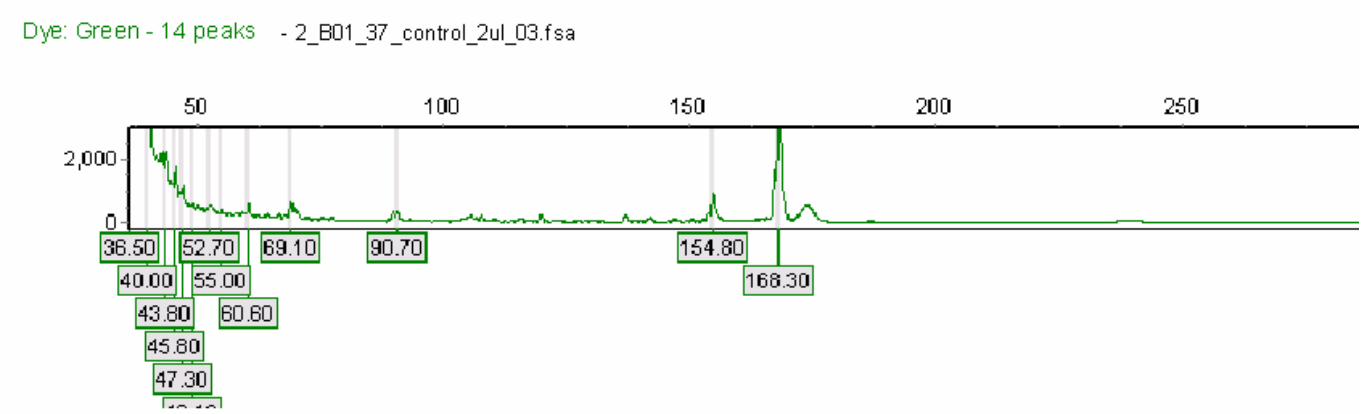
Figure 20. *Fragment analysis results for ARMS Patient #4, Primer 37 with electropherogram and peak table.*

Dye: Green - 5 peaks - 2_D05_ARMS_5_37_07.fsa



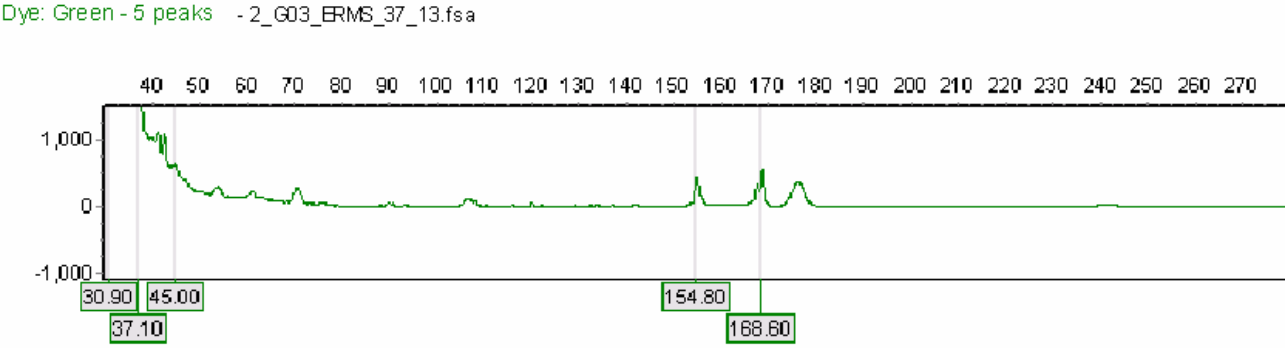
No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	37.10	4446	39862			1.0	Unknown	500.0	
2	45.00	507	5547			1.0	Unknown	2.3	
3	70.60	654	11018			1.0	Unknown	6.4	
4	154.80	316	2881			1.0	Unknown	8.9	
5	168.50	1236	9705			1.0	Unknown	129.7	

Figure 21. *Fragment analysis results for ARMS Patient #5, Primer 37 with electropherogram and peak table.*



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	35.00	7052	45223			1.0	Unknown	408.9	
2	36.50	7546	117513			1.0	Unknown	302.1	
3	40.00	8766	63976			1.0	Unknown	500.0	[<SAT (Repaired)>]
4	43.80	2258	17471			1.0	Unknown	104.6	
5	45.80	1740	12469			1.0	Unknown	157.0	
6	47.30	1166	8479			1.0	Unknown	59.7	
7	49.10	602	4295			1.0	Unknown	10.0	
8	52.70	558	10166			1.0	Unknown	2.0	
9	55.00	394	2791			1.0	Unknown	6.1	
10	60.60	575	4245			1.0	Unknown	36.3	
11	69.10	679	4353			1.0	Unknown	64.0	
12	90.70	382	2343			1.0	Unknown	28.8	
13	154.80	884	6906			1.0	Unknown	68.4	
14	168.30	4817	35291			1.0	Unknown	500.0	

Figure 22. *Fragment analysis results for CONTROL sample (genomic DNA) with Primer 37 with electropherogram and peak table.*



No	Size	Height	Area	Marker	Allele	Difference	Quality	Score	Comments
1	30.90	7034	73699			1.0	Unknown	386.2	
2	37.10	5093	44949			1.0	Unknown	500.0	
3	45.00	630	10027			1.0	Unknown	2.0	
4	154.80	446	3950			1.0	Unknown	20.6	
5	168.60	555	4205			1.0	Unknown	36.5	

Figure 23. *Fragment analysis results for ERMS Cell line (*ATCC#: CCL 136*), Primer37 with electropherogram and peak table.*

DISCUSSION

The microsatellite results for all sample types were normal or within the percentage range based on the genotypes detailed on the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/). However, significant results from this study indicate that ARMS patient#2 (Figure#4) when analysed by microsatellite analysis exhibited a definite mutation for primer 5 as compared to the other 4 ARMS patients, the ERMS cell line sample and the control. Patient #2 sample may possess microsatellite instability, (MSI) which is noted for many other types of tumors [Van Dyke et al., 1994]. The presence of the additional microsatellite (Figure#4) is associated with genomic instability, giving rise to mutations that involve the addition or subtraction of one or two repeat units. This may or may not be dependent on the site of this locus on the *PAX7* gene.

There was a deletion observed in the ERMS cell line by analysis with Primer 16 (Figure#15), which is located at the 3'of intron 5. This may be an aberration cause by the immortalization process of the RD cells. The particular cell line used, ATCC – CCL-136™, is isolated from muscle tissue of a female *Homo sapiens* fetus and consists of spindle cells and large multinucleated cells. The medium in which these adherent cells are stored is Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose, 90%; fetal bovine serum, 10% and DMSO, 5% at a temperature of 37° Celsius. DMSO can inhibit certain types of reactions (i.e. Big Dye Terminator reactions used in DNA sequencing) which may explain why the peak which is present in the other sample types including the control, is absent in the ERMS sample.

Recessive oncogenic alterations typically lead to the biallelic inactivation of tumor suppressor genes [Cavenee et al., 1983]. Alterations such as homozygous and heterozygous deletions or gene conversions are thought to be among the most common genetic abnormalities in epithelial cancers and their detection using LOH analysis is becoming common and essential to the discovery of genes targeted by such events [Lindblad-Toh et al., 2000]. Oligonucleotide microarrays, capable of simultaneously determining the genotype of 1494 SNPs, have been used to map regions of LOH in small cell lung, breast, bladder, and prostate cancer [Primdahl et al., 2002; Schubert et al., 2002; Dumur et al., 2003; Hoque et al., 2003; Lieberfarb et al., 2003]. In small cell lung cancer, detection of regions of LOH using SNP arrays was shown to be comparable with LOH detection using microsatellite markers but required cancer cell purity of ≥90% [Schubert et al., 2002].

Regions of LOH, usually arising as a result of either hemizygous deletion or gene conversion events, are typically defined as stretches of chromosomal areas where all heterozygous and thereby informative alleles are rendered homozygous in the cancer. The boundaries of such regions of LOH are defined by either the presence of retained heterozygous alleles, the ends of chromosomal arms, or the centrosome. This classical definition assumes that all data points are completely accurate and that all polymorphic alleles are mapped correctly within the genome [Van Dyke et al., 1994; Davis et al., 1997].

Although previous research has shown where mutations within introns can affect phenotypes [White et al., 1995; Howe et al., 1997; Girard et al., 2000; Sun et al.,2000; Mattick et al., 2001; O'Keefe, 2002; Howe et al.,2003; Goodarzi et al., 2005; Beauchemin et al., 2006] the novelty of these finding is merely a start. At this point in time the significance of finding a mutation in one patient and a deletion in one ERMS cell line is not known but may prove to be vital for future testing of these sarcomas and may ultimately enable improved diagnosis and outcomes in patients with Alveolar Rhabdomyosarcoma and Embryonal Rhabdomyosarcoma.

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