



## Final Report

### OBJECTIVE

The goal of this project was to genotype two different single nucleotide polymorphisms (SNPs) in 50 test subjects. These polymorphisms included 1) a point mutation in the human Apo E gene (334 T C), and 2) a point mutation in human leptin gene (19 G A). Taqman based SNP genotyping assays from Applied Biosystems Inc. were used for this analysis.

### MATERIALS

#### **Samples (provided by Client)**

Saliva samples in Oragene collection kit.  
Samples for 20 subjects.

#### **Reagents**

TaqMan Genotyping Master Mix (Applied Biosystems, Inc.)

Taqman SNP assays (Applied Biosystems, Inc.)

- Apo E - Assay ID: C\_904973\_10; dbSNP ID: rs7412
- Leptin - Assay ID: C\_15966471\_20; dbSNP ID: rs2167270

#### **Instruments and Software**

NanoDrop 100-ND Spectrophotometer

7900HT Sequence Detection System (Applied Biosystems, Inc.)

### PROTOCOL

#### *Genomic DNA Isolation and Purification*

Genomic DNA was purified from a fraction of the saliva samples collected in Oragene collection kits. The Oragene DNA purification protocol was used. An additional 70% ethanol wash of the final DNA pellet was added to the procedure because preliminary testing showed a benefit of an additional wash of the DNA. The DNA was resuspended in nuclease-free water. The quantity and quality of the purified DNA was determined by absorbance spectroscopy and measuring the absorbance of the DNA at 230nm, 260 nm, and 280nm.

#### *Genotyping of Apo E and leptin point mutations with Taqman assays*

Genomic DNA from each sample was PCR amplified with primers that flank the polymorphic site and two oligonucleotide probes. These probes bind to a region that is internal to the PCR primers, their sequences differs at one base and each is labeled with a different fluorescent molecule (i.e., VIC or FAM). In the assay, a probe will bind the genomic DNA and the DNA polymerase will cleave the fluorescent label on the probe during the PCR amplification. The freed label will now fluoresce and its emissions can be measured. Each assay included water negative controls. Genomic DNA, PCR primers, probes and PCR reaction buffers were combined and PCR amplified with 50 cycles. At the end of the PCR amplification, a single fluorescence measure was taken in each well for both fluor (i.e., VIC and FAM).

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### SUMMARY AND INTERPRETATION

Genotyping was conducted on 20 subjects for two different SNPs. DNA was successfully purified from saliva collected in the Oragene kit. There was a minimum of 30 ng/ $\mu$ l DNA (mean 158 ng/ $\mu$ l  $\pm$  15, std error) from all samples in a 50  $\mu$ l volume. The quality of the DNA by 260/280 ratio ( $>$  1.7) was good, although the 260/230 ratio (0.5 - 1.2) was less than optimal. Preliminary testing of a subset of samples showed that two 70% ethanol washes of the final DNA preparation was necessary to improve 260/230 ratios. The genomic DNA purified from these saliva samples worked well for these SNP assays.

A Taqman assay (Applied Biosystems Inc.) was used to interrogate the Apo E (334 T C) and leptin (19 G A) SNPs in 20 subjects. All the samples yielded signals for one or both probes, or alleles. Water negative controls failed to yield a significant signal in these assays. The raw and computer interpreted data for the Apo E and leptin SNPs are given in Tables 1-2. For the Apo E (334 T C) SNP, 7 subjects were homozygous for the C base or allele, 5 subjects were homozygous for the T allele, and 8 subjects were heterozygous. For the leptin (19 G A) SNP, 5 subjects were homozygous for the G base or allele, 2 subjects were homozygous for the A allele, and 13 subjects were heterozygous. A plot of these data is given in Figures 1-2. A summary of the genotypes is provided in Table 3.

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Table 1. Genotype data from the 7900HT Sequence Detection System for the Apo E (334 T C) SNP analysis of 20 subjects.

Well	Sample Name	Marker Name	Allele X Rn	Allele Y Rn	Call	Genotype	Quality Value	Passive Ref
1	1	ApoE	4.0127	1.0979	ApoE_VIC	T	100	1928.21
2	2	ApoE	4.0547	1.1216	ApoE_VIC	T	100	1937.23
3	3	ApoE	3.4048	7.1479	Both	Het	100	1647.34
4	4	ApoE	3.3945	7.1702	Both	Het	100	1579.53
5	5	ApoE	0.3567	8.6899	ApoE_FAM	C	100	1504.29
6	6	ApoE	3.3577	6.9803	Both	Het	100	1570.66
7	7	ApoE	0.3596	9.1813	ApoE_FAM	C	100	1398.76
8	8	ApoE	3.4897	7.2564	Both	Het	100	1438.98
9	9	ApoE	0.5122	9.1498	ApoE_FAM	C	100	1465.29
10	10	ApoE	0.4780	9.6233	ApoE_FAM	C	100	1361.72
11	11	ApoE	0.3035	9.2126	ApoE_FAM	C	100	1444.41
12	12	ApoE	0.4981	9.1943	ApoE_FAM	C	100	1486.94
25	13	ApoE	3.8936	0.9977	ApoE_VIC	T	100	1509.88
26	14	ApoE	3.5796	6.9281	Both	Het	100	1289.16
27	15	ApoE	3.7942	7.9296	Both	Het	100	1205.74
28	16	ApoE	3.6882	7.5329	Both	Het	100	1261.65
29	17	ApoE	3.6385	7.4882	Both	Het	100	1445.22
30	18	ApoE	0.6477	9.7080	ApoE_FAM	C	100	1474.43
31	19	ApoE	3.4519	0.9824	ApoE_VIC	T	100	1204.71
32	20	ApoE	3.8531	1.4547	ApoE_VIC	T	100	1204.56
85	water	ApoE	0.2053	0.4381	Undetermined		100	2676.63
86	water	ApoE	0.1769	0.4425	Undetermined		100	2364.82
87	water	ApoE	0.1716	0.4319	Undetermined		100	2126.07

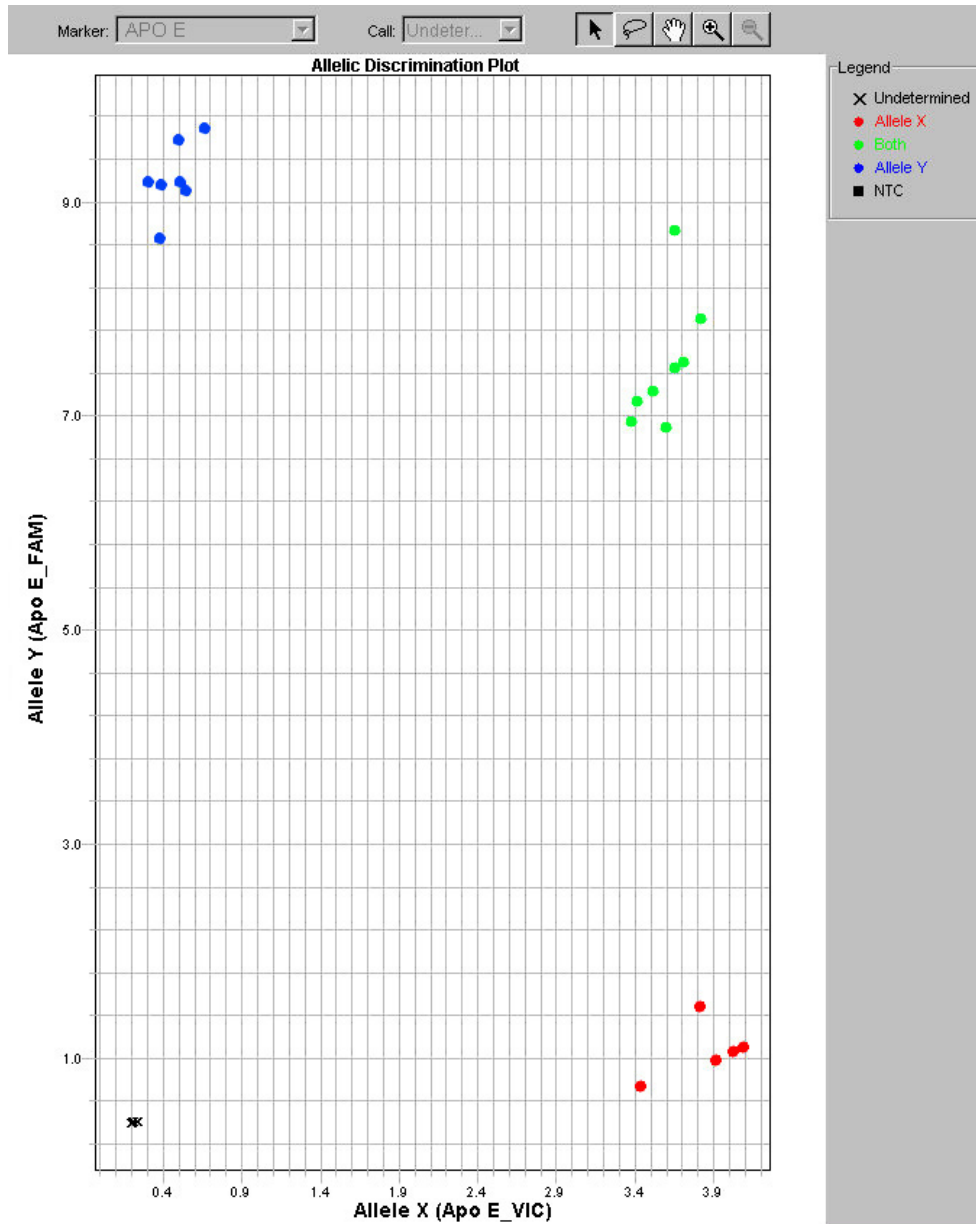
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Table 2. Genotype data from the 7900HT Sequence Detection System for the Leptin (19 G A) SNP analysis of 20 subjects.

Well	Sample Name	Marker Name	Allele X Rn	Allele Y Rn	Call	Genotype	Quality Value	Passive Ref
1	1	Leptin	0.08831	1.50591	COMT_FAM	G	100	2003.75
2	2	Leptin	0.07891	1.57272	COMT_FAM	G	100	2166.88
3	3	Leptin	0.65080	1.11241	Both	Het	100	2102.09
4	4	Leptin	0.63890	1.12823	Both	Het	100	2147.98
5	5	Leptin	0.58859	0.92836	Both	Het	100	2189.56
6	6	Leptin	0.64387	1.12650	Both	Het	100	2133.46
7	7	Leptin	0.63019	1.00465	Both	Het	100	2180.23
8	8	Leptin	0.63525	1.15407	Both	Het	100	2051.46
9	9	Leptin	0.06124	1.51675	COMT_FAM	G	100	2142.02
10	10	Leptin	0.81591	0.42755	COMT_VIC	A	100	2113.30
11	11	Leptin	0.06402	1.55908	COMT_FAM	G	100	2097.32
12	12	Leptin	0.68151	1.18165	Both	Het	100	2222.80
25	13	Leptin	0.58001	1.08839	Both	Het	100	1651.92
26	14	Leptin	0.55913	0.88872	Both	Het	100	1801.01
27	15	Leptin	0.67841	1.25221	Both	Het	100	1630.59
28	16	Leptin	0.66641	1.15660	Both	Het	100	1737.11
29	17	Leptin	0.62801	1.13732	Both	Het	100	1742.92
30	18	Leptin	0.06770	1.59803	COMT_FAM	G	100	1815.30
31	19	Leptin	0.6535	0.9623	Both	Het	100	2003.75
32	20	Leptin	0.6625	0.4781	COMT_VIC	A	100	2166.88
85	water	Leptin	0.10148	0.25844	Undetermined		100	2729.05
86	water	Leptin	0.22396	0.29210	Undetermined		100	2573.22
87	water	Leptin	0.17575	0.26801	Undetermined		100	2575.23
88	water	Leptin	0.09373	0.37190	Undetermined		100	1929.88

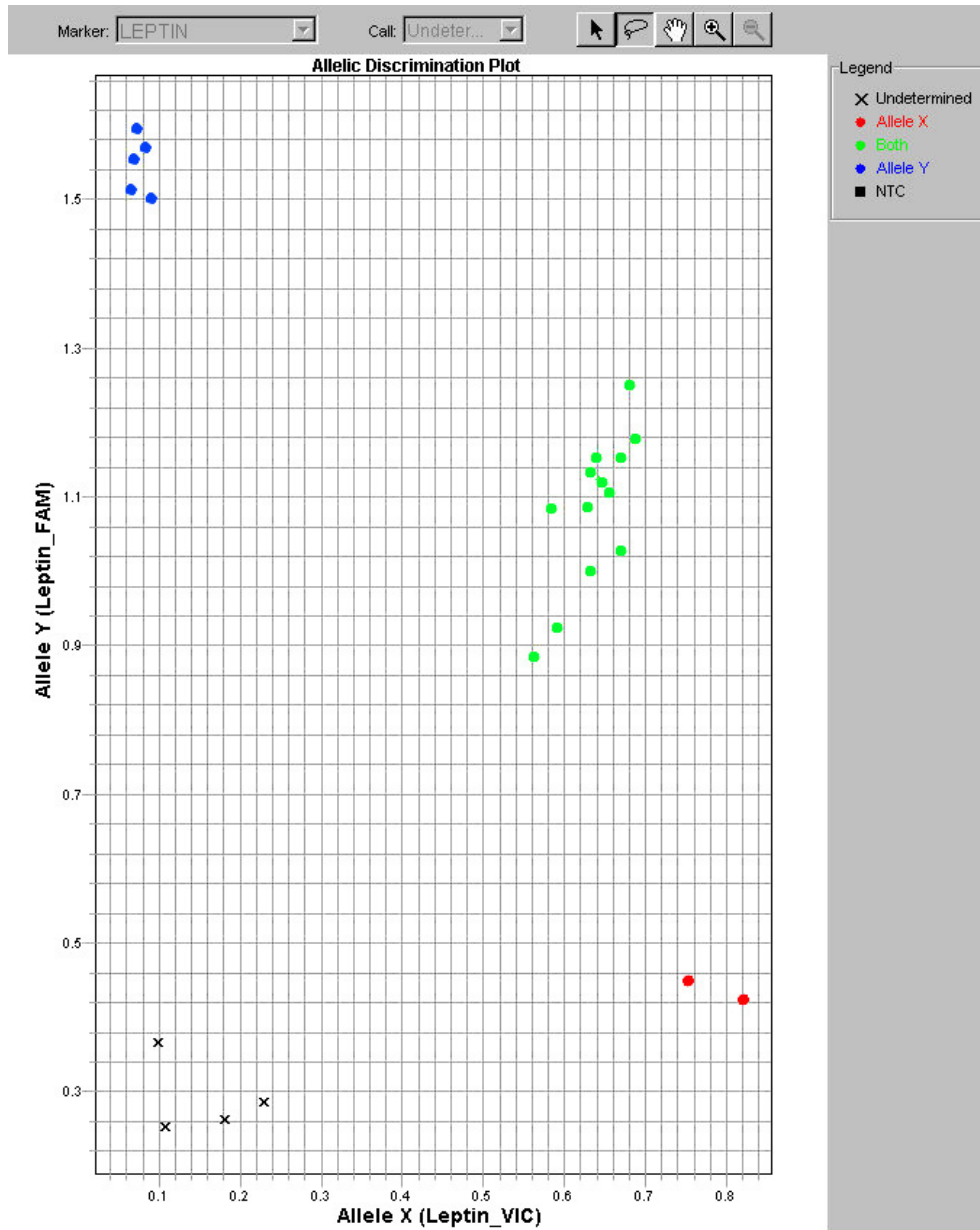
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Figure 1. Summary plot of the Apo E (334 T C) genotype for 20 subjects.



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Figure 2. Summary plot of the Leptin (19 G A) genotype for 20 subjects.



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Table 3. Genotype summary of Apo E (334 T C) and Leptin (19 G A) SNP analysis in 20 subjects.

<u>Subject</u>	<u>Apo E</u>	<u>Leptin</u>
1	T	HT
2	T	G
3	Het	HT
4	Het	HT
5	C	HT
6	Het	G
7	C	HT
8	Het	HT
9	C	HT
10	C	HT
11	C	A
12	C	A
13	T	G
14	Het	Het
15	Het	Het
16	Het	Het
17	Het	Het
18	C	G
19	T	A
20	T	A