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Project Name

XXXXXXXXXXXX

Document

Final Report

Analysis

Genotyping

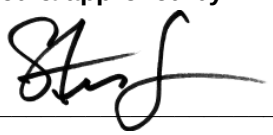
Researcher

Name and Address

Date

Month XX, 20XX

Reviewed & approved by:



Steve Granger, Ph.D, Chief Scientific Officer

Salivary DNA Genotyping - Final Report

Study Objective:

The goal of this project was to isolate high quality genomic DNA from 24 salivary samples and genotype them for several single nucleotide polymorphisms (SNPs).

Materials:

24 frozen SalivaBio Oral Swabs containing human saliva samples were received in swab storage tubes (SSTs). Saliva was tested for hormones prior to DNA isolation. The corresponding hormone testing roster number is XXXXX.

Methods:

DNA isolation from SalivaBio Swabs

A Salimetrics developed proprietary method was used for Genomic DNA extraction from the saliva samples. Purity and yield for each sample was measured by absorbance of the purified DNA at A260 and A280 by spectrophotometry. DNA quality was considered acceptable when A260:A280 ratio was at or above 1.8. Samples were aliquoted and stored at -20 C until analysis.

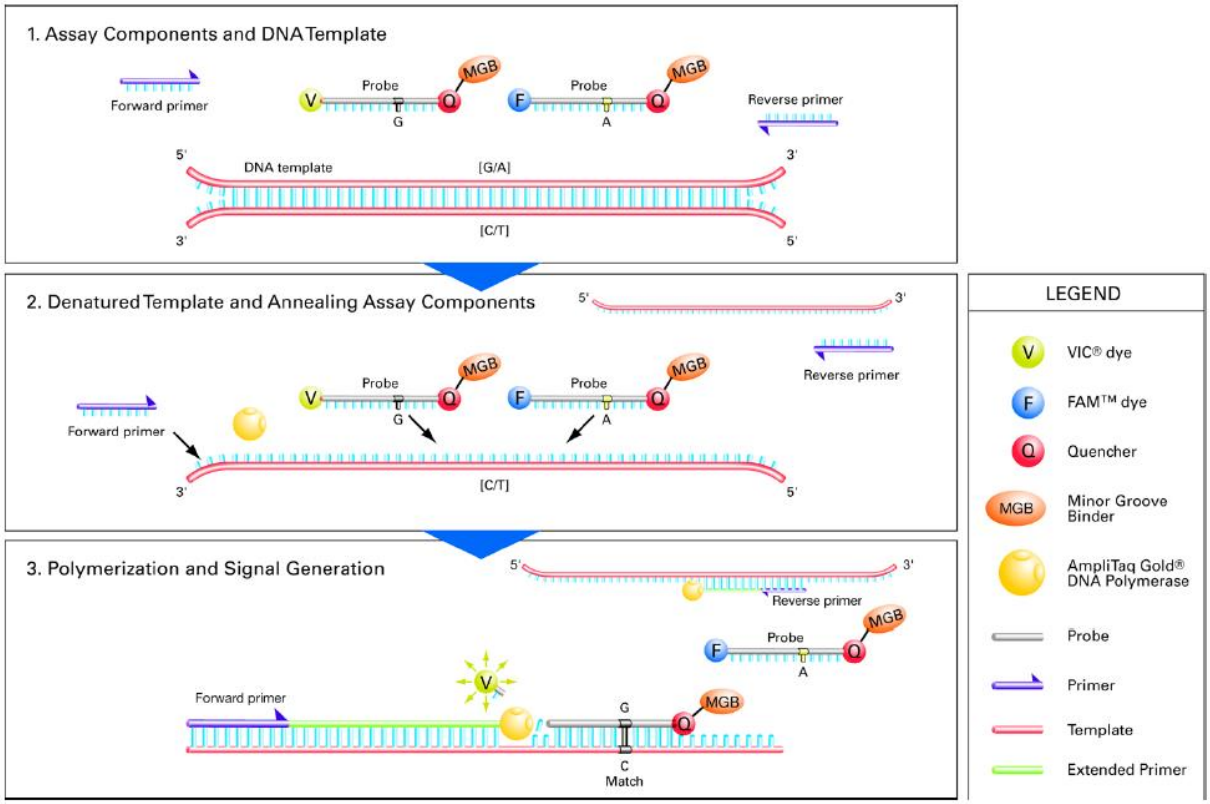
SNP Assays

Taqman® SNP Genotyping Assays (Applied Biosystems/LifeTech) were used to amplify and detect the two alleles for the following polymorphisms:

Table 1. SNP database information

Assay ID	dbSNP	Gene Symbol	Gene Name	Context Sequence [VIC/FAM]
Custom Assay ID: AH01F70	rs41423247	BCL1; NR3C1	nuclear receptor subfamily 3; group C; member 1 (glucocorticoid receptor)	CACCAATTCCTCTCTTAAAGAGATT[G/C]ATCAG CAGACATAACTTGTCTACTT
C___1594392_10	rs2070951	NR3C2	nuclear receptor subfamily 3; group C; member 2	ACTGTGGTAGCCTTTGGTCTCCATC[C/G]CTAA CAAATAAATTTACATTAAAAA
C___2255420_10	rs737865	COMT	catechol-O-methyltransferase	GCTTTTTGGATTTTTCCAGCCAGGG**[A/G]**TTTT TGTGTCCTGTTGCTTTTTATT
C__11592758_10	rs6265	BDNF-AS;BDNF	BDNF antisense RNA;brain-derived neurotrophic factor	TCCTCATCCAACAGCTCTTCTATCA[C/T]GTGTT CGAAAGTGTCAGCCAATGAT

For each SNP analysis, PCR amplification was performed by an Applied Biosystems (ABI) 7500 Real-Time PCR machine using sequence-specific DNA primers and TaqMan PCR universal mastermix. To detect each allele, TaqMan DNA probes, containing fluorescent reporter dyes at their 5' ends and non-fluorescent quenchers at their 3' ends, were used. As extension of the SNP primers occurred into the region containing the bound probes, reverse transcriptase exonuclease activity cleaved the probes, separating the 5' fluorescent tags from the 3' non-fluorescent quenchers, thereby producing a fluorescence signal detected by the ABI 7500 Real-Time PCR instrument. This principle is illustrated in the diagram below.


1. Assay Components and DNA Template
Forward primer
Probe
MGB
V
Q
G
Probe
MGB
F
Q
A
Reverse primer
5'
DNA template
[G/A]
3'
3'
[C/T]
5'
2. Denatured Template and Annealing Assay Components
5'
3'
Reverse primer
Forward primer
Probe
MGB
V
Q
G
Probe
MGB
F
Q
A
3'
[C/T]
5'
3. Polymerization and Signal Generation
5'
3'
Reverse primer
Probe
MGB
F
Q
A
V
Forward primer
G
MGB
Q
C
Match
LEGEND
V
VIC® dye
F
FAM™ dye
Q
Quencher
MGB
Minor Groove Binder
AmpliTaq Gold® DNA Polymerase
Probe
Primer
Template
Extended Primer

Summary and Interpretation:

DNA Isolation

DNA was successfully extracted from (24 of 24, 100%) saliva samples. The DNA quality and quantity data along with a summary of the genotyping results are represented in Table2.

Table 2. DNA data summary

Roster ID	Sample ID	Conc. (ng/uL)	260/280	260/230	rs41423247	rs6265	rs737865	rs2070951
	031	9.00	1.73	1.04	G/G	C/T	A/A	C/C
	032	39.44	1.81	1.17	G/C	C/T	A/A	C/G
	033	18.96	1.71	0.95	G/G	C/C	A/A	C/C
	034	43.73	1.83	1.19	G/C	C/T	A/A	C/G
	037	13.40	2.05	1.50	G/C	C/C	A/A	G/G
	038	14.58	1.55	0.76	G/G	C/C	A/A	G/G
	042	19.44	1.81	1.17	G/C	C/C	A/G	C/C
	043	59.96	1.96	1.90	G/C	C/T	G/G	C/G
	044	21.74	1.75	1.04	G/G	C/C	A/A	G/G
	045	24.73	1.97	1.60	G/C	C/C	A/G	C/C
	046	24.32	1.83	0.88	G/C	C/C	A/G	C/G
	047	53.51	1.86	1.45	G/C	C/T	A/A	C/G
	048	9.49	2.05	1.03	G/G	C/T	G/G	C/C
	049	20.30	1.70	0.83	G/G	C/T	A/A	C/G
	050	21.24	1.58	0.80	G/G	C/C	A/A	C/C
	051	6.26	1.55	0.71	G/C	C/T	G/G	C/G
	052	6.97	1.64	0.74	G/C	C/C	A/A	C/G
	053	5.82	1.89	0.64	G/C	C/C	A/A	C/G
	054	9.24	1.63	0.58	G/G	C/C	A/G	G/G
	055	40.30	1.83	1.54	G/C	C/C	A/A	G/G
	056	24.43	1.97	1.69	G/C	C/T	A/A	C/G
	057	15.18	1.76	1.01	G/G	T/T	A/G	C/G
	058	27.31	1.90	1.32	G/C	C/C	A/A	G/G
	059	25.56	1.93	1.50	G/G	C/C	A/G	C/C

SNP Assays

NR3C1 (BCL-1): rs41423247

A TaqMan Custom SNP Genotyping assay from LifeTech/ABI was used to investigate the BCL-1: rs41423247 polymorphism for all 24 subjects, using the custom primer set from Spijker, 2009. 24 (100%) samples yielded results. The allele data for this SNP is given in Table 4. An allelic discrimination plot of this data is displayed in Figure 1. For the non-protein coding BCL-1: rs41423247 SNP, 10 subjects are homozygous for the nucleotide base G ("G" Allele) and 0 subjects are homozygous for the nucleotide base C ("C" Allele) and 14 subjects are heterozygous, with a genotype containing both the nucleotide bases C and G.

The measured genotype frequencies corresponded to the Hardy-Weinberg equilibrium (HWE) (N=24, x^2=4.07, df=1, p=0.0.04). Summary results are given on Table 10. (X^2 = chi squared; p = p value of chi squared; df = degrees of freedom). If $p < 0.05$ – data is not consistent with Hardy-Weinberg equilibrium.

BDNF: rs6265

A Taqman SNP Genotyping Assay was used to investigate the BDNF rs6265 SNP for 24 subjects. 24 samples (100%) yielded results for one or both alleles. The raw and computer interpreted data for this SNP is given in Table 5. An allelic discrimination plot of this data is shown in Figure 2. For the BDNF SNP 14 subjects were homozygous for the C allele,1 were homozygous for the T allele and 9subjects were heterozygous.

The measured genotype frequencies corresponded to the Hardy-Weinberg equilibrium (N=24, x^2=0.0.09, df=1, p=0.74).

COMT: rs737865

Taqman SNP Genotyping Assay was used to investigate the COMT rs7373865 SNP for 24 subjects. 24 samples (100%) yielded signals for one or both alleles. The raw and computer interpreted data for this SNP is given in Table 6. An allelic discrimination plot of this data is shown in Figure 3. 3 subjects were homozygous for the G allele, 15 subjects were homozygous for the A allele, and 6 subjects were heterozygous.

The measured genotype frequencies corresponded to the Hardy-Weinberg equilibrium (N=24, x^2=0.2.67, df=1, p=0.10).

NR3C2: rs2070951

A TaqMan SNP Genotyping assay from LifeTech/ABI was used to investigate the NR3C2 MR-2GC: 2070951 polymorphism for all 24 subjects. 24 (100%) samples yielded results. The allele data for this SNP is given in Table 7. An allelic discrimination plot of this data is displayed in Figure 4. For the non-protein coding NR3C2 MR-2GC: 2070951 SNP,6 subjects are homozygous for the nucleotide base C ("C" Allele) and 6 subjects are homozygous for the nucleotide base G ("G" Allele). 11 subjects are heterozygous, with a genotype containing both the nucleotide bases C and G.

The measured genotype frequencies corresponded to the Hardy-Weinberg equilibrium (N=24, x^2=.04, df=1, p=0.83).

Reagents:

DNA Isolation

Genomic DNA Mini Kit (cat. # XXX).

SNP Reagents

TaqMan® Universal PCR Master Mix (LifeTech/ABI cat. # 4303337)
TaqMan® SNP Genotyping Assays (LifeTech/ABI cat. # 4351379)
LifeTech Assay ID and dsSNP data listed in Table 1 below.

Footnotes:

Concurrent with the subject samples tested, several control samples were also assayed to provide data quality assurance. These controls include:

- "Negative Template Controls" (NTCs) or "Blanks", which contain all assay reagents and water instead of DNA template;
- "Empty" samples which are treated the same as all subject samples during extraction, but contain no saliva;
- Positive Controls, which are samples with a known genotype for the polymorphisms of interest. These include samples labeled "ProMega control".
- For quality assurance some samples may be repeated.
  In all cases, the assay results for the NTCs and "Empty" samples reported "NR" or "Undetermined," indicating no DNA template was present. All positive controls were checked to their respective reference genotype and found to agree. Repeated samples gave consistent results. For visualizing allelic discrimination plots print in color.

References:

Shen GQ[1], Abdullah KG, Wang QK. The TaqMan method for SNP genotyping. Methods Mol Biol. 2009;578:293-306.

Wendland JR, Moya PR, Kruse MR, Ren-Patterson RF, Jensen CL, Timpano KR, Murphy DL. A novel, putative gain-of-function haplotype at SLC6A4 associates with obsessive-compulsive disorder. *Hum Mol Genet.* 2008 Mar 1;17(5):717-23.

Spijker AT, Rossum EFC, Hoencamp E, DeRijk RH, Haffmans J, Blom M, Manenschihn L, Koper JW, Lamberts SWJ, Zitman FG,. Functional polymorphism of the glucocorticoid receptor gene associates with mania and hypomania in biopolar disorder. *Bipolar Disorders.* 2009: 11:95-101.

Table 3. Allele Frequency Information

	rs41423247	rs6265	rs737865	rs2070951
Homozygous	C/C (3)	C/C (20)	A/A (9)	C/C (9)
Heterozygous	G/C (10)	C/T (7)	A/G (16)	C/G (11)
Homozygous	G/G (14)	T/T (0)	G/G (2)	G/G (7)

	rs41423247	rs6265	rs737865	rs2070951
X^2	0.34	0.60	2.00	0.88
X^2 test P value	0.56	0.44	0.16	0.35

1. If P < 0.05 - not consistent with HWE. With 1 degree of freedon (df = 1).
2. Not accurate if <5 individuals in any genotype group.

Michael H. Court (2005-2008)

Table 4. BCL-1 SNP rs41423247 data

Roster ID	Sample ID	Allele1 Delta Rn	Allele2 Delta Rn	Quality(%)	Call	Method
	035	1.36	0.08	98.33	Homozygous G/G	Auto
	036	1.51	0.07	98.33	Homozygous G/G	Auto
	064	1.55	0.08	98.33	Homozygous G/G	Auto
	067	1.22	1.37	98.33	Heterozygous G/C	Auto
	068	1.61	0.10	98.33	Homozygous G/G	Auto
	079	1.01	1.20	98.33	Heterozygous G/C	Auto
	080	1.56	0.10	98.33	Homozygous G/G	Auto
	081	1.33	0.28	98.33	Homozygous G/G	Auto
	082	1.54	0.07	98.33	Homozygous G/G	Auto
	083	1.58	0.09	98.33	Homozygous G/G	Auto
	084	0.05	1.85	98.33	Homozygous C/C	Auto
	085	1.46	0.10	98.33	Homozygous G/G	Auto
	086	1.11	1.24	98.33	Heterozygous G/C	Auto
	087	1.62	0.14	98.33	Homozygous G/G	Auto
	088	1.56	0.14	98.33	Homozygous G/G	Auto
	089	0.09	1.97	98.33	Homozygous C/C	Auto
	090	1.05	1.14	98.33	Heterozygous G/C	Auto
	091	1.22	1.36	98.33	Heterozygous G/C	Auto
	092	1.30	1.41	98.33	Heterozygous G/C	Auto
	093	1.69	0.09	98.33	Homozygous G/G	Auto
	094	1.68	0.12	98.33	Homozygous G/G	Auto
	095	1.66	0.10	98.33	Homozygous G/G	Auto
	096	1.06	1.14	98.33	Heterozygous G/C	Auto
	097	0.05	1.97	98.33	Homozygous C/C	Auto
	098	1.22	1.33	98.33	Heterozygous G/C	Auto
	099	1.15	1.24	98.33	Heterozygous G/C	Auto
	100	1.26	1.35	98.33	Heterozygous G/C	Auto
	NTC	0.04	1.77	98.33	Undetermined	Auto
	Promega control	1.27	1.34	98.33	Heterozygous G/C	Auto

Figure 1. BCL-1 SNP rs41423247

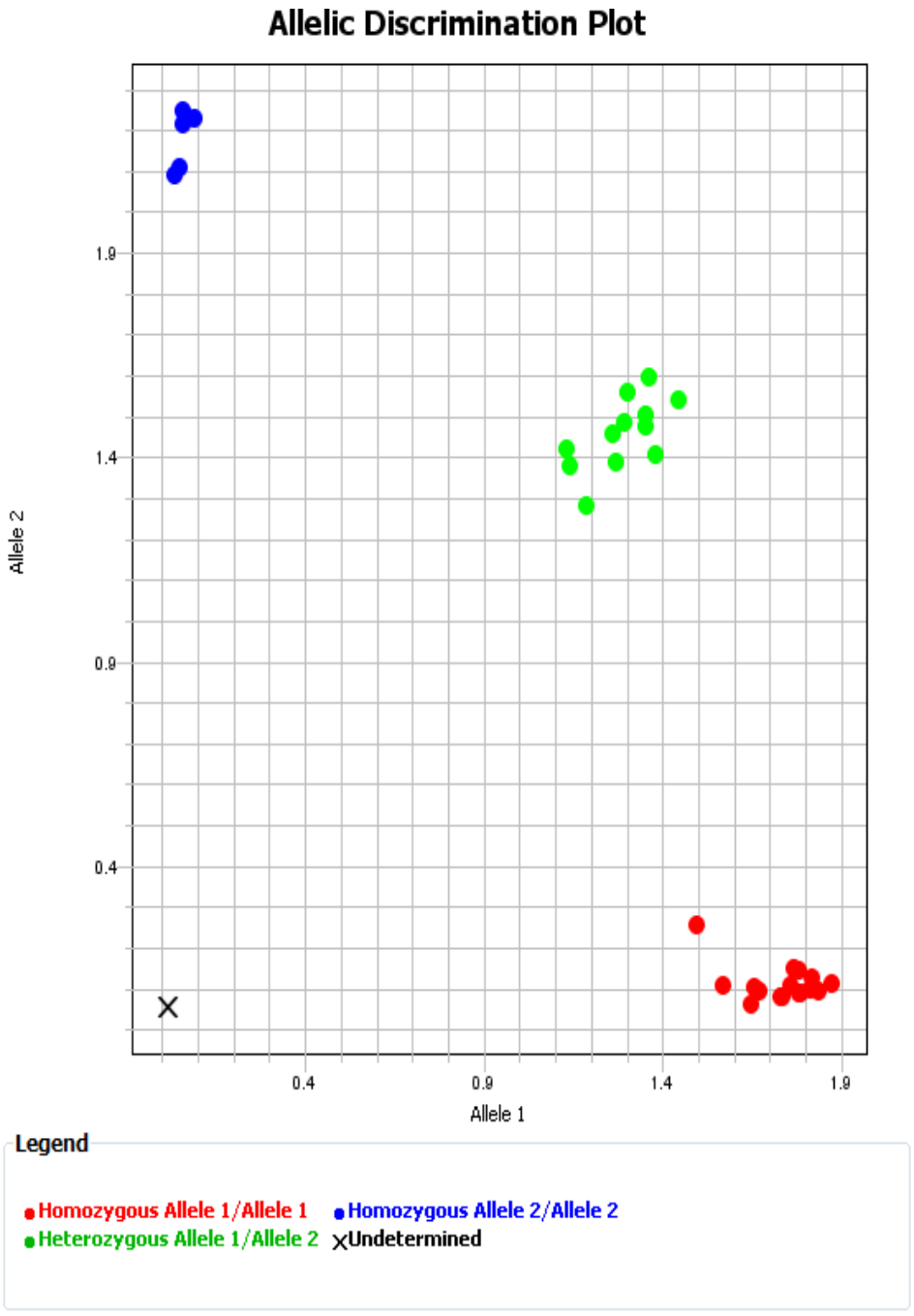


Table 5. BDNF SNP rs6265 data

Roster ID	Sample ID	Allele1 Delta Rn	Allele2 Delta Rn	Quality(%)	Call	Method
	035	2.32	0.11	98	Homozygous C/C	Auto
	036	2.29	0.09	98	Homozygous C/C	Auto
	064	2.32	0.13	98	Homozygous C/C	Auto
	067	1.72	1.27	98	Heterozygous C/T	Auto
	068	2.44	0.15	98	Homozygous C/C	Auto
	079	2.08	0.11	98	Homozygous C/C	Auto
	080	2.24	0.13	98	Homozygous C/C	Auto
	081	1.45	1.04	98	Heterozygous C/T	Auto
	082	1.83	1.42	98	Heterozygous C/T	Auto
	083	2.32	0.13	98	Homozygous C/C	Auto
	084	2.35	0.14	98	Homozygous C/C	Auto
	085	2.15	0.14	98	Homozygous C/C	Auto
	086	2.18	0.13	98	Homozygous C/C	Auto
	087	3.19	0.24	98	Homozygous C/C	Auto
	088	2.20	0.14	98	Homozygous C/C	Auto
	089	2.09	0.13	98	Homozygous C/C	Auto
	090	2.14	0.09	98	Homozygous C/C	Auto
	091	1.82	1.33	98	Heterozygous C/T	Auto
	092	2.18	0.11	98	Homozygous C/C	Auto
	093	1.56	1.16	98	Heterozygous C/T	Auto
	094	1.66	1.19	98	Heterozygous C/T	Auto
	095	2.19	0.09	98	Homozygous C/C	Auto
	096	1.95	0.10	98	Homozygous C/C	Auto
	097	2.14	0.12	98	Homozygous C/C	Auto
	098	2.31	0.10	98	Homozygous C/C	Auto
	099	2.14	0.11	98	Homozygous C/C	Auto
	100	1.52	1.12	98	Heterozygous C/T	Auto
	NTC	0.03	0.03	99	Undetermined	Auto

Figure 2. BDNF rs6265

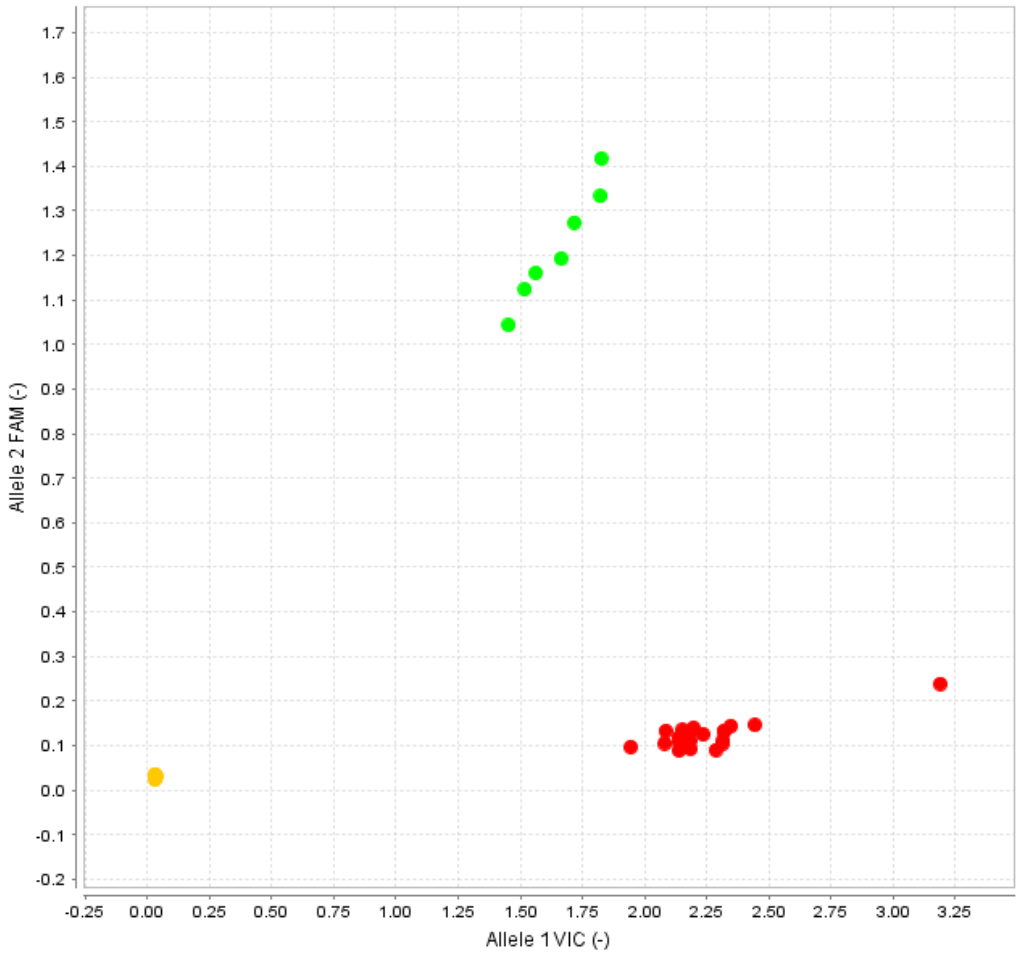


Table 6. COMT SNP rs737865 data

Roster ID	Sample ID	Allele1 Delta Rn	Allele2 Delta Rn	Quality(%)	Call	Method
	035	3.91	4.82	98	Homozygous A/A	Auto
	036	3.14	4.50	98	Heterozygous A/G	Auto
	064	3.09	4.59	98	Heterozygous A/G	Auto
	067	3.69	5.67	98	Heterozygous A/G	Auto
	068	4.07	5.31	98	Homozygous A/A	Auto
	079	3.38	5.25	98	Heterozygous A/G	Auto
	080	3.41	6.08	98	Homozygous G/G	Auto
	081	4.03	5.01	98	Homozygous A/A	Auto
	082	3.64	5.67	98	Heterozygous A/G	Auto
	083	3.17	4.55	98	Heterozygous A/G	Auto
	084	3.42	5.34	98	Heterozygous A/G	Auto
	085	3.63	4.08	98	Homozygous A/A	Auto
	086	3.52	5.41	98	Heterozygous A/G	Auto
	087	3.90	4.91	98	Homozygous A/A	Auto
	088	3.78	5.89	98	Heterozygous A/G	Auto
	089	4.05	5.26	98	Homozygous A/A	Auto
	090	3.36	5.25	98	Heterozygous A/G	Auto
	091	3.95	5.16	98	Homozygous A/A	Auto
	092	2.93	5.88	97	Homozygous G/G	Auto
	093	3.51	5.32	98	Heterozygous A/G	Auto
	094	3.75	5.75	98	Heterozygous A/G	Auto
	095	3.91	4.73	98	Homozygous A/A	Auto
	096	3.19	3.32	98	Homozygous A/A	Auto
	097	3.68	5.55	98	Heterozygous A/G	Auto
	098	3.49	5.26	98	Heterozygous A/G	Auto
	099	3.64	5.57	98	Heterozygous A/G	Auto
	100	3.66	5.50	98	Heterozygous A/G	Auto
	NTC	0.03	0.03	99	Undetermined	Auto

Figure 3. COMT SNP rs737865

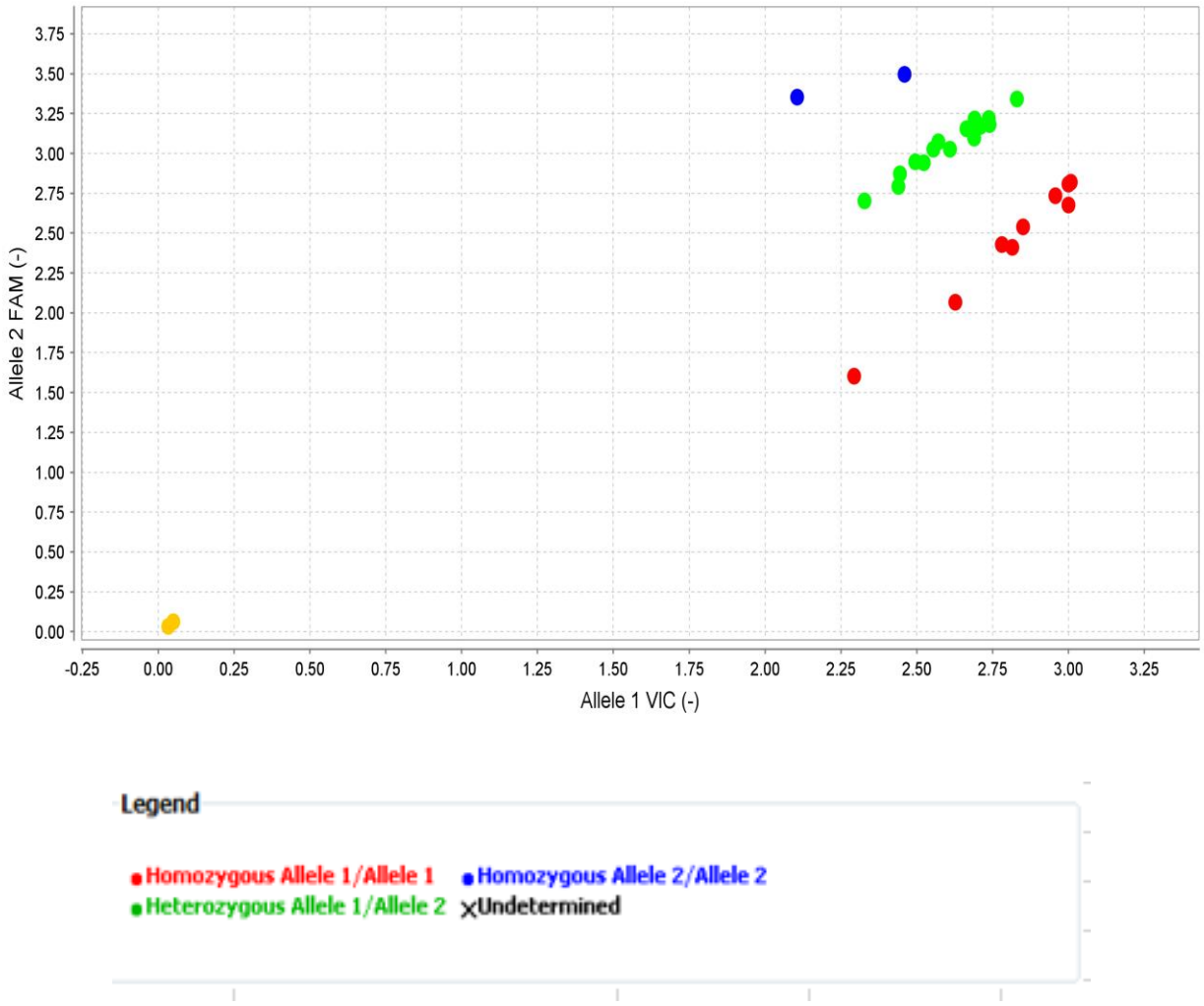


Table 7. MR-2G/C SNP rs2070951 data

Roster ID	Sample ID	Allele1 (G) Delta Rn	Allele2(C) Delta Rn	Quality(%)	Call	Method
	035	0.05	1.62	98.35	Homozygous G/G	Auto
	036	0.91	1.36	98.35	Heterozygous C/G	Auto
	064	0.68	1.02	98.35	Heterozygous C/G	Auto
	067	1.25	0.11	98.35	Homozygous C/C	Auto
	068	1.53	0.12	98.35	Homozygous C/C	Auto
	079	0.46	0.71	98.35	Heterozygous C/G	Auto
	080	1.27	0.13	98.35	Homozygous C/C	Auto
	081	0.06	1.51	98.35	Homozygous G/G	Auto
	082	0.06	1.71	98.35	Homozygous G/G	Auto
	083	0.57	0.77	98.35	Heterozygous C/G	Auto
	084	0.92	0.11	98.35	Homozygous C/C	Auto
	085	0.67	0.13	98.35	Homozygous C/C	Auto
	086	0.76	0.10	98.35	Homozygous C/C	Auto
	087	0.07	1.79	98.35	Homozygous G/G	Auto
	088	0.06	1.67	98.35	Homozygous G/G	Auto
	089	0.73	1.13	98.35	Heterozygous C/G	Auto
	090	0.42	0.64	98.35	Heterozygous C/G	Auto
	091	0.84	1.14	98.35	Heterozygous C/G	Auto
	092	0.60	0.90	98.35	Heterozygous C/G	Auto
	093	0.82	1.17	98.35	Heterozygous C/G	Auto
	094	0.06	1.74	98.35	Homozygous G/G	Auto
	095	0.67	1.05	98.35	Heterozygous C/G	Auto
	096	0.94	0.69	N/A	Homozygous C/C	manual
	097	0.71	1.13	98.35	Heterozygous C/G	Auto
	098	0.08	1.64	98.35	Homozygous G/G	Auto
	099	1.25	0.10	98.35	Homozygous C/C	Auto
	100	1.23	0.11	98.35	Homozygous C/C	Auto
	NTC	0.03	0.09	100.00	Undetermined	Auto
	Promega control	1.01	1.29	98.35	Heterozygous C/G	Auto

(*retest result)

Figure 4. MR-2GC SNPrs2070951