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Project Name

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Document

Final Report

Analysis

Genotyping

Researcher

Name and Address

Date

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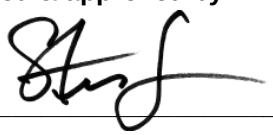
Reviewed & approved by:



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 variable number tandem repeats (VNTRs).

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.

VNTR Assays

The genomic regions of interest were PCR amplified using VNTR-specific fluorescent-labeled primers. The amplified fragments were then analyzed by capillary electrophoresis to detect the number of repeats present for the VNTR investigated. The number of repeats is used to categorize the VNTR genotype of each sample.

5-HTTLPR

The Human Serotonin Transporter Gene linked polymorphism, 5-HTTLPR 22 base pair repeat (44 base pair insertion/deletion), located in the promoter region of the SLC6A4 gene, was investigated by following the method reported by Wendland et al. 2008.

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 1. DNA data summary

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

VNTR Assay

For the investigation of the 5HTTLPR VNTR, primers were obtained from Applied Biosystems to target the 22 basepair repeat sequence. 24 (100%) samples yielded results. Raw and interpreted data for this VNTR is given on Table 8.The most commonly seen variants of the 5HTTLPR VNTR are the S (14-repeat) and L (16-repeat) alleles. All three genotypes were represented: L/L (n=2; 8.3%), S/L (n=9; 38%), and S/S (n=13; 54%). The measured genotype frequencies corresponded to the Hardy-Weinberg equilibrium (N=24, x^2=0.04, df= 1, p=0.85). . Summary results are given on Table 10.

The distribution of alleles for the 5HTTLPR polymorphism is different among different ethnic populations. The frequencies of the S/L alleles have been reported as follows in the given populations: Caucasian S=45%, L=55%; White-Hispanic S=48%, L=52%; African-American S=21%,L=79%; Chinese S=77%, L=23%; and Japanese S=82%, L=18%.

Reagents:

DNA Isolation

Genomic DNA Mini Kit (cat. # XXX).

VNTR Analysis

Human Serotonin transporter (SLC6A4) 5HTTLPR L/S repeat
5' Fluorescent labeled oligos (LifeTech/ABI cat. # 450007)
5HTTLPR Forward: 5'-TCCTCCGCTTTGGCGCCTCTTCC-3'
Reverse: 5'-TGGGGGTTGCAGGGGAGATCCTG-3'
HotStar Taq Plus DNA polymerase kit – (Qiagen cat. #203605)

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 2 VNTR 5HTTLPR data

Roster ID	Sample ID	Allele 1	Allele 2	Size 1	Size 2	Height 1	Height 2
	035	L		509.09		32219	
	036	L		509.03		31862	
	064	S		466.29		32258	
	067	S	L	466.38	509.27	32110	17271
	068	L		509.23		31749	
	079	S	L	466.45	509.28	12832	7113
	080	S	L	466.35	509.45	22212	9388
	081	L		509.16		32363	
	082	S	L	466.13	509.34	32012	21858
	083	S		466.16		32274	
	084	L		509.31		32174	
	085	L		509.23		19695	
	086	S	L	466.52	509.19	21063	11710
	087	S	L	466.52	509.35	26028	13159
	088	S	L	466.45	509.26	31856	17762
	089	L		509.13		32273	
	090	S	L	466.42	509.12	11118	6184
	091	L		508.88		32231	
	092	L		509.19		32240	
	093	S		465.97		32147	
	094	S	L	466.45	509.37	27552	13549
	095	S		466.45		31578	
	096	L		509.33		15709	
	097	S	L	466.45	509.47	26154	11747
	098	S	L	466.39	509.41	18051	8742
	099	S	L	466.64	509.31	22327	11880
	100	S		466.37		9591	

(*retest result)