



University
of Dundee

From GWAS to Functional Studies in South Indian Population

By

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Outline...

- ❑ Introduction
- ❑ Sample Genotyped so far
- ❑ Illumina HTS Assay Chemistry and Genome Studio
- ❑ The Problem Of Early Onset Type 2 Diabetes In Indians
- ❑ Work Plan Ahead
- ❑ Conclusion

Introduction

- Date of joining – April (Yoga Prabhu) and May (Sneha) 2019. PhD registered at Madras University (February 2020)

- Tentative month of PhD submission is February 2023

- For the past 12-13 months, we are into data generation:
 - DNA isolation by Phenol-Chloroform method
 - Genome-Wide Association Studies (Illumina Global Screening Array v2.0)
 - Functional genomics

Samples genotyped so far...

❑ **FREEZE 1-** Retrospective Samples

- Total samples genotyped- 6056 (done by Dr. Liju Samuel and Mr. Sathish Natarajan)

❑ **FREEZE 2-** CURES samples, CURES follow up and Retrospectives samples

- Total samples genotyped- 3108

❑ **FREEZE-3-** Prospective, TREND and NGT (DCLIP and Super control)

▪ Prospective samples: (n=2804)

- T2D samples for which clinical and biochemical parameters are screened, patients with 3 visits, Carl Zeiss retinal Imaging

▪ TREND samples: (n=672)

- >18 years, around Rural villages from Chennai, Kanchipuram districts

- NGT and T2D samples with retinal images

▪ NGT (DCLIP and Super Control): (n=321)

❑ **Isolated >1400 DNA samples and Genotyped >2000 samples from Freeze 2 and Freeze 3**

Stage 1

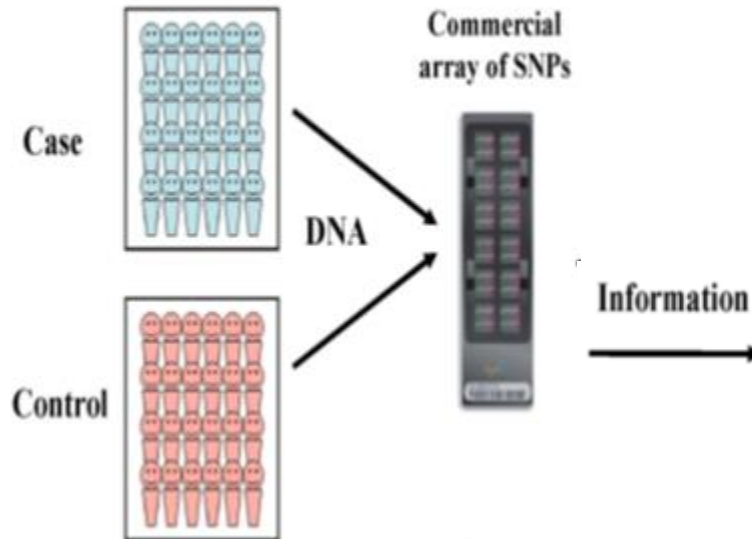
Stage 2

Stage 3

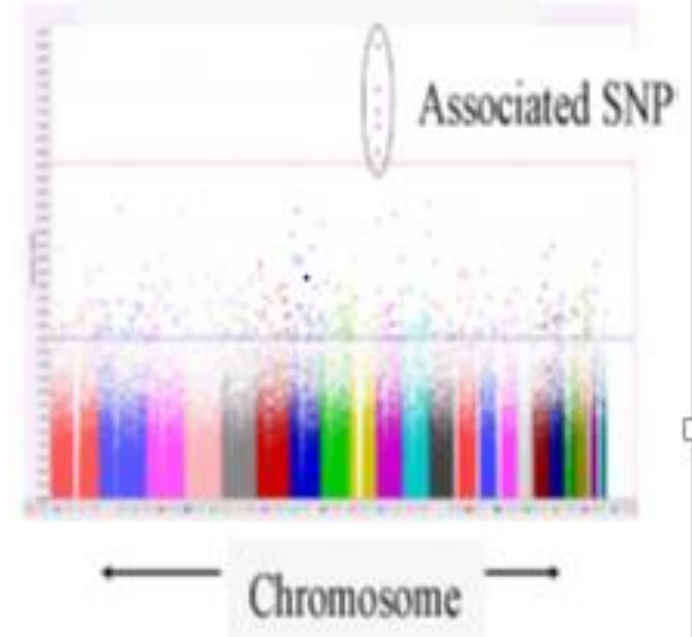


Phenotyping

Genotyping



Mapping



- ❖ Quality and Quantity Check using Nano drop
 - A260/280 – 1.75 to 1.8,
 - >50ng DNA

Features of Infinium HTS assay

- The Infinium Global Screening Array-24 v2.0 (GSA) BeadChip is an advanced genotyping array that provides a cost-effective solution for population-scale genetic studies, variant screening, and precision medicine research.
- Combines highly optimized multiethnic genome-wide content, clinical research variants, and QC markers

Feature	Description
Species	Human
Total No.of. Markers	6,65,608
Capacity for custom bead types	50,000
No.of. Samples per bead chip	24
DNA input requirement	200ng
Assay chemistry	Infinium HTS assay
Scan time per sample	HiScan- 2.0 min per sample iScan- 2.5min per sample
Support System	iScan or HiScan system software



Infinium Assay Work Flow

Genomic DNA (200 ng) | Day 1

1 Amplify and incubate DNA



Day 2

2 Fragment amplified DNA



3 Precipitate and resuspend



4 Prepare BeadChip



5 Hybridize samples on BeadChip



Day 3

6 Extend/stain samples on BeadChip



7 Image BeadChip



8 Autocall genotypes and generate reports



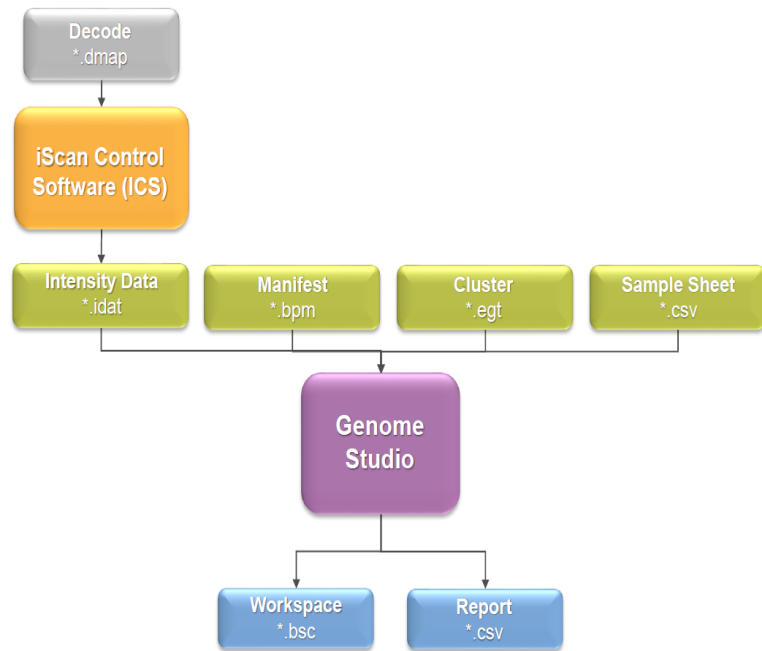
* Indicates stain in red channel

* Indicates stain in green channel

Genome studio software

- The Infinium Global Screening Array-24 v2.0 (GSA) BeadChip is an advanced genotyping array that provides a cost-effective solution for population-scale genetic studies, variant screening, and precision medicine research.

GenomeStudio Genotyping Analysis



The screenshot shows the GenomeStudio Genotyping Analysis software interface. The main window displays a 'Full Data Table' with columns for Index, Name, Address, Chr, Position, GenTrain Score, and various fraction values (Frac A, C, G, T). A 'SNP Graph' window is open, showing a plot of Norm R vs Norm Theta for MitoA13106G. A 'Samples Table' window shows a list of samples with their IDs and genders. An 'Errors Table' window is also visible. A 'Log' window at the bottom shows the software's activity log, including messages like 'Project Load is complete'. Annotations with orange arrows point to the 'Graph Window', 'Project Window', 'Samples Table', 'Data Table', and 'Log Window'.

Index	Name	Address	Chr	Position	GenTrain Score	Frac A	Frac C	Frac G	Frac T
1	MitoA1004...	9048...	M	10045	0.7260	0.317	0.203	0.119	0.361
2	MitoA1055...	9058...	M	10551	0.7038	0.307	0.287	0.089	0.317
3	MitoA1125...	9039...	M	11252	0.7038	0.302	0.356	0.119	0.223
4	MitoA1146...	9019...	M	11468	0.7220	0.277	0.312	0.153	0.257
5	MitoA1181...	9047...	M	11813	0.6955	0.272	0.347	0.124	0.257
6	MitoA1230...	9065...	M	12309	0.7161	0.318	0.318	0.114	0.249
7	MitoA1310...	9011...	M	13106	0.6879	0.257	0.376	0.144	0.223
8	MitoA1326...	9014...	M	13264	0.7128	0.297	0.312	0.134	0.257
9	MitoA1378...	9059...	M	13781	0.7357	0.312	0.376	0.094	0.218
10	MitoA1423...	9069...	M	14234	0.7433	0.356	0.342	0.069	0.233
11	MitoA1458...	9069...	M	14583	0.7312	0.436	0.312	0.074	0.178
12	MitoA1521...	9031...	M	15219	0.6644	0.267	0.297	0.168	0.267
13	MitoA1524...	9060...	M	15245	0.6849	0.257	0.317	0.153	0.272
14	MitoA1530...	9044...	M	15302	0.4777	0.277	0.337	0.139	0.248
15	MitoA1575...	9075...	M	15759	0.7220	0.312	0.332	0.099	0.252
16	MitoA1592...	9020...	M	15925	0.7038	0.387	0.244	0.114	0.259
17	MitoA1616...	9010...	M	16163	0.6955	0.343	0.353	0.100	0.204

Image Courtesy:
 Dr. Liju Samuel
 Illumina Genome studio Analysis Software Manual

**How do I leverage our GWAS results, a
goldmine of wealth ? ???**

A Partial list of the rare variants in Indian EOT2D

Total EOT2D		310
Variants in MODY Genes	<i>HNF4A</i>	1
	<i>GCK</i>	2
	<i>HNF1A</i>	5
	<i>PDX1</i>	10
	<i>HNF1B</i>	1
	<i>NEUROD1</i>	4
	<i>KLF11</i>	13
	<i>PAX4</i>	8
	<i>BLK</i>	9
	<i>ABCC8</i>	10
	<i>KCNJ11</i>	7
	<i>APPL1</i>	5
	<i>WFS1</i>	21
	TOTAL	96

My Broad Objectives

1. To screen genetic markers in South Indian population using Genome-wide association studies in Type II diabetes
2. To compare the rare variants of EOT2D from Monogenic Panel Genes with identified variants from GWAS data and characterize and validate the effect of the identified rare variant from Monogenic Panel Genes *in vitro*
3. To construct Polygenic Risk score for Early Onset Type II diabetes

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