TEACHER'S ACTIVITY REPORT 2016-2017.

FACULTY: Science DEPARTMENT: Zoology IQAC ACTIVITY No: SVC/2016-17/ZOO/MV/1

NAME OF THE ACTIVITY: Workshop on "MOLECULAR CHARACTERIZATION OF HUMAN SAMPLES TO IDENTIFY DISEASE RISK LOCI"			
DATE	**FACULTY**	**DEPARTMENT/COMMITTEE**	**COORDINATOR NAME**
7th-8th April, 2017	Science	Zoology	Dr. P. S. Dhanaraj Dr. Mansi Verma
TIME	**VENUE**	**NUMBER OF PARTICIPANTS**	**NATURE: Outdoor/Indoor**
9:30 AM onwards	Zoology Lab	30	Indoor
SUPPORT/ASSISTANCE:	Sri Venkateswara College		

BRIEF INFORMATION ABOUT THE ACTIVITY (**CRITERION NO. - 2,3,5**):

TOPIC/SUBJECT OF THE ACTIVITY	**Workshop on "MOLECULAR CHARACTERIZATION OF HUMAN SAMPLES TO IDENTIFY DISEASE RISK LOCI"**
OBJECTIVES	The Zoology department of Sri Venkateswara College organized workshop on "Molecular Characterization of Human Samples to identify Disease risk Loci" sponsored by DBT Star Scheme in collaboration with Public Health Foundation of India (PHFI, Gurgaon) from 7-8th April 2017. The workshop was intended to provide introduction to basic molecular techniques to graduate students and covered following topics: 1. Introduction to molecular markers 2. Introduction to Polymerase Chain Reaction and Restriction Digestion 3. Laboratory exercises- PCR, restriction digestion, SNP detection
METHODOLOGY	Human DNA samples were provided by Dr. Aastha Aggarwal, Research Scientist and Assistant Professor, PHFI. Following steps were implemented to learn about molecular markers: 1. General Introduction to topic 2. Polymerase Chain Reaction (Theory) 3. PCR (wet lab) 4. Restriction Digestion and Gel Electrophoresis (Theory) 5. Preparation of gel (wet lab) + discussion 6. Gel Electrophoresis (checking of PCR product- wet lab) 7. Restriction Digestion (wet lab) 8. Introduction to molecular markers (Theory) 9. Preparation of gel (wet lab) + discussion 10. Gel Electrophoresis (genotyping- wet lab)

OUTCOMES	Students were happy to perform experiments together with Dr. Aastha Aggarwal. They learnt about PCR and used human samples for tracing markers responsible for obesity. They perform restriction digestion and ran the samples on gel. Students were excited to observe amplification of genes in some patients suffering from obesity.

PROOFS & DOCUMENTS ATTACHED (Tick mark the proofs attached):

Notice & Letters	**Student list of participation √**	**Activity report**	**Photos**	Feedback form
Feedback analysis	News clip with details	Certificate	Any other	**Manual √**

IQAC Document No:	Criterion No:	Metric No:
Departmental file no	IQAC file No: SVC/2016-17/ZOO/MV/1	

NAME OF TEACHER & SIGNATURE	NAME OF HEAD/ COMMITTEE INCHARGE & SIGNATURE	IQAC COORDINATOR (SEAL & SIGNATURE)
Dr. Mansi Verma	Dr.Mansi Verma	

For Reference

Criterion I	Curricular Aspects (planning & Implementation)		Criterion V	Student Support & Progression
Criterion II	Teaching Learning & Evaluation		Criterion VI	Governance
Criterion III	Research, Innovations & Extension		Criterion VII	Institutional Values & Best Practices
Criterion IV	Learning Resources and Infrastructure			

WORKSHOP

on

"Molecular Characterisation of Human Samples to Identify Disease Risk Loci"

— conducted by —

Dr. Aastha Aggarwal
Research Scientist and Assistant Professor
Centre for Chronic Conditions and Injuries (CCCI),
Public Health Foundation of India

— organised by —

The Department of Zoology,
Sri Venkateswara College

DATE: 7TH-8TH APRIL, 2017
TIME: 9:30AM ONWARDS
VENUE: ZOOLOGY HONOURS LABORATORY, SRI VENKATESWARA COLLEGE

Committee Members

Patron
Dr. P. Hemalatha Reddy

Convenor
Dr. P.S. Dhanaraj

Co-Convenor
Dr. Mansi Verma

Advisory Committee:

Mrs. Ramaa Sinha
Dr. Anita Verma
Dr. Om Prakash

Organizing Committee:

Dr. Vartika Mathur
Dr. Rajendra Phartyal
Dr. P. Jayaraj
Dr. Kh. Bronson Kumar
Dr. Ajaib Singh
Dr. Vagisha Rawal
Dr. Manoj Jaiswal
Dr. Riyaz Bakshi

Workshop on "MOLECULAR CHARACTERIZATION OF HUMAN SAMPLES TO IDENTIFY DISEASE RISK LOCI"

Programme

Time	Topic
Day 1	
9:30AM-10:00 AM	General Introduction to topic
10:00AM -11:00AM	Polymerase Chain Reaction (Theory)
11:00AM -12:30PM	PCR (wet lab)
12:30PM -13:30PM	Lunch Break
13:30PM-14:30 PM	Restriction Digestion and Gel Electrophoresis (Theory)
14:30 PM -15:30PM	Preparation of gel (wet lab) + discussion
15:30 PM -17:00PM	Gel Electrophoresis (checking of PCR product- wet lab)
Day 2	
9:30 AM -10:30 AM	Restriction Digestion (wet lab)
10:30AM -11:30AM	Introduction to molecular markers (Theory)
11:30AM -12:30PM	Preparation of gel (wet lab) + discussion
12:30 PM -13:30PM	Lunch Break
13:30 PM -14:30PM	Gel Electrophoresis (genotyping- wet lab)
14:30 PM -15:30PM	Discussion

A. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction is a technique to amplify target genomic regions. It is an in vitro method for the enzymatic synthesis of specific DNA sequence using oligonucleotide primers that hybridize to opposite strands through complimentary base pairing and flank the region of interest in the target DNA. It allows the exponential amplification of target DNA sequence through repeated cycles of DNA synthesis. Each molecule of target DNA synthesized acts as a template for the synthesis of new target molecules in the next cycle.

The technique is a rapid, inexpensive and simple means of producing relatively large numbers of copies of DNA molecules from minute quantities of source DNA material in a simple enzyme reaction, even when the source DNA is of relatively poor quality. It was developed by Kary Mullis in 1983.

1. Required Components and Reagents

Ingredient	Function
10X Buffer	Maintains the pH of the PCR reaction required for optimum activity and stability of the *Taq* DNA Polymerase
deoxy Nucleotide Triphosphates (dNTPs)	• Provides energy required for the process to take place • Provides the building blocks of DNA- nucleotides (dATP, dGTP, dTTP and dCTP). It is important to add equal amounts of each nucleotide to the master mix to prevent mismatch bases
Magnesium Chloride ($MgCl_2$)	• Acts as a catalyst for optimum activity of *Taq* polymerase • Incorrect $MgCl_2$ concentration reduces or stops the activity of the enzyme
Primers	A pair of oligonucleotides or short single-stranded pieces of DNA (20-30 bases) that bind to the opposite DNA strands on either side of the sequence to be amplified by complementary base-pairing, allowing *Taq* DNA polymerase to identify the location and initiate incorporation of the deoxynucleotides by providing the free 3′-OH required for covalent

	extension.
***Taq* DNA Polymerase**	It is a thermostable enzyme obtained from *Thermus aquaticus*, a hot spring bacterium. It adds dNTPs to the DNA template and synthesises a new strand complementary to the original strand. It lacks 3′→5′ proofreading exonuclease activity.
Template DNA	The DNA molecule containing the target sequence to be amplified

2. Procedure

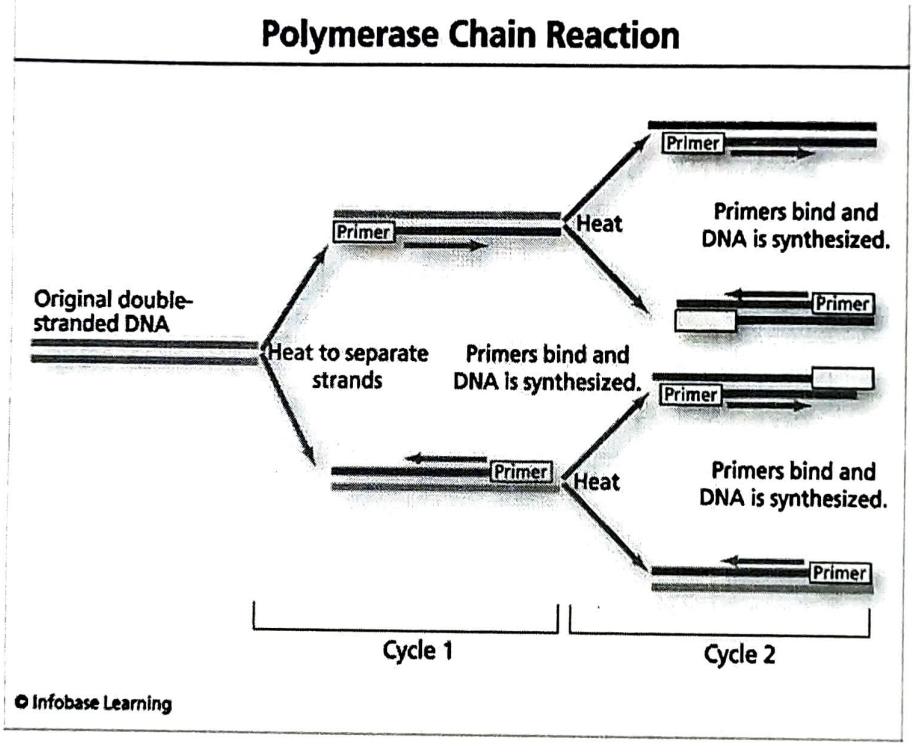
There are three major steps in a PCR which take place at different temperatures. This temperature variation is provided by a pre-programmed automated thermocycler that heats and cools the reaction tubes placed in it to the precise temperature required for each step of the reaction, in a very short time. A repetitive series of the three steps results in exponential accumulation of the DNA sequence of interest. The three steps are as follows:

Denaturation: The double stranded DNA molecule (DNA template) is destabilized under the influence of temperatures as high as 94°C and the DNA molecule melts open into single stranded DNA capable of being copied by the DNA polymerase. All enzymatic reactions stop at this stage such as extension from a previous cycle. Prior to the first cycle, during an initialization step (Initial Denaturation), the PCR reaction is heated to a temperature of 94-96°C and this temperature is then held for 1-9 minutes. This first hold is employed to ensure that most of the DNA template gets denatured.

Annealing: The reaction is cooled to a temperature that allows binding of the primers to the 3′ end of the single-stranded DNA without permitting the double helix to reform between the template strands. Locations where the primer sequence exactly complements the sequence of the template, strong ionic bonds are formed thereby allowing DNA synthesis in the next step. Primers that are in position of 'no exact match' dissociate from the template in the next cycle when the temperatures are raised. The temperature at which this step takes place depends upon the melting temperature of the primers that is affected by its sequence, length and GC content. The temperature may vary between 50°C and 70°C.

Extension or Elongation: The polymerase, directed by the position of the primers, copies the intervening target sequence using the single-stranded DNA as template through complementary base pairing in 5' to 3' direction. It adds bases to the 3' end of the primer by condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand. 72°C is the ideal working temperature for the DNA polymerase. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified.

A ***final elongation*** step of 5-15 minutes (depending on the length of the DNA template) after the last cycle may be used to ensure that any remaining single-stranded DNA is fully extended. A final hold of 4-15°C for an indefinite time may be employed for short-term storage of the reaction.



Polymerase Chain Reaction

3. PCR Protocol

Reagents Required: Taq Buffer, Taq enzyme, MgCl2, dNTPs, Forward Primer, Reverse Primer;

Other Components: DNA, Milli Q water

Plastic ware: PCR tubes/ strips/ plate and seal, 10µl sterile tips, 20-100µl sterile tips, 2ml vial

Equipment: Micro-pipettes, vortex, centrifuge, PCR machine

Miscellaneous: tissue, discard beaker, ice

Samples: Human DNA isolated from blood samples collected from general population

Marker: *MC4R* (rs17782313)

Reaction Mix:

Reagent/ Component	Requirement (in µl)	
	1X	
PCR Mix		
Taq Buffer (10X)	1	
$MgCl_2$ (15mM)	0.6	
dNTPs (200µM)	1	
Forward Primer (25ng/µl)	1	
Reverse Primer (25ng/µl)	1	
Taq enzyme (1U//µl)	0.34	
Milli Q water	3.06	
Sub Total	**8**	
DNA	2	
Total	**10**	

***PCR Cycles*:**

1. *Initial Denaturation:* 94°C for 5 min
2. *Denaturation:* 94°C for 45 sec
3. *Annealing:* 60°C for 45 sec
4. *Extension:* 72°C for 45 sec

   Cycles 2-4 to be repeated 35 times
5. *Final Extension:* 72°C for 5 min
6. *Final Hold:* 4°C infinity

***Expected Band size*:** 330bp

4. Checking PCR Product (through gel electrophoresis)

Prior to carrying out any further analysis it should be confirmed if the amplimer (amplified DNA fragment obtained from PCR) is indeed the required one by comparing its molecular size with a standard DNA ladder and visualising it through electrophoresis.\

B. Restriction Digestion

Restriction Digestion is a technique used for cleaving DNA molecules at specific locations. This is achieved by use of Type II restriction endonucleases/ restriction enzymes. These enzymes cut DNA at specific sites, called restriction sites, at which particular short sequences of bases are present. These enzymes recognize distinct and specific nucleotide sequences called recognition sequences, usually 4-12 base pairs long, and then cut at specific location called restriction site. Several enzymes can share restriction sites.

In case of Restriction Fragment Length Polymorphism (RFLP) markers, the amplified DNA sequence is subjected to restriction digestion. After ensuring that the PCR product is of required molecular size, enzyme mix is made by mixing the concerned enzyme and its buffer in a fresh tube. The enzyme mix is then immediately added to the PCR products and the samples incubated at specific temperature for specific duration. Restriction digestion is followed by agarose gel electrophoresis to determine genotype of the sample.

Restriction Digestion Protocol for MC4R (rs17782313)

Reagents Required: Enzyme Buffer, enzyme;

Other Components: PCR product, Milli Q water

Plastic ware: PCR tubes/ strips/ plate and seal, 10µl sterile tips, 20-100µl sterile tips, 2ml vial

Equipment: Micro-pipettes, vortex, centrifuge, Air Incubator

Miscellaneous: tissue, discard beaker, ice

Enzyme: *Ava*I (*Eco*88I) (Fast Digest)

Reaction Mix:

Reagent/ Component	**Requirement (in µl)**	
	1X	
Enzyme Mix		
Enzyme Buffer	2	
Enzyme	1	
MilliQ Water	2	
Sub Total	5	
PCR Product	10	
Total	15	

Incubation Conditions:

Temperature: 37°C

Time: 15 minutes

Expected Band sizes: 330bp, 299bp, 31bp

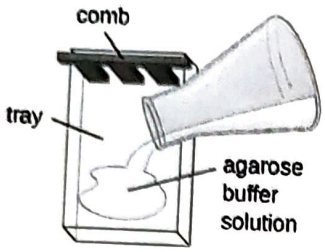
C. Genotyping

Genotyping is the process of determining base pairs present at a particular locus (also known as genotype) in a sample. In humans, who are diploid organisms, genotyping for a particular locus results in information about base pairs present at the two homologous chromosomes for that locus. These are known as alleles.

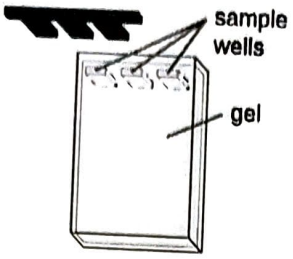
Genotype of the target genomic region is determined using Agarose gel electrophoresis. Gel electrophoresis refers to the technique in which macromolecules, either nucleic acids or proteins, are forced across a span of gel, motivated by an electric current. The macromolecules are separated on the basis of size, electric charge and other physical properties. Agarose gels are commonly used for analyses involving DNA. Agarose is a natural colloid extracted from sea weed. It is very fragile and easily destroyed by handling. Agarose gels are usually used to separate DNA fragments ranging from a few base pairs (50 bp or so) to several kilo bases (10,000 bp or so). Accordingly, it is usually used at concentrations between 0.7% and 3%. Higher is the concentration of the gel lower is the porosity required for proper separation of low molecular weight substances and vice versa.

Agarose gels are formed by suspending dry agarose in aqueous Tris Borate EDTA (TBE) buffer and boiling the mixture until a clear solution formed. Ethidium bromide (EtBr) is then added to the solution and the solution is poured into a preset gel plate. EtBr used in the gel gives fluorescence under UV rays, permitting visualisation of the series of DNA bands spread across the gel according to their size. Once the gel is set, it is taken out and submerged in TBE buffer in electrophoretic tank. Samples are then loaded, mixed with loading dye, into the wells. A commercially available ladder containing DNA fragments of known size is also loaded and the gel is run at a constant voltage till the time separation of bands is achieved. The gel is then visualised under UV rays and the results are documented.

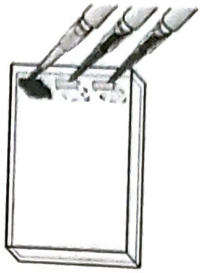
1. An agarose and buffer solution is poured into a plastic tray. A comb is placed into the tray on one end.



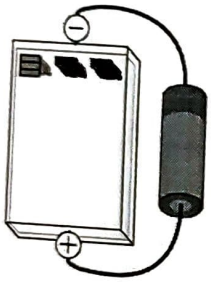
2. The agarose polymerizes into a gel as it cools. The comb is removed from the gel to form wells for samples.



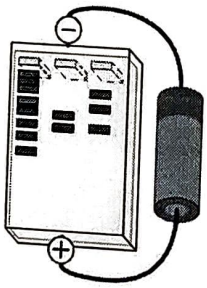
3. DNA samples colored with a tracking dye are pipetted into the wells.



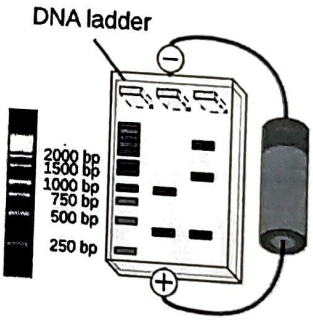
4. The tray is placed into a chamber that generates electric current through the gel. The negative electrode is placed on the side nearest the samples. The positive electrode is placed on the other side.



5. DNA has a negative charge and will be drawn to the positive electrode. Smaller DNA molecules will be able to travel faster through the gel.



6. One well, called a DNA ladder, will contain DNA fragments of known sizes. This ladder is used to determine the sizes of other samples.



(a)

Agarose Gel Electrophoresis

D. Interpretation of Results

***MC4R* (rs17782313)**

Bands	Annotation	Genotype
330bp	- - or 1 1	TT
299bp, 31bp	+ + or 22	CC
330bp, 299bp, 31bp	+ - or 12	TC

SNP rs17782313 is a known risk locus for obesity.

Overweight and obesity is a condition characterized by Body Mass Index (BMI) >25 kg/m^2. It is a metabolic condition caused due to imbalance in energy intake and expenditure. There are several phenotypes (observable traits) that look at various aspects of obesity. These are weight, BMI, waist-hip ratio (WHR), fat mass, lean mass, skin folds. Its prevalence is on rise due to rapid and widespread lifestyle changes such as increased intake of unhealthy diets (energy-dense foods rich in fat) and decreased levels of physical activity. It is a risk factor for various chronic conditions such as cardiovascular diseases (eg- heart diseases, stroke), diabetes, cancers etc. According to latest round of National Family Health Survey (NFHS-4), around 21% women and 18% men in the age range of 15-49 years have BMI $\geq$ 25 kg/m^2.

Obesity is a common chronic condition (also known as multifactorial or complex conditions), implying that it is influenced by several factors including environmental and genetic risk factors and their interaction. Obesity can be monogenic (Mendelian inheritance pattern and rare) or polygenic (common and less severe). *Polygenic or multigenic obesity is caused by mutations in several genes, each of which has a small influence on manifestation of obesity*. Obesity risk is 2-8 times higher for a person with family history of obesity ("it tends to run in families"). Starting from twin studies, evidence has emerged from various genetic studies that common obesity is influenced by a number of genetic variants. These variants *indicate* a person's risk of developing

obesity but are *not diagnostic* of the condition. These variants interact with each other and with environmental factors to *predispose* an individual (increase his risk) of developing the condition.

A search for Genome Wide Association Studies (GWASs) in GWAS catalog results in 62 studies and 1905 associations. Majorly 58 loci contribute to polygenic obesity. Of these *FTO, PCSK1, MC4R, CTNNBL1* are the most significant. Together all these variants explain ~1.7% variance in BMI which is quite low.

rs17782313 is an intergenic single nucleotide polymorphism lying 188 kb downstream of the Melanocortin-4 Receptor (MC4R) gene and is located at 18q21.32. It is associated with increased feeling of hunger, increased snacking, decreased satiety, and increased total, fat and protein energy intake. Each mutant allele is associated with an increased risk of higher BMI. The magnitude of this risk will vary between populations. A genotype 'CC' means that the individual has 2 copies of mutant allele and has twice the risk of 'X' unit increased BMI than 'TC' genotype.

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Protocol for Preparation of Tris Borate EDTA (TBE) Buffer

Chemical	Quantity
Tris (Hydroxymethyl) aminomethane $NH_2.C(CH_2OH)_3$ salt	54 g
Ethylene Diamine Tetra Acetic acid (EDTA)	2.9 g
Boric Acid	27.5 g
The chemicals are dissolved in 500ml distilled water. The solution is then stored at 4°C.	

List of Participants

S.No.	Name	Course
1	Garima	PhD student
2	Anwesha	TZH
3	Niharika	TZH
4	Divya	TZH
5	Tejaswini	TZH
6	Uma	TZH
7	Saumya	TZH
8	AJN Vats	TZH
9	Mrigya	SZH
10	Yukti	SZH
11	Soham	SZH
12	Ananya	SZH
13	Monika	SZH
14	Rohit	SZH
15	Himanshu	SZH
16	Aparna	SZH
17	Saiyami	SZH
18	Prerna	SBS
19	Pallavi	SBS
20	Dilsher	SBS
21	Rohit Goyal	SBS
22	Monika	SBS
23	Sandeep	SBS
24	Aakanksha Dogra	SBS
25	Harshit	SBS
26	Vipin	TBS
27	Abdus	TBS
28	Reema	TBS
29	Renuka	TBS
30	Aditi	TBS

SRI VENKATESWARA COLLEGE
(University of Delhi)

Internal Quality Assurance Cell

This is to certify that the Activity report (Teacher/Department /Society/Association) has been submitted for documentation to IQAC, Sri Venkateswara College, University of Delhi.

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