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Avadi, Chennai, Tamil Nadu – 600 054

List of students undertaking Internship during the Academic Year 2022 – 2023

Program Name : Biotechnology

Program Code :214

INTERNSHIP DETAILS (2022 – 2023)

S.No.	Register Number	Name	Year	Course	Name of the Organization	Duration
1.	112720214007	Issac Winston.J	II	Molecular Techniques to study Disease-Biology with special reference to Diabetes	Madras Diabetes Research Foundation	July 2022 to August 2022

Head of the Department



MADRAS DIABETES RESEARCH FOUNDATION

ICMR CENTRE FOR ADVANCED RESEARCH ON DIABETES

Affiliated to the University of Madras & Deakin University, Australia & recognized as a Scientific and Industrial Research Organisation (SIRO) by the Department of Scientific and Industrial Research, Ministry of Science & Technology, Govt. of India.

19th September, 2022

Certificate of Proficiency

This is to certify that Mr. J. ISSAC WINSTON, student of DEPARTMENT OF BIOTECHNOLOGY, ST. PETER'S COLLEGE OF ENGINEERING AND TECHNOLOGY, Chennai has successfully completed the Two-months (July-August 2022) Summer Research Fellow Program (SRFP) on the topic "Molecular Techniques to study Disease-Biology with special reference to Diabetes" at the Madras Diabetes Research Foundation (MDRF), Chennai under my mentorship. During this tenure, the student has acquainted himself on the state-of-the-art cell and molecular biology techniques & developed research aptitude on how these techniques could be ideally applied to study and investigate the molecular pathogenesis of diabetes and its vascular complications. His conduct, character and research aptitude are very good.

Yours Sincerely,

Dr.M.Balasubramanyam
ICMR Emeritus Scientist

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INTERNSHIP REPORT

“Molecular Techniques to study Disease-Biology with Special Reference to Diabetes”

By

J. ISSAC WINSTON
DEPARTMENT OF BIOTECHNOLOGY
ST. PETER'S COLLEGE OF ENGINEERING AND TECHNOLOGY
CHENNAI.



GUIDE & MENTOR:

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ICMR EMIRETUS SCIENTIST

MADRAS DIABETES RESEARCH FOUNDATION (MDRF), Chennai

Brief note on the outcome of my SRFP Internship:

At the outset I would like to express my deep gratitude to the Science Academy for giving me an opportunity to undergo SRFP Internship. I sincerely thank Dr.M.Balasubramanyam, ICMR Emeritus Scientist, Madras Diabetes Research Foundation (MDRF), Chennai for his mentorship during my SRFP tenure. During my two-months internship period, I had the opportunity to learn basics and advanced technologies of new-biology areas pertaining to investigations that aim to unravel the complex pathogenesis of diabetes and its complications. I got an opportunity to visit the labs, particularly cell and molecular biology, vascular biology and molecular genetics and exposed myself to several hands-on techniques so as to elaborate my research aptitude.

I am also fortunate to attend a distinguished talk by an international expert on the topic Aldehyde dehydrogenase, an emerging drug target of diabetic cardiometabolic complications. Which gave me a clarity on the role of ALDH2 that is linked to the reduction of chronic alcohol -mediated myocardial hypertrophy in diabetic patients. This talk exposed me to learn how preclinical research will pave way for clinical investigations as a follow-up and thereby development of newer therapeutic opportunities. I have also participated as a delegate in the 9th edition of Dr. Mohan's International Diabetes Update-2022. It was a three-day virtual workshop (22-25 July 2022) in which various national and international faculties gave their talks/case presentations/ panel discussion on current trends in diabetes prevention, diagnosis, management and what is precision medicine. This exposure is really an eye-opener for me to develop my research aptitude.

Finally, I sincerely attest that the Summer Research Fellowship Program has given me an opportunity to learn more, expanded my quest for scientific research, and gave me a directionality to progress further in my career advancement.

ABBREVIATIONS

DNA -Deoxyribo Nucleic Acid	RFLP - Restriction Fragment Length Polymorphism
RNA - Ribo Nucleic Acid	NCBI - National Centre For Biotechnology Information
EDTA - Ethylene Diamine Tetraacetic Acid	mRNA - Messenger RNA
SDS - Sodium Dodecyl Sulphate	mi RNA - Micro RNA
HCl - Hydrochloric Acid	AMV - Avian Myeloblastosis Virus
MgCl - Magnesium Chloride	RT - Reverse Transcriptase
TE buffer - Tris EDTA buffer	MMR - Multi Mode Reader
NaCl - Sodium Chloride	SSB - Sample Solubilizing Buffer
dNTP - Deoxynucleotide Triphosphates	DM - Diabetes Mellitus
dATP - Deadenosine Triphosphates	NCD - Non Communicable Disease
dGTP - Deguanosine Triphosphate	NRK - Normal Rat Kidney cells
dTTP - Dethymidine Triphosphate	MODY - Maturity Onset Diabetes of the Young
dCTP - Decytidine Triphosphate	LADA - Latent Autoimmune Diabetes in Adults
AGE - Agarose Gel Electrophoresis	FPCD - Fibrocalculous Pancreatic Diabetes
DMEM - Dulbecco's Modified Eagle Medium	DME - Diabetic Macular Edema
RPMI - Roswell Park Memorial Institute medium	DRN - Diabetic Retinal Neurodegradation
DMSO - Dimethyl Sulfoxide	SARS Cov2 - Severe Acute Respiratory syndrome Corona virus 2
FBS - Fetal Bovine Serum	T1DM - Type1 Diabetes Mellitus
PBS - Phosphate Buffer Saline	T2DM - Type2 Diabetes Mellitus
cDNA - Complementary DNA	OGTC - Oral Glucose Tolerance Test
RT-PCR - Real Time reverse transcriptase Polymerase Chain Reaction	RBST - Random Blood Sugar Test
RIPA - Radioimmunoprecipitation Assay	GTC - Glucose Tolerance Test
NaVo4 - Sodium Orthovanadate	GDM - Gestational Diabetes Mellitus
SDS - Sodium Dodecyl Sulphate	
PAGE - Poly Acrylamide Gel Electrophoresis	
APS - Ammonium Per Sulfate	
TBST - Tris Buffered Saline	
BSA - Bovine Serum Albumin	
HRP - Horse Radish Peroxidase	
RBC - Red Blood Cells	
WBC - White Blood Cells	
UV - Ultra Violet rays	
SNPs - Single Nucleotide Polymorphism	
PCR - Polymerase Chain Reaction	
DKD - Diabetic Kidney Disease	
ESRD - End Stage Renal Disease	

Abstract

Diabetes mellitus (DM) is a chronic metabolic Non Communicable Disease (NCD), has attained a great epidemic proportions worldwide about 537 million cases. India is one of the epicentres of global diabetes epidemic and has the second highest number of more than 75 million individuals as of 2021. It is a heterogeneous disorder characterized by hyperglycemia, hypoglycemia as a consequence it also further induces complications like diabetic neuropathy, nephropathy, vasculopathy and retinopathy etc. Therefore the screening and diagnostic analysis of Diabetes paves way for proposal of research aptitude and advanced achievements in the field of Diabetology. The primary focus of my appraisal is to grasp knowledge in the basic molecular techniques involved in disease analysis and relate them with diabetes and its complications. Even there are several methods for diabetes diagnosis such as OGTC, GTC, RBST etc, the molecular techniques implied in diabetology study such as analysis of SNPs using PCR- RFLP used in predicting an individual's response to certain drugs, toxins and risk of developing disease, Cell culture technique in mass production of Normal Rat Kidney (NRK) cells used for relative research between diabetes and its complications, RNA isolation and complementary DNA (cDNA) synthesis used for performance of RT-PCR for comparative studies of two genes with respect to diabetology.

Protein analysis which is crucial in advancing knowledge about diseases, discovery of biomarkers, drug discovery and development of therapeutics implemented by their extraction (Bradford method), estimation and their expressions using western blot which provides a relative progression in Diabetology. Hence learning these basic molecular techniques paves a way to correlate Diabetes with current pandemic situations like Covid-19 and accomplish new advancements in future in the field of Diabetology.

Keywords: Diabetes Mellitus, Non-Communicable Disease, hyperglycemia, hypoglycemia, Diabetology, neuropathy, nephropathy, vasculopathy, retinopathy, OGTC, GTC, RBST, Normal Rat Kidney cells (NRK), cDNA, RT-PCR, SNPs, Covid-19.

Literature Review

Diabetes Mellitus (DM) is the most common metabolic disorder arises due to elevated sugar levels in the blood. Nowadays due to an unhealthy-lifestyle diabetes has emerged as a devastating disorder and affects almost individuals of all age group. It is characterized into Type-1, Type-2, Gestational, MODY, FCPD and LADA etc. Type 1 Diabetes (T1DM) results from the destruction of pancreatic- β cells that is mediated by the immune system (Ilonen, Lempainen and Veijola, 2019). Mostly children or adult people were affected by T1DM in which insulin is absent or extremely low leads to hyperglycemia. In type 2 diabetes, the response to insulin is diminished and this is defined as insulin resistance (Goyal and Jialal, 2022). Insulin resistance develops from obesity and aging as a consequence of imbalance between insulin levels. Gestational diabetes mellitus (GDM) among pregnant women increase the risk of both short term and long term complications, such as birth complications, babies large for gestational age

(LGA) and Type2 DM for both mother and offspring (Rasmussen et al., 2020). MODY is a heterogeneous disorder identified by non-insulin dependant diabetes at young age (under 25Y/o). These cases are important to efficiently and accurately diagnose, given the clinical implications of syndromic features, cost-effective treatment regimen and the potential impact on multiple family members(Broome et al., 2021). Fibrocalculus Pancreatic Diabetes (FCPD) is a unique form of diabetes secondary to non- alcoholic calcific pancreatitis. The onset is usually in childhood with recurrent abdominal pain. By adolescence and adulthood, patients have large pancreatic calculi. LADA is a specific form of type1 diabetes which develops in the 4th or 5th decade of life. Individuals suffering from LADA are lean, prone to ketosis and have poor c-peptide levels during diagnosis. Several complications associated with diabetes such as diabetic neuropathy, nephropathy, retinopathy and myocardial effects are researched and related using numerous molecular techniques. Therefore the knowledge of basic molecular techniques involved in diabetology is extremely crucial in order to proceed further in research studies. Pancreatic fat is linked to reduced insulin secretion, atleast under specific circumstances such as prediabetes low BMI and increased risk of type 2 diabetes mellitus(Wagner et al., 2022).

Diabetes mellitus has profound effects on multiple organ system; however the loss of vision caused by diabetic retinopathy might be one of the most impactful in patients life(Antonetti, Silva and Stitt, 2021). In DR, vision loss is due to Diabetic Macular Edema (DME) and it may also result in Diabetic Retinal Neurodegradation (DRN). Micro vascular complications of T2DM assist in Diabetic Kidney Disease (DKD), End Stage Renal Disease (ESRD) and erectile dysfunction. Sexual dysfunction is an often-overlooked microvascular complication of T2DM, with a complex pathogenesis originating from endothelial dysfunction(Faselis et al., 2020). These complications can be analyzed through variousprotein expression studies which help in identification of biomarkers, drug discovery and developmentof therapeutics. There is a great risk of evolution of pandemics like Covid-19 because it was discovered that SARS Cov-2 could be induced through T2DM. With the rising prevalence of obesity, diabetes has come to an increasing awareness of their impacts on infectious diseases, including increased risk of various infections, post infection complications and mortality from critical infections(Zhou et al., 2021). Patients with covid-19 related diabetes are called “Covi DIAB”

Despite the fact that these complications influence human organ system, there are certain remedies for controlling Diabetes. Metformin is one of the most popular oral glucose lowering medications, widely considered to be the optimal initial(first line) therapy for T2DM.(Sanchez-Rangel and Inzucchi, 2017) which helps to control the amount of glucose in the blood and also increases body's response to insulin. But even with the availability of oral glucose-lowering drugs, insulin supplementation was often needed to achieve good glucose control level in T2DM through insulin therapy(Aschner, 2020). Stem cell based clinical trials for Diabetes Mellitus is an intraportal allogenic cadaveric islet transplantation that has been shown a promising therapy for patients with T1DM (de Klerk and Hebrok, 2021).Therefore the molecular techniques could be the key to success in the future scope of diabetology and these molecular techniques are primary elements that might play a vital role in creating a disease-free world.

Objective

Primary Objective:

To acquire knowledge on the basic molecular research techniques implied in analysis of disease with respect to diabetes mellitus

Secondary Objective:

To implement these techniques and co-relate them with diabetes and its complications

Materials and Methodology

Isolation of genomic DNA using phenol-chloroform method

Reagents used

10x lysis buffer (1litre)

Ammonium chloride (NH ₄ Cl)	41.8g
Potassium carbonate(KHCO ₃)	4.6g
H ₂ O	1000ml

The components were added and made up to **1000ml** in **2x** conc

500mm or 0.5m EDTA

EDTA-186.1 g in 800ml H₂O

Ph-7.5

Adjust ph to 8.0 using conc HCl

H₂O-1000ml

10% SDS

10 g-SDS

H₂O-1000ml

Proteinase k

Stock-100mg in 5ml

Reaction-20mg in 1ml

Ammonium acetate

Ammonium acetate-96.35g

H₂O-250ml

Tris buffer (ph 8) Tris HCl-121.4g , H₂O-700ml and Make upto 1000ml

TE Buffer-1M Tris HCl -5ml

0.5M of EDTA - 1ml

Make upto final volume 500ml

Chemical	Role in DNA isolation
EDTA	Acts as an chelating agent and blocks the activity of the DNase enzyme
MgCl ₂	Protects DNA from mixing with other cell organelles
NaCl	Prevents DNA denaturation
SDS	It is an anionic detergent that denatures cell membrane protein
TE buffer	Dissolves DNA
Tris	It maintains the pH of the solution and also permeables the cell

Quantification of genomic DNA using nanodrop spectrophotometer method

Sample-0.5-2ml

Purified or autoclaved water

Blanking solution-TE buffer

Selection of restriction enzymes and Designing of primers for PCR-RFLP

NCBI website, Primer Blast/ Primer 3 and Reverse complement page web

Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLP)

Reagents for PCR

10x PCR buffer

Taq polymerase

Master mix (dNTPs, MgCl₂, buffer)

Forward and reverse primers

Sterile water

Template DNA

Content	Volume (µl)
PCR master mix (dNTP- dATP,dCTP,dGTP,dTTP),MgCl ₂ ,buffer	24
Sterile H ₂ O	24
Forward primer (5' CAGAGCATGGACAGGGAG 3')	4
Reverse primer (5' GCAACTCCTCATGGCTGAGGTCTC 3')	4
Sample DNA	4
Total	60

Reagents for Agarose Gel Electrophoresis (AGE)

TE buffer

Tris HCl (pH 8.0)	10mm
EDTA	1mm

10X Stock solution

Tris base	108g
Boric acid	55g
0.5m EDTA	40ml
pH	8.0

Gel loading dye

Bromophenol blue	0.25%
Xylene cyanol	0.25%
Glycerol	50%
EDTA	1mol
pH	8.0

Acrylamide/bis acrylamide stock solution (29:1)

Prepared as an unpolymerized 30% solution in H₂O

Make upto 100ml with water

Acrylamide	29g
N-Methylene bisacrylamide	1g

Cell culture Techniques

Conditions

pH	7-7.4
Osmolality	280-320 mOsmol/kg
Temperature	35-37°C
CO ₂ Incubation	5%

Basic composition for Animal Cell Culture

Even animal cell culture media differs in their complexity most includes

Amino acids	0.1-0.2mM
Vitamins	1 mM
Salts	150mM
KCl	4-6mM
CaCl ₂	1mM
Glucose	5-10mM

Commonly used media

- Dulbecco Modified Eagle's Medium (**DMEM**)
- Roswell Park Memorial Institute (**RPMI**)

DMEM has twice the concentration of amino acids and four times the amount of vitamins as EMEM. Original formulation contains

Glucose	4500mg/L
Reduced sodium bicarbonate	1500mg/L

DMEM Medium preparation

Phenol red(maintained at pH 7.2)	Indicator
Antibiotics	10ml/L
Sodium bicarbonate (Ph 7.2-7.4)	3.7g/L

10g of DMEM in powdered form is mixed with Double-distilled water and made upto 1L final volume

10% Fetal Bovine Serum

Dimethyl Sulfoxide (DMSO)

Trypsin

Cell counting using Hemocytometer

$$\text{Concentration (cells)} = \frac{\text{No of cells counted}}{\text{No of squares count}} \times \text{dilution factor}$$

Viability of cells

While adding the reagent **tryphan blue**

Dead cells- turn dark blue

Viable cells-exclude

$$\text{Cell viability formula} = \frac{\text{no of viable(unstained cells)}}{\text{Total no of cells}} \times 100$$

Isolation of RNA by Trizol method

1xPhosphate Buffer Saline (PBS)

Sodium Chloride(NaCl)	8g
Potassium Chloride(KCl)	0.2g
Sodium Phosphate Dibasic	1.44g
Potassium Phosphate Monobasic	0.245g
pH	7.4

These components were added initially in 800ml distilled water and then made up to 1000ml

Trizol

Consists of **phenol, guanidinium** and **isothiocyanate**

Chloroform

For phase separation

Isopropanol

Precipitation

Complementary DNA (cDNA) synthesis

RNA template preparation

5x reaction buffer	4 μ l
2.5 mmol dNTPs	1.6 μ l
1 X RT primer	1 μ l
100 AMV Reverse transcriptase	0.5 μ l
RNA	2 μ l
RNase free H ₂ O	20 μ l

PCR master mix preparation

PCR Reaction Mix Components	Volume for 1 reaction	Volume for 6 reactions
2x SYBR Green	10 μ l	60 μ l
Forward primer	1 μ l	6 μ l
Reverse primer	1 μ l	6 μ l
Deionized water	4 μ l	24 μ l
cDNA sample	4 μ l	24 μ l
Total	20 μ l	120 μ l

Quantification of cDNA by RT - PCR Technique

PCR buffer with dNTPs	20 μ l
Forward primer	1 μ l
Reverse primer	1 μ l
Taq polymerase	0.1 μ l
cDNA	3 μ l

Extraction and Estimation of Proteins

Cell lysate

Protease inhibitor

Radioimmunoprecipitation buffer (RIPA)

1M Tris HCl	2.5ml
NaCl	0.402g
NP 40	0.5ml
EDTA	0.2ml
Sodium orthovanadate (NaVO ₄)	0.1ml

Sonication

Sonication is a method in which ultrasonic waves are passed into the cell culture in order to break the cell membrane and release proteins into solution.

Frequency used-**8Hz**

Bradford Reagent

Add the following components

Comassive brilliant blue G-250	100mg
95% ethanol	50ml
85% phosphoric acid	100ml

490µl of Bradford reagent and 10µl of the sample are added to the Bradford assay plates or wells and the colour change is observed

The wells are read at **590nm** using a Multi Mode Reader (MMR)

ANALYSIS OF PROTEIN EXPRESSIONS USING WESTERN BLOT

Sodium Dodecyl Sulfate - Poly Acrylamide Gel Electrophoresis (SDS-PAGE) 10% stacking gel

30% PAGE	2.55ml
1M Tris HCl (pH-6.8)	1.875ml
10% Ammonium Persulfate (APS)	0.15ml
10% Sodium Dodecyl Sulfate(SDS)	0.15ml
TEMED	0.015ml
Double distilled H ₂ O	10.2ml
Total Volume	15ml

10% Separating gel

30% acrylamide	5.3ml
Tris Base pH(8.8)	4ml
10% SDS	160 μ l
10%APS	160 μ l
TEMED	16 μ l
Double distilled water	6.3ml

Sample Solubilizing Buffer 10ml of 4x stock

1M Tris HCl (pH-6.8)	2.5ml
0.1% Bromophenol Blue	0.8ml
100% glycerol	4ml
SDS	1g
14.3 M β - Mercapitoethanol	2ml
Double distilled H ₂ O	0.5ml

Adjust the final volume to 10 ml using double distilled water and it is converted into 1x concentration for reaction

Transfer Buffer 1x

Tris HCl	3.03g
Glycine	14.4g

Make upto final volume 1000ml

SDS Buffer 1x

Tris base	3.03g
Glycine	14.4g
SDS	1g
Distilled H ₂ O	1L

Add the following components and make up to final volume 1000 ml

1x Tris Buffered Saline (TBST)

Tris HCl	20mM
NaCl	150mM
Tween 20 detergent	0.1%

Make up to 500ml using H₂O

5% Bovine Serum Albumin (BSA)

0.5 ml of BSA is used for blocking the antibodies from binding to the membrane non-specifically

Primary Antibody

1:10000 ratio (ie.5 μ l) was added and washed overnight

Secondary Antibody

5-6ml is added for our required blot and washed for 3 times

Chemiluminescence (luminon)	500 μ l
Substrate	500 μ l

These components were added to H₂O in order to make the membrane chemiluminant

Isolation of DNA by phenol-Chloroform method

DNA is the primary requirement of molecular biology techniques and it depends on the high purity and concentration of the extracted DNA. The isolation of DNA was a two day process in which blood samples collected from patients were transferred to tubes and subjected to **2x lysis buffer (twice the volume of blood)** in order to remove the **RBC'S** as we isolate DNA from WBC. The sample was mixed in a rotor at **2500rpm** for **10minutes** and held on at cold room for **25minutes**. Centrifugation was carried out at **2000rpm** and the supernatant was discarded. Subsequently the remaining pellet was added with **1ml(1000µl)** salt, EDTA, **0.1µl** of SDS and **10µl** protenase k to separate DNA from RNA, lipids, proteins etc. All these contents were mixed in a cyclomixer and stored for overnight incubation at **37°C**. On Second day a mixture of phenol (twice the volume) and chloroform: isoamyl alcohol was added to promote partitioning of lipids and cell debris to organic phase, leaving DNA in the aqueous phase. The reaction followed by mixing of contents at rotor for **10mins** and centrifugation at **2000rpm** for two times. The sample was moved in a fresh falcon tube and added with **0.5µl** of **ammonium acetate** followed by ethanol wash to precipitate the DNA. Thread like DNA structure appeared and was spooled out and mixed with **500µl** of **1xTE buffer** for storage or experimental use.



Spooling out of DNA
(Source: MDRF, Chennai)

Quantitative Analysis of Genomic DNA using Nanodrop Spectrophotometer method

Nanodrop spectrophotometer works on the principle of **Beer's law** that there is usually a quantitative relationship between solute concentration and the intensity of the transmitted light i.e. the more concentrated the specimen is, the less light is transmitted through it. Quantification was done by turning on nanodrop software and selecting the dsDNA application. Consequently **1µl** of blanking **TE buffer** was pipetted to the lower pedestal and the arm was lowered. Blank option was selected for the measurements to complete and followed by the sample solution to measure the sample concentration.

Just after the sample concentration was measured, the reported values are displayed. **Absorbance at 260/280nm** UV light due to the aromatic base moieties within their structure. Purines (thymine, cytosine, and uracil) and pyrimidines (adenine and guanine) both have peak absorbance at 260 nm and 280 nm thus making them standard for quantitating nucleic acid sample. Therefore the samples are measured at **260/280nm** to acquire the purity level of the sample.

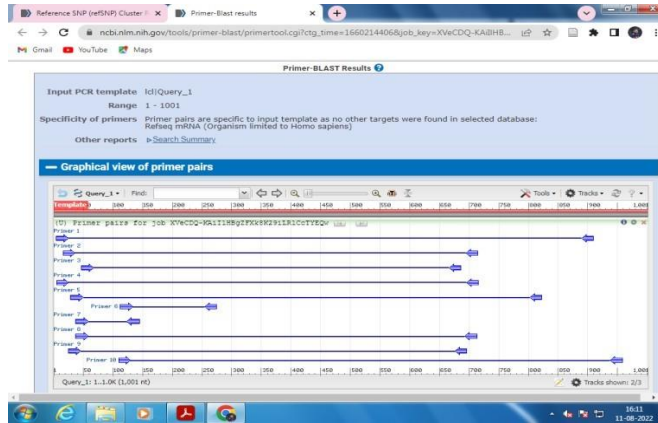
Nucleic Acid Type	Approximate at 260/280 ratio
Pure DNA	1.8
Pure RNA	2.0
Pure Proteins	0.57



Quantification of DNA using Nanodrop Spectrometer

Selection of restriction enzymes and designing of primers for PCR-RFLP

PCR-RFLP always starts with the designing of an optimum primer pair and finding the restriction enzymes that will identify the SNPs in the PCR-amplified product. The suitable forward and reverse primer is selected from primer blast and NCBI software. The suitable primer for SNP **rs731236** was designed by searching the **SNP ID** in NCBI and switched to classical site for downloading fasta sequence. After that downloaded sequence was copied and pasted in the primer blast software and suitable primers are selected based on **primer length, product size, base pair length etc.** Eventually the acceptable primers are selected for our PCR product



AATAGAAGGAGGGAAGCTGACGTGGTCTGGGCTACAGAGGTAGAGTGTGCCAGGAATGG
 CCTTTTGGAGGAAGACCTTTTAAGCTGTTATCCAAGGATCAGTAAGAGTCTGGCAAAGAT
 AGCAGAGCAGAGTTCCAAGCAGAGGGAGCACAGATGTGAAGGCTGGTGGCCAGAGAGCAT
 GGCGCATCGGGACGCTGAGGGATGGA**CAGAGCATGGACAGGGAGCAA**GGCCAGGCAGGG
 ACAGGGCCAGGTGCGCCCATGGAAGGACCTAGGTCTGGATCCTAAATGCACGGAGAAGTC
 ACTGGAGGGCTTTGGGGCCAGGCAGTGGTATCACCGGTCAGCAGTCATAGAGGGGTGGCCT
 AGGGGGTGTCTGCCGTTGAGTGTCTGTGTGGGTGGGGGGTGGTGGGATTGAGCAGTGAGGG
 GCCAGCTGAGAGCTCCTGTGCCTTCTTCTCTATCCCCGTGCCACAGATCGTCTGGGGTG
 CAGGACGCCGCGCTGAT**Y**GAGGCCATCCAGGACCGCCTGTCCAACACACTGCAGACGTAC
 ATCCGCTGCCGCCACCCGCCCCCGGGCAGCCACCTGCTCTATGCCAAGATGATCCAGAAGC
 TAGCCGACCTGCGCAGCCTCAATGAGGAGCACTCCAAGCAGTACCGCTGCCTCTCCTTCCA
 GCCTGAGTGCAGCATGAAGCTAACGCCCCTTGTGCTCGAAGTGTTTGGCAATGAGATCTCC
 TGACTAGGACAGCCTGTGGCGGTGCCTGGGTGGGGCTGCTCCTCCAGGGCCACGTGCCAGG
 CCCGGGGCTGGCGGCTACTCAGCAGCCCTCCTACCCCGTCTGGGGTTCAGCCCTCCTCTG
 CCACCTCCCCTATCCACCCAGCCATTCTCTCTCCTGTCCAACCTAACCCTTTCTGCGGGC
 TTTCCCCGGTCCCTT**GAGACCTCAGCCATGAGGAGTTGC**TGTTTGTGACAAAGAAACC
 AAGTGGGGGCAGAGGGCAGAGGCTGGA

SNP ID rs731236

Forward Primer -5'-CAG AGC ATG GAC AGG GAG CAA-3'

Reverse Primer- 5'- GCA ACT CCT CAT GGC TGA GGT CTC-3'

Reverse Complement- 5'-GAGACCTCAGCCATGAGGAGTTGC-3'

PCR Product size- 745bp

Restriction Enzyme - Taq I

5'.....T CG A..... 3'

3'.....A G C T..... 5'

Polymerase Chain Reaction- Restriction Fragement Length Polymorphisms (PCR-RFLP)

PCR-RFLP is based on endonuclease digestion of PCR-amplified DNA. The specific restriction endonuclease recognizes and cleaves the DNA in the region of the point mutation of the PCR product.

DNA Amplification using PCR

DNA amplification was carried out for four samples (**four reactions**). Four Samples were taken in microfuge tubes each with **1µl** of the solution. Consequently PCR **mastermix (dNTPs, MgCl₂, Buffer, Taq DNA Polymerase etc)** of **24µl** was added to the tubes followed by the forward (**4µl**) and reverse (**4µl**) primers designed using NCBI software. Then **24µl** of sterile water is added to the reaction mixture and subsequently ran in a Thermocycler for an hour.

The reaction that takes place in a thermocycler

Initial Denaturation	5min	95°C
Denaturation	0.5-1min	95°C
Annealing	0.5-1 min	55-65°C
Extension	1 min	72°C
Number of Cycles	25-30 min	
Final Extension	5 min	72°C

And almost continues for more than an hour till the resultant DNA was amplified and the desired product with product size was acquired.

Analysis of PCR products using Agarose Gel Electrophoresis (AGE)

After PCR was carried out **4µl** of the amplified DNA digested by the TaqI restriction enzyme was loaded to the **2%** agarose gel. In addition DNA marker of the 50bp ladder was also loaded parallel to the sample in a separate lane. The gel was run at 150 volts for 45 mins-1 hour with constant current and was

visualized using a UV transilluminator and the results recorded. By analyzing the cleaved fragments on the gel and the optimal DNA size marker the genotype was established.

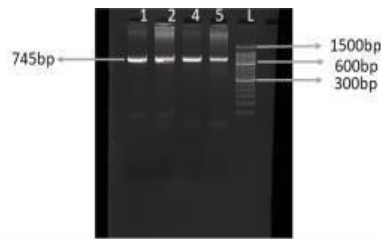


Figure 3 : Analysis of PCR products by agarose gel electrophoresis
 Lane 1 – 745bp
 Lane 2 – 745bp
 Lane 4 – 745bp
 Lane 5 – 745bp
 Lane L- Molecular weight marker (50bp Ladder)

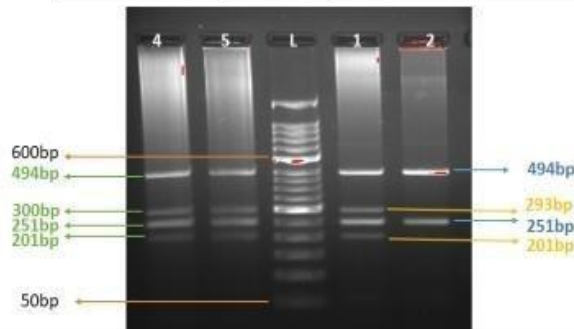


Figure 4: PCR-Restriction fragment length polymorphism digestion of (rs731236) using Taq I in 2% Agarose gel
 Lane 4- Genotype CT 494bp+293bp+251bp+201bp
 Lane3- Genotype CT 494bp+293bp+251bp+201bp
 Lane L- Molecular weight marker (50bp Ladder)
 Lane 2- Genotype CT 494bp+293bp+251bp+201bp
 Lane 1- Genotype TT 494bp+251bp

Analysis of PCR products by agarose gel electrophoresis

PCR-RFLP digestion of rs73136 using TaqI in 2% Agarose gel
 (Source: MDRF Chennai)

Cell Culture Techniques

The culture of animal cells and tissues is a generally and widely used technique that involves the isolation of cells, tissues, and organs from animals and growing them in an in vitro or artificial environment. The term culture means to keep cells alive and grow in appropriate medium that simulates the natural conditions. The cells are cultured from connective tissues such as fibroblasts, skeletal, cardiac, epithelial tissues neural cells, endocrine cells and many types of tumor cells.

Media Preparation (DMEM)

Dulbecco 'Modified Eagle' Medium (DMEM) is the commonly used medium for adherent cells subculture. The media is in a powdered form comprises of **glucose, sodium bicarbonate (3.7g/L), phenol and sodium pyruvate (110g/L)**. It was then added with **antibiotics (10ml)** to remove anti- bacterial, fungal and viral infectants present in the medium. The final volume is made upto **1L** using dissolved H₂O and the pH of the medium should be at **7.4**. Once the media was prepared it was subjected to filtration using media filterer. The filtered medium was then incubated at **37°C** overnight and checked for contamination.

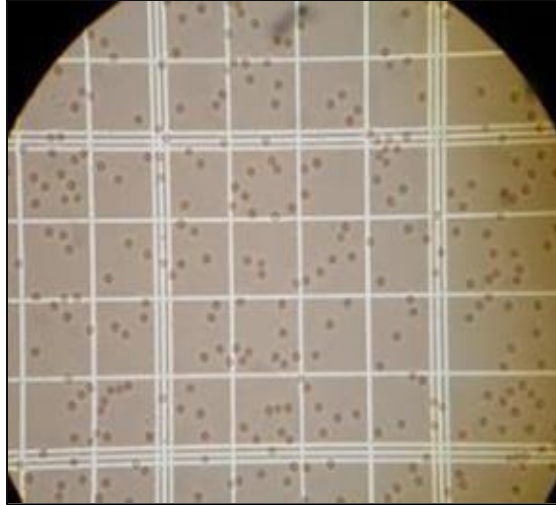
Subculture of adherent cell

Subculture begins with the discarding of old medium from the flask. The flask was washed with Serum free medium to remove the old media traces. Later Trypsination was done by addition of trypsin to stop enzymatic action and break the cell membrane as trypsin was toxic to cell lines. Incubation was done at **37°C** for about 2-3 mins and followed by addition of complete medium to reduce the toxicity of trypsin. Centrifugation was carried out for 5 minutes almost at 500-700 rpm and the supernatant was discarded as the cell lines were attached at the bottom of the falcon tube. Centrifuged cell lines were suspended in the prepared media at **1:3** ratio if the media is full in the culture flask and allowed to culture (increase in cell).

Cell Counting

The numbers of cells cultured in the plates were estimated using a Hemocytometer

$$\begin{aligned}\text{Concentration (cells)} &= \frac{\text{No of cells counted} \times \text{dilution factor}}{\text{No of squares count}} \\ &= 53 \times 1.5 \times 10^4 \\ &= 8.05 \times 10^6 \text{ cells/ml}\end{aligned}$$



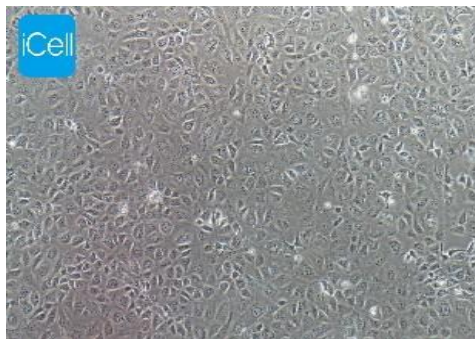
Cell counting using Hemocytometer
(Source: MDRF, Chennai)

Cell Viability

Cell viability refers to the amount of cells that are alive or dead in a cell culture. Two reagents erythrosin B and tryphan blue were used to verify cell viability. Both tryphan blue and Erythrosin B stains are actively excluded by the viable cells but are taken up and retained by dead cells which lack an intact membrane.

Cell viability formula

$$\frac{\text{No of viable (unstained cells)} \times 100}{\text{Total number of cells}}$$



Subculture of Normal Rat Kidney (NRK) - epithelial cells
(Source: MDRF, Chennai)

Isolation of RNA by Trizol method

DAY -1

Homogenisation

The growth media was removed from the cell culture and **1ml** of Trizol reagent per 2.5×10^6 cells are added to lyse the cells. Incubation was done for 5 minutes at room temperature followed by addition of **400µl** of chloroform which helps in phase separation. One more incubation was done for 15 mins at room temperature. Centrifugation was done for 15 minutes at 12000xg (**rcf-rotational centrifugal force**) at **4°C** and the mixture appears to a lower red interphase and a colourless upper phase. The aqueous phase containing RNA was transferred to a new tube by pipetting the solution out. Then RNA was precipitated by addition of **0.5ml** isopropanol and incubated overnight at **-20°C**.

DAY-2

The sample was thawed on ice for 10 minutes and centrifuged for 20 minutes at 12000xg at 4°C. Total RNA precipitate was formed like a white gel-like pellet at the bottom of the tube. The supernatant was discarded using a micropipette. The pellet was resuspended in 1ml of 75% ethanol and centrifuged for 5 minutes at 7500xg at 4°C. The supernatant was discarded once more and the pellet was resuspended in RNase free water. Incubation was done using water bath at 55-60°C for 15 minutes and proceeded to downstream applications or stored in -80°C for further use.

Quantification of RNA by Nanodrop Method

Nanodrop spectrophotometer was switched on and RNA option was selected in the Nanodrop software. 1µl of blanking RNase free water solution was pipetted on the lower pedestal and BLANK option was tapped. After blank measurements are done both pedestals are cleaned and 1µl of sample solution was pipetted into the lower pedestal and MEASURE option was tapped. When the sample measurements have completed the spectrum and reported values are displayed. The **260/280** ratio shows the purity of the sample RNA



Quantification of RNA by Nanodrop Method
(Source: MDRF, Chennai)

Complementary DNA (cDNA) Synthesis

Complementary DNA (cDNA) is synthesized from a single-stranded RNA (eg Mrna OR miRNA) template in a reaction catalyzed by enzyme reverse transcriptase. cDNA is often used to clone eukaryotic genes in prokaryotes. The steps involved in synthesis of cDNA are,

Steps	Temperature	Time
Primers	25°C	10 minutes
Reverse transcription	42°C	50 minutes
Enzyme inactivation	70°C	15 minutes

RNA Template Preparation

RNA templates are prepared using 5x reaction buffer (4 μ l), 2.5mmol dNTPs (1.6 μ l), 1x RT Primer (1 μ l), 100U AMV-Reverse transcription (0.5 μ l), RNA (2 μ l) and RNase free H₂O. Conditions to be maintained as,

Incubation	42°C for 60 minutes
RT-Inactivation	70°C for 5 minutes

The genomic DNA was removed thereby isolation of m-RNA by trizol method and further RNA template was prepared for above condition. Additionally the prepared RNA template undergoes PCR

Steps	Time and Temperature
Initial Denaturation	95°C for 5 minutes
Denaturation	95°C for 1minutes
Annealing	58°C for 1 minutes
Extension	72°C for 2 minutes
Final extension	72°C for 3 minutes

These processes are continuously carried out for 30-40cycles

Quantification of cDNA by RT-PCR Technique

Fluorescence based Real-Time reverse transcriptase polymerase Chain Reaction (RT-PCR) is one of the key technologies in the genomic era, making it an ideal method for mRNA detection. The progress of DNA amplification during the PCR can be monitored in the “real time” by measuring the mission of fluorescent “flash” during the amplification. The reaction rate can be measured continuously or determined at a point in time during the exponential amplification phase. Reverse transcriptase converts RNA to cDNA and cDNA amplification is done by PCR, a Real - Time calculation of multiple changes in amplification products. The reagents are thawed and the mastermix was mixed by swirling the tube. The number of reactions required for each assay was calculated based on sample numbers. The PCR reaction mix was prepared and mixed in appropriate volumes to the wells of the PCR plate followed by deionized water, primers and cDNA. Reaction mix was added to the 96-well microtiter

plate and sealed with PCR film and Sealer. Subsequently the plates were placed in a Real - Time thermocycler and the conditions suitable for RT-PCR are set to begin the process.

Process Involved in RT-PCR

Cycling Conditions	Time and Temperature
Preincubation	95°C for 120 seconds
2 Step Amplification	60°C for 60 seconds
Melting	95°C for 1 second
Cooling	50°C for 30 seconds

The following steps are carried out for 2 hours with more than 30-40 cycles

Formula for Calculating Gene Expression

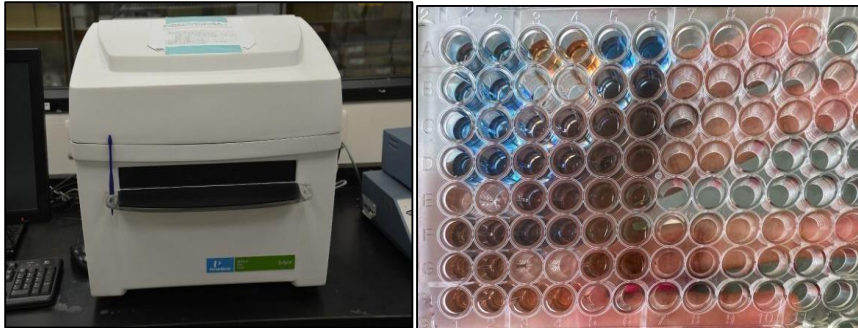
1. $\Delta Ct = Ct (\text{gene of interest}) - Ct (\text{housekeeping gene})$
2. $\Delta\Delta Ct = \Delta Ct (\text{treated sample}) - \Delta Ct (\text{untreated sample})$
3. Fold gene expression = $2^{- (\Delta\Delta Ct)}$

Extraction and Estimation of Proteins

Protein extraction is the process of isolating proteins from samples of cell cultures, whole tissues or biological fluids. Protein can be extracted through various methods such as physical, chemical and mechanical methods. If the intracellular proteins are required chemical reagents such as detergents can be used to break the phospholipids cellular membrane. Cell lysate is a process of homogenizing the cell cultures to extract the desired proteins. The cell culture was washed with cold Phosphate Buffer Saline to maintain osmotic pressure of the cells followed by **Radioimmunosensitivity Assay (RIPA)** buffer (100 μ l) for rapid cell lysis and stabilization of proteins. Subsequently 80 μ l of protease inhibitor was added to the culture as protease inhibitors prevent degradation of proteins and to obtain best possible protein yield. Cell scraper was used to dislodge the cells and further lysis was done using sonicator at **8Hz**. Centrifugation was done at 12250 rcf at 4°C for 20 minutes and the supernatant was removed and stored at 20°C.

Estimation of protein using Bradford's reagent

Bradford assay, a calorimetric protein assay which is based on absorbance shift in the Comassive dye when the previously red form comassive reagent changed and stabilized into comassive blue by binding of proteins. Already extracted samples were taken from -20°C and $10\mu\text{l}$ of samples added in wells and thawed before use. Subsequently $490\mu\text{l}$ of Bradford's reagent was added to the samples and mixed using vortex mixer for few minutes. Comassive dye colour change was observed from red to blue which confirms the presence of proteins and the Samples were read at 590nm using MMR. Concentration of the protein samples were analyzed using Calibration curve.



Estimation of protein using Bradford-method
(Source: MDRF, Chennai)

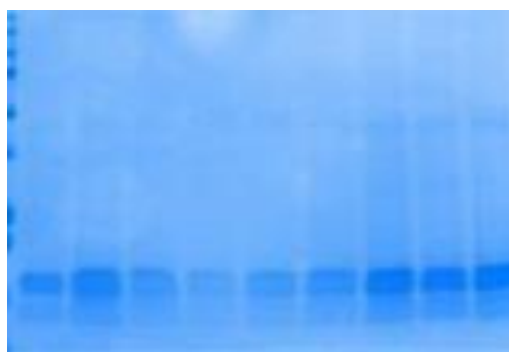
ANALYSIS OF PROTEIN EXPRESSIONS USING WESTERN BLOT

Protein Separation using SDS-PAGE

SDS-PAGE is an extensive method used for the separation of proteins from the given samples using poly acrylamide gel in which proteins are separated based on their molecular weight as polymerization occurs between the acrylamide and bisacrylamide monomers and forms the two gels used for separation (stacking and separating gel). Ammonium persulfate and TEMED used for gel preparation assists in polymerization. Both 5% stacking (Ph-6.8) and separating gel (Ph-8.8) were prepared to run the samples with protein. Principally lowest % of gel was prepared to separate high molecular weight proteins and the change in pH in gel preparation was for large and small pore formation in gels which separates the proteins based on their molecular weight. Sample was prepared according to required concentration (ie. 40ng, 30ng and 35ng) and Sample Solubilizing Buffer (SSB) buffer was used to make equal volumes of all samples. (i.e. if sample volume- $4\mu\text{l}$ and $1\mu\text{l}$ of SSB buffer). Subsequently centrifugation was done for few minutes at 550 rcf and boiled at 90°C for 10 minutes to break the peptide bonds. Further centrifugation was carried out at 550rcf for 5 minutes to eliminate bubbles and run at electrophoresis tank at 50V. The gel begins to run from positive to negative charges as we induce negative charge to proteins using SDS. Once the sample progress to separating gel the voltage was increased to 55V and allowed to run and followed by preparation of transfer buffer and methanol addition (changes membrane from hydrophobic to hydrophilic) for membrane transfer.

Membrane Transfer

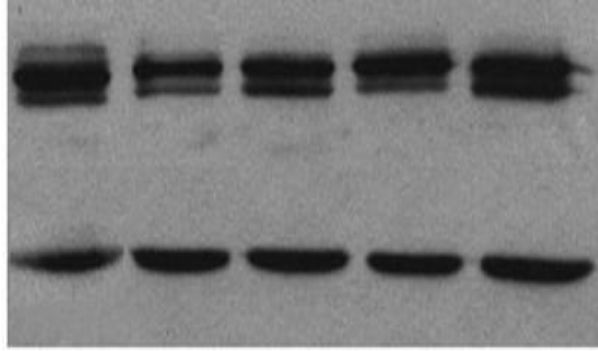
Membrane was submerged in transfer buffer for few minutes and the gel ran through SDS-PAGE was collected and cut at specific site according to the required protein's molecular weight. The sliced gel portion was placed in the negative side and placed on a transfer tank and membrane transfer was carried out overnight at 50V. The membrane transfer was achieved through wet transfer.



Wet Membrane transfer
(Source: MDRF, Chennai)

Blocking of antibodies and protein analysis using Western Blot

After membrane transfer the transferred membrane was washed using **wash buffer** (Tris HCl+H₂O) for 3 times orderly to remove the unbound antibodies present in the membrane so that antibodies bound to the desired protein remain intact. Subsequently Blocking was performed using **5% Bovine Serum Albumin (BSA)** and washed using **Tris Buffered saline (TBST)** with 0.1% Tween detergent three times. Later primary antibody (1:1000) 5 μ l was added to the membrane and allowed for TBST wash for three times continuously. Then secondary antibody was added and washed several times, followed by TBST wash for three times. Eventually Chemiluminescence stuff (luminon), substrate and water were made upto volume of 500 μ l and added to the membrane followed by analysis using UV transilluminator. Protein expressions among the samples were analyzed using the image formed by the transilluminator and the relation between them was analyzed. Expressions of the proteins **FN - 220kDa**, **CTH - 45kDa**, and **CBS - 63kDa** were analyzed through the following image.



Protein expressions of FN, CTH and CBS proteins in the samples
(The denser bands represent presence of elevated protein levels)
(Source: MDRF, Chennai)

Discussion

This report is a humble effort of elucidating the basic concepts of molecular techniques that can be effectively applied to understand the expanding molecular pathogenesis of diabetes and its complications.

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I feel thankful to got selected by the ‘**INDIAN ACADEMY OF SCIENCES’ SUMMER RESEARCH FELLOWSHIP PROGRAM (SRFP-2022)** and pursue my research in Diabetology at **MADRAS DIABETES RESEARCH FOUNDATION (MDRF)** Chennai. I am beholden to the Indian Academy of Sciences for giving me this opportunity and surely this has been a great learning experience for me to work in such a renowned institute.

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Program Name: Chemical Engineering

Program Code: 203

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S.No	Register No	Name of the Student	Internship	Internship Duration
1	112720203001	BALAJI S	CIPET	27-07-2023 - 28-07-2023
2	112720203002	JAGADESH M	CIPET	27-07-2023 - 28-07-2023
3	112720203004	LOKESH KUMARAN M N	CIPET	27-07-2023 - 28-07-2023
4	112720203006	PAVITHRA C	CIPET	27-07-2023 - 28-07-2023
5	112720203008	SWETHA E	CIPET	27-07-2023 - 28-07-2023
6	112720203009	YOGESHWARAN R S	CIPET	27-07-2023 - 28-07-2023

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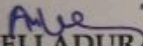
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During the Internship training period, the performance of the trainee was found good.


VELLADURAI A.
Head of the Department
CAD/CAM/CAE Centre


RAVICHANDRAN. A
Head of the Department
Vocational Training Centre



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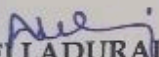
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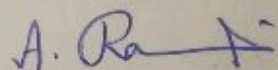
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Vocational Training Centre



मुख्यालय : सिपेट, गिण्डी, चेन्नै - 600 032. **Head Office :** CIPET, Guindy, Chennai - 600 032.

केन्द्र : अहमदाबाद, अमृतसर, औरंगाबाद, अगरतला, बर्दी, बालासोर, बेंगलुरु, भोपाल, भुवनेश्वर, चन्द्रपुर, चेन्नई, देहरादून, गुरुग्राम, गुवाहाटी, ग्वालियर, हैदराबाद, हाजीपुर, हल्दिया, इम्फाल, जयपुर, कोच्चि, कोरबा, लखनऊ, मदुरै, मुरथल, मैसूरु, रायपुर, राँची, वलसाड, वाराणसी एवं विजयवाडा
Centres : Ahmedabad, Amritsar, Aurangabad, Agartala, Baddi, Balasore, Bengaluru, Bhopal, Bhubaneswar, Chandrapur, Chennai, Dehradun, Gurugram, Guwahati, Gwalior, Hyderabad, Hajipur, Haldia, Imphal, Jaipur, Kochi, Korba, Lucknow, Madurai, Murthal, Mysuru, Raipur, Ranchi, Valsad, Varanasi & Vijayawada

**सिपेट : पेट्रोकेमिकल्स तकनीकी
संस्थान (आईपीटी)**

रसायन एवं पेट्रोसायन विभाग
रसायन एवं उर्वरक मंत्रालय, भारत सरकार
गिण्डी, चेन्नै - 600 032.
फोन : 91-44-2225 4701 (6 लाइन)
फैक्स : 91-44-22254707
ई-मेल : chennai@cipet.gov.in
वेबसाइट : www.cipet.gov.in



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**CIPET : INSTITUTE OF PETROCHEMICALS
TECHNOLOGY (IPT)**

Department of Chemicals & Petrochemicals
Ministry of Chemicals & Fertilizers, Govt. of India
Guindy, Chennai - 600 032.
Phone : 91-44-2225 4701 (6 Lines)
Fax : 91 - 44 - 22254707
E-mail : chennai@cipet.gov.in
Website : www.cipet.gov.in

CIPET/CHN/VTC/INTERNSHIP TRG/2022-23/302

28.07.2022

INTERNSHIP CERTIFICATE

This is to certify that **Ms. PAVITHRA.,** D/o. Mr. Chandran S., student of B.Tech (Chemical Engineering) from St. Peter's College of Engineering and Technology, Chennai has completed the two day "INTERNSHIP" from 27.07.2022 to 28.07.2022 at Central Institute of Petrochemicals Engineering & Technology, Guindy, Chennai 600 032.

During the Internship training period, the performance of the trainee was found good.

VELLADURAI A.
Head of the Department
CAD/CAM/CAE Centre

RAVICHANDRAN. A
Head of the Department
Vocational Training Centre



मुख्यालय : सिपेट, गिण्डी, चेन्नै - 600 032. **Head Office :** CIPET, Guindy, Chennai - 600 032.

केन्द्र : अहमदाबाद, अमृतसर, औरंगाबाद, अगरतला, बदी, बालासोर, बेंगलुरु, भोपाल, भुवनेश्वर, चन्द्रपुर, चेन्नई, देहरादून, गुरुग्राम, गुवाहाटी, ग्वालियर, हैदराबाद, हाजीपुर, हल्दिया, इम्फाल, जयपुर, कोच्चि, कोरबा, लखनऊ, मदुरै, मुरथल, मैसूर, रायपुर, राँची, वलसाड, वाराणसी एवं विजयवाडा

Centres : Ahmedabad, Amritsar, Aurangabad, Agartala, Baddi, Balasore, Bengaluru, Bhopal, Bhubaneswar, Chandrapur, Chennai, Dehradun, Gurugram, Guwahati, Gwalior, Hyderabad, Hajipur, Haldia, Imphal, Jaipur, Kochi, Korba, Lucknow, Madurai, Murthal, Mysuru, Raipur, Ranchi, Valsad, Varanasi & Vijayawada

सिपेट : पेट्रोकेमिकल्स तकनीकी
संस्थान (आईपीटी)

रसायन एवं पेट्रोसायन विभाग
रसायन एवं उर्वरक मंत्रालय, भारत सरकार
गिण्डी, चेन्नै - 600 032.
फोन : 91-44-2225 4701 (6 लाइन)
फैक्स : 91-44-22254707
ई-मेल : chennai@cipet.gov.in
वेबसाइट : www.cipet.gov.in



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CIPET : INSTITUTE OF PETROCHEMICALS
TECHNOLOGY (IPT)

Department of Chemicals & Petrochemicals
Ministry of Chemicals & Fertilizers, Govt. of India
Guindy, Chennai - 600 032.
Phone : 91-44-2225 4701 (6 Lines)
Fax : 91 - 44 - 22254707
E-mail : chennai@cipet.gov.in
Website : www.cipet.gov.in




CIPET/CHN/VTC/INTERNSHIP TRG/2022-23/301


28.07.2022

INTERNSHIP CERTIFICATE

This is to certify that Mr. YOGESHWARAN R.S., S/o. Mr. Ramesh D., student of B.Tech (Chemical Engineering) from St. Peter's College of Engineering and Technology, Chennai has completed the two day "INTERNSHIP" from 27.07.2022 to 28.07.2022 at Central Institute of Petrochemicals Engineering & Technology, Guindy, Chennai 600 032.

During the Internship training period, the performance of the trainee was found good.


VELLADURAI A.
Head of the Department
CAD/CAM/CAE Centre


RAVICHANDRAN. A
Head of the Department
Vocational Training Centre



मुख्यालय : सिपेट, गिण्डी, चेन्नै - 600 032. Head Office : CIPET, Guindy, Chennai - 600 032.

केन्द्र : अहमदाबाद, अमृतसर, औरंगाबाद, अगर्तला, बदी, बालासोर, बेंगलुरु, भोपाल, भुवनेश्वर, चन्द्रपुर, चेन्नई, देहरादून, गुरुग्राम, गुवाहाटी, ग्वालियर, हैदराबाद, हाजीपुर, हल्दिया, इम्फाल, जयपुर, कोच्चि, कोरबा, लखनऊ, मदुरै, मुरथल, मैसूरु, रायपुर, राँची, वलसाड, वाराणसी एवं विजयवाड़ा
Centres : Ahmedabad, Amritsar, Aurangabad, Agartala, Baddi, Balasore, Bengaluru, Bhopal, Bhubaneswar, Chandrapur, Chennai, Dehradun, Gurugram, Guwahati, Gwalior, Hyderabad, Hajipur, Haldia, Imphal, Jaipur, Kochi, Korba, Lucknow, Madurai, Murthal, Mysuru, Raipur, Ranchi, Valsad, Varanasi & Vijayawada



St. PETER'S

COLLEGE OF ENGINEERING & TECHNOLOGY

Affiliated to Anna University | Approved by AICTE | ISO 9001:2015 Certified | NAAC with 'A' Grade
Avadi, Chennai, Tamilnadu – 600 054

List of Students Undertaking Internships during the Academic Year 2022-2023

Program Name: Civil Engineering

Program Code: 103

INTERNSHIP DETAILS (2022 – 2023)

S.NO	Register Number	Name of the Student	Name of the Company	Duration
1	112719103001	Hamshawarthini M.	JPS Construction	12.07.2022 – 09.08.2022
2	112719103002	Hepsiba T.	JPS Construction	12.07.2022 – 09.08.2022
3	112719103003	Krishna Kumar P.	Kula Aqua Consultant Pvt. Ltd.	12.07.2022 – 09.08.2022
4	112719103005	Sathish. R	Kula Aqua Consultant Pvt. Ltd.	12.07.2022– 09.08.2022
5	112719103006	Sweatha A.	JPS Construction	12.07.2022 – 09.08.2022
6	112719103007	Vikey A.	JPS Construction	12.07.2022 – 09.08.2022
7	112719103301	Gokulan P.	Babu & Associates	23.07.2022- 14.08.2022

Head of the Department

PRINCIPAL

JPS CONSTRUCTION

No.1391, Thaila Nagar, Matchuvadi - Post,
PUDUKKOTTAI - 622 004.

☎ : 04322-270660 Cell : 94433 81336, 98424 28133
e.mail : jpsconstruction2021@gmail.com

Date :

HIGHWAYS DEPARTMENT
Construction & Maintenance Wing,
Thiruvallur Division.

CERTIFICATE OF INTERSHIP TRAINING

This is Certified that **Ms.M.HAMSHAWARTHINI**, Roll No.112719103001 studying B.E., (Civil Engineering) in St. Peter's College of Engineering & Technology, Avadi, Chennai - 600 054, has participated and completed the Inplant Training for the period from **12.07.2022 to 09.08.2022.**

She was kept trained under the CMRDP work of "Widening from Two Lane to Four Lane and Improvements at km 26/7 - 30/9 of Walajabad - Sungawarchatiram - Keelacherry Road including Construction of Storm Water Drain at Km.26/8 - 27/4 (L/R), Km.30/4 - 30/8 (L/s) & 30/4 - 30/9 (R/s), Widening of RCC Box Culvert at Km.26/10, 27/10 (i) & (ii), 28/2, 28/4 (i) & (ii), 28/8 (i) & (ii), 28/10 (i) & (ii), 29/8 & 30/6 and Reconstruction of RCC Box Culverts at Km.29/4".

She was placed in various Civil Engineering works associated with the above work. During this training period, her participation and activities are **good.**

For JPS CONSTRUCTION


Managing Partner



GSTN : 33AAQFJ0289
PAN : AAQFJ0289E

JPS CONSTRUCTION

No.1391, Thaila Nagar, Matchuvadi - Post,
PUDUKKOTTAI - 622 004.

☎ : 04322-270660 Cell : 94433 81336, 98424 28133
e.mail : jpsconstruction2021@gmail.com

Date :

HIGHWAYS DEPARTMENT
Construction & Maintenance Wing,
Thiruvallur Division.

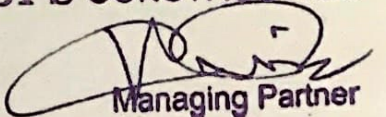
CERTIFICATE OF INTERSHIP TRAINING

This is Certified that **Ms.T. HEPSIBA**, Roll No.112719103002 No.112719103001 studying B.E., (Civil Engineering) in St. Peter's College of Engineering & Technology, Avadi, Chennai - 600 054, has participated and completed the Inplant Training for the period from **12.07.2022 to 09.08.2022**.

She was kept trained under the CMRDP work of "Widening from Two Lane to Four Lane and Improvements at km 26/7 - 30/9 of Walajabad - Sungawarchatiram - Keelacherry Road including Construction of Storm Water Drain at Km.26/8 - 27/4 (L/R), Km.30/4 - 30/8 (L/s) & 30/4 - 30/9 (R/s), Widening of RCC Box Culvert at Km.26/10, 27/10 (i) & (ii), 28/2, 28/4 (i) & (ii), 28/8 (i) & (ii), 28/10 (i) & (ii), 29/8 & 30/6 and Reconstruction of RCC Box Culverts at Km.29/4".

She was placed in various Civil Engineering works associated with the above work. During this training period, her participation and activities are **good**.

For JPS CONSTRUCTION


Managing Partner

Kula Aqua Consultant Pvt Ltd

No :181, M.G.Y. Babu Street, Janaki Nagar, Valasaravakkam, Chennai- 600 087, India
Tel. No. 044 4863 7077 Mobile: +9190032 83535 www.kulaaqua.in Email : kulaaqua@gmail.com

Date: 18.11.2022

Certificate

To whomsoever it may concern, this is to certify that **P.Krishna Kumar (112719103003)** of St.Peter's college of engineering and technology has undergone training in our concern during 12-07-2022 to 09-08-2022. He has been exposed to various construction materials and tests and Basics of AutoCad software in this period. He has been found sincere and dedicated in the period of training. We wish him success in all his future endeavour.

For Kula Aqua Consultant Pvt. Ltd.,



Mr. R. Kulasekaran
(Director)



Kula Aqua Consultant Pvt Ltd

CIN: U45309TN2017PTC118814
GST: 33AAGCK7807E1ZO

No :181, M.G.Y. Babu Street, Janaki Nagar, Valasaravakkam, Chennai- 600 087, India
Tel. No. 044 4863 7077 Mobile: +9190032 83535 www.kulaaqua.in Email : kulaaqua@gmail.com

Date: 17.11.2022

Certificate

To whomsoever it may concern, this is to certify that **R.Sathish (112719103005)** of St.Peter's college of engineering and technology has undergone training in our concern during 12-07-2022 to 09-08-2022. He has been exposed to various construction materials and tests and Basics of AutoCad software in this period. He has been found sincere and dedicated in the period of training. We wish him success in all his future endeavour.

For Kula Aqua Consultant Pvt. Ltd.,



[Handwritten Signature]
Mr. R. Kulasekaran
(Director)



GSTN : 33AAQFJ0289
PAN : AAQFJ0289E

JPS CONSTRUCTION

No.1391, Thaila Nagar, Matchuvadi - Post,
PUDUKKOTTAI - 622 004.

☎ : 04322-270660 Cell : 94433 81336, 98424 28133
e.mail : jpsconstruction2021@gmail.com

HIGHWAYS DEPARTMENT
Construction & Maintenance Wing,
Thiruvallur Division.

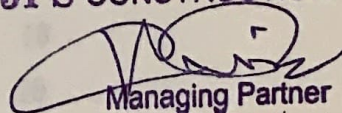
Date :

CERTIFICATE OF INTERSHIP TRAINING

This is Certified that **Ms.A. SWEATHA**, Roll No.112719103006 No.112719103001 studying B.E., (Civil Engineering) in St. Peter's College of Engineering & Technology, Avadi, Chennai - 600 054, has participated and completed the Inplant Training for the period from **12.07.2022 to 09.08.2022**.

She was kept trained under the CMRDP work of "Widening from Two Lane to Four Lane and Improvements at km 26/7 - 30/9 of Walajabad - Sungawarchatiram - Keelacherry Road including Construction of Storm Water Drain at Km.26/8 - 27/4 (L/R), Km.30/4 - 30/8 (L/s) & 30/4 - 30/9 (R/s), Widening of RCC Box Culvert at Km.26/10, 27/10 (i) & (ii), 28/2, 28/4 (i) & (ii), 28/8 (i) & (ii), 28/10 (i) & (ii), 29/8 & 30/6 and Reconstruction of RCC Box Culverts at Km.29/4".

She was placed in various Civil Engineering works associated with the above work. During this training period, her participation and activities are **good**.

For **JPS CONSTRUCTION**

Managing Partner



GSTN : 33AAQFJ0289E
PAN : AAQFJ0289E

JPS CONSTRUCTION

No.1391, Thaila Nagar, Matchuvadi - Post,
PUDUKKOTTAI - 622 004.

☎ : 04322-270660 Cell : 94433 81336, 98424 28133
e.mail : jpsconstruction2021@gmail.com

Date :

HIGHWAYS DEPARTMENT
Construction & Maintenance Wing,
Thiruvallur Division.

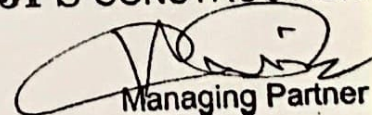
CERTIFICATE OF INTERSHIP TRAINING

This is Certified that **Mr.A. VIKEY**, Roll No. 112719103007 studying B.E., (Civil Engineering) in St. Peter's College of Engineering & Technology, Avadi, Chennai - 600 054, has participated and completed the Inplant Training for the period from **12.07.2022 to 09.08.2022**.

He was kept trained under the CMRDP work of "Widening from Two Lane to Four Lane and Improvements at km 26/7 - 30/9 of Walajabad - Sungawarchatiram - Keelacherry Road including Construction of Storm Water Drain at Km.26/8 - 27/4 (L/R), Km.30/4 - 30/8 (L/s) & 30/4 - 30/9 (R/s), Widening of RCC Box Culvert at Km.26/10, 27/10 (i) & (ii), 28/2, 28/4 (i) & (ii), 28/8 (i) & (ii), 28/10 (i) & (ii), 29/8 & 30/6 and Reconstruction of RCC Box Culverts at Km.29/4".

He was placed in various Civil Engineering works associated with the above work. During this training period, his participation and activities are **good**.

For **JPS CONSTRUCTION**


Managing Partner

No. 113/3, New Market Street,
Choolaimedu High Road, Chennai - 94,
Email : bpluserchitects@gmail.com

Dated: 23.08.2022

Certificate

To Whomsoever it may concern, this is to certify that P. Gokulan(112719103301) of Peter's college of Engineering and technology has undergone training in our concern during 23.07.2022 to 14.08.2022. He has been exposed to various construction materials and tests in this period. He has been found sincere and dedicated in the period of training. We wish him success in all his future endeavors.

Truly yours

V. Babu
for Babu & Associates



V. Babu
Architect

V. BABU, M. Arch.
CA 2012/48200
No. 1133, New Market Street,
Choolaimedu High Road, Chennai - 94



St. PETER'S COLLEGE OF ENGINEERING AND TECHNOLOGY

(Approved by AICTE New Delhi, Affiliated to Anna University, Chennai,
Accredited by NAAC with 'A' Grade and ISO 9001:2015 Certified Institution)
College Road, Avadi, Chennai - 600 054.

Phone 044-26558091 26558092 Website www.spcet.ac.in e-mail spcet2008@gmail.com

Dr.K. Purushothaman, M.E., Ph.D
Principal

Date: 04.06.2022

To

The General Manager
CodeBind Technologies
107, N Usman Rd,
Postal Colony, T. Nagar,
Chennai, Tamil Nadu 600017

Dear Sir,

Warm Greetings from St. Peter's College of Engineering and Technology, Chennai.

St. Peter's College of Engineering and Technology is managed by Lakshmi Saraswathi Education Trust .It was started in the year 2008. The College is affiliated to Anna University of Technology, Chennai and approved by the All India Council for Technical Education (AICTE), New Delhi. The Department of Electronics and Communication was established in the year 2008-2009 with the basic objective of providing a quality learning environment for the students with qualified teachers, state-of-the art facilities in the field of Electronics and Communication Engineering.

The college aims to develop in to a top class professional institution in the years to come thus providing training to students to take up challenging assignments and contribute to national and international development. We have been always imparting a high standard of practical training so that students have to undergo Internship Training, which enable them to familiarize with the industrial environment. We request you to kindly accord permission to Christo Jeniston J (112720106001), ECE student to undergo internship training.

Our student shall obey all your company rules and regulations and shall maintain the highest order of discipline during the time period

We look for your kind permission for the above internship training to our student.

Thanking you,




PRINCIPAL
St. Peter's College of Engineering & Technology
Avadi, Chennai-600 054.

Yours Sincerely,

PRINCIPAL



St. PETER'S COLLEGE OF ENGINEERING AND TECHNOLOGY :: CHENNAI

(Approved by AICTE, New Delhi, Affiliated to Anna University, Chennai.
Accredited by NAAC with 'A' Grade and ISO 9001:2015 Certified Institution)

List of students undertaking internships during the Academic Year 2021-2022

Program Name: Electronics and Communication Engineering Program Code: 106

INTERNSHIP DETAILS (2021-2022)

S. No.	Register Number	Name of the Student	Name of the Company	Duration
1	112720106001	J.CHRISTO JENISTON	Codebind Technologies	04.08.22-08.08.22


PRINCIPAL




PRINCIPAL
St. Peter's College of Engineering & Technology
Avadi, Chennai-600 054.

<codeblind/>TM

{Technologies}

Solution Without Compromise!



CERTIFICATE OF COMPLETION

CERTIFICATE NUMBER

CERTIF042005220801

This certificate is awarded to J. CHRISTO TENISTAN,
 who has **undergone Implant** Training in EMBEDDED SYSTEMS,
 from 24.08.2022 to 08.08.2022 at **CodeBind Technologies**,
 Chennai. During the course of training period, the conduct of the
 trainee was found to be Good.



Training Facilitator

ASLH

Issuing Authority

[Signature]

Head Office - Nagercoil, Chennai

Branch Office -

Coimbatore

Tiruvly

044 - 43304239



PRINCIPAL

[Signature]
 St. Peter's College of Engineering & Technology
 Avadi, Chennai-600 054.



St. PETER'S COLLEGE OF ENGINEERING AND TECHNOLOGY :: CHENNAI

(Approved by AICTE, New Delhi, Affiliated to Anna University, Chennai.
Accredited by NAAC with 'A' Grade and ISO 9001:2015 Certified Institution)

List of Students undertaking internships during the Academic Year 2022-2023

Program Name: Electrical and Electronics Engineering

Program Code: 105

INTERNSHIP DETAILS (2022 – 2023)

S.NO	Register Number	Name of the Student	Name of the Company	Duration
1.	112719105002	MALINI.M	JOY TECHNOLOGY	09.1.23 – 07.4.23
2.	112719105007	UMAPATHY.B	JOY TECHNOLOGY	09.1.23 – 07.4.23
3.	112719105006	SHAKTHIVEL.S.M	UNIQ TECHNOLOGY	25.1.23 - 31.3.23
4.	112719105003	MOHAMMED HUSSAIN M.R	PLASMIC HEATRODS	06.2.23 - 22.2.23
5.	112719105701	MOHAMMED MUZZAMIL.I	PLASMIC HEATRODS	06.2.23 - 22.2.23

Head of the Department

PRINCIPAL



St. PETER'S COLLEGE OF ENGINEERING AND TECHNOLOGY

(Approved by AICTE, New Delhi, Affiliated to Anna University, Chennai,
Accredited by NAAC with 'A' Grade and ISO 9001:2015 Certified Institution)
College Road, Avadi, Chennai - 600 054.

Phone 044-26558091, 26558092

Website www.spcet.ac.in

e-mail : spcet2008@gmail.com

Date: 02.12.2022

To
The HR Manager,
Joy Technologies,
No:2/88 Seevaram Village,
OMR Perungudi,
Chennai, Tamil Nadu 600096

Dear Sir,

Warm Greetings from St.Peter's College of Engineering and Technology,
Chennai.

St.Peter's College of Engineering and Technology is managed by Lakshmi Saraswathi Educational Trust. It was started in the year 2008. The college is affiliated to Anna University of Technology Chennai and approved by the All India Council for Technical Education (AICTE), New Delhi.

The college aims to develop in to a top class professional institution in the years to come thus providing training to students to take up challenging assignments and contribute to national and international development. We have been always imparting a high standard of practical training so that students have to undergo **Internship Training**, which enable them to familiarize with the industrial environment. We request you to kindly accord permission to the following students of Electrical and Electronics Engineering Department to undergo internship training.

1. Ms.M.Malini (112719105002)
2. Mr.B.Umapathy(112719105007)

Our students shall obey all your company rules and regulations and shall maintain the highest order of discipline during the training period.

We look for your kind permission for the above internship training to our students.

Thanking You,

Yours Faithfully,

PRINCIPAL

April 07, 2023

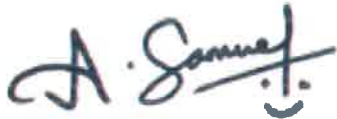
TO WHOM IT MAY CONCERN

We certify that **Malini M** has successfully completed our **Digital Marketing Internship** program from **Jan 09, 2023 to April 07, 2023**.

During this time, She performed tasks around digital marketing including but not limited to social media content creation and email campaigning.

She displayed professional traits during his internship period and managed to complete all assigned tasks as requested. She was hardworking, dedicated and committed.

We wish her all the best for his future endeavors.



Authorized Signatory
Samuel Darwin
Chief Executive Officer

21-April-2023

To Whom It May Concern

We certify that **Umopathy B** has successfully completed our digital marketing internship program from January 09, 2023 to Apr 07, 2023.

During this time, he performed tasks around digital marketing including but not limited to social media content creation and email campaigning.

He displayed professional traits during his internship period and managed to complete all assigned tasks as requested. He was hardworking, dedicated and committed.

We wish him all the best for his future endeavors.



Authorized Signatory
Samuel Darwin
Chief Executive Officer



St. PETER'S COLLEGE OF ENGINEERING AND TECHNOLOGY

(Approved by AICTE, New Delhi, Affiliated to Anna University, Chennai,
Accredited by NAAC with 'A' Grade and ISO 9001:2015 Certified Institution)
College Road, Avadi, Chennai - 600 054.

Phone 044-26558091, 26558092 Website : www.spcet.ac.in e-mail : spcet2008@gmail.com

Date: 09.01.2023

To
The HR Manager,
Uniq Technologies,
North Usman Road,
T-Nagar,
Chennai, Tamil Nadu 600017

Dear Sir,

Warm Greetings from St.Peter's College of Engineering and Technology, Chennai.

St.Peter's College of Engineering and Technology is managed by Lakshmi Saraswathi Educational Trust. It was started in the year 2008. The college is affiliated to Anna University of Technology Chennai and approved by the All India Council for Technical Education (AICTE), New Delhi.

The college aims to develop in to a top class professional institution in the years to come thus providing training to students to take up challenging assignments and contribute to national and international development. We have been always imparting a high standard of practical training so that students have to undergo **Internship Training**, which enable them to familiarize with the industrial environment. We request you to kindly accord permission to the following students of Electrical and Electronics Engineering Department to undergo internship training.

1. Ms.S.M.Shakthivel (112719105006)

Our student shall obey all your company rules and regulations and shall maintain the highest order of discipline during the training period.

We look for your kind permission for the above internship training to ourstudents.

Thanking You,

Yours Faithfully,

PRINCIPAL

REFERENCE NO: INV-003998

DATE: 24th January 2023

TO WHOMSOEVER IT MAY CONCERN

This is to certify that **SM SHAKTHIVEL (Register no:112719105006) B.E (Electrical and Electronics Engineering)** in **St.Peter's College of Engineering and Technology Chennai**, has been permitted to do his internship with project in our organization.

His Internship period: **25th January 2023 to 31 March 2023**

After the successful completion of his internship, he will get the completion letter from our organization.

Thanks & Regards,



(HR Head)



Date: 25.01.2023

To
The Technical Manager,
Plasmic Heatrods
No;9, Bethel street,
Thirumullaivoyal,
Chennai, Tamil Nadu 600062

Dear Sir,

Warm Greetings from St.Peter's College of Engineering and Technology, Chennai.

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The college aims to develop in to a top class professional institution in the years to come thus providing training to students to take up challenging assignments and contribute to national and international development. We have been always imparting a high standard of practical training so that students have to undergo **Internship Training**, which enable them to familiarize with the industrial environment. We request you to kindly accord permission to the following students of Electrical and Electronics Engineering Department to undergo internship training.

1. Mr.M.R.Mohammed Hussain (112719105003)
2. Mr.I.Mohammed Muzzamil(112719105701)

Our students shall obey all your company rules and regulations and shall maintain the highest order of discipline during the training period.

We look for your kind permission for the above internship training to ourstudents.

Thanking You,

Yours Faithfully,

PRINCIPAL



☎ +919840074899
✉ plasmicheatrods@gmail.com
🌐 www.plasmicheatrods.com

CERTIFICATE OF INTERSHIP

This internship program certificate is proudly awarded to **MR. M.R MOHAMMED HUSSAIN**, Fourth year **B.E(EEE)**, Student of **St.Peter's College Of Engineering and Technology** for his outstanding completion of the internship in **FURNACE AND HEATING ELEMENTS** at **PLASMIC HEATRODS** from **06.02.2023** to **22.02.2023**. During the period of his internship program with us he was found punctual, Hardworking and inquisitive. We wish him all success.

FOR PLASMIC HEATRODS

Authorized Signatory
TECHNICAL MANAGER

PLASMIC HEATRODS

Mfrs of: All kinds Of Industrial Heaters, Equipments & Control Panel
9, Bethel Street, Cholambedu, Thirumullaivoyal, Chennai-600 062
GST NO. 33ADYPJ4882A1ZD



+919840074899

plasmicheatrods@gmail.com

www.plasmicheatrods.com

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Mfrs of: All kinds Of Industrial Heaters, Equipments & Control Panel
9, Bethel Street, Cholambedu, Thirumullaivoyal, Chennai-600 062
GST NO. 33ADYPJ4882A1ZD

INSPIRISYS/HRD/OL/2023/N2470

Mar, 02, 2023

Mr. S Narayanan
No: 21, Agara Street,
Thiruvallur,
Tamil Nadu - 602001

OFFER LETTER

Dear Narayanan,

With reference to your application and the subsequent interview you had with us, we are pleased to offer you employment in **Inspirisys Solutions Limited** as per the terms and conditions given in the subsequent paragraphs.

We bring to your kind attention that you will be put on a training programme immediately upon appointment to make you adequate and compatible to the work atmosphere along with specialised hands on experience to meet the standard of work provided by the company which entails considerable expenses, that enhances your market value as a professional.

In accordance with company policy and guidelines you are required to sign a contractual agreement along with a surety/guarantor accepting and agreeing to work with the company for a minimum period of **Thirty months** inclusive of the above stated training, failing which you will be liable to make a payment of Rs. 1, 20,000 as liquidated damages towards expenses of training and breach of contractual period. You are put on notice that signing of this agreement is a mandatory and precedent clause for your employment with the company, therefore you may consider this offer before signing of the acceptance letter. Compliance of the same shall be strictly executed once the acceptance is received from you.

Your initial place of posting will be at **Chennai** and Effective date of appointment will be **03rd, March, 2023**

1. SALARY & BENEFITS

Your salary and other emoluments are given in the **Annexure**.

2. DESIGNATION & GRADE

Your designation will be **Trainee Engineer Software** and the equivalent grade in accordance with our policies will be **EL-100**.

SERVICE RULES

- a) Your employment with Inspirisys Solutions Limited is full time and you shall not engage in any commercial business or pursuit on your own account or as an agent for others during the course of employment.

- b) You are required to seek permission from the management before you undertake any course of study.
- c) You are required to treat all information and official correspondence as confidential. You shall not at any time or times, without the consent of the company disclose, divulge or make public except under legal obligation, by word of mouth or otherwise, details of manufacturing processes, software development, technical know-how, security arrangements, administration, accounts or any other dealings of the company known to you in the course of your service or otherwise.
- d) As per company Policy, you shall be required to give necessary undertakings to the company.
- e) You shall work under the supervision of such officer/s as may be decided by the company from time to time. You shall diligently and faithfully carry out instructions given to you to the best of your power, skill and ability in the best interests of the company.
- f) You shall keep the company informed of any change in your residential address or civil status.
- g) You shall be responsible for the safekeeping and return in good condition and order of all the company's property which may be in your use, custody or charge.
- h) The retirement age as per the company's policy is **55 years**. The date of birth given by you and taken on record is **12-Jan-2001**
- i) You shall abide by the rules and regulations of the company which are in force and/or which may be framed from time to time.
- j) You shall regularly check the internal policies of the company and abide by such policies.

PROBATION & JOINING FORMALITIES

- a) You shall be on probation for a period of **Twelve months** with effect from your date of joining with the company.
- b) During the period of probation, you will not be entitled to any leave with pay other than casual leave. However, you will be eligible for PF Contribution and ESI contribution (if applicable) by the Company and on confirmation you shall be eligible for Privilege Leave as per the rules of the company.
- c) In case you wish to resign from the services of company during the probation period you are to give one month's notice or compensation in lieu of notice as decided by company. This notice is mandatory and cannot be relaxed for any reasons whatsoever. However the company can terminate your services during probation without any notice.

- d) Should your work be found satisfactory at the end of probation, your appointment will be confirmed in writing. Unless so confirmed in writing, you shall continue to be on period of probation. The probation period is extendable at the sole discretion of the management.
- e) You are required to maintain yourself in a state of medical / physical, mental fitness and ensure annual medical checkups. Any neglect on your part in this regard may render your service liable for termination.

3. OTHER TERMS & CONDITIONS

- a) You are liable to be transferred to any place of business or department of the company at the discretion of the management.
- b) You are liable to be posted abroad depending on the needs of the business. In the event of such posting, all allowances and benefits will be applicable based on the Foreign Travel Policy and the following employment norms are applicable.
 - (i) You are responsible to comply with local laws prevalent in the country of posting. Any legal expenses due to non-adherence of local laws if attributable to the company, besides visa related issues will be borne by the company.
 - (ii) Employees will be forbidden to submit resignation in the midst of project when posted abroad. All travel related expenses that included air ticket, visa expenses etc. will be borne by the employee in case of any such resignation.
- c) Your employment separation process is governed by the following rules:
 - (i) It is mandatory to serve two months' notice period in case of resignation of your service after confirmation. Salary in lieu of notice period is not acceptable.
 - (ii) You are not entitled for any leave other than eligible casual leave at the time of serving the notice period in case of resignation.
 - (iii) If the above stated mandatory clauses are not complied with and you leave the company without serving the notice period, the company shall be entitled to withhold your service certificate for such period and claim damages against you for the said term.
 - (iv) The company has the right to terminate your employment after an internal inquiry in case of any disciplinary proceedings taken up against you.
 - (v) In case of termination of your services by the company other than the above clause you will be paid two-month salary in lieu of notice period.
- d) If during the period of employment with the company, you make an invention or discovery or improvement to either of the above, connected to any of the articles manufactured and/or services provided by or dealt with by the company, or in respect of any processes or methods etc., pertaining to those mentioned above, whether patented or otherwise, employed by the company, it is agreed that the same shall be owned solely by the company or its customers as the case may be.

- e) You shall not, during the course of your employment with the company, undertake or attempt to seek any engagement, employment and / or consultancy service or accept any assignment in any other organization anywhere in the world unless authorized by the company in writing
- f) If at any time in the opinion of the company, which shall be final, your performance is found to be below acceptable / satisfactory levels or you become insolvent or are found guilty of dishonesty, disobedience, misappropriation, theft, fraud, disorderly behavior, negligence, indiscipline, absence from duty without permission or of any conduct considered by the company as detrimental to its interests or of violation of one or more terms of this appointment, your services may be terminated without notice.
- g) At the time of joining, you are requested to produce the Original Certificates and copies in proof of:
 - Qualification.
 - Age, Identity and address.
 - 5 passport size color photographs.
 - Relieving Order and Experience certificate from previous employer. (if applicable)
- h) In case your written acceptance of this offer is not received within 2 days of issue, this offer will be treated as withdrawn and cancelled, without any further reference to you.
- i) In case you do not report for duty within 2 days of the joining date indicated by you in the acceptance letter, the offer will be treated as withdrawn and cancelled, without any further reference to you.

4. OFFER VALIDITY:

- a) This offer is valid only if you do not have any standing backlogs as on the date of joining Inspirisys Solutions Limited.
- b) This offer is valid only if you join **within 30 days** from the date of completion of the degree course.

We welcome you to **Inspirisys Family** and look forward to a long, successful and mutually beneficial relationship.

With Best Wishes,
For INSPIRISYS SOLUTIONS LIMITED

A handwritten signature in black ink, appearing to read "Isaac", written over a horizontal line.

ISAAC SATHISH KUMAR
SENIOR MANAGER - TALENT ACQUISITION

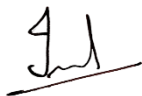
ANNEXURE

Compensation Package		
Name	S Narayanan	
Grade	EL-100	
Designation	Trainee Engineer Software	
Location	Chennai	
COMPONENTS	Per Month	Per Annum
Basic	13,199	158,388
HRA	8,799	105,588
Total Gross (A)	21,998	263,976
Company Contribution		
Provident Fund	1,584	19,008
Gratuity *	634	7,608
ESI	200	2,400
Bonus **	584	7,008
TOTAL (B)	3,002	36,024
TOTAL CTC (A +B)	25,000	300,000

*Gratuity is payable as governed by the Gratuity policy of the company.

**Declaration of bonus is subject to availability of profit computed in accordance with the Payment of Bonus Act.

For INSPIRISYS SOLUTIONS LIMITED



ISAAC SATHISH KUMAR
SENIOR MANAGER - TALENT ACQUISITION

Acceptance of Offer

I have read, understood and accept the above-mentioned terms and conditions and I will join duty on _____

Signature: _____

Date: _____

OFFER LETTER

24-January -2023

GOBISRI. M
St.Peters college – Chennai

Dear Gobisri. M,

Congratulations! With reference to the interview with us for a career in our organization, we are pleased to inform you that you have been selected for free training in our organization as software trainee.

You will be joining and performing your training at our office in Chennai. You will be on provided training for a period of 4 months from the date of your joining. On software testing and development and you will never been charged even a single rupee in the period of this training. After the training you will be assisted for placements in different companies based on your performance.

On the date of joining, you would be required to submit the following documents with this letter.

1. College ID card copy.
2. Aadhar card copy.
3. One passport size photo.

Welcome to our Organization!

We are looking forward to have you onboard on or before the month of February at Techveel.

Sincerely,



CEO & Founder Techveel

Hereby, I accept the offer and Read related terms and conditions. I will join on
(_____)

OFFER LETTER

24-January -2023

KARUNAGARAN G
St.Peters college – Chennai

Dear Karunakaran G,

Congratulations! With reference to the interview with us for a career in our organization, we are pleased to inform you that you have been selected for free training in our organization as software trainee.

You will be joining and performing your training at our office in Chennai. You will be on provided training for a period of 4 months from the date of your joining. On software testing and development and you will never been charged even a single rupee in the period of this training. After the training you will be assisted for placements in different companies based on your performance.

On the date of joining, you would be required to submit the following documents with this letter.

1. College ID card copy.
2. Aadhar card copy.
3. One passport size photo.

Welcome to our Organization!

We are looking forward to have you onboard on or before the month of February at Techveel.

Sincerely,



CEO & Founder Techveel

Hereby, I accept the offer and Read related terms and conditions. I will join on
(_____)

112721205004



112721205006



112721205013

