Report on workshop (5th -8th December, 2016)

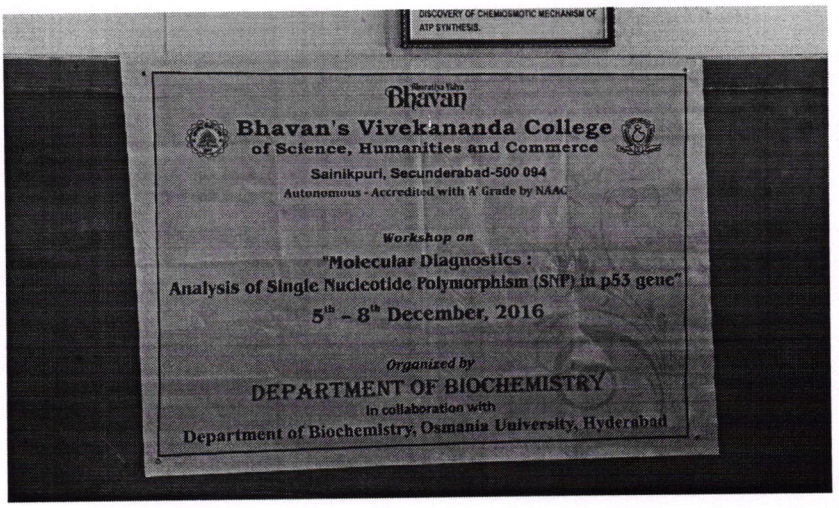
The department of Biochemistry conducted a workshop on" Molecular Diagnostics: Analysis of Single Nucleotide Polymorphism in p53 gene". This was organized in collaboration with the Department of Biochemistry, Osmania University from 5th to 8th of December, 2016.

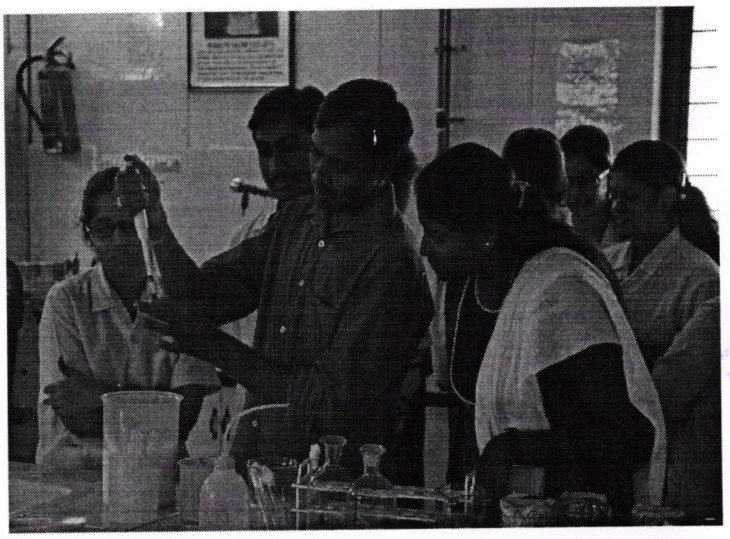
Dr. Manjula Bhanoori, Asst Prof from the department of Biochemistry, OU was the resource person for this workshop. Research scholars of Dr. Manjula's lab, Mr.Venkat Reddy and Ms. Swapna have helped in providing the hands-on-experience to the students. The experiments carried out in the workshop were DNA isolation from blood samples, analysis of p53 polymorphism amongst the DNA samples by PCR amplification and RFLP techniques.

This workshop has provided the students in-depth practical knowledge and hands-on- experience in carrying out the above said techniques. The workshop was concluded with a valedictory function. Principal Prof. Y.Ashok addressed the students and gave away participation certificates.

A. Lai Dade

Head, Dept. of Bio-Chemistry
Bhavan's Vivekananda College
Sainikpuri, Secunderabad-500 094





A. Lai Yadi
Head, Dept. of Bio-Chemistry

2016-17
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To,
The Principal,
Bhavan's Vivekananda College,
Sainikpuri, Secunderabad.

Respected Sir,

Subject: Permission to organize workshop on "Molecular Diagnostics: Analysis of single nucleotide polymorphism (SNP) in p53 gene" in collaboration with Dept. of Biochemistry, O.U-reg.

The Department of Biochemistry is planning to organize a workshop on "**Molecular Diagnostics: Analysis of single nucleotide polymorphism (SNP) in p53 gene"** for students of M.Sc (Biochemistry). The objective of this training programme is to expand the technical skills of the students in recent areas of molecular diagnostics which have high practical importance. This kind of training and exposure will definitely help the students to face the interviews and improve their employment opportunities in the area of clinical diagnostics. This workshop is planned to be organized in collaboration with Dr.Manjula Bhanoori, Assistant Professor, Department of Biochemistry, Osmania University from 5th -8th December,2016.

The estimated cost for conducting this training programme is Rs.29,815/-which is for procuring chemicals and for technical support.

Thanking you,
Yours faithfully

A. Sai Padma 6/10/2016.
Dr.A.Sai Padma
Head, Dept. of Biochemistry,
BVC.

Bharatiya Vidya
Bhavan

BHAVAN'S VIVEKANANDA COLLEGE
OF SCIENCE, HUMANITIES AND COMMERCE
Sainikpuri, Secunderabad-500 094
Autonomous- Accredited with 'A' Grade by NAAC

Workshop on

"Molecular Diagnostics: Analysis of Single Nucleotide Polymorphism in p53 gene"

5th – 8th December, 2016

Organized by

DEPARTMENT OF BIOCHEMISTRY

In collaboration with
Department of Biochemistry,
Osmania University, Hyderabad

Resource persons for the workshop on

"Molecular Diagnostics: Analysis of single nucleotide polymorphism (SNP) in p53 gene"

in collaboration with Dept. of Biochemistry, O.U

5th- 8th December, 2016.

Resource person Mr.Venkat Reddy, O.U demonstrating DNA extraction

Resource person Ms.S.Swapna, O.U demonstrating PCR technique

Aim: To identify SNP (Single Nucleotide Polymorphism) variation in exon 4 of P53 gene to distinguish Arg allele and Pro allele.

METHODOLOGY

1.Collection of Blood Samples: Peripheral blood samples (5 ml) would be collected from all the subjects in EDTA (Ethylene diamine tetra acetic acid) coated vacutainers and plasma would be removed followed by storage at -20 °C until further analysis performed.

2. ***DNA isolation from blood:*** Genomic DNA will be extracted from 1 ml of EDTA anti-coagulatedwhole blood by salting out method.

Equipment and Reagents required for DNA isolation

Equipment: Centrifuge (REMI), centrifuge tubes (10ml), water bath, micro pipettes, microtips (20-200µl and 100-1000 µl), Eppendorfs tubes (1.5ml).

Reagents: 5ml of whole blood

Solution A: Sucrose-10M, $Mgcl_2$-1M Tris-HCl (pH: 8)-1M, 10% of Triton X-100.

Solution B: NaCl-1M Tris-HCl (pH 8)-1M Na-EDTA (pH 8)-0.5M and 5% SDS (Sodium Dodecyl Sulphate).

Solution C: 100gm of Sodium perchlorate wouldbe added in 142 ml autoclaved double distilled water (5M sodium perchlorate).

PCA Solution: Phenol, Chloroform and Isoamyl alcohol in ratio of 25:24:1

CA Solution: Chloroform and Isoamyl alcohol in the ratio of 24:1.

TE Buffer for dissolving DNA: 10mM Tris HCl, 1mM EDTA; pH 8.0.

PROCEDURE

Cell preparation (25°C)

To the 1ml whole blood collected in EDTA, 4ml of solution A would be added and mixed by inverting the capped tube gently for about a minute. The mixture centrifuged at 3000rpm for 5min. The supernatant will be discarded without disturbing the pellet.

Cell lysis (25°C)

To the pellet, 350ml of solution B would be added and vortexed till the solution became homogeneous. The homogenate would be transferred to a 1.5ml tube.

Deproteination (25°C)

100ml of solution C would be added followed by 600ml of chilled chloroform. The mixture will be made homogeneous by mixing. Centrifugation at 6500rpm for 10min separated the layers.

DNA extraction(25°C)

The upper aqueous phase would be collected and equal volume of PCA would be added. The solution would be mixed thoroughly and spun at 7000rpm for 10min to separate the phases. This procedure of PCA treatment would be repeated again with the collected aqueous phase. It is then subjected to CA treatment in a similar manner.

DNA precipitation (25°C)

To the aqueous phase from the CA treatment, one-tenth volume of 3M sodium acetate would be added. To precipitate the DNA, 2.5 volume of chilled ethanol would be added.

DNA washing

The spooled DNA would be washed twice with 70% ethanol and after drying, will be dissolved in appropriate volume of TE buffer.

DNA quantification

Concentration of DNA would be measured using spectrophotometric as well as densitometric methods. A unit of absorbance at 260nm is considered equivalent to 50mg/ml concentration. The DNA quantities will also measure using the SYNGENE software after analyzing the band intensities of an ethidium bromide stained gel.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is an invitro amplification process, in which amplification of a single or few copies of target DNA mixture across several orders of magnitude take place, generating thousands to millions of copies of a particular DNA sequence. It is a primer mediated enzymatic amplification of DNA sequences. The process involves amplification of DNA template using a thermo-stable DNA polymerase enzyme (Taq DNA polymerase) which catalyzes the buffered reaction in the presence of an oligonucleotide primer pair and four deoxynucleoside triphosphates (dNTPs).

Procedure

The PCRs would becarried out in 10ml volume using the appropriate specific primer pair (Table1). The primers will be designed from the gene sequences using the 'Primer3 Plus' software. The parameters used were length of 20-28 bases, 45-55% GC content, 55-65°C annealing temperature and a difference of not more than 1°C between the melting temperatures (Tms) of forward and reverse primers. The obtained primer sequences would be screened for non-specificity by BLAST and for self complementarity. The PCR mixture contained 40 - 50ng of genomic DNA, 0.5mM each primer, 200mM each dNTPs, 1X PCR buffer, 2.5mM $MgCl_2$ and 1.25U of Taq DNA polymerase(Table 2). After denaturation at 94°C for 5min, the PCR would be performed for 35 cycles of 30 sec at 94°C, 1min at 56°Cannealing temperature and 1 min at 72°C(Table 3). Each PCR willbe setup with a positive and a negative control. The PCR products would be analyzed by 1.5% agarose gel.

AGAROSE GEL ELECTROPHORESIS

Agarose gel electrophoresis (1.5 %) is used to check amplification of the PCR product.

Reagents

Agarose (electrophoresis grade)

1X TBE (89 mM Tris base, 89 mM acetic acid, 2 M EDTA, PH 8.0)

Ethidium bromide 1µg/µl

6X loading buffer [Bromophenol blue (0.03%) and xylene cyanol FF (0.03%)]

Procedure

300 mg of Agarose would be added to 15ml of 1X TAE buffer and boiled in a microwave oven. The agarose melts and dissolves in the buffer.

★ The mould (gel tray) would be set ready by sealing both ends. The comb will be inserted within the mould.

★ 1 µl of ethidium bromide would be added to the boiled agarose and mixed properly.

★ The boiled liquefied agarose would be cooled to ≈40°C temperature and poured on to the mould without formation of air bubbles.

★ After the gel set, the comb and sealing would be removed from the mould.

★ The gel would be placed in the running buffer in the horizontal electrophoresisunning unit and allowed for pre run at 150V for 5 min.

★ The PCR products (5µl) would be mixed with 6X loading buffer (1µl) and loaded in each well.

★ DNA ladder (50 bp) would be loaded in the rightmost well of the gel.

★ Electrophoretic run would be performed at 100V for 45 min.

★ DNA bands would be visualized under trans-illuminator.

Table 1. Primers

Reagent (concentration)	Volume
10X PCR Buffer	1 µl
$MgCl_2$ 1.5 mM	0.8 µl
dNTPs (10 mM of each)	0.5 µl
Primer forward (10 pmole/ µl)	0.2 µl
Primer Reverse (10 pmole/ µl)	0.2µl
Ampli Taq DNA polymerase (0.25U)	0.2 µl
Milli-Q grade water	3.1 µl
Template DNA	4 µl
Total	10 µl

Table 3: PCR cycling conditions

1	Initial denaturation	94°C for 5 minutes
2	Denaturation	94°C for 40 seconds
3	Annealing	56°C for 30 seconds
4	Extension	72°C for 50 seconds
5	Final extension	72°C for 10 minutes

35 cycles of step 2 to 4

Procedure

The DNA fragments with the Arg72Pro SNP of p53would be amplified by PCR using specific primers

10 mL of PCR product would be digested with 10 units of restriction enenzyme BstUI (New England Biolabs) at 60°C for 1-3h. DNA fragments would be electrophoresed through a 3 % agarose gel and stained with ethidium bromide. The "Arg72" allele is cleaved by BstUI and yields two small fragments (169 bp and 127 bp). The "Pro72" allele is not cleaved by BstUI and has a single 296 bp band. The heterozygote contains three bands (169, 127, and 296 bp) (Fig. 1).

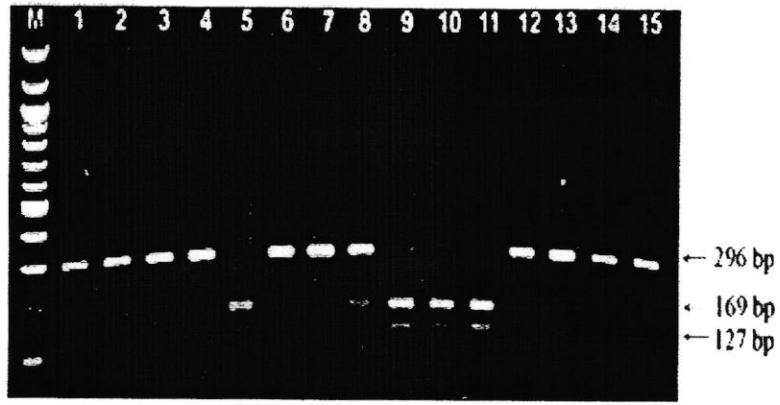

Fig. 1: Arg72Pro polymorphism of p53 by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis: Lanes 1–4, 6, 7, 13, and 15 represent homozygous proline alleles (Pro/Pro); Lanes 5, 9, 10, and 11 represent homozygous arginine alleles (Arg/Arg); and Lanes 8, 12, and 14 represent heterozygous alleles (Arg/Pro). The molecular weight marker is shown in the left part of the gel.