GIRRAJ GOVERNMENT COLLEGE (A)

NIZAMABAD

(NAAC Accredited with "B")

"Hands on training on Polymerase Chain Reaction"

One Day Workshop

On 7th January-2022

Organized by
Department of Zoology

Girraj Government College (Autonomous) Nizamabad

Department of Zoology

Detailed report on one day workshop

1.**Title : *"Hands on training on Polymerase Chain Reaction"***

2. **Date** : 7th January 2022

3. **Venue** : College e- classroom

4**. Resource person**: Dr.Surya Prakash Goud Ponnam, Assistant Professor, Dept. of Molecular Biology and Biotechnology from Tezpur Central University , Assam

5. **Faculty involved in organizing the workshop**:

1. Mr. G.Srinivas Reddy HOD - Convener
2. Mr. M .Chandra Kumar, Assistant Professor of Zoology - Organizing Secretary
3. Mr. Devi Das, Assistant Professor of Zoology - Co-convener
4. Dr. Rafia Yasmeen, Assistant professor of Zoology Co-convener
5. Mr. Deepak, Contract lecturer in Zoology
6. Dr. Sunil, Guest lecturer in Zoology
7. Mrs. Lavanya, Guest faculty in Zoology
8. Mrs.Farhana Begum, Guest faculty in Zoology
9. Mr.S.Abbu, Guest faculty in Zoology

6. **Number of student beneficiaries: 113**
7. **Number of staff beneficiaries:14**

ONE - DAY WORKSHOP

on

HANDS ON TRAINING ON

POLYMERASE CHAIN REACTION (PCR)

07-01- 2022

Organized by

Dept. of Zoology
Girraj Govt. College (A), Nizamabad

WORKSHOP SCHEDULE

S. No	Session	Name of the Resource Persons
	9:30 to 10.00 am	**Registration**
1	10:00 to 11:15 am	Inauguration Inaugural words by Chief Patron An overview of activities of Dept. of Zoology by Convenor
2	11:15 to 11:30 am	Tea break
3	11:30 to 1:00 pm	**Invited talk** **Title: POLYMERASE CHAIN REACTION (PCR)** Resource Person: **Dr. Surya Prakash Goud. Ponnam** M.Sc (UoH)) Ph.D (LVPI-UoH) Assistant Professor Department of Molecular Biology and Biotechnology Tezpur University (Central.Univ.) , ASSAM.

4	1:00 to 2:00 pm	Lunch
5	2:00 to 3:45 pm	**Hands On Training (Technical Session)**
	3:45 to 4:15 pm	Question & Answer session Students feedback
	4:15 to 4:30 pm	Tea – Break
	4:30 to 5:00pm	**Valedictory**

REGISTRATION FORM

ONE - DAY WORKSHOP

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Girraj Govt. College (A), Nizamabad

Name:

Category: Student/Research Scholar/Faculty

Gender: Male/Female

Department:

College/University:

Email:

Mobile:

Applicant's Signature

AGENDA

Day-1 (07-01-2022)

Inviting Dignitaries by Dr.Rafia Yasmeen , Asst. Professor of Zoology

1. **Preside over the workshop** : Dr. E. Lakshmi Narayana
   Principal, Girraj.Govt.College(A) , Nizamabad
2. **Resource Person** : Dr.Surya Prakash.G. Ponnam, Asst.Professor
   Dept. of Molecular Biology and Biotechnology
   Tezpur University (Central .Univ.) Assam.
3. **Prayer** : 1)
   2)
   3)
4. **Chief Patron Welcoming Address** : Dr. E. Lakshmi Narayana
   Principal, Girraj.Govt.College(A) , Nizamabad
5. **Objectives of the Workshop** : By G.srinivasa Reddy, Convenor
6. **Chief Guest's Profile** : By M.Chandra Kumar , Organizing Secretary
7. **Invited talk** : Dr.Surya Prakash.G. Ponnam,Asst.Professor
   Dept. of Molecular Biology and Biotechnology
   Tezpur University (Central .Univ.) Assam.
8. **Vote of thanks** : Sri.Devidas
9. **Students Feedback** :
10. **Lunch**
11. **Post Lunch Session** : Hands on training (Technical Session)
12. **Vote of Thanks** : Dr.Sunil
13. **Tea Break**
14. **Student interactive Session / Feedback**:
15. **Valedictory /Felicitation to Resource Person**

REPORT ON WORKSHOP :

Workshop was inaugurated on 7th Jan 2022 at 10:00 a.m. in the college e- classroom with the National song Vandemataram in the presence of Principal Dr E. Lakshmi Narayana, president of the event.

Resource person Dr Surya Prakash ponnam goud was the chief guest, Organizing committee members, staff and students were also present for the inaugural session

Convenor Sri.G. Srinivasa Reddy had presented the aims and objectives and importance of conducting the workshop.

Aims and objectives of the workshop

1. To enlighten the students about polymerase chain reaction technique and reverse transcriptase polymerase chain reaction technique, which is abbreviated as RT PCR that is used nowadays as a major tool to diagnose covid-19.
2. To bring awareness among the students of life sciences about the corporate needs and how to grab the opportunities using these latest techniques

3. To improve the competency in the subject content of the students to face the competition of global standards
4. To develop expertise in the practical and to provide field knowledge
5. To enlighten the students of life sciences about the contemporary changes occurring in the society e because of the impact of globalization privatization and liberalization

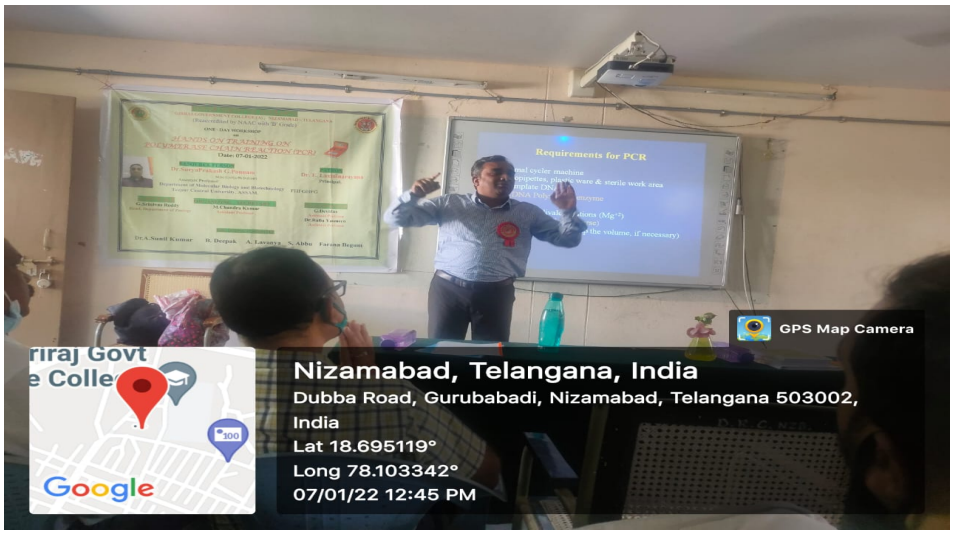
Organizing secretary Sri M. Chandra kumar, has introduced the resource person for the Presentation

Presidential message:

At the outset Dr E Lakshmi Narayana Principal and President of the workshop reiterated the need of the hard work among the students to face competition with Global standards. He advised the students to make use of all the facilities available in the Department of Zoology. He also emphasized the need of organizing such workshops to create zeal and enthusiasm among students . He advised us to conduct more workshops in the near future .

Invited talk and chief guest message:

Dr Surya Prakash ponnam, Assistant professor from Tezpur Central University Assam interacted with the students and faculty. He enlightened the importance of Polymerase Chain Reaction technique in gene amplification and Reverse transcriptase Polymerase Chain Reaction technique in diagnosis of coronavirus during this pandemic situation. He provided hands-on training on PCR technique.



The handout was provided to the students on the above mentioned techniques.

Students feedback:

Analysis of the feedback gave satisfaction to the organizers . Most of the participants had opinion that the aims and objectives of the workshop were achieved

They have passed positive remarks about the resource person

They also stressed the need for organizing Sach workshops in your future

Students participation in Workshop:

Participation Certificates Distribution:

Later vote of thanks was given by Mr Devidas, Assistant professor of Zoology. He thanked the Principal Dr Lakshmi Narayana on behalf of the organizing committee for providing financial assistance to conduct the workshop.

He also thanked the participants for their patience during this pandemic situation.

Hand Out on Polymerase Chain Reaction (PCR)

PCR or Polymerase Chain Reaction is a technique used in molecular biology to create several copies of a specific DNA segment in vitro .This technique was developed in 1983 by Kary Mullis, an American Biochemist. PCR has made it possible to generate millions of copies of a small segment of DNA. This tool is commonly used in the Molecular Biology and Biotechnology laboratories.

Principle of PCR

The PCR technique is based on the enzymatic replication of DNA. In PCR, a short segment of DNA is amplified using primer mediated enzymes. DNA polymerase synthesizes new strands of DNA complementary to the template DNA. But DNA polymerase can only add nucleotides to the pre-existing 3'-OH group. Therefore, a primer is required for DNA de novo synthesis.

Components of PCR

A component of PCR constitutes the following:

1. **DNA Template**– the DNA of interest from the sample.
2. **DNA Polymerase**– Taq Polymerase is used, which is isolated from Thermus aquaticus. It is thermo stable and does not denature at very high temperatures. It is most active around 70°C (a temperature at which a human or E. coli DNA polymerase would be nonfunctional).
3. **Oligonucleotide Primers**- These are the short stretches of single-stranded DNA complementary to the 3' ends of sense and antisense strands. They are usually around 20 nucleotides in length. Two primers are used in each PCR reaction, and they are designed so that they flank the target region.
4. **Deoxyribonucleotide triphosphate**– These provide energy for polymerization and are the building blocks for de novo synthesis of DNA. These are single units of bases.
5. **Buffer System**– Magnesium and Potassium provide optimum conditions for DNA denaturation and annealing. It is also important for fidelity, polymerase activity, and stability.

PCR Steps

The PCR involves three major cyclic reactions:

Denaturation :

Denaturation occurs when the reaction mixture is heated to 94℃ for about 0.5 to 2 minutes. This breaks the hydrogen bonds between the two strands of DNA and converts it into a single-stranded DNA.

The single strands now act as a template for the production of new strands of DNA. The temperature should be provided for a longer time to ensure the separation of the two strands.

Annealing :

The reaction temperature is lowered to 54-60℃ for around 20-40 seconds. Here, the primers bind to their complementary sequences on the template DNA.

They serve as the starting point for the synthesis of DNA.

The two separated strands run in the opposite direction and consequently there are two primers- a forward primer and a reverse primer.

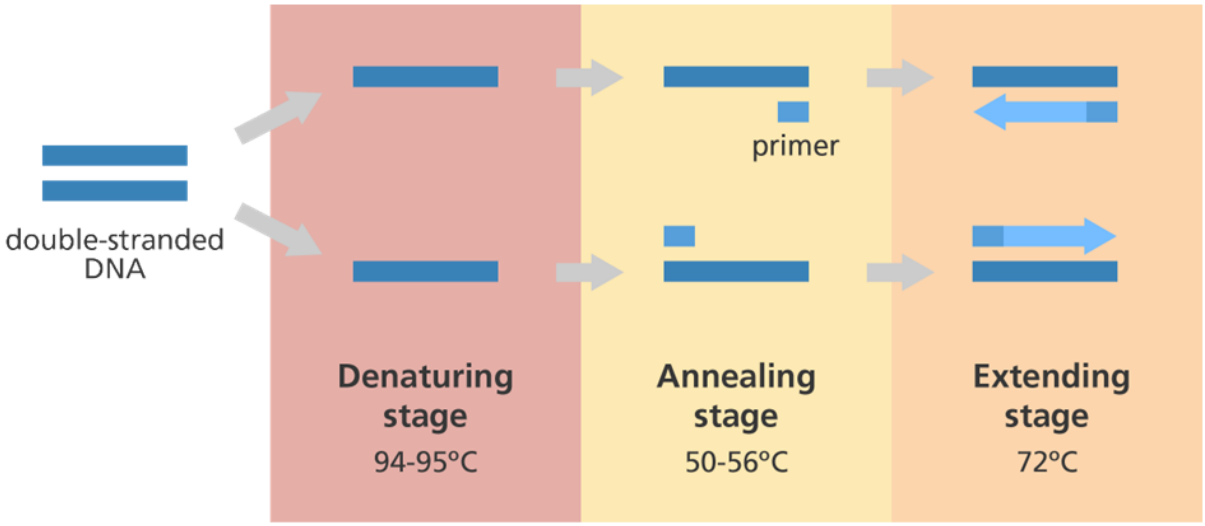
Elongation:

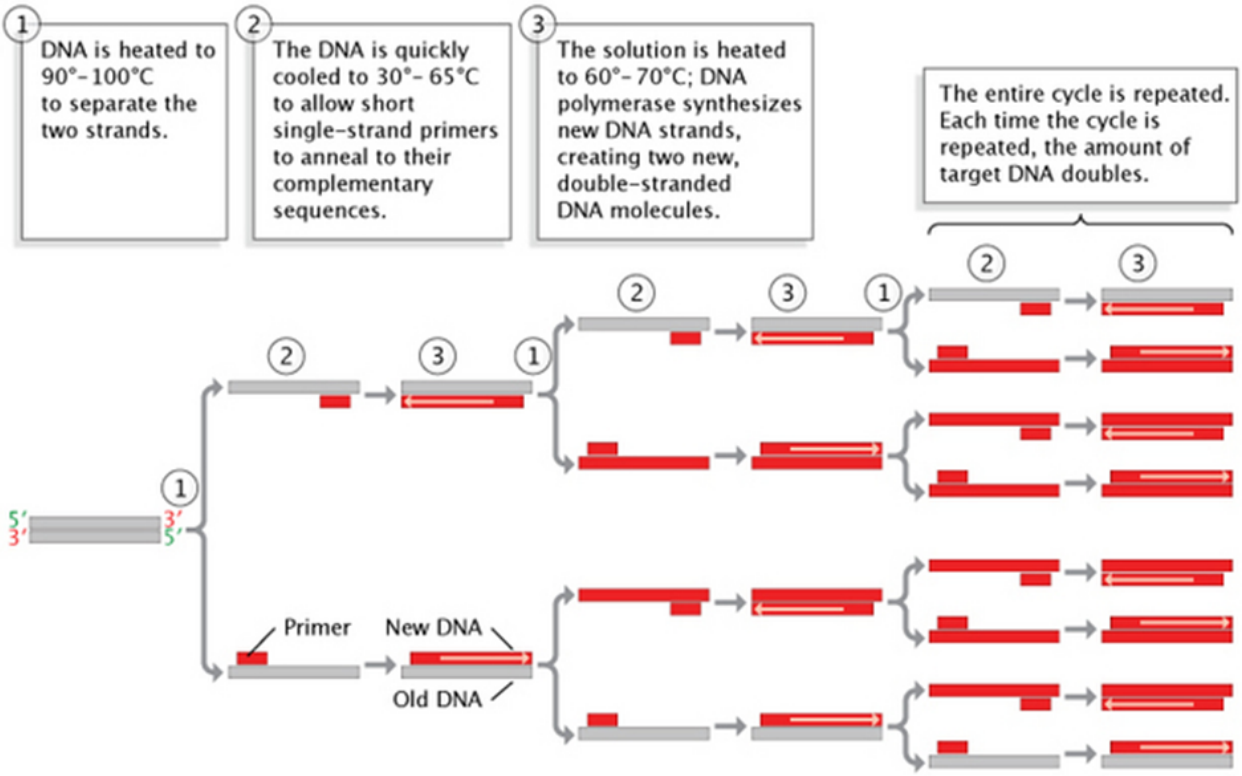
At this step, the temperature is raised to 72-80℃. The bases are added to the 3’ end of the primer by the Taq polymerase enzyme.

This elongates the DNA in the 5’ to 3’ direction. The DNA polymerase adds about 1000bp/minute under optimum conditions.

Taq Polymerase can tolerate very high temperatures. It attaches to the primer and adds DNA bases to the single strand. As a result, a double-stranded DNA molecule is obtained.

These three steps are repeated 20-40 times in order to obtain a number of Copies of DNA of interest in a very short time period.





Types of PCR

PCR is of the following types:

Real-time PCR

In this type, the DNA amplification is detected in real-time with the help of a fluorescent reporter. The signal strength of the fluorescent reporter is directly proportional to the number of amplified DNA molecules.

Nested PCR

This was designed to improve sensitivity and specificity. They reduce the non-specific binding of products due to the amplification of unexpected primer binding sites.

Multiplex PCR

This is used for the amplification of multiple targets in a single PCR experiment. It amplifies many different DNA sequences simultaneously.

Quantitative PCR

It uses the DNA amplification linearity to detect, characterize and quantify a known sequence in a sample.

Arbitrary Primed PCR

It is a DNA fingerprinting technique based on PCR. It uses primers the DNA sequence of which is chosen arbitrarily.

Applications of PCR

The following are the applications of PCR:

Medicine

- Testing of genetic disease mutations.
- Monitoring the gene in gene therapy.
- Detecting disease-causing genes in the parents.

Forensic Science

- Used as a tool in DNA fingerprinting.
- Identifying the criminal from millions of people.
- Paternity tests

Research and Genetics

- Compare the genome of two organisms in genomic studies.
- In the phylogenetic analysis of DNA from any source such as fossils.
- Analysis of gene expression.
- Gene Mapping

PCR Reaction Mixture Components:

1. Assemble all reaction components on ice and quickly transfer the reactions to a thermo cycler. The final volume should be 50 µL.

2. Thaw all reagents on ice
3. Assemble reaction mix into 50 µl volume in a thin walled 0.2 ml PCR tube.
4. Add reagents in following order: water, buffer, dNTPs, MgCl2, template primers, Taq polymerase.
5. Gently mix by tapping the tube. Briefly centrifuge to settle tube contents.
6. Prepare negative control reactions without template DNA.
7. Prepare a positive control reaction with a template of known size and with appropriate primers.

Component	Final Concentration/amount
water	to 50 µL
buffer	1 x
Taq polymerase	0.05 units/µL
dNTP mix	200 µM
MgCl2	0.1-0.5 mM

Forward primer	0.1-0.5 µM
Reverse primer	0.1-0.5 µM
template	200 pg/µL
DMSO (optional)	1 to 10% w/v

PCR steps

Program your thermocycler for your PCR reaction using the following guidelines:

Step	Temp	Time	# of cycles
Initial Denaturation	94°C	5 min	
Denaturation	94°C	30 sec	30-35
Primer Annealing	T_m-5°C	45 sec	
Extension	72°C	1 min per kb	
Final Extension	72°C	5 min	

Analyze the results of your PCR reaction via gel electrophoresis.

Primer Designing

The first step to a successful gene amplification is designing your primer

- Length of 18-24 bases
- 40-60% G/C content
- Start and end with 1-2 G/C pairs
- Melting temperature (Tm) of 50-60°C
- Primer pairs should have a Tm within 5°C of each other
- Primer pairs should not have complementary regions

Step 1: Look up your gene you wish to amplify

Step 2: Use your base pair information to build a set of primers within the range of your gene

Step 3: There are number of sites that offer the primer designing services (Example: IDT,Invitrogen, primer3 etc) or design your primer manually.

Step-4: Go to the selected primer designing tool in the chosen site.

Step-5: Analyze all the parameter results.

Step-6: Finally, also check manually for any errors if present and confirm the primers
Length of 18-24 bases

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Girraj. Govt. College (A) Nizamabad
Department of Zoology
FEEDBACK

ON

One workshop on "Hands on training on Polymerase Chain Reaction (PCR)"

held on 07th January-2022.

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1. Name of the Resource Person :
2. Designation and Address :
3. Subject Content of the Resource Person : Excellent/Very Good/ Good/ Satisfactory
4. Presentation : Excellent/Very Good/ Good/ Satisfactory
5. Registration and Reception : Excellent/Very Good/ Good/ Satisfactory

6. Seating Arrangement : Excellent/Very Good/ Good/ Satisfactory

7. Hospitality : Excellent/Very Good/ Good/ Satisfactory

8. Any other suggestions :

Date: **Signature**

Certificate :

ONE – DAY WORKSHOP
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HANDS ON TRAINING ON
POLYMERASE CHAIN REACTION (PCR)
07Th JANUARY 2022

Organized by
Departments of Zoology
Girraj Government College (Autonomous), Nizamabad

CERTIFICATE

This is to certify that Dr / Prof. / Smt. / Sri _____ of _____ has participated in One-Day work shop on "Hands on Training on Polymerase Chain Reaction (PCR)" held on 07Th January, 2022, organized by the Department of Zoology, Girraj Government College (A), Nizamabad.

G.Srinivas Reddy
Convenor

M.Chandra Kumar
Organizing Secretary

Dr.E.Laxmi Narayana
Principal & Patron