

# Sri Lakshmi Narayana Institute of Medical Sciences

Date: 05.06,2018

From

Dr. PammySinha Professor and Head, Department of Pathology, Sri Lakshmi Narayana Institute of Medical Sciences,

Bharath Institute of Higher Education and Research,

Chennai.

To

The Dean,

Sri Lakshmi Narayana Institute of Medical College, Bharath Institute of Higher Education and Research, Chennai.

Sub: Permission to conduct value-added course: Polymerase Chain Reaction: Principle, Technique and Applications

Dear Sir,

With reference to the subject mentioned above, the department proposes to conduct a valueadded course titled: Polymerase Chain Reaction: Principle, Technique and Applicationsfor II year Allied Health Sciences students from JULY-SEPT 2018. We solicit your kind permission for the same.

Kind Regards din't Dr.PammySinha

### FOR THE USE OF DEANS OFFICE

Names of Committee members for evaluating the course:

The Dean: Dr. Sugumaran. A

The HOD: Dr. PammySinha

The Expert: Dr. Sivaganesh @ Porko. G

SIM TORANGE BOTTON VILLACE, DESTRACAS ESTA MESTORES, C.

PUDENCIALISMY COST DOS Aceistani Professor Department of Profesion Department

The committee has discussed about the course and is approved.

(Sign &Seal) STREETIN HARRISH HELLIGH IN THE WEST SCHOOLS Subject Expert

(Sign &Seal)

(Sign &Seal)

PROPESSOR & NEAD, DEPT, OF PAYHOLOGY SKILAKSRMI NARAYAN INSTITUTE O MEDICAL SCIENCES, PRIDUCHERBY - BUR BUT.



# Sri Lakshmi Narayana Institute of Medical Sciences

OSUDU, AGARAM VILLAGE, VILLIANUR COMMUNE, KUDAPAKKAM POST, PUDUCHERRY - 605 502.

[Recognised by Medical Council of India, Ministry of Health letter No. U/12012/249/2005-ME ( P -II ) dt. 11/07/2011 ]

[Affliated to Bharath University, Chennai - TN ]

### Circular

12.06.2018

Sub: Organising Value-added Course: Polymerase Chain Reaction: Principle, Technique and Applicationsreg

With reference to the above mentioned subject, it is to bring to your notice that Sri Lakshmi Narayana Institute of Medical Sciences, **Bharath Institute of Higher Education and Research** is organizing Polymerase Chain Reaction: Principle, Technique and Applicationsfor II year Allied Health Sciences students from JULY-SEPT 2018. The course content and is enclosed below.

The application must reach the institution along with all the necessary documents as mentioned. The hard copy of the application should be sent to the institution by registered/ speed post only so as to reach on or before 30.06.2018. Applications received after the mentioned date shall not be entertained under any circumstances.

Encl: Copy of Course content

Dean

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SELECTION MENTER RESIDENCE SERVICE VERSEN OCOOU. ACABAMA VIDIACIE.

KOCDAPASIGAM POGT, PUDUCHETARY - 805-502

# **COURSE PROPOSAL**

Course Title: POLYMERASE CHAIN REACTION - PRINCIPLE, TECHNIQUE & APPLICATION for II year AHS students

### CourseObjective:

At the end of the course, the students should be able to know and describe

1. PRINCIPLE of PCR

2. TECHNIQUE of PCR

3. APPLICATION of PCR

To assess target II year Allied Health Sciences students towards the PCR and getting their feedback.

CourseOutcome: POLYMERASE CHAIN REACTION - PRINCIPLE, TECHNIQUE & APPLICATION

Course Audience: II year Allied Health Sciences

Course Coordinator: Dr.PammySinha

Course Faculties with Qualification and Designation:

1. Dr. Sivaganesh @ Porko. G, Assistant professor, Pathology

2. Dr.J.Priyadharisini, Assistant professor, Pathology

3. Dr. A.Manoharan, Assistant professor, Pathology

### Course Curriculum/Topics with schedule

SINo	Date	Торіс	Time	Faculty	Hours
1,	7.07.2018	Introduction	1.30-	Dr. Sivaganesh @	2.5
			4.pm	Porko.G	hrs
2.	14.07.2018	Steps of PCR	1.30-	Dr.J.Priyadharisini	2.5 hrs
۷,			4 pm		
	21.07.2018	Essential	1.30-	Dr. A.Manoharan	2.5 hrs
3.		Ingredients of PCR	4 pm		
4.	28.07.2018	Procedure	1.30-	Dr.J.Priyadharisini	2.5 hrs
		riocedure	4 pm		
	4.08.2018	Equipment	1.30-	Dr. Sivaganesh @	2.5 hrs
5.		-	4 pm	Porko.G	
	11.08.2018	Thermal Cycling	1.30-	Dr. A.Manoharan	2.5 hrs
6.		, omg	4 pm		2.5 (#3
7.	18.08.2018	Trunca - CDCD	1.30-	Dr.J.Priyadharisini	2.5 hrs
_ /.		Types of PCR	4 pm		2,0 ,5
	25.08.2018	Applications of PCR	1.30-	Dr. Sivaganesh @	2.5 hrs
8.			4 pm	Porko.G	
		Practical Class			-
9.	1.09.2018	Procedure	1.30-	Dr. A.Manoharan	2.5 hrs
			4 pm		

10.	8.09.2018	Equipment&Thermal Cycling	1.30- 4 pm	Dr.J.Priyadharisini	2.5 hrs
11.	15.09.2018	Troubleshooting	1.30- 4 pm	Dr. A.Manoharan	2.5 hrs
12	22.09.2018	Assessment and feedback	1.30- 4.pm	Dr. Sivaganesh @ Porko.G	2.5 hrs
		Total			30hrs

### REFERENCES:

- 1. PranabDey Basic and Advanced Laboratory Techniques in Histopathology and Cytology-Springer (2018)
- 2. Molecular Diagnostics in Cytopathology\_ A Practical Handbook for the Practicing Pathologist-Springer International Sinchita Roy-Chowdhuri, Paul A. VanderLaan, John M. Stewart, Gilda da Cunha Santos
- 3. Henry's Clinical Diagnosis and Management by Laboratory Methods, 23e-Elsevier (2016)Richard A. McPherson MD MSc, Matthew R. Pincus MD PhD

### **VALUE ADDED COURSE**

### 1. Name of the programme& Code

Polymerase Chain Reaction: Principle, Technique and Applications & PA08

2. Duration & Period

30 hrs& JULY - SEPT 2018

3. Information Brochure and Course Content of Value Added Courses

Enclosed as Annexure- I

4. List of students enrolled

Enclosed as Annexure- II

5. Assessment procedures:

Short answer questions- Enclosed as Annexure- III

6. Certificate model

Enclosed as Annexure- IV

7.No. of times offered during the same year:

One

- 8. Year of discontinuation: 2019
- 9. Summary report of each program year-wise

	Value Added Course- JULY - SEPT 2018								
SI. No	Course Code	Course Name	Resource Persons	Target Students	Strength& Year				
1	PA08	PCR	Dr. Sivaganesh @ Porko. G	H AHEY	JULY - SEPT 2018				

10. Course Feed Back

Enclosed as Annexure- V

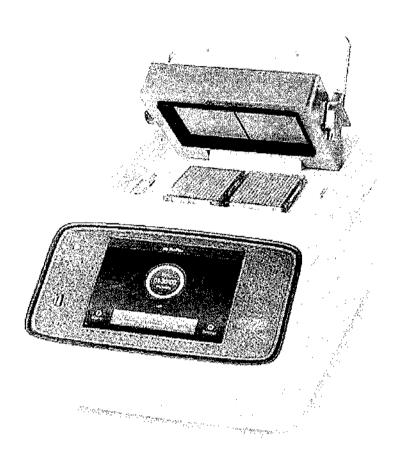
RESOURCE PERSON 6

Assistant, Prote-

**COORDINATOR** 

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# POLYMERASE CHAIN REACTION – PRINCIPLE, TECHNIQUE & APPLICATION



PARTICIPANT HAND BOOK

### **COURSE DETAILS**

Particulars	Description
Course Title	POLYMERASE CHAIN REACTION – PRINCIPLE,
	TECHNIQUE & APPLICATION
Course Code	PA08
Objective	1. Introduction
:	2. Steps of PCR
	3. Essential Ingredients of PCR
	4. Procedure
	5. Equipment
	6. Thermal Cycling
	7. Troubleshooting
	8. Types of PCR
	9. Applications of PCR
	PCR in research activities
opportunities	
Key Competencies	On successful completion of the course the students will
	have skill in PCR techniques
Target Student	II AHS students
Duration	30hrs JULY-SEPT 2018
Theory Session	20hrs
Practical Session	10hrs
Assessment	SHORT ANSWER QUESTIONS
Procedure	

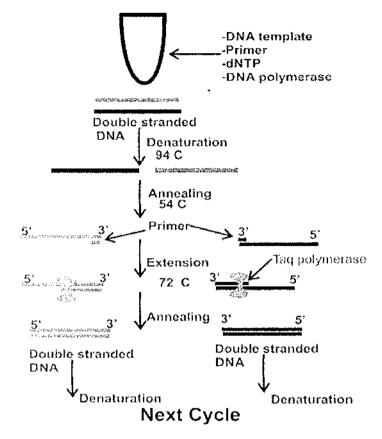
### Introduction

Polymerase chain reaction (PCR) is one of the most important techniques in molecular pathology. With the help of PCR, the single or the pieces of target DNA can be amplified many folds. This technique is now an integral part in every modern laboratory for both the diagnosis and research use.

PCR acts like a "molecular photocopying" machine and amplifies the specific target DNA.

The basic principles of PCR are:

- Double-stranded target DNA is made into single-stranded DNA by applying heat.
- Two oligonucleotide strands or primers are added. The oligonucleotide strand binds with its complimentary DNA strand to the 3' ends.
- The DNA strand is now extended with the help of DNA polymerase (Taq polymerase). This polymerase enzyme incorporates the nucleotides in the DNA to make it elongated.
- The cycle is repeated



(Denaturation step, 94 °C: DNA is heated to 94 °C to make it single stranded. Only 1–2 min are given to this heating process in each cycle.

- Annealing, 54 °C: The temperature is rapidly cooled. In this lowered temperature, the primer quickly anneals with the respective site of DNA. With the help of Taq polymerase, the reaction starts at the primer-DNA template site.
- Extension, 72 °C.
- The complementary nucleotides are attached from the 3' to 5' end of DNA.

  There is an exponential increment in the number of genes in each cycle. At least 30 cycles of denaturation-annealing- extension is done in each PCR

### **Essential Ingredients of PCR**

- *Primers*: The primers are the small pieces of artificially made DNA strands that are actually
- the complementary strands of the 3' end of each strand of target DNA. Primers usually consist of 20–30 nucleotides.
- DNA polymerase (Taq polymerase): Taq polymerase is a type of DNA polymerase enzyme that extends the new DNA strand. It combines at the end of the primer and then sequentially adds new nucleotides to the DNA strand at 3' end complementary to the target DNA. High temperature (94 °C) is needed to separate the

double-stranded DNA. Ordinary DNA polymerase breaks down in this temperature. However, the Taq polymerase has the unique characteristic to work efficiently in higher temperature. This Taq DNA polymerase is extracted from the bacteria *Thermusaquaticus*. These bacteria live in hot spring and can survive there.

- Deoxynucleotide triphosphates (dNTPs): Deoxynucleotide triphosphates (dNTPs) are dATP, dCTP, dGTP, and dTTP. These are the raw material or the basic building blocks of the new DNA strands. The Taq polymerase captures these dNTPs from the working solution and attach them to the terminal part of the primers to extend the DNA chain.
- The target DNA from the sample: The target DNA is extracted from the sample.
- Buffer solution: It provides the optimum chemical environment for the reaction to occur.
- *Magnesium chloride (MgCl2)*: Magnesium chloride works as a cofactor of the Taq polymerase enzyme.

### Equipment

- Thermal cycler
- PCR tubes and caps
- Ethanol-resistant marker
- Micropipettes set

### Thermal Cycling

- Close the cap of the PCR tubes and then put them in the thermal cycler. Standard Steps
- Initial denaturation: At 94 °C for 1 minute
- Denaturation: At 94 °C for 30 seconds
- Annealing: 50–60 °C for 30 seconds: The temperature may vary depending on the primer used. The temperature of annealing stage should be 3 to 4 °C lower than the melting point (Tm) of the primers.
- Extension: 72 °C for 1 minute per kilobase of the PCR product
- Final extension: 72 °C for 10 minute
- Termination: The reaction is terminated by chilling the mixture to 4 °C.

Cycling Time The PCR thermal cycle rapidlyheats and cools the PCR reagent mixture. The cycling time depends on (1) size of the DNA template and (2) G-C content of DNA. The number of the thermal cycler is usually set as 25–30 cycles. If the thermal cycle is increased more than 35, then too many unwanted DNA products may be produced.

The product is calculated as MMf = 2N

Mf = final number of DNA molecule, M = initial number of DNA molecule, N = number of PCR cycle

### **Purification of the Amplified Product**

The following measures are taken to purify the PCR products from the reaction solution:

• Agarose gel electrophoresis of the product:

Agarose gel electrophoresis is done from the portion of the PCR product to verify the validity of the test.

- *Cloning of products*: In this technique further PCR is done to confirm the PCR product. This is done when the gene is present in very tiny amount.
- Sequencing of products: This is done by automated sequencer machine to analyse the sequence of DNA formed as PCR product.

### **Troubleshooting**

The various problems in PCR are described here:

- 1. No amplification of DNA: The possible causes may be:
- Too small amount of DNA template: If there is very less amount of DNA template, then there may not be adequate amplification. The amount of DNA template should be increased in such condition.
- *Too stringent reaction condition*: If the reaction condition is kept as very strict, then there may not be any amplification of PCR products.
- Reagent is not added: One or more reagents may not be added in the reaction mixture. The whole reaction should be done again by carefully adding the reagents.
- Reagents are not in optimum concentration or expired: The reagents should be madefresh, and one new reagent is changed at atime to find out which reagent createdproblem.
- Denaturation temperature is either too high or too low: In such condition changethe temperature 1°C low or high at a time.
- Primer annealing temperature is very high: In such case, lower the temperature 2 °C at a time.
- *Primer dimer formation*: Two primers may self-anneal or anneal with each other. In this case the gel electrophoresis shows a small product of less than 100 base pair.

Addition of DMSO may solve this problem. Alternatively hot start thermal cycling may resolve this issue.

- Suboptimal number of thermal cycle: Suboptimal number of thermal cycle may produce less amount of PCR product. In such condition, increase 5–10 more number of thermal cycle.
- Faulty primer: The primer may be faulty and therefore PCR may not function at all. In such case, redesign the primer.
- 2. *Non-specific product*: Non-specific product may be formed in PCR, and in this case multiple bands with variable lengths are seen in gel electrophoresis. This may be due to:
- *Too less stringency in PCR*: Too less stringent condition generates unwanted DNA products in PCR.
- Too much DNA template: Too much DNA template may produce undesired product in PCR.
- Too many thermal cycles: In such case, reduce the number of thermal cycle 5–10.
- Magnesium concentration is very high: Adjust the concentration and make it low.
- Faulty primer: Redesign the primer.
- Carry over contamination: Change the place of PCR.

### Types of PCR

- 1. *Direct PCR*: This is the standard PCR technique as has been described in the previous section.
- 2. Reverse transcriptase PCR (RT-PCR): In case of RT-PCR, at first cDNA is prepared from RNA of the target sample. This cDNA is thenamplified by PCR technique.
- 3. Asymmetric PCR: In this technique unequal concentration of primers is used. The great excess of primers is used for the targeted DNA strand that we need to

amplify. As the reaction proceeds, only the adequate amount of primer in the reaction mixture produces the particular DNA strand in excess. Therefore ultimately single-stranded DNA (ssDNA) is formed as PCR product. As the reaction is slow and goes on arithmetically, so many more cycles are needed in this technique. Asymmetric PCR is used for DNA sequencing and hybridization as only one strand is needed in such conditions.

- 4. Hot start PCR: Normally DNA polymerase acts in the room temperature and even in the ice pack. Thereby there always remains the possibility of spurious products. In hot start PCR technique, the DNA polymerase is unreactive at the lower temperature and works only at higher temperature. This is done by conjugating an inhibitor with the polymerase enzyme, and in the higher temperature, the inhibitor is free from the polymerase enzyme and allows it to work.
- 5. In situ PCR: In case of in situ PCR, the reaction takes place within a cell on the glass slide. The PCR product is accumulated within the cell, so it is possible to locate the origin of the amplified DNA. The specially designed PCR machine is used to put the slide within it.
- 6. *Inverse PCR*: Inverse PCR (IPCR) amplifies anonymous DNA sequence. It helps to identify the flanking DNA sequence of the genome outside the boundary of the known target sequence.
- 7. Single-strand conformation polymorphism (SSCP):

sequence

The basic principle of SSCP is that the single-stranded DNA has a specific conformation. Any alteration of the single base change due to mutation may lead to different migration pattern of the single-stranded DNA, and therefore in electrophoresis one can distinguishwild-type DNA from mutant DNA.

8. Real-time PCR: Real-time PCR is also known as quantitative PCR (qPCR) as it constantly monitors the quantity of the amplified DNA in the reaction process. In case of qPCR, the amplified DNA is fluorescently

labelled, and the emitted fluorescent is directly proportional to the amount of the amplified fluorescent dye. Therefore in each cycle, the amount of the product can be directly monitored, and it is also possible to quantitate the initial amount of the target DNA in the sample.

9. *Nested PCR*: In case of nested PCR, more than two pairs of primers are used for DNA amplification. The first PCR is a conventional PCR, and the primer is used for the DNA template of the sample. In secondary

PCR the product of the first PCR is used as the target of the second set of primers. The DNA sequence of secondary PCR is different, and therefore there is no chance of undesired PCR product formation.

### **Applications of PCR**

The various applications of PCR in clinical area are described below:

- (a) DNA sequencing: PCR is often used in case of DNA sequencing.
- (b) *Diagnosis of infection*: PCR is widely used for the diagnosis of viral, bacterial and parasiticinfection. As mentioned before Q-PCRcan quantitate the viral load in the body.

PCR rapidly detects the tuberculosis within a few hours, whereas the culture of mycobacteria takes few weeks to develop. As a sensitive technique PCR is able to detect tuberculosis in the early latent phase.

- (c) Diagnosis and prognostic information on cancer: PCR technique is extensively used in he field of cancer:
- Mutation detection: PCR is applied to detect mutation of the oncogenes and tumor suppressor gene such as mutation in p53, c-myc, ras gene, etc..
- *Chromosomal changes*: PCR helps to identify the specific chromosomal changes such as chromosomal translocation, gene rearrangement, loss of heterozygosity, etc.
- *Monoclonality detection*: PCR can detect B and T cell gene rearrangement and thereby can prove the monoclonality in doubtful case of lymphoma.

- *Minimal residual disease*: In case of follow- up of a cancer case, PCR particularly Q-PCR can detect and quantitate certain genetic change to detect any minimal residual disease of a patient.
- (d) Genetic diseases: PCR technique is very helpful to detect various genetic diseases such as Down's syndrome, cystic fibrosis, Gaucherec disease, etc. The main advantage of PCR technique is that it can bypass the aggressive placental bed biopsy to detect these inherited diseases. The minute amount of foetal cells collected from the mother's blood or cervical mucosa are enough to reach at a diagnosis (e) Forensic pathology: PCR technique is helpfulin forensic pathology in different ways:
- To detect paternity of the child
- To identity of the corpse or mutilated body
- To identify the criminal from the crime site and biological materials of the criminal.
- (f) Gene therapy: PCR helps to engineer the specific gene to introduce in the diseased person to cure various diseases

VALUE ADDED COURSE
Polymerase Chain Reaction: Principle, Technique and Applications & PA08

List of Students Enrolled: JULY - SEPT 2018

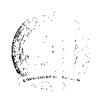
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No	Name of the Student	Roll No	Signature				
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2	MOHANA. A	PM17 CPT 194	10001				
3	NEVETHA.R	PM17 CPT 195	1 Mint				
4	NOYA ANN OOMMEN	PM17 CPT 196	Burell				
5	PADMAVATHI. T	PM17 CPT 197	Alema.				
6	PRABHUL CHANDRAN.V	PM17 CPT 198	aum.				
7	RASIGA. M	PM17 CPT 199	Min				
8	RESHMI	PM17 CPT 200	Cima.				
9	SRAVAN .S .RAJ	PM17 CPT 201	8 cm				
10	SREERAJ.R	PM17 CPT 202	Tom				
11	SRI RAMYA.S	PM17 CPT 203	Sllvy.				
12	SRUTHY SUDHEER	PM17 CPT 204	s. Comilla				
13	SURIYAMOORTHI. V	PM17 CPT 205	Pint.				
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RESOURCE PERSON

COORDINATOR

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# SRI LAKSHMI NARAYANA INSTITUE OF HIGHER EDUCATON AND RESEARCH

Annexure - III

## Polymerase Chain Reaction: Principle, Technique and Applications

### Short answer questions

Course Code: PA08

LANSWER ALL THE QUESTIONS	(5X2=10 marks)		
1. What are the steps in PCR?			
2. What are the types in PCR?			
3. What is the principle of PCR?			
4. List 5 frouble shooting steps in PCR			
5. Name some applications of PCR		·	
	• • .		
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POLYMERASE CHAIN REACTION: PRINCIPLE, SKEERAJ. R
TECHNIQUE AND
APPLICATION 17-CP ROSE

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- (i) Denaturation Step. 1400: DNA is heatedly and to make all single stranded only. 1-2. cycle.
- (ii) Annealing suic the temperature is repially. cooled in this towered temperature the Primer quickly annoals with the respective site of DNA mite het help of Too.
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(iii) Extinsion, 72°

- 2, Type of per:
- (i) Direct pep. This is the standard pep techiques as has been described in the promions Section
  - (i) RALLESSE transcriptuse PCR (PT-PCR): in Carse of PT-POR First DNA vis propored from PNA 197 the torget sample this DNA is thermplified by PCR. Lechnique.
- 3. Porinciple of PCR:

# STILLARSHMI NATAYATA INSTITUTE OF MEDICAL Sciences

This is to certify that \_\_\_\_\_

PADMANATHI. T

\_ has

actively participated in the Value Added Course on Polymerase Chain Reaction: Principle,

Technique and Applications held during JULY-SEPT2018 Organized by Sri Lakshmi

Narayana Institute of Medical Sciences, Pondicherry- 605 502, India.

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Dr. Sivaganesh @ Porko. G

RESOURCE PERSON

Dr. Pammy Sinha

COORDINATOR

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Narayana Institute of Medical Sciences, Pondicherry- 605 502, India.

Dr. Sivaganesh @ Porko. G

RESOURCE PERSON

Dr. Pammy Sinha

**COORDINATOR** 

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## Student Feedback Form

Cours APPL	e Name: POLYMERASE CHAIN REA JCATION	CTION	– PRIN	NCIPLE	, ТЕСН	NIQUE	&	
Subje	ct Code: <u>PA08</u>							
Name	of Student: MOHANA . N-			R	oll No.: _	PI	M17 CP	17 194
	We are constantly looking to improve							,
evalua	ations, comments and suggestions will hel						5 (3 ) (3 )	ou.
SI. NO	Particulars	1	2	3	4	5		
1	Objective of the course is clear	-		<u> </u>		.,,_,		
2	Course contents met with your expectations						}	
3	Lecturer sequence was well planned	· ••		-				
4	Lectures were clear and easy to understand		<u> </u>			1		
5	Teaching aids were effective							
6	Instructors encourage interaction and were helpful		<u></u>					
7	The level of the course				-			
8	Overall rating of the course	1	2	3	(4)	5		
* Rating	: 5 – Outstanding; 4 - Excellent; 3 – Good; 2–	Satisfact	ory; 1-	l Not-Satisf	actory			
Sugges	stions if any:				Walan.			

Date: 22/9/18

Signature

# Student Feedback Form

Cours APPI	se Name: POLYMERASE CHAIN REA LICATION	CTION	- PRII	NCIPLI	Е, ТЕСН	NIQUE	&
Subje	ct Code: <u>PA08</u>						
Name	of Student: KESAVEL + V	·		1	Roll No.:	PMI	10TAT 212
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SI. NO	Particulars	1	2	3	4	5	
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2	Course contents met with your expectations		-				
3	Lecturer sequence was well planned	<del>  -</del> -	ļ <u>.</u>	<del> </del>			
4	Lectures were clear and easy to understand	<u> </u>					
5	Teaching aids were effective	<u> </u>					
6	Instructors encourage interaction and were helpful	<u> </u>					
7	The level of the course						
8	Overall rating of the course	1	2	3	(4)	5	
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Date: 22-09-18

Signature

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Date: 22.09.2018

From

Dr.Sivaganesh @ Porko. G Assistant Professor Department of pathology Sri Lakshmi Narayana Institute of Medical Sciences Bharath Institute of Higher Education and Research, Chennai.

Through Proper Channel

To

The Dean, Sri Lakshmi Narayana Institute of Medical Sciences Bharath Institute of Higher Education and Research, Cheunai.

Sub: Completion of value-added course: POLYMERASE CHAIN REACTION - PRINCIPLE, TECHNIQUE & APPLICATION

Dear Sir.

With reference to the subject mentioned above, the department has conducted the value-added course titled: "Polymerase Chain Reaction: Principle, Technique and Applications for II year Allied Health Sciences students from JULY-SEPT 2018. We solicit your kind action to send certificates for the participants, that is attached with this letter. Also, I am attaching the photographs captured during the conduct of the course.

Dr. Sivagan esa (a) Porko, Grofosso Uspanish at Language Co. es Charach Karasan in the man the control of the co

Encl: Certificates

**Photographs** 

