

V.M.V.V Sangha's

V.M. K. S. R. Vastrad Arts Science And V.S
Bellihal Commerce college, Hungund

Department of Botany Year 2021-2022

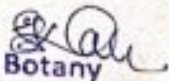
Project reports by B.sc VI semester students



Principal

Vijaya Mahantappa Krupaposhit
S.R.Vastrad Arts, Science & V.S.Bellihal
Commerce College, Hungund-587111

Sl.no	Student Name	Reg.No	Project topic
1	Amaresh kumar	S1937006	Transgenic plants
2	Aruna k	S1937009	Polymerase chain reaction
3	Bharat	S1937018	Transgenic plants
4	Chanchal	S1937020	Transgenic plants
5	Deepa	S1937022	Transgenic plants
6	Kavita	S1937037	Transgenic plants
7	Meharunnisa	S1937051	Transgenic plants
8	Nagaraj	S1937054	Transgenic plants
9	Prem	S1937066	Transgenic plants
10	Reshma	S1937072	Transgenic plants
11	Sangeeta	S1937076	Transgenic plants
12	Satish	S1937078	Transgenic plants
13	Sharanbasava	S1937082	Transgenic plants
14	Shilpa	S1937085	Polymerase chain reaction
15	Shridhar	S1937089	Transgenic plants
16	Tejaswini	S1937098	Transgenic plants
17	Vijayalaxmi	S19370104	Transgenic plants


 Botany
 Head of the Department
 V.M.K.S.R Vastrad Arts, Commerce And
 Science College, Hungund Dist: Baealkr:



V M K S R VASTRAD ARTS, SCIENCE,
V S BELLIHAL COMMERCE COLLEGE
HUNGUND

PROJECT REPORT

College roll no: 06

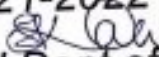
Examination seat no: S1937018

CERTIFICATE

This is to certify that Mr: Bharata N Badiger of BSc 5th semester has satisfactorily completed the project report in Botany subject as prescribed by Rani Chennamma University Belagavi.

During year 2021-2022

Examiner:


Head Dept of Botany

- 1) 
- 2) 



V M K S R VASTRAD ARTS, SCIENCE &
V S BELLIHAL COMMERCE COLLEGE
HUNGUND

**AIMS & STRATERIES FOR
DEVELOPMENT OF TRANSGENIC
PLANT**

PROJECT DONE BY

BHARATA N BADIGER

BSc 5th Sem

Botany

Rani Chennamma University Belagavi

2021-2022

Aims and strategies For Development Transgenic Plant

Introduction of Transgenic plant :

The plants in which a functional foreign gene has been incorporated by any biotechnological methods that generally not present in plant are called transgenic plant . A Number of transgenic plants carrying genes for trails of economic importance have either been released for commercial cultivation or under field trials .

There are several methods which are used in gene transfer

- 1) Electroporation
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Initially some plants were produced by using repoeter gene Later on several gene for known trails of economic importance were incorporated into many crop plants .

In some cases antisense RNA genes have been introduced to inhibit expression of existing genes in a desirable manner

All these approaches led to the development of transgenic Crop plants of economic importance .

More than 1000 field trial tests with transgenic Crop plant have been conducted some of the commercially grown transgenic crop plants in developed countries are Flavr Savri & Endless summer tomatoes Freedom II Squash High - lauric rapeseed (canola and Round up Ready Soyabean etc

- ❖ In 1994 the European Union approved by the US Environmental protection Agency making it the Country's first pesticide producing crop
- ❖ In 1995 Canola with modified Oil composition (Calgene) ,(calgene) ,Bt Cotton (Monsanto) glyphosate resistant Soybeans (Monsanto) Virus -resistant squash (Asgrow) and additional delayed ripening tomatoes (DNAP, Zeneca/ Peto ,and Monsanto) were approved.
- ❖ In 2000 - Vitamin A -Enriched golden rice ,was the first food with increased nutrient value .

Modification of DNA using genetic Engineering techniques

Aim is to introduce a new trait to the plant



The inserted Sequence is known as the transgene

The purpose of inserting a combination of genes in a plant so as to make it as useful and productive as possible

Examples in food crops include resistance to certain pests ,diseases ,or Environmental conditions ,reduction of spoilage, or resistance to chemical treatments or improving the nutrient profile of the crop .

Examples in non food crops include production of pharmaceutical agents biofuels and other industrially useful goods as well as for bioremediation.

Desirable genes may provide features such as higher yield or improved quality ,pest or disease resistance or tolerance to heat ,cold and drought

Transgenic technology enables plant breeders together in one plant useful genes form a wide range of living sources

Generate more useful and productive crop varieties containing new combinations of genes

Transgenic plant

The plant containing an inserted foreign DNA is called transgenicplant .

The process of production of a transgenic plant is transgenesis .

The Following are some transgenic plants

- Transgenic plants with nif genes
- Transgenic tobacco plants
- Transgenic tobean

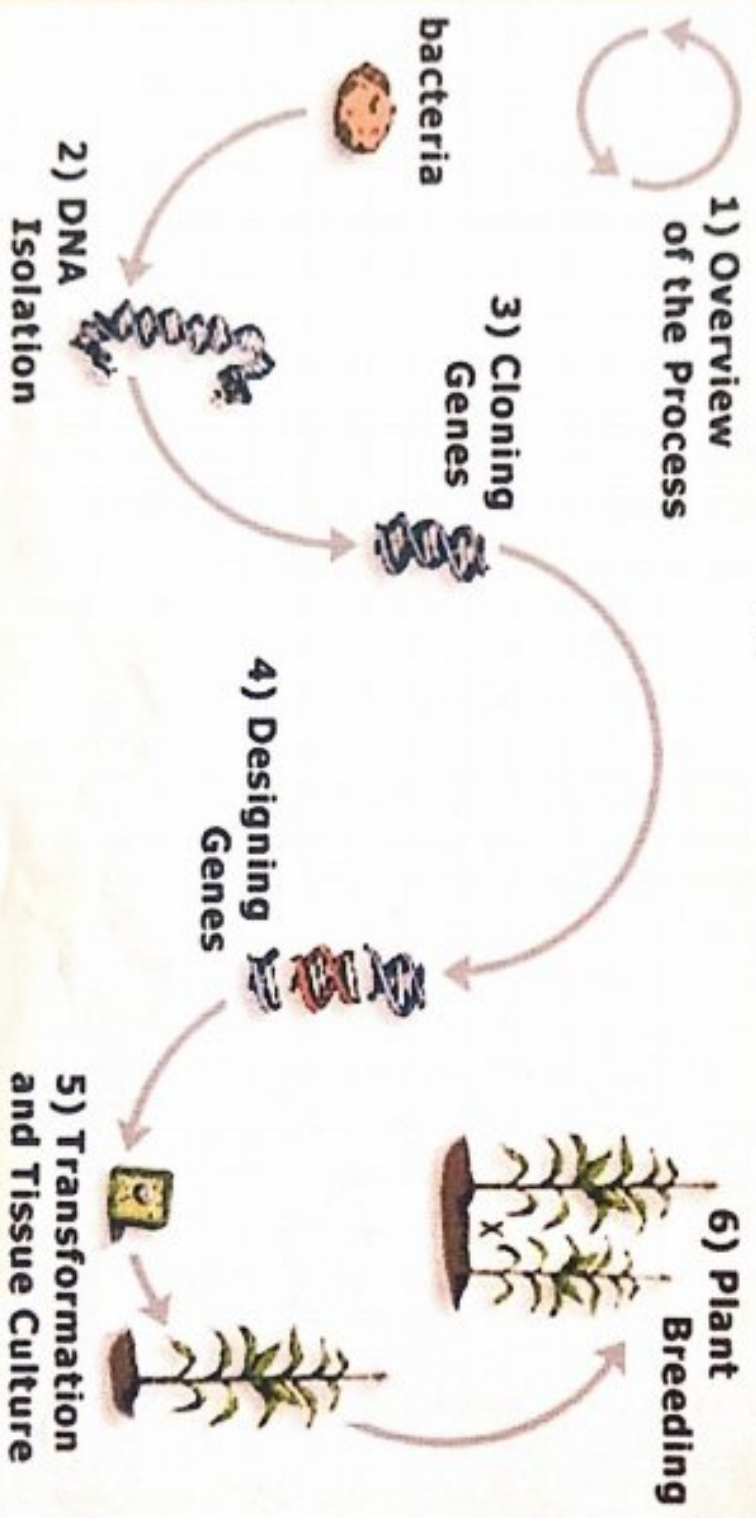
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- The Desired DNA and plasmids are treated with a restriction Enzyme. The cleaved desired DNA and plasmid are mixed. DNA ligase Enzyme is added to this mixture. Now the desired DNA is joined with the plasmid to produce a recombinant DNA .
- The recombinant DNA is introduced into the host plant cell. The host cell is developed into a plant by tissue Culture technique. This plant is called transgenic plant .
- Plants containing introduced DNA are known as transgenic plants or genetically Engineered plants .they have acquired a new trait form the introduced DNA inherit the trait for many generations .

The Following Characters of transgenic plant

a) Herbicide Resistance

Transgenic Plants



- b) Insect Resistance
- c) Virus Resistance
- d) Improved storage proteins
- e) Improved oils and fats
- f) Male sterility
- g) Altered flower colours
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Herbicide Resistant plants

- Many transgenic plants with herbicide resistance have been developed by using genetic engineering .
- Such transgenic plants tolerate the herbicides and be safe in the field ,when the herbicides are applied in the field .

EX :- Glyphosate resistant petunia ,tobacco ,tomato

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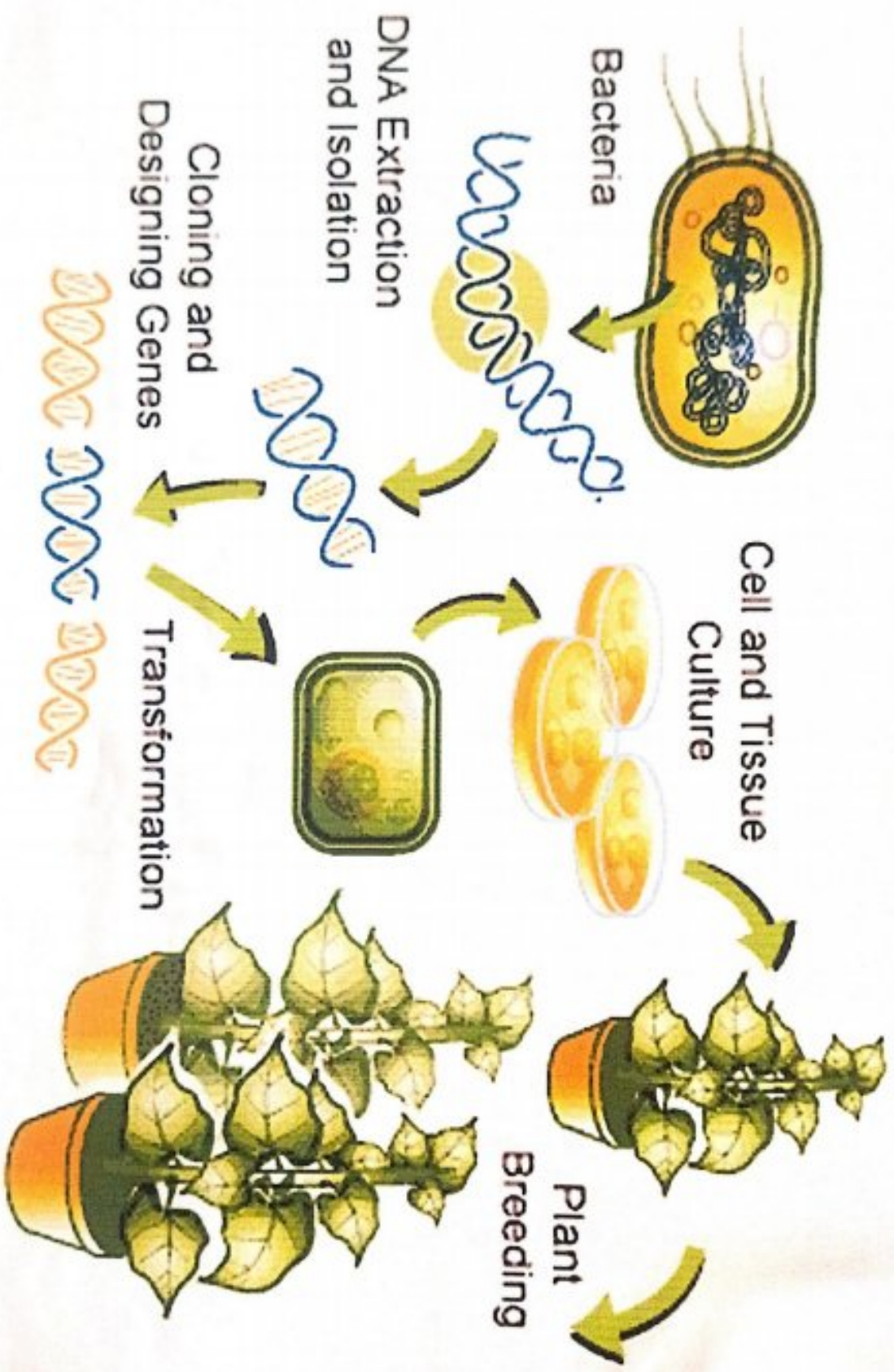
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V M K S R VASTRAD ARTS, SCIENCE,
V S BELLIHAL COMMERCE COLLEGE
HUNGUND

PROJECT REPORT

College roll no: 36

Examination seat no: S1937006

CERTIFICATE

This is to certify that Mr: Amaresh M Kumbar of BSc 5th semester has satisfactory completed the project report in Botany subject as prescribed by Rani Chennamma University Belagavi.

During year 2021-2022

Examiner:

S. Ka
Head Dept of Botany

1) *H. K. K.*
15/2/22

2) *D. K. K.*
15/2/22



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HUNGUND

**AIMS & STRATERIES FOR
DEVELOPMENT OF TRANSGENIC
PLANT**

PROJECT DONE BY

AMARESH M KUMBAR

BSc 5th Sem

Botany

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2021-2022

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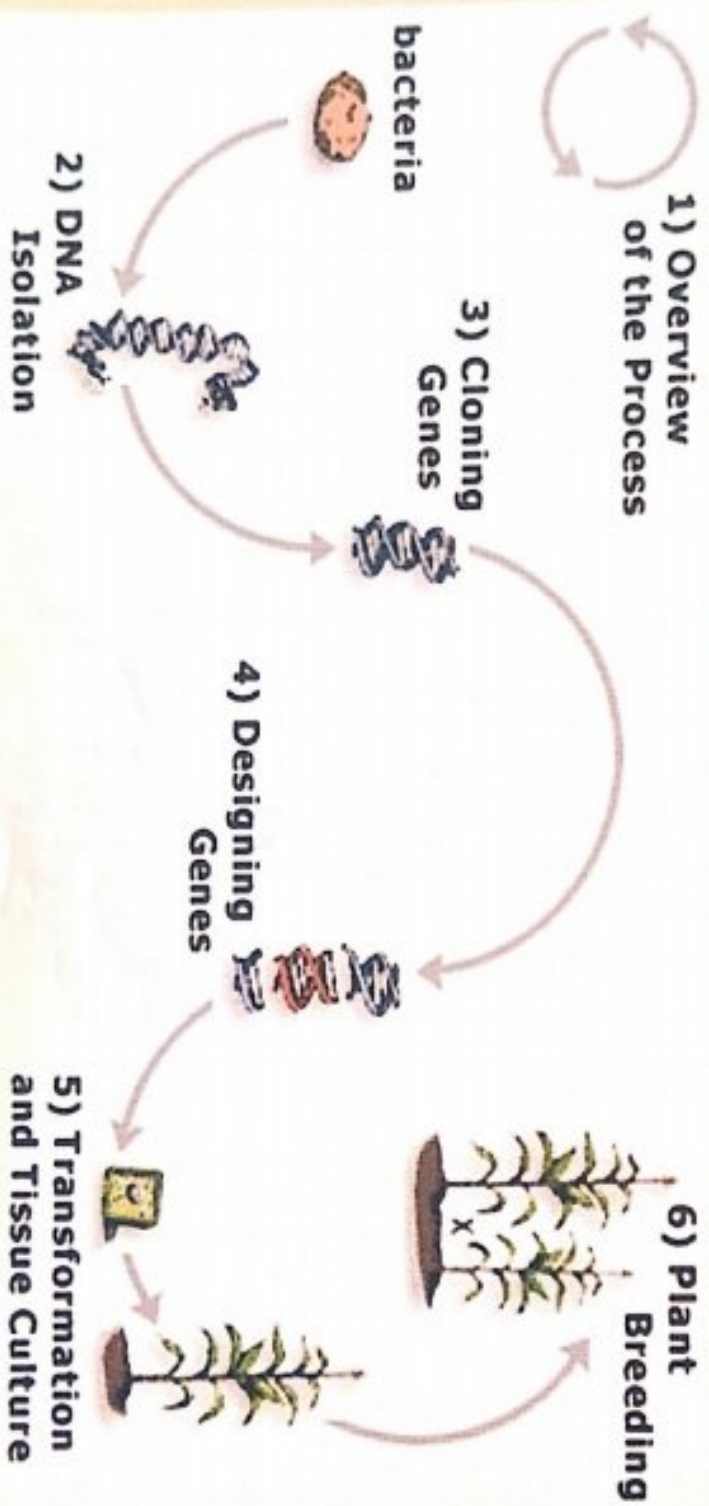
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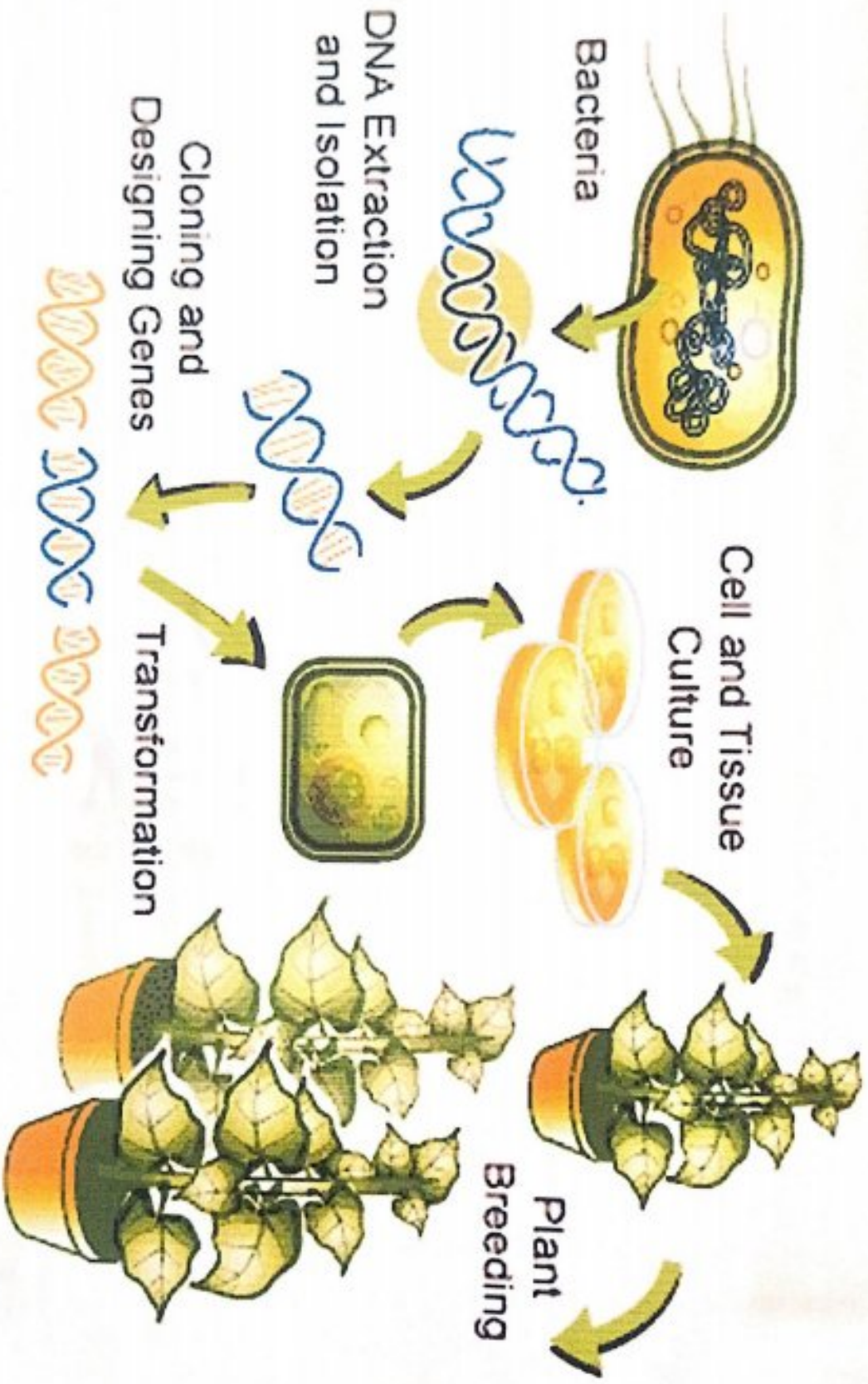
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DEVELOPMENT OF TRANSGENIC
PLANT**

PROJECT DONE BY

KAVITA S MADAR

BSc 5th Sem

Botany

Rani Chennamma University Belagavi

2021-2022



V M K S R VASTRAD ARTS, SCIENCE,
V S BELLIHAL COMMERCE COLLEGE
HUNGUND

PROJECT REPORT

College roll no: 04

Examination seat no: S1937037

CERTIFICATE

This is to certify that Mr: KAVITA S MADAR of BSc 5th semester has satisfactory completed the project report in Botany subject as prescribed by Rani Chennamma University Belagavi.

During year 2021-2022

Examiner:

S. S. S.
Head Dept of Botany

- 1) *K. S. S.*
15/12/22
- 2) *A. S. S.*
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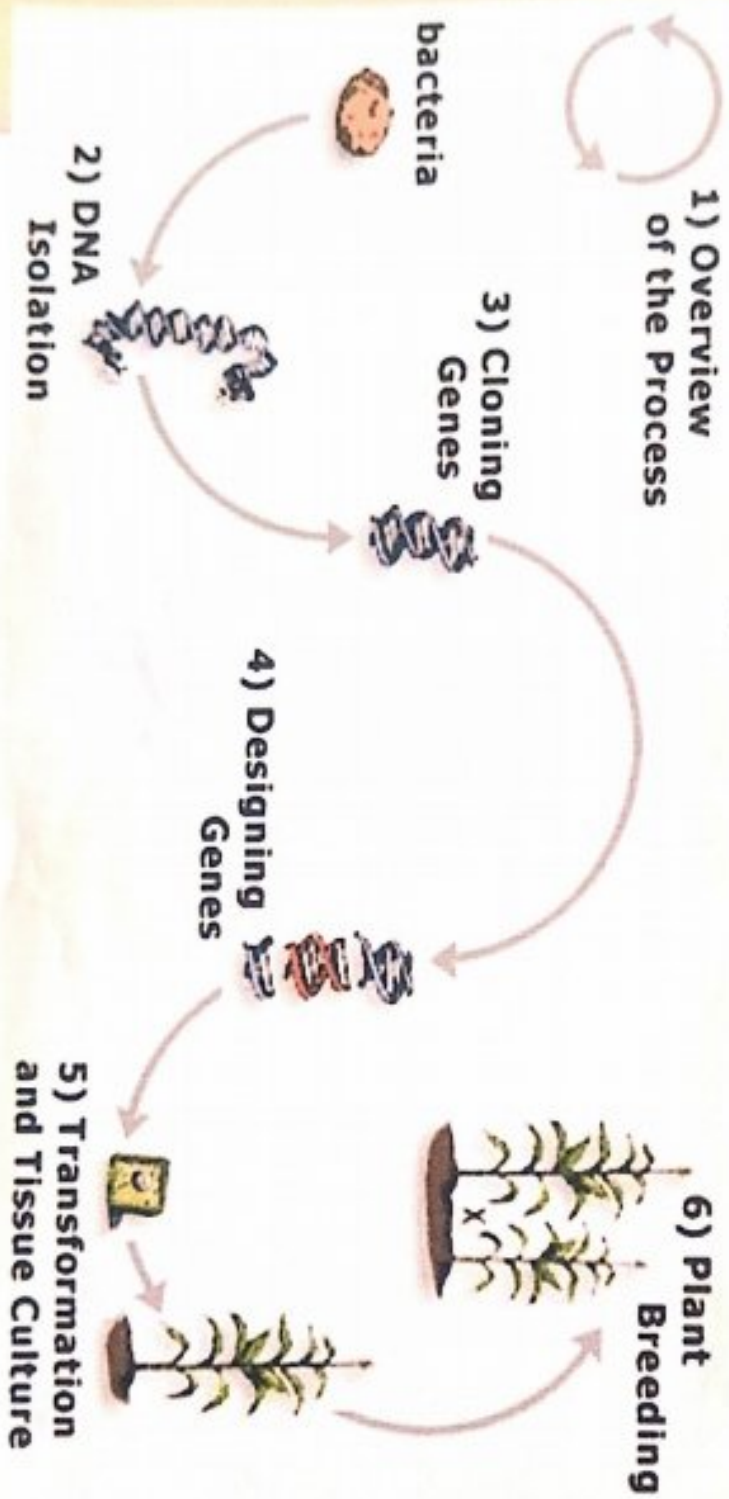
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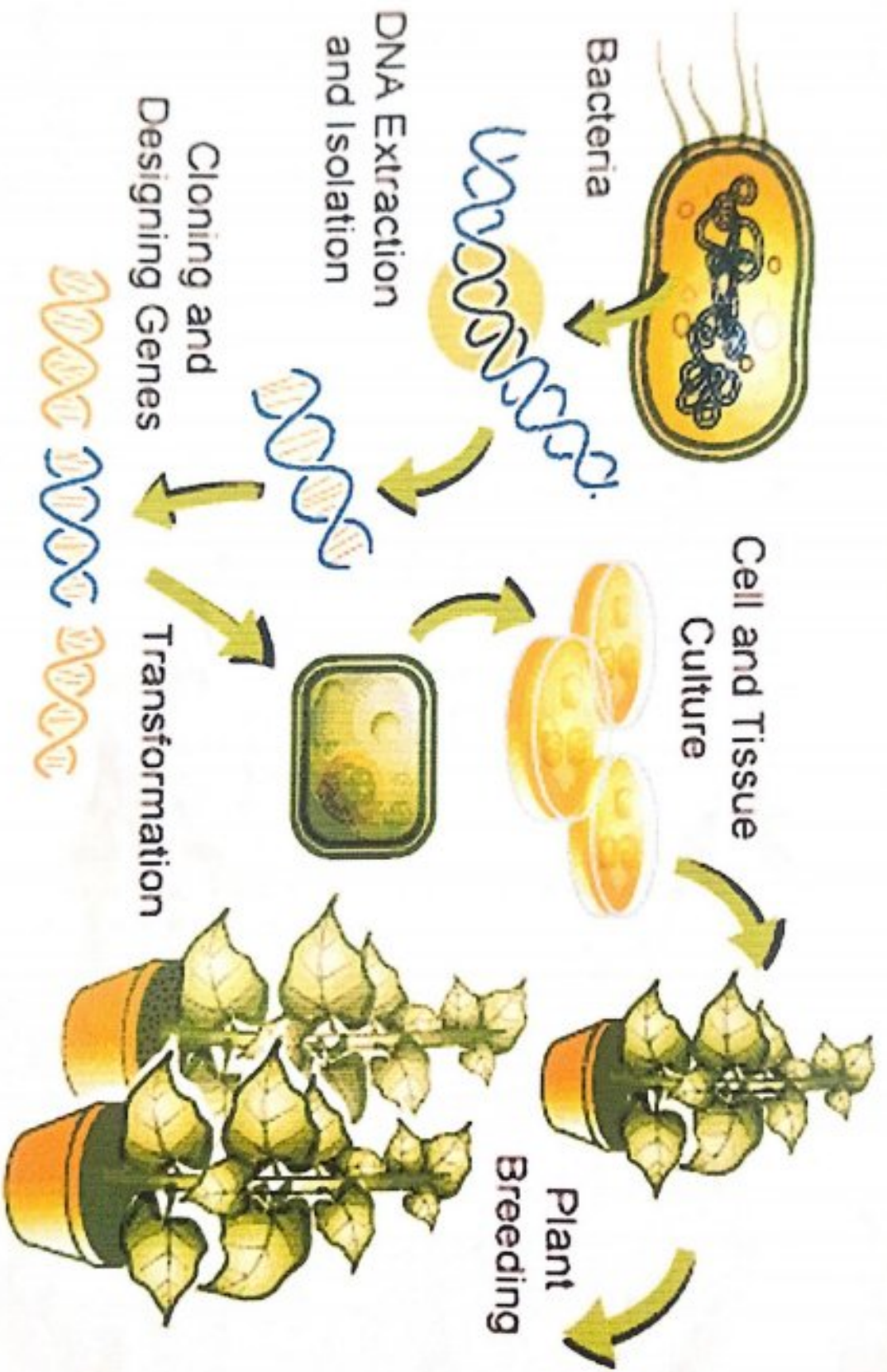
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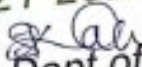
College roll no: 85

Examination seat no: S1937022

CERTIFICATE

This is to certify that Miss: Deepa Hampannavar of
BSc 5th semester has satisfactory completed the
project report in Botany subject as prescribed by
Rani Chennamma University Belagavi.

During year 2021-2022


Head Dept of Botany

Examiner:

- 1) 
16/12/22
- 2) 
16/12/22



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BSc 5th Sem

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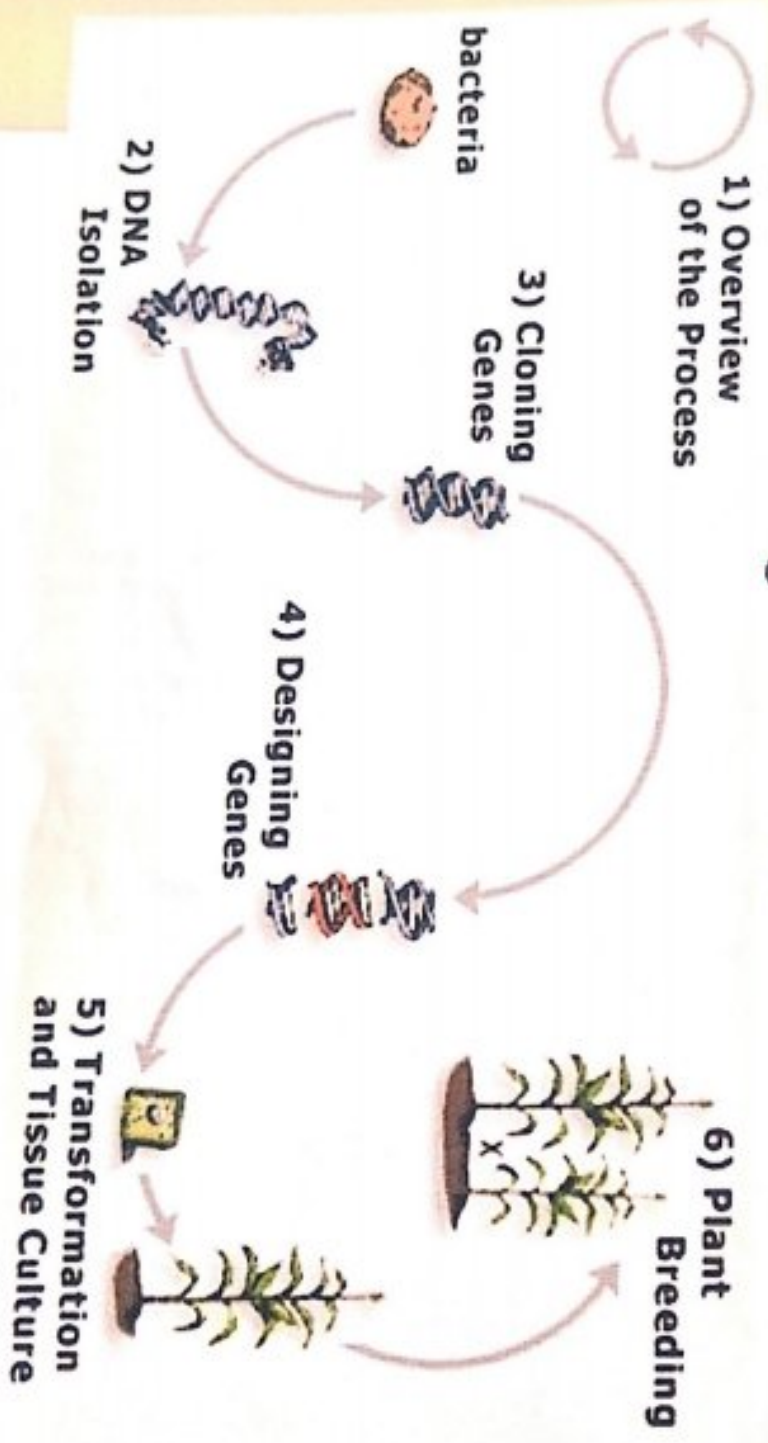
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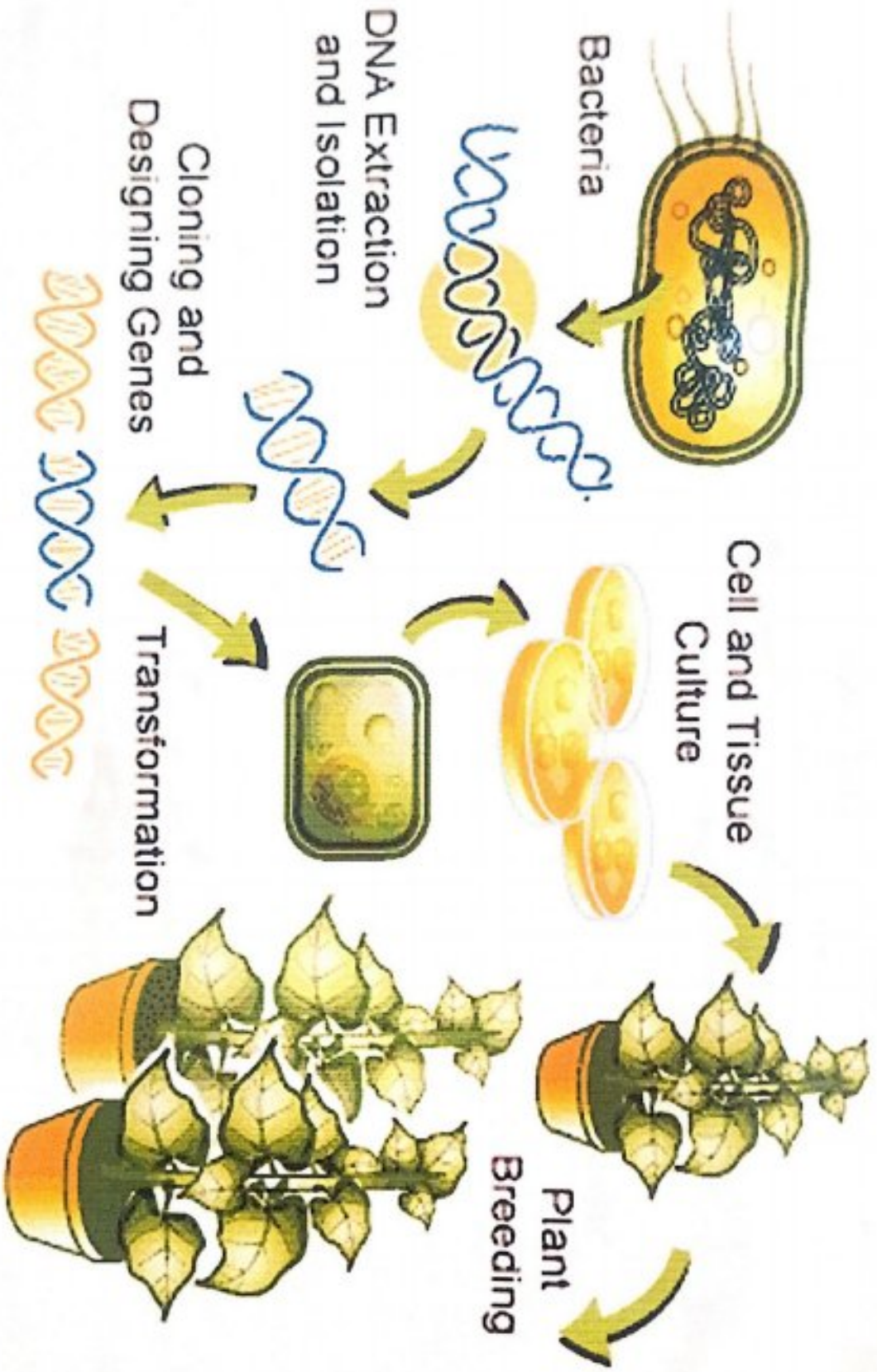
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V S BELLIHAL COMMERCE COLLEGE
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PROJECT REPORT

College roll no: 53

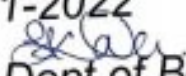
Examination seat no: S1937020


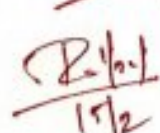
CERTIFICATE

This is to certify that Miss:chanchalaS Vaishnavof BSc
5th semester has satisfactory completed the project
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During year 2021-2022

Examiner:


Head Dept of Botany

- 1) 
15/2/22
- 2) 
15/2



V M K S R VASTRAD ARTS, SCIENCE &
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**AIMS & STRATERIES FOR
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PLANT**

PROJECT DONE BY

CHANCHAL S VAISHNAV

BSc 5th Sem

Botany

Rani Chennamma University Belagavi

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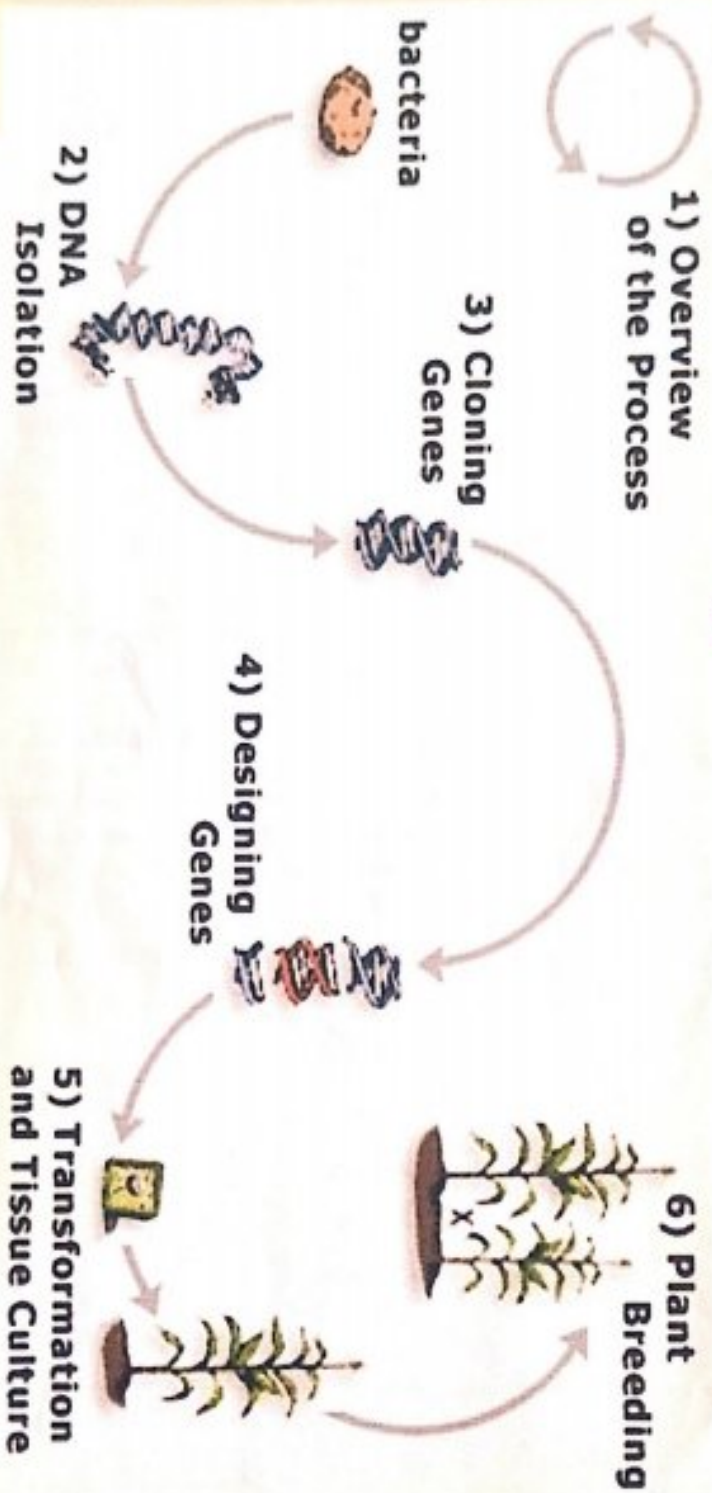
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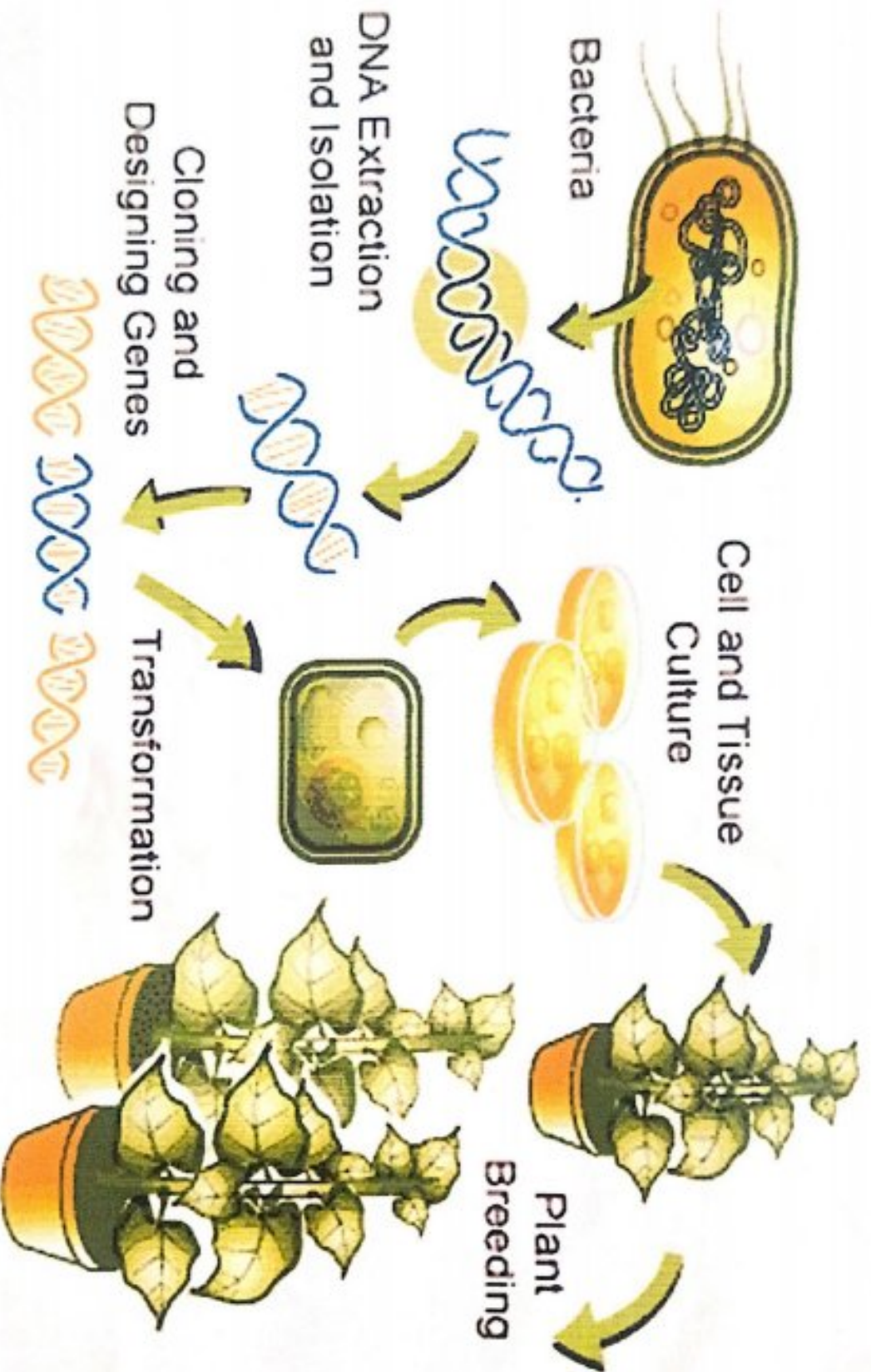
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HUNGUND**

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PROJECT DONE BY

Meharunnisa Talikoti

BSc 5th Sem

Botany

Rani Chennamma University Belagavi

2021-2022



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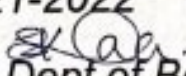
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CERTIFICATE

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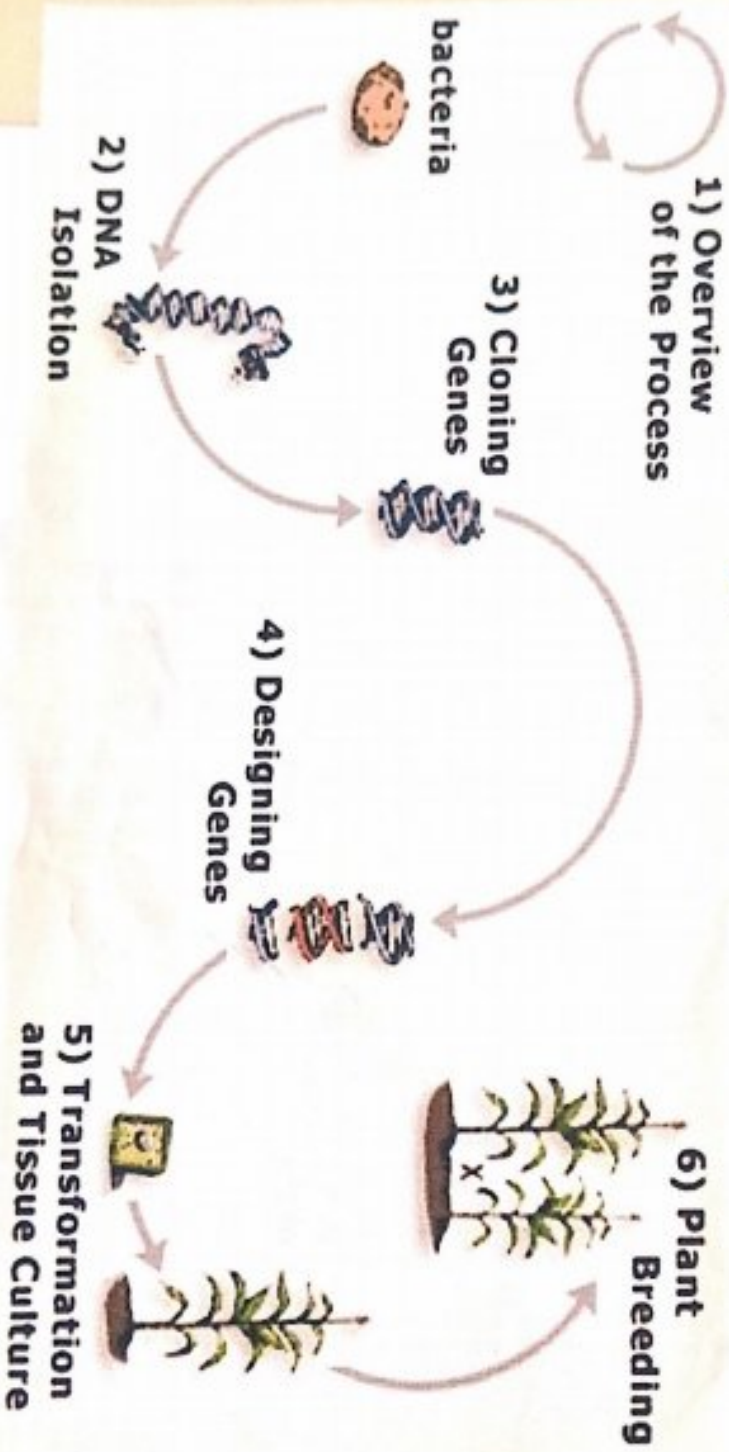
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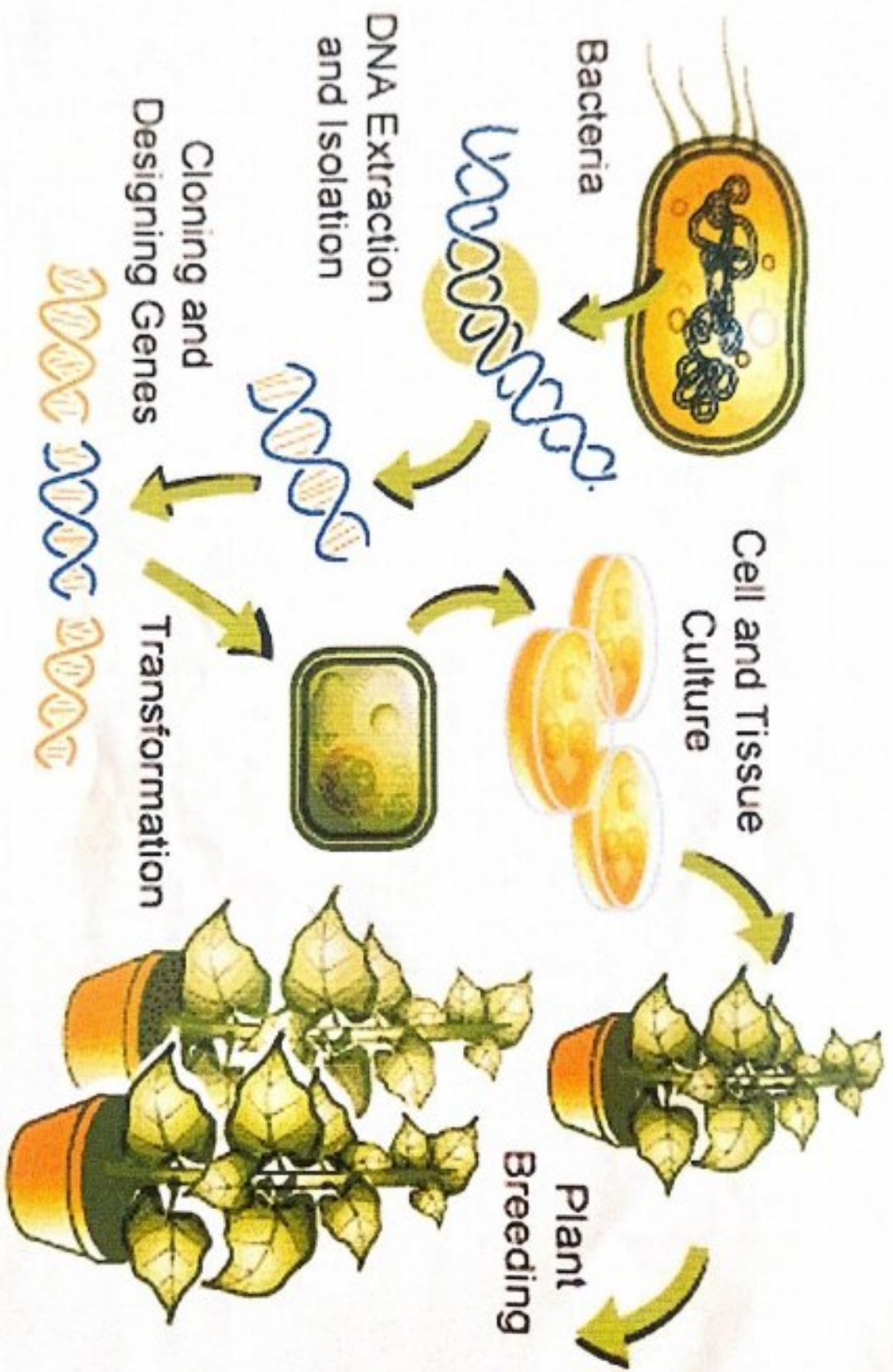
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College roll no: 48

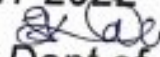
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CERTIFICATE

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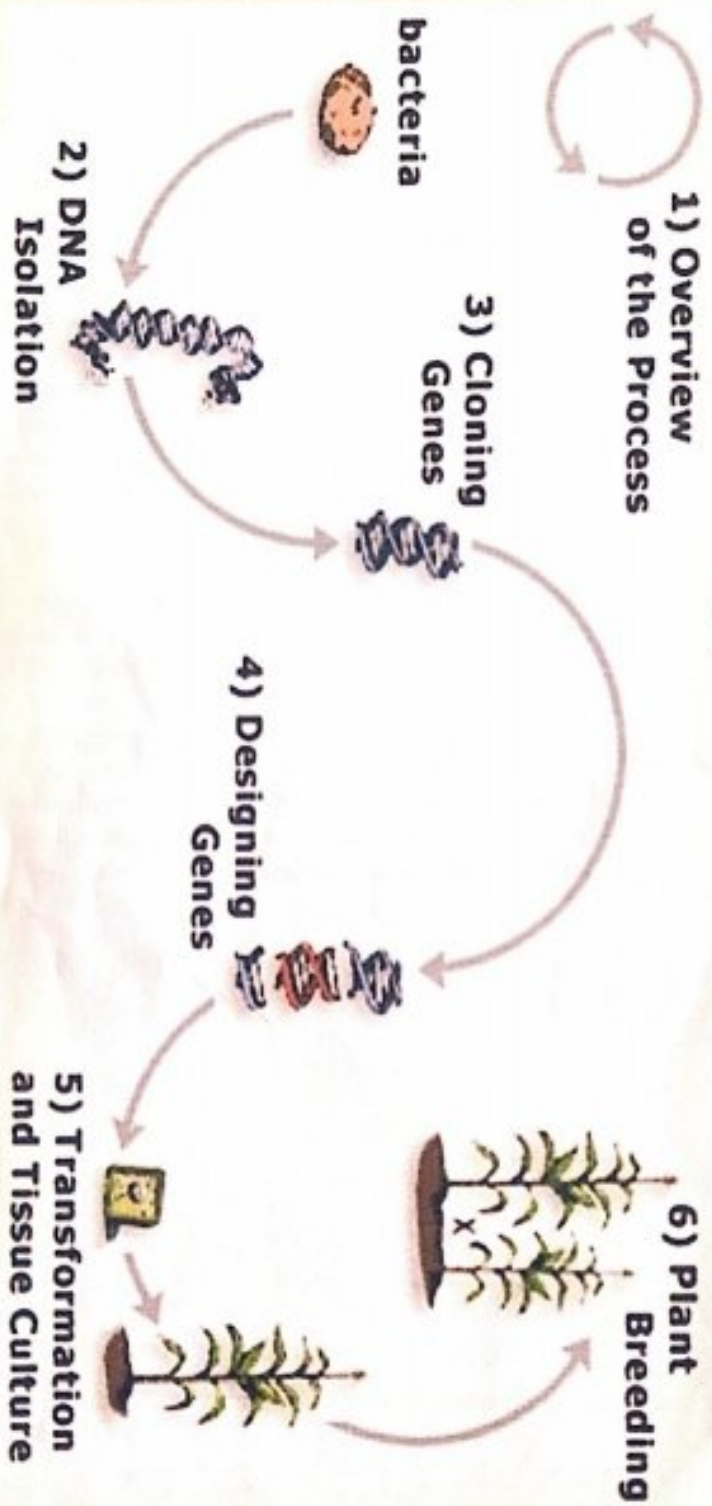
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V M K S R VASTRAD ARTS, SCIENCE,
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HUNGUND

PROJECT REPORT

College roll no: 22

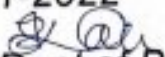
Examination seat no: S1937072

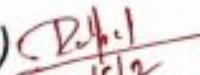
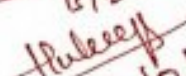
CERTIFICATE

This is to certify that Miss: Reshma S Hosamath of BSc
5th semester has satisfactorily completed the project
report in Botany subject as prescribed by Rani
Chennamma University Belagavi.

During year 2021-2022

Examiner:


Head Dept of Botany

- 1) 
15/2
- 2) 
15/2/22



V M K S R VASTRAD ARTS, SCIENCE &
V S BELLIHAL COMMERCE COLLEGE
HUNGUND

**AIMS & STRATERIES FOR
DEVELOPMENT OF TRANSGENIC
PLANT**

PROJECT DONE BY

RESHMA S HOSAMATH

BSc 5th Sem

Botany

Rani Chennamma University Belagavi

2021-2022

Aims and strategies For Development Transgenic Plant

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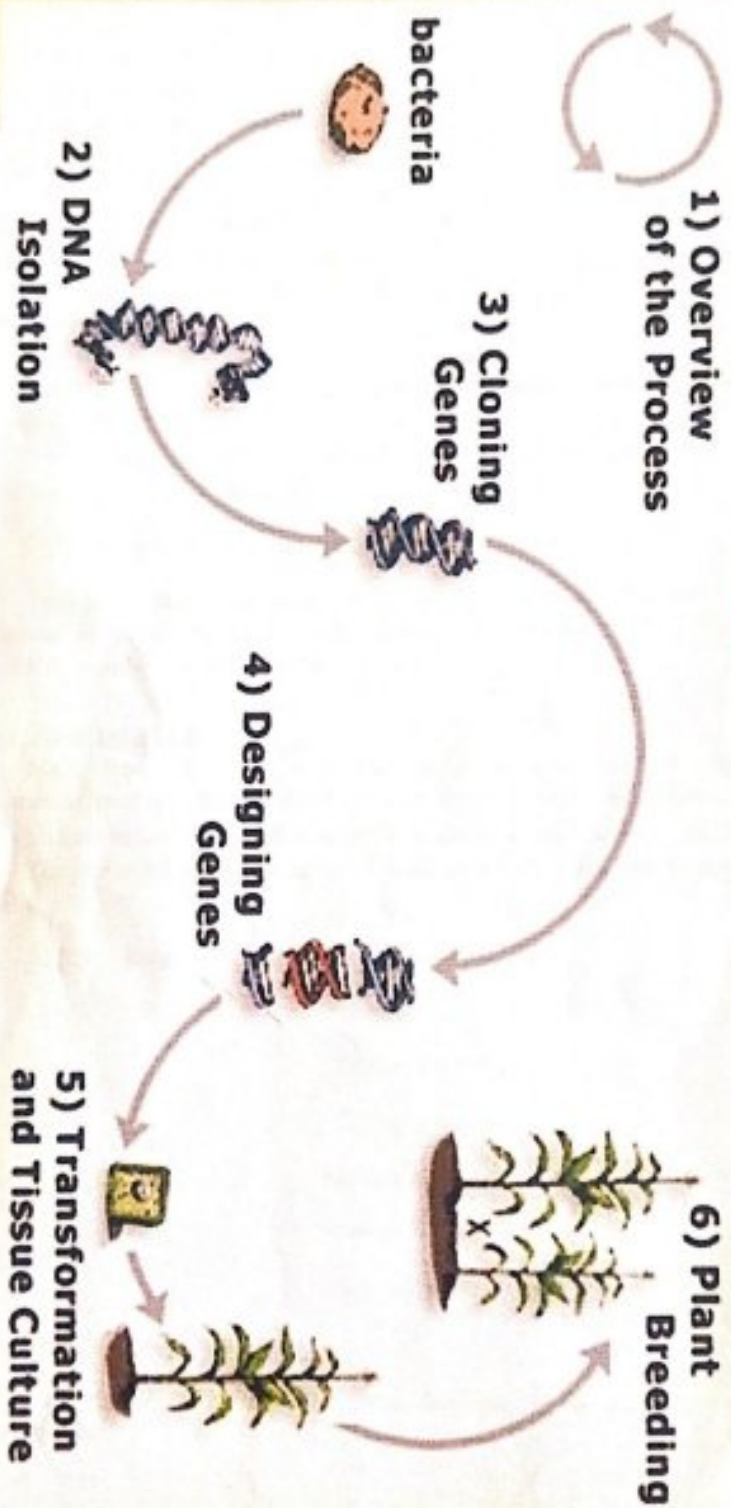
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Transgenic Plants



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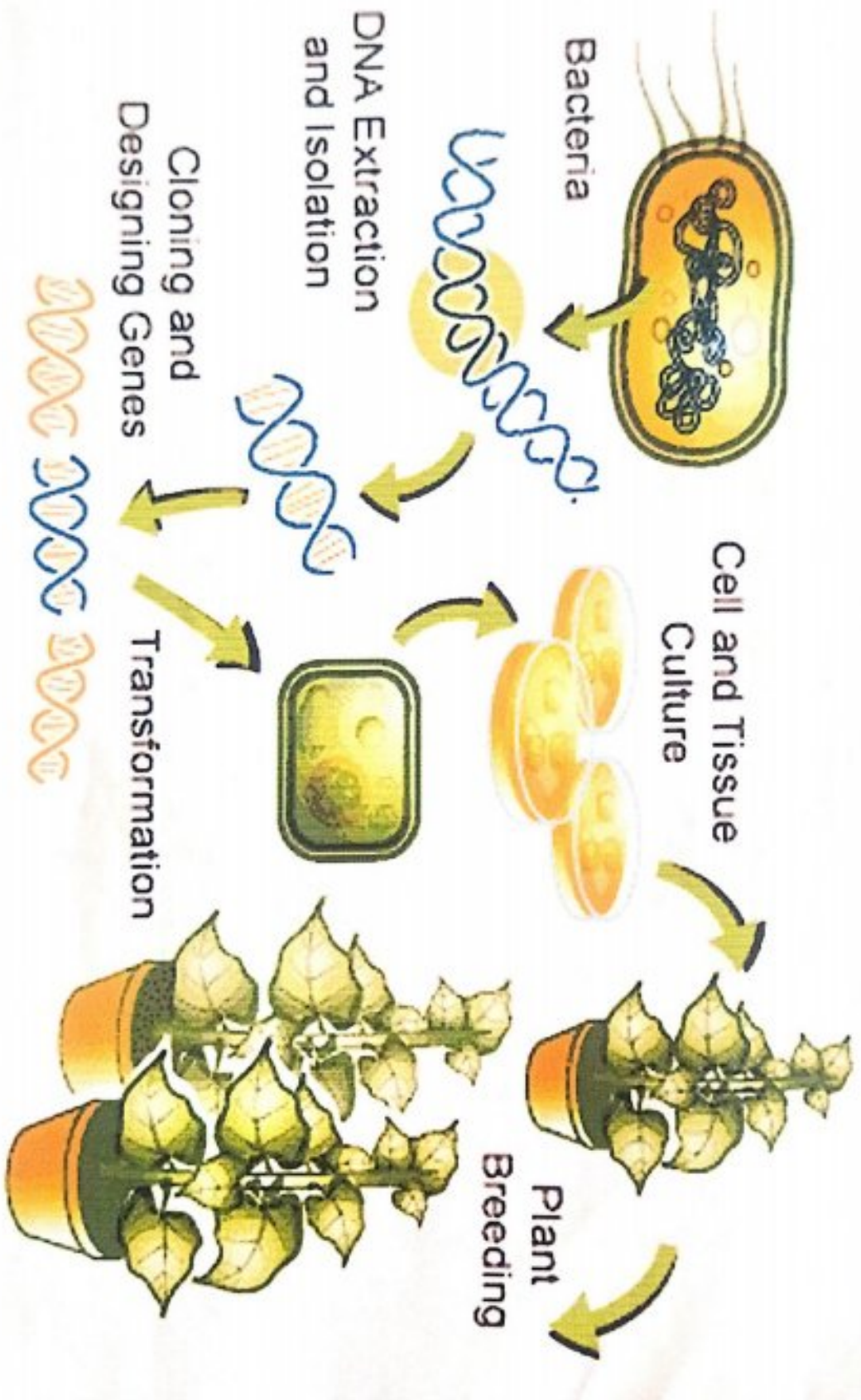
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HUNGUND

AIMS & STRATERIES FOR
DEVELOPMENT OF TRANSGENIC
PLANT

PROJECT DONE BY

SANGEETA Y WALIKAR

BSc 5th Sem

Botany

Rani Chennamma University Belagavi

2021-2022



M K S R VASTRAD ARTS, SCIENCE,
V S BELLIHAL COMMERCE COLLEGE
HUNGUND

PROJECT REPORT

College roll no: 51

Examination seat no: S1937076

CERTIFICATE

This is to certify that Miss: Sangeeta y walikar of BSc 5th semester has satisfactory completed the project report in Botany subject as prescribed by Rani Chennamma University Belagavi.

During year 2021-2022

Examiner:

Head Dept of Botany

1) 

2) 
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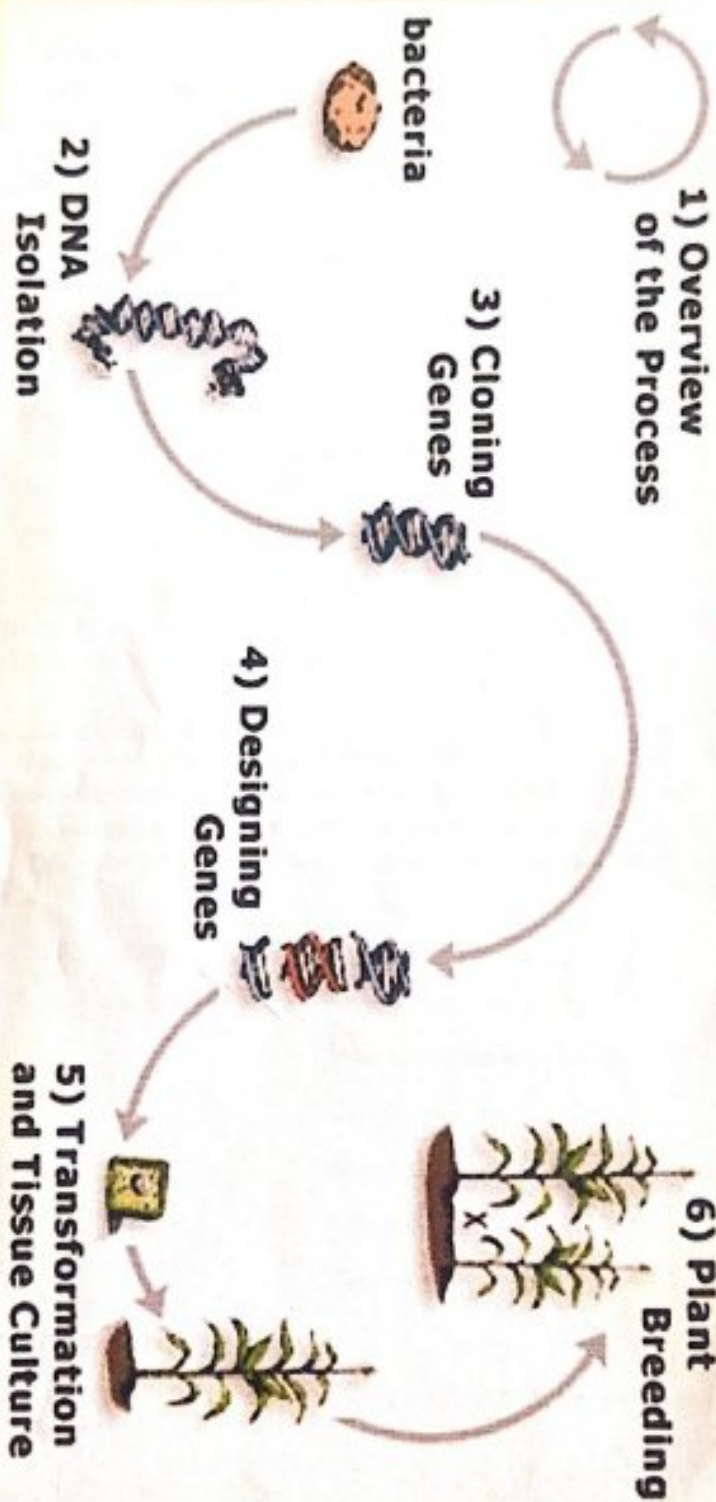
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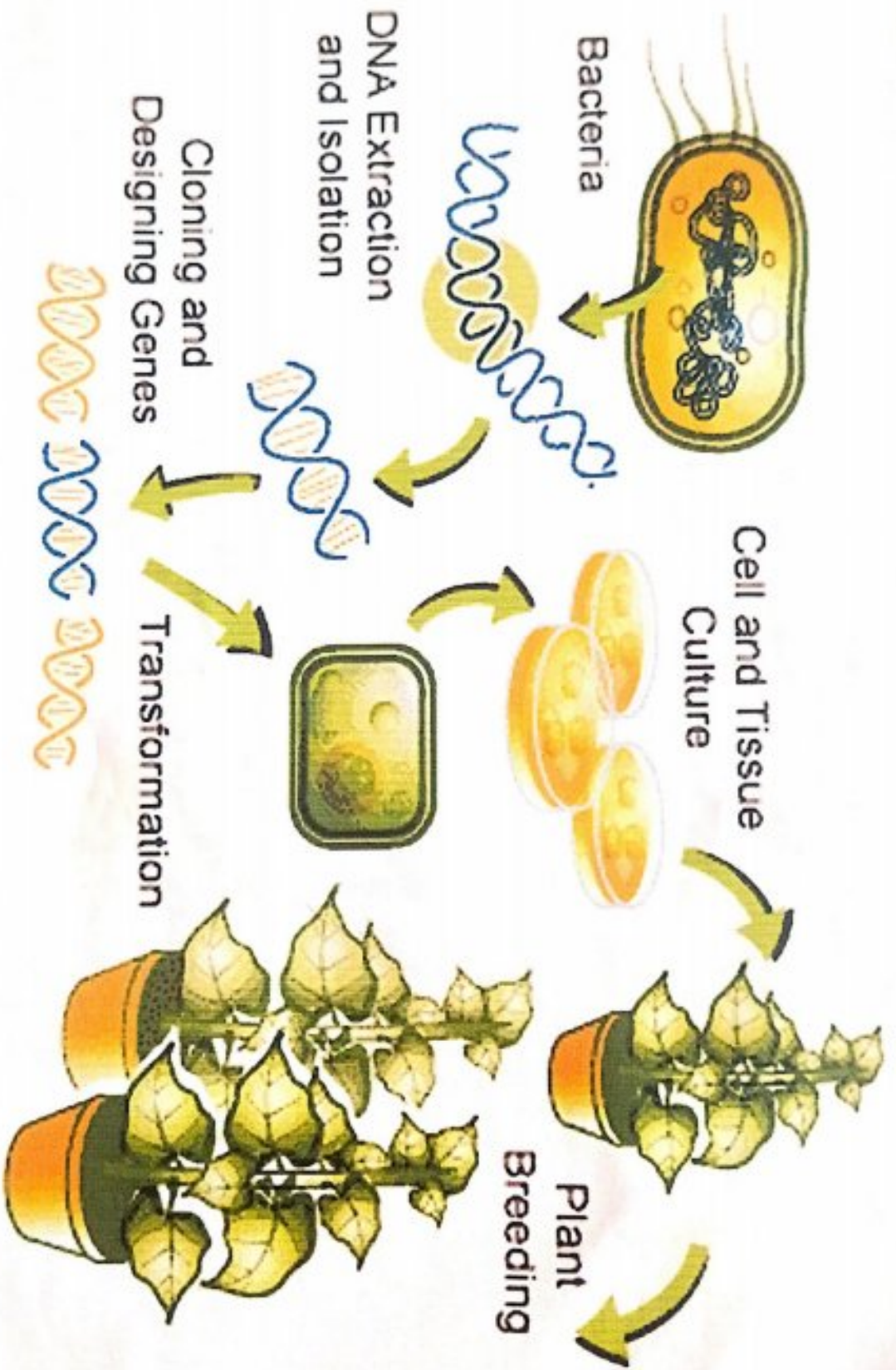
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PROJECT REPORT

College roll no: 47

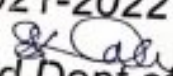
Examination seat no: S1937078

CERTIFICATE

This is to certify that Mr. Satish H B of BSc 5th semester has satisfactory completed the project report in Botany subject as prescribed by Rani Chennamma University Belagavi.

During year 2021-2022

Examiner:


Head Dept of Botany

1) 
14/2/22

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**AIMS & STRATERIES FOR
DEVELOPMENT OF TRANSGENIC
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PROJECT DONE BY

Satish H B

BSc 5th Sem

Botany

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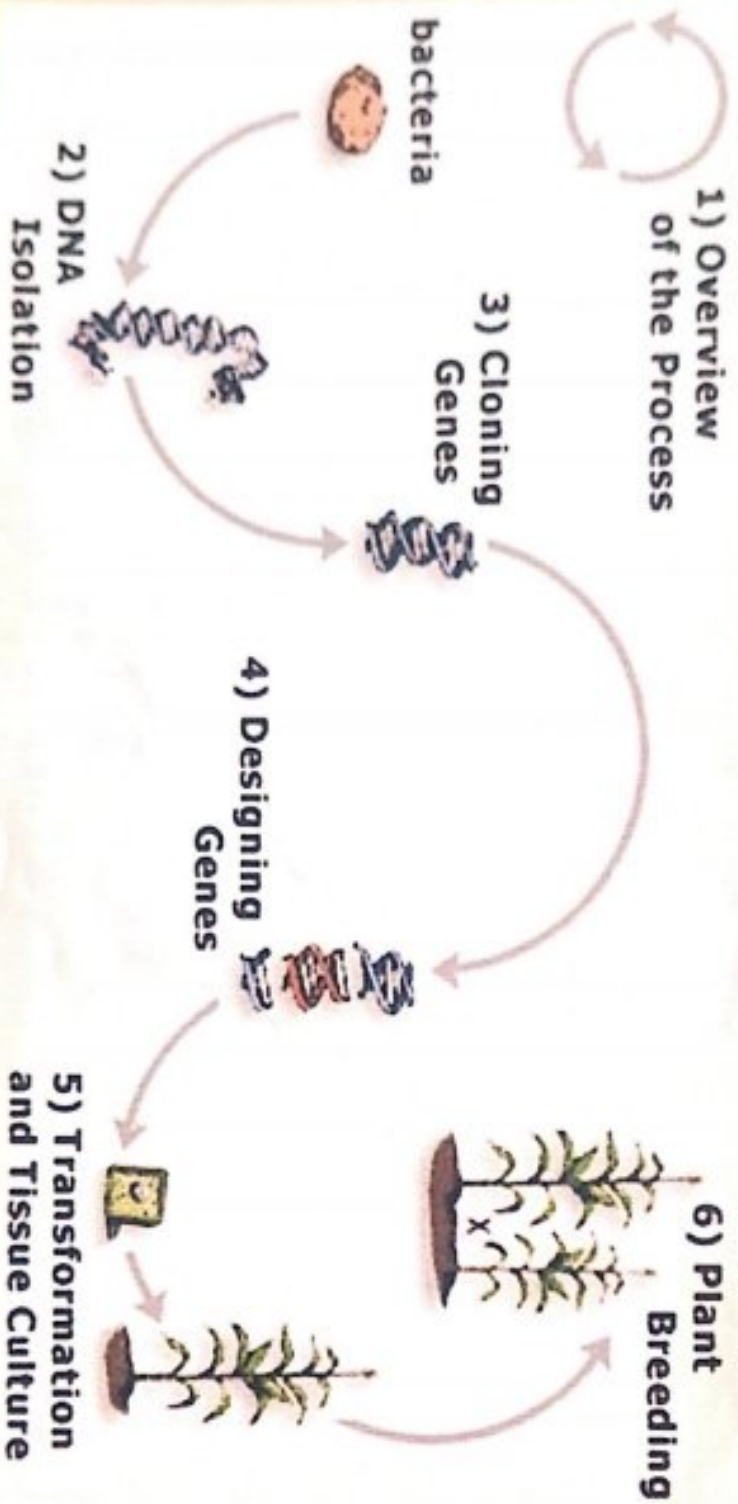
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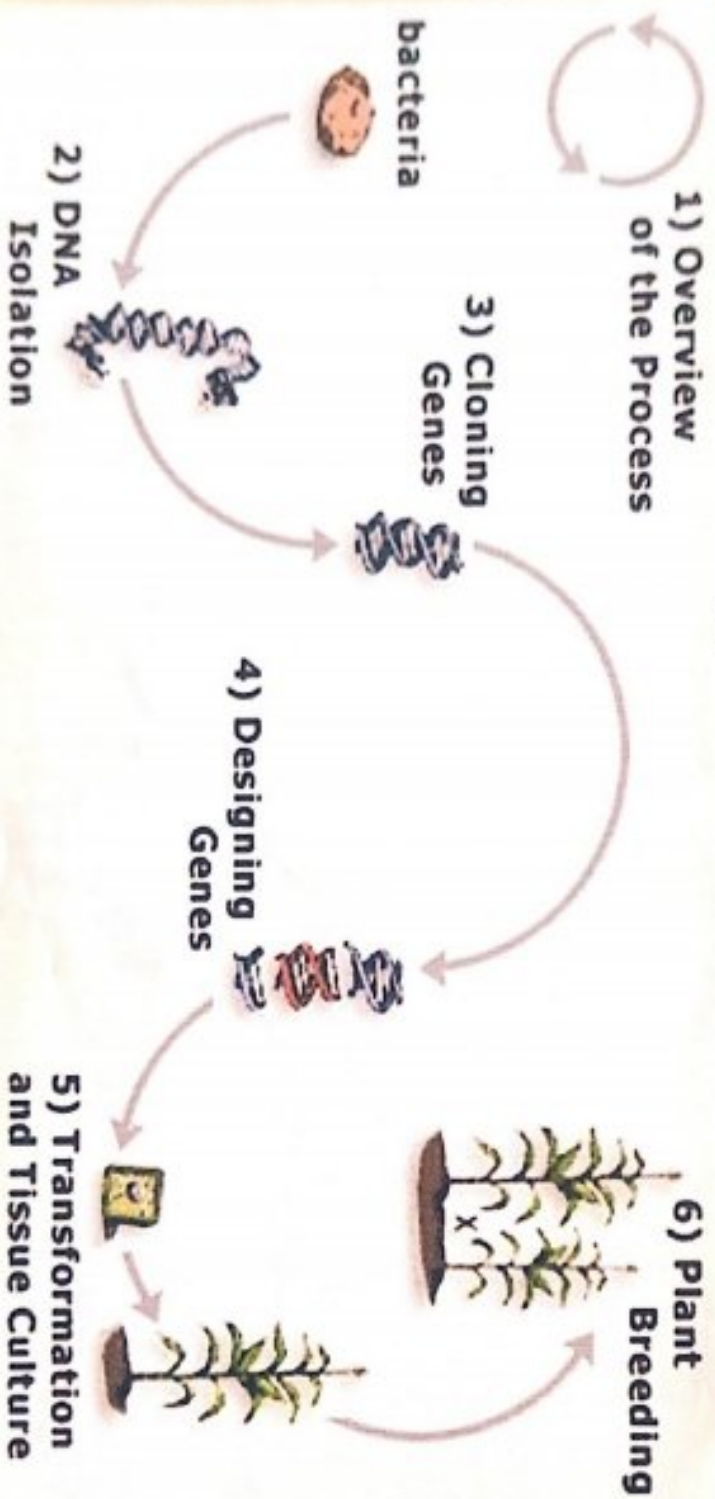
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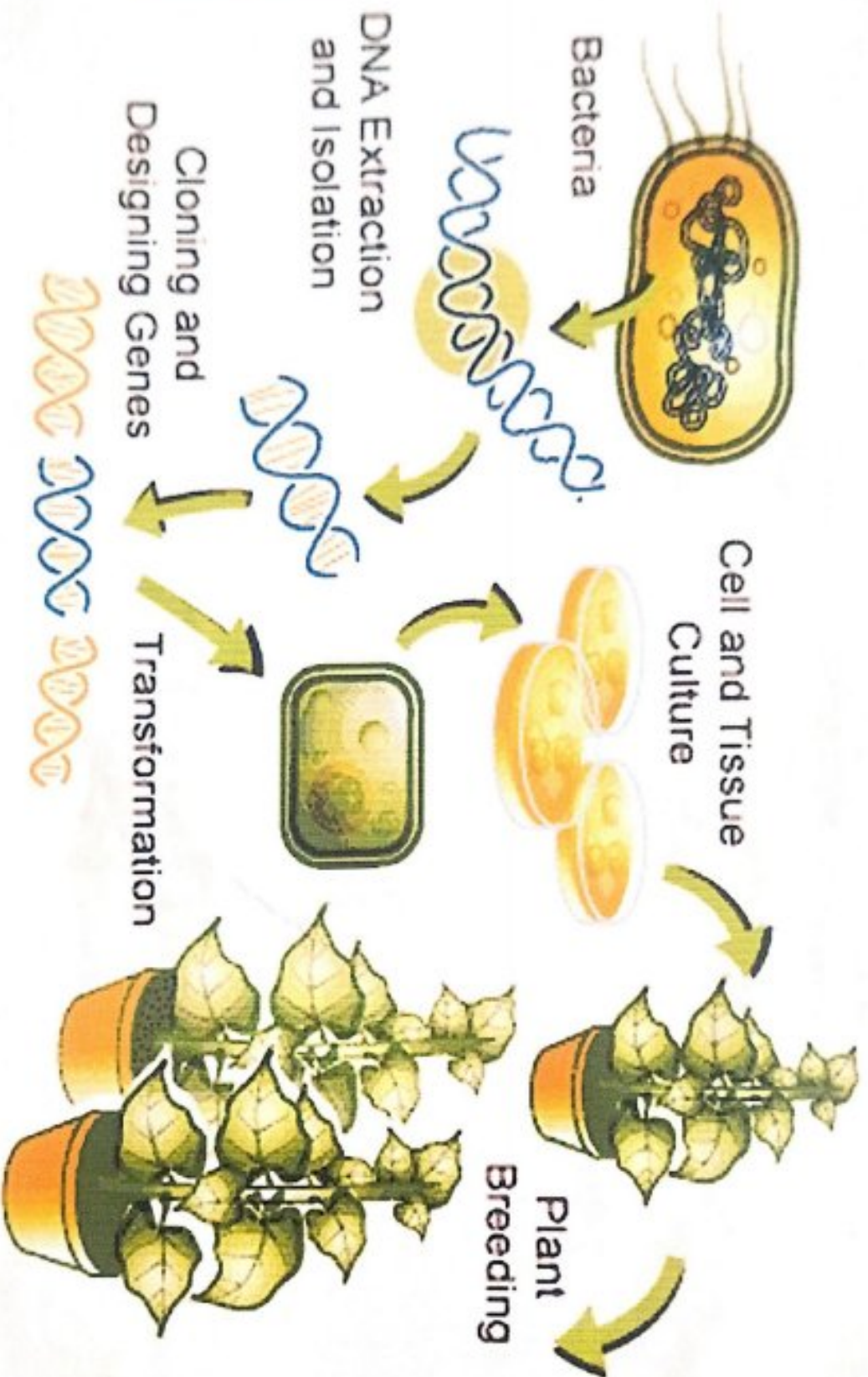
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**V M K S R VASTRAD ARTS, SCIENCE,
V S BELLIHAL COMMERCE
COLLEGE HUNGUND
PROJECT REPORT**

College roll no: 78

Examination seat no: S1937082

CERTIFICATE

This is to certify that **Mr: Sharanbasava S Hiremath** of BSc 5th semester has satisfactory completed the project report in Botany subject as prescribed by Rani Chennamma University Belagavi.

During year 2021-2022

Examiner:

S. K. A.
Head Dept of Botany

- 1) *H. K. S.*
14/2/22
- 2) *B. K. S.*
14/2/22



**V M K S R VASTRAD ARTS, SCIENCE &
V S BELLIHAL COMMERCE COLLEGE
HUNGUND**

**AIMS & STRATERIES FOR
DEVELOPMENT OF TRANSGENIC
PLANT**

PROJECT DONE BY

SHARANABASAVA S HIEMATH

BSc 5th Sem

Botany

Rani Chennamma University Belagavi

2021-2022

Aims and strategies For Development Transgenic Plant

Introduction of Transgenic plant :

The plants in which a functional foreign gene has been incorporated by any biotechnological methods that generally not present in plant are called transgenic plant. A Number of transgenic plants carrying genes for trails of economic importance have either been released for commercial cultivation or under field trials.

There are several methods which are used in gene transfer

- 1) Electroporation
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- ❖ In 1994 the European Union approved by the US Environmental protection Agency making it the Country's first pesticide producing crop
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Modification of DNA using genetic Engineering techniques

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The purpose of inserting a combination of genes in a plant so as to make it as useful and productive as possible

Examples in food crops include resistance to certain pests, diseases, or Environmental conditions, reduction of spoilage, or resistance to chemical treatments or improving the nutrient profile of the crop.

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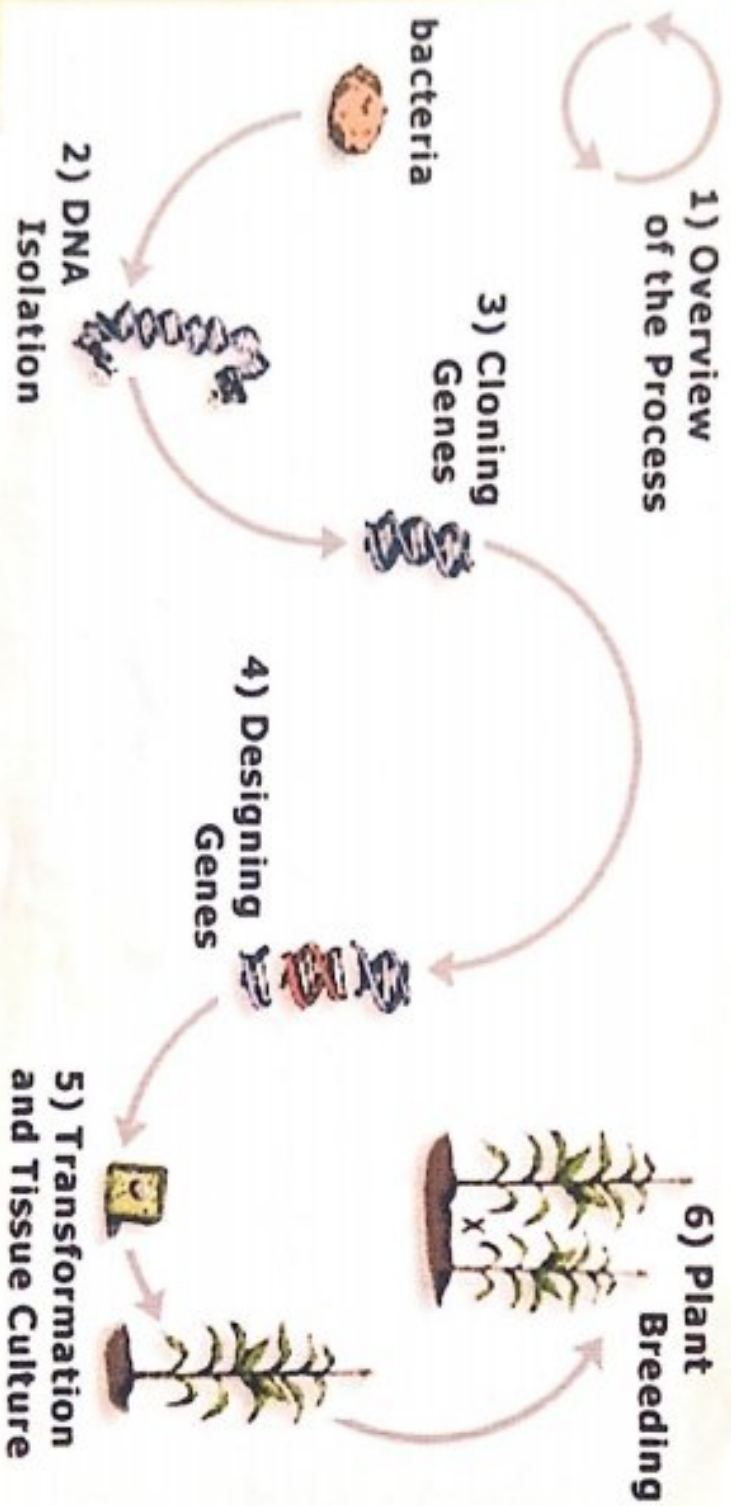
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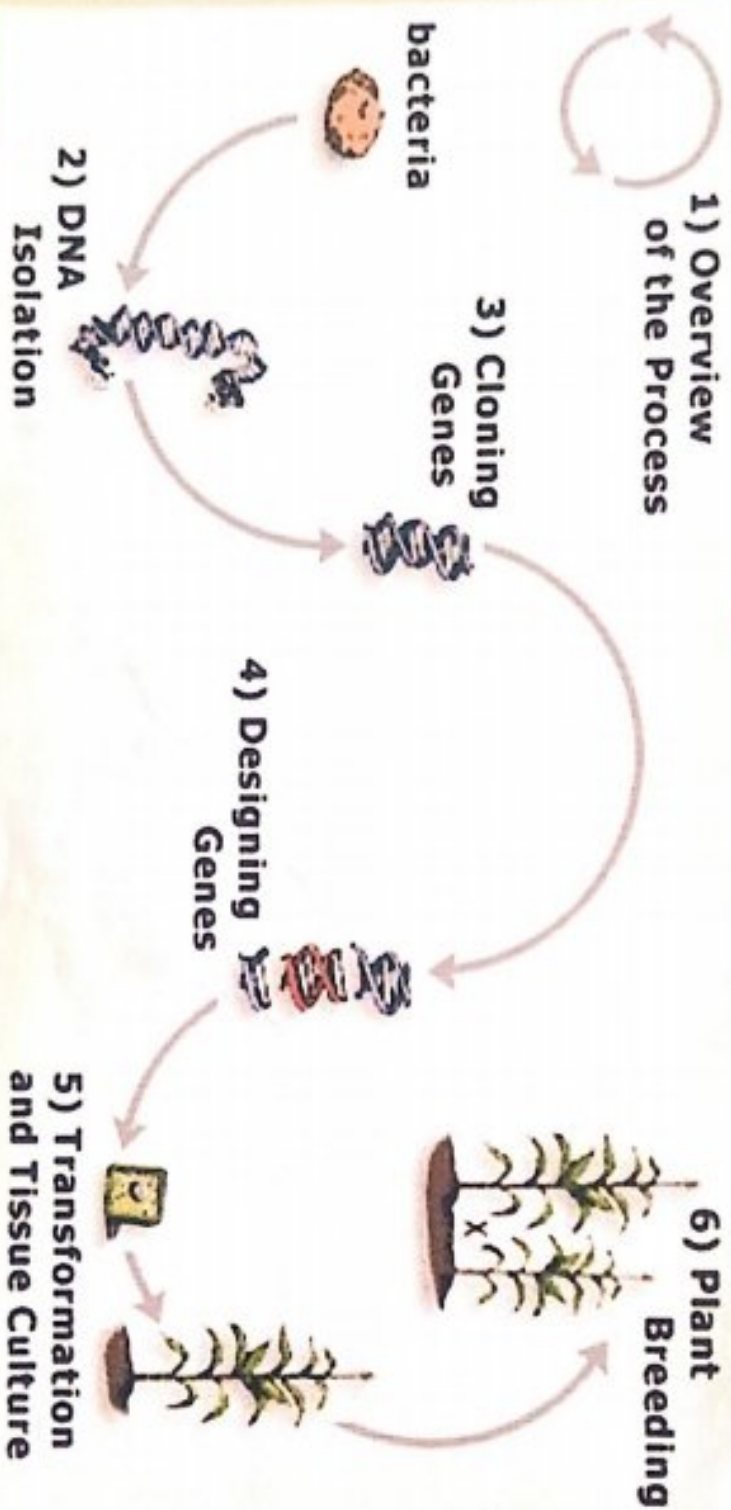
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a) Herbicide Resistance

Transgenic Plants



Transgenic Plants



- b) Insect Resistance
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- d) Improved storage proteins
- e) Improved oils and fats
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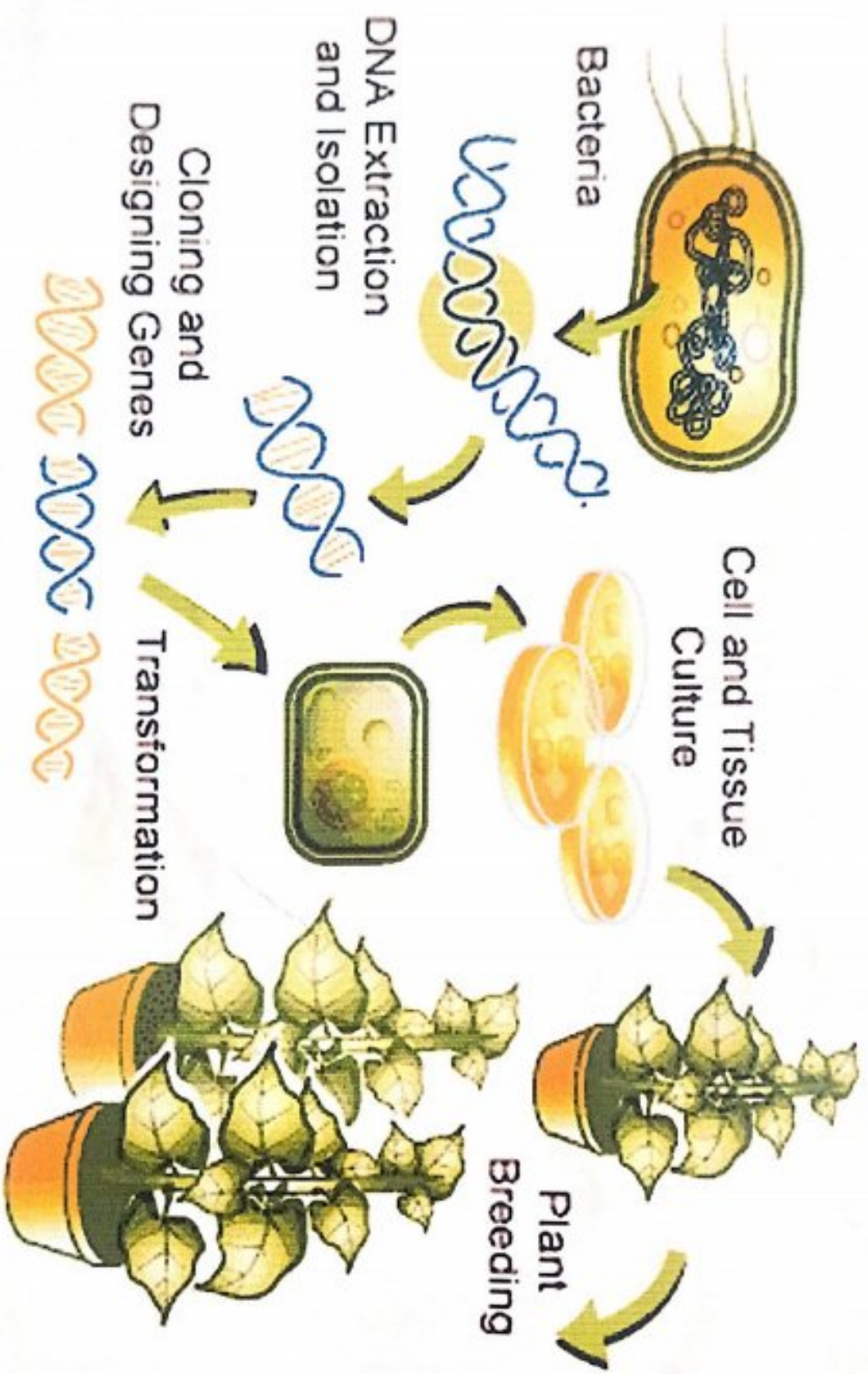
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College roll no: 01

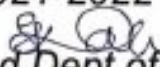
Examination seat no: S1937085

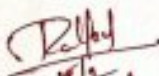

CERTIFICATE

This is to certify that Miss: Shilpa S Tarival of BSc 5th semester has satisfactorily completed the project report in Botany subject as prescribed by Rani Chennamma University Belagavi.

During year 2021-2022

Examiner:


Head Dept of Botany

- 1) 
15/2
- 2) 
15/2/22



V M K S R VASTRAD ARTS, SCIENCE &
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PROJECT DONE BY

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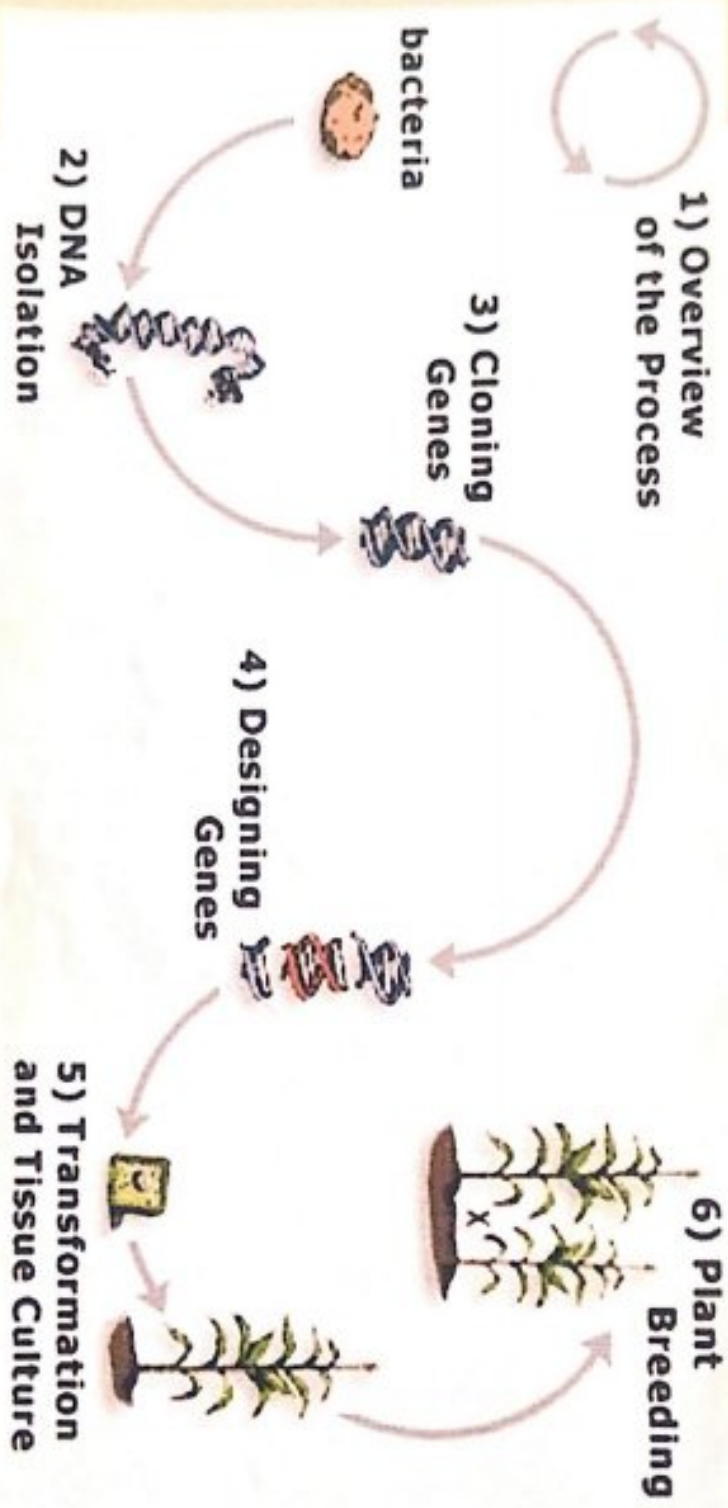
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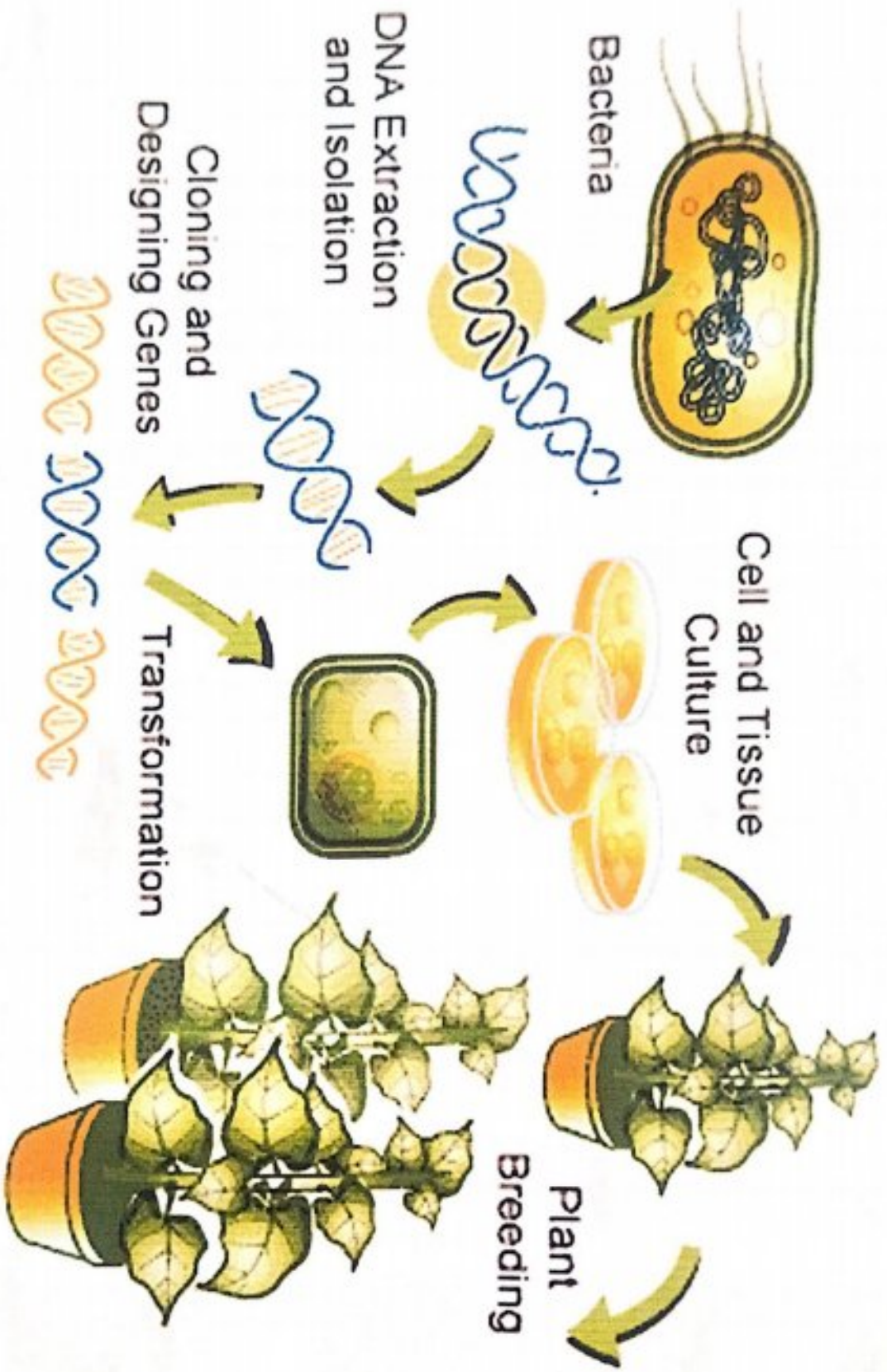
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**V M K S R VASTRAD ARTS, SCIENCE,
V S BELLIHAL COMMERCE
COLLEGE HUNGUND
PROJECT REPORT**

College roll no: 25

Examination seat no: S1937089

CERTIFICATE

This is to certify that **Mr: Shridhar Patil** of BSc 5th semester has satisfactory completed the project report in Botany subject as prescribed by Rani Chennamma University Belagavi.

During year 2021-2022

Examiner:

S. K. A.
Head Dept of Botany

- 1) *H. K. S.*
15/12/22
- 2) *(R. K. S.)*
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**V M K S R VASTRAD ARTS, SCIENCE &
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HUNGUND**

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PROJECT DONE BY

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BSc 5th Sem

Botany

Rani Chennamma University Belagavi

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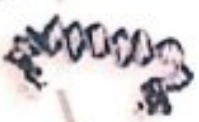
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2) DNA
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6) Plant
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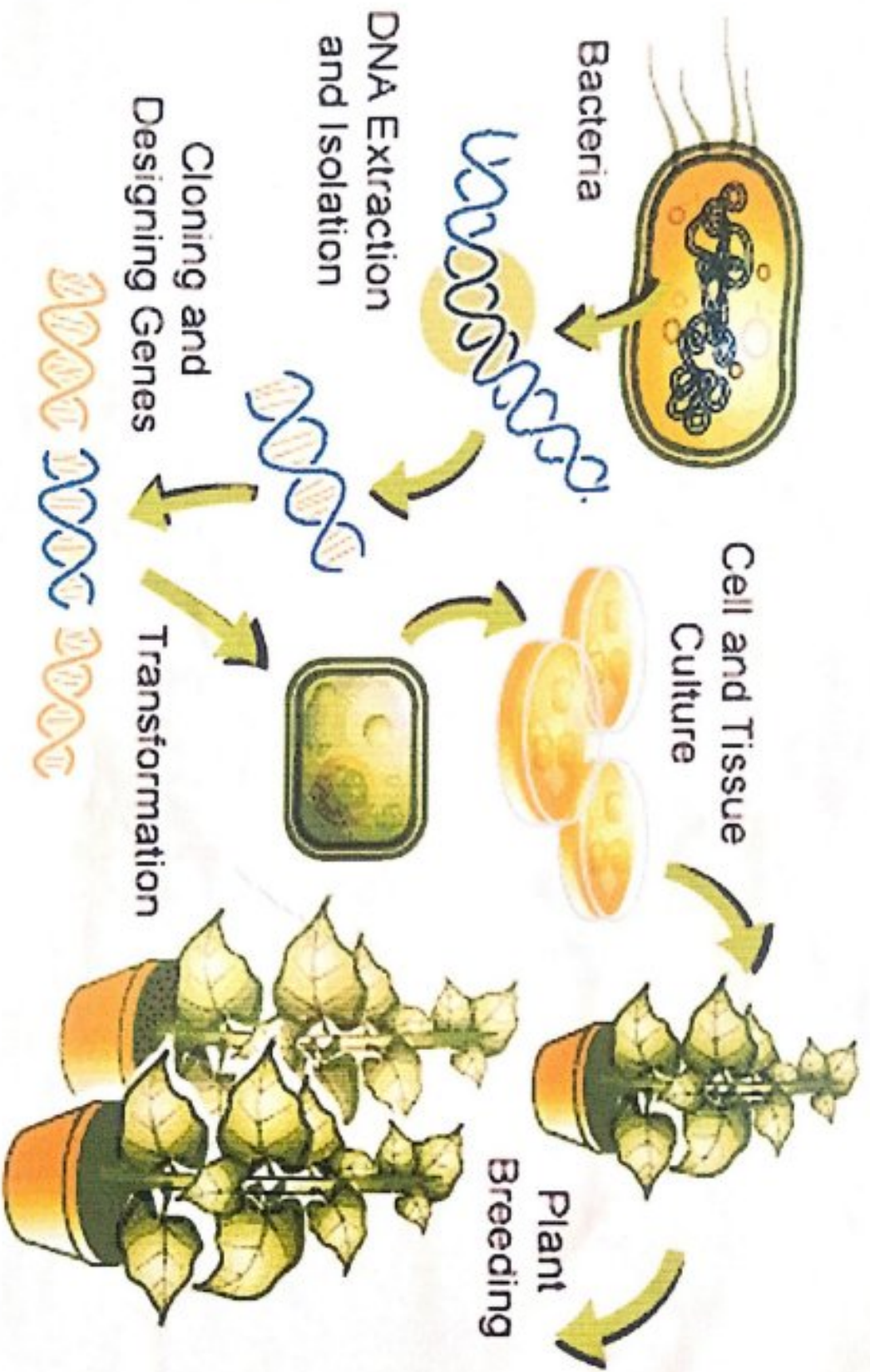
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College roll no: 21

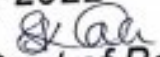
Examination seat no: S1937098

CERTIFICATE

This is to certify that Miss: Tejashwini S Kirasur of BSc
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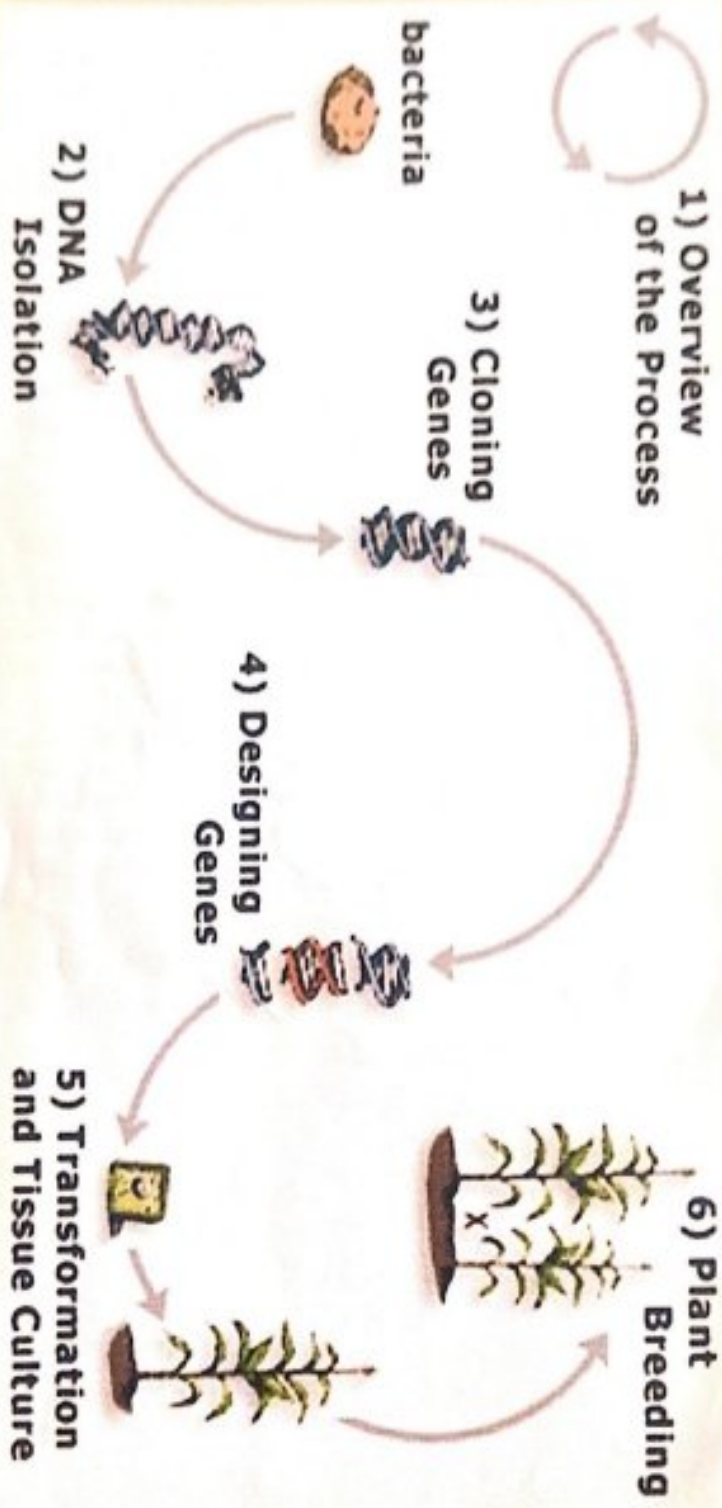
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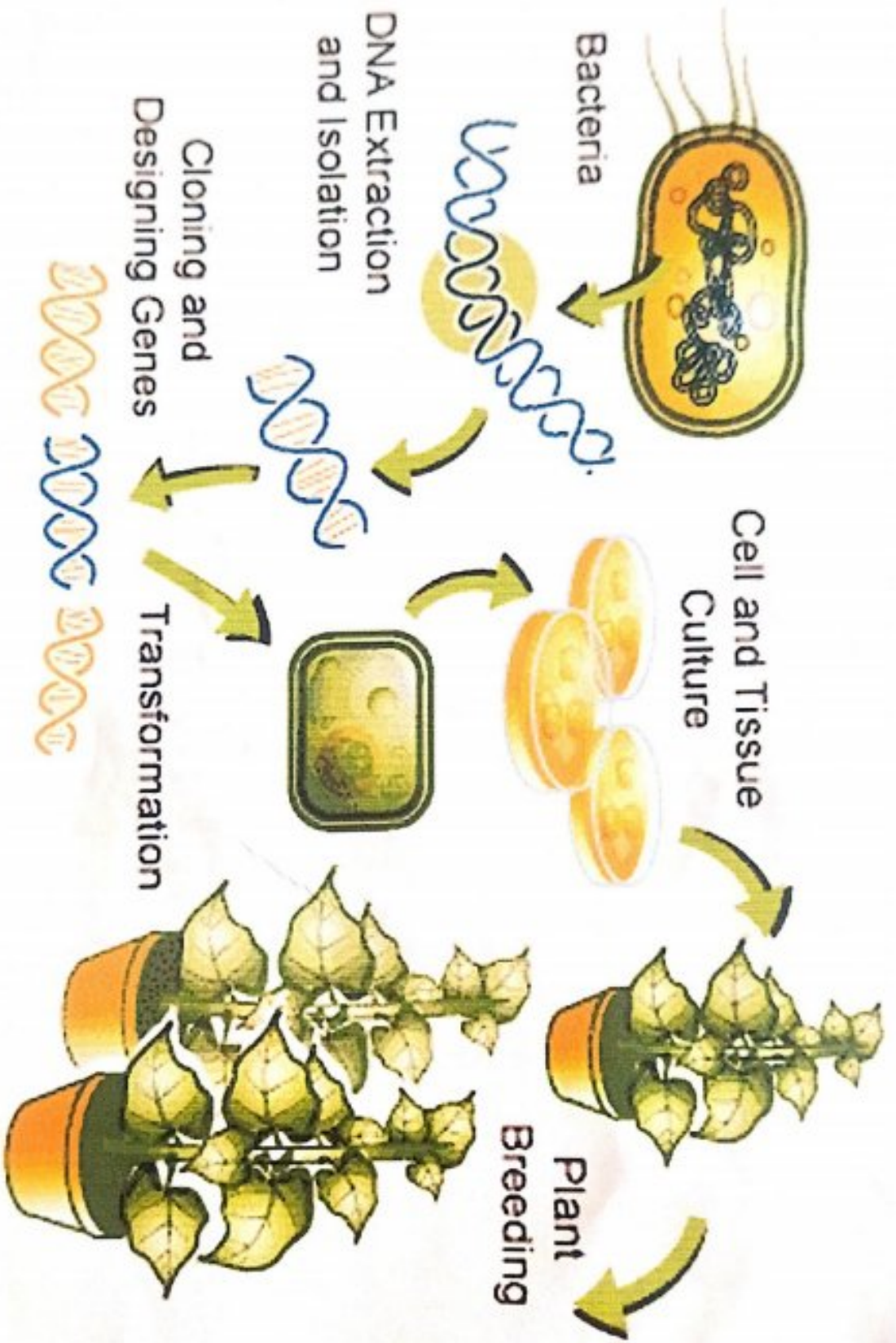
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**V M K S R VASTRAD ARTS, SCIENCE &
V S BELLIHAL COMMERCE COLLEGE
HUNGUND**

**AIMS & STRATERIES FOR
DEVELOPMENT OF TRANSGENIC
PLANT**

PROJECT DONE BY

VIJAYALAXMI PATTAR

BSc 5th Sem

Botany

Rani Chennamma University Belagavi

2021-2022



V M K S R VASTRAD ARTS, SCIENCE,
V S BELLIHAI COMMERCIAL COLLEGE
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PROJECT REPORT

College roll no: 66

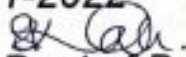
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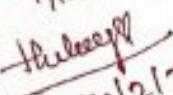
CERTIFICATE

This is to certify that Mrs: Vijayalaxmi Pattar of BSc 5th semester has satisfactorily completed the project report in Botany subject as prescribed by Rani Chennamma University Belagavi.

During year 2021-2022

Examiner:


Head Dept of Botany

- 1) 
14/12
- 2) 
14/12/22

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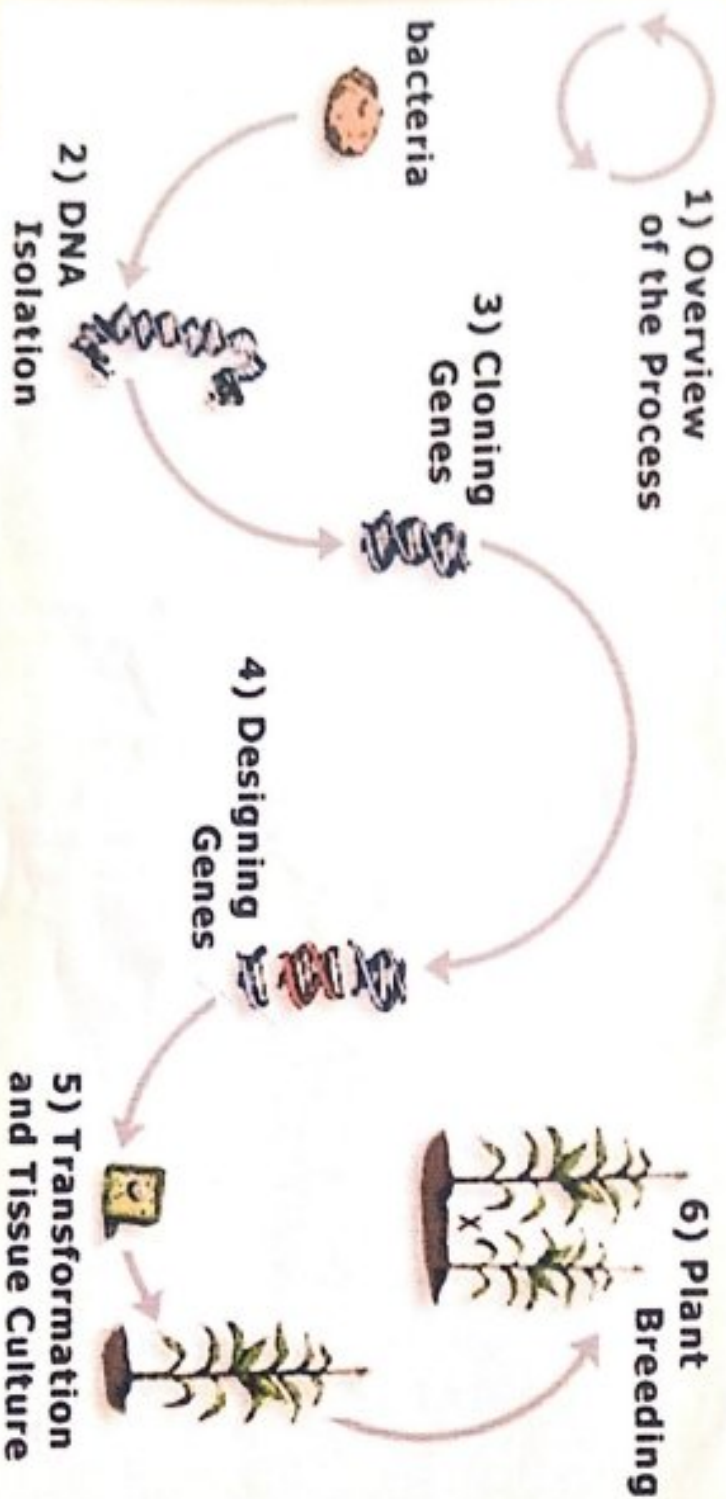
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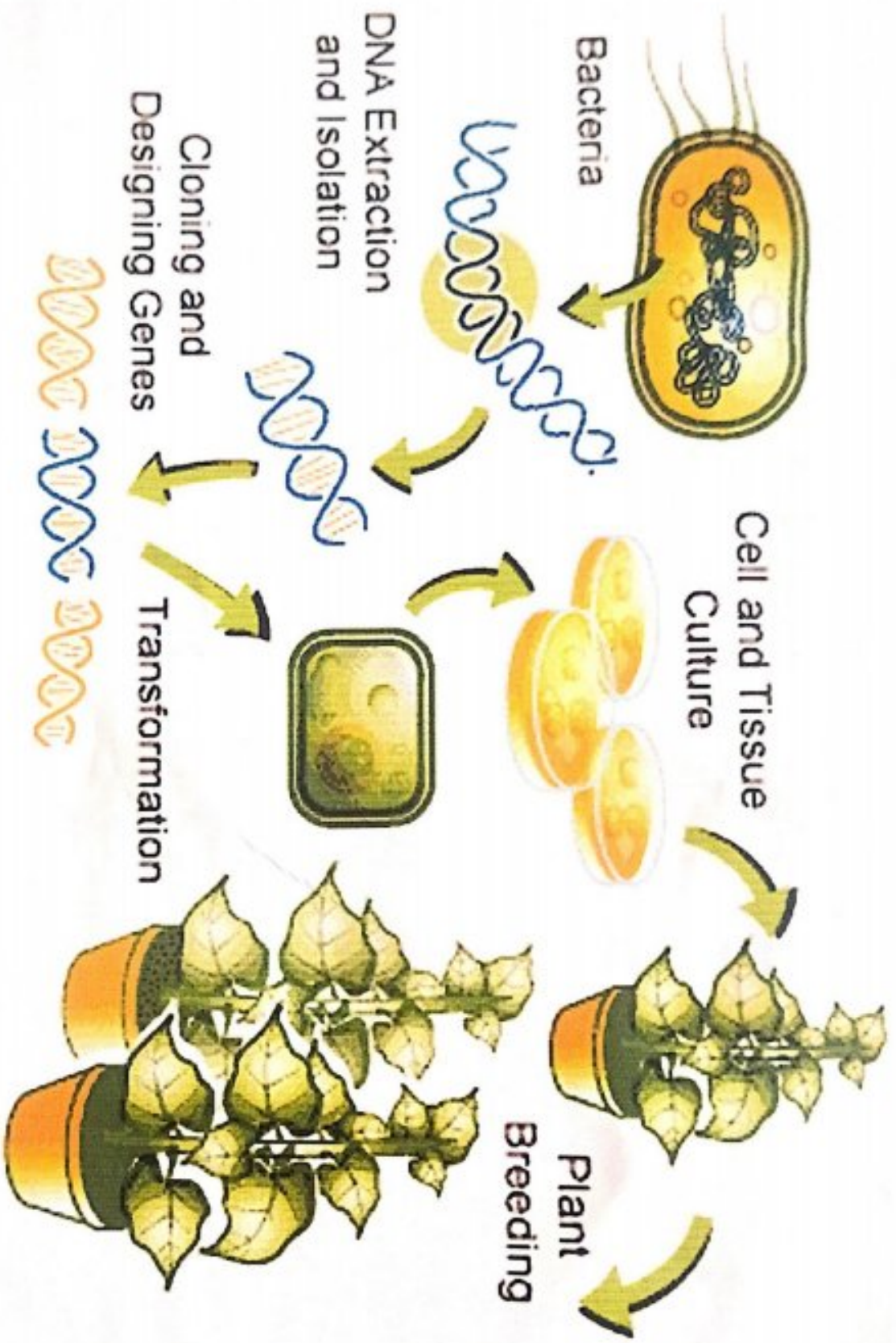
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PROJECT REPORT

College roll no: 58

Examination seat no: S1937009

CERTIFICATE

This is to certify that Miss: Aruna k Gantiof BSc 5th semester has satisfactory completed the project report in Botany subject as prescribed by Rani Chennamma University Belagavi.

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Examiner:

[Signature]
Head Dept of Botany

- 1) *[Signature]*
15/2
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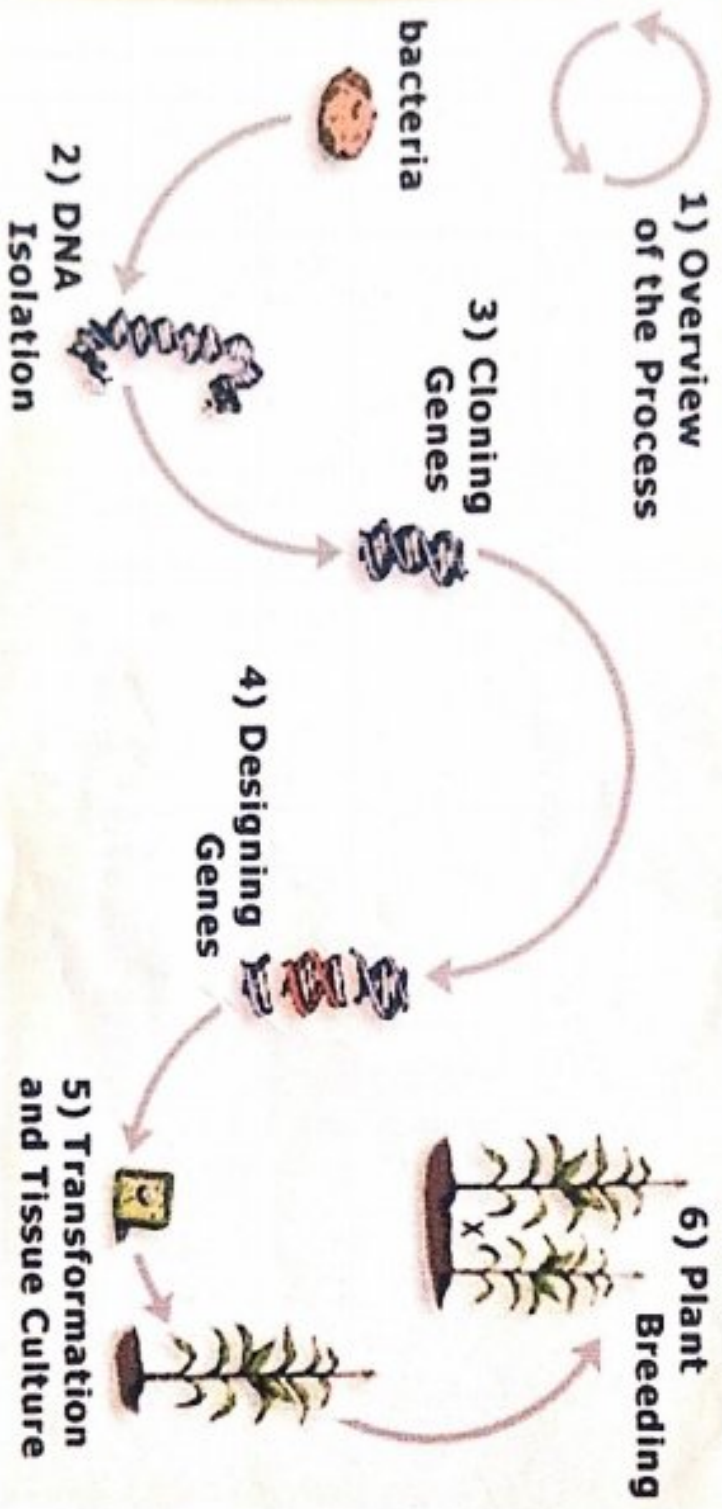
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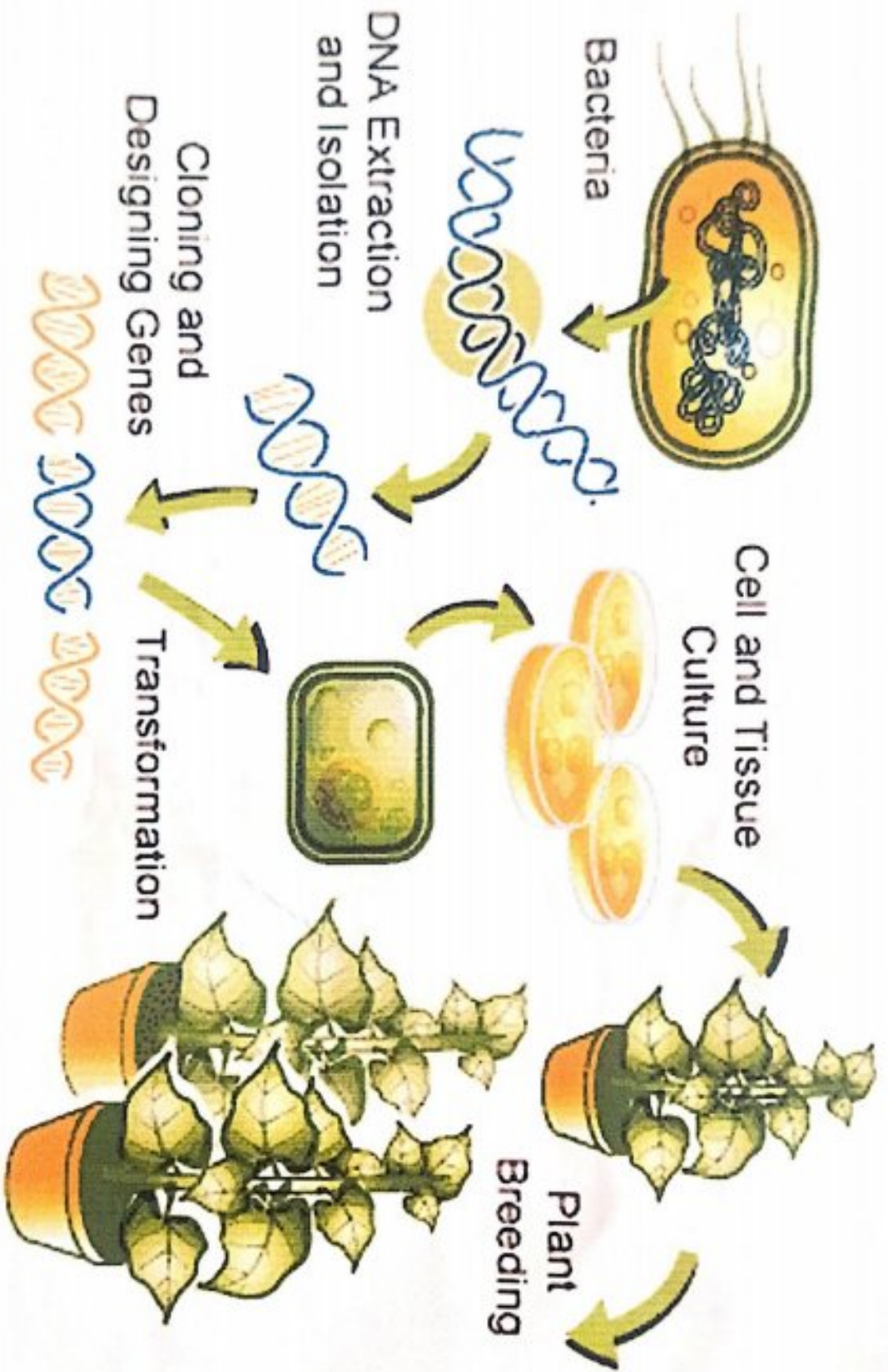
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POLYMERASE CHAIN REACTION

PROJECT DONE BY

ARUNA GANTI

BSC 6TH SEMESTER

BOTANY

RANI CHENNAMMA UNIVERSITY BELAGAVI

2021-2022

POLYMERASE CHAIN REACTION

INTRODUCTION: The polymerase chain reaction technique developed by Kary Mullis in 1985. Is extremely powerful. It generates microgram quantities of DNA copies of desired DNA. DNA segment present even as

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single copy in the initial preparation in matter of few hours. The PCR process has been completely automated & compact thermal cyclers are available in market. The PCR carried out in invitro technique. It utilizes a DNA preparation containing the desired segment to be amplified 2 nucleotide primers., specific i.e complementary to the 3' borders. Triphosphates viz, TTP, DCTP, DATP, TGTP & heat stabled DNA polymerase. Ex: Taq(isolated from bacterium thermos aquaticus), Pfu(from pirococcus Furioses), & Vent(from thermococcus litoralis) polymerases. Pfu & Vent polymerase are more efficient than Taq polymerase.

PCR is cell free amplification technique for synthesizing multiple identical copies of any DNA of interest. The double stranded DNA of interest is denatured to separate into 2 individual strands. Each strand is allowed to hybridize with a primer (renaturation). The primer template duplex is used for DNA synthesis (DNA polymerase). Denaturation, renaturation & synthesis are repeated again & again to generate multiple forms of target DNA.

DEFINITION OF PCR: PCR is laboratory technique for rapidly producing (amplifying) millions to billions of copies of specific segment of DNA.

COMPONENTS OF PCR:

1) DNA Template: DNA template is DNA target sequence. DNA template is DNA molecule that contains DNA region to be amplified, segment we are concerned which is target sequence.

2) DNA Polymerase: DNA polymerase sequentially adds nucleotides complementary to template strand at 3' -OH of bound primers & synthesizes new strands of DNA complementary to target sequence. The most commonly used DNA polymerase is Taq DNA polymerase (from *Thermus aquaticus*, a thermophilic bacterium) because of high temperature stability. Pfu DNA polymerase (from *pyrococcus furiosus*) also used widely because of higher fidelity.

3) Oligonucleotide primer: Oligonucleotide made up of 2'-deoxyribonucleotides are molecules used in polymerase chain reaction. These are referred to as primers & are used to massively amplify a small amount of DNA. The primer binds to specific DNA sequence & DNA polymerase is used to extend the oligonucleotide & replicate the complementary strand.

4) Deoxyribonucleotide triphosphate: dNTP stands for deoxyribonucleotide triphosphate employed in PCR to expand the growing DNA strand. dATP, dTTP, dGTP, dCTP are four common dNTP's used in PCR. dNTP are used in the chain termination method to stop expansion of DNA synthesis. The ingredients of PCR, RT-PCR, DNA sequencing that helps to grow the DNA or DNA amplification.

5) Buffer system: PCR is carried out in a buffer that provides a suitable chemical environment for activity of DNA polymerase. PCR buffer consist 3 ingredients namely; a) tris hcl: which control the pH.

b) *KCl*: which help the primer in attachment on DNA single strand in annealing step.
work on DNA stability.

c) Gelatin:

TYPES OF PCR

1) Real time PCR
Quantitative PCR
cell PCR

4) Multiplex PCR

5) Nested PCR

6) Fast cycling PCR

2)

3) Single

1) REAL TIME PCR STEPS

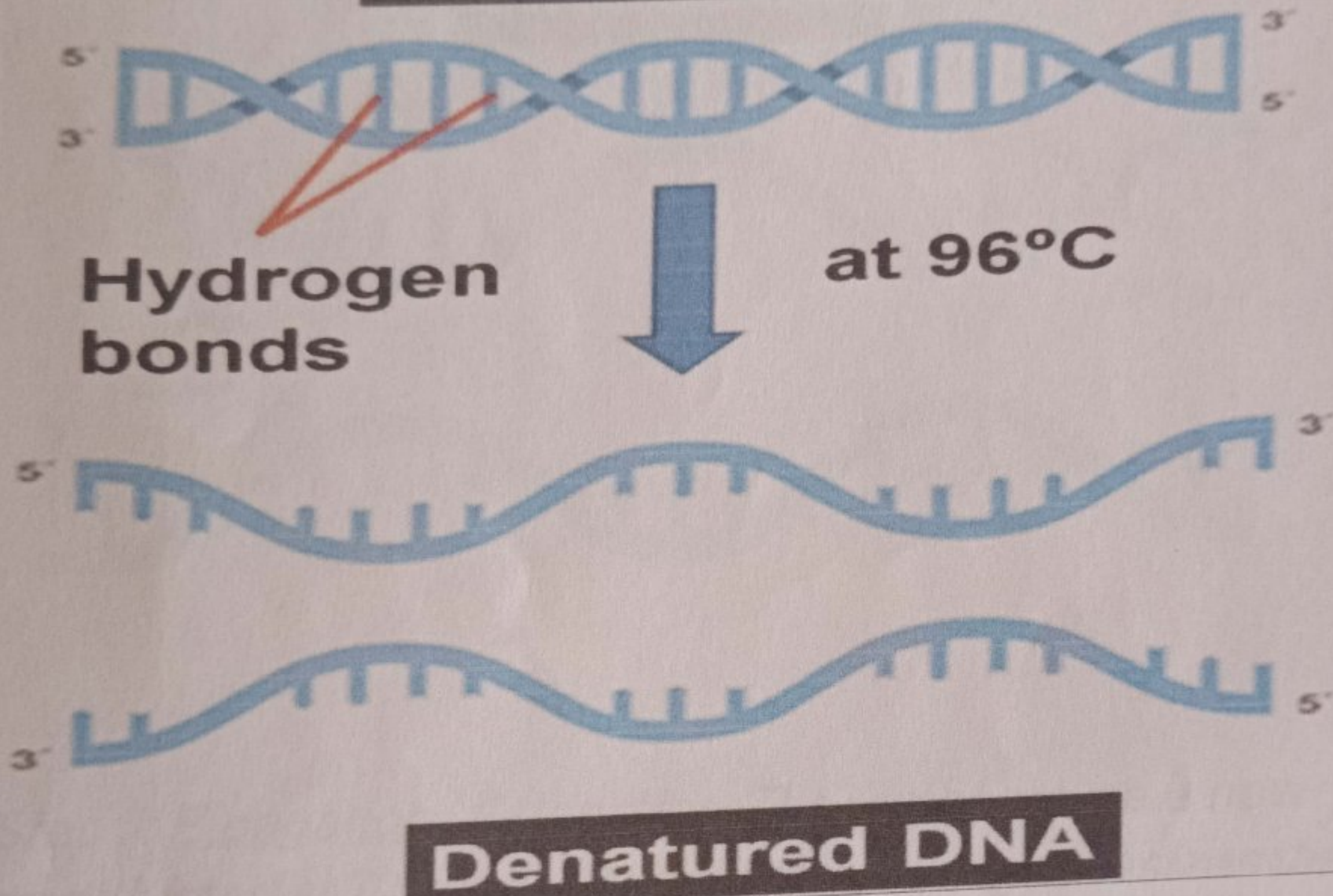
At the start of PCR, the DNA from which segment is to be amplified on excess of two primer molecules, four deoxyribonucleotide triphosphate & DNA polymerase are mixed together in reaction mixture. The following operations are perforated sequentially....

Step 1: Denaturation: The desired DNA is heated to high temperature at 94°C in 0.5-2 minutes, which assures single stranded DNA. It results in the separation of the two strands of DNA, each of which would function as a template for synthesis of new molecule of DNA, this is

called denaturation.

Denaturation step in PCR

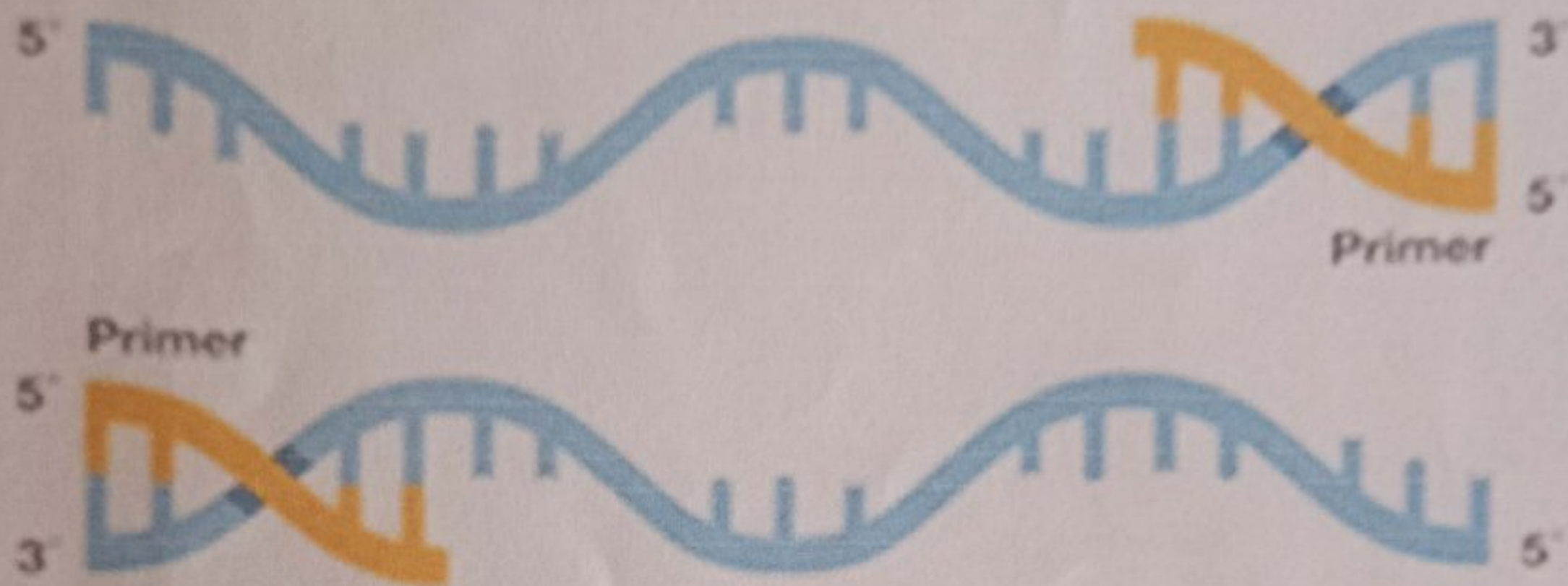
Template DNA



Step 2: Annealing: The mixture is now cooled to temperature about 54°C - 60°C , at 2 minutes that permits annealing of primer to complementary sequences in DNA. These sequences are located at 3'-ends of two strands of desired segment. This step is called annealing. The annealing temperature depends on length & G C content of primer being used.

Annealing step in PCR

Annealing



Step 3: Elongation / Extension: The temperature is now so adjusted at 72°C that DNA polymerase synthesizes the complementary strands by utilizing 3'-OH of primers. This reaction is same as occurs in vivo during replication of leading strand a DNA duplex. The primers are extended towards each other so that DNA segment lying between two primers is copied. This is ensured by employing primers complementary to 3' ends of segment to be amplified.

In case Taq polymerase the optimum temperature for synthesis between 70°C-75°C the temperature of reaction mixture is adjusted to temperature.

This situation has following additional advantages between 70°C-75°C base pairing between about 20bases

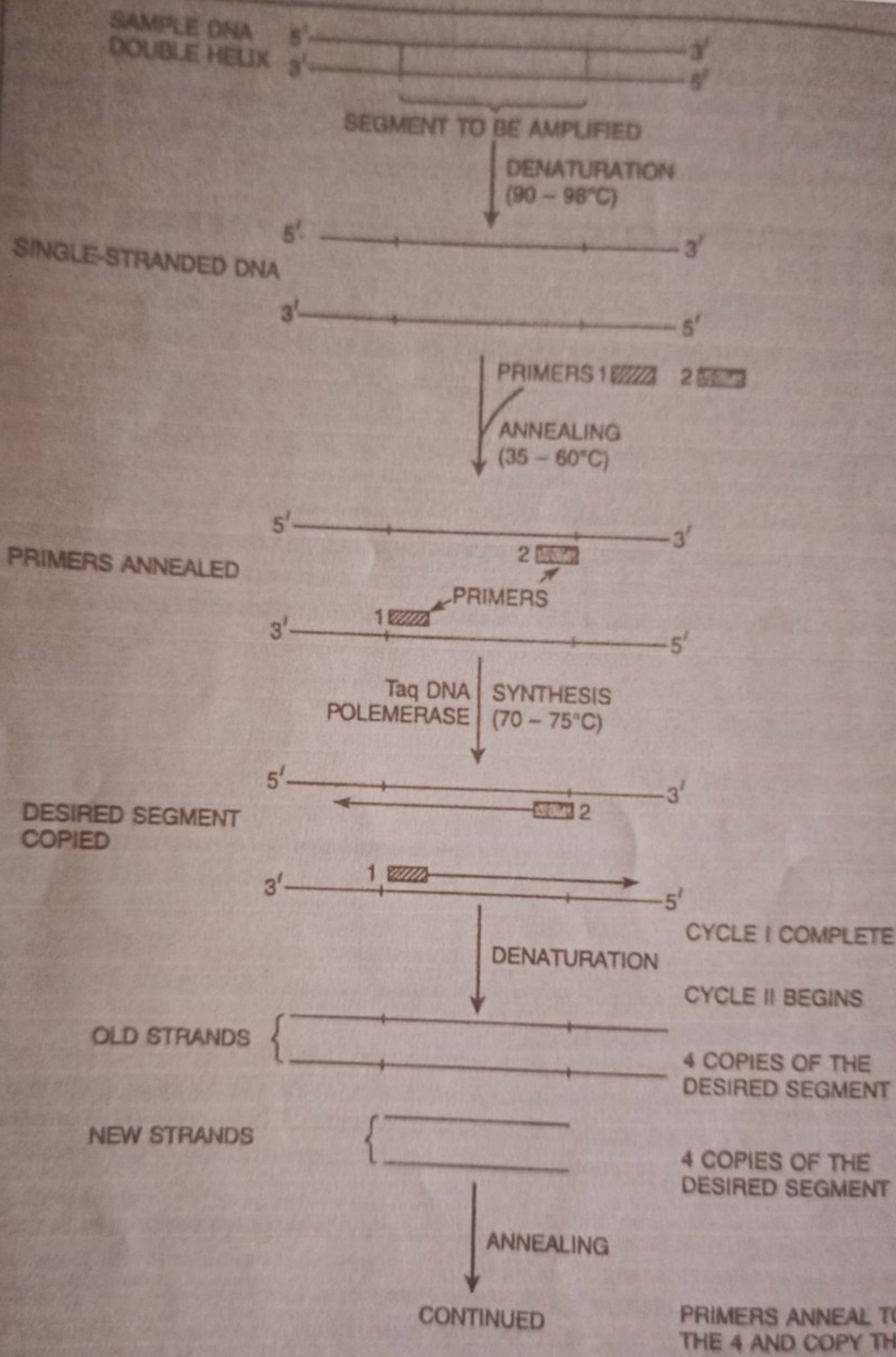
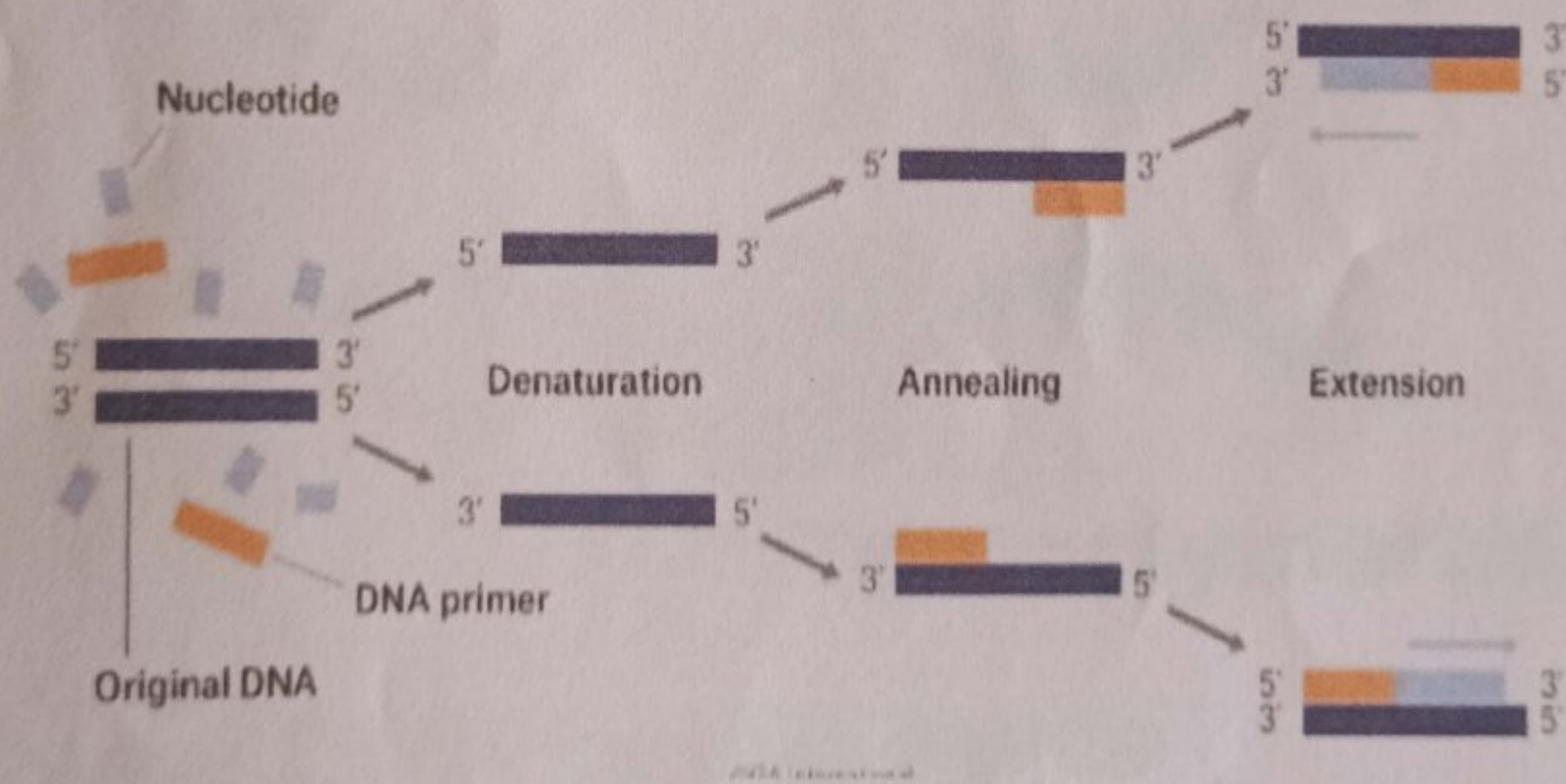


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long primers & DNA is much more specific than at 37°C, the optimal temperature for E-coli DNA polymerase. This minimizes chances of annealing of primers to imperfectly matched sequences & thereby amplification of unwanted DNA; the specificity of annealing is further increased by selecting appropriate conditions like ionic strength primer length. The completion of step 3 completes 1st cycle of amplification each cycle may take few minutes.

Extension step in PCR

Extension



Taq polymerase

New DNA being made

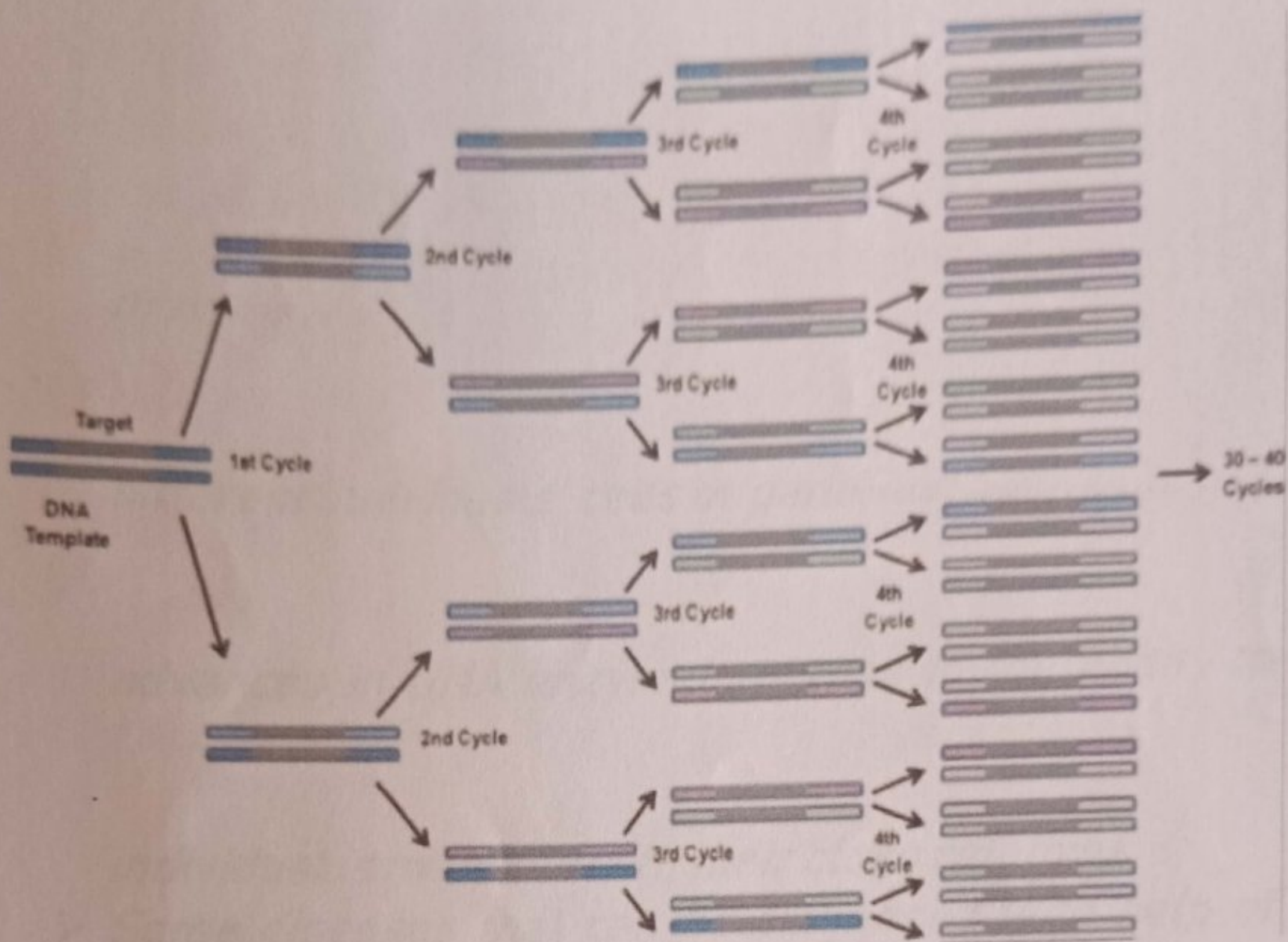
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VARIATION OF PCR

PCR is highly versatile technique it has been modified in variety of way to suit specific situation & appreciations. Some important variations of PCR as follows:-

- PCR can be used to amplify sequence flanking (located on either side) a DNA segment border sequences of which known as inverse PCR.*
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**) It can be used selectively amplify desired cDNA molecule from among mixture of DNA as (RT-PCR).*

APPLICATIONS OF PCR

- *PCR can be used to determine sex of embryos.*
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CONCLUSION

PCR is highly accurate & rapid method for duplicating genetic material. PCR is not only vital in clinical laboratory by amplifying small amounts of DNA for STD detection, but it also important for genetic predisposing for defects such as factor V Leiden. It is very helpful way to replicate DNA & diagnose certain diseases. It is very realizable way to test & analyze the DNA & is also very efficient in sense that it only takes few hours. The PCR technology can also employed in law enforcement, genetic testing of animal stocks & vegetable hybrids, drug screening along with many more areas.



**V M K S R VASTRAD ARTS, SCIENCE,
V S BELLIHAL COMMERCE COLLEGE
HUNGUND**

PROJECT REPORT

College roll no: 01

Examination seat no: S1937085

CERTIFICATE

This is to certify that Ms: Shilpa Tarival of BSc 6th semester has satisfactory completed the project report in Botany subject as prescribed by Rani Chennamma University Belagavi.

During year 2021-2022

Examiner:

Ali
Head Dept of Botany

- 1) *Rohit* 24/2/22
- 2) *Heeloo*

Botany
Head of the Department
V.M.K.S.R. Vastrad Arts, Commerce and
Science College, Hungund Dist: Bagalkot



VMKSR VASTRAD ARTS, SCIENCE &

VS BELLIHAL COMMERCE COLLEGE

HUNGUND

POLYMERASE CHAIN REACTION

PROJECT DONE BY

SHILPA TARIVAL

BSC 6TH SEMESTER

BOTANY

RANI CHENNAMMA UNIVERSITY BELAGAVI

2021-2022

POLYMERASE CHAIN REACTION

INTRODUCTION: The polymerase chain reaction technique developed by Kary Mullis in 1985. Is extremely powerful. It generates microgram quantities of DNA copies of desired DNA. DNA segment present even as single copy in the initial preparation in matter of few hours. The PCR process has been completely automated & compact thermal cyclers are available in market. The PCR carried out in invitro technique. It utilizes a DNA preparation containing the desired segment to be amplified 2 nucleotide primers, specific i.e complementary to the 3' borders.

Triphosphates viz, TTP, DCTP, DATP, TGTP & heat stabled DNA polymerase. Ex: Taq (isolated from bacterium *thermos aquaticus*), Pfu (from *pirococcus Furioses*), & Vent (from *thermococcus litoralis*) polymerases. Pfu & Vent polymerase are more efficient than Taq polymerase.

PCR is cell free amplification technique for synthesizing multiple identical copies of any DNA of interest. The double stranded DNA of interest is denatured to separate into 2 individual strands. Each strand is allowed to hybridize with a primer (renaturation). The primer template duplex is used for DNA synthesis (DNA polymerase). Denaturation, renaturation & synthesis are

repeated again & again to generate multiple forms of target DNA.

DEFINITION OF PCR: PCR is laboratory technique for rapidly producing (amplifying) millions to billions of copies of specific segment of DNA.

COMPONENTS OF PCR:

- 1) DNA Template: DNA template is DNA target sequence. DNA template is DNA molecule that contains DNA region to be amplified, segment we are concerned which is target sequence.
- 2) DNA Polymerase: DNA polymerase sequentially adds nucleotides complimentary to template strand at 3' -OH of bound primers & synthesizes new strands of DNA complementary to target sequence. The most commonly used DNA polymerase is Taq DNA polymerase (from *Thermus aquaticus*, a therophillic bacterium) because of high temperature stability. Pfu DNA polymerase (from *pyrococcus furiosus*) also used widely because of higher fidelity.
- 3) Oligonucleotide primer: Oligonucleotide made up of 2'-deoxyribonucleotides are molecules used in polymerase chain reaction. These are referred to as primers & are used to massively amplify a small amount of DNA. The primer binds to specific DNA sequence & DNA

polymerase is used to extend the oligonucleotide & replicate the complementary strand.

4) Deoxyribonucleotide triphosphate: dNTP stands for deoxyribonucleotide triphosphate employed in PCR to expand the growing DNA strand. dATP, dTTP, dGTP, dCTP, are four common dNTP's used in PCR. dNTP are used in the chain termination method to stop expansion of DNA synthesis. The ingredients of PCR, RT-PCR, DNA sequencing that helps to grow the DNA or DNA amplification.

5) Buffer system: PCR is carried out in a buffer that provides a suitable chemical environment for activity of DNA polymerase. PCR buffer consist 3 ingredients namely; a) tris hcl: which control the ph.
b) kcl : which help the primer in attachment on DNA single strand in annealing step. c)
Gelatin: work on DNA stability.

TYPES OF PCR

- | | |
|---------------------|---------------------|
| 1) Real time PCR | 4) Multiplex PCR |
| 2) Quantitative PCR | 5) Nested PCR |
| 3) Single cell PCR | 6) Fast cycling PCR |

1) REAL TIME PCR STEPS

At the start of PCR, the DNA from which segment is to be amplified on excess of two primer molecules, four

deoxyribonucleotide triphosphate & DNA polymerase are mixed together in reaction mixture. The following operations are performed sequentially....

Step 1: Denaturation: The desired DNA is heated to high temperature at 94°C in 0.5-2minutes, which assures single stranded DNA. It results in the separation of the two strands of DNA, each of which would function as a template for synthesis of new molecule of DNA, this is called denaturation.

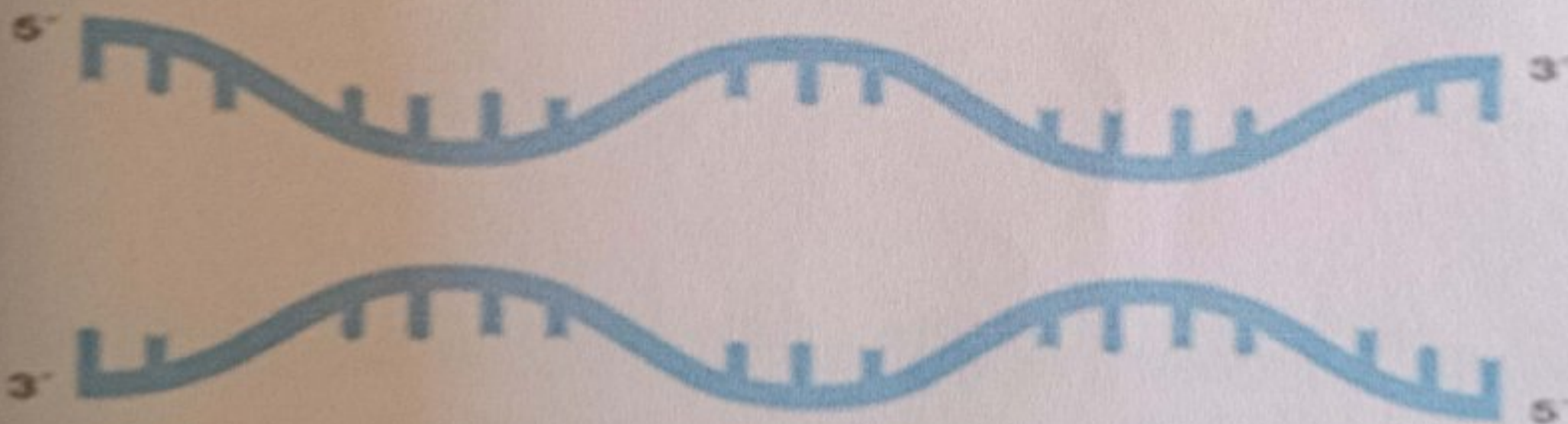
Denaturation step in PCR

Template DNA



Hydrogen bonds

at 96°C



Denatured DNA

Step 2: Annealing: The mixture is now cooled to temperature about 54°C - 60°C , at 2minutes that permits

annealing of primer to complementary sequences in DNA. These sequences are located at 3'-ends of two strands of desired segment. This step is called annealing. The annealing temperature depends on length & G C content of primer being used.

Annealing step in PCR

Annealing



Step 3: Elongation | Extension: The temperature is now so adjusted at 72^oc that DNA polymerase synthesizes the complementary strands by utilizing 3'-OH of primers. This reaction is same as occurs in vivo during replication of leading strand a DNA duplex. The primers are extended

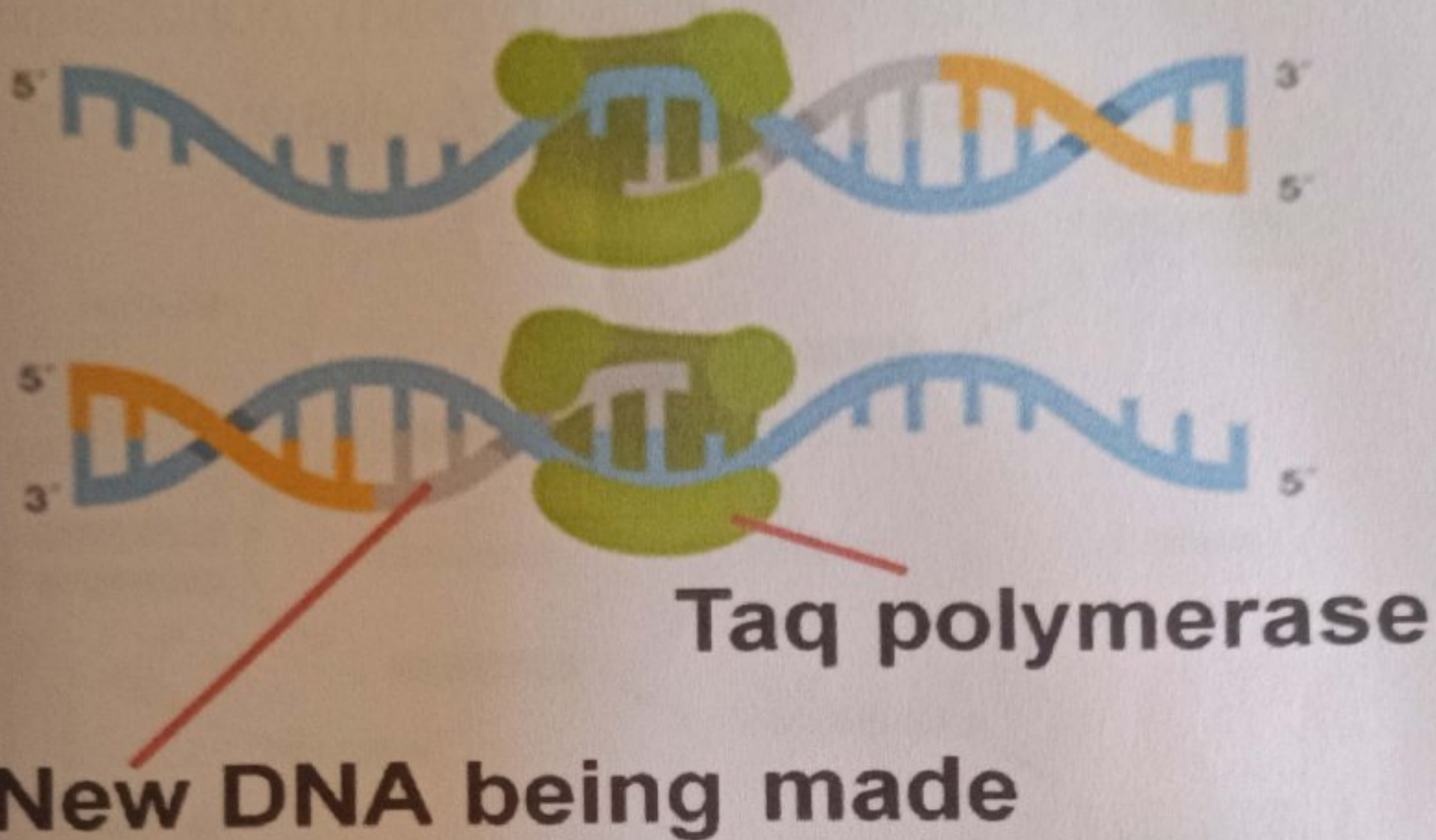
towards each other so that DNA segment lying between two primers is copied. This is ensured by employing primers complementary to 3' ends of segment to be amplified.

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This situation has following additional advantages between 70°C - 75°C base pairing between about 20 bases long primers & DNA is much more specific than at 37°C , the optimal temperature for E-coli DNA polymerase. This minimizes chances of annealing of primers to imperfectly matched sequences & there by amplification of unwanted DNA ; the specificity of annealing is further increased by selecting appropriate conditions like ionic strength primer length. The completion of step 3 completes 1st cycle of amplification each cycle may take few minutes.

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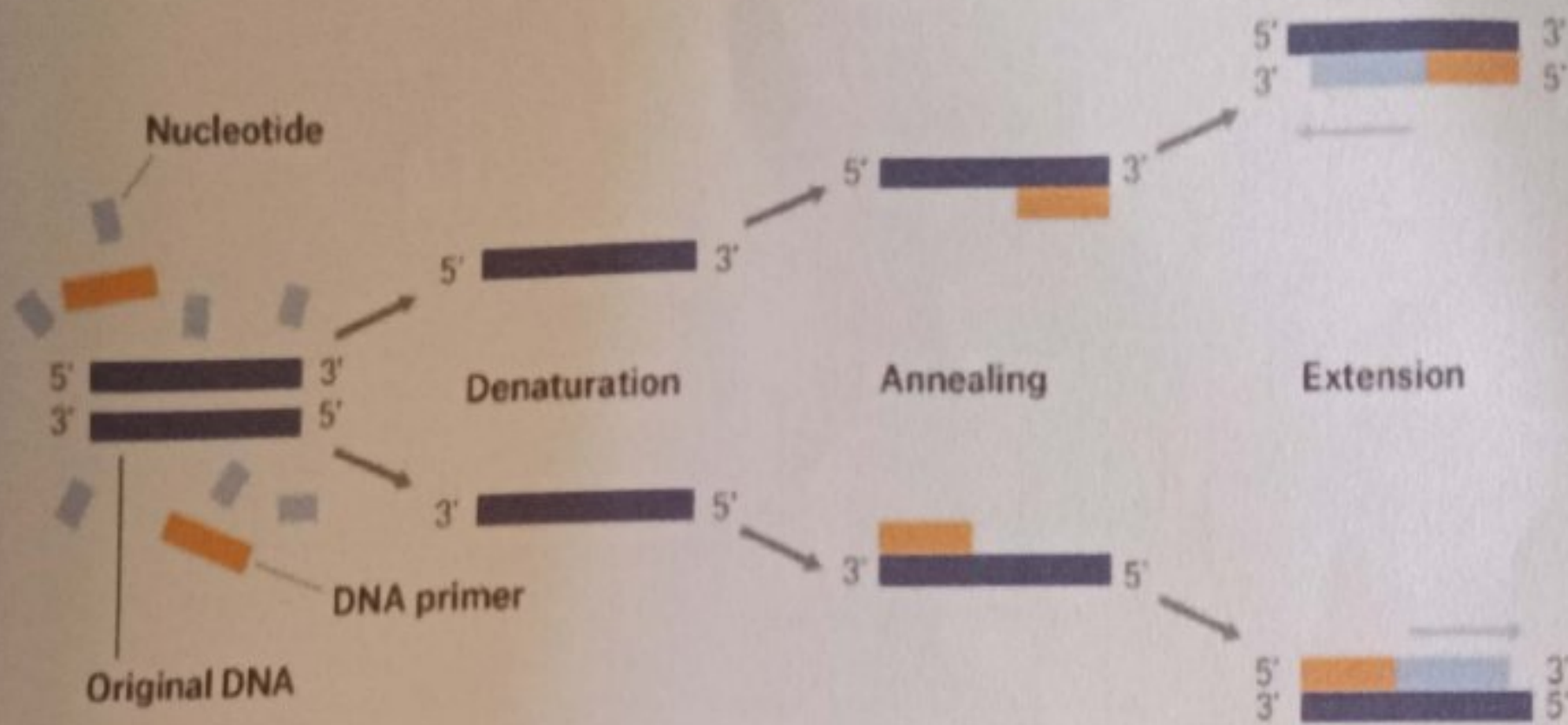
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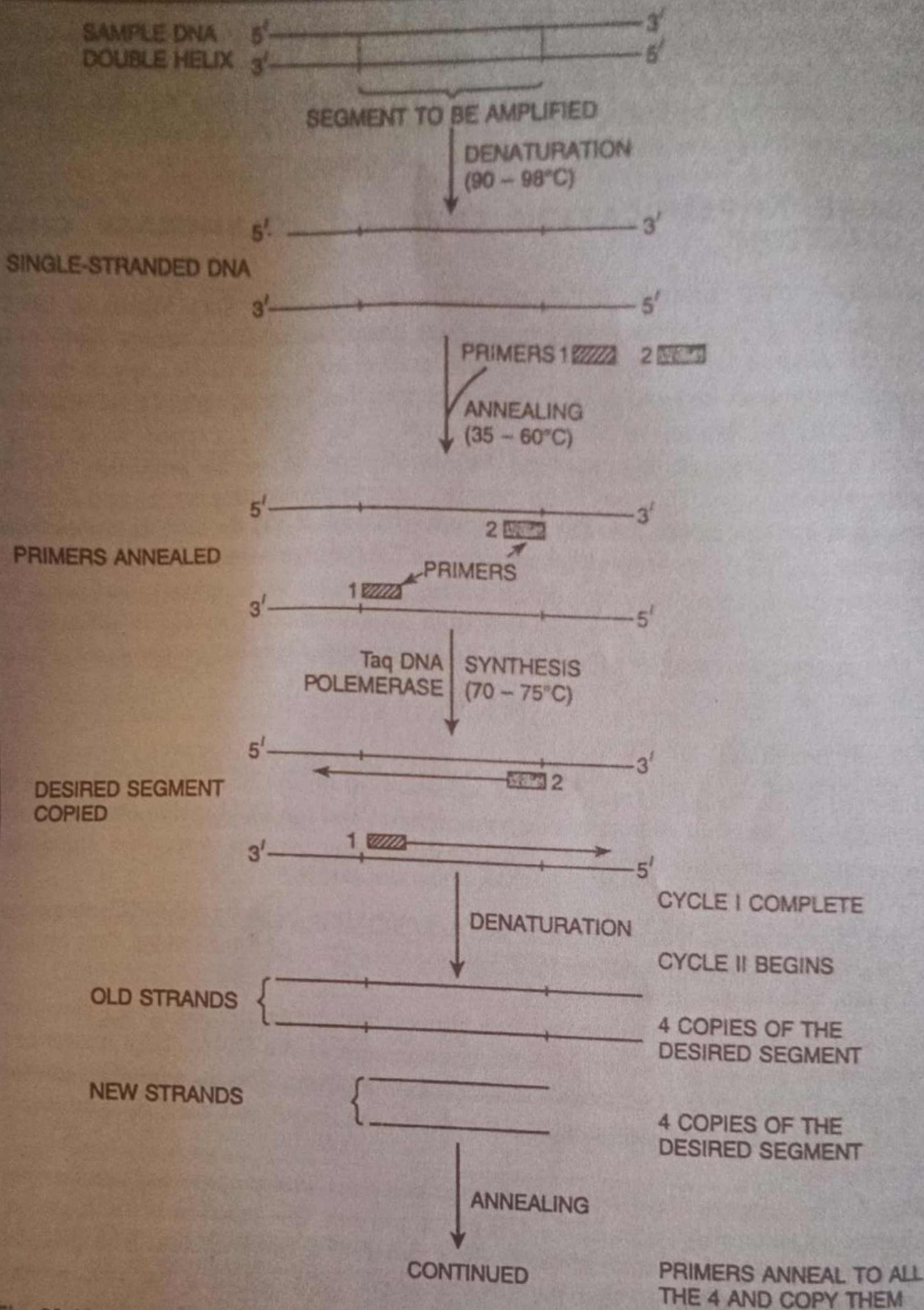


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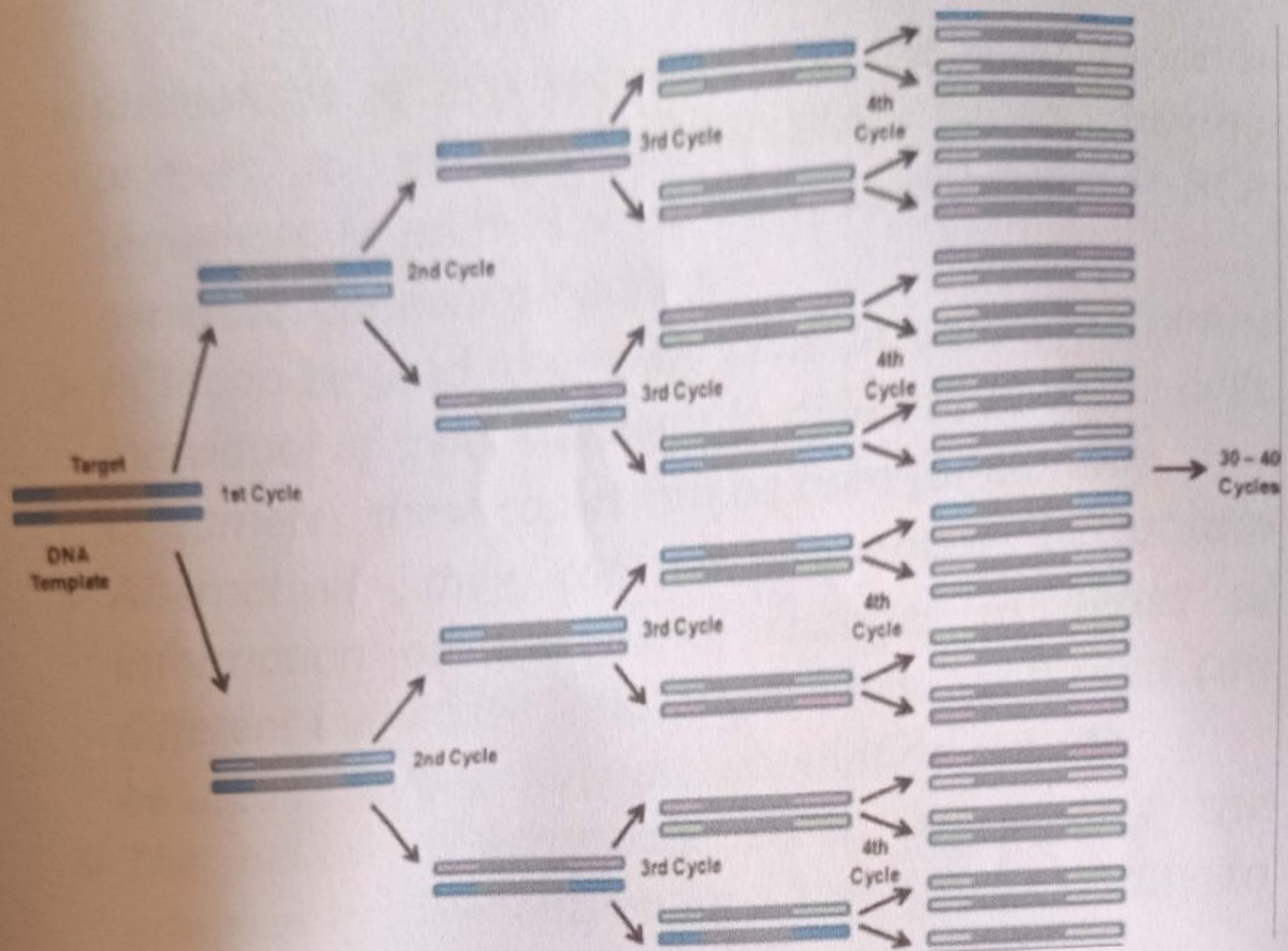
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nucleotides of any sequence can be used random primers to amplify polymorphic DNA's having sequences to primers used. Such application of PCR generates random amplified.

- PCR can be used to amplify gene present in different individual of species & even in different somatic cells or gametes, these copies can be used for cloning.
- Alternatively they can be sequenced to obtain information on mutational changes in genes of different individuals' cells or gametes. Such data can be used in disease diagnosis population growth.
- The ability to synthesize short oligos and the advances in DNA enzymology lead to the ability to amplify DNA sequences.
- PCR can also used in parental testing, where an individual is matched with their close relatives.
- Some diseases that can be diagnosed with help of PCR; Huntington's disease, Cystic fibrosis, Human immunodeficiency virus.
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