ISBN: 978-81-69967-06-8

PRACTICAL HANDBOOK OF MOLECULAR BIOLOGY AND MOLECULAR DIAGNOSTICS

ZOOLOGY (B. Sc. III, Sem V)

Prof. (Dr.) Kavita A. Gajare

AS PER NEP-2020 (2.0)

SYLLABUS OF SHIVAJI UNIVERSITY, KOLHAPUR

(IMPLEMENTED FROM JUNE 2026)

Bhumi Publishing

Published by: BHUMI PUBLISHING

PRACTICAL HANDBOOK OF
MOLECULAR BIOLOGY AND MOLECULAR DIAGNOSTICS

Zoology (B. Sc. III Sem. V)

(ISBN: 978-81-69967-06-8)

AS PER NEP-2020 (2.0) SYLLABUS OF SHIVAJI UNIVERSITY, KOLHAPUR

(IMPLEMENTED FROM JUNE 2026)

Author

Prof. (Dr.) Kavita A. Gajare

Head,

Department of Zoology,

The New College, Kolhapur, M.S., India

Bhumi Publishing

2026

Title: Practical Handbook Molecular Biology and Molecular Diagnostics

Author: Prof. (Dr.) Kavita A. Gajare

First Edition: September 2026

ISBN: 978-81-69967-06-8

Published by:

Bhumi Publishing

BHUMI PUBLISHING

Nigave Khalasa, Tal – Karveer, Dist – Kolhapur, Maharashtra, INDIA 416 207

E-mail: bhumipublishing@gmail.com

PREFACE

The Practical Handbook of Molecular Biology and Molecular Diagnostics has been prepared as a comprehensive and student-friendly resource for B.Sc. III Semester V students of Zoology, in accordance with the NEP-2020 (2.0) syllabus of Shivaji University, Kolhapur, implemented from June 2026. The handbook is designed to strengthen students' understanding of fundamental molecular biology concepts through systematic practical exercises, laboratory procedures, observations, and interpretations.

Molecular biology and molecular diagnostics have become essential components of modern biological sciences, providing powerful approaches for understanding genetic material, gene expression, molecular interactions, and disease detection. Practical exposure to these techniques enables students to connect theoretical knowledge with laboratory-based applications and develops essential skills in scientific observation, experimentation, documentation, and analysis.

The handbook covers important practical aspects related to molecular biology and molecular diagnostics, with emphasis on laboratory-oriented learning. Each practical is presented in a structured manner, including relevant principles, objectives, requirements, procedures, observations, calculations wherever applicable, and expected outcomes. The content has been organized to facilitate easy understanding and effective laboratory execution by undergraduate learners.

Special attention has been given to clarity, accuracy, and stepwise presentation so that students can confidently perform experiments under appropriate laboratory guidance. The handbook also aims to encourage scientific thinking, analytical abilities, and responsible laboratory practices.

It is hoped that this handbook will serve as a useful companion for students and teachers, supporting effective implementation of the revised curriculum and promoting hands-on learning in molecular biology and molecular diagnostics.

- Author

Shivaji University. Kolhapur

B. Sc. III Sem. V Zoology (NEP 2.0)

Major Practical V Based on

Molecular Biology & Molecular Diagnostics

(2 Credits: 60 Hours)

Sr. No.	Title of the Practical
1.	Estimation of DNA
2.	Agarose Gel Electrophoresis
3.	To determine the Sequence of the Newly Synthesized DNA Strand During Replication.
4.	To identify the promoter region in the given sequence of DNA
5.	To identify the transcription initiation +1 region on a DNA sequence
6.	To determine the mRNA Sequence from the given Gene Sequence
7.	To identify the Open Reading Frame (ORF) in a given mRNA Sequence
8.	To identify 3' and 5' splice sites in the given mRNA sequence
9.	To deduce the Amino Acid Sequence from the given ORF of mRNA using the dictionary of the Genetic Code
10.	To deduce at least one possible sequence of ORF using a sequence of amino acids and using the dictionary of the genetic code
11.	To study the following techniques (using simulation) a. Southern blotting b. Northern blotting c. Western blotting
12.	To calculate the GC percentage in a given sequence of at least 200 nucleotides
13.	To arrange the given sequences from lower to higher according to the time required for denaturation in PCR a. Based on GC content b. Based on length
14.	To analyse the photographs of FISH
15.	To identify Molecular Diagnosis of a Matching DNA Sample Using DNA Fingerprinting Gel Analysis

Practical 1: Estimation of DNA

Aim: To estimate the quantity of DNA in a given sample

Principle: In the presence of acid, deoxyribose in DNA forms hydroxy-2-methyl-1,4-dicarbonyl, which reacts with diphenylamine to give a blue color that can be read at 595 nm or with a red filter on a colorimeter.

Requirements:

Reagents

- Buffered Saline - 0.5M NaCl in 15mM Trisodium Citrate pH7.0 (Dissolve 0.441gm Trisodium Citrate and 2.922 gm Sodium Chloride in 100ml distilled water).
- Standard DNA - Dissolve 10 mg calf thymus DNA (Commercially available) in 100 ml buffered saline (100 µg/ml).
- Diphenylamine solution – 1 g diphenylamine powder dissolved in 100ml glacial acetic acid and add 2.5ml concentrated H_2SO_4

Equipment: Water bath, Spectrophotometer or colorimeter

Glassware: Test tubes, beakers, pipettes

Procedure:

1. Prepare a standard DNA solution by dissolving 10mg calf thymus DNA in 100 ml Buffer saline having a concentration of 100 µg/ml).
2. Take six clean test tubes. Label three test tubes as S1, S2, S3 and the other three as T1, T2, T3.
3. Pipette 3ml of standard DNA solution into the S1, S2, S3.
4. Pipette 3 ml of unknown DNA solution into T1, T2, and T3.
5. Add 5ml diphenyl amine reagent to each tube.
6. Prepare a blank tube by adding 3ml distilled water and 5ml diphenyl amine reagent.
7. Mix well and keep all test tubes in a boiling water bath for 10 minutes.

Additions table:

Reagents	**Blank**	**Standard** (100 µg/ml DNA solution)			**Unknown** **Unknown DNA sample**		
		S1	**S2**	**S3**	**T1**	**T2**	**T3**
DNA Sample	3ml DW	3ml	3ml	3ml	3ml	3ml	3ml
Diphenylamine reagent	5ml	5ml	5ml	5ml	5ml	5ml	5ml
	Mix well and keep in a boiling water bath for 10 min						
	Allow it to cool. Measure at 595nm or with a red filter						

- A blue colour will develop.
- Set the spectrophotometer at 595nm or the colorimeter at the red filter.
- Set zero OD using a blank.
- Read OD of all samples.
- Calculate the average for the standard and unknown.

(One can draw a standard graph of DNA for estimation of DNA)

Observation table:

Samples	OD	Average OD
S1		
S2		
S3		
T1		
T2		
T3		

- Calculate the concentration of the unknown using the following formula.

Concentration of Unknown DNA sample = $\frac{OD\ of\ Unknown\ X\ Concentration\ of\ Standard}{OD\ of\ Standard}$ µg/ml

Result: The concentration of DNA in the given Sample is ……

Practical 2: Agarose Gel Electrophoresis (Demonstartion)

Aim: To separate DNA fragments by agarose gel electrophoresis

Principle:

DNA and RNA have phosphate in their backbone. The phosphate group is negatively charged. So, DNA and RNA are negatively charged molecules. They move towards the positively charged anode during electrophoresis.

Agarose gel acts as a molecular sieve. Smaller molecules run faster through the pores of the sieve. Thus, the molecules are separated according to their size, with smaller fragments near the anode and larger fragments near the loading well. Bands of separated DNA/RNA fragments can be observed under a UV Transilluminator using bromophenol blue.

Requirements:

Chemicals:

- Agarose
- TAE: 40 mM Tris, 20 mM acetic acid, and 1 mM EDTA (pH 8.0 to 8.5)
- DNA or RNA sample
- DNA ladder/ molecular weight marker
- Loading dye: Bromophenol Blue in glycerol
- Visualizing dye: Ethidium Bromide

Equipment:

- UV transilluminator
- Electrophoresis unit
- Power pack and electric supply

Other: Microfuge tubes, Micropipettes with disposable tips, beakers, pipettes, etc

Procedure:

Preparation of TAE buffer

Prepare a stock solution (50X) by adding

Add 242 g Tris base and 57.1 mL glacial acetic acid to 100 mL of 0.5 M EDTA (pH 8.0)

Raise the volume to 1L with deionized water.

Working Buffer: Dilute the stock solution to 1X (1ml stoke solution raise to 50ml)

Preparation of Agarose gel:

1. Add 1 g of agarose powder is mixed in 100 mL of TAE (1X) buffer.
2. Heat the solution to dissolve the agarose.
3. Allow it to cool to 40^{o}C.

4. Add 0.5 µg/ml ethidium bromide.
5. Pour the gel into the gel tray of the electrophoresis unit by closing it on both sides with stopper tape.
6. Place the comb and allow it to cool and solidify.

Caution: Ethidium bromide is a carcinogen. Use proper gloves while handling it and the gel.

Setting the electrophoresis unit:

1. Fix the tray with the gel in the electrophoresis unit by removing the stopper tapes.
2. Keep the wells near the cathode (Black electrode)
3. Pour the TAE (1X) buffer into the unit to fill the chamber and flood over the gel.
4. Remove the comb and view the wells.
5. Set the electrodes in the unit. Confirm the electrodes have dipped in the buffer.
6. Connect the red and black cables to the anode and cathode, respectively.

Preparation of Loading sample

1. The loading dye: add 3ml glycerol and 25mg bromophenol blue in 7ml deionized water.
2. Mix the DNA sample with the loading dye.

Loading the sample:

1. Load 10 to 30 µl DNA samples containing 10 to 100ng DNA into the wells using a micropipette.
2. Insert the tip of the micropipette deep into the well and slowly load the sample to avoid spilling.
3. Add a DNA ladder sample in the first or last well for reference.

Running electrophoresis:

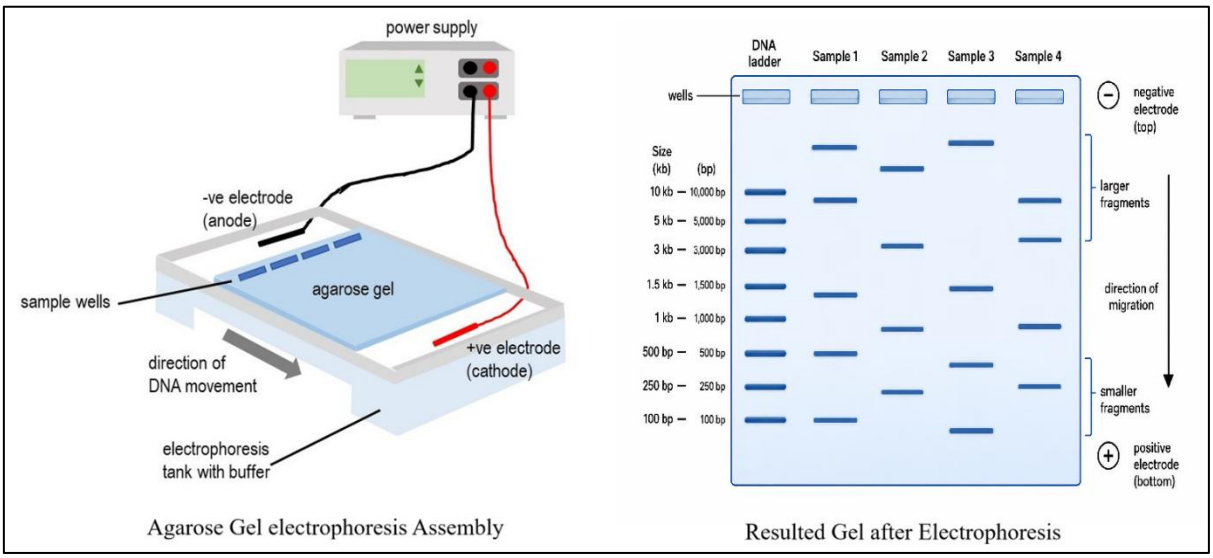
1. After sample loading, connect the red and black cables to the respective slots of the power pack.
2. Switch on the current. Set the power pack to 120 V.
3. After a few minutes, observe the electrodes. Small emerging bubbles indicate proper running of the electrophoresis.
4. Observe the bromophenol blue running in the gel.
5. End the electrophoresis when the bromophenol blue reaches the other end.

Visualizing the gel:

1. After ending the electrophoresis, carefully remove the gel from the tray. Put the gel on the surface of the UV transilluminator.

2. The ethidium bromide stain specifically binds to DNA. It appears orange under UV.
3. So, the DNA bands appear orange under UV.

Diagram:



Results:

The DNA fragments are separated according to their sizes. The smaller fragments run faster and reach near the anode, while larger fragments run more slowly and remain near the wells. (The given sample has been separated into..... bands of size ranging from ..... to .....)

Practical 3: To determine the Sequence of the Newly Synthesized DNA Strand During Replication

Aim: To determine the sequence of the primer and newly synthesized strand using the sequence of the template DNA.

Background:

DNA replication is the synthesis of a replica of DNA using DNA as a template. During replication, DNA polymerase adds complementary dNTPs using the template strand in the 5' to 3' direction. T is added opposite A, A is added opposite T, G is added opposite C and vice versa.

The primer is an RNA segment added by the enzyme primase for making 3'-OH available for DNA polymerase. It also uses the DNA template but adds NTPs and U opposite A.

Requirement: Sequence of the template strand with properly denoted 5' and 3' ends.

Determine a sequence of a 20-nucleotide RNA primer and a 50-nucleotide newly synthesized strand using the given DNA template.

Procedure:

1. Find the 3' end of the template. Add 20 complementary nucleotides. Add U opposite A.
2. Continue to add complementary nucleotides, but add T opposite A

Sequence 1

5'CGTTTGAAATCCCGCTTCGGTTGGGGACTGACTGTGGCGA

CGACGGTGCTTCATGCCTGCCGTAAGATCGAGCAGTTGCGT

GAAGAGAGCCACGATATCAAAGAAGATTTTTCAAATTTAAT

CAGAACATTGTCATC 3'

Sequence 2:

5’GTGTCACTTTCGCTTTGGCAGCAGTGTCTTGCCCGATTGCA

GGATGAGTTACCAGCCACAGAATTCAGTATGTGGATACGCCC

ATTGCAGGCGGAACTGAGCGATAACACGCTGGCCCTGTACG

CGCCAAACCGTTTTGT3’

Sequence 3:

3’CCTCGATTGGGTACGGGACAAGTACCTTAATAATATCAATG

GACTGCTAACCAGTTTCTGCGGAGCGGATGCCCCACAGCTG

CGTTTTGAAGTCGGCACCAAACCGGTGACGCAAACGCCAC

AAGCGGCAGTGACGAGCAACGTCGCGGCCCCTGCACAGGT

CAGGTTG5’

Sequence 4:

3’CGCGCGTAAATTTGGCGTACGTAGCGCTATGCCGAC

ATCATATCCGTGAAATCTTCTCGCGCTACACCTCGCGC

ATTGAACCGTTGTCACTGGATGAGGCTTATCTCGATG

TCACCGATAGCGTCCATTGCCACGG5’

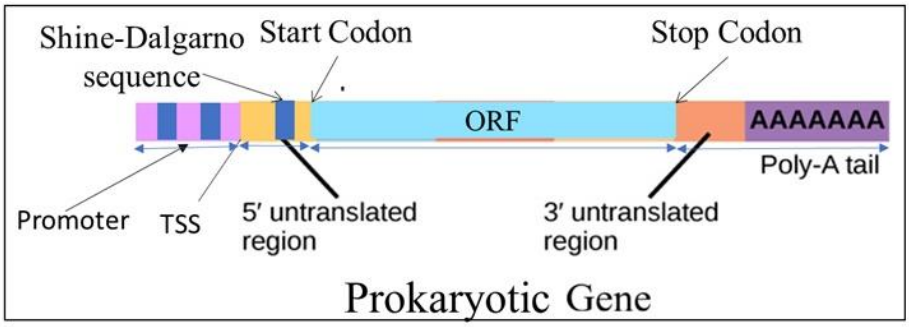
Practical 4: To identify the promoter region in the given sequence of DNA

Aim: To search and identify a promoter region in a sequence of gene.

Requirement: Sequences of a complete prokaryotic gene including the promoter and properly defined template and non-template strands.

Background:

Components of a prokaryotic gene



A prokaryotic gene has a regulatory sequence upstream of the +1 site. It is the site for RNA polymerase binding; the following are the components of a promoter region in prokaryotes.

- 5’ Upstream Element: 20 bp AT-rich
- -35 Box: 6bp (TTGACA)
- Spacer: 13 to 17 bp
- -10 Box: 6 bp –(TATAAT)
- Discriminator: 5-8bp GC rich
- Transcription Start Site (TSS): Adenine (A)

Procedure:

1. Confirm the given sequence is of the non-template strand. Otherwise, draw the complementary sequence if the template strand sequence is provided.
2. Locate an AT-rich stretch near the 5’ end of the gene.
3. Search for the -35 TTGACA sequence. Highlight and label it.
4. Search for the -10 TATAAT sequence 13 to 17 base pairs downstream. Highlight and label it.
5. Find the ‘A’ 5 to 8 bp downstream from the 3’ end of TATAAT. Mark it as TSS.
6. The AT-rich stretch, -35 element, the spacer, -10 element and the discriminator up to the -1 (base immediately upstream of the TSS) together is the promoter region.
7. Mark the promoter region.

Gene sequence 1: Non-template strand

5’AAAAAAATAAATTAAATTATTTGACATTATATTATTTATTATAATC
GCGCCAAATTAATTAAAGGAGGTAAAAAATTATGGCCAGCAGAGGC
GTAAACAAGGTTATTCTCGTTGGTAATCTGGGTCAGGACCCGGAAGT
ACGCTACATGCCAAATGGTGGCGCAGTTGCCAACATTACGCTGGCTA
CTTCCGAATCCTGGCGTGATAAAGCGACCGGCGAGATGAAAGAACA
GACTGAATGGCACCGCGTTGTGCTGTTCGGCAAACTGGCAGAAGTG
GCGAGCGAATATCTGCGTAAAGGTTCTCAGGTTTATATCGAAGGTCA
GCTGCGTACCCGTAAATGGACCGATCAATCCGGTCAGGATCGCTACA
CCACAGAAGTCGTGGTGAACGTTGGCGGCACCATGCAGATGCTGGG
TGGTCGTCAGGGTGGTGGCGCTCCGGCAGGTGGCAATATCGGTGGTG
GTCAGCCGCAGGGCGGTTGGGGTCAGCCTCAGCAGCCGCAGGGTGG
CAATCAGTTCAGCGGCGGCGCGCAGTCTCGCCCGCAGCAGTCCGCTC
CGGCAGCGCCGTCTAACGAGCCGCCGATGGACTTTGATGATGACATT
CCGTTCTGAAAAAGGCCGCGGCCGTTTT
CGGCCGCGGCCTTTTTTTT3’

Gene sequence 2: Non-template strand

5’AAAAATAAATTAAATTAATTGACATTATATTAAATTTATTATAATC
CGGCCATATTAATTAAAGGAGGTAAAAAATTATGAATGAGTTCAAG
AGGTGTATGCGCGTGTTTAGTCATTCTCCCTTTAAAGTACGGTTAATG
CTGCTCTCTATGTTGTGCGATATGGTCAACAACAAACCGCAGCAAGA
TAAACCTTCCGATAAATAGAAAAGGCCGCGGCCGTTTTCGGCCGCGG
CCTTTTTTTT3’

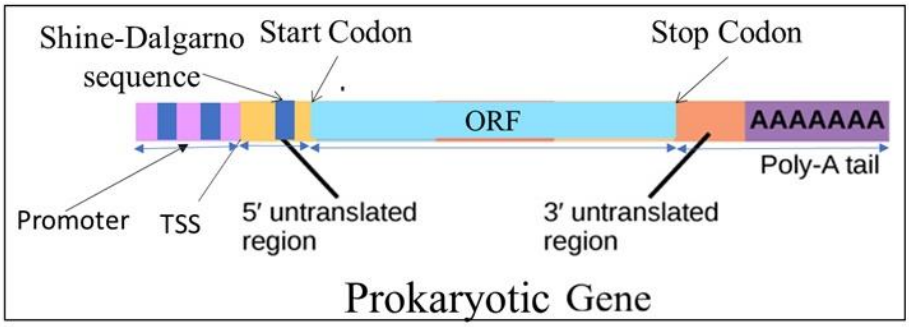
Practical 5: To identify the transcription initiation +1 region on a DNA sequence

Aim: To search and identify the Transcription Start Site (TSS) or +1 site

Requirement: Sequences of a complete prokaryotic gene including the promoter and properly defined template and non-template strands.

Background:

Components of a prokaryotic gene



A prokaryotic gene has a regulatory sequence upstream of the +1 site. It is the site for RNA polymerase binding; the following are the components of a promoter region in prokaryotes.

- 5’ Upstream Element: 20 bp AT-rich
- -35 Box: 6bp (TTGACA)
- Spacer: 13bp (13 to 17 bp)
- -10 Box: 6 bp –(TATAAT)
- Discriminator: 6bp (5-8bp) GC rich
- Transcription Start Site (TSS): Adenine (A)

Procedure:

1. Confirm the given sequence is of the non-template strand. Otherwise, draw the complementary sequence if the template strand sequence is provided.
2. Locate an AT-rich stretch near the 5’ end of the gene.
3. Search for the -35 TTGACA sequence. Highlight and label it.
4. Search for the -10 TATAAT sequence 13 to 17 base pairs downstream. Highlight and label it.
5. Find the ‘A’ 5 to 8 bp downstream from the 3’ end of TATAAT. Mark it as +1/ TSS.

Gene sequence 1: Non-template strand

5’AAAAAAATAAATTAAATTATTTGACATTATATTATTTATTATAATC
GCGCCAAATTAATTAAAGGAGGTAAAAAATTATGGCCAGCAGAGGC
GTAAACAAGGTTATTCTCGTTGGTAATCTGGGTCAGGACCCGGAAGT
ACGCTACATGCCAAATGGTGGCGCAGTTGCCAACATTACGCTGGCTA
CTTCCGAATCCTGGCGTGATAAAGCGACCGGCGAGATGAAAGAACA
GACTGAATGGCACCGCGTTGTGCTGTTCGGCAAACTGGCAGAAGTG
GCGAGCGAATATCTGCGTAAAGGTTCTCAGGTTTATATCGAAGGTCA
GCTGCGTACCCGTAAATGGACCGATCAATCCGGTCAGGATCGCTACA
CCACAGAAGTCGTGGTGAACGTTGGCGGCACCATGCAGATGCTGGG
TGGTCGTCAGGGTGGTGGCGCTCCGGCAGGTGGCAATATCGGTGGTG
GTCAGCCGCAGGGCGGTTGGGGTCAGCCTCAGCAGCCGCAGGGTGG
CAATCAGTTCAGCGGCGGCGCGCAGTCTCGCCCGCAGCAGTCCGCTC
CGGCAGCGCCGTCTAACGAGCCGCCGATGGACTTTGATGATGACATT
CCGTTCTGAAAAAGGCCGCGGCCGTTTT
CGGCCGCGGCCTTTTTTTT3’

Gene sequence 2: Non-template strand

5’AAAAATAAATTAAATTAATTGACATTATATTAAATTTATTATAATC
CGGCCATATTAATTAAAGGAGGTAAAAAATTATGAATGAGTTCAAG
AGGTGTATGCGCGTGTTTAGTCATTCTCCCTTTAAAGTACGGTTAATG
CTGCTCTCTATGTTGTGCGATATGGTCAACAACAAACCGCAGCAAGA
TAAACCTTCCGATAAATAGAAAAGGCCGCGGCCGTTTTCGGCCGCGG
CCTTTTTTTT3’

Practical 6: To determine the mRNA Sequence from the given Gene Sequence

Aim: To determine the mRNA sequence from the given gene sequence

Requirement: Sequences of a prokaryotic gene with properly defined template and non-template strands.

Background:

Prokaryotic mRNA starts at the transcription start point (TSS) and ends with poly A. It includes a 5' untranslated region (5'UTR), at least one Open reading frame (ORF) and a 3'UTR.

5'UTR:

It is a region downstream of the TSS and upstream of the start codon of the ORF. It is a non-coding region for initial ribosome binding. It has a ribosome-binding Shine-Dalgarno sequence complementary to a part of the 16S rRNA of the small subunit of the ribosome. It includes…

- Leading Spacer: 3 to 10bp
- Shine-Dalgarno: 7bp AGGAGGT
- Downstream Spacer: 5-9 bp
- Start Codon: ATG (AUG in mRNA)

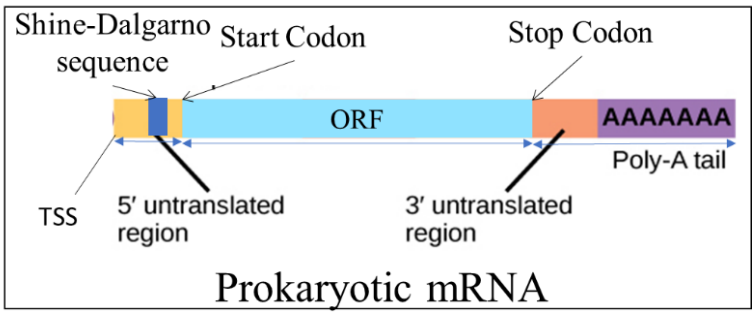
ORF

It is a sequence of triplet codons that codes for a sequence of amino acids in a protein. It starts with the initiation codon 'ATG' (AUG in mRNA) and ends with a stop codon TAA, TAG or TGA (UAA, UAG, or UGA in mRNA)

3'UTR:

It is the region downstream of the stop codon. It is also a non-coding region and plays an important role in gene regulation. If the gene has an intrinsic terminator, then the 3'UTR includes…

- Stop Codon: TAA/TAG/TGA
- Spacer: 4bp
- GC-Rich Stem - Loop - GC-Rich Stem: 26bp
- Poly-U Tail: 8 T -3'



Procedure:

1. Confirm that the given sequence is the non-template strand. Otherwise, draw the complementary sequence if the template strand sequence provided.
2. Find the TSS as described in the previous practical. Mark it as +1.
3. Find the Shine-Dalgarno sequence AGGAGGT 3 to 10 nucleotides downstream of the +1 site.
4. Find the start codon ATG about 6 nucleotides downstream of the Shine-Dalgarno sequence.
5. Mark all Triplet codons after ATG until a stop codon.
6. Find the GC-rich inverted repeat on the intrinsic terminator followed by a stretch of T.
7. Convert the sequence of DNA to a sequence of mRNA from TSS to Poly T by replacing T with U

Gene sequence 1: Non-template strand

5’AAAAATAAATTAAATTAATTGACATTATATTAAATTTATTATAATCCGG

CCATATTAATTAAAGGAGGTAAAAAATTATGAATGAGTTCAAGAGGTG

TATGCGCGTGTTTAGTCATTCTCCCTTTAAAGTACGGTTAATGCTGCTC

TCTATGTTGTGCGATATGGTCAACAACAAACCGCAGCAAGATAAACCT

TCCGATAAATAGAAAAGGCCGCGGCCGTTTTCGGCCGCGGCCTTTTTT

TT3’

Gene sequence 2: Non-template strand

5’AAAAAAATAAATTAAATTATTTGACATTATATTATTTATTATAATCGCG

CCAAATTAATTAAAGGAGGTAAAAAATTATGGCCAGCAGAGGCGTAA

ACAAGGTTATTCTCGTTGGTAATCTGGGTCAGGACCCGGAAGTACGCT

ACATGCCAAATGGTGGCGCAGTTGCCAACATTACGCTGGCTACTTCCG

AATCCTGGCGTGATAAAGCGACCGGCGAGATGAAAGAACAGACTGA

ATGGCACCGCGTTGTGCTGTTCGGCAAACTGGCAGAAGTGGCGAGCG

AATATCTGCGTAAAGGTTCTCAGGTTTATATCGAAGGTCAGCTGCGTA

CCCGTAAATGGACCGATCAATCCGGTCAGGATCGCTACACCACAGAA

GTCGTGGTGAACGTTGGCGGCACCATGCAGATGCTGGGTGGTCGTCA

GGGTGGTGGCGCTCCGGCAGGTGGCAATATCGGTGGTGGTCAGCCGC

AGGGCGGTTGGGGTCAGCCTCAGCAGCCGCAGGGTGGCAATCAGTT

CAGCGGCGGCGCGCAGTCTCGCCCGCAGCAGTCCGCTCCGGCAGCG

CCGTCTAACGAGCCGCCGATGGACTTTGATGATGACATTCCGTTCTGA

AAAAGGCCGCGGCCGTTTT CGGCCGCGGCCTTTTTTTT3’

Practical 7: To Identify the Open Reading Frame (ORF) in a given mRNA Sequence

Aim: To identify the Open Reading Frame (ORF) in a given mRNA Sequence

Requirement: A sequence of mRNA

Background:

An mRNA sequence has a 5' UTR, an open reading frame (ORF) and a 3'UTR. The ORF starts with the start codon AUG and ends with a stop codon. Any one of UAA, UAG or UGA may be present in the given sequence.

Procedure:

1. Scan the given mRNA sequence from the 5'end to find the start codon AUG.
2. Confirm it by finding the Shine-Dalgarno sequence about 6 nucleotides upstream of the AUG.
3. Mark the triplet codons after the start codon until you find one of the stop codons.
4. Mark the start and stop codons.
5. The region from the start codon to the stop codon is the ORF.

Sequence 1

5'AAAUUAAUUAAAGGAGGUAUAAAUUUAUGGCCAGCAGAGGCGU
AAACAAGGUUAUUCUCGUUGGUAAUCUGGGUCAGGACCCGGAAGU
ACGCUACAUGCCAAAUGGUGGCGCAGUUGCCAACAUUACGCUGGC
UACUUCCGAAUCCUGGCGUGAUAAAGCGACCGGCGAGAUGAAAGA
ACAGACUGAAUGGCACCGCGUUGTGCUGUUCGGCAAACUGGCAGA
AGUGGCGAGCGAAUAUCUGCGUAAAGGUUCUCAGGUUUAUAUCGA
AGGUCAGCUGCGUACCCGUAAAUGGACCGAUCAAUCCGGUCAGGA
UCGCUACACCACAGAAGUCGUGGUGAACGUUGGCGGCACCAUGCA
GAUGCUGGGUGGUCGUCAGGGUGGUGGCGCUCCGGCAGGUGGCAA
UAUCGGUGGUGGUCAGCCGCAGGGCGGTUGGGGUCAGCCUCAGCA
GCCGCAGGGUGGCAAUCAGUUCAGCGGCGGCGCGCAGUCUCGCCC
GCAGCAGUCCGCUCCGGCAGCGCCGUCUAACGAGCCGCCGAUGGAC
UUUGAUGAUGACAUUCCGUUCUGAAAAAGGCCGCGGCCGUUUUCG
GCCGCGGCCUUUUUUU3'

Sequence 2

AUUAAUUAAAGGAGGUAAAAAAUUAUGAAUGAGUUCAAGAGGUG
UAUGCGCGUGUUUAGUCAUUCUCCCUUUAAAGUACGGUUAAUGCU
GCUCUCUAUGUUGUGCGAUAUGGUCAACAACAAACCGCAGCAAGA
UAAACCUUCCGAUAAAUAGAAAAGGCCGCGGCCGUUUUCGGCCGC
GGCCUUUUUUUU3’

Practical 8: To identify 3' and 5' splice sites in the given mRNA sequence

Aim: To find the 5' splice site, 3' splice site and the branch point in a given sequence.

Requirement: A non-template strand sequence of an intron with flanking sequences of exons.

Background:

Eukaryotic pre-mRNA has some non-coding sequences apart from the 5'UTR and 3'UTR alternated with the coding sequences. These non-coding sequences are called introns and the coding sequences are called exons. In RNA processing, the introns are removed by the process of splicing. In this process, the pre-mRNA is cut precisely at the 5' splice site and the 3' splice site of each intron-exon junction followed by the joining of exons. Mis-splicing by a single nucleotide may lead to a frameshift. So, identification of the splice site is the most critical step in the splicing process.

Key for identification of 5' splice site: 5' Splice site has GT and can be identified by the following key.

5'M A G | G T R A G T 3'

- M = A or C
- **R** = A or G (Purines)
- **GT** = The invariant, mandatory core dinucleotide.
- | 5' Splice site

Key for identification of the 3' Splice site: The 3' Splice site has AG and the key for identification is an upstream stretch of pyrimidines followed by AG.

Key for identification of the branch point: The branch point is an 'A' playing important role in the splicing mechanism. It is 18 to 40 nucleotides upstream of the 3' Splice site. Key for the branch point is …

5'Y N Y T R <u>A</u> Y 3'

- <u>A</u> Branch Point
- **Y** = Pyrimidine (C or T/U)
- **R** = Purine (A or G)
- **N** = Any nucleotide

Procedure:

1. Scan the sequence to find the dinucleotide GT.
2. Apply the key for identification of the 5' Splice site to confirm the splice site.

3. After confirming, highlight the key features of the 5' splice site and mark the 5’splice site with an arrow.
4. Scan the sequence further to find a stretch of pyrimidines and locate the dinucleotide AG as the 3’ splice site.
5. Highlight the 3’ AG and mark the 3’splice site with an arrow.
6. To locate the branch point, scan the sequence 18 to 40 nucleotides upstream of the 3’ splice site to locate an ‘A’ that fulfils the key requirements.
7. Highlight the branch point ‘A’ and its key features.

Sequence:

5’ATGCGTACTGCAATCGACTAGCTAGCATCGATCGATCGATCAGG
TGAGTAGCCTAGCGCCAGGACCCGGAAGTACGCTACATGCCAAA
TGGTGGCGCAGTTGCCAACATTACGCTGGCTACTTCCGAATCCTG
GCGTGATAAAGCGACCGGCGAGATGAAAGAACAGACTGAATGGC
ACCGCGTTGTGTACTGACTCACCCCTTTTCTTTTCTTTCCTAGGTC
ACTGCACTAGCTAGCTAGCATCGATCGATCGATCGACT3'

Practical 9: To deduce the Amino Acid Sequence from the given ORF of mRNA using the dictionary of the Genetic Code

Aim: To deduce the Amino Acid Sequence from the given ORF of mRNA using the dictionary of the Genetic Code

Requirement: An ORF and the dictionary of the Genetic code.

Background:

The ORF is a coding region of mRNA. It codes for an amino acid sequence of a protein. The genetic dictionary gives the genetic codes and the amino acids assigned to them. The genetic code is non-ambiguous. Using the dictionary, the sequence of mRNA can be read to deduce the amino acid sequence.

Procedure:

1. Find the start codon, AUG.
2. Mark the triplet codons downstream of AUG up to the stop codon.
3. Write the amino acid assigned to the start codon, fMet.
4. Find the amino acids assigned to each triplet codon and write them in the same sequence as the codons.

The genetic dictionary

	U		C		A		G		
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

Sequence of ORF:

5’AUGGCCAGCAGAGGCGUAAACAAGGUUAUUCUCGUUGG

UAAUCUGGGUCAGGACCCGGAAGUACGCUACAUGCCAAA

UGGUGGCGCAGUUGCCAACAUUACGCUGGCUACUUCCGA

AUCCUGGCGUGAUAAAGCGACCGGCGAGAUGAAAGAACA

GACUGAAUGGCACCGCGUUGTGCUGUUCGGCAAACUGGCA

GAAGUGGCGAGCGAAUAUCUGCGUAAAGGUUCUCAGGUU

UAUAUCGAAGGUCAGCUGCGUACCCGUAAAUGGACCGAU

CAAUCCGGUCAGGAUCGCUACACCACAGAAGUCGUGGUG

AACGUUGGCGGCACCAUGCAGAUGCUGGGUGGUCGUCAG

GGUGGUGGCGCUCCGGCAGGUGGCAAUAUCGGUGGUGGU

CAGCCGCAGGGCGGTUGGGGUCAGCCUCAGCAGCCGCAGG

GUGGCAAUCAGUUCAGCGGCGGCGCGCAGUCUCGCCCGCA

GCAGUCCGCUCCGGCAGCGCCGUCUAACGAGCCGCCGAUG

GACUUUGAUGAUGACAUUCCGUUCTGA

Practical 10: To deduce at least one possible sequence of an ORF using a sequence of amino acids and using the dictionary of the genetic code

Aim: To deduce at least one possible sequence of ORF using a sequence of amino acids and using the dictionary of the genetic code

Requirements: An amino acid sequence with properly denoted N and C terminals and dictionary of genetic code

Background:

The genetic dictionary gives the genetic codes and the amino acids assigned to them. The amino acid sequence can be used to find the mRNA (ORF) sequence. But the genetic code is degenerate. So, there may be more than one possible sequences. The N-terminal amino acid is the first amino acid. It is coded by the first codon at the 5' end of the ORF.

Procedure:

1. Locate the N and C terminals of the given amino acid sequence.
2. Find the N-terminal amino acid in the genetic dictionary and write the code assigned to the amino acid.
3. Mark it as the 5' end to the first nucleotide of the codon.
4. Add the codes for the next amino acids to its 3' side according to the sequence of the amino acids.
5. If an amino acid has more than one codon, choose any one of them.

The genetic dictionary

	U		C		A		G		
U	UUU UUC	Phe	UCU UCC UCA UCG	Ser	UAU UAC	Tyr	UGU UGC	Cys	U C
	UUA UUG	Leu			UAA UAG	Stop Stop	UGA UGG	Stop Trp	A G
C	CUU CUC CUA CUG	Leu	CCU CCC CCA CCG	Pro	CAU CAC	His	CGU CGC CGA CGG	Arg	U C A G
					CAA CAG	Gln			
A	AUU AUC AUA	Ile	ACU ACC ACA ACG	Thr	AAU AAC	Asn	AGU AGC	Ser	U C
	AUG	Met			AAA AAG	Lys	AGA AGG	Arg	A G
G	GUU GUC GUA GUG	Val	GCU GCC GCA GCG	Ala	GAU GAC	Asp	GGU GGC GGA GGG	Gly	U C A G
					GAA GAG	Glu			

The amino acid sequence 1:

N-fMet-Ala-Arg-Lys-Gly–Ser-Gln-Val-Tyr-Ile–Glu–Gly–Gln-

Leu- Arg-Thr-Arg-Lys-Trp-Thr-Ilu-Gly–Pro-Cys–Gln-Pro-

Gln-Lye-Ser-Trp-Gly-Gln-Pro-Gln-Gln-Pro-Gln-Gly-Ala-Gln-C

Amino acid sequence 2:

N-fMet-Asp-Gln-Ser-Gly-Gln-Asp-Arg-Tyr-Thr-Thr-Glu-Val-Val-

Val-Asn-Gly-Gly-Thr-Met-Gln-Met-Leu-Gly-Gly-Arg–Gln-Gly-Gly

–---

Gly-Ala-Pro-Ala–Gly-Gly-Asn-Ilu-Gly–Gln-C

Practical 11: To study the blotting techniques (using simulation)

Aim: To arrange the Southern, Northern, and Western blotting setup using simulation

Requirements:

Plastic tray, Glass platform, Whatman filter papers, blotting paper towels, agarose gel/a simulation of a gel, a simulation of nitrocellulose paper, weight, buffer.

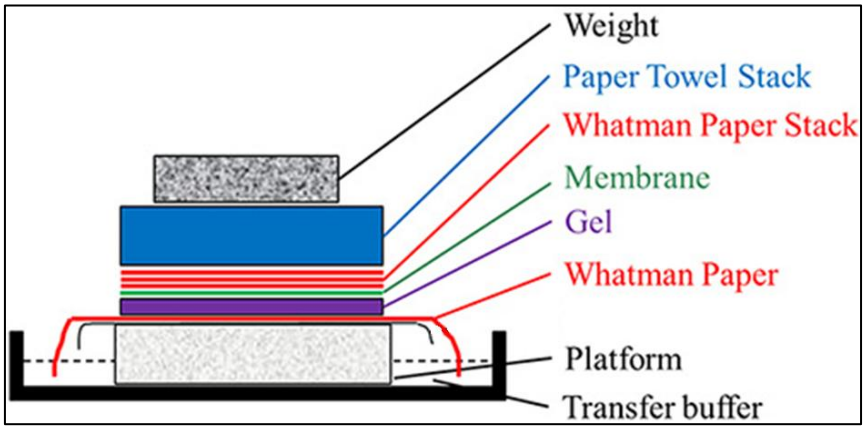
Background:

The DNA, RNA or protein molecules are separated by gel electrophoresis. The gels are fragile and difficult to handle for analysis. So, the bands of separated molecules are transferred onto a sturdier membrane, the nitrocellulose/nylon/polyvinylidene difluoride (PVDF) membrane. Thus, the separated bands can be stored for a longer time and the analysis is safe and easy.

Procedure:

1. Take a plastic tray and place a glass platform in it.
2. Place a Whatman filter paper with wicks on either side, which are dipped in the buffer in the tray.
3. Place the simulated gel in the Whatman filter paper.
4. Place the simulated nitrocellulose membrane of the same size and shape as the gel onto the gel.
5. Place some Whatman filter papers on it and place some filter paper towels on the Whatman filter papers.
6. Place a weight on the top.
7. Pour some buffer into the plastic tray, enough to dip the Whatman paper wicks.
8. The buffer gradually siphons through the Whatman filter paper wick, then through the gel, carrying the bands to the nitrocellulose membrane.

Diagram:



Results: The bands are transferred onto the membrane.

Practical 12: To calculate the GC percentage in a given sequence of at least 200 nucleotides

Aim: To calculate the percentage of GC content in the given DNA sequence

Requirements: DNA sequence (single strand or double strand), calculator

Background:

DNA is a double-stranded structure. The two strands are bound together by hydrogen bonds between the nitrogen-bases in nucleotides. Adenine pairs with thymine with two hydrogen bonds and guanine pairs with cytosine with three hydrogen bonds. So, G≡C requires more energy to break than T=C. The GC content of the DNA affects the denaturation time. Higher GC content in a DNA fragment increases the time for denaturation. DNA fragment with lower GC content requires less is the time for denaturation. So, for deciding the approximate time required for denaturation in PCR setting, the GC content of a DNA fragment is calculated.

Procedure:

1. Divide the nucleotides in one strand of DNA into groups of ten.
2. Find the number of G and C in each group.
3. Sum the G and C counts of all groups.
4. Count the total number of nucleotides in the same strand.
5. Calculate the percentage of GC content by applying the formula-

$$\% \text{ GC content} = \frac{Number\ of\ G\ and\ C\ residues\ in\ a\ DNA\ strand}{Total\ Number\ of\ the\ nucleotides\ in\ same\ strand} X\ 100$$

DNA strand sequence:

5’AAGGCTCCATCATGCGCCTGGGTGAAGACCGTTCCATGGATGTGG
AAACCATCTCTACCGGTTCGCTTTCACTGGATATCGCGCTTGGGGCA
GGTGGTCTGCCGATGGGCCGTATCGTCGAAATCTACGGACCGGAATC
TTCCGGTAAAACCACGCTGACCTGCAGGTGATCGCCGCAGCGCAGC
GTGAAGGTAAAACCT3’

Result: The % GC content of the given DNA sequence is ……

Practical 13: To arrange the given sequences from lower to higher according to the time required for denaturation in PCR

a) Based on GC content

Aim: To arrange the DNA fragments according to the time required for denaturation based on GC content.

Requirements: 5 different sequences of DNA fragments of the same length with different GC contents

Background:

In double-stranded DNA, adenine pairs with thymine with two hydrogen bonds, and guanine pairs with cytosine with three hydrogen bonds. So, G≡C requires higher energy to break, while A=T requires less energy. So, the DNA fragment with higher GC content requires a higher temperature and duration for denaturation during PCR. The thermocycler program has to be set accordingly.

Procedure:

- Calculate the % GC content of each DNA fragment as in the previous practical.
- Arrange the DNA fragments from lower GC content to higher GC content.
- The order for denaturation duration is the same as the order of GC content.

The DNA fragment sequences:

1. ACTGGATATCGCGCTTGGGGCAGGTGGTCTGCCGATGGGCCGTATCGTCG
2. GTTATCGTCGTTGACTCCGTGGCGGCACTGACGCCGAAAGCGGAAATCGA
3. TAAAATCATTCATGTGGATATGGACTGCTTTTTCGCCGCAGTGGAGATGCG
4. TTCGGCGACCCTCCGCGCCCAGGAACGCCGCCAGACACTCGGCAACGAGC
5. TATTTTGTGCGATATAATCAACAACAAATTGCAACAAGATAAACCTTCCA

Calculation:

Formula: % GC content = $\frac{Number\ of\ G\ and\ C\ residues\ in\ a\ DNA\ strand}{Total\ Number\ of\ the\ nucleotides\ in\ same\ strand} X\ 100$

% GC content for fragment 1

% GC content for fragment 2

% GC content for fragment 3

% GC content for fragment 4

% GC content for fragment 5

Order of fragments from lower GC% to higher GC%

…….<……..<……<……<…….

Order of denaturation duration from lower to higher

…….<……..<……<……<…….

Result:

The order of DNA fragments given, from lower duration for denaturation to higher duration, is…

…….<……..<……<……<…….

b) Based on length

Aim: To arrange the DNA fragments according to the time required for denaturation based on their lengths.

Requirements: 5 different sequences of DNA fragments with different lengths.

Background:

In double-stranded DNA, the two strands of DNA are bound together by hydrogen bonds between the nitrogen bases of nucleotides. For denaturation of DNA, these hydrogen bonds have to be broken. Longer is the DNA fragment, the higher the energy required for denaturation. So, longer fragments require a higher temperature and duration in PCR.

Procedure:

1. Count the number of nucleotides in each given fragment of DNA.
2. Arrange the fragments from shortest to longest.
3. The denaturation duration is small for shorter fragments and longer for longer fragments.
4. The arrangement of the fragments according to length is the same for the duration of denaturation, from shorter to longer.

The DNA fragment sequences:

1. GTTATGAAAGTTGACTCCGTGGCTGCG
2. GTTAGGCTGACGCC
3. GTTATCGTCGTTGACTCCGTGGCGGCACTGACGCCGAAAGCGGAAATCGA
4. GTTATCGTCGGGCTGACGCCGAAAGCGGAAATCGA
5. GTTATCGTCGTTGACTCCGTGGCTGACGCCGAAAGCGGAAATCGA

Length of

Fragment 1=…… bp

Fragment 2=…… bp

Fragment 3=…… bp

Fragment 4=…… bp

Fragment 5=…… bp

Arrangement of DNA fragments from Shorter to longer

…….<……..<……<……<…….

Order of denaturation duration from lower to higher

…….<……..<……<……<…….

Result: The order of DNA fragments given, from lower duration for denaturation to higher duration, is…

…….<……..<……<……<…….

Practical 14: To analyse the photographs of FISH

Aim: To analyse the photographs of fluorescent in situ hybridization (FISH) results.

Requirements: Coloured photographs of the FISH results

Background:

FISH is a technique where fluorescent probes are used to find specific chromosomes or DNA sequences complementary to the probes in a cell. The bound probes can be seen under a fluorescent microscope after washing away unbound probes.

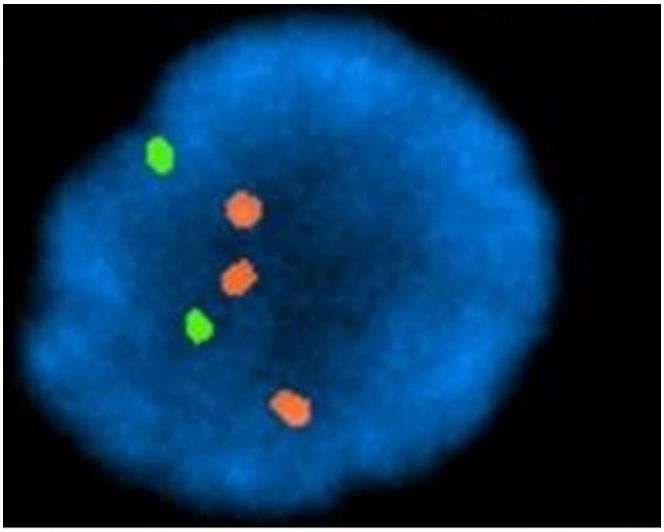
Procedure for FISH (This procedure is not expected to be performed by the students)

- For FISH, a squash or smear of cells is taken on a clean glass slide.
- The slides are fixed using methanol, acetic acid or formalin.
- To increase the permeability of the cells for the probes, slides are treated with a mild enzyme or detergent.
- The DNA in the cell is denatured by heat denaturation or treatment with formamide.
- The slides are incubated at 37°C in a dark, humid chamber overnight in a buffer containing the specific probes.
- The excess probes are washed out using saline sodium citrate (SSC) at room temperature for 2 min.
- The slides are air-dried and counterstained with DAPI (4,6-diamidino-2-phenylindole)
- These slides are visualized and photographed under a fluorescence microscope.
- The photographs are used for diagnosis.

Results:

Identify the photographs and diagnose the disorder

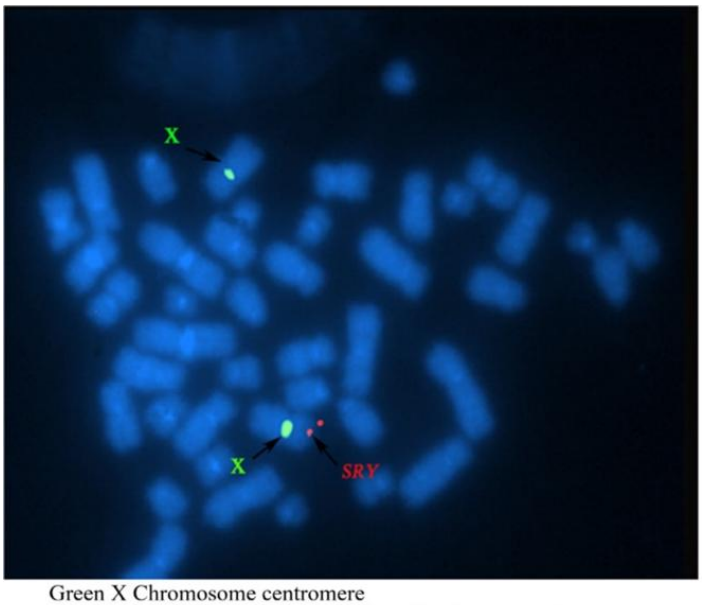
Photograph 1:



Orange Chromosome 21 Green Chromosome 18

Result: Photograph 1 shows ……

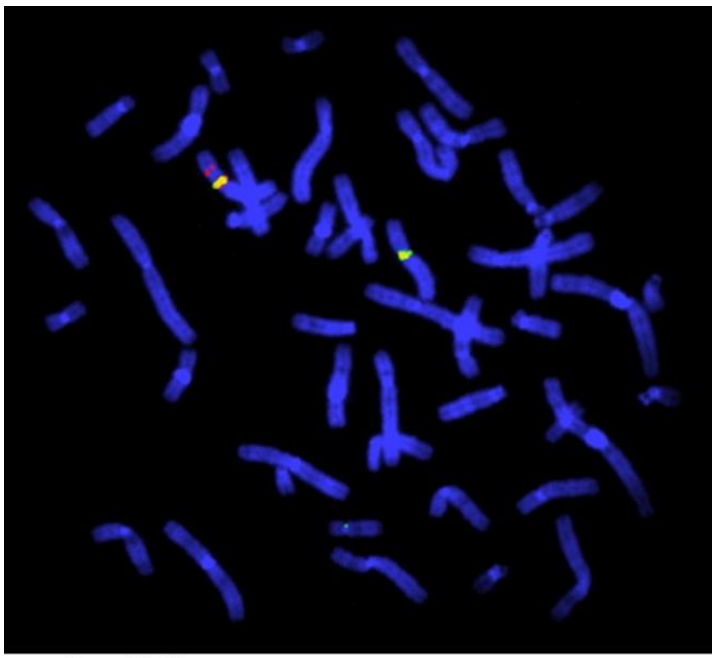
Photograph 2:



Green X Chromosome centromere
Orange SRY (Sex determining region of Y chromosome)

Result: Photograph 2 shows ……

Photograph 3:



Green X Chromosome centromere
Orange DMD mutant gene

Result: Photograph 3 shows ……

Practical 15: To identify the molecular diagnosis of a Matching DNA Sample Using DNA Fingerprinting Gel Analysis

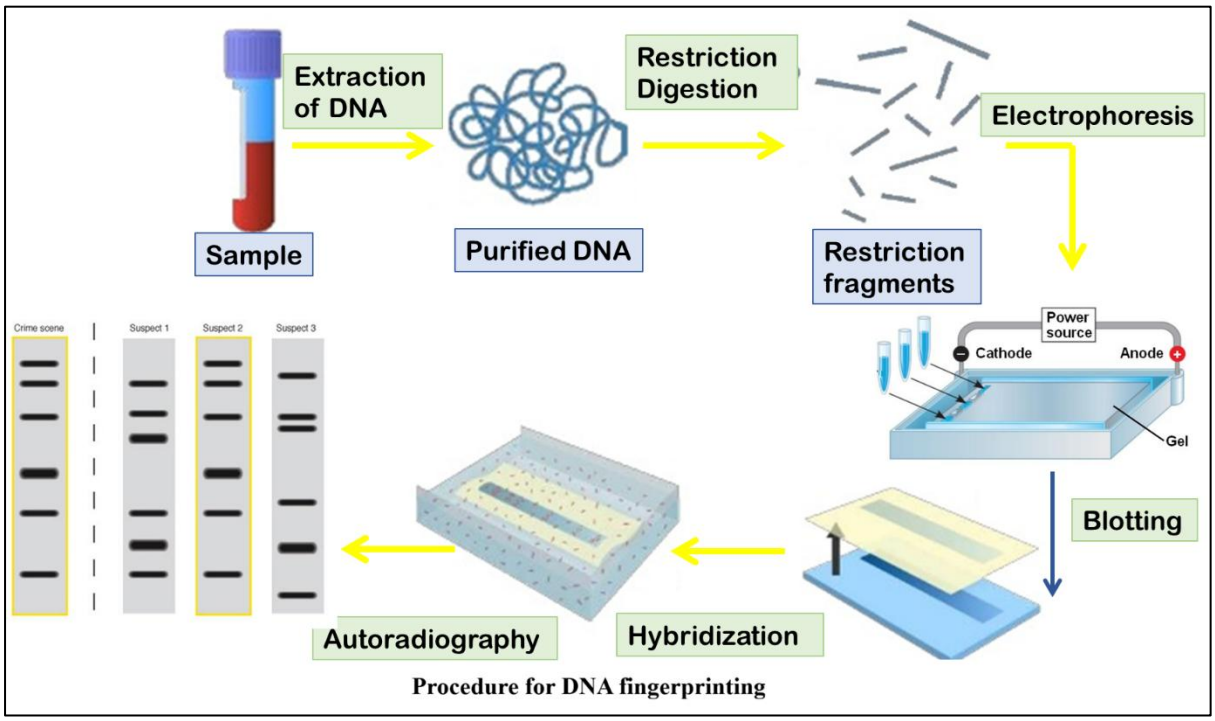
Aim: To read the DNA fingerprint gel and identify the match.

Requirements: Photographs of DNA fingerprint result gels.

Background:

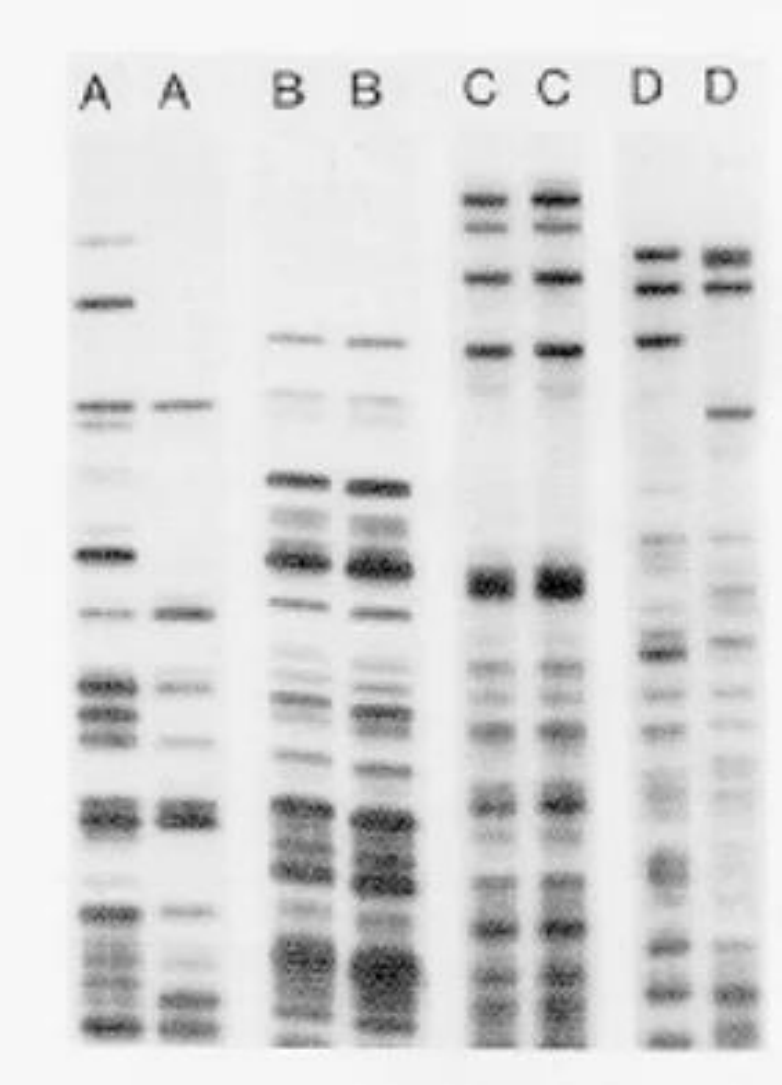
A laboratory technique used to identify individuals based on unique patterns in their DNA sequences. DNA fingerprinting was invented in 1984 by Professor Sir Alec Jeffreys. It is also called DNA typing, DNA profiling, genetic fingerprinting, genotyping, or identity testing. The individual variation is due to the Variable number of tandem repeats (VNTR), which includes minisatellites and microsatellites. This technique is based on restriction fragment length polymorphism (RFLP)

Procedure: (This procedure is not expected to be performed by the students)



Procedure for DNA fingerprinting

Photograph 1: shows DNA fingerprints of twin pairs. By observing the gel, identify the pairs of identical and non-identical twins.

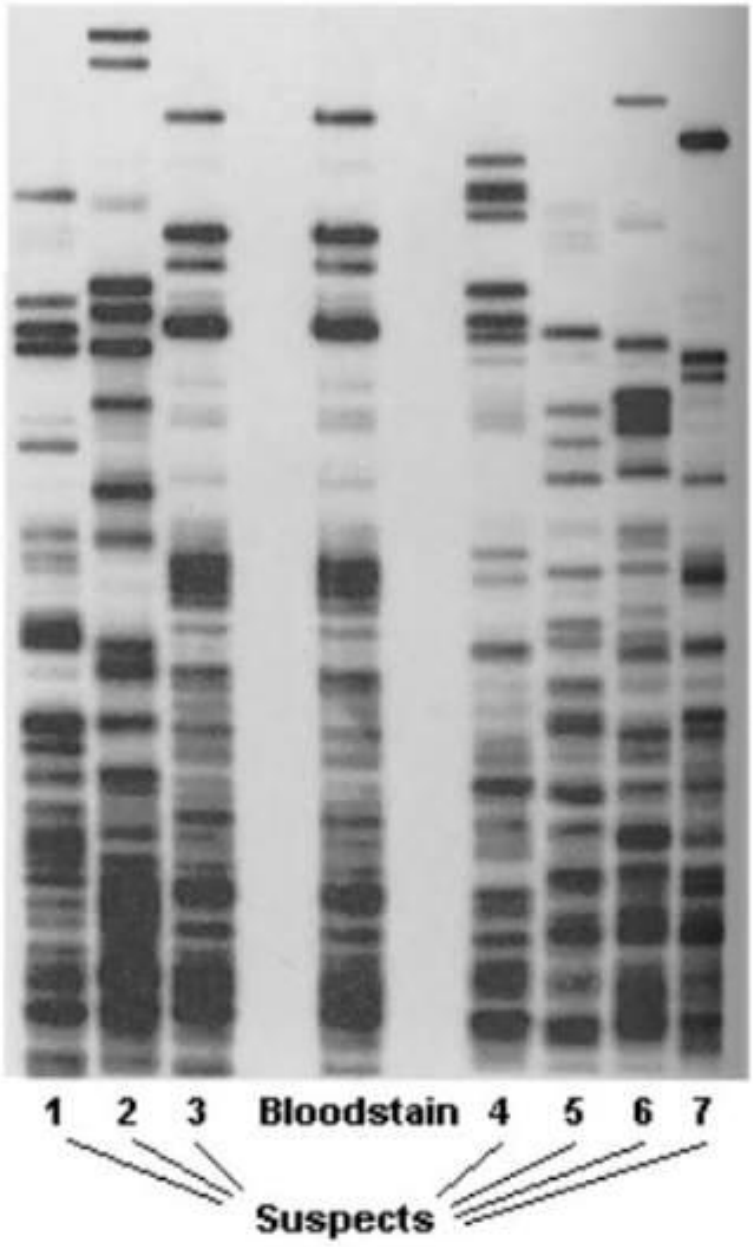


Result: In the given Photograph 1,

DNA Fingerprints of identical twins are ……

DNA Fingerprints of Non-identical twins are ……

Photograph 2: This is a photograph of a DNA fingerprinting gel with a sample from the crime scene and suspects. Find the victim/ criminal by observing the gel.



Result: In the given Photograph 2, the DNA fingerprint of victim/ criminal no. …… matches the evidence at the crime scene.

Practical Handbook Molecular Biology and Molecular Diagnostics

For B. Sc. III, Sem V AS PER NEP-2020 (2.0) SYLLABUS OF SHIVAJI UNIVERSITY, KOLHAPUR (IMPLEMENTED FROM JUNE 2026)

ISBN: 978-81-69967-06-8

About Author

Dr. Kavita Amrut Gajare is an Professor and Head of the Department of Zoology at The New College, Kolhapur. She holds a B.Sc. in Zoology, M.Sc. in Zoology with specialization in Cell Biology, SET in Life Sciences, and a Ph.D. in Zoology from Shivaji University, Kolhapur. Her doctoral research focused on the protective effect of Bacopa monniera Linn extracts on the mouse brain during ageing, reflecting her specialization in cell biology and broader research interest in gerontology. With over 25 years of teaching experience at undergraduate and postgraduate levels, she has served at the Government Vidarbha Institute of Science and Humanities, Amravati, Rajaram College, Kolhapur, and The New College, Kolhapur. Dr. Gajare has published 42 research papers in national and international journals, presented 20 papers at conferences, authored three books and a book chapter and a design patent granted by the Government of India. She has completed research projects funded by UGC and DST and has guided Ph.D. and M.Phil. scholars. She has undertaken training in cell and molecular biology, animal tissue culture, bioinformatics, karyotyping, FISH, and innovative pedagogies. She has also contributed to academic administration through various institutional and university committees, including BOS Zoology and IAEC, and is a life member of professional organizations.

Bhumi Publishing

Bhumi Publishing,
Kolhapur, Maharashtra, India
bhumipublishing@gmail.com
www.bhumipublishing.com

ISBN 978-81-69967-06-8
9 788169 967068