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Table showing the Characters of Blood Group :

Blood group	antigen	Antibody	Acceptable Blood	Donate blood	Genotype
A-group	A	anti - B	A, O	A, AB	$I^A I^A / I^A i$
B-group	B	anti - A	B, O	B, AB	$I^B I^B$ (or) $I^B i$
AB-group	AB	—	A, B, AB, O	AB	$I^A I^B$
O-group	—	anti A and anti B	O	AB, AB, O	ii

Blood group	Antigen on red cells	Antibody in plasma	Rhesus factor
A^+	A	B	present
A^-	A	B	absent
B^+	B	A	present
B^-	B	A	absent
AB^+	A and B	None	present
AB^-	A and B	None	absent
O^+	None	A and B	present
O^-	None	A and B	absent

1. Identification of Blood Groups and blood Grouping:-

Introduction :-

Human Blood Contains specific proteins which facilitates Blood grouping. According types of blood groups and identified is A, B, AB and O basing on the presence of the A and B antigens on the red blood Cells.

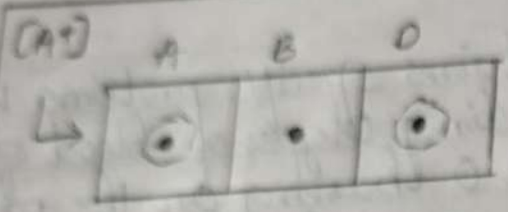
The A type has only the A-antigen and the B-Blood type has only B-antigen. The AB blood type has (neither) Both A and B antigens and the O blood type has neither. A nor B antigens and the O blood type has That is, a person with A blood type will have anti B-antibodies, and a person with B blood type will have anti (B/A antibodies. A person with AB blood type passess no antibodies. but a person with O Blood type will have both anti A and anti B antibodies.

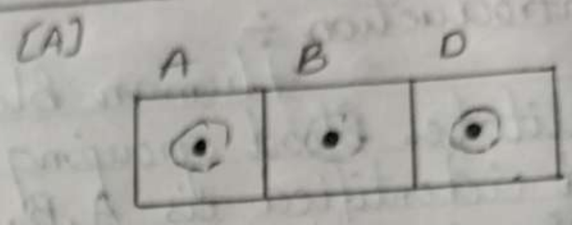
Although the distribution of the person four ABO blood type varies between racial groups, O is most Common and AB is the least Common.

procedure :-

Slide method is the Common and Simple method to identify blood groups. Here a prick is made over the finger with a sterilized needle after wiping it with spirit Cotton. Then the finger is required for flow of blood. The first drop is wiped and then two drops of blood is over the clean slide one on either end.

Teacher' Signature :


(A+)
A
B
D


[A]
A
B
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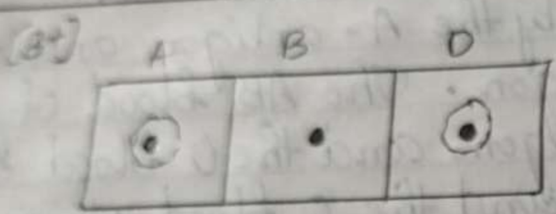
(B+)
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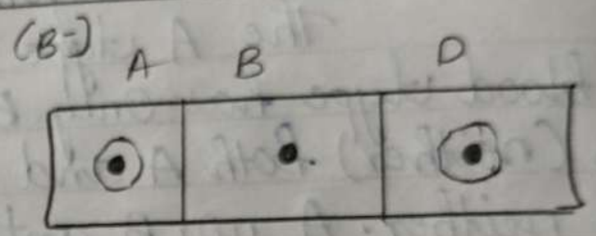
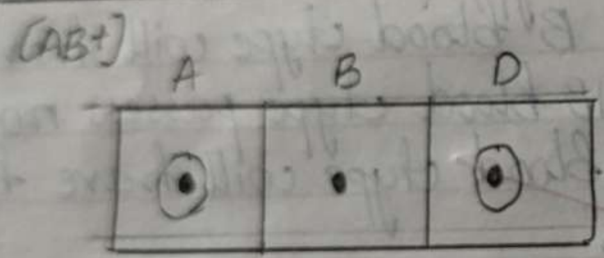
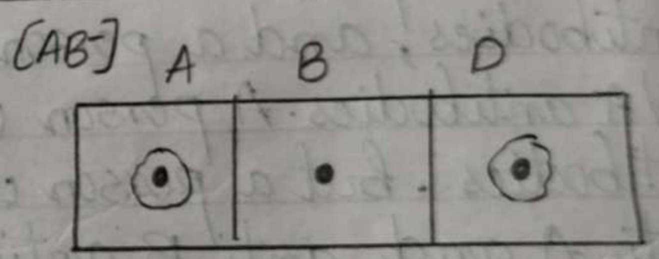
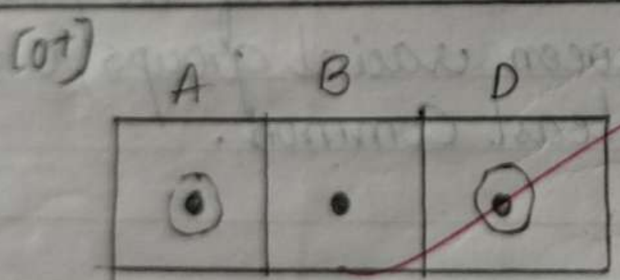
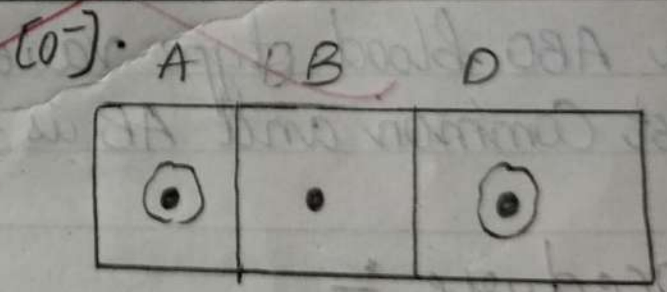

(B-)
A
B
D


(AB+)
A
B
D


[AB-]
A
B
D


[O+]
A
B
D


[O-]
A
B
D

One drop of anti 'A' Serum is added to the left drop of blood. Another anti 'B' Serum is added to the right drop of blood. Then the drops are mixed throughly with the help of a glass and rod. Blood group can be decided after 5 to 10 min of mixing by observing agglutination.

The ABO grouping is the first test done on blood when it is tested for transfusion.

A- Group :- Agglutination with anti A Serum (L^+):
No agglutination with anti B Serum (R^-).

B- Group :- Agglutination with anti B Serum (R^+):
No agglutination with anti A and B Sera (L^-R^+)

AB- Group :- Agglutination with A and B Sera (L^+R^+)

O- Group :- No agglutination with A and B Sera (L^-R^-)

Blood group	Left side blood drop + anti 'A' Serum	right slide Blood drop + anti 'B' Serum
A - Group	+	−
B - Group	−	+
AB - Group	+	+
O - Group	−	−

"+" agglutination results in dumping of blood cells.
"−" agglutination never produce dumping.

Another blood groups drop is taken over a fresh slide and to it, anti D Serum is added and throughly mixed if dumping is seen after 5-10 min then it is Rh +ve and no dumping is seen then Rh-ve.

Result: Agglutination is found in AD Blood group 'A+'

A B D

Teacher' Signature :

2. Veneral disease Research Laboratory test [VDRL]:-

Aim:- The VDRL is a screening test for sexually transmitted infection syphilis caused by the spirochete bacterium treponema pallidum.

2. It is a non-treponemal test, which detects antibodies IgM and IgG antibodies to lipoidal material released from damaged host cell as well as lipoprotein-like material and possibly cardidipin released from the treponemes. These antibodies are traditionally reffered to as reagins.

3. The VDRL test is thus slide micro flocculation test used to screen for syphilis in addition to more specific tests used to diagnosis the disease.

4. VDRL test becomes positive 1-2 weeks after appearance of chance. The test becomes reactive (50-75%) in the table phase of primary syphillis becomes highly reactive (100%) in the secondary syphillis and reactivity decreases (75%) there after.

Requirements of VDRL test:-

patient's serum, water bath, freshly prepared cardiolipin antigen, VDRL slide, mechanical rotator, pippetes and hypodermic syringe with unbeveled needle and microscope known reactive and non-reactive serum controls are also required.

principle of VDRL test :-

The veneral disease research labora-tory (VDRL) test are slide microflocculation test that detect antibodies produced against antigens released by damaged host cells in patient's suffering from syphilis.

2. It uses antigens containing 0.03% cardiolipin, 0.21% lecithin and 0.9% cholestrol. the antigen suspended in a buffered saline solution forms flocculates when combined with lipodial antibodies in serum fluid from syphillis patient's.

3. For the test at first a drop of antigen is placed on a slide and then a drop of serum is added to it. the slide is rotated to mix the content. In case of positive test flocc-ulation occurs which are read using a microscope dumping (or) agglulation indicates reactive specimen (or) presence of autoantibody in patient's specimen while non-reactive specimens appear as homogenous suspension.

procedure of VDRL test :-

The test can be performed with qualitatively and quanlitatively those tests that are reactive by qualitative test are subjected to quanlitative test to determine the antibody titres

Qualitative Method :-

1. patient Serum is inactivated by heating at 56°c for 30 min in a water bath to remove Specific inhibitors (such as Complement).

2. VDRL antigen Suspension (Colloidal Suspension of tissue Cardiolipid (or) Chemically Synthesized Cardiolipin), Controls and Samples are brought to room temperature.

3. One drop (50ul) of the test Specimen positive and negative Controls is pipetted anto Separate reaction Circles of the disposable Slide.

4. A drop of diluted antigen Suspension is added to the measured volume of Specimen, positive and negative Controls.

5. Using 2 mixing Sticks the test Specimen and the reagents VDRL is mixed such that it throughly Separats uniformly Over the entire reaction Circle.

6. The Slide gently rotated and Continously either manually (or) On a mechanical rotator at 180 r.p.m

7. Flocculation is checked microscopically using 10x Objective and eye piece at about 8 minutes.

Result :

1. positive test :
pressure of antigen antibody dumps in the Centre of the periphery of the test circle indicates positive VDRL test.

2. Negative test :
Absence of antigen antibody dumps indicated by a Smooth, even light grey appearance with no ~~appeo~~ aggregates.

All reactive and weakly reactive Serum requires serial dilution to estimate antibody titer, The titer is reported as the reciprocal of the highest dilution. Which shows a positive test result.

3. Radial immuno diffusion :

Immuno diffusion is a technique for the detection (or) measurement of antibodies and antigen's by their precipitation which involves diffusion through a substance such as agar (or) gel agarose, simply it denotes precipition in gel.

* It refers to any of the several techniques for obtaining a precipitates between an antibody and this specific antigen. This can be achieved by :

1. (a) Suspending antigen / antibody in a gel and letting the other migrate through it from a well or.

2. (b) Letting both antibody and antigen nigrate through the gel from sepdrate well such that they from an area of precipitation.

Radial immuno diffusion (RID) (or) mancini method is also known as mancini immuno diffusion (or) single radial immuno diffusion assay. It is a single diffusion technique where by a solution containing the antigen is placed into well in a gel (or) agar surface evenly impregnated with antibody.

Principle of Radial Immuno diffusion :

* Radial immuno-diffusion is a type of precipitation reaction it is thus based on the principle of the precipitin curve which states that antigen - antibody interact formings

visible Cross linked precipitate when the proper ratio of antigen to antibody is present.

* In the test antibody is incorporated into agar and poured into a glass plate to form a uniform layer circular well are at into the agar and antigen is introduced into the wells.

* Specific antigen to the impregnated antibodies diffuse through the agar in all directions from the well and react with the antibody present forming visible precipitate (or) a precipitin ring. Ring shaped bands of precipitates from Concentracally around the well indicating reaction.

* The diameter of the precipitate ring formed Corresponds to the amount of antigen in the Solution.

procedure of Radial immuno diffusion :-

1. An agar Containing an appropriate antiserum (antibody) is poured in plates.

2. Carefully circular wells are cut and removed from the plates.

3. A Single (or) Series of standards Containing known Concentration of antigen are placed in Separate wells, while Control and "Unknown" Sample are placed in other remaining wells.

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4. As the antigen diffuses radially a ring of precipitate will from in the area of optimal antigen - antibody Concentration

5. The ring diameters are measured and notes.

6. A Standard Curve is prepared using the ring diameter of the Standards Versus their Concentrations. This Curve is then used to determine the Conc. of Control and unknown Samples.

Objectives of Radial Immuno diffuses :-

The mancini immuno diffusion test may be carried out with one (or) more of the following objectives:

1. To detect antigen - antibody Complexes.
2. Describe the circumstance under which ag ab Complexes precipitate act.
3. Determine relative Concentration of antigens.

Result interpretation of Radial immuno diffusion :-

1. The presence of a precipition ring around the antigen wells indicates specific antigen - antibody interaction.

2. Absence of preciption ring suggest absence of reaction.

3. The greater amount of antigen of the well the farther the ring will form from the well.

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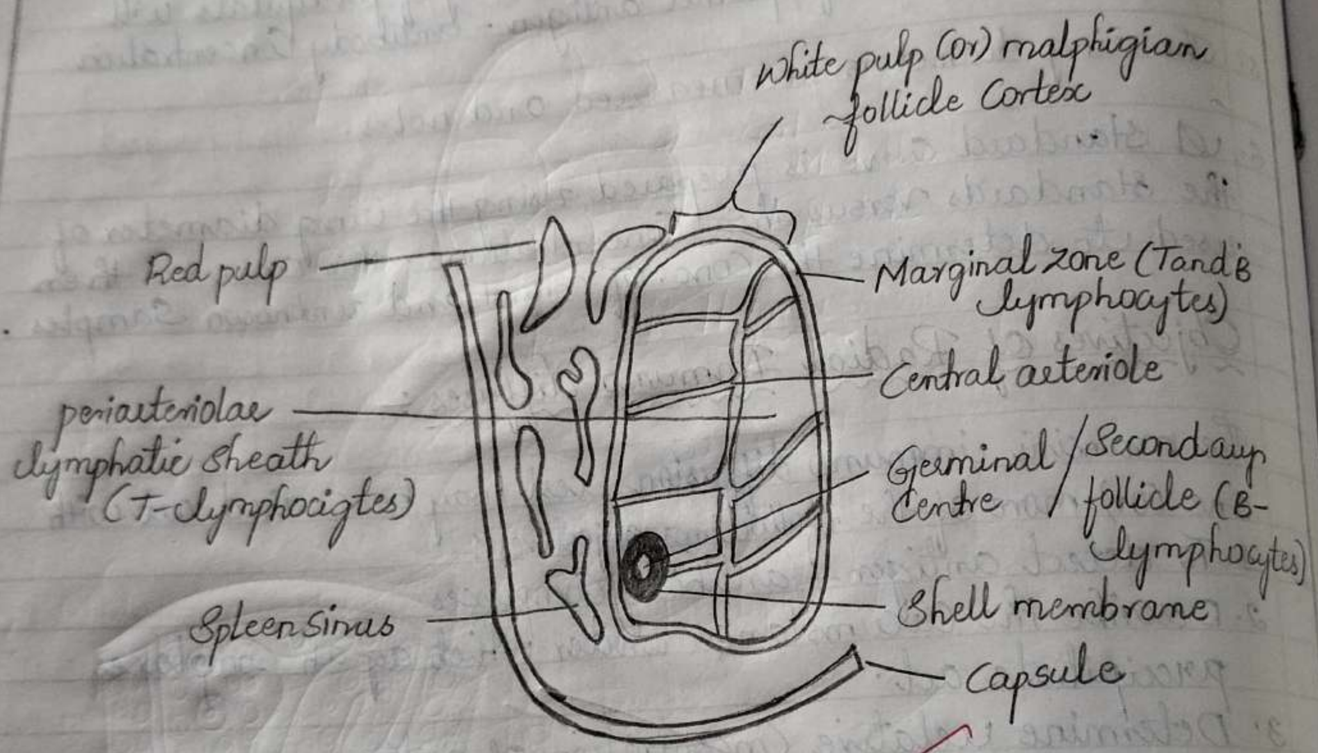

T.S. Of Spleen

Histological Study of Spleen, Thymus and Lymph node :-

[A] Spleen :-

It is located under the left side of the ribcage, next to the stomach as a small and red.

(i) It is an important Constituent of the reticuloendothelical System of body.

(ii) It is the largest tender ball like structure in body's lymphatic system.

(iii) Haemoglobin is broken down into heam and globin by spleen.

(iv) Besides, it assists liver and helps in maintaing the composition of blood.

(v) The red pulp of the spleen serves the circulatory system by filtering the blood, and acting as a recycling station for the red blood cells, It is a storage point for other important blood Components. it makes up the majority of tissue in most healthy spleens. the red pulps Consist of Connective tissue Called Cords of Billroth. The red pulp stores (WBC) special cells Called "Splenic Cells" which stores digest and transport RBC.

(vi) The write pulp of the spleen is the tissue that perform the Spleen's immune functions. it Consists of a layering of different tissues and nodules each of which perform distinct functions for the immune system.

→ Make antibodies to make the immune System strong.

→ Breaks down waste products from dead Cells.

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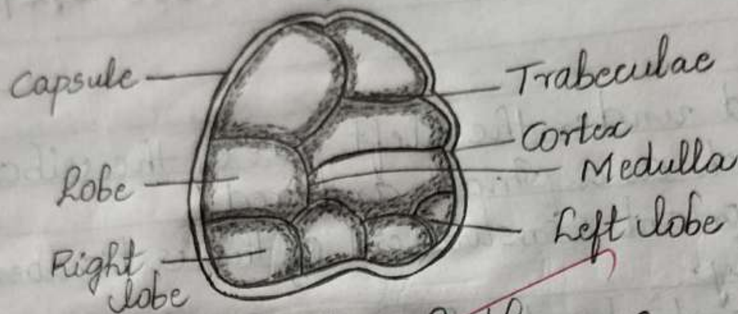
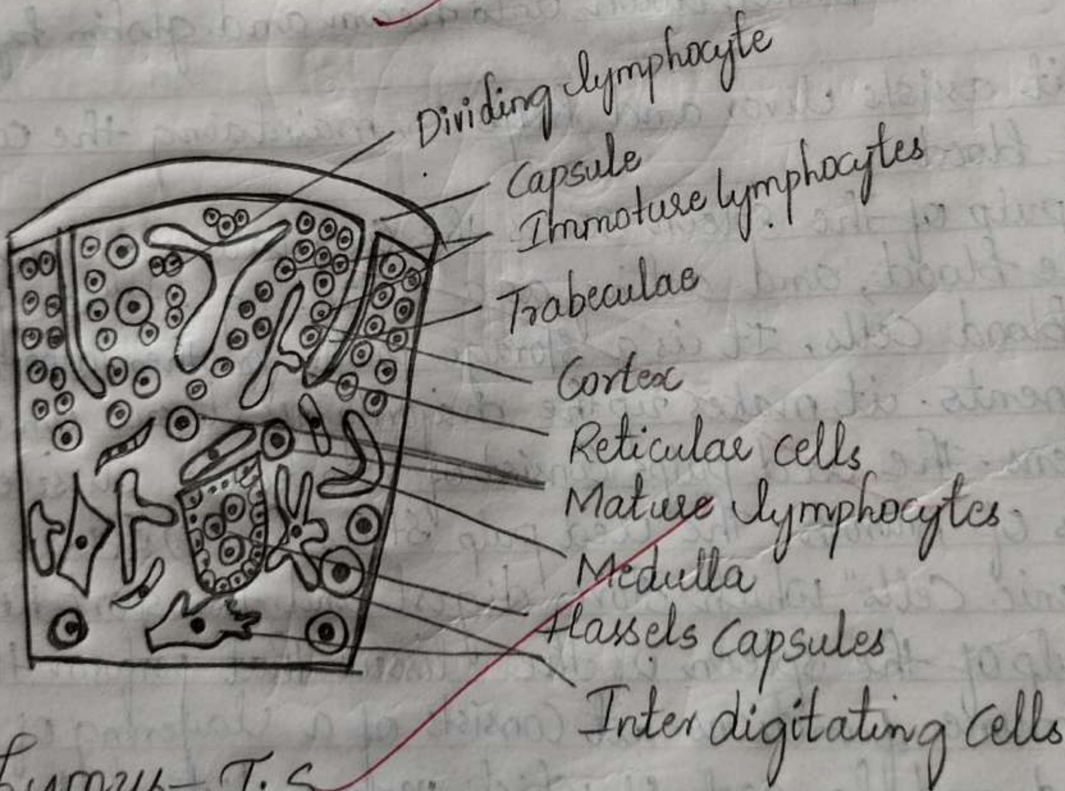


T.S. Of thymus



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Thymus- T.S

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(B) Thymus Gland :-

(i) In human Thymus is located in mediasternum between lungs.

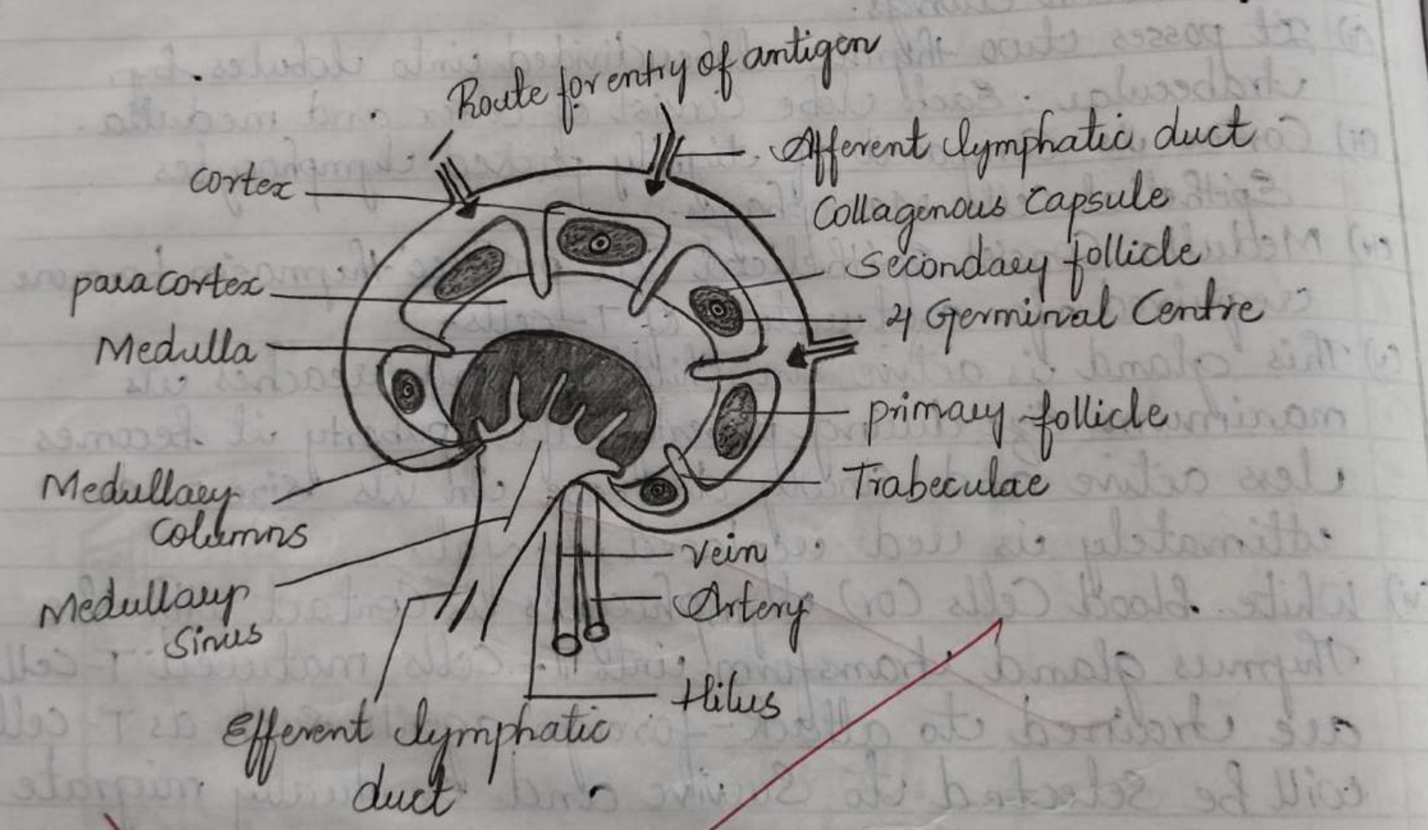
(ii) It posses two thymic lobes divided into lobules by trabecular. Each lobe Consist of Cortex and medulla.

(iii) Cortex is Composed of tightly packed lymphocytes Epithelial Cells Macrophages.

(iv) Medulla Consist Epithelical cell produce thymosin hormone required for maturation of T-cells.

(v) This gland is active in Childhood and reaches its maximum Size during puberty. after puberty it becomes less active and slowly decrease in its size and ultimately is red replaced by fat.

(vi) White blood Cells (or) lymphocytes is Contact with the Thymus gland transform into T-cells matured T-cells are trained to attack-foreign agents such as T-cells will be Selected to Survive and eventually migrate to the medulla.



Human Lymphnode

(c) Lymph nodes ÷

These are the masses of lymphatic and Connective - tissue located on the capillaries either singly as lymph gland (or) in clusters (tonsils) Some of the common lymph nodes are - axillary nodes (in armpits) genital (inguinal) nodes (in region) cervical nodes (in neck region) inter coasted node (in chest region) lumbar node (in lumbar region) iliac nodes in pelvic region) and payer's patches (in small intestine) Besides these lymphatic nodes a number of them are also present near major blood vessels, specially dorsal aorta. Tonsils ÷ are Cluster of lymph nodes, they are very often Called as policemen. adenoid tonsils degenerate often 7 year of age causing inflammation called Tonsilities.

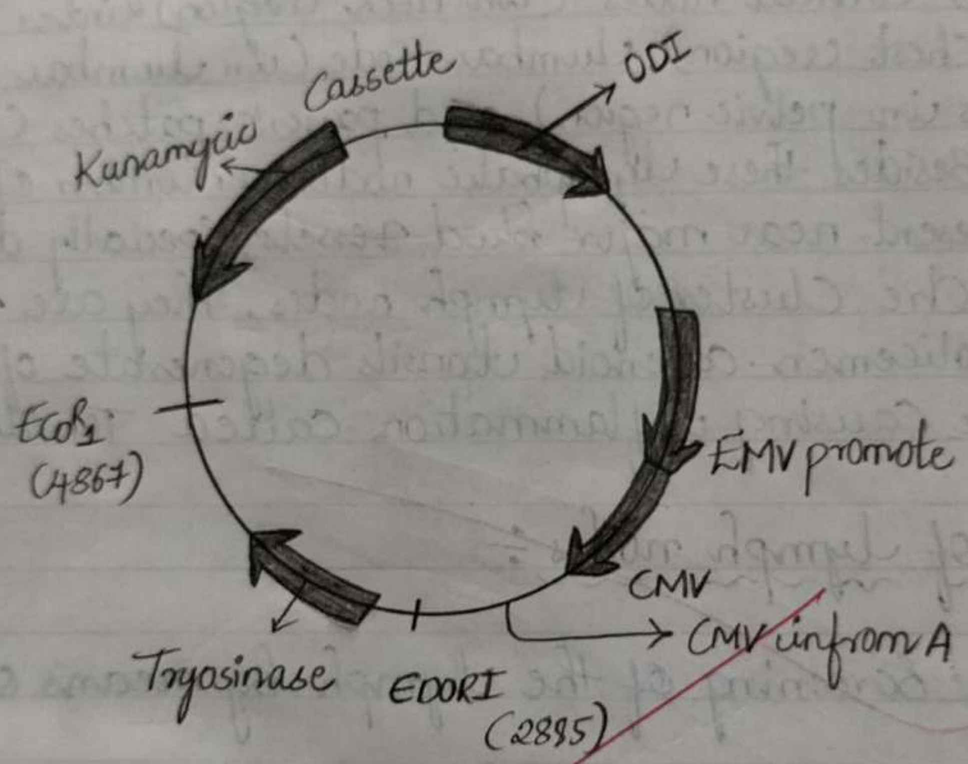
* Functions of lymph nodes ÷

→ They make screening of the lymph by means of phagocytic activity.

→ They serve a great defensive role against bacterial infections.

→ They carry out immunological respones. they help in elaboration of antibodies into circulation.

→ lymph nodes produce globulin.


Cassette
ODI
Kunamycin
EcoR1
(4867)
EMV promote
CMV
CMV infrom A
Tryosinase
EcoRI
(2885)

Study the following techniques through photograph (or) virtual lab:

(I) (a) Identification of vectors:

* vector defination: Vectors are the Carrier DNA's into which foreign DNA's (or) genes of interest are spliced to make a r-DNA vectors along with the inserted DNA (or) the foreign DNA are then introduced into appropriate host cell and are maintained for study (or) expression.
* Characteristics of an ideal vector: An ideal vector should be small in size with a single site for restriction endonuclease.

A region of replication and 1-2 genetic masks vectors are of two categories viz.

1. Cloning vectors
2. Expression vectors.

1. Cloning vector:

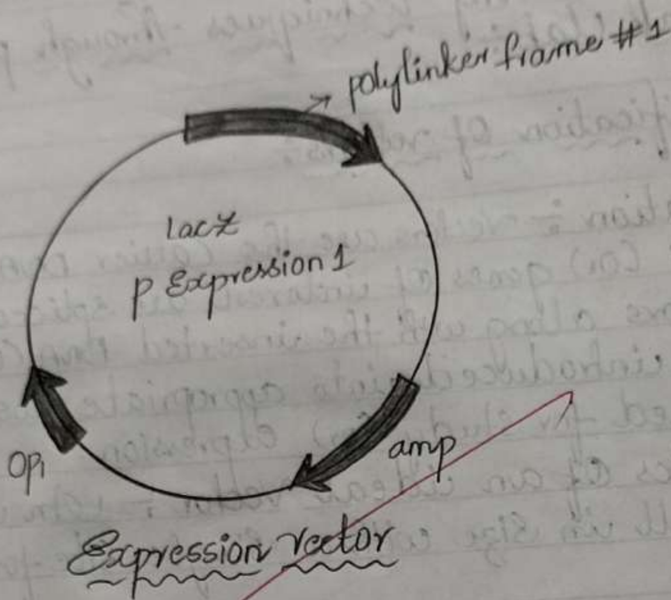
Vectors are using for obtaining millions of copies of cloned DNA segment cloning vector are used for creating genomic library.

→ Cloning vectors are mostly commerical

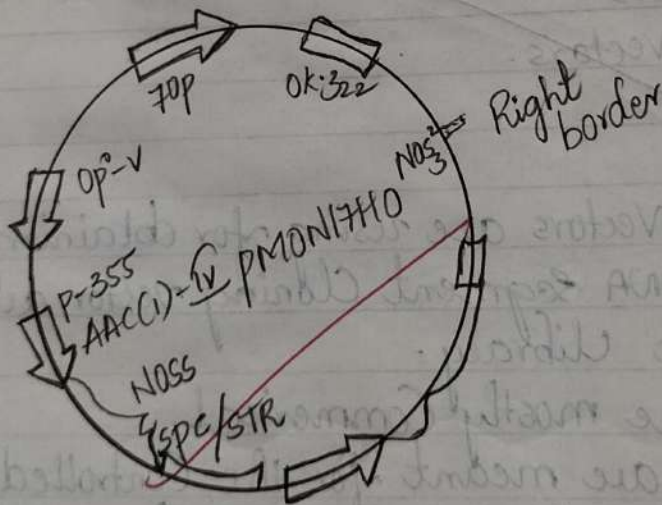
→ Expression vectors are meant for the controlled expression of a particular gene inside a convenient host (Eg: E,coil]

→ Control of expression is very important to insert the target DNA. Expression site controlled by a particullar promote.

→ Some commenly used promoters are T7 promoters and lac promoters (old promoter)



Expression Vector



plasmid as a Cloning

* Mostly cloning vector process :-

1. Restriction site engineered out of them
2. A synthetic multiple cloning site (smcs) inserted containing many restriction sites.
3. A selectable marker such as antibiotic resistance
4. A functional site of origin of replication (ORI)
5. "vir" gene for plant transformation
6. Integrase site for chromosomal insertion
7. "LacZ" a fragment for a complementation.
8. Blue-White selection and reporter gene facilitating the production of recombinant proteins (eg:- Green-fluorescent protein [GFP] (or) to the glutathione S-transferase)

They allow the expression of cloned gene to give the product. These are used for transform to generate trans genic plant, animal (or) microbe

→ Antibodies generated to a protein are used to clone the encoding CDNA.

→ Here the bacteria transcribe and translate YEP and use an antibody probe to find the clony of cells expression vector passes.
(i) a poly-linker for cloning (ii) an origin of replication
(iii) an ab resistance gene (iv) an ab resistance gene

→ The poly-linker is located in the lacZ gene
(1) Allows sub-cloning from one vector backbone to another (2) every sub-cloning reaction maintain the appropriate reading frame (3) Support site specific recombinant. plasmids are extra chromosomal ds, circular, self replicating DNA molecules. They can be cleaved at a self with RE Enzyme.

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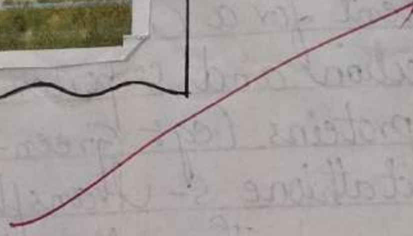
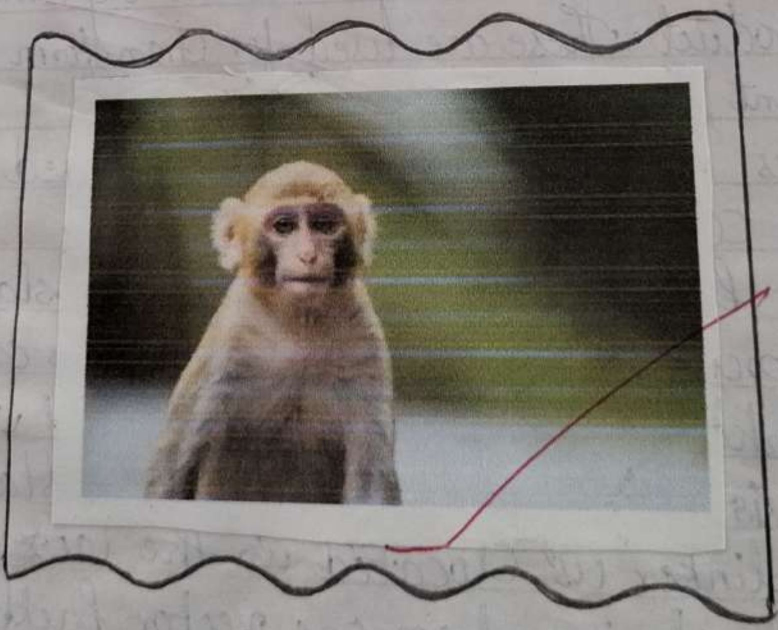
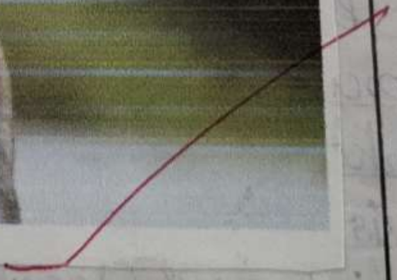






(B) Identification of Transgenic animals :

* Transgenic Rabbit :

→ Alba, the EGFP (Enhanced Green flurescent protein) Bunny
→ Created in 2000 as a transgenic artwork.

* Transgenic Monkey :

→ ANDi was the first transgenic monkey, born in 2000.
→ "ANDi" Stands for "inserted DNA" spelled backwards
→ An engineered virus was used to insert the harmless gene for green fluorescence protein (GFP) into ANDi's rhesus genome.
→ ANDi proves that transgenic primates can be and can be Express a foreign gene delivered into their genome.

* Transgenic livestock :

→ Bioreactors whose cells have been engineered to Synthesis marketable proteins.
→ More economical than producing desired protein in cell Culture.

* Transgenic Cattle :

→ Transgenic Cows are made to produce lactoferrin and interferons in their milk.
→ pirion free Cows resistant to (move) mad Cow disease.

* Transgenic Sheep :

→ For good quality wood production.

* Transgenic goat :

→ Goat that Could Express tissue plasminogen activator, anti thrombin III, Spider Silk etc in milk.

* Transgenic Mouse :

Alzheimer's mouse

1. In the brain of alzheimer's patients, dead nerve cells are entangled in a protein called amyloid.
2. Mouse made by introducing amyloid precursor gene into fertilized egg of mice.

Oncomouse :

1. Mouse model to study Cancer.
2. Made by inserting activated Oncogenes.

Smart mouse :

1. Biological model engineered to overexpress NR 2B receptor in the Synaptic pathway.

2. This makes the mice learn faster like juveniles throughout their lives.

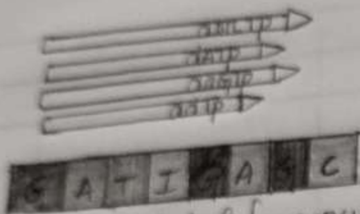
Transgenic animals :

1. Mice :- used to study human immune System.

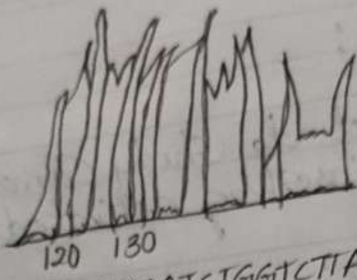
2. Chickens :- more resistant to infections

3. Cows : increased milk supply and leaner meat.

4. Goats, sheep and pigs :- produce human proteins in their milk.

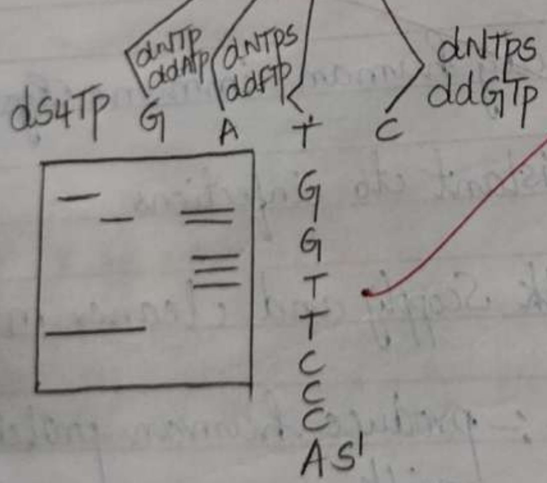


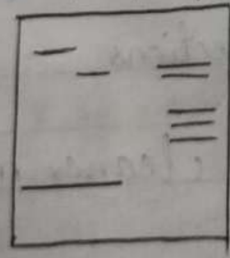
dye- labelled dideoxynucleotide are also generate DNA fragments of different lengths.



Sager method of DNA Sequencing

Sanger ddNTP Chain Tremination Sequencing





: Flow Chart for Sanger Method of DNA Sequencing:

(c) DNA Sequencing:

* Summary:
The Sanger method, also refferred to as dideoxynucleotide sequencing (or) chain termination sequencing is based on the use of dideoxynucleotide (ddNTp) is addition to the normal nucleotides (dNTp) found in DNA dideoxynucleotide are essentially the same as nucleotides except they contain a hydrogen group on the 3' carbon instead of a hydroxyl group (-OH). These modified nucleotides when integrated into a DNA sequence, prevent the addition of futher nucleotids thus stopping the elongation of the DNA chain. This occurs because a phosphodiester bond cannot form between the dideoxynucleotide and the next incoming nucleotide and thus the DNA chain is termed terminated.

Following steps are the outlines of the procedures of the Sanger method.

1. The region of DNA to be sequenced is amplified in some way and then denaturated to produce SSDNA.
2. A sequencing primer is annealed to the SSDNA.

3. Dideoxynucleotide chain termination DNA sequencing then takes advantages of the fact that a growing chain of nucleotides extending in the 5' to 3' direction will terminate if instead of a conventional deoxynucleotide, a 2'3' dideoxynucleotide becomes incorporated by performing four separate reactions each containing a DNA polymerase and a small amt of one of the four dideoxynucleotide in addition to all four dideoxynucleotide four separate sets of chain terminated fragments can be produced.

4. Following the replication / termination step, these chain can terminated fragments will remain bound to the SSDNA molecules which has acted as a template. By heating these partially double stranded molecules and adding a denaturation agent such as formamide the single stranded chain termination molecules can be released from their template and separated using high resolution denaturing gel electrophoresis.

5. The sequencing of the original region of DNA is then finally reduced by examing the relative positions of the dideoxynucleotide chain termination products in the four line of denaturing gel.

(c) DNA finger printing :-

* Introduction :
Genome of every individual has a unique set of repeated DNA sequence. These can be identified by a wide range of restriction enzyme, of species by (solution) Souther blot analysis of restriction Enzyme, digits of genomic DNA using the DNA probe sequences visualization of unique pattern of sequences provides a DNA finger print.

* protocol:
1. Isolation of Genomic DNA :-
The cell pellet is lysed and treated with ribonuclease A and proteinase K. DNA is purified from the lysate byp Extration with phenol and chloroform. (Eth) ethanol is used to ppt DNA which is recovered by Centrifiguration The pellet is dried and dissolved.

2. Restriction Enzyme digestion of DNA :
An Excess of hindi restriction Enzyme is used to digest Then DNA overnight at 37 ± 1°c. A DNA sample with a known "finger print" is digested in parallel with the test sample as a control for restriction Enzyme.

3. (a) Agarose Gel Electrophoreses :-
The DNA digest is divided into two equal aliquotes to provide for a repeat, if necessary and Electrophorased

under standard Conditions in a prepared 0.7% agarose gel Control DNA digest and mole. wt markers are sun on each gel. Each gel photographed over UV light.

3. (b) Southern blotting:
DNA fragments in the gels are rendered Single Stranded by a Sequence of acid, alkali and neutral DNA fragments are transformed to nylon filter by (b). Capillary action using a high salt Solution.

4. (a) DNA probes: The standard probe used in an Oligonucleotide representing the Core Sequence of the 33.15 probe with a Chemicalnescent reporter System (5,6). The labelled probe is Supplied by (Zeneca) and is prepared under GMP Condition.

(b) Hybridization of DNA probes to southern blotting: Southern filters are pre-hybridization in 0.5M Na_2HPO_4, 0.1% SO at 50°C AU labelled probe is hybridised to the filters in hybridization Solution for 20 min at 50°C Non-Specifically bound probe is removed by a Series of Stringency Washes.

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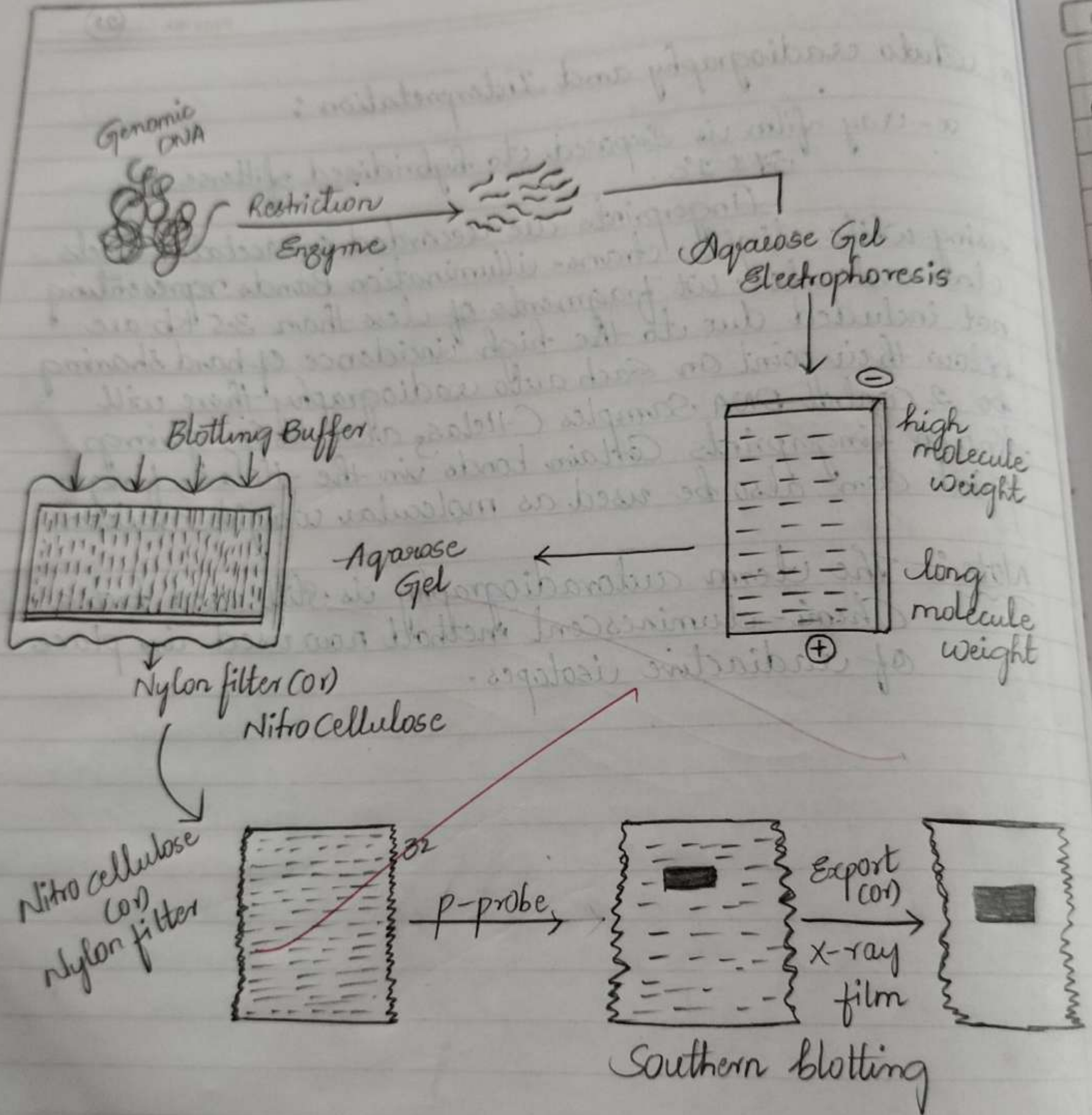
5. Auto radiography and Interpretation :

x-ray film is Exposed to hybridised filterse at 37 ± 1°c.

Fingerprints are recorded on acetate sheets using white light trans-illumination Bands representing lab molecules. wt fragments of less than 3.5 kb are not included due to the high incidence of hard sharing below their point on Each auto radiography there will be 2 control DNA Samples (Helas3 and KS62) givings known fingerprints certain bands in the Helas3 finger print can also be used as molecular weight marker.

Note :- The term autoradiography is still used but a Chemi-luminescent method now used in place of radiactive isotopes.

Teacher' Signature :



Southern blotting

gens are also belong to iso antigens. ... like brain, kidney

(E) Southern blotting :

* principle :-

(i) Southern Blotting is an example of RFLP (restriction fragment length polymorphism). it was developed by fordwald M Southern (1975). Southern blotting is a hybridization technique for identification of particular size of DNA from the mixture of other similar molecules. this techniques is based on the principle of separation of DNA fragment by of Electrophoreses and identification by labelled probe hybridization.

(ii) Separated DNA fragment after transfering an nylon membrane the desired DNA is detected using specific DNA probe that is a short (100-500bp), SSDNA, the

(iii) A hybridisation probe is a short (100-500bp) SSDNA, the probe are labelled with a marks so that they can be detected after hybridisation.

* procedure / steps :

1. Restriction digests : by RE enzyme and amplification by (R) PCR
2. Gel Electrophoresis : SDC gel Electrophoresis
3. Denaturaturation : Treating with HCl and NaOH
4. Blotting
5. Baking and Bocking with Cassin in BSA.
6. Hybridization using labelled probes
7. Visualization of autodiagram.

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are confined to ... only. The organs like brain, kidney,

Step-I ÷ Restriction Digest ÷

(i) The DNA is fragmentized by using suitable restriction enzyme RE cuts the DNA at specific site generating fog+.

(ii) The no. of fragments of DNA obtained by restriction digest of is amplified by PCR.

Step-II : Gel Electrophoresis ÷

(i) The desired fragments of DNA is separated by this method.

Step-III Denaturation ÷

(i) The SDS gel after electrophoresis is then soaked in alkali (NaOH) (or) acid (HCl) to denature the dsDNA-fragment.

(ii) DNA stands get sepparated.

Step-IV Blotting ÷

(i) The separate stands of DNA is then transfored to the positively charged nylon membrane by blotting process.

Step V ÷ Baking and Blocking ÷

(i) After the DNA of insert bound on the membrane it is baked on autoclave to fix in the membrane.

(ii) The membrane is then treated with Cossein (or) Braine Serum albumin (BAS) which saturated all binding site of membrane.

Step: VI : Hybridization with labelled probes ÷

(i) The DNA bound to membrane is treated with labelled probe.

(ii) The labelled probe contains the complementary sequences to the gene of interst.

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(iii) The probe bind with Complementary DNA on the membrane. Since all other non-specific binding site on the membrane Since all other non-specific binding site on the membrane has been blocked by BSA (or) Casein.

Step (vi): Visualization by autodiagram:

(i) The membrane bound DNA labelled with probe can be visualized under autodiagram with given pattern of band.

* Application of Southern blotting:

1. Southern blotting technique is used to detect DNA in given Sample.
2. DNA finger printing is an example of southern blotting.
3. Used for patternity testing, criminal, identification victim identification.
4. The isolate and identify desire gene of interest.
5. Used in restriction fragment length polymorphism.

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are confined ... only. The organs like brain, kidney,

(F) Westhern Blotting:

* Introduction:-

Westhern Blotting is an important technique used in cell and molecular (Blotting) Biology. By using a western blot specific proteins can be identified from a complex mixture of protein extracted from cells. The techniqrue includeds three steps to complete the task:-
① Separation by size ② transfer to a solid support and ③ marketing target protein using a proper primary and secondary antibody to visualize.

Lysis to extract protein
protein can be extracted either from tissue (or) from (protein) adherent cell.

(A) protocol for extraction from adhrent cells:-
1. Wash cell in the tissue culture flask (or) dish by adding cold phosphate buffered saline (PBS) and rocking gently discard PBS.
Note:- Keep tissue culture dish on ice throughout.
(2) Add PBS and use a cell scraper to dislodge the cell pippette the mixture into microcentrifuge tubes.
(3) Centrifuge at 1500 RPM for 15 min and discard the supernant.
(4) Add 180 ul of ice cold cell lysis buffer with 20 ul fresh protease inhibitor mix.
Note:- It protein concentration is not high energy at the end, it is advised to repeat the prodeure with a higher proportion of protease inhibitor mix.

(5) Incubate for 30 min on ice and the clarify the lysate by spinning at 1200mp for 10 min at 4°c
(6) Transfer supernatant (or protein mix) to a (form) fresh tube and store on ice (or) forzen at 20°c (or) 80°c
(7) measure the concentration of protein using a spectrophotometer.
(B) Electrophoresis:-
To perform SDS-PAGE Experiment the following materials are needed.
1. power supplies: power supplies convert AC to DC current
2. Electrophoresis: Thus are various types of chambers sold by supplies. Be sure that the SDS-PAGE gel fits well in the Electrophoresis chambers.

* Reagents:-

(i) SDS-PAGE gels: These can be prepared in the lab (or) pure chase them precast from commerical stores. Be sure that pure cast gel fitt well into the Electrophoresis chambers.
(ii) proteins sample: cell lysates (or) protein mixtures (BSA and ovalbumin) can be diluted 1 to 1 using 2× SDS 0.01%. sample buffer (125Mm Trisis : 20% Glycerol) 4% SDS-PAGE bromophenol blue pH 6.8) and boiled for 10min. in addition to SDS, a reading agents such as dithiotheritol (DTT) (or) (2-meter) 2-mercaptoethanol is usually added to reduse disulfide linkage overcoming some forms of tertiary protein foldage.

ns are also belong to iso antigens.

3. protein ladder :- Reference protein ladder will allow to determine the location of protein of intrest on its molecular size.
4. Running Buffer : protein samples loaded on SDS-PAGE gel will be run SDS-PAGE running buffer (0.25m Tris 19.2m) Glycine, 1.0% SDS - pH 8.3.
5. Staining buffer : Once the Electrophoresis is over SDS-PAGE gel will be stained in porcaus solution (0.1% w/v) ponceaus 5% acetic acid)
6. Destaining buffer : ponceaus stain also binds to SDS-PAGE gel SDS-PAGE destin solution / methanol 50ml acetic acid 1ml D.H_2O 43ml) is to destain solution ponceaus from the gel protein bands can be visualizied by naked eyes (or) document by imaging systems.
7. PBS buffer 2% peptone 0.5% yeast Extract, 10MM NaCl 2mmKcl 10MM, $MgCl_2$, 10MM $MgSO_4$ 20MM glucose.

* protocol :-

1. pour the running buffer into the Electrophorator.
2. place gel inside the Electrophorator and connect to a power supply. when connecting to the power source always connect red to red and black to black.
3. Make sure buffer covers the gel completely and remove the combo carefully.
4. load marker (6 ul) followed by sample (5ul) into each well.
5. Run the gel with low voltage (60v) for separating gel use higher voltage (140v) for stocking gel.

Expt. No.
Date

(c) Electrotransfer:-

① Cut 6 filter sheets to fit the measurement of the gel and one poly vinylidence fluriode (PDVE) membrane with the dimension.

2. wet the sponge and filter paper (interface) in transfer buffer and wet the PVrE membrane in methanol
3. Separate glass plates and retrieve the gel.
4. Create a transfer sandwhich by keeping the following one over the other as follows.

Sponge
3- Filterpaper
Gel PDVE
3- Filterpaper

Tip:- Ensure there are no air bubbles between the gel and PVDE membrane and sequence and extra liquid).

5. Relocate the sandwhich to transfer apparatus which should be placed on ice to maintain 4°C add Towbin transfer buffer (25MM Tris, 192Mm glycine, 0.1% SDS 20% methanol) to apparatus and ensure that the sandwhich is covered with the buffer place electrodes on top of the sandwhich ensuring that the PVDE membrane is between the gel and a positive electrode.
6. Transfer and run for one 90 min (Tip: the running time should be proportional to the thickness of gel so this may be reduced to 45 min, for 0.75 mm gels.

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Expt. No. Date

Additional requirment :-

(a) phasphate - Buffered Saline (PBS):
100Mm Sodium phosphate monohydrate 0.262g
dibasic Sodium phasphate .... 1.15g, 0.9% Nacl 9.0g
double distilled water to make upto 1 .... 1L.

(b) Tween PBS (TPBS) :-
PBS with 0.1% Tween 20
Tris - Buffered Saline (TBS)
100mm Tris/HCl 12.11g
Double distilled water to make upto 90ml
Nacl ..... 9g.

(c) TBS (TTBS) ....
TBS with 0.1% Tween 20

(d) primary antibodies :
Ab directed against ovalbumin and BSA.

(e) Secondary antibodies :
Radio labelled Secondary antibodies.

(f) Alkaline phosphate Assay Buffer :
0.1M macl ..... 5.8g ; 5mm $MgCl_2$ 0.476g
100mm Tris ...... 12.11g ; Tris Hcl 12.11g

(g) Tris - Hcl buffer pH 9.5 .... 100Mm Tris 12.1g/1L

(h) BCIp stock ...... BCIp (disodium salt) 50mg and 100% dimethyl formamide - 1ml

(i) NBT Stock .... 50mg NBT per ml in 70% dimethyl formatide

(j) DAB/$NiCl_2$ visulization Stock Solution .... DAB ..... 40ml and double distilled water to make upto 7ml.

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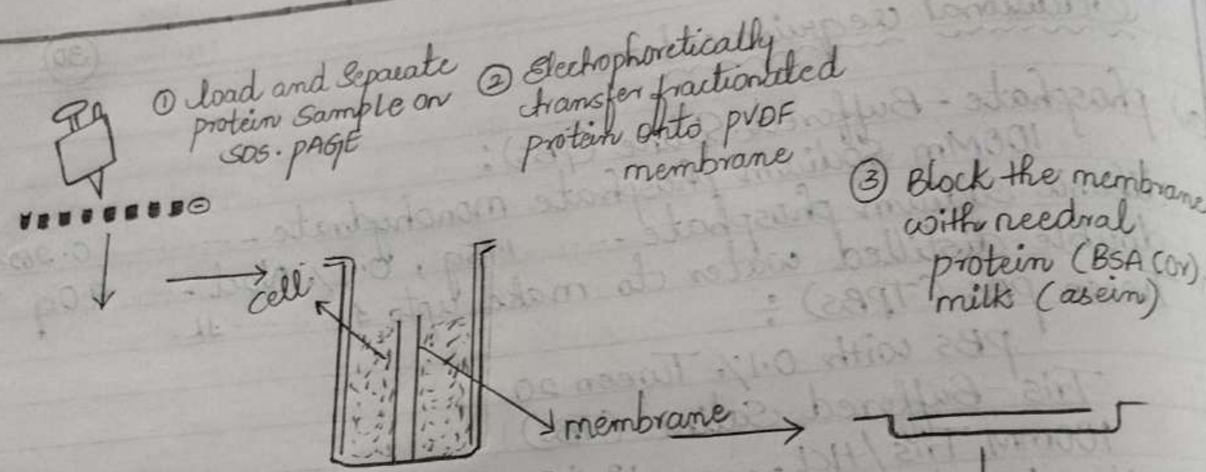


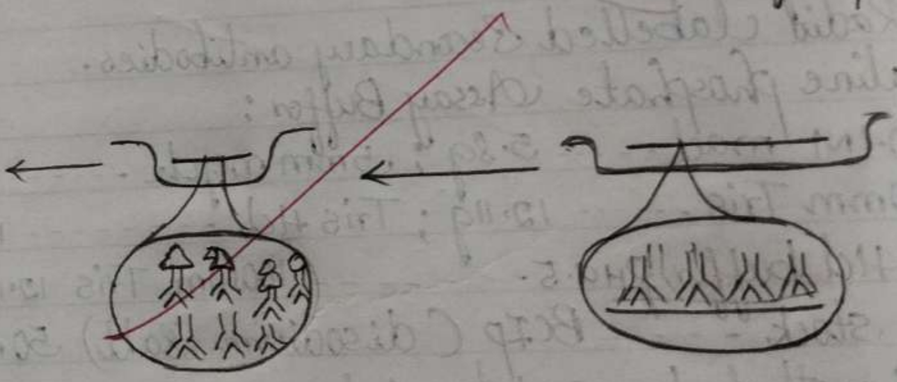
⑥ Incubate the blot with Chemiluminescent HRP substrate and expose film

⑤ Incubate the membrane with HRP labelled Secondary a specific to primary antibody

④ Incubate membrane with primary antibody specific to target protein

WESTERN BLOT :

- KD8
- 148
98
- 60
- 52
- 45
- 36
- 22



(k) $NiCl_2$ stock ... $NiCl_2$ 80mg and double distilled water to make up to 7 ml.
(l) 3% hydrogen peroxide : 3ml of hydrogen peroxide.
(m) peroxide assay Buffer (pH. 7.5) 12.1g of 100 Mm Tris.

* protocol :-

1. Incubate the membrane in TTBS blocking Buffer 100ml for 1 hour.
2. Incubate for 30-60 min, use (apparatus) appropriate dilution of I° Ab's 1:2000 - 1:10.000 and fellow manufactures instructions.
3. Wash in 100ml TTBS for 10 min, thrice
4. Transfer and incubate the membrane with biotinylated Secondary antibodies 2mg/ml : one drop of 2.5/50 ml TTBS for 30-60 min.
5. prepare avidin - Biotin : Ap/HRP Complex 90ml of reagent A (avidin + 90ml of reagents B (voctastain) with 100 ml TTBS.
6. Wash in 100ml TTBS for 10min Thrice
7. Incubate in avidin - Biotin - Ap (or) HRP Complex for 30 min.
8. Wash in 100ml + TTBS for 10 min thrice
9. Develop the membrane and visualize the Coloured bands.

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(iii) Demonstration of PCR/Virtual lab:

place a well plate into the ice bucket as a holder for the 0.2ml thin walled PCR tubes. Allowing PCR reagents to be added into cold. 0.2ml thin walled PCR tubes will help prevent nuclease activity and non-specific priming.

Reagents: Sterile water, 10x PCR Buffer dNTP'S, $MgCl_2$ primers of template DNA.

procedure: pipette the following PCR reagents in the following order into a 0.2ml thin walled PCR tubes.

Sinces Experiments should have at least a negative control and possibly a positive control it is benefical to set up a master mix in a 1.8ml micro centrifuge tube

Factor affecting PCR:

(1) primer:

* PCR reaction needs two primers, a forward and a reverse primer.

* primers are synthesized oligonucleotide usually ranging from 15-30 bases long.

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* primers are designed such that at 3'end they do not have more than two bases complementary to each other as this results in PRIMER-DIMER formation.

* The G+C contents is in the range of 40-60%.

* The melting temperature (TM) of both forward and reverse primer is usually same.

(ii) Amount of Template DNA :-

* Optimal amount of template DNA usually in nano gram high concentration inhibit (or) results in non-specific amplification.

* Taq DNA polymerase.

* Taq DNA polymerase is 94KD. Thermostable DNA polymerase isolated from thermus aquaticus.

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Application :-

1. Forcinic Science : DNA fringerprinting, paternity testing and Criminal identification.
2. Diagnosis : Molecular identification of micro-organisms
3. Evolution Study : Evolutionary Biology.
4. Fossil Study : paleontology
5. Gene : Expression and Cloning
6. gene Sequencing
7. vaccine production by r-DNA technology
8. Drug discovery
9. Mutation Study
10. Human genome project.

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