Toppersnotes
Unleash the topper in you

CSIR-NET

Council of Scientific & Industrial Research

LIFE SCIENCE

VOLUME – 3

FUNDAMENTAL PROCESSES

SIERRA INNOVATIONS

CONTENT

PCR TECHNIQUE

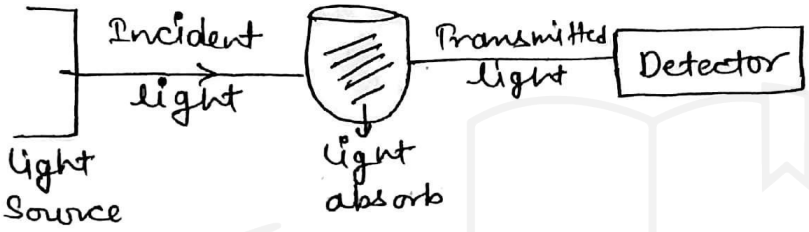
(Polymerase chain reaction)

→ Does not obey 2^n rule Invitro method

2^n 1985 Kerry mullis

→ First desire product is obtain in 3rd round.

* Strengency :-

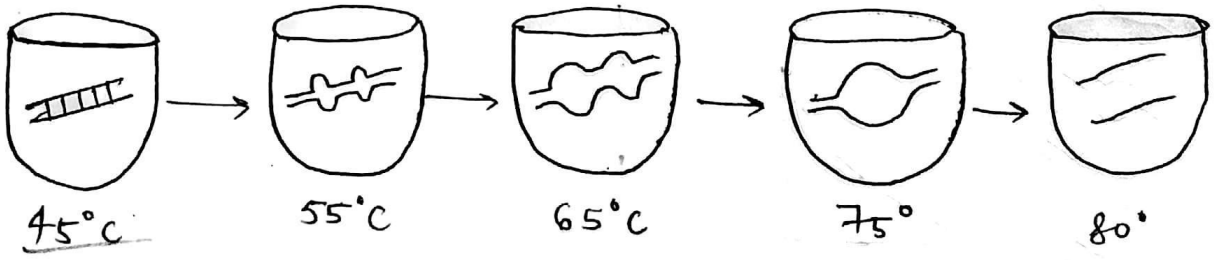


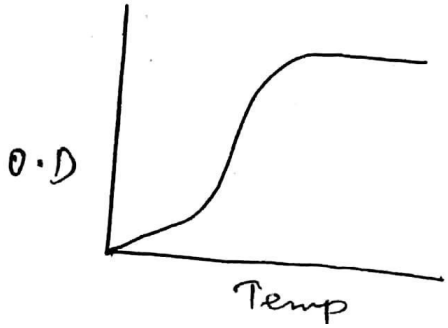
→ Incident light Transmitted light से अधिक होती है।
अब Detector द्वारा light detect नहीं होती। इसका मतलब molecule complete light को absorb कर चुका है

→ RNA, DNA से अधिक light absorb करता है।

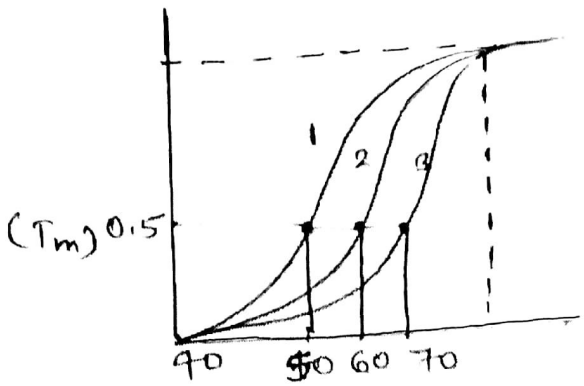
→ Molecule atoms से मिलकर बने होते है।
atoms में e^- के द्वारा light को absorb किया जाता है।

→ DNA degraded - 80°C but Water - 100°C





Temp ↑es होने पर DNA degrade होता जाता है।



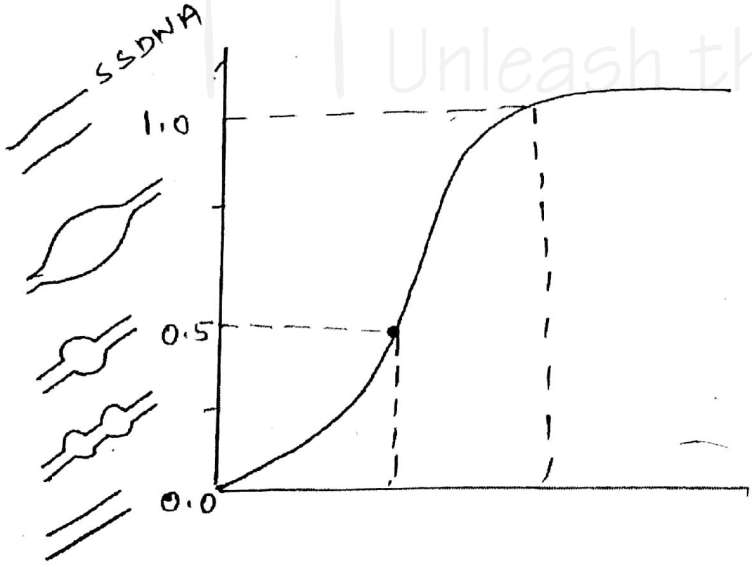
Tm = Half life of O.D

→ Temp at which 50% DNA & Half DNA is melted

G≡C Tm ↑es
A=T Tm ↓es

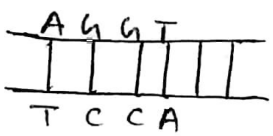
→ Single stranded DNA absorb light more than Double Stranded DNA because SSDNA the e⁻ are not involve in H-bonding formation with its complementry strand. so e⁻ are free so they absorb more then bounded e⁻is of DS DNA.

→ When we melt the DNA O.D of DNA ↑es

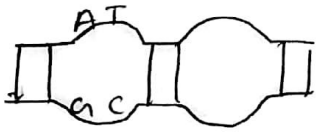


→ GC Rich melt on high temp & AT Rich melt on normal temp.

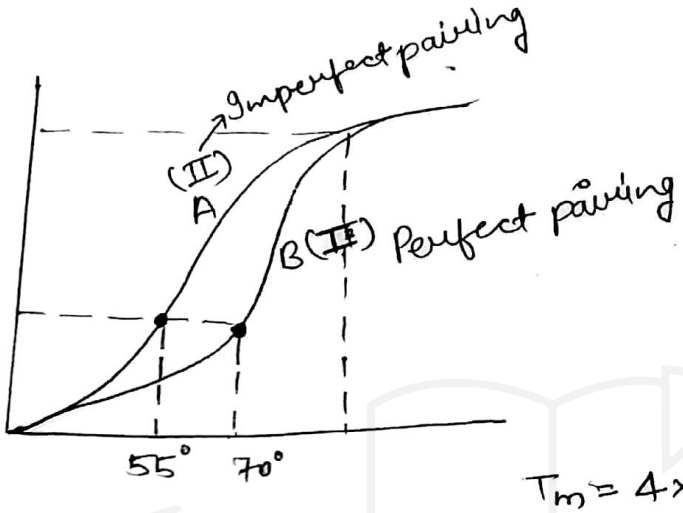
3



(I)

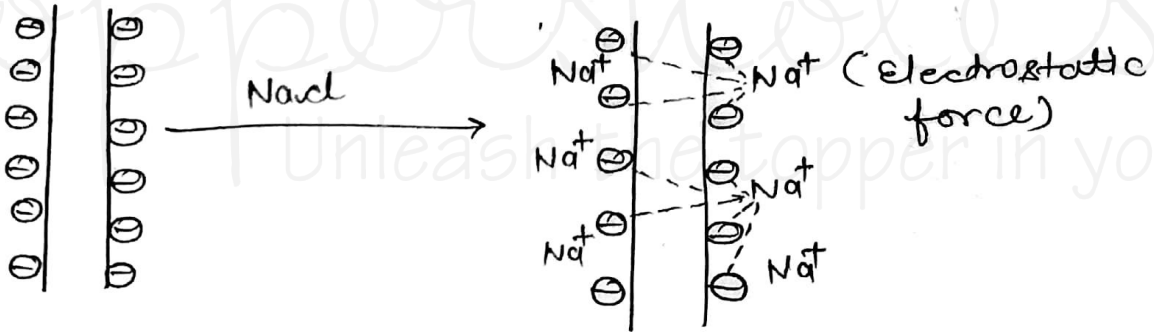


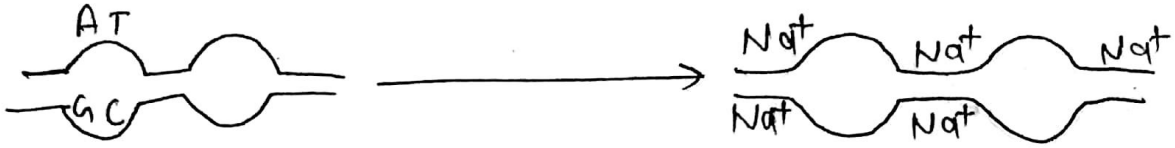
(II)



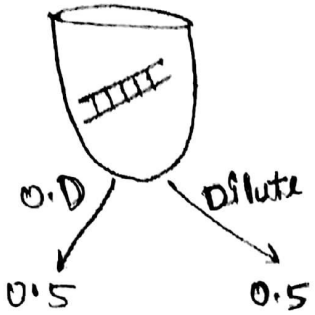
$T_m = 4 \times (G+C) + 2 \times (A+T)$

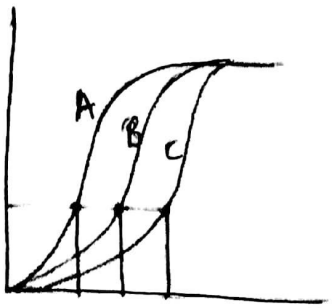
→ Now we added salt on DNA then Na^+ binds with DNA & DNA melting point is increases.





→ By adding salt temp is ↑es & Imperfect pairing look like perfect pairing



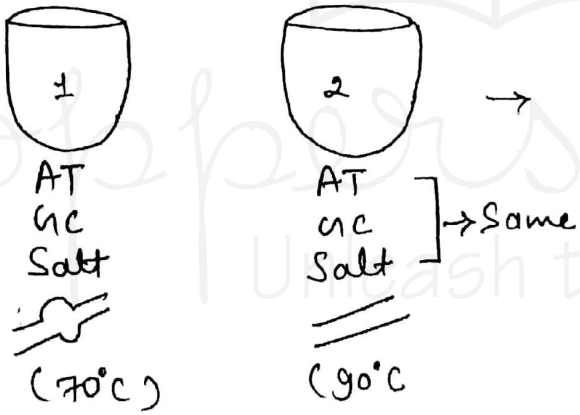


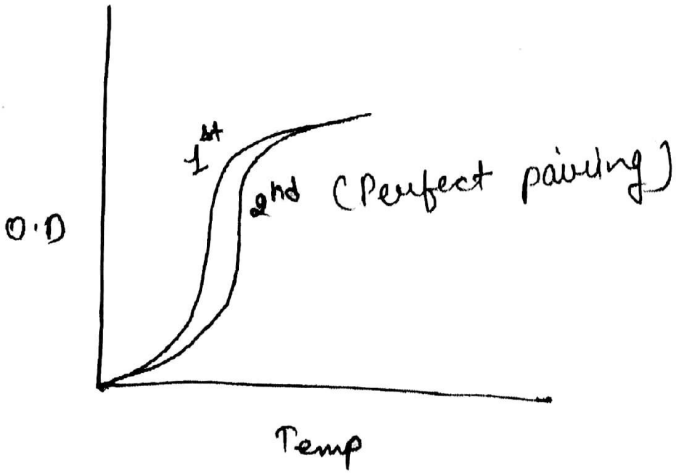
Salt conc^{n}

A < B < C

High Strengency

High Temp, Low Salt





✡ Primer designing :-

5' ATGAT ..... GCGCTAG 3'

Primer – ① 5' ATGAT 3' (same 5'-3')
② 5' CTAGCGC 3'
(2nd is complementry to 3' end)

→ When primer is design DNA to RNA & RNA to protein primer is easily identified but primer is design by protein. It is not (100% accuracy) because for one amino acid 6 codon is used.

5' ACTATGATCTAA 3' DNA

↓

AGUAUGAUCUAA RNA (m-RNA)

↓ 100% accuracy

Met Met (Protein)

Primer is 17-30 nucleotide long

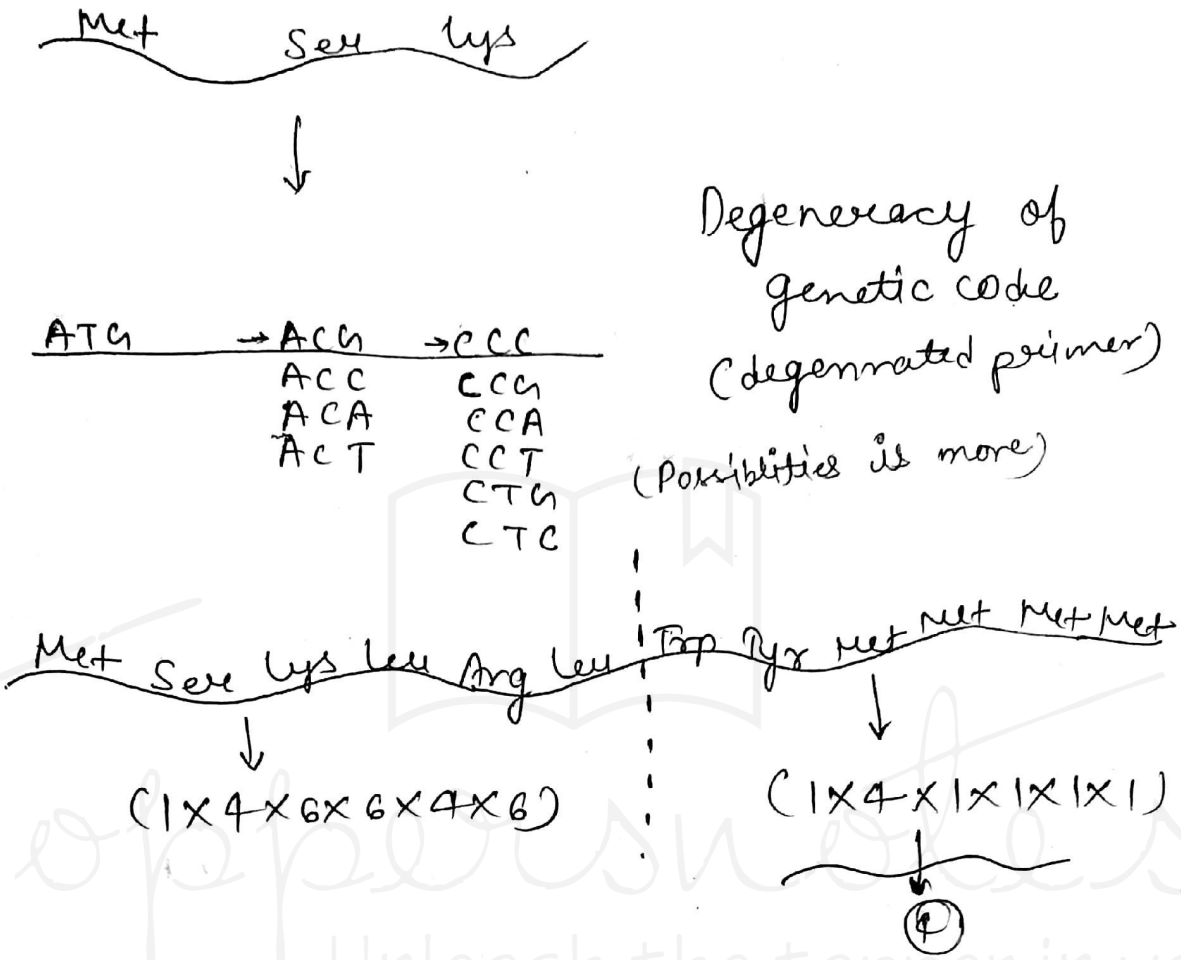
1 codon = 3 nt

= AUGCCCGGGAUGACCAGG

6 codon = 3 nt
= 6×3 = 18

17 nt = 6×3 = 18 nt

→ one amino acid code by more than one codon.



→ The strech of protein / the part of protein which gives minimum number of possibilities of primer is choose in the primer designing

☆ RAPD PCR
(Random amplified Polymorphic DNA)

→ Also as RAPID PCR

No. of cells (DNA) Obtained from crime spot is less →  DNA → To amplify the DNA

↓

Primer is required

↓

Primer is designed after DNA seq. ← Universal Primer

Universal primer = 10 nt (Random primer)

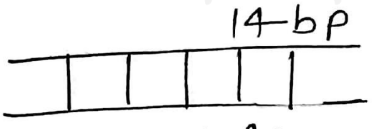
GAAAATTTTC

RAPD PCR

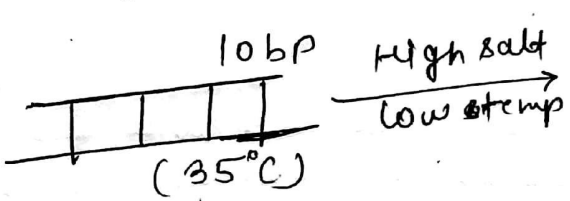
10^6 = 10 cutter not use in bacteria

10^9 = 10 cutter use

⇒ Low stringency
Low Temp, High salt



(Melt on 68°C Temp)



Temp ↑es

Universal primer :-
It is complementary to nucleotide seq. that are very common in a particular set of DNA molecule cloning vectors.

→ They are able to bind to a wide variety of DNA Templates

→ RAPD (RAPID PCR)

→ In RAPD PCR a polymorphic DNA is amplified by the help of universal primer.

→ The length of universal primer is 10 nt.

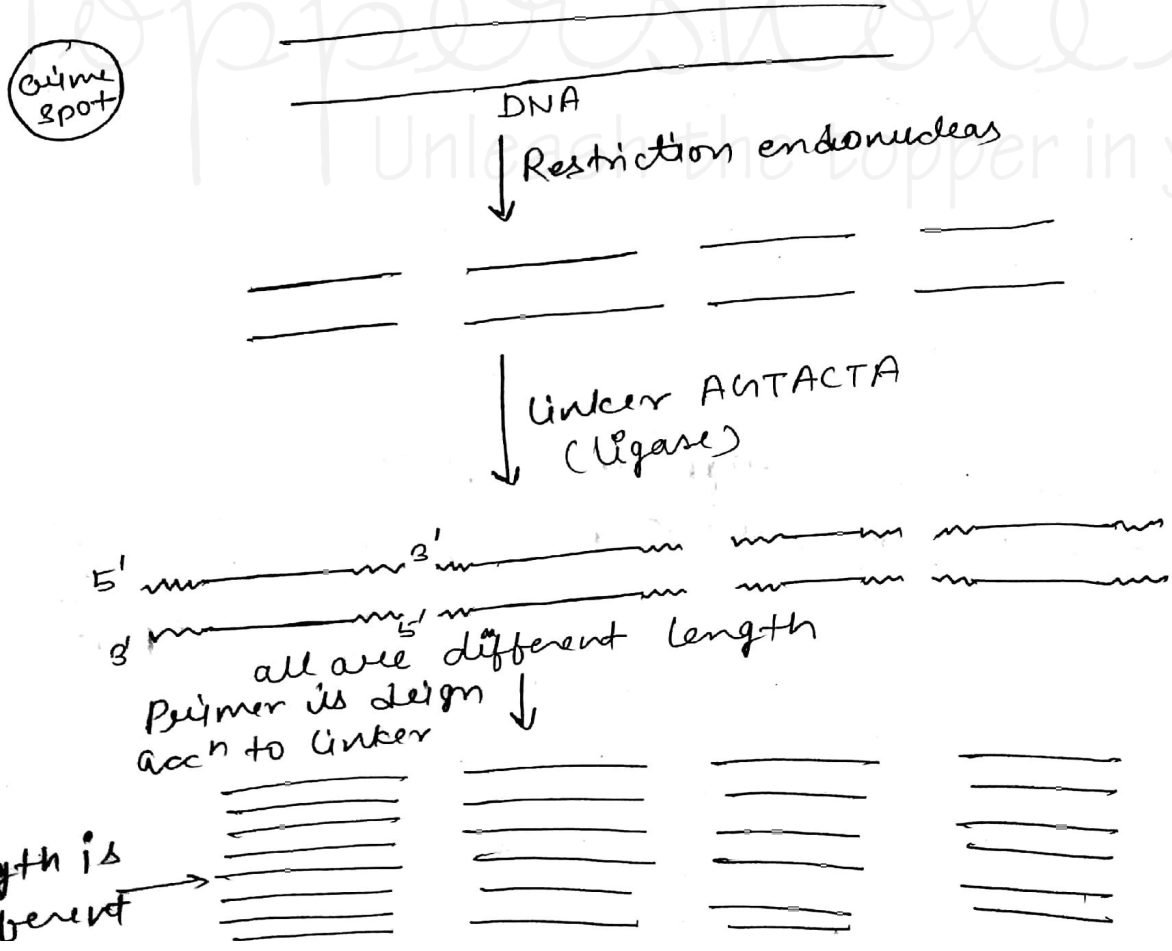
→ The size of Human genom is 10^9

$$= \frac{10^9}{10^6} = \frac{1000000000}{1000000} = 10^3 \ (1000)$$

→ So this 10 nt primer binds at 1000 sites at is specific primer becoz of the binding of 1000 sites the polymorphic DNA is randomly amplified. hence called as RAPD. & also ell as Arbitrary PCR.

★ A.F.L.P PCR :-

(Amplified fragment length polymorphism)



RAPD
AFLP > PCR RFLP – Southern blotting
SSR

→ AFLP Involve 2 set of PCR
In first set primer is design from the linker sequence.
→ This allows amplification of all the restriction fragments
→ In 2nd set of PCR specific primers are design which are internal to linker / Restriction site
→ Linker is a known DNA seq.
→ In 2nd set of AFLP PCR a particular DNA is amplified.

* Constituent of PCR :-

→ Target DNA
→ Two oligonucleotide primer, forward primer & Reverse prime.
→ All dNTP N = A/T/G/C
→ Thermostable DNA polymerase buffer, mg^{2+}

Formulla –

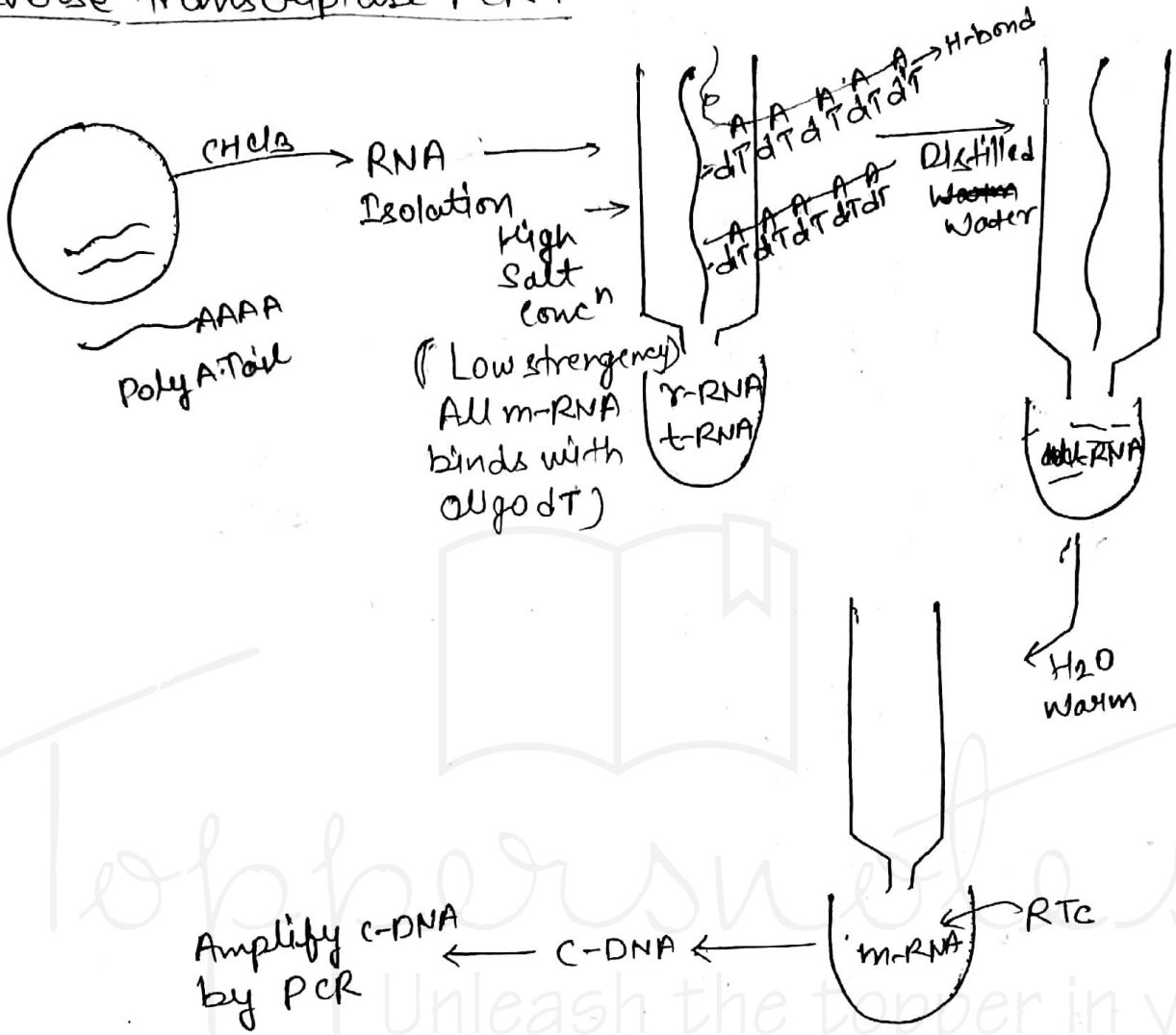
$$Tm = 4(G+C) + 2(A+T)$$

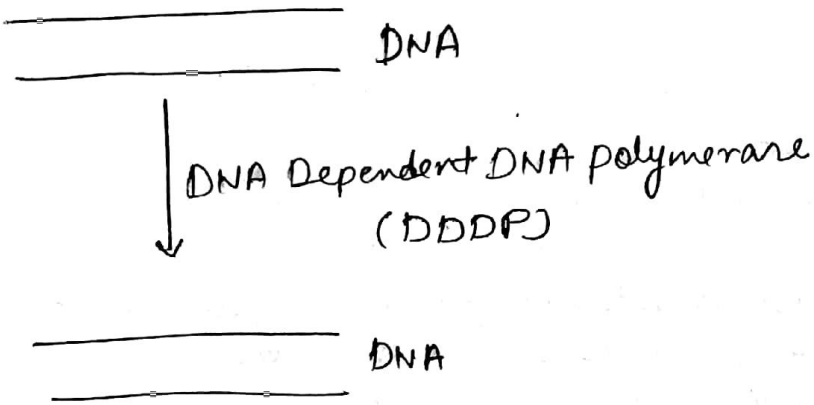
PCR Product :-

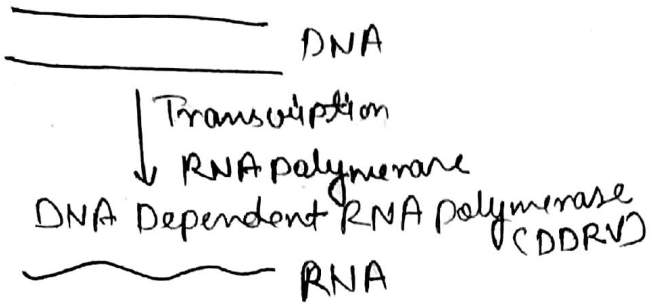
$$\text{Initial amount of DNA} \times (1 + \text{efficiency})^{\text{No. of cycle}}$$

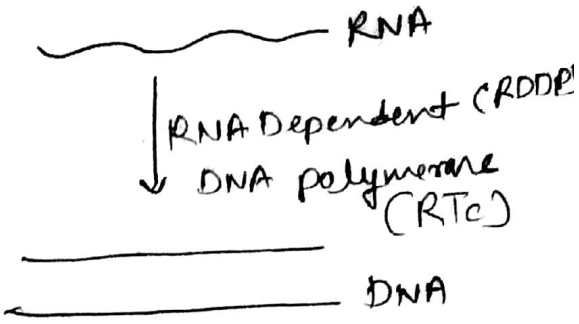
→ Taq DNA polymerases is obtained from Thermus aquaticus & is thermostable up to 94°C with an optimum working temp of 80°C other thermostable DNA poly- currently used in PCR are PFU (Pyrococcus furiosus), Bst E (Bacillus stearothermophilus) & Tth (Thermus thermophilus)

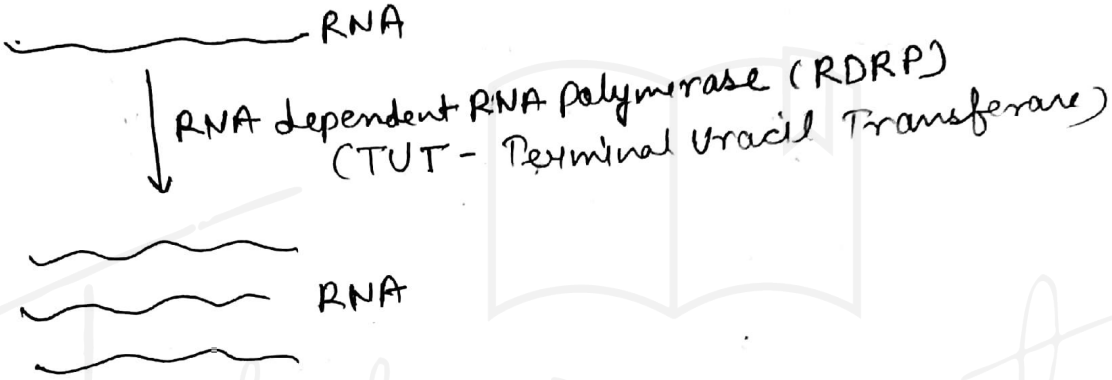
Reverse transcriptase PCR :-

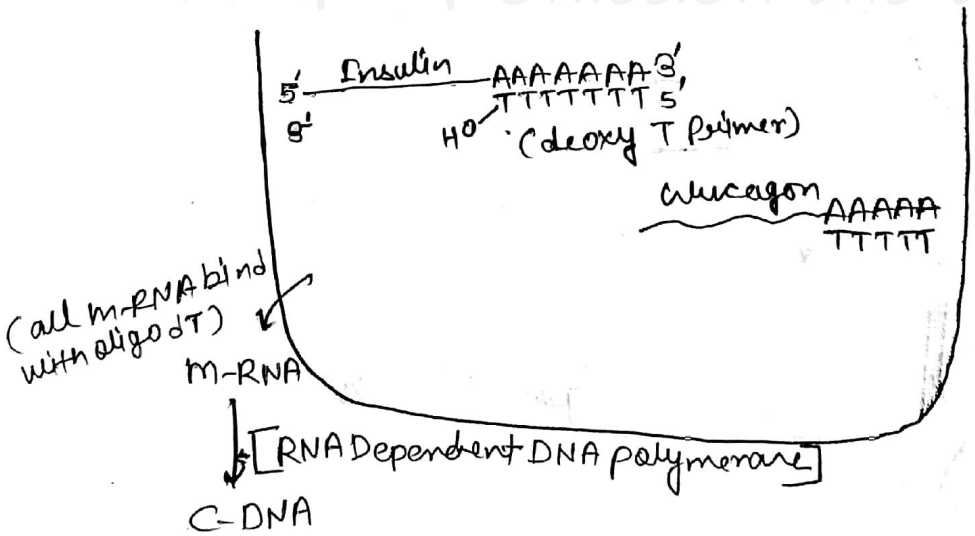


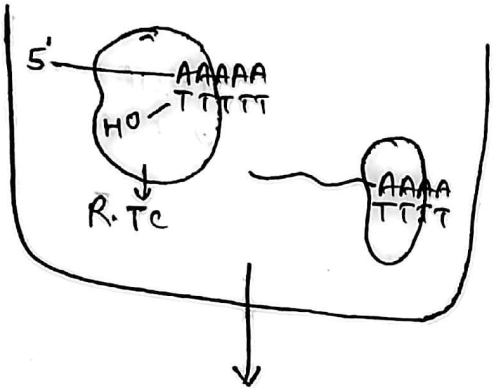






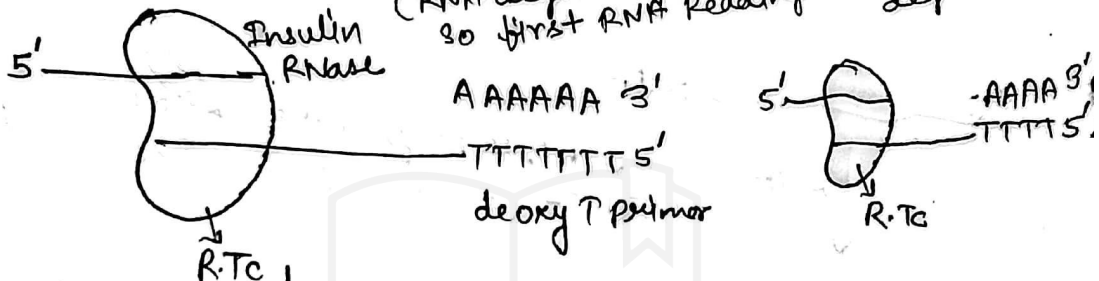




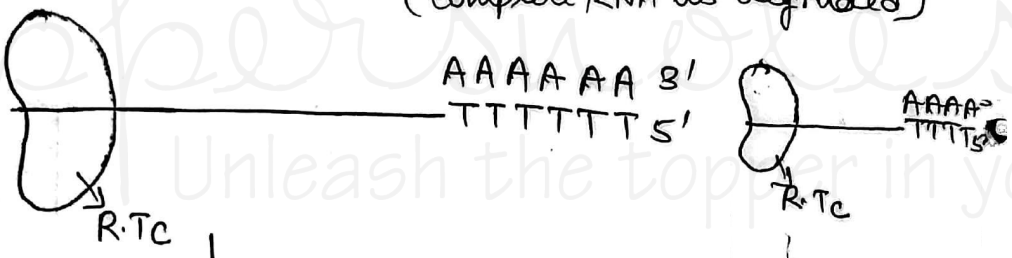


→ Poly A-Tail is not degraded

(RNA degrading activity is present so first RNA Reading & second RNA degrading)



(complete RNA is degraded)

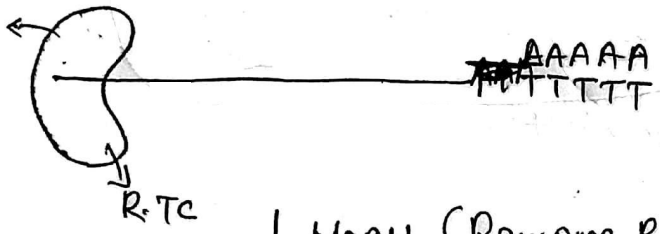


Activities present

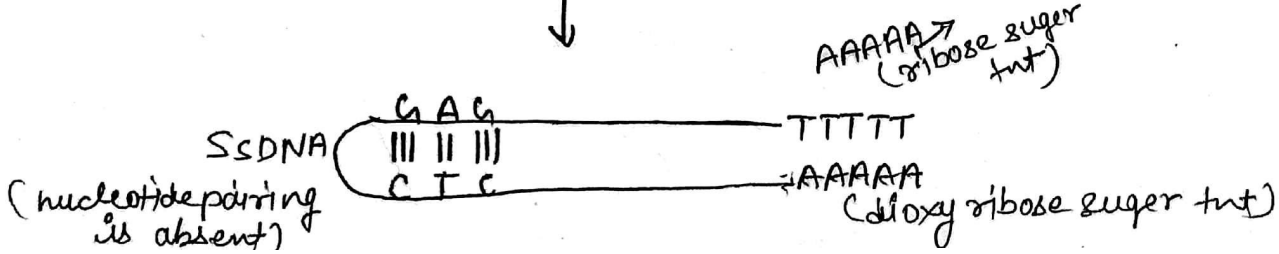
1.) RDDP

11.) RNase

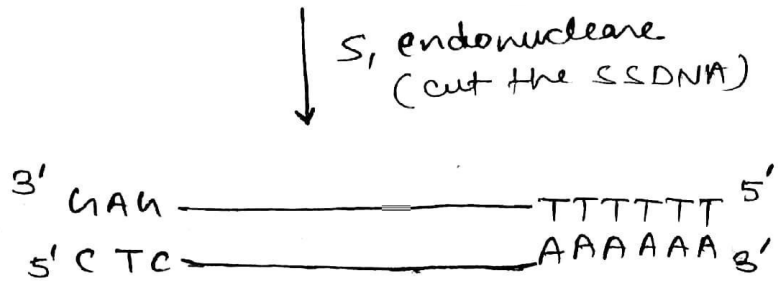
111.) DDDP



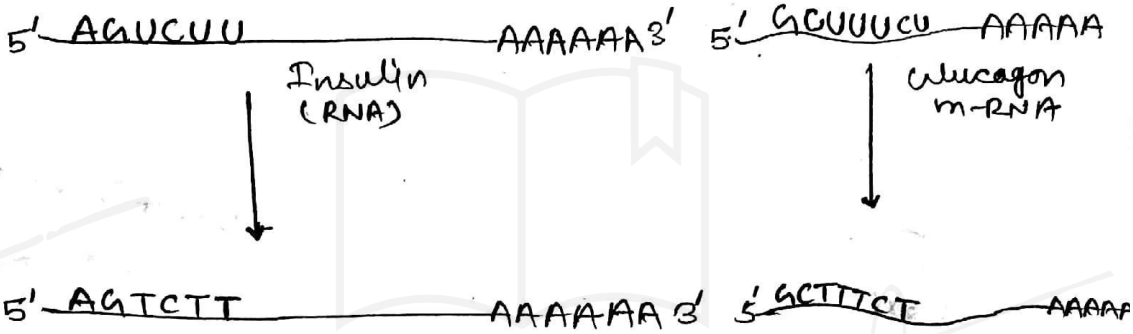
NaOH (Remove RNA Adenine)



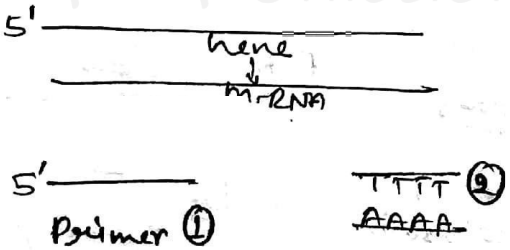
(nucleotide pairing is absent)



* Primer designing in RT-PCR :-



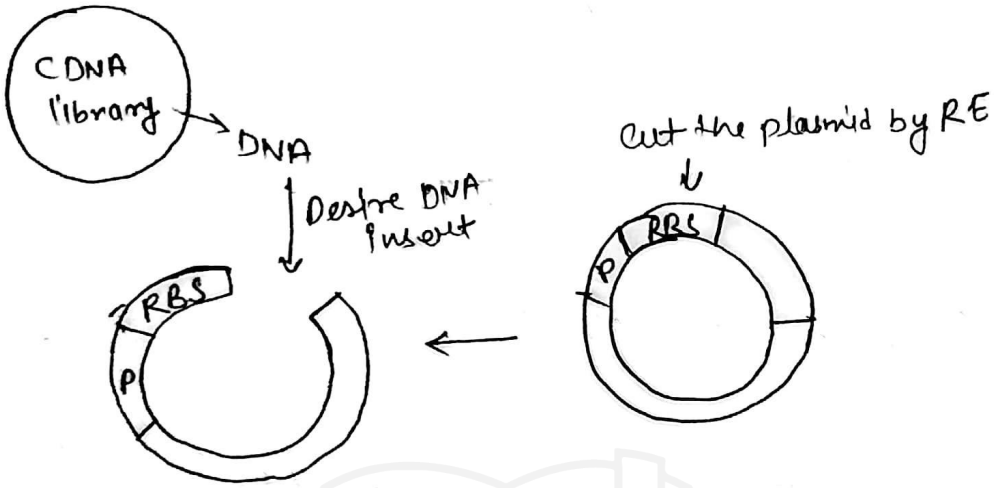
→ Both 2nd primer is same (oligo dT) so in RT-PCR only one primer is need (5'-3')

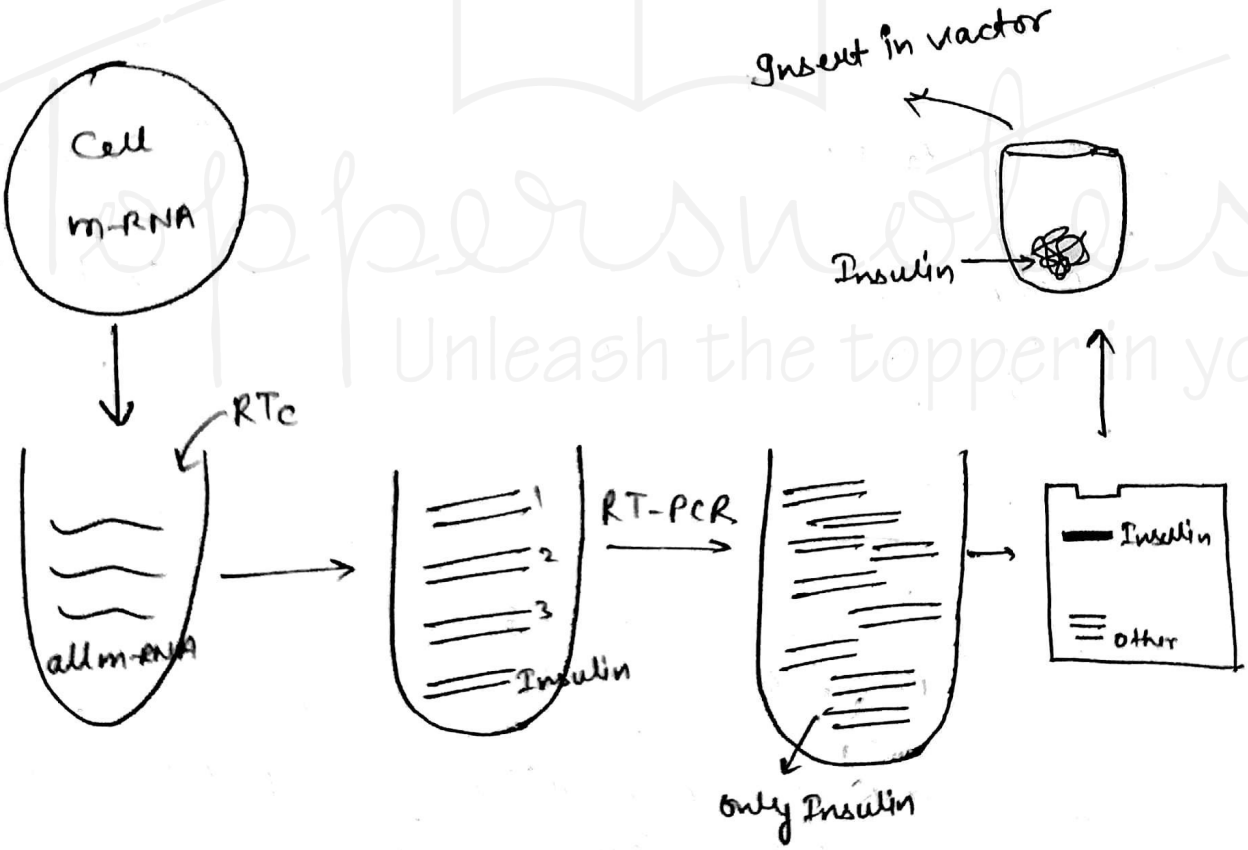


→ In RT-PCR one primer is gene specific & second primer is oligo dT primer

→ The reverse transcriptase convert all m-RNA into c-DNA. but in RT-PCR only the specific gene is amplify. using specific primer.

✯ Applications of RT-PCR :-



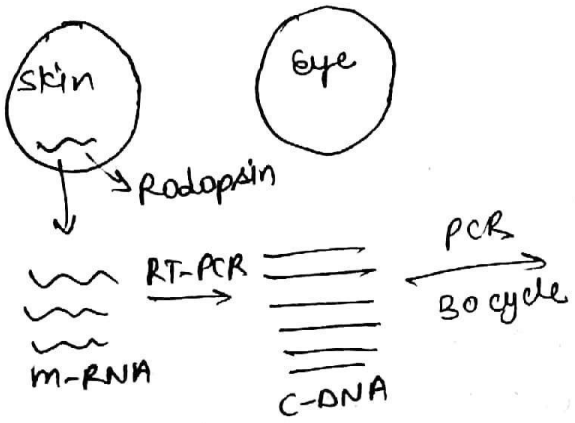


→ Desire gene के लिए C-DNA library & RT-PCR दोनों use किया जाता है।

→ RT-PCR detect the m-RNA in low concentration

eg.

(1)



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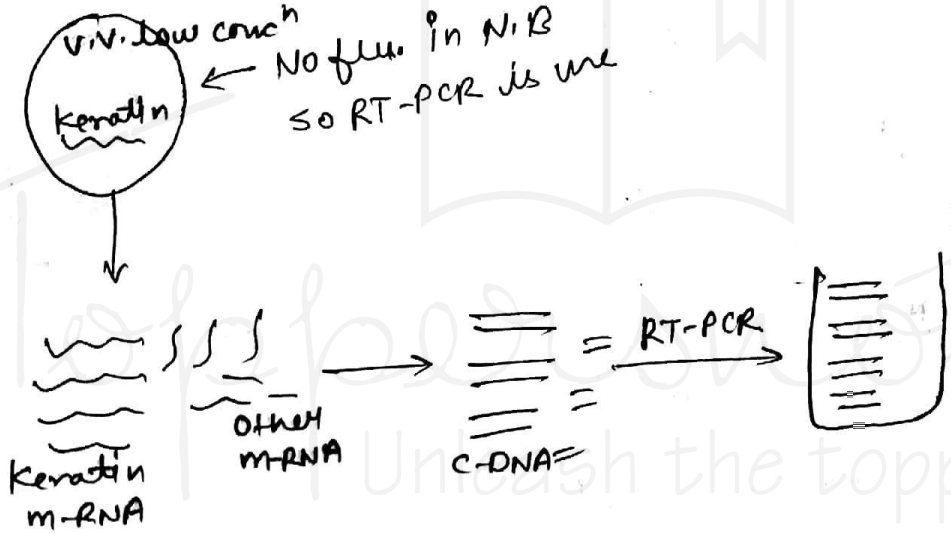

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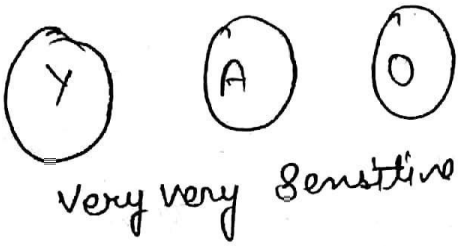

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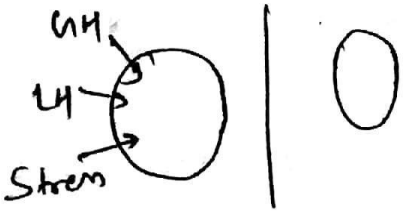

In very very low conc^n RT-PCR is use