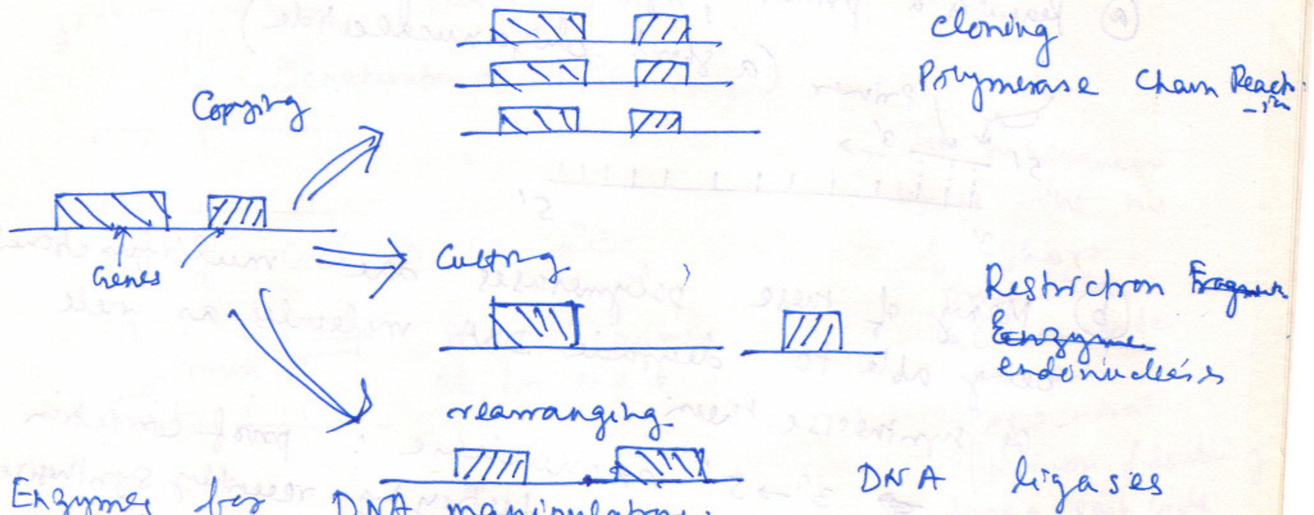
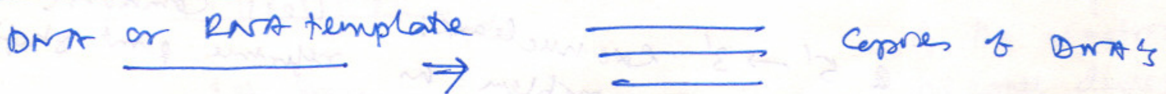
DNA Editing.



Enzymes for DNA manipulations:

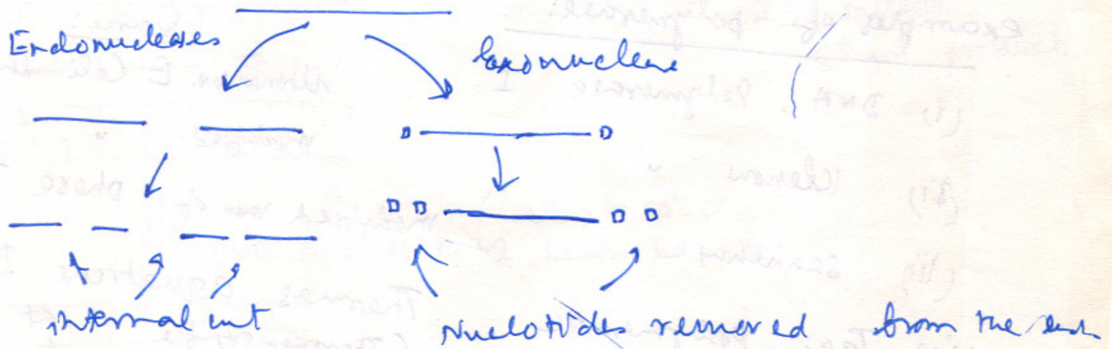
- DNA polymerases: (synthesis)

DNA or RNA template → Copies of DNAs

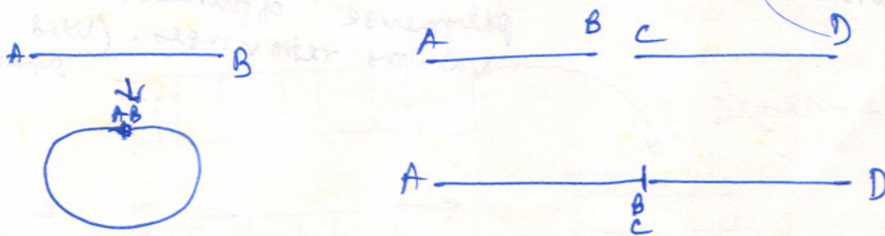


5' → 3' direction

- Nucleases (cutting)



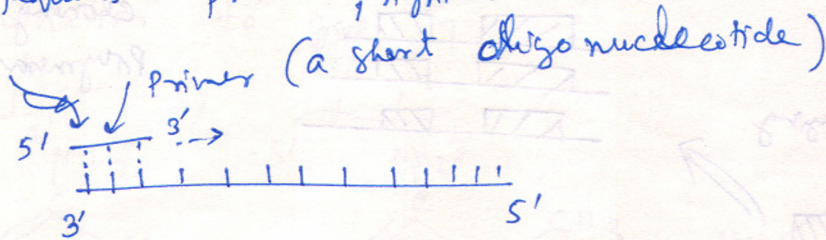
- Ligases:



- End modification enzymes: means of labelling DNA molecules with radioactive & other markers.

DNA polymerases:

(a) Requires a primer, synthesis takes place from 5' to 3' direction
Primer (a short oligonucleotide)



(b) Many of these polymerases are multifunctional, being able to degrade DNA molecules as well as synthesise them.

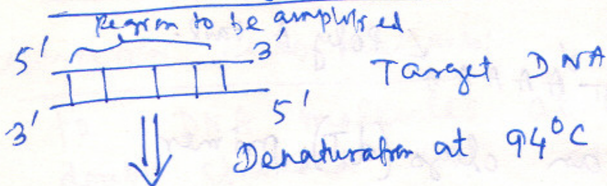
- 3' → 5' exonuclease: proof correction at 3' end by destroying recently synthesised nucleotide.

- 5' → 3' exonuclease: Less common. Causes problem as reference point shifts in DNA sequencing.

examples of polymerase:

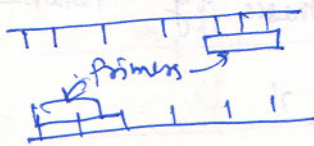
		Source:	Working Temp.
(i)	DNA Polymerase I	unmodified E Coli enzyme	37°C (i)–(iii)
(ii)	Klenow "	modified "	
(iii)	sequenase	modified ver of phase T7 DNA polymerase I.	
(iv)	Taq Polymerase	Thermus aquaticus DNA polymerase I (Thermo-stable at 75°C or above; optimum fn. at 72°C.	
(v)	Reverse transcriptase	RNA-dependent DNA polymerase obtained from various retroviruses. (Used for cDNA synthesis).	

The polymerase chain reaction (PCR).

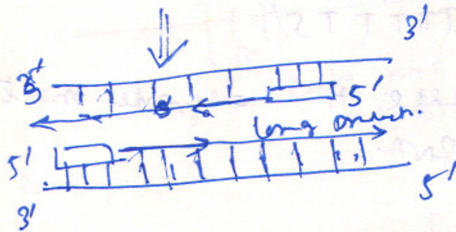


Target DNA

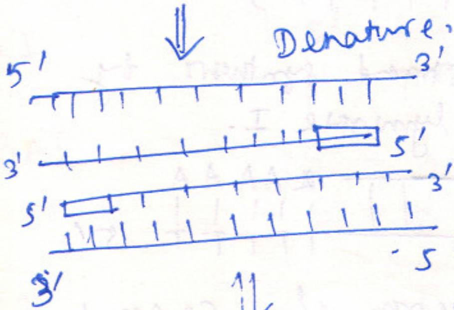
Denaturation at 94°C



Cool to 50-60°C.



Pair of primers at two ends.



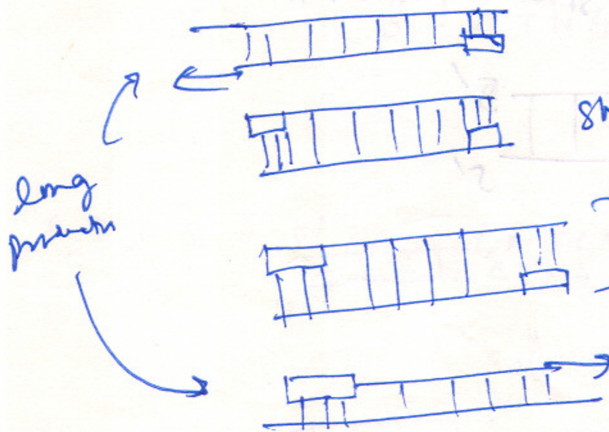
DNA synthesis at 72°C

1st. Stage,

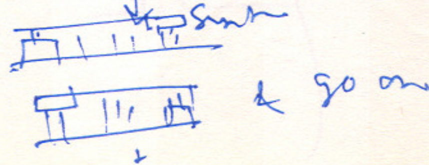
Denature,

(Cooling to 50°-60°c & heating to 72°C)

DNA synthesis.



Denature

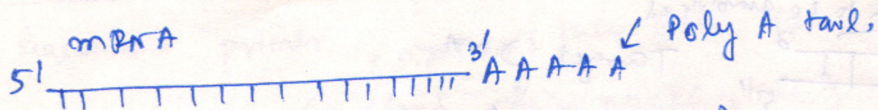


& so on

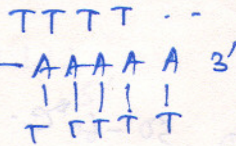
In subsequent cycles the no. of short products accumulate in an exponential fashion (doubling during each cycle until one of the components of the reaction becomes depleted).

After 30 cycles: 250 million short products.

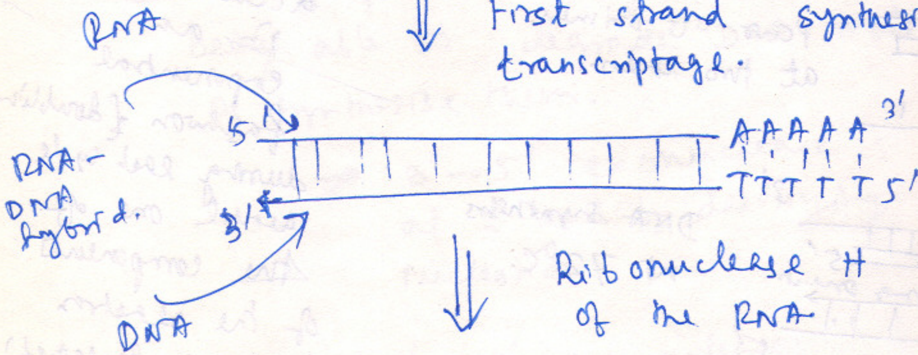
(*) Reverse transcriptase for cDNA synthesis :



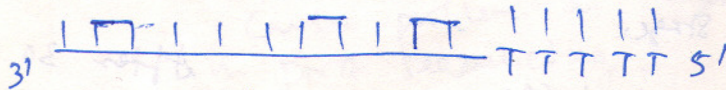
Add an oligo (dT) primer



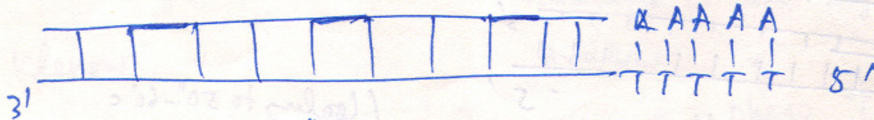
First strand synthesis by reverse transcriptase.



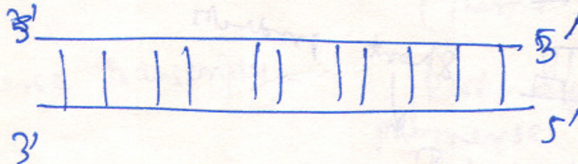
Ribonuclease H degrades most of the RNA



Second strand synthesis by DNA polymerase I.

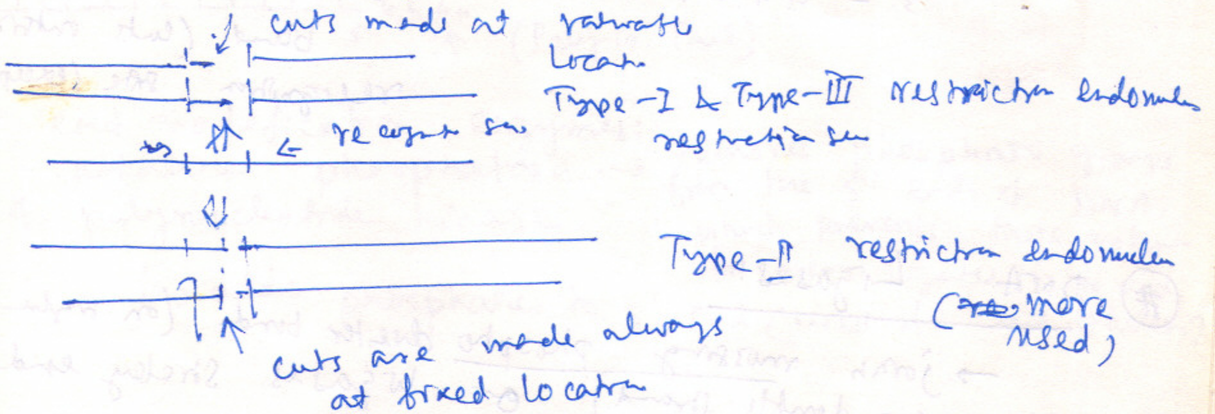


Completion of second strand synthesis.



Nucleases:

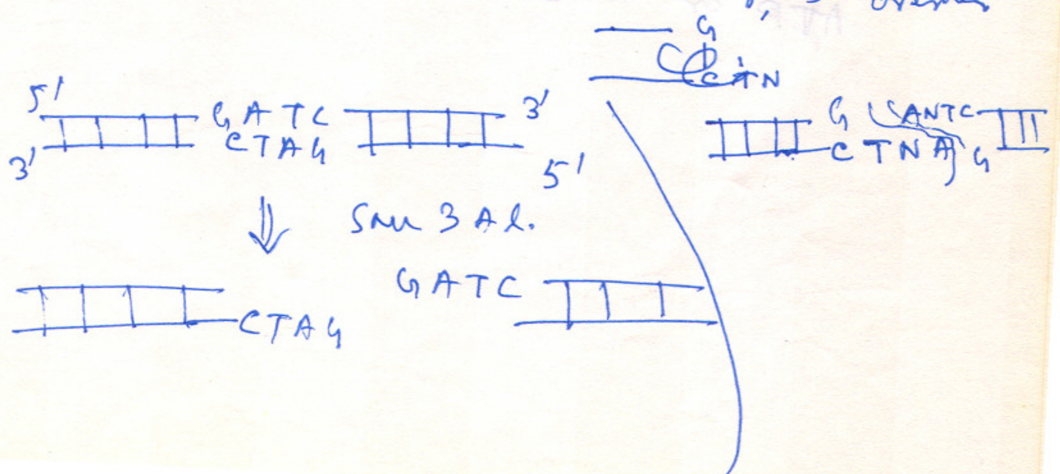
Restriction endo nucleases: Enzymes that bind to DNA molecules at a specific sequence & make double stranded cut at or near the sequence (known as recognition sequence).



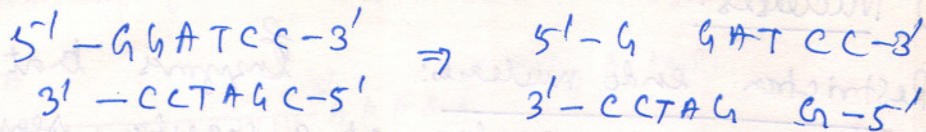
Some examples:

Enzyme	Recognition Seq.	End sequence
AluI	5'-AGCT-3' 3'-TCGA-5'	blunt end 5'-AG : CT-3' 3'-TC : GA-5'
Sau3AI	5'-GATC-3' 3'-CTAG-5'	5'- : GATC-3' 3'- CTAG : -5' Sticky 5' overhang
HinfI	5'-GANTC-3' (N = any nucleotide) 3'-CTNAG-5'	5'-G : ANTC-3' 3'-CTNA : G-5' Sticky, 5' overhang

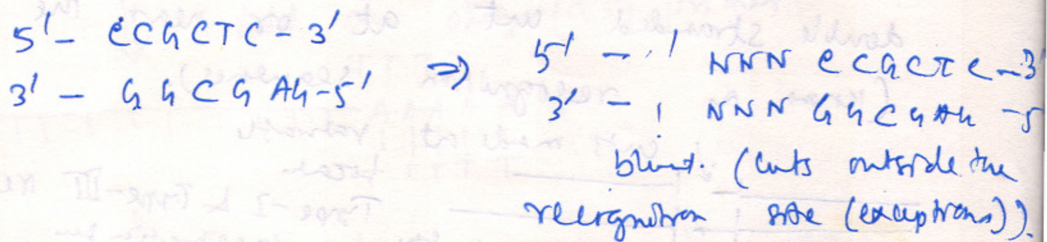
e.g.



BamH1

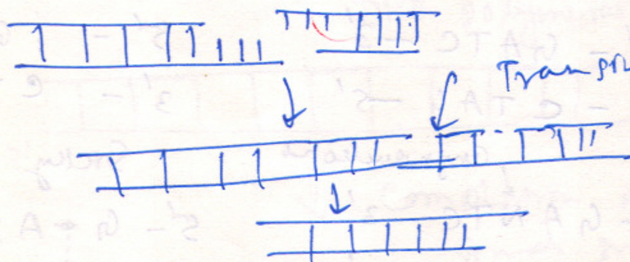


BsrB1



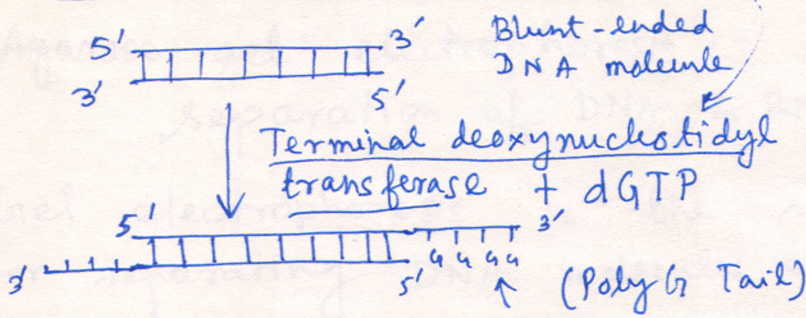
DNA Ligases:

→ joins missing phospho diester bonds (on single strand or double strand) or ligases sticky ends of restriction fragments.



Reactions require energy provided by adding ATP or NAD.

(#) End-modification enzymes:



Template-independent DNA polymerase

Other end modification enzymes:

alkaline phosphatase → removes phosphate groups from the 5' ends of DNA, which prevents these molecules from being ligated to one another

T4 polynucleotide kinase

↳ adds phosphates to 5' end (used for end labelling)