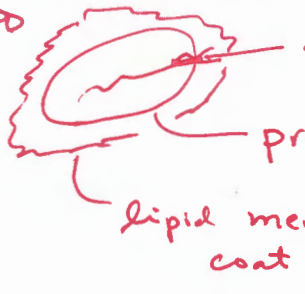
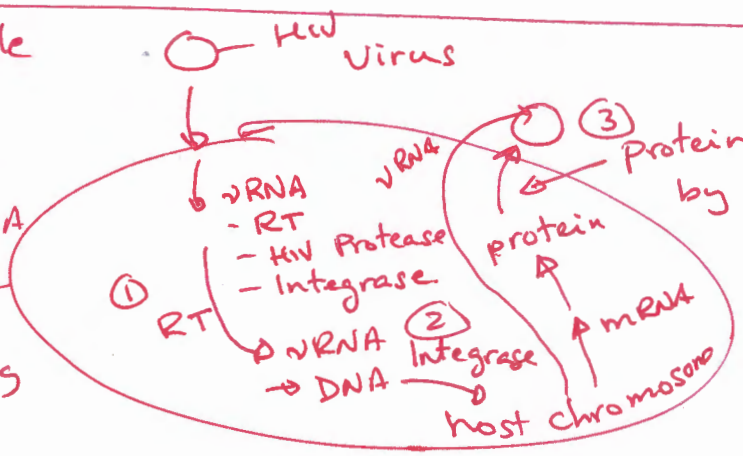
Virus → cloned gene → sequence cloned gene.



Non living (= dead)
Can't reproduce on their own.

HIV - Life cycle

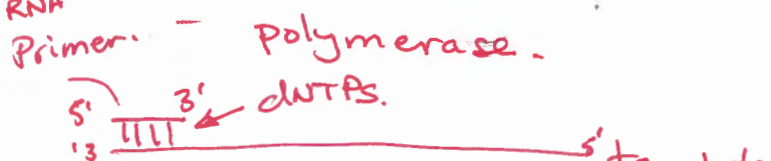


Retrovirus.
RNA → DNA → mRNA → protein

1, 2, 3 good drug targets
a) unique to virus.
b) essential for viral replication.

fold (assemble) into virus.

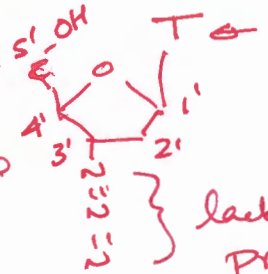
RT = Reverse transcriptase.



- 5'→3' polymerase activity.
- RNA or DNA template. → vRNA → ssDNA → dsDNA → integrate
Lacking 3'→5' exonuclease ⇒ NO error correction.
i) mutations in vRNA
ii) changes drug targets
aa in the
iii) drug resistance.

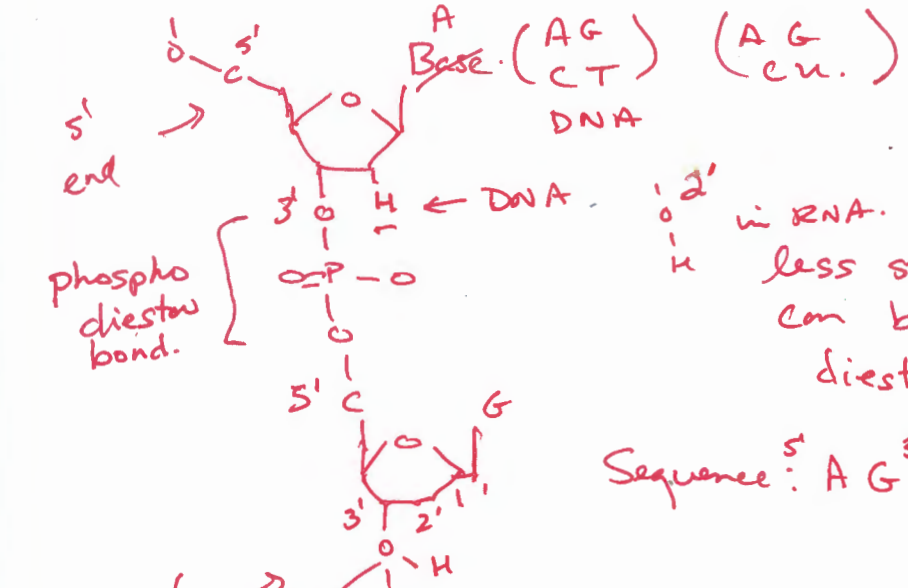
Inhibitors

Competitive
- Similar to substrate
- non-functional



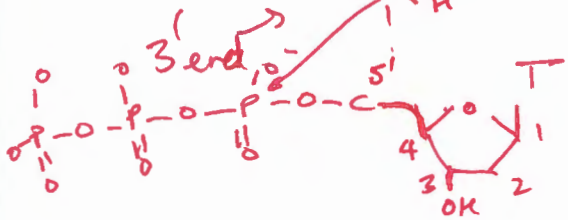
Allosteric.
- bind elsewhere
- change shape of active site.

Nucleic Acids & DNA.



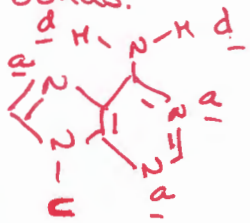
2' in RNA.

less stable : oxygen can break the phospho-diester bond.



- base added
- pyrophosphate.

H-bonds.



purine.

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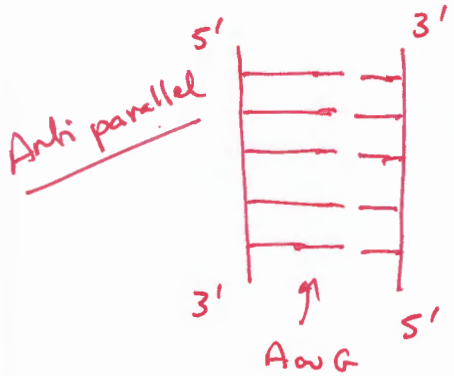
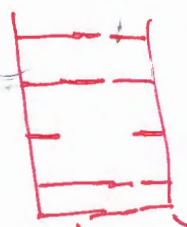
ds DNA (ds RNA) - basepairing rules A = T, G ≡ C





① H-bonding

② Purine - pyrimidine pair (same size)

purine + pyrimidine fits well.

grooves

minor

major.

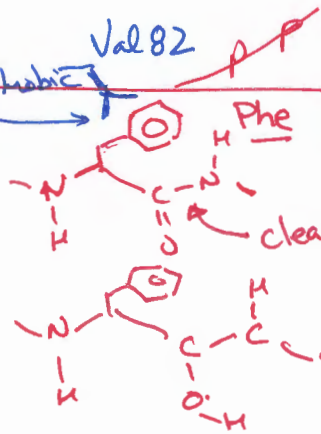
HIV Protease.

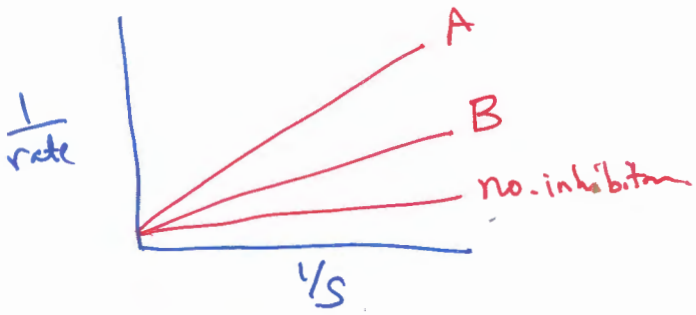
Val 82 → Specificity

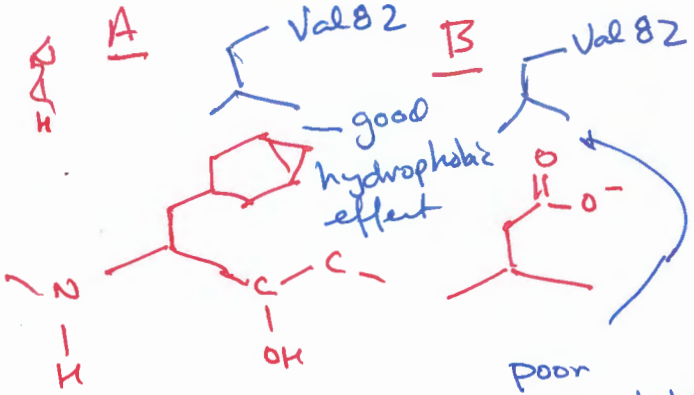
homodimer

Asp + Asp = cleave the peptide bond

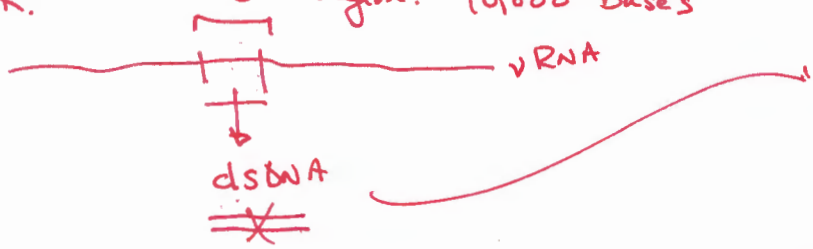
perform the chemistry





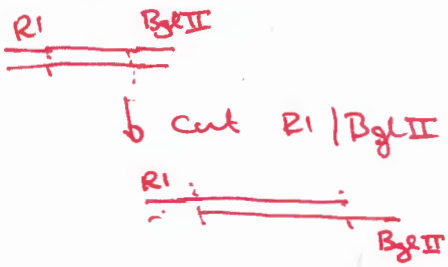


PCR. target region. 10,000 bases



target sequence.

- A) Sequence
- B) express protein in bacteria study pure protein



EcoR1

GAATTC
CTTAAG

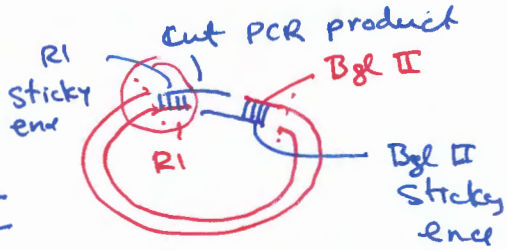
AATTC
G

R1 sticky end

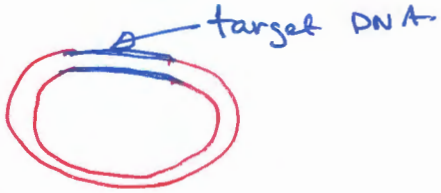
BglII

CGATCG
GCTAGC

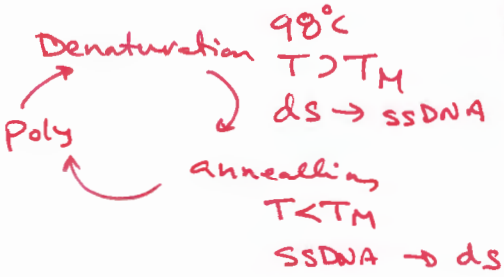
GATCG
C



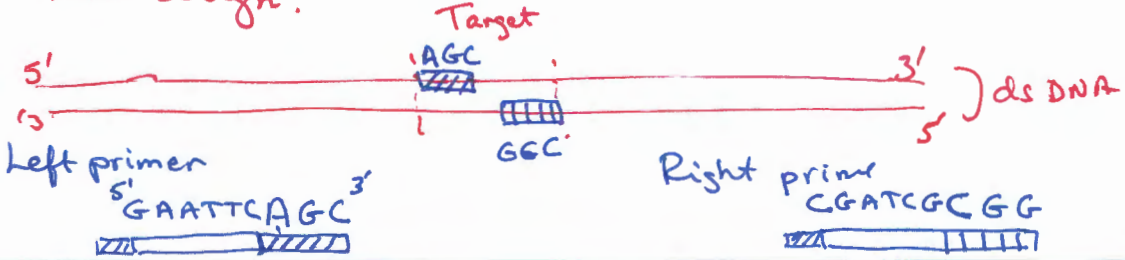
DNA ligase



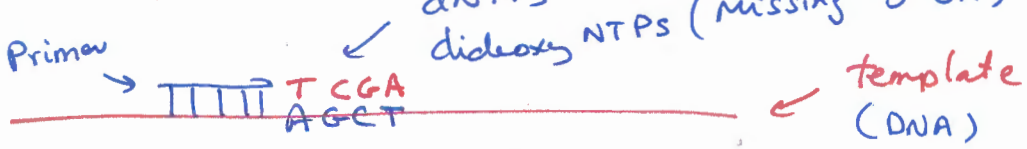
A) Steps



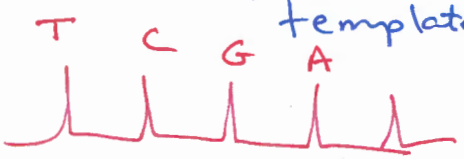
B) Primer design.



DNA Sequencing



- generate all possible DNA molecules
  - i) begin at same location
  - ii) end known base. (ddNTP colored)
- Separate by size.
  - Smallest → largest
  - = order of base added to primer
  - reading 5' → 3' direction from primer (sequence that is complementary to template)



Identifying mutations.

~~DNA~~ ~~AAAAGTTT~~T

Protein

only correct reading frame ⇒ gives known protein sequence

W.T. → DNA AAA ATG TTT TAA GC

Protein Met Phe (stop)

G TTT TAA GC

DNA Seq

Phe → blastn

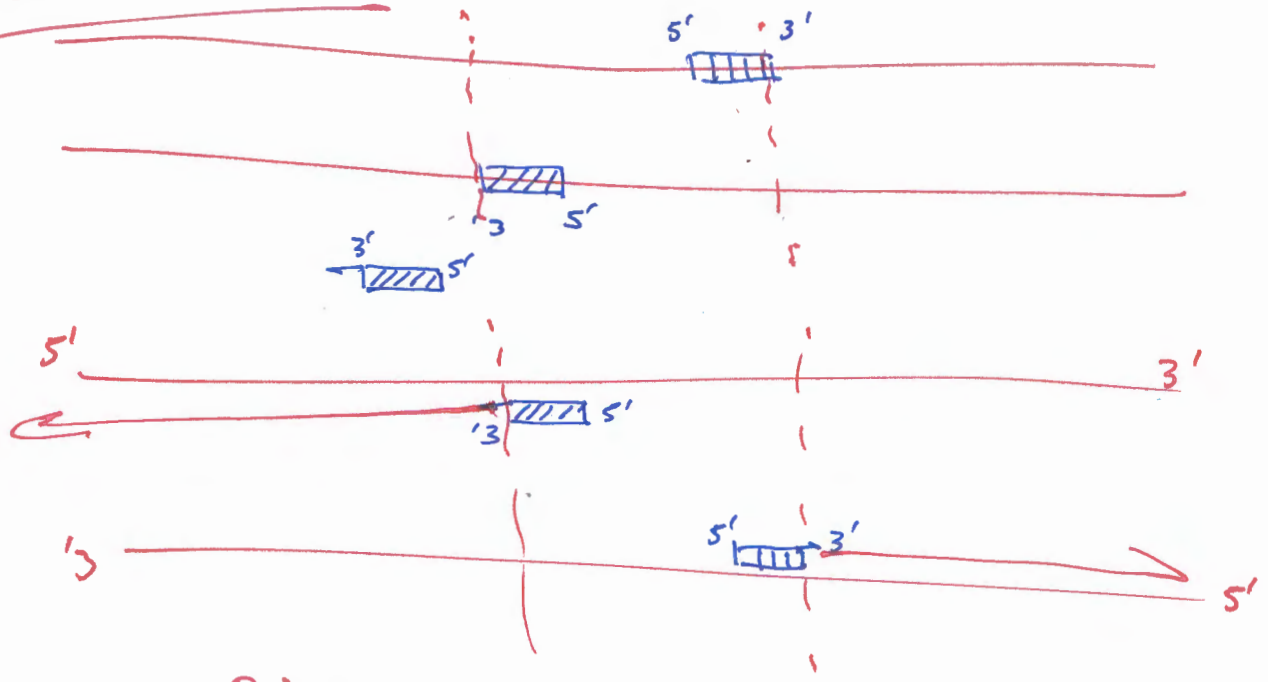
W.T. G|TTT|TAA|GC

Mut G|TAT|TAA|GC

Tyr.

1. Align WT seq read on entire wild-type sequence
2. Use known reading frame to determine correct reading frame for your seq read.
3. Use same reading frame for mutant.
4. Find mutation.

Wrong Primers



Correct Primers.