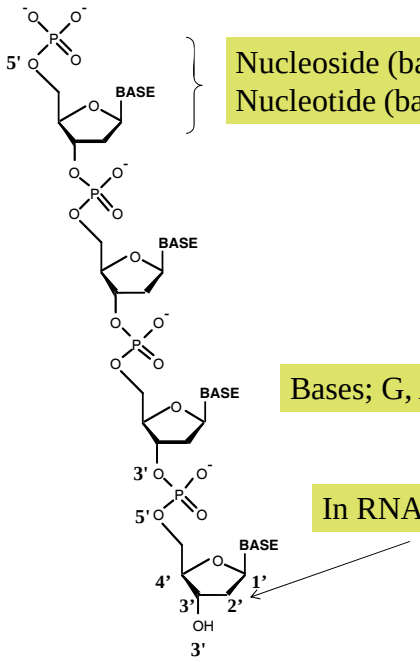
Recombinant DNA

- "Making a recombinant DNA" refers to the creation of new combinations of DNA segments not found together in nature.

  ~ "Cloning a gene"
- The isolation and manipulation of genes allows for more precise genetic analysis as well as practical applications in medicine, agriculture, and industry.



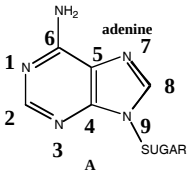
Nucleoside (base + sugar)
Nucleotide (base + sugar + phosphate)

Bases; G, A, C, T (U in RNA)

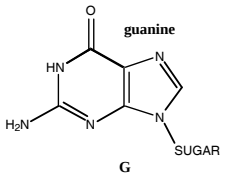
In RNA there is a 2'-OH group

Bases

PURINES

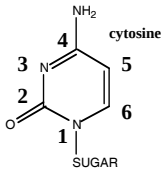


A

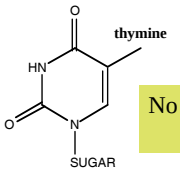


G

PYRIMIDINES



C



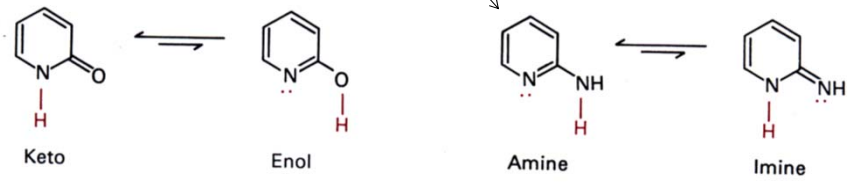
T

No methyl group in uracil

Nucleosides: adenosine, guanosine, cytidine, thymidine (uridine)

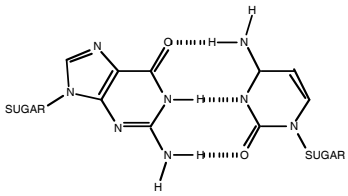
Tautomerism

Dominant forms at physiological pH

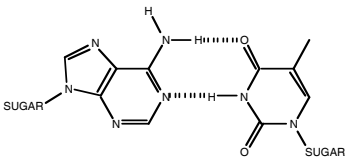


Watson-Crick Base-pairs

10.6 Å

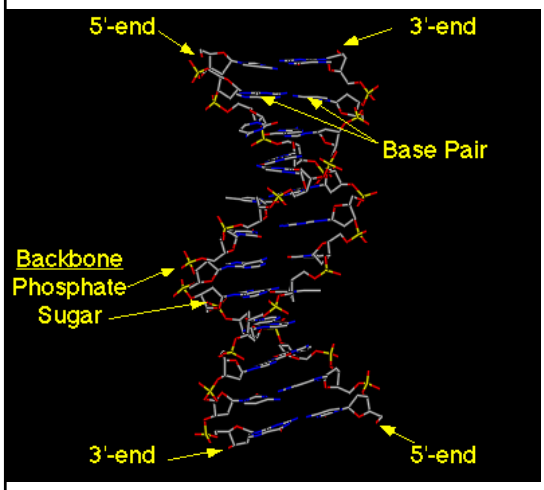


G-C



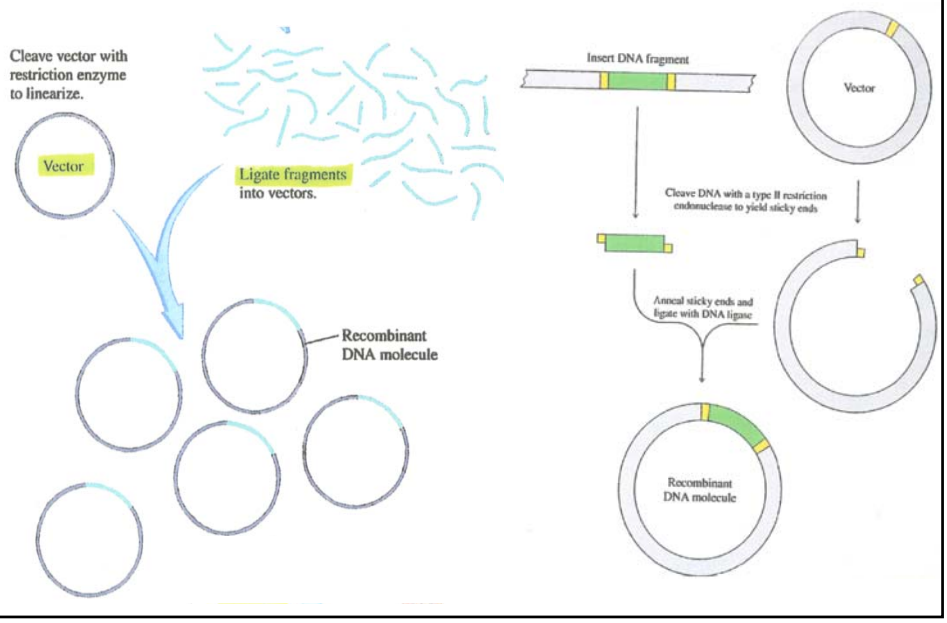
A-T

DNA double helix



- Sugar-phosphates on outside, exposed to solvent
- Bases point inwards forming Watson-Crick pairs
- Right-handed, antiparallel double helix
- Base-pairs are stacked but offset
- 34 Å per 10 base pairs in one turn of helix

Molecular Cloning

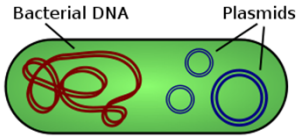


Problem: How to get enough DNA?

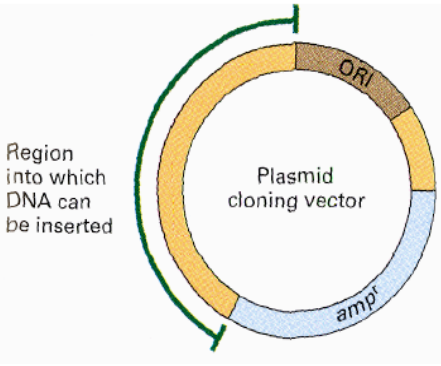
- 10 L *E. coli* culture contains at most 0.1mg ***(~1.5 X 10^{-10} moles!)*** of any 1000 bp length chromosomal DNA.
  - Separation, isolation, and purification would yield much less.

  * Similarities and differences between bacterial chromosome DNA vs. plasmid DNA?
- Getting large amounts of eukaryotic DNA is even more difficult.

Plasmid Vectors



- Plasmid: Small (<200 kb), circular DNA molecules that occur naturally in bacteria and replicate **independently**.



- Vector: Engineered plasmids that contain:
•Replication elements (high copy #)
•Resistance markers
•Cloning sites
•Other elements (e.g., promoters for mammalian expression i.e., to get proteins).
*distinguish this from getting DNA.