DNA
- Adenosine / Guanine / Cytosine / Thymine
- Sugar: 2-deoxyribose
- Phosphate linear chain linkage

RNA
- Adenine / Guanine / Cytosine / Uracil
- Sugar: ribose
- Phosphate linear chain linkage

Structure of Nucleic Acids
- Linkage: mononucleotides are linked via phosphodiester bridges between the 5' and 3' hydroxyl group
  - Bases are NOT attached covalently to one another
  - Structures are typically written L to R, 5’ to 3’ unless otherwise stated
- Nonenzymatic Hydrolysis of Nucleic Acids
  - Strong Acidic Hydrolysis of DNA / RNA results in purines removed from sugar
    - purine bases / pyrimidine nucleosides / deoxyribose or ribose / P*
  - Strong Basic Hydrolysis
    - Products: nucleoside monophosphates
    - Of DNA DOES NOT OCCUR - lacks 2'-hydroxy group
  - Important for separating RNA (n) / DNA (stable base)

DNA Double Helix: both prok and euk cell DNA has a double helix wrapped around the same axis
- Right handed, o-helix
- Backbone: phosphodiester bonds and deoxyribose sugar on outside of molecule
  - Polar Groups:
    - P* project to outside, interacting with (+) charged (@ physiological pH) proteins
    - (-) P* (@ physiological pH) complexed with cations (Na/K/Ca) to stabilize helix
  - Nonpolar Groups: interior of the molecular structure, water excluded
- Bases project to the interior of helix, with planes parallel to one another and stacked perpendicular to long axis of strand
- Van der Waals forces contribute to stability of the helix
- Base Pairs: bases of strands form hydrogen bonds with bases of the other - shows complementary relationship
  - A=T  G=C  Pairings NOT in equal distribution
  - A=T weaker than G=C, so sections that are A=T rich can be easier separated
- Strands are complimentary (not the same nt sequence) and antiparallel (one runs 5’-3’, one runs 3’-5’)
- 2 Periodicities one at 3.4 Å (parallel bases separation) and 34 Å (one complete turn of the helix)
  - B: 10bp to complete one turn of double helix
- Major and Minor Grooves: site of regulatory protein binding (major), with major larger (22Å) than minor (12Å)
  - Formed by the angle of glycosyl bonds (>180° = major, <180° = minor)
  - Strands do not need to be separated for protein to read DNA sequence
- Stability: normally a flexible chain, below interactions provide stabilization
  - Hydrophobic & electronic interactions (Van der Waals) between stacked bases - shielded from aq environment
  - Hydrogen bonds between nitrogen bases of complementary strands (A=T  G=C)
  - Ionic: (-) P* and polar sugar exposed to aq environment
  - Keep strands from collapsing by keeping (-) charges separated from one another
- Melting: DNA in solution and then slowly increase temperature in the lab
  - Hypochromic Effect: when DNA heated to the melting temp, and an increase in UV light absorbance occurs
    - Stacked bases in dsDNA shield UV absorption
    - Melting causes UV to increase absorbance by bases - monitor melting based on absorbance in soln
    - Heating causes H-bonds to disrupt, 2 strands separate
      - A=T melts at lower temps than G=C
  - Annealing: H-bond formation allows dsDNA to form complementary single strand
- Confirmations of the Double Helix
  - Same base pairing rules apply, but may function differently in gene regulation / expression
  - Changes in shape of minor / major grooves affect protein binding
  - B: most common, described by Watson-Crick, 10bp
  - C: not found physiologically, 9bp / turn (33Å)
  - A: 11bp / turn (28Å), N-bases not perpendicular but tilt 20°, forms under low hydration conditions
    - RNA only forms A in double stranded form – due to ribose structure
  - Z: 12bp/turn, L-handed helix/ staggered zig-zag backbone/ bases found on the outside
    - Occurs in vivo in alternating pur-pyr stretches (specifically G-C rich) / favored in regions of methylation of deoxycytidine in GC rich regions
    - Stim Expression of Genes: proteins bind specifically to Z-DNA & may function in gene regulation
  - B & Z can be present in one segment without separating strands
- Segments of adenosine in a row can bend helix - 6 in a row can cause a bend of 18°
  - May be a feature recognized by DNA binding proteins – expression blocked in regions with a bend
Cloning DNA with Restriction Endonucleases

Laboratory Chemistry of Nucleic Acids

- **Selection of a Vector**
- **Isolation of DNA to be Cloned**

- **Enzymatic Hydrolysis (Nucleases)** – metabolism within the cell
  - **Exonuclease**: eats DNA/RNA one base at a time starting from a specific end
    - 5': presented with 5'-hydroxyl on terminal nucleotide to remove form polynucleotide chain
    - 3': presented with free 3'-OH on terminal nucleotide to remove from polynucleotide chain
  - **Endonuclease**: eat the interior of a polynucleotide, specific for 5' or 3' side of phosphodiester bond
    - Can cut ssDNA or dsDNA depending on nuclease

- **Restriction Endonuclease**: recog specific base sequence in foreign DNA for degradation & cleave both strands
  - Found in bacteria and not in eukaryotes – restrict growth of bacterial viruses
  - Host modifies DNA so that they do not attack host DNA
  - Cuts at defined sequences and then allows for annealing with same enzyme produced ends
    - Cut at certain sequence and then put it back together
  - Beneficial: chromosome structure / creating recombinant DNA / isolating genes / sequencing DNA molecules
  - **Palindrome**: reads the same way forward and backward – many cut at these sequences
    - Creates specific ends of DNA and assist cloning & creating specific size DNA fragments

- **Cloning DNA with Restriction Endonucleases**
  - **Recombinant DNA**: linking restriction fragments from one organism with another’s DNA
    - DNA of organism 1 linked to vector (bacterial / viral fragment propagated in host organism)
  - **Procedure**
    - DNA fragment from organism of interest obtained from genomic / cDNA library
    - Fragment introduced into plasmid (small circular DNA from bacteria)
    - Recombinant DNA introduced into bacterial cell via transformation
    - Bacteria with engineered plasmid selected for via Ab & isolated
    - Bacteria replicate and DNA cloned
  - **Selection of a Vector** – How can we propagate this DNA fragment?
    - Possess a number of sites cleaved by different restriction enzymes
    - **Selectable marker**: Genes that encode for resistance to Ab – allows for selection of bacteria
    - Internal signals (promoters, etc) permit insert to be transcribed & translated into protein
  - **Sticky Ends**: H-bonding of complementary extensions to facilitate DNA fragment ligation
    - Ligation = formation of phosphodiester bonds via enzymes
    - SE created from blunt ends which have Linker Sequences added to them
    - **Linker Sequences**: joined covalently to ends of DNA & contain restriction enzymes – convert to specific restriction enzyme type
  - **Isolation of DNA to be Cloned** – to isolate specific gene, screen a DNA Library (contains fragments of DNA)
    - Single DNA molecule (pure fragment of DNA) not often used, usually isolate a gene for specific protein
      - DNA Source: genomic DNA digest (genomic library) or DNA from mRNA (cDNA library)
      - Library consists of transformed cells bearing different fragments of DNA or cDNA - coding
- Genomic Library for isolating a specific gene – has non-coding regions in it
  - cDNA is just the coding region and will have introns removed to generate protein
  - Vector: DNA to be inserted is small (6-10kb)
    - λ phage vectors can handle up to 20kb – infects *E.coli*
    - COSmids: can handle up to 50kb
      - Plasmids with DNA sequences (cos sites) allowing plasmid to package into bacteriophage coat protein
      - Allows efficient expression of the library, replicated as a plasmid & insert
- Cloning: constructed by cloning all DNA of genome of an organism
  - Larger vectors used for fewer colonies to clone entire genome / genes of interest obtained complete (3’ & 5’ flanking sequences)
  - Insert DNA from partially digesting genomic DNA with restriction endonuclease (Sau3A)
  - Separated fragments separated by electrophoresis & removed for cloning
  - Random genomic DNA fragments ligated to λ phage vectors cut with BamHI
  - λ bacteriophage genomic library: random DNA ligated to λ genome arms
    - infect bacteria and propagate
    - Screening of library allows you to look at human genome
      - Conducted via hybridization with a probe for sequence of interest
  - Human Genome Project done with bacterial artificial chromosomes (BACs) which can accommodate up to 300kb
- Eukaryotic DNA Sequences
  - Highly repetitive: localized @ centromere & participates in alignment during mitosis
    - aka satellite DNA and not transcribed
    - More of a structural role in chromosome formation
  - Moderately Repetitive: both transcribed and untranscribed sequences
    - Ex. rRNA rubunits / tRNAs / histone proteins
    - Randomly repeated with untranscribed spacer regions between them
    - Several hundred to several thousand per genome required to satisfy need of cell

![Diagram](image-url)