Chapter 2

Key Concepts:

- **Nucleases** (核酸酶): enzymes that degrade nucleic acids
  - **Endonucleases** (内切核酸酶): split a polynucleotide chain by hydrolyze (水解) internal bonds
  - **Exonucleases** (外切核酸酶): must from the end of chain
  - **restriction endonucleases** (限制性内切核酸酶): usually called restriction enzymes (限制酶)
    - recognize a specific DNA sequence
    - cut, or restrict, at or near that sequence.
- DNA sequencing:
  - **dideoxy sequencing** (双脱氧测序, 1977): first generation
  - **next generation of sequencing** (NGS, 2005): also called second-generation NGS
  - **third-generation NGS systems** (recently)
- **polymerase chain reaction** (PCR, 聚合酶链反应, Kary Mullis 1983):
  - amplicon (扩增子): within 40 thermal cycles of an idealized PCR reaction, a single template DNA molecule generates approximately 10^{12} amplicons—more than enough to go from an invisible target to a clearly visible fluorescent dye–stained (荧光染色) product.
  - reverse transcriptase PCR (RT-PCR): allow for RNA templates to be converted to cDNA and then subject to regular PCR
  - applications:
    - identify individuals from residual DNA on crime scene evidence as small as cigarette butts, smudged fingerprints, or a single hair.
    - Evolutionary biologists have made use of PCR to amplify DNA from wellpreserved samples
- DNA Microarrays (微阵列):
  - **gene expression profiling**: measure expression level of mRNA quantitatively.
  - **single nucleotide polymorphisms (SNP, 单核苷酸多态性)**: single nucleotide substitutions at a specific genetic locus.
    - missense mutation (错义突变): SNP creates a mutation within a gene involved in the metabolism of a drug.
- **Chromatin immunoprecipitation** (ChIP, 染色质免疫沉淀阵列): allows researchers to detect the presence of any protein of interest at a specific DNA sequence in vivo (在体内).
  - ChIP-seq: allow a researcher to obtain a genome-wide footprint of all of the binding sites of the protein of interest (e.g. transcript factor).
- **Transgenic** (转基因): An organism that gains new genetic information from the addition of foreign DNA
  - replace defective genes by functional genes

- The most powerful techniques for changing the genome use gene targeting to delete (knockouts) or replace (knock-in) genes by homologous recombination.