Introduction to molecular biology
   Combining genetics, biochemistry, structural chemistry
Information flow in biological systems:
   The Central Dogma
   The Sequence Hypothesis
Macromolecular structures and properties
   DNA
      History : Identification of DNA as the genetic material
      Chemical composition
      Structural features: Basepairing, Antiparallel structure
      Nomenclature
      Denaturation and annealing
      G+C content
   RNA
      Chemical composition
      Structure
   Protein
      Primary, secondary, tertiary and quaternary structural features
Some useful facts:
   Average MW of a base pair=660
   Size of average bacterial gene=1000bp
   Average protein size=30kDa
   Average MW amino acid=110Da
The Genetic Code:
   Transcriptional units
   Gene-protein relationships
   Role of tRNA, tRNA synthetases
   Ribosome structure
   Degenerate genetic code
   Signals for translation initiation and termination
Molecular cloning methods
   Restriction enzymes
      Site recognition, cleavage
      Frequency of occurrence of sites
      Where they come from; modification
   Joining DNA fragments with DNA ligase
   Transformation
      Chemical treatment
      Electroporation
Vectors
   Plasmids
      Features
      Examples
Phages

Advantages
Selecting recombinants
  Use of alkaline phosphatase to reduce vector background
  Blue/white color screen
  Hybridization
  Immunological methods
Other vectors:
  Cosmids
  ssDNA phage vectors
  Phagemids
Polymerase Chain Reaction (PCR)
  Basics
  Applications

Techniques in molecular biology
  Molecular separations
    Gel Electrophoresis
    Chromatography
  Importance of radioactive labels
  Methods for labeling nucleic acids
  Southern blotting
  Northern blotting
  Site-directed mutagenesis
  Restriction Mapping
  Mapping ends of RNA
    S1 nuclease mapping
    Primer extension
  Reporter gene expression
  Methods for analyzing protein-DNA interactions
    Gel mobility shifts
    DNasel footprinting

Genomics
  DNA sequencing methods
  Sanger
  Maxam Gilbert
  Automation
  Shotgun approaches
  Sequencing Large genomes

In silico workshop
  Constructing recombinant plasmids in silico
  Restriction digestion in silico
  Translation into amino acid sequences in silico
  Identifying open reading frames in silico
  Analyzing G+C content in silico
  Start and stops of genes in silico

Prokaryotic transcription machinery
  Initiation, elongation, termination
RNA polymerase structure
\[ \alpha \beta (\text{core}) + \sigma (\text{specificity}) \]

Promoters
-10, -35, consensus
UP elements and \( \alpha \)-CTD

Melting DNA (closed complex \( \rightarrow \) open complex), abortive initiation

Termination
\( \rho \)-dependent
\( \rho \)-independent (stem-loop structure)

Regulation of transcription in prokaryotes
Terms & Concepts: Operon. Regulon
Lac operon
Organization of the operon
Diauxic growth
Negative control by repressor
Mechanism of repression
Inducer
Positive control by cAMP and CAP
Interaction of CAP with \( \alpha \)-CTD
Role of DNA bending
Maltose operon; regulation; alternative binding sites
Arabinose operon; regulation; repression; repression loop; autoregulation
Trp operon
Regulation by repressor
Sensing trp as co-repressor
Regulation by attenuation (in E. coli)
Alternative secondary structures of mRNA leader
Sensing trp as charged tRNATrp
Role of termination

Global regulation
Use of alternative sigma factors
SPO1
Heat shock response
Use of alternative RNA polymerases
Phage T7
Utilizing the T7 system for regulated expression in E. coli.
Vectors such as pET21a
Strains such as BL21(DE3).

Protein-DNA interactions in prokaryotes
Structural features
Origins of DNA binding specificity
Contributions of the protein
Contributions of the DNA

Transcription in eukaryotes
Machinery
Three RNA polymerases
Identification
Functions
Common features
Unique features
Structural properties
Post-translational modification
Promoters
Roles of promoter elements for each polymerase
Locations of the promoter elements
Regulatory vs. core promoter elements
General transcription factors for each polymerase
Models for assembly
Functions of the individual factors
Common themes
Regulation
Properties of eukaryotic activators
Structural features
DNA binding domains
Dimerization domains
Activation domains
Modular structure of activators
Coactivators
Major classes
Properties
Interactions with the general transcription machinery
Mechanisms of activation
Targets of activators
Steps in initiation that are affected
Importance of coactivators
Chromatin
Structural components
Histones
Structural features
Modification
DNA
Packaging states
Effects on transcription
Strategies used by factors to contend with chromatin
Chromatin remodeling and modification factors
RNA Processing
Types of modification and evidence for each
Splicing
Alternative splicing
Capping
Polyadenylation
Importance of each to gene expression
RNA signals involved
Protein factors involved
Involvement of snRNAs
Mechanisms of RNA processing