

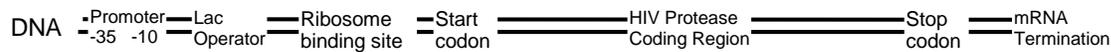
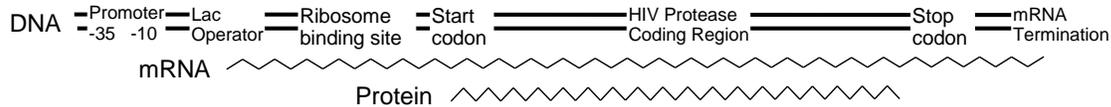
**Exam Coverage: Lectures 18-27**

**A. Expression of Proteins in Bacterial using Plasmids:**

You should be familiar with the overall steps in the construction of the plasmid, e.g.

- 1) PCR used to amplify the DNA and add restriction sites to the end of the PCR product.
- 2) Cutting with restriction enzymes to make sticky ends on the PCR product and the plasmid
- 3) Ligation to join the two DNA fragments.
- 4) Transformation into bacteria
- 5) Selection for plasmid containing cells by growth in the presence of an antibiotic.

You should understand the role and organization of all of the DNA sequence elements on the protein expression plasmid:



mRNA production (DNA to mRNA)	X	X					X
Protein production (mRNA to protein)			X	X	—————codons—————>	X	

**Summary of expression features:**

**Origin of replication:** Overall mechanism of DNA replication:

Role of the following enzymes in DNA replication:

- Helicase
- Gyrase (topoisomerase)
- Single stranded binding protein
- Pol III
- Pol I
- Primase
- Ligase

Leading versus lagging strand synthesis.

Topoisomerase inhibitors in the treatment of disease, e.g. cancer.

**Antibiotic resistance gene:**

- Codes for a protein that can inactivate an antibiotic.
- Allows for selection of bacteria that contain the plasmid as these can grow in the presence of the antibiotic.
- Mechanism of antibiotic resistance of penicillin (inhibition of bacterial cell wall synthesis).

**Promoter:**

- Site of RNA polymerase binding
- Sequence specific recognition of -35 and -10 region by sigma factor
- Overall process of polymerization: DNA template, NTPs polymerized in the 5' to 3' direction

**Lac Operator:**

- Binding site for lac repressor protein, prevents generation of mRNA when lac repressor is bound
- Inducers (lactose, IPTG) cause allosteric change in the lac repressor – causing it to leave the DNA.
- An example of sequence specific recognition of DNA – hydrogen bonding to the edge of a basepair.

**B. Gene Regulation in General:**

- Repressor proteins (e.g. lac repressor)
- Activator proteins
- Bacterial operons – multiple coding sequences on one mRNA, coordinate regulation.
- Enhancer sequences in eukaryotic cells.

**C. Protein synthesis:**

- Overall structure of the ribosome, 30s, 50s subunits, role of each subunit, exit tunnel
- Charging of tRNAs with amino acids (correct amino acid is added to the correct tRNA).
- Pairing of codon on mRNA with anticodon on tRNA.
- Positioning of Met codon due to spacing from the ribosome binding site.
- Cycle of adding new amino acids.
- Role of stop codon in release of protein.
- Inhibition of protein synthesis by antibiotics – general modes of action. Come prepared to answer questions like: “If an antibiotic X blocks the exit tunnel – what steps in protein synthesis can still occur, which cannot.”

**D. mRNA Processing – Eukaryotic cells.**

- polyA addition to 3' end: effects stability of mRNA, transport out of the nucleus.
- RNA splicing: removal of introns, joining of exons.
- Alternative splicing: variation in number of exons in the final mRNA. Exon order remains the same.
- Mechanism of splicing: Branch point causes break at 5' splice site, 5' splice site joins to 3' splice site.
- Effect of mutations on mRNA splicing.

**E. Carbohydrates:**

- Ketose versus aldose,
- 5 carbon sugar –ribose, found in nucleic acid
- 6 carbon sugars– glucose
- Ring formation – generation of new chiral center -  $\alpha$  or  $\beta$ .
- Disaccharides: general structure, glycosidic bond.
- Storage polysaccharides (starch, glycogen). Shorthand nomenclature for linkage (e.g.  $\beta(1-4)$ ).
- Structural polysaccharides: Cellulose
- Bacterial cell wall structure, in general terms (linear polymers of modified glucose, crosslinked by proteins). Mechanisms of inhibition by penicillin.
- Describe the molecular basis of penicillin action, penicillin resistance ( $\beta$ -lactamase) and the dual role of penicillin and clavulanic acid in killing bacteria.

**F. Lipids & Membranes:**

- You should be able to recognize the chemical structure of fatty acids, waxes, phospholipids, cholesterol.
- Understand the relationships between the hydrophobic effect and spontaneous formation of micelles and bilayers.
- Describe how cholesterol affects the fluidity of lipid bilayers.
- Describe the properties of biological membranes in terms of the different components.
- Be able to distinguish between transport proteins and signaling membrane proteins.
- You should know the function of the following structures in the cell:
  - ribosome
  - rough endoplasmic reticulum
  - golgi
- Osmotic effects: Be able to predict how the cell volume will change if the cell is placed in solutions that have a different salt (or carbohydrate) concentration than what is inside the cell.
- Mechanism by which proteins are exported out of the cell. Including the leader sequence (for secreted proteins) and the membrane anchor-sequence (for membrane bound proteins).

**G. Cholesterol metabolism disease.**

- Normal regulation of cholesterol (uptake of LDL, feedback regulation)
- Endocytosis of LDL + LDL receptor → endosome
- Genetic defect in LDL receptor = no feedback, more cholesterol made than necessary.
- Treatment with statins that inhibit (competitive) one step in cholesterol biosynthesis pathway.

**H. Cell signaling** - You should know the difference between a kinase and a phosphatase.

Three main types of signaling:

## 1. G-protein

- a) Hormone binds to G-protein coupled receptor
- b) GTP replaces GDP, activates G-protein
- c) Adenyl cyclase activated, cAMP produced
- d) Protein kinases activated, phosphorylated form of some enzymes are turned on, e.g. glycogen phosphorylase, releasing glucose from glycogen.

## 2. Tyrosine kinase activation:

- Receptor chains in the membrane are monomers.
- Hormone induces dimerization, activating tyrosine kinases, which then phosphorylate a cascade of other proteins.
- HER2 breast cancer – overproduction of growth hormone receptor – uncontrolled cell growth.
- Treatment with antibody (trastuzumab), which prevents hormone from activating receptor.

## 3. Direct binding to proteins that affect transcription (production of mRNA)