

## Chapter 9 Homework Assignment

- We will not cover the entire chapter. Please use the lecture notes and the Review Sheet for testable material
- I have decided to alter the homework assignment for Chapter 9. The following problems will be due once we finish the chapter:

**3, 4, 7, 8, 10**

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## DNA-Based Information

*Of all the natural systems, living matter is the one which, in the face of great transformations, preserves inscribed in its organization the largest amount of its own past history.*

- Emile Zuckerkandl and Linus Pauling

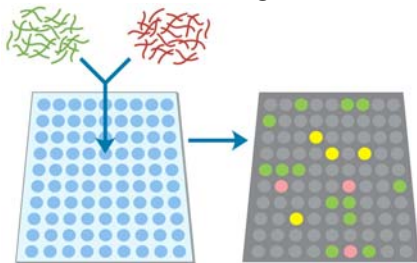
**-omics ...is the study of...:**

- **Genomics:** the full complement of an organism's genes.
- **Proteomics:** the full complement of an organism's proteins:
- **Transcriptomics:** an organism's RNA transcribed from its DNA
- **Metabonomics:** an organism's metabolite profiles
- **Structural genomics:** the 3-D structures of an organism's proteins and RNAs
- **Pharmacogenomics:** the interaction between genes and gene products and medications. How an individual's genetic inheritance affects the body's response to drugs.

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## Chapter 9 DNA-Based Information Technologies



## DNA Cloning: The Basics

- Understanding of any complex problem usually begins by breaking that problem into smaller, less complex units.
- This method is also used by the biochemist who isolates and studies the individual components of a biological system, its DNA, RNA, proteins, etc.
- However, the sheer amount of DNA found in living cells makes the process very challenging.
- Think about trying to find a very small needle in a VERY large haystack
- Technologies have since been developed that allow us as scientists to locate, isolate, prepare and study small segments of DNA derived from much larger chromosomes.
- These techniques of **DNA cloning** led the way in the development of all of the -omics fields!



Paul Berg Herbert Boyer Stanley N. Cohen

**Very Smart Guys**

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## DNA Cloning: The Basics

- DNA Cloning deals with separating a specific gene or DNA segment from a larger chromosome or DNA vehicle, attaching it to a smaller molecule of carrier DNA and then replicating this modified DNA  $10^3$  or  $10^6$  times.
- Cloning of DNA has five general steps:
  - Cutting the DNA at a precise location
  - Selecting a small molecule of DNA capable of self-replication
  - Joining two DNA fragments covalently
  - Moving this **recombinant** DNA from the test tube into a host cell
  - Selecting of identifying host cells that contain the recombinant DNA
- These methods are collectively called **recombinant DNA technologies** or **genetic engineering**

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## DNA Cloning: The Basics

### Cutting the DNA

Chromosomal DNA

Cleavage site

Recognition sequences

Cleavage site

EcoRI restriction endonuclease

PvuII restriction endonuclease

Sticky ends

Blunt ends

Enzyme	Site
ClaI	A T C G A T
EcoRI	G A A T T C
FnuAI	G A A T T C
HaeIII	G A G C C
HindII	G T T A A C
HindIII	C A T A T C
HindIII	A A A G C C T T
PstI	C T C A G C
PstI	G A A G C C

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Berg et al. "Biochemistry" 5th Ed, Figure 9.33

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## DNA Cloning: The Basics

### Cutting the DNA

- Restriction endonucleases** are the DNA cutters for genetic engineering who recognize and cut DNA at locations specific to each enzyme
- These enzymes were discovered by Werner Arber, who found that they served as an immune response in bacteria (chewed up foreign DNA)
- These enzymes recognize specific sequences of DNA called **Palindromes**
- There are three types of enzymes:
  - Type I:** cleaves 400 – 7000 bp from the recognition site
  - Type II:** cleaves adjacent to or within the recognition site **Most Used! WHY?**
  - Type III:** cleaves 25 to 27 bp from the recognition site

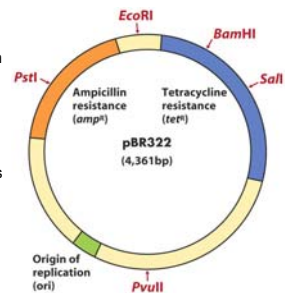
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## DNA Cloning: The Basics

### Cloning Vectors

- Cloning Vectors** are DNA vehicles that are capable of recombination and self-replication, and include:
  - Plasmids (One we are concerned with here!)
  - Bacteriophages
  - Bacterial (or Yeast) Artificial Chromosomes (BACs and YACs)
- Plasmids** are small circular DNA molecules that replicated separately from their host cell's chromosome
- While they do occur naturally, scientists have engineered plasmids for specific use as vehicles for recombinant DNA technologies



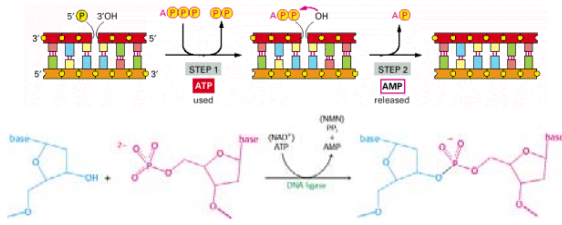
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## DNA Cloning: The Basics

### Joining the DNA

- Once the gene of interest and the cloning vehicle have both been cut, the two must be joined to form the recombinant DNA
- The enzyme responsible for this ligation is **DNA Ligase**



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Alberts *et al.* "Mol. Biol. of the Cell" 4<sup>th</sup> Ed, Figure 5.14  
Berg *et al.* "Biochemistry" 5<sup>th</sup> Ed, Figure 27.28

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## DNA Cloning: The Basics

### Selection

- Identification of cells containing the vector molecule requires engineering or **selection** of the vector molecule to contain a suitable marker gene whose expression provides a means of identifying cells containing it.
- Two popularly used marker gene systems are based on:
  - Antibiotic resistance genes:** A host cell strain is chosen that is sensitive to a particular antibiotic, often ampicillin, tetracycline or chloramphenicol. The corresponding vector has been engineered to contain a gene which confers resistance to the antibiotic. After transformation, cells are plated on agar containing the antibiotic to rescue cells transformed by the vector.
  - $\beta$ -galactosidase gene complementation.** The host cell is a mutant which contains a fragment of the  $\beta$ -galactosidase gene but cannot make any functional  $\beta$ -galactosidase. The vector is engineered to contain a different fragment of the  $\beta$ -galactosidase gene. After transformation by the vector, functional complementation occurs resulting in active  $\beta$ -galactosidase which can be assayed by acting on a colorless substance, Xgal (5-bromo, 4-chloro, 3-indolyl  $\beta$ -D-galactopyranoside), to make a blue product.

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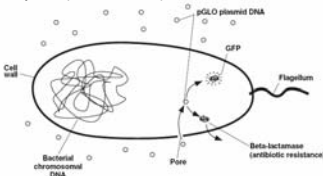
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## DNA Cloning: The Basics

### Transfer of DNA into the Cell

- Transformation** is the *uptake of foreign DNA*, usually in the form of a circular plasmid
- There are multiple methods for transformation, including:
  - Electroporation**
    - Electrical shock makes cell membranes permeable to DNA
  - Calcium Chloride/Heat Shock**
    - Chemically-competent cells uptake DNA after heat shock

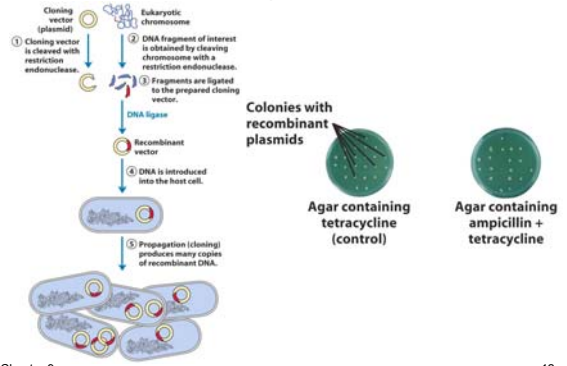
Transformation Animation



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## DNA Cloning: The Basics



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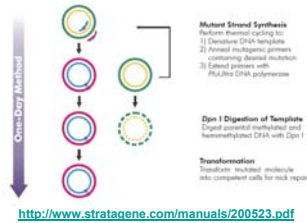
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## DNA Cloning: The Basics

### Site-Directed Mutagenesis

- **Site-Directed Mutagenesis** is a technique that allows for the alteration of a single amino acid by changing the DNA of the gene that encodes a target protein.
- Basically, you design a primer that contains one or two nucleotide changes
- These changes are transferred into daughter DNA molecules via replication
- The mutated daughter DNA molecules are then templates for transcription which produces an mRNA strand.
- This strand is then translated into protein which should contain the target AA mutation.



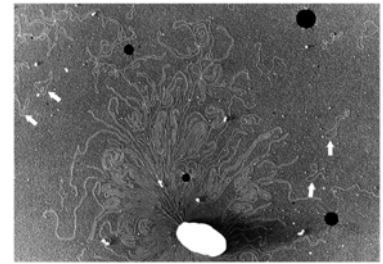
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## Genes to Genomes

### Genomes and Chromosomes

From a lysed *E. coli* cell:

- The *E. coli* genome consists of a single chromosome which is a double-stranded circular DNA molecule with **4,639,221** base pairs.
- *E. coli* also contain smaller circular DNA molecules that are free in the cytosol (plasmids); see white arrows in figure.



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## Genes to Genomes

### Terms

- **Chromosome:** Component in the cell that contains genetic information
- **Plasmid:** Circular DNA molecule that replicates separately from the host chromosome
- **Genome:** The complete set of genes for an organism
- **Proteome:** The entire protein complement encoded by an organism's genome
- **DNA cloning:** Cutting out a piece of DNA from the genome and inserting into a plasmid vector.
- **Recombinant DNA:** A DNA molecule comprising covalently linked segments from two or more sources

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## Genes to Genomes

### Genomes and Chromosomes

From a human:

- The human genome consists of 22 chromosomes (times 2) plus an X and a Y, or two X chromosomes (46 total).
- Eukaryotic chromosomes are complex, consisting of DNA and protein.
- The human genome contains 700 times more DNA than the *E. coli* genome:  **$3 \times 10^9$**  (3 billion) base pairs.



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**Why do you think there are more base pairs here?**

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## Genes to Genomes Genomes

- Complete genome sequencing for many organisms (mostly microorganisms) has been accomplished.
- The resulting field of genomics concerns the study of genes on a cellular scale.
- This has been made possible through technological advances in DNA sequencing...

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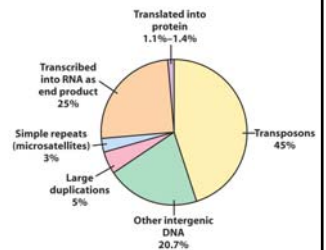
### Mammalian genomes Sequenced and in progress

Human (3000 Mb; complete)  
 Mouse (2500 Mb; complete)  
 Cow (3000 Mb)  
 Armadillo (3000 Mb)  
 Lesser hedgehog  
 African elephant (3000 Mb)  
 Opossum  
 Rabbit (3500 Mb)  
 Chimp (3100 Mb)  
 Dog (2400 Mb)  
 Rat (2800 Mb)

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## Genes to Genomes Human genome project results

- Estimated 27,894 genes
- ~1.1% in **exons**
- 1/1000 bp differ between individual humans: **SNPs** (single nucleotide polymorphisms)
- From SNPs arise human variety.
- <1% of SNPs are expected to impact protein function.
- Thus, thousands of genetic variations contribute to human diversity (not millions!)



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## Genes to Genomes Human Genome Project

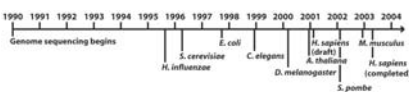
- The human genome project was initiated in 1989 with the goal of sequencing the 3 billion base-pair human genome in 15 years.
- The National Institutes of Health and the Department of Energy instituted the joint project. 20 centers contributed.
- There was great skepticism that this could be accomplished in a reasonable amount of time.
- In 1998, the company Celera genomics formed to sequence the human genome.
- Celera and the HGP concurrently announced the human genome draft in 2001. The genome was completed in 2004.



Francis S. Collins J. Craig Venter

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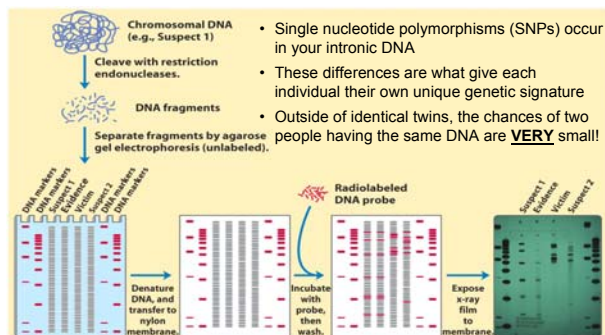
More Very Smart Guys



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## Genes to Genomes Forensics and SNPs

- Single nucleotide polymorphisms (SNPs) occur in your intronic DNA
- These differences are what give each individual their own unique genetic signature
- Outside of identical twins, the chances of two people having the same DNA are **VERY** small!



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## Genes to Genomes Human Genome Project



Fruit fly: 13,000 genes



Human: 28,000 genes.

The surprisingly small number of genes in the human genome (~ 100,000 expected; < 30,000 identified) was a major surprise from the project.

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## Genomes to Proteomes Proteomics

- **Proteomics** is the determination and analysis of the complete complement of proteins expressed by a genome
- There remain thousands of proteins in each eukaryotic cell about which we know nothing (> 40% in the human genome!).
- Characterizing the proteome is a much larger task than the genome. This links genes to function:
  - **Phenotypic function** describes the effects of a protein on an entire organism
  - **Cellular function** describes the network of interactions with other proteins in the cell
  - **Molecular function** describes the precise biochemical activity of a protein, including details such as the reactions an enzyme catalyzes or ligands a receptor binds
- There are three main pathways to investigating protein function:
  - Sequence and structural comparison with genes and proteins of known function
  - Determination of when and where a gene is expressed
  - Investigation of the interactions of the protein with other proteins

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## Genes to Genomes Human Genome Project

The modest number of genes indicates we must look elsewhere to explain the human complexity.

- Gene regulation, modification (*i.e.* methylation)
- Chromosomal modifications
- Location, quantity, timing of transcription
- Tissue-specific protein expression
- Roles (regulatory, other) of intronic DNA
- RNA splicing
- RNA roles in gene expression
- RNA editing (changes made to mRNA)
- Translational control (at ribosome)
- Alterations in protein-protein interactions

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Venter et al., *Science* (2001)291, 1304-1351.

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## Genomes to Proteomes Comparative Genomics

- **Comparative genomics** is the comparison among genes and proteins of known function.
- This method utilizes sequence and structural relationships and is aided by the increasing availability of genomics data
- **Orthologs**: Genes of different species but possessing a clear sequence and functional relationship to each other.
- **Paralogs**: Genes within an organism with a sequence and structural relationship.
- Conserved gene order on a chromosome is its **synteny**

Human 9	Mouse 2
EPB72	Epb7.2
PSMB7	Psmb7
DNM1	Dnm
LMX1B	Lmx1b
CDK9	Cdk9
STXBP1	Stxbp1
AK1	Ak1
LCN2	Lcn2

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## Genomes to Proteomes

### Cellular Expression Patterns

For genes with no identifiable relationships to known genes, other approaches need to be applied.

- Determining which tissues a gene is expressed in or what circumstances trigger the appearance of a gene product can provide insight into the function
- 2-D gel electrophoresis coupled with mass spectrometry allow for the analysis of the appearance of particular proteins from different tissues
- This appearance can be the function of development or from tissues treated in different ways.



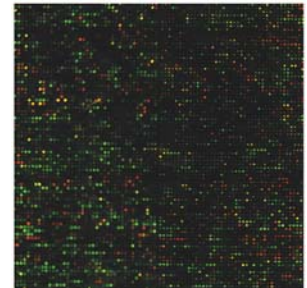
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## Genomes to Proteomes

### Cellular Expression Patterns

- Each spot in this microarray contains DNA from one of the 6,200 genes in the yeast genome.
- The different colors indicate conditions under which the genes are expressed.
- Here, green spots represents mRNAs abundant early in development, red RNAs are abundant later in development.



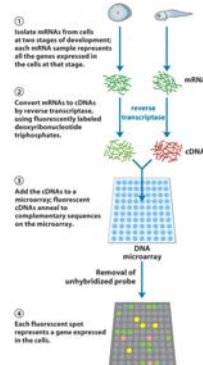
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## Genomes to Proteomes

### Cellular Expression Patterns

- DNA Microarrays (aka DNA Chips) allow for the rapid and simultaneous screening of many thousands of genes
- Here, an array of DNA segments from known genes are amplified and placed on a solid surface (usually by a highly precise robot!)
- These arrays are pre-designed with the content of each spot known
- This array can answer questions such as which genes are expressed at a given developmental stage,



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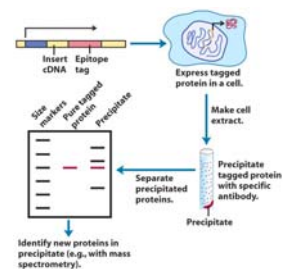
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## Genomes to Proteomes

### Probing Protein Interactions

Analysis of protein-protein interactions also can reveal important information about a protein's function and its role in the cell.

- A cDNA library can be constructed in which each gene is contiguous with (fused to) an epitope tag
- This tag allows researchers to pull out the protein of interest using an immobilized antibody that binds the tag
- If the protein interacts with any other protein within the cell, this second protein can be isolated with the tagged protein
- Purification techniques then identify the second protein



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## Genome Alterations

### Genetic Based Approaches to Treating Disease

- Once a human disease gene has been characterized, molecular genetic tools can be used to dissect gene function and explore the biological processes involved in the normal and pathogenic states.
- The resulting information can be used to design novel therapies using conventional drug-based approaches.
- In addition, molecular genetic technologies have recently provided a variety of novel therapeutic approaches that can be categorized into two broad groups, depending on whether the therapeutic agent is a gene product/vaccine or genetic material.

**Once the target gene has been identified, what do you think we can do to decrease its detrimental effects?**

**What if there are multiple genes involved in the disease process?**

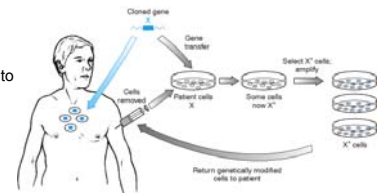
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## Genome Alterations

### Gene Therapy

- An essential component of classical **gene therapy** is that cloned genes have to be introduced and expressed in the cells of a patient in order to overcome the disease.
- Practically, this usually involves targeting the cells of diseased tissues. However, deliberate targeting of unaffected cells (such as enhancement of the immune system) may be preferred in some approaches
- Two major general approaches are used in the transfer of genes for gene therapy: transfer of genes into patient cells outside of the body (*ex vivo*) or inside the body (*in vivo*).



Strachan and Read "Human Molc Gen 2" Figure 22.3  
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## Genome Alterations

### Recombinant Proteins & Genetically Engineered Vaccines

- Here the therapy is to deliver proteins or vaccines which have been produced by genetic engineering instead of traditional methods. Methods involve:
  - **Expression cloning of normal gene products** — cloned genes are expressed in microorganisms or transgenic livestock in order to make large amounts of a medically valuable gene product;
  - **Production of genetically engineered antibodies** — antibody genes are manipulated so as to make novel antibodies, including partially or fully *humanized antibodies*, for use as therapeutic agents;
  - **Production of genetically engineered vaccines** — includes novel cancer vaccines and vaccines against infectious agents.

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