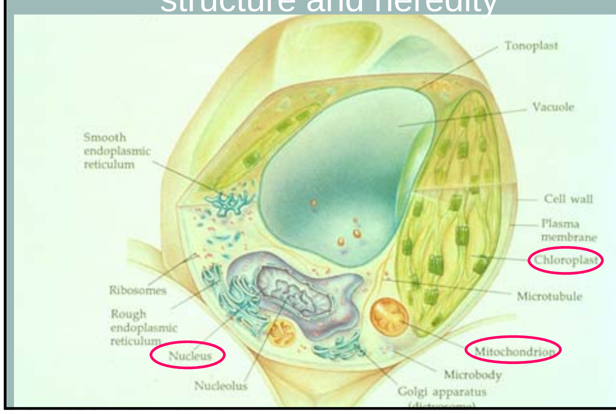
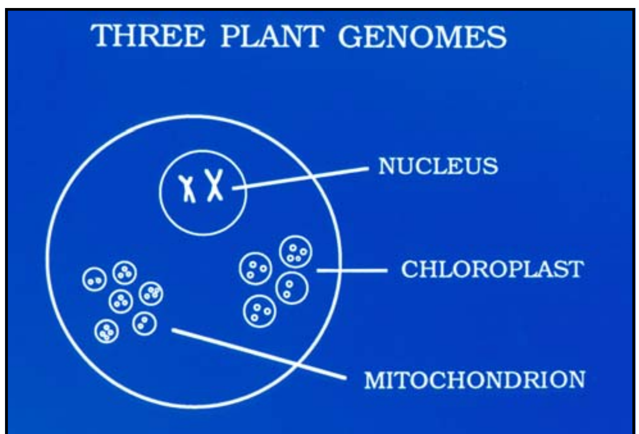
A few basics on genes and genomes related to GMOs and the issues

Whole genome concepts

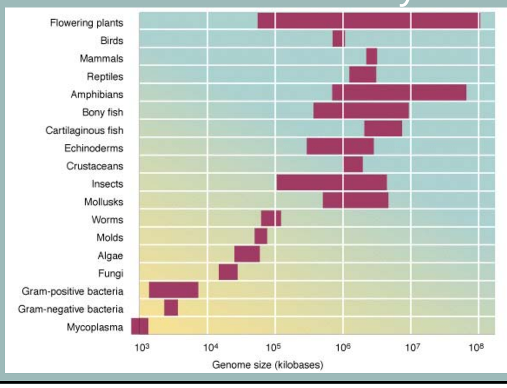
Cells are highly complex in structure and heredity





A genome refers to a complete set of genetic material in a cell or part of a cell – such as all nuclear chromosomes, all chloroplast DNA molecules

Nuclear genome size varies widely

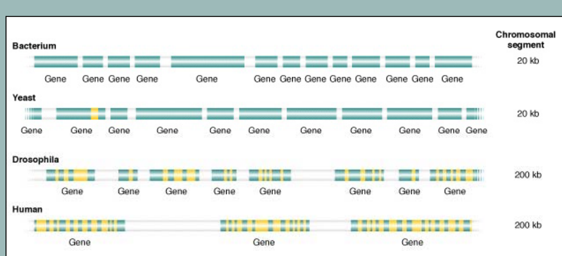


Examples of nuclear genome size variation among plants

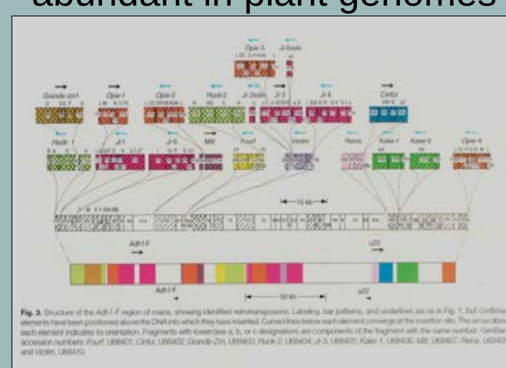
Haploid genome sizes given

• *Arabidopsis thaliana*	120 Mbp (120,000,000 bp)
• Poplar	480 Mbp
• Rice	450 Mbp
• Soy	1,100 Mbp
• Maize	2,500 Mbp
• Barley	5,000 Mbp
• Hexaploid wheat	16,000 Mbp
• *Fritillaria (*lilly family)	>87,000 Mbp

Gene density varies widely



Old transposable elements abundant in plant genomes

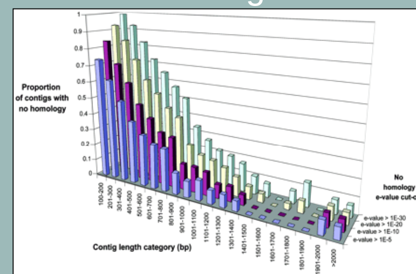


San Miguel et al., *Science* 1996 274: 765-768

Plant genomes – complex and fluid

- Nuclear – diploid or higher
  - Diploid – two copies
  - Polyploid – many copies; aneuploid – partial copies
  - Most plant genomes result from polyploid events, then act as diploids over time
    - Many copies of most genes, which diverge or lose function over evolution = gene families! Many genes with similar/overlapping functions
    - Related concept of redundancy in genomes and development – gene knock-outs often without effects
  - **Much non-critical DNA where genes can insert**

Plants exhibit extensive conservation of gene content



Loblolly pine and *Arabidopsis thaliana* differ greatly in form, ecological niche, evolutionary history, and genome size. Yet most genes of substantial length have an *Arabidopsis* gene homolog. Kirst et. al. 2003.

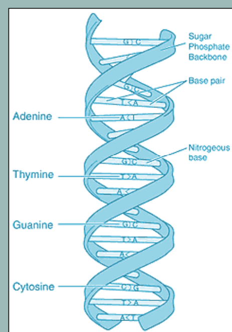
Gene concepts and basic methods

What is a gene?

- Segment of a chromosome that allow a cell to produce a specific function or molecule
- Inferred either of two ways
  - via genetics (inheritance of variants) or
  - physical analysis (DNA sequence information)
- DNA segment of defined function

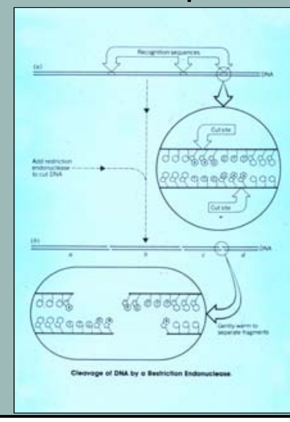
Basic DNA structure

- Exists as 2 complementary strands containing adenine (A), thymine (T), cytosine (C), or guanine (G)



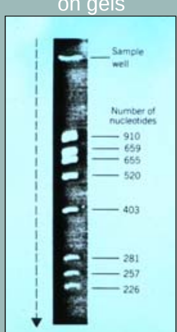
How are genes cut and spliced?

Restriction enzymes allow DNA to be cut at specific sequences

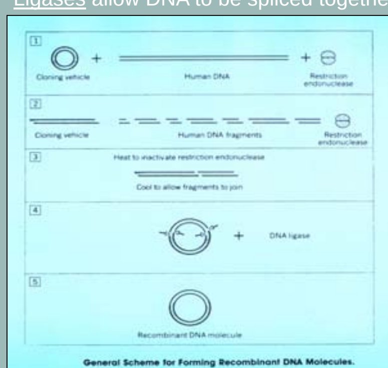


How are genes cut and spliced?

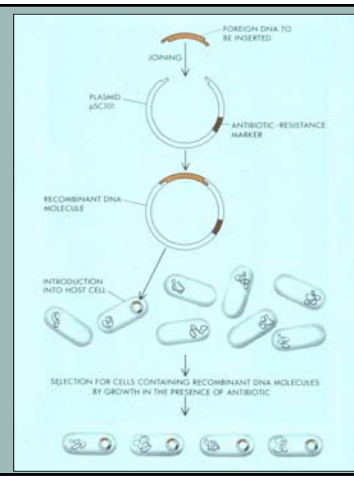
Cut fragments are separated on gels



Ligases allow DNA to be spliced together



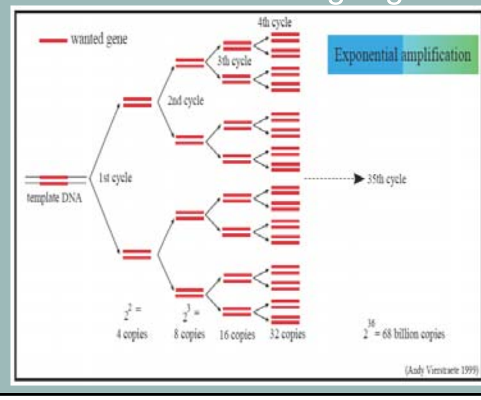
Gene cloning allows lots of DNA for specific genes to be made, required for study and manipulation



The polymerase chain reaction allows gene cloning without bacteria

- Extremely sensitive
  - Amplifies target DNA in repetitive cycles, thus called “chain reaction”
  - Capable of detecting DNA from a single cell
  - Basis of most DNA forensic procedures and other kinds of DNA fingerprinting and mapping
- Basis of most sensitive systems for detecting “contaminating” transgenic DNA
  - Can be highly quantitative
  - Alternative, lower cost but lower sensitivity = antibody, ELISA systems for detecting specific proteins – more widely used
  - Both methods get difficult with mixed and highly processed food

Polymerase chain reaction makes lots of DNA of a target gene



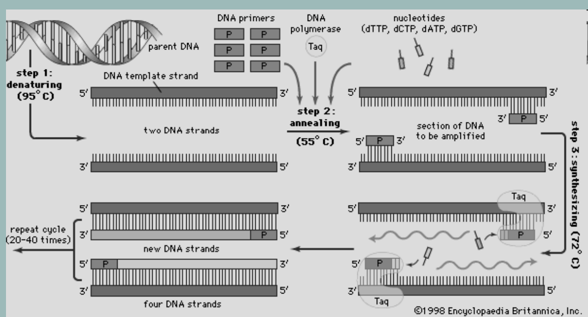
Primers give gene-specificity in PCR

- A primer is a single stranded DNA fragment that is synthesized by a machine
- They anneal to single stranded DNA, hybridizing as in the double-helix base pairing
- Fragments of 16-25 bp are usually specific to single genes in large genomes
- Need two different primers for each gene targeted for amplification

The PCR Process: Components of the reaction

- Template DNA = The sample to amplified from
- Primers = Short, specific segments of DNA, provide SPECIFICITY
- dATP, dTTP, dCTP, dGTP = Building blocks of DNA as its synthesized
- Thermostable DNA polymerase to build the new strand
- Buffer and salts (KCl, MgCl2)

Overview of PCR process



Promoters

- They determine under what environmental conditions, in what cells, and to what level a gene is expressed
- They can be excised from coding region and transferred to other genes
- Many function over a wide phylogenetic distances (e.g., all dicot plants)

Examples of promoter:gene combinations produced via recombinant DNA methods

Promoter (controls expression)	Gene (encodes protein)
^{35}S-CAMV (plant virus)	**Phenolic pathway enzyme** (bacteria)
Herbicide tolerant	
Pollen sac (tobacco)	**RNA degrading enzyme** (bacteria)
Male-sterile (hybrids)	
FMV (plant virus)	**Insect toxin protein** (Bt bacteria)
Insect resistant	
Tomato Fruit 5X	**Lycopene pathway enzyme** (tomato)
Improved nutrition	

How much transgenic DNA in a GM crop?

- Example of soy, diploid genome of 2.2 Giga-base pairs (2.2 x 10^9 bp)
- One transgene insert, ~3 genes = ~10,000 bp (10 kb)
- 10 kb / 2.2 Gb = 0.00000045 = 4.5 x 10^{-7} = .45 ppm = 0.000045%
- 1 ppm = 1 foot in 189 miles
  - 1 ppm = 1 foot in 1 million feet
  - Thus 1 million feet / 5,280 feet = 189 miles
- Thus ½ ppm = 1 foot in 378 miles

Summary of genomic-molecular genetic concepts

- Large number of genes highly conserved in function, DNA sequence
  - Most species share very large numbers of genes
- Easy to study presence of any specific gene, even at very low level, using PCR (polymerase chain reaction)
  - Unwanted gene flow easy to monitor
- Genes can be dissected into protein coding and expression controlling regions (promoters)

Discussion questions

- Why are genomes so complex and variable?
- How does a gene within a living plant differ from one in a test tube or a bacterium?