Genetic Manipulation
Part 2

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Genetic Manipulation
DNA Modification: Gene Insertion

- Remember **chromosomal crossover?**
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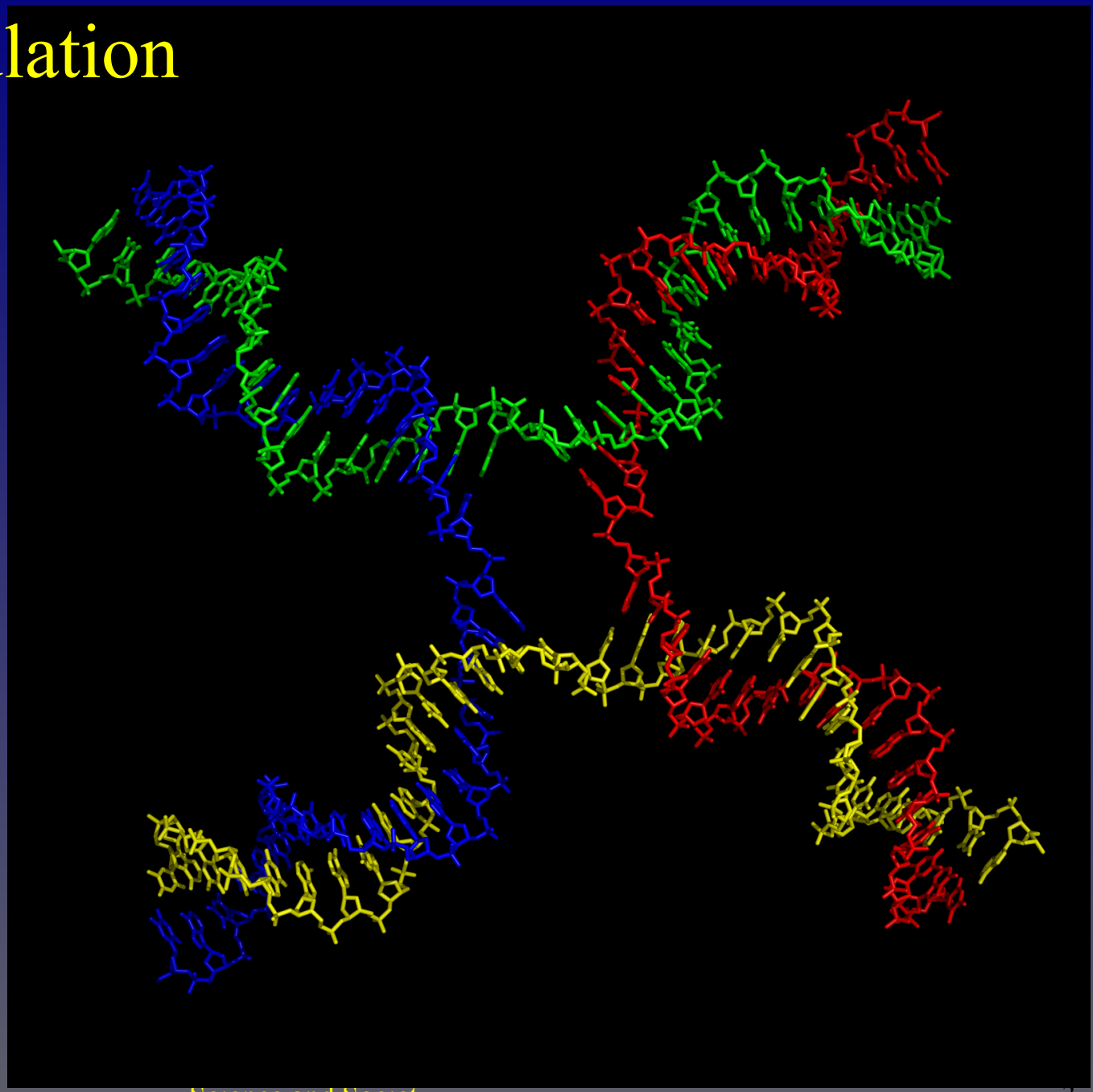
- In "gene targeting," homologous recombination is used to replace one gene with another.

Genetic Manipulation

DNA Modification And Construction of Synthetic Genes

Homologous recombination occurs at Holliday junctions

Recombination



Genetic Manipulation
DNA Modification: Gene Insertion

- Prepare a segment of DNA in bacteria or yeast with:
  the desired gene,
  a reporter gene, and
  a selectable marker gene

- Reporter gene – something that can be detected

- Selectable marker – something that allows selection of the organisms with the new gene: in bacteria, a drug resistance gene

Genetic Manipulation
DNA Modification: Gene Insertion

- Insert the new genes into embryonic stem cells, e.g. from mice along with the homologous recombination system
- The new gene replaces the existing one
- Select stem cells with the new gene
- Inject them into mouse embryos
- Select mice in which the modified cells formed the reproductive system, breed them

Genetic Manipulation
STEM Cells

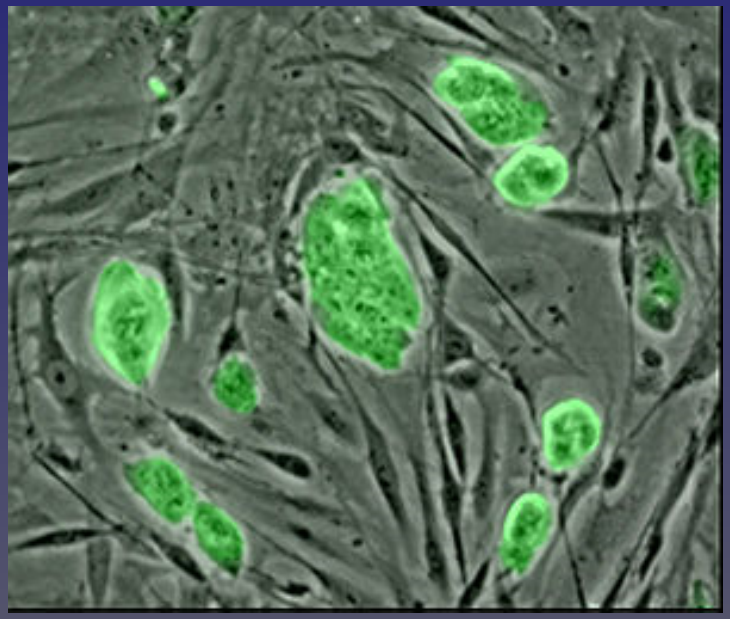
- A STEM cell (or stem cell) is one that has the ability to duplicate itself through mitotic cell division and can differentiate into a diverse range of specialized cell types.
- Bone marrow, skin, muscle (adult stem cells)
- Embryos (embryonic stem cells)

Genetic Manipulation
STEM Cells

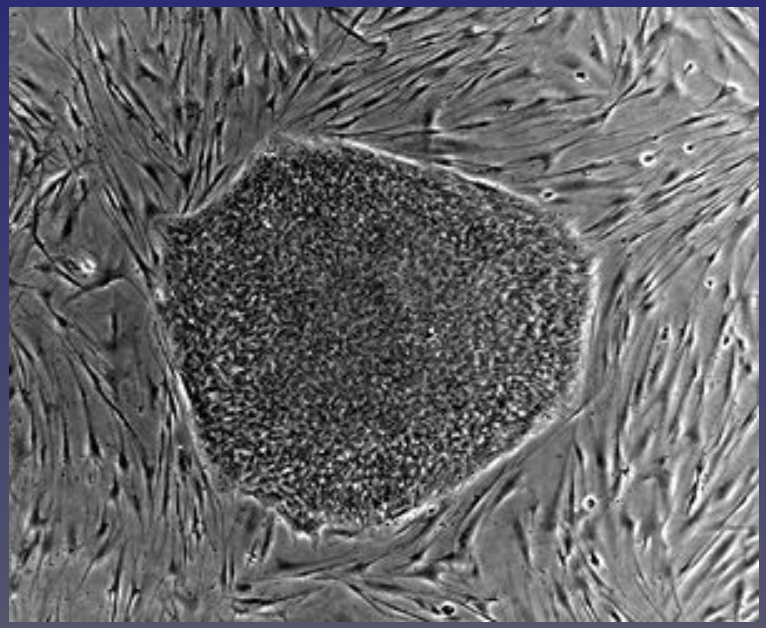
- Totipotent stem cells are produced from the fusion of an egg and sperm cell and by the first few divisions of the fertilized egg. These cells can differentiate into embryonic and extraembryonic (e.g. placenta) cell types.
- Pluripotent stem cells are the descendants of totipotent cells and can differentiate into any embryonic cells
- Multipotent stem cells can produce only cells of a closely related family of cells (e.g. marrow stem cells differentiate into red blood cells, white blood cells, platelets, etc.).
- Unipotent cells can produce only one cell type, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells).

Genetic Manipulation
Embryonic STEM Cells

- To remain undifferentiated, embryonic stem cells must be grown in the presence of very specific factors.
- Human embryonic stem cells are grown in a dish with mouse fibroblasts (embryonic connective tissue) and *fibroblast growth factor*



Mouse stem cells



Human stem cell mass on background of mouse fibroblasts

Genetic Manipulation
Adult STEM Cells

- Pluripotent stem cells do not exist in adults
- Mouse fibroblasts can be treated to become pluripotent.
- Therefore, research on adult stem cells is not equivalent to the use of embryonic stem cells.
- Certain treatments can reprogram an adult stem cell to become pluripotent, but current methods involve an oncogene (cancer-causing gene), and thus cannot be used in therapy. Note that cancer is infinitely self replicating like stem cells!

Genetic Manipulation
Reprogramming Adult STEM Cells

- Why are adult cells not pluripotenr?
- Certain genes have been “turned off.”
- Mechanisms:
  - Histone rearrangement due to DNA modification, e.g. addition of a methyl (CH_3) group; several other direct histone modifiers
  - Destruction of the gene's mRNA
- Genes or other molecular agents must be added to the adult cell to reverse the silencing mechanisms

Genetic Manipulation
Genetic Engineering: Cloning

- Reprogram an adult stem cell
- Replace the nucleus of a fertilized egg with the nucleus of the reprogrammed cell
- Place the fertilized egg in a host
- Wait

Genetic Manipulation
Genetic Engineering: Cloning

- Dolly, a Finn Dorset sheep was the first mammal cloned from an adult cell, was born and lived for six years. Her chromosomes displayed some abnormalities
- Hundreds of embryos failed before her success
- We have no way of guaranteeing that reprogramming is 100% complete
- Animals that have been cloned:
  Carp, Cats, Cattle, Deer, Dog, Ferret, Fruit Flies, Goat, Gaur, Horse, Mice, Mouflon, Mule, Pig, Rabbit, Rat, Rhesus Monkey, Sheep, Water Buffalo, Wolf

Genetic Manipulation

Genetic Engineering: Cloning

James Symington, a former police officer who lives in West Los Angeles, is surrounded by the five clones of his deceased dog, Trakr. Trakr found the last surviving victim of the 9/11 attack. The clones were a gift from BioArts International

Genetic Manipulation

Genetic Engineering: Eugenics

- Eugenics: the improvement of human hereditary traits through various forms of intervention.
- Eugenics was supported by Theodore Roosevelt, Woodrow Wilson, Margaret Sanger, George Bernard Shaw, Winston Churchill and other upstanding citizens.
- Eugenics gained a bad reputation from Nazi programs to achieve a "racially pure" population, including forced sterilization and euthanasia
- Forced sterilization of mentally ill and mentally handicapped and prohibitions on marriage of the "feeble minded" was practiced in the US

Genetic Manipulation
Genetic Engineering: Eugenics

- Modern eugenic practice includes:
  - Use of a different term
  - Screening of couples for genetic-based diseases such as Tay-Sachs
  - Privacy of personal genetic information
  - Genetic Information Nondiscrimination Act (2008)

- As new methods to alter genetics become safe and effective, other techniques are likely to be generally available

- A single cell can be removed from an eight-cell embryo without disturbing its development. The removed cell could be analyzed for any genetic disease or for other traits.

Genetic Manipulation
Epigenetics

- While nuclear DNA is the overwhelming source of inherited characteristics, other factors exist
  - Mitochondrial DNA (only from mother)
  - Cell differentiation (possible errors)
  - Difference in inherited chromosomes from mother and father when only one is expressed (genetic imprinting)
  - Other environmental factors that affect gene expression

Genetic Manipulation
Diagnostics and Research

- Determination of a person's genome can reveal disposition to breast cancer, disorders of hemostasis, cystic fibrosis, liver diseases and many others
- The gene for HLA-B27 disposes one to "seronegative spondyloarthropathies"
  (you're more likely to have psoriatic arthritis)
- If the gene could be silenced, the disease might be alleviated
- What genes are active in cancers?

Genetic Manipulation
Human Genome Project

- The *Human Genome Project* was begun in 1990 with the goal of determining the entire human DNA sequence
- While the original *NIH* project was underway, a parallel effort was started by private company, *Celera Genomics*

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- The results were released by both groups in 2003 with loose ends finished in 2006

- About 8% ignored due to probable irrelevance (extreme repetition)

- 3 Billion nucleotides

HG View

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ORIGIN
  1 ccagccttca gccggagaac cgtttactcg ctgctgtgcc catctatcag caggctccgg
 61 gctgaagatt gcttctcttc tctcctccaa ggtctagtga cggagcccgc gcgcggcgcc
121 accatgcggc agaaggcggt atcgcttttc ttgtgctacc tgctgctctt cacttgcagt
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Genetic Manipulation
Summary

- The last sixty years have taken us from almost complete ignorance of the molecular basis of genetics to a deep understanding and methods to manipulate it.
- Much remains to be learned and done
- Like any new technology, it has the potential for good and bad uses
- The intricacy of the mechanisms are beautiful and many are universal. They show our connection to all living things.
- Over 98% of our DNA is shared with chimpanzees. Many genes are identical.
- Humans have 23 chromosomes. Chimps have 24. Why?