Learning Unit #1
The Age of DNA - What is Genetic Engineering

Themes
1. Age of DNA, Genetic Engineering, Genomics, & Mammalian Cloning
2. What do genes look like - DNA demonstration
3. DNA into the Home - DNA Testing - Age of DNA
4. Genetic Engineering into the Home - Cloning - Age of DNA
5. Genetic Engineering into the Home - Cloning - The House - What do these Experiments tell us about Unity in Gene Processes?
6. How does the Scientific process work - No opinions!
7. Other Examples of Genetic Engineering - the Plant, Insect, Cloned Monkey, Super Horse, Drosophila Mouse, Six-Finger Mouse, SCID Bone Marrow Therapy
8. Asilomar Conference - History & Mating - DNA
9. What is Genetic Engineering & What Affects Society?
10. Genetic Engineering - Anything New?
11. Plant Crop Engineering/Valuable Demonstration
12. Genetic Mutations, DNA, Alleles
13. Genetics of Mankind/Eugenics
14. Era of Genetics
15. Era of Mammalian Cloning/Stem Cells/Reproductive
We Live in the Era of DNA, Genetic Engineering, Whole Genome Sequencing & Genomics, Mammalian Reproduction & Cloning, and the SYNTHESIS of These Technologies!!!
DNA DEMONSTRATION

1. What does DNA look like?
2. How can you conduct an "experiment" to "touch" DNA?
3. Hypothesis testing - Which flask has the DNA? How test prediction?
April 4th...

DNA PERFUME

by Bijan

DNA...
it's not just a perfume... it's gene therapy.

WE LIVE IN THE AGE OF DNA
The Age of the Gene
DNA Comes Into Your Home!

We have begun to control our biological destiny!

WHAT DOES DNA LOOK & FEEL LIKE?
An Age of DNA that comes into the home....

Do it yourself DNA testing to find family history!!

YOU will have a DNA test this quarter!
Now the Genetic Testing Really Begins

It Starts With a Single Drop of Blood Taken From Each Newborn

And Ends When Scientists Predict Everyone's Physical and Mental Future

Human red blood cells. Magnification: 19,600x
A Hypothetical Genetic Case Study

FAMILY ONE
Dominant Genetic Disease X

FAMILY TWO
Dominant Genetic Disease X

Offspring Possibilities

How Can DNA Testing be Used to Determine Whether the Parents and Children Carry the “Defective” Gene?
Do you want to know your future?

Every state in the country requires that infants be tested for a list of obscure diseases. Before long, some states could move on to DNA testing of all newborns. Now is the time to decide a critical question: How much do we want to know and when do we want to know it?

By Jeff Wheelwright
Photography by Catherine Ledner
DNA TESTING FAMILIES FOR CARRIERS OF "DEFEKTIVE" GENES!

Figure 22.7 Detection of the sickle-cell hemoglobin mutation by Southern blot analysis of genomic DNAs cut with restriction enzyme MstII.
DNA Confirms Infected Cow’s Origin

Next in the inquiry into a Washington state case of mad cow disease is a focus on feed.

BY JOHANNA NEUMAN
Times Staff Writer

WASHINGTON — DNA tests have confirmed that the Holstein found last month to be infected with mad cow disease originated in Alberta, Canada, U.S. Department of Agriculture officials said Tuesday.

The DNA testing on the cow and her offspring, as well as earlier-reported records showing that the cow had been sold by an Alberta farmer disposing of his dairy herd, "makes us confident in the accuracy of this trace-back," said W. Ron DeHaven, the department’s chief veterinarian.

The confirmation, based on DNA tests at two laboratories — one in the United States and one in Canada — leaves unanswered the question of how the cow from a farm in Washington state became infected. Officials will now concentrate on the feed used by the cow’s original owner in Alberta.

Dr. Brian Evans, chief veterinary officer for the Canadian Food Inspection Agency, said on a USDA conference call Tuesday that investigators would also try to determine whether the feed source for the Holstein was the same as that for an Alberta cow diagnosed with mad cow disease in May.

Scientists believe that bovine spongiform encephalopathy, or BSE, the brain-rotting illness commonly known as mad cow disease, can be transmitted to cattle that eat feed containing the remains of infected cows. In the past, leftovers of slaughtered animals — including the brain and the spinal cord, which are believed to harbor the source of the infection — were ground up and used in animal feed.

In 1997, the U.S. and Canada banned the use of the remains of ruminants, or cud-chewing animals, in feed used for cattle, but both North American cows diagnosed with BSE — the one discovered in Canada in May and the one found in the United States in December — were born several months before that ban went into effect. The human form of the illness, variant Creutzfeldt-Jakob disease, has been associated with consumption of food made from BSE-infected animals.

Agriculture Secretary Ann M. Veneman announced Dec. 23 that a cow slaughtered Dec. 9 had tested positive for BSE. The cow was tagged for testing because it was a "downer" cow that was unable to walk to slaughter. The cow’s meat products had already been distributed before Veneman’s announcement, primarily to retail outlets in Washington and Oregon.

While officials recalled the meat, it is not known how much was recovered.

Veneman has since announced a series of reforms to bolster U.S. defenses against BSE, including a ban on accepting downer cows for slaughter and a rule that would hold all meat products from an animal tested for disease until results are completed.

But after Tuesday’s announcement of the DNA results confirming the cow’s origin, some producers said the Agriculture Department had moved too slowly to determine the source of the infection.

"They knew the leads pointed back to Canada, and if they had made the announcement immediately, it might have mitigated a great deal of our loss," said John Locke, executive director of R-CALF USA, a national association of cattle producers.
THE AGE OF GENETIC ENGINEERING COMES INTO THE HOME.

Genetically Engineered Zebra Fish.

State Takes Dim View of GloFish, Bans Sale

By KENNETH R. WEISS Times Staff Writer

RED ZEBRA: GloFish are implanted with a gene from sea anemones.

State Game Panel Bans Sale of GloFish

Glowing review: watchdogs want tighter rules for transgenic pets.

Pet Glo Fish!!
Using a Jellyfish Gene to Make Animals and Plants Glow!!!

Green Fluorescence Protein
Making a "GloFish"

......Using Genetic Engineering &
A Jellyfish Gene!!!
A "GloFish" Embryollll!
A "GloFish!!!!"
What do these experiments tell us about what our gene can do? About unity and genetic processes?

How about a "GloMouse!!!"
How About "GloMice!!!"
Note - The same gene is active in a fish, fly, mouse, a plant - what does this imply about our genes & genetic processes?
What Do These GloGenes Experiments "Say" About Unity of Genetic Processes?

What is the Hypothesis?

What are the Predictions?

What Experiment(s) to Test Predictions?
A Peer Reviewed?
Have the Data Been Verified?
What are the Experimental Data?
How Test Hypothesis?
What are the Predictions?
What is Your Hypothesis?
What are the Observations?
Scientific Process
Other Examples of Genetic Engineering - Bacterial Gene into a Plant

What About Inserting Bacterial Genes Into Higher Organisms To Produce a Result With Significant Applications??
How to Make an Insect-Resistant Plant

1. Isolate bacterial gene that produces protein toxic against certain insects

2. Insert Bt gene and a "marker" gene into cells

3. Identify cells with Bt and "marker" genes

4. Allow cells to grow into plants. Plants now produce toxins against insect pests

Bacterial Gene → Plant
Insect Resistance with Bt Control

Genetic Engineering a Plant to Resist Worms!!!
What do Farmers Say About This Technology?

Max Smith
Farmer
What Else Can Be Done With Genetic Engineering?
How About a "GloMonkey!!!"

Using red fluorescence protein
WHAT ABOUT A GLO MONKEY—ANDZ?

Jelly fish gene → monkey!

MONKEY BUSINESS

A tiny primate with a gene from a jellyfish raises scientists’ hopes—and some serious ethical questions.

How can this technology help human beings?

Are there ethical issues in genetically engineering monkeys? Humm? Has it been done?
MORE EXAMPLES OF THE POWER OF GENETIC ENGINEERING
DNA → SPECIFIC TRAIT

1. Super Mouse
2. XX μ → O
3. Obese Mouse
4. SCID - Severe Combined Immuno deficiency
   → Human Gene Therapy
Human Growth Hormone Gene Can Be Engineered into A Mouse

1. Eggs X Sperm → Pronuclei
   - Mouse egg just after fertilization, before fusion of pronuclei
   - Plasmid injected into pronucleus
     - Plasmid carrying the gene for human growth hormone
     - Micropipette

2. Egg implanted into "foster mother mouse"
   - "Foster mother mouse" gives birth
     - Extract DNA from tissue biopsy
       - DNA fragment encoding mouse growth hormone
       - DNA fragment encoding human growth hormone

3. Analyze by PCR or hybridize with probe for plasmid by Southern blotting

To produce?
GIGANTIC MICE FROM EGGS INJECTED WITH GROWTH HORMONE GENES
Animals can be genetically engineered with new genes that specify new traits.

Human Gene → Mouse!

**INJECTION OF FERTILIZED EGG**

![Diagram of egg injection](image)

**Transgene DNA**

- Pure DNA representing a specific gene for growth hormone

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**Figure 15-31** Transgenic mouse. The mice are siblings, but the mouse on the left was derived from an egg transformed by injection with a new gene composed of the mouse metallothionein promoter fused to the rat growth hormone structural gene. (This mouse weighs 44 g, and its untreated sibling 29 g.) The new gene is passed on to progeny, in a Mendelian manner, and so is proven to be chromosomally integrated. (R. L. Brinster)

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**We are entering the Era of "Designer" Organisms!**

**ALGO** → **GloFish, ANDi!**

**SAME TECHNOLOGY!!!**
Males v Females differ by only the presence or absence of the Y chromosome (simplistically!)

The normal diploid chromosome number of a human being is 46, 22 pairs of autosomes and two sex chromosomes. The autosomes are grouped by size (A, B, C, etc.), and then the probable homologues are paired. A normal woman has two X chromosomes and a normal man, shown here, an X and a Y.

What genes are on the Y chromosome?

How do you "naturally" obtain a XY ?

XX or ?

The Human Gene For Maleness

CAN ...
The human Sry gene can make a female (XX) mouse into a male.

**FIG. 3** Analysis of adult sex-reversed transgenic mouse m33.13. a, PCR analysis of genomic DNA from m33.13 (lane 3), showing Sry and control (myogenin) bands. No band corresponding to Zfy-1 was seen, demonstrating the lack of a Y chromosome; this result was confirmed by Southern blotting using Y-chromosome probes Y3535 (ref. 40) and SaI (ref. 41) (not shown). Normal XX female and XY male littermates (33.9, lane 2 and 33.17, lane 1) are shown for comparison. **M**, marker bands (1.018, 5.10, 396, 344, 298, 220, 201, 154 and 134 base pairs). b, External genitalia of mice 33.17 (left) and 33.13 (right). c, Histology of testis sections from mice 33.17 (left) and 33.13 (right). Bar, 90 μm.

METHODS. For PCR analysis, 0.1 μg genomic DNA was added to a 50-μl reaction mixture containing 1.5 mM each dNTP, 50 mM Tris–HCl pH 9.15 mM ammonium sulphate, 7 mM MgCl₂, 0.05% Nonidet P-40, 0.5 U Taq polymerase (Amersham), and 500 ng each oligonucleotide primer. Amplification consisted of 30 cycles of 94 °C for 5 s, 65 °C for 30 s and 72 °C for 30 s in a Techne PCR-2 thermocycler. An 8-μl aliquot was electrophoresed on a 2% agarose–TBE gel. Primers for Sry were (5'-3') TCTAGAGTGGCCAAACACGAG and CATGACACACCCACCAACA (indicated as triangles in Fig. 1) and for Zfy-1, CCTATTGGATGACTGCAATTTATG and GACTAGACATGTCTTACATGTCGAC. Myogenin primers corresponded to nucleotides 656–675 and 882–901 of the rat complementary DNA sequence. PCR products were 441, 160 and 245 bp, respectively. Tissues were processed for histological examination as described in Fig. 2 legend.

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The "ground state" of human development is a female! Need one gene to switch development into a male.

1. Eve had to have lost a Y chromosome from Adam's rib! or Eve gave rise to men!
Goats can be turned into "factories" to produce medically-important human proteins.

**Human Gene → Goats!**

**Diagram:**
- Goat gene for milk production
- 3' Human tPA gene
- 3' Recombinant tPA gene
- Inject recombinant DNA into a fertilized egg nucleus
- Implant embryo into uterus of a goat
- Some of the offspring will carry the tPA gene
- Milk is collected from lactating transgenic goat
- tPA is extracted from milk

**Annotations:**
- tPA = tissue plasminogen activator
  - dissolved blood clots & prevents heart attacks!
- Natural?
- Any different than breeding cattle? Cows? For maximum food production?
CORRECTING GENETIC DEFECTS IN HUMANS USING GENETIC ENGINEERING

**Human Gene → Humans!**

1. Isolated somatic cells from the patient are homozygous for the defective allele.
2. A copy of the normal allele is inserted into viral DNA.
3. Isolated somatic cells are infected with the virus containing the recombinant DNA.
4. The viral DNA carrying the normal allele inserts into the patient's somatic cell chromosome.
5. Somatic cells containing the normal allele are cultured.
6. Cultured cells are injected into the patient.
7. Symptoms are relieved by expression of the normal allele.

**Viral DNA**

**Normal allele**

**Recombinant DNA**

**Somatic cell**

**Virus**

**Well patient**

**CORRECTING SCID - Severe Combined Immunodeficiency**

Using the ADA gene

Adenosine Deaminase Gene → Nucleic Acid Metabolism

**Human Gene Therapy is a 20-Year-Old Technology**
Figure 2.10 The Flow of Genetic Information in Cells

DNA is copied into RNA during the process of transcription. RNA directs the synthesis of proteins during translation. Through proteins, genes control the metabolic and physical properties or traits of an organism.

1. Genes can "work" independently of other genes - unique units!
2. Basic Genetic Processes Universal
   a. DNA Replication
   b. DNA → RNA → Protein
3. Basic Genetic Processes can be used to engineer/transfer genes from one organism to another - stably
The process of gene to trait is the "same" in all organisms!

**Universal Process!**

**Translating the Genetic Code Into Proteins is a Conserved Process**

**Replication**

**Transcription (RNA synthesis)**

**Translation (protein synthesis)**

**Ribosome**

**Protein**

What is the "Big" Implication of this for Biology?

Can intervene in this process in living cells?

All organisms use the same processes and "rules" to generate traits!! And the same molecules/chemistry is involved!
What is Genetic Engineering & What does it do?
DNA Cloning or Genetic Engineering
Uses Natural Processes of Living Cells to Isolate Single Genes

- Introduce DNA into host cell
- Isolate cells with cloned gene
- Produce protein from cloned gene

Gene A now replicated in host cell
- Bacterial cell produces human protein - recognizing human gene as its own.

Implications.

Gene A now in host "chromosome"

Ingenously engineered bacterial chromosome to have a human gene.

Produce lots of genes or protein.

Genetically engineered bacterial chromosome to have a human gene.
"Why" Clone Genes From the Genome of an Organism?

1. **Purify** Individual Gene from the Genome —— Separate from rest of Genes

2. **Amplify** the Gene to obtain enough DNA to Study and/or Engineer

3. **Use** the cloned Gene to:
   a. **Study** Gene Structure & Function!! **THE Major use**
   b. Use to make Pharmaceuticals
   c. Use in animal & plant Gene Therapy
   d. Use to Diagnose diseases
   e. Use to Correct diseases
   f. Use to Identify individuals
   g. Use to Convert cells into others

Gene Engineering has lead to new knowledge about how living cells function and for applications that improve all of our lives!
The average atomic mass of one base pair is 635 daltons (a dalton is 1/12 the mass of a carbon atom).

The β globin gene is approximately 2000 bp in length.
So, the atomic mass of the β globin gene is:

2000 bp ×
635 daltons/bp
= 1.27 × 10^6 daltons

Mass of β globin gene in an adult human

There are two copies of the β globin gene per cell.
There are 10^{13} cells per individual.
So, the total atomic mass of β globin DNA per individual is:

1.27 × 10^6 daltons/gene ×
2 genes/cell ×
10^{13} cells/individual
= 2.54 × 10^{19} daltons

If there are 6.02 × 10^{23} daltons per gram, then:

\[
\frac{2.54 \times 10^{19} \text{ daltons}}{6.02 \times 10^{23} \text{ daltons/gram}} = \frac{0.000042 \text{ grams}}{0.0042 \text{ g}} = 0.0000097 \text{ grams} = 0.00002 \text{ mg}
\]

\[42 \mu g \beta \text{ globin DNA per human}\]

Mass of β Globin DNA in Adult Human vs. 1-liter Culture of E. coli Carrying β Globin Gene on Plasmid

The average atomic mass of one base pair is 635 daltons (a dalton is 1/12 the mass of a carbon atom).

The β globin gene is approximately 2000 bp in length.
So, the atomic mass of the β globin gene is:

2000 bp ×
635 daltons/bp
= 1.27 × 10^6 daltons

Mass of β globin gene in a liter of E. coli

There are 500 copies of the β globin gene per cell.
There are 5 × 10^{11} cells per liter.
So, the total atomic mass of β globin DNA per liter is:

1.27 × 10^6 daltons/gene ×
500 genes/cell ×
5 × 10^{11} cells/liter
= 3.175 × 10^{20} daltons

If there are 6.02 × 10^{23} daltons per gram, then:

\[
\frac{3.175 \times 10^{20} \text{ daltons}}{6.02 \times 10^{23} \text{ daltons/gram}} = \frac{0.0000527 \text{ grams}}{0.00527 \text{ mg}} = 0.000001 \text{ grams} = 0.00002 \text{ mg}
\]

\[527 \mu g \beta \text{ globin DNA per liter!}\]

Can produce 10^6 more β globin gene in a 1-liter bacterial culture than in the entire human body using gene engineering methods!
The cow chymosin gene is cloned and amplified in bacteria, leading to an infinite amount of chymosin to make cheese!
IN ITS SIMPLEST FORM

GENETIC ENGINEERING MEANS...........

1. Isolating a gene from a chromosome of an organism and

2. Cloning (Replicating identical copies) the gene in bacterial cells (cloning one/gene in cells - not cell/organism cloning

3. To: (1) Study ALL that gene
   (2) Ultimately find out what it does

Using bacteria as factories to produce large amounts of ONE gene for study

But the use, benefits, and implications are much, much bigger!
The Era of DNA Manipulation
Means.............

1. DNA/Genes can be cloned/isolated from any organism.

2. DNA segments of any kind and from any organisms can be combined.

3. Engineered gene/DNA molecules can be re-inserted into the cells of any organism to make to work—whole genomes or "organisms" can be synthesized!

4. There are no genetic limits—All of Biology uses the same rules!

We have known how to manipulate genes for 35 years!

The Implications are enormous!
THE AGE OF DNA AND GENE CLONING HAS AFFECTED SOCIETY IN MANY WAYS!

AND WE HAVE JUST BEGUN!

1. Basic understanding of living processes! What is life? What is the basis of biological diversity?
2. Basic understanding of genes
4. Agriculture: higher yielding crops
5. Business/Commerce: biotech industry
6. The law/Forensics
   - Patents
   - Identification
   - Privacy issues
7. Anthropology
   - Human origins/diversity = unity of humanity
8. Evolution
   - Where did we come from?
9. Philosophy/Religion: how do we view ourselves in relation to God/nature (e.g., synthetic genomes)
Novel Applications of Genetic Engineering/Recombinant DNA Technology

Fig. 14.1 The different ways that recombinant DNA technology has been exploited.

Fig. 1.1 The impact of gene manipulation on the practice of medicine.
Genetic Engineering Technology has led to many legal and ethical issues.

1. Patenting living organisms, cells, & genes?
2. Regulating "Experimentation" - recombinant?
   DNA, stem cells, transgenic plants and animals
3. Regulating release of genetically modified organisms into environment - crops, farm animals, mosquitoes
4. Genetic Testing - genetic data bases, voluntary, involuntary, newborn screening, criminals, suspects
5. Genetic Discrimination - insurance, workplace, society
6. Eugenics - Genetic Enhancement
7. Reproductive Rights - genetic enhancement "child" wrongful birth suits
8. Gene Therapy - correcting genetic disorders
9. Gene Testing Companies - liabilities
10. Human Cloning - Reproductive Rights - Regulate?
11. Synthetic Genomes - what is life?
Issues that need to be resolved by informed public choices

Genetic Testing

Gene Therapy

Genetic Privacy

Genetic Discrimination

Genetically Engineered Food

And need to be guided by sound science!!
1. If you could choose traits for your baby, would you choose to.
   a. Rule out a genetic disease 74%
   b. Ensure greater intelligence 8%
   c. Influence height or weight or hair/eye color 2%
   d. Select gender (male or female) 5%
   Choose All: 10%

2. Should parents with a genetic disease be required to test their children to
determine whether they are carriers or have the disease?
   a. Yes 50%
   b. No 50%

3. If you carried a gene for an incurable fatal disease, would you have your unborn
child tested for the disease?
   a. Yes 90%
   b. No 10%

4. If the test showed that the baby would have the fatal disease, would you consider
ending the pregnancy through abortion?
   a. Yes 69%
   b. No 31%

5. Should a child that is born with a genetic disease be allowed to sue their parents
for failing to test for the disease that is causing them so much "misery;" i.e., sue
for wrongful life liability?
   a. Yes 15%
   b. No 85%

6. Should the government regulate gene therapy – that is, altering genes to cure or
prevent diseases – by passing specific legislation at the state and/or federal levels?
   a. Yes 70%
   b. No 30%

7. Should the state and/or federal government regulate funding for stem cell
research?
   a. Yes 59%
   b. No 41%

8. Should the state and/or federal government ban the cloning of human beings?
   a. Yes 50%
   b. No 50%

9. Should cloning of human embryos be permitted to obtain patient-specific stem
cells to cure diseases such as diabetes, Parkinson's, and muscular dystrophy?
   a. Yes 82%
   b. No 18%
10. Should insurance companies have access to your genetic records or DNA fingerprints without your permission?
   a. Yes 5%
   b. No 95%

11. Should every individual who is arrested for a crime be required to have their DNA fingerprinted and deposited in a National Criminal DNA database?
   a. Yes 64%
   b. No 36%

12. Should the government regulate the engineering of animals and plants for use in agriculture and/or medicine?
   a. Yes 78%
   b. No 22%

13. Should employers be able to obtain access to employees’ genetic records and/or DNA without permission?
   a. Yes 2%
   b. No 98%

14. Should employers be allowed to require their employees to undergo DNA testing?
   a. Yes 30%
   b. No 70%

15. Should the police be allowed to collect DNA information gathered from suspected criminals as they currently do with fingerprints?
   a. Yes 66%
   b. No 35%

16. Is it a good or bad idea for the FBI to create a DNA database with information gathered from suspected criminals and crime scenes throughout the country?
   a. Good Idea 78%
   b. Bad Idea 22%

17. If parents choose to give birth to a child with a genetic disease, should the parents or society pay for the health care for their child?
   a. Parents 73%
   b. Society 27%

18. Over the past 30 years how many genetic engineering "disasters" have occurred?
   a. 100 24%
   b. 0 41%
   c. 10 34%

19. If you could undergo gene therapy and change any of your genetic traits (e.g., eye color, hair color, skin color, presence/absence of body hair, etc.) without any adverse affects, would you do it?
   a. Yes 46%
   b. No 54%

20. If food were labeled as genetically engineered, would you buy it for yourself or your family?
   a. Yes 75%
   b. No 25%
21. Are the building blocks of genes made of DNA, RNA, or proteins?
   a. DNA 55%
   b. RNA 0%
   c. Proteins 45%

22. How many years ago was DNA discovered?
   a. 50 68%
   b. 100 28%
   c. 10 4%

23. Should genetically enhanced food be labeled?
   a. Yes 90%
   b. No 10%

24. Is organically grown food more nutritious than food grown using conventional agriculture?
   a. Yes 31%
   b. No 69%

25. How many genes have you eaten within the last 24 hours?
   a. 0 8%
   b. 1,000 8%
   c. 10,000,000 or more 84%

26. Have you tasted any foods that were produced by genetic engineering within the last week?
   a. Yes 84%
   b. No 16%

27. What year are you in school?
   a. First 34%
   b. Second 24%
   c. Third 27%
   d. Fourth 15%
   e. Fifth 0%

28. Are you a science or a non-science major?
   a. Science 34%
   b. Non-Science 66%

29. Have you ever had an exciting, dynamic science class that made you think that "science is neat, fascinating, and important for society?"
   a. Yes 79%
   b. No 21%

30. How many hours do you watch of television a week?
   a. 0 hrs. 17%
   b. 1-3 hrs. 58%
   c. 4-7 hrs. 23%
   d. 7-10 hrs. 2%
   e. 10+ hrs.

31. How many hours do you spend listening to, watching, or reading the news a week?
   a. 0 hrs. 10%
   b. 1-5 hrs. 70%
   c. 5-15 hrs. 20%
Issues Raised by Genetic Engineering Technology - like all new technologies society & people are affected.

Science-philosophy arguments concerning genetic engineering

<table>
<thead>
<tr>
<th>Categorical Argument</th>
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<tbody>
<tr>
<td>Some human activities such as genetic engineering are fundamentally reprehensible. Developing this technology, &quot;man plays God&quot; and claims competencies beyond his capacities, degrading nature to the course of his technical manipulations.</td>
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<tr>
<th>Pragmatic Argument</th>
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<td>The key objective of genetic engineering is to reduce the suffering of diseased individuals. The procedures which are applied must, however, be safe, and the patient must be able to decide if he or she wishes to apply genetic diagnosis or therapy.</td>
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<tr>
<th>Social Policy Argument</th>
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<td>The social effects of genetic engineering cannot be estimated. In genetic therapy, wrong priorities are chosen, better prophylaxis would be more desirable. We start down a slippery slope that will lead us involuntarily to inhumane practices towards the next generations (&quot;eugenics bottom up&quot;)</td>
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Problematic areas of genetic research

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<tr>
<th>Topic</th>
<th>State of the art</th>
<th>Regulation or trend</th>
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<tbody>
<tr>
<td>Cloning of humans</td>
<td>Cloning of animals possible</td>
<td>not permitted</td>
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<tr>
<td>Use of embryonic stem cells</td>
<td>Growing expertise</td>
<td>permitted, but regulated</td>
</tr>
<tr>
<td>Artificial insemination, sexing, surrogate mothers</td>
<td>State of the art in animals</td>
<td>Artificial insemination permitted, sexing and surrogate mothers forbidden</td>
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<tr>
<td>Prenatal diagnosis</td>
<td>Cytological methods established, DNA-based diagnosis partially established</td>
<td>Permitted, abortion permitted after medical indication</td>
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<td>Identifying genetic risks by genetic screening</td>
<td>Possible for some monogenic diseases</td>
<td>Under debate if one gene defect is predictive and if diagnosis is acceptable for incurable diseases; strict data protection required towards employers, insurance companies</td>
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<tr>
<td>Knockout animals for drug research</td>
<td>Widely established</td>
<td>Generally accepted, but hotly debated by animal protection groups</td>
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<tr>
<td>Food and biopharmaceutical production using transgenic animals or plants</td>
<td>Many techniques established</td>
<td>Debated in view of consumer protection, animal protection, ecological consequences</td>
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<tr>
<td>Transgenic microorganisms or cell lines for production of biopharmaceuticals</td>
<td>Established</td>
<td>Widely accepted</td>
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Public acceptance of genetic engineering (survey 2001)

<table>
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<th>Agricultural plants</th>
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<td>Good</td>
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</table>

<table>
<thead>
<tr>
<th>Domestic animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
</tr>
<tr>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biopharmaceuticals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
</tr>
<tr>
<td>41</td>
</tr>
</tbody>
</table>

Why it is important to understand the science behind genetic engineering!!

That's what this class is about!
Breeding and cultivation of plants have taken place over thousand of years.

Genetic engineering is not new.

Crops of Egypt 400 B.C.
PLANT BREEDING/CLASSICAL GENETIC ENGINEERING DEMONSTRATION

1. What is the origin of genetic engineering?

2. Who were the first individuals to manipulate genes & organisms?

3. What plants & animals were engineered & how?
Breeders Have Selected For Variability in Plant Control Genes To Generate Novel Crops.

How Are These Plants Related?

Breeding For Parts of Plants!

What is Being Manipulated?
What did the early "Genetic Engineers" select for to generate these vegetables?

Genes? How do we know genes exist?
Gene variants? Mutations?
Are genes controlling these traits?
Do we now know what they are?
Figure 2-4 The seven character differences studied by Mendel. [After S. Singer and H. Hilgard, The Biology of Plants. Copyright 1978 by W. H. Freeman and Company.]

- Round or wrinkled ripe seeds
- Yellow or green seed interiors
- Purple or white petals
- Inflated or pinched ripe pods
- Green or yellow unripe pods
- Axial or terminal flowers
- Long or short stems

Figure 2-5 Mendel's cross of purple-flowered ♀ × white-flowered ♂ yielded all purple-flowered progeny.
CATTLE BREEDING IN EGYPT 4000 YEARS AGO!

Manipulating Existing Genetic Variability

Variability Brought About by Chance Mutation!
Biologists chase down pooches' genetic and social past

A Shaggy Dog History

Dog father. Dogs might have evolved from an ancestor of this Chinese wolf.

15,000 years ago in Europe Asia

Genetic Variability

Can only arise by selecting for existing variability

What are the genetic differences? How did they arise?

Science

Common pedigree. From Chihuahuas (left) to Great Danes, dogs of all shapes and sizes have common ancestors.
The dog has its day

Hans Ellegren

Domestication and selective breeding have transformed wolves into the diversity of dogs we see today. The sequence of the genome of one breed adds to our understanding of mammalian biology and genome evolution.
MAJOR CROPS WERE ENGINEERED FROM NON-PRODUCTIVE WILD RELATIVES 15,000 YEARS AGO!

Regions Where Major Crops Were Established

Breeding involves gene manipulation!

Using existing gene variability!
Corn And Its Ancestor Teosinte

Note Differences in Plant Architecture
Yet They Are The Same Species

ONLY 5 GENES CAUSE THESE PLANTS TO BE DIFFERENT!
Now know what they are!
Domestication of Wheat

Diploid
14 Chromosomes

Einkorn wheat
(AA)

Goat grasses
(BB)

(Tetraploids
28 chromosomes

Emmer, macaroni,
wheat, etc.
(AAAB)

Note Difference
in Grain Number

Hexaploids
42 chromosomes

Bread wheats
(AAABBDD)
Domestication of Wheat

Diploid
14 Chromosomes

Einkhorn wheat
(AA)

(BB)

Goat
grasses

(TDD)

Tetraploids
28 chromosomes

Emmer, macaroni,
wheat, etc.
(AABB)

Note Difference
in Grain Number

Hexaploids
42 chromosomes

Bread wheats
(AABBD)

(AABBDD)
Domesticating Crops Caused Increased Seed Size

Elder

Sunflower

Squash

Wild  Crop

10,000 Years Ago....
Genetic Engineering for Big Seeds

WT

ap2-10

J. Okamuro
D. Jofuku
UC Santa Cruz

...2006
Domesticating crops caused an increase in seed head size 10,000 years ago.

Domesticated Foxtail Millet

Wild Foxtail Millet
Genetic Engineering for Organ Size

35S:ANT

Bob Fischer
UC Berkeley
Breeding a "New Organism"

The problems with doing it the "Old Fashioned" way

Engineering A Novel Crop
By "Wide" Breeding

Cabbage (Brassica)  Radish (Raphanus)

X

"Head"

Storage Root

Karpechenko
1925

???
Engineering A Novel Crop
By "Wide" Breeding

Cabbage (Brassica)   Radish (Raphanus)

"Head"

Storage Root

Radish leaves!!!

RaphanoBrassica

Cabbage roots!!!

Karpechenko 1925 (R.I.P.!!)

Result shows the unpredictability of classical breeding approaches.
Breeding uses natural variability of genes as raw material.

**Tomato Genetic Diversity**

How does this variability allow us to introduce new traits inherited?

Diversity generated by mutations in a gene that change its chemical sequence and slightly alters its function.
Alternative Forms of the Same Gene Lead to Genetic Diversity

What is the relationship between the mutant and normal gene?

This is also the basis of genetic variability in all organisms — including humans — the "raw material" for DNA testing!
What are the origins of using genetics to "improve" mankind?
"Engineer Humans!!"
Genetics was re-discovered only 100 years ago!

Mendelism: The Basic Principles of Inheritance


1909 - Johannsen first used the term Gene
**Table 9.3: Common Inherited Human Diseases**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Molecular and Cellular Defect</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUTOSOMAL RECESSIVE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sickle-cell anemia</td>
<td>Abnormal hemoglobin causes deformation of red blood cells, which can become lodged in capillaries; also confers resistance to malaria.</td>
<td>1/625 of sub-Saharan African origin</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>Defective chloride channel (CFTR) in epithelial cells leads to excessive mucus in lungs.</td>
<td>1/2500 of European origin</td>
</tr>
<tr>
<td>Phenylketonuria (PKU)</td>
<td>Defective enzyme in phenylalanine metabolism (tyrosine hydroxylase) results in excess phenylalanine, leading to mental retardation, unless restricted by diet.</td>
<td>1/10,000 of European origin</td>
</tr>
<tr>
<td>Tay-Sachs disease</td>
<td>Defective hexosaminidase enzyme leads to accumulation of excess sphingolipids in the lysosomes of neurons, impairing neural development.</td>
<td>1/1000 Eastern Europe Jews</td>
</tr>
<tr>
<td><strong>AUTOSOMAL DOMINANT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huntington's disease</td>
<td>Defective neural protein (huntingtin) may assemble into aggregates causing damage to neural tissue.</td>
<td>1/10,000 of European origin</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>Defective LDL receptor leads to excessive cholesterol in blood and early heart attacks.</td>
<td>1/122 French Canadians</td>
</tr>
<tr>
<td><strong>X-LINKED RECESSIVE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duchenne muscular dystrophy (DMD)</td>
<td>Defective cytoskeletal protein dystrophin leads to impaired muscle function.</td>
<td>1/3500 males</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>Defective blood clotting factor VIII leads to uncontrolled bleeding.</td>
<td>1–2/10,000 males</td>
</tr>
</tbody>
</table>

Archibald Garrod - 1902

Phenylketonuria
Figure 14.10 Inherited human disorders with defects in phenylalanine-tyrosine metabolism: phenylketonuria, tyrosinosis, tyrosinemia, alkaptonuria, and albinism. All five disorders are caused by autosomal recessive mutations. The mutations, which result in the synthesis of inactive enzymes, block phenylalanine-tyrosine metabolism at the steps indicated.
And attempts have been made to "select" out "bad" genes in Man....

**Eugenics**

Directed genetic change in Man

1. **Positive** - Add "good" genes
2. **Negative** - Remove "bad" genes

Question: (Can this ever be done completely?)

By:

- Preventing individuals from having children (negative)
- Encouraging individuals with the "correct" traits to have children (positive... or is it??) or using gene therapy/enhancement in the future?!?

Question:

Don't we all do this to a certain extent?

Are there "good" v "bad" genes?
By the outbreak of the First World War, sterilization laws were in such

The courts had also declared unconstitutional not only the stringent

The opportunity to press a test case arose that June, when a seventeen-year-old girl named Carrie Buck, who seemed definable as a "mental imbecile," was committed to the Virginia Colony for Epileptics and Feeble-minded at Danville.

Carrie's mother, Emma, had lived at the Colony since 1910 and was also
certified to be feebleminded. Carrie herself had conceived a child out of
wedlock, and shortly before her commitment, she gave birth to a daughter.

Vivian: Carrie was given the Stanford revision of the Binet-Simon I.Q. test
and was found to have a mental age of only five years, well within Henry
Goddard's definition of "moron." Carrie's mother was found to have a
mental age of slightly under five years. Thus, according to these results,
there was mental deficiency in two successive generations. If Vivian could
be shown to be feebleminded too, Carrie would be a perfect subject for a
state of the Virginia sterilization statute. In September 1924, the Colony's
board of directors ordered Carrie Buck sterilized, and a court-appointed
guardian initiated legal proceedings by appealing the order in a suit on
behalf of the superintendent of the Colony, Albert S. Priddy. 19

In preparing their case, Virginia officials consulted Harry Laughlin at
the Eugenics Record Office. Laughlin examined the pedigrees of Carrie,
his mother, and her daughter, and information about them given him by
Colony officials, and—without ever having seen them in person—provided
an expert deposition that Carrie's alleged feeblemindedness was primarily
hereditary. Carrie and her forebears, Laughlin submitted, "belong to the
shiftless, ignorant, and worthless class of anti-social whites of the South."

At the time of Laughlin's deposition, however, there was no evidence at all
that Vivian was mentally defective. To clarify the matter, Caroline E.
Wilhelm, a Red Cross worker who had placed Vivian in a foster home, was
prevailed upon to examine her there. At the initial hearing, in the Circuit
Court of Amherst County, she testified that there was "a look" about Vivian
(whom at the time of the visit was seven months old) which was "not quite
normal." Evidence also came from Arthur Eustisbrook of the Eugenics Re
cord Office, who had subjected Vivian to a mental test for an infant and
concluded that she was below average for a child her age. In the court
proceeding, Eustisbrook testified that the feeblemindedness in the Buck line
conformed to the Mendelian laws of inheritance, and the judge upheld the
sterilization order. 20

The case—now known as Buck v. Bell, because Priddy had in the
meanwhile died and been replaced as the defendant by the Colony's new
superintendent, John H. Bell—was carried to the Virginia Supreme Court
of Appeals in 1925, and the sterilization order was again upheld. In April
that it was argued before the United States Supreme Court. Carrie's defense
counsel, I. F. Whitehead, a one-time member of the board of directors of
the Colony, attacked the sterilization statute, warning that under this type of
law "a reign of doctors will be inaugurated and in the name of science
new classes will be added, even races may be brought within the scope of
such a regulation and the worst forms of tyranny practiced." Nevertheless,
the Court was persuaded not only that Carrie Buck and her mother were
"feebleminded" but also—because Vivian was, too (or so all the experts
said)—that the feeblemindedness was hereditary. The Court, whose members
ship ranged in political conviction from William Howard Taft to Louis D.
Brandeis, upheld the Virginia statute by a vote of eight to one. The sole
dissenter was Justice Pierce Butler, a conservative, and he kept his minority
opinion to himself. The decision declared that sterilization on eugenic
grounds was within the police power of the state, that it provided due
process of law, and that it did not constitute cruel or unusual punishment.

The Court's opinion was written by Justice Oliver Wendell Holmes, an
enthusiast of science as a guide to social action, who managed to find
a link between eugenics and patriotism. "We have seen more than once that
the public welfare may call upon the best citizens for their lives. It would
be strange if it could not call upon those who already sap the strength of
the State for these lesser sacrifices... in order to prevent our being
swamped with incompetents. The principle that sustains compulsory
vaccination is broad enough to cover cutting the Fallopian tubes."

The Court's opinion, however, restrained the use of sterilization
against people of "unfitness," meaning they had to meet the legal definition
of a "mental imbecile." The Court held that sterilization was not "enough"

Eugenicists naturally rejoiced at Buck v. Bell. For some years prior to
the decision, the American Eugenics Society had promoted what it thought
might be a constitutional revision of the faulty sterilization statutes. Apart
from procedural and technical changes, the revisions centered on making
the laws eugenic rather than punitive in intent. After Buck v. Bell, what was
constitutional was clear. By the end of the nineteen-twenties, sterilization
laws were on the books of twenty-four states, with the South no longer a
regional exception. (Though now severely restricted by federal regulation,
they are still on the books in twenty-two states today.) The laws were not
uniformly enforced, but Carrie Buck was sterilized soon after the Court's

Buck v. Bell generally stimulated either favorable, cautious, or—most
critically—no editorial comment. Few if any newspapers took notice of
the impact of the decision on civil liberties in the United States. The I.Q.
tests used in the Buck case have long since been discredited as indicators
purely of general intelligence. With regard to the allegedly hereditary
nature of mental defect in the Buck line, it is of interest that Carrie's
daughter Vivian went through the second grade before she died of an
infectious disorder in 1932. Her teachers reportedly considered her very
bright. 21

190 IN THE NAME OF EUGENICS 111
BUCK v. BELL
274 U.S. 200 (1927).

MR. JUSTICE HOLMES delivered the opinion of the Court.

This is a writ of error to review a judgment of the Supreme Court of Appeals of the State of Virginia, affirming a judgment of the Circuit Court of Amherst County, by which the defendant in error, the superintendent of the State Colony for Epileptics and Feeble Minded, was ordered to perform the operation of salpingectomy upon Carrie Buck, the plaintiff in error, for the purpose of making her sterile. The case comes here upon the contention that the statute authorizing the judgment is void under the Fourteenth Amendment as denying to the plaintiff in error due process of law and the equal protection of the laws.

Carrie Buck is a feeble minded white woman who was committed to the State Colony above mentioned in due form. She is the daughter of a feeble minded mother in the same institution, and the mother of an illegitimate feeble minded child. She was eighteen years old at the time of the trial of her case in the Circuit Court, in the latter part of 1924. An Act of Virginia, approved March 20, 1924, recites that the health of the patient and the welfare of society may be promoted in certain cases by the sterilization of mental defectives, under careful safeguard, &c.; that the sterilization may be effected in males by vasectomy and in females by salpingectomy, without serious pain or substantial danger to life; that the Commonwealth is supporting in various institutions many defective persons who if now discharged would become a menace but if incapable of procreating might be discharged with safety and become self-supporting with benefit to themselves and to society; and that experience has shown that heredity plays an important part in the transmission of insanity, imbecility, &c. The statute then enacts that whenever the superintendent of certain institutions including the above named State Colony shall be of opinion that it is for the best interests of the patients and of society that an inmate under his care should be sexually sterilized, he may have the operation performed upon any patient afflicted with hereditary forms of insanity, imbecility, &c., on complying with the very careful provisions by which the act protects the patients from possible abuse.

The superintendent first presents a petition to the special board of directors of his hospital or colony, stating the facts and the grounds for his opinion, verified by affidavit. Notice of the petition and of the time and place of the hearing in the institution is to be served upon the inmate, and also upon his guardian, and if there is no guardian the superintendent is to apply to the Circuit Court of the County to appoint one. If the inmate is a minor notice also is to be given to his parents if any with a copy of the petition. The board is to see to it that the inmate may attend the hearings if desired by him or his guardian. The evidence is all to be reduced to writing, and after the board has made its order for or against the operation, the superintendent, or the inmate, or his guardian, may appeal to the Circuit Court of the County. The Circuit Court may consider the record of the board and the evidence before it and such other admissible evidence as may be offered, and may affirm, revise, or reverse the order of the board and enter such order as it deems just. Finally any party may apply to the Supreme Court of Appeals, which, if it grants the appeal, is to hear the case upon the record of the trial in the Circuit Court and may enter such order as it thinks the
Circuit Court should have entered. There can be no doubt that so far as procedure is concerned the rights of the patient are most carefully considered, and as every step in this case was taken in scrupulous compliance with the statute and after months of observation, there is no doubt that in that respect the plaintiff in error has had due process of law.

The attack is not upon the procedure but upon the substantive law. It seems to be contended that in no circumstances could such an order be justified. It certainly is contended that the order cannot be justified upon the existing grounds. The judgment finds the facts that have been recited and that Carrie Buck “is the probable potential parent of socially inadequate offspring, likewise afflicted, that she may be sexually sterilized without detriment to her general health and that her welfare and that of society will be promoted by her sterilization,” and thereupon makes the order. In view of the general declarations of the legislature and the specific findings of the Court, obviously we cannot say as matter of law that the grounds do not exist, and if they exist they justify the result. We have seen more than once that the public welfare may call upon the best citizens for their lives. It would be strange if it could not call upon those who already sap the strength of the State for these lesser sacrifices, often not felt to be such by those concerned, in order to prevent our being swamped with incompetence. It is better for all the world, if instead of waiting to execute degenerate offspring for crime, or to let them starve for their imbecility, society can prevent those who are manifestly unfit from continuing their kind. The principle that sustains compulsory vaccination is broad enough to cover cutting the Fallopian tubes. Jacobson v. Massachusetts, 197 U.S. 11. [Three generations of imbeciles are enough.]

But, it is said, however it might be if this reasoning were applied generally, it fails when it is confined to the small number who are in the institutions named and is not applied to the multitudes outside. It is the usual last resort of constitutional arguments to point out shortcomings of this sort. But the answer is that the law does all that is needed when it does all that it can, indicates a policy, applies it to all within the lines, and seeks to bring within the lines all similarly situated so far and so fast as its means allow. Of course so far as the operations enable those who otherwise must be kept confined to be returned to the world, and thus open the asylum to others, the equality aimed at will be more nearly reached.

Judgment affirmed.

Mr. Justice BUTLER dissents.

What about 14th Amendment?
“Life, liberty & happiness without due process of law”
Buck vs. Bell
Themes → Ethics → Implications → Eugenics
www.dnai.org
EGG DONOR NEEDED

$50,000.00

Caucasian Egg Donor Needed
For Loving Family

All Expenses Paid

Free Medical Screening

You Must Be At Least 5'7"
Have A 1300+ Sat Score
Possess no major family medical issues

For More Information
Please Email Darlene:
TomEsquire@aol.com

Of fax inquiries to: 1-619-234-8881

Hitt & Pinkerton, Attorneys at Law
(1-800-264-8828)

But don't we "gain" in this? :)[11]

This is Eugene! 21st Century Style!
Directed Genetic Change

a. Classical Breeding - new gene combinations

b. Molecular Genetic Engineering - DNA technology
   (1) Reconstructing genes
   (2) Modifying genes
   (3) Synthesizing genes
   (4) Combining genes from different organisms
   (5) Cross species barrier! Mouse gene → plants!
   (6) Synthesizing whole genomes!!

Altering Genetic Makeup of an organism for:

(1) Basic Science
(2) Medicine
(3) Agriculture
(4) Environment
(5) Biology Factories
(6) The Law
(7) Commerce
...

LIMITATIONS OF CLASSICAL BREEDING/ENGINEERING

1. Limited to genes of organisms that interbreed or severe ethical issues with "Man"

2. Only can make new gene combinations with existing genes -- genes created by "natural" mutations. Can't predict outcome. Karazelenko

3. Can't make existing genes "better" -- just better combinations of existing genes -- new combinations of gene terms/alternatives.

4. Only useful for obvious traits -- ones that can be observed visually (e.g., Seed Size)

5. Time -- limited by generation time of organism do introduce "wild" forms of a gene into a crop or farm animal -- slow

E.g., crops x domesticated animals breed over 100's x 1000's of years!
Using DNA Technology to Genetically Engineer Organisms Has Unlimited Potential

1. Any gene from any organism can be used in any organism — No breeding barrier!

2. New genes can be created — Genes that produce new proteins or that work better
   (New genetic variability!!)

3. Existing genes can be switched on and in "places" they are normally off or vice versa!
   Gene regulation can be altered! Gene pathways can be controlled!

4. Speed — Can happen within a generation
   — Very quickly (e.g., human DNA engineering or gene therapy)

5. Genes or pieces of genes can be used from any genome/organism — Only limited
   by rules of life! of the gene’s chemistry!

6. Ability to change, alter, manipulate, control
   the genetic "blueprint" of any organism —
   NO biological limitation — Follow rules of biology!
Classical breeding combines many genes with unpredictable consequences.

TRADITIONAL PLANT BREEDING

Plant Breeding Combines Many Genes At Once

<table>
<thead>
<tr>
<th>Traditional Line</th>
<th>Commercial Variety</th>
<th>New Variety</th>
<th>Many Genes Transferred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desired Gene</td>
<td>(Many Crosses)</td>
<td>Desired Gene</td>
<td></td>
</tr>
</tbody>
</table>

PLANT BIOTECHNOLOGY

Biotechnology Adds A Single Gene

<table>
<thead>
<tr>
<th>Desired Gene</th>
<th>Commercial Variety</th>
<th>New Variety</th>
<th>One Gene Transferred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene Transfer (one generation)</td>
<td>Desired Gene</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Molecular breeding/Engineering is controlled and uses one characterized gene at a time!
The ERA of Genomics will enable us to have access to **ALL** genes of every living organism on the Earth!

- To understand biology —
- To use to engineer new gene combinations —
- To use for the benefit of mankind (e.g., new drugs, better crops, novel industrial processes, etc.!)
Genome Sequencing Using Computers and Robotics

Separating Fluorescing DNA Fragments By Size

Laser Detection of Fluorescing Nucleotides

Computer Visualization of DNA Sequence

Specific Order » Specific Function!!

IT IS POSSIBLE TO ISOLATE AND SEQUENCE EVERY GENE IN A GENOME!

NEED TO CLONE DNA SEGMENT FIRST
The Genomes of all major classes of organisms have been sequenced including humans!

Figure 9.21
Relative sizes of genomes if they were printed at 25,000 characters per page and bound in 1500-page volumes. One volume would contain about as many characters as a telephone book 2.5 inches thick. The E. coli genome would require about 200 pages, yeast 500 pages, and so forth.

By 2010 (or sooner) all of the genes of each major group of organisms on Earth will have been isolated, sequenced, and their functions revealed.

All genes in these organisms have been identified — e.g., mouse vs. human have same genes!
**SEQUENCED GENOMES**

1. Many Viruses
2. Hundreds of bacteria including E. coli and many human bacterial pathogens
3. Many Molds including Yeast
4. Important plants such as Rice & Arabidopsis which is a broccoli relative
5. Many Animals including nematode, fruit fly, mosquito, chicken
6. Close Relatives of humans including mouse, rat, & chimpanzee, dog
7. Human

*We will learn about our genetic origins & what makes us different from a chimpanzee or mouse - only 1% DNA difference!*
The sequence reveals all the genes in the E. coli cell - but not the function!

Figure 9.24
Diagram of the DNA sequence organization of Escherichia coli strain K-12. The coordinates are given in base pairs as well as in minutes on the genetic map. The coding sequences are shown as gold and yellow rays, which are transcribed in a clockwise (gold) or counterclockwise (yellow) direction. Green and red arrows denote genes for transfer RNAs or for ribosomal RNAs, respectively. The gold rays of the "sunburst" are proportional to the degree of randomness of codon usage in the coding sequences. Genes with the longest rays use the codons in the genetic code almost randomly. The origin and terminus of DNA replication are indicated. Bidirectional replication creates two "replicores." The peaks on the circle immediately outside the sunburst indicate coding sequences with high similarity to previously described bacteriophage proteins. [Courtesy of Frederick R. Blattner and Guy Plunkett III. From F. R. Blattner et al. 1997. Science 277: 1453.]
AND ALL OF YOUR GENES CAN BE STUDIED FOR THEIR ACTIVITY IN CELLS COLLECTIVELY!

EXPERIMENTAL FIGURE 9-35 DNA microarray analysis can reveal differences in gene expression in yeast cells under different experimental conditions. In this example, cDNA prepared from mRNA isolated from wild-type Saccharomyces cells grown on glucose or ethanol is labeled with different fluorescent dyes. A microarray composed of DNA spots representing each yeast gene is exposed to an equal mixture of the two cDNA preparations under hybridization conditions. The ratio of the intensities of red and green fluorescence over each spot, detected with a scanning confocal laser microscope, indicates the relative expression of each gene in cells grown on each of the carbon sources. Microarray analysis also is useful for detecting differences in gene expression between wild-type and mutant strains.

DNA Chip
Find which genes are active
2.3. Cancer genes
- Cancer genes
- Heart disease genes
- Obesity genes
- Hypertension genes
- Aging genes
etc., etc.

IT'S A NEW ERA OF BIOLOGY!
The Ultimate Outcome of Genome Projects

1. **All** the genes of major organisms isolated & identified. Use these genes to combine them for any purpose (medicine, agriculture).

2. **All** of the functions of genes in the cells of major organisms revealed. What they do to specity traits.

3. The regulatory networks or wiring that controls gene activity from "birth" to "death" revealed. How a child is formed from a fertilized egg cell!

4. The DNA functions & networks that direct cells to develop into complex organisms revealed our biological destiny!!

5. The relationships between the DNA/genes of all organisms revealed - what makes a "man a man" and a "mouse a mouse?"

→ (Immortality?)
Venter Cooks Up a Synthetic Genome in Record Time

Generating a synthetic genome by whole genome assembly: \( \phi X174 \) bacteriophage from synthetic oligonucleotides

Hamilton O. Smith, Clyde A. Hutchison III*, Cynthia Pfannkoch, and J. Craig Venter*

Institute for Biological Energy Alternatives, 1901 Research Boulevard, Suite 600, Rockville, MD 20850

Fig. 4. Plaques of syn\( \phi X \)-A. There appear to be several plaque morphologies: small plaques with sharp borders, medium-sized plaques, and large plaques with fuzzy borders.

What does this experiment say about living processes?
Ethical Considerations in Synthesizing a Minimal Genome


"The prospect of constructing minimal and new genomes does not violate any fundamental moral precepts or boundaries, but does raise questions..."

Will it be possible to create "life" beginning with a genome sequence?

1. Create new organisms to study critical life processes—origins of life, bacterial evolution, control of cell metabolism, etc.

2. Designer bacteria for specific tasks—e.g. breakdown of environmental toxins

3. How does this experiment change our views of what life is? Or does it?
The ERA of Mammalian Reproduction & Cloning combined with Genetic Engineering opens up a whole new set of possibilities.
**CLONING DOLLY THE SHEEP**

**EXPERIMENT**

**Question:** Are differentiated animal cells totipotent?

**METHOD**

1. Cells are removed from the udder of a Dorset ewe.
   - Dorset sheep (#1)

2. An egg is removed from a Scottish blackface ewe.
   - Scottish blackface sheep (#2)

3. The nucleus is removed from the egg.

4. Udder cells are deprived of nutrients in culture to halt the cell cycle prior to DNA replication.

5. The udder cell and enucleated egg are fused.

6. Stimulating mitotic inducers causes the cell to divide.

7. An early embryo develops and is transplanted into a receptive ewe.

8. The embryo develops and Dolly is born.
   - Dorset sheep, genetically identical to #1

**RESULTS**

- Differentiated animal cells are totipotent in nuclear transplant experiments.

---

**What does this say about the genetic potential of cells?**

---

**Question**
ORGANISMS THAT HAVE BEEN CLONED

1. Plants
2. Frogs
3. Mice
4. Rats
5. Sheep (Dolly)
6. Goats
7. Mules
8. Cattle
9. Horses
10. Pigs
11. Cats (cc = copy cat) - Dogs
12. Monkeys (ANDI - inserted DNA)
13. Humans ?!

Leading to Ethical Issues & New Opportunities (e.g., curing human disorders, saving endangered species, etc.)
GENETICALLY ENGINEERED CLONES CAN BE MADE.

FIGURE 11.11
Transgenic cattle produced by cloning with fetal cells. (a) Fibroblasts are obtained from a fifty-five-day-old bovine fetus. The fibroblasts are totipotent muscle and tendon cells arising early in the fetal stage. (b) The fibroblasts are cultivated in nutritious medium Petri dishes and modified with foreign genes. (c) Then the nucleus, with its genetically altered DNA, is removed from the cell, and the nucleus is implanted into an egg cell lacking a nucleus. (d) The egg cell with its new nucleus is encouraged to multiply and form an embryo. (e) Embryos are implanted to surrogate mothers, and (f) some months later, transgenic calves are born. They are clones because they have originated from single cells, and they are transgenic because all their cells bear foreign genes.

COMBINING GENETIC ENGINEERING + HEMALİON CLOTHING! IMPLICATIONS?
The Potential Use of Embryonic Stem Cells in Medicine

Human embryonic stem cells can be cultured in the laboratory and induced to differentiate. Their use as transplants to replace damaged tissues is under intensive investigation.
**Figure 11.20 Reproductive Cloning and Therapeutic Cloning**

In reproductive cloning, the goal is to produce a cloned baby. In therapeutic cloning, stem cells that are genetically identical to the cells taken from a patient are produced to provide patient-specific stem cell therapy.
<table>
<thead>
<tr>
<th></th>
<th>Embryonic Stem Cells</th>
<th>Adult Stem Cells</th>
<th>Therapeutic Cloning (Somatic Cell Nuclear Transfer)</th>
<th>Reproductive Cloning</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Final or &quot;end&quot; product</strong></td>
<td>Undifferentiated stem cells (isolated from fetal or embryonic tissue such as an embryo at the blastocyst stage) growing in culture</td>
<td>Undifferentiated stem cells (isolated from adult tissue such as bone marrow cells) growing in a culture dish</td>
<td>Undifferentiated stem cells growing in a culture dish (obtained from the person who will also serve as the recipient of these cells)</td>
<td>&quot;Cloned&quot; human</td>
</tr>
<tr>
<td><strong>Purpose/application</strong></td>
<td>Source of stem cells for research and for treating human disease conditions such as replacing diseased or injured tissue</td>
<td>Source of stem cells for research and for treating human disease conditions such as replacing diseased or injured tissue</td>
<td>Source of stem cells that are genetically matched to recipient for treating human disease conditions such as replacing diseased or injured tissue</td>
<td>Create, duplicate, or replace a human by producing an embryo for implantation, leading to the birth of a child</td>
</tr>
<tr>
<td><strong>Surrogate mother required</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Human created</strong></td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Time frame</strong></td>
<td>A few weeks of growth in culture</td>
<td>A few weeks of growth in culture</td>
<td>A few weeks of growth in culture</td>
<td>9 months, the duration of a normal biological pregnancy (after growth of the embryo in culture)</td>
</tr>
</tbody>
</table>