HC 70A
Winter 2005
Professor Bob Goldberg

Lecture #1 1/10/05
The Age of DNA - What is Genetic Engineering

Revised Notes/ Hand-out 1/12/05

THEMES

1. Age of DNA, Genetic Engineering, Genomics, & Mammalian Reproduction
2. DNA Demonstration
3. DNA Comes into Play - Some Basic Genetic Engineering
4. Glycogen, Clocky, GloMouse - What do these experiments tell us about unity in genetic processes? What do they tell us about what genes do?
5. What's a hypothesis → prediction → test → approach to test?
6. Other examples of Genetic Engineering - Cloned, Insect R Plants, Glo Monkey, Super Mouse, Vote Mouse, Human Gene Therapy (Scid disease)
7. What is Genetic Engineering? Purpose?

Genetic Engineering - Anything New? Classical breeding vs. the first Genetic Engineering Experiments!

Plant/Vegetable Mutation/ Genetic Variability Demonstration
What is NC 70A all about?

ERA of Genomics & Mammalian Reproduction 10/5
We Live in the Era of....

DNA

Genetic Engineering

Whole Genome Sequencing & Genomics

Mammalian Reproduction & Cloning

And the SYNTHESIS of These Technologies!!!
1. What Does DNA look like?
2. How can we perform an "experiment" to "touch" DNA?
3. Hypothesis Testing - Which flask has the DNA? How test prediction?
WE LIVE IN THE AGE OF DNA

The Age of the Gene

DNA Comes Into Your Home!

April 4th...

DNA

PERFUME

by Bijan

DNA...

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

We have begun to control our biological destiny!

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

DO-IT-YOURSELF DNA

If you've tried and tried but your family tree is still just a seedling, mail-order DNA testing may be for you. Comparing your genetic profile with those of other genealogy buffs—and potential relatives—can provide new leads. For $149 and up, Family Tree DNA will give you a list of 25 markers (or genetic traits) you carry, based on a swab from the inside of your cheek. For a bit more—$220 and up—Oxford Ancestors (oxfordancestors.com) will check 10 markers and tell you which "Seven Daughters of Eve" clan you belong to. If that's too steep, the Molecular Genealogy Research Project will test 250 markers for free. Run by Brigham Young University, it hopes to create a worldwide database. The catch: the data must be kept anonymous. In other words, the project will create a map of ancestry lines—not an individual report for you.

SCIENCE

FAMILY TREE DNA
No blood—just swab your cheek. $149 and up.
DNAfamilytree.com

MOLECULAR GENEALOGY RESEARCH PROJECT
Uses mouthwash. Free.
molecular-genealogy.byu.edu

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
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 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, left-over parts 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 Lockie, 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!!!!!"
How About a GloFly!!!!!!
How About a "GloMouse!!!"
How About “GloMice!!!”

MICE EXPRESSING GFP
A GloPlant With the Same Jellyfish Gene!!!
What Do These GloGene Experiments “Say” About Unity of Genetic Processes?

What is the Hypothesis?

What are the Predictions?

What Experiment(s) to Test Predictions?
What About Inserting Bacterial Genes Into Higher Organisms To Produce a Result With Significant Applications?

**Southern California Checklist**

- **PROTECT CABBAGE CROPS.** The minute you plant a brassica, squadrons of cabbage white butterflies seem to descend on it to lay their eggs. The easiest way to thwart them is to cover your cabbage crops with row covers right from the start. The next best option is spraying with *Bacillus thuringiensis* to kill the young caterpillar larvae.
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
Genetic Engineering a Plant to Resist Worms!!!

INSECT RESISTANCE with Bt

CONTROL Bt
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
MONKEY BUSINESS

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

HOW TO MAKE A MONKEY SHINE

1. Using recombinant-DNA technology, scientists stitched the jellyfish gene into a crippled virus.
2. Then they infected 224 monkey eggs with the virus, hoping it would insert the gene into the eggs' DNA.
3. The eggs were fertilized in test tubes, resulting in 128 embryos.
4. Forty of the healthiest embryos were implanted into 20 surrogate mothers, resulting in five successful pregnancies.

ANDI the rhesus monkey was conceived and born with an extra gene taken from a jellyfish. Here's how it was done:

How can this technology help human beings?

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

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

1. **Fertilization**
   - Female mouse (♀) and male mouse (♂) cross.
   - Eggs (♀) and sperm (♂) combine.
   - Pronuclei form.

2. **Plasmid Injection**
   - Plasmid carrying the gene for human growth hormone is injected into pronucleus using micropipette.

3. **Egg Implantation**
   - Egg implanted into "foster mother mouse".

4. **Birth**
   - "Foster mother mouse" gives birth.

5. **DNA Extraction**
   - DNA extracted from tissue biopsy.

6. **Analysis**
   - DNA fragment encoding mouse growth hormone.
   - DNA fragment encoding human growth hormone.

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

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 39 g.) The new gene is passed on to progeny, in a Mendelian manner, and so is proven to be chromosomally integrated. (R. L. Brinster)

We are entering the Era of "Designer" Organisms!

ALGO -> CLOFISH, AND:
SAME TECHNOLOGY!!!
Males vs. Females Differ by only the Presence or Absence of the Y Chromosome (simplistically!)

19-2. 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 gene are on the Y chromosome?

How do you "naturally" attain a XY? XY or ?

The Human Gene for Maleness CAN --------
Make a "Female" Mouse Be a Male!

What does this say about human/Nice??

Making a Male Mouse
The "ground state" of human development is a female! Need ONE gene to switch development into a Male.

Eve had to have lost a Y chromosome from Adam'srib, or Eve gave rise to Adam!
Mice can be engineered to be obese!

Mouse weighed down by genetics

Implications...
Goats can be turned into "factories" to produce medically important human proteins.

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.

tPA = tissue plasminogen activator
   → dissolves blood clots & prevents heart attacks!
Correcting Genetic Defects in Humans using Genetic Engineering

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.

Human Gene Therapy is a 20-year-old technology.

Corr enticing SCID - Severe Combined Immunodeficiency Using the ADA gene
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
- Information
- DNA
- Transcription (RNA synthesis)
- Information
- mRNA
- Translation (protein synthesis)
- Ribosome
- Protein

The reason "why" genetic engineering is possible.

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

All organisms use the same processes and "rules" to generate traits! And the same molecules/chemistry is involved!

Can intervene in this process in living cells.
What is Genetic Engineering & What does it do?
DNA Cloning or Genetic Engineering uses natural processes of living cells to isolate single genes.

- **Source DNA**
- **Cloning vector**
- **A genome with 1000s of genes**
- **Enzymatic fragmentation**
- **Enzymatically linearize**
- **Vector DNA**
- **Join target DNA and cloning vector**
- **DNA construct**
- **Introduce DNA into host cell**
- **Isolate cells with cloned gene**
- **Protein encoded by cloned gene**
- **Gene A news replicated as host cell divides**

**Produce**
- Lots of Gene A (for protein)
- Genetically engineered bacterial chromosome to have a human gene

**Implications**
- Bacterial cell produces human protein - recognizes human gene on its own
"Why" Clone Genes From the Genome of an Organism?

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

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

- **Use** the cloned Gene to:
  1. **Study** Gene Structure & Function
  2. **Use** to make **Pharmaceuticals**
  3. **Use** in animal & plant **Gene Therapy**
  4. **Use** to **Diagnose** diseases
  5. **Use** to **Correct** diseases
  6. **Use** to **Identify** individuals
  7. **Use** to **Convert** cells into **Tissue**

Gene Engineering has lead to new knowledge about how living cells function and new applications that improve all of our lives!
DNA CLONING ALLOWS US TO
Isolate, Manipulate & Study
INDIVIDUAL GENES

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

The \( \beta \) globin gene is
approximately 2000 bp in length
So, the atomic mass of the \( \beta \) globin gene is:
2000 bp
\times
635 daltons/bp
= \( 1.27 \times 10^6 \) daltons

Mass of \( \beta \) globin gene in an adult human
There are two copies of the \( \beta \) globin gene per cell

There are
10^19 cells per individual

So, the total atomic mass
of \( \beta \) globin DNA per individual is:
\( 1.27 \times 10^6 \) daltons/gene
\times
2 genes/cell
\times
10^19 cells/individual
= \( 2.54 \times 10^{25} \) daltons

If there are
6.02 x 10^{23} daltons per gram, then:
\[ \frac{2.54 \times 10^{25} \text{ daltons}}{6.02 \times 10^{23} \text{ daltons/gram}} = 0.000042 \text{ grams} \]
= 0.042 mg

\( 0.042 \text{ mg} = \text{globin DNA per human} \)

Mass of \( \beta \) Globin DNA in Adult Human vs. 1-liter Culture of E. coli Carrying \( \beta \) 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 \( \beta \) globin gene is
approximately 2000 bp in length
So, the atomic mass of the \( \beta \) globin gene is:
2000 bp
\times
635 daltons/bp
= \( 1.27 \times 10^6 \) daltons

Mass of \( \beta \) globin gene in a liter of E. coli
There are 500 copies of the \( \beta \) globin gene per cell

There are
5 x 10^{11} cells per liter

So, the total atomic mass
of \( \beta \) globin DNA per liter is:
\( 1.27 \times 10^6 \) daltons/gene
\times
500 genes/cell
\times
5 x 10^{11} cells/liter
= \( 3.175 \times 10^{20} \) daltons

If there are
6.02 x 10^{23} daltons per gram, then:
\[ \frac{3.175 \times 10^{20} \text{ daltons}}{6.02 \times 10^{23} \text{ daltons/gram}} = 0.000527 \text{ grams} \]
= 0.527 mg

\( 0.527 \text{ mg} = \text{globin DNA per liter} \)

Can produce 10X more \( \beta \) globin DNA in a 1-liter bacteria culture than in the entire human body using gene
engineering methods!
Even Cheesemaking is Helped by Our Cloning & Genetic Engineering.

**Composition of milk**

<table>
<thead>
<tr>
<th>Component</th>
<th>Milk (%)</th>
<th>Whey (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>~88</td>
<td>~94</td>
</tr>
<tr>
<td>Fat</td>
<td>~3.4</td>
<td>~0.5</td>
</tr>
<tr>
<td>Protein</td>
<td>~3.3</td>
<td>~1</td>
</tr>
<tr>
<td>Casein</td>
<td>~2.6</td>
<td>~</td>
</tr>
<tr>
<td>Lactose</td>
<td>~4.8</td>
<td>~</td>
</tr>
</tbody>
</table>

**Processing of milk**

- Sour milk products → starter cultures → milk → lactase → reduced-lactose milk products → oil-in-water emulsion with mixed micelles from α-, β- and κ-casein
- Cheese curd → whey → lactase → animal feed, fermentation raw material
- Hydrolysis of the polar region of κ-casein by chymosin (rennin) leads to destruction of micelles, resulting in coagulated milk (salted out by Ca²⁺)
- Lactose and whey protein → lactose syrup

**Manufacture of chymosin**

- Native:
  - Stomachs of young animals
  - Cutting, activation at pH ≤ 5
  - Extraction: salt water, 14 d
  - Purification: ultrafiltration, standardization
  - 200U/kg stomach

- Microbial:
  - Preculture: high-yield mutants of Mucor miehei or M. puellus
  - Bioreactor: dextrose syrup, soy meal, 30°C, 72 h
  - Purification: separation of mycelium, reverse osmosis, precipitation
  - 5000U/m³ in 72 h

- Recombinant:
  - Recombinant microorganism: Escherichia coli
  - Bioreactor: maltodextrins, 37°C, 36 h
  - Purification: isolation of inclusion bodies, Triton-X100/EDTA, urea/alkali-extract, ion-exchange chromatography, acid treatment
  - 20000U/m³ in 36 h

100X more in a bacterial culture!

The cow chymosin gene is cloned in bacteria, leading to an infinite amount of chymosin to make cheese!

What about religious issues, kosher laws?
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 dna/gene in cells - not cell/organism cloning)

To: (1) Study A/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 kinds and from any organisms can be combined.

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

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**
3. **Medicine → Diagnosis & Treatment of Diseases**
4. **Agriculture → Higher Yields of Crops**
5. **Business/Commerce → Biotech Industry**
   - **The Law/Forensics**
     - Patients → Identification → Privacy Issues
6. **Anthropology**
   - Human origins/diversity vs. unity of humanity
7. **Evolution**
   - Where did we come from?
8. **Philosophy/Religion** → How we view ourselves in relation to God & nature
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, and genes.
2. Regulating "Experimentation"—recombinant DNA, stem cells, transgenic plants and animals.
3. Regulating release of genetically modified organisms into the environment—crops, farm animals, mosquitoes.
5. Genetic Discrimination—insurance, workplace, society.
11. Synthetic Genomes—what is life?
Issues that need to be resolved by informed public choices

**What People Think**

**Genetic Testing**
- Rule out a fatal disease: Yes 60%, No 40%
- Ensure greater intelligence: Yes 33%, No 67%
- Influence height or weight: Yes 12%, No 88%
- Determine sex: Yes 14%, No 86%
- Should parents with genetically linked diseases be required to test their children for them? Yes 39%, No 55%

**Gene Therapy**
- Should the government regulate gene therapy—that is, altering genes to cure or prevent diseases? Yes 62%, No 38%
- Cloning of whole animals? Yes 47%, No 53%
- Using genetic testing to pick the traits in unborn children? Yes 46%, No 54%

**Genetic Privacy**
- Should insurance companies have access to your genetic record or DNA without permission? Yes 6%, No 94%
- Should employers be able to obtain access to employees' genetic records or DNA without permission? Yes 5%, No 95%

**Genetic Discrimination**

**Genetically Engineered Food**
- Should genetically engineered food be labeled as such? Yes 81%, No 19%
- If food were labeled as genetically engineered, would you buy it for yourself or your family? Yes 28%, No 72%

*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 fatal disease 78%  4% No Answer
   b. Ensure greater intelligence 14%
   c. Influence height or weight 4%
   d. Determine sex 0%

2. Should parents with genetically linked diseases be required to test their children for them?
   a. Yes 53%  2% No Answer
   b. No 45%

3. If you had the gene for an incurable life-threatening disease, would you have your unborn child tested for the disease?
   a. Yes 86%  2% No Answer
   b. No 12%

4. If the test showed that the baby would have the disease, would you consider ending the pregnancy through abortion?
   a. Yes 50%  2% No Answer
   b. No 48%

5. Should the government regulate using gene therapy—that is, altering genes to cure or prevent diseases?
   a. Yes 76%  4% No Answer
   b. No 20%

6. Should the government regulate cloning of animals?
   a. Yes 79%  2% No Answer
   b. No 19%

7. Should the government regulate using genetic testing to pick the traits in unborn children?
   a. Yes 77%
   b. No 23%

8. Should insurance companies have access to your genetic record or DNA without permission?
   a. Yes 2%
   b. No 98%

9. Should employers be able to obtain access to employees’ genetic records or DNA without permission?
   a. Yes 2%
   b. No 98%
10. Should the police be allowed to collect DNA information gathered from suspected criminals as they currently do with fingerprints?
   a. Yes 73%
   b. No 27%

11. 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 69% 2% No Answer
   b. Bad Idea 29%

12. Do you do your own grocery shopping?
   a. Yes 66% 2% No Answer
   b. No 31%

13. If food were labeled as genetically engineered, would you buy it for yourself or your family?
   a. Yes 69% 6% No Answer
   b. No 25%

14. Should genetically engineering food be labeled as such?
   a. Yes 90% 6% No Answer
   b. No 4%

15. What year are you in school?
   a. First 31%
   b. Second 21%
   c. Third 27%
   d. Fourth 17%
   e. Fifth 4%

16. Where do you live?
   a. Home 13% 3% No Answer
   b. Dorms 40%
   c. Off Campus Apartments 44%

17. How many hours do you watch television a week?
   a. 0 hrs. 19% 2% No Answer
   b. 1-3 hrs. 56%
   c. 4-7 hrs. 19%
   d. 7-10 hrs. 0%
   e. 10+ hrs. 4%

18. How many hours do you spend listening to, watching, or reading the news a week?
   a. 0 hrs. 0% 2% No Answer
   b. 1-3 hrs. 71%
   c. 4-7 hrs. 23%
   d. 7+ hrs. 4%
Issues Raised by Genetic Engineering Technology - like all new technologies, society & people are affected.

Science-philosophy arguments concerning genetic engineering

categorical argument
Some human activities such as genetic engineering are fundamentally reprehensible. Developing this technology, "man plays God" and claims competencies beyond his capacities, degrading nature to the course of his technical manipulations.

pragmatic argument
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.

social policy argument
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 ("eugenics bottom up")

Problematic areas of genetic research

<table>
<thead>
<tr>
<th>topic</th>
<th>state of the art</th>
<th>regulation or trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>cloning of humans</td>
<td>cloning of animals possible</td>
<td>not permitted</td>
</tr>
<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>
</tr>
<tr>
<td>prenatal diagnosis</td>
<td>cytological methods partially established</td>
<td>permitted, abortion permitted after medical indication</td>
</tr>
<tr>
<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>
</tr>
<tr>
<td>knockout animals for drug research</td>
<td>widely established</td>
<td>generally accepted, but hotly debated by animal protection groups</td>
</tr>
<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>
</tr>
<tr>
<td>transgenic microorganisms or cell lines for production of biopharmaceuticals</td>
<td>established</td>
<td>widely accepted</td>
</tr>
</tbody>
</table>

Public acceptance of genetic engineering (survey 2001)

- agricultural plants: 29%, 27%, 22%, 12%, 10%
- bacteria: 43%, 23%, 7%, 17%, 10%
- domestic animals: 13%, 32%, 31%, 14%, 10%
- biopharmaceuticals: 91%, 28%, 11%, 13%, 11%

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 original 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?
Breeding uses natural variability of genes as raw material.

**Tomato Genetic Diversity**

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

mutations result in genetic diversity!!!

what is the relationship between the mutant & normal gene?

This also the basis of genetic variability in all organisms - including humans & the "raw material" for DNA testing!
Farm animals were also "engineered" by breeding wild relatives.

Figure 11.2 The ancient Egyptians were successful cattle breeders. This miniature stable, which dates from about 2000 BC, shows longhorn cattle. Other cattle breeds had short horns or no horns. (Metropolitan Museum of Art, Rogers Fund and Edward S. Harkness Gift, 1920)

Cattle breeding in Egypt 4000 years ago!

Manipulating existing genetic variability

Variability brought about by chance mutations!
A Shaggy Dog History

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

15,000 years ago in Europe Asia

Common pedigree. From Chihuahuas (left) to Great Danes, dogs of all shapes and sizes share common ancestors.

Genetic variability

Trace using DNA testing!

Can only arise by selecting for existing variability

What are the genetic differences? How did they arise?

MAJOR CROPS WERE ENGINEERED FROM NON-PRODUCTIVE WILD RELATIVES 10,000 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!
Domestication of Wheat

Diploid
14 Chromosomes

Einkhorn wheat
(AA)

Goat grasses
(BB) (DD)

Tetraploids
28 chromosomes

Emmer, macaroni,
wheat, etc.
(AABB)

Hexaploids
42 chromosomes

Bread wheats
(AABBD)

Note Difference
in Grain Number
Selected for genes controlling size of seeds. Because that's what was consumed—

We now know what these genes are!
Genetic Engineering for Big Seeds

WT

ap2-10

J. Okamuro
D. Jofuku
UC Santa Cruz
Domesticating Crops Caused Increase Seed Head Size

Foxtail Millet

Wild  Domesticated

Breeding increased size of seeds & number of seeds
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)

"Head"  
Storage Root

Karpechenko 1925

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

Cabbage (*Brassica*) X Radish (*Raphanus*)

"Head" x Storage Root

Radish leaves!!!

*RaphanoBrassica*

Cabbage roots!!!

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

Result shows the unpredictability of classical breeding approaches.
What are the origins of using genetics to “improve” Mankind? “Engineer Humans!!”
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**

**Skeptonurie**
GARROD—SOME HUMAN DISEASES CAN BE DUE TO INHERITED DEFECTS IN METABOLISM

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.

REVOLUTIONARY AT TIME—GENETICS AFFECTS HUMAN DISEASE!  

(66)
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

By: Preventing individuals from having children (negative)

or

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

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

Are there "good" or "bad" genes?
better “left behind in the cast-off junk of ignorant efforts, with which the past is filled.”  

By the outbreak of the First World War, sterilization laws were in such dispute as to have been de facto suspended in their operation in a number of states. All the courts had also declared unconstitutional not only the stringent Iowa statute but less sweeping measures in six other states. Advocates of eugenic sterilization, frustrated at the legal impasse, wanted to take the issue to the Supreme Court. In Virginia, eugenacists helped draw up a sterilization statute, passed by the legislature in March 1924, that was designed to meet the constitutional objections. The opportunity to press a test case arose that June, when a seventeen-year-old girl named Carrie Buck, who seemed definable as a “moral imbecile,” was committed to the Virginia Colony for Epileptics and Feebleminded, in Lynchburg.  

Carrie’s mother, Emma, had lived at the Colony since 1919 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 nine years, well within Henry Goddard’s definition of “moron.” Carrie’s mother was found to have a mental age of slightly under eight 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 test 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 Carrie’s behalf against the superintendent of the Colony, Albert S. Priddy.  

Preparing their case, Virginia officials consulted Harry Laughlin at the Eugenics Record Office. Laughlin examined the pediatrics of Carrie, her mother, and her daughter, and information about them given 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 deficient. 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 (who at the time of the visit was seven months old) which was “not quite normal.”  

Evidence also came from Arthur Estabrook of the Eugenics Record 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, Estabrook testified that the feeblemindedness in the Buck line conformed to the Mendelian laws of inheritance, and the judge upheld the sterilization order.  

The case—now known as Buck v. Bell, because Priddy had in the meantime 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 1927 it was argued before the United States Supreme Court. Carrie’s defense counsel, J. P. Whitehead, a onetime 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—that Vivian was, too (or so all the experts said)—that the feeblemindedness was heritable. The Court, whose membership 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.” With deliberate punch Holmes asserted: “Three generations of imbeciles are enough.”  

Eugenacists 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
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, 187 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.
EGG DONOR NEEDED

Caucasian Egg Donor Needed
For Loving Family

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

Free Medical Screening
All Expenses Paid

$50,000.00

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)
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 \( \rightarrow \) plants!
   6. Synthesizing whole genomes!!

Altering Genetic Makeup of an Organism for:

1. Basic Science
2. Medicine
3. Agriculture
4. Environment
LIMITATIONS OF CLASSICAL BREEDING/ENGINEERING

1. Limited to genes of organisms that interbreed. Severe ethical issues with "man."

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

3. Can't make existing genes "better" -- just better combinations of existing genes -- new combinations of gene forms/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 to introduce "wild forms of a gene into a crop or farm animal -- slow.

E.g., crops & domesticated animals bred over 100's & 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.

3. Existing genes can be switched on 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 ADH engineering or gene therapy)

5. Genes or pieces of genes can be used from any species/organism — only limited by rules of life or 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. Desired gene (many crosses) results in a new variety with many genes transferred.

PLANT BIOTECHNOLOGY

Biotechnology adds a single gene. Gene transfer (one generation) results in a new variety with one gene transferred.

Molecular breeding/engineering is controlled and uses one characterized gene process 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.)!
We live in the Genomics Era - The Age of the Genome!!

Nuclear fission
Five-dimensional energy landscapes

Seafloor spreading
The view from under the Arctic ice

Career prospects
Sequence creates new opportunities

Genetic Engineering gave "Birth" to this Era!!
IT IS POSSIBLE TO ISOLATE AND SEQUENCE EVERY GENE IN A GENOME!

Genome Sequencing Using Computers and Robotics

Separating Fluorescing DNA Fragments By Size

Laser Detection of Fluorescing Nucleotides

Computer Visualization of DNA Sequence

1. What makes gene unique
2. What if Gene
3. How it works in cell

Specific Order = Specific Function in cell
The Genomes of all Major Classes of Organisms Have Been Sequenced Including Humans!

Phage λ
50 kb
2 pages

Escherichia coli
(bacteria)
4.7 Mb
200 pages

Saccharomyces cerevisiae
(yeast)
12.5 Mb
500 pages

Caenorhabditis elegans
(nematode)
Arabidopsis thaliana
(plant)
100 Mb
3 volumes

Drosophila melanogaster
(fruit fly)
165 Mb
5 volumes

1 kb = 1,000 bases
1 Mb = 1,000,000 bases

Human being
3000 Mb
80 volumes
25 kb per page
1500 pages per volume
(2 inches thick)

Chimpanzee

Rat

Chicken

Rice

Butterfish

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 & human have same genes!
SEQUENCED GENOMES

1. Many Viruses
2. Hundreds of bacteria including E. coli & 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
7. Human

We will learn about our genetic origins, what makes us different from a Chimpanzee vs. Mouse - only 1% DNA difference!
The Sequence Reveals all the genes in the Circle... but not the function!

E. coli
K-12 MG1655
4,639,221 bp

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 bars, 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 "replichores." 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.]
An 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.

Find which genes are active where e.g., 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 and 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 specify 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 or 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?"
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 - origin 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.
2. An egg is removed from a Scottish blackface ewe.
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.
9. Dorset sheep, genetically identical to #1

**RESULTS**

Scottish blackface sheep (#3)

**Conclusion:** Differentiated animal cells are totipotent in nuclear transplant experiments.

What does this say about the genetic potential of cells?
ORGANISMS THAT HAVE BEEN ClOWed

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)
12. Monkeys (Avoi - inserted ovum)
13. Humans ?!

Leading to Ethical Issues & new opportunities (e.g., curing human disorders, saving endangered species, etc.)
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.
Human Stem Cells can potentially be genetically engineered.

1. The early embryo, or blastocyst, is cultured in a nutrient medium.
2. The outer layer collapses and the inner cell mass is freed from the embryo. Chemicals are added to disaggregate the inner cell mass into smaller clumps.
3. Each clump grows into a colony.
4. Special differentiation factors are added to colonies in separate containers.
5. Deliver differentiated cells to damaged tissues.

Correct genetic disorder (e.g., diabetes) and replace with normal engineered pancreas!

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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 tissue is under intensive investigation.
What About Human Cloning?

And combining with Genetic Engineering!!

THE MAKING OF A HUMAN CLONE

[EXCLUSIVE]

DAYS INSIDE A MAVERICK EMBRYO LAB

Embryos? Adult Human Beings?
Genetically Engineered Cloned Human Embryos?
Is a "clone" human?

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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 “end” 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>“Cloned” 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>
FILM
Cutting & Splicing of DNA

History: The discovery of genetics to DNA to Double Helix to DNA Cloning