

# Reprogenetics: Options

Lecture 9

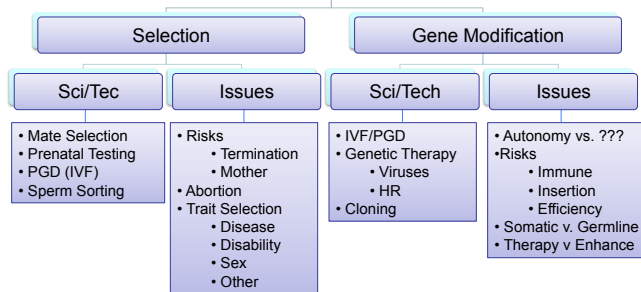
Genetics & Society  
Honor 3215, Fall 2008  
Bryan Benham

## Why Reprogenetic Options?

- Increase individual autonomy
  - Reproductive autonomy
  - Parental autonomy
- Risks
  - “New Eugenics”
  - Moral Expressivism
  - Social Inequalities

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### Reprogenetic Options



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## Selection

- Mate Selection
- Prenatal Testing
- PGD (w/IVF)
- Sperm Sorting (w/IVF, Sex)

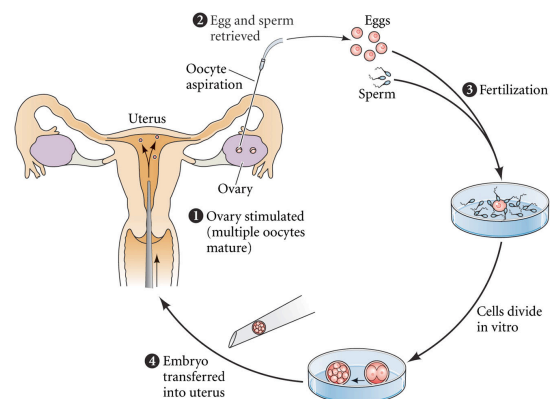
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## Genetic Manipulation



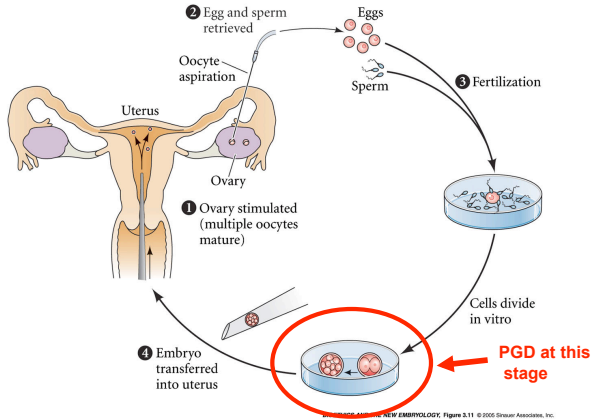
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## IVF: *In vitro* Fertilization



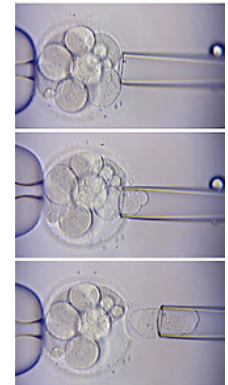
BIOETHICS AND THE NEW EMBRYOLOGY, Figure 3.11 © 2000 Sinauer Associates, Inc.

## IVF: *In vitro* Fertilization



## PGD: Pre-implantation Genetic Diagnosis

- At the 4- to 8- cell stage of the embryo (~day 3), two cells are removed for analysis.
- Embryo continues to develop normally\* because cells are totipotent.
- Results available (~2 days) prior to implantation.



\*Cows and mouse results indicate, and success in humans is consistent with these results.

Image: <http://yalefertilitycenter.org/patient/pgd.html>

## Genetic Manipulation

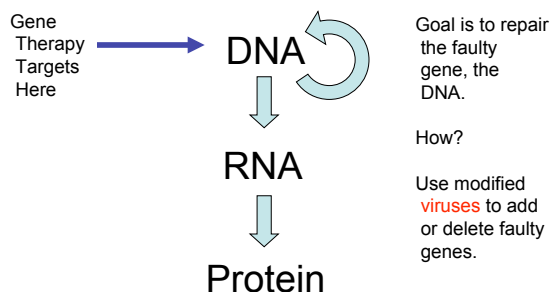


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## Gene Therapy: The Basic Idea

- **Gene therapy:** modifying or adding functioning gene to a person so that those genes regain functionality.
  - **Somatic gene therapy:** therapy that targets cells of the body, not germ-line cells. This form is actively pursued: more than 1,000 clinical trials and more are on-going. (SCID, other blood diseases)
  - **Germ-line gene therapy:** therapy that targets reproductive cells (sperm and eggs) thus affecting future generations. Currently not pursued, and often legislation acted against this form of gene therapy.
- Most common: viral vectors (modified to insert "new" gene).

## Gene Therapy: The Basic Idea



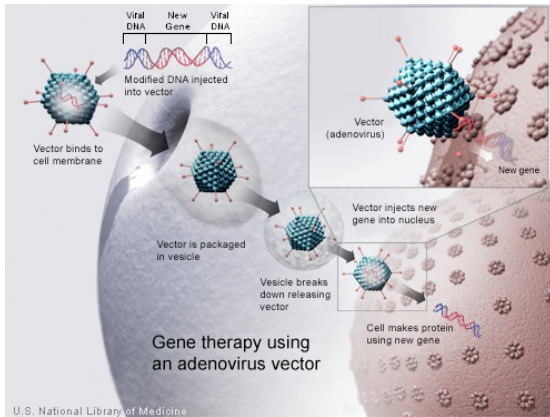
## Gene therapy viruses

Two Types:

**Non-integrating**  
e.g., adenovirus

**Integrating**  
e.g., retrovirus

- Insert into the cell but don't actually combine with the genome of the person
- Capitalize on the protein making ability of cells **temporarily**.
- Risks:
  - not stable
  - immune response
- Insert into the genome of the person
- Capitalize on the protein making ability of cells in a more **permanent** manner.
- Risks:
  - Insertional mutagenesis
  - Immune response



[http://ghr.nlm.nih.gov/info/gene\\_therapy/showprocedures](http://ghr.nlm.nih.gov/info/gene_therapy/showprocedures)

## Concerns with Viruses

- Integrating (retroviruses)
  - Insertional mutagenesis
  - Immune response
- Non-integrating (adenoviruses)
  - Immune response
  - Unstable

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## “Homologous Recombination”

- Site specific insertion of DNA
- Also known as: gene targeting or knockout technology
- Developed by Mario Capecchi

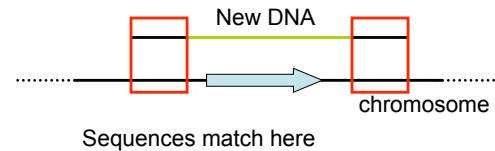


<http://learn.genetics.utah.edu/features/knockout/>

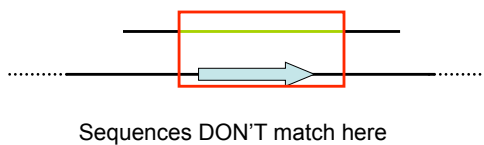
You are Invited  
Lecture on Social Responsibility in Science:  
Thursday, Nov. 13, 4:00PM at HSEB 1750

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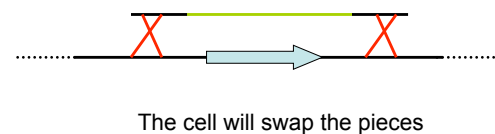
## Knockout technology



## Knockout technology



## Knockout technology

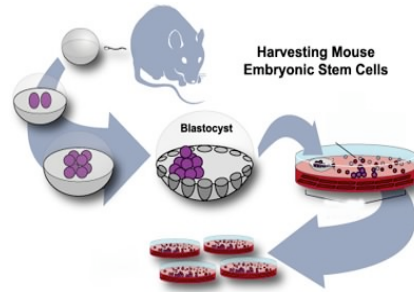


# Knockout technology

This can add, delete or replace genes.

Very precise (if you know the gene...)

# Knockout technology



1. Harvest the inner cellular mass (in purple) which are pluripotent ES cells.
2. Inject desired transgene (plus an antibiotic resistance gene or marker gene), and multiply.
3. Kill off those ES cells w/out antibiotic resistance; leaving the ES cells with inserted transgene to proliferate.
4. THEN: Inject those transgenic ES cells into early stage embryos (blastocyst) and implant...

[http://www.openbiosystems.com/collateral/genomics/images/Cells%20and%20cell%20lines/ES\\_cell\\_isolation.jpg](http://www.openbiosystems.com/collateral/genomics/images/Cells%20and%20cell%20lines/ES_cell_isolation.jpg)

See next slide...

**The New York Times** October 8, 2007

### Gene Targeting

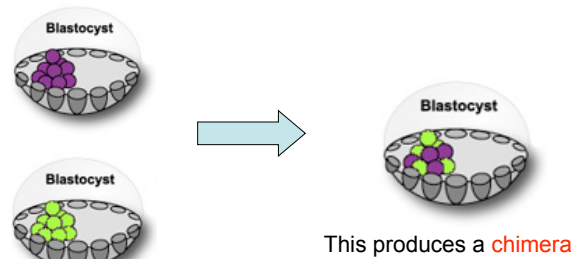
Three scientists were awarded the Nobel Prize in medicine yesterday for developing a technique for creating mice with specific genetic mutations.

The diagram shows the process of gene targeting in mice. It starts with a donor mouse embryo from which stem cells are harvested. These stem cells are then cultured and a targeted gene (containing a modified or deactivated gene) is introduced. The modified stem cells are then injected into a blastocyst and implanted into a surrogate mother. The resulting offspring are chimeric, containing both normal and genetically modified cells. Finally, breeding these chimeric mice results in normal and genetically modified offspring.

1. **STEM CELL CULTIVATION** Embryonic stem cells are removed from the embryo of a normal mouse.
2. **INTRODUCTION OF TARGETED GENE** DNA containing a modified or deactivated gene is introduced. The few stem cells that take up the new gene are allowed to proliferate.
3. **STEM CELL INJECTION** The modified stem cells are injected into a blastocyst and implanted in a surrogate mother, which gives birth to a chimeric mouse that contains both normal and genetically modified cells.
4. **BREEDING** The chimeric mouse is mated with a normal mouse. Offspring include both normal mice and mice with the new gene in every cell.

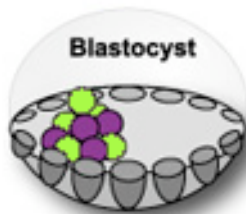
Illustration: The Lasker Foundation; Nobel Committee

# Cells from embryos can be mixed

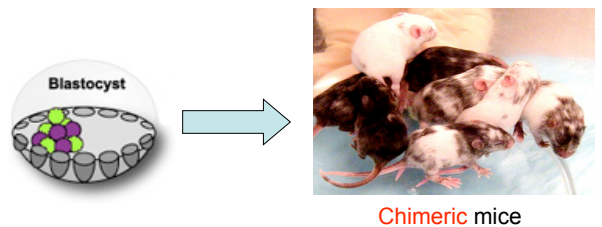


# Chimeras

- The DNA never mixes (they are not hybrids).
- The cells grow, divide and communicate with each other during development as one organism.



# Cells from a blastocyst can become ANY cell in the body



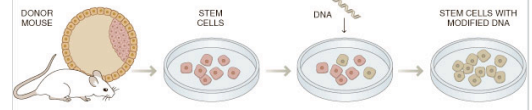
# Chimeras

- Cells from different species can be mixed and they will grow together into one organism. Chimeras show that early embryonic cells respond to signals in their environment to fill any role in an organism.
- Some humans have been found that are natural chimeras (Twins and Mothers). Some of their tissues have one genotype, the rest of their tissues have a different genotype.



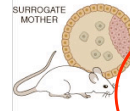
## Gene Targeting

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4. **BREEDING** The chimeric mouse is mated with a normal mouse. Offspring include both normal mice and mice with the new gene in every cell.

Sources: The Leuker Foundation; Nobel Committee.

THE NEW YORK TIMES  
The New York Times

# Concerns with HR

- Inefficient
  - ZFN: Zink Finger Nuclease
    - A protein engineered to identify DNA sequence 1000x more efficiently
- Results in Chimeras, not transgenics
  - Unlikely to force breed chimera humans
  - Unlikely to help in all cases (Dominant?)

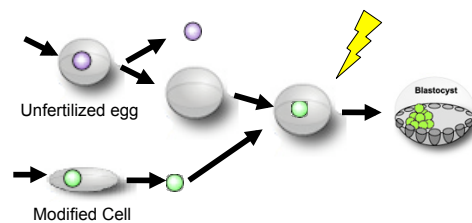
# Genetic Manipulation



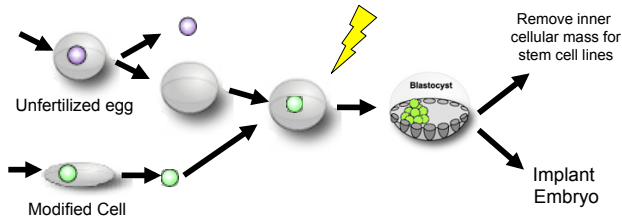
# Cloning

- PGD and Gene Therapy can be combined with reproductive Cloning to avoid making chimeras.
- Recipe:
  - Take modified genome in nucleus of cell. Insert into enucleated egg.
  - Add electricity.
  - Insert into womb once cell division proceeds normally.

# Somatic Cell Nuclear Transfer (SCNT): “cloning”



## Therapeutic vs. Reproductive



## Reproductive cloning



Dolly  
Rosalind 1996



Millie, Christa, Alexis, Carrel and Dotcom  
PPL Therapeutics 2000



CC  
Texas A&M 2001



George and Charlie  
Adv. Cell Tech 1999



Snuppy  
S. Korea 2005

## Concerns with Clones

- Efficiency is low (Dolly took over 500 eggs)
- Safety (“premature aging” and other problems)
- Legal prohibition on reproductive cloning.

## Genetic Manipulation



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## Concerns with Reproductive Genetic Manipulations

- Increase reproductive autonomy?
  - “New Eugenics” & Moral Expressivism
  - Therapy vs. Enhancement (which traits?)
  - Somatic vs. Germline modification
- Safety:
  - IVF, PGD, Gene Therapy, and Cloning are risky
  - Knowledge of genomics is limited
- Cost: IVF is expensive
  - Social inequalities?
  - Slippery Slope: genetic haves vs. have-nots

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## Green’s Punnett Square

	Treatment	Enhancement
Somatic Modification	Somatic Treatment (X-SCD gene therapy)	Somatic Enhancement (Gene doping in sports)
Germline Modification	Germline Treatment (Removing disease from family line)	Germline Enhancement (“Superathletes”)

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# Green's Punnett Square

	<b>Treatment</b>	<b>P R E V E N T I O N</b>	<b>Enhancement</b>
<b>Somatic Modification</b>	Somatic Treatment (X-SCD gene therapy)		Somatic Enhancement (Gene doping in sports)
<b>Germline Modification</b>	Germline Treatment (Removing disease from family line)		Germline Enhancement (“Superathletes”)

# Reprogenetic Options

