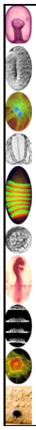
Anderson - Cloning Quote



The people who make policy decisions should damned well know what they are talking about before they make the decisions. There is nobody who is an expert on cloning who would be afraid after seeing "Attack of the Clones."

Kevin J. Anderson

Evidence for Genomic Equivalence

Possibilities for cellular differentiation mechanism:

1. During development, cells discard genes not needed in their later development.

   Corollary: Only germ cells retain all genes.

   (A few examples known of chromosomal loss during development: Antibody genes in immune cells.)

2. Differentiated cells retain all genes, but only use some.

Evidence for Genomic Equivalence

Early evidence:

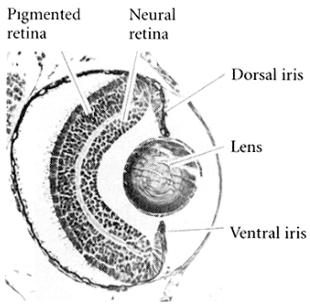
Cytogenetics

- all cells appeared to have same chromososmes

Metaplasia

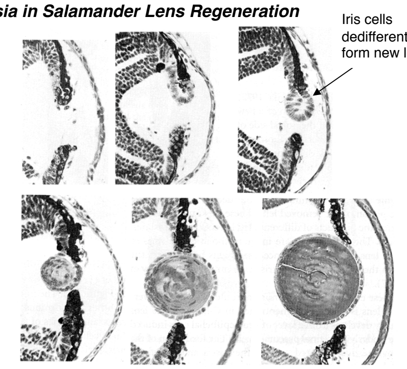
- differentiated cells 'de-differentiate,' generate other differentiated cell types
  (seen in regeneration, also some cancers)

Metaplasia in Salamander Lens Regeneration



Normal Unoperated Eye

Metaplasia in Salamander Lens Regeneration



Lens fully regenerated

Evidence for Genomic Equivalence

The ultimate evidence: ***Animal Cloning***

reproducing a complete organism from a single differentiated adult nucleus

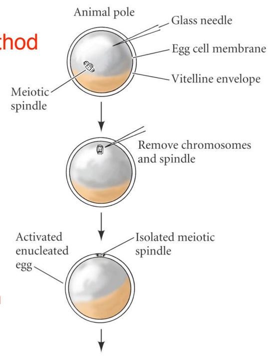
First accomplished with frogs:

Briggs & King (1950's) - work with *Rana*

Gurdon (1960's) - work with *Xenopus*

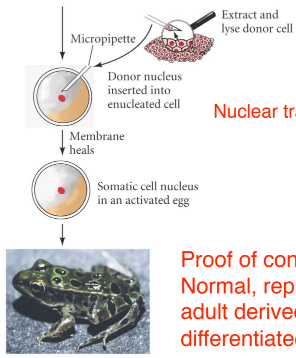
Figure 4.5(1) ***Procedure for Transplanting Blastula Nuclei into Activated Enucleated* Rana pipiens *Eggs***

Frog cloning method



Egg Enucleation

Figure 4.5(2) ***Procedure for Transplanting Blastula Nuclei into Activated Enucleated* Rana pipiens *Eggs***

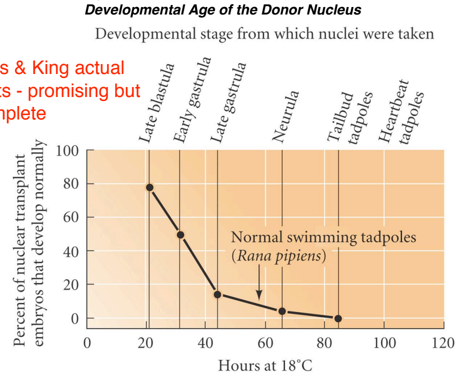


Nuclear transplantation

Proof of concept: Normal, reproductive adult derived from differentiated nucleus

Figure 4.6 ***Percentage of Successful Nuclear Transplants as a Function of the Developmental Age of the Donor Nucleus***

Briggs & King actual results - promising but incomplete



Briggs & King Results

Keywords: Totipotent

Pluripotent

- Progressive loss of nuclear potency
- Significant pluripotency exists in embryonic nuclei

John Gurdon's cloning experiments

- used *Xenopus* vs. *Rana*
- used serial transplantation of embryonic nuclei
- used donor and host nuclei of different genotypes

Figure 4.7 ***A Clone of*** **Xenopus laevis** ***Frogs***

Gurdon + *Xenopus* + serial transplantation: ultimate success - cloned frogs

The re-emergence of cloning:

Dolly the cloned sheep, 1997

Figure 4.8(1) ***Cloned Mammals, Whose Nuclei Came From Adult Somatic Cells***

Dolly with offspring

DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 4.8 (Part 1) Sinauer Associates, Inc.

The re-emergence of cloning:
Dolly the cloned sheep, 1997

Mammalian cloning, previously thought impossible, achieved by Wilmut et al.

Technical achievement:
Sheep mammary cells in G_0 fused with enucleated egg

Reignites long-dormant debate about cloning

Figure 4.8(2) ***Cloned Mammals, Whose Nuclei Came From Adult Somatic Cells***

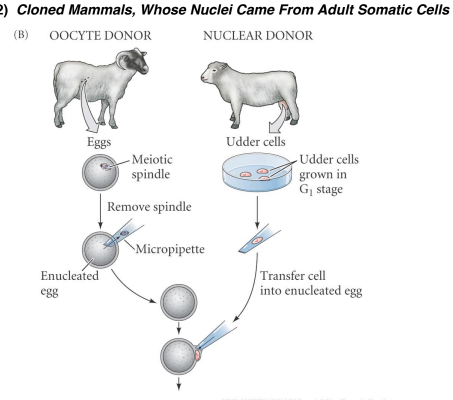
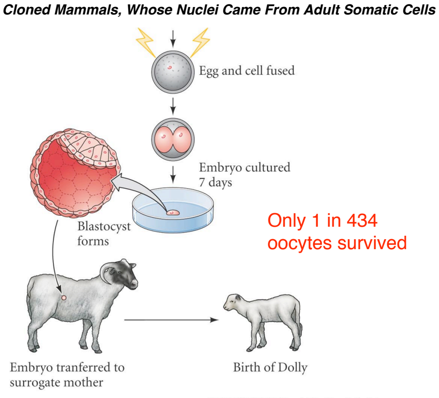


Figure 4.8(3) ***Cloned Mammals, Whose Nuclei Came From Adult Somatic Cells***



Only 1 in 434 oocytes survived

Other mammals cloned in quick succession after 'Dolly' (1997)

1998 - Cow, Mouse
2000 - Goat, Pig
2002 - Cat
2003 - Rat, Mule, Horse
2004 - Deer
2005 – Dog

Nov 2007 – Rhesus monkey
nuclear transfer blastocysts

Rhesus monkey nuclear transfer blastocysts

nature International weekly journal of science

Nature , | doi:10.1038/nature06357; Published online 14 November 2007

Producing primate embryonic stem cells by somatic cell nuclear transfer

J Byrne, D Pedersen, L Clepper, M Nelson, W Sanger, S Gokhale, D Wolf & S Mitalipov

Derivation of embryonic stem (ES) cells genetically identical to a patient by somatic cell nuclear transfer (SCNT) holds the potential to cure or alleviate the symptoms of many degenerative diseases while circumventing concerns regarding rejection by the host immune system. However, the concept has only been achieved in the mouse, whereas inefficient reprogramming and poor embryonic development characterizes the results obtained in primates. Here, we used a modified SCNT approach to produce rhesus macaque blastocysts from adult skin fibroblasts, and successfully isolated two ES cell lines from these embryos. DNA analysis confirmed that nuclear DNA was identical to donor somatic cells and that mitochondrial DNA originated from oocytes. Both cell lines exhibited normal ES cell morphology, expressed key stem-cell markers, were transcriptionally similar to control ES cells and differentiated into multiple cell types in vitro and in vivo. Our results represent successful nuclear reprogramming of adult somatic cells into pluripotent ES cells and demonstrate proof-of-concept for therapeutic cloning in primates.

Cloned mammals have problems

BBC NEWS WORLD EDITION

You are in: Science/Nature

Friday, 14 February, 2003, 20:28 GMT

Dolly the sheep clone dies young

Dolly the Sheep was born in 1996

Dolly the sheep, who became famous as the first mammal to be cloned from an adult cell, has died.

The news was confirmed on Friday by the Roslin Institute, the Scottish research centre which created her.

A decision was taken to "euthanase" six-year-old Dolly after a veterinary examination showed that she had a progressive lung disease, the institute said in a statement.

Dolly became the first mammal clone when she was born on 5 July 1996.

She was revealed to the public the following year.

> **She was not old - by sheep standards - to have been put down**
> Dr Patrick Dixon, expert on ethics of human cloning

Post-mortem

Dr Harry Griffin, from the institute, said: "Sheep can live to 11 or 12 years of age and lung infections are common in older sheep,

WATCH/LISTEN ON THIS STORY
The BBC's Pallab Ghosh
"Her ultimate legacy is the start of a scientific revolution"

See also:
- 14 Feb 03 | Science/Nature Goodbye Dolly
- 14 Feb 03 | Science/Nature Scientists wait for Dolly's cause of death
- 04 Jan 02 | Science/Nature Cloned sheep Dolly has arthritis
- 04 Jan 02 | UK Head-to-head: Cloning
- 10 Sep 98 | Science/Nature How Dolly became a cash cow
- 30 May 00 | Science/Nature Listen to public, says Dolly scientist
- 27 May 99 | Science/Nature Is Dolly old before her time?
- 21 Dec 97 | Science/Nature First there was Dolly...

Internet links:
- Roslin Institute

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Top Science/Nature stories now:
- Date for first Australians
- Fifth closest star discovered
- Mona Lisa smile secrets revealed
- The gene that maketh man?
- Gravity wave detector all set

Or not…

First Cloned Mouse Dies Of Old Age

Cumulina Was Two Years, Seven Months Old

HONOLULU May 10, 2000

E-MAIL STORY | PRINT STORY | SPHERE | SHARE | TEXT SIZE: A A A

One of these mice may be Cumulina. Who can tell? **(AP)**

(CBS) Cumulina, the world's first cloned mouse, has died of old age.

The University of Hawaii medical school said that Cumulina died in her sleep last Friday of natural causes. The mouse was two years, seven months old - about seven months above average. That corresponds to age 95 in human years, the university said in a statement.

The mouse made headlines when the results of the distinctive cloning technique of Dr. Ryuzo Yanagimachi's team were reported in the journal *Nature* in July 1998.

The University of Hawaii medical school said that Cumulina died in her sleep last Friday of natural causes. The mouse was two years, seven months old – about seven months above average. That corresponds to age 95 in human years, the university said in a statement.

Cloning long dead animals?

Proceedings of the National Academy of Sciences of the United States of America

Production of healthy cloned mice from bodies frozen at −20°C for 16 years

Sayaka Wakayama[a], Hiroshi Ohta[a], Takafusa Hikichi[a], Eiji Mizutani[a], Takamasa Iwaki[b], Osami Kanagawa[c], and Teruhiko Wakayama[a,1]

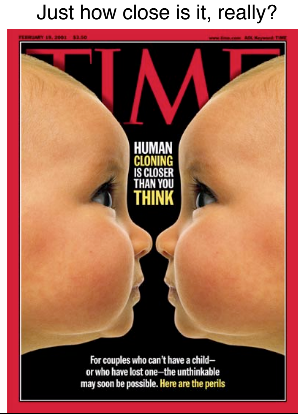
PNAS November 11, 2008 vol. 105 no. 45 17318–17322

Abstract

Cloning animals by nuclear transfer provides an opportunity to preserve endangered mammalian species. However, it has been suggested that the "resurrection" of frozen extinct species (such as the woolly mammoth) is impracticable, as no live cells are available, and the genomic material that remains is inevitably degraded. Here we report production of cloned mice from bodies kept frozen at −20 °C for up to 16 years without any cryoprotection. As all of the cells were ruptured after thawing, we used a modified cloning method and examined nuclei from several organs for use in nuclear transfer attempts. Using brain nuclei as nuclear donors, we established embryonic stem cell lines from the cloned embryos. Healthy cloned mice were then produced from these nuclear transferred embryonic stem cells by serial nuclear transfer. Thus, nuclear transfer techniques could be used to "resurrect" animals or maintain valuable genomic stocks from tissues frozen for prolonged periods without any cryopreservation.

brain cloning cryopreservation nuclear transfer reprogramming

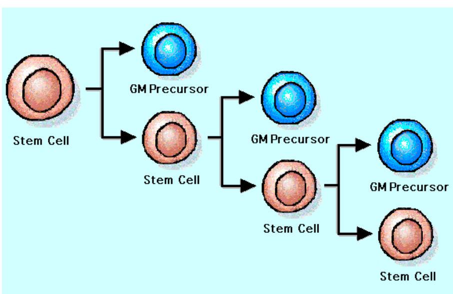
Just how close is it, really?



a closely related topic:

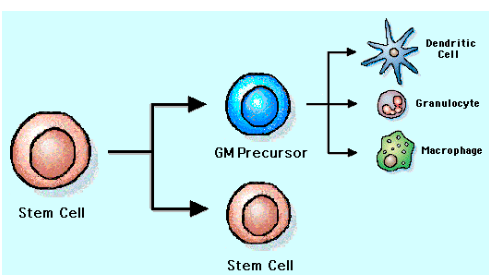
Embryonic Stem Cells

Stem cells



Stem cells can divide indefinitely...

Stem cells



Stem cells can divide indefinitely,
and give rise to differentiated cell types

Typical Stem Cell Populations

- Hematopoietic stem cells (in bone marrow and circulation)
- Epidermal stem cells
- Intestinal crypt cells
- Germ cells (producing sperm and eggs)

Problems with Adult Stem Cells

- Rare
- Difficult to identify and isolate
- Limited potential to make other cell types (plasticity)
- Limited capacity for self-renewal

If only we were newts.

Before Amputation | 0 7 21 25 32 42 72

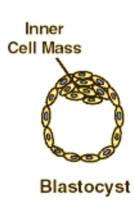
Days Post-Amputation

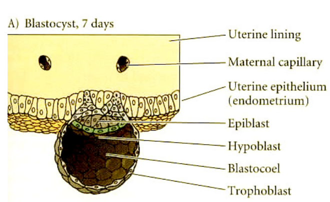
Stem cells offer the hope of replacing, renewing, regenerating or repairing any cell, tissue or organ in the body.

The Ultimate Stem Cell

The fertilized egg (zygote) is
totipotent -
capable of giving rise to every cell type found in the body, and to all extraembryonic tissues
(i.e., the placenta etc.)

Pluripotent Embryonic Stem Cells come from the **Inner Cell Mass** or **Epiblast** of the early embryo

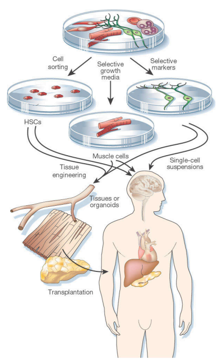


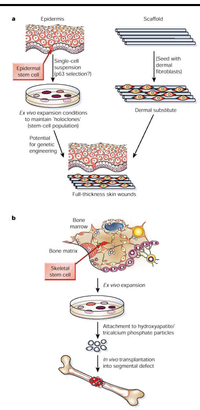


Pluripotent Stem Cells

- Capable of giving rise to all body cell types
- Unable to generate trophoblast-derived placental tissues
- Maintain normal genetic makeup (karyotype)
- Capable of indefinite self-renewal
- Can remain undifferentiated (without signal)

Using Embryonic Stem Cells

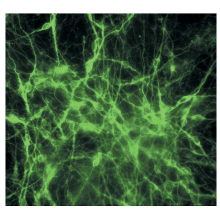




Using Embryonic Stem Cells

Potential therapeutic uses of stem cells to repair the nervous system

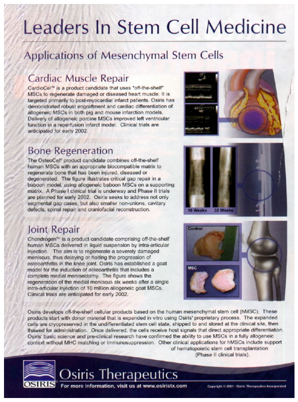
- Parkinson's disease
- Huntington's disease
- Spinal cord injury
- Stroke
- Multiple sclerosis



Dopaminergic nerve cells derived from mouse ES cells

Stem Cell-derived Therapies are Here

OSIRIS



Problems with Embryonic Stem Cells

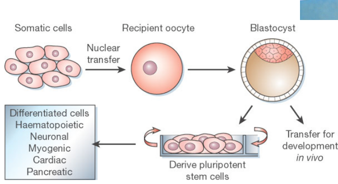
Ethical issues of human embryo manipulation and destruction

Safety concerns with most/all stem cells:

- Immune system rejection of grafts
- Regulation of growth and differentiation
- Transmission of infectious agents
- Long-term safety and efficacy

Solving Technical Problems

Therapeutic cloning (but, new ethical issues)



Genetic manipulation of ES cells to avoid immune system rejection of grafts

Other options: iPS cells

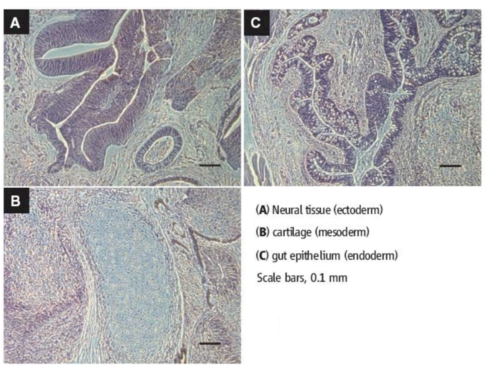
www.sciencemag.org SCIENCE VOL 318 21 DECEMBER 2007

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,[1,2]* Maxim A. Vodyanik,[2] Kim Smuga-Otto,[1,2] Jessica Antosiewicz-Bourget,[1,2] Jennifer L. Frane,[1] Shulan Tian,[3] Jeff Nie,[3] Gudrun A. Jonsdottir,[3] Victor Ruotti,[3] Ron Stewart,[3] Igor I. Slukvin,[2,4] James A. Thomson[1,2,5]*

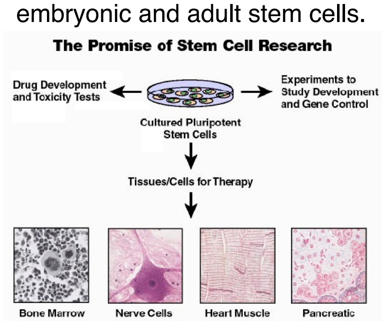
Somatic cell nuclear transfer allows trans-acting factors present in the mammalian oocyte to reprogram somatic cell nuclei to an undifferentiated state. We show that four factors (*OCT4*, *SOX2*, *NANOG*, and *LIN28*) are sufficient to reprogram human somatic cells to pluripotent stem cells that exhibit the essential characteristics of embryonic stem (ES) cells. These induced pluripotent human stem cells have normal karyotypes, express telomerase activity, express cell surface markers and genes that characterize human ES cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers. Such induced pluripotent human cell lines should be useful in the production of new disease models and in drug development, as well as for applications in transplantation medicine, once technical limitations (for example, mutation through viral integration) are eliminated.

iPSC-derived tissues in teratomas



(A) Neural tissue (ectoderm)
(B) cartilage (mesoderm)
(C) gut epithelium (endoderm)
Scale bars, 0.1 mm

The coming years will show the promise and controversies of human embryonic and adult stem cells.



Scientific Uses of Embryonic Stem Cells

Scientific Uses of Embryonic Stem Cells

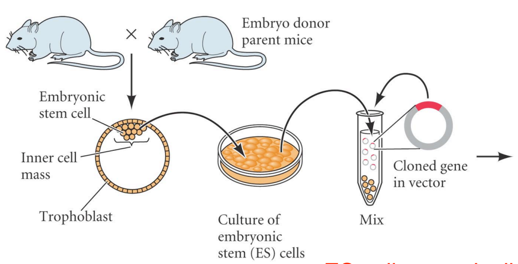
Creation of **Transgenic** Organisms

[Transgenic: having experimentally altered genetic material by transfer of DNA from an external source.]

Method:

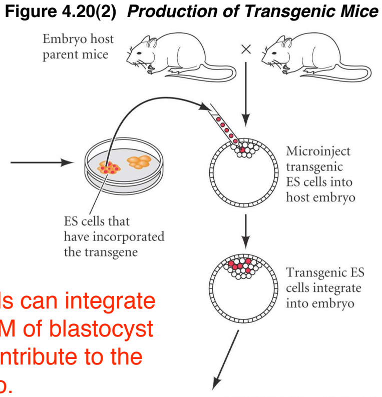
- ES cells isolated, genetically altered *in vitro*
- ES cells re-inserted into embryo to create chimera.
- Chimera reproduces to create pure transgenic

Figure 4.20(1) ***Production of Transgenic Mice***



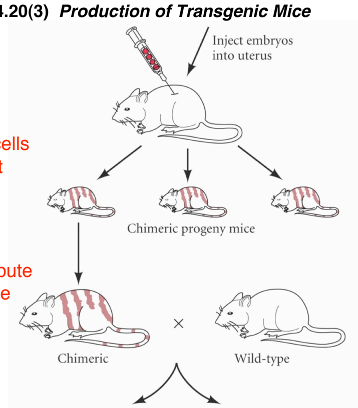
ES cells genetically altered *in vitro*

Figure 4.20(2) ***Production of Transgenic Mice***



ES cells can integrate into ICM of blastocyst and contribute to the embryo.

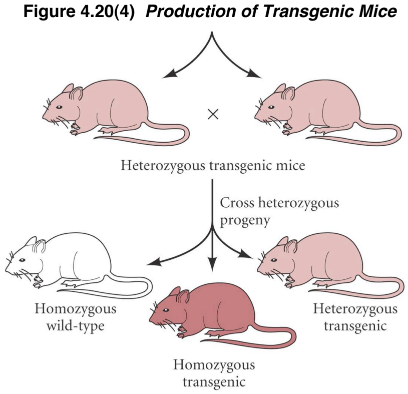
Figure 4.20(3) *Production of Transgenic Mice*



Chimera has cells of two different genotypes

If ES cells contribute to germline, some gametes are transgenic

Figure 4.20(4) *Production of Transgenic Mice*



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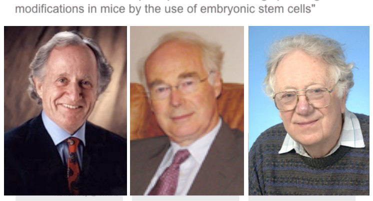
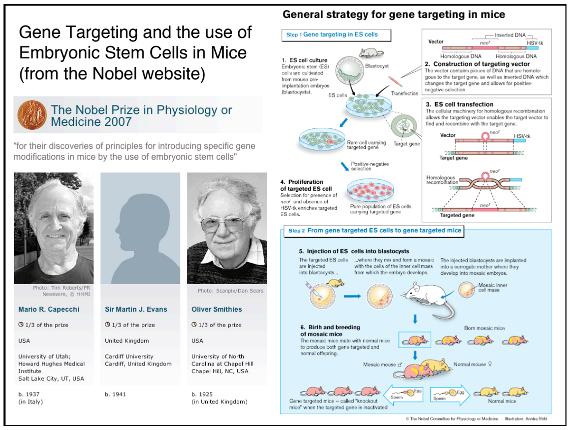
The Nobel Prize in Physiology or Medicine 2007

"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"



Mario R. Capecchi	Sir Martin J. Evans	Oliver Smithies
1/3 of the prize	1/3 of the prize	1/3 of the prize
USA	United Kingdom	USA
University of Utah; Howard Hughes Medical Institute Salt Lake City, UT, USA	Cardiff University Cardiff, United Kingdom	University of North Carolina at Chapel Hill Chapel Hill, NC, USA
b. 1937 (in Italy)	b. 1941	b. 1925 (in United Kingdom)


Gene Targeting and the use of Embryonic Stem Cells in Mice (from the Nobel website)
The Nobel Prize in Physiology or Medicine 2007
"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"
Photo: Tim Roberts/PR Newswire, © HHMI
Photo: Scanpix/Dan Sears
Mario R. Capecchi
1/3 of the prize
USA
University of Utah; Howard Hughes Medical Institute Salt Lake City, UT, USA
b. 1937 (in Italy)
Sir Martin J. Evans
1/3 of the prize
United Kingdom
Cardiff University Cardiff, United Kingdom
b. 1941
Oliver Smithies
1/3 of the prize
USA
University of North Carolina at Chapel Hill Chapel Hill, NC, USA
b. 1925 (in United Kingdom)
General strategy for gene targeting in mice
Step 1 Gene targeting in ES cells
1. ES cell culture
Embryonic stem (ES) cells are cultivated from mouse pre-implantation embryos (blastocysts).
Blastocyst
ES cells
Transfection
Vector
Inserted DNA
HSV-tk
Homologous DNA
Homologous DNA
2. Construction of targeting vector
The vector contains pieces of DNA that are homologous to the target gene, as well as inserted DNA which changes the target gene and allows for positive-negative selection
3. ES cell transfection
The cellular machinery for homologous recombination allows the targeting vector enables the target vector to find and recombine with the target gene.
Rare cell carrying targeted gene
Target gene
Positive-negative selection
4. Proliferation of targeted ES cell
Selection for presence of neo and absence of HSV-tk enriches targeted ES cells.
Pure population of ES cells carrying targeted gene
Homologous recombination
Targeted gene
Step 2 From gene targeted ES cells to gene targeted mice
5. Injection of ES cells into blastocysts
The targeted ES cells are injected into blastocysts...
...where they mix and form a mosaic with the cells of the inner cell mass from which the embryo develops.
The injected blastocysts are implanted into a surrogate mother where they develop into mosaic embryos.
Mosaic inner cell mass
6. Birth and breeding of mosaic mice
The mosaic mice mate with normal mice to produce both gene targeted and normal offspring.
Born mosaic mice
Mosaic mouse ♂
Normal mouse ♀
Sperm
Egg
Gene targeted mice – called "knockout mice" when the targeted gene is inactivated
Normal mice
© The Nobel Committee for Physiology or Medicine
Illustration: Annika Röhl

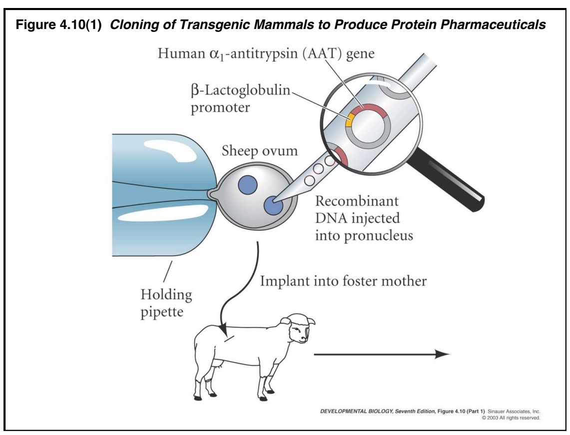


Figure 4.10(1) Cloning of Transgenic Mammals to Produce Protein Pharmaceuticals
Human α1-antitrypsin (AAT) gene
β-Lactoglobulin promoter
Sheep ovum
Recombinant DNA injected into pronucleus
Holding pipette
Implant into foster mother
DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 4.10 (Part 1) Sinauer Associates, Inc. © 2003 All rights reserved.

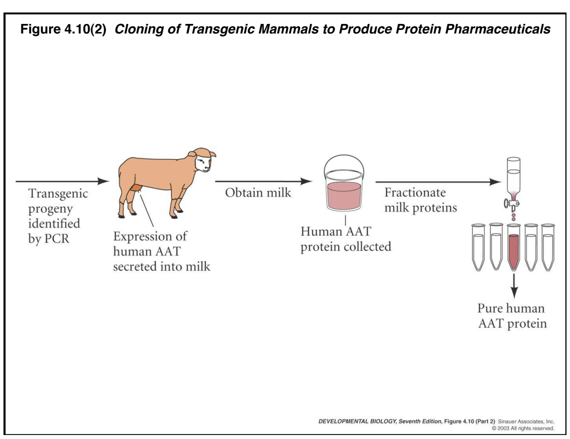


Figure 4.10(2) Cloning of Transgenic Mammals to Produce Protein Pharmaceuticals
Transgenic progeny identified by PCR
Expression of human AAT secreted into milk
Obtain milk
Human AAT protein collected
Fractionate milk proteins
Pure human AAT protein
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