Human Embryonic and Pluripotent Stem Cells and Cloning

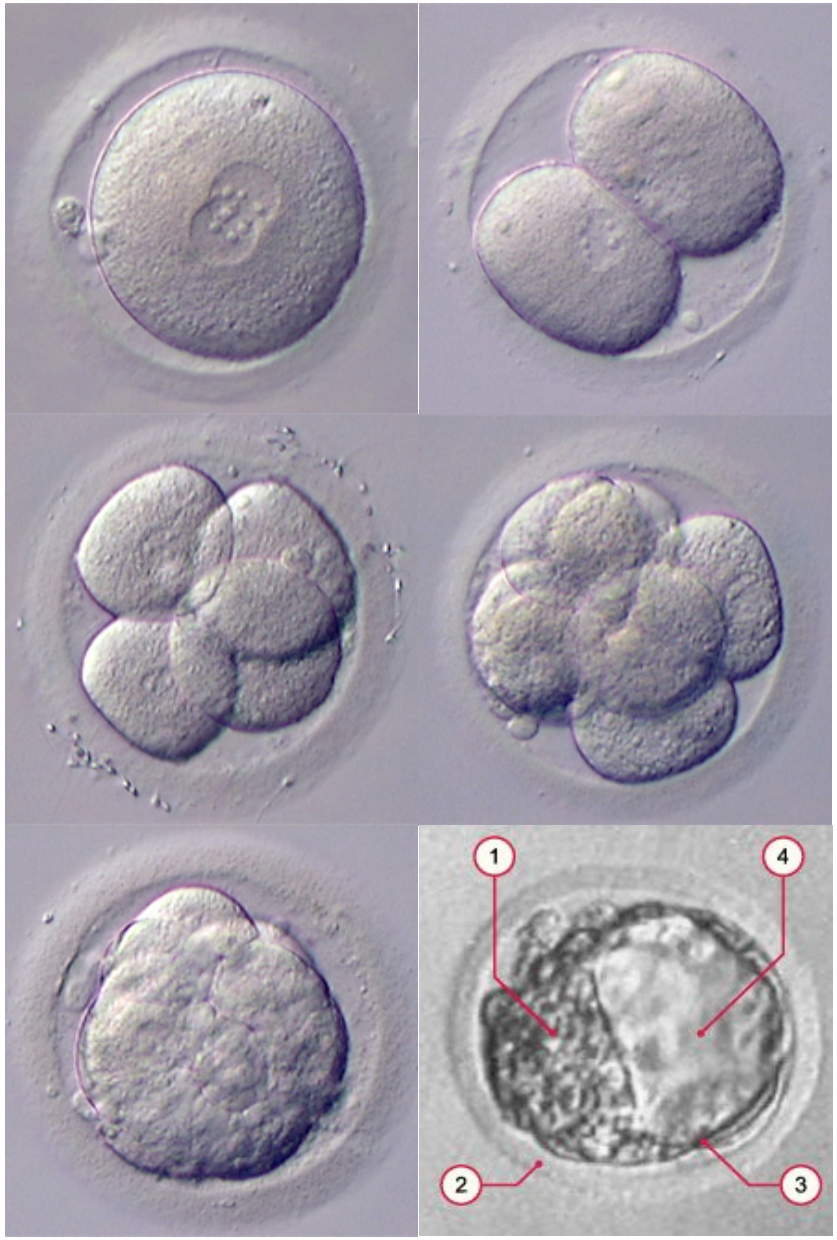
Techniques:

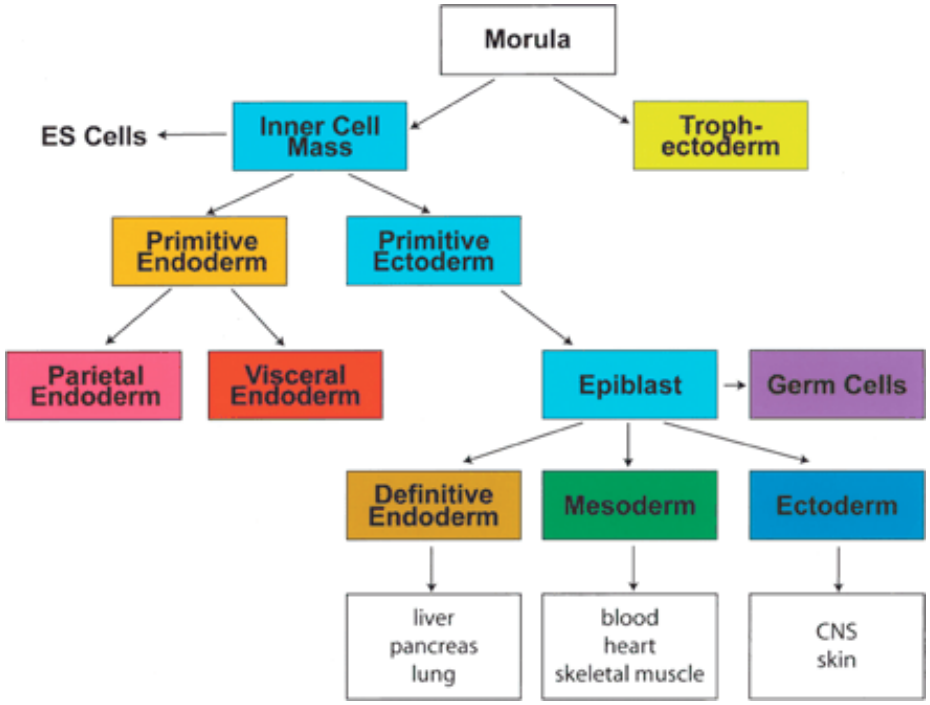
Embryo/Blastocyst-Derived

Nuclear Transplantation (Dolly type)

Induced Pluripotent Cells

Early Development and the Pluripotent Inner Cell Mass





http://www.biology-online.org

Blastocyst and inner cell mass

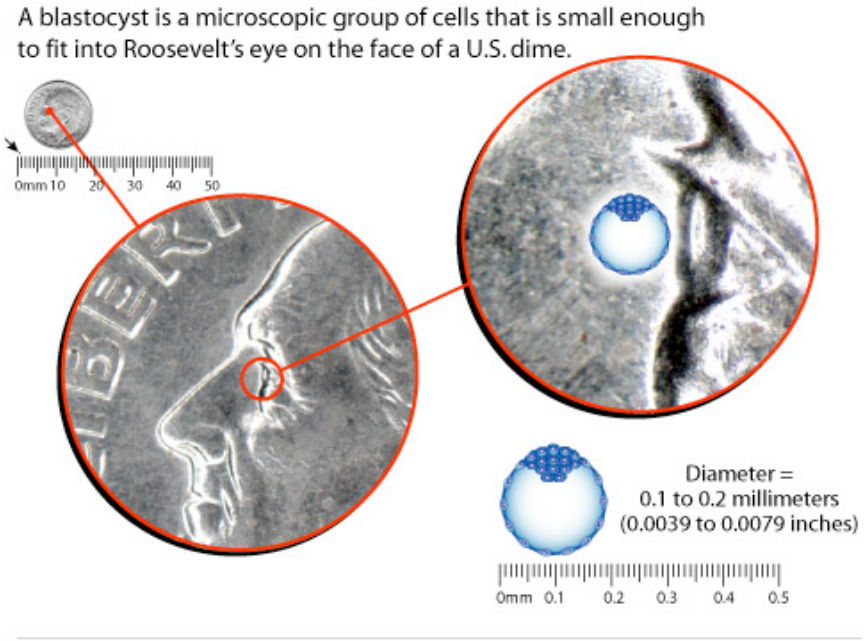
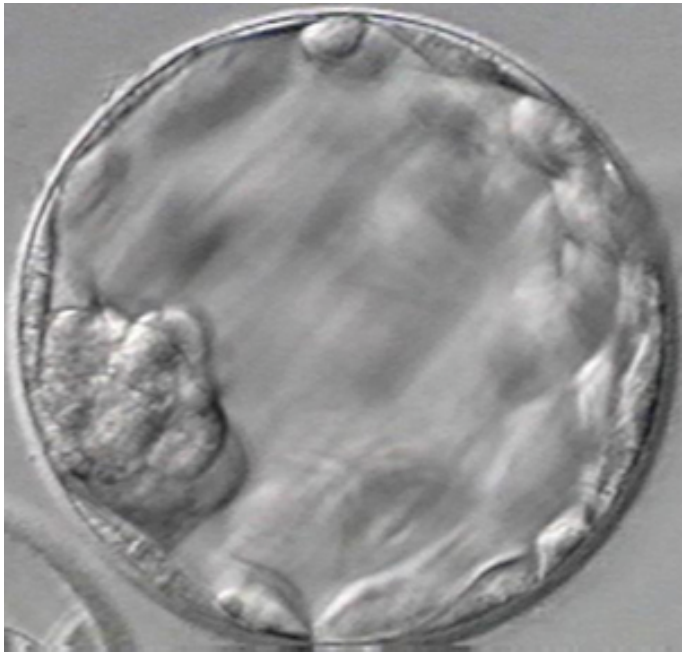


Illustration by Cell Imaging Core of the Center for Reproductive Sciences.



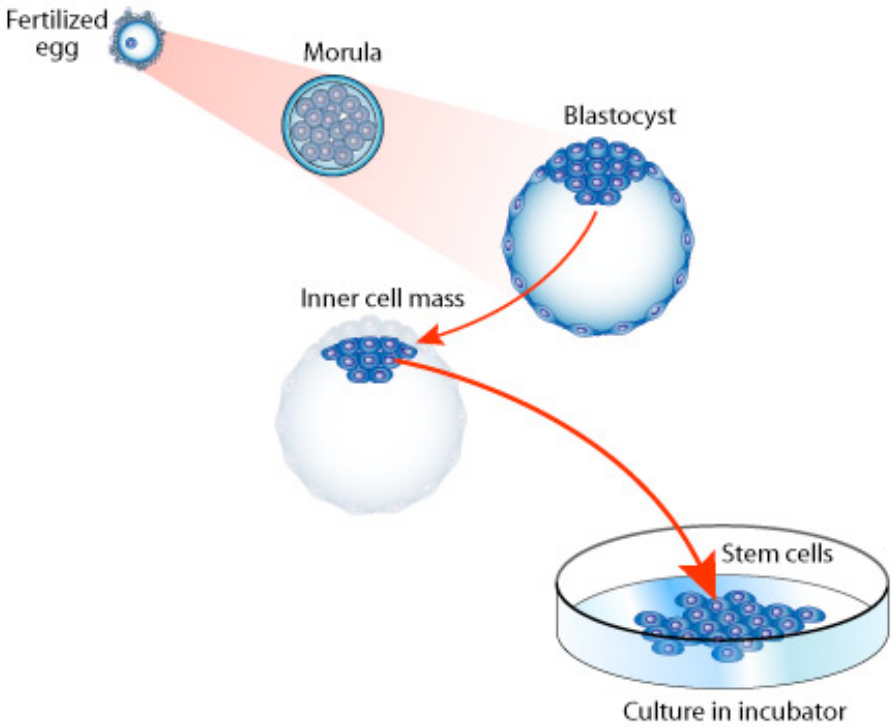


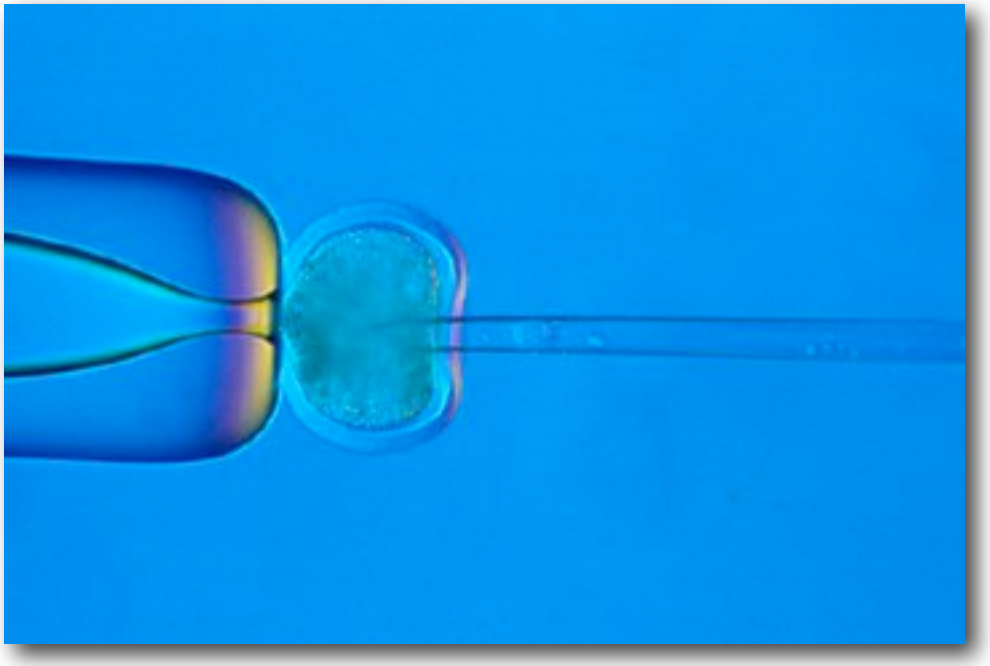
Illustration by Cell Imaging Core of the Center for Reproductive Sciences.

Embryo-derived ES cells

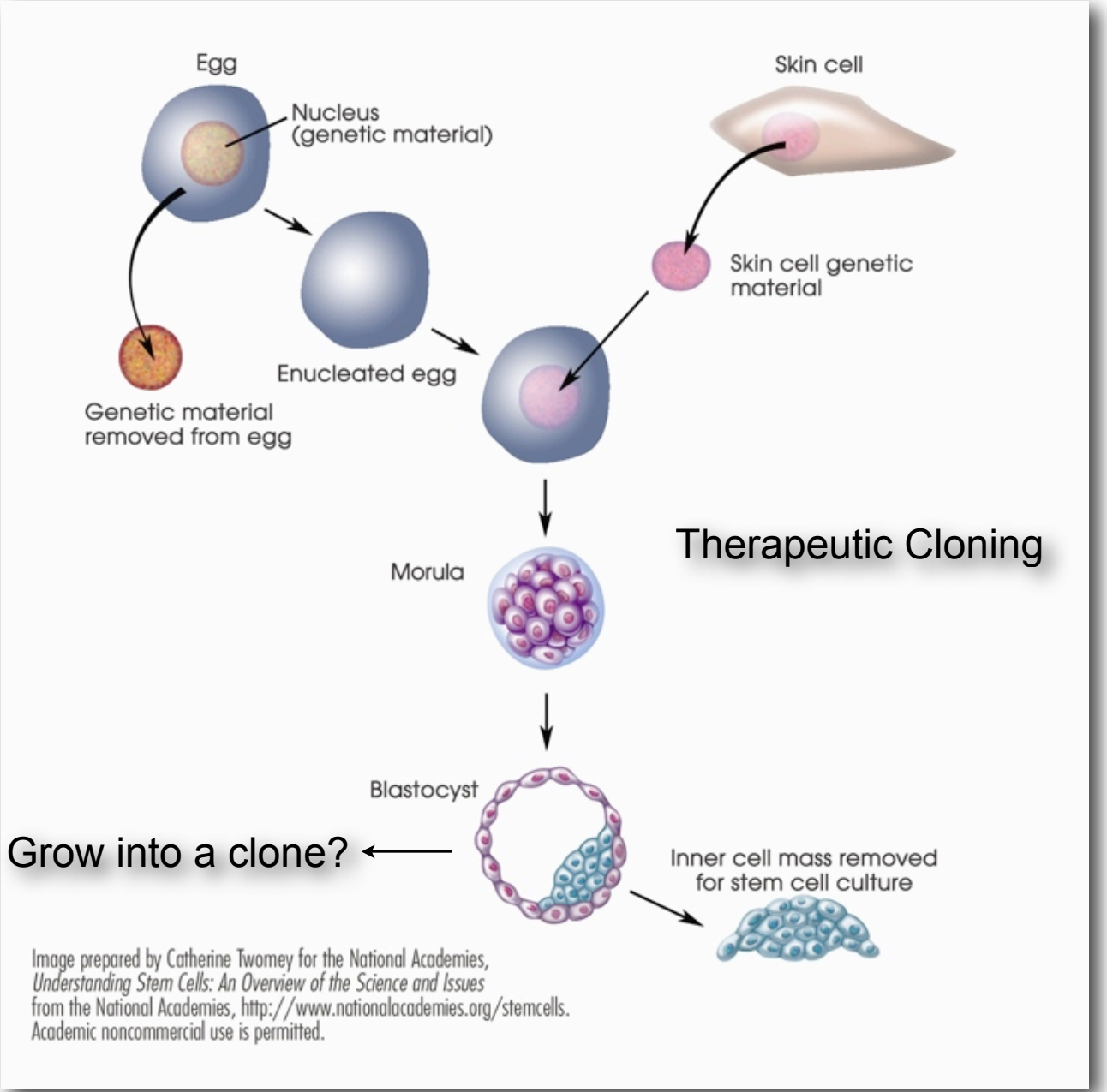
Pros:
• Derived from tissue most comparable to the “real” pluripotent stem cells

Cons:
• You need human blastocysts (IVF clinics, “egg harvesting”)
• The genetic makeup is different form the patient you would like to use them in
• Until last week use and funding was severely limited by federal regulations

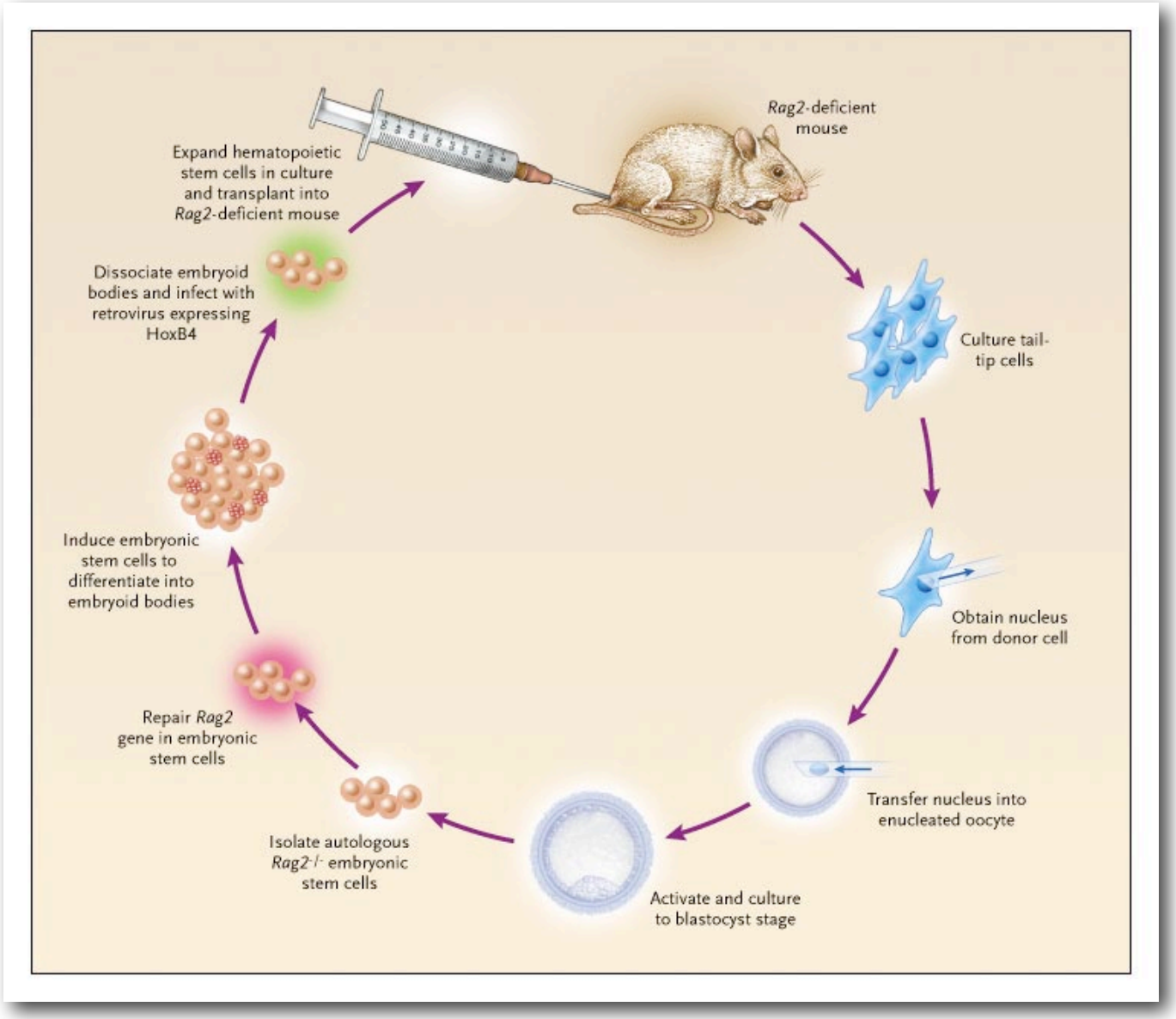
Nuclear Transplantation



Nuclear Transplantation



Therapeutic Cloning



Nuclear transfer

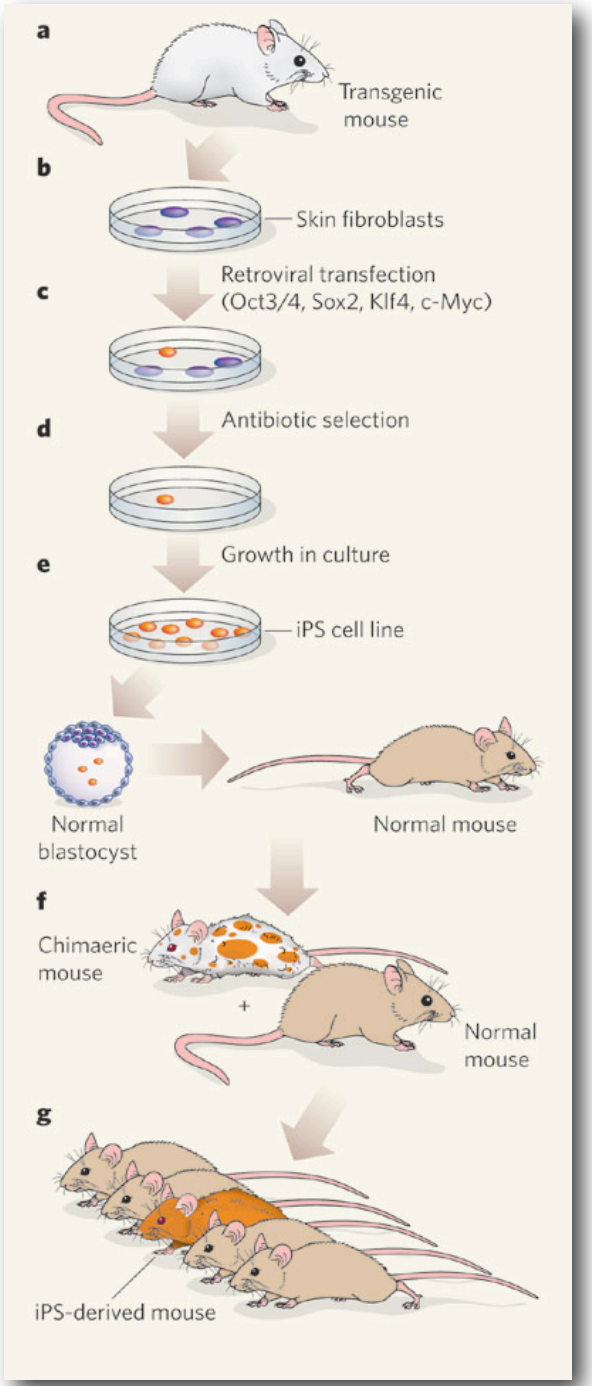
Pros:

- ES cells have the same genetic makeup as the donor of the nucleus and could be better for transplantation purposes
- No destruction of normal (human) blastocyst is required

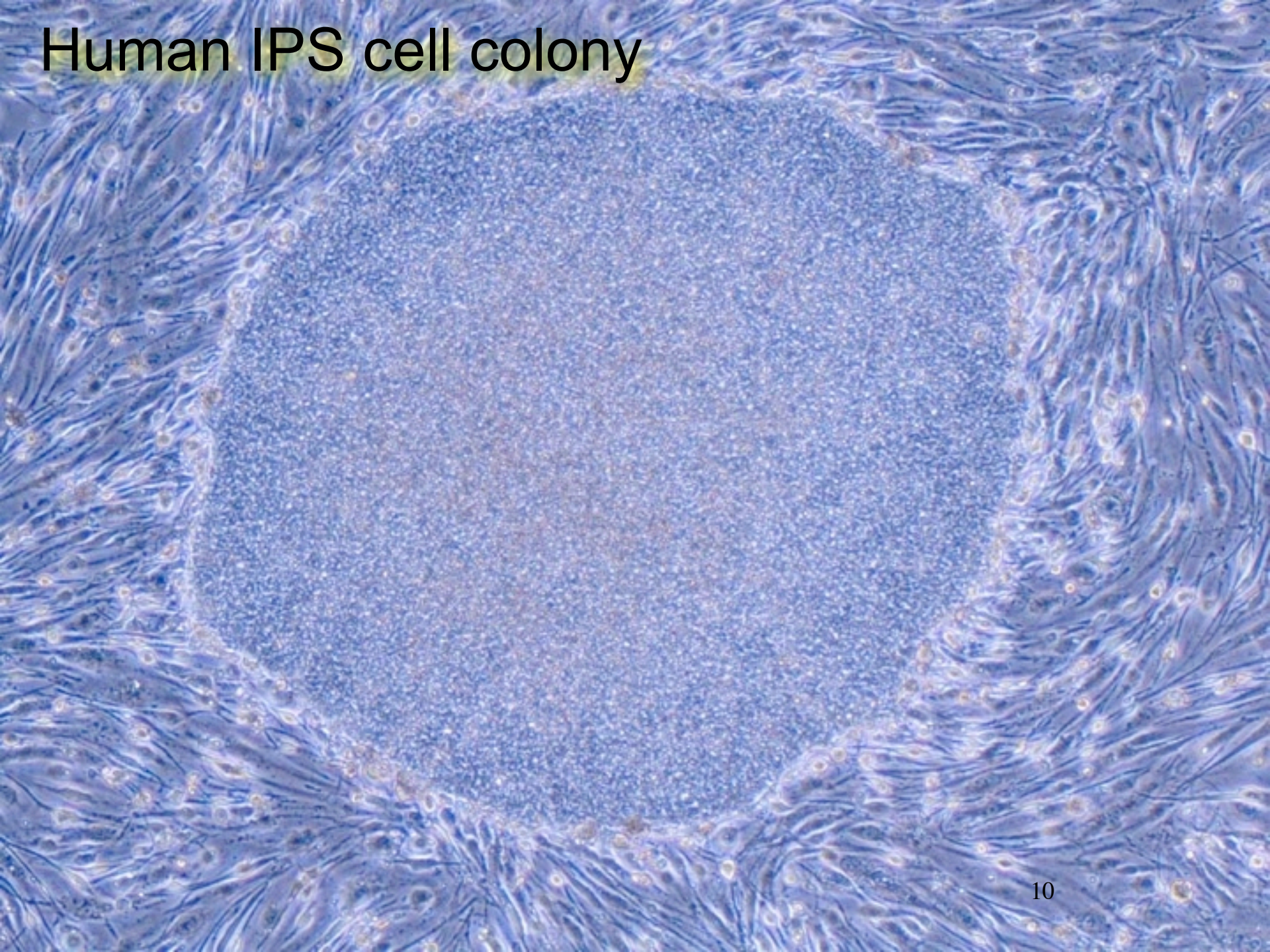
Cons:

- Nucleus is “old” and is likely damaged and altered
- Donor eggs are needed
- Process is VERY inefficient, you need a lot of eggs, transfers and pseudopregnant females to get one single clone

Induced Pluripotent Stem Cells



Human IPS cell colony



IPS cells

Pros:

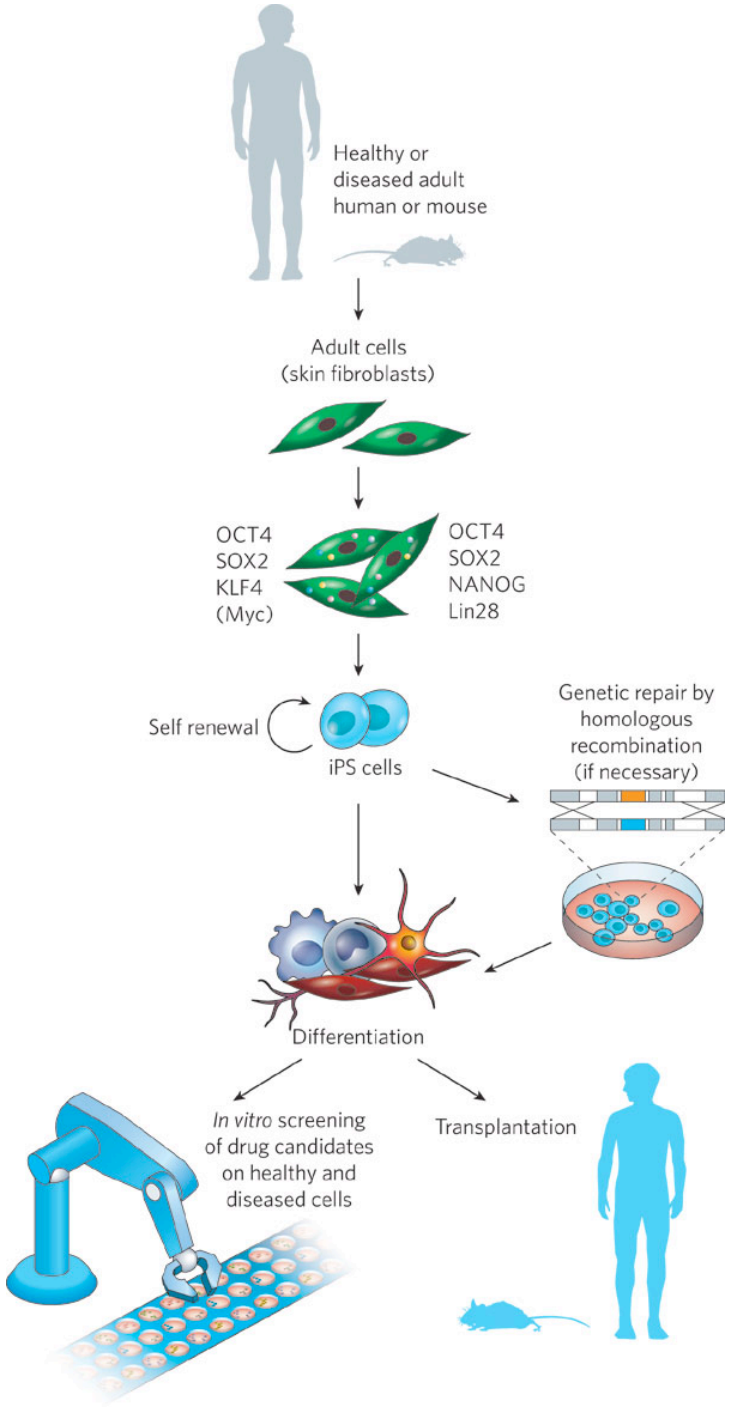
- IPS cells have the same genetic makeup as the donor of the nucleus and could be better for transplantation purposes
- No destruction of normal human blastocyst is required
- No eggs are needed
- Process is very efficient
- Likely the best source for “personalized regenerative medicine”

Cons:

- Nucleus is “old” and is likely damaged and altered
- Genetic manipulation is required (cancer?)

The future of IPS cells?

- Make your “own” rejuvenated tissues
- Repair defective genes



How About Clones?

What are clones?

Distinct organisms with identical DNA formed via asexual reproduction

monozygotic twins (0.2% of the human population) are clones

- **ES cells/IPS cells:**

  Blastocyst injections

  works in mice likely in other animals
- **Nuclear Transplantation**

  Dolly and all others

Famous first nuclear transfer products:
Dolly, Snuppy and Copy Cat

Why is my clone sick?

1. High failure rate of the cloning process

0.1% - 3% success rate.

2. Problems during later development

Cloned animals tend to suffer from "Large Offspring Syndrome" (LOS)

3. Abnormal gene expression patterns

Complete reprogramming is needed for normal or near-normal development. Incomplete programming will cause the embryo to develop abnormally or fail.

4. Telomeric differences

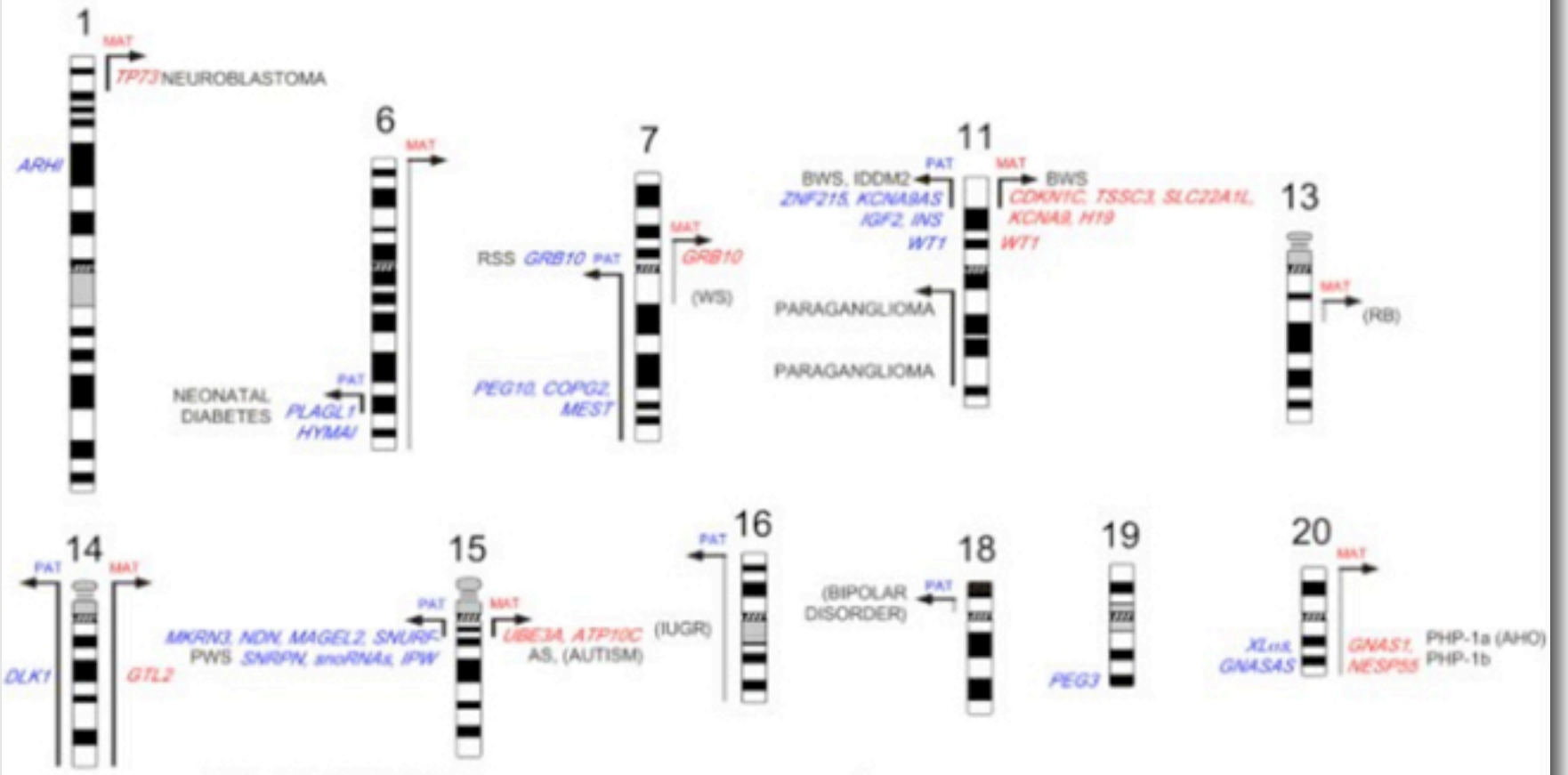
As cells divide, their chromosomes get shorter as a natural part of aging. In cloned animals, the telomeres are sometime longer, but sometimes not….

Imprinting

- In placental mammals, the DNA inherited via the egg is differently expression activity than the DNA inherited via the sperm
- Imprinting can be lost during life

Human imprinting map

http://greallyoffice.aecom.yu.edu/



RSS: Russell-Silver syndrome
WS: Williams syndrome
BWS: Beckwith-Wiedemann syndrome
IDDM: Insulin-dependent diabetes mellitus
RB: Retinoblastoma
PWS: Prader-Willi syndrome
AS: Angelman syndrome
IUGR: Intrauterine growth retardation
PHP: Pseudohypoparathyroidism

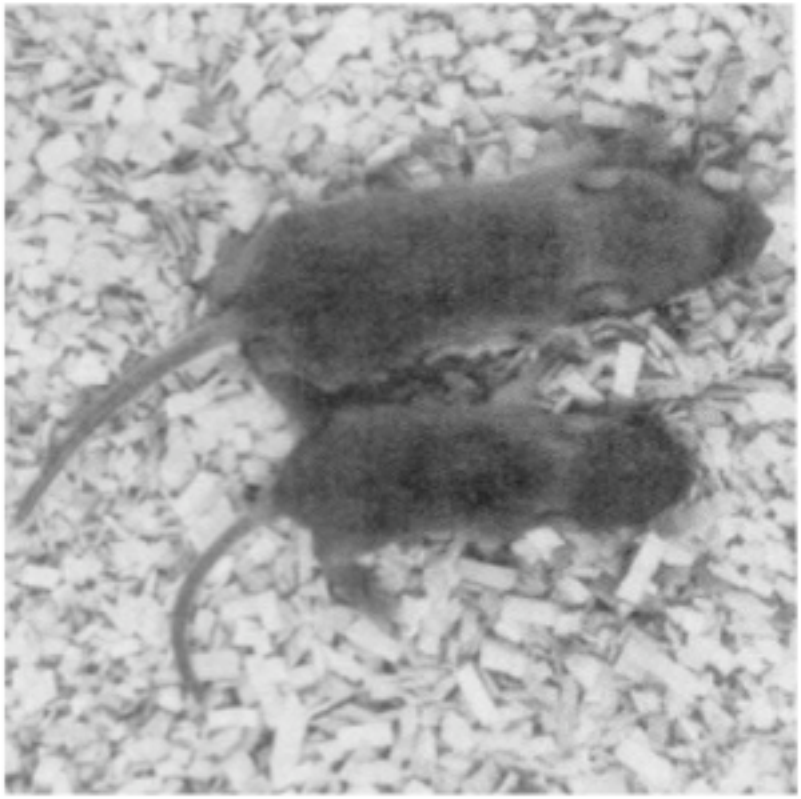
| Imprinted phenotypic effect clear

| Possible Imprinted phenotypic effect

PAT ← Evidence for paternally expressed gene(s)

MAT → Evidence for maternally expressed gene(s)

The genetics of Insulin Related Growth Factor-II (IGF-II)



IGF-II

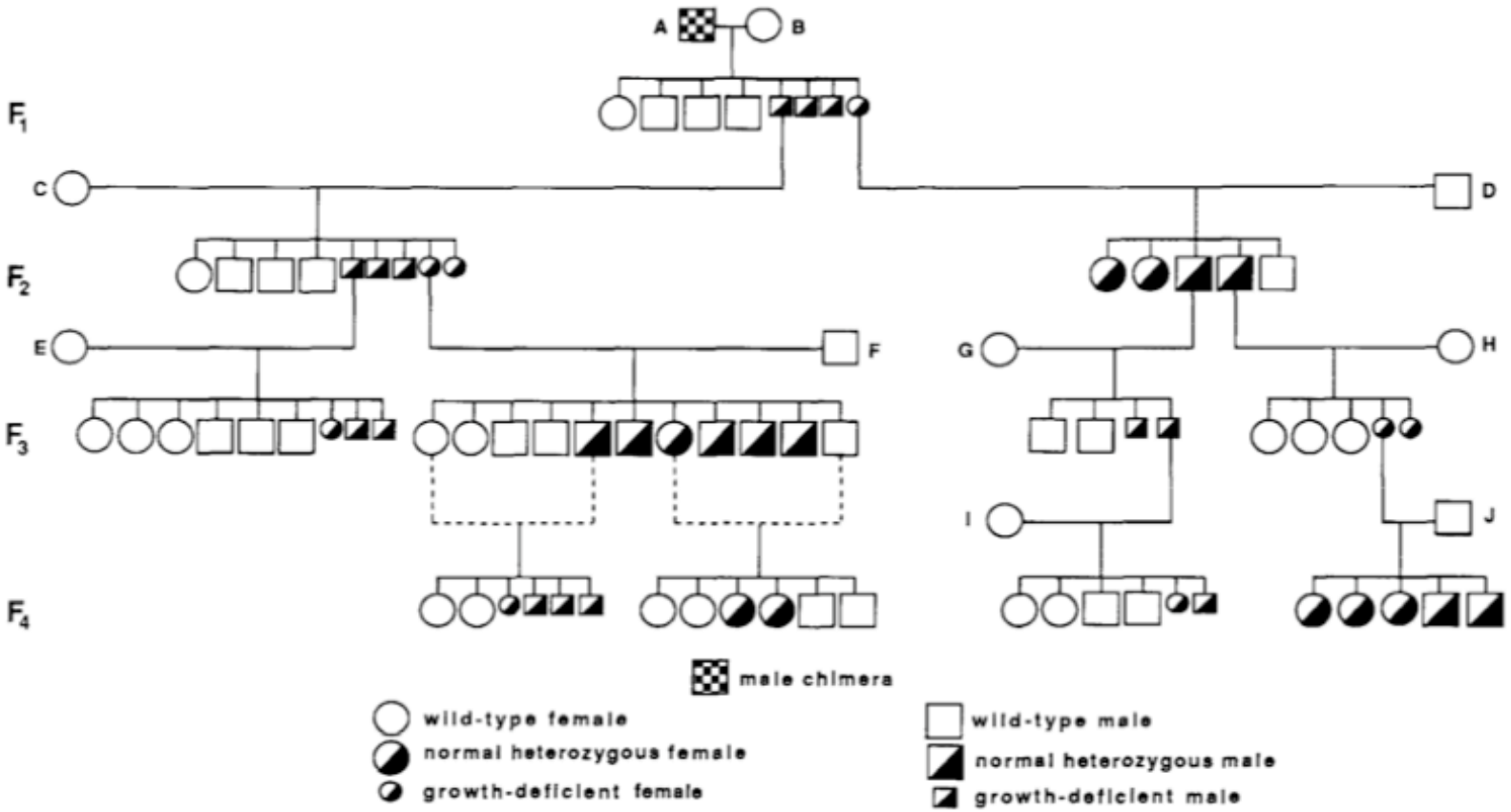


Figure 2. Partial Pedigree of IGF-II Mutant Animals

Four generations of offspring from a complete germline male chimera (animal A; DeChiara et el., 1990) are shown in a partial pedigree. The genetic backgrounds of the animals used in these crosses were: A, C, D, G, H, I, and J, 129/Sv//Ev; B, (C57BL/6J × DBA/2)F1; C, MF1; E and F, C57BL/6J. Solid and dashed lines represent outcrosses and intercrosses, respectively.

Clones are big.Why? Loss of Imprinting

Imprinting Trivia

- Are Marsupials imprinted?
- Are Birds?
- Are Monotremes?

Sec. 302. Prohibition on human cloning

(a) IN GENERAL- It shall be unlawful for any person or entity, public or private, in or affecting interstate commerce, knowingly--

(1) to perform or attempt to perform human cloning;
(2) to participate in an attempt to perform human cloning; or
(3) to ship or receive for any purpose an embryo produced by human cloning or any product derived from such embryo.

(b) IMPORTATION- It shall be unlawful for any person or entity, public or private, knowingly to import for any purpose an embryo produced by human cloning or any product derived from such embryo.

(c) PENALTIES-

(1) CRIMINAL PENALTY- Any person or entity that violates this section shall be fined under this title or imprisoned not more than 10 years, or both.
(2) CIVIL PENALTY- Any person or entity that violates any provision of this section shall be subject to, in the case of a violation that involves the derivation of a pecuniary gain, a civil penalty of not less than $1,000,000 and not more than an amount equal to the amount of the gross gain multiplied by 2, if that amount is greater than $1,000,000.

(d) SCIENTIFIC RESEARCH- Nothing in this section restricts areas of scientific research not specifically prohibited by this section, including research in the use of nuclear transfer or other cloning techniques to produce molecules, DNA, cells other than human embryos, tissues, organs, plants, or animals other than humans.'

The Neanderthal sequence is complete. Let's make one or two!

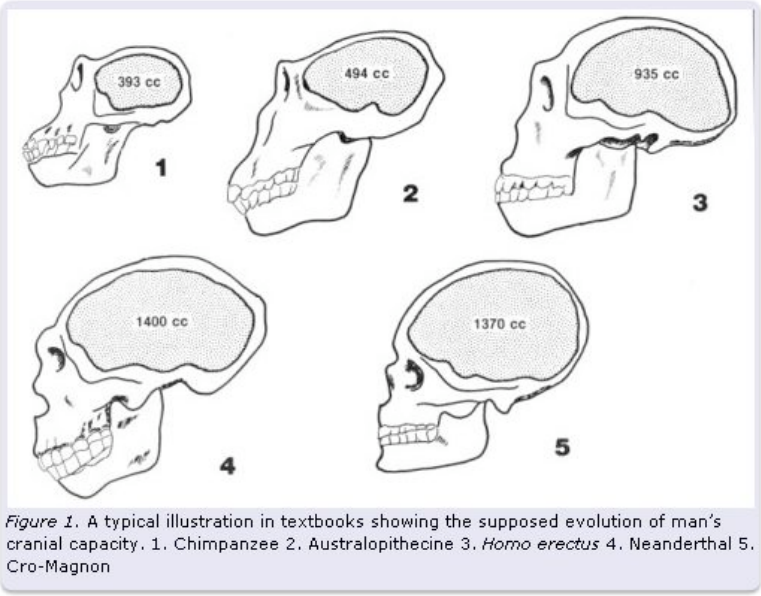
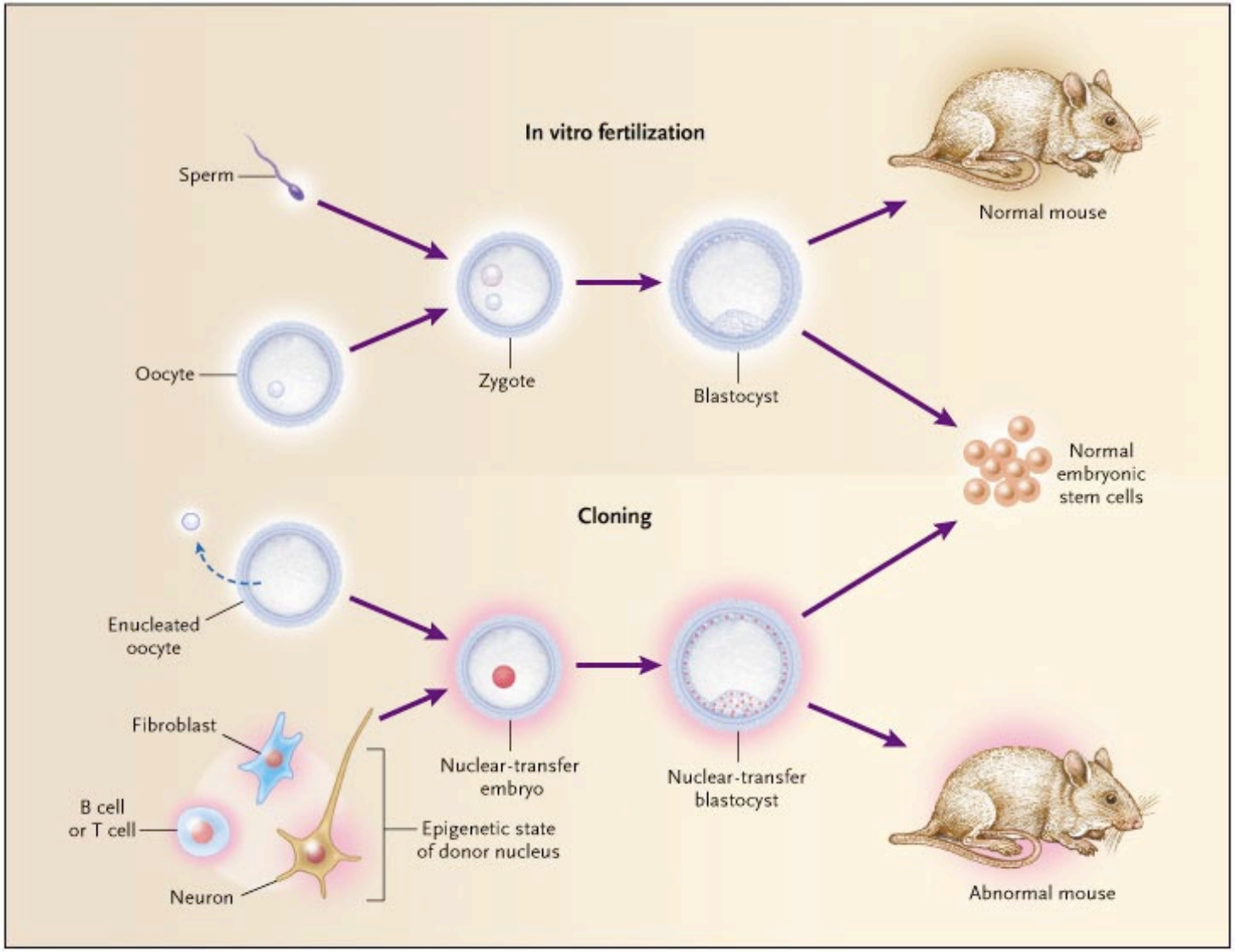
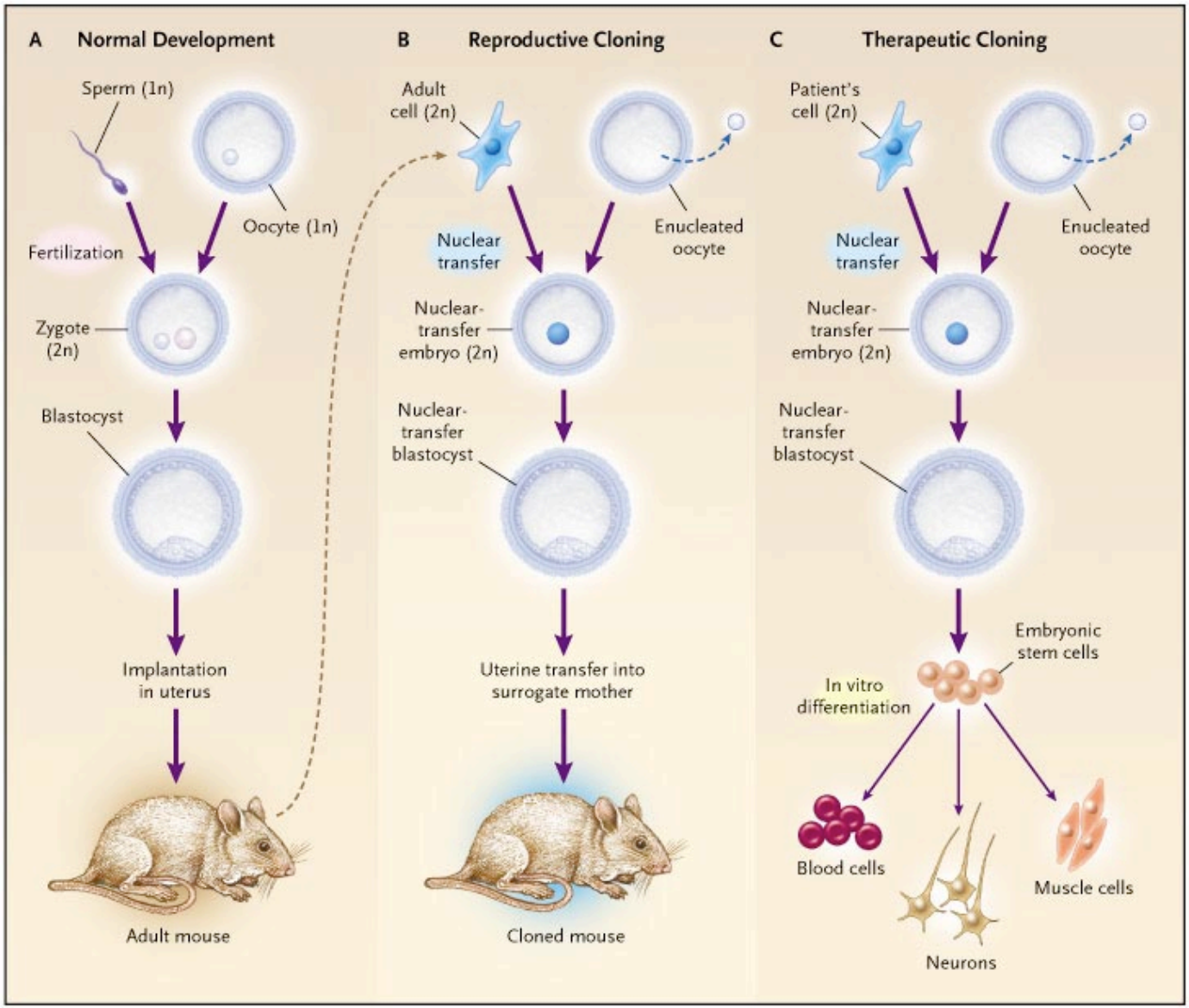


Figure 1. A typical illustration in textbooks showing the supposed evolution of man's cranial capacity. 1. Chimpanzee 2. Australopithecine 3. *Homo erectus* 4. Neanderthal 5. Cro-Magnon

Directed breeding?
Human Egg/Chimp Egg/Blastocyst?
Human/Chimp Carrier?
Human Upbringing?
Put them in a zoo/deserted island?
Educate?
Mate?


In vitro fertilization
Sperm
Oocyte
Zygote
Blastocyst
Normal mouse
Normal embryonic stem cells
Cloning
Enucleated oocyte
Fibroblast
B cell or T cell
Neuron
Epigenetic state of donor nucleus
Nuclear-transfer embryo
Nuclear-transfer blastocyst
Abnormal mouse


A Normal Development
Sperm (1n)
Oocyte (1n)
Fertilization
Zygote (2n)
Blastocyst
Implantation in uterus
Adult mouse
B Reproductive Cloning
Adult cell (2n)
Enucleated oocyte
Nuclear transfer
Nuclear-transfer embryo (2n)
Nuclear-transfer blastocyst
Uterine transfer into surrogate mother
Cloned mouse
C Therapeutic Cloning
Patient's cell (2n)
Enucleated oocyte
Nuclear transfer
Nuclear-transfer embryo (2n)
Nuclear-transfer blastocyst
Embryonic stem cells
In vitro differentiation
Blood cells
Muscle cells
Neurons