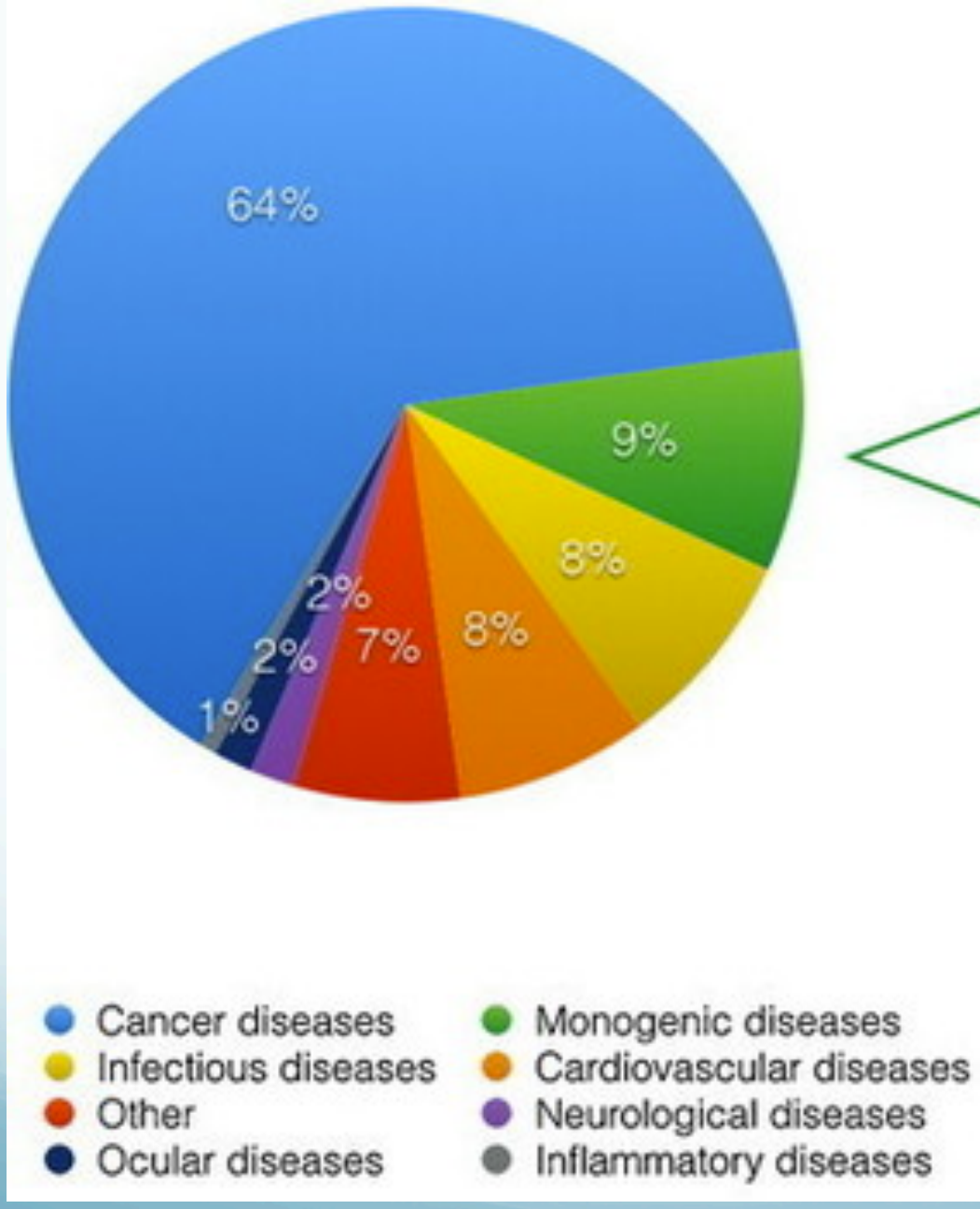
evolving
GENE THERAPY

Janoš Terzić

DAVID VETTER



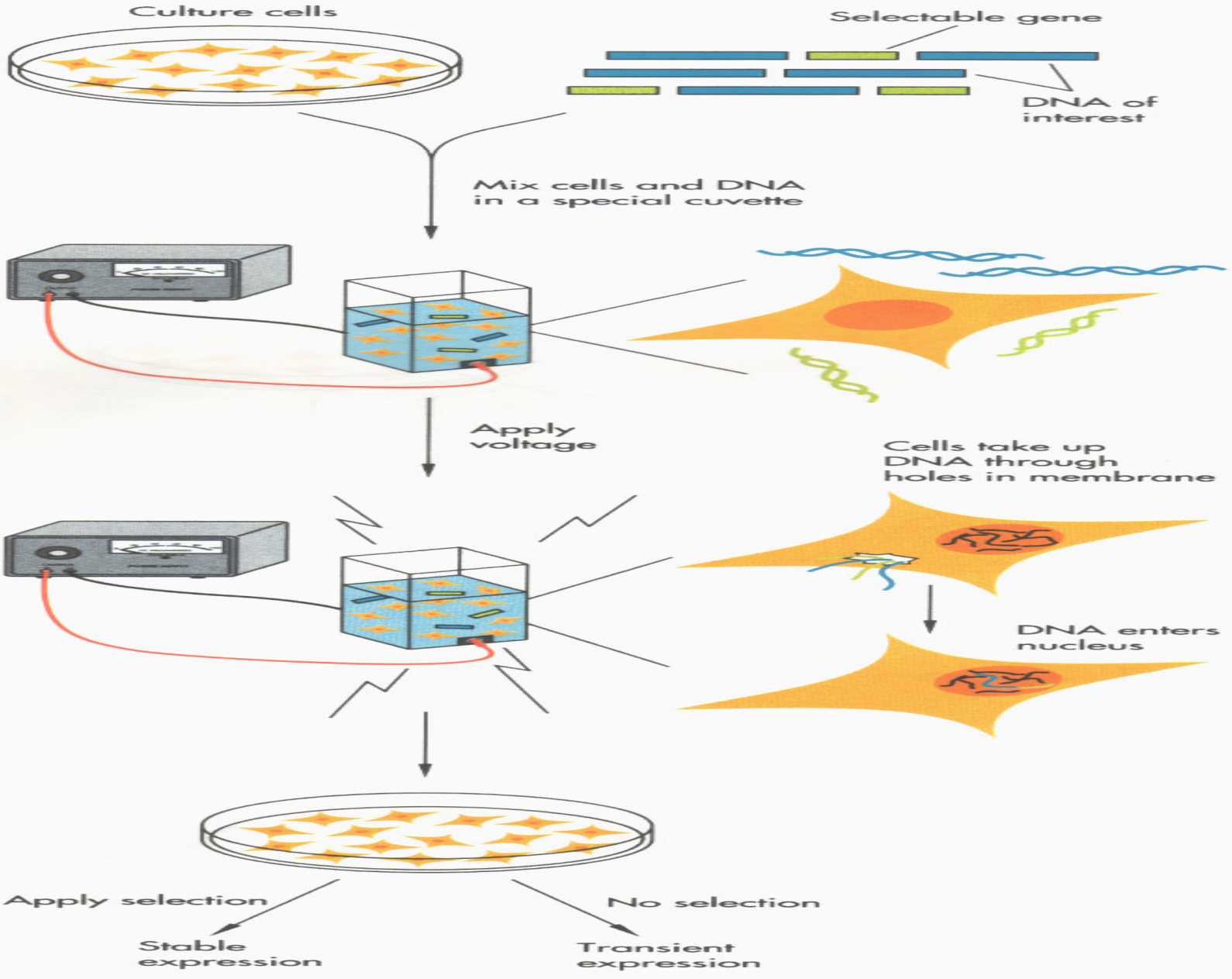
Monogenic disorders

- Adrenoleukodystrophy
- α-1 antitrypsin deficiency
- Becker muscular dystrophy
- β-thalasssaemia
- Canavan disease
- Chronic granulomatous disease
- Cystic fibrosis
- Duchenne muscular dystrophy
- Fabry disease
- Familial adenomatous polyposis
- Fanconi anaemia
- Galactosialidosis
- Gaucher's disease
- Gyrate atrophy
- Haemophilia A and B
- Hurler syndrome
- Huntington's chorea
- Junctional epidermolysis bullosa
- Late infantile neuronal ceroid lipofuscinosis
- Leukocyte adherence deficiency
- Limb girdle muscular dystrophy
- Lipoprotein lipase deficiency
- Mucopolysaccharidosis type VII
- Ornithine transcarbamylase deficiency
- Pompe disease
- Purine nuccleoside phosphorylase deficiency
- Recessive dystrophic epidermolysis bullosa
- Sickle cell disease
- Severe combined immunodeficiency
- Tay Sachs disease
- Wiskott-Aldrich syndrome

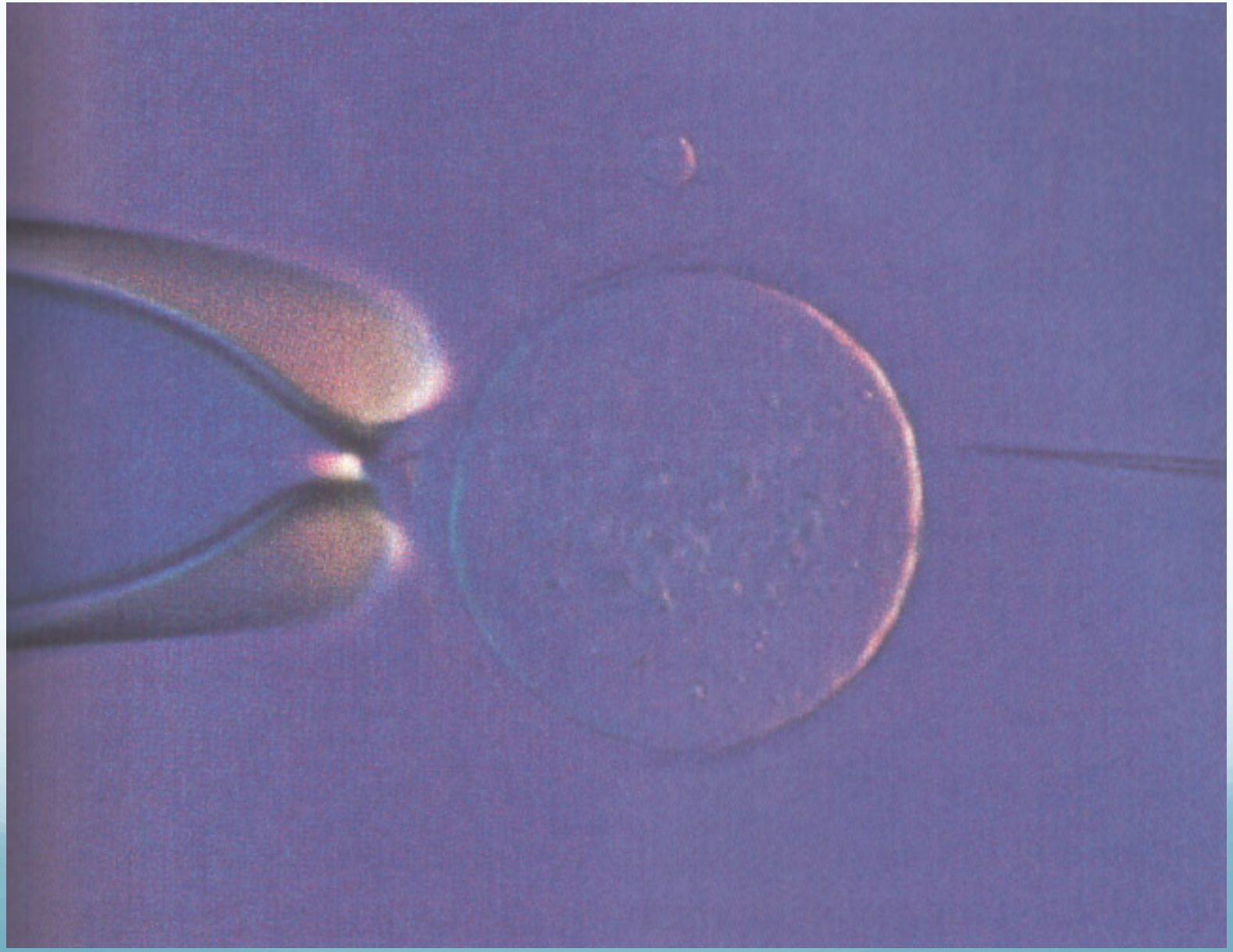
PREREQUESTS

- **Somatic cells**
- **Finished experiments on cells and animal models**
- **Only for some medical conditions**

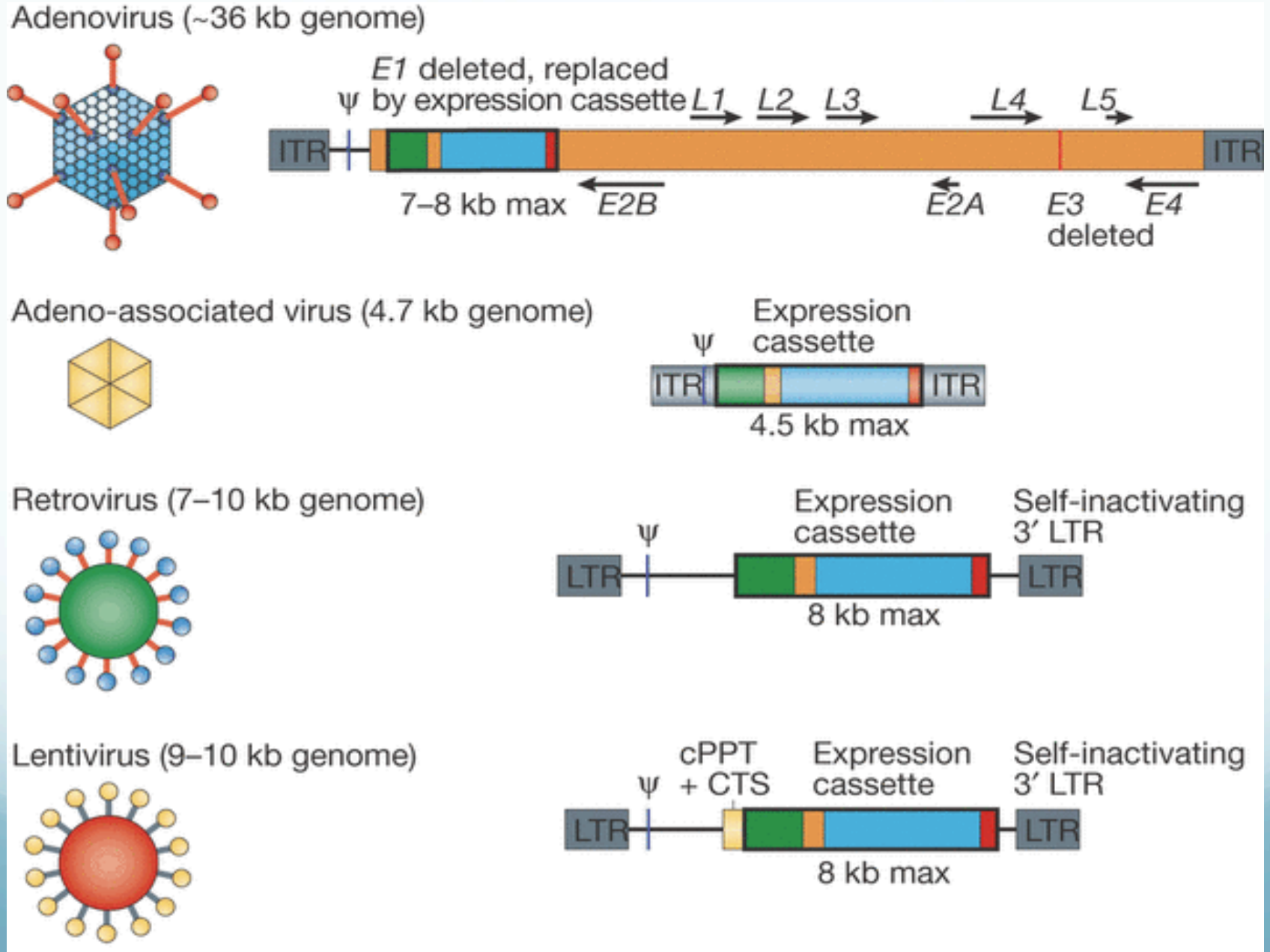
Electroporation



Microinjection

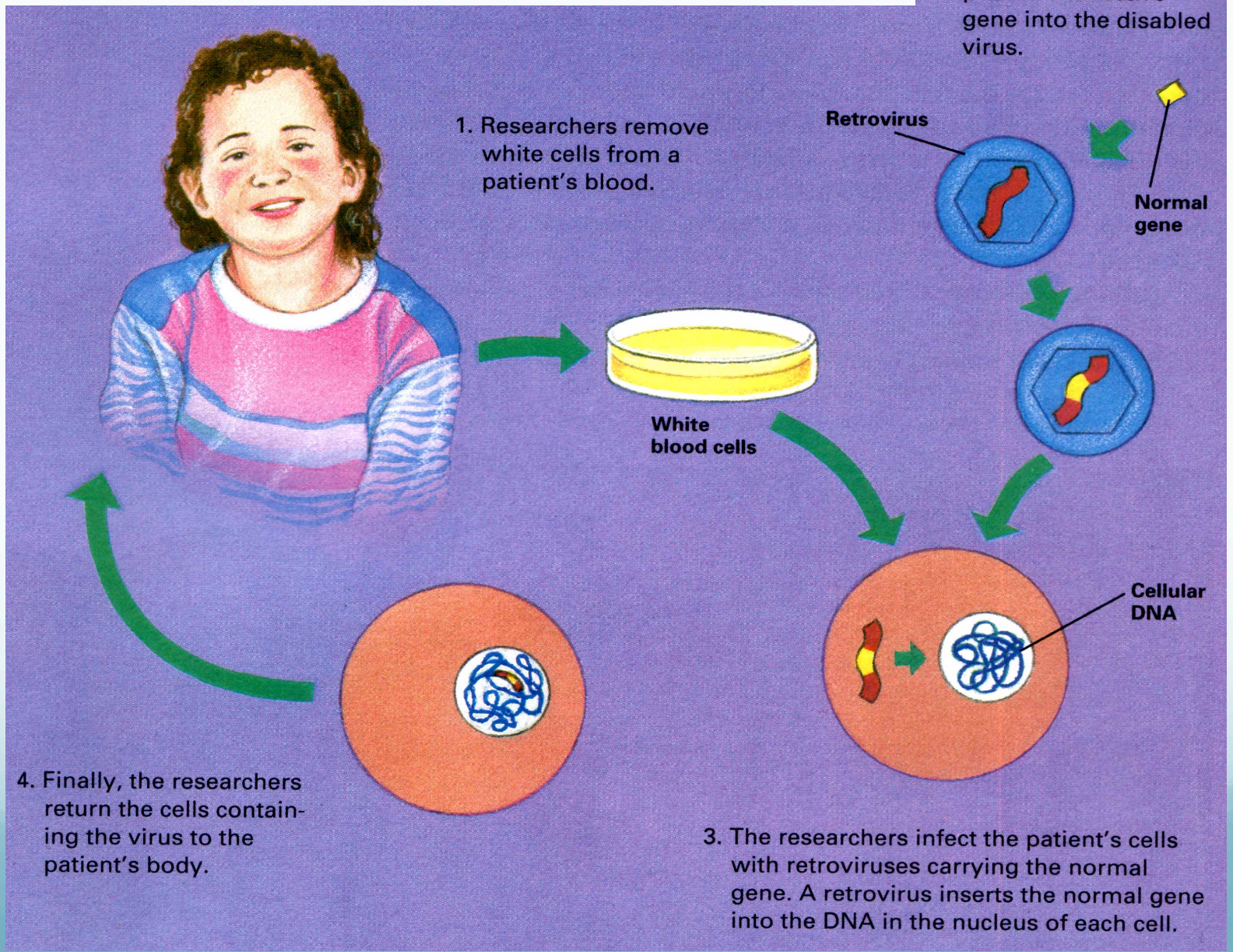


Viral vectors



1991.

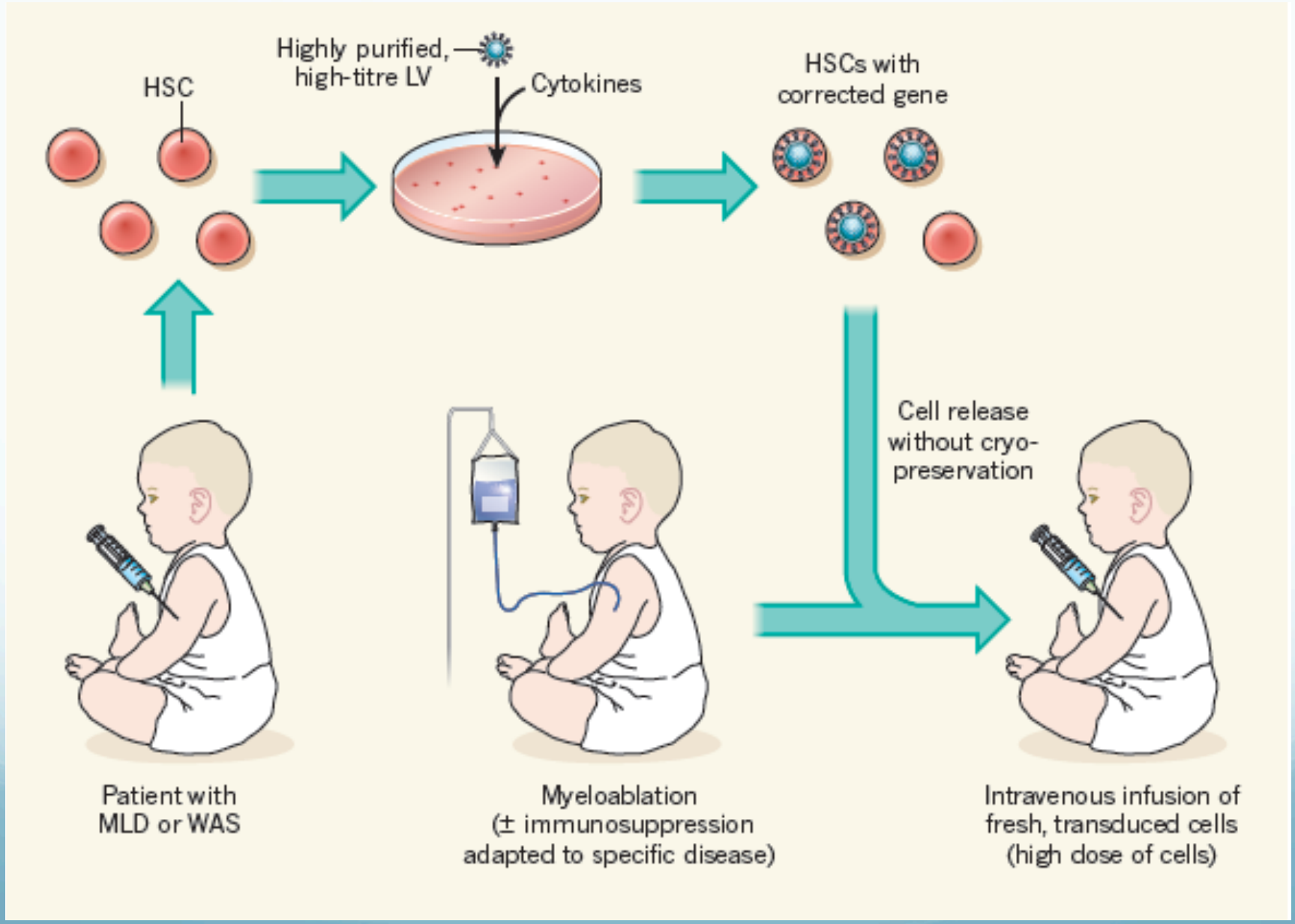
First patient: ADA deficiency



Jesse Gelsinger

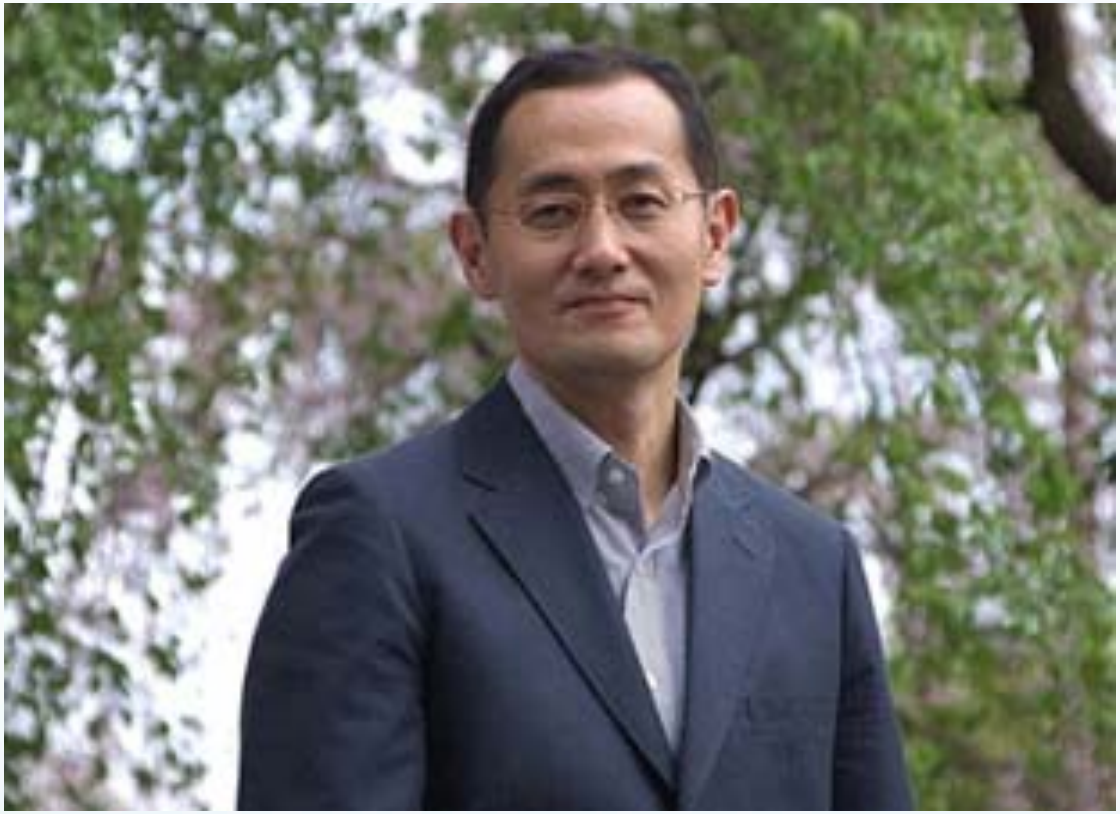
was the first person publicly identified as having died (1999)
in a clinical trial for gene therapy. He was 18 years old.

In 2013.


HSC
Highly purified, high-titre LV
Cytokines
HSCs with corrected gene
Cell release without cryo-preservation
Patient with MLD or WAS
Myeloablation (± immunosuppression adapted to specific disease)
Intravenous infusion of fresh, transduced cells (high dose of cells)

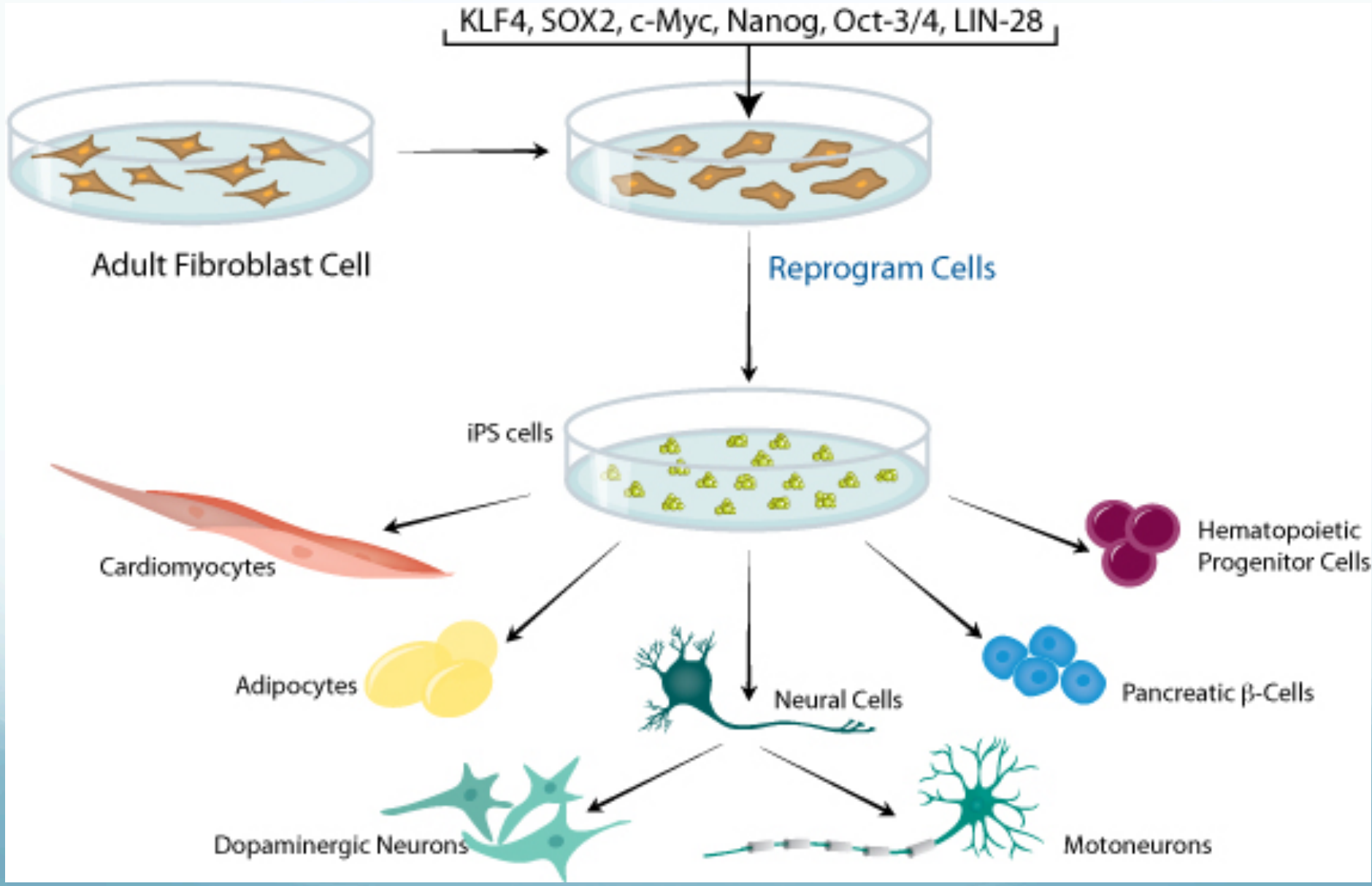
- **Metachromatic leukodystrophy**
  - Destruction of white brain matter - leukodystrophy
  - *Ex vivo* therapy with lentiviral vectors
  - 60% blood cells with vector (1 vector per cell)
  - Arilsulfatase-A: 10 x higher then in healthy people
  - Disease onset was late for 7 to 21 months
- **Wiscott-Aldrich syndrome**
  - 25%-50% k. stanica eksprimira vektor
- **Lentiviral vectors**
  - Accepts big genes
  - Easy to isolate and efficient transduction
  - Safety – integrates gene near HMGA2 gene

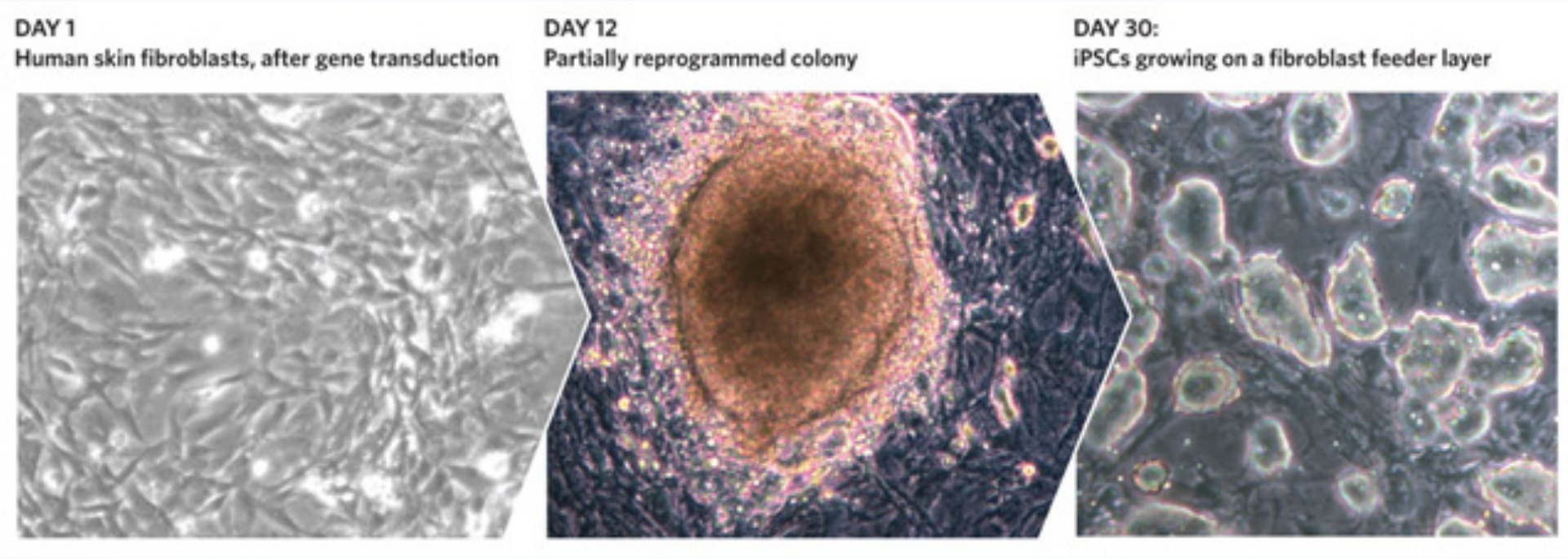
iPS
induced pluripotent stem cells



SHINYA YAMANAKA

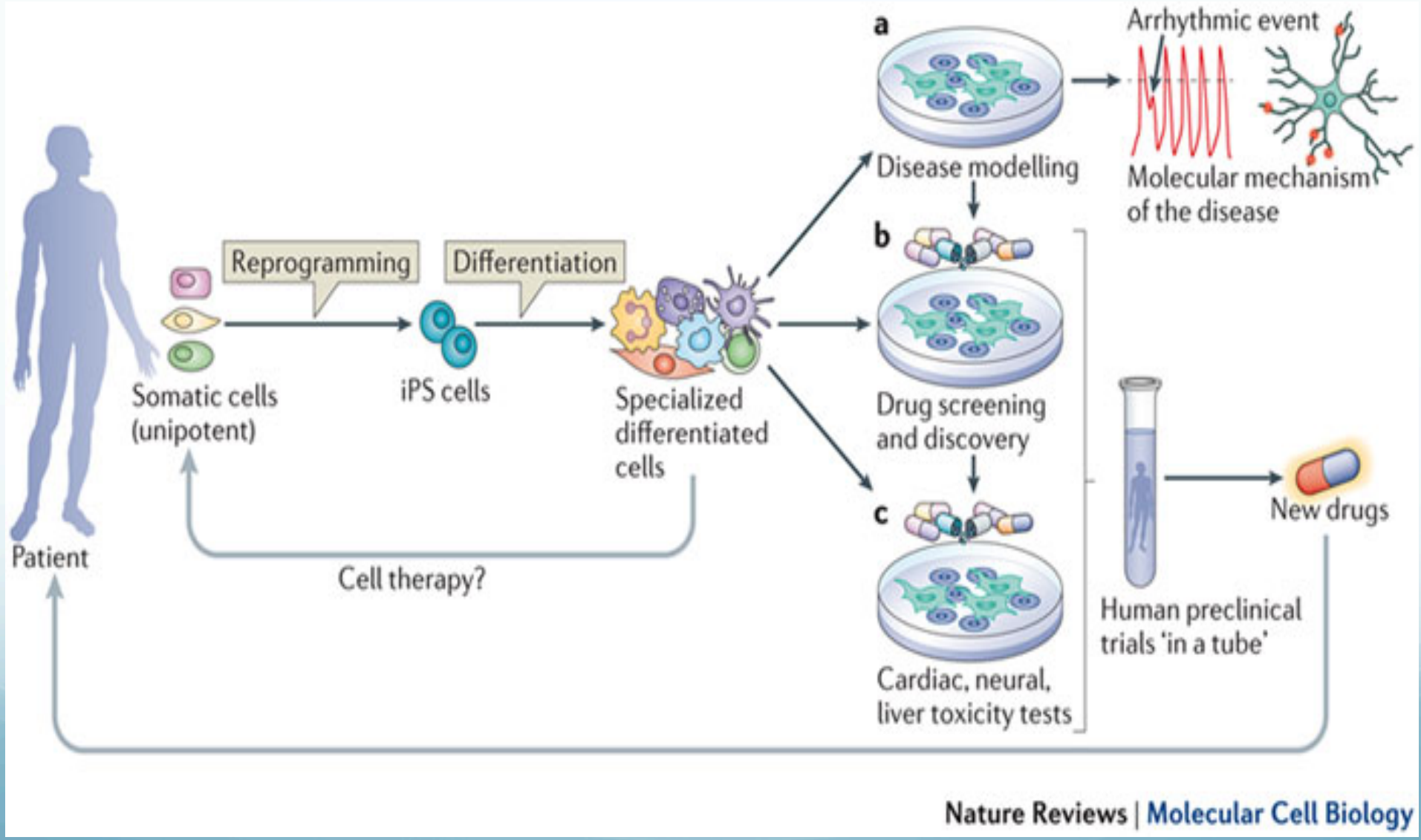
iPS production





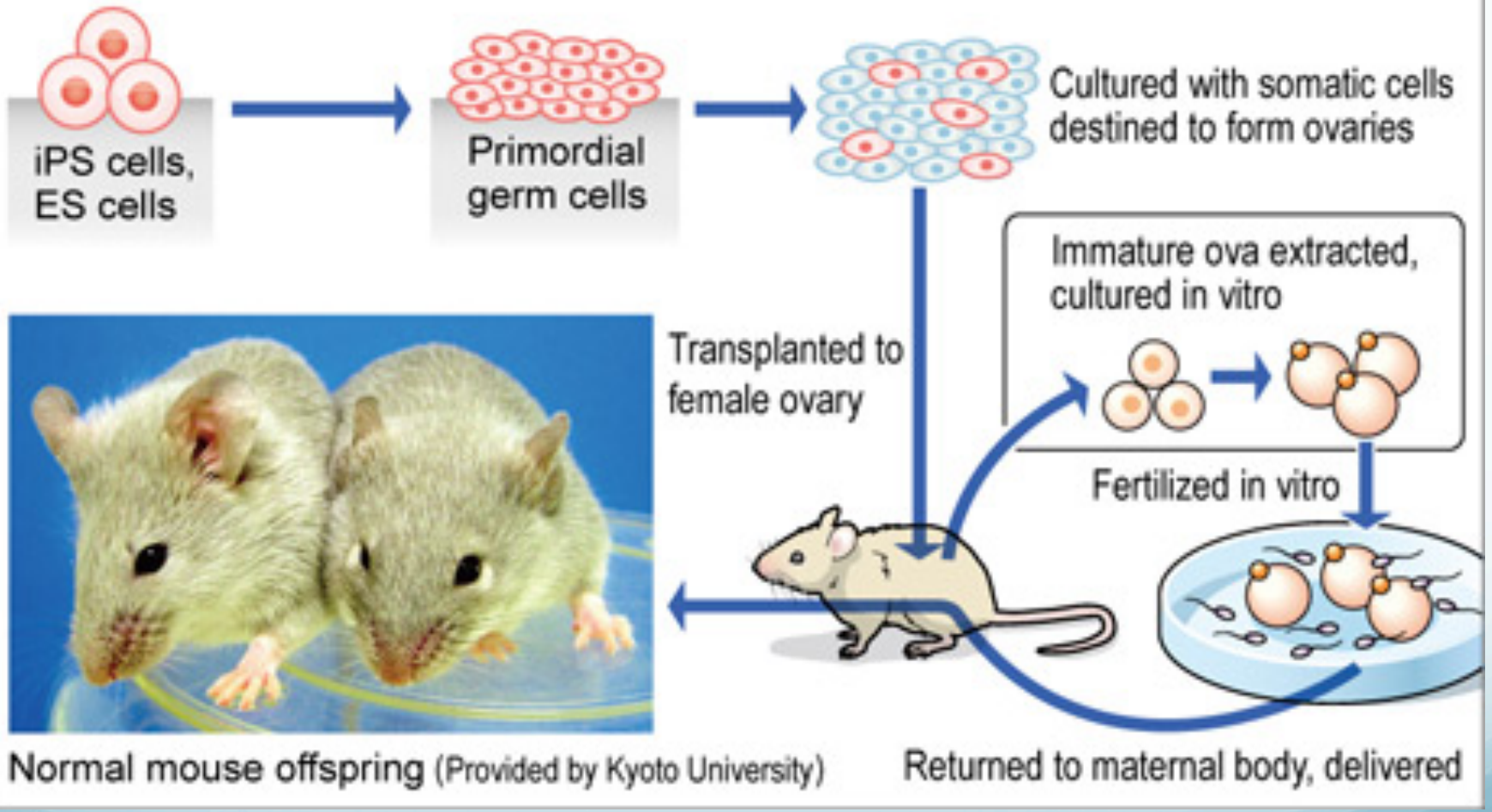
iPS *under microscope*

Possible use of iPS



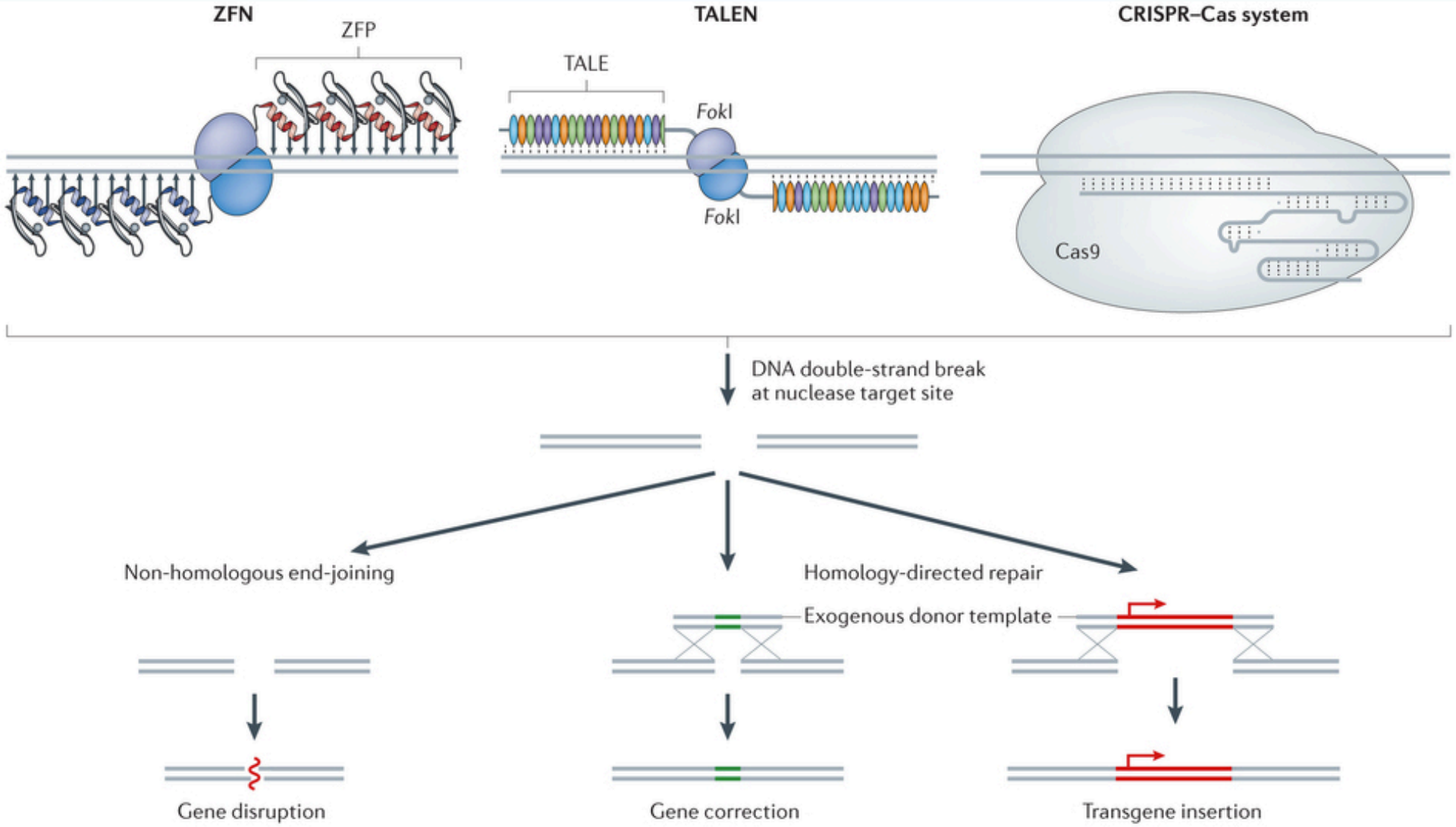
The Asahi Shimbun

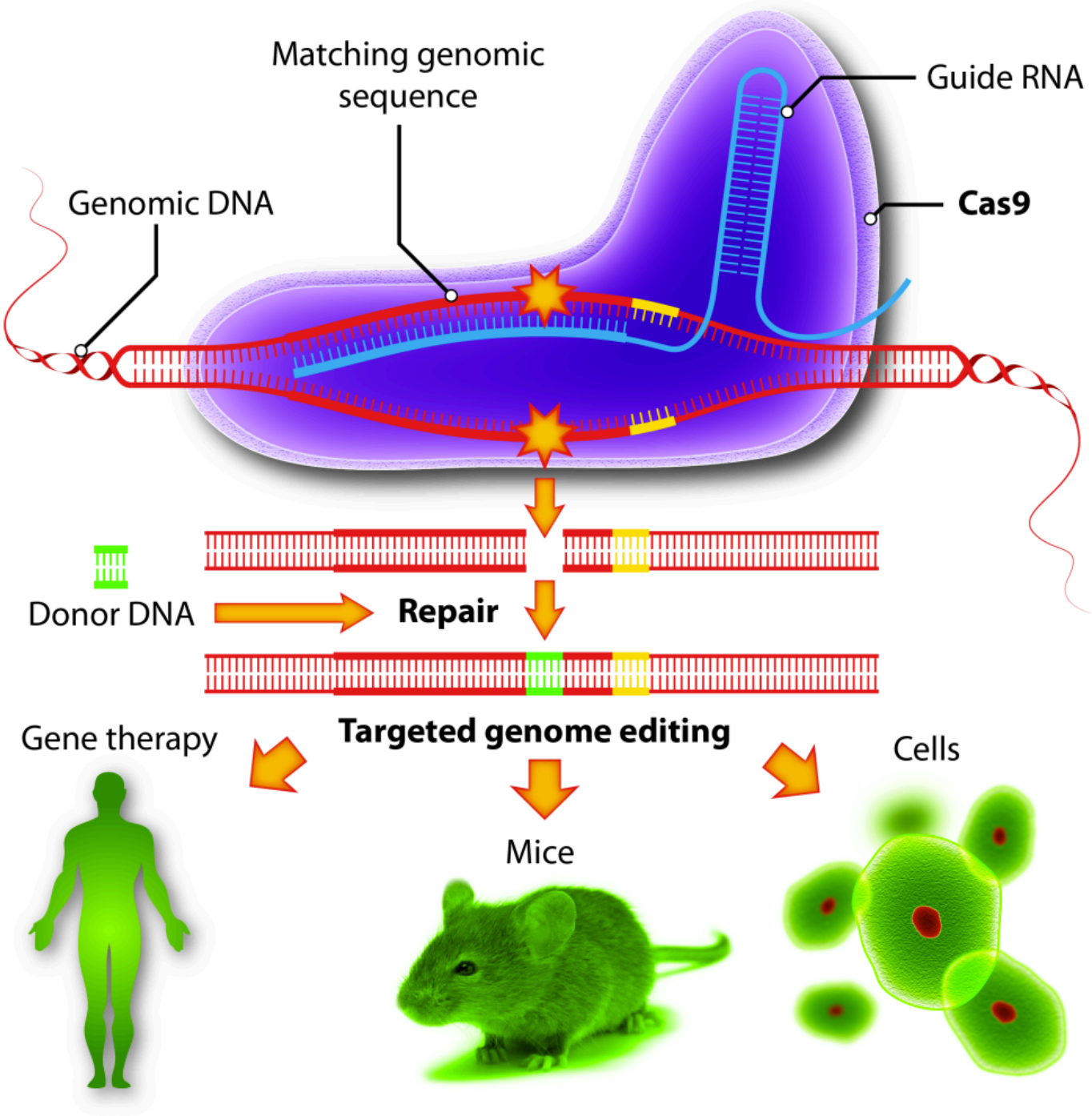
How scientists generated ova from iPS cells

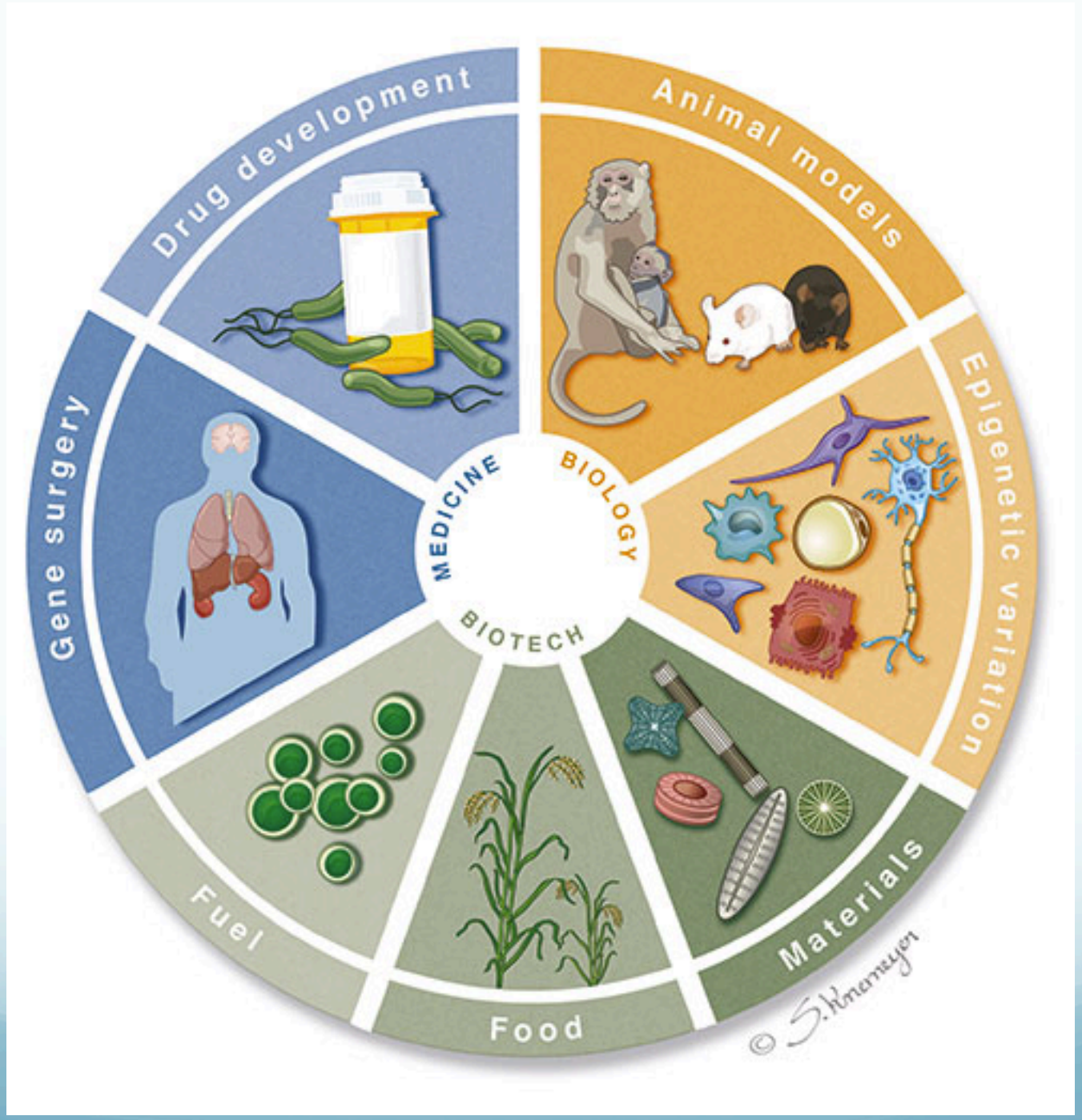


Gene editing

Gene editing technologies

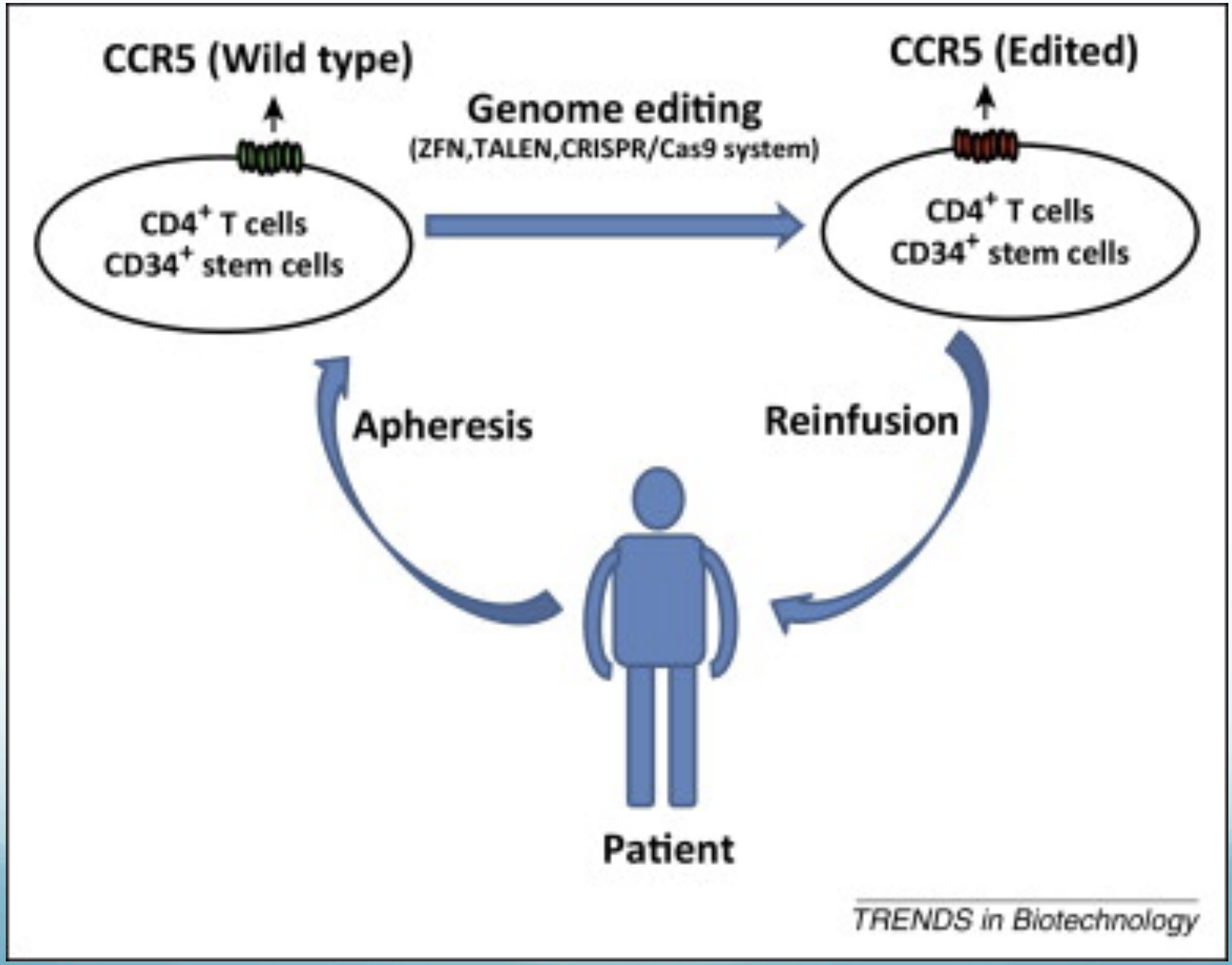



Matching genomic sequence
Guide RNA
Genomic DNA
Cas9
Donor DNA
Repair
Gene therapy
Targeted genome editing
Cells
Mice


Drug development
Animal models
Gene surgery
Epigenetic variation
MEDICINE
BIOLOGY
BIOTECH
Fuel
Food
Materials
© S. Kmenecyan

First human gene – editing therapy

- NEJM Dec 2014.
- 12 people with HIV
(over 70 patients treated to date)
- ZNF
- 50% of patients do not take antiviral TH


CCR5 (Wild type)
CCR5 (Edited)
Genome editing
(ZFN,TALEN,CRISPR/Cas9 system)
CD4+ T cells
CD34+ stem cells
CD4+ T cells
CD34+ stem cells
Apheresis
Reinfusion
Patient
TRENDS in Biotechnology

Matt Chappell had his immune cells gene edited

Matt Chappell

Two years without therapy (most of his adult life he was HIV positive)

Haemophilia

- **Healthy gene for factor IX under albumin promoter in monkeys**

- **September 2017. : "green light" for human trial**

Leukemia

- **Edited donor cells to avoid GVHR**

&

- **Donor cells resistent to chemotherpy**

Retinitis pigmentosa

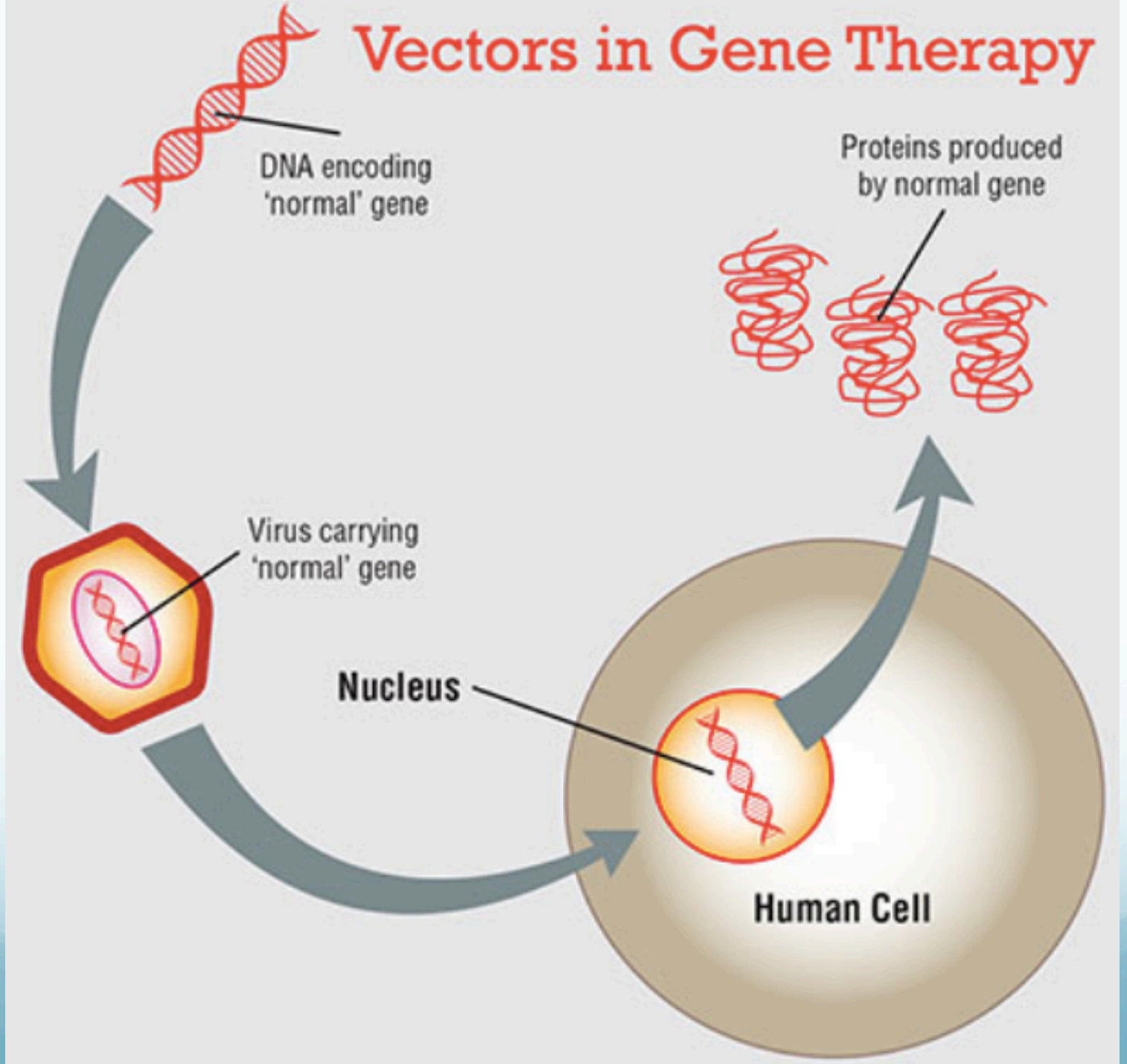
- **Editing of RPE65 improved light sensitivity in all 21 patients**

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 NOVEMBER 2, 2017 VOL. 377 NO. 18

Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy

J.R. Mendell, S. Al-Zaidy, R. Shell, W.D. Arnold, L.R. Rodino-Klapac, T.W. Prior, L. Lowes, L. Alfano, K. Berry, K. Church, J.T. Kissel, S. Nagendran, J. L'Italien, D.M. Sproule, C. Wells, J.A. Cardenas, M.D. Heitzer, A. Kaspar, S. Corcoran, L. Braun, S. Likhite, C. Miranda, K. Meyer, K.D. Foust, A.H.M. Burghes, and B.K. Kaspar

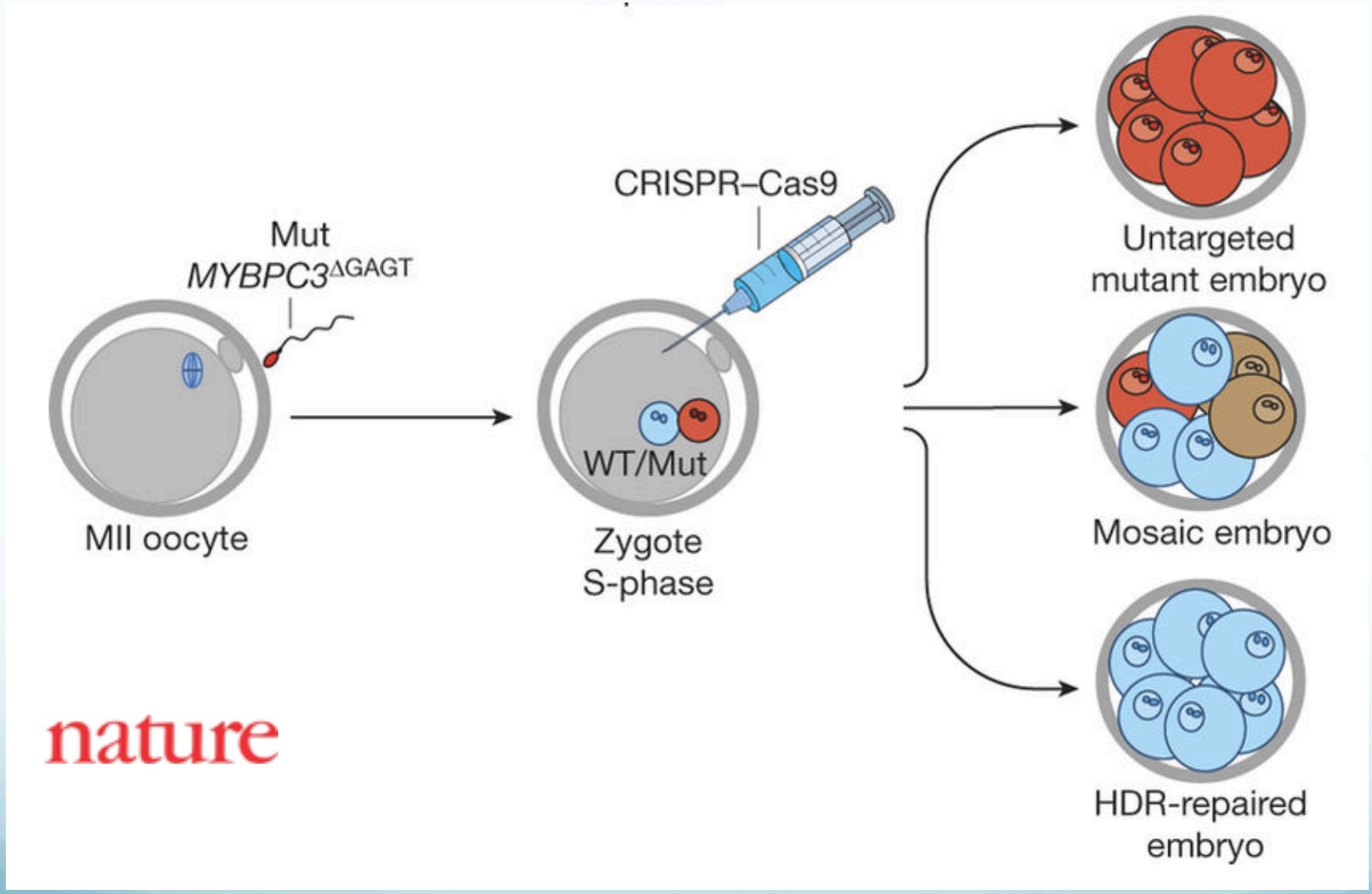

Vectors in Gene Therapy
DNA encoding 'normal' gene
Proteins produced by normal gene
Virus carrying 'normal' gene
Nucleus
Human Cell

Evelyn Villarreal

Gene editing of human zygote

Gene editing of human zygote

- **August 2017.**
- **Oregon Health and Science University**
- **Gene MYBPC3**
- **CRISPR-Cas9**


Mut
MYBPC3ΔGAGT
MII oocyte
CRISPR–Cas9
WT/Mut
Zygote
S-phase
Untargeted
mutant embryo
Mosaic embryo
HDR-repaired
embryo

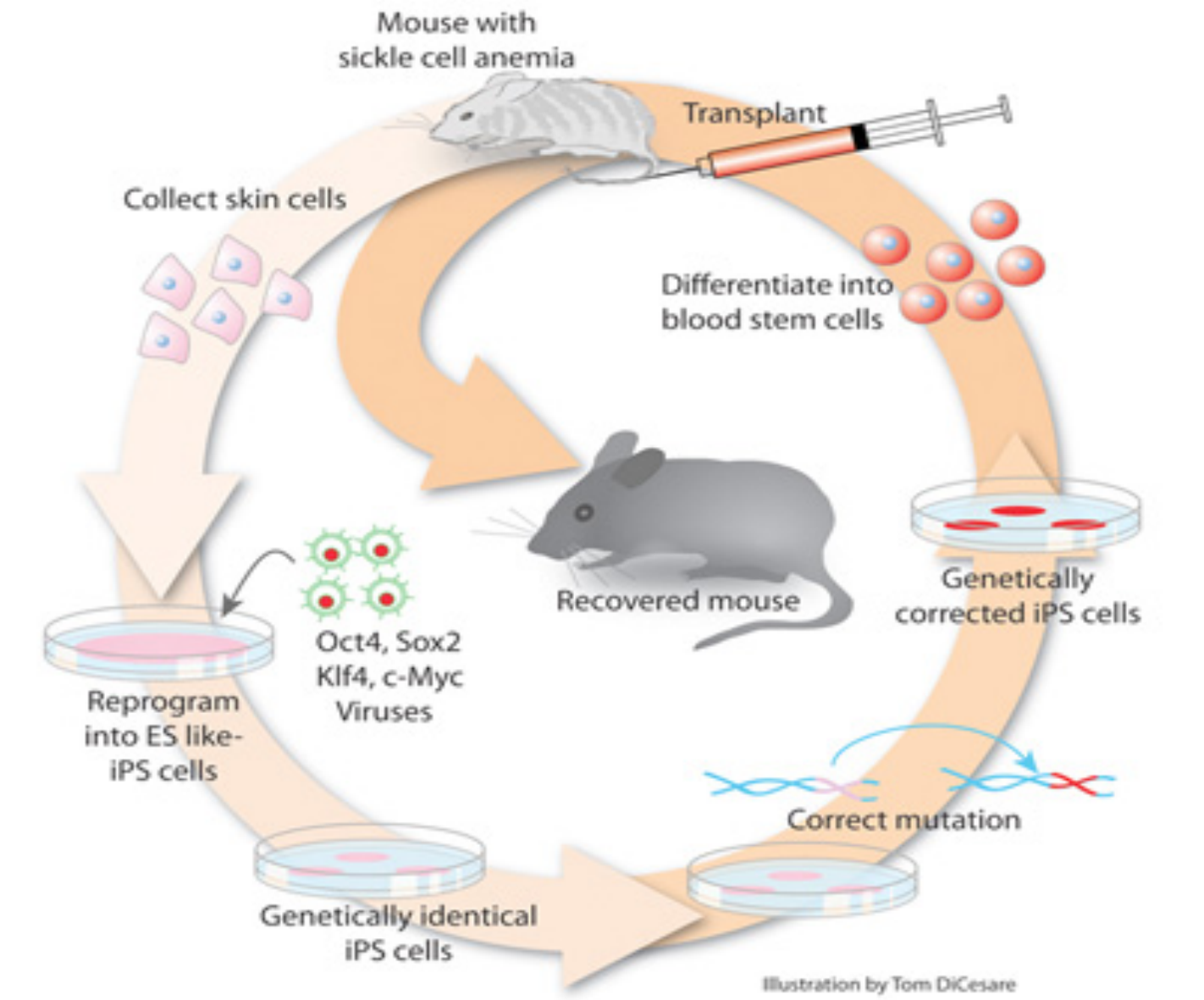
nature

70%

First gene edited human by CRISPR–Cas9

- **November 2016., China (soon in USA)**
- **Lung cancer**
- **Gene PD-1 was modified**

iPS + CRISPR


Mouse with
sickle cell anemia
Transplant
Collect skin cells
Differentiate into
blood stem cells
Recovered mouse
Genetically
corrected iPS cells
Oct4, Sox2
Klf4, c-Myc
Viruses
Reprogram
into ES like-
iPS cells
Correct mutation
Genetically identical
iPS cells
Illustration by Tom DiCesare

HUMAN GENOME

15 February 2001

nature

www.nature.com

the human genome

Nuclear fission
Five-dimensional energy landscapes

Seafloor spreading
The view from under the Arctic ice

Career prospects
Sequence creates new opportunities

naturejobs
genomics special

16 February 2001

Science

Vol. 291 No. 5507
Pages 1145–1434 $9

THE HUMAN GENOME

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

Prior HGP

- **Linkage analysis of large femilies, confirmation on animal models...**
- **Around 1000 (out of 7000) single gene disorders were identified (Huntington d., cystic fibrosis etc) by year 2000**

Importance of HGP

- Molecular mechanisms of diseases
- Multigenetic disorders
- Microbial genetis
- Antropology, evolution, migration
- Agriculture

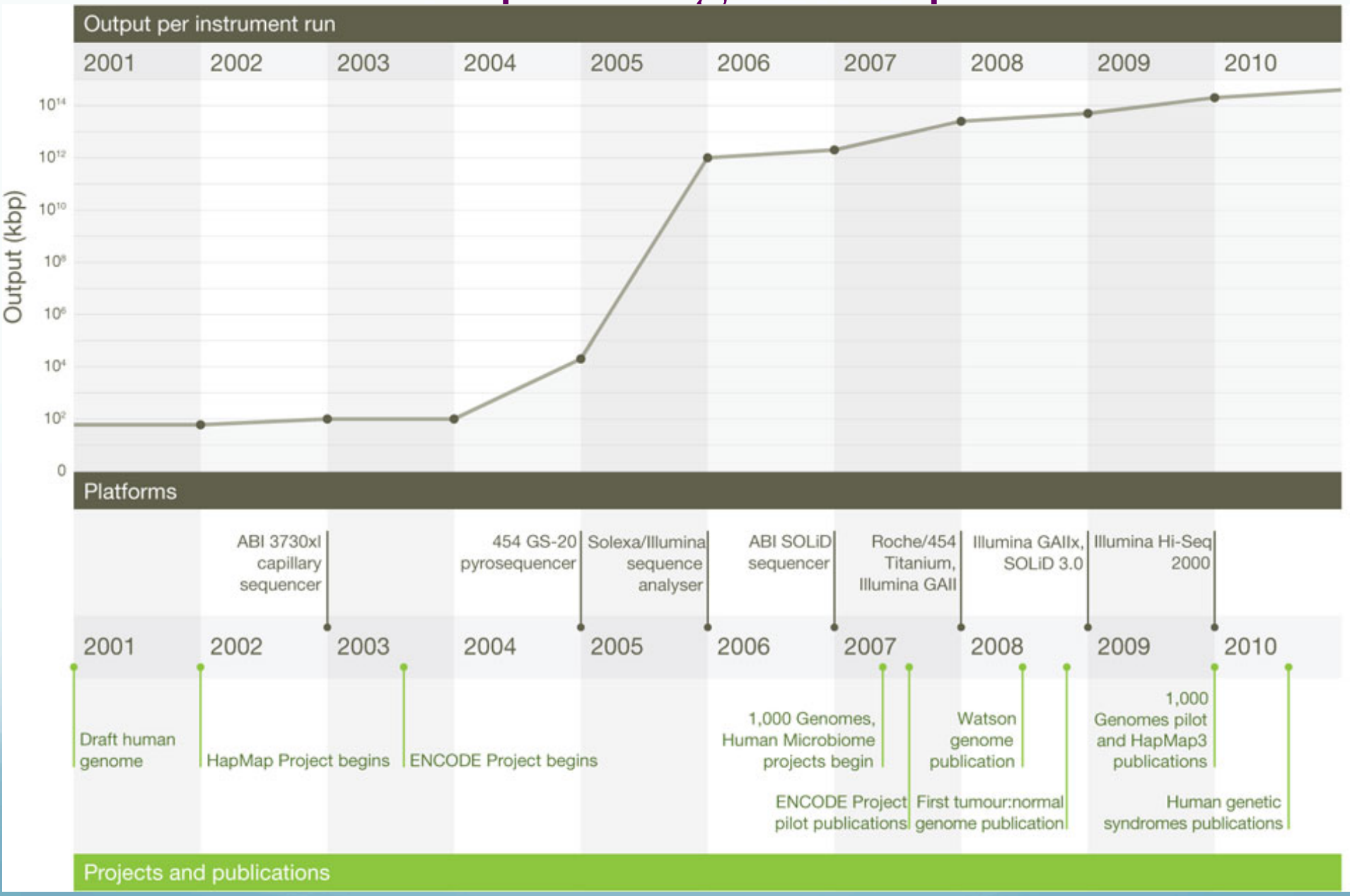
Medical impact

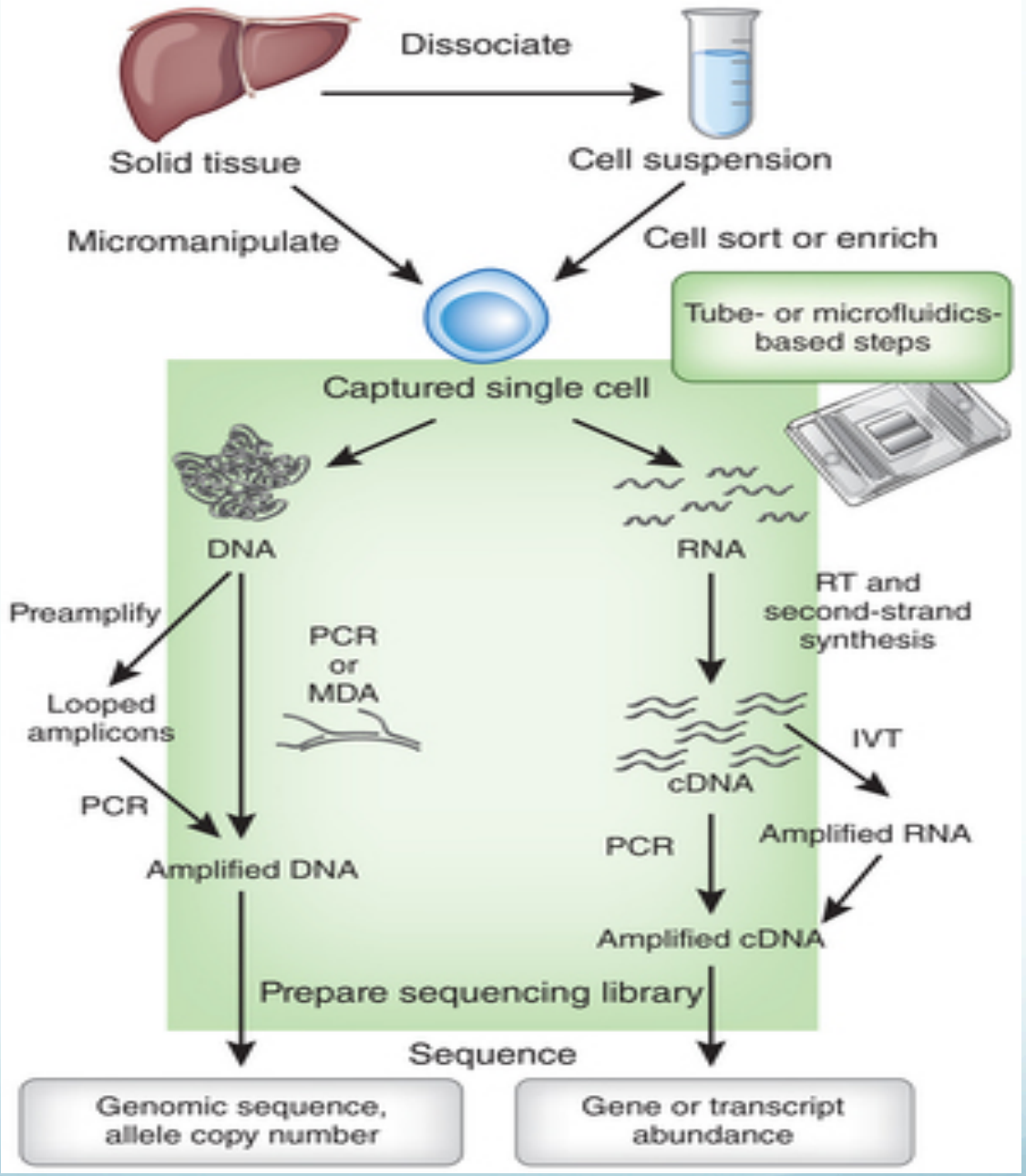
- *Better diagnosis and prognosis*
- *Genotype then therapy*
- *Health risks in %*

Novel scientific disciplines

- Proteomics
- Bioinformatics
- GINA 21.05.2008.

DNA sequencing development



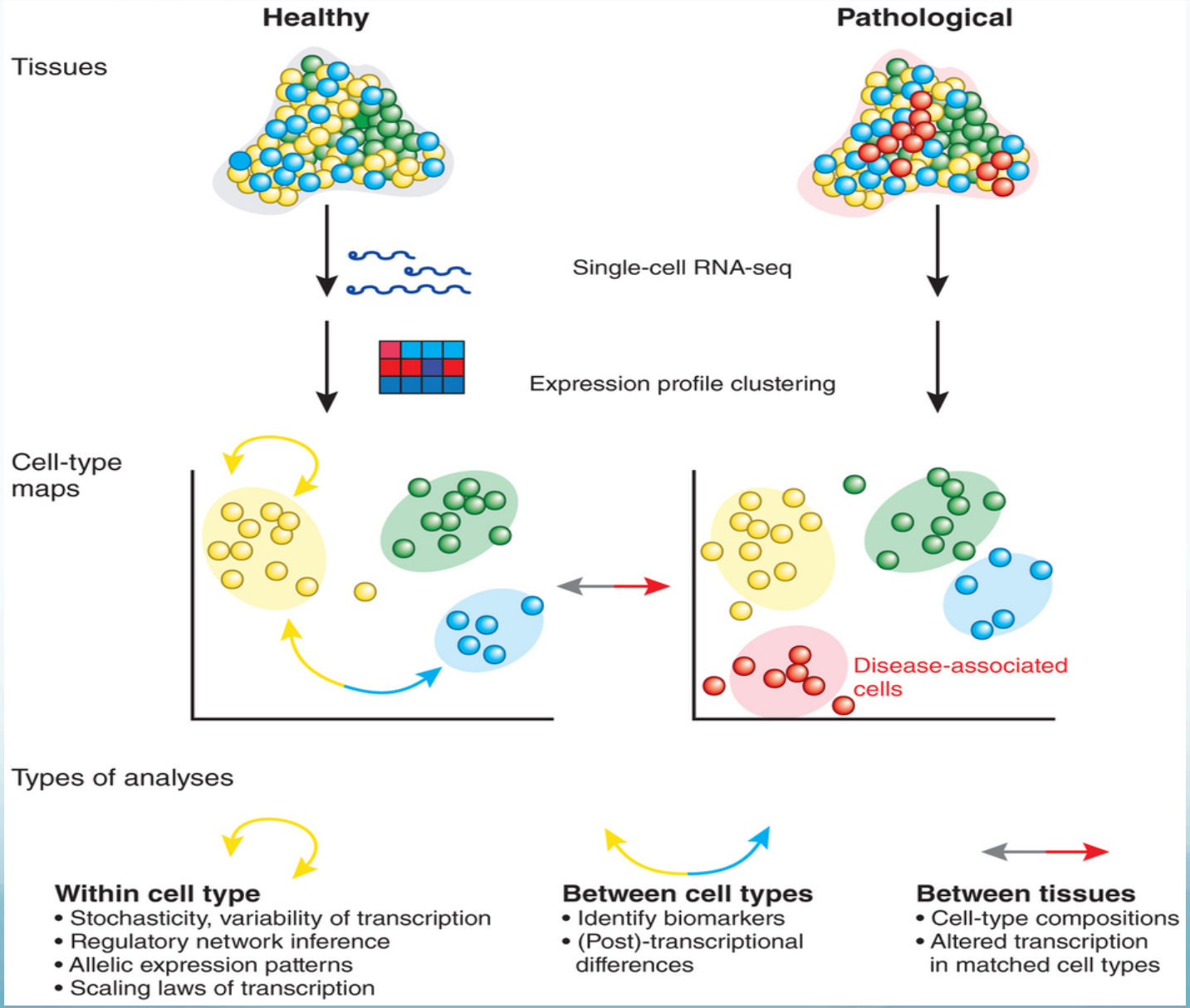


SINGLE-CELL SEQUENCING

Single-cell sequencing

- DNA and RNA
- On average between 5000 and 15000 active genes per cell
- Prenatal dg, cancer etc.

Biological diversity



After HGP i

- 5% of our genome is conserved, but only 1,1% are protein coding genes
- Remaining 4 % are regulatory elements and RNA genes
- Around 90% euchromatin is transcribed
- Pseudogenes (around10% genes has it, regulatory role)

After HGP ii

- Gene number: around 21.000
- Gene with most exons - 363 is TITIN
- Longest gene is DISTROFIN 2,4 M bp
- Very active genes have a few introns
- 9% of genes are overlapping (usually second strand is transcribes)

After HGP iii

- Biological world started as a RNA

  Followed by proteins – due to their adjustability, and a DNA due to its stability

- H. Sapiens and C. elegans have 21 and 20 thousand genes respectively, and it seems that our complexity comes from RNA and proteins

ENCODE I
Encyclopedia of DNA Elemnts

Goal: To determine function of human DNA

Who: 440 scientists on 147 cell types

RESEARCH (24 types of experiments):

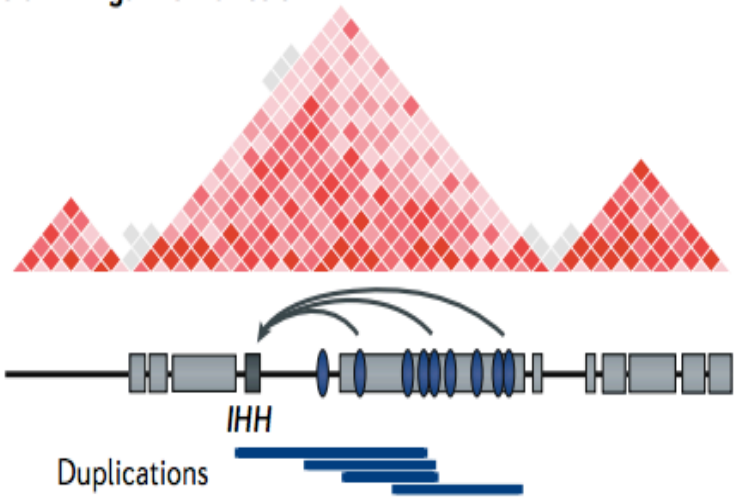
- Histone modifications (13/60)
- Binding of transcription factors (120/1800)
- Distant chromosomal interactions
- Promoter region quantification

ENCODE II

- **80% of our genome has some sort of functional activity !**
- 70,000 promoters and 400,000 *enhancers*
- Around 50bp is common for 1000 promoters
- 2,9 millions of DNaseI sites, and 3700 of them in all cell types

Topologically associated domains

a Intra-TAD gain of function



Phenotype

Duplications of enhancer elements cause preaxial synpolydactyly of feet

Examples

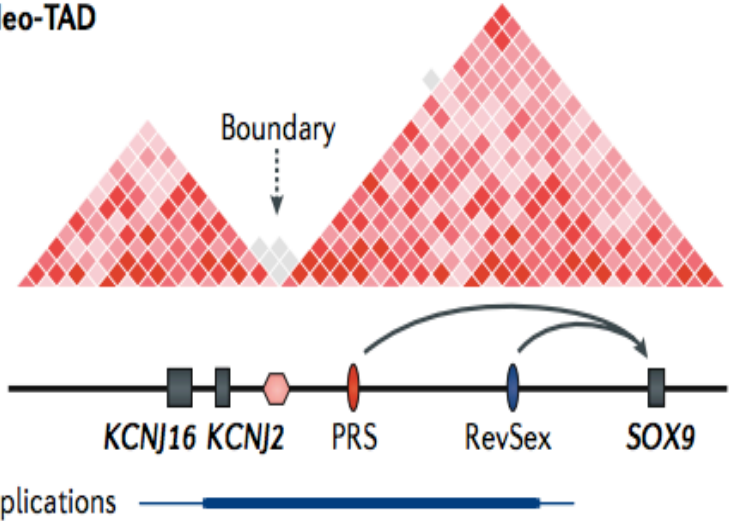
Gain of function:

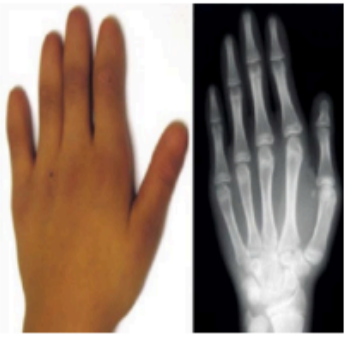
- *SOX9* locus: duplications of gonad enhancer cause 46,XX sex reversal
- *BCL6* locus: duplications of super enhancers cause B cell lymphomas
- *SHH* locus: duplications of limb enhancer causes polydactyly

Loss of function:

- *PAX6* locus: aniridia
- *DLX5* and/or *DLX6* loci: split hand foot malformation
- *SOX9* locus: deletions of gonad enhancer cause 46,XY sex reversal

b Neo-TAD





Cooks syndrome: Duplications of TAD boundary, *KCNJ2* and *KCNJ16* cause aplasia of nails and short digits

- *FGF2* locus: colorectal cancer
- *PRDM6* locus: medulloblastoma

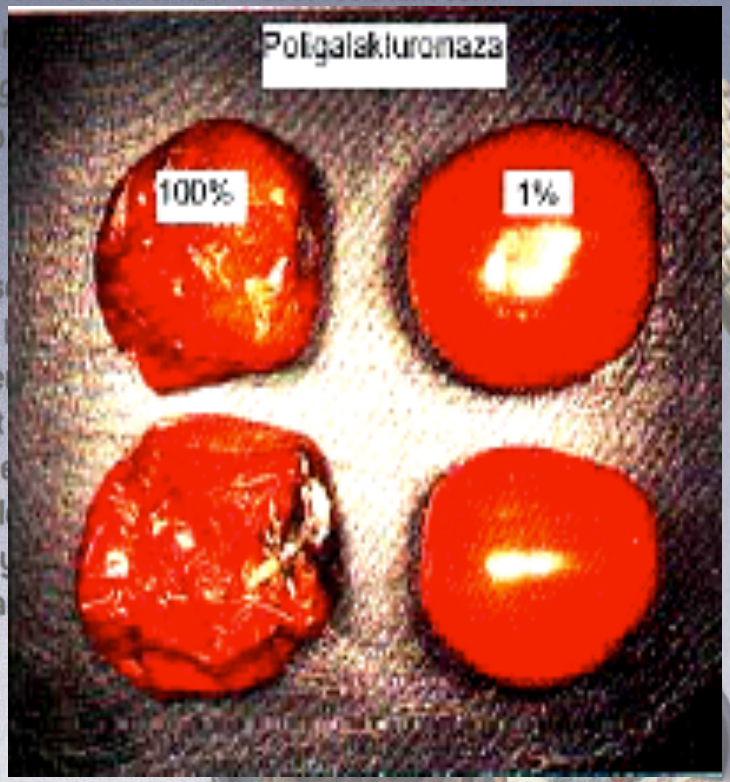
G M O

HOW TO MAKE A GENETICALLY MODIFIED PLANT

Manufacturers can produce genetically modified plants in different ways. The diagram below presents a highly simplified version of how insect-resistant corn might be made. Insect-protected GM pla
gene from the bacterium *Bacillus thuring*
cells to produce a protein that is toxic to
benign to most other creatures.

1 From the bacterium *Bacillus thuringiensis*, isolate the gene that directs cells to produce a protein toxic to certain insects

2 Try to ins
cells the
a "marker" ge
flag cells that
up the Bt gene
markers shiel
being killed b
antibiotic or a



DESTRUCTIVE PEST

BT TOXIN

4 Allow the genetically altered cells to grow into plants. Those plants—and crops derived from their seeds—produce the Bt toxin in their cells. As insect pests susceptible to the toxins dine on the plants, they die instead of destroying the crop

BACILLUS THURINGIENSIS (BT)

TOXIN GENE FROM BACTERIUM

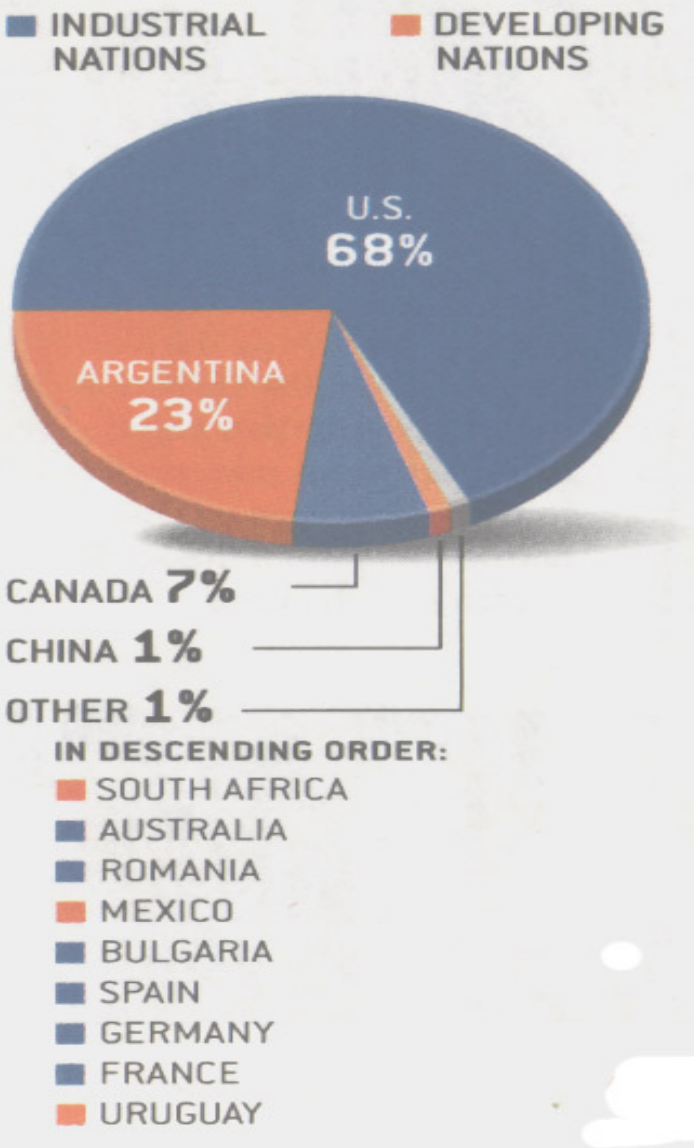
MARKER GENE

DYING PLANT CELL THAT DID NOT TAKE UP GENES

PLANT CELL THAT DID TAKE UP GENES

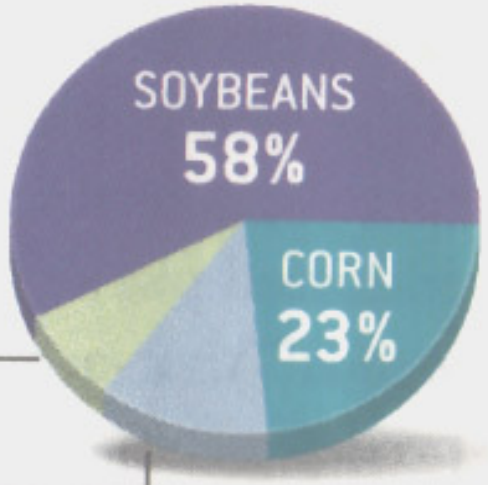
DEAD PESTS

COUNTRIES PRODUCING GM CROPS IN 2000

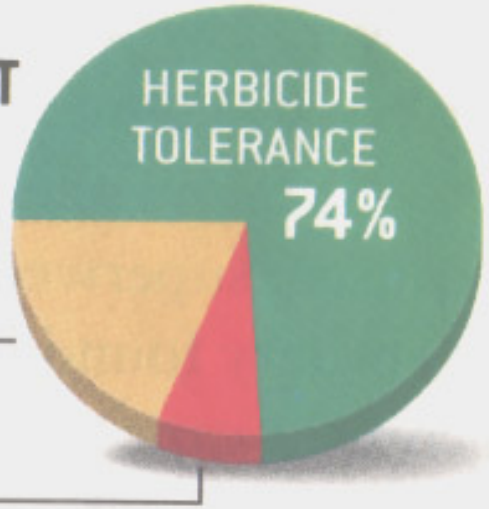


PERCENT OF TOTAL GM AREA

BY TYPE . . .



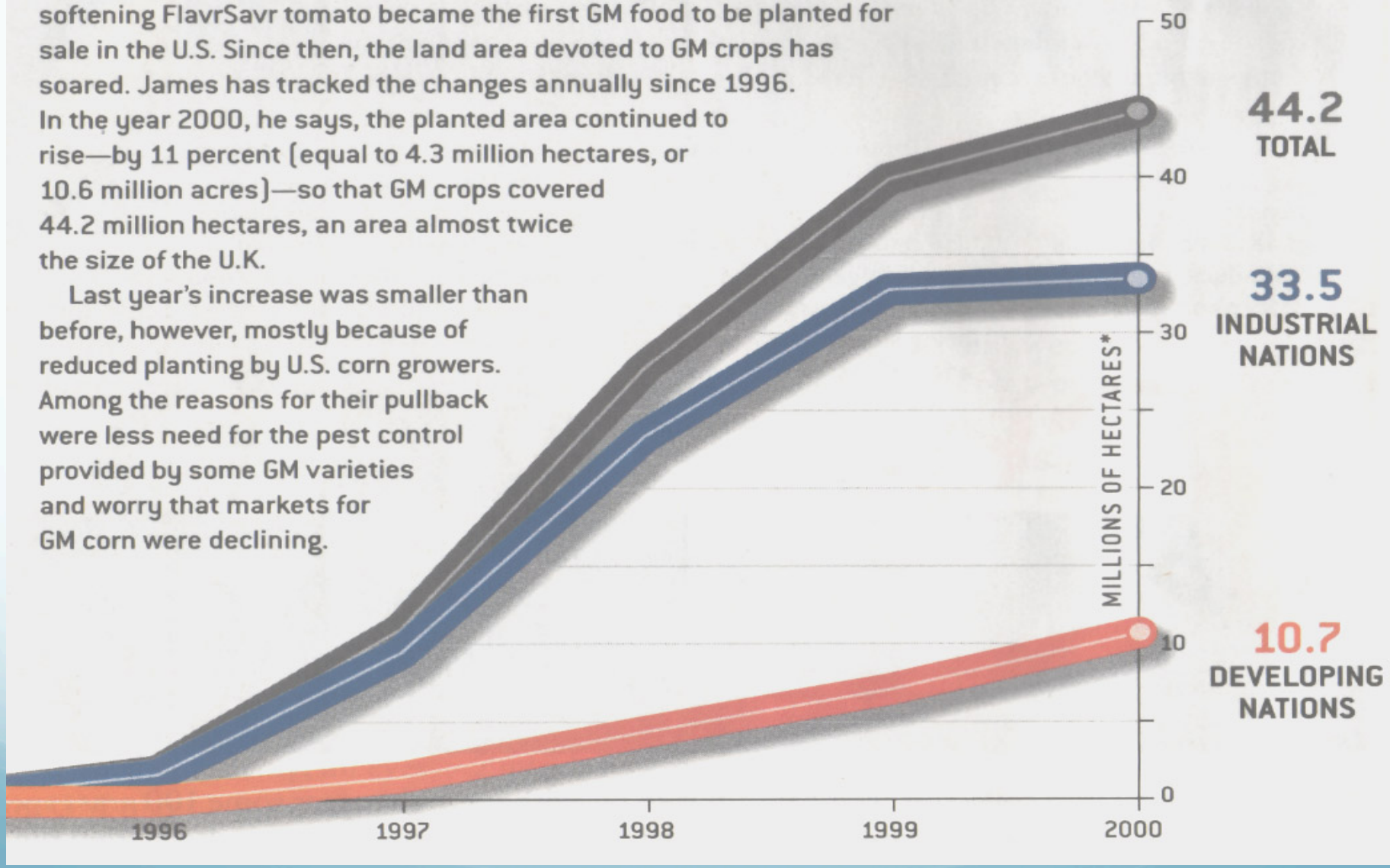
. . . AND TRAIT



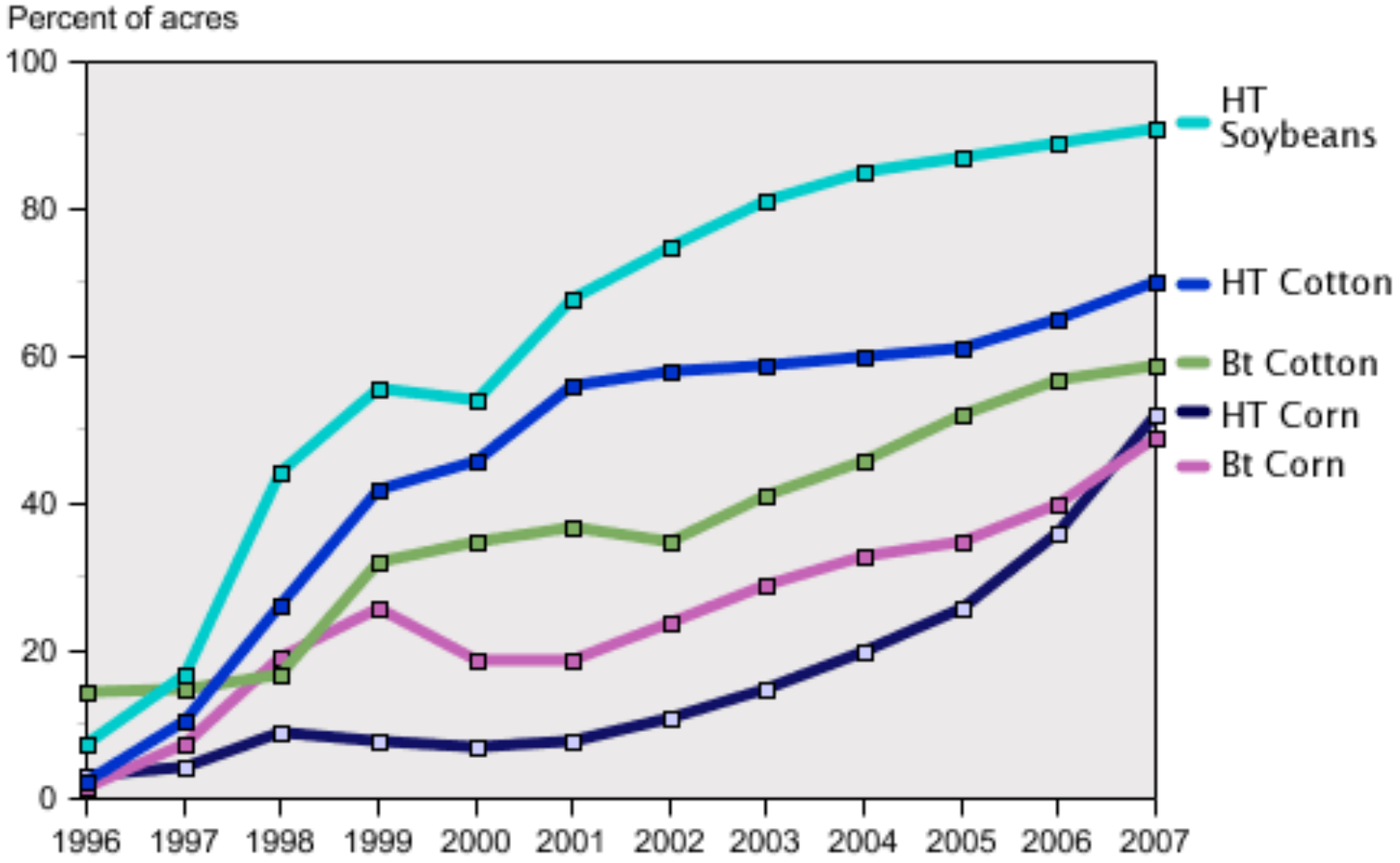
Figures may not add up to 100% because of rounding.

Commercial planting of genetically modified crops began in China with tobacco in 1992, according to Clive James of the International Service for the Acquisition of Agri-biotech Applications. In 1994 the slow-softening FlavrSavr tomato became the first GM food to be planted for sale in the U.S. Since then, the land area devoted to GM crops has soared. James has tracked the changes annually since 1996. In the year 2000, he says, the planted area continued to rise—by 11 percent (equal to 4.3 million hectares, or 10.6 million acres)—so that GM crops covered 44.2 million hectares, an area almost twice the size of the U.K.

Last year's increase was smaller than before, however, mostly because of reduced planting by U.S. corn growers. Among the reasons for their pullback were less need for the pest control provided by some GM varieties and worry that markets for GM corn were declining.



Adoption of genetically engineered crops grows steadily in the U.S.



Note: Data for each crop category include varieties with both HT and Bt (stacked) traits.
Source: 1996-1999 data are from Fernandez-Cornejo and McBride (2002). Data for 2000-07 are avalable in the ERS data product, Adoption of Genetically Engineered Crops in the U.S., tables 1-3.

Are you buying Frankenstein food?
THE RED ZONE
THE AMBER ZONE
THE GREEN ZONE
THE GREEN BRANDS
FRANKENSTEIN FOOD FIASCO
By DAVID DERBYSHIRE
Science Correspondent
Looks familiar: these cloned sheep at the Roslin Institute in Edinburgh are seen primarily as a means to
We modify their genes at our peril
Food technology: A new breed of eco-warrior is challenging the big corporations by taking direct action
Wheatfields turn into war zones
By CHARLES ARTHUR
Technology Editor
OUR RIGHT TO SAY NO TO GMOs
GMO AWARE
NO GMOs
iddle-class activists
Finance
News Analysis Agribusiness is running scared from GM foods
Test fields of conflict

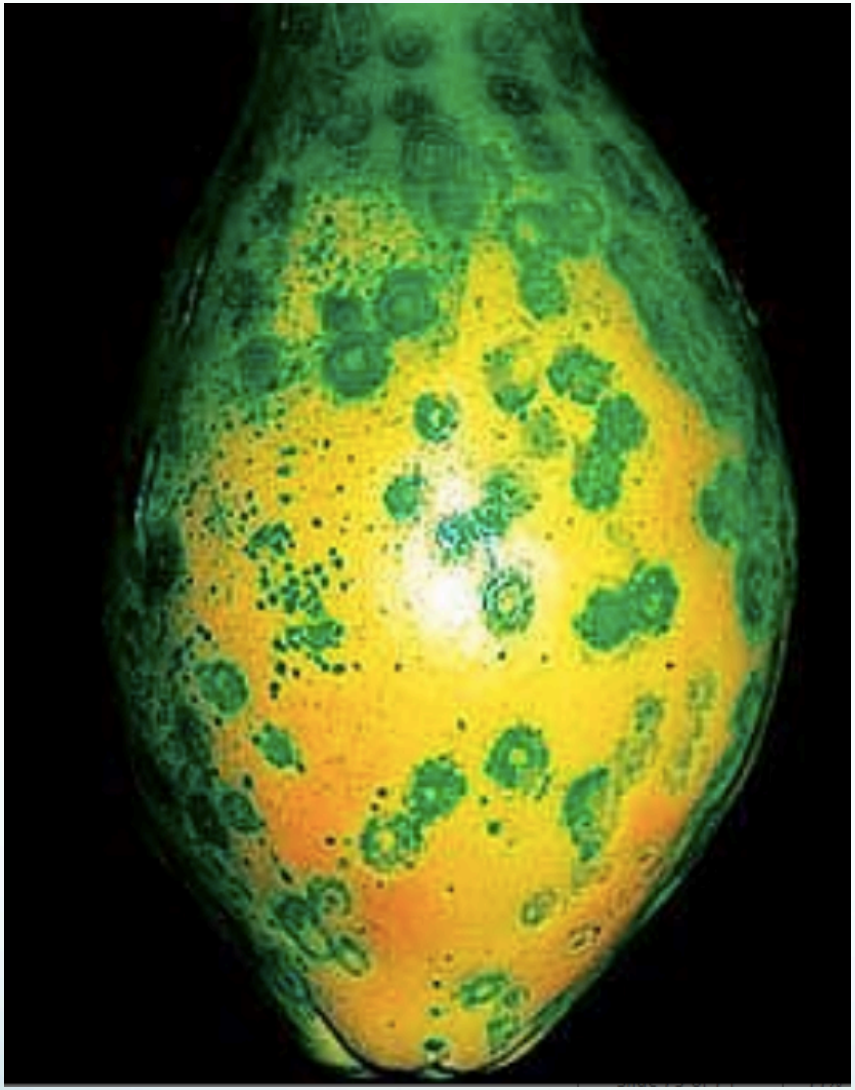
Monarch butterflies have become a focus of worry.

THREE WORRIES

1 INNOCENT CREATURES WILL BE HURT by insecticides built into many GM crops.	2 SUPERWEEDS WILL ARISE as genes that give crops the ability to kill insect pests or to withstand herbicides find their way into weeds.	3 GM CROPS WILL SUDDENLY FAIL because insect pests will evolve tolerance to built-in insecticides and because weeds will evolve immunity to herbicides sprayed over fields of herbicide-tolerant GM plants.
What the research says: Laboratory studies indicate that nontarget insects, such as monarch butterflies, could be harmed, but field studies suggest that the risk is small.	*What the research says:* Studies have found no superweeds, but anecdotal reports have surfaced. Because pollen from GM plants can often fertilize weedy relatives of those plants, GM crops should not be grown near such relatives.	*What the research says:* No failures have been documented, but they are likely to occur. Critics and proponents of GM crops disagree over the adequacy of current preventive measures.

- 2 000 000 /y. of kids die or suffer developmental defects

- In Uganda 30 % of calories from bananas
- *Xanthomonas* bacterium but there is GMO resistant variety (gene from pepper)



- Papaya in Hawaii was viraly infected (ringspot virus)
- Now in USA 80% papaya are GMO

Laureates Letter Supporting Precision Agriculture (GMOs)

June 29th 2016

To the Leaders of Greenpeace, the United Nations and Governments around the world

The United Nations Food & Agriculture Program has noted that global production of food, feed and fiber will need approximately to double by 2050 to meet the demands of a growing global population. Organizations opposed to modern plant breeding, with Greenpeace at their lead, have repeatedly denied these facts and opposed biotechnological innovations in agriculture. They have misrepresented their risks, benefits, and impac and supported the criminal destruction of approved field trials and research projects.

152 Laureates Supporting Precision Agriculture (GMOs)

Peter Agre	2003	Chemistry
Zhores I. Alferov *	2000	Physics
Sidney Altman	1989	Chemistry
Hiroshi Amano	2014	Physics
Werner Arber	1978	Medicine
Richard Axel	2004	Medicine
David Baltimore	1975	Medicine

152 Nobel laureates supports GMO

thank you !