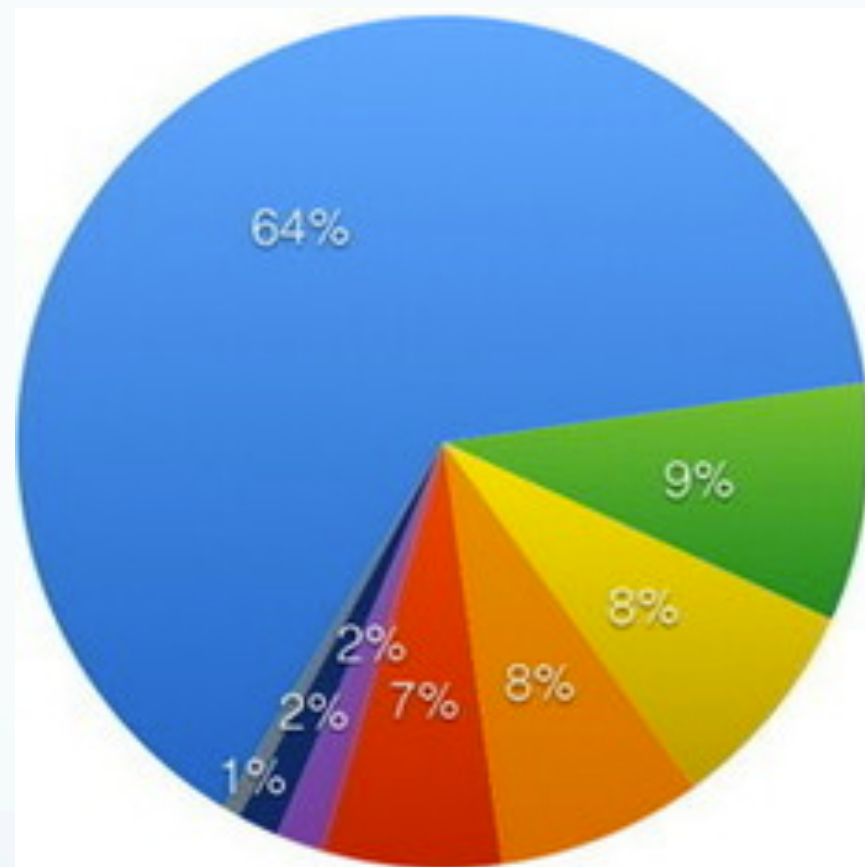


evolving
GENE THERAPY

Janoš Terzić



DAVID VETTER



- | | |
|-----------------------|---------------------------|
| ● Cancer diseases | ● Monogenic diseases |
| ● Infectious diseases | ● Cardiovascular diseases |
| ● Other | ● Neurological diseases |
| ● Ocular diseases | ● Inflammatory diseases |

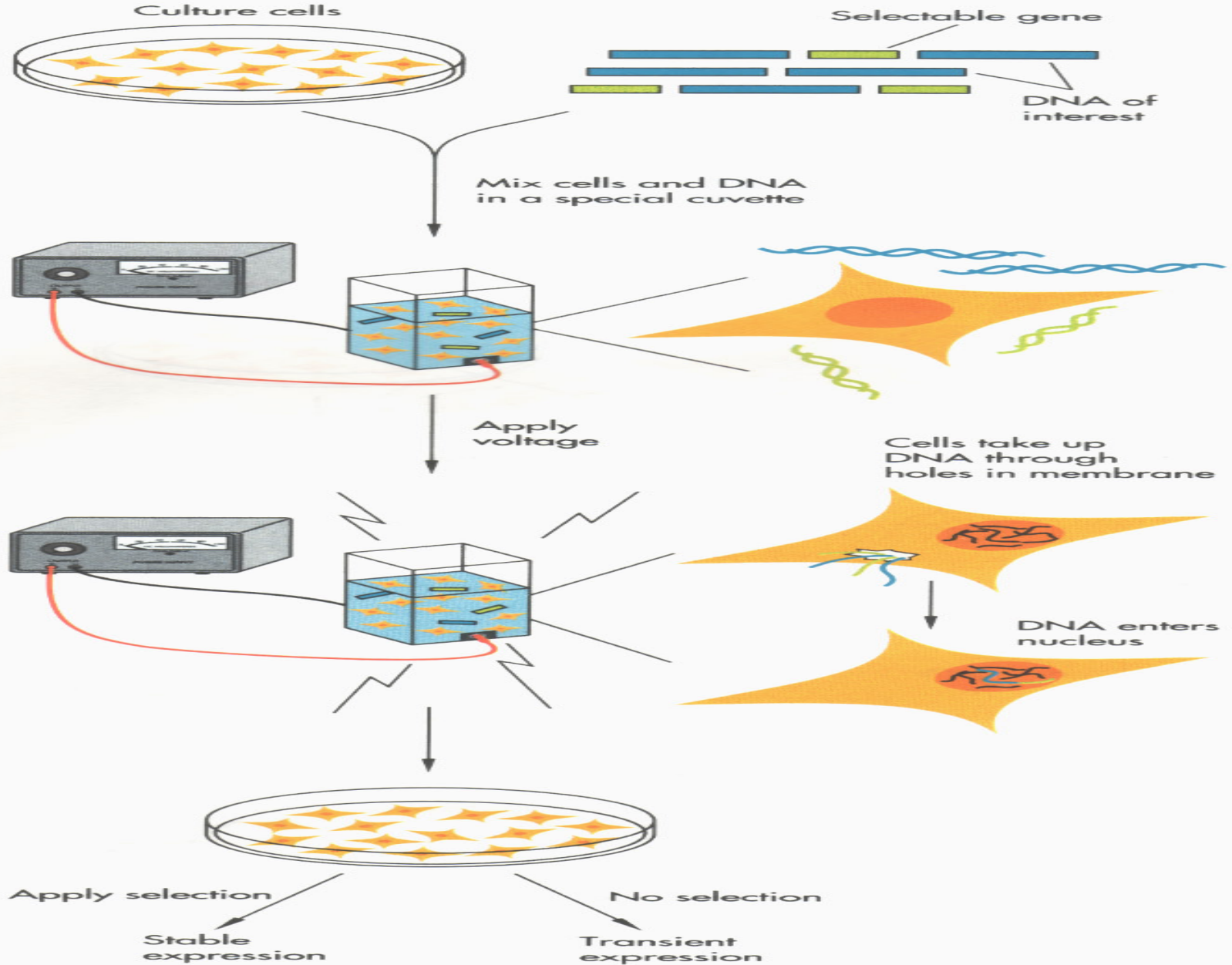
Monogenic disorders

- Adrenoleukodystrophy
- α -1 antitrypsin deficiency
- Becker muscular dystrophy
- β -thalassaemia
- 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 nucleoside phosphorylase deficiency
- Recessive dystrophic epidermolysis bullosa
- Sickle cell disease
- Severe combined immunodeficiency
- Tay Sachs disease
- Wiskott-Aldrich syndrome

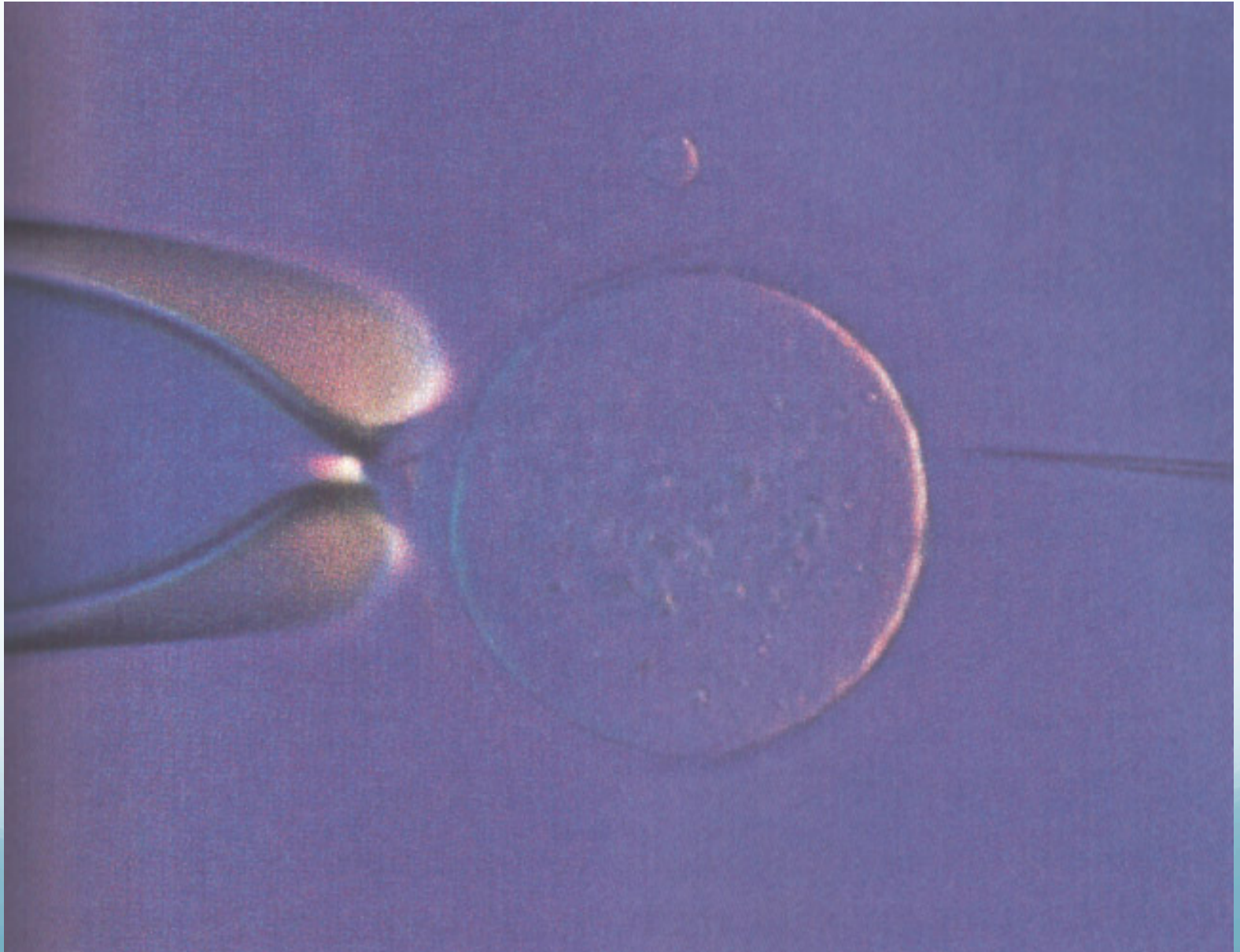
PREREQUESTS

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

Electroporation

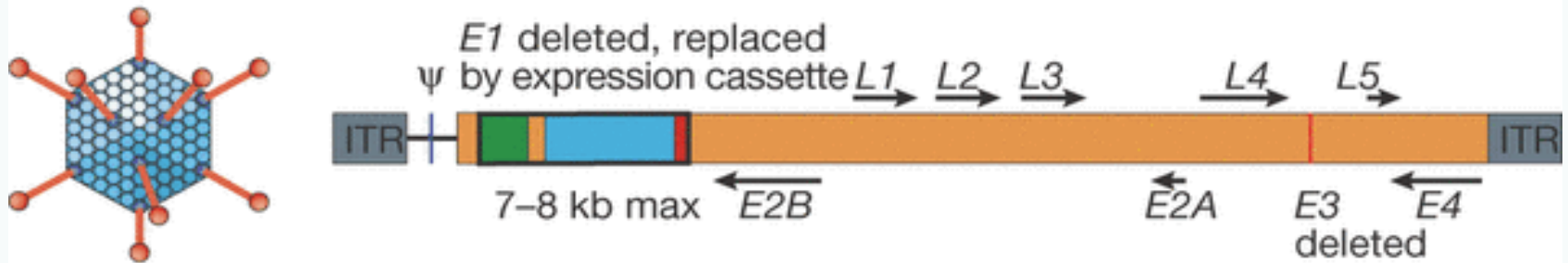


Microinjection

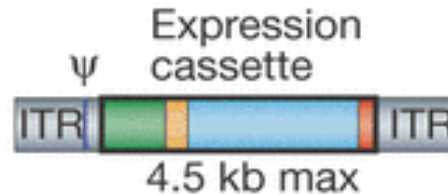


Viral vectors

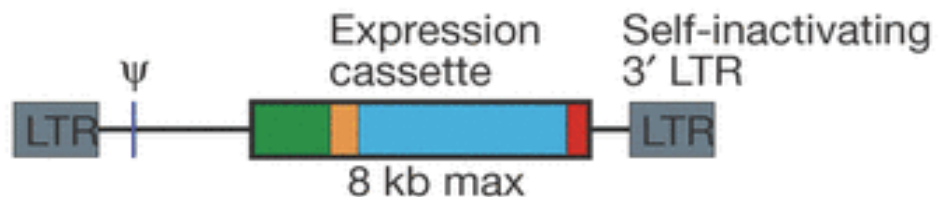
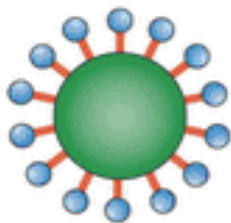
Adenovirus (~36 kb genome)



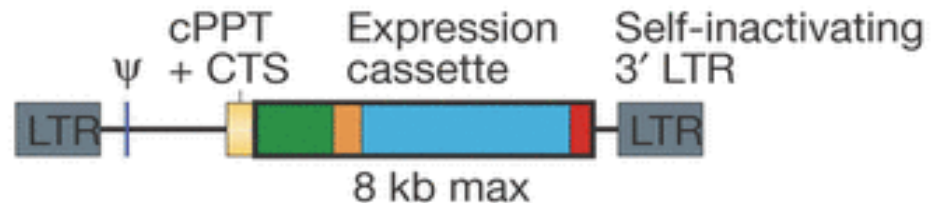
Adeno-associated virus (4.7 kb genome)

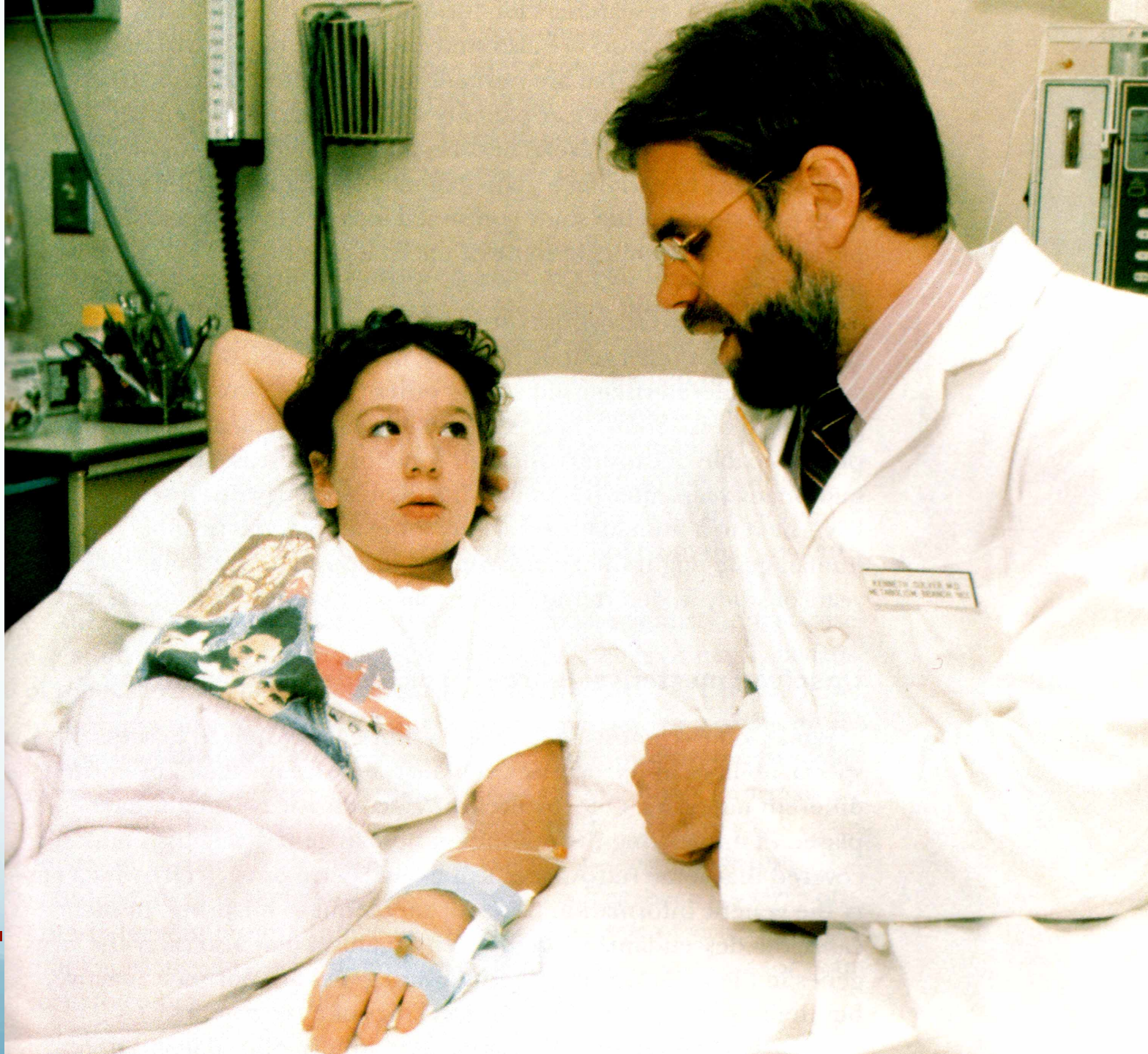


Retrovirus (7–10 kb genome)



Lentivirus (9–10 kb genome)

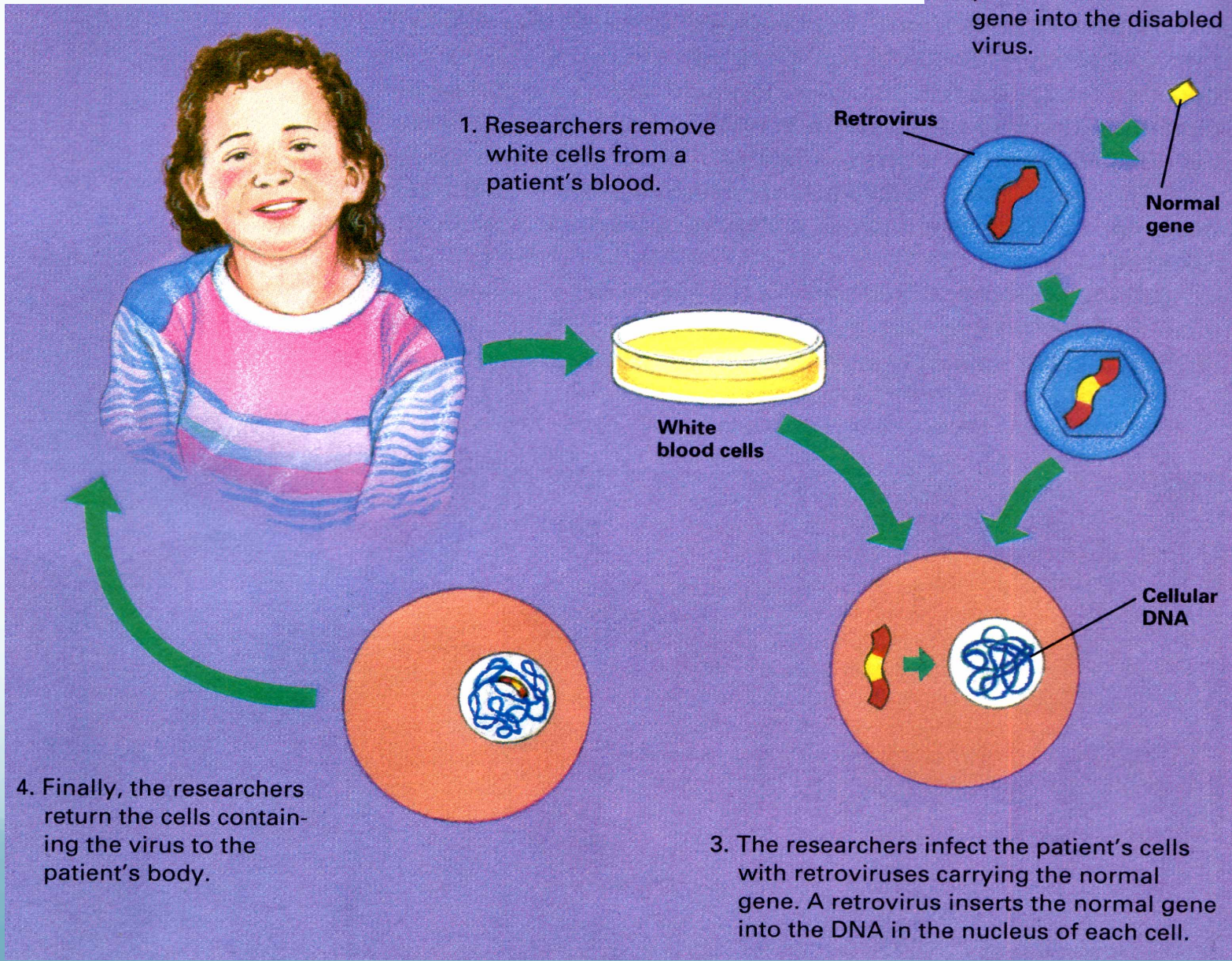


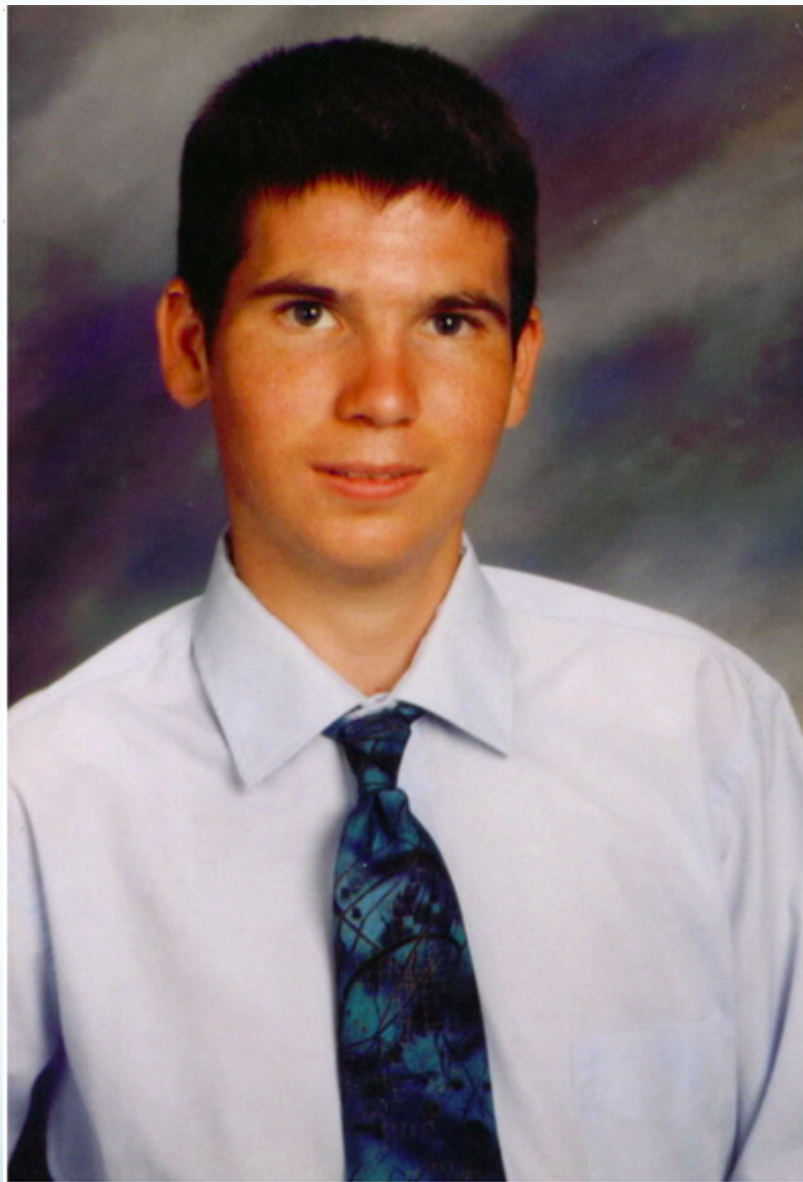


1991.

First patient: ADA deficiency

2. The scientists disable a retrovirus so that it is unable to cause disease. Then they splice a normal copy of the patient's defective gene into the disabled virus.

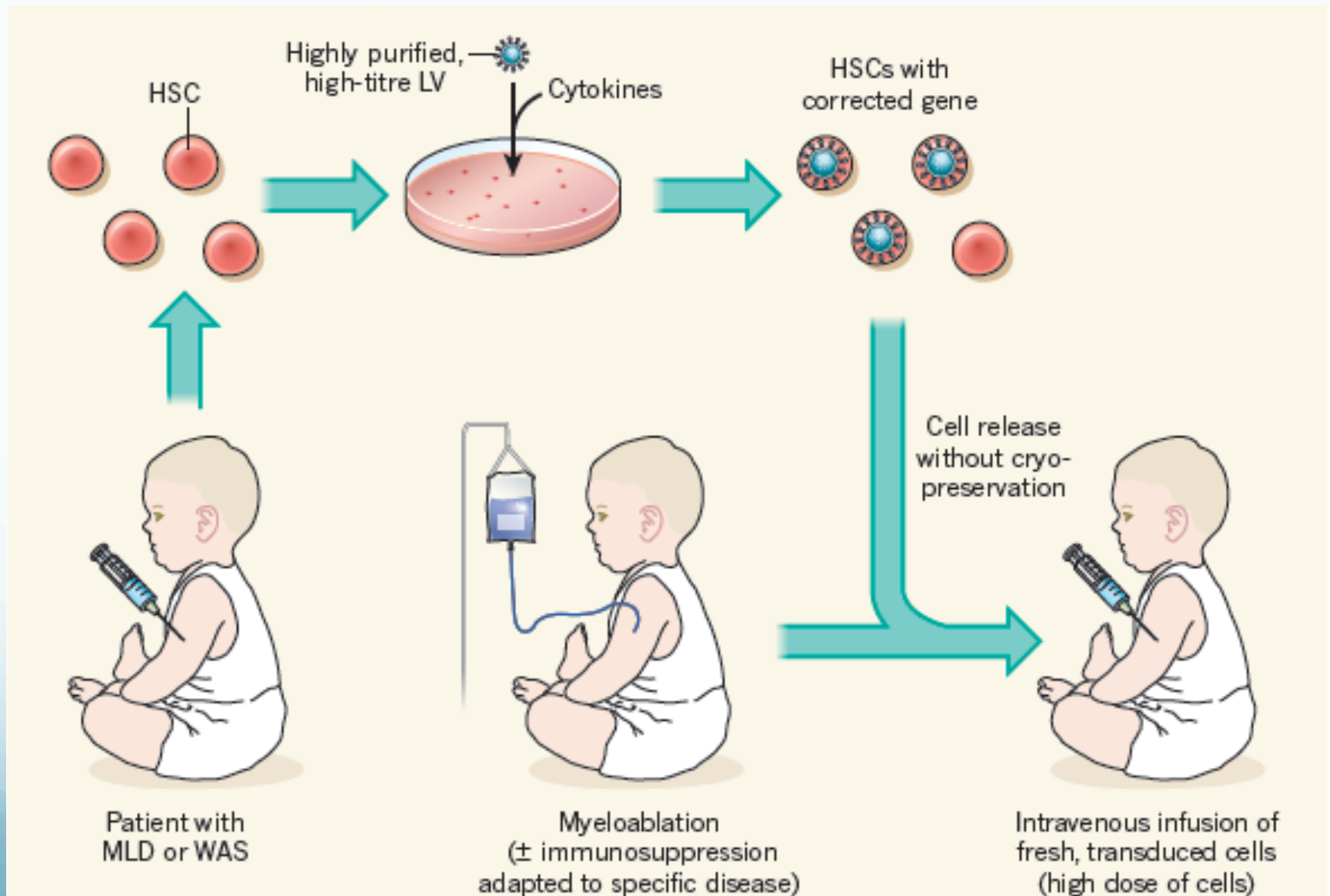




Jesse Gelsinger

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

In 2013.



- **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**

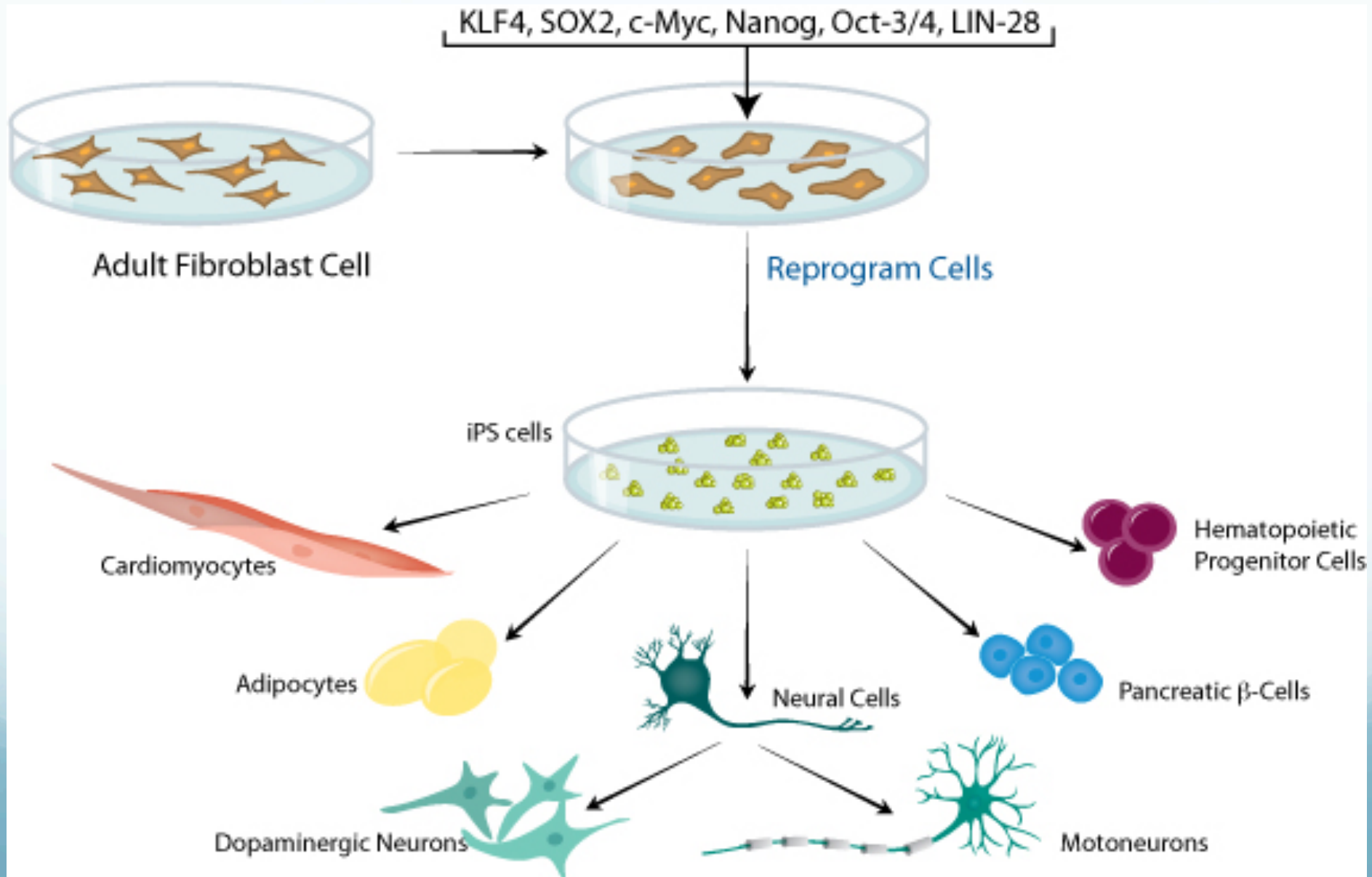
- Accepts big genes
- Easy to isolate and efficient transduction
- Safety – integrates gene near HMGA2 gene

iPS
induced pluripotent stem
cells

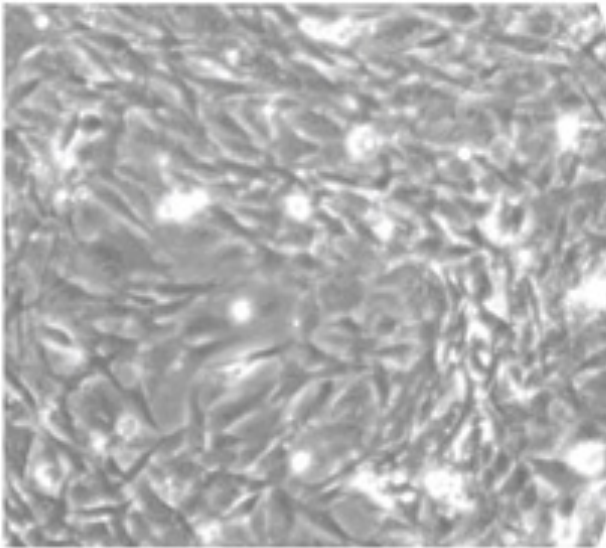


SHINYA YAMANAKA

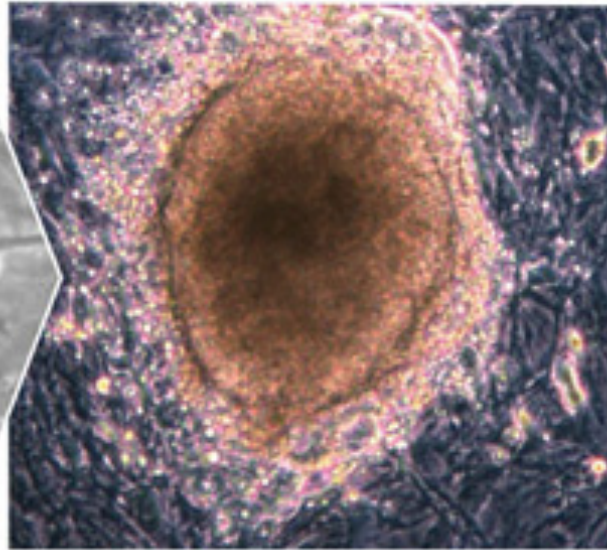
iPS production



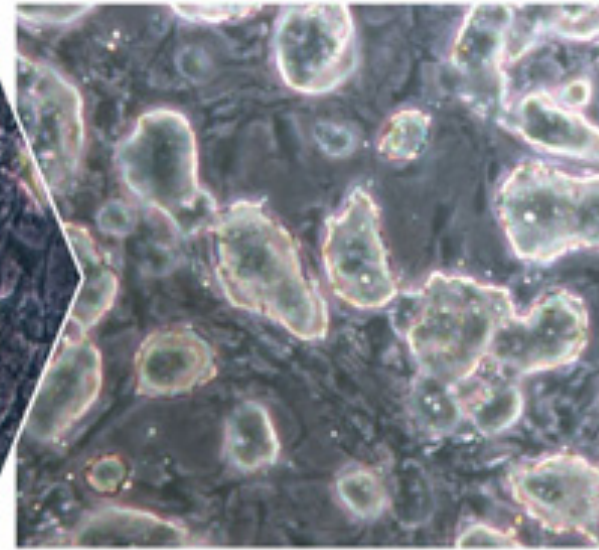
DAY 1
Human skin fibroblasts, after gene transduction



DAY 12
Partially reprogrammed colony

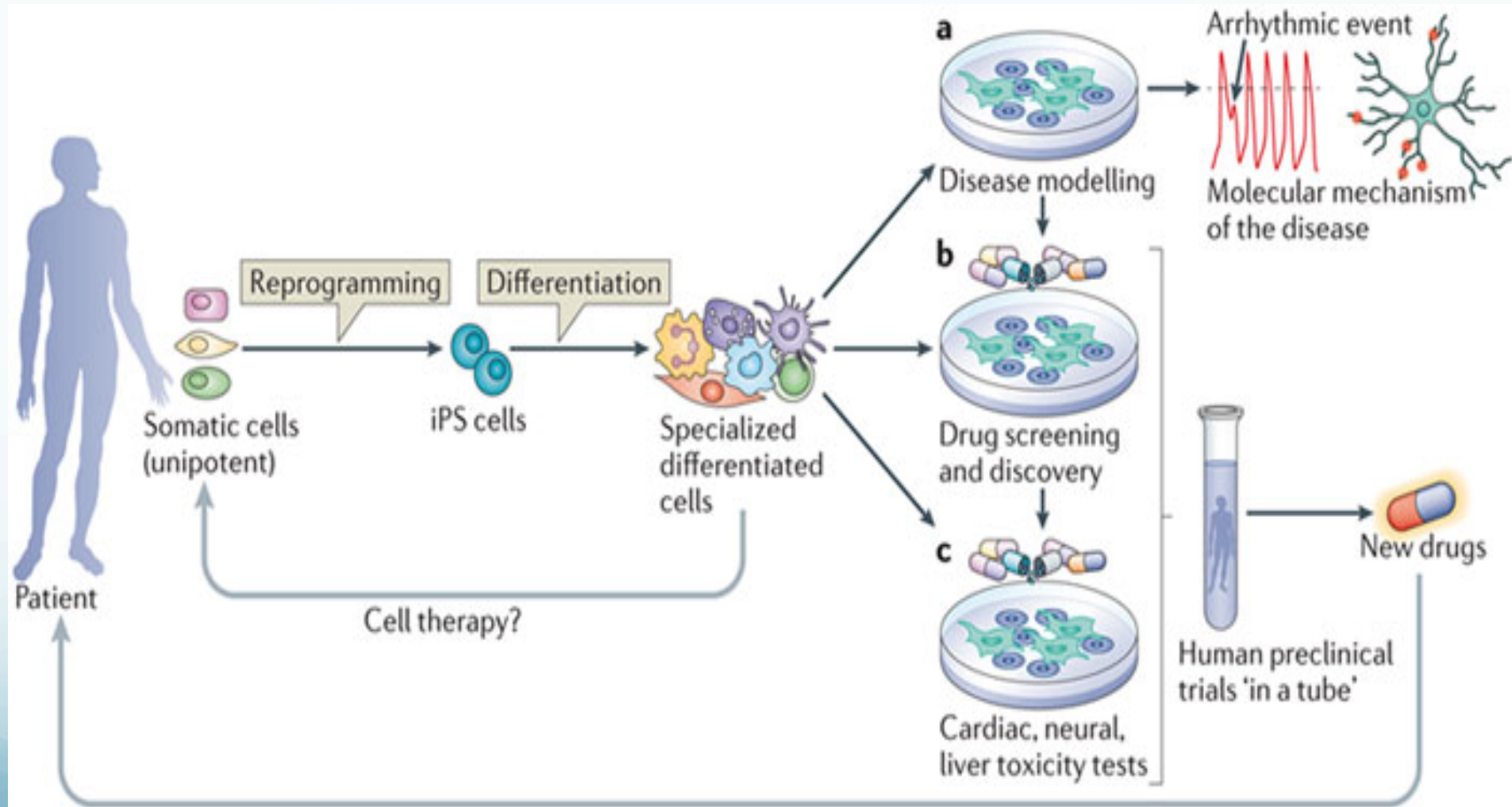


DAY 30:
iPSCs growing on a fibroblast feeder layer

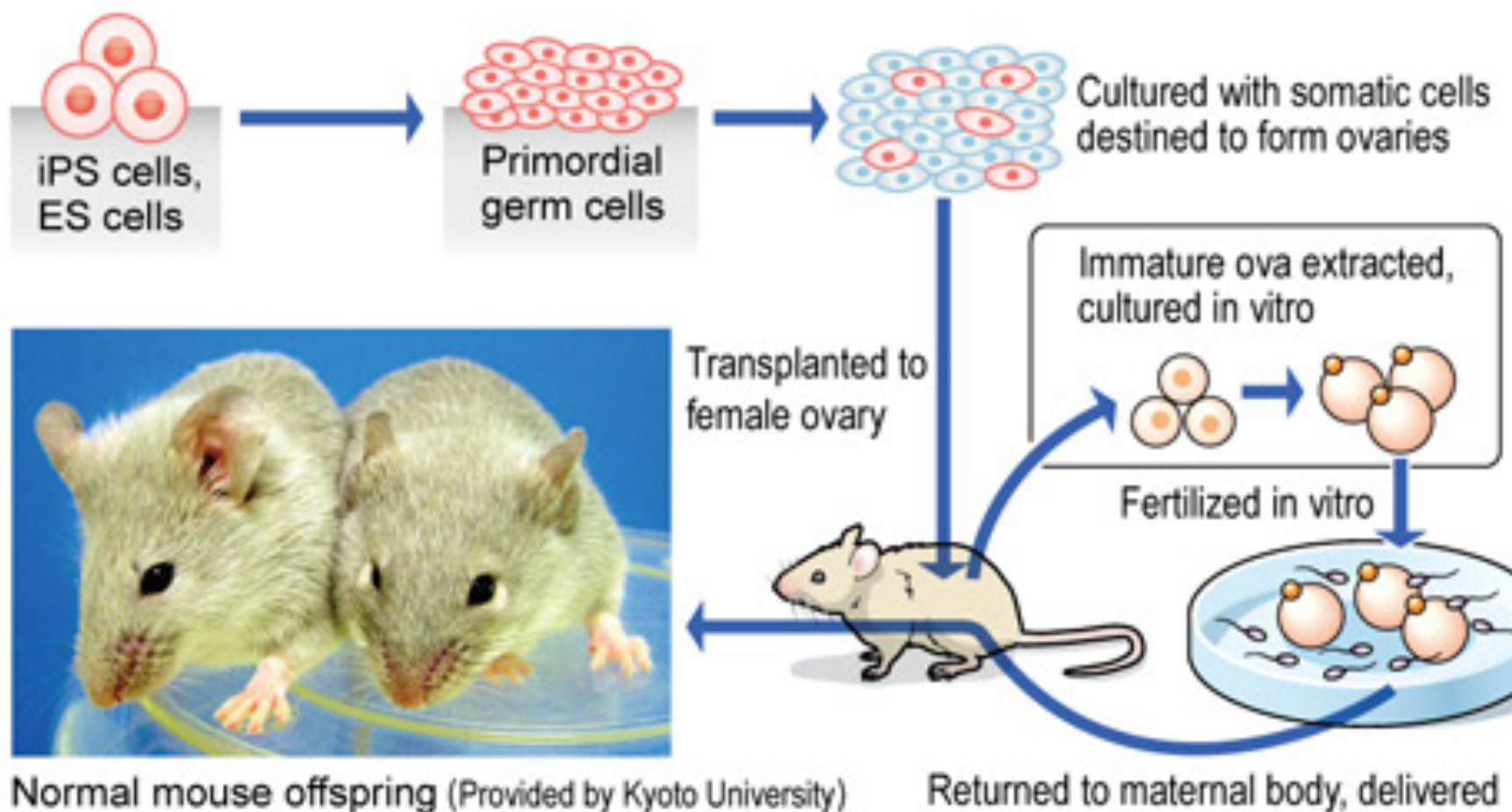


iPS under microscope

Possible use of iPS

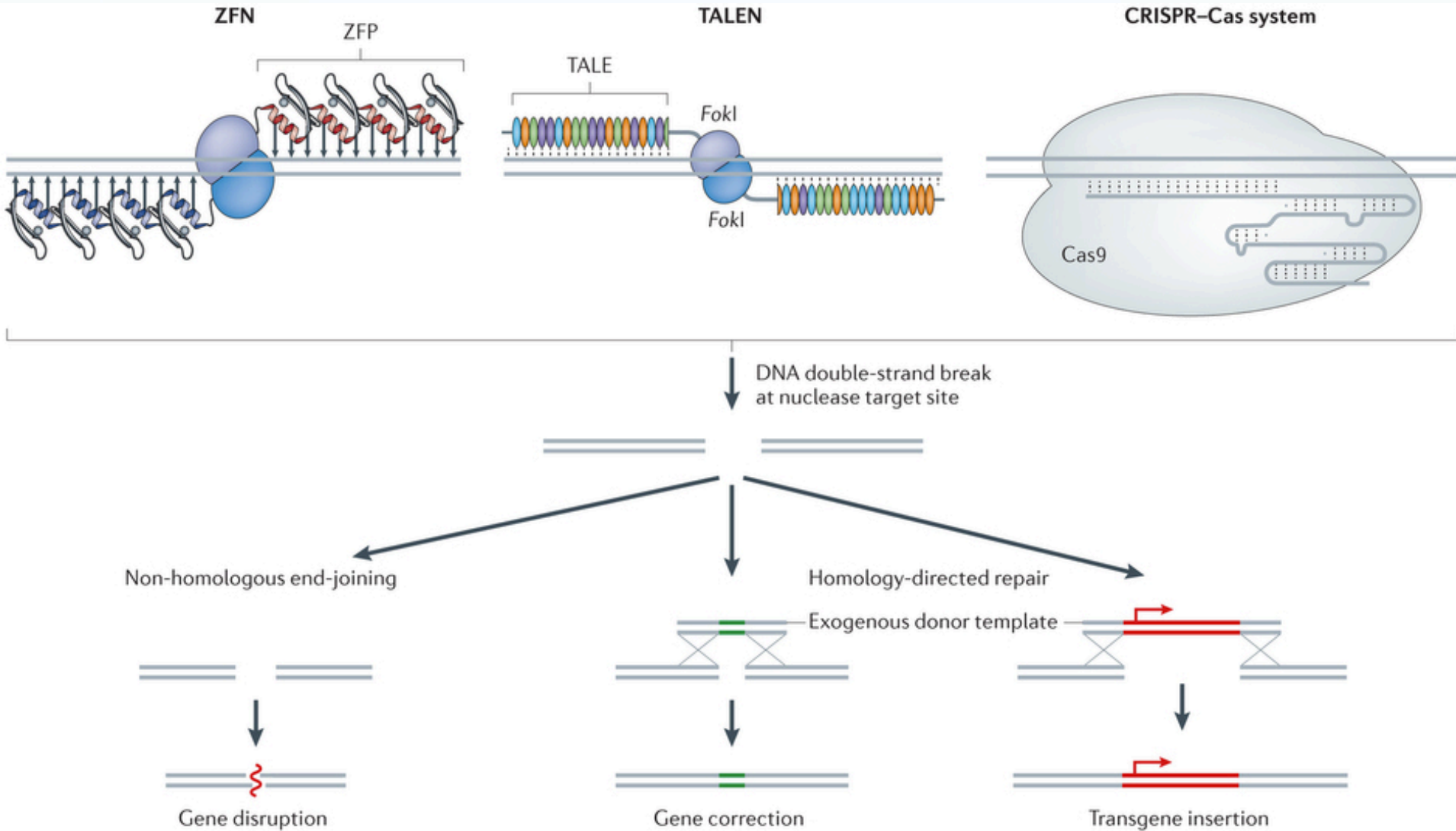


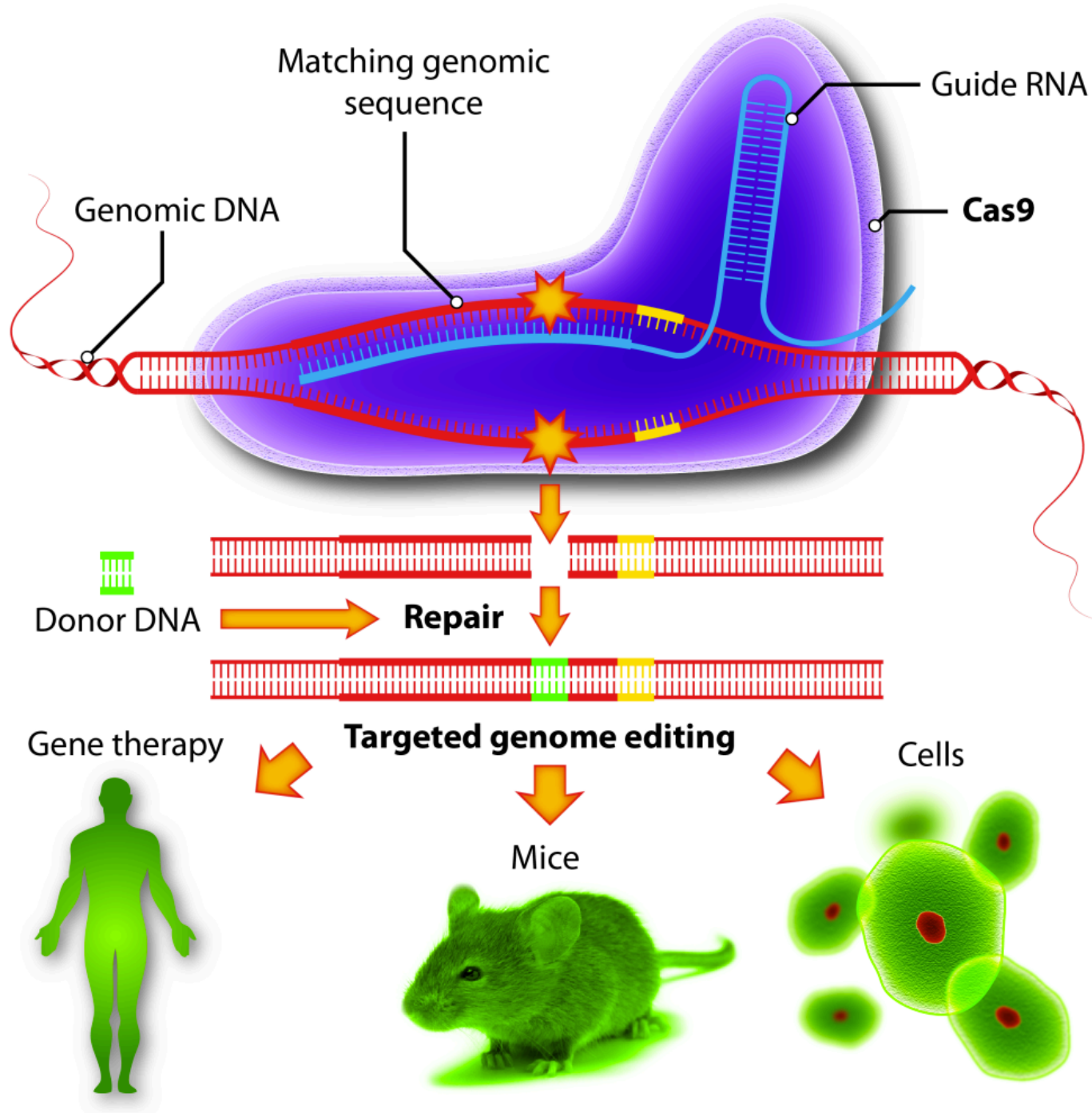
How scientists generated ova from iPS cells

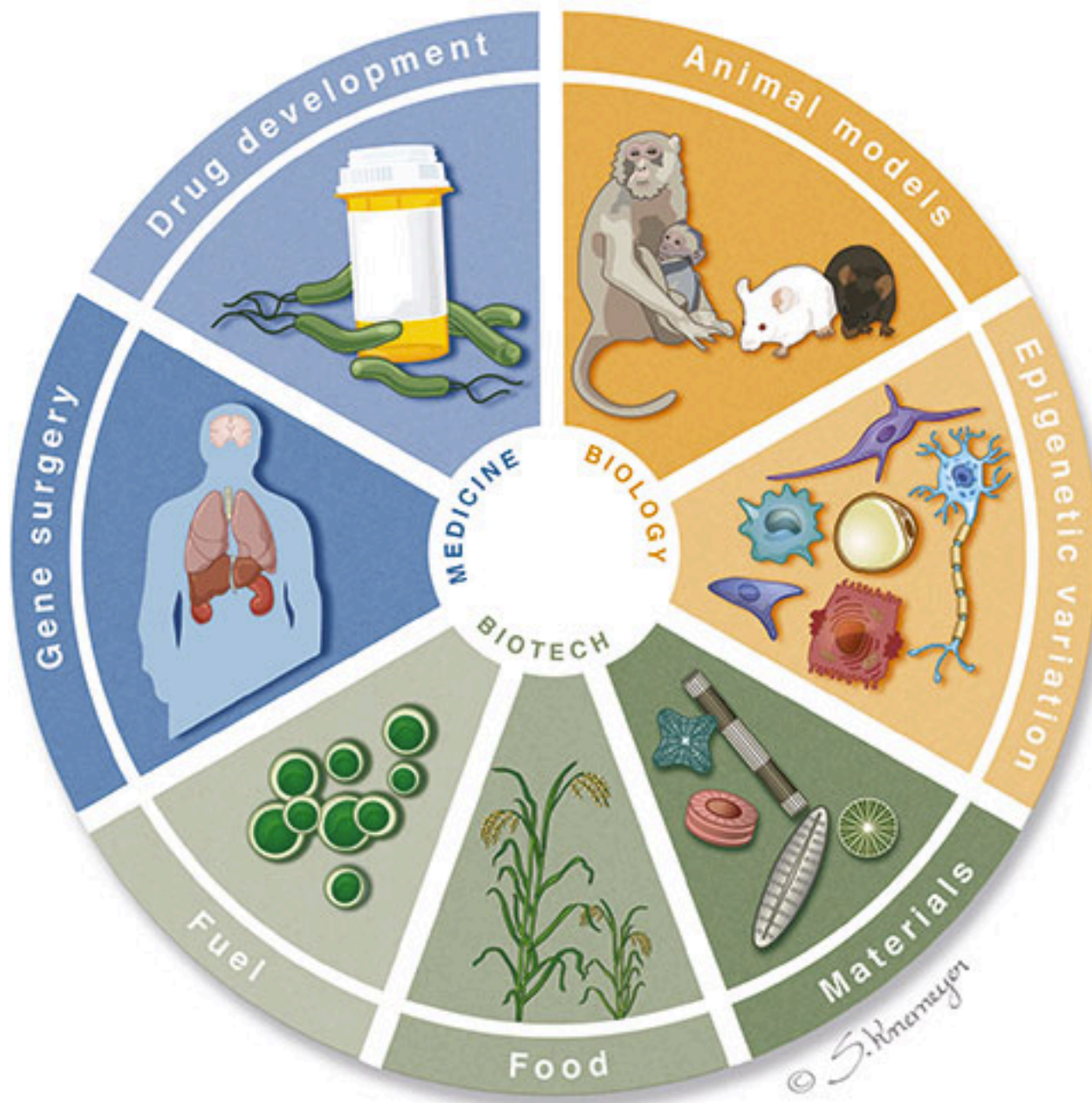


Gene editing

Gene editing technologies

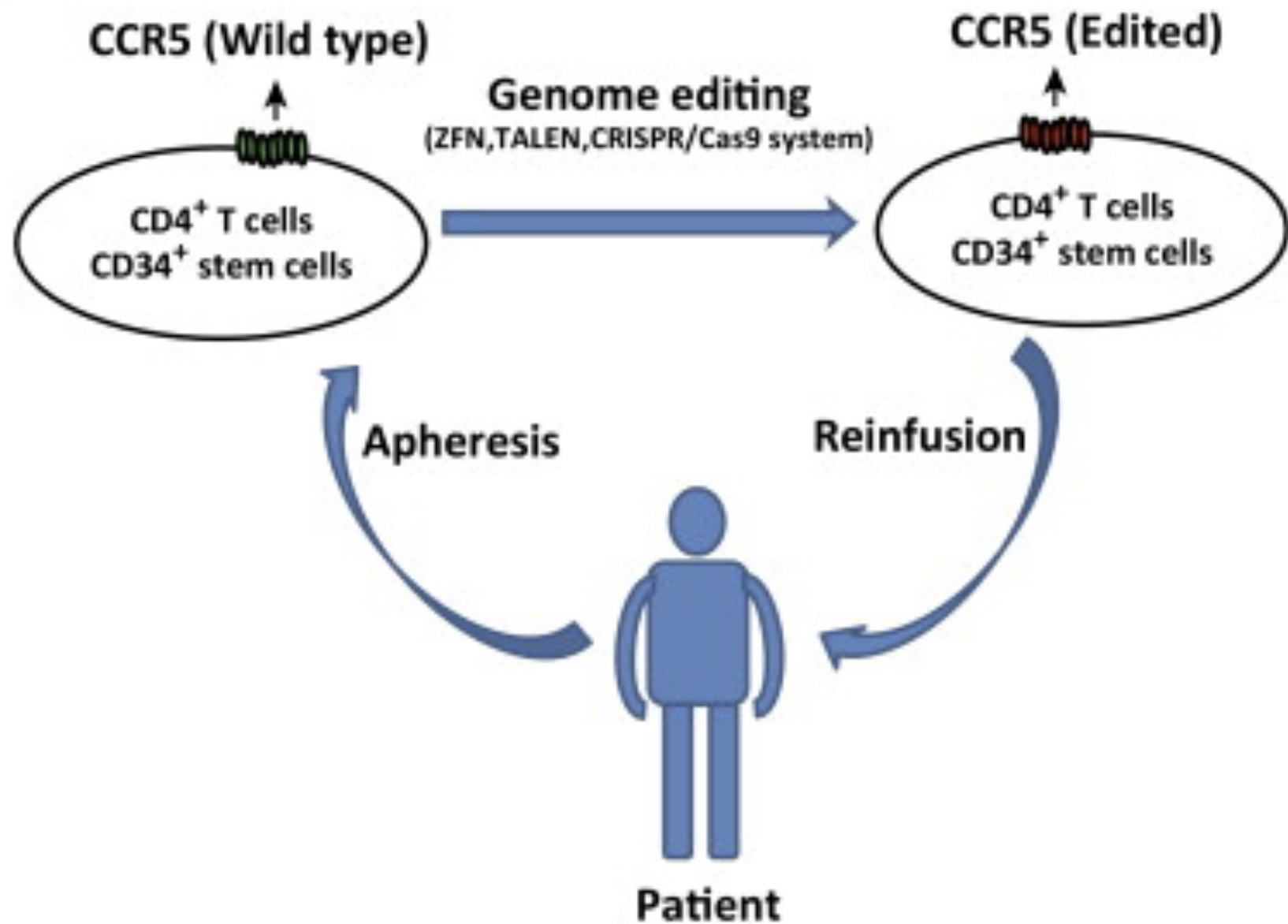






First human gene – editing therapy

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





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 resistant to chemotherapy

Retinitis pigmentosa

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

The NEW ENGLAND JOURNAL *of* MEDICINE

ESTABLISHED IN 1812

NOVEMBER 2, 2017

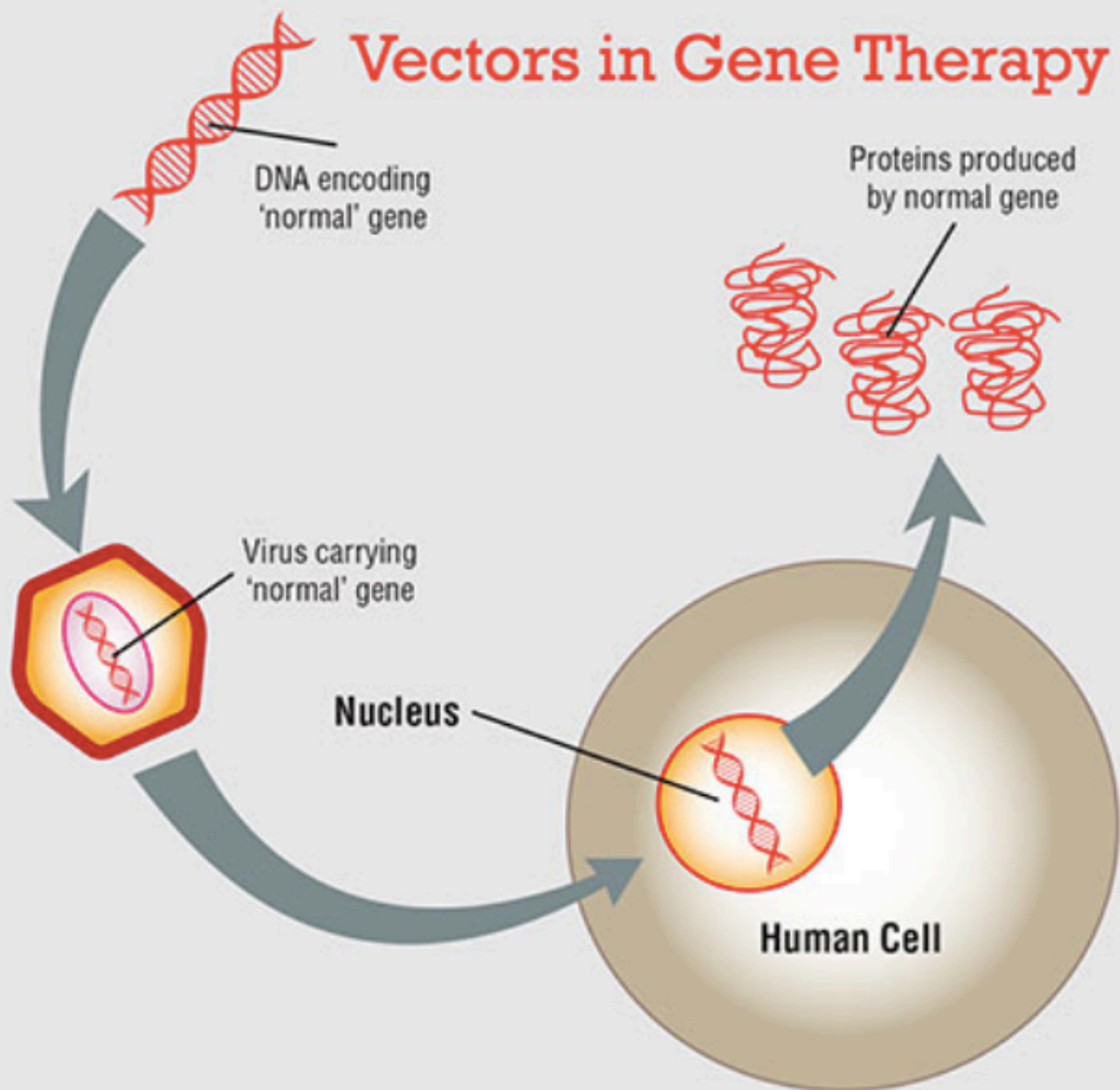
VOL. 377 NO. 18

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





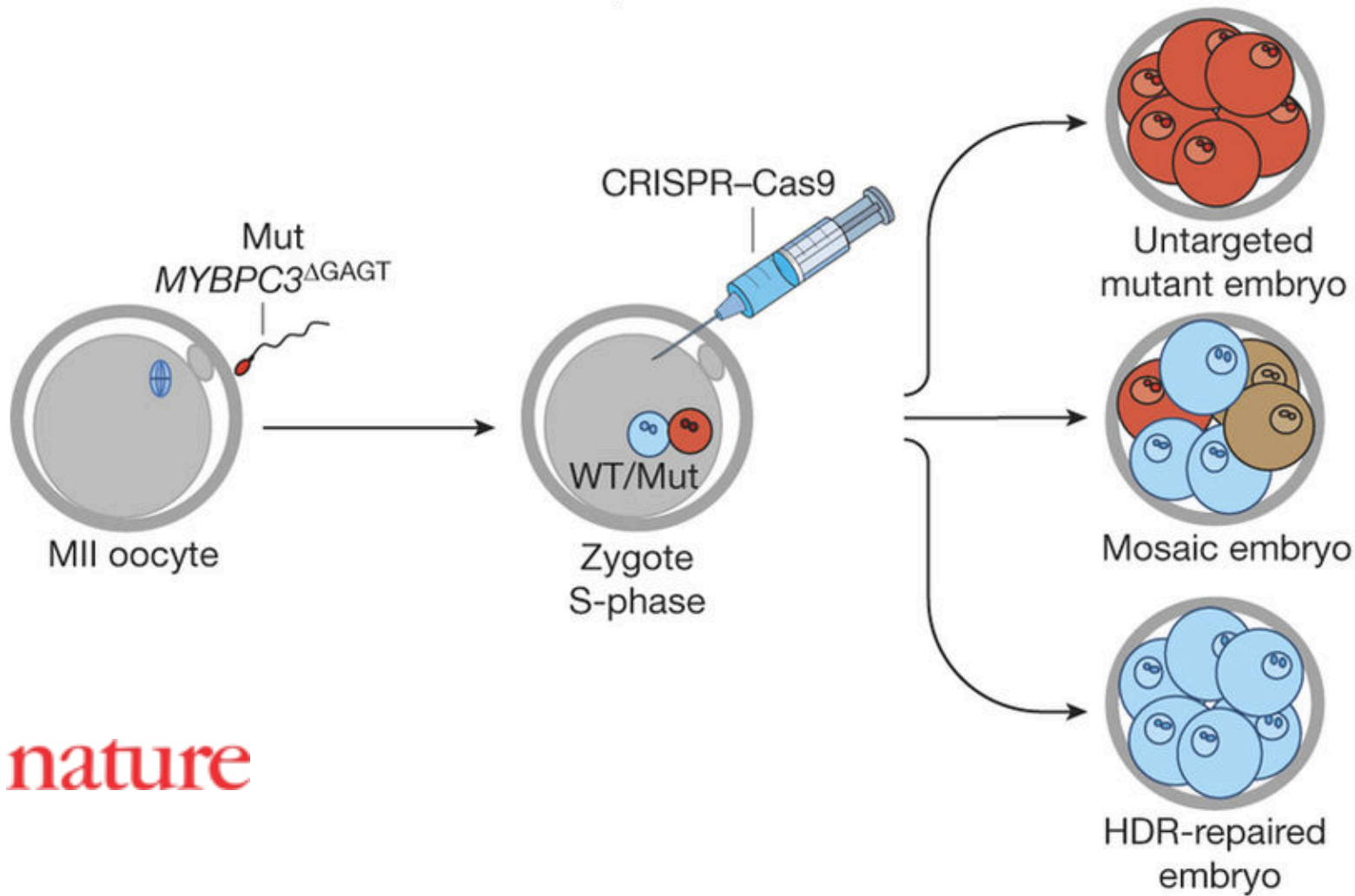
Evelyn Villarreal

Gene editing of human zygote



Gene editing of human zygote

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



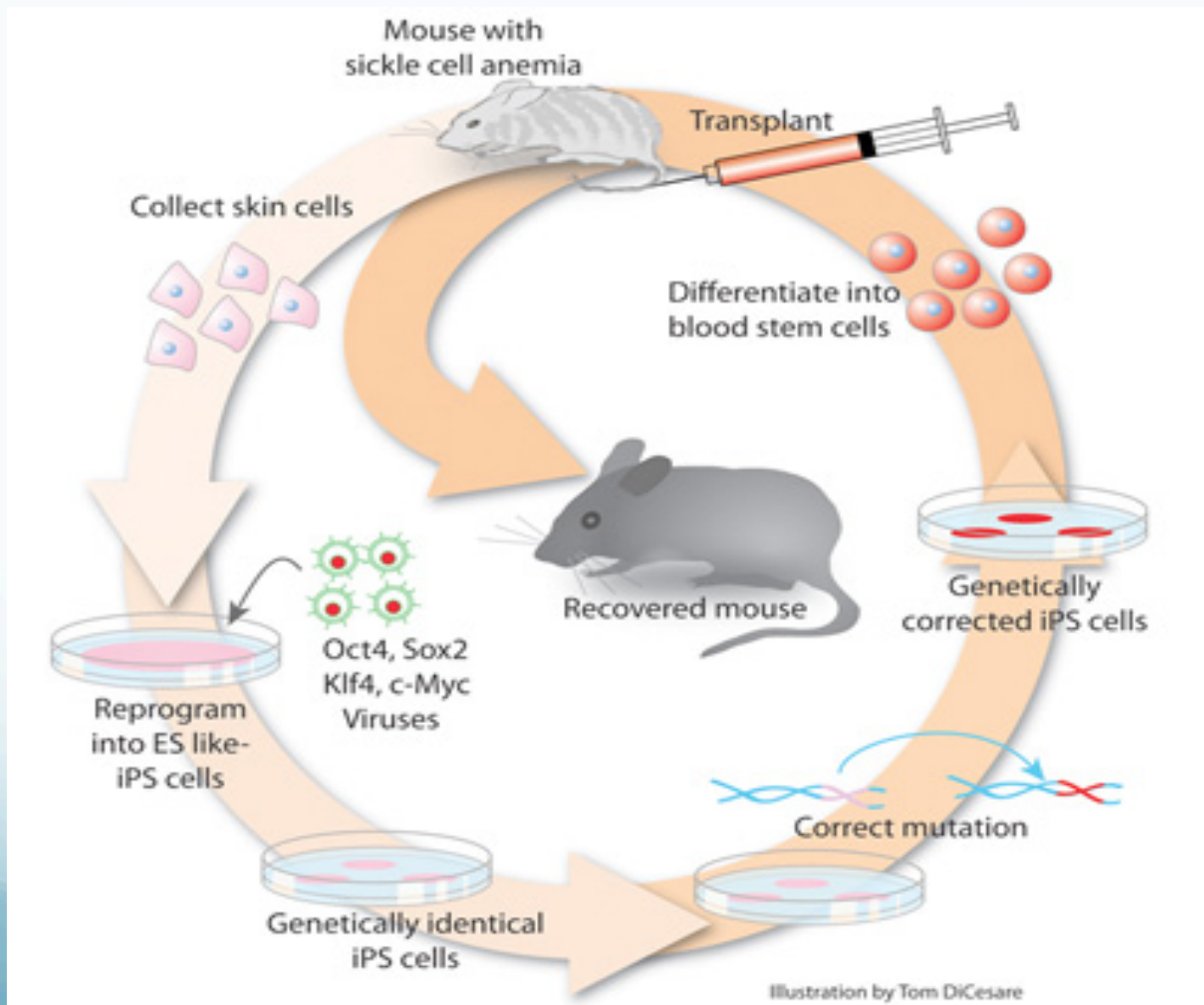
nature

70%

First gene edited human by CRISPR-Cas9

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

iPS + CRISPR



HUMAN GENOME

15 February 2001

nature

ISSN 0028-280X

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 families, 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

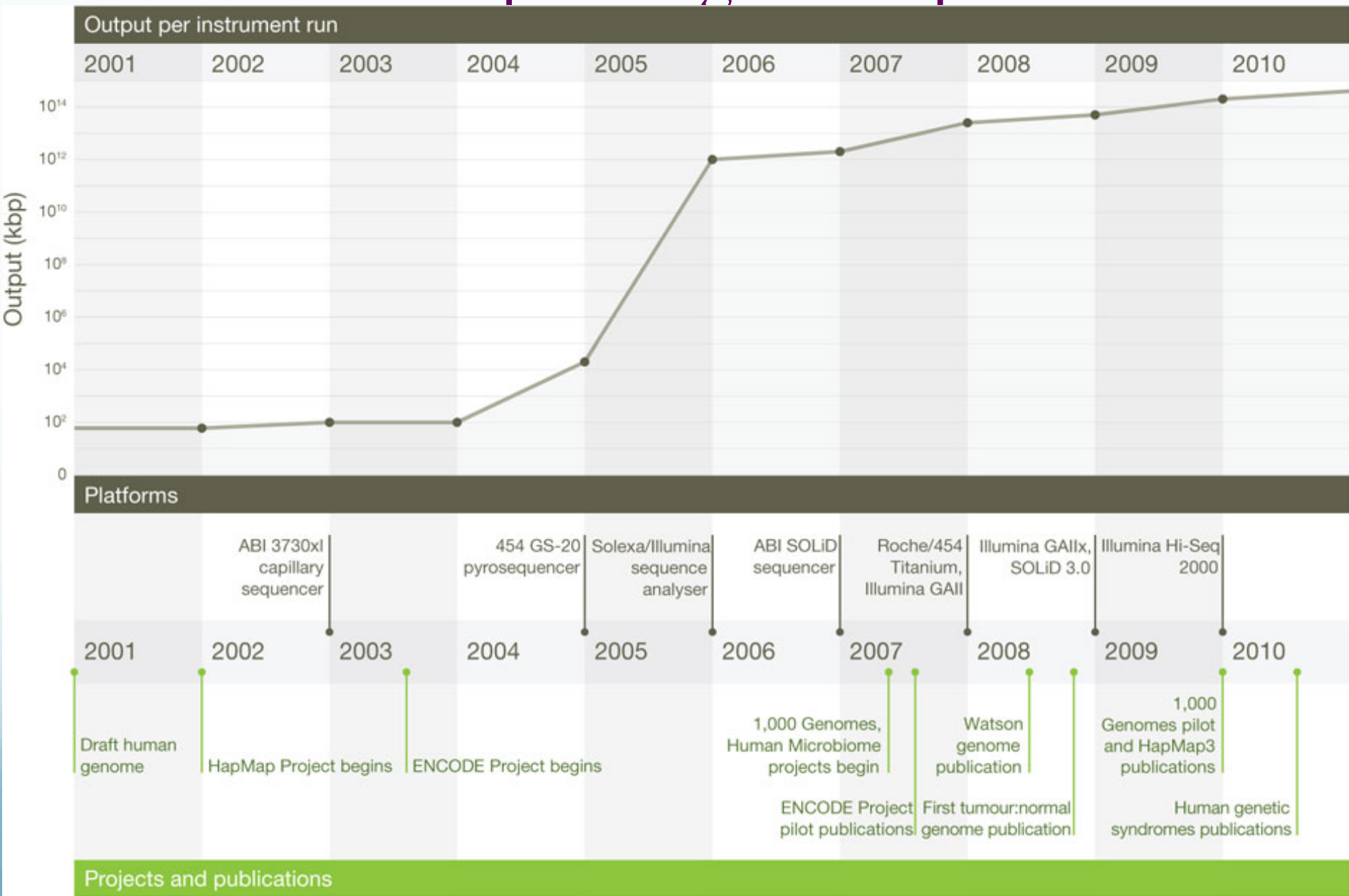
Medical impact

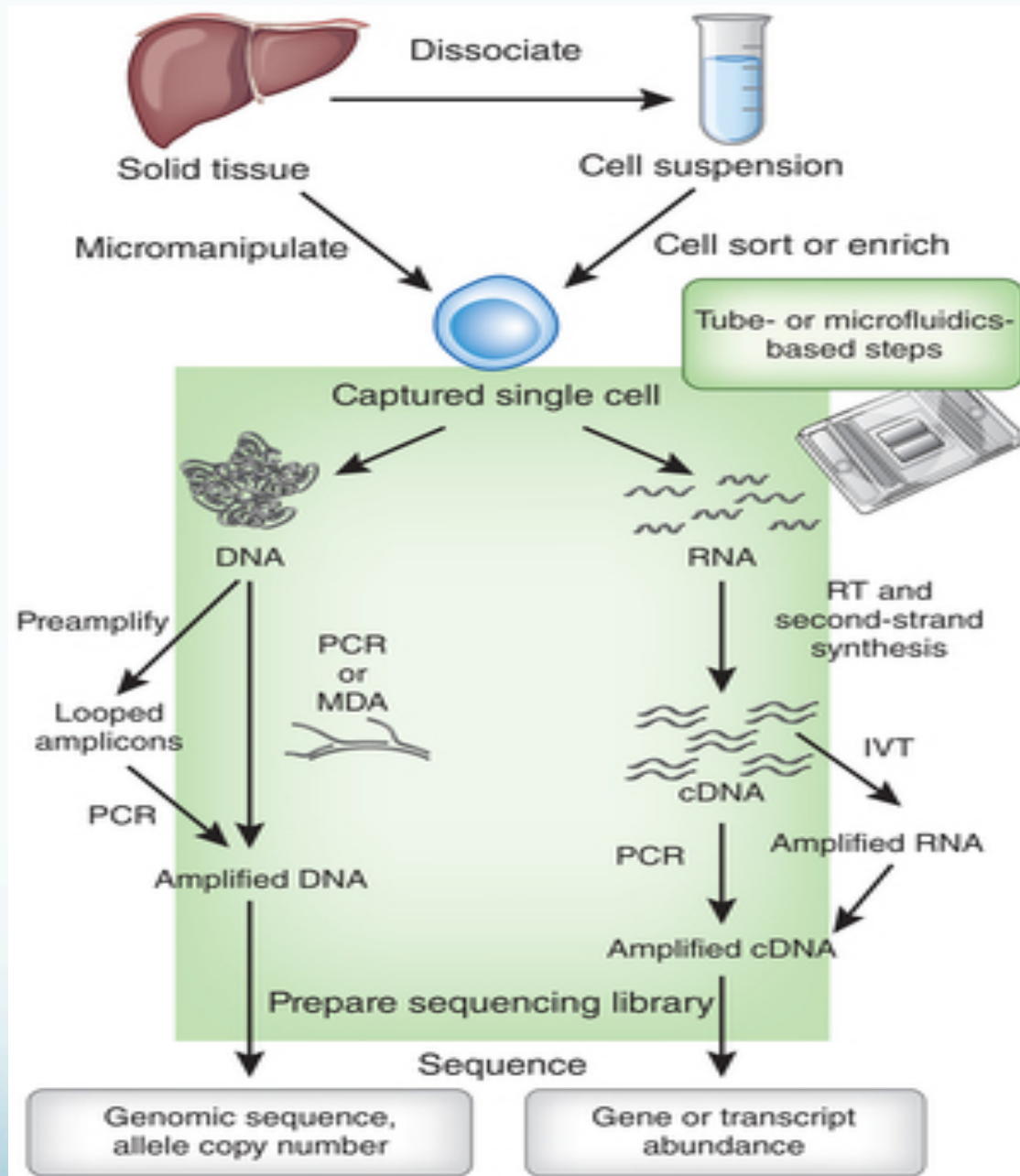
- *Better diagnosis and prognosis*
 - *Genotype then therapy*
 - *Health risks in %*

Novel scientific disciplines

- Proteomics
- Bioinformatics
- GINA 21.05.2008.

DNA sequencing development



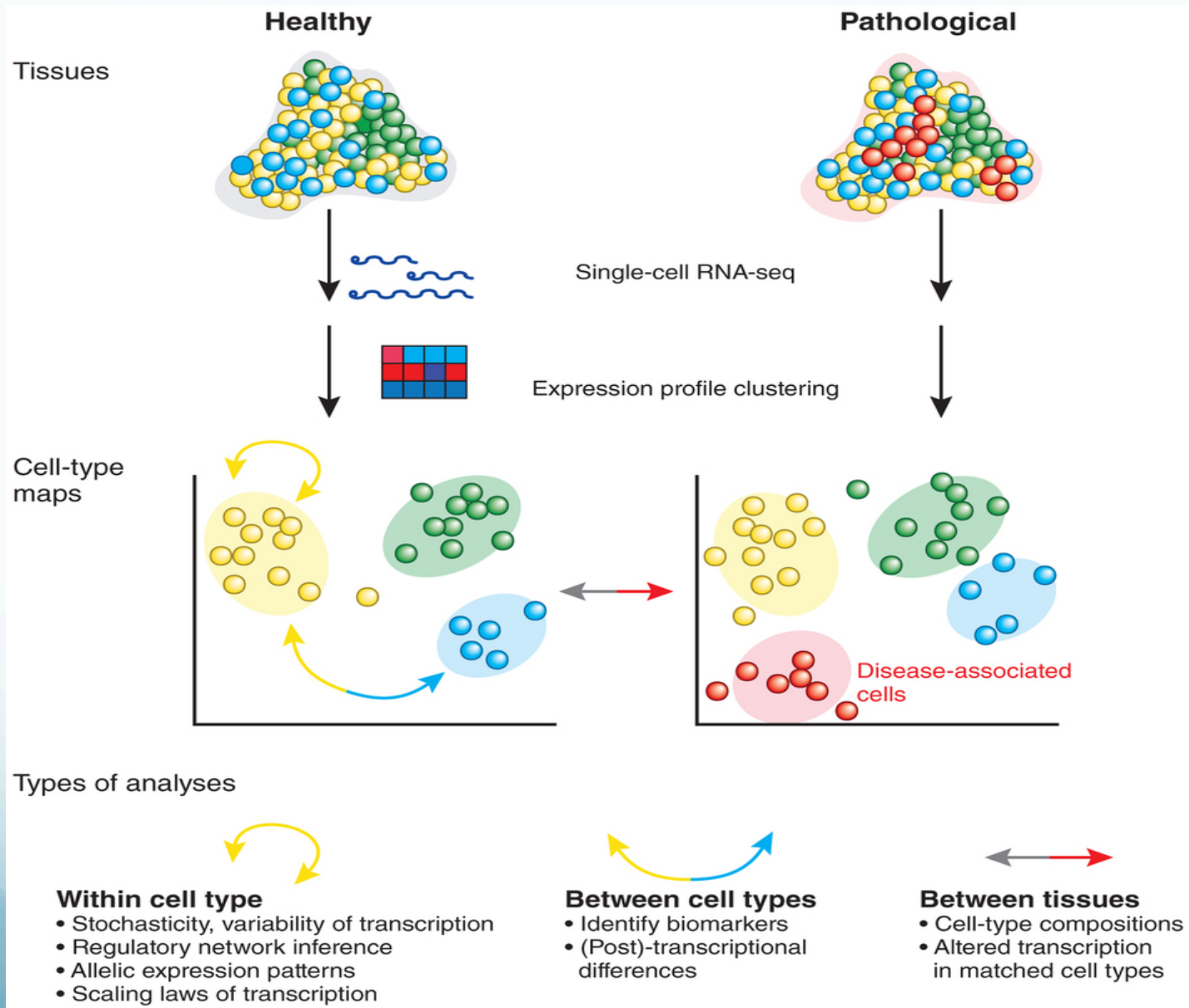


SINGLE-CELL SEQUENCING

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 (around 10% 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 Elements

Goal: To determine function of human DNA

Who: 440 scientists on 147 cell types

RESEARCH (24 types of experiments):

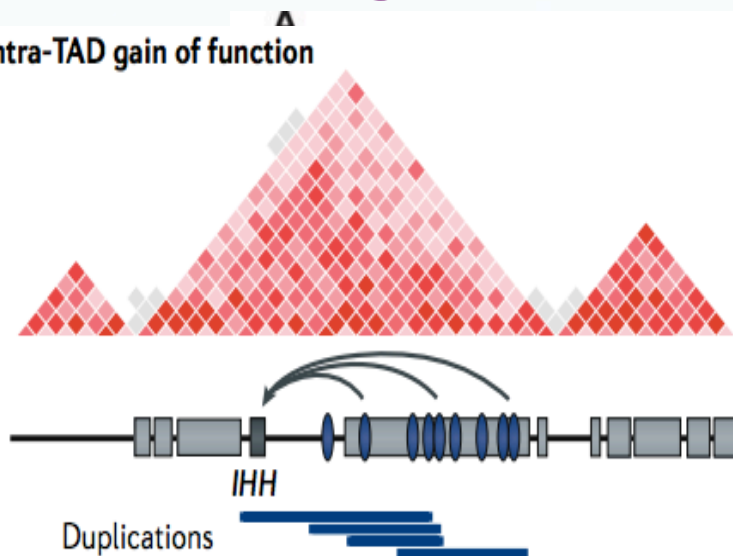
- Histone modifications (13/60)
- Binding of transcription factors (120/1800)
- Distant chromosomal interactions
- Promoter region quantification

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

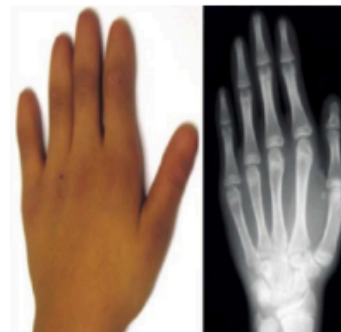
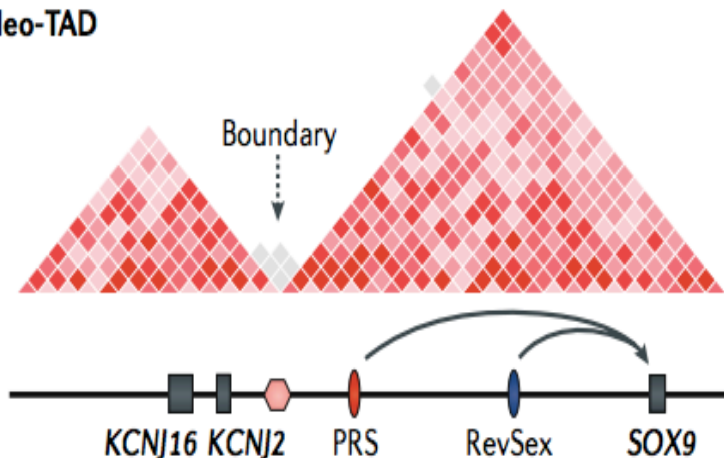
Gain of function:

- *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

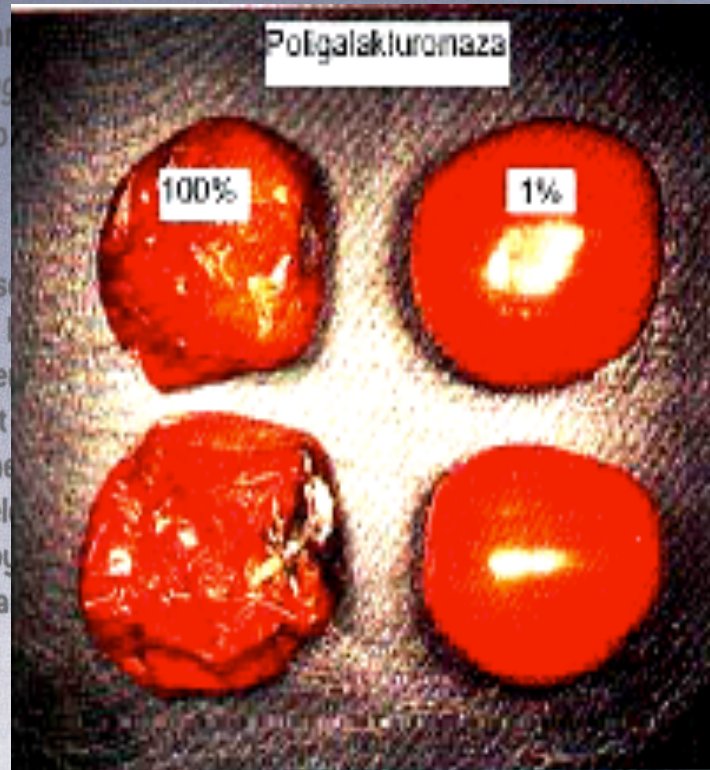


HOW TO MAKE A GENETICALLY MODIFIED PLANT

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 plant the gene from the bacterium *Bacillus thuringiensis* cells to produce a protein that is toxic to insects. The gene is 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 insert the gene into plant cells the gene a "marker" gene flag cells that have taken up the Bt gene markers shield the cells from being killed by antibiotics 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

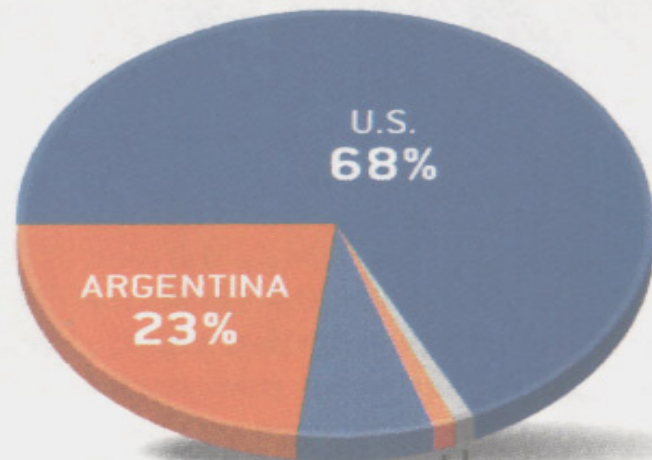
PLANT CELL THAT DID
TAKE UP GENES

DEAD PESTS

COUNTRIES PRODUCING GM CROPS IN 2000

INDUSTRIAL NATIONS

DEVELOPING NATIONS



CANADA 7%

CHINA 1%

OTHER 1%

IN DESCENDING ORDER:

- SOUTH AFRICA
- AUSTRALIA
- ROMANIA
- MEXICO
- BULGARIA
- SPAIN
- GERMANY
- FRANCE
- URUGUAY

PERCENT OF TOTAL GM AREA

BY TYPE ...

CANOLA

6%

COTTON

12%

SOYBEANS

58%

CORN

23%

... AND TRAIT

INSECT RESISTANCE

19%

BOTH

7%

HERBICIDE TOLERANCE

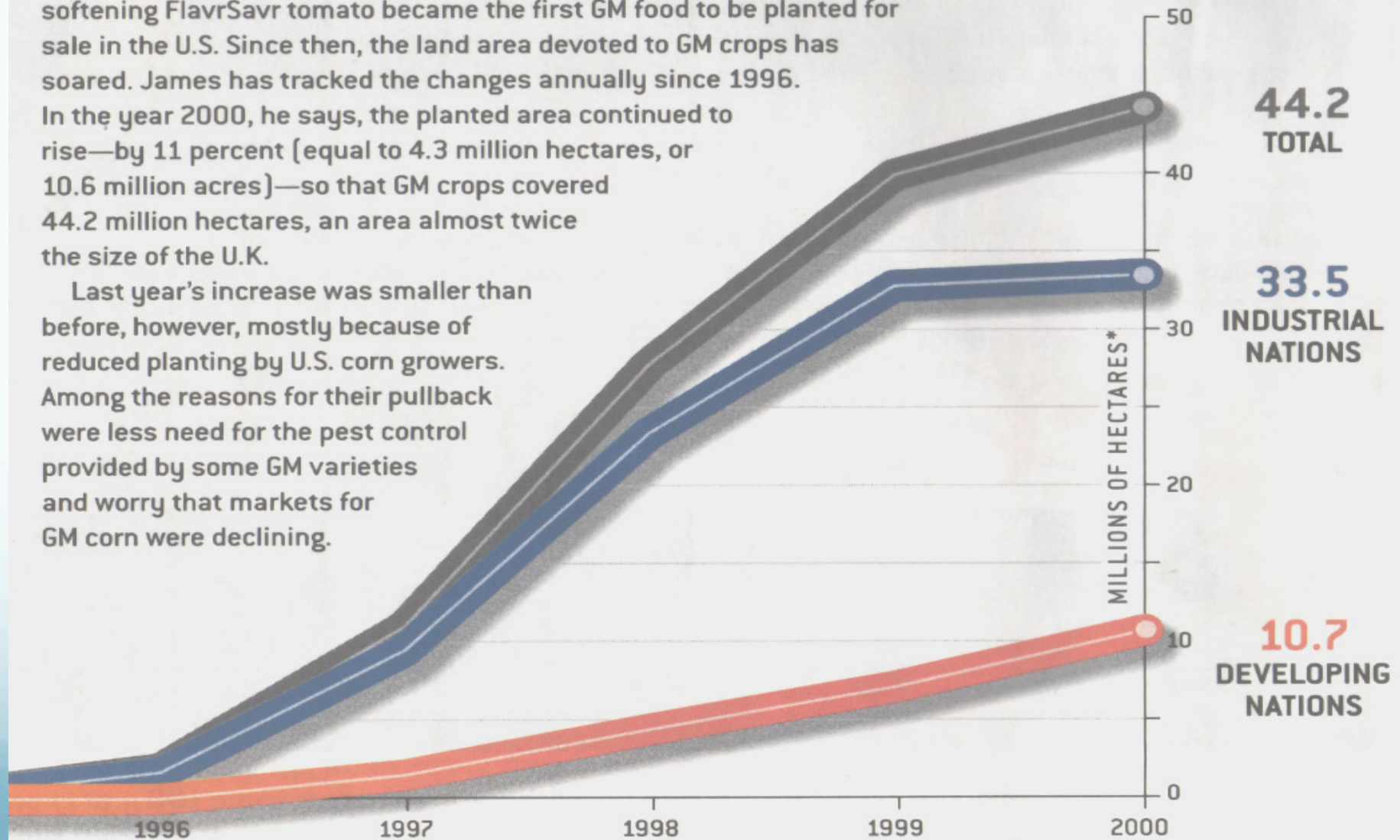
74%

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.

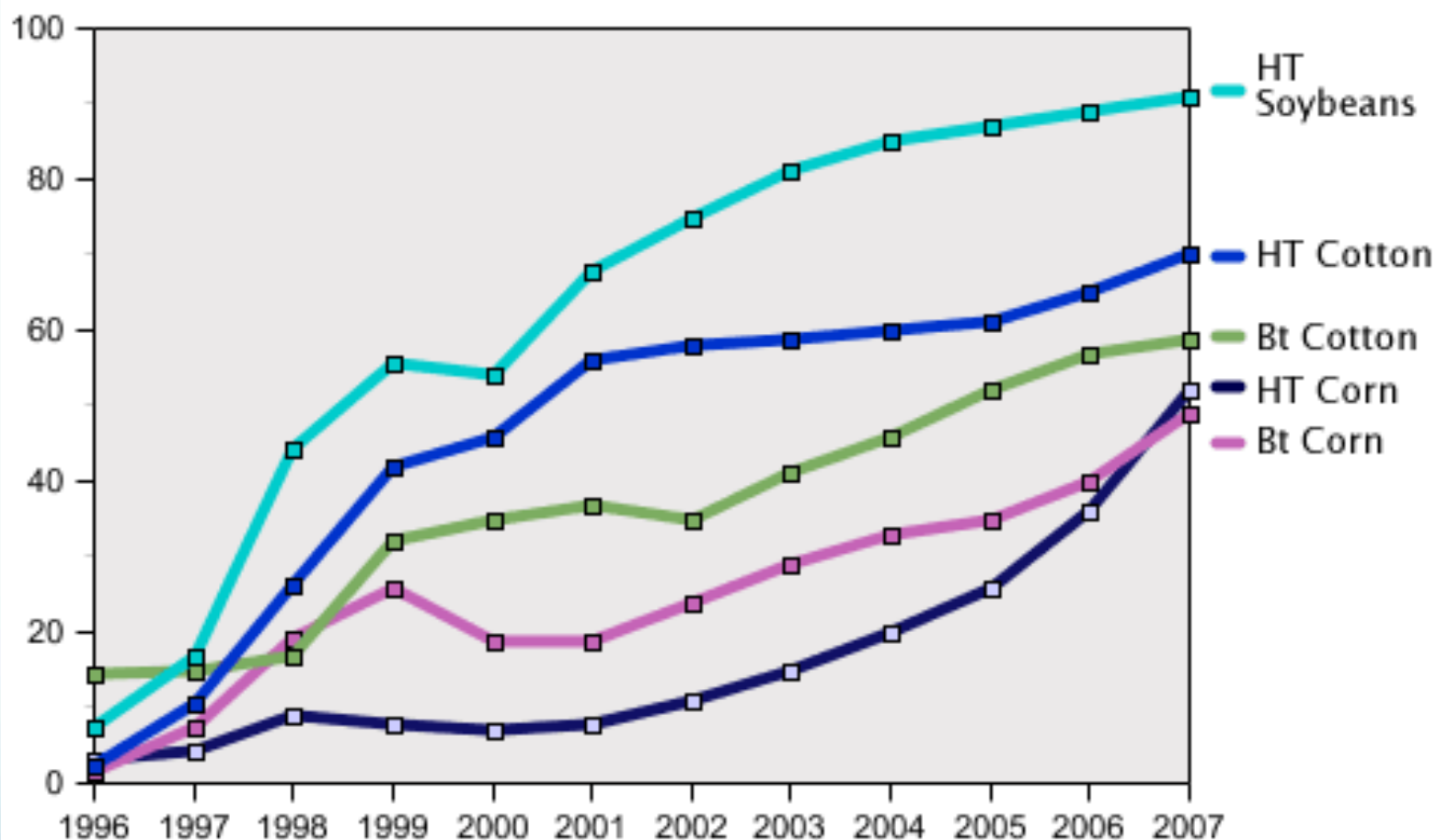
Last year's increase was smaller than before, however, mostly because of reduced planting by U.S. corn growers. Among the reasons for their pullback were less need for the pest control provided by some GM varieties and worry that markets for GM corn were declining.

GLOBAL AREA OF GM CROPS



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

Percent of acres



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 available in the ERS data product, Adoption of Genetically Engineered Crops in the U.S., tables 1-3.

As you move around a supermarket or smaller food shops, it's helpful to know which areas are most likely to conceal products that may have been tampered with so that you can be on your guard. Not all shopping zones are the same. For example, certain categories of food — fresh meat, for example — you can afford to be a bit more relaxed about, while in others — such as sandwiches — extreme vigilance is called for. Below are some tips to help you identify categories or types of food and drink where you need to be extra vigilant.

RED ZONE
not the food and drinks
contain GM ingredients.
reduced-fat foods
is imported from the US
riches
meats, both meat and vegetarian
soy-based ones.
Chinese)
als containing soya
ducts containing soya
k and noodles
at, fish, poultry or
ealent, breaded, battered
mb
meats

- sausage rolls
- savouries and quiches
- (vegetarian)
- creamy mousses (such as salmon or tuna)
- dips and salads
- hard cheese, cheese
- flavoured yoghurts
- frozen yoghurt and non-dairy desserts
- margarine and spreads
- sauces and stir-fry pastes (such as tomato)
- baked goods (especially flavoured breads)

and coils
such as hot chocolate
and chocolate confectionery
packet soups
ices (savory and sweet
ousses mixes)
and sweet spreads (such
chocolate)
lips (such as cheese and
drinks and meal
into
ula milk

SM-FREE...

markets wallow in a sea of conventional, wholefood or 'natural' foods. However, wholefood shops are the wholefood trade has set its standards. It has done this with a full pedigree to guarantee the wholefood sector has as many familiar companies stand out. It is incorporated in the 'green' label for you to consult on the shelves these days stock more to be free from any form of genetic

THE AMBER ZONE

It is possible that the foods and drinks in this zone will contain GM ingredients.

- Bread and rolls
- Sweet and savoury breads (e.g. croissants, muffins, scones, pikelets)
- Artificial flavourings (e.g. vanilla or peppermint essences)
- Flavoured teas, fruit teas and herbal infusions
- Tinned fruit
- Tinned vegetables
- Tinned fish packed in oil
- Any food fortified with vitamin B2 (riboflavin) (breakfast cereals, baby food and soft drinks)
- Any yeast for baking
- Dried and wet baby food

THE GREEN ZONE

You can be sure that, for the time being at least, none of the foods or drinks in this zone have been genetically modified in any way.

- Fruit and vegetables, fresh or frozen (but *GM* whole tomatoes, on sale in the pipeline)
- Meat, poultry and game, fresh or frozen, just butchered and cleaned
- Fish and shellfish, fresh or frozen, but not processed fish fingers or processed seafood such as crab sticks
- Eggs in shells
- Coffee (beans, ground and instant)
- Natural teas and herbal infusions without flavourings, either loose or in teabags
- Real cocoa powder

TRAN FOO

00

By DAVID DERBYSHIRE
Science Correspondent

[illegible]

helping the U.S. firm

Safe shopping: Using this guide can help you determine whether your food and drink purchases are likely to have been genetically modified

Use this section to get more information about 'branded' foods. These are products carrying brand names of individual companies as opposed to those that carry the supermarket's own label. This section looks at familiar branded foods that could be genetically modified or could become so in the near future and gives each brand a red/amber/green rating*

RED The company sees some potential benefits in GM food and is prepared to use GM ingredients and derivatives. Its foods are more likely to contain GM ingredients

AMBER The company's foods could be genetically modified. Companies in this category have no clear stance for or against genetic modification. They either cannot guarantee all their products or can give only a qualified GM-free guarantee "for the time being."

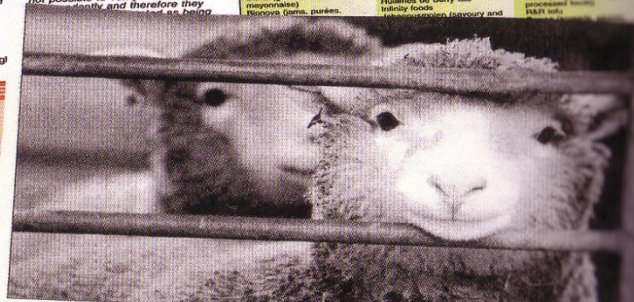
GREEN The company never knowingly sources genetically modified

ingredients or derivatives. Its foods are highly unlikely to contain GM ingredients or derivatives and would only do so through unintentional contamination.

**This rating is based on policy and statements of intent supplied by the company or its parent company. It is not possible to verify these claims independently and therefore they are presented as being unverified.*

[illegible]

Aero	Echo
After Eight	Elmlea
Altora	Fab lollipops
Alpen (all types)	Flake
Astros	Flora
Batclifore	Fox's biscuits
Bird's Eye Walls	Fox's glacier min
Black Magic	Freddo
Blue Band	Fry
Boost	Fry's confection
Butter sausages	Frulibix



Looks familiar: these cloned sheep at the Roslin Institute in Edinburgh are seen primarily as a means to genetic improvement.

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

ONE OF the most intense commercial battles of recent years found a focus yesterday at the Royal Show in Toronto, in a patch of wheat covering an area the size of two table tennis tables. It looked no different from any other wheat, except that it had its own 24-hour security guard.

What the guards are protecting is actually invisible. The wheat has been genetically modified (GM) to contain extra gluten genes – so it is stronger, has a taller stalk and bouncier. But that makes it a prime target for the groups aiming to uproot such crops. So for this year GM plants have been torn up at almost 30 of 200 experimental sites around Britain. To keep the wheat

1111

BY CHARLES ARTHUR
Technology Editor

in ten food products contain soya or soya oils or soya lecithin. From September, the food will have labels: "Does not contain genetically modified elements", or: "Contains genetically modified elements".

The Laboratory of the Government Chemist (LGC) is one of two UK organisations offering DNA tests to identify whether foods contain GM elements. Recently it hosted a crowded industry session with speakers from the supermarket Sainsbury's and Rank Hovis McDougall, which makes all products.

ly-modified crops such as oilseed rape are the latest target for eco-protest groups.

Middle-class activists on the

Finance

News Analysis Agribusiness is running scared from GM foods

Test fields of conflict

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.

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.

2 SUPERWEEDS WILL ARISE
as genes that give crops the ability to kill
insect pests or to withstand herbicides
find their way into weeds.

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.

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:
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.



Transgenic rice forming β -carotene (provitamin A)

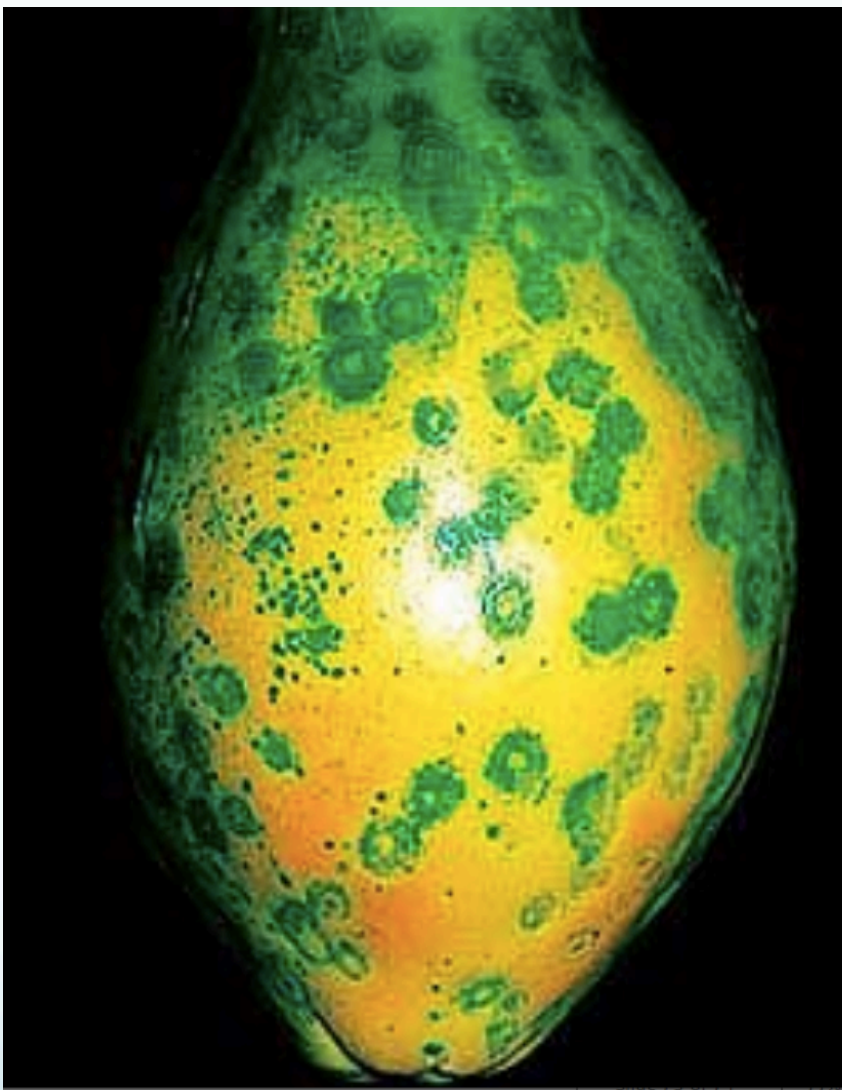


nany)

- 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 virally 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 impacts 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 !