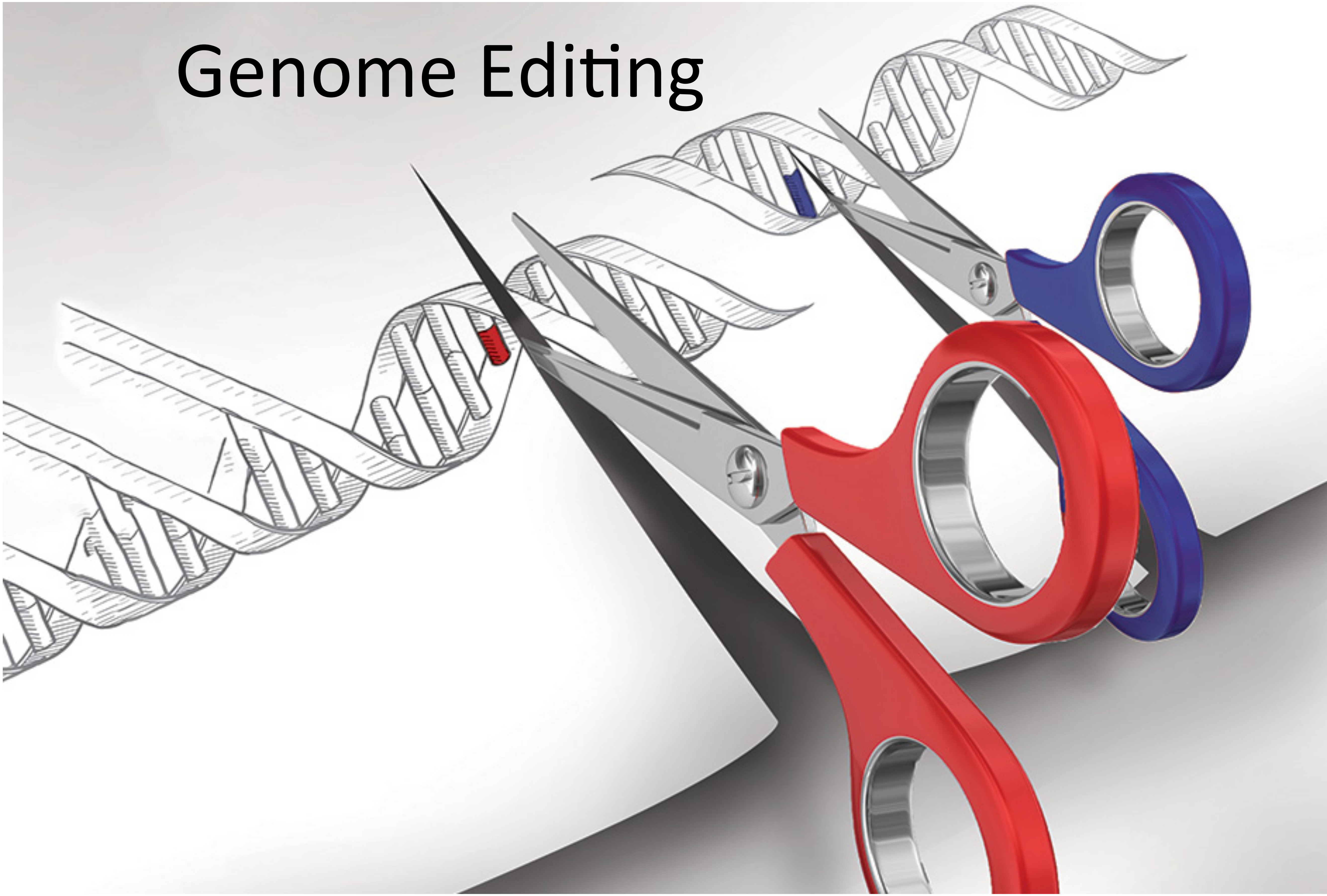

Genome Editing

THE NEW YORKER

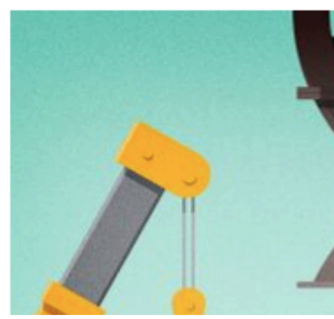
ANNALS OF SCIENCE | NOVEMBER 16, 2015 ISSUE

THE GENE HACKERS

A powerful new technology enables us to manipulate our DNA more easily than ever before.

BY MICHAEL SPECTER

At thirty-four, Feng Zhang is the youngest member of the core faculty at the Broad Institute of Harvard and M.I.T. He is also among the most accomplished. In 1999, while still a high-school student, in Des Moines,



U.S.News & WORLD REPORT NEWS

Geneticists Urge Caution on Breakthrough Technology of Gene Editing

Group issues statement allowing basic, but limited, research.

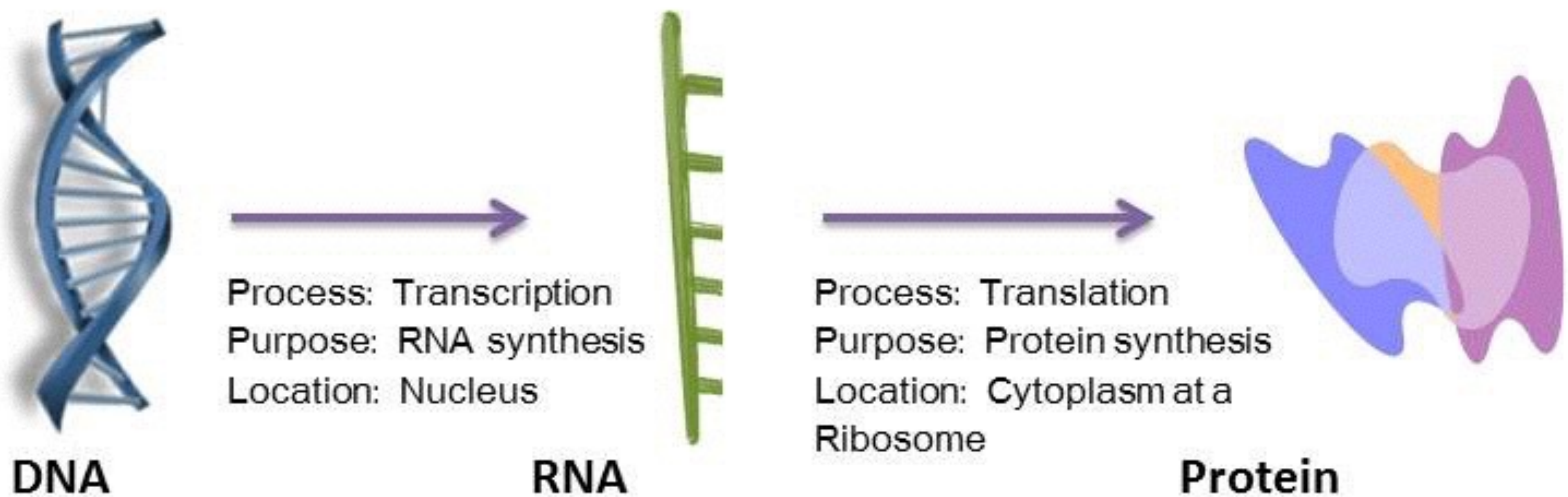
The Crispr Quandary

SCIENCE

Scientists Seek Moratorium on Edits to Human Genome That Could Be Inherited

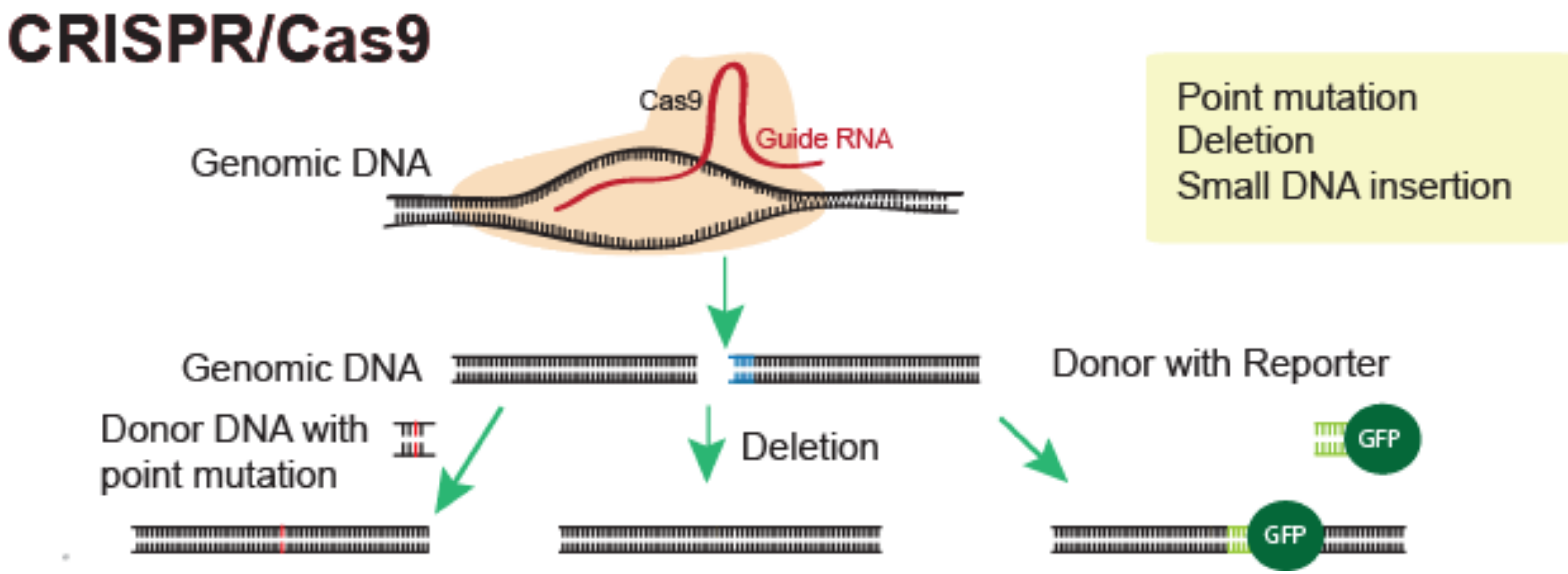
By NICHOLAS WADE DEC. 3, 2015

The Central Dogma



DNA contains the original codes for making the proteins that living cells need. mRNA is a copy of a gene located on the DNA molecule. mRNA will leave the nucleus of the cell and the ribosome will read its coding sequences and put the appropriate amino acids together.

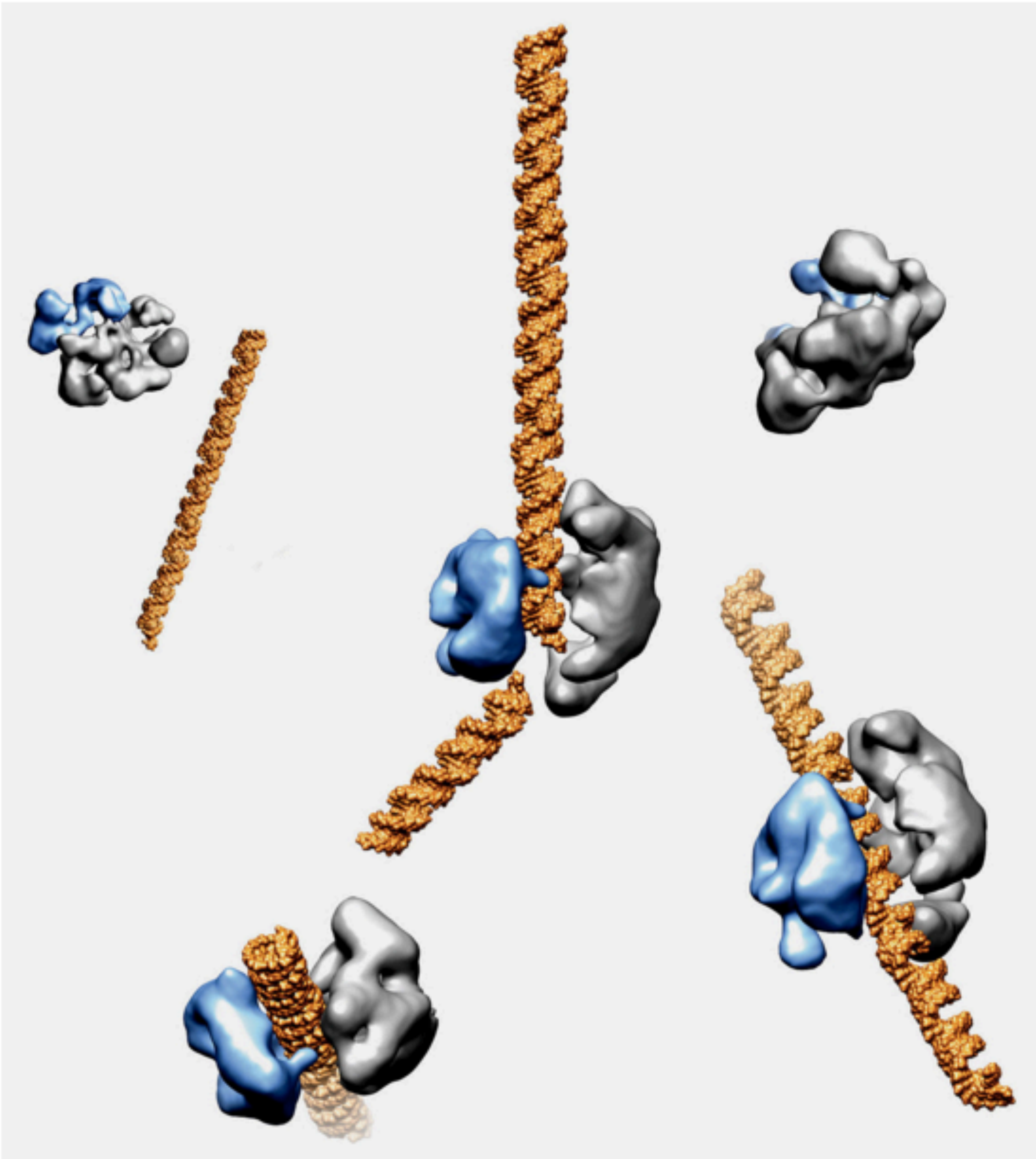
How CRISPR/Cas9 works



The Cas9 nuclease (scissors) cuts DNA.
The "guide RNA" tells Cas9 where to make the cut.
The cell repairs the damage but often makes mistakes, causing mutations.

http://www.appliedstemcell.com/services/animal-models/knock-in-mouse-crispr/

Cas9 in action, cutting DNA



The enzyme Cas9, shown in blue and gray, can cut DNA, in gold, at selected sites, as seen in this model from electron microscope images.

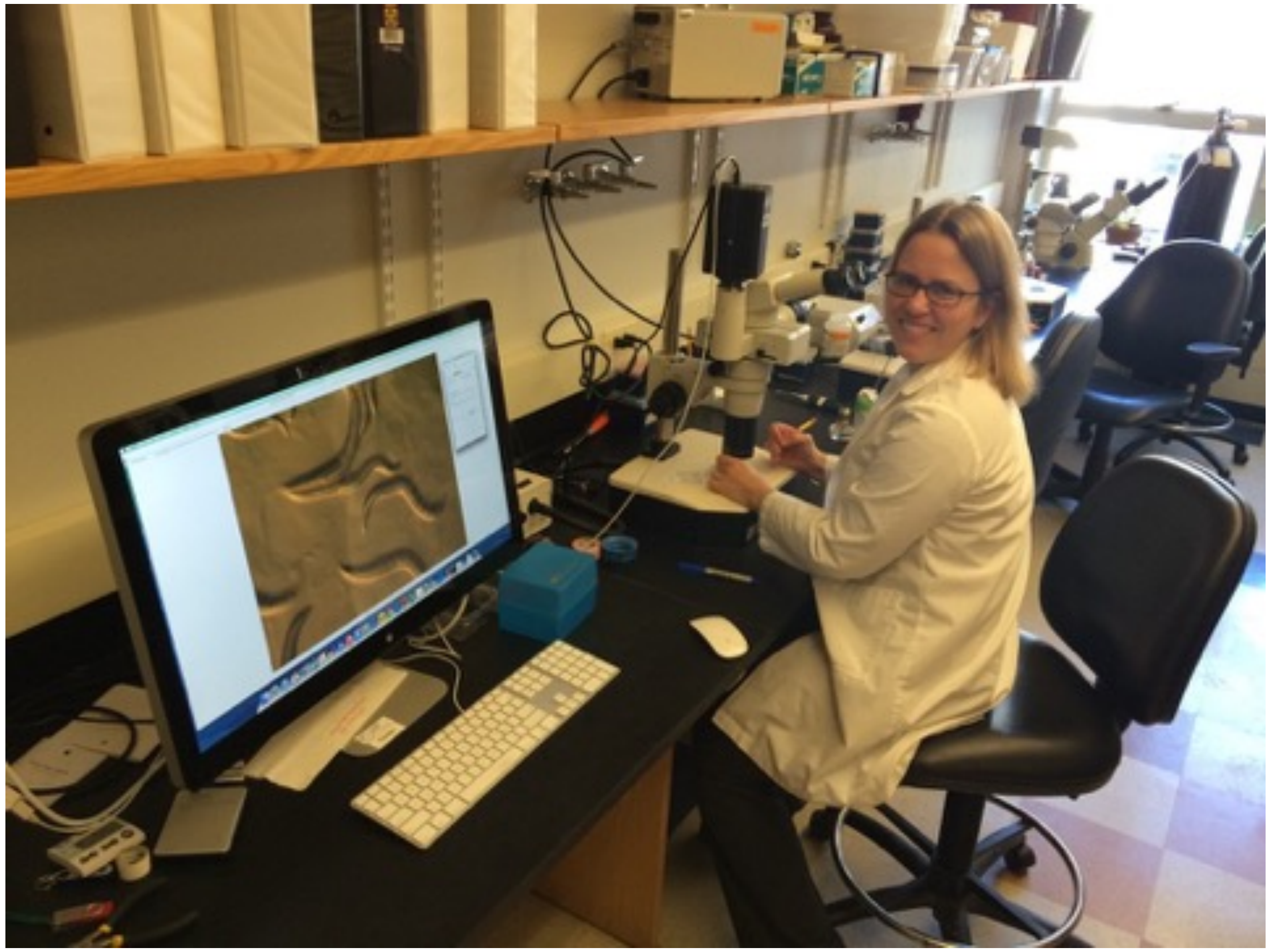
David Taylor and Jennifer Doudna

http://www.nytimes.com/2014/03/04/health/a-powerful-new-way-to-edit-dna.html

Ok, but what's it for?

Science

increasing our understanding of how genes produce phenotypes



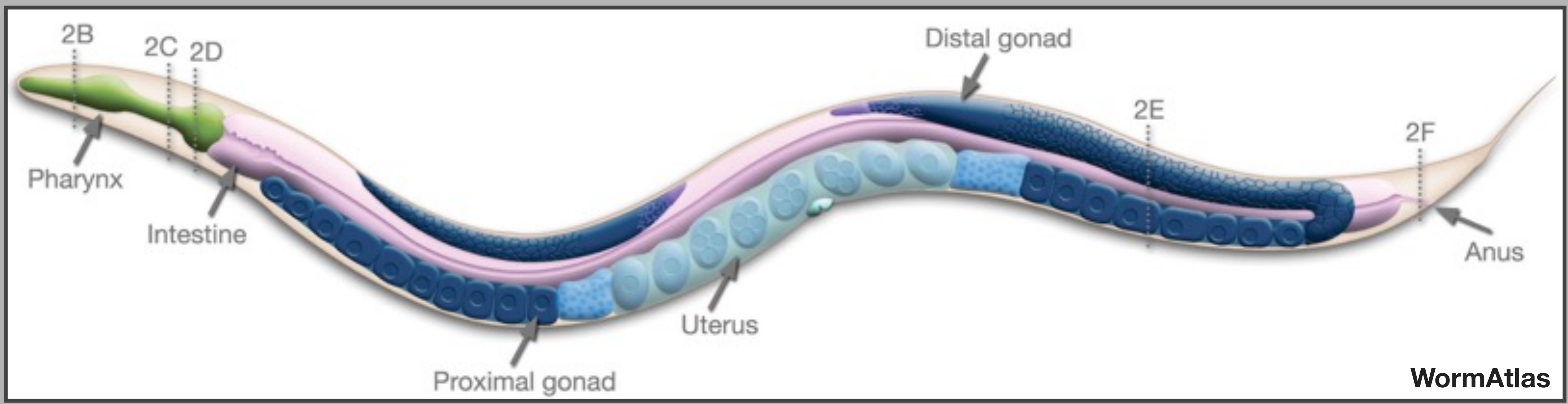
Agriculture

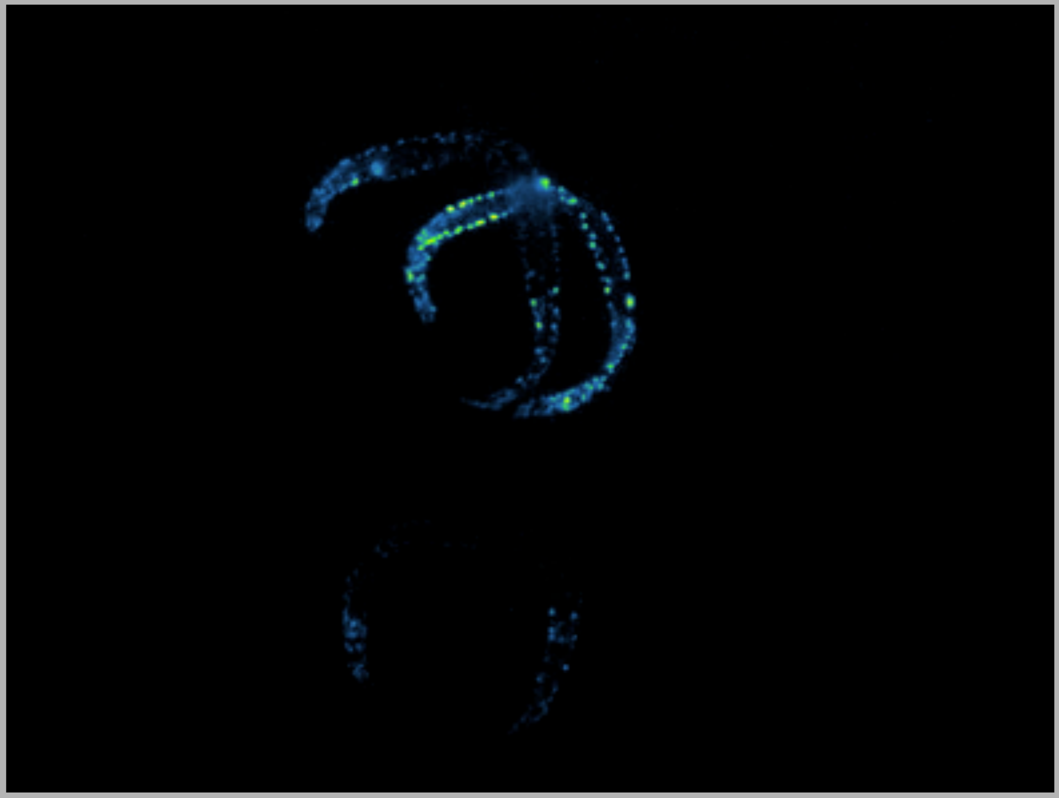
- increased yields
- increased quality
- disease resistance

Medical applications

- animal models for testing potential therapies
- producing pharmaceuticals
- gene therapy – correcting the mutations that cause disease

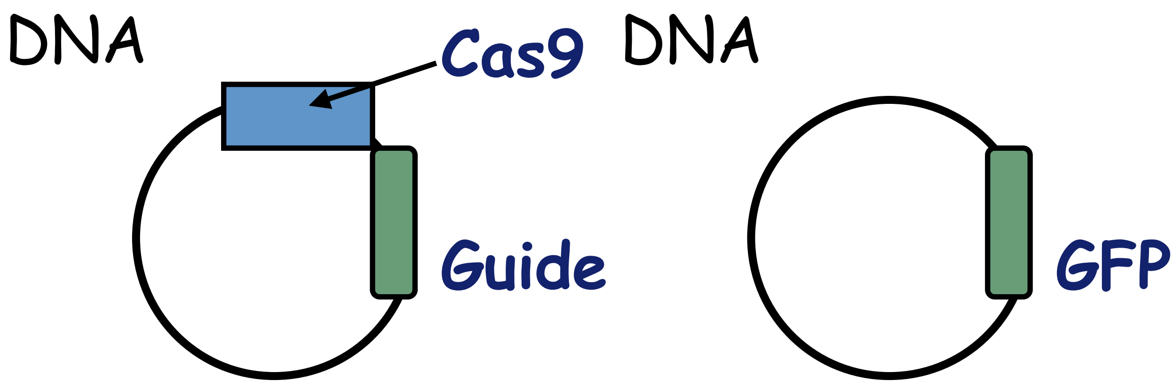
The worm *C. elegans* - transparent, tractable, quick



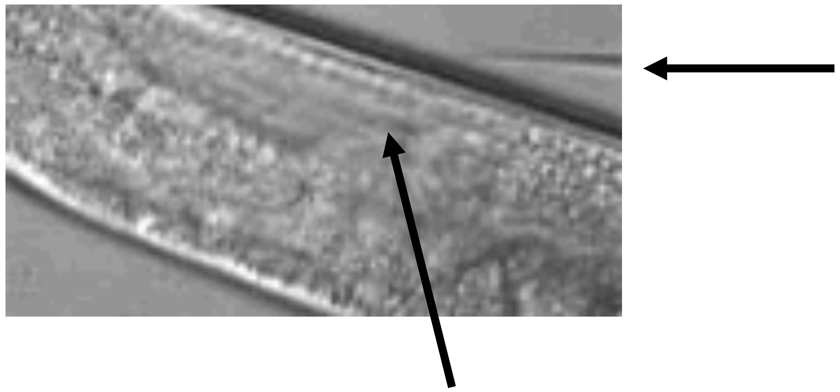


How to modify the genome of a worm

1. Get DNA encoding Cas9 and the guide + whatever you want to add



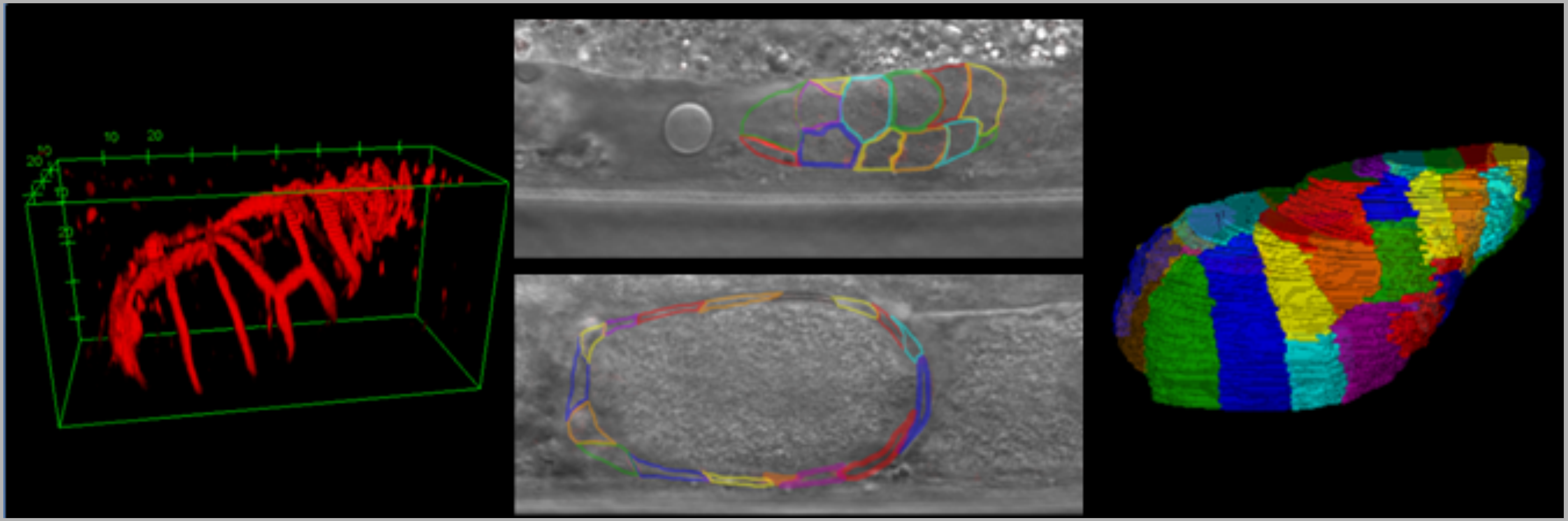
2. Inject the DNA into a worm



needle

3. Look for transgenic worm progeny!

How do animals make functional tubes?



Labeled cells by CRISPR-ing in a red fluorescent protein.
Watching in 4D as the tube forms and functions.

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Potential applications in agriculture

Plants
Heat, drought, pest, stress and disease resistance
Improved nutrition

Animals
Faster than traditional breeding for desired traits
Increased yield, decreased time to market, etc.

Article | OPEN

Efficient Generation of Myostatin Mutations in Pigs Using the CRISPR/Cas9 System

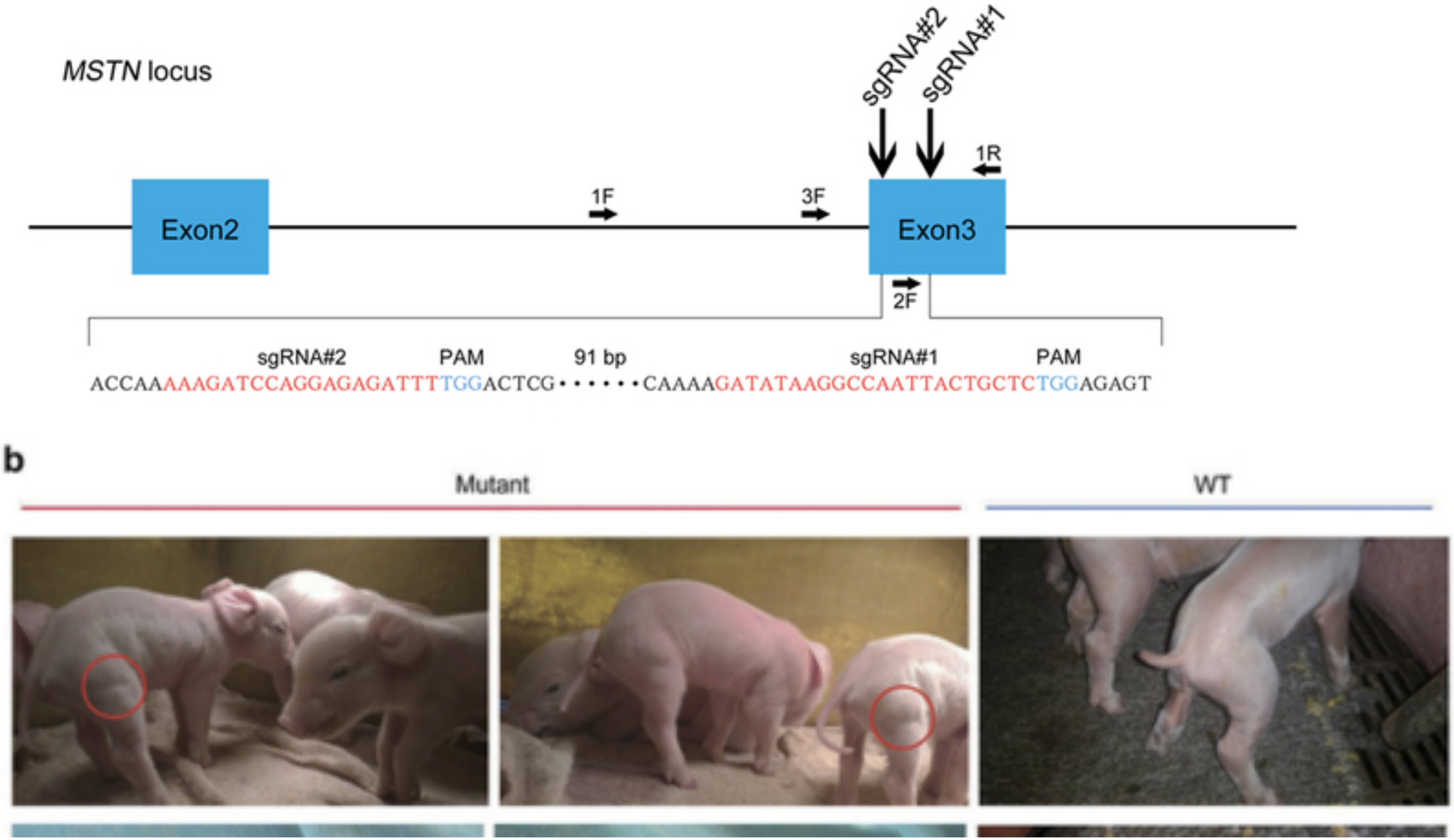
Kankan Wang, Hongsheng Ouyang, Zicong Xie, Chaogang Yao, Nannan Guo, Mengjing Li, Huping Jiao & Daxin Pang

Scientific Reports **5**, Article number: 16623 (2015)
doi:10.1038/srep16623
Download Citation
Genetic engineering | Transgenic organisms

Received: 14 April 2015
Accepted: 16 October 2015
Published online: 13 November 2015

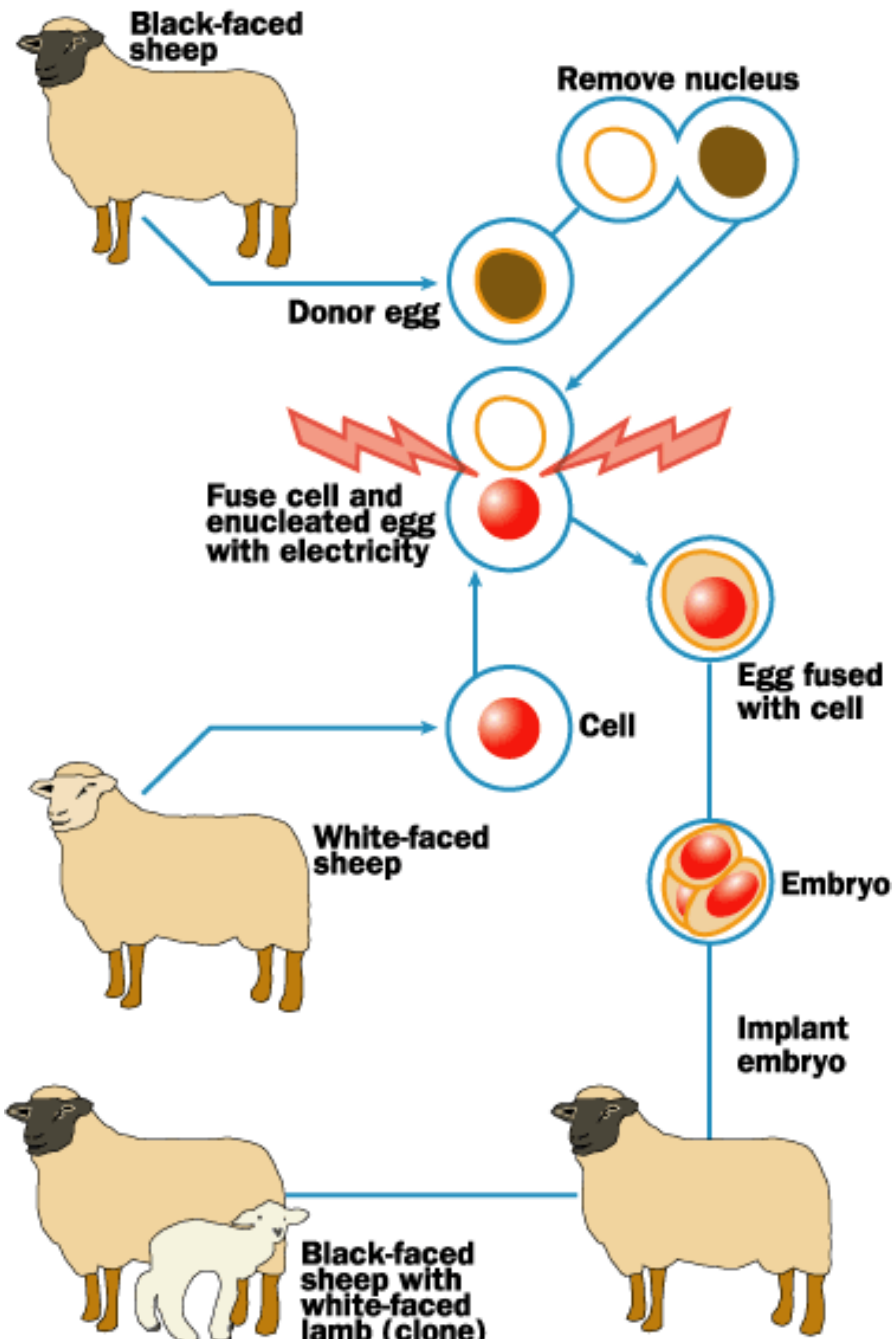
http://www.nature.com/articles/srep16623

The myostatin mutant pig grows A LOT of muscle



http://www.nature.com/articles/srep16623

The myostatin pig is a clone (made by SCNT - somatic cell nuclear transfer).



In the case of the myostatin pig, the nucleus used to make the clone came from a CRISPRed pig cell line.

Dolly was born 20 years ago! In 1996!

from "How stuff works"

Concerns and questions about genetic modification of plants and animals

Many CRISPR concerns are the same as those raised by GMOs and corporate agriculture

- Distrust of corporate agriculture and/or scientists.
- Intellectual property issues and concerns.
- Unintended ecological consequences.
- Local impacts on traditional farming practices.
- Decreased variety of production - loss of heirloom varieties.
- Concerns about ethical treatment of animals, animal quality of life.
- Concerns about adequacy of safety testing.
- Labeling/informed consent.

- Conception of the natural.
- Hubris - should *Homo sapiens* be reshaping nature?

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https://www.ipscell.com/2015/12/top-10-list-of-crispr-predictions-for-new-year/

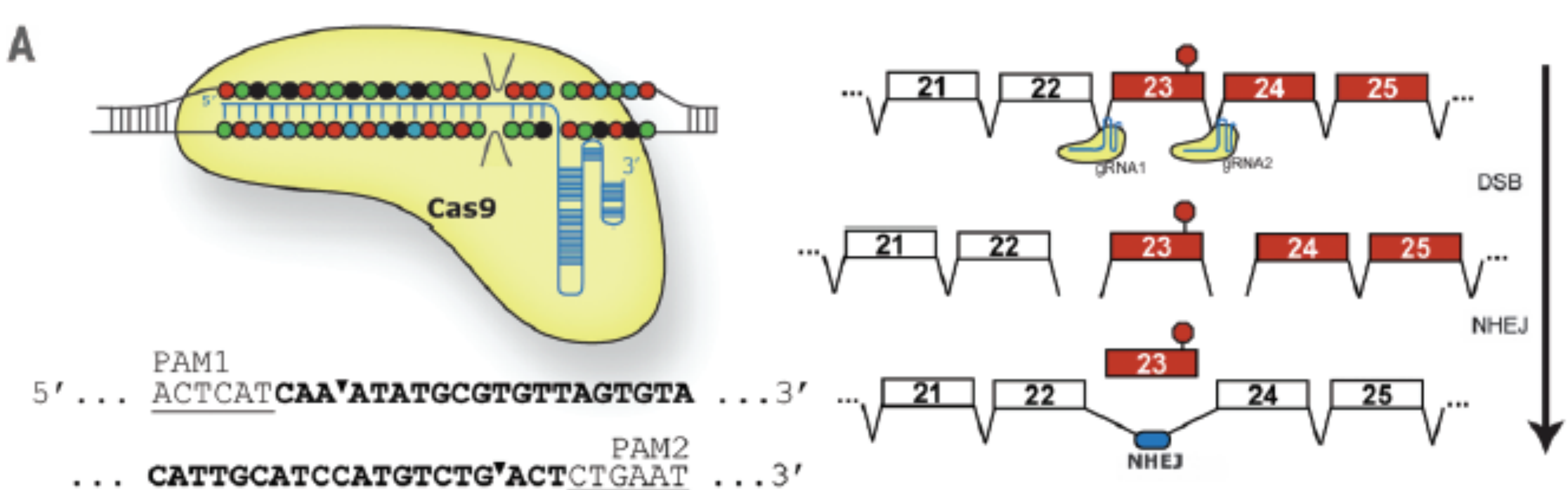
Improved model and (mouse) treatment for muscular dystrophy

Science. 2015 Dec 31. pii: aad5143. [Epub ahead of print]

In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy.

Nelson CE[1], Hakim CH[2], Ousterout DG[1], Thakore PI[1], Moreb EA[1], Rivera RM[3], Madhavan S[1], Pan X[2], Ran FA[4], Yan WX[5], Asokan A[3], Zhang F[6], Duan D[7], Gersbach CA[8].

Here's how they took out the mutation in the dystrophin gene that was causing the muscular dystrophy



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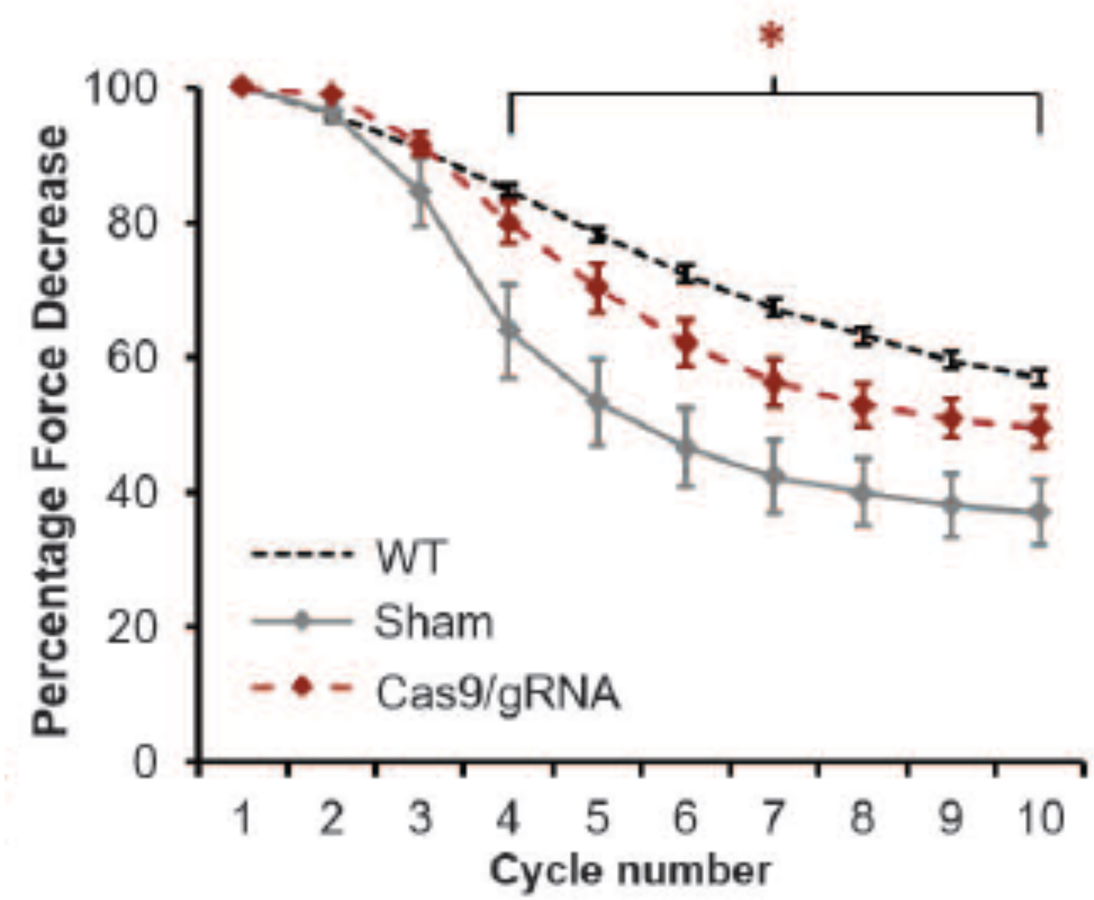


When the authors injected Cas9 and guide RNA into the adult mouse, they were able to improve muscle function.

Muscle response to cyclic stress wasn't completely restored, but it was better than before.

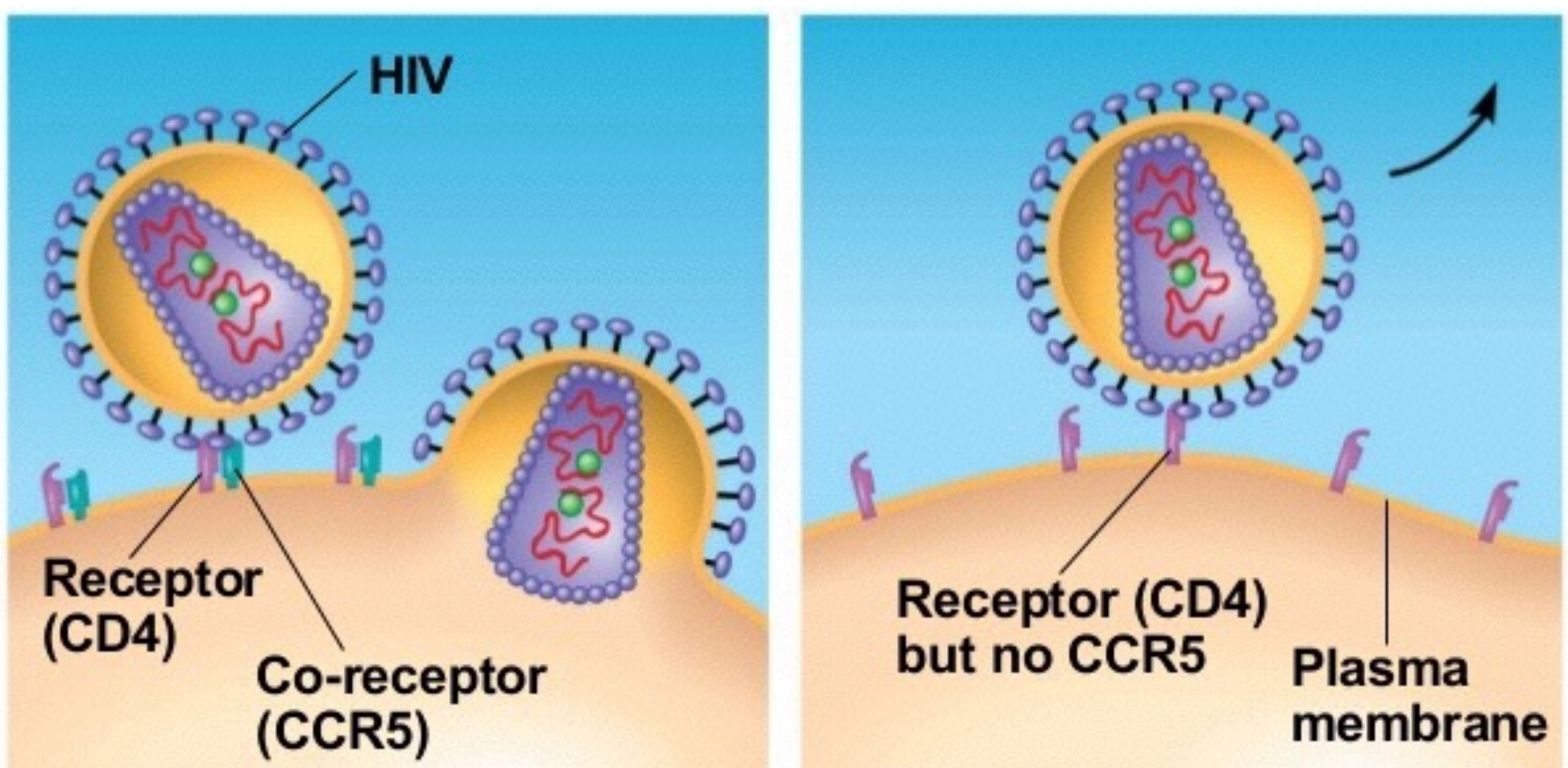
Modifying human genes - an effective treatment for HIV

J Gen Virol. 2015 Aug;96(8):2381-93. doi: 10.1099/vir.0.000139. Epub 2015 Apr 8.

Inhibition of HIV-1 infection of primary CD4+ T-cells by gene editing of CCR5 using adenovirus-delivered CRISPR/Cas9.

Li C[1], Guan X[1], Du T[2], Jin W[1], Wu B[3], Liu Y[2], Wang P[1], Hu B[1], Griffin GE[4], Shattock RJ[5], Hu Q[6].

Figure 7.11



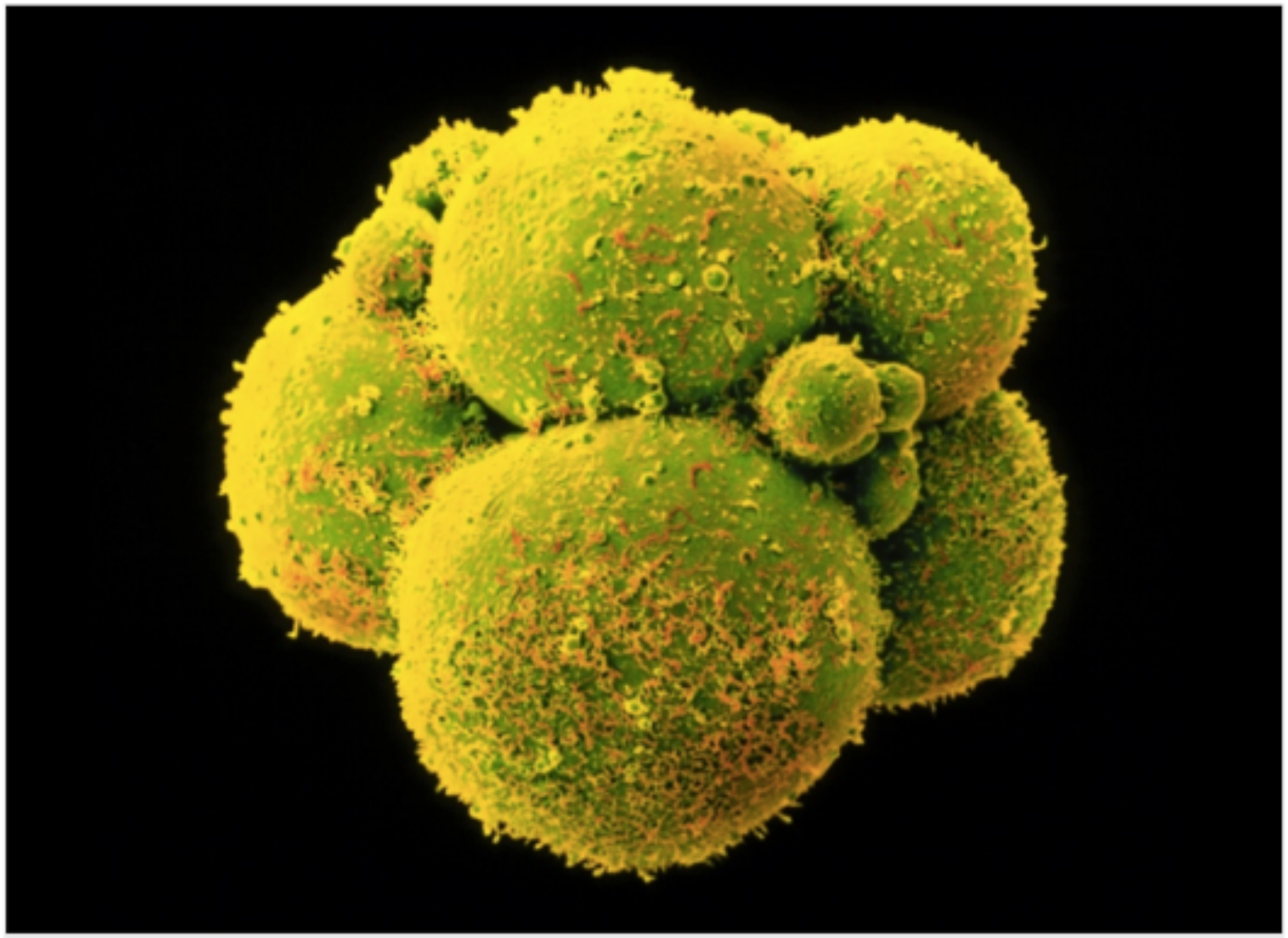
HIV can infect a cell that has CCR5 on its surface, as in most people.

HIV cannot infect a cell lacking CCR5 on its surface, as in resistant individuals.

Modifying HIV patient bone marrow stem cells so they no longer express CCR5 = HIV resistance

http://www.slideshare.net/5alod/campbell-biology-9th-edition-chapter-7-slides-31358677

Modifying human embryos - clearly possible, but should we?



Dr. Yorgos Nikas/SPL

Human embryos are at the centre of a debate over the ethics of gene editing.

In 2015, a Chinese group used CRISPR of human embryos to correct a mutant gene which causes β-thalassaemia, a potentially fatal blood disorder.

The procedure was not efficient: of 86 embryos injected, only a small fraction (~10%) were correctly modified.

Other practical concerns include off target effects (mutations elsewhere in genome) and unknown success rates following implantation (these embryos were not implanted in moms)

http://www.nature.com/news/chinese-scientists-genetically-modify-human-embryos-1.17378

Modifying human embryos - ethical concerns

Modifying human embryos - ethical concerns

Potential for improving the human condition…but…
-Insufficient knowledge and unintended consequences
-(In)adequacy of informed consent
-Risks borne by those with no say in the decision, including future generations
-General questioning of the wisdom of modifying human genomes at all
-Questions about how to define a "desirable" or "superior" trait in a human
-Potential for misuse (spectre of eugenics, weaponization)
-Economic issues

Equal access to care/ health technologies
Expensive - best use of limited funds

Further reading and resources

CRISPR: The good, the bad and the unknown
http://www.nature.com/news/crispr-1.17547

International Summit on Human Gene Editing
https://innovativegenomics.org/international-summit-on-human-gene-editing/

In Scientific American
http://www.scientificamerican.com/article/new-discovery-moves-gene-editing-closer-to-use-in-humans/

http://www.scientificamerican.com/article/biologists-create-more-precise-molecular-scissors-for-genome-editing/

Statement on Genome Editing Technologies and Human Germline Genetic Modification
http://www.hinxtongroup.org/hinxton2015_statement.pdf