Gene Editing: History and Development

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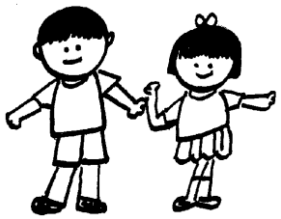
What is gene editing

Parents



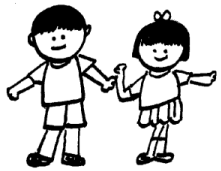
Children

Parents

In embryo

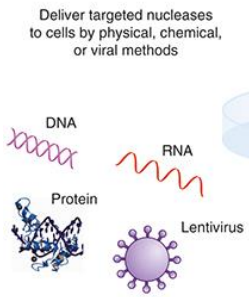
Gene editing

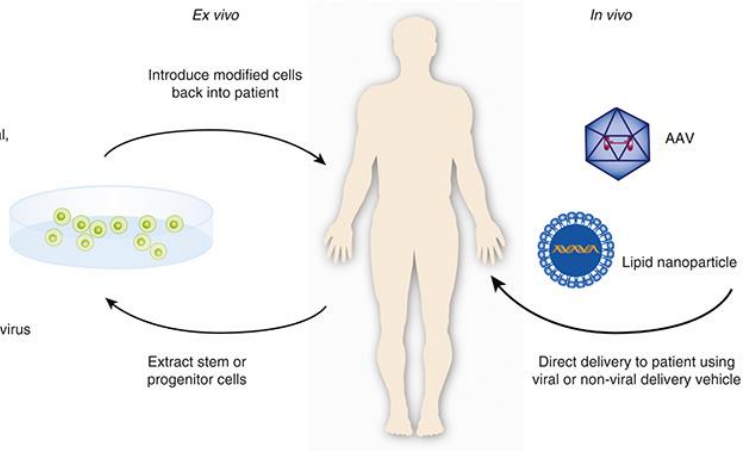
Modification to genome



Children

(adapted from Maeder and Gersbach, 2016)





In adult

1) Modify cells ex vivo
2) Modify cells in vivo

History of gene editing

DNA carries genetic information

Fred Griffith's experiment

Avery Macleod McCarty's experiment

Hershey-chase's experiment

Watson and Cricks

Structure of DNA and mechanism of replication

Ability to manipulate DNA

Improvement of molecular cloning technique In 1970s
i.e. site-directed mutagenesis

1989 First knock-out mice
Recombination-based method

Ability to alter trait in complex animal

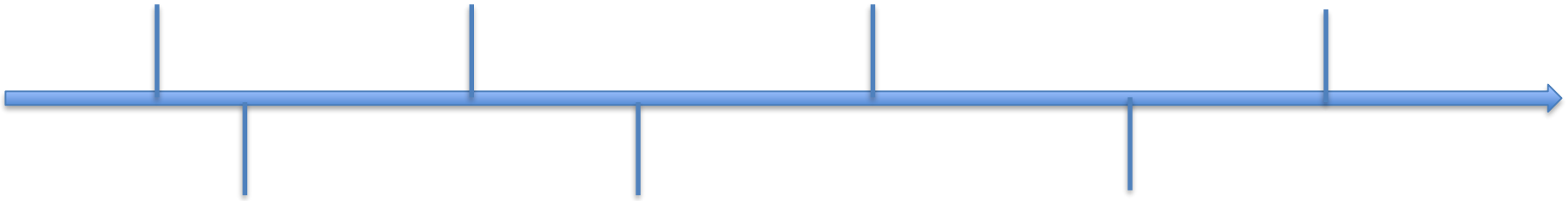
- Obtain sequence of human genome
- Understand the relationship between genes and phenotypes in human

2003:
- Completion of Human Genome Project
- ENCODE launched in 2003

2011 TALEN

Application on genome editing

2013 CRISPR



Gene therapy vs gene editing

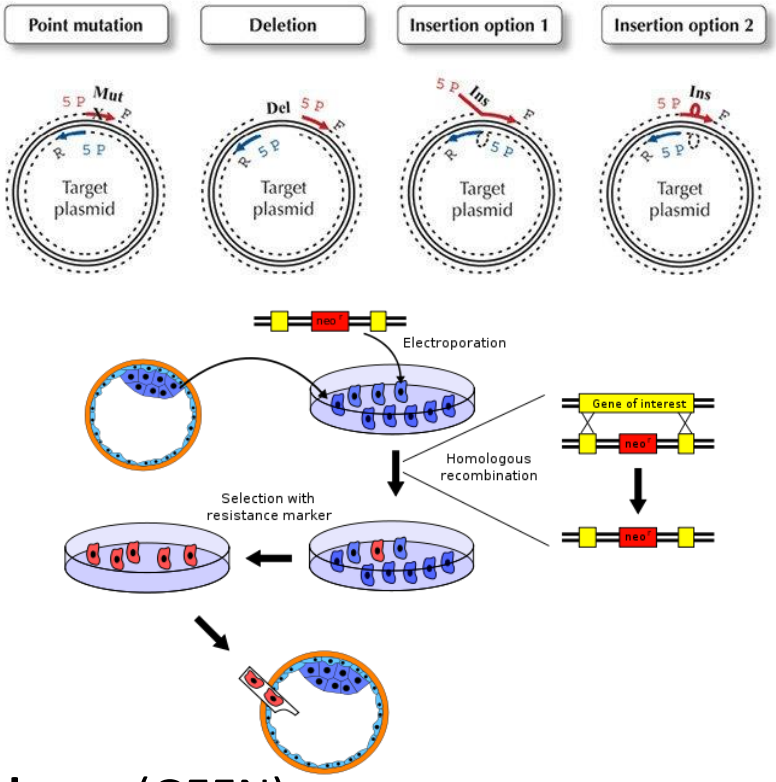
- Both are approaches to **introduce exogenous DNA sequences into host**
- Differences
  - Gene therapy
    - Gene carried on a vector (i.e. adenovirus or retrovirus) is introduced into human cell. Can be transient or permanent.
  - Gene/Genome editing
    - Modifying (i.e. addition, deletion or replacement) host genome **at specific site**.

How

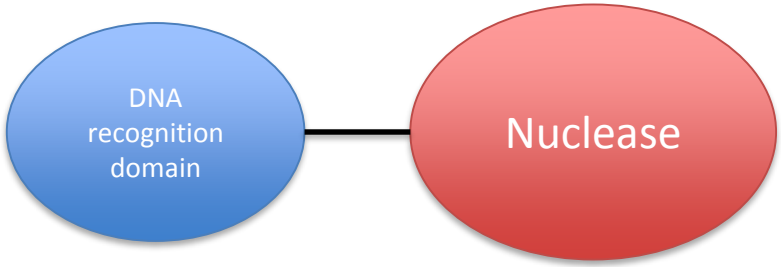
to edit?

Approaches in modifying DNA sequence

- **Old approaches:**
  - Site directed mutagenesis (in simple organism)
  - Recombination based methods (i.e. yeast, mouse)



- **Latest approach:**
  - Genome editing with **<u>engineered nuclease</u>** (GEEN)

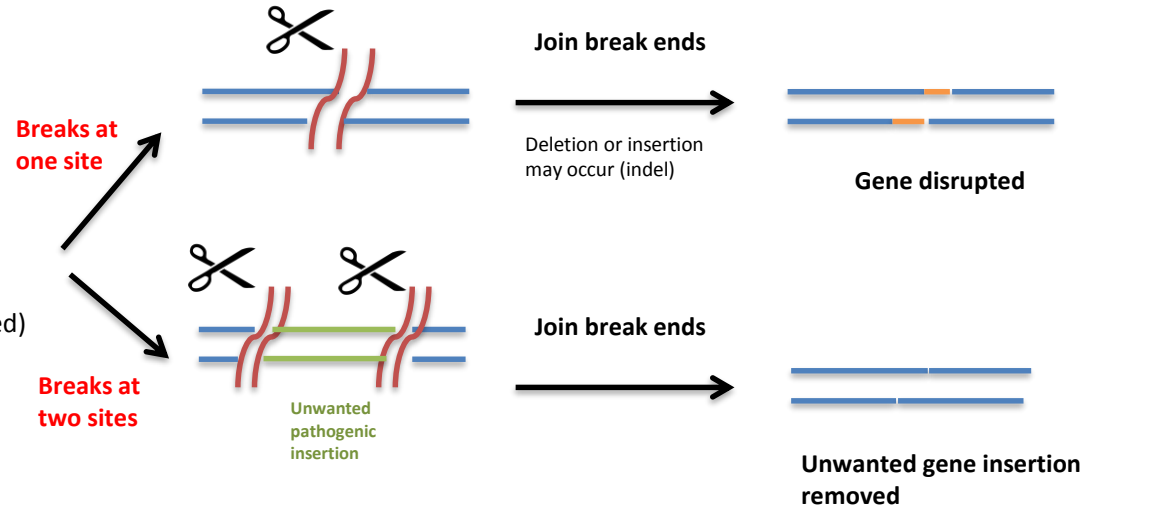


Nuclease generates break and then…

Two ways of DNA repair

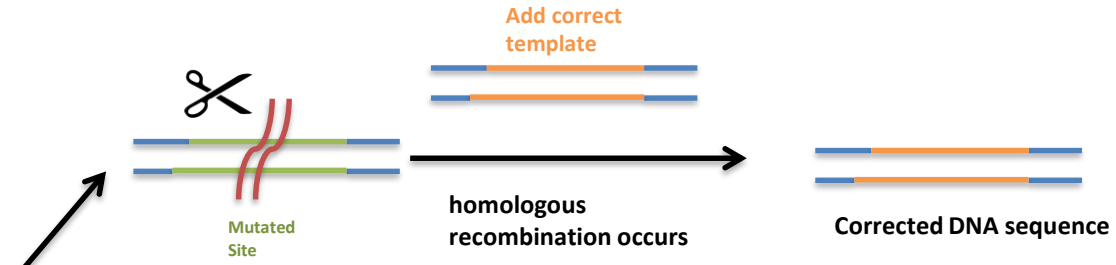
Non-homologous End joining (NHEJ)

Join both break ends
(no homologous recombination needed)



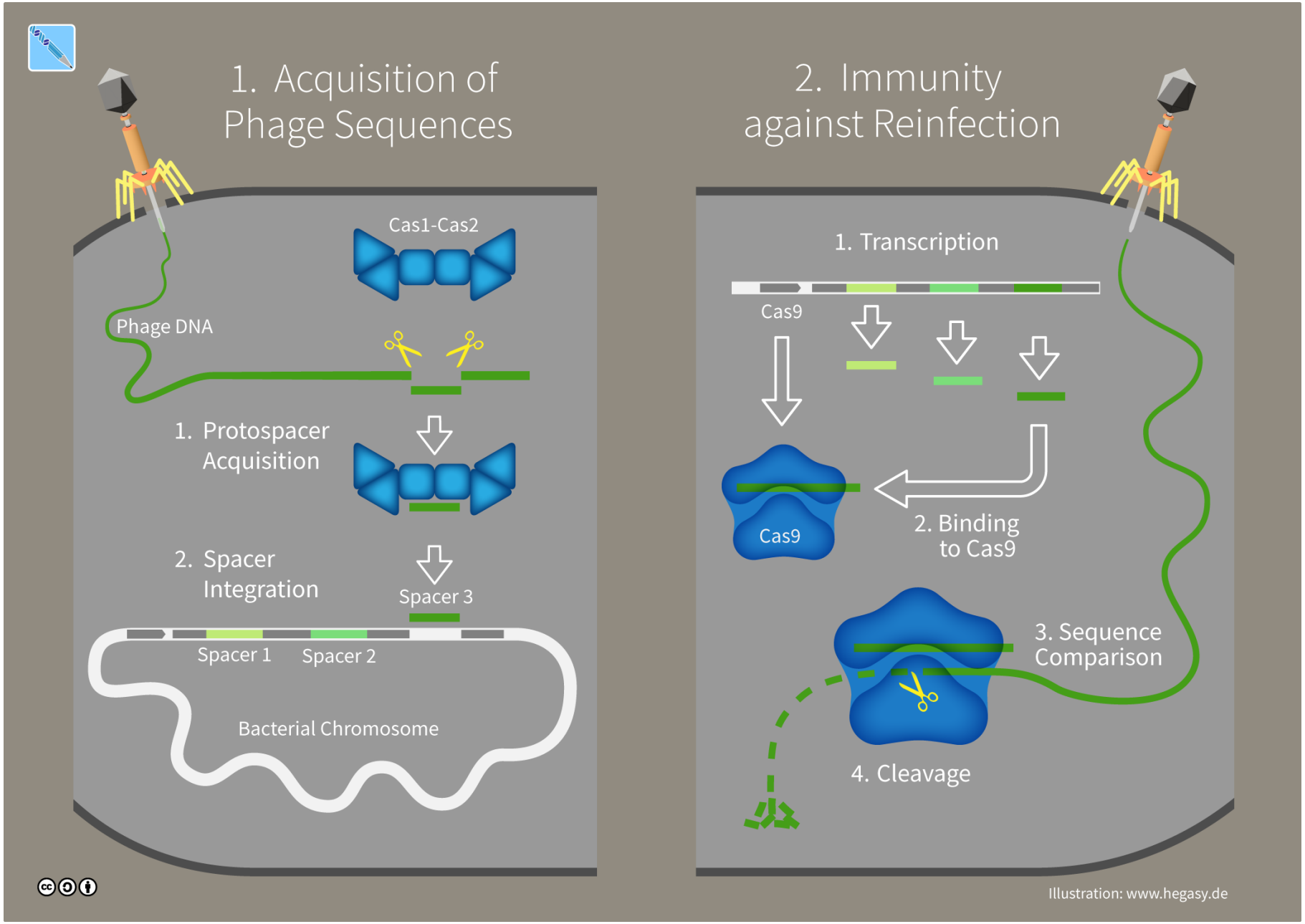
Homologous recombination (HDR)

Cause homologous recombination at break site

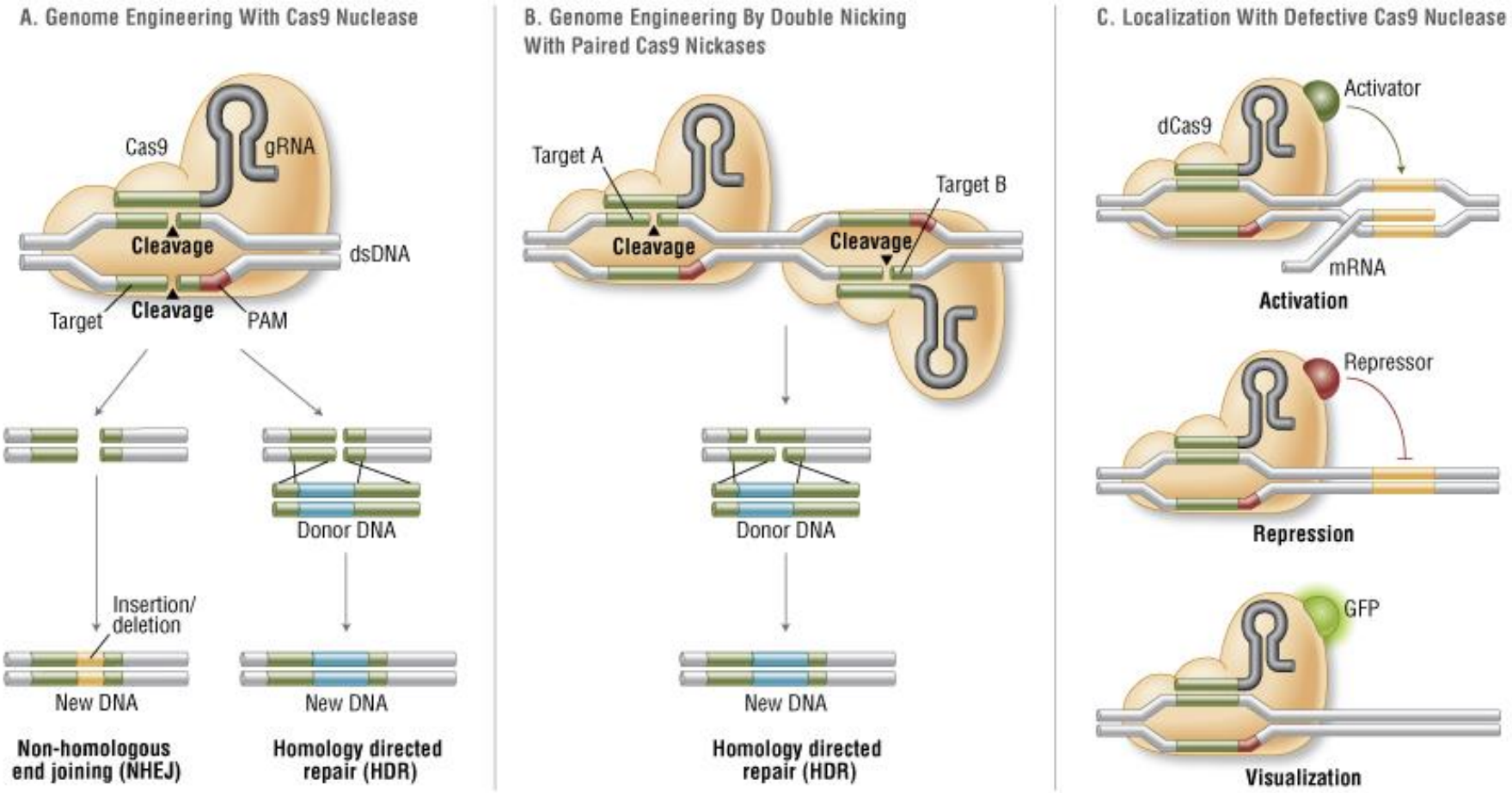


CRISPR – Cas9 system

- CRISPR: **c**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats
- Cas9: **c**rispr **a**ssociated **p**rotein 9, an RNA-guided endonucleases
- Repetitive elements found in Bacteria and Archaea in mid-1990s
- Bacterial or archael **adaptive immunity** against invasion by foreign genetic elements from bacteriophages or plasmids


1. Acquisition of Phage Sequences
Cas1-Cas2
Phage DNA
1. Protospacer Acquisition
2. Spacer Integration
Spacer 3
Spacer 1
Spacer 2
Bacterial Chromosome
2. Immunity against Reinfection
1. Transcription
Cas9
Cas9
2. Binding to Cas9
3. Sequence Comparison
4. Cleavage
Illustration: www.hegasy.de

Applications of CRISPR-Cas9 system



https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology

Why CRISPR?

	Other methods (e.g. TALENs)	CRISPR
Targeting efficiency	1-50%	>70% in zebrafish and plants, 2-5% in iPSC, 78% in one-cell mouse embryo
Nuclease	Engineered specifically depending on target	Only need to design the gRNA
Versatility	Low	High (guide RNA can be designed readily for genomic screening)
Cost	High	Low

A modified form of CRISPR nuclease may be used to edit RNA.

nature biotechnology

DNA-guided genome editing using the *Natronobacterium gregoryi* Argonaute

Feng Gao[1], Xiao Z Shen[2], Feng Jiang[1], Yongqiang Wu[1] & Chunyu Han[1]

The RNA-guided endonuclease Cas9 has made genome editing a widely accessible technique. Similar to Cas9, endonucleases from the Argonaute protein family also use oligonucleotides as guides to degrade invasive genomes. Here we report that the *Natronobacterium gregoryi* Argonaute (NgAgo) is a DNA-guided endonuclease suitable for genome editing in human cells. NgAgo binds 5′ phosphorylated single-stranded guide DNA (gDNA) of ~24 nucleotides, efficiently creates site-specific DNA double-strand breaks when loaded with the gDNA. The NgAgo–gDNA system does not require a protospacer-adjacent motif (PAM), as does Cas9, and preliminary characterization suggests a low tolerance to guide–target mismatches and high efficiency in editing (G+C)-rich genomic targets.

F. Gao et al. Nature Biotechnol.34, 768–773; 2016
Hebei University of Science and Technology

An alternative way to CRIPSR to edit human genome was proposed in 2016, yet its authenticity is in doubt.

Paper was retracted in August 2017.

nature International weekly journal of science

NATURE | NEWS

Replications, ridicule and a recluse: the controversy over NgAgo gene-editing intensifies

As failures to replicate results using the CRISPR alternative stack up, a quiet scientist stands by his claims.

David Cyranoski

08 August 2016 | Updated: 09 August 2016

Gene-Edited Animals

- A breed of Dalmatians lacks a working copy of a gene needed to clear uric acid
- Dog breeder told US **FDA** his plan to fix the single mutation with CRISPR in Jan 2017
- In February, **FDA** declared modified dogs **cannot be sold or even given away**
- FDA determined that, **the portion of an animal's genome that has been intentionally altered, whether mediated by rDNA or modern genome editing technologies, is a drug because it is intended to alter the structure or function of the animal** and, thus, subject to regulations.

"We cured this disease, but the FDA won't let us."

- David Ishee

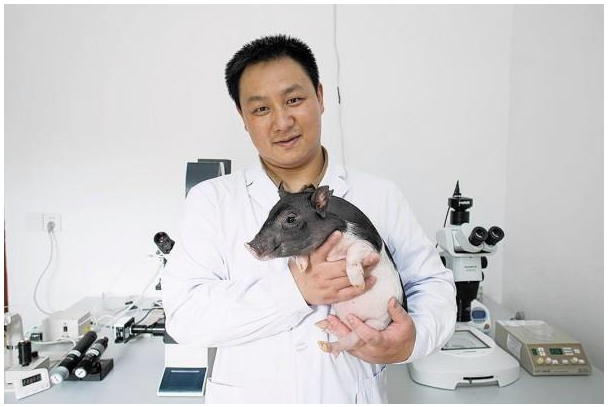
Risk of misuse

Scientific Innovation

https://www.technologyreview.com/s/603530/a-biohackers-plan-to-upgrade-dalmatians-ends-up-in-the-doghouse/

Gene-Edited Animals

- In 2015, BGI (華大基因) showcased '**micropigs**' in Shenzhen with intention of making them commercially available with US$1,400 per animal
- **TALENs** (transcription activator-like effector nucleases) was used to disable growth hormone receptor gene in fetal cells
- In mid 2017, however, BGI officials say they will not be selling the pigs
- May be due to negative public sentiment in GMO and uncertainty in the regulations



Bama pigs, which weigh only 35-50 kg, are useful in studies of gut microbiota

Conventional Breeding
≈
Gene-edited Pets
?

https://www.technologyreview.com/s/608207/china-genomics-giant-drops-plans-for-gene-edited-pets

Ethical issues

- Ethical to gene edit animal? Sold it for research purpose? Sold it for pet?
- How is this compared to generating genetic-engineered animal?
- How is this compared to generating animal of required trait by breeding?
- Are we too human centred in the judgement?

Examples of clinical trials

Identifier	Phase	Title	Status as of October 2015
NCT00842634	Phase 1	Autologous T Cells Genetically Modified at the CCR5 Gene by Zinc Finger Nucleases SB-728 for HIV	Completed
NCT01044654	Phase 1	Phase 1 Dose Escalation Study of Autologous T Cells Genetically Modified at the CCR5 Gene by Zinc Finger Nucleases in HIV-Infected Patients	Completed
NCT01082926	Phase 1	Phase I Study of Cellular Immunotherapy for Recurrent/Refractory Malignant Glioma Using Intratumoral Infusions of GRm13Z40-2, An Allogeneic CD8+ Cytolitic T Cell Line Genetically Modified to Express the IL 13-Zetakine and HyTK and to be Resistant to Glucocorticoids, in Combination With Interleukin-2	Completed
NCT01252641	Phase 1/2	Study of Autologous T Cells Genetically Modified at the CCR5 Gene by Zinc Finger Nucleases in HIV-Infected Subjects	Completed
NCT02225665	Phase 1/2	Repeat Doses of SB-728mR-T After Cyclophosphamide Conditioning in HIV-Infected Subjects on HAART	Active
NCT01543152	Phase 1/2	Dose Escalation Study of Cyclophosphamide in HIV-Infected Subjects on HAART Receiving SB-728-T	Recruiting
NCT02500849	Phase 1	Safety Study of Zinc Finger Nuclease CCR5-modified Hematopoietic Stem/Progenitor Cells in HIV-1 Infected Patients	Recruiting

(adapted from Maeder and Gersbach, 2016)

The First Man to Have His Genes Edited Inside His Body in November 2017

- For curing Hunter syndrome, a genetic disorder that causes a range of symptoms including joint stiffness, breathing problems, and developmental delay
- Iduronate-2-sulfatase in liver cells edited by Zinc finger nuclease

https://www.theatlantic.com/science/archive/2017/11/sangamo-first-gene-editing-in-body/545960/

2017 – Oregon USA

Targeted a mutation in *MYBPC3* gene that cause the heart muscle to thicken

2017 – Guangzhou, China

Targeted point mutation in *HBB* gene that leads to β-thalassaemia (‘base editing’)

First to edit out the mutation responsible for a ‘recessive’ disease

In total **EIGHT** studies were published reporting gene editing in human embryos, **FIVE** since August 2017.

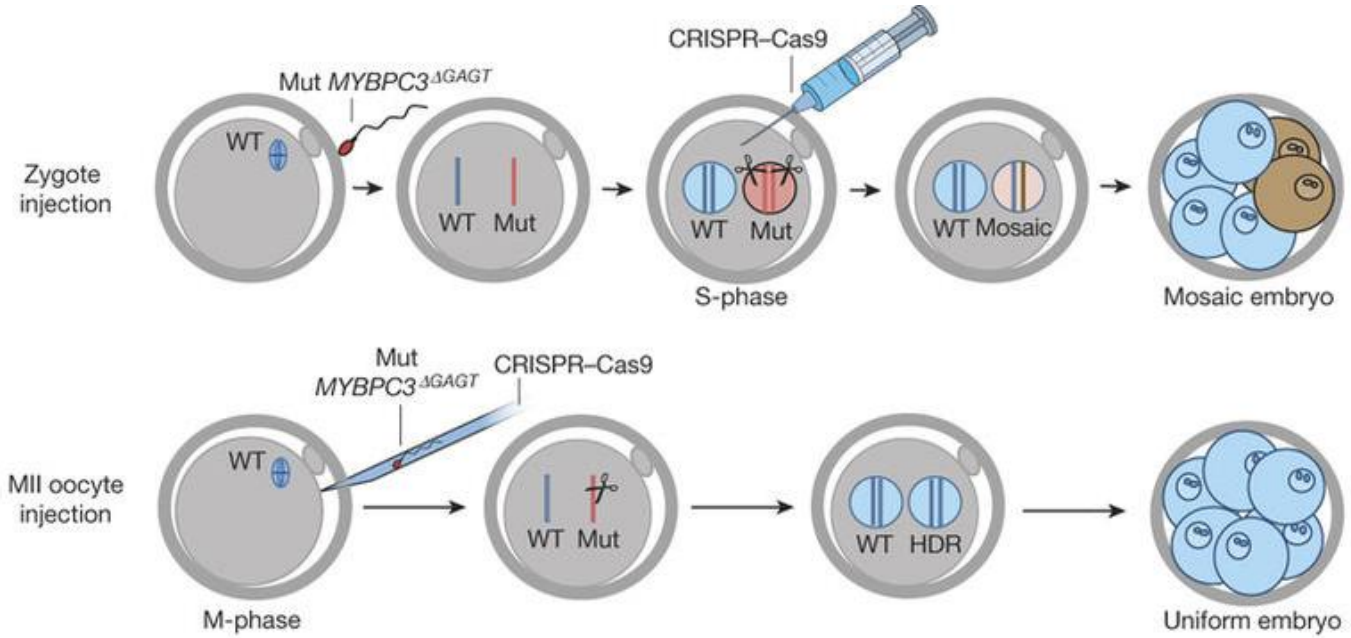
Closer Look – Human Embryos Editing

- Single mutation in cardiac myosin-binding protein C (***MYBPC3***) can cause hypertrophic cardiomyopathy, which leads to heart failure

- Shoukhrat Mitalipov's team from Oregon Health and Science University isolated human single cell embryos and injected CRISPR/Cas9 constructs to target the mutation in the *MYBPC3* gene

Closer Look – Human Embryos Editing

- Two approaches were used



Genome editing occurs when sperm contains a single mutant copy, eliminating mosaicism

- The article claims **72.4%** of the resulting embryos had only the WT *MYBPC3*

DIY Kit and self-experimentation

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Home > All Products > DIY Bacterial Gene Engineering CRISPR Kit

Home > Gene Engineering Kits > DIY Bacterial Gene Engineering CRISPR Kit

www.the-odin.com/diy-crispr-kit/

DIY Bacterial Gene Engineering CRISPR Kit

$159.00

Shipping: Calculated at checkout

Save

6 product reviews

Quantity: 1

Add To Cart | Add to Wishlist

Product Description

For holiday gifts we stop shipping on Dec. 20th. Understand that the kit contains perishable material that shouldn't be left at room temperature for longer than 1 week after receiving the kit or it can start to go bad. If long-term storage is required leave a note in the comments and we will include a redeemable coupon for the perishable materials.

Need a holiday gift idea?

http://www.the-odin.com/diy-crispr-kit/

DIY Kit and self-experimentation

- Privately-funded labs, community labs, or even individuals can now perform **DIY** non-institutional biotechnology with ease
- **Low cost** and **simplicity** of CRISPR have opened DIY labs to gene-editing
- FDA had stressed in Nov 2017 that the sale of gene therapy kits is **illegal**
- However, it may not stop the sale and self-experimentation
  - Example: removal of myostatin gene by CRISPR

DIY Kit and self-experimentation

- Myostatin, a gene for regulating muscle growth
- Josiah Zayner, a biochemist, injected himself with CRISPR system to remove myostatin gene

- J. Zayner: "*This is the first of many people who will change their genomes. This will happen for medical reasons, for science, athletics or maybe just because people wanted to or were bored.*“

- Other biohackers are getting ready to tinker with their own genes

https://www.newscientist.com/article/mg23631520-100-biohackers-are-using-crispr-on-their-dna-and-we-cant-stop-it/

https://lambsharbinger.wordpress.com/2016/07/21/first-crispr-trial-in-humans-is-reported-to-start-next-month-stat/
http://www.leuvenmindgate.be/news/genome-editing-is-picking-up-pace
https://www.zazzle.com/crispr+gifts

Ethical issues

- Less controversial in using gene editing for treatment?
- Acceptable for the change of germ line
- Acceptable for non-clinical changes?

- Can gene editing be **effectively** regulated???

Thanks for your attention