The Gene Editing Revolution

Brett M. Sansbury, Ph.D.
Leader of Discovery Research
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IN THE LAB

A giant gorilla and a winged wolf: Does 'Rampage' get the science of CRISPR right?

By MEGAN THIELKING @meggophone *and* ANDREW JOSEPH @DrewQJoseph / APRIL 12, 2018

We here at STAT cover CRISPR a lot. But it's not every day we get to cover Dwayne "The Rock" Johnson.

The Rock and the genome-editing technology meet in a new movie, "Rampage," coming out Friday. Through a freak accident, a gorilla, a wolf, and a crocodile ingest some CRISPR complexes. The animals — whose genomes become edited to make them stronger, bigger, faster, and more aggressive — soon wreak havoc on the city of Chicago

George the gorilla and Rock "The Dwayne" Johnson in "Rampage."
WARNER BROS. PICTURES

It's packed with action, gratuitous destruction, and an anti-poaching message, along with at least a dozen references to CRISPR, some of which are even accurate, say STAT reporters (and amateur movie critics) Megan Thielking and Andrew Joseph, who saw an advanced screening this week. Here are their thoughts — both scientific and cinematic — on the film. This conversation contains spoilers.

ANDREW: ...On a different note, this did make me think of a common movie plot point. As our colleague Damian Garde wrote last year, Hollywood loves a biopharma villain. There's clearly some fear among the public about what scientists can do, particularly when it comes to rewriting the code of life. I do wonder if this movie will be people's introduction to CRISPR. Are they going to go home and Google it and see headlines invoking "designer babies"? Is this just going to make people scared of CRISPR, even if they know that it probably won't lead to a mutant crocodile that can King-Kong-style crawl up the Sears Tower? This is totally made up, but I imagine the marketing people at CRISPR Therapeutics are discussing this right now!

The Nobel Prize in Chemistry 2020

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2020 to

Emmanuelle Charpentier
Max Planck Unit for the Science of Pathogens, Berlin, Germany

Jennifer A. Doudna
University of California, Berkeley, USA

"for the development of a method for genome editing"

Genetic scissors: a tool for rewriting the code of life

Emmanuelle Charpentier and Jennifer A. Doudna have discovered one of gene technology's sharpest tools: the CRISPR/Cas9 genetic scissors. Using these, researchers can change the DNA of animals, plants and microorganisms with extremely high precision. This technology has had a revolutionary impact on the life sciences, is contributing to new cancer therapies and may make the dream of curing inherited diseases come true.

Researchers need to modify genes in cells if they are to find out about life's inner workings. This used to be time-consuming, difficult and sometimes impossible work. Using the CRISPR/Cas9 genetic scissors, it is now possible to change the code of life over the course of a few weeks.

"There is enormous power in this genetic tool, which affects us all. It has not only revolutionised basic science, but also resulted in innovative crops and will lead to ground-breaking new medical treatments," says Claes Gustafsson, chair of the Nobel Committee for Chemistry.

As so often in science, the discovery of these genetic scissors was unexpected. During Emmanuelle Charpentier's studies of *Streptococcus pyogenes*, one of the bacteria that cause the most harm to humanity, she discovered a previously unknown molecule, *tracrRNA*. Her work showed that tracrRNA is part of bacteria's ancient immune system, *CRISPR/Cas*, that disarms viruses by cleaving their DNA.

Charpentier published her discovery in 2011. The same year, she initiated a collaboration with Jennifer Doudna, an experienced biochemist with vast knowledge of RNA. Together, they succeeded in recreating the bacteria's genetic scissors in a test tube and simplifying the scissors' molecular components so they were easier to use.

In an epoch-making experiment, they then reprogrammed the genetic scissors. In their natural form, the scissors recognise DNA from viruses, but Charpentier and Doudna proved that they could be controlled so that they can cut any DNA molecule at a predetermined site. Where the DNA is cut it is then easy to rewrite the code of life.

Since Charpentier and Doudna discovered the CRISPR/Cas9 genetic scissors in 2012 their use has exploded. This tool has contributed to many important discoveries in basic research, and plant researchers have been able to develop crops that withstand mould, pests and drought. In medicine, clinical trials of new cancer therapies are underway, and the dream of being able to cure inherited diseases is about to come true. These genetic scissors have taken the life sciences into a new epoch and, in many ways, are bringing the greatest benefit to humankind.

Emmanuelle Charpentier, born 1968 in Juvisy-sur-Orge, France. Ph.D. 1995 from Institut Pasteur, Paris, France. Director of the Max Planck Unit for the Science of Pathogens, Berlin, Germany.

Jennifer A. Doudna, born 1964 in Washington, D.C, USA. Ph.D. 1989 from Harvard Medical School, Boston, USA. Professor at the University of California, Berkeley, USA and Investigator, Howard Hughes Medical Institute.

Prize amount: 10 million Swedish kronor, to be shared equally between the Laureates.
Further information: www.kva.se and www.nobelprize.org
Press contact: Eva Nevelius, Press Secretary, +46 70 878 67 63, eva.nevelius@kva.se
Expert: Claes Gustafsson, +46 70 858 95 21, claes.gustafsson@medkem.gu.se, Chair of the Nobel Committe for Chemistry

The Royal Swedish Academy of Sciences, founded in 1739, is an independent organisation whose overall objective is to promote the sciences and strengthen their influence in society. The Academy takes special responsibility for the natural sciences and mathematics, but endeavours to promote the exchange of ideas between various disciplines.

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THE ROYAL SWEDISH ACADEMY OF SCIENCES

Emmanuelle Charpentier
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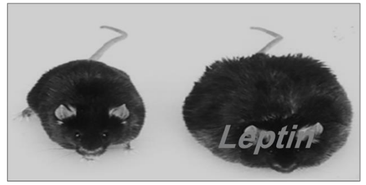
Editing?

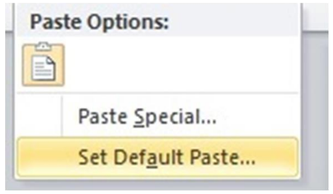
Gene Editing?

Cutting

Deleting
Knockout

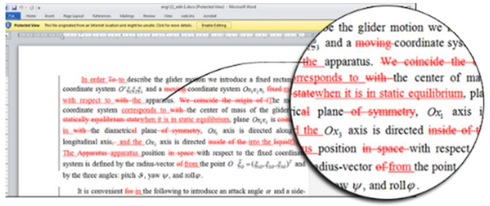


Pasting



Inserting
Transgenic

Changing



Replacing
Knock-in

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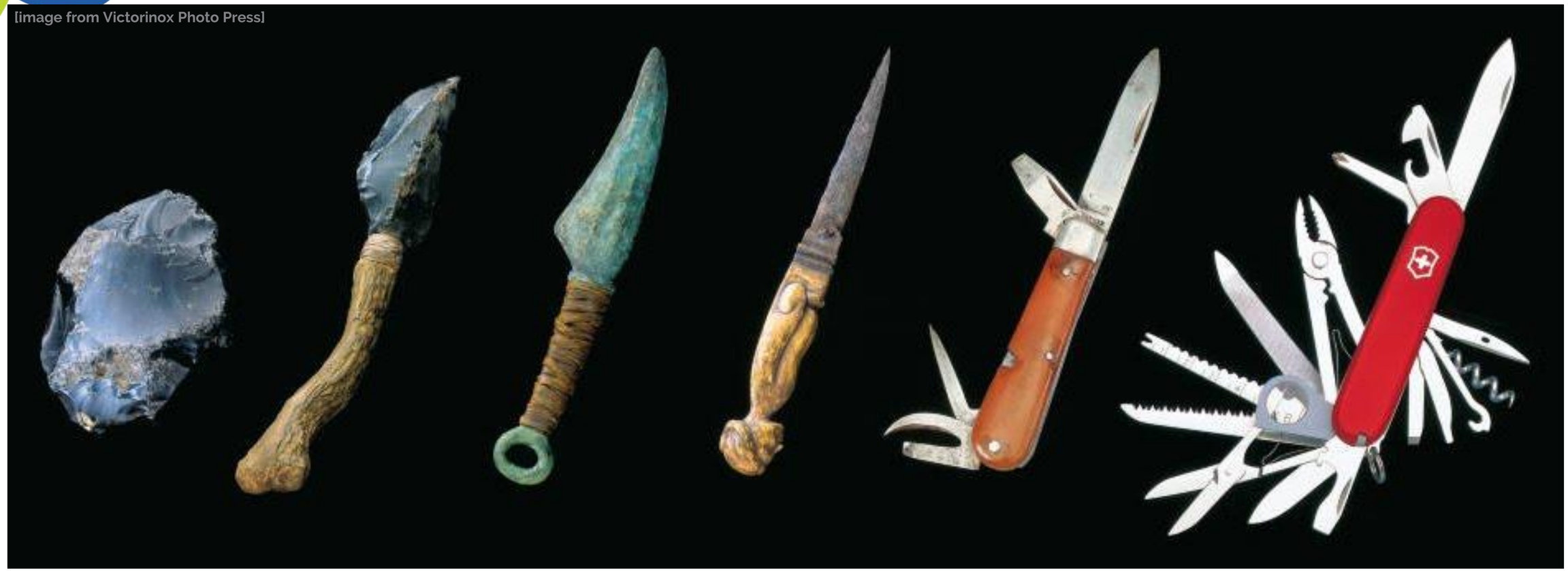
Evolution: Swiss Army Knife

[image from Victorinox Photo Press]



Evolution of the Gene Editing Toolkit

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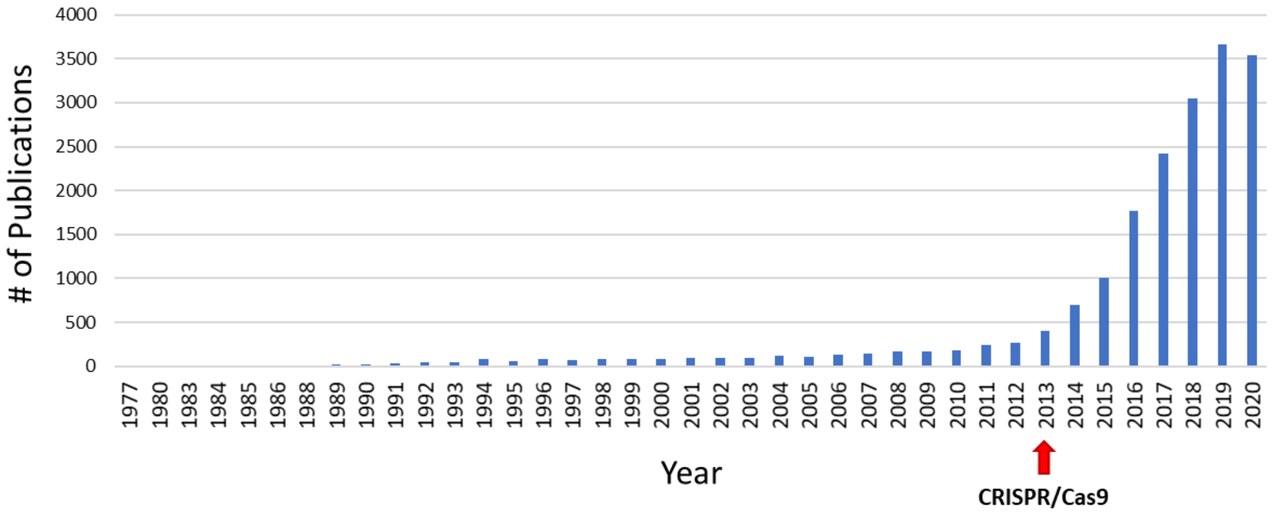
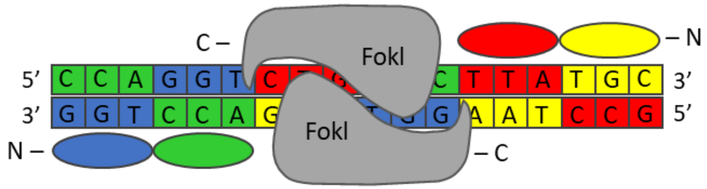
Overview of Gene Editing Technologies

1860s - The discovery of DNA by Friedrich Miescher
1953 - Watson and Crick uncover double helix structure
1980s - Mutagenesis using modified viruses developed
1990s - Gene Editing using Zinc Finger Nucleases
2011 - Gene Editing using TALENs
2013 - Gene Editing with CRISPR/Cas9

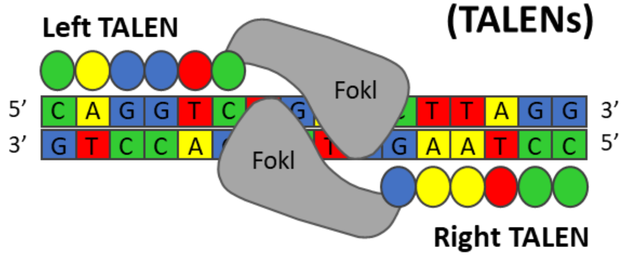
Gene Editing Publications by Year



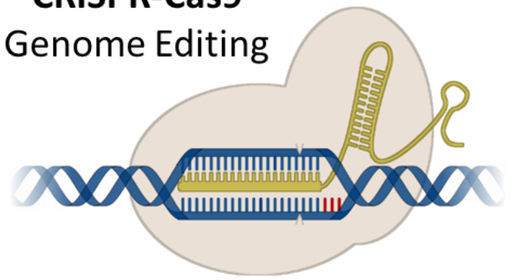
Zinc-finger Nucleases **(ZFNs)**



Transcription-like Effector Nucleases **(TALENs)**



CRISPR-Cas9
Genome Editing



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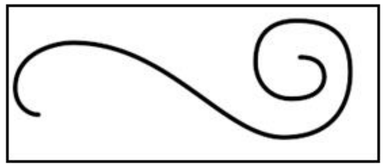
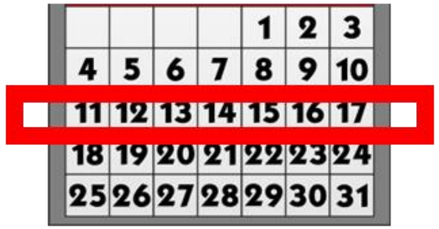
	ZFNs	TALENs	CRISPR/Cas9
Designing			
Building			
Speed			
Scalability	Difficult	Possible	Readily possible

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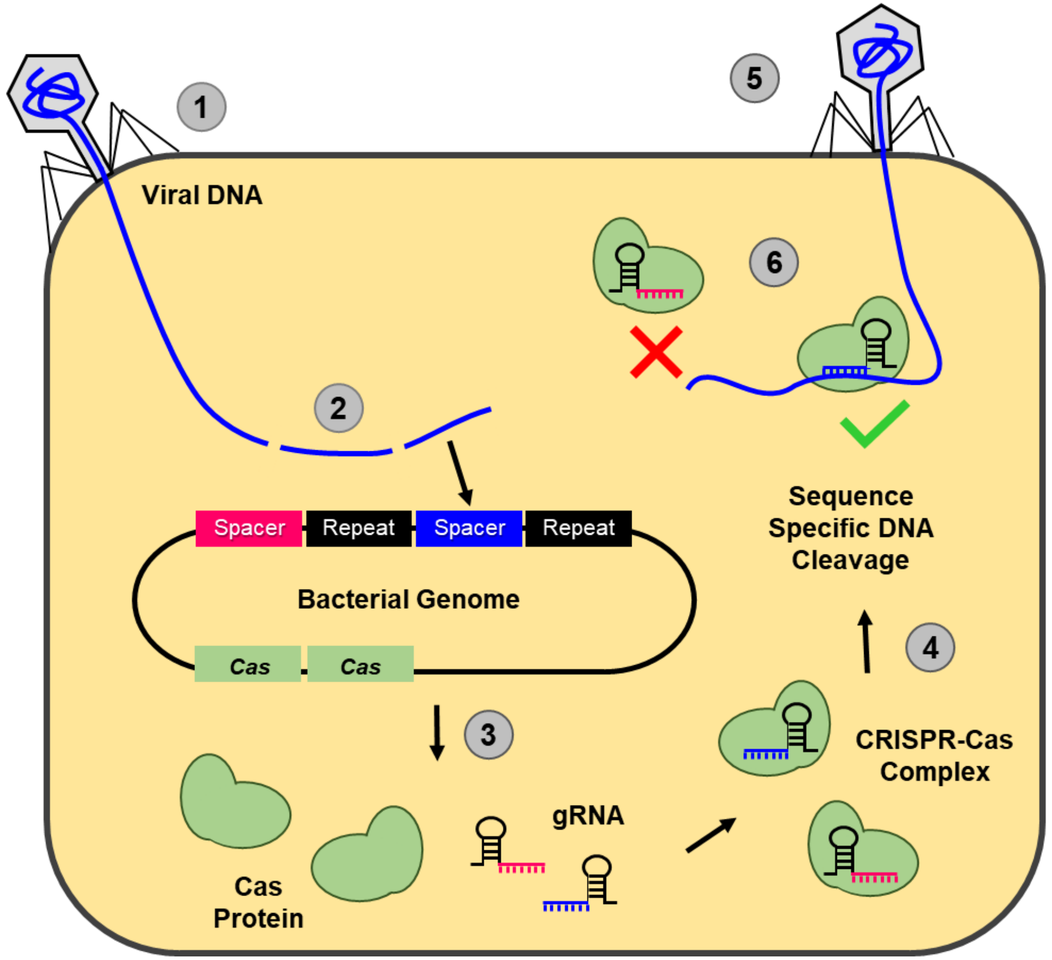
Adaptive Immunity in Bacteria



1. Viral Infection
2. Viral DNA Integration into Host Genome
3. CRISPR components are Produced
4. Formation of tracr:crRNA-Cas Complex
5. Viral Re-Infection
6. CRISPR-directed DNA Cleavage

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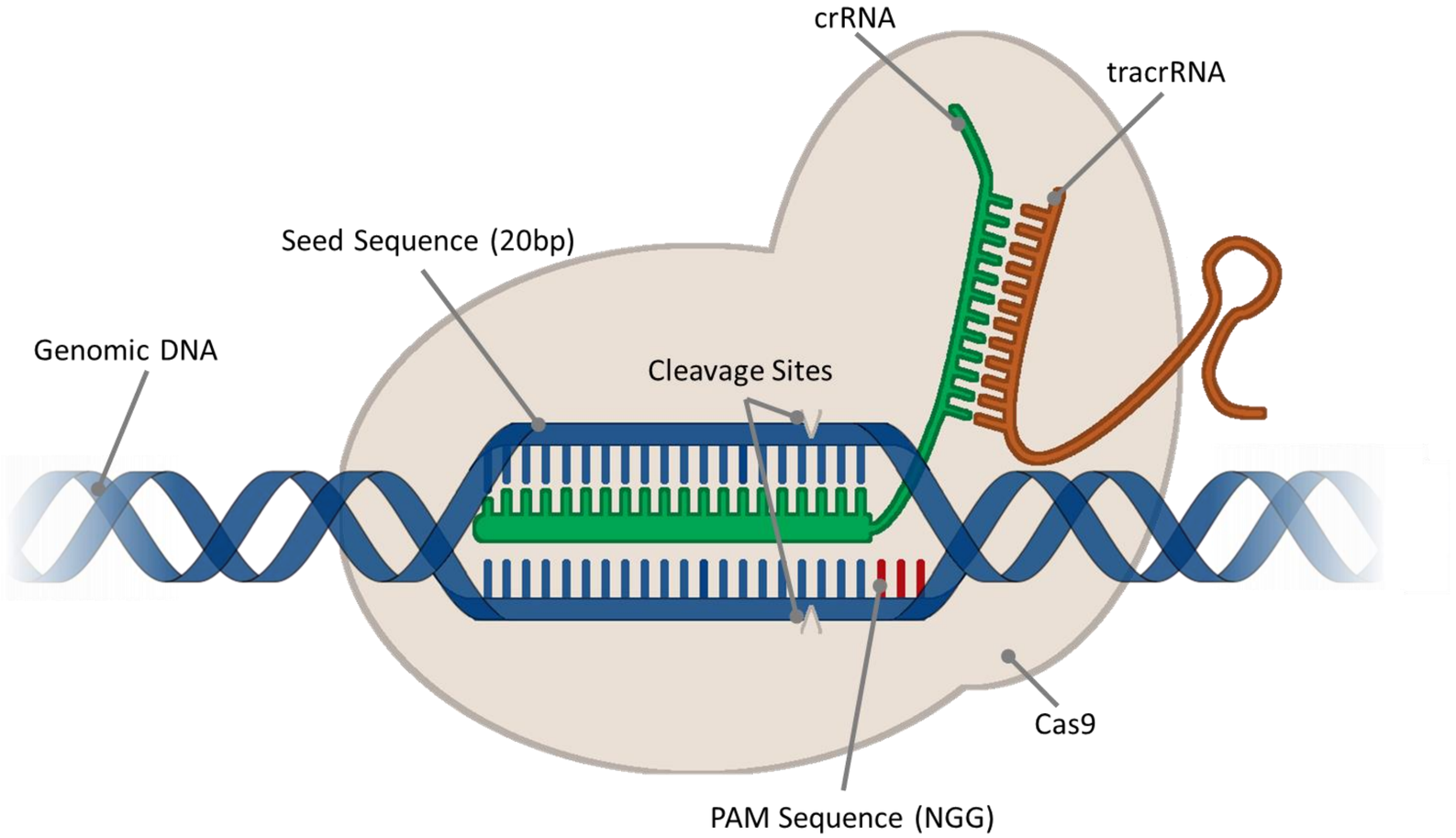
Overview of CRISPR-Cas9 Complex



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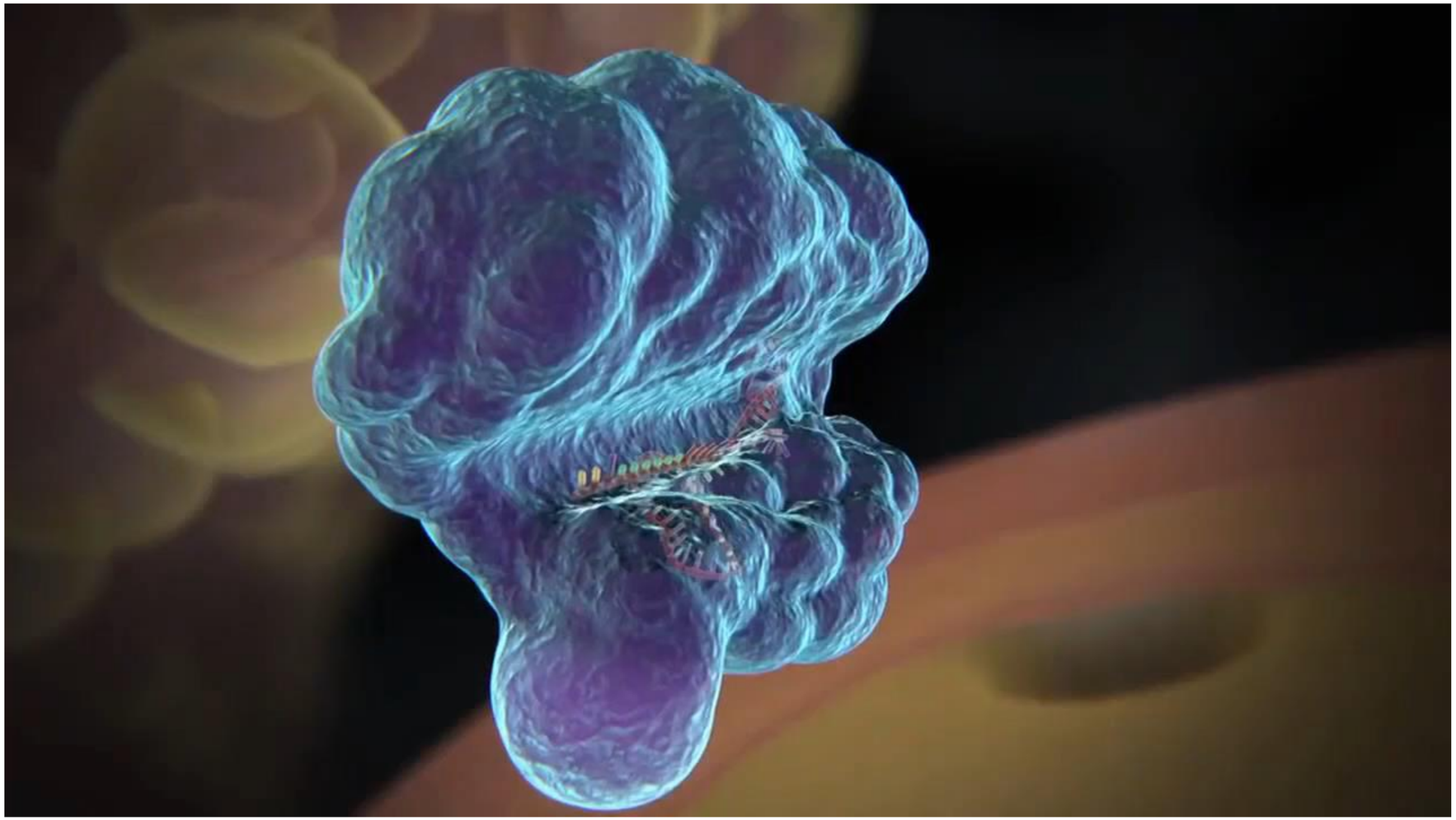

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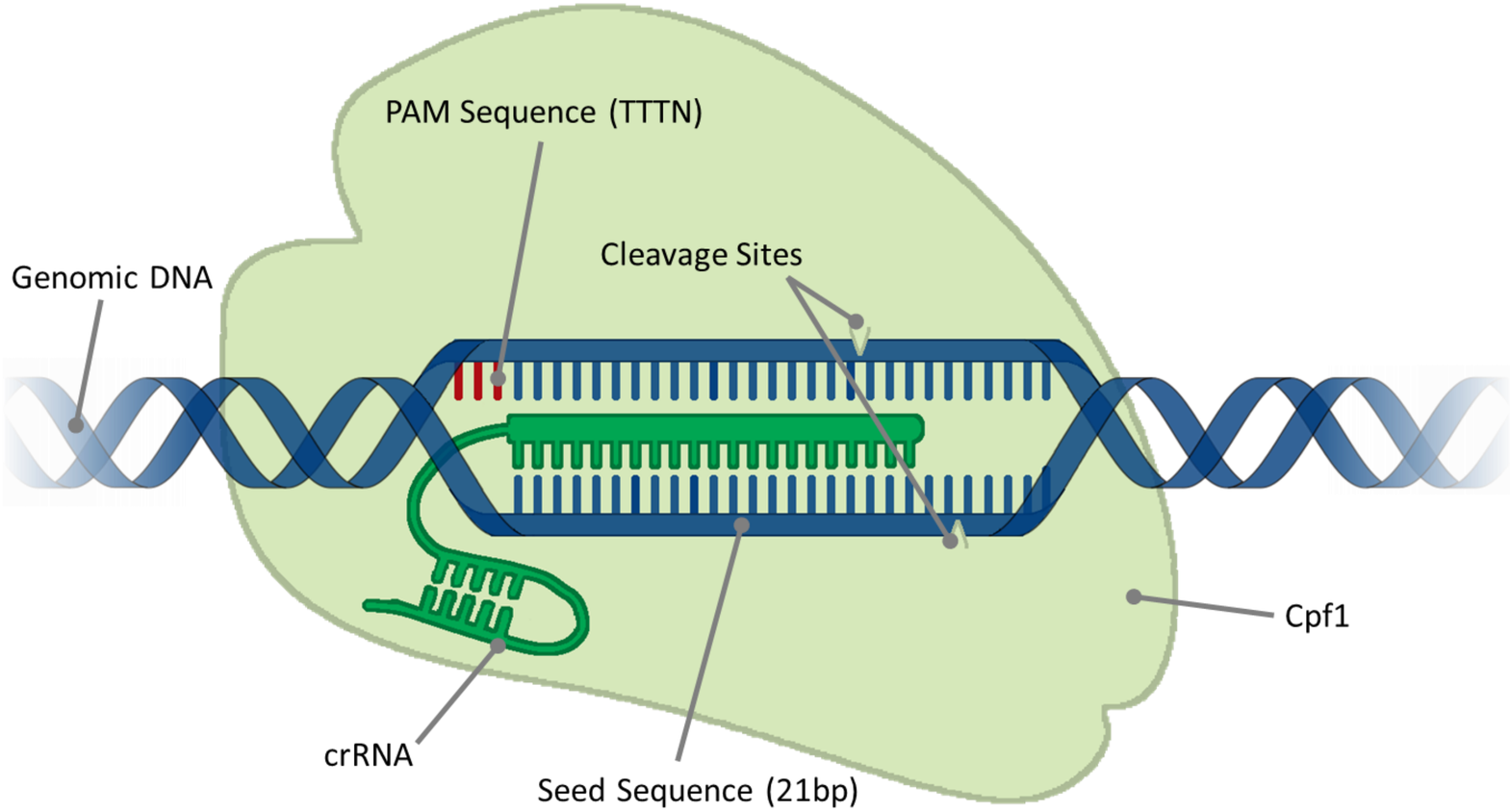
Overview of CRISPR-Cas12a Complex



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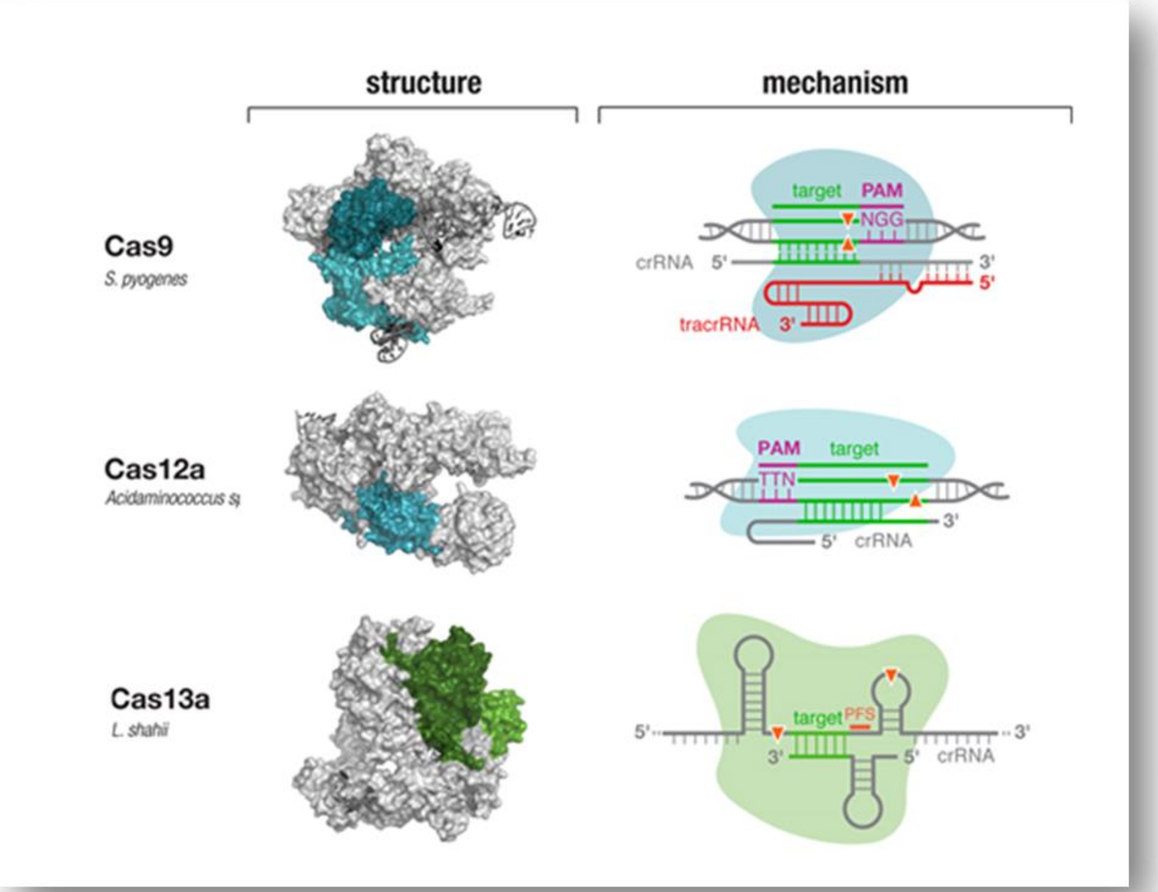
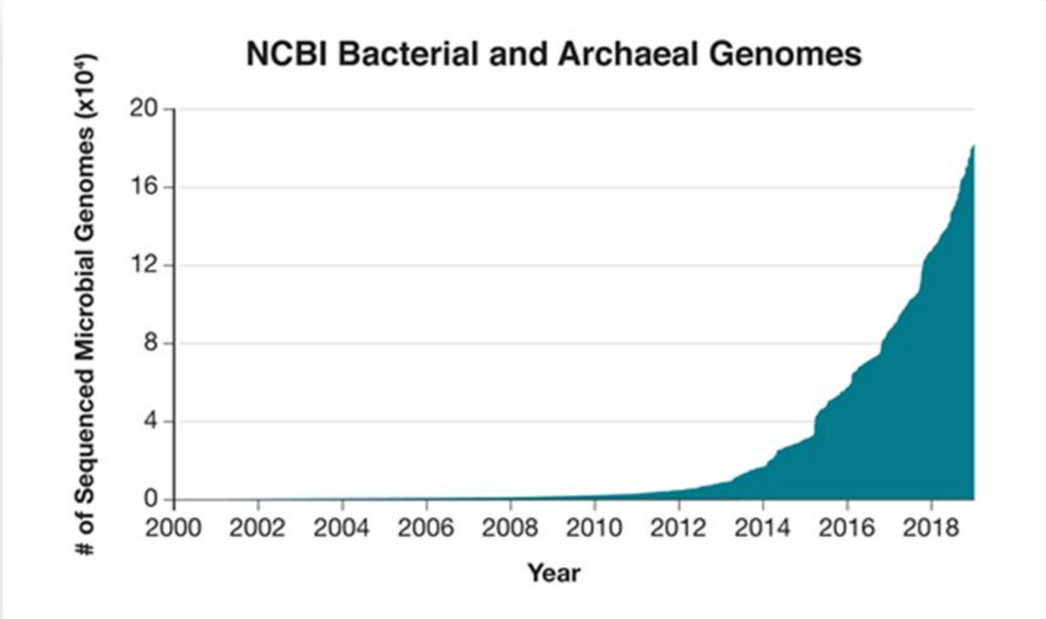
CRISPR-Cas Gene Editing Technology





Zhang F. Development of CRISPR-Cas systems for genome editing and beyond. Q Rev Biophys. 2019;52:e6.

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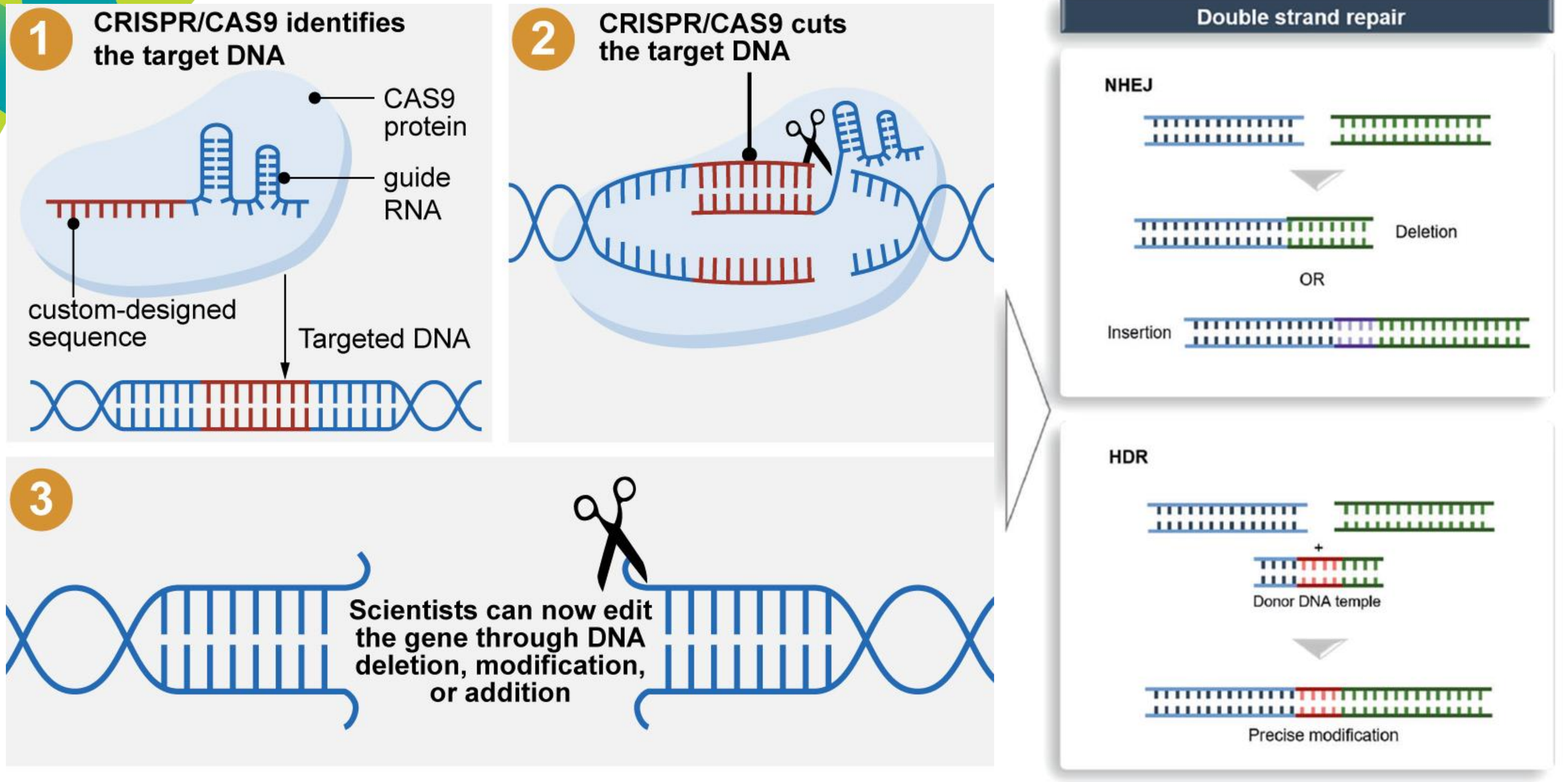

Source: GAO. | GAO-20-478SP

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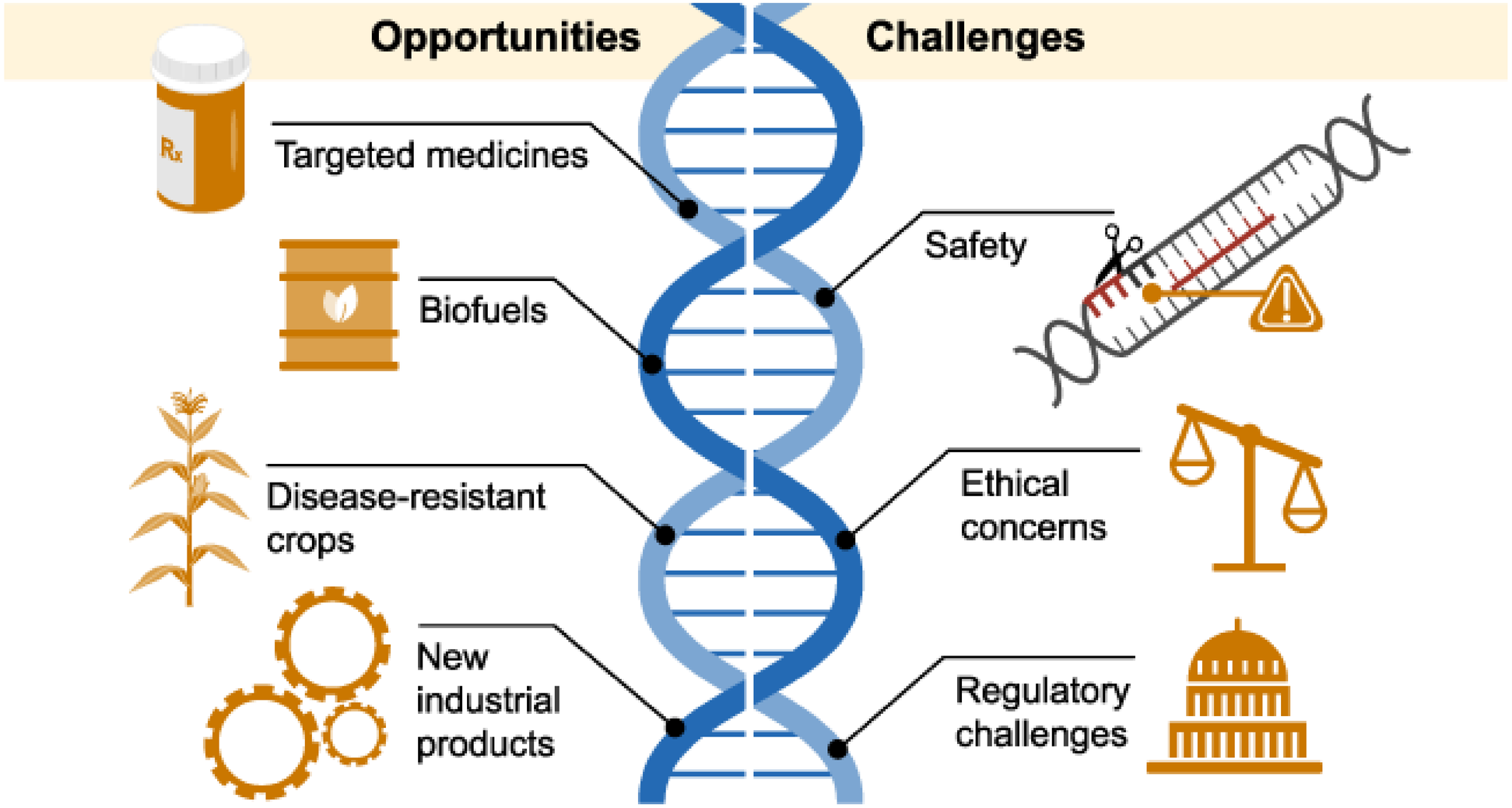
Current Limitations & Restrictions of Gene Editing

- **Off-site mutagenesis.** Large genomes may consist of DNA sequences with very close similarity to the target site which may also be modified during targeted gene editing.
- **Introduction of unintended indels at target site.** Following DNA breakage the cellular repair process can introduce unpredictable secondary mutations.
- **Competing NHEJ/HDR responses.** A major challenge to clinical applications has been to enhance HDR-mediated precise modifications while decreasing NHEJ-induced indel production during gene editing.

Kang et al. Addressing challenges in the clinical applications associated with CRISPR/Cas9 technology and ethical questions to prevent its misuse. *Protein & Cell*. 2017;8(11):791-795.



Source: GAO. | GAO-20-478SP

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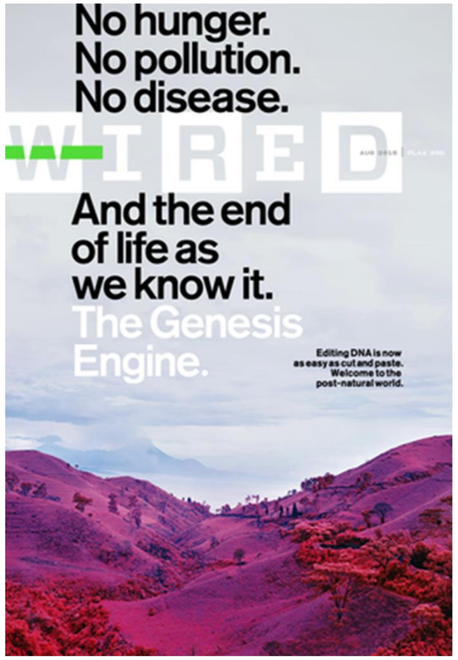
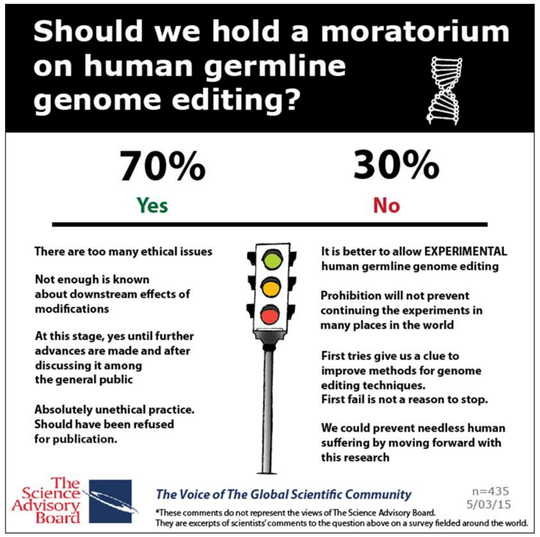
Risks?





Statement for the Record

Worldwide Threat Assessment of the US Intelligence Community

Senate Armed Services Committee

James R. Clapper

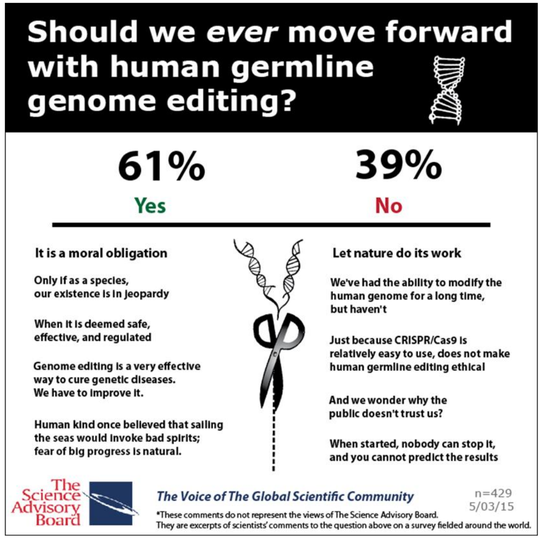
Director of National Intelligence

February 9, 201

TO CRISPR OR NOT TO CRISPR?

Examining the ongoing ethical debate surrounding embryonic gene editing

EVOLUTION
GLOBAL TALENT ATTRACTION



Courtesy of Kavita M. Berger

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NEWS • 26 NOVEMBER 2018

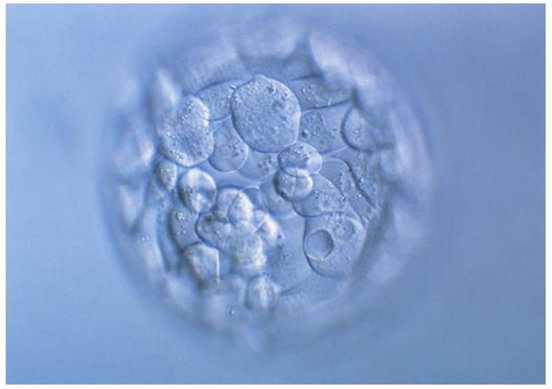
Genome-edited baby claim provokes international outcry

The startling announcement by a Chinese scientist represents a controversial leap in the use of genome editing.

A Chinese scientist claims to have helped make the world's first genome-edited babies — twin girls, who were born this month. The announcement has provoked shock and outrage among scientists around the world.

He Jiankui, a genome-editing researcher at the Southern University of Science and Technology of China in Shenzhen, says that he impregnated a woman with embryos that had been edited to disable the genetic pathway HIV uses to infect cells.

In a video posted to YouTube, He says the girls are healthy and now at home with their parents. Sequencing of the babies' DNA has shown that the editing worked, and altered only the target gene, he says.



A Chinese scientist claims that twin girls have been born whose genomes were edited at the embryo stage.
Credit: Pascal Goetgheluck/Science Photo Library

The scientist's claims have not been verified through independent genome testing, nor published in a peer-reviewed journal. But, if true, the twins' birth would represent a significant — and controversial — leap in the use of genome editing. Until now, the use of these tools in embryos has been limited to research, often to investigate the benefit of using the technology to eliminate disease-causing mutations from the human germ line. But some studies have reported off-target effects, raising significant safety concerns.

HIV's entry point

Documents posted on China's clinical-trial registry show that He used the popular CRISPR–Cas9 genome-editing tool to disable a gene called *CCR5*, which encodes a protein that allows HIV to enter a cell.
Genome-editing scientist Fyodor Urnov was asked to review documents that described DNA sequence analysis of human embryos and fetuses edited at the *CCR5* locus for an article in MIT Technology Review. "The data I reviewed

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HIV's entry point

Documents posted on China as9 genome-editing tool to disable a gene called *CCR*

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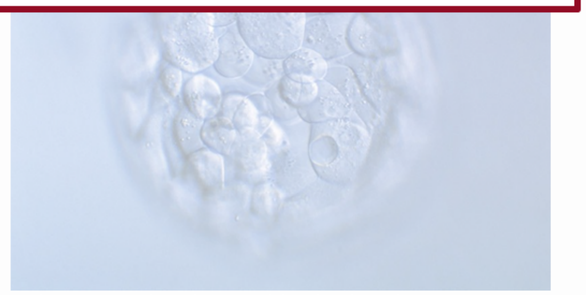
"I was really horrified and stunned when he described the process he used… It was **so inappropriate** on so many levels."

-Jennifer Doudna, Founder of Caribou Biosciences, Inc.



A Chinese scientist claims that twin girls have been born whose genomes were edited at the embryo stage.
Credit: Pascal Goetgheluck/Science Photo Library

"This experiment exposes healthy normal children to risks of gene editing for **no real necessary benefit**."

Julian Savulescu, director of the Oxford Uehiro Centre for Practical Ethics at the University of Oxford

"There is, at present, **no unmet medical need** that embryo editing addresses."

Fyodor Urnov, Altius Institute for Biomedical Sciences, Seattle WA

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NEWS • 28 NOVEMBER 2018 • CORRECTION 30 NOVEMB

CRISPR-baby Scientist H

He Jiankui gives talk about controversial claim of

today at a gene-editing summit in Hong Kong to explain his experiment. He delivered his talk amid threats of legal action and ne scientific community and beyond,

ted his work publicly, outside a handful of e. Scientists welcomed the fact that he lk left many hungry for more answers, and that He's claims are accurate.

elieve him," says Robin Lovell-Badge, a t completely convinced."

cally modified embryos and implanted them in women. He explained how he verified the gene edits — and revealed that another woman is possibly pregnant with a gene-edited embryo.

Lovell-Badge, like many other scientists, s an in-depth comparison of the parents' ar Many scientists faulted He for a lack of tra a landmark, and potentially risky, project. "I'm happy he came, but I was really horri Doudna, a biochemist at the University of technique that He used. "It was so inappr

"The genetically edited infant incident reported by media **blatantly violated China's relevant laws and regulations**. It has also violated the ethical bottom line that the academic community adheres to. It is **shocking and unacceptable**."

-Xu Nanping, Vice Minister, Ministry of Science and Technology of the PRC

"[The NIH] does not support the use of gene-editing technologies in human embryos… This work represents a deeply disturbing willingness by Dr. He and his team to flout international ethical norms. The need for… setting limits for this kind of research, now being debated in Hong Kong, has never been more apparent."

Francis Collins, Director of the National Institutes of Health

"It is impossible to overstate **how irresponsible, unethical and dangerous** this is at the moment. There was a worrying **lack of oversight or scrutiny** of his clinical plans before he started human experiments and a complete **lack of transparency** throughout the process."

Kathy Niakin, Francis Crick Institute, London

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Gene Editing: Potential Applications & Current Research

Drug Development

- Eliminate HIV
- Cancer immunotherapy
- Repair genetic blindness

Animal Models

- Model human disease
- Universal transplant organs
- Huntington's disease

Agriculture

- Control pesticide resistance
- Sustainable, storable foods
- Accelerated growth crops

Gene Drives

- Disease prevention
- Eliminate malaria
- Control invasive species

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DIVERSITY OF TARGETS FOR THERAPEUTIC GENOME EDITING (Maeder & Gersbach 2016)

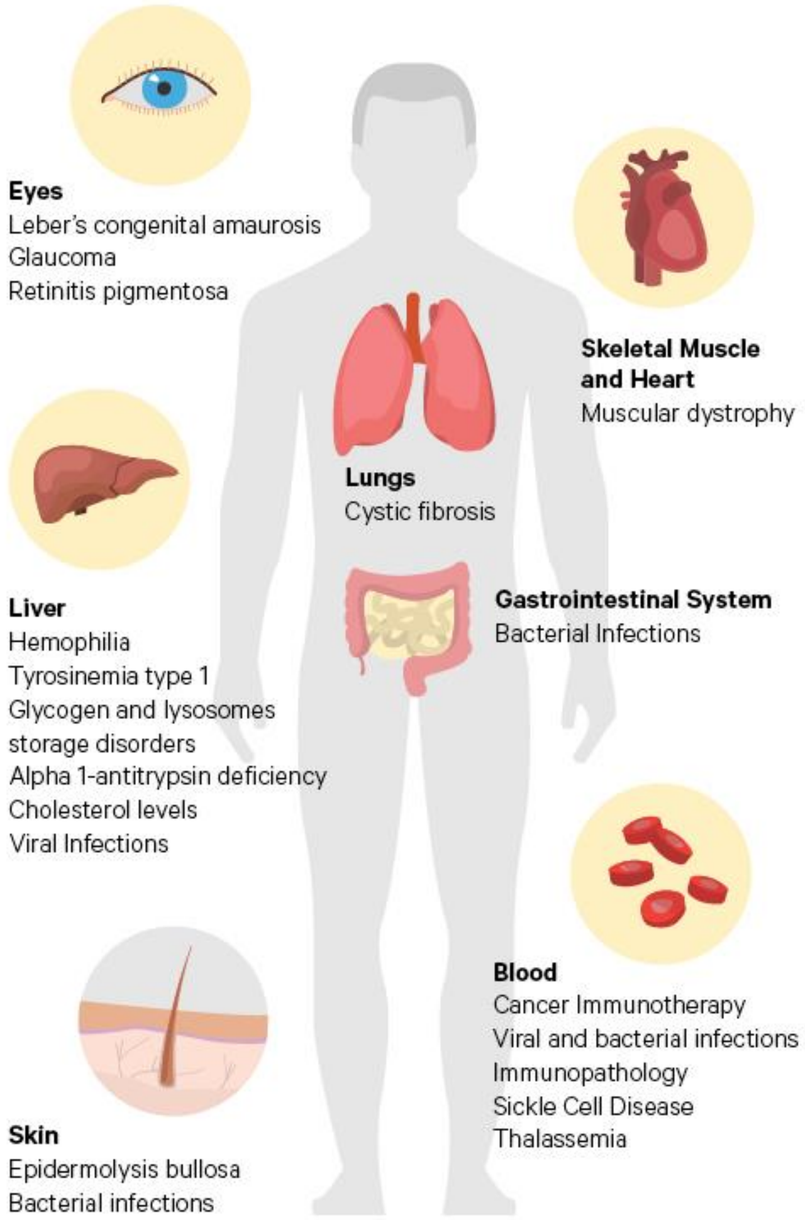

Table 2. Clinical trials of gene editing in the treatment of human diseases.

Platform	Disease applications	Target	Edited cells	Delivery	Sample size	Phase	Trial number
ZFN	HIV-1 infection	CCR5	CD4+ T cells	Adenovirus	12	I	NCT00842634
	HIV-1 infection	CCR5	CD4+ T cells	Adenovirus	19	I	NCT01044654
	HIV-1 infection	CCR5	CD4+ T cells	Adenovirus	21	I/II	NCT01252641
	HIV-1 infection	CCR5	CD4/CD8 T cells	Adenovirus	26	I	NCT01543152
	HIV-1 infection	CCR5	CD4/CD8 T cells	mRNA	12	I/II	NCT02225665
	HIV-1 infection	CCR5	CD4+ T cells	mRNA	14	I	NCT02388594
	HIV-1 infection	CCR5	CD4+ T cells	mRNA	30	I/II	NCT03666871
	HIV-1 infection	CCR5	CD4+ T cells	mRNA	12	I	NCT03617198
	HIV-1 infection	CCR5	CD34+HSPCs	mRNA	18	I	NCT02500849
	HPV-induced cervical precancerous lesions	HPV16/18 E7	Epithelial cells	DNA	20	I	NCT02800369
	Mucopolysaccharidosis I	IDS gene	Hepatocytes	AAV	9	I/II	NCT03041324
	Mucopolysaccharidosis II	IDUA gene	Hepatocytes	AAV	3	I/II	NCT02702115
	Hemophilia B	Factor IX gene	Hepatocytes	AAV	12	I	NCT02695160
	β-Thalassemia	BCL11A gene	CD34+HSPCs	mRNA	6	I/II	NCT03432364
	Recurrent/refractory malignant glioma	IL13Ralpha2	CD8+T cell	Injection	6	I	NCT01082926
TALEN	Relapsed/refractory B-ALL	CD52, TRAC	CAR T cells	Lentivirus	18	I	NCT02808442
	HPV-related cervical intraepithelial neoplasia	HPV16/18 E6/E7	Epithelial cells	Plasmid	40	I	NCT03226470
TALENs and CRISPR/Cas9	HPV-related cervical intraepithelial neoplasia	HPV16/18 E6/E7	Epithelial cells	Plasmid	60	I	NCT03057912
CRISPR/Cas9	AML	CD123, TRAC	CAR T cells	mRNA	162	I	NCT03190278
	Metastatic non-small cell lung cancer	PDCD1	T cells	DNA	12	I	NCT02793856
	Castration-resistant prostate cancer	PDCD1	T cells	DNA	Withdrawn	I	NCT02867345
	Muscle-invasive bladder cancer	PDCD1	T cells	DNA	Withdrawn	I	NCT02863913
	Advanced esophageal cancer	PDCD1	T cells	DNA	16	I	NCT03081715
	Metastatic renal cell carcinoma	PDCD1	T cells	DNA	Withdrawn	I	NCT02867332
	HIV-1 infection with ALL	CCR5	CD34+ HSPCs	Liposome and electroporation	5	I	NCT03164135
	EBV-positive cancers	PDCD1	T cells	DNA	20	I	NCT03044743
	Relapsed refractory multiple myeloma, melanoma, synovial sarcoma, and myxoid/round cell liposarcoma	NY-ESO-1, TRAC PDCD1	T cells	Lentiviral and electroporation	18	I	NCT03399448
	Relapsed or refractory CD19+ leukemia and lymphoma	TRAC, B2M	CAR T cells	Lentiviral and electroporation	80	I/II	NCT03166878
	Relapsed or refractory CD19- leukemia and lymphoma	CD19 and CD20 or CD22, TRAC	CAR T cells	Lentiviral and electroporation	80	I/II	NCT03398967
	Mesothelin-positive multiple solid tumors	PDCD1 and TRAC	CAR T cells	Lentiviral DNA	10	I	NCT03545815
	Mesothelin-positive multiple solid tumors	PDCD1 and TRAC	CAR T cells	Lentiviral DNA	10	I	NCT03747965
	Metastatic gastrointestinal epithelial cancer	CISH	TIL	Electroporation	Withdrawn	I/II	NCT03538613
	T cell leukemia or lymphoma	CD7, CD28	CAR T cells	–	21	I	NCT03690011
	Neurofibromatosis type 1	NF1	iPSCs	DNA	20	I	NCT03332030
	β-Thalassemia	HBB gene	iHSCs	–	12	I	NCT03728322
	β-Thalassemia	BCL11A gene	CD34+ HSPCs	–	45	I/II	NCT03655678
	Sickle cell disease	BCL11A gene	CD34+HSPCs	–	45	I/II	NCT03745287
	LCA10	CEP290 gene	Photoreceptor cells	AAV	18	I/II	NCT03872479

ZFN zinc-finger nuclease, *CCR5* chemokine receptor 5, *HSPCs* hematopoietic stem/progenitor cells, *IDS* iduronate 2-sulfatase, *IDUA* α-L-iduronidase, *BCL11A* mouse B cell lymphoma factor 11A, *B-ALL* B acute lymphoblastic leukemia, *TRAC* T cell receptor alpha chain, *TALEN* transcription activator-like effector nuclease, *CRISPR* clustered regularly interspaced short palindromic repeat, *AML* acute myeloid leukemia, *PDCD1* programmed cell death 1, *NF1* neurofibromatosis type 1, *TIL* tumor-infiltrating lymphocytes, *iPSCs* induced progenitor stem cells, *iHSCs* induced hematopoietic stem cells, *LCA10* Leber congenital amaurosis type 10, *CEP290* centrosomal protein 290, *AAV* adeno-associated virus

Trials Using CRISPR

Trials involving cancer highlighted in yellow

Status	Conditions
Unknown	Human Papillomavirus-Related Malignant Neoplasm
Recruiting	Tuberculosis, Pulmonary
Recruiting	HIV-1-infection
Recruiting	Solid Tumor, Adult
Recruiting	Beta-Thalassemia|Thalassemia|Genetic Diseases, Inborn|Hematologic Diseases|Hemoglobinopathies
Recruiting	Multiple Myeloma|Melanoma|Synovial Sarcoma|Myxoid/Round Cell Liposarcoma
Recruiting	Leukemia Lymphocytic Acute (ALL) in Relapse|Leukemia Lymphocytic Acute (All) Refractory|Lymphoma, B-Cell|CD19 Positive
Recruiting	Sickle Cell Disease|Hematological Diseases|Hemoglobinopathies
Recruiting	B-cell Malignancy|Non-Hodgkin Lymphoma|B-cell Lymphoma
Not yet recruiting	Thalassemia
Recruiting	Solid Tumor, Adult
Active	Kabuki Syndrome 1
Recruiting	Gastrointestinal Infection
Recruiting	B Cell Leukemia|B Cell Lymphoma
Recruiting	High Grade Ovarian Serous Adenocarcinoma|Stage III Ovarian Cancer AJCC v8|Stage IIIA Ovarian Cancer AJCC v8|Stage IIIA1 Ovarian Cancer AJCC v8|Stage IIIA2 Ovarian Cancer AJCC v8|Stage IIIB Ovarian Cancer AJCC v8|Stage IIIC Ovarian Cancer AJCC v8|Stage IV Ovarian Cancer AJCC v8|Stage IVA Ovarian Cancer AJCC v8|Stage IVB Ovarian Cancer AJCC v8
Recruiting	B Cell Leukemia|B Cell Lymphoma
Completed	Esophageal Cancer
Recruiting	Neurofibromatosis Type 1|Tumors of the Central Nervous System
Not yet recruiting	T-cell Acute Lymphoblastic Leukemia|T-cell Acute Lymphoblastic Lymphoma|T-non-Hodgkin Lymphoma
Active	Metastatic Non-small Cell Lung Cancer
Recruiting	Stage IV Gastric Carcinoma|Stage IV Nasopharyngeal Carcinoma|T-Cell Lymphoma Stage IV|Stage IV Adult Hodgkin Lymphoma|Stage IV Diffuse Large B-Cell Lymphoma
Recruiting	Blindness|Leber Congenital Amaurosis 10|Vision Disorders|Eye Diseases|Eye Diseases, Hereditary|Eye Disorders Congenital|Retinal Disease|Retinal Degeneration
Recruiting	Malignant Hyperthermia

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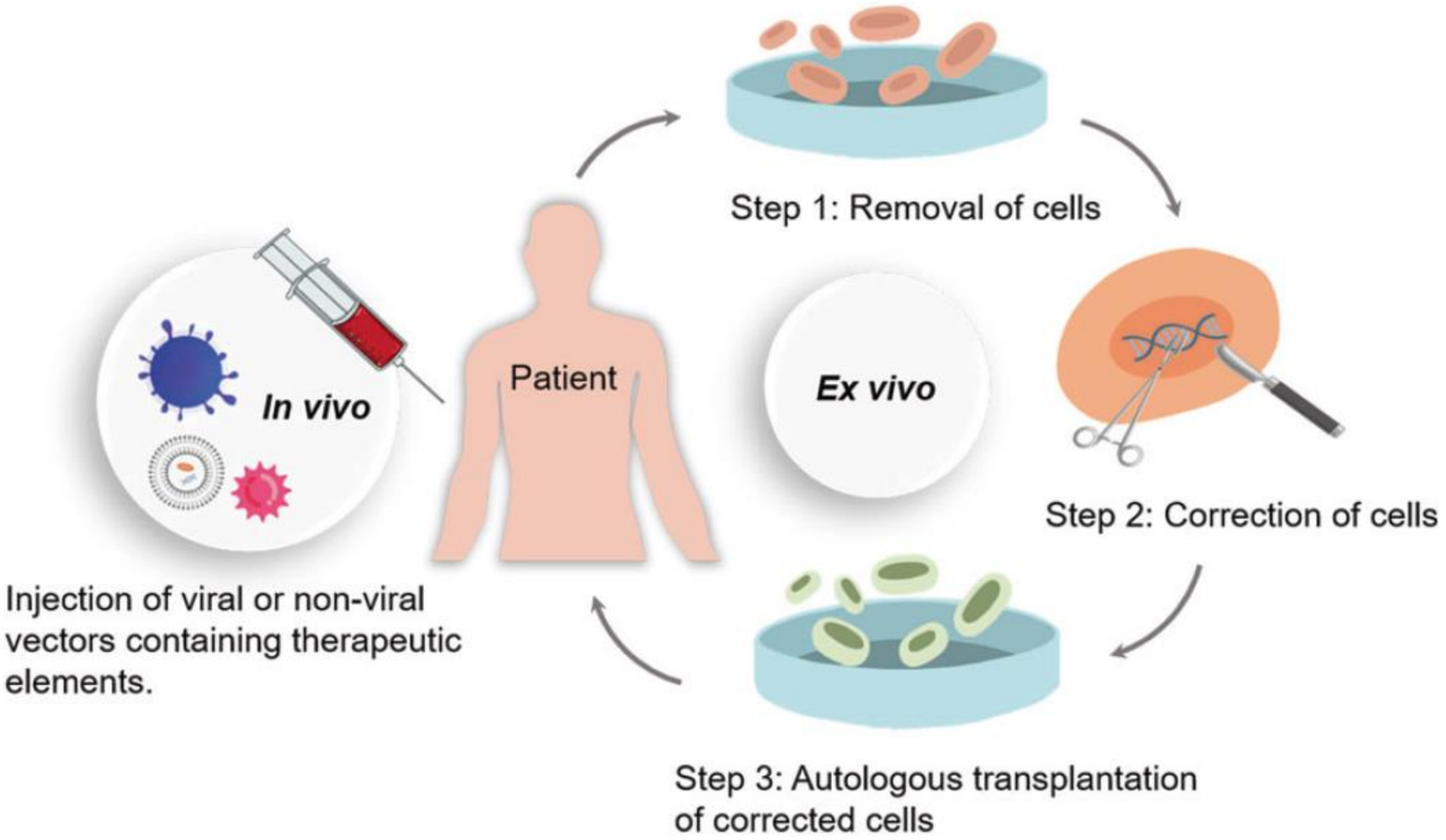
Ex vivo and *in vivo* genome editing for clinical therapy



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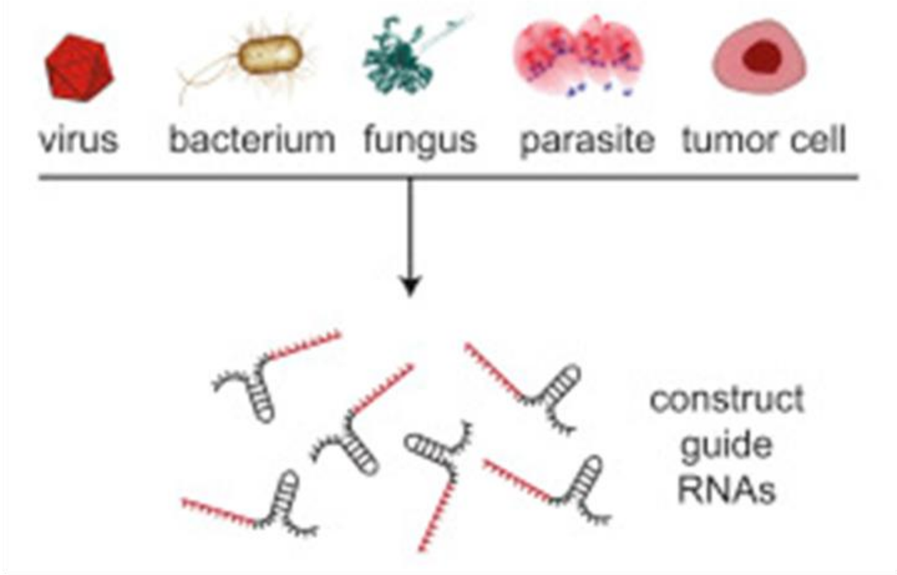
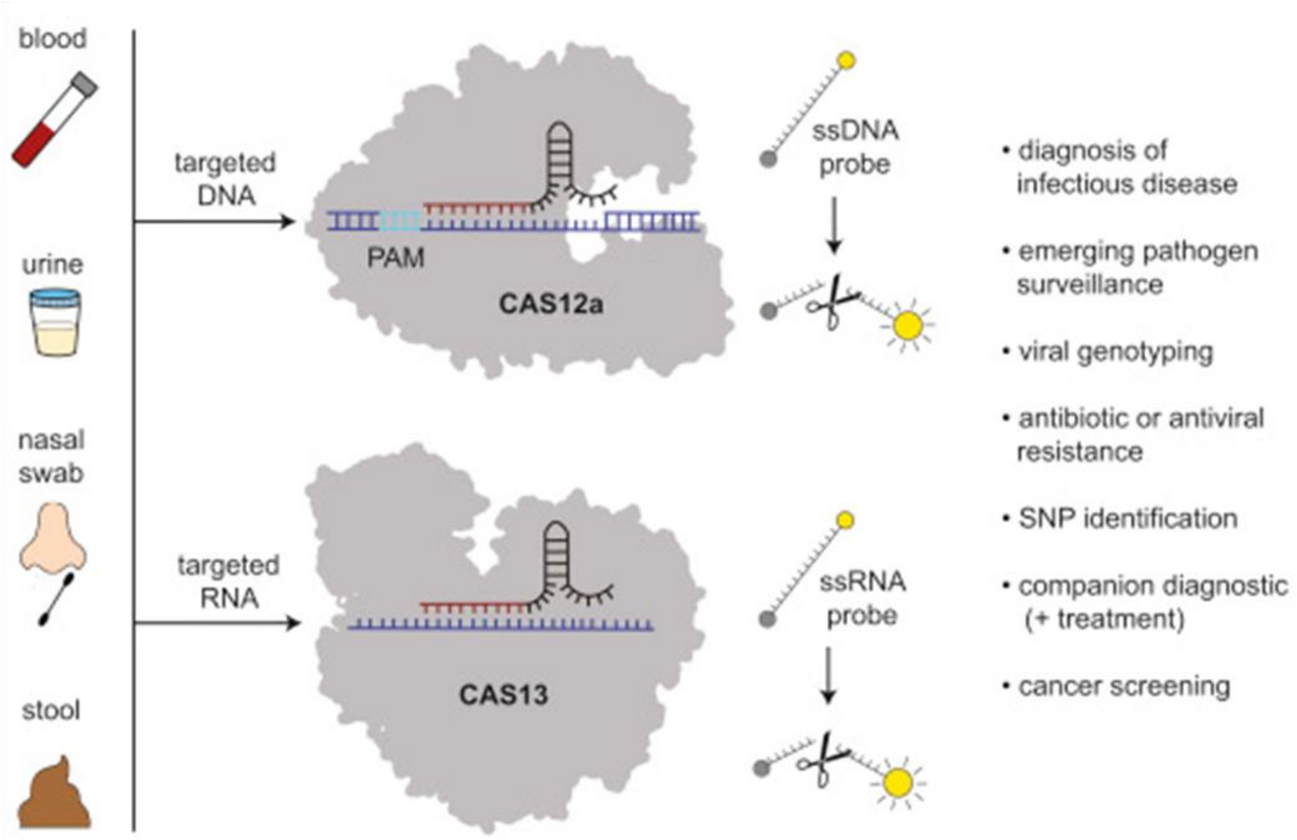
Infectious Disease Diagnostics with CRISPR



After collection of clinical samples in a point-of-care setting, such as the patient bedside, medical office, hospital ward, or in the field, the Cas12a or Cas13-based assay can be performed directly from the sample in under 2 hours, without the need for a separate DNA or RNA extraction step.



10.1016/j.chom.2018.05.016

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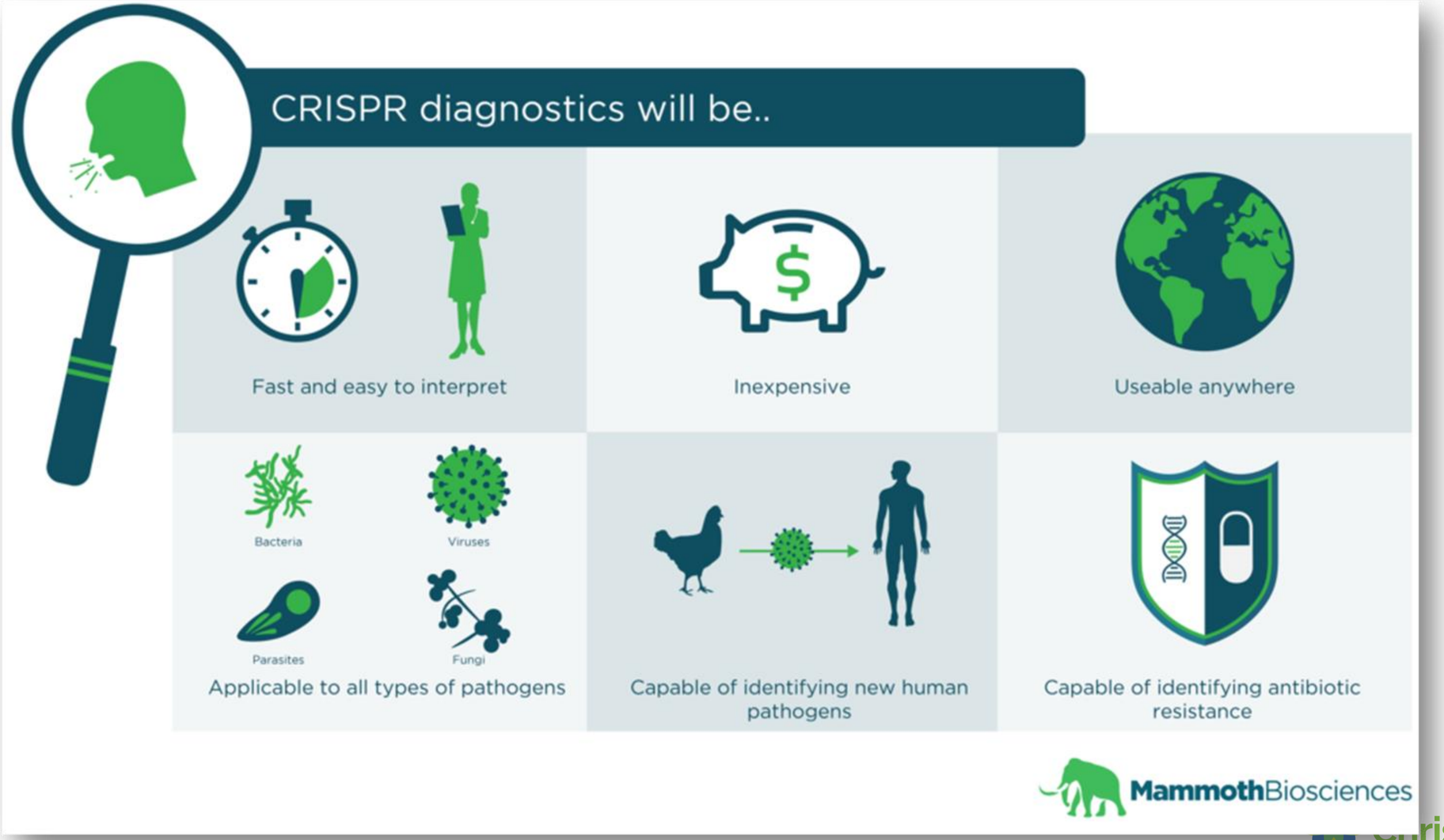
Infectious Disease Diagnostics with CRISPR



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Mammoth Biosciences Collaborates with GSK Consumer Healthcare to Develop COVID-19 Test Using CRISPR-Based Platform

Driven by the gap in COVID-19 testing, the collaboration aims to create a fully disposable, rapid and handheld test for consumers

“Mammoth and GSK Consumer Healthcare have begun work on the COVID-19 test and are aiming to have a device submitted for FDA Emergency Use Authorization (EUA) review before the end of 2020. The test will then be made available to US healthcare facilities that diagnose or treat COVID-19, and are eligible to use EUA-approved tests, with the goal of having it subsequently available over-the-counter to consumers. Following the development of a COVID test, both companies intend to explore how they can use the DETECTR™ technology platform to develop other types of diagnostics for consumer use. ”

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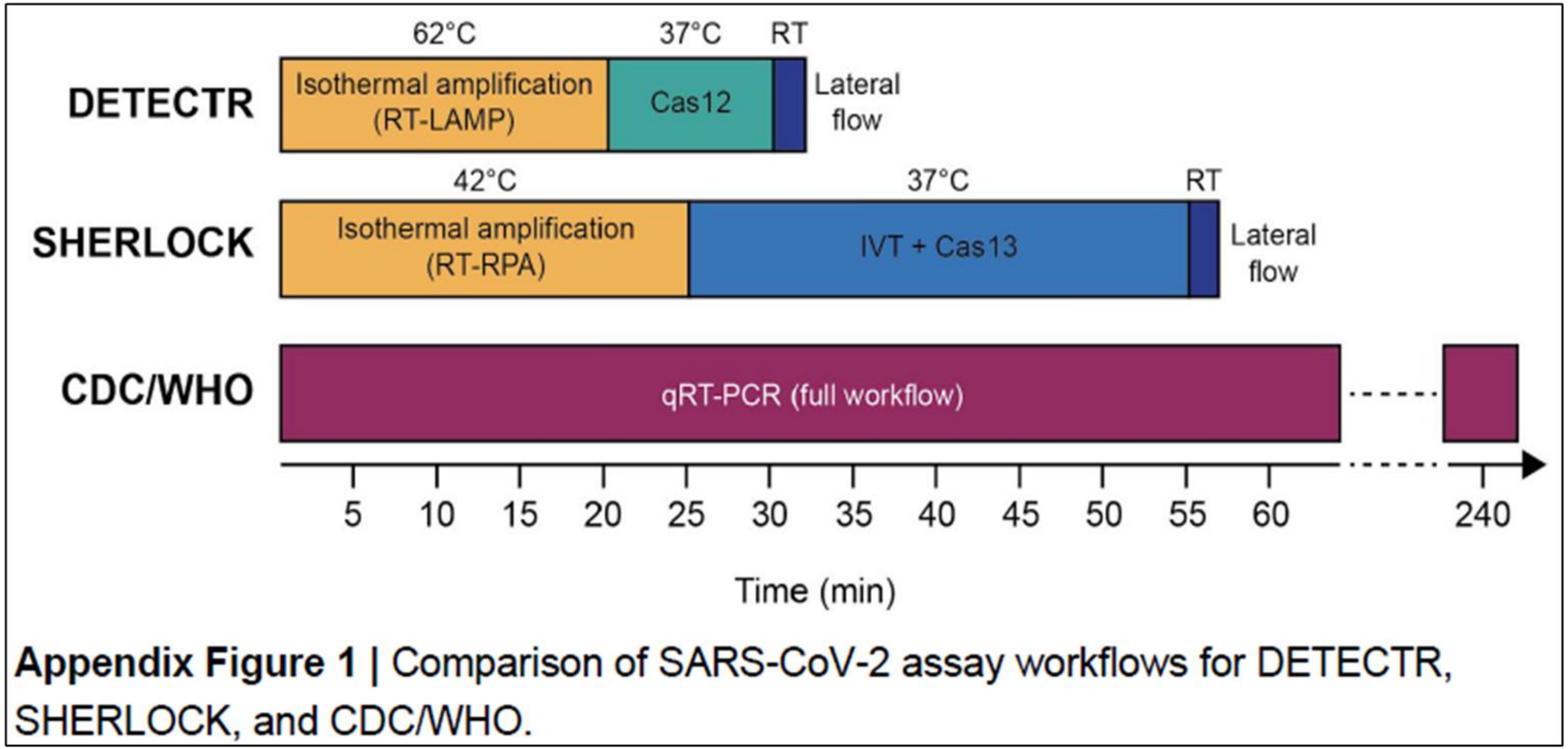
SARS-CoV-2 Workflow Comparison



Appendix Figure 1 | Comparison of SARS-CoV-2 assay workflows for DETECTR, SHERLOCK, and CDC/WHO.

Broughton, J. P.; Deng, W.; Fasching, C. L.; Singh, J.; Charles, Y.; Chen, J. S.; Biosciences, M.; Francisco, S. S.; Francisco, S.; Francisco, S. A Protocol for Rapid Detection of the 2019 Novel Coronavirus SARS-CoV-2 Using CRISPR Diagnostics : SARS-CoV-2 DETECTR. ***2020****, 1–9*

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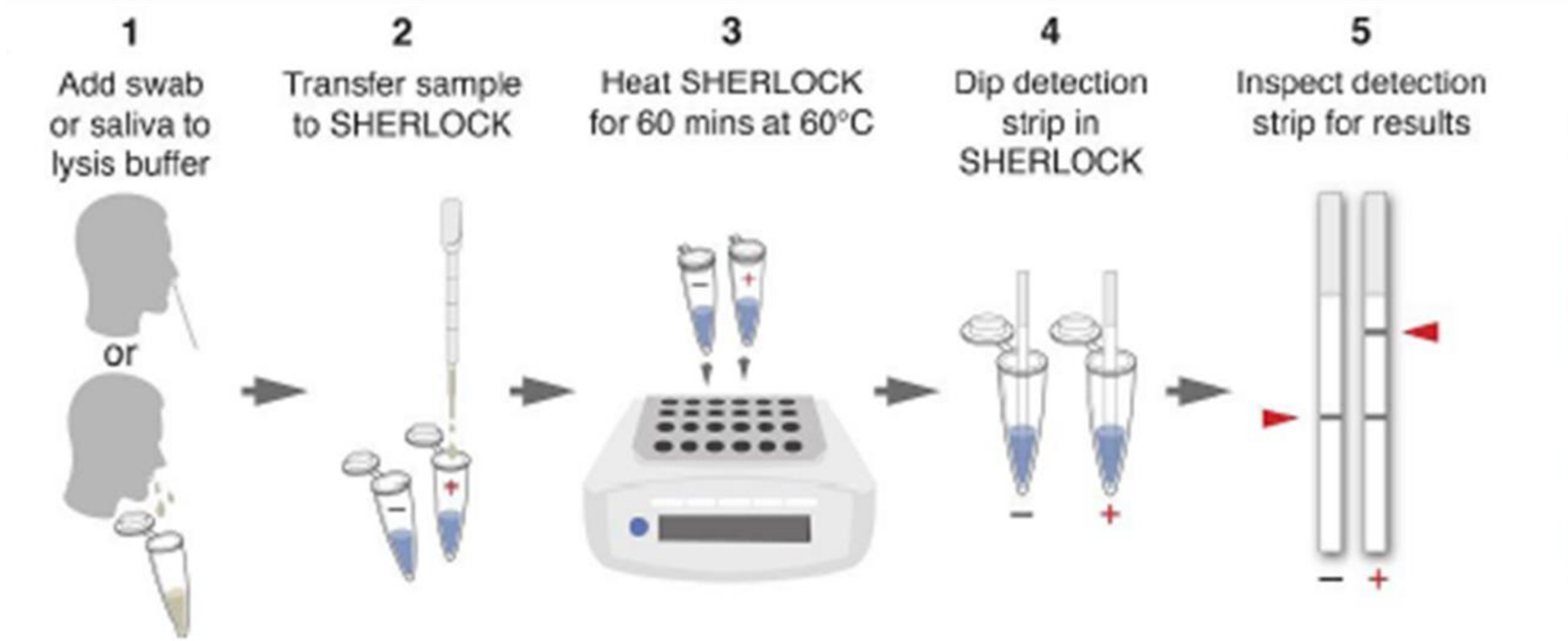
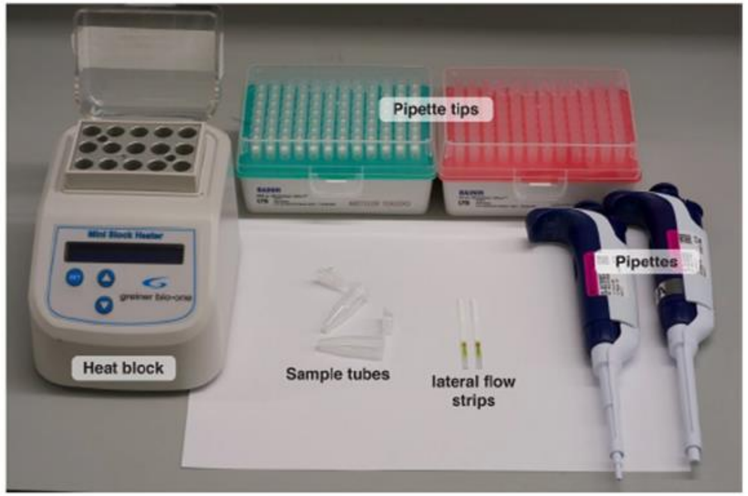
Gene Editing Institute

Sherlock 2.0 Point of Care Testing (STOPCovid)





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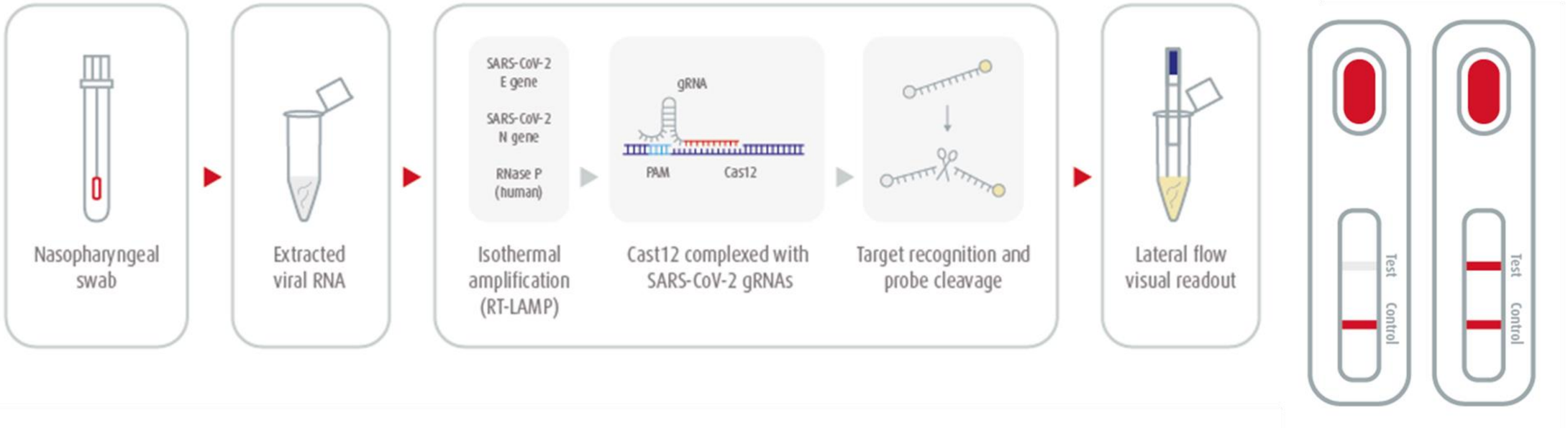
Endonuclease-Targeted CRISPR Trans Reporter (DETECTR)



[MEDRXIV. MARCH 27, 2020]

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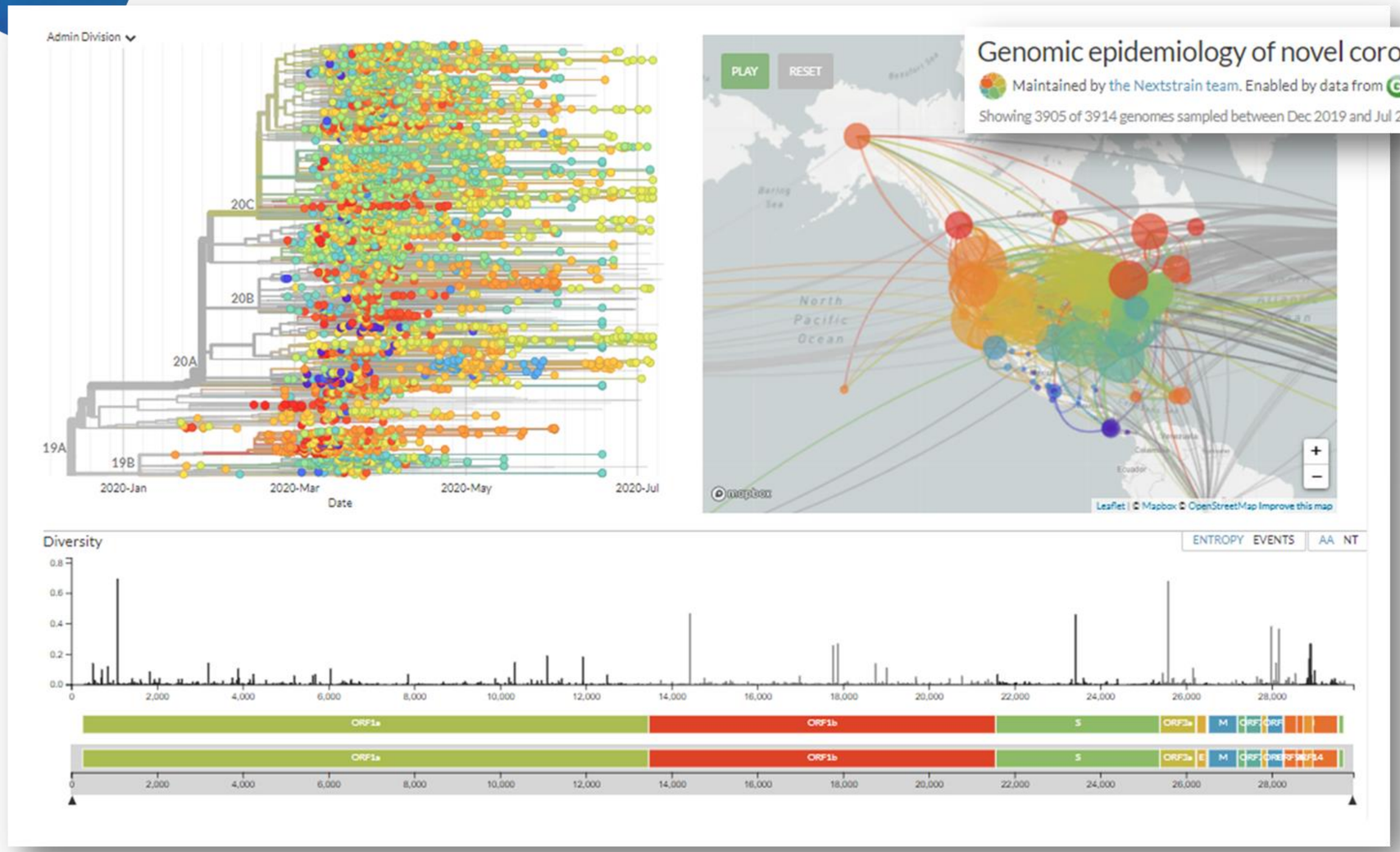
Geographical Hotspot Mutation Distribution



USA/MN-GMF-09331/2020
Nucleotide mutations: T4051C, G10981T
No amino acid mutations
Divergence: 9.892
Date: 2020-07-05
Admin Division: Minnesota
Author: Craig S. Richmond et al B
GISAID EPI ISL: 489903
Click on tip to display more info

USA/WA-S832/2020
No nucleotide mutations
Divergence: 7.909
Date: 2020-04-16
Admin Division: Washington
Author: Chu et al et al A
GISAID EPI ISL: 450002
Click on tip to display more info

USA/MO-UW-771/2020
Nucleotide mutations: G198A, A1445G, C3218A, C6723T, A16541C, T21458C, T25550A, C28748T
AA mutations:
ORF1a: K394E, Q985K, T2153I
ORF1b: N1025T, I2664T
ORF3a: L53H
Divergence: 15.854
Date: 2020-07-06
Admin Division: Missouri
Author: Gage Moreno et al B
GISAID EPI ISL: 495476
Click on tip to display more info

USA/MI-UM-20065001042/2020
Nucleotide mutations: G12514T, G14250T, C18698T, A26927G, A27533G
AA mutations:
ORF1b: L261F, A1744V
ORF7a: H47R
Divergence: 10.893
Date: 2020-05-05
Admin Division: Michigan
Author: Valesano et al. et al
GISAID EPI ISL: 495566
Click on tip to display more info

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Sickle Cell Disease

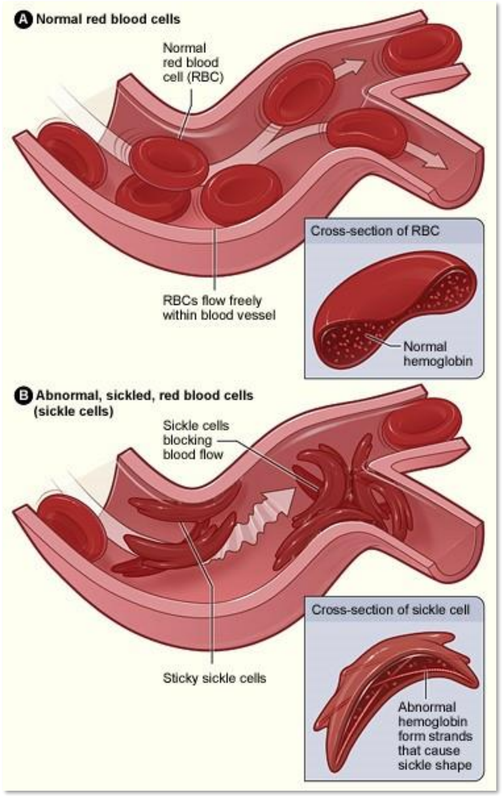
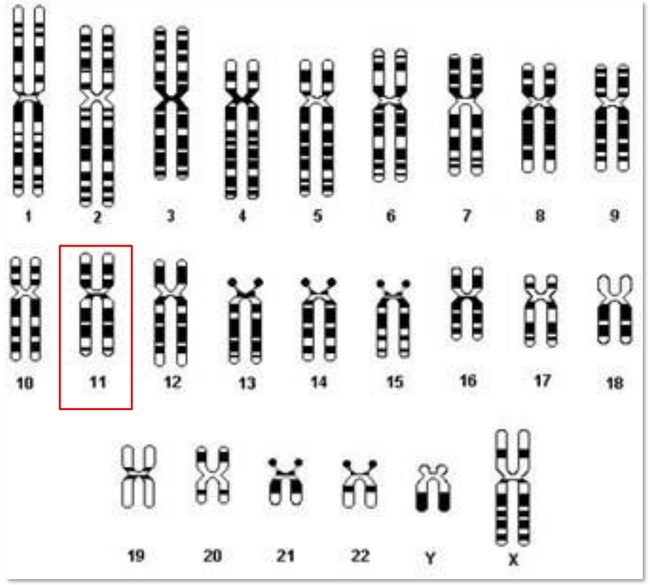
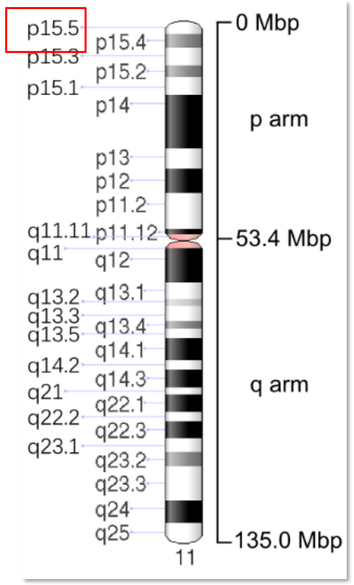


Figure from National Institute for Health, Sickle Cell Disease





Modified from Georgia Gwinnett College Genetic Disease Project

Wild-type β-Globin

CTGACTCCTGAGGAGAAGTC

GACTGAGGACTCCTCTTCAG

Sickle Cell β-Globin

CTGACTCCTGTGGAGAAGTC

GACTGAGGACACCTCTTCAG

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Hope for the Future

Flipping the Switch: Initial Results of Genetic Targeting of the Fetal to Adult Globin Switch in Sickle Cell Patients

Erica B. Esrick, Christian Brendel, John P Manis, Myriam A Armant, Helene Negre, Colleen Dansereau, Marioara Felicia Ciuculescu, Stephanie Patriarca, Brenda Mackinnon, Heather Daley, Shanna Richard, Daniela Abriss, Renee Maxwell, Stephen Braunewell, Lauryn Christiansen, Sarah Nikiforow, Jerome Ritz, Maureen Achebe, Olivier Negre, Leslie E. Lehmann, Matthew M Heeney, Alessandra Biffi, and David A. Williams

Blood 2018 132:1023; doi: https://doi.org/10.1182/blood-2018-99-116733

The Washington Post
Democracy Dies in Darkness

Health & Science

Gene therapies could transform the treatment of sickle cell disease

The New York Times

These Patients Had Sickle-Cell Disease. Experimental Therapies Might Have Cured Them.

wbur | On Point

Gene Therapy Advances Offer Hope For Sickle-Cell Disease Cure 17:09

BIOTECH

STAT+

Novel gene therapy 'switch' for sickle cell disease shows encouraging results in pilot trial

By ADAM FEUERSTEIN @adamfeuerstein / DECEMBER 1, 2018

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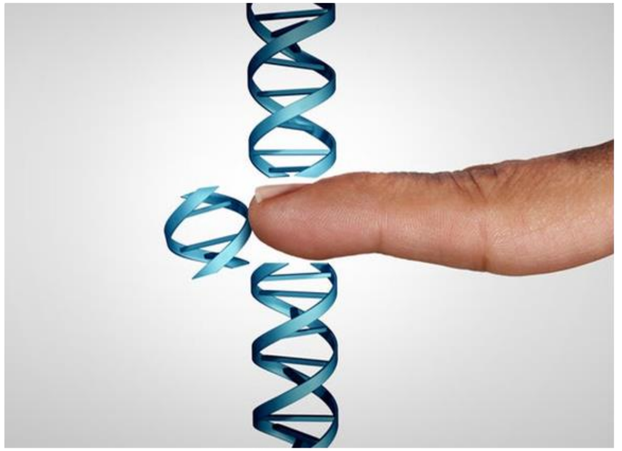
SCIENTIFIC AMERICAN.

A reprint from
Observations
Opinion, arguments & analyses from guest experts and from the editors of Scientific American

Potential Inequities in New Medical Technologies

Even if we solve the ethical questions about using breakthrough techniques such as CRISPR, will all patients have equal access?

By Eric B. Kmiec, Jonathan Marron on March 28, 2020



Credit: Getty Images

As headline-catching new technologies emerge—like tools to "edit" our DNA—researchers, doctors, patients and the general public are excited about the future of medicine and the research that informs its practice. For some, there are obvious and critical conversations taking place about the ethics of this research, including how we do it (think "CRISPR babies" in China) and the potential for edits (intentional or otherwise) that could be passed on to future generations.

These conversations are important, but they can overshadow another equally important question. Will all patients have equal access to these new technologies?

That question requires us to look back at the blemished history of medical research and health care that has resulted in some patients—based on their race, gender, income and other factors—having poorer access to health care and poorer health in general. In some cases, this is because the system neglected them. In others, because the system blatantly abused them. The question we must ask is: Will new treatments continue or even worsen **deeply rooted disparities? Or will we lay the groundwork for future treatments that benefit all people equitably?**

The history of medical research and health care is critical to understand here. Black Americans, for example, have been subjected to a long list of historical exploitations, producing an understandable and widespread legacy of mistrust in the health sector. These range from the appalling use of enslaved women to create modern gynecological treatments, to the now infamous Tuskegee experiments, in which scientists observed the effects of untreated syphilis in young black men, to the story of Henrietta Lacks' cancer samples being used without her knowledge.

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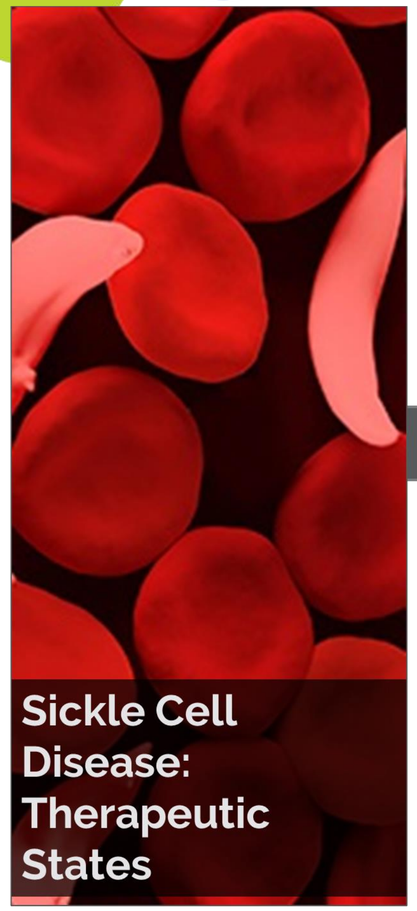
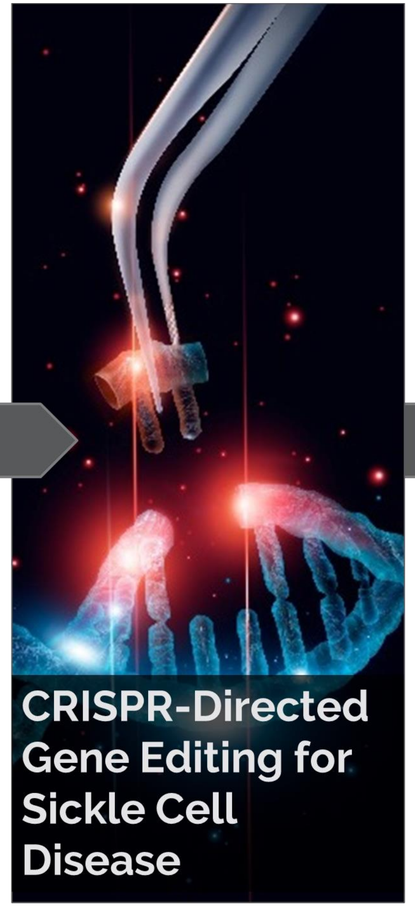
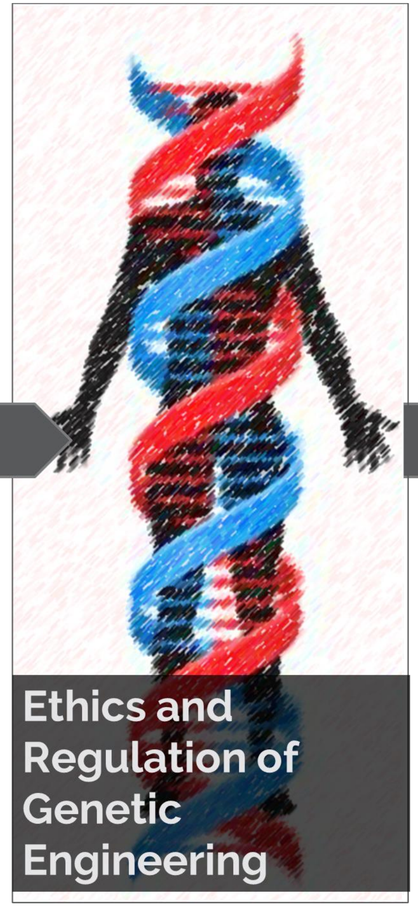
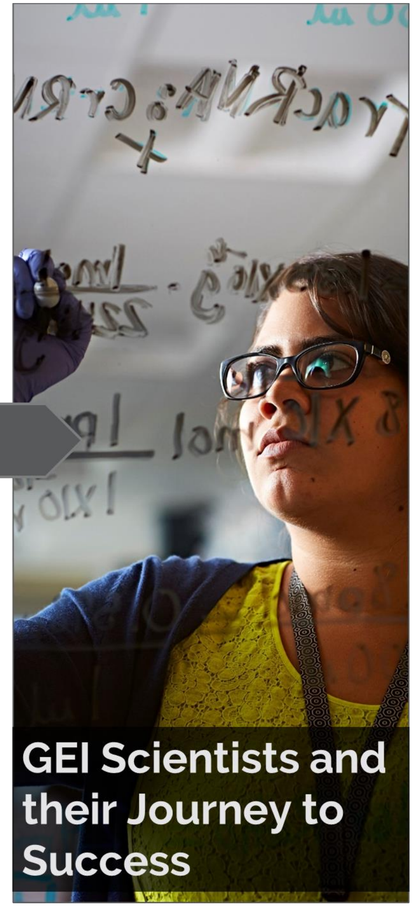

Sickle Cell Disease: Therapeutic States


CRISPR-Directed Gene Editing for Sickle Cell Disease


Laboratory Demonstration of CRISPR-Cas Editing for Sickle Cell


Gaining Trust in Breakthrough Technologies


Ethics and Regulation of Genetic Engineering


GEI Scientists and their Journey to Success

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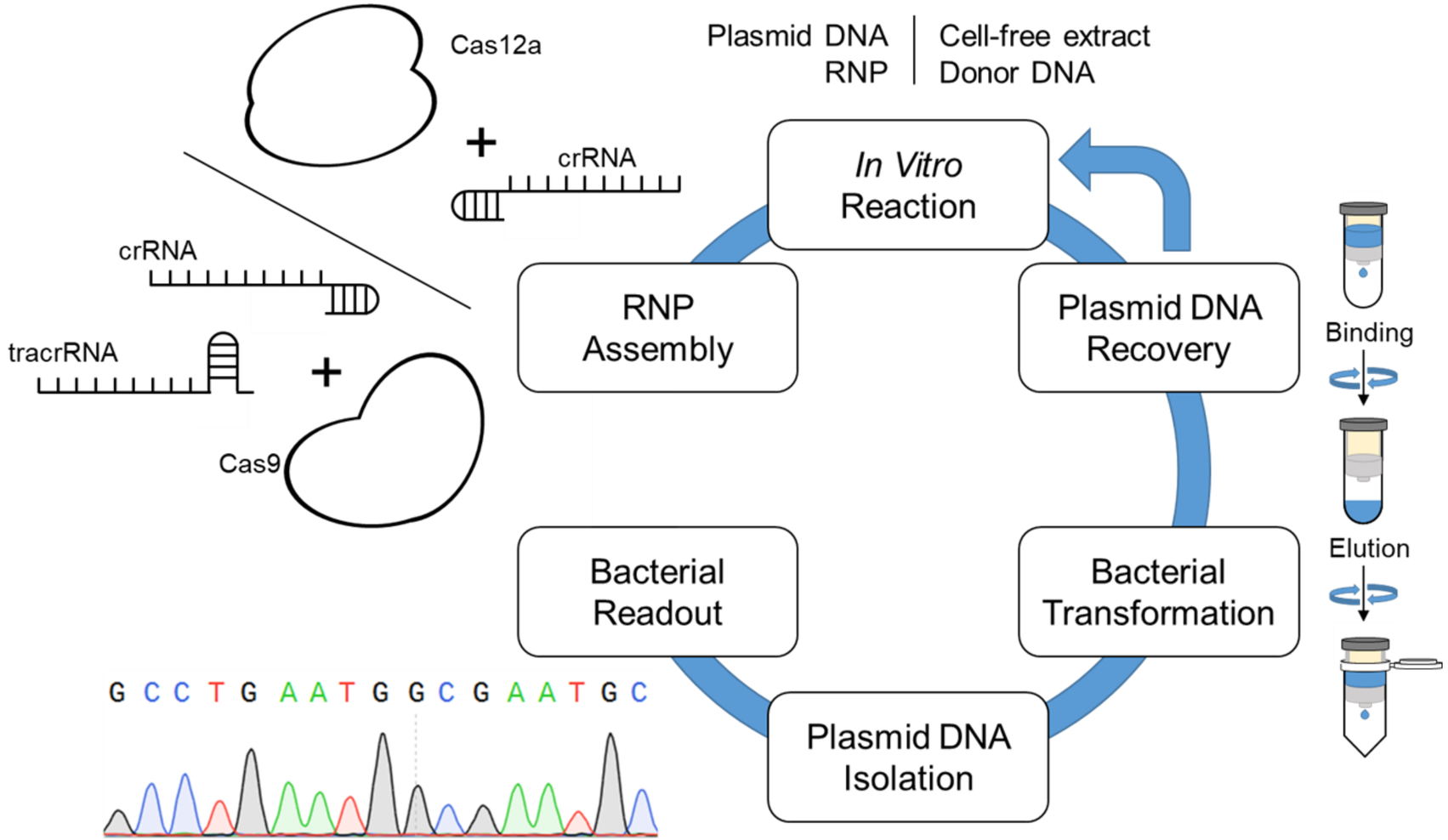
In Vitro Gene Editing



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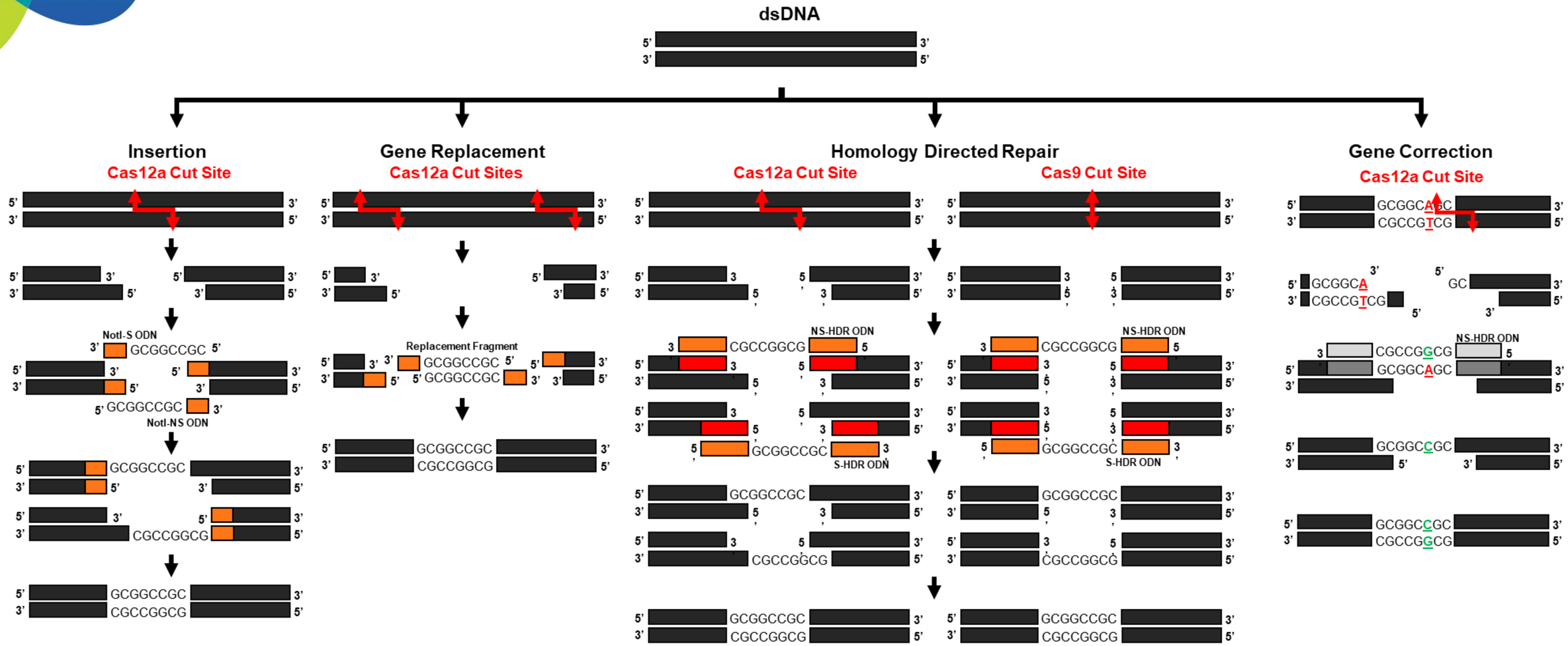
In Vitro Gene Editing



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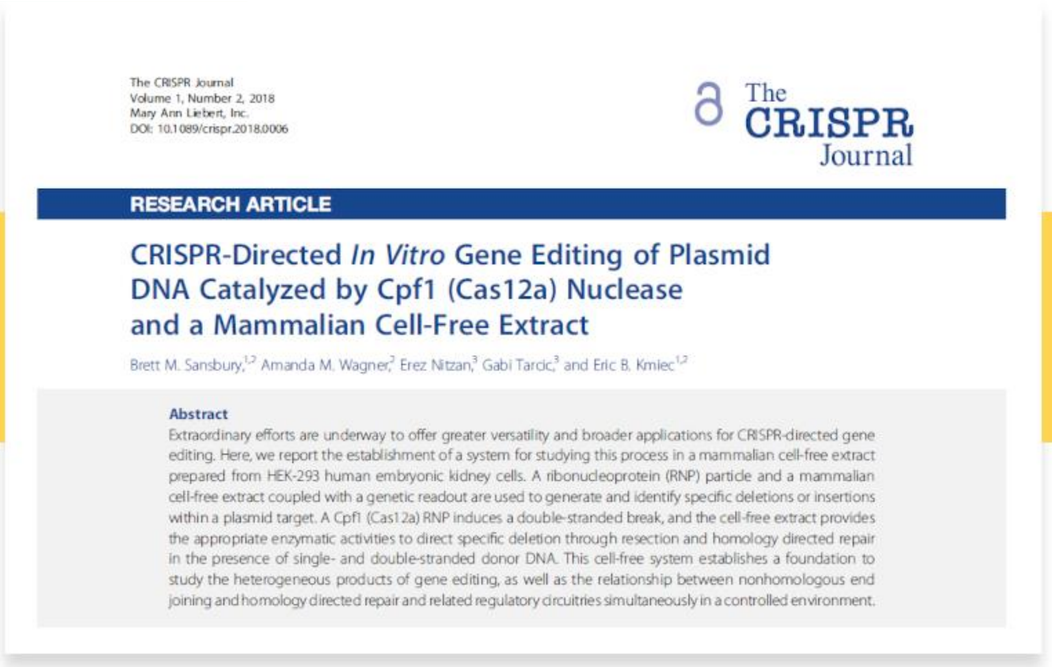


The CRISPR Journal
Volume 1, Number 2, 2018
Mary Ann Liebert, Inc.
DOI: 10.1089/crispr.2018.0006

The CRISPR Journal

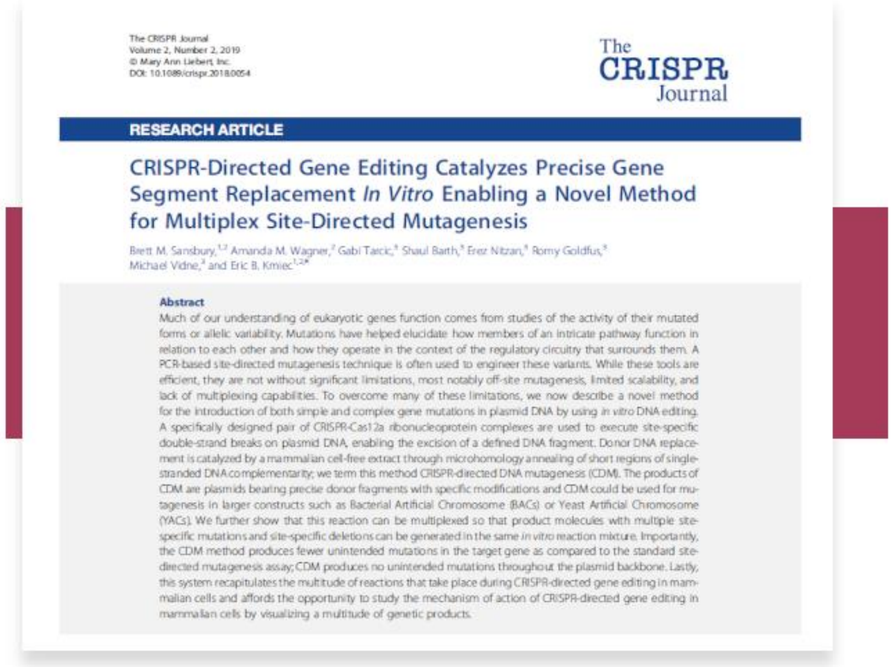
RESEARCH ARTICLE

CRISPR-Directed *In Vitro* Gene Editing of Plasmid DNA Catalyzed by Cpf1 (Cas12a) Nuclease and a Mammalian Cell-Free Extract

Brett M. Sansbury,[1,2] Amanda M. Wagner,[2] Erez Nitzan,[3] Gabi Tarcic,[3] and Eric B. Kmiec[1,2]

Abstract

Extraordinary efforts are underway to offer greater versatility and broader applications for CRISPR-directed gene editing. Here, we report the establishment of a system for studying this process in a mammalian cell-free extract prepared from HEK-293 human embryonic kidney cells. A ribonucleoprotein (RNP) particle and a mammalian cell-free extract coupled with a genetic readout are used to generate and identify specific deletions or insertions within a plasmid target. A Cpf1 (Cas12a) RNP induces a double-stranded break, and the cell-free extract provides the appropriate enzymatic activities to direct specific deletion through resection and homology directed repair in the presence of single- and double-stranded donor DNA. This cell-free system establishes a foundation to study the heterogeneous products of gene editing, as well as the relationship between nonhomologous end joining and homology directed repair and related regulatory circuitries simultaneously in a controlled environment.



The CRISPR Journal
Volume 2, Number 2, 2019
© Mary Ann Liebert, Inc.
DOI: 10.1089/crispr.2018.0054

The CRISPR Journal

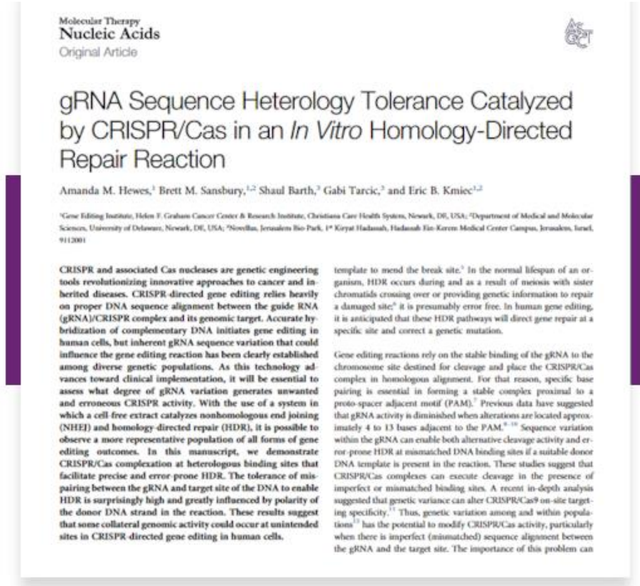
RESEARCH ARTICLE

CRISPR-Directed Gene Editing Catalyzes Precise Gene Segment Replacement *In Vitro* Enabling a Novel Method for Multiplex Site-Directed Mutagenesis

Brett M. Sansbury,[1,2] Amanda M. Wagner,[2] Gabi Tarcic,[3] Shaul Barth,[3] Erez Nitzan,[3] Romy Goldfus,[3] Michael Vidne,[3] and Eric B. Kmiec[1,2,*]

Abstract

Much of our understanding of eukaryotic genes function comes from studies of the activity of their mutated forms or allelic variability. Mutations have helped elucidate how members of an intricate pathway function in relation to each other and how they operate in the context of the regulatory circuitry that surrounds them. A PCR-based site-directed mutagenesis technique is often used to engineer these variants. While these tools are efficient, they are not without significant limitations, most notably off-site mutagenesis, limited scalability, and lack of multiplexing capabilities. To overcome many of these limitations, we now describe a novel method for the introduction of both simple and complex gene mutations in plasmid DNA by using *in vitro* DNA editing. A specifically designed pair of CRISPR-Cas12a ribonucleoprotein complexes are used to execute site-specific double-strand breaks on plasmid DNA, enabling the excision of a defined DNA fragment. Donor DNA replacement is catalyzed by a mammalian cell-free extract through microhomology annealing of short regions of single-stranded DNA complementarity; we term this method CRISPR-directed DNA mutagenesis (CDM). The products of CDM are plasmids bearing precise donor fragments with specific modifications and CDM could be used for mutagenesis in larger constructs such as Bacterial Artificial Chromosome (BACs) or Yeast Artificial Chromosome (YACs). We further show that this reaction can be multiplexed so that product molecules with multiple site-specific mutations and site-specific deletions can be generated in the same *in vitro* reaction mixture. Importantly, the CDM method produces fewer unintended mutations in the target gene as compared to the standard site-directed mutagenesis assay; CDM produces no unintended mutations throughout the plasmid backbone. Lastly, this system recapitulates the multitude of reactions that take place during CRISPR-directed gene editing in mammalian cells and affords the opportunity to study the mechanism of action of CRISPR-directed gene editing in mammalian cells by visualizing a multitude of genetic products.



Molecular Therapy
Nucleic Acids
Original Article

gRNA Sequence Heterology Tolerance Catalyzed by CRISPR/Cas in an *In Vitro* Homology-Directed Repair Reaction

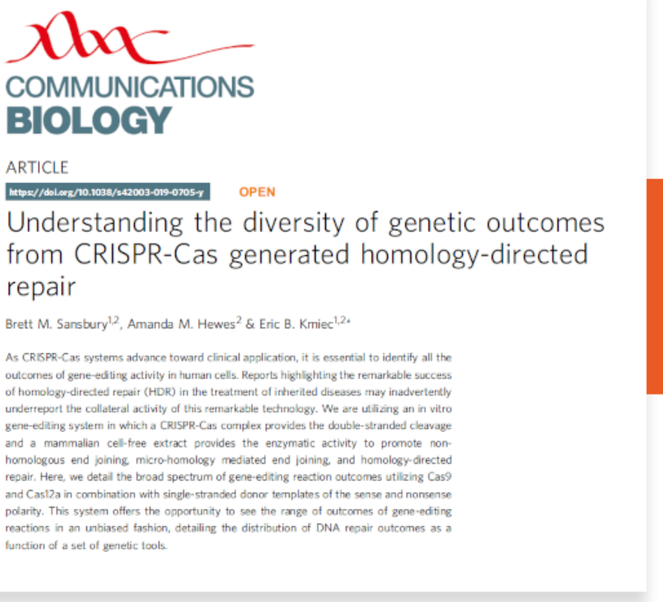
Amanda M. Hewes,[1] Brett M. Sansbury,[1,2] Shaul Barth,[3] Gabi Tarcic,[3] and Eric B. Kmiec[1,2]

[1]Gene Editing Institute, Helen F. Graham Cancer Center & Research Institute, Christiana Care Health System, Newark, DE, USA; [2]Department of Medical and Molecular Sciences, University of Delaware, Newark, DE, USA; [3]Novellus, Jerusalem Bio Park, 1st Kiryat Hadassah, Hadassah Ein-Kerem Medical Center Campus, Jerusalem, Israel, 9112001

CRISPR and associated Cas nucleases are genetic engineering tools revolutionizing innovative approaches to cancer and inherited diseases. CRISPR-directed gene editing relies heavily on proper DNA sequence alignment between the guide RNA (gRNA)/CRISPR complex and its genomic target. Accurate hybridization of complementary DNA initiates gene editing in human cells, but inherent gRNA sequence variation that could influence the gene editing reaction has been clearly established among diverse genetic populations. As this technology advances toward clinical implementation, it will be essential to assess what degree of gRNA variation generates unwanted and erroneous CRISPR activity. With the use of a system in which a cell free extract catalyzes nonhomologous end joining (NHEJ) and homology-directed repair (HDR), it is possible to observe a more representative population of all forms of gene editing outcomes. In this manuscript, we demonstrate CRISPR/Cas complexation at heterologous binding sites that facilitate precise and error prone HDR. The tolerance of mispairing between the gRNA and target site of the DNA to enable HDR is surprisingly high and greatly influenced by polarity of the donor DNA strand in the reaction. These results suggest that some collateral genomic activity could occur at unintended sites in CRISPR-directed gene editing in human cells.

template to mend the break site.[7] In the normal lifespan of an organism, HDR occurs during and as a result of meiosis with sister chromatids crossing over or providing genetic information to repair a damaged site;[8] it is presumably error free. In human gene editing, it is anticipated that these HDR pathways will direct gene repair at a specific site and correct a genetic mutation.

Gene editing reactions rely on the stable binding of the gRNA to the chromosome site destined for cleavage and place the CRISPR/Cas complex in homologous alignment. For that reason, specific base pairing is essential in forming a stable complex proximal to a proto-spacer adjacent motif (PAM).[9] Previous data have suggested that gRNA activity is diminished when alterations are located approximately 4 to 13 bases adjacent to the PAM.[9–15] Sequence variation within the gRNA can enable both alternative cleavage activity and error-prone HDR at mismatched DNA binding sites if a suitable donor DNA template is present in the reaction. These studies suggest that CRISPR/Cas complexes can execute cleavage in the presence of imperfect or mismatched binding sites. A recent in-depth analysis suggested that genetic variance can alter CRISPR/Cas9 on-site targeting specificity.[15] Thus, genetic variation among and within populations[17] has the potential to modify CRISPR/Cas activity, particularly when there is imperfect (mismatched) sequence alignment between the gRNA and the target site. The importance of this problem can



COMMUNICATIONS BIOLOGY

ARTICLE

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Understanding the diversity of genetic outcomes from CRISPR-Cas generated homology-directed repair

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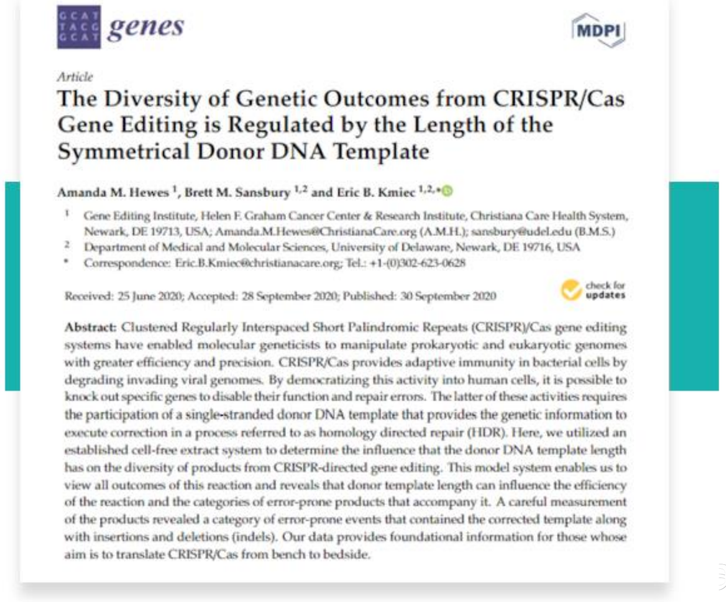
As CRISPR-Cas systems advance toward clinical application, it is essential to identify all the outcomes of gene-editing activity in human cells. Reports highlighting the remarkable success of homology-directed repair (HDR) in the treatment of inherited diseases may inadvertently underreport the collateral activity of this remarkable technology. We are utilizing an in vitro gene-editing system in which a CRISPR-Cas complex provides the double-stranded cleavage and a mammalian cell-free extract provides the enzymatic activity to promote non-homologous end joining, micro-homology mediated end joining, and homology-directed repair. Here, we detail the broad spectrum of gene-editing reaction outcomes utilizing Cas9 and Cas12a in combination with single-stranded donor templates of the sense and nonsense polarity. This system offers the opportunity to see the range of outcomes of gene-editing reactions in an unbiased fashion, detailing the distribution of DNA repair outcomes as a function of a set of genetic tools.



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The Diversity of Genetic Outcomes from CRISPR/Cas Gene Editing is Regulated by the Length of the Symmetrical Donor DNA Template

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Abstract: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas gene editing systems have enabled molecular geneticists to manipulate prokaryotic and eukaryotic genomes with greater efficiency and precision. CRISPR/Cas provides adaptive immunity in bacterial cells by degrading invading viral genomes. By democratizing this activity into human cells, it is possible to knock out specific genes to disable their function and repair errors. The latter of these activities requires the participation of a single-stranded donor DNA template that provides the genetic information to execute correction in a process referred to as homology directed repair (HDR). Here, we utilized an established cell-free extract system to determine the influence that the donor DNA template length has on the diversity of products from CRISPR-directed gene editing. This model system enables us to view all outcomes of this reaction and reveals that donor template length can influence the efficiency of the reaction and the categories of error-prone products that accompany it. A careful measurement of the products revealed a category of error-prone events that contained the corrected template along with insertions and deletions (indels). Our data provides foundational information for those whose aim is to translate CRISPR/Cas from bench to bedside.

Application of *In Vitro* Gene Editing System

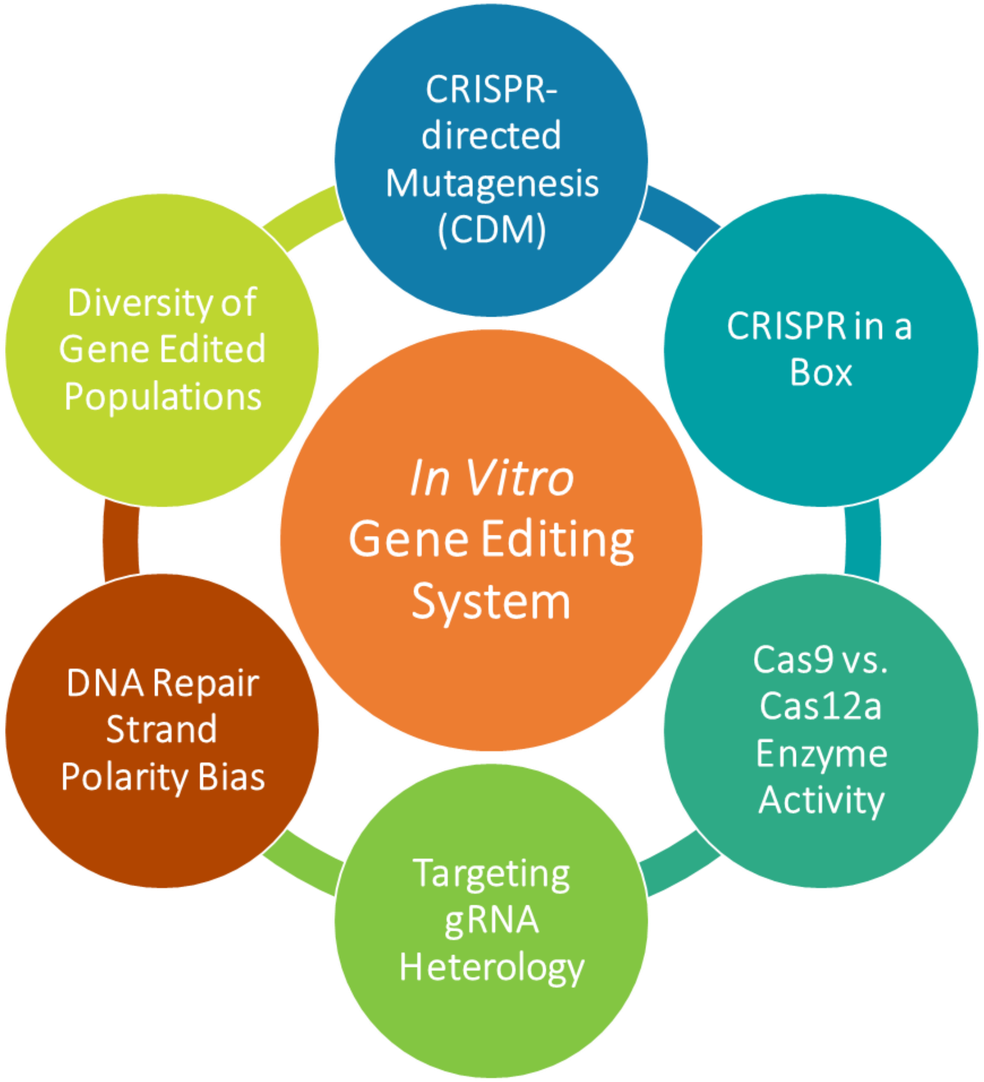

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Table 1. Comparison of ZFN, TALEN and CRISPR/Cas9 platforms.

	ZFN	TALEN	CRISPR/Cas9
Recognition site	Zinc-finger protein	RVD tandem repeat region of TALE protein	Single-strand guide RNA
Modification pattern	Fok1 nuclease	Fok1 nuclease	Cas9 nuclease
Target sequence size	Typically 9–18 bp per ZFN monomer, 18–36 bp per ZFN pair	Typically 14–20 bp per TALEN monomer, 28–40 bp per TALEN pair	Typically 20 bp guide sequence + PAM sequence
Specificity	Tolerating a small number of positional mismatches	Tolerating a small number of positional mismatches	Tolerating positional/multiple consecutive mismatches
Targeting limitations	Difficult to target non-G-rich sites	5′ targeted base must be a T for each TALEN monomer	Targeted site must precede a PAM sequence
Difficulties of engineering	Requiring substantial protein engineering	Requiring complex molecular cloning methods	Using standard cloning procedures and oligo synthesis
Difficulties of delivering	Relatively easy as the small size of ZFN expression elements is suitable for a variety of viral vectors	Difficult due to the large size of functional components	Moderate as the commonly used SpCas9 is large and may cause packaging problems for viral vectors such as AAV, but smaller orthologs exist

ZFN Zinc-finger nuclease, *TALEN* Transcription activator-like effector nuclease, *CRISPR* Clustered regularly interspaced short palindromic repeat