genome editing

A QUICK TECHNICAL WALK THROUGH

PAWAN K.DHAR
School of Biotechnology
Jawaharlal Nehru University
New Delhi

https://upload.wikimedia.org/wikipedia/commons/8/8b/Ácido_desoxirribonucleico_%28DNA%29.png

Restriction Enzyme

BLUNT END RESTRICTION SITE

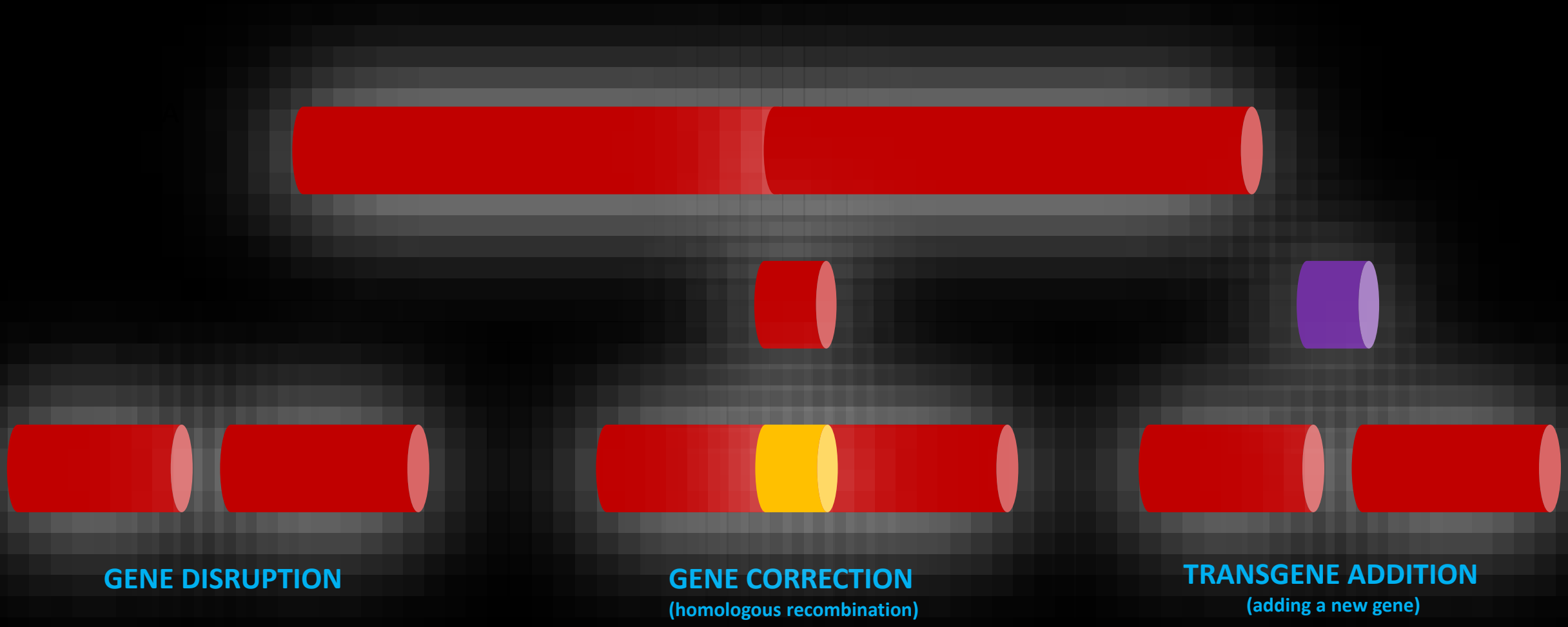
Cutting the DNA

STICKY END RESTRICTION SITE

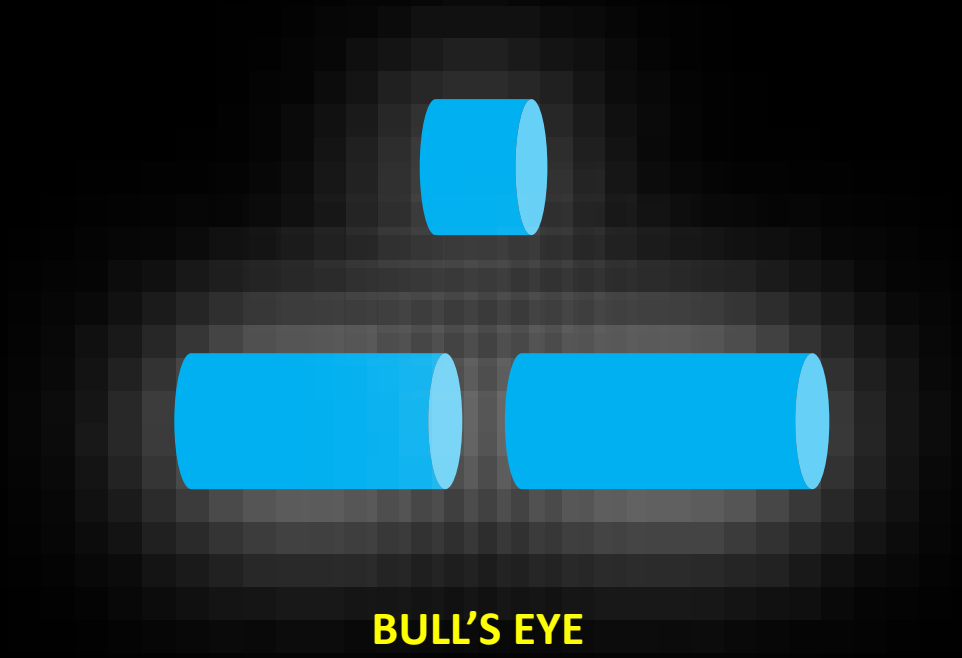
GAATTC
CTTAAG

Cutting the DNA

slicing DNA leads to several possibilities

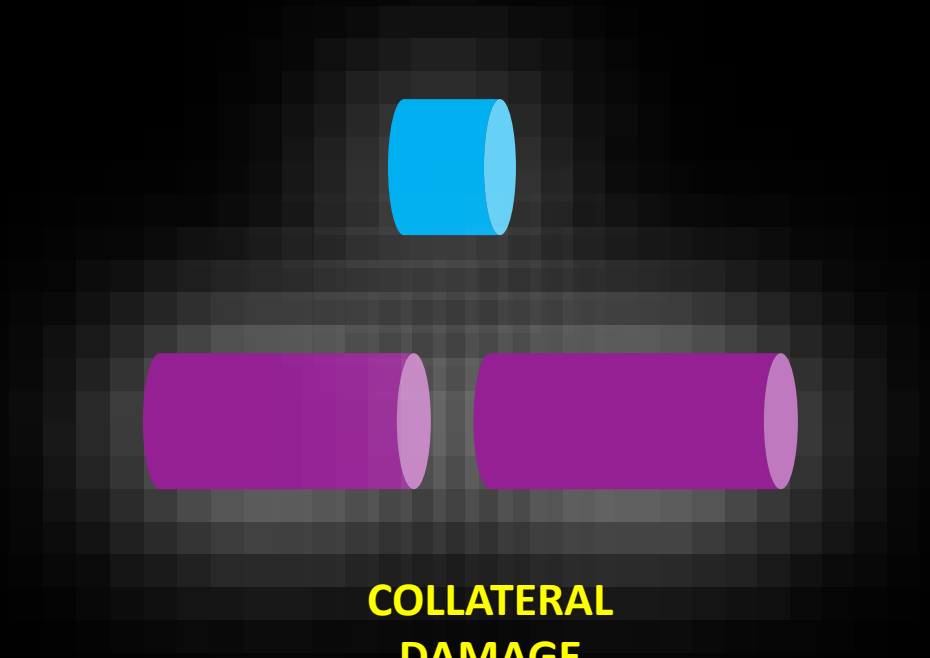


Challenge ONE : To achieve targeted integration



BULL'S EYE

(LOW OCCURENCE)



COLLATERAL DAMAGE

(MORE FREQUENT)

CHALLENGE ONE : Targeted integration

Frequency of cutting the DNA is 4^n

EcoRI: 6 base recognition sequence $4^6 = 4096$

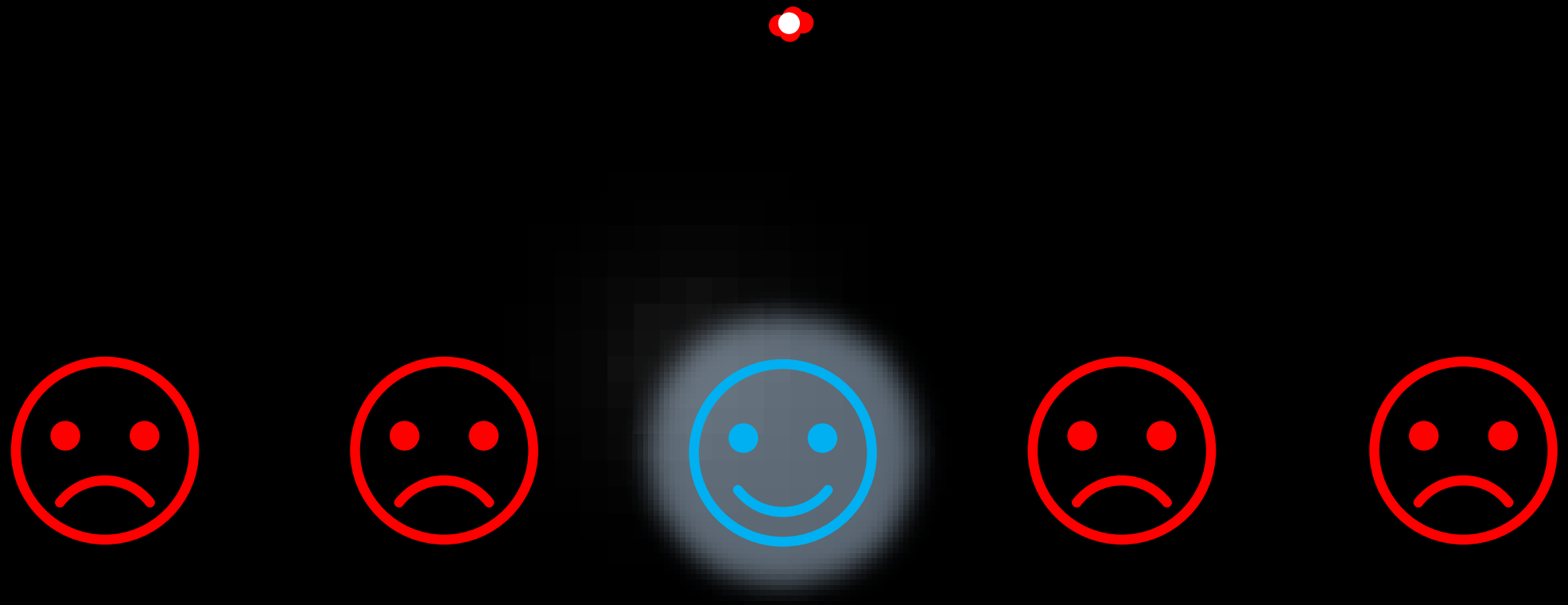
E. Coli genome length ~ 4.6 million bp

assuming uniform distribution of restriction sites

4, 600, 000 / 4096

1123

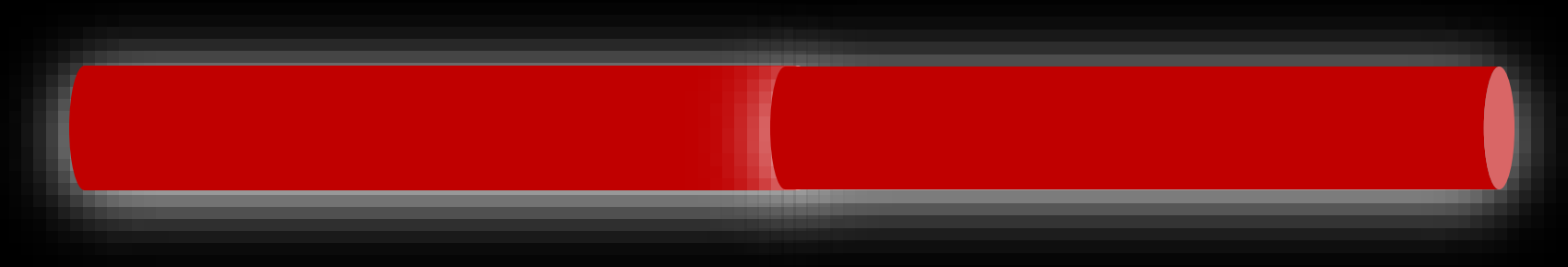
Challenge TWO : To make cellular uptake more efficient

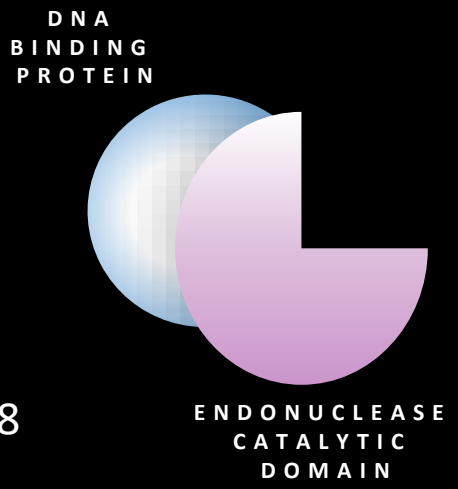


TOOLS FOR GENOME SURGERY

ZINC FINGER NUCLEASES
ZFNs

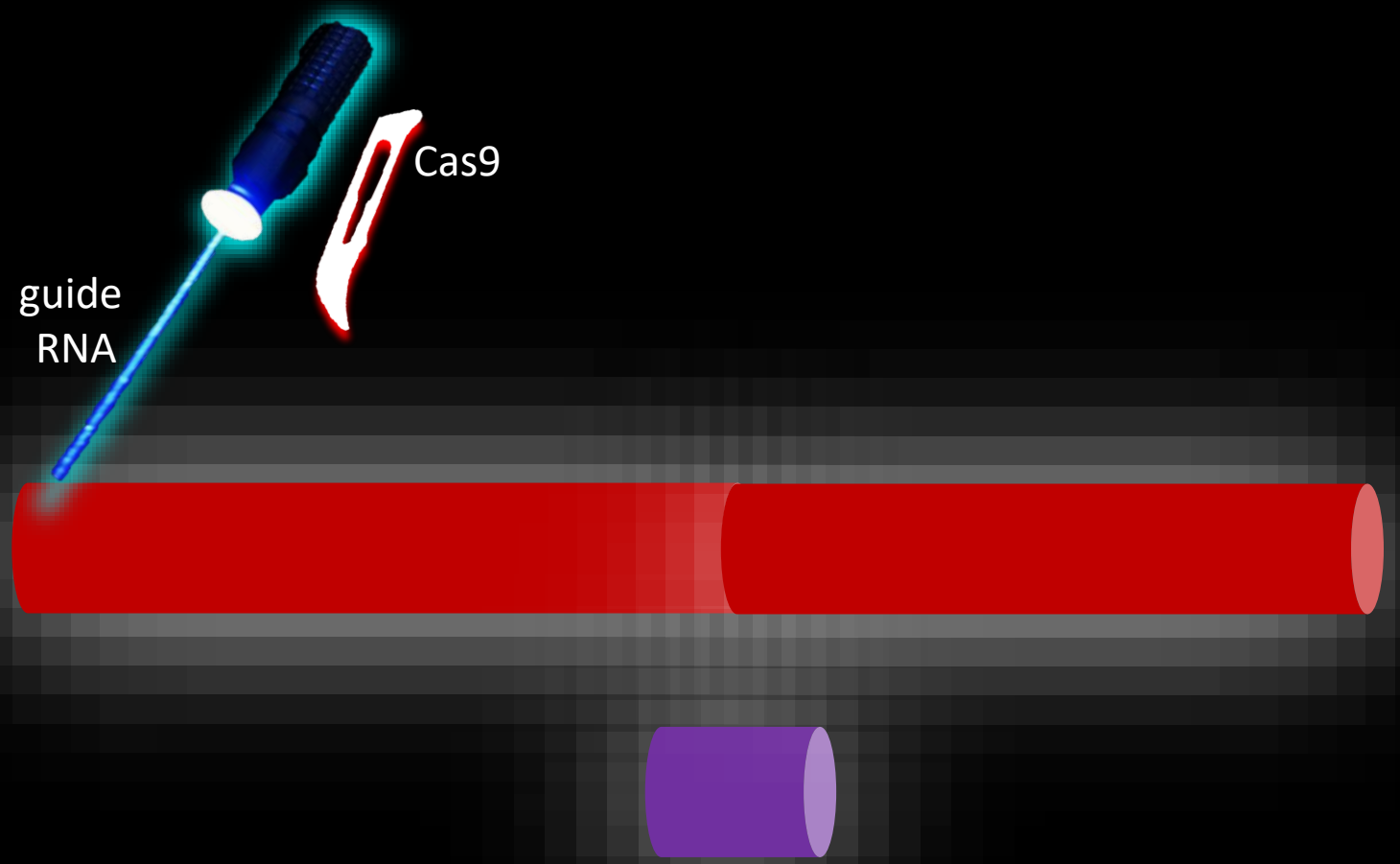
TRANSCRIPTION ACTIVATOR
LIKE EFFECTOR NUCLEASES
TALENs





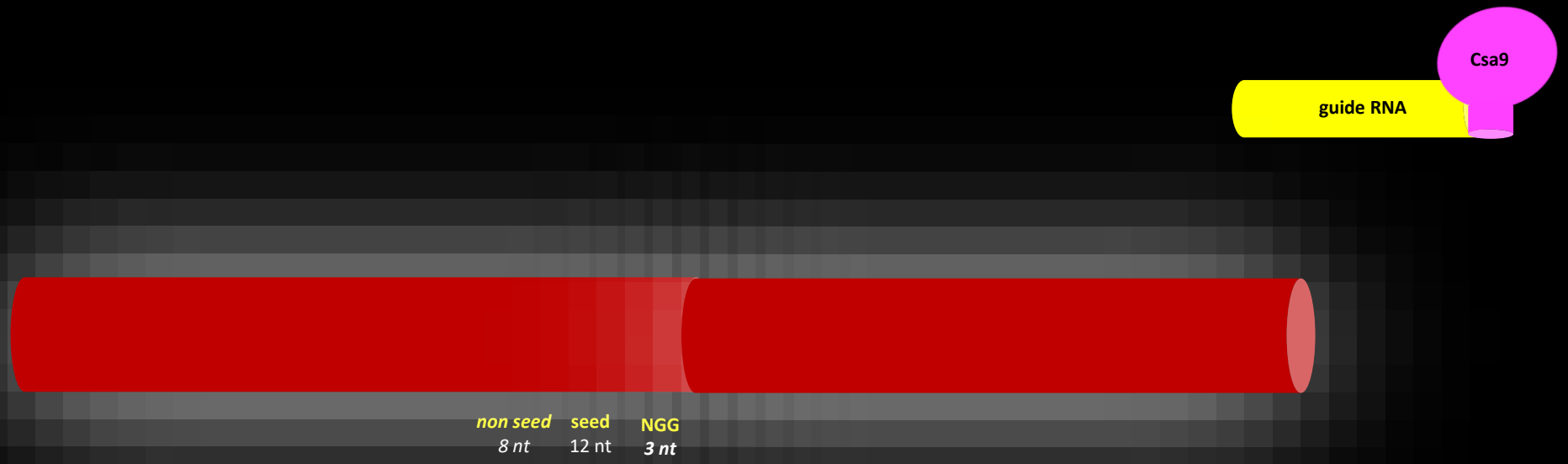
Latest tool in genome editing

CRISPR CAS9



detailed description

CRISPR CAS9

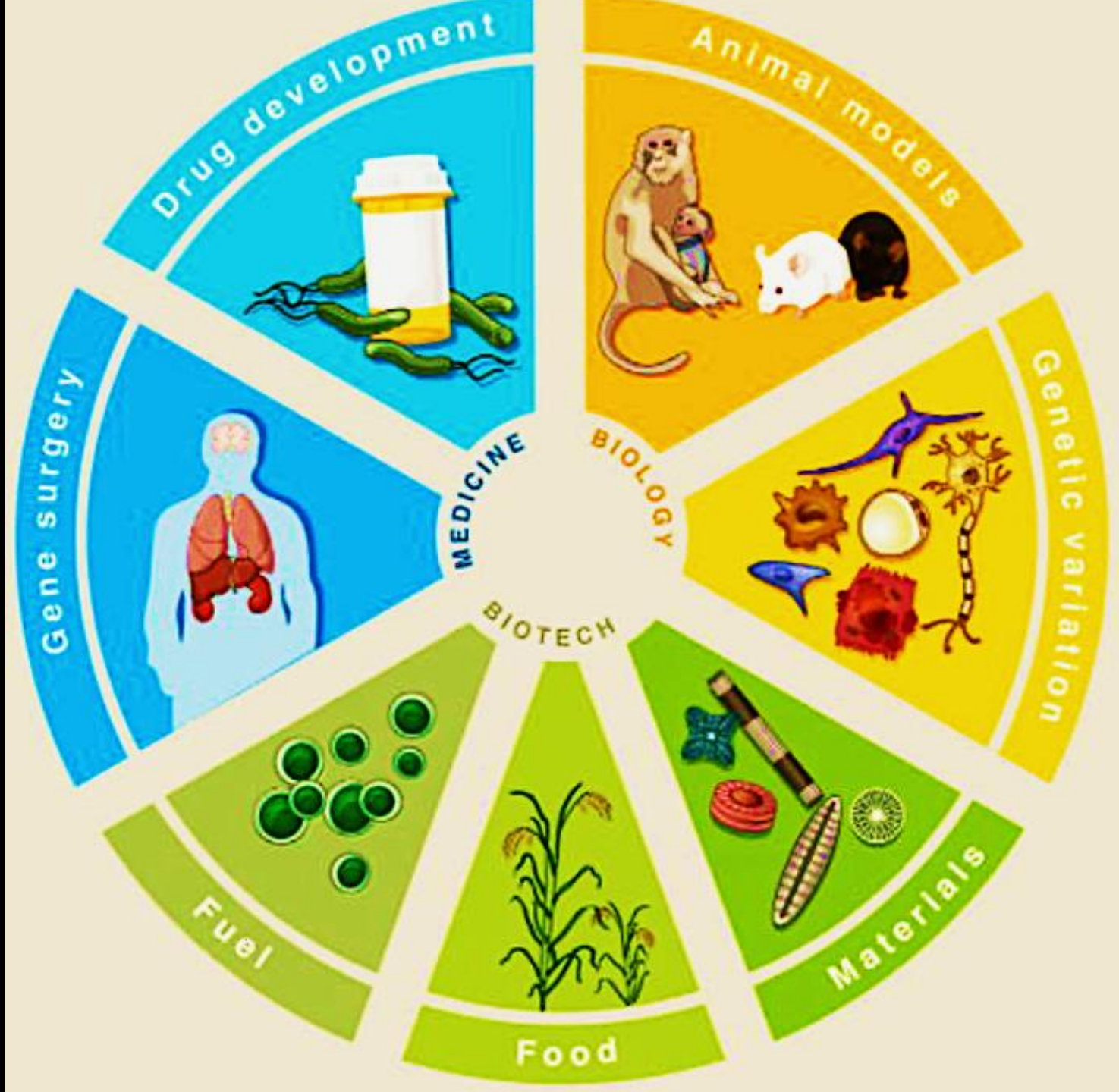


Protospacer
20 nt

PAM
Protospacer
Adjacent
Motif

Applications of Genome Engineering

Hsu et al 2014. Cell 157, 1262-78



July 2020

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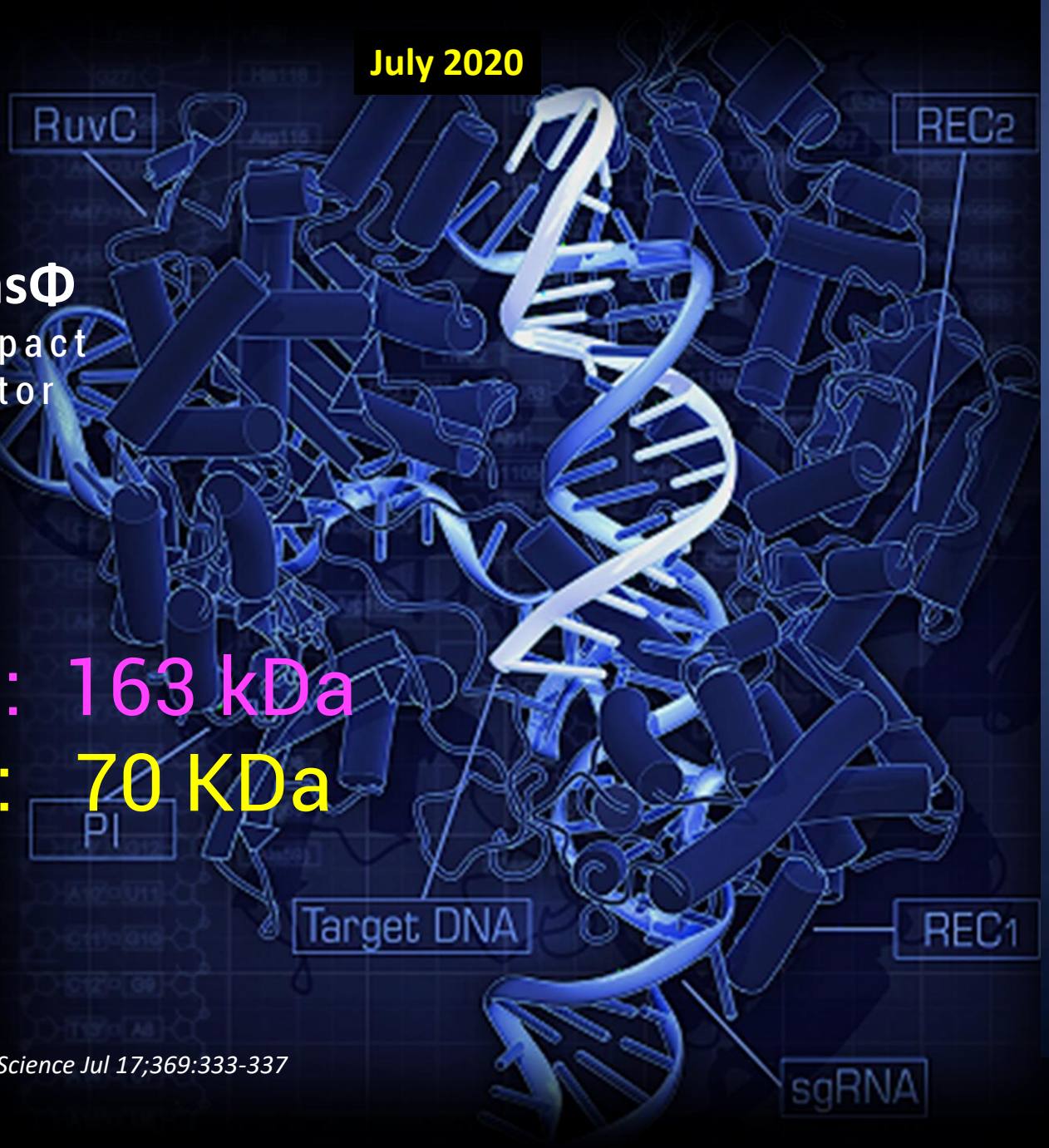
CRISPR-CasΦ

a hypercompact genome editor



Cas9 : 163 kDa

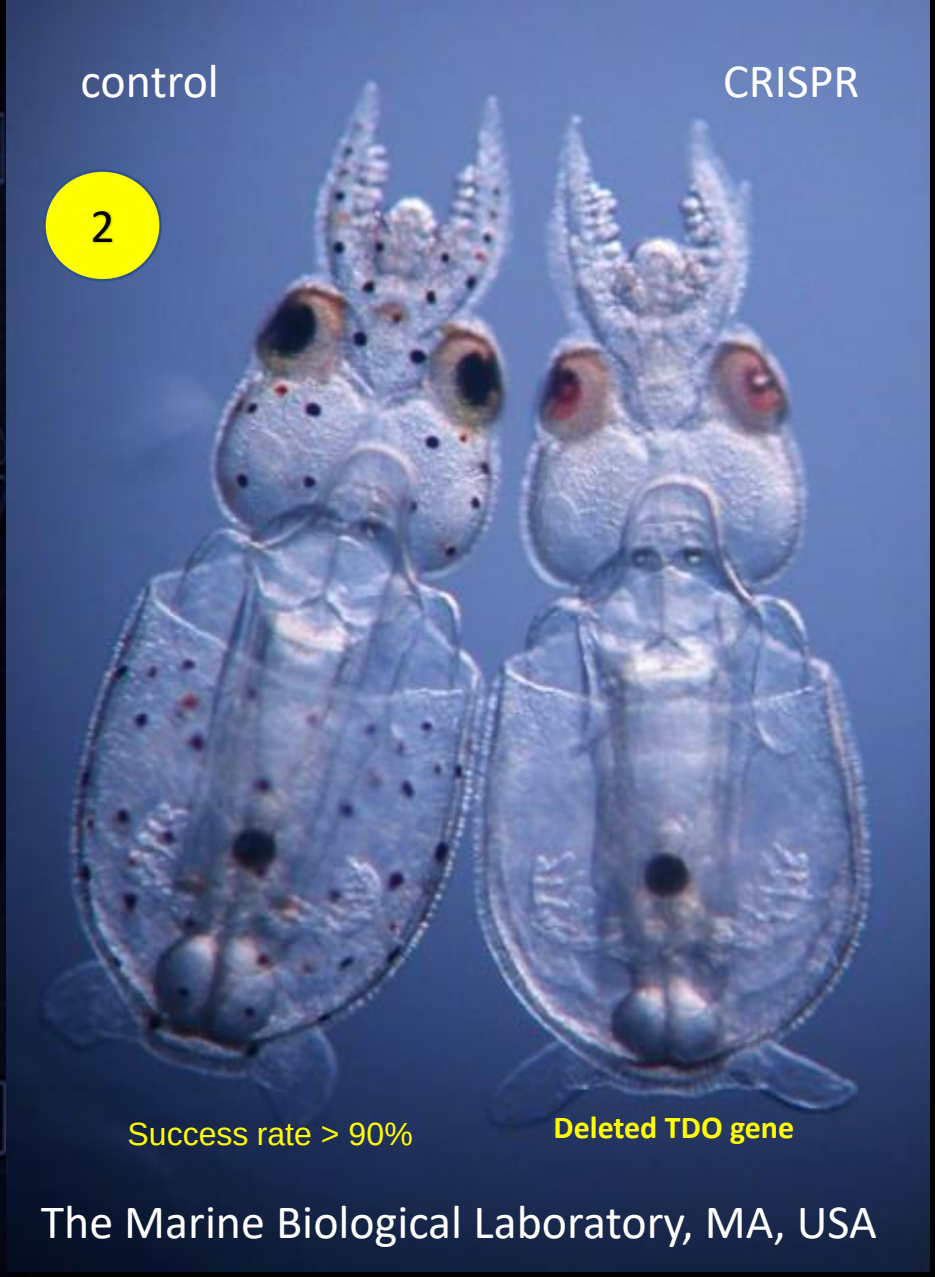
CasΦ : 70 KDa

2020 Science Jul 17;369:333-337

control

CRISPR

2



Success rate > 90%

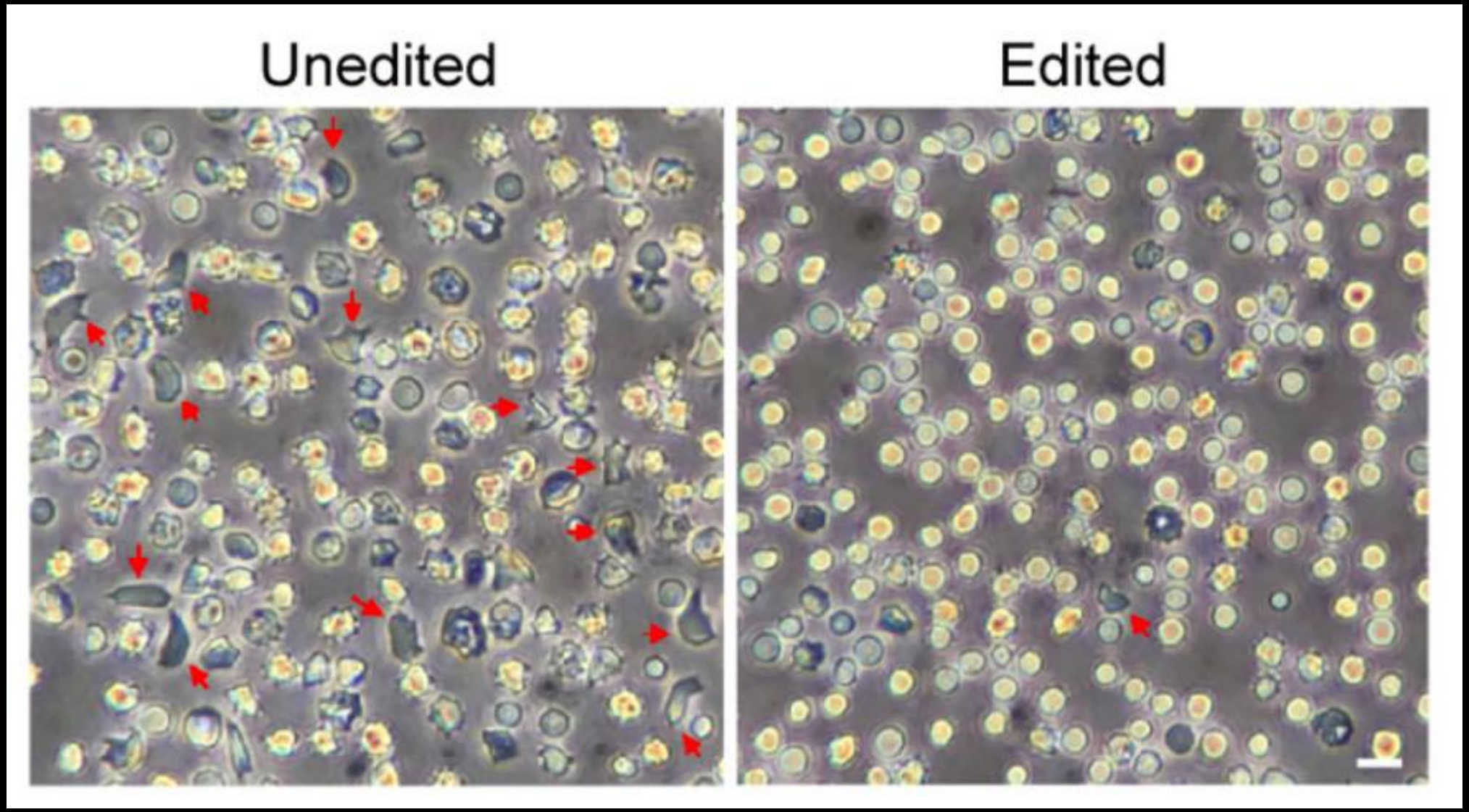
Deleted TDO gene

The Marine Biological Laboratory, MA, USA

Karen Crawford et al. Highly Efficient Knockout of a Squid Pigmentation Gene. Current Biology, ***July 30, 2020***

3

A CRISPR approach to treating sickle cell disease



Wu et al Nat Med 2019: 25, 776-83

4 Repurposing bacterial toxins for DNA surgery

CRISPR FREE GENOME EDITING

DddA : double-stranded DNA cytidine deaminase

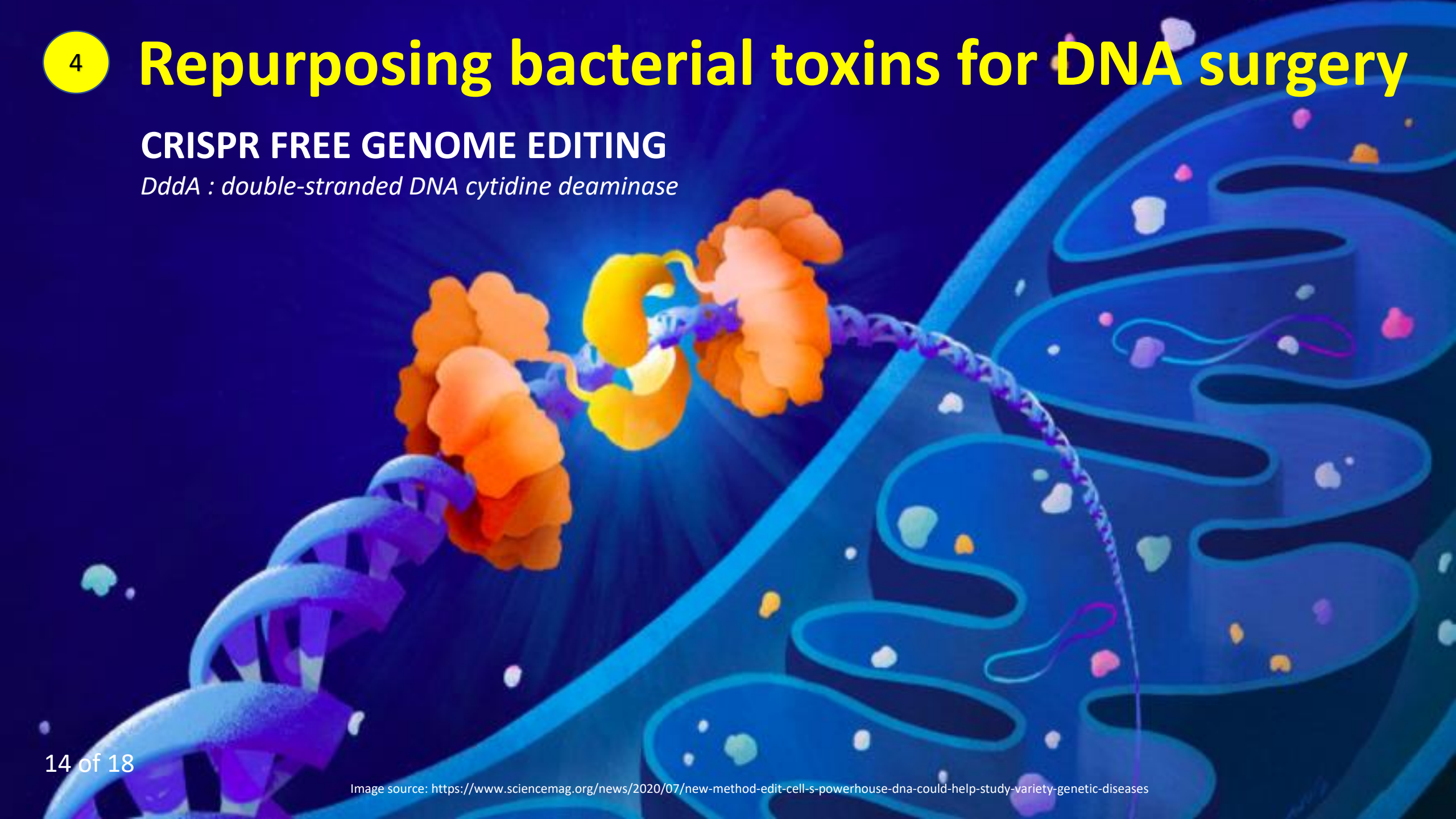


Image source: https://www.sciencemag.org/news/2020/07/new-method-edit-cell-s-powerhouse-dna-could-help-study-variety-genetic-diseases

5 Human genome editing

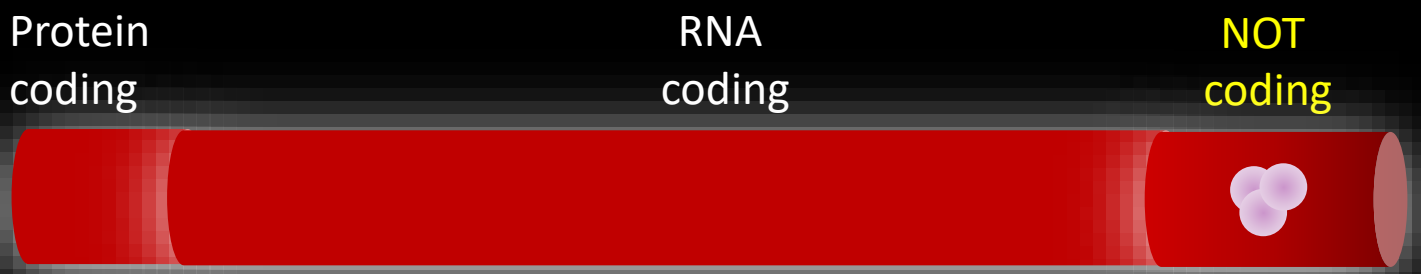
International Commission on the Clinical Use of Human Germline Genome Editing

Expert Advisory Committee developing Global Standards for Governance and Oversight of Human Genome Editing

Used CRISPR-Cas9 to reduce the transmission efficiency of malaria parasites in mosquitoes

Prof. Shailja Singh
Jawaharlal Nehru University

Singh et al. Proc Natl Acad Sci U S A. 2019: 116(35): 17498–17508



Dhar et al 2009
Joshi et al 2013
Raj et al 2015
Shidhi et al 2015
Joshi et al 2016
Varughese et al 2017

Making functional proteins from the non expressing sequences of the genome

antimalarial

Anti breast cancer
Anti Alzheimers
Anti bacterial
Anti fungal

tREP: tRNA encoded peptide

Anti leishmania
(patent filed, publication coming very soon)

FORESIGHT BIOTECH Pvt. Ltd.
Jawaharlal Nehru University, New Delhi

Identified novel Cas like molecules from **Intergenic sequences** of model organisms

patent : under process

Anti Cas proteins

Thank you

dharlab.net