

Clustered Regularly Interspaced Short Palindromic Repeats

CRISPR Technology: From Prokaryotic Origins to Eukaryotic Applications

AN OVERVIEW OF CRISPR'S JOURNEY IN GENETIC ENGINEERING

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Scientists Find Form of Crispr Gene Editing With New Capabilities

A common bacterium contains molecules that target RNA, not DNA. If it can be harnessed for use in humans, the process may lead to new forms of bioengineering.

Gene Editing Spurs Hope for Transplanting Pig Organs Into Humans

Geneticists have created piglets free of retroviruses, an important step toward creating a new supply of organs for transplant patients.

Scientists Can Design 'Better' Babies. Should They?

Advances in reproductive technology have put genetic choices within reach of prospective parents. But critics warn of ethical peril.

CRISPR in the News: The New York Times

As D.I.Y. Gene Editing Gains Popularity, 'Someone Is Going to Get Hurt'

After a virus was created from mail-order DNA, scientists are sounding the alarm about the genetic tinkering carried out in garages and living rooms.

1.

SCIENCE

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June 3, 2016

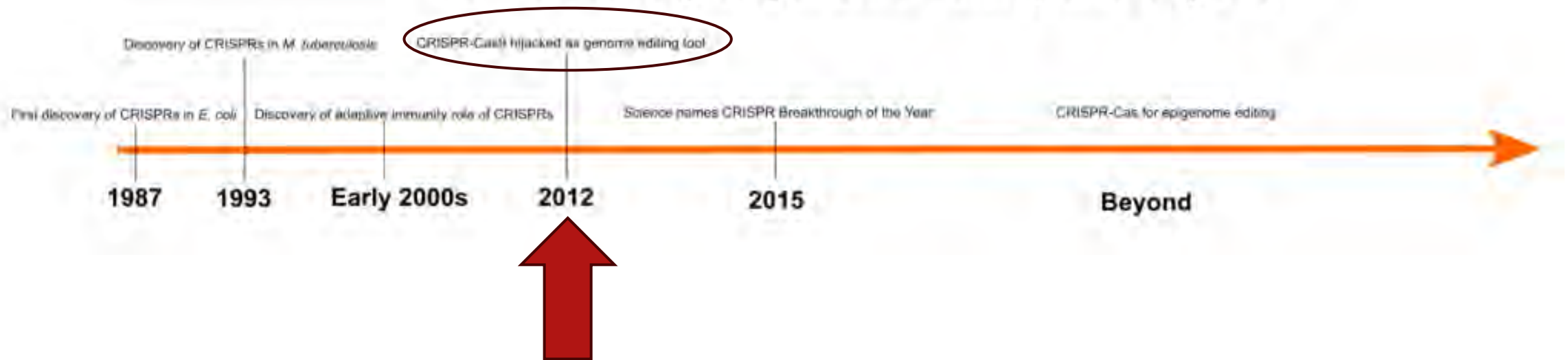
U.S.

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June 10

The CRISPR timeline: from discovery to genome editing and beyond



1987

• discovery of CRISPR clustered repeats in *E. coli*

Identification

1993

• discovery of CRISPR clustered repeats in *M. tuberculosis*

Structural and functional characterization

2000

• discovered, that CRISPR families are widespread in prokaryotes

2002

• identification of *cas* genes
• coined the CRISPR acronym

2005

• foreign origin of spacers revealed

2007

• evidence for bacterial CRISPR adaptive immunity

2008

• CRISPR system type III-A targets DNA

2009

• classification of CRISPR systems into three types

2011

• tracrRNA and crRNA form a duplex structure in association with Cas9

2010

• Cas9 cleaves target DNA within a protospacer

2012

• *in vivo* characterization of DNA targeting by Cas9

2014

• crystal structure of Cas9

2013

• CRISPR-Cas9 gene editing achieved in mammalian cells

2017

• first CRISPR germline editing in implanted human embryos

• first CRISPR clinical trial for treatment against HIV-1

2018

• first CRISPR clinical trial for cancer immunotherapy

2020

• Nobel Prize for CRISPR-Cas9 genome editing

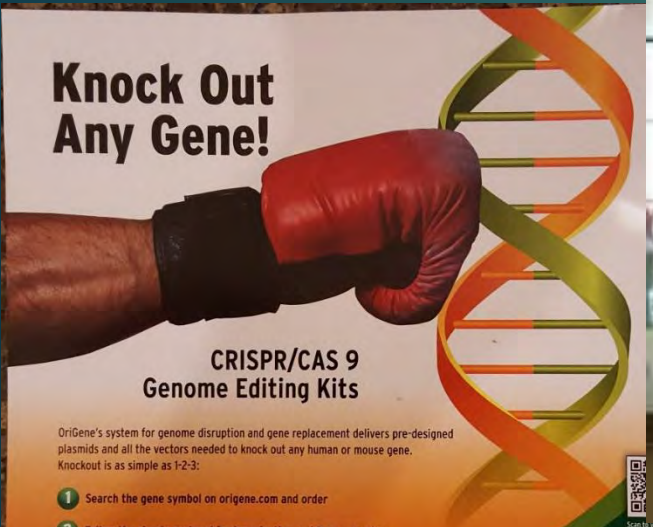
• first *in vivo* CRISPR clinical trial for treatment against blindness

2019

The CRISPR-CAS 9 System: Early Applications in Eukaryotes and a Patent Dispute



Knock Out Any Gene!



CRISPR/CAS 9 Genome Editing Kits

Origene's system for genome disruption and gene replacement delivers pre-designed plasmids and all the vectors needed to knock out any human or mouse gene. Knockout is as simple as 1-2-3:

- 1 Search the gene symbol on origene.com and order
- 2 Follow the simple protocol for transfection and Puro selection
- 3 Validate the knockout

Kit Components Include:

- 2 guide RNA vectors to ensure efficient cleavage
- Donor vector with pre-designed homologous arms
- Knockin GFP-Puro for selection
- Scramble gRNA as negative control included

Come to OriGene, the trusted molecular biology expert, for your CRISPR needs.

ORIGENE

www.origene.com/CRISPR-CAS9



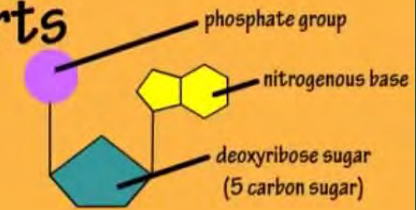
SO: HOW
DOES
CRISPR
WORK??

First, a primer on DNA

structure of nucleic acids: DNA & RNA

DNA - deoxyribonucleic acid

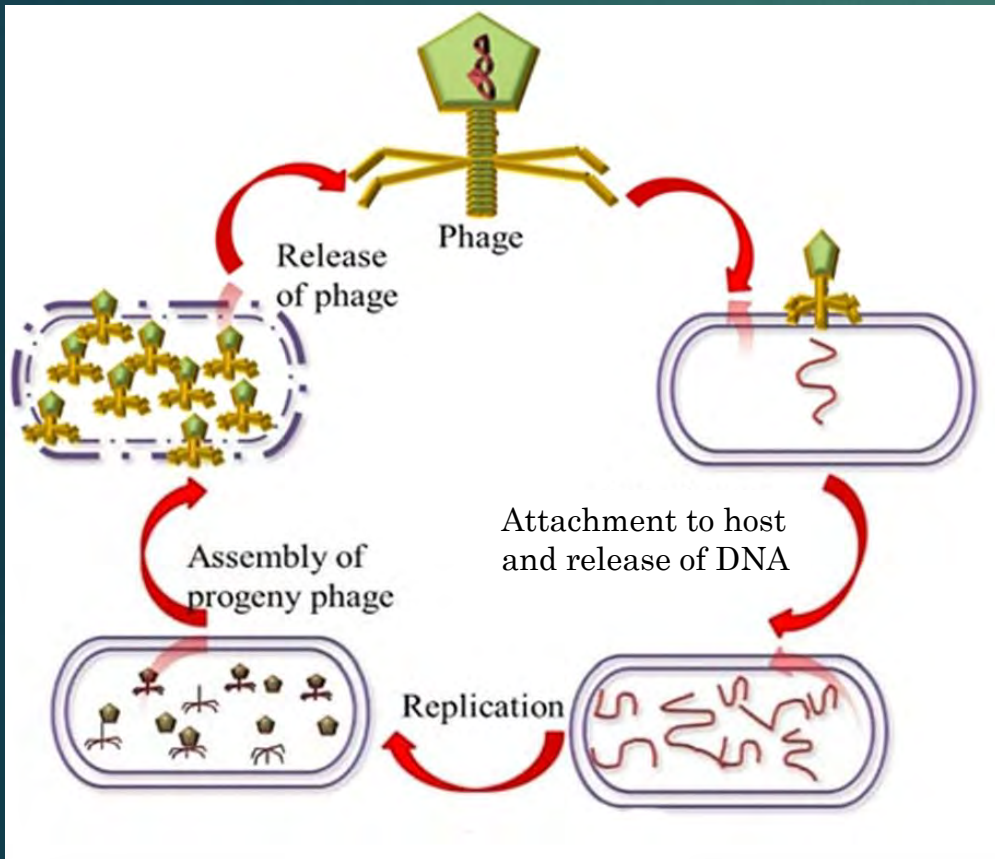
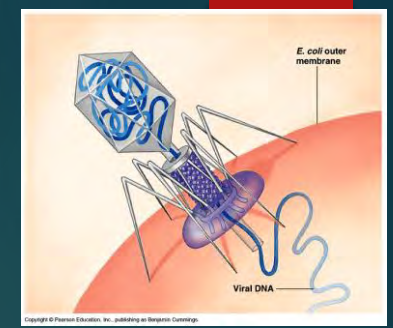
3 parts



What's the takeaway from this??

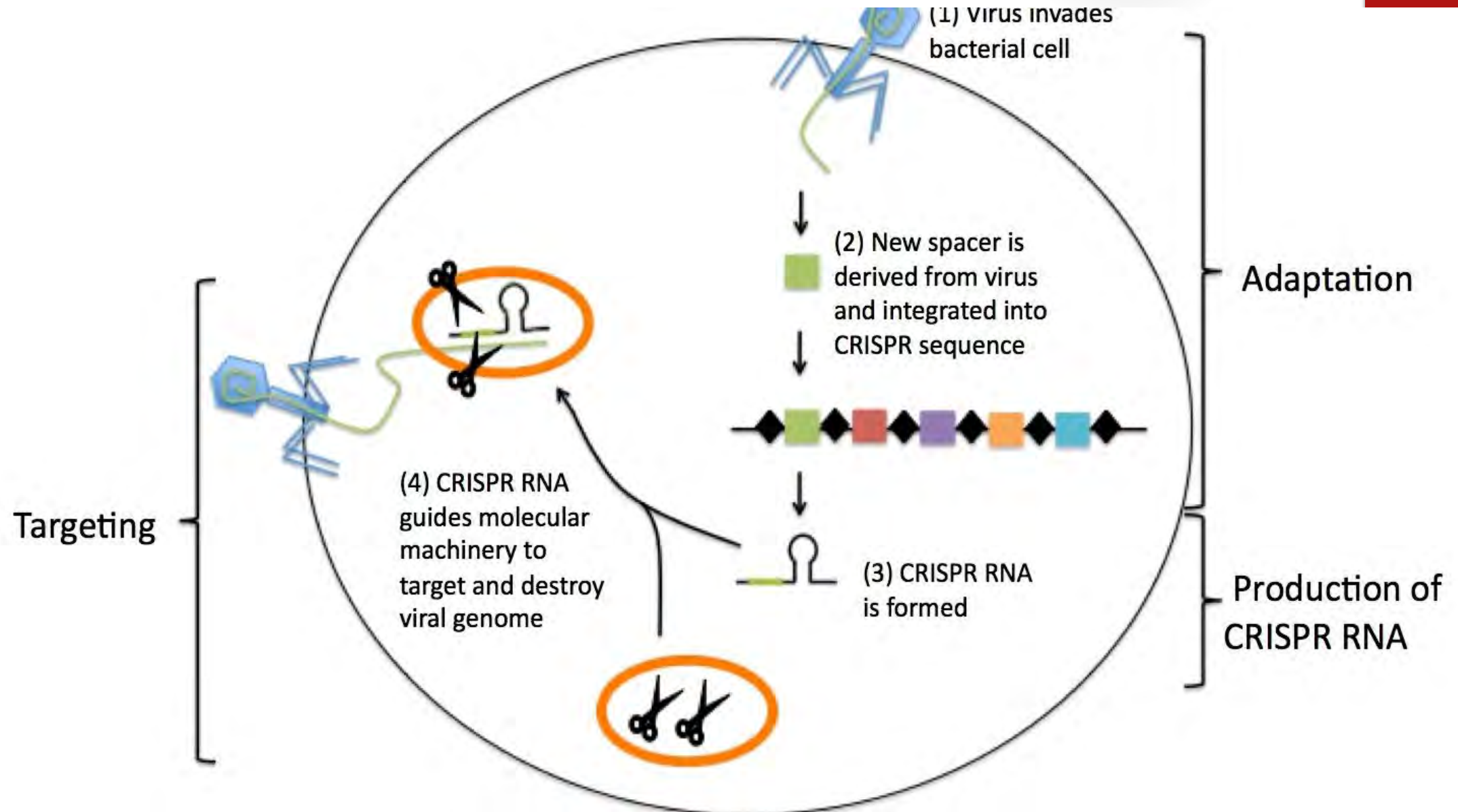
- ▶ **Base pairing in DNA is unique:**
 - ▶ Adenine *always* bonds to Thymine
 - ▶ Guanine *always* bonds to Cytosine
- ▶ **Genes are sequences of DNA.** The code in a gene lies in the sequence of base pairs.
- ▶ **So, if you know the sequence in a gene or even a part of the sequence, you can design a “probe” with complementary bases which will seek out that gene in the nucleus.**
 - ▶ This is the basis of PCR and other molecular techniques such as ancestry tracing.
 - ▶ *It is also the basis of CRISPR.*

CRISPR In Prokaryote Cells

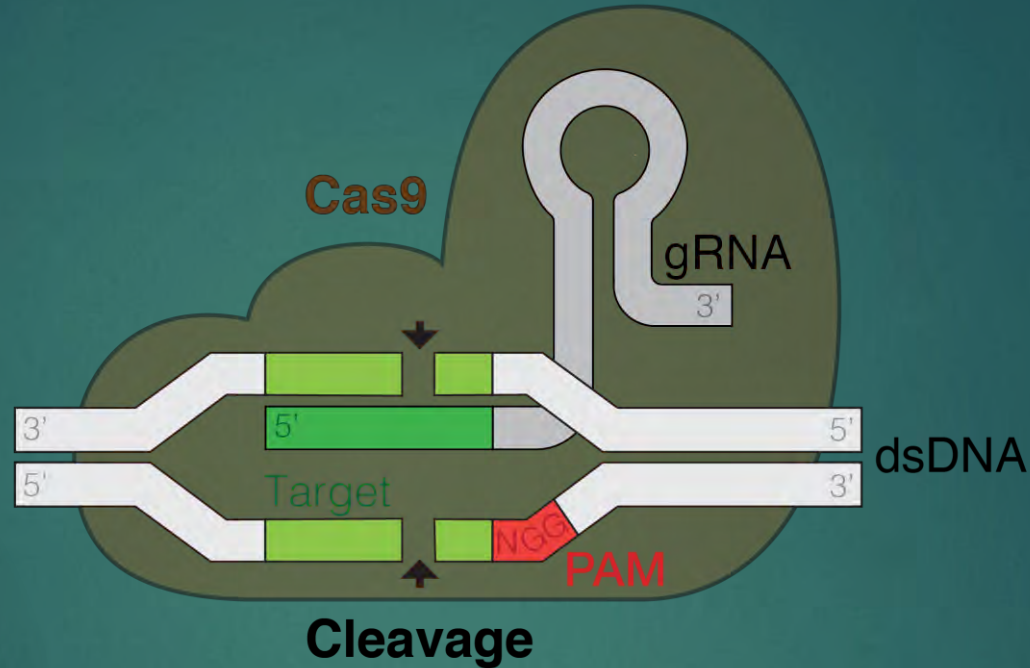


- ▶ Viruses act by inserting their nuclear (genetic) material (DNA or RNA) into the cell.
- ▶ The viral genetic material takes over the protein and nucleic acid synthetic machinery of the cell to make more viruses.
- ▶ Eventually the new viruses erupt from the cell, destroying it, and go on to infect other cells.
- ▶ Viruses that attack bacterial cells are called *bacteriophages*.
- ▶ **CRISPR is based on the bacterial cell's "immune" or defensive system to protect against viruses (or allospecific plasmids).**

How CRISPR works in prokaryotes



Role of the PAM*



- ▶ Cas9 will not cleave the protospacer sequence unless there is a PAM sequence adjacent to the site on the bacterial or viral DNA. The spacer in the bacteria's own CRISPR loci does not contain a PAM sequence and will thus not be cut by the nuclease. But the protospacer in the invading virus or plasmid does contain the PAM sequence and will thus be cleaved by the Cas9 nuclease.

*Protospacer Adjacent Motif

CRISPR in action



Taking advantage of CRISPR system:

