Clustered Regularly Interspaced Short Palindromic Repeats

CRISPR Technology: From Procaryotic Origins to Eukaryotic Applications

AN OVERVIEW OF CRISPR'S JOURNEY IN GENETIC ENGINEERING

Leathem Mehaffey

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.

June 3, 2016

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SCIENCE

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U.S.

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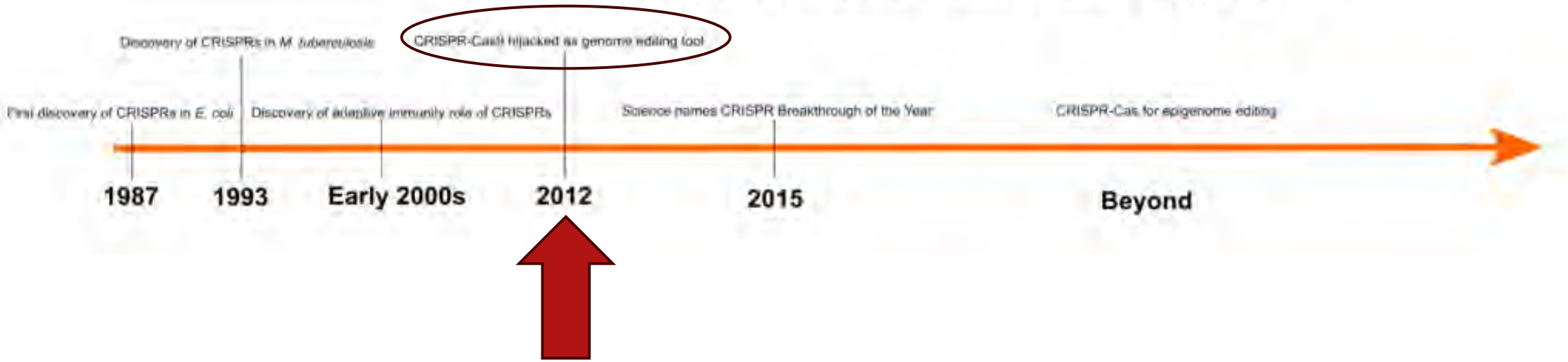
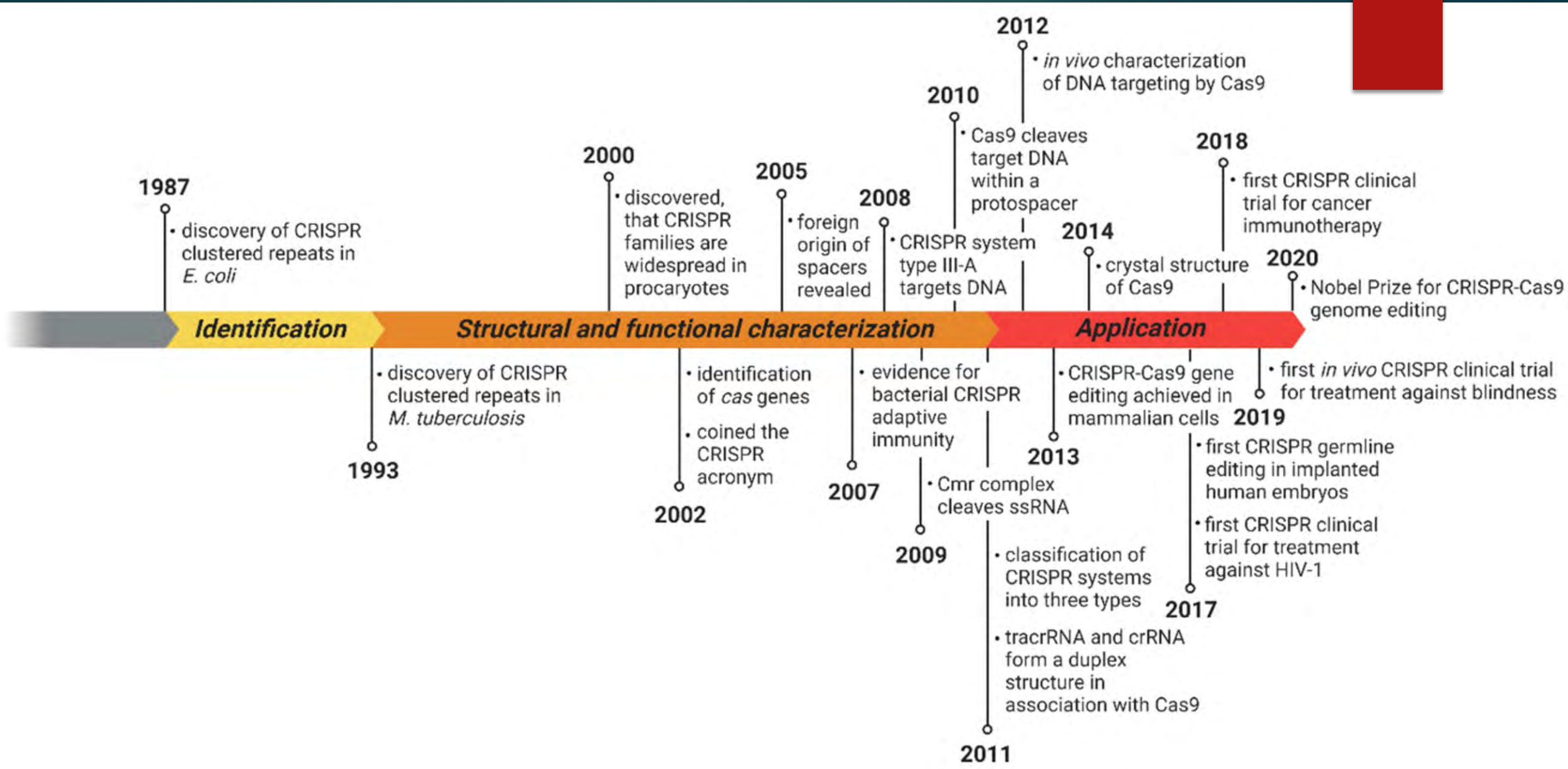
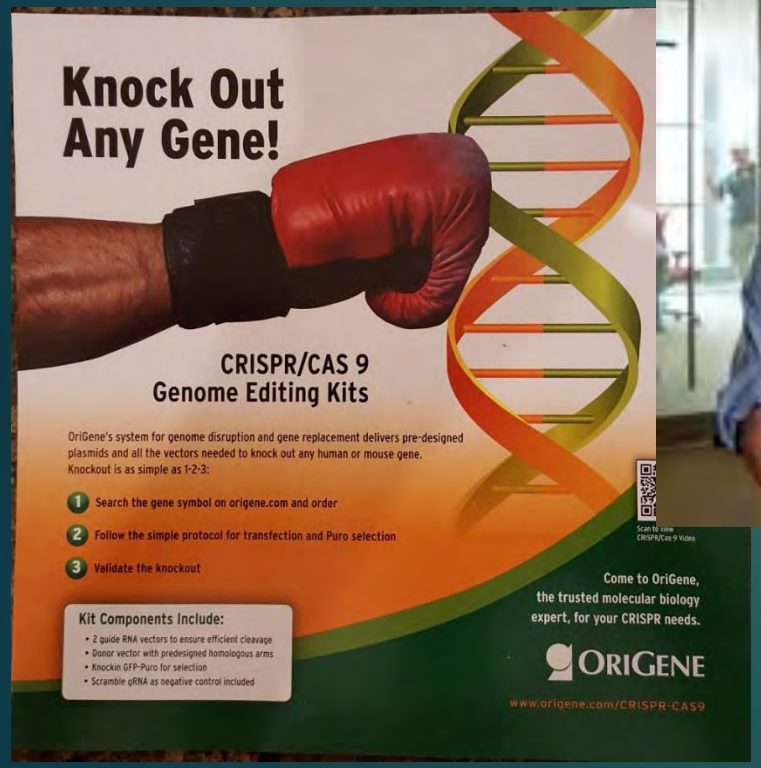
June 10

The CRISPR timeline: from discovery to genome editing and beyond




1987
discovery of CRISPR clustered repeats in E. coli
1993
discovery of CRISPR clustered repeats in M. tuberculosis
2000
discovered, that CRISPR families are widespread in procaryotes
2002
identification of cas genes
coined the CRISPR acronym
2005
foreign origin of spacers revealed
2007
2008
CRISPR system type III-A targets DNA
evidence for bacterial CRISPR adaptive immunity
2009
Cmr complex cleaves ssRNA
2010
Cas9 cleaves target DNA within a protospacer
2011
classification of CRISPR systems into three types
tracrRNA and crRNA form a duplex structure in association with Cas9
2012
in vivo characterization of DNA targeting by Cas9
2013
2014
crystal structure of Cas9
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
2019
CRISPR-Cas9 gene editing achieved in mammalian cells
first in vivo CRISPR clinical trial for treatment against blindness
2020
Nobel Prize for CRISPR-Cas9 genome editing
Identification
Structural and functional characterization
Application

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

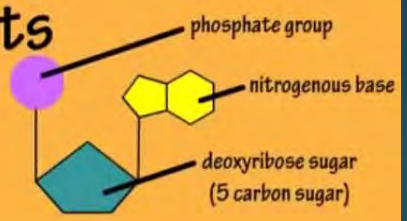


SO: HOW DOES CRISPR WORK??

First, a primer on DNA

structure of nucleic acids: DNA & RNA

3 parts

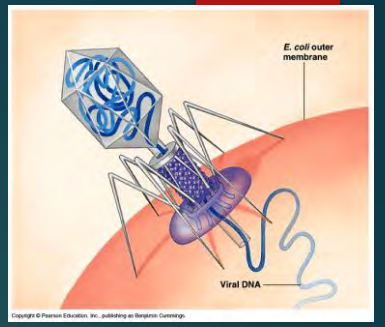


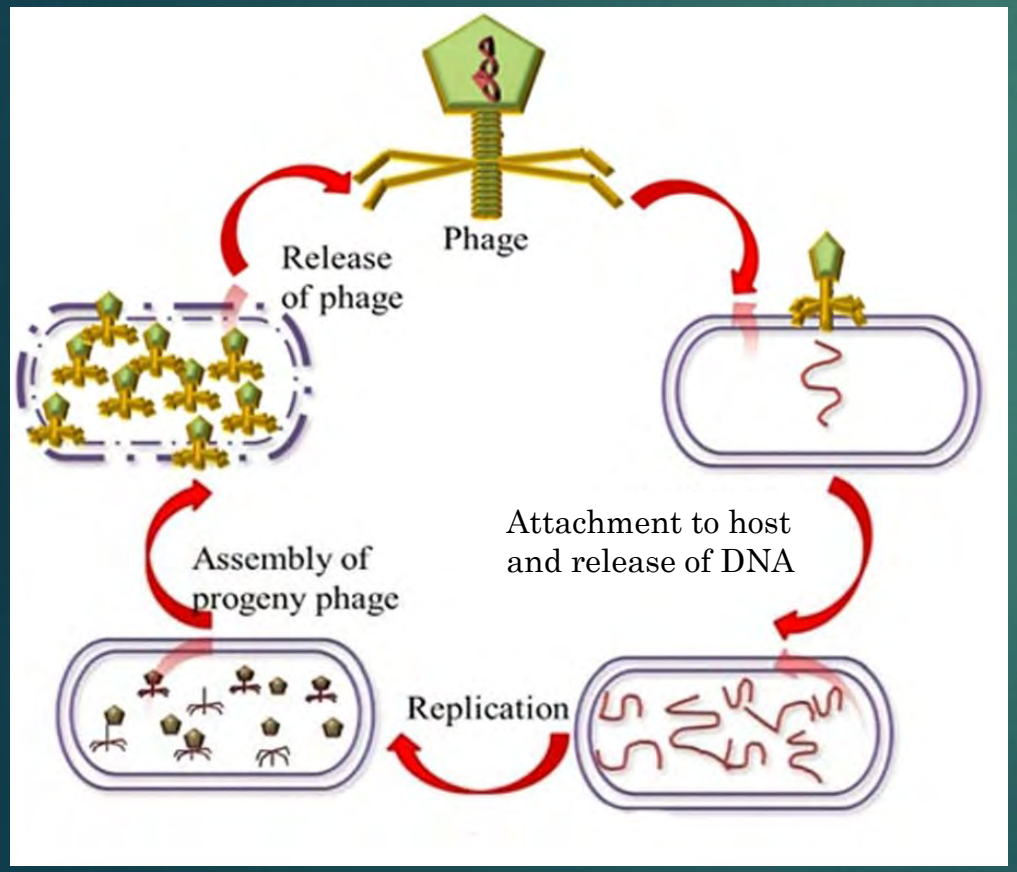
DNA - deoxyribonucleic acid

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*.

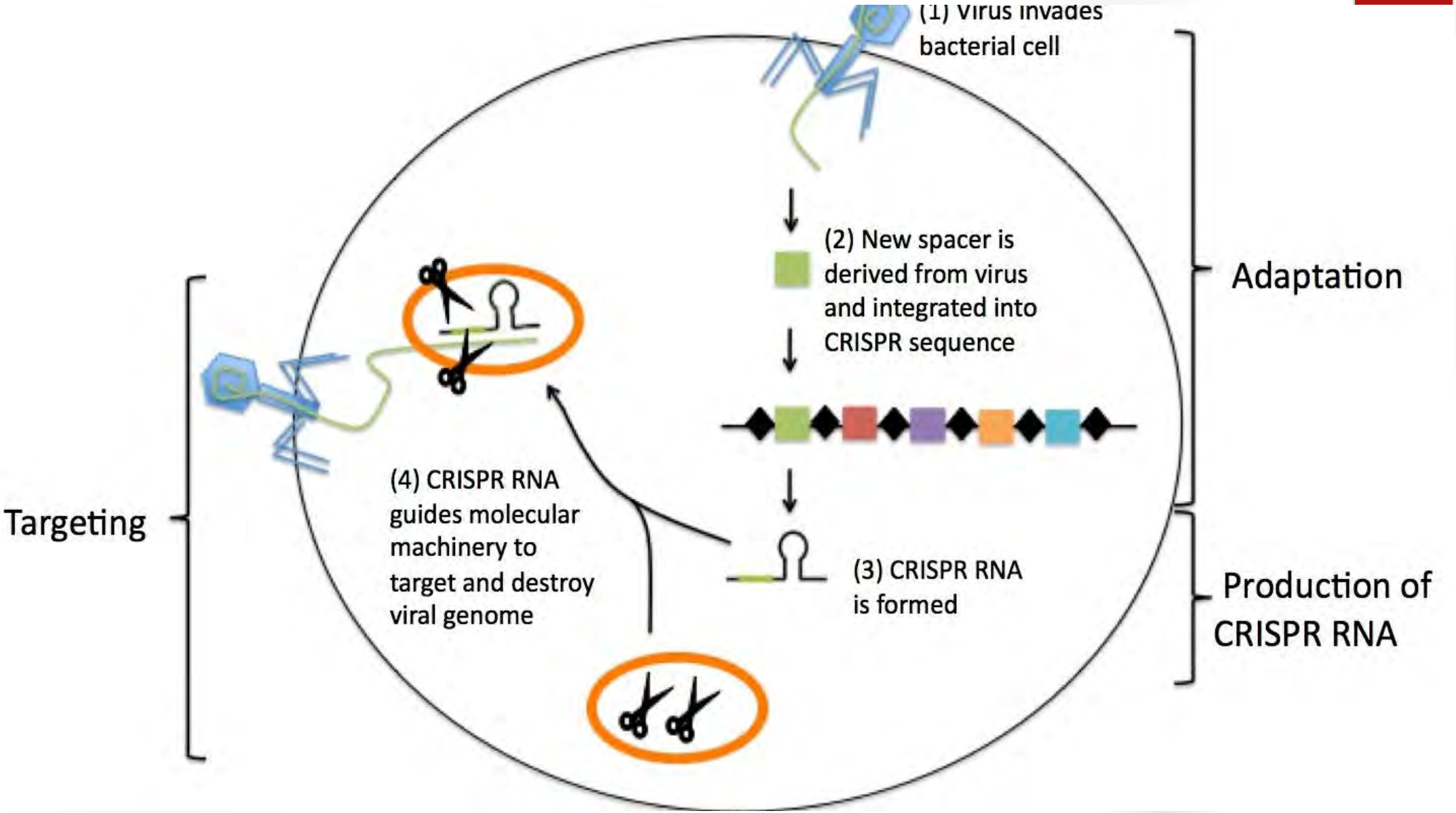
CRISPR In Prokaryote Cells



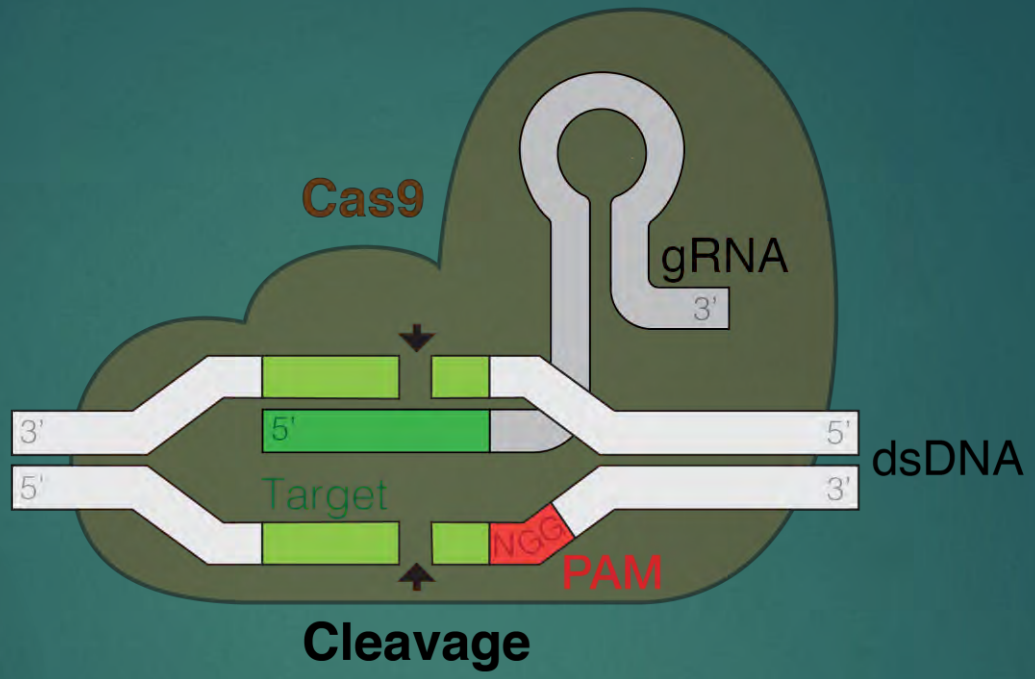


- Viruses act by inserting their nuclear (genetic) material (DNA or RNA) into the cell.
- The viral genetic material takes over the protein and nucleic acid synthetic machinery of the cell to make more viruses.
- Eventually the new viruses erupt from the cell, destroying it, and go on to infect other 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



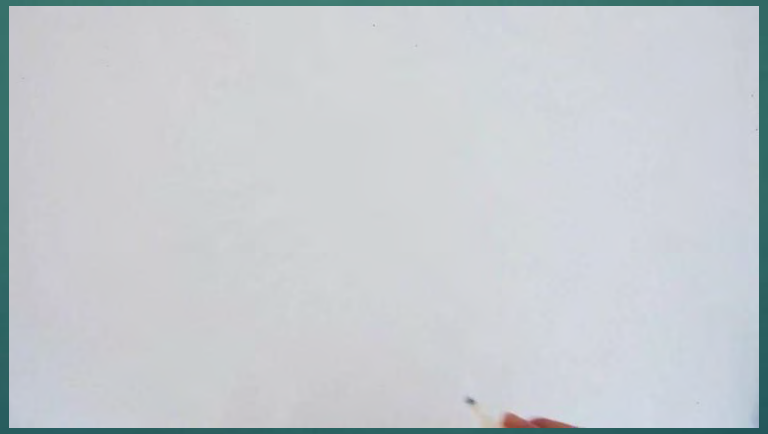
Role of the PAM*



- 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: