



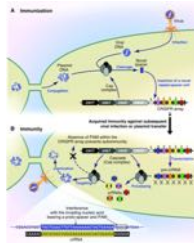




CRISPR

An adaptive immune defense mechanism in archaea and bacteria.

- Uses RNA to direct degradation of foreign genes containing perfect or near perfect identity to a guide sequence (**gRNA**, ~20 nt) within the RNA.
- **Cas9**: the most commonly used nuclease for CRISPR-based genome editing.
- **PAM motif**: A sequence of 5'-NGG-3' just downstream of the target sequence.
- Two possible modes of repair following target cleavage:
 - Non-homology end joining (**NHEJ**) - commonly introduces mutations.
 - Homologous recombination (**HR**) - can be utilized to generate specific mutations, insertions, etc.



Horvath and Barrangou, 2010
<https://www.youtube.com/watch?v=MnYppm1stIs>

A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity

Wenbin Dou^{1,2}, Richard D'Alagni^{1,2}, Sean Parker¹, Michael Rosen¹, Jennifer A. Doudna^{1,2,3}, Elizabeth Charpentier^{1,2}
¹Lawrence Livermore National Laboratory, ²University of California, ³Howard Hughes Medical Institute

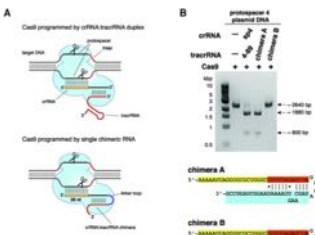


Figure 5

"We propose an alternative methodology based on RNA-programmed Cas9 that could offer considerable potential for gene-targeting and genome-editing applications."

Multiplex Genome Engineering Using CRISPR/Cas Systems

Lu Cong^{1,2}, F. Ann Ran^{1,2}, David Cox², Shouling Liu^{1,2}, Robert Barretto², Anne Haber², Patrick D. Hsu^{1,2}, Doudna BW¹, Wangen Jiang¹, Luciano A. Marraffini¹, Feng Zhang^{1,2}

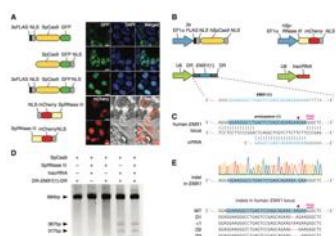
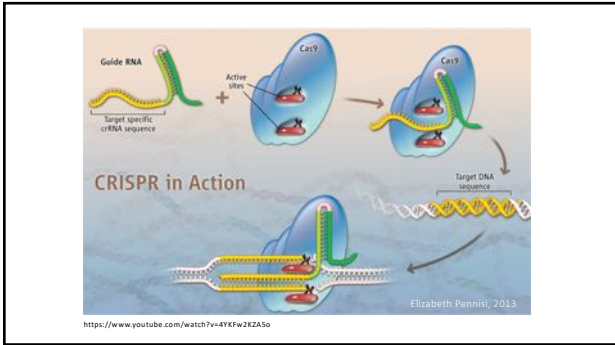
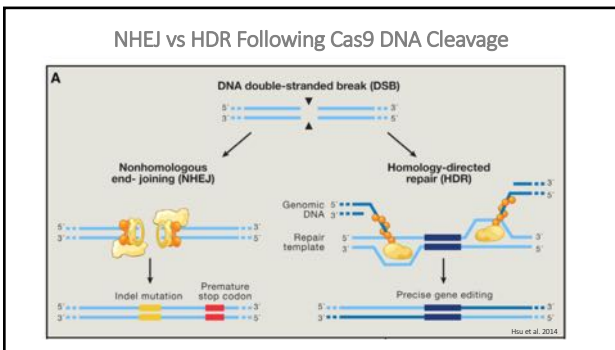
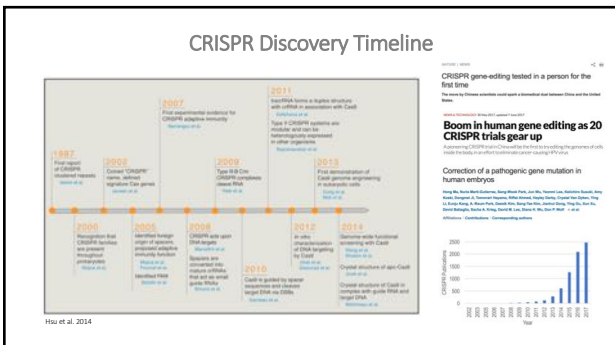


Figure 1





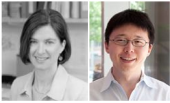



Patenting CRISPR

<https://www.google.com/patents/US20140068797>
<https://www.google.com/patents/US8697359>

Bitter fight over CRISPR patent heats up
 Unusual battle among academic institutions holds key to gene-editing tool's future use.

Heidi Ledford
 12 January 2016


Ding, ding, ding! CRISPR patent fight enters next round

By Janelline L. on 26, 2017, 7:00 AM

CRISPR PATENT DISPUTE HIGHLIGHTS

August 2012	December 2012	February 2013	March 2013	16th March 2013	October 2013	April 2014	2014/2015	February 2015	January 2016
Jinek et al. (2012) Doudna and Charpentier's CRISPR-Cas9 paper was published (available online - June 2012)	provisional patent filed by Zhang team; no work began in 2011	Cong et al. (2013) Zhang's CRISPR-Cas9 paper was published (available online - January 2013)	Doudna and Charpentier search for a patent with a priority date - May 2012	file to insert rule changed to "first to file" by the AIA	Zhang filed for accelerated prosecution of a patent for the use of CRISPR-Cas9 in eukaryotic cells; priority date referring to the provisional patent application from December 2012	USPTO granted patent to Zhang	Doudna revised the initial patent application, broadening the claims	Shoukang Liu's email to Doudna	patent interference proceedings started

www.peerscientist.com



<https://www.youtube.com/watch?v=liboHEQumDgc>

Exercise

Design a guide RNA to mutate the *C. elegans* gene *daf-2*.

- Examine the sequence of *C. elegans daf-2* on wormbase and select an ~200 nt exonic region to target for editing.
- Option 1: Use the guide RNA tool at <http://crispr.mit.edu> to design a guide RNA.
 Option 2: Use the IDT tool: https://www.idtdna.com/site/order/designtool/index/CRISPR_SEQUENCE
- Visit IDT to order guide RNA and Cas9 protein.
<https://www.idtdna.com/site/order/oligoentry/index/crispr>
<https://www.idtdna.com/pages/products/crispr-genome-editing/alt-r-crispr-cas9-system>
