
CRISPR
EVERYWHERE

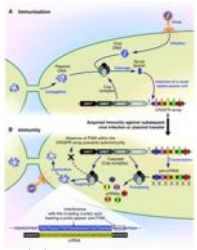

TIME
The Gene Machine
No hunger.
No pollution.
No disease.
The Economist
Editing humanity
The prospect of genetic enhancement


http://static6.businessinsider.com

CRISPR

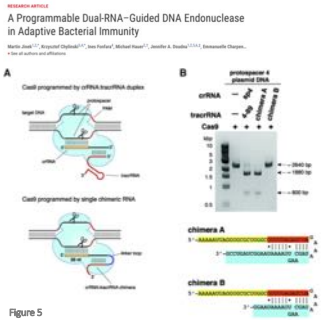
An adaptive immune defense mechanism in archaea and bacteria.

- 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.

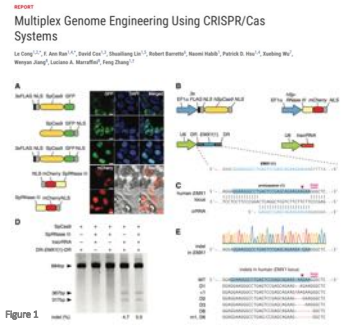


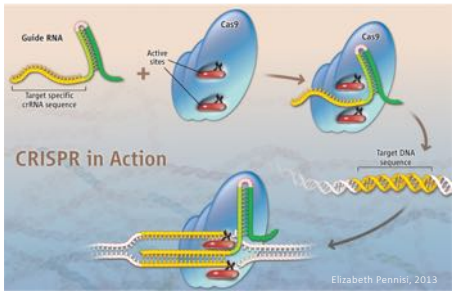
Horvath and Barrangou, 2010

https://www.youtube.com/watch?v=MnYppmstxIs



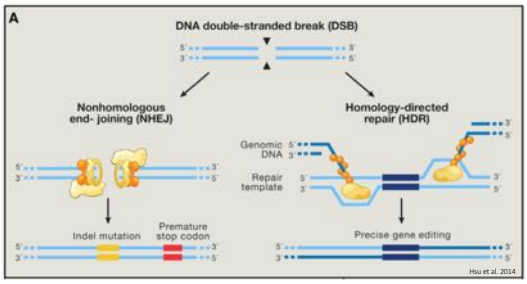
"We propose an alternative methodology based on RNA-programmed Cas9 that could offer considerable potential for gene-targeting and genome-editing applications."



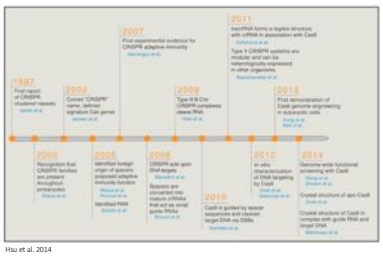


https://www.youtube.com/watch?v=4YKFw2KZA5o

NHEJ vs HDR Following Cas9 DNA Cleavage



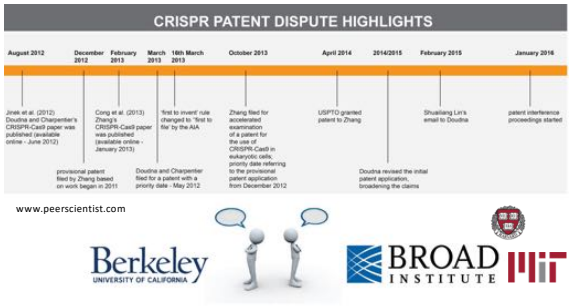
CRISPR Discovery Timeline



Hsu et al. 2014

Patenting CRISPR

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



www.peerscientist.com

Berkeley UNIVERSITY OF CALIFORNIA

https://www.youtube.com/watch?v=IboHEQumDGc

Exercise

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

1. Examine the sequence of *C. elegans daf-2* on wormbase and select an ~200 nt exonic region to target for editing.

2. 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

3. 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