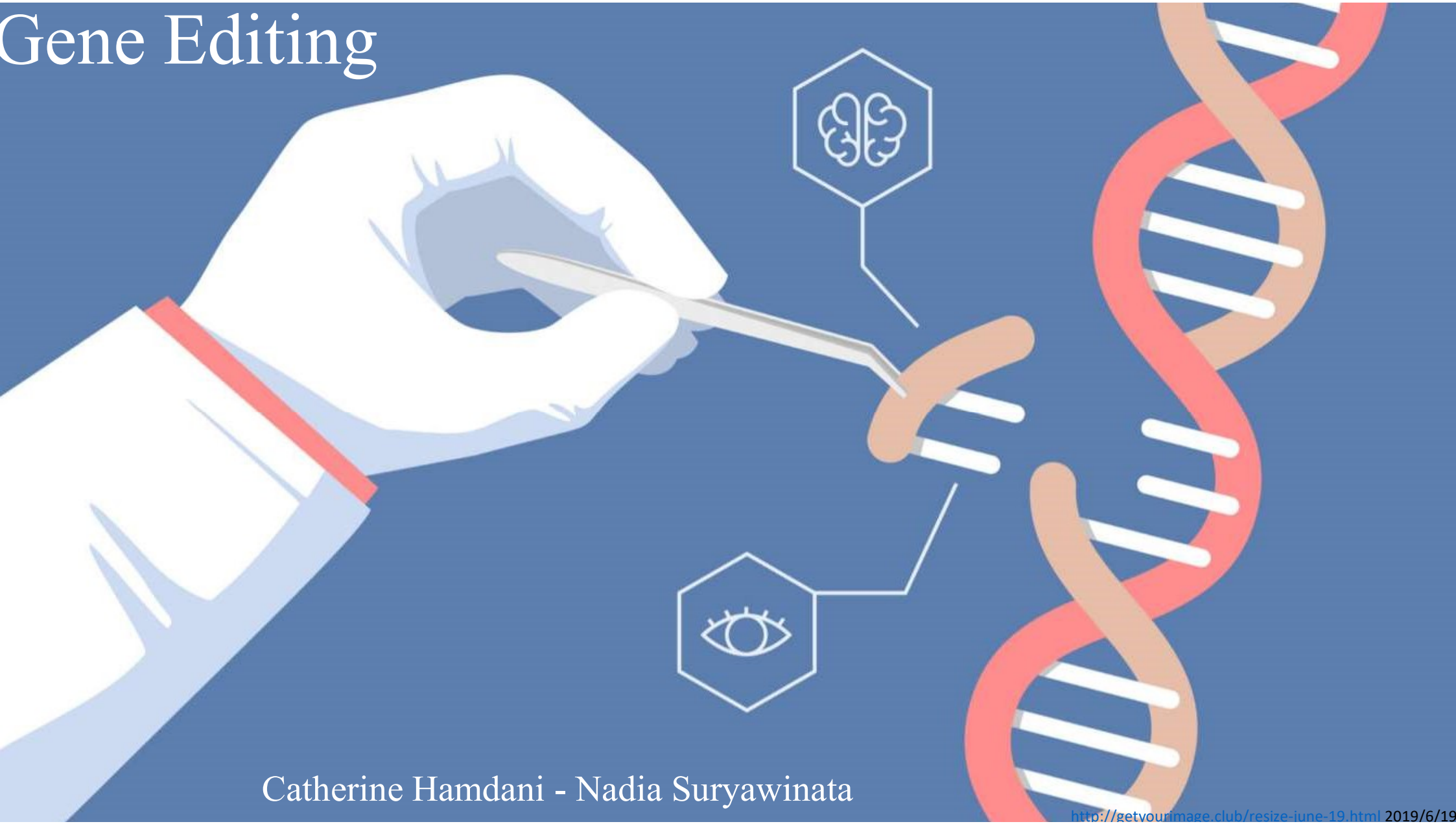
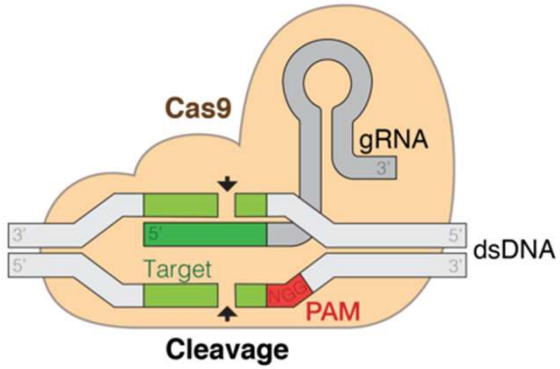
Gene Editing



Catherine Hamdani - Nadia Suryawinata

What is Gene Editing?

A type of genetic engineering where genes are inserted, deleted, or modified in an organism
→ CRISPR-Cas9



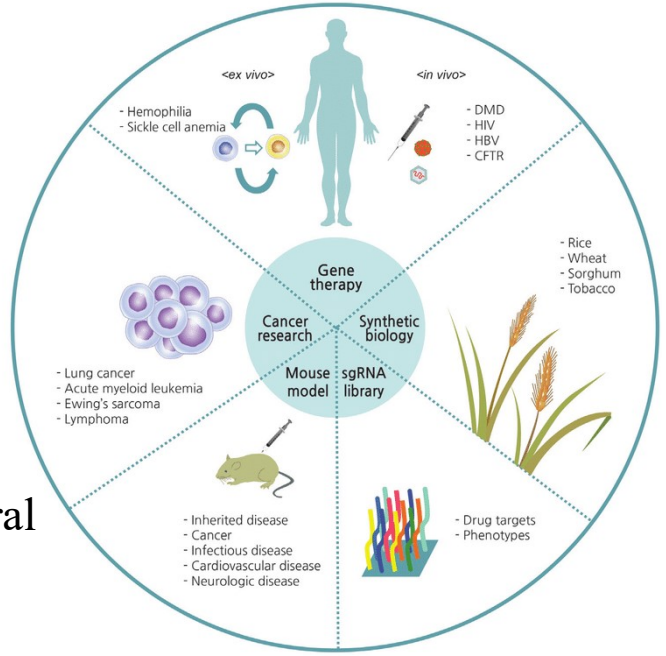
http://junq.info/wp-content/uploads/2019/03/JUNQ-9-1.pdf 2019/6/24

Purpose?

- Curing illnesses caused by genetic errors → gene therapy
- Wiping out certain species from the world
- Creating illness resistant organisms
- Possibility of 'designer babies'

How was it found?

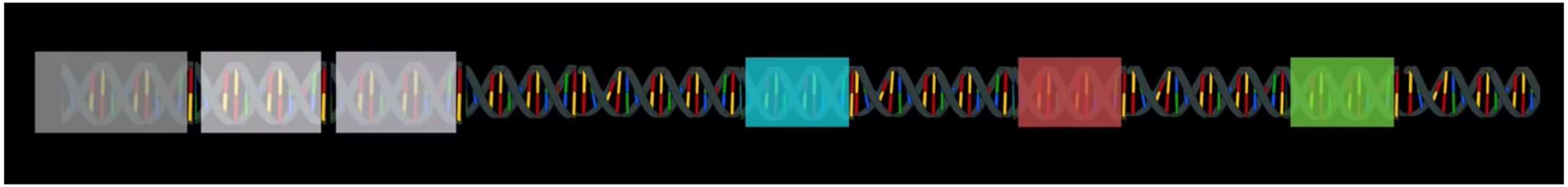
- A naturally occurring mechanism in prokaryotes to fight off viral infections
- Scientists found that the mechanism could be controlled



https://www.researchgate.net/figure/Overview-of-gene-editing-and-its-applications-Genetic-defects-can-be-corrected-via-gene_fig2_312025175 2019/6/24

CRISPR , *the bank of memories*

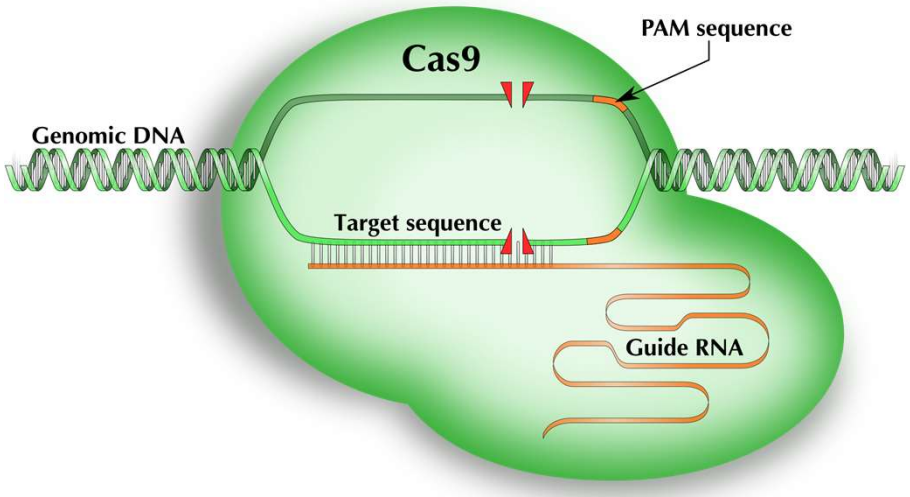
- CRISPR is a DNA sequence in prokaryotic organisms ex. bacteria
- In bacteria, it is made of DNA from viruses that attacked the bacteria, and is used to detect DNA from similar virus.
- Composed of short and palindromic sequences, with ‘spacer’ DNAs in between, and Cas genes
- Spacer DNA are DNA from viruses that attacked previously.
- When a virus attacks, Cas genes will be transcribed to make Cas complex, and transcribe crRNA from spacer DNA.



https://www.youtube.com/watch?v=MnYppmstxIs 2019/11/20

Cas9 Complex (CRISPR associated protein 9)

- An enzyme composed of a guide RNA (sgRNA) that guides Cas9 to the correct sequence of DNA
- It will bounce along the DNA until the sgRNA can match with the specific sequence on DNA
- It unwinds the DNA, binds to it, then Cas9 protein will cut it, creating a double strand break.

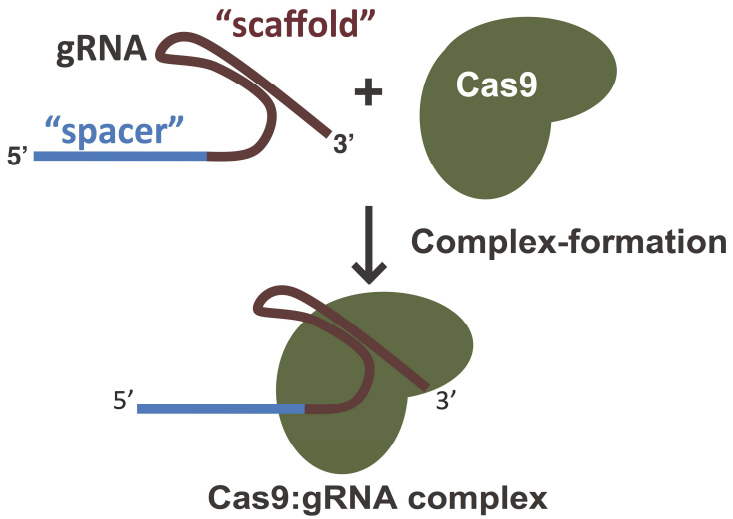


http://onlineresize.club/pictures-club.html 2019/6/24

https://on.ge/story/30964-%E1%83%95%E1%83%98%E1%83%9C-%E1%83%90%E1%83%A0%E1%83%98%E1%83%A1-cas9-%E1%83%93%E1%83%90-%E1%83%A0%E1%83%9D%E1%83%92%E1%83%9D%E1%83%A0-%E1%83%9B%E1%83%A3%E1%83%A8%E1%83%90%E1%83%9D%E1%83%91%E1%83%A1-crispr 2019/6/24

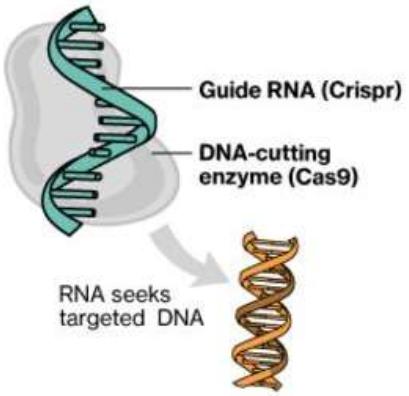
Guide RNAs (sgRNA)

- Main job is to guide the Cas9 protein to the correct place on the DNA
- 2 parts:
  - Scaffold sequence: necessary for Cas binding
  - Spacer



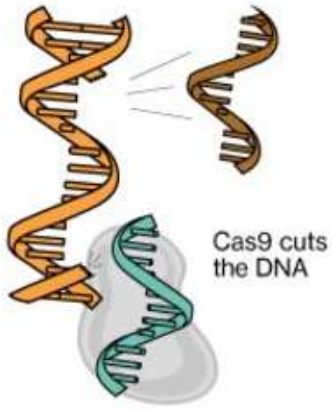
https://blog.addgene.org/components-of-crispr/cas9-our-new-crispr-101-ebook 2019/6/24

How do scientists use this mechanism?



Scientist construct an artificial guide RNA with about 20 bases to match with the sequence they want to cut.

→

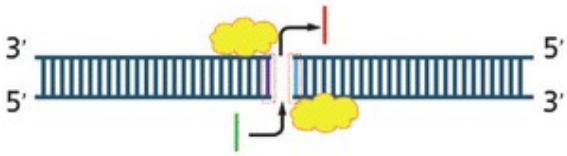


DNA cut by Cas9

→

Double strand break

→

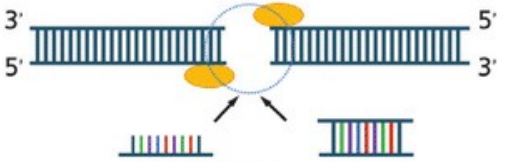


NHEJ

(Gene disruption by small insertions or deletions)

Natural DSB repair mechanism, most likely non-homologous end-joining

- Might cause mutations



HDR

(Gene correction or insertion by recombination with donor template)

Scientist can insert a DNA template for the broken DNA to repair with

- Scientists can insert any information sequence they want

https://germination.ca/who-owns-crispr/ 2019/11/20

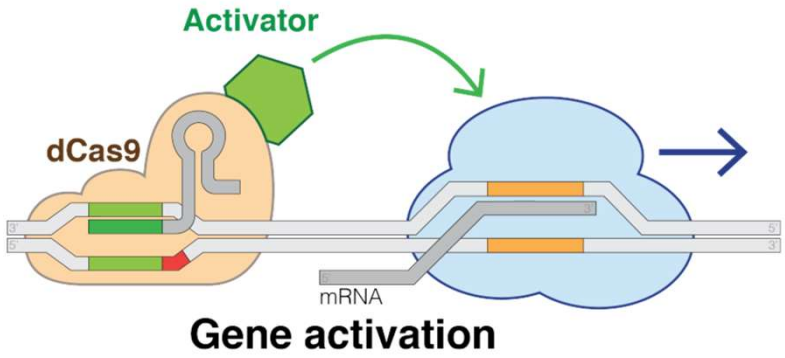
Application: Activation/ Knockdown

- Using “dead” versions of Cas9 (dCas9) → cannot cut DNA, but still able to target sequence

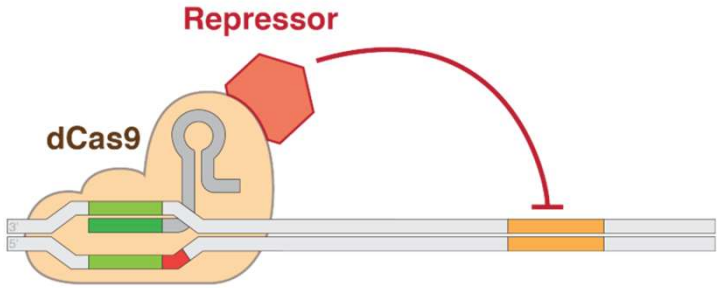
-Various regulatory factors are added to dCas9 → turn genes on /off

-Target site is methylated → inhibits transcription

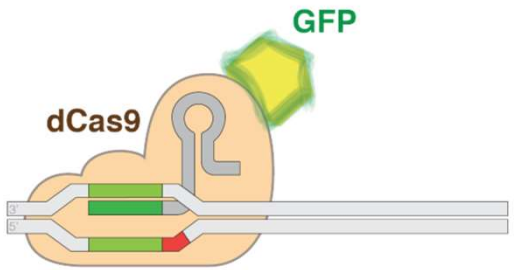
-guide RNA targets the promoter



Gene activation



Gene repression



Visualisation

https://ui-ex.com/download.html 2019/6/24