CRISPR Lab

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Materials

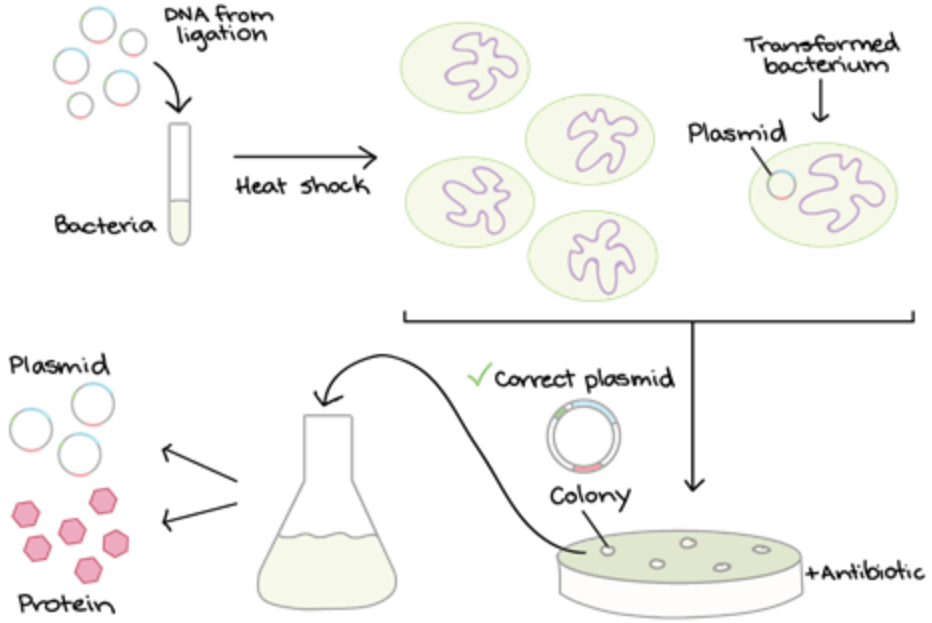
- 1 tube of competent cells (labelled “MG M”)
- Ice
- 42c water
- DNA (CRISPR + Donor plasmid)
- Pipette + tips
- 1 mL of LB
- gloves

Transformation

1. Take 1 uL of DNA (CRISPR + Donor plasmids) from me and mix with your comp cells
2. Put tube of comp cells with DNA on ice for 15 min
3. Put the tube into the 42C water bath for 45 seconds
4. Put the tube on ice for 2 minutes
5. Add 1 mL of LB and incubate for 1 hour (recovery)
6. Plate on antibiotic (ampicillin)

Transformation Overview

- Transformation is the process by which a cell takes up foreign DNA



Transformation

1. **Take 1 uL of DNA (CRISPR + Donor plasmids) from me and mix with your comp cells**
2. Put tube of comp cells with DNA on ice for 15 min
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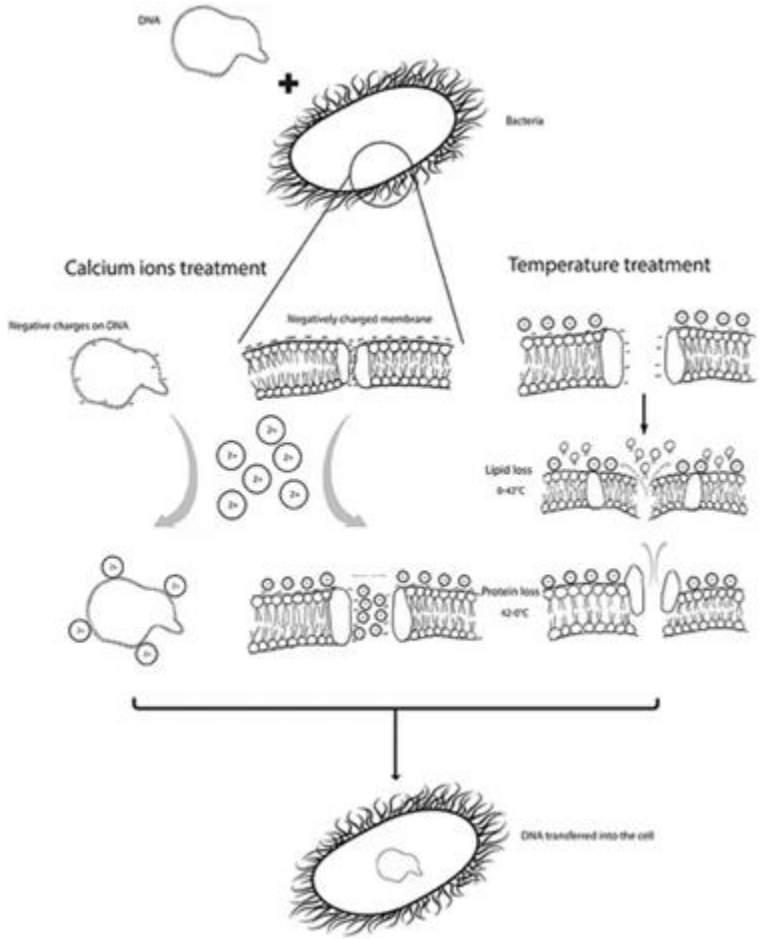
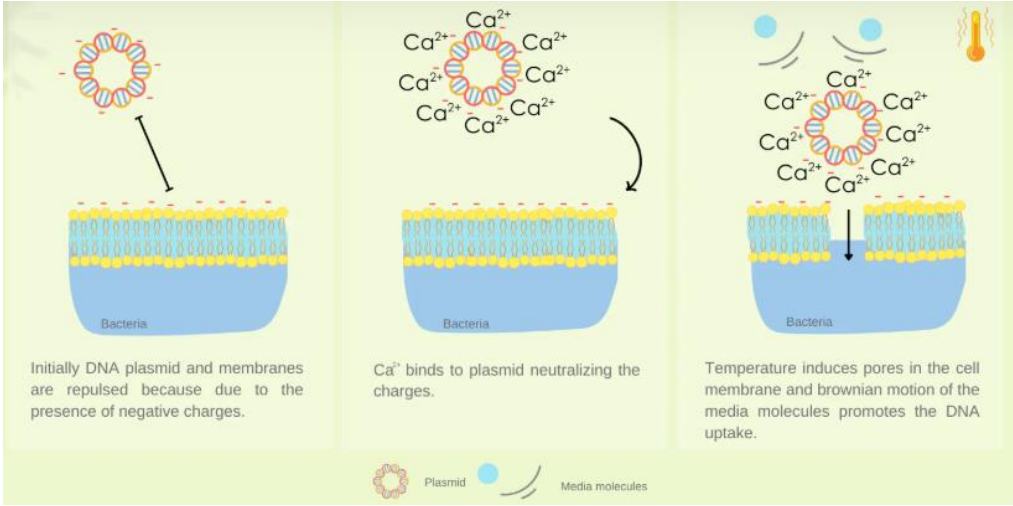

DNA
Bacteria
Calcium ions treatment
Temperature treatment
Negative charges on DNA
Negatively charged membrane
Lipid loss
0-42°C
Protein loss
42-0°C
DNA transferred into the cell

Role Play

1. Ice for 15 min.
2. Heat Shock for 45 sec.
3. Ice for 2 min.



- Which *E. coli* cell took in the recombinant plasmid?
- Which *E. coli* cell was transformed?
- What happens to the *E. coli* cell that was not transformed?

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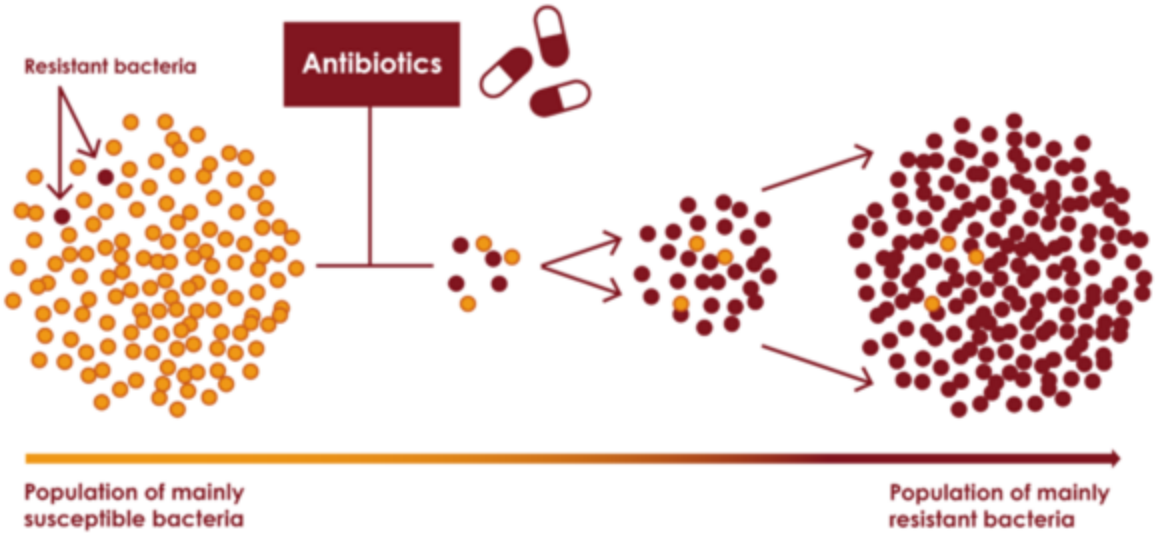
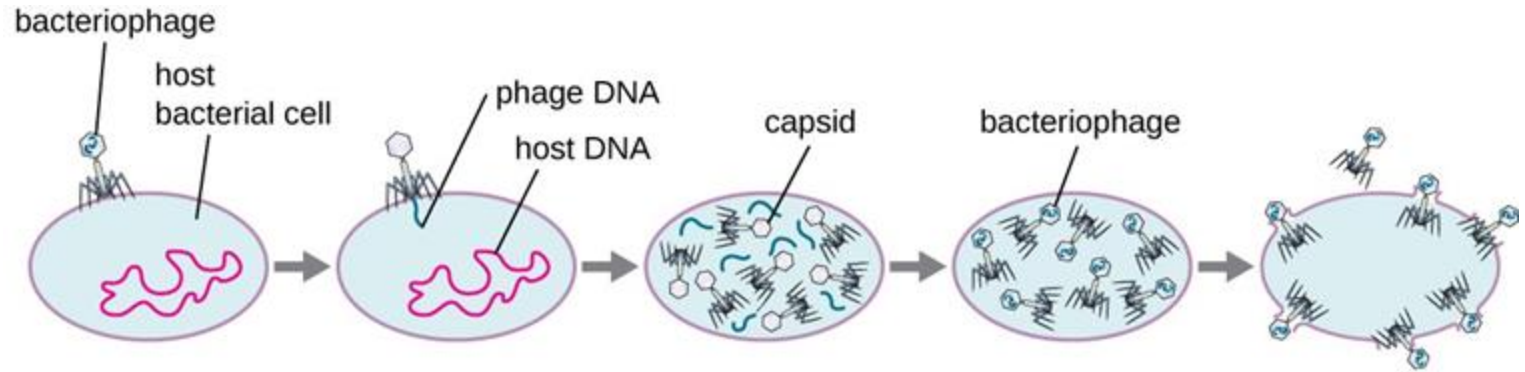
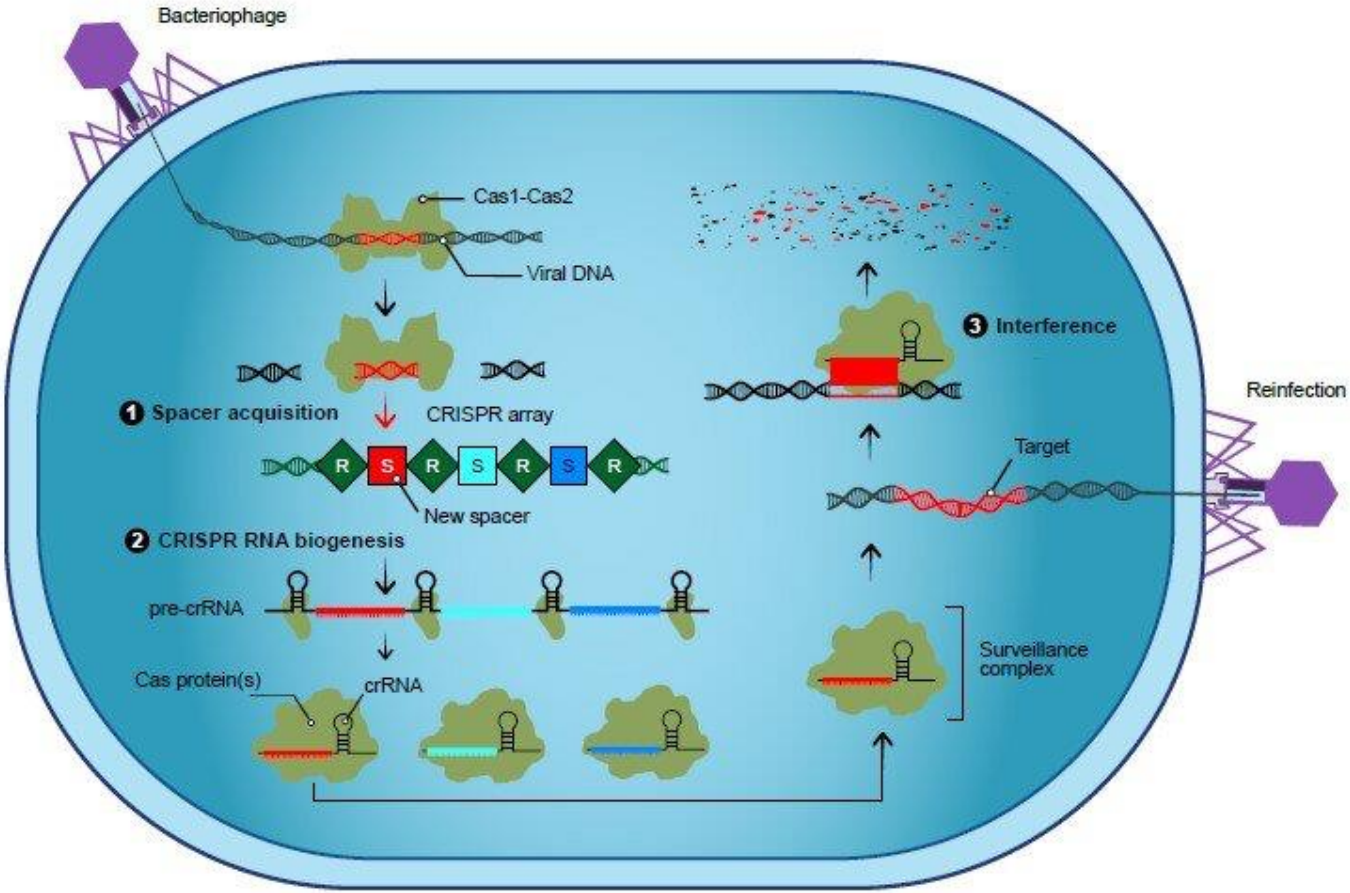

Resistant bacteria
Antibiotics
Population of mainly susceptible bacteria
Population of mainly resistant bacteria

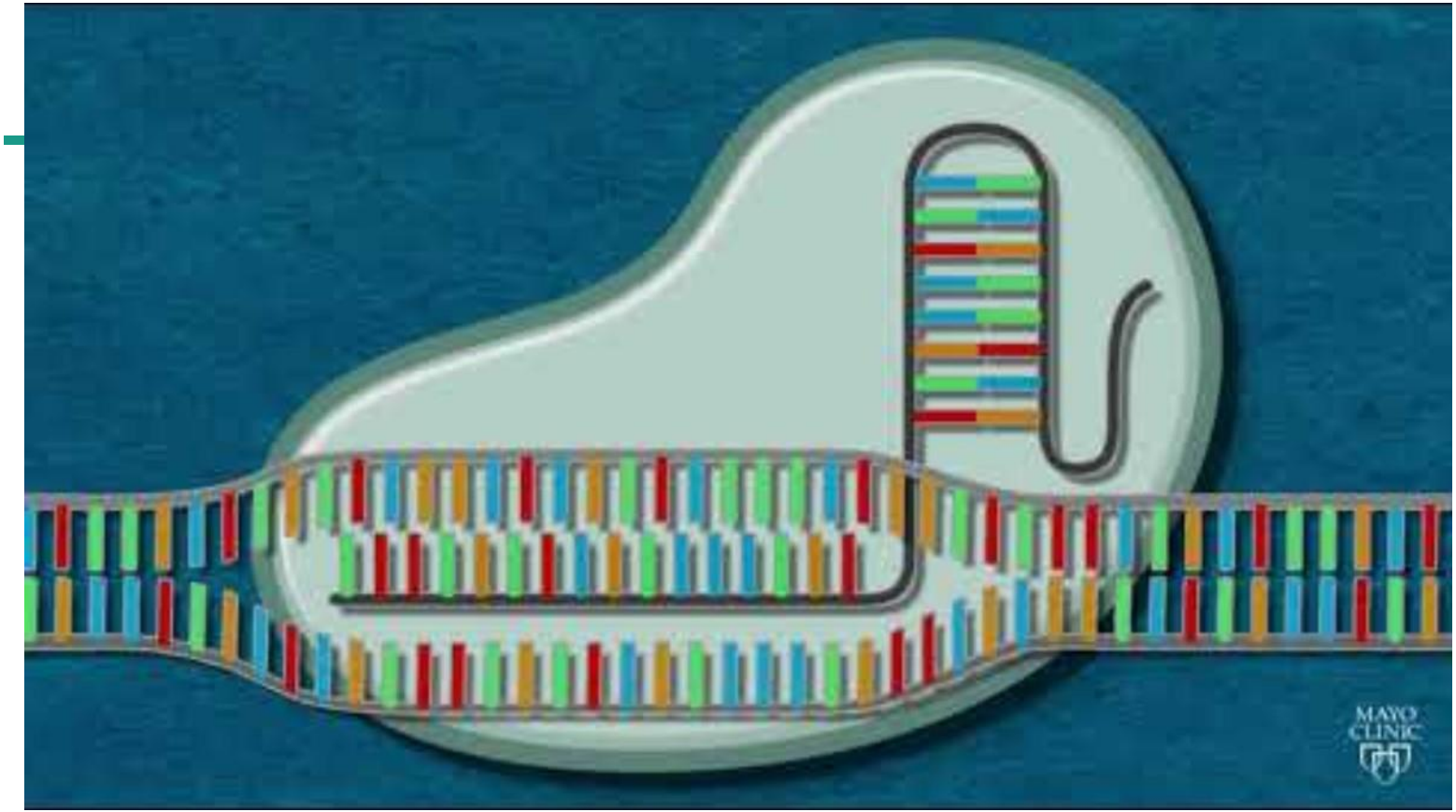
What is the meaning of life?

What is the goal of life?

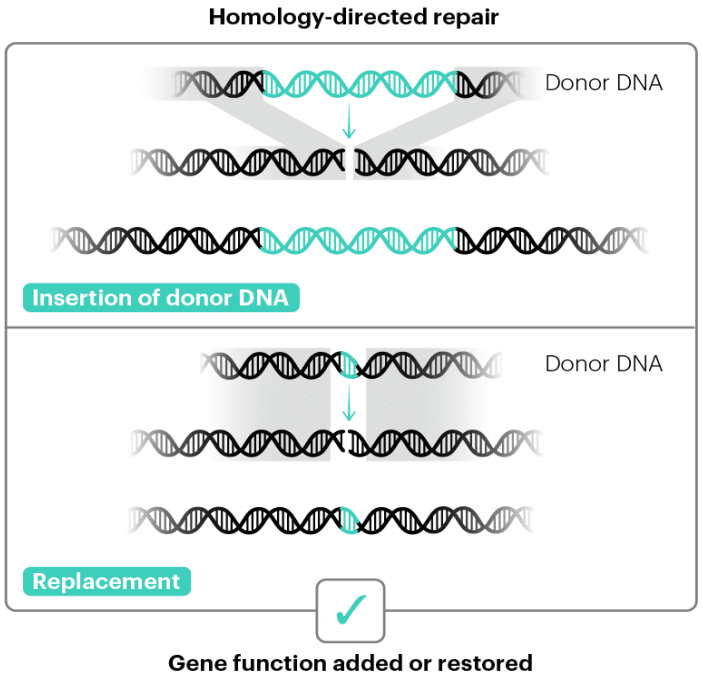
Viruses and bacteria have been in battle for millenia




Bacteriophage
Cas1-Cas2
Viral DNA
1 Spacer acquisition
CRISPR array
R
S
R
S
R
S
R
New spacer
2 CRISPR RNA biogenesis
pre-crRNA
Cas protein(s)
crRNA
3 Interference
Surveillance complex
Target
Reinfection

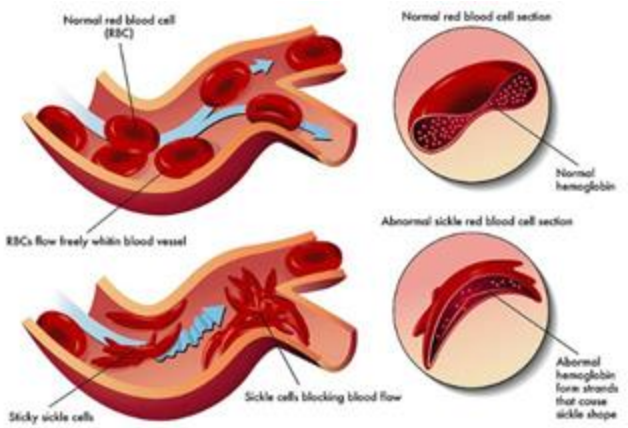

MAYO
CLINIC

How can we insert DNA sequences using CRISPR?

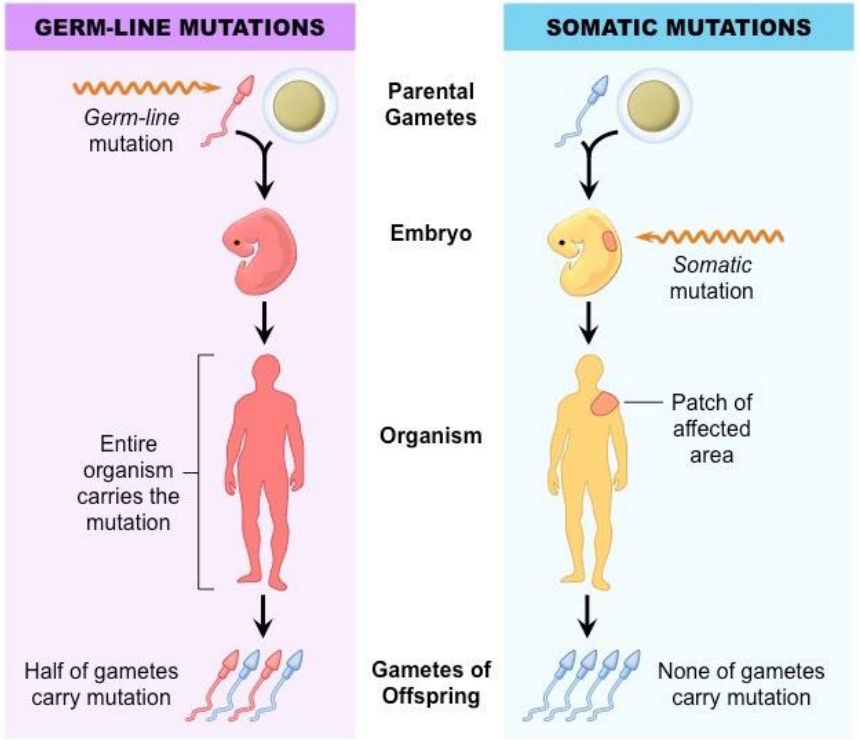


What are some applications of CRISPR?

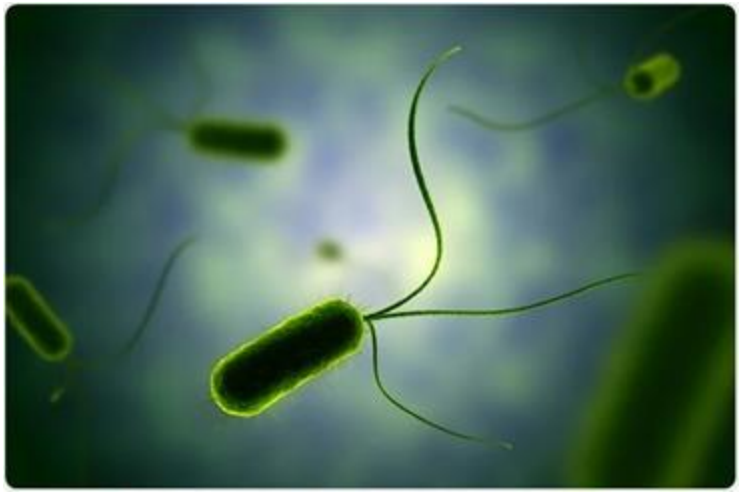
1. **In bacteria:**
   a. Quick and easy genome editing to make bacteria express the genes you want them to
2. **In plants:**
   a. Can make crops resistant to pathogens or extreme environmental conditions
3. **In humans:**
   a. **Diabetes** – dysfunctional insulin producing cells can be edited and replaced in a patient
   b. **Sickle cell disease** – replenishing normal red blood cells
      i. Casgevy 1st approved in December 2023
   c. Research is ongoing for applications in **cancer** and more – almost exclusively involving somatic editing



Somatic vs Germline editing (in humans)



Goal of CRISPR in this lab



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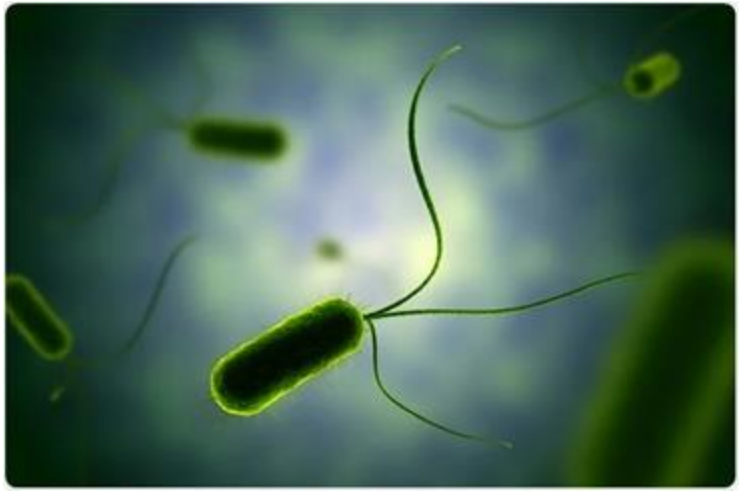
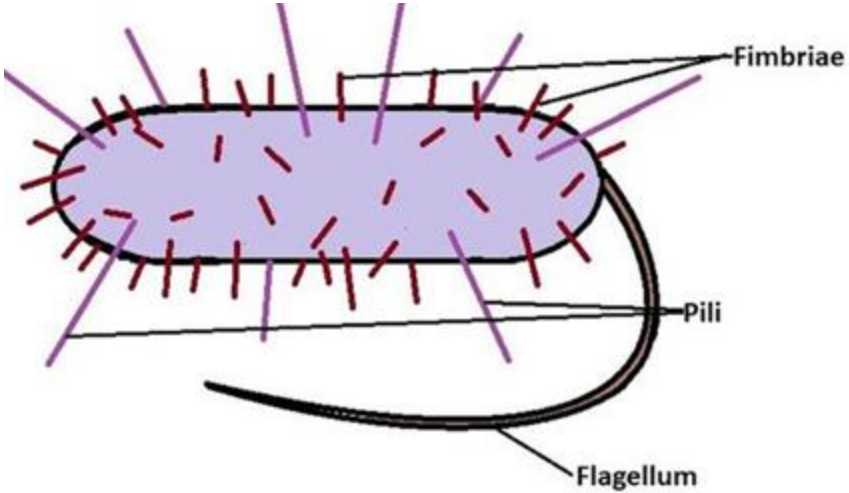
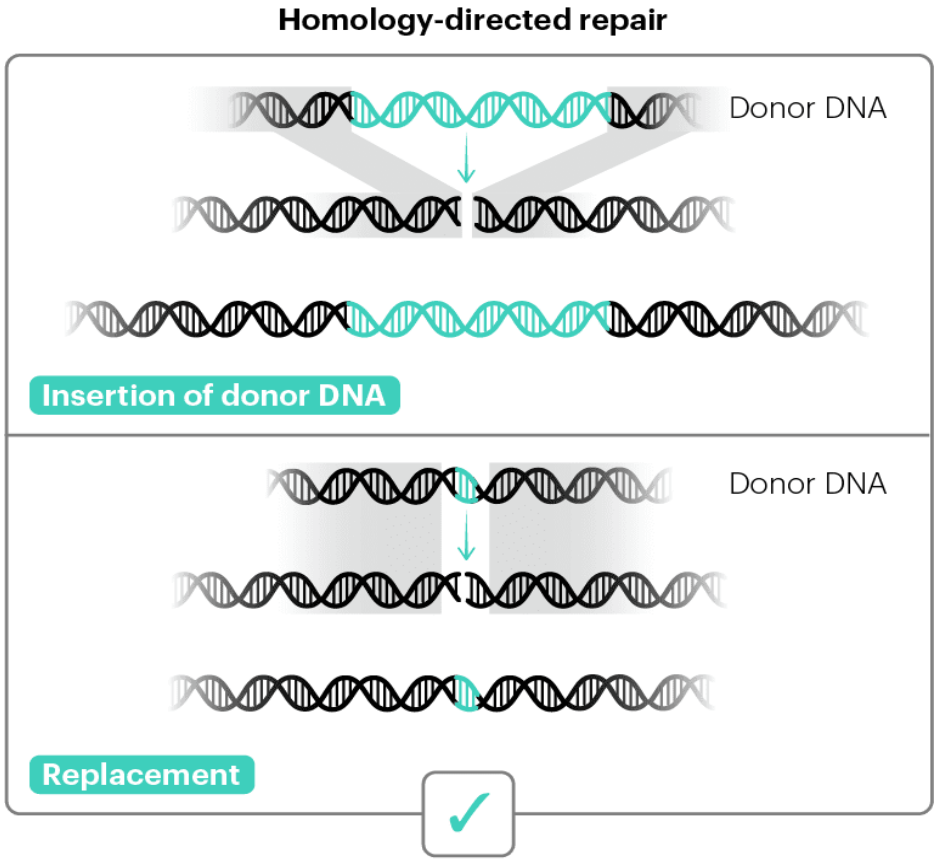


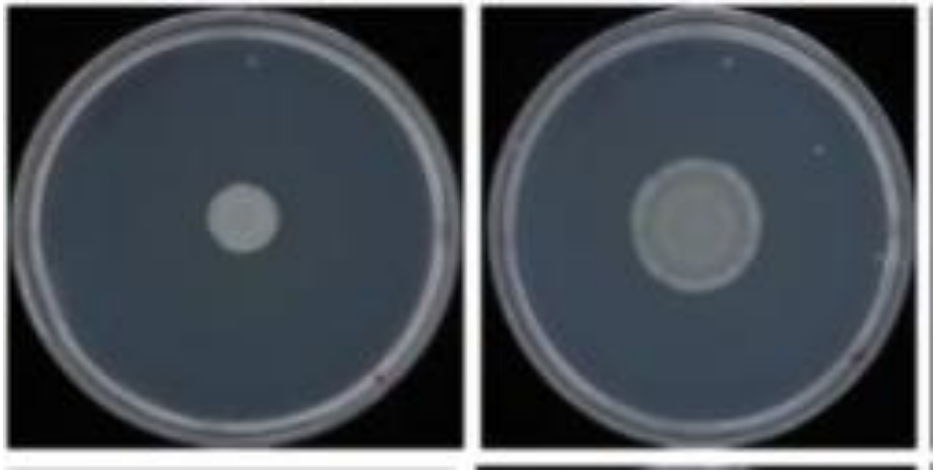


A mutation in the FilC gene disables the flagella. We are going to fix that with CRISPR.

How are we doing CRISPR in this lab?


Homology-directed repair
Donor DNA
Insertion of donor DNA
Donor DNA
Replacement
✓

How will we know it is successful?



Bioethics

Presentation created with the help of NYU Langone High School's Bioethics Project lesson plans

What is ethics?

- What comes to mind when you think of the word *ethics?* Can you think of a time in the past 24 hours that you had to think, “what should I do?”
- Ethics is a systematic inquiry into the nature of right and wrong actions (human moral rules), undertaken with the goal of improving human existence
- An important distinction in the study of ethics is **facts** vs **values**
  - **Facts** attempt to describe the way things are, ie: “the sky is blue” or “male blue jays are bigger than female blue jays”
  - **Values** are ideas about the way you want things to be or the way you think things should be, ie: “people shouldn’t chew with their mouths open” or “desiring money is wrong”
  - Facts, whether they are absolutely true or not, are value-neutral -- facts attempt to describe but never attempt to evaluate
- Ethics is only concerned with values! **Let us be respectful of everyone’s values.**

What is bioethics?

- Bioethics is the application of these moral and ethical discussions to medicine, biology, and health
- A classic example involves the resuscitation debate:
  - *A man aged 58 suffered a massive heart attack while shoveling snow. Medical emergency personnel arrived at the scene within minutes and resuscitated him. During those minutes, however, his brain was starved of oxygen, resulting in permanent brain damage. He is now unconscious and surviving on life support, without which his heart will stop. What should his family do?*
- **Key ethical questions here:**
  - Is turning off a patient's respirator morally equivalent to killing the patient?
  - Does it matter what the man wanted for his life before the heart attack? (some people sign legally-binding DNR, or do-not-resuscitate forms)
  - Do the emotional and financial effects on the family of the person on life support matter?
  - What role should medical professionals play in these types of scenarios?

Let’s get into some case studies

01. CRISPR-edited babies

02. Genetic testing and 23andMe

CRISPR-edited babies

- November 2018: He Jiankui announced that he made the world's first genome-edited babies
  - An example of **germline editing**
- He claimed that he utilized CRISPR technology to alter the CCR5 gene in a set of embryos in an attempt to provide immunity to HIV, that he then implanted into a woman
  - They are now 5 years old and have no reported problems with their growth
- Many scientists around the world were shocked and said that gene editing of embryos that would result in pregnancy needs to be prohibited

Discussion Questions

1. Were He Jiankui's actions justified?
2. In what situations is it ok to use CRISPR, and what is too far?
3. Who gets to decide what the regulation should be? What do we do if not everyone agrees?

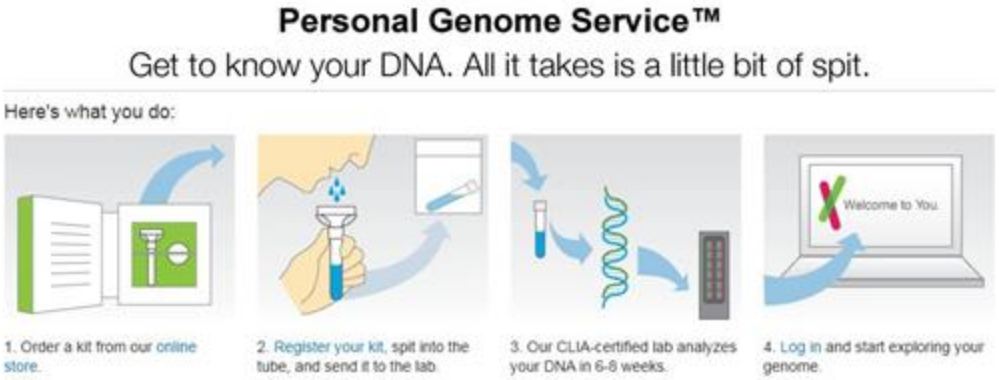
What actually happened

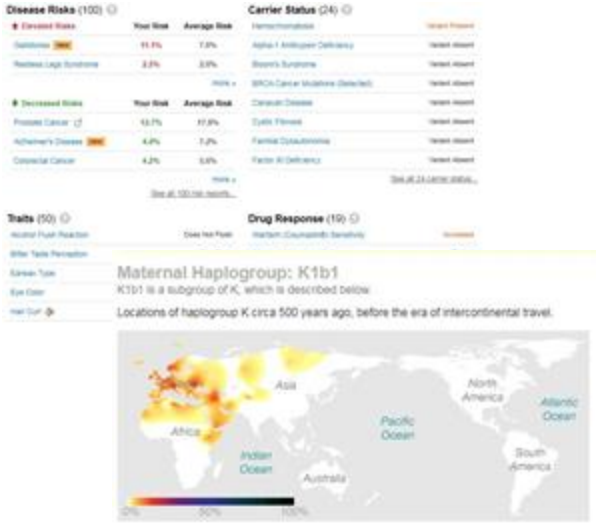
- He Jiankui was jailed for 3 years by the Chinese government
- World Health Organization formed the Expert Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing
    - Say that **germline** (heritable) human genome editing should be banned
    - **Somatic** human genome editing is ok
- However, some people with genetic disorders (such as Duchenne muscular dystrophy) support him and want him to develop a cure for their disease

Case study 2

Genetic Testing + 23andMe

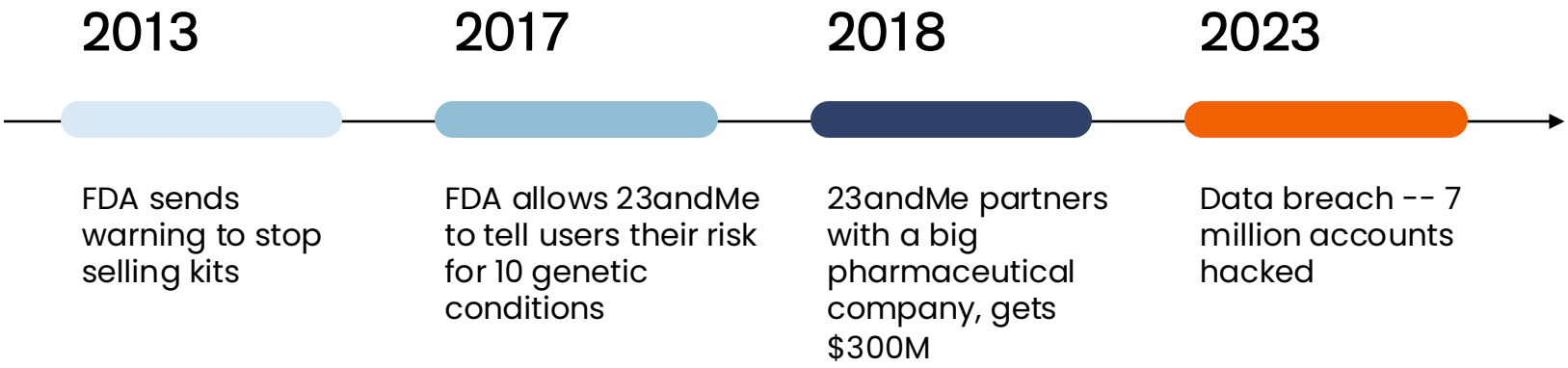
- Founded in 2007, 23andMe is a genetic testing company that allows you to spit in a tube, mail your saliva, and get it **DNA sequenced** to find out about your genetic ancestry and health risks
- Also has data from 10 million users who have consented to have their genetic data used for research purposes, which it is now using to develop drugs





singularityweblog.com

23andMe



2024: facing 30+ lawsuits

23andMe data breach

- October 2023: data breach!
- Information stolen: full names, usernames, profile pictures, date of birth, sex, genetic ancestry results, relationship labels, family trees, and self-reported location
- 23andMe said *only* 0.1% of users (14,000) profiles were hacked
- However, because of DNA Relative and Family Tree links between profiles, **7 million** profiles were compromised
    - (no comment from 23andMe)
- Hackers specifically targeted Ashkenazi Jews and users of Chinese descendants with the intent to sell this information
    - Basis for the class action lawsuit

https://www.wired.com/story/23andme-breach-sec-update/

Discussion Questions

1. Who “owns” genetic data?
2. Should there be more protections on genetic data than on other types of personal information?
3. Is it ethical for 23andMe to use patient data for drug research and profit from it?
4. Would you feel comfortable getting a 23andMe test done?

Open access to genetic data caught the Golden State killer

- Between 1974 and 1986, Joseph James DeAngelo committed at least 13 murders, 51 rapes, and 120 burglaries across California

- In 2016, his distant relatives submitted their DNA to a genetic genealogy platform (GEDmatch), which was traced back to him by the police through a family tree containing 1000 people
- He was finally arrested in 2018

- **Does this case change any of your opinions on genetic data privacy?**

More bioethics cases for you to read about

- **Henrietta Lacks** – her cells were used to start the immortal HeLa stem cell line that is currently used in a lot of research, but without her consent
- **Tuskegee experiment** – 400 black men with syphilis were recruited into a study and were not offered treatment when it became available, because the researchers wanted to track the disease progression until death
- **Theranos fraud case** – a company started by Elizabeth Holmes that defrauded investors and lied about the science behind their technology