CRISPRkit

An Overview

An accessible CRISPR technology can offer new learning experiences to grade school students

BACKGROUND:

CRISPR gene editing/regulation technology is revolutionizing both life science research and healthcare, and is becoming a pillar for future medicines, known as ‘gene therapy’.

PROBLEM:

Despite widespread use in academia, logistical and financial limitations prevent the use of CRISPR in school settings or in the public domain.

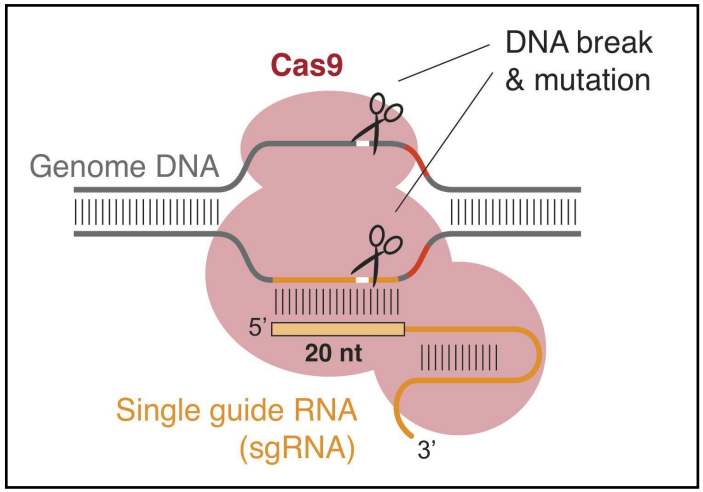
SOLUTION:

Design a CRISPR kit that is accessible, which overcomes resource limitations as an education tool for promoting broad accessibility in genetic engineering teaching.

CRISPRkit

What's CRISPR?

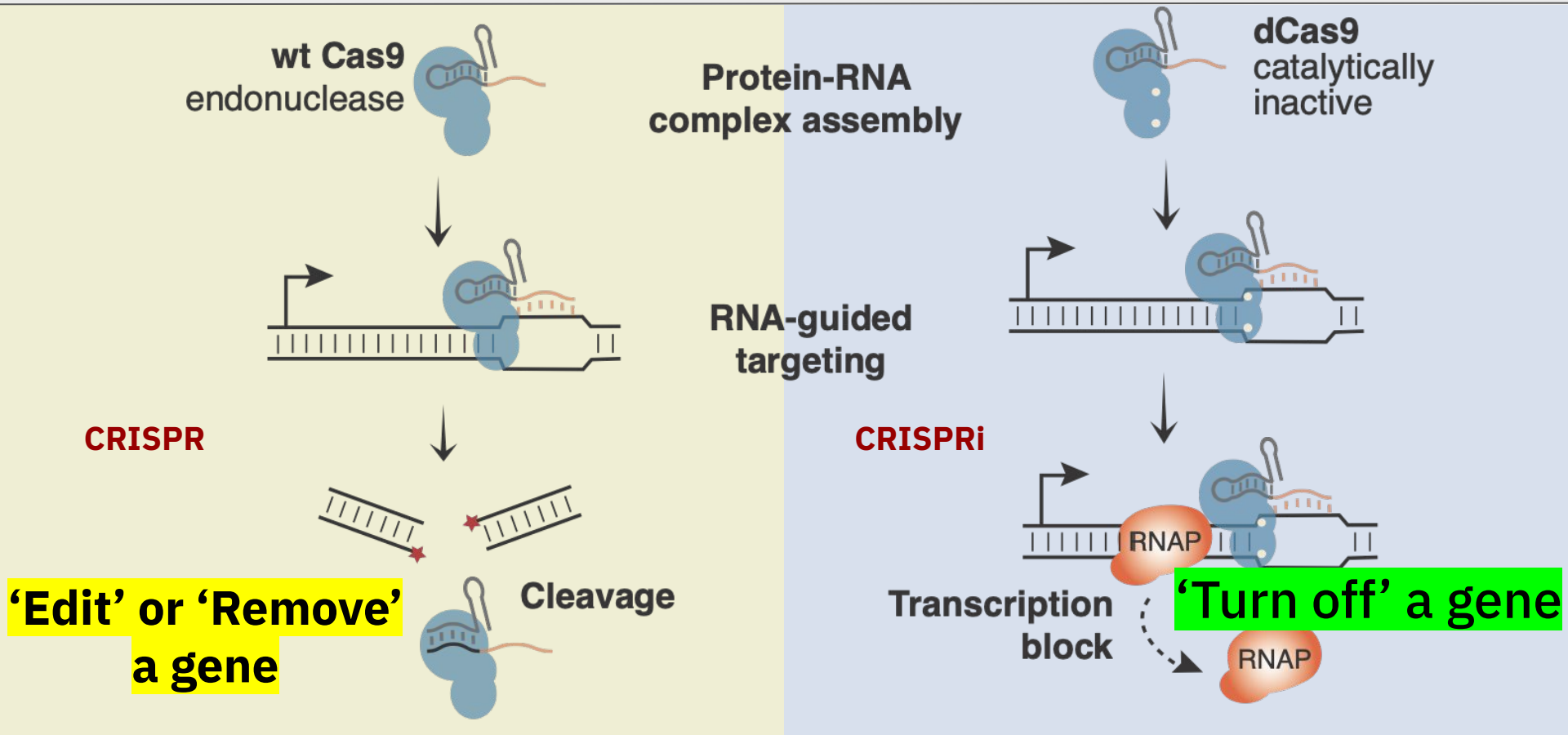
CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats



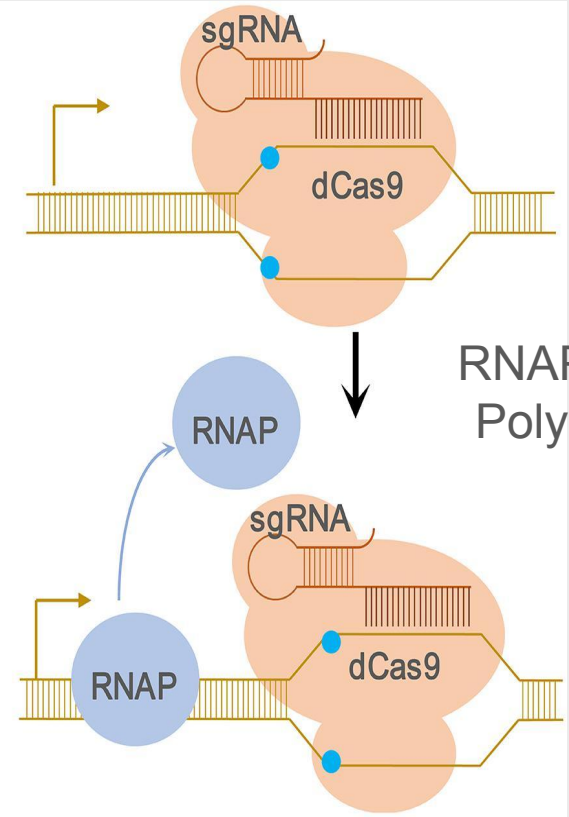
1. **Genome DNA**
   a. Target DNA - the DNA we would like to cut and introduce a mutation to
2. **sgRNA**
   a. A ~20bp RNA that forms complementary base pairing with the target DNA - think of it as a GPS system that recognises the target DNA and guides the Cas9 protein to cut the target DNA
3. **Cas9**
   a. A protein that forms a complex with the sgRNA. After the complex is formed, the sgRNA will lead the complex to the target DNA, and Cas9 will cut the target DNA by inducing a double-stranded break

CRISPRKIT

CRISPR has been used for gene editing or modulation (in research and clinics)



Introducing CRISPRi and dCas9



CRISPRi

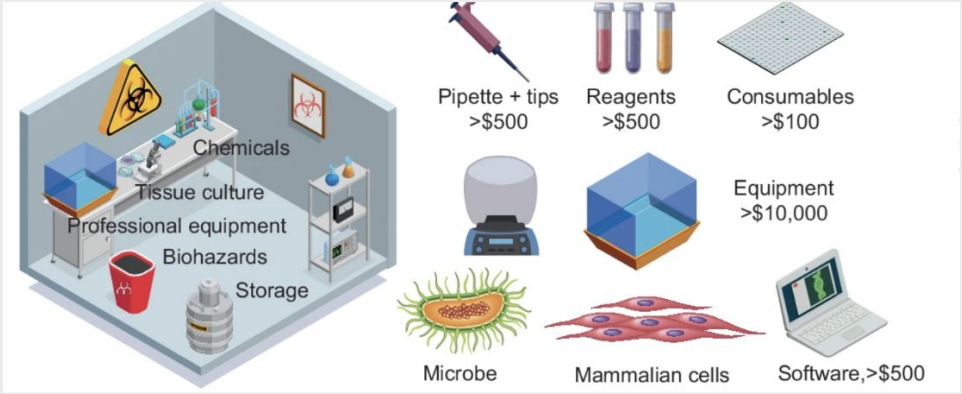
Benefits of using dCas9

- Reduces gene expression by blocking RNAP access to genome to initiate transcription
- Temporary and Reversible
- Dimmer vs On/Off Switch
- Less Complications

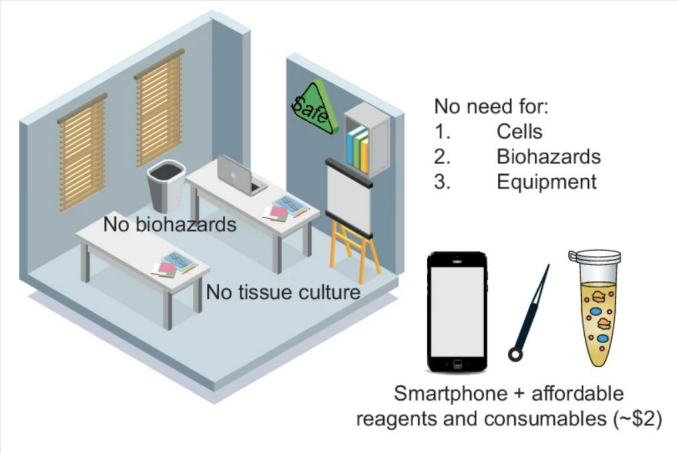
CRISPRkit is Accessible

- Affordable
- Safe
- Robust
- Anyone can use

Normal CRISPR Experiment in Lab Setting (~$20,000 +)

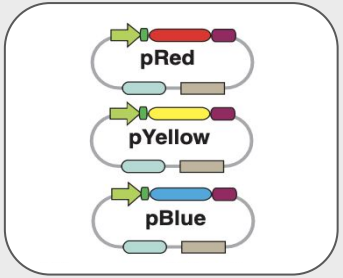


Our CRISPRkit Design (~$2)

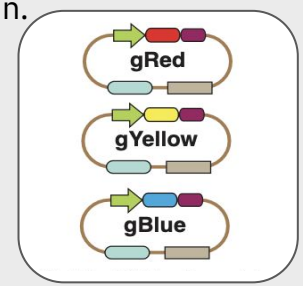


CRISPRkit

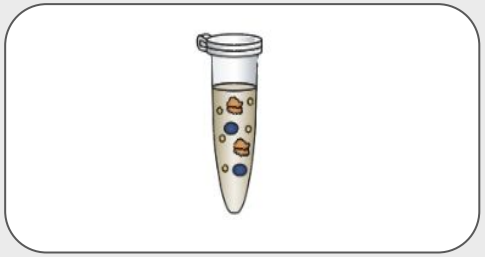
Components of CRISPRkit



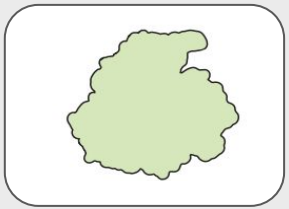
1. **Target Gene Plasmids**
   a. The target DNA we are trying to edit / repress with CRISPR. Could be any enzyme, or pigmentation protein.



2. **gRNA Plasmids**
   a. The 'GPS' system that finds the target DNA



Cell free system
a. An E.Coli extract solution where we add the **target gene plasmid**, **gRNA plasmid**, and **dCas9 protein** in. The cell-free machinery allows for transcription and translation of the plasmids for CRISPRi to occur



3. **dCas9 protein**
   a. Forms complex with gRNA, binds to target DNA, and blocks RNA Polymerase from accessing DNA for transcription

CRISPRkit

Let's Combine this knowledge into CRISPRkit...

- Here's our general workflow (Taking a Red Chromoprotein as example)

Components

R Chromoprotein Plasmid + **sgRNA plasmid Targeting R** (sequence **complementary** to R Chromprotein) + **dCas9 protein**

Expression System

Cell free system

Expected output

Gene repression achieved - R chromprotein not expressed

YES CRISPRi

R Chromoprotein Plasmid + **sgRNA plasmid Targeting another gene** (sequence **NOT complementary** to R Chromprotein) + **dCas9 protein**

Cell free system

No gene repression - R chromoprotein is expressed

NO CRISPRi