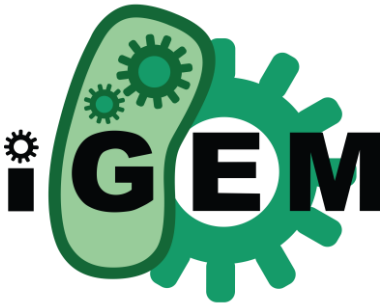
CRISPR-Cas System

Team NUS_Singapore-Sci
5th October 2018



CONTENT

1. Introduction to CRISPR-Cas System
2. Application of CRISPR-Cas9
    - Gene editing and beyond
    - Case Study
3. Other CRISPR family Proteins
    -Nucleic acid detection
    -RNA editing
4. Ethical Concerns

Discovery of CRISPR

Francisco Mojica

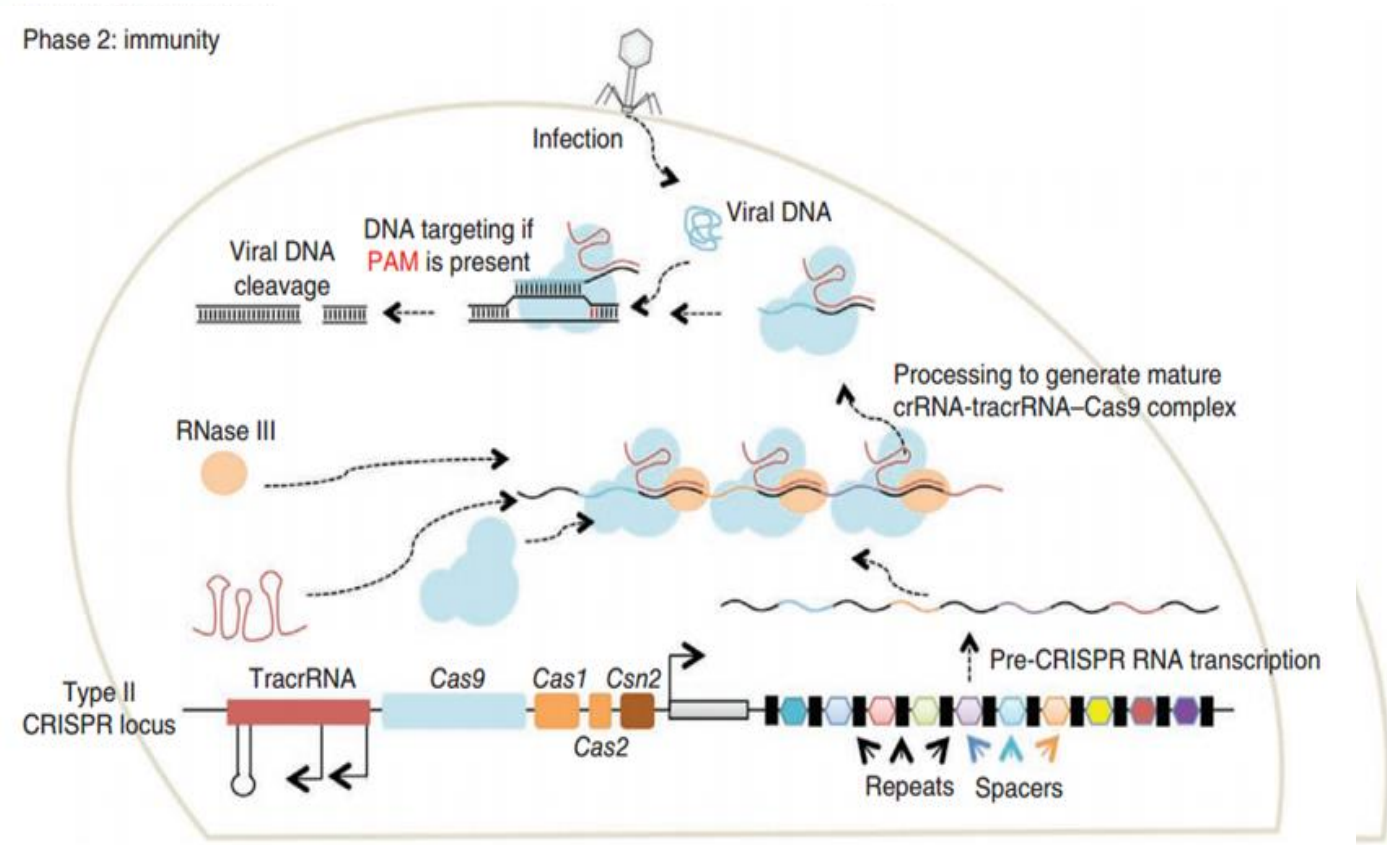
- First discovered in archaea and later in bacteria
- Part of the bacteria immune system against invading viruses
- Comprise repeating sequences, with “spacer” sequences in the middle

Natural Function of CRISPR: Immune Response with Memory in Bacteria

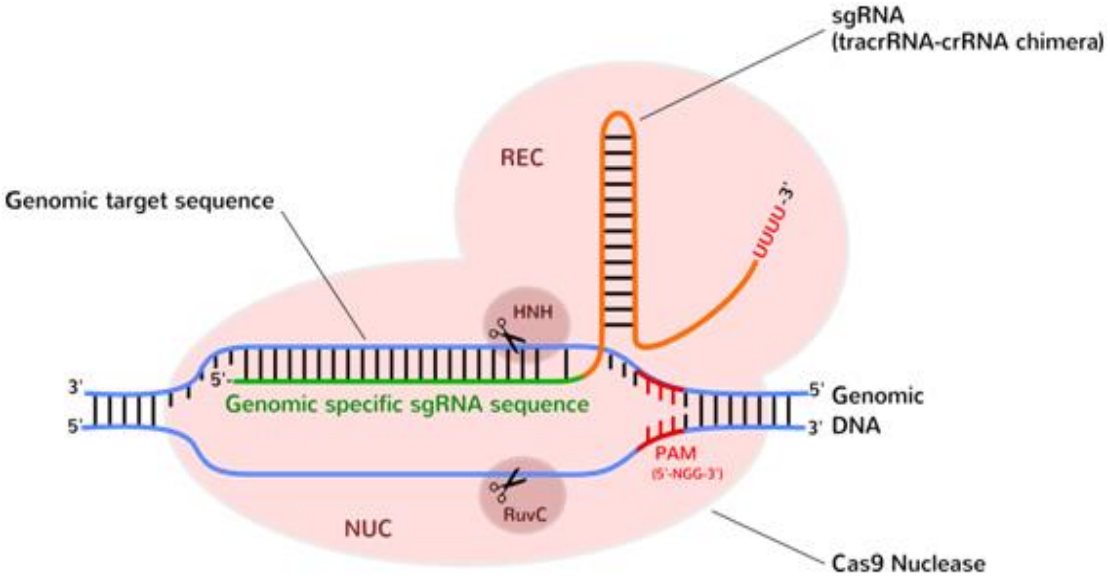
- "Spacer" sequences
  - When phage injects its DNA into bacteria cell, a short sequence is inserted between the CRISPR sequence of bacterial DNA
  - Constitute a library of pathogens encountered by bacteria
  - Pass down this information to offspring

Bacteria use CRISPR-Cas system to recognise viral infections and protect themselves from renewed infection → Memory

Natural CRISPR Pathway

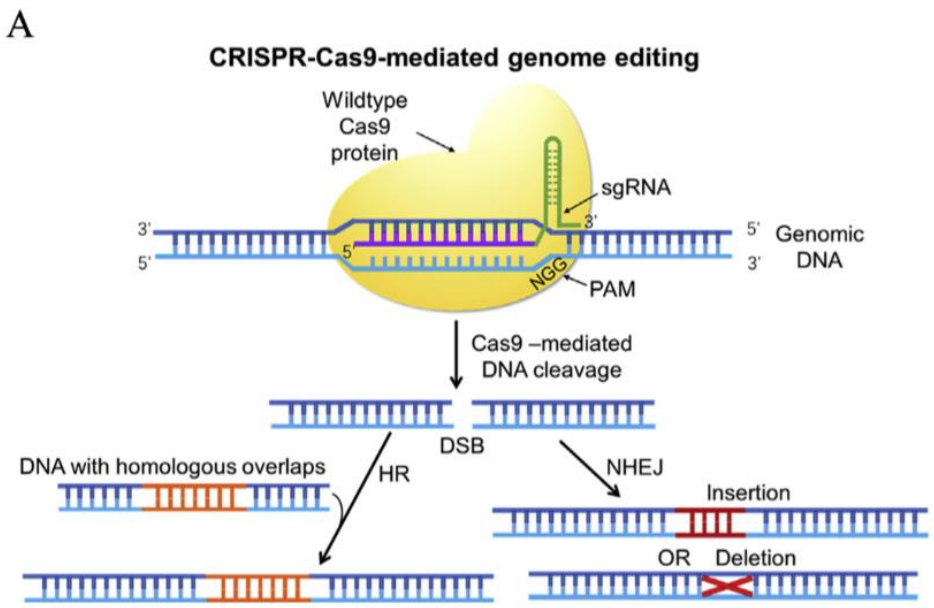


CRISPR Gene Editing



- In gene editing, a **single guide RNA (gRNA)** is created
- Behaves as tracrRNA to recognise region of interest
- **PAM**: 3-5 nucleotide sequence serve as binding signal for Cas9
- Allow formation of DNA-RNA hybrid

CRISPR Gene Editing



Cas9 cuts both DNA strands using

1. Non-homologous end joining (NHEJ)

2. Homology directed repair

Result in

- Gene silencing
- Insertion of new gene products

What about dCas?

CRISPR-Cas genome editing and Beyond? (1:37)

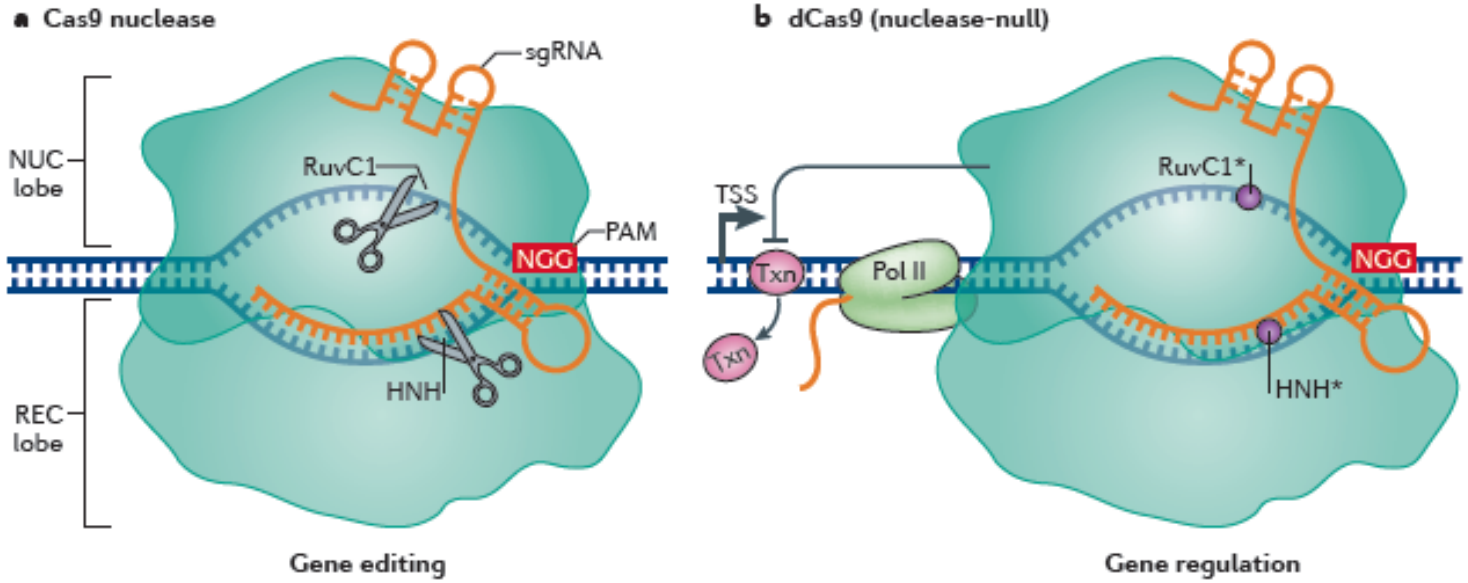
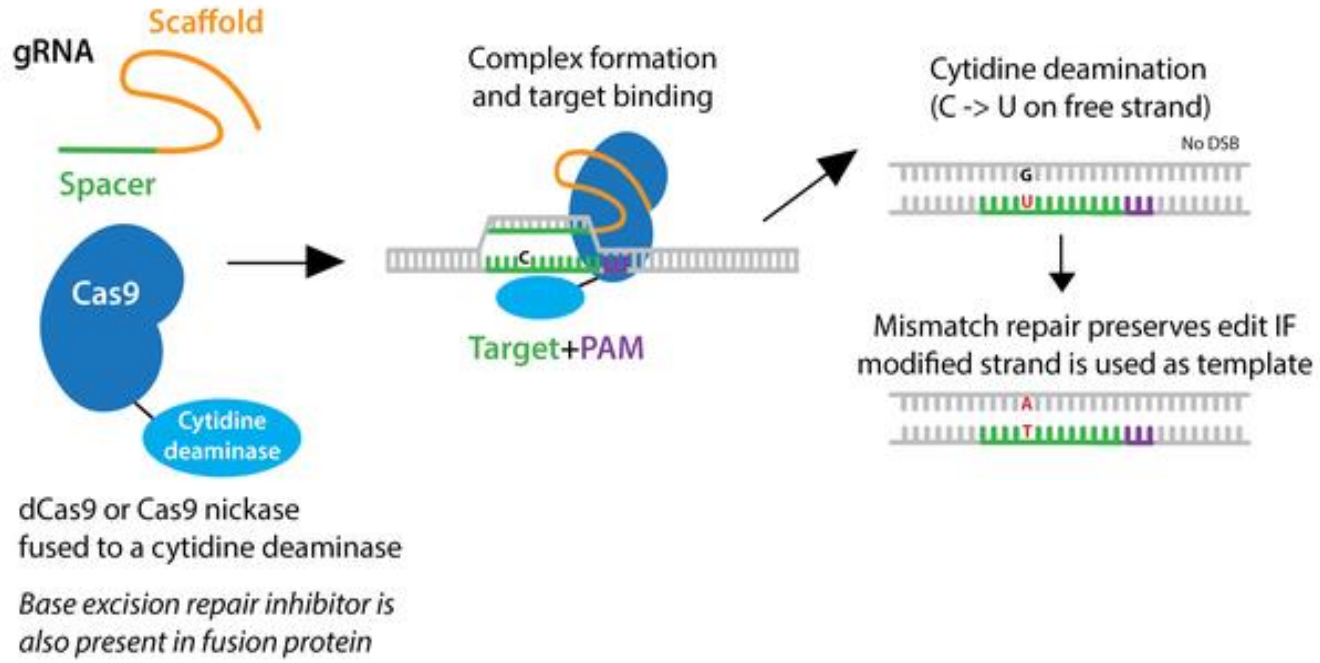


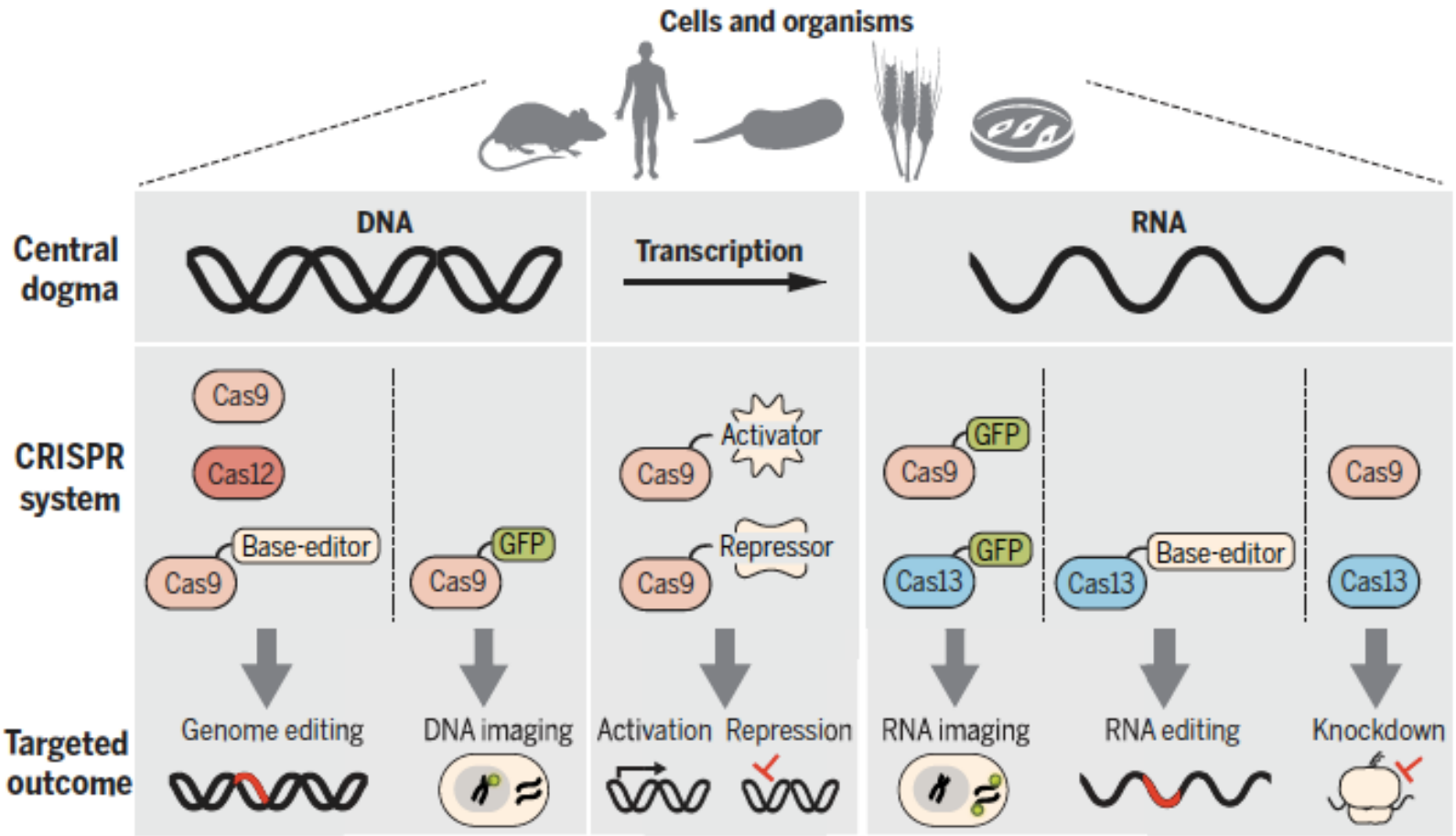
Figure 1 | **Gene editing versus gene regulation using *Streptococcus pyogenes* Cas9 and dCas9. a** | The *S. pyogenes*

- Introducing mutations into the S. pyogenes Cas9 in its two nuclease domains, HNH and RuvC.
- The resulting nuclease-deficient (dCas9) is unable to cleave DNA but retains the ability to specifically bind to DNA when guided by a sgRNA.

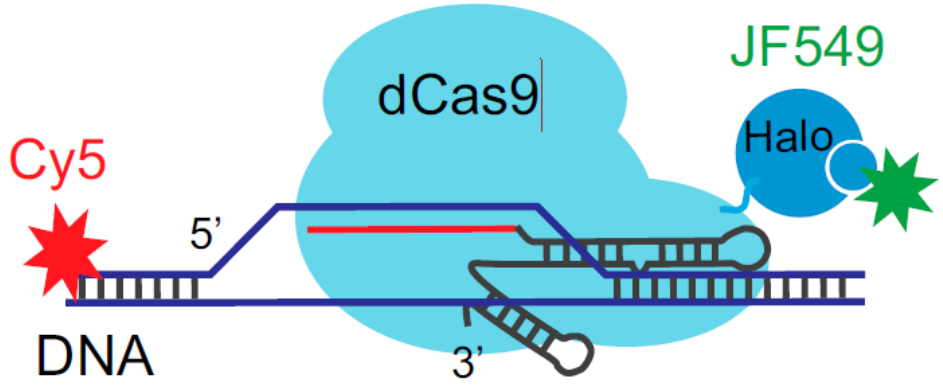
CRISPR-dCas9 Base Correction

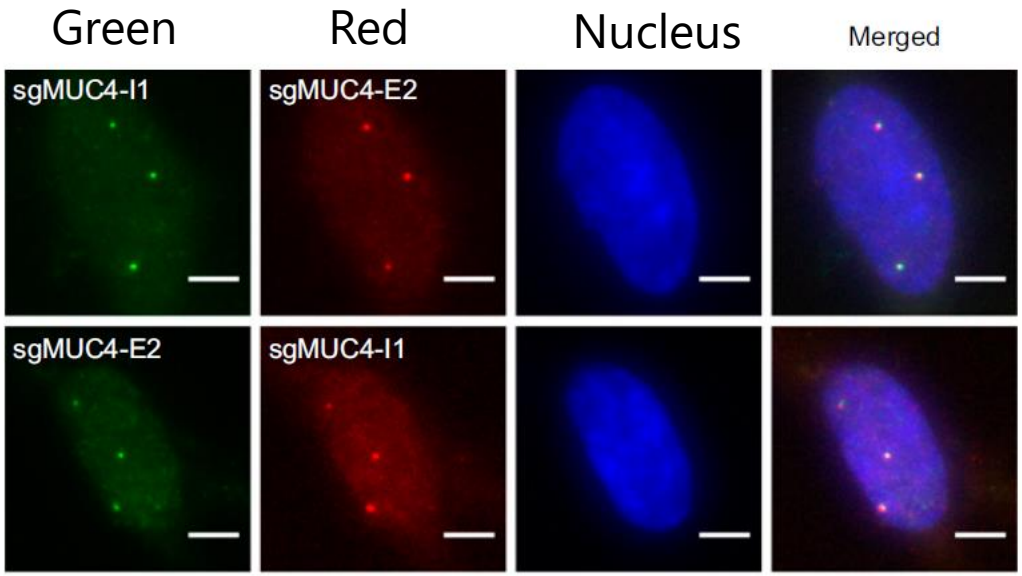


Summary of CRISPR-Cas system (applications)



In situ Labelling using CRISPR-Cas9





Deng, Wulan, et al. "CASFISH: CRISPR/Cas9-mediated in situ labeling of genomic loci in fixed cells." Proceedings of the National Academy of Sciences 112.38 (2015): 11870-11875.

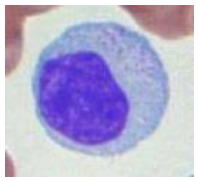
Clinical Trial

Phase I Trial of Autologous T Cells Engineered to Express NY-ESO-1 TCR and Gene Edited to Eliminate Endogenous TCR and PD-1

For Who?	Melanoma, Synovial sarcoma and Multiple myeloma patients, for whom there are no effective therapies
Purpose	Assess the safety and dosage of a novel intervention
End Points	Safety, feasibility, clinical assessment of anti-tumor responses and survival, as well as an examination of T cell bioactivity, immunogenicity
Possible Side Effects	Graft-versus-host disease, where the therapeutic T cells that kill the cancer also attack the patient's organs

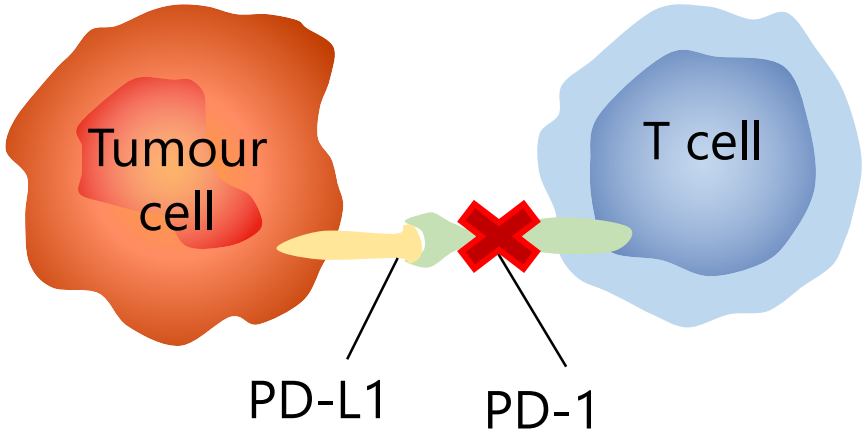
Baylis, F., & McLeod, M. (2017). First-in-human Phase 1 CRISPR Gene Editing Cancer Trials:Are We Ready? *Current Gene Therapy*, *17*(4), 309–319. http://doi.org/10.2174/1566523217666171121165935

Clinical Trial



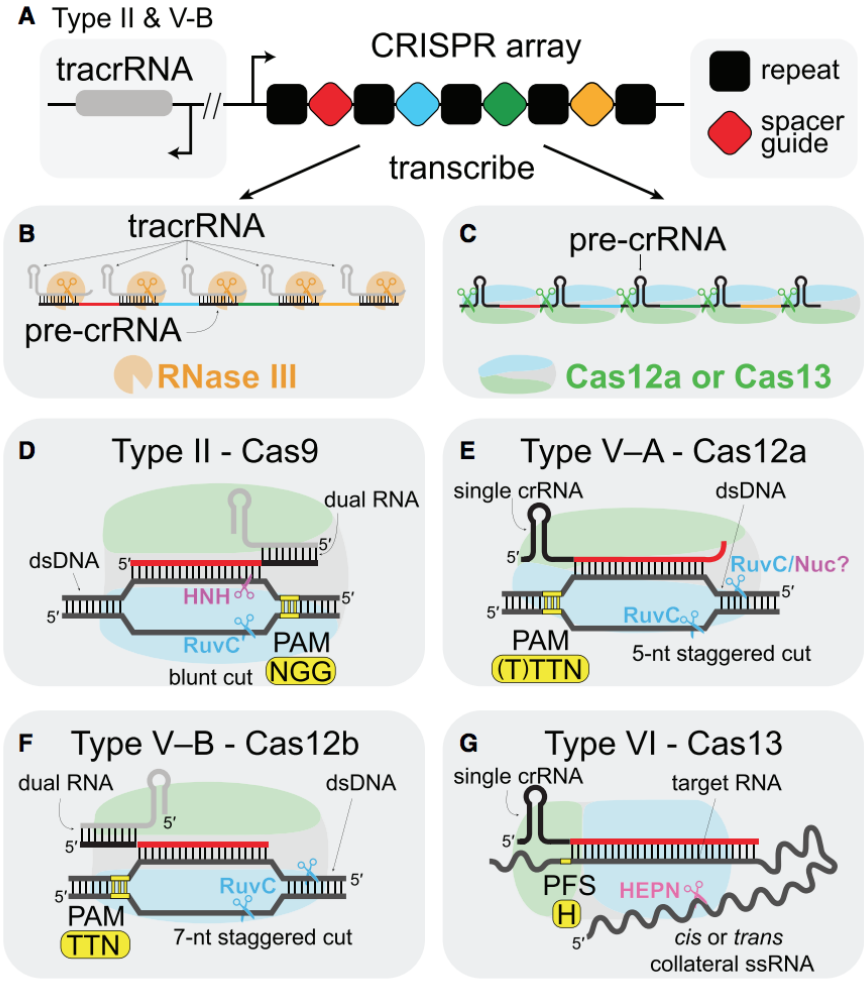
Collection of Peripheral Blood Lymphocytes

Engineer T cells' genome such that their activity wouldn't be suppressed by tumour cells



Patients undergo lympho-depleting chemotherapy and then, receive a single dose of CRISPR edited T cells

Other CRISPR Family proteins



Cas 13a, b, c: type VI CRISPR-associated RNA-guided ribonu clease (RNase)

-Cas 12a (Cpf1): type V-A CRISPR effector RNA-guided DNA endonuclease (DNase)

Discussion Question

Background:

Mosquito transmitted Viral Disease

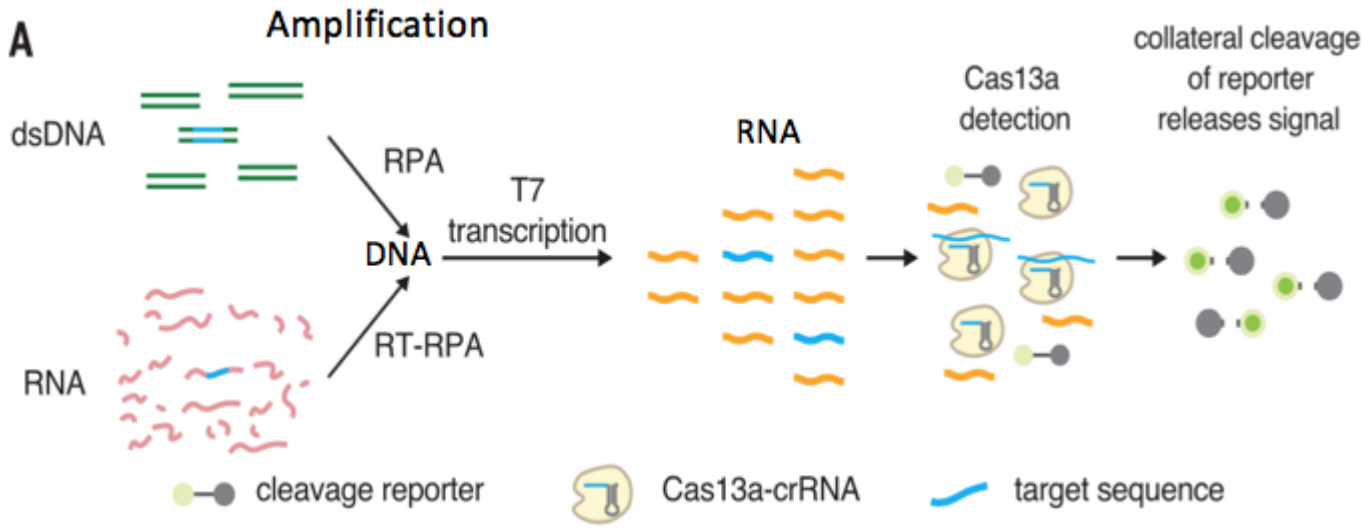
- Dengue Fever
- Zika Virus: Microcephaly of baby
- What do you think are the features that a diagnostic device should have ?

Rapid, sensitive detection

Portable, visual readout

Nucleic Acid detection: SHERLOCK (2017,2018)

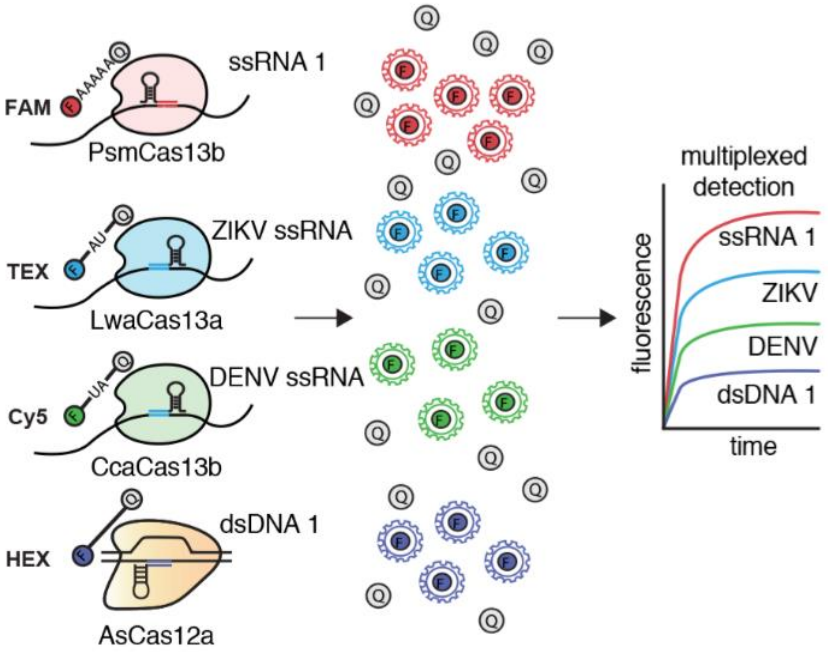
Specific High-Sensitivity Enzymatic Reporter UnLOCKing



Detect DENV, ZIKV from human liquid biopsy (blood, saliva, urine)

Detect EGFP mutation in lung cancer

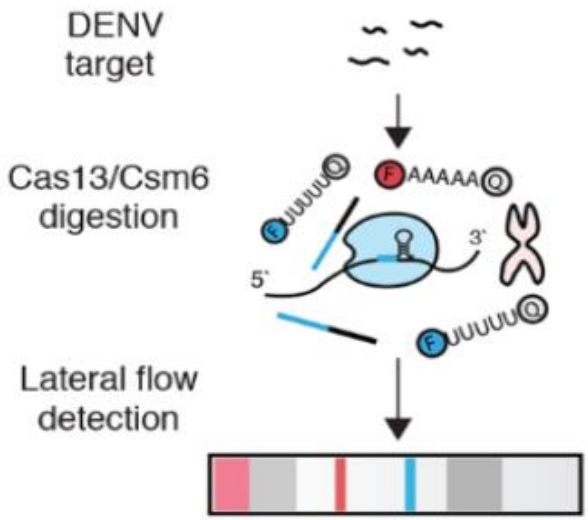
Further improvement on SHERLOCK : Multiplex detection : 4 targets at one time

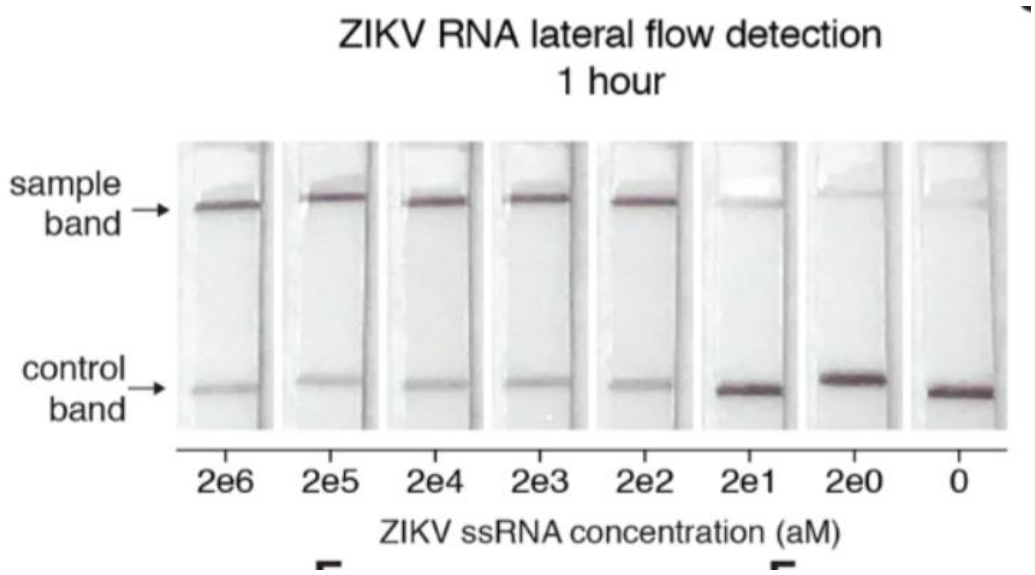


Challenge: Same Cas13 will cleave reporter no matter which target it encounters

Solution: Unique dinucleotide cleavage preference of different Cas13a and Cas13b family proteins-- independent detection

Further improvement on SHERLOCK : Instrument Free Detection

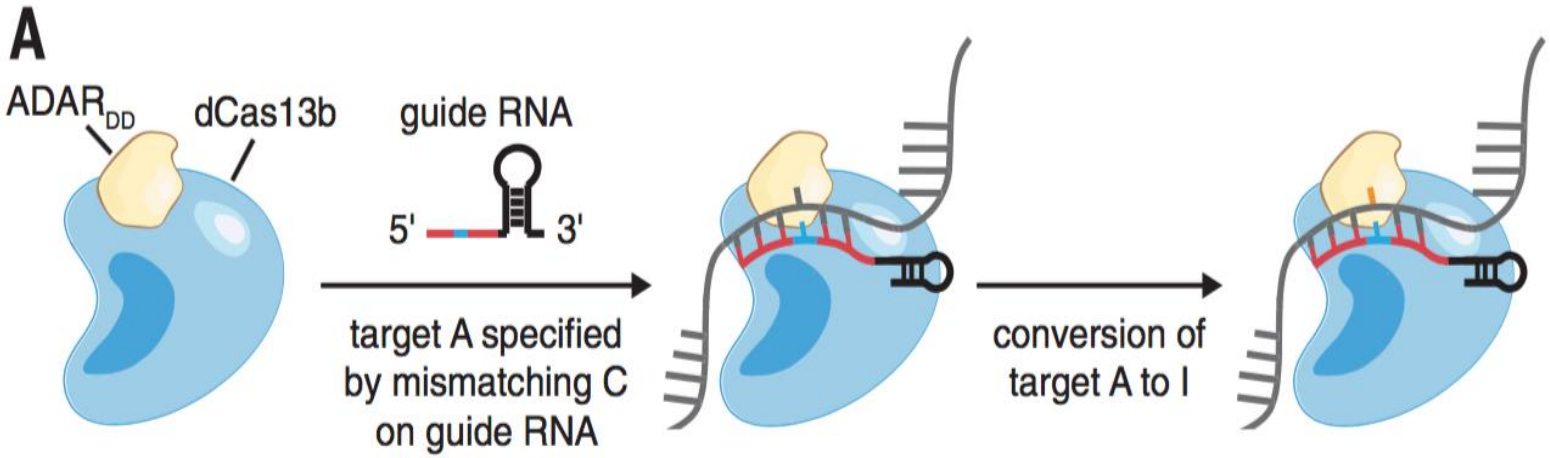




-Portable system, not reliant on fluorescent detector system anymore

-90min sensitivity to 2aM for DENV and ZIKV ssRNA

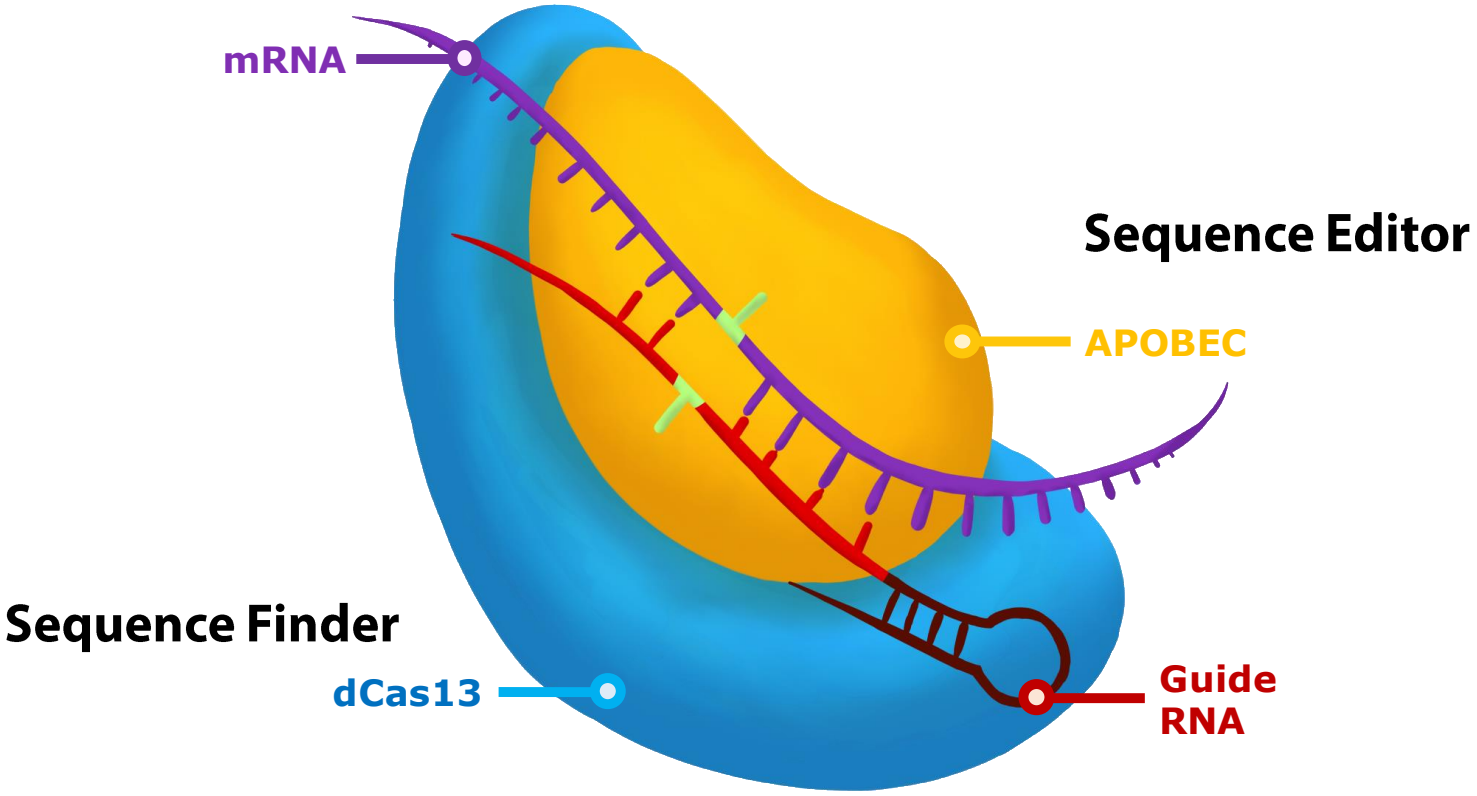
Cas13b: RNA editing (2017)



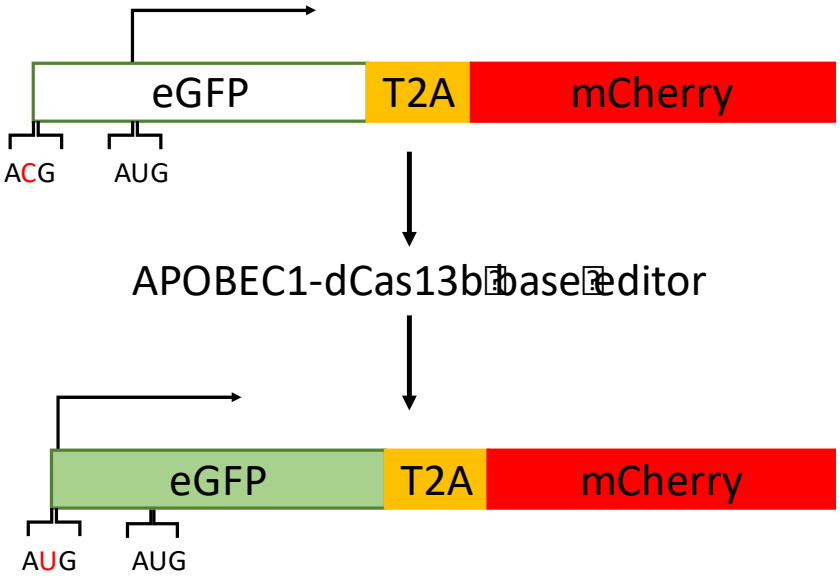
Change protein expression level without changing Genome!

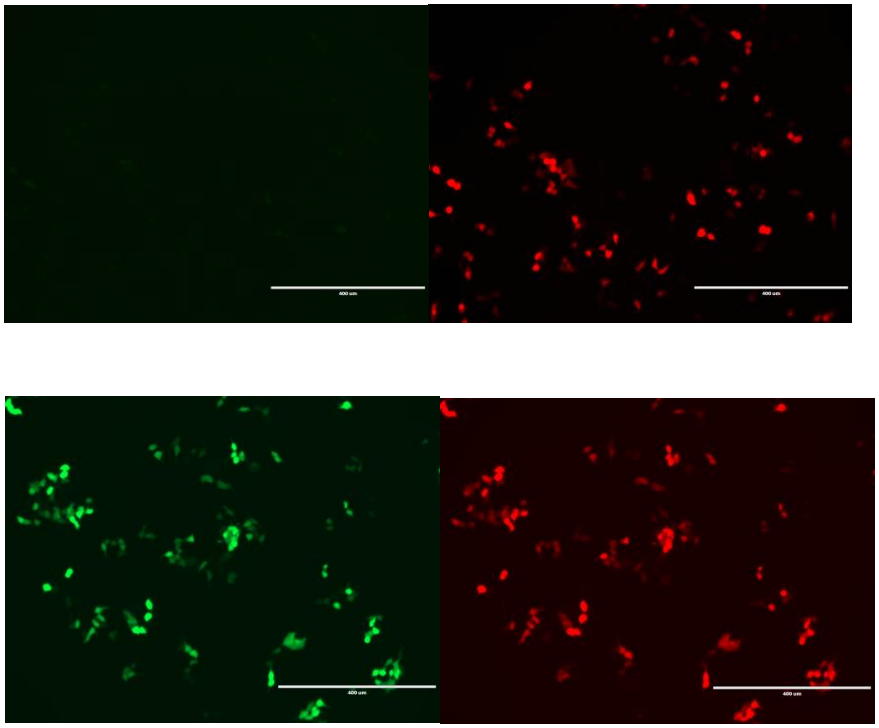
Cas13b: RNA editing (our iGEM project)

dCas13-**APOBEC** guided Cytosine to Uracil RNA editing



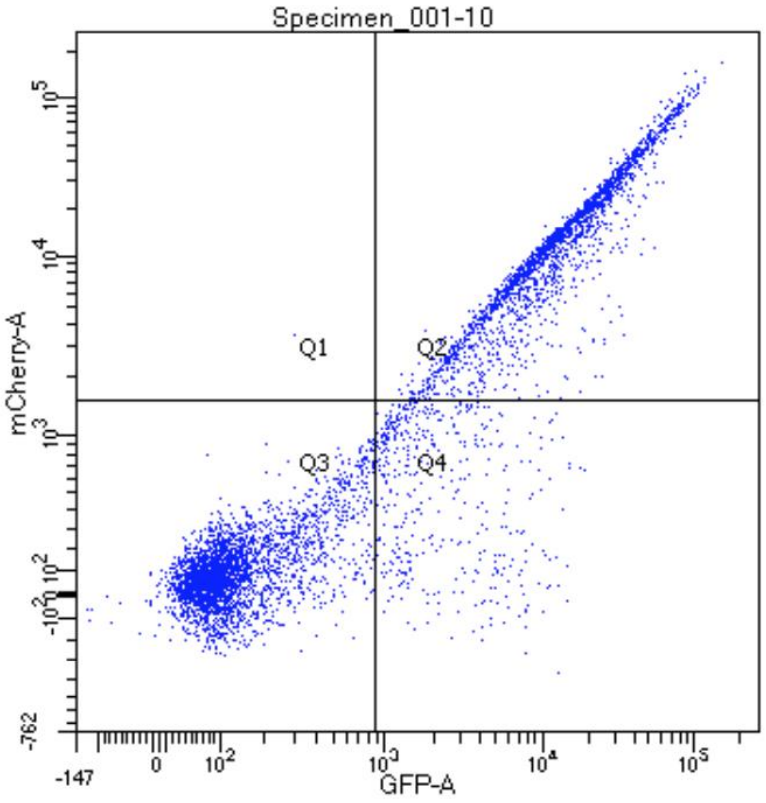
Cas13b: RNA editing (our iGEM project)



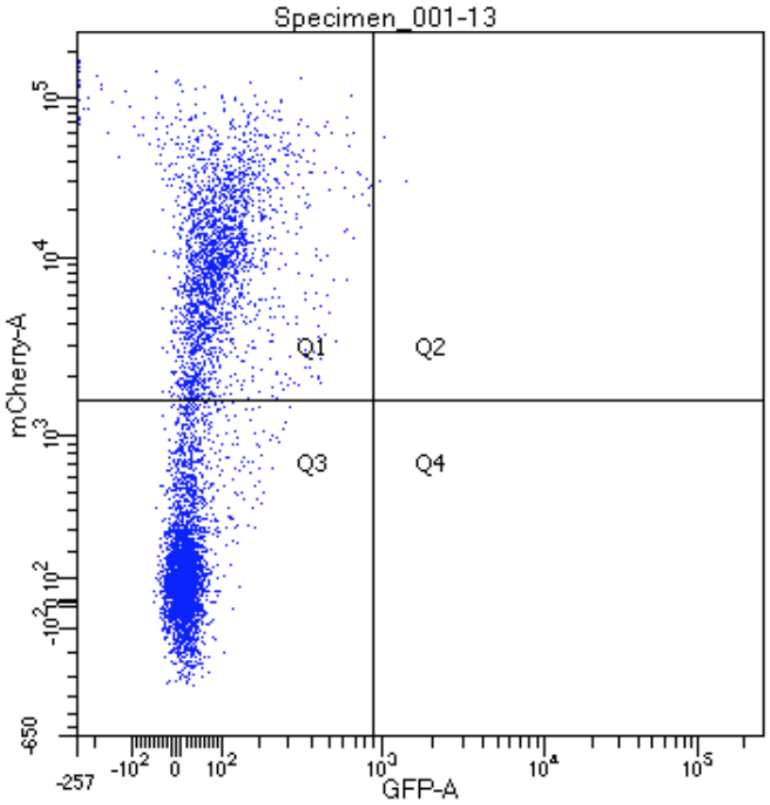


Cas13b: RNA editing (our iGEM project)

Wild type eGFP-T2A-mCherry



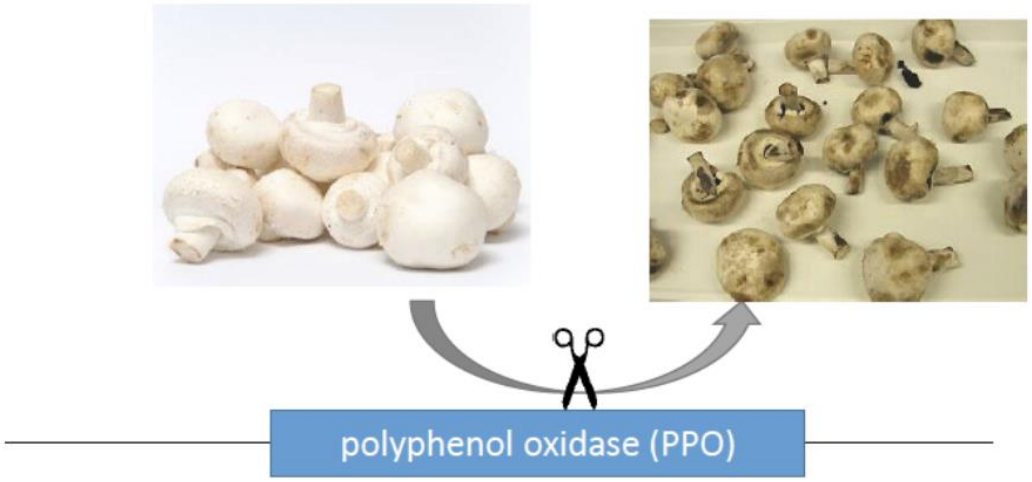
ACG-mut



CRISPR not consider as GMO?

NATURE | NEWS

Gene-edited CRISPR mushroom escapes US regulation



- Polyphenol oxidase (PPO) causes browning of mushrooms during storage
- CRISPR to introduce mutations to 1 out of 6 *PPO* genes
- 30% reduced activity
- Prolonged storage time

Waltz, Nature 2016

NATURE | NEWS

Gene-edited CRISPR mushroom escapes US regulation

- US Department of Agriculture (USDA) will not regulate a CRISPR modified mushroom (*"No foreign DNA present"*)
- Cultivated and sold without passing through the agency's regulatory process
- First CRISPR-edited organism to be approved

Waltz, Nature 2016

Ethical issues - Safety

- Off- target sequences
- Mosaicism

How do we increase specificity of editing?

What is a good threshold for risk?

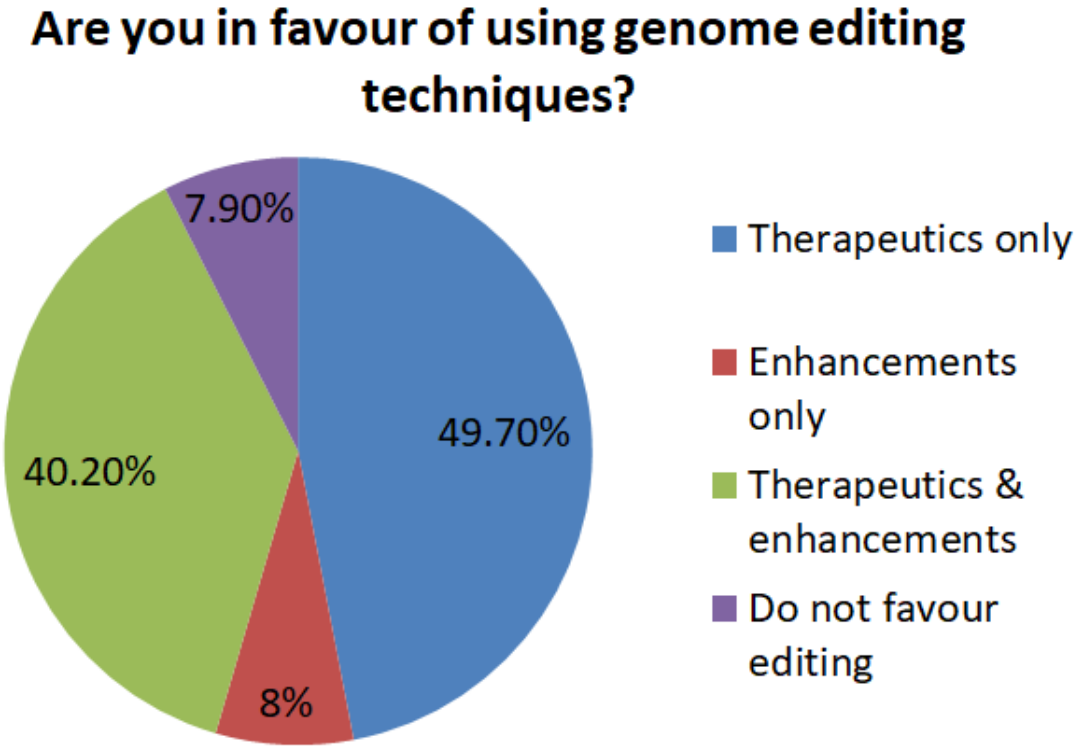
Ethical issues – Justice & Equity

- Accesibility issues
- Possible abuse of technology for enhancements
- Perpetuating disparities

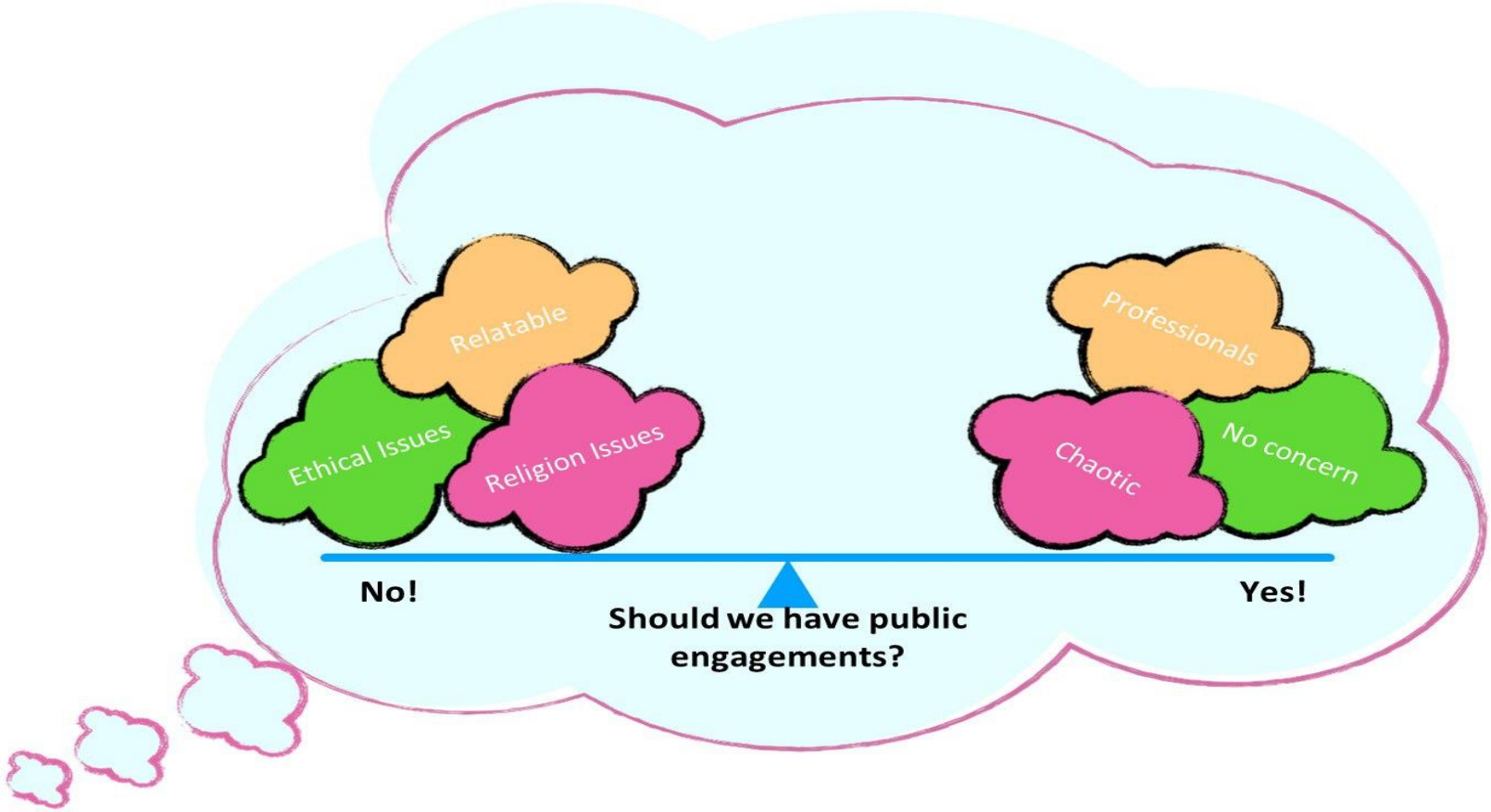
What will genetic enhancement refer to?

Is it possible to make a baby smarter at birth?

Survey Results

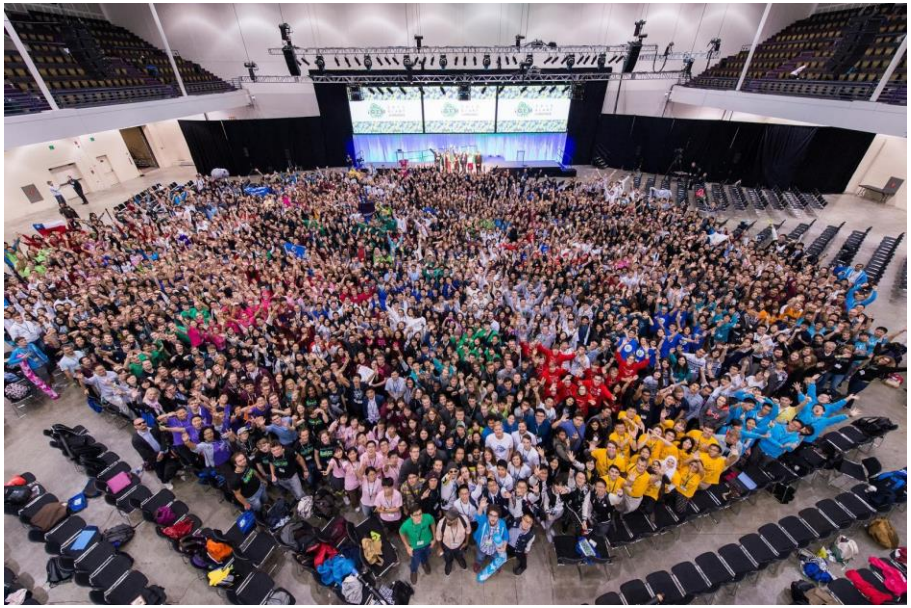


Survey Results



What is iGEM

- The premiere synthetic biology competition for students all over the world.
- Present in October in **Boston, USA**



Our Team

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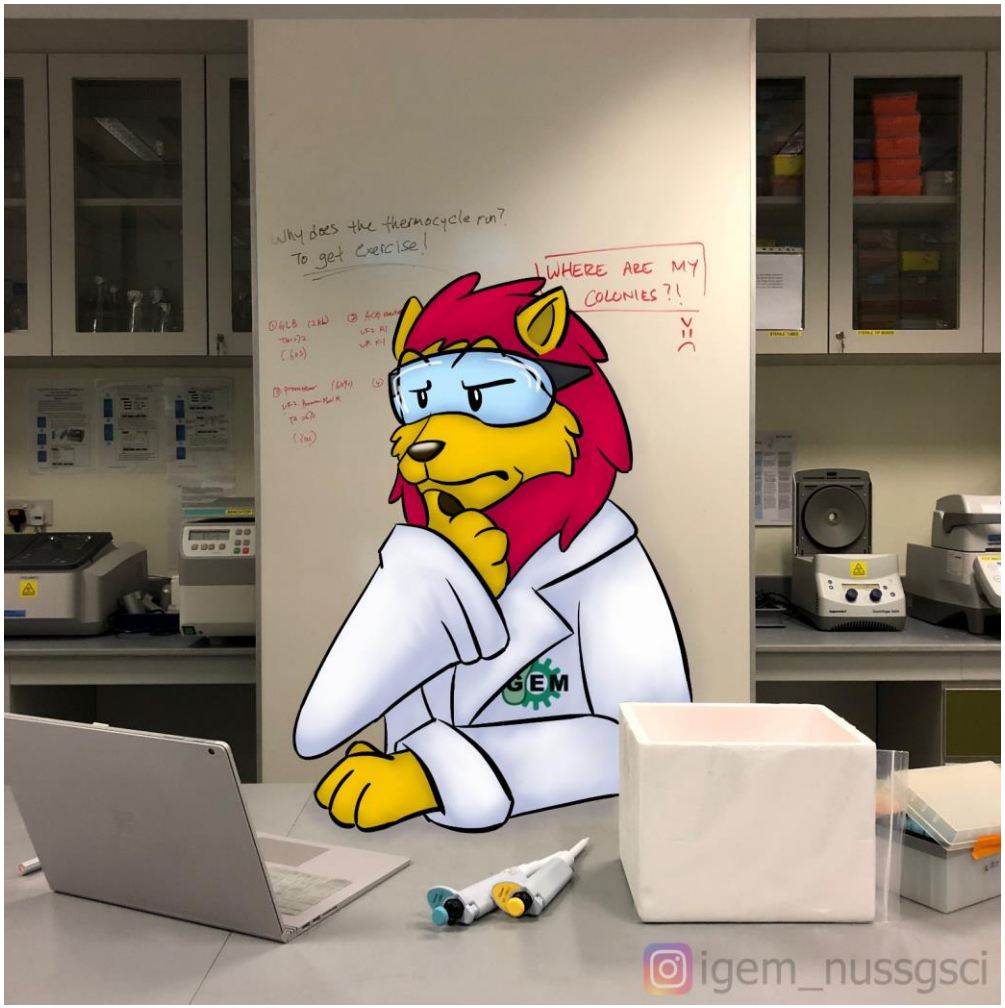

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