iGEM Global
CRISPR Conference

iGCC
iGEM GLOBAL CRISPR CONFERENCE

naMOOste

iGEM IISER Kolkata

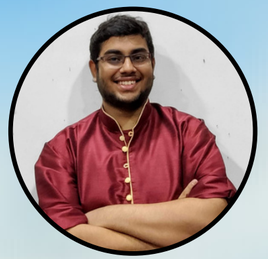
Tackling Sub-Clinical Bovine Mastitis using SynBio

iGEM IISER KOLKATA

iGEM

Promega

The Team

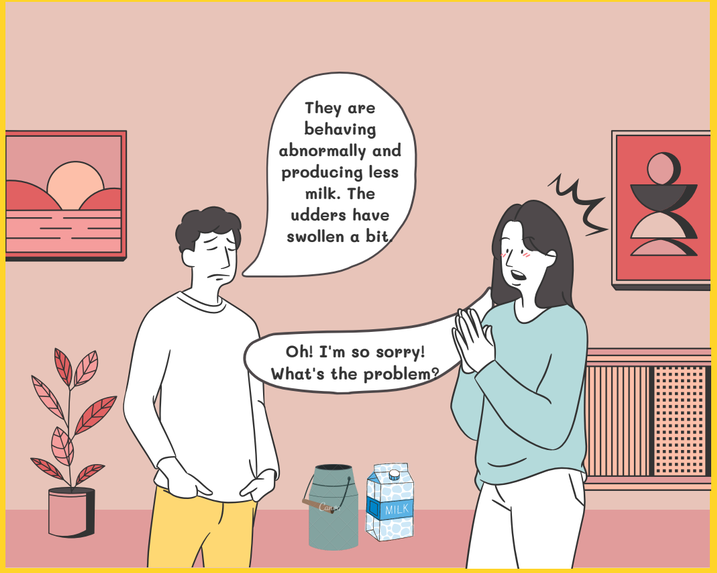


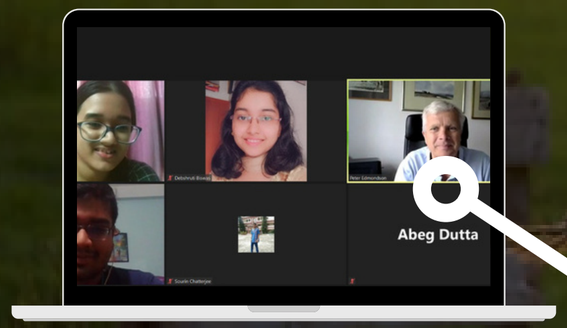






HOW WE MET BOVINE MASTITIS





Veterinary Scientists

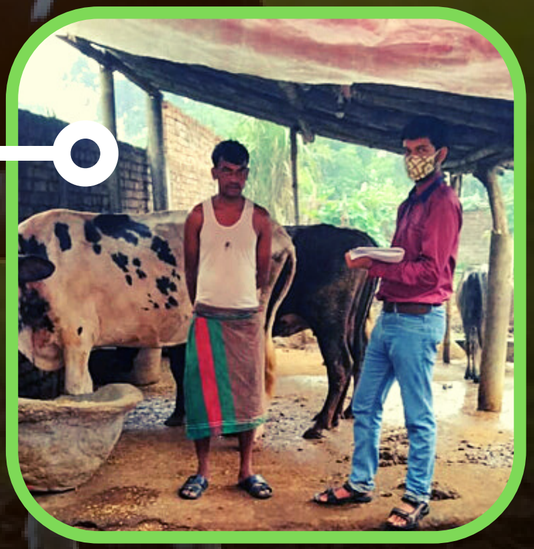
Local milkpersons

Veterinary Doctors

Contacting stakeholders

Small scale dairy farmers

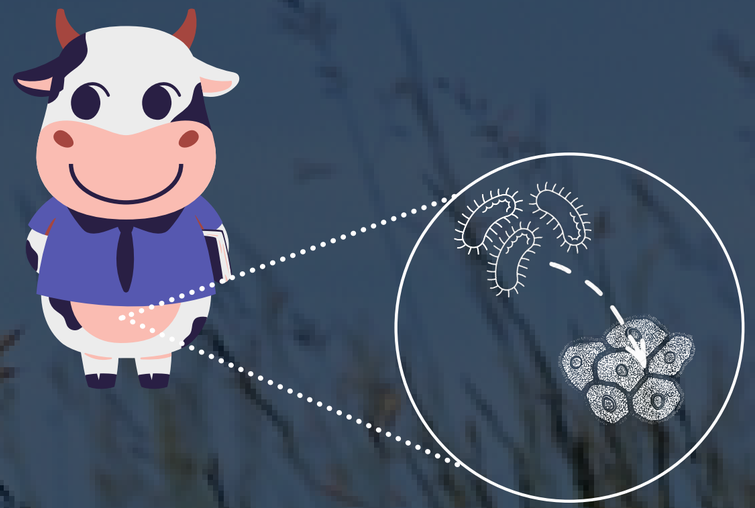
Large scale dairy farmers



Bovine Mastitis

Inflammation of Parenchyma of mammary glands

pathogens invade the udder tissue of mammary glands - multiply - attack host immune system



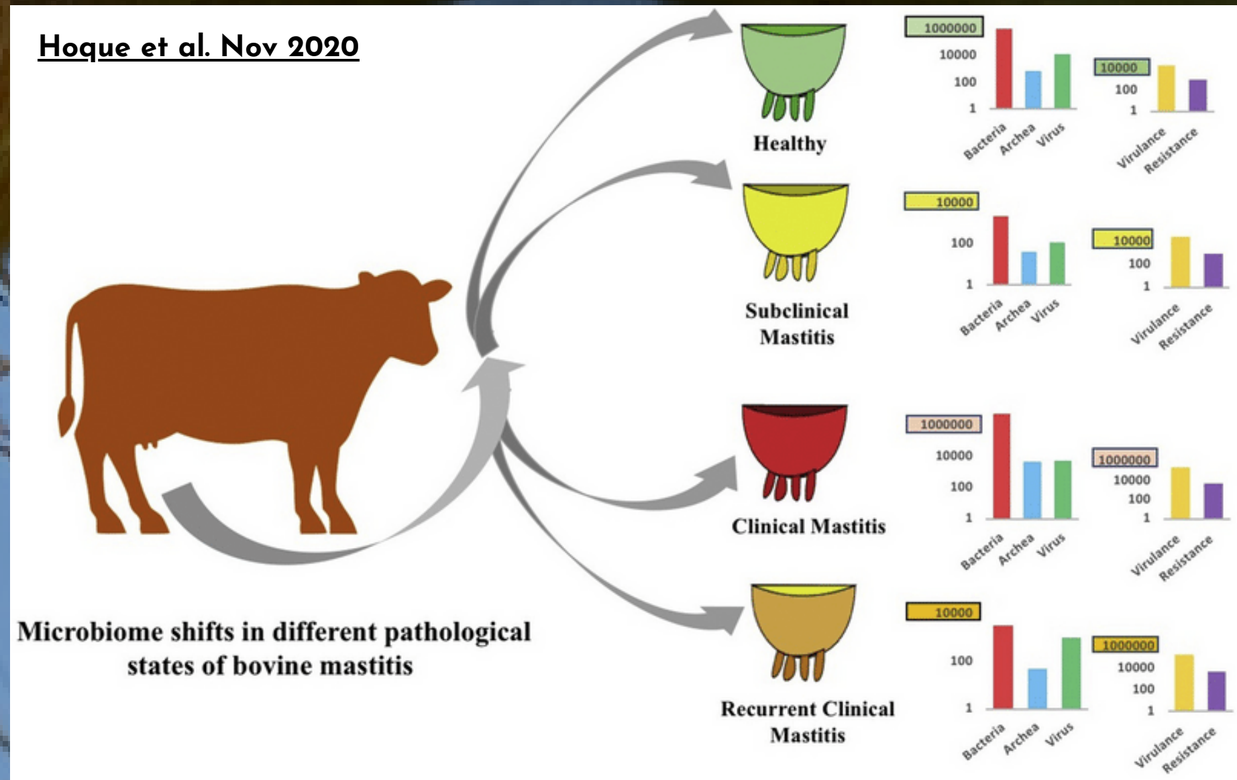
Bovine Mastitis

Inflammation of Parenchyma of mammary glands

pathogens invade the udder tissue of mammary glands - multiply - attack host immune system

Bovine Mastitis

Stages



Bovine Mastitis

Pathogens

Staphylococcus aureus

Streptococcus uberis

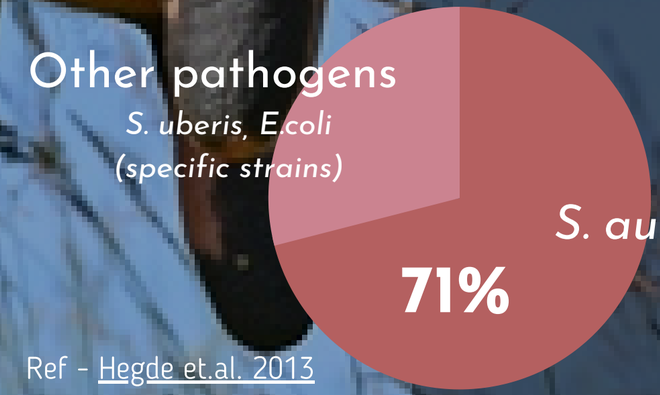
Corynebacterium spp.

Klebsiella spp.

Ref - Birhanu et.al. 2017

Bovine Mastitis

Pathogens



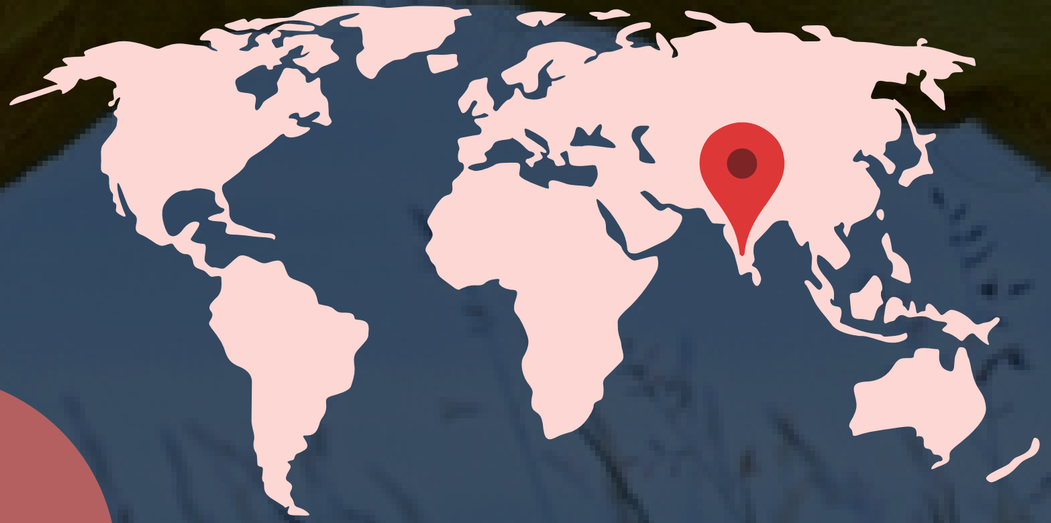
Ref - Hegde et.al. 2013

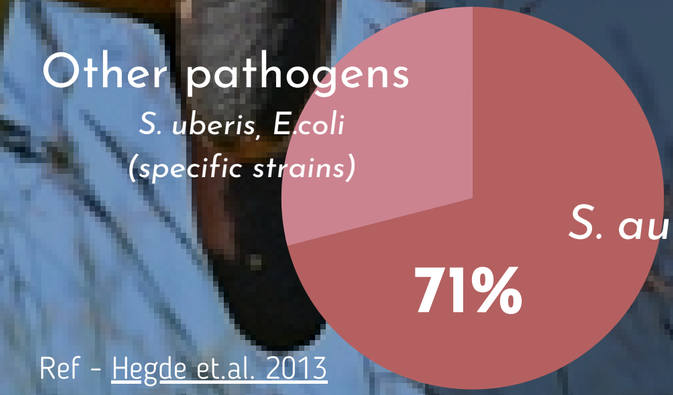
Bovine Mastitis

Dr. Indranil Samanta

Dr. Peter Edmondson

Pathogens





Ref - Hegde et.al. 2013

Problems faced due to bovine mastitis

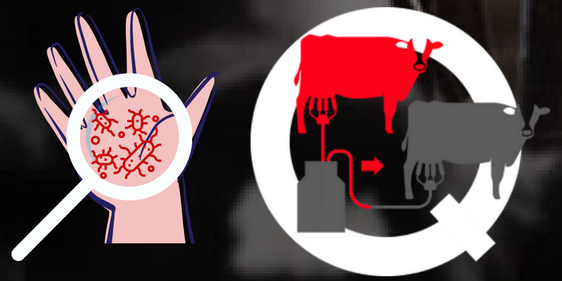
TRANSMISSION

DETECTION

TREATMENT

ECONOMIC LOSSES

- No visible symptoms in early stage for detection
-



Problems faced due to bovine mastitis

TRANSMISSION

DETECTION

TREATMENT

ECONOMIC LOSSES

redness
pain
heat
hardness

clots
pus
flakes
watery
appearance

Problems faced due to bovine mastitis

TRANSMISSION

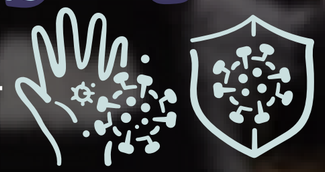
DETECTION

TREATMENT

ECONOMIC LOSSES

infection spread to humans

can lead to epidemics in future if left unchecked

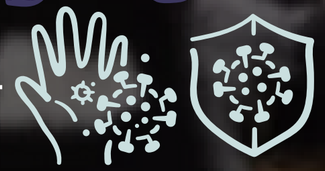


Problems faced due to bovine mastitis

TRANSMISSION

infection spread to humans

can lead to epidemics in future if left unchecked



DETECTION

- Current detection methods are ineffective
- Cheap, easy to use, portable kits unavailable for timely detection

TREATMENT

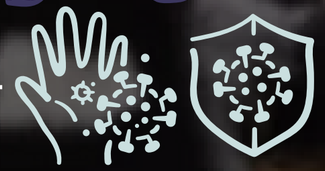
ECONOMIC LOSSES

Problems faced due to bovine mastitis

TRANSMISSION

infection spread to humans

can lead to epidemics in future if left unchecked

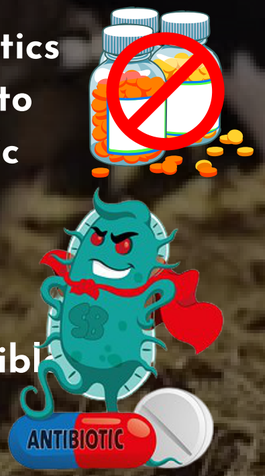


DETECTION

- Current detection methods are ineffective
- Cheap, easy to use, portable kits unavailable for timely detection

TREATMENT

- NO to Antibiotics
- Very difficult to treat in chronic stage
- Antibiotic resistance
- Costly inaccesibl treatment



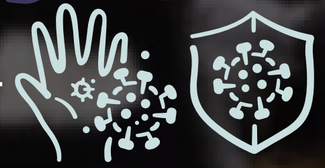
ECONOMIC LOSSES

Problems faced due to bovine mastitis

TRANSMISSION

infection spread to humans

can lead to epidemics in future if left unchecked



DETECTION

- Current detection methods are ineffective
- Cheap, easy to use, portable kits unavailable for timely detection

TREATMENT

- NO to Antibiotics
- Very difficult to treat in chronic stage
- Antibiotic resistance
- Costly inaccesibl treatment

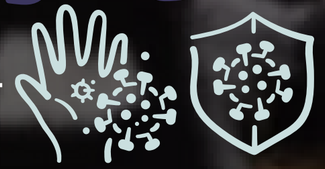
ECONOMIC LOSSES

Problems faced due to bovine mastitis

TRANSMISSION

infection spread to humans

can lead to epidemics in future if left unchecked

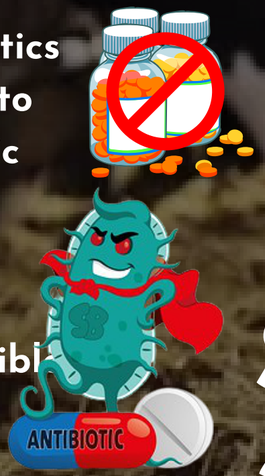


DETECTION

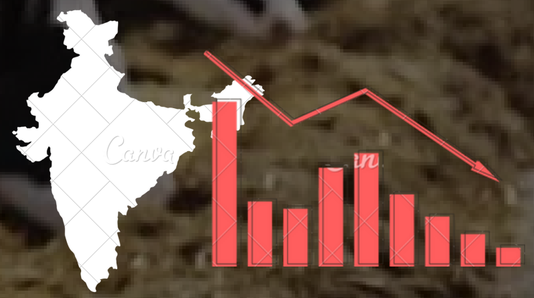
- Current detection methods are ineffective
- Cheap, easy to use, portable kits unavailable for timely detection

TREATMENT

- NO to Antibiotics
- Very difficult to treat in chronic stage
- Antibiotic resistance
- Costly inaccesibl treatment



ECONOMIC LOSSES



$300 Million

2.58L less milk per day per mastitis affected cow

"Prevention is better than cure"

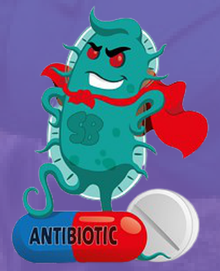
Diagnosis

Easy to use
Point of care

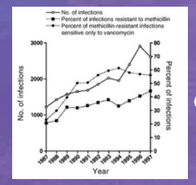
Cheap

Portable

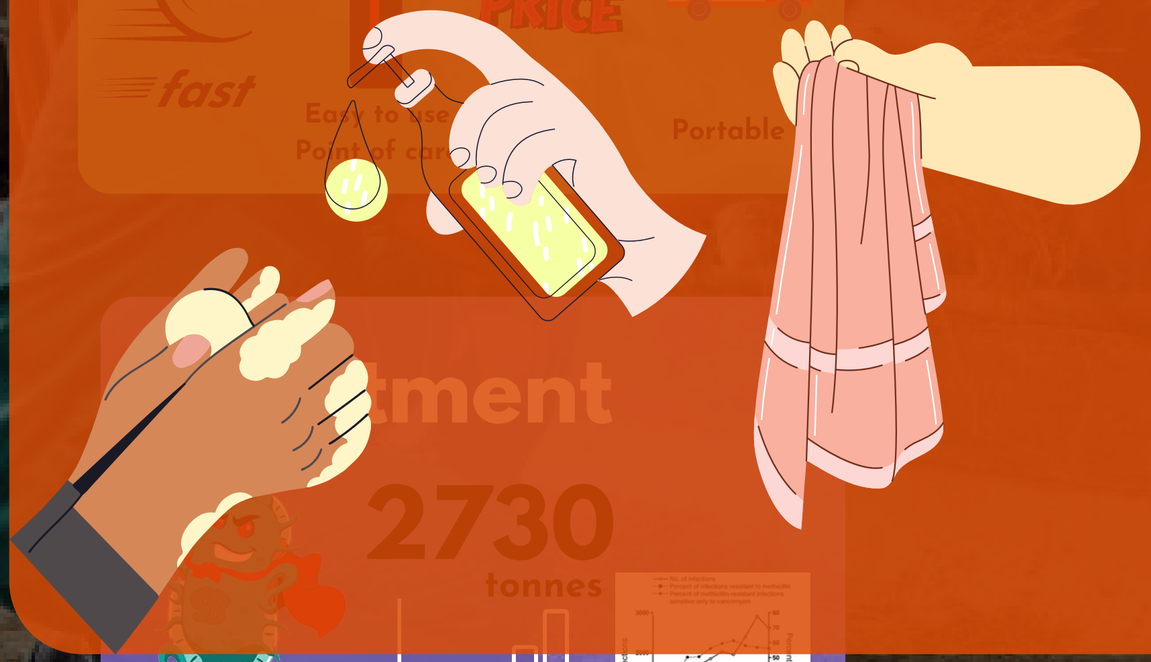
Treatment



2730
tonnes



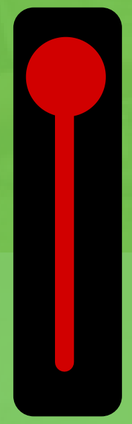
"Prevention is better than cure"



"Prevention is better than cure"

Diagnosis

Easy to use
Point of care



Cheap

Portable

DETECTION

Detecting the disease

bta - miR-7863

Staph. aureus

24 folds

bta - miR-21-3p

Strep. uberis

2.5 folds

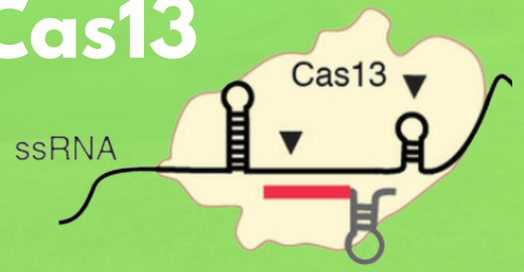
miRNA-223

Staph. aureus
Strep. uberis

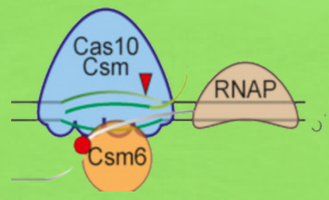
What we intend to use

Sherlock V2

Cas13

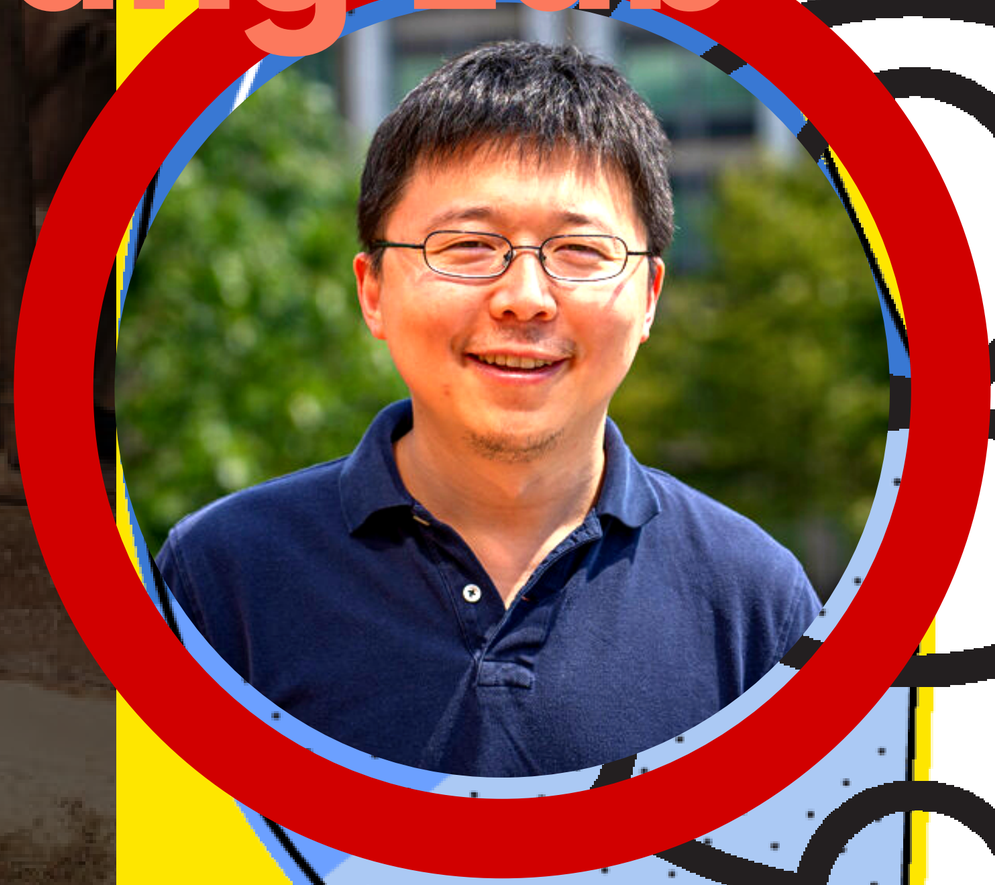


Csm6



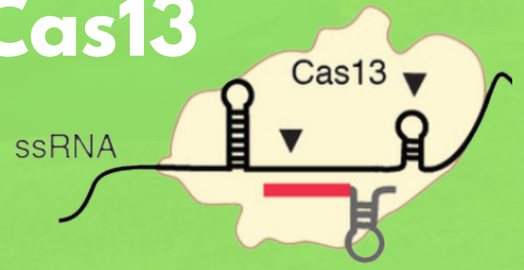
What we intend to use

Zhang Lab

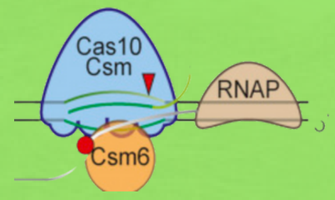


Sherlock V2

Cas13

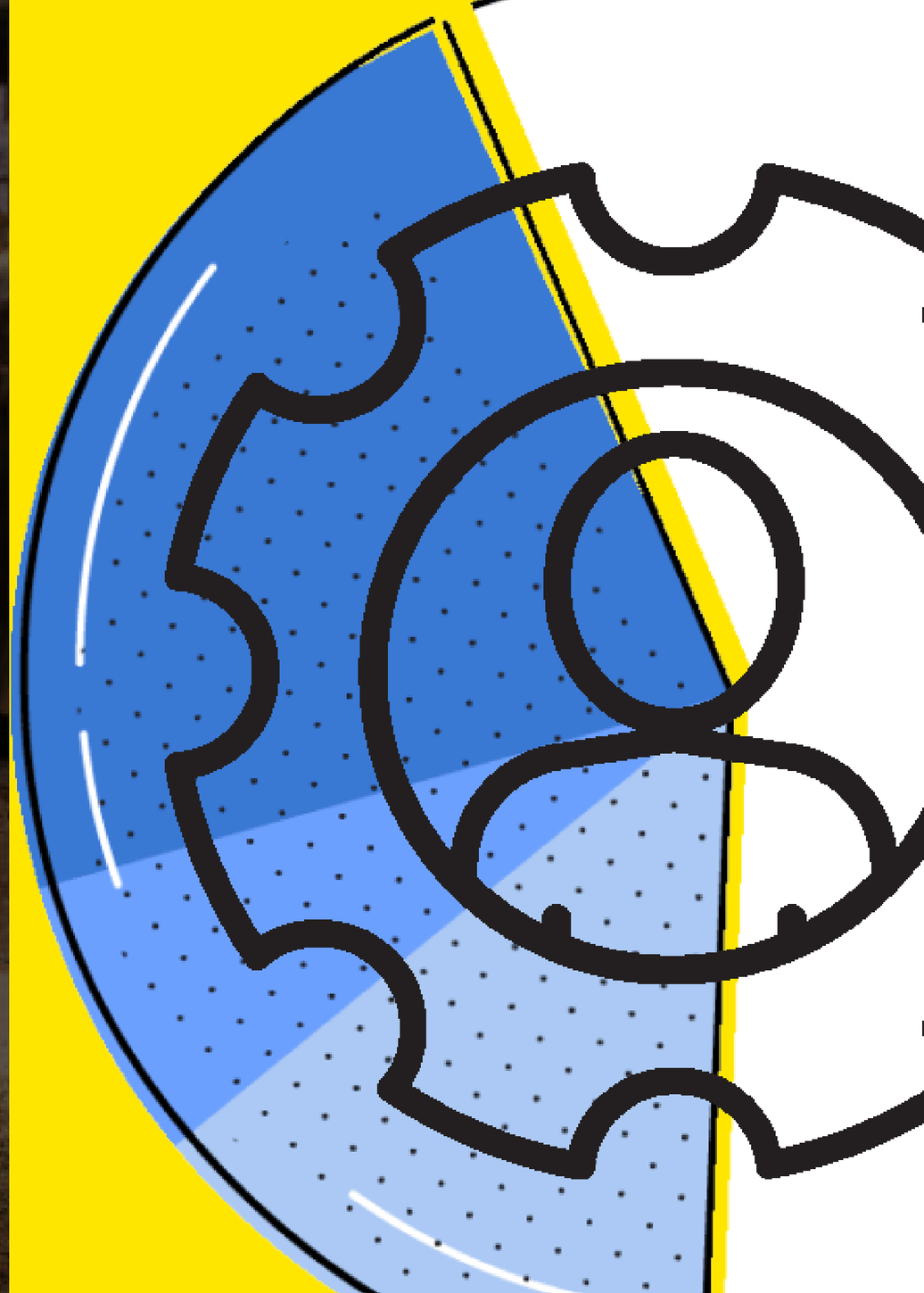


Csm6

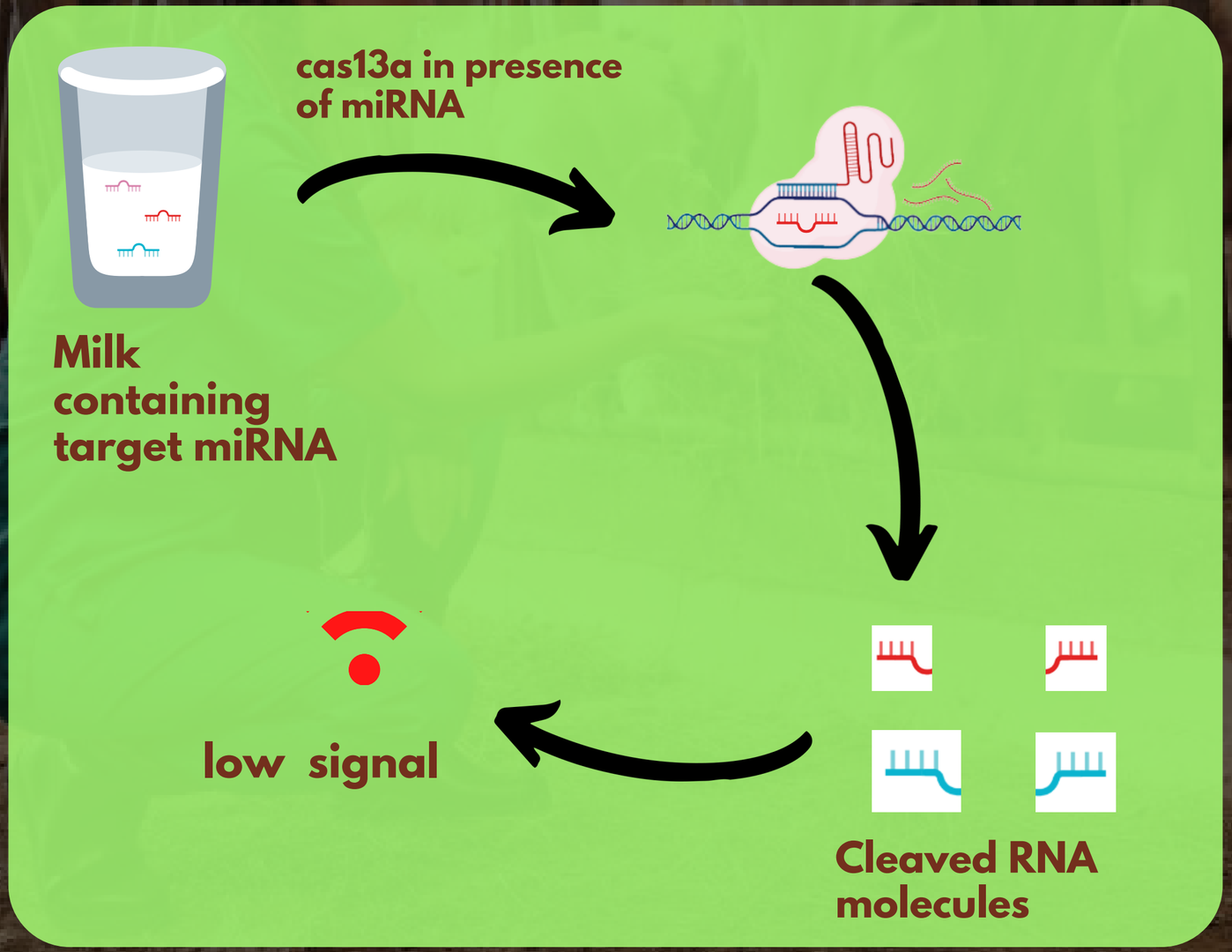


Usage of Cas13a

1. Cas13a is a **non specific** RNA cleavage enzyme.
2. When it encounters the **target miRNA**, it gets activated and **cleaves all types of RNA molecules**
3. The cleavage residues generate **hex adenylates** containing **2'-3' cyclic phosphate ends.**



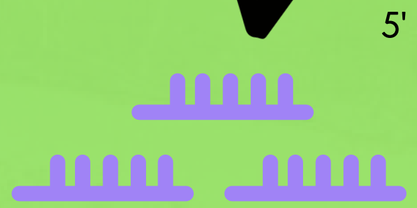
Graphical abstract



Usage of Csm6

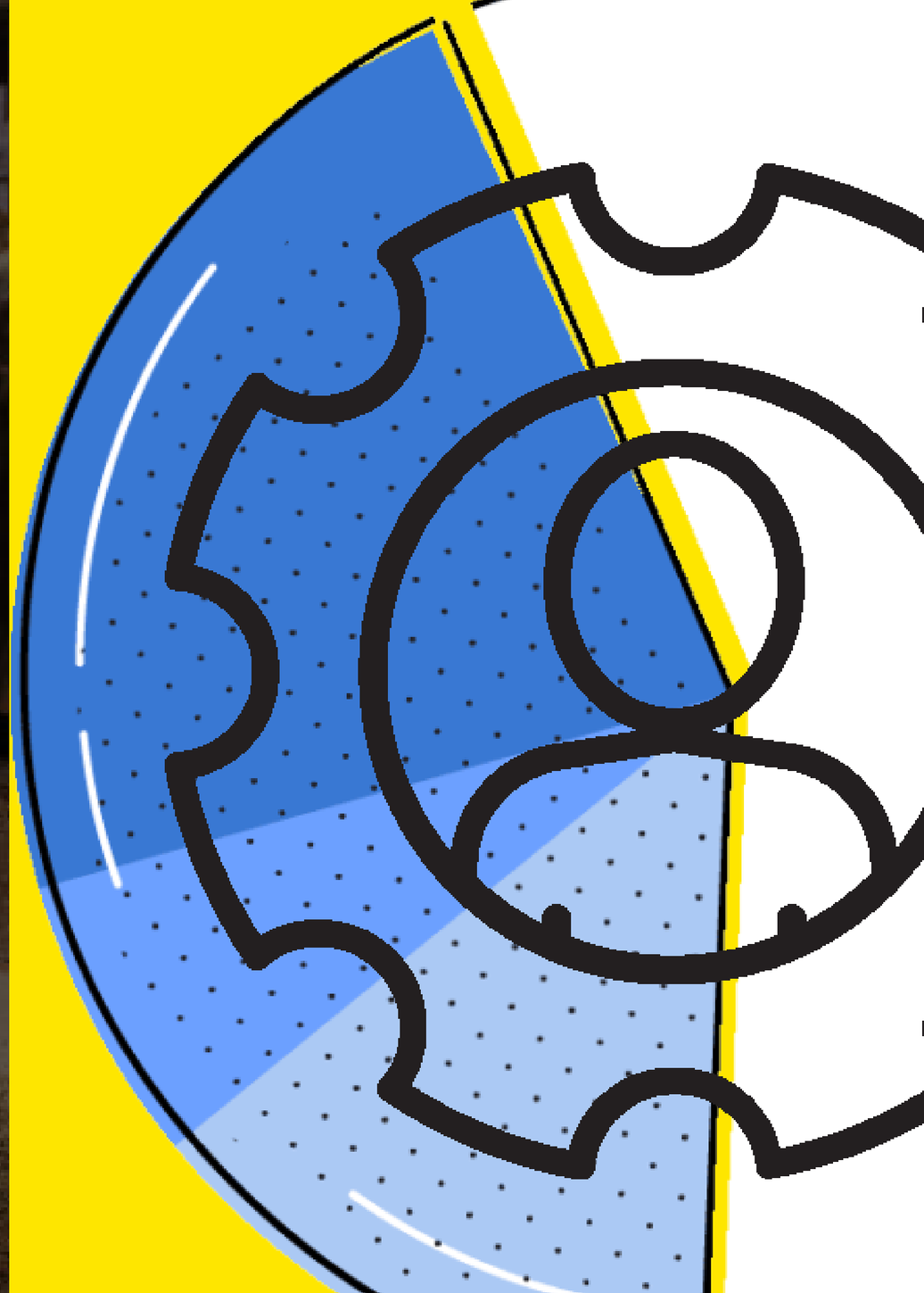
cleavage residues generate **hex adenylates** containing 2′-3′ cyclic phosphate ends.

activate Csm6 complex



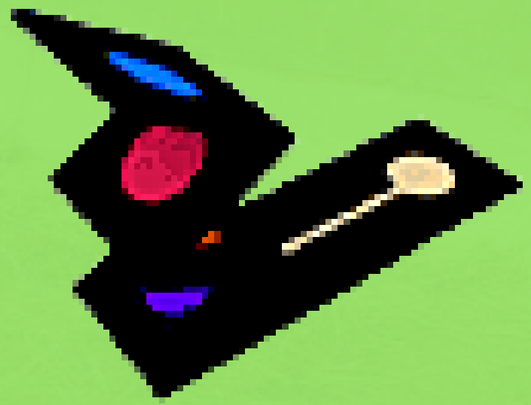
More RNA Cleavage molecules

signal amplified



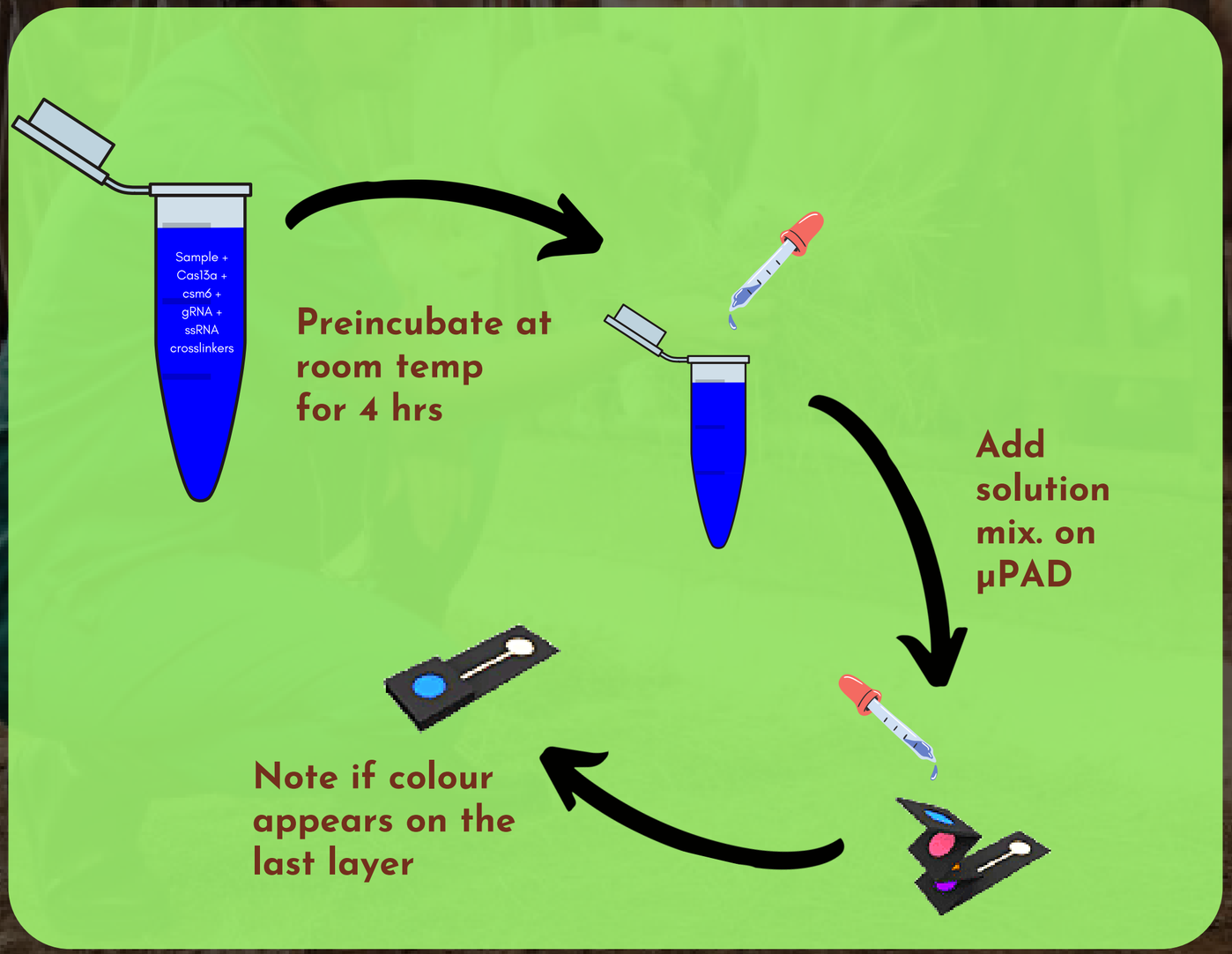
Implementation

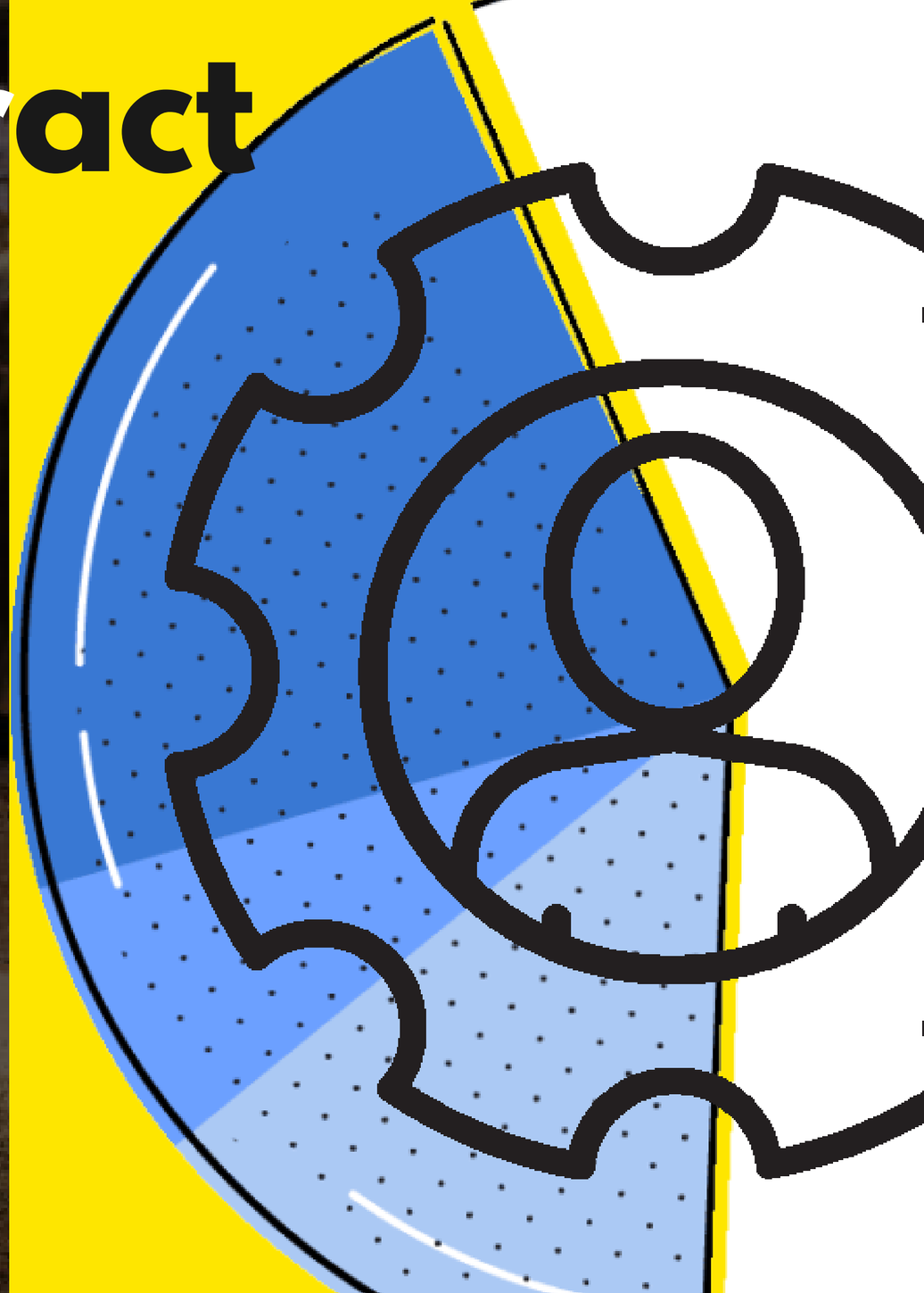
Colorimetric detection
using μPAD



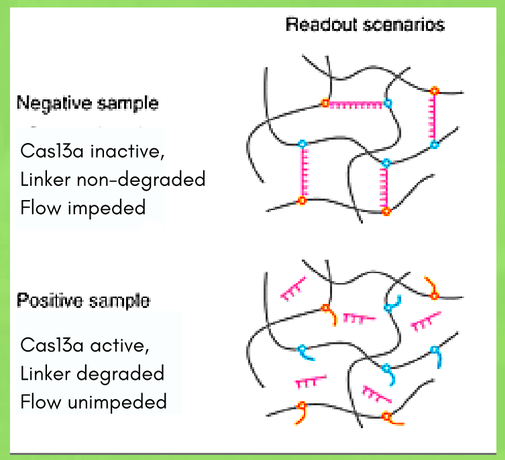
Collins Lab

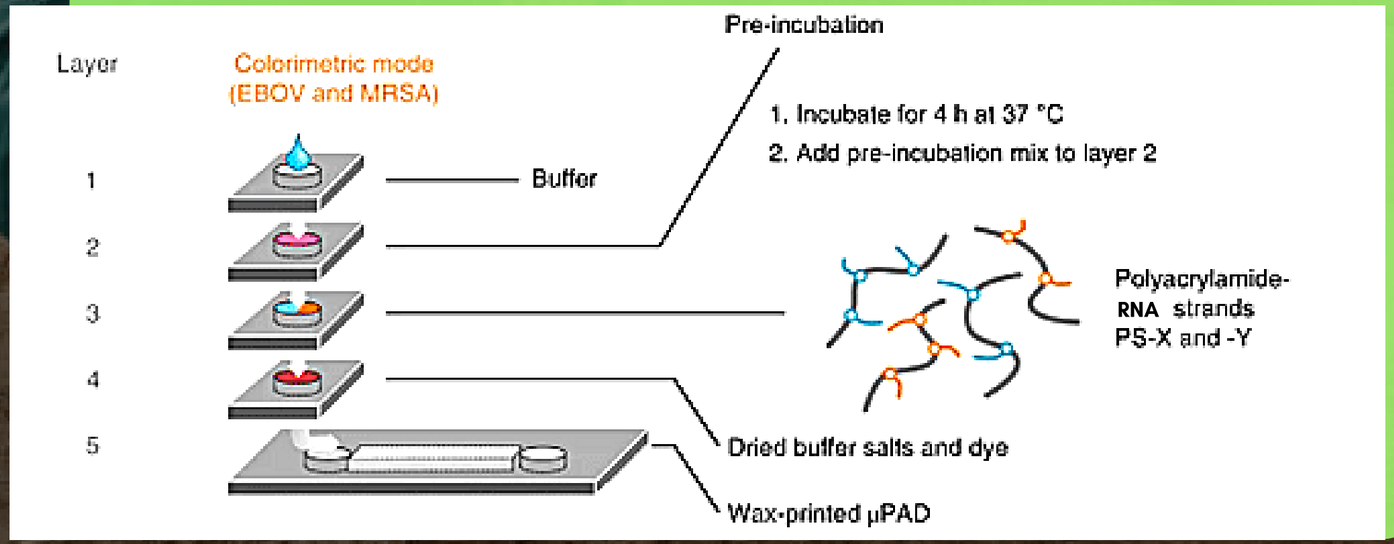
Graphical Abstract

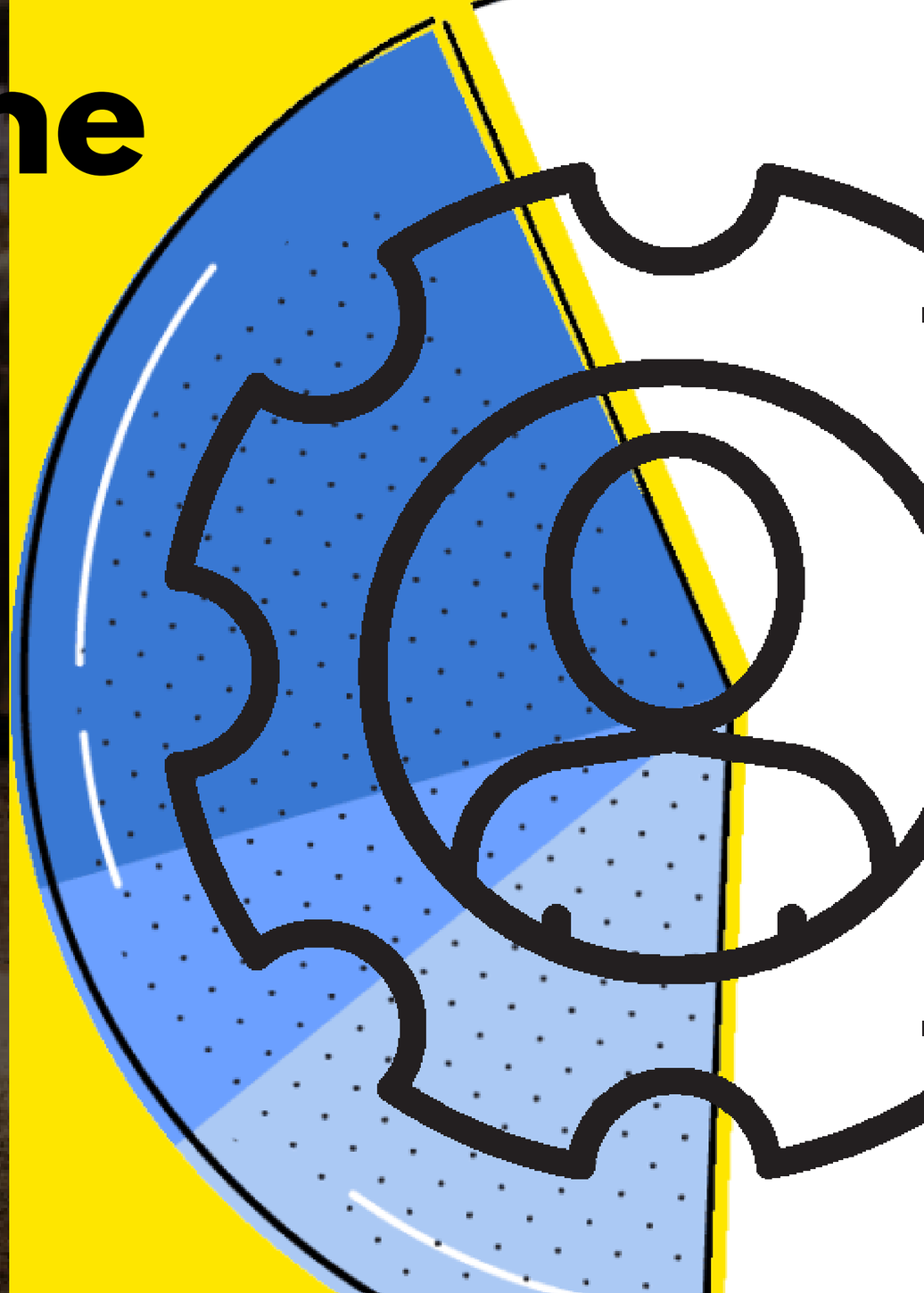




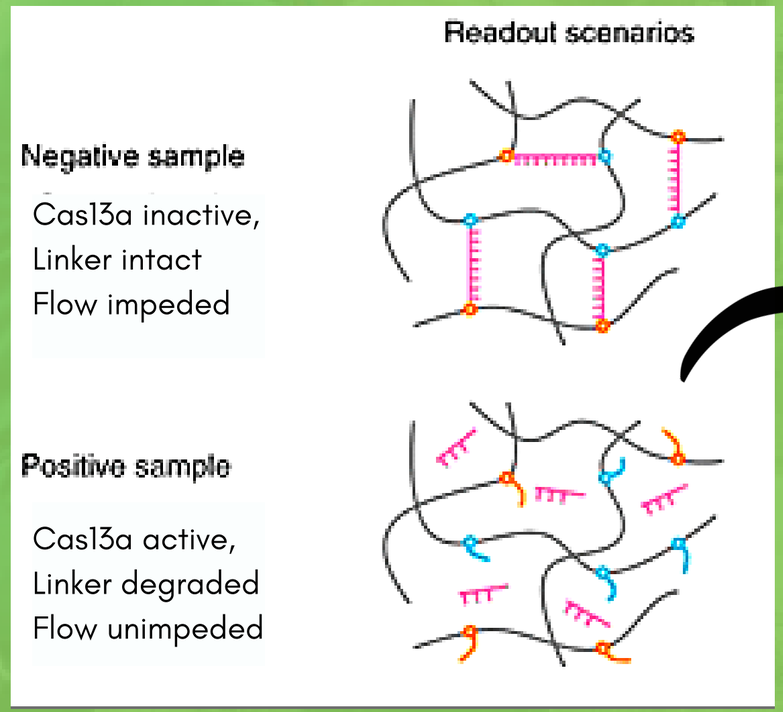
Breakdown of the μPAD







Readouts


Readout scenarios
Negative sample
Cas13a inactive,
Linker intact
Flow impeded
Positive sample
Cas13a active,
Linker degraded
Flow unimpeded


Negative
Positive

Closing the loop

Fast

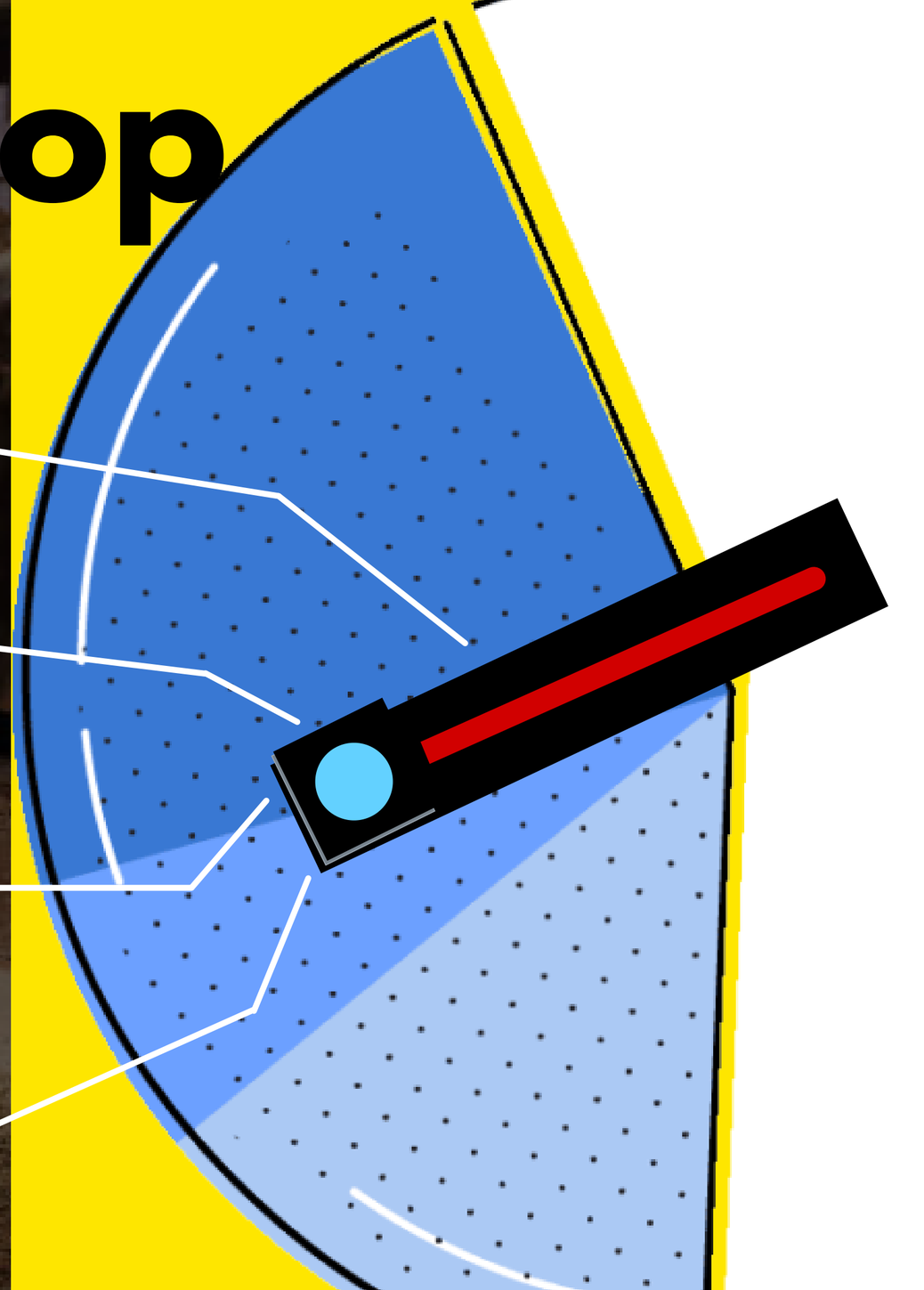
Highly sensitive, accurate, safe

due to efficient transcleavage activity

Portable

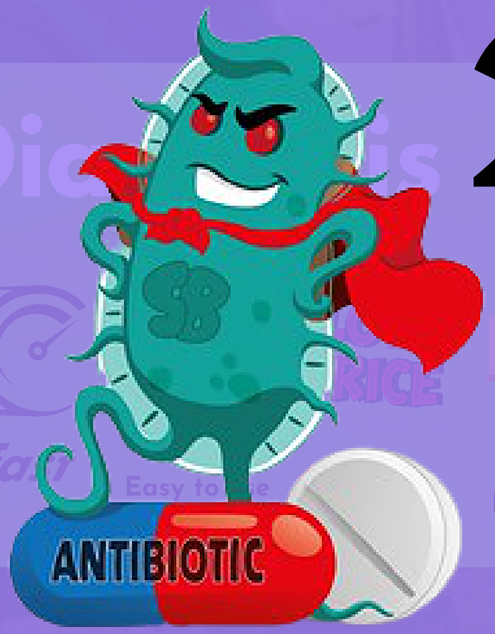
Cheap, Easy to use

Can be stored at room temperature



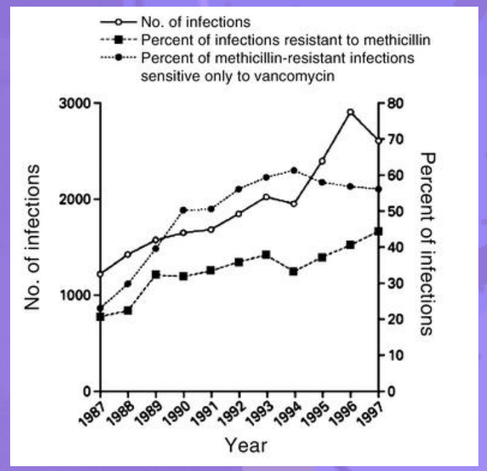
"Prevention is better than cure"

Treatment



2730
tonnes

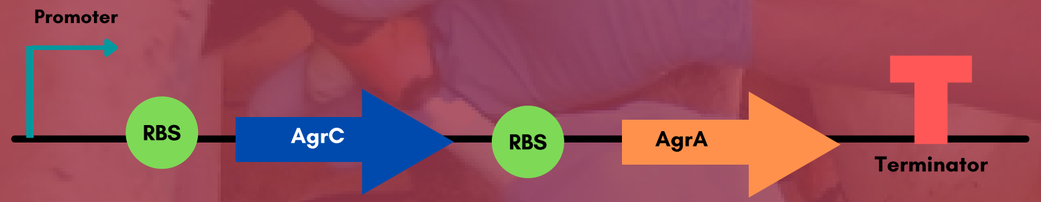
Ref - Lowy, 2003

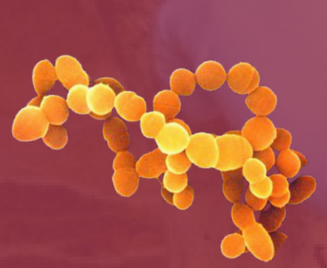


Treatment

Genetic Circuit to be cloned

Two component AgrC-AgrA signal transduction system



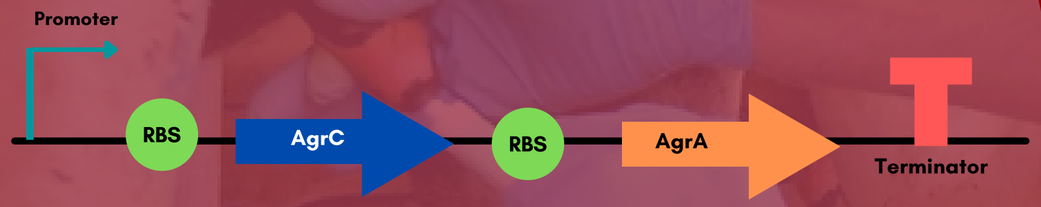


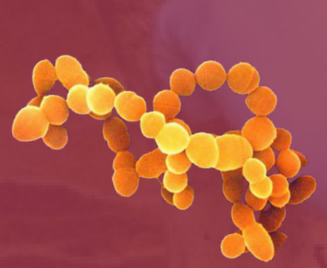
Lactococcus lactis LMG 7930

Treatment

Genetic Circuit to be cloned

Two component AgrC-AgrA signal transduction system





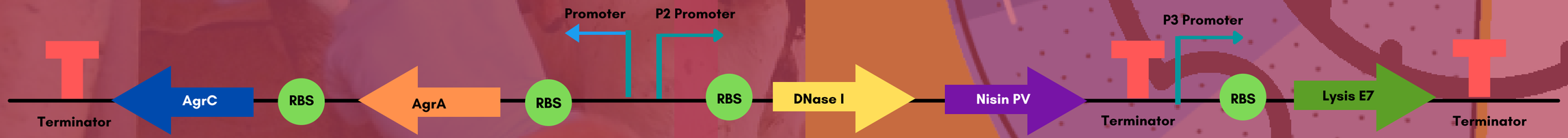
Lactococcus lactis LMG 7930

Dr. Amirul Islam Mallick

Treatment

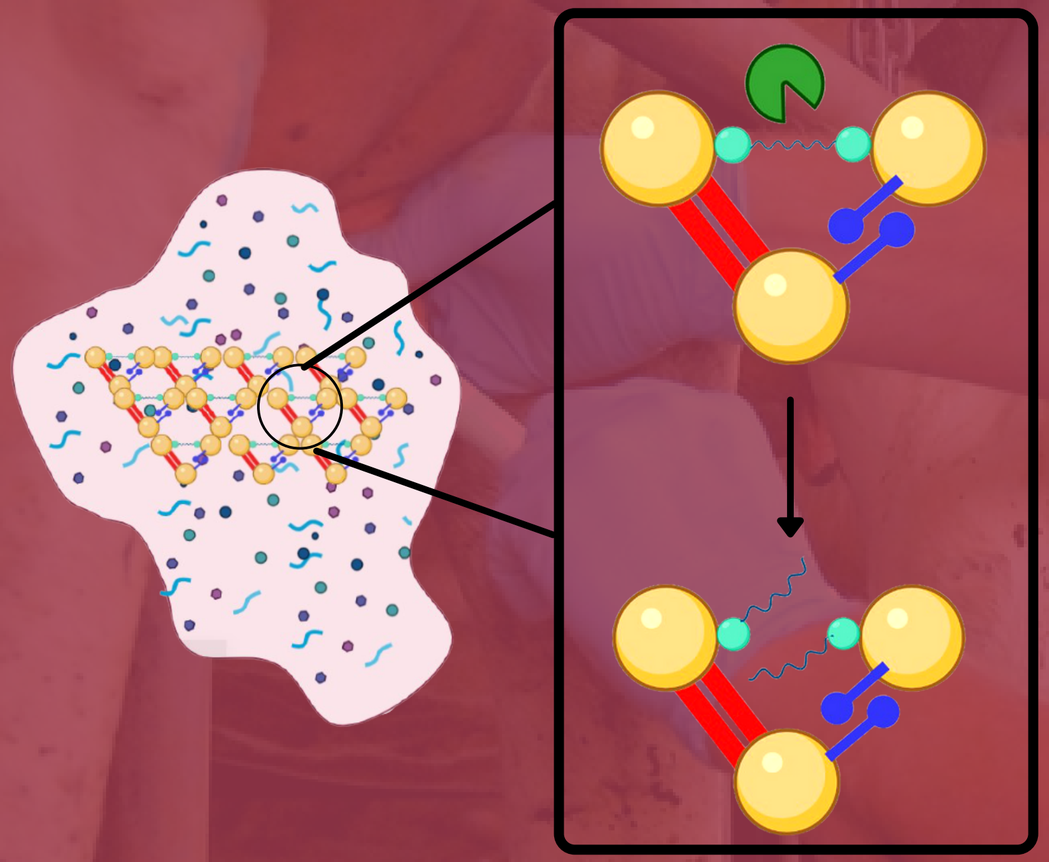
Our Genetic Circuit

AgrA from AgrA-AgrC system will activate P2 & P3 promoter to facilitate the release of DNaseI, NisinPV & LysisE7.



Treatment

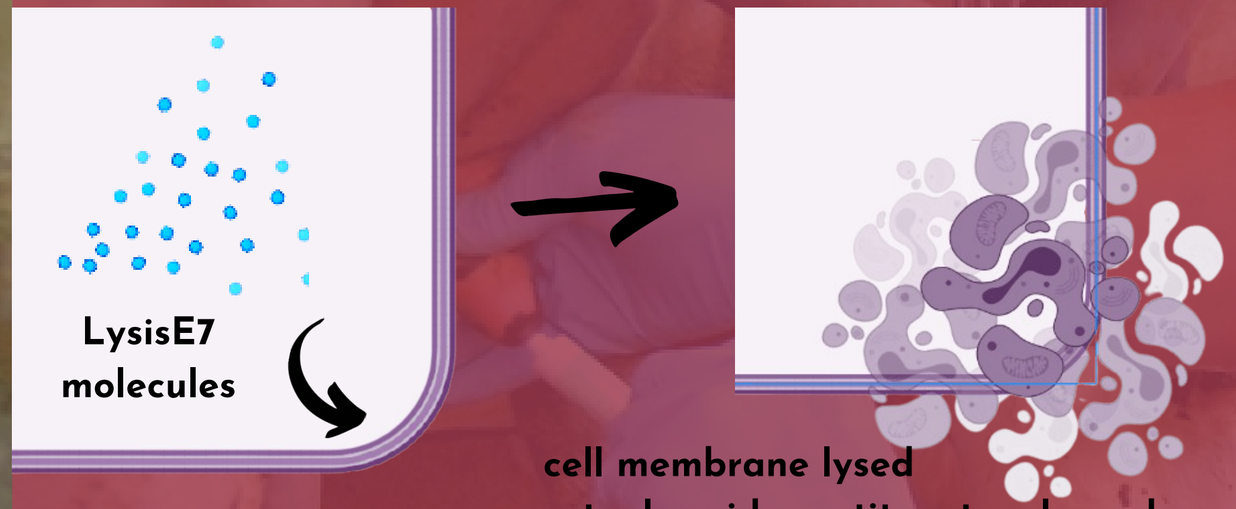
DNaseI - to degrade biofilm



Dr. Peter Edmondson

Treatment

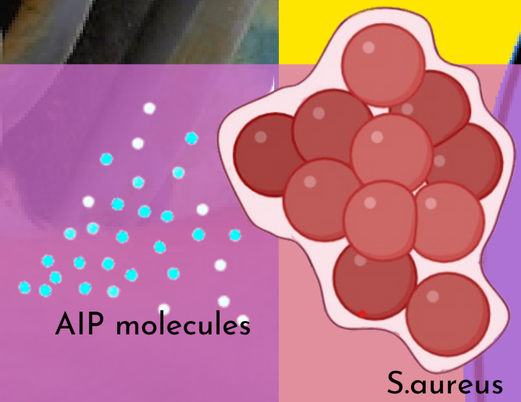
Lysis E7 - Kill Switch

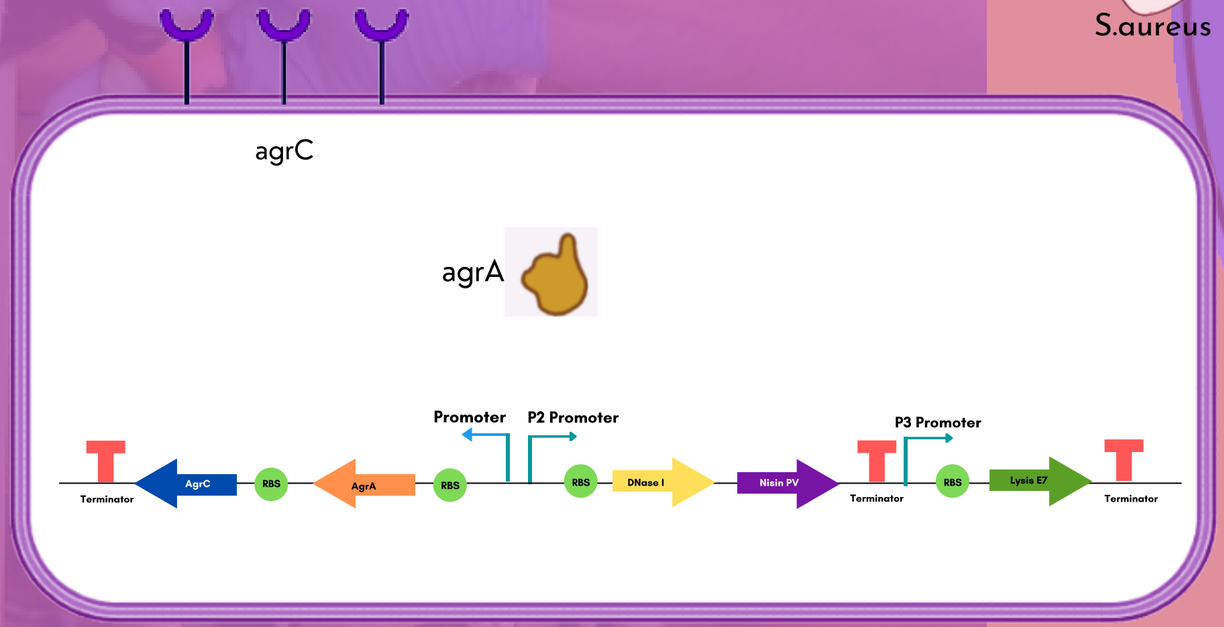


Treatment

How our GMO works

Presence of S.aureus in udder is marked by higher concentration of AIP, which will activate the quorom sensing unit of our GMO

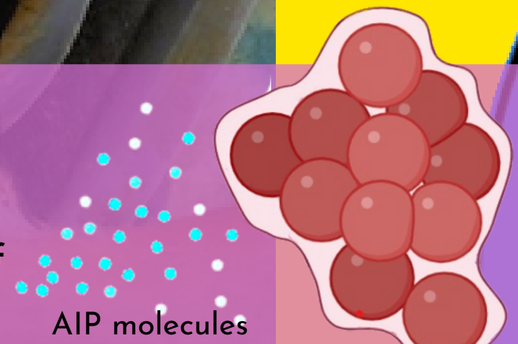


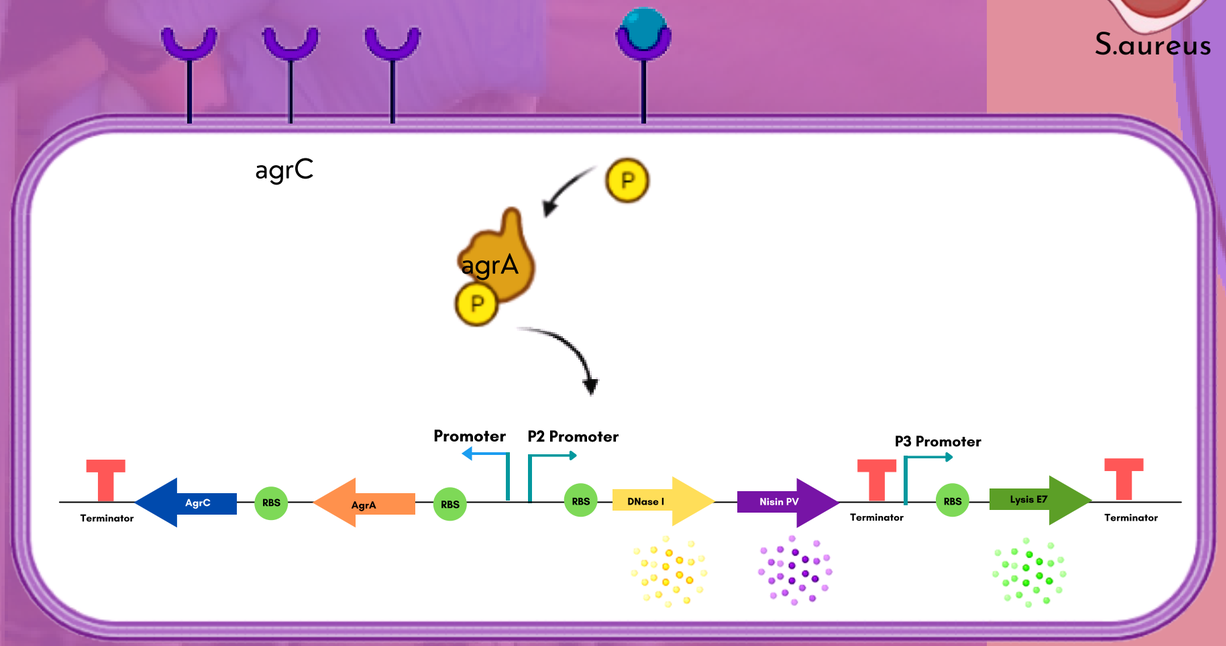


Treatment

How our GMO works

AIP molecules bind to the AgrC receptors which in turn phosphorylates AgrA molecules further switching on the P2 promoterThis leads to synthesis of DNaseI, NisinPV and LysisE7 molecules.Rate of synthesis of these proteins are fine tuned based on promoter affinity

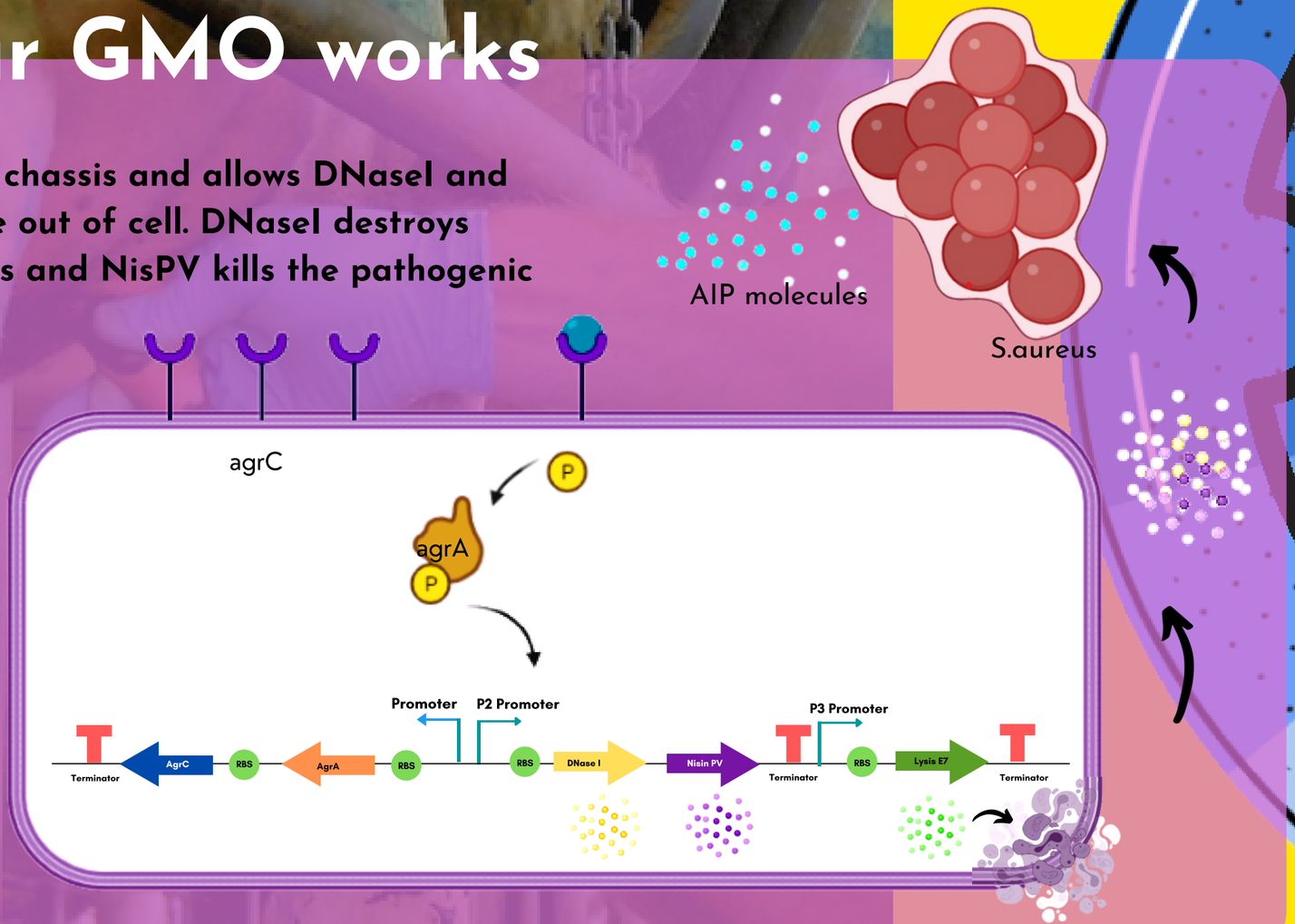




Treatment

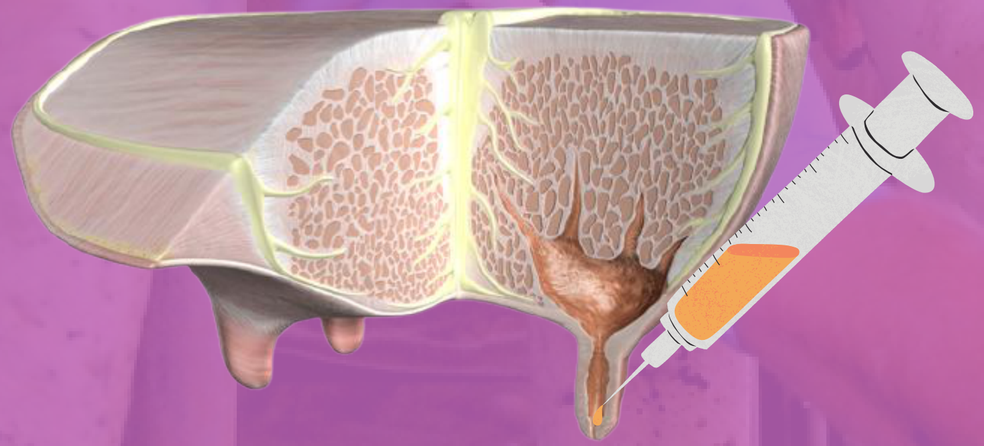
How our GMO works

Lysis E7 lyses the chassis and allows DNaseI and NisinPV to escape out of cell. DNaseI destroys biofilm of S.aureus and NisPV kills the pathogenic cells.



Implementation

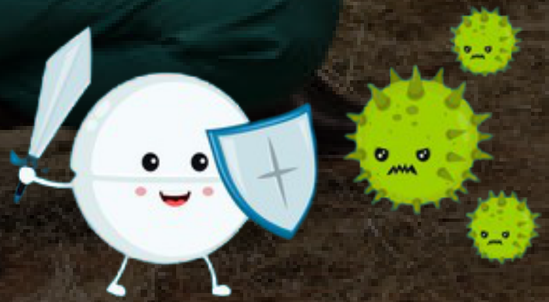
Intramammary injection in udder tissue through the teats of the cow



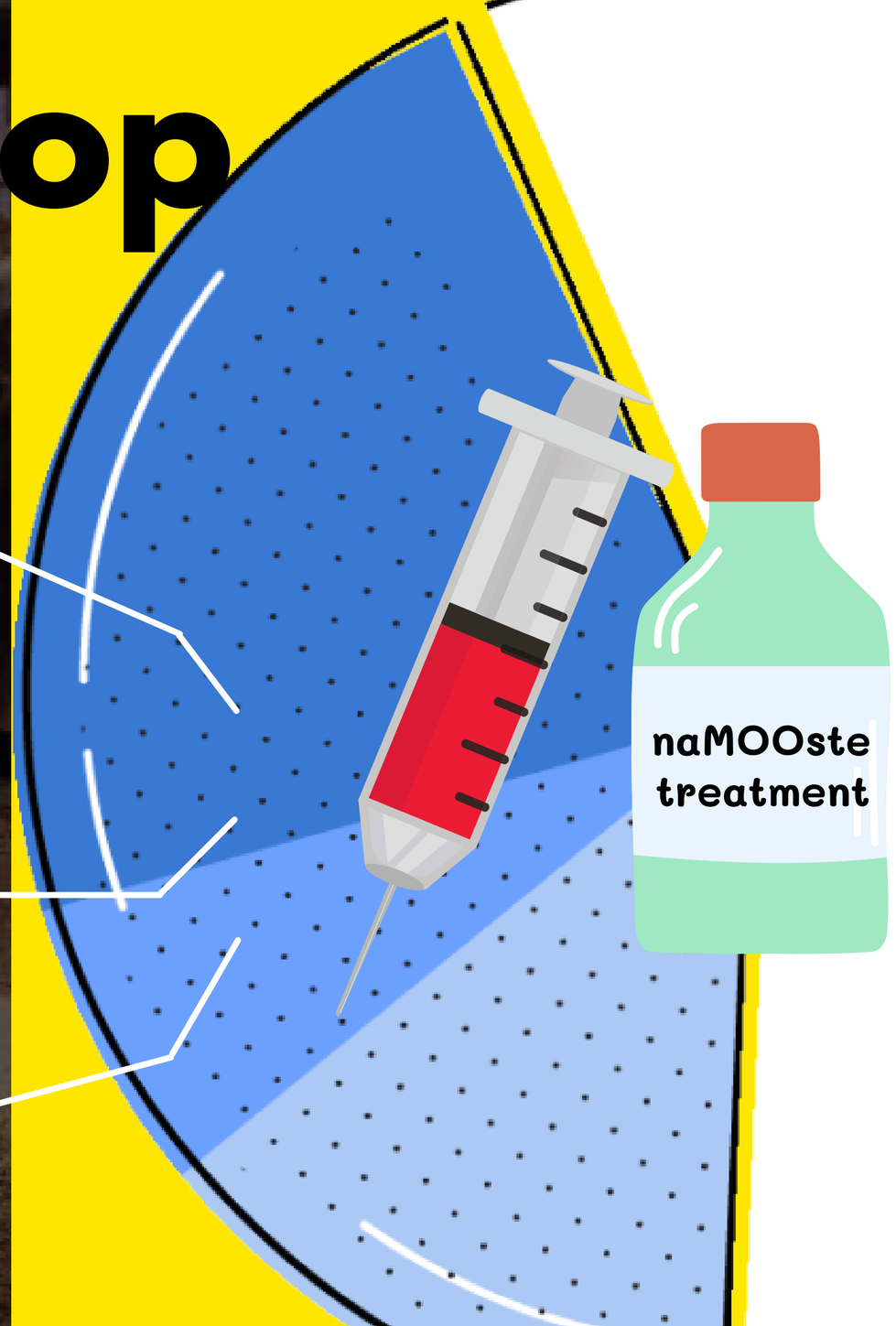
Closing the loop

Reduce Antibiotic Usage

Slowdown evolution of AMR in pathogens



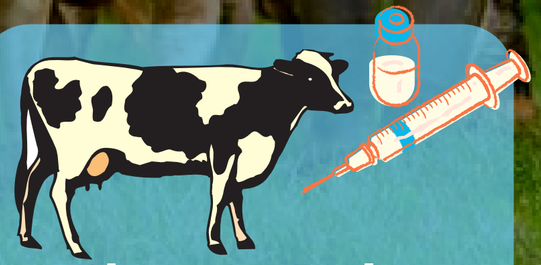
Can kill AMR pathogens



Reaching out to stakeholders

introducing inputs from scientists while project development

Lab testing

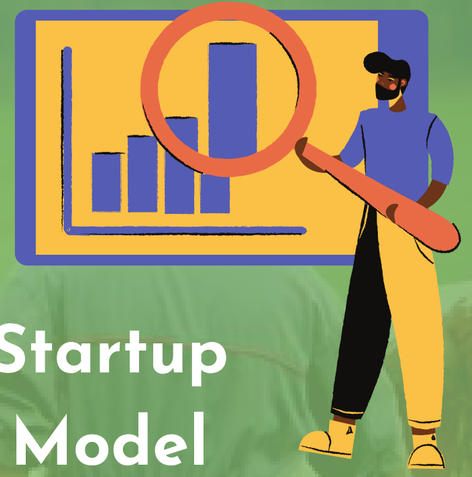


Phase trials

Aware & educate farmers

Bio Safety

Check

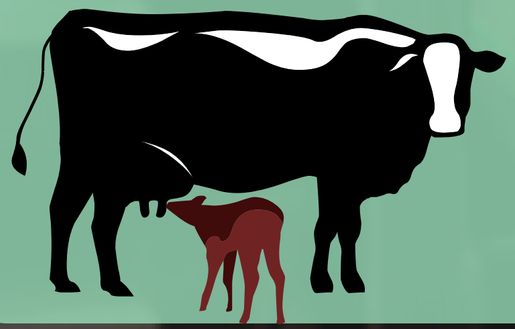


Startup Model

Educate & Aware general mass

naMOOste can do wonders...

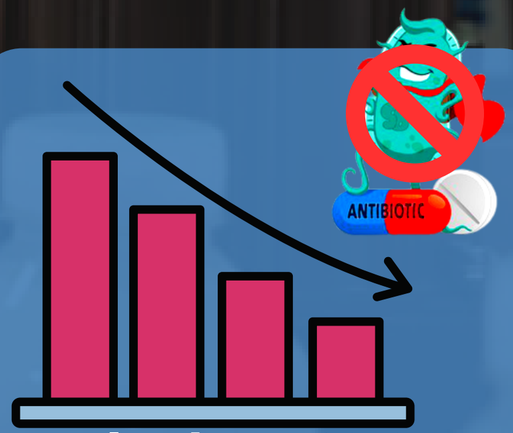
Save many bovines



increase milk production

safe milk to drink

reduce chances of spreading infection in humans ingesting milk products

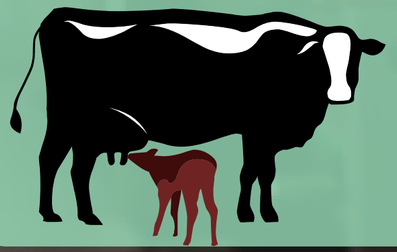


slacken AMR evolution

naMOOste can do wonders...

BOOST ECONOMY

Save many bovines

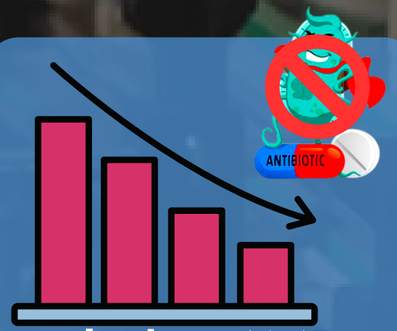


increase milk production

safe milk to drink

reduce chances of spreading infection in humans ingesting milk products

slacken AMR evolution



Thank You

IGEM IISER Kolkata

@igem.iiserk

@iGEM_IISERKol

team.igem@iiserkol.ac.in