HYBRID
LAB NETWORK

CRISPR-Cas9 editing of *Streptomyces* genome

Marta Vaz Mendes

Bioengineering and Synthetic Microbiology | BSM

With the support of the Erasmus+ Programme of the European Union

Project received funding from the European Union´s ERASMUS+ PROGRAMME HIGHER EDUCATION STRATEGIC PARTNERSHIP – KA203 under the Grant Agreement N° 2019-1-PT01-KA203-061449

HYBRID
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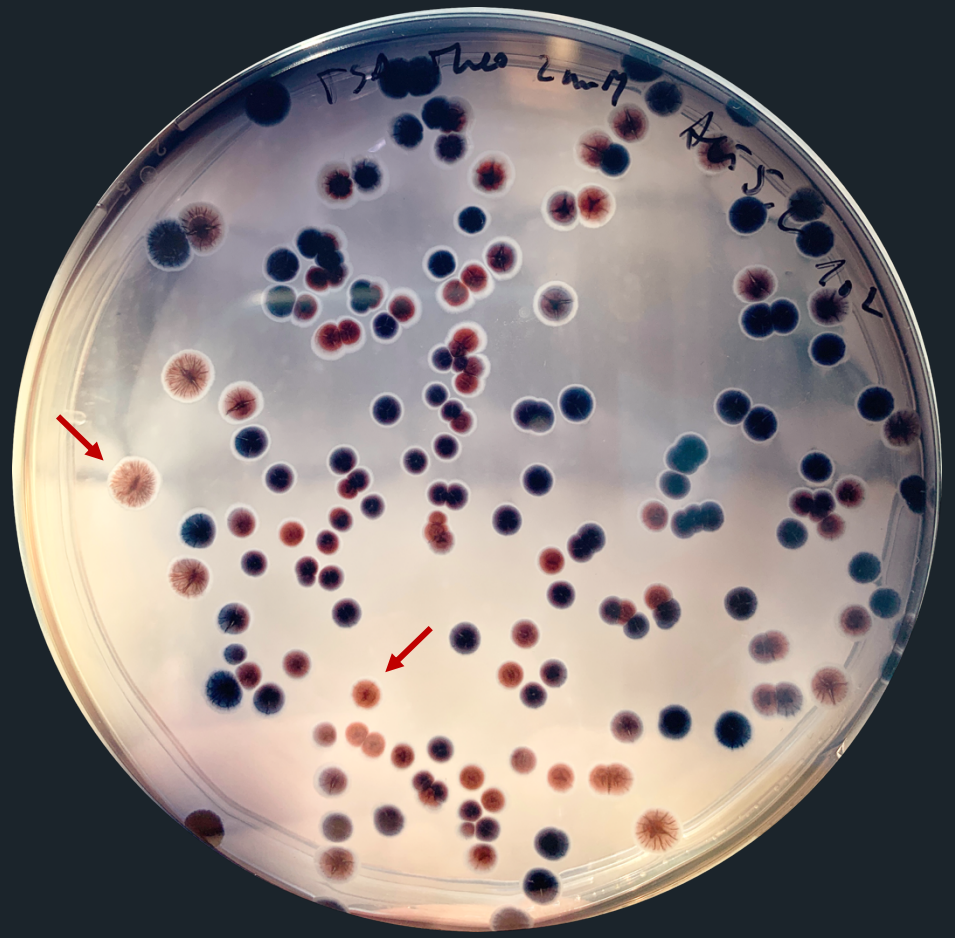
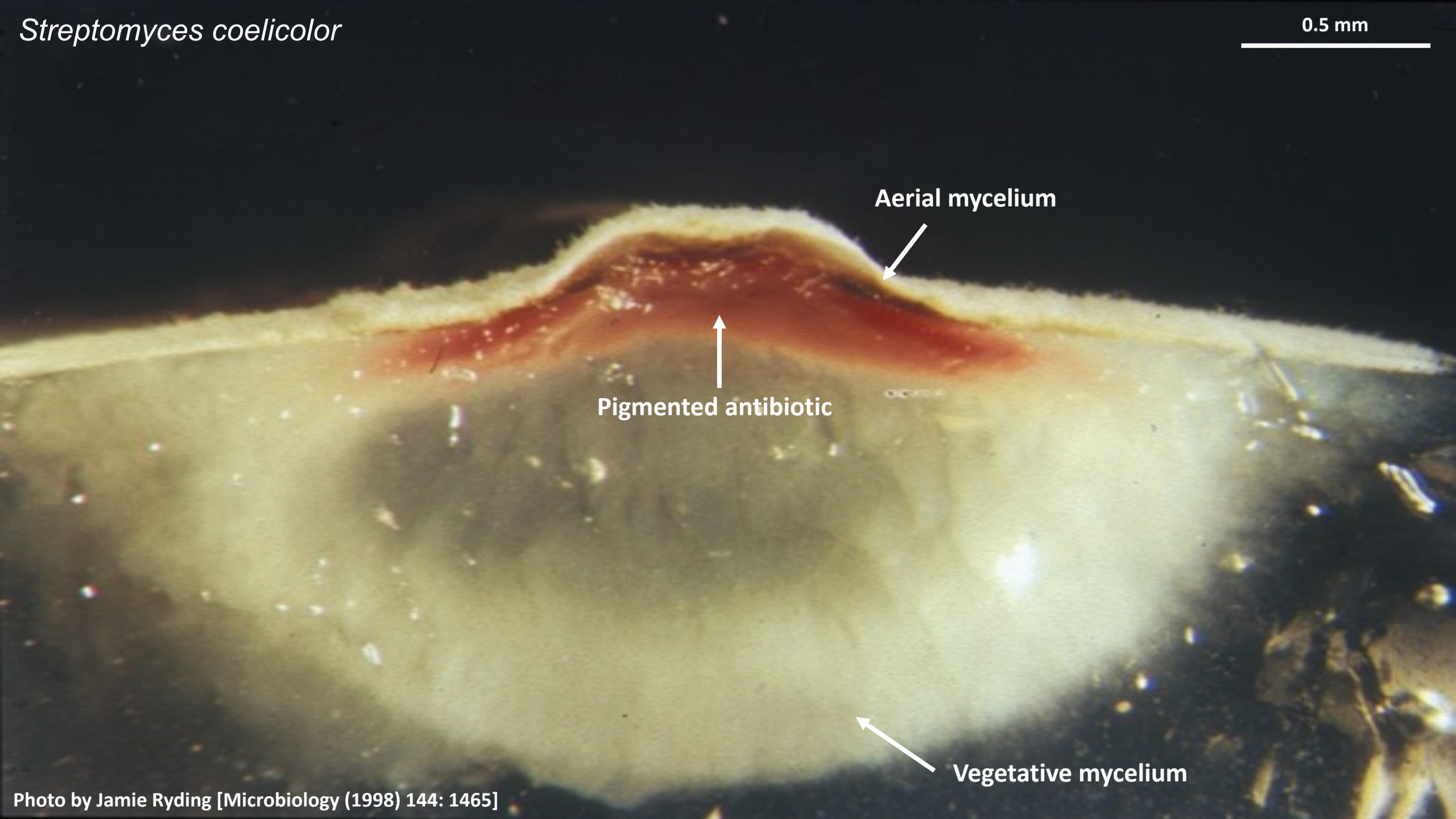
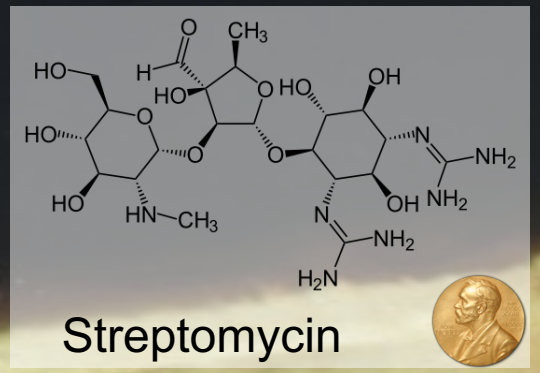
WT

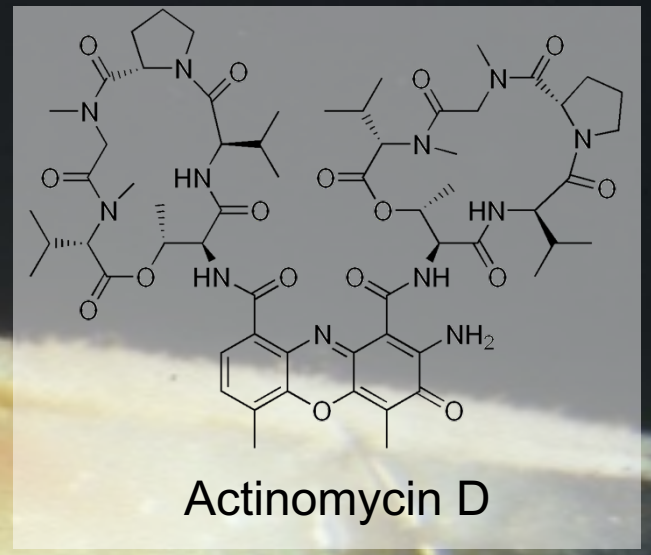


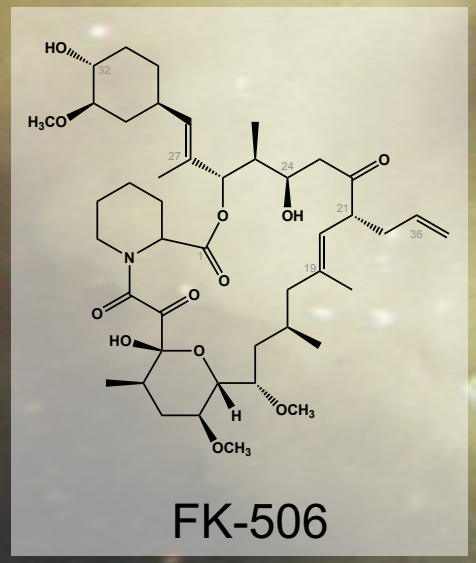
Cas9-edited

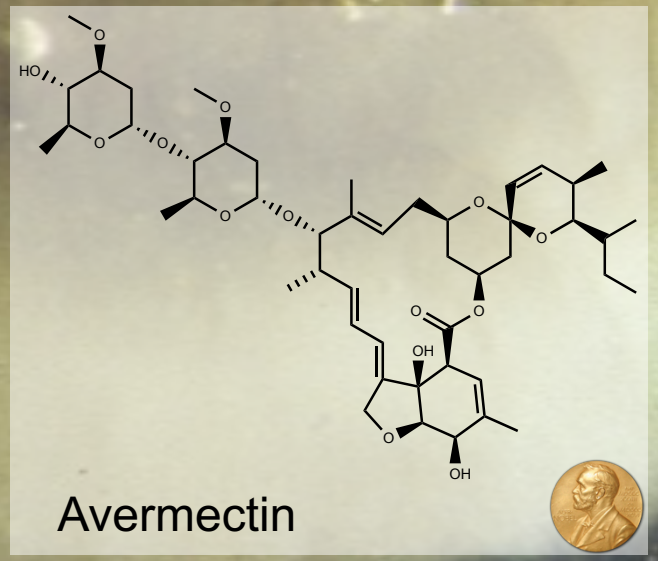

Streptomyces coelicolor
0.5 mm
Aerial mycelium
Pigmented antibiotic
Vegetative mycelium
Photo by Jamie Ryding [Microbiology (1998) 144: 1465]

Actinobacteria produce an enormous diversity of natural compounds









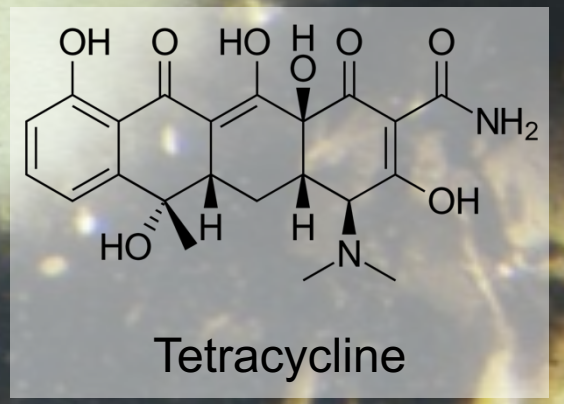
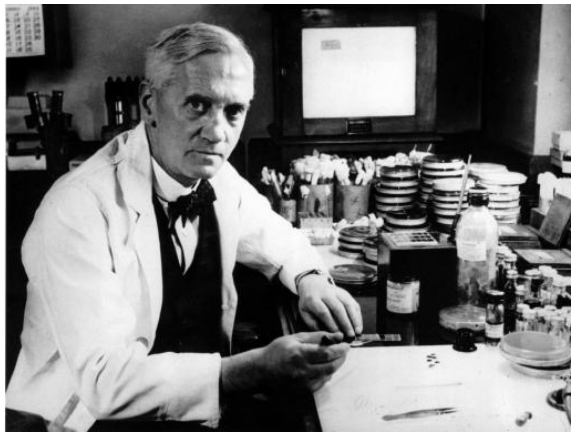
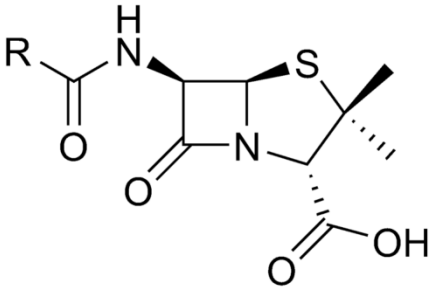


Photo by Jamie Ryding [Microbiology (1998) 144: 1465]

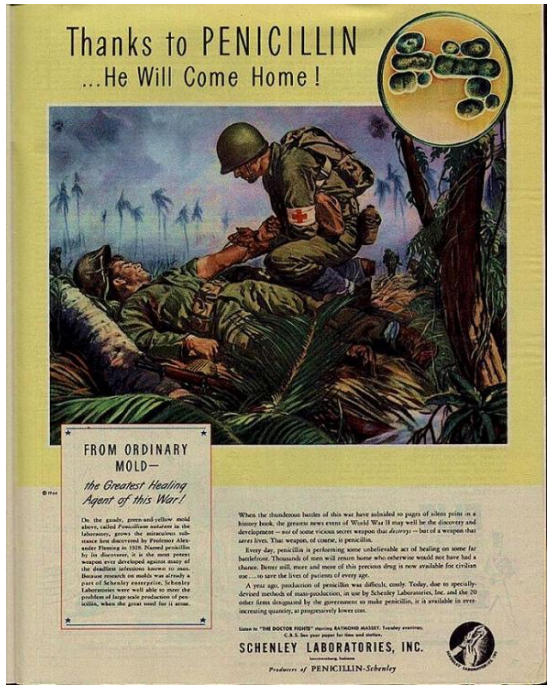
Long time ago…



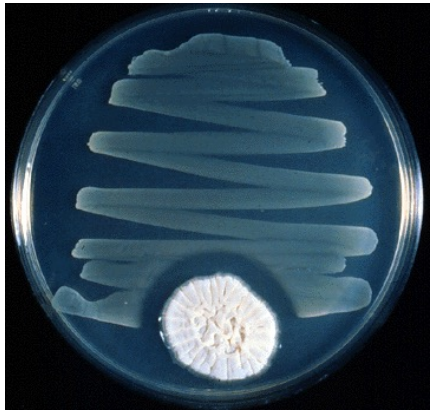
Alexander Fleming



Penicillin (1928)

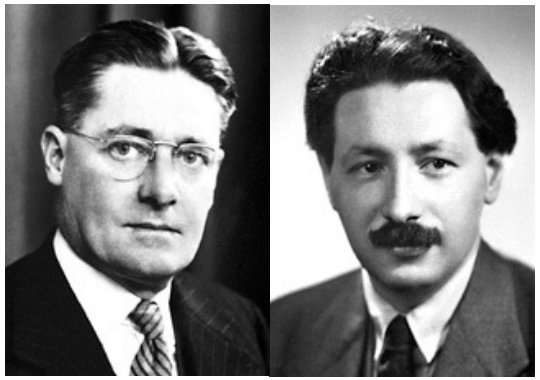


Howard Florey and Ernst Chain

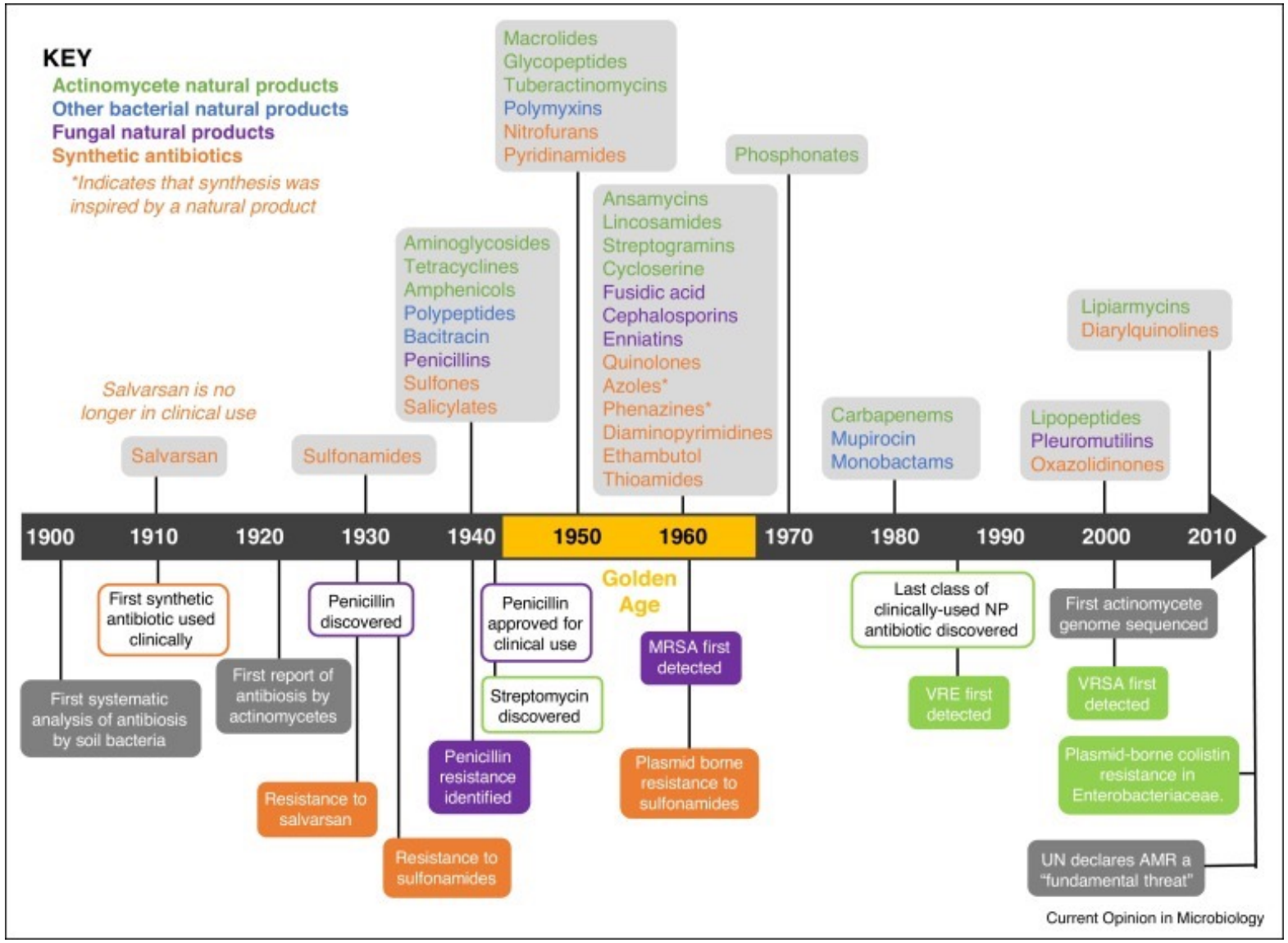


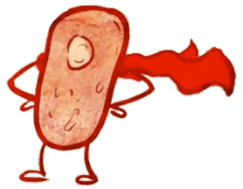
Penicillium notatum

"for the discovery of penicillin and its curative effect in various infectious diseases“ (1945)



Antibiotic discovery





[doi: 10.1016/j.mib.2019.10.008]

BBC

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NEWS

Health

Antibiotic resistance plan to fight 'urgent' global threat

24 January 2019

Drug-resistant superbugs are as big a threat as climate chan
will say as he unveils a new five-year plan to tackle the probl

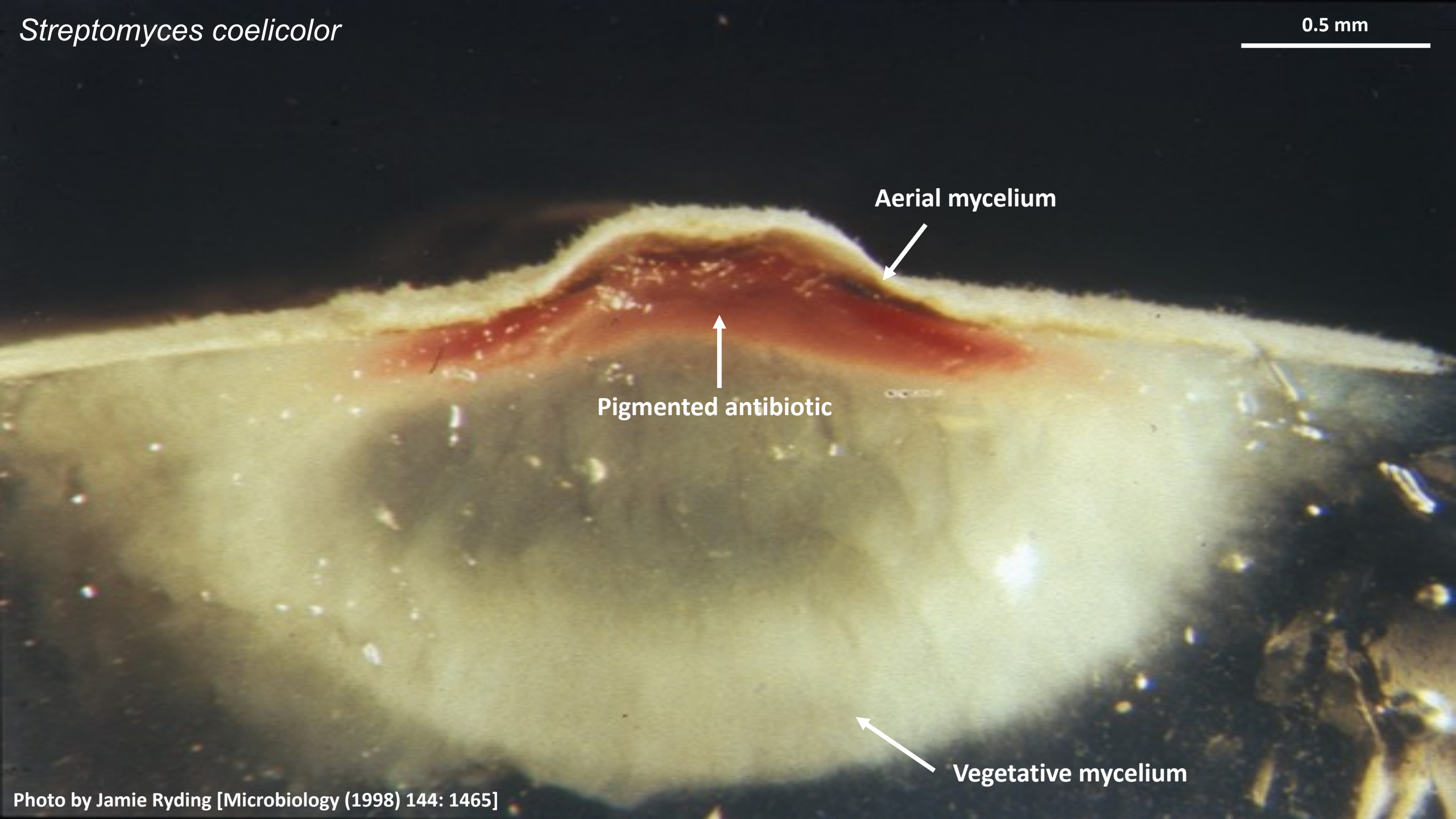
MUTANT E.COLI IS IN BRITAIN

Health

Drug resistance likely to kill 400,000 Canadians by 2050, report predicts

Problem already costs the national health-care system $1.4B a year

Laura Kane · The Canadian Press · Posted: Nov 12, 2019 8:16 AM ET | Last Updated: 3 hours ago


Streptomyces coelicolor
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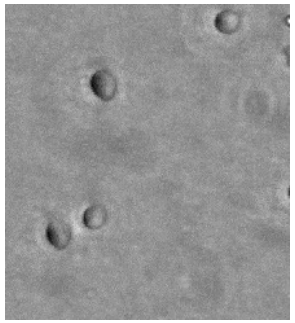
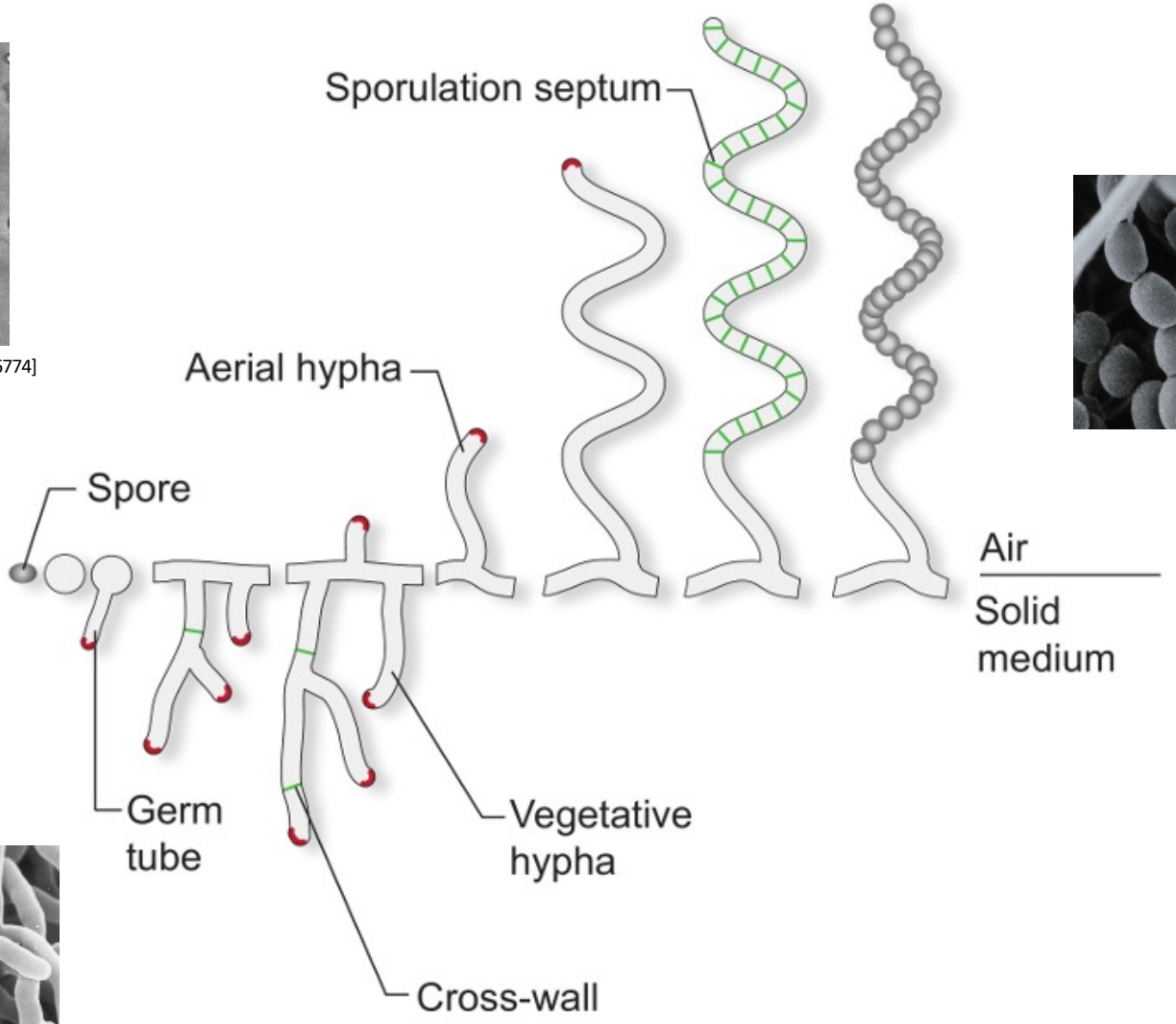
Pigmented antibiotic
Vegetative mycelium
Photo by Jamie Ryding [Microbiology (1998) 144: 1465]

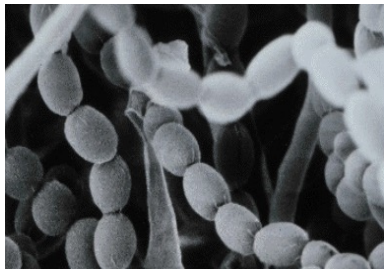
Scott Chimileski (http://www.scottchimileskiphotography.com/)

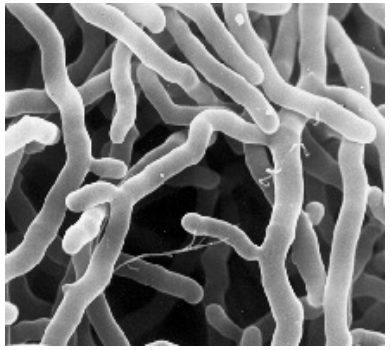
Streptomyces life cycle



[Jyothikumar *et al.* (2008) AEM, **74**: 6774]







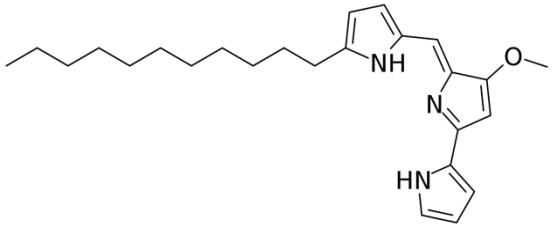
[Schlimpert *et al.*, 2016 – 10.3791/53863]

Streptomyces coelicolor - A case of colors

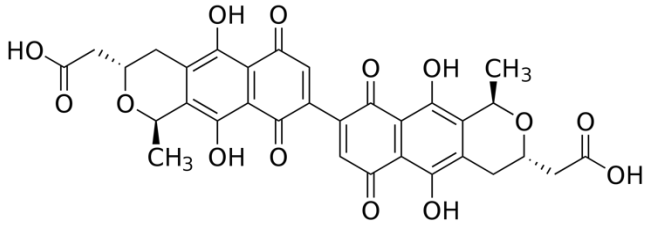
Science Photo Library

Science Photo Library

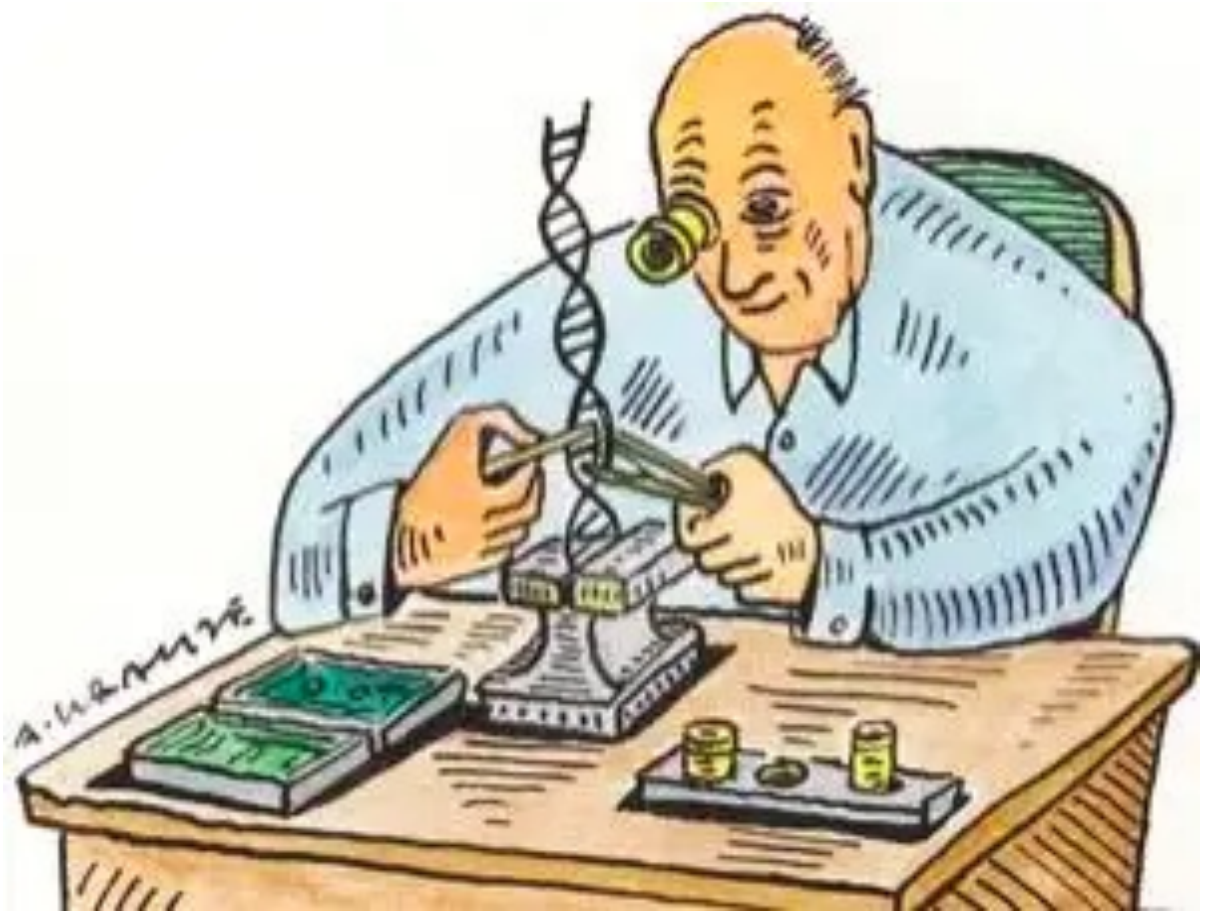
Undecylprodigiosin



Actinorhodin



CRISPR-*cas9* as a tool for gene editing

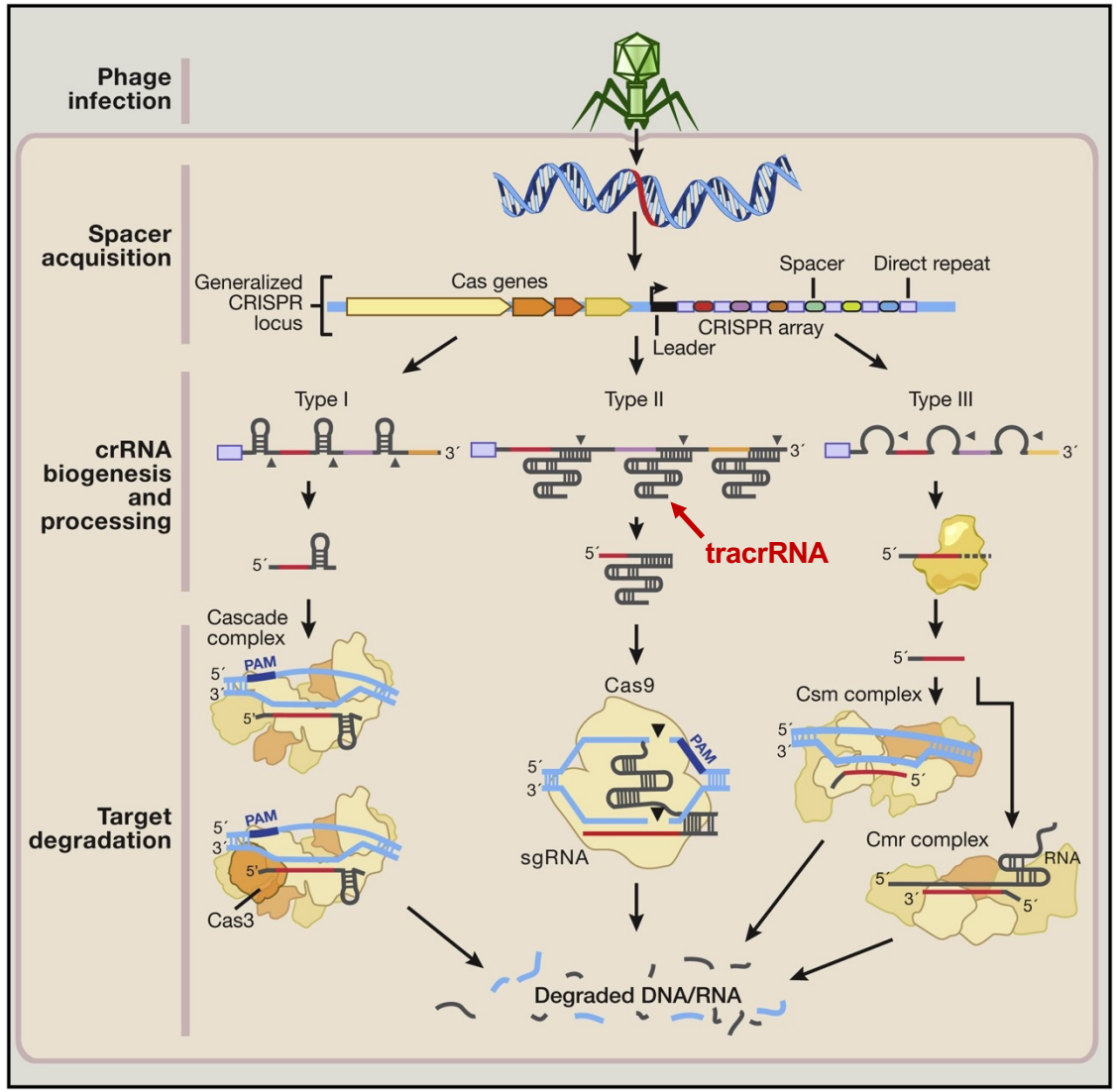


YouTube

Kurzgesagt – In a Nutshell

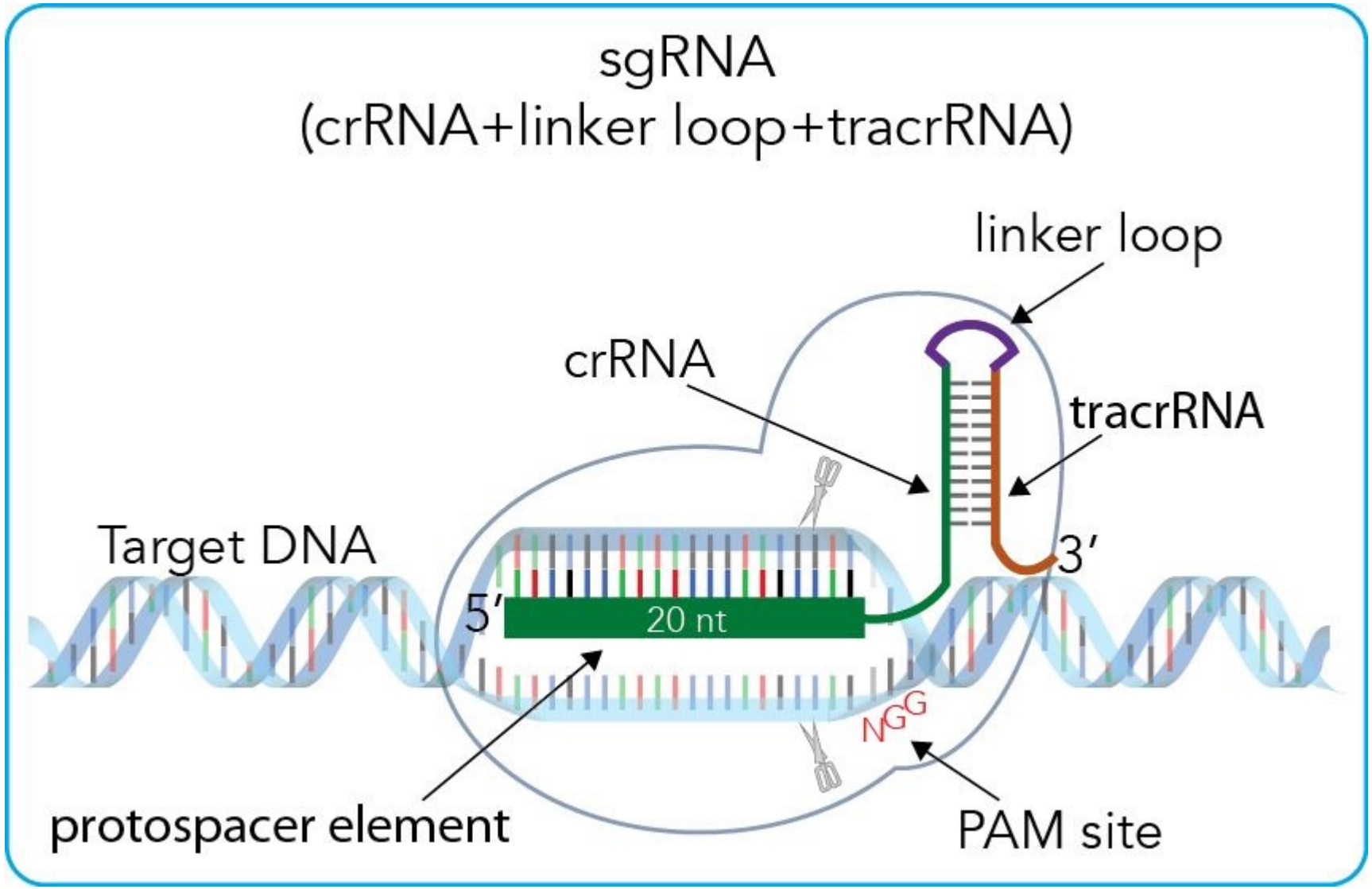
14,5 M de subscritores

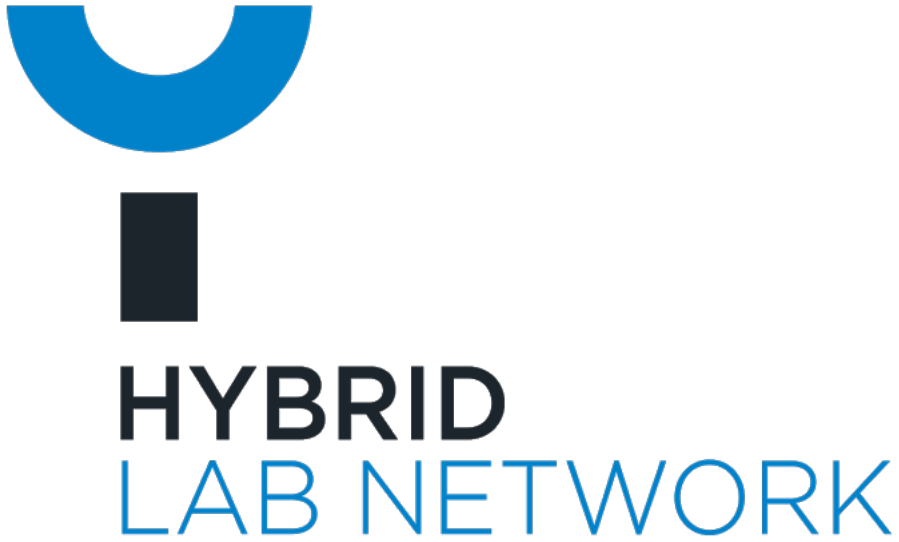
CRISPR-mediated immunity in bacteria



crRNAs – CRISPR RNAs
tracrRNA – *trans*-activating CRISPR RNA

[doi: 10.1016/j.cell.2014.05.010]


sgRNA
(crRNA+linker loop+tracrRNA)
linker loop
crRNA
tracrRNA
Target DNA
3′
5′
20 nt
NGG
protospacer element
PAM site



CRISPR-Cas9 editing of *Streptomyces* genome

The protocol explained

Showcase – turning off the blue pigment

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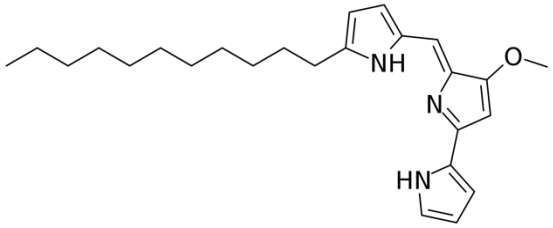
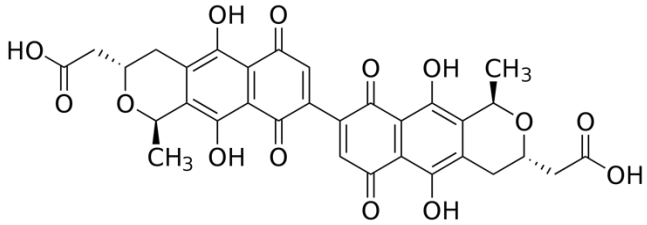
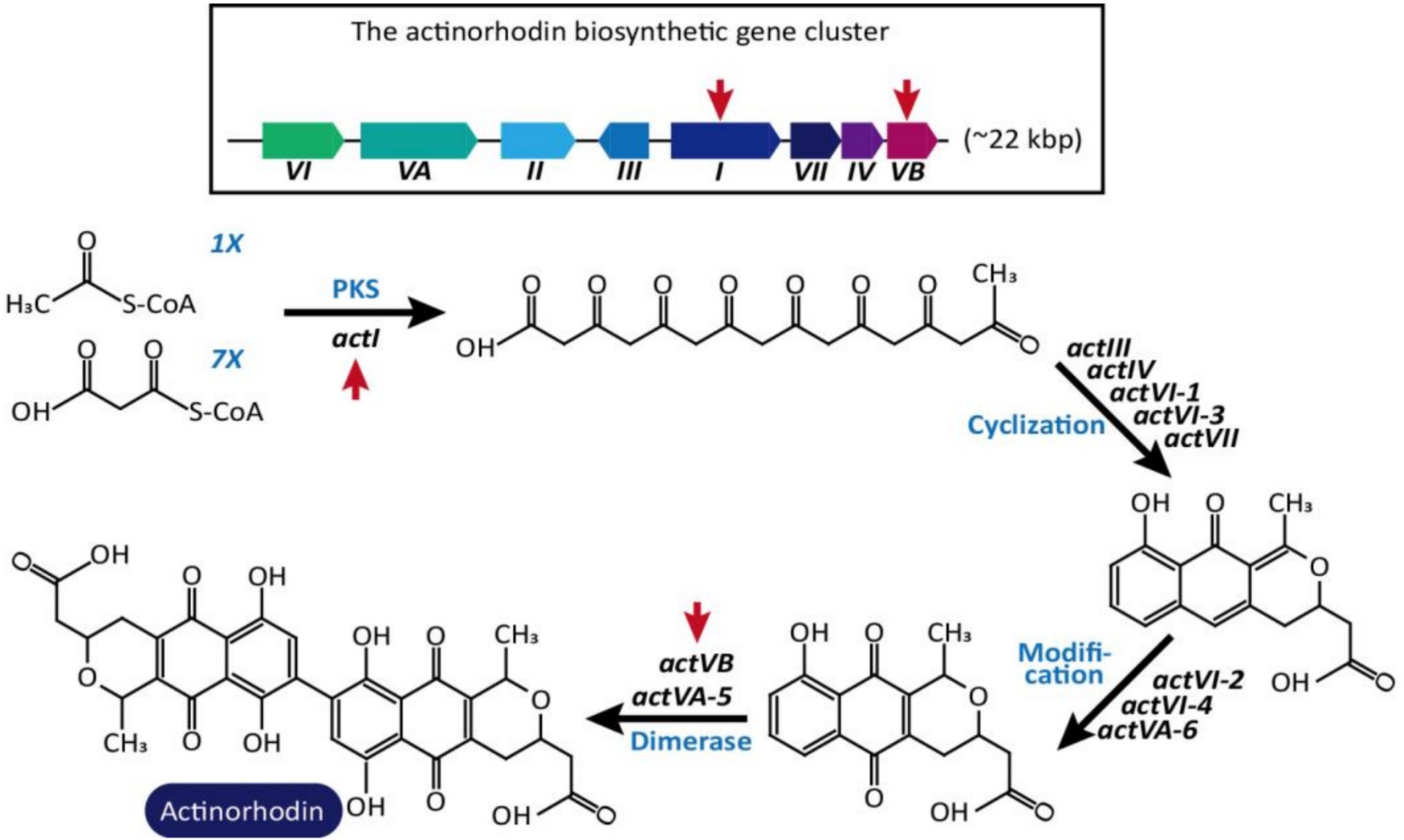
Undecylprodigiosin



Actinorhodin



Actinorhodin biosynthetic pathway



[doi: 10.3390/ijms19041089]

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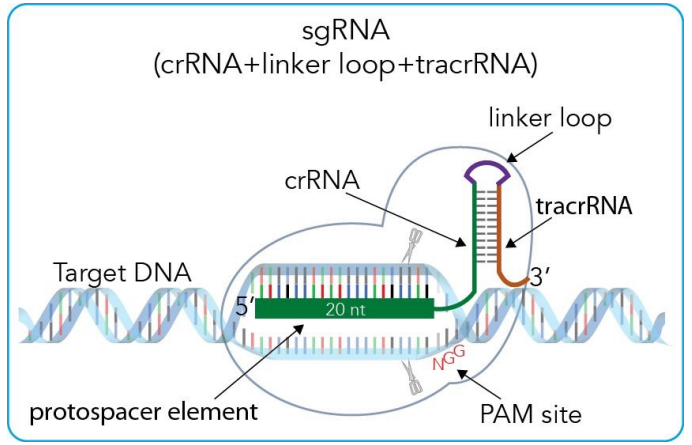
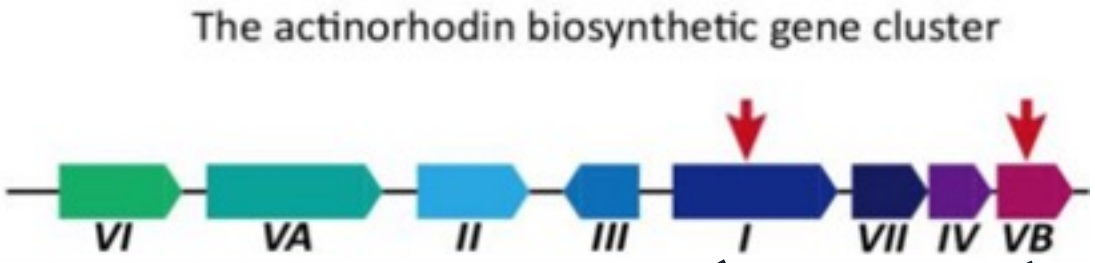
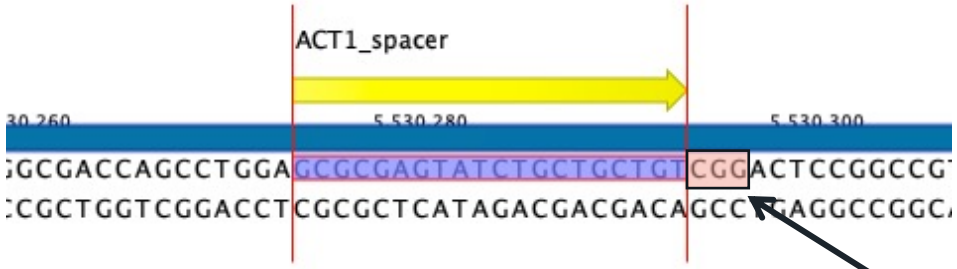
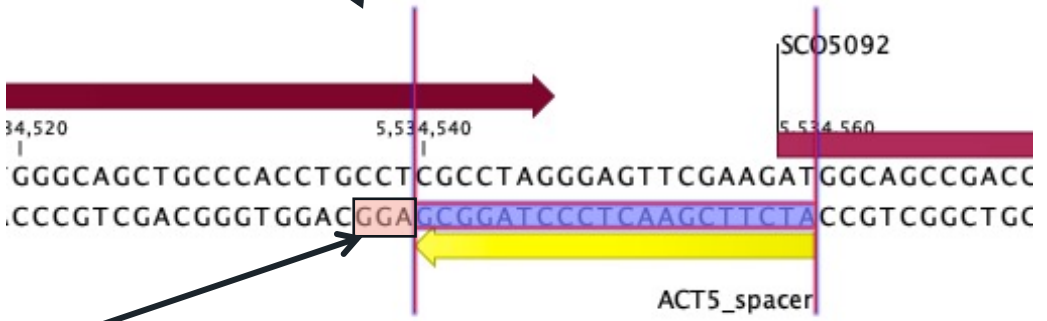
B. Protospacer selection and sgRNA generation





Genome analysis





PAM site

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LAB NETWORK

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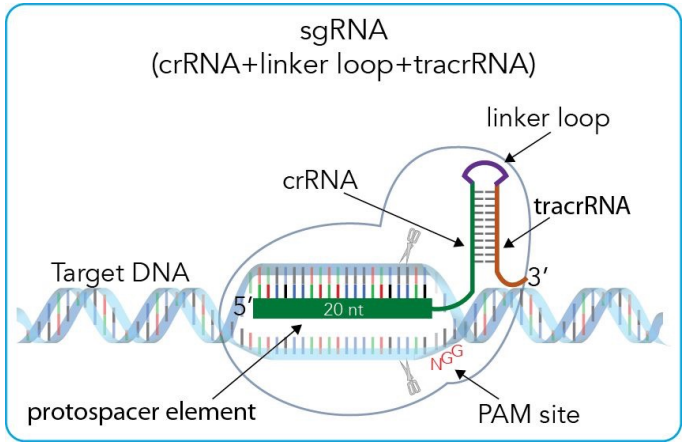
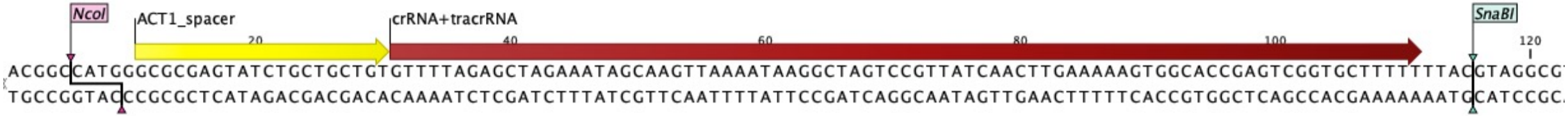


- sgRNA generation by PCR

  NcoI - [spacer + crRNA + linker loop + tracrRNA] – SnaBI (122 bp)



- Cloning fragment into pCRISPR-TT vector NcoI-SnaBI

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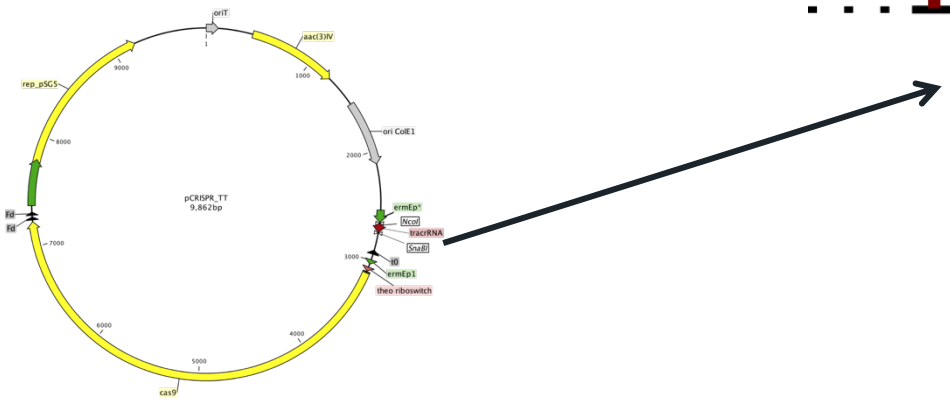
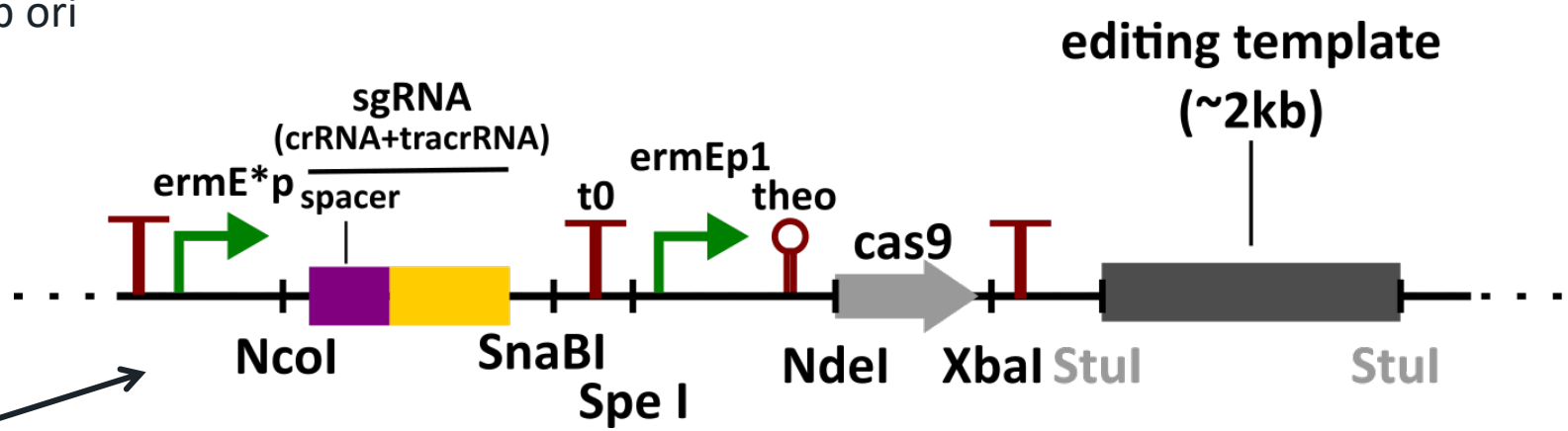
C. Construction of CRISPR plasmid

- Cloning fragment into pCRISPR-TT vector NcoI-SnaBI
  - Apra^r
  - E. coli & Streptomyces rep ori





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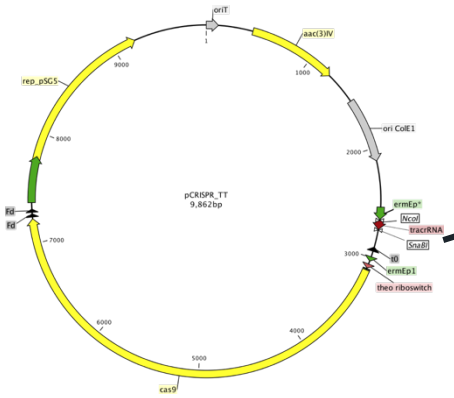

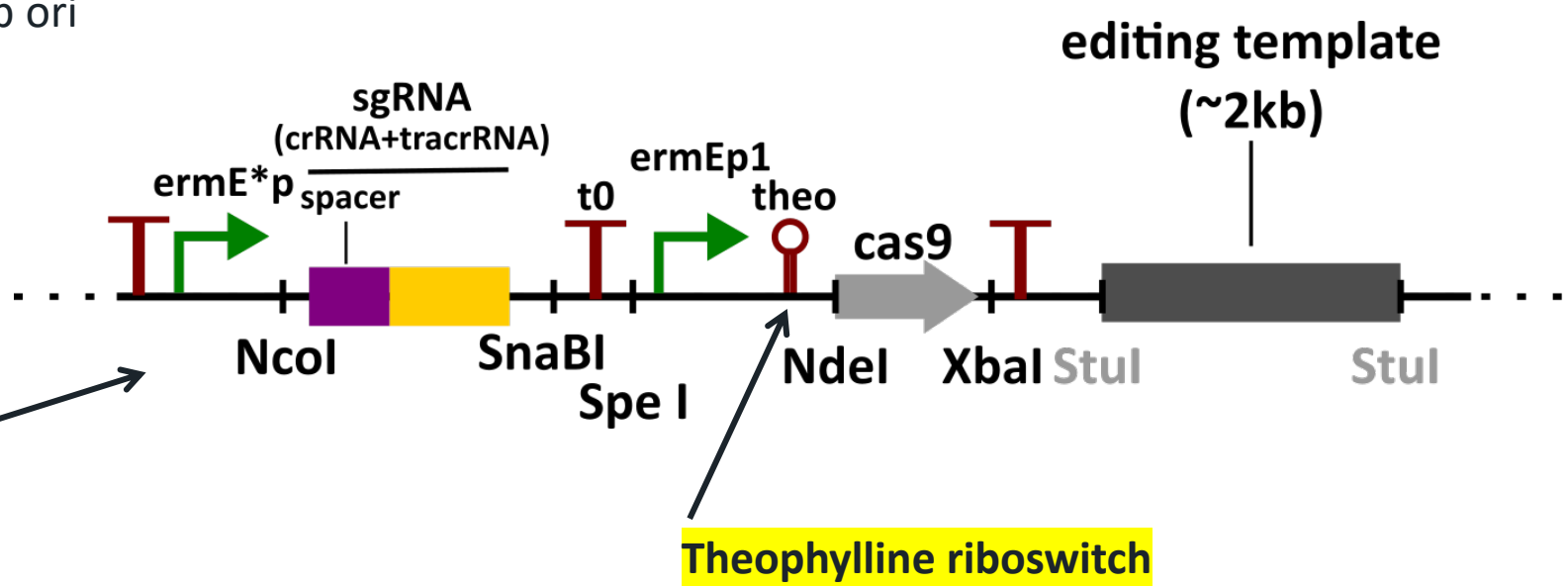

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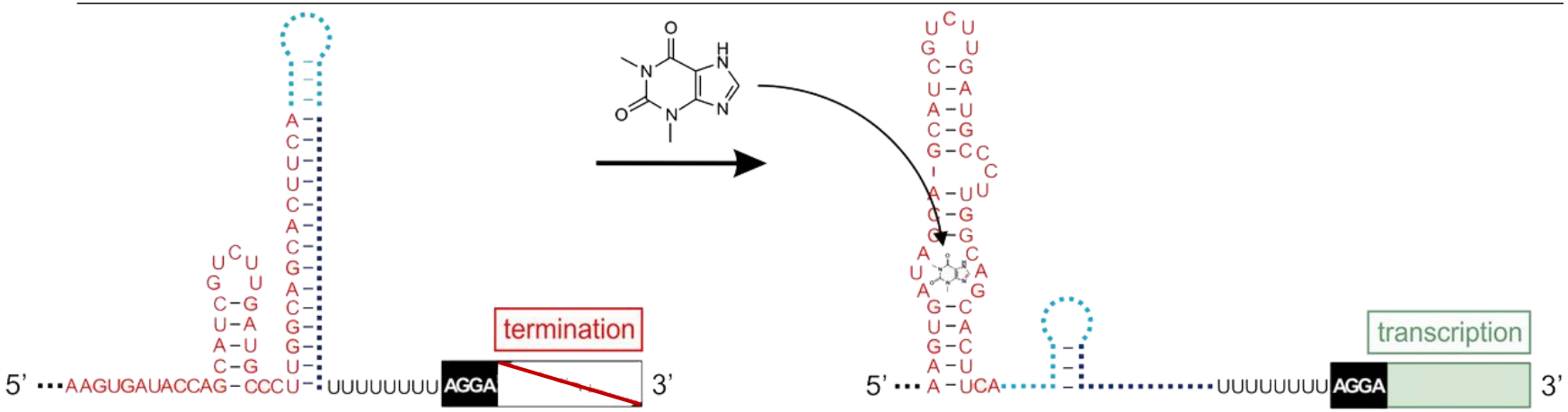
Theophylline riboswitch



Transcription OFF

Transcription ON

[doi: 10.1093/nar/gks1330]

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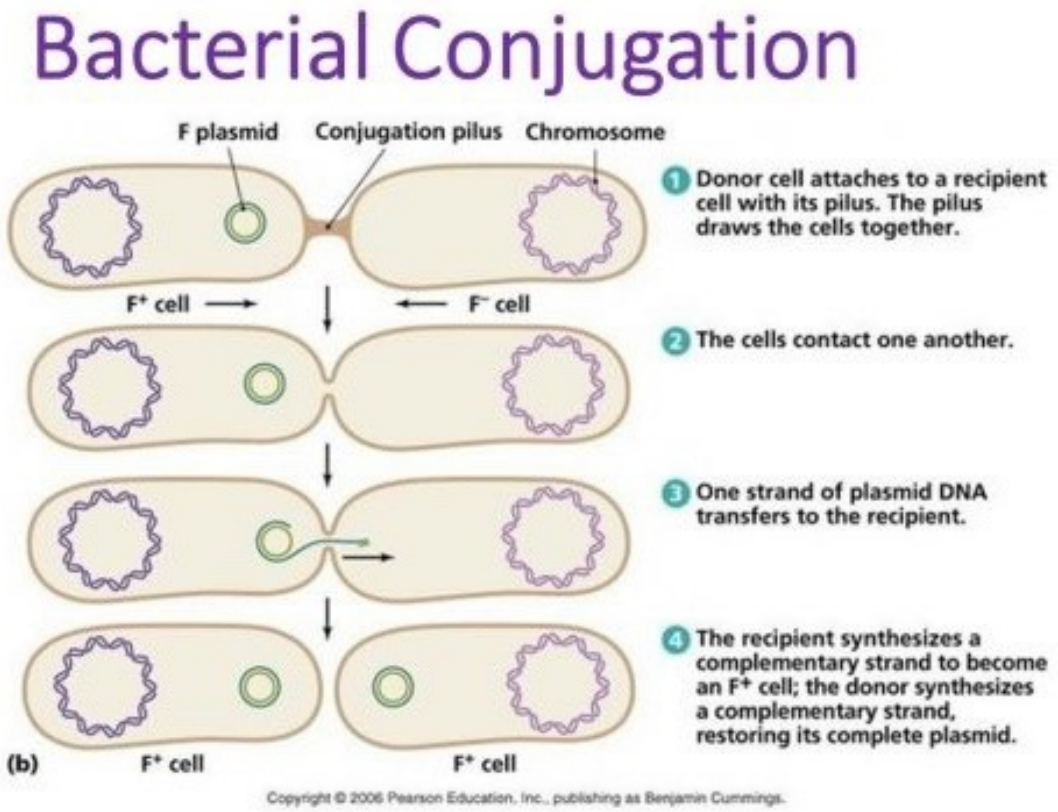
D. transfer of CRISPR plasmid into *S. coelicolor* by intergeneric conjugation

- Conjugation *E. coli* – *S. coelicolor*



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C. Induction of cas9 expression

- plating the *S. coelicolor* harbouring the CRISPR plasmid in media with theophylline

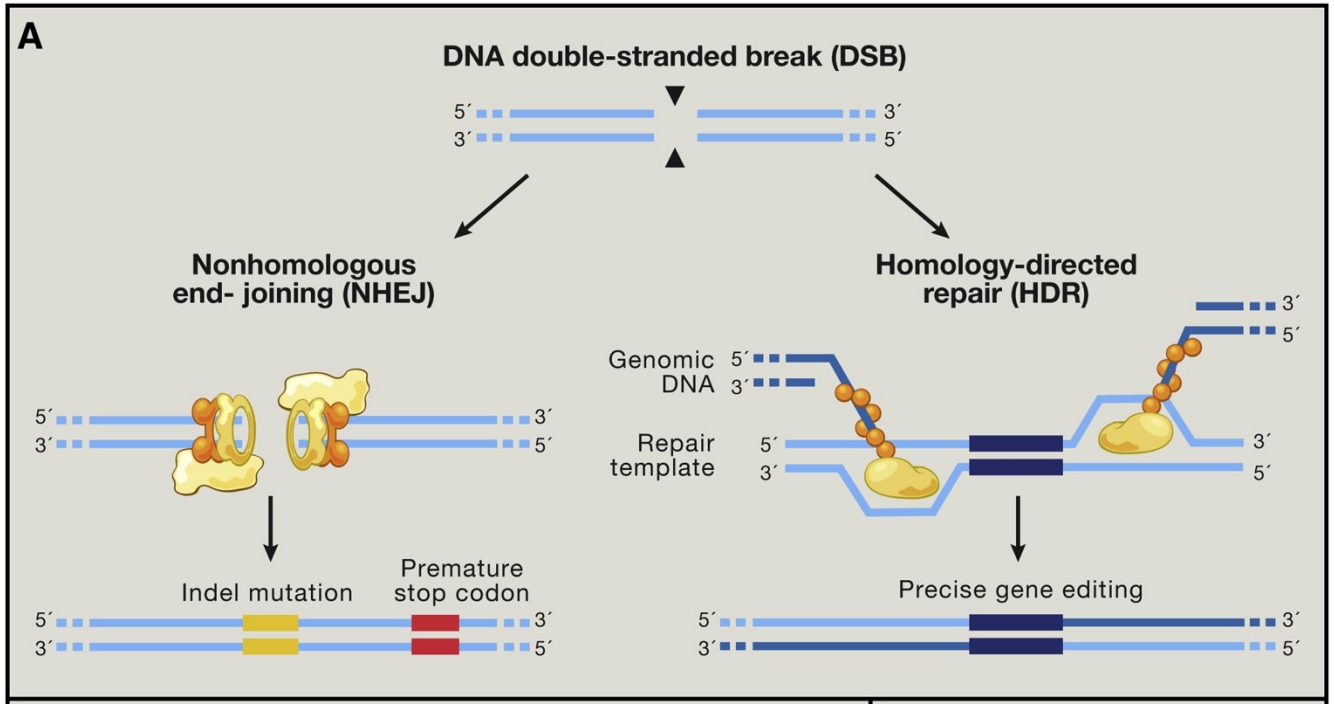
  → induction of theophylline riboswitch → induction of *cas9 expression*

  → different media, different pigment production profile

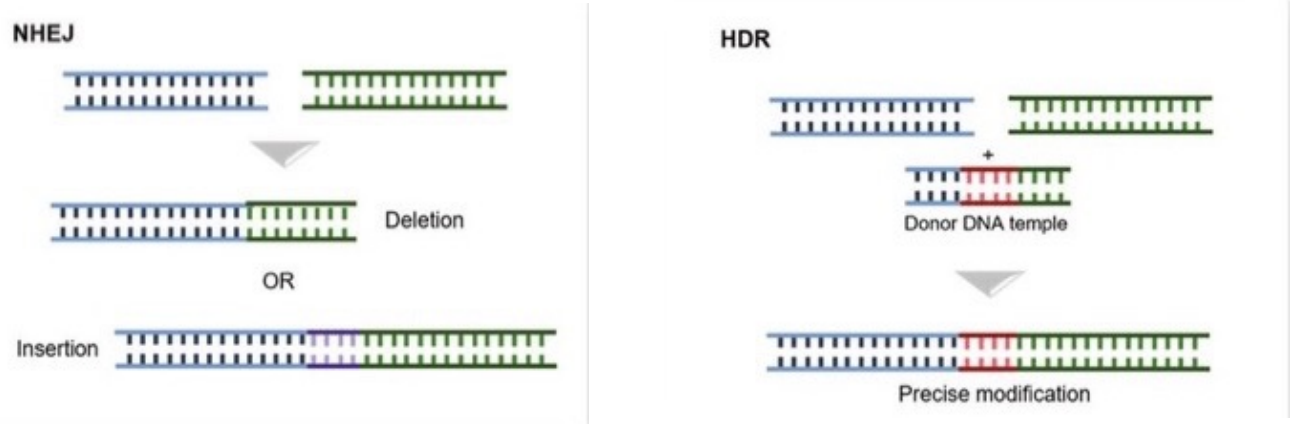
  → presence of Cas9 results in the generation of DNA breaks at the sgRNA location.

  How the bacteria repairs the breaks?

Double strand repair – NHEJ vs HDR



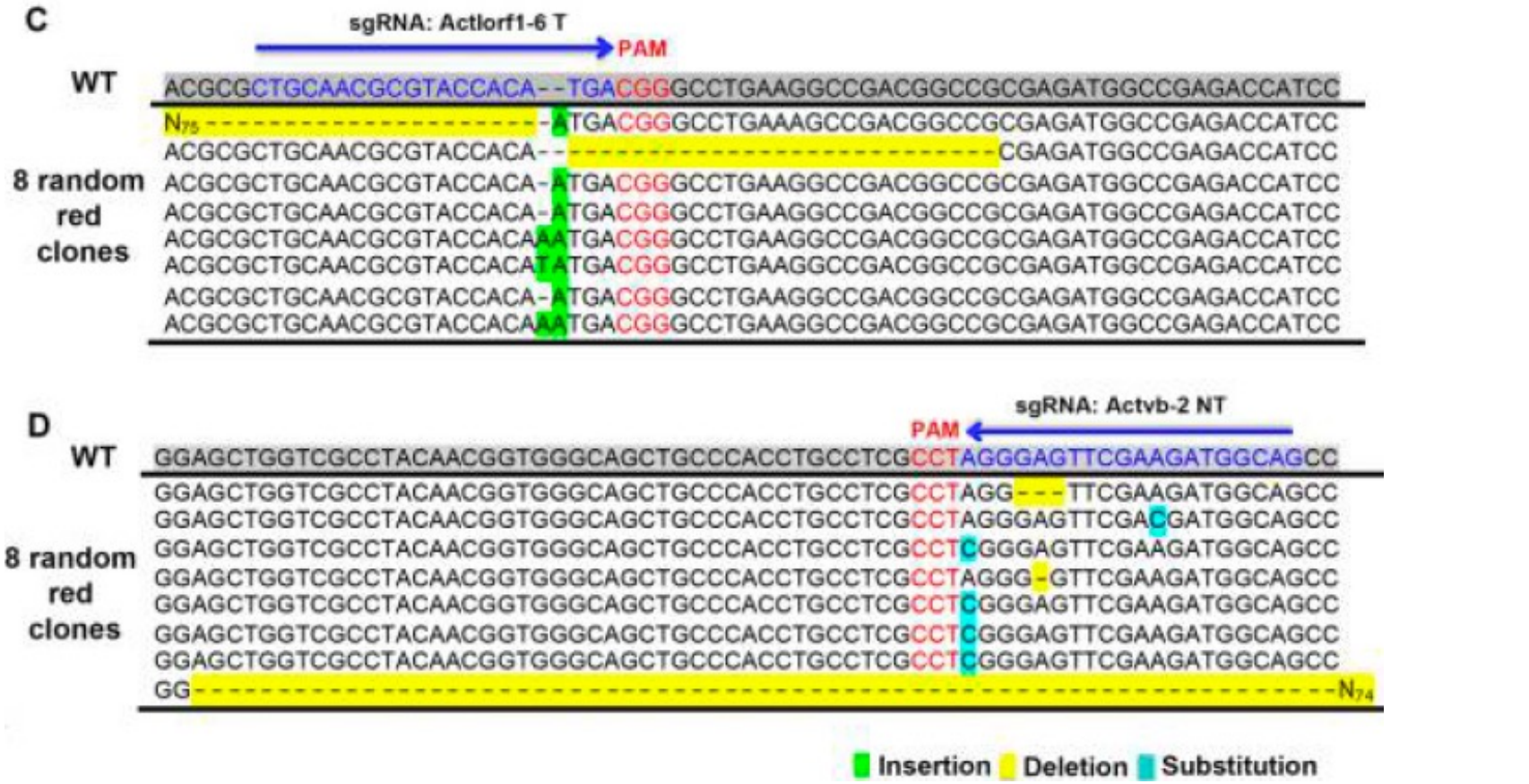
[doi: 10.1016/j.cell.2014.05.010]



HYBRID
LAB NETWORK

ACT1

ACT5



[doi: 10.1021/acssynbio.5b00038]

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What do you need to plate the spore suspension

- To work on sterile conditions → use a bunsen burner (or similar)
- Media plates (TSA + 5 mM theo)
- spore solution (WT,
- spreader
- pipette – 100 µL

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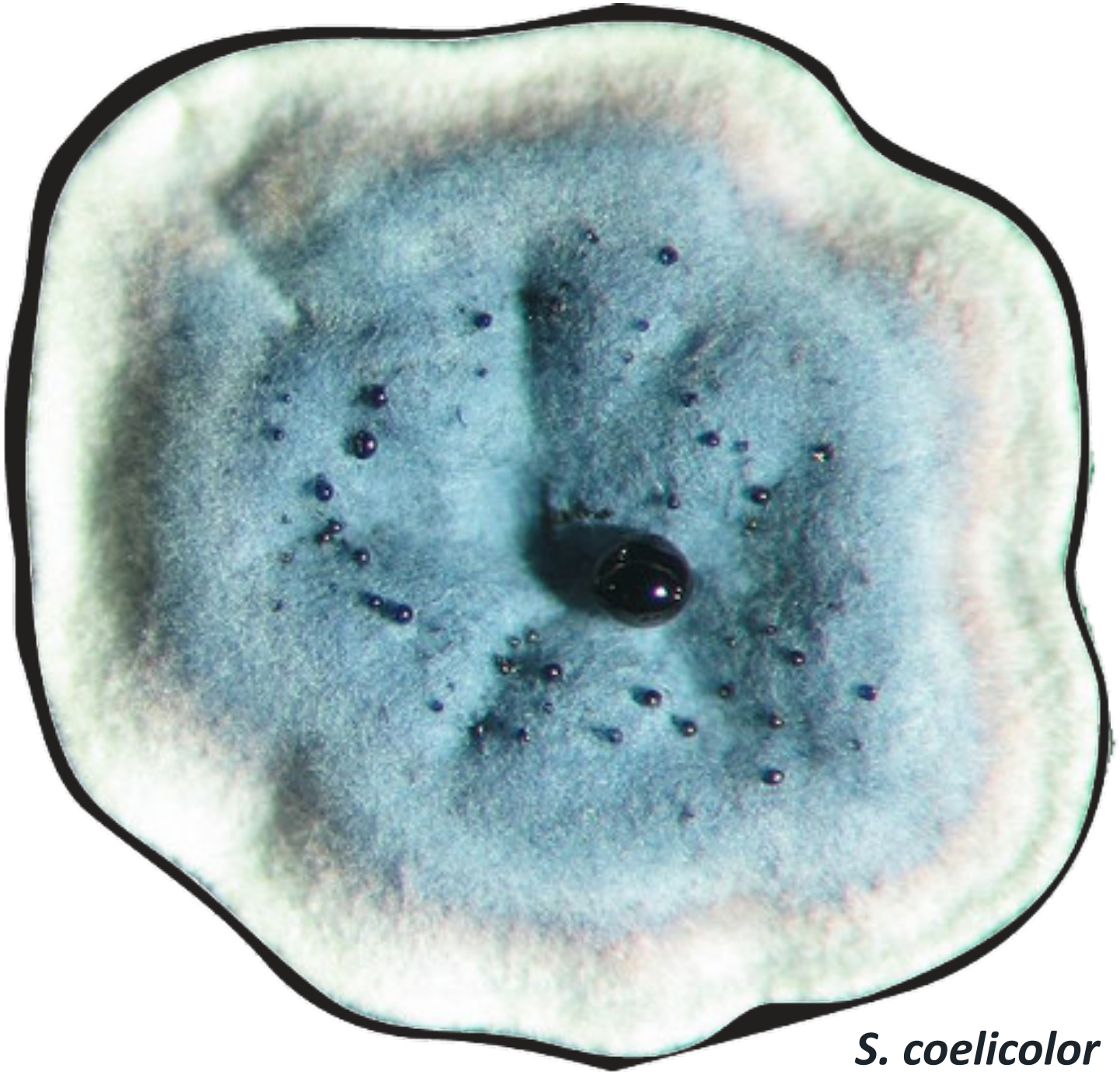

S. coelicolor

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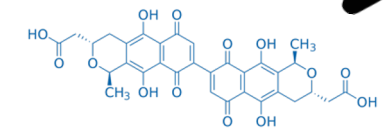
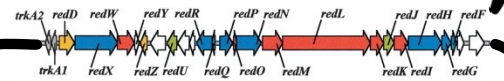
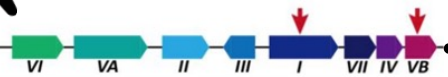
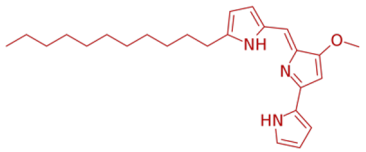


Actinorhodin







Undecylprodigiosin

HYBRID
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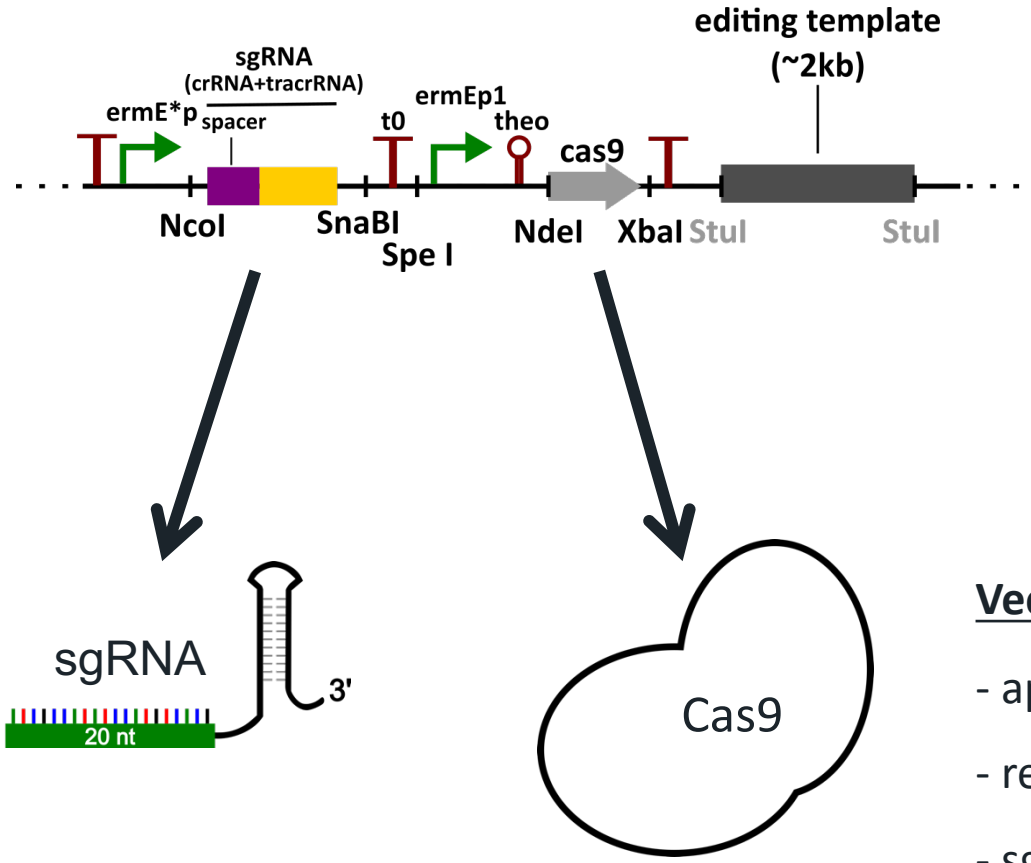
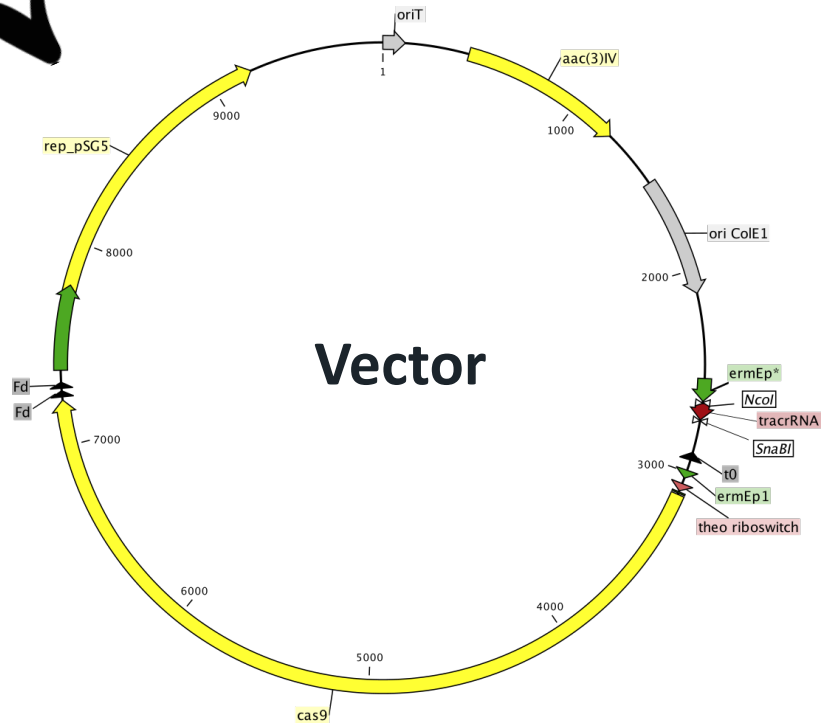
sgRNA generation



Vector features:

- apramycin – vector selection
- replication origin
- sgRNA
- *cas9*
- transfer origin



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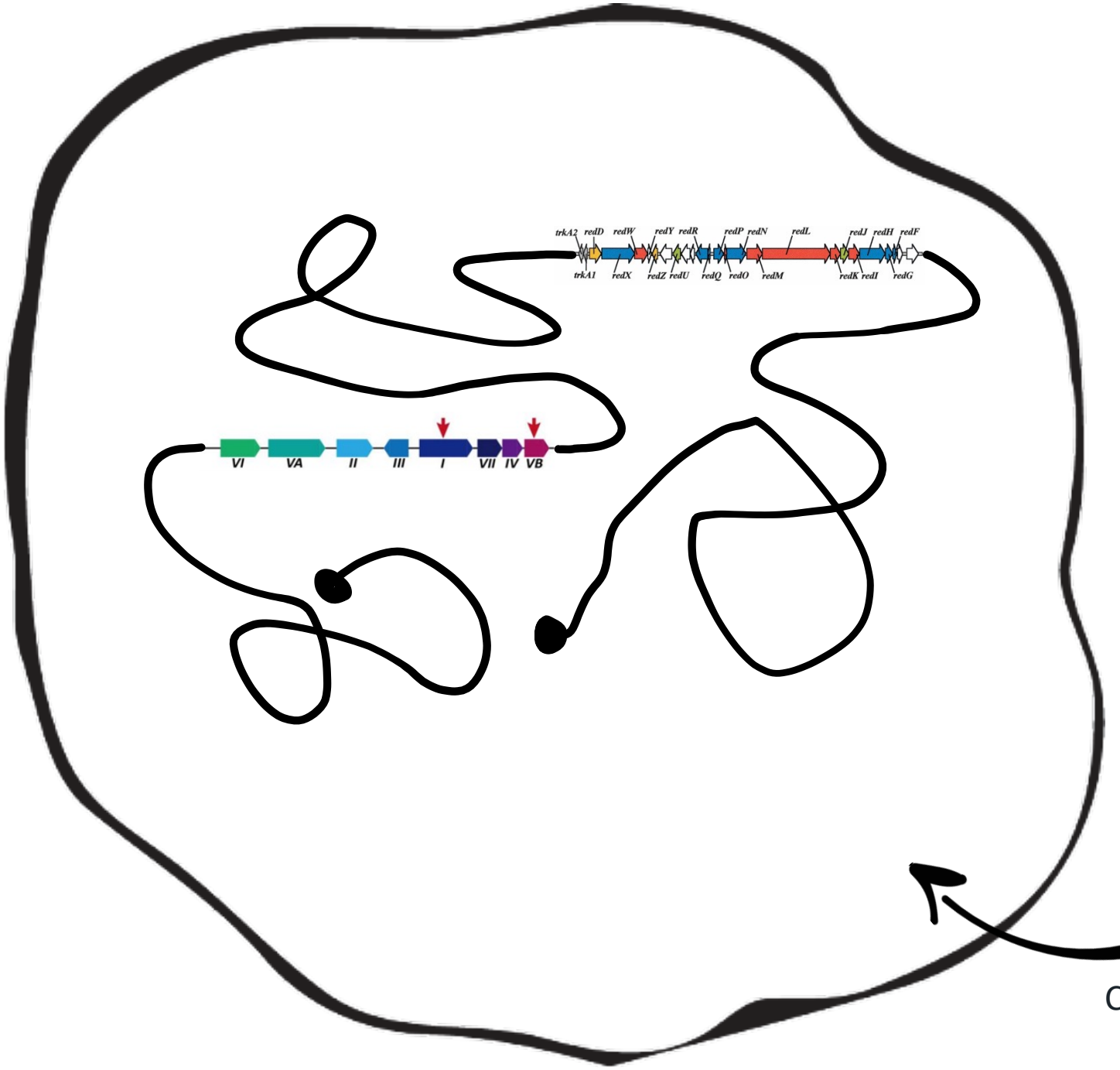

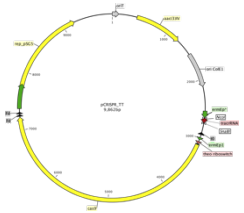

Conjugation

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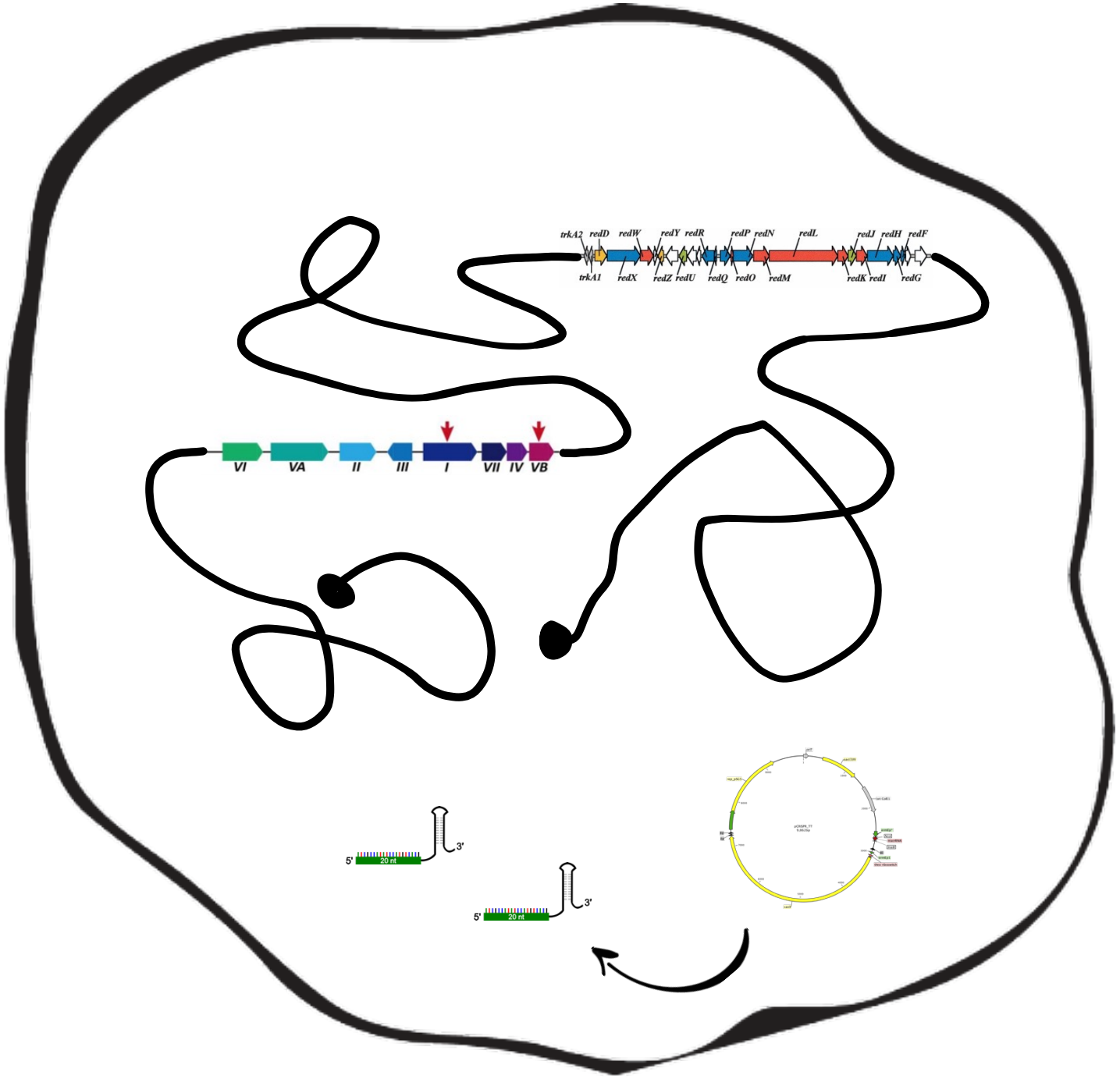
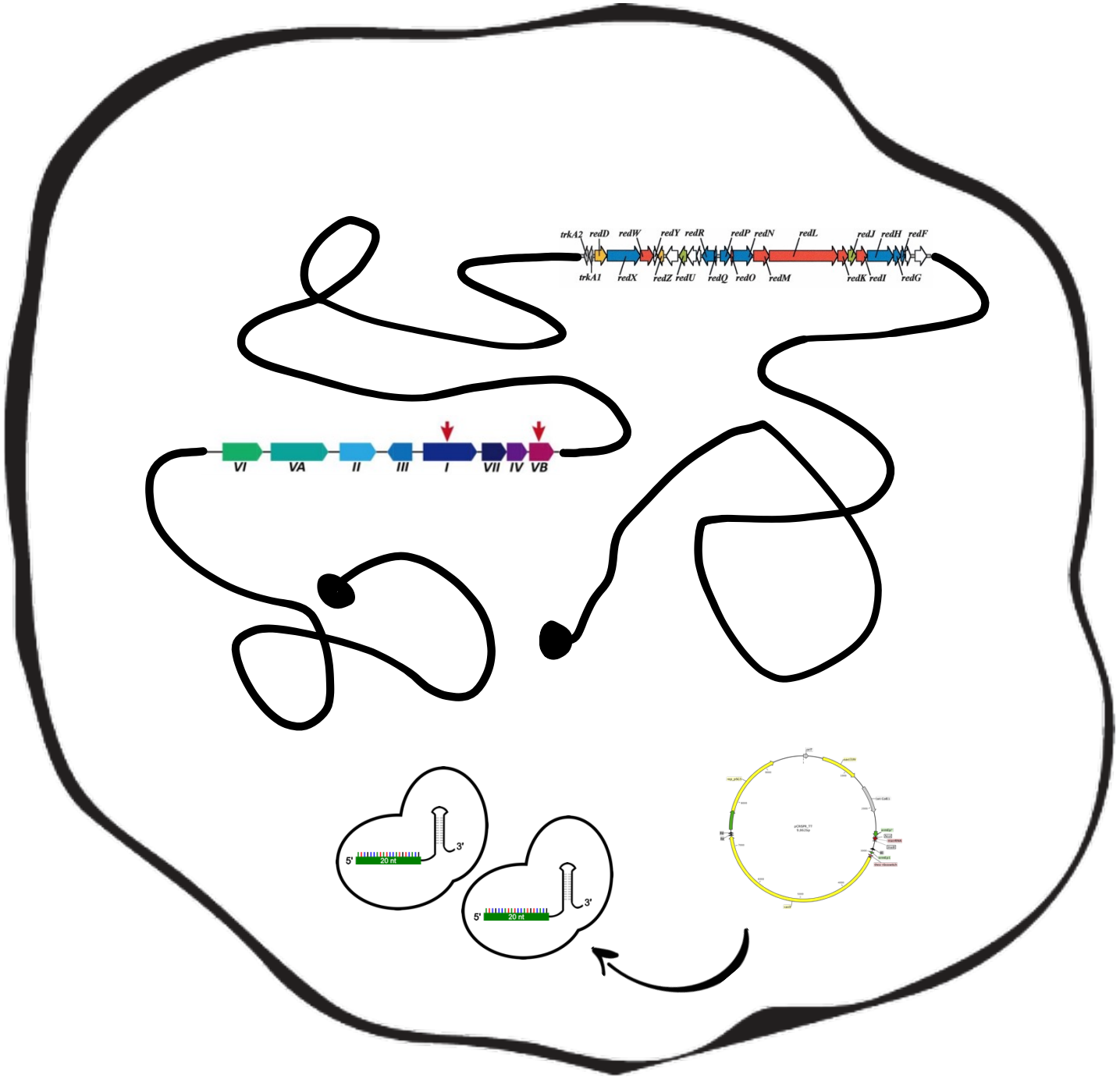


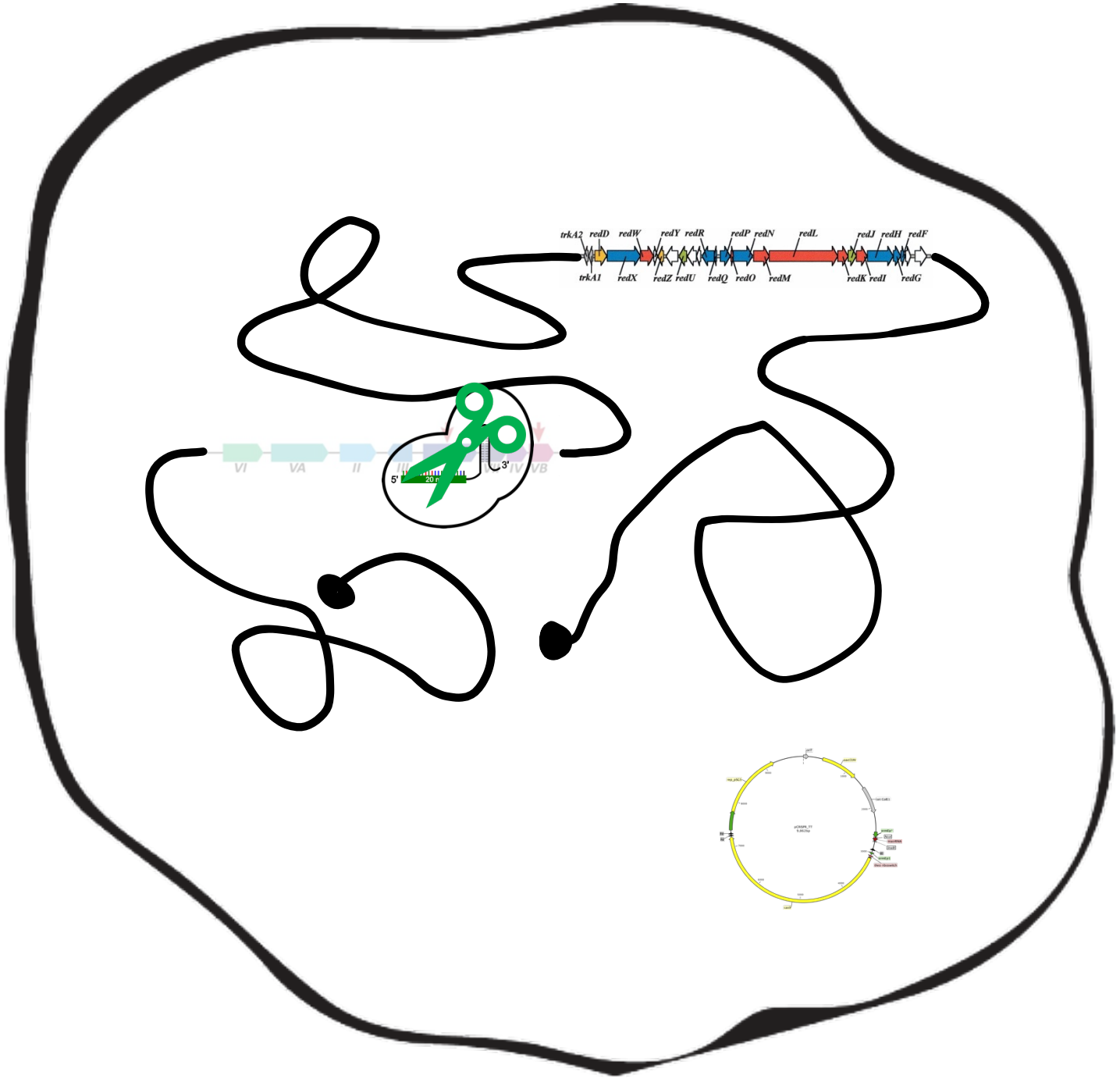
Apramycin^{r}

HYBRID
LAB NETWORK



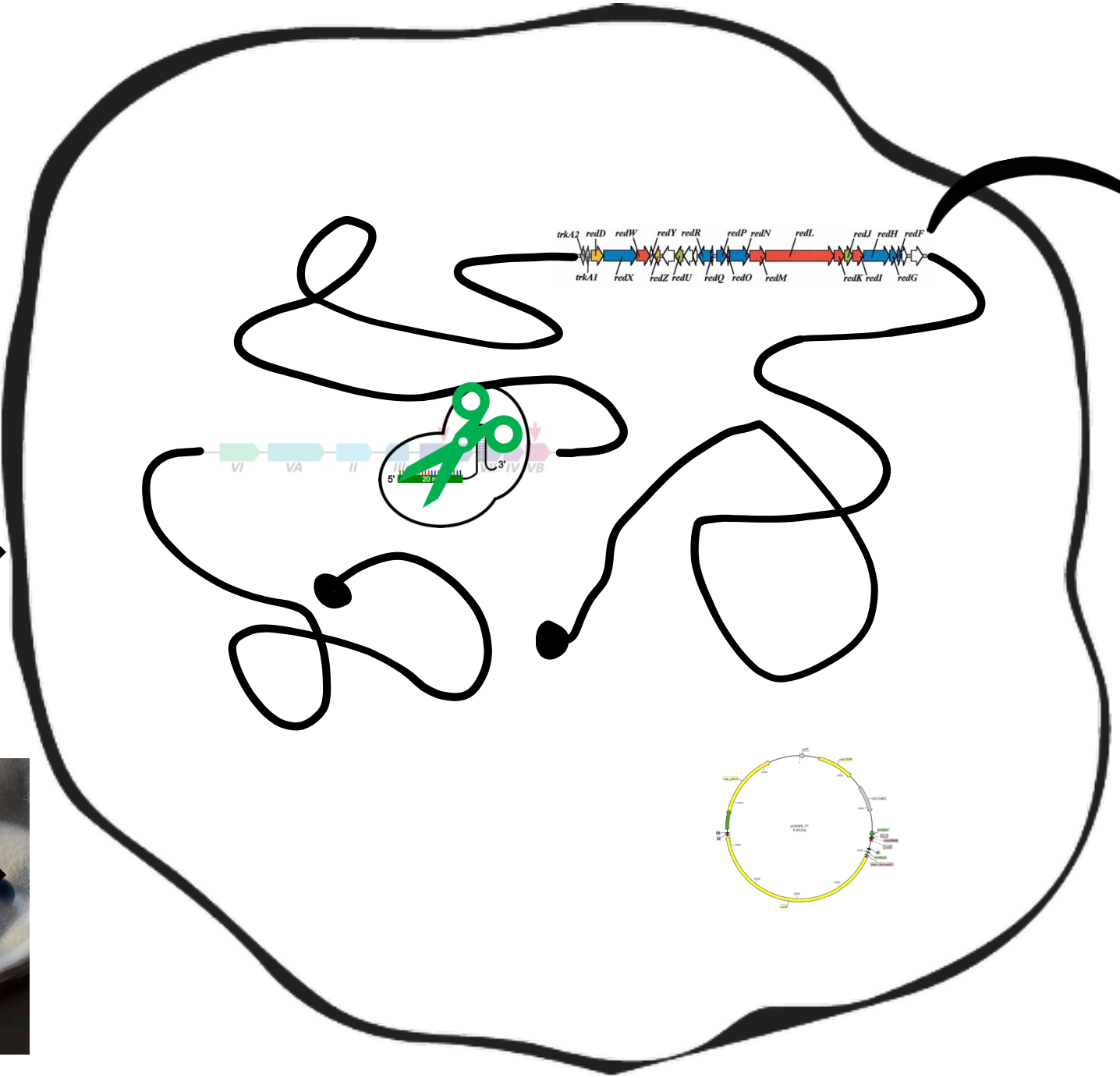
+ theophylline
Apramycin^{r}

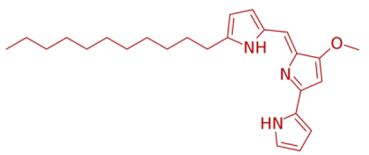
HYBRID
LAB NETWORK



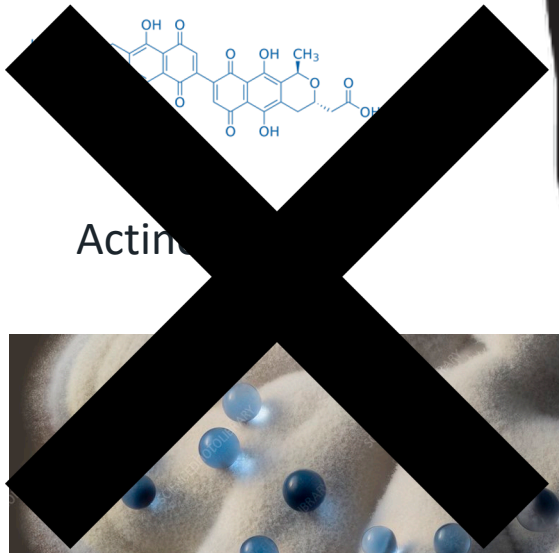
+ theophylline
Apramycin^{r}

HYBRID
LAB NETWORK





Undecylprodigiosin



Actin

+ theophylline

Apramycin^{r}

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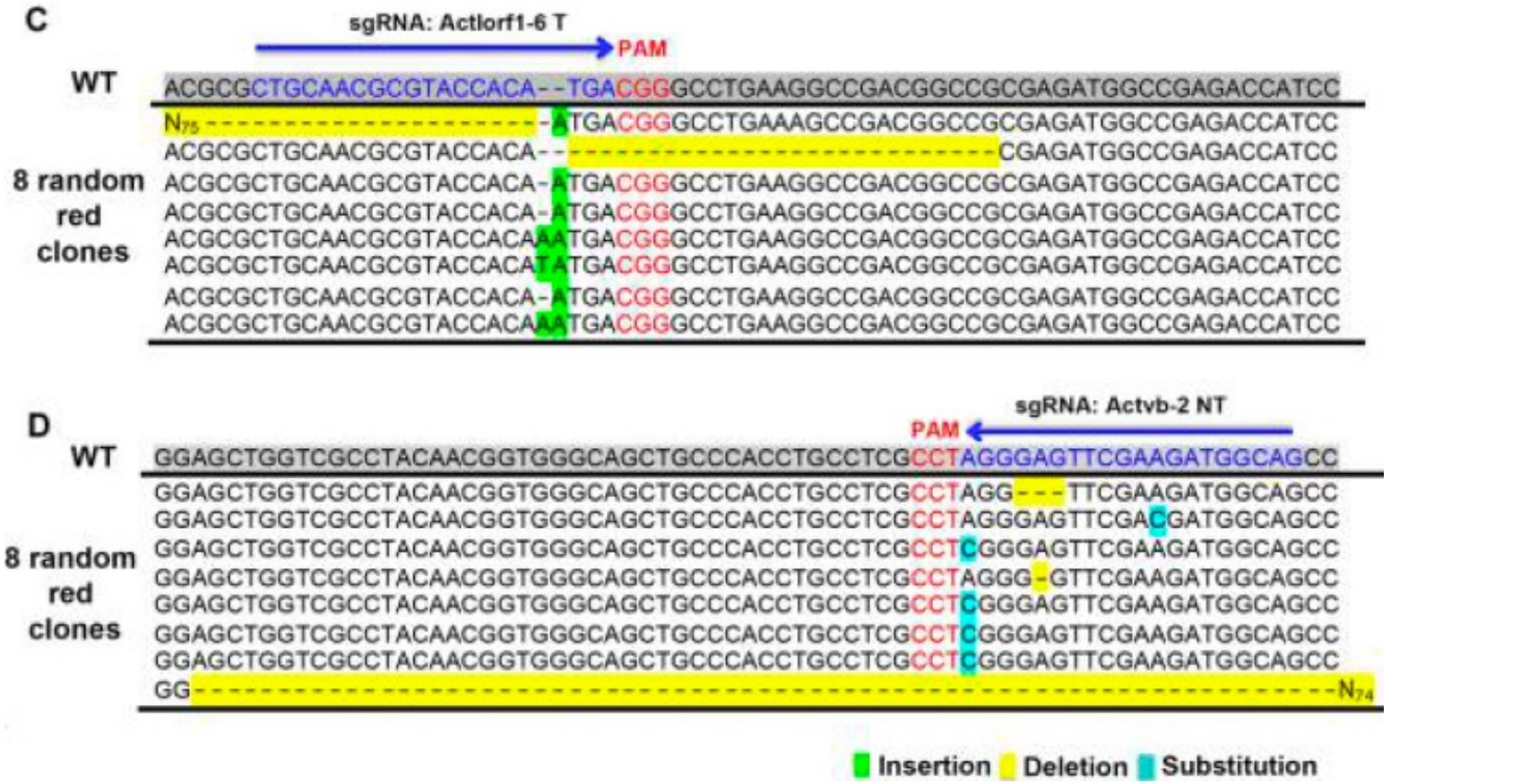
ACT1

ACT5



[doi: 10.1021/acssynbio.5b00038]

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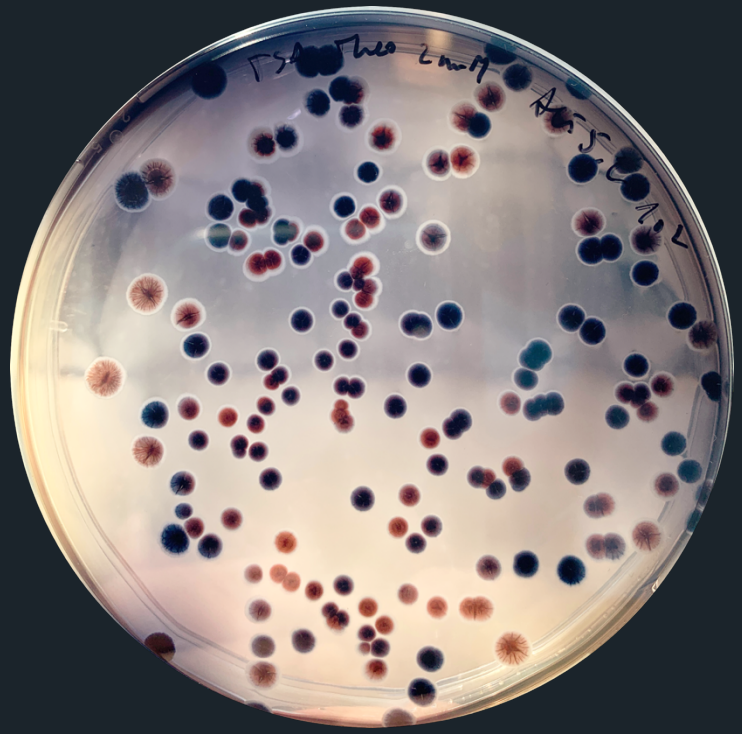
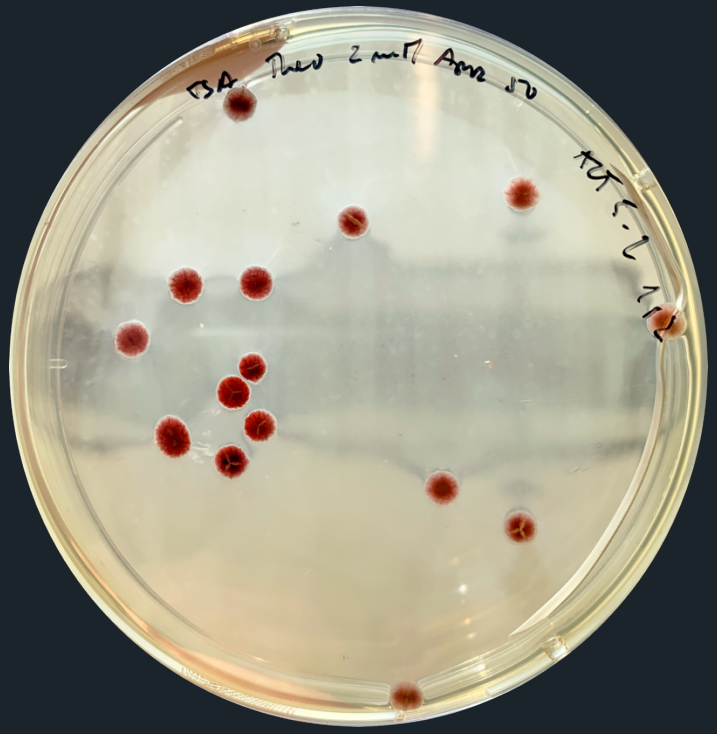
Theo 2mM

wild-type



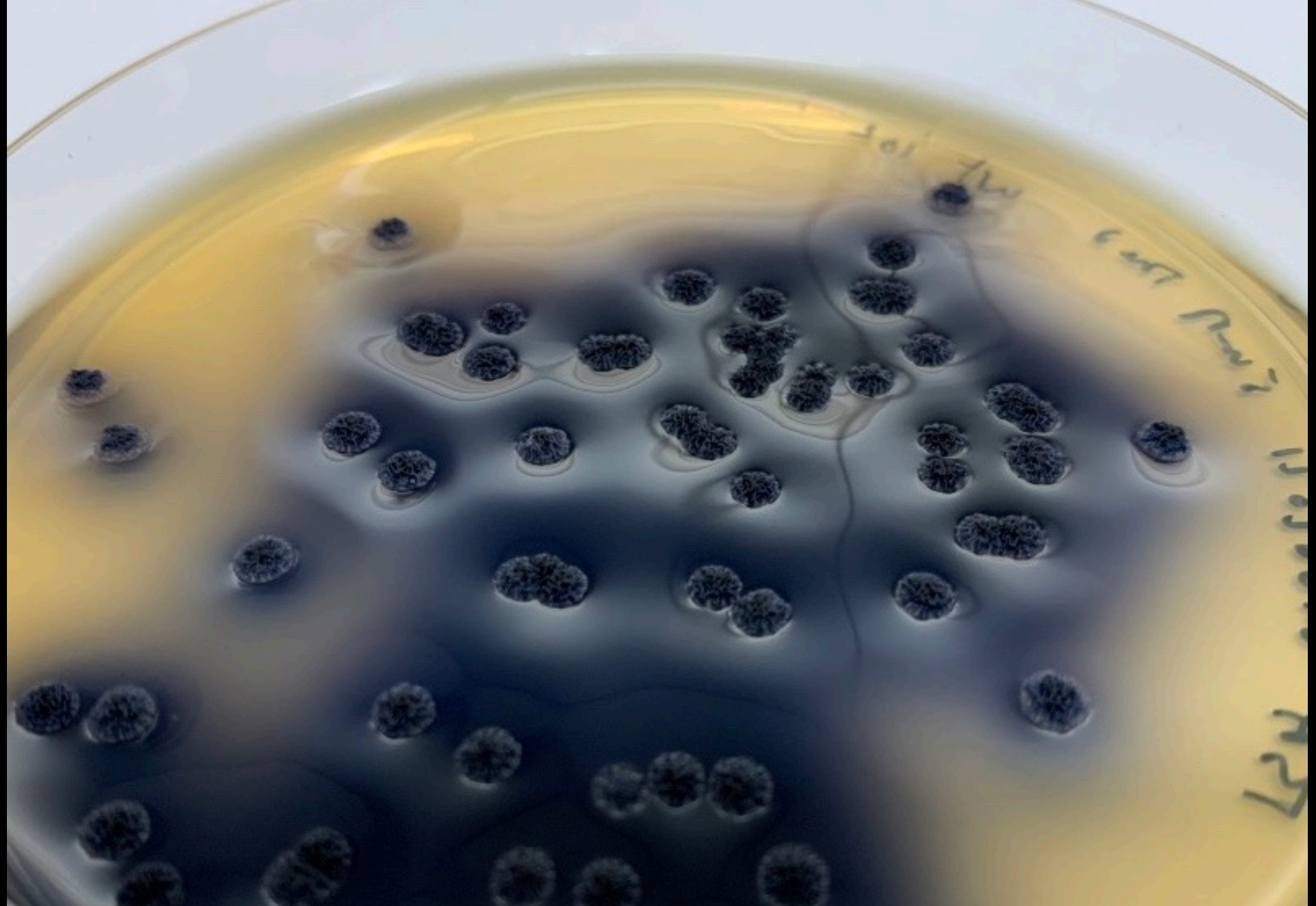
Theo 2mM

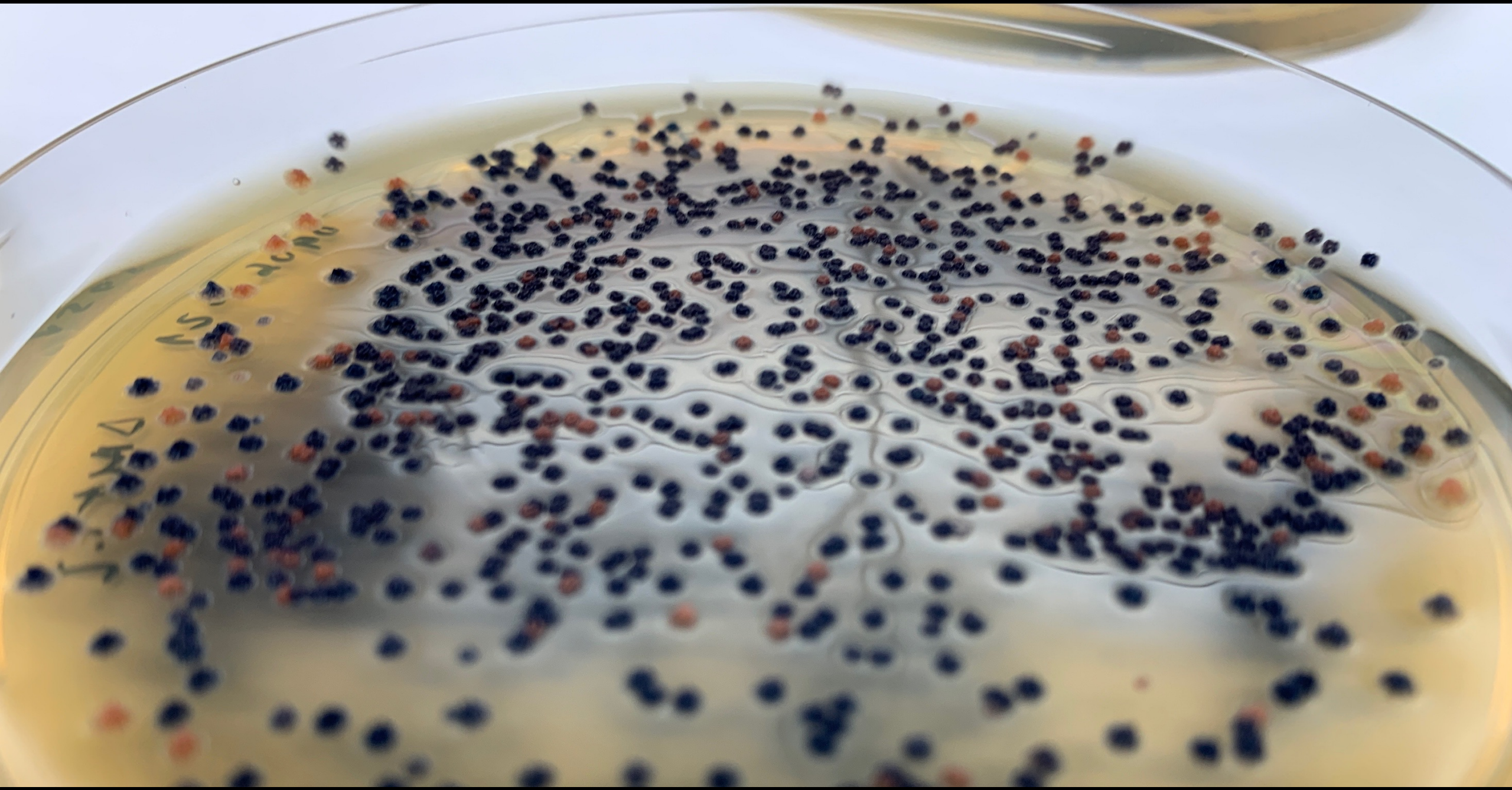
ΔACT 5.2

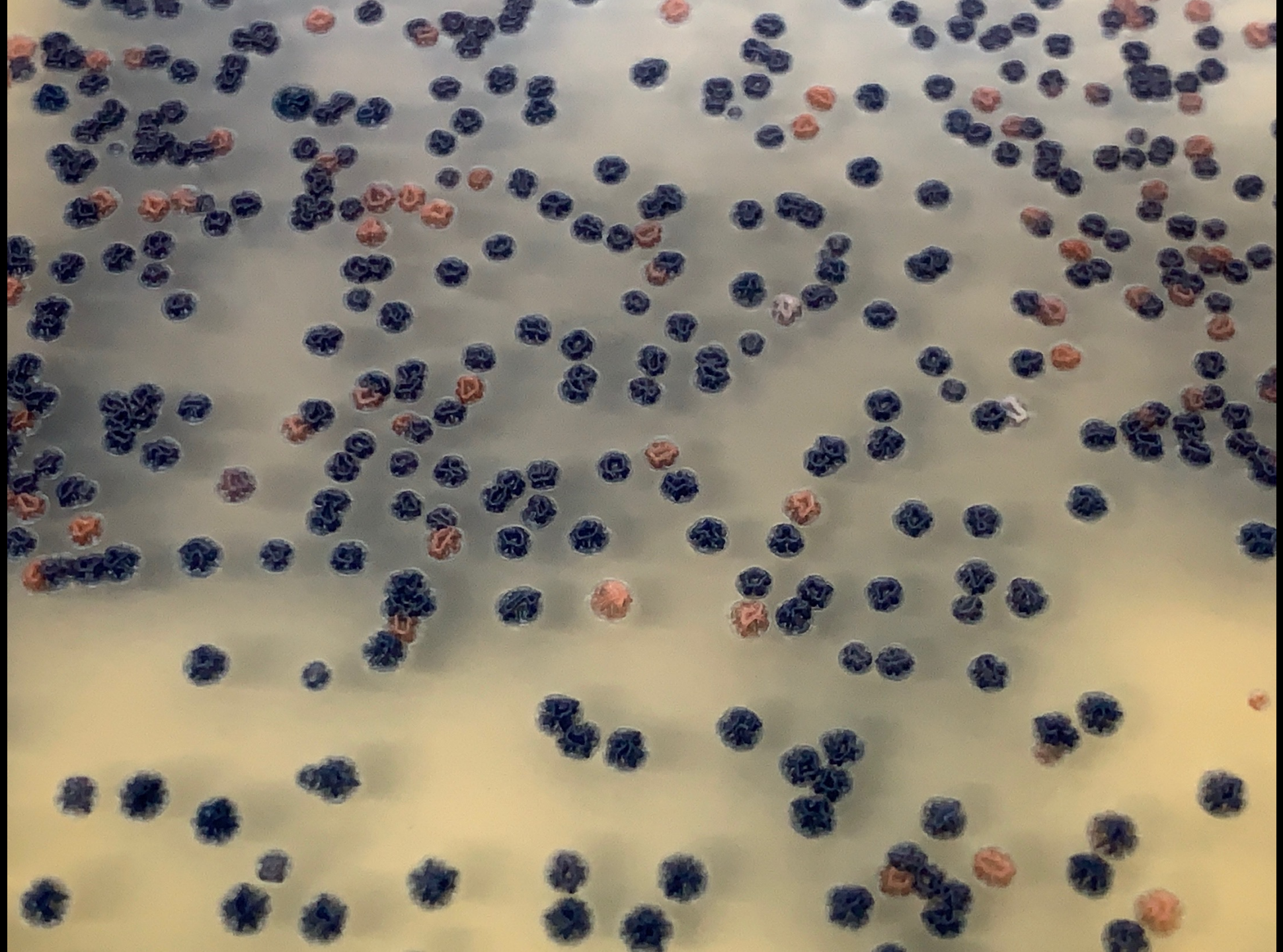


Theo 2mM + Apra 50 µg/mL

ΔACT 5.2







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Thank you!

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Aalto University
School of Arts, Design
and Architecture

ALMA MATER
EUROPAEA

IBMC

waag

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