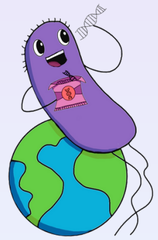
Synthetic Biology



Free Coli
iGEM 2021

iGEM 2021

The Australasian SynBio Challenge

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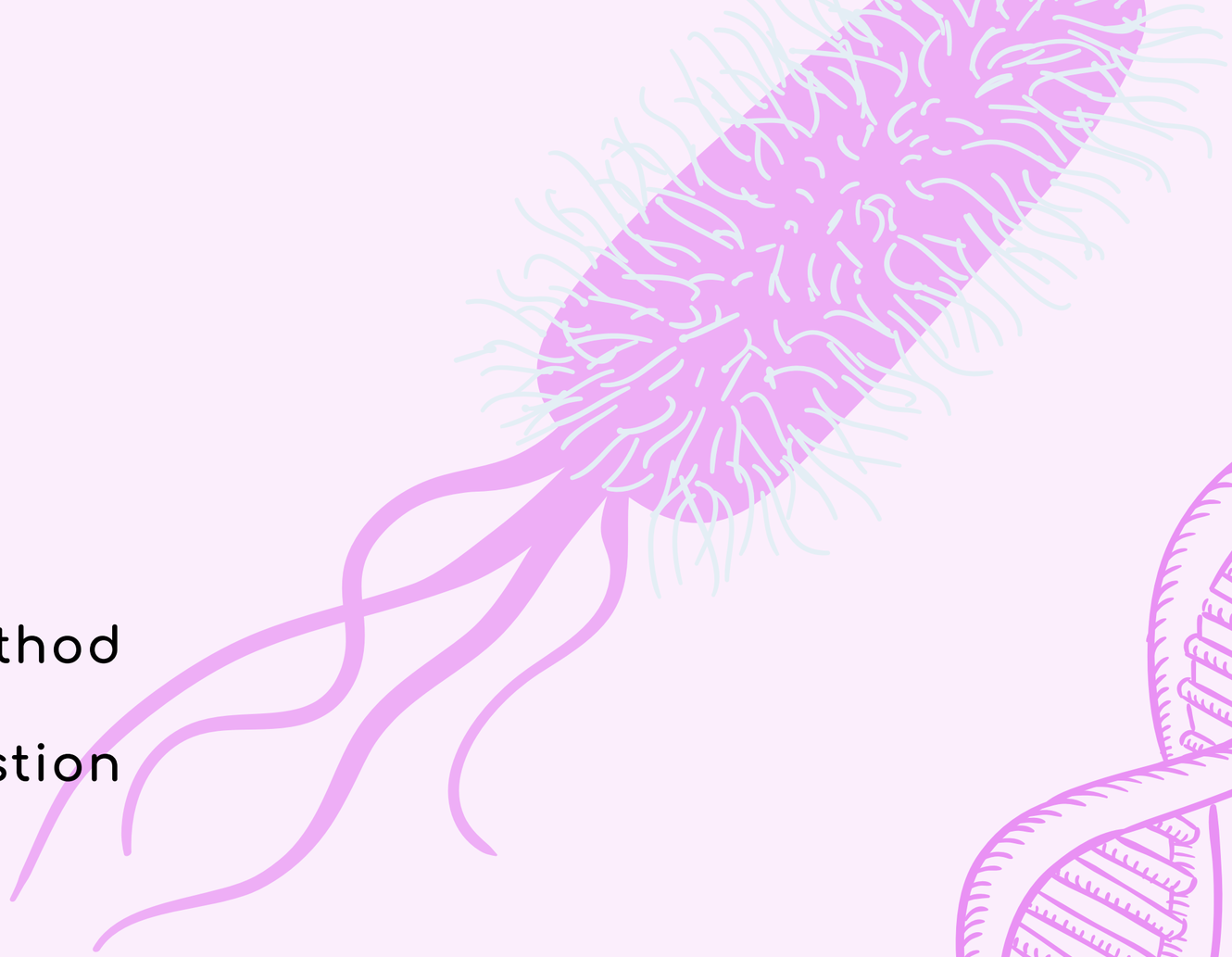
Overview

1. Introduction
2. What is Synthetic Biology?
3. Recapping the Scientific Method
4. Developing a Research Question
5. Formulating a Hypothesis
6. Developing Predictions
7. Designing an Experiment



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Q & A

Please send any questions you have on synthetic biology, genetic engineering, iGEM, or studying science at University - we will endeavour to answer throughout the presentation or at the end.

Go to www.menti.com and use the code 6235 4649?

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iGEM 2021

The International Genetically Engineered Machine (iGEM) Foundation is a global non-profit dedicated to advancing synthetic biology, education and competition.

The iGEM Competition asks multidisciplinary teams of university students to redesign an organism or biological system to build a better world by solving problems with the help of synthetic biology.

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University of Sydney Past Projects

2019 - Genetically engineering E. coli to produce psilocybin enzymes

2017 - Designed and produced stable, efficient and low-cost insulin

2016 - Designed a fruit ripeness biosensor (won global runner-up)

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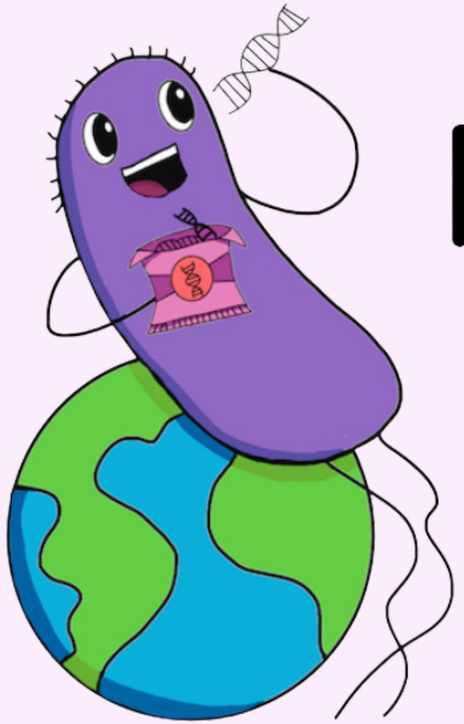
Improving accessibility to synthetic biology by engineering a naturally transformable Escherichia coli



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What is Synthetic Biology?

Synthetic biology = redesigning organisms to produce something useful, like medicine, food or fuel

1. E. coli cells designed to make insulin to treat diabetes
2. Microbes designed to clean up pollutants
3. Rice designed to produce vitamin A to treat nutritional deficiency

The future of....

Health &
Medicine

Agriculture &
Food

Environment &
Biocontrol

Industrial
Biotechnology

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Show transcript

Unlocking Australia's $27 billion Synthetic Biology opportunity

This new report identifies that synthetic biology has the potential to unlock $27 billion in revenue and 44,000 jobs annually for Australia by 2040. This includes $19 billion for food and agriculture and $7 billion for the health and medicine sectors.

Read the report

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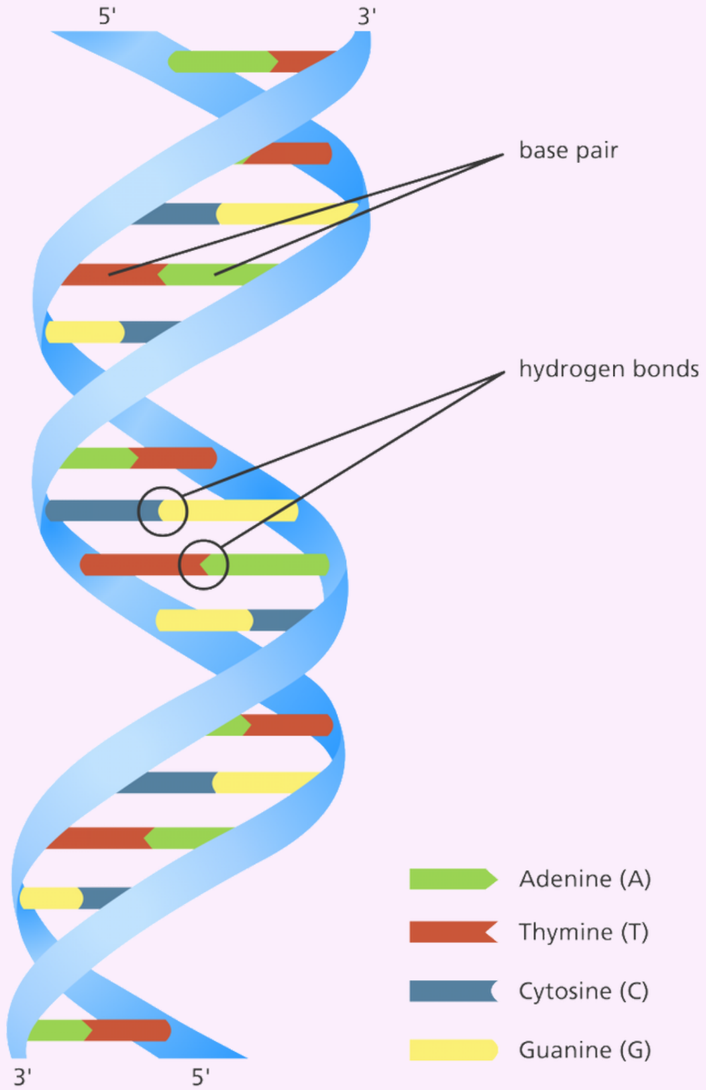
How do you redesign an organism?

Go to www.menti.com and use the code 8431 5148?

To redesign an organism, you need to change its DNA

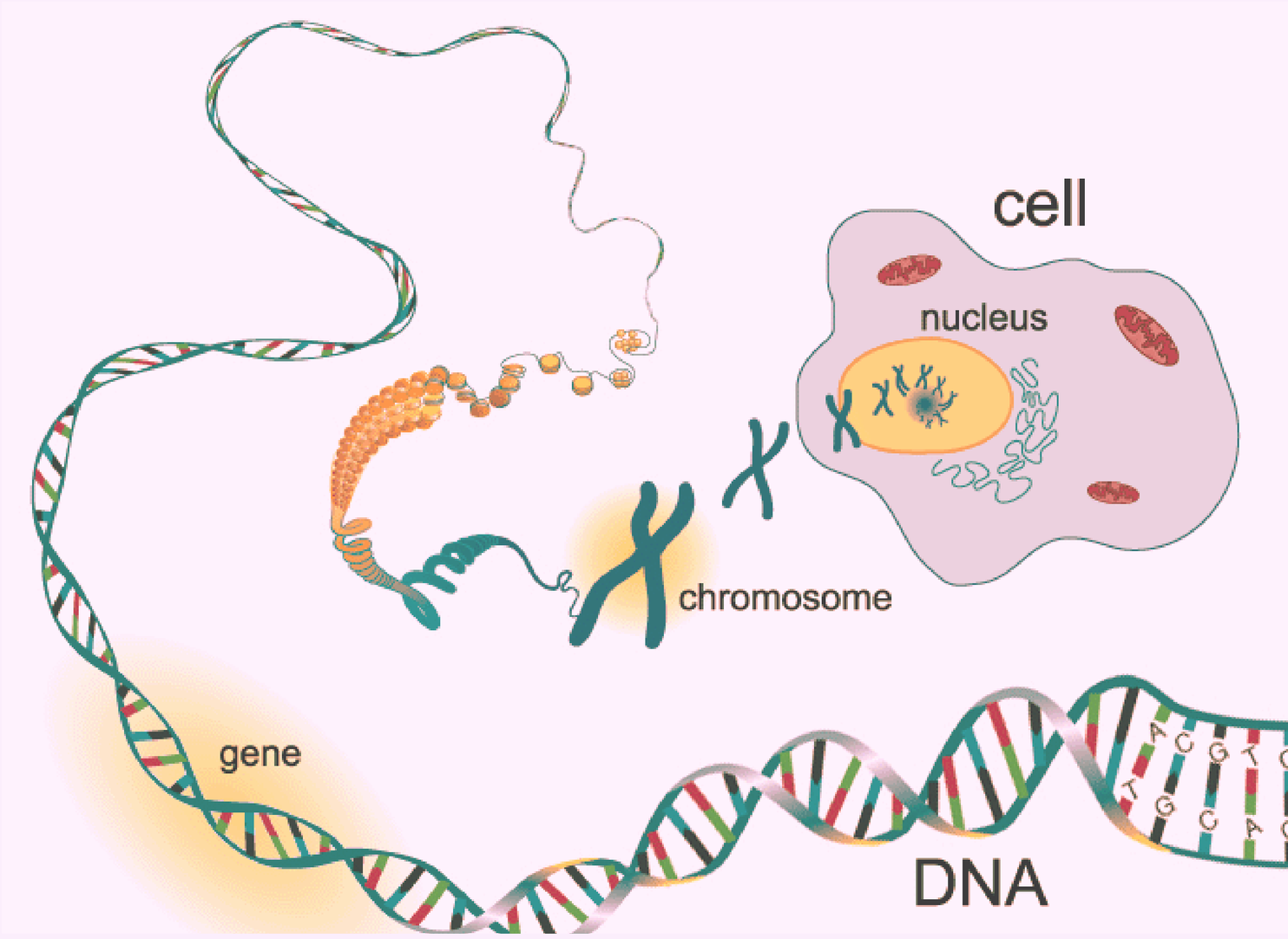


DNA is a molecule carrying genetic instructions for the development, functioning, growth and reproduction of organisms (and many viruses)

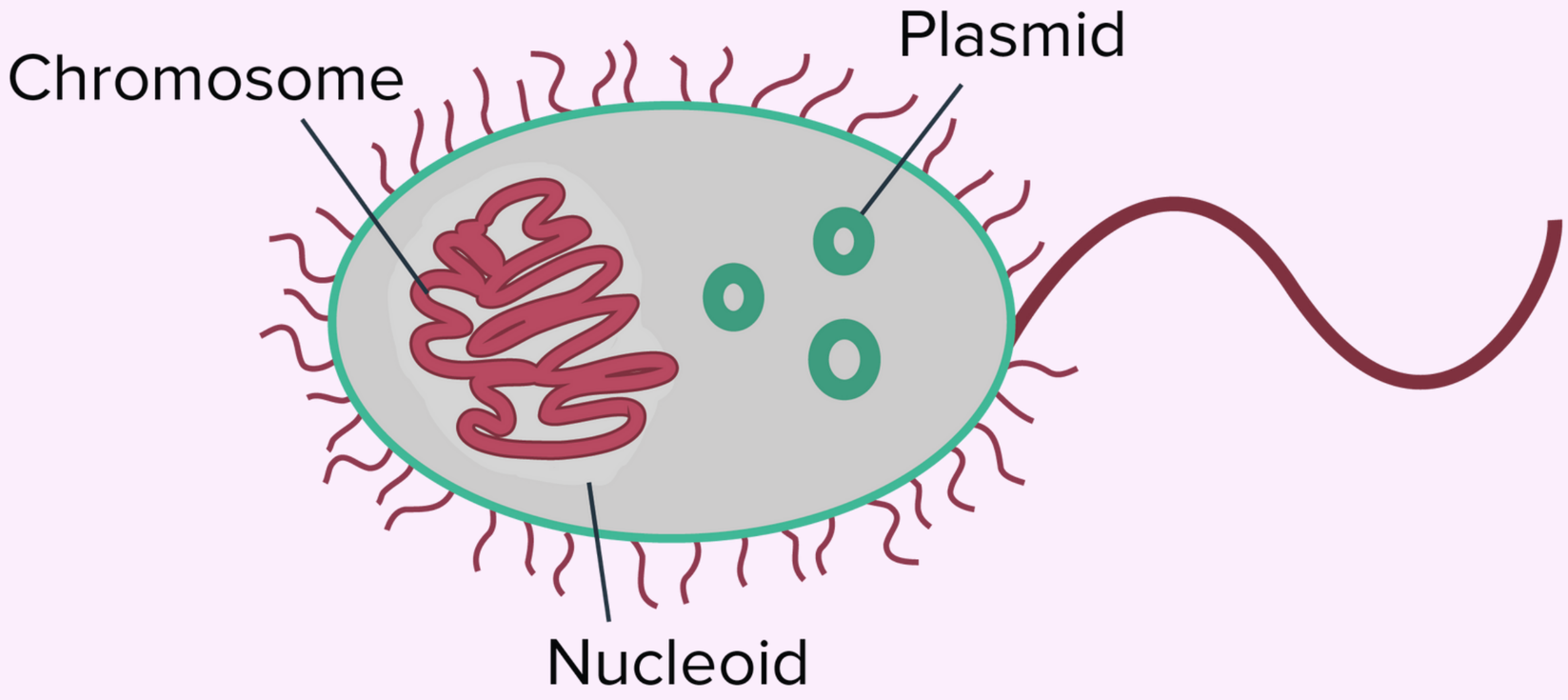
DNA is stored in chromosomes...

Humans (eukaryotes) have 23 pairs of chromosomes, one in each pair inherited from each of our parents.

Bacteria (prokaryotes) like E. coli only have one circular chromosome!



Prokaryotes reproduce via binary fission - the unicellular organism divides into two daughter cells, each with the same chromosome (and identical genetic material) as its parent



Imagine that your DNA is a book containing all of the instructions for your body's development, function and regulation...

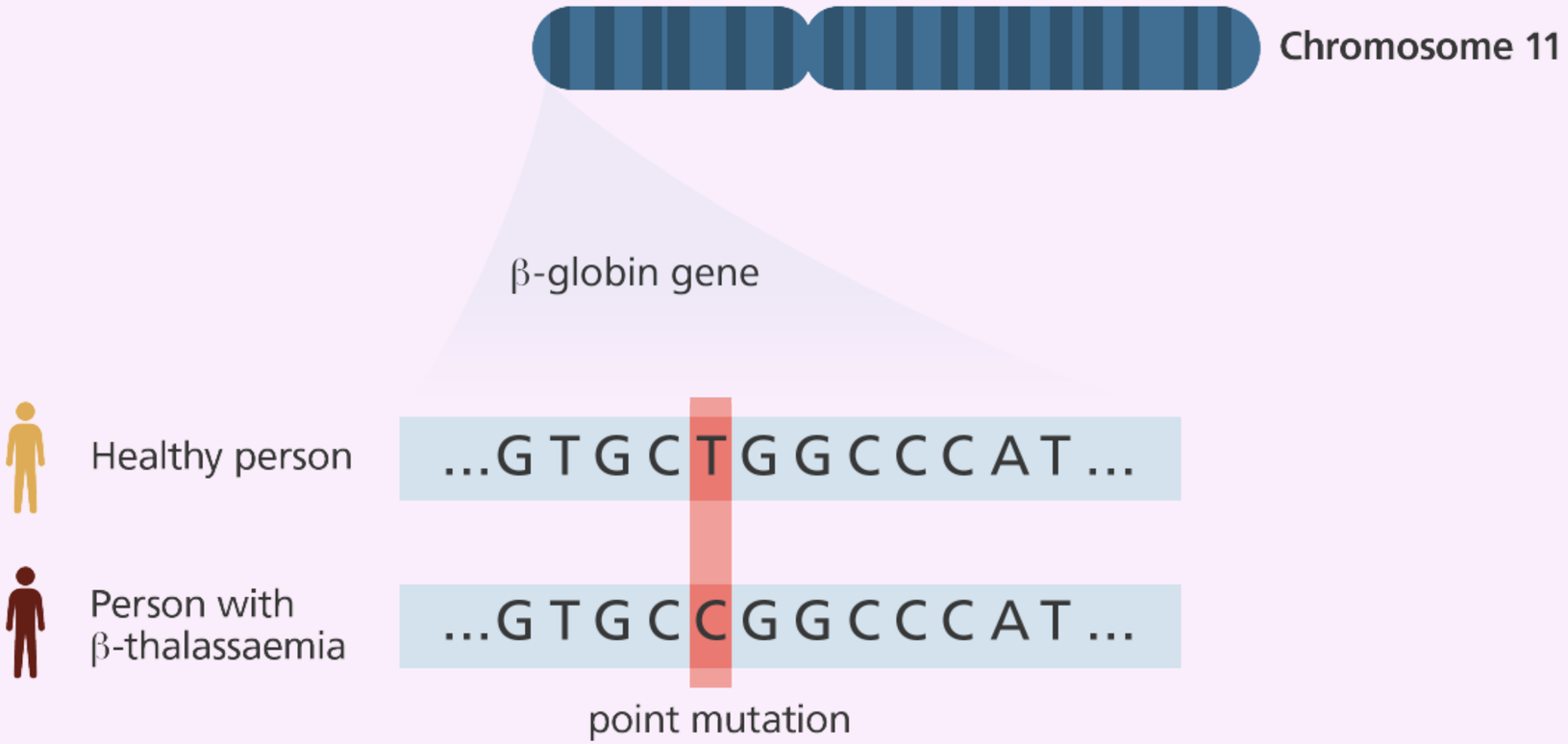
The book is written in the nucleic acid alphabet: A for adenine, T for thymine, C for cytosine and G for guanine.

Every word is a gene - a sequence of As, Ts, Cs and Gs that can be read to give the instructions for specific proteins - these genes are called protein coding genes.

>NC_000011.10:c5227071-5225464 Homo sapiens chromosome 11, GRCh38.p13 Primary Assembly
ACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCATCTGACTCCTGA
GGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGC
AGGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGACAGAGAAG
ACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATTGGTCTATTTTCCCACCCTTAGGCTGCTGG
TGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGG
CAACCCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGAC
AACCTCAAGGGCACCTTTGCCACACTGAGTGAGCTGCACTGTGACAAGCTGCACGTGGATCCTGAGAACT
TCAGGGTGAGTCTATGGGACGCTTGATGTTTTCTTTCCCCTTCTTTTCTATGGTTAAGTTCATGTCATAG
GAAGGGGATAAGTAACAGGGTACAGTTTAGAATGGGAAACAGACGAATGATTGCATCAGTGTGGAAGTCT
CAGGATCGTTTTAGTTTCTTTTATTTGCTGTTCATAACAATTGTTTTCTTTTGTTTAATTCTTGCTTTCT
TTTTTTTTCTTCTCCGCAATTTTTACTATTATACTTAATGCCTTAACATTGTGTATAACAAAAGGAAATA
TCTCTGAGATACATTAAGTAACTTAAAAAAAAACTTTACACAGTCTGCCTAGTACATTACTATTTGGAAT
ATATGTGTGCTTATTTGCATATTCATAATCTCCCTACTTTATTTTCTTTTATTTTTAATTGATACATAAT
CATTATACATATTTATGGGTTAAAGTGTAATGTTTTAATATGTGTACACATATTGACCAAATCAGGGTAA
TTTTGCATTTGTAATTTTAAAAAATGCTTTCTTCTTTTAATATACTTTTTTGTTTATCTTATTTCTAATA
CTTTCCCTAATCTCTTTCTTTCAGGGCAATAATGATACAATGTATCATGCCTCTTTGCACCATTCTAAAG
AATAACAGTGATAATTTCTGGGTTAAGGCAATAGCAATATCTCTGCATATAAATATTTCTGCATATAAAT
TGTAACTGATGTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCCAGCTACCATTCTGCTTTTATTTT
ATGGTTGGGATAAGGCTGGATTATTCTGAGTCCAAGCTAGGCCCTTTTGCTAATCATGTTCATACCTCTT
ATCTTCCTCCCACAGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCACTTTGGCAAAGAATTCA
CCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCTGGTGTGGCTAATGCCCTGGCCCACAAGTATCA
CTAAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACT
GGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAA

The sequence for the human beta globin gene

We can edit the instructions by changing one or multiple basepairs - this is called creating a mutation.



Developing a Research Question: Free Coli

Synthetic biology and genetic engineering will be key players in the fight against global problems like pandemics, genetic diseases and climate change

Wealthy developed nations with greater access to research infrastructure, education and a skilled workforce are leading the way in syn bio R&D

1. How can we address this inequity? How can we make participation in syn bio R&D cheaper and more accessible?
2. Can we improve a foundational technology (like E. coli) to achieve this goal?

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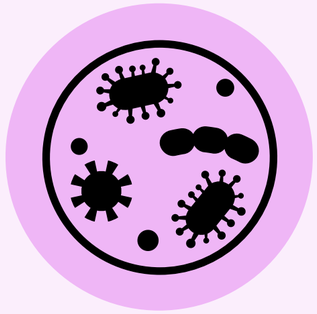
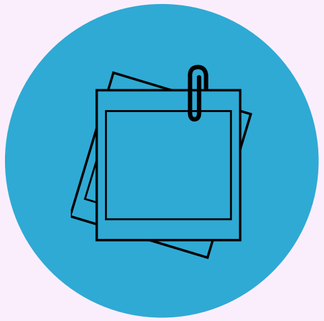


Foundational Technologies: Escherichia coli

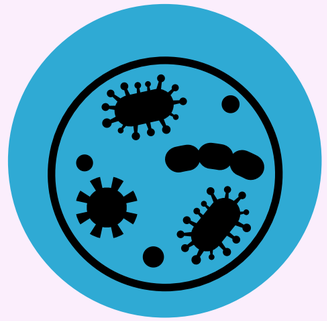
E. coli is the preferred host organism for synthetic biology work like cloning, making recombinant (redesigned) DNA or producing proteins for therapeutics (like insulin)

PC1 compliant



Well researched

Large toolkit



Rapid growth

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Introducing competent E. coli

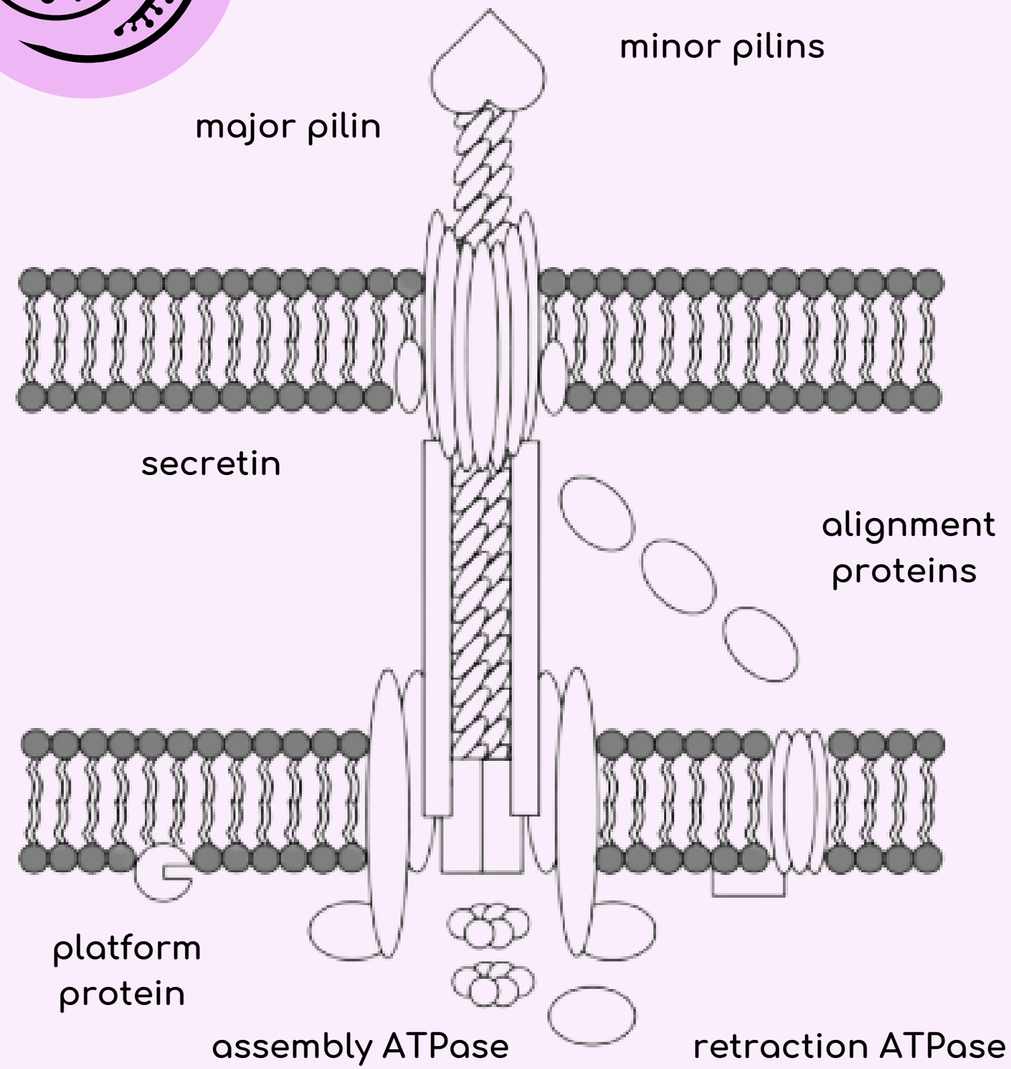

Figure 1. Schematic of gram-negative bacterium's type IV pilus

Competence is the ability of a cell to take up foreign DNA from its environment.

It is found in bacteria such as A. baylyi, but not E. coli.

Competent bacteria use Type IV Pili to pull DNA into the cytoplasm.

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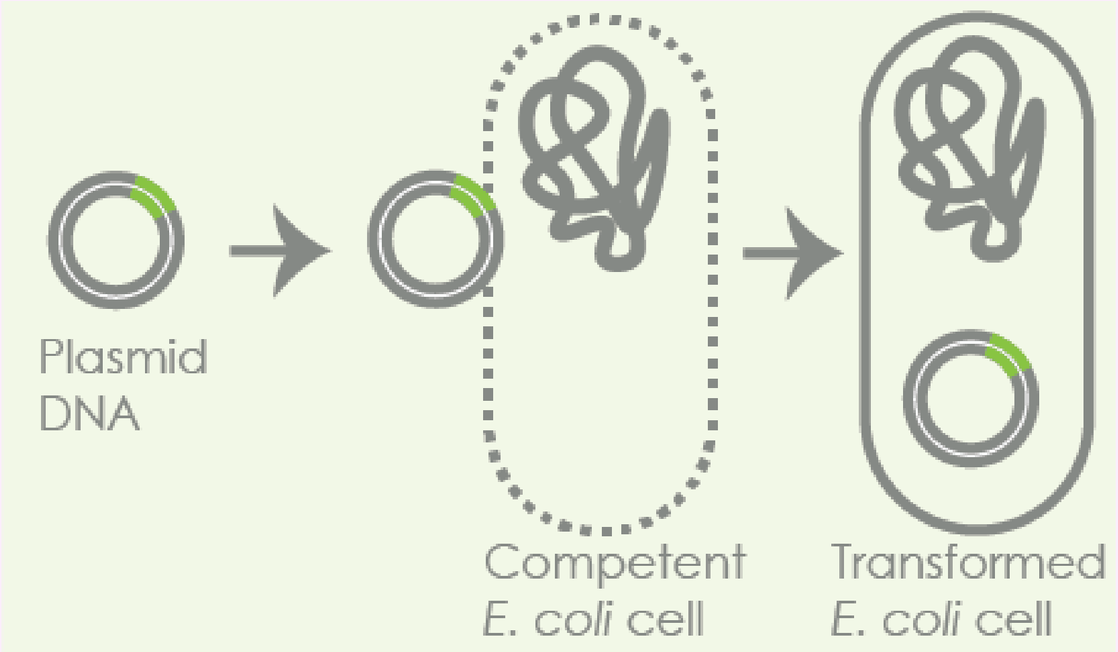
Introducing competent E. coli

Transforming naturally incompetent E. coli cells requires chemical treatment or electroporation.

Manufacturing chemically or electrocompetent E. coli cells is an accessibility barrier to synthetic biology research.



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Developing a Research Question: Literature Review

1. Can we improve a foundational technology (like E. coli) to make participation in syn bio R&D cheaper and more accessible?

We had to hit books to find answer our initial questions:

- Why isn't E. coli naturally competent?
- What genes do competent bacteria have that give them this ability?
- How many competence genes are there?
- Does E. coli have these genes?
- If so, why don't they work?

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Literature Review

PLOS one

OPEN ACCESS Freely available online

Natural DNA Uptake by *Escherichia coli*

Sunita Sinha*, **Rosemary J. Redfield**

Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada

Abstract

Escherichia coli has homologues of the competence genes other species use for DNA uptake a competence and transformation have never been detected. Although we previously showed t by the competence regulator Sxy as in other gamma-proteobacteria, no conditions are kn expression. We have now tested whether the competence gene homologues encode a fur and whether DNA uptake leads to recombination, by investigating the effects of plasmid competence in a wide variety of *E. coli* strains. High- and low-level *sxy* expression alone di of the strains tested, despite varying the transforming DNA, its concentration, and the measurements of uptake of radiolabelled DNA were below the limit of detection, detected when recombination functions were provided by the lambda Red recombin *E. coli sxy* expression can induce natural DNA uptake and that *E. coli*'s competenc machinery. However, the amount of transformation cells undergo is limited b inefficient DNA processing/recombination.

Type IV pili: dynamics, biophysics and functional consequences

Lisa Craig[1]*, *Katrina T. Forest*[2]* and *Berenike Maier*[3]*

Abstract | The surfaces of many bacteria are decorated with long called type IV pili (T4P), dynamic filaments that are rapidly pool of pilin subunits. Cycles of pilus extension, bind phenomenally diverse array of functions, includ formation. On the basis of recent develop molecular architecture of the T4P m insights into the assembly and approaches have reveal respond to external stim

MicroReview

Type IV secretion in Gram-negative and Gram-positive bacteria

...n[1]* Peter J. Christie[2]
... Steffen Backert[4]**
...y, Beuth University of ... Berlin, Germany.
...cular Genetics, ... Houston,

of bacterial recipient cells in the vicinity. Here, we summarize recent advances in our knowledge of 'paradigmatic' and emerging systems, and further explore how new basic insights are aiding in the design of strategies aimed at suppressing T4SS functions in bacterial infections and spread of antimicrobial resistances.

Introduction

T4SSs represent a highly diverse superfamily of ...ystems found in many bacterial species. This ...presented at a functional level by an ... capacity of T4SSs to (i) recognize ... stranded (ss) DNA substrates

PNAS

DNA-uptake machinery of naturally competent *Vibrio cholerae*

Patrick Seitz and Melanie Blokesch[1]

Laboratory of Molecular Microbiology, Global Health Institute, School of Life Sciences, Swiss Federal Institute of Technology Lausanne (École Polytechnique Fédérale de Lausanne), CH-1015 Lausanne, Switzerland

Edited by John J. Mekalanos, Harvard Medical School, Boston, MA, and approved September 19, 2013 (received for review August 22, 2013)

Natural competence for transformation is a mode of horizontal gene transfer that is commonly used by bacteria to take up DNA from their environment. As part of its developmental program, so-called competence components of

tweezers, Hahn et al. showed that DNA binding and uptake also occur preferentially at the cell pole (9). It is unknown whether a polar localization pattern of the DNA-uptake machinery is universal for all naturally competent bacteria

1 **Pilus production in *Acinetobacter baumannii* is growth phase**

2 **dependent and essential for natural transformation**

3

Nina Vesel and Melanie Blokesch*

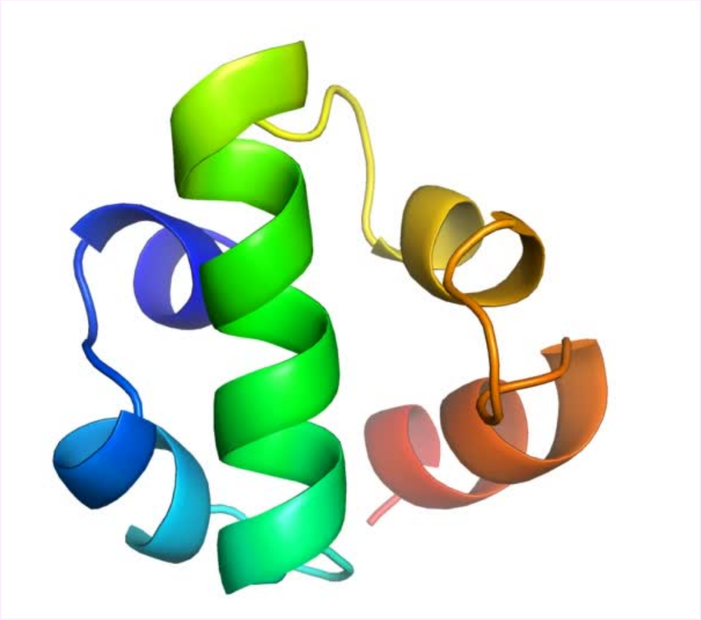
Laboratory of Molecular Microbiology, Global Health Institute, School of Life Sci...

Polytechnique Fédérale de L...

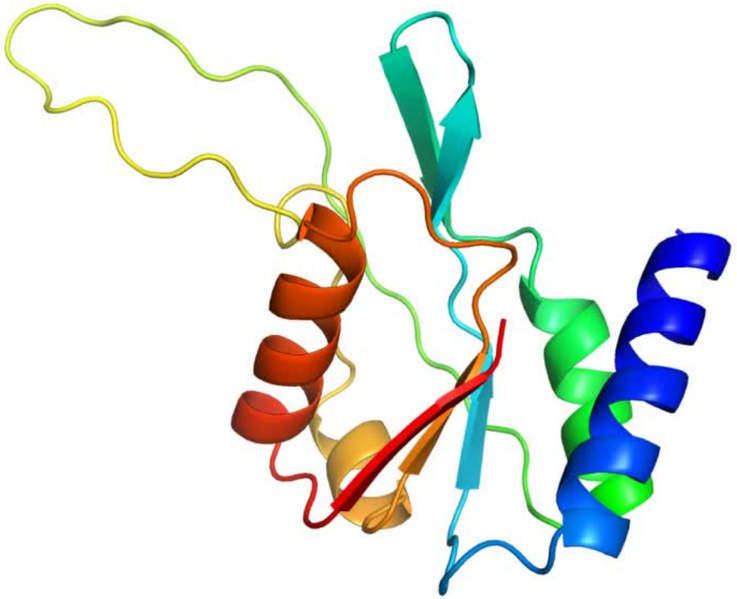
Natural Transformation Genes

- PilD - prepilin peptidase
- PilB - extension motor, ATPase
- PilC - inner membrane platform protein
- PilF - type IV pilus biogenesis/stability protein, motility
- FlmT - minor pilin, cell adhesion
- PilU - retraction motor, transport protein
- PilT - retraction motor, transport protein
- ComA - transmembrane ATPase
- ComEA - DNA-binding protein
- PilM/ComM - alignment complex protein
- PilN/ComN - alignment complex protein
- ComF - helicase/transferase
- PilE - minor pilin, protein transport
- ComE - minor pilin, cell adhesion
- ComC - competence, cell adhesion
- PilX - minor pilin
- ComB - minor pilin
- PilV - minor pilin
- FimU - minor pilin
- ComP - major pilin
- PilQ/ComQ - outer membrane secretin
- PilP/ComL - alignment complex protein
- PilO/ComO - alignment complex protein

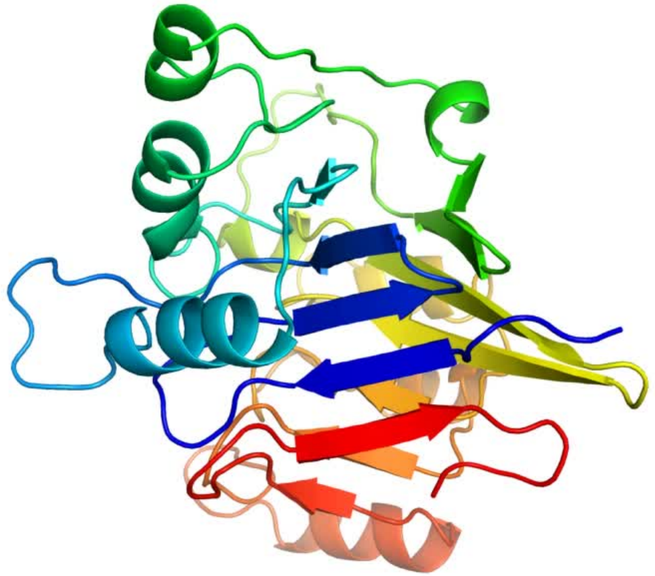
3D Protein Structure of the 1st Gene cluster



ComEA
DNA binding protein



ComF
Helicase/Translocase



ComA(ComEC)
Transmembrane
ATPase

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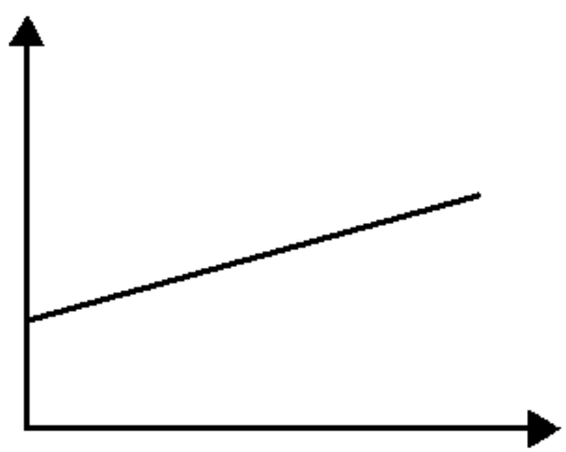
Developing Predictions: Modelling the Mathematics of Biology

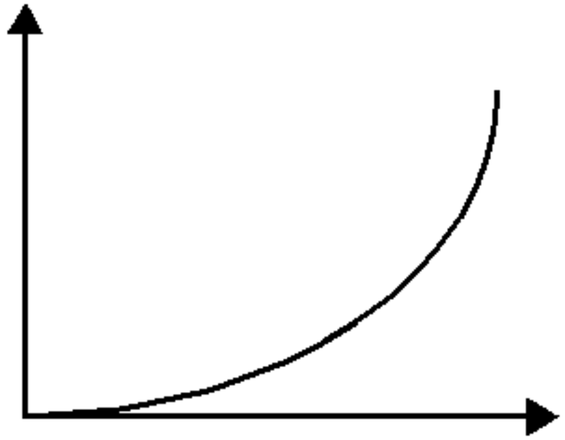
"All models are wrong, but some are useful." - George Box

Modelling tries to find patterns using mathematics or statistics that replicate the real world so that we can make predictions about it.

You have probably all come across some models in your maths or science classes such as linear models or non-linear models like parabolas and exponentials.



Linear relationship
Number of personnel vs. employee costs



Nonlinear relationship
Population growth over time

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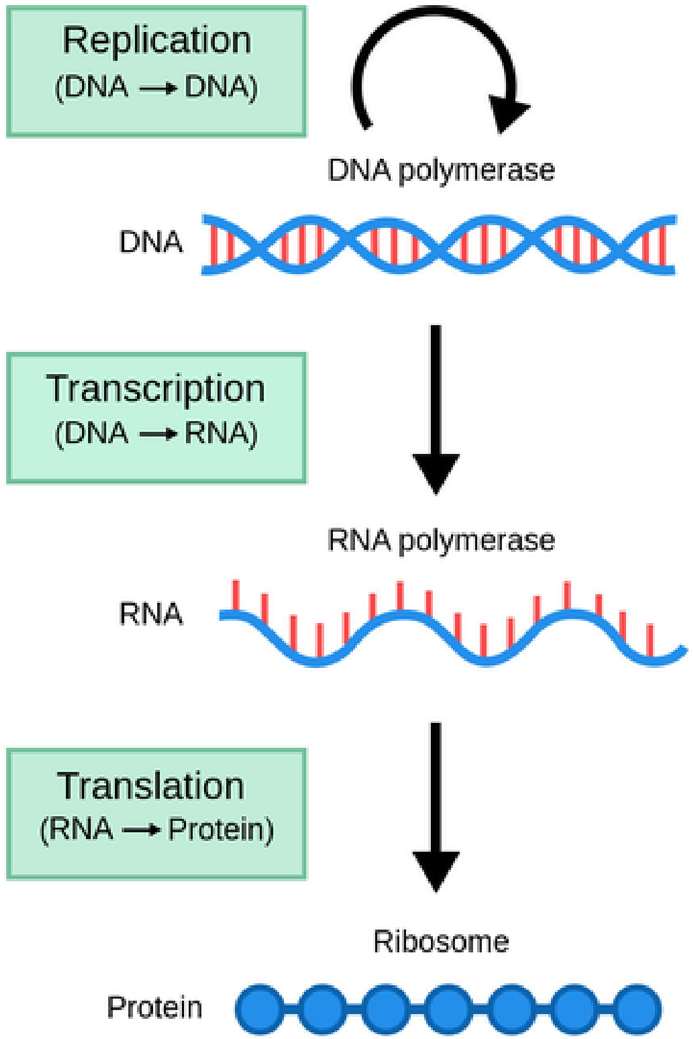
We needed to find patterns in gene expression levels so we could determine the best way to cluster our genes.

We used transcriptome data, which gives us information about the expression levels on an RNA level, and allows us to suggest the specific function in natural transformation in E. coli.

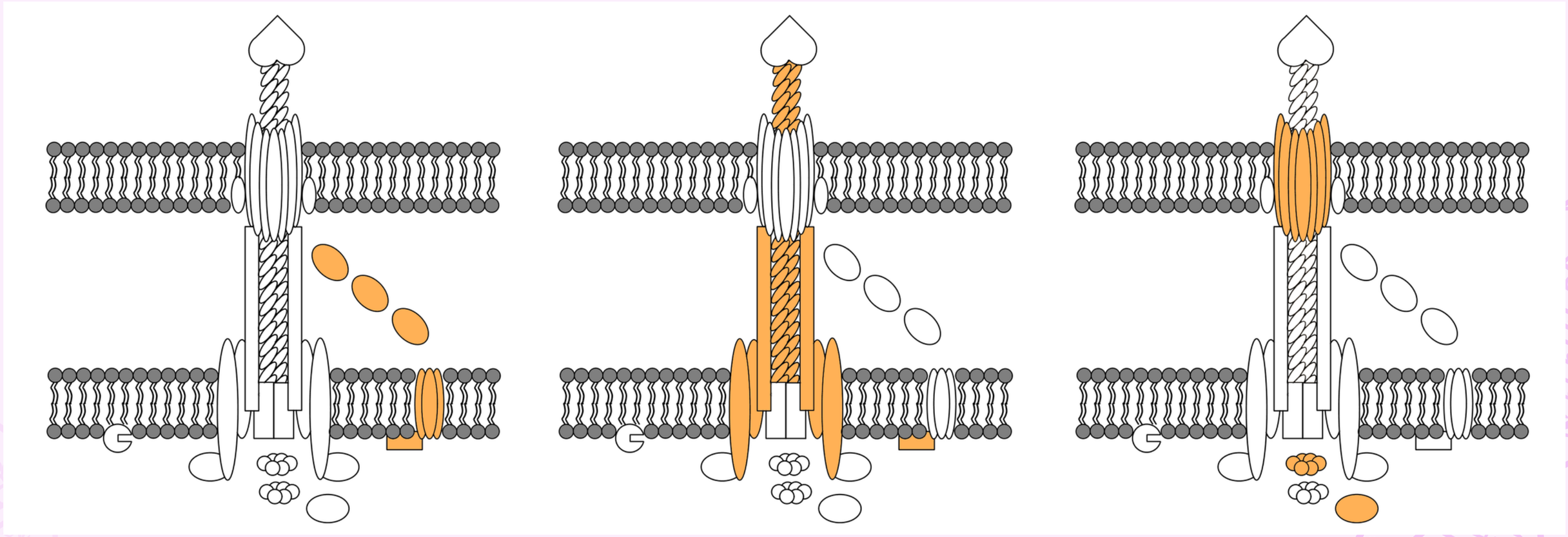
We have a number of tools to measure the concentration of RNA transcripts from a target gene - analysis of gene expression.

Measuring protein abundance can be a bit trickier.



We used a method of unsupervised machine learning called K-means clustering to allocate genes into clusters based on similarity.

Cluster	Genes	Total Length	Transcriptome Concentrations	Mean concentration	Standard deviation	Promoter Strength	Optimal Promoter Strength	Percentage Error
1	comea comf coma	3447	52 39 72	54.64	16.76	34	34.00	0.00
2	pilq pilb comb pilx	5519	78 88 102 94	90.52	10.21	34	56.32	39.63
3	pilt pilu fimt	2703	34 69 70	57.66	20.82	34	35.88	5.24
4	piln pilo pilp comf	2333	159 131 119 123	132.88	18.14	100	82.69	20.94
5	pilf pild	1660	686 542	613.76	101.78	100	381.92	73.82
6	pilm pilv come fimu pilc	3778	276 240 321 343 291	294.42	39.94	100	183.20	45.42
7	comp	443	6375	6374.78	NA	100	3966.74	97.48
8	comc	4352	261	260.79	NA	100	162.28	38.38



Cluster 1

Cluster 2

Cluster 3

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Designing an Experiment to Test Predictions

Unfortunately, Sydney's COVID-19 outbreak and the lockdown prevented us from conducting our laboratory research to test our predictions and validate our design.

We hope to hand down our research, design and predictions to a new generation of researchers so that they can test and validate our design...this could be you!

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What do we need to test?

1. Have we successfully inserted our gene clusters into the E. coli genome?
2. Are our natural transformation genes being transcribed as expected?
3. Are our natural transformation genes being translated as expected?
4. Are our natural transformation genes functioning as expected - have they built the type IV pilus DNA uptake machine?
5. How efficient are out Free Coli cells are taking up and integrating foreign DNA?

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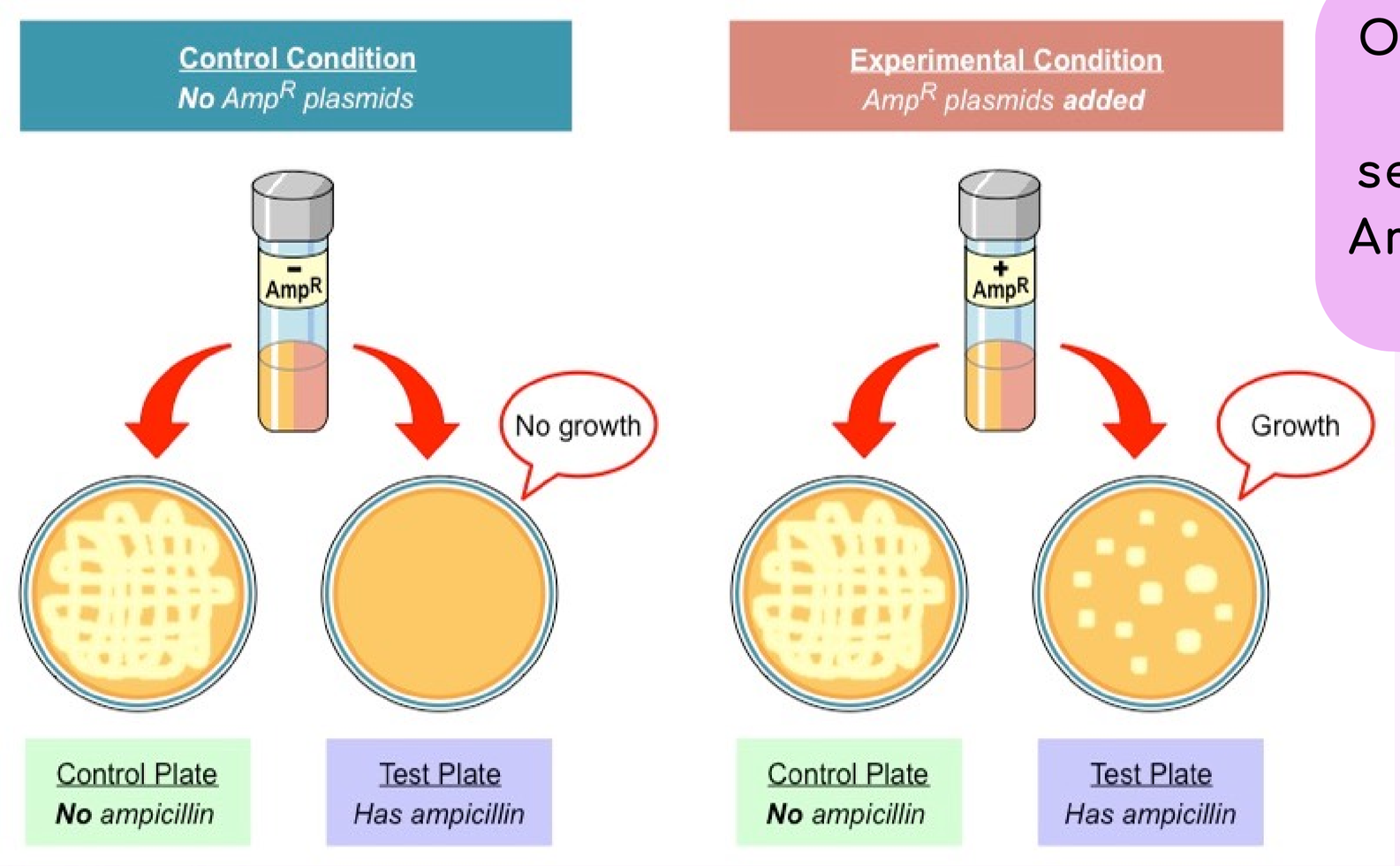
Testing Successful Insertion: Selection Markers



Our experiment design uses eight different selection markers: TpR, AmpR, fosC2, CmR, GmR, TcR, malS and qacE

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Novel Recombineering Strategy: The Problem

Three problems to solve:

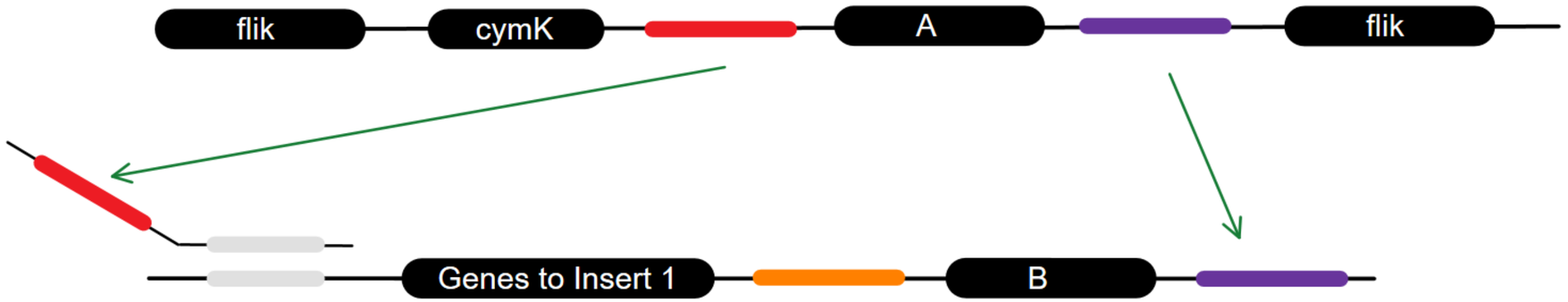
- We needed to use selectable markers with each cluster insertion, but we didn't want to these to remain in the cell.
- We wanted to be able to insert clusters in any order, in case one does not work.
- We wanted the clusters to be inserted at a designated area of the genome, not randomly.

Novel Recombineering Strategy: Babushka Blocks!

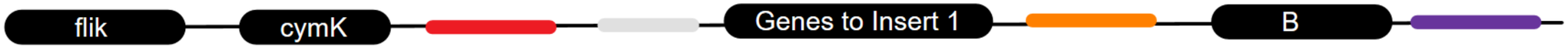
Solution: Babushka Blocks!

- We devised a recombineering strategy that allowed us to divide the genes over 8 gBlocks, and sequentially incorporate them into the E. coli chromosome.
- Key components:
  - Babushka blocks are inserted into each other, like Babushka dolls.
  - When inserted, they knock out the resistance marker in the previous block.
  - They can be inserted in any order with the help of primers.

Insert Cluster 1:



After Insertion:



Testing Successful Transcription: Transcriptomics

The transcriptome is the range of messenger RNA (mRNA) expressed by an organism.

It is a measure of gene expression.

Toolkit: Next gen sequencing and microarrays

Testing Successful Translation: Proteomics

The proteome is the proteins expressed by a genome at any one time - it is constantly changing a cells respond to environmental conditions.

Proteomics aims to identify and quantify all the proteins, but the level of protein we can detect in a cell is dependent on spatial and temporal dynamics - so we can only measure protein abudance (impacted by both polypeptide synthesis and degradation) not protein synthesis.

Toolkit: Western blot/Mass spectrometry

Q & A

Go to www.menti.com and use the code 62354649?

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Synbio Educational Survey

Help us inform the future of synbio education!

We will send the link to your teachers!

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