
iGEM
GLP.exe
TÜBINGEN

Overview

- What is iGEM?
- Our Team
- Our project
- Sponsors and Supporters

International Genetically Engineered Machine Competition (iGEM)

- International student competition in synthetic biology
- Started in January 2003 at the MIT
- One year for project completion
- Interdisciplinary self organised teams
- Not only wetlab work
- Collection of genetic parts for building biological devices and systems.
- Final Project presentation at the MIT, Boston

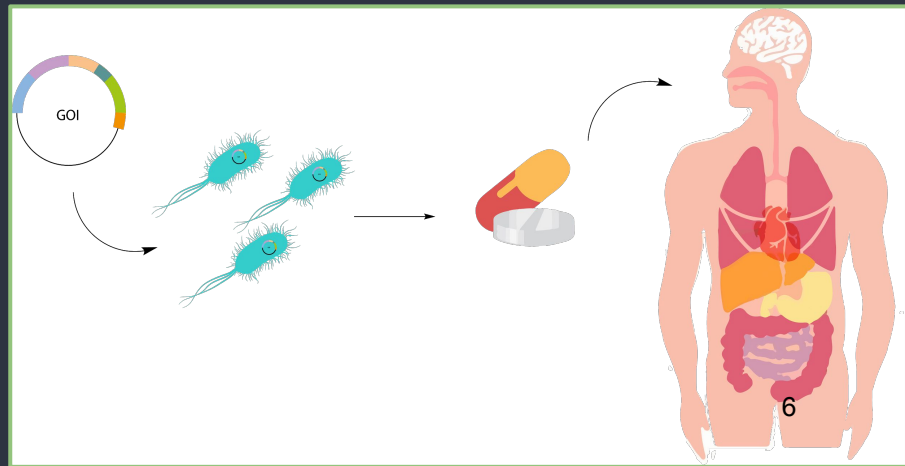
iGEM Tübingen 2019

iGEM Tübingen 2019; Team Organisation

- Weekly meeting and progress report
- Organisation through subteams
    - Finance
    - Human practice
    - Design
    - Social Media/Wiki
    - Safety
    - Drylab/Modeling
    - Wetlab

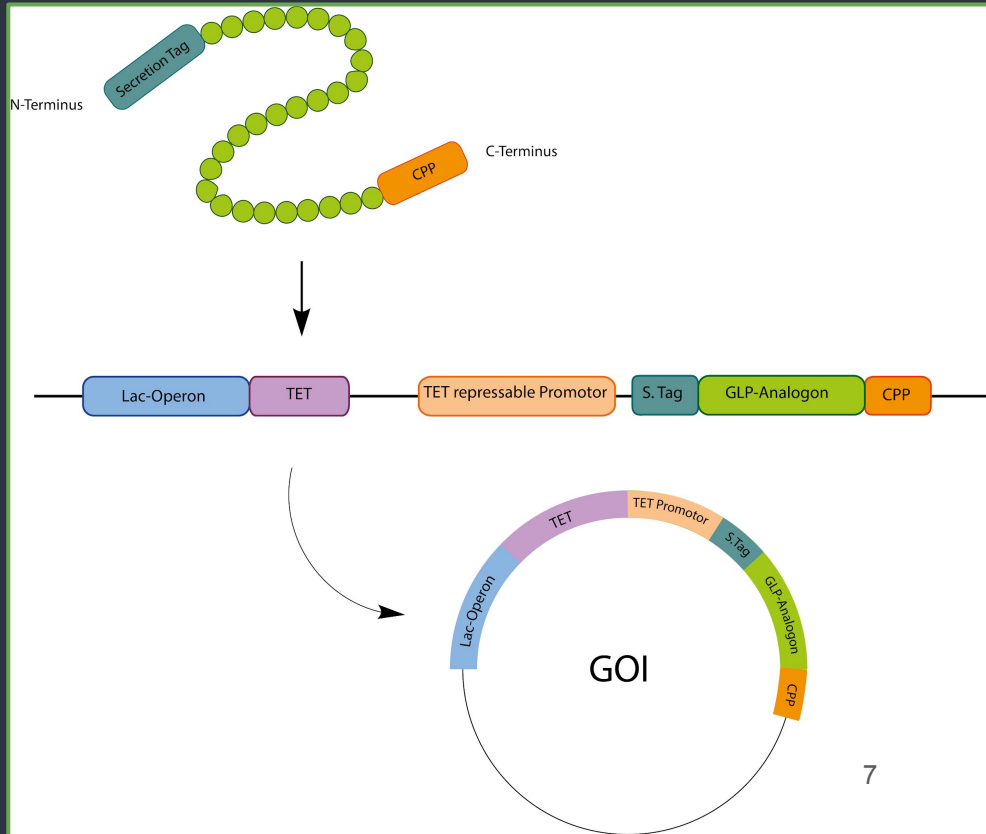
Project 2019

- Microbial chassis used as probiotic for Diabetes Type II
  - Glucose-dependent incretin secretion
- Cas3-based kill-switch
  - Regulated by environmental factors
- CPP Characterisation
  - Penetration vs Cytotoxicity
- Characterisation of *E.coli* Nissle 1917
  - RNA-Seq



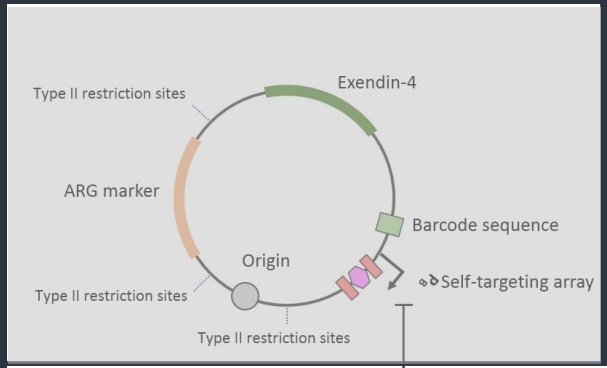
Incretin Secretion

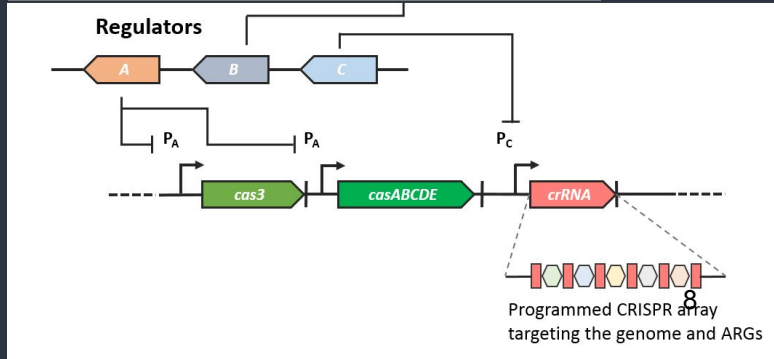
- Exendin-4 (GLP-1 analogon) secretion dependent on glucose
- Secretion Tag and cell penetrating peptide (CPP)



Kill-switch Mechanism

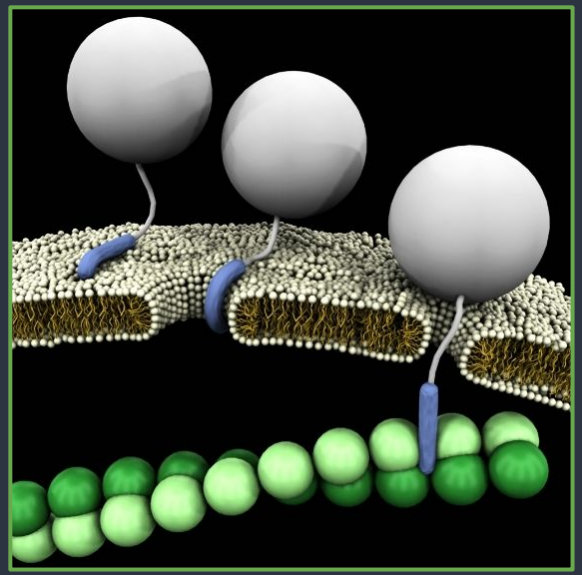
- Biocontainment
  - To prevent the bacterium and its DNA from escaping into the environment
- Based on Cas3
  - shredding the DNA, starting from the cut site
- Regulated by factors present only in the designated application area
  - Temperature
  - Fatty acids
  - Mucus-degradation products





Cell-penetrating Peptides

- Short peptides
- Efficiency evaluation of several CPPs
- Use the best to ensure our incretin can enter the enteric cells



[Kromhout et al. 2011]

Cell-penetrating Peptides - Software

- Manually curated data
  - ~ 250 data points
  - CPP sequence - fluorescence value
- Gradient Boosted Trees
  - Regression
- Identify most important features
  - Amino acids
  - Physico-chemical properties
- Predict cell penetrating efficacy for novel amino acids

E. coli Nissle Characterization

- Already approved as probiotic
  - Badly characterized
- Reaction to different environmental conditions and stress?
  - Human intestine?
  - Outside the intestine?
- Idea: Subject Nissle to various stress factors and examine transcriptome
  - Transcriptomic changes in our application area?
  - Heat shock proteins?
  - Pathways involved in stress response?

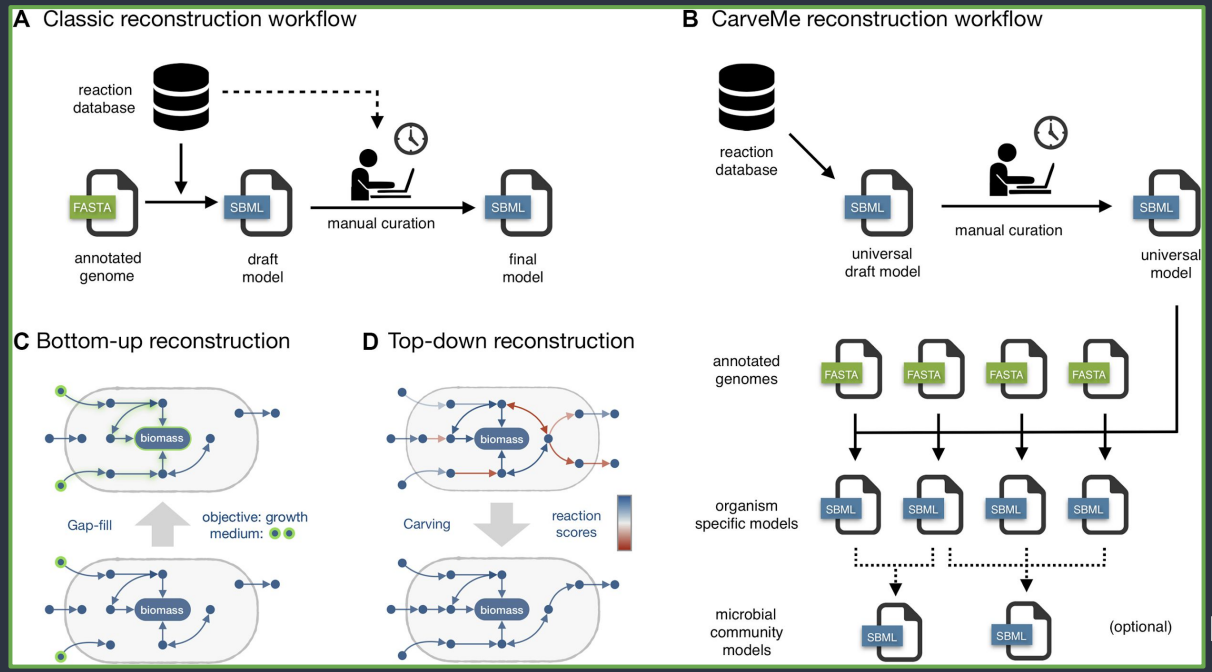
E. coli Nissle Characterization

- RNA-Seq
  - 48 Samples
    - 12 Conditions
    - 4 Replica per condition

Aerob	Anaerob
LB medium 37°C	LB medium aerob 37°C
LB medium 25°C	LB medium anaerob 37°C
LB medium 8°C	mGAM medium 37°C
LB medium pH 4 37°C	mGAM medium + Metformin 37°C
LB medium H_2O_2 37°C	mGAM medium + bile acids 37°C
LB medium dry freezed	mGAM medium + bacterial culture supernatant 37°C

E. coli Nissle Characterization

- No metabolic model available for *E. coli* Nissle
- CarveMe



[Machado et al, 2018]

E. coli Nissle Characterization

- Use novel metabolic model to:
  - Identify optimal growth medium
  - Model Interaction and competition between different microbial species
- SMETANA
  - MRO (metabolic resource overlap): Calculates how much the species compete for the same metabolites
  - MIP (metabolic interaction potential): Calculates how many metabolites the species can share to decrease their dependency on external resources
  - SCS (species coupling score): Measures the dependency of one species in the presence of others to survive
  - MUS (metabolite uptake score): Measures how frequently a species needs to uptake a metabolite to survive

[Machado et al, 2019; Zelezniak et al, 2015]

Human Practices

- Raising public awareness about GMOs, synthetic biology and our project
  - Cooperation with the Experimenta Heilbronn (high school student lab workshop)
  - Open discussion on biohacking with the Experimenta
  - Survey on Diabetes Type II
- Scientific exchange with experts
  - Dr. Lisa Maier (UKT), Dr. Latz (Uni Bonn), Prof. Fritsche (UKT), Dr. Timo Müller (Helmholtz Institute Munich)
- Implementation and promotion of the UN's Sustainable Development Goals in iGEM

Sponsors & Supporters

- AG Angenent (MPI, Uni Tübingen)
  - Prof. Dr. Lars Angenent
  - Dr. Pengfei Xia
  - Dr. Bastian Molitor
  - Patrick Schweizer
  - Sarah Schulz
- AG Wohlleben (Uni Tübingen)
- Prof. Dr. Klaus Harter (Uni Tübingen ZMBP)
- Angel Angelov, Christina Engesser (NCCT Tübingen)
- Dr. Vladimir Benes (EMBL)
- Dr. Lisa Maier (UKT)

Thank you for your attention!

Further information

igem-tuebingen.com

2019.igem.org/Team:Tuebingen