"Search and Destroy"

NTNU is B.A.C.K.  Bacterial Anti-Cancer Kamikaze

NTNU – Trondheim
Norwegian University of Science and Technology
Our project summarized:

Making bacteria kill cancer cells

Why?

...and how?
Why using *bacteria* to cure cancer is a good idea

• Challenges with traditional cancer treatment
  - Drug specificity
  - Drug delivery

• Bacteria are highly sensitive environmental sensors
  – Enables high specificity

• With genetic engineering:
  – In vivo drug synthesis
  – Directed drug delivery
How can bacteria cure cancer?

1. Location of cancer cells and toxin synthesis
2. Lysis activation
3. Destruction of host cell with toxin release
1. Location of cancer cells and anti-cancer toxin synthesis

- Tumor tissue is low in oxygen
- E. coli moves down an oxygen gradient [1]
- E. coli can actively seek out cancer cells
- Colicin E1 is a bacterial toxin
  - Constitutively expressed
  - Accumulated within the cell

The cell becomes a ticking colicin bomb

E. coli can actively seek cancer cells

2. Lysis activation

- Exploiting two properties of cancer cells:
  - Large oxygen consumption
  - Increased lactate production
- Tumor tissues are low in oxygen and high in lactate

Lysis triggered as a response to both factors
3. Destruction of host cell with toxin release

- Lactate and oxygen signals trigger activation of lysis system
- Bacterial host cell destroyed
- Accumulated colicin released

Cancer cell destruction

Bacterial Anti-Cancer Kamikaze
In detail
Confirmation of lysis induction

- BioBrick part: Enterobacteria phage T4 Lysis Device
  - Three genes coding for lysozyme, holin and antiholin
  - When coupled to a promoter, the device allows controlled cell lysis
  - Functionality confirmed by coupling to pBAD promoter and induction with arabinose
Confirmation of lysis induction
We built a colicin device

- Constitutive promoter with RBS
  - **BBa_K081005**
  - Strong constitutive promoter with strong RBS- for maximization of colicin expression

- Colicin E1 + immunity protein
  - Based on **BBa_K150009**
  - Coding sequences for colicin E1 and immunity protein
  - Submitted as **BBa_K822002**

![Diagram of colicin device]
Results for colicin synthesis

- The function of the colicin part was confirmed by two experiments
  - Part was placed under a constitutive promoter and transformed into *E. coli*
  - Cell cultures containing the colicin plasmid were lysed and added to other, colicin-free cell cultures
  - OD grew significantly slower than in the negative control cultures, indicating colicin production
Results for colicin synthesis

Graphs showing the OD at 578 and 600 nm over time for Colicin and Buffer, compared to a Negative control.
Other constructs

- **Constitutive YFP generator**
  - Submitted as **BBa_K82003**
  - YFP expressed under a constitutive promoter
  - Positive control for fluorescence experiments

- **LacI generator**
  - Submitted as **BBa_K82004**
  - LacI gene with RBS and a double terminator
  - For regulation of LacI repressed elements
Other constructs

- Test cut of BBa_K29006
  - None of the expected bands showed up
- Sequencing results did not match those from the Registry

- Test cut of BBa_K82004
  - Expected bands confirmed and highlighted in red
- Correct sequence confirmed
Modelling

- System was divided into three parts
  - lld promoter, vgb promoter and holin production
- The parts were then combined to a model of the full system
  - Approximately 60 reactions
- Results show that the amount of holin fluctuates, but does not reach critical levels
Collaboration

- Collaborated with Rose-Hulman Institute of Technology (RHIT)
  - We did stochastic modelling of their system
The iGEM Matchmaker

- Online tool
- Makes it easy for iGEM teams to find each other
- Used by several of the 2012 teams
- Ready to use for future competitions
- 350 hits since beginning of August this year
- Teams have collaborated because of it
Human practices

• We participated at Researchers' Night
• Event for high schools students at NTNU
  - 1100 students participated
  - Fully booked in four minutes
Human practices
Main achievements

- Submitted and characterized two parts
  - Colicin E1 + immunoprotein device
  - Constitutive YFP generator
- Improved the LacI generator part
- Created the iGEM Matchmaker, an online tool for facilitating collaboration between iGEM teams
- Modelled our own circuit as well as RHIT's
The future of Bacterial Anti-Cancer Kamikaze
The future of Bacterial Anti-Cancer Kamikaze

• We have not been able to conclusively show that bacteria can attack cancer cells
• Working hypothesis remains valid
• Some proof of concept:
  - Toxin synthesis
  - Controlled lysis
• Professional research is being done on the subject:
  - Most approaches seem to be similar to what we envisioned
  - Environmentally controlled invasion of cancer cells by engineered bacteria followed by toxin release
Thank you for your time!

We had fun!