Intrinsic Factor-y: An alternative treatment to Pernicious Anemia
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**Pernicious Anemia**

Type of Megaloblastic Anemia

Caused by B12 deficiency
Malabsorption due to lack of intrinsic factor (atrophic body gastritis)

Symptoms mainly on three systems
Blood, gastro-intestinal tract and nervous system

Frequency:
1 in 681 people for Turkey*
1 in 680 people for USA*

Treatments
Life long injection B12

*Based on data from [http://www.cureresearch.com/p/pernicious_anemia/stats-country.htm](http://www.cureresearch.com/p/pernicious_anemia/stats-country.htm)
B12 (Cobalamin)

- Water soluble vitamin
- Essential vitamin (must be taken by diet)
- Function as co factor in three enzyme process:
  - Conversion of homocysteine to Met
  - Conversion of Methylmalonic acid to succinyl coenzyme A
  - Conversion of 5-methyltetrahydrofolate to tetrahydrofolate

! DNA Synthesis
! Red blood cell production
Absorption of B12

- Three important protein in absorption mechanism:
  - Haptocorrin (R-protein)
  - Intrinsic Factor*
  - Transcobalamin-II
In case of Pernicious Anemia
autoantibodies to parietal cells and intrinsic factor
Autoreactivity of T cells
Loss of parietal cell
No IF production
Project

Production and secretion of IF in ileum.

Why?
Long-standing treatment to Pernicious anemia disease.
Module I: Secretion of GIF

Signal Peptidase

Type II Secretion Machinery

OmpA+GIF

Extracellular Matrix

Cytoplasm
The part

- Western Blot analysis from culture supernatant
Figure 1: Agarose gel results of parts double cut (Lane right to left: Empty, OmpA-GIF+B0015, OmpA-GIF, B0034, Marker)
Module 2: Purification and Characterization of Recombinant GIF

- Clon the gif gene downstream of GST-tag
- Analyzing B12 binding of recombinant GIF protein
Results

Figure 2: Agarose gel result of BamHI and XhoI double cut of plasmid isolated from colonies which are transformed with ligation product
Module 3: pH dependent expression of GIF

nhaA promoter – can be regulated by pH and nhaR protein

- In the intestine (pH is higher), nhaA promoter senses the pH change and activates gene expression.
The part

- Analyze expressed GIF protein with increasing pH by Western Blot
Results

Figure 3: Agarose gel results of DNA isolation from DH5α strain
Results

Figure 4: Agarose gel results of amplification nhaA promoter from DH5α strain via gradient PCR
Results

Figure 5: PCR amplification of nhaA promoter at 59°C annealing temperature
Module 4: Kill Switch

- Toxin-Antitoxin Module: MazE-MazF
The part
Modelling

Equations For Transcription

\[
\frac{d}{dt}[\text{MazE mRNA}] = N \cdot P \cdot \frac{1}{1 + \left(\frac{[\text{Glucose}]}{K_{\text{Glucose}}} \right) \cdot n_{\text{Glucose}}} - a_{\text{mRNA}}[\text{MazE mRNA}]
\]

\[
\frac{d}{dt}[\text{MazF mRNA}] = N \cdot P \cdot \frac{1}{1 + \left(\frac{[AI_2]}{K_{AI_2}} \right) \cdot n_{AI_2}} - a_{\text{mRNA}}[\text{MazF mRNA}]
\]

Equations for Translation

\[
\frac{d}{dt}[\text{MazE}] = t_{\text{mazE}}[\text{MazE mRNA}] - a_{\text{mazE}}[\text{MazE}]
\]

\[
\frac{d}{dt}[\text{MazF}] = t_{\text{mazF}}[\text{MazF mRNA}] - a_{\text{mazF}}[\text{MazF}]
\]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
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<tbody>
<tr>
<td>N</td>
<td>Plasmid copy number</td>
</tr>
<tr>
<td>P</td>
<td>Promoter Strength</td>
</tr>
<tr>
<td>k_d</td>
<td>Degradation Rate</td>
</tr>
<tr>
<td>K_a</td>
<td>Dissociation (Equilibrium) Constant</td>
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<tr>
<td>n_h</td>
<td>Hill Coefficient</td>
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<tr>
<td>t_s</td>
<td>Translation Rate</td>
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</table>
Results

Figure 6: EcoRI cut of first ligation reaction products
Problems in Project

- Implementation
  - Milk fat capsules
- Plasmid curing in small intestine
  - Selective pressure with hox-sox genes (BBa_K817015)
- Plasmid instability
  - Resolvase (BBa_K817018)
Future Plans

- Complete experiments and validation of prototype
- Mouse experiment (Gif^tm1(KOMP)Vlcg)
Human Practice

- Our Website
  - http://igem.itu.edu.tr/
- Social Media
  - Facebook
  - Twitter
    - https://twitter.com/ituigem
• Interview with people

-What is iGEM?

* What is synthetic biology?
• Interview with professional
  • A talk with Dr. Aslıgül Kendirci (Drug Development Director of Roche and also PDGA Country Head Turkey, Middle East and North Africa)

• Interview with one of the patient suffering from pernicious anemia

For more information: http://igem.itu.edu.tr/humanpractise.php
• Publicize iGEM and Synthetic Biology in our university, Istanbul and Turkey.
  • Article of ITU team in Science and Future Journal*

• Article of ITU team in ITU24 (University newspaper)**

Future Plans

- Cover a team song
- Continue to Publicize iGEM
Acknowledgment

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