Bio Engineered Bacteria for Cancer Treatment

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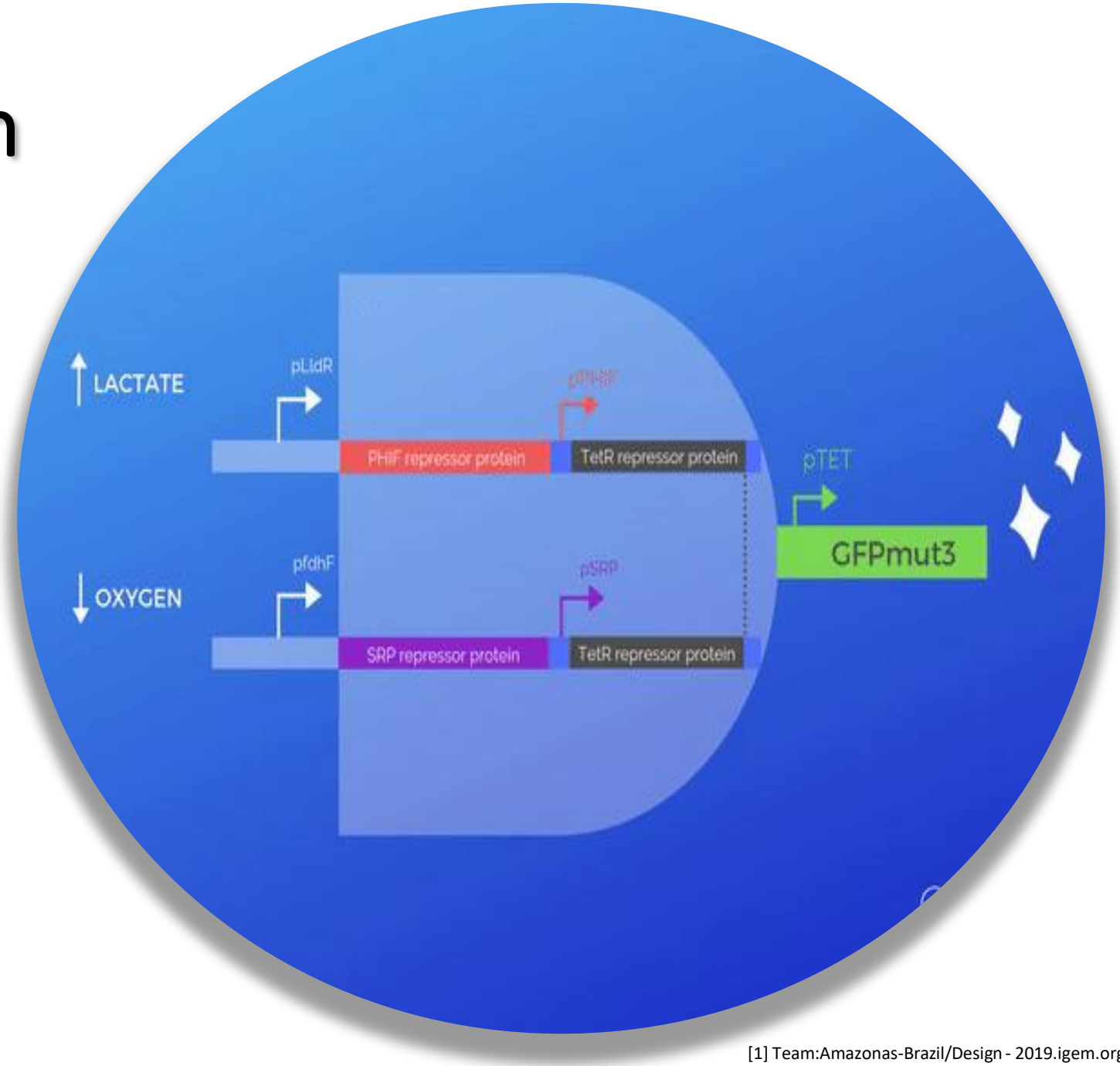
Topics of Discussion

- Problem Definition
- Treatment
- *E.coli* genetic engineering
- BioBricks design



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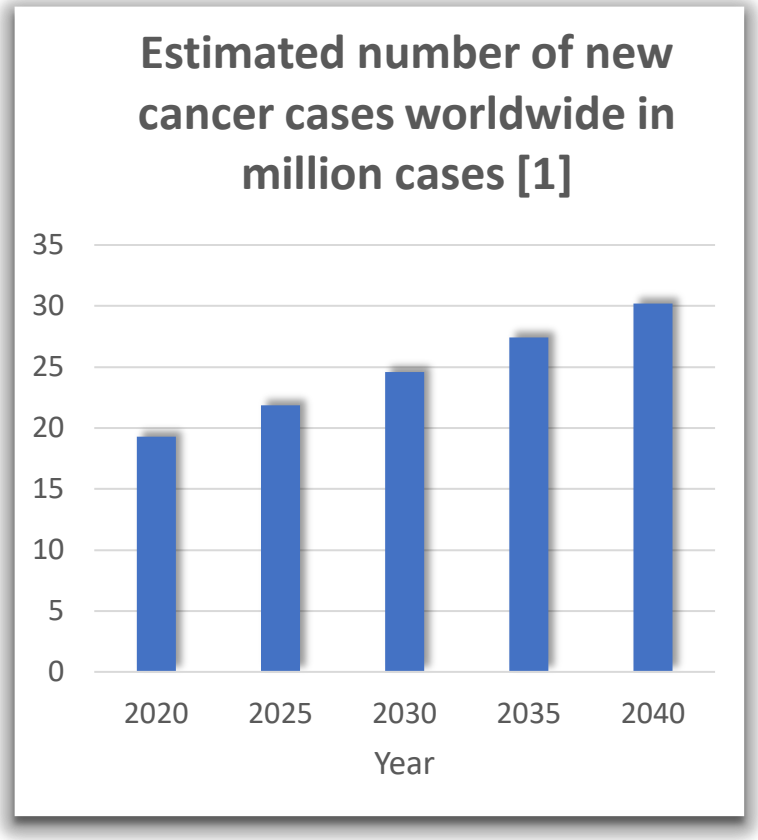
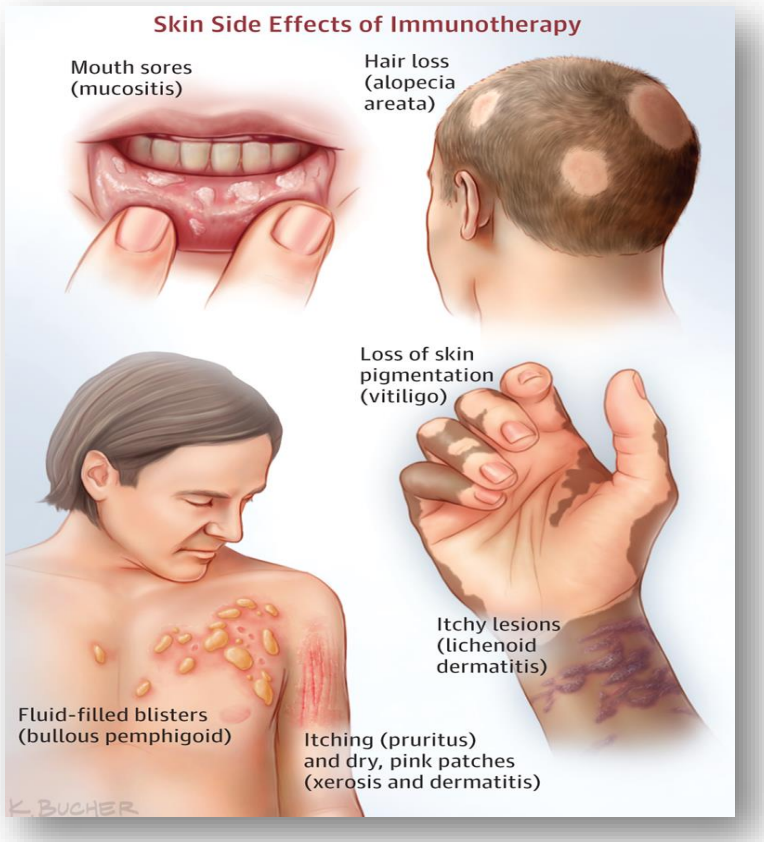
[1] Team:Amazonas-Brazil/Design - 2019.igem.org

Problem Definition



[1]



[2]

[3]

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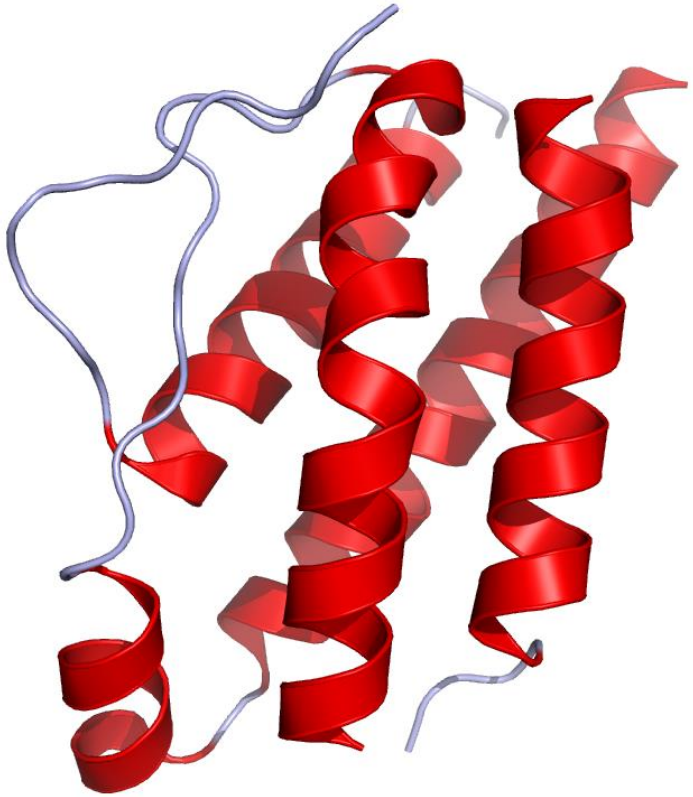
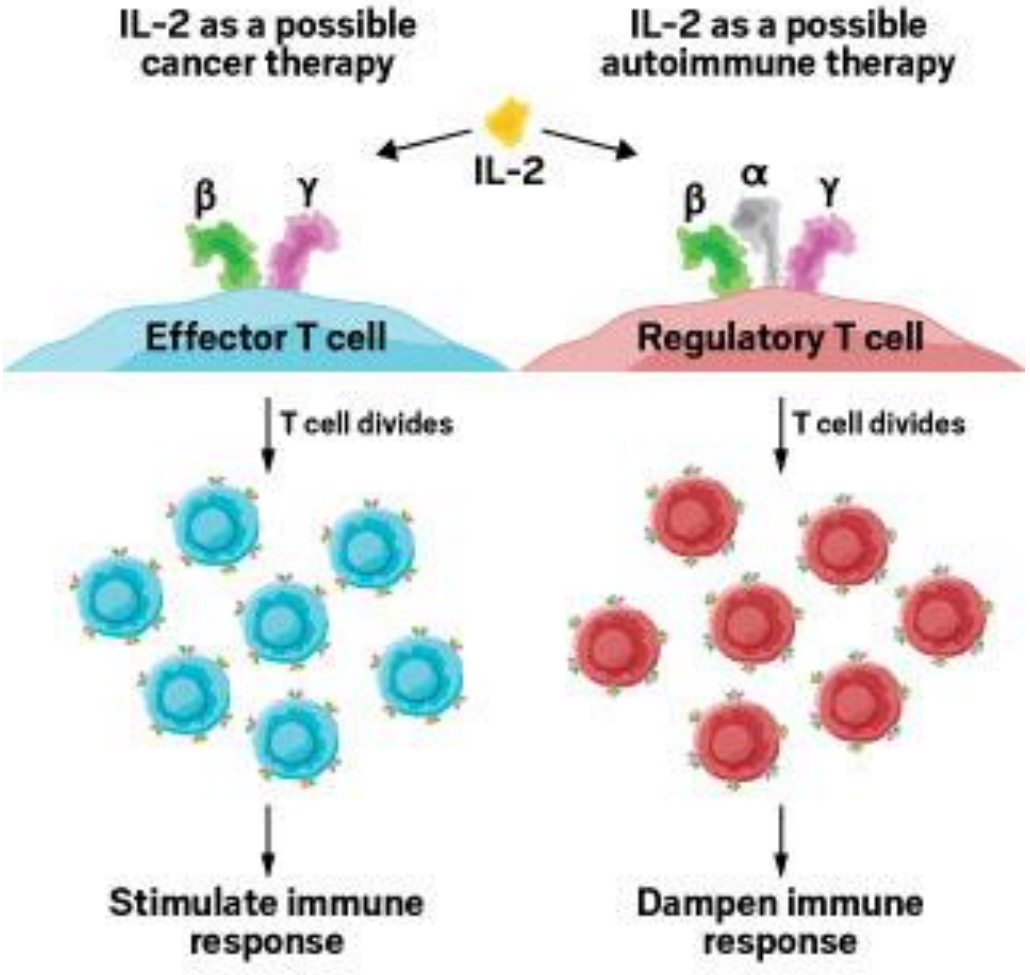
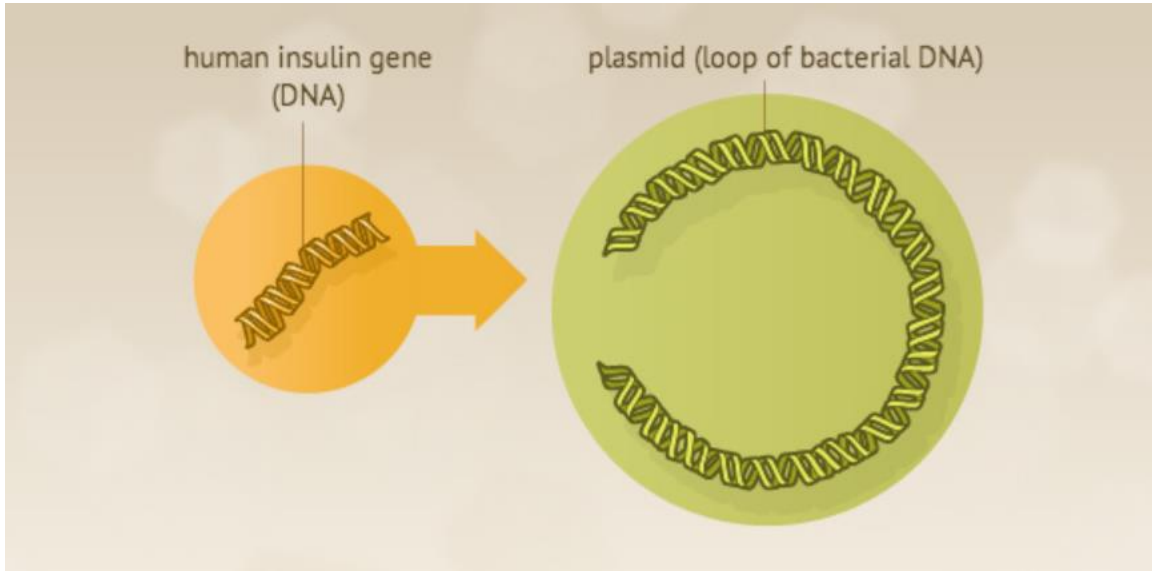
[1] https://www.statista.com/statistics/1031316/new-cancer-cases-forecast-worldwide/
[2] https://jamanetwork.com/journals/jamadermatology/fullarticle/2683521
[3] https://www.medicalnewstoday.com/articles/68511

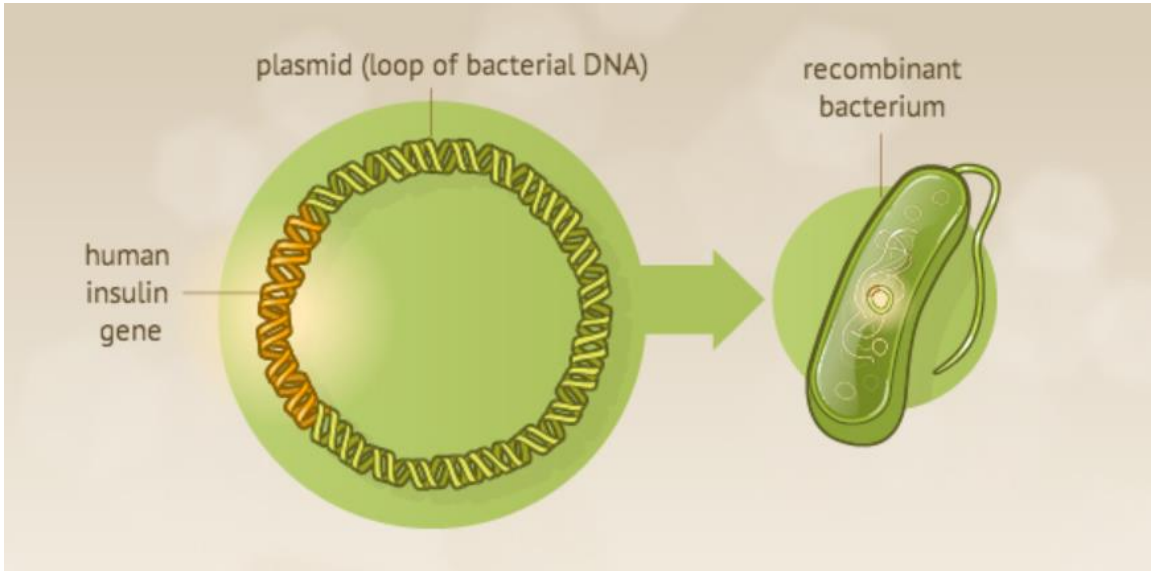
Interleukin-2 (Aldesleukin)



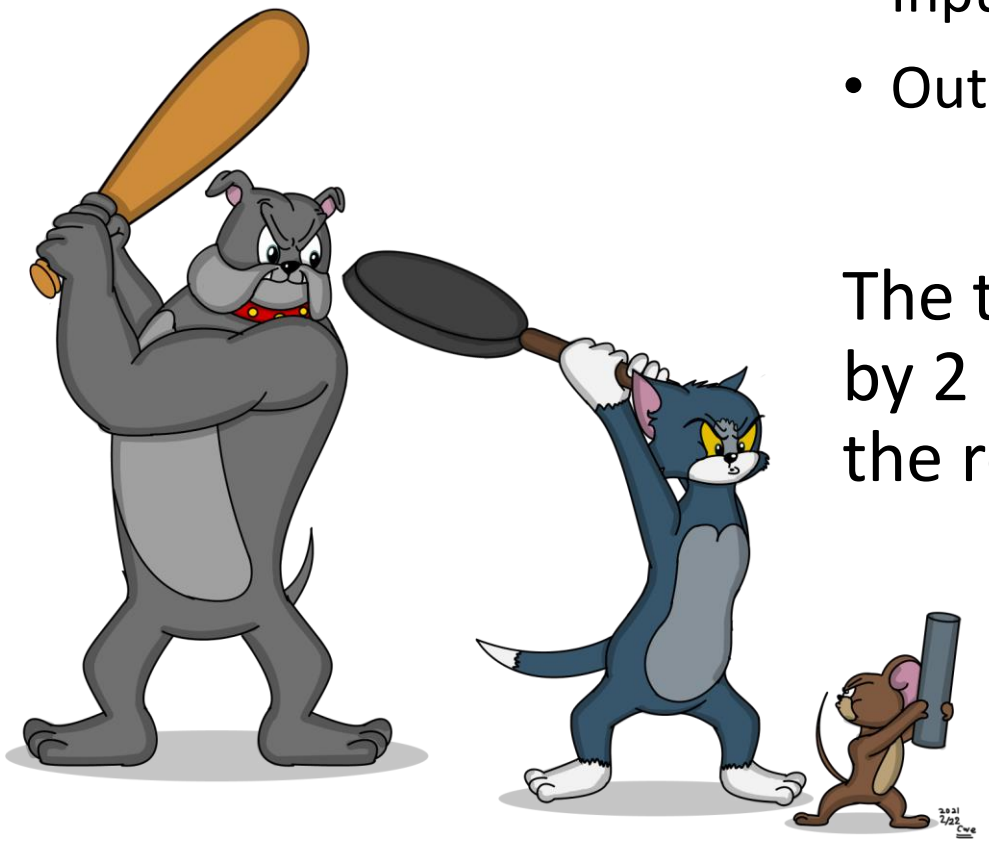


E. coli


human insulin gene
(DNA)
plasmid (loop of bacterial DNA)


plasmid (loop of bacterial DNA)
recombinant
bacterium
human
insulin
gene

Biobrick design



How does the system work?

- Inputs: hypoxia and high lactate
- Output: Aldeslukin (treatment)

The treatment gene is in constant repression by 2 different proteins, and our inputs cancel the repression state.

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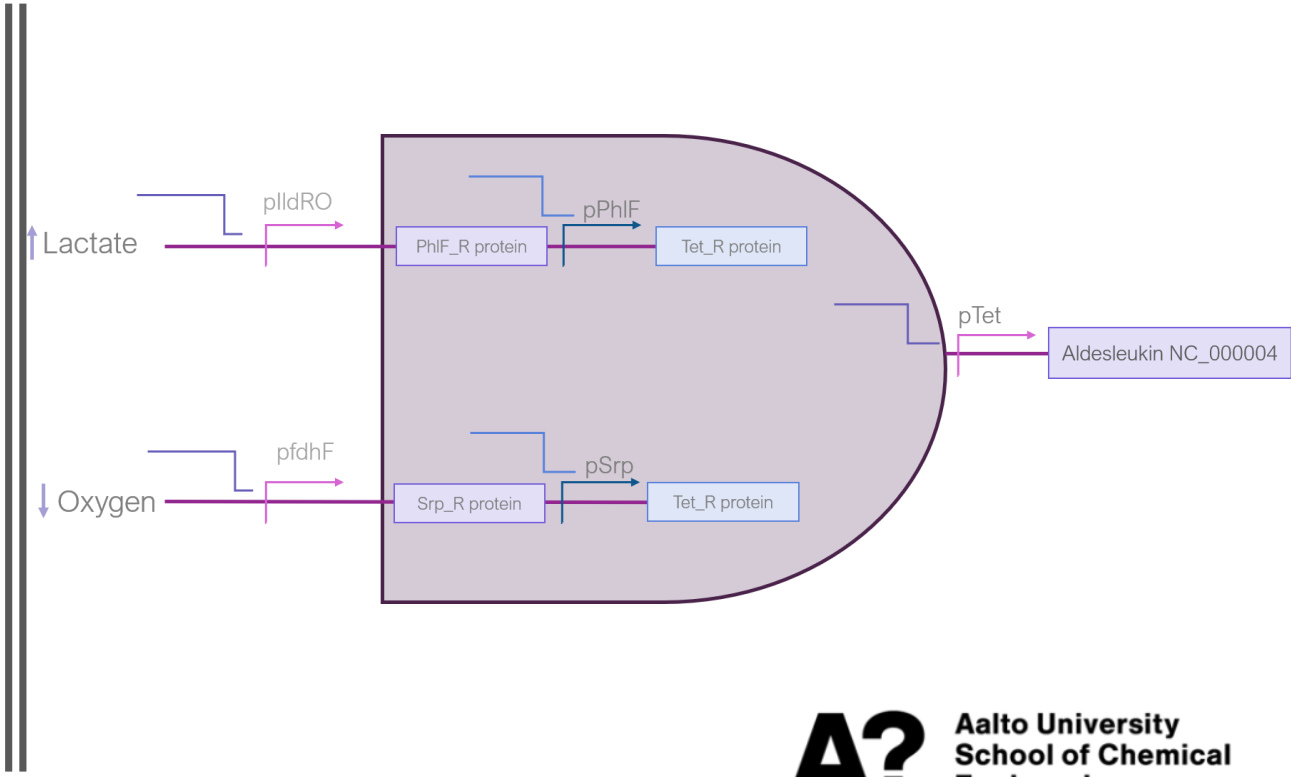
Truth table and circuit design

Lactate	Hypoxia	Output
0	0	0
1	0	0
0	1	0
1	1	1



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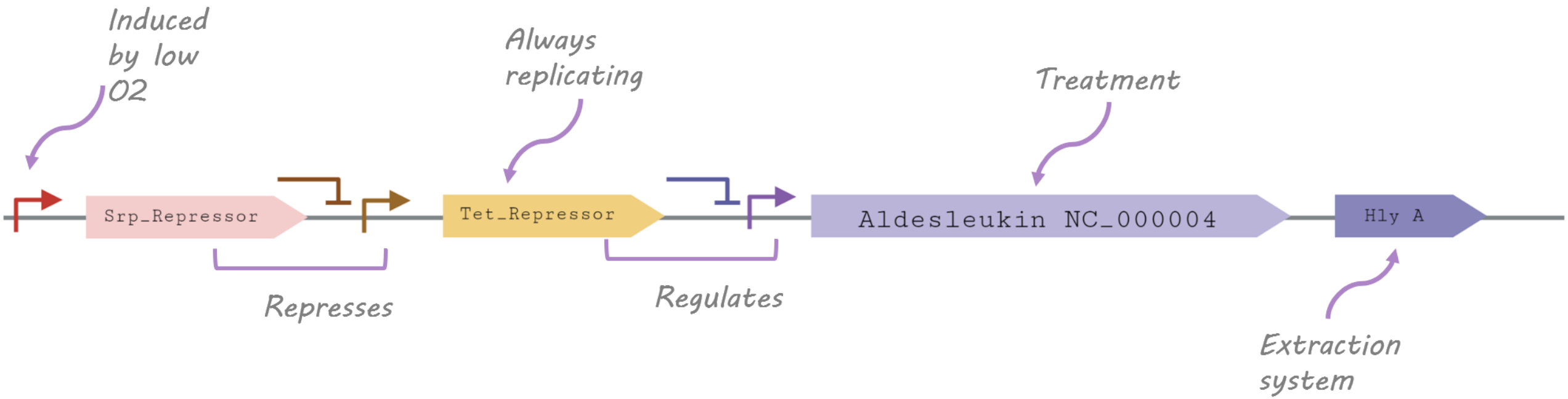
[7] https://2019.igem.org/Team:Amazonas-Brazil/Design

Hypoxia state circuit



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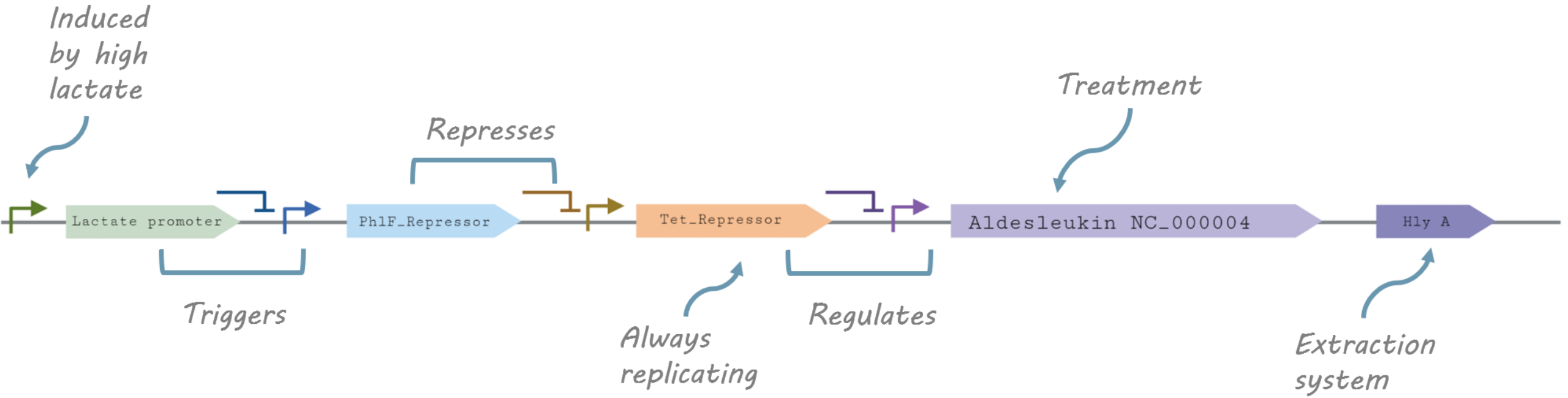
Lactate circuit



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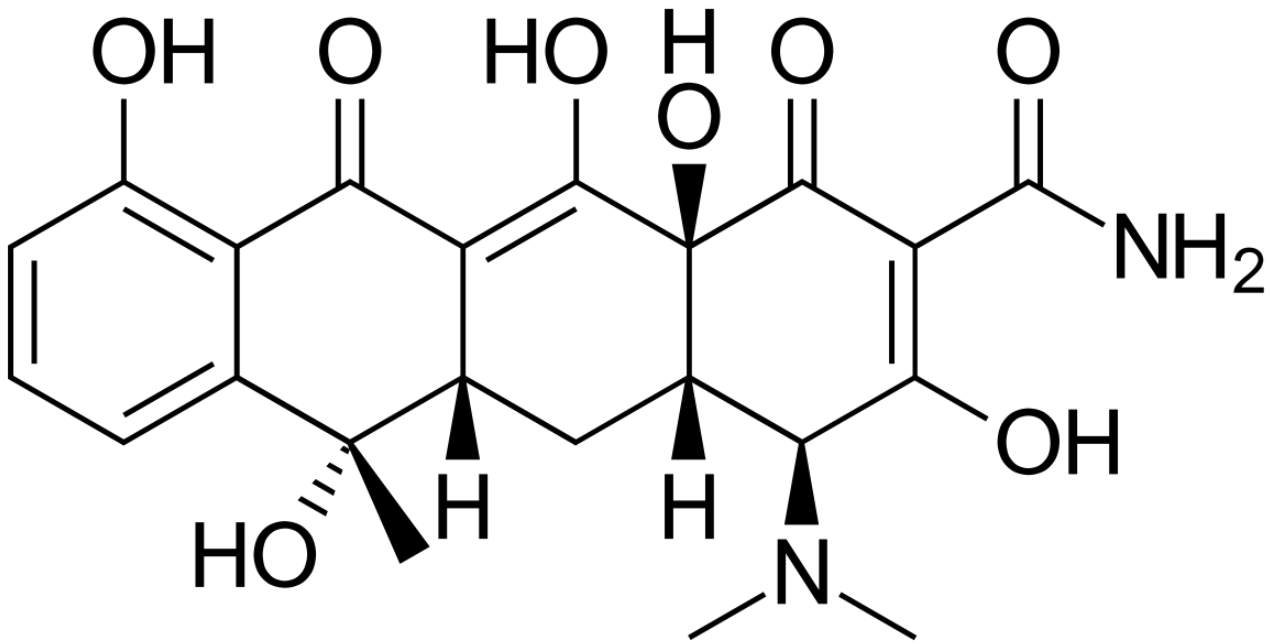
Final plasmid Design

- Cloned on pUC19 vector
  - totalling 8004 bp
  - Common for E. coli
  - With Amp resistance
  - 54 bp multiple cloning site polylinker

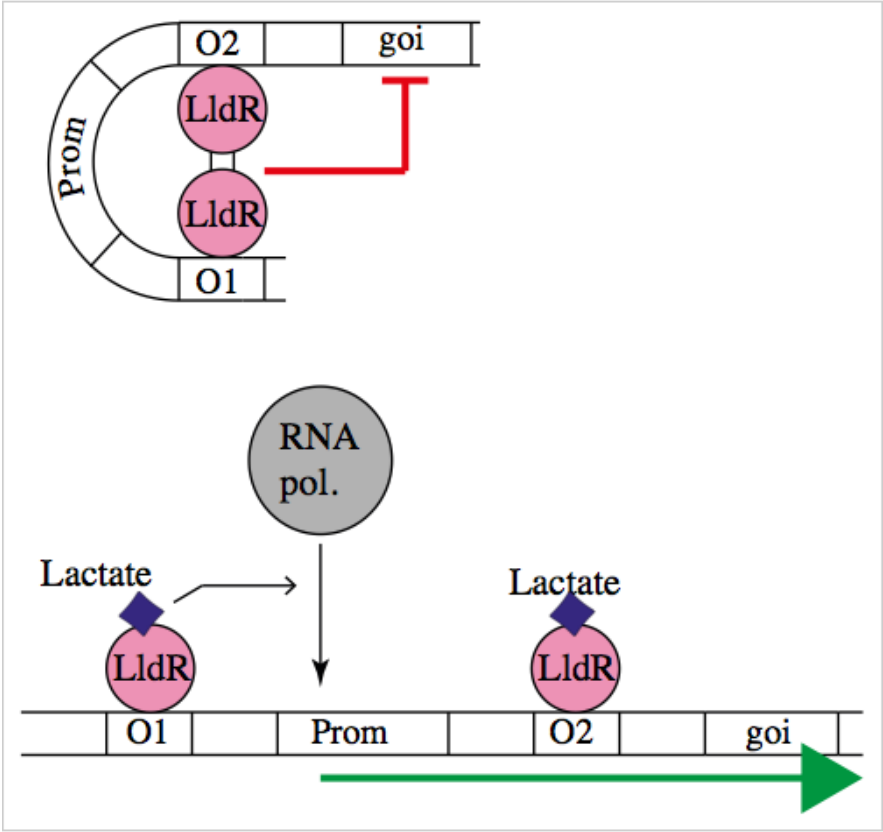
GCF_000001405.39 or TETRAN

- Human Tetracycline
- Encodes a member of the major facilitator superfamily of transporter proteins
- Efflux of organic anions, including the non-steroidal anti-inflammatory drugs indomethacin and diclofenac



promoter of LldR

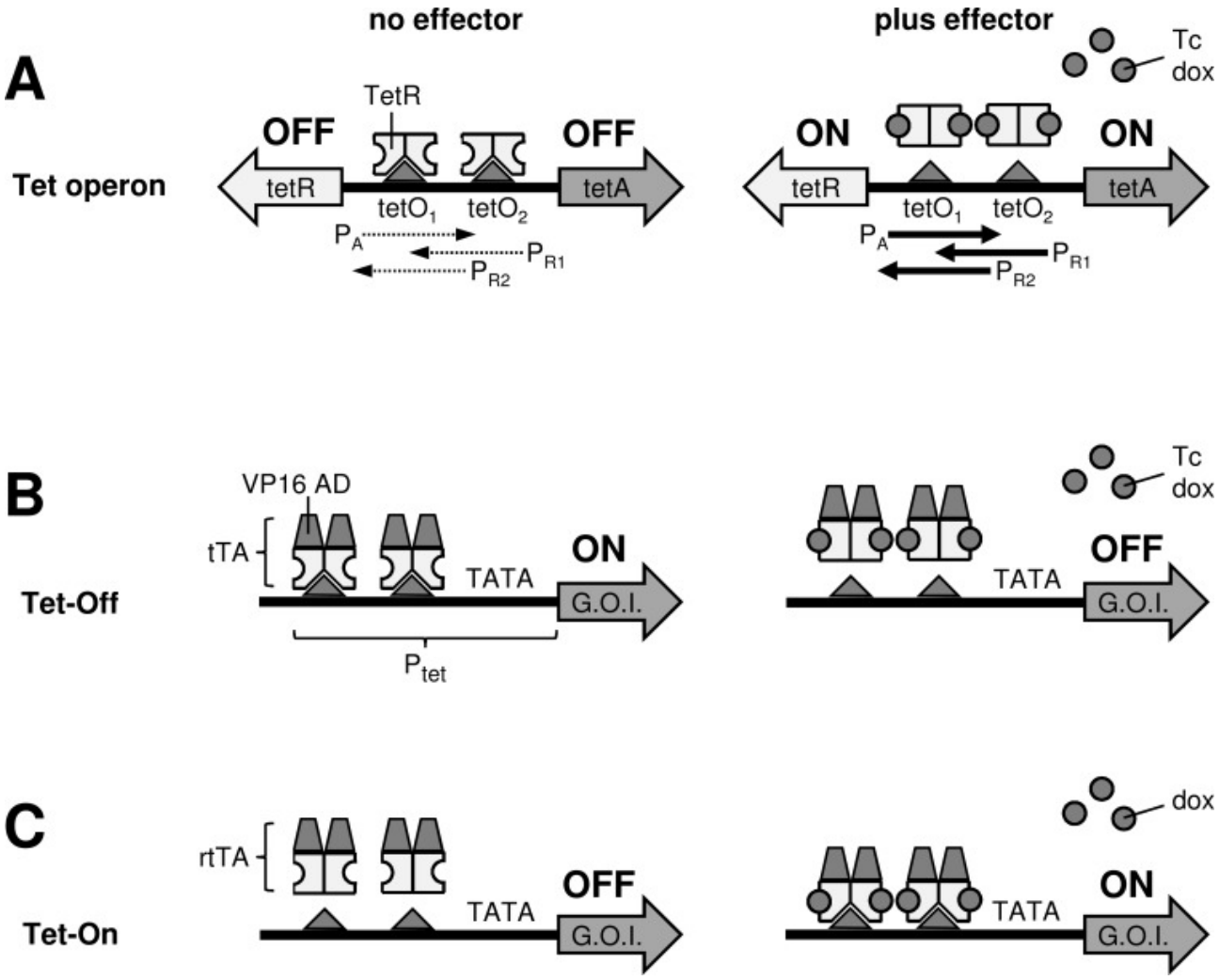
- Natural promoter with 2 operators
- It regulates the expression of the *lldPRD* operon
- Involved in L-lactate metabolism



[4] http://parts.igem.org/Part:BBa_K1847008

promoter TetR

Based on regulatory elements that control the activity of the tetracycline-resistance operon



[5] https://dx.doi.org/10.2174%2F1566523216666160524144041

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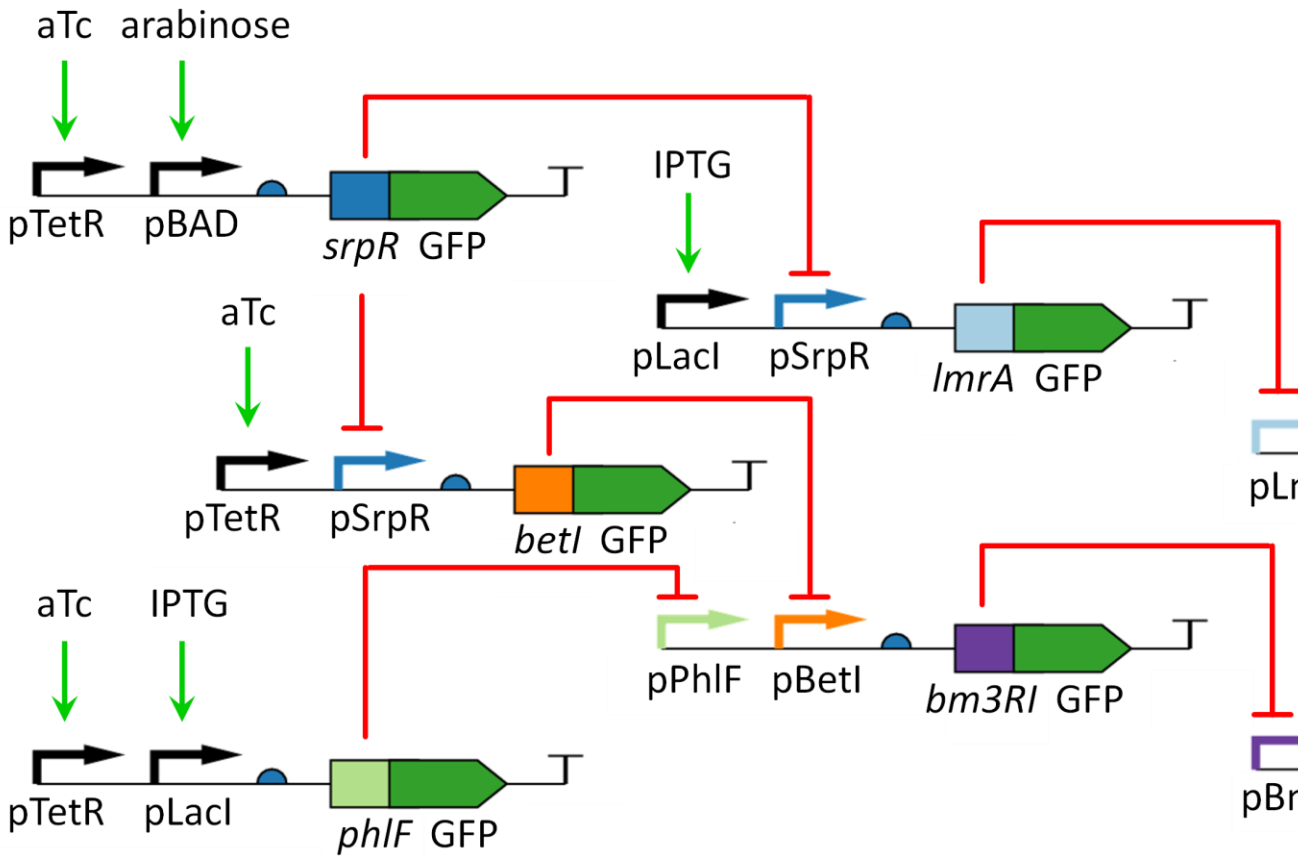
Other promoters

Usually used in E. coli vectors to produce GFP

- Promoter SrpR
  - 5000 RFU
- Promoter PhlF
  - Consecutive promoters family
- Promoter J23100
  - 2 different RBS
  - 10000 RFU

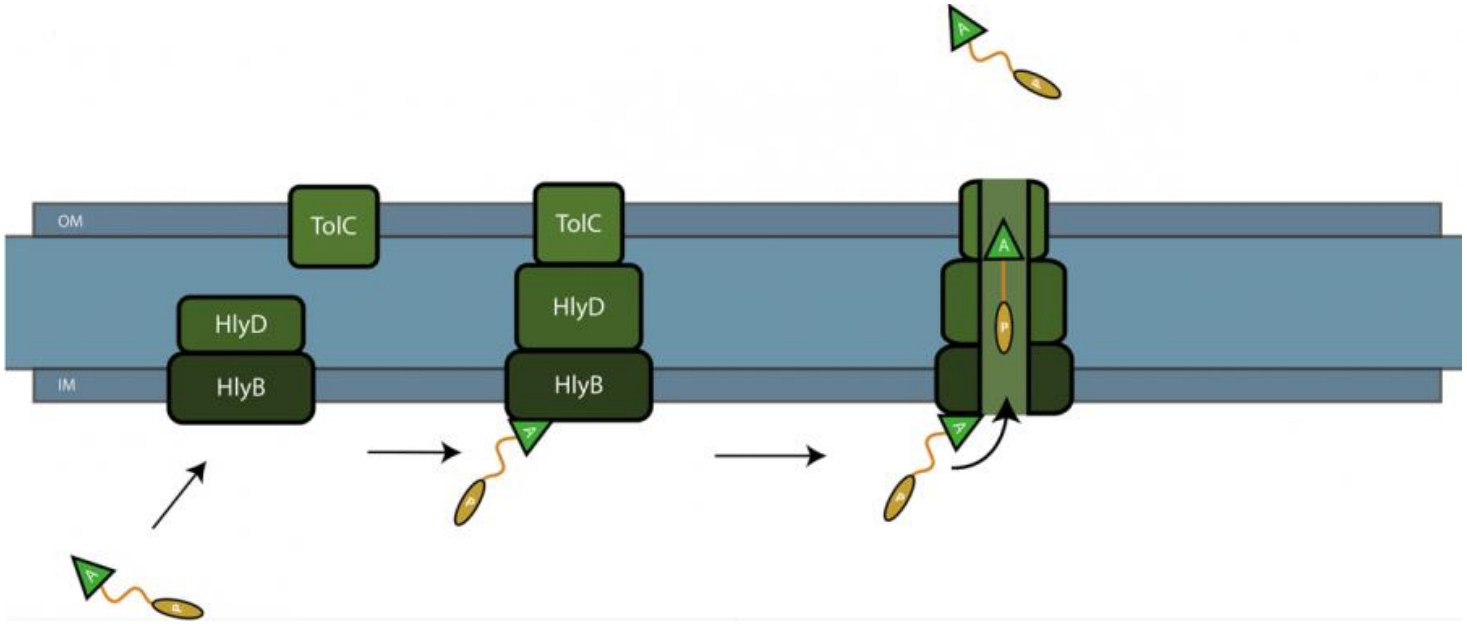


[6] http://2014.igem.org/Team:BostonU/Repressors

HlyA signal peptide

Carried by alpha-hemolysin extracellular media export system

- HlyB
- HlyD
- TolC



[8] http://parts.igem.org/Part:BBa_K2812006

Thank you for your attention

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- https://www.ncbi.nlm.nih.gov/gene/10227