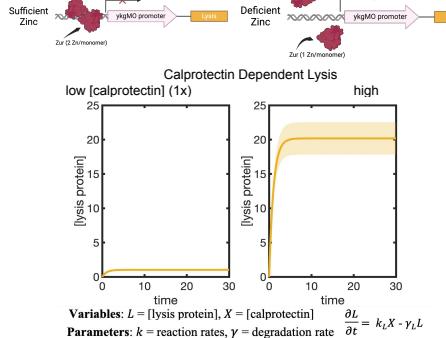
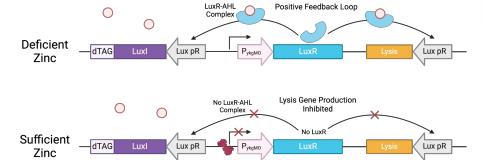




Calprotectin Dependent Lysis



Quorum Sensing Dependent on Calprotectin Concentration



- LuxR is only produced in the presence of calprotectin
- Therefore, lysis protein is only produced when there is calprotectin & high cell density

$$\frac{\partial I}{\partial t} = \beta_I + k_I \frac{C}{K_p + C} - \gamma_I I, \quad \gamma_I, K_p, \frac{k_{p,r}}{k_{p,f}}$$

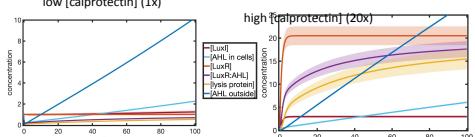
$$C = R \frac{A}{K_p + A}, \quad K_p = \frac{k_{p,r}}{k_{p,f}}$$

$$\frac{\partial A_{in}}{\partial t} = k_A I - \gamma_A A_{in} - k_{ex} \varphi A_{ex}, \quad \varphi \frac{V_{ex}}{V_{in}} \geq 1$$

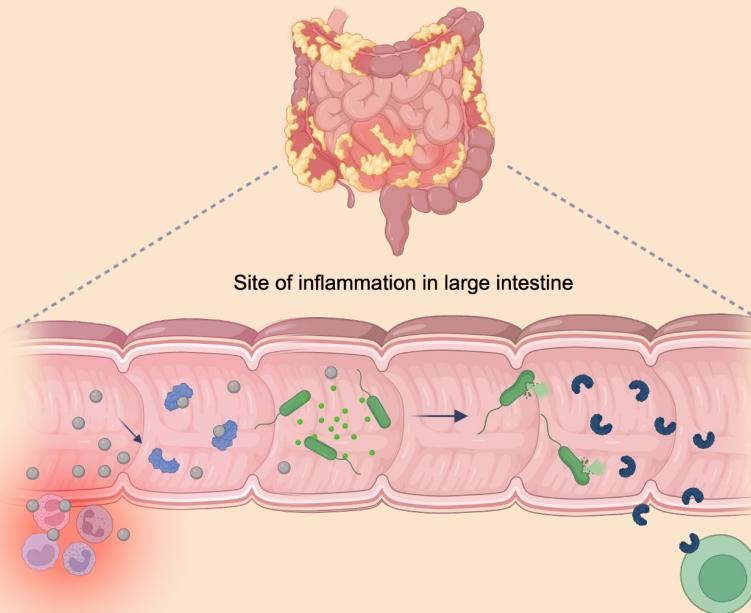
$$\frac{\partial R}{\partial t} = k_A X - \gamma_R R$$

$$\frac{\partial A_{ex}}{\partial t} = \frac{1}{\varphi} k_{in} \sum A_{in} - k_{ex} A_{ex}$$

$$\frac{\partial L}{\partial t} = k_L C - \gamma_L L$$



Our modeling results suggest a ~20% decrease in lysis protein production when calprotectin dependent quorum sensing is used instead of calprotectin dependence alone. However, slower lysing is a small tradeoff compared to how quorum sensing enables synchronous release of IL-10, which helps achieve a higher IL-10 concentration, which is important for the effectiveness of this therapeutic.

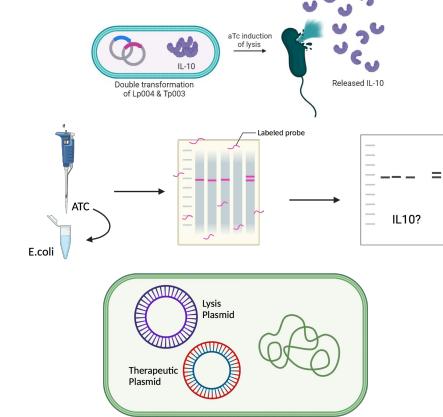


Abstract

An advanced therapeutic approach for treating inflammatory bowel disease (IBD) using a genetically engineered probiotic bacterium with a controlled lysing mechanism regulated by quorum sensing.

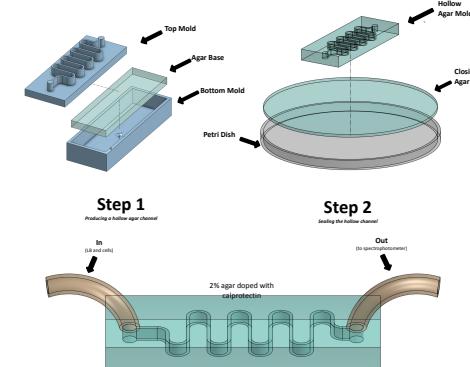
Quorum sensing enables bacterial populations to coordinate their behavior based on cell density, triggering a genetic response that induces the self-destruction of the bacteria. This controlled lysis ensures the precise release of interleukin-10 (IL-10), a potent anti-inflammatory cytokine, directly into the intestinal cells at the site of inflammation, ensuring targeted delivery of IL-10 where it is needed most and maximizing anti-inflammatory effects while minimizing systemic exposure and potential side effects.

Double Transformations



The successful IL-10 producing plasmid was double transformed with the successful Lysis gene plasmids into one cell. After administering aTc to trigger the lysis of *E.coli*, the sample's supernatant will be tested for the presence of released IL-10.

Microfluidic Model



Final Microfluidic Chamber

$$t_i = \frac{l_i w_i h_i}{Q}, \quad Q = A_i V_{avg}, \quad h_{Li} = f \frac{l_i}{D_{Li}} \rho_f \frac{V_{avg}^2}{2}$$

Left: Resident time of fluid in each zone of channel. Middle: Volumetric flow rate. Right: Head loss pressure

Therapeutic Model for Inflammatory Bowel Disease by Probiotic-Mediated Release of IL-10



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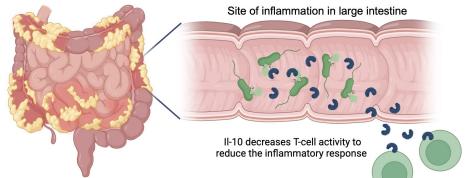
Lytic Delivery

Rebekah Choi, George Kopf, Camille Perez, Manna Sam, Kenzo Salazar, Beatrice Ramm

Department of Molecular Biology and Bioengineering, Princeton University

Introduction

Objective The Lysis team aims to construct genetic circuits in *E. coli* with different lysis genes native to bacteriophages under the control of an inducible promoter to characterize the mechanism of releasing of therapeutic.



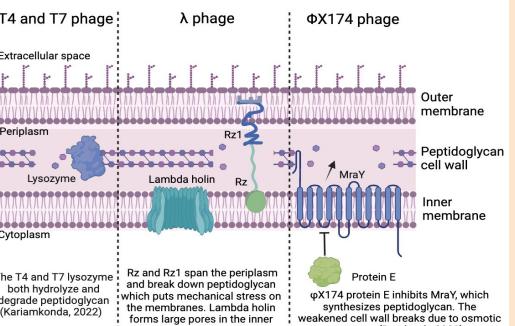
Overview The chosen method for delivering the therapeutic IL-10 is cell lysis. Although IL-10 is not yet approved for therapy due to its potential to induce harmful autoimmune responses, localized expression in mice has shown reduced adverse effects (Carlini et al. 2023). Instead of secreting IL-10, we opted for lysis because the molecule is too large to be secreted naturally by *E. coli*. Lysis also helps regulate the engineered probiotic population and prevents unpredictable mutations during bacterial reproduction. This approach ensures sufficient IL-10 release for effective treatment.

Carlini, V., Noonan, D. M., Abdalaleem, E., Goletti, D., Sansone, C., Calabrone, L., & Albini, A. (2023). The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. *Frontiers in Immunology*, 14, 116167. <https://doi.org/10.3389/fimmu.2023.116167>

Background

The five lysis genes tested were lambda phage holin, T7 phage lysozyme, T4 phage lysozyme, lambda phage Rz and Rz1, and ϕ X174 phage protein E.

Lysis Mechanisms of Different Phages



We wanted to characterize both the speed and sensitivity of the five lysis genes.

Since the phages' lysis proteins originate from are able to infect gram-negative bacteria, the team considered the safety of our engineered probiotic in the intestine. The team wants to ensure the selected lysis protein does not affect mammalian cells or other bacteria when in the intestine.

Bernhardt, T. G., Root, W. D., & Young, R. (2000). Genetic evidence that the bacteriophage ϕ X174 lysis protein inhibits cell wall synthesis.

Proceedings of the National Academy of Sciences of the United States of America, 97(8), 4297–4302. <https://doi.org/10.1073/pnas.97.8.4297>

Berry, J., S. M. Mekhora, E. J. Strickland, K. C., & Young, R. (2008). The final step in the phage life cycle: the Rz and Rz1 lysis proteins link the inner and outer membranes. Molecular Cell, 31(3), 315–320. <https://doi.org/10.1016/j.molcel.2008.01.020>

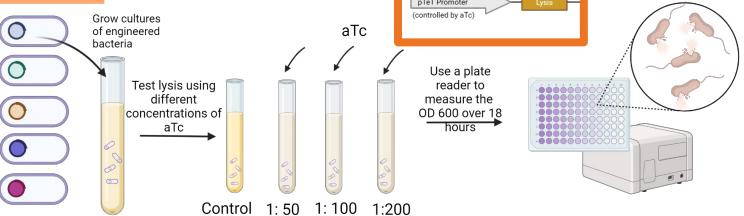
Karamikonda, M., Sharma, M., Gupta, P., & Poluri, K. M. (2022). Overexpression of bacteriophage T4 and T7 endolysins differentially regulate the metabolic fingerprint of host *Escherichia coli*. International journal of biological macromolecules, 221, 212–223.

<https://doi.org/10.1016/j.ijbiomac.2022.09.012>

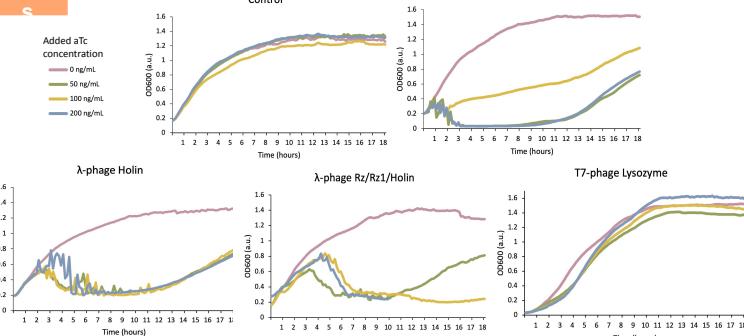
Characterization of Lysis Gene Efficiency

Methods

Experiment 1: Characterization of Lysis Genes



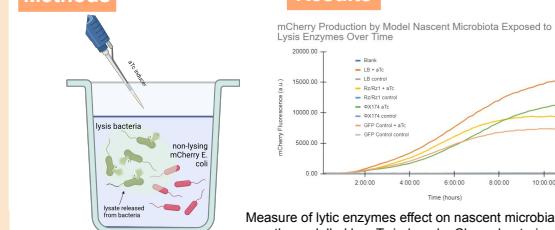
Results



Interaction with Nascent Microbiota

Methods

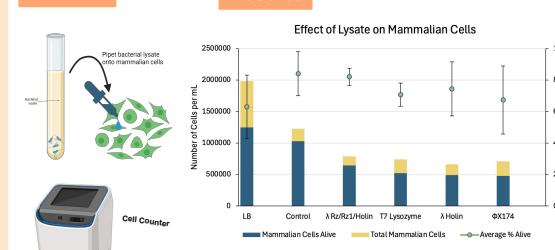
Results



Interaction with Mammalian Cells

Methods

Results

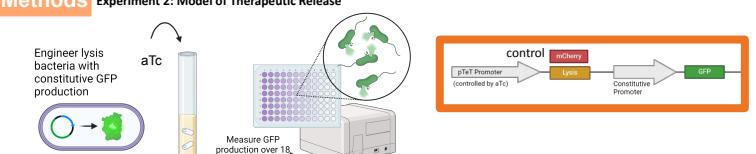


The lytic proteins in the lysate had minimal effect on the survival of mammalian cells.

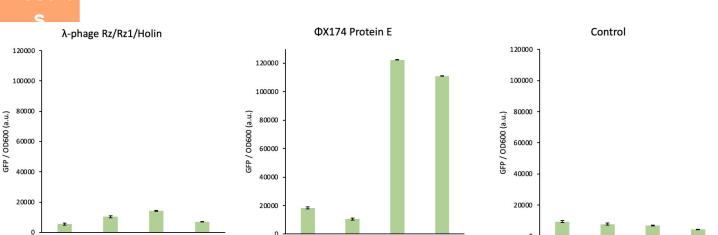
Model of Protein Release

Methods

Experiment 2: Model of Therapeutic Release



Results



Conclusion and Future Steps

Initial experimental results indicate that both the phiX and Rz1 genes effectively reduced OD of the engineered *E. coli* over time, demonstrating successful lysis of the engineered *E. coli* bacteria upon induction with aTc.

In experiment 2, we found that functional GFP was able to be released when the cell lyses. GFP is a model protein for the therapeutic protein that we hope to release.

Further experiments exploring cell interactions within intestines revealed that the engineered *E. coli* bacteria were not completely lysing mammalian cells or other *E. coli* strains, indicating that the lysis mechanism can target engineered *E. coli* without eliminating non-targeted cells. However, we observed that some mammalian cell growth was stunted with the addition of our bacterial lysate treatment.

To build upon this project we can measure how much IL-10 is produced using the different lysis genes and/or test the engineered bacteria with other types of gut microbiome bacteria.

Acknowledgements

This work is supported by Princeton Omenn-Darling Bioengineering Initiative, Jared Toettcher, Beatrice Ramm, Evan Underhill, and Beena Lad, members of the Toettcher Lab in Lewis Thomas Laboratory, Department of Molecular Biology at Princeton University, Integrated DNA Technologies, and Plasmidsaurus. This work contributes to international Genetically Engineered Machines synthetic biology research competition.

Therapeutic Model for Inflammatory Bowel Disease by Probiotic-Mediated Release of IL-10

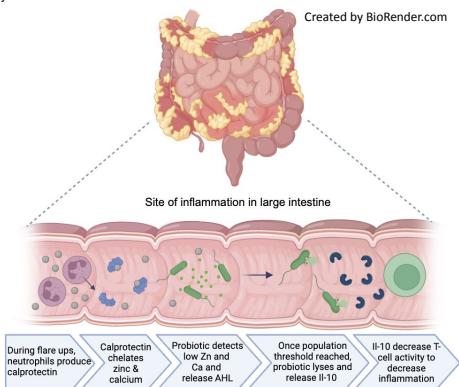


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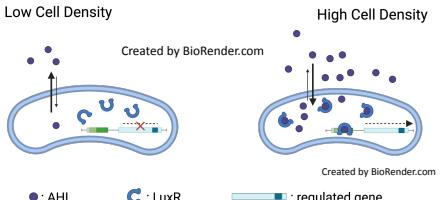
Introduction

Overview Quorum sensing (QS) enables bacteria to coordinate their behavior based on cell density via signaling molecules called autoinducers. This project uses QS to control the lysis of genetically engineered probiotic *E. coli*, releasing interleukin-10 (IL-10) at the inflammation site in the gut. By fluorescent intensity and optical density (OD) measurements, we aim to identify the density threshold that triggers bacterial lysis.



Objective The primary objective of the Quorum Sensing team is to engineer *E. coli* to conduct biomarker dependent quorum sensing (QS). By doing so, we aim to engineer probiotics that can distinguish between inflammatory and noninflammatory regions in the bowel and release IL-10 only in inflammatory regions.

Background Quorum sensing is a process of cell-to-cell communication that relays information about cell density. The target gene, which in our probiotics results in lysis, is only activated at high cell density.



Why incorporate quorum sensing?

For it to be effective, IL-10 must be released at a high enough concentration. Therefore, by using quorum sensing, the cells will lyse and release IL-10 synchronously to maximize treatment.

Why release IL-10 selectively?

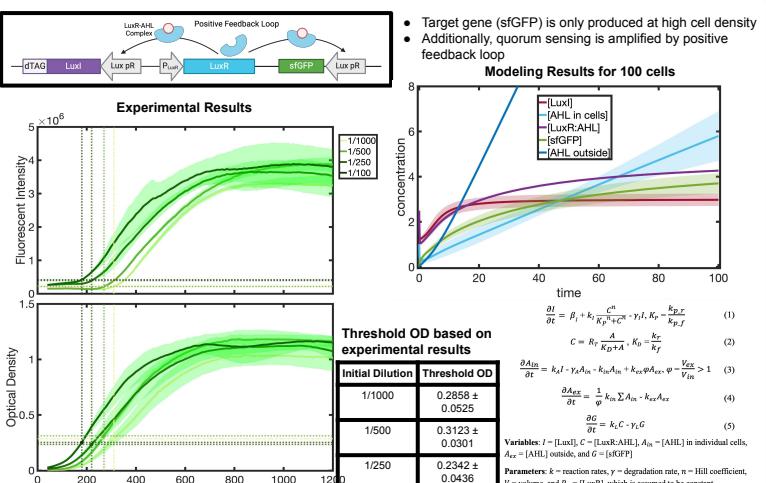
While IL-10 can decrease inflammation, it does so by decreasing T-cell activity. Therefore, to maintain homeostasis and a functional immune system, it is crucial that IL-10 is released only in the inflammatory sites. We will achieve this by controlling cell lysis through quorum sensing and the detection of calprotectin.

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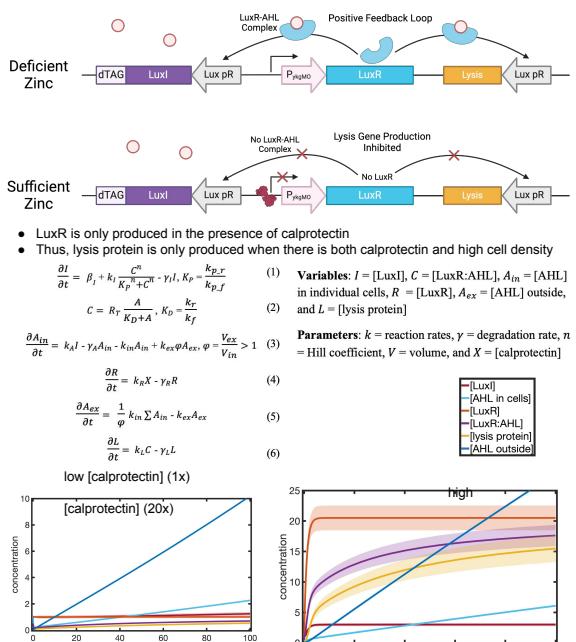
Quorum Sensing

Quorum Sensing Kinetics



Predictive Modeling Results

Quorum sensing controlled by calprotectin concentration



Conclusion and Future Steps

While the modeling result suggested that calprotectin dependent lysis produces a relatively higher concentration of lysate protein than QS-induced lysis, the synchronized lysis mechanism is still the preferred mechanism to ensure a high therapeutic load is delivered, with little tradeoff.

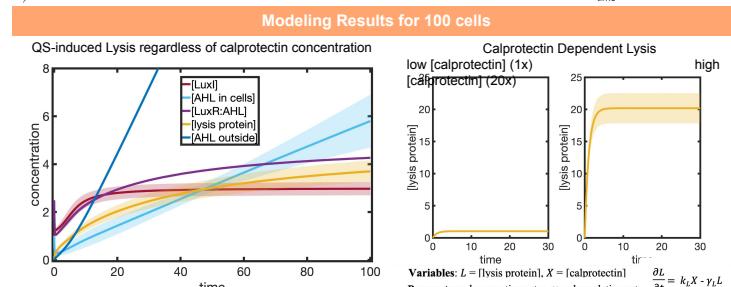
This supports our next step, to test the plasmid in a ± calprotectin environment and to insert the calprotectin-dependent ykgMO promoter into the QS-lysis plasmid. By replacing the LuxR promoter with the ykgMO promoter, LuxR will be produced with low zinc. Low zinc is correlated with high concentrations of calprotectin so LuxR can only activate the lysis gene when IBD is present.

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Therapeutic Model for Inflammatory Bowel Disease by Probiotic-Mediated Release of IL-10



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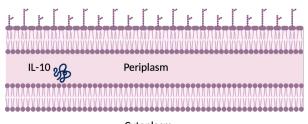
Introduction

Objective

The goal of the Therapeutics team is to constitutively produce IL-10 in the periplasm of *Escherichia coli* using different periplasmic signal sequences. Our therapeutic molecule- IL-10 would then be released in the lysis of the final bacterial model to relieve symptoms of Ulcerative Colitis.

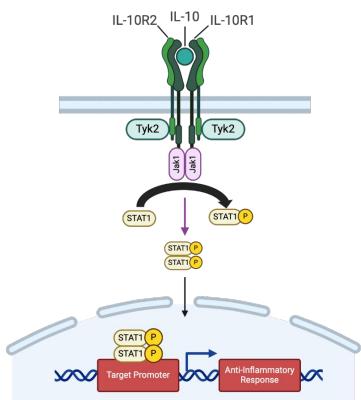
Overview

- Designed plasmid with a periplasmic signal sequence to direct IL-10 production in the periplasm of *E. coli*.
 - The periplasm reducing environment facilitates proper disulfide bond formation, crucial for IL-10's folding and functionality, mimicking conditions in mammalian cells.
- Microscopic imaging identified location of IL-10 and mCherry fluorescent protein
- Western blot confirmed the successful production of the therapeutic within the *E. coli*.



Background

- IL-10 is crucial for maintaining intestinal mucosal homeostasis.
 - It has potential as a therapeutic agent for inflammatory bowel disease (IBD).
- IL-10 binds to its receptor, activating JAK1 and Tyk2.
 - This activation leads to the stimulation of STAT3, a transcription factor important for anti-inflammatory responses.
- Previous studies support IL-10's use in treating IBD.
 - However, success has been limited due to inappropriate modes of administration.



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Beena Lad, Beatrice Ramm, Jared Toettcher, Evan Underhill

Department of Molecular Biology and Bioengineering, Princeton University

Therapeutics

Plasmid Design

Plasmids: Tp002—Tp004

constitutive promoter — signal peptide — IL-10

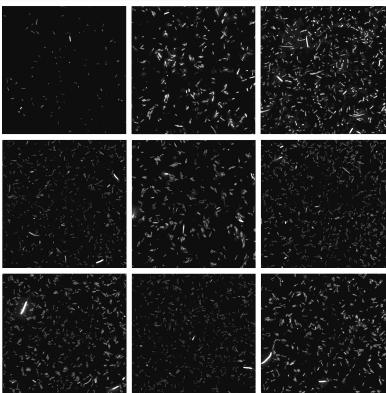
Plasmids: Tp005—Tp007

constitutive promoter — signal peptide — IL-10 — linker — mCherry

Periplasmic Signal Peptides: DsbA, TorA, PhoA

Imaging Results – Different Signal Peptides

Tp005: DsbA



Tp006: TorA

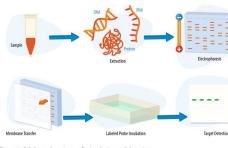
Tp007: PhoA

Tp005 - Tp007 on a 100x microscope to visualize the location of fluorescence in the bacteria. This imaging indicated that the signal peptide did not transfer both the mCherry and IL-10 into the periplasm, which could have been due to the size of the protein or the additional start codon in front of the mCherry gene sequence.

Western Blot Results – Production of IL-10

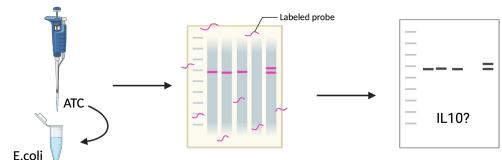
IL-10 length: ~18 kD
IL-10 + signal peptide length: ~22 kD

250 kD
150 kD
100 kD
75 kD
50 kD
37 kD
25 kD
20 kD
15 kD
10 kD



The image to the left shows a Western Blot with plasmids Tp002, Tp003, and Tp004 at OD of 0.7. The band located around the 20 kD ladder blot shows that our Tp003 produced IL-10 with the signal peptide.

Double Transformation



The successful IL-10 producing plasmid was double transformed with the successful Lysis gene plasmids into one cell. After administering aTc to trigger the lysis of *E. coli*, the sample's supernatant will be tested for the presence of released IL-10.

Conclusion and Future Steps

- The primary objective of the Therapeutics team was to successfully produce the anti-inflammatory cytokine IL-10 within the periplasm of bacterial cells
- This cytokine was intended to be released upon cell lysis, forming the basis of a novel therapeutic approach for alleviating symptoms of ulcerative colitis.
- The goal was to optimize the production and functional deployment of IL-10, enhancing its therapeutic potential.
- Results highlighted the significant potential of bacterial-based cytokine delivery as a therapeutic strategy for ulcerative colitis.

Future Goals

- Refinement of Targeting Methods:
- Refine methods for targeting and localizing therapeutic proteins like IL-10 within bacterial cells to enhance efficacy and release upon cell lysis.
- Explore alternative signal peptides and optimize expression conditions for consistent periplasmic localization.

Broader Applications:

- Adapt the bacterial model for the treatment of a broad spectrum of diseases beyond ulcerative colitis.
- Tailor the approach to deliver therapeutic proteins or small molecules for treating cancers, ulcerative colitis, and other conditions requiring localized delivery of therapeutic agents.
- Develop a versatile platform capable of addressing diverse therapeutic needs, providing targeted and efficient treatment options for a wide array of diseases.

References

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