Johns Hopkins Engineering

Immunoengineering

Immunoengineering—Allergy and Autoimmunity

Cell Engineering

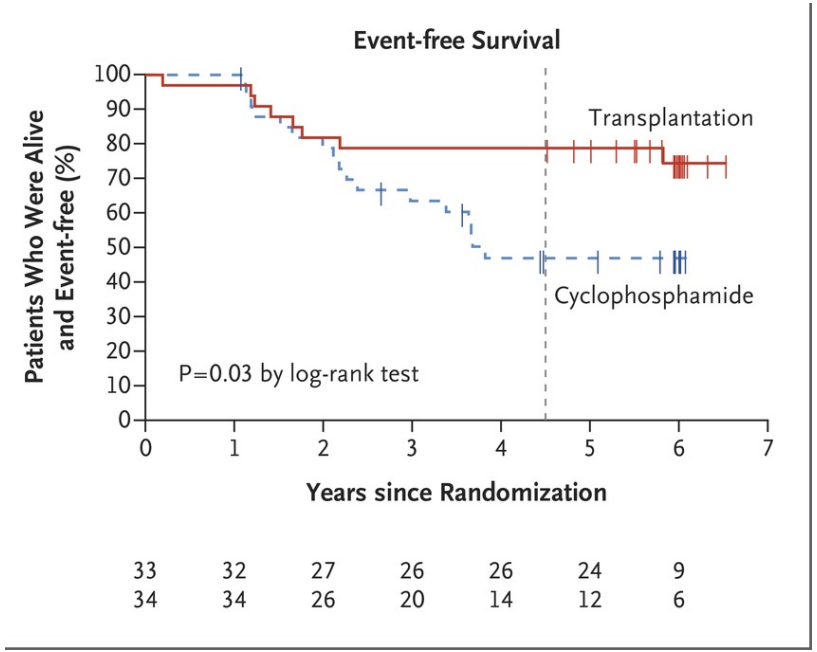
JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING

General Outline

- **Engineered Cells**
- **Engineered Microbes**
- Engineered Proteins
- Engineered Genetic Material

Stem Cell Transplantation for Scleroderma

- Reset the immune system
- Significant increase in patient survival and limiting relapse
- Compared to conventional immunosuppressive chemotherapy - cyclophosphamide

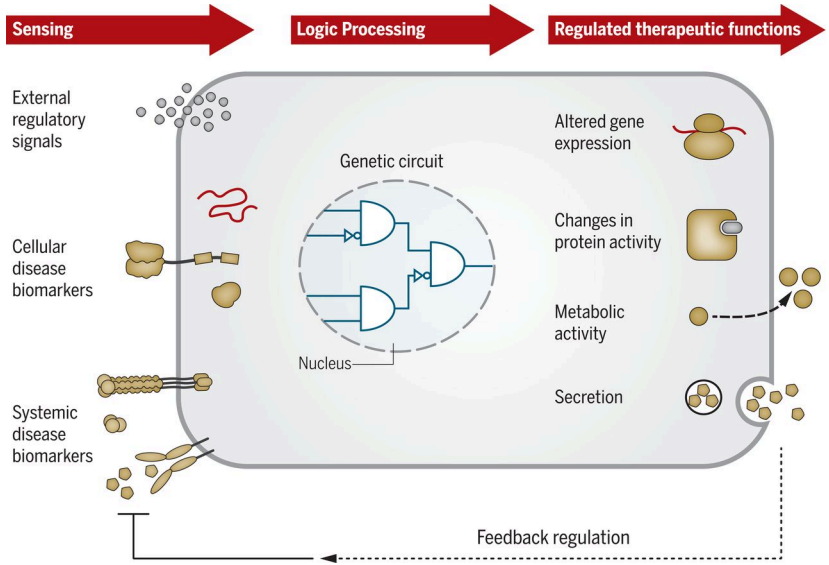


Sullivan, Keith M., et al. "Myeloablative Autologous Stem-Cell Transplantation for Severe Scleroderma." *New England Journal of Medicine* 378.1 (2018): 35-47.

Cellular Engineering Background

Advantages

- Overcomes challenges of biologics and small molecules
- Potential for a dynamic response that can regulate therapeutic:
  - Dose
  - Timing
  - Localization
- Act as a living sensor for diagnostics

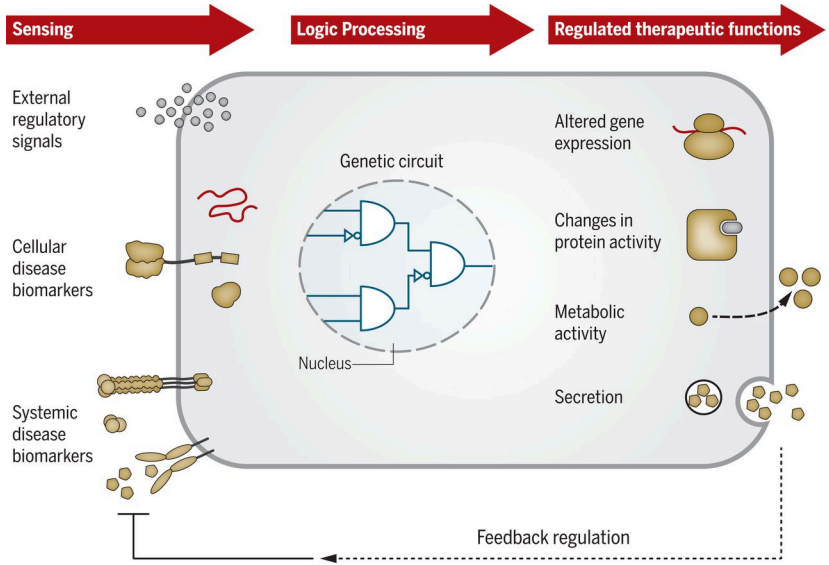


Kitada, Tasuku, et al. "Programming gene and engineered-cell therapies with synthetic biology." *Science* 359.6376 (2018): eaad1067.

Cellular Engineering Background

Disadvantages

- Lack of control (e.g. CAR T cells)
- Need to create many circuits to find one that works
- Cell type and source
- Immune reaction
- Nucleic acid delivery



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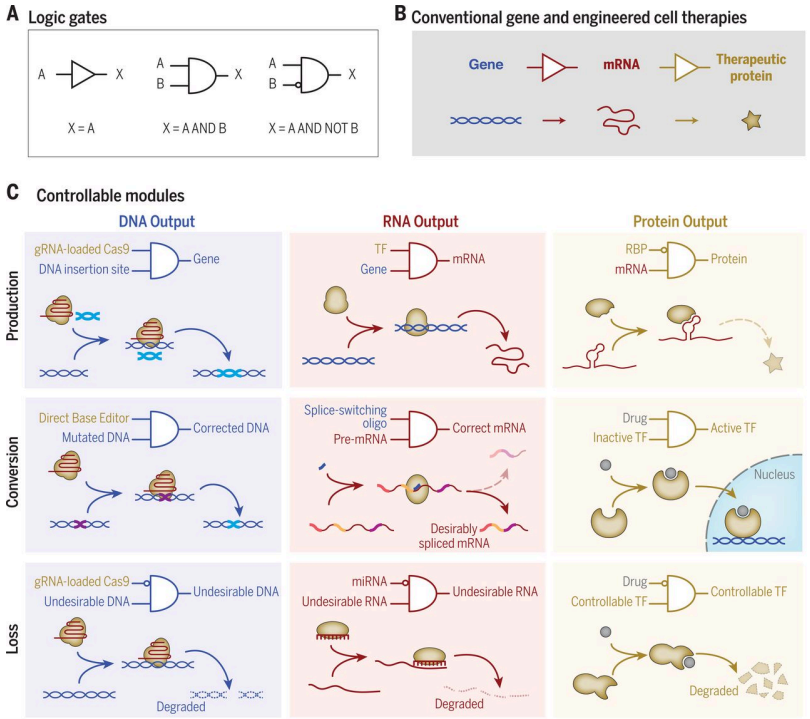
Synthetic Biology and Genetic Circuits

Engineering Approaches

- Computer-aided design
- Modularity & Abstraction
- Feedback control



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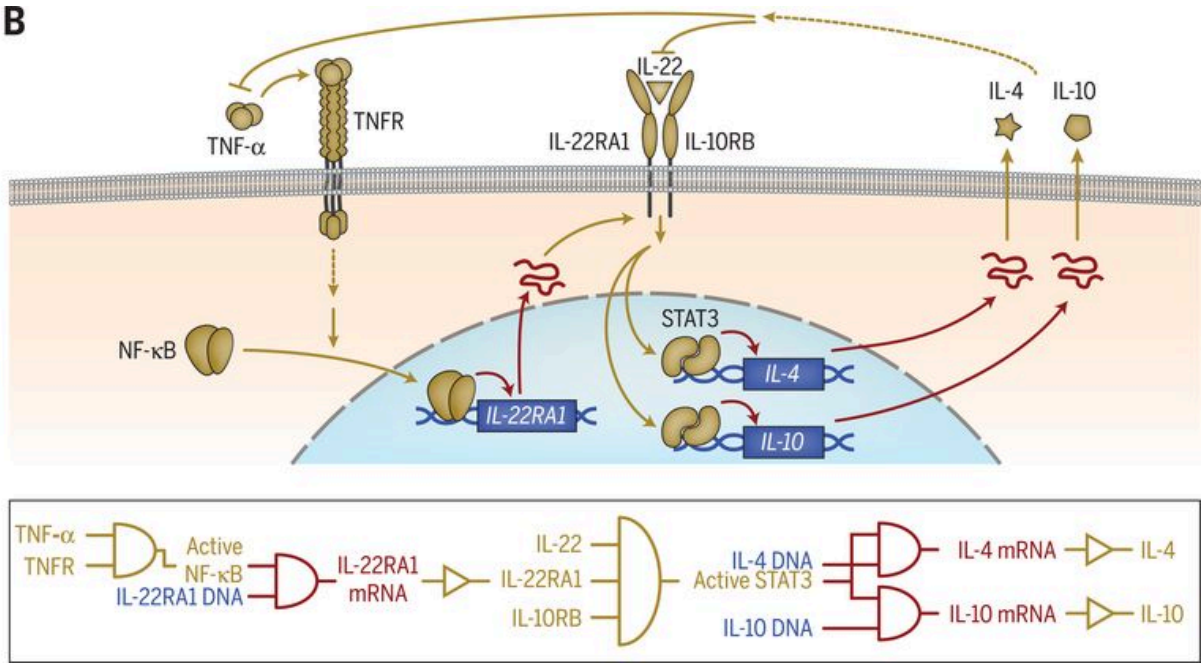
Genetic Circuits Example - Psoriasis

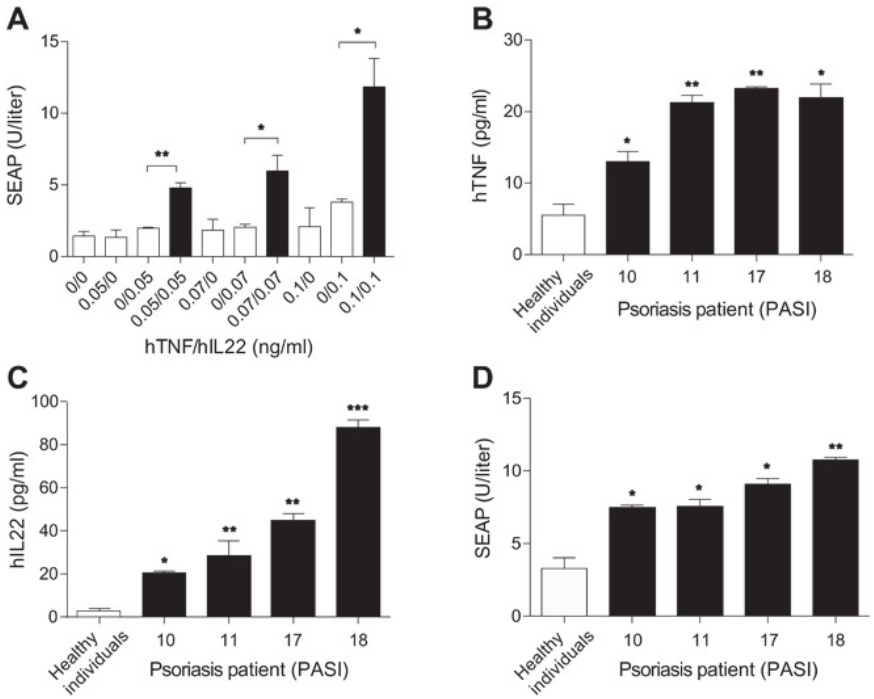
- TNFa & IL-22 upregulated
  - Target of many current therapies
- IL-4/10 help control
  - Also a therapeutic option
- AND gate for dual production



Kitada, Tasuku, et al. "Programming gene and engineered-cell therapies with synthetic biology." *Science* 359.6376 (2018): eaad1067.

Genetic Circuits Example - Psoriasis

- Control Psoriasis in vitro and in vivo in mice compared to conventional treatment
  - Need to be encapsulated in alginate gel
- Designer cell responds to cytokine levels from psoriasis patients



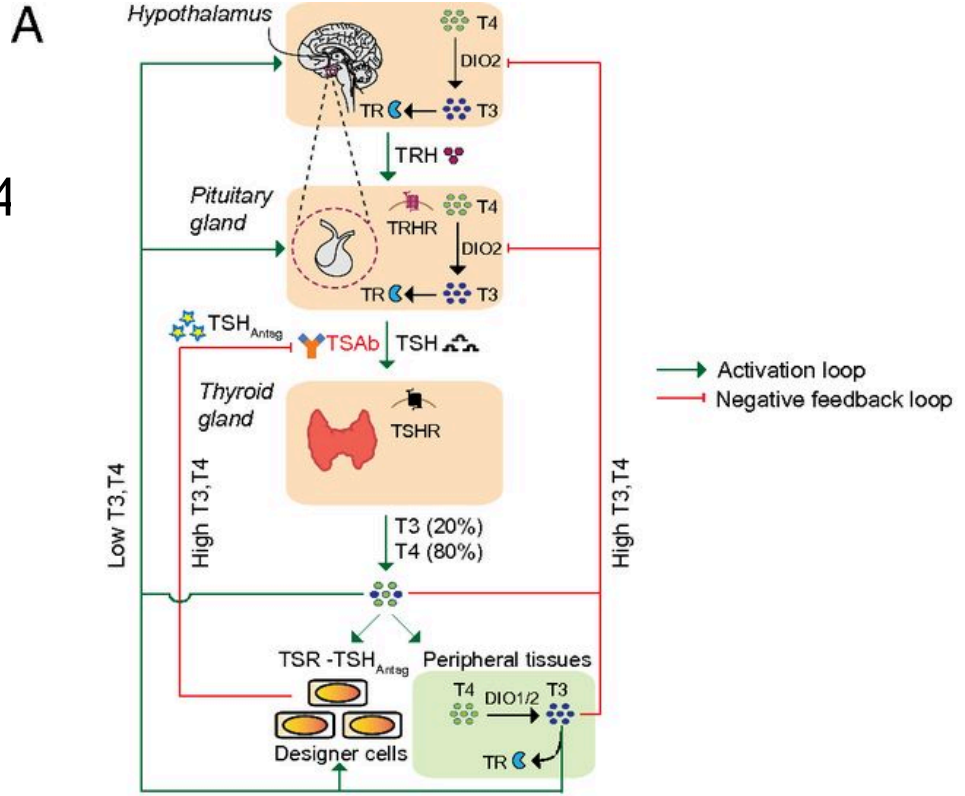
Schukur, Lina, et al. "Implantable synthetic cytokine converter cells with AND-gate logic treat experimental psoriasis." *Science translational medicine* 7.318 (2015): 318ra201-318ra201.

Genetic Circuits Example – Grave's Disease

- Low T3/4
  - TSH release from pituitary gland
    - Stimulate thyroid to release T3/4
    - Negative feedback from T3

  levels to suppress TSH release in hypothalamus and pituitary gland

- Graves Disease = constitutively activate T3/4 production and disrupt negative feedback loop

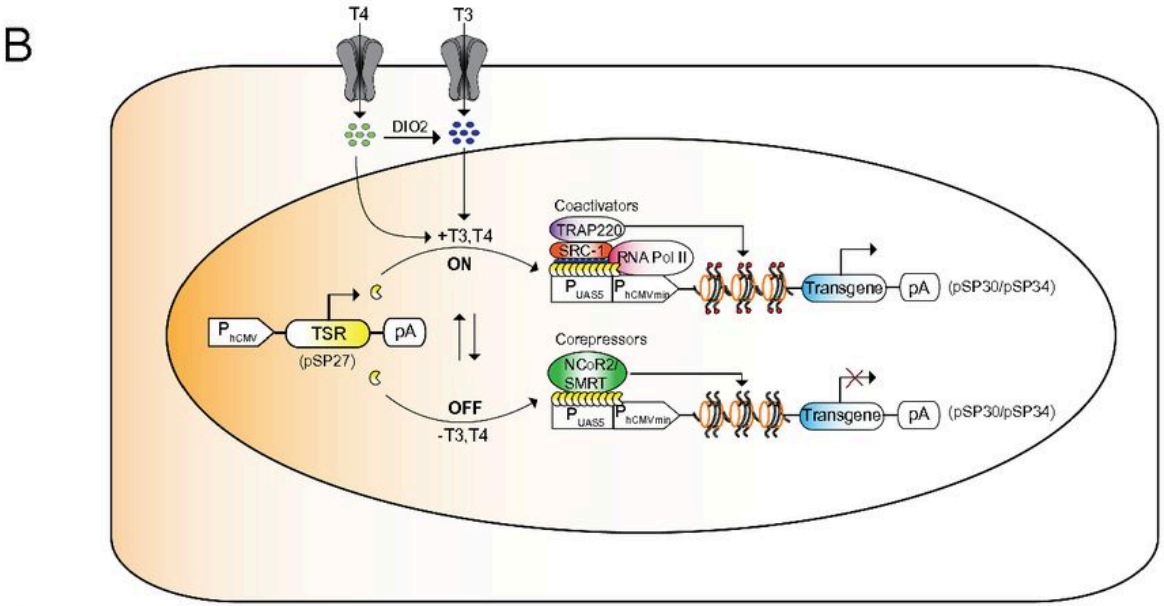


Saxena, Pratik, et al. "Synthetic gene network restoring endogenous pituitary–thyroid feedback control in experimental Graves' disease." *Proceedings of the National Academy of Sciences* 113.5 (2016): 1244-1249.

Genetic Circuits Example – Grave's Disease

Designer cell

- Input is T3 sensor
  - Which turns ON gene expression if signaled
- Output are TSH antagonists
- Regulated by what it is therapeutically targeting



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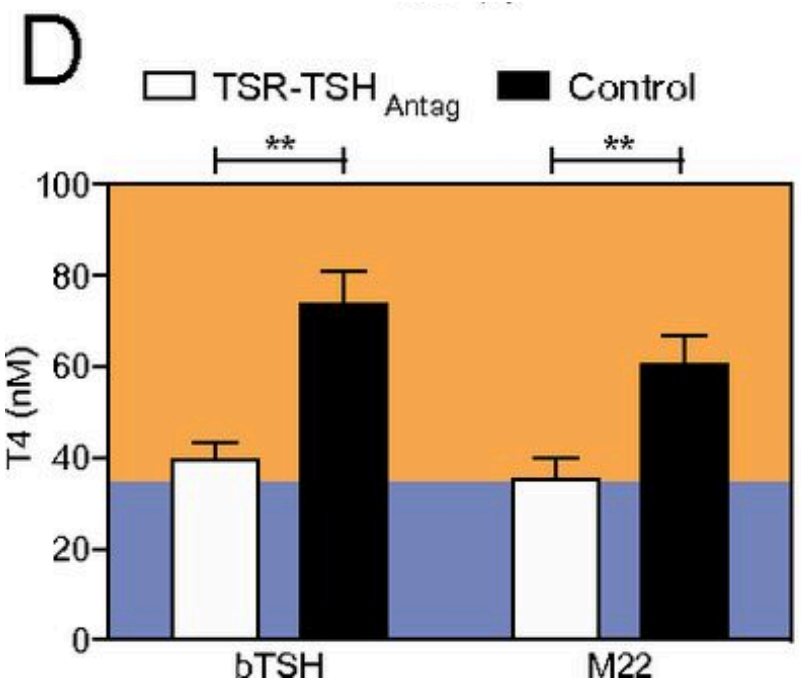
Genetic Circuits Example – Grave's Disease

Designer cell

- Control T4 levels in mice with hyperthyroidism
- Need to be injected in alginate spheres



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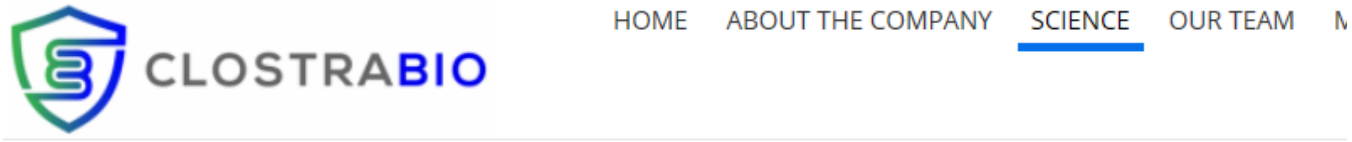
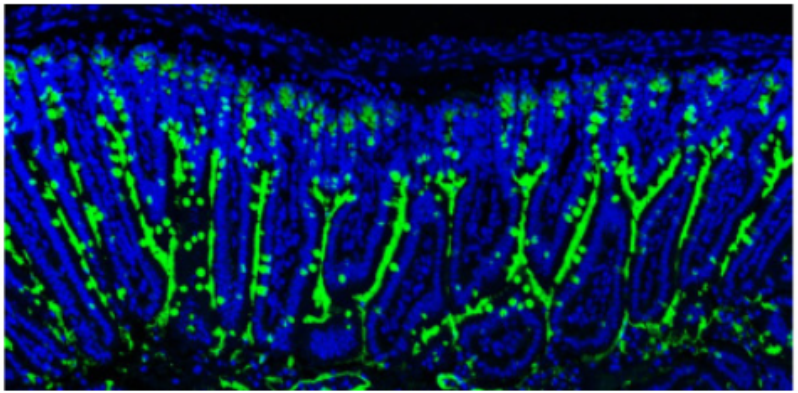
Restoring Protective Microbiota



Start up around treating food allergies with adding bacteria to the microbiota

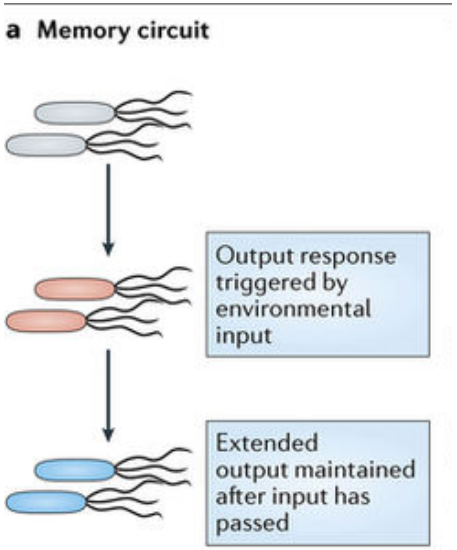


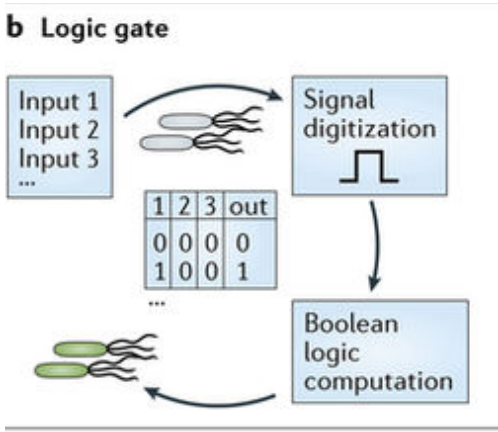
http://www.clostrabio.com/science.html

Genetic Engineering for bacteria

Advantages

- More straightforward circuit design
- Ability of transfer
- Availability and production of cells



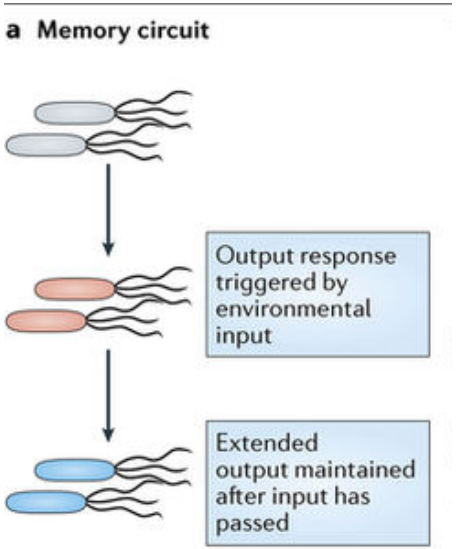


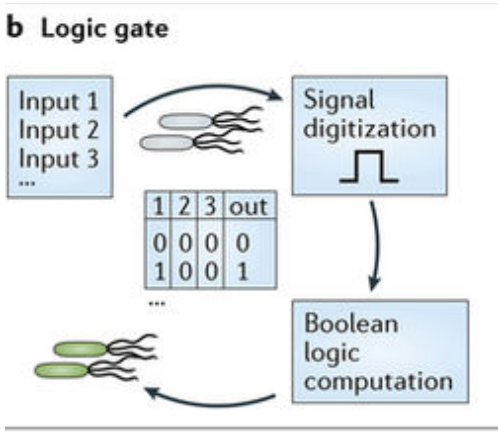
Riglar, David T., and Pamela A. Silver. "Engineering bacteria for diagnostic and therapeutic applications." *Nature Reviews Microbiology* (2018).

Genetic Engineering for bacteria

Disadvantages

- Safety and Regulation
- Preclinical screens
- Stability
  - Growth rate
  - Loss of function
  - Rate of mutations

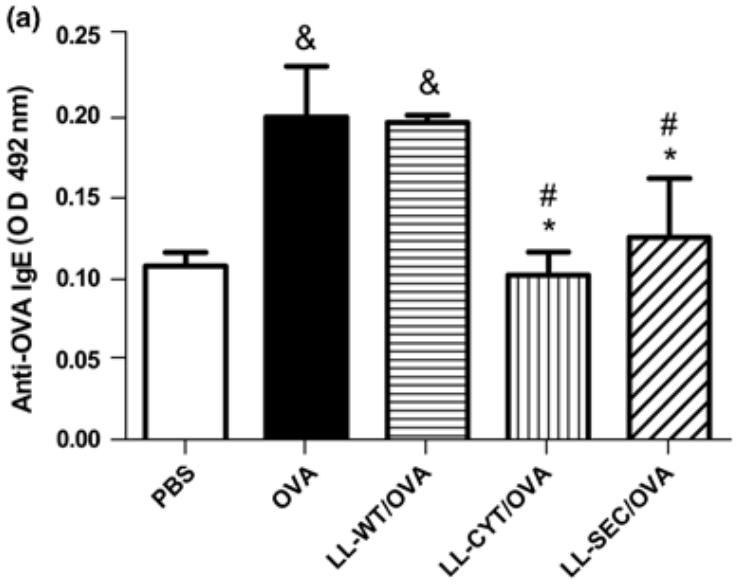




Riglar, David T., and Pamela A. Silver. "Engineering bacteria for diagnostic and therapeutic applications." *Nature Reviews Microbiology* (2018).

Bacterial Engineering Example – Allergies

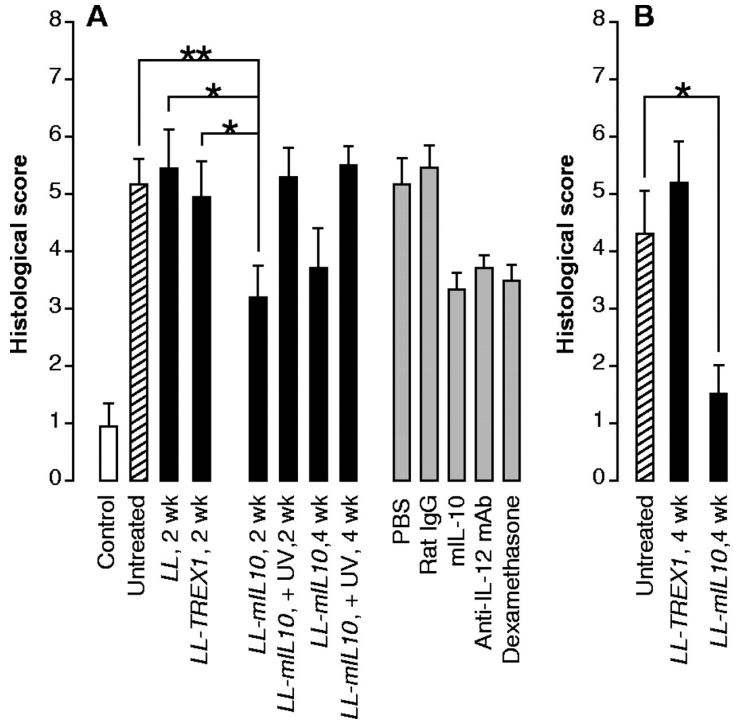
- *Lactococcus lactis - GRAS*
- Engineered to express IL-10
  - Downregulate immunoflammatory and used in treatment
- Decreased IgE levels to sensitized antigen



Marinho, Fábio Antônio Vitarelli, et al. "An intranasal administration of Lactococcus lactis strains expressing recombinant interleukin-10 modulates acute allergic airway inflammation in a murine model." *Clinical & Experimental Allergy* 40.10 (2010): 1541-1551.

Bacterial Engineering Example – Chron's disease

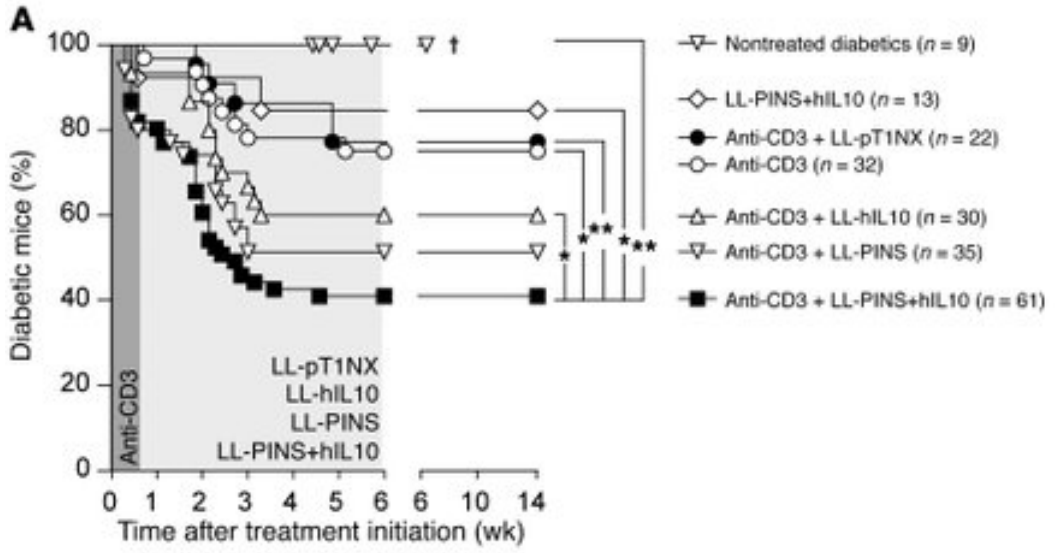
- *Lactococcus lactis* – IL-10
- Reduction of 50% of disease score for mice



Steidler, Lothar, et al. "Treatment of murine colitis by Lactococcus lactis secreting interleukin-10." *Science* 289.5483 (2000): 1352-1355.

Bacterial Engineering Example – Diabetes

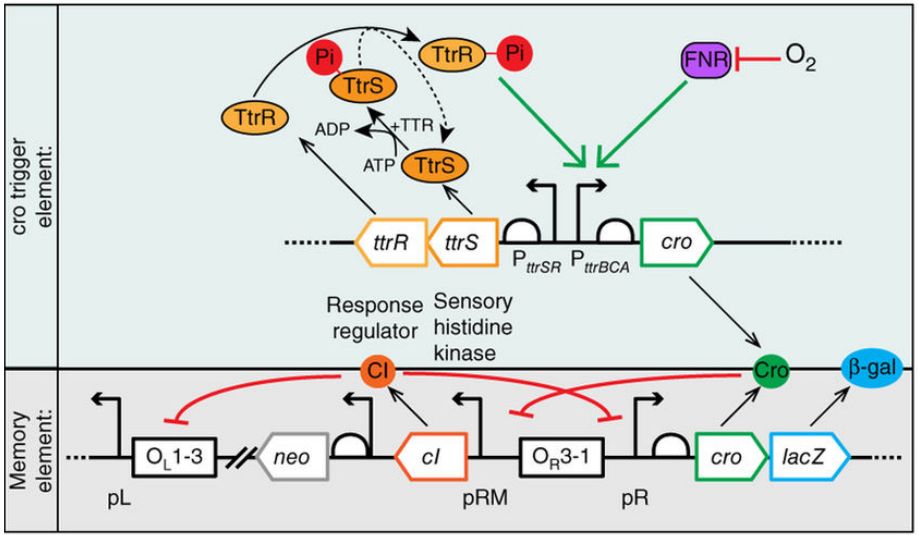
- *Lactococcus lactis* – IL-10 & proinsulin autoantigen
- Reduce Diabetes in 66% of mice
- Combination with low dose of systemic anti-CD3



Takiishi, Tatiana, et al. "Reversal of autoimmune diabetes by restoration of antigen-specific tolerance using genetically modified Lactococcus lactis in mice." *The Journal of clinical investigation* 122.5 (2012): 1717-1725.

Engineered Bacteria to Persist

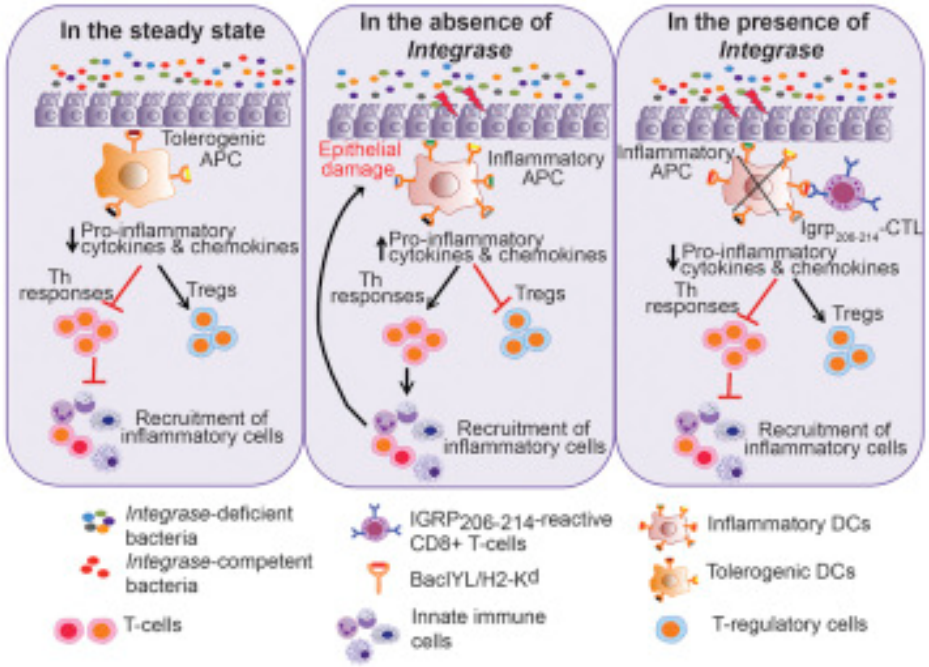
- Detect tetrathionate for indicator of inflammation in the gut
- Stable in gut for up to 6 months



Riglar, David T., et al. "Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation." *Nature biotechnology* 35.7 (2017): 653.

Bacterial Mimitopes – A potential way for therapy?

- The *Bacteroides* integrase encodes a low-avidity mimotope of IGRP206-214
- The microbial epitope recruits diabetogenic CD8+ T cells to the gut
- Crossreactive CD8+ T cells suppress colitis by targeting gut DCs
- Suppression of colitis is MHC class I-, Itgb7-, and perforin-dependent



Nanjundappa, Roopa Hebbandi, et al. "A Gut Microbial Mimic that Hijacks Diabetogenic Autoreactivity to Suppress Colitis." *Cell* 171.3 (2017): 655-667.

Comparison

Mammalian Cells

+ Ability for systemic administration

- Difficult to genetically engineer

+ May require less engineering because functionally similar

- Difficult to maintain cells and lack of available cell sources

Bacteria Cells

- Limited areas of influence (bacterial restricted sites)

+ Easier to genetically engineer

- Different biology may not be compatible (protein modifications)

+ Easier to grow cells and obtain cell lines

Challenges Facing Cellular Engineering

- Safety
- Control
- Administration and Persistence
- Cost
- Standardization