Engineering Synthetic Biotic Medicines To Secrete Human Proteins and Short Chain Fatty Acids as a Versatile Platform To Treat Inflammatory Bowel Disease

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Summary

- Synthetic Biotic medicines are live, non-pathogenic *E. coli* Nissle (EcN) bacteria designed with drug-like properties.
- Our synthetic biology platform allows the engineering of probiotic strains with versatile and disparate modalities that can sense and influence inflammatory pathways in vivo including:
  - *In vivo* environmental biosensors that detect inflammatory signals, e.g., nitrate
  - Short chain fatty acid production that can alter inflammatory signaling
  - Production and secretion of human proteins, including cytokines
- These innovations are key to the development of gut-targeted therapeutics for inflammation-based pathologies such as Inflammatory Bowel Disease (IBD).

EcN protein expression is engineered to respond to environmental signals in vivo

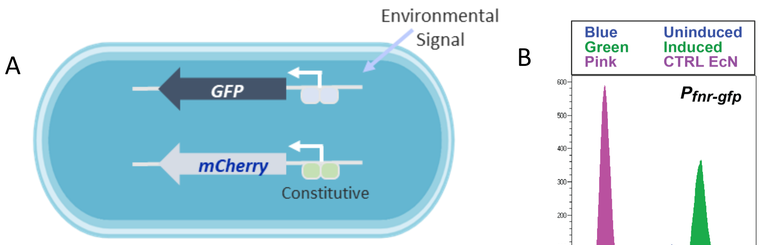

Figure 1. A. Design and induction of a biosensor strain engineered to constitutively express mCherry and express GFP upon sensing an in vivo environmental signal. **B.** In vitro FACS analysis of a biosensor EcN strain responding to low oxygen conditions (P_{fnr} = fnrS promoter).

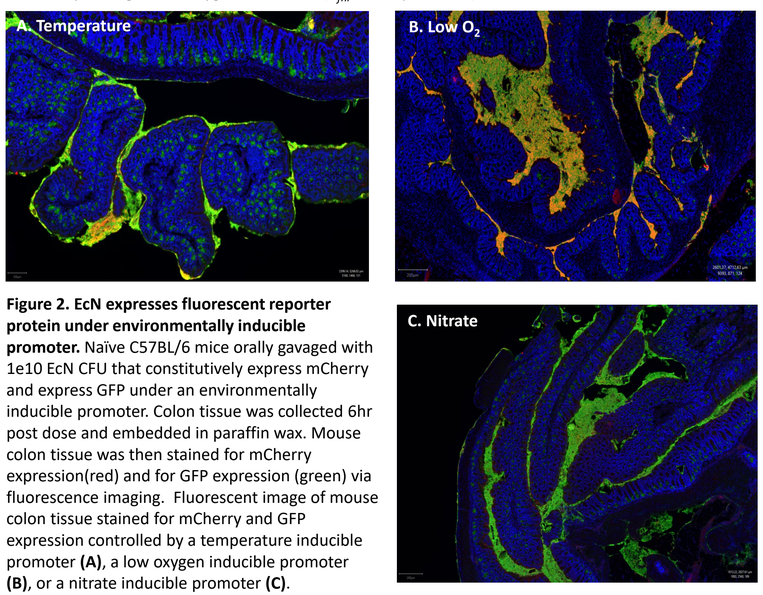

Figure 2. EcN expresses fluorescent reporter protein under environmentally inducible promoter. Naïve C57BL/6 mice orally gavaged with 1e10 EcN CFU that constitutively express mCherry and express GFP under an environmentally inducible promoter. Colon tissue was collected 6hr post dose and embedded in paraffin wax. Mouse colon tissue was then stained for mCherry expression(red) and for GFP expression (green) via fluorescence imaging. Fluorescent image of mouse colon tissue stained for mCherry and GFP expression controlled by a temperature inducible promoter **(A)**, a low oxygen inducible promoter **(B)**, or a nitrate inducible promoter **(C)**.

Engineering EcN to produce Short Chain Fatty Acids

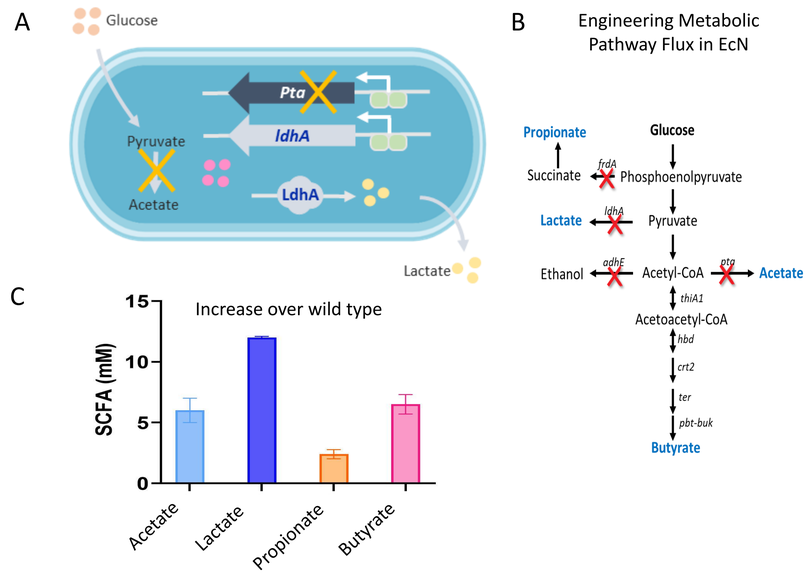

Figure 3. A. Schematic of EcN-Lac. The lactate producing strain was engineered by removing the *pta* gene from the acetate producing pathway and introducing a plasmid containing the *E. coli ldhA* gene under the control of an inducible promoter. **B.** Schematic of SFCA metabolic pathways that can be altered for SCFA production. Red Xs denote possible genes modified for a butyrate producing strain. **C.** In vitro measurement of excess SCFAs produced from engineered EcN strains compared to wild type EcN. Dark blue var denotes EcN-Lac.

EcN-Lac increases Tregs during anti-CD3-induced small intestine inflammation

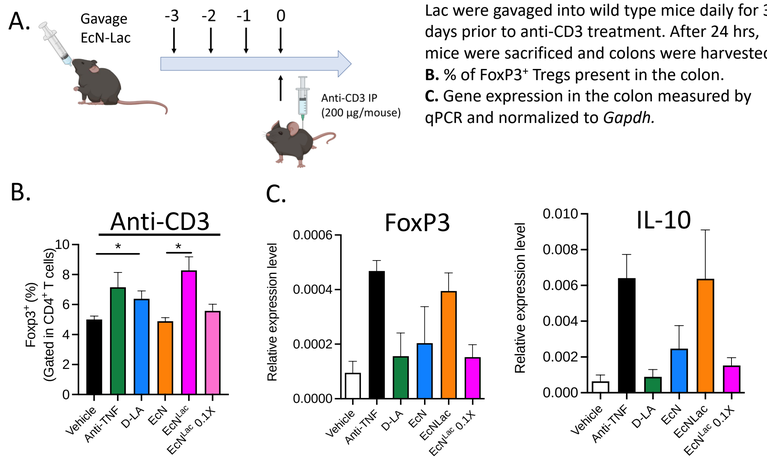

Figure 4. A. Mouse study design. 1e10 CFU EcN-Lac were gavaged into wild type mice daily for 3 days prior to anti-CD3 treatment. After 24 hrs, mice were sacrificed and colons were harvested. **B.** % of FoxP3+ Tregs present in the colon. **C.** Gene expression in the colon measured by qPCR and normalized to *Gapdh*.

Development and characterization of EcN-IL-22, an integrated hIL22 Secreting Strain

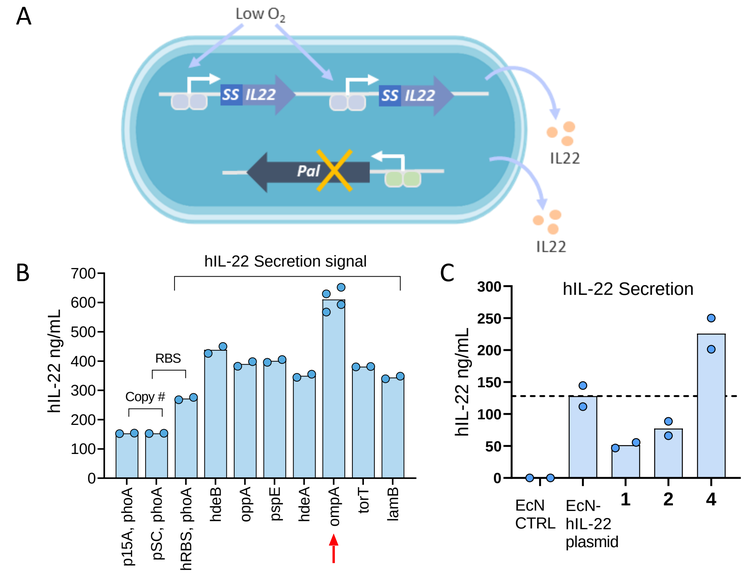

Figure 5. A. Schematic of EcN-IL-22. Multiple copies of IL-22 fused to a secretion signal (SS) are integrated into the EcN chromosome. Removal of the *pal* gene results in a Diffusible Outer Membrane (DOM) phenotype for enhanced secretion from EcN. **B.** ELISA assay comparing the affect of plasmid copy number, ribosome-binding site (RBS) and signaling peptide on hIL-22 secretion normalized to 1e8 CFU/5hrs. hRBS, high affinity RBS **C.** IL-22 production from integrated strains containing 1, 2, or 4 copies of integrated hIL-22 with the ompA signaling peptide. The 4-copy integrant is designated EcN-IL-22.

hIL22 secreted by EcN induces IL22 dependent gene expression in vivo

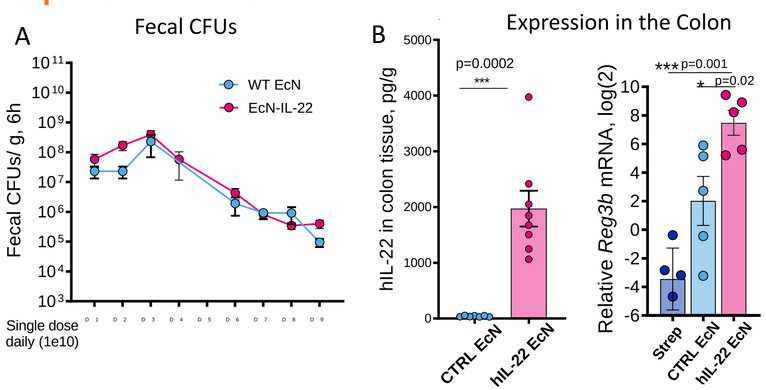

Figure 6. A. EcN-IL-22 is viable and biologically active *in vivo.* EcN-IL-22 numbers decrease with time in naïve mice with Strep in drinking water. **B.** At day 4, high levels of secreted hIL-22 are detected in the colon by ELISA. In colon tissue, *in vivo* target engagement of bacterially-secreted IL-22 is detected as upregulation of the IL-22-dependent biomarker, Reg3b.