Introduction
Cardiovascular disease is the leading cause of death in the United States. The disease is highly associated with atherosclerosis, the hardening of plaque in one’s arteries. The way the human gut microbiota process L-carnitine, a compound found in high levels in red meat, leads to the formation of the proatherogenic substance Trimethylamine N-oxide.

Goal
The University of Illinois Team has made it our goal to create a probiotic to outcompete the gut bacteria for L-carnitine, thus preventing TMAO formation in the gut and reducing one’s risk for cardiovascular disease.

Materials & Methods
We engineered *Escherichia coli* strain Nissle 1917 to uptake and breakdown L-carnitine. All genes came from *Pseudomonas aeruginosa*. Nissle has been tested and used for over 80 years as a safe probiotic.

**Breakdown**
- **Carnitine Dehydrogenase** (BBa_K1205000)
  - Breaks down L-carnitine into 3-Dehydrocarnitine

**Uptake**
- CbcVW (BBa_K1205002)
  - Integrated transporter
- CaiX (BBa_K1205001)
  - Helps direct L-carnitine to transporter

Conclusion
Based on our results, we can infer the successful function of all enzymes and transporters cloned into our engineered Nissle 1917. Through the successful expression in Nissle 1917 of the CBCwv transporter complex, the CaiX L-carnitine binding protein, and the Carnitine Dehydrogenase (CDH), we found that extracellular L-carnitine uptake increased drastically when compared to wild type Nissle 1917. In the intestinal environment, this successful take up and breakdown of L-carnitine will avert extracellular L-carnitine from being used as a substrate for TMA production and ultimately decrease the risk of atherosclerosis in patients.

Verification in Nissle 1917
- **Bba_k1205000**, which encodes for Carnitine Dehydrogenase, showed activity during spectrophotometric assays. The protein was validated by measuring NAD+ reduction, a result of L-carnitine degradation.

Probiotic Delivery
- The engineered Nissle 1917 was grown in alginate beads and then inserted into pill capsules. To ensure delivery to the small intestine, our target area, the pills were given an enteric coating. Experiments were run to show pill breakdown in different pH.

Safety
- Our team developed a method for detecting EtBr contamination. By adding food dye to your EtBr solution, EtBr is now visible. This dye has no effect on the gel picture.