

Team 99 Bottles Tech Spec

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Project Goal



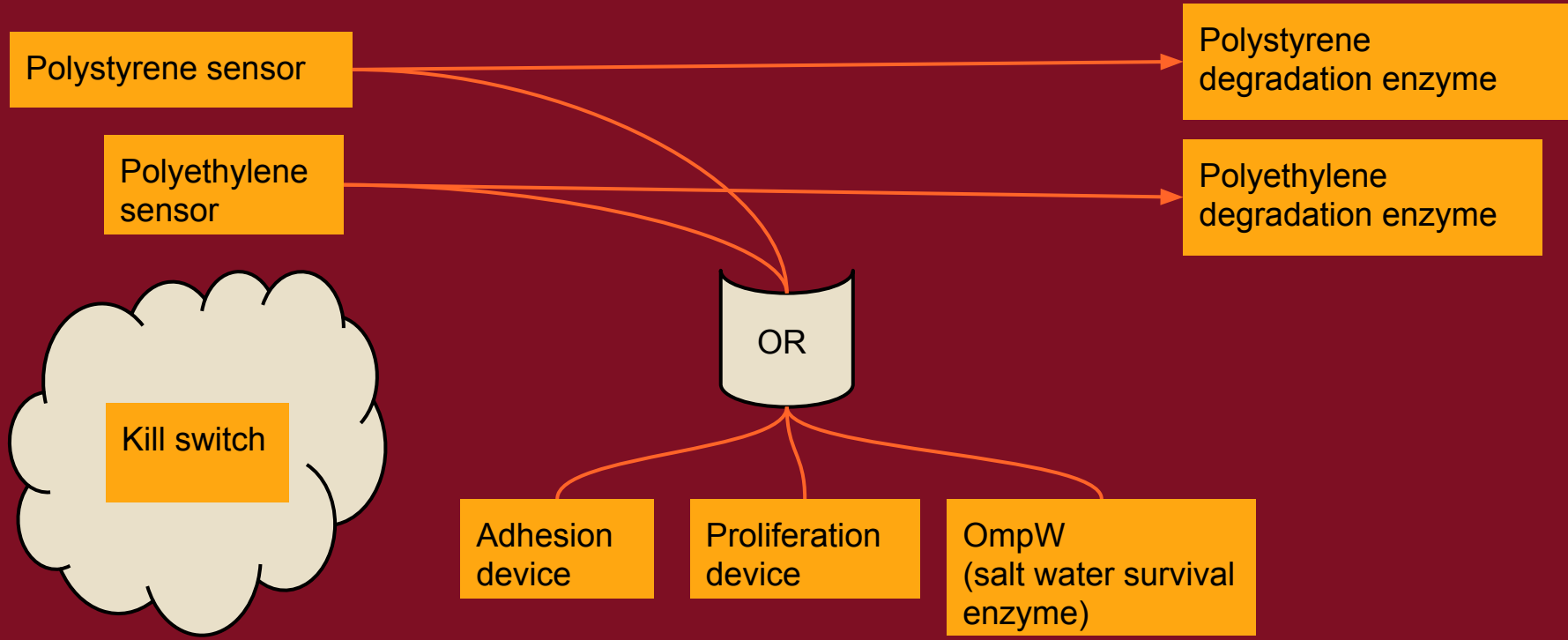
Develop an engineered system in *E. coli* to degrade existing plastics in oceans.

Impact

- Clean areas polluted with plastic, especially beaches and plastic islands
- Preserve marine ecosystems



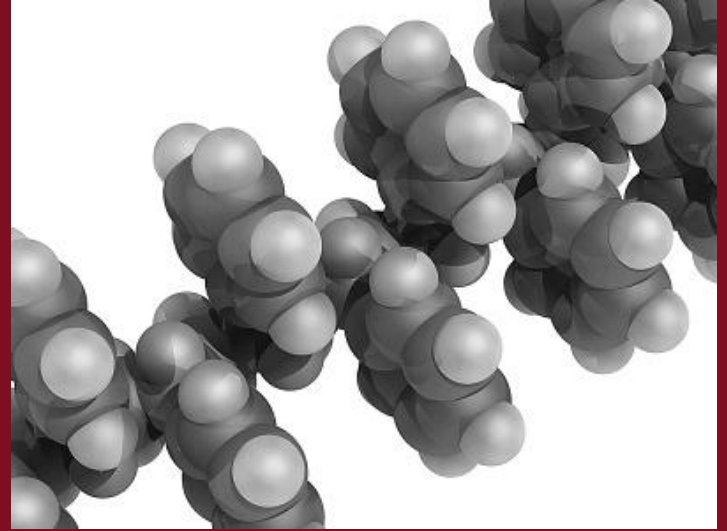
Device-Level Diagram



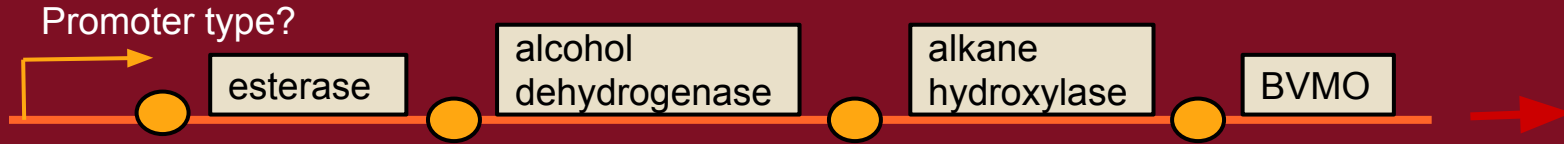
Parts: Plastic Sensor

Problem: Most plastics are too big to enter an E. coli cell

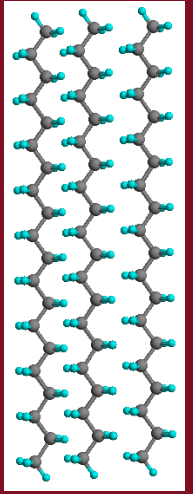
Solution: Secrete low levels of degradation enzymes. When the end-products of degradation are detected, secrete a lot more enzymes



Parts: Polyethylene Decomposer

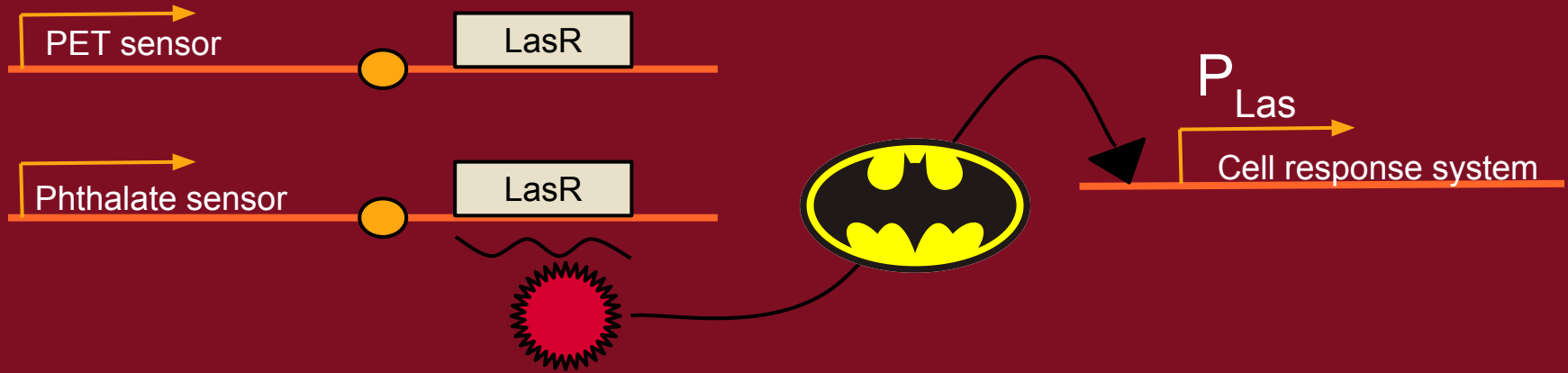


polyethylene



- Activated when it receives a signal from the plastic sensor
- All enzymes needed to decompose a specific plastic will be encoded on one polycistronic DNA sequence.

Parts: OR gate



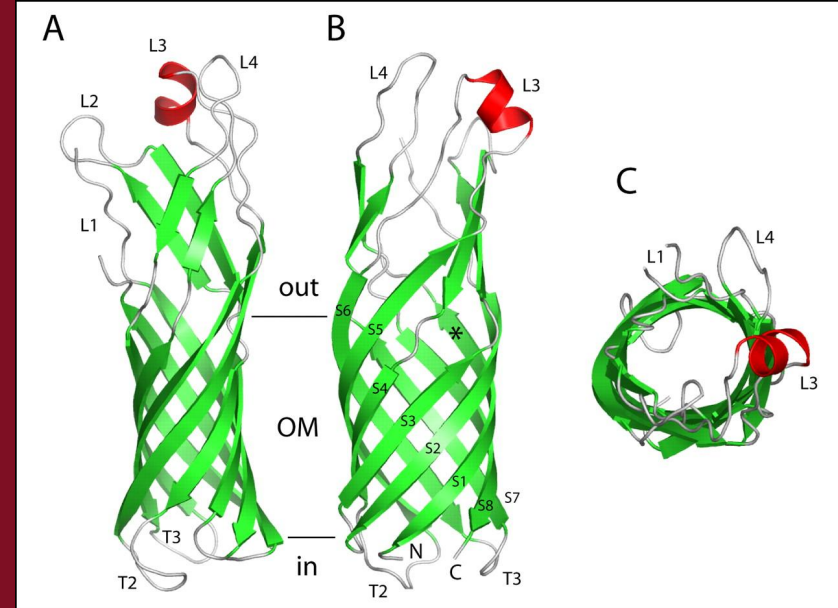
All plastic sensors will encode the activator LasR, which stimulates the P_{Las} promoter.

Parts: Proliferation and Adhesion

- Enhance natural adhesion properties of E. coli
 - surface-activated platelets
 - Haven't yet been tested in E. coli
 - upregulate expression of hydrophobic proteins on membrane
- Still searching for enzyme to promote proliferation

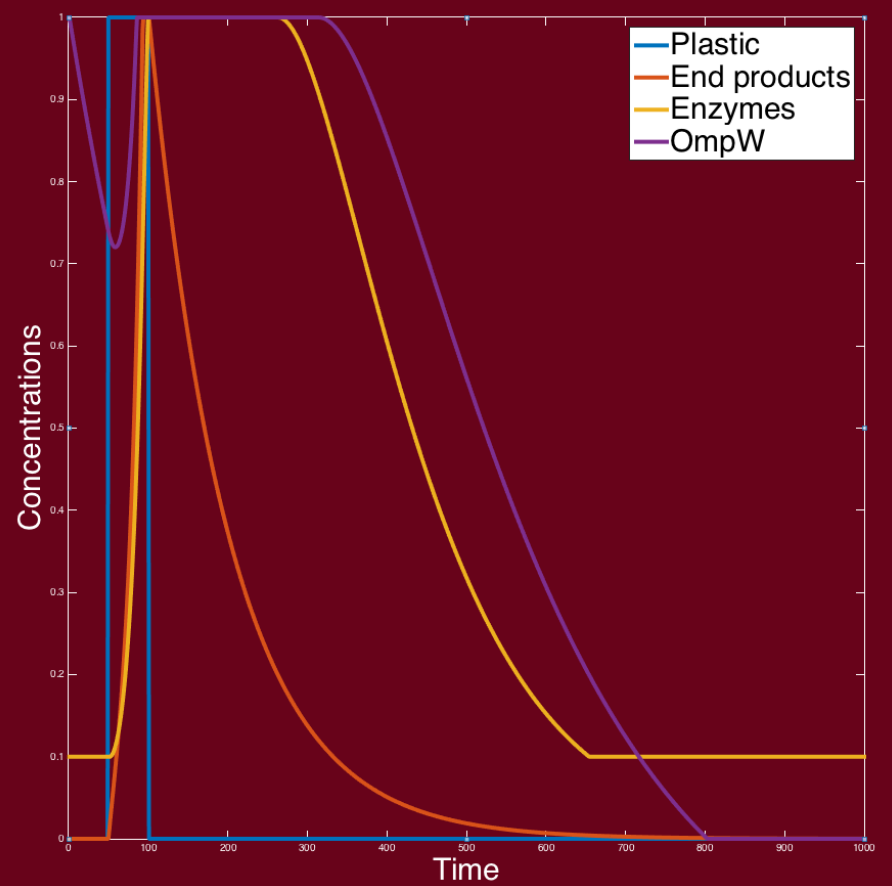
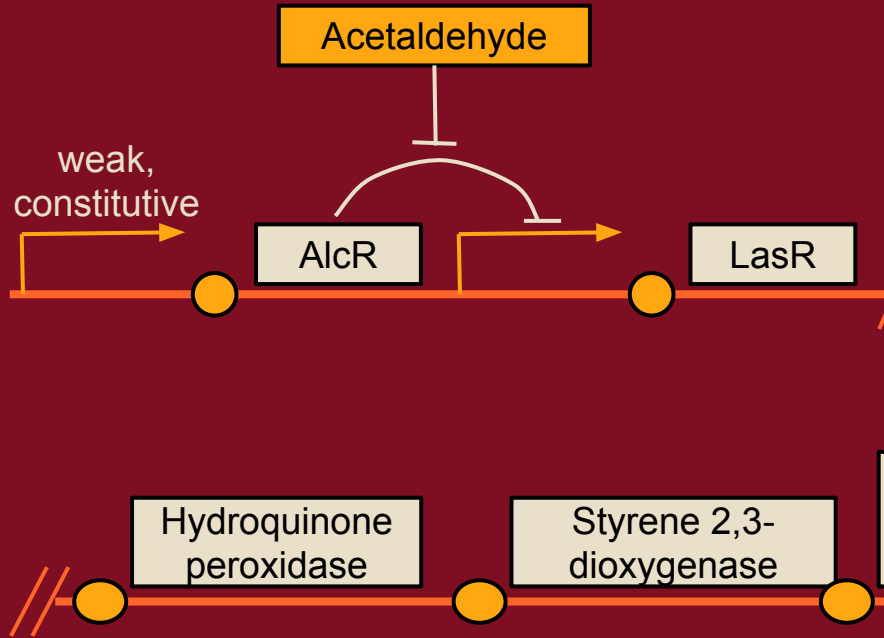
Parts: Slow death

- OmpW allows *E. Coli* to survive in salty seawater
 - Only produce OmpW when plastics are detected
- Without plastics, the cells quickly sink to Davy Jones' Locker



OmpW- a salt pump

Assembly and Timing Diagram



Parts: Kill Switch

Silencing ribosomal RNA (rRNA)

- Used in making proteins and therefore essential to E. coli survival
- rRNA is species-specific, so other organisms will not be harmed

Method: RNA interference (RNAi)/antisense RNA

- Added antisense RNA will bind to rRNA and prevent it from working

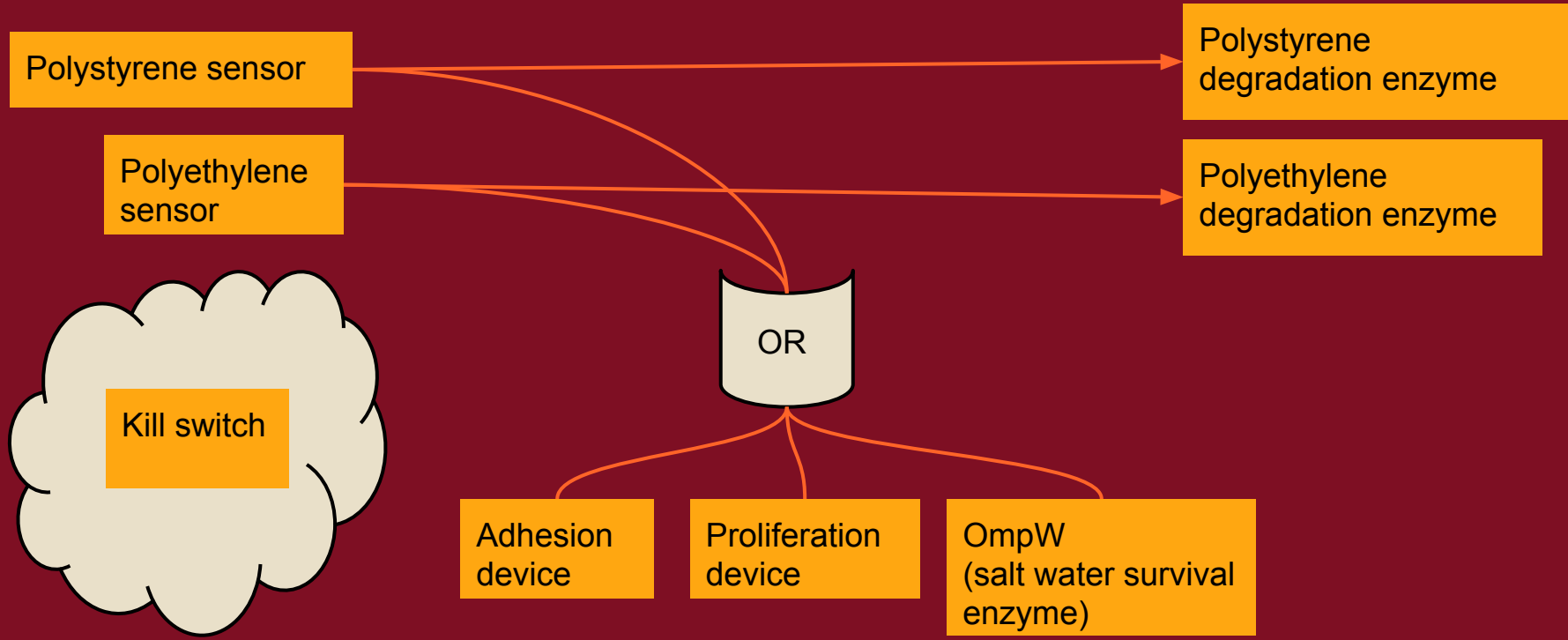
Testing and Debugging

- Test effectiveness and safety of decomposition enzymes
- Select for cells that have received our plasmid with an antibiotic
- Use fluorescent reporter genes such as GFP, YFP, mCherry, etc. to ensure that our promoters work when exposed to their respective signal

Concerns

- E. coli may not detect plastic quickly enough to survive
- System may be too complex
- Decomposition enzymes may have unforeseen effects on E. coli
- Biofilms may be hard to get rid of
- RNAi for the kill switch may be expensive or difficult to produce and apply

Summary



Decision

GO!!!