Regulated gene expression is an essential part of the synthetic biologist's toolkit. Promoters can respond to specific inputs (e.g., Lac operator expression in the presence of IPTG, via the Lac repressor) or general inputs (induction of stress responses systems via alternative sigma factors that sense changes in global cell state). These sigma factors activate transcription by binding to nucleotide signatures at the -10 and -35 boxes of their cognate promoters. We set out to design, construct, and validate a BioBrick library of IPTG-inducible alternative-σ promoters for Ecoli. We determined the consensus of alternative-σ promoters for Ecoli, with varying degrees of similarity to the consensus.

### Methods

**Designing a LacO + σ hybrid promoter**

We obtained the consensus of alternative-σ promoters for Ecoli and inserted the appropriate -10 and -35 boxes. We then cloned these promoters upstream of a YFP expression construct (BBa_E0430), and measured expression changes when IPTG, stress, or both were applied. We classified responses to these two inputs into multiplicative, AND, and OR types. We found that the sigma-24 promoters respond to both IPTG and heat-shock, but are induced at a much greater level when both stimuli are presented (AND). In contrast, the sigma-28 promoter responds to both IPTG and starvation, but expression). We then cloned these promoters upstream of a YFP expression construct (BBa_E0430), and measured expression changes when IPTG, stress, or both were applied. We classified responses to these two inputs into multiplicative, AND, and OR types. We found that the sigma-24 promoters respond to both IPTG and heat-shock, but are induced at a much greater level when both stimuli are presented (AND). In contrast, the sigma-28 promoter responds to both IPTG and starvation, but expression)

**Measuring responses to IPTG**

We quantify the response by averaging fluorescence per cell value over three timepoints after applying stress.

**Possible types of responses to IPTG + stress**

1. IPTG and starvation were applied by direct synthesis, and ligated into a YFP expression construct.

**Promoter synthesis and YFP expression constructs**

17 hybrid promoters were obtained by direct synthesis, and ligated into a YFP expression construct.

**Growth, stress, and measurement protocols**

- Growth: exponential phase
- Stress: heat shock, stationary phase
- Measurement protocols
  - YFP / cell
  - Cell density: 514/527 nm
  - °C (glu) pH

**StressKit: where can we use these new parts?**

- Use general physical cue to regulate gene expression: a heat shock sensor in BR-compatible expression vector
- Turn on StressWatch sensors to keep track of all the stresses your precious bacterial colony has been through!