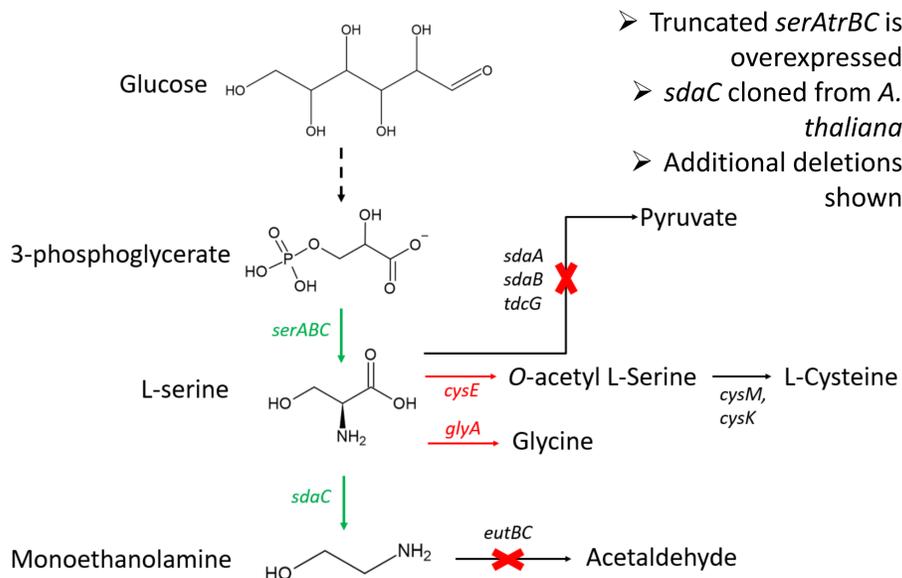




Bioproduction of Monoethanolamine

- Monoethanolamine (MEA) can achieve post-combustion capture of carbon dioxide via gas scrubbing, but current production methods are derived from fossil fuels.
- **Bioproduction** of MEA by *E. coli* via serine decarboxylase (*sdc*) from the precursor L-serine is achievable but has limitations. Serine diverts to the production of amino acids glycine and cysteine via serine hydroxymethyltransferase (*glyA*) and serine acetyltransferase (*cysE*).

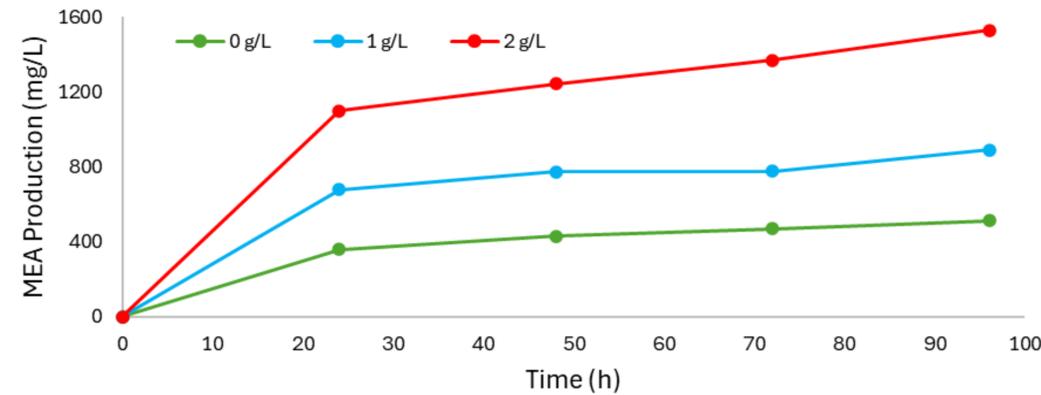
MEA Metabolism from Glucose



CRISPR Interference of *glyA* and *cysE*

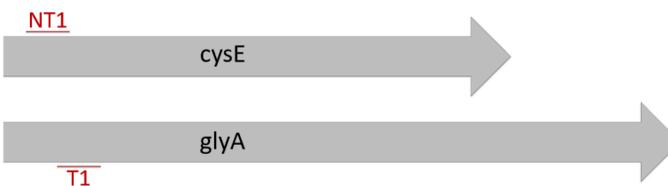
- Gene knockout eliminates the competing pathways, but forces an auxotrophy for glycine and cysteine
 - Modulating repression using CRISPRi/Cas12a is an alternative that could prevent this

MEA Production w/ Serine Supplementation



- *E. coli* MG1655 K-12 $\Delta sdaA$, $\Delta sdaB$, $\Delta tdcG$, $\Delta eutBC$ with pSerAtrBC/pSdaC and supplemented with 0,1, and 2 g/L serine
- Increasing MEA production suggests limitation by pre-cursor availability

CRISPRi Plasmid Design

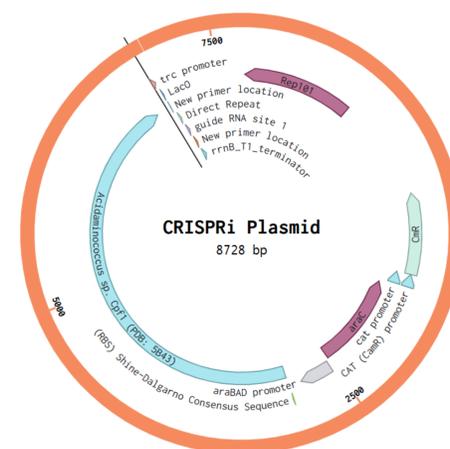


Gene	Location	Efficiency	Target Strand
<i>cysE</i>	143 bp	71	Lagging
<i>glyA</i>	311 bp	76	Leading

- Chop-chop tool used to calculate off-target binding/efficiency
- Gold Gate Assembly used to assemble array and Cas12a backbone (pBbs8c-ddcpf1)

Guide RNA (gRNA) primer selection:

- Length: 23 bp
- PAM Sequence: TTTV



Results

- *cysE* CRISPRi plasmid successfully cloned and transformed into Serine/MEA producing strain

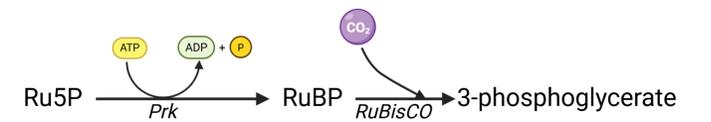
Future Directions

1) Improving MEA Production

- High-performance Liquid Chromatography (HPLC) to analyze MEA production
- Identify new CRISPRi gRNA with varying efficiency to modulate repression

2) Improving L-Serine Availability

- Integrating *serAtrBC* in *E. coli* MG1655 K-12 $\Delta sdaA$, $\Delta sdaB$, $\Delta tdcG$, $\Delta eutBC$
- Introduce *prk*-RuBisCO shunt to increase 3-phosphoglycerate



Created in <https://BioRender.com>

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