



Chimeragenesis of plant MBOATs to define substrate selectivity

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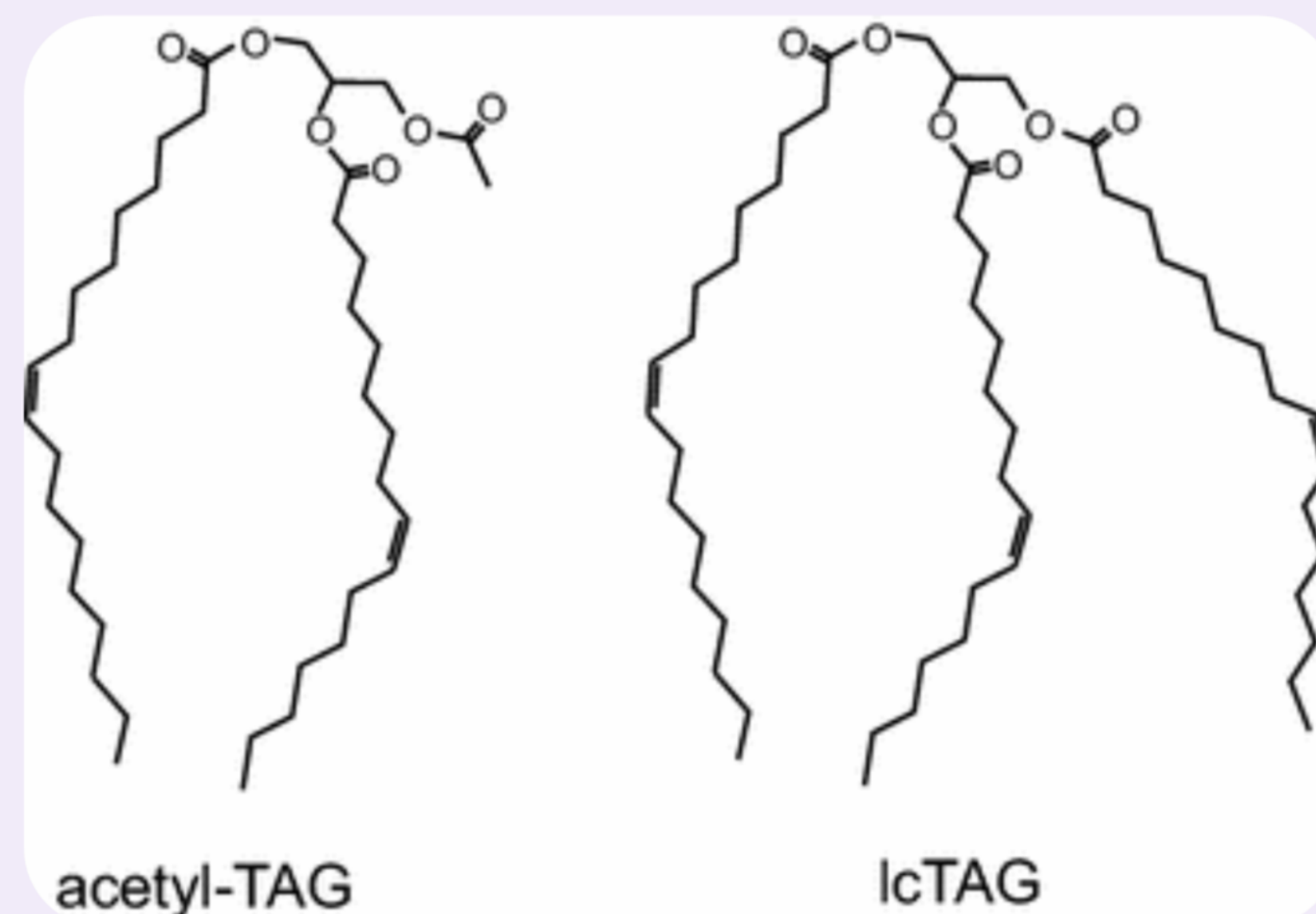
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Introduction

- TAG molecules (lcTAG) are a type of lipid where three fatty acids are attached to a glycerol molecule
- MBOATs are membrane bound enzymes that transfer an acyl group/fatty acid onto a molecule
- **SaDGAT** catalyzes the synthesis of lcTAG by transferring a third fatty acid to the *sn*-3 position
- **EfDAcT** catalyzes the synthesis of acetyl-TAG by transferring an acetyl group to the *sn*-3 position
- 64 chimeras were made of SaDGATs and EfDAcTs amino acid sequences by exchanging chunks of amino acids between the two enzymes. Of these chimeras, 17 showed a general change in TAG production from a previous experiment



Objective

To determine whether creating chimeras of SaDGAT and EfDAcT has an influence on the selectivity of these 17 novel enzymes by quantitating the amount and type of TAGs produced

Methods



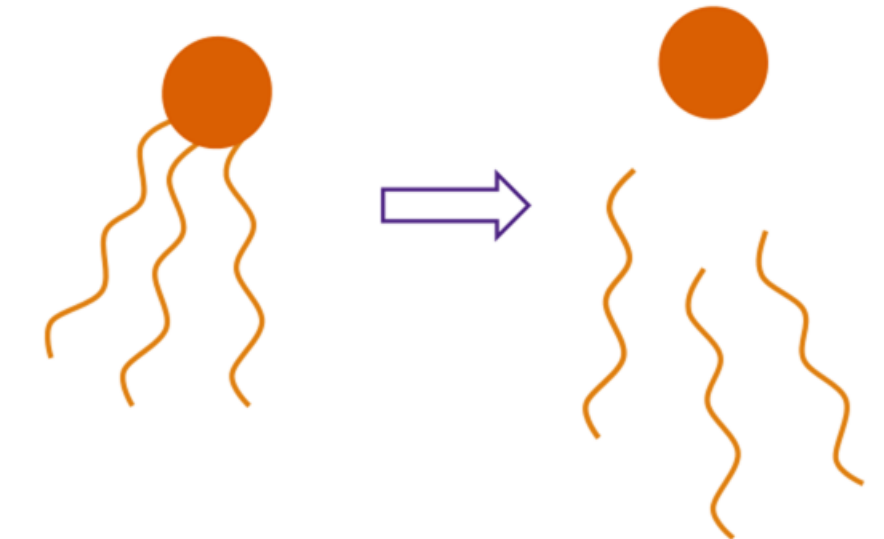
1 Culture the 17 chimera strains onto yeast plates

2 Create liquid cultures in SC-URA medium and incubate for 2 days

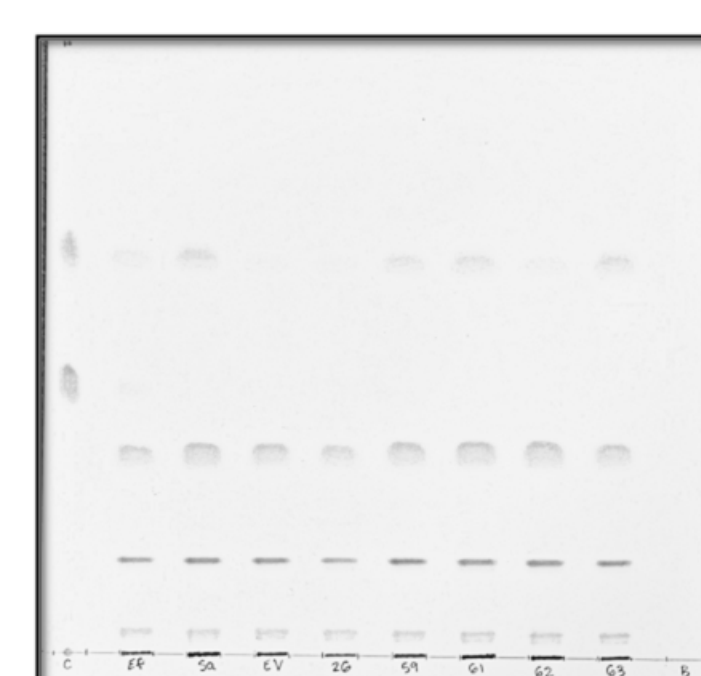
3 Measure the OD and perform a lipid extraction



6 Quantitation with Gas Chromatography



5 Convert lipids into FAMES to prep for Gas-Chromatography



4 Run extract on a TLC plate to separate lcTAG and acetyl-TAG

Results

Figure 1
TLC Plate

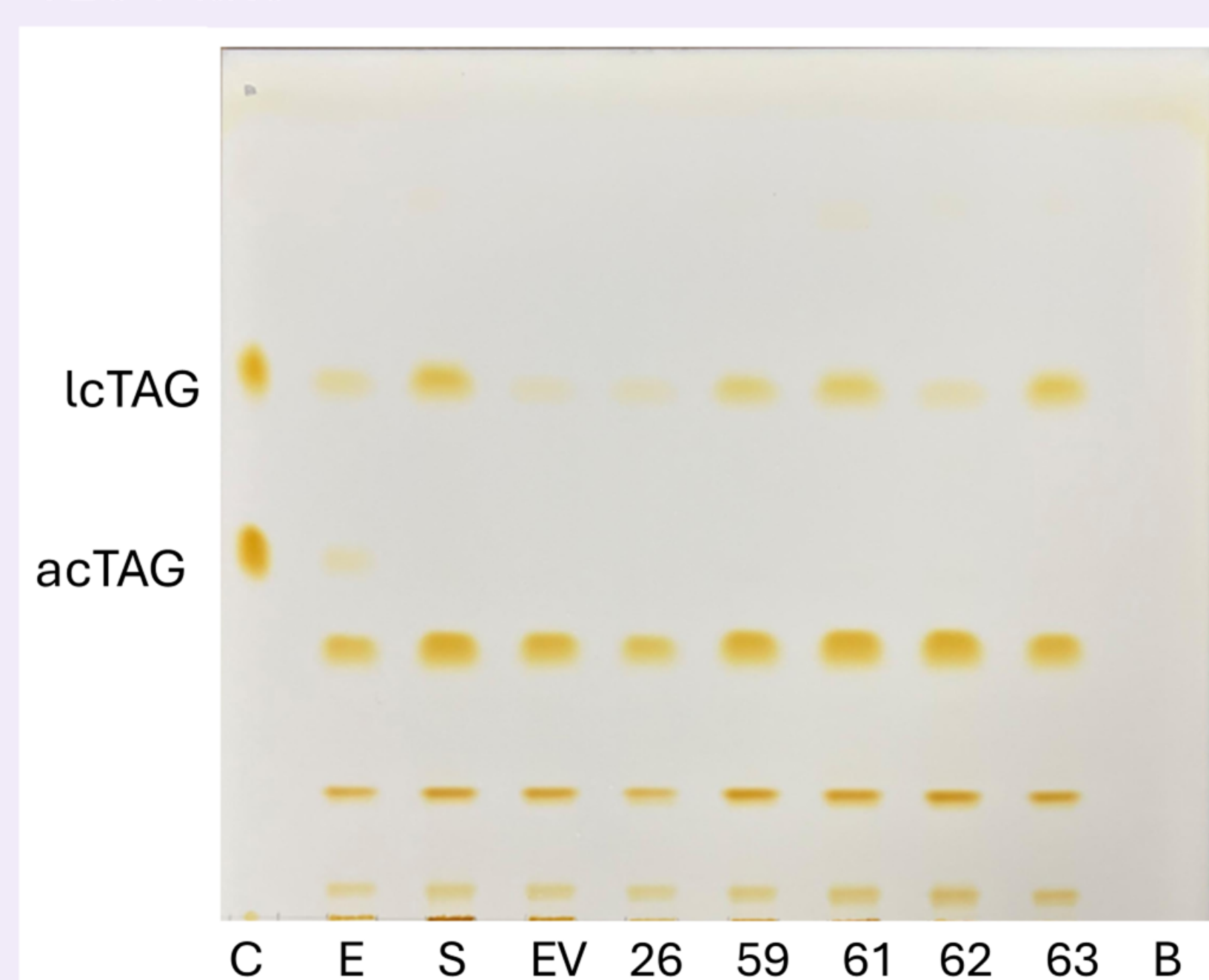
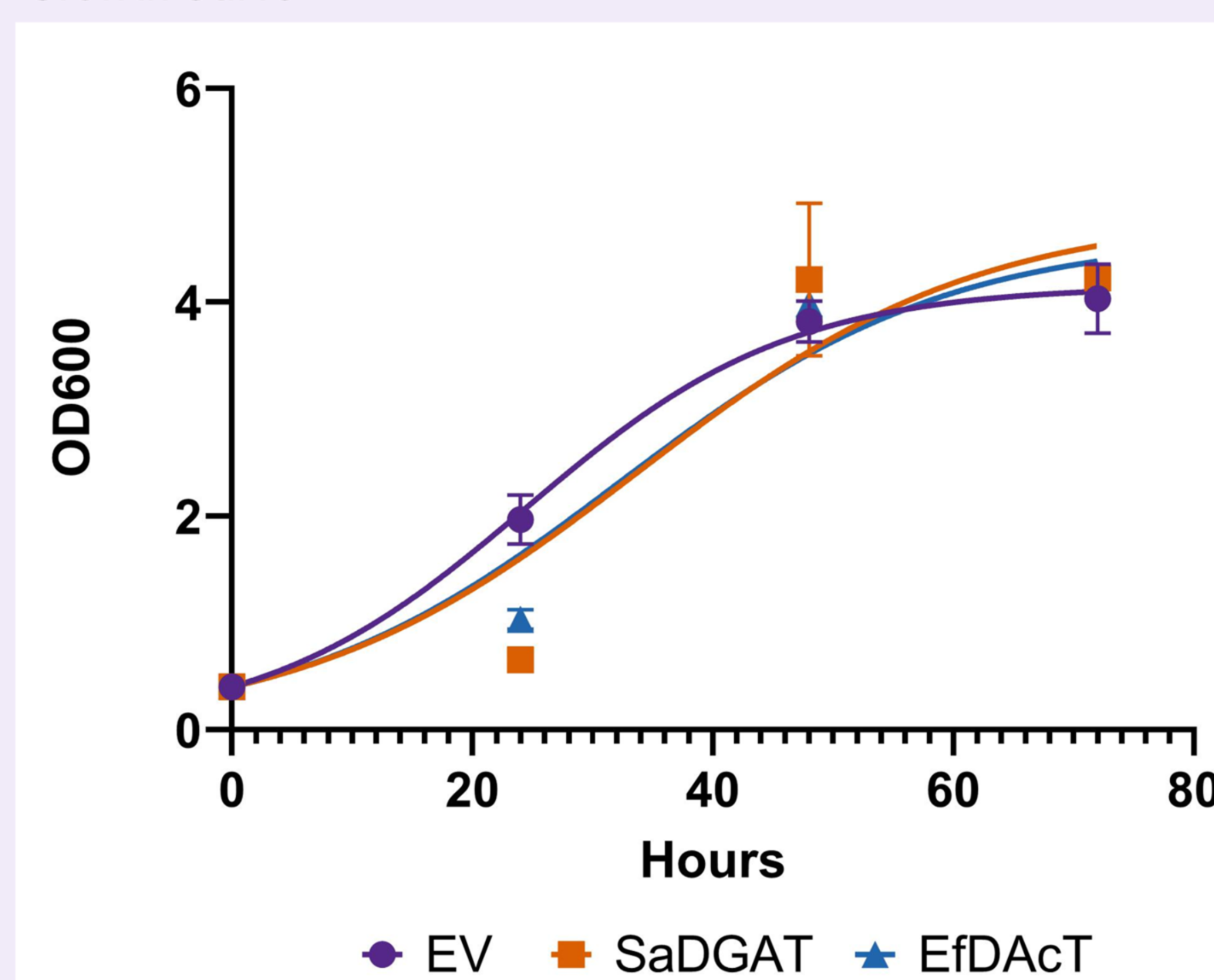


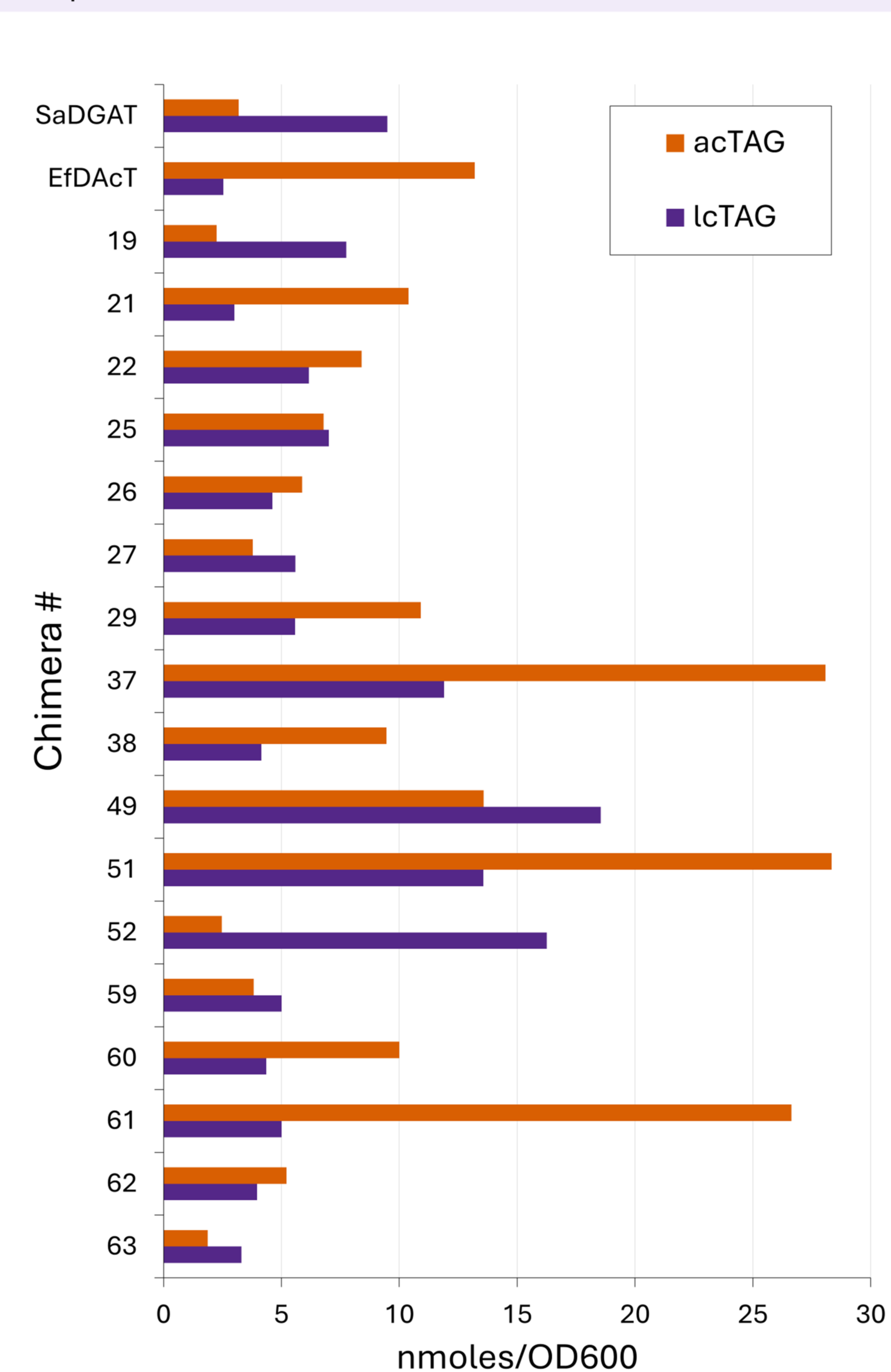
Figure 2
Growth Curve



Cultures of EfDAcT, SaDGAT, and the EV (empty vector) were incubated in a 30°C shaker. The absorbance (OD600) was measured with a UV-Vis Spectrophotometer in 24-hour intervals over the span of 3 days

Figure 3

Comparison of TAG Production Between Chimeras

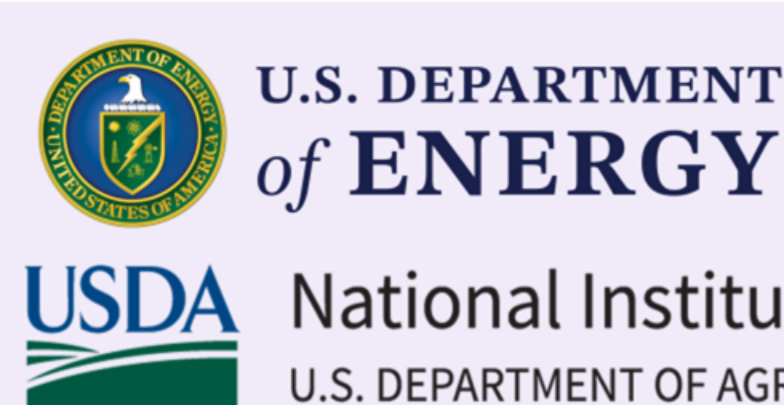


Bar graph comparing the lcTAG and acTAG production of EfDAcT control, SaDGAT control, and 17 chimeras

Conclusion

- The type and amount of lipids produced varied between chimeras, showing that the changes in the amino acid sequences influenced lipid production
- **Drawbacks:** There were high levels of background using TLC, so we are exploring using mass spectrometry to measure the lipids directly
- **Future Direction:** This data will be put into a machine learning model that will help us determine what parts of the amino acid sequence affect TAG production

Acknowledgments



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