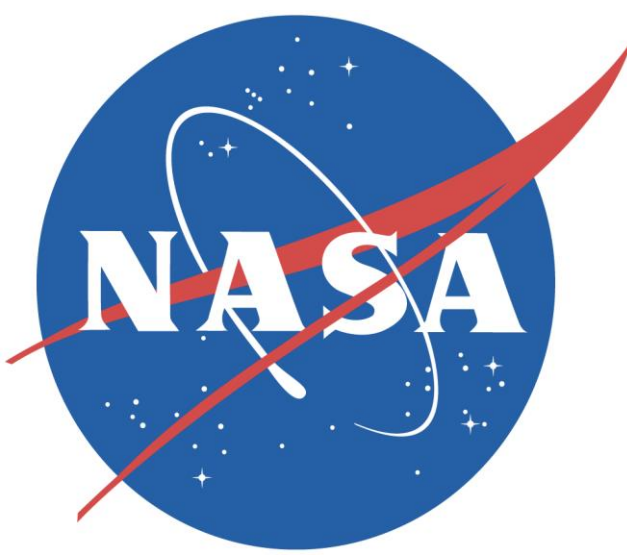
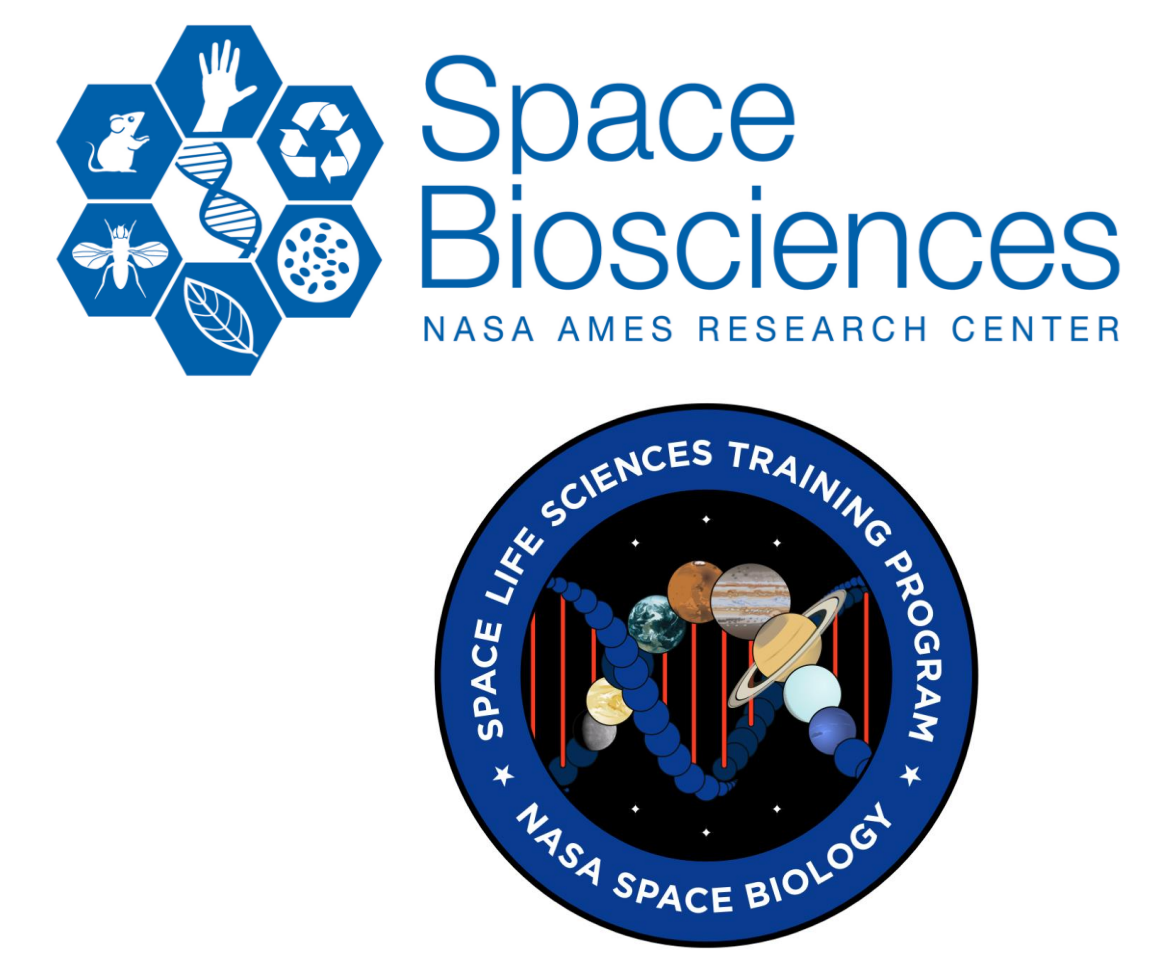




Precision Fermentation: Relieving the Crabtree Effect in *S. cerevisiae* Through Genetic Engineering



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Abstract

Nutrient degradation in the current NASA pre-packaged food system poses significant challenges to crew health in missions beyond low earth orbit as certain vitamins and nutrients have been shown to degrade during extended storage, and resupply and fresh food items cannot be readily provided. BioNutrients (BN) uses synthetic biology in an effort to supplement the NASA food system by delivering *in situ* production of nutrients and therapeutics produced by genetically engineered microbes. One yeast *S. cerevisiae* strain utilized in BN is genetically modified to produce two types of antioxidants: zeaxanthin and β -carotene. Zeaxanthin is a non-provitamin A carotenoid and has been implicated in eye health and vision performance, and β -carotene is a provitamin A carotenoid. Microbial production of high-value nutrients can aid our understanding and address the issue of nutrient degradation and loss in the NASA food system.

Although *S. cerevisiae* is an excellent candidate for a long shelf life, on-demand nutrient production system, its space applications are limited by the Crabtree effect where respiration is shut down in favor of fermentative metabolism when glucose is present in high concentrations, causing the release of unwanted ethanol. Current and likely future life support systems are negatively impacted by ethanol and ethanol release is strictly limited.

To improve feasibility of using *S. cerevisiae*, we explored ways to reduce the Crabtree effect, thereby limiting its ethanol production. Specifically, we used CRISPR-cas9 to genetically modify various genes in this pathway.



Fig 1: BioNutrients-1 Gen-0 bioreactor system, photo credit NASA

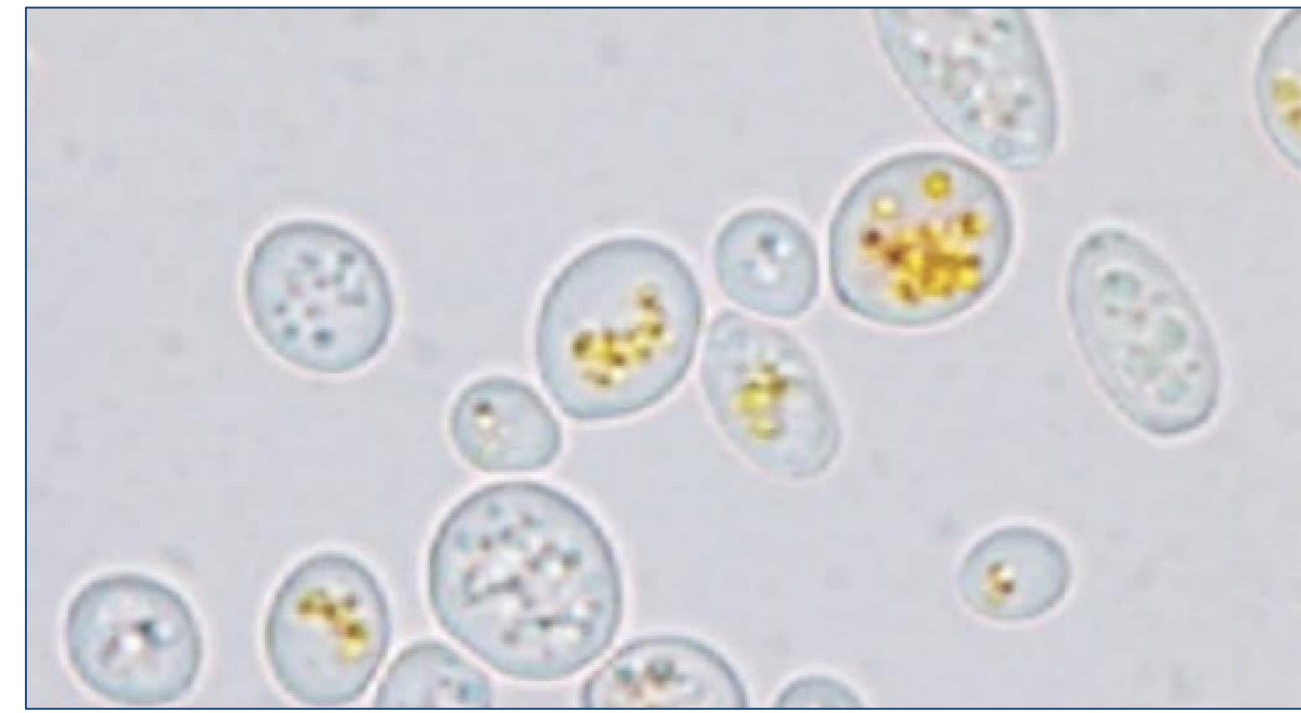


Fig 2: BioNutrients-1 *S. cerevisiae* strain producing β -carotene. Image taken with 100x objective.

Introduction

- Nutrient degradation in the current NASA pre-packaged food system poses significant challenges to crew health in missions beyond low earth orbit [1]
- BN proposed to use synthetic biology to supplement the NASA food system by delivering *in situ* production of nutrients via genetically engineered microbes [2]
- S. cerevisiae* is utilized in BN to produce carotenoids: zeaxanthin and β -carotene [2]
- S. cerevisiae*'s space applications are limited by the Crabtree effect, in which respiration is shut down in favor of fermentative metabolism when glucose is present in high concentrations, causing the release of ethanol, both reducing the conversion of glucose into the desired product, and negatively impacting the water recovery system of the ISS [3]
- Crabtree effect reduces *S. cerevisiae*'s productivity and restrains volume and timing of yeast fermentation [4]

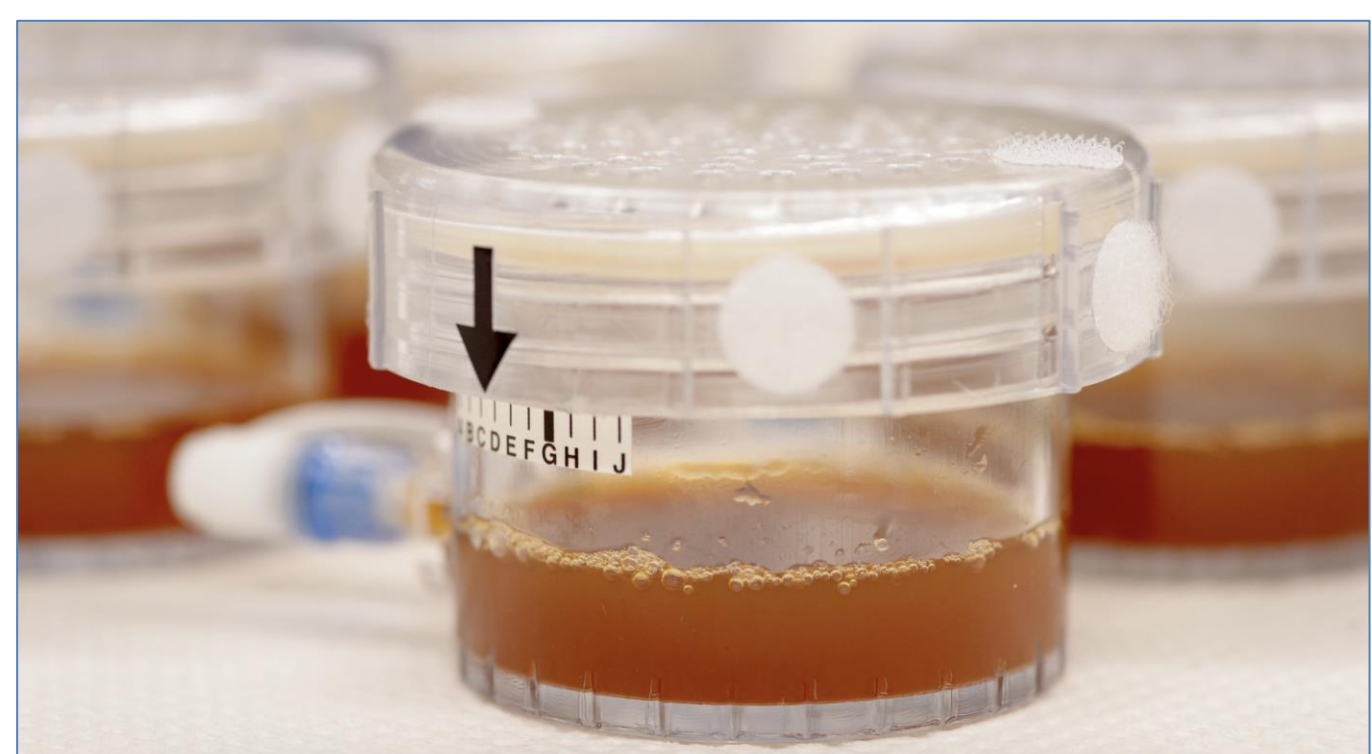


Fig 3: BioNutrients-1 Ground Control Experiment, photo credit NASA



Fig 4: Astronauts on the International Space Station, photo credit NASA

Objectives

- To improve feasibility of using *S. cerevisiae* as a biomanufacturing organism by reducing the Crabtree effect, thereby limiting its ethanol production and increasing carotenoid production

- #1: Enhance carotenoid production
- #2: Reduce ethanol production

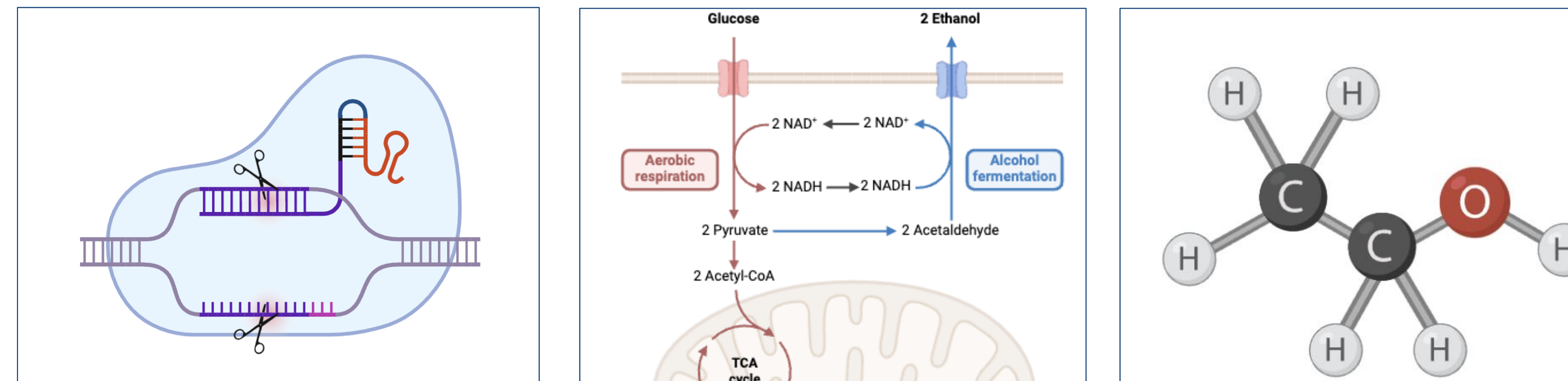


Fig 5: Project Objectives and Workflow, created with BioRender.com

Materials and Methods

- Identified gene targets: glucose sensors *RGT2* & *SNF3*
- Made custom guide RNA using site-directed mutagenesis
- Made custom repair DNA using PCR
- Transformed CRISPR/Cas 9 components into carotenoid-producing and Wildtype (WT) *S. cerevisiae*

Gene	RGT2	SNF3
Function	Glucose sensors	
Mutation	Double deletion	
Effect	- Reduced fermentation rate - Slowed consumption of glucose - Reduced ethanol production - Increased biomass (which may result in greater bioproduction)	

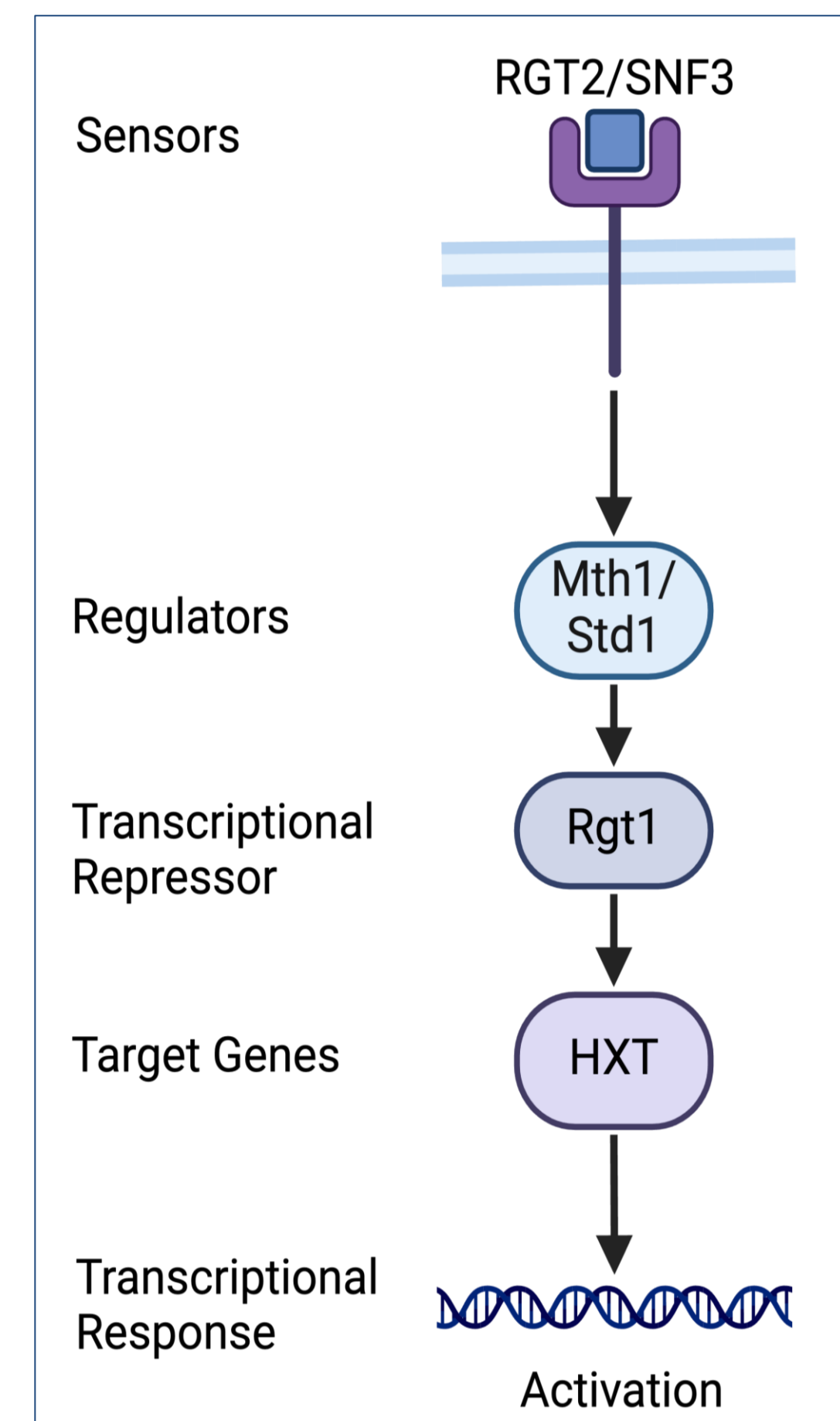


Fig 6: *RGT2/SNF3* Glucose Sensing Pathway, created with BioRender.com

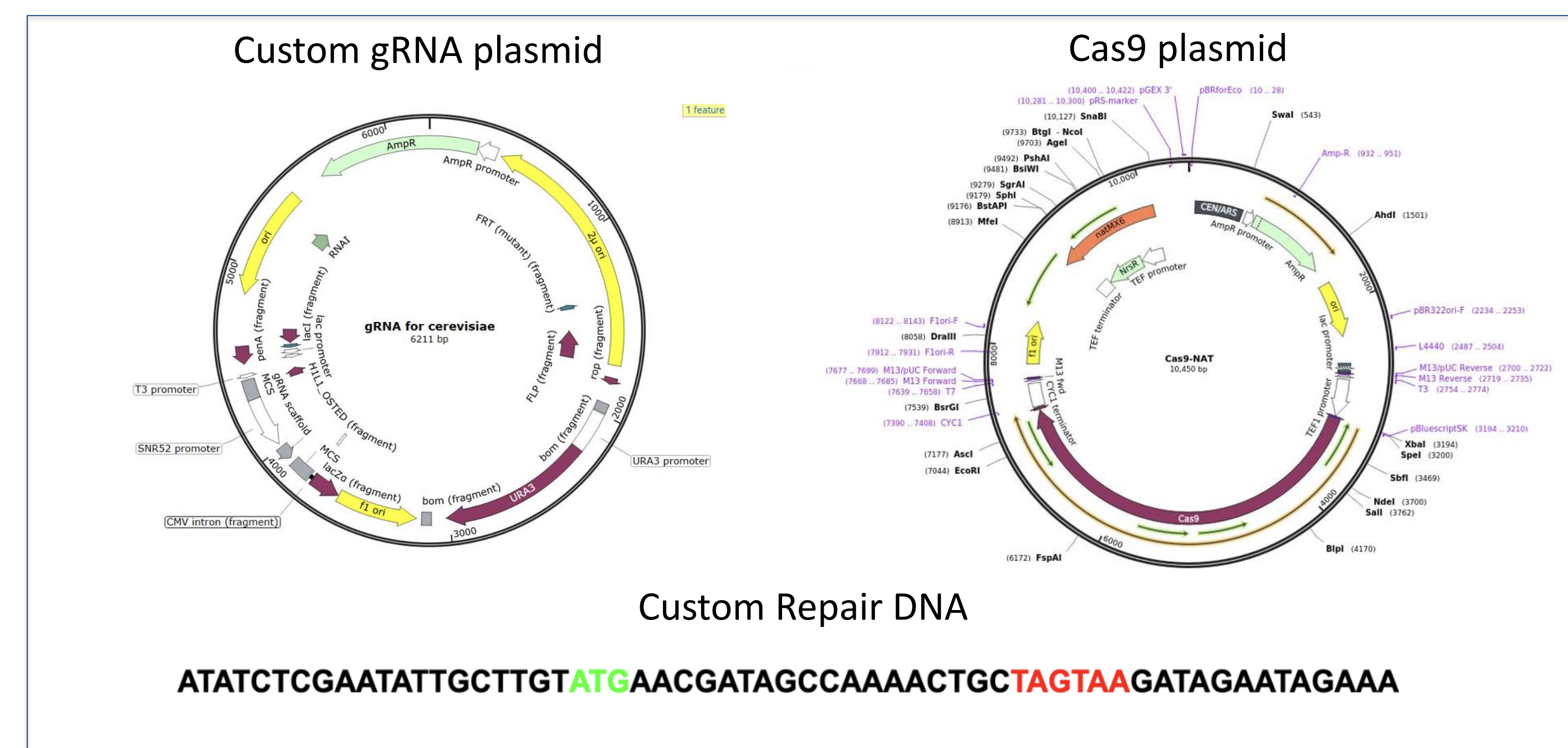


Fig 7: CRISPR/Cas9 Components for Transformation, created with BioRender.com

Results

- Successfully knocked out *SNF3* and *RGT2* in both WT and carotenoid-producing strains
- Confirmed via sequencing

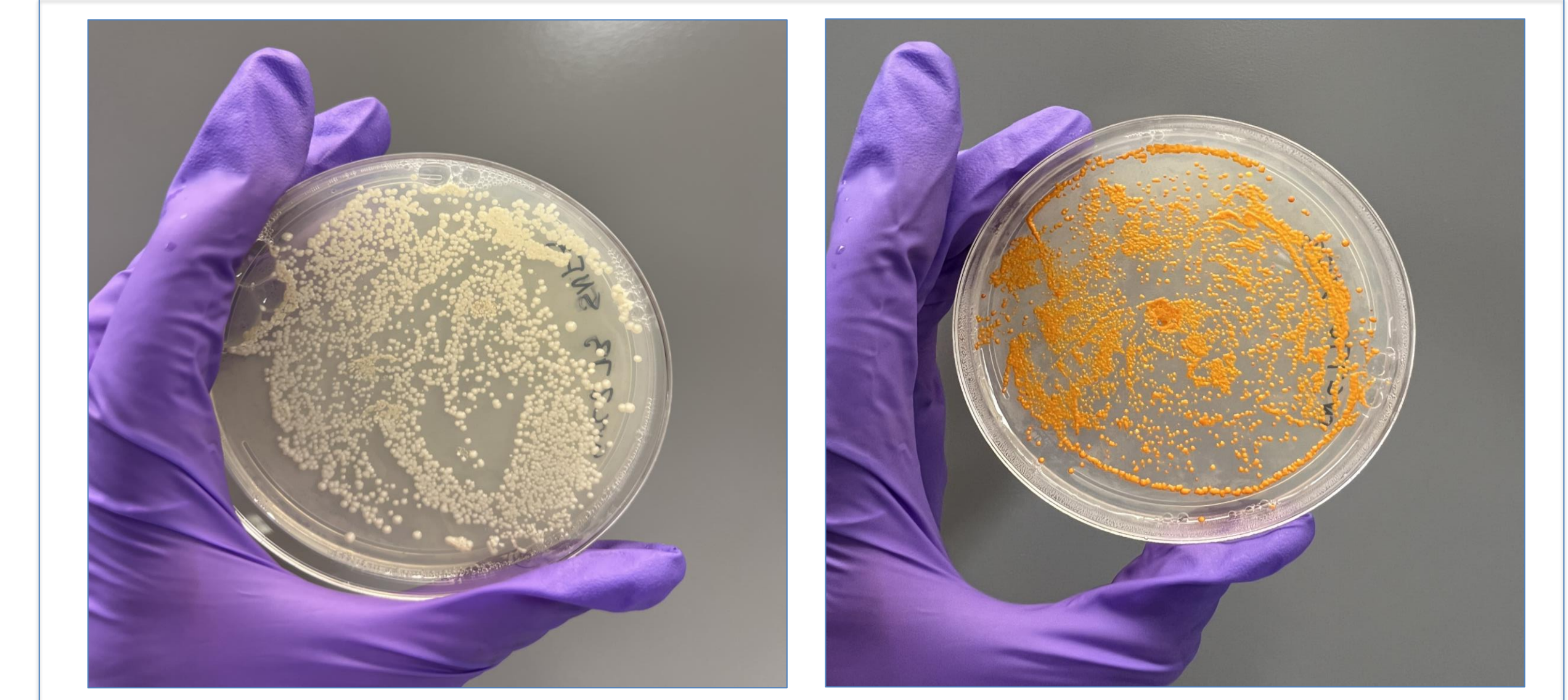


Fig 8: *S. cerevisiae* Host Strain (left) and Engineered β -carotene-Producing Strain (right)

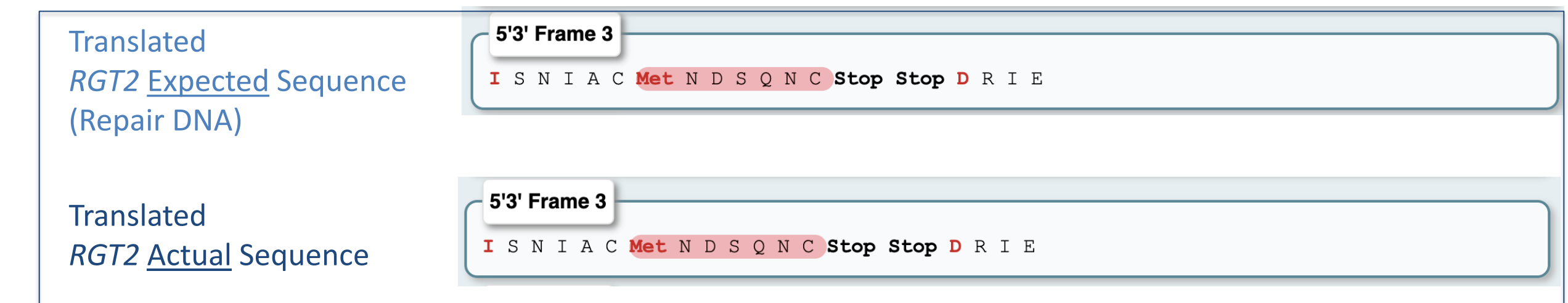


Fig 9: Sequencing Results to Confirm *RGT2* Deletion

Discussion

- Successful construction of *RGT2* & *SNF3* double deletion strains
- If mutated strains exhibit desired phenotypic effects, this would increase *S. cerevisiae*'s feasibility in space, resulting in improved protection of life support systems and more robust biomanufacturing capabilities

Future Directions

- Analyze ethanol production using High Performance Liquid Chromatography (HPLC) of WT vs engineered strains to confirm reduced ethanol production
- Analyze growth and carotenoid production by testing growth kinetics (plate reads and biomass) in WT vs engineered strains to confirm increased carotenoid production

References & Acknowledgements

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NASA Space Biosciences SLSTP: <https://www.nasa.gov/ames/research/space-life-sciences-training-program>

NASA Space Biology Program: <https://science.nasa.gov/biological-physical/programs/space-biology>

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More information on the BioNutrients missions can be found here: [What is BioNutrients? – NASA](#) and [BioNutrients: A Five-Year Experiment in Space Nears Completion - NASA](#)