Video Transcript:

Hello everyone. I am Guo-Hsuen Lo, and today I will be presenting “Engineering a photosynthetic yeast through endosymbiosis”. This is a project that I have researched under Dr. Mehta’s lab with other colleagues Jason, Noah, and Stanley. I thank them for giving me the opportunity to present.

From the extensive studies on the eukaryotes, the endosymbiotic theory provides considerable evidence that chloroplasts, as well as other organelles such as mitochondria, are bacterial in origin. Some of these evidence are from DNA sequencing comparison that chloroplasts have their own circular DNA, and their own ribosomes have different subunits than their host cells. The goal of this research is to replicate the early stages of endosymbiotic model to understand probable events that led to the creation of chloroplasts. We have chosen S elongatus as our symbionts and yeast cells (S cerevisiae) as our hosts to try to build a synthetic chloroplast generated through symbiosis with a model eukaryote.

In order to verify the capability of endosymbiosis, the hosts and the symbionts must go through mutations such that they cannot survive without depending on another. The experimental approach involves making mutations to S. elongatus and then introducing them to the yeast cytosol. Namely, we can delete key biosynthetic genes and express other genes ectopically from neutral sites. So far, we have made mutants that can secrete ATP and mutants that can secrete glucose. Both of these are retained in the cytosol of yeast because we use mutant yeast that don't have a properly-functioning mitochondrion and are thus respiration-deficient. They cannot survive in medium without exogenous glucose, unless they receive the metabolite from the symbiont

For the DNA homologous recombination to occur, it can be achived by delivering a suicide plasmid into S. elongatus. This can be done conveniently through conjugation, a transfer of genetic material by direct cell-to-cell contact. It can be done between different cells, so it is easy to first transform the plasmid into competent E coli with pilus. We have evidence that a partial recombination can happen at key biosynthetic genes of S elongatus by a suicide plasmid carrying the same starting and ending region as its target. A partial recombination occurs because S elongatus naturally

carries multiple copies of its chromosomes, so they must undergo a pressure selection in order to create a homozygous mutant.

We have recorded the results of endosymbiosis based on concentration of ATP and glucose, the microscopic observation of the mutants, and the recombination of S. elongatus symbionts. After the recombination of S elongatus and its fusion with yeast cells, the results showed that the mutant can significantly secrete ATP when challenged with ADP. Under the induction of sodium chloride, the mutants that express the invertase gene can break down sucrose to produce glucose. Fluorescent microscope observations can highlight the existence of S elongatus living inside the yeast cells. The chimera has shown to have partially-restored-respiration-competency, and the PCR analysis detected a homologous recombination happened at the lysine biosynthesis gene lysA.

To create a long-lasting photosynthetic yeast cell, the key will be to discover the optimal auxotrophs that can result in a consistent symbiotic relationship between the host and the symbionts. We will look further into generating a variety of s elongatus which are auxotrophic for a specific amino acid provided by yeast. We will explore optimal expression of SNARE proteins that prevents the symbionts from destruction of the host. As we move forward with the research, we aim to show DNA transfer between the host and the symbionts to mimic the chloroplasts that we see today. Finally, we aim to reconstitute natural product biosynthesis in chimeras and power synthesis with light.