Genetic Suppressor Screen in Search of Novel Sec6 Interacting Proteins in *Saccharomyces cerevisiae*

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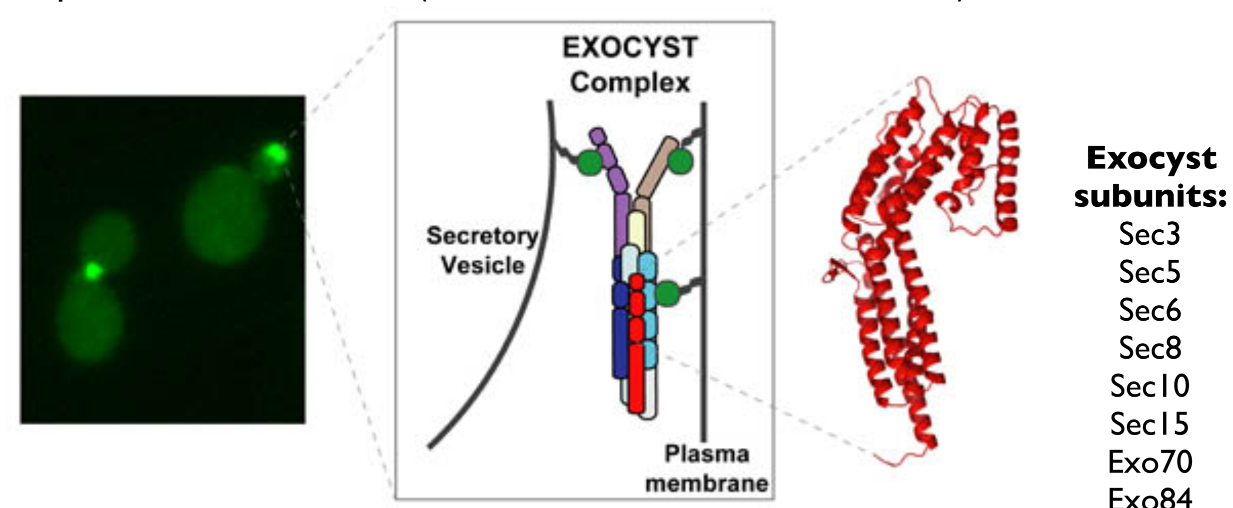
Abstract

Polarized protein secretion is a fundamental process for all eukaryotic cells involving many steps mediated by hundreds of proteins. The eight-subunit-complex, named exocyst, is essential for the proper delivery of secretory vesicles to the plasma membrane, and our interest lies specifically on the Sec6 subunit. Earlier work showed temperature sensitive mutants of *SEC6*, which have altered amino acid clusters on the protein surface, resulted in severe growth and secretion defects at 37°C (Songer and Munson, 2009). Interestingly, analyses of exocyst assembly in these mutant backgrounds revealed that the complex was fully assembled with all eight subunits at 37°C, yet the whole complex mislocalized from expected sites of secretion. We currently hypothesize that Sec6 has an important anchoring function for exocyst, and that the mislocalization of the complex stems from disruption of Sec6's surface interaction with unknown factor(s) on the plasma membrane. In order to identify the potential anchoring factors, we employed a genetic screen using a genomic library. The screen design allows us to identify genes that compensate the growth defect of *sec6-49* cells at 37°C, suppressing the mutation and allowing mutant cells to grow. We successfully isolated approximately 30 plasmids that allowed *sec6-49* cells to survive the temperature shift, and DNA sequencing of these plasmids was initiated to identify candidate genes. We found several putative suppressor candidates and are currently validating the suppression of individual candidate genes. Once we confirm the true suppressors, we plan to explore the functional relationship with Sec6 further. Overall, the isolation and characterization of novel anchoring proteins will shed light on mechanistic details of Sec6 and exocyst function, which is critical for understanding mechanistic details of quality control in the secretory pathway in higher eukaryotes.

Introduction

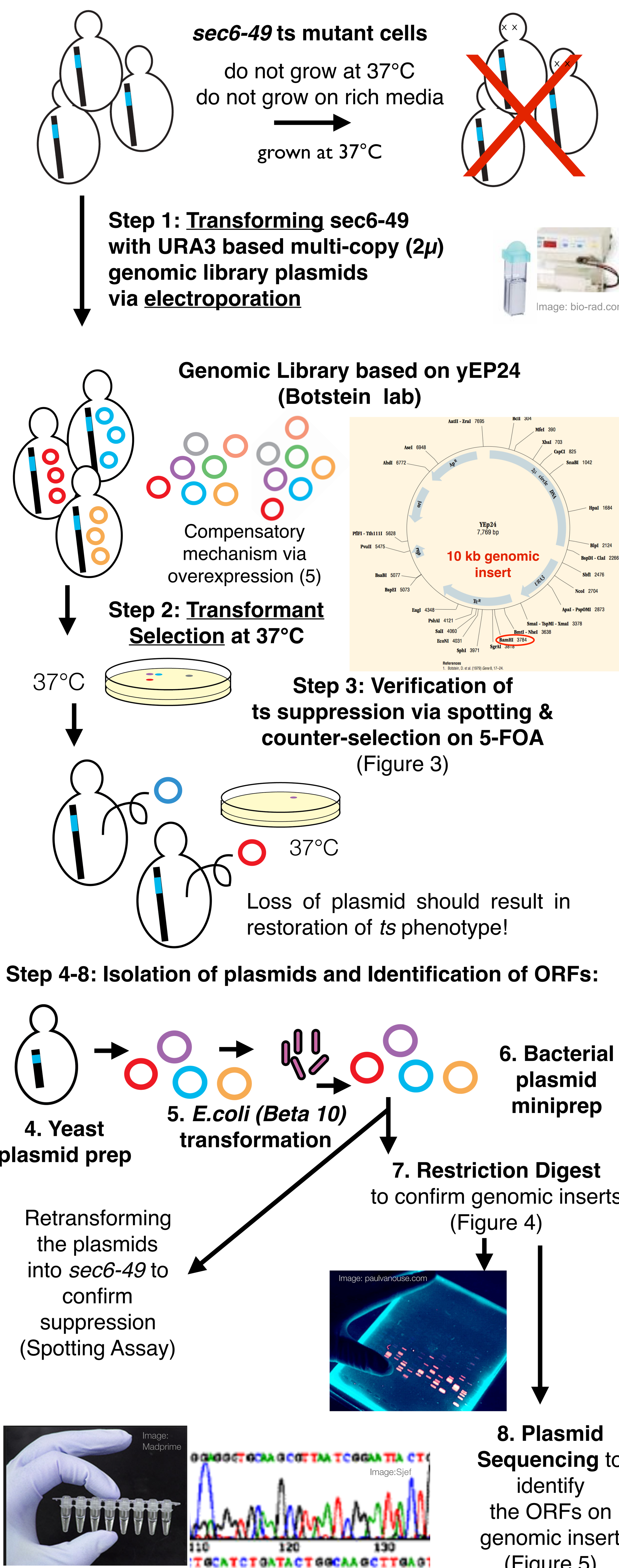
Protein secretion is a fundamental process for all organisms, from yeast to higher eukaryotes. Each step of the pathway is heavily regulate by many proteins based on specific protein-protein interaction that execute vesicle generation, transport, targeting and delivery to the target membrane. The Exocyst complex is a highly conserved octomeric protein complex that mediates a step in polarized vesicular trafficking (TerBush DR *et al.,* 1996), and the complex was originally discovered and characterized in *Saccharomyces cerevisiae.* The exocyst plays diverse roles in a variety of cellular functions by interacting with different sets of binding partners within specific functional contexts, and mutation and malfunction of the exocyst components are implicated in many human diseases such as ciliopathies and cancer (Martin-Urdinoz M *et al.,* 2016).



Adapted from Munson M. and Novick P., *Nat Struct Mol Biol.* 2006

Sec6, our protein of interest, is an essential, 88kDa protein shown to anchor the complex to the sites of secretion, and two highly conserved surface amino acid "patches" in the C-terminus are mutated, the cells display temperature sensitivity (Songer J *et al.,* 2009). In *sec6-49* mutant, L418, Y422, W433, Q470, Q474, and V478 residues on the protein surface were changed to Alanine (Songer J *et al.,* 2009). Our goal is to identify novel interactors of Sec6p, which may potentially link the exocyst complex to the SNARE protein Sec9p on the plasma membrane. The genes whose overexpression compensate the temperature sensitivity of sec6-49 mutants may serve as Sec6 interacting proteins that have not been characterized.

Overview of the Screen



Results

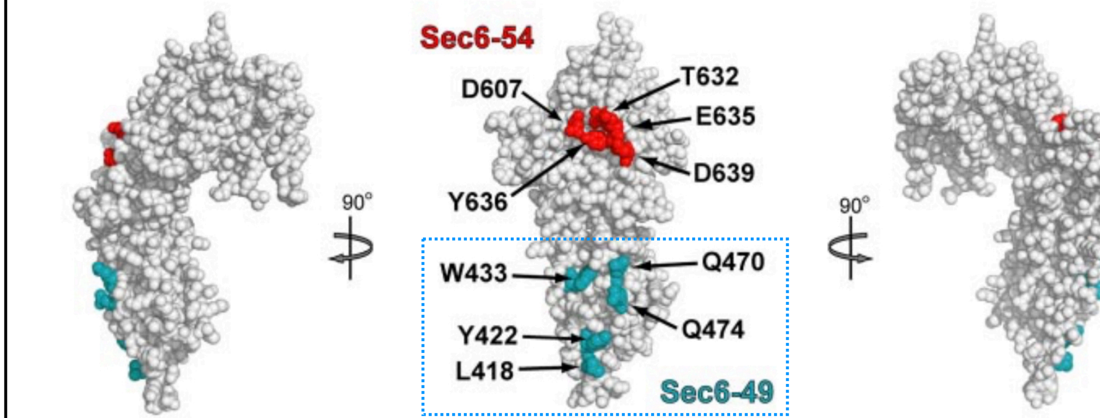


Figure 1. A Model showing two conserved amino acid patches
Songer and Munson, *Mol Biol Cell*, Vol. 20: 973-982 (2009)

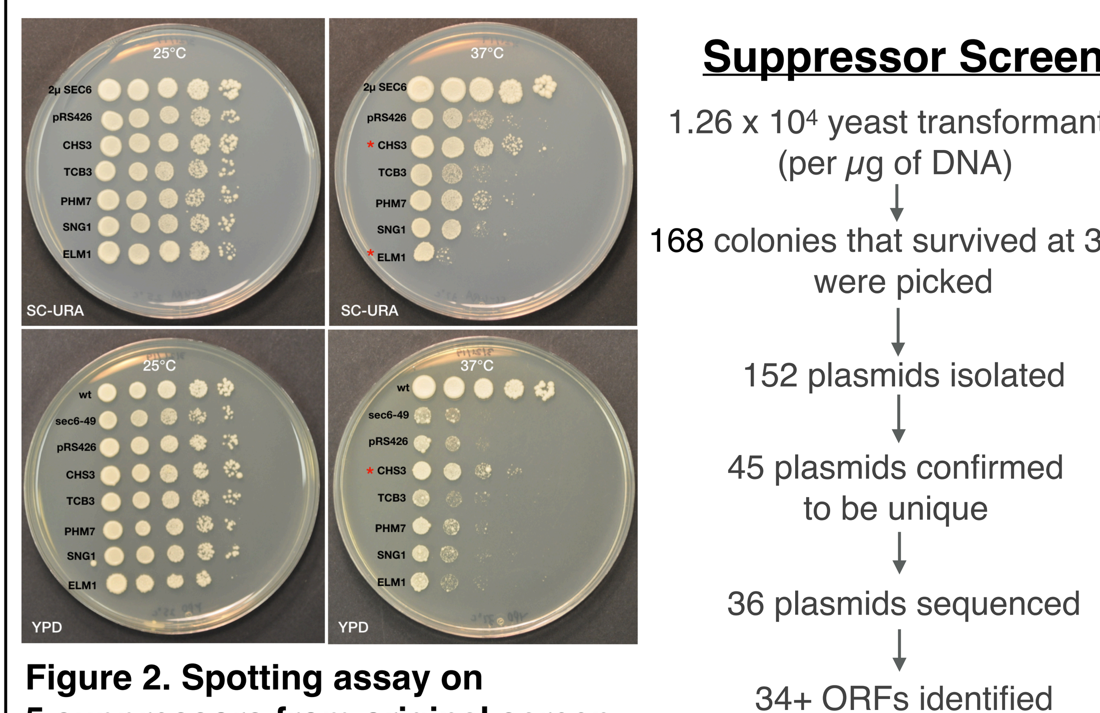


Figure 2. Spotting assay on 5 suppressors from original screen

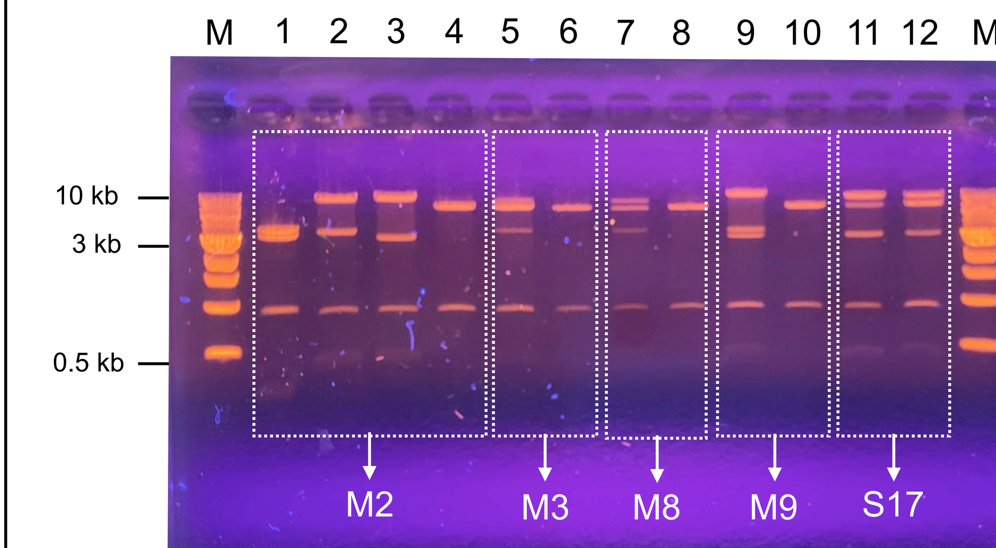


Figure 3. Restriction digest (EcoR V digest shown here) of unique bacterial plasmids from M series and S17 from previous screen used for comparison

References

1. TerBush DR, Maurice T, Roth D, Novick P. (1996). The Exocyst is a multiprotein complex required for exocytosis in *Saccharomyces cerevisiae. The EMBO Journal.* 15(23): 6483-6494.
2. Martin-Urdiroz M, Deeks MJ, Horton CG, Dawe HR, Jourdain I. (2016 Apr 12). The Exocyst Complex in Health and Disease. *Front Cell Dev Biol.* 4:24.
3. Songer, J. and Munson, M. (2009 Feb1). Sec6p anchors the assembled Exocyst at sites of secretion. *Mol Biol Cell.* (20): 973-982.
4. Creutz, CE, Snyder, SL, Schultz, TA. (2004). Characterization of the yeast tricalbins: membrane-bound multi-C2-domain proteins that form complexes involved in membrane trafficking. *Cell Mol Life Sci.* 61(10):1208-20.
5.Elbing, K, McCartney, PR, Schmidt, MC. *(2006).* Purification and characterization of the three Snf1-activating kinases of *Saccharomyces cerevisiae. Biochem J.* 393(Pt 3):797-805.
6.Koehler CM and Myers AM. (1997). Serine-threonine protein kinase activity of Elm1p, a regulator of morphologic differentiation in *Saccharomyces cerevisiae. FEBS Lett.* 408(1):109-14.

Acknowledgments

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Results

Suppressor Screen 3

1.5 x 10⁵ yeast transformants (per μg of DNA)

150 randomly selected suppressor candidates

Replica plated and selected 10 colonies

10 confirmed plasmids to be unique

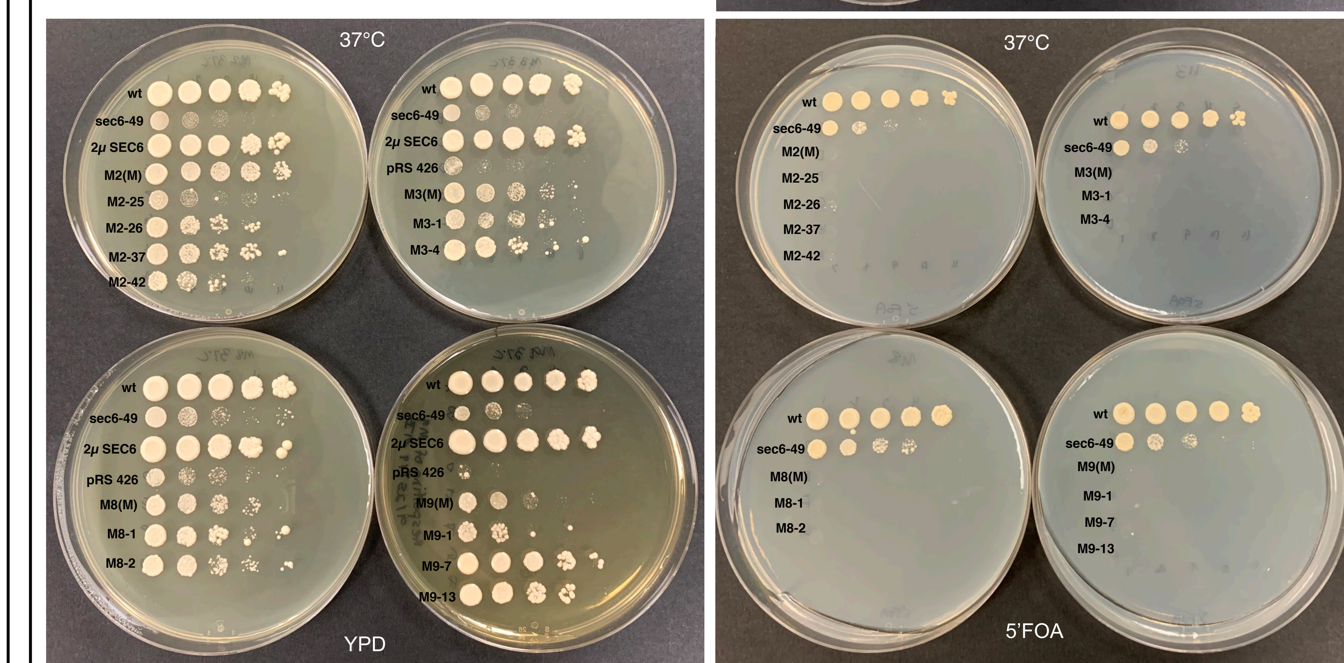

Figure 4. Spotting assay on 10 new suppressors from third round of screen: 0.2 OD unit of each strain (OD_{600} 0.4-0.8) was serially diluted, manually spotted and grown for 72 hours prior to image capture.

ORF	Localization	Attributes
LSB5	Plasma membrane	Endocytosis
STE50	Cytoplasm	Protein kinase regulator activity
FUS1	Plasma membrane	Cortical protein anchoring at mating projection tip
YTP1	Plasma membrane	Integral membrane protein with unknown functions
SRO9	Polysomes; cytoplasm	Regulation of translation

Table 1. Suppressor candidates from screen 2 and 3 plasmid sequencing to be studied further in future cloning and spotting assays (Based on yeast genome database)

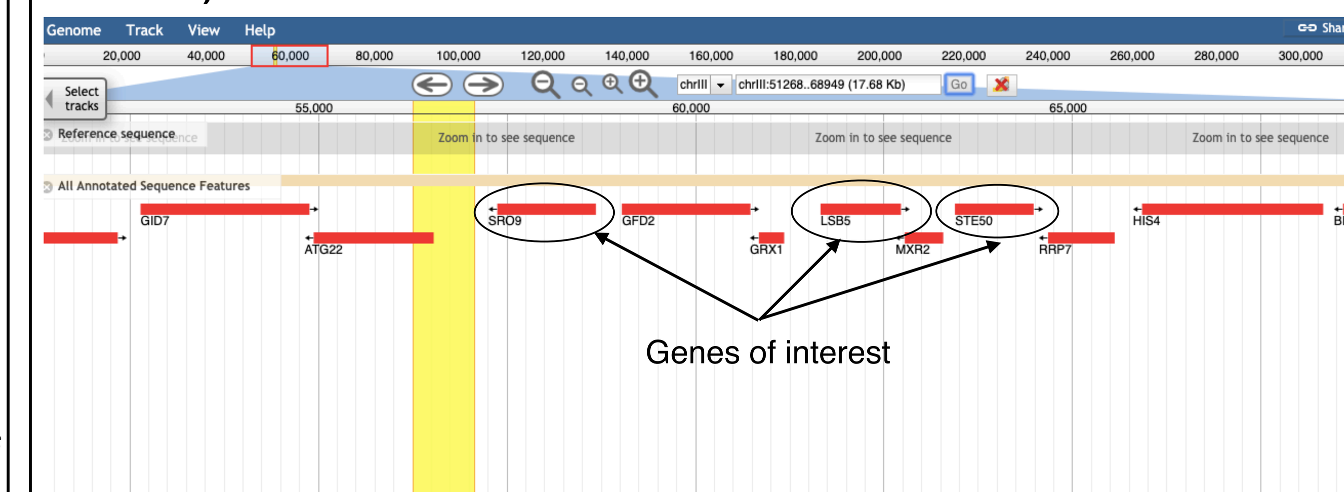

Figure 5. S1 plasmid sequencing results and ORFs via BLAST

Conclusion and Future Direction

- Completion of candidate suppressor plasmid sequencing
- Identify ORFs and genes of interest through *S. cerevisiae* genome database search and literature
- Clone each individual candidate gene into 2μ plasmid and transform into *sec6-49* mutant to validate growth suppression
- Additional biochemical and genetic experiments to explore the functional relationship between the suppressor genes and Sec6