Potential Roles for Long Non-Coding RNAs in the Regulation of Mating-Type Switching in *Ogataea polymorpha*

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Abstract

Sexual reproduction is a risky process can lead to DNA damage or decrease a genome's chance of survival, as only half of an organism's genome is passed onto its progeny. Thus, organisms who sexually reproduce have evolved highly regulated signaling mechanisms to ensure reproduction and mating proceed smoothly. Mating-type switching is an example of regulated sexual reproduction found in many yeast species. In budding yeast, two mating-types, **a** and α mate with each other. When a yeast cell has no viable mating partner, it performs mitosis to form two identical cells. The mother cell then goes through a chromosomal inversion event and switches to the opposite mating-type, so the mother and daughter cell can mate. This inversion is extremely risky and can result in DNA damage or cell death. To mitigate risk, mating-type switching is regulated by multiple signal cascades to prevent something from going awry during switching. The transcription factor *STE12* has been identified as an important agent in the regulation of these signal cascades, and is necessary and sufficient to induce switching. Our research focuses on switching in the yeast *Ogataea polymorpha*, which is distantly related to the model yeast, *Saccharomyces cerevisiae*. In this research, we investigated the downstream pathway of *STE12*-mediated mating-type switching in *O. polymorpha*. We looked for putative long non-coding (lnc)RNA (non-coding RNAs longer than 200 base pairs) molecules involved in the regulation of the switching signaling cascade. We induced switching in α cells and performed RNA-seq analysis to identify lncRNAs regulated by *STE12*. We created a bioinformatic pipeline to identify novel transcripts upregulated by *STE12*. We found 5 putative lncRNAs in the set of novel transcripts that are upregulated by *STE12* and may have a role in switching regulation. In the future, we need to investigate the functions of the 5 lncRNAs to see if they work in the signal response cascade that regulates mating-type switching.

O. polymorpha Mating-Type Switching Through Genomic Reorientation of the *MAT* Locus

Fig. 1. Yeast Sexual Processes. Asexual or sexual lifecycle (including mating-type switching of an isolated spore) of a yeast cell leading to sporulation. Pink = α cells, green = **a** cells.
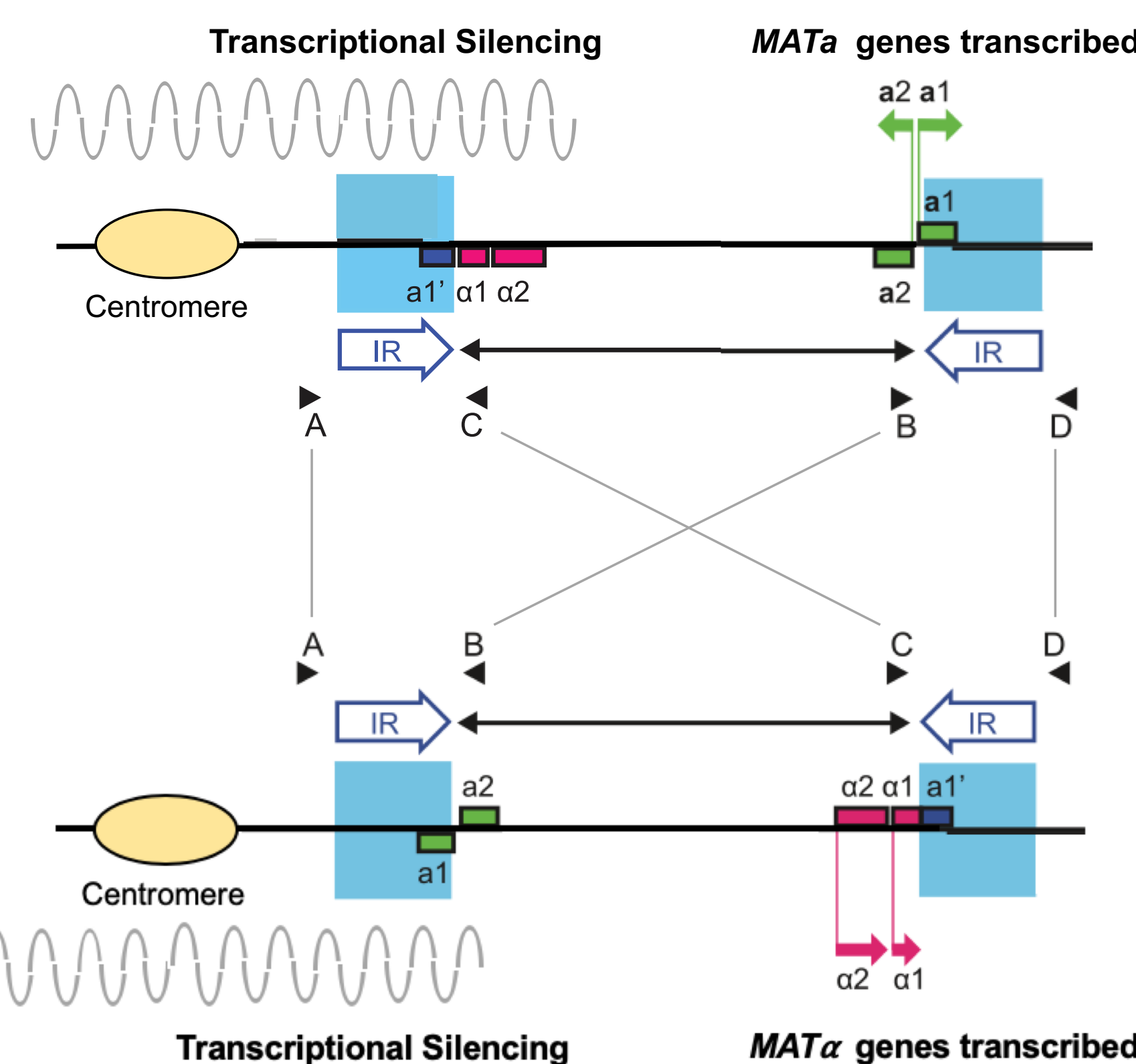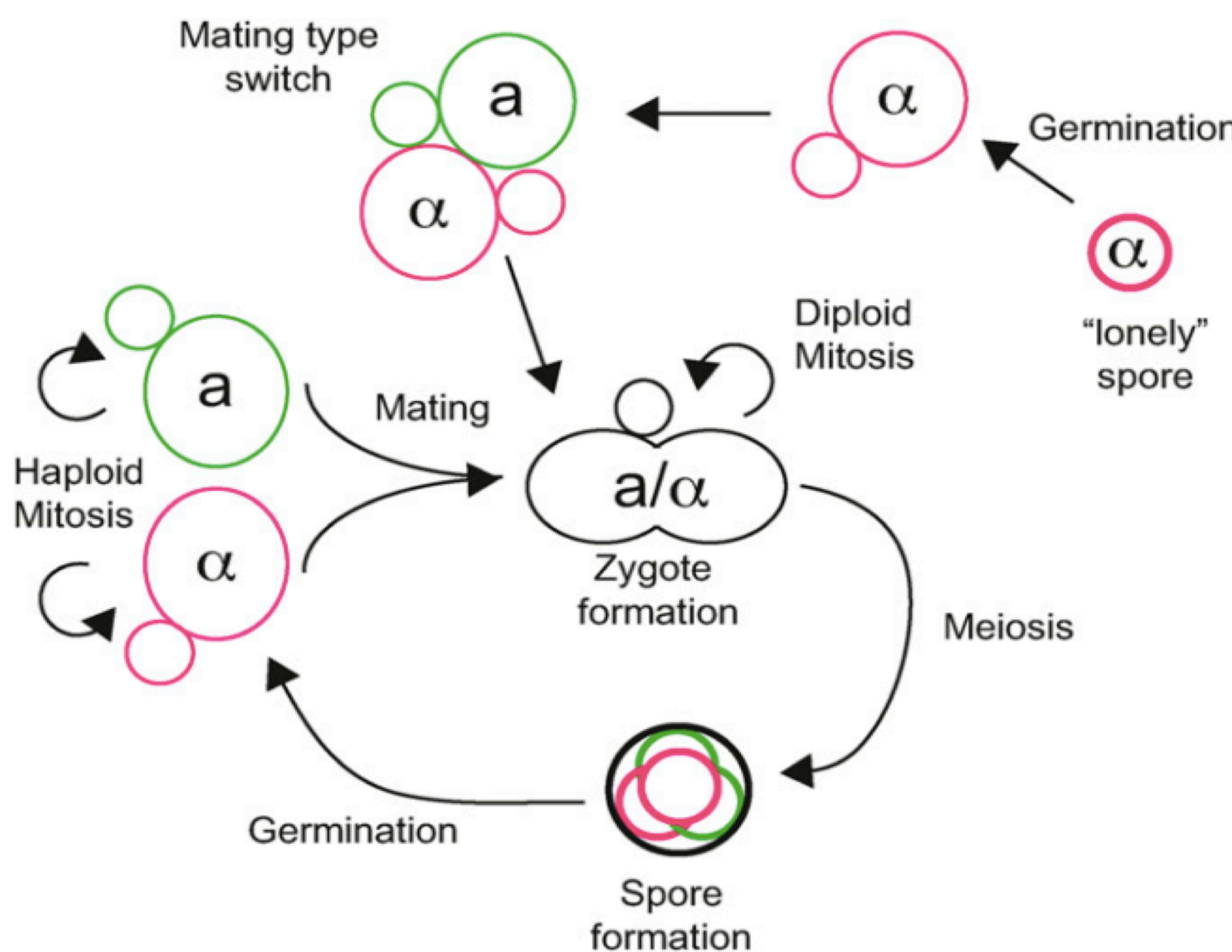

Fig. 2. *Mating-Type (MAT)* Locus on Chromosome 3. *O. polymorpha MAT* locus in both the **a** and α orientation. Blue boxes are inverted repeat sequences. Primers are labelled and grey lines show the primer reorientation after inversion. Pink represents MATα, green represents MAT**a**.

Hypothesis: Long Non-coding RNAs regulate the progression of Mating-Type Switching following *STE12* overexpression.

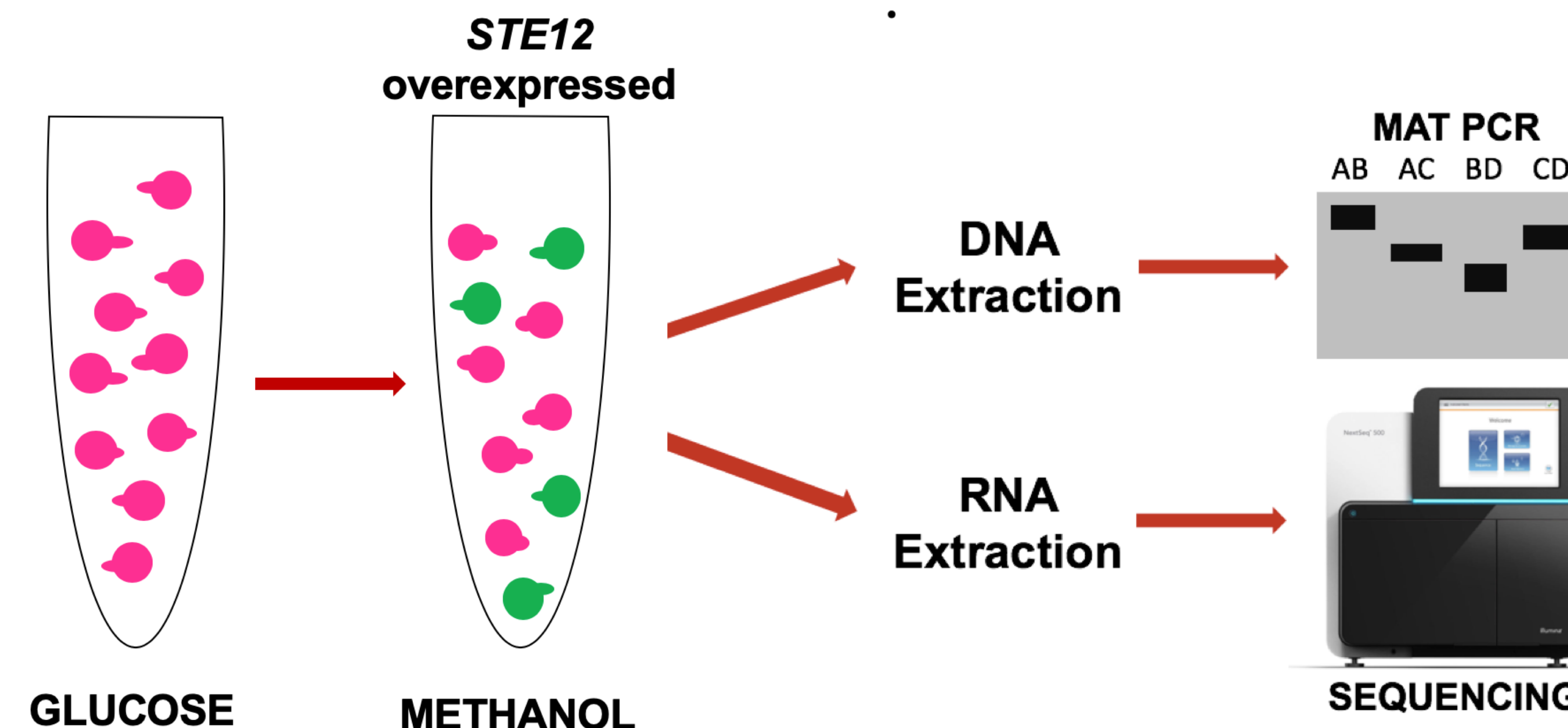## Overexpression of *STE12* Induces Mating-Type Switching

Fig. 3. Overexpressing *STE12* Induces Mating-Type Switching in *O. polymorpha*. Overexpression of STE12 was induced using a methanol-inducible promoter, which led to switching. DNA and RNA were extracted from the cells. PCR was run on the DNA to determine *MAT* locus orientation. RNA was sequenced using an Illumina NovaSeq at the University of Colorado Anschutz Medical Campus Genomics and Microarray Core Facility.

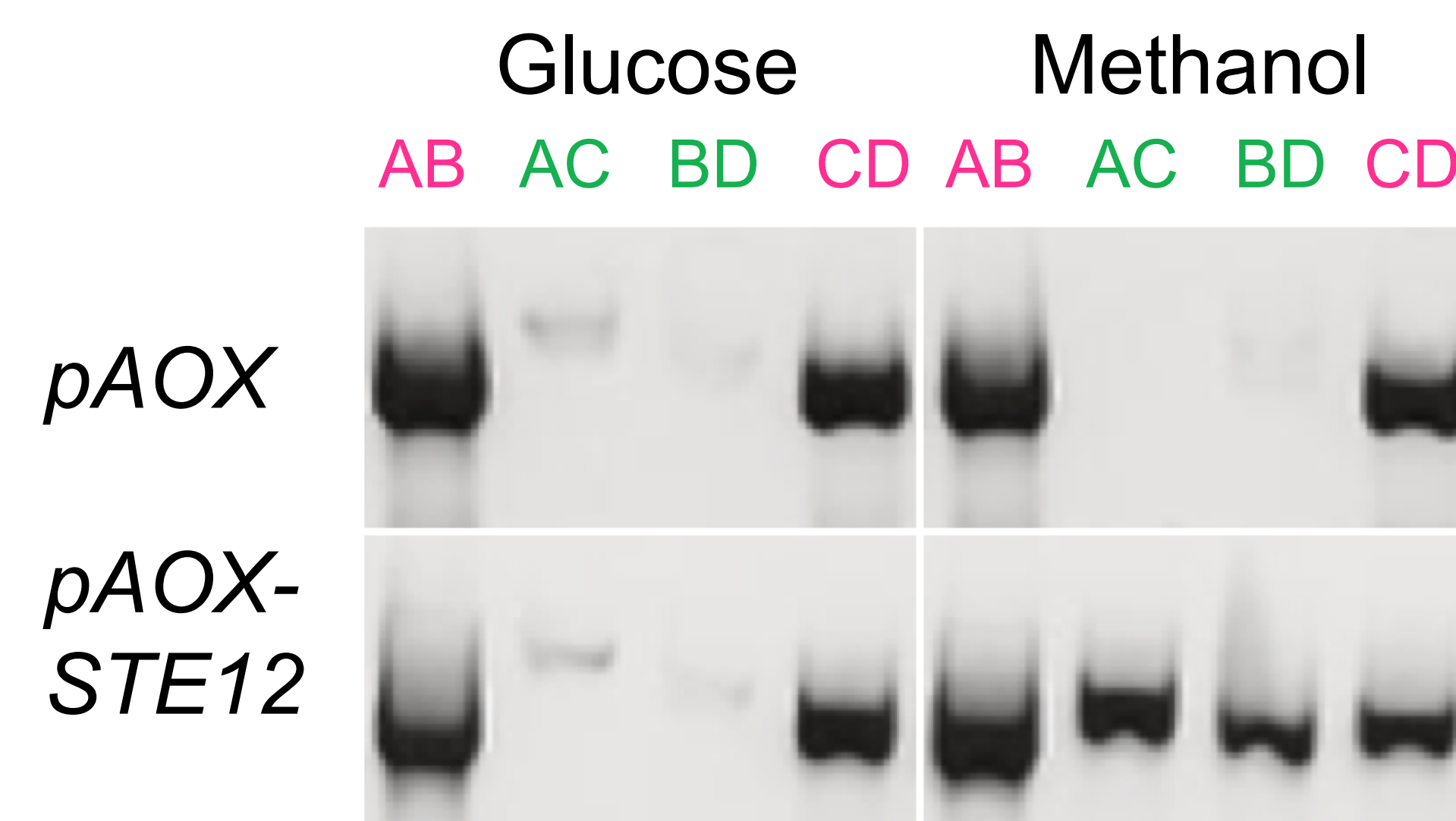## Switching Occurred in *MATα* Cells Overexpressing *STE12*

Fig. 4. α Cells in Methanol Performed Mating-Type Switching. PCR results from the DNA extraction showing that strains overexpressing *STE12* (*pAOX-STE12*) performed Mating-Type Switching, while strains lacking the *STE12* overexpression vector (*pAOX*) did not perform MTS. PCR Primer combinations are listed at the top.
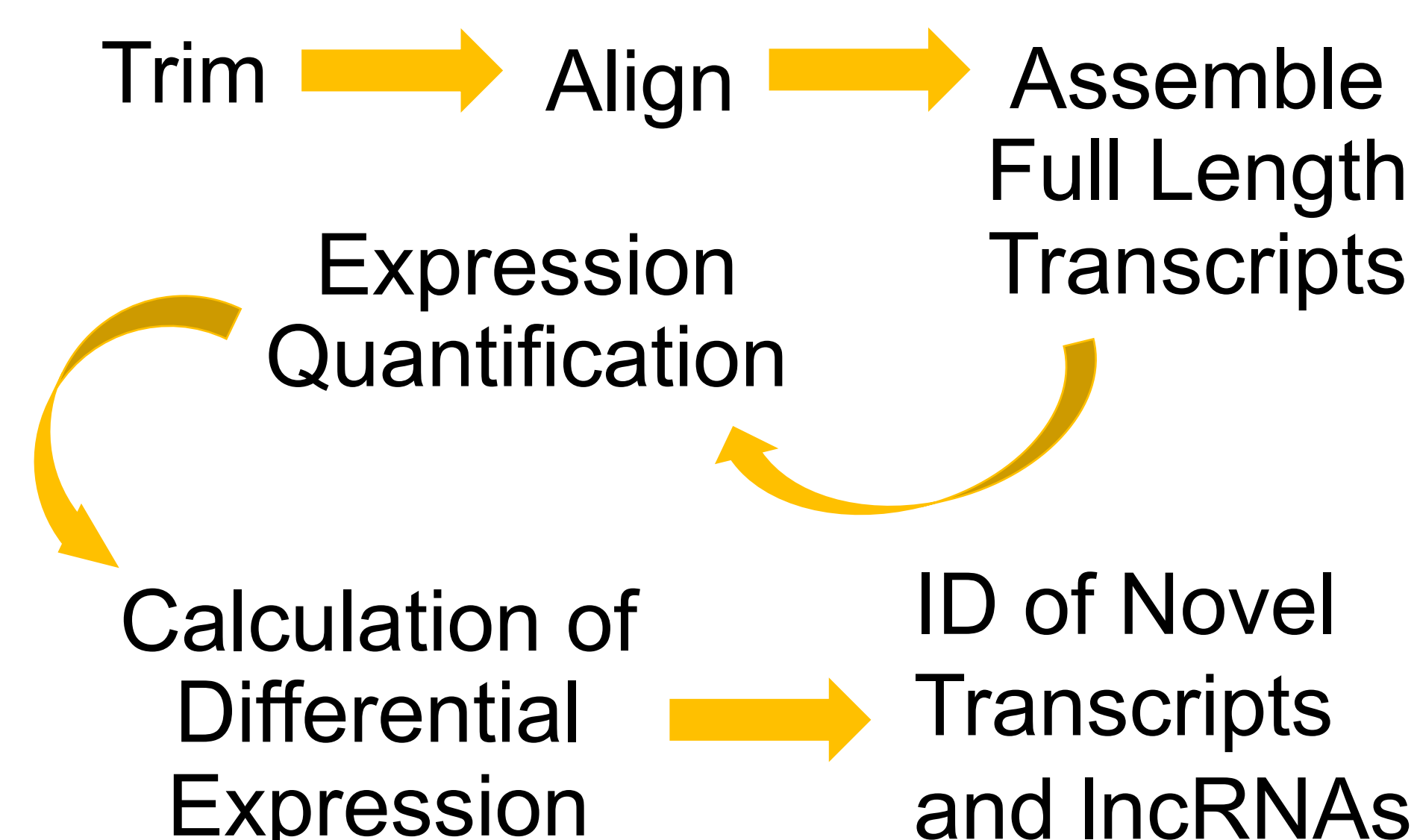
Identification of Long Non-Coding RNAs

Fig. 5. Bioinformatic Workflow Used to Identify and Analyze Long Noncoding RNAs. Most programs used were available on the Galaxy Server (Penn State). Once upregulated novel transcripts were identified, their nucleotide sequence was analyzed using the Coding Potential Calculator created by the Center for Bioinformatics to analyze coding potential.

Validation of Coding Sequences Upregulated by *STE12* in Total RNA-seq

Table 1. *STE12* Upregulates Mating Genes.

Gene Name	Function	MATα total RNA pAOX-STE12 vs. pAOX	MATα mRNA pAOX-STE12 vs. pAOX
MFalpha1	α factor pheromone	8.53	4.03
BAR1	protease that cleaves α factor	6.09	6.75
FUS3	MAP kinase involved in mating	5.84	5.71
MFa	a pheromone	5.59	5.56
GPA1	α subunit pheromone receptors	5.52	6.23
STE3	receptor for a-factor	5.36	5.74
STE12	trans. factor, pheromone response	5.00	5.88
CDS:62574	unknown	5.00	4.36
OPOL_16726	prenylcysteine lyase	4.62	4.43
FAR1	cell cycle arrest to pheromones	4.27	4.30

STE12 Upregulates Five Potential Long-Non Coding RNAs

Table 2. The Total RNA-Seq Dataset Contains 7 *STE12* Regulated Novel Transcripts.

Transcript Name	Log₂ (Fold Change)	Length	Coding Potential Score	Coding or Non-Coding
MSTRG.6170	3.51	3096	-1.03047	Non-Coding
MSTRG.74	2.77	17772	6.8913	Coding
MSTRG.1933	2.2	4869	-1.01701	Non-Coding
MSTRG.2246	1.97	12190	3.76732	Coding
MSTRG.2241	1.73	4250	-0.274634	Weak Non-Coding
MSTRG.1617	1.57	1917	-1.04476	Non-Coding
MSTRG.1283	1.54	952066	-32.5407	Non-Coding

*Blue Denotes Non-Coding Transcripts

Future Directions

- Identify lncRNAs Secondary Structures
- Identify Targets of lncRNAs
- Analyze *cis* versus *trans* gene regulation by lncRNAs
- Knockdown or knockout lncRNAs and analyze differential expression of regulated genes.

Works Cited.
Hanson, Sara, et al. *PLOS*. Genetics, 2017.
Hanson, Sara. et al. *PNAS*. 2013.
Kong, Zhang. et al. *Nucleic Acids Research*. 2007
Pertea, Micheal. et al. *Nature Protocols*. 2016.
https://www.illumina.com/systems/sequencing-platforms.html