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R06. Identification of the Cellular Pathways Targeted by Two Antifungal Natural Products Using RNA-Seq Analysis

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IDENTIFICATION OF THE CELLULAR PATHWAYS TARGETED BY TWO ANTIFUNGAL NATURAL PRODUCTS USING RNA-SEQ ANALYSIS

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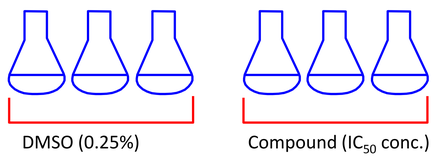
RNA-SEQ ANALYSIS OVERVIEW

Experimental Strategy



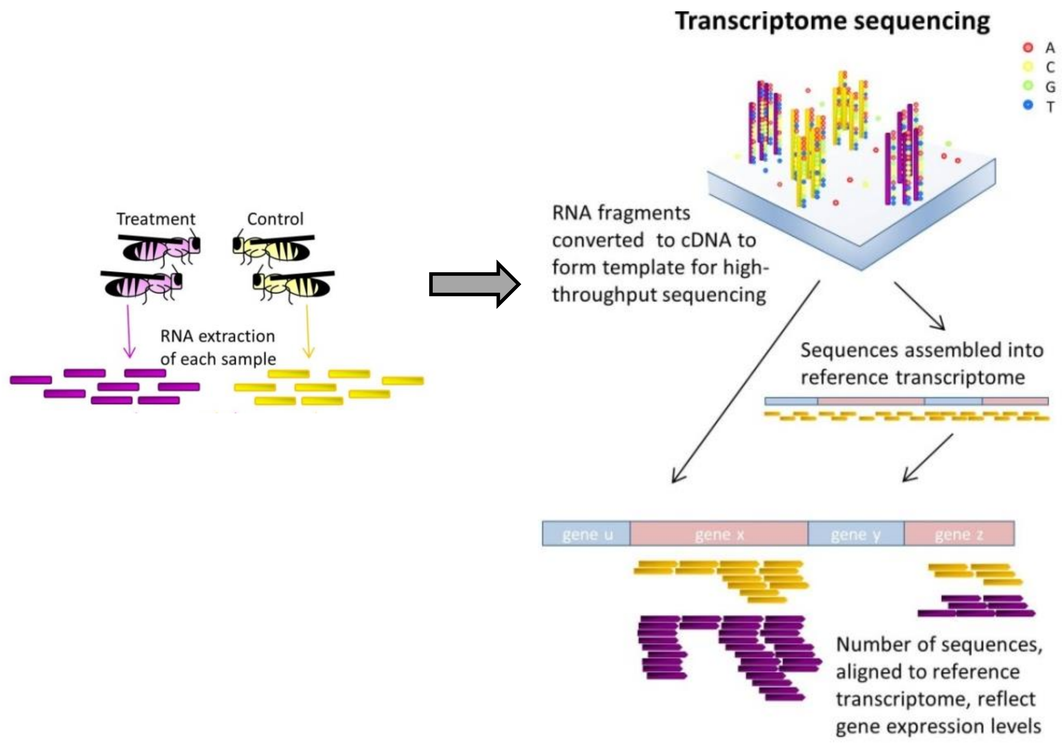
Drug treatment of yeast cells for 1.5 doublings (~ 4.5 h)

Isolate RNA

Prepare sequencing library

Perform RNA sequencing
ID differentially expressed genes

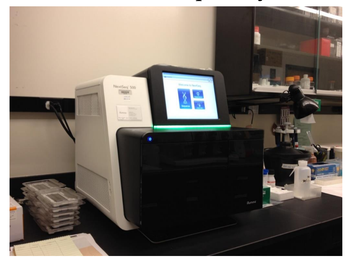
RNA-Seq Technology



From Wertheim, B. (2012) Beyond the gene list: Exploring transcriptomics data in search of gene functions, trait mechanisms, and genetic architecture. In "Functional Genomics", ed. Meroni, G., InTech, DOI: 10.5772/48239.

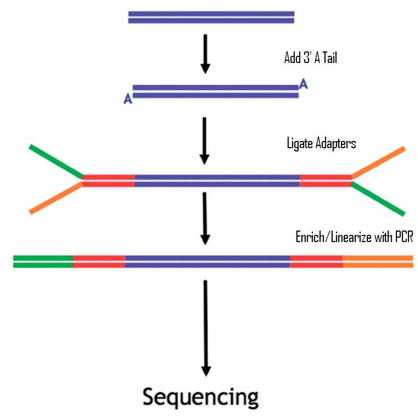
Sequencing Instrument

Illumina NextSeq 500 System



Sample Preparation

Prepare cDNA from mRNA



RESULTS

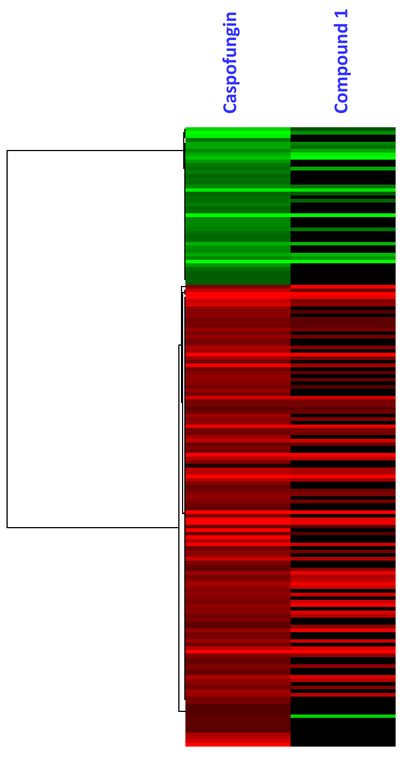
COMPOUND 1

A Plant-Derived Steroidal Alkaloid Potentially Targets the Fungal Cell Wall

Functional Categorization of 252 Genes Upregulated by Compound 1

GOID	GO_term	Cluster frequency	Background frequency	P-value
71554	**cell wall organization or biogenesis**	**35 out of 252 genes, 13.9%**	**198 out of 7165 background genes, 2.8%**	**6.22E-13**
5975	carbohydrate metabolic process	30 out of 252 genes, 11.9%	221 out of 7165 background genes, 3.1%	9.23E-08
9628	response to abiotic stimulus	19 out of 252 genes, 7.5%	172 out of 7165 background genes, 2.4%	0.00649

Compound 1 Response is Similar to Response of Cell Wall Targeting Drug Caspofungin



Significance:
- Caspofungin is only effective against *Candida* species, not against *Cryptococcus* species.
- Compound 1 has very potent activity against *Cryptococcus* species.
- Cell wall targeting antifungals are fungal specific and less toxic.

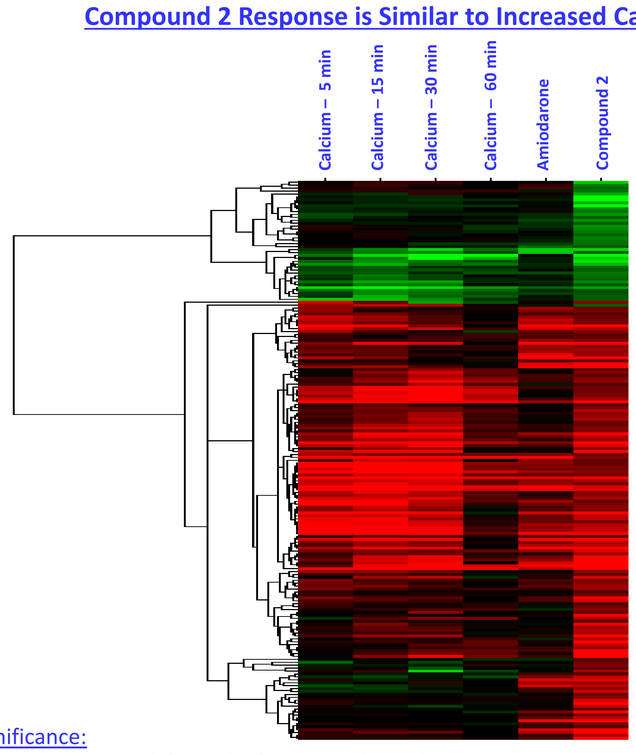
COMPOUND 2

A Microbe-Derived Cyclic Peptide Potentially Targets Fungal Calcium Homeostasis

Functional Categorization of 290 Genes Upregulated by Compound 2

GOID	GO term	Frequency	Genome Frequency
8150	biological process unknown	77 out of 290 genes, 26.6%	1133 of 6433 genes, 17.6%
71554	cell wall organization or biogenesis	32 out of 290 genes, 11.0%	198 of 6433 genes, 3.1%
42221	response to chemical	29 out of 290 genes, 10%	446 of 6433 genes, 6.9%
5975	carbohydrate metabolic process	21 out of 290 genes, 7.2%	198 of 6433 genes, 3.1%
6811	**ion transport**	**20 out of 290 genes, 6.9%**	**263 of 6433 genes, 4.1%**
6629	lipid metabolic process	19 out of 290 genes, 6.6%	296 of 6433 genes, 4.6%
43934	sporulation	17 out of 290 genes, 5.9%	133 of 6433 genes, 2.1%
6605	protein targeting	16 out of 290 genes, 5.5%	307 of 6433 genes, 4.8%
51321	meiotic cell cycle	16 out of 290 genes, 5.5%	282 of 6433 genes, 4.4%
55085	transmembrane transport	14 out of 290 genes, 4.8%	234 of 6433 genes, 3.6%
23052	signaling	14 out of 290 genes, 4.8%	245 of 6433 genes, 3.8%
6468	protein phosphorylation	12 out of 290 genes, 4.1%	197 of 6433 genes, 3.1%

Compound 2 Response is Similar to Increased Calcium Response



Significance:
- Current antifungal drugs don't target calcium homeostasis.
- Calcium plays important roles in virulence of fungal pathogens.

ACKNOWLEDGEMENTS
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