

Rapid Development of Molecular Resources for a Freshwater Mussel Using RNA-Seq

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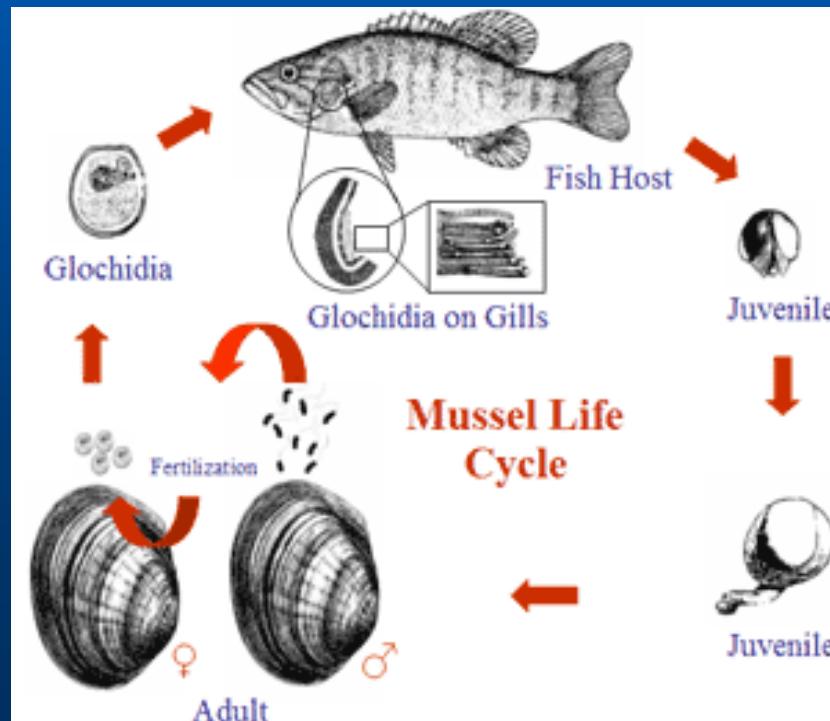


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Background

- Freshwater mussels: 297 taxa
- One of the most endangered groups in the world
- Southeast hot spot of diversity





Introduction

Information are NOT available for:

- **Understanding of mussel assemblages**
- **Spatial arrangements of populations**
- **Naturally occurring sex ratios and male spawning contributions**
- **Genetic variability within wild populations**





Species of interest

Villosa — Endangered members

V. fabalis



V. vanuxemensis umbrans



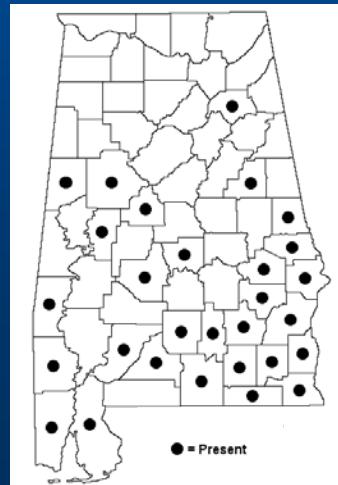
V. Nebulosa



V. choctawensis



Villosa lienosa The most wide-spread mussel in Alabama



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Mussel Genomic Resources

	Nucleotide sequences
Freshwater unionid	10,102
<i>Villosa</i>	104
<i>Villosa fimbialis</i>	18
<i>Villosa vanuxemensis umbrans</i>	9
<i>Villosa choctawensis</i>	1
<i>Villosa lienosa</i>	8



Study Aims

- Gene identification and annotation
- Marker mining for downstream analysis
- Gene expression profiling under heat stress



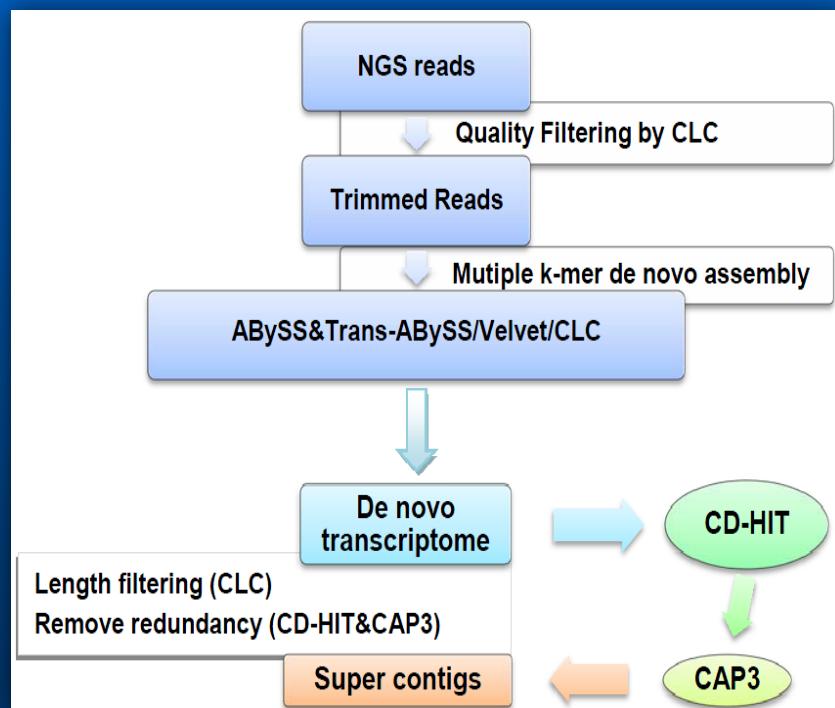
Methods

- Sample collection and heat stress treatment
 - Collect from Chewacla Creek
 - Acclimatization
 - Heat stress
- RNA and DNA isolation (mantle, gill, muscle)
- Illumina HiSeq 2000 sequencing
- Data analysis and validation

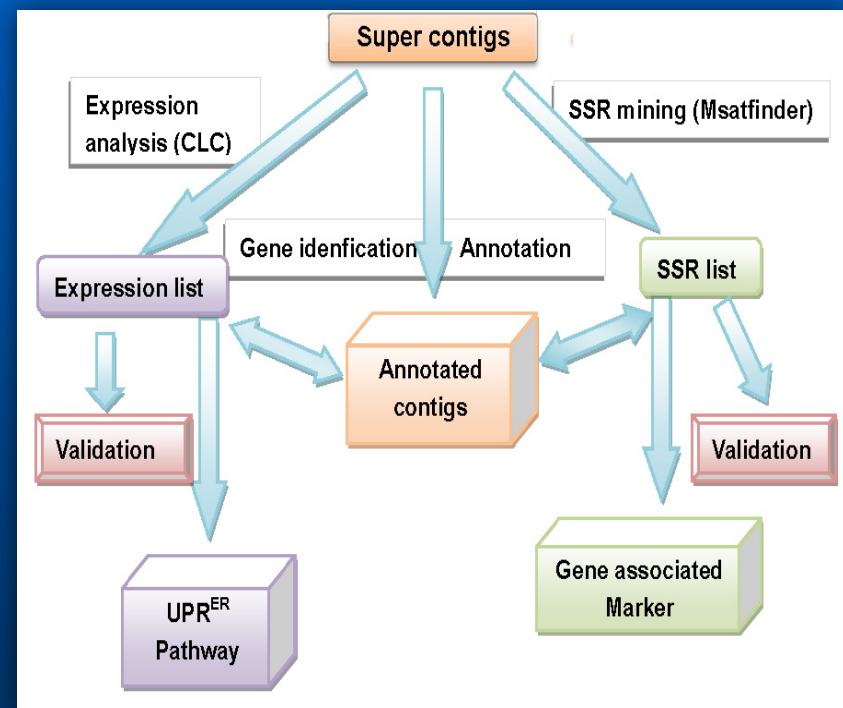




Data analysis and validation



Best assembly selection



Contig analysis



The Assembly Results

	Trans-ABySS	Velvet	CLC
K-mer	47	7	1
Contigs($\geq 100\text{bp}$)	1,806,746	501,764	1,311,097
Avg length (bp)	619	440	266
N50 (bp)	1191	1198	305
Contigs after length filtering($\geq 200\text{bp}$)	1,193,893	356,346	438,104
% contigs after length filtering	64.3%	71.0%	33.4%
Avg contig length after length filtering (bp)	860.3	552.7	521.1
Contigs ^a	1,177,801	229,188	438,070
Avg length (bp) ^a	856.4	612.0	521.2
Contigs ^b	778,234	193,016	437,283
Avg length (bp) ^b	707.5	627.9	521.8
Reads mapped to final reference (%)	148,378,497 (91.4%)	120,921,555 (74.5%)	138,638,637 (85.4%)

^a After CD-HIT-EST ^b After CD-HIT-EST+ CAP3



Gene Identification and Annotation

	Putative gene matches	Annotated contigs $\geq 500\text{bp}$	Unigene matches	Quality unigene matches
UniProt	111,049	73,569	22,785	12,791
Invertebrate	213,755	146,899	32,537	17,564
Nr	248,059	180,224	46,043	23,742
GO terms	98,419	65,681	26,328	N/A





Differentially Expressed Genes

	Total	Up	Down	Avg read number	Fold change>5	Fold change>10
Contigs	1934	1158	776	109.3	422	98
Unique genes	604	371	233	128.4	88	12





Microsatellite Mining and Validation

SSR mining

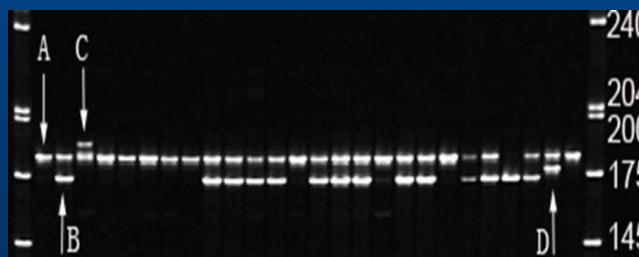
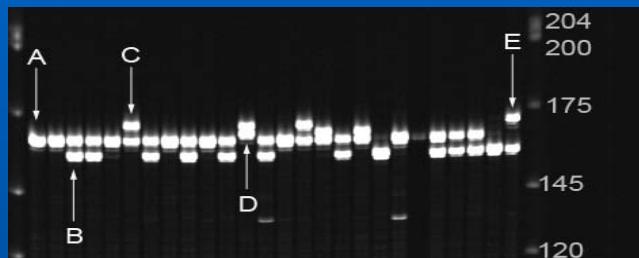
Total number of sequences examined	778,234
Total size of examined sequences (bp)	550,600,555
Total number of identified SSRs	65,086
Total number of SSRs with primers	36,582
Contigs containing SSRs with primers	31,960

Distribution of SSRs in different repeat types

Di-nucleotide	34,719
Tri-nucleotide	19,834
Tetra-nucleotide	10,285
Penta-nucleotide	162
Hexa-nucleotide	86

Summary of SSRs validation

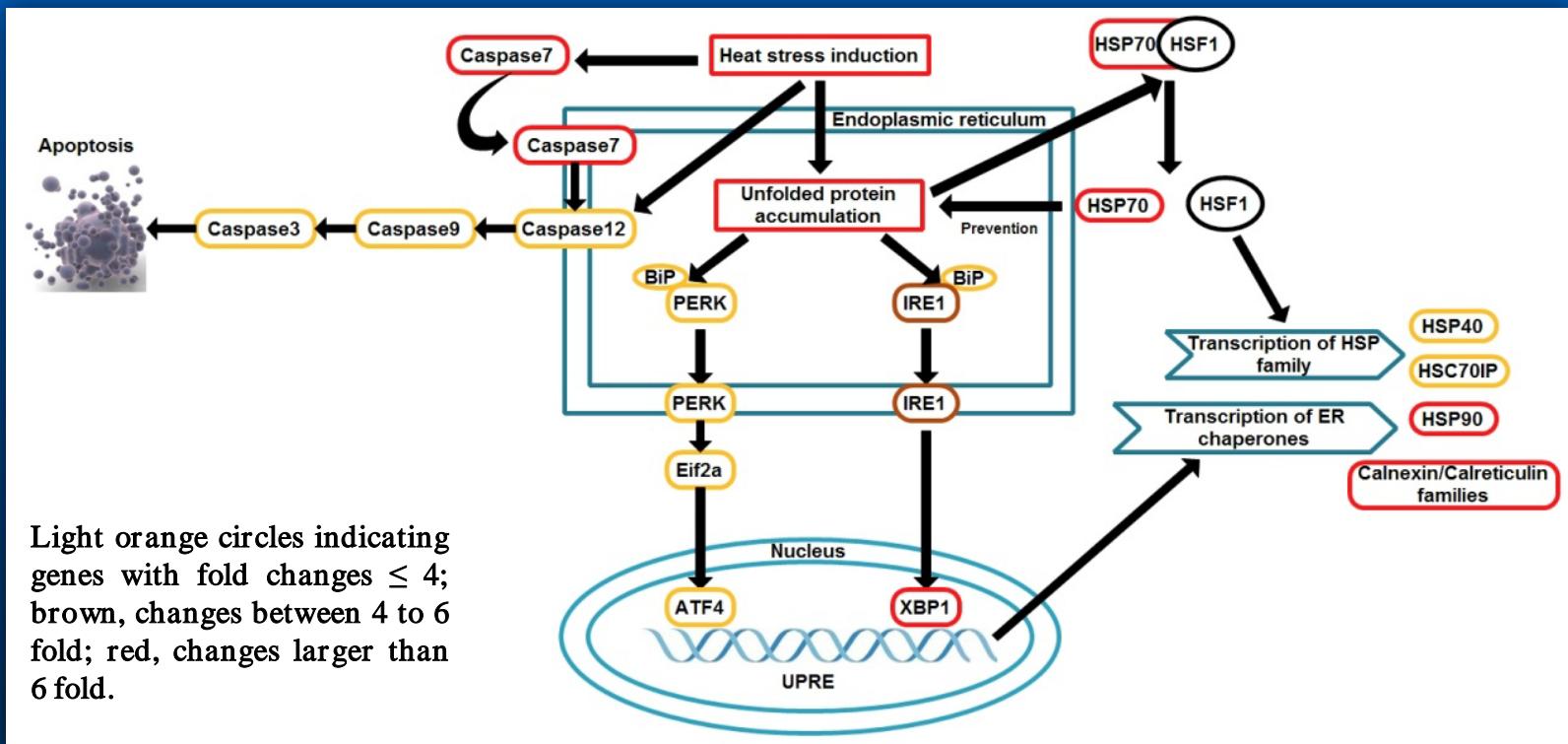
Individuals	26
Total markers	47
Amplified markers	44
Polymorphic markers	16





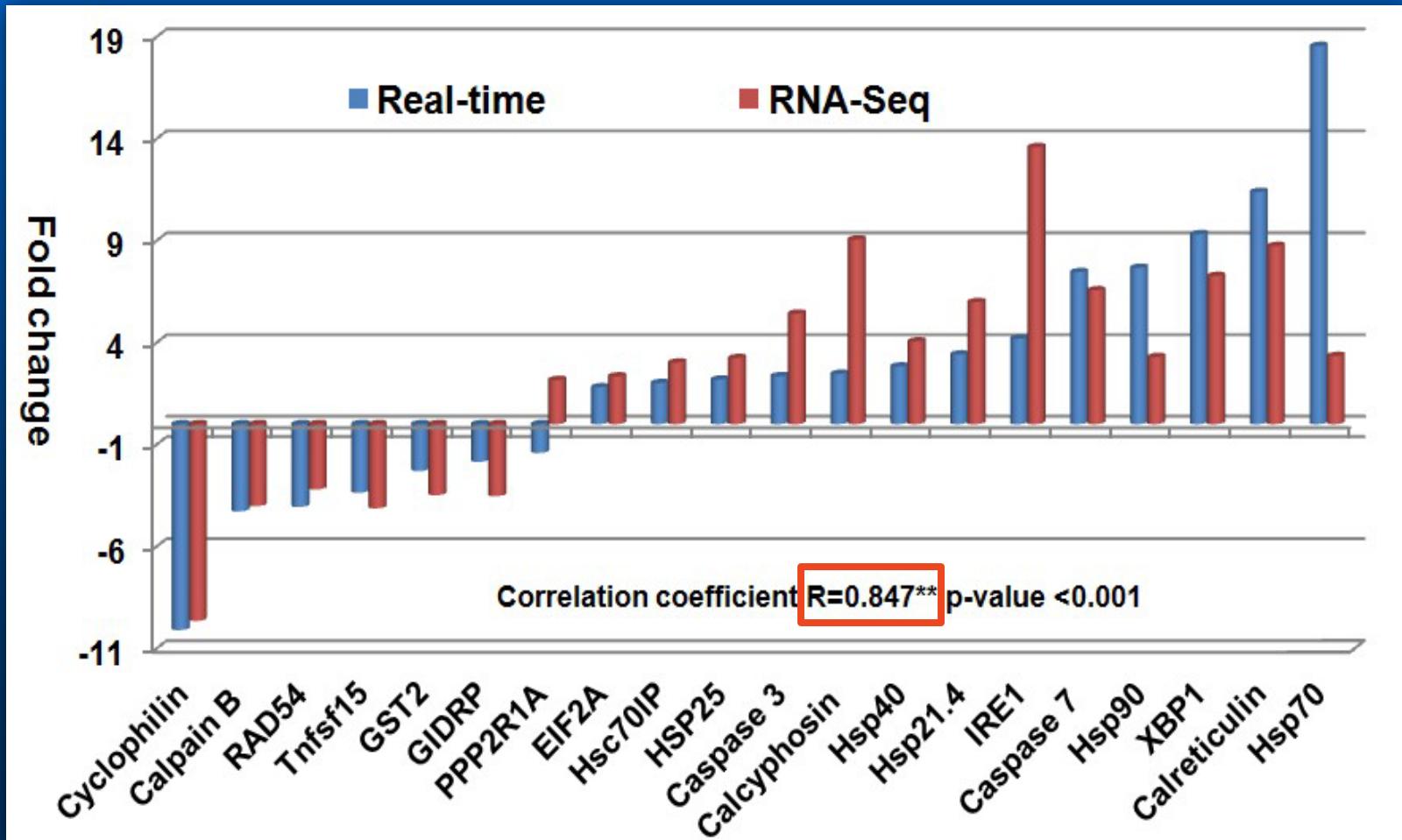
Pathway analysis

UPR^{ER} Pathway: Heat-shock response and endoplasmic reticulum unfolded protein response





Real-time PCR Validation





Summary

- A simple, economical approach for rapid generation of large-scale molecular resources for the freshwater mussel, *V. lienosa*.
- A substantial portion (over 46,000 unique gene matches) of the mussel transcriptome in high-quality contigs encoding both tens of thousands of genes and microsatellites.
- Use of mussels exposed to elevated temperatures allowed enrichment for stress-related genes and identification of differentially expressed pathways relevant to heat stress.



Acknowledgments

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Thank you! Any questions?



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