Biology, Ecology, and Management of Brown Marmorated Stink Bug in Orchard Crops, Small Fruit, Grapes, Vegetables, and Ornamentals

Funding

USDA United States Department of Agriculture

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Collaborating Institutions

University of Delaware

ars

Penn State

The State University of New Jersey Rutgers

Northeastern IPM Center

Washington State University

Cornell University

OSU Oregon State University

University of Maryland

VirginiaTech

NC State University

StopBMSB.org

Genetic studies of BMSB

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Pick Lab Objectives

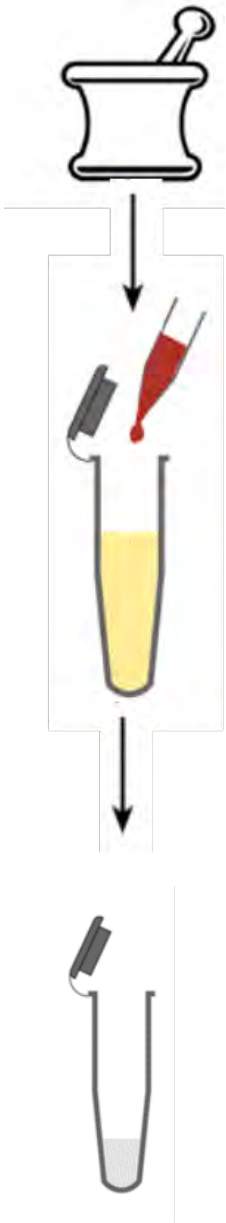
Subobjective 1.5. **Genetic** studies of BMSB

Subobjective 2.2.8. Establish **RNAi** technology to control BMSB.

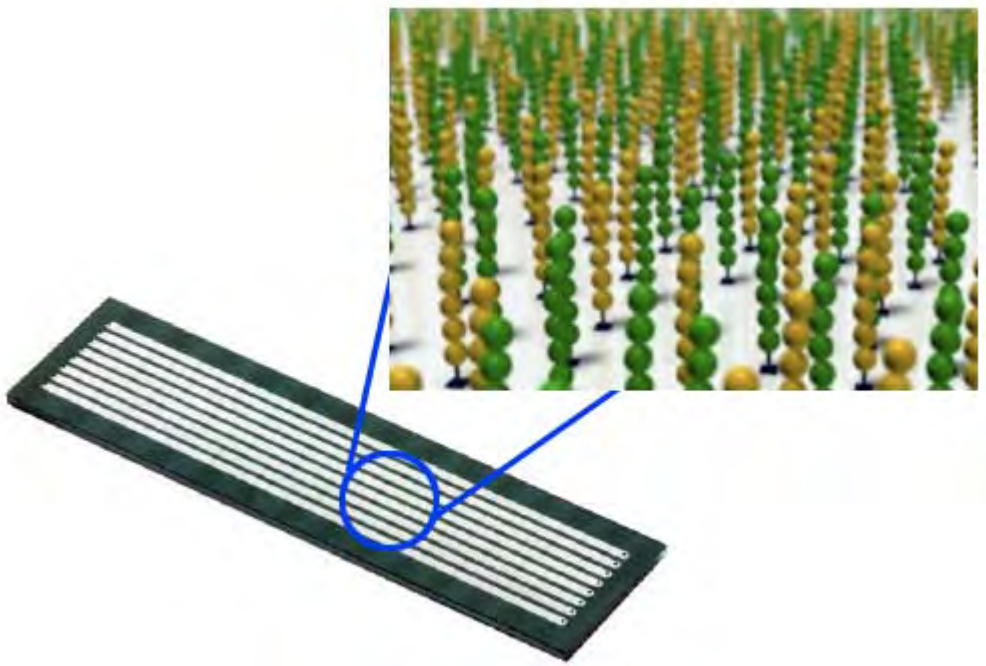
Subobjective 1.5. Genetic studies of BMSB

- Goal: identify **all** BMSB genes
  - Learn about basic biology
  - Identify genes that are novel in BMSB
  - Identify genes that are candidates for use in pest control
- Approach: isolate and sequence BMSB mRNA
  - Collaborators for RNA-seq, Julie Dunning Hotopp, Institute for Genome Sciences, UMAB

mRNA Extraction from all life stages



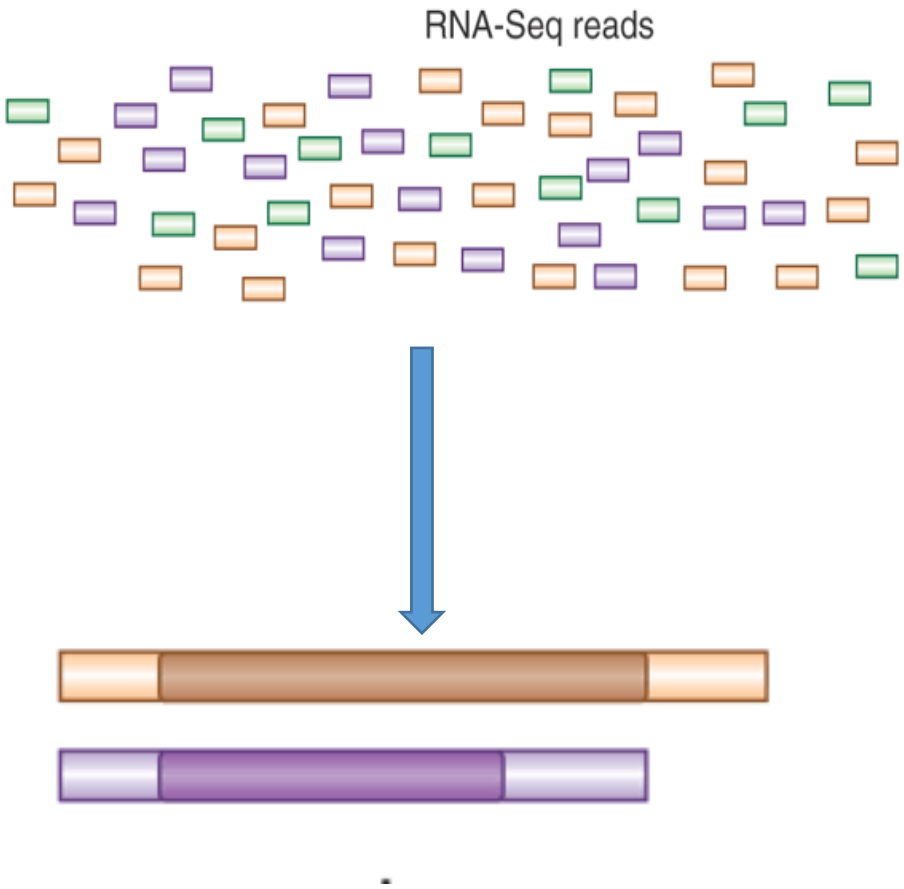
High-throughput Illumina Sequencing (RNA-seq)



Illumina.com

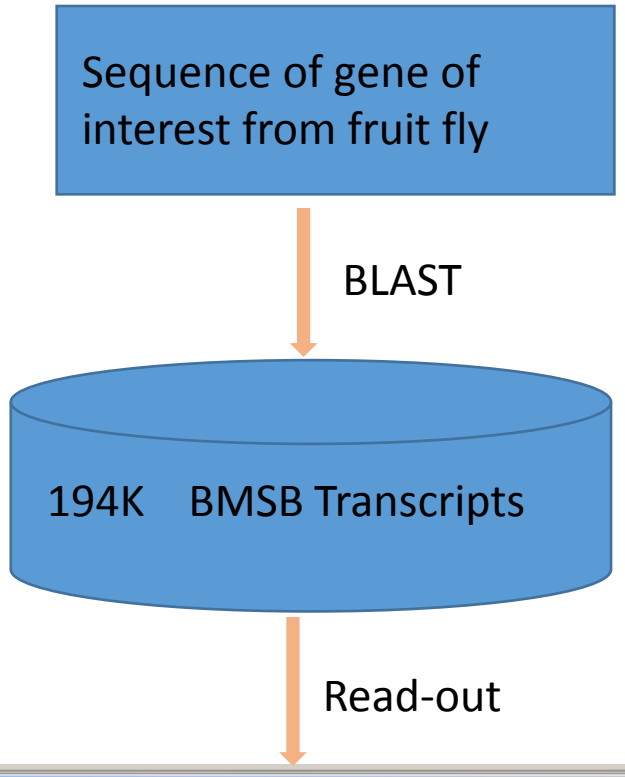
Generates millions of short sequence reads

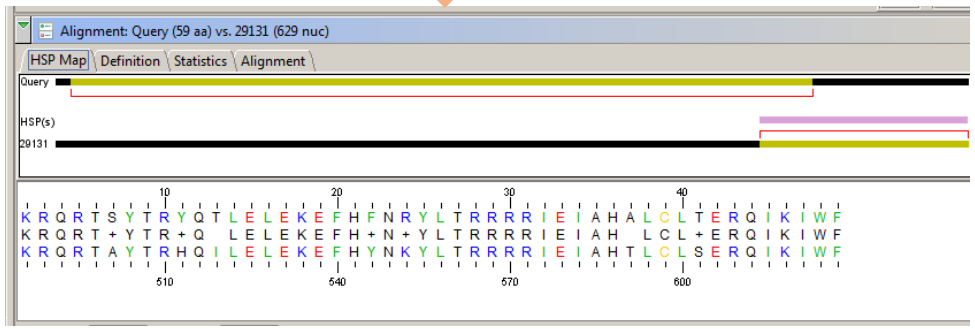
De novo RNA assembly with Trinity



- 194,729 transcripts were assembled from 196,233,912 reads
- 50,599 transcripts were >1000 bp

Identify genes in the Trinity Assembly





- To find a particular gene we want to study from more than 194K transcripts, We used BLAST
- Sequence of gene of interest from fruit fly was used as bait (or query)
- Sequences similar to gene of interest identified by BLAST
- Gene from BMSB identified
- Sequence confirmed with RT-PCR
- Sequence information used for RNAi

Insecticide resistance-related genes

Table 3: Insecticide resistance-related genes transcribed in BMSB and other insects. Inside parentheses is shown the percentage of these genes in the corresponding genome.

Gene function	BMSB	T. castaneum	A. pisum	P. humanus
Cytochrome P450 domain proteins	143 (0.49%)	121 (1.23%)	74 (0.44%)	89 (0.36%)
Carboxylesterases	112 (0.41%)	150 (0.51%)	74 (0.44%)	83 (0.34%)
Glutathione S-transferase	42 (0.15%)	38 (0.39%)	34 (0.20%)	41 (0.17%)
Superoxide dismutases	20 (0.07%)	6 (0.06%)	5 (0.03%)	5 (0.02%)
Catalases	4 (0.01%)	4 (0.04%)	1 (0.01%)	1 (0.00%)
Glutathione peroxidases	7 (0.03%)	3 (0.03%)	1 (0.01%)	3 (0.01%)
Ascorbate peroxidases	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total number of ORFs in each	29148	9848	16879	10774

Summary of Genetic studies of BMSB

Isolate and sequence BMSB mRNA

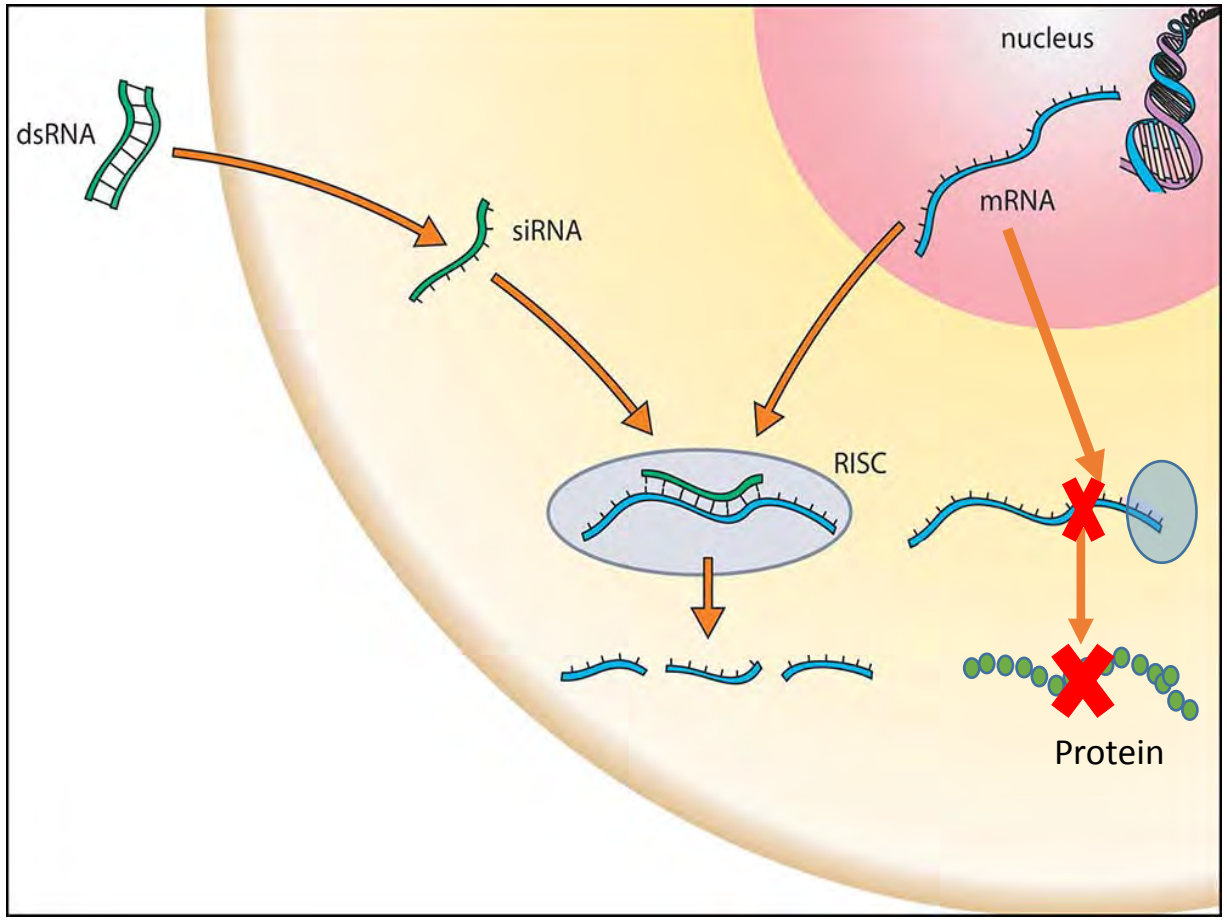
- Transcriptome sequence complete
- Many genes related to insecticide resistance were found.
- Manuscript in preparation

Current plans:

- Identify genes of interest for pest control
- Ideas for genes of interest?

Subobjective 2.2.8. Establish RNAi technology to control the invasive BMSB.

How does RNAi work



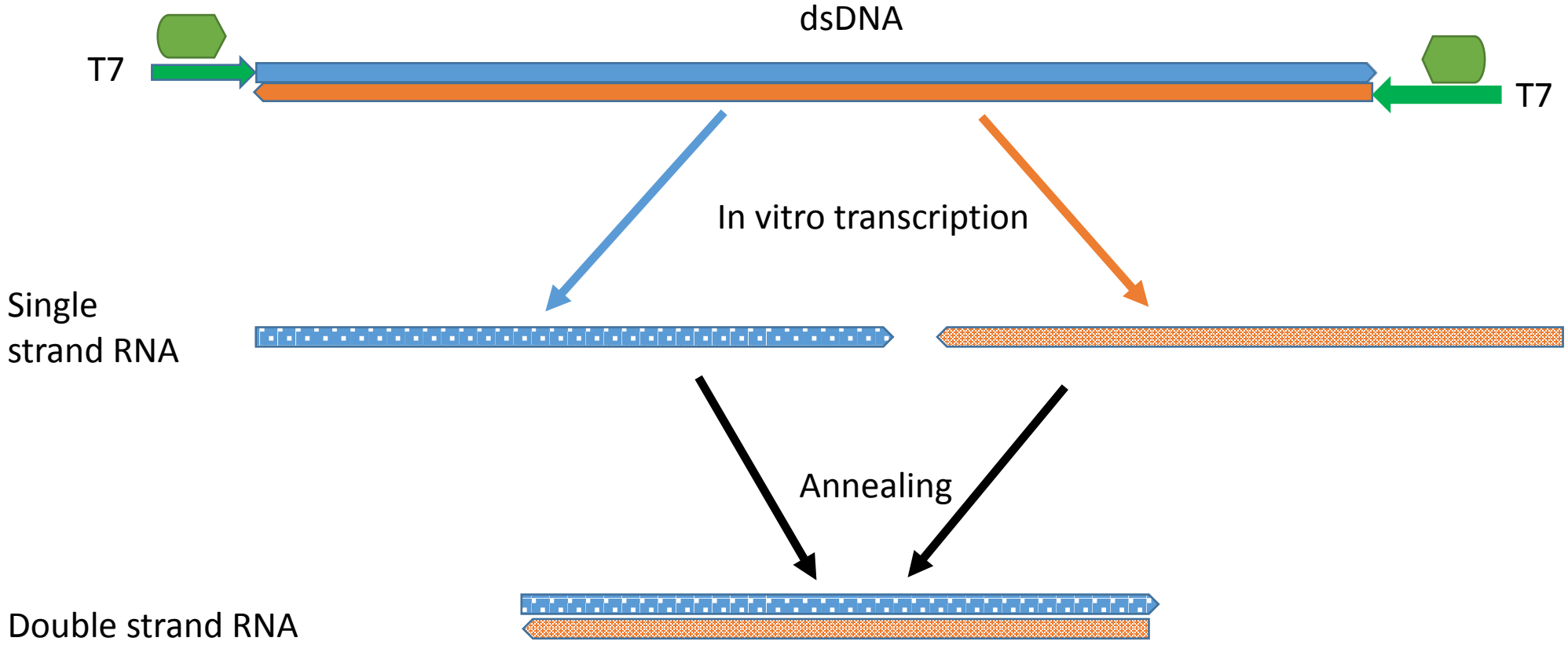
Modified from Wikipedia.com

- Genetic information in DNA is translated into mRNA, then protein.
- dsRNA causes mRNA of the gene it matches to be destroyed
- Functions of the protein are lost
- Different phenotypes depend on function of the genes/proteins.

Goals of RNAi studies--From a Pest Control view

- Choose genes that are important for survival, fertility and development, so we can kill the bug or block their reproduction.
- Genes that will only effect BMSB
- To establish the method: Genes cause phenotypes that one would not expected to see in wild type.

Making dsRNA



RNAi Delivery by Injection into Adults

dsRNA delivered to female BMSB by injection

Target different types of genes

- Inject parent and expect death or decreased fitness
- Inject parent and see defect in offspring (dead or deformed)
- The effect can last for a few weeks.

BMSB *v-ATPase* RNAi Preliminary

- v-ATPase dsRNA or injection buffer were injected into the abdomen of adult BMSB.

Results:

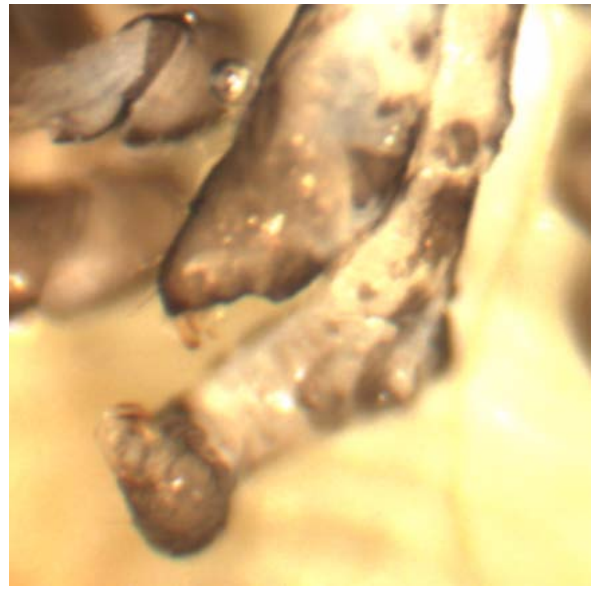
- **v-ATPase group**, three out of six dead within 24 hours
- **Buffer only group**, zero out of 5 dead
- All the surviving bugs still alive after ten days.
- One day Fasting experiment **v-ATPase group**, three out of three dead; **Buffer only group**, one out of five dead

Tentative conclusion: Targeting v-ATPase could decrease fitness

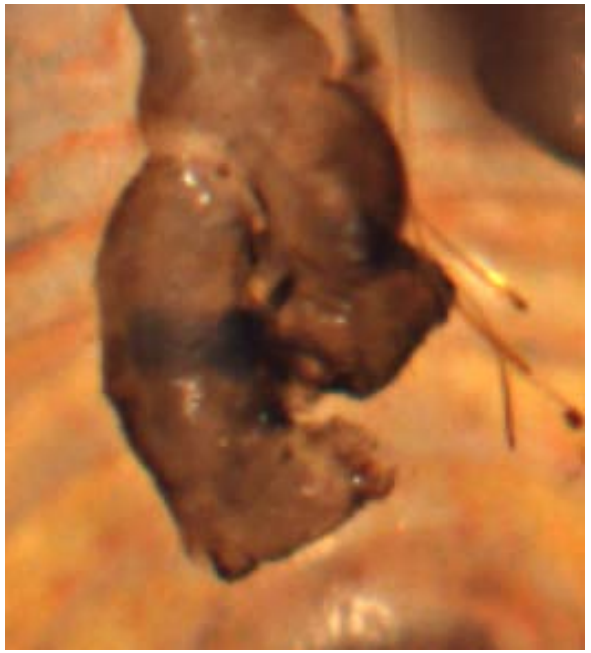
Scr RNAi causes abnormalities of BMSB mouthparts in offspring

Wild Type

SCR Parental RNAi



Scr RNAi – another example



Subobjective 2.2.8. Establish RNAi technology to control the invasive BMSB - summary

- RNAi is effective in BMSB
- RNAi injected into females causes defects in offspring
- RNAi gave expected phenotype for Scr with 100% penetrance
- Preliminary effectiveness of a second RNAi target
- Currently testing additional genes

Thanks!

- Galen Dively and his whole lab
- Paula Shrewsbury
- Nancy Harding
- Panagiotis Ioannidis
- Julie Dunning Hotopp