

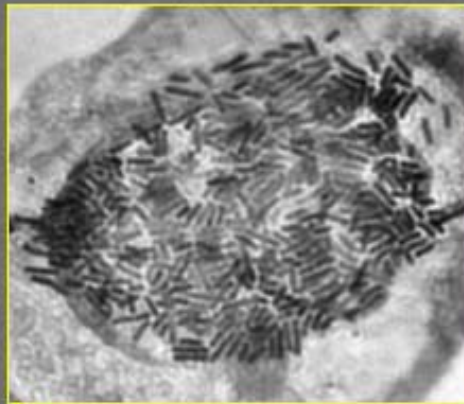
Generation and Analysis of an EST dataset from recovering axenic dauer juveniles of *Steinernema carpocapsae*

Zoe Mulroy Hehir



A special organelle (vesicle) inside the nematode provides an excellent environment for the bacteria to survive between infection cycles.

Xenorhabdus nematophila inside vesicle



Free-living infective
juvenile nematode :
Steinernema carpocapsae

Killing without Symbiotic Bacterium

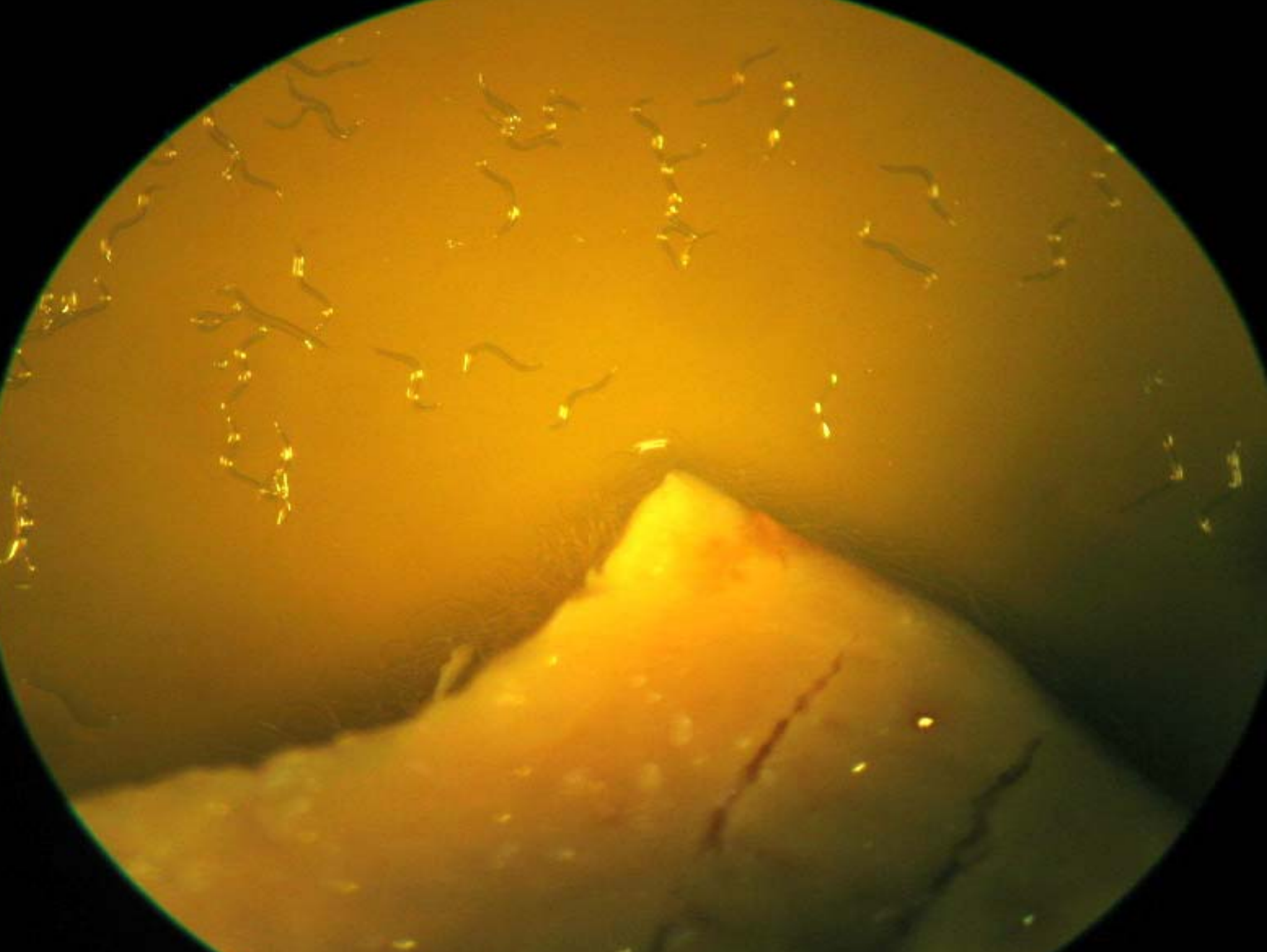
- Boemare *et al.* 1983. Axenic *S. capocapsae* can infect and kill their insect host
- *Heterorhabditis* spp. require their bacterium *Photobabdu* to kill their host
- Genes that encode insecticidal proteins. These genes are usually encoded in the genome of the bacterial symbiont in other EPN pathosystems

Aim

- Identify strains of *S. carpocapsae* that are highly virulent to the host
- Identify tissues in the recovering nematode that are transcriptionally active
- To generate an EST library
- Identify novel genes encoding proteins toxic to the insect host

S. carpocapsae cultured axenically

Strain	Origin
Breton	France
All	USA
A10	USA
DD136	USA
C4A	China



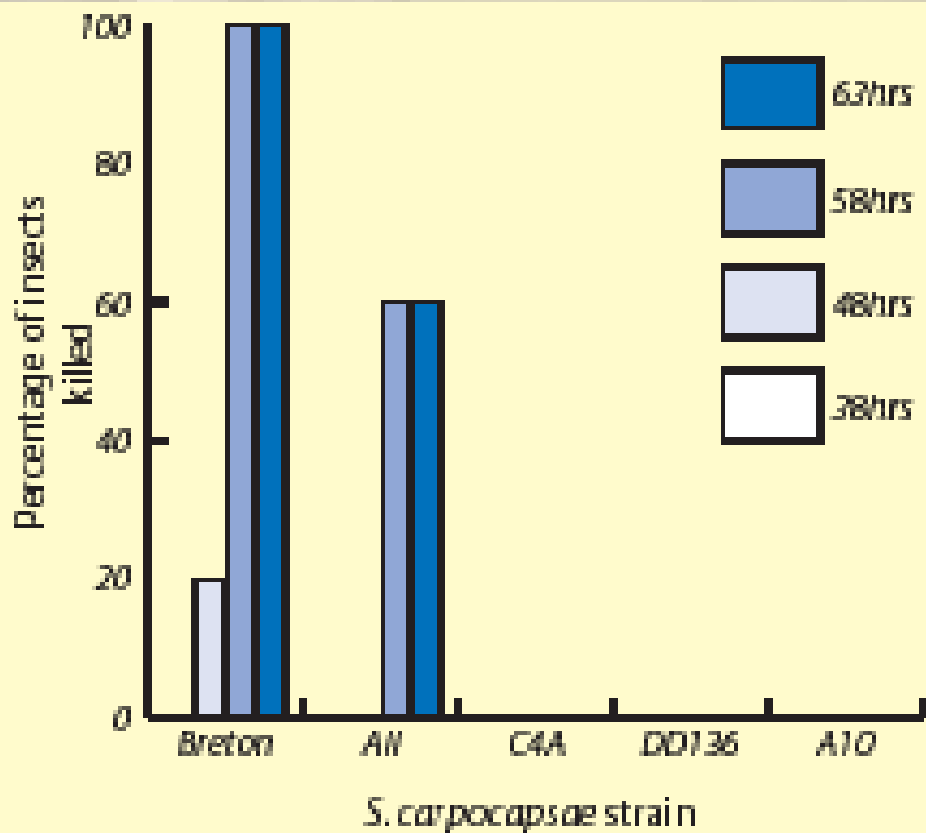
Virulence Tests

Virulence tests were conducted on *G. mellonella*

Dauer juveniles were injected at doses of 1, 5 and 10 DJ/*G. mellonella*

The strain Breton was found to be the most virulent killing 100% of exposed insects at a dose of 1 DJ/*G. mellonella* within 53 hrs

This kill rate is comparable with the nematode bacterial complex



Virulence of *S. carpocapsae* strains against *Galleria mellonella*

- Graph shows percentage of *G. mellonella* killed at a dose of 1 DJ/*Galleria*
- Breton found to be the most virulent killing 100% of exposed insects within 53hrs

Transcriptionally active Tissues

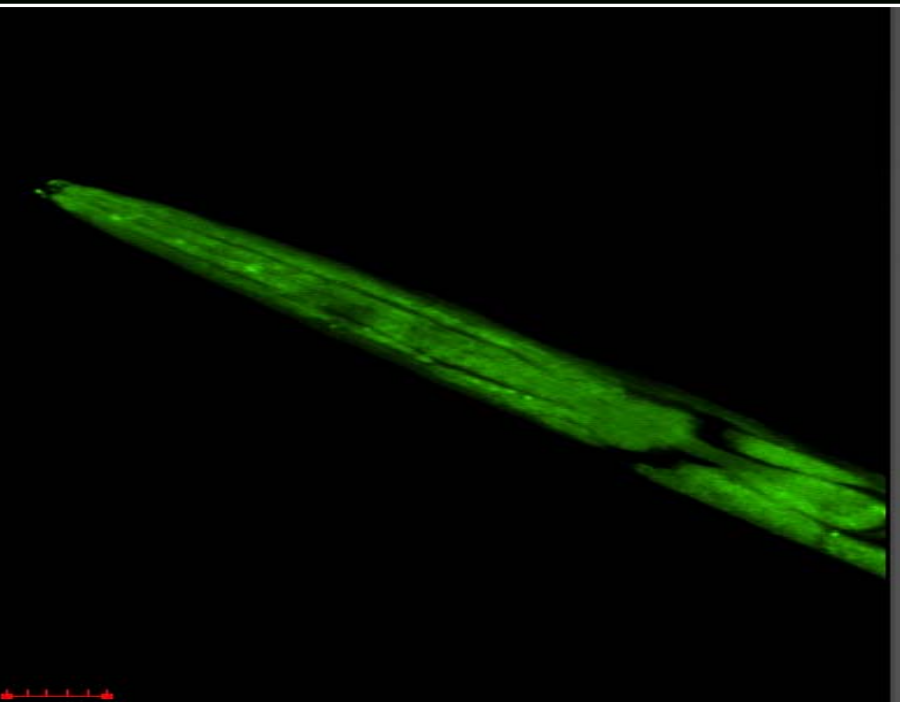
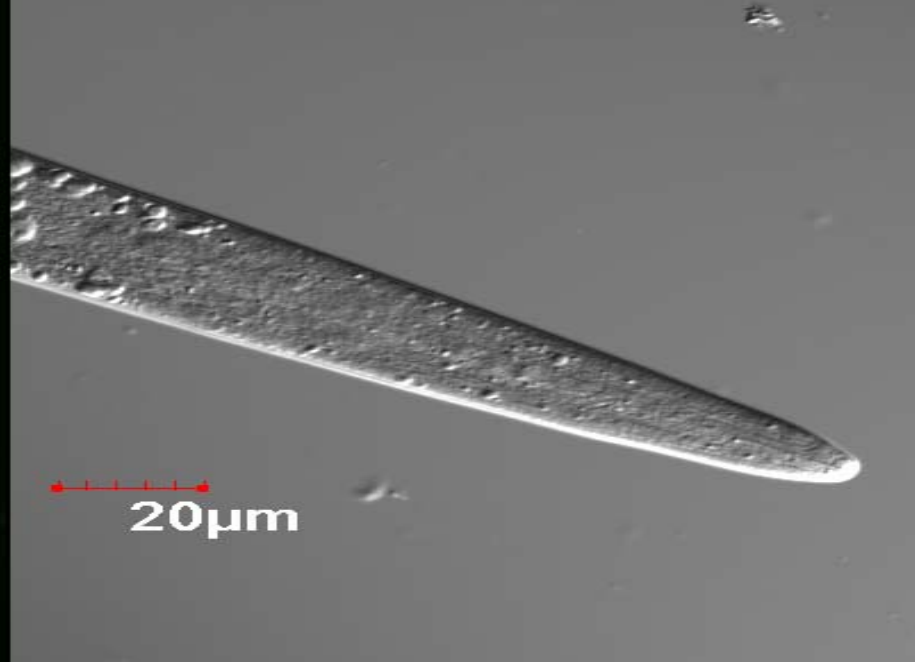
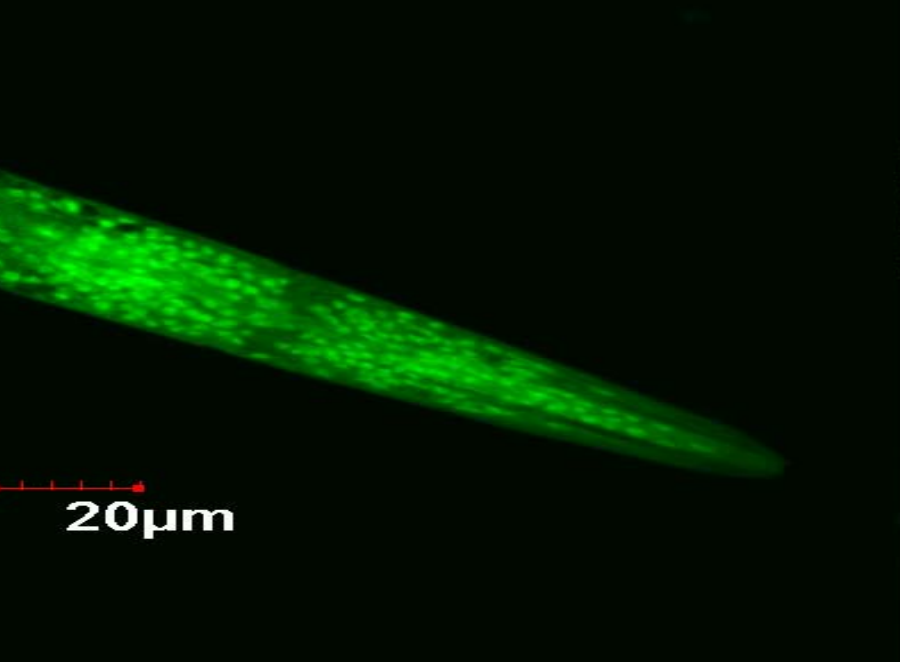
Fluorescent dye that emits light when bound to RNA

Binding of SYTO-12 to a particular cell or tissue is indicative of high levels of transcription within that tissue

Stain bound to pharyngeal glands and genital primordia, indicating that these tissues are transcriptionally active

Activity in the gland cells suggests that these cells produce large quantities of secreted proteins that may encode insecticidal toxins at this stage

Presence of RNA was highest 4 hrs after recovery



Creating a cDNA library

A cDNA library was made using the Long-Distance PCR method described in the Creator™ SMART™ cDNA library construction kit (BD Biosciences)

Over 1×10^6 primary recombinants were present after cloning

Colony PCR

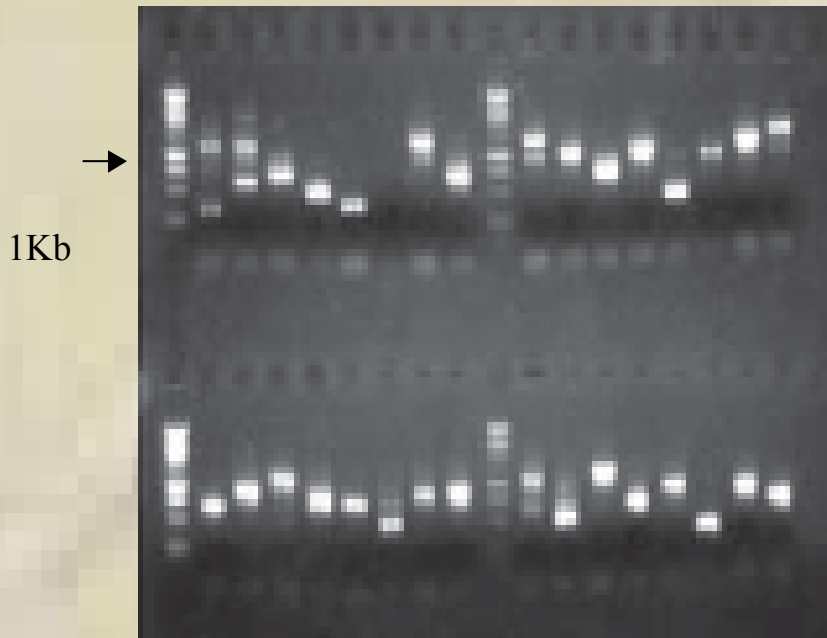


Fig. 1 ColonyPCR reaction examining insert sizes in *S. carposapae* cDNA library. Markers (lanes 1 and 10 in each panel) are Promega 1Kb ladder. The arrow indicates the 1Kb fragment in this ladder

Creating a cDNA library

- The transformed cells were then plated onto 20cm² LB chloramphenicol agar plates at a density of approximately 3000 colonies per plate
- In total 15,360 bacterial colonies were then picked into 384 well plate using a Q-bot robot (Genetix)
- The bacterial cultures were grown in the plates in freezing medium and stored at -80°C before use
- The library was duplicated for security

Plasmid Preparations and Sequencing

- A Biomek 2000 liquid handling robot transferred the bacterial stocks from the 384 well plates to 96 well plates
- The plasmids were purified from alkaline lysed bacterial suspensions using Millipore 96 well clearing and binding plates
- 96 well plates were run on an ABI 3730 capillary sequencer

Results

- 4608 clones were sequenced from this library, from the sequenced ESTs 269 contigs were assembled
- Batch BLAST analysis in Bioinformatics unit at SCRI. This includes contig analysis followed by searches against DNA (BLASTN), and protein databases (BLASTX) and BLASTN searches against dbEST a NCBI

Stress Resistance

- Heat shock proteins
- Glutathione s-transferase

Transport

- Amino acid transporter
- Lipid binding proteins
- Fatty acid binding proteins

Growth

- Elongation factor
- TGF- β

Detoxification

- UDP - Glucotransferase
- Transthyretin
- Cytochrome P450

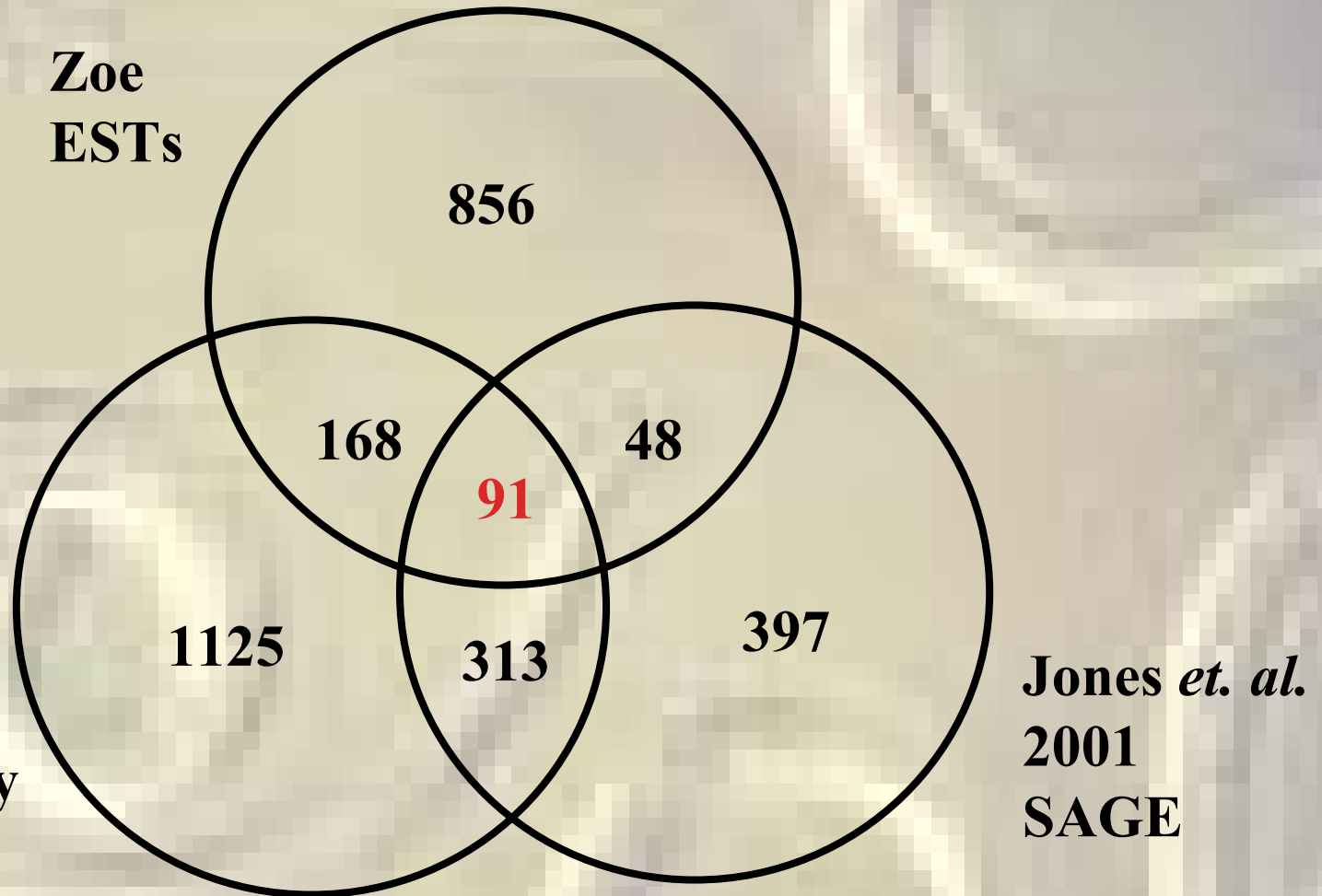
Metabolism

- Zinc finger proteins
- Calmodulin
- Proteosomes
- Ubiquitin

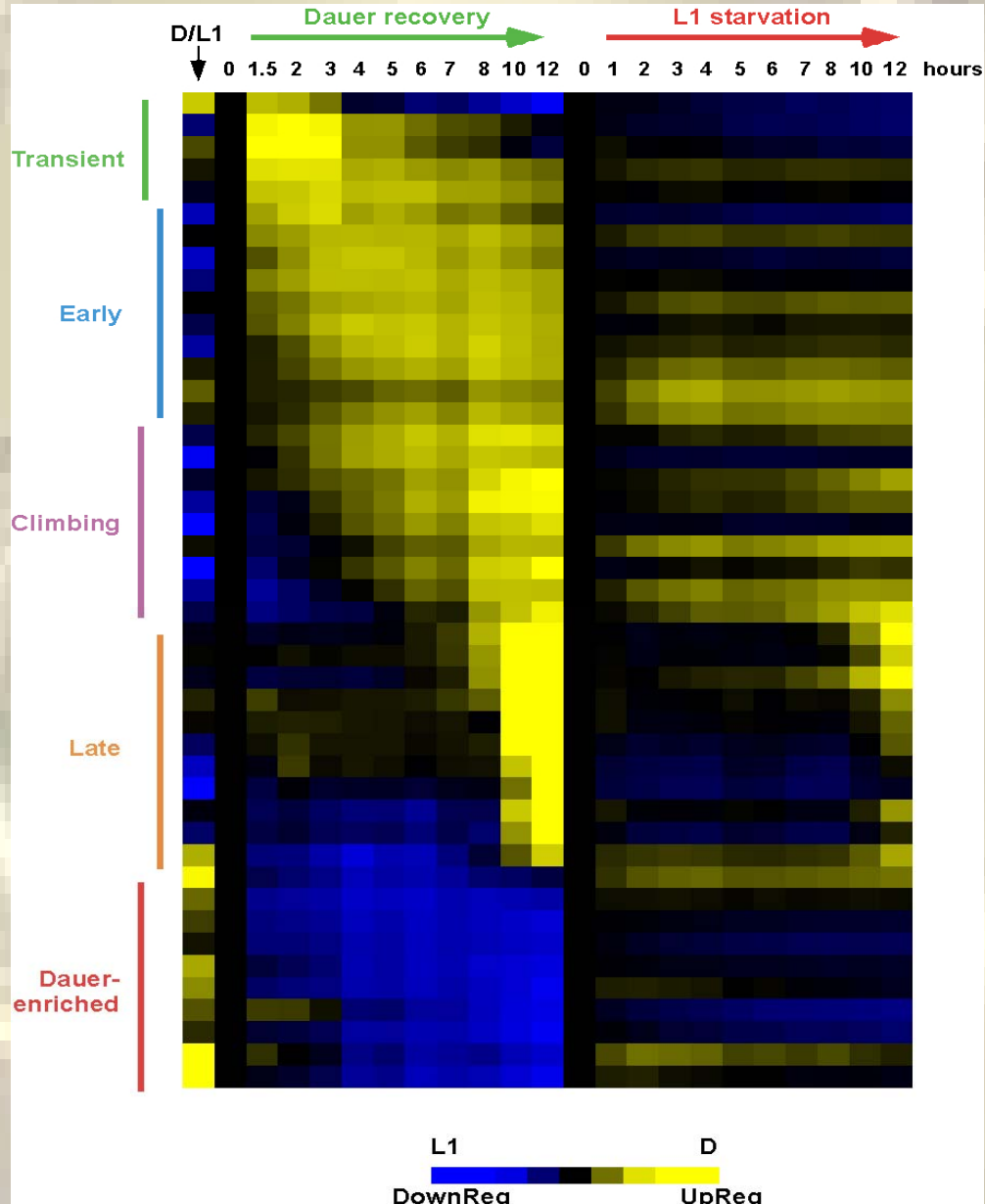
Signal Transduction

- Insulin growth factor
- Transforming growth factor

A Database Comparison



Wang and Kim Microarray Analysis



Kegg Analysis

Kegg Regulatory Pathways	Enzymes
1. Metabolism	237
Carbohydrate Metabolism	36
Energy Metabolism	49
Lipid Metabolism	29
Nucleotide Metabolism	26
Amino acid Metabolism	68
Glycan Biosynthesis and Metabolism	2
Metabolism of Cofactors and Vitamins	13
Biosynthesis of Secondary Metabolites	2
Xenobiotics Biodegradation and Metabolism	12
2. Genetic Information Processing	129
Transcription	7
Translation	58
Folding Sorting and Degradation	8
Replication and Repair	2
3. Environmental Information Processing	4
Signal Transduction	4

Future Work

- cDNA microarray analysis

- The EST library has been microarrayed onto high density BAC filters

- Non-recovered vs recovered

- Virulent strains vs non-virulent

Future Work

- Investigation into the novel genes
- Full length sequencing of genes of interest & subcloning into a bacterial expression host
- Injection of recombinant bacteria into *G. mellonella*
- In situ hybridization

Acknowledgements

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