

TURBO TALKS



Molecular Detection of eukaryotic pathogens in esophageal cancer

Jianzhen Xu, Shantou University medical college, China

Research goal: To investigate whether or not eukaryotic pathogen infection play a role in esophageal cancers

How? using next-generation sequencing through a polymerase chain reaction (PCR)-based analysis of 16S rRNA genes(metagenomics)

Samples/Model System: biopsy

The challenge: bioinformatics analysis

Workshop goals: To learn how EuPathDB can assist in designing the project and analyzing the data



Generation of transgenic Plasmodium parasite

Jianlin Jiang, Emory University

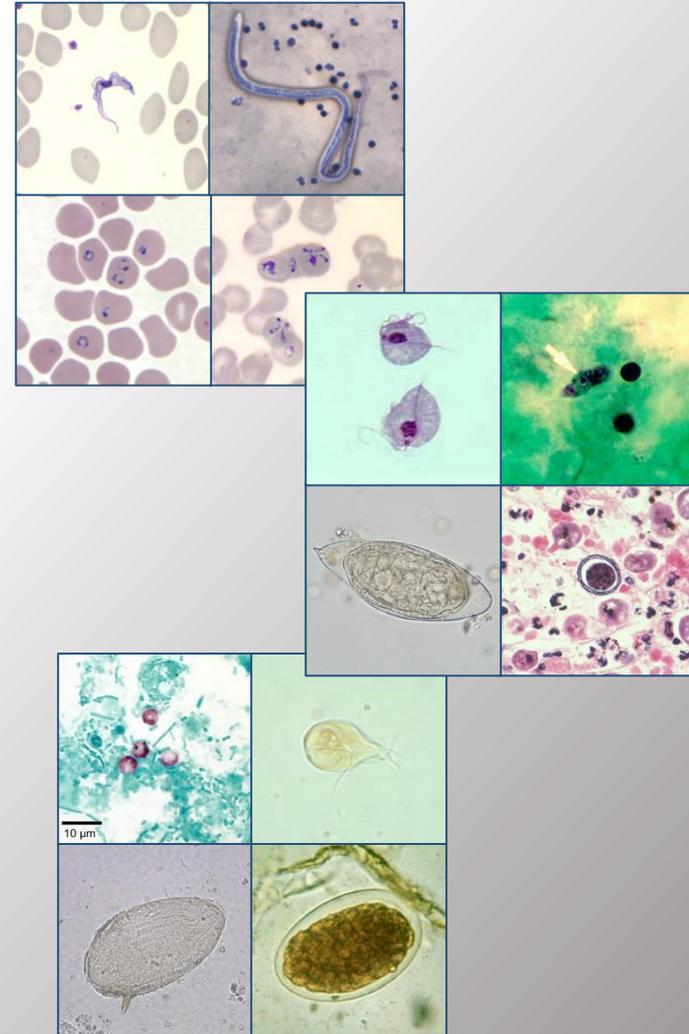
Research goal: Integration of fluorescent gene expression cassette into parasite genome

How? double cross over recombination, or Cas9 technique

Samples/Model System: Monkey in vivo transfection

The challenge: Want to download all homologous gene and PCR primer verification

Workshop goals: To learn hoe to find homologous gene and design gene specific PCR primer



Characterization of *Toxoplasma gondii* Tgj1 Type I Hsp40

Jonathan Munera-Lopez, National University of General San Martín (Argentina)

Research goal: Identify the biological role of Tgj1 in *Toxoplasma gondii*

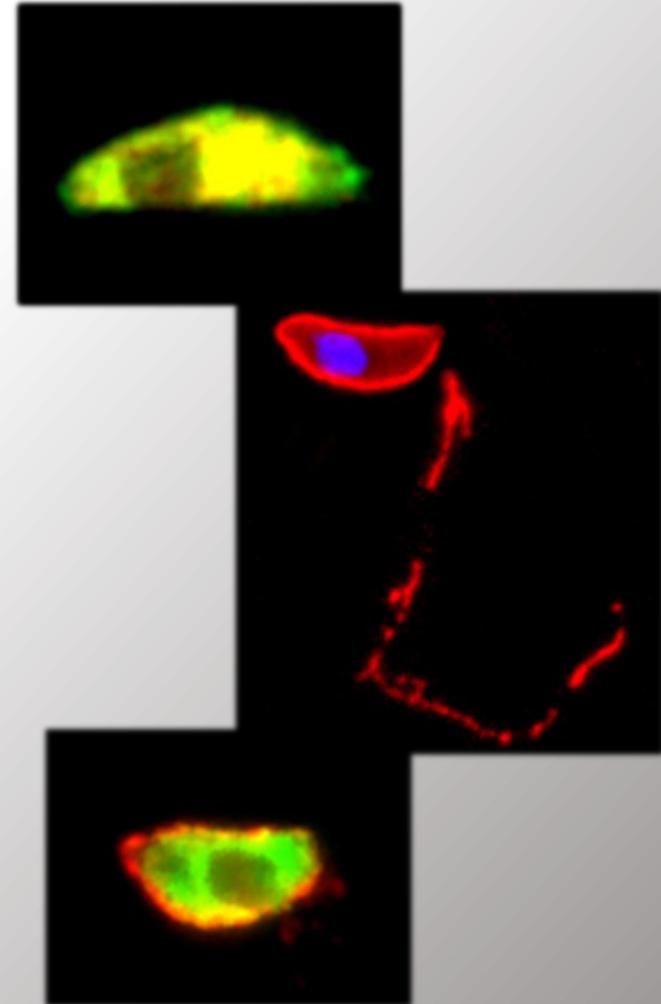
How? Localization and functional studies by endogenous tagging, CRISPR Knock-outs and invasion assays.

Samples/Model System:

Toxoplasma gondii

The challenge: Determine if Tgj1 is involved in invasion.

Workshop goals: Develop efficient search strategies and analysis of the outcome.



Epidemiology and Population Biology of Grape Downy Mildew in Georgia

Cheng-Fang Hong, University of Georgia

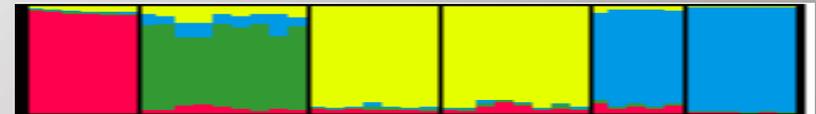
Research goal: Monitor downy mildew epidemics and clarify the importance of sexual reproduction and population biology of downy mildew pathogen in GA.

How? CAPS analysis of ITS and β -tubulin gene and SSR markers.

Samples: Vineyard field samples, e.g. infected tissues, leaf litters.

The challenge: Integrate and interpret information obtained from experiments and translate into knowledge.

Workshop goals: To learn how EuPathDB can assist in analyzing my results.



Grapevine downy mildew: population diversity and role of sexual reproduction in epidemic dynamics in subtropical regions

Meyrielle Pires de Camargo, University of São Paulo/University of Georgia

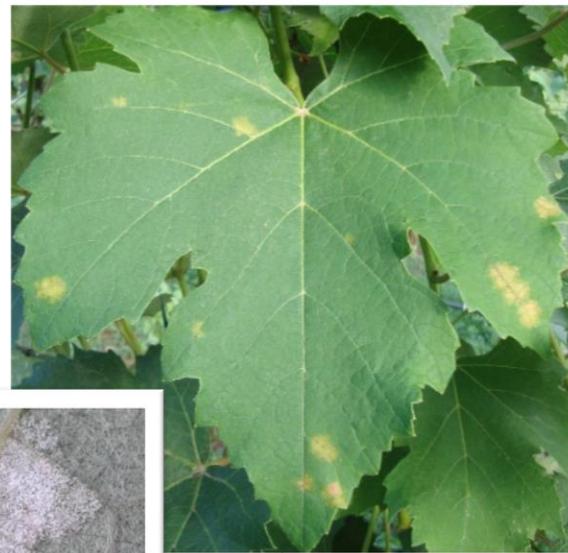
Research goal: Investigate the population diversity of *P. viticola* from GA-USA and SP-BR, to understand the role of primary and secondary infections in downy mildew epidemics and verify the presence of cryptic species of *P. viticola*.

How? SSR analysis; CAPS and DNA sequencing (ITS, β -tubulin);

Samples/Model System: single-lesions of *P. viticola* from grapevine leaf tissues

The challenge: Data analysis and interpretation of results

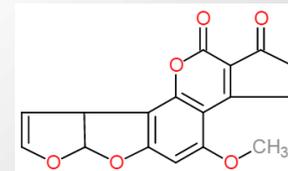
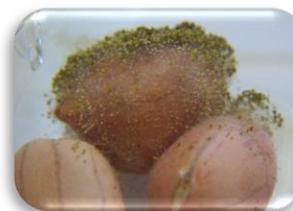
Workshop goals: Acquire knowledge of different database resources to improve data analysis of my research



Aflatoxin biosynthesis in *Aspergillus flavus* and its relationship to environmental oxidative stress.

Jake Fountain, University of Georgia

Dr. Gaurav Agarwal, University of Georgia



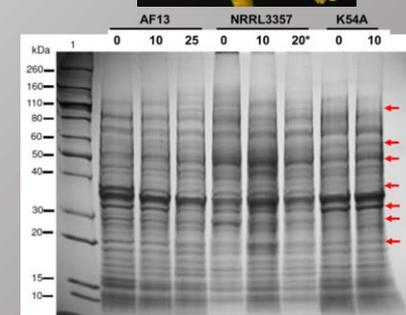
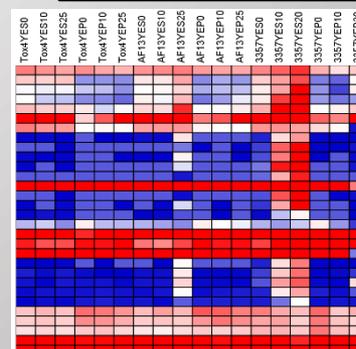
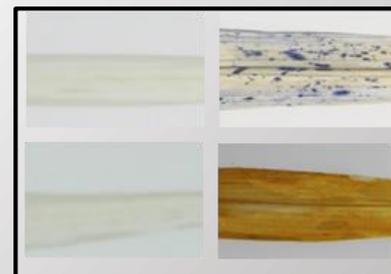
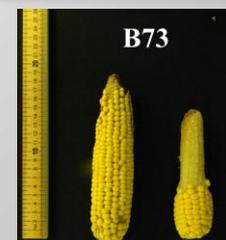
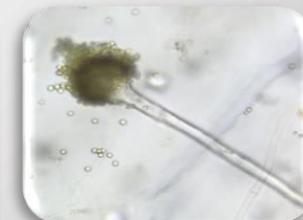
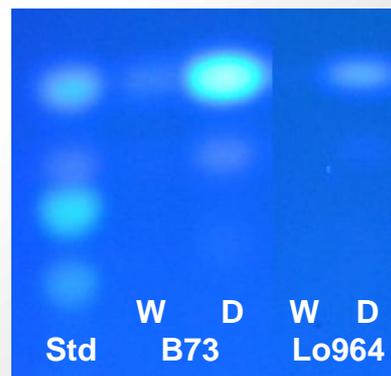
Research goal: Investigate the biological role of aflatoxin biosynthesis, particularly under environmental stress conditions, and to examine host plant responses to drought stress.

How? RNA sequencing and iTRAQ proteomics of the pathogen under increasing levels of stress. Metabolomics of host plant tissue.

Samples/Model System: *A. flavus* mycelia and maize kernel tissues.

The challenge: Identifying connections between pathways to determine coordinated responses to stress.

Workshop goals: To learn how EuPathDB and FungiDB can assist in analyzing my results and informing my research.



Unraveling *Toxoplasma gondii* and *Trypanosoma cruzi* Glycome

Elisabet Gas (PhD), Chris West lab., BCMB Dept., UGA

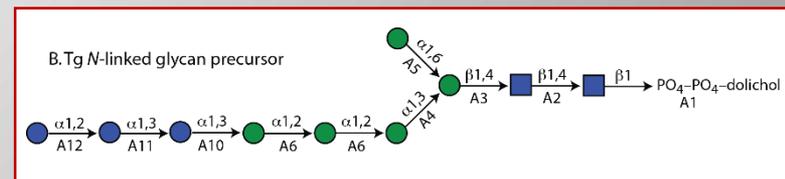
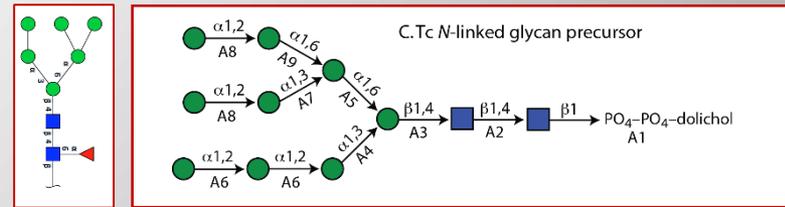
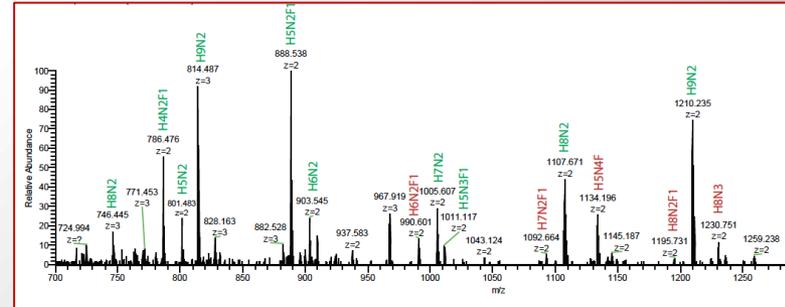
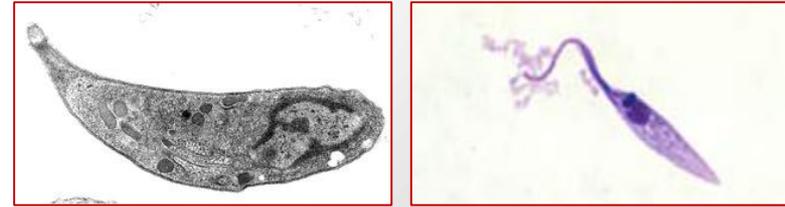
Research goal: to generate glycan libraries for *T. gondii* and *T. cruzi*

How? Developing a glycan pipeline analysis that allows automatic glycan detection and structure assignment

Samples/Model System: *T. gondii* tachyzoites and *T. cruzi* epimastigotes from cell culture

The challenge: to predict the effect of glycosyltransferase KO genes on the glycome

Workshop goals: to learn how EuPathDB can help in analyzing these results, predicting gene function and integrating metabolic pathway data



Streamlined Deletion of *Toxoplasma* Glycogenes Using CRISPR/Cas9

H. Travis Ichikawa, University of Georgia

Research goal: Establish an efficient and cost effective method to generate a deletion mutant library of *Toxoplasma gondii* for the community.

How? Predict *Toxoplasma gondii* glycogenes and delete them by CRISPR/Cas9 system.

The samples: Lab strains of *Toxoplasma gondii*, Type I, II and III.

The challenge: Accurate prediction of glycogenes and development of an efficient and cost effective method for deletion mutagenesis.

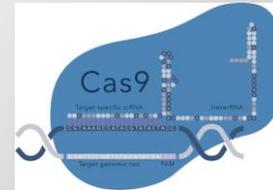
Workshop goals: To learn how EuPathDB can assist in understanding predicted glycogenes better and further developing the project to ask formulated biological questions.



Glycogenes prediction



gRNA/Cas9 plasmid designing

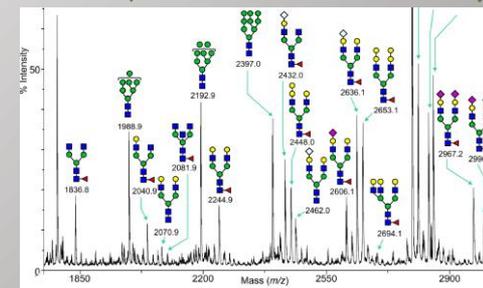
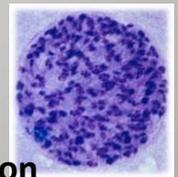


Deletion mutagenesis

Cloning of mutants



Brdyzoite differentiation



Glycomics analysis

Investigating *C. albicans* genome plasticity during growth within the host, Jen Mason, Emory University

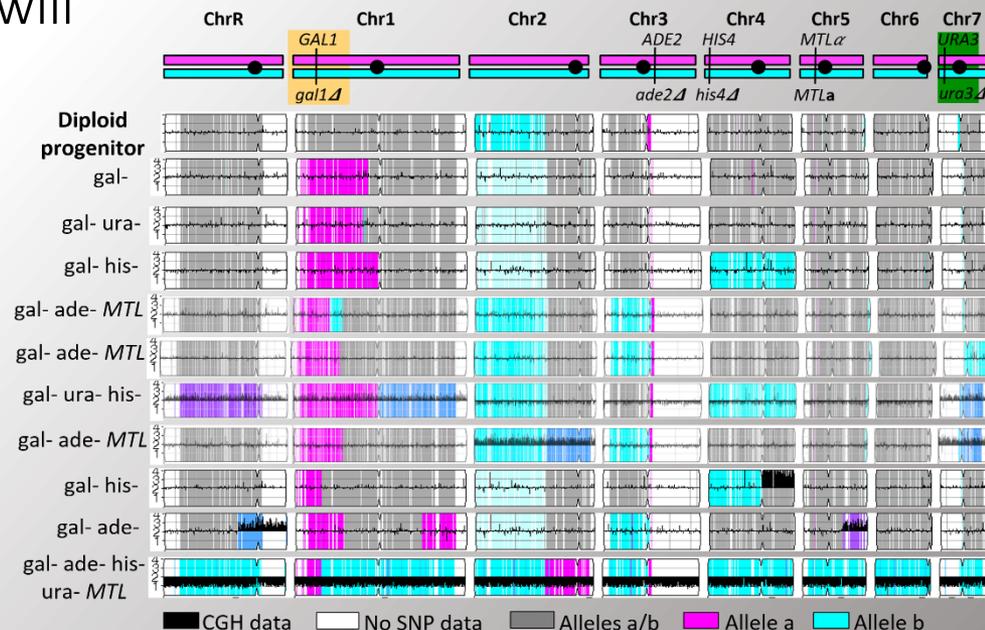
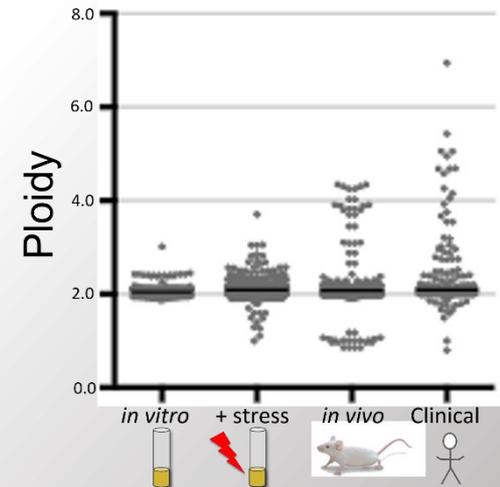
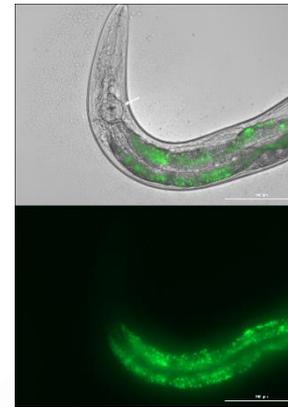
Research goal: Determine how growth within the host influences *C. albicans* genome plasticity in comparison to what is detected *in vitro*

How? LOH, SNP/CGH arrays, flow cytometry, sequencing

Samples/Model System: *C. albicans* will be propagated in a *C. elegans* host

The challenge: Developing the *C. elegans* system to model host interactions with *C. albicans*

Workshop goals: To learn new tools/technologies that can assist in data collection and analysis



■ CGH data □ No SNP data ▒ Alleles a/b ■ Allele a ■ Allele b

Enhanced *Cryptosporidium hominis* genome annotation

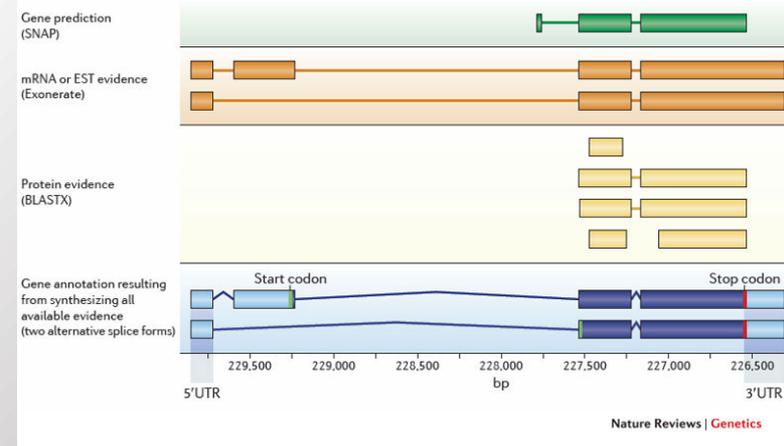
Yiran Li, University of Georgia

Research goal: Generate new annotations for *Cryptosporidium hominis* and provide enhanced functional and metabolic resources for other downstream analysis.

How? Use available experimental data as evidence to generate new annotation for newly sequenced genomes.

The challenge: Lack of physical map and expression data

Workshop goals: To learn how data are organized in EuPathDB and how to dig informative data for my research



Evolution of Multi-copy Gene Families (Dyneins and Sugar Transporters) in the Apicomplexa

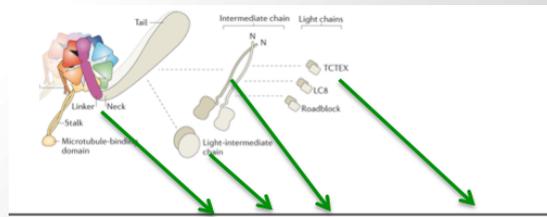
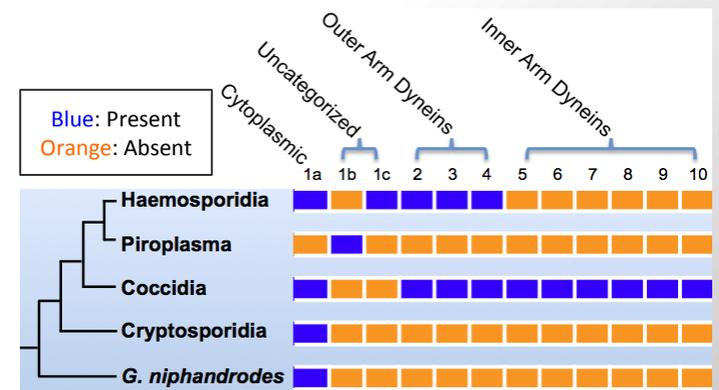
Ousman Mahmud, University of Georgia

Research goal: Characterize gene gain and loss patterns within the Apicomplexa and the biological significances of these patterns

How? Evolutionary analyses: Orthology clustering, Phylogenetics, etc. Data mining of functional data. Wet bench: Epitope Tagging

Samples/Model System: NGS datasets: genomes, transcriptomes, proteomes, etc. Wet bench: *Toxoplasma gondii*

Workshop goals: Learn about the latest EuPathDB tools and datasets that may be of use to my research



Species	DHC	DLIC	DIC	DLC		
				LC8	Roadblock	TCTEX
<i>P. berghei</i>	6	1	3	4	2	4
<i>P. cynomolgi</i>	6	1	3	4	2	4
<i>P. falciparum</i>	6	1	3	4	2	4
<i>P. knowlesi</i>	6	1	3	4	2	4
<i>H. tartakovskyi</i>	6	1	3	3	2	3
<i>B. bovis</i>	1	nf	nf	1	nf	nf
<i>B. bigemina</i>	1	nf	nf	1	nf	nf
<i>B. microti</i>	nf	nf	nf	1	nf	nf
<i>T. annulata</i>	1	nf	nf	1	nf	nf
<i>T. parva</i>	1	nf	nf	1	nf	nf
<i>T. equi</i>	1	nf	nf	1	nf	nf
<i>T. orientalis</i>	nf	nf	nf	1	nf	nf
<i>E. tenella</i>	10	1	3	5	2	2
<i>N. caninum</i>	10	1	3	6	2	3
<i>T. gondii</i>	10	1	3	6	2	4
<i>H. hammondi</i>	10	1	3	6	2	3
<i>S. neurona</i>	9	1	3	6	2	4
<i>C. muris</i>	1	1	1	1	1	1
<i>C. parvum</i>	1	1	1	1	1	1
<i>C. hominis</i>	1	1	1	1	1	1
<i>G. niphandrodes</i>	1	nf	nf	3	nf	1
<i>C. vella</i>	12	1	8	6	3	5
<i>V. brassicaformis</i>	15	2	8	9	2	5

Apicomplexan Dynein Subunit Copy Number

Illustration of Apicomplexan Dynein Heavy Chain Phylogeny

Genomics-based laboratory surveillance and reference diagnosis for *Cyclospora cayetanensis*

Yvonne Qvarnstrom, CDC

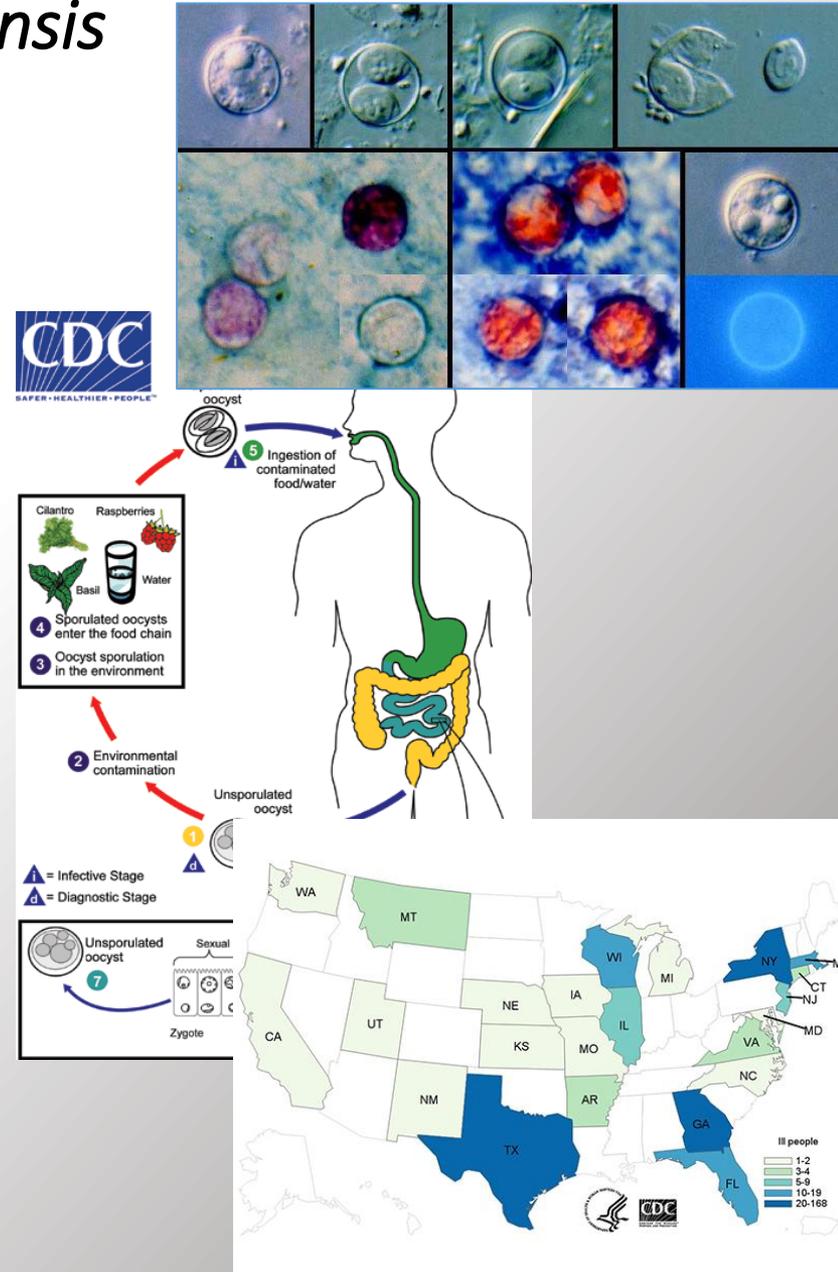
Research goal: Develop a typing method and better diagnostic tests to improve outbreak response

How? Genomics, transcriptomics and proteomics

Samples: Oocysts purified from human stool samples

The challenge: No reference genome available. Extremely little material to work with.

Workshop goals: To learn how EuPathDB works and if it could be a platform for the *Cyclospora* data.



Acquisition and Utilization of Host Macromolecular Nutrients by *Toxoplasma gondii*

Paige Teehan, Clemson University

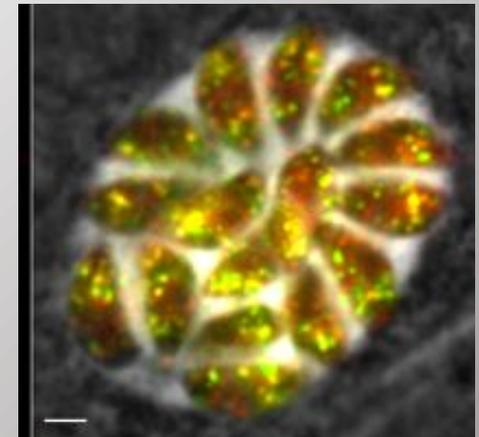
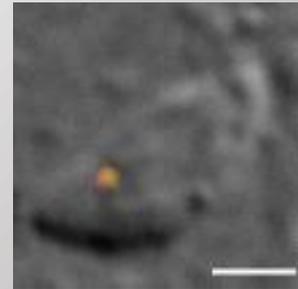
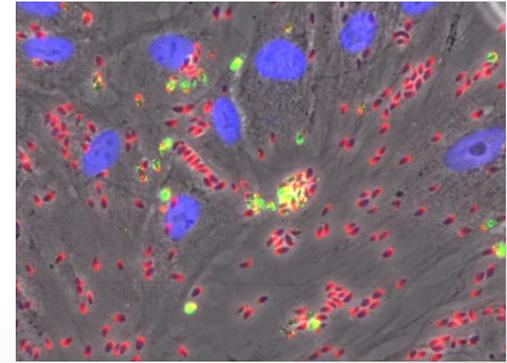
Research goal: Characterize the central role of the VAC/PLV in the utilization of host nutrients

How? By using a global epitope tagging strategy to identify more VAC/PLV-associated peptidases

Samples/Model System: *T. gondii* grown in HFF cells

The challenge: Correctly placing epitope tags to identify new genes without disrupting their function

Workshop goals: To learn about features of EuPathDB and ToxoDB that will assist in advancing my research



How *T. brucei* sense and respond to environmental glucose

Yijian (Evan) Qiu, Clemson University

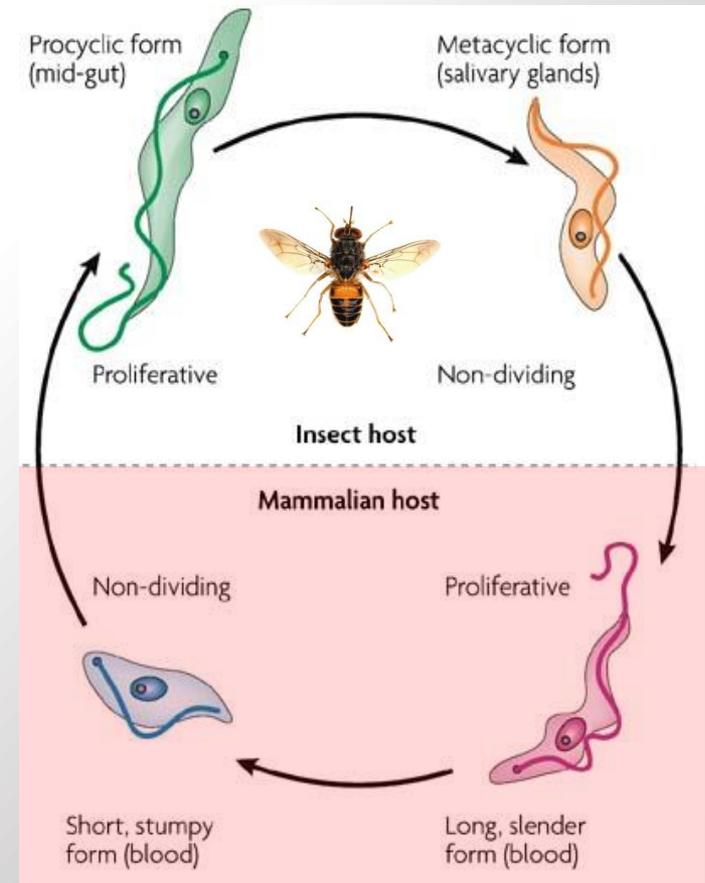
Research goal: Study the mechanisms enable the parasites to respond to difference in availability of glucose in the suite of niches they occupy

How? Perform RNAseq and RT-PCR on trypanosomes growing in different glucose levels

The samples: Cultured blood stream form and procyclic form parasites

The challenge: Blood stream form cannot live in the absence of glucose; Metacyclic form not easy to grow

Workshop goals: To learn how to EuPathDB to combine different datasets in analyzing my results



Modified from Lee et al., Nature reviews, 2007

Identification of a Pex3 homolog in *Trypanosoma brucei*

Logan Crowe, Clemson University

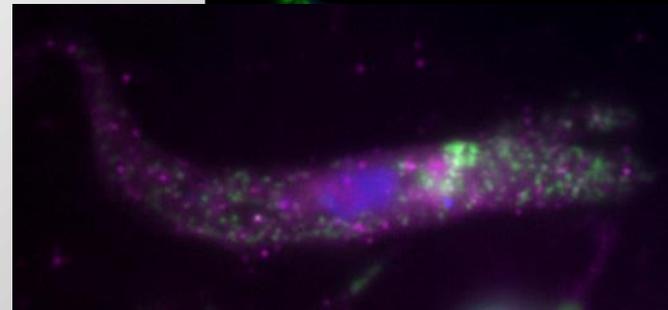
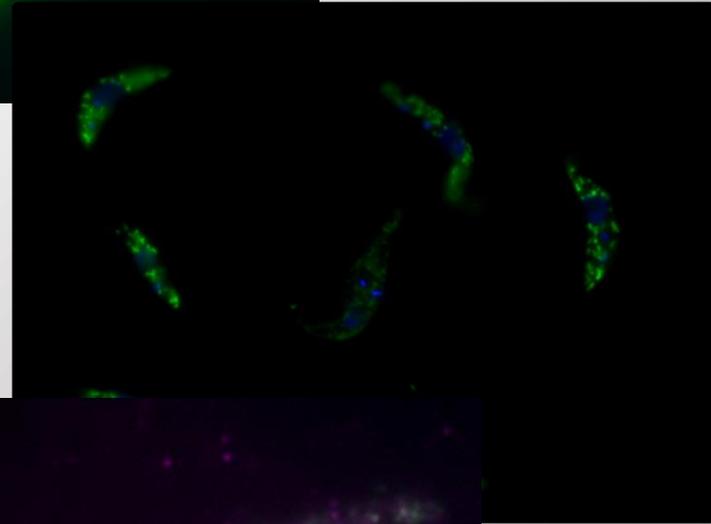
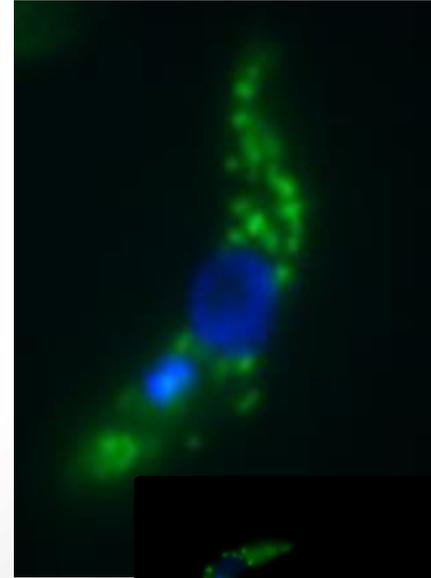
Research goal: Identify *T. brucei* Pex3

How? Sequence similarity to known Pex3s, functional characterization of putative Pex3, localization

Samples/Model System: *Trypanosoma brucei*

The challenge: There is low sequence similarity in peroxins, making identification difficult

Workshop goals: Learn better methods for identifying genes beyond BLAST



A DNA Sequence Based Surveillance System for Cryptosporidiosis



Kevin Tang, CDC, Atlanta, GA

Research goal: Develop DNA sequence based surveillance system to reduce the transmission of Cryptosporidium in the United States

How? Use NGS sequencing for Cryptosporidium genotyping and identification.

The samples: Stool specimens

The challenge: Purification of Cryptosporidium pathogen and differentiating pathogen DNA in clinical samples from host or other contaminated eukaryotic DNA

Workshop goals: To learn how EuPathDB can assist in analyzing the sequence data



Calcium entry and calcium uptake research in *Toxoplasma* calcium stores

Le Chang, University of Georgia

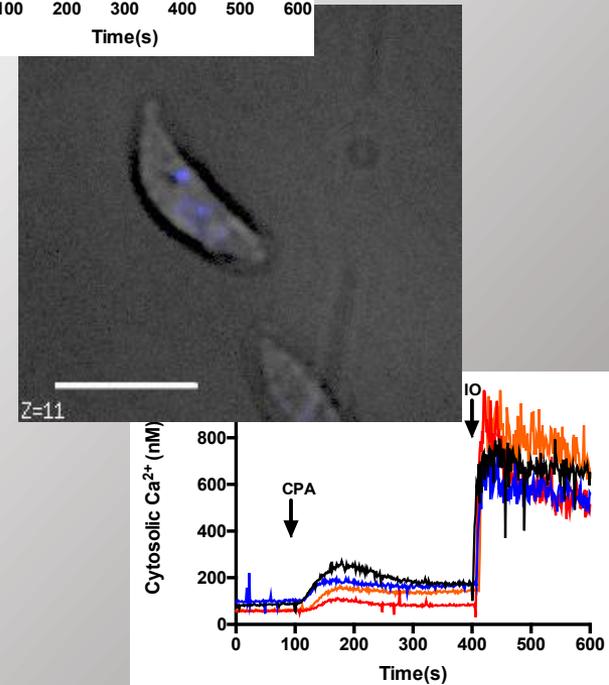
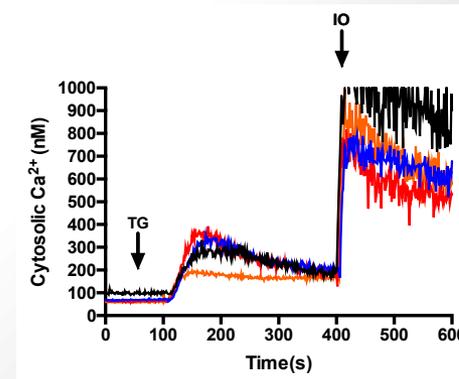
Research goal: By measuring calcium indicators fluorescence modification to figure out how calcium entry and calcium uptake in toxoplasma.

How? First, to load toxoplasma with different indicators or dyes in different part of the parasite, then use fluorimeter to measure modification of these indicators. Then by adding different chemical reagent to start research.

Samples/Model System: toxoplasma

The challenge: toxoplasma calcium stores

Workshop goals: To learn how to use EuPathDB to search essential gene in a efficiency way to assist my research.



Discovery and Characterization of Novel Glycosyltransferases that Modify *Toxoplasma* Skp1 in the Cytoplasm (Kazi Rahman)

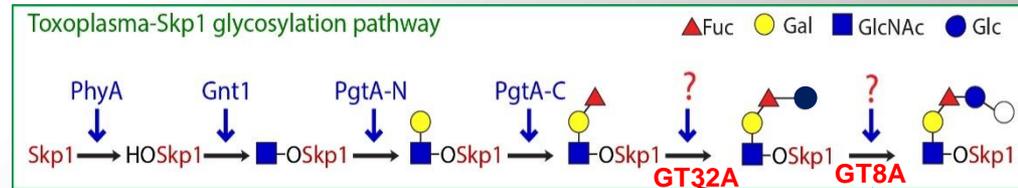
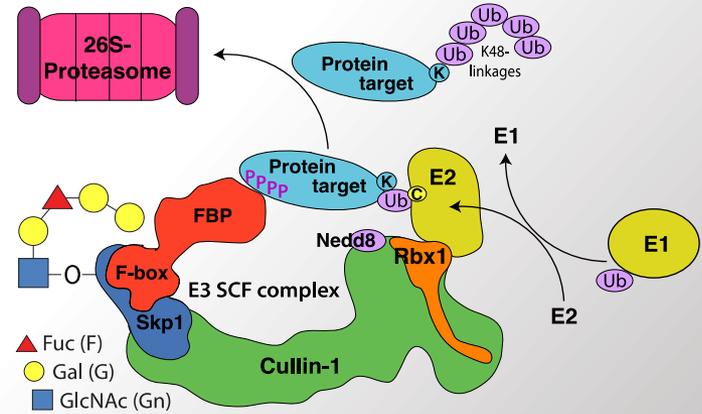
Research goal: Identification and Characterization of glycosyltransferases that transfer terminal disaccharide on *Toxoplasma* Skp1.

How? Bioinformatics-based prediction of entire glycosyltransferases from *Toxoplasma* and selection of putative Skp1 modifying glycosyltransferases based on evolutionary and functional analysis

The samples: *Toxoplasma* tachyzoites

The challenge: Prediction of glycosyltransferase domains and their orthologs due to low sequence homology

Workshop goals: To learn how EuPathDB can assist in identifying the evolutionary related glycosyltransferases that potentially modifies Skp1 in other organisms.



- Toxoplasma* proteome (~8400 proteins)
- Putative Glycosyltransferases (45)
- Cytosolic glycosyltransferases (10)

Biochemical and genetic analysis of a predicted glycogenin homolog in *Toxoplasma gondii*.

Msano Mandalasi, University of Georgia

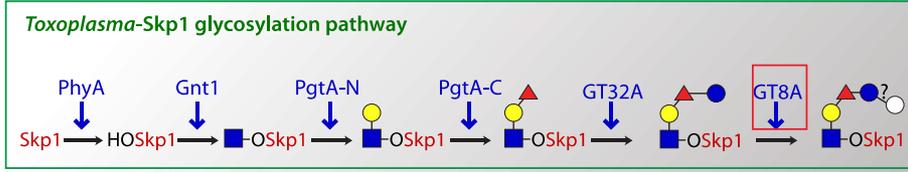
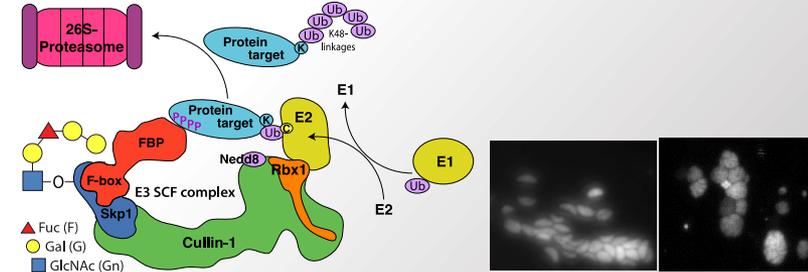
Research goal: Analyze and characterize function and structure of GT8A in Type I RH and Type II Me49.

How? Disrupt GT8A by CRISPR/Cas 9.

Samples/Model System: *Toxoplasma gondii* RH WT and Me49 rfp.

The challenge: Complementing GT8A by CRISPR/Cas 9 on UPRT locus.

Workshop goals: To learn how to identify gene elements from ToxoDB in making constructs for complementation in knock-out strains.



green names- glycogenin like
blue- GT8a-like

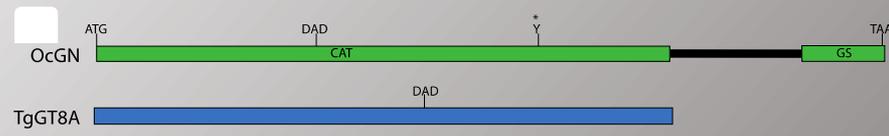
```
Oc 1-----MTDQAFVTLTNDAYAKGALVLSLKHQHRTRRLLAVLTPQVSDTMRKALEIVDFEV---ITVDILD-SGDSAHLTLMKRPEL
Hm 1-----MTDQAFVTLTNDAYAKGALVLSLKHQHRTRRLLAVLTPQVSDSMRKVLETVDFEV---IMVDVLD-SGDSAHLTLMKRPEL
Tg 1-----MSPRYAYATLLTDNSFYVEALLKSLKATKTPYPVLLLTSDVSOSTIKALVYQRKA [ 97 ]RLVGSVA-YPKAERDTCVPVQWD
Nc 1-----MSPRYAYATLLTDNSFYVEALLKSLKATKTPYPVLLLTSGVVSOSTLKALHRRRT [ 93 ]RLVGSIA-YPEKERNKCPVSWKN
```

```
Oc  GVTLKRLHC-WSLTQYSKCVFMDADTLVLI-ANIDDLFE-REELSAAPDPGPFDCFNSGVFVYQPSVETYNOLLHVA-S-EQ-GSFDGDDQG
Hm  GVTLKRLHC-WSLTQYSKCVFMDADTLVLI-ANIDDLFE-REELSAAPDPGPFDCFNSGVFVYQPSVETYNOLLHVA-S-EQ-GSFDGDDQG
Tg  --CFEKLKRVNEQVDEIVIVYVADDCIVL-RPVDELFLRQPLPAFAFDIFPPDKFNAGVAVLKPDLGEYGNMVAVA---ERLFSYDGDGDTG
Nc  --CFEKLKRDWEQVDEIVIVYVADDCIVL-GPVDELFLRKLPAFAFDIFPPDKFNAGVAVLKPDLGEYGRMIAAI---ERLFSYDGDGDTG
```

```
Oc  LLNTEFPNSWATTD-IRKHLFFIYNL--SSISI-VSYLPAFK-AFG-ANAKVVHFLGQTKFVWNYTYDTKTK [ 5 ]GHDPTMTHPQFLNVMMDFIT
Hm  LLNTEFPNSWATTD-IRKHLFFIYNL--SSISI-VSYLPAFK-VFG-ASAKVVHFLGRVVKVWNYTYDPKTK [ 5 ]AHDPTMTHPEFLNMMNIFT
Tg  FLNAYFSSWYENA-AGARLPPRYNALRRLYHMTYSSRKGYN-DAV-KPIKILHFCSSPKFWEQ-----PAKTDLELWVKVFL
Nc  FLNAYFSSWYESS-AGARLPPRYNALRRLYHMTYCSHKGYN-NAV-KPIKILHFCSSPKFWEQ-----PAKTDLELWVKVFL
```

```
# metal binding
$ present in all GT8 sequences and catalytically essential
+ sugar nucleotide binding
* autoglucosylation site in glycogenin

Oc: Oryctolagus cuniculus
Hm: Homo sapiens
Tg: Toxoplasma gondii
Nc: Neospora caninum
```



CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated gene 9) system in *Trypanosoma Cruzi*.

Teresa Cruz-Bustos, CTEGD, University of Georgia

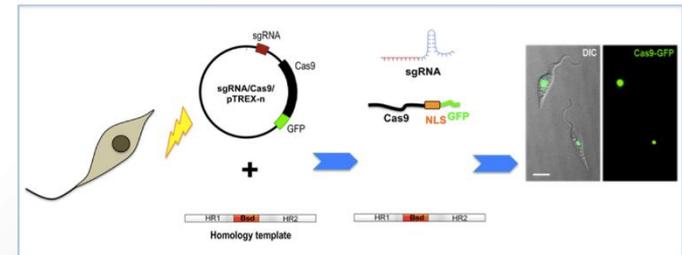
Research goal: gene editing using CRISPR/Cas9 system for disrupting genes and the homology recombination technique in *Trypanosoma Cruzi*.

How? PCR, cloning, transfection and drugs selection of Parasites

Samples/Model System: *Trypanosoma cruzi*

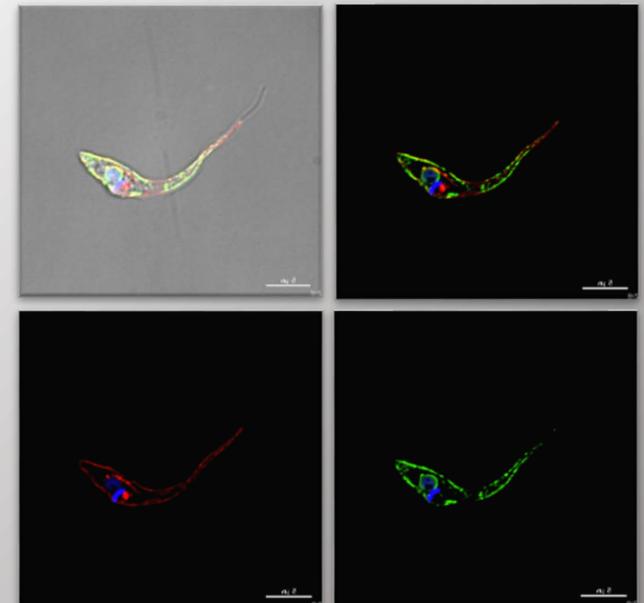
The challenge: using CRISPR/Cas9 to silence genes with the purpose of study the functionality of this genes of *T.cruzi* some of them are related with the mechanosensor response and other involved in osmotic response

Workshop goals: To learn how EuPathDB can assist in analyzing genes and informing my research



The method was significantly improved after including a homology template for DNA repair

Lander et al., 2015. mBio 6(4): e01012-15



Effect of Sporulation Conditions on Fitness of *A. fumigatus*

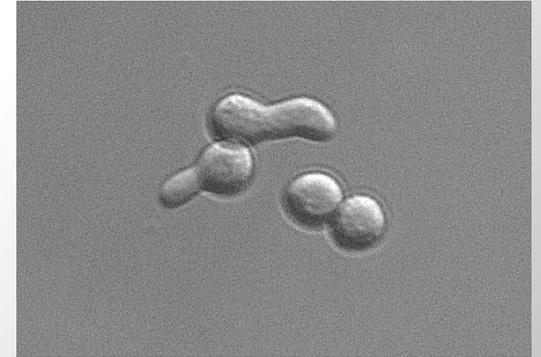
Rosemary Wills, University of Georgia

Research goal: Investigate whether sporulation conditions affect the germination and growth of fungal pathogen *Aspergillus fumigatus*

How? Produce conidia under different conditions and assess germination potential, hyphal growth rates, and dormant spore transcriptomes.

The samples: *Aspergillus fumigatus* Af293

Workshop goals: To learn to use EuPathDB to analyze gene, protein, and transcript sequences.



Advanced Molecular Techniques Course

David Peterson, CTEGD, UGA

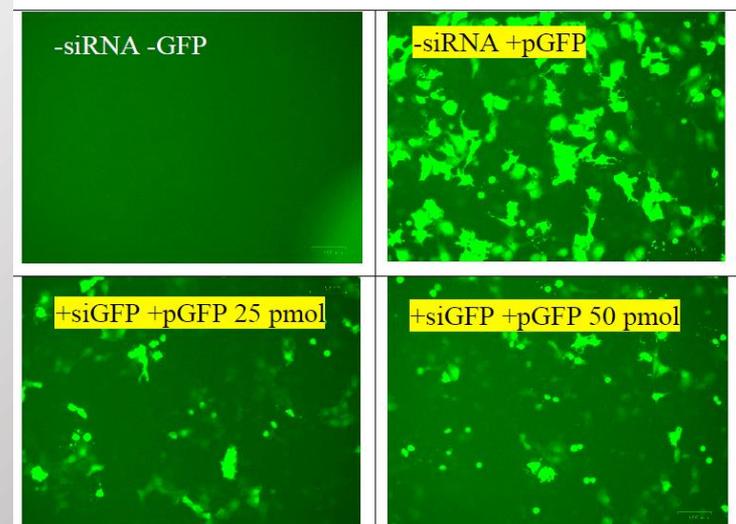
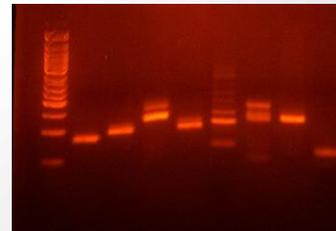
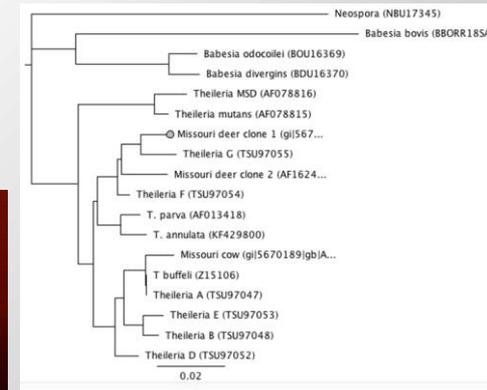
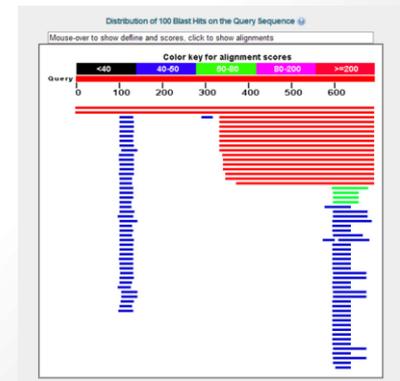
Teaching goal: Revise bioinformatics related content in course to keep topics up to date and relevant

How? Use examples from published research and online tools that can be adapted to in class exercises

Samples/Model System: Anything in EuPathDB that can be used to illustrate solutions to research questions

The challenge: Create useful and relevant class exercises that demonstrate the utility of a well designed genomic analysis platform

Workshop goals: To gain a better understanding of the array of tools within EuPathDB



Determining the Link between Calcium Influx and Motility in *Toxoplasma gondii*

Stephen Vella, University of Georgia

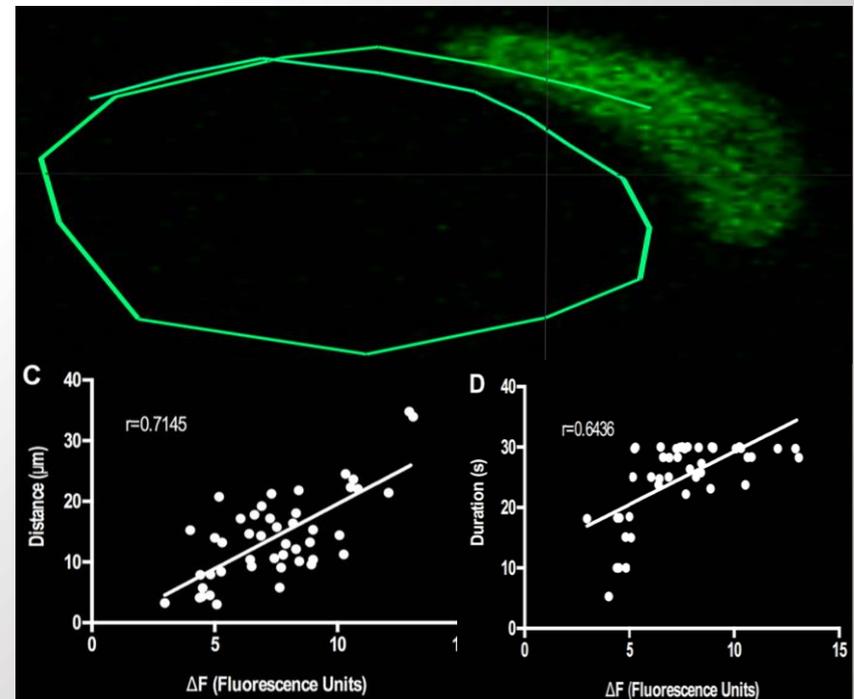
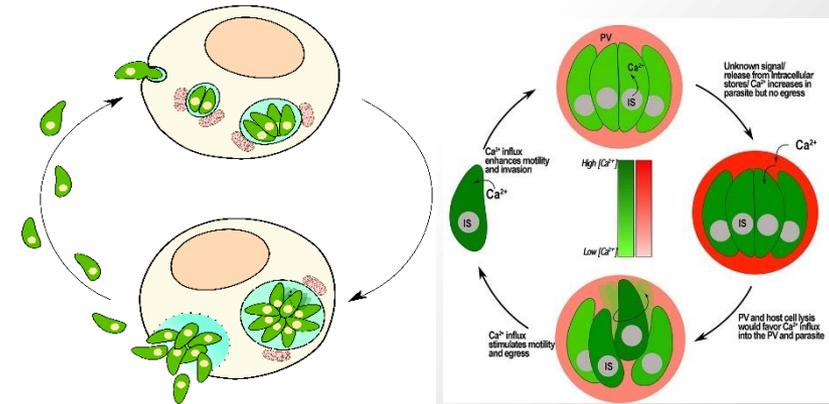
Research goal: Determining the role of calcium influx and calcium oscillations in stimulating the switch from a non-motile state to a motile state in *T. gondii*

How? Using live-cell imaging and fluorometry determine the contribution of Intracellular Calcium Stores versus Extracellular Calcium Influx in stimulating downstream lytic cycle processes such as motility

Samples/Model System: *T. gondii* tachyzoites expressing Genetically Encoded Calcium Indicators and/or loaded with calcium indicators. Calcium release will be stimulated using pharmacological drugs

The challenge: The molecules and their role involved in calcium signaling in *T. gondii* are currently unknown.

Workshop goals: Learn how to navigate ToxoDB more fluently in search of possible genetic targets.



Environmental (“E”) effects on vector (“G”) & parasite (“G”) interactions.

Ash Pathak & Courtney Murdock, Dept. of Infectious Diseases, Ecology & the CTEGD, UGA, Athens

Research goal: Using next-gen tools such as RNA-seq in combination with hypothesis-driven experiments in the laboratory, but ultimately in field conditions.

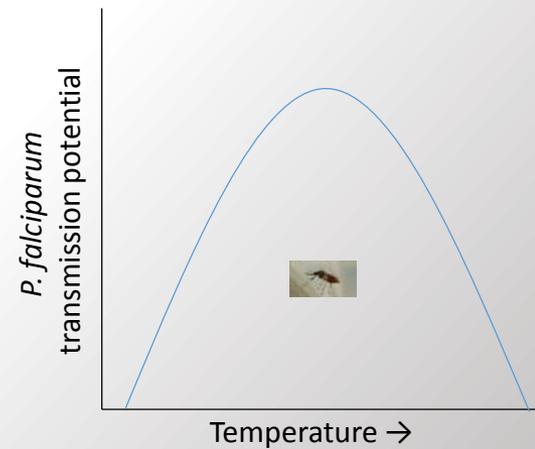
Samples/Model System: 1) *An. stephensi* and *P. falciparum*, 2) *Aedes* spp. and arboviruses. Samples: mosquito midguts, salivary glands and whole carcasses.

The challenge: 1) Identifying & navigating databases without a degree in bioinformatics or computer science, 2) determining to what extent I trust the outputs and 3) linking individual outputs and/or patterns of outputs to biological function.

Workshop goals: 1) to verify that databases aren’t rocket science, 2) identify potential gene targets for further validation and functional analyses.



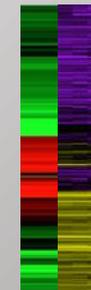
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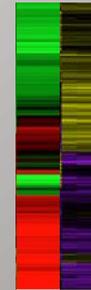
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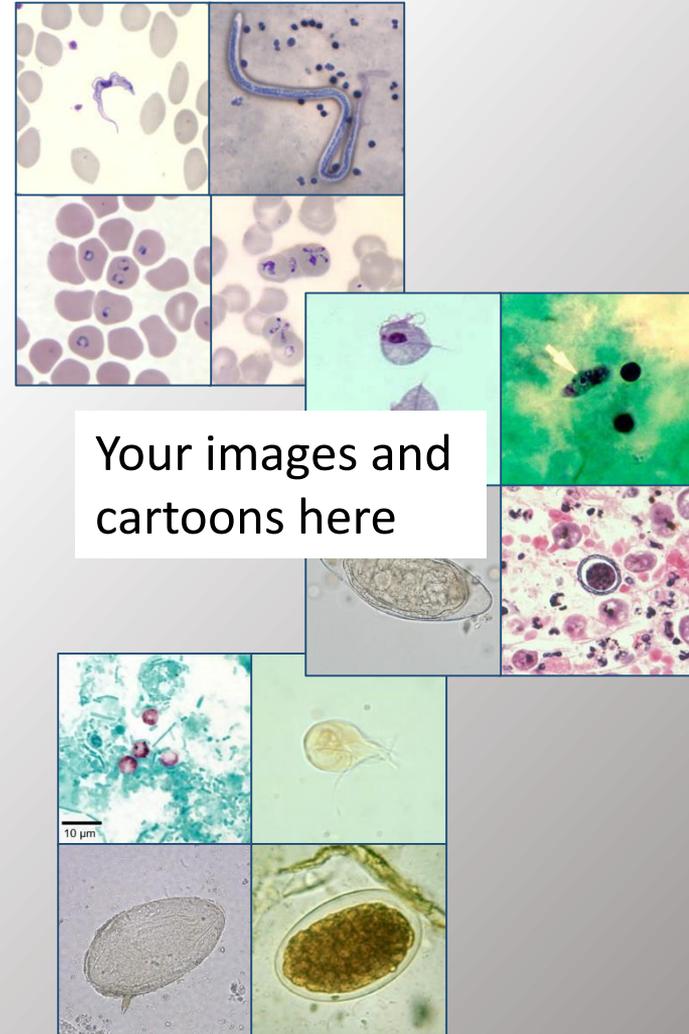


Kristen Knipe, Biotechnology Core Facility Branch Centers for Disease Control and Prevention

Research goal: Because I work in the Core Facility, there is no one specific research project. We have customers working with *Plasmodium*, *Leishmania*, and *Cryptosporidium* spp.

The challenge: Most of the analysis we do now is with bacterial species.

Workshop goals: To enhance my knowledge and understanding of EuPathDB and parasitic genome analysis.



Annotation of *Cryptosporidium baileyi*

Shelton Griffith – University of Georgia

Research goal: Annotate the *Cryptosporidium baileyi* genome to have a comparative model to compare to human-infecting Cryptosporidiosis because the *C. baileyi* genome can complete its life cycle experimentally tractable chicken eggs unlike *C. parvum/hominis*.

How? I will annotate the genome using publically available data found on NCBI and CryptoDB.

The challenge: The challenge is getting the most complete annotation possible using the data this is available to me.

Workshop goals: To become an expert using CryptoDB.



Malaria Host-Pathogen Interaction Center (MaHPIC) and Host Acute Models of Malaria to study Experimental Resilience (HAMMER)

Allison Hankus, Emory University

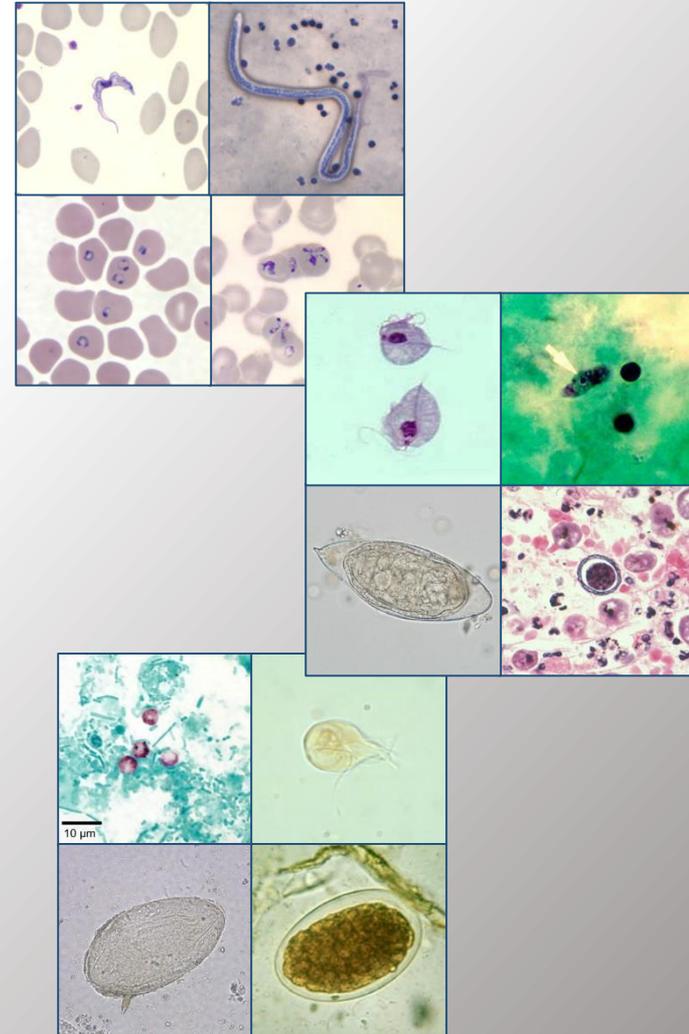
Research goal: Identify novel targets in the *Plasmodium* host-pathogen interaction from a system biology approach.

How? Perform transgenic experiments targeting genes of interest in the *Plasmodium knowlesi* genome.

Samples/Model System: Non human primate models.

The challenge: Applying a systems biology approach to understand expression variation through the various *Plasmodium* lifecycles.

Workshop goals: To become more familiar with EuPath/PlasmoDB to assist in future proteomics and genomics research.



Identification of the candidate genes for TSWV resistance in peanut

Gaurav Agarwal, University of Georgia, Tifton (E-mail: ga23981@uga.edu)

Research goal: To develop a high density genetic map and fine map the QTL for tomato spotted wilt virus (TSWV) resistance

How? By SNP identification using the whole genome re-sequencing (WGS) of the parents (10x to 12x) and the RIL population individuals (3x to 5x)

The samples: Leaf samples of the parental genotypes and the RIL population

The challenge: To identify the recombination break points and define the recombination bins to be used as markers for marker trait association

Workshop goals: To learn how EuPathDB can assist in analyzing my results and informing my research

