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## Introduction

**The collaboration**. The Microbial Genome Annotation Network (MGAN<sup>1</sup>) is collaborating with the Genomics Education National Initiative (GENI<sup>2</sup>) project and Openmods<sup>3</sup> (Fig. 1) to provide authentic and inexpensive research opportunities to high school and undergraduate students in the field of microbial genome analysis. The available technology includes: 1) an online toolkit that consolidates publically available bioinformatics tools into a single platform, and 2) online collaboration tools and wet-lab resources (e.g., bacterial strains, primers and plasmids) for functional genomics studies.

#### **Defining gene/genome annotation and functional genomics**.

Gene annotation is the process of using computer technology to predict a biological function for a gene or region of a chromosome. There are many different bioinformatics tools currently available, which makes choosing among them a daunting task for a novice annotator. Functional genomics is the process of performing wetlab experimentation to confirm the function predictions. Numerous approaches are described in the published literature. GENI and GENI-ACT<sup>4</sup> make it easier to implement an authentic research experience in genome analysis by providing training and faculty resources.

**3** steps to an authentic research experience in genome analysis. Step 1: Find a microbe whose genome has been sequenced, but for which little published work is available. Start with IMG or NCBI. Step 2: Choose an annotation approach. A few of the annotation questions that one might ask using GENI-ACT are these:

- Is the original (automated) gene call accurate?
- Have the genes in a particular region of a chromosome been accurately called?
- Does my organism-of-interest possess the genes for a particular pathway, process or cellular structure?
- What is a predicted function of a gene identified as "hypothetical"?
- Does a particular gene in the chosen bacterial genome show evidence of being acquired by horizontal gene transfer?
- Other questions. A myriad of other questions can be asked that utilize some or all of the bioinformatics tools associated with GENI-ACT.

Step 3: Collect experimental evidence to support the function prediction. GENI focuses on the complementation approach.

**Purpose**. This poster describes the GENI and GENI-ACT systems, faculty resources, and faculty development opportunities that will enable one to implement a microbial genome analysis project.



# **GENI and GENI-ACT projects provide authentic undergraduate research experiences** in genome analysis

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#### $\Gamma^5$ , the GENI Annotation Collaboration Toolkit (GENIanding to include access to all sequenced genomes in and 3) allowing for multi-instructor collaborations.

Figure 2. GENI-ACT contains course management features (Panel A), and the ability to assign multiple genes from multiple organisms to the same students through an online lab notebook format (Panel B). Panel A is a screenshot of the course management page: students can work individually or a team; and teaching assistants have access to student work. Panel B is a screenshot of the entry into the lab notebook feature: students can be assigned one or multiple genes from different microbes; students can work individually or in teams on the same project.

### eni-act

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Gene Locus	Organism	Lab Notebook	Remove Annotation
Smon_0084	Streptobacillus moniliformis DSM 12112 CP001779	Lab Notebook	<u>Remove</u> <u>Annotation</u>
Mrub_0004	Meiothermus ruber DSM 1279 CP001743	Lab Notebook	<u>Remove</u> <u>Annotation</u>
EcolC_1778	Escherichia coli ATCC 8739 CP000946	Lab Notebook	<u>Remove</u> <u>Annotation</u>

Figure 3. Screenshot of the GENI-ACT online lab notebook.

The GENI-ACT lab notebook is organized into modules that use a variety of publically available bioinformatics tools and databases (e.g., NCBI, GenBank, BLAST, CDD, T-COFFEE, TMHMM, SignalP, PSORT-B, Phobius, JGI's Integrated Microbial Genomes (IMG), TIGRFAM, Pfam, PDB, KEGG, MetaCyc, ExPASy Enzyme, IMG Gene Context, GC Heat Map, Phylogeny.fr, Rfam). Collectively, these programs and databases are used to make function predictions of putative open reading frames.

GENI-ACT has the following features:

instructions on how to use the different bioinformatics programs

• the flexibility to ask a variety of research questions

• a modular design that allows for flexibility in using any combination of modules, as determined by the project question

• faculty development opportunities – instructors and students are learning contemporary bioinformatics tools

student work is publishable online and available to other **GENI-ACT** users

A

As a follow-up to gene annotation studies, the GENI project provides detailed instructions for subsequent functional genomics studies, which is the wet-lab complement to the bioinformatics analysis. Panel A in Fig. 4 outlines the approach of cloning a geneof-interest using ligase independent cloning and its subsequent complementation using an appropriate *E. coli* Keio null strain.<sup>5</sup>



B

Figure 4. Overview of the ligase independent cloning and complementation approach used in the GENI system<sup>2</sup> (Panel A), and the confirmation of *Meiothermus ruber* gene Mrub\_2778 as acetylglutamate kinase/argB (Panel B) in the arginine biosynthesis pathway. Mrub\_2778 and *E. coli* argB were cloned into the pKt-1 vector and transformed into the E. coli argB null strain JW5553-1. Plate Sector 1 = WT *E. coli*, Sector 2 = null *E. coli* strain; Sector 3 = pKt1 in null strain; Sector 4 = E. *coli* argB-pKt1 in null strain; Sectors 5&6 = twoMrub2778-pKt1 clones in E. coli null strain.

## **Training and contact information**

MGAN provides training workshops for the GENI-ACT platform. Contact Lori Scott at loriscott@augustana.edu.

Contact Brad Goodner at GoodnerBW@hiram.edu for GENI-ACT instructor accounts and information about an upcoming functional genomics workshop in the summer of 2015 at Hiram College, OH.

Contact Katie Houmiel at houmik@spu.edu for instructional resources associated with GENI.

## Acknowledgements

Many people have contributed to the development of the tools described in this poster, but only a few could be listed due to space limitations. Please see the various websites for a more comprehensive list of collaborators.

Literature cited accessed 6/13/14) 9/23/14)

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sciences curriculum. PLoS Biol. 8(8):e1000448. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2919421/ (Accessed 6/13/14) <sup>6</sup>Baba, T., T. Ara, M. Hasegawa, et al. 2006. Construction of Escherichia coli K-12 in-frame, singlegene knockout mutants: the Keio collection. Mol Syst Biol 2:1-1; E. coli null strain provided by the Coli Stock Center (http://cgsc.biology.yale.edu/Strain.php?ID=99802)

## **Functional genomics using GENI**



<sup>1</sup>Microbial Genome Annotation Network (MGAN) website at <u>http://mgan-network.org/index.html</u>

<sup>2</sup>Genomics Education National Initiative (GENI) website at <u>http://www.geni-science.org/</u> (accessed

<sup>3</sup>Openmods website at <u>http://www.openmods.org/</u> (accessed 6/13/14) <sup>4</sup>GENI-Annotation Collaboration Tool (GENI-ACT) website at <u>http://www.geni-act.org/</u> (accessed

<sup>5</sup>Ditty JL, Kvaal CA, Goodner B, et al. 2010. Incorporating genomics and bioinformatics across the life