

BACKGROUND

- Have a gene involved in neurological disease, its function unclear
- Knockout is lethal, so...
 - Designed a conditional knockout (cKO) mouse where gene is deleted from non-neuronal cell type in the brain
- Behavioral phenotype: obsessive-compulsive disorder
- We know the gene product regulates transcription
- Hypothesis: Changes in gene expression are responsible for phenotype

MODULE RESEARCH GOALS

1. Sample preparation

Extract total RNA from optic nerves of test (cKO) and control (+/Cre;+/+ and +/+;flox/flox) animals and send for sequencing.

2. Computation

Get list of differentially expressed genes.

3. Lead generation

Pare list to manageable number of candidate genes (top 20) for follow-up by qRT-PCR.

STUDENT LEARNING GOALS

1. Experimental design

Students will become familiar with the major anatomical features of the brain (cortex, cerebellum, hippocampus, striatum, thalamus, hypothalamus, prefrontal cortex)

Students will be able to explain how a conditional knockout is designed and made.

Students will be able to explain the current deep sequencing technologies (specifically, Illumina)

STUDENT LEARNING GOALS

2. Working with large datasets

Students will become comfortable managing large datasets

Students will become familiar with the Galaxy platform for managing datasets

Students will have the option of learning script code

Students will understand the principles of filtering data and be able to justify their choices for the filtering of data

STUDENT LEARNING GOALS

3. Application

Students will identify the genes whose transcription has changed

Students will be able to use gene ontology software to identify pathways whose constituent genes have substantial changes

Students will take one of the genes from the putative target list and identify its key features including tissue expression, function, family members, isoforms, domain structure, etc.

STUDENT LEARNING GOALS

4. Communication

Students will be able to assemble a coherent oral description of the Galaxy workflow

Students will be able to clearly communicate the design of the experiment, including the design of the conditional knockout animal

Students will have a final written report on one of the putative genes (see Student Learning Goals #3)

CORE COMPETENCIES

1. Apply the Process of Science

Phenotype → Transcriptional Changes →
RNA-Seq → Genes

2. Use Quantitative Reasoning

Statistics

Managing large data sets

3. Use Modeling and Simulation

Modeling disease state through pathways

CORE COMPETENCIES

4. Tap Into the Interdisciplinary Nature of Science

Molecular Biology → Behavior → Disease/
Medicine → Statistics → Computation

5. Ability to Communicate and Collaborate with Other Disciplines

Crowd sourcing

Gene report/Final project

Liberal Arts Symposium potential

CORE COMPETENCIES

6. Understand the Relationship Between Science and Society

Genetic component of neurological behavior

Using mouse models for human disease

GCAT-SEEK SEQUENCING REQUIREMENTS

- Any compatible RNA-seq platform
 - Illumina used here
 - 454, SOLiD also possibilities

ASSESSMENT

Experimental design

Weekly quizzes

3-4 page report

RNAseq workflow

Compare next-gen and microarrays

Summary of RNAseq paper

Working with large datasets/application

Examine the effect of changing parameters

Oral presentation: report back to group

10-page gene report

TIME LINE OF MODULE (1 SEMESTER)

Week	Monday	Wednesday	Friday
1	Biology background	Next-gen technologies	Experimental design
2	<i>Student presentations on an RNAseq paper</i>		
3	RNA and transcription	RNA extraction	Bioanalyzer Library construction*
4	Tuxedo workflow, Galaxy	Processing sequences, examine output	Changing parameters, submit history
5	<u>Lecture</u> : <i>de novo</i> txtome sequencing	Begin <i>de novo</i> assembly	Submit <i>de novo</i> assembly
6	<u>Lecture</u> : mapping reads	Map against reference transcriptome	
7	Compare <i>de novo</i> assembly with reference txtome		Learn/run Cuffmerge
8	Learn/run Cuffdiff	<u>Lecture</u> : Stats	<u>Lecture</u> : GO
9	Learn/run GO	Gene lists, analysis of hits, database tools	

REFERENCES

Note: The module described uses a conditional knockout (cKO) mouse which has not yet been published. Therefore, we have intentionally left the details vague enough to protect our research interests, yet providing enough detail for an interested researcher to substitute our cKO mouse with his/her organism of choice for RNA-seq. When our paper on the cKO mouse has been accepted, we will update this file to reflect the citation and all relevant background material.