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Don't Drink and Fly: Alcohol Resistance Behavior in Drosophila Informs us about Genes that Contribute to Alcoholism

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Many undergraduate students understand that model organisms are important for understanding how biology works, but may not make the connection that animal models such as *Drosophila melanogaster* can be used to understand such human conditions as Alcohol Use Disorder (AUD) and Fetal Alcohol Syndrome (FAS). To address this knowledge gap, we introduced an inquiry-based laboratory module in which students perform hands-on Ethanol Behavior Mobility Assays (EMBAs) using flies with either different Alcohol Dehydrogenase (ADH) alleles or different developmental exposure to ethanol. The lab module contains a bioinformatic component for students to explore the evolutionary conservation of the ADH gene between flies and humans. The implementation of this exercise in a sophomore/junior-level Genetics course led to a high level of student satisfaction and a more integrated view of the role of model organisms in studying AUD and FAS. Funding acknowledgements: ABLE Roberta Williams Laboratory Teaching Initiative Grant and NSF HBCU-UP TIP Grant # 1912188.

Keywords: model organism, *Drosophila melanogaster*, alcoholism, fetal alcohol syndrome, behavioral assay, inquiry-based learning

Introduction

While biology laboratory classrooms often introduce undergraduate students to a variety of organisms and lecture about how these organisms can be used as models, students often do not connect that model organisms such as the common fruit fly, *Drosophila melanogaster*, can facilitate understanding of human diseases including Alcohol Use Disorder (AUD) and Fetal Alcohol Syndrome (FAS). To ameliorate this knowledge gap, a two-course, inquiry-based module was developed for a sophomore/junior-level course that meets once per week for 1 hour and 50 minute sessions. The hands-on portion of the laboratory is simple enough to use with students of different STEM disciplines including Chemistry and Physics freshman-level students. However, the preparation time involved may be prohibitive to large freshman-level laboratories unless support staff is available to expand and sex-sort fly strains for both the AUD-based and FAS-based exercises and the pre-ethanol exposure for the FAS-based lab. Expansion of fly stocks needs to begin 1 month in advance and it is not very time consuming; neither is exposing developing larvae to alcohol. However, depending on the instructor's background and familiarity with fruit flies, the sex-sorting of flies a day or two ahead of the class session can take up to 3 hours of intensive stereoscope work to prep full sets (4 vials) of flies for student groups. In contrast, the bioinformatic portion of the lab should translate well to larger class sizes as virtually no preparation time is needed for that portion of the module series.

Laboratory Learning Objectives

Students participating in the experience will have the following three learning objectives for the 2-3 day laboratory module:

- Observe differences in behavioral responses of fruit fly strains with variant ADH alleles using Ethanol Mobility Behavior Assay (EMBA) to measure Sedation Time 50 (ST50) and Sedation Time 100 (ST100)
- Use the FlyBase bioinformatics tool to investigate the gene function and conservation between flies and humans
- Explain the evolutionary basis for why model organisms can be used to inform us about human diseases including Alcohol Use Disorder (AUD) and Fetal Alcohol Syndrome (FAS).

Student Time Investment

Students at North Carolina Central University had ample time to observe the ethanol-induced behaviors for at least 1 set of either male or female flies and perform Excel analysis (calculations and graph generation) within a 2-hour lab period. To include the FlyBase bioinformatic exercise, it typically required either time outside the classroom or a second class period; hence, the two-day modular approach. Most universities have a 3-hour lab period and will likely have time for students to assay a set of males and a set of females in addition to completing the FlyBase bioinformatic exercise in one lab session. To also complete the Adh adult fly lab, a separate lab day was required. Therefore, the series of exercises in this publication may take as little as 2 days at institutions where there is a 3-hour laboratory session and as many as 3 days for institutions having only a 2-hour laboratory period as shown in Table 1.

Table 1. Flexible time needed dependent on portion of modules covered: 1, 2 or 3 lab sessions.

	1-Lab	**2-Labs**	**3-Labs**
Protocol:	Control strain assay	Adh alleles assay	FlyBase activity
Inquiry:	Which gender more resistant to alcohol?	Which Adh allele is most resistant to alcohol (AUD)?	How does the fly Adh gene compare to human Adh gene?
Discussion:	Females bigger; increased resistance = AUD	The Adh^S allele is resistant; enzyme metabolism slower (s)	They are 54% identical, 71 % positive; research paper on varying human Adh alleles
Optional:	FlyBase activity	FlyBase activity	n/a

Student Outline

Alcohol Module Series: Investigating Alcohol Use Disorder and Fetal Alcohol Syndrome in Fruit Flies

Name: _____ **Section:** _____

Overall Learning Objectives

1. Observe differences in behavioral responses of fruit fly strains with variant ADH alleles using Ethanol Mobility Behavior Assay (EMBA) to measure Sedation Time 50 (ST50) and Sedation Time 100 (ST100)
2. Use the FlyBase bioinformatics tool to investigate the gene function and conservation between flies and humans
3. Explain the evolutionary basis for why model organisms can be used to inform us about human diseases including Alcohol Use Disorder (AUD) and Fetal Alcohol Syndrome (FAS).

Introduction

Over the next few weeks, you will have the opportunity to gain hands-on experience with live animals to measure intoxicated fly behavior. In week 1, you will be provided with varying genetic backgrounds (different **Alcohol Dehydrogenase (ADH) alleles**) and in week 2 you will use adult flies that have been reared in a chronic, alcohol-rich environmental serving as a model for Fetal Alcohol Spectrum Disorder (FASD) which includes Fetal Alcohol Syndrome (FAS).

Alcohol Use Disorder and the Alcohol Resistance Phenotype

According to the National Institute of Alcohol Abuse and Alcoholism (NIAAA), an estimated 88,000 people a year die from alcohol-related behaviors, which makes alcohol consumption the third leading cause of preventable death in the United States. Alcohol consumption at social gatherings is a social norm; however, depending on your DNA content, this "normal" behavior could easily turn into a debilitating disease. Alcohol Use Disorder (AUD) is defined as a chronic relapsing brain disease which is 50-60% genetically based. One in 8 Americans suffer from an AUD. Thus, it is a fairly common, life-altering disease (https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/understanding-alcohol-use-disorder). A behavior associated with AUD is called the "**alcohol resistance" phenotype**. Humans who are resistant to the "buzz" feeling from small amounts of alcohol will consume greater amounts of alcohol to achieve the feeling they seek. In other words, there are groups of people that are resistant to the effects of alcohol due to their genetic make-up. So, they drink more alcohol, leading them to abuse the drug. Thus, an alcohol resistance phenotype is associated with **Alcohol Use Disorders (AUD),** which include alcoholism.

Why flies?

To investigate the genes and molecular pathways that contribute to AUD, researchers turn to **model organisms** including the common fruit fly, *Drosophila melanogaster*. Did you know that fruit flies are naturally attracted to alcohol? It's true! Multiple *Drosophila* species typically dine on fermenting fruit. What caused fruit to ferment? You guessed it: yeast. During the fermentation, the yeast produce, among other products, ethanol! Our tiny fruit fly friends are, thus, attracted to the delicious aroma of alcohol AND they have some tolerance to certain percentage of alcohol. Interestingly, *D. melanogaster* respond to alcohol similarly to humans with an initial period of hyperactivity followed by sedation. While humans and fruit flies may look vastly different, the differences are due to ~a 25% genetic difference. Surprisingly, these tiny fruit flies contain approximately 75% of the human disease-related genes in their genome. In other words, genes responsible for a whole host of human diseases are **conserved** (which means they have very similar DNA/protein coding sequences) in the humble bug. Diseases including Diabetes, Parkinson Disorder, sleep disorders, cancer, jet lag, alcoholism and many more can be studied in fruit flies. Since the genes found in fruit flies and humans are **evolutionarily conserved**, we can apply information we learn from these model organisms and apply it to human health concerns.

Function of the Alcohol Dehydrogenase (ADH) Gene Product

The product of the alcohol dehydrogenase (ADH) gene is an enzyme that converts alcohol (ethanol) to acetaldehyde in the cytoplasm, especially in liver cells. The acetaldehyde is then transported into the mitochondria where the acetaldehyde gene product converts the incredibly toxic acetaldehyde to acetic acid. Acetic acid can then enter the Krebs Cycle to produce energy for the cell (**see Figure 1**).

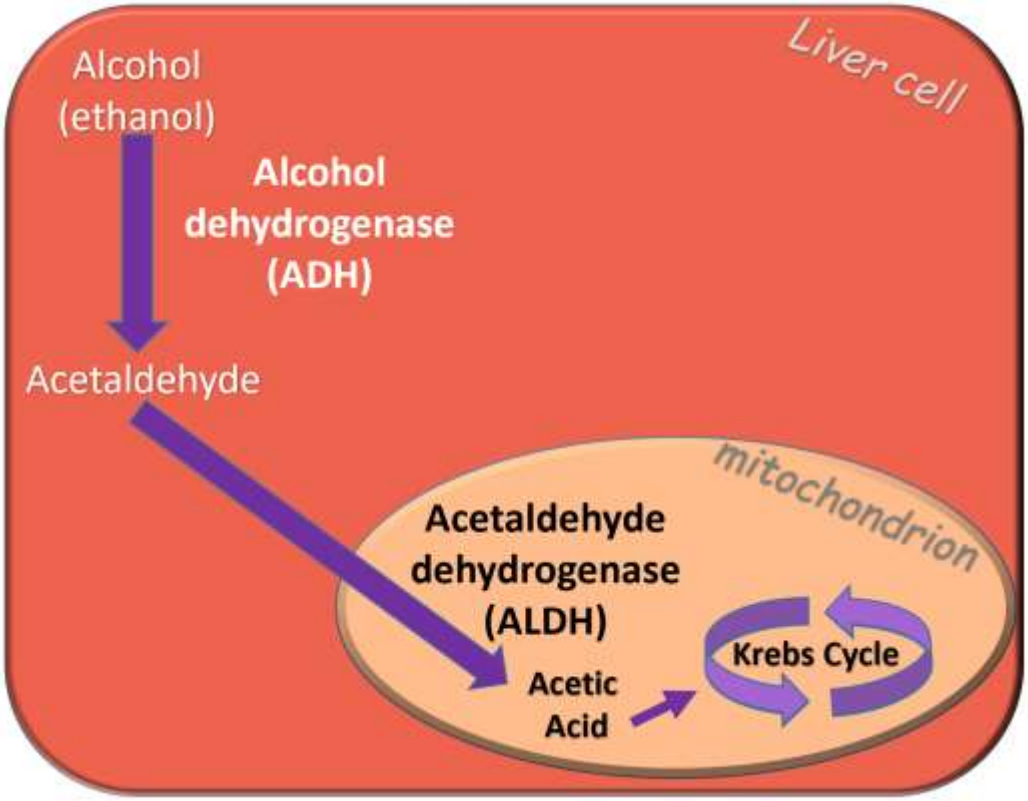

Figure 1. Alcohol is metabolized in liver cells using enzymes: cytoplasmic alcohol dehydrogenase and mitochondrial acetaldehyde dehydrogenase.

The Alcohol Dehydrogenase (ADH) Gene Allele affects alcohol metabolism

In humans, there are different alleles of the alcohol dehydrogenase (ADH) gene. Alteration of the ADH allelic sequence can impact the function of the alcohol dehydrogenase enzyme produced from the gene. For example, humans with the ADH1B or ADH1C alleles produce enzymes that rapidly convert alcohol substrate into the toxic acetaldehyde product (Edenberg, 2007). The conversion is so fast that the acetaldehyde dehydrogenase (ALDH) enzyme cannot keep up. This results in the person being sensitive to alcohol with phenotypes like the "flush response," where the face turns red and the person stops drinking the alcohol as a result of the unpleasant response. Conversely, the ADH1C*2 allele is associated with resistance to the effects of alcohol. People with this allele can drink more alcohol without feeling the effects and therefore, drink more and more alcohol to obtain the "buzz." This ADH allele is associated with Alcohol Use Disorder (AUD) or alcoholism (Montane-Jane et al., 2006). Presumably, the alcohol dehydrogenase enzyme produced by the ADH1C*2 gene metabolizes the alcohol substrate to the toxic aldehyde product at a slower rate. Thus, humans have different responses to the same amount of alcohol due to differences in their genotype which contributes to their enzymatic phenotype. Since the enzymes in the cell metabolize alcohol at different rates, it impacts the overall behavior humans display in response to similar alcohol intake.

ADH alleles in *Drosophila* (fruit flies)

The *Drosophila melanogaster* genome also encodes an alcohol dehydrogenase enzyme with various alleles that affect the behavioral response of the fruit flies to the same amount of alcohol. In this lab exercise, you will empirically discover the impact of the ADH alleles on fruit fly behavior using the Ethanol Mobility Behavioral Assay (EMBA).

DAY 1) EMBAs: Control Strain either Oregon R or w^{1118}

Pre-lab Videos:

1. How to Expose flies to ethanol demonstration video: https://youtu.be/rBuxZglfQ8g
2. JOVE article video A Simple Way to Measure Ethanol Sensitivity in Flies | Protocol (jove.com)

Background: Your instructor has reared flies for 10-14 days. As the adult flies emerged from the pupal cases, adult flies were collected and aged from 1-5 days and then sorted by the two sexes (male and female) into food vials.

Prediction: **Make a prediction: Which sex will be more resistant to the alcohol (i.e. take longer to become sedated)? _____**

Instructions: Run the EMBA assay as indicated by the protocol on the adult flies your instructor has provided and fill in the data table 1 below.

Protocol:

1) First, record the room temperature on your data table or sheet of paper.

2) Next, you will see a clean **cotton plug** sitting on your lab bench for each assay you will do. These are the plugs that you will be putting your dyed ethanol on.

3) Set a p1000 at **500 µL**.

4) Locate the **colored 100%, 200-proof Ethanol (EtOH) [color due to food coloring]**.

5) **Pipette 500 µL of the blue-tinted EtOH onto ONE side of each plug**.

6) You will have to quickly switch out the clean plug for the ethanol plug. Make sure to place the colored, ethanol-soaked side of the plug face down into the vial!

7) Get ready to press 'start' on your timer (can use cell phone) immediately once both vials of flies are exposed to the EtOH.

8) Observe the flies.

9) At 1-minute intervals, record the number of flies that are NOT mobile in the column below or your own sheet of paper. Make sure to indicate the sex (either male or female) in the top row, as well as the number or allele of your experimental strain.

   RULE of THREE: Sometimes flies will appear sedated at a time point, but then will get back up and walk around. If this occurs and you have fewer flies at a later time point than you did at an earlier time point, then just count the number sedated and record it. This is when we go with the

'RULE of THREE': when the same number of flies are sedated for 3 consecutive minutes, count the first of the series of 3 as the time sedated.

10) Enter the data onto a piece of paper while in class and transfer to an Excel Worksheet outside of class as shown in a video tutorial.

11) Report the ST50 and ST100 that you have collected on the bottom of your worksheet.

12) Class data will be posted for you to complete calculations on the AVERAGE Sedation Time (ST50) when ½ of the flies have been sedated and Sedation Time 100 (ST100) when 100% of the flies in the ethanol exposure chamber have been sedated. You will calculate the average sedation times for each fly sex for tested in the class.
13) Make sure to record the following for each Ethanol Mobility Behavior Assay (EMBA) you run.
    a. **Temperature in room: _____**
    b. Strain of fly you are assaying (circle 1): w^{1118} or Oregon R

DATA TABLE 1. Record the number of flies that are sedated each minute of the assay..

Time (minutes)	Control Male w1118	Control Female w1118
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		

Control male flies ST 50 = _____ (1/2 flies sedated)
Control female flies ST 100 = _____ (all flies sedated)

EMBA Day 2: Comparing two genetically different strains

Learning Objectives Part 1:

1. **Reiterative learning of Ethanol Mobility Behavior Assay (EMBA) using two genetically different fly strains.**
2. **Collect data into Excel and enter into an Excel Worksheet**
3. **Determine and report the ST50 and ST100 for <u>your</u> EMBA**
4. **Using class data (posted after everyone submits their observational data), calculate:**
    a. **The average ST50 and ST100 for each sex and each fly strain**
    b. **The Standard Deviation from the Average for ST50 and ST100**
    c. **The statistical significance for**
        i. **Comparing female w^{1118} to female Adh^S, Adh^F, and/or Adh^{N1} strain;**
        ii. **Comparing w^{1118} males to male Adh^S, Adh^F, and/or Adh^{N1} strain;**
        iii. **Comparing w^{1118} females to w1118 males**
        iv. **Comparing Adh^S, Adh^F, and/or Adh^{N1} females to Adh^S, Adh^F, &/or Adh^{N1} males.**

Protocol:

1.) First, record the room temperature on your data table or sheet of paper.

2.) Next, you will see a clean **cotton plug** sitting on your lab bench for each assay you will do. These are the plugs that you will be putting your dyed ethanol on.

3.) Set a p1000 at **500 µL**.

4.) Locate the **colored 100%, 200-proof Ethanol (EtOH) [color due to food coloring]**.

5.) **Pipette 500 µL of the blue-tinted EtOH onto ONE side of each plug**.

6.) You will have to quickly switch out the clean plug for the ethanol plug. Make sure to place the colored, ethanol-soaked side of the plug face down into the vial!

7.) Get ready to press 'start' on your timer (can use cell phone) immediately once both vials of flies are exposed to the EtOH.

8.) Observe the flies.

9.) At 1-minute intervals, record the number of flies that are NOT mobile in the column below or on your own sheet of paper. Make sure to indicate male or female in the top row, as well as the number of your experimental strain.

10.) Enter the data into an Excel Worksheet outside of class as shown in a video tutorial

11.) Report the ST50 and ST100 that you have collected on the bottom of your worksheet.

12.)Class data will be posted for you to complete calculations on the AVERAGE Sedation Time (ST50) when ½ of the flies have been sedated and Sedation Time 100 (ST100) when 100% of the flies in the ethanol exposure chamber have been sedated. You will calculate the average sedation times for each fly sex for each Adh strain tested in the class.

13.)Make sure to record the following for each Ethanol Mobility Behavior Assay (EMBA) you run.

a. **Temperature in room: _____**
b. **Fly SEX assayed (circle 1**): **male or female**
c. **Adh strain of fly you are assaying (circle 1):** Adh^F , Adh^S or Adh^{N1}
d. **Make predictions:**
    i. **Which sex do you think will be more resistant to the alcohol? Why?**
    ii. **How do you think your Adh allele will respond to the alcohol? Be more resistant than the control or more sensitive than the control? Why?**

14.) Optional: Your instructor may opt to run a tolerance assay by providing you with flies that have been exposed to ethanol once. You will expose them a second time to determine if their sedation time changes. If it takes the flies longer to become sedated, then the flies will be 'tolerant' to the effects of alcohol.

DATA TABLE 1. Record the number of flies that are sedated each minute of the assay. The first few minutes will be '0'.**Copy this page for each sex and/or Adh strain you assay.**

Time (minutes)	Control Fly Strain (w^{1118})	Adh__ fly strain
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
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22		
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26		
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28		
29		
30		
31		

W1118 ST 50 = _____ (1/2 flies sedated)
W1118 ST 100 = _____ (all flies sedated)

Adh ____ST 50 = _____ (1/2 flies sedated)
Adh____ ST 100 = _____ (all flies sedated)

Example EMBA Data Collected for Adh^S -containing Drosophila melanogaster strain.

Class Data Table 2. Fall 2020 Ethanol Mobility Behavior Assay (EMBA) Data. Sedation Time of 50% (ST50) and ST100 EMBA Data comparing w^{1118} genotype to Adh^S genotype of sex-sorted *Drosophila melanogaster (Dmel)* organisms. Room temperature was 23 ºC.

Female Dmel flies				
	ST50		**ST100**	
	w^{1118}	**AdhS**	**w^{1118}**	**Adh^S**
Student #1	27	42	63	68
Student #2	17.5	30	25	41
Student #3	23	44	56	56
Student #4	17.5	21	28	38
Student #5	17.5	39	23	52
Student #6	21	38	41	46
Student #7	11	25.5	35	39
Student #8	14.5	42	21	51
Student #9	14	18.5	28	32
MALE Dmel FLIES				
	ST50		**ST100**	
	w^{1118}	**Adh^S**	**w^{1118}**	**Adh^S**
Student #10	12	15	18	22
Student #11	8.5	12	24	24
Student #12	6	11	9	19
Student #13	7	15	12	22
Student #14	7	15	16	30
Student #15	17	12.5	25	23
Student #16	20	33	40	41
Student #17	12	27	28	36

Ethanol Assay Worksheet: Crunching & Graphing Numbers

You all will be calculating and sharing data with each other.

1) First, figure out your ST100 (in minutes) for each strain in your assay. The ST100 is the time at which 100% of your flies are sedated (or stationary). So, **for example**, if all 4 of your male wild type flies are sedated at 15 minutes, your ST100 would be 15. You will figure out your ST100 for both wild type and experimental strains. Please make sure to write down your experimental strain in the blank shown below (highlighted in red). **ALSO, please make sure you label independent values for males and females! You can fill in below.**

-Control, w^{1118} Wild type male ST50: _____ mins

- Experimental $Adh^{N1, S \text{ or } F}$(circle 1 allele) male ST50 (_____): _____ mins

- Control, w^{1118} Wild type female ST50: _____ mins

- Experimental $Adh^{N1, S \text{ or } F}$(circle 1 allele) female ST50 (_____): _____ mins

- Control, w^{1118} Wild type male ST100: _____ mins

- Experimental $Adh^{N1, S \text{ or } F}$(circle 1 allele) ST100 (_____): _____ mins

- Control, w^{1118} Wild type female ST100: _____mins

- Experimental $Adh^{N1, S \text{ or } F}$(circle 1 allele) female ST100 (_____): _____mins

2) Follow the JovE video to interpolate your ST50 data as needed using the attached graph paper.

3) Next, we will calculate the AVERAGE ST50 and ST100. You will need the class data for this! You will type your data in an Excel workbook at the instructor's bench and the Class Data Table 2 will be posted on the Blackboard.

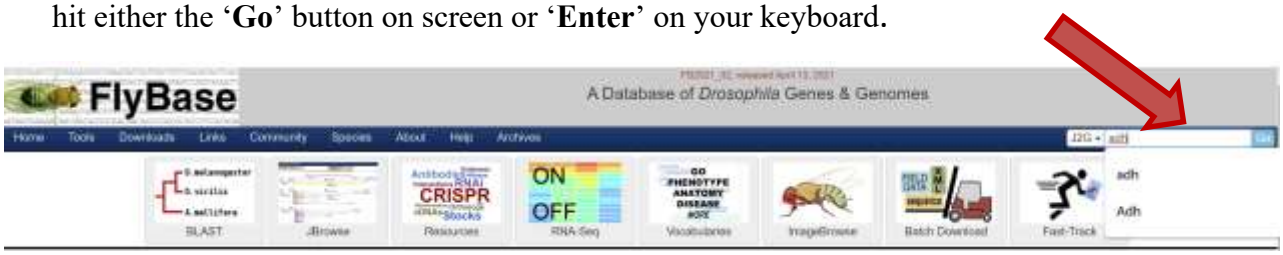
4) Lastly, graph your **averages** on the attached page or in Excel as demonstrated in the **Excel Tutorial Video** posted on Blackboard. Label your y-axis as time in minutes. Make sure to label each strain on the x axis. You must have your wild type control included in this graph.

Questions to answer and discuss:

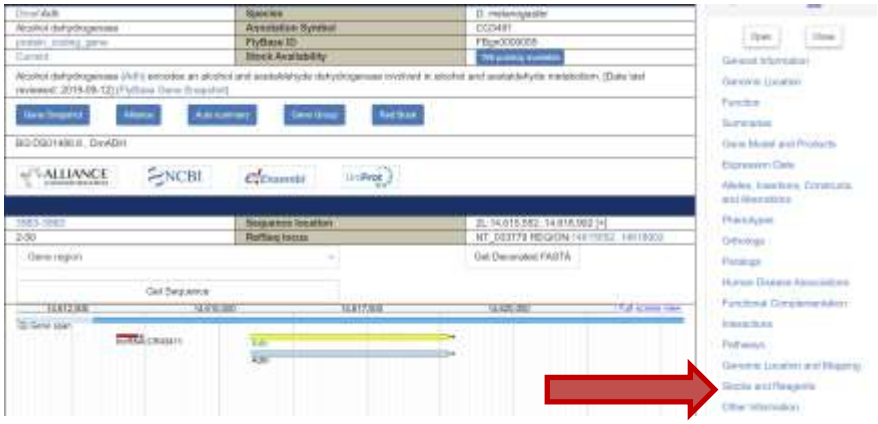
1. Name at least 2 criteria that qualify a fruit fly as immobile.
2. What is the importance of putting dye in the ethanol used for this assay?
3. On average, which strain of flies lasted longer before sedation? The control flies, or experimental flies?
4. On average, which sex of fly took more time to become sedated? Males? Females? Neither: each sex took equal time? Did the results match your prediction? Explain why one sex might take longer to become sedated:
5. When it takes longer to become sedated, is the organism sensitive or resistant to alcohol?

Day 3 Bioinformatics on the Alcohol dehydrogenase (Adh) Gene

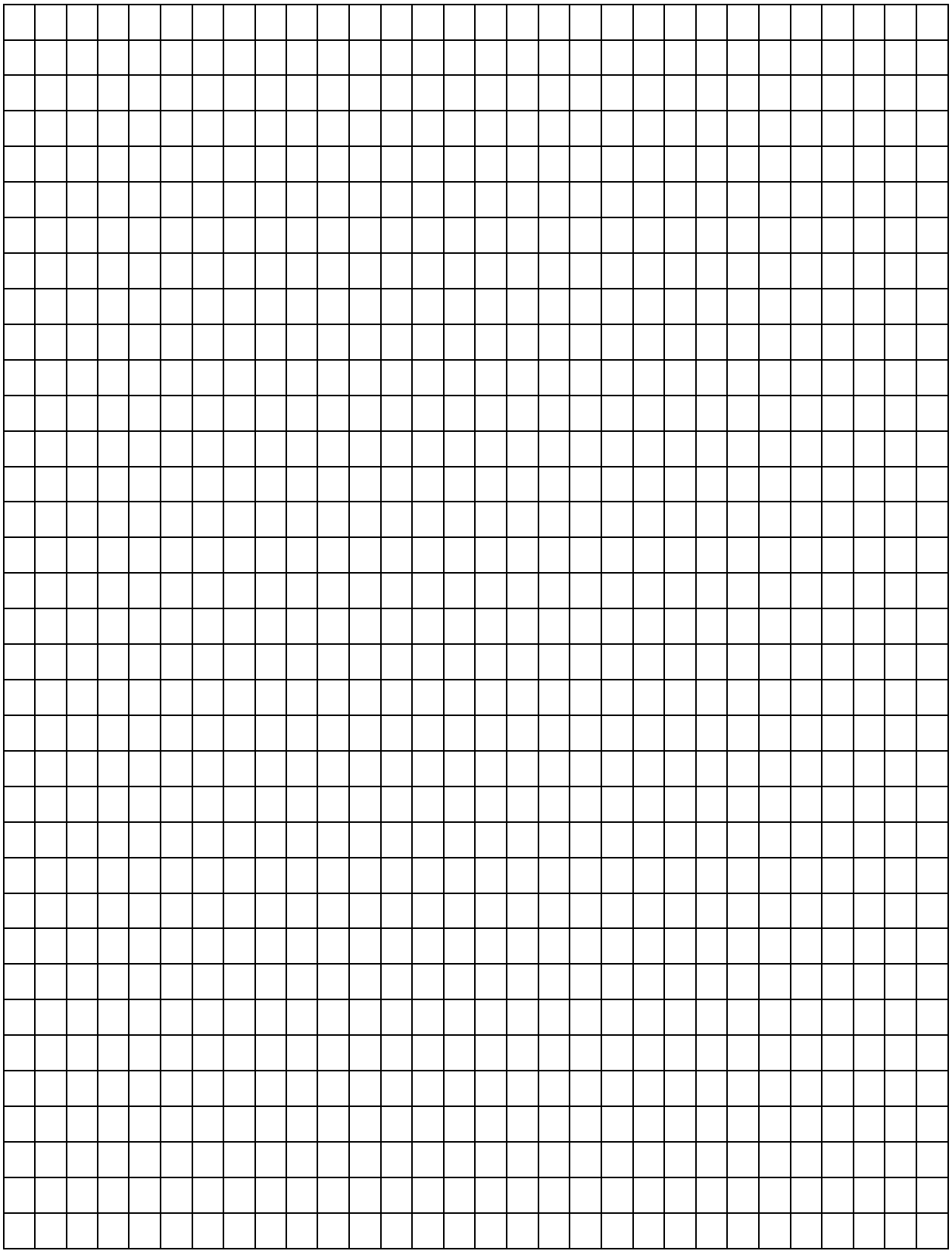
1. Using FlyBase at http://flybase.org/ place 'adh' in the upper right-hand search box and then hit either the '**Go**' button on screen or '**Enter**' on your keyboard.



2. You will get the Adh gene page on FlyBase (as shown below). On the right-hand menu, select the 'Stocks and Reagents' link.



3. Find your Adh allele in the fly stocks, click on the active links and find out about your allele(s). Read about each Adh allele that was assayed by the class. Write a few sentences explaining whether your prediction(s) correspond with the results and, based on what you learn about your allele in FlyBase, explain why or why not.



Day 3: FlyBase: Alcohol Dehydrogenase (ADH) Gene in Alcohol Use Disorder in Flies and Humans.

Learning Objectives:

1. **Use FlyBase including BLAST**
2. **Compare ADH in humans and flies**
3. **Analyze ModENCODE expression data of ADH gene.**

Instructions: Answer questions using the LAST PAGE of this document which has the ANSWER SHEET on 1 page. Submit ONLY your answer sheet to the Blackboard Submission Portal.

Pre-Lab videos to Watch:

1. **Dr. Key's Introduction to FlyBase:** **https://youtu.be/XhyXc63dhhE**
2. **FlyBase for Undergrads video:**

https://www.youtube.com/watch?v=_oL8h47fOuQ

3. **Johns Hopkins BLAST tutorial:**

https://www.youtube.com/watch?v=HXEpBnUbAMo

Lab Computer Work

1. Go to flybase.org
2. In the 'Jump to Gene' box, insert the letters "**ADH**" and hit 'Go'

Figure 1. FlyBase search engine text box.

3. On the menu found on the right side just under the 'Jump to Gene' box, select on the 3rd link down named: "**Function**" (red arrow in Figure below). This will take you to the GO Summary Ribbon. Look below the **GO Summary Ribbon at the table.**

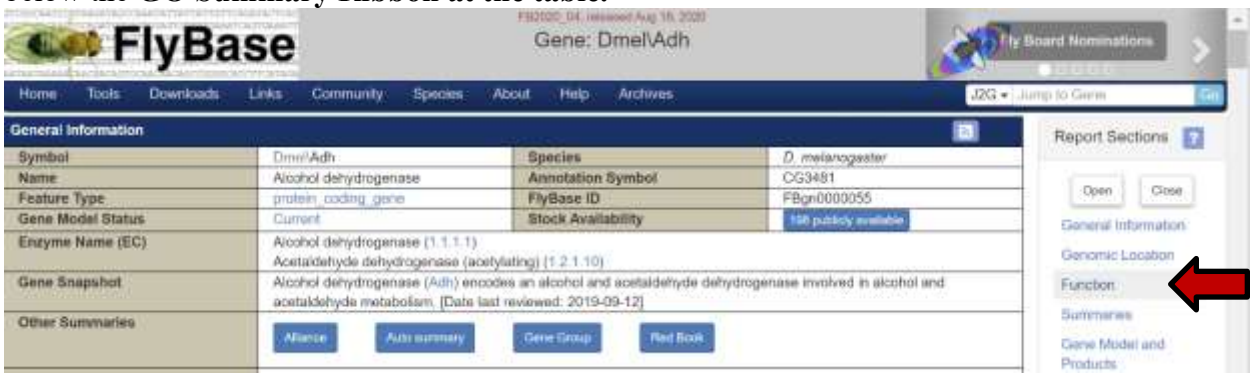


Figure 2. FlyBase right-hand menu.

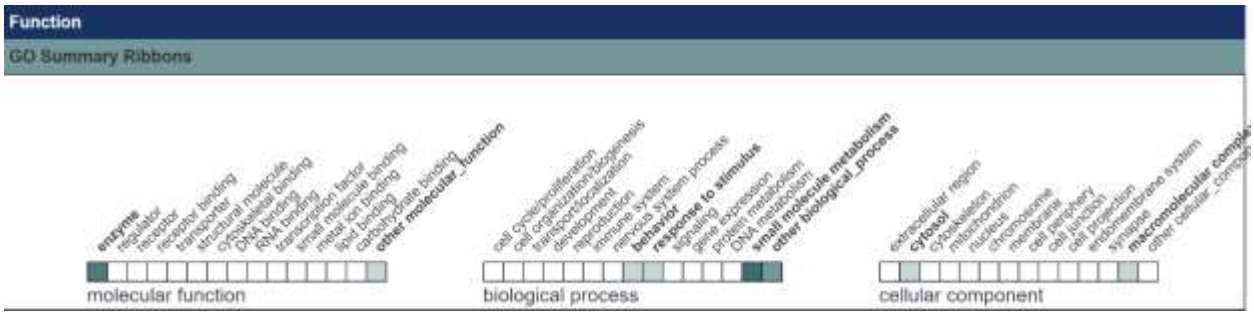


Figure 3. FlyBase GO Summary Ribbons: Ribbon 1 = molecular function, Ribbon 2 = biological processes, Ribbon 3 = cellular component.

Q#1: ***SATA (Select-All-That-Apply). Using the Ribbons found under the 'GO Summary Ribbons',*** *to see the* '**Molecular Functions' Ribbon.** Place a checkmark in each blank below that corresponds to the **ADH** gene's molecular function:

____ enzyme
____ receptor
____ transcription factor
____ small molecule binding
____ lipid binding
____ other molecular function

Q#2: Use the mouse to hover over the '**enzyme' button** under **Molecular Function** graphic and write exactly the 3 names of the enzymes that appear in the drop-down box:

a. _____

b. _____

c. _____

Q#3: ***SATA.*** Look again under the GO Summary Ribbons and place a checkmark in the blanks below that correspond to the **Biological Processes** of the **ADH** gene product:

____ cell cycle /proliferation
____ cell organization/biogenesis
____ cellular transport/localization
____ development
____ immune system
____ behavior
____ nervous system process

Q#4: ***SATA.*** Under the GO Summary Ribbons and check blanks corresponding to terms related to the **ADH** gene **Cell Components**:

____extracellular region
____ membrane
____ cell projection
____ neuron/synapse
____ Cell junction

4. Scroll down or hyperlink down to the "**Orthologs**" menu to expand it (red arrows).

Figure 4. Orthologs menu on ADH FlyBase website.

Q#5: What is the name of the **HUMAN gene** that is an ortholog to the Drosophila? **ADH** gene_____

5. Click the 'Alignment' link to open up the comparison of the human and fruit fly orthologous gene products: the proteins.

6. Looking at the protein-protein alignment, answer questions **Q6, Q7**, and **Q8** below.

Protein Alignment: Adh and HPGD

Sequence 1: NP_001027266.1 **Gene:** Adh **FlyBaseID:** FBgn0000055 **Length:** 256 **Species:** Drosophila melanogaster
Sequence 2: NP_000851.2 **Gene:** HPGD **HGNCID:** 5154 **Length:** 266 **Species:** Homo sapiens

Alignment Length: 249 **Identity:** 67/250 (27%)
Similarity: 118/250 (47%) **Gaps:** 24/250 (10%)

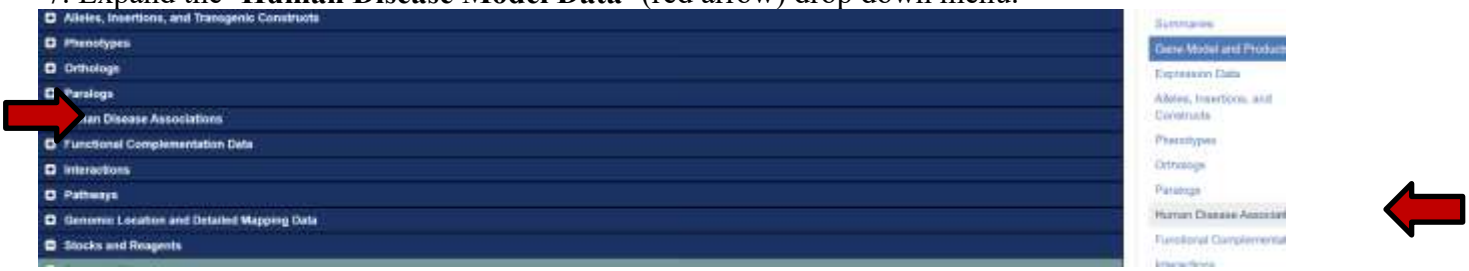
```
Fly      7 NKNVIFVAGLG-GIGLDTSKELLKRDLKNLVILD-----RIENPAAIAELKAINPKVTVTFYPYD 65
           |..|..|.|.. |||...:.:||.:..| :.:::|     .:::..||:.|  ...|:.|: |...|
Human    4 NGKVALVTGAAQGIGRAFAEALLLKGAK-VALVDWNLEAGVQCKAALDE--QFEPQKTL-FIQCD 64

Fly     66 VT--VPIAETTKLLKTIFAQLKTVDVLINGAGILDDHQIERTIAVNYTGLVNTTTAILDFWDKRK 129
           |.  ..:.:|.:.:...|.:|   |:|:|.||:.:|...|:|:.:|...:::.|...||:..|:.
Human   65 VADQQQLRDTFRKVVDHFGRL---DILVNNAGVNNEKNWEKTLQINLVSVISGTYLGLDYMSKQN 126

Fly    129 GGPGGIICNIGSVTGFNAIYQVPVYSGTKAAVVNFTSSLAKLAPI--TGVTAYTVNPGITRTTLV 191
           ||.|||.|:|.|:|....:|.|||..|:..:|.||.|.|..|.:  :|||....:.||...|.::
Human   127 GGEGGIIINMSSLAGLMPVAQQPVYCASKHGIVGFTRSAALAANLMNSGVRLNAICPGFVNTAIL 191

Fly     192 HT------FNSWLDVEPQVAEKLLAHPTQPSLACAENFVKAIELNQ-NGAIWKL 238
           .:      ...:::.:..:.:.:..:........|...:..||.:. ||||.|:
Human   192 ESIEKEENMGQYIEYKDHIKDMIKYYGILDFPLIANGLITLIEDDALNGAIMKI 245
```

Q#6: What is the length of each protein: Drosophila Adh _____ a.a. and Human HPGD_____ a.a.

Q#7: What is the percent amino acid (a.a.) Identity: ____ and what does 'Identity' mean? _____ What symbols in the alignment indicate 'identity' amino acids (same amino acid in both species)? _____

Q#8: What is the percent a.a. Similarity: _____ and what does 'Similarity' mean? _____ What symbols in the alignment indicate 'similar' amino acids (in the same biochemical group)? _____

7. Expand the '**Human Disease Model Data'** (red arrow) drop down menu.

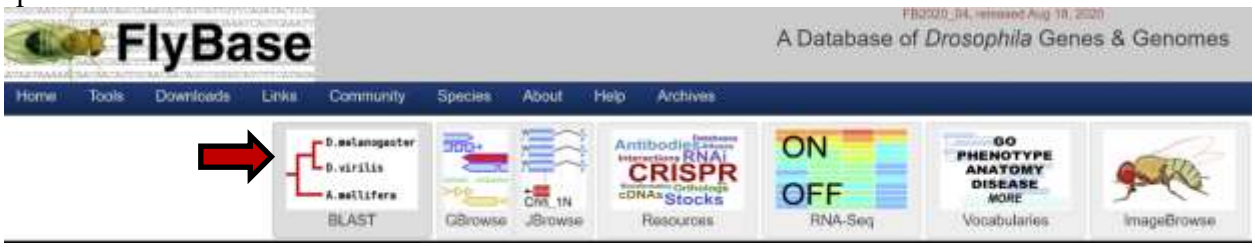


Q#9: List the 1 **human** disease that mutation of the ADH gene is linked to:

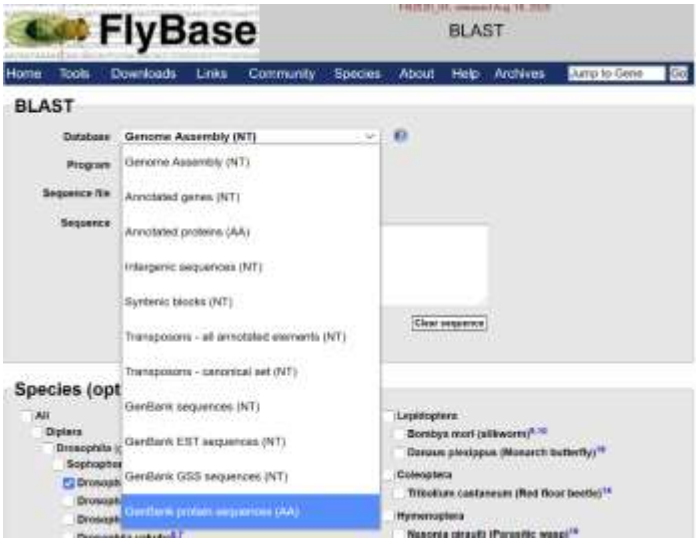
8. Copy the below sequence for HUMAN Alcohol Dehydrogenase 1a (ADH1a); displayed in FASTA format.

>sp|P07327|ADH1A_HUMAN Alcohol dehydrogenase 1A OS=Homo sapiens OX=9606 GN=ADH1A PE=1 SV=2
MSTAGKVIKCKAAVLWELKKPFSIEEVEVAPPKAHEVRIKMVAVGICGTDDHVVSGTMVT
PLPVILGHEAAGIVESVGEGVTTVKPGDKVIPLAIPQCGKCRICKNPESNYCLKNDVSNP
QGTLQDGTSRFTCRRKPIHHFLGISTFSQYTVVDENAVAKIDAASPLEKVCLIGCGFSTG
YGSAVNVAKVTPGSTCAVFGLGGVGLSAIMGCKAAGAARIIAVDINKDKFAKAKELGATE
CINPQDYKKPIQEVLKEMTDGGVDFSFEVIGRLDTMMASLLCCHEACGTSVIVGVPPDSQ
NLSMNPMLLLTGRTWKGAILGGFKSKECVPKLVADFMAKKFSLDALITHVLPFEKINEGF
DLLHSGKSIRTILMF

9. From the FlyBase Home Menu, select the Drosophila **BLAST** icon to link out to the Drosophila-specific BLAST tool.

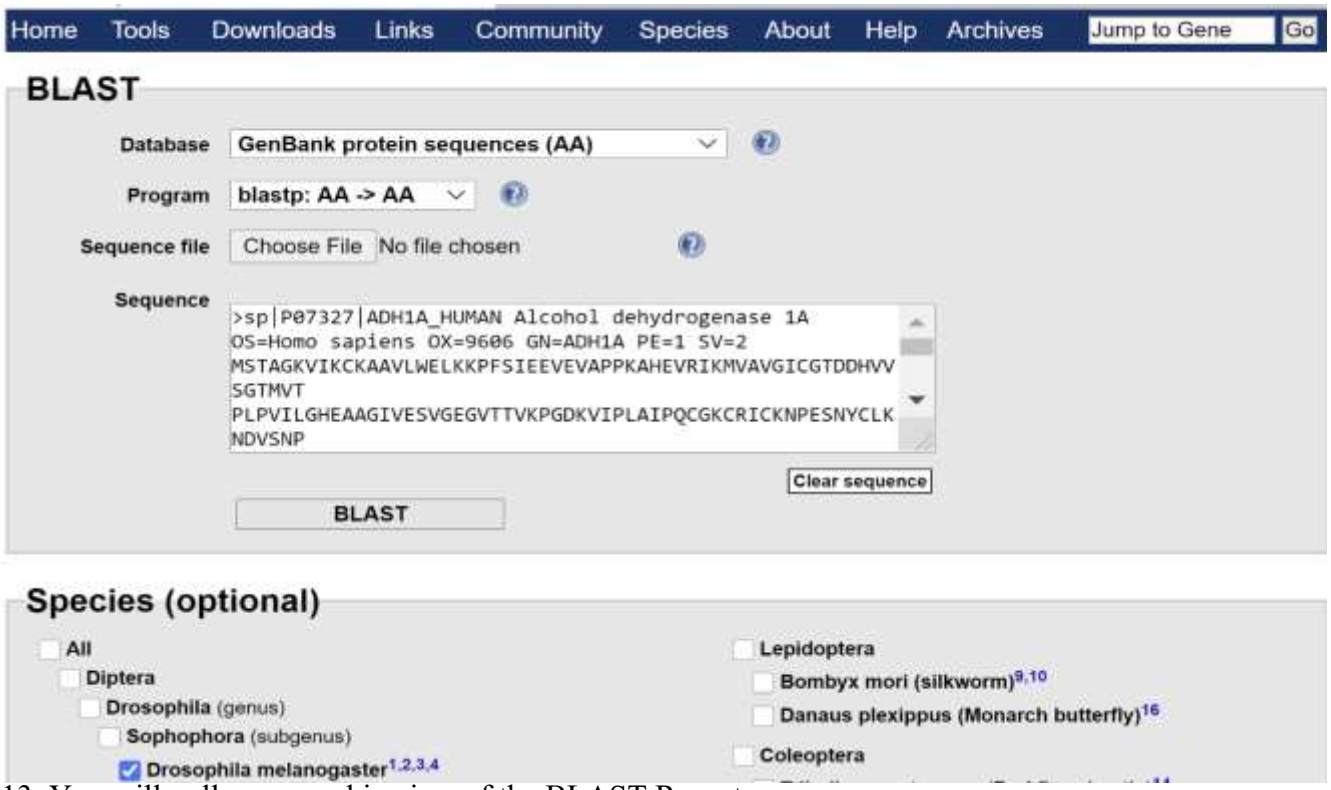


10. Click on the '**Database**' **drop down arrow** and select "**GenBank Protein Sequences (AA)".**



11. Then paste the *Homo* sapiens alcohol dehydrogenase 1a FASTA protein sequence you copied above into the '**Sequence' window.**

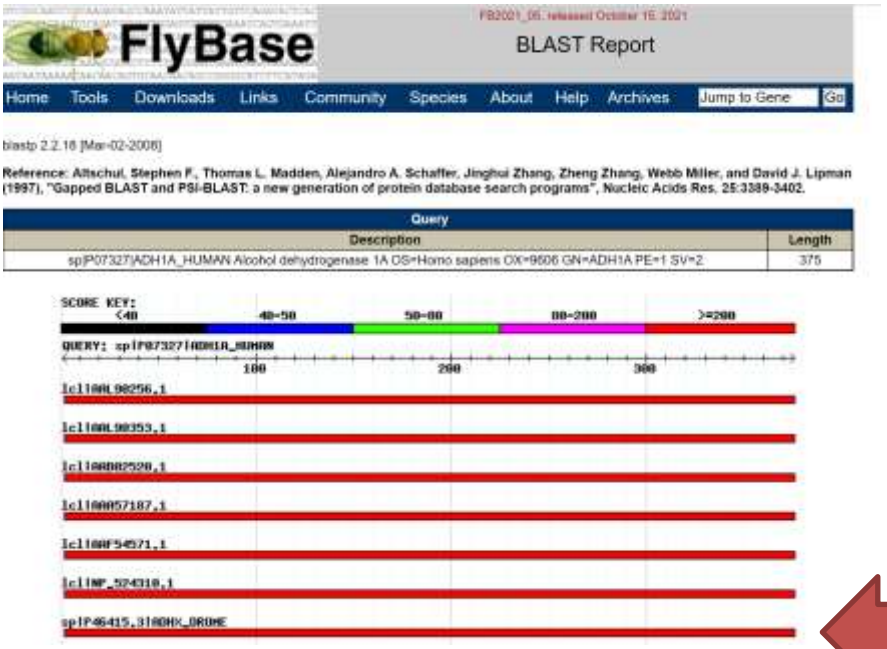
12. Finally, **hit the 'BLAST'** button as highlighted in the next snapshot.



13. You will pull up a graphic view of the BLAST Report.
14. Click on the **5th red line**… which is one of the BLAST hits.

Q#10: What is the name of the *Drosophila* protein that is identified in the BLAST report?

_____ _____



5th "hit"

Human and fly ADH alignment

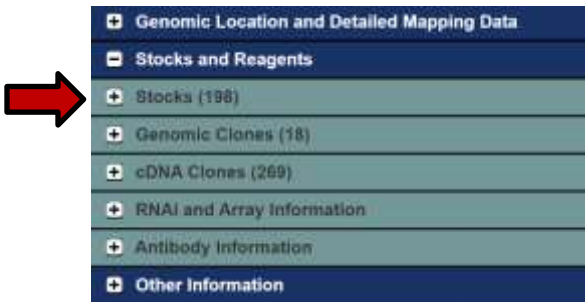
>sp|P46415.3|ADHX_DROME RecName: Full=Alcohol dehydrogenase class-3; AltName: Full=Alcohol dehydrogenase class-III; AltName: Full=Glutathione-dependent formaldehyde dehydrogenase; Short=FALDH; Short=FDH; Short=GSH-FDH; AltName: Full=Octanol dehydrogenase; AltName: Full=S-(hydroxymethyl)glutathione dehydrogenase
Length = 379

HSP # = 1 , **Score =** 417.157 bits (1071) , **Expect =** 3.26302e-116
Identities = 202 / 377 (53.6%) , **Positives =** 267 / 377 (70.8%) , **Gaps =** 3 / 377 (0.8%)

Subject FASTA

```
Query:   2          STAGKVIKCKAAVLWELKKPFSIEEVEVAPPKAHEVRIKMVAVGICGTDDHVVSGTMVTP   61
                    +T GKVI CKAAV WE KKP  IE++EVAPPKAHEVRIK+ A G+C TD   +GS
Subject: 3          ATEGKVITCKAAVAWEAKKPLVIEDIEVAPPKAHEVRIKITATGVCHTDAFTLSGADPEG   62

Query:   62         L-FVILGHEAAGIVESVGEGVTTVKPGDKVIPLAIFQCGKCRICKNPESNYCLKNDVSNP   120
                    L FV+LGHE AGIVESVGEGVT  K GD VI L IPQC +C+ CK+ ++N C E  ++
Subject: 63         LPFVVLGHEGAGIVESVGEGVTNFRAGDHVIALYIPQCMECKPCRSGKTNLCQKIRLTQN   122

Query:   121        QGTLQDGTSRFTCRGKPIHHFLGISTFSQYTVVEDMAVAKIDAASPLEKVCLIGCGFST   180
                     G + +GTSR +C+ + + HF+G STF++YTVV + ++ KI+  +PLEKVCL+GCG STG
Subject: 123        AGVMPEGTSRLSCRGQQLFHFMYTSTFAEYTVVADISLTKINEKAPLEKVCLLGCGISTG   182

Query:   181        YGSAVNVAKVTPGSTCAVFGLGGVGLSAIMGCKAAGAARIIAVDINKDKFAKAKELGATE   240
                    YG+A+N AKV  GSTCAV+GLG VGL+  +GCK AGA +I  +DIN DKF  AK+ G T+
Subject: 183        YGAALNTAKVEAGSTCAVWGLGAVGLAVGLGCKKAGASKIYGIDINPDKFELAKKFGFT   242

Query:   241        CINPQDY--KKPIQEVLKEMTDGGVDFSFEVIGRLDTMMASLLCCHEACGTSVIVGVPPD   298
                     +NP+D   K  IQ  L ++TDGG D++FE IG ++TM ++L   H+  GTSV++GV
Subject: 243        FVNPKDVAEKGSIQNILIDLTDGGFDYTFECIGNVNTMRSALEATHKGWGTSVVIGVAGA   302

Query:   299        SQNLSMNPMLLLTGRTWKGAILGGFKSKEQVPKLVADFMAKKFSLDALITHVLPFEKINE   358
                     Q +S  P  L+ GR WKG+  G++ S   VPKLV D++ K  +D  ITH LP  +INE
Subject: 303        GQEISTPPFQLVFGRVWKGSAFGGWRGVSVDVPKLVSDYLEKDLLVDEFITHELPLSQINE   362

Query:   359        GFDLLHSGKSIRTIINF   375
                    FDL+H G+SIR+I+ +
Subject: 363        AFDLMHKGESIRSIIKY   379
```

Q#11: If you wanted to study alcoholism in the fruit fly model organism, **how many different mutant ADH stocks** could you **order** from the Bloomington Stock Center ….



[hint: Stock (###) and if you want to see the all make sure to hit the blue box that says "**More stocks available…" at Bloomington]?** _____

Q#12: Analyzing the BLAST hit above:

a. Record the identity score: _____

b. Record the positive score:_____

c. Record the E-value: _____

d. Is it likely that fruit flies have an alcohol dehydrogenase gene coding for the protein? Justify your answer using **Expect value (E-value)** and the percent positives in your answer.

Follow this link https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3860432/
to a research paper about Human ADH genes and answer the below questions:

Q#13: There is 1 ADH gene in fruit flies, how many different ADH genes are in humans?

Q#14:. Discussion: What are some of the reasons that studying the genes associated with alcohol use disorders (AUD) is easier in fruit flies as compared to human?

ANSWER sheet for FlyBase ADH Exercise

Student name: _____ Date: _____

Q#1:

_____ enzyme
_____ receptor
_____ transcription factor
_____ small molecule binding
_____ lipid binding
_____ other molecular function

Q#2:

a. _____

b. _____

c. _____

Q#3:

_____ cell cycle /proliferation
_____ cell organization/biogenesis
_____ cellular transport/localization
_____ development
_____ immune system
_____ behavior
_____ nervous system process

Q#4:

_____ extracellular region
_____ membrane
_____ cell projection
_____ neuron/synapse
_____ cell junction

Q#5:_____
Q#6: _____
Q#7: _____
Q#8: _____
Q#9: _____
Q#10: _____
Q#11: _____
Q#12: _____
Q#13: _____
Q#14: _____

References

https://www.cdc.gov/ncbddd/fasd/data.html

https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/understanding-alcohol-use-disorder

Edenberg, H.J. The Genetics of Alcohol Metabolism: Role of Alcohol Dehydrogenase and Aldehyde Dehydrogenase Variants. Alcohol Res Health. (30) 1: 5-13 (2007)

Maples, T. and A. Rothenfluh. A Simple Way to Measure Ethanol Sensitivity in Flies. J. Vis. Exp. (48), e2541, doi:10.3791/2541 (2011).

Montane-Jaime, K., Moore, S. Shafe, S., Joseph, R., Crooks, H. Carr, L., and C.L. Ehlers. ADH1C*2 allele is associated with alcohol dependence and elevated liver enzymes in Trinidad and Tobago. Alcohol. 39 (2): 81-86 (2006).

Pandey, U. B., and C. D. Nichols. “Human Disease Models in Drosophila Melanogaster and the Role of the Fly in Therapeutic Drug Discovery.” Pharmacological Reviews, vol. 63, no. 2, 2011, pp. 411–436., doi:10.1124/pr.110.003293.

Materials

For the bioinformatics and calculation days, students will need a computer with Internet access for FlyBase bioinformatics questions (Day 1) and worksheet (Day 3). Excel program. LCD projector and computer are required for in person instructor presentations. However, pre-lab video tutorials and a pre-lab quiz have been created to reduce instructional time in the lab and maximize "hands-on" time for students.

Adult Behavioral Assays (EMBA)	Nutri-fly food	66-113	Genesee Scientific	381.45
	Narrow Vials with flugs (assays performed in the vials and the white flugs make flies visible)	32-109BF	Genesee Scientific	278.15

.

Reagents, Flies & Shipping costs	100% ethanol (200-proof) – 500 mL	AC-61509-50000	Fisher Scientific	2*65.22 = **$130.44**
	Control (w1118) & Adh flies	See Appendix A.	Bloomington Stock Center	8*$17 per strains = **$136**
	shipping	As needed	Sigma, Genesee, Bloomington	$200 estimate

Each student group (groups of two are recommended) will need two ethanol exposure chambers (consisting of a narrow vial that has ½ of a fly plug (flug from Genesee Scientific) or buzz plug (ThermoFisher Scientific) pushed to the bottom (without the food shown in the JOVE video protocol). Including the described exposure chamber student groups will need:

- 2 Ethanol exposure chamber
- 4 whole flugs
- P1000 & tips or disposable pipette to measure 500 μL
- 15 or 50 mL conical of 200 proof EtOH (dyed with food coloring
- Timer
- 8-10 control flies (male or female) in a food vial
- 8-10 Adh allele flies (male or female) in a food vial
- OR
- 8-10 control flies reared on chronic exposure during 5-day larval developmental period (in a food vial)
- One recycled Styrofoam ice box

For a class of 24 students to assay 1 sex per group you will need

- 24 ethanol exposure chambers (2 per student group; 12 groups in the class)
- 48 whole flugs/buzz plugs/vial closures
- 192-240 of each sex-sorted control fly strain and experimental strain (either ADH allelic variants or ethanol-exposed flies)
- 12 p1000s and appropriate tips
- 12 conical tubes with 200 proof (100%) ethanol dyed with food coloring
- 12 timers (or have students use cell phones)
- At least 1 thermometer to provide room temperature on the whiteboard.
- 12 recycled white Styrofoam ice boxes

Notes for the Instructor

The major challenges of this laboratory include: making sure to start expanding the flies well in advance and the labor-intensive sex sorting of flies a day or two before the class performs the assays. Another challenge is timing flies that eclose from the alcohol-treated pupal cases versus the untreated pupal cases. It is recommended to stagger the vial ages of the untreated organisms so as to have treated and untreated adult flies that are all between the ages of 1-5 days old. Finally, it is recommended that you have trained T.A.s and/or helper undergraduate students to assist students in flipping the flies from food vials into the ethanol exposure chambers and then exchanging dry plugs with the ethanol-containing plugs.

Assessment data was collected which demonstrated that students had positive attitudinal gains and knowledge gains by participating in this 3-day lab module focused on ethanol-induced behavior and using the FlyBase Bioinformatic database to study the different ADH alleles in *Drosophila* (see Appendix A Figure A-1 and A-2) .

Appendix B contains information on Materials and Methods to prep the wet-lab portions of the ethanol-ADH allele modules and how to organize and analyze collected data.

Appendices C and D contain two Power Points to supplement the printed lab. The first presentation was given during the ABLE workshop in June 2021 and includes YouTube links to videos recorded for pre-lab viewing and also links to YouTube posted videos of Ethanol Mobility Behavior Assays (EMBAs) that students who are in quarantine or otherwise remote can observe and use with the worksheets published in this article. The second presentation in Appendix D is completely recorded on YouTube and the URL for this introductory video is also provided.

Appendix E lists questions used for 1.) post-module survey and 2.) pertinent Cumulative Lab Exam questions.

Cited References

https://www.cdc.gov/ncbddd/fasd/data.html

https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/understanding-alcohol-use-disorder

Edenberg HJ. 2007. The Genetics of Alcohol Metabolism: Role of Alcohol Dehydrogenase and Aldehyde Dehydrogenase Variants. Alcohol Res Health. 30 (1): 5-13.

Maples T, Rothenfluh A. 2011. A Simple Way to Measure Ethanol Sensitivity in Flies. J. Vis. Exp. (48), e2541, doi:10.3791/2541.

Montane-Jaime K, Moore S, Shafe S, Joseph R, Crooks H, Carr L, Ehlers CL. 2006. ADH1C*2 allele is associated with alcohol dependence and elevated liver enzymes in Trinidad and Tobago. Alcohol. 39(2): 81-86.

Pandey UB, Nichols CD. 2011. Human Disease Models in Drosophila Melanogaster and the Role of the Fly in Therapeutic Drug Discovery. Pharmacological Reviews, 63(2): 411–436, doi:10.1124/pr.110.003293.

Acknowledgments

Thank you very much to the peer reviewers of this laboratory manuscript whose comments improved the resulting publication. Many thanks to all of the BIOL3100 Genetics students at North Carolina Central University for participating in assessments. I am grateful for the ABLE Roberta Williams TIG (this paper is aim #1 still have aims #2 &#3 to finish developing) and NSF HBCU-UP TIP Grant # 1912188.

About the Authors

Author Jenni Echeverria served as Teaching Assistant in the BIOL3100 Genetics course while she earned her Masters of Science in the Biological and Biomedical Science Department at North Carolina Central University. She now works as a Research Technician at the Duke University Immunology and Virology Quality Assessment Center (IVQAC) in the Duke University Vaccine Institute, where she has been majorly involved in Covid-19 testing and vaccine research.

Cathy Silver Key has been the instructor for the sophomore-/junior-level required Genetics course at North Carolina Central University (an HBCU) since 2005. She also teaches 4 other courses: 1.) the sophomore-level Molecular Biology of Cells (summer only); 2.) a newly developed CURE Drosophila Behavioural Genetics (DaBuGs) courses (NSF funded), 3.) the senior-level Inquiries in Developmental Biology course in Spring semesters, and 4.) Graduate Genetics in Fall semesters. She also maintains a research lab where she happily trains Underrepresented Minority (URM) students to become future researchers and professionals.

Appendix A: Science Education Data

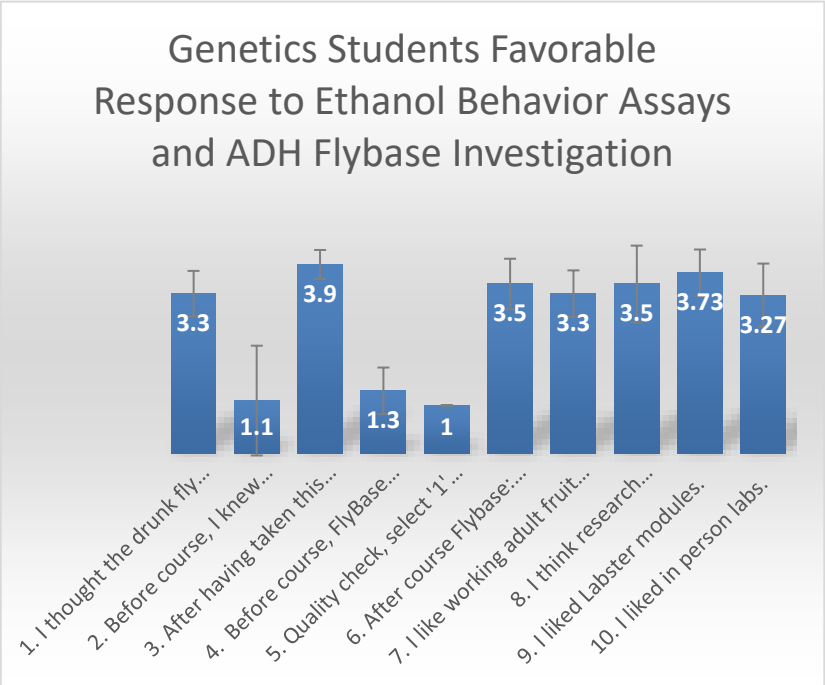


Figure A-1. Student responses to lab 3-day model on fly ethanol behavior assay and FlyBase Bioinformatic exercise. See Appendix E for full questions.

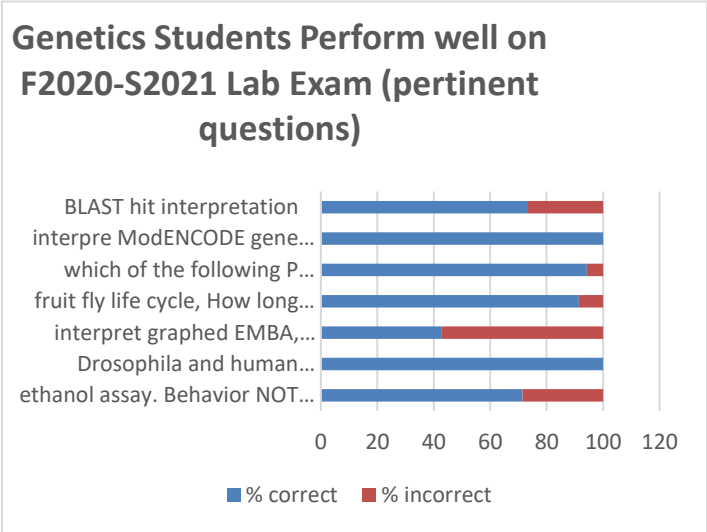


Figure A-2. Student performance on Cumulative Lab Exam suggests that most learning objectives for the 3-Day ethanol behavior and bioinformatic labs were met. One area for improvement is interpretation of graphed data. See Appendix E for full questions.

Appendix B: Materials and Methods

Table A-1. Alcohol Dehydrogenase (ADH) Drosophila Bloomington Drosophila Stock Center*

Strain	Catalog Number	Phenotype	Genotype
Control w^{1118} strain	5905	Normal behavior, white eyes	w^{1118}; Adh^{+}
Control Oregon R	5	Normal behavior, red eyes	Oregon-R-C, Adh^{+}
Adh^{F} allelic strain	6040	Sensitive to ethanol, red eyes	Adh[F] Nucleotide substitution: A1490C Amin acid changes: K192T
Adh^{N1} allelic strain	3976	Sensitive to ethanol, red eyes	Adh[N1] Nucleotide change: G14616681A Reported amino acid change: G92E
Adh^{S} allelic strain	4062	Resistant to ethanol, white eyes	w[1118]; Adh[S] Nucleotide change:C8826A Amino acid change:T192K|Adh-PA

*** Website for Bloomington Drosophila Stock Center (BDSC): https://bdsc.indiana.edu/ For Canadian professors/instructors, you will need to submit Import Permission Documentation to order the flies.**

Food for Drosophila stocks. Dr. Key's lab makes 1 L at a time of Nutri-fly food (Cat# 66-113) from Genesee Scientific (https://geneseesci.com/) supplemented with Proprionic Acid as per the manufacturer's instructions. Each 1 L pack makes 1 tray of narrow food vials with about 5 mL of food per vial. Food is cooked ahead of time, covered with cheesecloth to prevent random fruit flies from entering food vials while the food cools. Food should cure for 48-72 hours and then either be covered with Geneseal for Narrow Vials (Genesee Sci. Cat # 59-166) and placed in 4°C or sprinkled lightly with dry yeast and cotton flugs inserted (store at either room temperature or at 4°C). Instant food from Carolina Biologicals can be used instead of cooked food (Formula 4-24 Instant Drosophila Medium, Blue, 1L; Catalog # 173210).

Fly Maintenance and Expansion. Maintenance of flies and sex-determination is described on the FlyMove website (http://flymove.uni-muenster.de/). In general, flies in narrow vials should be turned over into new food once per week if flies are kept at room temperature (once every 2 weeks if flies are at 18°C). Always keep one vial with larvae and pupae until the newly turned over vial has larvae. To expand the flies, you will want to turn flies over twice per week, keeping the flies in an incubator set to 25°C with a container of water set in the bottom of the incubator for humidity. For a class of 20-25 students where each student will handle their own vial of flies, begin the expansion 1.5 months ahead of the date needed.

Prepping flies for Ethanol Behavior Mobility Assay (EMBA). Clear all adult flies from vials 1-4 days before needed for students. One to 2 days ahead of the student "hands-on" date, anesthetize flies with CO_2 (or FlyNap) and sex sort the flies, placing 8-10 flies per vial. Flies should be no more than 5 days old for the assay.

Preparing the Ethanol Exposure Chambers. Obtain a narrow cotton vial closure called buzz plugs or flugs. Cut in half using a razor blade. Push one half down into a narrow vial using a large dry erase marker. Plug the top of the container with a whole buzz plug or flug.

Performing the ethanol RESISTANCE assay. Watch the JOVE video (Maples and Rothenfluh, 2011; A Simple Way to Measure Ethanol Sensitivity in Flies | Protocol (jove.com)) and Cathy Silver Key's video tutorial to learn how to perform the assay https://youtu.be/rBuxZglfQ8g .

Performing the ethanol TOLERANCE assay. The JOVE video in the Maples and Rothenfluh article also presents how to do the tolerance assay. Basically, the flies are exposed to the ethanol for the amount of time that it takes for 50% of the flies in the vial to become sedated. Then allow the flies to recover from the ethanol exposure for 4 hours. Using a fresh ethanol exposure chamber, expose the recovered flies to the same amount of ethanol (500 μL of 100% 200 proof) as before. For this repeat ethanol exposure, observe how long it takes for 50% of the flies to become sedated: sedation time 50 (ST50). If it takes more time for the flies to become sedated during the second exposure, then the flies are said to have developed TOLERANCE to ethanol.

Appendix C: Major Workshop PowerPoint Presentation

Virtual Conference of the
Association for Biology Laboratory Education

ViABLE2021

Major Workshop:
"Don't Drink and Fly: Alcohol Resistance Behavior in *Drosophila* informs us about Human Genes that Contribute to Alcoholism"

June 17, 2021

S. Catherine Silver Key and Jennifer Echeverria
@ North Carolina Central University

This work is funded in part by a seed grant from an ABLE Roberta Williams TIG and in part by an NSF HBCU-UP TIP: EU-STEM DaBuGs Grant # 1912188

1

About NCCU (where lab implemented)

NORTH CAROLINA CENTRAL UNIVERSITY
TRUTH AND SERVICE
1910
Durham, North Carolina
James E. Shepard, Founder

- ✓ HBCU
- ✓ ~8,000 students
- ✓ 300 Biological & Biomedical Science
- ✓ BIOL3100(Genetics) required course

2

Teaching the Lab in 1, 2 or 3 Two-Hour Lab Sessions

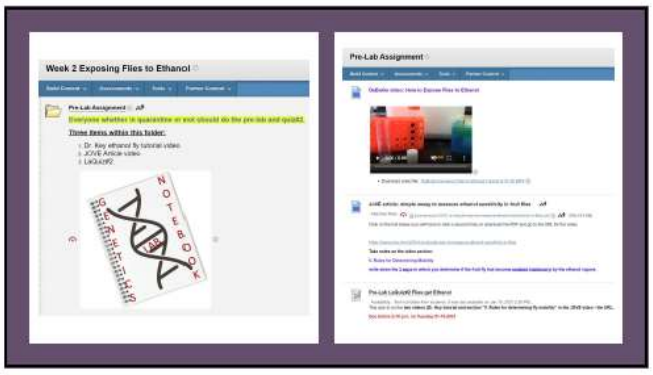
1-Lab Session Model	2-Lab Session Model	3-Lab Session Model
Protocol: w1118 control strain(1 or 2 vials per student, male and/or female **Inquiry:** which gender of flies will be more resistant to effects of alcohol? **Presentation/Discussion:** Increased resistance to the effects of alcohol contributes to Alcohol Use Disorders (AUD) **Optional:** FlyBase activity on Alcohol Dehydrogenase	**Protocols:** Do the male vs. female lab; then do the Alcohol Dehydrogenase (ADH) lab with different ADH alleles. **Inquiries:** **1.** Which gender more resistant to alcohol? 2. Which ADH allele could lead to AUD? **Presentation/Discussion:** Increased resistance to the effects of alcohol contributes to Alcohol Use Disorders (AUD) **Optional:** FlyBase activity on Alcohol Dehydrogenase	**Protocols:** **1.**Do the male vs. female lab; 2. then do the Alcohol Dehydrogenase (ADH) lab with different ADH alleles. 3. FlyBase activity on Alcohol Dehydrogenase **Inquiries:** **1.** Which gender more resistant to alcohol? 2. Which ADH allele could lead to AUD? 3. Is the ADH gene similar in humans compared to flies? What can we learn about ADH expression patterns in flies and apply to humans? **Presentation/Discussion:** Increased resistance to the effects of alcohol contributes to Alcohol Use Disorders (AUD); ADH variance contributions; expression data suggest about ADH more or less resistant?

3

Workshop Outline

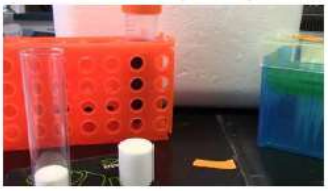
- How to Introduce to the Ethanol Mobility Behavior Assay (EMBA) to students
- The EMBA Kit
- Perform/view the EMBA
- *BREAK*
- Crunching data and generating the graph
- Bioinformatics FlyBase worksheet
- Student feedback and learning gains

4



5

Pre-Lab Video Tutorial #1:
Dr. Key Demonstrates Exposing Flies to Ethanol



Unlisted video on YouTube: https://youtu.be/rBuxZgIfQ8g

6

Pre-Lab Video Tutorial #2: Journal of Visualized Experiment (JOVE) Article

7

URL for the JOVE Maple and Rothenfluh article: [A Simple Way to Measure Ethanol Sensitivity in Flies | Protocol (jove.com)]

Pre-Lab Quiz: Only 4 questions based on the two video tutorials.

8

Workshop Outline

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9

Workshop Outline

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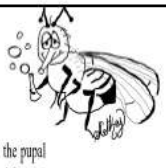
- Student feedback and learning gains

10



DAY 1) EMBAs: Control Strain either Oregon R or w^{1118}

Background: Your instructor has reared flies for 10-14 days. As the adult flies emerged from the pupal cases, adult flies were collected and aged from 1-5 days and then sorted the two genders (male and female) into food vials.

Prediction: Make prediction: Which gender will be more resistant to the alcohol (i.e. take longer to become sedate)? _____

Instructions: Run the EMBA assay as indicated by the protocol on the adult flies your instructor has provided and fill in the data table 1 below.

Protocol:

11

EMBA Day 2: Comparing two genetically different strains

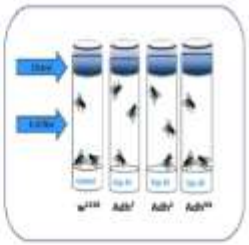
Learning Objectives Part 1:

1. **Reiterative learning of Ethanol Mobility Behavior Assay (EMBA) using two genetically different fly strains.**
2. **Collect data into Excel to and enter into an Excel Worksheet**
3. **Determine and report the ST50 and ST100 for your EMBA**
4. **Using class data (posted after every submits their observational data), calculate:**
   a. **The average ST50 and ST100 for each gender and each fly strain**
   b. **The Standard Deviation from the Average for ST50 and ST100**
   c. **The statistical significance for**
      i. **Either female w^{1118} compared to female Adh^{S}, Adh^{1}, and/or Adh^{S1} strain;**
      ii. **comparing w^{1118} males to male Adh^{S}, Adh^{F}, and/or Adh^{S1} strain;**
      iii. **Comparing w^{1118} females to w1118 males**
      iv. **Comparing Adh^{S}, Adh^{F}, and/or Adh^{S1} females to Adh^{S}, Adh^{F}, &/or Adh^{S1} males.**

12

In person 'Intoxicated Fly Assays'

1. Flip flies from food vial into the ethanol exposure chamber
2. Quickly cap the top with a flug
3. To a separate flug, pipette 500 uL ethanol (dyed blue)
4. Start timer
5. Tap every minute and record observations for 10 s each tap



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w1118 Control EMBAs on YouTube

- Female vs Male Part 1: https://youtu.be/wCZDhDiGNA8
- Female vs Male Part 2: https://youtu.be/d7-rMVQn2jw
- Female vs Male Part 3: https://youtu.be/XGJVmBM8rEc

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ADH variant ethanol assays on YouTube

- Male ADH variant assay on YouTube:
  - Part 1: https://www.youtube.com/watch?v=JZWDMe6C_fs
  - Part 2: https://youtu.be/Rmp0Xkufswz8
- Female ADH variant assay on YouTube:
  - Part 1: https://youtu.be/zGtS6WZHXJw
  - Part 2: https://youtu.be/l6W9LTlVV5w

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Workshop Outline

- How to Introduce to the Ethanol Mobility Behavior Assay (EMBA) to students
- The EMBA Kit
- Perform/view the EMBA
- *BREAK*
- Crunching data and generating the graph
- Bioinformatics FlyBase worksheet
- Student feedback and learning gains

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EMBA Kit Contents

18

Female vs Male Part 1: https://youtu.be/wCZDhDiGNA8
Female vs Male Part 2: https://youtu.be/d7-rMVQn2jw
Female vs Male Part 3: https://youtu.be/XGJVmBM8rEc

Male ADH variant assay on YouTube:
- Part 1: https://www.youtube.com/watch?v=J2WDMe6C_fs
- Part 2: https://youtu.be/Rmp3Xku5wz8

Female ADH variant assay on YouTube:
- Part 1: https://youtu.be/zGtS6WZHXJw
- Part 2: https://youtu.be/I6W9LTJVVSw

EMBA Kit Packed Up

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DaBuGs Video Excel Tutorial#1: 'Crunching the Numbers'

Also available on Unlisted YouTube: https://youtu.be/[illegible]

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DaBuGs Video Excel Tutorial #2: Graphing EMBA Data

Tutorial video also available on unlisted YouTube: https://youtu.be/[illegible]

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Workshop Outline

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Day 3: FlyBase: Alcohol Dehydrogenase (ADH) Gene in Alcohol Use Disorder in Flies and Humans

Learning Objectives:

1. **Use FlyBase including BLAST**
2. **Compare ADH in humans and flies**
3. **Analyze ModENCODE expression data of ADH gene.**

Instructions: Answer questions using the LAST PAGE of this document which has the ANSWER SHEET on 1 page. Submit ONLY your answer sheet to the Blackboard Submission Portal.

Pre-Lab videos to Watch:
1. Flybase for Undergrads video:
https://www.youtube.com/watch?v=_oL8h47fOuQ

2. BLAST tutorial:
https://www.youtube.com/watch?v=HXEp8nUbAMo

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Workshop Outline

- How to Introduce to the Ethanol Mobility Behavior Assay (EMBA) to students
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- Student feedback and learning gains

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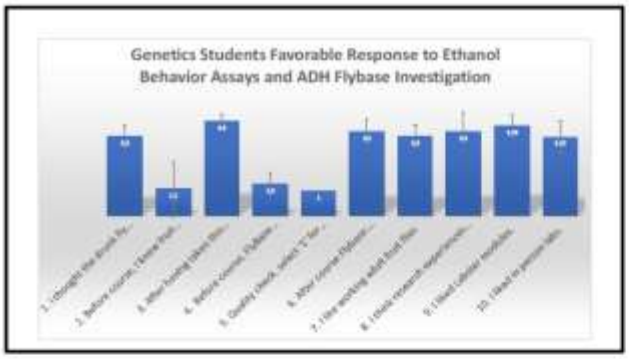

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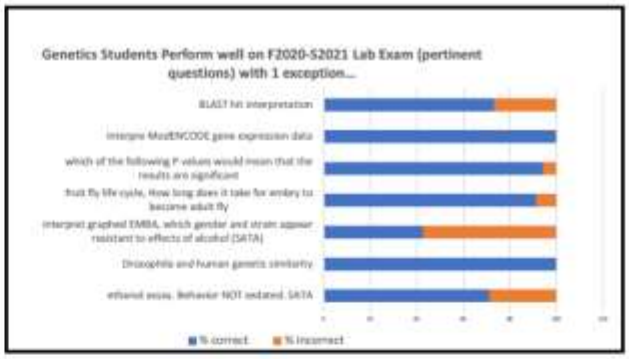

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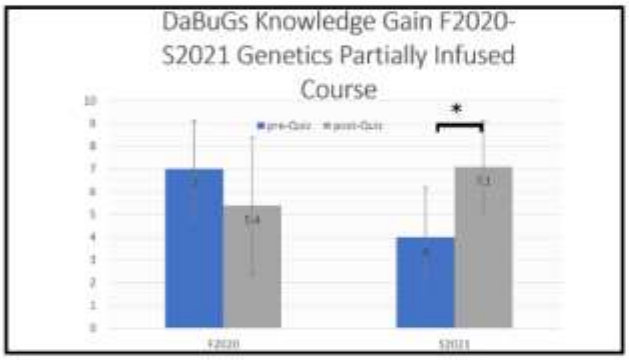

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Appendix D: PowerPoint to Introduce Students to Flies and ADH gene

1

This Power Point presentation available on Unlisted YouTube URL: https://youtu.be/JiGBF4eYbbs

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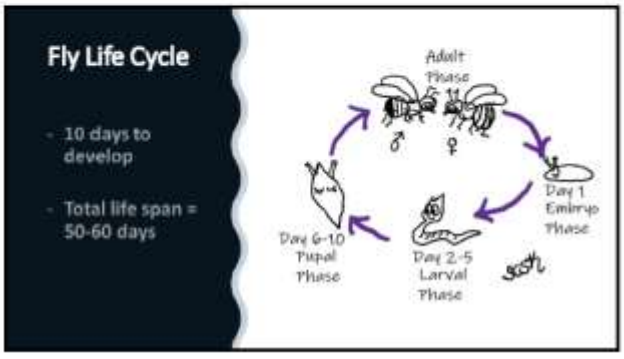

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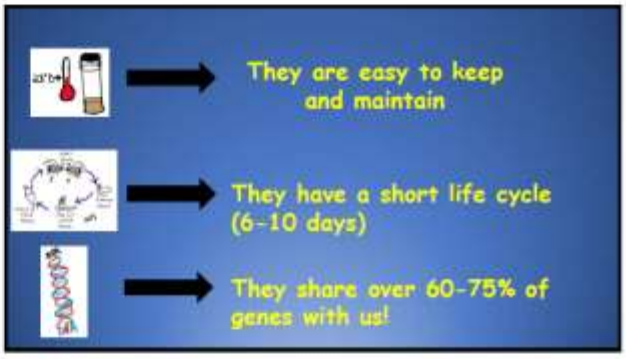
What do we know about fruit flies?

7

8

Why use flies?

9



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Alcohol Use Disorder (AUD)

11

Alcohol Use Disorder (AUD) Definition from NIH

- Problem drinking that becomes severe is given the medical diagnosis of "alcohol use disorder" or AUD. AUD is a chronic relapsing brain disorder characterized by an impaired ability to stop or control alcohol use despite adverse social, occupational, or health consequences.

Alcoholism is an AUD

https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/alcohol-use-disorders

12

Genotype/Phenotype leading to AUD

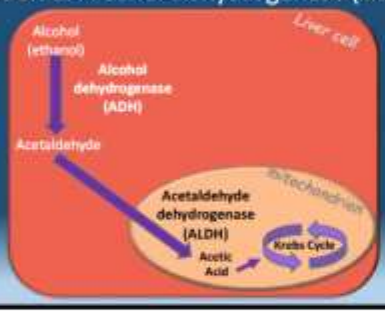
- Genetic difference
- Different alcohol metabolism
- Alcohol Resistant Behavior: less of a 'buzz' and therefore drink more and more to reach the 'buzz' feeling

13

What does the ADH (alcohol dehydrogenase) gene do?

14

Function of Alcohol Dehydrogenase (ADH)



15

Why Should We Care?

- 1 in 8 Americans suffer from Alcohol Use Disorder (AUD)
- Alcohol responsible for over 88,000 deaths.

16

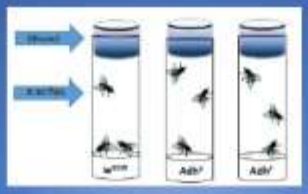
How to study Alcoholism in flies...

17

How do we use fruit flies in the lab to study alcoholism in humans?

18

Ethanol Mobility Behavior Assay (EMBA)



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Translational Research

From BENCH to BEDSIDE

20

So how do we use these results?

Differences ADH alleles and other genes

Differences Ethanol-induced behaviors

Translational Research

(Bioinformatics FlyBase.org)

Improve AUD Treatment

21

NOTE!
URLs to Videos posted on YouTube
on how to do the assay and crunch the data
provided in the Manuscript Appendices!

22

References

Scholz, Henrike, et al. "The Hangover Gene Defines a Stress Pathway Required for Ethanol Tolerance Development." Nature, vol. 436, no. 7052, 2005, pp. 845–847.

Maples, Thomas, and Adrian Rothenfluh. "A Simple Way to Measure Ethanol Sensitivity in Flies | Protocol." JoVE (Journal of Visualized Experiments), 19 Feb. 2011, www.jove.com/video/254/a-simple-way-to-measure-ethanol-sensitivity-in-flies.

Pandey, U. B., and C. D. Nichols. "Human Disease Models in Drosophila Melanogaster and the Role of the Fly in Therapeutic Drug Discovery." Pharmacological Reviews, vol. 63, no. 2, 2011, pp. 411–436.

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Appendix E: Assessment Questions

Pertinent Lab Exam Questions:

12. **Multiple Choice: About how similar are Drosophila (fru...**

Question	About how similar are Drosophila (fruit flies) to humans at the genetic level? (Hint: AUD video lecture)
Answer	✓ 60-75% similar
	10-25% similar
	45-55% similar
	99% similar

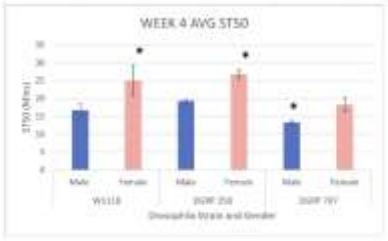
13. **Multiple Answer: Ethanol assay. When being exposed to...**

Question	Ethanol assay. When being exposed to ethanol, what counts as mobile (NOT sedated). Select all answers that are true.
Answer	✓ Able to walk across or up a surface
	✓ Able to turn themselves over
	Able to move their legs as they stay in place
	Able to move their wings as they stay in place

14. **Multiple Answer: Studying the graphed Ethanol Mobility...**

Question

Studying the graphed Ethanol Mobility Behavioral Assay (EMBA) data below, which gender(s) and strain(s) of flies appear RESISTANT to the effects of alcohol? (select as many as are correct. It could be as few as 1 and as many as 4)



Answer

male DGRP-399

male DGRP-707

female DGRP-399

female DGRP-707

21. Multiple Choice: Which of the following P values would...

Question	Which of the following P values would mean that the results are significant?
Answer	0.001
	0.1
	0.06
	0.6

13. **Multiple Choice: The below alignment shows the results...**

Question

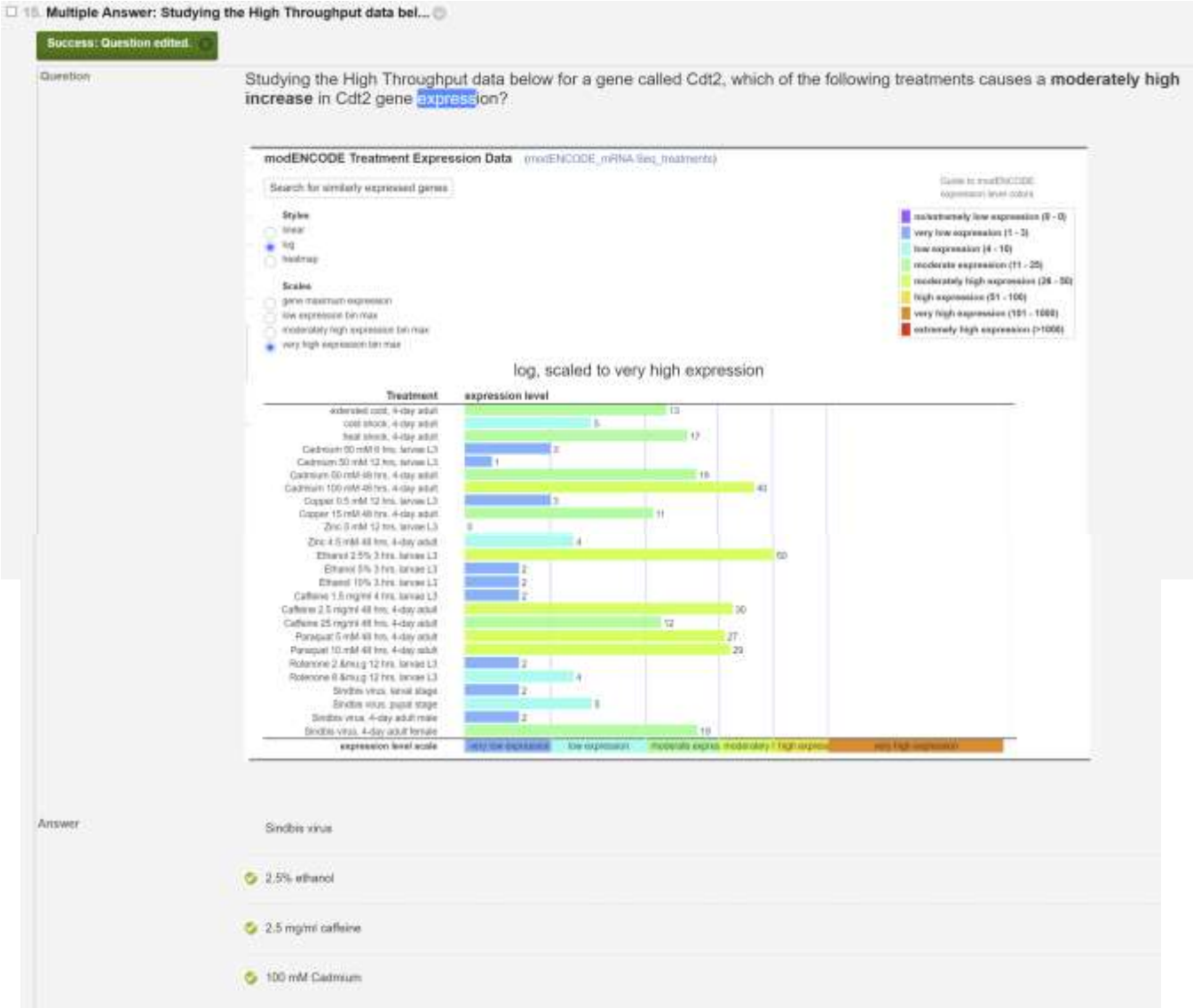
Answer

yes, because the E value is '0' (zero) which means there is no chance that the two sequences are different

yes, because the gap percent is low (<15%)

no, because the number of positive amino acids are just a little more than half

no, because the number of identical amino acids are just a little more than a million



Post-survey questions:

Likert survey. Circle a number for each statement. 'n/a' means not applicable. '1' = strongly disagree; '5' = strongly agree.

1. I interacted with Mr. Saliim frequently.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
2. I feel confident in using TinkerCAD.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
3. I was satisfied with my TinkerCAD design and 3D print out.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
4. I thought the drunk fly assay were interesting.				
	Strongly disagree	Disagree	Agree	Strongly agree

n/a	1	2	3	4
5. **<u>Before</u>** I took this course, I **knew fruit flies** could be used to **study alcoholism**.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
6. **<u>After</u>** having taken course, I **now know that flies** can be used to study **alcoholism**.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
7. **<u>Before</u>** I took this course, I knew how to **use FlyBase**.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
8. Quality check, select '1' for the response to this statement.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
9. **<u>After</u>** having taken course, I **now know** how to look up a human **gene** in flies using **FlyBase**.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
10. I liked working with adult fruit flies.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
11. I liked working with fruit fly larvae.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
12. I liked using the **Labster modules**.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
13. I liked having **in person labs**.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
14. I spend more time as a student than I do as an employee (at a **job**).				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4
15. I think having research experiences are important for professional and/or graduate school application.				
	Strongly disagree	Disagree	Agree	Strongly agree
n/a	1	2	3	4

Write any additional comments here (and/or on the back page):

Appendix F

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit **http://www.ableweb.org/.**

Papers published in *Advances in Biology Laboratory Education: Peer-Reviewed Publication of the Conference of the Association for Biology Laboratory Education* are evaluated and selected by a committee prior to presentation at the conference, peer-reviewed by participants at the conference, and edited by members of the ABLE Editorial Board.

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