**Drosophila-based genetics project lab.**

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**TA preparation instructions**

Experiment 1: Phenotypic characterization of mitochondrial haplotypes and mitochondrial mutations.

This experiment is divided in four parts:

a) *Study of the developmental time strains of flies*.- A week before the experiment, the instructor/TA will collect eggs from the following fly strains: wild type (*OreR;OreR*), *simW501;OreR*, and *sdhBEY12081* and place around 30 eggs in a vial for each strain. Collect eggs following the method described in (JoVE Science Education Database). Prepare 1 vial per strain per pair of students, in total 12 vials of each strain for a class of 24 students. In a week, students will count the number of larvae, pupae and adults if any. The strain *simW501;OreR* is known to have slower development than the wild type (*OreR;OreR*) control (Holmbeck et al., 2015; Montooth et al., 2010) (see examples Figure 1). Therefore, lower number of larvae and pupae are found in this strain.

To our knowledge there is not published research describing the developmental time of the strain *sdhBEY12081*. The allele *sdhBEY12081* is homozygous viable but not fertile. *sdhBEY12081* is located on the second chromosome of the *Drosophila melanogaster* genome (Walker et al., 2006) and is maintained in a heterozygous stateover the balancer chromosome *CyO* described in flybase ([www.flybase.org](http://www.flybase.org)). This balancer does not have a marker for larval or pupal stages, therefore we were not able to differentiate homozygous larvae or pupae from heterozygous. Studying the developmental time of the *sdhBEY12081/CyO* stock, we showed that this strain produced a higher number of flies in pupae than those in the control (see examples Figure 1). Although there are many variables that can explain this result, we like to suggest heterozygous advantage as a potential explanation in class, so that students can relate to what is covered in the genetics lecture. In a planned future course improvement, *sdhBEY12081* chromosome will be placed over a balancer chromosome with larvae and pupae markers such as GFP tagged *CyO* balancer (Casso et al., 2000) . The lack of GFP expression will allow us to discriminate homozygous *sdhBEY12081* from heterozygous individuals.

*b and c) Climbing assay and Flight assay*.- 11 to 16 days before the experiment is scheduled, the instructor/TA will breed flies of the three strains. Prepare 10 vials per strain. For each vial, breed 5 males and 5 females in 5 ml of fly food. Empty the adults after four days of mating. Since the allele *sdhBEY12081 (sdhB)* cannot be kept in homozygosis*,* breed three times more flies than those needed for the wild type and the *simW501;OreR* (around 30 vials). As explained above, the allele *sdhBEY12081* is located on the second chromosome of *Drosophila melanogaster* genome and it is maintained by the balancer chromosome *CyO*. The *CyO* chromosome has a mutation in the gene *Curly* that produces a curvature of the wings. Hence, homozygous *sdhBEY12081* individuals can be sorted by the presence of a straight wings phenotype. Collect adults one day or two days before the day of the experiment. The day of the experiments, each pair of students will be provided with 1 vial per strain. Each vial will have 10 adult flies. All the flies should be around the same age (±1 to 4 days) flies per vial per strain.

Perform the climbing experiment first as explained in the lab manual, and then use the flies from the climbing experiment and perform the flight assay. After the flight assay, the flies cannot be reused as they will be trapped on the walls of the cylinder (Figure 2 and 3).

d) *In vitro activity of succinate dehydrogenase.-* For this experiment the instructor/TA will sort 20 females per strain per vial. Each pair of students will be provided with a vial for each strain. Adult females can be obtained from the same vials that produced the adults used for the climbing and flight assays. To ease the preparation process, the *sdhBEY12081*allele can be used in heterozygosis since the activity of the succinate dehydrogenase is significantly decreased in heterozygous individuals as compared to the wild type control (Figure 4).

Experiment 2: Analysis of inheritance

Each pair of students will be provided with 5 virgin females of the double marker *If/Cyo ; MKRS/TM6B* (lab manual page 19) and 5 males *sdhBEY12081* . In the lab manual, *sdhBEY12081* males are described as homozygous. We used heterozygous males *sdhBEY12081* but kept the genotype in the lab manual as homozygous since explicitly writing the heterozygous genotype: *sdhBEY12081* /CyO will give away that the location of the *sdhBEY12081* allele is on the second chromosome. To be sure that this change won’t affect the rest of the experiment, the instructor or TA will carefully confirm that students selected individuals with regular sized eyes, curled wings and short bristles from the F1 cross.

A description of how to select virgins is provided in (Stocker and Gallant, 2008) or in the youtube video (<https://www.youtube.com/watch?v=zC04KcfllL4>).

Experiment 3: Molecular characterization of haplotypes by PCR-RFLP

For this experiment, the TA or instructor will prepare two agarose gels, one for gel electrophoresis confirmation of the Polymerase Chain Reaction (PCR) product, and the second for confirmation of the restriction fragment length polymorphism (RFLP) reaction. The expected fragment after PCR amplification is about 980 bp. The enzyme used for RFLP, RsaI, will cut *D. melanogaster* PCR product in several fragments, the largest of which would be 760 bp, but won’t cut the *D. simulans* product (Figure 5).

Experiment 4: Independent project

For this experiment we provide the students with a list of previously amplified Drosophila stocks that we have in my lab (please see material suggested).

**References**

Casso, D., Ramı́rez-Weber, F.-A., and Kornberg, T.B. (2000). GFP-tagged balancer chromosomes for Drosophila melanogaster. Mech. Dev. *91*, 451–454.

Holmbeck, M.A., Donner, J.R., Villa-Cuesta, E., and Rand, D.M. (2015). A Drosophila model for mito-nuclear diseases generated by an incompatible tRNA-tRNA synthetase interaction. Dis. Model. Mech. *8*, 843–854.

JoVE Science Education Database. Essentials of Biology 1: yeast, Drosophila and C. elegans. Drosophila melanogaster Embryo and Larva Harvesting and Preparation.

Montooth, K.L., Meiklejohn, C.D., Abt, D.N., and Rand, D.M. (2010). Mitochondrial-nuclear epistasis affects fitness within species but does not contribute to fixed incompatibilities between species of Drosophila. Evolution *64*, 3364–3379.

Stocker, H., and Gallant, P. (2008). Getting started : an overview on raising and handling Drosophila. Methods Mol. Biol. *420*, 27–44.

Walker, D.W., Hájek, P., Muffat, J., Knoepfle, D., Cornelison, S., Attardi, G., and Benzer, S. (2006). Hypersensitivity to oxygen and shortened lifespan in a Drosophila mitochondrial complex II mutant. Proc. Natl. Acad. Sci. U. S. A. *103*, 16382–16387.

**Tentative schedule for 2.5hrs 2X/week**

| **Class** | **Expt 1:** | **Expt 2:** | **Expt 3:** | **Expt 4- Project** | **Due dates** |
| --- | --- | --- | --- | --- | --- |
| 1/26 | Introduction.  Overview of *D. melanogaster.*  Developmental time. |  |  |  |  |
| 1/28 | Climbing assay.  Flying assay. | Introduction.  Set up F1 cross. |  |  |  |
| 2/2 | Succinate Dehydrogenase. |  |  |  |  |
| 2/4 | Graph results.  Experiment 1 overview.  Lab report discussion. | Clear F1 parents |  | Introduction  literature search & project development |  |
| 2/9 |  | Set up F2 cross. |  | article discussion | **Written article**  **response** |
| 2/11 |  |  | DNA extraction  Set up PCR |  |  |
| 2/16 | **1st Lab report due** | Clear F2 parents | Gel electrophoresis  and RFLP |  | **1st Lab report due** |
| 2/18 |  |  | RFLP gel electrophoresis.  Discussing results | Article discussion |  |
| 2/23 | **Article response**  **Oral Presentation** | | | | **Article Oral**  **Presentation** |
| 2/25 |  | F2 climbing assay  Identify chromosome  location of *sdhBEY12081* |  | Work on Proposal  Presentation |  |
| 3/2 | **Proposal**  **presentation** | | | | **Proposal**  **presentation** |
| 3/4  To  4/27 |  |  |  | Work on project |  |
| 4/29 |  |  |  | Work on project | **Lab notebook** |
| 5/4 |  |  |  | Work on project |  |
| 5/6 | **Oral presentation** | | | | **Oral presentation** |
| 5/8 | **Final lab report due** | | | | **Final lab report** |

**Tentative schedule for 3hrs 1X/week**

| **Class** | **Expt 1:** | **Expt 2:** | **Expt 3:** | **Expt 4- Project** | **Due dates** |
| --- | --- | --- | --- | --- | --- |
| 1/26 | Introduction.  Overview of *D. melanogaster.*  Developmental time.  Climbing assay  Flying assay |  |  |  |  |
| 2/2 | Succinate Dehydrogenase. | Introduction.  Set up F1 cross. |  |  |  |
| 2/9 | Graph results.  Experiment 1 overview. | Clear F1 parents |  | Introduction  literature search & project development |  |
| 2/16 | Lab report discussion. | Set up F2 cross. |  | article discussion | **Written article**  **response** |
| 2/23 | **1st Lab report due** | Clear F2 parents | DNA extraction  Set up PCR |  | **1st Lab report due** |
| 3/2 |  |  | Gel electrophoresis  and RFLP | Article discussion |  |
| 3/9 | **Article response**  **Oral Presentation** | | | | Article Response  Oral presentation |
| 3/16 |  | F2 climbing assay  Identify chromosome  location of *sdhBEY12081* | RFLP gel electrophoresis.  Discussing results |  |  |
| 3/23 | **Proposal**  **presentation** | | | | **Proposal**  **presentation** |
| 3/30  To  4/27 |  |  |  | Work on project |  |
| 4/31 |  |  |  | Work on project | **Lab notebook** |
| 5/7 |  |  |  | Work on project |  |
| 5/14 | **Oral presentation** | | | | **Oral presentation** |
| 5/17 | **Final lab report due** | | | | **Final lab report** |

**Material suggested for the experiments (aligned for the tentative schedule 2.5 hrs. 2X/week)**

| **Date** | **Expt. 1:** | **Expt. 2:** | | | **Expt. 3:** | **Project** |
| --- | --- | --- | --- | --- | --- | --- |
| 1/26 | Developmental time. **Each table:**  2 vials of control, w501 and sdhb  2 Sharpies, 2 funnels, 2 chambers with flyNap,  2 rolls of tape | NO preparation | | | NO preparation |  |
| 1/28 | Climbing assay. **Each table**  6 clean empty vials with cotton stopper. 1 Ruler and 1 permanent marker, 1 roll of tape.  Flying assay. **Each table:**  2 500-ml graduated cylinders  1 long pair of forceps, 2 funnel, 1 beaker with  Paraffin oil, a bunch of cotton.  2 vials of control, w501 and sdhb with 10 flies  each | Collecting virgin females  sdH or IF/CyO; MKRS/TM6b | | |  |  |
| 2/2 | Succinate Dehydrogenase.  **PREPARE MIF and MRB (500ml of each for both**  **Classes. Could be prepared the week before)**  **50mM Succinate (50 ml each class),**  **2mM DCPIP (8 ml each class),**  **2mM Decylubiquinone (8 ml each class)**  **BUFFERS BEFORE CLASS**  Micropipettors, tips,  1 96-well plate /**class,**  18 1.5ml Eppendorf tubes/**table**  6 blue pestles /**table**  **Ice buckets**  **Funnels** |  | | |  |  |
| 2/4 |  | Clear F1 parents  permanent marker, funnels, | | |  |  |
| 2/9 |  | Set up F2 cross.  2 Sharpies, 2 funnels,  2 chambers with flyNap,  2 rolls of tape | | |  |  |
| 2/11 |  |  | | | Water bath set to 55C  DNA extraction: [PureLinkTM Genomic DNA mini kit (Invitrogen #K1820-01)].  **Each table:**  12 clean tubes, 6 blue pestles,  6 columns, 12 collecting tubes,  1ml EtOH 100%, 4ml Wash buffer 1,  4ml Wash buffer 2, 0.5ml Elution Buffer  1ml of sterile water  180 ul proteinase K  Lysis Buffer  Set up PCR. **Each table**  Ice buckets,  2 1.5ml tubes, 8 PCR tubes,  2.5ml water, 50 ul 10X Buffer,  20 ul dNTPS  Freezer box  Primers, enzymes will be distributed  during class |  |
| 2/16 | **1st Lab report due** | Clear F2 parents  Sharpies, funnels, | | |  |  |
| 2/18 |  |  | | | Gel electrophoresis and RFLP  1% agarose gel (1X TBE), space for  48 samples + ladders, stained  with SYBR safe  Gel box with 1X TBE buffer  Power supply  100 bp ladder  Incubator at 37C  **Each Table:**  8 1.5ML empty vials, 100ul loading dye  60 ul water, 40 ul buffer 4, enzymes  and BSA will be distributed during class |  |
| 2/23 | **Article response**  **Oral Presentation** | | | | | |
| 2/25 |  | Sort F2 progeny  by marker.  F2 climbing assay  Identify chromosome  location of *sdhBEY12081* | | | RFLP gel electrophoresis.  1% agarose gel (1X TBE), space for  48 samples + ladders, stained with SYBR safe  Gel box with 1X TBE buffer  Power supply  100 bp ladder |  |
| 3/2 | **Proposal**  **presentation** | | | | | |
|  | | |  |

**Buffers preparation**

**Mitochondrial Isolation Buffer (MIB)**

|  |  |  |
| --- | --- | --- |
| **Reagent** | **MW** | **For 500ml** |
| 225mM Mannitol | 182.17 | 20.4 g |
| 75 Mm Sucrose | 342.3 | 12.98 g |
| 10mM MOPS | 209.26 | 1.05 g |
| 1mM EGTA | 380.4 | 0.38 g |
| 0.5% BSA` |  | 2.5 g |

pH it to 7.2. Initial pH will be acidic (around 3)

**Mitochondrial Respiration Buffer (MRB)**

|  |  |  |
| --- | --- | --- |
| **Reagent** | **MW** | **For 500ml** |
| 225mM Mannitol | 182.17 | 40.8 g |
| 75 Mm Sucrose | 342.3 | 12.98 g |
| 10 mM KCL | 74.55 | 0.373 g (or 5ml of 1M stock-for stock add 74.55g in 500ml dH2O) |
| 10mM Tris HCL | 157.6 | 0.8 g (or 5ml of 1 M stock –for stock add 60 g in 500ml dH2O) |
| 5 mM KH2PO4 | 136.1 | 0.340 g (or 2.5ml of 1M stock-for stock add 34.03 g in 250 ml dH2O) |

pH it to 7.2. Initial pH will be slightly basic (around 8)

**Buffer for succinate dehydrogenase in vitro activity**

|  |  |  |
| --- | --- | --- |
| **Reagent** | **MW** | **For 100ml** |
| 45mM NaH2PO4 | 119.98 | 0.539 g |
| 0.1mM EDTA | 372.24 | 2.9 mg (or 1 ml or 10mM stock-for stock add 0.29g in 100 ml dH2O) |
| 1.2 mg/ml BSA |  | 120 mg |

**Rest of reagents**

Succinate: freeze the aliquots and can be used for both classes

DCPIP: wrap in aluminum foil, keep at RT and can be used for both classes

Decylubiquinone: prepare 2mM solution from frozen 40mM stock solution

|  |  |  |
| --- | --- | --- |
| **Reagent** | **MW** |  |
| 50mM succinate | 270.2 | **For 10ml:** 135 mg in dH2O distribute 0.5ml/table. Freeze the rest in aliquots of 1ml |
| 2mM DCPIP | 290.0 | **For 10 ml**: 5.8 mg in dH2O. Distribute 1ml/table |
| 2 mM Decylubiquinone | 322.4 | **For 8 ml:** Make a stock of 40mM (6.4mg in 0.5ml **ETOH**) then add 35 l of the stock to 665ul of **ETOH**). Protect from light and freeze the rest of the stock. Distribute 100l/table (protected from light). Keep in the dark. |

**Fly strains**

Individual fly strains can be obtained by contacting the corresponding authors of the following publications.

All the strains can be requested from:

Eugenia Villa-Cuesta

Assistant Professor of Biology

Adelphi University, Science Building 111

1 South Avenue, Garden City, NY, 11530

*OreR; OreR*🡪 (Holmbeck et al., 2015; Montooth et al., 2010)

*simW501;OreR* 🡪 (Holmbeck et al., 2015; Montooth et al., 2010)

s*dhBey12081*🡪 (Walker et al., 2006)

**Additional mutant Drosophila lines offered to the students**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **strain** | **symbol** | **phenotype** | **type of allele** |  |
| *white* | *w* | eyes white | recessive, sex-linked |  |
| *singed* | *sn* | bristles curly | recessive, sex-linked |  |
| *Bar* | *B* | eyes bar-shaped | dominant, sex-linked |  |
| *black* | *b* | body black | recessive, autosomal |  |
| *brown* | *bw* | eyes brown | recessive, autosomal |  |
| *sepia* | *se* | eyes sepia | recessive, autosomal |  |
| *ebony* | *e* | body dark | recessive, autosomal |  |
| *Wrinkled* | *W* | wrinkled wings | dominant, autosomal |  |
| *Antennapedia* | *Antp* | antenna transformed into leg | dominant, autosomal |  |
| *apterous* | *ap* | no wings | recessive, autosomal |  |
|  |  |  |  |  |
| All of the strains are available from Carolina Biological Supply Company, Burlington, NC. | | | | |
|  |  |  |  |  |

**Supplementary resources to the Drosophila lab manual**

Introductory readings to help instructors and students with fly handling and conventional practices for rearing *Drosophila*:

Greenspan, R.J. (2004). Fly Pushing: The Theory and Practice of Drosophila Genetics (CSHL Press).

Stocker, H., and Gallant, P. (2008). Getting started : an overview on raising and handling Drosophila. Methods Mol. Biol. *420*, 27–44.

Both resources can be found in the following links: [(Stocker and Gallant, 2008)](https://www.zora.uzh.ch/707/1/Stocker_2007.pdf) and [(Greenspan, 2004)](https://books.google.com/books?id=CgtIr1V0zxAC&printsec=frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false).