Biology 2250 Laboratory 4

2011

DNA Extraction of *Drosophila melanogaster* and Analysis of Human Karyotypes

Exercise 1: Extracting DNA from *Drosophila* fruit flies

A common technique used in genetic research today is the extraction of DNA from various organisms, both eukaryotic and prokaryotic. Extraction is often the first step involved in genetic research and is ultimately used for many reasons such as to study gene(s), the determination of nucleotide and/ or amino acid sequences, cloning, running a PCR reaction, diagnosing genetic disorders and diseases, as well as the production of many copies of genes or proteins for treating diseases, and in the use of recombinant DNA technology or genetic engineering.

As such, there are many different techniques (called protocols) available to extract DNA, the exact procedure of which depends on the type of organism used and the type of DNA to be extracted. For instance, the extraction of plasmid DNA versus genomic DNA in bacteria use different protocols, so one must use the proper protocol in order to get the desired type of DNA extracted. There are similarities in protocols used as well though, as all must be able to effectively prepare the organism for removing the DNA which involves breaking open cells, removing cell and organelle membranes, isolating the DNA, etc. Therefore, often similar types of chemicals, steps, etc., are used in the techniques.

In this laboratory exercise, you will extract DNA from fruit flies (*Drosophila melanogaster*) using a protocol commonly used in *Drosophila* genetic research. This will allow you to use practical lab techniques such as centrifuging, pipetting, etc., as well as allowing you to think critically about the procedure and the outcomes.

Materials:

Disposable dissecting gloves
10 Frozen Fruit flies (*Drosophila melanogaster)*
1.5 ml Eppendorf tubes
Eppendorf rack
P-20 microliter pipettor
P-200 microliter pipettor
P-1000 microliter pipettor
Yellow tips for pipettors

Blue tips for pipettors
Discard container for used pipettor tips
Fumehood
37º C Hot water bath
microcentrifuge
-20º C freezer
Squishing Buffer (100µl Tris HCL pH 8.0, 20µl EDTA, 50µl 5M NaCl, sterile H_20)
Proteinase K
Phenol
24 Chloroform: 1 isoamyl alcohol
Sodium acetate
95% Ethanol
70% ethanol
Distilled water
3- 50ml beakers
Permanent marker
Ice bucket with ice
1 ml disposable pipettes
Blue pestle sticks

Procedure: Work in teams of 2. **(Gloves must be worn at all times!!)**

1. Obtain a 1.5 ml Eppendorf tube containing 10 frozen *Drosophila*. Place it in the ice bucket until the flies are ready to be used.

2. In a separate 1.5ml tube, add 1ml Squishing buffer and 8.7µl of 23mg/ml Proteinase K.

3. Transfer 500µl of the Squishing buffer/Proteinase K solution to the Eppendorf tube containing the fruit flies.

4. Squish flies with a blue pestle stick and then place the tubes in the 37ºC hot water bath for 30 minutes. Be sure to label your tube.

5. After removing the tube from the hot water bath, go to the fumehood and add 250µl of Phenol and 250µl of 24 Chloroform: 1 isoamyl alcohol to the fly mixture. Invert the tube to mix. Place the tube in the ice bucket until ready to be used.

6. Centrifuge the tubes at 10,000 rpm for 10 minutes, being sure to balance your tube with another team. **NOTE: If you need to add or remove liquids during balancing, this has to be done in the fumehood!!**

7. While waiting for the centrifugation process, add 1ml **ice cold** 95% ethanol, followed by 10µl sodium acetate, to a new 1.5 ml Eppendorf tube. Place it in the ice bucket until ready to use.

8. After centrifuging, **in the fumehood** collect ~ 80% (~390µl) of the top layer of the liquid in the centrifuged tube and place in the Eppendorf tube containing the ethanol and sodium acetate. **Be careful not to collect any of the debris below that top layer!** Dump the remaining liquid in the organic dump in the fumehood and discard the old tube in the plastics dump.

9. Place the tube in the -20ºC freezer for 30 minutes (NOTE: if we were going to use the DNA after extracting it, normally it would have to be placed in the freezer for at least 1 hour). Remove and centrifuge at 10,000rpm for 15 minutes.

10. After centrifuging, CAREFULLY decant the liquid from the tubes in the organic dump in the fumehood.

11. Add 250µl of 70% ethanol to the tube. Centrifuge again at 10,000rpm for 3 minutes.

12. Using the P-1000 pipettor, remove 230µl of the ethanol, being careful not to disturb the pellet. Then, with a P-20 pipettor, careful remove as much of the remaining 20µl of the ethanol as you can without disturbing the DNA pellet. Let the tubes air dry allowing the remaining ethanol to evaporate.

13. Resuspend the DNA pellet in 500µl distilled water.

Normally after this, you would store the extracted DNA in a -20ºC freezer until it was needed.

Lab 4- Exercise 1 Questions: NAME:_____ Lab Slot_____

1. What did the DNA suspension look like? **1 mark**

   _____
   _____
   _____

2. Can you tell with certainty that you have DNA? _____Why or why not? **1 mark**

   _____
   _____
   _____

   If not, what else could be in the suspension? **1 mark**

   _____
   _____
   _____

3. What could you do to find out if you only had DNA as your final product? **2 marks**

   _____
   _____
   _____
   _____
   _____

4. What are 3 uses of extracted DNA? **1 mark**

   _____
   _____
   _____
   _____

A common problem that many students have when writing laboratory reports is their lack of understanding as to why certain steps and/or chemicals are used when doing an experiment. In an effort to get you thinking about the procedure that was used to extract DNA from *Drosophila*, the following questions are focused on some of the techniques, equipment and chemicals that were used in order for you to gain an understanding of how DNA was able to be isolated. You may use reliable internet sources to find your answers.

5. a) What is the purpose of the buffer? **2 marks**

_____
_____
_____
_____
_____

b) Why is EDTA used in the squishing buffer? **2 marks**

_____
_____
_____
_____
_____

c) Why is NaCl used in the squishing buffer? **2 marks**

_____
_____
_____
_____

6. What is Proteinase K and what is its function in DNA extraction techniques? **2 marks**

_____
_____
_____
_____

7. Why do you squish the flies? **2 marks**

_____
_____
_____

8. What is the purpose of incubating the fly material at 37 degrees for 30 minutes? **2 marks**

_____
_____
_____
_____
_____

9. Why is phenol and chloroform used? **4 marks**

10. Why is centrifugation required? **2 marks**

11. a) What is the purpose of the 95% ethanol? **2 marks**

b) What is the purpose of the 70% ethanol? **2 marks**

12. What is the purpose of sodium acetate? **2 marks**

Exercise 2: Analysis of Human Karyotypes.

A karyotype is the set of chromosomes of an organism or a cell and is often seen as a size-ordered alignment of chromosome pairs in a chart. Karyotyping is an integral part of the study of cytogenetics, which is the microscopic study and analysis of the genetic characteristics of chromosomes. A karyotype is used in human genetics because it connects chromosomes to symptoms and traits of many human syndromes and diseases.

A major reason for looking at karyotypes is for people thinking of becoming parents to show whether or not they have any chromosomal changes that could be passed on to their offspring. Karyotypes are also used to diagnose existing conditions in offspring, which could be the result of changes in chromosomes during gamete formation or during cell division of the early embryo.

The karyotyping procedure usually involved using blood (however other cells can be used). Whichever cell is used however, it must be undergoing mitosis and ideally be in metaphase, so that the chromosomes are replicated, condensed, and easily seen with a microscope.

Preparing a karyotype involves taking the mitotic cells and culturing, fixing and staining these cells. With a microscope, cells in which all of the chromosomes are easily distinguishable are found and photographed. The photograph is enlarged and cut up into individual chromosomes. Homologous pairs are identified and arranged in order by size into the karyotype. The exception is the sex chromosomes, which are last on the karyotype. While this is a common procedure that is still often used today, there are now automatic chromosome analyzers which uses a camera, a computer, and a microscope, which is quicker and more accurate, as well as other karyotyping techniques such as spectral karyotyping, which uses fluorescent dyes that bind to specific regions of chromosomes and Array-based karyotyping, which uses extracted DNA.

How to Analyze a Karyotype:

In order to determine the pairs of chromosomes, it is based on chromosomal size, location of centromere and banding patterns on the chromosomes.

In terms of **size**, chromosomes are generally placed onto a chart or analysis sheet in 7 groups labeled A-G, with the largest chromosome pairs in A and the smallest in G (with the exception of the sex chromosomes, which are placed last).

The **centromere** is visible as a constriction at a certain location on the chromosome and divides the chromosomes into 2 arms. The shorter arm is called “p”, while the longer arm is known as “q”. In terms of centromere location, there are 3 positions possible:

a) Telocentric- at one end
b) Acrocentric- near the end
c) Metacentric- in the middle

Banding patterns are seen as stripes on the chromosomes due to special staining techniques of euchromatin and are used to help compare chromosomes because the locations and the sizes of these bands are specific to each chromosome. There are different banding patterns used, which are based on the staining procedures used. For example, G-bands are darkly

stained and contain a high concentration of A-T bases, whereas R-bands are the reverse and contain a high concentration of G-C bases.

Therefore, size, location and banding patterns are used for karyotyping because two chromosomes that belong to the same pair should be of the same approximate size, have their centromeres in the same location and should have the same banding patterns.

Types of changes in chromosomes:

1) Changes (known as rearrangements) in the structure of a chromosome:

a) A **deletion** (del) – a loss of a fragment (and therefore the loss of genes) of a chromosome during cell division
b) A **duplication** (dup) – a doubling of a segment of a chromosome caused by that segment joining the homologous chromosome, causing that segment to repeat
c) An **inversion** (inv) – a reversal of the direction of a fragment of a chromosome
d) A **translocation** (t) – the movement of a chromosomal segment to another (non-homologous) chromosome

2) Changes in chromosome number, mostly caused by Nondisjunction, results in an abnormal number of chromosomes (called **Aneuploidy**) in cells. Types of aneuploidy cells that are normally diploid cells include:

a) **Nullisomic** cells: have one chromosome pair missing, as in the notation 2n-2
b) **Monosomic** cells: has only one chromosome of a certain chromosome pair, as in the notation 2n-1
c) **Trisomic** cells: have one extra chromosome, as in the notation 2n +1.
d) **Tetrasomic** cells have an extra chromosome pair, as in the notation 2n+2

It should be noted that when dealing with aneuploidy conditions involving the sex chromosomes, the notation involves listing the copies of the sex chromosomes and if one is missing, putting an "O" in its place. Examples include XYY, XXY, XO, etc.

Procedure – Work in Pairs

1. Each student pair will be supplied with photocopied digital images of 2 human karyotypes, one of which is a normal female or male and the other a karyotype of an individual with a genetic disorder or syndrome. Record on your Karyotype Analysis Sheet the Subject Letter that is located on the top of your sheet of chromosomes.

2. **Completing one karyotype at a time** carefully cut out each chromosome (leave a bit of space around each chromosome instead of cutting exactly on the margin of the image).

3. Using the diagram of known numbered chromosomes provided as a basis of comparison, match the homologous pairs using size, location of the centromere and the banding patterns if present.

4. Arrange and glue or tape the pairs on the karyotype analysis sheet in order from longest to shortest, with the exception of the sex chromosomes, X and Y, which are last. NOTE: the X chromosome is of medium length, somewhat between chromosomes 4 and 5 while the Y is one of the smaller chromosomes, comparable to chromosome 14. Be sure to place the chromosomes in the same orientation as they are found on the sheet of known chromosomes.

5. Interpret the karyotypes.

How to Interpret a Karyotype:

Once you've placed all your chromosomes on the karyotype analysis sheet, you have to correctly write the **notation** for the karyotype. This notation includes: total number of chromosomes, the sex chromosomes as well as any extra or missing autosomal chromosomes.

For example, 45, XX, -13 would be a person with 45 chromosomes, is female and is missing a chromosome 13.

If there are rearrangements, these are noted as well. For example, del (4q-) would mean a deletion in the q arm of chromosome 4. Note the "-"sign. A + or – is placed after the chromosome number and the symbol for the arm where an increase or decrease has occurred. Other notations include something like: t (2; 5). This would be a translocation between chromosomes 2 to 5 and inv (2) would be an inversion on chromosome 2. **Note:** if one of the rearranged chromosomes is a sex chromosome it is listed first, otherwise the chromosome with the lowest number is listed first.

You will also be asked to make a diagnosis, if applicable of any abnormalities observed from the karyotype and using your textbook or a reliable internet site, the symptoms of any genetic disorders found.

Some Possible Human Disorders/ Syndromes:

1. Triple X Syndrome: this is due to an extra copy of chromosome X in a female
2. Klinefelter Syndrome: this is due to an extra copy of chromosome X in a male
3. Turner Syndrome: this is due to a loss of chromosome X or Y
4. Down Syndrome: this is due to an extra copy of chromosome 21
5. Cri du Chat Syndrome: this is due to a deletion of the tip of the p arm of chromosome 5, specifically bands 5p15.2 and 5p15.3.
6. Patau Syndrome: this is due to an extra copy of chromosome 13
7. Jacobs Syndrome: this is due to an extra copy of chromosome Y in a male
8. Edwards Syndrome: this is due to an extra copy of chromosome 18
9. Fragile X Syndrome (also known as Martin-Bell Syndrome): this is due to a large number (between 200 to 1300) of duplications of a repeated 3 base pair sequence (CGG) on the FMR1 gene on the fragile site of the X chromosome on the q arm.