

Fruitful DNA Extraction

Activity adapted from “Human Body Exploration” by Kalumuck @ the Exploratorium

We have discussed how **morphological** characters, such as bone structures or the presence or absence of a placenta, can be used to determine the evolutionary relationships between organisms. These morphological characters are part of an organism’s **phenotype**, which is any observable quality of an organism, such as its morphology or behavior.

We can also use **molecular** characters to determine evolutionary relationships. One of the most common types of molecular data is DNA sequence data (we call this molecular data because we are studying DNA molecules). Because DNA mutations accumulate over evolutionary time, the same gene in different individuals or species may be made up of slightly different DNA sequences. This sequence of nucleotide bases is called an organism’s **genotype**. This molecular data can be used to determine evolutionary relationships because as mutations accumulate over time, closely related organisms will have very similar genotypes while more distantly related organisms will have DNA sequences that have fewer base pairs in common.

In this lab, we will be extracting DNA from kiwi fruit. Extracting DNA from an organism is the first step in determining its genotype. Typically, scientists will look at the sequences of one or several genes in the organisms they are studying. This will allow them to compare hundreds or thousands of nucleotides to assess relatedness. These molecular characters can then be combined with morphological characters to more accurately determine evolutionary relationships.

The basics and beyond:

DNA stands for _____ . It is the genetic material present in all organisms, from bacteria to humans. The monomer (subunit of the nucleic acid DNA) is a _____, which consists of a nitrogen-containing base, a sugar, and a phosphate group. Hundreds of thousands of these are linked together to form a chain. Two chains are paired together (with complementary bases (A-T and C-G) matching up) and twisted in a double helix to form a finished DNA strand. In Eukaryotes, one DNA strand in combination with proteins makes up one _____ (hint: you have 46).

The bare essentials of extracting DNA out of cells (these are in order):

1. To get DNA out of the cells, you need to break down the cell membranes and walls (fruit comes from plants – it has cell walls).
2. You need to degrade the proteins found in the fruit. This is to:
 - (1) separate the DNA from the proteins in the chromosomes.
 - (2) keep the protein from interfering with later steps.
3. You need to bind the DNA together so that it can be removed from the solution.
 - To do this, you need to neutralize the DNA’s charge with salt ions, which will allow the DNA to cluster together.
 - Clustering of DNA can be accomplished in other ways too - by adhering it to a silica membrane, for example.
4. Removing DNA from the solution
 - By adding a solution that the DNA is not soluble with (such as alcohol).

Procedure- Work in pairs.

1. Heat a hot water bath to 60 degrees C.
2. Prepare DNA extraction solution
 - A. Dissolve 2 g salt in 90 ml water in a 200 ml beaker.
 - B. Add 10 ml of detergent and stir gently.
3. Peel the kiwi fruit and cut into chunks.
4. Measure 30 g kiwi chunks.
5. Mash your kiwi thoroughly with a plastic fork.
6. Place the mashed kiwi in a beaker.
7. Pour 40 ml DNA extraction solution into the beaker with the kiwi.
8. Add 1tsp meat tenderizer.
9. Place the beaker with the fruit and extraction solution into a 60 degree C hot water bath
 - A. Use a thermometer and make sure it is not too hot and not too cold!!!
10. Incubate the kiwi in the hot water for 12 minutes. Stir occasionally to distribute the heat.
11. Transfer the kiwi beaker to the ice bath for 5 minutes and stir occasionally.
12. While the extraction is cooling, set up your filtration (for this part, work in groups of 2 – you will split the digested kiwi solution)
 - A. Place a funnel over a clean 200 ml flask. Fold the filter (as demonstrated) to fit the funnel.
13. Pour the cooled extraction mixture into the filter-lined funnel. **We will have to let the kiwi filter overnight.**

The next day:

14. Prepare an ice bath to cool the ethanol.
15. Thoroughly swirl the filtrate (the filtered kiwi fruit and extraction solution)
16. Pour about 5 ml of filtrate into a test tube.
17. **GENTLY** layer about 10 ml of ice cold ethanol on top of the filtrate.
 - Gently pour it down the side of the test tube while you hold it at an angle.
18. Let the solution sit for 2 minutes without disturbing it.
 - A white precipitate should form IN THE ALCOHOL LAYER. ... this is.... DNA.
 - If you like, you can collect it with a wire hook, stirring rod, or dropper. It will appear as a slimy white mucus.