



THE STUFF THAT GENES ARE MADE OF *Extracting DNA from living cells*

*"...These our actors,
As I foretold you, were all spirits and
Are melted into air, into thin air;
And like the baseless fabric of this vision,
The cloud-capped towers, the gorgeous palaces,
The solemn temples, the great globe itself,
Yea, all which it inherit, shall dissolve;
And, like this insubstantial pageant faded,
Leave not a rack behind. We are such stuff
As dreams are made on, and our little life
is rounded with a sleep."*

The Tempest IV, i, 148



PURPOSE

To separate DNA from the other components of living cells so that the DNA is tangible and can be seen. Similar steps and reagents are used in research labs to purify DNA for genetic analysis and sequencing.

INTRODUCTION

DNA is a long thin molecule contained in the nucleus of all **eukaryotic** cells (where is it found in **prokaryotic** cells?).

The structure of the DNA molecule gives it the ability to self-replicate and to carry instructions for the formation of proteins. In other words, DNA is the stuff that genes are made of.

One way to understand new ideas is by making analogies. After you read through this lab, try imagining that in the passage quoted above, Shakespeare was writing about cells instead of plays. How many ways can you make the lab fit the Shakespeare?

MATERIALS & EQUIPMENT

- | | |
|---|---|
| <input type="checkbox"/> One fresh onion, medium size | <input type="checkbox"/> Meat tenderizer containing papain (3.0 gm) |
| <input type="checkbox"/> Cutting board | <input type="checkbox"/> Cheesecloth or large coffee filter |
| <input type="checkbox"/> Kitchen knife for dicing onion (or food processor) | <input type="checkbox"/> Plastic transfer pipet |
| <input type="checkbox"/> Thermometer | <input type="checkbox"/> Large funnel |
| <input type="checkbox"/> Distilled water (200 ml) | <input type="checkbox"/> Blender |
| <input type="checkbox"/> Water bath at 60°C (Hot Pot) | <input type="checkbox"/> Balance |
| <input type="checkbox"/> Dishwashing detergent (10 ml) | |
| <input type="checkbox"/> Ice bath | |
| <input type="checkbox"/> Table salt (1.5 gm) | |
| <input type="checkbox"/> 2 beakers (1000 ml) | |
| <input type="checkbox"/> EDTA (5 ml of 0.5M) | |
| <input type="checkbox"/> Spoon, wooden or plastic | |
| <input type="checkbox"/> Graduated cylinder, 100 ml | |
| <input type="checkbox"/> Graduated cylinder, 50 ml | |

Per person or group:

- Glass rod or wooden stick
- Tube, 15 ml
- 6 ml ice-cold ethanol, 95%
- Plastic transfer pipet
- Rack or beaker to hold 15 ml tubes

PROCEDURE

Part I: Extracting the DNA (done collaboratively)

1. Divide into 4 teams and do the following:

FIRST TEAM

- Obtain onion, cutting board, and knife.
- Coarsely chop onion into pieces such as you might use in spaghetti sauce (thumbnail size); don't chop too finely.
- Place chopped onion into an empty 1000 ml beaker.



SECOND TEAM

- Put 75 ml distilled water in a 100 ml graduated cylinder.
- Measure and add to the graduated cylinder:
 - 5 ml 0.5M EDTA
 - 1.5 g table salt (NaCl)
- Measure and add to the solution above:
 - 10 ml detergent
- Mix gently to avoid foaming.
- Add (gently, to avoid foaming) distilled water to a total volume of 100 ml.

THIRD TEAM

- *NOTE: This solution should be made shortly before it is used. If you are not spooling the DNA today, wait and make this solution just before spooling the DNA.*
- Put 30 ml distilled water in a 50 ml graduated cylinder.
- Measure and add to the graduated cylinder:
 - 3.0 gm of meat tenderizer
- Swirl or stir to mix thoroughly, but avoid vigorous shaking, which can denature (inactivate) the protease activity.
- Add distilled water to a volume of 50 ml.

FOURTH TEAM

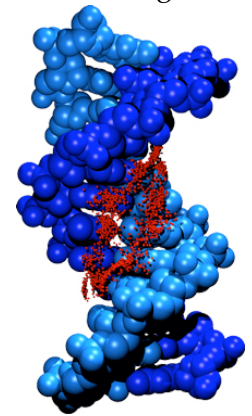
- Line large funnel with four layers of cheesecloth, or with coffee filter folded to fit. Place in a 1000 ml beaker.
- Wet cheesecloth or filter with distilled water.
- Check water level and temperature of waterbath. It should be around 60°C.

2. Pour detergent/salt/EDTA solution (prepared by second team) over chopped onions.
3. Place onion mixture in 60°C water bath for 10-12 minutes.
Take turns stirring mixture, pressing onions against side of beaker to disrupt the tissue and help lyse the onion cells.
The hot water bath softens the cell walls and membranes, so the DNA is released. It also further denatures (deactivates) the enzymes in the mixture that can degrade DNA. More is not better, longer heating can denature the DNA.
4. While onion mixture is incubating in 60°C water bath, read ahead to find out what's going on:

Detergent: Both the cell and its nucleus are bound by membranes made of lipids (fats/oils) with many proteins embedded in them. The detergent breaks the membranes apart, just as detergent dissolves the grease on dishes. This breaking of membranes is called "lysing." (The product "Lysol" gets its name from its advertised ability to lyse the cell membranes of bacteria, thus acting as a disinfectant.)

EDTA: Cells contain many enzymes. Nucleases are enzymes that break down DNA if it is not protected inside the nucleus. When we lyse the nuclear membranes, we expose

DNA to these enzymes. EDTA is a binding agent that prevents nucleases from working by binding divalent cations (Mg^{++} and Ca^{++}) that these enzymes need to do their jobs. (If you read the ingredients of some packaged foods, you will find EDTA listed as a preservative, for this same reason: it prevents enzymes from degrading the food.)



Salt: The phosphates that form the "backbone" of the DNA molecule are negatively charged in a water (aqueous) solution. Negative charges repel each other, yet we want the DNA molecules to crowd together so they can be precipitated from the solution in the final step. The addition of table salt solves this problem. When salt dissolves in water, it forms positively charged Na^+ ions that will "shield" the negative charges of the DNA phosphates. Salt also helps reproduce the environment in the cells (~0.15 M or 1% salt) which increases the stability of the DNA in the solution.

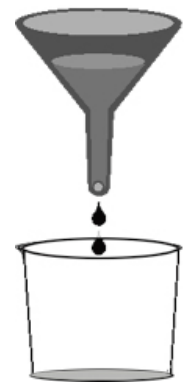
Meat tenderizer: DNA is not the only large molecule in the cell; cells also contain proteins. There are more proteins in a cell than there is DNA. To help separate the DNA from the proteins, we use enzymes. Meat tenderizer contains an enzyme called papain. Papain breaks proteins into small pieces (this is how papain tenderizes meat, by breaking down the protein muscle fibers). Papain is NOT deactivated by EDTA, so it will work in our solution.

Ethanol: DNA is soluble in water/aqueous solutions, but not in ethanol concentrations higher than about 65%. When the ethanol is added to the DNA filtrate, DNA should precipitate, appearing as a whitish mass between the alcohol and filtrate. The other cell contents (degraded proteins, lipids, etc.) do not precipitate out of solution in this concentration of ethanol. The colder the ethanol, the better the DNA precipitation.

5. Cool onion mixture in ice bath for 5 minutes.
While the mixture cools, stir gently and press onion pieces against sides of beaker. The ice water cools the DNA solution. DNA degrades more quickly at warm temperatures.

6. Pour mixture in blender and blend for
1 minute on low speed, then
30 seconds on high speed.
This mechanical action breaks down remaining cell membranes, releasing the cells' contents into the liquid. However, if it is done too much, it also shears the long DNA fibers.

7. Pour mixture slowly into funnel with cheesecloth.
Let liquid filter through into beaker.
This may be a slow process and can be left to continue overnight in a refrigerator.
Alternatively, cheesecloth or filter can be gently squeezed to speed filtration. Try not to get foam into filtrate, though.



Part II: Spooling the DNA (done by each person or each group of 2-3).

8. Place about 2 ml of the onion filtrate in a 15 ml tube.
9. Add about 1 ml of the meat tenderizer solution (prepared by third lab group) to the tube. Swirl *gently* to mix and let the papain (protease) digest the proteins for a couple of minutes.
10. Add 6 ml **ice-cold** ethanol to your tube.
Pour the ethanol slowly down the side of the tube, or add it slowly down the side of the tube with a plastic transfer pipet.
Ethanol is less dense than your aqueous onion solution. Ethanol should float in a layer on top of the onion solution.
DO NOT stir or swirl! You do not want these layers to mix.
11. Let the tube sit undisturbed for 2-3 minutes as the DNA precipitates out of solution. It is important not to shake the tube.
12. Spool the DNA
Once you have a whitish mass of precipitating DNA, place your glass rod or wooden stick into the tube so that the end pokes into this mass.
Gently twirl the rod or stick. Long strings of precipitated DNA should spool around the rod so you can lift it out.
13. Follow instructions for lab clean up, and wash your hands at the end of lab.

Want to know more about DNA?

Check out these websites.

DNA Learning Center at the Cold Spring Harbor Laboratory
www.dnalc.org

Genetic Science Learning Center University of Utah Dept. of Human Genetics
<http://gslc.genetics.utah.edu/>