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BACKYARD BIOLOGY

Kitchen Counter DNA Lab

Extract, purify, and experiment with the blueprint of life.

By Dr. Shawn

DNA is perhaps the most extraordinary structure in all creation. Its famous double helix is the longest molecule known and regulates the life processes in every cell on Earth. Even more, the code that DNA carries is the actual blueprint of life itself. The human recipe, for example, consists of roughly 3 billion molecular bits of information laid out in a precise sequence. Perhaps most amazingly, this miraculous winding staircase directly links every creature on Earth to our ancient and common past — far back to when evolution first began shaping the biological forms that would ultimately populate the world we know today. By examining the differences between the DNA in our bodies and that in other organisms, we can tell when our species diverged from chimps, apes, and even primordial fish.

The properties of this massive molecule are so mysterious and wondrous that most folks assume only the enlightened priesthood of laboratory biologists can extract and study it. Not so. In fact, anyone can extract, purify, and experiment with DNA at home.

When released from a cell, DNA typically breaks up into filaments. In solution, these strands have a slight negative electric charge, which makes for some very useful chemistry. For example, the more negative sections of one DNA strand will tend to attract the more positive regions of another. This causes DNA molecules to clump together and fall out of solution. However, if salt is added, its positive ions are attracted to the DNA's negative charges, effectively neutralizing them. This stops the fragments from adhering and keeps them floating in solution.

So, by controlling the salt concentration, anyone can make DNA fragments either disperse or clump together. And therein lies the critical secret of separating DNA from cells and manipulating it at home.

ISOLATING THE DNA

Extraction

Here's how it works. First, you'll need a salty solution, called a buffer, into which DNA can dissolve. Next, you'll need to break open a bunch of cells and let their molecular "guts" seep out into your buffer. Then, you'll want to add a special enzyme that will destroy unwanted molecules, such as proteins, which would otherwise contaminate your results. Finally, you'll have to reduce the salt concentration enough to cause the DNA molecules to clump together and fall out of solution.

Step One: Build the Buffer

First, you'll need to whip up your buffer (see recipes on next page). Pour 120ml (about 4 oz.) of distilled or bottled water into a clean glass container. Add the table salt and baking soda, and stir vigorously. After they have dissolved completely, stir in the detergent. Shampoos and liquid laundry detergents that contain sodium lauryl sulfate (check the label) work well.

Next, add the tenderizer by wetting a toothpick, inserting it into the meat tenderizer, and transferring it to the buffer. Meat tenderizer contains an enzyme called papain that breaks up proteins so they won't come out with the DNA. Pineapple juice and contact lens cleaning solution also contain protein-busting enzymes, so, alternatively, you can add a drop of one of these two liquids.

Lastly, because DNA degrades fast (sometimes in a matter of minutes), you'll want to slow the pace of destruction by chilling the buffer in a bath of crushed ice. If the buffer becomes cloudy, you've chilled it too much. In that case, warm it just enough to clear it.

Step Two: Get the DNA

For a source of DNA, try the pantry. You can get great results with raw onions, garlic, bananas, or tomatoes. But it's your experiment; choose your own personal favorite fruit, veggie, meat (fresh or frozen), or fungus.

Once you've secured your DNA source, you'll need to process its cells to extract their organic molecules. First, use a knife to dice the material into small pieces. Put the material into a blender and pour in just enough distilled or bottled water to cover the chunks. Then break up (or *lyse*, as biologists say) the cells by pulsing the blades in short bursts until you've blended the material into a slushy mass. This will rip open some of the cells directly and expose many more cell walls and nuclei to the detergent's attack.

Finally, you need to leach out the organic molecules. Place 5ml (1 tsp) of the minced mush into a

For the buffer: Distilled or bottled water (glass 1) 120ml (about 4 oz), **salt** 1.5 grams (¼ tsp), **baking soda** 5 grams (1 tsp), **liquid laundry detergent, dish detergent, or shampoo (glass 2)** not soap — look for sodium lauryl sulfate on the label, 5ml (1 tsp), **crushed ice** to chill the buffer, **meat tenderizer, pineapple juice, or contact lens cleaning solution** just a dollop.

For a source of DNA: Anything with living cells or cells preserved by freezing such as fruits, vegetables, legumes, fungi, meat from the butcher shop (a frozen cow tongue works great!), bone marrow from soup bones, etc.

To extract the DNA: Isopropyl (rubbing) alcohol (glass 3) with no additives and as concentrated as possible. Chill the bottle in the freezer before you begin.

Sundries: A drinking glass to mix the buffer, **small narrow glass container** (preferably with straight walls; a test tube is ideal, but a shot glass will do) to extract the DNA, **narrow drinking straw** to add the alcohol, a **graduated test tube** (or a plain one and a ruler with a centimeter scale) to measure the DNA, **glass swizzle stick** to remove the DNA.

clean container. Mix in 10ml (2 tsp) of your chilled buffer. Swirl *gently* for 2 minutes, and the guts of the shattered cells will separate into the buffer intact. If you stir too vigorously, you'll break up some of the DNA.

Step Three: Dump the Gunk

Next, you'll want to separate the solid gunk from the molecule-laden soup. This is best done with a centrifuge. If you don't have access to one, you can always build one yourself from an old kitchen blender. (To learn how, check out "A Kitchen Centrifuge" on my Tail-Kicking Downloads page scifair.org). If you choose this route, spin at low speed for 2 minutes.

If you don't have a centrifuge lying about and don't want to build one, there are simpler options, such as the toe of an old nylon stocking. Just cut 6 inches off the foot, drop the toe into a clean drinking glass or jar, stretch the fabric across the opening, and pour your molecular broth through. The stretch fabric will cling to the glass and the fine mesh makes a wonderful filter.

Step Four: Extract the DNA

When you've extracted the liquid from the gunk, carefully pour at least 5ml (1 tsp) of the fluid into a narrow vessel, such as a clean shot glass, clear plastic vial, or test tube. (If you're using a vessel larger than a test tube, you'll need more fluid. Use enough to fill the container at least one-quarter full.) You are now ready to coax the DNA molecules to stick together and fall out of the solution.

Remember, the DNA is only suspended in the buffer because salt ions prevent these giant negatively charged molecules from sticking together. Now, you're ready to reduce the salt concentration enough to let the DNA molecules clump together and fall out of solution.

Remove your chilled alcohol from the freezer. Along the inside of the container, you'll need to carefully pour about the same amount of alcohol as you have buffer, so that the alcohol gently settles on *top* of your DNA-laden buffer. To do this, dip a narrow drinking straw into the alcohol bottle and then block off the top of the straw with your finger to capture some alcohol. Remove the straw, tilt the glass, and touch the tip to the inside of the glass. Then, simply let the alcohol flow down along the side. Because the alcohol is less dense than the buffer, it will float on top. If you have a very steady hand, you can also do this step by gently decanting the alcohol into the container by pouring the solution down along a pencil into the container.

Where the 2 liquids meet, a gelatinous sludge will suddenly appear. That sludge is DNA!

At this point, you should see 3 distinct layers: the alcohol on the top, the DNA sludge directly below that, and the buffer on the bottom. The DNA should appear as stringy filaments that stick together. If, instead, it appears as chunky pieces of floating debris, something happened to break up the molecules. You'll still be able to measure its volume, but you may not be able to remove it for study.

Buffer Banter

In the lab, scientists often use the detergent sodium dodecyl sulfate, or SDS, to extract DNA from cells. SDS is also common in shampoos and household detergents, where it goes by the name sodium lauryl sulfate.

Scientists also use table salt – pure sodium chloride – without additives. Morton ("When it rains, it pours") adds calcium silicate to its brand of salt to prevent caking in high humidity. But too many calcium (or magnesium) ions can react to lace your buffer with a white "soap scum," especially if you use a soap rather than a detergent. You can use a liquid soap, but you'll need a salt with no calcium or magnesium compound added (read the label). Water softener salts (both sodium chloride and potassium chloride) work well. Otherwise, use detergent and table salt.

Scientists also use distilled water but bottled water will work just fine. Just don't use tap water because it's loaded with undesirable ions and (often) worse, chlorine, which destroys DNA on contact.

Dyeing DNA

Some dyes bind directly to DNA. Adding a drop or two to the solution will stain the transparent sludge so you can easily see your entire harvest. The safest of these for home use is methylene blue. It is non-toxic, and since it is used to treat certain illnesses in fish, you can find it at many well-stocked aquarium supply stores. It usually comes as a 2.3% solution. The proper DNA stain is about a 1% solution, so you'll want to dilute it by adding an equal amount of bottled or distilled water.

TAKING IT FURTHER

DNA Experimenting

WELCOME TO THE WORLD OF MOLECULAR BIOLOGY

There are two types of experiments that are particularly easy to do, and I recommend that even the most adventurous experimenters start with one of these: discovering how much DNA can be extracted from different organisms under different circumstances, and exploring the conditions that cause DNA to degrade.

Measuring the amount of DNA you've extracted from a sample couldn't be simpler. First, measure the inner diameter of your straight-walled container holding the DNA. Once you know that number, just measure the thickness of the sludge. With that information, it's easy to calculate the volume of DNA you've produced: the equation is $V = \Pi D^2 T/4$, where D is the inner diameter of the vessel and T is the thickness of the layer of DNA sludge. Next, divide the volume of extracted DNA by either the volume or the mass of the material it came from. The simplest way to do that is to accurately measure how much mush you put into your buffer, and then process all of the buffer to extract every scrap of the DNA that leached into it. If you have an accurate scale, weigh your sample before processing it. If not, just measure the volume of the material before you blend it.

Example: Suppose you processed 5g of onion into 10ml of buffer and extracted 1ml of DNA. How much DNA did you get from each gram of onion? Easy! Just divide what you got by what you started with: 1ml DNA / 5g onion = 0.2ml/g.

You can also run experiments with the DNA itself. Usually, the first step is to remove the DNA sludge. It takes a little practice, but you can do it using a clean glass and a swizzle stick. Gently insert the stick through the layer of alcohol and swirl it very slowly in the same direction, with the tip suspended just below the top of the buffer solution. Longer pieces of DNA will spool into the glass, leaving smaller molecules behind.

After a minute of swirling, slowly pull the stirrer up through the alcohol. This will make the DNA adhere to the end of the stick, where it will appear as a transparent, viscous, "snotlike" clump clinging to the tip.

If you now resuspend the molecules in a fresh batch of buffer, you can expose them to chemicals, sunlight, temperatures, or anything else that might break up the DNA. Make a new batch exactly as before and chill it, but don't bother adding the detergent. Submerge the swizzle stick and gently agitate for several minutes as the DNA dissolves into the buffer. Then divide the buffer equally into 2 clean glass containers. Expose one — your test sample — to whatever agent you wish to test. Leave the other — your control— alone. Then process both as quickly as possible and compare the amount of DNA you can extract from each buffer. The difference between the volumes from your test and control is a measure of how much damage your agent does to DNA.

If this sounds too easy, keep in mind that DNA is fragile stuff and it can be affected by lots of subtle things that might escape your notice. Getting consistent results takes practice, so make sure you vary the exposure and that your plotted data shows a regular behavior before drawing any conclusions.

COLD STORAGE

It's actually easy to store your DNA for later use. Just place the "snot bulb," swizzle stick and all, in a container filled with ice-cold isopropyl alcohol and put it in the freezer. Your DNA will keep almost forever.

DR. SHAWN'S DNA EXTRACTION KIT

As a special service to MAKE readers, Dr. Shawn has assembled a kit that contains everything you need to perform at least 20 DNA experiments, including laboratory-grade SDS detergent and chemicals, a fast-flow filter, a generous amount of nontoxic DNA stain, a protein-busting enzyme, a shatterproof graduated test tube, a laboratory-style glass DNA extraction rod, and complete instructions. The cost is $27 + $4 shipping (U.S. only). You may send a check to: 5600 Post Rd. Suite 114-341, East Greenwich, RI 02818, or call (401) 398-7001, or order online at scifair.org.

Dr. Shawn (Shawn Carlson, Ph.D.) is a MacArthur Fellow and the founder and executive director of the Society for Amateur Scientists. To learn more about the society, visit sas.org.