

Step Three: Separate the Fluid From the Solids

Filter: Next you must separate the cellular debris from the molecule-laden soup. To do so, use the fast-flow nylon filter included with this Experimenter's Kit.

The fine nylon mesh makes a wonderful filter. Stretch the open end over a clean beaker.

Let the contents stand and drain on their own for about 30 seconds or so until the fluid has stopped dripping. Then discard.

When you've completed your experiment, don't forget to gently wash the filter in warm soapy water. If properly cared for, your filter should last a very long time.



The fast-flow nylon filter makes it easy to separate the cellular matter from the buffer.

Warning: Do not force the fluid through the filter by squeezing it with your hand. If you do, you will drive some of the cell matter into the solution and contaminate your DNA.

When You've Liberated the Liquid From the Cellular Matter...

The clear filtered liquid contains the buffer chemicals plus lots of organic molecules—DNA, proteins (which are being broken down by the Protein Destroyer enzyme), RNA, carbohydrates and others—that leached out of the cells. Remember, the DNA stays dissolved only because the salt ions in the buffer prevent the negatively charged DNA molecules from sticking together. Now, you're ready to reduce the salt concentration to let those molecules clump up and "precipitate" (pre-cip-i-tate) out of the solution.

Step Four: Extract the DNA

- **Transfer 5 milliliters** (one tsp) of the filtered buffer to the plastic graduated test tube.
- **You should have chilled** your rubbing alcohol in your freezer so that it is now ice cold. If not, do so now and toss out your sample. The DNA won't last until it's chilled. If so, get the squeeze bottle out of the freezer.
- **Carefully deposit** about 5 milliliters of the chilled alcohol on top of the DNA solution by tilting the test tube as shown and gently squeezing the plastic bottle. This will allow the alcohol to stream slowly down along the inside of the test tube so it flows onto the buffer gently. Alcohol is less dense than the buffer and so it will float on top.

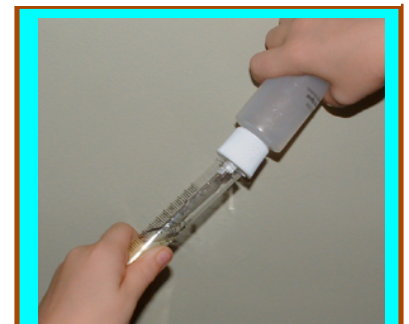
Where the two liquids meet, a gelatinous sludge should appear.

That sludge is DNA!

At this point, you should see three distinct layers; the extraction agent (top), the DNA sludge (middle), and the buffer (bottom).

NOTE: Both RNA and protein molecules can also be extracted in the same way as DNA. That's why this Experimenter's Kit includes our Protein Destroyer. This enzyme is what scientists call a protease (pro-te-ase)—it destroys protein on contact. However, you'll find no Rnase (enzymes that degrade RNA) here. Why not? Because, believe it or not, RNA-busting chemicals are so abundant in nature and so hearty that they naturally occur nearly everywhere, even in distilled water! In fact, it's hard to prepare a

solution that *doesn't* have them. So your DNA should be nearly pure.



When the Extraction Agent is made to flow gently on to the top of the buffer, a layer of DNA sludge appears between them.

