Student activity: Extracting your own DNA

Materials
- Micropipettes or graduated transfer pipettes
  If you don’t have micropipettes, you can use calibrated / graduated disposable plastic transfer pipettes. On these pipettes the ‘stalk’ is graduated, allowing volumes of less than 1 ml to be transferred with sufficient accuracy for this experiment.
- Disposable culture loops or buccal swabs
- Small Falcon tube or a test tube with a bung or cap
  Falcon tubes are calibrated test tubes with screw caps. If you don’t have them, just use normal test tubes.
- Water bath at 40°C (optional)
- Disinfectant solution
- Lysis solution
- Proteinase K solution
- Sodium acetate solution
- Cold ethanol or isopropyl (rubbing) alcohol (keep in the freezer until required)

Procedure
1. Place 1 ml lysis solution in your Falcon tube or test tube.
2. Vigorously scrape a loop or swab around the inside of your cheeks and across your tongue.
3. Place the loop or swab in the lysis buffer and mix it around to dislodge your cells.
4. Put your loop or swab in the disinfectant.
5. Repeat steps 2-4 twice more to ensure you get plenty of cells. Use a new loop or swab each time.
6. Add 20 µl (or 1 drop if you are using transfer pipettes) of proteinase K to your tube.
7. Cap the tube and invert it a couple of times to mix.
8. Incubate the mixture in the water bath or at room temperature for 10 min.
9. Add 100 µl sodium acetate.
10. Cap your tube and shake well to mix.
11. Add 3 ml cold ethanol.
12. Cap the tube and invert it very slowly to mix.
13. Your DNA should appear as a whitish stringy precipitate.
Safety note:
The solutions can irritate eyes and skin, so wear a lab coat, safety glasses and gloves. Saliva can carry diseases; only handle your own loops or swabs and put used items in the disinfectant.

Disposal: liquids can be poured down the sink with plenty of water. Used loops or swabs can be placed in normal waste after disinfecting for 15 minutes.

Questions for discussion
- What does ‘lysis’ mean? How does this help extract the DNA?
- The lysis buffer contains a detergent called SDS. Using your knowledge of cell structure, what do you think the detergent does?
- Inside cells, DNA is found tightly coiled up and bound to a variety of proteins. Which step helps to release the DNA from the proteins?
- What does the last step tell you about the solubility of DNA in both salty water and ethanol?
- How could you confirm that the white precipitate really is DNA?

Extension activities
- Compare this method of extracting DNA with the simpler methods using frozen peas (Madden, 2006) or kiwi fruit. How do they differ? Which one works best? Can you explain why? Can you find out which method is closest to the method that professional geneticists use?
- Simply extracting someone’s DNA is not enough to tell if they have a predisposition for obesity. What other tests would have to be done? Find out more about the techniques used in genetic research.
- In many countries, parents who carry serious genetic conditions like cystic fibrosis or haemophilia can opt for pre-implantation genetic diagnostics to avoid having children that carry the disease. Do you think this procedure should be available for parents who have a genetic predisposition for obesity? Do your classmates agree with you?

Sourcing and preparing the reagents

Lysis solution (50 ml)
1. Prepare tris-buffered saline (TBS) according to manufacturer’s instructions or standard recipe.
   TBS can be purchased as a ready-made solution, in tablet form or made up from scratch.
   Safety note: a ready-made sodium dodecyl sulphate (SDS) solution is recommended as powdered SDS is harmful if inhaled. If powdered SDS is used, the teacher should prepare the solution, wearing a mask and using the fume hood.
   See also the general Science in School safety note on page 57.
2. If using ready-made 10 % SDS solution, add 5 ml SDS to 45 ml TBS.
3. If using powdered SDS, dissolve 0.5 g in 50 ml TBS.

3 M Sodium acetate solution (for 50 ml)
1. Dissolve 12.3 g anhydrous sodium acetate in 50 ml distilled water.
2. Add dilute HCl to adjust to pH 5.2.
3. Store in the fridge until required.

Proteinase K (100 µg / ml)
1. Dissolve 1 mg proteinase K in 10 ml tris-buffered saline.
2. Only a very small amount of the enzyme is required, so you might want to make up a smaller volume if you have a sufficiently accurate balance. Simply adjust the quantities accordingly.
3. Store in the freezer until required.

Disinfectant solution
Suitable disinfectants include 0.015 M sodium hypochlorite solution, 1 % Virkon® solution or 5 % domestic bleach. After soaking for at least 15 min, the loops can be transferred to a plastic bag (wear gloves) and disposed of with normal waste.

To help you find the necessary reagents, a list of product numbers for Sigma-Aldrich is given in table 2. However, you will also be able to source them from other suppliers.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Sigma-Aldrich product number</th>
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<tbody>
<tr>
<td>Tris buffered saline (TBS)</td>
<td>Tablets: T5030 or 94158</td>
</tr>
<tr>
<td>Sodium dodecyl sulphate (SDS)</td>
<td>Powdered: L3771 10% solution: 71736</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>S2889</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>P6556</td>
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</tbody>
</table>

Table 2: Sigma-Aldrich product numbers for the reagents used in this protocol