DANAEXTRACTOR SALIVA 2

1. OBJETIVE OF THE EXPERIMENT

This kit allows each student individually to isolate their own DNA from a sample of their saliva.

2. KIT INCLUDES

COMPONENT	AMOUNT	STORE
LYSIS SOLUTION	30 ml	Room Temperature
SODIUM ACETATE SOLUTION	6 ml	Room Temperature
ABSOLUTE ETHANOL	80 ml	**4ºC**
PROTEINASE K 50 µg/ml	2 x 275 µl	**-20ºC**
TUBE 15 ml	25 units	Room Temperature
PASTEUR PIPETTE 1 ml	25 units	Room Temperature
PASTEUR PIPETTE 3 ml	25 units	Room Temperature

Required and non-supplied material:

A) Micropipette or micropipettes of 20 µl to 200 µl and the corresponding tips.

B) Micropipet or micropipettes of 200 µl to 1000 µl and the corresponding tips.

C) Gloves, goggles and gowns.

3. PRACTICE

3.1 METHODOLOGY

1. Get the sample.

The first step is to obtain the cells from which we will extract the DNA. These cells are obtained very easily by scratching the tongue and the inner walls of the mouth.

2. Release the contents of the cells.

A) **Lysis**: this is the process by which the cell and nuclear membranes are broken. This is achieved with detergents that allow to release the DNA in the solution.
B) **Unpacking the DNA**: we used Proteinase K, an enzyme that degrades proteins that were bound to DNA and kept it compacted. So when proteinase K acts, the DNA decompresses and at the same time degrades other organic molecules in our sample, such as DNases, enzymes whose function is to degrade DNA.
C) **Neutralize the DNA load**: this process is performed using a salt, sodium acetate. Na+ ions bind to the phosphate groups of DNA that have a negative charge.
3. Precipitate the DNA

The final step is the precipitation of DNA that will allow us to make visible the invisible. For this we use ethanol. The DNA is soluble in water, but when we add ethanol it unwinds and precipitates. After adding the ethanol, we observe white threads that begin to appear in suspension. It's our DNA.

3.2 PROTOCOL

1. Obtaining a good sample of saliva **is the most important step** in the process. The objective is to obtain samples of saliva where we find the largest possible number of cells containing a large amount of DNA.

It is important not to drink or eat anything within 30 minutes of sampling.

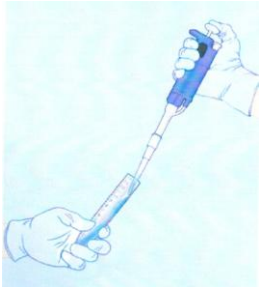
Secrete 1.5 ml saliva in a glass or other suitable container (not supplied). To facilitate obtaining the largest number of cells possible to scratch the inside of the mouth, palate and cheeks, and even teeth for 1 minute, to detach the cells from these areas before secreting the saliva.

2. Collect 1 ml of saliva using the 1 ml pasteur pipette and place its contents in the 15 ml tube.

3. Using a 200-1000 µl micropipette or 1 ml pasteur pipette (not supplied) add **1ml of Lysis solution** to the 15 ml tube containing the saliva.

4. Cover the tube well and mix the contents by inverting the tube.

5. Using a micropipette add **20 µl of Proteinase K**. Hold the pipette on the wall of the tube and let the liquid slide through the wall.



6. Cover the tube well and mix the contents by inverting the tube.

7. Incubate for 10 minutes and shake every 3 minutes.

8. Add 200 µl of Sodium acetate solution. Cover the tube well and mix the contents by inverting the tube.

9. Using the 3 ml pasteur pipette add **3 ml of the Ethanol, which should always be cold at 4°C (very important)**. Position the pipette tip into the inner tube wall **and allow the liquid to slip very slowly**.

10. Incubate for 2 minutes without shaking.

11. Cover the tube well and mix its contents **by inverting the tube very slowly**.

12. The DNA will appear as a whitish precipitate. We observe white strands that begin to appear in suspension. **It's our DNA**. Ideally, it appears as a single compact white strand but often breaks down and many small white strands appear.