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Saliva DNA Extraction & Purification using ORAgene Kit

Step1: Saliva Sample Collection & Incubation

1. Before starting, clean mouth by rinsing with water. Wait for at least 1 minute before spitting your sample. **Label your name or initial on the white lid.**

2. Spit your saliva into the Blue Oragene Container. Spit until the amount of liquid saliva (not bubbles) reaches the level shown in picture below

3. Tighten the white lid very firmly.

4. Gently mix your saliva.

5. Incubate the sample at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours.

User Tips:

- Do NOT remove plastic film from the big white lid.
- To make more saliva, close your mouth and wiggle your tongue or rub your cheeks.
- Some people may find it hard to spit so much saliva. It is easier to spit more if you place ¼ tsp of plain white sugar on your tongue.
- Finish spitting within 30 minutes.

Step 2: Saliva DNA Extraction & Purification (For 1 ml sample)

1. Transfer 1000 µL of the mixed Oragene/ saliva sample to a 1.5 mL microcentrifuge tube.
2. For 1000 µL of Oragene/saliva, add 40 µL (1/25th volume) of Oragene Purifier (OG-L2P, supplied) to the microcentrifuge tube and mix by vortexing for a few seconds.
3. Incubate on ice for **10** minutes.
4. Centrifuge at room temperature for **5** minutes at 13,000 rpm (15,000 × *g*).
5. Carefully transfer the clear supernatant with a pipette tip into a fresh microcentrifuge tube. Discard the pellet containing impurities.
6. To 1000 µL of supernatant, add 1000 µL (i.e., an equal volume) of room-temperature 95-100% ethanol. Mix gently by inversion 10 times.
7. Allow the sample to stand at room temperature for **10** minutes to allow the DNA to fully precipitate.
8. Place the tube in the microcentrifuge in a known orientation. Centrifuge at room temperature for **2** minutes at 13,000 rpm (15,000 × *g*).
9. Carefully remove the supernatant with a pipette tip and discard it. Take care to avoid disturbing the DNA pellet.

   (Optional: If the pellet didn't show, add the following steps:

   *9a. carefully add 250 µl of 70% ethanol. Let stand at room temperature for **1** minute.*

   9b. completely remove the ethanol without disturbing the pellet.)
10. Add 100 µL of TE buffer to dissolve the DNA pellet. Vortex for at least 5 seconds. For better results, add one of the following steps:

    a) Additional vigorous pipetting and vortexing, and/or

    b) Incubation at 50°C for 1 hour with occasional vortexing, and/or

    c) Incubation at room temperature for 1-2 days