

Laboratory protocol for manual purification of DNA from 500 μ L of Oragene[®]/saliva sample[†] using the Agencourt DNAdvance kit

Introduction

This protocol details the purification process using Agencourt's DNAdvance kit for saliva collected using Oragene[®] self-collection kits.

Please note that modifications to this protocol are necessary for automated processing with the Agencourt DNAdvance method for Biomek 96.

Agencourt strongly recommends using aerosol-barrier (filter) pipette tips when performing the Agencourt DNAdvance purification.

Procedure

1. Collect saliva sample with the Oragene self-collection kit and incubate at 50°C for 1 hour.

Note that the incubation step can be performed any time after saliva collection, and before DNAdvance purification. Each Oragene kit should provide enough saliva for 6 to 8 \times 500 μ L samples.

2. Transfer 500 μ L of sample into a new 1.5 mL microcentrifuge tube or 1.2 mL storage plate (Thermo Fisher, Cat. No. AB-1127) for manual processing. Use a 2.2 mL storage plate for automated processing (Thermo Fisher, Cat. No. AB-0661).
3. Add 200 μ L of Bind1 buffer and pipette mix 10 times or until mixed well.
4. Shake Bind2 bottle until bead particles are re-suspended well in solution.
5. Add 340 μ L of Bind2 buffer in each well and pipette mix 15 times or until mixed well.

During this step, DNA binds to the magnetic particles. When mixing manually, use a mix volume that is slightly less than the total volume in the well and pipette slowly to minimize the formation of air bubbles. Air bubbles can trap magnetic beads and prevent them from being pulled to the bottom of the plate, thus decreasing yield.

6. Incubate the plate at room temperature for 1 minute.
7. Place the sample plate on an Agencourt SPRIPlate Super magnet for 8 minutes to separate.
8. Aspirate and discard the supernatant while the plate is situated on the magnet.

When aspirating, place the pipette at the center of the well to avoid disturbing the ring of magnetic beads. If you are having trouble transferring clear supernatant without beads, it is ok to leave the last 10 μ L to 20 μ L in the well.

9. Take the plate off the magnet. Add 700 μ L of 70% ethanol and pipette mix 20 times or until the magnetic beads are re-suspended from the bottom of the well.

Make fresh 70% ethanol for each extraction. Pipette mix until the magnetic beads are back in suspension. Try to eliminate any bead clumping with thorough tip mixing.

10. Place the plate back on the magnet for 2 minutes, or until the solution clears.
11. Aspirate and discard the supernatant while the plate is situated on the magnet.

[†] Saliva samples were collected with Oragene[®]-DNA or Oragene[®]-DISCOVER.

