SOP#112: Agencourt DNA extracdtion from saliva colleted in Genotek Oragene kits

1. PURPOSE:

This protocol describes the manual purification of DNA using Agencourt's DNAdvance kit for saliva collected using Oragene® self-collection kits.

2. MATERIALS

2.1 Reagents and supplies

Material	Vendor	Cat#
Saliva samples collected from Oragene self-collection kit		
1.5mL microcentrifuge tubes	Varies	Varies
1.2mL storage plate	Thermo Fisher	AB-1127
P1000 pipetteman and filter tips	Varies	Varies
P200 pipetteman and filter tips	Varies	varies
Agentcourt SPRIPlate Super magnet	Alpaqua	A32782
Bind1 buffer	Beckman-Coulter Genomics	Part of Agencourt DNAdvance kit (Cat# A48705)
Bind2 buffer	Beckman-Coulter Genomics	Part of Agencourt DNAdvance kit (Cat# A48705)
Elution buffer	Beckman-Coulter Genomics	Part of Agencourt DNAdvance kit (Cat# A48705)
70% Ethanol	Varies	Varies
Hard-Shell® 96-Well PCR Plates	Bio-Rad	HSP9901
Agarose	Varies	Varies

2.2 Equipment

50°C water bath
4°C fridge or cold room
-80°C freezer
Nanodrop 2000 Spectrometer
Gel boxes
Power supply
Gel imager

3. EXPERIMENTAL PROCEDURES

3.1 Sample Preparation

3.1.1. Collect saliva samples with the Oragene self-collection kit. Samples can be stored at room temperature for at least 3 years.
3.1.2. Incubate samples at 50°C for 1 hour. Samples can be stored at room temperature for at least 3 years after this step.
3.1.3. Enter the sample IDs to the 96 well plate map and print a hard copy.
3.1.4. Transfer 500 µL of saliva sample into a 1.2mL storage plate according to the plate map.

3.2 Binding

3.2.1. Add 200 µL of Bind1 buffer to each well and mix by pipetting 10 times or until mixed well.
3.2.2. Shake Bind2 buffer bottle to until the bead particles are re-suspended well.
3.2.3. Add 340 µL of Bind2 buffer in each well and mix by pipetting 15 times or until mixed well. Pipette slowly to avoid air bubbles.
3.2.4. Incubate the plate at room temperature for 1 minute.
3.2.5. Place the sample plates on an Agencourt SPRIPlate Super magnet for 8 minutes to separate.
3.2.6. Aspirate and discard the supernatant while the plate is still situated on the magnet. Avoid disturbing the ring of magnetic beads; it's okay to leave the last 10 – 20 µL.

3.3 Ethanol wash

3.3.1. Remove plate off magnet. Add 700 µL of 70% ethanol to each well and mix by pipetting 20 times to re-suspend beads from the bottom of the well. Try to eliminate any bead clumps with tip mixing.
NOTE: Make fresh 70% ethanol for each extraction.
3.3.2. Place the plate back on the magnet for 2 minutes, or until the solution clears.
3.3.3. Aspirate and discard the supernatant while the plate is still situated on the magnet. Avoid disturbing ring of magnetic beads.
3.3.4. Repeat steps 3.3.1 through 3.3.3 two more times.
3.3.5. Remove as much of the final ethanol wash as possible before adding the elution buffer.

3.4 Elution

3.4.1. Remove the plate off the magnet. Add 50 µL of elution buffer to each well and mix by pipetting 10 times or until the magnetic beads are re-suspended completely from the bottom of the well.
3.4.2. Place the plate back on the magnet for 3 minutes, or until the solution clears.

3.5 Storage

3.5.1. Label a clean 96-well plate as the source DNA plate for the specific study. Transfer 40 µL of supernatant to the labeled 96-well plate for storage. Slowly aspirate to avoid disturbing the ring of beads; If beads are aspirated during transfer, dispense the sample back into the plate and incubate for another 5 minutes before repeating aspiration.
3.5.2. Quantify DNA by measuring OD260/OD280. Enter the DNA concentration into the sample manifest and calculate the amount of source DNA and elution buffer needed to make the normalized plate, based on the template.
3.5.3. Label a new clean 96-well plate as the normalized DNA plate. Normalize DNA to 40 ng/ul in a new 96-well plate, with well positions matching the source DNA plate.

3.5.3. Check DNA integrity by running 3 ul of the normalized DNA on 0.8% agarose gels. Exclude degraded samples in the final telomere length analysis report.
3.5.2. Store DNA at -80°C for further use.

REVISION HISTORY

DATE	AUTHOR	REVISION #	REVISION REASON
March 13, 2018	Calvin Wu	A	Initial Release
Jan 8, 2018	Jue Lin	B	Added DNA quantification and quality check. A few other minor revisions