Purification of DNA from nasal swab samples using the Performagene PG-AC4 Kit

1 Purpose / Introduction

1.1 To purify DNA using the Performagene PG-AC4 kit from nasal swab samples collected from animals using PG-100 nasal swabs.

2 Equipment/Reagents/Materials

PG-AC4 reagent package: PG-L2P purifier

Microcentrifuge capable of running at 15,000 × *g*

Air or water incubator at 50°C (Note: The false bottom tube will float in a water incubator, therefore an air incubator may be preferred.)

Ethanol (95% to 100%) at room temperature

DNA buffer: TE (10 mM Tris-HCl, 1mM EDTA, pH 8.0)

(Optional) Glycogen (20 mg/mL) (e.g., Invitrogen Cat. No. 10814-010)

Ethanol (70%) at room temperature

5M NaCl solution

3 Principle

3.1 To purify DNA from a 0.5 mL aliquot of a sample that has been collected and preserved in Performagene chemistry with the PG-100 collection kit. The sample is first heated to release the DNA and inactivate inhibitors. A purifier is then added to precipitate impurities and inhibitors,

purifier is then added to precipitate impurities and inhibitors, which are removed by pelleting leaving the DNA in suspension. The DNA is then collected by precipitation using NaCl and washed to remove any residual impurities and then used down stream for genotyping or other DNA analysis.

4 Procedure

1. Mix the sample by shaking vigorously for 5 seconds.
2. Incubate the sample in a 50°C air incubator for a minimum of 2 hours, or in a 50°C water incubator for a minimum of 1 hour.
3. Removal of collection sponge: Remove the cap and press the collection sponge against the inside of the tube to extract as much of the sample as possible. Discard sponge and cap.
4. Transfer 500 µL of the mixed Performagene sample to a 1.5 mL microcentrifuge tube. (TRANSFER THE REMAINDER OF THE SAMPLE TO A NEW 1.5ml MICROCENTRIFUGE TUBE AND STORE AT -20°C)
5. Add 20 µL (1/25th volume) of PG-L2P purifier to the microcentrifuge tube and mix by vortexing for a few seconds.
6. Incubate sample on ice for 10 minutes.
7. Centrifuge at room temperature for 5 minutes at 15,000 × *g*.
8. Carefully transfer the clear supernatant with a pipette tip into a fresh microcentrifuge tube. Discard pellet.
9. To 500 µL of supernatant, add 25 µL (1/20th volume) of 5M NaCl, mix.
10. To 500 µL of supernatant , add 600 µL of room temperature 95% to 100% ethanol. Mix gently by inversion 10 times.
11. (Optional) Add Glycogen at 20mg/ml as a carrier to increase visibility of the pellet.
12. Allow the sample to stand at room temperature for 10 minutes to allow the DNA to fully precipitate.
13. Place the tube in the centrifuge in a known orientation (DNA pellet may not be visible after centrifugation). Centrifuge at room temperature for 2 minutes at >15,000 × *g*.

14. Carefully remove the supernatant with a pipette tip and discard it. Take care to avoid disturbing the DNA pellet.
15. Carefully wash the DNA by adding 250 µLof 70% ethanol. Let stand for 1 minute at room temperature. Remove the ethanol with a pipette tip without disturbing the pellet.
16. Centrifuge for 6 seconds to pool any remaining ethanol, remove with a pipette tip.
17. Add 100 µL of DNA buffer (e.g. TE buffer) to dissolve the DNA pellet. Vortex for at least 5 seconds. Let sit at room temperature overnight to ensure complete rehydration of the DNA.
18. DNA can now be quantified and used in downstream applications or stored at -20°C.

5 Maintenance

5.1 The temperature of incubators and freezers should be checked on a regular basis to ensure they are in correct working order.

5.2 Buffers and solutions should be kept fresh and discarded when they become old.

6 Health and Safety

6.1 PG-L2P Purifier contains Potassium Chloride refer to the MSDS.