Genorise Urine DNA Extraction Kit 200

This kit is to isolate total DNA including genomic and mitochondrial DNA from 2 ml of total urine and is for 200 applications. If a smaller or larger sample volume is employed, the reagent volumes should be proportionally decreased or increased. This kit can significantly improve quality and quantity of DNA and is much cheaper than the similar products ($310/200 preps).

Materials provided in the kit:
250 ml 5 x Lysis Solution
400 ml Protein Precipitation Solution
200 ml DNA Hydration Solution

Materials required but not provided in the kit:
Proteinase K, prepare at 20 mg/ml and store at -20ºC
Glycogen, prepare at 20 mg/ml and store at -20ºC
Isopropanol (2-propanol)/Ethanol
15 ml conical tube/1.7 ml microcentrifuge tube

Protocol

Cell Lysis

1. Take 2 ml urine sample to a 15 ml conical tube. Add 0.5 ml of 5 x Lysis Buffer and 13 µl of 20 mg/ml Proteinase K solution, vortex for 20 sec.
2. Incubate at 55ºC for 1 hr to overnight until the cell lysate becomes completely clear (disrupted).

Protein precipitation

1. Cool sample to room temperature by placing on ice for 1 min.
2. Add 0.9 ml of Protein Precipitation Solution to the lysate.
3. Vortex samples at high speed for 20 sec and place sample into an ice bath for 5 min.
4. Centrifuge at 3,000 x g for 30 min.

DNA Precipitation

1. Pour the supernatant containing DNA into a new 15 ml conical tube and centrifuge at 3,000 x g for 20 min.
2. Pour the supernatant to a new conical tube; repeat centrifugation until no pellet is seen.
3. Pour the supernatant containing DNA into a new 15 ml conical tube containing 3.4 ml 100% isopropanol and 7 µl of 20 mg/ml Glycogen solution.
4. Mix the samples by inverting 50 times and incubate at room temperature for 10 min.
5. Centrifuge at 3,000 x g for 30 min. The DNA may or may not be visible as a small white pellet, depending on yield.
6. Pour off the supernatant and drain the tube briefly on clean absorbent paper. Add 3 ml of 70% ethanol and invert the tube several times to wash the DNA pellet.
7. Centrifuge at 3,000 x g for 30 min, and carefully pour off the ethanol.
8. Invert and drain the tube on clean absorbent paper, completely remove the liquid residue by a pipette, and finally allow airing dry for 5 min.

DNA Hydration

1. Add 20 µl DNA hydration solution.
2. Resuspend the DNA pellet by a pipette for 5 times and rehydrate the DNA by incubation for 10 min at room temperature.
3. Vortex briefly and pulse spin before use, and store at -20°C.

Following DNA precipitation, if you observe a big pellet with a color other than white, we recommend you repeat this protocol with a start volume of 0.5 ml of DNA isolate and 1 hr cell lysis. It is normal if you do not see a white pellet after isopropanol precipitation, but you should see it after 70% ethanol wash.
Air drying of DNA pellet should not exceed 5 min.