Puregene DNA isolation from ears

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This is a protocol for extracting DNA from a mouse ear punch for genotyping.

Keywords: mouse ears DNA extraction

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DNA Purification from 0.5-2.0 mg solid tissue (Expected yield range 0.3-1.5 µg DNA)

Cell Lysis

1. Dissect tissue sample quickly and freeze in liquid nitrogen. Store at -70° to -80°C. Fresh tissue may also be used. Work very quickly and keep tissue on ice at all times including when tissue is weighed.
2. Add 0.5-2.0 mg (0.0005-0.002 g) frozen ground tissue or fresh tissue to a 1.5 ml tube containing 100 µl Cell Lysis Solution, remove from ice, and homogenize thoroughly using a microfuge tube pestle. Place sample back on ice until next step.
3. Incubate lysate at 65°C for 15 minutes. Alternatively, if maximum yield is required, 0.5 µl proteinase K Solution (20 mg/ml) may be added to the lysate. Mix by inverting 25 times and incubate at 55°C for 3 hours to overnight, until tissue particulates have dissolved. If possible, invert tube periodically during the incubation.

RNase Treatment

1. Add 0.5 µl RNase A Solution (4 mg/ml) to the cell lysate.
2. Mix the sample by inverting the tube 25 times and incubate at 37°C for 15-60 minutes.

Protein Precipitation

1. Cool sample to room temperature.
2. Add 33 µl Protein Precipitation Solution to the RNase A-treated cell lysate.
3. Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate. Place sample on ice for 5 minutes.
4. Centrifuge at 13,000-16,000 x g for 3 minutes. The precipitated proteins will form a tight pellet. If the protein pellet is not visible, repeat Step 3 followed by incubation on ice for 5 minutes, then repeat Step 4.

DNA Precipitation

1. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml centrifuge tube containing 100 µl 100% isopropanol (2propanol). If the DNA yield is expected to be low (<1 µg), add a DNA carrier such as Gentra Glycogen Solution (0.5 µl glycogen 20 mg/ml) to the 100 µl isopropanol.
2. Mix the sample by inverting gently 50 times.
3. Centrifuge at 13,000-16,000 x g for 5 minutes.
4. Pour off supernatant and drain tube on clean absorbent paper. Add 100 µl 70% Ethanol and invert tube several times to wash the DNA pellet.
5. Centrifuge at 13,000-16,000 x g for 1 minute. Carefully pour off the ethanol. Pellet may be loose so pour slowly and watch pellet.
6. Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.

DNA Hydration

1. Add 20 µl DNA Hydration Solution (20 µl will give a concentration of 50 ng/µl if the total yield is 1 µg DNA).
2. Rehydrate DNA by incubating sample 1 hour at 65ºC and/or overnight at room temperature. If possible, tap tube periodically to aid in dispersing the DNA.
3. Store DNA at 4°C. For long-term storage, store at -20°C or -80°C.

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2/03• 00440 Rev B

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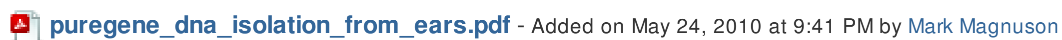
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