Puregene DNA isolation from tails

This is a protocol for extracting DNA from mouse tails for genotyping.

Keywords: tails mouse DNA extraction

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DNA purification from 5-10 mg mouse tail tissue (Expected yield range 10-75 µg DNA)

Cell Lysis

1. Chill a 1.5 ml tube containing 300 µl Cell Lysis Solution on ice. Please note that the solution will turn cloudy.
2. Place 5 mm (5-10 mg) fresh or frozen mouse tail tissue (minced if possible) into the chilled Cell Lysis Solution.
3. Add 1.5 µl proteinase K Solution (20 mg/ml) to the sample and mix by inverting 25 times. Incubate at 55ºC overnight or until tissue has dissolved. If possible, invert tube periodically during the incubation.

RNase Treatment (optional)

1. Add 1.5 µl RNase A Solution to the cell lysate.
2. Mix sample by inverting the tube 25 times and incubate at 37ºC for 15-60 minutes.

Protein Precipitation

1. Cool sample to room temperature.
2. Add 100 µl Protein Precipitation Solution to the cell lysate.
3. Vortex at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.
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 microfuge tube containing 300 µl 100% isopropanol (2propanol).
2. Mix the sample by inverting gently 50 times.
3. Centrifuge at 13,000-16,000 x g for 1 minute; the DNA will be visible as a small white pellet.
4. Pour off the supernatant and drain tube on clean absorbent paper. Add 300 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 for 10-15 minutes.

DNA Hydration

1. Add 50 µl DNA Hydration Solution (50 µl will give a concentration of 500 µg/ml if the total yield is 25 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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Attachment

puregene_dna_isolation_from_tails.pdf - Added on May 24, 2010 at 9:44 PM by Mark Magnuson

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