PUREGENE®

Protocol

PUREGENE® DNA Purification Kit

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** (2-propanol). 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 65°C 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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