PUREGENE®

Protocol

# PUREGENE® DNA Purification Kit

## DNA Purification Protocol For 1-2 Million Cells

### Expected Yield Range 5-10 µg DNA

### Cell Lysis

1. Add 1-2 million cells in balanced salt solution or culture medium to a 1.5 ml tube.
2. Centrifuge at 13,000-16,000 x g for 5 seconds to pellet cells. Remove supernatant leaving behind 10-20 µl residual liquid.
3. Vortex the tube vigorously to resuspend the cells in the residual supernatant. This greatly facilitates cell lysis in Step 4 below.
4. Add 300 µl **Cell Lysis Solution** to the resuspended cells and pipet up and down to lyse the cells. Usually no incubation is required; however, if cell clumps are visible after mixing, incubate at 37°C until the solution is homogeneous. Samples are stable in **Cell Lysis Solution** for at least 2 years at room temperature.

### RNase Treatment

1. Add 1.5 µl **RNase A Solution** 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 100 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.
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 incubating 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 1.5 ml microfuge tube containing 300 µl **100% Isopropanol** (2-propanol).
2. Mix the sample by inverting gently 50 times.
3. Centrifuge at 13,000-16,000 x g for 1 minute. The DNA should be visible as a small white pellet.
4. Pour off supernatant and drain tube on clean absorbent paper. Add 300 µl **70% Ethanol** and invert the 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 50 µl **DNA Hydration Solution** (50 µl will give a concentration of 200 ng/µl if the total yield is 10 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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