Genomic DNA Extraction Using Serapure

"Serapure" is the name given to home-brew AMPure using SeraMag beads.

Solutions

Cell Lysis Buffer

100 mM NaCl
100 mM Tris-Cl pH 8.0
25 mM EDTA pH 8.0
0.5% SDS

Autoclave the NaCl, Tris and EDTA before making the buffer. Filter sterilize.

Proteinase K (20 mg/mL)

RNAse A (4 mg/mL)

Ethanol (100% and 70%)

10 mM Tris-Cl, pH 8.0

Method

Cell Lysis

1. Place ~3 mg of tissue in a 1.5-mL microcentrifuge tube containing 100 µL of Cell Lysis Solution (see recipe above). Macerate the tissue as much as possible. Add 5 µL each of Proteinase K and RNaseA. *Note*: 100 µL is less than the typical 300 µL used in other protocols. This smaller volume will allow you to do high-throughput extractions in microtiter plates. However, if you need to extract lots of DNA using more tissue, use 300 µL of Cell Lysis buffer, and add 540 µL of beads in the steps below.

2. Incubate at 55 C for at least a few hours or overnight. The tissue should be completely dissolved.

3. Cool the sample to room temperature.

Purification using Serapure

1. Add 180 µL of Serapure. Make sure the Serapure has been mixed well and all the beads have been suspended by vigorous vortexing prior to adding to your sample.

2. Mix by flicking the tube or gentle pipetting. The Serapure beads may clump; try not to disturb the clumping beads too much, but continue to mix.

3. Incubate at room temperature for five minutes to ensure the DNA binds to the beads.

4. Place the tube on a magnet rack and allow the beads to congregate for at least 2 minutes. The supernatant should be clear.

5. Remove the supernatant.

6. Wash twice with 70% Ethanol. When adding the ethanol, add it to the opposite side of the tube from where the beads are. Each wash only needs to last ~30 seconds.

7. Remove as much ethanol as possible; aspirate if necessary.

8. Allow the beads to air dry for ~5 minutes *off the magnet*.

9. Resuspend in 50-200 µL of 10 mM Tris (pH 8.0). Make sure the beads resuspend completely; this may take some flicking or gentle mixing. Incubate at room temperature for 5 minutes.

10. Place the sample on a magnetic stand for two minutes, or until the beads have separated from the supernatant.

11. Remove the supernatant to a new tube. Your DNA is ready.

Note: If extremely high molecular weight DNA is needed (e.g. to build mate-pair libraries), please use phenol-chloroform extractions (and be gentle).