Protocol

A Silica Membrane-Based Method for the Isolation of Genomic DNA from Tissues and Cultured Cells

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INTRODUCTION

Silica chemistry has become the method of choice for the purification of high-quality genomic DNA from samples such as mouse-tail clippings, animal tissue, and tissue culture cells. The Wizard SV Genomic DNA Purification System uses a solid-phase silica-impregnated filter membrane for the purification of genomic DNA. The procedure consists of tissue disruption (if necessary), cell lysis, binding of DNA to the SV membrane, washing to remove residual contaminants, and elution of DNA in nuclease-free water. Sample and reagents can be driven through the membrane by either centrifugation or vacuum filtration. The procedure can be performed manually or on liquid-handling robots such as the Biomek FX and Biomek 2000 Laboratory Automation Workstations (Beckman Coulter). Individual minicolumns can be used for manual processing of low numbers of samples. Amplifiable DNA can be isolated from up to $5 \times 10^6$ cells, 20 mg of tissue, or from up to 1.2 cm of mouse tail without centrifugation of the lysates. Isolation from tissue or mouse tails requires an overnight digestion with proteinase K. The genomic DNA isolated with this system is of high quality and serves as an excellent template for PCR.

MATERIALS

Reagents

- PBS (for tissue cultured cells)
- Proteinase K (20 mg/ml) (for use with animal tissues)

Wizard SV 96 Genomic DNA Purification System (Promega; includes Binding Plate, 96-well deep-well plate, DNA elution plate, Nuclei Lysis Solution, 0.5 M EDTA, Wizard SV Lysis Buffer, Wizard SV Wash Solution, RNase A solution, and nuclease-free H$_2$O)

Source material, either tissues of cultured cells

Equipment

Liquid-handling robot (for automated procedure), such as the Biomek FX or Biomek, including:

- Three single-position labware automated labware positioners (ALPs) (Beckman Coulter)
- Four P250 tip rack assemblies (Beckman Coulter)
- 4x four-position labware ALPs (Beckman Coulter)
- 96-well Tip Wash ALP (Beckman Coulter)
- Adhesive plate sealers (foil)
METHOD

1. For preparation of DNA from animal tissues, begin this procedure at Step 2. When using cultured cells, wash the cells in 1x PBS and proceed directly to Step 6.

Preparation of Mouse Tail and Tissue Lysates for Automated Purification

2. Place a 0.5- to 1.2-cm mouse tail clipping or up to 20 mg of other tissue into each well of a 96-well, deep-well plate.

3. Prepare 275 µl of the Digestion Solution master mix for each sample as follows:

<table>
<thead>
<tr>
<th>Nuclei Lysis Solution</th>
<th>200 µl</th>
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<tbody>
<tr>
<td>EDTA (0.5 M)</td>
<td>50 µl</td>
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<tr>
<td>Proteinase K (20 mg/ml)</td>
<td>20 µl</td>
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<tr>
<td>RNase A solution (4 mg/ml)</td>
<td>5 µl</td>
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</tbody>
</table>

4. Add 275 µl of the master mix to each sample and cover the plate with an adhesive seal. Incubate overnight (16-18 hours) in a 55°C water bath.

If necessary, at this stage the sample lysate may be stored frozen at -70°C. Before proceeding with automated genomic DNA purification, frozen lysate must be warmed to 55°C for 1 hour.

5. If necessary, collect any undigested debris by centrifuging the plate at 2000g. Transfer the supernatant to a new 96-well, deep-well plate.

Automated Purification of Genomic DNA From Prepared Tissue Lysates

From this point onward, steps can be automated on a liquid-handling workstation without further user intervention.

6. Add 250 µl (150 µl for tissue cultured cells) of Wizard SV Lysis Buffer to each sample well and mix by pipetting up and down several times.

7. Transfer the lysates to the wells of the Binding Plate sitting in the vacuum manifold. Bind genomic DNA to the plate by applying a vacuum until all of the lysate has passed through the Binding Plate.
8. Add 1 ml of Wizard SV Wash Solution to each well of the Binding Plate and apply the vacuum until the wash solution passes through the Binding Plate.

9. Repeat Step 8 for a total of three washes.

10. After the wells have emptied, continue to apply vacuum for an additional 6 minutes to allow the binding matrix to dry.

11. Prepare the vacuum manifold assembly for elution. Move the Binding Plate and position a deep-well elution plate in the vacuum manifold. Then, position the Binding Plate on top of the elution plate in the same orientation so that the Binding Plate tips are centered on the deep-well elution plate.

12. Add 200 µl of nuclease-free H2O to each well of the Binding Plate, and incubate for 2 minutes at room temperature.

13. Apply the vacuum for 1 minute to elute purified genomic DNA from the SV 96 Binding Plate into the 96-well deep-well plate.

14. Repeat Steps 12 and 13 for a total elution volume of 400 µl.

15. Assess the yield and quality of Wizard SV-purified samples by spectrophotometric analysis at $A_{260}$ and $A_{280}$. Typical yield of high-molecular-weight genomic DNA is 5-20 µg, depending on sample source, with $A_{260}/A_{280}$ ratios of 1.7 ± 0.08. Purified samples can be sealed and stored at -20ºC or -70ºC.

**TROUBLESHOOTING**

**Problem:** Copurification of RNA with genomic DNA

[Step 15]

**Solution:** RNA that may have copurified with genomic DNA can be removed by adding 2 µl of RNase A solution per 250 µl of nuclease-free H2O before elution of genomic DNA from the column. After elution, incubate the purified genomic DNA at room temperature for 10 minutes. Alternatively, the RNase A Solution (2 µl) may be added after elution.

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**Caution**

**Proteinase K**

Proteinase K is an irritant and may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

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**Recipe**

**Phosphate-buffered saline (PBS)**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount to add (for 1X solution)</th>
<th>Final concentration (1X)</th>
<th>Amount to add (for 10X stock)</th>
<th>Final concentration (10X)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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NaCl 8 g 137 mM 80 g 1.37 M
KCl 0.2 g 2.7 mM 2 g 27 mM
Na₂HPO₄ 1.44 g 10 mM 14.4 g 100 mM
KH₂PO₄ 0.24 g 1.8 mM 2.4 g 18 mM

If necessary, PBS may be supplemented with the following:
CaCl₂•2H₂O 0.133 g 1 mM 1.33 g 10 mM
MgCl₂•6H₂O 0.10 g 0.5 mM 1.0 g 5 mM

PBS can be made as a 1X solution or as a 10X stock. To prepare 1 L of either 1X or 10X PBS, dissolve the reagents listed above in 800 mL of H₂O. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H₂O to 1 L. Dispense the solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle or by filter sterilization. Store PBS at room temperature.

Recipe

Proteinase K

(20 mg/ml) Purchase as a lyophilized powder and dissolve at a concentration of 20 mg/ml in sterile 50 mM Tris (pH 8.0), 1.5 mM calcium acetate. Divide the stock solution into small aliquots and store at -20°C. Each aliquot can be thawed and refrozen several times but should then be discarded. Unlike much cruder preparations of protease (e.g., pronase), proteinase K need not be self-digested before use.