

Protocol for Genomic DNA Isolation from Mouse Tail/Animal Tissue or Cultured Cells

Description

The following protocol is for genomic DNA isolation from cultured cells or animal tissue using the Genomic DNA Purification Kit (Catalog #79020). For complete instructions, refer to the Technical Manual (Document #10000005432).

Directions

1. Prepare cell lysate from mouse tail or tissue, or from tissue culture cells, as indicated below.

Mouse Tail or Animal Tissue Lysate

- a) Prepare Digestion Solution as indicated in Table 1. Mix thoroughly and store on ice.

Table 1. Preparation of Digestion Solution

Components	Volume per Sample
Tissue Lysis Solution	200 μ L
EDTA	50 μ L
Proteinase K Solution	20 μ L
RNase A Solution	5 μ L
Total Volume	275 μ L

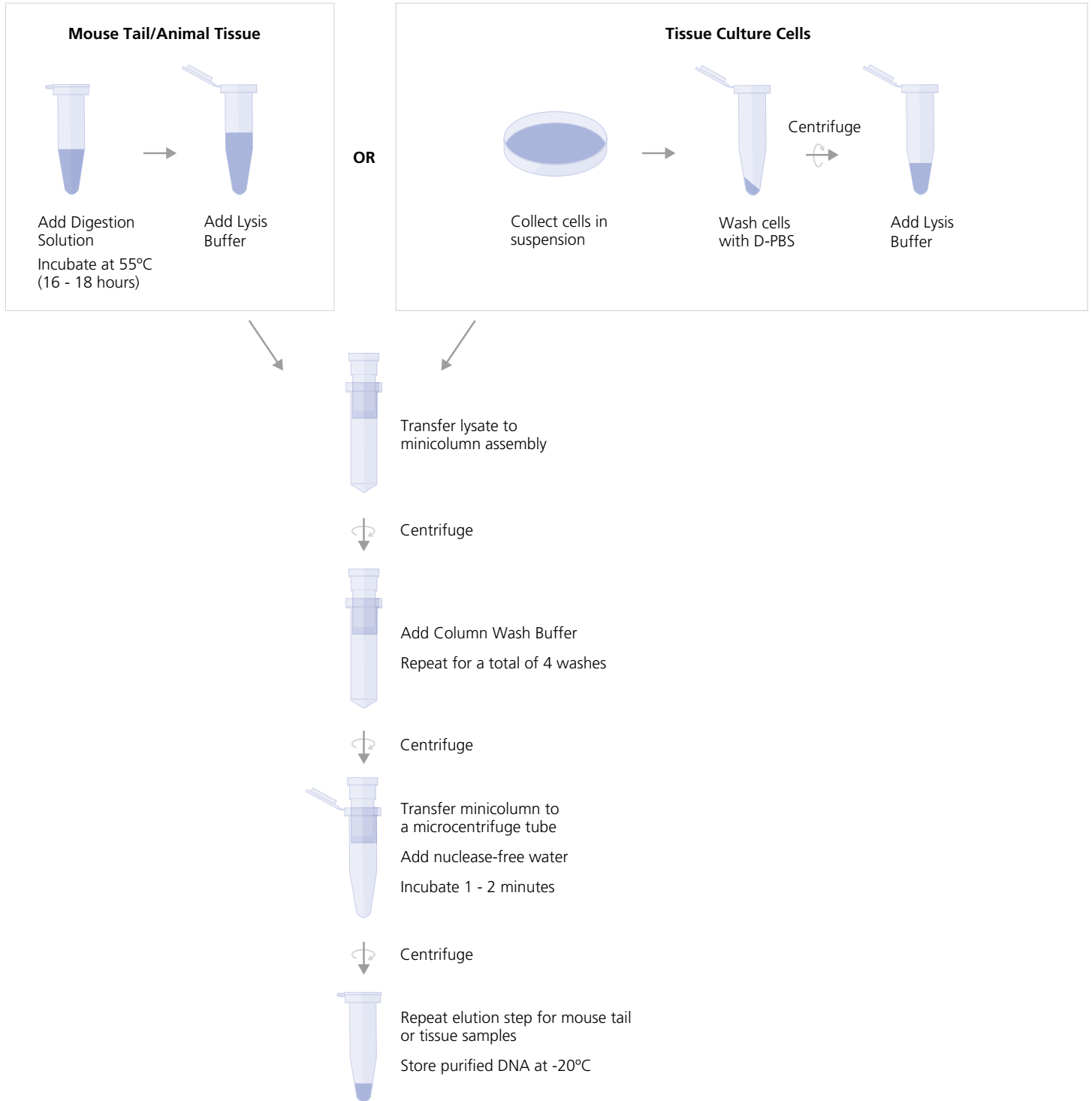
- b) Cut a 0.5 - 1.2 cm length of mouse tail from the tip or weigh up to 20 mg of tissue sample in a clean DNase-free 1.7 mL microcentrifuge tube.
- c) Add 275 μ L Digestion Solution to each tube.
- d) Incubate the sample tubes overnight (16 - 18 hours) in a 55°C heating block or water bath.
- e) Add 250 μ L Lysis Buffer to each sample. Vortex to mix.
- f) Proceed to step 2 for DNA isolation.

Tissue Culture Cell Lysate from Cell Suspension

- a) Collect 1×10^4 to a maximum of 5×10^6 cells. Wash the cells once with D-PBS.
 - b) Add 150 μ L Lysis Buffer to the washed cells. Mix by pipetting up and down.
 - c) Proceed to step 2 for DNA isolation.
2. Insert minicolumn into Collection Tube.
 3. Transfer lysate sample to the minicolumn assembly.
 4. Centrifuge at 13,000 x g for 3 minutes. Remove the minicolumn from the Collection Tube and discard the liquid. Reinsert the minicolumn in the Collection Tube.
 5. Add 650 μ L Column Wash Buffer (with ethanol added). Centrifuge at 13,000 x g for 1 minute. Remove the minicolumn from the Collection Tube and discard the liquid. Reinsert the minicolumn in the Collection Tube.
 6. Repeat step 5 for a total of 4 washes.
 7. Empty the Collection Tube and place the minicolumn back in the tube. Centrifuge at 13,000 x g for 2 minutes to dry the membrane.
 8. Carefully transfer minicolumn to a new labeled 1.7 mL microcentrifuge tube.
 9. Add 250 μ L nuclease-free water to the minicolumn. Incubate at room temperature for 1 - 2 minutes. Centrifuge at 13,000 x g for 1 minute. For **mouse tail or animal tissue lysates**, proceed to step 10. For **tissue culture lysates**, proceed to step 11.
 10. Add an additional 250 μ L nuclease-free water to the minicolumn. Incubate at room temperature for 1 - 2 minutes. Centrifuge at 13,000 x g for 1 minute.
 11. Discard minicolumn and store purified DNA at -20°C.

Note: For mouse tail or animal tissue lysates, elution volume will be approximately 500 μ L. For tissue culture cell lysates, elution volume will be approximately 250 μ L. This is the recommended elution volume for optimal DNA yield. A lower elution volume will concentrate the DNA but may decrease total yield.

Protocol for Genomic DNA Isolation



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