TECHNICAL MANUAL

DNA Isolation Using the Genomic DNA Purification Kit

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1.0 Introduction

The Genomic DNA Purification Kit uses a membrane-based system to quickly purify genomic DNA from mouse tails, animal tissues, and cultured cells. Up to 20 mg of tissue (mouse tail or animal tissue) or between 1 x 10^4 and 5 x 10^6 tissue culture cells can be processed per purification. The genomic DNA isolated with this system is of high quality and serves as an excellent template for downstream applications such as agarose gel electrophoresis, restriction enzyme digestion, and PCR analysis.

The Genomic DNA Purification Kit is based on the ability of DNA to bind silica membranes in the presence of chaotropic salts. Tissue or cells are first lysed in detergents, then the DNA is isolated by microcentrifugation to force the lysate through the membrane while the DNA binds to the silica on the membrane surface. Ethanol-based washing then removes the salts, and the DNA is eluted in nuclease-free water.

2.0 Materials, Reagents, and Equipment

2.1 Genomic DNA Purification Kit (Catalog #79020)

The components listed below are available as part of the Genomic DNA Purification Kit and are not available for individual sale.

Refer to the Product Information Sheet (PIS) for the Genomic DNA Purification Kit (Document #10000005435) for component storage and stability information; the PIS is also available at www.stemcell.com, or contact us to request a copy.

COMPONENT NAME	COMPONENT #	SIZE
DNA Minicolumns	79021	250 Columns
Collection Tubes	79022	250 Tubes
Column Wash Solution	79023	185 mL
Tissue Lysis Solution	79024	50 mL
RNase A Solution	79025	1 mL
Nuclease-Free Water	79026	150 mL
EDTA*	79027	30 mL
Lysis Buffer*	79028	50 mL

*Please refer to the Safety Data Sheet (SDS) for hazard information.

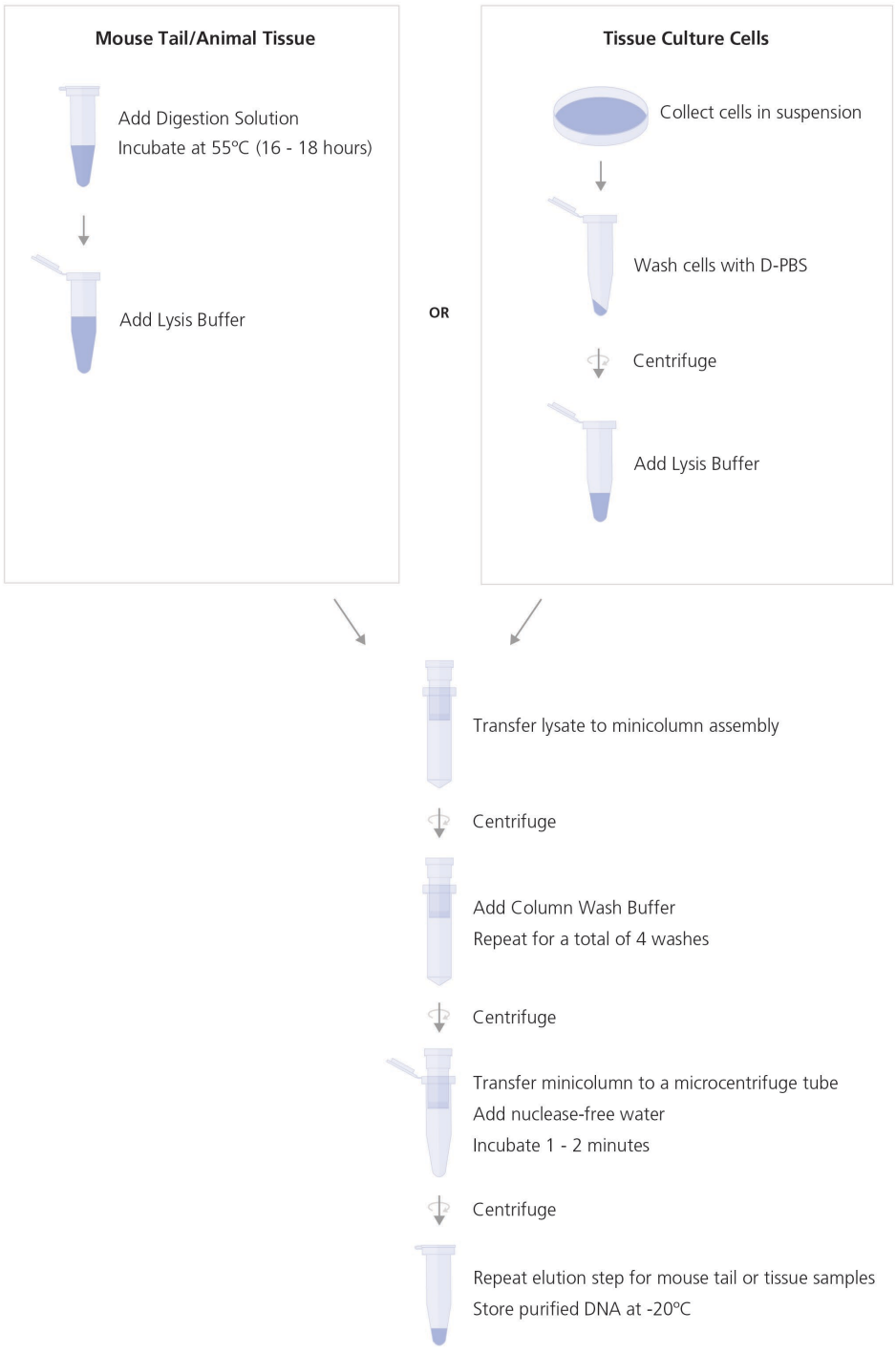
2.2 Additional Required Materials and Reagents

PRODUCT	CATALOG #
95% Ethanol, RNase-free	---
Microcentrifuge tubes, 1.7 mL	e.g. 38089
For mouse tail clippings and animal tissues:	
Proteinase K Solution	79016
55°C heating block or water bath	---
For tissue culture cells:	
D-PBS (Without Ca++ and Mg++)	37350

2.3 Equipment

- Biosafety cabinet certified for Level II handling of biological materials
- Microcentrifuge
- Pipettor and sterile tips
- -70°C freezer
- Refrigerator (2 - 8°C)

3.0 Protocol Diagram



4.0 Preparation of Reagents

Before performing cell lysis and the DNA purification protocol, prepare Column Wash Buffer as follows:

1. Add 315 mL of 95% ethanol to 185 mL of Column Wash Solution. Mix thoroughly.
2. Label the bottle to indicate that ethanol has been added.
3. Store Column Wash Buffer at 15 - 25°C, tightly capped.

5.0 Preparation of Cell Lysate

The following protocols are for preparing cell lysates from mouse tail clippings or animal tissues (section 5.1), and from tissue culture cells (section 5.2).

5.1 Mouse Tail or Animal Tissue Lysate

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

Table 1. Preparation of Digestion Solution

COMPONENT	VOLUME PER SAMPLE	VOLUME FOR N SAMPLES
Tissue Lysis Solution	200 µL	(N + 1) x 200 µL
EDTA	50 µL	(N + 1) x 50 µL
Proteinase K Solution	20 µL	(N + 1) x 20 µL
RNase A Solution	5 µL	(N + 1) x 5 µL
Total Volume	**275 µL**	**(N + 1) x 275 µL**

2. Cut a 0.5 - 1.2 cm length of mouse tail from the tip. Alternatively, weigh up to 20 mg of tissue sample. A 1.2 cm mouse tail clipping weighs approximately 20 mg.
3. Cut the mouse tail clipping or tissue sample into two equally sized pieces and place them in a 1.7 mL microcentrifuge tube.

   Note: Tissue mass cannot exceed the recommended amount, or the minicolumn will clog. Mouse tail clippings must be from within the terminal 2 cm of the mouse tail. Samples further from the tip of the tail contain more cartilaginous material that will clog the minicolumn.
4. Add 275 µL of Digestion Solution to each sample tube. The sample should be completely covered with solution. If the sample is not completely covered, cut the tissue into smaller pieces.
5. Incubate the sample tubes overnight (16 - 18 hours) in a 55°C heating block or water bath. It is not necessary to shake the tubes during incubation.

   Note: Lysates must be warm for the DNA purification protocol.
6. Optional: After overnight digestion, centrifuge samples at 2000 x *g* to pellet any undigested hair or cartilage. Transfer supernatant to a new 1.7 mL microcentrifuge tube.
7. Add 250 µL of Lysis Buffer to each sample. Vortex to mix.

   Note: If the lysate cannot be processed immediately, store at -80°C. Frozen lysates must be thawed and warmed at 55°C for 1 hour before continuing with DNA purification.
8. Immediately proceed with DNA purification (section 6.0).

5.2 Tissue Culture Cell Lysate

The following protocols are for lysing cells from adherent or suspension cultures. Use at least 1 x 10^4 cells to a maximum of 5 x 10^6 cells per purification. The number of cells may need to be optimized for cell type and function.

For harvesting and lysing adherent cells, proceed to section 5.2.1. For lysing cells in suspension, proceed to section 5.2.2.

5.2.1 Adherent Cells

For adherent cells grown in multi-well plates, a yield of up to 1 x 10^6 cells/well is possible, depending on cell type and well size.

1. Aspirate cell culture medium. Wash cells in the tissue culture plate with D-PBS. Remove D-PBS.
2. Harvest cells using appropriate dissociation method. Collect cells in a sterile centrifuge tube and centrifuge cell suspension at 300 x *g* for 5 minutes. Remove and discard supernatant.
3. Add D-PBS to wash cells. Centrifuge at 300 x *g* for 5 minutes. Remove as much supernatant as possible and discard.
4. Add 150 µL Lysis Buffer to the cell pellet. Mix by pipetting up and down.

   Note: If the cell lysate will not be used immediately, store at -80°C. Frozen lysates must be thawed and warmed at 55°C for 1 hour before continuing with DNA purification.
5. Proceed to DNA purification (section 6.0).

5.2.2 Suspension Cells

If starting with a frozen cell pellet, begin at step 3 by adding Lysis Buffer to the frozen pellet.

1. In a sterile centrifuge tube, centrifuge cell suspension at 300 x *g* for 5 minutes. Remove and discard supernatant.
2. Add D-PBS to wash cells. Centrifuge at 300 x *g* for 5 minutes. Remove as much supernatant as possible and discard.
3. Add 150 µL Lysis Buffer to the cell pellet. Mix by pipetting up and down.

   Note: If the cell lysate will not be used immediately, store at -80°C. Frozen lysates must be thawed and warmed at 55°C for 1 hour before continuing with DNA purification.
4. Proceed to DNA purification (section 6.0).

6.0 DNA Purification

The following protocol is for DNA purification of mouse tail or animal tissue lysates, or tissue culture cell lysates. Prepare sample as described in section 5.1 or 5.2. Wear gloves throughout the protocol, and change gloves frequently. Keep tubes closed whenever possible.

1. Prepare one minicolumn assembly for each lysate sample; an assembly consists of a minicolumn placed in a Collection Tube. Label the collection tube and place the assembly in a microcentrifuge tube rack.
2. Transfer the entire lysate sample to a minicolumn assembly.
3. Centrifuge the assembly at 13,000 x *g* for 3 minutes. If some lysate remains on the column, centrifuge again at 13,000 x *g* for 1 minute.
4. Remove the minicolumn from the tube and discard the liquid in the tube. Place the minicolumn back in the tube.
5. Add 650 µL of Column Wash Buffer to each minicolumn assembly.
6. Centrifuge at 13,000 x *g* for 1 minute.
7. Remove the minicolumn from the tube and discard the liquid in the tube. Place the minicolumn back in the tube.
8. Repeat steps 5 - 7 for a total of 4 washes of the minicolumn.
9. 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.
10. Remove the minicolumn and place in a new labeled 1.7 mL microcentrifuge tube for elution.
11. Add 250 µL of nuclease-free water (at room temperature or heated to 65°C) to the minicolumn. Incubate at room temperature (15 - 25°C) for 1 - 2 minutes.
12. Centrifuge the assembly at 13,000 x *g* for 1 minute.
13. For **mouse tail or animal tissue lysates**, proceed to step 14. For **tissue culture cell lysates**, proceed to step 16.
14. Remove the assembly from the centrifuge. Add an additional 250 µL of nuclease-free water to the minicolumn. Incubate at room temperature (15 - 25°C) for 1 - 2 minutes.
15. Centrifuge the assembly at 13,000 x *g* for 1 minute.
16. Remove the minicolumn from the elution tube and discard. Cap the elution tube containing purified DNA and store at -20°C to -80°C.
    - For **mouse tail or animal tissue lysates**, elution volume will be approximately 500 µL. This is the recommended elution volume for optimal DNA yield. A lower elution volume will concentrate the DNA but may decrease total yield.
    - For **tissue culture cell lysates**, elution volume will be approximately 250 µL. This is the recommended elution volume for optimal DNA yield. A lower elution volume will concentrate the DNA but may decrease total yield. A higher elution volume dilutes the DNA and does not improve yield.

7.0 Troubleshooting

PROBLEM	POSSIBLE CAUSE	SOLUTION
Low A_{260} (Low DNA yield)	Tissue lysate stored at -20°C to -80°C, or undergone multiple freeze-thaw cycles. Lysate that has been frozen may have a decreased amount of genomic DNA.	For optimal performance, purify the DNA as soon as the lysate is prepared.
	Tissue culture cells are low in genomic DNA; genomic DNA yield may vary depending on the number of cells used.	Increase the amount of starting material to a maximum of 20 mg of tissue or 5 x 10^6 tissue culture cells.
	Lysis Buffer not added to samples.	Ensure Lysis Buffer is added to all samples.
	Frozen lysates were not thawed and warmed to 55°C.	Process tissue lysates as soon as they are removed from 55°C incubation. If samples have cooled, place them back at 55°C for 1 hour, then continue with purification.
	Steps not followed correctly or wrong reagents used.	The DNA purification protocol steps must be followed in order, ensuring the correct reagents are used at each step. This ensures that the DNA remains bound to the membrane during the purification process.
	Ethanol not added to Column Wash Solution.	Prepare Column Wash Buffer as described in section 4.0 before beginning the DNA purification protocol.
RNA contamination	RNase A Solution was not added to the Digestion Solution; RNA was copurified with genomic DNA from tissue culture cells.	Add 2 µL of RNase A Solution to the final eluate and incubate at room temperature for at least 10 minutes.
Clogged column	Lysate too concentrated or viscous.	• Dilute lysate with Lysis Buffer until it is easy to pipette; apply the entire lysate to the column. • Do not allow lysate to cool after incubation at 55°C; if lysate has cooled, place back at 55°C for 1 hour, then continue with purification.
	Too much tissue sample was used in the lysate preparation.	Use a maximum of 20 mg of mouse tail or animal tissue for lysate preparation.
	Too many cells were processed.	Use a maximum of 5 x 10^6 cells.
	Mouse tail clipping treated with Proteinase K Solution contains a lot of undigested hair or cartilage.	• Mouse tail clipping must be collected from within the terminal 2 cm of the tail tip. • After proteinase K digestion, centrifuge the sample at 2000 x *g* to pellet undigested sample. Transfer supernatant to a new 1.7 mL microcentrifuge tube prior to adding Lysis Buffer.

PROBLEM	POSSIBLE CAUSE	SOLUTION
Incomplete digestion of tissue samples	Proteinase K Solution not added to Digestion Solution.	Prepare the Digestion Solution according to Table 1 (section 5.1).
	Too much tissue sample was used in the lysate preparation.	A maximum weight of 20 mg of mouse tail or animal tissue can be used for lysate preparation.

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