illustra
tissue & cells
genomicPrep Mini Spin Kit

For the rapid extraction and isolation of genomic DNA from animal tissues and cultured mammalian cells

Product booklet

Codes: 28-9042-75 (50 purifications)
28-9042-76 (250 purifications)

Page finder

1. Legal

Product use restriction

The components of the **illustra™ tissue & cells genomicPrep Mini Spin Kit** have been designed, developed, and sold for research purposes only. They are suitable for *in vitro* use only. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is the responsibility of the user to verify the use of the **illustra tissue & cells genomicPrep Mini Spin kit** for a specific application as the performance characteristic of this kit has not been verified to any specific species.

Contact your GE Healthcare representative for the most current information (see back cover for contact details).

http://www.gelifesciences.com

Amersham Place, Little Chalfont,
Buckinghamshire. HP7 9NA UK.

2. Handling

2.1. Safety warnings and precautions

Warning: For research use only.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Suitable protective clothing such as laboratory overalls, safety glasses, and gloves should be worn. Care should be taken to avoid contact with skin or eyes; if contact should occur, wash immediately with water (see Material Safety Data Sheet and/or safety statements for specific recommendations).

Warning: This protocol requires the use of Ethanol

The chaotrope in the Lysis buffer type 4 is harmful if ingested, inhaled, or absorbed through the skin, and can cause nervous system disturbances, severe irritation, and burning. High concentrations are extremely destructive to the eyes, skin, and mucous membranes of the upper respiratory tract. Gloves should always be worn when handling this solution.

Use of this product with cells, tissue, or tissue products should be considered biohazardous. Follow appropriate safety procedures while using this kit and when handling DNA isolated from these sources. Waste effluents from this kit should be decontaminated with bleach or detergent-based method. Decontamination with bleach may be reactive resulting in foam and emission of

ammonia gas and should be carried out in an exhaust hood. Consult local safety regulations for safe disposal of all waste.

2.2. Storage

All kit components should be stored at room temperature (20–25°C) upon arrival. Once reconstituted, store Proteinase K at 4°C.

2.3. Expiry

For expiry date please refer to outer packaging label. Proteinase K reconstituted in DNase-free water is stable for 4 months or until expiry on outer packaging label, when stored at 4°C.

3. Components

3.1. Kit contents

Identification	Pack Size Cat. No.	10 purifications sample pack	50 purifications 28-9042-75	250 purifications 28-9042-76
	Proteinase K, lyophilized powder	1 vial (10 mg)	1 vial (30 mg)	2 vials (2 × 30 mg)
	Lysis buffer type 1	1.5 ml	6 ml	27 ml
	Lysis buffer type 4	12 ml	60 ml	2 × 165 ml
	Wash buffer type 6	1.5 ml (Add 6 ml absolute Ethanol before use)	6 ml (Add 24 ml absolute Ethanol before use)	30 ml (Add 120 ml absolute Ethanol before use)
	Elution buffer type 5	3 ml	12 ml	60 ml
	illustra tissue & cells mini columns	10	50	5 × 50
	Collection tubes	10	50	5 × 50

Refer to the Certificate of Analysis for a complete list of kit components. GE supplies a wide range of buffer types across

the illustra nucleic acid purification and amplification range. The composition of each buffer has been optimized for each application and may vary between kits. Care must be taken to only use the buffers supplied in the particular kit you are using and not use the buffers supplied in other illustra kits e.g. the Lysis buffers type 1 & 4 supplied in the illustra tissue & cells genomicPrep Mini Spin Kit are not the same as the Lysis buffer type 2 supplied in the illustra bacteria genomicPrep Mini Spin Kit.

In order to avoid confusion and the accidental switching of buffers between kits, a numbering system has been adopted that relates to the entire range of buffers available in the illustra purification range. For example there are currently 14 Lysis buffers in the illustra range, 6 Wash buffers and 8 Elution buffers, denoted by Lysis buffer type 1–14, Wash buffer type 1–6 and Elution buffer type 1–8, respectively. Please ensure you use the correct type of Lysis, Wash and Elution buffer for your purification.

3.2. Materials to be supplied by user

Chemicals:
Dulbecco's Phosphate Buffered Saline Solution (PBS)
Absolute Ethanol
RNase A, lyophilized powder (20 mg/ml)
Disposables:
2 ml DNase free microcentrifuge tubes (snap-cap)

3.3. Equipment needed

Microcentrifuge that accommodates 1.5 ml microcentrifuge tubes.
Vortex mixer
Water bath or heating block at 70°C

Recommended homogenizer for genomic DNA purification from animal tissues:
Hand-held motor-driven homogenizer (Kimbel, part no. 749540 or equivalent) with probe (part no. 0090)

4. Description
4.1. Introduction

The **illustra tissue & cells genomicPrep Mini Spin kit** is designed for rapid extraction of genomic DNA from various animal tissues and cultured mammalian cell lines. The protocols for extraction of genomic DNA from mammalian cell lines and animal tissue utilize the same buffers. Although the protocols are rapid, they have been designed to minimize shearing, resulting in high quality intact genomic DNA.

The kit utilizes Lysis buffers in combination with Proteinase K to release and de-proteinate genomic DNA (1). Genomic DNA is then bound onto a silica membrane in the presence of a chaotropic solution (2). Contaminants are removed during the Wash & Dry step and DNA is eluted with Elution buffer type 5. The entire procedure can be completed in as little as 90 minutes, to yield genomic DNA with a purity and quality that is compatible with most molecular biology applications, including cloning, restriction enzyme digestion, PCR amplification and genotyping.

The tissue and cell Lysis buffers type 1 & 4 have been optimized to extract DNA from several tissue types such as liver, kidney, and mouse tails. Typical yields are 0.5–1.5 µg genomic DNA per mg of tissue. 5–50 mg tissue can be used per mini-prep. The kit is designed to give consistent recovery of genomic DNA with high purity (A_{260}/A_{280} = approximately 1.8).

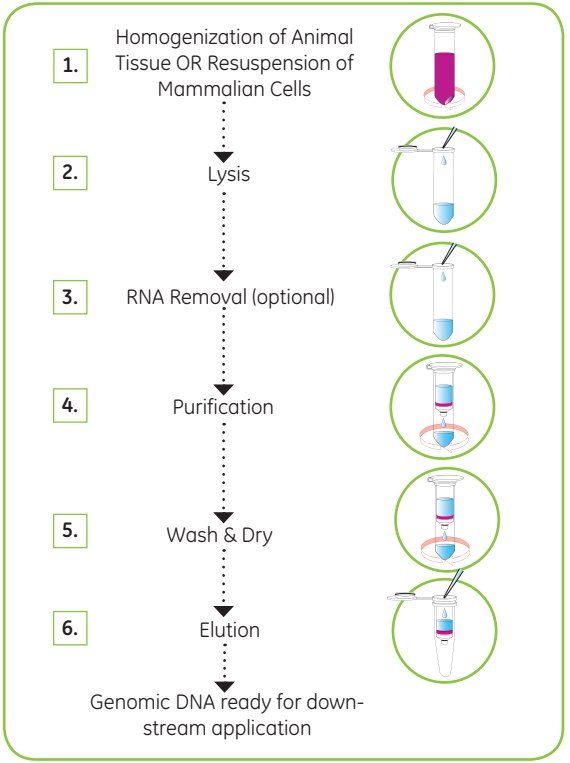
The complete homogenization of animal tissue is critical. Following homogenization, the tissue should be uniformly suspended in solution and free of any visible clumps. Hand-held motor-driven homogenizers perform better than a mortar and pestle, especially when working with rat or mouse kidney and tail tissues (see section 3.3 for details of a hand held homogenizer shown to give good results with this kit).

Genomic DNA can also be isolated from cultured mammalian cell lines using this kit . Between 1×10^6 and 5×10^6 cells are required. The yields vary according to cell type and growth state. Typical yields are 10–13 µg from 5×10^6 CHO cells, and 40 µg from the lung fibroblast cell line MRC5.

The kit contains sufficient reagents and columns for 50 (28-9042-75) and 250 (28-9042-76) purifications.

4.2. The basic principle

Use of the illustra tissue & cells genomicPrep Mini Spin Kit involves the following steps (images have been shown for the purification of genomic DNA from mammalian cells only):



Step	Comments	Component
1. Homogenization of Animal Tissue OR Resuspension of Mammalian Cells	Use of hand-held homogenizer gives more consistent yield.	
2. Lysis	Cells are lysed in presence of Lysis buffer type 1 and Proteinase K.	**Lysis buffer type 1** **Proteinase K**
3. RNA Removal (optional)	RNA is removed by RNase A.	**Lysis buffer type 4**
4. Loading	Chaotropic salt in Lysis buffer type 4 promotes the binding of genomic DNA to the novel silica membrane.	**illustra tissue & cells mini column & Collection tube** 
5. Wash & Dry	Lysis buffer type 4 contains a chaotropic salt that removes protein and other contaminants from membrane bound genomic DNA. The ethanolic Wash buffer type 6 removes residual salts and other contaminants and dries the silica membrane at the same time.	**Wash buffer type 6**
6. Elution	Genomic DNA is eluted in a low ionic strength buffer.	**Elution buffer type 5**

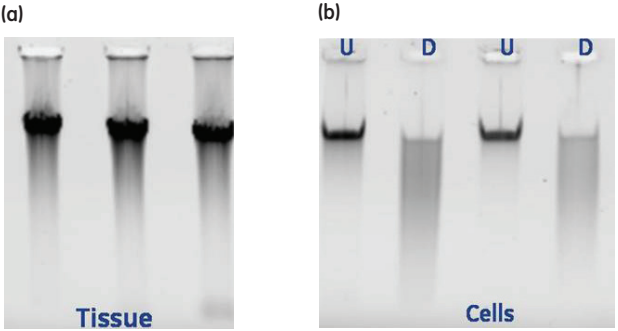
4.3. Product specifications

Sample Type	Tissue	Cultured cells
Sample Input Size	5–50 mg of animal tissue	Up to 5×10^6 cultured cells
Elution Volume	200 µl	200 µl
Number of Steps	5	5
Binding Capacity	> 35 µg	> 35 µg
Yield	0.5–1.5 µg genomic DNA/mg of animal tissue*	10–20 ug of genomic DNA (from 5×10^6 cells)
Purity (A_{260}:A_{280})	> 1.75	> 1.75
Time/prep	90 minutes	45 minutes
Product Size	> 20 kbp	> 20 kbp

*Values shown derived from rat liver samples; actual yields will vary depending on tissue type used.

4.4. Typical output

Figure 1. Gel characteristics of purified genomic DNA from (a) rat liver and (b) cultured CHO cells



The high quality genomic DNA extracted from CHO cells shown in figure (b) was successfully digested using the HindIII restriction enzyme (wells denoted with D).

Table 1 below shows typical yield of genomic DNA from obtained from several types of animal tissue, as determined by UV spectrophotometry (A_{260}). All preparations were treated with RNase.

Table 1. Genomic DNA yield from various animal tissue types

Tissue type	Amount (mg)	DNA Yield (µg)
Rat liver	10	13.8
Rat liver	10	12.8
Rat liver	15	18.9
Rat kidney	15	16.3
Rat kidney	15	20.3
Mouse tail	15	11.7

Table 2 shows below gives typical yields of genomic DNA obtained from various mammalian cell lines. All preparations were treated with RNase.

Table 2. Genomic DNA yield from tested mammalian cell lines

Cell Line	Cell Number	DNA Yield
CHO cells	5×10^6	13.9 µg
CHO cells	3×10^6	8.8 µg
Kidney 293 cells	5×10^6	22.1 µg
MRC5	5×10^6	43.6 µg

5. Protocol

External factors which may affect the quality of genomic DNA isolated from tissue are outlined in section 6.2.

Note: Buffers and columns are NOT transferable between GE Healthcare kits, e.g., the composition of the Lysis buffers type 1 & 4 in the tissue and cells genomicPrep Mini Spin Kit is not the same as the Lysis buffer type 10 in the blood genomicPrep Mini Spin Kit and the tissue & cell mini columns are not the same as the blood mini columns.

Use of icons

The Key below describes the purpose of the icons used throughout the protocol booklet.

This icon is used to highlight particularly critical steps within the protocol that must be adhered to. If this advice is not followed it will have a detrimental impact on results.

This icon is used to highlight technical tips that will enhance the description of the step. These tips may indicate areas of flexibility in the protocol or give a recommendation to obtain optimum performance of the kit.

5.1. Preparation of working solutions

See sections 3.2. & 3.3 for Materials & Equipment to be supplied by user.

Proteinase K

Dissolve the supplied lyophilized **Proteinase K** in DNase-free water. Add 1.5 ml of DNase-free water to the vial of **Proteinase K** in kit 28-9042-75 or 1.5 ml to EACH vial of **Proteinase K** in kit 28-9042-76. Sample pack users, please add 500 µl DNase-free water to the supplied vial of **Proteinase K**. Final concentration is 20 mg/ml. Vortex to dissolve. Once reconstituted, store **Proteinase K** at 4°C.

Lysis buffer type 1

Ensure no precipitate is visible in the bottle containing **Lysis buffer type 1**. If necessary, warm the buffer in a 56°C water bath for 2–3 minutes. **Lysis buffer type 1** should be stored at room temperature (20–25°C).

Wash buffer type 6

Add Absolute Ethanol to the **Wash buffer type 6** before use.

To the **Wash buffer type 6** in kit code 28-9042-75 add 24 ml Absolute Ethanol, and to the **Wash buffer type 6** in kit code 28-9042-76 add 120 ml Absolute Ethanol before use. To the **Wash buffer type 6** in the 10 purifications size sample pack, please add 6 ml of Absolute Ethanol.

Mix by inversion and indicate on the label, by ticking the box, that this step has been completed. Store upright and airtight at room temperature (20–25°C).

RNase A (user supplied)

Prepare a stock solution of RNase A in DNase-free water (to give a final concentration of 20 mg/ml) prior to use. e.g., to a vial containing 10 mg of RNase A add 500 µl of DNase-free water.

Elution buffer type 5

Remove required volume of **Elution buffer type 5** from supplied bottle (200 µl per sample) into a separate 1.5 ml microcentrifuge tube. Pre-heat to 70°C prior to use in Elution step.

5.2. Protocol for extraction of genomic DNA from animal tissues

1. Homogenization of Animal Tissue

a. Using a sterile blade, weigh out 5–50 mg animal tissue. Slice and transfer it to the bottom of a 2 ml microcentrifuge tube. Keep the tubes on ice until you are ready to proceed to step 2.

5–50 mg tissue sample

Note: Use 2 ml microcentrifuge tubes in order to reduce spills during homogenization. Up to 50 mg of tissue can be used per purification, but the yield of genomic DNA may begin to plateau when purifying genomic DNA from greater than 20–25 mg of tissue. Therefore, if genomic DNA is to be extracted for example from 50 mg of tissue, split the tissue into two × 25 mg portions and perform two separate purifications to maximize genomic DNA recovery.

When working with mouse or rat tails, place thin tail slices into a mortar, cover with liquid nitrogen and crush the tails into very small pieces with a pestle. After the liquid nitrogen has fully evaporated (see section 6.2.), transfer the sample into a microcentrifuge tube and proceed to step b.

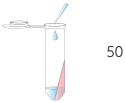
b. Add 1 ml of PBS.

c. Spin for 1–2 minutes at maximum speed (16 000 × g).

Note: Actual spin time required depends on the tissue type and weight of sample.

d. Discard supernatant by aspiration or by inverting the tube taking care not to disturb the sample.

e. Add 50 µl of PBS.



f. Homogenize the tissue completely into solution.

Note: This step usually takes 2-3 minutes per sample. A hand-held motor-driven homogenizer (Kimbel, part no. 749540 or equivalent) is recommended over a mechanical pestle. The protocol is optimal with 50 µl of PBS. Do not increase PBS volume; otherwise DNA yield may be reduced.

g. Spin 10 seconds at 2 000 × g to bring contents to the bottom of tube.

10 seconds
2 000 × g

Note: For calculation of RPM from RCF, please see section 6.1.

2. Lysis

a. Add 50 µl of **Lysis buffer type 1**

50 µl Lysis buffer type 1

b. Add 10 µl of **Proteinase K** . Vortex for 15 seconds.

10 µl Proteinase K,

c. Incubate for 1 hour at 56°C.

1 hour 56°C

Note: During the incubation step, remove required volume of Elution buffer type 5 from supplied bottle (200 µl per sample) into a separate 1.5 ml microcentrifuge tube. Pre-heat to 70°C prior to use in Elution step.

d. Spin briefly for 10 seconds at 2 000 × g to bring contents to the bottom of tube.

10 seconds
2 000 × g

3. RNA Removal (optional)

a. Add 5 µl of 20 mg/ml RNase A solution. Incubate for 15 minutes at room temperature (RT).

(Optional)
5 µl RNase A
15 minutes RT

Note: RNase treatment is strongly recommended as most animal tissues contain large quantities of RNA.

4. Purification

a. Add 500 µl of **Lysis buffer type 4** . Vortex for 15 seconds.

500 µl Lysis buffer type 4

b. Incubate for 10 minutes at room temperature.

10 minutes RT

c. For each purification that is to be performed, place one tissue & cells mini column inside one Collection tube.

d. Apply each sample to a separate column

e. Spin for 1 minute at 11 000 × g. Discard the flowthrough.

Note: Do not overload the column. The maximum volume that can be loaded is 720 µl.

Note: The entire sample should flow through the column. If any of the columns clog, spin at 11 000 × g for a further 15–30 seconds to clear the residue and proceed with Wash & Dry step.

5. Wash & Dry

a. Add 500 µl of Lysis buffer type 4 ▭.

500 µl Lysis buffer type 4

b. Spin for 1 minute at 11 000 × g. Discard the flowthrough.

1 minute
11 000 × g

c. Place the column back inside the Collection tube.

d. Apply 500 µl of **Wash buffer type 6** ▭.

500 µl Wash Buffer type 6

e. Spin for 3 minutes at 11 000 × g. Discard the Collection tube.

3 minutes
11 000 × g

Note: Prior to proceeding to the Elution step make sure the columns are completely dry. Any liquid visible inside the column should be completely removed by an additional spin.

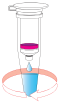
6. Elution

a. Transfer the column to a fresh 1.5 ml DNase-free microcentrifuge tube (user supplied).

b. Add 200 µl of pre-warmed **Elution buffer type 5** to the center of the column. Incubate for 1 minute at room temperature.

Note: Elution buffer type 5 must be pre-warmed to 70°C to maximize DNA recovery.

c. Spin for 1 minute at 11 000 × g to collect the purified genomic DNA.



Note: A second elution step will increase yield by approximately 25%, but will reduce concentration.

d. Analyze the isolated DNA on an 0.8% agarose gel. The size should be > 20 Kbp.

e. For short term storage, place genomic DNA at 4°C. For long term storage, aliquot sample and store at -20°C. Do not subject samples to repeat freeze-thaw cycles.

5.3. Protocol for isolation of genomic DNA from mammalian cell lines

1. Resuspension of Mammalian Cells

a. Transfer 1–5 × 10^6 cultured cells to microcentrifuge tube(s). Spin cells for 1 minute at 2 300 × g. A visible cell pellet will appear at the bottom of the tube. Discard supernatant. If cells were grown in suspension or removed by scraping alone from Petri dishes or flasks, proceed directly to step 2. Lysis

1–5 × 10^6 cultured cells
1 minute
2 300 × g

Note: For calculation of RPM from RCF, please see section 6.1.

b. For attached cells removed by trypsin treatment ONLY:
Wash the cell pellet with PBS as follows:
Add 1 ml PBS. Resuspend cells by pipetting up and down. Spin for 1 minute at 2 300 × g. A visible cell pellet will appear at the bottom of the tube. Discard supernatant.

Cells harvested with trypsin ONLY:
1 ml PBS
1 minute
2 300 × g

2. Lysis

a. Add 100 µl of **Lysis buffer type 1** and re-suspend cells completely by pipetting up and down followed by vortexing for 15 seconds

100 µl Lysis buffer type 1

b. Add 10 µl of Proteinase K (20 mg/ml) to each sample. Vortex again for 15 seconds.

10 µl Proteinase

c. Incubate samples for 15 minutes at 56°C followed by 2 minutes at 70°C.

15 minutes 56°C
2 minutes 70°C

Note: During the incubation, remove required volume of **Elution buffer type 5** from supplied bottle (200 µl per sample) into a separate 1.5 ml

microcentrifuge tube. Pre-heat to 70°C prior to use in Elution step.

d. Spin cells for 10 seconds at 2 000 × g to bring contents to bottom of tube.

10 seconds
2 000 × g

3. RNA Removal (optional)

a. Add 5 µl of RNase A (20 mg/ml) to each sample and incubate for 15 minutes at room temperature (RT).

optional)
5 µl RNase A
15 minutes RT

Note: RNase treatment is strongly recommended as most laboratory grown cells contain large quantities of RNA.

4. Purification

a. Add 500 µl **Lysis buffer type 4** ▭.

500 µl Lysis buffer type 4

b. Incubate for 10 minutes at room temperature.

10 minutes RT

c. Spin 10 seconds 11 000 × g to bring contents to bottom of tube.

10 seconds
11 000 × g

d. For each purification that is to be performed, place one tissue & cells mini column inside one Collection tube.

e. Apply each sample to a separate column

f. Spin for 1 minute at 11 000 × g. Discard the flowthrough.

1 minute
11 000 × g

Note: Do not overload the column. The maximum volume that can be loaded is 720 µl.

Note: The entire sample should flow through the column. If any of the columns clog, spin for a further 15–30 seconds at 11 000 × g to clear the residue and proceed with Wash & Dry step.

5. Wash & Dry

a. Apply 500 µl of **Lysis buffer type 4** to column.

b. Spin for 1 minute at 11 000 × g. Discard the flowthrough by emptying the Collection tube.

c. Place the column back inside the Collection tube.

d. Apply 500 µl **Wash buffer type 6** .

e. Spin for 3 minutes at 11 000 × g. Discard the Collection tube.

Note: Prior to proceeding to the Elution step, make sure the columns are completely dry. Any liquid visible inside the column should be completely removed by an additional spin.

6. Elution

a. Transfer the column to a fresh 1.5 ml DNase-free microcentrifuge tube (user supplied).

b. Add 200 µl of pre-warmed **Elution buffer type 5** directly onto the center of the column. Incubate the columns for 1 minute at room temperature.

c. Spin for 1 minute at 11 000 × g to collect the purified genomic DNA.

Note: A second elution step will increase yield by approximately 25%, but reduce concentration.

d Analyze the DNA on 0.8% agarose gel. The size should be > 25 Kbp.

e. For short term storage, place genomic DNA at 4°C. For long term storage, aliquot sample and store at -20°C. Do not subject samples to repeat freeze-thaw cycles.

6. Appendices

6.1. RPM to RCF calculation

The appropriate centrifugation speed for a specific rotor can be calculated from the following formula:

$RPM = 1\,000 \times \sqrt{(RCF/1.12r)}$

Where RCF = relative centrifugal force; r = radius in mm measured from the centre of the spindle to the bottom of the rotor bucket; and RPM = revolutions per min.

e.g. if an RCF of 735 × g is required using a rotor with a radius of 73 mm, the corresponding RPM would be 3 000.

6.2. Tissue homogenization considerations

Efficient homogenization of tissue samples is important for high DNA yield. Hand-held battery powered homogenizers are relatively inexpensive, and recommended for consistent high yields. Highly fibrous and bony tissues, such as kidney and tails, should be pulverized in liquid nitrogen inside a mortar and pestle that has itself been pre-chilled with liquid nitrogen (3) as outlined below.

1. Weigh an appropriate amount (5–50 mg) of animal tissue in a clean weighing boat. Slice the tissue into small pieces while keeping the tissue sample on ice.
2. Transfer the tissue slices to a pestle that has been pre-chilled with liquid nitrogen. Slowly pour liquid nitrogen to cover the entire material. Put the pestle on a bench top and slowly begin crushing the tissue samples to fine powder with the aid of a mortar. It may be necessary to add liquid nitrogen a few times (e.g. if the liquid nitrogen evaporates quickly) to completely pulverize the tissue samples.

3. After evaporation of remaining liquid nitrogen, transfer the crushed tissue into a 2 ml microcentrifuge tube and add PBS. Continue with homogenization as outlined in the standard protocol 5.2 above.

6.3. Estimation of cell density

Do not exceed 5×10^6 cells per sample when purifying genomic DNA from cultured mammalian cells. Genomic DNA yields drop when silica columns begin to experience clogging (seen at 1×10^7 cells). Cell density should be estimated using an automated cell counter (e.g. Coulter) or counted under a microscope using a standard hemocytometer (for example, Hausser Scientific, catalogue number 1483). If sample total cell count exceeds 5×10^6, spilt sample into two and proceed with two genomic DNA preparations.

Follow the guidelines below for measuring cell density using a hemocytometer.

1. Clean a hemocytometer and the short coverslip thoroughly and wipe clean with Ethanol (this step is not necessary if using disposable hemocytometers)
2. If working with adherent cells, treat the cells with trypsin and wash once with PBS.
3. Re-suspend cells in an appropriate volume of PBS to give approximately 1×10^6 cells/ml. Make sure the cells are completely re-suspended without any visible clumps.
4. Add 10 µl of re-suspended cells to the hemocytometer, making sure the solution spreads completely under the coverslip (by capillary action).
5. Place the hemocytometer under a light microscope, focus on one grid and the cells using lowest magnification and begin counting cells only at the four corner squares and the middle square (3). Count all cells except those touching the middle lines at the bottom

and right. Aim to have 50–100 cells per grid. If cell count is > 150/grid, it is advisable to dilute the cells, clean the hemocytometer and re-count cells.

6. Add the number of cells in a total of ten grids and multiply by dilution factor supplied with your particular hemocytometer to give the number of cells/ml of PBS.

6.4. Estimation of yield and purity

Purified genomic DNA concentration should be determined by UV spectrophotometry (A_{260}) and by agarose gel electrophoresis through comparison with a known standard. The reliable range of A_{260} data should be determined for individual spectrophotometers. Generally, for spectrophotometers with a 1 cm path length, A_{260} readings should lie between 0.1 and 1.0 and appropriate dilutions (5 to 50 ng/µl) should be analyzed. For Nano-Drop™ spectrophotometers, absorbance readings between 1 and 10 are reliable.

The UV spectrophotometric ratio A_{260}/A_{280} provides information regarding the purity of genomic DNA. A purity ratio of 1.7 to 1.9 indicates that the genomic DNA is pure for all standard molecular biology applications. If the ratio is lower than 1.7, the purified genomic DNA might contain some protein impurities. Similarly, if the ratio is higher than 1.9, the genomic DNA might contain some RNA impurities.

1 OD unit (A_{260}) is equivalent to approximately 50 µg/ml double-stranded DNA.

Yield = A_{260} × 50 µg/ml × 0.2 ml = the total µg of purified genomic DNA in the sample.

6.5. Troubleshooting guide

This guide may be helpful in the first instance, however if problems persist or for further information please contact GE Healthcare technical services. Visit http://www.gelifesciences.com for contact information.
Alternatively log onto http://www.gelifesciences.com/illustra

Problem: Genomic DNA yield was low

Possible cause	Suggestions
Elution buffer type 5 not pre-warmed to 70°C	• Before commencing protocol, aliquot required volume of elution buffer type 5 (200 µl per sample) into a 1.5 ml microcentrifuge tube. Pre-heat to 70°C in a water bath or heated block.
	Isolation of genomic DNA from animal tissue
Homogenization of tissue incomplete	• Use a hand-held motor homogenizer. • When using mouse or rat tails, crush thin slices of tails in liquid nitrogen with a pestle and mortar before homogenization (see section 6.2).
Tissue sample old or subjected to repeat freeze/thaw cycles	• For best results, use fresh tissue samples
Incorrect volume of PBS or Lysis buffer type 1 used	• Follow the protocol carefully. The ratio of PBS: Lysis buffer type 1 is critical
Column clogged due to overloading	• Do not use more than 25 mg tissue as starting material for each sample. If necessary, split the tissue between two preparations

Problem: Genomic DNA yield was low

Possible cause	Suggestions
Isolation of genomic DNA from mammalian cell lines	
Incomplete resuspention of cell pellet in Lysis buffer type 1	• Make sure cells are completely resuspended by pipetting up and down and vortexing for 15 seconds. Check for absence of cell pellet
Incorrect cell numbers used as starting material	• Check between 1–5 × 10^6 cells were used as starting material • Use 3–5 × 10^6 cells for optimal recovery. • Do not exceed 1 × 10^7 cells, as yields may drop

Problem: Poor purity of isolated DNA

Possible cause	Suggestions
Too much tissue or too many cells used per sample	• Ensure correct amounts used
Failed to perform (i) Wash with Lysis buffer type 4 or (ii) Proteinase K digestion	• Repeat isolation, taking care to complete all steps

Problem: Poor purity of isolated DNA

Possible cause	Suggestions
Performed Proteinase K digestion at room temperature not 56°C	• For optimal performance, Proteinase K digestion should be carried out at 56°C
Failed to perform RNase A digestion	• RNase treatment is strongly recommended when working with mammalian cell lines

Problem: Restriction enzymes fail to cut isolated DNA

Possible cause	Suggestions
Failed to complete Wash & Dry step	• Repeat isolation taking care to complete a.–d. of the Wash & Dry step.
Sub-optimal digestion conditions used	• Use 50–100 U of high unit-concentration restriction enzyme per µg of genomic DNA in the digest. Set-up reaction in 50–100 µl volume and incubate the digest overnight (16 hours).
EDTA interfering with digestion	• EDTA can chelate magnesium ions required for restriction enzyme function. Elute using pre-warmed DNase-free water, instead of Elution buffer type 5

6.6. Related products

A full range of Molecular biology reagents can be found on the GE Healthcare website and in the catalogue.

Application	Product	Product Number	Pack sizes
Kits containing ready-to-use mix for PCR amplification	illustra Hot Start Master Mix	25-1500-01	100 reactions
	illustra PuReTaq Ready-To-Go PCR Beads	27-9557-01	96 reactions in 0.2 ml tubes/plate
	FideliTaq PCR Master Mix Plus (2 ×)	E71182	100 reactions
	FideliTaq PCR Master Mix Plus	E71183	100 reactions
Premixed nucleotides for PCR amplification	illustra DNA Polymerization Mix	28-4065-57	10 µmol
	illustra DNA Polymerization Mix	28-4065-58	40 µmol (4 × 0.5 ml)

Application	Product	Product Number	Pack sizes
Premixed nucleotides for PCR amplification	illustra PCR Nucleotide Mix	28-4065-60	500 µl
	illustra PCR Nucleotide Mix	28-4065-62	1 ml
Preparation of PCR products for automated sequencing	ExoSAP-IT™	US78200	100 reactions
Sequencing reaction kits optimized for MegaBACE DNA analysis system	DYEnamic ET Terminator Cycle Sequencing Kits	US81050	100 templates
	DYEnamic ET Terminator Cycle Sequencing Kits	US81060	1000 templates

7. References:

1. Aljanabi, S.M. & Martinez, I., *Nucl*. Acids Res. 25, 4692-4693 (1997).

2. Vogelstein, B. & Gillespie, D., *Proc*. *Natl*. *Acad*. Sci. USA 76, 615 (1979).

3. Sambrook, J & Russell, D. W., Molecular Cloning, A Laboratory Manual , chapter 6, (2001).

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28-9042-75

29275353 AG 06-2017

Quick Reference Protocol Card

illustra™ tissue & cells genomicPrep Mini Spin Kit

28-9042-75 (25 purifications)
28-9042-76 (250 purifications)

A. Protocol for extraction of genomic DNA from animal tissues

• Ensure 20 mg/ml Proteinase K and RNase A available • Ensure no precipitate present in Lysis buffer type 1. • Ensure ethanol added to Wash buffer type 6 • Ensure Elution buffer type 5 pre-heated to 70°C

:Homogenize :Add :Spin :Incubate

1. Homogenization of Animal Tissue

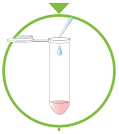
- 5–50 mg animal tissue into 2 ml microcentrifuge tube
- 1 ml PBS
- 2 minutes 16 000 × g; discard supernatant
- 50 µl PBS
- Homogenize (hand-held homogenizer recommended)
- 10 seconds 2 000 × g

2. Lysis

- 50 µl Lysis buffer type 1
- 10 µl Proteinase K; vortex 15 seconds
- 1 hour 56°C
- pre-heat Elution buffer type 5 (200 µl per purification)
- 10 seconds 2 000 × g

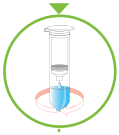
3. RNA Removal (optional)

- 5 µl 20 mg/ml RNase A
- 15 minutes room temperature



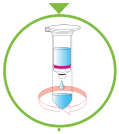
4. Purification

- 500 µl Lysis buffer type 4; vortex 15 seconds
- 10 minutes room temperature
- Transfer sample to tissue & cells mini column inside collection tube
- 1 minute 11 000 × g; discard flowthrough



5. Wash & Dry

- 500 µl Lysis buffer type 4
- 1 minute 11 000 × g; discard flowthrough
- 500 µl Wash buffer type 6
- 3 minutes 11 000 × g; discard Collection tube



6. Elution

- Transfer column to a new 1.5 ml DNase-free microcentrifuge tube
- 200 µl pre-warmed Elution buffer type 5
- 1 minute room temperature
- 1 minute 11 000 × g; retain flowthrough
- Store purified genomic DNA at -20°C

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28-9042-75
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Quick Reference Protocol Card

illustra™ tissue & cells genomicPrep Mini Spin Kit

28-9042-75 (25 purifications)
28-9042-76 (250 purifications)

B. Protocol for extraction of genomic DNA from mammalian cell lines

• Ensure 20 mg/ml Proteinase K and RNase A available • Ensure no precipitate present in Lysis buffer type 1. • Ensure ethanol added to Wash buffer type 6 • Ensure Elution buffer type 5 pre-heated to 70°C

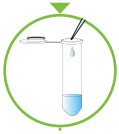
:Homogenize :Add :Spin :Incubate

1. Re-suspension of Mammalian Cells

- Count: 1-5 × 10^6 cells; pipet into microcentrifuge tube
- 1 minute 2 300 × g; discard supernatant
- If cells treated with trypsin:
- 1 ml PBS
- 1 minute 2 300 × g; discard supernatant

2. Lysis

- 100 µl Lysis buffer type 1; re-suspend with pipet; vortex 15 seconds
- 10 µl Proteinase K; vortex 15 seconds
- 15 minutes 56°C
- 2 minutes 70°C
- Pre-heat Elution buffer type 5 (200 µl per purification)
- 10 seconds 2 000 × g

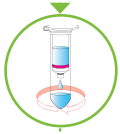


3. RNA Removal (optional)

- 5 µl 20 mg/ml RNase A
- 15 minutes room temperature

4. Purification

- 500 µl Lysis buffer type 4
- 10 minutes room temperature
- 10 seconds 11 000 × g
- Transfer sample to tissue & cells mini column inside collection tube
- 1 minute 11 000 × g; discard flowthrough



5. Wash & Dry

- 500 µl Lysis buffer type 4
- 1 minute 11 000 × g; discard flowthrough
- 500 µl Wash buffer type 6
- 3 minutes 11 000 × g; discard Collection tube

6. Elution

- Transfer column to a new 1.5 ml DNase-free microcentrifuge tube
- 200 µl pre-warmed Elution buffer type 5
- 1 minute room temperature
- 1 minute 11 000 × g; retain flowthrough
- Store purified genomic DNA at -20°C

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