CHAPTER THIRTEEN

Isolation of Genomic DNA from Mammalian Cells

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Abstract

The isolation of genomic DNA from mammalian cells is a routine molecular biology laboratory technique with numerous downstream applications. The isolated DNA can be used as a template for PCR, cloning, and genotyping and to generate genomic DNA libraries. It can also be used for sequencing to detect mutations and other alterations, and for DNA methylation analyses.

Methods in Enzymology, Volume 529
ISSN 0076-6879
http://dx.doi.org/10.1016/B978-0-12-418687-3.00013-6

1. THEORY

In general, isolation of genomic DNA from mammalian cells involves cell lysis, removal of proteins and other cellular contaminants, and organic extraction, followed by recovery of DNA. Typically, mammalian cells are lysed using a detergent-based buffer, which solubilizes lipids, thus disrupting the integrity of cell membranes. This releases cellular components into solution. Proteinase K is then added to facilitate the digestion and removal of proteins from the cell lysates. Next, organic extraction is carried out, in which a mixture of phenol, chloroform and isoamyl alcohol is added. DNA separates into the aqueous phase, while most other contaminants separate into the organic phase. An optional treatment with RNase A ensures that the sample is free from RNA contamination. DNA is then recovered by ethanol precipitation.

2. EQUIPMENT

Centrifuge
Incubator (50 °C)
Microcentrifuge
Vortex mixer
Magnetic stir plate
Micropipettors
Pipet-aid
15-ml Conical centrifuge tubes
1.5-ml Microcentrifuge tubes
Micropipettor tips
Beaker, 1 l
Magnetic stir bars
10-ml pipettes
pH indicator paper
Aluminum foil

3. MATERIALS

Sterile deionized water
Tris base
Hydrochloric acid (HCl)
Sodium chloride (NaCl)
EDTA disodium ($Na_2EDTA.2H_2O$)

Sodium dodecyl sulfate (SDS)
Proteinase K
Phenol
8-hydroxyquinoline
Chloroform
Isoamyl alcohol
Ammonium acetate (NH_4OAc)
Ethanol
Phosphate buffered saline (PBS)
RNase A (optional)

3.1. Solutions & buffers

Step 2 Lysis Buffer

Component	Final Concentration	Stock	Amount
NaCl	100 mM	1 M	1 ml
Tris–HCl, pH 8.0	10 mM	50 mM	2 ml
EDTA, pH 8.0	25 mM	250 mM	1 ml
SDS	0.5%	10%	0.5 ml
Proteinase K	0.1 mg ml^{-1}	1 mg ml^{-1}	1 ml

Add sterile water to 10 ml. Add proteinase K fresh before use

Step 3 Buffered Phenol

Component	Stock	Amount
Phenol		500 ml
8-hydroxyquinoline		0.5 g
Tris–HCl, pH 8.0	50 mM	Variable

See preparation step for instructions to make it up

Phenol Extraction Buffer

Component	Amount
Buffered phenol	25 ml
Chloroform	24 ml
Isoamyl alcohol	1 ml

Mix well. Wrap container in aluminium foil to protect from light

Step 4 7.5 M ammonium acetate

Dissolve 57.8 g ammonium acetate in 100 ml (final volume) of sterile deionized water.

70% Ethanol

Mix 70 ml 100% ethanol and 30 ml sterile deionized water.

TE Buffer

Component	Final concentration	Stock	Amount
Tris–HCl, pH 8	10 mM	50 mM	2 ml
EDTA	1 mM	250 mM	40 µl

Add sterile deionized water to 10 ml

4. PROTOCOL

4.1. Preparation

Make the buffered phenol. Add 0.5 g 8-hydroxyquinoline to a glass beaker containing a stir bar. Add 500 ml phenol and 500 ml 50 mM Tris–HCl, pH 8.0.

Cover with aluminium foil to protect light-sensitive reagents from oxidation.

Stir for 10 min at room temperature, allowing the phases to separate.

Decant most of the upper aqueous phase into an appropriate waste container. Carefully remove the remainder with a 10-ml pipette. Add another 500 ml 50 mM Tris–HCl, pH 8.0. Stir and decant aqueous phase as before. Check the pH of the lower phenol phase with pH paper. Repeat equilibrations with 50 mM Tris–HCl, pH 8.0 until the pH of the phenol phase reaches 8.0.

Add 250 ml of 50 mM Tris–HCl, pH 8.0. Store at 4 °C in either a brown glass bottle or a clear glass bottle wrapped in aluminium foil to protect from light.

Have cells ready to extract DNA.

4.2. Duration

Preparation	Variable
Protocol	About 2 days

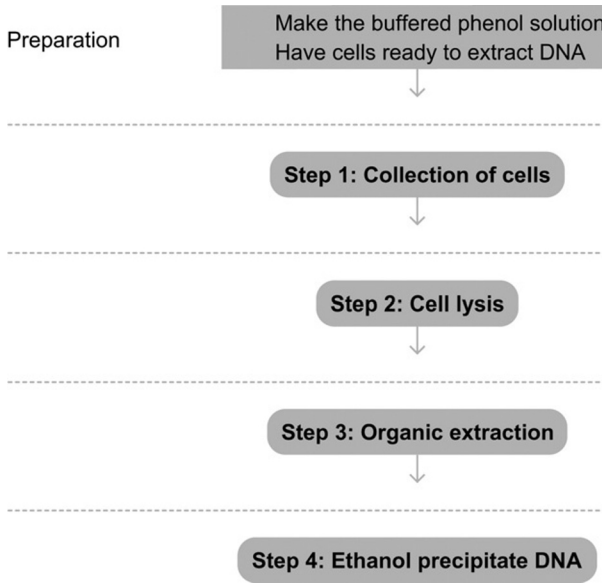

Figure 13.1 Flowchart of the complete protocol, including preparation.

4.3. Caution

Phenol is highly corrosive. It should be handled with care and should be opened only in a fume hood. Used phenol should be disposed appropriately according to chemical safety regulations, not down the sink.

See Fig. 13.1 for the flowchart of the complete protocol.

5. STEP 1 COLLECTION OF CELLS

5.1. Overview

Cells grown in culture are harvested and washed.

5.2. Duration

20 min

1.1 Collect cells in a 15-ml conical centrifuge tube. If starting with tissue culture cells in suspension, directly collect suspension in a conical tube. If starting with adherent cells in culture, trypsinize and collect cells in a conical tube.

1.2 Determine the number of cells collected.

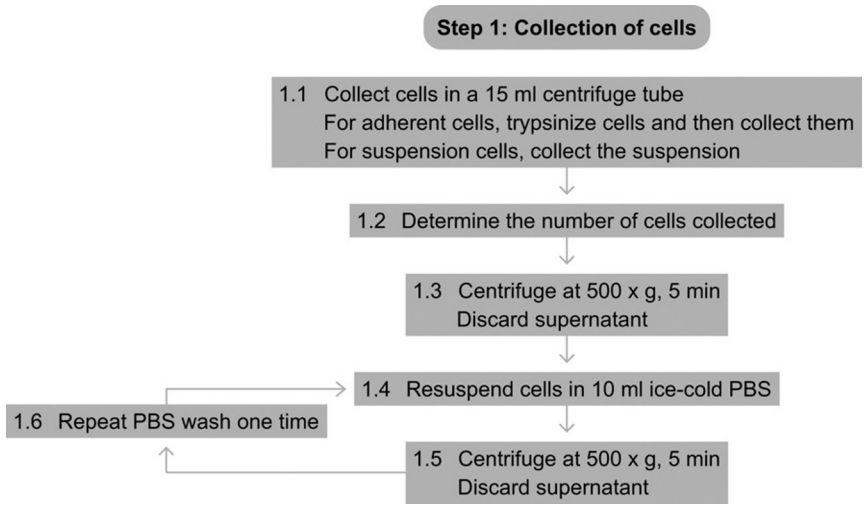

Figure 13.2 Flowchart of Step 1.

1.3 Centrifuge at 500 × g for 5 min. Discard supernatant.
1.4 Resuspend in 10 ml ice-cold PBS.
1.5 Centrifuge at 500 × g for 5 min. Discard supernatant.
1.6 Repeat PBS wash (Steps 1.4 and 1.5).
See Fig. 13.2 for the flowchart of Step 1.

6. STEP 2 CELL LYSIS

6.1. Overview

A detergent-based buffer is used to disrupt cell membranes and release DNA, protein, and other cell components. Proteinase K removes protein contaminants (see also Lysis of mammalian and Sf9 cells).

6.2. Duration

12–18 h

2.1 Resuspend cells in a suitable amount of Lysis Buffer. In general, use 1 ml of buffer per 10^8 cells.
2.2 Transfer to a 1.5-ml microcentrifuge tube. Vortex the samples.
2.3 Incubate at 50 °C for 12–18 h.
See Fig. 13.3 for the flowchart of Step 2.

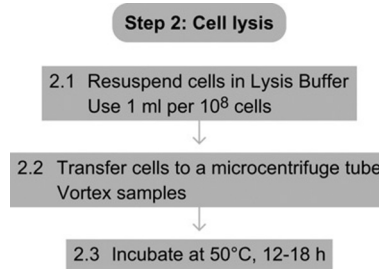

Figure 13.3 Flowchart of Step 2.

7. STEP 3 ORGANIC EXTRACTION

7.1. Overview

Organic extraction is used to separate DNA from other cellular contaminants.

7.2. Duration

10 min

3.1 Add an equal volume of phenol extraction buffer to the lysed cell suspension.

3.2 Vortex for 10 s.

3.3 Centrifuge at $2000 \times g$ for 5 min in a microcentrifuge at room temperature.

3.4 Transfer the upper aqueous phase, which contains DNA, to a new microcentrifuge tube. Determine the volume of the aqueous phase obtained.

3.5 Optional: Add RNase A to a final concentration of 20 $\mu g\ ml^{-1}$. Incubate sample at 37 °C for 20 min.

See Fig. 13.4 for the flowchart of Step 3.

8. STEP 4 ETHANOL PRECIPITATION

8.1. Overview

Ethanol is used to precipitate DNA from solution.

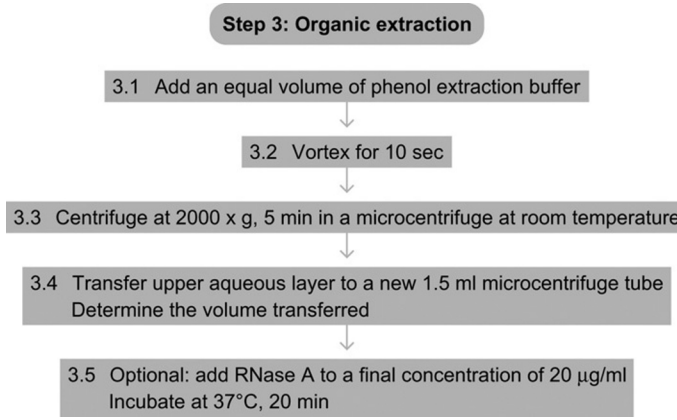

Figure 13.4 Flowchart of Step 3.

8.2. Duration

40 min

4.1 Add 0.5 volumes of 7.5 M ammonium acetate and 2 volumes of 100% ethanol. Vortex.

4.2 Centrifuge at $2000 \times g$ for 10 min in a microcentrifuge at room temperature.

4.3 Remove supernatant carefully, without disrupting DNA pellet.

4.4 Add 1 ml of 70% ethanol and invert tube several times to wash the DNA pellet.

4.5 Centrifuge at $1700 \times g$ for 5 min in a microcentrifuge at room temperature.

4.6 Remove supernatant. Uncap the tube and allow the pellet to air-dry for 10–15 min.

4.7 Resuspend DNA in TE buffer or sterile water.

8.3. Tip

Resuspension in TE buffer stabilizes the DNA for long-term storage. However, the EDTA in the TE buffer may interfere with certain downstream applications, in which case, resuspension in water is preferred.

See Fig. 13.5 for the flowchart of Step 4.

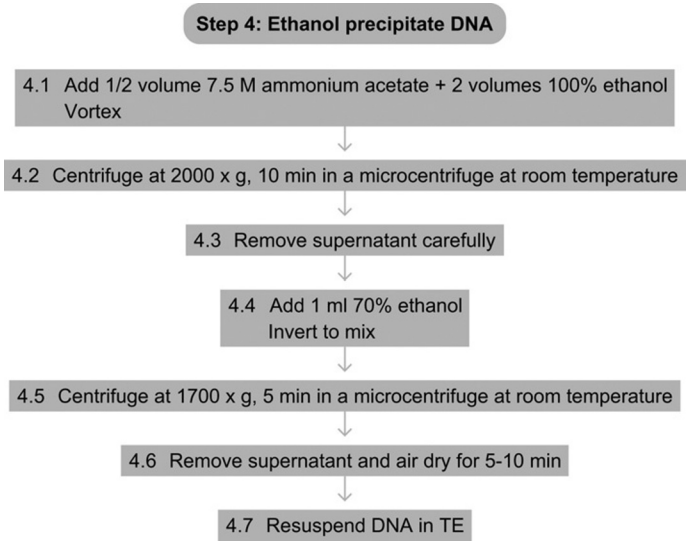

Figure 13.5 Flowchart of Step 4.

REFERENCES

Related Literature

Ausubel, F. M., et al. (1992). Preparation and analysis of DNA. *Short Protocols in Molecular Biology*: *vol. 2.1*. New York: Wiley.

Referenced Protocols in Methods Navigator

Lysis of mammalian and Sf9 cells