

Extraction of genomic DNA from tissue culture cells

Isolating high-molecular weight genomic DNA is essential for PCR, hybridization, and other molecular analyses where degraded DNA may introduce allelic dropout or signal bias.

This protocol yields genomic DNA fragments ranging from 20–30 kbp without the need for phenol/chloroform extraction or alcohol precipitation. It is optimized for adherent or suspension mammalian cell lines. Procedures for blood, fresh or fixed tissues are not covered.

Risk assessment

- Work with human-derived material or transgenic cell lines (BSL-2)
- ▷ Wear gloves, safety glasses, lab coat



Reviewed: Nov 11, 2023

Procedures

>> Preparation of cell lysates

- | | |
|---|---|
| <input type="checkbox"/> 7U/μL RNase A | <input type="checkbox"/> Cell lysis buffer, 1 mL (R) |
| <input type="checkbox"/> 0.6–0.8U/μL Proteinase K | <input type="checkbox"/> Buffer PB (Binding buffer), pH 4.8 |

- (1.) Prepare 10 μL proteinase K in a sterile microcentrifuge tube.
- (2.) Harvest 1×10^6 – 5×10^6 cultured cells by trypsinization or equivalent method. Wash pellet in PBS.

Hint: For adherent cells in a 96-well plate, wash with PBS, directly add 25 μL lysis buffer containing 2 μL proteinase K. Incubate 60 min at 55 °C in a thermocycler, then inactivate for 10 min at 95 °C. The cleared lysate can be used directly for PCR.

- (3.) Resuspend pellet in 200 μL PBS and transfer to tube containing proteinase K.
- (4.) *Optional:* Add 4 μL RNase A. Mix briefly. Incubate 2 min at room temperature.
- (5.) Add 200 μL cell lysis buffer. Vortex to obtain a homogenous solution.
- (6.) Incubate at 55 °C for 10 min to complete protein digestion.
- (7.) Add 200 μL Buffer PB. Mix thoroughly.

>> DNA purification

- (1.) Apply the lysate on a silica membrane spin column. Centrifuge at $11\,000 \times g$ for 1 min or use a vacuum manifold.
- (2.) Wash the silica membrane with 650 μL Buffer PE.
- (3.) Repeat the wash step once more.
- (4.) Dry membrane by centrifugation at $11\,000 \times g$ for 2 min.
- (5.) Place the spin column into a clean microcentrifuge tube.
- (6.) Apply 25–200 μL 5 mM Tris pH 8.0. Incubate 1 min. Elute by centrifugation at $11\,000 \times g$ for 1 min.

Storage of genomic DNA

- (1.) Store at 4 °C or at room temperature for up to six months under slightly basic conditions (pH 8.0) or at –20 °C for long-term storage.

Hint: Genomic DNA tolerates up to twenty freeze-thaw cycles without degradation.

Quality assurance: Check DNA concentration after storage at 4 °C or room temperature to compensate for evaporation.

Analyses

- Determine concentration and purity by measuring absorbance at 260 nm, 280 nm, and 230 nm.

Nucleic acid	A260 = 1.0	A260/A280	A260/A230
dsDNA	50 ng/ μ L	1.8–1.9	2.0–2.2

Note: Extinction ratios vary with composition and pH. A260/A280 may shift 0.2–0.3 with pH.

- Resolve 100 ng DNA on a 0.8–1.0% agarose TAE gel to verify size and integrity.

Troubleshooting

DNA purification

In Step 6:

- Low DNA recovery
 - Use low-salt elution buffer between pH 7.0 and pH 8.5.

Recipes

Cell lysis buffer

Amount	Ingredient	Stock	Final
500 μ L	Tris hydrochloride (Tris-Cl), pH 8.0 ◇ R0056	1 M	10 mM
100 μ L	EDTA, pH 8.0	◇ R0017 0.5 M	1 mM
250 μ L	Triton™ X-100	◇ R0057 10%	0.05%
To 50 mL	Water, reagent-grade		

Store at room temperature.

Cell lysis buffer

10 mM Tris-Cl, 1 mM EDTA, 0.05% Triton™ X-100

Date:

Sign:

R0142

Change log

2023-11-11 Benjamin C. Buchmuller Adaptation as SOP.

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