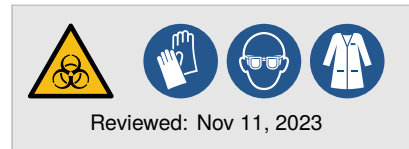


Extraction of genomic DNA from tissue culture cells

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.




This is a bench card. Full protocol available online.


Procedures

>> Preparation of cell lysates


- | | |
|--|---|
| <input type="checkbox"/> R0040 7 U/μL RNase A | <input type="checkbox"/> R0142 Cell lysis buffer, 1 mL |
| <input type="checkbox"/> 0.6–0.8 U/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. 
- (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. 

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

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🔗 [Recipe \(available online\)](#) 🛠️ [Troubleshooting \(available online\)](#) 📖 [Notes \(available online\)](#)

