BECKMAN COULTER
Life Sciences

DNA extraction from cell culture using GenFind V3

Researchers working with cultured cell lines and primary cells and researchers working on genome editing technologies may use this protocol.

Please reference current GenFind V3 IFU for product information (Part Number C34880, C34881)

Purpose

This protocol provides a method for extracting DNA from cultured cells (≤ 2 million cells). Data shown shows the efficacy of this procedure with 1.4 million Jurkart cells, an immortalized line of human T lymphocyte cells.

Materials Used

Material	Part Number	Supplier
2 ml 96-well plate	609681	Beckman Coulter
PBS, pH 7.4	10010023	Thermo Fisher Scientific
Nuclease-free water (Molecular Grade)	AM9932	Thermo Fisher Scientific
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
Microcentrifuge tubes 1.5 mL	357448	Beckman

Protocol

1. **Lysis**
    a. Add **200 µL** of **1 x PBS** to fresh or frozen cell pellet
    b. Mix by pipetting up and down 10 times, or until thoroughly mixed
    c. Add **500 µL** of **Lysis (LBB)**
    d. Add **30 µL** of **Proteinase K (PK)**
    e. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    f. **Incubate** the plate for **15 minutes** at **37°C**
2. **Bind**
    a. Vortex to fully resuspend the **Bind (BBB)**
    b. Add **300 µL** of **Bind (BBB)** to the plate
    c. **Incubate** the plate for **5 minutes** at **room temperature**
    d. Place the plate on a **magnet** for **10 minutes** (or until supernatant is clear)
    e. Remove and discard the supernatant without disrupting the beads
    f. Remove the plate from the magnet
3. **Wash**
    a. Add **800 µL** of **Wash (WBB)** to plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the plate on a **magnet** for **10 minutes** (or until supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads

e. Remove the plate from the magnet
f. Repeat steps 3.a-3.e for a total of **2 washes**
g. Add **1.6 mL** of **Wash (WBC)** to plate
h. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
i. Place the plate on a **magnet** for **6 minutes** (or until supernatant is clear)
j. Remove and discard the supernatant without disrupting the beads
k. Remove the plate from the magnet
l. Repeat steps 3.g-3.k for a total of **2 washes**

4. **Elute**

a. Add **40 µL** of **nuclease free water** to plate
b. Incubate the plate for **2 minute** at **room temperature**
c. Place the plate on a **magnet** for **2 minute** (or until supernatant is clear)
d. Remove and **Save** the supernatant without disrupting the beads

Example Data

Data shown below resulted from isolated genomic DNA (gDNA) from 1.4 million Jurkart. DNA yield and purity was assessed by a NanoDrop (Thermo Fisher Scientific) (Table 1). The genomic integrity of the three replicates was assessed on an Agilent Genomic DNA Screen Tape (Agilent). The DIN scores, which represent the amount of gDNA degradation, were all above 8.4 indicating highly intact gDNA (Figure 1).

	Cell Number	Conc. (ng/µL)	Yield (µg)	A260/A280	A260/A230
Jurkat cells (Sample A)	1.4 x 106	323.8	13.0	1.98	2.16
Jurkat cells (Sample B)	1.4 x 106	319.9	12.8	1.97	2.15
Jurkat cells (Sample C)	1.4 x 106	281.5	11.3	1.99	2.19
Average	1.4 x 106	308.4	12.3	1.98	2.17

Table 1. Concentration and purity of DNA extracted from 1.4 million Jurkat cells. Total average DNA yield from 3 replicates was 12.3 µg. High DNA purity meets requirements for many downstream applications.

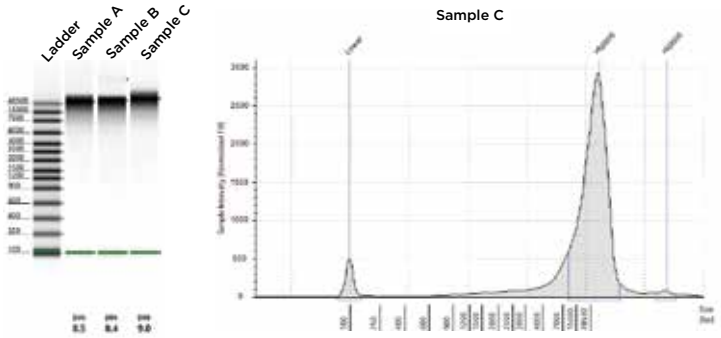

Figure 1. An Agilent Genomic DNA Screen Tape of DNA extracted from 1.4 million Jurkat cells. The gel indicates highly intact DNA predominately >48.5 kb with the DIN scores at the bottom. To the right of the gel is the electropherogram of Sample C.

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