BECKMAN COULTER Life Sciences

GenFind V2 Supplemental Protocol: Isolation of gDNA from Large Number of Cells

Researchers working on cultured cells who want to extract DNA from over million cells may use this protocol.

Please reference current GenFind V2 protocol for product information (Product Number: A41497, A83078 or A41499).

Purpose

Next-generation sequencing (NGS), with its high-throughput technology, has revolutionized genomic study. Obtaining intact, high quality, and concentrated genomic DNA (gDNA) is the first and one of the most critical steps for NGS. Recently, increased number of researchers have reported that a viscous cell lysate significantly decreases gDNA quality and quantity. This protocol provides a method for extracting intact, high quality gDNA, while being scalable and offering the ability to be automated on Beckman Coulter liquid handler.

Table 1. Materials Used

MATERIAL	PART NUMBER	SUPPLIER
Tris buffer solution, pH 9 (1 M)	E694	VWR Chemicals
DTT (1 M)	646563	Sigma
Triton™ X-100	X100	Sigma
Benzonase® Nuclease	sc-202391	Santa Cruz
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
PBS, pH 7.4	10010023	ThermoFisher Scientific
Nuclease-free water (Molecular Grade)	AM9932	ThermoFisher Scientific
Tube Magnet (1.5, 1.7, and 2ml)	A29182	Beckman Coulter
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
Microcentrifuge tubes 1.5 mL	357448	Beckman
1.2mL 96-well plate	AB1127	ThermoFisher Scientific

Table 2. 10x Benzonase nuclease buffer

REAGENT	FINAL
Tris buffer solution, pH 9 (1 M)	20 mM PH:9
DTT (1 M)	100 mM
Triton™ X-100	1%
Benzonase nuclease	6.25 U
Total	60 µL

Protocol

1. **Prepare**
    a. Resuspend frozen or fresh cell pellet in 1xPBS.
    b. Aliquot cell solution with desired cell number to 1.2 mL 96-well plate.

2. **Lysis**
    a. The volume of **1XPBS and cell suspension** should be **140 µL**.
    b. **Mix** the cell suspension with Benzonase nuclease buffer.
        - Cell suspension 140 µL
        - Benzonase nuclease 6.25U
        - 10X Benzonase buffer 60 µL
        - Final 200 µL
    c. Add **400 µL** Lysis Buffer and **9 µL** of 96 µg/µL **Proteinase K** to the samples immediately after Benzonase nuclease treatment.
    d. Gently pipette tip **mix** 10 times or until well mixed.
    e. Incubate the samples at room temperature for 15 minutes.

3. **Bind**
    a. Invert the **Binding Buffer** bottle 20 times to ensure complete resuspension of magnetic particles before using.
    b. Add **300 µL Binding Buffer** to the samples.
    c. Gently pipette **mix** 10 times or until well mixed.
    d. **Incubate** the plate at **room temperature** for **5 minutes** to bind.
    e. Place the sample plate on a magnet Plate for 10 minutes to separate.
    f. Aspirate off the supernatant and discard while the plate is situated on the magnet.
    g. Take the plate off the magnet.

4. **Wash 1**
    a. Add **800 µL** of **Wash Buffer 1** to the plate.
    b. Pipette **mix** 10 times or until the magnetic beads are resuspended from the bottom of the well.
    c. Place the plate back on the magnet for 10 minutes, or until the solution clears.
    d. Aspirate and discard the supernatant while the plate is situated on the magnet.
    e. Repeat steps 4a through 4d for a second wash with the Wash Buffer 1.
    f. Take the plate off the magnet.

5. **Wash 2**
    a. Add **500 µL** of **Wash Buffer 2** to the plate.
    b. Pipette **mix** 10 times or until the magnetic beads are resuspended from the bottom of the well.
    c. Place the plate back on the magnet for 5 minutes, or until the solution clears.
    d. Aspirate and discard the supernatant while the plate is situated on the magnet.
    e. Repeat steps 5a through 5c for a second wash with the Wash Buffer 2.
    f. Take the plate off the magnet.

6. **Elute**

   a. Add **40 µL** of **nuclease free water** to the plate.
   b. **Mix** by pipetting up and down 10 times (or until thoroughly mixed).
   c. **Incubate** at **60°C** for **2 minutes** while shaking at **300 rpm**.
   d. Place on magnet for 5 minutes.
   e. Remove and save the supernatant without disrupting the beads.

Result

2 million HCT116 cells yield 21 µg DNA, however, only half of the DNA was extracted from 4 million cells due to the viscous cell lysate.

Treatment with Benzonase nuclease significantly increased DNA yield with 4 million cells while maintaining high DNA integrity.

This method solved the viscosity problem while being scalable and offering the ability to be automated on Beckman Coulter liquid handler.

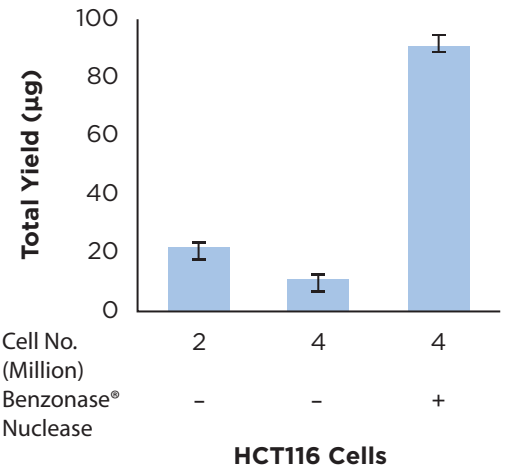
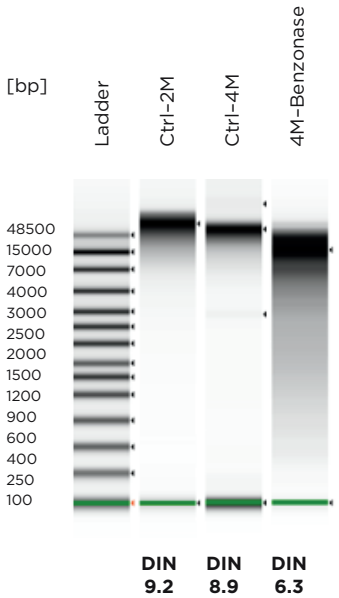


Figure 1. Yield form different cell number treat with or without Benzonase nuclease. Without Benzonase nuclease treatment, 2 million cells yield about 20 µg DNA, when cell number doubles, the yield decreased almost half due to the viscosity. However, with Benzonase nuclease treatment, the gDNA yield from 4 million cells significantly increased.



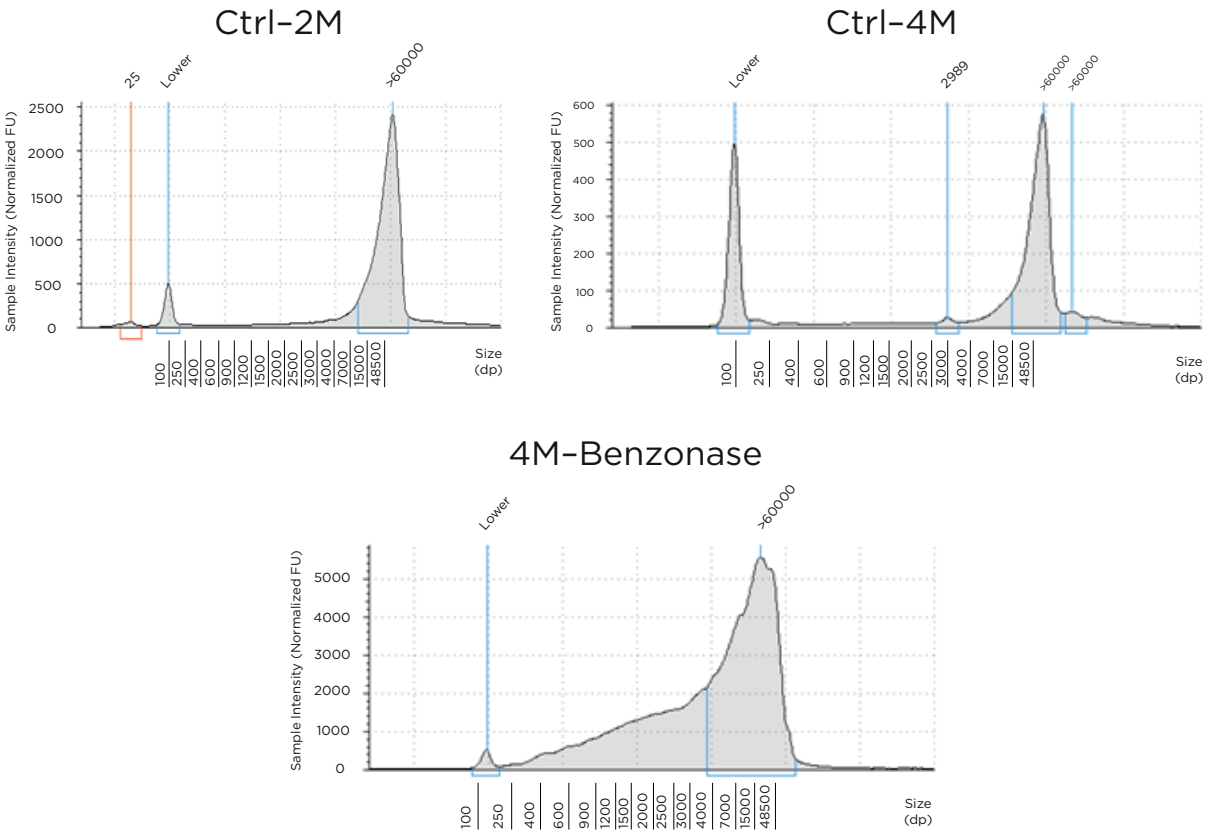


Figure 2. Quality of the gDNA extracted from the cells. The integrity of high molecular weight gDNA is compatible with NGS when determined by Genomic DNA ScreenTape assay (Agilent).