BECKMAN COULTER Life Sciences

DNA extraction from fresh/frozen tissue using a GenFind V3 Kit

Researchers working on fresh/frozen tissue who want to extract DNA and use the same kit as other sample types may use this protocol. Please reference current GenFind V3 IFU for product information (Part Number C34880, C34881)

Purpose

This provides a solution that allows a user to use one kit to extract DNA from multiple sample types with small modifications in the workflow.

Materials Used

Material	Part Number	Supplier
1.2 mL 96-well plate	AB1127	Thermo Fisher Scientific
100% Ethanol (Molecular Grade)	AB00138	American Bio
Nuclease-free water (Molecular Grade)	AM9932	Thermo Fisher Scientific
2 mL 96-well plate	609681	Beckman Coulter Life Sciences
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
Stainless Steel Beads, 5 mm	69989	Qiagen

Protocol

1. **Sample Preparation**
    a. Cut off 10 mg of tissue and place in a 1.2 mL 96-well plate
2. **Lysis**
    a. Add **1 stainless steel** bead to the plate
    b. Add **100 µL** of **Lysis (LBB)** to the plate
    c. Add **100 µL** of **nuclease-free water** to the plate
    d. Bead beat at **1200 rpm** for **30 seconds**
    e. Rest for **30 seconds**
    f. Bead beat at **1200 rpm** for **30 seconds**
        i. This step will vary by tissue type and should be optimized for each tissue
    g. Spin sample at **3,000 rpm** for **3 min**
    h. Transfer **200 µL** of the **supernatant** of the **sample** to a 2 mL 96-well plate
    i. Add **400 µL** of **Lysis (LBB)** to the sample plate
    j. Add **30 µL** of **Proteinase K (PK)**
    k. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    l. **Incubate** the sample plate for **30 minutes** at **37°C**

3. **Bind**
    a. Vortex to fully resuspend the **Bind (BBB)**
    b. Add **300 µL** of **Bind (BBB)** to the sample plate
    c. **Incubate** the sample 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 sample plate from the magnet
4. **Wash**
    a. Add **800 µL** of Wash **(WBB)** to the sample 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 4.a - 4.e for a total of **2 washes**
    g. Add **1.6 mL** of **Wash (WBC)** to the sample 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**
5. **Elute**
    a. Add **40 µL** of **nuclease-free water** to plate
    b. **Incubate** the plate for **2 minutes** at **room temperature**
    c. Place the plate on a **magnet** for **2 minutes** (or until supernatant is clear)
    d. Remove and **Save** the supernatant without disrupting the beads

Example Data

Data shown below are from 16.6mg of mouse lung tissue. The three samples represent three technical replicates. DNA yield and purity were assessed on a NanoDrop (Thermo Fisher Scientific) (Table 1). Genomic DNA (gDNA) integrity was assessed on an Agilent Genomic DNA ScreenTape (Agilent) (Figure 1). The DIN scores, which represent gDNA degradation, were all above 8 indicating low levels of gDNA degradation.

Sample	Conc. (ng/µL)	Yield (µg)	Yield/mg of tissue (µg)	260/280	260/230
A	220.0	8.8	0.53	1.92	2.13
B	186.4	7.5	0.45	1.90	1.94
C	146.7	5.9	0.35	1.92	2.02

Table 1. The concentration, yield, yield per milligram of tissue, and DNA purity of DNA extracted from mouse lung tissue

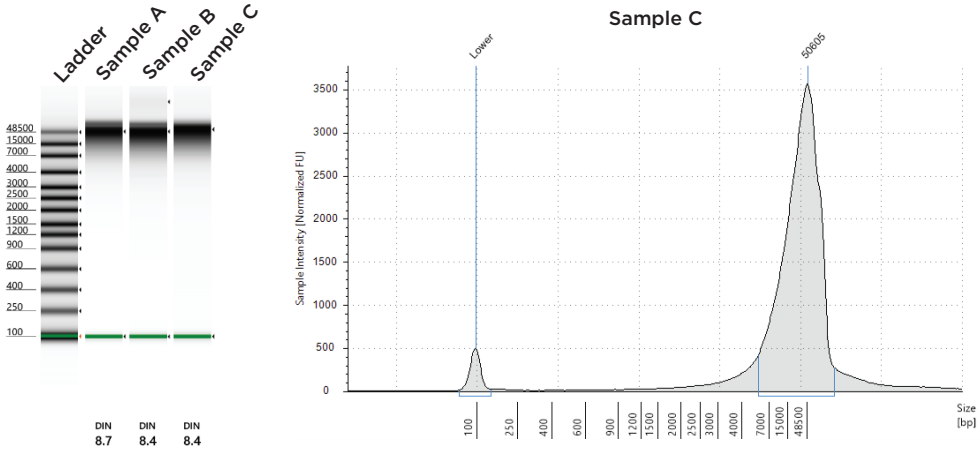

Figure 1. An Agilent Genomic DNA ScreenTape of DNA extracted from 16.6 mg of mouse lung tissue. Left figure, is the gel of all three samples and the right figure is an electropherogram of Sample C. The DIN scores are at the bottom of the gel.

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