BECKMAN COULTER
Life Sciences

Formalin-fixed and non-paraffin-embedded tissue DNA extraction

This method is applicable for scientists that need to extract DNA from formalin-fixed tissue.

Purpose

Due to fragmentation of the DNA and cross-linked protein-DNA complexes during the tissue formalin fixation process, getting any amount of DNA from formalin-fixed and non-paraffin-embedded tissue can be a big challenge. Here we demonstrated a DNA extraction protocol from formalin-fixed and non-paraffin-embedded brain tissue using FormaPure DNA XL kit. In this protocol, protein-DNA cross-links are reversed using heat.

Materials Used

Material	Part Number	Supplier
FormaPure XL DNA	C35996	Beckman Coulter
Microcentrifuge tubes 1.5 mL	357448	Beckman Coulter
Tube Magnet (1.5, 1.7, and 2ml)	A29182	Beckman Coulter
SPRIPlate 96R Ring Super Magnet Plate	A32782	Beckman Coulter
1.2 mL 96-well Plate	AB1127, or equivalent	Thermo Fisher
Ethanol	AB-00138	American Bioanalytical
DNase I (RNase-free)	AM2222 or AM2224	Thermo Fisher
Nuclease-free water (Molecular Grade)	AM9932	Thermo Fisher
Microcentrifuge	NA	NA
Tissuelyser	NA	NA
Hand homogenizer	NA	NA

Protocol

1. **Homogenization/Lysis**
    a. Transfer the fixed tissue from the original container into a 1.5mL tube. Wash the fixed tissue with **1 mL** 1x PSB for **30sec**. Repeat for a total of **2 washes**
    b. Hydrate the fixed tissue with gradient of **Ethanol** (from 100%, 95%, 90%, 80%, 70%, to 50%) and finally in **H_2O**
    c. Transfer **30 mg** fixed tissue to a 1.5 mL centrifuge tube or 96-well plate
    d. Add **200 µL LBA** and **30 µL Proteinase K** to the tissue
    e. Homogenize the tissue use either tissuelyser (steel ball). Adjust the speed and time to make sure of proper tissue homogenization. After homogenization, remove the steel ball. Hand homogenizer is also recommended if low throughput is desired.
    f. Centrifuge the sample at **12000 rpm** for **1 min**
    g. Transfer the supernatant to a new well or tube. Leave the non-homogenized tissue at the bottom of the tube or plate
    h. **Incubate** the sample at **60°C overnight**

2. **Decrosslinking**
    a. **Incubate** the sample for **60 min** at **80°C**
    b. Remove the sample from the heat source
3. **RNase A Treatment**
    a. Add **5 µL** of **RNase A** to the sample
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. **Incubate** for **5 min** at **room temperature**
4. **Bind**
    a. Add **300 µL** of **BBA** to the sample
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
    c. **Incubate** for **5 min** at **room temperature**
    d. Place the sample on a **magnet** for **10 min** (or until supernatant is clear)
    e. Remove and discard the supernatant without disrupting the beads
    f. Remove the sample from the magnet
5. **Wash**
    a. Add **400 µL** of **Wash buffer WBA** to the sample
    b. Mix by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the sample on a **magnet** for **10 minutes** (or the supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the sample from the magnet
6. **Ethanol Wash**
    a. Add **750 µL** of freshly prepared **80% ethanol** to each sample
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the sample on a magnet for 10 minutes (or the supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the sample from the magnet
    f. Air dry the samples on the magnet for 10 min
7. **Elute**
    a. Add **40 µL** of **nuclease free water** to the sample
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. **Incubate** for **2 minutes** at **60°C**
    d. Place the sample on a magnet for **5 minutes** (or until supernatant is clear)
    e. Remove and **save** the supernatant without disrupting the beads

Results

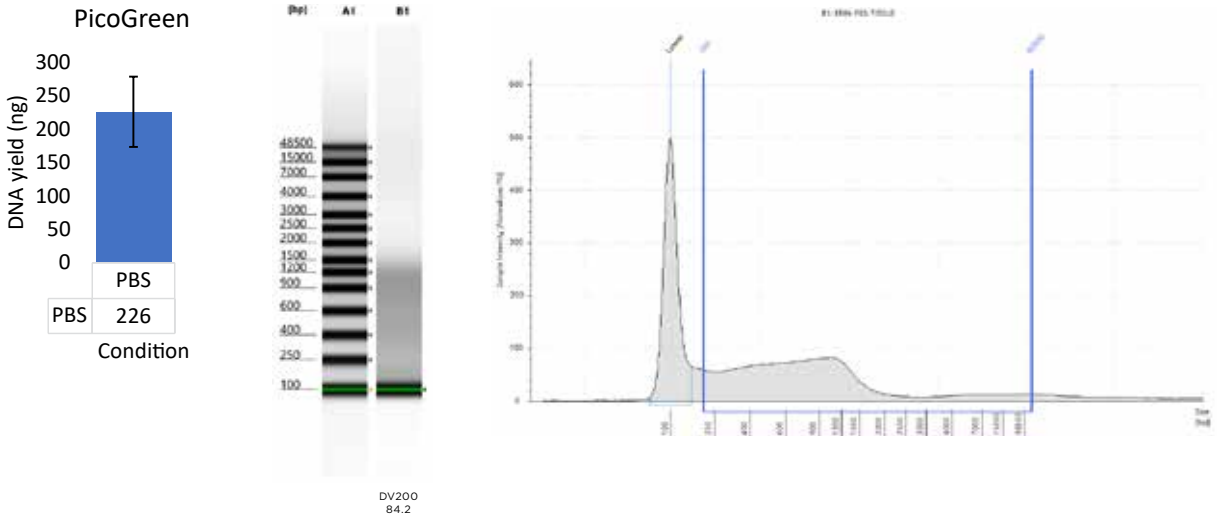

Figure. Formalin-fixed and non-paraffin-embedded brain tissue (customer sample) derived DNA yield and integrity using FormaPure DNA XL. Left: DNA yield was measured by Quant-iT™ PicoGreen® dsDNA Assay. Middle: DNA integrity and DV200 were measured by gDNA TapeStation. Extraction experiments were done in triplicate.

AAG-5267SP04.19