APPLICATION NOTE

Nucleic acid extraction from liver tissue: An integrated and automated workflow solution.

Summary

Nucleic acid extraction from tissue commonly precedes downstream applications like quantitative PCR and Next Generation Sequencing, which are methods utilized to drive genomic-based research. The Omni Bead Ruptor Elite™ bead mill homogenizer and optimized 2 mL Bead Kits provide power and efficiency in sample homogenization releasing analytes of interest, while the chemagic™ 360 nucleic acid extractor automatically extracts genomic material from homogenized samples at low, medium and high throughput demands.

Herein, we show integration of the Omni Bead Ruptor Elite bead mill homogenizer with the chemagic 360 nucleic acid extractor and JANUS® G3 automated workstation platforms by creating a workflow that incorporates sample preparation of tissue, custom-integrated liquid handling and automated nucleic acid extraction and provides the end user with nucleic acid suitable for further genomic analysis.

Materials and methods

Equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor Elite bead mill homogenizer 2 mL Tube Carriage (Cat # 19-373)

For research use only. Not for use in diagnostic procedures.

Omni Bead Ruptor Elite Bead Mill Homogenizer

- Omni Bead Ruptor Elite bead mill homogenizer 48 position 2 mL Tube Carriage (Cat # 19-378)
- Hard Tissue Homogenizing Mix 2.8 mm Ceramic Beads (Cat # 19-628)

Methods

Sample preparation

For sample preparation, 450 mg of murine liver tissue was added to a 2 mL Hard Tissue Homogenizing Mix tube (Cat # 19-628) along with 1 mL of phosphate buffered saline (PBS), pH 7.2 (Gibco, Cat # 20012027). Tissue samples were weighed out with a tolerance ± 5 mg. The liver samples were homogenized on the Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E) at 4.5 m/s for 30 seconds. After homogenization, the lysate was centrifuged at 10,000 xg for 5 minutes to pellet cell debris.

Workflow integration

The Omni Bead Ruptor Elite bead mill homogenizer can process 24 or 48, 2 mL tubes at one time using either the 24 or 48-position 2 mL tube carriage (Cat # 19-373) (Cat # 19-378), respectively. After homogenization, the lysate is manually transferred into chemagen 24 or 96 well plates, depending on throughput. Alternatively, both 2 mL tube carriages have been formatted in-house with the capability to attach to a custom-designed support on the JANUS G3 deck, along with Winprep integration as a custom Labware file. With this custom integration, automated liquid handling of lysate direct from the 24 or 48-position carriages into desired lysate plates is facilitated as a part of chemagic 360 automated nucleic acid extraction workflows.

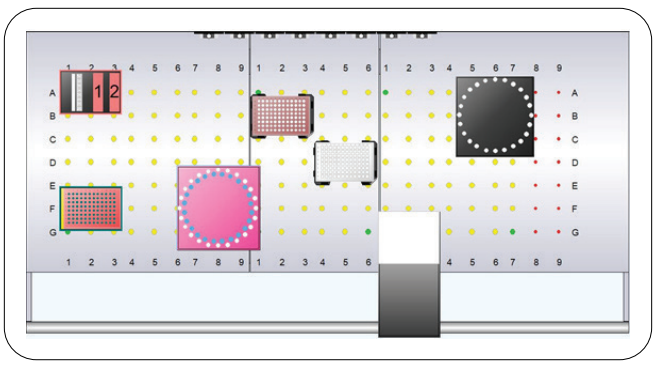

Figure 1: Example Winprep deck layout showcasing the 24 position 2 mL tube carriage (top right, black) and 48 position 2 mL tube carriage (bottom left, pink).

Nucleic acid extraction and analysis

After centrifugation step, 100 µL of tissue lysate was transferred to 12 separate wells of a 96-well plate (Revvity, Cat # CMG-555-15) using the JANUS G3 automated workstation and customized Reagent Liquid Transfer Winprep program. Similarly, 100 µL Lysis Buffer provided with the chemagic Tissue DNA Kit (Cat # CMG-723) was transferred to each well containing the lysate. Next, 6 µL of Proteinase-K, included in the chemagic Tissue DNA kit, was manually transferred to all wells along with 5 µL RNase A (Thermo Scientific, Cat # EN0531). The plate was then incubated at 37 °C for 10 minutes. During this time, Elution Buffer and Magnetic Beads were transferred into respective plates using a Reagent Liquid Transfer Protocol on the JANUS G3 automated workstation. After incubation, the Tissue DNA extraction was carried out using the chemagic 360 nucleic acid extractor.

After completion of the chemagic 360 extraction protocol, eluted nucleic acid concentration and integrity was determined by A_{260}/A_{280} spectrophotometry.

Quantitative PCR

Ten microliters of eluted Liver DNA was added to qPCR mix (BioRad, Cat # 1725122) along with 5 µM of forward and reverse 18S primers. The 18S gene was targeted with forward primer 5′ - CAG CAG CCG CGG TAA TTC C – 3′, reverse primer 5′ - CCC GTG TTG AGT CAA ATT AAG C – 3′ yielding a product size of 676 bp. An 18S-positive DNA extract was used as the positive control along with nuclease-free water for the negative control. Reactions were loaded into the BioRad CFX Connect Real Time Instrument (BioRad Cat. # 1855201), amplified for 40 cycles and visualized via gel electrophoresis.

Results

We evaluated the chemagic Tissue DNA kit using the Omni Bead Ruptor Elite bead mill homogenizer for sample preparation of liver tissue. As a result, homogenized liver tissue yielded an average DNA yield of 2.5 µg. Spectrophotometric data obtained from eluate also indicates that eluted DNA is pure and free of contaminants. Quantitative PCR amplifying eukaryotic 18S ribosomal subunit showed an average Cq value of 20.76, revealing that eluted DNA is intact and suitable for downstream analysis (Table 1).

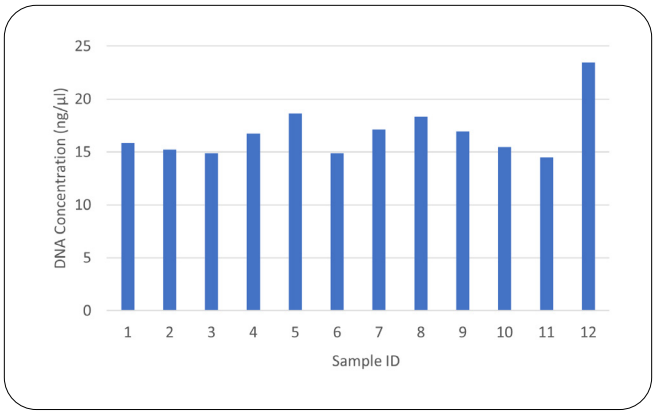

Figure 2: Liver DNA concentration from 12 samples. Lysate from samples 1-12 was prepared with Pro-K and RNase A. Average A_{260}/A_{280} from samples 1-12 was 1.90.

Table 1: 18S qPCR Cq values from extracted Liver DNA

Sample	Cq
Liver 1	21.45
Liver 2	21.21
Liver 3	21.10
Liver 4	21.23
Liver 5	20.56
Liver 6	20.41
Liver 7	20.82
Liver 8	21.08
Liver 9	20.93
Liver 10	20.75
Liver 11	20.32
Liver 12	19.24
Positive extract control	22.01
Negative control	39.07

Conclusions

In this proof-of-concept application note, we have integrated the Omni Bead Ruptor Elite bead mill homogenizer and chemagic 360 nucleic acid extractor for isolation of DNA from liver tissue. The Omni Bead Ruptor Elite bead mill homogenizer and 2 mL Hard Tissue Homogenizing Mix was shown to be an effective solution for sample preparation of liver tissue, producing a homogenate suitable for downstream automated nucleic acid extraction. After a 70-minute automated extraction, the resulting DNA was high-yield and free of contaminants and proven suitable for qPCR analysis. Furthermore, the Omni Bead Ruptor Elite bead mill homogenizer can accommodate a variety of sample preparation throughput demands with either 24 or 48-position 2 mL tube carriages. Either manually or using custom-integrated JANUS G3 liquid handling capabilities, homogenate transfer to chemagic 24 or 96-well lysate plates is accomplished.

Ordering information

Equipment	Catalog number
Omni Bead Ruptor Elite bead mill homogenizer	19-042E
Omni Bead Ruptor Elite bead mill homogenizer 2 mL Tube Carriage	19-373
Omni Bead Ruptor Elite bead mill homogenizer 48 position 2 mL Tube Carriage and Finger Plate	19-378
Hard Tissue Homogenizing Mix 2.8 mM Ceramic Beads	19-628

Authors

Gabriella Ryan, M.S.
Rodney Nash, Ph.D

Revvity, Inc.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com