APPLICATION 202111

Nucleic Acid Extraction from HepG2 Cells: An Integrated Solution Utilizing the Bead Ruptor Elite™ Homogenizer, chemagic™ 360 Nucleic Acid Extractor and JANUS® G3 Automated Workstation

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Bead Ruptor Elite™ Bead Mill Homogenizer

Summary

Applications featuring Next Generation Sequencing, quantitative PCR, or other genomics-based research using *in-vitro* cultured cells requires a nucleic acid extraction upstream of aforementioned methods. The Bead Ruptor Elite™ Bead Mill Homogenizer and 2 mL bead kits are a streamlined sample preparation solution that provides efficiency in cell lysis, releasing nucleic acid for extraction on the chemagic™ 360 automated nucleic acid extractor.

Herein, we outline integration of the Bead Ruptor Elite™ Bead Mill Homogenizer with the chemagic™ 360 nucleic acid extractor and JANUS® G3 automated workstation, creating an end-to-end solution for sample preparation of *in-vitro* cultured cells and nucleic acid extraction workflows.

Materials and Methods

Equipment

- Bead Ruptor Elite™ Bead Mill Homogenizer (Cat # 19-042E)
- Bead Ruptor Elite™ Bead Mill Homogenizer 2 mL Tube Carriage (Cat # 19-373)
- Bead Ruptor Elite™ Bead Mill Homogenizer Finger Plate (Cat # 19-370)
- Bead Ruptor Elite™ Bead Mill Homogenizer 48 position 2 mL Tube Carriage (Cat # 19-378)
- Bead Ruptor Elite™ Bead Mill Homogenizer 48 position 2 mL Tube Carriage Finger Plate (Cat # 19-370-248)
- Hard Tissue Homogenizing Mix 2.8 mm Ceramic Beads (Cat # 19-628)

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Materials and Methods

HepG2 Cell Culture and Cell Counting

HepG2 cells (ATCC, Cat # HB-8065) were grown in DMEM (Gibco, Cat # 11965118) supplemented with 7% FBS (Gemini Bio, Cat # 900-108), 1% L-glutamine (Gemini Bio, Cat # 400-106) and 1% Penicillin:Streptomycin (Gemini Bio, Cat # 400-109) on tissue culture treated 75 cm^2 flasks (Corning, Cat # 430641U). Cells were grown to confluency at 37°C supplemented with 5% CO_2, and were detached from the plate using Accutase (Gemini Bio, Cat # 400-158). HepG2 cells were counted using the Cell Size assay on the Moxi GO II instrument (Orflo, Cat # MXG102). Cells were diluted to a final concentration of $3x10^5$ cells/mL.

Sample Preparation

For sample preparation, 1 mL of HepG2 cells was added to a 2 mL Hard Tissue Homogenizing Mix tube (OMNI, Cat # 19-628). The HepG2 samples were homogenized on the Bead Ruptor Elite™ Bead Mill Homogenizer (OMNI, 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 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 (OMNI, Cat # 19-373) (OMNI, 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.

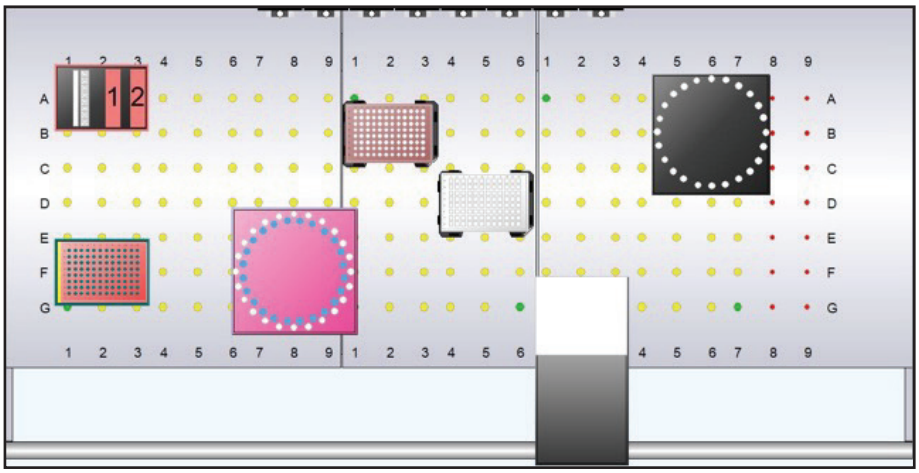

Figure 1. Example Winprep deck layout showcasing the 24 position 2 mL tube carriage (top right, black) and 48 position 2 mL tube carriage (bottom left, pink).

Nucleic Acid Extraction and Analysis

After centrifugation step, 100 µl of cell lysate was transferred to 12 separate wells of a 96-well plate (PerkinElmer, 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 (PerkinElmer, 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 HepG2 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

The outlined experiments using the chemagic™ Tissue DNA kit and Bead Ruptor Elite™ Bead Mill Homogenizer for sample preparation resulted in nucleic acid that is suitable for downstream PCR analysis. Eluted nucleic acid had an average yield of 3.9 µg. Amplified HepG2 DNA had an average Cq value of 16.16, revealing that eluted DNA is intact and compatible with downstream analysis (Table 1).

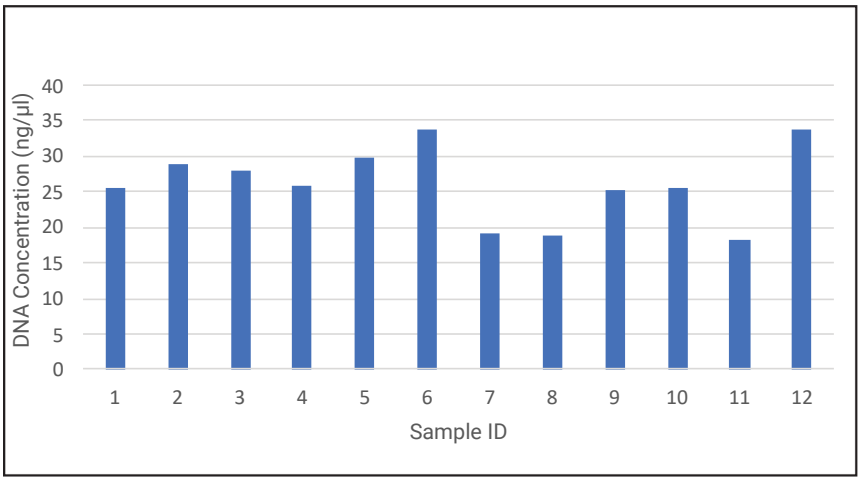

Figure 2. HepG2 DNA concentration from 12 samples. Lysate from samples 1-12 was prepared with Pro-K and RNase A. Average A_{260}/A_{280} from samples 1-12 was 2.21.

Sample	Cq
HepG2 1	16.05
HepG2 2	16.32
HepG2 3	15.08
HepG2 4	16.04
HepG2 5	15.28
HepG2 6	15.75
HepG2 7	16.35
HepG2 8	16.49
HepG2 9	16.11
HepG2 10	16.01
HepG2 11	16.02
HepG2 12	18.46
Positive Extract Control	22.01
Negative Control	39.07

Table 1. 18S qPCR Cq values from extracted HepG2 DNA.

Conclusions

In the enclosed application note, we have showcased proof-of-concept integration capabilities of the Bead Ruptor Elite™ Bead Mill Homogenizer and chemagic™ 360 automated nucleic acid extractor for isolation of DNA from *in-vitro* grown HepG2 cells. The Bead Ruptor Elite™ Bead Mill Homogenizer and 2 mL Hard Tissue Homogenizing Mix was demonstrated as a solution for sample preparation of *in-vitro* cultured cells, producing a homogenate suitable for downstream automated nucleic acid extraction. After a 70-minute extraction, eluted DNA was high-yield and intact, proving suitable for downstream analysis via qPCR. Additionally, the 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. Using either manual methods or custom-integrated JANUS® G3 liquid handling, homogenate transfer and extraction preparation is achieved.

Ordering Information

Equipment	Catalog Number
Bead Ruptor Elite™ Bead Mill Homogenizer	19-042E
Bead Ruptor Elite™ Bead Mill Homogenizer 2 mL Tube Carriage	19-373
Bead Ruptor Elite™ Bead Mill Homogenizer Finger Plate	19-370
Bead Ruptor Elite™ Bead Mill Homogenizer 48 position 2 mL Tube Carriage	19-378
Bead Ruptor Elite™ Bead Mill Homogenizer 48 position 2 mL Tube Carriage Finger Plate	19-370-248
Hard Tissue Homogenizing Mix 2.8 mm Ceramic Beads	19-628

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