APPLICATION NOTE

High yield DNA extraction protocol from bovine liver utilizing the Omni Bead Ruptor Elite bead mill homogenizer with automated extraction on the chemagic 360 nucleic acid extractor.

Summary

The processing of human and animal tissue samples for molecular analysis has become a cornerstone of research efforts across a multitude of disciplines. In particular, the processing of soft tissues, such as liver samples, is proving critical for everything from drug development research to anatomy research examining embryology and stem cell development. Nucleic acid extraction is one of the commonly performed processes employed when preparing these samples for downstream analysis, allowing researchers to produce high qualities and quantities of DNA and/or RNA from their samples.

A critical component of nucleic acid extraction has proven to be the sample preparation as the initial step of the workflow, wherein the tissue specimen is dissociated and cells are lysed to expose their genetic material. Though there are multiple methodologies to prepare tissue samples for nucleic acid extraction, one method that has proven to be highly reproducible for sample preparation is mechanical tissue lysis via bead mill homogenization. When utilizing mechanical homogenization of tissues, a consistent and uniform homogenate of liver samples can be produced in preparation for the next steps in processing. Utilizing the Omni Bead Ruptor Elite™ bead mill homogenizer, we demonstrate the consistent homogenate produced when processing bovine liver samples in preparation for nucleic acid extractions.

Omni Bead Ruptor Elite bead mill homogenizer

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

Equally important in the nucleic extraction workflow is reproducibility of high qualities and quantities of nucleic acid extractions. Automation has been demonstrated to dramatically improve the reproducibility of these workflows, reducing human error, and ensuring precise protocol replication from sample to sample. One technology currently in the field for the automation of nucleic acid extractions is the chemagic™ 360 nucleic acid extractor. Utilizing patented magnetic bead technology; the chemagic 360 instrument allows for a completely automated nucleic acid extraction process. The resultant purified nucleic acids are then ready for a variety of downstream activities such as PCR or next generation sequencing.

However, automated nucleic acid extraction protocols still require sample preparation prior to introduction of the sample into the workflow. The chemagic 360 suggests 10 mg of tissue or less to be added to their device which has been processed by either enzymatic or mechanical lysis. As denoted above, enzymatic digestion of tissues introduces additional reagents to the workflow which may have an impact on tissue quality and downstream analysis. The sample preparation step prior to automated extraction is a point in the workflow which can introduce human error or inconsistencies between samples, especially if utilizing chemical or enzymatic digestion reliant on the user to produce digested tissues for insertion into the chemagic 360 instrument. In an effort to produce consistent lysed tissue samples we propose the utility or mechanical lysis on the Omni Bead Ruptor Elite bead mill homogenizer. With the utility of automated extraction workflows dependent on full tissue lysis prior to nucleic acid extraction, it is critical to ensure a robustly reproducible process which provides consistent full lysis of tissue samples for downstream use.

Herein we demonstrate the utility of Omni Bead Ruptor Elite bead mill homogenizer in combination with the chemagic 360 instrument for a robustly reproducible workflow resulting in high qualities and quantities of DNA from bovine liver samples. This protocol prescribes a complete workflow from sample lysis through automated DNA extraction with a focus on complete sample homogenization and reproducibility in comparison to enzymatic lysis.

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)
- 2 mL Hard Tissue Homogenizing Mix 2.8 mm Ceramic Beads (Cat # 19-628)
- chemagic 360 (Cat # 2024-0020)
- chemagic DNA Tissue10 Kit H96 (Cat # CMG-723)

Procedure

Thirty two, 2 mL screw capped tubes prefilled with ceramic bead beating media (Cat # 19-628) were filled with 750 µL of chemagen tissue lysis buffer (Cat # CMG-805). Approximately 5 g of bovine liver was thawed following storage at -20 °C. This liver was then partitioned into 100 g (+/- 1 g) samples via manual scalpel dissection. Each sample was then loaded into a previously prepared 2 mL tube for processing. Once each sample was loaded into their respective tubes, 18 µL of Proteinase K (Cat # CMG-723) and 7.5 µL of RNase A (Thermo Fisher, Cat # EN0531) were added.

All tubes were then processed using the Omni Bead Ruptor Elite bead mill homogenizer at a speed of 5 m/s for 30 s. After processing, the tubes were centrifuged at 9000 rpm for 10 minutes. Following centrifugation all tubes were incubated at 37 °C for 30 minutes.

After incubation 200 µL of each sample was loaded into a Revvity 2 mL Riplate (Cat # CMG555-15) in triplicate. This plate was then loaded into a chemagic 360, and the "standard tissue" protocol was run using the chemagic DNA Tissue10 H96 kit (Cat # CMG-723). After completing the protocol, the resulting DNA from each processed sample in the plate was then quantified via a Nanodrop 2000 (Thermo-Scientific, Cat #ND2000CLAPTOP). The samples were evaluated for quantity and quality by recording total yield and A_{260}/A_{280} ratio for evaluation of the extracted DNA.

This process was completed on three separate days and the results were then statistically compared to one another via a 2 tailed paired t-test in order to evaluate reproducibility.

Results

Nucleic acid concentration from the three trails were averaged and recorded on the Nanodrop demonstrating concentrations of 102.86, 101.81, and 100.68 ng/µL, respectively. Standard deviation was then determined across the entire population and a relative standard deviation (RSD) value was derived from those values for each day of testing, resulting in RSDs of 10, 14 and 12 % respectively. The average values of each run were then analyzed using a two tailed paired t-test. The t-test showed no significant difference between trials 1 vs 2, 1 vs 3, and 2 vs 3 with p values of 0.7, 0.4, and 0.7.

The average A_{260}/A_{280} ratio of all samples determined by the nano drop was found to be 1.78. This is around the recommended value for high quality, or "pure", DNA according to Thermo-Scientific protocol. High quality DNA such as this will aid in producing more accurate results in downstream applications.

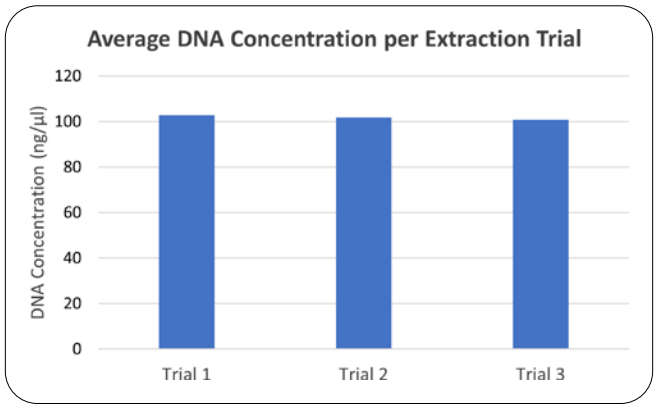

Figure 1: Average nucleic acid concentration per trial as shown in ng/µL. Each trial had the complete concentrations averaged and statistically evaluated as discussed in the methods section.

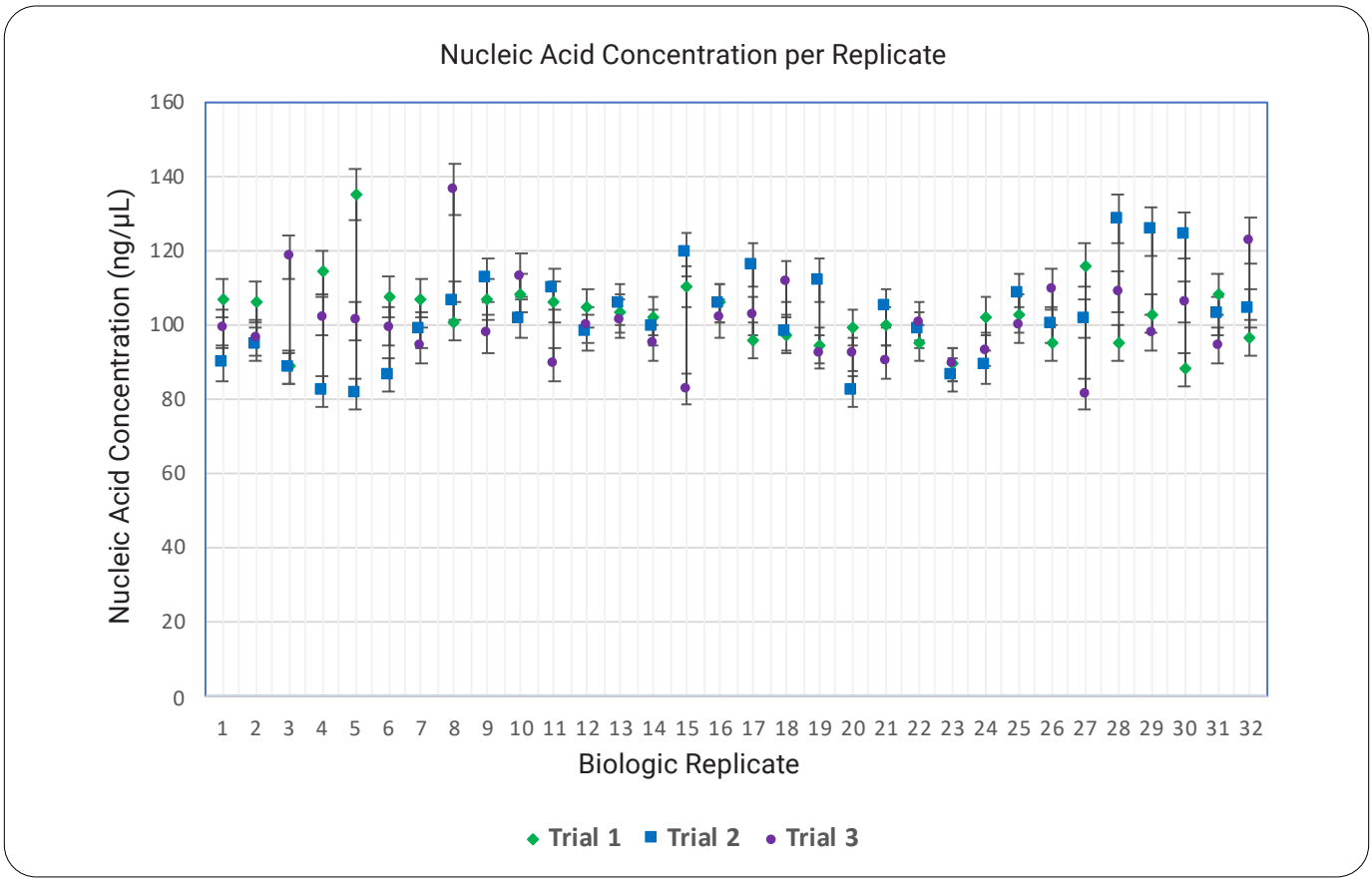

Figure 2: Distribution nucleic acid concentrations per replicate. The green diamonds represent the obtained nucleic acid concentrations in trial 1, the blue squares represent the nucleic acid concentrations in trial 2, and the purple circles represent the nucleic acid concentrations in trial 3. All concentrations were run in triplicate, allowing for addition of the error bars in this figure to show how close the agreeability of the three technical replicates in each trial run.

Conclusion

Herein, no significant difference ($p < 0.05$) was found between the three runs of the proposed extraction workflow when obtaining purified DNA from frozen bovine liver samples. Based upon this data we show the combination of the Omni Bead Ruptor Elite bead mill homogneizer and chemagic 360 to be a highly reproducible workflow for nucleic acid extractions in preparation for downstream genomic applications. Furthermore, we show how integrating the Omni Bead Ruptor Elite bead mill homogenizer into the chemagic 360 workflow allows for much larger samples to be processed into high quality DNA. This will save time prepping samples, allow for a larger variety of samples to be processed and ensure the quality of extracted nucleic acids.

Authors

Madelyn M Carlson
Caleb M Proctor, M.S.
Zachary P Morehouse, D.O., M.S.
Rodney J Nash, Ph.D.

Revvity, Inc.

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

For a complete listing of our global offices, visit www.revvity.com