spex® sampleprep

App Note

Comparison of the Effectiveness and Recovery of DNA Extraction Methods: Manual Homogenization vs the Spex Genomax® Homogenizer

The ability to effectively homogenize biological tissues and disrupt cells is essential to produce sufficient quality and quantity of nucleic acids for DNA and RNA studies. In addition, many samples require homogenization or particle size reduction to create representative samples usually due to their overall heterogeneous nature or state as received by a laboratory. Homogeneity is the state of being of uniform composition throughout the sample, whereas heterogeneity lacks uniformity in one or more characteristics.

Laboratory or analytical samples must be processed into a form which allows for extraction, processing or digestion for further testing. In biological samples, homogenization can aid in the speed of processing by disrupting the biological tissue and lysing the cells, thereby allowing for the extraction of genetic material. The most common method for obtaining a homogeneous sample is grinding or comminution, which leads to an increase in accuracy and decrease in uncertainty.

In the laboratory, some scientists use manual or semi-automated methods for tissue processing which can be time consuming and produce samples that are not thoroughly homogenized. The implementation of automated homogenization and processing allows for higher throughput of samples, as well as faster and improved recovery of DNA/RNA from biological materials.

This study will demonstrate the efficiency of using an automated homogenization method as a function of time savings, as well as an improvement in the recovery and extraction of genetic material. In previous studies using the Spex Geno/Grinder®, pesticide extraction of agricultural and environmental products was found to greatly increase recovery, while the processing time for sample preparation was significantly reduced.

In this new study (a joint project conducted by Spex and HoppeSyler), a manual pellet mixer and automated homogenizer (Spex Genomax) were used to disrupt a variety of biological tissues prior to genomic DNA (gDNA) isolation. The isolated gDNA was then used for downstream sensitive applications to determine the effectiveness of manual versus automated homogenizers for use in analytical and molecular biology research.

Methods and Materials

The workflow focused on three processes: 1. tissue homogenization; 2: DNA extraction; and 3. DNA isolation.

Tissue Homogenization

Sixty milligrams of fresh tissue were added to 500 µL of the DNA binding buffer and 20 µL of proteinase K (20 mg/mL) from the Spex DNAmax Animal Genomic DNA Isolation kit. The homogenization and processing of tissues was performed as per manufacturer protocol. The samples were then incubated for 15 minutes at 55 ºC with gentle shaking at 700 rpm. After the proteinase K digestion, 20 µL of RNase A was added to the lysate and the sample was incubated at room temperature for five minutes before 200 µL of > 95% ethanol was added. The lysate was then mixed vigorously via a vortexer for five seconds.

DNA Extraction and Column Wash

After vortexing, 700 µL of the lysate was loaded onto the silica spin column provided with the DNAmax kit and centrifuged at 13,000 x g for one minute at room temperature. The flow-through was discarded and 500 µL of wash buffer A (with ethanol added) from the kit was added to the column and again centrifuged for three minutes. The resulting flow-through was discarded.

DNA Isolation and Elution

The column was centrifuged for an additional one minute and the collection tube was discarded. To the column, 100 µL of EB or TE buffer (pH 8.0) was added and allowed to sit for three minutes at room temperature before centrifuging again for one minute. The flow-through containing purified gDNA was collected for analysis and qPCR.

Manual versus Genomax: Nucleic Acid Isolation and Recovery

In the first study to compare manual versus automated grinding, samples of various animal tissue (beef loin, chicken breast and mouse heart) were processed to homogenize, extract and isolate gDNA. Samples processed using manual homogenization showed, on average, processing times over 200 minutes (close to 2 ½ hours) compared to less than ½-hour processing time using the Spex Genomax (Figure 1). Processing time is decreased by over 86% using the automated Genomax methods.

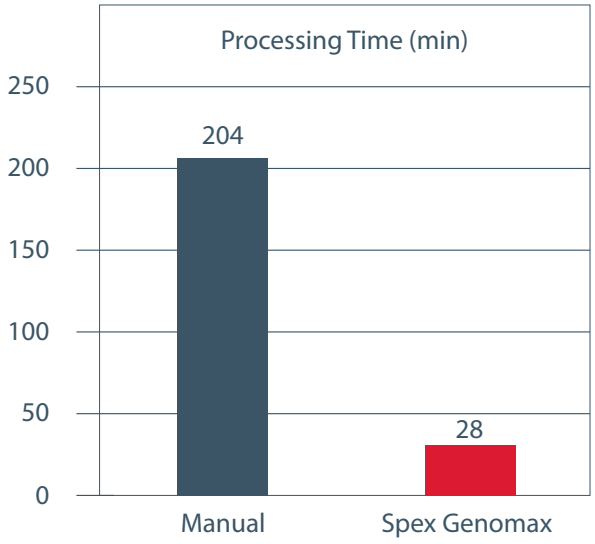

Figure 1. Processing time for gDNA homogenization,
extraction and isolation with manual and Genomax processing.

Three type of animal tissues (beef loin, chicken breast and mouse heart) were compared for the quality and quantity of genetic material recovered. Beef loin and chicken breast represent different density animal muscle tissues. Mouse heart represents fibrous animal organ tissue, which is tough to homogenize. Traditionally, dense and fibrous tissues are the most difficult to homogenize and produce the least amounts of recoverable gDNA. After processing using manual methods and the Genomax, gDNA was isolated and tested for concentration using UV/VIS spectroscopy (Figure 2).

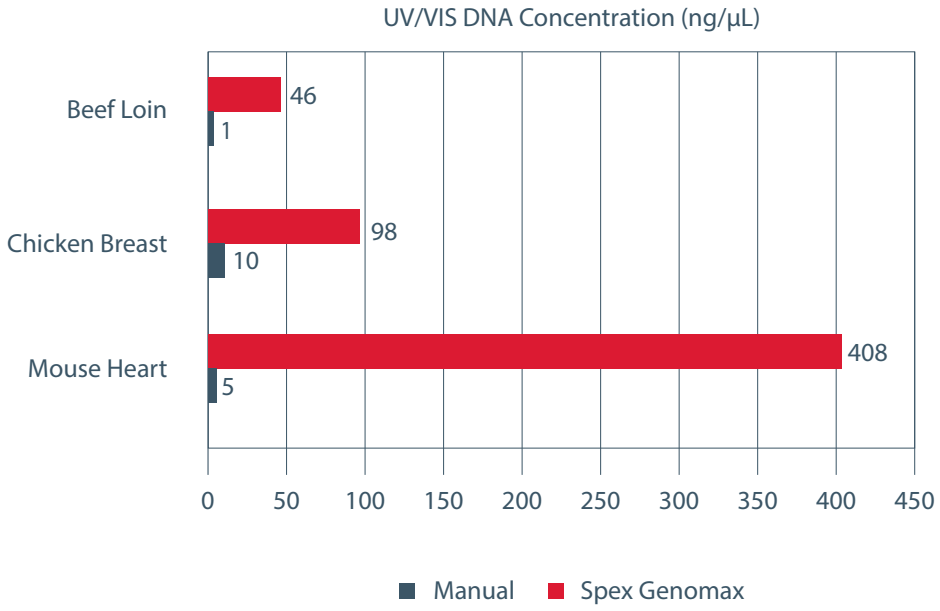

Figure 2. DNA recovery in various animal tissue using manual and Genomax methods.

Manual methods often produced incomplete extraction and isolation of DNA, while automated homogenization with the Genomax produced complete homogenization and efficient extractions as seen in the electrophoresis gels (Figure 3).

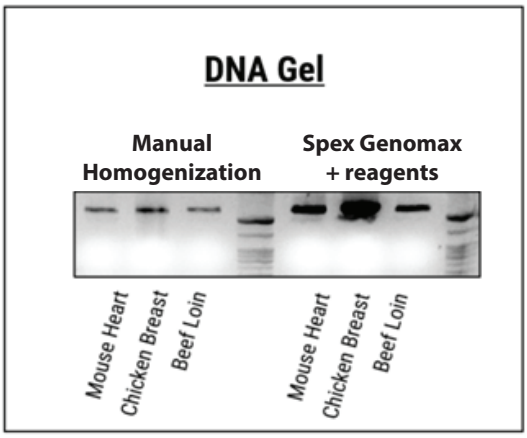

Figure 3. Gel electrophoresis of DNA processed using manual versus automated homogenization using the Genomax.

In a follow-up to this first study, 30 mg of mouse heart was homogenized using a commercial manual pellet mixer versus the Spex Genomax homogenizer. The mouse heart was homogenized using the manual homogenizer according to the manufacturer's recommendations, while the tissue in the Genomax was homogenized with three cycles of homogenization at 1,500 rpm for 1 minute per cycle, with a 20-second pause between cycles. The DNA was isolated with the Spex DNAmax kits and prepared for qPCR analysis using primers encompassing the mouse *Ostβ* promoter or *Hic1* gene body. Each analysis was performed in duplicate. There was found to be a 35-fold increase in yield from the Genomax compared to the manual homogenization. In fact, the Genomax samples produced strong curves at each sample size when using 0.25 µL to 1 µL of the isolated gDNA, whereas the gDNA isolated using the manual homogenizer generated amplification curves with 1 µL (Figure 4).

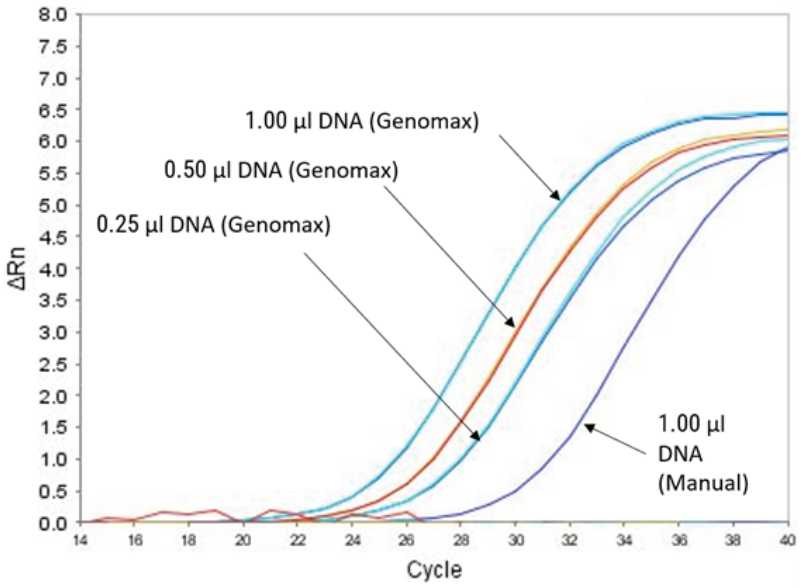

Figure 4. Replication of mouse organic solute transport β promoter. Chromosome 9, 65330248 – 65330323 (75 base pairs) with Genomax and manual processing.

The amplification of target genes when using smaller volumes of gDNA (0.25 µL and 0.5 µL) proved successful with the Genomax, whereas gDNA isolated via manual processing failed to produce any signal (Figure 5).

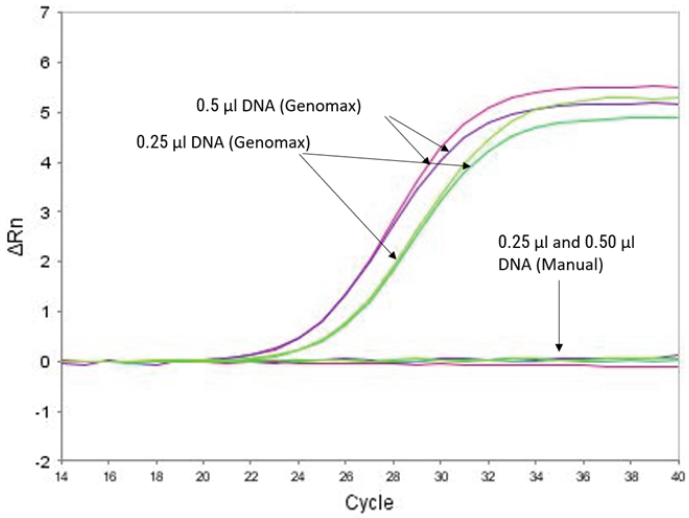

Figure 5. Manual versus Genomax replication of low sample volume of mouse hypermethylated in cancer 1 gene body. Chromosome 11, 75058091 – 75058200 (109 base pairs).

Conclusions

The automation of homogenization and cell lysis increases the efficiency of the process in both time and resulting gDNA extraction. The quality, quantity and replication of the target gDNA is significantly superior with automated processes such as the Genomax, especially when combined with the Spex DNAmax extraction kits. Overall, the time to process the samples is greatly reduced to less than half an hour using the Genomax and yields more replicable copies of genetic material at lower sample volumes.

spex.com

Phone: +1.732.549.7144 • +1.800.LAB.SPEX
Fax: +1.732.603.9647
spexsales@antylia.com

Connect with us