APPLICATION NOTE #20146

Drosophila melanogaster Protein and DNA Extractions Using the Bead Ruptor Elite

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Drosophila melanogaster is a commonly used organism in many research studies. A reliable method for protein and nucleic acid extraction is a critical step toward understanding the molecular processes that occur in *Drosophila*. This study demonstrates a fast and efficient method of extracting proteins and DNA from *Drosophila* using the Bead Ruptor Elite bead mill homogenizer.

Materials and Methods

For protein extractions, 30 mg to 50 mg of whole *Drosophila melanogaster* were placed into 2ml polypropylene screw cap tubes containing 1.4 mm ceramic beads (Cat #19-627) along with one milliliter of 50 mM Tris-HCl, pH 7.6. The samples were placed in a 2 mL tube carriage (Cat #19-010) and disrupted in the Bead Ruptor Elite for 30 seconds at 6.45 m/s. One milliliter of the homogenate was placed in a fresh 1.5ml microtube and centrifuged at 12,000 rpm for 10 minutes. The supernatant was removed and placed into clean 1.5ml microtubes. 10 µL of each protein extract was mixed with 10 µL of Laemmli sample buffer and incubated at 90°C for 5 min. Proteins were then separated by electrophoresis on a 4-20% Tris Glycine SDS PAGE gel at 200 V for 30 minutes. Protein concentrations were analyzed at 280 nm using a Nanodrop spectrophotometer.

For DNA extractions, 30 mg of whole *Drosophila melanogaster* were placed into 2 ml reinforced tubes with 1.4 mm ceramic beads. 220 µL of TL buffer from the Omega E.Z.N.A. Tissue DNA Kit was added to the tube and samples were disrupted in the Bead Ruptor Elite at 6 m/s for 30 seconds. The entire homogenate was transferred to a clean 1.5 ml microtube and the Omega E.Z.N.A Tissue DNA Kit protocol was followed for the reaming of the procedure. 25 µL of OB Protease Solution was added to the homogenate and vortexed thoroughly. Samples were incubated at 37°C for one hour. Samples were then centrifuged at 12,000 rpm for five minutes. The resulting supernatants were transferred to clean 1.5 mL microtubes and 220 µL of BL buffer was added to each sample and incubated at 70°C for ten minutes. 220 µL of 100% ethanol was then added and samples were transferred to a HiBind DNA Mini Column. Samples were centrifuged at 12,000 rpm for one minute. The filtrate was discarded and 500 µL of HBC buffer was added to the binding columns and centrifuged at 12,000 rpm for one minute. The DNA bound on the column was washed twice with 700 µL of DNA Wash Buffer and centrifuged for one minute. DNA was eluted in 50 µL of DDH2O water. 2 µL of diluted genomic *Drosophila* DNA was mixed with 2 µL of Tris/Borate/EDTA (TBE)/Urea sample buffer and separated by electrophoresis on a 5% TBE precast gel at 200 V for 30 minutes. DNA concentrations were analyzed at 260nm using a Nanodrop spectrophotometer.

Results

Homogenization of *Drosophila melanogaster* can be difficult and time consuming. The use of bead mill homogenization significantly decreases the effort and time needed for homogenization. The Bead Ruptor Elite's versatility allows it to homogenize samples ranging from the softest tissues such as liver to some of the toughest tissues such as skin. With a maximum throughput of twenty four samples per run, the Bead Ruptor Elite is amenable to studies involving high sample numbers and which require a high level of reproducibility.

Herein, we examined the extraction efficiency of the Bead Ruptor Elite for homogenization of *Drosophila melanogaster*. Both DNA and protein extractions were performed. The average protein concentration from a 30 mg sample and 50 mg sample of *Drosophila* was 16.7 mg/ml and 18.1 mg/ml respectively. The average DNA concentration from a 30 mg sample of *Drosophila* was 344 ng/µL. A_{260}/A_{280} values indicated high DNA purity and electrophoresis analysis revealed a high degree of genomic DNA integrity with little DNA shearing (Figure 1). Protein extraction efficiency was further evaluated by gel electrophoresis in four replicates. Lane to lane variation was minimal and abundant proteins were observed over a high molecular weight range.

Conclusion

The Bead Ruptor Elite's effectiveness for homogenizing small organisms such as *Drosophila melonogaster* was demonstrated. Complete homogenization was achieved in thirty seconds with high analyte yields.

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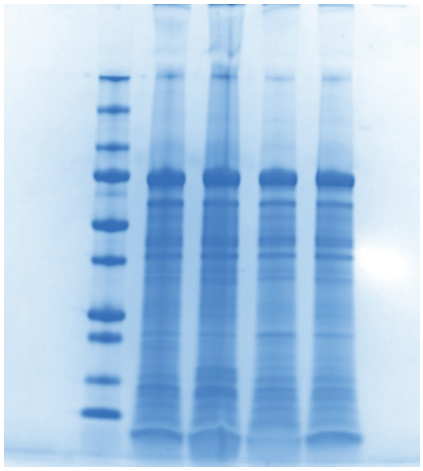

Figure 1 *Drosophila* protein extracts analyzed by SDS PAGE. Lane 1: Protein ladder. Lane 2-3: 30 mg of *Drosophila* homogenate. Lane 4-5: 50 mg of *Drosophila* homogenate

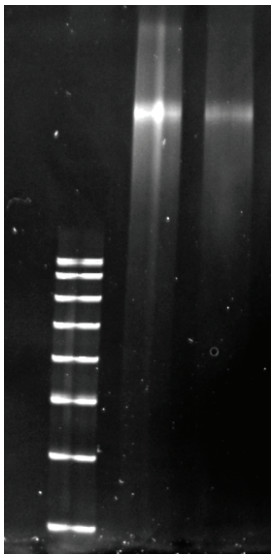

Figure 2 *Drosophila* genomic DNA analyzed by 5% polyacrylamide gel. Lane 1: 100bp DNA ladder. Lane 2-3: DNA from 30 mg of *Drosophila* homogenate

Omni Bead Ruptor Elite

Part Numbers Referenced **Bead Ruptor 24**

Motor Unit: 19-040E

Bead Ruptor – 2 mL Carriage Kit: 19-010-310

2 mL Pre-Filled Hard Tissue Homogenizing Mix: 19-627

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