Northwestern Review

Volume 1 | Issue 1 Article 12

2016

Analysis of Alternative Storage Conditions for DNA Recovery from Field Samples

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Abstract

As ecologists increasingly employ molecular methods, they find that tried and true preservation solutions (e.g. ethanol or formalin) may not be optimal when samples are targeted for genetic analyses. Before traveling to remote sample sites, researchers need to consider which preservation methods are likely to yield the largest quantity and highest quality of DNA based on their travel times and field conditions. They also need to consider whether they will have access to preservatives at remote sites and whether those preservatives can be safely transported. To determine which preservation methods would most reliably preserve tissue for genetic analysis under a range of field conditions, we examined total DNA recovery from female fruit flies (Drosophila melanogaster) individually held in various solutions (70% ethanol; 2% SDS, 100 mM EDTA; 1% SDS, 50 mM EDTA; 0.66% SDS, 33 mM EDTA; Zymo© lysis buffer; Zymo Xpedition© lysis buffer) at three different temperatures (22oC, 4oC and -20oC) for varying lengths of time (1 day, 4 weeks, and 8 weeks). We predicted that insects held in Zymo Xpedition© buffer would yield the overall highest DNA recovery since this buffer was designed for field collected animal tissue. We also predicted that variation in DNA recovery from insects held in different solutions would increase with preservation time and holding temperature. Although we observed significant differences in total DNA recovery from some of our samples, no trends were identified. Preliminary band quality analyses of PCR products utilizing stored DNA as template for amplification of the mCOI gene generally indicated decline in product quality as storage time increased. Future work will focus on better quantifying stored DNA quality and examining the relationship between total DNA recovered and overall DNA quality.

About the Author

Authors Alison Schutt, Emily Stricklin, Britta Ten Haken, and Joseph Tolsma are currently students at Northwestern College.

An environmental scientist, Dr. Furlong holds a doctorate in ecology, evolution and marine biology. Her research and publications have been in the fields of stream ecology, entomology and biogeography, and she has experience as a biological consultant. Furlong teaches Introduction to Environmental Science, General Biology, Invertebrate Zoology and Aquatic Ecology. She has also been a stream ecology instructor for the Creation Care Study Program in Belize.

Dr. Tolsma's research efforts in cancer genetics and cell biology have been widely published in scientific journals and have received a number of awards. A Northwestern College alumna, she holds a doctorate in microbiology/immunology/virology from Northwestern University. During a sabbatical, she worked on a cell and molecular genetics textbook for non-science majors, as well as an adult Sunday school curriculum on genetic technologies. She has been a part of several symposia on bioethical issues surrounding genetic technologies, such as stem cells, cloning and genetic testing. Her current laboratory research extends her interest in genetics to populations in a study of mayfly genetic variation in Northwest Iowa watersheds and her interest in cancer cell biology with a project that measures the anti-proliferative effects of plant extracts and chemicals in those extracts on human tumor cells in vitro. She is the 2015 recipient of Northwestern's annual Teaching Excellence Award.

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Abstract

As ecologists increasingly employ molecular methods, they find that tried and true preservation solutions (e.g. ethanol or formalin) may not be optimal when samples are targeted for genetic analyses. Before traveling to remote sample sites, researchers need to consider which preservation methods are likely to yield the largest quantity and highest quality of DNA based on their travel times and field conditions. They also need to consider whether they will have access to preservatives at remote sites and whether those preservatives can be safely transported. To determine which preservation methods would most reliably preserve tissue for genetic analysis under a range of field conditions, we examined total DNA recovery from female fruit flies (*Drosophila melanogaster*) individually held in various solutions (70% ethanol; 2% SDS, 100 mM EDTA; 1% SDS, 50 mM EDTA; 0.66% SDS, 33 mM EDTA; Zymo© lysis buffer; Zymo Xpedition© lysis buffer) at three different temperatures (22ºC, 4ºC and -20ºC) for varying lengths of time (1 day, 4 weeks, and 8 weeks). We predicted that insects held in Zymo Xpedition© buffer would yield the overall highest DNA recovery since this buffer was designed for field collected animal tissue. We also predicted that variation in DNA recovery from insects held in different solutions would increase with preservation time and holding temperature. Although we observed significant differences in total DNA recovery from some of our samples, no trends were identified. Preliminary band quality analyses of PCR products utilizing stored DNA as template for amplification of the mCOI gene generally indicated decline in product quality as storage time increased. Future work will focus on better quantifying stored DNA quality and examining the relationship between total DNA recovered and overall DNA quality.

Introduction

With molecular biological techniques rapidly becoming a standard component of ecological studies, scientists are rethinking strategies for specimen preservation in the field. Traditionally, it was most important to use a preservative solution that maintained structural characteristics but, if molecular biological analysis is the goal, structure is less important than choosing a preservative solution that maintains the integrity of specimen DNA. Ethanol solutions (typically 70%) and formaldehyde preserve specimen structural integrity and also may protect the integrity of DNA but are difficult to obtain at remote sites and can be problematic to transport due to their flammability and toxicity. Pokluda et al. (2014) proposed alternative preservative solutions containing various concentrations of sodium dodecyl sulfate (SDS) and ethylenediaminetetraacetic (EDTA) acid. Their paper provided intriguing evidence that these solutions may be as effective at preserving insect DNA as 70% ethanol, however their experimental design did not allow quantitative evaluation of DNA yield. To obtain clearer evidence that alternative preservative solutions were as or more effective than ethanol at maintaining DNA integrity in insect specimens, we designed a quantitative experiment. We reared genetically identical (wild type), female *Drosophila melanogaster* as a uniform organism and placed those organisms in six different solutions (70% ethanol; 2% SDS, 100 mM EDTA; 1% SDS, 50 mM EDTA; 0.66% SDS, 33 mM EDTA; Zymo© lysis buffer; Zymo Xpedition© lysis buffer), at three different temperatures (22ºC, 4ºC and -20ºC) , for three different times (1 day, 4 weeks, and 8 weeks). After isolating DNA we measured the amount of DNA recovered using a Nanodrop 2000 © Spectrophotometer. We also measured the quality of the DNA by determining if it was amplifiable by PCR and assessing band quality of the amplified PCR product.

Materials and Methods

Specimen Selection and Storage

Wild-type *Drosophila melanogaster* were offspring of those purchased from Carolina Biologicals (Burlington, NC). Flies were anesthetized with FlyNap (Carolina Biologicals, Burlington, NC), female flies were selected, and placed in microfuge tubes containing 70% ethanol, 2% SDS 100 mM EDTA, 1% SDS 50 mM EDTA, 0.66% SDS 33 mM EDTA, Zymo Xpedition© Lysis/Stabilization Buffer (Zymo Research, Irvine, CA), or Lysis Buffer (ZR Tissue and Insect DNA Prep, Zymo Research, Irvine, CA). Specimens, in solution, were placed at -20ºC, 4ºC, or 22ºC for 24 hours, 4 weeks, or 8 weeks.

DNA Isolation

After storage for the allotted time, DNA was isolated from the *Drosophila melanogaster* specimens using ZR Tissue and Insect DNA MiniPrep (Zymo Research, Irvine, CA) for all specimens except those in Xpedition Lysis/Stabilization Buffer. DNA from specimens in Xpedition Buffer was isolated using Xpedition Tissue and Insect MiniPrep (Zymo Research, Irvine, CA). Purified DNA was quantitated by measuring the 260/280 wavelength ratio with a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, Grand Island, NY). Significant differences in total DNA amounts from samples held under varied storage conditions were determined by one-way ANOVAs and significant subsets with the Tukey HSD Post Hoc Test.

Amplification by PCR

A 710-bp fragment of the mCOI gene was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAGATATTGG-3') and HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer, et al, 1994). PCR conditions used were 60 ng of DNA, 2.5 uM $MgCl_2$, 0.5 mM dNTP mix (Promega, Madison, WI), 1x Go-Taq buffer, 1 µL Go-Taq polymerase (Promega, Madison, WI) in a total volume of 50 µl. Reactions were amplified in 35 cycles at the following temperatures: 1 minute at 95ºC, 1 minute at 40ºC, 1.5 minutes at 72ºC with a final extension of 10 minutes at 72ºC followed by holding the samples at 4ºC. PCR products were visualized by loading 20 µl of the PCR reaction on 1% agarose gels run in TAE buffer at 100 mV. Bands were imaged using a Bio-Rad Chemidoc MP Imager (Bio-Rad, Hercules, CA).

Estimation of DNA Quality via Band Analysis

60 ng of template DNA was used in each PCR reaction. DNA quality was estimated by analyzing the quantity of PCR product produced. Five different individuals ranked band intensity relative to the molecular weight standards from 0 (no band) to 3 (more intense than molecular weight standards). Differences in band quality at different storage times (1 day vs. 4 weeks) were statistically assessed with two-tailed Ttests.

Results

These preliminary results are a work-in-progress. We isolated DNA from a uniform population of lab-reared female, wild-type *Drosophila melanogaster* held in six different storage solutions at three different temperatures for three lengths of time. For each treatment we extracted DNA from 5 flies. Total DNA collected varied considerably, ranging from 10-2505 ng. Although the range was large, mean differences in total DNA were rarely significant (Figures 1 and 2). We successfully amplified a 710-bp fragment of the mCOI gene from the majority of our 24 hour and 4 week DNA samples. Using the brightness of the PCR products on a gel as an indicator of DNA quality (Figures 3 and 4), we observed an overall decline in DNA quality with greater storage time for most treatments (Figure 3). Regardless of storage conditions our DNA template produced a PCR product with an intensity that suggests it is could be sequenced more than 50% of the time. Template isolated from specimens stored in various solutions, regardless of time or temperature, produced quality PCR products between 63 and 75% of the time. When considering only temperature, samples stored at -20ºC resulted in template DNA that produced sequence-quality bands 80% of the time. That percentage dropped to 66% at 4ºC and 53% at 20ºC. Samples stored for only 1 day resulted in PCR products that were sequence-quality 79% of the time while extending storage to 4 weeks reduced that to 57%.

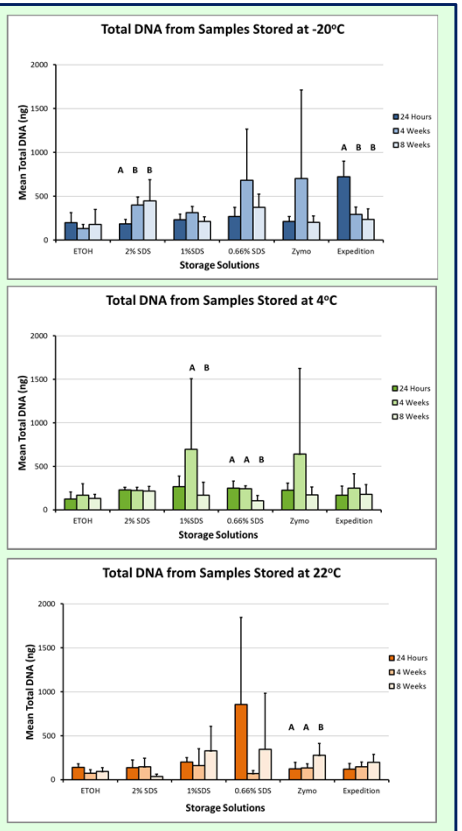

Figure 1. Mean total DNA isolated from *Drosophila melanogaster* (n=5) stored at three different temperatures, for three different lengths of time, in six different solutions (ETOH = 70% Ethanol; 2% SDS = 2% SDS, 100 mM EDTA; 1% SDS = 1% SDS, 50 mM EDTA; 0.66% SDS = 0.66% SDS, 33 mM EDTA; Zymo = Zymo© lysis buffer, Expedition = Zymo Xpedition© lysis buffer). Within each solution type, significantly different means are indicated by different letters (ANOVA p<0.05, Tukey HSD p<0.05). Standard deviations are represented by error bars.

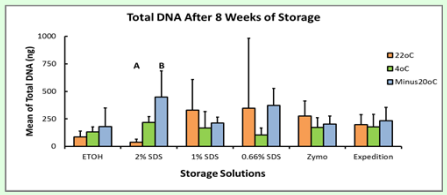

Figure 2. Mean of total DNA after 8 weeks of storage at 3 temperatures in different storage solutions (ETOH = 70% Ethanol; 2% SDS = 2% SDS, 100 mM EDTA; 1% SDS = 1% SDS, 50 mM EDTA; 0.66% SDS = 0.66% SDS, 33 mM EDTA; Zymo = Zymo© lysis buffer, Expedition = Zymo Xpedition© lysis buffer). Total DNA differed significantly in 2%SDS 50mM EDTA (ANOVA p<0.05). The significantly samples are indicated by different letters (Tukey HSD p<0.05). Standard deviations are represented by error bars.

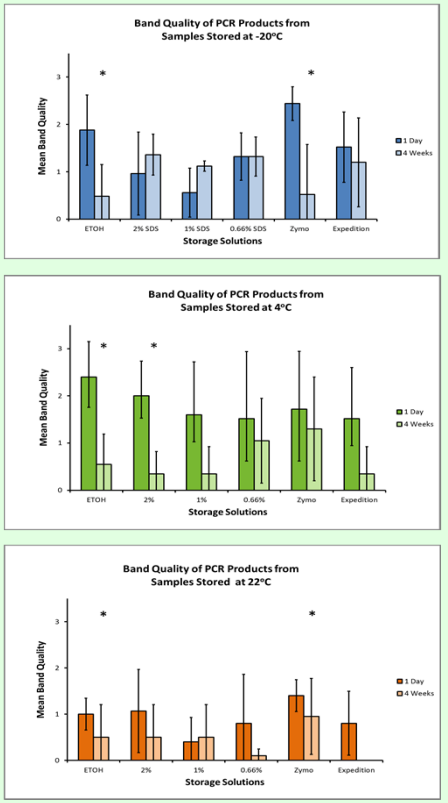

Figure 3. Mean band quality of PCR products stored in various solutions generally declined with increasing storage time (1 day vs. 4 weeks), however differences were rarely significant. Significantly different means are indicated with asterisks (Ttest p< 0.05). Standard deviations are indicated by error bars. Storage solutions: ETOH = 70% Ethanol; 2% = 2% SDS, 100 mM EDTA; 1% = 1% SDS, 50 mM EDTA; 0.66% = 0.66% SDS, 33 mM EDTA; Zymo = Zymo© lysis buffer, Expedition = Zymo Xpedition© lysis buffer.

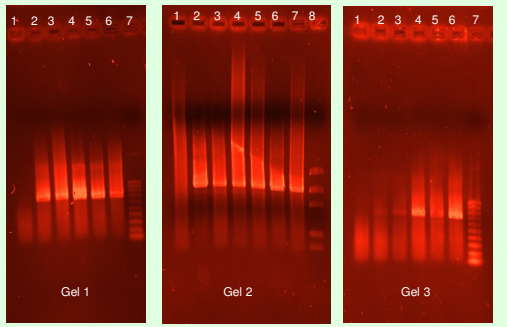

Figure 4. Representative PCR products amplified from *Drosophila melanogaster* DNA isolated after specimens were stored under various conditions. Gel 1: Samples stored in Zymo lysis buffer at -20ºC for 24 hours. Lane 1: no DNA control; Lanes 2-6: replicate samples. Gel 2: Samples stored at 4ºC for 24 hours. Lane 1: no DNA control; Lane 2: 70% ethanol; Lane 3: 2%SDS, 100 mM EDTA; Lane 4: 1% SDS, 50 mM EDTA; Lane 5: 0.66% SDS, 33 mM EDTA; Lane 6: Zymo Expedition Buffer; Lane 7: Zymo lysis buffer. Gel 3: Samples stored in Zymo Xpedition Buffer at -20ºC for 24 hours. Lane 1: no DNA control. Lanes 2-6: replicate samples. Lanes 7, 8, and 7 represent MW standards.

Discussion

Our purpose in tackling this project was very practical. We were having problems finding ethanol in remote locations and transporting samples preserved in ethanol. We were asking if there were more portable preservation options and that might yield better results in our genetic analyses. After reading Pokula et al. (2014), we were eager to try some of their suggested alternatives. In addition, we were curious about buffers offered by Zymo©. However, if these alternatives are "good," we wanted to quantify "how good" before going out into the field again. Using replicates from a very uniform population (lab-reared, female, wild-type *Drosophila melanogaster*), we hoped to eliminate some of the variability inherent in "wild caught" insects. By comparing and quantifying total DNA and quality of DNA yield from specimens held in various storage solutions and under a variety of storage conditions, we hoped to find the best options for our own field collections.

We predicted that, regardless of the preservation solution, the quantity and quality of DNA recovered from our samples would decline with increasing storage time and increasing temperature. In addition, we assumed that the Zymo© Xpedition lysis buffer would give the best results overall since it was advertised as specially formulated for field preservation under a wide range of conditions.

The DNA recovered from our experiment was remarkably variable in quantity (Figures 1 and 2) and although some trends are evident, very few significant differences in treatment were observed. We saw evidence that ethanol preservation gives mediocre DNA recovery under a variety of conditions, but so do the other preservation fluids. In particular, we noted that the Zymo© Xpedition lysis buffer did not provide superior results. We also noticed that, while increasing storage time may result in decreased yield, this was not always the case (Figure 1). Likewise, lower storage temperature tended to give higher, but rarely significantly higher, yields (Figure2). We believe that variation in sample processing (tissue bashing) may mask the treatment effects.

Since the goal of our own research is the recovery of high quality DNA for amplification and sequencing, we found the results of the band analyses particularly interesting (Figure 3) and plan to expand this part of our study to include eight week samples. Furthermore, our DNA quality assessment is somewhat subjective and we plan to quantify it more precisely. We will measure the concentration of PCR products amplified from the same amount of template DNA (60 ng) with the NanoDrop 2000 Spectrophotometer and quantify the bands amplified using densitometry measurements of bands with the Biorad ChemiDoc MP Imager. This will allow us to more fully explore the relationship between quantity and quality of DNA isolated from specimens held in under various conditions.

Additionally, we wonder if our highly variable DNA quantities were related to the extraction procedure rather than the preservation of our specimens. We experimented with various extraction procedures including bashing specimens with Zymo© bashing beads by hand, using a Disrupter Genie©, vortexing by hand, and shaking bashing bead tubes in a rack. Visual examination of the specimens after different disruption methods suggested that this step was highly variable. We are working on increasing the consistency of this step in the DNA isolation process before we repeat this experiment on more variable populations of insects.

As our field season approaches, we know that we need to keep our specimens cold and process them in a timely manner for best results. However, we also now know that a few days at room temperature will not doom our efforts and that we can confidently use inexpensive and portable alternatives to ethanol.

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Acknowledgements:
Funding for this project was provided by Northwestern College Scholarship Grants (Tolsma) and a Northwestern College Endowed Research Fellowship (Furlong). We are grateful to Steve Bogaard and the Biology and Chemistry departments at Dordt College for allowing us to use their NanoDrop 2000 Spectrophotometer. The 2015 BIO 202 class provided skilled hands during a particularly busy DNA isolation day.