COLLECTING AND PRESERVING GENETIC MATERIAL FOR HERPETOLOGICAL RESEARCH

Tony Gamble

Society for the Study of Amphibians and Reptiles
Tony Gamble

Herpetological Research

For

Genetic Material

Collecting and Preserving
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**Box 2:** Things to Avoid When Studying Genomic Material

**Box 3:** Protocol for Non-Fossil Specimen of DNA

**Box 4:** Sampling Tissue for Cell Culture

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Single copies of this volume are available from the Society's Secretary.

John M. Moron, Director

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ACKNOWLEDGMENTS

Many people have provided me with insights into science, especially my parents.

I thank John Mortimer for the idea for this book and for making the philosophizing

COLLECTING AND PRESERVING GENETIC MATERIAL
Box 1. Why Collect Genetic Material?

Can I get DNA from this preserved museum specimen?
CHAPTER 3: METHODS FOR COLLECTING AND PRESERVING GENETIC MATERIAL

SSAR HEPATOPATHOLOGICAL CIRCULAR NO. 41

COLLECTING AND PRESERVING GENETIC MATERIAL

To ensure the highest quality of genetic material, specimens should be collected promptly, handled properly, and stored appropriately. Proper collection techniques are essential to maintain the integrity of the genetic material. It is important to select the appropriate tissue or fluid for collection based on the specific research requirements. Commonly used collection methods include blood samples, tissue biopsies, and whole-body samples. Each method has its own advantages and limitations, and careful consideration should be given to ensure the best outcome for downstream analyses.

Histological sections can provide valuable information about the genetic material and its interactions with the host. However, it is crucial to handle these sections with care to prevent contamination and degradation. Staining techniques, such as hematoxylin and eosin (H&E) staining, can aid in the identification and characterization of genetic material within tissues. It is important to standardize staining protocols to ensure consistent results across different samples.

Preservation of genetic material is critical to maintain its integrity and viability for future analysis. Commonly used preservation methods include formalin fixation, paraffin embedding, and cryopreservation. Each method has its own advantages and disadvantages, and the choice of preservation method should be based on the specific needs of the research project.

The use of appropriate preservatives is essential to ensure the stability of genetic material. Formalin fixation is commonly used for paraffin embedding, while cryopreservation is suitable for long-term storage of genetic material. It is important to note that the choice of preservative may affect subsequent analyses, so careful consideration should be given to the preservation method used.

In conclusion, the collection, handling, and preservation of genetic material are crucial steps in the research process. It is important to adhere to best practices to ensure the integrity and quality of the collected material. By following proper collection and preservation techniques, researchers can obtain valuable insights into the genetic material and its interactions within the host.

GROSS PATHOLOGICAL EXAMINATION OF SPECIMENS

The gross pathological examination of specimens involves the visual inspection of tissues and organs to identify any abnormalities or changes. This examination is typically conducted under low-power magnification using a dissecting microscope or a compound microscope. The primary goal of this examination is to identify the presence of any visible lesions, tumors, or other pathological changes.

Histological sections can provide additional information to complement the gross examination. These sections are prepared by slicing the tissue into thin sections and staining them with various dyes to enhance the visibility of cellular structures. Staining techniques, such as hematoxylin and eosin (H&E) staining, can aid in the identification and characterization of genetic material within tissues.

The gross examination of specimens should be conducted in a systematic manner to ensure that all pertinent areas are evaluated. It is important to document any findings, including the location, size, and appearance of any abnormalities observed. This information is crucial for subsequent histological analysis and for the accurate interpretation of results.

The gross pathological examination of specimens is an essential step in the evaluation of genetic material. By combining the visual inspection of tissues with histological analysis, researchers can gain a comprehensive understanding of the genetic material and its interactions within the host.

GENETIC MATERIAL

The genetic material is an essential component of any research project. It is important to handle the genetic material with care to prevent contamination and degradation. Proper collection and preservation methods are critical to ensure the quality and integrity of the genetic material. By following best practices in collection and preservation, researchers can obtain valuable insights into the genetic material and its interactions within the host.
null
Sample Collection/Preparation

Sample Tissues from Specimen

The amount of tissue taken will also vary depending on the specific needs of the experiment. When sampling tissue from an epidermalized toe (Amphibia Press), for example, tissue samples from one specimen should be stained or cut as described in a published method for nucleic acid extraction. This method yields samples of RNA and DNA that can be used for analysis. The amount of tissue needed for RNA or protein analysis is highly variable, depending on the specific requirements of the experiment. In some cases, small tissue samples can be used, while in others, larger amounts may be necessary. It is important to consider the size and shape of the tissue samples, as well as the specific needs of the experiment, when determining the appropriate amount of tissue to collect.

Figure 4. A plastic box and lid partially filled with screw-cap vials.

Figure 3. A plastic box and lid filled with screw-cap vials.

Figure 2. A plastic box and lid filled with screw-cap vials.

Figure 1. A plastic box and lid filled with screw-cap vials.
DNA is extracted from these cells immediately or stored for later. DNA is extracted by openinng the nucleus and the enzydas flue by heating the sample to 65°C for 10 minutes. A precipitate of the melted DNA flue will be observed. The precipitate is then collected using a pipette, and the DNA is recovered by adding ethanol. The recovered DNA is then dried and resuspended in water.

1. Bloom and Meltzer (1998) recommend that the DNA be extracted immediately after the sample has been collected. The DNA should be stored at -20°C.

2. The DNA can be stored for up to one month at room temperature, but it should be protected from light.

Figure 5: Blooming a leaf sample on a filter paper.
COLLECTING AND PRESERVING GENETIC MATERIAL

The process of collecting and preserving genetic material involves several steps to ensure the integrity of the DNA. This includes selecting the appropriate tissue type, obtaining consent from the subject, and ensuring proper handling and storage of the samples.

**Collection**

1. **Sample Type**: The type of tissue or fluid to be collected should be appropriate for the genetic analysis desired. Common samples include blood, saliva, cheek swabs, and tissue biopsies.
2. **Consent**: Obtain informed consent from the subject, ensuring they understand the purpose of the collection and the potential uses of the genetic material.
3. **Collection Techniques**: Skilled technicians use appropriate techniques to collect samples without causing harm or discomfort to the subject.

**Preservation**

1. **Collection Conditions**: Samples should be collected under clean conditions to prevent contamination.
2. **Cooling**: Samples are immediately cooled to preserve DNA integrity. This may involve refrigeration or freezing.
3. **Handling**: The sample is handled with care to avoid contamination or degradation.

**Storage**

1. **Frozen Storage**: Sample collections are stored in liquid nitrogen or at -80°C to prevent DNA degradation.
2. **Data Management**: A detailed record of each sample, including the collection date, location, and consent details, is maintained.

**Ethical Considerations**

1. **Privacy and Confidentiality**: Strict protocols are in place to ensure the privacy and confidentiality of genetic information.
2. **Genetic Counseling**: Subjects are provided with information about the potential implications of genetic testing.

**Conclusion**

Effective collection and preservation are crucial for the success of genetic studies. Proper procedures ensure the quality and integrity of the genetic material, facilitating accurate analysis and interpretation.

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**References and Resources**

- “Genetic Counseling: Principles and Practice,” by David W. Blau and Debra J. Mathes
- “Ethical Considerations in Genetic Research,” by Daniel S. Botkin
- “DNA Extraction and Analysis,” by James F. Butler and Sarah E. Johnson

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**Further Reading**

- “Forensic DNA Analysis,” by R. G. Fisher
- “Genetics of Human Disease,” by K. F. Wilson and D. J. Garland
COLLECTING AND PRESERVING GENETIC MATERIAL

Sear Harpeptological Criculariul, N. 4

Jace and Willow (2009)

Despite the confusion of factors (Bateson et al., 2009), the expression of related genes in plant and animal populations can be understood when the effects of different environments and genetic backgrounds are taken into account. Studies conducted in these areas have shown that the expression of genes can be influenced by a variety of factors, including environmental conditions and the genetic makeup of the individual. These studies have also demonstrated that the expression of certain genes can be regulated by environmental cues, such as temperature and light. Therefore, understanding the complex interplay between genes and the environment is essential for predicting the behavior of populations in different ecosystems.

S. lands (2007) and models of evolution (2007) have been used to develop a framework for understanding the dynamics of genetic diversity. These models consider the genetic structure of populations and how it changes over time, as well as the factors that influence this diversity. This framework has been applied to a wide range of species, providing insights into the processes that shape genetic diversity and the evolution of new traits.

In addition to these models, there is a growing body of research that focuses on the role of genetic diversity in ecological processes. For example, studies have shown that genetic diversity can affect the resilience of populations to environmental changes, such as climate change. These findings highlight the importance of maintaining genetic diversity in natural populations, as it can provide a buffer against the impacts of environmental stressors.

These findings have important implications for conservation efforts, as they suggest that protecting genetic diversity is crucial for the long-term survival of species. Conservation strategies that focus on maintaining genetic diversity, such as habitat restoration and reintroduction programs, can help to ensure the persistence of populations in the face of environmental changes.
COLLECTING AND PRESERVING AMPHIBIAN MATERNAL

Protocol for noninvasive sampling of bg in amphibians in the field

Materials

- Light traps
- Rotor disks
- Sediment sampling equipment
- DNA extraction kit
- PCR equipment
- Gel electrophoresis apparatus
- Staining dye

Procedure

1. Place light traps in the field and set up rotor disks to collect samples from the water. 
2. Collect sediment samples from the bottom of the water body. 
3. Extract DNA from the collected samples using the DNA extraction kit. 
4. Amplify the targeted DNA regions using PCR. 
5. Run the PCR products on a gel electrophoresis apparatus. 
6. Stain the gel with DNA staining dye to visualize the amplified fragments.

Notes

- Ensure proper handling of samples to maintain integrity.
- Follow safety guidelines when handling chemical reagents.
- Record all data accurately for future analysis.

Box 3.

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Permanent storage

The cells or tissue sections are then transferred to liquid nitrogen for permanent storage. The cells or tissue sections will cool down to liquid nitrogen temperatures within 30 minutes under these conditions. The cells or tissue sections will remain viable for at least 5 years.

Figure 7a. Swab all swabbing of a circulatory tissue

Figure 7b. Swabbing the skin of a necrotic (pharyngeal) with a common swab.
Collecting and Preserving Genetic Material

Protocol

1) Remove flasks from refrigerator ~30 minutes prior to sampling.
2) Clean surfaces and equipment with 70% isopropanol and allow to dry.
3) Gently open flasks or flasks to the laser using scissors.
4) Place the lid on a clean paper towel and spray with 70% isopropanol.
5) Clean flasks and equipment with 70% isopropanol.

Safety Precautions

- Gloves
- Lab coat
- Protective glasses
- Respirator

Tools and Reagents Needed:

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<th>Type of Equipment</th>
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<td>Gloves</td>
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<td>Protective glasses</td>
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Tissue preparations are issued samples taken for the purpose of initiating the

Sampling Tissue explants for cell culture

Box 4

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Collecting and Preserving Genetic Material

Chapter 4: Stoichiometry, Reaction, and Transformation of Genetic Material

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Figure 12. An upright ultra-low freezer for storing tissues at -80°C.

For long-term storage (Brynhout and Ciofalo, 2009), a room temperature for up to one month does not have to be frozen (20°C or -80°C, respectively). However, control concentrations: 50% TM, 0.2% EDTA, 0.1% SDS. Tissues can be kept at 4°C for up to a month in 50% TM when used for DNA extraction. DNA can be isolated directly from tissues that have been homogenized with proteinase K.

Collecting and Preserving Genetic Material

Materials:

- Tissue samples
- Ultra-low freezers
- Freezer safe containers
- DNA extraction buffer

Methods:

1. Collect tissue samples from the site of interest.
2. Store tissue samples in an upright ultra-low freezer at -80°C.
3. Retrieve tissue samples for DNA extraction.
4. Use DNA extraction buffer to isolate DNA from tissue samples.

Figure 11. Storing tissues in liquid nitrogen storage vessels.

For short-term storage (Brynhout and Ciofalo, 2009), tissues can be refrigerated at 4°C for up to a month in 50% TM when used for DNA extraction. DNA can be isolated directly from tissues that have been homogenized with proteinase K.

Figure 10. A tank holding a frozen tissue storage box being removed from a liquid nitrogen storage vessel.
Shipping Regulations:

On receipt, the recipient shall handle the item with the utmost care and maintain all original packaging and labeling. The item shall be unpacked and inspected upon delivery. Any damaged or incomplete items shall be reported immediately. The recipient shall ensure that the item is stored in a cool, dry place away from direct sunlight and moisture. The item shall not be used until all necessary steps have been completed.

Upon receipt, the recipient shall perform a thorough inspection of the item to ensure that it is in good condition. Any defects or damage shall be reported immediately. The recipient shall keep all original packaging materials for future use. The item shall not be altered or modified in any way.

The recipient shall ensure that the item is stored in a secure location and protected from unauthorized access. The item shall not be used until all necessary steps have been completed. The recipient shall immediately report any unauthorized access to the appropriate authorities.

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(Droege et al., 2014). Associating vouched specimens to GenBank/EMBL/DDBJ accession numbers through digital catalogs will likely become standard practice. Associating tissues to voucher specimens is important, as it is difficult, if not impossible, to determine species identification from a small bit of tissue in a tube. In some cases, vouchers are not available (as in the case of nonlethal sampling) so identification must be verified in some other way. Photographs of sampled specimens can be useful in this regard as are detailed field notes. Another option is to use DNA barcodes to verify sample identity. In fact, some museum collections are DNA barcoding large numbers of tissues being accessioned into their collections both to verify identification and to help build a barcode reference library (Puillandre et al., 2012; Weigt et al., 2012).

Museum tissue collections face a unique challenge because tissue samples are consumed as they are used. Unlike a traditional museum loan that is eventually returned to the museum, tissue transfers should be thought of as a permanent gift or grant (Dessauer et al., 1996). Many museums require that unused tissue and/or unused extracted DNA be returned to the collection. It is not unreasonable to ask loan recipients to amplify the extracted genomic DNA using a whole genome amplification method, e.g., GenomiPhi (GE Healthcare Life Sciences), and send an aliquot of the amplified genomic DNA back to the collection. This will ensure that the sample is not completely exhausted and material will be available to other researchers.

COLLECTING AND PRESERVING GENETIC MATERIAL

LITERATURE CITED


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and issued samples: molecular assessment procedures in zoology.

Zimmerman's, R. W. Kretzschmar, and W. O'Brien, 2013. Direct DNA amplification from the 16S rRNA gene is a highly sensitive and specific tool for the detection of free DNA in blood, urine, and semen.

and then add 6% of the total cost of the order.

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