

# Wildlife Forensics and Conservation Research with the Freezer/Mill®



**APPARATUS:** CG-500 Freezer/Mill

**APPLICATION:** Cryogenic Grinding For Pulverizing Challenging Samples

## Introduction

**Wildlife forensics and conservation research** require precise sample preparation techniques for analyzing biological materials such as pangolin scales, elephant teeth and skulls, and tiger skin and hair. These biological specimens are essential for **forensic investigations, species identification, and conservation initiatives** because they provide genetic, isotopic, and structural information that can help trace the origin of animals, determine cause of death, and identify victims or perpetrators in wildlife trafficking cases.

Due to the **hardness and thermal sensitivity** of these materials, traditional grinding methods often result in sample degradation, DNA shearing, or inconsistent particle sizes. **Cryogenic grinding**, using a **Freezer/Mill®**, offers a reliable and reproducible method for pulverizing these challenging samples while preserving their molecular integrity.

This method enhances **DNA extraction, stable isotope analysis, and forensic identification**, all of which are critical for wildlife conservation, enforcement of anti-trafficking laws, and improving analytical precision. Unlike conventional grinding, cryogenic milling prevents heat-induced degradation, ensures sample purity, and yields uniform particle sizes, making it the preferred approach for processing tough biological materials.

## Materials and Methods

### Equipment and Materials

- Cryogenic Freezer/Mill (Cole-Parmer SamplePrep CG-500)
- Liquid Nitrogen (196 °C) for Cryogenic Freezing
- Stainless Steel Grinding Vials and Impactors – Item Number 6803
- Wildlife Sample Types – Pangolin Scales, Elephant Skull and Teeth, Tiger Skin and Hair
- Personal Protective Equipment (PPE) – Cryogenic Gloves, Face Shield, Lab Coat



## Sample Readiness and Preparation

Wildlife-derived materials, including pangolin scales, elephant teeth and skull fragments, and tiger skin and hair, were first cleaned to remove surface contaminants that could interfere with analysis. The samples were then trimmed into appropriately sized pieces to fit securely within stainless steel grinding vials.

## Cryogenic Freezing

Prepared samples were placed directly into stainless steel vials along with suitable impactors and securely sealed. These vials were then submerged in liquid nitrogen (-196 °C) for a minimum of 10 minutes. Freezing the samples within the vials ensured consistent brittleness across all material types, minimized handling, and prevented potential contamination or thermal degradation prior to grinding.

## Instrument Setup and Sample Grinding

Grinding parameters—including pre-cool time, grinding cycle duration, rest time and rate—were customized based on the hardness and structure of each sample type. The samples were then cryogenically ground under controlled impact and friction to yield a fine, homogeneous powder suitable for analytical procedures.

## Sample Extraction and Storage

Post-grinding, the powdered material was carefully extracted using pre-cooled tools to maintain sample integrity and prevent moisture condensation.

## Sample Types, Challenges and Grinding Parameters

### Pangolin Scales (Keratin-Based Structure)

#### Challenges:

- Hard keratin composition makes mechanical grinding difficult.
- Heat generated during grinding can degrade DNA and affect analysis.

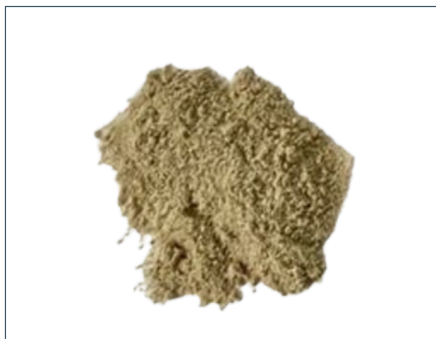
#### Freezer/Mill Solution:

- Cryogenic grinding eliminates heat buildup, preserving keratin integrity.
- Produces fine powder for DNA extraction and mass spectrometry.

## Grinding Parameters:

Sample Type	Pre-Freezing Time	Grinding Time	Rate	Sample Size	Cycles	Rest Time
Pangolin Scales	10 min	2 min	15 cps	10 g	2	2min

## Before and After - Pangolin Scales



## Elephant Skull and Teeth (Dense Bone Material)

### Challenges:

- High mineral content makes mechanical grinding inefficient.
- Manual grinding can result in inconsistent particle size and contamination.

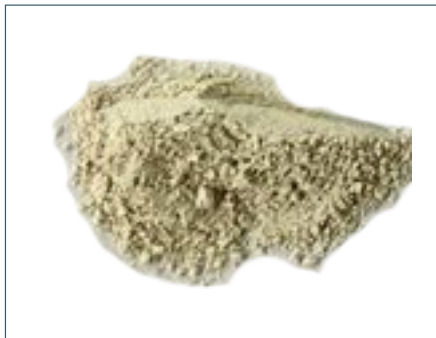
### Freezer/Mill Solution:

- Ensures uniform, fine bone powder for stable isotope analysis and species identification.
- Minimizes contamination and preserves structural integrity.

## Grinding Parameters:

Sample Type	Pre-Freezing Time	Grinding Time	Rate	Sample Size	Cycles	Rest Time
Elephant Skull and Teeth	10 min	2 min	15 cps	10 g	2	2min

## Before and After - Elephant Skull



## Before and After - Elephant Teeth



## Tiger Skin and Hair (Keratin and Pigment-Based Material)

### Challenges:

- Difficult to grind uniformly without DNA loss.
- Heat and oxidation can degrade pigments and proteins.

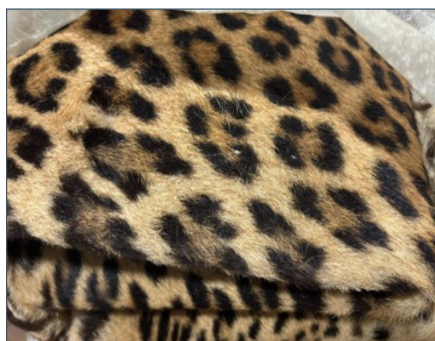
### Freezer/Mill Solution:

- Maintains protein and DNA integrity, preventing degradation.
- Produces fine particulate for forensic hair comparison and genetic testing.

### Grinding Parameters:

Sample Type	Pre-Freezing Time	Grinding Time	Rate	Sample Size	Cycles	Rest Time
Tiger Skin and Hair	10 min	2 min	15 cps	1 g	2	2min

## Before and After - Tiger Skin



## Before and After - Tiger Hair



## Results and Discussion

Cryogenic grinding plays a crucial role in improving the quality and reliability of wildlife forensic analysis. It leads to higher **DNA extraction** yields compared to manual pulverization, enabling more dependable results in genetic investigations. The method also preserves stable isotope signatures, which are essential for tracking the **geographic origin and dietary habits of species**—key factors in conservation research. Furthermore, the uniform and fine powders produced through cryogenic grinding enhance **forensic identification**, allowing for more accurate species verification and stronger evidence in cases of **illegal wildlife trade**. Importantly, the sealed and cold processing environment **minimizes contamination**, **ensuring consistent and accurate analytical outcomes** across all sample types.

## Conclusion

The Freezer/Mill is an essential tool for wildlife forensic and conservation research, enabling efficient **DNA extraction, protein profiling, and isotopic studies**. Its cryogenic grinding capability preserves sample integrity, supporting law enforcement against **wildlife trafficking**. Compared to conventional grinding methods, cryogenic grinding offers superior **precision, uniformity, and contamination control**, making it the preferred choice for forensic applications.

## Additional Resources

The following research article is recommended as supplemental references to strengthen the scientific relevance and application breadth of the Freezer/Mill in wildlife forensics and conservation research.

1. Nuwer, Rachel. 2015. "Forensic Tools Battle Ivory Poachers." Scientific American, April 13, 2015.  
<https://www.scientificamerican.com/article/forensic-tools-battle-ivory-poachers/>.

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