To the editor: Seeds are the reproductive structures of plants and are often components of the everyday diet (1). For this reason, seeds can become relevant evidence in forensic science to aid in establishing the cause and manner of death and for determining if legal action is appropriate for a wrongful death. Regardless of whether or not the decedent was poisoned or the death was due to accident or suicide, the forensic scientist still must link information from the autopsy back to a source and time of ingestion. Most plant-related deaths include accidental and intentional poisoning, or drug overdoses from plant-derived illicit substances and natural herbal remedies obtained from unreliable sources (1-5).

Traditional forensic techniques for the analysis of stomach contents involve the use of a light microscope to observe unknown samples from the stomach and compare to known reference samples (1,6-10). Often a comparison microscope that allows for the simultaneous visualization of the plant cell walls on the slides with both unknown and reference samples is helpful for plant species identification. Since plant cells have a sturdy cellulose wall around the cell membrane, they can retain an identifiable shape even after digestion. Even better, plant seeds are often completely indigestible and so can be useful for assessing ingested plant material even if those materials have passed through the stomach into the intestines and are otherwise unrecognizable (1,6-10).

One relatively new approach to identification of stomach contents is the use of DNA to identify a plant species from an ingested seed and subsequently link the ingested seeds back to a source plant by a technique called amplified fragment length polymorphism (AFLP) analysis (1,11). This method has been used routinely for plant breeding programs to identify DNA molecules that can be associated with a desirable feature of the organism (eg, disease resistance or increased protein content). AFLP creates a “complex DNA band pattern” that characterizes a particular plant, so that one may barcode a single individual within a population by its DNA pattern. It is a molecular method that involves 1) DNA extraction, 2) two separate cycles of polymerase chain reaction (PCR) amplification, and 3) detection by DNA fragment length analysis. All of these methods are used routinely in forensic laboratories for human identification, and with some minor modifications, can be used for plants (1,8-13).

We investigated the benefits and limitations of performing DNA analyses on plant seeds recovered after ingestion in order to define when it would be valuable to pursue DNA analyses in addition to microscopic tests on seeds. To this end, a variety of seeds were ingested from tomato, both from fresh
sources and from processed foods. Microscopic and AFLP tests were performed to determine (in theory) if plant DNA barcodes could be generated to link plant material from the stomach back to the scene of a last meal. The stomach is a harsh acidic environment that would not normally favor DNA.

We used 12 different brands of commercial tomato products. There were 5 brands of canned tomatoes including “Hunt’s,” “Weis,” “Big Top,” “Del Monte,” and “Contadina”. The 7 brands of spaghetti sauces included “Prego,” “Newman’s Own,” “Healthy Choice,” “Francesco Rinaldi,” “Barilla Basilico,” “Classico,” and “DelGrosso.” Seeds were separated manually from other materials in the can or from feces then collected by washing through a wire mesh sieve, rinsed clean with sterile water, and examined under the microscope.

Individual seeds were ground to a fine powder in liquid nitrogen with a mortar and pestle. DNA chemical extractions were performed as recommended by the manufacturer of DNeasy Plant Mini Kits (Qiagen, Inc., Valencia, CA, USA). This simple plant DNA extraction procedure yields pure total DNA ready for PCR amplification within two hours. AFLP analysis to generate plant DNA barcodes for each sample was performed as recommended by the manufacturer of the AFLP Plant Mapping Kit (Applied Biosystems, Inc., Foster City, CA, USA). The data generated from the AFLP method was analyzed using Genotyper 2.5 software (Applied Biosystems).

Our data from three replicates of DNA extractions per type of tomato seed revealed that any form of commercial processing (e.g., cooking or canning) will negatively affect the yield of DNA from seeds. However, very importantly, any fresh plant seeds (e.g., eaten fresh and not cooked) will yield sufficient quantity and quality of DNA to perform a DNA test (Figure 1). Peak patterns from multiple seeds are sufficiently diverse and complex to allow for a unique band pattern per each seed tested which is important to link a seed back to a source (i.e., for most species with sufficient genetic diversity, a unique DNA pattern can be generated and variety specific markers identified) (8,9).

Our results show that if the plant seed was not exposed to a heat source via cooking prior to ingestion, it is possible to use our plant DNA extraction and typing methods to generate a unique molecular signature. The ability to do this may enable investigators to use science to establish a genetic linkage between the victim and the crime scene through what was eaten for the last meal (1,14-16).

The methods used for our investigation are standard and accepted methods in the field of plant molecular research. The equipment we used is also standard for many forensic and research laboratories that routinely perform DNA methods for tracing lineages. Some considerations for future work include: 1) an expected range of DNA amounts recovered from different plant species based on their size and weight; 2) determining which seeds will not withstand

![Figure 1](image_url). A representative AFLP signature from a single tomato seed. The complexity of the peak pattern can be adjusted using different polymerase chain reaction (PCR) amplification primer sets supplied in the AFLP Plant Mapping Kit so that each seed sample can be individualized to a unique barcode. The y-axis is relative fluorescence units (RFU, height of the peak; 0-2000 RFU as a standard height range); the x-axis is the size of the DNA fragment (0-500 bases as a standard fragment size range).
the digestive process, as well as tomato and pepper seeds that have a tougher outer seed coat; and 3) developing species databases for ready comparison to evidentiary samples to quickly identify the plant variety. The envisioned applications for this technology include identification and potential sourcing of contaminant seeds found in spices, cereals, and herbal teas or traditional herbal medicines that have not been altered by substantive commercial heating or processing.

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