

## Sample Analysis

*Although the Nation's forensic laboratories generally have the policies, systems, and tools to collect, extract, amplify, and analyze many biological samples, most would not be able to handle the number of samples associated with a mass fatality event. This chapter offers an overview of processes involved in the DNA typing of a large number of samples in a relatively short period. See appendix H for a more rudimentary discussion of DNA analysis.*

A forensic laboratory's mass fatality plan should include large-scale collection and extraction procedures, alternate analytical methods for particularly challenging samples, automation for handling high-volume analyses, and quality assessment tools for interpreting results. The plan also should consider work and storage spaces, including sample accessioning and processing areas that have sufficient bench space and biological containment hoods.

Laboratories may plan to use robotics in batch analysis in a mass fatality identification. In the World Trade Center (WTC) identification effort, robotics was essential in handling the quantity of samples. It is important for laboratory directors to note, however, that there is likely to be a steep learning curve with such new procedures. Therefore, advance planning is important.

As was the case after the 9/11 attacks, the environmental conditions to which samples are exposed can compromise the quantity or quality of extractable DNA. Of course, the quality of biological samples will be incident specific, ranging from good quality, high molecular weight to highly degraded. Therefore, DNA-typing methods need to be robust.

### Sample Collection

Although all components of the DNA identification process are important, sample collection may be the most critical and frequently overlooked. In the urgency to identify the victims, there may be little attention paid to how the remains are

collected. Planning can have a great impact on the quality and quantity of typable DNA. To standardize the collection materials—which, in turn, will simplify the extraction process—the laboratory manager should be involved in the sample collection process.

Protocols for chain-of-custody documentation in collecting evidence and handling samples must be a part of a laboratory's mass fatality plan. This is important not only for scene reconstruction and quality control, but also for any subsequent legal proceedings. As in any situation with potential judicial implications, it is critically important to use the best forensic practices in collecting and preserving samples. Improper preservation methods can lead to the loss of typable DNA and the potential compromise of data that is necessary for a positive identification.

A mass fatality plan should provide for the collection of personal items from family members and others. After a mass fatality event, family members will be eager to provide samples to help identify a loved one. In a smaller incident, family reference samples may be easier to collect and

**We knew immediately that hundreds of environmentally challenged samples would not yield full 13-locus STR profiles and would therefore require extra laboratory effort. So, early on, we explored alternate, novel technologies to help turn samples with only partial profiles into those that would allow solid victim identifications. In doing so, we carefully considered issues regarding genetic linkage of markers, and also agreed that only methods meeting NYS validation standards would be used.**

*Frederick Bieber*

analyze than a victim's personal items. However, in a larger event, it may be more efficient to use personal items for identification, assuming sufficient quantities of DNA can be recovered from a personal effect and its sole use by the victim can be assured.

As noted in prior chapters of this report cellular material can be derived from hair, stamps, envelopes, toothbrushes, razors, and unwashed clothing. If personal effects are used in a mass fatality identification effort, it is advisable to collect several samples, if possible, as some will be better suited for analysis than others. It can be challenging to develop instructions for submission of a victim's personal items, including a way to ensure that only the victim used the item. Also, it is important to keep in mind that a family's emotional attachment to a loved one's personal item may be strong.

It also may be necessary to collect reference samples from around the world. In this case, it may be helpful to consult with professionals who work at paternity testing laboratories with remote sample collection experience.

Three sample forms that may provide general guidance are included with this report: Personal Items Submission Form (appendix B), Family and/or Donor Reference Collection Form (appendix C), and the Family Tree Form (appendix D).

Needless to say, it should always be considered that a personal item may contain the DNA from someone other than the victim/purported owner. That is why the Sample Personal Items Submission Form (appendix B) solicits detailed information regarding everyone who may have used the item. To prevent misidentification of remains due to the presence on the personal item of DNA from other contributors, the DNA profile recovered from the personal item should, if possible, be compared to the DNA profiles of family members to ensure that the proper biological relationship exists between the DNA on the personal item and the DNA from the family members.

## Sample Storage

Work and storage space must accommodate sample accessioning and processing, including sufficient bench space and safety hoods. An

estimate of the number of potential samples should be made so that sufficient storage space can be assured (see exhibit 4). Soft tissue samples need to be stored in ultra-low-temperature freezers. In addition to securing appropriate freezer space, additional refrigerators may be needed to store samples during the extraction and analysis phases. If sample recovery at the disaster site is a long-term process, tissue decomposition will become a factor in planning for sufficient storage space.

Depending on the conditions at the disaster site, larger portions of tissue may be needed to compensate for degradation as time passes during the collection process. In the case of bone, for example, a few cubic centimeters may (under optimal conditions) be adequate for analysis, but an entire femur may be required in more compromised situations. Not only do larger samples require more storage space, but extraction procedures may require modification to accommodate larger sample sizes.

Following the WTC attacks, other laboratories offered to assist the Office of the Chief Medical Examiner (OCME). Such offers are likely to occur after any future mass fatality incident. If appropriate chain of custody, accessioning, and other infrastructure concerns are addressed, outsourcing may be considered. Obviously, however, if samples are sent to other laboratories at any stage of the analysis, the same quality control and chain-of-custody practices must be maintained (see chapter 14, *Quality Control*).

## Short Tandem Repeat (STR) DNA Amplification and Analysis

In general, polymerase chain reaction (PCR) issues in a mass fatality identification effort are no different than in any other situation, except for the greater number of samples. Although different analytical approaches may eventually be required to make identifications, it is most expedient to use familiar and well-established technologies (i.e., short tandem repeat (STR) typing) as the method of first analysis. In fact, many disaster samples may be wholly typable by STR analysis.

It should be remembered when performing extractions, however, that additional testing may be needed; therefore, extraction techniques that will accommodate other testing methods—such as mitochondrial DNA (mtDNA) sequencing—should be considered.

After extraction, the template DNA is subjected to PCR, which is particularly useful for analyzing materials that may contain degraded DNA. A typical PCR requires three steps and is based on specific annealing and extension of oligonucleotide primers (two per marker) that flank a defined target DNA segment. The template DNA to be amplified by the PCR is first denatured, usually by heating the sample to 95 degrees Centigrade.

After denaturation, the two primers hybridize to the separated strands at a given locus. Primer annealing is accomplished by lowering the temperature to a defined point, typically between 45–65 degrees Centigrade. The next phase in the PCR process, primer extension, is generally carried out at 72 degrees Centigrade, the temperature at which *Thermus aquaticus* DNA polymerase can most effectively copy the original template DNA by extending the primers and making complementary copies of the original template DNA. These three steps (denaturation, primer annealing, and primer extension) represent a single PCR cycle.

Upon repeated cycles of the PCR, an exponential accumulation of a discrete DNA fragment containing the genetic marker of interest is achieved. Thus, PCR generates large amounts of specific DNA sequences from relatively small (picogram or nanogram) quantities of genomic DNA. Amplification of target sequences of DNA is primarily a technique to prepare the sample for typing.

Only a limited template may be available, and inhibitors to PCR may further reduce the yield of PCR product. Efforts should be made to optimize the components of the PCR to overcome the vagaries of environmental contamination. Some practices used by laboratories during routine analyses—using reduced reaction volumes, for example—may not be appropriate when samples are compromised. A larger reaction volume may dilute inhibitors to the point that the PCR can be successful. Additional enhancements to reduce the impact of inhibitors, such as Bovine Serum Albumen, may be considered part of the protocol

for maximizing DNA yields from compromised samples.

## Alternative Testing Methods

In the WTC identification effort, the OCME relied on the recommendations of the Kinship and Data Analysis Panel (KADAP) regarding new identification methods for analyzing compromised samples. In considering additional typing technologies and strategies, the KADAP considered the sufficiency of extracted material to support all attempted technologies, as well as any quality control issues that might arise. The KADAP also considered how to handle the statistical approach using other technologies, including linkage and haplotype/genotype comparisons.

**Mitochondrial DNA Analysis.** STRs reside in the human cell nucleus; outside the nucleus, in the cytoplasm, are mitochondria. Mitochondria are subcellular organelles that contain an extra chromosomal genome separate and distinct from the nuclear genome. Human mitochondrial DNA differs from nuclear DNA in that it is a closed, circular (rather than linear) molecule; it is smaller, consisting of approximately 16,569 base pairs; it is maternally inherited; it does not undergo recombination; and it is present in high copy number in a cell.

The maternal inheritance and lack of recombination characteristics are particularly helpful in identifying human remains. Associations can be made or refuted where known maternal relatives are the reference sample sources, even if they are several generations removed from the victim.

The primary advantage of using mtDNA (as opposed to nuclear DNA analysis) on compromised samples is the high copy number of mtDNA molecules in a cell. When the amount of extracted DNA is very small or degraded (as can be the case in mass disaster tissue samples of bone, teeth, and hair), an identification is more

**The KADAP served as a model for scientific collaboration and peer review of method validation under the challenging and stressful conditions of responding to a mass fatality disaster.**

*Anne Walsh*

likely using mtDNA analysis than using the polymorphic markers found in nuclear DNA.

In the WTC identification effort, a number of samples could not be typed sufficiently with STR loci to identify the source with a high degree of confidence. In these cases, mtDNA sequencing was attempted to increase the discrimination power. Although the extraction process for mtDNA typically requires a relatively clean environment, this was not possible in the WTC identification effort, due to the number of samples. However, reasonable precautions were taken, including a reduction in the number of amplification cycles (28 or 29 instead of the typical 36). This reduced contamination issues, although at the expense of the sensitivity of detection.

Although not as informative as a battery of autosomal STR loci, a unique mitotype may be sufficient to make an identification, if the victims are from a closed population. The mitotype can be used to group individuals into smaller categories, narrowing the candidate pool. It may then be possible for a less informative partial STR profile to become a unique identifier within the mtDNA subcategory. Screening by mtDNA sequencing would be possible because of the availability of high-throughput analysis, coupled with software that automatically interprets mitotypes.

**Repositioning Primers.** In the WTC identification effort, recovered DNA was often too degraded and fragmented to produce STR results with standard commercial STR kits. However, by repositioning the primers so that they resided closer to the repeat region, the amplified product (or amplicon) was made smaller than some of the fragmented DNA template molecules, thus making genetic characterization of the sample possible for more STRs than when using traditional typing. These STR miniplexes were invaluable for analyzing the more degraded samples, and, in fact, results were obtained for some samples at loci that were not typable using commercially available kits.

The general assay procedure for the miniplex test used in the identification of WTC victims was similar to that used for forensically validated STRs. After evaluating the methods, reagents, and validation data, the KADAP determined that no additional equipment and training was necessary.

### Single Nucleotide Polymorphism Analysis.

The PCR amplicon size can be further reduced by amplifying regions that contain a class of genetic markers known as single nucleotide polymorphisms (SNPs). Although an abundant supply of SNPs exists for identity testing, most SNPs are biallelic and, therefore, not as informative for identity testing as STR loci. However, because the amplicon size can be reduced 60–80 base pairs in length, DNA that is degraded beyond the limits of STR typing may be typable.

In the WTC identification effort, an SNP typing method was validated for the more difficult-to-type samples. In fact, identifications that otherwise would not have been possible were made using this technology. Combining the features of a chip array, the primer extension assay, and universal tags, the multiplex assay method was carried out in a flat-bottom microplate, in which each well contained a total of 16 individual antitag sequences for 12 SNPs and 4 controls. (Basically, each PCR primer, about 45 bases long, is comprised of a 25-base-long segment that is complementary to the area immediately adjacent to the SNP extension site and a 20-base-long sequence—that is, the tag sequence—that is complementary to an antitag sequence attached to the bottom of a well.)

Using that process, the SNP extension product was transferred after PCR and allowed to hybridize in the array of antitags. A fluorescent detection system allowed typing of the two possible alleles at the SNP site by comparing signals from fluorescent dyes used to label the two different allelic products in the PCR extension reaction. With this technology, identifications were made on some very compromised samples that otherwise would not have been possible to identify.