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Microsatellite Markers in Biobanking: A New Multiplexed Assay

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Microsatellites, or MSATs, offer a fast and cost-effective way for biobanks to establish a biospecimen genetic profile. Importantly, this genetic profile can be used to authenticate multiple submissions derived from the same individual as well as biospecimens derived from the same original sample submission over time. While the Certificate of Confidentiality provided by the National Institutes of Health offers some meaningful protection to prevent the disclosure of potentially identifiable information to entities within the United States, we consider, in this study, the potential to offer additional protection to participants who choose to donate to biobanks by minimizing the use of forensic Combined DNA Index System (CODIS) MSAT markers in biobanking. To this end, we report the design and validation of a new multiplexed MSAT assay that does not include CODIS markers for use in biobanking operations and quality control management.

Keywords: microsatellite, biobanking, biospecimen authentication

Introduction

The ubiquity of repetitive elements in eukaryotic genomes has contributed to their extensive characterization and cataloging.[1] Microsatellites (MSATs), also known as short tandem repeats (STRs), comprise the subset of these elements with repeat sequences shorter than 14 bp in length. MSAT methodology emerged in the 1980s through efforts to leverage individual human genetic variation data to construct a unique genetic profile or genetic ''fingerprint.''[2] Since that time, MSATs have been utilized in a wide range of applications, including population genetics,[3] forensic testing,[4] cell line authentication,[5,6] paternity testing in humans,[7] animals[8] and plants,[9] genetic mapping,[10,11] and biobanking.[12] MSATs are found across the human genome, exhibit relatively high levels of allelic variability in humans, and are inherited according to known principles of human genetics.[13]

Biospecimen genetic profiling

From the biospecimen perspective, there are two primary applications of MSAT methodology that we consider in more detail, biobanking and forensic testing. Biobanking involves the collection, processing, storage, and distribution of biological samples, including but not limited to blood, tissue, cell cultures, and DNA.[14] There are several advantages to the use of MSATs in biobanking. First, when multiple samples collected from the same individual are submitted to a biobank, MSAT profiling remains the fastest and most cost-effective way to compare genetic variation in DNA extracted from each submission vial to confirm that all of the samples originated from the same individual. Indeed, the data collection, storage, and analysis of MSATs are all more cost effective and time efficient relative to newer array-based or next-generation sequencing-based assays, especially when multiplexed.[15,16] Moreover, this value is especially relevant to older, historical biobanks that include thousands or tens of thousands of submissions with (or without) already established MSAT profiles. Second, biobanks that store MSAT profiles for each biospecimen can also compare profiles between submissions to identify potential duplicate submissions and submissions from related individuals. Third, initial MSAT profiles collected at submission can be stored and compared against MSAT profiles collected from derived products that are produced through downstream sample processing to ensure biospecimen identity and integrity over time. Fourth, MSAT profiles of

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derived biospecimens that are distributed back to the research community can be compared against the submission MSAT profile to confirm that every externally distributed sample is derived from the intended original submission. These third and fourth use cases are especially relevant for cell culture biobanking. Studies of lymphoblastoid cell line transformation,[17] passaging, and expansion[18] demonstrate negligible impact on genome-wide genetic variation over time. However, the extensive documentation of mischaracterized and misidentified cell line use in research demonstrates the critical importance of maintaining consistent measures of cell line integrity and quality control throughout biospecimen processing.[19–21]

Forensic applications benefit from MSAT profiling in a similar manner. In these cases, MSAT profiles are typically compared between crime scene biospecimens and potential victim-derived biospecimens, potential suspect-derived biospecimens, or MSAT profiles already stored in forensic databases.[4,22] The Federal Bureau of Investigation (FBI) has typically used 13 STR loci for forensic profiling in the United States, although this was recently expanded to 20 loci, and Interpol uses 12 loci in the EU.[23,24] The FBI maintains the Combined DNA Index System (CODIS), which comprises MSAT profiles of DNA samples collected from crime scenes and from individuals for potential matches.[25]

Ethical issues raised by MSAT usage

Due to a long history of marginalization, exploitation, and abuse of black communities, indigenous peoples, and other communities of color by researchers, there is understandable mistrust within those communities of those of us who conduct research.[26,27] Relatedly, there is also a lack of representation of these communities in genetic research,[28,29] limiting what we can know and understand of the genetic diversity within these communities and the benefits that research can offer them. When the language we use to describe people/samples in research is the language of law enforcement, trust is more likely to be harmed than rebuilt, and efforts to improve equity in research will be frustrated.

Given the increasing use of genetic information from noncriminal contexts by law enforcement to identify crime scene suspects, there is an additional concern about the use and potential misuse of genetic information that has been collected for other applications,[30,31] including research. Some protection of these data and the individuals from whom they were derived is offered by the National Institutes of Health (NIH) through Certificates of Confidentiality (COCs), which are issued automatically for all NIH-funded projects that use identifiable information collected from participants, and can be requested by projects that are not funded by the NIH.[32] The NIH COC protects against the disclosure of identifiable participant information ''in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding''[32]; however, there are very few instances in which this protection has been tested in court.[32] While a COC might minimize the risk of misuse of CODIS MSATs collected from research participant biospecimens, it cannot address the concerns about trust. An additional protection that can be offered by biobanks to research participants who agree to donate biospecimens is the minimization of use of CODIS markers in biospecimen processing, quality control and integrity analyses, and authentication—describing samples in a language that is unique to research and not shared by law enforcement.

In this study, we describe a new MSAT assay for use in biobanking operations and quality control management that minimizes risk of data misuse by law enforcement by excluding the CODIS MSATs that are used by law enforcement in the United States.

Methods

In 1997, Coriell scientists implemented an MSAT assay comprising six tetranucleotide repeats that are polymorphic in humans for biobanking applications (THO-1, D5S592, D10S526, vWA31, D22S417, and FES/FPS). Since 2004, Coriell has used an ABI 3730 fluorescent sequencer with 48 capillaries to assay this set of MSATs. This instrument is higher throughput and allows more flexible fluorochrome selection for assaying multiplexed sets of MSATs relative to the older ABI 377 that was previously employed. By using several fluorochromes, labeled primers can be multiplexed at the polymerase chain reaction (PCR) step, or combined in a single capillary run after PCR.

This standard set of six MSATs is run with a primer pair targeting the amelogenin allele on the X and Y chromosomes. The amplicon size from the amelogenin gene on the X and Y chromosomes differ; thus, male samples generate two different-sized amplicons, and female samples generate two amplicons of the same size.[33] This feature, therefore, allows a single multiplex PCR assay to generate both a DNA fingerprint for biospecimen identity profiling and a determination of sex. The assay is routinely run on DNA extracted from each submission vial (most commonly whole blood) as well as on each derived biospecimen (most commonly transformed or expanded lymphoblastoid cell lines). This assay can also detect as low as 10% cross-contamination from other samples; in particular, the presence of more than two alleles at a given MSAT locus indicates cross-contamination. We note that when large-scale data, such as genome-wide single nucleotide polymorphism microarray data or next-generation whole genome sequencing data, are available, several more sensitive methods to identify cross-contamination are available.[34–36]

For the past decade, Coriell has employed a supplemental panel of MSATs, the ''Identifiler Plus'' (the AmpFLSTR™ Identifiler™ Plus PCR Amplification Kit by Thermo Fisher Scientific), for added discrimination in the subset of cases where all standard six MSAT loci match between apparently distinct individuals. The Identifiler Plus marker set contains 15 STRs, of which 13 overlap core CODIS loci. More recently, in an effort to minimize the inclusion of CODIS markers in ongoing quality control efforts, Coriell has developed a new in-house supplemental panel of six MSATs.

MSAT assay development

One of the members of the NIGMS Human Genetic Cell Repository (NIGMS Repository) Scientific Advisory Committee, Andrew J. Sharp, PhD, shared a list of over 600 tetranucleotide MSATs with relatively high heterozygosity in human whole genome sequencing data. We prioritized candidates for our new assay based on those with the largest number of observed alleles. Of those 38 candidates, we used

the Primer3 tool[37–39] to predict the range of PCR product size, GC content, primer melting temperature (T_m), and the degree of self-complementarity of each primer. We further used the Thermo Fisher Scientific Multiple Primer Analyzer to identify potential primer dimerization between primer pairs. After computational predictions were evaluated, we chose eight MSAT primer pairs to assess in the laboratory. Primers resulting in overlapping allele size ranges were tagged with distinct fluorochromes. Each of these primer pairs were first run in an individual PCR to confirm successful PCR amplification; seven of these primer pairs successfully amplified individually and were tested together in a multiplex PCR; and six of these primer pairs successfully amplified in a multiplexed PCR. A range of annealing temperatures were compared (58°C, 60°C, 62°C, 64°C, and 66°C), and the 64°C annealing temperature resulted in the best PCR performance. Supplementary Table S1 includes additional information on multiplex PCR conditions, and Supplementary Table S2 contains additional details on each primer pair.

All of this initial primer testing was performed on three NIGMS Repository DNA samples (NA06990, NA10859, and NA07057). MSAT allele sizes were called using Thermo Fisher Scientific's GeneMapper™ software. As shown in Supplementary Table S2, one MSAT marker pair lies on the same chromosome, and the markers are over 13 Mb apart. When comparing the locations among the standard and new MSAT assay markers, two additional MSAT marker pairs lie on the same chromosome, each over 30 Mb apart. While linkage disequilibrium (LD) in humans has been documented over regions as large as 1 Mb,[40] the majority of LD regions across human genomes are much smaller and consistent with our assumption of relative independence across MSAT markers included in our standard and new MSAT assays.

No IRB approval was needed to conduct this study.

Results

Assay implementation

For the current study, we leveraged data from the NINDS Human Genetics Resource Center (NINDS Repository), one of the largest biorepositories at Coriell with over 48,000 unique submissions. Each of the six standard MSAT markers has sufficiently high heterozygosity in the NINDS Repository sample data (0.78, 0.83, 0.90, 0.81, 0.84, and 0.70, respectively), such that the probability of identical ''profiles'' in unrelated individuals with this marker set is theoretically one in one million (when assuming independence among markers).[41] We calculate similar theoretical discriminatory power for the new set of six MSAT markers using the same approach,[41] assuming unrelated individuals and independence among markers, and using the heterozygosity information shared by Dr. Sharp; however, we do not have enough data to estimate the empirical discriminatory power of this marker set.

In practice, all of the NINDS Repository samples have been assayed for the standard MSAT 6-plex, and the Molecular Biology Laboratory at Coriell has documented several matches (Table 1). Two of these matched pairs reflect comparisons between family members, while the remaining eight matched pairs occurred between apparently unrelated individuals. Three of these matched pairs occurred between individuals with distinct self-reported gender, while the remaining seven matched pairs occurred between individuals with the same self-reported gender. The results from the new MSAT assay demonstrate the ability to distinguish all 10 NINDS Repository matched pairs that previously required a second MSAT assay (in this case the Identifiler Plus assay) to differentiate (Table 1). This discrimination power holds even for related individuals. The siblings from family NINDS0760 can be distinguished by one allele for four of the six new MSATs, and the siblings from family NINDS5712 can be distinguished by one allele for two of the six new MSATs. Of the remaining eight pairs of individuals that shared profiles for all six of the standard MSAT assay, no more than one of the new assay's MSATs is shared between individuals. Therefore, in cases where the standard MSAT assay is unable to distinguish apparently unrelated or related but distinct individuals, adding the new MSAT assay offers additional and adequate discrimination power without using any additional CODIS markers.

Discussion

MSAT assays offer a fast and cost-effective way[15,16] for biobanks to establish a genetic fingerprint of each biospecimen submission to serve as a reference against other submissions as well as against biospecimens derived from the same submission to ensure sample authentication and sample integrity over time. We have routinely used a multiplexed assay of six MSATs to establish genetic profiles for each biobank submission. While the theoretical discriminatory power of this standard assay is one in one million, in practice we occasionally identify matching profiles between biospecimens collected from distinct people. We previously utilized the Identifiler Plus assay for additional resolution in these cases; however, this assay includes 13 CODIS markers and the NIGMS Repository Scientific Advisory Committee in collaboration with the NIGMS Repository team at Coriell felt an alternative that did not rely on MSAT markers used by law enforcement would offer added protection to individuals who contribute samples to biorepositories.

We developed and validated a new multiplexed MSAT assay to replace our reliance on the Identifiler Plus assay to discriminate samples that could not be distinguished by our standard MSAT 6-plex assay alone. We leveraged existing data from over 48,000 research participants that have donated biospecimens to the NINDS Repository and identified a subset of 10 submission pairs from distinct individuals with matching profiles using our standard MSAT assay. The results from our new MSAT assay demonstrate the ability to distinguish all 10 NINDS Repository matched pairs that previously required the Identifiler Plus assay to differentiate (Table 1). This discrimination power holds even for the subset of related individuals included in Table 1. Therefore, in cases where our standard MSAT assay is unable to distinguish apparently unrelated or related but distinct individuals, adding this new MSAT assay offers additional and adequate discrimination power without reliance on the Identifiler Plus assay.

More generally, we have found that there are more than enough polymorphic MSATs in the human genome to use for biobanking applications without the need to rely on CODIS markers. In this study, we share the development and validation of a new multiplexed MSAT assay that

Table 1. Microsatellite Comparisons

Repository ID	*Gender*	*Family ID*	*Relationship to proband*	*THO-1*	*D5S592*	*D10S526*	*VWA31*	*D22S417*	*FES/FPS*	*STR_101818*	*STR_109310*	*STR_1285164*	*STR_222658*	*STR_308935*	*STR_359667*
ND08549	F	NINDS0760	Daughter	159 159	178 186	246 254	149 153	173 185	224 228	300 356	291 303	158 194	325 351	197 213	378 390
ND08550	M	NINDS0760	Son	159 159	178 186	246 254	149 153	173 185	224 228	300 356	303 343	158 194	351 361	161 213	390 402
ND36045	F	NINDS4814	Daughter	159 163	182 186	194 254	149 153	173 185	220 224	340 350	335 343	194 194	325 351	165 217	334 378
ND44362	F			159 163	182 186	194 254	149 153	173 185	220 224	304 308	291 339	194 194	349 353	141 145	326 378
ND03713	M			159 167	178 178	202 246	153 157	185 185	224 224	312 360	303 343	194 194	325 349	165 217	330 330
ND14295	M	NINDS1306	Proband	159 167	178 178	202 246	153 157	185 185	224 224	344 344	295 335	194 234	325 349	197 197	362 398
ND10753	M	NINDS1063	Spouse	159 175	182 186	194 246	153 157	173 173	220 224	348 356	295 347	150 194	325 349	169 169	330 364
ND43103	F			159 175	182 186	194 246	153 157	173 173	220 224	320 324	331 343	194 194	349 353	145 221	350 394
ND11464	M			163 175	178 182	198 202	149 153	173 185	228 228	348 348	295 299	194 230	349 351	141 169	382 382
ND43406	M			163 175	178 182	198 202	149 153	173 185	228 228	304 340	291 331	194 194	325 349	209 217	326 382
ND14406	F			163 175	182 186	194 202	153 153	173 193	220 228	344 364	299 303	194 194	325 325	157 205	334 364
ND43372	F			163 175	182 186	194 202	153 153	173 193	220 228	340 342	299 327	194 226	325 325	165 197	350 364
ND38417	M	NINDS5712	Sibling	167 175	178 182	190 190	145 149	177 177	224 224	308 312	295 299	150 194	325 349	201 225	330 382
ND41175	M	NINDS5712	Proband	167 175	178 182	190 190	145 149	177 177	224 224	308 312	295 299	150 194	325 349	201 217	330 376
ND31713	M			171 175	178 178	194 242	141 149	173 177	224 228	304 324	299 299	166 234	325 401	169 169	358 382
ND45055	M			171 175	178 178	194 242	141 149	173 177	224 228	340 344	299 303	154 194	349 397	165 213	378 386
ND36600	M	NINDS4727	Sibling	175 175	182 190	194 194	149 153	177 189	224 228	328 340	295 331	150 226	337 349	141 165	378 386
ND43425	F			175 175	182 190	194 194	149 153	177 189	224 228	304 340	327 339	194 194	325 393	157 209	334 370
ND07492	F			175 175	186 190	238 246	149 153	185 197	224 228	320 344	295 299	194 194	325 389	193 201	390 402
ND14800	F			175 175	186 190	238 246	149 153	185 197	224 228	340 360	327 335	158 210	325 393	169 229	354 374

demonstrates discriminatory power between apparently unrelated as well as between related individuals without the inclusion of CODIS markers.

Authors' Contributions

Designed study: G.S., N.T., and L.S. Collected data: G.S. Analyzed data: L.S., G.S. Wrote article: G.S., D.M., S.S-E., D.R., N.T., and L.S.

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Author Disclosure Statement

No conflicting financial interests exist.

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Supplementary Material

Supplementary Table S1
Supplementary Table S2

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