TECHNICAL NOTE

Terry L. Laber,[1] B.S.; James T. Iverson,[1] B.S.; James A. Liberty,[1] B.S.; and Staci A. Giese,[1] B.S.

The Evaluation and Implementation of Match Criteria for Forensic Analysis of DNA

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ABSTRACT: This study describes a method for establishing match criteria used in forensic DNA typing. The validity of applying different match criteria based upon the molecular weight of a DNA band is discussed. The match criteria presented allow visually matching DNA patterns to be confirmed by computer assisted image analysis over the entire range of the sizing ladder.

Approximately 5000 intragel and 5000 intergel comparisons were made between the restriction fragment length polymorphism (RFLP) DNA band sizes obtained from casework, mock cases, and environmentally insulted samples and the band sizes obtained from their corresponding bloodstain standards (controls). Analyses of these data suggested that fragments located in different molecular weight size regions of an analytical gel required different match criteria for assessing a visual match. The results of these analyses support the use of the following match criteria: Intragel 0.5–10 kb = ±1.7%, 10–15 kb = ±3.2%, 15–22.6 kb = ±5.8%; Intergel and blind control 0.5–10 kb = ±3.0%, 10–15 kb = ±4.2%, 15–22.6 kb = ±10.0%; and human cell-line K562 and the monomorphic locus D7Z2 = ±2.5%. Each match criterion was also evaluated with respect to the distance in millimeters between matching bands throughout the 0.5–22.6 kb molecular weight size range. Applying these match criteria to different gel regions has been shown to be valid and reliable in comparisons conducted on more than 10,000 validation samples, in over 500 forensic cases and in more than 200 searches of a criminal sexual offender (CSO) database containing over 5000 individuals.

KEYWORDS: forensic science, deoxyribonucleic acid (DNA), restriction fragment length polymorphism (RFLP), match criteria, intragel, intergel, forensic casework

DNA typing of single-locus highly polymorphic variable number of tandem repeats (VNTRs) by restriction fragment length polymorphism (RFLP) has become the preeminent method for identifying potential sources of biological evidence in many forensic laboratories [*1–11*]. An individual's DNA profile can be linked to forensic biological specimens by one of several methods. The most common method involves making a direct comparison between DNA patterns derived from forensic evidence specimens and DNA patterns derived from known blood samples obtained from candidates developed through traditional investigative means. A less common method, but increasingly significant, is searching DNA databases containing profiles obtained from known criminal offenders or casework evidence. The former method has been in use for several years. DNA database searches and comparisons have only recently become a viable method for identifying repeat offenders from forensic biological evidence.

While the establishment of databases containing DNA profiles of criminal offenders has only been in use a short time, there is increasing evidence that these databases will play an important role in the future. As of January 1995, over thirty states had adopted some form of legislation requiring DNA typing of convicted criminal offenders [*12*]. The Federal Bureau of Investigation (FBI) is currently establishing the Combined DNA Index System (CODIS) which will facilitate the storing and searching of DNA profiles at the local, state and national levels. The Minnesota Forensic Science Laboratory (MFSL), a pilot laboratory of CODIS, has been receiving blood samples from convicted criminal sexual offenders (CSO) since January of 1990. To date, more than 5000 CSO DNA profiles, employing six VNTR loci, have been entered into the MFSL CSO database. This database has been searched approximately two hundred times involving non-suspect investigations and has been successful in identifying a suspect on eight separate occasions. In addition, DNA profiles derived from several different cases have been used to link and identify serial offenders. In one notable example, a series of sixteen sexual assaults were linked to one individual. Six of these sixteen assaults were also shown to involve a second individual.

To evaluate potential matches between an individual's DNA profile and the DNA profile derived from an evidentiary sample, a laboratory must establish match criteria which reflect the resolution of the profiling system. This paper describes how match criteria were established, evaluated and implemented at the MFSL. Development of the MFSL match criteria has allowed match declarations to be objectively and quantitatively confirmed over the entire range of the 0.5–22.6 kilobases (kb) molecular weight size marker (Gibco BRL, Gaithersburg, MD).

In performing forensic DNA typing, an examiner compares two DNA patterns and declares a match, a non-match or an inconclusive result. When declaring a match between two DNA patterns, a two-

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[1]Forensic Scientists, Minnesota Bureau of Criminal Apprehension, Forensic Science Laboratory, St. Paul, Minnesota.

The evaluation and implementation of intragel and intergel forensic match criteria for the objective quantitative confirmation of deoxyribonucleic acid (DNA) visual matches spanning the entire range of the molecular weight size marker.

pronged approach has been used [13]. First, a visual examination of the autoradiogram(s) allows the experienced examiner to subjectively compare two individual DNA patterns and declare a match/nonmatch. Visually matching patterns are then further evaluated by computer assisted image analyses. The results of these analyses are subjected to the laboratory's match criteria as a means of applying an objective quantitative evaluation of the match. Factors within each laboratory, such as electrophoretic resolution, protocol, and type of equipment affect the extent of RFLP band measurement imprecision upon which the match criteria are based. Since measurement imprecision varies from laboratory to laboratory, it is necessary that each laboratory establish its own match criteria for the confirmation of a visual match.

Two DNA samples from a common origin will generally show a smaller range of size variation when analyzed on the same gel (intragel) than when analyzed on separate gels (intergel). For this reason, two sets of match criteria were established; intragel and intergel. Both were derived empirically from repetitive measurements and comparisons of RFLP band sizes generated from bloodstain control and forensic type samples of common origin.

The intragel match criteria are used to confirm a visual match by computer assisted image analysis when two samples are analyzed on the same gel. The intergel match criteria serve three different functions. First, they constitute the numerical matching rules used to confirm a visual match by computer assisted image analysis when two samples are analyzed on different gels. Second, they establish the appropriate window for searching a population database in order to determine the proportion of the population that could be potential contributors of an evidentiary sample. Third, the intergel match criteria are used to establish the appropriate window for searching criminal databases for matches.

Match criteria were also established for the different controls routinely analyzed on each gel. These include the K562 cell line and internal blind control samples. In addition, a match criterion was established for the monomorphic locus D7Z2.

Each match criterion was also evaluated with respect to the distance in millimeters between matching bands throughout the 0.5–22.6 kb molecular weight size range. The intragel match criteria that were derived from the empirical data in this study were compared to the intragel match criteria that had been established and in use by the MFSL prior to the initiation of this study.

Materials and Methods

All samples in this study were analyzed on LE agarose 12 × 20 cm gels (in the absence of ethidium bromide) following HAE III restriction digestion according to the MFSL RFLP protocol for DNA analysis [14]. Six polymorphic VNTR probes were utilized in this study: YNH24 (Promega; D2S44), MS1 (Cellmark; D1S7), V1 (Lifecodes; D17S79), TBQ7 (Promega; D10S28), CMM101 (Promega; D14S13) and pH-30 (BRL; D4S139). The monomorphoric probe MGB7 (Oncor; D7Z2) which identifies a repeat 2731 base pair (bp) α satellite DNA fragment [15] was also used.

Approximately 5000 size comparisons were made between each of the following: 1) K562 human cell line band sizes obtained from casework, population and CSO gels and the MFSL K562 mean band sizes and 2) D7Z2 band sizes obtained from casework, population and CSO samples and the D7Z2 published band size value of 2731 bp.

Approximately 5000 intragel and 5000 intergel comparisons were made between the band sizes obtained from casework (bloodstains and vaginal swabs), mock cases (bloodstains, vaginal swabs and semen stains) and environmentally insulted samples that were previously described [14] and band sizes obtained from their corresponding bloodstain standards (controls).

The distance separating two matching bands at various regions of the molecular weight size marker was determined and used to further evaluate the match criteria. This was accomplished by first sizing the 1 mm spaced lines (bands) on a clear plastic ruler placed between two lanes containing BRL size markers that had been previously separated on an analytical gel. Utilizing the individual ruler band sizings, the average number of base pairs per millimeter was determined for various base pair size ranges throughout the BRL size marker. Next, the number of base pairs between two matching bands for a given match criterion was determined for the same base pair size ranges. The number of base pairs between two matching bands was divided by the base pairs per millimeter for that base pair size range. This permitted the maximum distance (in millimeters) between two matching bands to be approximated in any molecular weight size range and still have the two bands fall within a given match criterion.

Results and Discussion

Initially the MFSL employed one match criterion of ±2.5%.[2] Data generated while using this match criterion indicated that a match criterion of ±2.5% was inadequate for assessing band matches above 10 kb. Therefore, the percent differences[3] between corresponding sample and control sizings were evaluated at intervals of 0–2, 2–4, 4–6, 6–8, 8–10, 10–15 and 15–22.6 kb. As a result, it was determined that if three match criteria, ±1.7%, ±3.2% and ±5.8% were utilized for the regions of 0–10, 10–15 and 15–22.6 kb respectively, the assessment of band matches could be achieved over the entire range of the size marker. This intragel match criteria has been in use at the MFSL for the past four years. The purpose of this study, in which hundreds of additional specimens were analyzed, was to: 1) reevaluate the MFSL intragel match criteria; 2) develop intergel match criteria; and 3) establish additional match criteria to be used in evaluating band sizes obtained from control samples and a monomorphic locus probe.

K562 Cell Line

The greatest difference observed across six VNTR loci between the K562 RFLP band sizes obtained from casework, population and CSO gels, and the MFSL K562 mean band size values, was 2.48% (Table 1). Approximately 90% of the K562 band sizes were within 1% of the mean. No significant skewing of the percent difference values (positive or negative) was indicated (+0.06%). The average difference between K562 band sizes and the mean was 0.38% for 786 casework comparisons, 0.50% for 902 population comparisons and 0.54% for 3060 CSO comparisons. From the variation observed, the match criterion for the K562 cell line control was established at ±2.5%.

[2] The percentages are applied to each band, i.e., the total variation between matching bands is twice the stated value.

[3] Specimen allele band size minus standard allele band size divided by the standard allele band size multiplied by 100.

TABLE 1—*Maximum percent differences observed between K562 RFLP band sizes obtained from casework, population, and CSO gels and the MFSL K562 mean band size values. Also shown are the number of comparisons made and the K562 mean band size values.*

Loci	Total number of comparisons	Greatest percent difference from K562 mean band size values			MFSL K562 mean band size values	
		Case work	CSO	Population	Band 1 (bp)	Band 2 (bp)
D1S7	872	1.72	2.46	1.09	4588	4238
D2S44	888	1.03	2.37	1.17	2910	1793
D4S139	860	1.80	2.47	2.35	6478	3451
D10S28	876	1.76	2.26	1.93	1762	1194
D14S13	428	0.91	2.20	1.22	1643	—
D17S79	824	0.98	2.48	1.57	1976	1524

Monomorphic Probe MGB7 (Locus D7Z2)

The greatest difference observed between the D7Z2 band sizes obtained from casework, population, mock case and CSO samples, and the published D7Z2 band size of 2731 bp, was 2.50% (Table 2). The mean band size ranged from 2739 bp (+0.3%) for population samples to 2745 bp (+0.5%) for CSO samples. Therefore, the monomorphic locus band sizes obtained from a variety of forensic type samples were consistent with the published value of 2731 bp. The match criterion for the monomorphic locus (D7Z2) was established at ±2.5%.

Intragel Match Criteria

The intragel match criteria were derived empirically from comparisons of RFLP band sizes obtained from casework, mock cases and environmentally insulted samples with the band sizes obtained from their corresponding bloodstain standards (controls) analyzed on the same gel. Approximately 4600, 400 and 100 intragel comparisons were made in the 0.5–10, 10–15 and 15–22.6 kb regions of the gel respectively. Table 3 summarizes the data obtained from these intragel comparisons. The currently employed MFSL match criteria of ±1.7%, ±3.2% and ±5.8% are slightly less or equal to those suggested by the empirical data in this study ±1.9%, 3.2% and 6.1% respectively, and are, therefore, conservative in evaluating intragel matches. As a result, the data in this study strongly support the continued use of the currently employed MFSL intragel match criteria.

TABLE 2—*Maximum percent differences observed between D7Z2 band sizes obtained from population, mock case, CSO and casework samples and the D7Z2 standard value of 2731 base pairs. The observed average D7Z2 values are also shown and compared to the standard D7Z2 value.*

Sample type	Count	Maximum percent difference from standard	Observed average D7Z2 values in base pairs ([a])
Population	767	1.94	2739 (0.3%)
Mock Cases	345	1.60	2742 (0.4%)
CSO	4191	2.50	2745 (0.5%)
Casework	361	1.50	2740 (0.3%)

[a]Percent difference from standard D7Z2 value.

Intergel Match Criteria

The intergel match criteria serve to confirm a visual match between two samples analyzed on different gels and to establish the appropriate window used to search databases. The intergel match criteria should take into account both forensic specimen measurement variation and database measurement variation. The database variation represents the maximum variation observed when database samples (blood samples) have been analyzed repeatedly on different gels. The forensic variation represents the maximum variation observed when forensic specimens are analyzed repeatedly and compared to known blood samples. In determining the MFSL intergel match criteria, the forensic and database variations were determined separately and then combined to yield the maximum variation that one would expect to see in comparing forensic and database samples. Since the intergel match criteria was determined from the addition of the total maximum ranges of the two variations, it was felt that a reasonable and conservative intergel match criteria was produced. The authors acknowledge that alternative methods may yield equally valid intergel match criteria, however, this method has been verified by its application in actual intergel casework comparisons and in numerous database searches.

Intergel Forensic Specimen Measurement Variation

The intergel forensic specimen measurement variation was derived empirically from over 5000 comparisons between band sizes obtained from mock cases and environmentally insulted specimens and the mean band sizes obtained from their corresponding bloodstain standards. Each of the standard's mean band sizes were determined by analyzing nine separate bloodstains that were prepared from a liquid blood sample. Each bloodstain was analyzed on a separate gel and sized by two different scientists. By using the mean band sizes for the standards, the variation due to the forensic specimens could be closely approximated.

Approximately 4500, 500 and 150 intergel comparisons involving forensic specimens were made in the 0.5–10, 10–15 and 15–22.6 kb regions of the gel respectively. Table 4 summarizes the data obtained from these intergel comparisons. The portion of the total intergel match criteria that can be attributed to the forensic specimen measurement variation is shown in the far right column.

Intergel Database Measurement Variation

The intergel database measurement variation was derived empirically from over 2500 comparisons of band sizes obtained from analyzing duplicate database samples on separate gels.

Approximately 2200, 200 and 100 intergel database sample comparisons were made in the 0.5–10, 10–15 and 15–22.6 kb regions of the gel respectively. Table 5 summarizes the data obtained from these intergel comparisons. The portion of the total intergel match criteria that can be attributed to the database measurement variation is shown in the far right column.

Intergel Match Criteria Determination

Combining the variation attributable to the forensic specimens (±3.1%, ±4.4% and ±10.0%) and database samples

TABLE 3—*Intragel match criteria determinations from the comparisons of specimens and standards at three base pair ranges. Also shown is the maximum differences, standard deviation, mean percent difference between positive and negative values, and the mean difference between specimens and standards.*

Base pair size range (KB)	Number of comparisons	Maximum differences (total)	Standard deviation	Mean of all positive and negative values	Average difference between specimens and standards	Empirical match criteria
0.5–10	4600	+3.67% and −3.89% (7.56%)	0.62	+0.1%	0.5%	±1.9%[a]
10–15	400	+6.17% and −6.72% (12.89%)	2.09	+0.2%	1.6%	±3.2%
15–22.6	100	+15.28% and −9.31% (24.59%)	4.21	+0.6%	3.0%	±6.1%

[a]In deriving the match criterion, the maximum difference in repeated measurements (7.56%) is assumed to arise from equal variation in the two most extreme bands, i.e., +3.8% and −3.8%, or ±1.9% around each band.

TABLE 4—*Intergel forensic specimen measurement variation determinations from the comparison of between specimens and standards at three base pair ranges. Also shown is the maximum differences, standard deviation, mean percent difference between positive and negative values, and the mean difference between specimens and standards.*

Base pair size range (KB)	Number of comparisons	Maximum differences (total)	Standard deviation	Mean of all positive and negative values	Average difference between specimens and standards	Forensic specimen measurement variation
0.5–10	4500	+2.86% and −3.43% (6.29%)	0.47	+0.06%	0.4%	±3.1%
10–15	500	+3.83% and −4.90% (8.73%)	1.22	−0.2%	1.0%	±4.4%
15–22.6	150	+12.03% and −7.75% (19.78%)	3.10	+0.6%	2.4%	±10.0%

TABLE 5—*Intergel database measurement variation determinations from the comparison of duplicate database samples at three base pair ranges. Also shown is the maximum differences, standard deviation, mean percent difference between positive and negative values, and the mean difference between duplicate database samples.*

Base pair size range (KB)	Number of comparisons	Maximum differences (total)	Standard deviation	Average difference between duplicate samples	Database measurement variation
0.5–10	2200	(5.33%)	0.60	0.6%	±2.7%
10–15	200	(8.21%)	1.43	1.3%	±4.0%
15–22.6	100	(18.85%)	3.42	3.3%	±9.4%

(±2.7%, ±4.0 and ±9.4%), the empirical data suggested intergel match criteria (mean of the variation around the forensic band and database band) of ±2.9%, ±4.2 and ±9.7% for the 0.5–10, 10–15 and 15–22.6 kb regions of the gel respectively (Table 6). Based on this empirical data, the MFSL established its intergel match criteria for these regions of the gel at ±3.0%, ±4.2% and ±10.0%.

The match criteria adopted by the MFSL provide a reasonable, conservative means for estimating the frequency of matching alleles in a population database. Also, these match criteria help to ensure that potential matching DNA profiles are not missed when searching criminal databases. In our experiences, these slightly larger match criteria are insignificant in magni-

TABLE 6—*Intergel match criteria determined by combining intergel forensic specimen variation and database measurement variation.*

Base pair size range (KB)	Forensic specimen measurement variation	Database measurement variation	Empirical match criteria (mean variation)
0.5–10	±3.1%	±2.7%	±2.9%
10–15	±4.4%	±4.0%	±4.2%
15–22.6	±10.0%	±9.4%	±9.7%

tude to be of any consequence in confirming intergel visual matches, because matches occur over several highly polymorphic loci and additional loci can be employed to confirm matches. In our laboratory intergel matches are very rarely necessary, because known samples are either run on the same gel with the evidence, or the evidence is retested on the same gel when a suspect is identified at a later time.

Established Match Criteria—Distance

The MFSL intergel and intragel match criteria were compared graphically to a ±2.5% match criterion (Fig. 1). The maximum allowed distance in millimeters between two matching bands for each of the three match criteria were compared at various base pair regions of the gel. The graph of the distance between two matching bands at various base pair size ranges utilizing a ±2.5% match criterion shows the distance between matching bands decreases rapidly between 10 kb and 22.6 kb. As a result, bands that have migrated nearly identically (within 0.5 mm) could fall outside the ±2.5% match window. This shows that fragments with increasingly large differences in measured size will be visually indistinguishable and, therefore, a larger window is needed to assess these visual matches. The data supports the use of a ±3.2% and ±5.8% intragel window and a ±4.2% and ±10.0% intergel window for assessing bands for this purpose. In our laboratory, where six polymorphic loci are routinely employed, the ability to assess bands above 10 kb has been shown to add valuable sizing information in approximately 25% of our cases. Prior to establishing match criteria above 10 kb, this information could not be utilized. Figure 1 illustrates that even with the expanded match windows, the separation between bands above 10 kb generally must be less, than the separation between bands below 10 kb, in order to fall within established match criteria. This shows that the established match criteria above 10 kb are actually very stringent. This is further illustrated and summarized in Fig. 2. Also shown in Fig. 2 is the application of intergel and intragel match criteria in confirming visual matches. (The average match window distance (in mm) shown in Fig. 2 is for comparative purposes only, and is not advocated for use in declaring matches.)

DNA Profile Frequency Determinations

The size of the window used in searching population databases for statistical purposes is derived from the intergel match criteria. However, the size of the search window applied will depend upon the statistical method used for calculating RFLP pattern frequen-

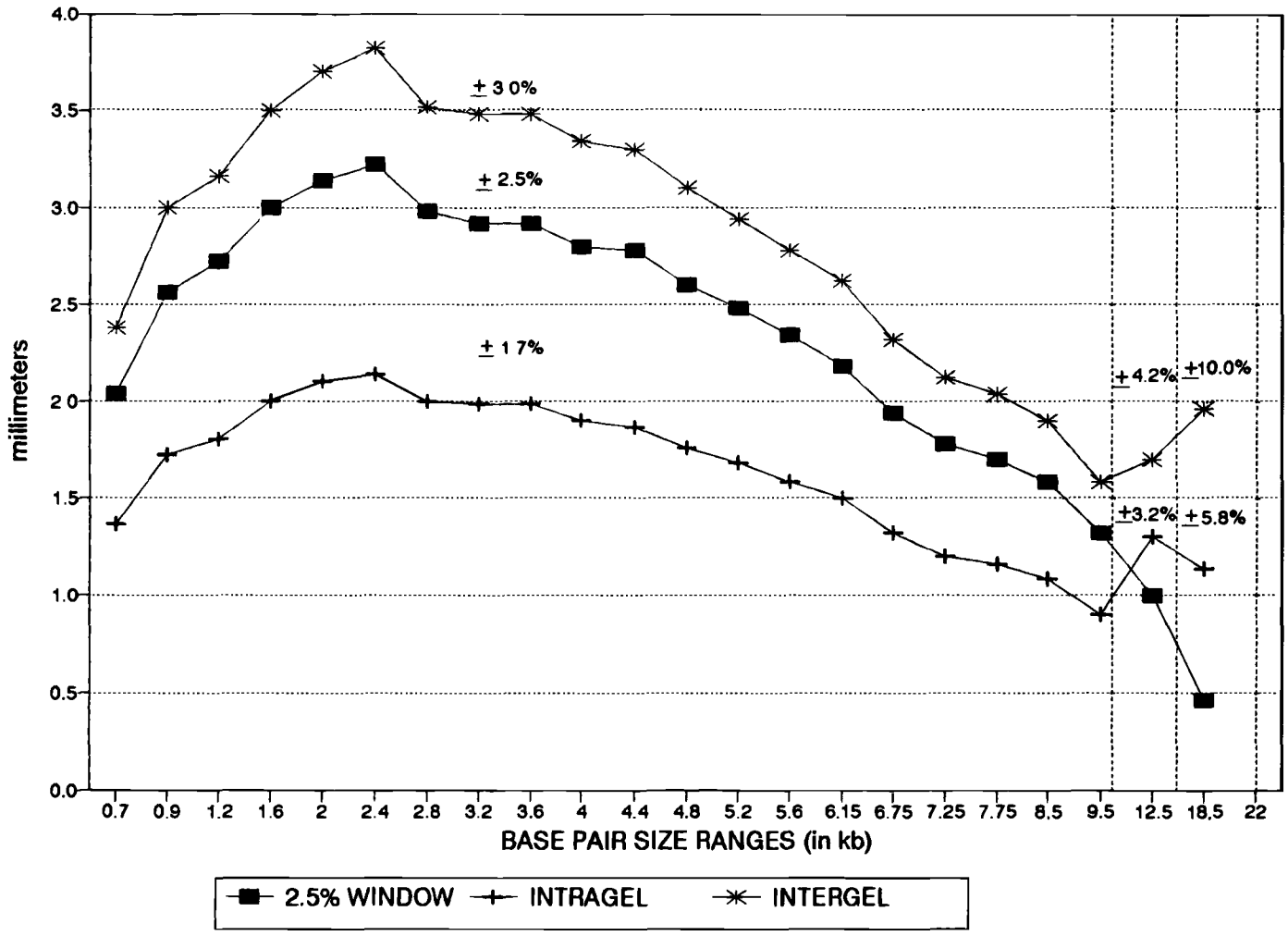

FIG. 1—*The Minnesota Forensic Science Laboratory's intragel and intergel match criteria compared to a ± 2.5% match criterion. Each data point represents the maximum allowed distance in millimeters between two matching bands for each of the three match criteria at various base pair regions of the gel.*

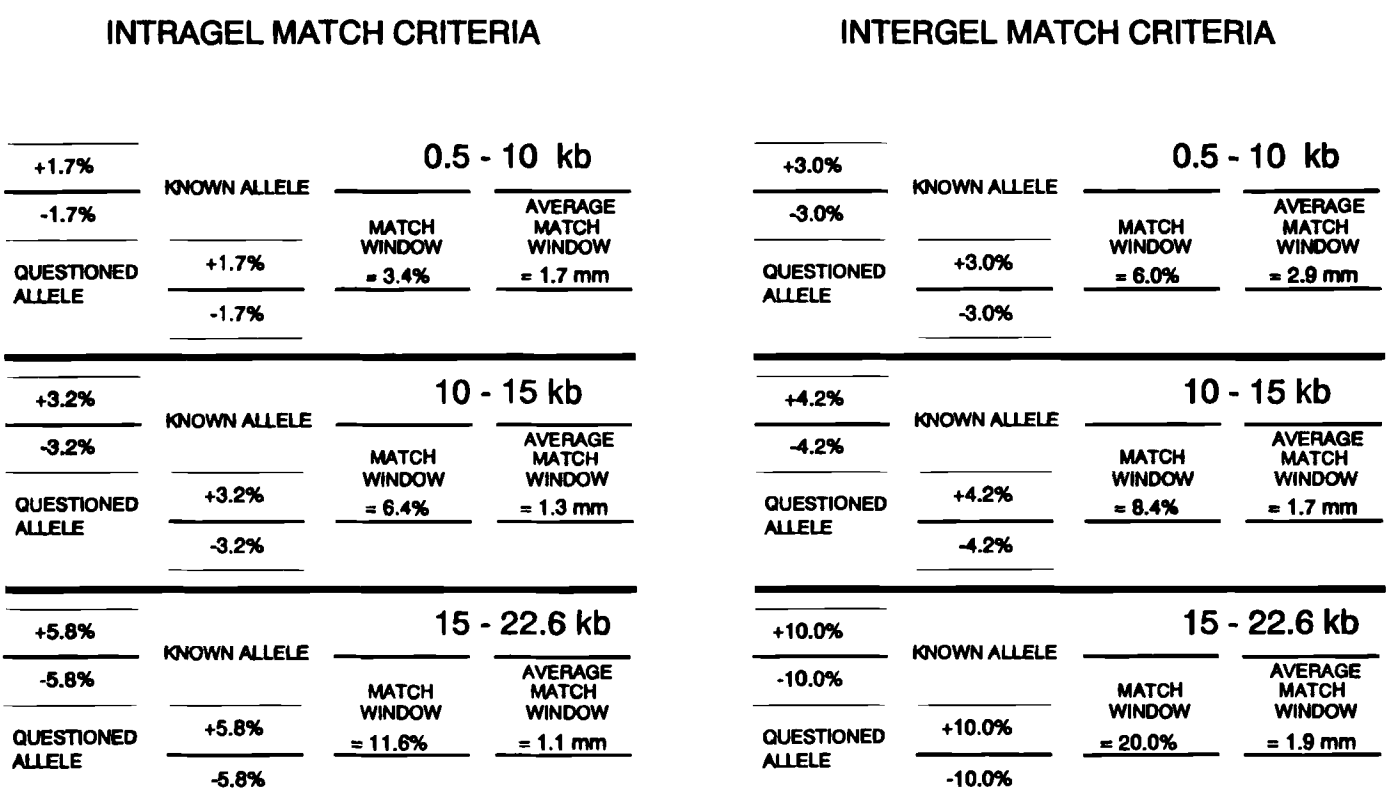

FIG. 2—*An illustration demonstrating the use of intragel and intergel match criteria in determining if two questioned bands fall within established match criteria.*

cies: 1) If the fixed bin approach is used, when the allele from the questioned sample falls within a particular bin, it is assigned that bin's frequency. If after applying the appropriate intergel match criteria to the questioned allele (i.e., ±3% if the allele is in the 0.5–10 kb range) and the allele's range overlaps an adjacent bin, the bin with the higher frequency is selected for statistical purposes [*13*]; 2) If the fixed bin interim ceiling approach is used in accordance with TWGDAM consensus, the allele frequency is computed as described above for the fixed bin approach [*16*], however, if the fixed bin interim ceiling approach is used in accordance with NRC recommendations, the allele frequency is computed by adding the frequencies of all bins that contain any alleles that fall within the window specified by the laboratory's intergel match criteria [*17*]; and 3) If the floating bin approach is used, the intergel match criteria is used to establish a window around the questioned band and each band in the population database. Any population band whose window overlaps the window around the forensic band is counted for statistical purposes. Therefore, when applying the floating bin approach, the total window will be four times the forensic intergel match criterion [*16*]. For example, for an intergel match criterion of ±3.0%, this means a floating window of ±6.0%, for a total window of 12%.

Conclusion

This study demonstrates that suitable match criteria can be established for the confirmation of visual matches over the entire range of the sizing ladder. In addition, the established match criteria provide appropriate windows for searching population databases to determine the proportion of a population that could be potential contributors of an evidentiary sample. Establishing these criteria increases the amount of usable data for forensic RFLP analysis, while maintaining the conservatism desired in forensic testing. Application of these criteria on over 10,000 samples, in more than 500 forensic cases and in over 200 searches of our criminal sexual offender database has shown them to be both practical and valid.

The findings described in this paper clearly demonstrate that consistent and reliable VNTR RFLP profiles can be obtained and compared when samples are analyzed from the same or different electrophoresis gels. The data also confirm the validity of comparing and declaring matches between casework and database generated DNA profiles.

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Address requests for reprints or additional information to
Terry Laber
Minnesota Forensic Science Laboratory
Biology Section
1246 University Ave.
St. Paul, MN 55104