Match and Binning RFLP Procedures of Cellmark
Historical Summary and Current Procedures

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To evaluate any evidence that has been evaluated by the RFLP technique requires a quantitative match criterion. There are many different match criteria currently in use by different forensic laboratories. Even laboratories that by and large use the FBI techniques have often adopted a quantitative match criterion that differs from the FBI's. In this document the current Cellmark match criterion will be summarized along with their method for constructing floating bins. Copies of the Cellmark protocol are also included at the end of the document. Secondly, the match criterion that Cellmark originally used around 1988 and 1989 is reviewed and shown to differ from the current match criteria. Lastly, documentation and discussion of the application of Cellmark's match criteria are provided and shown to be inconsistent with Cellmark's written protocol and standard practice in the forensic community.

Current Match Criteria and Floating Bin Technique

Appendix A contains copies of Cellmark's current match criterion and method for constructing floating bins. From this we note the following. Cellmark has a different quantitative match criterion for compared samples that have been analyzed on the same gel and autorad (intragel) *vs*. those run on different autorads and gels (intergel). In general the intergel match criteria is wider than the intragel match criteria. This is a reasonable state of affairs since there is good empirical evidence that identical samples run on different gels are more likely to be separated by a greater distance than samples on the same gel.

For evidence and known samples on the same gel (most frequent type of comparison) the match criteria for profiles with three or more single-locus probe results (most frequent type of sample) is that they be no more than two resolution units apart. A resolution unit is a term that is original and unique to Cellmark. Originally they were defined as the number of base pairs that cover one millimeter on an autorad (see appendix B). Effectively, Cellmark has created 13 intervals (shown below) with different match criteria. From the table below it is clear that as the size of the DNA fragment gets larger the match criteria gets wider. This is also reasonable since the measurement error is larger for large DNA fragments than it is for small DNA fragments.

Cellmark Resolution Units Defined

Size Range, in kilobases	Resolution unit expressed as a % of band size at midpoint
2.04-3.00	1.15%
3.01-4.07	1.2%
4.08-5.09	1.4%
5.10-6.11	1.7%
6.12-7.13	1.95%
7.14-8.14	2.25%
8.15-9.16	2.35%
9.17-10.18	2.6%
10.19-11.2	2.9%
11.21-12.22	3.4%
12.23-14.33	3.6%
14.34-19.19	4.2%
19.20-25	5.15%

As an example two compared bands that fall in the range 6.12-7.13 kb could differ by no more than 3.9% of their midpoint (assuming intragel samples with three or more single-locus probes).

Once samples have been declared to match the next step in the process is to determine frequencies of these profiles. For these samples what is required is an estimate of how common or rare **matching** DNA fragments are in the population of interest. The Cellmark protocol states that a floating bin should be constructed that is one resolution unit greater than the match criteria. Thus, for intragel matches with three or more single-locus probes this means that the size of the floating bins should be ± 3 resolution units.

Original Cellmark Match Criteria

Appendix B contains a document that was provided to the defense in the 1989 case of California *vs.* Axell, to describe the Cellmark match criteria. At that time Cellmark's match criterion was more conservative. An intragel match required that compared bands fall within one resolution unit and integel comparisons within two resolution units. Additionally, the floating bins size was set to exactly the same size as the match criteria.

Sometime around 1991 Cellmark changed the match criteria and floating bin definitions. The original floating bin sizes summarized in the appendix B memo was criticized as too small. The reason for this is that when floating bins are constructed one then, in effect, searches a database for matching bands. A database can be viewed as a collection of many samples and a large number of different gels and autorads. Thus, according to Cellmark's own definition of matches the search through a database should employ a window that is at least 2 resolution units in size. Whether this criticism was important for the ultimate change in the Cellmark protocol is unclear but nevertheless, Cellmark did make the appropriate change when they increased the size of the floating bin relative to their match criteria.

Inconsistent Application of these Protocols

In case work Cellmark adjusts the size of their floating bins depending whether the match is good or bad. This type of adjustment is not described in their protocol nor is it supported by published research. Worse yet Cellmark is the only forensic laboratory that makes this type of adjustment. In particular if an intragel match has one or more bands with a difference of less than 2 resolution units but greater that 1 resolution unit, Cellmark follows their written protocol. In other words they construct floating bins of three resolution units on all bands to compute frequencies. If all bands in the profile are less than 1 resolution unit different, then Cellmark computes frequencies based on two resolution unit floating bins. In other words the relative rarity of matching profiles is determined by whether the analysts had a good day or a bad day in the lab. This is of course nonsensical. The match criterion is what guides how common or rare matches are. It does not matter if a particular case has evidence and known samples which are exactly the same size or are all at the two resolution limit, the frequency of finding matches in the population is not affected.

The absurdity of this practice is illustrated dramatically in the case of Colorado *vs* Harlan. In this case several samples were run on a single gel. On set of samples required a two resolution unit match criterion and the other set a one resolution unit match criterion. The report from this case is reproduced in appendix 3. A summary of the evidence and contrasts is given below.

Evidence and Known Samples (Colorado vs. Harlan)

Evidence Samples		
01		Vaginal swab
	X1	Female cells
	X2	Male fraction
03		Gun stain
04		Sweat pants
Knowns		
05		Victim
06		Suspect Harlan

Cellmark called a match between 03 and 05 and between 01X1 and 05 using a one resolution unit match criteria. They then constructed three resolution unit floating bins and the five-locus match probability was 1 in 6.5 million (Caucasian). Cellmark also declared a match between 06 and 01X2 but stated this was with a one resolution unit match criteria. Thus, the frequency for this match was computed using two resolution unit floating bins and was reported to be 1 in 590 billion. Although the profiles are different (05 vs. 06) the major reason for the huge difference is the different size floating bins.

ZENECA

CELLMARK DIAGNOSTICS

Title : Match Criteria for Forensic Cases

File Location : SOPs

Originator : R. Cotton
L. Forman

Modification Number : 0

Date of Issue : JAN 0 1 1992

Approvals

Function	Signature	Date
Laboratory Director	David W. Stern	12-20-91
Quality Assurance	Kathleen Sheridan	12/19/91

Date Written : August 7, 1991

Title : Match Criteria for Forensic Cases

File Location : SOPs | **Originator : R. Cotton, L. Forman**

Modification Number : 0 | **Date of Issue :** JAN 0 1 1992

1. DNA fragment sizes (also known as DNA band sizes) obtained with the Bio Image Visage 60 are used in conjunction with the database program to determine band matches. The following criteria have been established as necessary to determine whether DNA banding patterns from different samples match. NOTE: See Appendix A for the definition of a resolution unit.

   a. **Match Criteria for intragel comparison:** The calculated DNA fragment sizes from the two samples must agree within plus or minus one resolution unit when only the single-locus cocktail autoradiograph is available or within plus or minus two resolution units when at least three individual single-locus probe results are available for analysis.

   b. **Match Criteria for intergel comparison:** The calculated DNA fragment sizes from the two samples must agree within plus or minus two resolution units when only the single-locus cocktail autoradiograph is available or within plus or minus three resolution units when at least three individual single-locus probe results are available for analysis.

2. Following the instructions for appropriate data entry, the database program is used to obtain band size ranges for matching.

3. When comparing band patterns among samples, results can usually be classified as one of four types:

   a. **Match:** All bands present in the unknown sample have matching bands in the standard sample.

   b. **Partial Match:** The banding patterns obtained from the standard and the unknown are very similar, and the bands in common match by the match criteria. This includes those cases where partial DNA banding patterns are obtained from the unknown due to small quantities of DNA or degradation of the sample.

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c. **Inconclusive:** No banding pattern was obtained from either the standard sample or the unknown sample or the banding pattern was unsuitable for comparison. Banding patterns which have shifted outside the match criteria and some cases of partial degradation may also be included in this section.

d. **No Match:** The bands present in the unknown do not have matching bands in the standard sample(s) and the samples are suitable for comparison.

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CELLMARK DIAGNOSTICS

Title: Statistical Analysis of DNA Band Patterns for Forensic Cases

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File Location : SOPs

Originator : R. Cotton
L. Forman

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Approvals

Function	Signature	Date
Laboratory Director	Robin Cotton	8-25-95
Quality Assurance	[signature]	8-25-95

Date Written : August 7, 1991 **P037**
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Modified By: Lisa Forman, Robin Cotton, Charlotte Word
c:\sops.all\p037.01

Title: Statistical Analysis of DNA Band Patterns for Forensic Cases

File Location : SOPs

Originator : R. Cotton
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Modification Number : 1

Date of Issue : 8/28/95

1. For identity testing:

   Following the instructions for appropriate data entry, frequencies for each band are obtained as a printout from the database program. When determining band frequencies, the bin size used is one resolution unit greater than the resolution unit used in matching. (In other words, when a discrete band size is entered into the database program, the calculated frequency is the sum of the frequencies of all bands in the relevant database that fall within the match criteria resolution unit range PLUS one additional resolution unit range.) Calculating frequencies for samples with matching DNA band patterns is customarily done as follows: (NOTE: See Appendix A for additional information about calculations and Appendix B for additional information about the database program and sample database program printouts forf HinfI and Appendix C for HaeIII.)

   a. Individual single-locus probes (SLPs) are used to generate a probability statistic based on the frequency of a phenotype in a population. Using the relevant population database, the heterozygosity value (2pq) for the known bands is calculated for each probe. When this value can be determined for each of the SLPs, they are multiplied together to produce the overall frequency of all loci. The inverse of the allele frequency is the probability of phenotypic identity, i.e., it is an estimate of the likelihood that any two unrelated people would share this pattern at all loci.

   b. In a situation where only a single-locus cocktail (SLC) and no individual SLPs are available, a cocktail frequency is calculated using the appropriate population

database. For each band observed, the frequency for all loci in that cocktail are summed. The summed freqencies for each of the observed bands is then multiplied together. This value is multiplied by 2^n (where "n" is the number of loci used in the cocktail, and represents the observation of heterozygosity at each of those loci). This statistic considers that each band in the SLP cocktail is a member of a heterozygous set even though a total of two bands per probe in the cocktail may not be visible.

c. In cases in which some, but not all individual SLPs could be determined, the following method uses the assigned SLPs combined with the unassigned SLPs (i.e., a cocktail): Frequencies are determined from the appropriate database for bands identified by known SLP as in part (a.). A cocktail frequency is calculated for those bands of unassigned origin as in part (b.). The summed cocktail bands are multiplied together with the frequencies for the assigned bands, and then multiplied by 2^n as in part (b.) to arrive at a probability based on bands from assigned and unassigned loci.

NOTE (for HinfI): When comparing matching banding patterns between DNA from evidence and standard samples, the frequency of the DNA banding pattern is calculated for every lane that has a band pattern. These frequencies are compared and the lowest one is reported.

2. For biological relationship testing:

a. Using SLPs: Single-locus probes can be used in estimating the probability that a genetic relationship exists between biological evidence and one or more known

CELLMARK
DIAGNOSTICS

TO: Bruce Budowle

DATE: June 14, 1989

FROM: Robin W. Cotton

SUBJECT: Match Criteria and Bin Definition

Establishing criteria for band matching and defining DNA fragment size ranges for use in determining allele frequencies are necessary procedures for quantitative assessement of DNA typing data. To accommodate the change in resolution inherent in electrophoresis, we have defined the resolution capability of our gel system in 1kb increments for the length of the gel. These data are used to define a match vs. non-match of two samples and to establish frequency binning criteria for intra- and inter-gel pattern comparisons.

The ability of the gel system to show a distinct separation of two bands whose sizes are close together decreases from the bottom to the top of the gel. The local limit of resolution in our gel system is defined by measuring the mm distance between successive bands of the 1kb ladder (BRL) and dividing this measurement into the difference in BP between the two adjacent bands. This figure is expressed as a percentage of the midpoint (in BP) of each range and estimates the number of BP resolved in 1 mm distance of the gel.

$$\frac{\dfrac{F_i - F_{i-1}}{M_i - M_{i-1}}}{\dfrac{F_i - F_{i-1}}{2} + F_{i-1}}$$

F = fragment size in BP
M = migration distance in mm

= resolution limit expressed as a % of midpoint of interval

In making a comparison between two DNA patterns from the same gel, the DNA fragment sizes are calculated using the method of Elder and Southern. Two sizes are said to match if one is within either plus or minus one resolution limit of the other. In terms of the original measurements of migration distance this requirement corresponds to a 1 mm distance between compared bands. When a comparison of patterns on two separate gels is necessary, the bands being compared must fall within 2 times the local limit of resolution.

The frequencies of all bands in the range $\pm$ the resolution limit are summed to calculate the allele frequency of a given band. Measurements of two matching patterns are made by two scientists and the most conservative final frequency is reported.

002483

REPORT OF LABORATORY EXAMINATION

ZENECA

July 6, 1994

CELLMARK DIAGNOSTICS

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Re: Cellmark Case No. F941085
CBI Laboratory Case No. D94-0462

It was previously stated in the Report of Laboratory Examination dated April 27, 1994, that using the four single-locus probes MS1, MS31, MS43, and g3, a DNA banding pattern was obtained from the vaginal swab which was similar to the DNA banding pattern obtained from the blood swatch labelled Rhonda Maloney. Further testing using the four single-locus probes sequentially has shown that this DNA banding pattern obtained from the vaginal swab matches the DNA banding pattern obtained from the blood swatch labelled Rhonda Maloney.

It was also stated in the Report of Laboratory Examination dated April 27, 1994, that using the four single-locus probes MS1, MS31, MS43, and g3, the DNA banding pattern obtained from the green material cutting labelled sweatpants was similar to the DNA banding pattern obtained from the blood swatch labelled Rhonda Maloney and that further testing was ongoing. Further testing using the four single-locus probes sequentially has shown that the DNA banding pattern obtained from the green material cutting labelled sweatpants matches the DNA banding pattern obtained from the blood swatch labelled Rhonda Maloney.

It was further stated in the Report of Laboratory Examination dated April 27, 1994, that using the four single-locus probes MS1, MS31, MS43, and g3, a DNA banding pattern was obtained from the vaginal swab which matches the DNA banding pattern obtained from the blood swatch labelled Robert Harlan, and the DNA banding pattern obtained from the stained swatches labelled gun matches the DNA banding pattern obtained from the blood swatch labelled Rhonda Maloney.

Further testing using the single-locus probe YNH24 (D2S44) was performed on the vaginal swab, the labial swab, the stained swatches labelled gun, the green material cutting labelled sweatpants, the blood swatch labelled Rhonda Maloney, and the blood swatch labelled Robert Harlan.

Using the probe YNH24, three DNA bands were obtained from the vaginal swab. One of these three bands matches the one DNA band obtained from the blood swatch labelled Rhonda Maloney. The remaining two DNA bands obtained from the vaginal swab match the two DNA bands obtained from the blood swatch labelled Robert Harlan.

Using the probe YNH24, one DNA band was obtained from the green material cutting labelled sweatpants. This DNA band matches the one DNA band obtained from the blood swatch labelled Rhonda Maloney.

Using the probe YNH24, no DNA bands were obtained from the labial swab or from the stained swatches labelled gun. The inability to visualize bands may be due to an insufficient quantity of DNA isolated from these samples.

Using the five single-locus probes sequentially, the approximate frequencies in the Caucasian, African American, and Western Hispanic populations of the DNA banding pattern obtained from the vaginal swab and the blood swatch labelled Robert Harlan are as follows:

Population Database	Frequency
Caucasian	1 in 590 billion
African American	1 in 1.8 billion
Western Hispanic	1 in 860 billion

Using the five single-locus probes sequentially, the approximate frequencies in the Caucasian, African American, and Western Hispanic populations of the DNA banding pattern obtained from the vaginal swab, the green material cutting labelled sweatpants, and the blood swatch labelled Rhonda Maloney are as follows:

Population Database	Frequency
Caucasian	1 in 6.5 million
African American	1 in 80 million
Western Hispanic	1 in 46 million

Using the four single-locus probes (MS1, MS31, MS43, and g3) sequentially, the approximate frequencies in the Caucasian, African American, and Western Hispanic populations of the DNA banding pattern obtained from the stained swatches labelled gun and the blood swatch labelled Rhonda Maloney are as follows:

Population Database	Frequency
Caucasian	1 in 2.6 million
African American	1 in 18 million
Western Hispanic	1 in 12 million

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