National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2390

DNA Profiling Standard

Standard Reference Material (SRM) 2390 is intended for (1) standardization of forensic and paternity quality assurance procedures for Restriction Fragment Length Polymorphisms (RFLP) testing using *Hae*III restriction enzymes, and (2) instructional law enforcement or nonclinical research purposes. It is **NOT** intended for any human/animal clinical diagnostic use.

SRM 2390 was originally certified in 1992 using measurements performed during 1990 through 1991 [1]. While RFLP allelic band size is nominally equal to the number of nucleotide base pairs (bp) in a DNA fragment, measured band size is a protocol-defined quantity [2]. The RFLP measurement protocols recommended by the Royal Canadian Mounted Police [3], the U.S. Federal Bureau of Investigation [4], and the Scientific Working Group for DNA Analysis Methods [5] are sufficiently similar to provide validly comparable values [6]. This certificate updates the band size values of the original SRM 2390 certification to reflect the evolution of forensic practice from 1991 to 1998.

Each SRM 2390 unit consists of 20 components. Quantitative allelic band sizes are provided for human DNA from two sources: (1) the female cell line K562, and (2) the male source "TAW." Three different forms of material from the two sources are provided: (1) cell pellet, (2) extracted genomic DNA, and (3) a *Hae*III restriction digest "pre-cut" DNA. The remaining components are well-characterized consumable materials required for qualitative evaluation of the *Hae*III RFLP measurement process. These components include standards for quantifying extracted DNA by use of yield gels, a DNA ladder for band size determination, materials for labeling the DNA size ladder, a viral DNA marker for assessment of electrophoretic separation, and agarose that is compatible with all DNA components. See Components and Storage on page 2 of this certificate for a detailed listing of the components.

Expiration of Certification: When stored as specified in the Components and Storage section on page 2, the certified values should remain within their uncertainty limits for at least five years from the date of shipment. NIST will keep samples of this material under surveillance for five years after the date of last sale. If changes occur beyond the limits certified, NIST will notify purchasers.

The technical aspects of the recertification process were coordinated by D.J. Reeder formerly of the NIST Biotechnology Division. Material stability studies and coordination of the interlaboratory study were performed by K.L. Richie of the NIST Biotechnology Division. Data analyses was provided by D.L. Duewer of the NIST Analytical Chemistry Division.

Statistical consultation on aspects of these analyses was provided by H-k. Liu of the NIST Statistical Engineering Division.

The support aspects involved in the recertification of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert.

Gary L. Gilliland, Chief
Biotechnology Division

Gaithersburg, MD 20899
Certificate Issue Date: 2 February 2001
See Certificate Revision History on Last Page

Nancy M. Trahey, Chief
Standard Reference Materials Program

All measurements used in the recertification of allelic band sizes of SRM 2390 K562 and TAW components were performed by personnel of the laboratories listed in Appendix A.

Certified Band Sizes: Tables 1 and 2 list certified values (expected allelic band sizes and sizing uncertainty) for band size measurements of the cell pellet and extracted genomic SRM 2390 K562 and TAW DNA at six commonly probed genetic loci: D1S7 [7], D2S44 [8,9], D4S139 [10], D5S110 [11], D10S28 [12], and D17S79 [13]. Due to strong measurement-related correlation among allelic bands for a given sample, sizing uncertainty is defined for the pair of allelic bands at these six genetic loci. Two sets of certified values are provided: (1) a set reflecting measurement practice at the beginning of 1998, based only on results of a NIST-conducted interlaboratory comparison [14], and (2) a set reflecting measurement practice from 1991 through 1997 [15]. These certified allelic band size regions are presented in graphical form in Figures 1a through 1f.

Informational Band Sizes: Table 3 lists informational values for SRM 2390 K562 and TAW DNA band sizes at six less commonly probed genetic loci: D7S467 [16], D7Z2 [17], D8S358 [18], D14S13 [9], D17S26 [19], and DYZ1 [20].

NOTICE AND WARNING TO USER

The K562 cell line was derived from a human subject with a diagnosis of chronic myelogenous leukemia [19,20]. The TAW male DNA components are derived from blood cells from a healthy human; these materials have been tested and found to be nonreactive for hepatitis B surface antigen (HB_sAG) and HIV by FDA-approved testing. No test method can ensure that human-derived material does not contain infectious agents. **HANDLE AS IF CAPABLE OF TRANSMITTING DISEASE.**

Components and Storage: Each unit includes 20 components, 19 of which contain material that must be kept frozen.

Box A: (Store at -20 °C)

- #1 Molecular Weight Marker DNA
- #2 Molecular Weight Marker Dilution
- #3 Molecular Weight Marker Probe
- #4 DNA Klenow Fragment
- #5 Stop Solution
- #18 Adenovirus Visible Ladder
- #19 10X Buffer (used with components #12, #13, #15, #16, #17, and #18)

Attached to Box A: (Shipped at -20 °C, but store at room temperature)

- #20 Agarose

Box B: (Store at -20 °C)

- #6 250 ng DNA standard
- #7 100 ng DNA standard
- #8 50 ng DNA standard
- #9 25 ng DNA standard
- #10 12.5 ng DNA standard
- #11 6 ng DNA standard

Box C: (Store at -70 °C)

- #12 K562 Cell Pellet (3×10^6 cells)
- #13 K562 Undigested DNA (232 ng/µL)
- #14 K562 DNA, *Hae*III Digest (25 ng/µL, premixed with loading buffer)
- #15 TAW Male Cell Pellet (3×10^6 cells)
- #16 TAW Male Undigested DNA (200 ng/µL)
- #17 TAW Male DNA, *Hae*III Digest (25 ng/µL)

SOURCE AND ANALYSIS

Stability: All components except the three noted below were found unchanged from the original certification upon retesting in 1998. Due to long term storage and possible desiccation at –20 °C, component #1 may contain less than its stated 30 µL volume. The total amount of molecular weight marker DNA has neither changed nor degraded. If you have received an SRM with component #1 having reduced volume, reconstitute to 30 µL with distilled/deionized water.

While the band sizes for the precut SRM 2390 K562 and TAW DNA components are qualitatively the same as their non-precut analogues, they yield band sizes that are quantitatively distinguishable. The magnitude of the difference appears to have increased from 1991 to 1997 [14]. Therefore, components #14 and #17 should be used for qualitative evaluations only.

Source of Material: The TAW male materials and the 10X buffer were provided by Analytical Genetic Testing Center, Inc., Denver, CO. The K562 female materials and all other components of the SRM were provided by Life Technologies, Inc., Rockville, MD.

Certified Values for Forensic Practice as of 1997: The certified allelic band sizes in Table 1 are derived from analyses performed at 20 forensic laboratories from Fall 1997 through Spring 1998. The participants in this study are listed in Appendix A. These participants were requested (1) to use as many components of the SRM 2390 unit as were compatible with their routine RFLP analysis systems, (2) to analyze the SRM 2390 K562 and TAW materials using as close to their routine casework protocols as possible, and (3) to report allelic band sizes for all genetic loci they routinely probe. Many participants supplied copies of the autoradiograms and/or chemilumigrams resulting from their analyses. Images of a "representative" set of chemilumigrams are provided in Appendix B.

Because these data are representative of a maximum of 20 laboratories, the sizing uncertainty is expressed as a 95 %/95 % tolerance region. NIST certifies, with 95 % confidence, that 95 % of all measurement pairs at a given locus that are validly characteristic of forensic practice on or about the end of 1997 will be bounded by the given ellipse. In addition to numeric specification in Table 1, these tolerance ellipses are defined by the thin black line in Figures 1a through 1f. A detailed description of the analysis and interpretation of results from this interlaboratory comparison exercise has been published [14].

Certified Values for Forensic Practice from 1991 through 1997: The certified allelic band sizes in Table 2 are based upon all relevant RFLP band sizing data available, including non-SRM 2390 K562 information from proficiency tests conducted from 1991 through 1997 and well documented empirical relationships [6,23-25].

Since these certified values are based upon a large body of data, the sizing uncertainty is expressed as a 99 % tolerance interval. NIST certifies that 99 % of all measurement pairs, at a given locus that are validly characteristic of forensic practice from 1991 through 1997, will be bounded by the given ellipse. In addition to numeric specification in Table 2, these tolerance ellipses are defined by the thick black line in Figures 1a through 1f. A detailed description of the analysis and interpretation of results from this interlaboratory comparison exercise has been published [15].

Table 1. Certified Values for "1997-Equivalent" Band Size Measurements of SRM 2390 Cell Pellet and Extracted Genomic Components

			K562					TAW				
Locus	n_{97}	$K^{n_{97}}_{bi_{95/95}}$	$\overline{X}_1$ (bp)	$\overline{S}_1$ (bp)	$\overline{X}_2$ (bp)	$\overline{S}_2$ (bp)	$\overline{R}$	$\overline{X}_1$ (bp)	$\overline{S}_1$ (bp)	$\overline{X}_2$ (bp)	$\overline{S}_2$ (bp)	$\overline{R}$
D1S7	20	11.9	**4585**	29.9	**4237**	23.6	0.88	**7773**	63.2	**6886**	41.3	0.82
D2S44	20	11.9	**2905**	19.5	**1788**	14.3	0.81	**3711**	23.2	**1288**	8.7	0.65
D4S139	20	11.9	**6474**	49.2	**3440**	22.2	0.78	**10854**	117	**8185**	64.7	0.71
D5S110	17	13.2	**3700**	23.2	**2926**	17.4	0.85	**3343**	20.4	**1444**	11.5	0.60
D10S28	19	12.3	**1754**	11.4	**1180**	9.7	0.83	**3935**	24.5	**1788**	10.2	0.82
D17S79	15	14.5	**1979**	14.8	**1514**	11.1	0.88	**1753**	11.2	**1515**	11.7	0.95

Two allelic bands are expected for each of these six genetic loci. Designate the larger of the two band size measurements at a given locus as x_1 and the smaller as x_2. With 95 % confidence, 95 % of "1997-valid" $\{x_1,x_2\}$ measurement pairs for the cell pellet and extracted genomic components of SRM 2390 will be within the 95 %/95 % tolerance ellipse:

$$\frac{\left(\frac{x_1-\overline{X}_1}{\overline{S}_1}\right)^2+\left(\frac{x_2-\overline{X}_2}{\overline{S}_2}\right)^2-2\overline{R}\left(\frac{x_1-\overline{X}_1}{\overline{S}_1}\right)\left(\frac{x_2-\overline{X}_2}{\overline{S}_2}\right)}{1-\overline{R}^2}\le K^{n_{97}}_{bi_{95/95}}$$

where n_{97} is the number of participants reporting data for each genetic locus.

Details on the construction of these tolerance ellipses and estimation of the critical values are provided in Reference [14]. Figures 1a through 1f state these certified band sizes (the location of the "×" in the figures) and 95 %/95 % tolerance region (the region inside the black ellipse) for these six loci in graphical form.

The DNA Advisory Board STANDARD 9.5 states, "The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST Standard Reference Material or standard traceable to a NIST standard." *We suggest that each user of this SRM plot their own band size measurements for each locus using an appropriate computer graphics system or a copy of the enclosed figures.* Preparation of such plots is an appropriate "check [of] DNA procedures." See Reference [14] for further details.

Table 2. Certified Values for "1991 to1997-Equivalent" Band Size Measurements of SRM 2390 DNA Components

		K562					TAW				
Locus	$K_{bi_{99}}$	$\overline{X}_1$ (bp)	$S(\overline{X}_1)$ (bp)	$\overline{X}_2$ (bp)	$S(\overline{X}_2)$ (bp)	$R(\overline{X}_1,\overline{X}_2)$	$\overline{X}_1$ (bp)	$S(\overline{X}_1)$ (bp)	$\overline{X}_2$ (bp)	$S(\overline{X}_2)$ (bp)	$R(\overline{X}_1,\overline{X}_2)$
D1S7	14.2	**4583**	39	**4234**	36	0.82	**7768**	82	**6881**	65	0.69
D2S44	14.2	**2912**	19	**1792**	13	0.69	**3712**	26	**1291**	12	0.42
D4S139	14.2	**6505**	50	**3447**	22	0.54	**10862**	174	**8200**	91	0.64
D5S110	14.2	**3720**	24	**2941**	22	0.79	**3343**	24	**1444**	13	0.48
D10S28	14.2	**1758**	12	**1185**	11	0.63	**3929**	28	**1788**	14	0.50
D17S79	14.2	**1984**	14	**1522**	13	0.72	**1755**	14	**1517**	13	0.68

With high confidence, 99 % of "1991 to 1998-equivalent" $\{x_1,x_2\}$ measurement pairs for K562 and TAW DNA will be within the 99 % tolerance ellipse:

$$\frac{\left(\frac{x_1-\overline{X}_1}{S(\overline{X}_1)}\right)^2+\left(\frac{x_2-\overline{X}_2}{S(\overline{X}_2)}\right)^2-2\overline{R}\left(\frac{x_1-\overline{X}_1}{S(\overline{X}_1)}\right)\left(\frac{x_2-\overline{X}_2}{S(\overline{X}_2)}\right)}{1-R(\overline{X}_1,\overline{X}_2)^2}\le K_{bi_{99}}$$

Details on the construction of these tolerance ellipses and estimation of the critical values are provided in References [14] and [15]. Figures 1a through 1f state these certified band sizes (the center of the plot) and 99 % tolerance region (the region inside the black ellipse) for these six loci in graphical form.

Table 3. Information Values for Additional Loci

		K562		TAW	
Locus	n_{97}[a]	$\overline{X}_1$ (bp)	$\overline{X}_2$ (bp)	$\overline{X}_1$ (bp)	$\overline{X}_2$ (bp)
D7S467	5	**4677**	**3217**	**4496**	**4339**
D7Z2[b]	1	**2736**		**2747**	
D8S358	1	**5878**	**1303**	**3417**	**2383**
D14S13[c]	2	**1642**		**1579**	
D17S26	1	**4823**	**1358**	**5514**	**4852**
DYZ1[d]	1			**3571**	

[a] the number of participants in the 1997 to 1998 NIST conducted interlaboratory comparison exercise who reported data for each genetic locus. Where more than one participant reported data, the information values are the median of the band sizes reported.

[b] human-specific monomorphic locus having a known sequence of length 2731 bp

[c] both K562 and TAW are apparent homozygotes at this locus

[d] Y chromosome locus

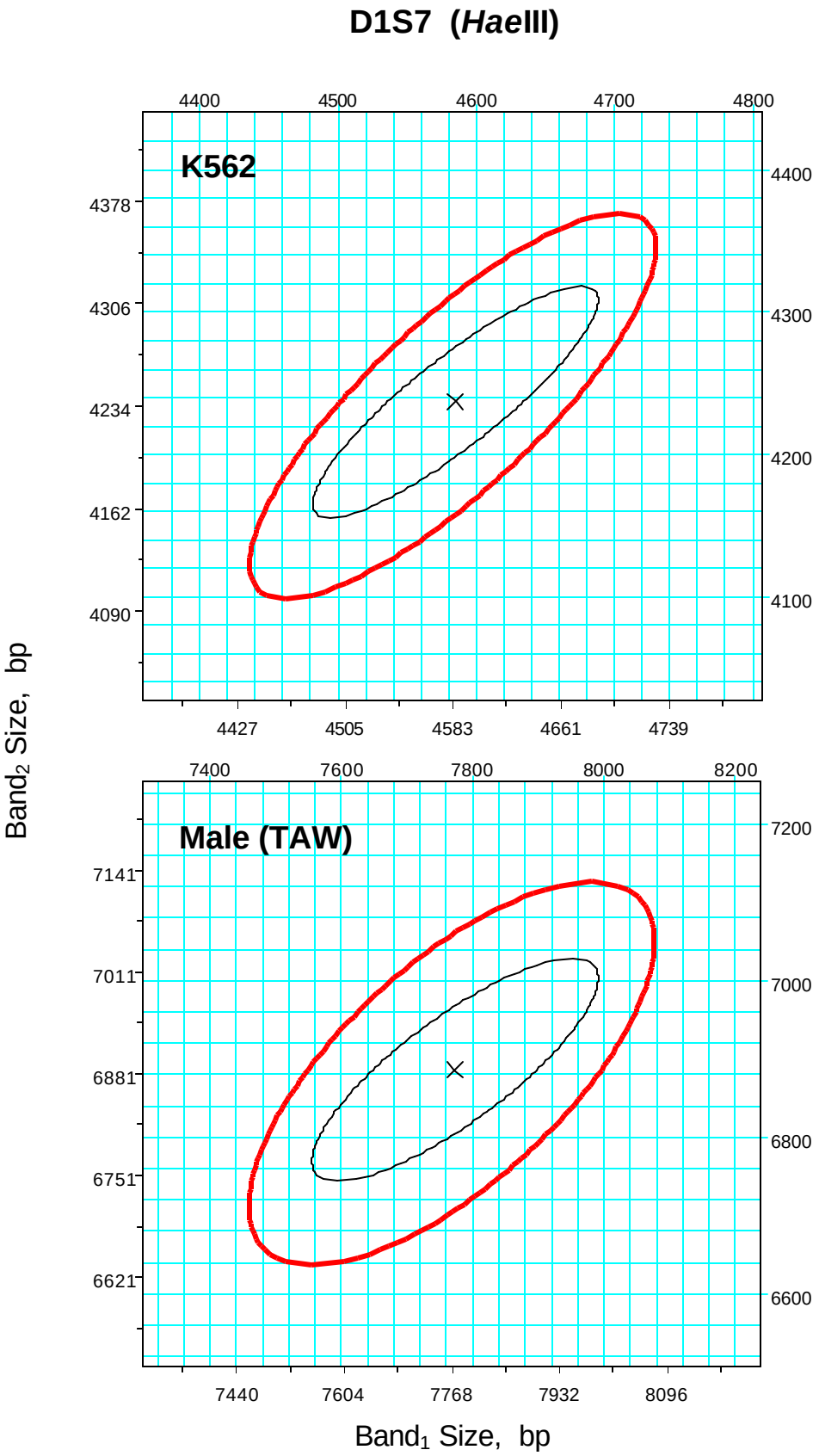

Figure 1a. D1S7 Tolerance Ellipses

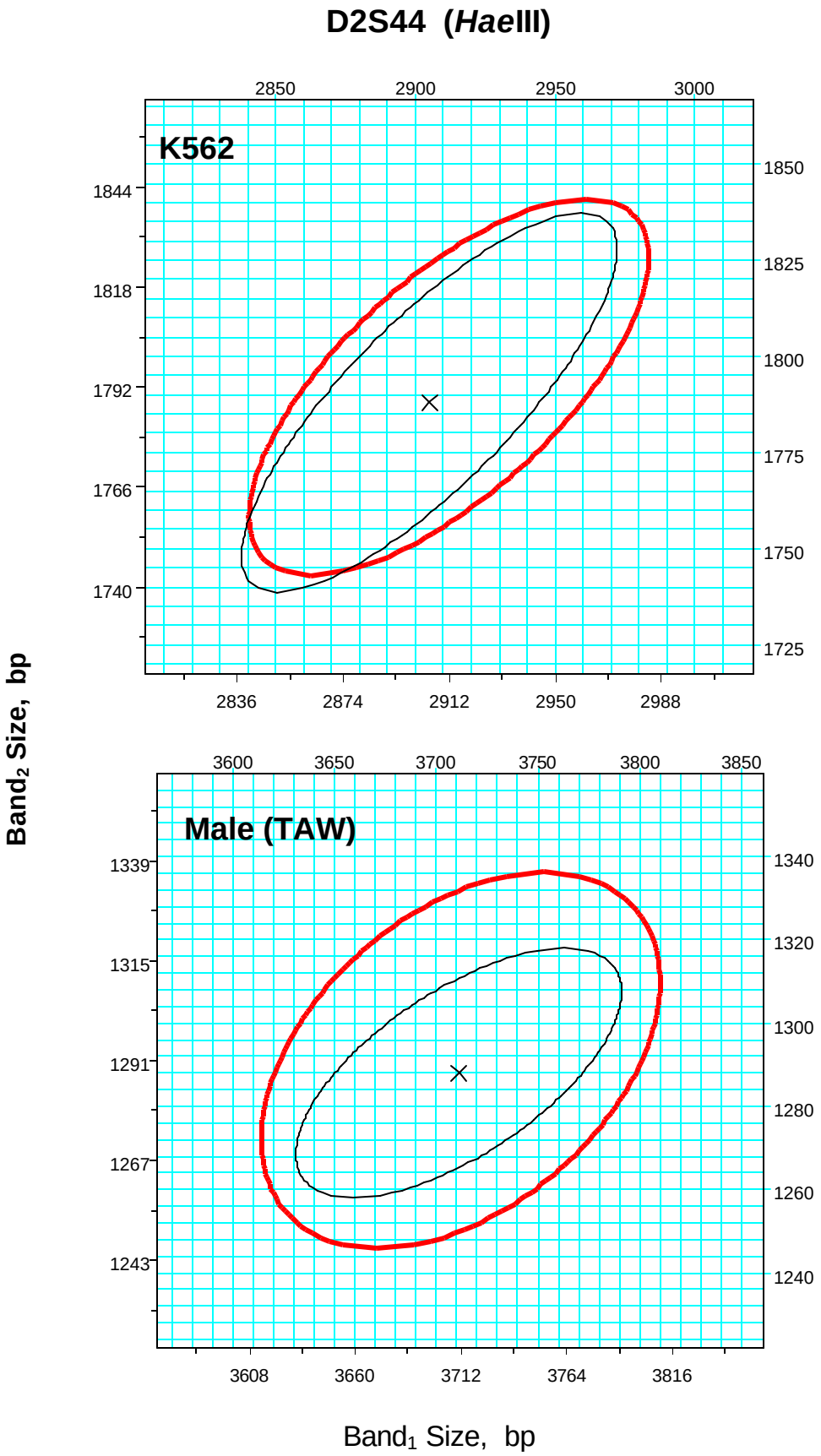

Figure 1b. D2S44 Tolerance Ellipses

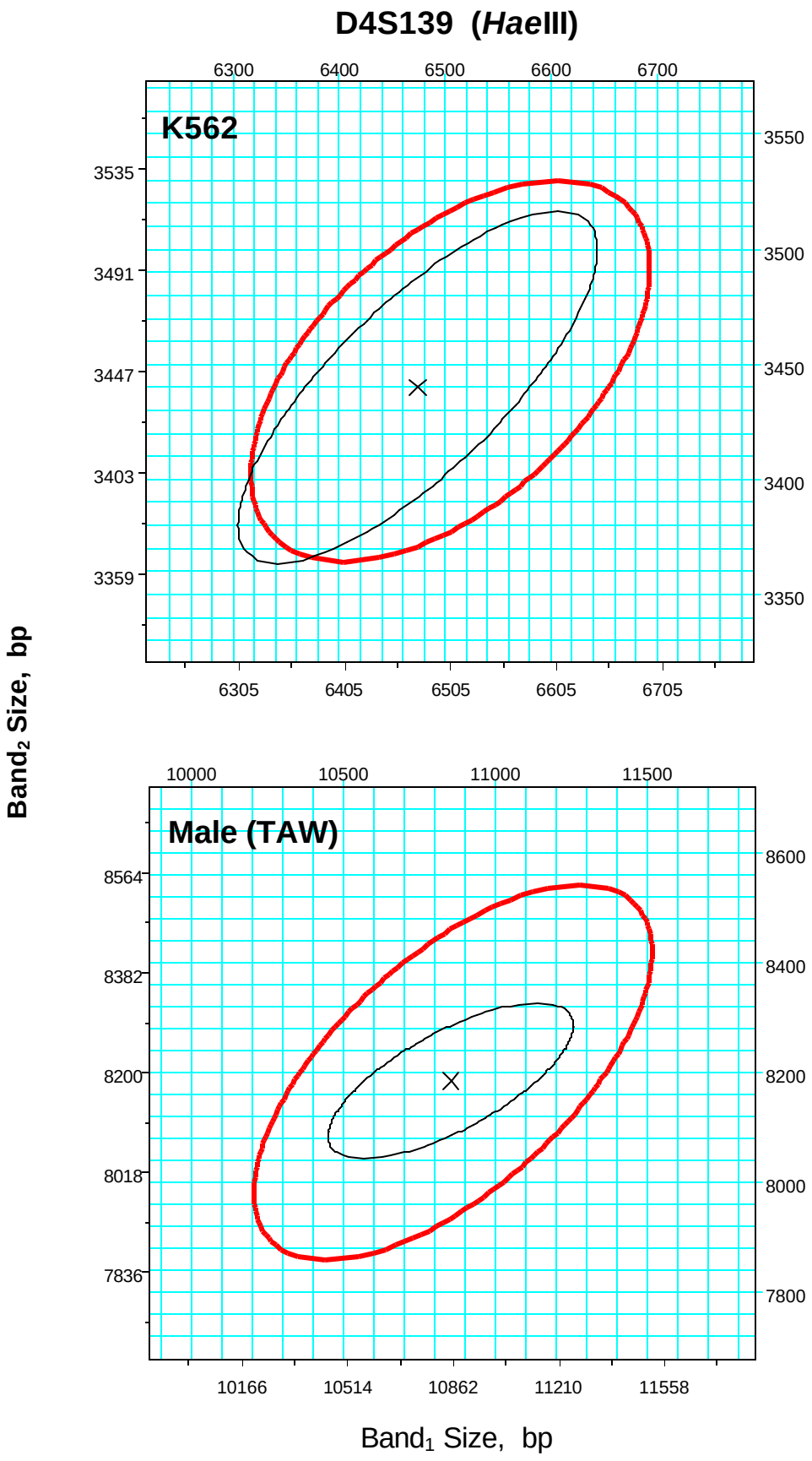

Figure 1c. D4S139 Tolerance Ellipses

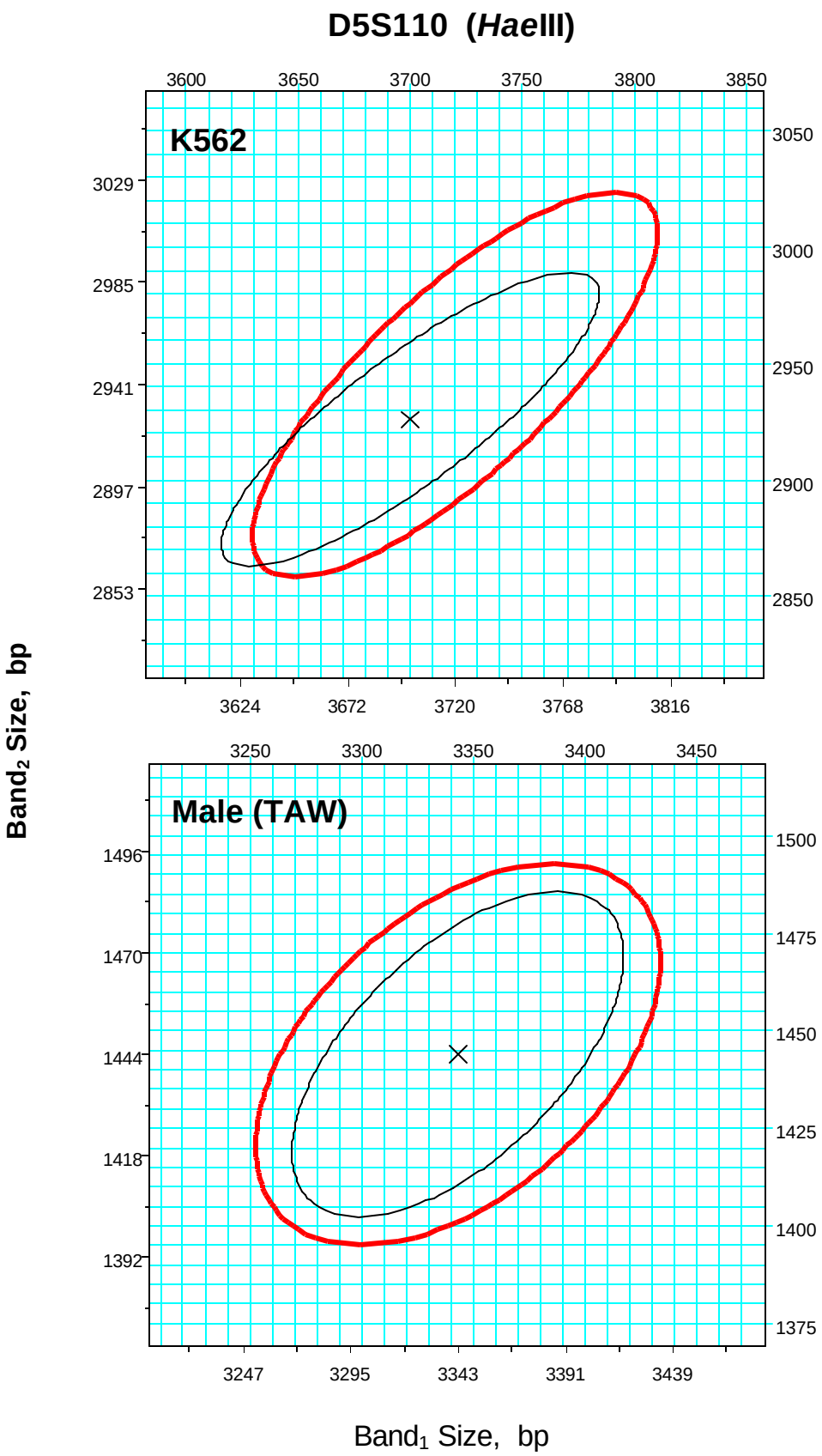

Figure 1d. D5S110 Tolerance Ellipses

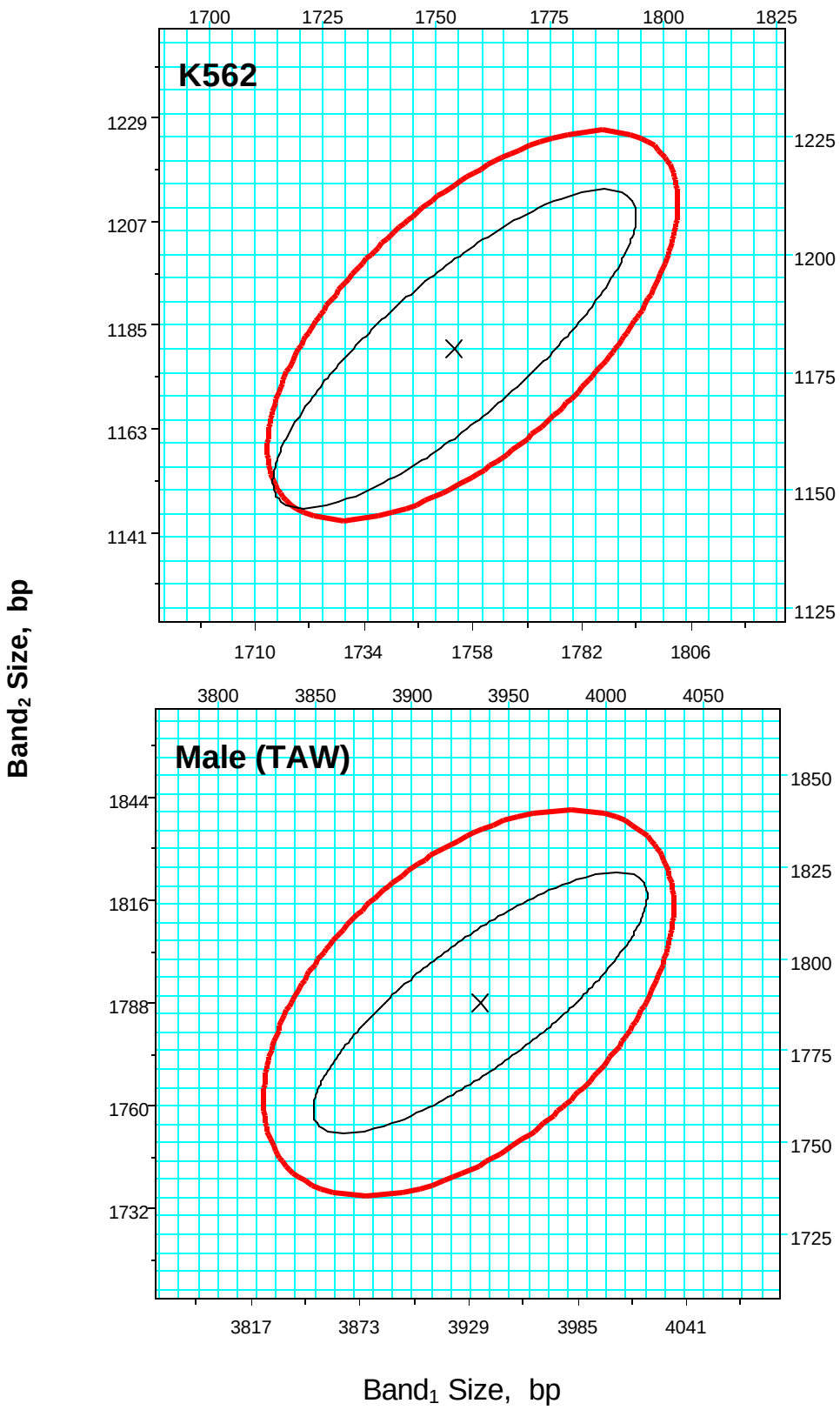

Figure 1e. D10S28 Tolerance Ellipses

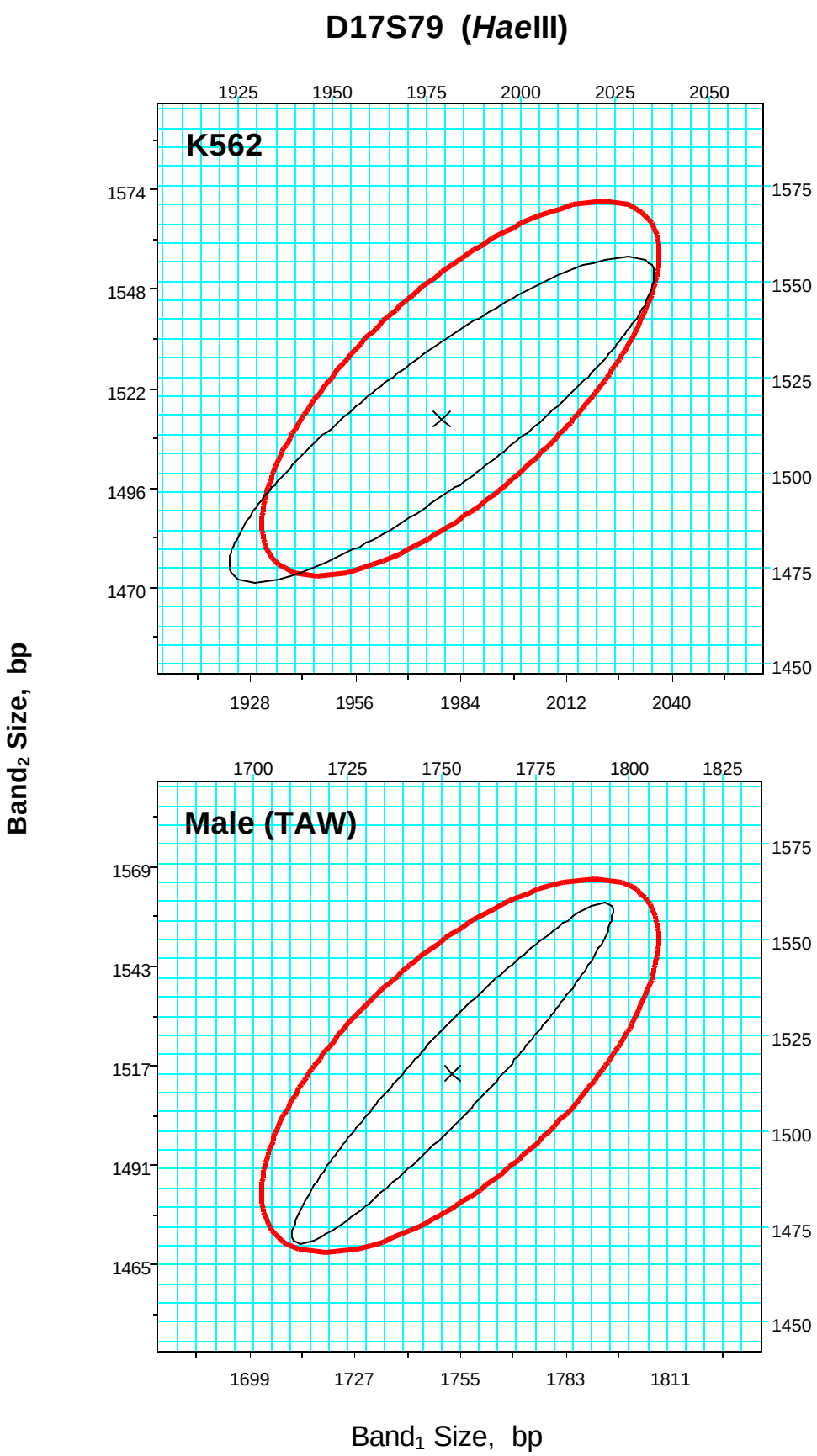

Figure 1f. D17S79 Tolerance Ellipses

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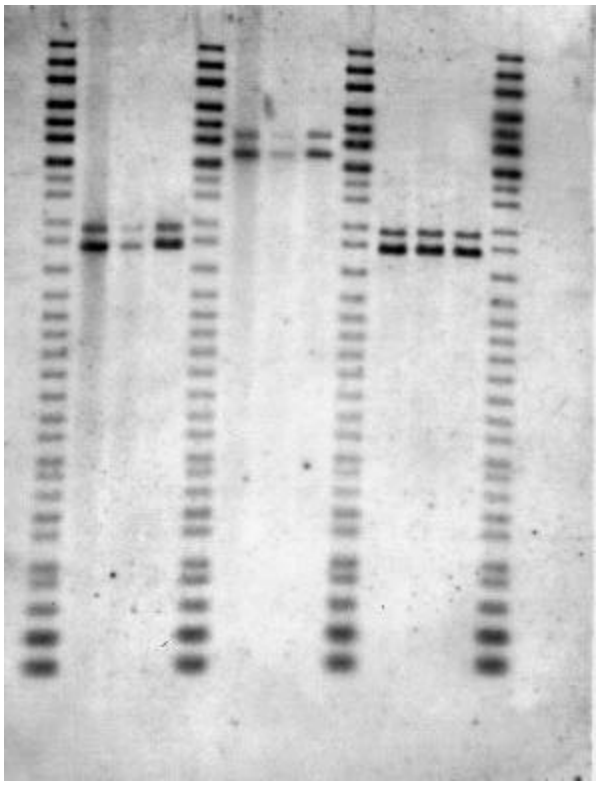
Certificate Revision History: 2 February 2001 (This technical revision reports updates of the band size values of the original SRM 2390 to reflect the evolution of forensic practice from 1991 to 1998.); 10 August 1992 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet http://www.nist.gov/srm.

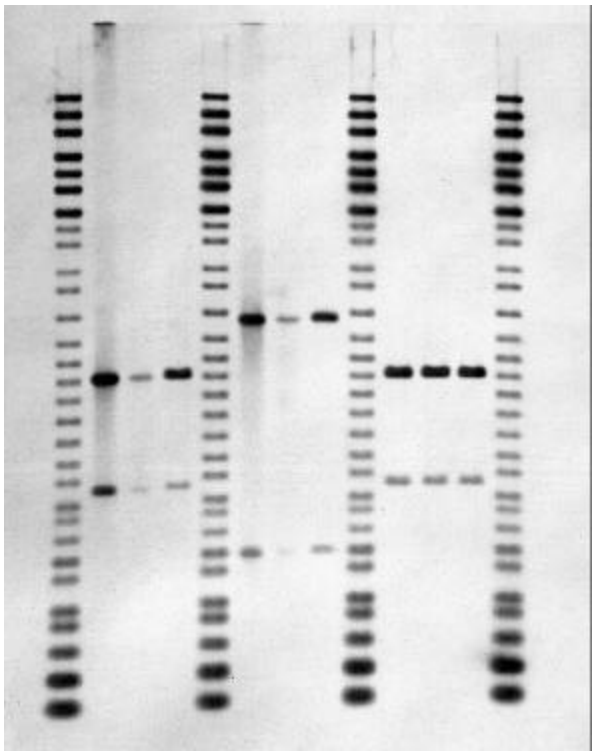
APPENDIX A. Laboratories Participating in the Recertification of SRM 2390

Arizona Department of Public Safety Crime Laboratory DNA Unit, Phoenix, AZ
Broward County Sheriff's Office, Crime Laboratory, Ft. Lauderdale, FL
Connecticut State Police Forensic Science Laboratory DNA Unit, Meriden, CT
Illinois State Police, Forensic Science Center at Chicago, Chicago, IL
Illinois State Police, Springfield Forensic Science Laboratory, Springfield, IL
Indianapolis–Marion County Forensic Services Agency, Indianapolis, IN
Kentucky State Police Crime Laboratory, Frankfort, KY
LabCorp, Forensic Identity Testing, RTP, NC
Maryland State Police Crime Laboratory, Pikesville, MD
Metro-Dade Police Department Crime Laboratory Bureau, Miami, FL
Minnesota Department of Public Safety Bureau of Criminal Apprehension, St. Paul, MN
New York State Police Forensic Investigation Center, Albany, NY
North Carolina State Bureau of Investigation Crime Laboratory, Raleigh, NC
Orange County Sheriff-Coroner Department DNA Laboratory, Santa Ana, CA
Pennsylvania State Police DNA Laboratory, Greensburg, PA
South Carolina Law Enforcement Division DNA Laboratory, Columbia, SC
Vermont Forensic Laboratory, Department of Public Safety, Waterbury, VT
Washington State Patrol Crime Laboratory, Seattle, Seattle, WA
Washoe County Sheriff's Office Crime Laboratory, Reno, NV
Wisconsin State Crime Laboratory, Milwaukee, Milwaukee, WI

APPENDIX B. Images of Representative Chemilumigrams



Locus D1S7



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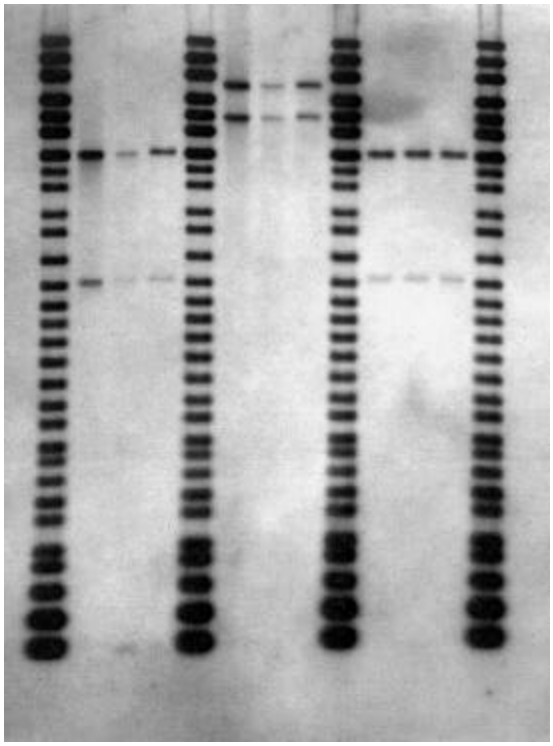

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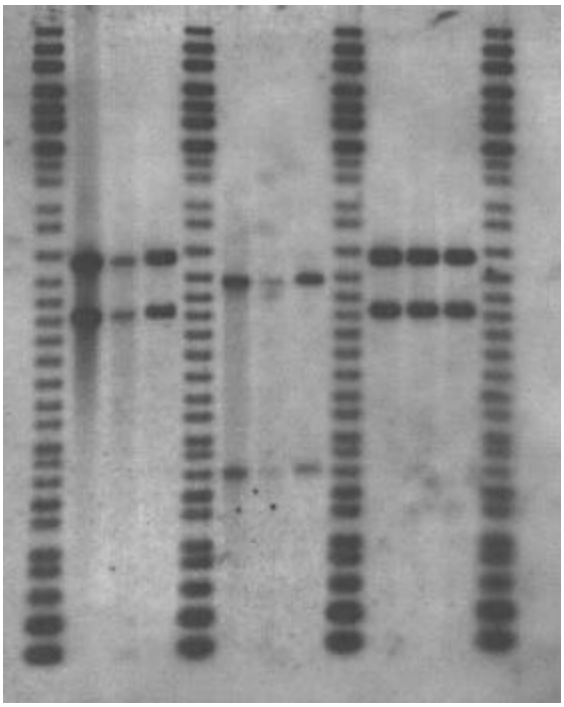

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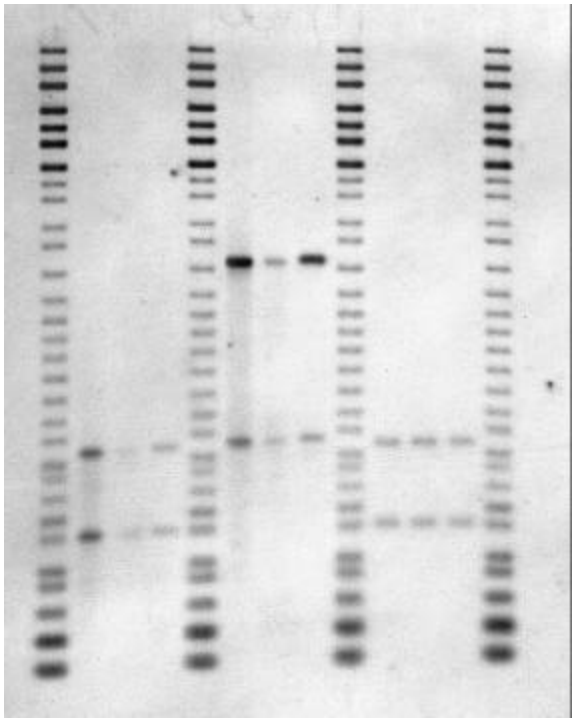

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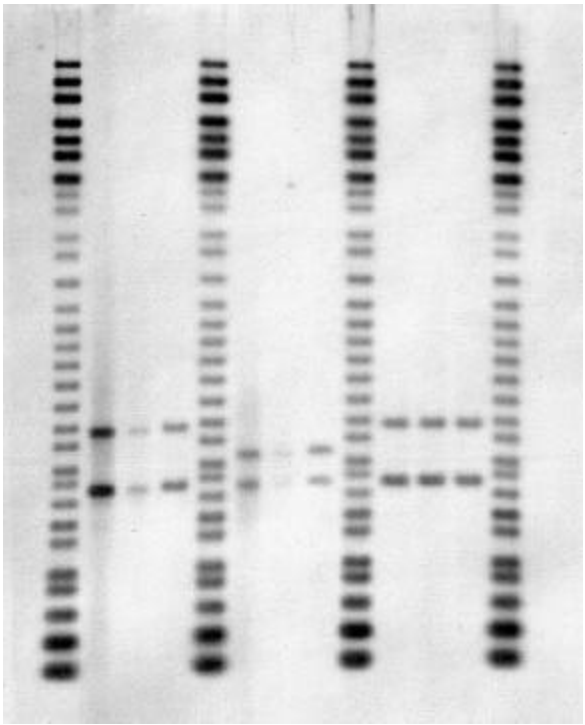


Locus D17S79

Annex

Standard Reference Material® 2390

DNA Profiling Standard

Standard Reference Material (SRM) 2390 contains 20 components designed to help laboratories who are performing DNA profiling by Restriction Fragment Length Polymorphism (RFLP) methods.

Each component is included to allow laboratories to verify that their own analysis system is operating within proper controls.

The following is a brief summary of the function of each component:

Components #1 to #5: consist of a DNA Analysis Marker System. This system is used for proper sizing of DNA bands revealed through Southern blotting applications with 32P-labeled probes.

Component #20: is a highly purified agarose with low electroendosmosis. It has been functionally tested with the rest of the components in the set.

Components #6 to #11: consist of a set of human genomic DNA samples to be used as standards for a yield gel. The reagent concentrations range from 250 ng to 6 ng.

Component #19: is a 10X loading buffer to be diluted 1:1 and used as a 5X concentration for use in testing unknown DNA samples.

Component #18: is an adenovirus visible ladder to be used as a marker lane to assure that the electrophoretic procedures are performing correctly.

Components #12 to #14: consist of K562 DNA (female) in three forms: #12 approximately 3 million cells; #13 pre-extracted genomic DNA; #14 genomic DNA predigested with Hae III restriction enzyme (pre-mixed with loading buffer).

Components #15 to #17: consist of DNA (male) in three forms: #15 approximately 3 million cells; #16 pre-extracted genomic DNA; #17 genomic DNA predigested with Hae III restriction enzyme.

Details and instructions for use of each component are given on the following pages.

OVERVIEW

SRM 2390 can be used in several different ways depending on the laboratory's needs in quality assurance. Components of the SRM are intended for use to assure that each step of a protocol for DNA profiling is functioning properly.

DNA samples are optimally analyzed using the protocol similar to the one used by the Federal Bureau of Investigation (FBI). This protocol and the relevant SRM component used in checking this step consists of:

a) extracting DNA from the cell pellet - Components #12 and #15

b) quantifying the amount of DNA extracted using the set of quantitative controls supplied with the SRM - Components #6 to #11

c) cutting the DNA with a Hae III restriction enzyme - Components #13 and #16

d) testing the restriction process by gel electrophoresis - Components #14 and #17

e) running all samples by electrophoresis on an analytical gel with Tris Acetate EDTA buffer and appropriate viral DNA markers for assessing proper separation - Components #1 to #5; #18; #20

f) blotting the separated DNA onto a nylon membrane using an alkaline transfer solution

g) hybridizing the sample DNA to radioactively-labeled or chemiluminescent DNA probes

h) exposing the membrane to x-ray film

i) imaging the developed autoradiogram with a computerized imaging system to determine band sizes - Components #1 to #5 and certified band sizes

Laboratories that use a "working DNA standard" on a daily basis, such as K562 or another source of DNA, qualify their working standards in a lane on an analytical gel and perform band sizing for their materials using the SRM set. Such calibrated working standards can then be deemed "traceable to NIST standards."

In a quality assurance role, the SRM may be used to verify that various components, such as Hae III enzyme, agarose, extraction procedures, and yield gel results, etc., are functioning properly by direct comparison to the appropriate component. For example, a laboratory's properly functioning extraction protocol and restriction digestion procedure using Hae III should give equivalent results as those presented by the pre-digested DNA samples.

DNA Analysis Marker System*

Components #1 to #5

Storage Conditions: -20 °C

Description: The DNA Analysis Marker System* is designed for use in Southern blotting applications that use ^{32}P-labeled probes. The system includes an unlabeled ladder of ? DNA fragments to be electrophoresed in a lane of an agarose gel. The system also includes reagents for making a ^{32}P-labeled probe for visualization of the marker fragments. This probe should be included in the hybridization solution. **DO NOT USE WITH OTHER LABELED PROBES THAT CONTAIN ? DNA SEQUENCES.**

Component		Amount
#1	Marker DNA for electrophoresis; ? DNA fragments, 10 % glycerol, 20 µM EDTA, 10 mM Tris-HCl (pH 7.5)	30 µL
#2	Marker DNA Dilution Buffer; 10 µM Tris-HCl (pH 7.5), 10 % glycerol, 0.02 % bromophenol blue, 20 µM EDTA	70 µL
#3	Probe Labeling Solution; 0.41µM DNA probe, 11 µM each dATP, dGTP, dTTP, 11 mM Tris-HCl (pH 7.9), 66 mM NaCl, 7.3 mM MgCl, 6.6 mM 2-mercaptoethanol	90 µL
#4	Large Fragment of DNA Polymerase I (Klenow fragment); 3 units/µL in 0.l M potassium phosphate (pH 7.0), 10 mM 2-mercaptoethanol, 50 % (v/v) glycerol	30 units
#5	Stop solution; 20 mM EDTA	100 µL

Quality Control: 3 µL of Component #1 was diluted with 7 µL of Component #2 and electrophoresed in a 11 cm x 16 cm 1.0 % agarose gel in Tris-acetate buffer for 16 h at 30 V. Tie DNA was transferred to a Biodyne® B nylon membrane by capillary transfer in 0.4 M NaOH for 6 h. The membrane containing the marker DNA was hybridized with 0.5 to 1.0 x 10^4 dpm/mL probe for 18 h in 0.3 M NaCl. 0.015 M sodium phosphate (pH 7.0), 0.0015 M EDTA, 10 % polyethylene glycol, 7 % sodium dodecyl sulfate (SDS). The membrane was then washed twice in a solution containing 0.3 M NaCl, 0.03 M sodium citrate, and 0.1 % SDS at room temperature, followed by a 10 min wash at 65 °C in a solution containing 0.015 M NaCl, 0.0015 M sodium citrate, and 0.1 % SDS. All fragment bands were distinct and sharp on a 3-day autoradiograph using X-OMAT® film with a Cronex® Lightning Plus intensifying screen[1] at -80 °C.

*Patent # 5,316,908 (Life Technologies, Inc.**)**

INSTRUCTIONS FOR USE OF COMPONENTS #1 THROUGH #5

Electrophoresis of Marker DNA: The concentration of Marker DNA is adjusted so that 3 µL of Component #1 will generate a good signal in a 3-day exposure by the assay given above.

1. Add 3 µL of Component #1 to sufficient Component #2 to give 10 µL total volume (7 µL).
2. Heat the marker at 65 °C for 5 min.

Load heated marker on an agarose gel in a lane adjacent to the other samples. If a stronger signal is desired, i.e., for shorter film exposure times, the amount of Marker DNA may be increased.

[1]Certain commercial equipment, instruments, or materials are identified in this certificate (or report, if applicable) in order to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Labeling Marker Probe with ^{32}P

1. In a clean microcentrifuge tube place:
   - 44 µL Component #3 (Labeling Component)
   - 5 µL a ^{32}P-dCTP (10 mCi/mL, 3000 Ci/mmol)
   - 1 µL DNA Polymerase Large Fragment (Component #4, 3 units)
2. Incubate for 1 h at 23 °C to 25 °C. =:50 % of the radioactivity should be incorporated and may be monitored by spermine for TCA precipitation.
3. Terminate the reaction by the addition of 50 µL Stop Buffer (Component #5).
4. Denature the probe by boiling 10 min.
5. Transfer the tube containing the probe from the boiling water to an ice bath. Leave the probe on ice for 5 min.
6. Microcentrifuge the probe for 2 min. This step not only brings down liquid from the sides of the tube, but also pellets denatured protein, etc., that causes background during the hybridization. In NIST's experience, this is the only purification of the probe that is necessary. Although further purification is not recommended, the unincorporated nucleotides may be removed by gel filtration.
7. The Marker Ladder can be hybridized at the same time as other samples for analysis. Add 1 µL of labeled marker probe per mL of hybridization solution, along with other probe(s).

Size Ladder
(Component #1)

NOTE: The marker fragment bands can be easily identified by counting from the gap in the marker pattern at approximately 1 kilobase (kb).

(Three day exposure of this lot of marker)

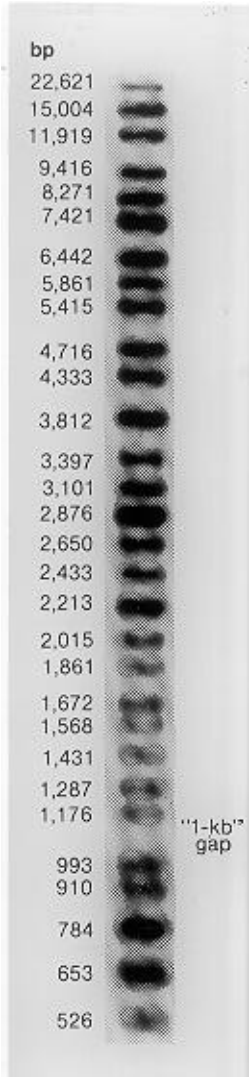
Fragment Sizes (base pairs)

22,621
15,004
11,919
9,416
8,271
7,421
6,442
5,861
5,415

4,716
4,333
3,812

3,397
3,101
2,876
2,650
2,433
2,213
2,015
1,861
1,672
1,568
1,431
1,287
1,176
"1-kb" gap
993
910

784
653
526



Biodyne® is a registered trademark of Pall BioSupport Company, Glen Cove, NY.
X-OMAT™ is a trademark of the Eastman Kodak® Company, Rochester, NY.
Cronex® is a registered trademark of E.I. duPont deNemours and Company, Wilmington, DE.

Yield Standard (Components #6 through #11)
and Loading Buffer (Component #19)

Storage Conditions: Reagents may be stored at 4 °C for (3 to 6) months or at -20 °C for long-term storage. Reagents may look turbid due to 0.1 % SDS. Turbidity may be eliminated by warming reagents to 37 °C for (5 to10) min.

Summary and Explanation of Components: The components enable the user to run four yield gels, with up to 34 unknown samples per gel. The set provides all the necessary controls and reagents to evaluate the quality and quantity of extracted DNA.

Reagents: Reagents are stored in 10 mM Tris, 0.2 mM Na_2EDTA and 0.1 % SDS. With the exception of the loading buffer, all of the reagents have been premixed with 5X loading buffer, such that 6 µL of standard or known DNA contain 4 µL of reagent and 2 µL of 5X loading buffer. The following reagents are included:

Component	Supplied	Volume
#6	250 ng standard (6 µL = 250 ng DNA) (male)	25 µL
#7	100 ng standard (6 µL = 100 ng DNA) (male)	25 µL
#8	50 ng standard (6 µL = 50 ng DNA) (male)	25 µL
#9	25 ng standard (6 µL = 25 ng DNA) (male)	25 µL
#10	12.5 ng standard (6 µL = 12.5 ng DNA) (male)	25 µL
#11	6 ng standard (6 µL = 6 ng DNA) (male)	25 µL
#19	10X loading buffer	300 µL

Materials Needed: pipette (1 to 100 µL), pipette tips, agarose, buffer (TAE or TBE) and ethidium bromide

Procedures: K562 DNA, Hae III digested was electrophoresed in a 1 cm x 16 cm gel in TAJE buffer for 16 h at 30 V. The DNA was transferred to a Pall Biodyne® B membrane in 0.5 M NaOH, 0.5 M NaCl for 4 h at room temperature. The DNA probe PH30 was labeled to a specific activity = 10^9 dpm/µg using a Random Primers DNA Labeling System. The membrane-immobilized DNA was hybridized to the probe according to the method of Budowle and Baechtel [3]. Hybridization was performed at a probe concentration of 5 x 10^5 cpm/mL (60 mL) at 65 °C for (16 to –24) h. Post-hybridization washes were in a solution containing 0.3 M NaCl, 0.03 M sodium citrate, and 0.1 % SDS at room temperature, followed by a solution containing 0.015 M NaCl, 0.0015 M sodium citrate, and 0.1 % SDS at 65 °C. The membrane was exposed to Kodak™ XAR film with Cronex® Lighting Plus intensifying screen at -80 °C.

Assays were performed by Life Technologies, Inc.

DIRECTIONS FOR USE:

NOTE: The set works under a wide variety of conditions. A suggested procedure is to perform electrophoresis at 50 V for 2 h or 200 V for 20 min on (0.8 to 1.0) % agarose. However, the laboratory routine yield gel procedure should be used.

1. Dilute the 10X loading buffer, Component #19, 1:1 with distilled water to make a 5X loading buffer.
2. Apply 6 µL each of 250, 100, 50, 25, 12.5 and 6 ng genomic DNA standard (Components #6 to #11) to lanes two through seven.
3. Add 2 µL loading buffer (5X) to 4 µL of each unknown DNA sample to be tested and load in remaining lanes.
4. Electrophorese under appropriate conditions and stain with ethidium bromide.

Agarose
Component #20

Storage Conditions: Store at room temperature

Description: This agarose (5 g, supplied by Life Technologies, Inc.) is highly purified with low electroendoosmosis (LE). The lot was functionally tested for its ability to generate sharp bands and low backgrounds in DNA typing assay. Note that this agarose is different from that used in the FBI protocols which calls for medium electroendoosmosis (ME) agarose.

Quality Control: DNA typing assay: A 1 % gel was prepared in TAE buffer. 10 µL K562 DNA, Hae III digested, was electrophoresed through the gel, transferred to a nylon membrane, and hybridized with DNA probe D4S139 (PH30) [7]. The bands were sized using the BRL DNA Analysis Marker System.

Specification: After an overnight exposure, only two bands are present within ± 2.5 % of the accepted allelic sizes [9].

Values Obtained: PH30 band #1: 6600 Kb PH30 band #2: 3437 Kb

Specification: 30 marker bands visible. No DNA background visible in lane.

Observed: 30 bands. No background.

- Gelling temperature of a 2 % (w/v) solution
  Specification: 36 °C to 42 °C
  Value Obtained: 36.5 °C

- Gel strength of a 1 % (w/v) solution
  Specification: = 1200 g/cm^2
  Value Obtained: 1386 g/cm^2

- Electroendoosmosis (-m_r)

  Specification: ≤ 0.11
  Value Obtained: 0.11

- Moisture
  Specification: ≤ 10 %
  Value Obtained: 6.5 %

- Sulfate
  Specification: ≤ 0.35 %
  Value Obtained: 0.10 %

Adenovirus Ladder
Component #18

Ladder Size (bp)
35 937
20 042
12 643
6085/6067
5051
4187/4131
3656/3613
2275
1475
1053
957
594

Description: This product is a cocktail of digested and undigested Adenovirus DNA. Fragments range in size from 594 bp to 35 937 bp. At the recommended loading, the 594 bp band should just be visible.

Use of Markers: Optimum signal is obtained by adding 185 ng of Visible Marker (Component #18) to a single lane on each gel. For use, take 10 µL of size marker and add 4 µL loading buffer (5X).

Load 14 µL per lane. Visible marker bands are detected after ethidium bromide staining of the agarose gel.

Concentration and Storage: The visible marker is supplied at 18.5 ng/µL in a mixture of 10 mM Tris and 0.2 mM Na_2EDTA with 0.1 % SDS. Reagents may be stored at 4 °C for (3 to 6) months or at -20 °C for long term storage. Reagents may look turbid due to 0.1 % SDS. Turbidity may be eliminated by warming reagents to 37 °C for (5 to 10) min.

Packaging and Usage: The visible size marker ladder is packaged at 10 µg of digested Adenovirus at a concentration of 18.5 ng/µL. This is enough for 34 gels, using one visible marker lane per gel using a loading of 185 ng.

K562 Cell Pellet, 3 x 10^6 Cells
Component #12

Storage Conditions: Store at -70 °C

Description: The DNA prepared from K562 cells is widely used as an allelic control in human DNA typing applications. The K562 cell line is derived from a pleural effusion of a patient with chronic myelogenous leukemia [5]. The cell line has been extensively studied and characterized for over 15 years, particularly for its erythroid properties [1,2,4,6,10]. Each lot of K562 cells is tested for genetic integrity by extracting the DNA from the cells and measuring the allele sizes obtained in a Southern blot assay with VNTR probes. This product is intended as a control for DNA typing applications and is not a viable source for cell culture stocks.

Quality Control: K562 DNA was isolated bv the protocol described in Reference [11]. One µg of isolated K562 DNA was then digested with endonuclease Hae III, electrophoresed through an agarose gel, transferred to a Biodyne® B nylon membrane, and hybridized with DNA probes D4SI39 (PH30) [7] and D2S44 (YNH24) [8]. The bands were sized using the BRL DNA Analysis Marker System.

Specification: After an overnight exposure, only two bands are present per probe within ± 2.5 % of the accepted allelic sizes [9].

Values Obtained:*	PH30 band #1: 6600 bp	YNH24 band #1: 2956 bp
	PH30 band #2: 3478 bp	YNH24 band #2: 1819 bp

*Initially tested by Life Technologies, Inc. Note that these are not the certified values.

Procedure: The K562 cell pellet was resuspended in 375 µL 0.2 M sodium acetate and vortexed for (1 to 2) s. 25 µL 10 % sodium dodecyl sulfate and 5 µL 20 mg/mL Proteinase K were then added. The suspension was vortexed 1 s and then incubated at 56 °C for 1 h. Phenol/chloroform/isoamyl alcohol (120 µL) was added, and the sample mixed by gently vortexing for 30 s. The phases were separated by centrifugation for 2 min. The aqueous (upper) phase was transferred to a clean tube, and the nucleic acid was precipitated by the addition of 1.0 mL cold (-20 °C) ethanol. After storage at -20 °C for 1 h, the DNA was collected by centrifugation for 30 s. The DNA pellet was dissolved in 180 µL 10 mM Tris pH 7.5 1 mM EDTA (TE) by incubating for 10 min at 65 °C. The DNA was precipitated again by adding 20 µL 2.0 M sodium acetate followed by 500 mL ethanol. Following centrifugation, the pellet was washed with 1 mL 70 % ethanol. The pellet was dried and dissolved by incubation in 200 µL TE overnight at 65 °C. The DNA was digested with Hae III (BRL Cat. No. 5205SA) using the conditions on the Product Profile®. The Hae III-digested DNA was electrophoresed in 1 % agarose gels. The DNA was transferred to Pall Biodyne® B membranes in 0.5 M NaOH, 0.5 M NaCl for 4 h at room temperature. The DNA probes PH30 and YNH24 were labeled to a specific activity > 10^9 dpm/µg using a Random Primers DNA Labeling System. Two identical membranes were prepared, and each was hybridized to one probe according to the method of Budowle and Baechtel [3]. Hybridization was performed at a probe concentration of 5 x 10^5 cpm/mL at 65 °C for (16 to 24) h. Post-hybridization washes were in a solution containing 0.3 M NaCl, 0.03 M sodium citrate, and 0.1 % SDS at room temperature, followed by a solution containing 0.015 M NaCl, 0.0015 M sodium citrate, and 0.1 % SDS at 65 °C. The membranes were exposed to Kodak XAR film with a Cronex® Lighting Plus Intensifying screen at -80 °C.

Assays were performed by Life Technologies, Inc.

K562 DNA, 25 µL
Component #13

Storage Conditions: Store at –70 °C

Storage Buffer: 10 mM Tris HCl (pH 7.5), 0.1 mM EDTA

Description: K562 DNA is a high molecular weight DNA prepared from the human cell line K562. This DNA is widely used as an allelic control in human RFLP analysis. Each lot is rigorously tested for chemical purity and performance in a Southern blot assay.

Quality Control: One µg K562 DNA was digested with endonuclease Hae III, electrophoresed through an agarose gel, transferred to a nylon membrane, and hybridized with DNA probes D4Sl39 (PH30) [7] and D2S44 (YNH24) [8]. The bands were sized using the BRL DNA Analysis Marker System (Cat. No. 4401SA).

Specification: After an overnight exposure, only two bands are present per probe within ± 2.5 % of the accepted allelic sizes [9].

Values Obtained:*	PH30 band #1: 6494 bp	YNH24 band #1: 2908 bp
	PH30 band #2: 3437 bp	YNH24 band #2: 1797 bp

*Initially tested by Life Technologies, Inc. These are **NOT** certified values.

The concentration of K562 DNA was determined by measuring the OD_{260}.

Specification: 0.2 µg/µL ± 0.05 µg/µl
Value Obtained: 0.232 µg/µL

The ratio of the OD_{260}/OD_{280} was determined in a UV spectrophotometer.

Specification: 1.7 to 1.9
Value Obtained: 1.88

One µg of DNA was inspected for RNA contamination by electrophoresis in a 1 % agarose gel and staining with ethidium bromide. The gel was then observed under UV light for the presence of stained material less than 300 bp.

Specification: No ethidium bromide stained material less than 300 bp
Observed: None

Procedures: Hae III digests of K562 DNA were electrophoresed in 1 % agarose gels. The DNA was transferred to Pall Biodyne® B membranes in 0.5 M NaOH, 0.5 M NaCl for 4 h at room temperature. The DNA probes PH30 and YNH24 were labeled to a specific activity = 10^9 dpm/ µg using a Random Primers DNA Labeling System. Two identical membranes were prepared, and each was hybridized to one probe according to the method of Budowle and Baechtel [3]. Hybridization was performed at a probe concentration of 5 x 10^5 cpm/mL (60 mL) at 65 °C for (16 to 24) h. Post-hybridization washes were in a solution containing 0.3 M NaCl, 0.03 M sodium citrate, and 0.1 % SDS at room temperature, followed by a solution containing 0.015 M NaCl, 0.0015 M sodium citrate, and 0.1 % SDS at 65 °C. The membranes were exposed to Kodak® XAR film with a Cronex® Lighting Plus intensifying screen at -80 °C

K562 DNA, Hae III digested, 100 µL- 25ng/µL
Component #14

Storage Conditions: Store at -70 °C

Storage Buffer: 10 mM Tris HCl (pH 7.5), 20 mM EDTA, 10 % glycerol, 0.02 % bromophenol blue

Description: K562 DNA is a high molecular weight DNA prepared from the human cell line K562. This DNA is widely used as an allelic control in human RFLP analysis. The K562 DNA used in the preparation of this product meets all of the quality control specifications for K562 DNA before use and is free from RNA and protein. This DNA is digested to completion with restriction endonuclease Hae III, purified, and dissolved in electrophoresis loading buffer. Each lot is rigorously tested for performance in a Southern blot assay.

Quality Control: 10 µL K562 DNA, Hae III digested, was electrophoresed through an agarose gel, transferred to a nylon membrane, and hybridized with DNA probes D4Sl39 (PH30) [7] and D2S44 (YNH24) [8]. The bands were sized using the BRL DNA Analysis Marker System.

Specification: After an overnight exposure, only two bands are present per probe within ± 2.5 % of the accepted allelic sizes [9].

Values Obtained:

PH30 band #1: 6494 bp	YNH24 band #1: 2940 bp
PH30 band #2: 3437 bp	YNH24 band #2: 1797 bp

Procedures: K562 DNA, Hae III digested, was electrophoresed in 1 % agarose gels. The DNA was transferred to Pall Biodyne® B membranes in 0.5 M NaOH, 0.5 M NaCl for 4 h at room temperature. The DNA probes PH30 and YNH24 were labeled to a specific activity = 10^9 dpm/ µg using a Random Primers DNA Labeling System. Two identical membranes were prepared, and each was hybridized to one probe according to the method of Budowle and Baechtel [3]. Hybridization was performed at a probe concentration of 5 x 10^5 cpm/mL at 65 °C for (16 to 24) h. Post-hybridization washes were in a solution containing 0.3 M NaCl, 0.03 M sodium citrate, and 0.1 % SDS at room temperature, followed by a solution containing 0.015 M NaCl, 0.0015 M sodium citrate, and 0.1 % SDS at 65 °C. The membranes were exposed to Kodak® XAR film with a Cronex® Lighting Plus intensifying screen at -80 °C.

Cell Pellet (Male Source), Volume = 10 µL
Component #15

Storage Conditions: Store at –70 °C

Cell Pellet: One tube of this control contains purified peripheral blood mononuclear cells from a single donor with about 3 x 10^6 cells in the pellet. The pellet should provide approximately 10 µg DNA after extraction using conventional procedures.

Male Genomic Human DNA
Component #16

Storage Conditions: As above

Description: This product consists of human genomic DNA from a single donor. It exhibits two distinct bands with the majority of the commercially available RFLP probes. It provides a control for the digestion of the sample with restriction endonuclease, electrophoresis conditions, hybridization, and detection system and for the detection of Y specific probes.

Use of Material: Add 100 ng to 500 ng of user-digested control genomic DNA to a single lane per gel. Choice of quantity is at the discretion of the user.

Concentration and Storage: Undigested control genomic DNA is supplied at 200 ng/µL in a mixture of 10 mM Tris, 0.2 mM and Na_2EDTA. Reagents may be stored at 4 °C for (3 to 6) months or at -20 °C for long-term storage.

Packaging and Usage: Undigested control genomic DNA is packaged as 5 µg undigested male genomic DNA at a concentration of 200 ng/µL. Amounts of undigested control genomic DNA, comparable to the evidence being tested, should be digested with each batch of samples. The digested control genomic DNA should be run in a lane adjacent to the predigested control genomic DNA.

Hae III Restricted Male Genomic DNA
Component #17

Storage Conditions: As above

Description: This product consists of male human genomic DNA, from a single donor, digested with the restriction enzyme Hae III. This product has two distinct bands with the majority of the commercially available RFLP probes. This product provides a control for the electrophoresis conditions, hybridization, and detection system. Further, as male DNA, it provides a positive control for the detection of Y probes.

Use of Material: The choice of quantity of this control is at the discretion of the end user. 25 ng (1 µL) to 500 ng (20 µL) of predigested control genomic DNA can be added to a single lane per gel.

Concentration and Storage: Predigested control genomic DNA is supplied at 25 ng/µL in a mixture of 10 mM Tris, 0.2 mM Na_2EDTA, and 0.1 % SDS. Reagents may be stored at 4 °C for (3 to 6) months or at -20 °C for long-term storage. Reagents may look turbid due to the presence of 0.1 % SDS. Turbidity may be eliminated by warming reagents to 37 °C for (5 to 10) min.

Packaging and Usage: Predigested control genomic DNA is packaged at 2.5 µg predigested genomic DNA in 100 µL at a concentration of 25 ng/µL. Amounts of digested control genomic DNA, comparable to the evidence being tested, should be run with each batch of samples. As a starting point, add 4 µL of loading buffer (10X) to 20 µL (500 ng), of predigested control genomic DNA and 25 µL of sterile deionized water, and load one sample per gel.

Suggested Sample Protocols

Yield Gel

Lane	Sample
1.	K562 cell extracted DNA
2.	male cell extracted DNA
3.	K562 genomic DNA
4.	male genomic DNA
5.	250 ng standard
6.	100 ng standard
7.	50 ng standard
8.	25 ng standard
9.	12.5 ng standard
10.	6 ng standard
11.	blank or laboratory sample
12.	blank or laboratory sample

Experience suggests that the genomic DNAs range from (3 to 4) µg/µL, so the user might want to adjust accordingly. In addition, the genomic DNA may require warming to 56 °C for (5 to 10) min.

Post-Restriction Test Gel

Lane	Sample
1.	blank
2.	blank
3.	K562 cell line extracted and cut
4.	K562 genomic DNA cut with Hae III
5.	K562 DNA supplied pre-cut with Hae III
6.	male cell line extracted and cut
7.	male genomic DNA cut with Hae III
8.	male DNA supplied pre-cut with Hae III
9	blank or laboratory sample
10.	blank or laboratory sample

Suggested Analytical Gel Orientation

Lane	Sample
1.	Visible Ladder (Adenovirus)
2.	blank or laboratory sample to be calibrated
3.	BRL DNA Analysis Marker System (Size Ladder)
4.	BRL K562 cell line extracted and cut with Hae III
5.	BRL K562 genomic DNA cut with Hae III
6.	BRL K562 pre-cut with Hae III
7.	BRL DNA Analysis Marker System (Size Ladder)
8.	male cell line extracted and cut with Hae III
9.	male genomic DNA cut with Hae III
10.	male genomic DNA pre-cut with Hae III
11.	BRL DNA Analysis Marker System (Size Ladder)
12.	blank or laboratory sample to be calibrated
13.	Visible Ladder (Adenovirus)
14.	blank

If running a 14-well system, leave one of the outer lanes blank. Perform electrophoresis, Southern blotting, and probing as usual.

Other configurations are possible if more than 14 wells are used. In that case, other DNA samples may be calibrated or checked for performance in the system.

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