National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 2390

DNA Profiling Standard

Standard Reference Material (SRM) 2390 is intended for use (1) in the standardization of forensic and paternity quality assurance procedures for Restriction Fragment Length Polymorphisms (RFLP) testing that uses HaeIII restriction enzymes and (2) for instructional law enforcement or non-clinical research purposes. It is not intended for any human/animal clinical diagnostic use. This SRM is composed of well-characterized human DNA in three forms from a female cell line (K562) and from a male source (TAW). It also contains DNA to be used for quantitative controls and a DNA ladder for size determination of DNA. Additional materials are included for labeling the DNA size ladder. A viral DNA marker set is included to assure that electrophoretic separations are properly performed. Agarose that has been pre-tested for compatibility with all DNA components is included. A unit is composed of 20 components, 19 of which contain frozen material. See section on page 2 of this certificate entitled "Description of Reactive Components" for a complete listing of the components.

Certified and Noncertified Band Sizes: Certified values for the sizes of each allelic band for five commonly used DNA probes are provided in Table 1 and are based on data obtained through extensive interlaboratory testing. A second procedure is included for verifying the accuracy of the calculated DNA band sizes. After computer imaging and band sizes are assigned, the size difference between band 1 and band 2 is calculated and compared to values listed in Table 2. Results should be within the designated uncertainty. The second procedure is only valid for K562 and the male DNA contained in this SRM. Noncertified values of band sizes for other probes are listed in Table 3 and are provided for information only.

NOTICE AND WARNINGS TO USER:

Warning: The DNA prepared from K562 cells was derived from a human subject with a diagnosis of chronic myelogenous leukemia. The TAW male DNA was derived from blood cells from a healthy human. The supplier of this material has tested the source material from which the male DNA was derived and found it to be nonreactive for hepatitis B surface antigen (HB_sAG) and HIV by FDA-approved testing. However, no test method can ensure that a product derived from human blood does not contain HIV, hepatitis, or other infectious agents. HANDLE AS IF CAPABLE OF TRANSMITTING DISEASE.

Expiration of Certification: Stored as specified in the section below, the certified values should remain within the uncertainty limits for at least 6 months from the date of shipment. NIST will keep samples of this material under surveillance for at least 6 months after the date of last sale. If changes occur beyond the limits certified, NIST will notify purchasers. In absence of such notification, the user should not use the material beyond 6 months after the date of purchase.

Storage: Store Box A and Box B at a temperature of -20 °C; store Box C at a temperature of -70 °C; store agarose component #20 at room temperature.

Use: Sample aliquots for analysis should be withdrawn immediately after opening the ampoules and should be processed without delay for the certified values in Table 1 to be valid within the stated uncertainty. Certified values are not applicable to material stored in ampoules that have been opened, even if they are resealed.

Gaithersburg, MD 20899
August 10, 1992

William P. Reed, Chief
Standard Reference Materials Program

The analytical determinations and technical measurements leading to the certification of this SRM were performed in the NIST Biotechnology Division of the Chemical Science and Technology Laboratory by K.L. Richie and D.J. Reeder.

Auxiliary testing and shipping protocols were coordinated by M.C. Kline of the Biotechnology Division.

The overall direction of the technical measurements leading to certification was under the chairmanship of D.J. Reeder, Leader, Biochemical Measurements Group and L.J. Powell, Chief, Biotechnology Division.

Consultation on the statistical aspects of the certification was provided by H-k Liu, S.D. Leigh, and K.R. Eberhardt of the Statistical Engineering Division.

The technical and support aspects involved in the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Standard Reference Materials Program by J.C. Colbert.

PREPARATION AND ANALYSIS

Description of Reactive Components: Twenty components are included in each unit, 19 of which contain frozen material.

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 for component #18 and for DNA digests (#12, #13, #15, #16 and #17)

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

Component #20 (packaged separately): Agarose (Shipped at -20 °C; store at room temperature)

Source of Material: The DNA used in the preparation of this SRM was obtained from a female (K562) cell line and from a male source (TAW). These materials and other components of the SRM were obtained from Life Technologies, Inc., Gaithersburg, MD and from Analytical Genetic Testing Center, Inc., Denver, CO. Each type of DNA consists of three forms: as a cell pellet, an extracted genomic DNA, and a HaeIII restriction digest (pre-cut DNA).

Interlaboratory Analysis: The certified and noncertified values for the bands for this SRM represent the pooled results from analyses performed at NIST and twenty-eight (28) collaborating laboratories. Laboratories participating in this study are listed in Appendix A. Results from all laboratories were included in the statistical analysis and the uncertainty statement. Participating laboratories were asked to provide calculated band sizes for locus D2S44 (YNH24) [1,2] and for as many other probes as they could work into their schedules. Additionally, a questionnaire was provided to allow further assessment of the variables associated with interlaboratory measurements. Completed autoradiograms were also requested so that resizing could be performed if necessary.

Of the twenty-nine laboratories participating in the certification, all reported results for D2S44 (YNH24). A total of thirty-four sets were examined and results tabulated because one laboratory's results were submitted in duplicate and another laboratory reported data from five separate runs. Nineteen laboratories reported results for D4S139 (PH30) [3]; 17 laboratories for D10S28 (TBQ7) [4]; 16 for D1S7 (MS1) [5]; and 12 for D17S79 (V1) [6].

Additional information on the interlaboratory studies conducted in the prototype development for this SRM can be found in reference [7]. Samples were analyzed using the protocol employed by the Federal Bureau of Investigation [8]. This protocol consists of a) extracting DNA from the cell pellet; b) quantifying the amount of DNA extracted using the set of quantitative controls supplied with the SRM; c) cutting the DNA with a HaeIII restriction enzyme; d) testing the restriction process by gel electrophoresis; e) running all samples by electrophoresis on an analytical gel with tris-acetate buffer in the presence of ethidium bromide and appropriate viral DNA markers for assessing proper separation; f) blotting the separated DNA onto a nylon membrane using an alkaline transfer solution; g) hybridizing the sample DNA to radioactively-labeled DNA probes; h) exposing the membrane to X-ray film; and i) imaging the developed autoradiogram with a computerized imaging system to determine band sizes.

CERTIFIED VALUES FOR DNA BAND SIZES OF SRM 2390

The certified values for this SRM were determined as part of an international collaborative study that was completed in early 1992. The program was initiated, supported and technically coordinated by NIST's Standard Reference Materials Program. As part of the protocol, each laboratory participating in the interlaboratory analysis performed the separations and probed with at least one of the listed DNA probes.

The certified values are given in Table 1 and Table 2 in DNA basepairs. Each certified value is the best estimate of the band sizes using the protocol specified by the Federal Bureau of Investigation.

VALUES FOR DNA QUANTIFICATION IN SRM 2390

Standards for quantifying extracted DNA by use of yield gels are contained in Box B. Each of the six tubes contains sufficient DNA to load the yield gel with 1μL of DNA (standard human genomic DNA) ranging from 250 ng to 6 ng. Samples were independently verified and tested by H. Merrick (Molecular Devices Corp., Menlo Park, CA) using the Threshold Total DNA Assay System and diluting the samples prior to analysis.

REFERENCES

1. Nakamura, Y., Gillilian, S., O'Connell, P., Leppert, M., Lathrop, G.M., Lalouel, J.M., and White, R. (1987a) Nucleic Acids Res. 15: 10073.

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7. Reeder, D.J. (1991) In: Proceedings for the International Symposium on Human Identification 1991. Promega Corporation pp 245-261.

8. Protocol for DNA RFLP Testing (1989), Forensic Science Research and Training Center, Federal Bureau of Investigation, Quantico, VA.

9. Statistical Intervals: A Guide for Practitioners (1991), Gerald J. Hahn & William Q. Meeker, John Wiley, New York.

Table 1. Certified Values

		DNA Source			
Locus (Probe)		K562 Band 1	K562 Band 2	Male Band 1	Male Band 2
D2S44 (YNH24)	Mean (in basepairs) Uncertainty	2907 ± 58	1791 ± 45	3720 ± 93	1299 ± 45
D4S139 (PH30)	Mean Uncertainty	6474 ± 160	3438 ± 78	10911 ± 661	8240 ± 324
D10S28 (TBQ7)	Mean Uncertainty	1757 ± 44	1182 ± 34	3935 ± 131	1794 ± 58
D1S7 (MS1)	Mean Uncertainty	4571 ± 101	4231 ± 118	7784 ± 319	6898 ± 243
D17S79 (V1)	Mean Uncertainty	1982 ± 65	1520 ± 55	1759 ± 66	1521 ± 58

The certified value for each band is the mean of the three means, across all laboratories in the study, based on the three preparations (cell, genomic and precut). The uncertainty is the half-width of a 95%/95% statistical tolerance interval for each band. A 95%/95% statistical tolerance interval is one that will, with 95% confidence, contain at least 95% of the relevant population. For each band, this refers to the population of individual results, for any of the three preparations, obtained by a population of laboratories similar to those in the interlaboratory study. [9]

Due to the high correlation between the two bands for each probe and source, the differences of the band sizes should be checked. Table 2 gives the certified differences and their uncertainties.

Table 2. Alternate Way to Assure Sizing Results

		DNA Source	
Locus (Probe)		K562	Male
D2S44 (YNH24)	Difference (in basepairs) Band 1 - Band 2	1117	2422
	Uncertainty (in basepairs)	± 39	± 78
D4S139 (PH30)	Difference Band 1 - Band 2	3037	2671
	Uncertainty	± 133	± 393
D10S28 (TBQ7)	Difference Band 1 - Band 2	575	2141
	Uncertainty	± 32	± 89
D1S7 (MS1)	Difference Band 1 - Band 2	340	886
	Uncertainty	± 89	± 138
D17S79 (V1)	Difference Band 1 - Band 2	461	238
	Uncertainty	± 28	± 22

Table 3. Information Values for Other Probes (Uncertified - no uncertainties given)

		DNA Source			
Locus (Probe)		K562 Band 1	K562 Band 2	Male Band 1	Male Band 2
D17S26 (EFD52)	Mean (in basepairs)	4866	1372	5589	4963
D7Z2 (Mono-morphic)	Mean	2735		2735	
DYZ1 (Male Specific)	Mean				3739

Appendix A. Laboratories participating in the certification of SRM 2390

LABORATORY	LOCATION
Arizona Dept. of Public Safety Crime Laboratory	Phoenix, AZ
Centre of Forensic Science	Toronto, Ontario, Canada
City of Fort Worth Police Department/Crime Laboratory	Ft. Worth, TX
Commonwealth of Virginia Crime Laboratory	Norfolk, VA
Connecticut Forensic Science Laboratory	Meriden, CT
FBI Laboratory	Washington, DC
Florida Department of Law Enforcement	Jacksonville, FL
GeneLex Corporation	Seattle, WA
Genescreen	Dallas, TX
Georgia Bureau of Investigation	Decatur, GA
Illinois State Police, Forensic Services	Springfield, IL
Kentucky State Police Forensic Laboratory	Frankfort, KY
Maryland State Police Crime Laboratory	Pikesville, MD
Metro-Dade Police Crime Laboratory	Miami, FL
Michigan State Police/DNA Laboratory	East Lansing, MI
Minnesota Forensic Science Laboratory	St. Paul, MN
Missouri State Highway Patrol	Jefferson City, MO
Nassau County Police Department	Mineola, NY
National Institute of Standards & Technology	Gaithersburg, MD
North Carolina Bureau of Investigation Crime Laboratory	Raleigh, NC
Orange County Sheriff's Coroner Office	Santa Ana, CA
Royal Canadian Mounted Police Forensic Laboratory	Ottawa, Ontario, Canada
South Carolina Law Enforcement Division	Columbia, SC
Southwest Institute of Forensic Science	Dallas, TX
Tarrant County Medical Examiner	Ft. Worth, TX
Texas College of Osteopathic Medicine	Ft. Worth, TX
Vermont Department of Public Safety	Waterbury, VT
Washington State Patrol Crime Laboratory	Seattle, WA
Washoe County Sheriff's Office	Reno, NV

Annex to the Certificate
for
SRM 2390, DNA Profiling Standard Certificate
dated
August 10, 1992

Gaithersburg, MD 20899
April 27, 1993
(Revision of Annex dated 12-3-92)

Thomas E. Gills, Acting Chief
Standard Reference Materials Program

INTRODUCTION

Standard Reference Material (SRM) 2390 contains twenty 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 #1-5: consist of a DNA Analysis Marker System. This system is used for proper sizing of DNA bands revealed through Southern blotting applications with ^{32}P-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 #6-11: 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 #12-14: 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 #15-17: 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 #6-11**

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 in the presence of ethidium bromide (generally added near the end of the run or, as in the FBI protocol, added at the beginning) and appropriate viral DNA markers for assessing proper separation - **Components #1-5; #18; #20**

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

g) hybridizing the sample DNA to radioactively-labeled 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 #1-5, and certified band sizes**

Laboratories that use a working DNA standard on a daily basis, such as K562 or other source of DNA, usually run their prepared standards in a lane on the analytical gel and perform band sizing using the SRM set. Calibrated or qualified 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, 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 - #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 lambda 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 LAMBDA DNA SEQUENCES.

Components	Amount
Component #1: Marker DNA for electrophoresis; lambda DNA fragments, 10 % glycerol, 20 mM EDTA, 10 mM Tris-HCL (pH 7.5)	30 µL
Component #2: Marker DNA Dilution Buffer; 10 mM Tris-HCL (pH 7.5), 10 % glycerol, 0.02 % bromophenol blue, 20 mM EDTA	70 µL
Component #3: Probe Labeling Solution; 0.4µM 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
Component #4: Large Fragment of DNA Polymerase I (Klenow fragment); 3 units/µL in 0.1M potassium phosphate (pH 7.0), 10 mM 2-mercaptoethanol, 50 % (v/v) glycerol	30 units
Component #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. The 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 0.5-1.0 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 at -80 °C.

*Patent Pending by Life Technologies, Inc.

Instructions for Use of Components #1 - #5

Electrophoresis of Marker DNA: The concentration of Marker DNA is adjusted so that 3 µL 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 (7µL).
2. Heat the marker at 65 °C for 5 min.
3. 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.

Labeling Marker Probe with ^{32}P

1. In a clean microcentrifuge tube place:
   44 μL Component #3 (Labeling Component)
   5 μL α^{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 23-25 °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

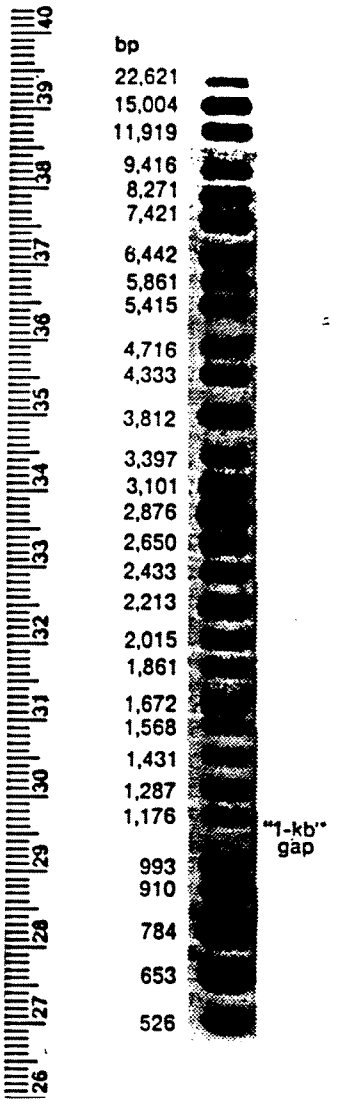
4,716
4,333
3,812

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 Co., 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 - #11) and Loading Buffer (Component #19)

Storage Conditions: Reagents may be stored at 4 °C for 3-6 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 5-10 min.

Summary and Explanation of Components: The components enable the user to run 4 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 (1-100 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 TAE 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 $\geq 10^9$ 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 5×10^5 cpm/mL (60 mL) at 65 °C for 16-24 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.

Biodyne• B is a registered trademark of Pall Biosupport Division.
Cronex• Lighting Plus is a registered trademark of E.I. duPont deNemours and Company.

(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. Yield gels are run in the presence of ethidium bromide.

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 - #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 electroendosmosis (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 2 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 - 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
6,085/6,067
5,051
4,187/4,131
3,656/3,613
2,275
1,475
1,053
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 3-6 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 5-10 minutes.

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 by the protocol described in "Procedures for the Detection of Restriction Fragment Length Polymorphisms in Human DNA", Forensic Science Research and Training Section, FBI Laboratory. 1 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 D4S139 (PH30) [7] and D2S44 (YNH24) [8]. The bands were sized using the BRL DNA Analysis Marker System.

Specification:	After an overnight exposure, only 2 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 1-2 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 μL 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 16-24 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.

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(Assays were performed by Life Technologies, Inc.)

K562 DNA, 25 μL (Component #13)

Storage Conditions: Store at -20 °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:

- 1 μg K562 DNA was digested with endonuclease Hae III, electrophoresed through an agarose gel, transferred to a nylon membrane, and hybridized with DNA probes D4S139 (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 2 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.; Note that 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 - 1.9
  Value Obtained: 1.88

- 1 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 16-24 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.

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K562 DNA, Hae III digested, 100 μL; 25ng/μL (Component #14)

Storage Conditions: Store at -20 °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 D4S139 (PH30) [7] and D2S44 (YNH24) [8]. The bands were sized using the BRL DNA Analysis Marker System.

  Specification: After an overnight exposure, only 2 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 16-24 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.

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(Assays were performed by Life Technologies, Inc.)

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

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)

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_2 EDTA. Reagents may be stored at 4 °C for 3-6 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)

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 3-6 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 5-10 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 DNA's range from 3-4 $\mu g/\mu L$, so the user might want to adjust accordingly. In addition, the genomic DNA may require warming to 56 °C for 5-10 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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