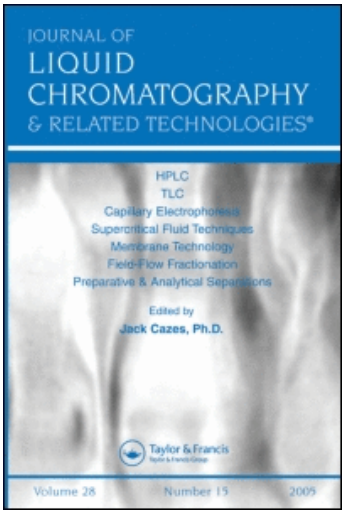

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

GENOTYPING OF A SHORT TANDEM REPEAT SYSTEM USING ION-PAIR REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Joseph M. Devaney[a]; James E. Girard[b]; Michael A. Marino[a]
[a] Transgenomic Inc., Gaithersburg, MD, U.S.A. [b] American University, Washington, DC, U.S.A.

Online publication date: 31 August 2001

J. LIQ. CHROM. & REL. TECHNOL., 24(13), 1997–2005 (2001)

GENOTYPING OF A SHORT TANDEM REPEAT SYSTEM USING ION-PAIR REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Joseph M. Devaney,[1,2,]* James E. Girard,[2] and Michael A. Marino[1]

[1] Transgenomic Inc., 11 Firstfield Road, Suite E, Gaithersburg, MD 20878, USA
[2] American University, 4400 Massachusetts Avenue, Washington, DC 20016, USA

ABSTRACT

Ion pair-reverse phase high performance liquid chromatography (IPRP HPLC) is a method that can be used for the genotyping of short tandem repeats (STRs) in human nuclear DNA. The genotyping of STRs is useful in human identification and parentage testing, gene mapping studies, cancer diagnostics, and diagnosis of hereditary diseases. IPRP HPLC offers an attractive method for STR analysis because of the short analysis time, and there is no need for the waste disposal associated with radioisotopic, enzyme-linked, or fluorescence detection systems. We evaluated the use of IPRP HPLC for the sizing and typing of STR alleles from the HUMTHOl locus on chromosome 11p15.5.

The IPRP HPLC conditions (column temperature, flow rate, and percent organic modifier per minute) were optimized for the

*Corresponding author. E-mail: jdevaney@transgenomic.com

separation of PCR products in the size range of 50-434 base pairs. Using the optimized separation conditions, the alleles of the HUMTHO1 system were sized in their native state (double stranded) with the use of internal markers. The analysis time for the HUMTHOl locus was less than 14 minutes, and the alleles could be peak captured for further examination such as sequencing.

INTRODUCTION

Short tandem repeats (STRs) are tandemly repeated units of sequence ranging between 2 and 7 basepairs (bps) in length that have high levels of heterozygosity, distinguishable alleles that are polymorphic based on the number of repeating units, and are amplified using the PCR reaction.[1-4]

One method to examine STRs is the WAVE® DNA Fragment Analysis System that employs ion-pair reverse-phase chromatography, that has been shown to give strict and reproducible size-based separation of DNA fragments from 40 up to 2,000 bp when analysis is in non-denaturing mode.[4-8]

The work presented here, demonstrates the use of WAVE® DNA chromatography technology in the separation, sizing, and typing of alleles from the locus HUMTHO1 located in intron 1 of the human tyrosine gene with a chromosomal position of **11**p15-15.5. The alleles were sized in their native state (double stranded). This locus is widely used in forensic and parentage assessment cases, therefore, it served as a great model for microsatellite sizing and type.

EXPERIMENTAL

Instrumentation

The WAVE® DNA Fragment Analysis System used for purification of markers and analysis of PCR products, is a Transgenomic non-metallic PEEK system with a variable wavelength detector set at 260 nm, a DNAsep® column (Transgenomic), and an autosampler with the capacity to handle 96 samples.

Chemicals

HPLC grade acetonitrile (ACN) (EM Science, Gibbstown, NJ) and triethylammonium acetate (TEAA) (Transgenomic, Inc., San Jose, CA, USA) were used to constitute the mobile phase.

STR Amplification

DNA amplification was performed as described in the Perkin-Elmer ABI PRISM STR protocol (Perkin-Elmer, Foster City, CA). The amplified tetrameric STR locus is HUMTHOl. This locus is amplified using primers from the Forensic Service of the British Home Office for use in forensic casework.

Chromatographic Conditions

The chromatographic conditions explored were column temperature, flow rate (mL/min), and percent acetonitrile per minute. For the optimization of acetonitrile (ACN) concentration, buffer B (containing 25% ACN) was increased from 40 to 65% of the buffer mixture (2.0% B per minute). Once the optimization was completed, the HPLC conditions for the sizing and typing of the

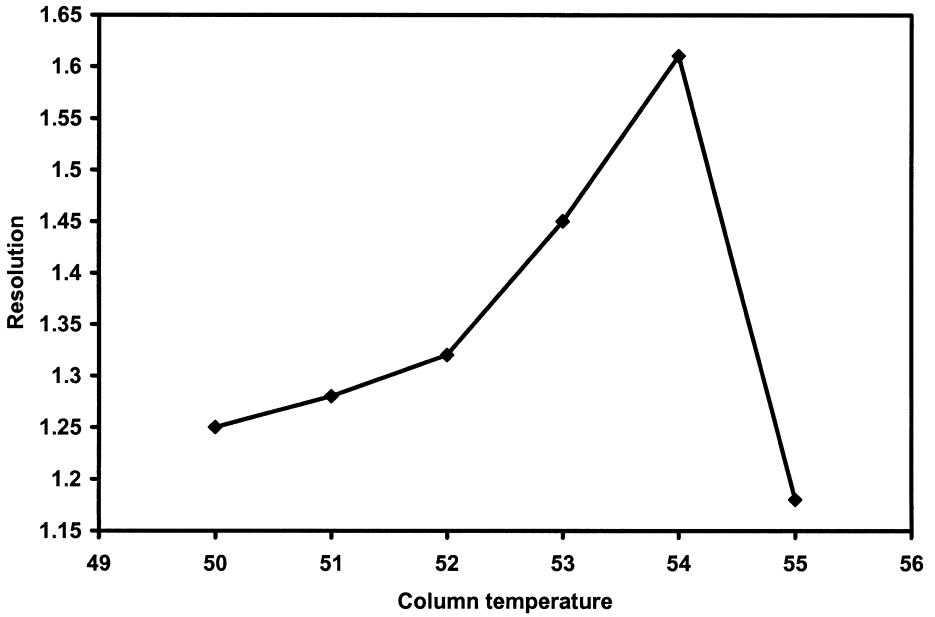

Figure 1. Resolution for 171-bp / 175-bp PCR products with increasing column temperature. The chromatographic conditions for the optimization of column temperature were a starting % B of 40 and the ending % B of 65 (2% B per min) with a flow rate of 0.9 mL/min.

HUMTHO1 locus were a column temperature of 54°C, a flow rate of 0.8 mL/min, and a 0.25% increase in ACN per minute. The starting and ending points for the linear gradient for the allele separations were 43% to 57% buffer B.

RESULTS AND DISCUSSION

The optimization of chromatographic conditions for the separation of HUMTHO1 alleles is shown in figures 1-3. As shown in figure 1, the column temperature was optimized by measuring the resolution between HUMTHO1 alleles consisting of 9 repeats (171-bp) and 10 repeats (175-bp). The optimal column temperature was 54°C and this column temperature was used for all subsequent analysis. Figure 2 shows the optimization of flow rate (mL/min), and the highest value of resolution between the 171-bp and 175-bp PCR products was at a flow rate of 0.8 mL/min.

Figure 3 shows the optimization of percent ACN per minute. The highest resolution value occurs at 1.0% of buffer B containing 25% ACN per minute (0.25% increase in ACN). The results of the optimization experiments were a

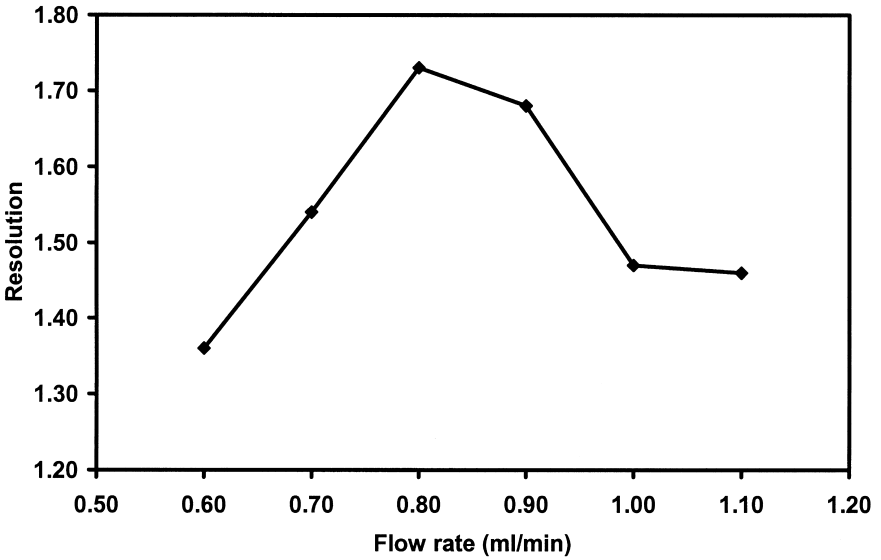

Figure 2. Resolution for 171-bp / 175-bp PCR products with increasing flow rate. The chromatographic conditions for the optimization of flow rate were a column temperature of 54°C and the same gradient conditions.

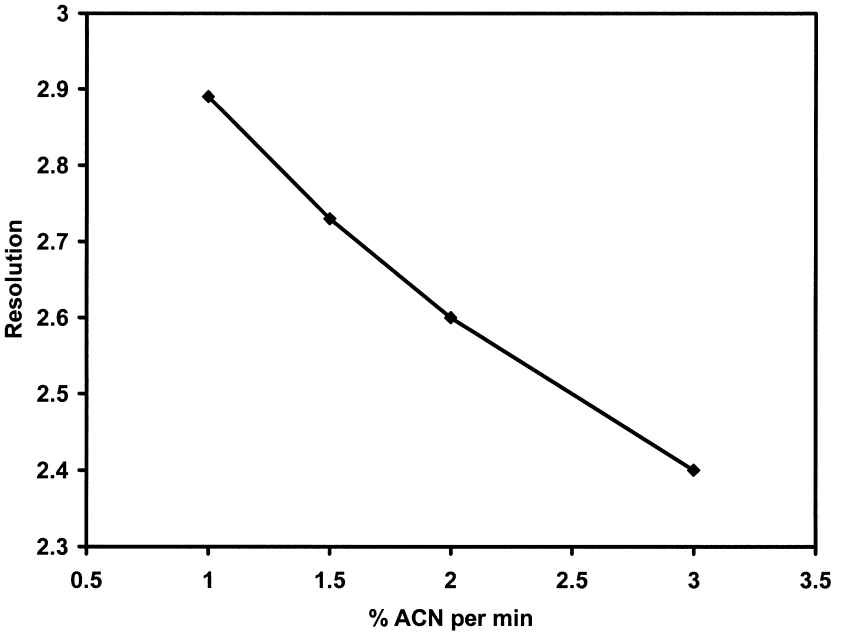

Figure 3. Resolution for 171-bp / 175-bp PCR products with increasing % Eluent B per minute. With the optimized IPRP HPLC conditions of column temperature 54°C and flow rate 0.8 mL/min.

column temperature of 54°C, a flow rate of 0.8 mL/min, and a 0.25% increase in ACN per minute (1% B per minute). These conditions were used for all sizing and typing experiments.

Figure 4 is the separation and sizing of an allelic ladder (HUMTHO1 alleles with types 6, 7, 8, 9, and 10) with markers (147-bp and 184-bp) surrounding the alleles. The sizing assay uses the markers to draw a line of regression. The alleles are fit to the line and a bp size is calculated. From the sizing of the allelic ladder, types can be assigned to calculated sizes. When unknown samples are analyzed and a size is calculated, an allele type can be assigned. Figure 5 shows an example of the DNA chromatography analysis of HUMTHO1 alleles (9, 9.3). The flanking standards are found at 11 min and 13.5 min, with bp sizes indicated on the chromatogram. Table 1 contains the sizing and typing of 15 unknown samples.

Table 2 shows the accuracy for the sizing procedure. The accuracy for the sizing assay was determined by comparing the calculated allele size with the size of the allele, as determined by sequencing. For all alleles, the sizing accuracy was from 0.47-bp for the 9.3 allele, to 1.68-bp for the 8 allele.

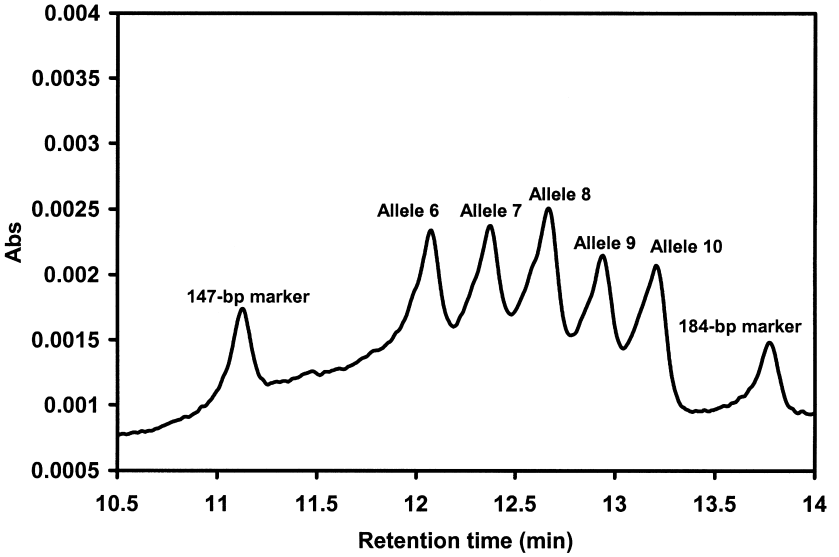

Figure 4. Separation of a HUMTHO1 allelic ladder using the Wave® chromatography system. The analysis conditions are located in the text.

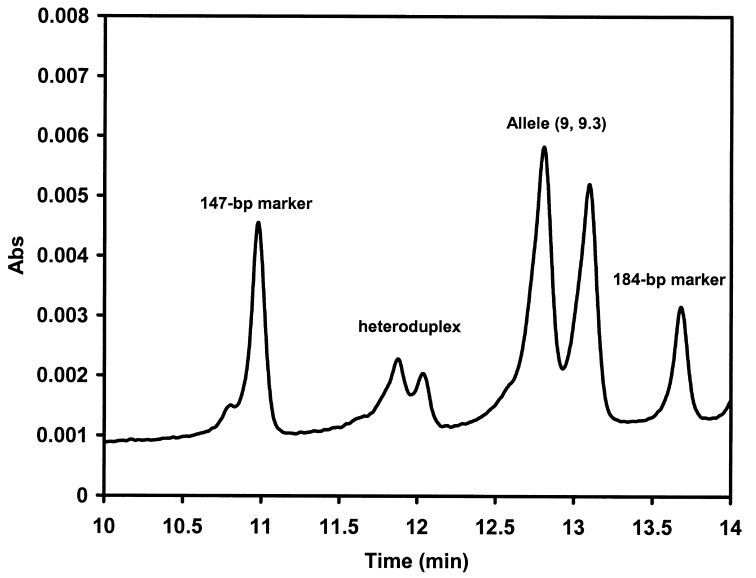

Figure 5. Typing of the HUMTHO1 locus using the Wave® system. The analysis conditions are located in the text.

Table 1. Allele Sizes and Types Calculated Using the 147 and 184-bp Markers

Sample	Calculated bp Sizes	Allele Type	Allele Type Using ABI 310 Genetic Analyzer
1	171.90, 173.95	9, 9.3	9, 9.3
2	168.73, 176.30	8, 10	8, 10
3	172.08, 176.05	9, 10	9, 10
4	173.89	9.3, 9.3	9.3, 9.3
5	159.66	6, 6	6, 6
6	164.53, 172.40	7, 9	7, 9
7	174.52	9.3, 9.3	9.3, 9.3
8	160.58, 168.90	6, 8	6, 8
9	164.00, 172.16	7, 9	7, 9
10	160.28, 172.34	6, 9	6, 9
11	164.14, 172.16	7, 9	7, 9
12	160.46, 172.57	6, 9	6, 9
13	160.29, 168.65	6, 8	6, 8
14	171.86, 174.78	9, 9.3	9, 9.3
15	164.40, 172.40	7, 9	7, 9

Table 2. Sizing Accuracy on the Wave® System

HUMTHO1 Allele	bp length	Calculated bp Length Using the Wave® System
6	159	160.19
7	163	164.41
8	167	168.68
9	171	172.42
9.3	174	174.47
10	175	176.22

Table 3. Sizing Precision on the Wave® System

HUMTHO1 Allele	N	Mean Calculated bp Size	Standard Deviation
6	12	160.16	0.40
7	8	164.21	0.18
8	6	168.76	0.13
9	16	172.18	0.24
9.3	12	174.26	0.40
10	4	176.17	0.18

The precision of a method is defined as the ability to obtain reproducible sizing of DNA fragments from injection to injection using an instrument. The sizing precision or standard deviation was determined for a set of thirty samples that were analyzed on different days and found to range as in Table 3. The standard deviation for the sizing of the alleles ranged from 0.13 to 0.40.

CONCLUSIONS

The separation of dsDNA on a HPLC with a DNAsep® column was optimized PCR-amplified alleles with respect to column temperature (54°C), flow rate (0.8 mL/min), and % ACN per min (0.25). With these optimal conditions, the resolution obtained for HUMTHO1 allele pairs ranged from 4.95 for a HUMTHO1 sample consisting of the allele pair 6, 10 (165 and 175-bp) to a resolution of 1.55 for a HUMTHO1 sample consisting of the allele pair 9, 9.3 (171 and 174-bp). These optimal conditions were used to size and type HUMTHO1 alleles in their natural state (double-stranded) with the use of RE-cleaved dsDNA as sizing markers.

Since the bracketing size markers were used as internal standards for each analysis, we were able to correct for any slight differences in the retention times that occurred from day to day analysis. In addition, the sample alleles can be isolated and purified for further analysis such as sequencing or cloning. The WAVE® system offers further advantages, including no post-PCR sample preparation, no requirement to label samples for detection, plus automation in both sample loading and analysis.

REFERENCES

1. Wallin, J.M.; Buoncristiani, M.R.; Lazruk, K.D.; Fildes, N.; Holt, C.L.; Walsch, P.S. J. Forensic Sci. **1998**, *43*, 854-870.
2. Noviello, C.; Courjal, F.; Theillet, C. C. Clin. Cancer Res. **1996**, *2*, 1601-1606.
3. Mansfield, D.C.; Brown, A.F.; Green, D.K.; Carothers, A.D.; Morris, S.W.; Evans, H.J.; Wright, A.F. Genomics **1994**, *24*, 225-233.
4. Thomson, J.A.; Pilotti, V.; Stevens, P.; Ayres, K.L.; Debenham, P.G. Forensic Sci. Int. **1999**, *100*, 1-16.
5. Huber, C.G.; Oefner, P.J.; Bonn, G.K. Anal. Biochem. **1993**, *212*, 351-358.
6. Huber, C.G.; Oefner, P.J.; Bonn, G.K. Anal. Chem. **1995**, *67*, 578-585.
7. Huber, C.G.; Oefner, P.J.; Preuss, E.; Bonn, G.K. Nucleic Acids Res. **1993**, *21*, 1061-1066.

8. Marino, M.A.; Devaney, J.M.; Smith, J.K.; Girard, J.E. Electrophoresis **1998**, *19*, 108-118.

Received November 23, 2000 Manuscript 5438
Accepted December 15, 2000