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STR Profiling of Human Cell Lines: Challenges and Possible Solutions to the Growing Problem

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Abstract

Genomic DNA preparations from 60 human cell lines from the National Cancer Institute (NCI), 48 human cell lines from American Type Culture Collection (ATCC) and 19 embryonic cell lines were profiled for autosomal short tandem repeat (STR) loci using the AmpF*l*STR® Identifiler® kit. Each DNA sample was profiled at least twice to ensure consistency and reproducibility of results. The resulting STR profiles were compared with the STR profiles in the database of ATCC. The allele calls for the common loci between the Identifiler® kit and the database were identical except for one DNA sample, which we attribute to amplification artifacts. We have observed a high percentage of the STR loci exhibiting allelic imbalance. Certain STR loci for some cell lines exhibited 3 or more alleles. This type of observation can result from a unique profile for a given cell line or as the result of clonotypic heterozygosity and is not necessarily due to contamination. Our study demonstrates that STR based technologies are useful for cell line authentication applications. These data, combined with data from other researchers, will enable the development of a standard genotyping protocol for cell line authentication.

Keywords: Cell line identification; Cell line authentication; Cell line contamination; STR; SNP; Cell line ID

Abbreviations: STR: Short Tandem Repeat; RFLP: Restriction Fragment Length Polymorphism; ISSR: Inter-simple Sequence Repeat; VNTR: Variable Number of Tandem Repeat; SNP: Single Nucleotide Polymorphism; CODIS: Combined DNA Index System

Introduction

Cell culture technology is a vital research tool used in diverse areas such as basic research, medicine, genetics, cell biology, vaccine development, reconstructive medicine, toxicity testing, and drug discovery, screening and development. The utility of cell lines became popular in different areas of science because of widespread availability and improvements in cell culture technologies. Though the history of cell culture dates back to 1885, HeLa was the first human cancer cell line to be established in culture in 1952 [1,2]. The cell lines used for biomedical research can be grouped into primary cell cultures, continuous cell cultures (established cell cultures), and stem cells. Cell line misidentification or contamination, similar to microbial contamination, are routine and obvious laboratory issues. However, from the literature it is evident that many cell biologists, unlike microbiologists, have not resolved these issues. The problem of cell line contamination and misidentification is highlighted in several articles, spanning several decades [3-7]. It is estimated that the results from studies published in as many as 20% of scientific publications using cultured cells could be of questionable significance [8] because 15 – 36% of the cell lines used were either misidentified or cross-contaminated with another cell line [9,10]. Dr. Roland M. Nardone submitted a letter in July 2007, signed by 18 other leading scientists, to the Secretary, US Department of Health and Human Services, highlighting the incidences and severity of this issue [7]. Lack of cell authentication results in wasted resources and impedes scientific progress, including drug discovery.

There are multiple sources of cell line contamination or misidentification [3-5,7]. Some common reasons are: inadvertent mixing of cell lines, mislabeling, over-subculturing, the pleiomorphic nature of the cells in culture and differential proliferation rates (resulting in a given strain taking over the culture). Lack of proper training of personnel handling the cell lines and transfer of unauthenticated cell lines from one investigator to another can compound the problem. Moreover, scientists may assume that changes in cell cultures resulted from the *in vitro* environment and a change in gene expression, rather than from mislabeling or the unintended introduction of an alien cell. The absence of distinctive morphological, biochemical and genetic markers for distinguishing one cell line from another can make identifying contamination difficult.

The need for cell line authentication was proposed as early as the 1970s by W. Nelson-Reese [11]. Confirmation of the identity and purity of cell lines is now becoming a prerequisite for distribution. All reputable cell banks now employ methods to confirm the identity and origin of the cell lines that they distribute [12]. Isoenzyme analysis, karyotyping, fluoroscent antibody staining, HLA typing, verification of species origin, and tests for microbial contamination are some of the methods for cell authentication and characterization [13-18]. The use of polymorphic genetic markers in the authentication of cell lines is becoming more popular because of their discrimination power and commercial availability of easy to use kits for this purpose. These genetic markers [19] are useful in genotyping assays such as restriction fragment length polymorphism (RFLP), retro-element insertion polymorphism [20], inter-simple sequence repeat (ISSR) [21],

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variable number of tandem repeat (VNTR) [22,23], single nucleotide polymorphism (SNP) [24] and short tandem repeat (STR) [25-30] for identification of cell lines. STRs with 2-6 bases have been routinely used in human identification laboratories for applications such as paternity testing, forensic casework, and the identification of victims of mass disaster for more than two decades [31-34].

In the past few years, application of STRs in human cell line identification has received a good deal of attention due to its usefulness in human identification [25-29]. Use of a SNP panel for human identification is an alternate genotyping system [35]. SNP genotyping offers several advantages over STRs such as the abundance (~9 million in the human genome), distribution throughout the genome (about 1 every 300 bp), 90% of human genetic variation come from SNPs, low mutation rate (1 x 10^{-9} per locus per generation), biallelic nature, high multiplex capability, and ease of data analysis. Though STRs are predominantly used for human identification, SNPs are commonly used for the identification of missing persons, victims of mass disaster, and resolving complex paternity cases [36]. The TaqMan Genotyping assays can detect single SNPs and the GenPlex™ HID system enables simultaneous amplification of 48 SNPs for such applications. The utility of this system in human identification has been demonstrated in multiple laboratories [35,37,38].

We describe the usefulness of the Identifiler® kit (for the analysis of 15 autosomal STR loci and Amelogenin) in human cell line authentication. The Identifiler® kit is currently used for human identification in forensic laboratories and provides a standard metric for human cell line authentication. Cell line authentication procedures will become relevant in source attribution for cultures in research laboratories and biological sample tracking in cell based therapeutics. These tools provide a standard methodology to assess the origin of a given cell, determine if contamination or clonotypic heterozygosity are an issue, as well as determine if there are unique genotypic features in a given cell line, particularly cancer cells. We also address several important considerations when analyzing genotyping data.

Materials and Methods

Genomic DNA from the panel of 60 human cancer cell lines (NCI-60) utilized in the National Cancer Institute's (NCI) anti-cancer drug screen was kindly provided by the NCI (National Cancer Institute, Bethesda, MD). Genomic DNA from 48 human cell lines was obtained from American Type Culture Collection (ATCC, Manassas, VA). Genomic DNA from 19 embryonic stem cell lines (provided by Dr. Uma Lakshmipathy, Invitrogen, Inc., Carlsbad, CA) was isolated with the DNeasy® Blood and Tissue Kit (QIAGEN, Valencia, CA). The cell lines are listed in Tables 1, 2, and 3 of the supplementary material.

The Quantifiler® Duo DNA quantification kit, Identifiler® kit, 7500 Real-time PCR System, 3130*xl* Genetic Analyzer and associated software, and GeneMapper® ID v3.2.1 software were from Applied Biosystems (Foster City, CA). PowerPlex® 16 was obtained from Promega (Madison, WI). All other chemicals used in this study were of analytical grade.

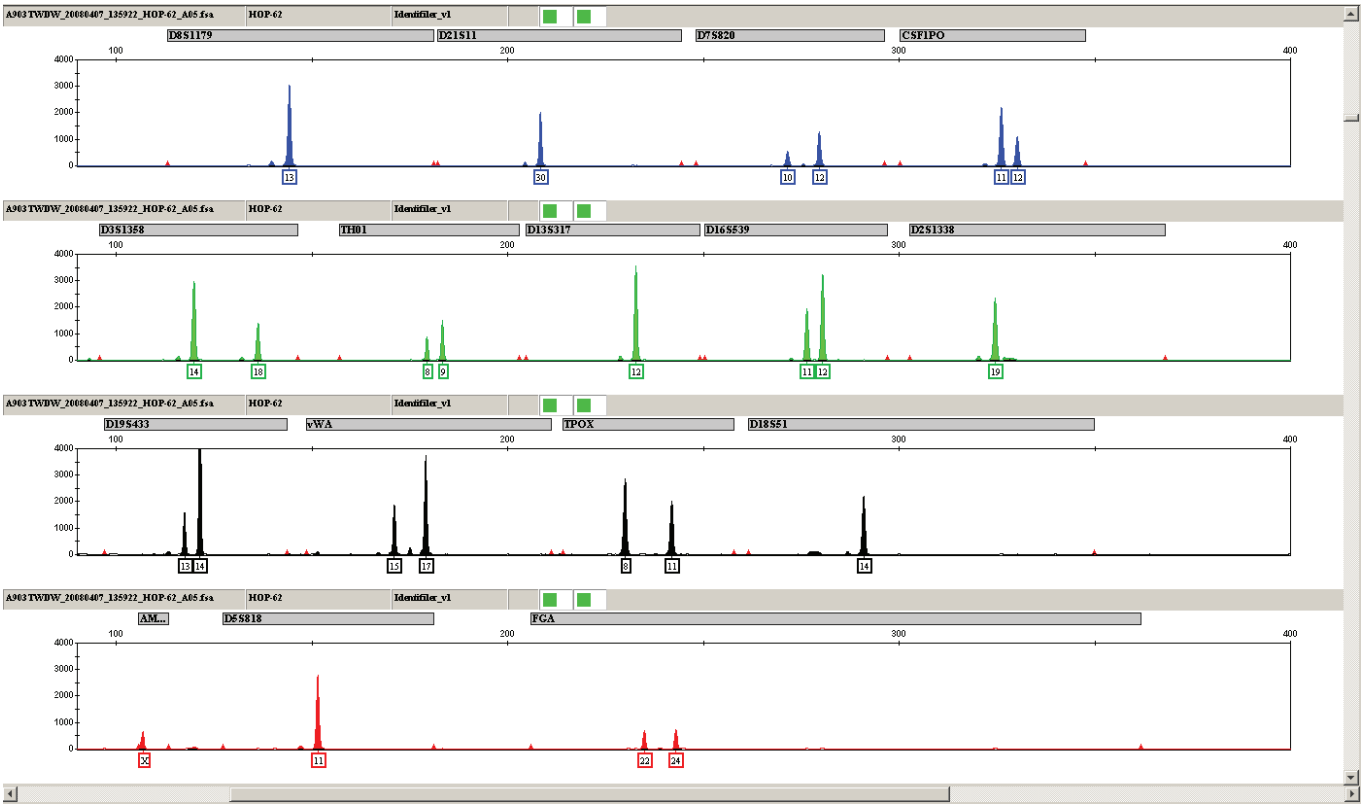

Figure 1: Typical STR profile with allele designation for genomic DNA extracted from the NCI cell line HOP-62. The horizontal axis represents the values in base pairs and the vertical axis represents the values in relative fluorescent units (RFU). The 6-FAM channel (blue) shows the loci D8S1179, D7S820, D21S11, and CSF1P0; the VIC channel (green) contains the loci D3S1358, TH01, D13S317, D16S539, and D2S1338; the NED channel (yellow) shows the loci D19S433, vWA, THO1, and D18S51; the PET channel (red) contains the FGA, D5S818, and Amelogenin.

Quantitation of human DNA

The quantity of human DNA in the preparations was determined with the Quantifiler® Duo DNA Quantification Kit (Applied Biosystems, Foster City, CA) using 2 µL of the DNA extract [39]. PCR was performed on the 7500 Real Time PCR System and the data were analyzed using 7500 System SDS Software v1.2.3 (Applied Biosystems, Foster City, CA).

STR analysis

The DNA samples were amplified using the Identifiler® kit as described previously [40,41]. The quantity of template DNA was 1 ng unless otherwise mentioned in the text. The amplified products were analyzed on a 3130*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA) and data analysis was performed using GeneMapper® ID Software v3.2.1 (Applied Biosystems, Foster City, CA) using 50 rfu (relative fluorescence units) as the threshold.

Results

We profiled genomic DNA from 60 human cell lines (NCI-60 cell lines), 48 human cell lines obtained from ATCC, and 19 embryonic stem cell lines. The genotypes are provided as supplementary data in Tables 1, 2, and 3, respectively. A typical Identifiler® profile from a cell line is presented in Figure 1. The genotype results obtained from our analysis were compared with the genotypes for 44 cell lines available from the ATCC website for the overlapping loci. Out of a total of 387 alleles from the 44 cell line DNA preparations, only one discrepant genotype was observed. The genotype for the D5S818 locus for 22Rv1 cell line DNA obtained in our laboratory was 11, 13, and is reported as 11, 12, 13 at the ATCC's website. The genotype 11, 13 at this locus was confirmed by re-amplification of this DNA preparation using 2 ng of template. Such comparison for the profiles generated from NCI-60 cell line DNA preparations could not be made because the genotypes generated at NCI for 22Rv1 were not available.

Table 4: STR profiles for NCI 60 cell lines exhibiting similar STR profiles.

Marker	Cell lines		Cell lines	
	NCI-ADR-RES	OVCAR-8	M14	MDA-MB-435
AMEL	X	X	X	X
CSF1PO	11	11	11	11
D13S317	12	12	12	12
D16S539	13	13	9,13	13
D18S51	14	14	13,17	13,17
D19S433	14,16	14,16	14,15	14
D21S11	28	28	30	30
D2S1338	19,23	19,23	19,24	19,24
D3S1358	18	16,18	14,16	14
D5S818	12	12	11,12	11,12
D7S820	12	12	8,10	8,10
D8S1179	10	10	13	13
FGA	20	20	21	21
TH01	7	7	6,7	6,7
TPOX	8	8	8,11	8,11
vWA	16,17	16,17	16,18	16,18

Table 5: STR profiles for the two ATCC cell lines.

Marker	Cell line	
	NCI-BL1395	NCI-H1395
AMEL	X	X
CSF1PO	12	12
D13S317	10,14	10,14
D16S539	11,13	11,13
D18S51	12,14	12,14
D19S433	13,16	13,16
D21S11	28,29	29
D2S1338	17,24	17,24
D3S1358	15,18	15
D5S818	12	12
D7S820	8	8
D8S1179	12,14	12,14
FGA	18,23	18,23
TH01	6,9.3	6,9.3
TPOX	8	8
vWA	14,17	14,17

Of the NCI cell lines studied, the genotype for the DNA from NCI-ADR-RES was identical to the profile from OVCAR-8 except for the locus D3S1358 (Table 4). The genotype for the DNA from M14 was identical to the profile from MDA-MB-435 except for loci D16S539, D19S433, and D3S1358 (Table 4).

ATCC cell lines NCI-BL1395 and NCI-H1395 were from the same individual, NCI-BL-H1395 was from the EBV transformed B lymphoblast and NCI-H1395 was from an adenocarcinoma. However, the STR profiles for the DNA preparations from these cell lines was different for D21S11 and D3S1358 loci when amplified by the Identifiler® kit (Table 5). The genotypes of DNA preparations obtained from ATCC and NCI were compared; the STR profile for five cell lines obtained from ATCC matched that of the DNA preparations obtained from NCI (Table 6).

We observed that the STR profiles of DNA from the NCI and ATCC cell lines had some unique features not usually present in human reference samples. Three examples are provided here. The profile generated from HCC38 cell line DNA (ATCC) exhibited high peak imbalance at multiple heterozygous loci (Figure 2a). The loci D8S1179, D21S11, D13S317, D16S539, and D2S1338 exhibited high peak imbalance (high heterozygote peak height ratio) of 30, 43, 34, 43, and 43%, respectively. In contrast, the alleles at D19S433, vWA, and TPOX loci exhibited good peak balance of 90, 95, and 87, respectively. The observation was confirmed by re-amplification (Figure 2b). The high peak imbalance pattern was also confirmed by amplification using the PowerPlex® 16 genotyping system (data not shown), which eliminates the primer binding and other PCR amplification related effects.

Figure 3a presents the profile for the HCC1143 cell line DNA wherein the allele 17 at the D18S51 locus was amplified at low amplitude compared to allele 14 resulting in higher peak imbalance. As a result, homozygous allele 14 was the genotype called. Allele 17 was recovered (above the analysis parameter threshold) using 2ng of template DNA (Figure 3b). Correct genotype for the HCC1143 cell line DNA at the D18S51 locus is 14, 17.

Multi-allele profiles were observed for a few cell lines. For example, the MOLT-4 profile has multiple alleles at the loci D21S11, D7S820, CSF1PO, FGA, and D18S51 (Figure 4). This multiple allele pattern was confirmed by repeat amplification using 2 ng of template DNA (data not shown). The multi allele pattern was also confirmed by amplification using the PowerPlex® 16 genotyping system (data not shown).

In a separate study, we profiled 19 embryonic stem cell lines analyzed in a previous study [42] using the Identifiler® kit (supplementary data (Table 3). All profiles were conclusive. Results confirmed that several stably-transfected derivatives of BG01V (coded with YW or YA) appropriately matched the parental line and that differentiated cultures (EB) matched undifferentiated cultures. STR typing proved to be a valuable method for tracking cellular genotype without being affected by minor genetic modification or differentiation.

The ability of STR profiling for detection of a mixture was investigated by generating mixture of DNA preparations from HCT15 and ACHN cell lines at different ratios up to 1:15. The results are summarized in Figure 5. Mixtures can be tracked by amplitude of unshared alleles. In general, it is possible to detect mixture of two individuals in a sample up to a ratio of 1:10.

Discussion

There are several advantages to using autosomal STRs in human identity testing. From a technical point of view, the amplicons are relatively short, the size difference between two alleles at a locus from a heterozygous individual is small, development of megaplex PCR for simultaneous amplification of 16 to 20 loci simultaneously and the techniques to separate fragments with single base length variation are relatively straight forward and kits and software are available commercially. As a tool for the identification of a unique person, STRs are ideal because they have a highly polymorphic nature, are scattered through out the genome and STRs that reside on different chromosomes and are not linked provide a unique genotype with high discrimination power [31-33]. A core set of 13 autosomal STR loci, termed CODIS (combined DNA index system) loci, have been selected for the generation of a database of convicted offenders in the USA, as

Table 6: STR profile concordance between the same cell line DNA obtained from ATCC and NCI.

Marker	Cell Line and Source									
	Hs578T		MDA-MB-231		PC-3		T-47D		MCF7	
	ATCC	NCI	ATCC	NCI	ATCC	NCI	ATCC	NCI	ATCC	NCI
AMEL	X	X	X	X	X	X	X	X	X	X
CSF1PO	13	13	12,13	12,13	11	11	11,13	11,13	10	10
D13S317	11	11	13	13	11	11	12	12	11	11
D16S539	9,12	9,12	12	12	11	11	10	10	11,12	11,12
D18S51	16	16	11,16	11,16	14,15	14,15	17	17	14	14
D19S433	14,15	14,15	11,14	11,14	14	14	14	14	13,14	13,14
D21S11	29,32.2	29,32.2	33.2	33.2	29,31.2	29,31.2	28,31	28,31	30	30
D2S1338	17,26	17,26	20,21	20,21	18,20	18,20	24	24	21,23	21,23
D3S1358	16,17	16,17	16	16	16	16	15,17	15,17	16	16
D5S818	11	11	12	12	13	13	12	12	11,12	11,12
D7S820	10	10	8,9	8,9	8,11	8,11	11	11	8,9	8,9
D8S1179	13	13	13	13	13	13	13	13	10,14	10,14
FGA	23,24	23,24	22,23	22,23	24	24	23	23	23,25	23,25
TH01	9,9.3	9,9.3	7,9.3	7,9.3	6,7	6,7	6	6	6	6
TPOX	8	8	8,9	8,9	8,9	8,9	11	11	9,12	9,12
vWA	17	17	15,18	15,18	17	17	14	14	14,15	14,15

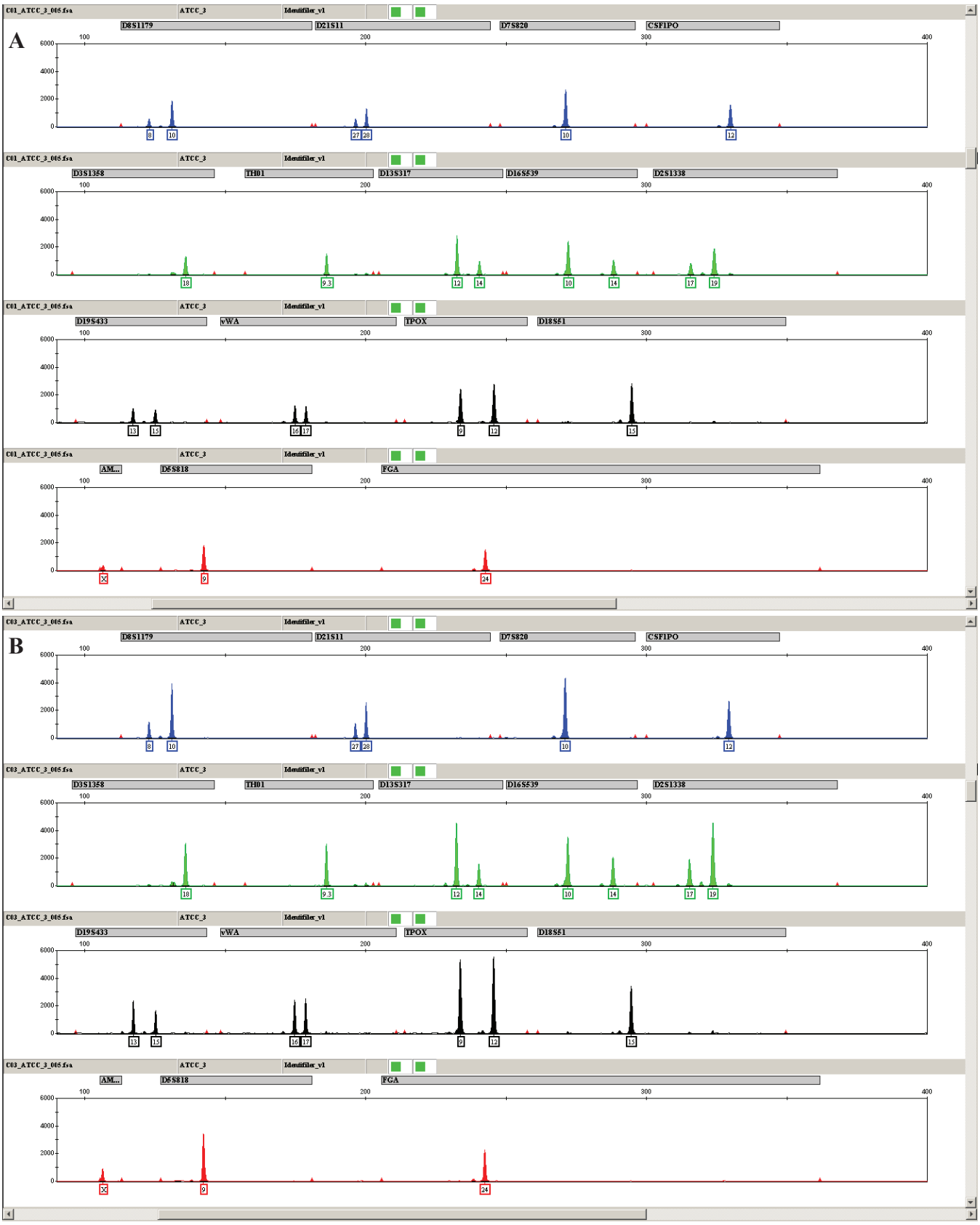

Figure 2: Identifiler® profiles of ATCC cell line HCC38. Genomic DNA from the cell line was amplified twice, (a) 1 ng DNA and (b) 2 ng DNA, to confirm the accuracy of STR genotyping.

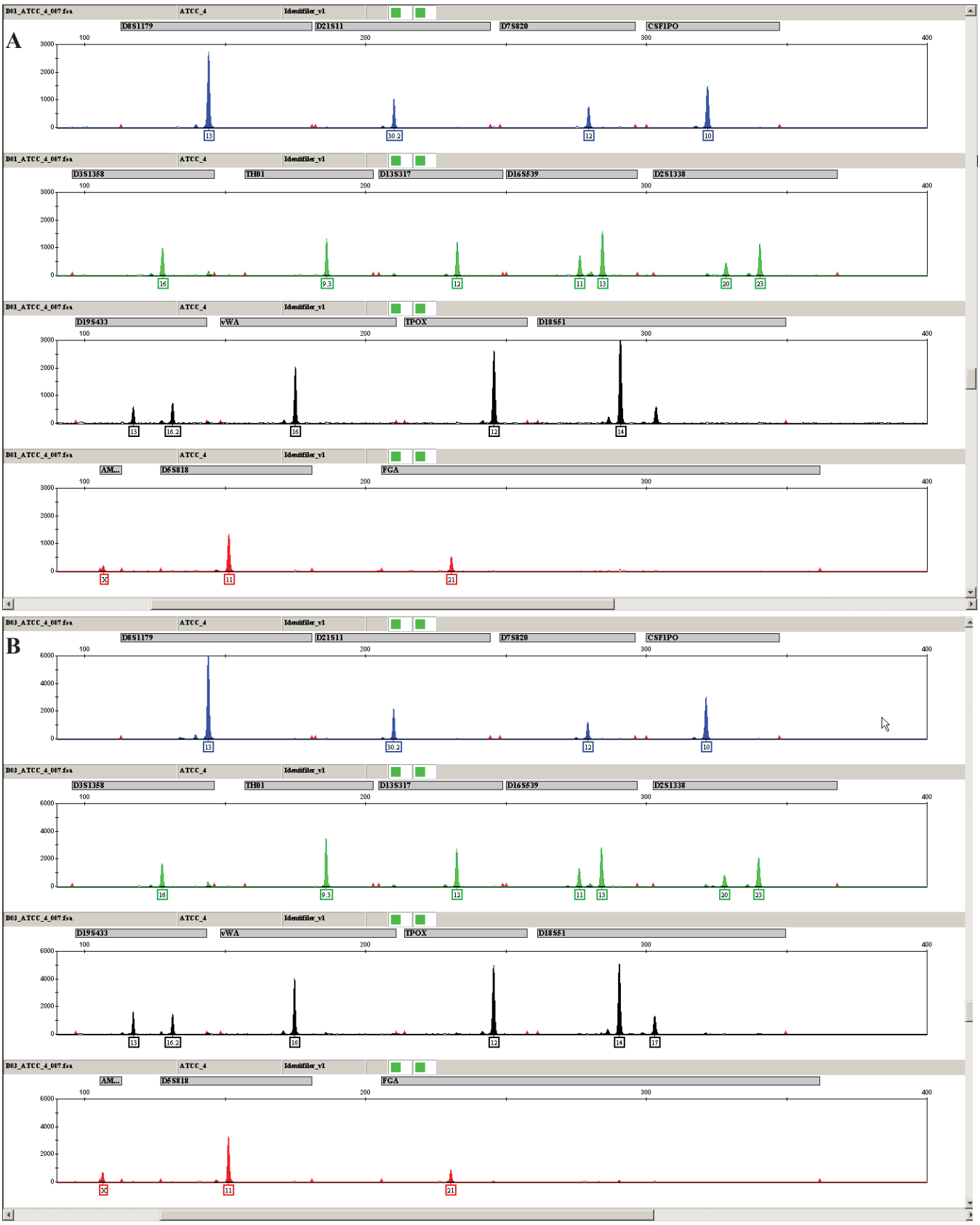

Figure 3: Identifiler® profiles of ATCC cell line HCC1143. Different amount of genomic DNA was used for PCR amplification, (a) 1ng DNA and (b) 2ng DNA.

well as over 25 other countries worldwide [33]. The CODIS database is managed by the Federal Bureau of Investigation (FBI). The Identifiler® kit enables simultaneous amplification of the 13 CODIS loci, D2S1338, D19S433 and sex determining marker Amelogenin. These loci are distributed over multiple chromosomes, thereby providing a high power of discrimination [41]. The probability of identity (P_i) values for the STR genotype from the Identifiler® kit is $1.32x10^{-18}$, $5.01x10^{-18}$, $7.65x10^{-18}$, and $3.62x10^{-17}$ for African American, US Caucasian, US Hispanic, and Native American population groups, respectively [41]. The Identifiler® kit is routinely used for genotyping applications in the forensic and human identification laboratories. Further, it is possible to obtain a complete STR profile from small quantities of DNA.

A comparison of five cell lines obtained from ATCC or NCI yielded identical STR profiles (Table 6). This confirms that quality control measures exist at these cell repositories. Obtaining a cell line from a reputable cell repository is, therefore, recommended. If this is not possible, authentication of the cell line is necessary. We observed a good correlation between the genotypes of the cell lines obtained in our laboratory and those available at the ATCC website except for the D5S818 locus for 22Rv1. The three allele pattern (alleles 11, 12, and 13) reported at the website of ATCC could be the result of an amplification artifact; allele 12 is probably a stutter product of allele 13. However, the possibility of genetic drift during sub-culturing cannot be ruled out. Parson et al. [26] have documented that the STR profiles of some cell lines change during long term culture as a result of clonotypic heterogeneity. The cell line pairs NCI-ADR-RES and OVCAR-8 and M14 and MDA-MB-435 probably originated from related individuals or these changes in genotype are result of mutations since the STR profiles are identical except for one and two loci, respectively (Table 4). These results suggest the use of 15 STR loci may be necessary to achieve the desired level of discrimination. The incremental cost of analysis of 15 STR loci over the fewer loci (e.g. 6 or 8) contributing from the cost of PCR reagents is marginal since the extraction, capillary electrophoresis, data analysis and analyst's time together to obtain a STR profile remains constant. It is important to note that ATCC cell lines NCI-BL1395 and NCI-H1395 originated from same individual but from two tumors (NCI-BL-H1395 was from the EBV transformed B lymphoblast and NCI-H1395 from adenocarcinoma) exhibited identical STR profile for 13 out of 15 STR loci (Table 5). Thus, it is important to track each cell line irrespective of the donor.

We observed a few unique patterns in the STR profiles for some cell lines. Below we elaborate more on the features of the analysis of these anomalies:

Heterozygote peak height ratio: DNA preparation from an uncontaminated cell line resembles a reference sample (single source sample) in human identification. One would expect that the two alleles at a heterozygous locus are amplified to a similar extent as measured by peak height. However, it is sometimes observed that the degree of amplification of the two alleles at a heterozygous locus varies. This differential amplification could be due to copy number variation at that loci, mutation at or near a primer binding region on one copy of the chromosome, the difference in the efficiency of amplification of the two alleles at each cycle (<5% variation in the efficiency of amplification during the initial PCR cycles can make a big difference in the final

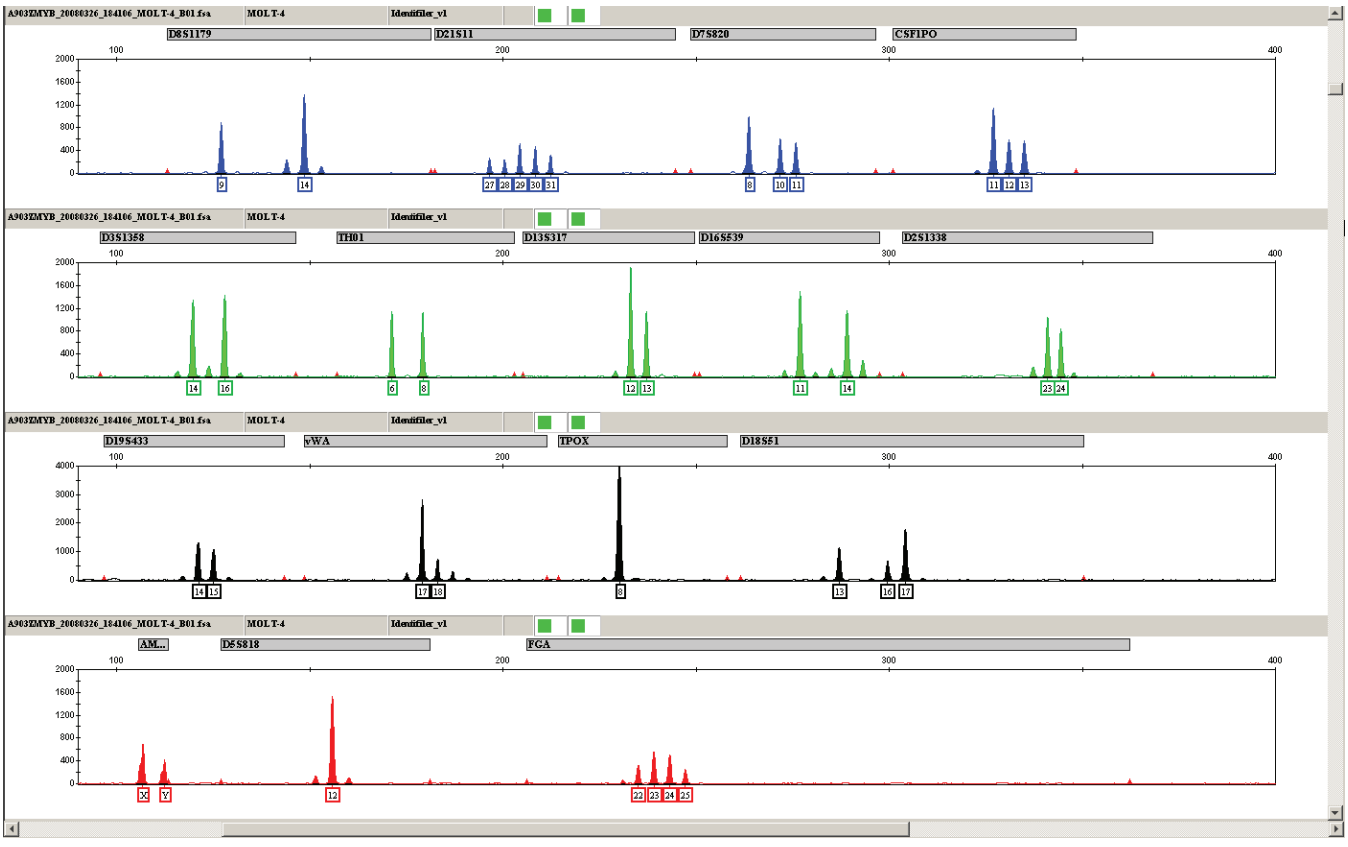

Figure 4: Identifiler® profiles of NCI cell line MOLT-4. Multiple allele (>2) pattern was observed for 5 loci.

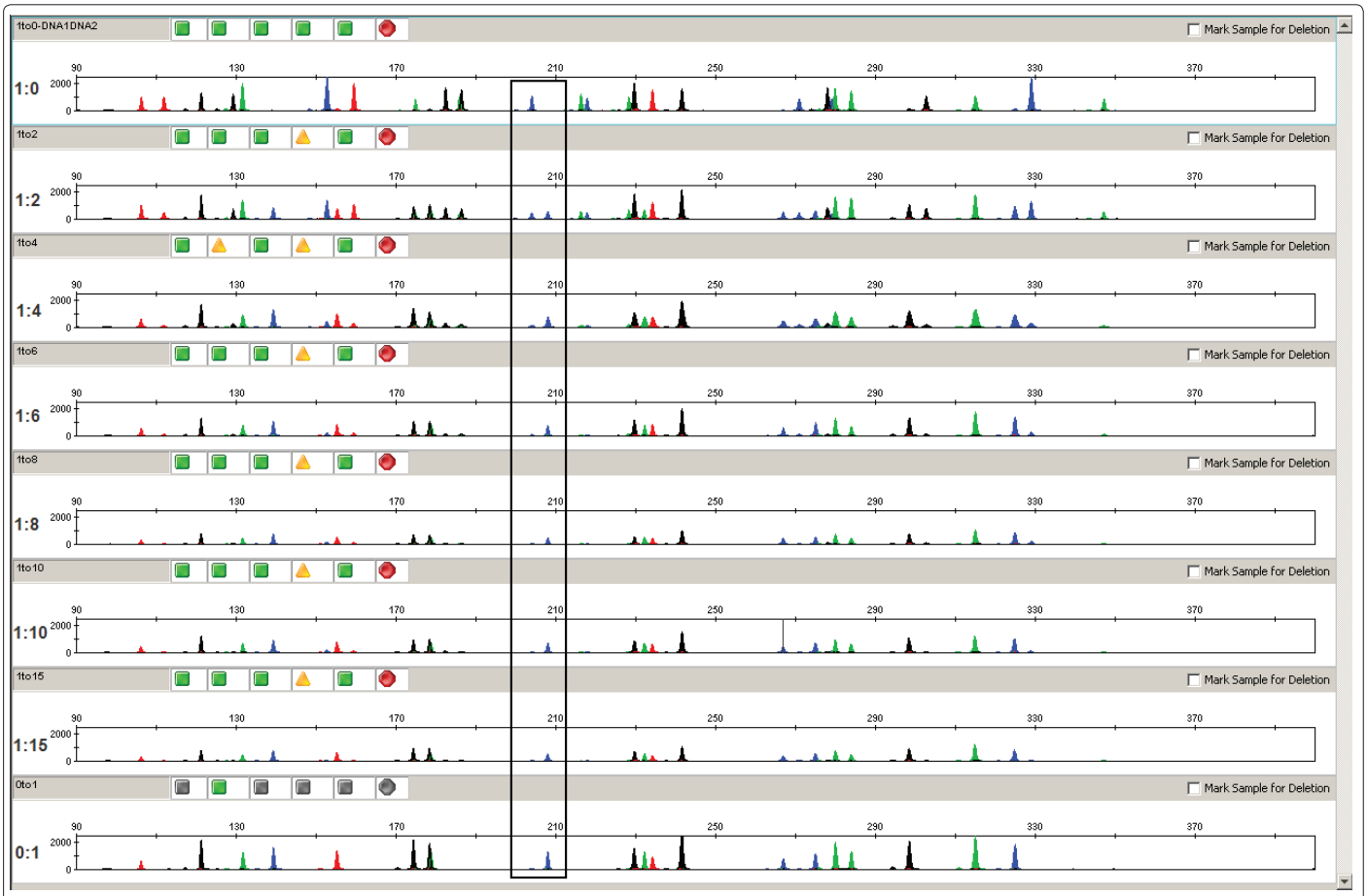

Figure 5: Detection of DNA mixtures. Mixture samples containing varying amounts of DNA from HCT15 and ACHN cell lines were prepared and amplified using the Identifiler® kit. The ratio of HCT15 and ACHN cell line DNA was 1:0, 1:2, 1:4, 1:6, 1:8, 1:10, 1:15 and , 0:1. The box denotes the allele changes at two loci at different DNA amount input ratio.

quantity of amplicons), number of copies in the template DNA (during dilution of the DNA extract the two copies of the genome may not get diluted to the same extent) or another, uncharacterized attribute of an individual. Acceptance criteria for a given reference sample is derived from internal validation studies by the human identification laboratory and may vary between laboratories. In general, a heterozygous peak height ratio of >50% is acceptable. However, the acceptable limit of heterozygous peak height ratio needs to be established for a given cell line, particularly for the cell lines exhibiting multiple allele patterns. It is important to note that this criterion does not apply to a mixed evidence sample. We have observed that many DNA preparations for the cell lines analyzed exhibited low heterozygote peak height ratios at multiple loci; e.g. the profile generated from the HCC38 cell line (Figure 2a and b). For ascertaining the genotype, it is recommended to quantify the human DNA with a human specific quantification method and perform two separate amplification reactions separated by time (not on the same plate) thereby minimizing the potential for amplification artifacts (Figure 2a and b).

Allele drop out: Allele dropout at a locus may result from poor amplification (peaks below the detection threshold) or failure of amplification. Use of low quantity template DNA often leads to allele drop out. When the HCC1143 cell line DNA was amplified using 1 ng of DNA, poor amplification of the allele 17 at D18S51 locus resulted in an erroneous genotype- 14 instead of 14 and 17. One approach to overcome such issues is to amplify DNA at two different template quantities (Figure 3a and b).

Multiple allele profile: Normally one expects either homozygous or heterozygous results at all loci from an individual donor. The presence of more than two alleles at one locus is reported in some individuals [31,33]. Multiple allele patterns at multiple loci, in general, are interpreted as a mixture profile in the human identification laboratory. DNA from the MOLT-4 cell line exhibited multiple alleles at 5 loci (Figure 4). Such profiles can be characteristic of a cell line due to clonotypic heterozygosity and is not necessarily the result of contamination. It is interesting that MOLT-4 is ~tetraploid [43] and exhibit microsatellite instability [44] which contributes to the observed multiple allele patterns. We analyzed the DNA preparations obtained from the indicated agencies and assume that the DNA obtained was from uncontaminated cell lines. Masters et al. [24] reported a three allele profile at the D21S11 locus for the HeLa53 cell line.

The unique features of a cell line such as low heterozygous peak height ratios or multiple alleles at a locus (>2) can be ascertained by performing two amplification reactions using 1 and 2 ng of template DNA. Performing two amplification reactions at two template DNA quantities enables the detection of amplification artifacts. Further, it is recommended to use a species specific quantification method (e.g. Quantifiler® kits for the human cell line DNA preparations) to obtain

high quality profiles.

Cell line misidentification or contamination is widely acknowledged to be a serious problem, resulting in false information in numerous studies published in the literature. The consequences are numerous such as research being performed based on faulty data, delay of important scientific discoveries, and wastage of valuable time and resources. The use of STR for identification purposes is an important part of the solution to this problem. However, use of these tools requires a thorough understanding of common amplification anomalies and how to interpret the resulting data. The American Type Collection Standards Development Organization Workgroup ASN-0002 has recently recommended use of STR profiling as a tool for cell line authentication [45].

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