Short Tandem Repeat DNA Typing Provides an International Reference Standard for Authentication of Human Cell Lines

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Summary
Cell lines have wide applications as model systems in the medical and pharmaceutical industry. Much drug and chemical testing is now first carried out exhaustively on in vitro systems, reducing the need for complicated and invasive animal experiments. The basis for any research, development or production program involving cell lines is the choice of an authentic cell line. Microsatellites in the human genome that harbour short tandem repeat (STR) DNA markers allow individualisation of established cell lines at the DNA level. Fluorescence polymerase chain reaction amplification of eight highly polymorphic microsatellite STR loci plus gender determination was found to be the best tool to screen the uniqueness of DNA profiles in a fingerprint database. Our results demonstrate that cross-contamination and misidentification remain chronic problems in the use of human continuous cell lines. The combination of rapidly generated DNA types based on single-locus STR and their authentication or individualisation by screening the fingerprint database constitutes a highly reliable and robust method for the identification and verification of cell lines.

Zusammenfassung: Short tandem repeat (STR) DNA Typisierung als internationale Referenztechnik für humane Zelllinien
In Anbetracht der Vielzahl von Erkrankungen des Menschen haben Zellkulturen als Modellsysteme in der medizinischen und pharmazeutischen Industrie stetig an Bedeutung gewonnen. Der Grund dafür ist unter anderem die Möglichkeit, neue Substanzen und Arzneimittel zunächst intensiv und unbegrenzt an in vitro Zellkulturen zu prüfen, sodass der Bedarf an entsprechenden Tierversuchen gesenkt werden kann. Für jede Forschung, Entwicklung oder biotechnologische Produktion ist es eine Hauptvoraussetzung, dass die eingesetzten Zelllinien authentisch sind. Die Prüfung der Identität von etablierten Zellkulturen wird heute mit Hilfe von Mikrosatelliten (short tandem repeats [STR]) durchgeführt. Eine auf Fluoreszenz basierende Polymerase Ketten Reaktion (PCR) ermöglicht zusätzlich zur Geschlechtsbestimmung die simultane Analyse von acht hochpolymorphen STR Orten und stellt damit eine schnelle und robuste Methode dar, die zur Überprüfung der Einzigartigkeit von DNA Mustern am besten geeignet ist. Die Auswertung unserer DNA Typisierungen zeigt, dass die Kreuzkontamination und falsche Identifikation von kontinuierlichen Zelllinien ein chronisches Problem sind. Die Kombination von schnell herzustellenden DNA Profilen und deren Abgleich in einer Fingerprint Datenbank stellen eine sehr sichere und zuverlässige Methodik für die Qualitätskontrolle in der Zellkultur dar.

Keywords: human cell lines, DNA typing, restriction length polymorphism, short tandem repeats, cross-contamination

1 Introduction

Mutation and tumour induction are results of toxic effects with severe consequences for human health, since they can be irreversible and harmful. The medical, pharmaceutical and even the cosmetic industries are committed to eliminate animal testing as soon as this is scientifically possible, but due to the limitations of single cells, no *in vitro* test can fully replace an existing *in vivo* animal test yet. Nevertheless, the establishment of human and animal cell lines bearing special characteristics has led to partial replacement of animal experiments by well accepted *in vitro* toxicity and allergy tests. Replacement of animal experiments by cell line tests will certainly expand in future. The value of *in vitro* tests depends how well they represent the *in vivo* system and on the strict quality control of the cell lines used. A lack of routine quality checks of cell lines leaves mix-ups or cross-contaminations undetected with far-reaching consequences. Today it has become indispensable to authenticate cell lines.

Discrimination among human individuals became possible by the use of restriction fragment length polymorphism

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(RFLP) of repetitive sequences within the human genomic DNA, which led to the concept of "DNA fingerprinting" (Jeffreys et al., 1985). The method is based on the phenomenon that genomes of higher organisms harbour many DNA regions that show multiallelic variation among individuals (Nakamura et al., 1987). The variability is so great that a few loci used in combination can distinguish between two individuals who are not identical twins by revealing a specific fingerprint. Sequence analysis demonstrated that the structural basis for polymorphism in these regions is the presence of tandem-repetitive, nearly identical DNA elements, which are inherited in a Mendelian way. Depending on the length of the repeats, repetitive sequences are classified into microsatellites, which include all short tandem repeats (STRs) with core sizes from 1 to 6 bp, and minisatellites consisting of 9 to >70 bp core sequences. Both categories of repeats can be governed by one definite locus or are spread all over the genome and belong to the single-locus systems (SLS) or multiple-locus systems (MLS), respectively (Dirks, 1999). Our aim was to establish a rapid, practical and reliable method with a high discrimination potential, which enables the *de novo* authentication of cell lines as well as the confirmation of cell line identity of different lots of the same line in case of replenishment of the distribution stock. Cell line authentication and early detection of contamination of one cell line with another should prevent false interpretation of results from experiments.

2 Material and methods

2.1 Cell lines and cell culture conditions

All cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and had been tested for authenticity by DNA fingerprinting and karyotypic markers previously. All cell lines were free of mycoplasma contaminations and were grown in appropriate media according to the instructions in the DSMZ catalogue (http://www.dsmz.de/mutz/mutzhome.htm). Cells were subjected to DNA isolation in their logarithmic growth phase with viabilities higher than 90%, as examined by trypan blue exclusion, to avoid DNA isolation from apoptotic cells.

2.2 Preparation of high molecular weight DNA

Cell culture suspensions containing 3-5 x 10^6 diploid cells were centrifuged in Eppendorf tubes at 2000 x g for 4 min. The supernatant was removed with a disposable pipette and discarded. The remaining pellet was resuspended carefully in 5 ml PBS using a pipette. After the washing step, the pellet was resolved completely in 200 µl PBS by vortexing. For isolation of the genomic DNA, the DNA extraction kit from Roche was used. 200 µl of solution I (guanidiniumhydrochloride) was added to the sample solution and mixed by pipetting. 40 µl proteinase K was added, mixed well using a vortex and incubated at 72°C for 10 min. 100 µl isopropanol was added to the sample, mixed well and the whole mixture was transferred to a filter tube

Tab. 1: Allele organisation and sizes of amplified human STR loci nucleotide range and number of known alleles of each STR locus (http://www.cstl.nist.gov/div831/strbase). Regular fragment sizes of alleles are printed in bold; variant alleles are printed in italics.

Allele	D5S818	D13S317	D7S820	D16S539	vWA	TH01	TPOX	CSF1PO	Amelogenin
3						169			
4						173			209 = X
5		164	212	266		177, *180*	220	287	215 = Y
6	114	168, *169*	216, *219*	270		181, *184*	224	291	
7	118	172, *173*	220, *223*	274		185, *188*	228	295	
8	122	176	224, *226*	278		189, *192*	232	299	
9	126	180	228, *229*	282		193	236	303, *304*	
9.3	*(129)*	*(183)*	*(231)*	*(285)*		196	*(239)*	*(306)*	
10	130	184	232, *233*	286	118	197, *200*	240	307, *310*	
11	134	188	236, *237*	290	122	201	244	311	
12	138	192	240, *241*	294	126	205	248	315, *316*	
13	142	196	244, *245*	298	130		252	319	
14	146	200	248	302	134		256	323	
15	150	204	252	306, *309*	138, *140*			327	
16	154				142, *144*			331	
17	158				146				
18					150, *152*				
19					154				
20					158				
21					162				
22					166				
23					170				

and spun for 1 min at 8000 rpm. The flow through was discarded, 500 µl of inhibitor removal buffer was added and the mixture was centrifuged again for 1 min at 8000 rpm. The flow through was discarded, 500 µl of wash buffer was added and spun again for 1 min at 8000 rpm. A new collection tube was attached and 200 µl of elution buffer preheated to 72°C was added and spun for 1 min at 8000 rpm. For maximum yield the elution step was repeated using 100 µl elution buffer. The purified genomic DNA concentration was adjusted to 10 ng/µl per sample and stored at 4°C.

2.3 Pre-requisites for DNA typing

General rules to avoid DNA carry-over contaminations were followed strictly: DNA extraction was carried out using equipment (pipettes, microcentrifuge, etc.) that was independent from the PCR set-up. DNA extraction, PCR reaction and PCR product analysis were performed in three separate laboratories. All reagents were stored in small aliquots to provide a constant source of uncontaminated reagents. New aliquot batches were tested and compared for quality prior to any use. No re-amplifications were carried out. The reactions were performed in a PCR working station or a hood capable of irradiating used pipettes, tips and tubes by UV-light. Gloves were worn during the whole procedure. The appropriate positive and negative controls (e. g. HeLa DNA and H_20, respectively) were employed in every test.

2.4 Hot start nonaplex fluorescence PCR DNA typing

The PCR amplification was modified from PowerPlex 1.2 multiplex from Promega Inc. (Madison, WI, USA), which contains eight STR systems plus gender determination. The amplification procedure and the parameters were optimised for 0.2 ml reaction tubes in an i-Cycler (Bio-Rad). The master mix was prepared for 25 µl per reaction of each sample plus one additional reaction. Per sample the components were: 10 pmol total primer (1 µl of a 10 µM solution), 2.5 µl 10 x Hot start PCR buffer (any supplier), 1 µl dNTP (5 µM), 0.2 µl (1 unit) hot start Taq polymerase (any supplier), 19.5 µl distilled water and 1 µl genomic DNA adjusted to 10 ng/µl. The genomic DNA was added after and independent from the mastermix. The PCR program was as follows 95°C for 3 min, 1 repeat; 94°C for 30 sec, 57°C for 30 sec, 72°C for 45 sec, 30 repeats; 60°C for 15 min, 1 repeat.

2.5 STR primer sequences and genomic location

D16S539, 16q22-24; non-coding region: 5´-GGG GGT CTA AGA GCT TGT AAA AAG; 5´-GTT TGT GTG TGC ATC TGT AAG CAT GTA TC.

D13S317, 13q22-q31; non-coding region: 5´- ACA GAA GTC TGG GAT GTG GAG GA; 5´-GGC AGC CCA AAA AGA CAG A.

D5S818, 5q21-q31; non-coding region, 5´-GGT GAT TTT CCT CTT TGG TAT CC; 5´-AGC CAC AGT TTA CAA CAT TTG TAT CT.

D7S820, 7q11.21-22; non-coding region, 5´-ATG TTG GTC AGG CTG ACT ATG; 5´-GAT TCC ACA TTT ATC CTC ATT GAC.

CSF1, 5q33.3-34; 3´-UTR of c-fms proto-oncogene for CSF-1 receptor gene, 5´-AAC CTG AGT CTG CCA AGG ACT AGC; 5´-TTC CAC ACA CCA CTG GCC ATC TTC.

TPOX, 2p23-2pter; intron 10 of human thyroid peroxidase gene, 5´-ACT GGC ACA GAA CAG GCA CTT AGG, 5´-GGA GGA ACT GGG AAC CAC ACA GGT TA.

TH01, 11p15-15.5; intron 1 of human tyrosine hydroxylase gene, 5´- ATT CAA AGG GTA TCT GGG CTC TGG; 5´-GTG GGC TGA AAA GCT CCC GAT TAT.

vWA, 12p12-pter; 3´-UTR of van Willebrandt factor gene, 5´-CTA GTG GAT GAT AAG AAT AAT CAG TAT GTG; 5´-GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG.

Amel, Xp22.1-22.3, Y, coding region of amelogenin, 5´-ACC TCA TCC TGGG CAC CCT GGT T; 5´-AGG CTT GAG GCC AAC CAT CAG.

Amelogenin is not an STR, but displays a 209 bp X-chromosome specific and 215 bp Y-chromosome specific fragment, respectively. Primers of non-coding regions were labelled with specific dye D2 (black) and primers within transcription units as well as amelogenin-specific primers were labelled with D3 (green), both from Beckman-Coulter.

2.6 Fragment detection and allelic STR lists

Aliquots of 1 µl of the amplification products were combined with 0.25 µl of an internal size standard (Size standard kit 400, Beckman-Coulter) in a total volume of 30 µl of sample loading solution on a microtitre plate. The samples were loaded automatically and analysed by the capillary electrophoresis system CEQ 8000 (Beckman-Coulter) using established fragment analysis parameters (Tab. 1). The fragment analysis software of CEQ 8000 enabled precise determination of detected alleles resulting in a genotype summary list.

3 Results

Genomic DNA from 550 human cell lines was analysed by STR profiling. No fragments were amplified in samples from animal cell lines, including genomic DNA from primates, as expected. All STRs used in the study harboured a tetranucleotide sequence motif, while intermediate sizes known as variant alleles have been observed for nearly all STR loci (alleles in italics in Tab. 1). The nonaplex PCR reaction was carried out using fluorescent dye-linked primers, resulting in labelled DNA fragments detected by capillary electrophoresis. The end result for each cell line was an individual electropherogram with each STR locus normally represented by two peaks in the appropriate colour (Fig. 1). The data were categorised according to their size in relation to an internal size standard, which was coloured differently and ran simultaneously in the same capillary. This analysis enabled every peak to be allocated a size corresponding to the number of repeat units present.

3.1 Similarity measurements and matching profiles

An algorithm was applied in order to compare the allelic profiles. Here the DNA profile in question (new accession line, external DNA typing service) was checked against every other DNA profile of reference lines already in the database.

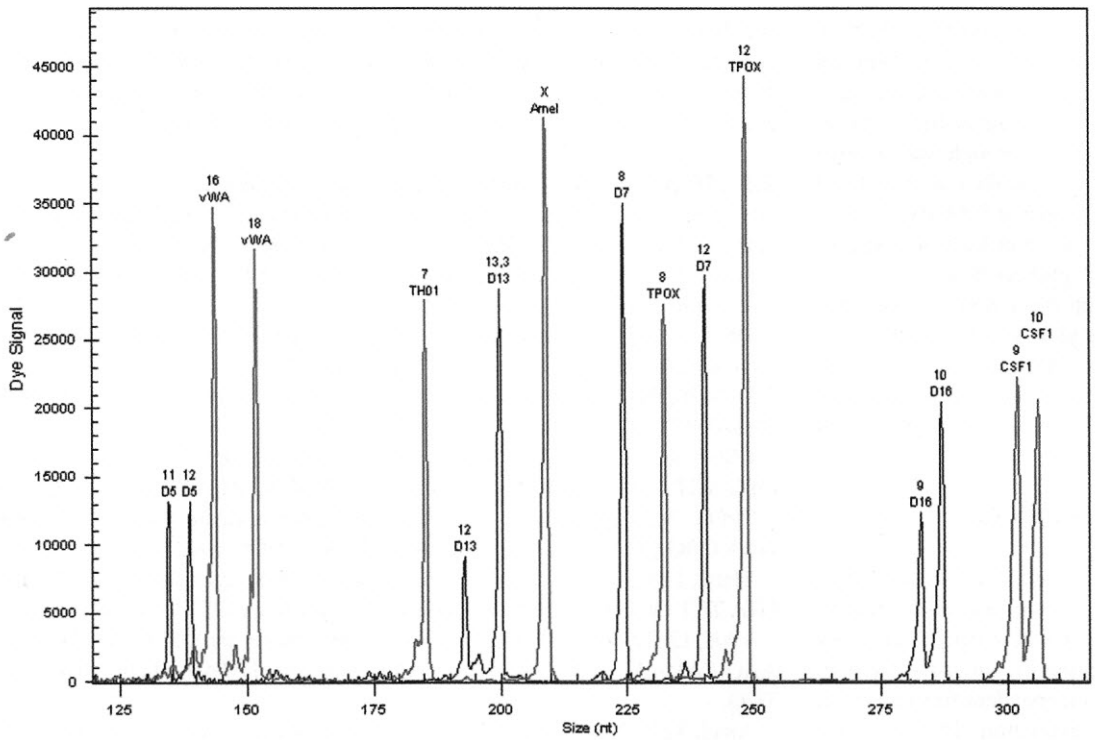

Fig.1: Electropherogram of the cervix carcinoma cell line HELA (ACC 057)
An electropherogram of nonaplex STR profiles obtained from genomic DNA of the cell line HELA (ACC 057) is shown. Dye signals in green belong to STR loci within transcription units of human genes, while black peaks belong to a group of STRs which are independent from coding regions. Numbers written above the STR loci indicate the respective alleles.

The number of identical alleles in the sample and reference profile was scored and expressed as a percentage of the total number of alleles in the tested DNA profile (Masters et al., 2001). Among the 550 cell lines subjected to DNA typing, 84 revealed no unique pattern, indicating an incidence of 15.3% false cell lines (http://www.dsmz.de/mutz/FalseLL.htm). In most instances, the false cell lines showed the same DNA fingerprint as another, already clearly authenticated cell line, while in some cases the profiles were closely related, differing by only one or two alleles. Closely related DNA profiles indicated a loss of heterozygosity (LOH), implying that no gain of additional alleles was observed. The identity of cross-contaminating cells was mostly restricted to a few well-known, classic cell lines (Tab. 2), which share the common characteristic of constitutively high proliferative activity. For an invading cell line, one cell division within approximately 24 h seems to be the pre-requisite for complete overgrowth in a long-term cell culture.

3.2 Detection of cross-contaminants

For normal human DNA, STR profiling will show two alleles at the STR loci, as expected for highly polymorphic loci in a diploid genome. Additional alleles at a locus are rarely seen in normal profiles, but can occur in case of trisomy, gene du-

Tab. 2: Most prolific contaminants in human cell lines.
All cell lines are widely used throughout the scientific community and are available from the major public cell line banks. Cytogenetic particulars are based on karyotypic data representing cell lines analysed at the DSMZ together with at least one independent sample analysed elsewhere.

Cell line	Year	Malignancy	Doubling	Contaminations	Karyotypic descriptors
CCRF-CEM	1964	T-ALL	24 h	10 x	t(8;9)(p11;p24) b
K-562	1970	CML-BC	30-40 h	10 x	tandem BCR-ABL fusion repeats
U-937	1974	hist. lymphoma	30-40 h	8 x	t(1;5)(p22;q3?), t(10;11)(p13;q14-21)
HL-60	1976	AML M2	25 h	7 x	dic(5;17)(q11;q11)
REH	1974	BCP-ALL	30-50 h	6 x	t(4;12;21;16)(q32;p13;q21;q24)
JURKAT	1976	T-ALL	25-35 h	3 x	del(2)(p23)
NALM-6	1976	BCP-ALL	36 h	3 x	t(5;12)(q33;p13)

plication or mixed populations of cells or hybrids. When most of the loci showed more than 2 peaks, this indicates a hybrid or mixture of cells. In all cases of detection of multiple alleles, an additional fingerprint system using 6 VNTR markers (Dirks et al., 1999) was carried out in order to verify population mixtures. Additionally, the suspicious cell lines were subjected to immunophenotyping experiments and karyotyping, which confirmed the cross-contamination using chromosomal markers. An example of an electropherogram of a cross-contaminated cell culture is given in Figure 2.

4 Discussion

In recent years the use of human and animal cell culture has undergone a major expansion from being a purely experimental procedure to becoming an accepted technological field. The development and validation of *in vitro* methods has led to the replacement and reduction of the use of animal models. Since most facilities culturing cells use multiple cell lines simultaneously, because of the complexity of experimental designs today and because of the fact that the broad use of cell lines in science and biotechnology continues to increase, the possibility of an inadvertent mixture of cell lines during the course of day-to-day cell culture is ever present.

Different approaches towards DNA fingerprinting of cell lines have been described: one application involves restriction fragment length polymorphism (RFLP) analysis by the Southern blot technique with STR probes, which bind to multiple hypervariable regions on many different chromosomes (Stacey et al., 1992; Häne et al., 1993). This time-consuming procedure results in blots with a complex pattern of bands, permitting a final distinction between different cell lines due to the high individualisation potential. Advantages of the use of fluorescence PCR-based STRs in combination with capillary electrophoresis over traditional RFLP techniques are, (i) that discrete alleles from STR systems may be used which, due to their smaller size, allows the differentiation of DNA fragments which differ by a single base pair, (ii) that determination of discrete alleles allows results to be compared easily between laboratories without binning, (iii) that smaller quantities of DNA, including degraded DNA, may be typed successfully using STRs, thus, the quantity and integrity of the DNA sample is less of an issue with PCR-based typing methods than with conventional RFLP methods, (iv) finally, that the time needed for full authentication is reduced from 4 days to less than 8 hours.

The mutation rate of a given set of STR markers is an important parameter, because increased mutations can give rise to new alleles and genotypes within a pure cell lineage. A study within the German population by Brinkmann et al. (1998) demonstrated that mutation rates

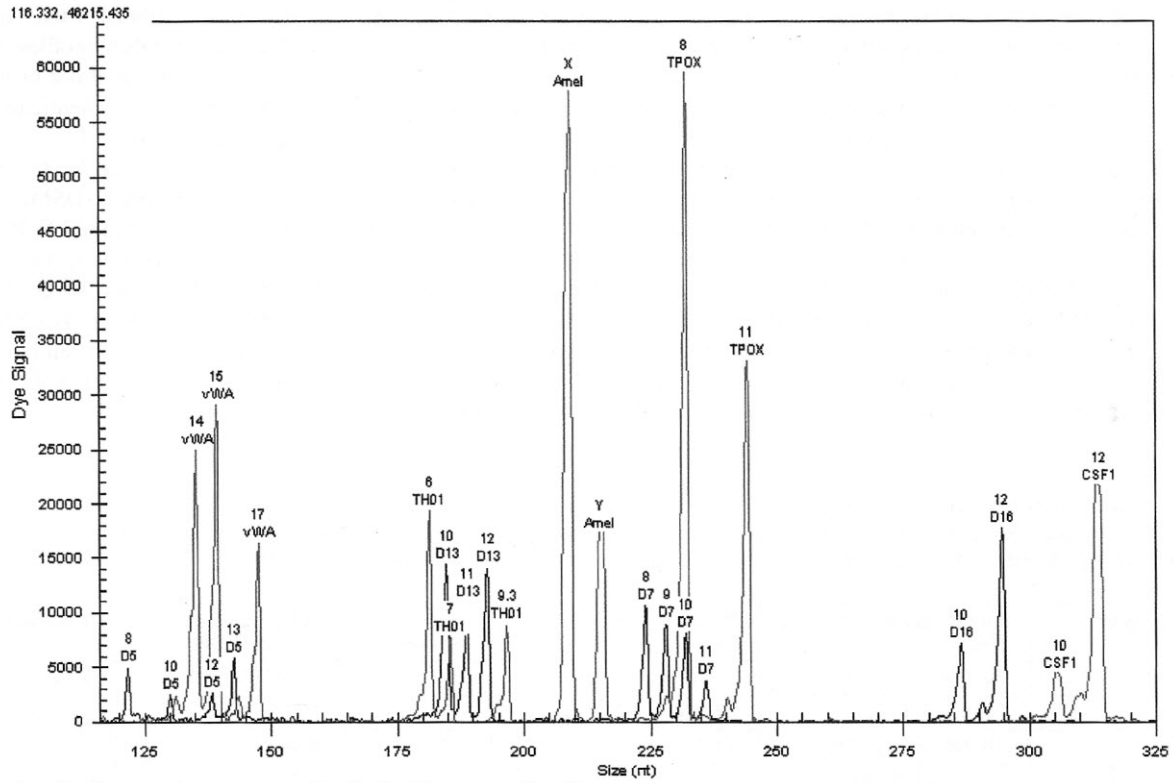

Fig. 2: Electropherogram of a cross-contaminated human cell culture
STR profile of a mixture of the human histiocytic lymphoma cell line U-937 (DSMZ ACC 005) with the Burkitt lymphoma cell line DAUDI (DSMZ ACC 078). The reference profiles are as follows: U-937: D5S818: 10, 12; D7S820: 10, 12; D13S317: 9, 11; D16S539: 12, 12; vWA: 14, 15; TH01: 6, 9.3; TPOX: 8, 11; CSF1PO: 10, 12; Amelogenin: X, X.
DAUDI: D5S818: 8, 13; D7S820: 8, 10; D13S317: 11, 12; D16S539: 10, 12; vWA: 15, 17; TH01: 6, 7; TPOX: 8, 11; CSF1PO: 12, 12; Amelogenin: X, Y.

of different loci differ by several orders of magnitude and that different alleles at one locus exhibit different mutation rates. In that study the mutation rates of nine STR loci were between 0 and 7 x 10^{-3} per locus per gamete and generation. The shared STR loci between Brinkmann's and our study are TH01 and vWA, carrying 0.000% and 0.199% mutations, respectively (table 3). Furthermore, overall mutation frequencies of the 8 autosomal microsatellite loci used in our study have been calculated by the American Association of Blood Banks (AABB) in the course of paternity testing studies (table 3), indicating that some STR loci are indeed hyper-variable and subject to high mutation rates. The mutation rates of the STR loci in table 3 are calculated from paternity tests, indicating a data background including meiotic events. Since VNTR and STR DNA elements are predominantly subjected to mutation during meiotic cell divisions (Wolff et al., 1988), it can be estimated, that mutation rates of STR loci in cell lines should be much lower because meiosis can be excluded. Our experience in DNA profiling of cell lines over a period of more than 10 years indicates no mutation in profiles of a given cell line at a low or very high passage number. Furthermore, the existence of subclones of different prominent cell lines like HELA, T24, HL60, K-562 etc. did not show any alteration with regard to their profiles, although the subclones displayed gains or losses in their characteristics (e. g. multidrug resistance). Finally, we have compared our database of DNA profiles with databases from ATCC (USA) and JCRB (Japan). We did recognise some loss-of-heterozygosity leading to missing alleles, but in no case any mutation of an STR was observed out of approximately 350 overlapping human cell lines. Taken together, the risk of detecting a false positive cell line due to mutated STR alleles should be negligible and has not been observed so far for cell lines.

Facilities with large holdings of cell lines need a rapid and secure authentication method in order to verify cell line authenticity during replenishment of the distribution stocks or to confirm the uniqueness of newly accessioned cell lines. The survey of 240 human cell lines submitted directly by their originators and subsequent comparisons of karyotypes and DNA profiles showed that 37 cell lines (15.3%) were misidentified at source (MacLeod et al, 1999). The result of this extended study demonstrates that cell line misidentification remains a chronic problem. The contaminants consist in almost all cases of long-established classic tumour lines. All are known for their minimal cultivation requirements and their high proliferative activity, most probably the main reasons for the wide-spread cross-contamination (Drexler & Matsuo, 1999). These findings suggest that misidentification is predominantly caused by overgrowth and that computer-aided searches in a cell line fingerprint database are necessary for an efficient and rapid detection of intraspecies cross-contamination.

In conclusion, we have adopted a simple and rapid method of DNA fingerprinting originally established for forensic purposes to the authentication of human cell lines. On the basis of the demonstrated suitability of a precise and accurate detection of STR alleles for cell lines, we have used this method for the establishment of an STR reference database comprising over 500 human cell lines, including most of the classic tumour cell lines. The high percentage of detected cross-contaminated or misidentified cell lines, although obtained most often directly from their originators, indicates that the cell line DNA profile database is a powerful tool for future cell line identification.

References

American Association of Blood Banks (AABB) (2003). URL at: http://www.cstl.nist.gov/biotech/strbase/ mutation.htm.

Brinkmann, B., Klintschar, M., Neuhuber, F. et al. (1998). *Am. J. Human Genet. 62*, 1408-1415.

Dirks, W. G., MacLeod, R. A. F., Jaeger, K. et al. (1999). First searchable database for DNA profiles of human cell lines: sequential use of fingerprint techniques for authentication. *Cell. Mol. Biol. 5*, 841-853.

Drexler, H. G., Dirks, W. G., MacLeod, R. A. F. et al. (2001). DSMZ Catalogue of Human and Animal Cell Lines. 8th Edition, Braunschweig, Germany.

Drexler, H. G., Dirks, W. G., Matsuo, Y. and MacLeod, R. A. F. (2003). False leukemia-lymphoma cell lines: An update on over 500 cell lines. *Leukemia 17*, 416-426.

Tab. 3: Mutation rates of human STR loci.
Apparent mutations observed at STR loci have been calculated by the American Association of Blood Banks (AABB) in the course of paternity testing. AABB, 2003 Annual Report, at http://www.cstl.nist.gov/biotech/strbase/mutation.htm.

STR System	Maternal meioses (%)	Paternal meioses (%)	Number of either	Total number of mutations	Mutation rate
CSF1PO	70/179,353 (0.04)	727/504,342 (0.14)	303	1,100/683,695	0.16%
TH01	23/189,478 (0.01)	29/346,518 (0.008)	23	75/535,996	0.01%
TPOX	16/299,186 (0.005)	43/328,067 (0.01)	24	83/627,253	0.01%
VWA	133/400,560 (0.03)	907/646,851 (0.14)	628	1,668/1,047,411	0.16%
D5S818	84/316,102 (0.03)	537/468,366 (0.11)	303	924/784,468	0.12%
D7S820	43/334,886 (0.01)	550/461,457 (0.12)	218	811/796,343	0.10%
D13S317	142/348,395 (0.04)	608/435,530 (0.14)	402	1,152/783,925	0.15%
D16S539	77/300,742 (0.03)	350/317,146 (0.11)	256	683/617,888	0.11%

Häne, B. G., Jäger, K. and Drexler, H. G. (1993). The Pearson product-moment correlation coefficient is better suited for identification of DNA fingerprint profiles than band matching algorithms. *Electrophoresis 14*, 967-972.

Jeffreys, A. J., Wilson, V. and Thein, S. L. (1985). Hypervariable minisatellite regions in human DNA. *Nature 314*, 67-73.

MacLeod, R. A. F., Dirks, W. G., Kaufmann, M. et al. (1999) Widespread intraspecies cross-contamination of human tumor cell line arising at source. *Int. J. Cancer 83*, 555-563.

Master, J. R., Thompson, J. A., Daly-Burns, B. et al. (2001). Short tandem repeat profiling provides an international reference standard for human cell lines. *Proc. Natl. Acad. Sci. 98*, 8012-8017.

Nakamura, Y., Leppert, M., O'Connell, P. et al. (1987). Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science 235*, 1616-1622.

Stacey, G., Bolton, B., Morgan, D. et al. (1992). Multilocus DNA fingerprint analysis of cell banks: stability studies and culture identification in human lymphoblastoid and mammalian cell lines. *Cytotechnology 8*, 13-20.

Wolff, R. K.; Nakamura, Y. and White, R. (1988). Molecular characterization of a spontaneously generated new allele at a VNTR locus: no exchange of flanking DNA sequence. *Genomics 3(4)*, 347-351.

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