Full Report

ibc Interdisciplinary Bio Central

Open Access, Open Review Journal

Development of a Simple Method to Determine the Mouse Strain from Which Cultured Cell Lines Originated

Kaori Yoshino[1], Kaoru Saijo[1], Chikako Noro[2] and Yukio Nakamura[1,*]

[1]Cell Engineering Division, RIKEN BioResource Center, Tsukuba, Ibaraki 305-0074, Japan
[2]Nihon University School of Medicine, Itabashi-ku, Tokyo 173-8610, Japan

Subject areas: Resources

Author contribution: K.Y., K.S. and C.N. performed experiments and analyzed data; Y.N. analyzed data and wrote the manuscript.

***Correspondence** and requests for materials should be addressed to Y.N. (yukionak@brc.riken.jp).

Reviewer: Atsushi Yoshiki, RIKEN BioResource Center, Japan;
Arihiro Kohara, National Institute of Biomedical Innovation, Japan

Editor: Yeonhee Lee, Seoul Women's University, Republic of Korea

Received December 14, 2010;
Accepted December 17, 2010;
Published December 29, 2010

Citation: Yoshino, K., et al. Development of a Simple Method to Determine the Mouse Strain from Which Cultured Cell Lines Originated. IBC 2010, 2:14, 1-9.
doi: 10.4051/ibc.2010.2.4.0014

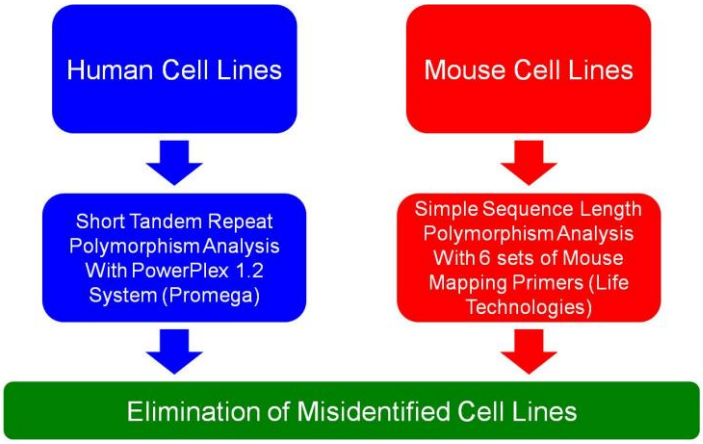
Funding: This work was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Competing interest: All authors declare no financial or personal conflict that could inappropriately bias their experiments or writing.

SYNOPSIS

Misidentification of cultured cell lines results in the generation of erroneous scientific data. Hence, it is very important to identify and eliminate cell lines with a different origin from that being claimed. Various methods, such as karyotyping and isozyme analysis, can be used to detect inter-species misidentification. However, these methods have proved of little value for identifying intra-species misidentification, and it will only be through the development and application of molecular biological approaches that this will become practical. Recently, the profiling of microsatellite variants has been validated as a means of detecting gene polymorphisms and has proved to be a simple and reliable method for identifying individual cell lines. Currently, the human cell lines provided by cell banks around the world are routinely authenticated by microsatellite polymorphism profiling. Unfortunately, this practice has not been widely adopted for mouse cells lines. Here we show that the profiling of microsatellite variants can be also applied to distinguish the commonly used mouse inbred strains and to determine the strain of origin of cultured cell lines. We found that approximately 4.2% of mouse cell lines have been misidentified; this is a similar rate of misidentification as detected in human cell lines. Although this approach cannot detect intra-strain misidentification, the profiling of microsatellite variants should be routinely carried out for all mouse cell lines to eliminate inter-strain misidentification.

.



An SSLP analysis using the 6 MIT markers described in this study was sufficient to distinguish the common and popular inbred mouse strains such as C57BL/6, BALB/c, C3H/He, 129/Sv and DBA/2. Although this approach cannot detect intra-strain misidentification, it should be routinely carried out for all mouse cell lines to eliminate inter-strain misidentification.

Keywords: cell bank, cross-contamination, microsatellite polymorphism, misidentification, quality control, short tandem repeat polymorphism, simple sequence length polymorphism

Introduction

Cultured cell lines have proved a valuable resource in all fields of the life sciences and have been utilized in many types of biological study. Currently, however, stringent analyses to check the identity of a cell culture are not always included as part of the culture protocol routine. This has led to misidentification or cross-contamination of cell lines going undetected. As a result, the published literature contains a number of reports that are based on wrongly identified cell lines[1]. Despite numerous publications warning of inter- and intra-species misidentification of cell lines[2-6], the problem of misidentification continues to occur at an extremely high rate[7-10]. Therefore, articles pointing out misidentification of cell lines continue to be published[11-14].

Inter-species contamination can be detected by various methods, such as karyotyping and isozyme analysis. However, it was not possible to detect intra-species misidentification prior to the development of molecular biology techniques that make use of the genetic differences between cell lines to facilitate their identification. One such method makes use of microsatellite polymorphisms to develop diagnostic profiles for cell lines[15]. Microsatellite polymorphisms result from differences in the numbers of a repeating unit of 1-7 base pairs; these variants are also called short tandem repeat (STR) polymorphisms or simple sequence length polymorphisms (SSLPs). These polymorphisms have been extensively used in forensic science. Gene profiling using STR polymorphisms (STR profiling) has been shown to be an efficient and reliable means for identifying individual human cell lines[16,17] and is now performed routinely in the major cell banks around the world. What about mouse cell lines; do they also suffer from problems of misidentification? This question prompted us to establish a method to authenticate the identities of mouse cell lines.

Results and Discussion

The mouse genome possesses a huge number of microsatellite polymorphisms, similarly to the genomes of humans and other mammalian species. The so-called MIT markers developed at the Massachusetts Institute of Technology (MIT) Whitehead Institute[18] in the mouse have been extensively developed and utilized in various fields of research. Information on microsatellite polymorphisms, including data on MIT marker sizes in 47 mouse strains, is publicly available on the Center for Inherited Disease Research Web site (http://www.cidr.jhmi.edu/mouse/mouse_resources.html). It is possible to amplify a number of polymorphic microsatellite loci using commercially available sets of primers. The PCR products are analyzed simultaneously with size standards using automated fluorescent detection techniques. The result is a simple numerical code that corresponds to the lengths of the PCR products amplified at each locus, and is accurate to less than one base pair.

Our aim was to establish a simple method that can be used for routine analysis in our cell bank work. Thus, we sought to establish a method using the smallest practical number of polymorphic loci. First, we screened more than 500 microsatellite primers covering the autosomes and the X chromosome and selected the 24 MIT markers that exhibited the most distinct differences between inbred mouse strains (Figure 1, Table 1), mostly larger than 10 bp on electrophoretic patterns[19]. We then performed an SSLP analysis of 40 mouse strains using the 24 selected MIT markers (Table 1) and concluded that 6 MIT markers would be sufficient to distinguish the common and popular inbred strains such as C57BL/6, BALB/c, C3H/He, 129/Sv and DBA/2 (Figure 2, Table 2).

An SSLP analysis, using the 6 MIT markers described above, has now been adopted by the Cell Engineering Division of the RIKEN BioResource Center (RIKEN Cell Bank) to exclude misidentification among the cultured mouse cell lines that we currently provide. However, this analysis can detect inter-strain but not intra-strain misidentification.

The screening of the RIKEN Cell Bank indicated that 97.7% (334 lines out of 342 lines) of the mouse cell lines were derived from

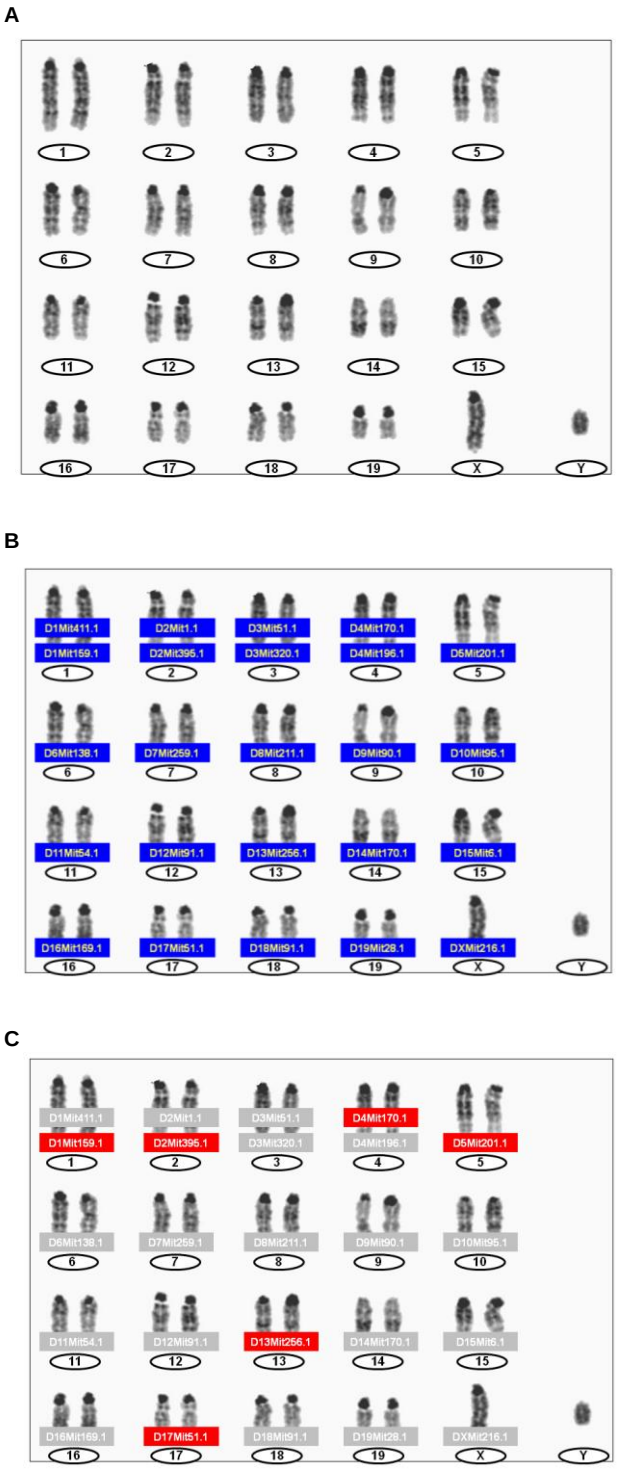
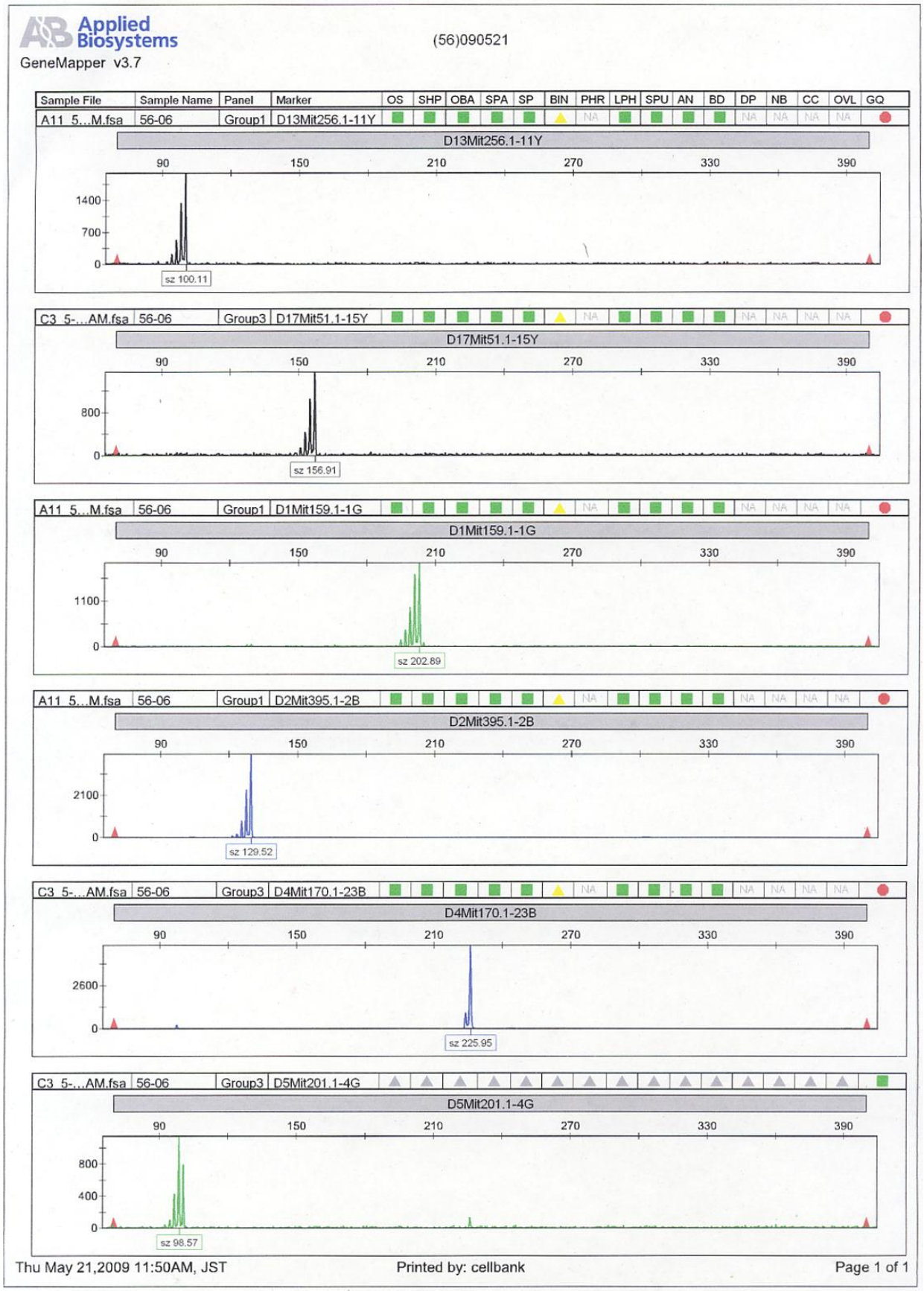


Figure 1. Distribution of the analyzed simple sequence length polymorphisms (SSLPs) loci in the mouse genome. (A) Karyotype of the mouse, 40XY. (B) The chromosomal distributions of the screened 24 loci are indicated. (C) The 6 loci selected as being sufficient for identification of common inbred mouse strains are indicated by the red background.

Table 1. Results of the SSLP analyses of 40 inbred mouse strains using the 24 loci indicated in Figure 1B

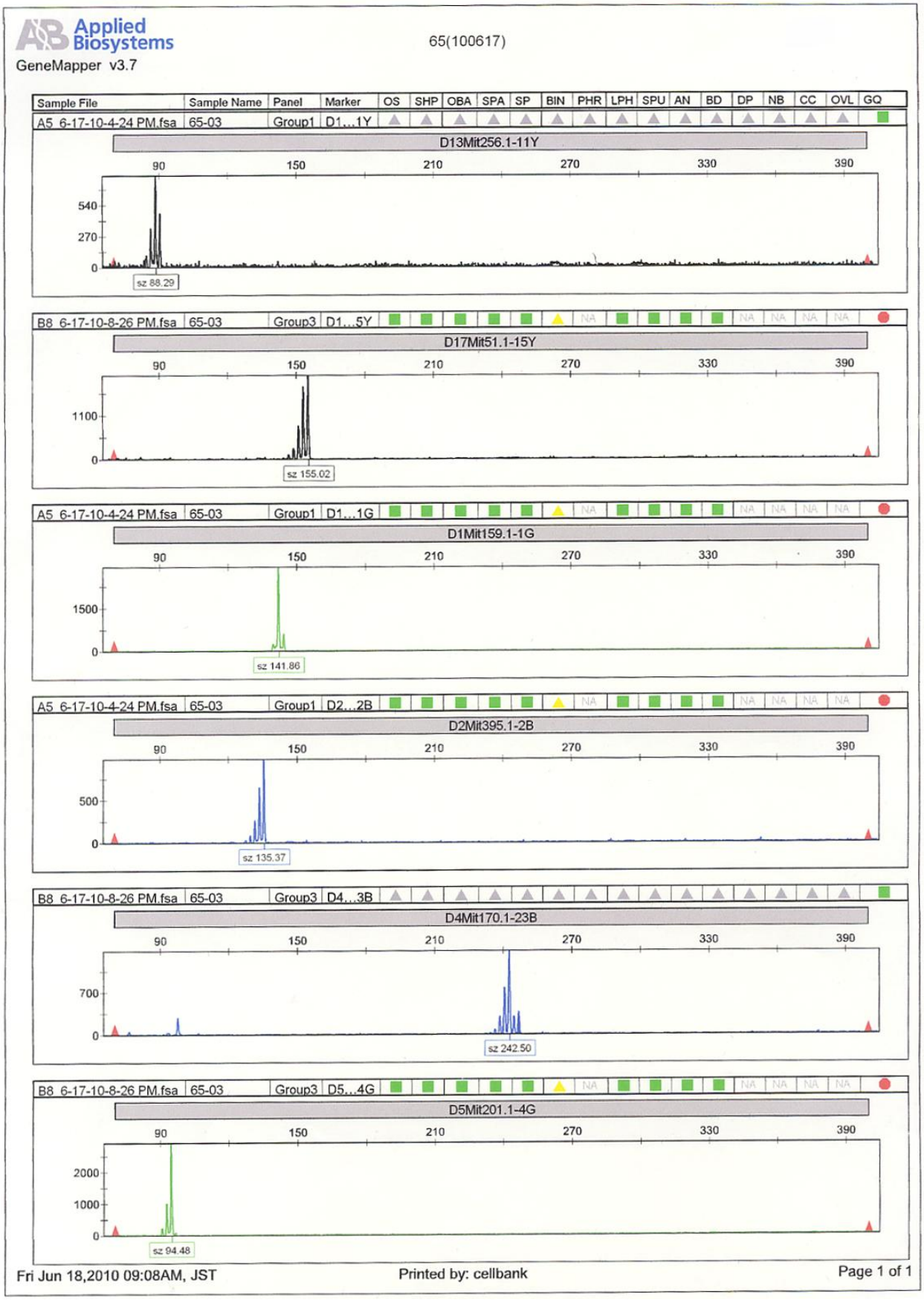
Strain Name	origin	Mit159_1	Mit411_1	Mit1_1	Mit385_1	Mit51_1	Mit320_1	Mit170_1	Mit196_1	Mit201_1	Mit138_1	Mit259_1	Mit211_1	Mit90_1	Mit95_1	Mit54_1	Mit91_1	Mit256_1	Mit170_1	Mit6_1	Mit169_1	Mit51_1	Mit91_1	Mit28_1	Mit216_1
C57BL/6JMs	Ms	203	104	217	130	240	110	226	104	98	132	241	150	110	201	148	153	100	203	134	234	157	132	153	126
C57BL/6J	JAX	203	104	217	129	240	110	226	104	98	132	241	150	110	201	148	153	100	203	134	234	157	132	153	126
C57BL/6J Jcl	Jcl	203	104	217	129	240	110	226	104	98	132	241	150	110	201	148	153	100	203	134	234	157	132	153	126
C57BL/6N Jcl	Jcl	203	104	213	129	240	110	226	104	98	132	241	150	106	201	148	153	100	203	134	234	157	132	153	126
C57BL/6N Cri	Cri	203	104	213	129	240	110	226	104	98	132	241	150	101	201	148	153	100	203	134	234	157	132	153	126
C57BL/10SnJ	Ms	203	104	213	130	240	110	226	104	98	196	241	160	106	201	148	153	100	203	134	234	157	132	155	126
C57L/J	Ms	142	97	213	130	240	110	226	104	98	132	217	150	106	181	148	153	100	188	134	234	163	130	157	126
C58/J	Ms	142	104	213	123	240	110	226	104	98	211	217	160	106	181	148	142	100	188	134	234	153	132	157	126
CBA/J	Ms	185	97	213	123	240	101	236	104	92	211	246	162	99	181	119	151	76	203	138	238	140	132	162	126
DBA/1J	Ms	142	97	213	135	256	101	236	104	92	207	246	160	103	181	144	153	76	202	138	234	153	132	162	126
DBA/2J	JAX	142	97	213	135	256	105	242	104	92	207	246	160	126	181	144	153	76	197	138	234	155	132	162	126
BALB/cJ	JAX	142	97	213	135	254	103	242	116	94	211	225	162	106	181	147	151	88	206	134	234	155	130	162	132
BALB/cANMs	Ms	142	97	213	135	254	103	242	116	94	211	225	164	106	181	147	151	88	204	134	234	155	130	162	132
BKR/J	Ms	203	97	213	161	256	103	236	116	104	211	225	164	106	193	119	153	76	206	134	234	140	132	162	126
C3H/HeJ	JAX	185	104	213	124	240	103	236	116	92	215	246	160	106	181	146	151	78	206	127	232	140	130	162	126
C3H/HeNCri	Cri	185	104	213	124	240	103	236	116	92	211	246	160	106	181	144	151	78	206	127	232	140	130	162	126
A/J	JAX	185	104	213	135	240	103	226	116	106	211	246	164	106	181	119	151	88	202	136	234	153	134	153	132
A/WySnJ	Ms	185	104	213	135	240	103	226	116	106	211	246	164	106	181	119	151	88	202	136	234	153	132	153	132
129/SvJ	Ms	191	104	215	157	246	103	226	116	94	211	239	162	106	181	147	142	76	202	108	234	163	132	153	132
SWJ	Ms	193	104	209	135	254	103	236	104	98	211	217	160	106	181	147	151	76	206	136	234	157	132	157	126
FVB/N	Jcl	191	104	224	161	254	108	234	104	102	211	244	160	112	181	148	150	88	200	134	260	142	124	162	128
SJL/J	JAX	203	104	219	161	254	108	242	104	106	211	246	160	108	181	147	149	88	202	134	234	153	130	153	126
SJL/JMs	Ms	203	104	219	161	254	108	242	104	106	211	246	160	108	181	147	149	88	202	134	234	153	130	153	126
SWR/J	Ms	203	97	213	161	256	103	232	104	104	215	244	162	106	181	148	150	92	203	131	262	142	130	162	126
CE/J	Ms	185	97	215	161	256	103	245	116	106	215	248	164	99	181	151	145	76	197	131	232	140	132	162	132
GRS/A	AichiCC	203	86	217	123	256	103	242	116	104	211	246	160	106	181	148	151	76	203	131	262	167	130	162	128
NOK/Nga	Nga	191	97	213	161	254	108	242	116	104	203	248	160	97	181	148	151	88	208	136	240	153	126	153	132
NON	Nga	191	97	213	135	254	103	242	116	92	207	225	160	106	181	147	151	100	203	131	240	157	130	153	132
NOY/Stm	Stm	185	97	213	123	256	103	232	104	98	207	244	160	106	181	147	151	100	203	131	262	153	124	153	132
NF1	Ms	142	97	226	129	326	99	226	104	92	344	217	160	128	201	119	114	100	220	108	278	170	130	155	135
CAST/Ei	Ms	168	111	229	138	232	91	247	106	102	236	227	182	107	187	135	114	90	174	108	311	165	142	160	136
CHD/Ms	Ms	144	97	230	140	288	105	228	106	108	249	219	156	112	201	123	114	92	204	108	286	172	227	176	118
HMI/Ms	Ms	166	97	228	100	238	91	249	104	98	221	258	191	121	185	173	114	86	174	117	311	220	147	160	139
KJR/Ms	Ms	142	97	228	142		87	226	110	108	284	217	175	124	201	121	114	98	204	108	236	149	120	172	133
M.MUS-NJL	Ms	176	106	224	142	230	91	226	106	100	280	217	141	84	197	139	114	90	197	106	266	151	138	172	121
MSM/Ms	Ms	142	97	222	129	313	89	226	104	96	332	217	168	124	204	121	114	92	218	108	278	170	120	155	140
M.spretus	RPCI	176	97	179	122	252	132	279	91	114	247	221	160	88	134	157	118	90	201	110	205	134	128	178	134
SPRET/Ei	JAX	180	97	190	124		106.5	269	91	114	266	213	185	95		151	116	90	205	110	204	134	115	170	139
PWK	RPCI	142	111	217	142	281	91	242	106	102	280	217	164	88	199	138	114	100	203	106	274	164	132	185	123
PBN/Ms	Ms	139	97	196	114		99	253	89	120	322	229	152	117		150	116	67	99	126		156	114	164	92

In each column (at each polymorphic locus), fragments of similar length are indicated by the same color.



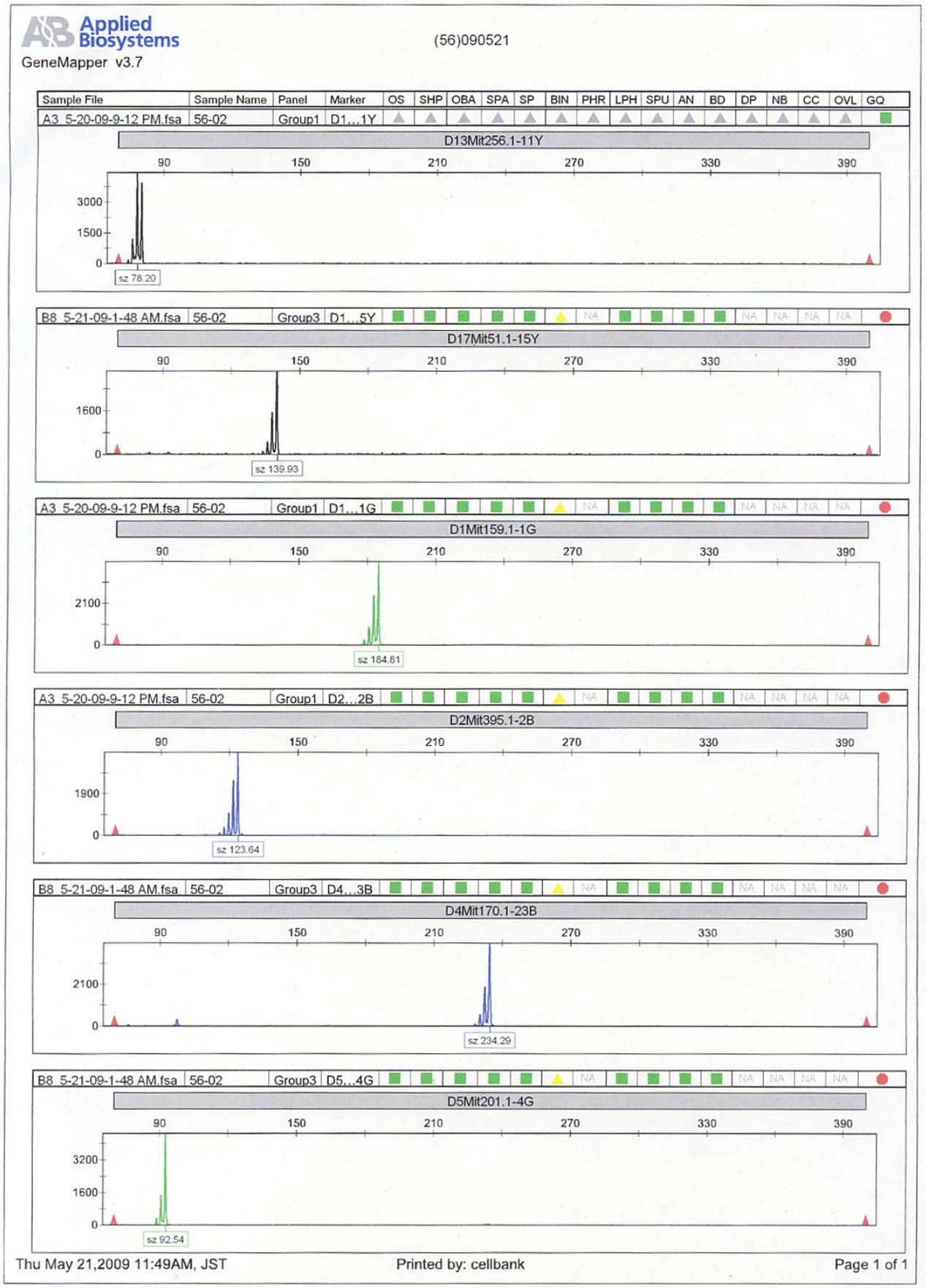
CH12F3-2A (RCB2809), C57BL/6

Figure 2A. Representative results from SSLP analyses using the 6 selected loci. (A) The CH12F3-2A cell line that is derived from the C57BL/6 mouse strain.



SP-2 (RCB3012), BALB/c

Figure 2B. Representative results from SSLP analyses using the 6 selected loci. (B) The SP-2 cell line that is derived from the BALB/c mouse strain.



L929 (RCB2619), C3H/He

Figure 2C. Representative results from SSLP analyses using the 6 selected loci. (C) The L929 cell line that is derived from the C3H/He mouse strain.

Table 2. SSLP analysis of 5 common and popular mouse strains using the 6 selected loci indicated in Figure 1C

Sample	D1 Mit159.1	D2 Mit395.1	D4 Mit170.1	D5 Mit201.1	D13 Mit256.1	D17 Mit51.1
C57BL/6	203.2	129.7	226.0	98.9	100.3	157.3
BALB/c	141.8	135.5	242.5	94.9	88.3	155.1
C3H/He	185.1	123.8	236.4	92.5	78.4	140.1
129/Sv	191.5	157.3	226.1	94.8	76.6	163.7
DBA/2	141.8	135.3	242.6	92.6	76.5	155.2

the common inbred strains (e.g., Table 3); the remaining 2.3% appeared to be derived from non-inbred mice. Cell lines derived from an F1 hybrid of two different strains show different alleles at each locus that correspond with those of their parental strains (Table 4). With regard to the cell line UV.CC3-11.1 (RCB2074), which was established from an F1 hybrid from the cross BALB/c x C3H/He, only the BALB/c allele at the D5Mit201.1 locus could be detected (Table 4). The C3H/He allele has been replaced by the BALB/c allele during culture, i.e., so-called loss of heterozygosity (LOH) appears to have occurred.

For some cell lines of uncertain provenance, we were able to identify the originating strains by SSLP analysis. For example, we found that LLC[20], PU5-18[21], and MBT-2[22] were derived from the C57BL/6, BALB/c, and C3H/He strains, respectively (Table 5). Interestingly, the LLC cell line had a deletion mutation at the locus D13Mit256.1 and only this mutated allele was detectable. Presumably, LOH had occurred subsequent to the deletion mutation.

Approximately 4.2% (14 lines out of 334 lines) of the mouse cell lines derived from common inbred strains were misidentified (Table 6), i.e., the strains were different from those claimed by the depositors of the cell lines. This rate of misidentification is similar to that reported for human cell lines[17]. As an example of misidentification, the cell line TSt-4 was registered as being C57BL/6-derived; however, SSLP analysis indicated that the cell line was derived from the BALB/c strain (Table 7). LOH following a deletion mutation was detected at the D17Mit51.1 locus of the TSt-4 cell line. Similarly, LOH following a deletion mutation was also detected in the MC3T3-E1[23] cell line at locus D1Mit159.2 (Table 8). In general, it is inevitable that aberrations such as point mutations, deletion mutations and LOHs accumulate in cell lines following long term culture. Thus, the optimum strategy is to culture the cell for as short period as possible not only in the cell bank but also for ordinary laboratory work.

Table 3. SSLP analysis of three C57BL/6-derived cell lines, B6mt-2, MEDEP-BRC5, and UV.B6-4.1 and three BALB/c-derived cell lines, RAW264, J774.1, and UV.BAL-7.1

Sample	D1 Mit159.1	D2 Mit395.1	D4 Mit170.1	D5 Mit201.1	D13 Mit256.1	D17 Mit51.1
C57BL/6	203.2	129.7	226.0	98.9	100.3	157.3
B6mt-2	203.2	129.8	226.0	98.7	100.3	157.0
MEDEP-BRC5	203.2	130.0	226.0	98.9	100.6	157.0
UV.B6-4.1	203.6	130.1	226.0	98.9	100.8	157.4
BALB/c	141.8	135.5	242.5	94.9	88.3	155.1
RAW264	141.8	135.3	242.5	94.6	88.3	155.0
J774.1	142.2	135.7	242.6	94.4	88.5	154.8
UV.BAL-7.1	141.8	135.3	242.4	94.6	88.2	155.1

The analyses authenticated the origins of the six cell lines.

Table 4. SSLP analysis of a mouse cell line derived from a (BALB/c x C3H/He) F_1 Mouse

Sample	D1 Mit159.1	D2 Mit395.1	D4 Mit170.1	D5 Mit201.1	D13 Mit256.1	D17 Mit51.1
BALB/c	141.8	135.5	242.5	94.9	88.3	155.1
C3H/He	185.1	123.8	236.4	92.5	78.4	140.1
UV.CC3-11.1	142.0 185.2	135.5 123.9	242.6 236.4	94.8	88.4 78.5	155.2 140.2

At locus D5Mit201.1 only the allele corresponding to that of BALB/c was detected.

Table 5. SSLP analysis of the LLC, PU5-18, and MBT-2 mouse cell line

Sample	D1 Mit159.1	D2 Mit395.1	D4 Mit170.1	D5 Mit201.1	D13 Mit256.1	D17 Mit51.1
C57BL/6	203.2	129.7	226.0	98.9	100.3	157.3
LLC	203.4	129.7	226.1	98.4	98.5	157.0
BALB/c	141.8	135.5	242.5	94.9	88.3	155.1
PU5-18	141.5	135.0	242.5	94.2	87.6	155.0
C3H/He	185.1	123.8	236.4	92.5	78.4	140.1
MBT-2	184.7	123.2	236.3	92.2	77.8	139.8

The mouse strains from which the cell lines originated were not registered by the depositors. The SSLP analysis indicated that they were derived from C57BL/6, BALB/c, and C3H/He, respectively.

Table 6. List of cell lines that the strains were different from those claimed by the depositors of the cell lines

RCB No.	Cell Name	Registered Strain	Result	Comment
RCB0792	T88-M	DBA/2J	C3H/He	Providing
RCB1144	DA-3	BALB/c	DBA/2	Providing
RCB2116	TSt-4	C57BL/6	BALB/c	Providing
RCB2117	TSt-4/G	C57BL/6	BALB/c	Providing
RCB2118	TSt-4/G-DLL1	C57BL/6	BALB/c	Providing
RCB2119	TSt-4/N	C57BL/6	BALB/c	Providing
RCB2120	TSt-4/N-DLL1	C57BL/6	BALB/c	Providing
RCB2633	MM46 CEA-2	C3H/He	Unknown	Providing
RCB2634	MM46-APR-MUC1 cl.1	C3H	Swiss	Providing
RCB2195	FVB-2	Swiss FVB	129/Sv	Stopped provision
RCB2196	ICRmt-1	ICR	Unknown	Stopped provision
RCB2617	MM46	C3H	Swiss	Stopped provision
RCB2632	BALB/3T3APR-MUC1 clone 16	BALB/c	Swiss	Stopped provision
RCB2647	BALB/3T3	BALB/c	Swiss	Stopped provision

Table 7. Misidentification of a cell line identified using SSLP analysis

Sample	D1 Mit159.1	D2 Mit395.1	D4 Mit170.1	D5 Mit201.1	D13 Mit256.1	D17 Mit51.1
C57BL/6	203.2	129.7	226.0	98.9	100.3	157.3
TSt-4	142.0	135.5	242.7	94.5	88.4	152.3
BALB/c	141.8	135.5	242.5	94.9	88.3	155.1

The mouse cell line TSt-4 was registered as a C57BL/6-derived cell line, but our SSLP analysis showed it was derived from the BALB/c strain.

Table 8. SSLP analysis of the mouse cell line MC3T3-E1

Sample	D1 Mit159.1	D2 Mit395.1	D4 Mit170.1	D5 Mit201.1	D13 Mit256.1	D17 Mit51.1
C57BL/6	203.2	129.7	226.0	98.9	100.3	157.3
MC3T3-E1	199.4	129.6	255.9	98.4	100.4	157.0

Loss of heterozygosity following a deletion mutation appears to have occurred at locus D1Mit159.1.

The C57BL/6N and C57BL/6J substrains were shown to have 11 single nucleotide polymorphisms (SNPs) in a study using mouse MD Linkage Panel 1449 SNPs (Illumina)[24]. It is therefore possible to distinguish cell lines derived from the C57BL/6N and C57BL/6J substrains using these 11 SNP.

The relatively high rate (4.2%) of misidentification of cell lines derived from common inbred mouse strains strongly suggests that intra-strain misidentification is also likely to have occurred. For example, although a cell line may be registered as being derived from colon cancer cells, an error may have occurred and the cell line was actually derived from another cancer (a similar phenomenon also applies to human cell lines). It is impossible to identify the originating tissue by microsatellite polymorphism analysis. In this context, a profiling analysis based on gene expression using many cell lines may be useful for authenticating the originating tissues of cultured cell lines.

Conclusion and Prospects

We have established a simple and reliable method to identify the common inbred mouse strains from which cultured mouse cell lines are derived. With respect to intra-strain misidentification, such as errors regarding the originating tissue type, it will be necessary to develop other analytic techniques, for example, gene expression profiling analysis. Other types of OMICS analysis, such as whole genome sequencing, will also be useful for authentication of cell lines. In this context, bioinformatics will become increasingly important for the quality control of cultured cell lines.

Materials and Methods

Mouse cell lines

All mouse cell lines that the Cell Engineering Division of the RIKEN BioResource Center (http://www.brc.riken.jp/lab/cell/english/) has collected, 342 cell lines in total, were subjected to SSLP analysis. We selected the latest preserved stock cells and those that were preserved immediately after deposition (token stock cells) for this analysis.

DNA preparation

DNA was prepared from approximately 2×10^6 cells using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany).

SSLP analysis

Multiplex PCR reactions for the SSLP analysis were carried out using the following fluorescent dye-linked primers (Mouse Mapping Primers, Life Technologies, Carlsbad, CA, USA): D1Mit159.1 (VIC) on chromosome 1, D2Mit395.1 (6-FAM) on chromosome 2, D4Mit170.1 (6-FAM) on chromosome 4, D5Mit201.1 (VIC) on chromosome 5, D13Mit256.1 (NED) on chromosome 13, and D17Mit51.1 (NED) on chromosome 17. VIC, 6-FAM, and NED are green, blue, and yellow fluorescent dyes, respectively.

PCR was performed with 2.4 □of genomic DNA (25 ng/ □) and 1.25 units AmpliTaq Gold (Applied Biosystems, Foster, CA, USA) in a 15 µl reaction volume using the GeneAmp PCR system 9700 (Applied Biosystems). Samples were amplified under the following conditions: an initial incubation at 95°C for 12 min was followed by 10 cycles of 94°C for 20 sec, 55°C for 20 sec, 72°C for 30 sec, and 20 cycles of 89°C for 20 sec, 55°C for 20 sec, 72°C for 30 sec, and finally incubation at 72°C for 10 min.

Labeled products were detected by electrophoretic size fractionation on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). A size control PCR was performed and the products were subjected to electrophoretic size fractionation as an internal control with each analysis. The end result for each cell line was an electropherogram with each allele represented as one or two peaks. As expected, one peak was detected at each locus in cell lines derived from inbred mouse strains. Samples that failed to give measurable peaks at all loci were reanalyzed using a different concentration of DNA or using newly prepared DNA.

Acknowledgements

This work was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

1. Stacey, G.N. (2000). Cell contamination leads to inaccurate data: we must take action now. *Nature* 403, 356.
2. Harris, N.L., Gang, D.L., Quay, S.C., Poppema, S., Zamecnik, P.C., Nelson-Rees, W.A., and O'Brien, S.J. (1981). Contamination of Hodgkin's disease cell cultures. *Nature* 289, 228-230.
3. Lavappa, K.S. (1978). Survey of ATCC stocks of human cell lines for HeLa contamination. *In Vitro* 14, 469-475.
4. Nelson-Rees, W.A., Daniels, D.W., and Flandermeyer, R.R. (1981). Cross-contamination of cells in culture. *Science* 212, 446-452.
5. O'Toole, C.M., Povey, S., Hepburn, P., and Franks, L.M. (1983). Identity of some human bladder cancer cell lines. *Nature* 301, 429-430.
6. Povey, S., Hopkinson, D.A., Harris, H., and Franks, L.M. (1976). Characterisation of human cell lines and differentiation from HeLa by enzyme typing. *Nature* 264, 60-63.
7. Dirks, W.G., MacLeod, R.A., and Drexler, H.G. (1999). ECV304 (endothelial) is really T24 (bladder carcinoma): cell line cross-contamination at source. *In Vitro Cell Dev Biol Anim* 35, 558-559.
8. MacLeod, R.A., Dirks, W.G., Matsuo, Y., Kaufmann, M., Milch, H., and Drexler, H.G. (1999). Widespread intraspecies cross-contamination of human tumor cell lines arising at source. *Int J Cancer* 83, 555-563.
9. Markovic, O., and Markovic, N. (1998). Cell cross-contamination in cell cultures: the silent and neglected danger. *In Vitro Cell Dev Biol Anim* 34, 1-8.
10. Scudiero, D.A., Monks, A., and Sausville, E.A. (1998). Cell line designation change: multidrug-resistant cell line in the NCI anticancer screen. *J Natl Cancer Inst* 90, 862.
11. American Type Culture Collection Standards Development Organization Workgroup ASN-0002. (2010). Cell line misidentification: the beginning of the end. *Nat Rev Cancer* 10, 441-448.
12. Chatterjee, R. (2007). Cell biology. Cases of mistaken identity. *Science* 315, 928-931.
13. Katsnelson, A. (2010). Biologists tackle cells' identity crisis. *Nature* 465, 537.
14. Phuchareon, J., Ohta, Y., Woo, J.M., Eisele, D.W., and Tetsu, O. (2009). Genetic profiling reveals cross-contamination and misidentification of 6 adenoid cystic carcinoma cell lines: ACC2, ACC3, ACCM, ACCNS, ACCS and CAC2. *PLoS One* 4, e6040.
15. Oldroyd, N.J., Urquhart, A.J., Kimpton, C.P., Millican, E.S.,

Watson, S.K., Downes, T., and Gill, P.D. (1995). A highly discriminating octoplex short tandem repeat polymerase chain reaction system suitable for human individual identification. *Electrophoresis* 16, 334-337.

16. Masters, J.R., Thomson, J.A., Daly-Burns, B., Reid, Y.A., Dirks, W.G., Packer, P., Toji, L.H., Ohno, T., Tanabe, H., Arlett, C.F., et al. (2001). Short tandem repeat profiling provides an international reference standard for human cell lines. *Proc Natl Acad Sci U S A* 98, 8012-8017.
17. Yoshino, K., Iimura, E., Saijo, K., Iwase, S., Fukami, K., Ohno, T., Obata, Y., and Nakamura, Y. (2006). Essential role for gene profiling analysis in the authentication of human cell lines. *Hum Cell* 19, 43-48.
18. Dietrich, W., Katz, H., Lincoln, S.E., Shin, H.S., Friedman, J., Dracopoli, N.C., and Lander, E.S. (1992). A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* 131, 423-447.
19. Moriwaki, K., Miyashita, N., Mita, A., Gotoh, H., Tsuchiya, K., Kato, H., Mekada, K., Noro, C., Oota, S., Yoshiki, A., et al. (2009). Unique inbred strain MSM/Ms established from the Japanese wild mouse. *Exp Anim* 58, 123-134.
20. Ralph, P., Moore, M.A., and Nilsson, K. (1976). Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. *J Exp Med* 143, 1528-1533.
21. Ralph, P., and Nakoinz, I. (1977). Direct toxic effects of immunopotentiators on monocytic, myelomonocytic, and histiocytic or macrophage tumor cells in culture. *Cancer Res* 37, 546-550.
22. Soloway, M.S. (1977). Intravesical and systemic chemotherapy of murine bladder cancer. *Cancer Res* 37, 2918-2929.
23. Sudo, H., Kodama, H.A., Amagai, Y., Yamamoto, S., and Kasai, S. (1983). In vitro differentiation and calcification in a new clonal osteogenic cell line derived from newborn mouse calvaria. *J Cell Biol* 96, 191-198.
24. Mekada, K., Abe, K., Murakami, A., Nakamura, S., Nakata, H., Moriwaki, K., Obata, Y., and Yoshiki, A. (2009). Genetic differences among C57BL/6 substrains. *Exp Anim* 58, 141-149.