Stem Cell Line Authentication and Contamination Detection

ABSTRACT

Cell line misidentification and contamination can have a profound effect on the results generated by cell line research. The U.S. National Institutes of Health recommends authenticating any cultured cells used in the laboratory. To aid in cell line authentication, Promega has developed the StemElite™ ID System, a ten-locus multiplex PCR system. This article demonstrates how the StemElite™ ID System can be used to identify a human stem cell line and to detect contamination from mouse feeder cells or other human cell lines.

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Introduction

Cell line misidentification and contamination have become important concerns for researchers. A recent *Science* article indicated that in some cases, laboratories have invested substantial time and effort researching cell lines that were revealed to be misidentified(1) . The situation has prompted the U.S. National Institutes of Health to issue a notice strongly urging researchers to authenticate cultured cells used in their laboratory(2) .

The StemElite™ ID System uses fluorescently labeled short tandem repeat (STR) genotyping, a mainstay of human forensic DNA profiling, to identify cells and detect human cell mixtures. The STR loci amplified by the StemElite™ ID System have been carefully selected to give optimum discrimination between cell lines, generating a random match probability of 1 in 2.92 x 10^9 for human cell lines not derived from a common donor line. The system also includes a mouse locus to detect contamination from mouse feeder layers. Confirmation of the identity and purity of a cell line using the StemElite™ ID System avoids wasted time and labor performing experiments on contaminated or misidentified cells.

Methods and Results

CELL LINE IDENTIFICATION: STEMELITE™ ID SYSTEM RESULTS FOR HUMAN EMBRYONIC STEM CELLS

Cell lines can be distinguished from one another by analyzing multiple STR loci. With each additional locus, the probability of the DNA profile of one cell line matching that of another decreases if the cells are not derived from the same parent cell line. The loci used in the StemElite™ ID System are those used in existing databases (e.g., American Type Culture Collection [ATCC]) with the additional locus D21S11 to increase overall resolving power. Genotypes generated using the StemElite™ System can be compared to those in existing databases or previously generated data to confirm cell line identity.

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Figure 1 shows a genotype for the H9 Human Embryonic Stem Cells (hESC), generated using the StemElite™ ID System. DNA was isolated from 1 × 10⁴ hESC using the Maxwell® Cell LEV DNA Purification Kit (Cat.# AS1140) and quantitated using the Quant-iT™ PicoGreen® dsDNA Reagent (Invitrogen, Cat.# P7589). Two nanograms of DNA was amplified with the StemElite™ ID System (Cat.# G9530), amplified products were analyzed using a 3130*xl* Genetic Analyzer (Applied Biosystems), and a genotype was generated.

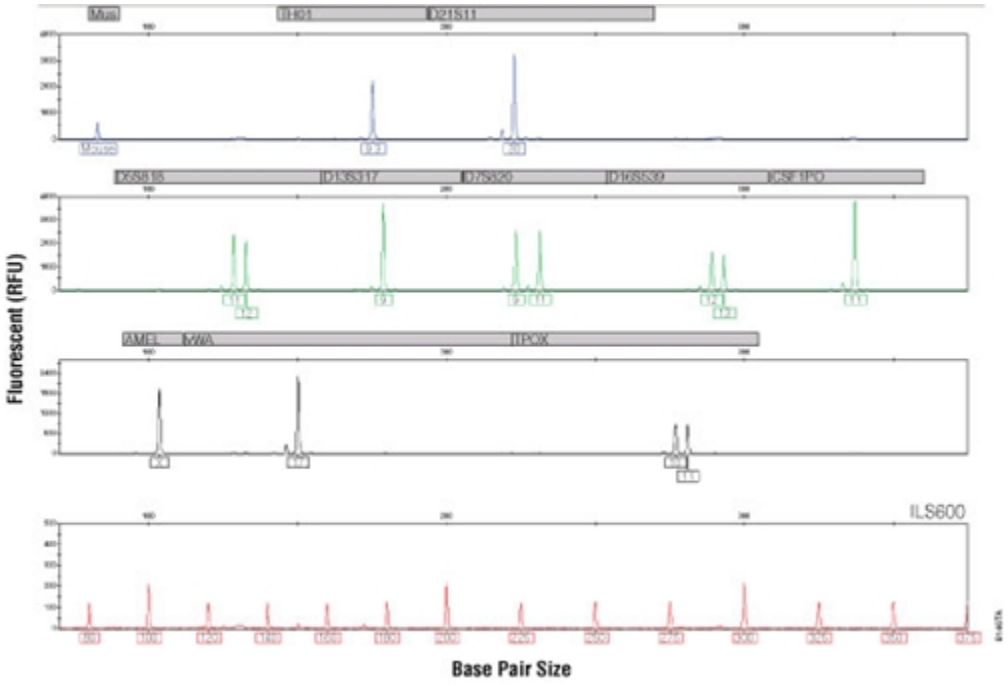

Figure 1. StemElite™ ID System electropherogram for the H9 human embryonic stem cell line. The StemElite™ ID multiplex amplification reaction was performed with 2ng of H9 hESC DNA. Amplification products were analyzed by capillary electrophoresis and products for each fluorescent channel graphed as base pair size versus fluorescence. Sizes were determined for each sample peak using the Internal Lane Standard 600 (Cat.# DG2611). An allelic ladder was analyzed along with the unknown samples and, like the samples, a size assigned for each peak.

Using genotyping software (GeneMapper® *ID*, Applied Biosystems), allele designations were assigned to the sized peaks within the allelic ladder. The software then compared the sample peaks with allelic ladder peaks and assigned an allele to each sample peak. The sample alleles are listed in a genotype table (Figure 2). The StemElite™ ID genotype was compared to a genotype for this cell line published by WICell and the National Stem Cell Bank. The genotypes matched, and the identity of the H9 hESC line confirmed.

Sample File	Marker	Allele 1	Allele 2
B01_StmEltID805_	Mus	Mouse	Mouse
B01_StmEltID805_	TH01	9.3	9.3
B01_StmEltID805_	D21S11	30	31
B01_StmEltID805_	D5S818	11	12
B01_StmEltID805_	D13S317	9	9
B01_StmEltID805_	D7S820	9	11
B01_StmEltID805_	D16S539	12	13
B01_StmEltID805_	CSF1PO	11	12
B01_StmEltID805_	AMEL	X	X
B01_StmEltID805_	vWA	17	17
B01_StmEltID805_	TPOX	10	11

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Figure 2. hESC StemElite™ ID Genotype. The GeneMapper® *ID* genotyping software assigned an allele to each sample peak shown in Figure 1. This genotype then can be compared to a published or archived genotype to confirm cell line identity.

MOUSE CELL CONTAMINATION: DETECTION OF MOUSE FEEDER CELLS IN HUMAN STEM CELL CULTURE

Human embryonic stem cell lines are often grown on mouse feeder cells. During passaging, the mouse feeder cells can be inadvertently transferred with the hES cells. Future applications will require pure lines free from mouse contamination. The StemElite™ ID System gives a qualitative measure of mouse cell contamination, indicated by the presence of a single peak in the electropherogram (Figure 3). Detection of mouse cells is then indicated on the genotype table.

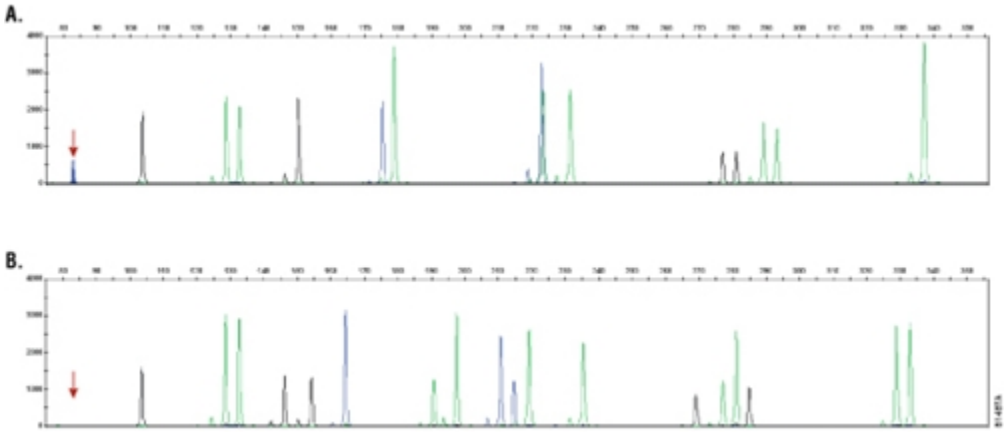

Figure 3. hESC StemElite™ ID Genotype table. The GeneMapper® *ID* genotyping software assigned an allele to each sample peak shown in Figure 1. This genotype then can be compared to a published or archived genotype to confirm cell line identity.

The sensitivity of the StemElite™ ID System to detect mouse contamination was tested by mixing a human immortalized cell line (HEK293) with increasing amounts of mouse cells (NIH 3T3). DNA was isolated using the Maxwell® Cell LEV DNA Purification Kit and quantitated using Quant-iT™ PicoGreen® Assay. DNA was amplified using the StemElite™ ID System, and the products analyzed. The results in Figure 4 show that mouse DNA was detected in cell mixtures contaminated with 1% mouse cells or more.

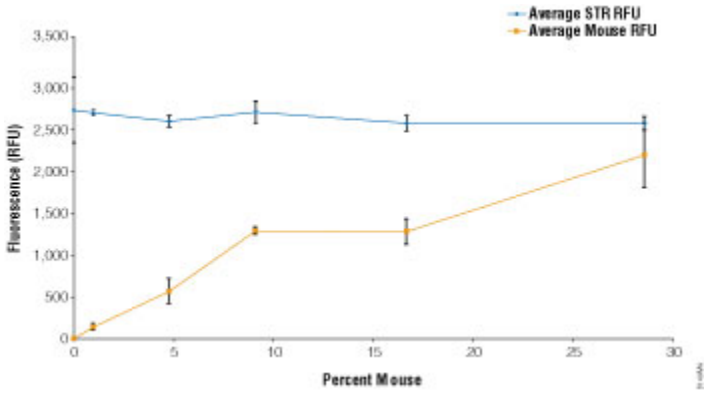

Figure 4. StemElite™ ID System detection of mouse cell contamination in human cell samples. An immortalized human cell line (HEK293) was mixed with increasing amounts of mouse cells (NIH 3T3). Samples were analyzed using the StemElite™ ID System, and the average peak height of the human-specific peaks was compared to the mouse-specific peak. Data represent the average of triplicate experiments.

HUMAN CELL CONTAMINATION: DETECTION OF HUMAN CELL MIXTURES

Many labs culture multiple cell lines concurrently, creating a risk for potential cross-contamination of one cell line with another. In StemElite™ ID System reactions, contamination is indicated by the presence of additional alleles at multiple loci compared to the pure culture genotype. In the following example, cell line contamination is mimicked by mixing hESC with HeLa human adenocarcinoma cells, then isolating the DNA. Figure 5 shows the contamination can easily be identified by comparing the StemElite™ ID genotype from the contaminated H9 hESC sample with the genotype from a pure H9 hESC population (e.g., previous experiment, Figure 2, or published genotype from the National Stem Cell Bank).

Samp	Marker	Allele 1	Allele 2	Allele 3	Allele 4	Height 1	Height 2	Height 3	Height 4
D05_	Mus	Mouse				1302			
D05_	TH01	7	9.3			213	5440		
D05_	D21S11	27	30			77	4748		
D05_	D5S818	11	12			3234	3070		
D05_	D13S317	9	12			5337	82		
D05_	D7S820	9	11			3056	2978		
D05_	D16S539	10	12	13		98	3010	2487	
D05_	CSF1PO	9	11			106	5983		
D05_	AMEL	X				2492			
D05_	vWA	17	18			2947	76		
D05_	TPOX	10	11			1611	1590		

Figure 5. StemElite™ ID genotype for hESC contaminated with a second human cell line. DNA was isolated from a mixture of 1 × 10^4 H9 hESC and 2 × 10^3 HeLa cells using the Maxwell® Cell LEV DNA Purification Kit and quantitated using Quant-iT™ PicoGreen® Assay. Two nanograms of purified DNA were then amplified with the StemElite™ ID System and analyzed using a 3130*xl* Genetic Analyzer (Applied Biosystems) and GeneMapper® *ID* software. The resulting genotype was compared to the genotype of H9 hESC shown in Figure 2. Peaks resulting from the contaminating HeLa cells are circled in red.

Conclusion

The StemElite™ ID System genotypes human cell lines, including human embryonic stem cells, and detects contamination of those cell lines. Cell line identity can be confirmed by comparing the StemElite™ ID System-generated genotype with a known genotype standard. Mouse cell contamination is also easily detected by the presence of a mouse-specific locus in the genotype. In addition, the StemElite™ ID System is able to detect the presence of human cell mixtures and thus prevent waste of time and resourses working on contaminated cell lines.

Acknowledgements

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REFERENCES

1. Chatterjee, R. (2007) Cell biology. Cases of mistaken identity. *Science* **315**, 928–31.
2. Ruiz Bravo, N. and Gottesman, M. (2007) Notice regarding authentication of cultured cell lines.

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