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OCCURRENCE AND STABILITY OF STR MICROVARIANT ALLELES IN ESTABLISHED HUMAN CELL LINES

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Abstract

Part of the ongoing quality control practice at the American Type Culture Collection (ATCC) is a short tandem repeat (STR) screening of all human cell lines. That intraspecific identity test, which examines polymorphic DNA regions, is conducted with PCR-based PowerPlex 1.2 kits, developed by Promega. The resulting allelic designation within a locus represents the number of tetranucleotide repeating units amplified from the template DNA. Occasionally, a microvariant allele—a DNA fragment having additions or deletions within a repeat—is detected. Microvariant verification is through either arithmetical means and/or via co-amplification with a sample known to have close but standard repeat alleles. In this presentation, microvariant occurrences are compared within members of these related cell lines: HeLa derivatives (cervical carcinoma) and contaminants; JURKAT derivatives (acute T-cell leukemia); and, RKO derivatives (colon carcinoma). All members of the HeLa family and HeLa-contaminants at ATCC expressed allele 13.3 at the D13S317 locus. In the cell line, I 9.2 (JURKAT lineage), a microvariant (in D7S820, allele 9.2) was detected in a cell line whose parent has a conventional profile. Members of the RKO family have highly differing STR profiles. The parent line, RKO, has a repeat number of 22 at the vWA locus, but the derivatives RKO-AS45-1 and RKO-E6 have 24 and 25 repeating units respectively, extending into the TH01 fragment size range. Furthermore, both of the RKO derivatives had microvariants emerge in the D16S539 locus. Regardless of the potential profile flexibility within a cell line family, all of the microvariants identified at ATCC to date are consistently expressed within the specific cell line. Thus, these microvariants are useful in cell line identity and, in the case of HeLa-contaminants, assist in the confirmation of suspected cell line mixtures.

Introduction

Background:

- Satellite DNA is used in forensics investigations because this genetic material is informative, stable, and polymorphic between individuals
- The Short Tandem Repeat (STR) profile—a list of alleles correlating to specific loci—is the genetic description of the cell line
- The factors determining the product length are the specific STR locus, primer binding sites, and the flanking regions between the primer site and the STR
- For human cell line identification, ATCC uses commercially available PowerPlex 1.2 kits by Promega Corporation, testing eight tetranucleotide STR loci (D16S539, D7S820, D13S317, D5S818, CSF1PO, TPOX, TH01, and vWA) and amelogenin for sex determination
- The probability of two random individuals sharing the same profile for the eight STR loci is less than one in 10^8
- Microvariants are formed by insertion and deletion mutations occurring within the STR or in the flanking regions. Point mutations are not detected through genotyping; researchers interested in that aspect may choose sequencing techniques. Depending on the nature of the mutation, alterations on the primer site may significantly decrease the product signal if not totally prevent amplification.

This presentation addresses three sets of microvariants detected at the ATCC:

- D13S317 allele 13.3 in the HeLa cell line, HeLa derivatives, and HeLa contaminants
- D7S820 allele 9.2 originating in I 9.2 (a derivative of JURKAT, which does not express this variant)
- Large vWA products extending outside of the normal range in the RKO family of cell lines

Methods

Cultured Cells

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DNA Extraction

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PowerPlex 1.2 PCR Reaction

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Electrophoretic Fractionation and Fragment Detection (ABI 310)

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GeneScan 3.1 Analysis (peak detection)

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Genotyper 2.0 Analysis (peak identification and analysis)

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Import into In-house Databank for Comparison (Paradox Macro)

Results

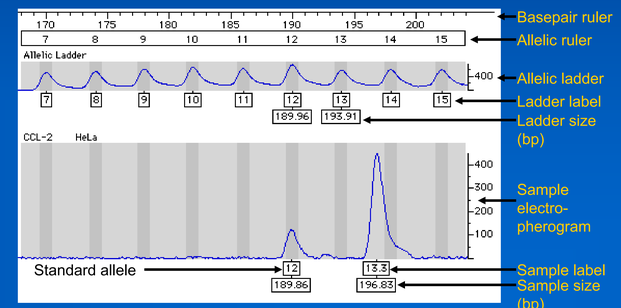

Figure 1. The alleles in HeLa (cervical carcinoma) at D13S317 are 12 and 13.3. The following equation was used to arithmetically confirm the microvariant (x):

x= |(size standard allele - size corresponding ladder) - (size off ladder allele - size ladder before off ladder allele)|

x= |(189.86-189.96) - (196.83-193.91)|

x= 3.02; with rounding to the nearest basepair, D13S317 off ladder allele = 13.3

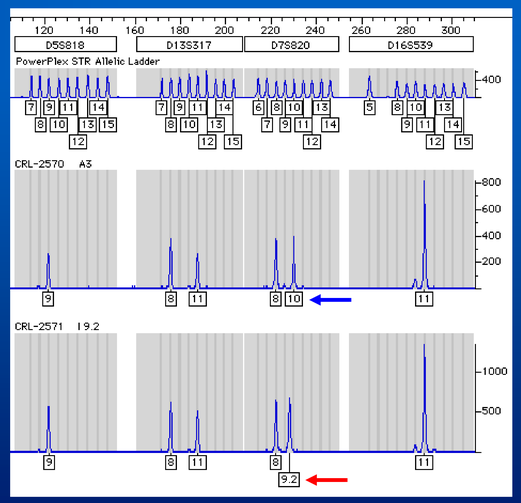

Figure 2. The A3 cell line (acute T cell leukemia, a wild-type JURKAT cell line) represents a normal profile. A microvariant emerges in the A3-derivative line, I 9.2 at D7S820 allele 9.2 from the parental allele 10. The derivative line arose after exposing A3 cells to the frameshifting mutagen, ICR-191.

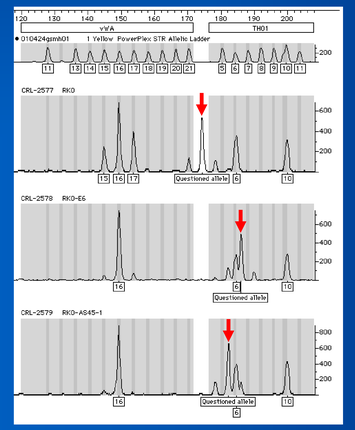

Figure 3A. The RKO family (colon carcinoma) has questionable alleles outside the vWA locus. For the transformed derivatives, RKO-E6 and RKO-AS45-1, one might call those alleles TH01 allele 6.2 and 5.2 respectively.

NOTE: the vWA alleles 15 and 17 are probably high, though reproducible, PCR artifacts.

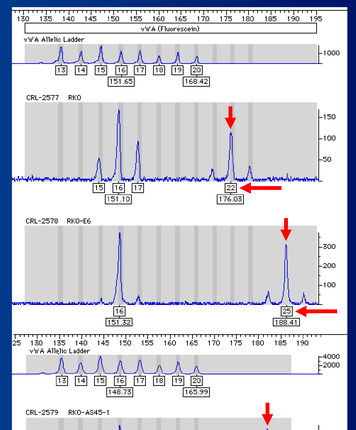

Figure 3B. The vWA Monoplex STR reactions of these lines reveals that the peaks represent vWA alleles.

Discussion

Microvariants are occasionally found in cell lines held at the ATCC. These stable mutations provide valid characterization data. For example, all 16 HeLa members held at the ATCC (including derivatives and contaminants) and no other cell lines tested to date have the D13S317 allele 13.3. With HeLa a notorious contaminant in early cell culture, this microvariant may be extremely useful in identifying any additional HeLa-mixtures yet to be identified. Preliminary sequence analysis indicates a guanine deletion in the eleventh AGAT repeat. While the sequence data are interesting, the exact nature and placement of this mutation is statistically inconsequential to genotype analysis.

The ATCC has seen only one origination of a microvariant from a standard profile cell line: the D7S820 allele 9.2 emerging in I 9.2 from A3. A frameshift mutagen was involved in creating the new cell line and the STR data suggests the ICR-191 intercalating molecule produced two deletions within the fragment region.

A second case of external factors influencing a cell line profile appears in the RKO family. Separate viral transformations of RKO led to the derivation of cell lines RKO-E6 and RKO-AS45-1. Whereas stressful conditions, such as transformations, have often produced a loss of heterozygocity, the manipulation of alleles is unusual. The second unexpected aspect about these profiles is the severe vWA allelic shift into the TH01 locus, especially since a parameter in commercial kit design is to prevent overlapping loci. The vWA alleles 22, 24, and 25 are not true *micro*variants, but an extreme variation nonetheless. This scenario could result in the inappropriate calling of an allele (such as mislabeling vWA alleles 24 and 25 as TH01 alleles 5.2 and 6.2). Dubious data may call for further experiments, such as MonoPlex reactions of the locus in question, to accurately resolve the problem.