

# CELL CULTURE FORENSICS OF CALU-3: A HUMAN LUNG EPITHELIAL CELL LINE

Human cell lines are used as model systems to investigate cellular and molecular processes occurring in humans to understand disease mechanisms and develop potential therapeutics. Cell cultures have been contaminated, mislabeled, or over-ridden by faster-growing cells for decades. One estimate made in 2006 suggested that nearly a third of human-tumor cell lines in one cell repository included cross contaminations, which is a significant problem because most human cell lines used in research projects are not tested to verify their identity. This project was designed to test the identity of lung epithelial cells used in NIH-funded research on cigarette-smoke-induced changes in epithelial cell function. Calu-3 cells are a human lung epithelial cell line derived from a pleural effusion in a patient with adenocarcinoma of the lung. Calu-3 cells were initially purchased from ATCC at passage 19. For identity testing, cultures at passages 23, 25 and 26 were screened. DNA was extracted from cells at 60%–80% confluence using the QIAamp DNA mini kit, which uses silica membrane technology. Cell line identification used cell culture forensics, which is based on the same technology as criminal DNA forensics. Ten polymorphic short-tandem repeat (STR) loci were amplified using the polymerase chain reaction and primers from the new Cell ID System. The length of the fragment produced for each locus reflects the number of repeats, 8–20 copies, present in that fragment. Fragment sizes, accurate to less than one base pair, were determined using the Applied Biosystems 3100 Genetic Analyzer. Using the forensic “product rule,” the genetic profile detected with all ten loci are predicted to have a random match probability of 1 in  $2.92 \times 10^9$ . The pattern detected in our Calu-3 samples matched that provided by ATCC, and therefore verify the identity of the Calu-3 cells maintained in our laboratory.

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## BACKGROUND

Cell lines are used in biological research to better understand human diseases and to find potential therapeutics. Cell lines were first introduced in 1951, when a woman by the name of Henrietta Lacks donated her cells, which were derived from her cervical cancer. Cell line contamination has been a problem ever since, costing researchers tens of millions of dollars on invalid research. Fortunately, there are ways to verify the identity of cell lines, cell culture forensics being the most efficient method. Calu-3 is a cell line used at the University of Alaska Anchorage to better understand smoke-induced changes in the human lung. This cell line has not yet been genetically linked back to the donor to prove that it is not contaminated. This project was designed to test the identity of lung epithelial cells used in NIH-funded research on cigarette smoke-induced changes in epithelial cell function.

## METHODS

### Tissue Culture

Calu-3 human bronchial epithelial cells were grown for approximately one week in Delbecco's Modified Eagles Media containing 10% fetal bovine serum, L-glutamine, and penicillin/streptomycin and maintained at 37°C and 5% CO<sub>2</sub>. Media was changed every 2–3 days.

### DNA Extraction

Cultures of Calu-3 cells were used at 60–80% confluence at passages 23, 25 and 26. DNA was isolated using the QIAamp DNA isolation kit (Qiagen,

Germantown, MD). This kit uses Silica Membrane Technology in a spin column format to purify the DNA.

### STR Amplification

STRs were amplified using the Cell ID System (Promega, Madison, WI). Briefly, 2 ng of genomic DNA from each sample was amplified using Taq Polymerase and optimized primers for the following ten loci: D21S11, TH01, TPOX, vWA, Amelogenin, CSF1PO, D16S539, D7S820, D13S317, D5S818. D21S11 and TH01 were labeled with fluorescein (FL). TPOX, vWA, and Amelogenin, were labeled with carboxy-tetramethylrhodamine (TMR). CSF1PO, D16S539, D7S820, D13S317 and D5S818 were labeled with 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE). Optimized thermocycler settings were provided by Qiagen. Using a combination of differences in fragment length and dyes, all loci were amplified and analyzed in a single reaction. DNA from K562 cells served as a positive control and reactions without DNA served as negative control. All experimental samples were run in triplicate.

### Fragment Analysis

The ABI3100 Genetic Analyzer along with GeneMapper software was used to analyze the amplified STR fragments to determine fragment length and the number of repeats in each fragment. 1 µl of each amplified product or Cell ID Allelic Ladder was mixed with an internal lane standard and HiDi formamide. All samples were run in triplicate. A custom bin file for GeneMapper 3.1 was generated using the Cell ID Allelic Ladder pattern generated to allow correct identification of frag-

ments and the numbers of repeats in each fragment.

## RESULTS

We successfully generated electropherograms for all samples amplified and analyzed. The STR peak heights within the electropherograms represented the fluorescence intensity of each amplified fragment present within the sample. This amount directly correlated to the amount of that specific allele of the STR locus present within the template DNA sample. The location of the peak along the x-axis of the electropherogram corresponded to its fragment size, which is influenced by the number of repeats present within the fragment. The STR locations and number of repeats in all our experimental samples followed the pat-

tern reported by ATCC. Therefore, the cell line analyzed is the Calu-3 cell line as purchased from ATCC.

## CONCLUSION

Because the number of repeats in our STR loci matched the number of repeats reported by ATCC for Calu-3, we were able to determine that the cells labeled as Calu-3 in our laboratory are indeed Calu-3 and are not contaminated with another cell line.

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## RESOURCES

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