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# Short Tandem Repeat (STR) Profiles of Commonly Used Human Ocular Surface Cell Lines

By

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**Short title:** STR profiles of cell lines

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## **Abstract**

### **Purpose**

To establish the short tandem repeat (STR) profiles of several human cell lines commonly used in ocular surface research.

### **Materials and Methods**

Independently DNA was extracted from multiple passages of three human corneal epithelial cell lines, two human conjunctival epithelial cell lines and one meibomian gland cell line, from different laboratories actively involved in ocular surface research. The samples were then subjected to STR analysis on a fee-for-service basis in an academic setting and the data compared against that in available databases.

### **Results**

The STR profiles for the human corneal epithelial cells were different among the three cell lines studied and for each line the profiles were identical across the samples provided by three laboratories. Profiles for the human conjunctival epithelial cells were different among the two cell lines studied. Profiles for the meibomian gland cell line were identical across the samples provided by three laboratories. No samples were contaminated by elements of other cell lines such as HeLa.

### **Conclusions**

This comprehensive study provides verification of STR profiles for commonly used human ocular surface cell lines that can now be used as a reference by others in the field to authenticate the cell lines in use in their own laboratories.

**Keywords:**

conjunctiva, cornea, epithelial, meibomian, short tandem repeat

## I. Introduction

Immortalized cell lines are extensively used in biomedical research, including eye research, as they are relatively cost effective, easy to use, can provide an unlimited supply of homogeneous material, and can circumvent ethical and biohazard (infectious) issues associated with the use of human tissue.<sup>1</sup> Whilst there are many benefits to the use of cell lines, researchers need to be aware of their limitations such as how well they mimic the primary cell of interest and effects of genetic drift over extended time in culture<sup>1</sup>. An additional major issue is one of misidentification. This may be due to simple human error such as incorrect labeling of a cell culture plate or flask during routine culture manipulation or be due to cross-contamination of one cell type by another.<sup>2</sup>

Cross-contamination was first recognized more than half a century ago, yet it is still an issue today.<sup>3</sup> The most common cross-contaminating cell line is the HeLa (human cervical adenocarcinoma) line and others include T-24 bladder carcinoma and HT-29 colon carcinoma cells.<sup>3</sup> The HeLa line was the first human cell line developed, and was derived, without consent, from cervical cancer cells from Henrietta Lacks in 1951.<sup>4</sup> HeLa cells are particularly robust and prolific and can rapidly over grow other cells. A recent study from China highlights the problem with 46% of 278 tumor cell lines being misidentified and close to 67% of these being cross-contaminated by HeLa cells.<sup>5</sup> Further it was recently documented that over 32,000 articles have been published that have used misidentified cell lines and it has been estimated that the extent of misidentification may be as much as one third of all cell lines.<sup>6</sup> Eye research is not immune from these issues. The most well documented examples concern uveal melanoma cell lines, several of which were thought to be of different backgrounds but

actually shared the same background (e.g. OCM3=OCM8) and some of which were found to be of cutaneous rather than ocular origin.<sup>7-9</sup> Misidentification has also been reported for RGC-5 cells, which were thought to be of rat retinal ganglion cell origin but recently were confirmed to be 661W cells, a mouse SV-40 T antigen transformed photoreceptor cell line.<sup>10</sup> Further “Chang conjunctival cells”<sup>10,11</sup> were found to be derived by HeLa contamination.<sup>13</sup> Disturbingly, there have been some twenty publications using Chang conjunctival cells since 2010, the most recent being 2016.<sup>14</sup>

Short tandem repeat (STR) DNA analysis has become the standard method for authentication of human cell lines<sup>15</sup> owing to the extensive experience of this technology for forensic purposes<sup>5</sup>, the simplicity of sample preparation and relatively low cost. Short tandem repeats, a type of microsatellite, are short sequences (typically 2-6 base pairs) of DNA that are repeated numerous times in a row, typically in non-coding regions of genes. STR profiling involves the use of specific primers for regions that flank microsatellite DNA to generate PCR amplicons which are then resolved by capillary electrophoresis, sized and converted into alleles and assigned a numeric value thus generating a unique profile of the number of repeats for specific STRs in that cell line.<sup>17</sup> Typical profile analysis involves simultaneous amplification of 15-17 STR markers (with eight being the minimum required for accurate discrimination) and amelogenin for sex determination.<sup>17</sup> The purpose of this study was to determine the STR profiles of several ocular surface cell lines and make the profiles available to the field in general so that other research groups have ready access to the information for authenticating the cells used in their laboratories.



## **II. Materials and Methods**

### **A. Cell Lines and DNA extraction**

Independent laboratories active in ocular surface research participated in the study. The following cell lines were profiled: SV40-immortalized human corneal epithelial cells (SV40-HCEC) developed by Araki-Sasaki et al.<sup>18</sup>; telomerase-immortalized human corneal epithelial cells (hTCEpi) developed by Robertson et al.<sup>19</sup>; telomerase-immortalized human corneal epithelial cells (HuCl-22/cdk4R/p53DD/TERT, abbreviated to HCLE) developed by Rheinwald et al.<sup>20</sup>; telomerase-immortalized human conjunctival epithelial cells (ConjEp-1/p53DD/cdk4R/TERT, abbreviated to HCjE) developed by Rheinwald et al.<sup>20</sup>; RSV-T transfected HC0597 human conjunctival epithelial cells developed by Ward et al. and reported originally as a conference abstract<sup>21</sup>; telomerase-immortalized human meibomian gland epithelial cells (HMGEC) developed by Liu et al.<sup>22</sup> Table 1 shows the passage number of the various cells tested and number of laboratories who independently submitted samples for analysis.

DNA was extracted either from cells being actively maintained under their normal growth conditions in culture or directly from frozen stocks stored in liquid nitrogen. Briefly, actively growing cells were lysed with DirectPCR® Lysis reagent (Viagen, Los Angeles, CA) in the presence of proteinase K, by incubating for a minimum of 3hr at 55°C followed by 1hr at 85°C. Isopropanol was used to precipitate the DNA. The DNA pellet was washed with 70% ethanol and allowed to air dry before being resuspended in 10mM Tris-0.2mM EDTA buffer. DNA was extracted from frozen stocks using a Qiagen DNA Easy Blood and Tissue Kit according to the manufacturer's protocol. Briefly, approximately  $10^6$  cells were lysed with 650  $\mu$ L Lysis Buffer. The resulting lysate (200

μL) was then digested with proteinase K, and genomic DNA was extracted according to the manufacturer's protocol for cultured cells. Each sample was eluted in 300 μL of LowTE buffer pH 8.0. Samples were diluted in HPLC-grade water to 1.5 ng/μL in preparation for sample amplification and fragment analysis. Samples were then shipped to the University of Arizona Genetics Core for analysis.

## **B. STR Profiling**

Profiling was performed by the University of Arizona Genetics Core (Tucson, AZ) using their standard methodology (<http://uagc.arl.arizona.edu/services/cell-line-authentication-human>), which utilizes the Promega PowerPlex 16HS assay to study 15 autosomal loci and amelogenin. The resulting data were compared with the DSMZ database (<https://www.dsmz.de/services/services-human-and-animal-cell-lines/online-str-analysis.html>) with 80% or greater identity at 8 core loci (TH01, D5S818, D13S317, D7S820, D16S539, CSF1PO, vWA, TPOX) and amelogenin being considered a match.<sup>17,23</sup> At least two passages from each cell line were analyzed with the exception of HC0597 for which only one passage was tested.

## **III. Results**

Table 2 shows the STR profiles obtained for each of the cell lines tested. All samples amplified indicating the human origin of the material and none were contaminated with material from other cell types including HeLa. As expected the profile for each cell line was different. None of the STR profiles matched with any other profile in the DSMZ database with the exception of the profile for the SV40-HCEC, which matched (with the

exception of D5S818, which is 13,13 in the database) to the profile for “HCE-T” corresponding to Reiken Cell Bank cell lines RCB1384 and RCB2280 indicating that our stocks of SV40-HCEC are the line developed by Araki-Sasaki et al.<sup>18</sup> and are free from cross-contamination. An additional source of confirmation came from a previously published STR analysis of this cell line in which the cells were also a match for RCB1384 and RCB2280.<sup>24</sup> This previously published profile matched that for SV40-HCEC shown in Table 2, except for the following loci: D18S51 (13,15) and CSF1PO (9,12).

The STR profiles for hTCEpi were identical for the four passages submitted for testing by three labs. For HCLE cells the profile of both passages tested was identical. For HCjE cells the profiles were identical (with two minor exceptions) for the seven passages from three labs. The exceptions were: at locus D3S1358 the result for one passage (p44) was 16,16; at locus D5S818 the result for one passage (p35) was 11,11. Notably the profile for the HCjE cells (Table 2) was distinct from that of Chang conjunctival cells (available at ATCC, [https://www.atcc.org/STR%20Database.aspx?geo\\_country=usa](https://www.atcc.org/STR%20Database.aspx?geo_country=usa)). For HMGEC cells identical profiles were obtained for the seven passages from three labs.

#### **IV. Discussion**

Finding that the common STR databases (e.g. ATCC, DSZM) have minimal data on ocular surface cells, our laboratories collaborated together to perform STR profiling on multiple passages of ocular surface cell lines. Of the six lines tested, only one, SV40-HCEC, matched to known lines in the available databases. This line of human corneal

epithelial cells was developed by Araki-Sasaki et al.<sup>18</sup> and has been extensively used in many laboratories around the world. As there is no standard nomenclature for this line, there is no easy way to determine precisely how many independent laboratories maintain this line and how many published studies have utilized the line. The latter probably exceeds more than 100 peer-reviewed publications. Our study utilized relatively early passage cells, which gave a match to the profile of the cells, in the DSMZ database. We also had the opportunity to compare our data on the SV40-HCEC cell line to one previously published study.<sup>24</sup> This also provided a match, although the profiles of our stocks were not identical – possibly due to genetic drift and microsatellite instability.<sup>23</sup>

None of the other lines tested matched with any other cell line in the DSMZ database. This was not unexpected given the rather specialized nature of ocular surface research and that most lines are available via the laboratory of origin rather than a commercial entity such as ATCC. The study confirmed that none of the specific stocks tested has been the victim of cross-contamination by other cell lines. In recent years the use of the hTCEpi line developed by Robertson et al.<sup>19</sup> has somewhat overtaken that of the SV40-HCEC line, although again the precise number of published studies using this line is difficult to determine. Rheinwald<sup>20</sup> and Gipson at the Schepens Eye Research Institute in Boston developed and characterized respectively the corneal and conjunctival cell lines, which became popularized by the work of Gipson and colleagues<sup>25</sup> with some 100 publications from several different laboratories using these telomerase modified cell lines. Although much less commonly used we also studied the HC0597 conjunctival epithelial cell line, which was developed and patented (to replace

the Draize rabbit eye test for evaluation of the eye irritation potential of products) by Ward et al. of The Gillette Company.<sup>26</sup> Another human conjunctival epithelial cell line, IOBA-NHC, was developed by Diebold et al.<sup>27</sup> and appeared promising at first. However it subsequently proved to be genetically unstable thus was not investigated here. The last line we studied was the more recently developed human meibomian gland epithelial cells,<sup>22</sup> which is in now under study by multiple laboratories in Europe, Asia and Australia in addition to the USA.

Given that many of the lines we investigated have been distributed to multiple labs around the world we hope these data are a useful resource for the field of ocular surface research. We strongly encourage all of our colleagues, but especially those working in multiuser culture facilities and where multiple lines are in use, to perform STR analysis on their own stocks of these cells to confirm their identity. Here we studied cell lines readily available in our laboratories. Ocular surface cell lines other than those studied here are no doubt in use in other laboratories across the world and we hope that our study will spur others to have additional lines profiled so that the community as a whole can be assured that we are all performing experiments with cells that are actually what we think they are.

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## Tables

**Table 1. Passage number of the various cells tested and laboratories that independently submitted samples for analysis**

| <b>Cell Line</b> | <b>Original Reference</b> | <b>Lab 1</b> | <b>Lab 2</b>                 | <b>Lab 3</b>         | <b>Lab 4</b> |
|------------------|---------------------------|--------------|------------------------------|----------------------|--------------|
| <b>SV40-HCEC</b> | 18                        | p15, p18     |                              |                      |              |
| <b>hTCEpi</b>    | 19                        | p37, p41     | p64                          | p67                  |              |
| <b>HCLE</b>      | 20                        |              |                              | p33, p43             |              |
| <b>HCjE</b>      | 20                        | p44, p48     | p32                          | p22, p23<br>p25, p35 |              |
| <b>HC0597</b>    | 21                        |              | p19                          |                      |              |
| <b>HMGEC</b>     | 22                        | p18, p24     | p4, p16,<br>p33, p53,<br>p54 |                      | p18, p24     |

Examples for reading the table: For SV40-HCEC one lab submitted two passages (p) for testing. For hTCEpi three labs submitted one or two passages for testing.

**Table 2 STR Profiles for the Human Ocular Surface Cell Lines Tested**

| <b>Loci</b>       | <b>SV40-<br/>HCEC</b> | <b>hTCEpi</b> | <b>HCLE</b> | <b>HCjE</b> | <b>HC0597</b> | <b>HMGEC</b> |
|-------------------|-----------------------|---------------|-------------|-------------|---------------|--------------|
| <b>D3S1358</b>    | 15, 17                | 17            | 15, 16      | 16, 18*     | 16, 17        | 15, 17       |
| <b>TH01</b>       | 7, 8                  | 7             | 6, 9        | 9           | 6, 9          | 6, 9.3       |
| <b>D21S11</b>     | 27, 30                | 32.2, 33.2    | 31          | 31, 34.2    | 28, 32.3      | 29, 30.2     |
| <b>D18S51</b>     | 13                    | 12, 15        | 14, 16      | 15          | 23, 24        | 12, 16       |
| <b>Penta E</b>    | 15, 16+               | 12, 15        | 7           | 12          | 12            | 5, 14        |
| <b>D5S818</b>     | 9, 13                 | 11            | 11, 12      | 11, 13**    | 12, 13        | 10, 13       |
| <b>D13S317</b>    | 8                     | 11, 12        | 10, 12      | 11, 12      | 11, 12        | 9, 14        |
| <b>D7S820</b>     | 10, 13+++             | 11, 12        | 10, 11      | 10, 12      | 10, 11        | 8, 11        |
| <b>D16S539</b>    | 9, 11                 | 9, 11         | 11, 13      | 12          | 10            | 8, 11        |
| <b>CSF1PO</b>     | 12+++                 | 11            | 12, 13      | 11          | na, na        | 10, 12       |
| <b>Penta D</b>    | 12, 15                | 12, 14        | 11, 16      | 9, 10       | 5, 9          | 8, 12        |
| <b>Amelogenin</b> | X                     | X, Y          | X, Y        | X, ***      | X             | X, Y         |
| <b>vWA</b>        | 18, 19                | 14, 17        | 16, 17      | 14          | 16, 19        | 16, 17       |
| <b>D8S1179</b>    | 11, 13                | 12, 13        | 11, 13      | 11, 12      | 13, 16        | 13, 15       |
| <b>TPOX</b>       | 8, 11                 | 8, 10         | 8           | 8, 11       | 8             | 8, 9         |
| <b>FGA</b>        | 20                    | 17, 15        | 19, 24      | 20, 24      | 22            | 23, 24       |

STR profiles were determined for three human corneal epithelial cell lines (SV40-HCEC, hTCEpi and HCLE, n=2, 4, 2 passages respectively); for two conjunctival epithelial lines

(HCjE and HC0597, n=7 and 1 passages respectively) and one meibomian gland cell line (HMGEc, n=7 passages, two labs coincidentally submitted the same passage numbers for testing, thus actually 9 samples were tested). For loci where there is only one number, only one peak was observed. + In one passage (p18) only one peak (at 16) was observed at this locus; ++ in one passage (p15) only one peak (at 10) was observed at this locus; +++ in one passage (p15) the result at this locus was 9,12. \*In one passage (p44) only one peak (at 16) was observed at this locus; \*\*in one passage (p35) only one peak (at 11) was observed at this locus. na=no amplification. \*\*\*

Cytogenetic examination of HCjE cells by the Center for Human Genetics at Boston University School of Medicine revealed that transformed cells from the male donor were monosomy X (data not shown).