TECHNICAL NOTE

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Recovery of DNA for Forensic Analysis from Lip Cosmetics*

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ABSTRACT: To obtain a reference DNA profile from a missing person, we analyzed a variety of personal effects, including two lip cosmetics, both of which gave full DNA profiles. Further investigations were undertaken to explore this previously unreported source of DNA. We have tested a range of brands and types of lip cosmetics. Our studies have revealed that lip cosmetics are an excellent source of DNA, with almost 80% of samples giving a result. However, artifacts are frequently observed in the DNA profiles when Chelex is used for the DNA extraction and additional DNA purification procedures are required to ensure that an accurate DNA profile is obtained.

KEYWORDS: forensic science, DNA extraction, polymerase chain reaction, DNA typing, Profiler Plus, human identification, short tandem repeat, D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, amelogenin, cosmetic, lipstick.

In missing persons cases, it is important to quickly obtain a reference DNA profile from the missing individual, so that DNA profiling comparisons may be made with any forensic specimens subsequently recovered. A variety of objects may be used for this purpose, including toothbrushes (1), fingernail clippings (2) and formalin fixed paraffin embedded tissues (3).

To obtain a reference DNA profile from a young woman who had gone missing, a variety of her personal effects were obtained, including two lipsticks. A full DNA profile was successfully obtained from both lipsticks, although several fluorescent artifacts were detected in the profiles. The girl's DNA profile was subsequently confirmed from other personal effects and also from the DNA profiles of the biological parents.

These results prompted us to further examine the utility of various lip cosmetics for obtaining a DNA profile in forensic examinations and to also investigate the nature of the anomalous fluorescent peaks and artifacts observed, as well as mechanisms by which the artifacts could be removed.

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Materials and Methods

All analytical methods, consumables and reagents employed in this study were identical to those routinely used for forensic casework.

Sample Collection

Thirty-eight lip cosmetics were obtained from eleven staff, relatives and friends. Each item was considered to be "in use" by the owner. In total, 25 different brands were tested, which included a wide range of colors and types, including balms, colors, glosses, glazes, a pencil and sunscreens. Each was gently sampled on and around the edge of the used surface with a sterile cotton swab.

DNA Extraction and Purification

DNA was extracted from the swabs with Chelex (BioRad, USA) (4) and quantitated using the QuantiBlot kit (Applied Biosystems) using the manufacturers recommendations. All extracts were stored at −20°C until required for profiling. After all of the samples were profiled, selected samples were further purified using the QIAquick PCR Purification kit (Qiagen GmbH, Hilden, Germany), as per the manufacturer's instructions.

DNA Profiling

DNA profiling was performed with the AmpFlSTR® Profiler Plus™ PCR Amplification Kit (Applied Biosystems) system and in accordance with the manufacturers recommendations, with the exception that the reaction volume was reduced to 25 μl. The kit analyzes nine short tandem repeat (STR) loci and a locus of the Amelogenin gene. The positive control DNA sample (included with the Profiler Plus kit) is AmpFlSTR Control DNA 9947A. Water was employed as a negative control for PCR. All amplifications were performed using the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) and the amplified product analyzed with an ABI 310 Capillary Electrophoresis Genetic Analyser. The profiles obtained from each lipstick were read without prior knowledge of the donors profile.

A minimum peak height of 50 RFU (relative fluorescent units) was required to interpret a heterozygous peak and 150 RFU was the cut-off for a homozygous peak. No maximum RFU value was applied. A sample was considered to display artifacts if abnormally shaped or abnormally placed peaks were detected, and that these

peaks were of sufficient height that they had the potential to mask real alleles.

Blood Doping Experiments

A lipstick was swabbed in the manner described above with three sterile cotton swabs. These three swabs were then doped with either 1, 3 or 5 μl of freshly collected whole human blood (EDTA tube). An additional three blank swabs were also doped with 1, 3 or 5 μl of the same blood. All six swabs were then submitted for DNA extraction and quantitation as described above.

Light Microscopy

A lip balm and a lipstick were smeared directly onto clean microscope slides and immediately stained with 0.01% Crystal violet solution for approximately 5 min. After being rinsed with distilled water and dried, a cover slip was placed on the slide. Photomicrographs were taken with a Nikon CoolPix 950 Digital Camera mounted on a Nikon Eclipse E600 microscope using a 10× objective and a 100× ocular lens.

Results and Discussion

Lip cosmetics are complicated substances that may be comprised of a wide variety of compounds. The bulk of the product is usually naturally occurring oils and/or waxes, which may account for up to 90% of the total weight. The color of lipstick is obtained by the use of one or more organic dyes, inorganic pigments such as iron oxide, fillers such as titanium dioxide (5) or pearling agents. They may also contain other compounds designed to impart gloss, to improve "wearability", to moisturize, to provide fragrance or to provide UV protection (5,6).

The fact that lipsticks fluoresce has been previously documented and forms the basis of a method for forensic characterization of lipsticks. Ehara and Marumo (7) describe a nondestructive analysis method that involved the detection of fluorescence of lipsticks illuminated with light at wavelengths of 350, 445 and 515 nm. The ABI Prism 310 Genetic Analyser operates at similar wavelengths, with the 10 milliwatt argon ion laser exciting dyes at 488 and 514 nm (8).

DNA Extraction and Purification

After Chelex extraction, many of the DNA extracts were distinctly pigmented. Following further purification with the QIAquick kit, these samples were colorless. Results of further experiments are detailed below.

DNA Profiling

A summary of the results of the DNA profiling is provided in Table 1. A full DNA profile (nine STR loci plus Amelogenin) was obtained in 17/38 (44.8%) of lip cosmetics tested. Only 8/38 (21%) failed to give a result. A mixed DNA profile was obtained from 6/38 (15.8%) cosmetics tested (data not shown). In four cases, the contaminating profile came from a male, suggesting that contamination arising from the sharing of the lipstick is unlikely to be the cause in each case. It is possible that male cellular material was deposited onto the lip cosmetic following kissing. In three-quarters of the cases where a Y chromosome was detected, the Y chromosome was the only additional allele detected in the profile and was generally very small in comparison to the DNA profile of the owner of the cosmetic, being <15% of the height of the X chromosome peak. There was no Y chromosome peak detected in the remaining two mixtures and the contamination may have arisen from sharing of the cosmetics between females. When alleles from a second person were detected at various STR loci, the contaminating alleles were identified as minor peaks in comparison to the alleles contributed by the owner, and were only detected as partial profiles (the contaminating alleles were not observed at all STR loci).

TABLE 1—*DNA profiles from lip cosmetics.*

Loci	Occurrence	Percentage
10	17	44.8%
6–9	8	21.0%
1–5	5	13.2%
0	8	21.0%
Total	38	100%

Blood Doping Experiments

The results of the doping experiment are presented in Fig. 1. While the DNA recovered from the 1, 3 or 5 μl of blood was easily detectable in the swabs without lipstick, quantifiable levels of DNA could not be detected in any of the swabs that also contained lipstick. The inability to quantitate the DNA recovered from the lipstick samples is not related to the amount of sample used, because the DNA recovered from swabs without lipstick was readily detected. From these results, it is clear that some substance within lip cosmetics, possibly the wax or oil component, inhibits the ability to quantitate the DNA, even in the presence of a large quantity of DNA. Proof that the ability to quantitate the DNA was being inhibited was obtained when it was observed that these same samples could be successfully quantitated following further purification with the QIAquick PCR purification kit (data not shown).

Fluorescent Artifacts

A wide variety of anomalous fluorescent peaks were detected in the DNA profiles, indicating that components of the lips cosmetics

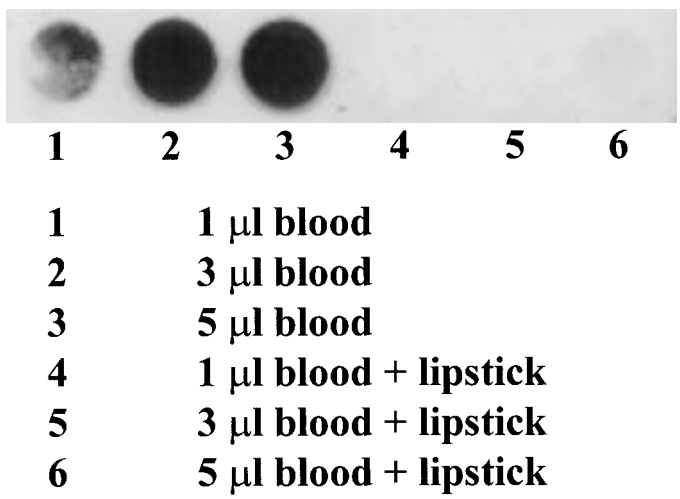

FIG. 1—*DNA quantitation of blood from swabs with or without lipstick. It can be seen that the ability to quantitate the DNA recovered from the swabs that were also in contact with a lipstick has been significantly compromised.*

are carried through the Chelex DNA extraction and amplification stages. This could be observed directly, as a number of the DNA extracts were seen to have a distinct pink or orange hue. However, while many of the extracts were pigmented, not all such extracts resulted in fluorescent artefacts occurring in the DNA profile, and artifacts in the DNA profiles were also observed from extracts that appeared colorless. The Blue and Green wavelengths were the most commonly affected, but artifacts were also observed in the Yellow region. The apparent molecular weights of the most common artifact were in the 140 to 160 bp range (Fig. 2), but artifacts were also observed below 90 bp and up to 305 bp. The occurrence of the fluorescent artifacts within the DNA profiles was reproducible following re-amplification and re-electrophoresis. The fluorescent artifacts were often many times larger than the allele peaks (Fig. 3) and had the potential to mask real alleles, especially at the D3S1358, vWA, D8S1179 and D5S818 loci. The Genotyper software did interpret some of the artefact peaks as alleles. Most of the artifacts were recorded as a series of "OL" (off ladder) alleles, with each data point above 50 RFU being called an OL allele. The large artifacts were recorded as alleles amongst multiple OL-alleles.

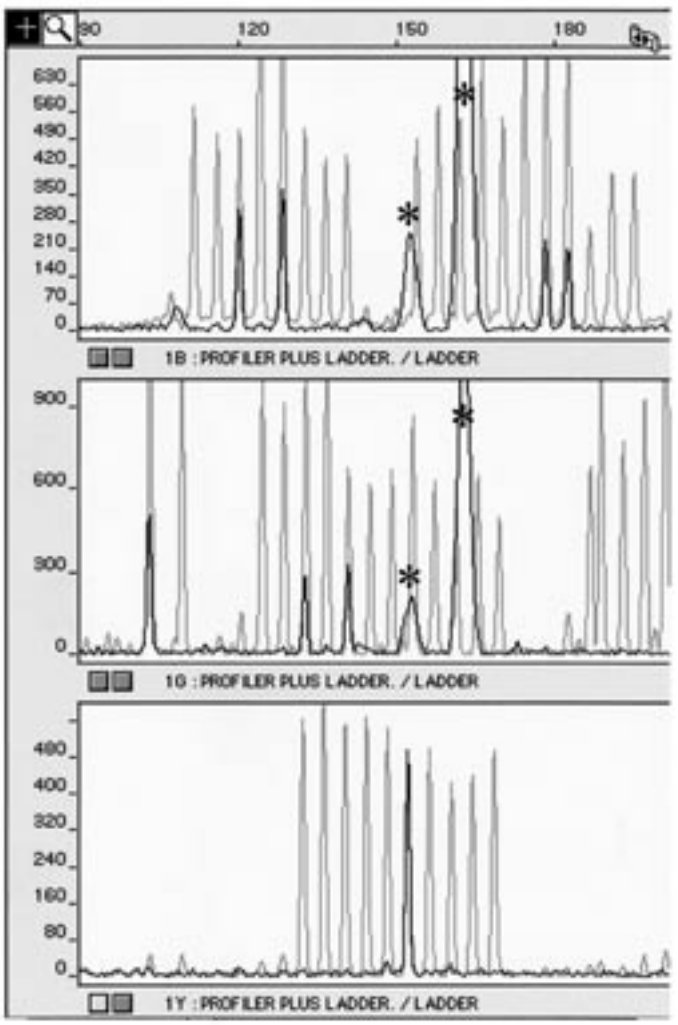

FIG. 2—*Most commonly observed artifacts. These artifacts are visible at an apparent size of 153 bp and 163.5 bp and are marked (*). The artifacts are visible in the top (blue) and middle (green) wavelengths, but not in the bottom (yellow) wavelength. In this case, the DNA profile of this individual may still be determined, although it may be seen that these artifacts have the potential to mask real alleles at the vWA and D8S1179 loci.*

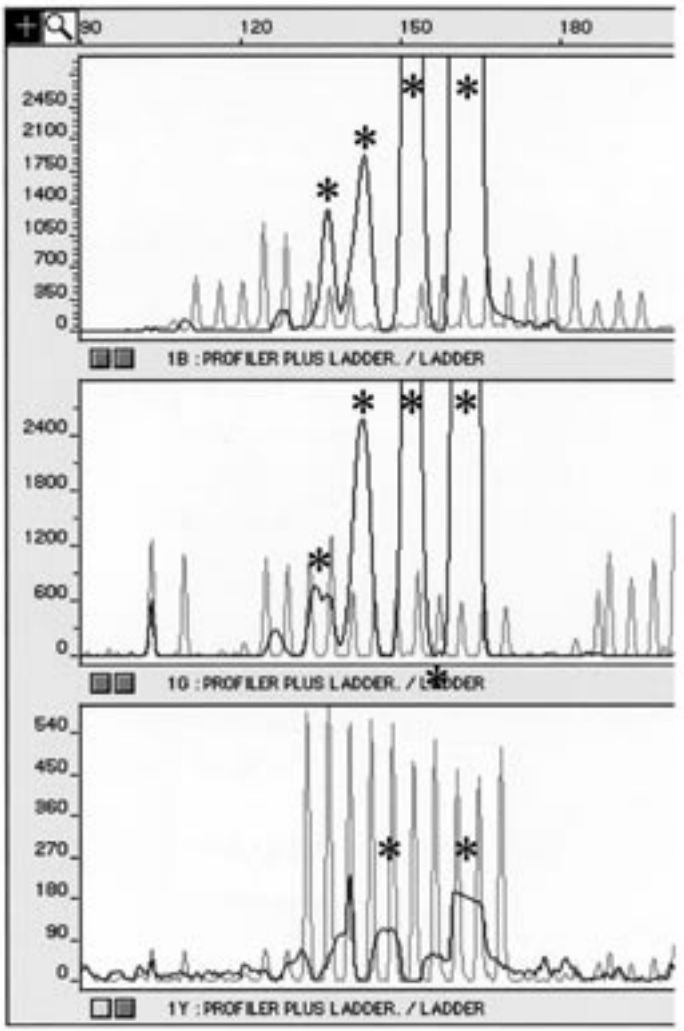

FIG. 3—*Very large fluorescent artifacts (*). In this example, the artifacts are present in all three wavelengths and are so large that they are off scale. Each has the potential to mask at least one real allele.*

Other Artifacts

Even when there were no obvious fluorescent artifacts present, there were a large number of anomalies frequently detected in the DNA profiles. Excessive allelic imbalance was commonly observed (Fig. 4). In many cases, the allelic imbalance was greater than 60%. This degree of imbalance is not observed under normal circumstances, and if present in a normal reference sample would result in a "not reportable" result being recorded at that locus. In one instance we observed total loss of a D5S818 allele, resulting in a known 10/12 heterozygote being mistyped as a 10/10 homozygote (Fig. 5). The occurrence of allelic imbalance and allelic dropout within a DNA profile was not reproducible following re-amplification of the same sample.

There was no obvious patterns to the artifacts. The most common fluorescent artifacts were detected in a range of brands, colors and types of lip cosmetic. Where several lip cosmetics from the same brand were tested (for example we tested five Revlon lipsticks), two showed fluorescent artifacts distinct from each other and the remaining three showed no artifact. The presence of artifacts of any type did not correlate with the ability

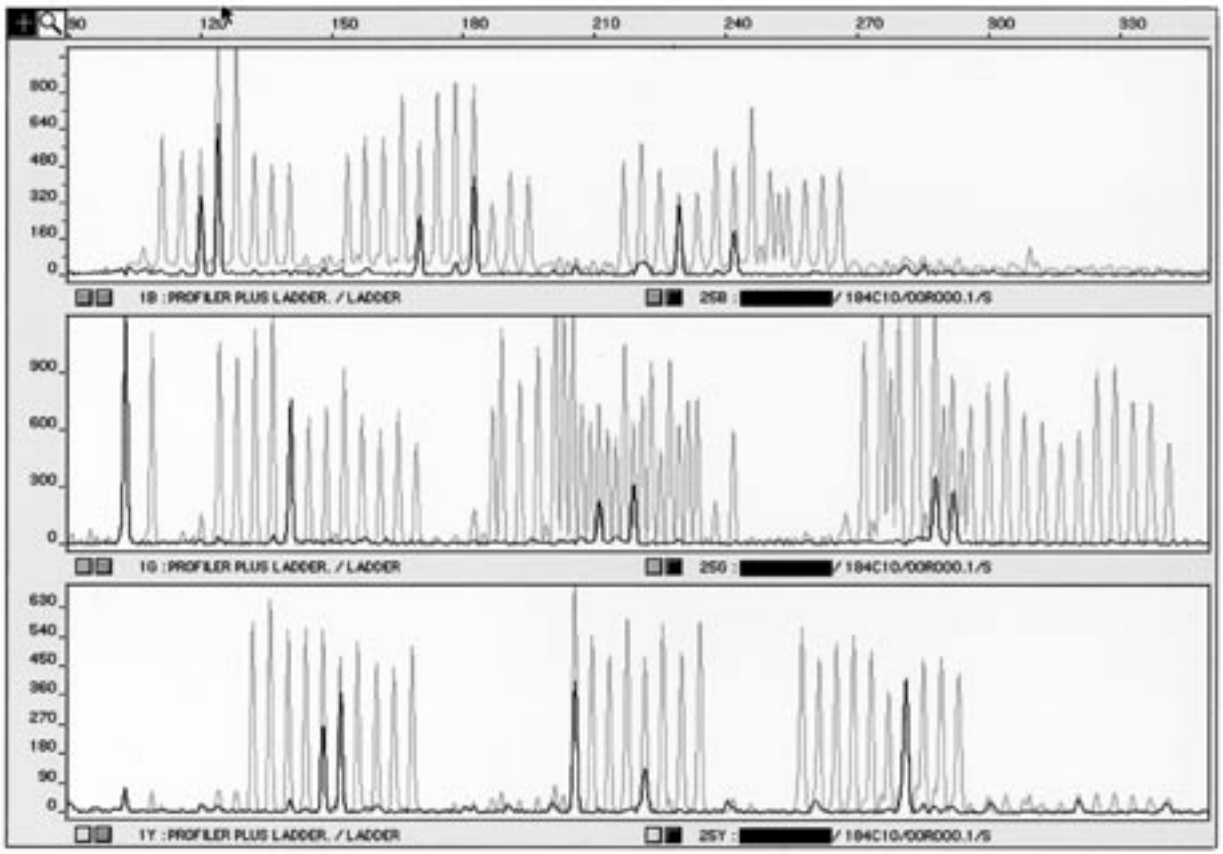

FIG. 4—*Excessive allelic imbalance. In this example, the individual DNA profile is readily visible, and no significant artifacts are visible. However, allelic imbalance is present at almost every locus (especially the D3S1358 and D13S317 loci). A DNA profile from a buccal swab showing this degree of allelic imbalance would not be acceptable.*

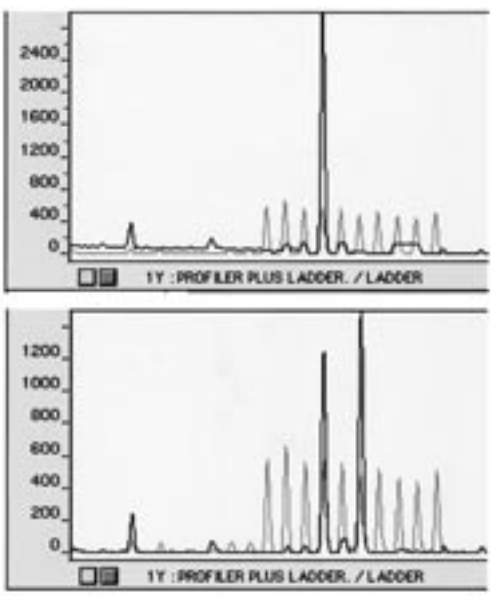

FIG. 5—*Total allelic loss. These are the results obtained at the D5S818 locus for DNA extracted from two different lipsticks used by the same person. As may be seen, the sample in the upper panel appears as a 10/10 homozygote, while alleles 10 and 12 are visible in the lower panel. Subsequent profiling of that individual with DNA extracted from a conventional buccal swab confirmed that the individual is indeed a 10/12 heterozygote at the D5S818 locus.*

to obtain a DNA profile. There was also no apparent correlation between:

- the presence or type of artifact and the lipstick color.
- the presence or type of artifact and the lipstick brand.
- the success rate in obtaining a DNA profile and the lipstick brand.
- the success rate in obtaining a DNA profile and the lipstick color.
- the success rate in obtaining a DNA profile and the lipstick owner.

At this time we have not been able to attribute the artifacts to any particular component of the lip cosmetics. As the number and type (if any) of fluorescent artifacts varied significantly between lip cosmetics, this suggests that the substances responsible for the fluorescent artifacts are not the base ingredients common to most lip cosmetics, but are more likely to be caused by the compounds that impart the color, consistency or specific function (e.g., sunscreen), as these are the components that will vary significantly between products. A detailed list of all lip cosmetics sampled, the profiling result obtained and the occurrence or absence of artifacts is presented in Table 2.

Effect of Purification

Following purification with the QIAquick kit, extracts could be successfully quantitated with the QuantiBlot system and the fluo-

TABLE 2—*Brand of lip cosmetic sorted according to DNA profiling result (number of loci) and presence of artifacts.**†

No. Loci	Artifacts	No Artifacts
10	Revlon Lipstick (A) (163) Clinique Lipstick (A) (163) Nutrimetics Lipstick (C) (152, 164) Coral Colours Lipstick (D) (163) Estee Lauder Lipstick (E) (<90, 143, 153, 164) Poppy Industries Lipstick (F) (162) Face New York Lipstick (F) (162)	Christian Dior Parfums Lipstick (B) Clinique Lipstick (E) Revlon All-Over Pencil (F) Cover Girl Lip Gloss (F) Natio Lip Colour (F) MAC Lipstick (F) Coral Colours Lipstick (G) Australian Cancer Society (Sunscreen) (H) Avon Lipstick (J) Chapstick Lip Balm (K)
9	Superdrug Lip Balm (G) (213) Avon Lipstick (H) (162) Revlon Lipstick (I) (181)	Estee Lauder Lipstick (C) Nutrimetics Lipstick (D) Suiseido Lip Gloss (F)
8	…‡	…
7	…	…
6	…	Nutrimetics Lipstick (C) Lipsmacker Lip Balm (G)
5	…	…
4	…	…
3	…	…
2	Clinique Lipstick (J) (180)	Banana Boat Lip Balm Sunscreen (I)
1	Classics by Tania Lipstick (D) (163) Estee Lauder Lipstick (F) (148) Maybelline Lipstick (H) (136, 143, 153, 165)	Chanel Extrait De Rouge (B)
0	Revlon Lipstick (E) (<90) Estee Lauder Lipstick (E) (163) Nectar Lip Balm (I) (140)	Revlon Colourstay Lipstick (A) Fiorucci Lip Balm (D) L'Oreal Rouge Pulp Liquid Lipcolour (F) Leichner Lip Glaze (J)
No.	17	21

* The letter in parentheses after each lip cosmetic identifies the owner (A–K).
† The numbers in parentheses after each item identifies the apparent size (bp) of the artifacts seen.
‡… indicates none seen for that category.

rescent artifacts were seen to disappear. These results clearly indicate that Chelex is not the optimal method for DNA extraction when dealing with samples that may be contaminated with lip cosmetics and that additional purification is required. The suitability of Qiagen columns for the isolation of DNA from forensic specimens has already been reported (9). In some instances, a DNA profile could be obtained that had not been previously detectable. The presence of allelic imbalance was seen to be reduced, but did not always disappear.

There were no differences observed between the lip balms and the lipsticks, either in the presence or absence of artifacts or the ability to obtain a DNA profile. Of the six lip balms tested, useful DNA profiles (six or more loci) were obtained from three and a poor result (two or fewer loci) detected in the remaining three. Interestingly, fluorescent artifacts were observed in two of the lip balms ("Superdrug" and "Nectar"), which have little or no color component in comparison to the lipsticks, suggesting that the fluorescent artifacts may not necessarily be related to the presence of the pigment compounds.

As was observed for the lipsticks, the ability to obtain a DNA profile from the lip balms was independent of the presence of the fluorescent artifacts. Of the two lip balms that showed fluorescent artifacts, no result was obtained from the "Nectar" lip balm while a nine locus result was obtained from the "Superdrug" lip balm. Further, no profile or artifacts were recovered from the "Fiorucci" Lip Balm, while a full profile without artifacts was obtained when the "Chapstick" Lip Balm was examined.

Light Microscopy

Abundant cellular debris was visible in smears made from both the lip balm and the lipstick. The majority of the epithelial cells were devoid of nuclear material although occasional nucleated cells were visible (Fig. 6) and are the most likely source of DNA available for profiling.

In conclusion, we have found that lip cosmetics represent an excellent source of DNA for use as a reference sample in missing or unidentified persons cases. However, fluorescent artifacts that have

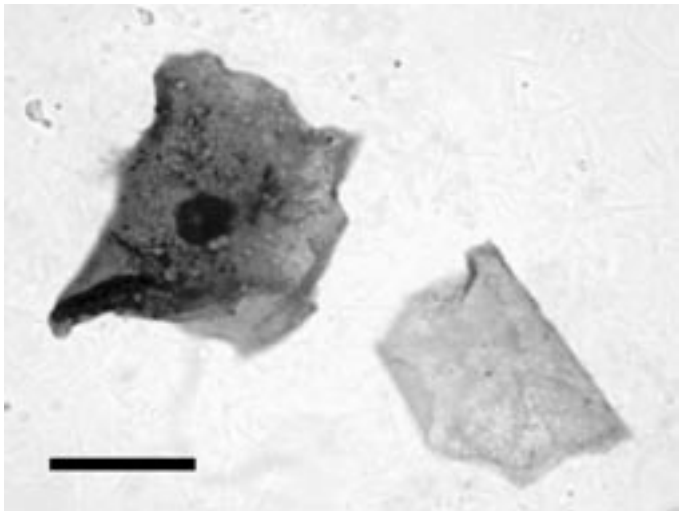

FIG. 6—*Light microscopic image of cells recovered from a lip cosmetic. Both a nucleated (left) and non-nucleated (right) cell are visible. Bar equals 10 μm.*

the potential to mask real alleles were seen in almost 50% of the samples when Chelex extraction was employed. Additional purification was required to ensure that they could be quantitated and amplified and the DNA profile interpreted reliably. There was also a high rate of nonreproducible allelic imbalance seen in the profiles generated, even when fluorescent artifacts were not observed.

Therefore, we suggest that extreme caution should always be exercised in the interpretation of any DNA profiles where lip cosmetics may be present, particularly if Chelex extraction methods are used. Care should also be taken when obtaining buccal swabs to ensure that lipstick does not contaminate the sample. As some lip cosmetic preparations are colorless (e.g., lip balms), care should be taken even if it does not appear that the individual is wearing any lipstick, or if the person being sampled is male. The presence of fluorescent artifacts in a DNA profile obtained from a buccal swab may be indicative of contamination by lip cosmetics and alternative DNA extraction methods may be required to alleviate the observed artifacts and obtain a reliable result, presumably by removing the substance(s) that cause inhibition and the fluorescent artifacts.

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