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Original scientific paper

MESSENGER RNA BASED SKIN IDENTIFICATION USING SKIN SPECIFIC MARKERS FROM FINGERPRINT IMPRESSIONS

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ABSTRACT. The study of forensic science may be complex especially in the areas of nucleic acids and trace evidence. Oftentimes, forensic scientists recover minute quantities of biological material from scenes therefore the need to generate the genetic profiles and identify the source of the material. The aim of this study is to determine the expression levels of skin-specific gene markers – *Lor*icrin (*LOR*), Corneodesmosin (*CDSN)* and Keratine 9 (*KRT9)*, on fingerprint impressions of individuals of specific age group and genders. Thumbprints were collected using labelled frosted microscope glass slides and cello tapes. Messenger RNA was extracted from the samples, converted to cDNA and amplified by qPCR with its specific primer sequences. In males, RNA yield was higher in the slide (26.96±8.68) compared to cello tape (18.32±4.52) while the reverse was the case in the females. Across gender, RNA purity and yield were higher in males than females. In males, *KRT9, LOR* and *CDSN* genes were more expressed using frosted slide (37.03±0.77, 40.46±2.66, 35.62±2.82) compared to cello tapes (35.33±0.3, 32.11±0.5, 35.28±0.86) respectively. In females, *LOR, CDSN* and *ACTB* genes were more expressed using frosted slides (36.26±0.8, 37.37±0.58, 26.63±0.12) compared to cello tapes (35.52±1.01, 35.57±3.22, 26.57±1.18) respectively. Across gender, *LOR, KRT9* and *ACTB* genes were more expressed in males than females while *CDSN* was more expressed in females than males. The expression levels of *CDSN* and *ACTB* genes were significantly ($p \leq 0.01$) correlated. This study shows that mRNA markers *LOR, CDSN* and *KRT9*, analysed via the RT-qPCR assays, are highly suitable for identifying skin cells even in small traces.

Keywords: skin-specific gene markers, *Lor*icrin, Corneodesmosin, Keratin 9, β-Actin, cDNA

INTRODUCTION

There is a strong trend in molecular forensics for the development of alternative techniques to identifying the cellular origin of biological samples and trace evidence collected at crime scenes (HALL *et al*., 2013). Information about the possible origin of biological samples of forensic traces is mostly ascertained via protein-based presumptive testing and DNA analysis. Messenger RNA-profiling has however emerged as an alternative strategy to

determine the biological origin of biological materials (ZUBAKOV *et al*., 2008). Additionally, in order for forensic scientists to overcome the limitations faced currently using for example classical body fluid identification approaches, the use of messenger RNA (mRNA) profiling has been suggested to supplant conventional methods for body fluid identification (JUUSOLA and BALLANTYNE, 2003). This ensures the production of unique gene expression patterns for each cell type which is evidenced by the presence and abundance of specific mRNAs (ALBERTS *et al*., 1994). Messenger RNA detection is a reliable method for identification of the most common biological samples taken from forensic cases (HAAS, *et al*., 2009; VISSER *et al*., 2011; KOHLMEIER and SCHNEIDER 2012). The variation within and between individual skin-specific mRNA as has been observed in some previously described cases, which complicate the determination of the presence of skin using certain markers, has thus pointed to the need for further investigation (HALL *et al*., 2013).

Skin is an additional forensically relevant cell type and three mRNA transcripts (*LOR*, *CDSN* and *KRT9*) had been reported to show high expression in skin samples relative to other forensically important ant cell types (VISSER *et al*., 2011). *LOR* encodes loricrin which is a component of the cornified cell envelope located in terminally differentiated epidermal cells. *CDSN* encodes corneodesmosin (HAFTEK *et al*., 2008) and it is involved in desquamation, the process which triggers shedding of the outer membrane layer of the skin (JACKSON *et al*., 1993). *KRT9* is a member of the superfamily of intermediate filament proteins expressed in all different epithelial cell types. *KRT9* is expressed only in the suprabasal cells of the epidermis and has previously been reported to be specifically expressed in palmar and plantar skin (SU *et al*., 2002). The reference gene *ACTB* (ß-actin), used here for normalising expression signals of skin-targeted mRNA markers is a major constituent of the contractile apparatus and one of the two non-muscle cytoskeletal actins. Actins are highly conserved proteins and are involved in ensuring cell motility, structure and integrity (VISSER *et al*., 2011). *LOR, CDSN* and *KRT9* are the markers used in the study and were selected due to their specificity and sensitivity in skin samplings. A positive control *ACTB* was used and it has been previously described and used as an endogenous control (LINDENBERGH *et al*., 2012). The aim of this study was to evaluate the expression levels of skin candidate genes, *LOR*, *CDSN* and *KRT9* on fingerprint impressions individuals of specific age groups and genders.

MATERIALS AND METHODS

Sampling

Thumbprint impressions were collected from twenty healthy individuals (10 males and 10 females) whose ages ranged from 17- 22 years and were grouped into four groups. The left and right thumbprints of 5 males and 5 females were taken on frosted slides while the left and right thumbprints of 5 males and 5 females were taken on cut rectangular transparent cello tape.

The subjects washed their hands with clean water without soap. Thereafter their hands were wiped clean using rubbing alcohol and then allowed to air dry for 30 minutes without contact with anything or anyone. Their left and right thumbprints were then taken with the use of an already labelled and sterilized frosted slides or cut pieces cello tape. The samples were immediately taken to the laboratory for analysis.

Materials used for this study

The materials used for the study were Pinpoint slide RNA isolation system 1 kit (Zymo Research®), LunaScript® RT SuperMix Kit (NEB #E3010), Luna® Universal qPCR Master Mix Kit (NEB #M3003), ASI Supreme™ Frosted Glass Microscope Slides (SM2576) and Scotch® transparent Cello tape.

Ribonucleic acid (RNA) extraction

Ribonucleic acid (RNA) was extracted from the samples collected using a Pinpoint slide RNA isolation system 1 kits (Zymo research®) following the manufacturer's instructions. The Pinpoint slide RNA isolation system 1 kit procedure involved two steps which were Pinpoint fractionation to recover fingerprint samples from glass slides and cut cello tapes and RNA extraction for total RNA recovery.

Spectrophotometric analysis

Ribonucleic Acid (RNA) quality and quantity were determined spectrophotometrically by analysing absorption ratios: A260/230 and A260/280 using a Nanodrop 1000 spectrophotometer (6305 JENWAY spectrometer).

Complementary DNA (cDNA) synthesis

Complementary DNA (cDNA) synthesis was carried out using the LunaScript RT SuperMix Kit (NEB #E3010). The cDNA synthesis reaction was prepared by adding 4 µl of LunaScript RT SuperMix (1X) to 10 µl of the RNA sample and made up to 20 µl with 6 µl of nuclease-free water. The reactions will then be incubated with primer annealing for 2 minutes at 25 °C, cDNA synthesis for 10 minutes at 55 °C and heat inactivation for 1 minute at 95 °C.

Real time quantitative polymerase chain reaction (RT-QPCR)

Real time quantitative PCR (RT-qPCR) was carried using the Luna Universal qPCR Master Mix Kit (NEB #M3003). Dye-based qPCR detection was prepared using 10 µl of Luna Universal qPCR Master Mix (1X), 0.5 µl of 10 µM forward primer (0.25 µM), 0.5 µl of 10 µM reverse primer (0.25 µM), 2 µl of cDNA products and 7 µl of Nuclease-free water. Initial denaturation (1 cycle) was carried out for 60 seconds at 95 °C, Denaturation (40-45 cycles) for 15 seconds at 95 °C; Extension (40-45 cycles) for 30 seconds at 60 °C; Melt curve (1 cycle) at 60-95 °C).

Statistical analysis

Data generated were analysed using IBM SPSS Version. 26 (IBM SPSS Inc., USA). Analysis of variance (ANOVA) was used to compare group means and mean differences were separated using Duncan Multiple Range Test at 5 % level of significance. Graphs were plotted using GraphPad 8.0.1 software. The comparative Ct method ($2^{-\Delta\Delta Ct}$ method) was used to analyse the expression level of the target genes according to LIVAK and SCHMITTGEN (2001) and RAO *et al*., (2013).

Primers used for the study

The primer sequences for the target genes and reference gene were designed on Primer blast and validated on Netprimer and Oligo analyzer. The primer sequences are shown in Table 1.

Table 1: Showing the primer sequences.

Genes	Primer sequences
Beta-Actin (*ACTB*)	Forward Primer - 5'- GACCTGTACGCCAACACAGT - 3' Reverse Primer - 5' -CTCCTTCTGCATCCTGTCGG - 3'
Corneodesmosin (*CDSN*)	Forward Primer - 5' - CTCCTTCTGCATCCTGTCGG - 3' Reverse Primer - 5' - GGAACCGGATGCACCTTGTA - 3'
Keratin9 (*KRT9*)	Forward Primer - 5'- AGTTGGAGGTGTTTTCCAGGG -3' Reverse Primer - 5' - TGGGGTTGGGAGGTAGTTGT -3'
***Loricin* (*LOR*)**	Forward Primer - 5'- TGGCTTTCCATTGGCTTCCA -3' Reverse Primer - 5' - AAGATCGTGCAGGGTGTGAG -3'

Ethical clearance

Informed written consent was obtained from all the study participants, and the study protocol was approved by the Health Research ethics committee of the College of Medicine, University of Lagos (CMULHREC Number: CMUL/HREC/O668/19).

RESULTS

In Table 2, *KRT9*, *LOR* and *CDSN* genes were more expressed in samples taken on a frosted slide (37.03 ± 0.77, 40.46 ± 2.66, 35.62 ± 2.82) than cut cello tapes (35.33 ± 0.3, 32.11 ± 0.5, 35.28 ± 0.86) amongst the male participants respectively. However, the reverse was the case for the *ACTB* gene which showed that the samples taken on cut cello tapes expressed more of this gene (27.05 ± 0.11) than the samples collected on frosted slides (26.94 ± 0.67). Amongst the female participants, *LOR, CDSN* and *ACTB* genes were more expressed in samples taken on frosted slides (36.26 ± 0.8, 37.37 ± 0.58, 26.63 ± 0.12) than those taken on cut cello tapes (35.52 ± 1.01, 35.57 ± 3.22, 26.57 ± 1.18) respectively. However, the reverse was the case for the *KRT9* gene which showed that the samples taken on cut cello tapes expressed more of this gene (35.4 ± 2.2) than the samples collected on frosted slides (34.76 ± 1.24). Across gender, *LOR, KRT9* and *ACTB* genes were more expressed in males than females while *CDSN* was more expressed in females than males.

Table 2. Descriptive statistics analysis output of the Cq values generated from RT-qPCR.

Candidate gene (cq)	Groups	Mean ± SEM	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
KRT9	**MS**	37.03 ± 0.77	34.8811	39.1684	34.65	38.71
	MT	35.33 ± 0.3	34.5047	36.1639	34.29	36.04
	FS	34.76 ± 1.24	31.3108	38.2178	30.82	38.60
	FT	35.4 ± 2.2	29.3026	41.5054	28.89	42.07
LOR	**MS**	40.46 ± 2.66	33.0742	47.8498	33.99	48.00
	MT	32.11 ± 0.5	30.7189	33.5091	31.02	33.92
	FS	36.26 ± 0.8	34.0396	38.4764	34.92	38.73
	FT	35.52 ± 1.01	32.7301	38.3139	32.25	38.50
CDSN	**MS**	35.62 ± 2.82	27.7842	43.4478	28.74	45.74
	MT	35.28 ± 0.86	32.8871	37.6809	32.50	37.95
	FS	37.37 ± 0.58	35.7654	38.9666	35.62	39.01
	FT	35.57 ± 3.22	26.6254	44.5066	26.96	44.96
ACTB	**MS**	26.94 ± 0.67	25.0915	28.7854	24.48	28.22
	MT	27.05 ± 0.11	26.7435	27.3640	26.76	27.34
	FS	26.63 ± 0.12	26.2826	26.9709	26.21	26.84
	FT	26.57 ± 1.18	23.2819	29.8504	22.05	28.73

MS = Male slide, FS = Female slide, MT = Male tape, FT = Female tape.

At 260/280 absorbance ratio (Table 3), samples which were taken on cut cello tapes yielded purer (1.96 ± 0.24) RNA than those collected on frosted slides (2.07 ± 0.21) in male while slide samples yielded purer (1.73 ± 0.02) RNA than cello tape samples (1.68 ± 0.13) in females. Also, RNA yield was higher in male slide samples (26.96 ± 8.68) than cello tape samples (18.32 ± 4.52) and the reverse was the case in the females. Across gender, RNA purity and yield were higher in males than females.

Table 3. Descriptive statistics analysis output of RNA spectrophotometric values.

	Groups	Mean ± SEM	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
Purity (260/280)	**MS**	2.07 ± 0.21	1.5	2.64	1.55	2.55
	MT	1.96 ± 0.24	1.31	2.62	1.46	2.78
	FS	1.73 ± 0.02	1.68	1.78	1.69	1.79
	FT	1.68 ± 0.13	1.31	2.06	1.48	2.21
Purity (260/230)	**MS**	0.26 ± 0.14	-0.13	0.64	0.03	0.76
	MT	0.12 ± 0.07	-0.08	0.32	0.03	0.40
	FS	0.19 ± 0.09	-0.05	0.43	0.03	0.40
	FT	0.19 ± 0.1	-0.09	0.46	0.02	0.43
Concentration (ng/µl)	**MS**	26.96± 8.68	2.86	51.07	7.2	55.1
	MT	18.32± 4.52	5.78	30.86	8.1	30.8
	FS	16.06± 2.34	9.57	22.56	8.1	20.8
	FT	18.66 ± 5.8	2.56	34.76	6.8	33.1

MS = Male slide, FS = Female slide, MT = Male tape, FT = Female tape.

Table 4 shows that there was no significant difference in Cq values of *KRT 9, CDSN* and the *ACTB* gene across gender and type of surfaces used. There was a significant difference ($p \leq 0.05$) between the expression of the *LOR* gene in samples collected on slides and cello tapes among the males. Also, there was a significant difference ($p \leq 0.05$) between the expression of the *LOR* gene in female samples collected cello tapes and male samples taken on slides.

Table 5 shows that there was no significant difference in spectrophotometric values of all extracted RNA samples at 260/280 and 260/230 absorbance ratio and in the concentration values across gender and type of surfaces used.

In Table 6, Pearson correlation analysis showed that there was a correlation between the expression levels of *CDSN* and *ACTB* genes at 0.01 level of significance. However, the *ACTB* expression level did not correlate with the expression levels of *KRT9* and *LOR* genes ($p>0.05$). Also, RNA Purity (260/230) correlated with concentration at 0.01 level of significance.

In Table 7, Spearman's rho correlation analysis showed that there was a correlation between the expression levels of *CDSN* and *ACTB* genes at 0.05 level of significance. Also, RNA Purity (260/230) correlated with concentration at 0.01 level of significance.

In Table 8, Kendall's tau-b correlation analysis showed that there was a correlation between the expression levels of *CDSN* and *ACTB* genes at 0.05 level of significance. Also, RNA Purity (260/230) correlated with concentration at 0.01 level of significance.

DISCUSSION

Forensic biologists have used fingerprint impressions to generate DNA profiles over the years (VAN OORSCHOT and JONES, 1997; TEMPLETON and LINACRE, 2014; SINELNIKOV and REICH, 2017; SUBHANI, *et al*., 2018; ROMANO *et al.*, 2019) yet, there is not a definitive test to determine the presence of skin epithelial cells in trace biological materials. Obtaining DNA profiles from trace biological evidence is routinely demonstrated with forensic 'touch DNA evidence samples. Oftentimes, genetic profiles obtained cannot be linked to the tissue source of the profiles and thus is usually rarely known (BUDOWLE *et al*., 2009; GILBERT, 2010).

Table 4: Multiple comparison showing Post-hoc tests of skin mRNA marker across gender and type of surfaces used.

Gene (cq)	Sample		Significance ($p \leq 0.05$)		
	Group	**Groups**	**Tukey HSD**	**Tukey LSD**	**Dunnett T3**
KRT9	**MS**	**MT**	0.81	0.38	0.36
		FS	0.63	0.25	0.58
		FT	0.82	0.40	0.97
	MT	**MS**	0.81	0.38	0.36
		FS	0.99	0.77	1.00
		FT	1.00	0.97	1.00
	FS	**MS**	0.63	0.25	0.58
		MT	0.99	0.77	1.00
		FT	0.99	0.74	1.00
	FT	**MS**	0.82	0.40	0.97
		MT	1.00	0.97	1.00
		FS	0.99	0.74	1.00
LOR	**MS**	**MT**	0.01*	0.001*	0.14
		FS	0.24	0.07	0.61
		FT	0.13	0.03*	0.49
	MT	**MS**	0.01*	0.001*	0.14
		FS	0.25	0.07	0.02
		FT	0.40	0.13	0.11
	FS	**MS**	0.24	0.07	0.61
		MT	0.25	0.07	0.02
		FT	0.99	0.73	0.99
	FT	**MS**	0.13	0.03*	0.49
		MT	0.40	0.13	0.11
		FS	0.99	0.73	0.99
CDSN	**MS**	**MT**	1.00	0.92	1.00
		FS	0.94	0.58	0.98
		FT	1.00	0.99	1.00
	MT	**MS**	1.00	0.92	1.00
		FS	0.91	0.52	0.35
		FT	1.00	0.93	1.00
	FS	**MS**	0.94	0.58	0.98
		MT	0.91	0.52	0.35
		FT	0.94	0.57	0.98
	FT	**MS**	1.00	0.99	1.00
		MT	1.00	0.93	1.00
		FS	0.94	0.57	0.98
ACTB	**MS**	**MT**	1.00	0.91	1.00
		FS	0.99	0.75	1.00
		FT	0.98	0.71	1.00
	MT	**MS**	1.00	0.91	1.00
		FS	0.97	0.67	0.16
		FT	0.96	0.62	1.00
	FS	**MS**	0.98	0.75	1.00
		MT	0.97	0.67	0.16
		FT	1.00	0.95	1.00
	FT	**MS**	0.98	0.71	1.00
		MT	0.96	0.62	1.00
		FS	1.00	0.95	1.00

* = ($p \leq 0.05$); MS = Male slide, FS = Female slide, MT = Male tape, FT = Female tape.

Table 5: Multiple comparison showing Post-hoc tests of spectrophotometric values of all extracted RNA samples at 260/280 and 260/230 absorbance ratio and the concentration values across gender and type of surfaces used.

Parameter	Sample Group	Groups	Post Hoc test		
			Tukey HSD	Tukey LSD	Dunnett T3
Purity (260/280)	MS	MT	0.97	0.67	1.00
		FS	0.51	0.18	0.55
		FT	0.41	0.13	0.57
	MT	MS	0.97	0.67	1.00
		FS	0.76	0.34	0.87
		FT	0.66	0.26	0.87
	FS	MS	0.51	0.18	0.55
		MT	0.76	0.34	0.87
		FT	1.00	0.86	1.00
	FT	MS	0.41	0.13	0.57
		MT	0.66	0.26	0.87
		FS	1.00	0.86	1.00
Purity (260/230)	MS	MT	0.78	0.36	0.93
		FS	0.97	0.65	1.00
		FT	0.96	0.63	1.00
	MT	MS	0.78	0.36	0.93
		FS	0.96	0.63	0.98
		FT	0.97	0.65	0.99
	FS	MS	0.97	0.65	1.00
		MT	0.96	0.63	0.98
		FT	1.00	0.99	1.00
	FT	MS	0.96	0.63	1.00
		MT	0.97	0.65	0.99
		FS	1.00	0.99	1.00
Conc. (ng/µl)	MS	MT	0.72	0.31	0.92
		FS	0.56	0.20	0.77
		FT	0.75	0.33	0.95
	MT	MS	0.72	0.31	0.92
		FS	0.99	0.79	1.00
		FT	1.00	0.97	1.00
	FS	MS	0.56	0.20	0.77
		MT	0.99	0.79	1.00
		FT	0.99	0.76	1.00
	FT	MS	0.75	0.33	0.95
		MT	1.00	0.97	1.00
		FS	0.99	0.76	1.00

MS = Male slide, FS = Female slide, MT = Male tape, FT = Female tape

Table 6: Pearson correlation analysis between the expression levels of the genes, RNA purity and concentration.

Parameter	Genes (cq)				Purity		Conc.
	KRT9	***LOR***	***CDSN***	***ACTB***	**(260/280)**	**(260/230)**	**(µg/µl)**
***KRT9* (cq)**	1	-0.003	-0.13	-0.25	0.25	0.17	0.19
		0.99	0.60	0.29	0.29	0.49	0.43
***LOR* (cq)**	-0.003	1	0.26	0.13	0.10	0.001	0.09
	0.99		0.28	0.59	0.69	1.00	0.72
***CDSN* (cq)**	-0.13	0.26	1	0.69**	-0.33	-0.20	-0.24
	0.60	0.28		0.001	0.16	0.41	0.31
***ACTB* (cq)**	-0.25	0.13	0.69**	1	-0.29	-0.11	-0.03
	0.29	0.59	0.001		0.21	0.66	0.89
Purity (260/280)	0.25	0.10	-0.33	-0.29	1	-0.06	-0.09
	0.29	0.69	0.16	0.21		0.81	0.72
Purity (260/230)	0.17	0.001	-0.20	-0.11	-0.06	1	0.82**
	0.49	1.00	0.41	0.66	0.81		0.00
Conc. (µg/µl)	0.19	0.09	-0.24	-0.03	-0.09	0.82**	1
	0.43	0.72	0.31	0.89	0.72	0.00	

** = Correlation is significant at the 0.01 level.

Table 7: Nonparametric Correlations (Spearman's rho correlation) analysis between the expression levels of the genes, RNA purity (260/280 and 260/230) and concentration.

Parameter	Genes (cq)				Purity		Conc. (µg/µl)
	KRT9	***LOR***	***CDSN***	***ACTB***	**(260/280)**	**(260/230)**	
***KRT9* (cq)**	1.00	-0.04	-0.29	-0.12	0.21	0.21	0.18
		0.89	0.22	0.62	0.37	0.38	0.46
***LOR* (cq)**	-0.04	1.00	0.13	0.09	0.17	0.01	0.12
	0.89		0.60	0.72	0.47	0.97	0.62
***CDSN* (cq)**	-0.29	0.13	1.00	0.49*	-0.22	-0.05	-0.15
	0.22	0.60		0.03	0.36	0.83	0.52
***ACTB* (cq)**	-0.12	0.09	0.49*	1.00	-0.27	0.01	0.11
	0.62	0.72	0.03		0.25	0.96	0.64
Purity (260/280)	0.21	0.17	-0.22	-0.27	1.00	-0.38	-0.36
	0.37	0.47	0.36	0.25		0.10	0.12
Purity (260/230)	0.21	0.01	-0.05	0.01	-0.38	1.00	0.88**
	0.38	0.97	0.83	0.96	0.10		0.00
Conc. (µg/µl)	0.18	0.12	-0.15	0.11	-0.36	0.88**	1.00
	0.46	0.62	0.52	0.64	0.12	0.00	

***** = Correlation is significant at the 0.05 level.
****** =Correlation is significant at the 0.01 level.

Table 8: Nonparametric Correlations (Kendall's tau-b correlation) analysis between the expression levels of the genes, RNA purity (260/280 and 260/230) and concentration.

	Genes (cq)				Purity		Conc.
Parameter	***KRT9***	***LOR***	***CDSN***	***ACTB***	**(260/280)**	**(260/230)**	**(µg/µl)**
***KRT9* (cq)**	1.00	-0.01	-0.21	-0.06	0.16	0.18	0.10
		0.95	0.19	0.70	0.33	0.29	0.54
***LOR* (cq)**	-0.01	1.00	0.13	0.04	0.13	0.02	0.05
	0.95		0.44	0.80	0.44	0.90	0.77
***CDSN* (cq)**	-0.21	0.13	1.00	0.39*	-0.13	-0.02	-0.12
	0.19	0.44		0.02	0.44	0.90	0.46
***ACTB* (cq)**	-0.06	0.04	0.39*	1.00	-0.21	0.01	0.10
	0.70	0.80	0.02		0.19	0.95	0.54
Purity	0.16	0.13	-0.13	-0.21	1.00	-0.29	-0.24
(260/280)	0.33	0.44	0.44	0.19		0.09	0.14
Purity	0.18	0.02	-0.02	0.01	-0.29	1.00	0.77**
(260/230)	0.29	0.90	0.90	0.95	0.09		0.00
Conc.	0.10	0.05	-0.12	0.10	-0.24	0.77**	1.00
(µg/µl)	0.54	0.77	0.46	0.54	0.14	0.00	

***** = Correlation is significant at the 0.05 level.
****** =Correlation is significant at the 0.01 level.

The inability to link the DNA profiles to the tissue source and prove that the DNA originated specifically from skin cells can lead to challenges against DNA evidence in court. Proper identification of the biological materials present forensic samples is important to the investigation, personalization and prosecution of a criminal offense and a misrepresentation of the nature of the evidence can have undue influence on the perception of the circumstance of the crime. Currently, there are no routinely validated methodologies used for the identification of skin epithelial cells. Utilization of mRNA profiling for the identification of forensically relevant biological fluids such as blood, semen, saliva, vaginal secretions and menstrual blood has been documented (JUUSOLA, and BALLANTYNE, 2003, 2005; HAAS *et al*., 2009). Hence, the need for the development and validation of an mRNA assay that targets highly overexpressed genes in skin in relation to other cell types usually encountered in crime scenes is crucial.

From the study, RNA yield ranged from 7.2 - 55.1 ng/µl in males and 6.8 - 33.1 ng/µl in females. Also, the RNA yield was higher in males than females. This agrees with the report of HANSON *et al*., (2011), who reported that the total input RNA should range from (5 – 25 pg) to be exquisitely sensitive to detect skin traces in evidentiary items.

KRT9, ACTB and *LOR* markers were more expressed in males than females while *CDSN* was more expressed in females than males. Across gender, *KRT9, CDSN* and *LOR* markers were more expressed than *ACTB.* These three target genes *KRT9, CDSN* and *LOR* were moderately expressed in the fingerprint impressions taken on frosted slides and cut cello tapes. Hence, this makes them suitable markers for skin cell identification and this agrees with the report of VISSER *et al*., (2011), documented that *CDSN, LOR* and *KRT9* genes showed strong over-expression in skin samples relative to samples from forensic body fluids and thus make them suitable markers for skin identification. However, HALL *et al*., (2013), reported that they failed to detect *KRT9* in any of the donors' soles (or arms) and only one participant indicated the presence of this marker in their palm. Hence, they suggested that these markers must be used with caution in the identification of skin cells in forensic samples. Additionally, GOMES *et al*., (2011) documented that they encountered detection problems for the *KRT9* gene which they linked to non-stringency of the pair of primers used and because preliminary results suggest a probable lower sensitivity of detection for *KRT9* in the analysed skin tissues. *LOR*

had the highest expression level (40.46 ± 2.66) and this agrees with the findings of HALL *et al*., (2013), who reported that *LOR* was also detected most frequently in the face and palm, as well as the leg; 61 %, 57 % and 64 % respectively. GOMES *et al*., (2011), also reported that *LOR* was a more stable and sensitive mRNA marker for human skin identification thus, *CDSN* and *KRT9* should not be used alone, but concomitant with *LOR*.

The difference between the expression levels of the *LOR* gene in male and cut cello tape samples was significant ($p \leq 0.05$). Also, a significant difference was observed between the female tape and male slide samples ($p \leq 0.05$). *CDSN* expression level correlated with the expression of *ACTB* ($p \leq 0.01$) but did not correlate with *LOR* and *KRT9*. HALL *et al*., (2013), documented that *CDSN* was observed to be the most sensitive marker, with the greatest expression rates detected in swabs taken from the forehead.

CONCLUSION

Messenger RNA (mRNA) profiling has been reported to be a good method used to identify most forensic materials obtained from forensic scenes. The mRNA markers *LOR*, *CDSN* and *KRT9*, analysed via the described qPCR assays, are highly suitable for identifying skin cells, including small traces of skin materials. Although the approach for skin identification introduced here can be improved upon to be more informative when applied on its own, however, it is recommended that the inclusion of other skin-targeted mRNA markers in multiplex systems, targeting other forensically relevant cell types should be used for further studies.

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