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(54) **Title:** METHODS FOR NON-INVASIVE COLLECTION OF SKIN CELLS FOR DNA ANALYSIS

(57) **Abstract:** A method for the detection of an aberration in DNA of skin cells. The method comprises contacting a skin site with a sterile swab, swabbing the skin site with the sterile swab to collect the skin cells, extracting DNA from the skin cells, and detecting the presence of the aberration in the DNA. In an alternate embodiment, the skin cells may be collected using a fine needle.

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METHODS FOR NON-INVASIVE COLLECTION OF SKIN CELLS FOR DNA ANALYSIS

FIELD OF THE INVENTION:

[0001] The present invention relates to methods for the collection of human skin samples for use in the diagnosis or characterization of disease, aging, or exposure to ultraviolet radiation. More specifically, the present invention provides a non-invasive method for the collection of skin samples for genotyping and the assessment of DNA damage caused, for example, by UV radiation.

DESCRIPTION OF THE PRIOR ART

[0002] Current methods for the collection of skin samples for use in the diagnosis or characterization of diseases, such as skin cancer, include invasive or painful methods that can cause substantial discomfort to the individual being tested. Examples of current methods for the collection of skin samples include punch biopsy, tapelift, and surgical excision. In addition to the discomfort caused by the current methods for skin collection, these methods must also be performed by a medical practitioner in order to be safely conducted. Further, the costs associated with these invasive test methods make it difficult to rapidly genotype and assess DNA damage for large populations of individuals. It would, therefore, be advantageous for there to exist a non-invasive skin collection methodology that may be conducted easily and rapidly in a home, clinical or cosmetic setting.

[0003] *Mitochondrial deletions associated with UV exposure*

[0004] The incidence of non-melanoma skin cancer (NMSC) is increasing in populations of European origin (Severi and English, 2004). For example, one million new cases are diagnosed each year in the USA (Wesson and Silverberg, 2003) and 65,000 in the UK (figures provided by Cancer Research. UK). NMSC accounts for around 90% of skin cancers and consists of basal cell and squamous cell carcinomas (BCC and SCC, respectively). BCCs are the most common form of NMSC and arise predominantly from the basal keratinocytes of the epidermis but also from cells in hair follicles and sebaceous glands. They are locally invasive but rarely metastasize. SCCs are also derived from basal keratinocytes; however, in contrast to BCCs, SCCs may metastasize. Compared with BCC, SCC shows the greatest increase with age and is concentrated in the elderly (Severi and English, 2004). The relative density of NMSC is highest on body sites usually exposed to the sun when outdoors such as

scalp, face, neck, and ears as defined by Armstrong (2004). SCC, however, differs appreciably from BCC in having a much lower density on body sites which are occasionally exposed to the sun such as shoulders, back, and chest as defined by Armstrong (2004).

[0005] Therefore, the major determinant of NMSC is the ultraviolet (UV) radiation component of sunlight that induces DNA damage. Importantly it is both the pattern (more continuous versus intermittent) and the cumulative amount of sun exposure that influences the development of NMSC (Armstrong and Kricker, 2001). To determine a reliable marker of cumulative UV exposure in human skin, the inventors and others have examined the novel idea of using mitochondrial DNA (mtDNA), rather than nuclear DNA, as a biomarker of UV-induced DNA damage (Pang et al, 1994; Berneburg et al, 1997; Birch-Machin et al, 1998; Birch-Machin, 2000).

[0006] The use of mtDNA damage as a biomarker for cumulative sun-exposure in human skin is a relatively new field of research and previous work has simply compared mtDNA damage to distinguish between sun-protected and sun-exposed skin (Pang et al, 1994; Berneburg et al, 1997; Birch-Machin et al, 1998). This approach is limited because NMSC is predominantly formed on body sites which are "usually" exposed to the sun when outdoors as opposed to sites that are "occasionally" exposed to the sun (Armstrong, 2004).

[0007] In the present Applicant's co-pending PCT application bearing publication no.WO/06/111029 (the contents of which are incorporated herein by reference), a 3895 bp deletion in human mitochondrial DNA (mtDNA) was identified as a biomarker of UV-induced DNA damage. This deletion was identified in the minor arc spanning nucleotides 547-4443. This deletion had previously been associated with Kearns Sayre Syndrome and Chronic Progressive External Opthalmoplegia (Moraes et al, 1995).

[0008] Examples in PCT publication no.WO/06/111029 demonstrate that that the frequency of occurrence of the 3895 bp mtDNA deletion is significantly different between body sites that are "usually" versus "occasionally" exposed to the sun. In addition, the examples demonstrated a link between the etiology of the 3895 bp deletion and the UV radiation component of sunlight by inducing the 3895 bp deletion *in vitro* with repetitive sub-lethal doses of a UVA+UVB light source. Skin samples for the examples provided in the PCT application were obtained by painful methods of skin collection previously known in the art.

[0009] It would be desirable to have a method for rapidly collecting skin samples and genotyping individuals to identify the presence of the 3895 bp and other mtDNA deletions associated with UV skin damage.

[0010] The present invention seeks to obviate or mitigate some or all of the above-mentioned problems associated with current methods for the collection of skin samples.

SUMMARY OF THE INVENTION

[0011] In one embodiment, the present invention provides a method for the detection of an aberration in DNA of skin cells comprising;

contacting a skin site with a sterile swab;

swabbing the skin site with the sterile swab to collect the skin cells;

extracting DNA from the skin cells;

detecting the presence of the aberration in the DNA.

[0012] In one aspect, the deletion being detected is the 3895 bp mtDNA deletion described below.

[0013] In another embodiment, the present invention provides a method for the non-invasive collection of DNA or mtDNA from skin cells for use in the detection of DNA or mtDNA biomarkers, the method comprising;

-contacting a skin site with a sterile swab;

-swabbing the skin site to collect the skin cells;

-extracting the DNA or mtDNA from the skin cells.

[0014] In another embodiment, the present invention provides a method for the detection of an aberration in DNA of skin cells comprising;

collecting skin cells from the dermis and/or epidermis by piercing through a tented layer of skin with a needle, without obtaining blood, to obtain a microscopic amount of dermal and/or epidermal tissue adhered to the core of the needle;

expressing the dermal and/or epidermal tissue from the core of the needle;

extracting DNA from the dermal and/or epidermal tissue;

detecting the presence of the aberration in the DNA.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] One or more embodiments of the invention will now be described by way of example only with reference to the appended drawings wherein:

[0016] Figure 1 shows real-time PCR data relating to the 3895 bp mtDNA deletion levels in skin samples collected from the nose and the heel using the method of the present invention.

[0017] Figure 2 shows real-time PCR data relating to levels of the 3895 bp mtDNA deletion in skin cells collected from various body sites using the method of the present invention.

[0018] Figure 3 shows real-time PCR data relating to levels of the 3895 bp mtDNA deletion in skin cells collected from various body sites using the method of the present invention.

[0019] Figure 4 is a gel showing the presence of amplification products present in samples collected from various non-invasive skin collection methods.

[0020] Figure 5 is a gel showing the presence of amplification products present in samples collected from various non-invasive skin collection methods.

DETAILED DESCRIPTION OF THE INVENTION

[0021] As used herein, "cycle threshold" (C_T) is the point at which target amplification of a nucleic acid sequence rises above background, as indicated by a signal such as a fluorescence signal. The C_T is inversely related to the quantity of the sequence being investigated.

[0022] As used herein, "diagnostic" or "diagnosing" means using the presence or absence of a mutation or combination of mutations as a factor in disease diagnosis or management. The detection of the mutation(s) can be a step in the diagnosis of a disease.

[0023] As used herein, "deletions" means removal of a region of DNA or mtDNA from a contiguous sequence of a nucleic acid. Deletions can range in size from one base to thousands of bases or larger.

[0024] As used herein, "mitochondria" means a eukaryotic cytoplasmic organelle that generates ATP for cellular processes.

[0025] As used herein, “mitochondrial DNA” or “mtDNA” is DNA present in mitochondria.

[0026] As used herein, "mutation" encompasses any modification or change in a DNA or RNA sequence from the wild type sequence, including without limitation point mutations, transitions, insertions, transversions, translocations, deletions, inversions, duplications, recombinations or combinations thereof. The modification or change of the sequence can extend from a single base change to the addition or elimination of an entire DNA or RNA fragment.

[0027] The present invention provides a non-invasive method for the collection of skin samples for genotyping or diagnostic tests. The method involves the use of a sterile swab, such as those used in the collection of buccal cells or cotton-tip swabs. The sterile swab is removed from its packaging and is rubbed on a skin site of interest. Preferably, the site is swabbed approximately 15 times in order to ensure that a sufficient number of skin cells are collected for genotyping or diagnostic purposes. Although the present invention is described below with reference to a specific example, the method may also be used to collect skin samples for the diagnosis or characterization of disease, aging, or exposure to ultraviolet radiation, and the identification of mutations associated therewith.

[0028] Following the swabbing of the skin, the swab is deposited into a sterile tube. Buffer may be added to the tube as necessary in order to maintain the integrity of the genetic material (i.e. DNA) contained therein. The DNA is then extracted utilizing well known methods in the art.

[0029] The method of the present invention may be used for widespread skin screening for both medical and cosmeceutical purposes. The method of the present invention may be used to measure various biomarkers associated with skin cancer (both non-melanoma skin cancer and melanoma). The ability to assess the level of DNA damage in an individual's skin due to UV radiation at any time point and from any external anatomical location provides the foundation for a unique and informative screening test for skin health.

[0030] The collection materials used in the method of the present invention may be packaged, depending on the desired application, into a consumer kit or a medical kit to be used in a clinical environment. Such kits could not only include the sterile swabs, but other materials necessary for genotyping (eg. the identification of mutations).

[0031] In example 1 provided below, one embodiment of the method of the present invention is used to collect skin cells for the quantification of biomarkers associated with damage caused by UV radiation. Specifically, the method of the present invention was used to collect skin samples for testing for deletions in the human mitochondrial genome, namely the 3895 bp mtDNA deletion identified in PCT application no.WO/06/111029. The 3895 bp deletion has a sequence corresponding to SEQ ID NO:1. The example shows that skin cells collected via the non-invasive method of the present invention provides sufficient mtDNA for obtaining results comparable to mtDNA obtained via previous skin collection methodologies.

[0032] In another embodiment of the present invention, a very small gauge needle (28 or 29 gauge) is used to collect skin cells for the purpose of genetic investigation. In this embodiment, skin cells are collected from the dermis and epidermis of a subject by piercing through a tented layer of the skin such that little or no blood is drawn, but a microscopic amount of dermal and epidermal tissue is adhered to the inner core of the needle. The skin may be tented by raising the skin using, for example, fingers, tweezers, or other forms of clamp. The skin material is contained in the needle until it is extracted for further processing (ie. DNA extraction). To express the skin sample from the needle, phosphate buffered saline is deposited into the column of the needle and then forced through with the plunger into a sterile tube. DNA is extracted utilizing well known methods in the art. As illustrated by example below, this minimally invasive method for the collection of a skin sample yields sufficient DNA or mtDNA for the assessment of DNA or mtDNA damage, for example, caused by UV radiation. As with the previous embodiment, this method of obtaining skin

samples is safe and painless. Further, as illustrated below, allows for sufficient DNA or mtDNA to be collected for conducting accurate assays.

[0033] Example 1: Analysis of 3895 bp human mtDNA deletion.

[0034] The method of the present invention was used to analyze the 3895 bp mtDNA deletion identified in PCT application no.WO/06/111029. Collection and extraction of the mtDNA was conducted as provided below.

[0035] 1. Skin samples were collected by swabbing a skin site approximately 15 times with a sterile swab. Skin samples were collected from heel (n= 41), nose (n= 43), inner arm (n= 20), ear (n= 5), shoulder (n= 5), buttock (n= 5), and back (n= 5).

[0036] 2. mtDNA was extracted using a commercially available kit (QiaAMP™ DNA Micro Kit, product no. 56304, *Qiagen*, Maryland USA) according to the manufacturer's protocol.

[0037] 3. Double stranded DNA was quantified using the HS-DNA Quant-it™ dsDNA HS Assay Kit (product no. Q32851, *Invitrogen*, California USA) on the Qubit™ Fluorometer (product no. Q32857), *Invitrogen*, California USA).

[0038] 4. The level of the 3895 bp deletion was then quantified by real-time PCR (rt-PCR) using the iQ Sybr Green Supermix™ (product no. 170-8882, *Bio-Rad*, California USA) and the following primers:
Forward 5'-CTGCTAACCCCATACCCCGAAAATGTTG-3' (SEQ ID NO: 2);
Reverse 5'-GAAGGATTATGGATGCGGTTGCTTGCGTGAG -3' (SEQ ID NO: 3).

[0039] In this example, the pair of amplification primers are used to amplify a target region indicative of the presence of the 3895 bp deletion. The forward primer overlaps a spliced region of mtDNA after deletion of the 3895 bp sequence has occurred (ie. a splice at a position between 547 and 4443 of the mtDNA genome). Therefore, extension of the overlapping primer to create the correct size amplification product can only occur if the 3895 bp section is deleted.

[0040] In the step of quantifying the 3895 bp deletion, the RT-PCR reaction was set up as follows:

12.5ul of iQ Sybr Green Supermix™;
350nmol forward primer (SEQ ID NO: 2);
350nmol reverse primer (SEQ ID NO: 3);
5ul of template (approximately 0.5ng dsDNA);
water to 25ul;

[0041] Cycling parameters:

Step 1. 95°C for 3 minutes;
Step 2. 95°C for 30 seconds;
Step 3. 67.5°C for 30 seconds;
Step 4. 72°C for 30 seconds;
Step 5. Plate Read

45 cycles of steps 2-5
Melting Curve 55-110°C reading every 3 seconds at 1°C intervals
Hold at 10°C for 10 minutes.

[0042] The results of these assays are shown in figures 1 to 3 and demonstrate a clear distinction between skin swabs taken from areas rarely exposed to sunlight/UV radiation (ie. heel and buttocks) and those usually exposed (ie. nose and ear). Levels of the 3895 bp deletion are significantly elevated in areas receiving a higher level of UV radiation such as the nose and shoulder when compared to areas generally protected from UV radiation such as the heel and the buttocks.

[0043] As shown in figures 1 and 2, the real time PCR cycle thresholds (C_T) for the 3895 bp deletion indicate that there is a higher incidence of the deletion in skin sites usually (nose or ear) or occasionally (shoulder or back) exposed to UV radiation compared to those sites that are rarely exposed (heel or buttocks).

[0044] Figure 3 shows that mtDNA collected from skin cells obtained from sites that are usually exposed to UV radiation (e.g. nose or ears) are characterized by increased levels of the 3895 bp deletion marker than mtDNA collected from skin cells obtained from sites rarely exposed to UV radiation (e.g. heel or inner arm).

[0045] These results also show the effectiveness of collecting skin samples in accordance with the present invention, in order to obtain sufficient mtDNA to conduct the assays. As such, the non-invasive skin collection methods of the present invention are similarly effective for obtaining mtDNA for analysis as invasive methodologies, for example, the methods used in the Applicant's PCT publication no.WO/06/111029.

[0046] Example 2: Comparison of Skin Collection Methods

[0047] Five different non-invasive skin collection methodologies were tested in order to identify which, if any, would yield sufficient quantity and quality of nucleic acids for molecular analyses such as quantitative real-time PCR. The five methods tested were:

- Tapelift using surgical tape;
- Biore® adhesive strip;
- Sterile swab wetted with 8% mandelic acid;
- Sterile swab wetted with distilled water; and
- Wax strip.

[0048] The tapelift, Biore strip and wax strip were applied to the surface of the skin following the application of 70% isopropanol to sterilize the area. Firm pressure was applied and then the tape or strip was removed quickly. The swabs were first deposited in a sterile solution of either 8% mandelic acid, or distilled water and then rubbed firmly on the skin site of interest after the skin had been cleaned with 70% isopropanol.

[0049] Following the collection of skin cells from 3 individuals each collection medium was deposited into 200ul phosphate buffered saline solution (PBS) and incubated overnight at 56°C.

[0050] All of the samples were then subjected to nucleic acid extraction using the Qiagen's QiaAMP™ DNA Mini Kit , buccal swab protocol (product no. 51304). The purified samples were quantified using the NanoDrop™ ND-1000 Spectrophotometer to determine if the extraction procedure was successful.

[0051] *Table 1 Determination of Quantity of DNA Extracted from Skin Collected by Five Non-invasive Methods*

Sample ID	DNA concentration ng/uL	A260nm	DNA Purity 260:280 Ratio
1 biore	-0.42	-0.008	0.48
1 swab with water	5.03	0.101	1.6
1 swab with mandelic acid	5.05	0.101	1.74
1 surgical tape	2.22	0.044	4.4
1 wax	3.52	0.07	1.69
2 biore	-0.02	0	-0.09
2 swab with water	2.74	0.055	1.97
2 swab with mandelic acid	4.9	0.098	1.82
2 surgical tape	2.99	0.06	1.78
2 wax	2.17	0.043	1.84
3 biore	-0.26	-0.005	0.24
3 swab with water	3.26	0.065	3.15
3 swab with mandelic acid	2.71	0.054	12.1
3 surgical tape	2.45	0.049	4.26
3 wax	2.67	0.053	4.25

[0052] When considering both nucleic acid concentration as well as the purity of the sample, the most consistent results were achieved for the swab samples using either water or mandelic acid as a wetting agent, or the wax samples.

[0053] Next, a PCR was performed on all samples to determine if amplification inhibitors were present or significant degradation of the sample had occurred during processing. Samples were amplified according to the following conditions:

Reagent	Final Concentration in Reaction
10X reaction Buffer	1X
dNTPs	0.4 mM each
BSA	1X
12s primer (forward)	0.4 uM
12s primer (reverse)	0.4 uM
Taq LA (Takara p/n RR002B	1.25 Units
Template	5 ul (of above concentration)
Water	To 25ul

[0054] The primers used were mitochondrial DNA primers having the sequences provided below:

12s primer sequence forward 5'-CGTTCCAGTGAGTTCACCCTC-3' (SEQ ID NO: 4)

12s primer sequence reverse R 5'-CACTCTTTACGCCGGCTTCTATT-3' (SEQ ID NO: 5)

[0055] The amplification reactions were cycled on a DNA Engine Tetrad (Bio-Rad) according to the following protocol:

1. 94°C for 2 minutes
2. 94°C for 30 seconds
3. 64°C for 30 seconds
4. 72°C for 30 seconds
5. Repeat steps 2-4 39 times
6. 4°C HOLD

[0056] Amplification products were then electrophoresed on a 2% agarose gel and stained with ethidium bromide. The amplification results are provided in figure 4, where the top half of the gel contains:

Lane 1 500ng 100bp GeneRuler SM0323 (Fermentas)

Lane 2 Biore from Individual 1

Lane 3 Swab with water from Individual 1

Lane 4 Swab with mandelic acid from Individual 1

Lane 5 Surgical tape from Individual 1

Lane 6 Wax from Individual 1

Lane 7 Biore from Individual 2

Lane 8 Swab with water from Individual 2

Lane 9 Swab with mandelic acid from Individual 2

Lane 10 Surgical tape from Individual 2

Lane 11 Wax from Individual 2

Lane 12 empty

Lane 13 Negative amplification control

Lane 14 Positive amplification control

Lanes 15-18 empty

[0057] And where the bottom half of the gel contains:

Lane 1 Biore from Individual 3

Lane 2 Swab with water from Individual 3

Lane 3 Swab with mandelic acid from Individual 3

Lane 4 Surgical tape from Individual 3

Lane 5 Wax from Individual 3

Lane 6 500ng 100bp GeneRuler SM0323 (Fermentas)

Lane 7 Biore extract negative control

Lane 8 Swab with water extract negative control

Lane 9 Swab with mandelic acid extract negative control

Lane 10 Surgical tape extract negative control

Lane 11 Wax extract negative control

Lane 12 empty

Lane 13 Negative amplification control (duplicate loading to Lane 13 above)

Lane 14 Positive amplification control (duplicate loading to Lane 14 above)

Lane 15-18 empty

[0058] Results

[0059] No mtDNA was amplified from mtDNA collected from skin cells harvested using the Biore strips. The swabs for both the water and the mandelic acid amplified, though the water swab amplified more brightly. The surgical tape amplified sporadically. The wax amplified brightly however the extract negative control in Lane 11 of the bottom half of the gel was contaminated likely as a result of the non-sterile nature or handling difficulties associated with the wax.

[0060] With all factors considered this example demonstrated that the use of a sterile swab is the preferred method of collection of a non-invasive skin sample. The swab can be dry or wetted with various liquids to facilitate collection or buffering of the sample.

[0061] Example 3: Comparison of Additional Skin Collection Methods

[0062] In this example five additional methods for the non-invasive collection of skin samples were tested in order to identify which, if any, would yield sufficient quantity and quality of nucleic acids for molecular analyses such as quantitative real-time PCR.

[0063] From a single individual, skin samples were collected twice using the following methods:

- scraping of skin using a sterile surgical blade
- scraping of skin using a wooden scraper

- sticky surface of an adhesive pad (CapSure™ Clean-up Pad, Arcturus)
- film from LCM MacroCap™ (Arcturus)
- heated film from LCM MacroCap™ (Arcturus)

[0064] The skin was first prepared by cleansing with a 70% isopropanol wipe. The wooden scraper and the surgical blade were passed firmly over the skin surface to remove skin cells and then deposited into a centrifuge tube. The adhesive pad and films were pressed firmly against the skin without rubbing to collect skin cells.

[0065] The multiple collections were processed using two different nucleic acid extraction methods. The first set was extracted using a proteinase K digestion as is well known in the art while the second set was extracted using the QiaAMP DNA Mini Kit (Qiagen 51304) .

[0066] The samples processed with the Qiagen kit were then quantified using the NanoDrop ND-1000 Spectrophotometer. Those in the PK digestion set were not as they were not cleaned up enough to facilitate this type of quantification.

[0067] *Table 2 Determination of Quantity of DNA Extracted from Skin Collected by Further Collection Methods (Qiagen extracted DNA)*

Sample ID	DNA concentration ng/uL	A260nm	DNA Purity 260:280 Ratio
AE Buffer	-1.09	-0.022	1.6
Woodscrape	2.06	0.041	0.95
Capsure	5.59	0.112	1.29
Blade	2.32	0.046	1.41
Blade -ve control	1.19	0.024	0.8
Capsure -ve control	2.47	0.049	1.2
Woodscrape -ve control	2.92	0.058	1.35
QIaGEN kIT -ve control	2.4	0.048	1.83

[0068] Samples were amplified according to the protocol provided in example 2. Amplification products were then electrophoresed on a 2% agarose gel and stained with ethidium bromide. Results are shown in figure 5, where the gel contains:

Lane 1 500ng of 100bp GeneRuler (SM0323)

Lane 2 Negative Amplification control

Lane 3 Positive amplification control

Lane 4 PK buffer wood scrape

Lane 5 PK buffer surgical blade scrape

Lane 6 PK buffer CapSure pad

Lane 7 PK buffer MacroCap

Lane 8 PK buffer MacroCap heated

Lane 9 PK buffer wood scrape negative extraction control

Lane 10 PK buffer surgical blade scrape negative extraction control

Lane 11 PK buffer CapSure negative extraction control

Lane 12 PK buffer MacroCap negative extraction control

Lane 13 PK buffer MacroCap heated negative extraction control

Lane 14 QiaAMP surgical blade scrape

Lane 15 QiaAMP CapSure pad

Lane 16 QiaAMP wood scrape

Lane 17 QiaAMP surgical blade scrape negative extraction control

Lane 18 QiaAMP CapSure pad negative extraction control

Lane 19 QiaAMP wood scrape negative extraction control

Lane 20 QiaAMP reagent negative control

[0069] The surgical blade scrape and the MacroCap™ were amplified using the PK buffer, while the CapSure™ pad amplified well using the QiaAMP™ kit. When compared to skin swabbing, the amount of mtDNA collected and the amount of amplified product obtained using the methods tested in this example were not found to be as effective.

[0070] *Example 4: Collection of Skin Samples using Needle*

[0071] Needles were used to collect skin samples from 5 different body sites of 9 individuals. The body sites included the eyebrow, earlobe, nape of the neck, hand, and heel. Using a needle as described above, the skin was pinched or tented between the thumb and forefinger of the sample collector's hand. The needle was passed through the skin, drawing little or no blood. The skin sample was extracted from the needle by depositing phosphate buffered saline into the column of the needle and then forcing the sample from the needle with a plunger into a sterile tube. DNA was then extracted from this volume containing the skin tissue using the QiaAMP™ DNA Mini Kit™ (Qiagen product no. 51304).

[0072] The samples were then amplified in order to identify the 3895bp mtDNA deletion. The reaction conditions and cycle parameters for this example were the same as for example 1 provided above. The results are presented in Table 3.

[0073] *Table 3: Results for Skin Samples collected via Needle*

Sample	Cycle Threshold C(t)	Sample	Cycle Threshold C(t)
Subject 1 left eyebrow	25.82	Subject 6 left eyebrow	22.15
Subject 1 left earlobe	25.98	Subject 6 left earlobe	19.87
Subject 1 neck	26.26	Subject 6 neck	25.87
Subject 1 right hand	26.73	Subject 6 right hand	27.91
Subject 1 right heel	27.93	Subject 7 left eyebrow	29.89
Subject 2 left eyebrow	30.85	Subject 7 left earlobe	19.18
Subject 2 left earlobe	25.6	Subject 7 neck	25.49
Subject 2 neck	23.92	Subject 7 right hand	23.41
Subject 2 right hand	27.01	Subject 7 right heel	27.05
Subject 2 right heel	35.55	Subject 8 left eyebrow	21.82
Subject 3 left eyebrow	29.64	Subject 8 left earlobe	21.32
Subject 3 left earlobe	23.51	Subject 8 neck	23.51
Subject 3 neck	24.52	Subject 8 right hand	16.35
Subject 3 right hand	22.47	Subject 8 right heel	20.57
Subject 4 left eyebrow	24.23	Subject 9 left eyebrow	25.09
Subject 4 left earlobe	23.64	Subject 9 left earlobe	26.76
Subject 4 neck	24.96	Subject 9 neck	27.74
Subject 4 right hand	25.93	Subject 9 right hand	25.24
Subject 4 right heel	23.58	Subject 9 right heel	22.39
Subject 5 left eyebrow	22.35		
Subject 5 left earlobe	24.39		
Subject 5 neck	22.06		
Subject 5 right hand	22.04		
Subject 5 right heel	22.6		

[0074] It is clear that the material obtained through this collection method is sufficient for molecular analyses such as real-time PCR. Specifically, the amplification product indicative of the 3895 bp mtDNA deletion has been detected and quantified as evidenced by Table 3. Therefore, the collection of skin samples via the needle collection method yields sufficient DNA for use in an assay of this kind.

[0075] Although the invention has been described with reference to certain specific embodiments, various modifications thereof will be apparent to those skilled in the art without departing from the spirit and scope of the invention as outlined in the claims appended hereto.

Claims:

1. A method for the detection of an aberration in DNA of skin cells comprising;

contacting a skin site with a sterile swab;

swabbing the skin site with the sterile swab to collect the skin cells;

extracting DNA from the skin cells;

detecting the presence of the aberration in the DNA.

2. The method according to claim 1 wherein the DNA is mitochondrial DNA (mtDNA).

3. The method according to claim 1 wherein the detection of the aberration is conducted using real-time PCR.

4. The method of claim 1 wherein the aberration is selected from the group consisting of deletions, substitutions, and insertions.

5. The method of claim 2 wherein the aberration is a 3895 bp mtDNA deletion between nucleic acids 546 to 4444 of the mtDNA genome.

6. A method for the detection of an aberration in DNA of skin cells comprising;

collecting skin cells from the dermis and/or epidermis by piercing through a tented layer of skin with a needle, without obtaining blood, to obtain a microscopic amount of dermal and/or epidermal tissue adhered to the core of the needle;

expressing the dermal and/or epidermal tissue from the core of the needle;

extracting DNA from the dermal and/or epidermal tissue;

detecting the presence of the aberration in the DNA.

7. The method according to claim 6 wherein the DNA is mitochondrial DNA (mtDNA).

8. The method according to claim 6 wherein the detection of the aberration is conducted using real-time PCR.

9. The method of claim 6 wherein the aberration is selected from the group consisting of deletions, substitutions, and insertions.

9. The method of claim 7 wherein the aberration is a 3895 bp mtDNA deletion between nucleic acids 546 to 4444 of the mtDNA genome.

10. The method of claim 5 wherein the needle is a 28 gauge or 29 gauge needle.

FIGURE 1

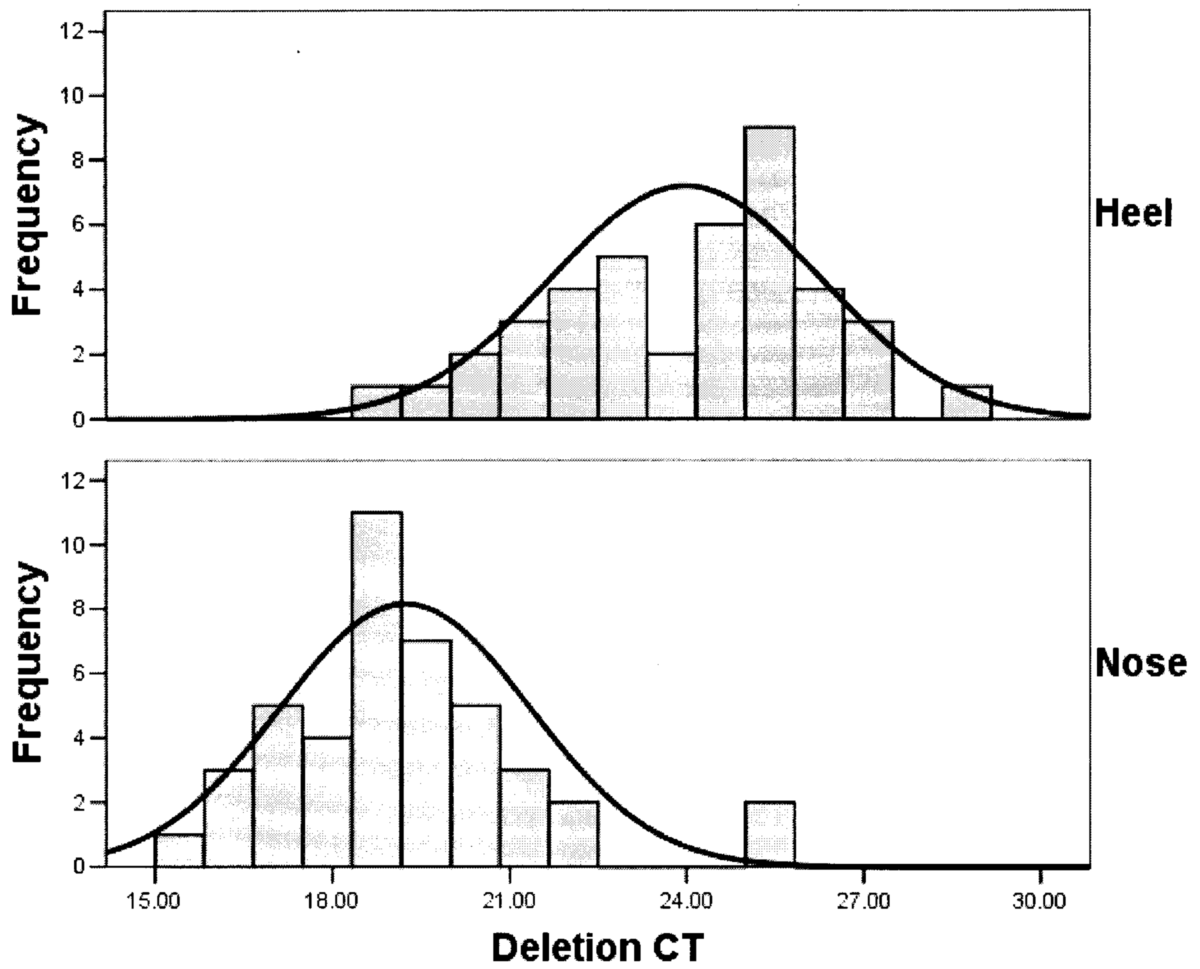


FIGURE 2

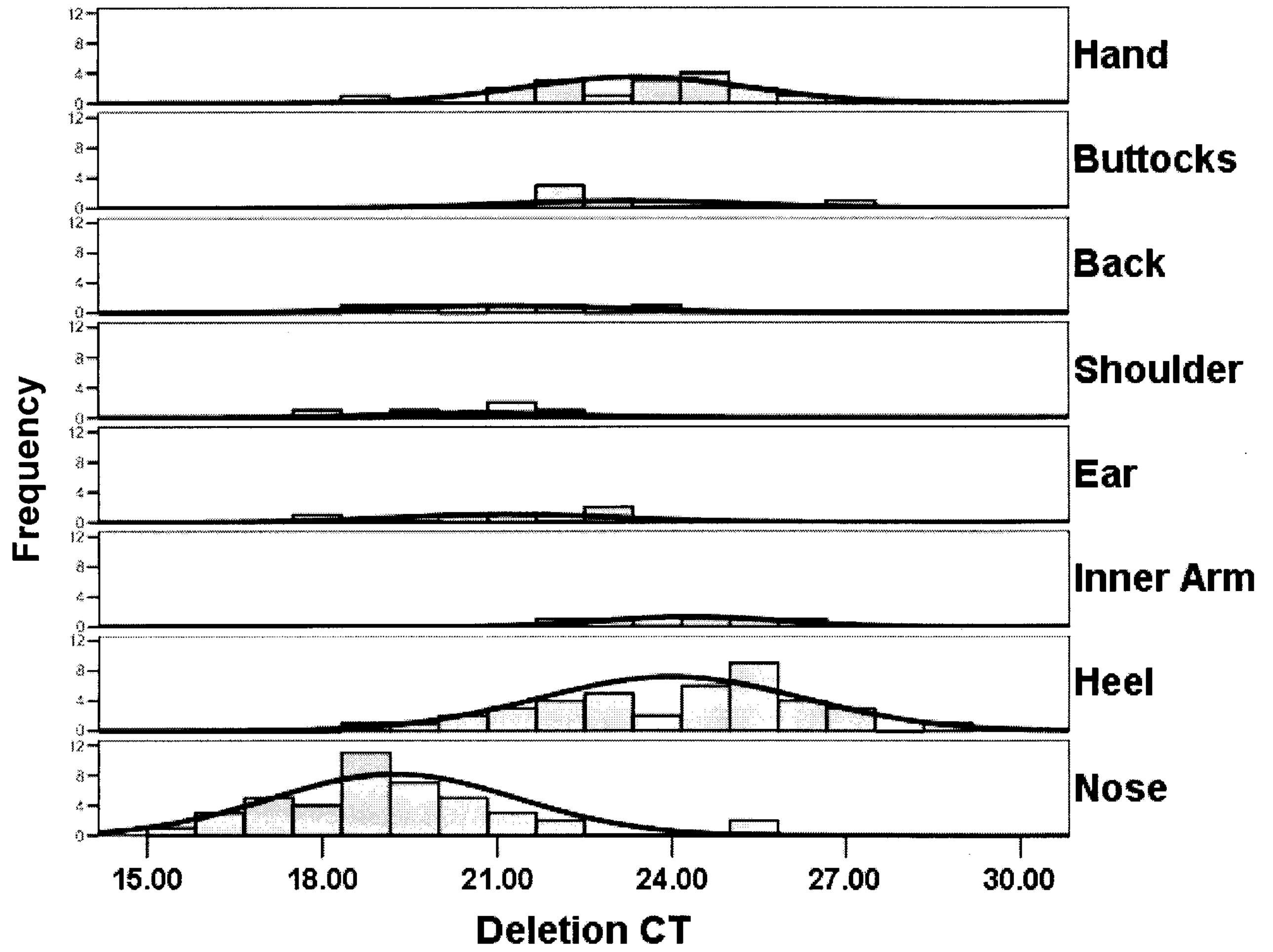


Figure 3

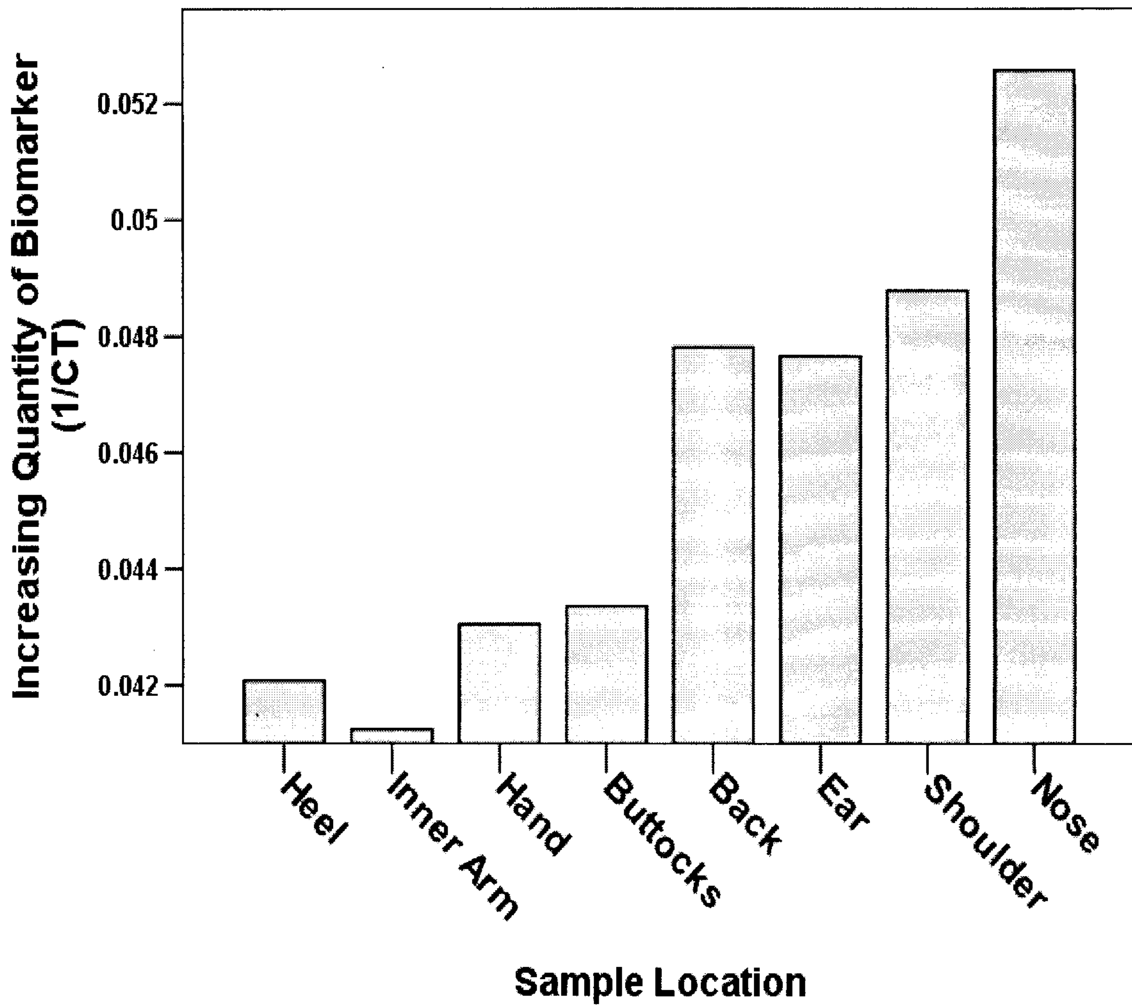
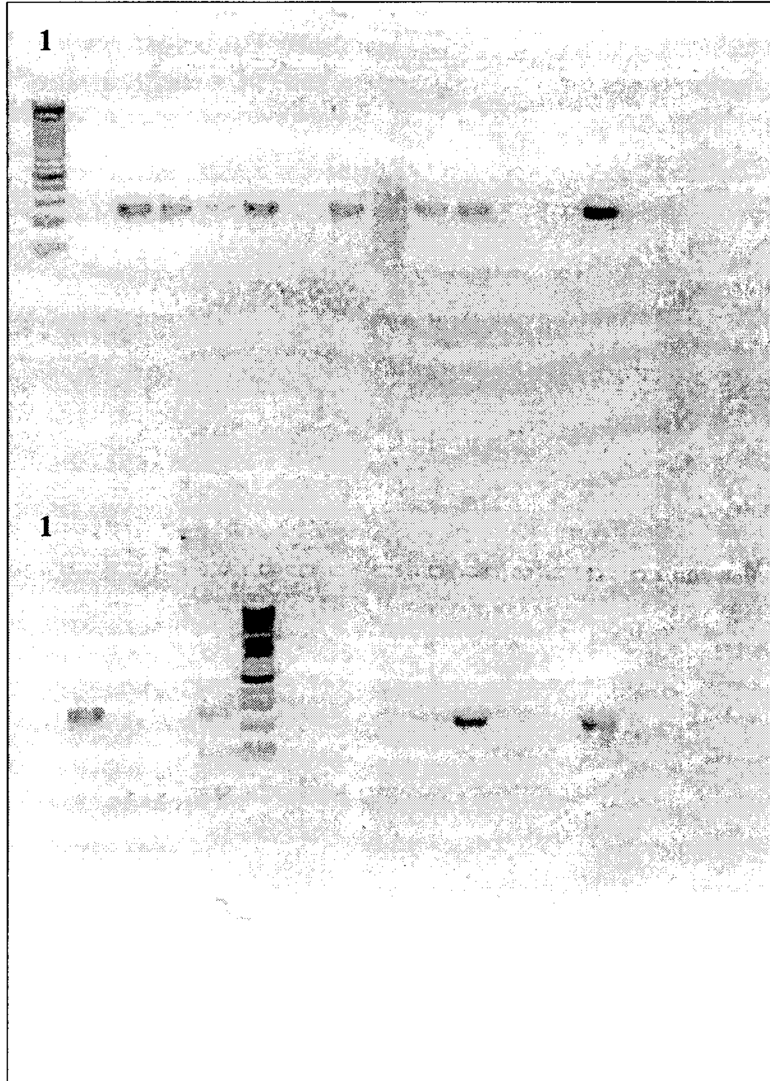
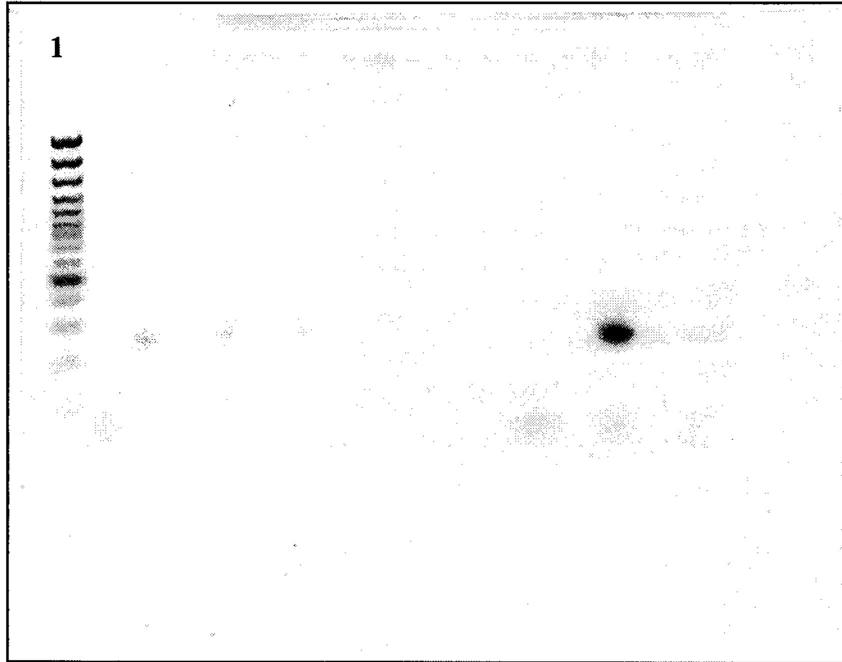


FIGURE 4



SUBSTITUTE SHEET (RULE 26)

Figure 5



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/001790

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C12Q 1/68*** (2006.01) , ***C12N 15/10*** (2006.01) , ***C12N 15/11*** (2006.01) , ***C12N 15/12*** (2006.01) , ***C12Q 1/24*** (2006.01) , ***C12P 19/34*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: ***C12Q 1/68*** (2006.01) , ***C12N 15/10*** (2006.01) , ***C12N 15/11*** (2006.01) , ***C12N 15/12*** (2006.01) , ***C12Q 1/24*** (2006.01) , ***C12P 19/34*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: CANADIAN PATENT DATABASE, DELPHION, USPTO/WEST, ESPACENET, STN / BAIZES, PUBMED;
Keywords: swab, isohelix swab, skin, skin cell, mitochondria, DNA, aberration, abrasion, dermis, epidermis, tented layer, needle.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	RICHARDS, B. et al. Multiplex PCR amplification from the CFTR gene using DNA prepared from buccal brushes/swabs. Hum. Mol. Genet. February 1993, Vol.2, No.2, pages 159-163, ISSN 0964-6906. See the entire document.	1-4, 6-9a, and 10 5 and 9b
X Y	von WURMB-SCHWARK, N. et al. Fast and simple DNA extraction from saliva and sperm cells obtained from the skin or isolated from swabs. Leg. Med. (Tokyo). May 2006, Vol.8, No.3, pages 177-181, ISSN 1344-6223. See the abstract; table 1; and page 181, left column.	1-4, 6-9a, and 10 5 and 9b

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 June 2008 (13-06-2008)	11 July 2008 (11-07-2008)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001-819-953-2476

Authorized officer

Qianfa Chen 819- 994-1374

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/001790

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 6-10
because they relate to subject matter not required to be searched by this Authority, namely :

   Claims 6-10, are directed to a diagnostic method involving surgical steps which the International Search Authority is not required to search under Rule 39.1 (iv) of the PCT. Regardless, this Authority has carried out a search based on the alleged effect(s) or purpose(s)/use(s) of the product defined in claims 6-10.

2. [ ] Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest [ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	SZIBOR, R. et al. Efficiency of forensic mtDNA analysis. Case examples demonstrating the identification of traces. Forensic. Sci. Int. September 2000, Vol.113, No.1-3, pages 71-78, ISSN 0379-0738. See the abstract; the introduction; page 73, right column, second paragraph to page 74, left column, second paragraph.	1-4, 6-9a, and 10 5 and 9b
X	US 6,291,171 B1 (RICCIARDI, R.P. and DE PHILLIPO, J.R.) 18 September 2001. See the entire document.	1 and 3
X	CLAY, J. DNA and family puzzles. LawNow. February/March 2004, pages 23 and 24. See the entire document.	1 and 2
Y	WO 2006/111029 A1 (PARR, R. et al.) 26 October 2006. See the abstract; page 11, line 35 to page 14, line 27; and table 4.	5 and 9b
A	SWEET, D. et al. An improved method to recover saliva from human skin: the double swab technique. J Forensic. Sci. March 1997, Vol.42, No.2, pages 320-322, ISSN 0022-1198. See the entire document.	1-10
A	WO 2006/089297 A1 (CARAGINE, T.A. et al.) 24 August 2006. See the entire document.	1-10

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2007/001790

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US 6291171 B1	18-09-2001	CA 2251785A1	22-05-1997
		EP 0956083A1	17-11-1999
		EP 0956083A4	05-07-2000
		US 5933644A	03-08-1999
		US 2001007750A1	12-07-2001
		WO 9718009A1	22-05-1997
WO 2006111029 A1	26-10-2006	AU 2006238390A1	26-10-2006
		CA 2606156A1	26-10-2006
		EP 1877559A1	16-01-2008
		KR 20080025040A	19-03-2008