ENVIRONMENTAL DNA:
REVIEWING ENVIRONMENTAL DNA, CONTAMINATION ISSUES
AND AN INVESTIGATION OF THE PREVALENCE OF BACKGROUND DNA
WITHIN MURDOCH UNIVERSITY

By
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Declaration

I declare that this manuscript does not contain any material submitted previously for the award of any other degree or diploma at any university or other tertiary institution. Furthermore, to the best of my knowledge, it does not contain any material previously published or written by another individual, except where due references has been made in the text. Finally, I declare that all reported experimentations performed in this research were carried out by myself, except that any contribution by others, with whom I have worked is explicitly acknowledged.

Signed: Supaneda Kolanski

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LITERATURE REVIEW OF
ENVIRONMENTAL DNA AND CONTAMINATION ISSUES
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Abstract
DNA profiling technology such as Short Tandem Repeat (STR) analysis has achieved worldwide public as well as professional acceptance due to a reliable means of individual identification and has had a significant impact on criminal justice systems. The approach has been employed in forensic disciplines for years for the purpose of individual identification in both cases of interrogation or exoneration of suspect(s). The ability to detect minute amount of trace DNA of the increased sensitivity of DNA analysis methodology not only allow investigator to amplify target DNA associated with the crime event, but also enables the discovery of contaminating DNA resulted from pre-existent environmental DNA at the scene of crime.
**Introduction**

Van Oorschot and Jones (1) reported that DNA profiles could be obtained from items that had been handled briefly and DNA profiles can be recovered by swabbing a surface that has been touched. Moreover, trace DNA can be retrieved from many everyday objects, such as briefcases, car keys, telephone handsets, bed sheets, shoe insoles, and firearms (1-4). In this review, ‘trace DNA’ refers exclusively to DNA that cannot be attributed to an identifiable body fluid (5). However, at amplification stage, trace DNA samples may be defined as ‘any sample which falls below recommended thresholds at any stage of the analysis, from sample detection through to profile interpretation, and cannot be defined by a precise picogram amount’ (6).

DNA profiling methods such as Short Tandem Repeat forensic DNA analysis has been utilized over a decade for the purpose of a retrieval of genetic profiles in order to identify sources of DNA (7). Low copy number (LCN) typing, particularly for current short tandem repeat (STR) typing, refers to the analysis of any sample that contains less than 200 picograms of template DNA (8). The sensitivity of DNA profiling system has significantly been increased due to the improvements in primer design, buffer composition as well as amplification conditions (6). As a consequence, the enhanced sensitivity effectively allows the amplification of incredibly small quantities of target biological material and due to trace DNA can be found everywhere, the improved profiling system simultaneously couples with the potential of detecting contaminant DNA which resulting in profiles containing multiple numbers of contributors (9). Several studies conducted a comparison between freshly invented and previous technologies including the one directed by Ballantyne, Poy (10). They proved that new methodology such as PowerPlex®21 has the ability to detect background DNA samples which were collected from surfaces within their laboratory whereas current system Profiler Plus is not able to.

This review aims to illustrate the deposition of trace DNA in environment along with factors that impact its deposition, persistence as well as transfer events. In addition, contamination issues together with a justification of future study are also discussed here.
Transfer of trace DNA

The deposition of trace DNA onto surfaces can be from either direct or indirect contact. Primary transfer (direct transfer) is not only when individual comes into contact with object, but also includes activities such as speaking, coughing, and sneezing within the vicinity of an item that may result in the transfer of DNA directly from an individual (11). Secondary transfer (indirect transfer) is the transfer of an individual’s DNA after deposition to another surface (12). Supposedly, DNA could be transferred even further as a result of subsequent contact situations and can be referred as ‘tertiary transfer’, ‘quaternary transfer’ and further transfer depending on number of transfers (see figure 1). However, published research on tertiary, quaternary or further transfer scenarios is limited nowadays. (13, 14).

![Diagram of DNA transfer](image)

Figure 1 demonstrates transfer events of trace DNA
Factors affecting deposition of trace DNA

- DNA “Shedding”

Lowe, Murray (15) investigated the ability of an individual to deposit his or her own DNA on an item. Eight persons were asked to wash their hands and then grip a sterile plastic tube for 10 seconds as well as 15 minutes. Subsequently, they found various percentages of profiles retrieved from touched item. The findings suggested the variation in the deposition or “shedding” of DNA between donors. As a result, the researchers defined the terms ‘good’ and ‘poor’ shedder based on the basis of the number of alleles that were recovered from a plastic tube after hand washing.

Furthermore, the skin condition of the shedder also plays a crucial role in relation to the degree of shedding. Bright and Petricevic (3) revealed that individuals with dried and flaky skin conditions on their hands, for examples, atopic dermatitis and psoriasis, deposited higher quantity and better quality DNA profiles than those without. Conversely, treatment of those skin conditions and activities prior to touching such as hand-washing could result in the reduction of available DNA for shedding.

- Type of surface

It is commonly believed that DNA is more readily deposited on some surfaces than others; rougher surfaces may collect more DNA than smooth surfaces, and the idea is supported by numbers of studies. For example, Daly, Murphy (16) showed that there was a significant difference in the amount of DNA recovered from three types of substrates such as wood, fabrics and glass. The maximum quantity of DNA was encountered from wood followed by fabric and then glass. In addition, they also found better quality profiles were also obtained from wood and fabrics than from glass. Consistently, Goray, Mitchell (17) encountered that the amounts of retrievable DNA deposited on cotton are markedly greater than for plastic irrespective of manner of contact.

Moreover, the results of the study from Fonnelop, Egeland (18) are consistent with those two investigations above. The authors conducted an observation of DNA transfer on wood, plastic tube and metal door handle. The outcomes suggested that DNA was readily transferred to wood and plastic whereas less was transferred to a metal door handle. Accordingly, there is a possibility that more absorbent surfaces will collect more DNA and assist the perseverance of trace DNA as skin cells adhere to, or are absorbed by, soft porous surfaces (cotton) more readily than hard and non-porous substrates (plastic).

- Nature of contact

Van Oorschot and Jones (1) found that the nature of contact appears to be a substantial factor contributing the deposition of DNA rather than the length of time the object is held. The results of
their study shown that similar amounts of DNA were recovered from a handled object from various duration time, suggesting that the majority of DNA transfer occurs at initial contact.

Correspondingly, current study from Fonnelop, Egeland (18) justified that it is not drastically dependent on the handling time due to most of the transfer can happen with a short period of contact. By investigating DNA profiles obtained from various handling times (30 and 10 seconds), the findings display that full donor profiles could be recovered from samples in all steps of the transfer chains (primary transfer to quaternary transfer) from both handling duration times.

According to both published studies, time does not appear to be the factor that can significantly affect the deposition of DNA since the outcomes suggest that large proportion of DNA will be located onto surface at the initial contact. In addition, types of contact such as passive, pressure as well as friction have also been investigated. Goray, Mitchell (17) observed transfer of dried skin cells on plastic and cotton. They found that friction contact between the two substrates significantly enhanced secondary transfer as it gives the highest percentage of DNA transfer compared to either passive or pressure contact. Likewise, for freshly deposited skin cells, the manner of contact had a notable impact on the percentage of DNA transferred, except for the case which plastic was a primary substrate and cotton was a secondary substrate. However, these experiments neglected to successfully assist in establishing the effect of friction on initial DNA position from touch due to the quantity of DNA that transferred onto the first substrate was not identified.

Factors affecting persistence of trace DNA
- Environmental factors
In addition to type of surface, persistence of DNA also depends on environmental factors as reported by Raymond, van Oorschot (19). The authors encountered that the amount of DNA recovered from buffy coat on the outdoor surfaces (window frame) as well as the surfaces were in a partly shady location and did not receive sunlight directly (vinyl bag), will significantly deteriorate over six weeks. Furthermore, they also illustrated that profiles could not be obtained from those surfaces after two weeks. In contrast, well-preserved samples (control) that kept in their laboratory were more robust and full profiles were retrieved after six weeks which was the longest time period tested in their experiments. This suggests that it is possible that profiles may be obtained from older samples (over six weeks) when DNA is kept in similar conditions (undisturbed in a cool and dark location). Their outcomes support the hypothesis that the possibility of recovering DNA from an outdoor surface dramatically decline over time and the researchers proposed that if a DNA profile is recovered from a similar location, it is likely to be from a recent contact. As a result, the authors concluded that the chance of recovering a DNA profile is substantially reduced over time. Although the study was
successful to demonstrate that the quantity of DNA decreases over time but it miscarried to explain in
details about what those environmental factors are and how they impacted the deterioration of DNA.

In addition to type of surface and environmental factors, since DNA profile is substantially reduced
over time, the preliminary amount of DNA has to be taken into account as in the similar
environments, greater amount of DNA would provide higher detection possibility than less amount of
DNA.

Transfer rates
Type of primary and secondary substrates, degree of moistness of biological material, manner of
contact, and the initial quantities of DNA transferred to an item influences the probability of detecting
secondary transfer (13, 18)

Goray, Mitchell (17) conducted an investigation of secondary DNA transfer of skin cells under
controlled test conditions. In their experiment, it was assumed that DNA transfer rates are
independent of the initial amount of deposit and this assumption is confirmed as they found there was
no insignificant difference in transfer rate between the largest and the smallest deposits within each
set of six replicates transfer event. In addition, the findings suggested that plastic (non-porous) as a
primary substrate incredibly facilitates transfer of DNA than cotton, and this transfer is variable
depending upon the secondary substrate. Vice versa, when the secondary substrate is non-porous
(plastic), the transfer rate was reduced to half for all biological samples and contact types.

Correspondingly, Lehmann, Mitchell (20) revealed that by using certain combinations of substrate,
biological material, and moisture, DNA can be transferred at least through six contact events whereas
some combinations produce none or incredibly small DNA transfer beyond the initial contact after
deposition. The authors monitored DNA transfer of three different biological materials: wet blood,
dry blood and touch DNA on glass and cotton. The findings illustrated that wet blood transferred
further than dry blood on either cotton or glass. On cotton substrate, full profiles can be obtained from
the first to the fourth substrate, but no alleles can be detected beyond this point where as dry blood
and touch DNA produced a full profile on the first substrate only. Additionally, dry blood produced a
partial profile on the second substrate. When glass was the substrate, wet and dry blood produced full
profiles from the first to the sixth substrate whereas touch DNA produced a full profile on the first
glass substrate only, and partial profiles on the second to the fifth substrate but cannot be detected
further.

van Oorschot, Goray (21) demonstrated that moistness is significant for the DNA transfer of all
biological samples as demonstrated in their study. The minimal transfers rates were observed when
moist samples (wet blood) is transferred from absorbent primary substrates, such as cotton or wool whereas a non-absorbent primary substrate such as plastic can greatly facilitate transfer event. The same study also observed that transfer events can be diminished when dry samples deposited on porous primary substrates.

Although it has been shown that the manner of contact intensely influences the percentage of biological material that is transferred as they found friction contact provides the significant level of useful alleles in comparison with pressure and passive (17), there is an exception when gloves are worn. It was revealed by Szkuta, Harvey (22) that the nature of contact had little influence on the retention of material by gloves and thus, the authors advocate that the composition of the vector (gloves) is another factor to consider when assessing transfer. In addition, the researchers also encountered that the transfer of touch DNA was highest with non-porous glass as the primary substrate followed by porous cotton as the secondary substrate which were concordant with (21) where the combination of non-absorbent primary and absorbent secondary gave the greatest transfer of touch DNA. However, when taking the vector into consideration, the greatest transfer of touch DNA was observed between non-porous glass and non-porous rubber (glove), followed by transfer between non-porous rubber (glove) and porous cotton.

**The relevance of DNA transfer at the crime scene**

As background contamination is unavoidable and must always be taken into consideration when processing the investigation, an association of DNA profiles recovered from the scene of crime in relation to the crime event itself needs to be interpreted. In this context, DNA transfer is described as either active (relevant) or passive (not relevant). ‘Active transfer’ is associated with direct transfer of DNA during the crime event itself. For example, perpetrator’s DNA profile is transferred to victim’s fingernails resulting from scratching during the incident. ‘Passive transfer’ is not associated with the crime event and results in the “background” distribution of DNA profiles that pre-exists the crime-scene. For instance, from environmental DNA at the scene, contaminated tools or consumables used during the process of investigation (23).

**Background DNA and Contamination issue**

Contamination is defined as ‘the introduction of DNA, or biological material containing DNA, to an exhibit at or after the point when a controlled forensic process starts’ (24). Its existence could be at any stages of the investigation: (a) prior to the crime has been committed; (b) in the interval between the crime and securing the crime scene; (c) during the investigation of the scene; or (d) within the laboratory (6).
The presence of trace DNA as background DNA in normal circumstances resulted from casual activities of innocent passer-by or individuals. Background DNA generally exists in all pervasive environment even before the crime has been committed and it can remain intact up to years after deposition depending on the environmental conditions (19). Subsequently, once the crime has been committed, background DNA may inadvertently be collected, profiled together with target DNA and subsequently, complicate the outcomes.

Within the forensic laboratory, an accumulation of cellular, purified and amplified DNA on surfaces of tools, equipment and consumables instigate an occurrence of contamination due to it may be unintentionally picked up and transferred to exhibits when examinations being operated (25). The principal sources of DNA contamination are (a) from individual to the exhibit/DNA sample; (b) from contaminated consumables to exhibit/DNA sample; and (c) cross-contamination from exhibit or DNA sample to DNA sample (24).

While DNA containing material is being transferred through contact, sufficient DNA still remaining on the vectors and may result in further transfer with subsequent contacts especially when dried blood is biological material that being contact. Furthermore, the possibility of the tools used during examination and surfaces becoming vectors for subsequent DNA transfer substantially increases if cleaning or replacement protocols are not strictly followed after use (26).

In addition, the relative risk of a particular vector causing contamination is dependent on a number of factors: (a) the area within the laboratory where the vector is used; (b) the amount of DNA deposited onto that vector; (c) the surface type of the vector; (d) the number of transfer steps between that vector and potential contact with an exhibit (27).

There are various studies conducted an assessment of the degree of background DNA on disposable glove surfaces during and after exhibit examination. The outcomes displayed that gloves are effective vectors as they carried a high level of contaminating DNA (9, 14, 18, 22, 26). Furthermore, DNA transfer was also encountered on other high-risk examination tools that regularly used in the lab, for instance, scissors and forceps especially when the vectors came into contact with blood and the tools were not cleaned adequately (9).

Moreover, contamination can also be introduced by manufacturer contaminated disposables or reagents with an obvious adverse effect on the results. A famous case study perfectly demonstrates a misled investigation caused by contamination - ‘Phantom of Heilbronn’ in Germany and Austria, in which sterile swab contamination during its manufacture caused police years to solve the issue and it linked 40 crime scenes incorrectly. In this case, even though sterilisation can eliminate bacteria,
viruses and fungi, it cannot destroy DNA of an innocent factory worker on cotton swabs. The police found her DNA matching series of crime ranging from common thefts to brutal murders as the swabs used in those cases were contaminated by this worker as a result, the police had to spend years to resolve the misled cases (28).

Besides, Taylor, Abarno (27) recently found that forensic case files can also carry detectable DNA. The results show a reasonable retrieval of DNA which would be expected as the high amount of contact with individuals across the workplace. In addition, items used most frequently such as chairs, phones, computer items, floors and communal office items such as water coolers also gave the highest recovery of DNA. The number of individuals detected in profiles is higher on items from more frequented areas. The same study also illustrates that non-sensitive areas within their lab (where an exhibit may pass through on the way to a sensitive area or where an exhibit may be stored exhibits, or samples taken from them, are sealed at all times in non-sensitive areas) more often gave profiles with higher contributor number (at least three contributors and can be up to five) due to the sites were cleaned less often.

In South Australia Police facilities, Henry, McGowan (29) found significant background DNA exists on a number surfaces and items. Investigation tools such as cameras posed the highest risk for DNA transfer with all samples tested yielding informative and highly mixed DNA profiles. In addition, the highest environmental DNA load was also found on benchtop, cupboard/drawer handles, and exhibit drying rails. The presence of high level of DNA might be due to those surfaces and items have not been cleaned appropriately and/or adequately. However, the issue can be solved by an implementation of staff elimination database.

To observe contamination event caused by police officer during criminal investigation which resulting in secondary DNA transfer from evidence bags, Fonnelop, Johannessen (30) show that a relatively high amount of DNA was detected on the outside of the bags and can be transferred to an exhibit during examination. However, contamination by secondary transfer from outside evidence bags is less likely and could only account for a small proportion of the observed police contamination.

Moreover, Goray and van Oorschot (31) conducted the study of DNA during social settings and found that individuals are also able to act as vectors for multiple transfer events of foreign DNA. Correspondingly, Fonnelop, Egeland (18) observed foreign DNA present on a person’s hand can be transferred onto a new object and follow the transfer chain to the third object nonetheless this was only a partial profile but gives support to the fact that under some conditions DNA can be transferred up to four times.
Mixed profiles
The DNA evidence given to the court is the scientist’s interpretation of the laboratory analysis, expressed in terms of a statistical calculation of the likelihood of a match between a crime scene profile and the accused’s profile. A mixed profile will reduce the statistical likelihood of a match, thus reducing the probative value of the evidence. That is, an accused may argue that their DNA is on the profile adventitiously. Depending on the other evidence, this may be sufficient to cast a reasonable doubt on the prosecution’s case (32). Mixed DNA samples from at least two contributors can be originated at the scene of crime or contamination during the process of investigation (33). Generally, the final results from the DNA analyses can be a full DNA profile, partial, blank, or mixed profile in case of containing two or more contributors. Frequently, instead of having a full profile, the profile can also lack information because alleles are missing or “drop-outs”. Conversely, extra alleles can be added to the profile or “drop-ins”. These are artefacts presented when performing analysis on samples with minute amounts of target DNA, degraded samples, samples with inhibitory compounds (or in combination) as well as the presence of contaminating DNA in samples (34).

Although Szkuta, Harvey (26) found that the level of contamination and subsequent contact by the vectors had no impact on the target sample, the examiners supposed that in casework scenarios where the suspect profiles are not known, profile interpretation becomes complicated by the addition of contaminating alleles and the probative value of the evidence may be affected. Agreeably, Gill (23) defined the terms “the hidden perpetrator effect” – when the perpetrator’s DNA is absent from the crime stain then the donor of the contaminant DNA becomes a suspect and the perpetrator is hidden. This incident leads to the “association fallacy” where an innocent individual may be implicated in a crime and his DNA wrongly attributed to a body fluid. If this is semen, for example, then it will compound the error further by suggesting the activity of sexual assault.

In addition, Gill (23) gave a definition “the naïve investigator” which established on the incident of wrongful arrest of Adam Scott due to the investigator found that his profile was the closest match to a crime stain in a national DNA database, the case was reported by Rennison (35). The man’s DNA profile was eventually traced to a contamination incident, but the case is outstanding because the match was adventitiously obtained from a search of the national DNA database.

Several high profile cases in Australia have potentially been involved with contamination issue, for instance, Farah Jama. In 2009, Jama was acquitted by the Victorian Court of Appeal after serving 16 months in prison following a conviction of rape in the County Court of Victoria. The case was unusual in that the conviction was based solely on DNA evidence, with no other evidence implicating the accused (36).
Contamination Prevention

Several approaches are applied throughout the investigation in order to prevent an occurrence of contamination. For example, Personal Protective Equipment (PPE), it has been commonly introduced for years as a routine basis within forensic casework throughout the investigation beginning from when the officer attending the scene of crime, to the laboratory where staff member performing exhibit examination. Additionally, findings from Rutty, Hopwood (11) confirmed that an appropriate application of PPE could effectively result in the minimization of potential DNA contamination of the crime scene.

Numbers of studies illustrate that item that being used in laboratory frequently act as vectors such as scissors or forceps, if they are not cleaned adequately (10, 22, 26). Hence, increasing density and frequency of cleaning, and/or replacement of items can reduce contamination risk of those tools. Additionally, encouragement amongst staff of the potential sources of contamination within the laboratory during examination is also recommended.

Recently, the world’s first International Standard on the manufacture of forensic consumables has been introduced to global forensic community in an effort to minimise the risk of occurrence of detectable human nuclear DNA contamination in products used to collect, store and analyse biological material for forensic purposes. ISO18385 provides guidance to manufacturers on how to minimise the risk of human DNA contamination in those products and as a consequence, the end users will be able to purchase consumables with confidence from manufacturers meeting the standard. Furthermore, not only the standard will assist investigators to avoid incident such as ‘Phantom of Heilbronn’ but also satisfy the requirements of their own accreditation to ISO/IEC 17025 certifying the competence of testing and calibration laboratories. Implementation of ISO18385 provides forensic scientists confidence that the products they use are fit for purpose and appropriate measures have been taken to significantly reduce contamination, thus diminishing the number of extraneous DNA contributing to DNA profiles which potentially making results simpler to interpret and finally, this leads to greater confidence in forensic science results by police and the courts (37).

Research gap

Although the investigation of trace DNA as background DNA has been conducted on many occasions, the majority of them was merely explored within laboratory and/or under controlled conditions where objects were decontaminated prior to the experiments operated and individuals or source of trace DNA were known and currently, the level of background DNA in general settings where environmental conditions, number of contacts, and the number of individuals cannot be controlled has not yet been investigated.
Conclusions

The presence trace DNA as background DNA can be either from direct or indirect contact. Shedding status of individual, type of substrate which DNA deposited on, as well as nature of contact are crucial factors that impact the deposition of trace DNA. Thus, it is impossible to declare who was the last person that came into contact with the touched item by considering barely quantities or quality of DNA left on the object due to individuals have varied shedding status as some of them might deposit high amounts of poor quality profile which provide a partial profile, conversely, some of them might produce less amounts of high quality DNA that give a useful profile.

Additionally, persistence of DNA is significantly dependent on type of substrate, environmental factors and most essentially, an initial amount of DNA. Even though trace DNA is deposited at the same time, greater amount of DNA would increase the possibility of detection than less quantity in the same environment as DNA could be collected on some type of surface especially by absorbent porous substrate or transferred further. Additionally, transfer rates establish on the combination of influences such as type of primary and secondary substrates (in secondary transfer event), moistness of biological material, manner of contact, and most importantly initial amount of DNA.

Nowadays, forensic scientists are dealing with extra numbers of contributors obtained from crime-related samples due to the enhanced sensitivity of new STR approaches which not only be able to detect minute amount of target DNA but also reveal contaminant DNA that presenting in the scene, consumables or laboratory. Although an occurrence of contamination can be reduced by stringent implementations of contamination prevention, the presence of background DNA resulted from innocent people is unavoidable.

It is undeniable that transfer events happen repeatedly in our environment due to individuals regularly shed their DNA as a consequence of routine activities and it remains as environmental DNA in both circumstances where rigorous cleaning procedures are applied daily or common areas which those procedures are abandoned. Subsequently, touched item, surface, or individual would unconsciously become a substrate and deposited DNA probably can be transferred further (secondary, tertiary or quaternary transfer) to another substrate, especially when the aspects such as type of substrates, moistness of biological material, and nature of contact facilitates the transfer events.

Since various studies prove that trace DNA is frequently present in environment and individuals can as vectors, it can be assumed that in the case when an offender who comes into contact with high frequented areas would act as a vector, carry numbers of others’ DNA and subsequently deposit those DNA of innocent people onto weapon or crime-related item/scene. As a result, there will be an occurrence of mixture profile and those background DNA could appear as major or minor
contributors. In addition, ‘the hidden perpetrator effect’ could probably arise especially if the offender is a poor shedder. Hence, an assessment of level of background DNA in uncontrolled settings where transfer events repetitively occur in public places such as door handles, handrails or vending machines, would demonstrate ruthlessness of background DNA in real-life situation and in what way immoral it is in profiling stage. The findings can be utilised to assist forensic scientists confirm the assumption that the presence of unknown profiles essentially obtained from environmental DNA and escalate level of awareness in interpretative process of investigation.
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THE PREVALENCE OF ENVIRONMENTAL DNA ACROSS MURDOCH UNIVERSITY
Abstract
DNA profiling technology has been employed in forensic science for years with the purpose of individual identification in cases of interrogation or exoneration of suspect(s). The ability to detect minute amount of trace DNA and the increased sensitivity of DNA analysis approaches, not only allows investigators to amplify target DNA associated with a crime event, but also enables the discovery of contaminating DNA resulted from pre-existing environmental DNA at the scene of crime. Therefore, these confounding DNA contamination could negatively influence outcomes. The aim of the study is to investigate the prevalence environmental DNA within Murdoch University by performing DNA quantification together with demonstrate the complexity of DNA profiles retrieved from various sites on campus. The findings intensely confirmed that frequently visited areas at Murdoch provide a mixed profile with a variety level of contributor numbers at each site while a full profile cannot be obtained.

Keywords: Forensic science, Environmental DNA, Background DNA, DNA profiling

Introduction
Locard’s Exchange Principle, ‘every contact leaves a trace’, is commonly known in forensic discipline. Thus, it is undeniable that crime scene exhibits are exceptionally crucial, as they may have some anticipated evidential value to an investigation, due to the interexchange between those exhibits and crime-related individuals, when the crime was being committed. In forensic casework, exhibits can range from trace samples to body parts and frequently extend to documents, photographs, besides other related case materials. The qualifying factor which makes an object an exhibit, is that it should also have some anticipated evidential value, which means that it could possibly be produced to a court or enquiry (1). Additionally, exhibit types vary depending on their nature as well as origins, and consequently its uniqueness offers distinctive answer for an investigation. For instance, bloodstains at crime scene would provide information about how blood is deposited on surfaces, and allow investigators to determine a cause or position where blood is originated from, while DNA derived from bloodstain samples would disclose a source(s) of blood. Hence, trace evidence such as DNA becomes utterly valuable, since they hold individual information of suspects and/or victims.

Frequently, it is unavoidable for investigators to deal with ‘trace DNA’ or ‘touch’ DNA in cases where bodily fluid is not available for individual identification purposes. Trace DNA or Low Copy Number (LCN) DNA refers to ‘any sample which falls below recommended thresholds at any stage of the analysis, from sample detection through to profile interpretation, and cannot be defined by a precise picogram amount’ (2).
Nowadays, the sensitivity of DNA profiling methodology has significantly been enhanced due to the improvements of primer design, buffer composition as well as amplification conditions (2). As a consequence, the increased sensitivity of freshly invented methodology, effectively not only allows the amplification of incredibly small quantities of biological material such as DNA, but also simultaneously couples with the potential of detecting contaminant DNA, which results in profiles containing multiple numbers of contributors (3). Several studies conducted a comparison between recent systems and previous technologies, including the one directed by Ballantyne, Poy (4), proved that contemporary methodology such as Promega PowerPlex®21, has the ability to detect background DNA samples which were collected from surfaces within their laboratory, whereas present used system Life Technologies Profiler Plus® is not able to.

The DNA evidence given to the court is the scientist’s interpretation of the laboratory analysis, expressed in terms of a statistical calculation of the likelihood of a match between a crime scene profile and the accused’s profile. A mixed profile will reduce the statistical likelihood of a match, thus falling the probative value of the evidence. That is, an accused may argue that their DNA is on the profile adventitiously. Depending on the other evidence, this may be sufficient to cast a reasonable doubt on the prosecution’s case (5). Several high-profile cases in Australia have potentially been involved with contamination issue, for instance, Farah Jama. In 2009, Jama was acquitted by the Victorian Court of Appeal after serving 16 months in prison following a conviction of rape in the County Court of Victoria. The case was unusual in that the conviction was based solely on DNA evidence, with no other evidence implicating the accused (5).

Gill (6) defined the terms “the hidden perpetrator effect” – when the perpetrator’s DNA is absent from the crime stain then the donor of the contaminant DNA becomes a suspect and the perpetrator is hidden. This incident leads to the “association fallacy” where an innocent individual may be implicated in a crime and his DNA wrongly attributed to a body fluid. If this is semen, for example, then it will compound the error further by suggesting the activity of sexual assault. Furthermore, Gill also gave a definition “the naïve investigator” which established on the incident of wrongful arrest of Adam Scott due to the investigator found that his profile was the closest match to a crime stain in a national DNA database, the case was reported by Rennison (7). The man’s DNA profile was eventually traced to a contamination incident, but the case is outstanding because the match was adventitiously obtained from a search of the national DNA database.

Mixed DNA samples from at least two contributors can be originated at the scene of crime or contamination during the process of investigation (8). Generally, the final results from the DNA analyses can be a full DNA profile, partial, blank, or mixed profile in case of containing two or more
contributors. Frequently, instead of having a full profile, the profile can also lack information because alleles are missing or ‘‘drop-outs’’, or vice versa, when extra alleles can be added to the profile or ‘‘drop-ins’’. These are artefacts present when performing DNA analysis on samples with minute amounts of DNA, degraded samples, samples with inhibitory compounds (or in combination) as well as the presence of contaminating DNA in samples (9).

Although Szkuta, Harvey (10) found that in considering the detectability of the contaminant sample, allele transfer and the corresponding average peak heights were slightly lower in the occurrence of a target sample compared to DNA-free. Whilst the detectability of transferred DNA declined slightly, the presence of any contaminating alleles within these profiles is still of concern. The target sample was consistently the major component within the profiles obtained. Hence, it can be inferred that the level of contamination and subsequent contact by the vectors had no impact on the target sample. However, in casework scenarios where the suspect profiles are not known, profile interpretation becomes complicated by the addition of contaminating alleles and the probative value of the evidence may be affected.

Contaminant DNA can be found at any stages of the investigation whether prior to the crime has been committed, during the investigation or within the laboratory (11). Within the forensic laboratory, an accumulation of cellular, purified and amplified DNA on surfaces of tools, equipment and consumables instigate an occurrence of contamination due to it may be unintentionally picked up and transferred to exhibits when examinations being operated (12).

Moreover, contamination can also be introduced by manufacturer contaminated disposables or reagents with an obvious adverse effect on the results. A famous case study perfectly demonstrates a misled investigation caused by contamination ‘Phantom of Heilbronn’ in Germany and Austria, in which ‘sterile’ swab contamination during its manufacture caused police years to solve the issue and it linked 40 crime scenes incorrectly. Even though sterilisation can eliminate bacteria, viruses and fungi, it cannot destroy DNA of an innocent factory worker’s on cotton swabs. In this case, her DNA was found matching series of crime ranging from common thefts to brutal murders as the swabs used in those cases were contaminated by this worker (13).

Prior to and during the crime has been committed, the presence of trace DNA as background DNA in normal circumstances developed from the deposition of trace DNA onto surfaces either direct or indirect contact. Primary transfer (direct transfer) is not only when individual comes into contact with object, but also includes activities such as speaking, coughing, and sneezing within the vicinity of an item that may result in the transfer of DNA directly from an individual (14). Secondary transfer (indirect transfer) is the transfer of an individual’s DNA after deposition to another surface (15).
Supposedly, DNA could be transferred even further as a result of subsequent contact situations and can be referred as ‘tertiary transfer’, ‘quaternary transfer’ and further transfer depending on number of transfers. However, published research on tertiary, quaternary or further transfer scenarios is limited nowadays (16, 17). Subsequently, touched item, surface, or individual would unconsciously become a substrate and deposited DNA probably can be transferred further to another substrate, especially when the aspects such as type of substrates, moistness of biological material, and nature of contact facilitates the transfer events.

It has been demonstrated in numerous published investigations that background DNA can be encountered in both circumstances where cleaning regimes stringently and regularly applied as well as locations where the rules are abandoned. Taylor, Abarno (18) recently found that case files can also carry detectable DNA. The results show a reasonable retrieval of DNA which would be expected as the high amount of contact with individuals across the workplace. In addition, items used most frequently such as chairs, phones, computer items, floors and communal office items such as water coolers also gave the highest recovery of DNA. The number of individuals detected in profiles is higher on items from more frequented areas. The same study also illustrates that non-sensitive areas within their lab (where an exhibit may pass through on the way to a sensitive area or where an exhibit may be stored exhibits, or samples taken from them, are sealed at all times in non-sensitive areas) more often gave profiles with higher contributor number (at least three contributors and can be up to five) due to the sites were cleaned less often.

In South Australia Police facilities, Henry, McGowan (19) found significant background DNA exists on a number surfaces and items. Investigation tools such as cameras posed the highest risk for DNA transfer with all samples tested yielding informative and highly mixed DNA profiles. In addition, the highest environmental DNA load was also found on benchtop, cupboard/drawer handles, and exhibit drying rails.

To observe contamination event caused by police officer during criminal investigation which resulting in secondary DNA transfer from evidence bags, Fonnelop, Johannessen (20) show that a relatively high amount of DNA was detected on the outside of the bags and can be transferred to an exhibit during examination. However, contamination by secondary transfer from outside evidence bags is less likely and could only account for a small proportion of the observed police contamination.

Moreover, Goray and van Oorschot (21) conducted the study of DNA during social settings and found that individuals are also able to act as vectors for multiple transfer events of foreign DNA. Correspondingly, Fonnelop, Egeland (22) observed foreign DNA present on a person’s hand can be transferred onto a new object and follow the transfer chain to the third object nonetheless this was
only a partial profile but gives support to the fact that under some conditions DNA can be transferred up to four times.

Although contamination risk can be reduced by increasing frequency of cleaning routine, the application of Personal Protective Equipment and an implementation of DNA-free consumable still background DNA shed from innocent people in general environment exists in all pervasive environment and it can remain intact up to years after deposition depending on the environmental conditions (23). Even though the investigation of trace DNA as background DNA has been conducted on many occasions, the majority of them was merely explored within laboratory and/or under controlled conditions where objects were decontaminated prior to the experiments operated and individuals or source of trace DNA were known and currently, the level of background DNA in general settings where environmental conditions, number of contacts, and the number of individuals cannot be controlled has not yet been investigated.

Since various studies prove that trace DNA is frequently present in environment and individuals can as vectors, it can be assumed that in the case when an offender who comes into contact with high frequented areas would act as a vector, carry numbers of others’ DNA and subsequently deposit those DNA of innocent people onto weapon or crime-related item/scene. As a result, there will be an occurrence of mixture profile and those background DNA could appear as major or minor contributors. In addition, ‘the hidden perpetrator effect’ could probably arise especially if the offender is a poor shedder. The study aims to assess background DNA levels in uncontrolled settings where transfer events repetitively occur in public places and demonstrate the callousness of DNA profiles retrieved in real-life situations, and in what way it could be immoral in profiling stage. The findings can be utilised to assist forensic scientists confirm the presence of unknown profiles essentially obtained from environmental DNA and escalate level of awareness in interpretative process of investigation.

**Materials and Methods**

**Sampling**

Full personal protective equipment (PPE) including coverall suit, mop cap, facemask, shoe covers, and doubled gloves were applied properly. Gloves were changed for each site prior to the collection. A drop of sterile water (Pfizer Water Steriluer) was deposited onto BBL™ CultureSwab™ EZ in order to perform single swab method. Samples were appropriately sealed and kept in 4 °C until DNA extraction commenced. Sampling areas chosen from frequently visited facilities and amenities at Economics, Commerce and Law Building (ECL) and the library on campus (see table 1). Moistened swab was used as a negative control and a buccal swab from the collector was a positive control.
Table 1 Displays the collection sites

<table>
<thead>
<tr>
<th>Sampling areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECL – handrail south (L)</td>
</tr>
<tr>
<td>Library – toilet door handle</td>
</tr>
<tr>
<td>ECL – handrail south (R)</td>
</tr>
<tr>
<td>Library - photocopier</td>
</tr>
<tr>
<td>ECL – handrail north (L)</td>
</tr>
<tr>
<td>Library – booth</td>
</tr>
<tr>
<td>ECL – handrail north (R)</td>
</tr>
<tr>
<td>Library – water fountain</td>
</tr>
<tr>
<td>ECL – lecture theatre 1</td>
</tr>
<tr>
<td>Library - microwave</td>
</tr>
<tr>
<td>ECL – lecture theatre 2</td>
</tr>
<tr>
<td>Library – vending machine</td>
</tr>
<tr>
<td>ECL – lecture theatre 4</td>
</tr>
<tr>
<td>Library – computer mouse</td>
</tr>
<tr>
<td>ATM A</td>
</tr>
<tr>
<td>Library – computer keyboard</td>
</tr>
<tr>
<td>ATM B</td>
</tr>
<tr>
<td>Positive control – author’s buccal swab</td>
</tr>
<tr>
<td>Beanbag</td>
</tr>
<tr>
<td>Negative control – sterile water</td>
</tr>
</tbody>
</table>

**DNA extraction and quantification**

DNA samples were subsequently extracted via QIAamp DNA Investigator Kit following the manufacturer protocols and the final elution volume of DNA was 40 uL. DNA quantification was performed using Quantifiler® Human DNA Quantification Kit operated in Applied Biosystems® ViiA™ 7 Real-Time PCR System. The final volume of PCR reaction was arranged to 12.5 uL. Thermal cycler conditions were set as 1x cycle: 10 minutes 95 °C, 50% ramp rate; 40x Cycles: 15 second 95°C 50% ramp rate, and 1 minute 60°C 65% ramp, where data capture point was.

**DNA amplification and profiling**

Samples with detectable DNA then profiled via Promega PowerPlex®21 using ProFlex™ PCR System. Thermal cycling conditions were set following manufacturer’s protocols. Detection of PCR products was achieved throughout capillary electrophoresis (Applied Biosystems® 3730xL Genetic Analyzer). Due to the limitation of resources, POP-4® polymer was replaced by POP-7™ polymer and CC5 ILS 500 was used as allelic ladders instead of WEN ILS 500. The manufacturer does not recommend the use of POP-7™ polymer as the artefacts may migrate within the fluorescein and JOE channels however this issue had seriously been taken into account during an interpretative process.

**Data analysis**

Fragments were analysed with SoftGenetics GeneMarker software version 1.95. An assessment of allelic ladders was performed in order to ensure that all peaks have been called correctly and the RFU cutoff was set at 50.
Results

Quantity of DNA

Standard curve with was plotted with an $R^2 = 0.995$. The majority of frequented places across Murdoch University covered with detectable amounts of DNA. Astonishingly, there was only one out of four handrails that provided measureable quantity of DNA. Lecture theatre 4 appeared to foster the highest quantity of DNA among three other lecture theatres as well as this observation. Low level of DNA was discovered from a beanbag and one of the ATM machines (ATM A) located nearby while all samples obtained from the library presented positive results (see table 2).

Table 2 shows qPCR results

<table>
<thead>
<tr>
<th>Location</th>
<th>DNA quantity (ng/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECL – left handrail (south)</td>
<td>undetermined</td>
</tr>
<tr>
<td>ECL – right handrail (south)</td>
<td>undetermined</td>
</tr>
<tr>
<td>ECL – left handrail (north)</td>
<td>undetermined</td>
</tr>
<tr>
<td>ECL – right handrail (north)</td>
<td>0.011</td>
</tr>
<tr>
<td>ECL – lecture theatre 1</td>
<td>0.473</td>
</tr>
<tr>
<td>ECL – lecture theatre 2</td>
<td>0.182</td>
</tr>
<tr>
<td>ECL – lecture theatre 4</td>
<td>1.042</td>
</tr>
<tr>
<td>ATM A</td>
<td>0.004</td>
</tr>
<tr>
<td>ATM B</td>
<td>undetermined</td>
</tr>
<tr>
<td>Beanbag</td>
<td>0.004</td>
</tr>
<tr>
<td>Library – toilet door handle</td>
<td>0.059</td>
</tr>
<tr>
<td>Library – photocopier</td>
<td>0.006</td>
</tr>
<tr>
<td>Library – booth</td>
<td>0.015</td>
</tr>
<tr>
<td>Library – water fountain</td>
<td>0.006</td>
</tr>
<tr>
<td>Library – microwave</td>
<td>0.160</td>
</tr>
<tr>
<td>Library – vending machine</td>
<td>0.072</td>
</tr>
<tr>
<td>Library – computer mouse</td>
<td>0.127</td>
</tr>
<tr>
<td>Library – computer keyboard</td>
<td>0.088</td>
</tr>
<tr>
<td>Positive control</td>
<td>10.967</td>
</tr>
<tr>
<td>Negative control</td>
<td>undetermined</td>
</tr>
</tbody>
</table>

STR typing

In open-air areas, DNA profiles including two contributors were present on the right handrail located at the north of the ECL buildings. A partial female profile was recovered from ATM A while a beanbag gave an incomplete profile of a male donor.

Within the ECL buildings, mixed profiles including a female as a dominance among two minor contributors obtained from lecture theatre 1 as up to five alleles exhibiting at two loci (D16S539 and THO1). At lecture theatre 2, a two-people mixture which appeared to have a female as a major contributor was recovered whereas a partial profile of a female retrieved from lecture theatre 4.
In the library, a profile recovered from toilet door handle displayed various contributors when there were as many as seven alleles exhibiting at the D1S1656 locus in addition to six alleles at six loci, suggesting there were at least three donors. Surprisingly, only a partial profile of a male can be obtained from a photocopier and the same trend occurred with a profile derived from a water fountain. At a booth table, a mixture of male and female contributors has been recovered, the profiles exhibiting four alleles at four loci (D3S1358, D18S51, D2S1338, D19S433).

Furthermore, an indistinguishable highly mixed profile was obtained from a microwave located in 24/7 common room. Seven alleles displayed at two loci (D1S1656, D2S1338) indicating there were up to four people and females appeared to be predominant. Whilst distinguishable profiles have been recovered from a vending machine placed nearby, demonstrating that two donors were likely to be females.

Additionally, a mixed profile gained from a mouse of a public computer, originated from three individuals due to there were up to six alleles presenting at three loci (D1S1656, D21S11, D12S391) and additionally, both male and female donated their profiles almost equally. While a keyboard from the same computer provided the same trend since there were as many as seven alleles exhibiting at six loci (D1S1656, D6S1043, D18S51, D2S1338, D8S1179, FGA). However, both mixtures from the mouse and keyboard were not distinguishable. Sampling sites together with number of alleles in each locus shown in table 3.
<table>
<thead>
<tr>
<th>Location</th>
<th>Number of allelic peaks exhibiting at the locus (loci)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>ECL – handrail north (R)</td>
<td>THO1</td>
</tr>
<tr>
<td>ECL – lecture theatre 1</td>
<td>-</td>
</tr>
<tr>
<td>ECL – lecture theatre 2</td>
<td>D8S1179</td>
</tr>
<tr>
<td>ECL – lecture theatre 4</td>
<td>-</td>
</tr>
<tr>
<td>ATM A</td>
<td>-</td>
</tr>
<tr>
<td>Beanbag</td>
<td>-</td>
</tr>
<tr>
<td>Library – toilet door handle</td>
<td>D13S317, CSF1PO, vWA, D7S820, FGA</td>
</tr>
<tr>
<td>Library – photocopier</td>
<td>-</td>
</tr>
<tr>
<td>Library – booth table</td>
<td>D3S1358, D18S51, D2S1338, D19S433</td>
</tr>
<tr>
<td>Library – water fountain</td>
<td>-</td>
</tr>
<tr>
<td>Library – microwave</td>
<td>D3S1358, THO1, D5S818</td>
</tr>
<tr>
<td>Library – vending machine</td>
<td>CSF1PO, THO1, D5S818, D8S1179</td>
</tr>
<tr>
<td>Library – computer mouse</td>
<td>D3S1358, Penta E, D16S539, D2S1338, CSF1PO, Penta D, vWA, D8S1179, FGA</td>
</tr>
<tr>
<td>Library – computer keyboard</td>
<td>D3S1358, D13S317, Penta E, D21S11, D19S433</td>
</tr>
</tbody>
</table>

Table 3 Demonstrates number of allelic peaks exhibiting at a locus (loci). Mixed profiles with two contributors were found at ECL handrail and lecture theatre 2 as four alleles exhibited one locus. While a mixture contained three contributors (five alleles at two loci). In addition, the largest number of allelic peak is seven suggesting that it could be up to four donors to the mixed profiles recovered from a toilet door handle, a microwave and a keyboard located in the library.
Discussion

Across Murdoch University, the majority of sampling areas (77%) on campus provided detectable DNA. The largest number of contributors encountered in this observation was four since up to seven allelic peaks attributed to the profiles obtained from a female toilet door handle, a microwave as well as a computer. Fewer number of contributors was recovered from the lecture theatres as students spent considerable duration time shedding their DNA during the classes undergoing.

Interestingly, incomplete profiles were retrieved from an ATM machine, a photocopier and a water fountain, this might be due to the manner of contact as the amenities require only the touch of fingertips in a short period of time during the use. Nevertheless, this circumstance did not apply to a microwave and a vending machine as severely mixed profiles have been recovered from them. For the microwave, the presence of mixed profile could possibly be due to the greasy from food covering all over the microwave control panel area which eased the translocation of dry skin cell (trace DNA) from individuals onto the surface while the buttons of the vending machine have a fair amount of space between themselves and the machine (4 mm approximately) where DNA could easily endure and accumulate subsequently.

In open-air areas where DNA could be partially exposed to elements (i.e. sunlight, heat or humidity), DNA degradation should definitely be taken into consideration as a mixture of two males obtained from a handrail gave extremely weak signal besides an absence of alleles at more than two loci and the similar trend also occurred with an incomplete male profile recovered from a beanbag. Additionally, the donors might be good shedders while others who previously came into contact with the handrail or beanbag might have a poor shedding status. Furthermore, the surface of handrail is smooth and non-porous which promotes further transfer events and this might be the cause why only one out of four handrails can be retrieved whereas a beanbag surface was rough which enables the assembly of DNA on the crease.

Female appeared to be a predominant contributor in the most profiles in this study and it might be due to the fact that male has higher metabolism rate than female (25) hence they generate more sweat and consequently resulting the skin nourished which decrease the possibility of DNA shedding. Cleaning routine is a vital factor that could generate the alterations of the DNA recovery here as some of participating locations might have been cleaned by staff prior to the sample collection.

DNA literally exists everywhere in our environment and the findings indicate that the variations of DNA in terms of quantity and quality, depend on the manner of contact, substrate type as well as conditions of the location. In forensic point of view when the case based solely on trace DNA, if the offender has come into contact with commonly touched areas especially when they have already
carried significant number of DNA donors, the possibility that the background DNA would cause complexity during the interpretation could potentially be arise. However, further investigation of transfer events from highly frequented spots onto an object is recommended in order to substantiate ‘the hidden perpetrator effect’.

Acknowledgement

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