

## Direct PCR in Forensic Science- An overview

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**ABSTRACT:** Direct PCR was first used in the field of microbiology, where it was more commonly known as colony PCR. Since then, many more applications of direct PCR has been described in other fields where it has aided in diagnosis of infectious diseases and in botany. Direct PCR is a technique where amplification is carried out on samples without prior extraction, purification or quantification. In forensic DNA, direct PCR is currently limited to amplifying blood and buccal stained FTA<sup>®</sup> cards using specially designed multiplex kits. These multiplex kits have improved buffer-polymerase systems which are more tolerant to inhibitors present on FTA<sup>®</sup> samples. In 2004, the UK government highlighted the need to continue improving police capabilities in the areas of recovering evidence and rapid analysis of body fluids and other relevant forensic samples. Based on these requests, several key drivers were identified to include the development of faster, cheaper and better forensic science, which is possible with direct PCR. The need for faster, cheaper, better forensic science also becomes more apparent with the increasing number of samples subjected for DNA analysis after the implementation of programmes such as the likes of the DNA Expansion Programme in the UK and the DNA Field Experiment in the US. However, just like any other scientific technique, direct PCR does have its pitfalls which could be overcome with several modifications to the conventional DNA profiling workflow.

**Keywords:** Direct PCR, colony PCR, DNA profiling

### Introduction

Direct PCR is a technique where samples are subjected to amplification without first having to go through the extraction process. Direct PCR has been widely used in molecular microbiology since 1989 [1], where it is more commonly known as colony PCR. Colony PCR is used as a rapid screening method for large numbers of bacterial cells for a gene of interest [2, 3]. It can be used to confirm success of ligation of a gene of interest into a plasmid and transformation of plasmids into bacterial cells. It has been known to be more cost effective and less time consuming compared to conventional miniprep techniques. To perform colony PCR, well isolated colonies are picked up by either using a pipette tip or a toothpick, suspended in either sterile distilled water or TE buffer and incubated at 95 °C for about 5 to 10 minutes [2]. An aliquot of the suspension is then subjected to PCR. It is also possible to skip the incubation step and suspend the colony in the PCR master mix and proceed for amplification. Besides immersion in a hypotonic solution (water), high temperatures during the initial hot start cycle will aid in rupturing bacterial cell walls and release the bacterial DNA/plasmid into the master mix to

be subsequently amplified. The same principle behind colony PCR is also applied in direct PCR.

Currently there are many applications to direct PCR. Direct PCR is used to identify target DNA sequence of pathogens in clinical samples to decide on the treatment strategy [4, 5]. Often diagnosis is achieved with standard PCR but even this takes time as the samples will first have to undergo extraction and purification. With direct PCR, rapid diagnosis followed by treatment is possible. A multiplex protocol for detection of virulent genes in *E.coli* in cases of severe food poisoning has also been found successful by using direct PCR [6]. Direct PCR has also been used in botany whereby plant DNA is amplified directly from the leaves [7, 8].

The use of direct PCR in forensic science is currently being explored after its introduction in molecular biology 20 years ago [1]. Direct PCR has been used to amplify buccal and bloodstained FTA<sup>®</sup> cards [9, 10], and various crime scene samples [11-13] especially those with blood and semen stains. Semen stains are quite common in sexual assault cases, where sperm cells are the main source of DNA from the (often male) offender. Unlike epithelial

cells, sperm cells need more stringent techniques to release their DNA due to their structure [14]. DNA contained in the sperm heads are tightly associated with a group of proteins called protamines, which make the sperm DNA highly condensed [14, 15]. This makes obtaining DNA from sperm cells challenging, however by increasing the incubation time at high temperature during the hot start cycle, it was possible to obtain DNA from sperm cells using direct PCR [13].

There are several concerns with direct PCR. Firstly, inhibitors present in samples such as blood, soil and denim may inhibit polymerase enzymes. Blood contains haematin and various other compounds which are known inhibitors to polymerase enzymes [16, 17]. Samples which have come in contact with soil might have humic acid which is also a known inhibitor [18, 19]. Samples deposited on denim usually pose problems in obtaining DNA profiles due to the presence of indigo dyes [20, 21]. There has been advancements in buffer-polymerase technology which can reduce the influence of these inhibitors on the PCR process [22]. An indication of the presence of inhibitors in the sample, such as the use Internal PCR controls (IPC) in multiplexes, could be useful to differentiate no profiles obtained due to insufficient template DNA and those caused by inhibitors, and to decide if purification is necessary in order to obtain a good quality DNA profile [23]. Secondly, the absence of a quantification step complicates STR analysis as most multiplexes work best within a narrow range. No profiles might be observed when amplifying less than 200 pg of DNA, whilst amplifying more than 2 ng might give rise to various artefacts that complicate interpretation [24, 25]. Without being able to find out how much DNA is in the sample, finding the right balance in DNA quantity would be an issue. That is why there are strict guidelines to follow when using commercial direct PCR kits available in the market.

The immergence of commercial direct PCR kits in the market in recent years has shown that there is potential for this technique to develop. Most of the kits target FTA<sup>®</sup> samples deposited with blood or buccal cells [26-28]. These kits are specifically designed to cater for database or paternity cases where the majority of samples analysed are FTA<sup>®</sup> cards. Components of FTA<sup>®</sup> cards include agents to lyses blood cells and preserve DNA from further degradation [29, 30]. These

components, if not removed prior to PCR can inhibit most DNA polymerases. The direct PCR kits have better buffer systems which allow the amplification of blood cells on FTA<sup>®</sup> without the need for prior washing [26, 27]. However, there have been reports of less satisfactory results obtained from buccal cells collected using Omni swabs indicating that these kits may not be as versatile in their application [31].

### Why use direct PCR?

In 2004, the UK government published the Police Science and Technology Strategy which highlighted the need to continue improving police capabilities in the areas of recovering evidence and rapid analysis of body fluids and other relevant forensic samples [32]. Based on the recommendations of this strategy, Mennell and Shaw [33] identified drivers for the improvements highlighted by this strategy which include the development of faster, better and cheaper forensic science. The speeding up of the investigative process leads to benefits such as increased public confidence in the process of investigation, reduced crime by catching offenders earlier and reduced overall cost of an investigation [33]. By subjecting forensic samples to direct PCR, it has the potential of obtaining DNA profiles faster, with an increased chance of obtaining a good quality DNA profile, and at a reduced cost compared to conventional DNA profiling procedures.

Most of the samples so far typed using direct PCR have been blood, semen or buccal cells. No reports have been obtained to date on the use of direct PCR to analyse low template DNA samples in forensic casework. When samples are subjected to extraction, there is a significant loss of DNA regardless of which extraction method is applied [34, 35]. When dealing with low template DNA where less than 100 pg of starting template is obtained, any further loss of DNA can considerably affect the quality of DNA profiles obtained. In a study carried out by Raymond *et al.* [36] where various touched items were subjected to conventional DNA profiling, almost half of the samples tested did not produce a DNA profile while only 8% of the samples gave full single DNA profiles (Figure 1). Factors that are thought to influence loss of DNA during extraction are the number of tube changes, the number of washing steps and the capacity of DNA to adsorb to matrices [35]. If it is possible to influence any one or more of those

factors, the loss of DNA can be significantly reduced. There is also an increased risk for sample contamination and transfer error

because of the increase in sample handling time during extraction.

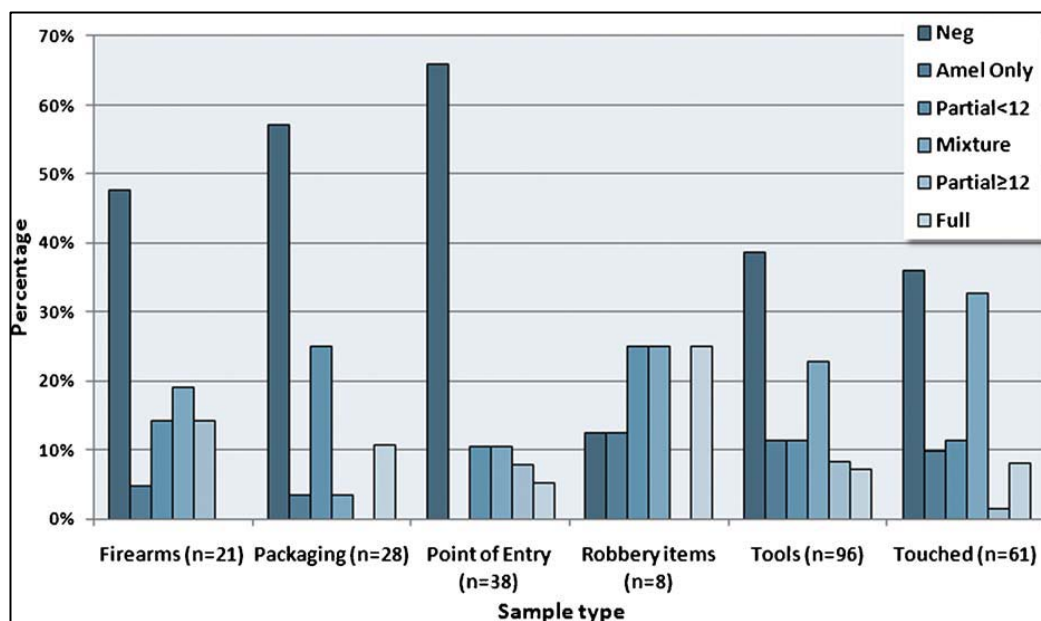


Figure 1: Completeness of DNA profiles obtained based on type of touch samples. Adapted in full from [36] (Reproduced with permission)

With direct PCR, there is no tube transfer and purification steps involved after the initial transfer of sample into the PCR tube [37]. Therefore, the loss of DNA associated with tube transfers and washings can be eliminated. Since the PCR tube is the only tube the DNA comes in direct contact with, there is minimal loss of DNA due to adsorption to polyethylene reaction tubes [38]. By subjecting DNA samples to direct PCR all three factors leading to loss of DNA can be minimised. Furthermore, reduction in tube transfers leads to the possibility of less handling errors such as contamination, transfer error or loss of samples [37, 39].

The number of cases subjected to forensic analysis in recent years has increased significantly [40]. The implementation of the DNA Expansion Programme in 2000 by the British Home Office saw a 74% increase in DNA material collected, a 76% increase in DNA submitted for processing, and a 32% increase in crime scene samples uploaded into the National Database over the course of the programme [41]. Following the success of the DNA Expansion Programme, the U.S. Department of Justice implemented a similar programme called the DNA Field Experiment to evaluate the expansion of DNA evidence collection and testing to the investigation of property crimes [42]. Prior to this, DNA

evidence was almost exclusively used to investigate violent criminal incidents [42]. Although no programmes such as the likes of the DNA Expansion Programme and the DNA Field Experiment has been officially introduced by the Malaysian Government, The Department of Chemistry Malaysia has seen an annual increase of about 20% on samples submitted for DNA analysis.

Since the implementation of the DNA Expansion Programme and the DNA Field Experiment, more samples are collected and submitted for DNA analysis especially those involving volume crimes like burglary and vehicle crimes [42]. Samples obtained from volume crimes involve body fluids and swabs of touched items from entry and exit points, searching the house, gathering items, tools and items left behind and disposing of items [42]. In a report put together by the US Department of Justice, it showed a significant increase in the number of cases received and the number of yearend backlogs from 2005 to 2009, Figure 2 [43]. The same report claims that the demand for DNA testing is rising due to the increased awareness of the potential value of DNA evidence [43]. There has been an increased request for analysis of 'touch DNA' samples because of the awareness that it is now possible to test smaller amounts of DNA [43]. By implementing direct PCR for these

samples, the amount of time it would take to extract, purify and quantify the sample can be eliminated altogether, and with faster turnaround for DNA analysis exceptionally quick arrests of offenders can be achieved [33].

Tilley and Ford [44] in 1996 were the first to raise the issue of processing DNA material from crime scene and recommended that the time taken should be reduced by both the police and the forensic science service providers in order to maximise the opportunities to solve crime with DNA evidence. Fast-tracking of investigations

involving DNA evidence has shown that it leads to more suspects being charged as a result of DNA matches [45]. Fast-tracking was a joint initiative between a UK police force and forensic science provider to speed up investigation of residential burglary offenses where DNA material had been recovered [45, 46]. With the implementation of this initiative, the duration of a burglary being reported and a suspect being charged was reduced from an average of 89 days to 45 days [45, 46]. With direct PCR, this duration could further be reduced as it eliminates the need for the extraction and quantification steps.

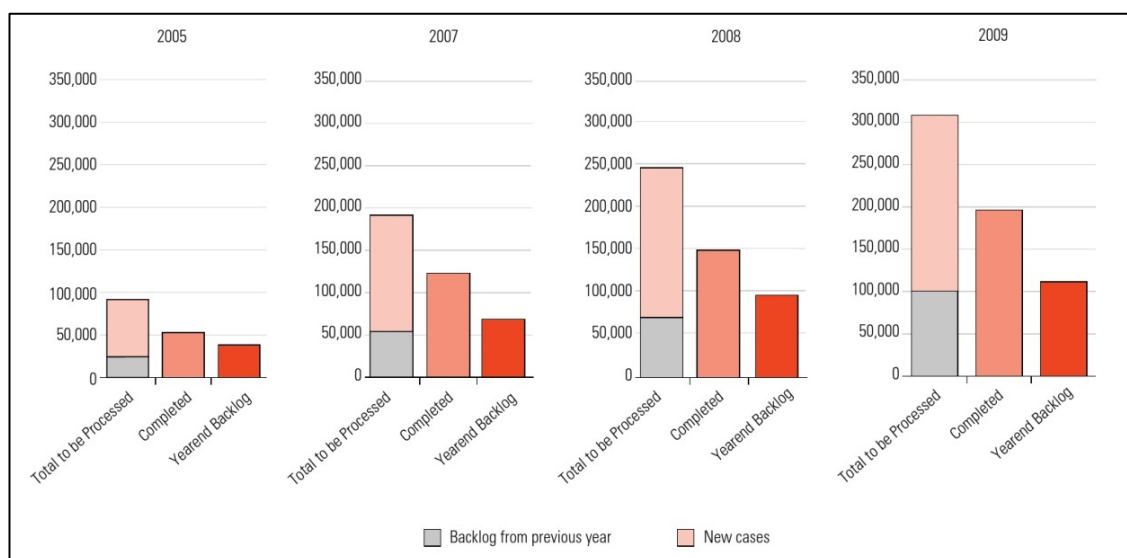


Figure 2: The number of cases and yearend backlogs from 2005 to 2009 in the US. Adapted in full with permission from [43]

Since the UK police force faced budget cuts of 20% after the government's spending review recently, costs involved for forensic analysis has been an issue [47]. Direct PCR is more cost effective as there is no need for expensive extraction, purification and quantification kits. Given that in most laboratories the principle cost lies in wages, the net labour time (*i.e.* the actual hands on time needed performing extraction, purification and quantification) is a good indication of the cost involved to generate a DNA profile [39]. A commercial extraction kit can cost around RM1000 to RM2000 for every 100 samples [48, 49], while a quantification kit can cost around RM3000 to RM6000 for every 300 samples [50-52]. The time involved to extract and quantify a batch of DNA samples can be anything from an hour to a few days, depending on the extraction methods used. If the net labour time is taken into account

together with consumable and reagent costs, these figures can increase significantly. If the extraction, purification and quantification processes are eliminated, which is possible with direct PCR, the amount of time and resources spent on a sample is reduced, and so would the net cost of processing each sample.

## Conclusion

After about 20 years being used in other fields, report of using direct PCR for database samples was first published in December 2009 [9]. Since then, the sudden explosion of commercial kits available in the market for direct PCR in recent years goes to show that there has been a significant demand for rapid analysis of forensic samples. It also indicates that direct PCR as a technique has the potential for further application and development in the field of forensic science.

The primary question that needs addressing is 'why is there a need for direct PCR in forensic DNA analysis?' The ever growing number of samples being submitted for DNA analysis coupled with the current economic climate has generated a dire need for 'faster, cheaper and better' forensic science which can be accomplished with direct PCR. The advantages of using direct PCR are as follows:

- ✓ *Faster*- The omission of the extraction and quantification steps reduces the overall time it takes to generate a DNA profile.
- ✓ *Cheaper*- The costs involved in purchasing expensive extraction and quantification kits can be reduced when using direct PCR.
- ✓ *Better*- Better DNA profiles could be obtained by using direct PCR because there is no loss of DNA associated with extraction protocols.

One of the disadvantages of using direct PCR is that the amount of DNA present in a sample is not known, therefore could suffer from artefacts associated with increased sample concentration. This may not be such an issue with low template DNA where the amount of DNA is very low, but with biological stains, this could be a problem if too much DNA is amplified. Another limitation of implementing direct PCR for forensic casework are for large items like garments, the exact area which has come in contact with the victim or perpetrator could be difficult to identify and therefore the area where the fibres should be recovered for PCR could prove to be difficult. In circumstances such as these, it would be recommended that the garment is swabbed and a portion of the swab be subjected to direct PCR for a better chance of obtaining a DNA profile.

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