Buletin SK 10(2)(2026): 86-95
*https://doi.org/10.17576/bsk-2026-10(2)-01* ISSN 2250-1852

**Artikel Asli / Original Article**

# The Band Patterns of RAPD-PCR Profiling for Comparative Study of Low-Quantity Human DNA (Hair Shaft) and E. coli

## Corak Jalur Profil RAPD-PCR Bagi Kajian Perbandingan DNA Manusia Berkuantiti Rendah (Batang Rambut) dan E. coli

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Received date: 7 July 2026
Revised date: 18 August 2026
Accepted date: 3 September 2026
Published date: 5 September 2026

## ABSTRACT

Forensic analysis of human hair shafts remains one of the biggest challenges, as DNA is often limited and degraded, frequently susceptible to microbial contamination. Traditional DNA typing is often inapplicable for such samples. For contaminated evidence, the RAPD approach offers a flexible and sequence-independent alternative to creating genetic profiles. This work assesses the applicability, reliability, and discriminatory power of RAPD in distinguishing the human DNA profile from a typical microbial contamination. The study developed random DNA profiles using human hair shaft DNA and compared the resulting banding patterns generated by E. coli as a model for non-human contamination, the microbiome. To assess the efficiency of RAPD, an experimental approach was used by the study, evaluating the influence of sample condition, primer choice, and PCR parameters on the quality and reproducibility of profiles obtained from both human hair samples and E. coli. Compared to the DNA profile obtained from E. coli, RAPD generates non-overlapping, unique banding patterns for human hair shaft DNA, proving its efficiency in discriminating the species under question. In addition to illustrating RAPD's capability for species-specific exclusion of microbial contaminants, this successful discrimination fills crucial gaps in the reproducibility validation of RAPD for human trace evidence processing. The results affirm that RAPD is a reliable, fast, and cheap initial screening method for problematic trace evidence. Responding to the need, as stated in recent literature, for validated rapid screening techniques, the research offers standardized guidelines that will advance the adoption of RAPD, particularly in resource-scarce forensic laboratories, toward supporting investigations in which traditional methods may fail

Keywords: RAPD, genetic profiles, hair shaft, microbial

## ABSTRAK

Analisis forensik rambut manusia kekal sebagai salah satu cabaran terbesar, kerana DNA sering terhad, terdegradasi, dan sering terdedah kepada pencemaran mikrob. Pemprofilan DNA tradisional sering tidak sesuai untuk sampel sedemikian. Bagi bukti yang tercemar, pendekatan RAPD menawarkan alternatif yang fleksibel dan bebas aturan untuk mencipta profil genetik. Kerja ini menilai kebolehgunaan, kebolehpercayaan dan kuasa diskriminasi RAPD dalam membezakan profil DNA manusia daripada pencemaran mikrob biasa. Kajian ini membangunkan profil DNA rawak menggunakan DNA rambut manusia dan membandingkan corak jalur yang terhasil daripada oleh E. coli sebagai model untuk pencemaran bukan manusia, iaitu mikrobiom. Untuk menilai kecekapan RAPD, pendekatan eksperimen telah digunakan oleh kajian ini, menilai pengaruh keadaan sampel, pilihan primer dan parameter PCR terhadap kualiti dan pengulangan profil yang diperoleh daripada kedua-dua sampel rambut manusia dan E. coli. Berbanding dengan profil DNA yang diperoleh daripada E. coli, RAPD menghasilkan corak jalur unik yang tidak bertindih untuk DNA batang rambut manusia, membuktikan kecekapannya dalam membezakan spesies yang dipersoalkan. Selain menggambarkan keupayaan RAPD untuk pengecualian spesifik spesies terhadap bahan cemar mikrob, diskriminasi yang berjaya ini mengisi jurang penting dalam pengesahan kebolehulangan RAPD untuk pemprosesan bukti surih manusia. Keputusan mengesahkan


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bahawa RAPD ialah kaedah saringan awal yang boleh dipercayai, pantas dan murah untuk bukti surih yang bermasalah. Menjawab keperluan, seperti yang dinyatakan dalam kesusasteraan terkini, untuk teknik saringan pantas yang disahkan, penyelidikan ini menawarkan garis panduan piawai yang akan memajukan penggunaan RAPD, terutamanya dalam makmal forensik yang kekurangan sumber, ke arah menyokong penyiasatan di mana kaedah tradisional mungkin gagal.

Kata kunci: RAPD, profil genetik, batang rambut, mikrobial

## INTRODUCTION

While funding and staffing have increased due to the demand for DNA databases, other forensic disciplines, such as hair and fiber analysis, have not seen proportional investment. This disparity is particularly concerning because hair evidence is one of the most common biological traces found at crime scenes. Whether shed naturally or forcibly removed, hairs can provide crucial leads. Yet, their degraded genetic material often complicates analysis, leaving their full potential untapped in many investigations (Liu et al. 2023). Hair shedding is a natural process, and it is normal for between 50 and 100 hairs to fall out per day (Gong & Li 2014). External forces, such as violent contact between suspects and rape or murder, can also cause hair to pull out. Due to their small size, hairs are easily overlooked at the crime scene and are not always cleaned up and destroyed by the suspects. However, most of the evidence consists of hair fragments without roots. As a major part of DNA is in the root, hair shafts are usually problematic samples in forensic analysis. For these reasons, hair DNA typing is directed at mitochondrial DNA (mtDNA), which is present in high copy number in each cell, instead of nuclear DNA analysis (Almeida et al. 2011). Recent progress in molecular biology has revitalised forensic hair analysis, making it possible to extract meaningful genetic information even from compromised samples. Among many DNA markers available, random amplified polymorphic DNA (RAPD) is the simplest and can be performed in a moderate laboratory for most of its applications. In addition, RAPDs can cover much of the genome and have the advantage that no prior knowledge of the genome under research is necessary (Babu et al., 2020).

Random Amplified Polymorphic DNA (RAPD) has emerged as a valuable tool for analysing degraded or limited DNA samples, such as hair, which often lack sufficient nuclear DNA for conventional methods like STR (Short Tandem Repeat) analysis (Williams et al., 1990). Lui et al.'s study on hair DNA is highly degraded and fragmented and may no longer have sufficient intact templates for polymerase chain reaction (PCR). The quality and quantity of mtDNA and nDNA (nuclear DNA) are the important parameters that primarily determine whether genotyping can be achieved or not. Based on Bourguignon et al's (2008) study, telogen hairs at the crime scene were classified into three groups based on the material attached to their roots: type 1, with no visible soft tissue; type 2, with some soft tissue; and type 3, with lots of soft tissue. Along with the hair shaft, in the proximal-to-distal direction, their changes were irregular. For forensic practice, most of the hairs obtained from crime scenes were of type 1 hairs, which lack visible root tissue and represent the most common finding; they cannot be successfully analysed using standard short tandem repeat (STR) genotyping methods. Even Type 2 and 3 hairs, which contain some cellular material, often yield insufficient DNA for reliable STR analysis. Consequently, forensic scientists frequently resort to mtDNA analysis as an alternative, despite its limitations in individual discrimination, due to its higher copy number and greater resistance to degradation compared to nDNA. When conducting idiomatic nDNA analysis, most hair shaft samples found at crime scenes are not informative for STR profiles, and one must turn to mtDNA or protein analysis. The mtDNA is typically the reserve since it is present in a greater copy number and is more resistant to degradation than nnDNA. MtDNA is also far more informative than nDNA STR for maternal generational or distant kinship relationships.

When the amount of human DNA is extremely low or highly degraded, also referred to as high microbial biomass samples, microbial DNA can become the predominant template in the reaction. Low microbial biomass samples can be easily overwhelmed by contamination from background DNA and are more prone to technical biases such as over-amplification during PCR (Selway et al. 2020). Microbiome represented the collection of all taxa that comprise microbial communities, including bacteria, fungi, and protists in the intestinal tract and their relation to microbes in the oral cavity and protozoan populations in sewage- contaminated environmental water. Microbiome diversity is unique for every individual. Each strain of bacteria has its own genome, which includes thousands of genes (Knight et al. 2017). Besides this, *E. coli (Escherichia coli)* is also a predominant facultative microbe of the gastrointestinal tract and a pioneer of the human gut, being one of the first bacteria to colonise neonates at birth (Martinson & Walk 2020). *E. coli* has a genome with unique features such as a strikingly ordered structure, remnants of many phage insertion sequences (IS), and a high transport capacity toward the cytoplasm. In this regard, *E. coli* has a vast portion of its genome that remains

uncharacterised for defined molecular pathways or roles, paving the way for future basic research that may have a significant impact on applied research and development. For biotechnological applications, growth optimisation is also a required feature of genetically modified organisms. For *E. coli*, it has been recently shown that growth can be manipulated by removing nonessential genomic sequences that can also lead to the design of important biotechnological strains for industrial purposes. In nature, many bacteria contain self- replicating DNA molecules that can be harnessed for molecular biology applications. *E. coli* plasmids were the first ones to be extensively modified for such purposes. (Idalia & Bernardo 2017).

Recent studies have shown that molecular methods such as RAPD can identify the species-specific genetic profile of human and *E. coli* DNA even when the DNA of either or both is present in very minute or degraded quantities. RAPD profiling provides a unique, reproducible pattern of bands for *E. coli*, which does not overlap with human DNA and therefore can confidently identify or exclude bacterial contaminants in forensic analyses. Other studies using advanced methodologies for distinguishing E. coli have reaffirmed these findings, using low-resource and non-invasive methods such as surface-enhanced Raman spectroscopy (SERS) combined with machine learning to rapidly distinguish human DNA signals from microbial DNA without ambiguity. As summarised by W. Liu et al. (2023), the principle of RAPD is that one single short oligonucleotide primer, which binds to many different loci, is used for amplifying random sequences from a complex DNA template. This implies that the fragment amplified through PCR is dependent on the length and size of both primer and target genome. RAPD technology allows for the rapid screening of DNA sequence-based polymorphisms at a very large number of loci. The RAPD technique does not require prior sequencing of DNA. Only low quantities of template DNA are required for RAPD because of the involvement of PCR. No sequence data are needed for the design of primers since random primers are commercially available (Nandani & Thakur 2014).

## MATERIALS AND METHODS

The study used two primary sources of DNA: eight distinct human hair shaft samples (representing trace evidence) and eight independent purified Escherichia coli *(E. coli*) culture preparations (representing microbial contamination). The human hair shaft samples were collected and prepared using the 1st BASE PrimeWay Genomic II DNA Extraction Kit, following the manufacturer's established protocol for challenging samples. The *E. coli* DNA control spots were obtained from prepared FTA cards (representing a standard non-human control or model microbial contamination).

### EXTRACTION OF DNA (HAIR SHAFT AND *E. COLI*)

Genomic DNA was extracted from the FTA card punches derived from the E. coli control spots. The extraction of hair shafts was performed using the 1st BASE PrimeWay Genomic II DNA Extraction Kit, following the manufacturer's established protocol for DNA. The extracted DNA was then quantified and stored at -20°C until use.

### SELECTION OF PRIMER

Two arbitrary decamer oligonucleotide primers were selected for the RAPD- Polymerase ChainReaction (PCR): OPB-20 (GGA CCC TTA C) and OPB-18 (CCA CAG CAG T). These primers were used universally for all human and *E. coli* samples, resulting in 16 amplification reactions per species (8 samples x 2 primers).

### PCR AMPLIFICATION

The bases (complementary to the template) are coupled to the primer on the3’end (the polymerase adds dNTPs from 5’to 3’, reading the template from 3’to5’side; bases are added complementary to the template). RAPD-PCR was performed with a total reaction volume of 25 µL per sample. All the reagents are mixed, the appropriate cocktails for the desired number of samples are made, and complete mixing is ensured by tapping the tube and quick spinning. About 1.0 µL of the template DNA is added directly into the PCR tube containing the volume about 18.75 µL of deionised water, 0.25 µL Taq DNA polymerase (0.05 U/ µL), 0.50 µL of DNTPs (0.2 mM), 2 µL RAPD Primer (0.4 µm), 2.50 µL of Taq Buffer (with MgCl2), 1 µL of Genomic DNA (or 2x FTA disc punches). The thermal cycling parameters were designed for low-stringency amplification, consisting of 30 cycles executed on a thermal cycler. The polymerase Chain Reaction (PCR) is a relatively simple but powerful technique that amplifies a DNA template to produce multiple copies of a specific DNA fragment in vitro. The initial phase consisted of denaturation at 95 °C for 3 minutes. Then the cycle starts with the denaturation phase at 95 °C for 30 seconds, primer annealing phase at 30 - 40 °C for 30 seconds, DNA extension phase at 72 °C for 1 minute, and repeated cycles. At the end of the cycles, a final extension period of 7 minutes was given at 72°C. The amplified products were stored at 4 °C.

### AGAROSE GEL ELECTROPHORESIS OF PCR AMPLIFIED DNA

After completion of the PCR programme, the products are separated in 1% agarose by dissolving 0.6g of agarose powder in 60 ml buffer. After heating and cooling, 1 µL of the fluorescent nucleic acid stain RedSafe was added to the sample. For each well, a loading mixture consisting of 5 µL of PCR product and 1 µL of gel loading dye was prepared and loaded. A molecular weight marker (5 µL DNA ladder and 1 µL dye) was loaded into the reference well. Electrophoresis was performed under constant voltage conditions, 100 volts for 50 minutes. Amplified products appear as blue colour bands under the UV Transilluminator due to the intercalation of staining dye. The resulting DNA banding patterns were visualised under ultraviolet (UV) light and digitally photographed for analysis and comparison of human versus *E. coli* profiles. All amplification products are found to be reproducible when reactions are repeated using the same reaction conditions.

## RESULTS

For human DNA (Fig. 1), primer OPB-18 generated intense, discrete bands at higher positions in the gel, corresponding to longer amplicons in the mid- to high-molecular-weight range (approximately 3000–8000 bp). This indicates efficient and relatively specific priming at multiple loci, with stable primer–template pairing at the selected annealing temperature. Similar size ranges and discrete RAPD bands are commonly associated with polymorphic but reproducible loci in genomic DNA fingerprinting studies. Meanwhile, for primer OPB-20, it showed faint amplification products and smearing. At 30.6°C, a clearer flow of banding pattern below the primer Tm, suggesting that sub-Tm conditions favour sufficient primer–template hybridisation without excessive mismatch. The temperatures above 32°C weaken binding between the primer and template, making it less stable as the reaction temperature approaches or surpasses the natural point at which primers dissociate from their targets during annealing (Violita et al., 2024). This high amount of GC content in the primer can impact the primer temperature melting (Tm) (Rodríguez-Lázaro et al., 2013). According to Thornton and Basu (2011), the Tm difference between primer pairs should not be more than1–2□C, and at the temperatures 30.6°C (G) and 30.0°C (H), the Tm difference from the melting point of both primers (32 °C).

Meanwhile, E. coli templates (Fig. 2.) amplified with both OPB-18 and OPB-20 produced faint, closely spaced bands located lower in the gel, indicating a predominance of shorter fragments in a relatively narrow size range. Such patterns are typical of bacterial RAPD profiles, where compact genomes and high densities of complementary primer sites lead to multiple short amplicons and complex, highly polymorphic banding. The observation of smear- like patterns at the identified optimal annealing temperatures (31.8°C for OPB-18 and 30.6°C for OPB-20) further reflects heterogeneous amplification from many adjacent priming sites, a feature frequently reported when RAPD is applied to diverse bacterial isolates. In bacteria, the compact genome and frequent proximity of complementary primer sites favour the amplification of shorter fragments and can produce complex patterns where bands cluster in a relatively narrow size range, enhancing discrimination among closely related strains (AlZubaidy 2019).

Taken together, the human RAPD profiles demonstrate fewer, longer, and more clearly resolved amplicons, consistent with a larger, less compact genome in which primer binding sites are more widely spaced, yielding distinct mid- to high-molecular- weight bands under partially optimised annealing conditions. In contrast, the *E. coli* patterns, characterised by shorter, closely spaced, and sometimes smeared bands, reflect the higher density of primer target sequences in a compact bacterial chromosome and the consequent production of overlapping short fragments. Similar contrasts between broad, discrete bands in higher eukaryotes and dense, fine-scale patterns in bacteria have been documented in RAPD-based diversity and fingerprinting studies. These results highlight that subtle adjustments of annealing temperature around the primer Tm are critical not only for maximising band clarity and reproducibility, but also for tailoring RAPD conditions to the structural characteristics of different genomes when the goal is species- or strain-level discrimination.

## DISCUSSION

The experiment focused on the amplification of human DNA and *E. coli* DNA using the RAPD-PCR technique with primers OPB18 and OPB20,which provided unique patterns, underlining the efficiency of RAPD in differentiating species. OPB-20 and OPB-18 primers are known for their efficiency in different species and are able to display a large number of polymorphic bands, which are useful in genetic differentiation techniques.Among the techniques widely used in genetic studies of both plant and animal species regarding genetic diversity are the utilisation of RAPD markers and the OPB series of primers. These primers are composed of 10 nucleotides and are known for their ability to target random spots in the genomic DNA of a species for amplification, making way for the development of polymorphic bands that are used in genotypic discrimination. The result that human DNA generated distinct mid-to-large molecular weight fragments (3000-8000 bp) is a direct outcome of the size and complexity of the eukaryotic

genome, which is appreciably larger and less dense than in the case of the prokaryotes. The choice of 10-base arbitrary primers like OPB-18 and OPB-20 is expected to decrease the possibility of complementarity between the hybridised sites within the possible "PCR- amplifiable" range. This might result in fewer and larger DNA fragments with enhanced resolution (Williams et al. 1990).

These are highly supported by the results that the size of DNA fragments, commonly accepted for the reproducible sites in DNA fingerprinting studies, generally fall within the mid-to-large molecular weight range (Mraidi and Lafta 2021; Pacheco et al. 2006). On the other hand, the patterns for *E. coli* showed close faint bands using both primers. The samples were characterised by banding patterns that were much shorter, denser, and often a smear. This has been attributed to the compact nature of the bacterial genome, which is marked by high gene density and minimal non-coding DNA. Such a nature allows frequent and proximal matching sequences for primers, hence giving a crowded profile of small fragments which tend to overlap and form a smear as observed in Alonso et al. 2004. Hence, the dominance of small fragments, as observed for bacterial DNA patterns because of the small size of their genomes with densely filled primer-binding sites (Mraidi & Lafta 2021). The variation in band resolution between human and *E. coli* genomes underscores the role of genome architecture in performing RAPD analysis. As seen, the small size of the bacterial genome, apart from increasing its overall amplicon density, makes band interpretations difficult, a common occurrence in RAPD analysis (Silva et al. 2017).

An examination of its size and architecture becomes important in this context when evaluating a RAPD profile. Results using primer OPB18, which produced strongly intense as well as unique bands with a large molecular weight index, suggest efficient as well as specific priming on several loci. This is an indication that the primers efficiently bind to sparsely clustered sites of binding as required for the large size of the genome of the eukaryote organisms (Chroboková et al. 2011). These observations confirm the effectiveness of RAPD analysis for the efficient identification of genomic differences as stated in previous literature Lin et al. 1996), (Qasem et al. 2015). However, the primer OPB-20 resulted in a very weak amplification product with a smear. This can be attributed to a non-optimal binding condition resulting in decreased binding specificity and stability, particularly at high temperatures above the binding site of the primers' melting temperature (Tm) (Chroboková et al. 2011). The fact that the results show clearer bands at a low annealing temperature supports the finding that modifications in this parameter can facilitate enough binding of the primer to the template, hence improving the binding clarity (Qasem et al. 2015; Chroboková et al. 2011). More precisely, the results support the notion in the current literature that high temperatures can cause non-favourable binding and a possible binding mismatch (Qasem et al. 2015). The role played by primer OPB-18 with a sequence of 5'-CCA CAG CAG T-3' and its GC content of 60% was significant in terms of producing intense and discrete bands for human DNA samples, especially with low template concentration. This was because the three hydrogen bonds found in G-C base pair interactions are more stable compared to A-T base pair interactions, especially with a temperature adjustment designed for effective annealing.

A designed annealing temperature close to 30.6°C and slightly differing from the melting temperature values of about 32°C is a reflection of the relative stability induced by the GC content, enabling effective hybridisation and interaction with degraded genomic DNA (Lin et al. 1996). This adaptability in annealing temperature aligns with findings from prior studies, which emphasize the necessity of fine-tuning PCR conditions to maximize amplification efficiency and specificity, further reinforcing the relationship between primer design, GC content, and PCR performance (Qasem et al. 2015). Moreover, the conditions in the polymerase chain reaction show that small-scale variations in the annealing temperatures close to the determined Tm value for primers can greatly affect the intensity of the banding patterns (Chroboková et al. 2011). An important factor to consider in this process is the optimal variation in the Tm values of primer pairs, which should not exceed 1-2°C in order to obtain optimal conditions for the amplification process, thus reiterating the requirement for strict temperature control in the RAPD method in order toobtain optimal band patterns for effective specie-strain differentiation (Chroboková et al. 2011).

The successful amplification of human DNA from the shaft of a human hair is an important milestone in the techniques developed in forensic analysis, emphasising the role of RAPD analysis in the challenging DNA samples that tend to be greatly degraded. Standard short tandem repeat analysis is likely to fail in such low-template situations, given the need for longer target sequences (Vivodík et al. 2017). But the primers required for RAPD techniques will tolerate a 10-bp mismatch for the purpose of extension; such tolerance is expected to greatly enhance the chances for appropriate binding even for the most degraded DNA samples (Zhang et al. 2009).

Though there has been little research work employing both primers for DNA in humans, some information about their use in specific genetic tests can be deduced from their efficiency in amplifying specific areas of a genome. According to research in molecular biology, OPB-18 is relatively preferable

in performing fine genetic analyses and studies of diversity because, on average, this primer tends to produce a higher number of polymorphic bands in very heterogeneous individuals (Molin et al. 2019). The other primer, OPB-20, is even more applicable in agriculture due to its particular banding pattern that facilitated more specific identification of agricultural plant varieties (Moreno et al. 2005).

Notably, the differences in the resolution of amplicons in the human and bacterial DNA profiles illustrate a crucial point in genomic research; the design of primers ought to address the complexities of the target genomes. The differences exemplify the significance of choosing conditions suitable for each organism's genomic makeup, which might be fundamental in biodiversity analysis and microbial identification (Asano et al. 2010). Taken collectively, our data provide supporting evidence for the hypothesis that changes in PCR conditions, especially the use of varying annealing temperatures, play paramount roles in optimising the ability of RAPD markers to be highly effective. The present study, therefore, not only underlines the importance of RAPD analysis in the study of genetic diversity, among other fields, but also the importance of systematic primer design, especially in the context of mixed genomic samples, in optimising the analysis of RAPD markers.

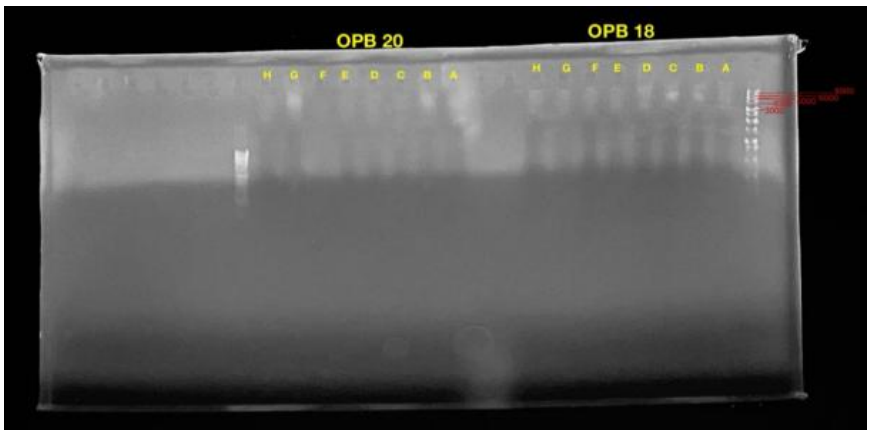



FIGURE1 Gel profile showing bands of human DNA from both primers (OPB-20 and OPB-18). Ladder 1kb, annealing temperature: 40.0°C (A), 39.1°C (B), 37.9°C (C), 36.3°C (D), 33.9°C (E), 31.8°C (F), 30.6°C (G), and 30.0°C (H)

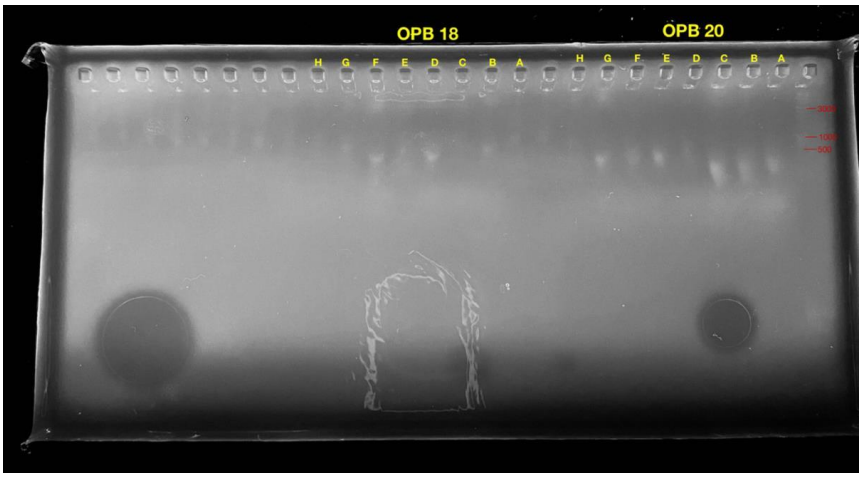



FIGURE 2 Gel profile showing bands of *E. coli* from both primers (OPB-20 and OPB-18). Ladder 1kb, annealing temperature: 40.0°C (A), 39.1°C (B), 37.9°C (C), 36.3°C (D), 33.9°C (E), 31.8°C (F), 30.6°C (G), and 30.0°C (H).

## CONCLUSION

In concluding the synthesis of findings, it is observed that human RAPD patterns generate fewer

and larger bands compared with *E. coli*, with bands appearing more pronounced and less densely packed compared with those found in *E. coli*, including smear patterns. These findings are justifiable due to the less compact nature and relatively larger size of a human genome compared with a bacterial genome, with more dispersed binding sites for primers compared with eukaryotic sites observed in bacterial DNA arrangements for RAPD analysis (Mraidi & Lafta 2021; Conner & Wood 2001). These variations observed among bands relate as expected to findings found among literature sources, with comparisons between eukaryotic and prokaryotic arrangements found in genes analysed via RAPD protocols. One of the most important aspects of the effective use of RAPD-PCR is based on the optimisation of parameters, specifically the annealing temperature of the primers. The optimal ranges for annealing temperatures are essential for the highest possible resolution of bands; however, the ranges identified must be properly cited for relevance (Herman et al. 2019). This is even more pertinent for the difference in prokaryotic (*E. coli*) versus eukaryotic (human) structure that affects DNA amplifications under different parameters (Chen et al. 2019).

Given its success, studies have presented standardised protocols for the utilisation of RAPD in forensic analysis. The availability of sequence-independent techniques is a definite advantage when dealing with contaminated or degraded samples and renders RAPD-PCR a necessary tool for forensic analysts facing resource limitations. This makes it a part of forensic practices, which comes in handy in cases where all other techniques have failed (Ramasoota et al. 2000). Following the success of RAPD-PCR in discriminating between human hair DNA and *E. coli* in this study, there are various future research avenues to further substantiate RAPD- PCR as an efficient means for forensic analytical purposes. Generally, the range of microbes needs to be extended to encompass greater microbial species analysis to better understand the discriminating potential of this technique. This can include further microbes like Staphylococcus skin bacteria, which would complement existing results well because hair samples are inherently mixed with one's skin bacteria (Ramasoota et al. 2000).

As for countering faint amplification and smudging in our hair shaft samples, there may be certain applications in investigating higher thermal cycle counts of 45 cycles to amplify poor-quality DNA or adding enhancers such as Bovine Serum Albumin (BSA) in counteracting inhibitory factors found in biological materials (Craven et al. 2010). A combination and comparison with more sensitive DNA visualisation techniques, like other fluorescent dyes, would improve the detection of faint bands obtained from RAPD, especially for shorter amplicons with tighter spacing found in a small bacterial genome. (Chen et al. 2019; AlZubaidy 2019). For the RAPD primer database to be extended beyond OPB-18 and OPB-20 primers, which have already been demonstrated in the course of this study and in the literature, the production of polymorphic interspecies templates may improve the resolution of human microbes even at the strain level (Ramasoota et al.2000; Wahyuni et al. 2024; Islam et al. 2007).

RAPD-PCR is found to possess great potential as a forensic tool, especially in the analysis of trace components in complex forensic samples. Although RAPD-PCR is accurate in tracing identity through DNA components, further investigation is required for improved application in the forensic environment. Above all, even though the research carried out manage to discriminate human DNA and *E. Coli*, but the limitations cannot be underestimated. In certain conditions, RAPD-PCR may not discriminate the samples and further analysis need to be taken.

## ACKNOWLEDGEMENT

The authors are thankful to the Program of Forensic Science, UKM, for facilitating the progress of this project

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