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Forensic Applications of Whole Genome Amplification

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FORENSIC APPLICATIONS OF WHOLE GENOME AMPLIFICATION

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Abstract

Whole genome amplification (WGA) has been touted as a possible technique to augment fragment analysis of STRs in amplifying low-levels of DNA recovered from crime scenes or items. Only LCN DNA analysis is routinely used as an additional amplification step; but this is not without problems, such as the increased incidents of stochastic variations.

A review of the literature was carried out in order to assess the latest research and to identify a potentially fit for purpose whole genome amplification technique.

There are three potential alternative techniques which show promise, miPEP, dcDOP-PCR and MDA used in conjunction with a macromolecular crowder; with the majority of studies investigating the effect of non-crowded MDA on a variety of stains, including degraded, low-level and stains containing mixtures of DNA.

A number of techniques are suggested for incorporation into STR analysis depending on the sample type as well as future strands of work.

Key words and expressions: *DNA, Forensic Genetics, WGA, MDA, miPEP*

1. Introduction

A review of the literature relating to the forensic applications of whole genome amplification (WGA) has been carried out. DNA profiling techniques are undoubtedly a valuable forensic investigations tool and the utilization of polymerase chain reaction (PCR) to amplify the amount of DNA present has been of significant use to forensic biology. However, where small amounts of DNA are recovered, the use of standard DNA profiling (utilising fragment analysis of short tandem repeats such as Applied Biosystems Second Generation Multiplex Plus system) [1] does not always suffice. A number of strategies have been suggested to overcome this problem; the most commonly used being low copy number (LCN) DNA analysis, the principle behind which is the increase in the number of PCR cycles from 28 to 34; with some additional modifications to the DNA profiling process. However, this increase in the number of PCR cycles does lead to some interpretational issues [2, 3].

A number of WGA techniques have been identified and investigated by various research groups, with each groups seeking to use different WGA techniques for various reasons. This review considers these areas of research and places them in context of each other and critically identifies areas that require further investigation as well as summarising the findings. Finally, a number of different WGA techniques are suggested for different sample types based on recently published research.

1.1. Interpreting DNA results

When interpreting DNA results it is often an assumption that the DNA profiled is from one individual. If a DNA profile is of good quality; for example, it has no more

than two alleles at each locus, the alleles are evenly balanced and there are minimal artefacts, then it may be reasonable to assume that the DNA profiled is from a single source. If there are more than two alleles at any one (or more) loci, this may indicate the presence of DNA from an additional source. This is straightforward;

PCR cycles was shown to be the optimal point (depending on which kit is used) at which a well amplified result could be obtained with minimal artefacts [4]. A recent report (2010) by Butler et al gives a good discussion of these artefacts [5]. Table 1 gives an overview of these artefacts/SVs.

Phenomenon	Description	Stochastic Variations	Mixture
Preferential Amplification	Where one allele at a locus has a significantly different peak area/height to its partner	Caused by failures to anneal during the early stages of PCR	A heterozygotic locus mixed with a homozygotic locus can give this appearance
Stutter Peaks	Where there is at least one additional peak present at a locus. Most commonly -4bp stutters (shorted than main allele, but can also observe -8bp and +4 bp stutters	Caused by slippage of the template strand or the complimentary strand during the extension phase of the PCR.	A major-minor DNA result can be characterised by no more than two relatively large with additional minor peaks than can be 4bp shorter or longer than the main peak
Allelic Drop In	Where there is an 'unexpected' extra allele at a locus	Can be due to the extra sensitivity of the LCN conditions	A second contributor can give rise to an extra allele at a locus (the uncertainty is increased if the two contributors are closely related)

Table 1: Showing the two main artefacts associated with DNA profiling results and how they can be indistinguishable between SVs and mixtures

however, if a DNA profile is weak or low-level (due to low amounts of DNA or degradation) then there are increased stochastic variations (SVs) [2]. This can then result in the assumption that there is a single contributor of DNA to the sample tested becoming unreasonable [4] and if excessive, the result then becomes less useful.

1.2. Stochastic variations

It has been shown that increasing the number of PCR cycles increases the amount of SVs and as such renders the DNA profiling results less robust. For the majority of STR profiling systems, ~28

1.3. Low copy number DNA analysis

Findley et al [6] proposed the increase in the number of PCR cycles from 28 to 34 in 1997. This study appears to have been carried out in response to a study by van Oorschot and Jones, also in 1997 [7] who managed to obtain touch DNA from pens and car keys, but this was limited as the amount of DNA required was greater than 1 ng and that only a single locus was used for analysis. Findley et al correctly identified that this was insufficient for forensic case work and reported a method for obtaining an SGM (the precursor to SGM+) profile from single cells. The main difference between the routinely used

method and the LCN method was mainly the increase in PCR cycles from 28 to 34, but also an adjustment of the primer concentration. This method has since been adopted by the UK Forensic Science Service and has been extensively validated by them [2]. It is commonly referred to as LCN DNA analysis.

For a more comprehensive overview of the issues relating to LCN DNA analysis consult the works by Gill et al 2000 [2] and more recently Budolwe et al 2009 [3].

An alternative method for increasing the amount of DNA prior to amplification is the use of whole genome amplification (WGA). The basic principle behind WGA is that the amount of genomic DNA present is increased before it undergoes the PCR process. There are a variety of WGA protocols such as primer extension pre-amplification (PEP) [8], degenerated oligonucleotide primed PCR (DOP-PCR) [9] and multiple displacement amplification (MDA) [10] and variations of such.

2. WGA Techniques

A raft of whole genome amplification techniques are in use in various medical research areas, such as fertility and cancer research. Such techniques include

Multiple displacement amplification [11]

Primer extension pre-amplification PCR (PEP-PCR) [8]

Improved PEP-PCR (I-PEP) [12]

- Degenerated oligonucleotide primed PCR (DOP-PCR) [9]

- Long products from Low DNA quantities DOP-PCR (LL-DOP-PCR) [13]

- Nested PCR [14]

2.1. Multiple Displacement Amplification

Multiple displacement amplification (MDA) appears to be the most popular method as most of the published research in the forensic uses of WGA has been carried out on MDA.

Blanco et al [10] reported the use of Φ29 DNA polymerase as being highly efficient for DNA synthesis. One of the principle reasons for this is the ability of the polymerase to cause strand displacement as part of the intrinsic polymerase process.

The Φ29 DNA polymerase is the enzyme at the heart of multiple displacement amplification, which is an isothermal method. This polymerase has an intrinsic proofreading activity; something that Taq polymerase lacks. These results in an error rate 100 times lower than Taq polymerase. However, unlike Taq polymerase, Φ29 polymerase is not resistant to high temperatures, therefore unsuitable for exposure to denaturing environments [10].

Random primers/Φ29 DNA polymerase complexes bind to parts of the denatured DNA. The polymerase then extends the primers until it reaches a double stranded DNA (such as that caused by an adjacent Φ29 DNA polymerase). The polymerase then displaces the newly formed DNA strand and continues with the primer extension. Further primers then bind to the displaced DNA strands and further extension takes place. This results in a 'hyper branched molecular structure' [15]. An overview of this process can be observed in Figure 1.

For a more comprehensive review on the non-forensic uses of MDA see Lovmar et al (2006) [16]. This also includes a discussion as to why MDA does not give uniform amplification.
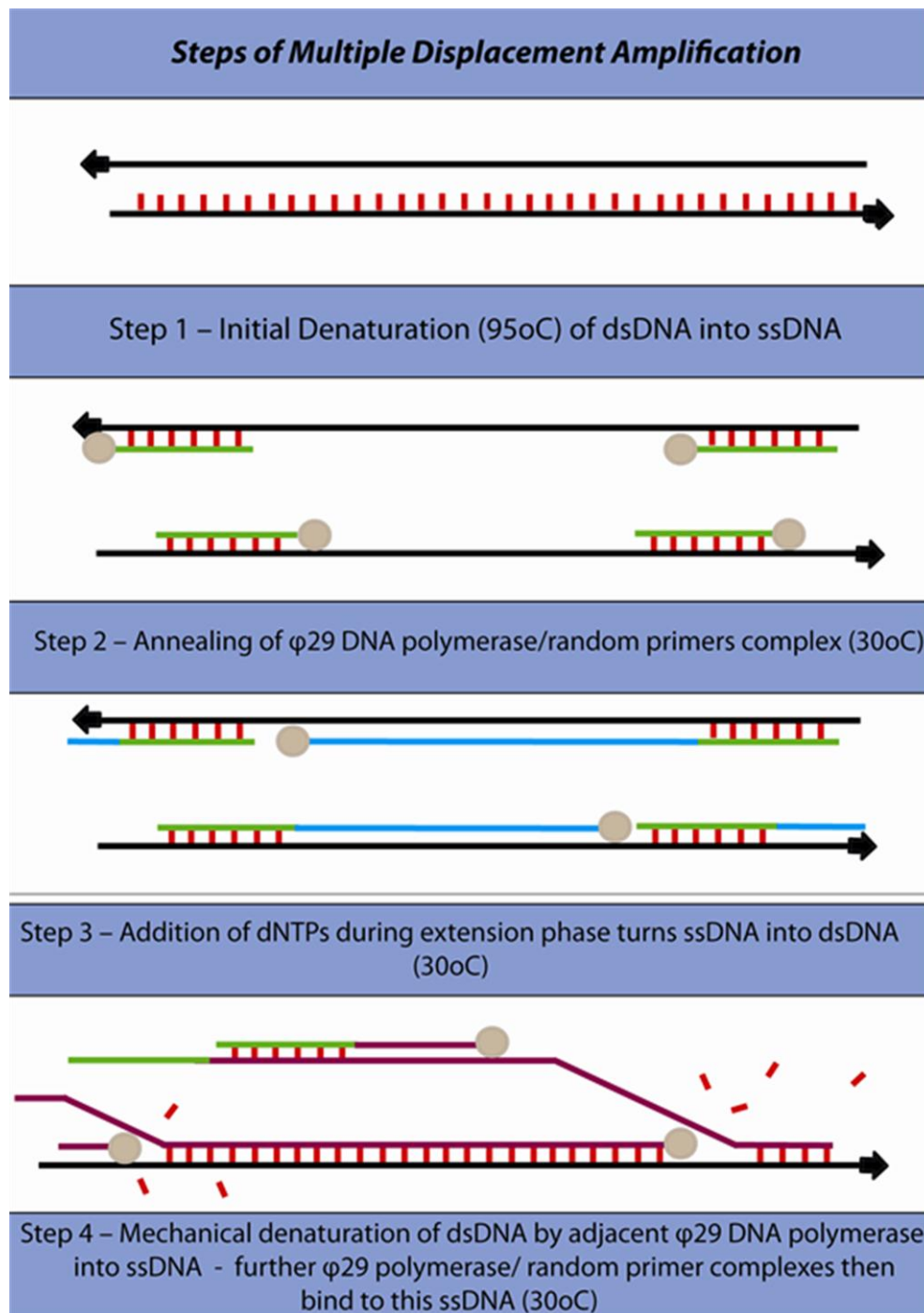
2.1.1. Forensic use of MDA

Fig. 1- This is a diagram showing the steps of multiple displacement amplification. [16]

A number of studies by Ballantyne et al [17-20] focussed on the use of MDA in a forensic context, with a view to improving success rates of STR profiling. The authors conducted an evaluation of two commercially available kits, one of which was PCR based (GenomePlex) and the MDA kit, GenomiPhi [18]. These were carried out on LCN DNA and artificially degraded DNA (using restriction enzymes). The results from the two kits were compared and in some cases GenomePlex performed better than GenomiPhi with respect to LCN DNA. Where the input amounts were 1 ng and 0.5 ng, GenomePlex amplified the samples further. However, when the input amounts were 0.1 ng and below, GenomiPhi

amplified further; at least when the DNA was quantified using the Quantifiler system. When the DNA was quantified using the Quantiblot, it appears that GenomePlex did not work at all, whereas when applied to GenomiPhi it indicated approximately the same levels as with the Quantifiler system. Spectrophotometry was also used, which is non-specific and indicated that GenomiPhi amplified more DNA than GenomePlex. The authors observed that from time to time the addition of the WGA step, be it GenomiPhi or GenomePlex, appears to result in a complete amplification failure, with some samples showing no DNA present or low-levels of DNA that are actually lower than the initial amount of DNA.

2.1.2. Effect of MDA upon profile quality

Ballantyne et al [18], not only considered the extent of amplification but also the effect of incorporating a WGA step on the quality of the results, looking at the number of alleles present, the extent of the stuttering and the amount of preferential amplification (allelic imbalance). At higher starting concentrations, the addition of WGA resulted in relatively similar quality of results. As the starting concentrations or initial amounts decreased, the quality of the results decreased with increased stuttering and allelic imbalances.

This study [18] also compared the two tests in relation to degraded DNA as opposed to LCN DNA. Following artificial degradation by restriction enzymes, which resulted in a significant reduction in the quality of the DNA results, both WGA steps were incorporated. For both kits, there was a dramatic improvement in the DNA profiling results, exhibited as an increase in discernable alleles. It was observed by Ballantyne et al [18] that this increase was concentration dependent, with less amplification with decreasing input amount, which would be expected. Ballantyne et al [18] also observed that upon amplification using GenomiPhi, the

prevalence of stutters and allelic imbalances were comparable to the prevalence of stutters and allelic imbalances upon the same sample prior to artificial degradation. However, this was not the case with GenomePlex, where it was worse. It can be seen from this study that the MDA based GenomiPhi was the better technique of the two.

A study by Schneider et al (2004) [21] looked at the effect of WGA upon SVs much closer. Rolling circle amplification (a precursor of MDA) was the WGA technique discussed and although not explicitly stated, MDA (GenomiPhi) appears to have been used as well. The findings indicated that reliable results could be obtained from a starting template of 500pg (in comparison with the optimum value of 1ng for SGM+). Drop outs occurred at 50 pg and were a common occurrence at 5 pg. This study carried out a useful role in evaluating the extent of SVs following WGA.

2.1.3. Effect of MDA upon mixed DNA results

One issue to consider with regards to a more sensitive DNA profiling test is the increased likelihood of profiling from more than one donor to the sample. Evaluating the significance of a contributor to a mixed DNA result requires a great deal of statistical analysis; using the peak area values obtained from carrying out SGM+ [22]. If the WGA amplification is not uniform, then this means that the incorporation of WGA can automatically render a mixed DNA result unusable.

A study by Thacker et al (2006) [23] addressed such an issue. Mixtures were artificially created in a range of mixing ratios (1:1, 1:3, 1:7 and 1:15) and the mixing proportions were verified using SGM+ without the MDA step. The mixing proportions were then calculated following the WGA step and compared with the mixing proportions obtained pre-WGA. The mixing proportions were maintained

for 1:1 and 1:3; however, for 1:7 and 1:15 there were problems when the starting template was at 1ng/µl; although this improved when the starting concentration was increased to 14 ng/µl. The authors in this study commented on the problems with interpreting the DNA results following MDA; mainly due to the increased prevalence of SVs.

Ballantyne et al [17] also considered the effect of incorporating an MDA step (GenomiPhi) upon mixed DNA results. It was shown that the addition of the MDA increased the number of minor contributor alleles throughout an extensive range of mixing ratios (from 1:1 to 1:1000). This increase was more pronounced as the mixing ratio decreases. However, the authors observed that it was more difficult to separate out the two contributors. Prior to the incorporation of the MDA step, it was easy to identify the major DNA profile and the minor contributor based upon differences in peak height. As observed previously by Ballantyne et al [18], the MDA step increased the amount of preferential amplification/allelic imbalance which meant that it was more difficult to identify which alleles were the major or the minor contributor, even when the mixing ratio was as low 1:1000.

Even though all the alleles present were correctly designated as compared to the controls, the increased prevalence of allelic imbalances rendered what previously could be classified a simple major/minor DNA result into a more complex result. It is still possible to compare a more complex result with an individual's DNA profile; however, the incorporation of the MDA step would most likely mean that any mixtures calculation that utilises peak height or peak area data would be unreliable. Ultimately, this would mean that any mixed DNA results obtained following the MDA step could not be presented in a court of law without any further improvement. Ballantyne et al,

attempted to address this by introducing 'molecular crowding' [17].

2.1.4. Molecular crowding and MDA

Molecular crowding is the use of high concentrations of macromolecules; which are thought to affect cellular reactions, such as those caused by DNA polymerases [24]. This high concentration is thought to affect the thermodynamics of the reaction resulting in increases in the binding of the polymerases.

When Ballantyne et al, [17] incorporated a molecular crowding reagent (either additional DNA or polyethylene glycol 400) in the MDA step, further amplification was observed when compared with the MDA step without PEG-400. In addition to this, the peak height ratios were increased, which allowed for the distinction between the major and the minor contributors. From this study, it appears that there is a very strong case for the use of 'crowded MDA' where there are DNA mixtures.

2.1.5. Use of MDA upon case work samples

A further study by Ballantyne et al [20], has applied this technique of crowded MDA and standard MDA to a number of non-probative samples from forensic case work. Such cases included blood stains, the seminal fraction from a rape case, samples from a balaclava, samples from the handle of a knife and from fingernail scrapings. In all cases, the use of crowded MDA increased the number of alleles profiled and improved the quality of the results. Whilst the use of non-probative case work samples lends a great deal of verisimilitude to the samples frequently encountered, the uncertainty as to the source(s) of the DNA on the swabs does mean that there is an element of doubt as to whether the DNA profiles obtained are truly representative of the contributing DNA. Thanks to the efforts of Ballantyne et al [17-20] it seems that out of the WGA

techniques available, crowded MDA is the closest to being accepted for use in routine case work. In order to achieve this, full validation studies incorporating blind samples (including varying mixing ratios) will need to be carried out at various forensic laboratories across the world.

2.1.6. MDA versus LCN DNA Analysis

Although most of these studies suggest that WGA could be a more efficient alternative to increasing the number of PCR cycles only one study actually compared a WGA technique with increasing the number of PCR cycles. The study by Lagoa et al in 2008 [25] carried out such an experiment. The amplification methods used were 28 cycles, 34 cycles, a 'WGA' step (which appeared to be MDA, although not explicitly stated) and, interestingly, nested-PCR. However, according to the author, it appears that the nested-PCR was performed after 28- cycles amplification using a miniSTR heptaplex kit.

The author's conclusion from this study is that increasing the number of PCR cycles is preferable to the use of MDA and nested-PCR upon latent fingerprints. This study was only carried out on latent fingerprints rather than any other body fluids and it only used the REPLI-g MDA kit from Qiagen (it was not specified whether this was the 'Midi-' or 'Mini-' kit). So whilst the study was interesting in that it compared 34 cycles amplification with MDA, it could have gone a lot further; for example it could have used the miPEP, reported as being more effective than MDA three years earlier, as the WGA technique of choice.

DNA from latent fingerprints appears to be the driving force for the incorporation of an additional amplification step, for example, a prior study by Sorensen et al in 2004 [26] appears to have investigated the use of MDA on DNA recovered from latent fingerprints on glass slides; but does not go on to discuss these results, focusing more on the ability of MDA to amplify cell lines. Interestingly, these authors appear to be the only ones to raise concerns about having a very sensitive DNA profiling test; given the background levels of DNA in the environment and the propensity of contamination.

Although various WGA techniques are widely used in medical research (such as fertility), there is limited published information regarding its use in forensic case work in that WGA techniques have been applied to non-probative case work samples [14], there does not appear to be any actual cases where WGA was been adopted. A number of publications do mention the potential value of WGA to profiling crime stains, but do not explore the issue in depth. One paper by Balogh et al in 2006 [27], explores the use of WGA on tissues and blood samples. Whilst this is interesting in that WGA is being applied in a forensic context, tissues and blood samples are not generally considered to contain low levels of DNA; even after a period of time has gone by; therefore, the use of WGA techniques would not usually be considered for such samples.

The publication by Balogh et al, [27] showed that whilst there was a demonstrable increase in the success rate for obtaining DNA profiles from blood and tissues, the success depends on the amount of DNA present in the first place, which is not an unexpected conclusion.

2.2. Modified improved PEP

Hanson et al 2005 [28] took the I-PEP method and adapted it further calling it a 'modified improved primer extension pre-amplification PCR' or miPEP. This modification appears to be made with the issues relating to forensic genetics firmly in mind as opposed to modifications being made with medical genetics in mind such as with I-PEP. This paper also agrees with Saiki et al 1998 [29] in that increasing the number of PCR cycles can lead to the Taq polymerase being less efficient and consequently resulting in increased SVs.

Hanson et al [28] also agreed with dismissing the idea of using Nested PCR due to the fact that the STR analysis consists of multiplexed primer pairs and the inherent technical difficulties of developing a compatible set of primers to allow such nesting to take place [28].

The main differences between I-PEP and miPEP appear to be the doubling of the PEP primer concentration from 20μM to 40μM; an increase in denaturing temperature from 92oC to 94oC; and the removal of the extension step during the PCR cycle. This modified protocol was applied to a wide range of samples; including varying starting concentrations of DNA (from 5 pg to 200 pg), different body fluids, environmental exposure and DNA from latent fingerprints.

The authors then carried out STR analysis using Profiler Plus following control samples, DOP-PCR, LL-DOP-PCR, MDA, I-PEP and miPEP. This was evaluated by identifying the lowest concentration from which at least one full STR profile could be obtained. Somewhat surprisingly, LL-DOP-PCR performed worse than the controls samples (in which no WGA step was incorporated) giving no DNA profiles at any template concentration. miPEP was reported as being the most effective WGA method with full DNA profiles being obtained from a starting template concentration of 5 pg. In order of effectiveness, LL-DOP-PCR did not perform as well as the others with no profile obtained, then DOP-PCR and MDA both at 100 pg, then I-PEP at 50 pg, no WGA step at 10 pg and finally miPEP at 5 pg. Significant preferential amplification was observed following all WGA methods. To place this in perspective, using standard STR profiling, the optimum concentration of DNA is 1 ng [30].

In summary, Hanson et al [28] appears to be saying that the incorporation of all WGA methods actually give a worse result; apart from the miPEP. This contradicts some of the other studies around; such as those by Ballantyne et al [17-20].

2.3. dcDOP-PCR

dcDOP-PCR is a variation of DOP-PCR that, like miPEP, has been developed with a forensic genetics purpose. The modifications made to DOP-PCR are the incorporation of a 10N degenerate primer rather than a 6N degenerate primer, the use of a higher quality Taq Polymerase (Platinum Taq High Fidelity) and an increase in the non-specific cycles from 5 to 12. In addition to the modifications of the DOP-PCR itself, there is also an incorporation of a post-PCR amplification step and an increase in the electro kinetic injection time during CE from 10 seconds to 20 [31].

However, Dawson Cruz [31] reported that there was no significant difference in the quality of the results between conducting post-PCR purification and without (p=0.6723); nor was there a significant difference between the changes in the electro kinetic injection time (p=0.4039).

dcDOP-PCR demonstrated a ~45% increase in the number of alleles presence when compared with DOP-PCR and ~34% increase when compared with standard STR without a WGA step. Dawson Cruz [31] also reported an improvement in data quality as well as a decreased prevalence of allelic drop in when compared with MDA and PEP.

This report did seek to compare dcDOP-PCR with other WGA techniques, such as MDA and PEP. However, the versions of the MDA and PEP tested were not the most up to date version. It would have been useful to compare dcDOP-PCR with crowded MDA and miPEP as well as LCN DNA analysis. Nevertheless, this technique does seem to be a promising WGA technique.

2.4. Nested PCR

This study's use of nested-PCR was of interest [25] in that whilst nested-PCR results appeared to be comparable with 34 cycles amplification, the authors dismissed this due to 'drawbacks' without clarifying what these were. The authors referred us to a study by Strom et al 1998 [32], in which nested-PCR was used on charred remains and 'minute' amounts of blood. However, this study only conducted nested-PCR on the Amelogenin locus. This study also referred to the technical difficulties in using nested-PCR in conjunction with a test that utilises eleven or more primer pairs in a multiplex. Presumably, it is these technical difficulties that Lagoa et al [25] refer to in dismissing the use of the heptaplex nested-PCR kit.

3. PCR vs. non-PCR techniques

PEP, I-PEP, nested-PCR and the DOP-PCR techniques are all PCR based; whereas the MDA technique is non-PCR based. The initial concern that appears to be expressed by a number of authors with the PCR based method is that the increased number of PCR reactions can lead to a poor quality result, in the similar way that having more than 28 cycles in the SGM+ system can lead to increased SVs. There is no particular apparent reason why a PCR based whole genome amplification method could provide a better result than, for example, using 50 PCR cycles, which would lead to further complications with respect to interpreting the results. Therefore, a non PCR based technique, such as MDA might be the preferred method.

However, a number of studies by Ballantyne et al [19, 20] have also reported that MDA leads to increased preferential amplification thus potentially rendering the results un-interpretable. However, little research appears to have been conducted which explores the effects of MDA on stutters. In theory, there should be very little or no effects of stuttering due to the use of MDA; however, this is not borne out by the research conducted by Ballantyne et al [19].

3.1. Comparison of I-PEP and MDA

A study, conducted by Barber et al [33], compared the use of I-PEP and MDA upon 'compromised forensic samples'. These compromised samples were DNA samples artificially degraded by using DNases and dried blood stains that were stored at room temperature for 16 months. The study also included mtDNA analysis on hair shafts. Ultimately, this study showed that both I-PEP and MDA increased the amount of DNA material present, but it decreases the overall size of the DNA shown as a decrease in molecular weight. The author then states that as a result of this, there will be more SVs following the incorporation of the WGA step. In comparing the two techniques, it was reported that MDA allowed for more amplification than I-PEP (10,000 fold as opposed to 1000-2000 fold). High molecular weight products were reported being present even in the negative controls; the authors attributed this to primer-dimers with extensions. It was noted that MDA produced further artefacts than I-PEP. A brief discussion was given as to the nature of these artefacts. It was noted that the artefacts in MDA appeared to be completely random with no consistent bands in between samples (including repetition of the same samples). This was thought to be due to the higher amplification ability of MDA.

No consideration appears to have been given to the possibility of contamination to the results. This is a feature common to most published research in this area.

3.2. Contamination

One of the issues associated with LCN DNA analysis and one that needs to be considered with respect to WGA, is that of contamination. An extensive review of LCN DNA analysis was carried out by Caddy [34] on behalf of the Forensic

Science Regulator in the United Kingdom. This review followed the collapse of a trial in which LCN DNA evidence was undermined. The review showed that the science behind LCN DNA was sound. However, it was observed that there was a lack of consistency with respect to DNA recovery at crime scenes as well as some confusion amongst the police forces as to when LCN DNA recovery procedures should be used.

This has implications for WGA techniques as the quality of the results produced do largely depend on the recovery of the DNA from the crime scenes. However, this issue cannot be fully investigated until the WGA techniques have been validated and accepted. Indeed, one area that does need some research is the method of recovering DNA samples for the purposes of undergoing WGA.

4. Conclusions

One view that is prevalent is that whole genome amplification could be a very useful tool for the forensic geneticist.

seem that the best WGA method to use for single source DNA profiles could well be the modified I-PEP suggested by Hanson et al [28] which is clearly PCR based. Whereas crowded MDA could be the technique of choice for mixed DNA results as suggested by Ballantyne et al [17].

One question that is often asked is 'could WGA replace 34 cycles amplification?' The short answer is no, but there are situations where it might be more appropriate to use WGA rather than 34 cycles. For example, if a DNA sample is old and/or degraded, it seems to be more appropriate to use 34 cycles amplification than WGA; but if the sample is low-level but of high quality (i.e. recent transient contact) then it might be more appropriate to use miPEP. Crowded MDA may be carried out on mixtures of DNA, but not until a more thorough investigation has been carried out using other amplification methods. Table 2 summarises which techniques might be the most appropriate technique given the sample types, based upon the published research.

Sample Type	Suggested Technique
Fresh abundant DNA	28 cycles STR analysis (e.g. SGM+)
Decayed abundant DNA	34 cycles STR analysis (e.g. LCN DNA analysis)
Fresh low-level DNA (touch DNA)	Incorporation of a WGA step (e.g. miPEP)
Decayed low-level DNA	mtDNA analysis (possibly incorporated with nested PCR)
DNA mixtures	Incorporation of a WGA step (e.g. Crowded MDA)

Table 2:- A summary of the types of stains and suggested techniques to be included.

However, there is a wide range of different WGA techniques available which can complicate matters. As well as WGA, there is also increasing PCR cycles and nested-PCR.

MDA appears to be the most popular WGA method for use in forensic science due to the abundance of research in this area, possibly because MDA is not PCR based and incorporates a proof-reading polymerase it could produce a more uniform amplification. However, it does

4.1. Further work

One thing that is clear is that much further work is required before WGA can be used in a court of law. A number of strands require further study, such as:

Comparison of miPEP, dcDOP-PCR and crowded MDA in terms of accuracy, sensitivity and quality of DNA profiling;

Comparison of the effectiveness of LCN DNA analysis, dcDOP-PCR, miPEP and crowded MDA upon degraded samples,

low-level fresh samples and mixtures of DNA;

Effects of miPEP upon mixtures of DNA in a similar manner as carried out by Ballantyne et al with respect to crowded MDA;

Accuracy of DNA profiling following incorporation of miPEP and crowded MDA using blind studies at a series of different laboratories to provide data for a full validation in order to move towards operational acceptance of said techniques;

Optimisation of the DNA profiling process following incorporation of the WGA step; for example, decreasing the number of PCR cycles from 28 to, for example, 22 would reduce the amount of SVs. However, would this be sufficient to raise the targeted sections sufficiently above background levels?

Carrying out the various WGA techniques upon naturally degraded samples in order to assess the reliability of data derived from artificially degraded samples. This should allow for a more robust assessment when deciding which test to carry out on old stains;

An investigation into the effects of dcDOP-PCR upon mixtures;

The use of crowded MDA upon mixtures of DNA from more than two individuals. If macromolecular crowding will result in the preferential amplification of one set of DNA, could this help modify the DNA results so that a 'clear contributor' can be identified from previously un-interpretable results?

If these strands are taken up and successfully carried out, then the use of crowded MDA, dcDOP-PCR or miPEP could then become part of a forensic genetics laboratory's repertoire. If the final strand, referring to the issue of mixtures of DNA from three or more individuals, shows promise, this could be a big breakthrough in terms of resolving un-interpretable results.

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