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Research article

Use of ‘‘AnyDirect PCR buffer’’ for PCR amplification of washed bloodstains: A case report

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Abstract

AnyDirect PCR buffer (BioQuest) permits to perform direct PCR from different kinds of forensic samples (blood, saliva, sperm, etc.) without any DNA purification step.

This is very useful in particular when working with small quantity of sample since it avoids the risk of loosing sample step by step as often happens with traditional DNA extraction procedures.

In the present casework we analyzed some bloodstains found by luminol test onto washed clothes and linen: all samples were analyzed either using the AmpFlSTRs Identifiler kit (Applied Biosystems).

Keywords: Washed bloodstains; Direct PCR; MiniSTRs

1. Introduction

DNA purification steps are time consuming and tedious, furthermore there is the risk of unexpected contamination during samples preparation.

In our previous study we validated the AnyDirect PCR buffer (BioQuest) that permits to perform direct PCR from different kinds of forensic samples (blood, saliva, sperm, etc.) without any DNA purification step. [1–3]. This is very useful in particular when working with small quantity of sample since it avoids the risk of loosing sample step by step as often happens with traditional DNA extraction procedures. Here we report a casework where we applied this procedure. During a quarrel at a disco exit, a boy was wounded by a knife. Some days after, investigators distrained some clothes in the house of a suspect who was identified by the victim. Clothes (referred as dressed by the suspect, the night of the murder) had been washed: by luminol test some bloodstains were found that were subjected to direct PCR amplification.

2. Material and methods

A 2 mm diameter punch from each bloodstain was directly put into the PCR reaction mix.

PCR reaction mix had the following composition [4]:

Buffer + $MgCl_2$: 5 × AnyDirect™ PCR buffer;
Primers: AmpFlSTR Identifiler (Applied Biosystems) that amplifies simultaneously 15 STRs loci (D19S433, D3S1358, D5S8118, D8S1179, vWA, TH01, D13S317, D21S11, TPOX, FGA, D7S820, D16S539, D18S51, CSF1PO, D2S1338 and Amelogenin;
dNTPs: dNTP mix (10 nmol each);
DNA polymerase: AmpliTaq Gold 2.5 U/μl.

As reference a blood sample from the victim was used: 0.5–1 μl of blood were put directly into the reaction mixture.

Different positive and negative controls were enclosed during the amplification steps.

PCR amplification has been performed using the following conditions:

Pre-heating step: 80 °C – 15 min; Initial denaturation:95 °C – 11 min;

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34 Cycles: denaturation: 94 °C – 1 min, annealing: 59 °C – 1 min, extension: 72 °C – 1 min;
Final extension: 60° – 60 min.

Amplified samples have been analyzed on an ABI PRISM 3130 multicapillary sequencer employing GeneMapper 3.2 software. For PCR fragment length determination, the allelic ladder from each kit as external standard and the internal size standards LIZ 500.

3. Results and discussion

We evaluated the quality of DNA profiles obtained using the ''AmpFlSTRIdentifiler'' (Applied Biosystems) amplification kit, with particular attention to the following points:

1. any potential interference due to the substrate,
2. peaks balance/imbalance,
3. peaks heights and area,
4. presence of allelic dropout,
5. presence of any artefact,
6. presence of preferential amplification,
7. reproducibility of results obtained.

DNA profile from the victim blood sample using direct PCR procedure showed no locus or allele dropout and was identical to the ones obtained by traditional purification (Chelex treatment) and amplification procedure, without any inhibition due to the effect of hemoglobin. With small traces like washed bloodstains, the direct procedure is strongly recommended in order to reduce the loss of material that inevitably happens with multi-steps traditional procedure. We observed no influence caused by the substrate when using a fragment of sample directly in the PCR mix: in comparison with other DNA extraction methods that often co-extract together with DNA even some inhibitors from the substrate, in this procedure no DNA inhibitors are released by tissues.

From bloodstains were obtained full/partial profiles: the quality was variable by sample, depending on the stain intensity, anyway no inhibition due to luminol effect was found, confirming that luminol does not interfere with DNA typing.

Bioquest PCR protocol suggests 30 PCR cycles, but, in our procedure, in order to improve the chance of success the number of PCR cycles was increased to 34. Moreover, in our experience, in all cases of weak signal five or 10 more cycles are required. No significant artefacts or high noise were observed due to the increased number of cycles, even if, obviously, precautions are required during the interpretation of data.

4. Conclusion

DNA typing showed that all tested bloodstains on the suspect clothes had the same DNA profile of the victim: so the suspect was convicted.

The good outcome of this casework confirms that direct PCR procedure could successfully replace the conventional DNA purification step, in particular when working with small samples, since it avoids the risk of loosing sample step by step, or when high throughput is required.

Conflict of interest

None.

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