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Biomek®-3000 and GenPlex in Forensic Genetics

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Biomek-3000 and GenPlex in Forensic Genetics

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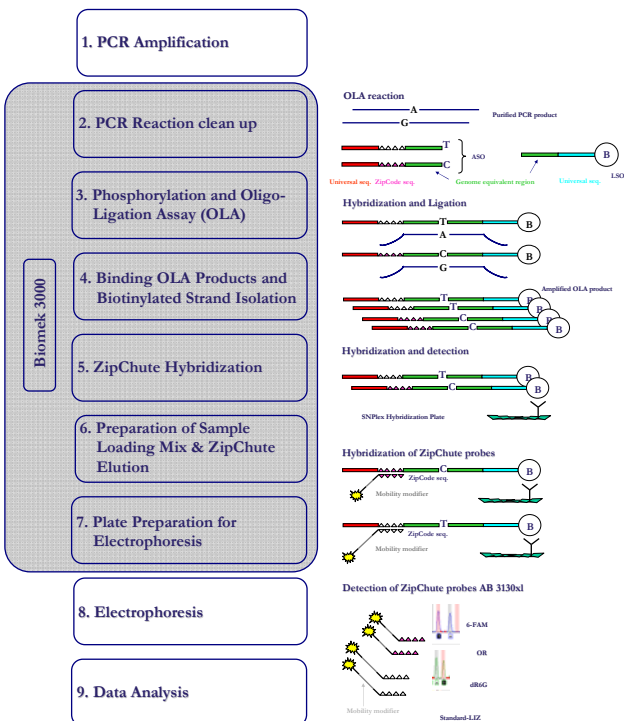
INTRODUCTION

SNP genotyping provides a supplement for conventional STR-based kits currently used for human identification. GenPlex (Applied Biosystems (AB)) is a SNP genotyping kit based on a multiplex of 48 informative, autosomal SNPs from the SNP*for*ID Consortium [1] plus the amelogenin gender marker [2].

Our objective was to setup, implement and validate a small and affordable, automated liquid handling robot for forensic case work samples (buccal swabs on, FTA® paper and Qiagen purified blood).

MATERIAL AND METHODS

The reaction scheme consisted of numerous steps (Figure 1) and was difficult to perform consistently without an automated liquid handler. All steps were performed in 96-well micro-titre plates. Automation was accomplished with a Biomek-3000 (Beckmann Coulter) automated liquid handler using five in-house developed methods. All methods allowed the user to select the number of subsequent injections on the capillary electrophoresis instrument (AB 3130xl, AB) enabling processing of both partial and full plates.

Figure 1. The GenPlex protocol.

1. PCR Amplification
2. PCR Reaction clean up
3. Phosphorylation and Oligo-Ligation Assay (OLA)
4. Binding OLA Products and Biotinylated Strand Isolation
5. ZipChute Hybridization
6. Preparation of Sample Loading Mix & ZipChute Elution
7. Plate Preparation for Electrophoresis
8. Electrophoresis
9. Data Analysis

Biomek 3000

OLA reaction
Purified PCR product
ASO
Universal seq. ZipCode seq. Genome equivalent region Universal seq. LSO

Hybridization and Ligation
Amplified OLA product

Hybridization and detection
SNPlex Hybridization Plate

Hybridization of ZipChute probes
ZipCode seq. Mobility modifier
ZipCode seq. Mobility modifier

Detection of ZipChute probes AB 3130xl
6-FAM
OR
dR6G
Mobility modifier
Standard-LIZ

RESULTS

The results obtained with 15 manually processed samples were compared to results obtained with the same samples processed on the Biomek-3000 (Figure 2). Full concordance between manually processed and automated processed samples were obtained in all genetic systems. Subsequently, a total of 286 samples were analyzed in duplicates with the GenPlex reaction using the Biomek-3000. Of the total of 572 samples, 97.6% resulted in a full profile. Full concordance was obtained between the two investigations of each sample. The results were further compared to those obtained from the same samples using a 49-plex PCR in combination with an ISO 17025 accredited SNaPshot® (AB) single base extension assay [3]. Full concordance of the results was obtained in all but one sample resulting in 99.99% concordance.

Figure 2. A representative sample manually processed and processed on the Biomek-3000.

Manual processing   Biomek-3000
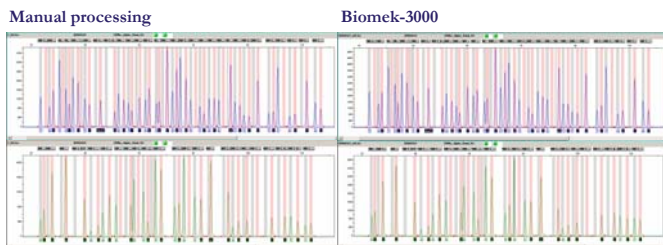
Sensitivity:
The sensitivity of the GenPlex reaction was evaluated with respect to allele calls. Qiagen purified DNA from two individuals were quantified using the Quantifiler HUM kit (AB) on a real time PCR instrument (AB 7900, AB). Five different DNA concentrations were tested in triplicates. Small amounts of template DNA could result both in a false homozygote or false heterozygote (Figures 3 and 4). Reproducible results were obtained with 250 pg DNA.

Figure 3. Sensitivity: Small amounts of template DNA resulted in false homozygotic allele calls.
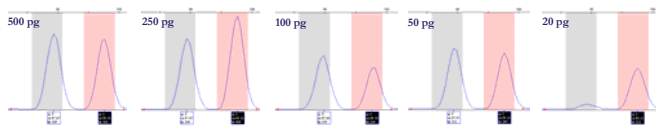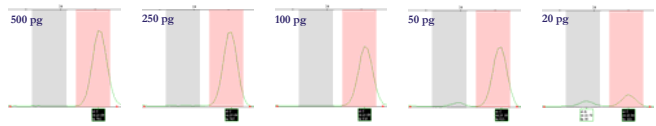
500 pg   250 pg   100 pg   50 pg   20 pg

Figure 4. Sensitivity: Small amounts of template DNA resulted in false heterozygotic allele calls.

500 pg   250 pg   100 pg   50 pg   20 pg

Mixtures:
The performance of the GenPlex reaction was evaluated using mixtures of homozygote and heterozygote samples in various ratios. Two samples previously analyzed with GenPlex were combined in the following ratios: 1:1; 2:1; 5:1; 10:1; 1:2; 1:5 and 1:10. A total of 1 ng of DNA was used as starting template in the subsequent PCR. The experiment was performed in triplicates. A cluster analysis was performed using GeneMapper v 4.0 (AB). As it can be seen in Figure 5, results obtained from a mixture or small amounts of DNA show a similar profile.

Figure 5. Cluster analysis performed with DNA mixtures and low amounts of DNA. Homozygote alleles are indicated with blue squares or red dots. Heterozygote alleles are indicated with green triangles. Samples with ratios in-between clusters are indicated with black crosses.

Sensitivity

SNP rs1382387   SNP rs1413212

Mixtures

SNP rs1382387   SNP rs1413212
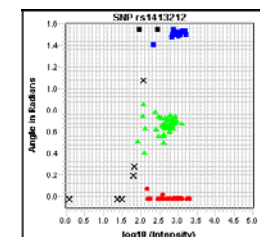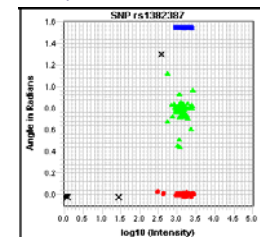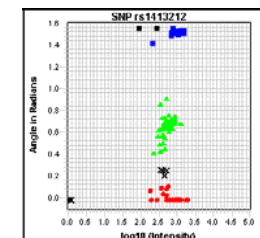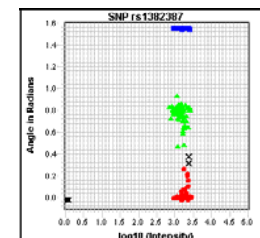
CONCLUSIONS

Overall, the results demonstrate that the Biomek-3000 can perform a series of complex reactions leading to highly consistent forensic genetic SNP typing results. Further key findings:

- Complete SNP profiles are reproducibly obtained using 250 pg input DNA.
- The performance of the GenPlex reaction is unreliable when the amount of DNA is below 100 pg.
- Some miscalled alleles were observed in samples with 50 and 20 pg of DNA.
- Cluster analysis can be used to identify mixtures.

REFERENCES

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