Application note
SFMP-0001

LGC Genomics

sbeadex® forensic PCR clean up

C. Proff - IfB-LGC GmbH, Cologne; F. Schubert - LGC Genomics GmbH, Berlin, Germany

Introduction

New approaches in forensic DNA typing technology mostly deal with the analysis of degraded and low copy number DNA with improvements being achieved by modifying PCR processes e.g. increase in the number of PCR cycles, reduction in reaction volume or the use of mini-STRs. This study comprised tests on low level DNA samples (artificial and routine casework) that were amplified (SGMplus, Life Technologies) with 28 cycles, and were subjected to two different PCR purification methods, one which was spin column based (Product A, Supplier A) and a modified protocol of the magnetic particle based sbeadex® forensic kit.

Aims of the study

- Suitability of sbeadex® chemistry for PCR product clean-up and concentration
- Comparison of sbeadex® chemistry with an alternative product
- Evaluation of the effects of PCR product enhancement.

Tests performed

50 µL SGMplus PCR samples were prepared in triplicate with 10, 20 and 40 pg of 007 control DNA. Single 50 µL PCR samples were prepared from 15 routine casework DNA extracts with observed DNA concentrations of 0 pg/µL to 19 pg/µL. The resulting 50 µL PCR product from each sample was processed as follows. 1 µL was run on the 3100 GeneticAnalyser to document the amplification result before clean-up / enhancement.

24 µL was treated according to a modified protocol of the sbeadex® forensic kit using an especially developed binding buffer. The remaining 24 µL was treated with Product A according to the manufacturer's protocol. For both PCR purification methods a 10 µL elution volume was used.

The purified PCR products were separated under three different electrophoresis conditions (see legend of Figure 1 to 3). The resultant DNA profiles were evaluated with GeneMapper® ID-X analysis software using peak detection threshold of 25 rfu. Median peak heights and allele numbers were calculated for every sample and purification / enhancement step as well as for the triplicate samples. Observations of possible additional / extra alleles were noted.

Results

During evaluation of the control DNA samples, PCR product concentration was shown to be more efficient with sbeadex® compared to Product A (Figure 1) with a 13 versus a 5 fold increase in signal intensity seen with the maximum electrophoresis conditions (2 µL / 10 sec / 3kV). Doubling the amount of PCR product used for electrophoresis resulted in a 1.2 to 1.4 fold increase in signal intensity. DNA typing success in terms of the number of detected alleles was comparable with both purification methods (Figure 2). Purification success was higher with Product A as cleaner backgrounds with less "dye-blobs" were observed.

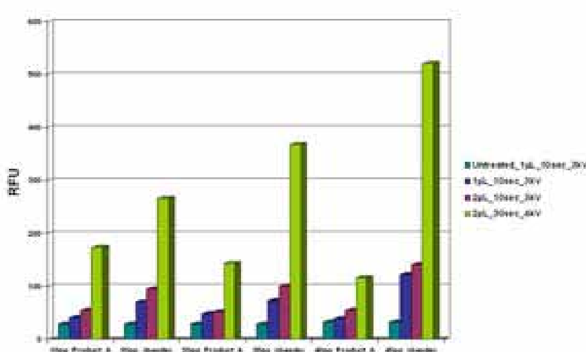

Figure 1: Shows the median signal intensities observed for all methods and electrophoresis conditions (for details see paragraph "Results")

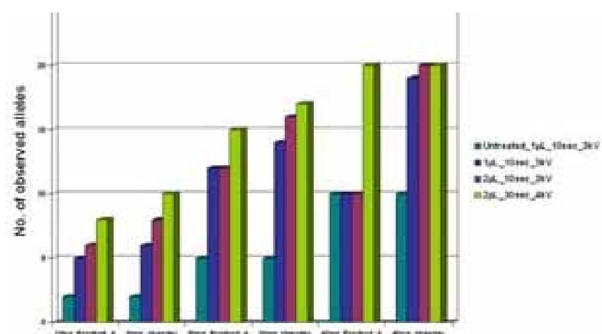

Figure 2: Shows the median number of alleles observed for all methods and electrophoresis conditions (for details see paragraph "Results")

During evaluation of the casework samples two samples were excluded from the data set due to capillary failure. The trend observed regarding signal intensity and typing success were confirmed with the casework sample data set i.e. purification with sbeadex® gave a greater fold increase in signal intensity compared to Product A. Typing success was comparable for both purification methods. Following sbeadex® purification some samples showed more alleles (1 to 6) compared to the Product A purified data set (Table 1). sbeadex® purified samples still tend to have dye blobs, the occurrence of these artefacts increases with every enhancement step.

Conclusions

Both sbeadex® and Product A are suitable methods to increase the information content of low level and compromised DNA samples in routine forensic casework. Higher median signal intensities with sbeadex® indicate a PCR product loss during PCR product purification with Product A probably due to higher affinity of DNA to beads than to silica columns and / or longer incubation times for binding and washing steps.

A dedicated post PCR purification work area is necessary to avoid PCR product cross contamination. The application is suitable only for low level DNA samples (0 - 20 pg/µL) otherwise overloading occurs.

In summary, the sbeadex® technology has shown to be an alternative product for rescuing forensic DNA samples which have low concentrations of PCR product after STR amplification (Figure 3).

Swabbed sample type	DNA conc. pg/µL	untreated 1µL_10sec_3kV		sbeadex® 2µL_30sec_4kV		Product A 2µL_30sec_4kV	
Gear stick	0	2	37.5	11	576	11	463
Piece of wood	0	-	-	12	578	12	142
Can edge	2	-	-	7	252	7	122
Glove	3	1	30	21	184	17	109
Blood stain	3	5	46	15	459	16	218.5
Cap	3	-	-	11	406	10	131.5
Cigarette butt	5	5	52	12	611.5	12	193
Screw	5	1	30	13	238	12	86
Blood stain	6	-	-	12	573	11	243
Torch	6	5	30	22	294.5	16	141.5
Woollen cap	8	3	30	18	255.5	15	167
Screwdriver	11	-	-	11	206	9	120
Metal part	19	-	-	22	443.5	20	179.5

Table 1: Number of alleles and median peak height (rfu) observed in the maximum post-PCR enhanced casework sample data set

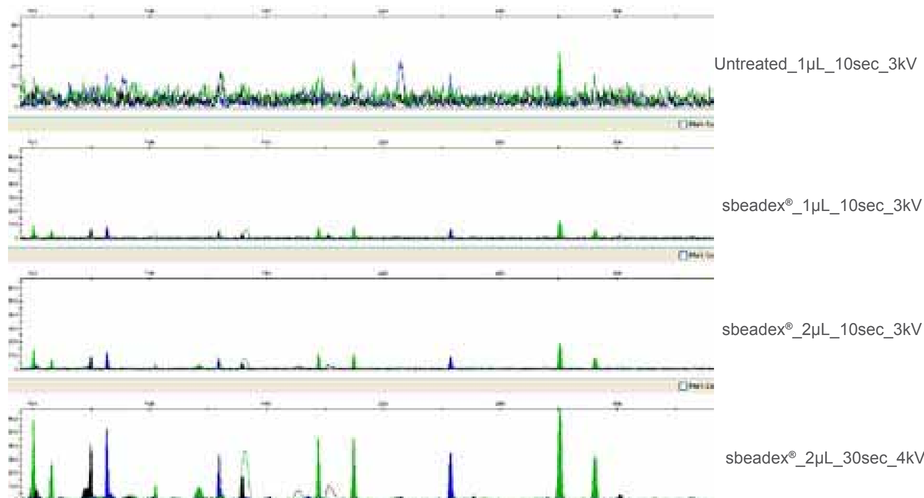

Figure 3:
Example data: sbeadex® purification and different enhancement steps for details see paragraph “Results“)

www.lgcgenomics.com

LGC Genomics
Germany
Ostendstr. 25 • TGS Haus 8
12459 Berlin

Tel: +49 (0)30 5304 2200
Fax: +49 (0)30 5304 2201
Email: info.de@lgcgenomics.com

United Kingdom
Unit 1-2 Trident Industrial Estate • Pindar Road
Hoddesdon • Herts • EN11 0WZ

Tel: +44 (0) 1992 470757
Fax +44 (0) 1438 900670
Email: info.uk@lgcgenomics.com

USA
100 Cummings Center • Suite 420H
Beverly • MA 01915

Tel: +1 (978) 232 9430
Fax: +1 (978) 232 9435
Email: info.us@lgcgenomics.com

0602.01-2013-03

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording or any retrieval system, without the written permission of the copyright holder. © LGC Limited, 2013. All rights reserved.