International Congress Series 1288 (2006) 507–509

www.ics-elsevier.com

Validation and evaluation of the ABI 3100 genetic analyser for use with STR analysis of buccal swabs—report of erroneous SGMplus® profiles caused by poor spectral calibration

S.C. Bardill *, T. Warren, I. Johnston, L.M. Johns, Jim Thomson

LGC, Runcorn, UK

Abstract. For many years, Applied Biosystems 377 DNA sequencers have been used in the production of short tandem repeat (STR) profile data for forensic human identification (HID) applications. These instruments provided a 36–96 lane, slab gel, based electrophoresis system. However accurate, using this system resulted in a relatively high level of re-work due to the inconsistent nature of the polyacrylamide gels used. The operation of the AB 377 was also labour intensive and hazardous due to the requirements for gel production. The development of capillary electrophoresis genetic analysers, e.g. AB 3100, suggested potential improvements in sensitivity, reliability, and flexibility. In this study, a comparison has been made between the AB 377 DNA sequencer and the AB 3100 genetic analyser when performing STR profiling of forensic buccal swabs. The criteria used to evaluate/validate the AB 3100 are outlined and the assessments made are described. Particular attention has been paid in describing an example of an SGMplus profile being assigned incorrectly due to poor spectral calibration on the 3100. © 2006 Published by Elsevier B.V.

Keywords: 3100; Stutter; Buccal; Forensic; Electrophoresis

1. Introduction

Capillary electrophoresis platforms such as the Applied Biosystems 3100 and 3130 genetic analysers have been widely adopted for STR analysis for forensic investigations.

* Corresponding author. LGC, Queens Road, Teddington, TW11 0LY, UK. Tel.: +44 20 8943 7610; fax: +44 20 8943 2767.

E-mail address: simon.bardill@lgc.co.uk (S.C. Bardill).

0531-5131/ © 2006 Published by Elsevier B.V.
doi:10.1016/j.ics.2005.12.001

Historically, slab gel sequencers such as the AB 1377 DNA sequencers have been used for this purpose and LGC, like other laboratories, has carried out extensive back-to-back comparisons of the two instruments before implementation of capillary platforms.

This validation demonstrated that overall, the capillary platforms offered improvements in sensitivity, reliability and flexibility, enhancing profile quality whilst reducing the operator time and the level of rework required.

However, importantly, in the course of this validation we identified three examples of incorrect SGM+ profiles being assigned using a 3100 analyser. The mis-assignments were characterised by the complete dropout of one allele in heterozygous TH01 loci. The cause of these mis-assignments was investigated and attributed to poor spectral calibration on the 3100 platform. The adoption of stringent criteria to validate spectral calibration is therefore recommended to minimise the possibility of such errors.

The back-to-back study also highlighted that the magnitude of stutter peaks observed on the capillary instruments is significantly higher than seen with the slab gel instrument. This has potential implications in mixture analysis where greater ambiguity may arise in some cases.

2. Mis-assignment of TH01 genotypes

217 of 220 samples run were designated correctly on the 3100. However, 3 remaining samples were designated incorrectly at the TH01 locus on the 3100. In all 3 cases, TH01 was wrongly assigned as a homozygote. The second allele peak was not visible, but in each case the 'missing' allele peak coincided exactly with a blue vWA peak (Fig. 1).

It was noted that all 3 samples were run in capillary 12 of one particular 3100 instrument, and all were found to be affected by a raised yellow background between clustered blue allele peaks. The profiles showed separated baselines in raw data and poor spectral compensation separated in the analysed data.

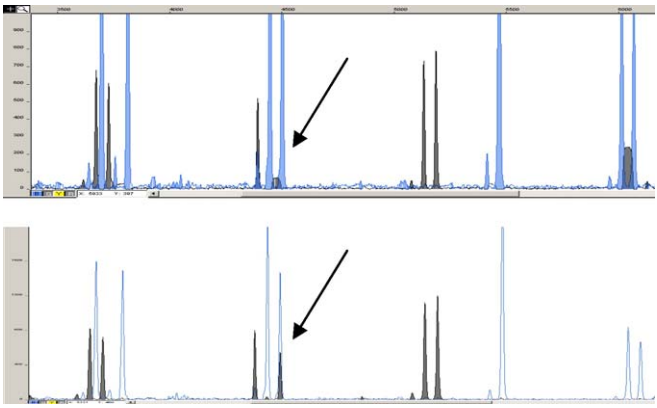

Fig. 1. Diagram showing the TH01 region from sample 8231 (profile mismatch sample). Top panel shows results from capillary with poor spectral calibration. TH01 9.3 allele is not visible. High background between blue peaks is indicative of poor spectral but overall profile quality is satisfactory. Bottom panel shows results from reanalysis of sample with good spectral calibration, indicating the TH01 9.3 allele clearly underlying the vWA allele peak.

Table 1
Differences in stutter ratio between 3100 and 377 data

Marker	Mean marker specific stutter ratio (3100), $N=3217$	Mean marker specific stutter ratio (377), $N=2670$
D3S1358	0.73	0.63
vWA	0.75	0.61
D16S539	0.57	0.49
D2S1338	0.84	0.76
AMEL	0	0
D8S1179	0.64	0.48
D21S11	0.70	0.55
D18S51	0.93	0.71
D19S433	0.88	0.73
THO1	0.31	0.23
FGA	0.83	0.66

It was surmised that poor spectral compensation had resulted in 'subtraction' of a genuine allele peak as though it was pull-up under the coincident vWA peak. After a new spectral calibration was performed on the instrument, no high background or other anomalies were reported and the genotypes matched those obtained from 377.

These anomalous results highlight an absolute requirement to ensure that a good spectral calibration is available for all capillaries in the array. A robust procedure to ensure good spectral calibration for all capillaries in any array has been developed and is performed after every new array installation.

3. Larger stutter peaks in capillary platform data

Distribution of stutter and *n*-peak percentages was investigated for each locus and S.D. calculated. All unambiguous stutter peaks and *n*-peaks above 50 rfu from both the 3100 and 377 sample sets were measured, recorded and logged as a percentage of the corresponding major allele peak area (Table 1).

A possible explanation for this increase in stutter peak magnitude across the platforms is that due to the increased sensitivity of the 3100, peaks are more defined above the baseline noise, which would account for the increased ratios. The increased sensitivity of the 3100 means that a truer representation of stutter peak magnitude is achieved. The implications of this are that the amount of template DNA put into the PCR reaction may have to be optimised as any error factor associated with the quantification method is highlighted by the 3100 instrument. An increase in the number of samples failing due to their stutter ratio being above the threshold value may also be observed, which could justify a move to using peak height as the main measure in calculating peak ratios as opposed to peak area.