LGC Genomics

Validation of custom sbeadex® forensic kit

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Background

Introducing a new technology into an existing laboratory can be time consuming and costly. Meeting accreditation requirements for lengthy validations can reduce capacity and consume experienced members of staff. LGC recently validated a new extraction chemistry within their Police and Criminal Evidence (PACE) subject sampling unit in the North West of England. For a high throughput lab dealing with hundreds of thousands of subject samples per year to very tight turnaround times, ceasing or adversely affecting production to develop and validate a new protocol on existing automation is not an option.

In 2006 LGC invested in a state of the art fully automated sampling line. The system performs DNA extraction, quantitation, concentration normalisation, PCR set-up, and electrophoresis plate set-up of forensic reference samples for loading to the UK National DNA Database. The automated sampling line utilises the latest Tecan technology including bespoke innovations and tailor made solutions based around magnetic bead chemistry, therefore making it difficult to change. However with LGC having their own proprietary magnetic particle extraction chemistry (sbeadex®) the task of developing and validating a new extraction protocol for the automated DNA extraction system was simpler and faster to achieve.

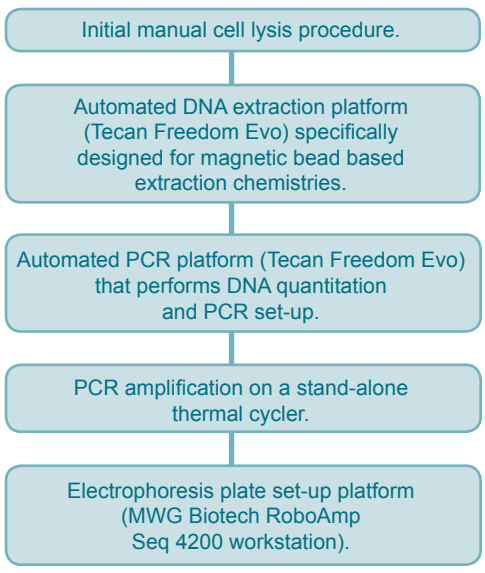

Figure 1: Flowchart demonstrating the automated processing line.

System set-up

The sbeadex® PACE protocol was developed and validated on the PACE DNA extraction robotic platform. This system consists of a Freedom Evo® 200 robot fitted with a 96 channel liquid handling unit (TeMo) and two RoMa plate manipulation arms. The deck is set-up with a magnet for bead collection and is configured for both 150 µL filtered and 200 µL non-filtered disposable tips.

Figure 2: The PACE DNA extraction robotic platform

Protocol development

LGC's experts from the PACE DNA and LGC Genomics teams adapted the current off the shelf sbeadex® forensic kit to fit the specifications of the PACE DNA extraction robotic platform. The absence of a heating element led to an optimised room temperature elution step, and a shortage of deck positions prompted the development of a reduced wash step protocol.

Apart from the necessary reagent volume changes and tip counting modifications the script remains the same, enabling the rapid switch between the previously validated magnetic bead extraction chemistry and sbeadex®.

'Existing capabilities of the robotic platforms were assessed and the in-house team of experts adapted the current sbeadex® forensic kit to meet the necessary requirements. The sbeadex® PACE protocol was finalised and the validation began two months after starting the evaluation'

Validation aims

- Maintain excellent quality results
- Not lengthen the run time on the automated system
- Not increase consumable costs
- Minimise changes to the systems configuration
- Minimise the project spend
- Be able to easily switch between chemistries

Validation

261 buccal samples were analysed back-to-back. To reflect the variation in sample storage conditions observed in PACE samples, these sample types were further broken down as shown in table 1. All samples were obtained from staff volunteer donors with known STR genotypes. These samples formed three complete automation batches of 87 samples each (89 including two negative extraction controls). The samples were dual processed using the ChargeSwitch Technology (CST) protocol and the new sbeadex® PACE protocol:

Buccal swab description	Storage conditions	Number	Comment
Fresh	Stored at 4°C for up to 10 days	181	Majority of samples received
Stored RT short term	Stored at room temperature for 10-20 days	40	Represents samples delayed in transit
Stored frozen long term	Stored at -20°C for 6 months – 5 years	40	Represents Reanalysis from B scrape

Table 1: Breakdown of sample types for back-to-back study.

The concentration of the resulting DNA extracts was measured by fluorescence using PicoGreen® dsDNA reagent (Life Technologies). Samples were amplified using SGM Plus® PCR kits (Life Technologies) and were run on capillary electrophoresis genetic analysers (Life Technologies) before being analysed with GeneMapper-ID analysis software (Life Technologies).

The validation criteria measured included:

- Variance of DNA yield - Mean DNA yield and distribution for each method and sample type were assessed. Optimal yield for subject samples is ~1 ng/µL with minimal variance
- Acceptability of positive and negative control samples
- Full concordance of all genotypes with the known donor STR profiles
- First time pass rate (FTPR) of the samples in the back to back study was measured for each condition
- STR profile quality. Measurements included peak magnitude, heterozygote balance, occurrence of stutter and N-peaks, over amplification and pull-up and number of samples noted as showing background or other artefact peaks were recorded.

The validation practical work was carried out with minimal disruption to the processing of the 'live' forensic samples. The nature of the protocol is such that a simple reagent change and selection of a new script within the software will enable the user to run the sbeadex® PACE protocol without the need to change any hardware, plastic ware or system set-up procedure.

Results

The resultant STR profiles were assessed against the validation criteria. The first time pass rate (FTPR) for each condition is described in table 2.

Buccal swab description	No.	FTPR CST (%)	FTPR sbeadex® (%)
Fresh	181	100	99.4
Frozen	40	100	97.5
Room temperature	40	100	100
Total	261	100	99.2
Frozen	40	100	97.5

Table 2: FTPR(%) for each protocol and storage condition.

The first time pass rate for the sbeadex® PACE data set was comparable to that of the CST data set. The occurrence of sample failures due to capillary electrophoresis problems were removed from the final FTPR calculation as these failures were not directly related to DNA extraction.

Overall two samples from the sbeadex® PACE dataset failed that could not be attributed to capillary electrophoresis. The first of the two failed to meet the criteria due to a stutter at three loci. The other sample was found to have a partial profile. Both samples passed after re-sampling.

The number of samples which have one or more loci falling below the acceptable peak height thresholds (75 RFU for heterozygote's and 250 RFU for homozygote's) were recorded for each condition and the average normalised peak heights for each locus were calculated (Figure 3).

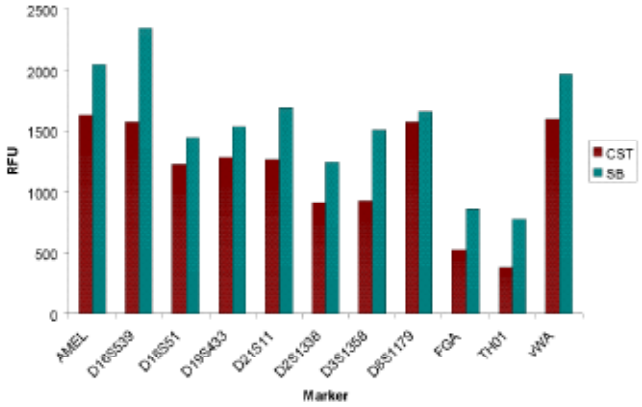

Figure 3: Average normalised peak height comparisons for each locus

The results show that the number of samples failing for sub-threshold peak heights was negligible and that the sbeadex® PACE data set displayed increased peak heights compared to CST in all loci.

Heterozygote balance between the sbeadex® PACE data set and the CST data set were compared by calculating the heterozygote peak area ratio (PAR). The sbeadex® PACE data had an average PAR of 1.0 compared to the CST data set which had an average PAR of 1.1. No samples within the sbeadex® PACE dataset failed to meet the technical standard for heterozygous balance.

There were no incidences of samples failing for the presence of artefact peaks, n-peaks or for poor background. Figure 4 shows a typical SGM Plus™ STR profile achieved using the sbeadex® PACE extraction protocol.

The results show that the sbeadex® PACE protocol is comparable to the existing CST chemistry currently being utilised.

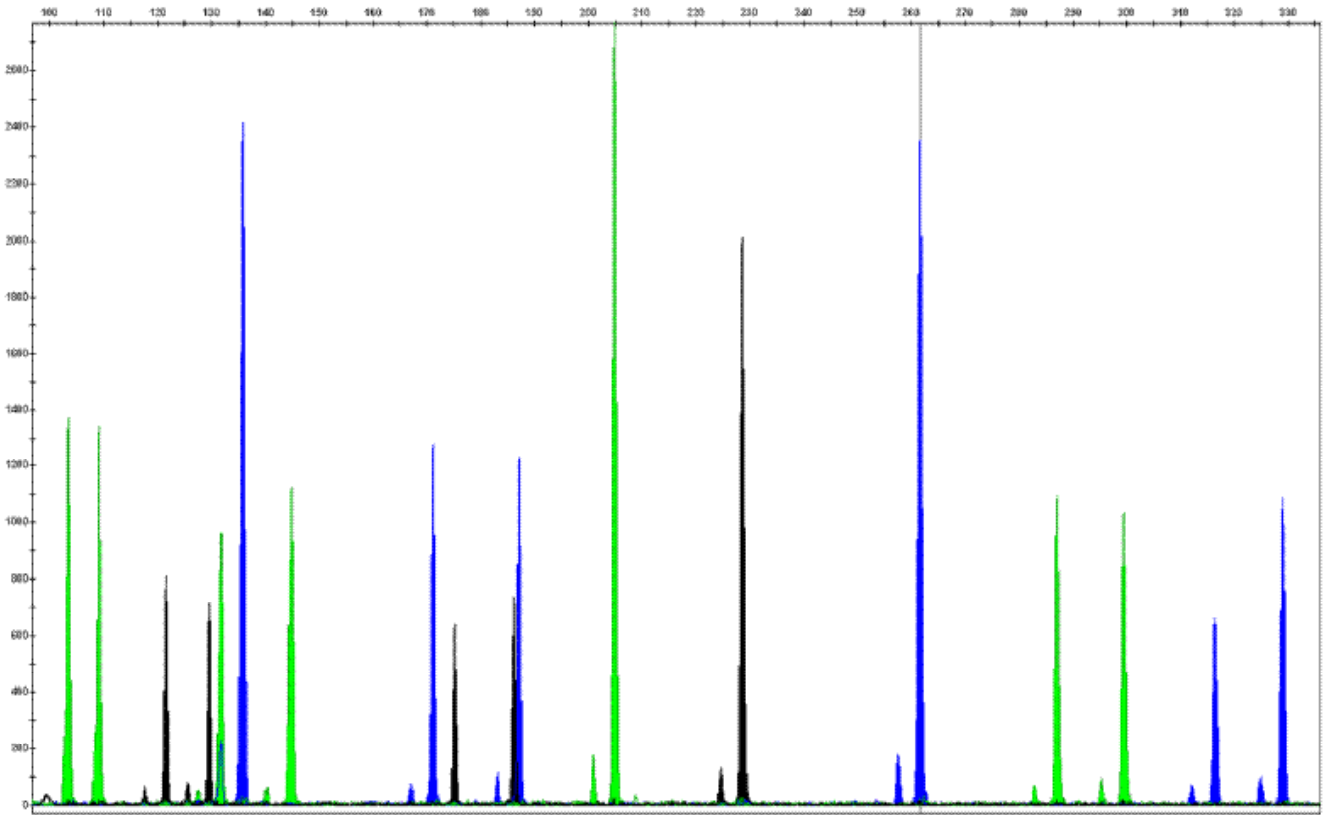

Figure 4: A typical STR profile generated from template DNA extracted using the sbeadex® PACE protocol

Change the easy way

The project met its aims delivering a validated robust new DNA extraction chemistry whilst also delivering a number of operational advantages over the previous validated magnetic bead DNA extraction chemistry:

- Maintained excellent quality results
- No change to up or downstream processing or sample tracking
- No change made to the configuration of the automated DNA extraction system
- No application support from the instrument manufacturer was necessary as in-house chemistry and automation expertise was utilised
- 11% reduction in extraction system run time
- 18% reduction in tip usage
- There was no disruption to the processing of PACE samples, with natural instrument downtime being used to perform the evaluation and validation experiments
- Ability to quickly and easily switch between two magnetic bead DNA extraction chemistries/ protocols
- Ability to purchase reagents in robot compatible plastic ware to allow direct 'plug-in' to the system. These bottles can be re-filled to reduce waste
- The optimisation, validation and training phases of the project were completed in 400 hours by one full time employee
- Time and cost savings were also realised during the training phase. As magnetic separation technology was already used extensive training programmes were reduced to workshop based learning in which 10 analysts were trained in four hours.

Conclusion

The performance of the sbeadex® PACE kit and protocol at recovering DNA from buccal swabs in line with the PACE DNA team's technical standards for loading DNA profiles to the UK National DNA Database is acceptable. The Tecan Freedom Evo extraction system within the PACE laboratory can be used to run the sbeadex® PACE protocol without any hardware modifications.

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