

## **AUTOMATED REACTION SETUP OF QUANTITATION PCR, STR AND CAPILLARY ELECTROPHORESIS ON THE QIAGILITY**

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The numbers of database as well as casework samples to be processed by forensic DNA laboratories are constantly growing. To cope with the increasing workload, manual procedures are more and more substituted by automated workflows. Besides allowing a higher sample throughput, automated solutions also help to improve the quality of results by reducing variability and avoiding user errors during multistep manual processes. Many laboratories have successfully automated their sample extraction work in the past years making use of various available low to high throughput systems. However, to shorten the overall turnaround time from sample to result further, improving the efficiency of downstream reaction setup becomes increasingly important.

The QIAgility is a compact benchtop instrument for automated setup of PCR reactions that is able to handle a wide variety of tube and plate formats. The instrument thus allows seamless integration into almost any existing laboratory workflow. The instrument has easy-to-use software that allows any user adapting setup protocols exactly to the requirements given by the extraction method used upstream and the PCR cyclers equipment present downstream. Optionally available UV light and HEPA filter help to reduce the risk of sample carry-over. The QIAgility performs the preparation of master mix from individual reaction components and dilutions of standard series and thereby provides a full walk away solution.

Protocols were developed for setup of Quantifiler™ and AmpF/STR® Identifiler™ PCR assays and the preparation of amplified products for capillary electrophoresis. The QIAgility Quantifiler™ reaction setup protocol performs a serial dilution of quantification standard DNA, prepares a master mix from individual kit components and distributes master mix, standards, samples and negative controls to a reaction plate. The instrument has a high pipetting precision, indicated by  $R^2$  values of standard curves of  $>0.99$ . Results from automated reaction setup were at least equivalent to that from manual reaction setup performed in parallel by highly skilled lab personnel. Pure buffer samples were processed along with positive samples in a checkerboard pattern; none of those negative control samples showed detectable levels of DNA. A protocol for setup of AmpF/STR® Identifiler™ PCR assays was developed that allows to load samples with any DNA concentration between 0 and 20 ng/μl without the need for making upfront adjustments. Samples are diluted automatically if necessary, and 1 ng of DNA is added to each reaction. For samples containing less than 100 pg/μl DNA the protocol uses the maximum volume possible. STR analyses of samples of a range of different concentrations show uniform peak heights after automated normalization. STR assay and capillary electrophoresis setup was performed using alternating patterns of positive and negative samples; again no signal above threshold was detected for any of the negative controls.

In conclusion, the automated reaction setup on the QIAgility system provides:

- fast and precise setup of common forensic PCR assays
- flexible integration into laboratory workflows
- high degree of process safety