

Processing challenging casework samples – new protocols for the QIASymphony® SP/AS



M. Scherer, B. Alsdorf, M. Breitbach, K. Matthaei, A. Schnur, N. Scholle, L. Bochmann, A. Prochnow, H. Engel
 QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

Introduction

The QIASymphony SP/AS instruments allow fully automated DNA extraction and PCR setup. Recently developed extraction protocols optimize performance with forensic samples. The Investigator® Advanced (ADV) protocol enhances DNA binding to magnetic particles thanks to a heating step prior to mixing. The High Efficiency (HE) protocol improves low-volume elution efficiency by eliminating any dead volumes that would typically reduce the overall yield. It is currently available for volumes from 30 to 80 µl. A new software package gives STR PCR setup normalization, allowing automatic adaptation of template amounts per reaction to a user-defined target based on imported concentration data. Minimum DNA template amounts can also be set, thus automatically excluding samples from downstream STR analysis if no reportable result is expected. This economical approach requires a sensitive and reliable quantification method to minimize the risk of false negatives. Results with Investigator Quantiplex Kits correlate well to the results from commercially available STR assays for samples close to the detection limit.



- Samples for:
- Forensic casework
 - Paternity testing
 - Reference testing

Human identification testing using the QIASymphony SP and AS.

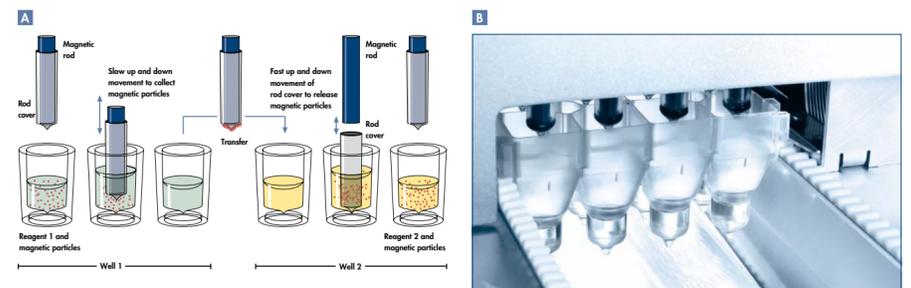
The QIASymphony SP and AS instruments

QIASymphony SP

- Magnetic bead-based DNA extraction
- Investigator Advanced (ADV) protocol for 200, 500, and 1000 µl lysate
- Flexible elution volumes of 30–200 µl buffer ATE or water

QIASymphony AS

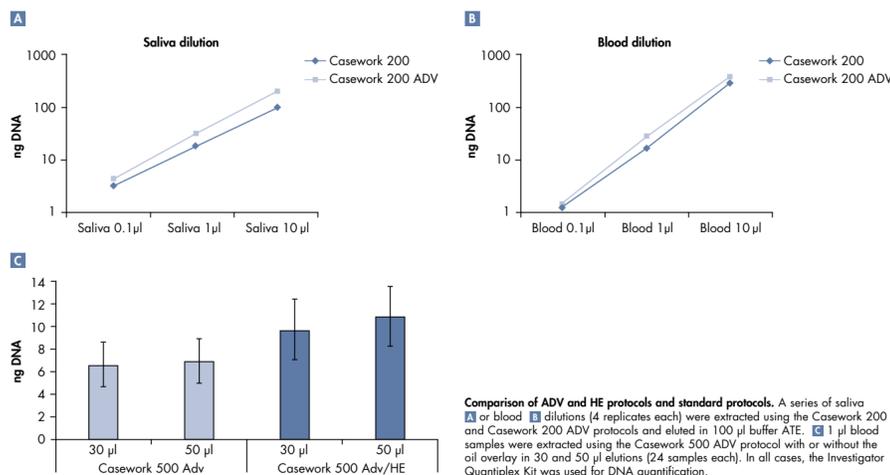
- Integrated reaction setup
- Normalization of PCR setup
- Flexible plate layout



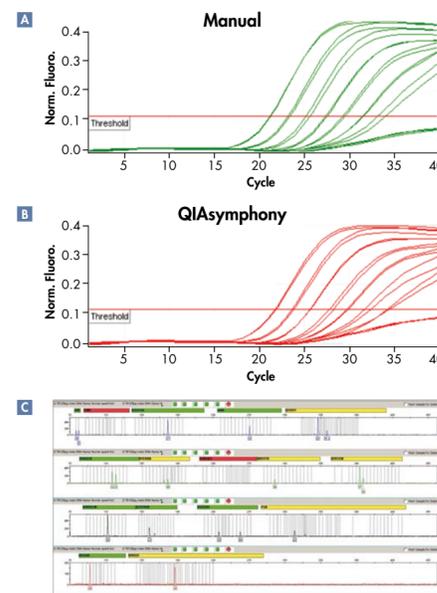
A Extraction principle of the QIASymphony SP. B Extractor with plastic accessories during a run.

Results: ADV and HE extraction protocols

The Investigator Advanced (ADV) protocol uses a longer heated binding step to increase the DNA yield. The sample, binding buffer, and magnetic beads are incubated on the lysis station before mixing on the extractor. For elution in volumes less than 100 µl, the High Efficiency (HE) protocol applies an oil overlay to prevent eluate being carried by the magnetic beads upon separation, thus reducing loss of purified DNA.



Results: Quantification

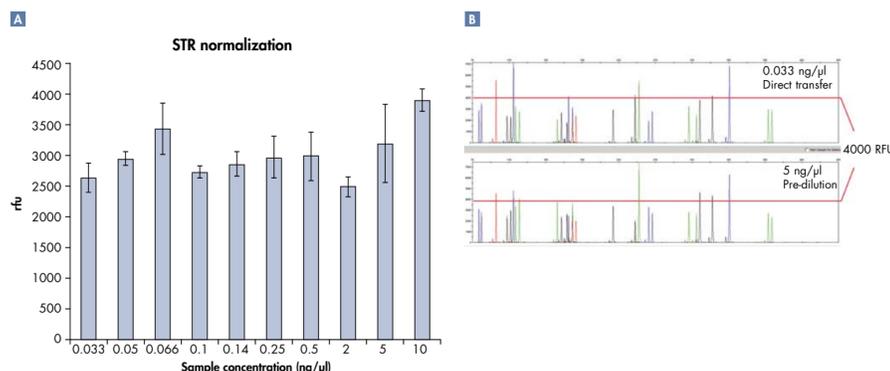


The QIASymphony AS provides high pipetting accuracy crucial for reliable DNA quantification. The instrument allows automated setup of quantification reactions and preparation of standard series. After quantification PCR, the concentration data can be uploaded to the QIASymphony instrument and used for normalization of STR PCR setup. A Data Handling Tool facilitates data transfer.

Comparison of manual and automated setup. Investigator Quantiplex Kit quantification standards and non-template controls were set up in duplicate manually and using the QIASymphony AS module. Real-time PCR was performed on a Rotor-Gene® Q with the standard protocol. Correlation of quantification and STR results. A DNA sample was quantified using the Investigator Quantiplex HYres Kit. The male DNA concentration was determined as 0.8 pg/µl. 15 µl template corresponding to 12 pg DNA was used for STR analysis with the Investigator ESSplex Plus Kit. As expected, a good partial profile of 19/29 alleles was obtained applying a 100 RFU threshold.

Results: Normalized STR setup

Software version 4.0 has a normalization feature for STR PCR setup on the QIASymphony AS, allowing the setting of template input to a user-defined value (e.g., 0.5 ng/reaction). Corresponding eluate volumes are automatically calculated based on imported quantification data. If required, eluates are pre-diluted in a dilution plate. Minimum DNA template amounts per reaction can also be defined to automatically exclude samples that will not give rise to loadable profiles (e.g., zero quants) from further analysis.

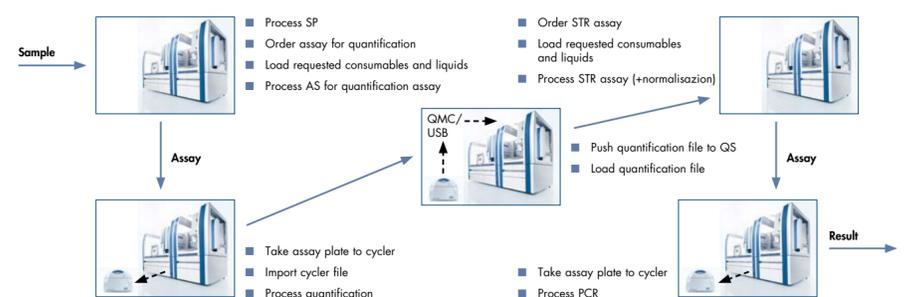


Normalized STR setup. A dilution series of DNA resulting in concentrations of 33 pg/µl to 10 ng/µl was prepared. Samples were loaded on the QIASymphony AS module (4 replicates each) and an Investigator ESSplex Plus reaction setup with a target input of 500 pg/reaction was performed. PCR was done on an Applied Biosystems® GeneAmp® 9700 instrument with Gold-plated 96-well Silver Block and capillary electrophoresis performed with a 3500 Genetic Analyzer. Comparison of average peak heights across all 16 ESSplex markers. Profile examples.

Conclusion

The QIASymphony SP/AS system functions within an integrated human identity testing workflow.

- Sensitive extraction of DNA from various casework and reference sample types
- Automatic transfer of purified DNA to AS module for quantification PCR setup
- Upload of quantification data and normalized setup of STR PCR



For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

All data generated in Hilden, Germany. Investigator STR PCR kits not available in the US and certain other countries.

Trademarks: QIAGEN®, QIASymphony®, Investigator®, Rotor-Gene® (QIAGEN Group); Applied Biosystems®, GeneAmp® (Life Technologies Corporation). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law. © 2013 QIAGEN, all rights reserved.