Unlocking Higher DNA Yields: The New EZ2® Connect Fx High Efficiency Extraction Protocols

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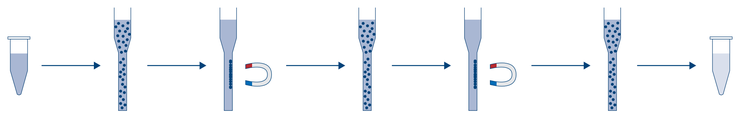
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Introduction and principle

The isolation of DNA of sufficient quantity and quality is a critical prerequisite for reliable forensic genetic analysis. Forensic samples encompass a broad range of biological materials and substrates and may contain only limited amounts of cellular material, fragmented DNA or substances that interfere with PCR amplification. Consequently, the extraction procedure must achieve efficient DNA recovery while removing cellular debris, proteins and potential inhibitors. This requirement is particularly important for trace, environmentally exposed or otherwise compromised samples, for which even small losses during purification may reduce the amount of amplifiable DNA available for downstream short tandem repeat (STR) analysis or NGS.

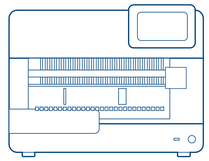
Magnetic-particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. DNA is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted in the user's choice of either water or TE buffer. The user can choose elution volumes between 20 µL and 200 µL.

This automated DNA extraction is a cornerstone of forensic workflows, requiring a delicate balance between throughput, purity and absolute yield. To address these requirements, a new suite of High Efficiency (HE) protocols was developed for the EZ2 Connect Fx Instrument, specifically optimized to maximize nucleic acid recovery without altering established chemistry or cartridge configurations.



The QIAGEN EZ2 Connext FX sample purification process.

Maximizing DNA yield



Background
Efficient automated DNA extraction is critical for maximizing STR profiling success, particularly from low-template, degraded, or challenging forensic samples.

Update
The new HE protocols maximize nucleic acid recovery without changing the established EZ2 Connect Fx workflow.

Protocol beta tested
- Trace HE
- Large volume HE
- Large volume RT HE

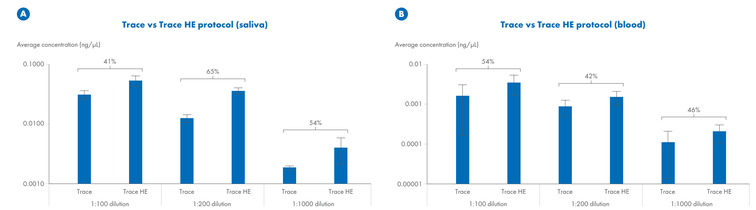
A developmental validation was undertaken, complemented by a collaborative, multi-laboratory beta-test. This assessed analytical performance, reproducibility and operational robustness of the newly developed EZ2 Connect Fx HE protocols relative to established reference protocols employing the EZ1&2® DNA Investigator Kit.

Automated extractions were performed across a range of sample matrices, including a five-point serial dilution series of biological fluids (blood and saliva) designed to simulate variable template input to challenge the extraction workflows for relevance in routine casework.

Multiple HE protocol variants were examined to determine whether protocol-specific modifications influenced DNA recovery, extract purity, processing consistency or compatibility with downstream molecular analysis.

Increased DNA from trace level samples

Efficient DNA extraction is essential for forensic analysis, particularly for low-template, degraded or otherwise challenging samples, where minor losses can compromise downstream quantification and STR profiling. To improve nucleic acid recovery, the HE protocols were developed for the EZ2 Connect Fx Instrument, and compared to standard existing protocols, provide superior yields of DNA from forensic samples.

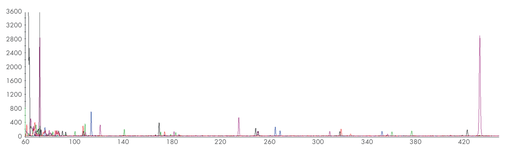


Dilution series of (A) saliva and (B) blood DNA samples. Samples 1:100, 1:200 and 1:1000 from a sample dilution series are shown. Data compared average DNA concentration per sample group (n=6) and increase (%) between the two different protocols and analyzed using the Investigator® Quantiplex® Pro Kit. Results show increased DNA yield across all dilution points.

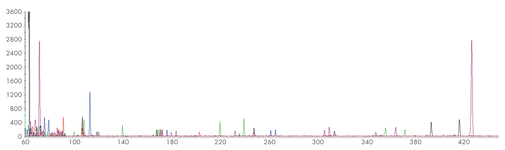
Improved DNA recovery: Minimizing profile dropout

The improvement in quantitative DNA yield was reflected in the downstream STR results. Across the dilution series, samples processed using the HE protocols showed enhanced allele recovery, with the greatest benefit observed at the lowest DNA input levels, where stochastic effects and allelic dropout are most likely to occur. For the most diluted samples, the HE protocols increased the proportion of recovered alleles by more than 30%, demonstrating that the additional DNA yield translated directly into more complete and informative STR profiles.

EZ2 Connect Fx Trace protocol



EZ2 Connect Fx Trace HE protocol



Combined electropherogram. Example DNA profiles for a 1:1000 saliva dilution processed with the standard EZ2 Trace protocol (upper panel) or the new EZ2 Trace HE protocol (lower panel). The DNA profile for samples processed using the HE protocol display more alleles and allelic peaks of greater peak height. Number of alleles counted above 100 RFU is shown.

Improved allele recovery.

Group	Trace		Trace HE	
	No. Alleles	Conc. (ng/µL)	No. Alleles	Conc. (ng/µL)
1:100	36	0.0276	36	0.0489
1:200	36	0.0145	36	0.0239
1:1000	22	0.0013	29	0.0016

Downstream STR analysis correlated directly with increased quantitative yields, resulting in enhanced allele recovery at the lower boundaries of the dilution series from samples processed with the Trace HE protocol.

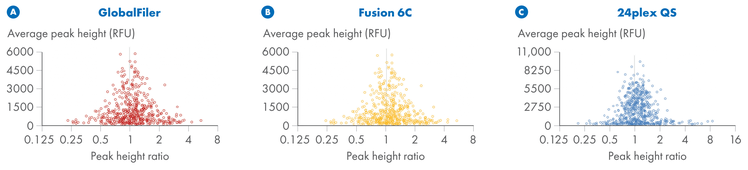
Downstream assay compatibility of EZ2 Connect Fx HE protocols

To determine the effect of the new HE protocols on the performance of subsequent STR amplification, extracts were generated using an elution volume of 100 µL and assessed using STR multiplex assays from different suppliers. Across the STR assays tested, peak height ratio distributions were comparable, indicating consistent intra-locus balance among the evaluated assays. At the lowest template level (1:1000 dilution), the observed increase in peak-height variability was consistent across assays and was attributable to the stochastic effects expected for low-template DNA analysis. No assay-specific bias was detected following implementation of the HE protocol updates. In particular, the data provided no evidence of PCR inhibition or extraction-related artefacts affecting profile balance. Collectively, these findings demonstrate that the updated EZ2 Connect Fx HE protocol improves DNA extraction yield from trace-level samples without compromising downstream STR amplification performance.

Average profile recovery across multiple assays.

Kit chemistry	Sample dilution	Avg. alleles	Expected (truth)	Recovery, %
GlobalFiler	1:100	46	46	100.00
	1:200	42	46	90.90
	1:1000	30	46	64.90
Fusion 6C	1:100	51	51	100.00
	1:200	45	51	88.90
	1:1000	33	51	64.10
24plex QS	1:100	45	45	100.00
	1:200	41	45	90.40
	1:1000	29	45	63.30

1:100, 1:200 and 1:1000 dilutions of a saliva serial dilution (n=6) processed using the new Trace HE protocol (elution volume 100 µL; 15 µL input volume for STR). Comparable average profile recovery across all three multiplexes (~100% at 1:100, ~90% at 1:200, ~64% at 1:1000).



STR peak heights of three autosomal STR kits. Graphs show the mean inter-locus peak height and the peak height ratio calculated for all alleles per STR marker. **A** GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific Inc.), **B** PowerPlex® Fusion 6C System (Promega Corporation), **C** Investigator 24plex QS Kit (QIAGEN).

Conclusions

The EZ2 Connect Fx High Efficiency (HE) protocols provide an effective strategy for increasing DNA recovery from low-template and challenging forensic samples. The higher quantitative yields translated directly into improved downstream STR performance, while retaining the established extraction chemistry and cartridge configuration of the EZ1&2 DNA Investigator Kit.

Increased DNA recovery: HE protocols generated higher quantitative DNA yields than the corresponding standard protocols across evaluated dilution series and multiple sample types

Improved low-template performance: The greatest benefit was observed at the lowest DNA input levels, where extraction losses and stochastic effects most strongly affect profile quality.

Enhanced STR allele recovery: At the highest sample dilutions, allele recovery increased by more than 30%, resulting in more complete and informative STR profiles.

Seamless workflow integration: The performance improvement was achieved without changes to the established EZ1&2 DNA Investigator chemistry or cartridge configuration, facilitating implementation on the EZ2 Connect Fx Instrument.

These findings indicate that the enhanced DNA recovery afforded by the HE protocols may mitigate allelic dropout and thereby increase the evidential value of low-template, degraded and otherwise compromised forensic samples.

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The EZ2 Connect Fx is intended for molecular biology applications in forensic, human identity, and paternity testing. This product is not intended for diagnosis, prevention, or treatment of a disease.

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Sample to Insight