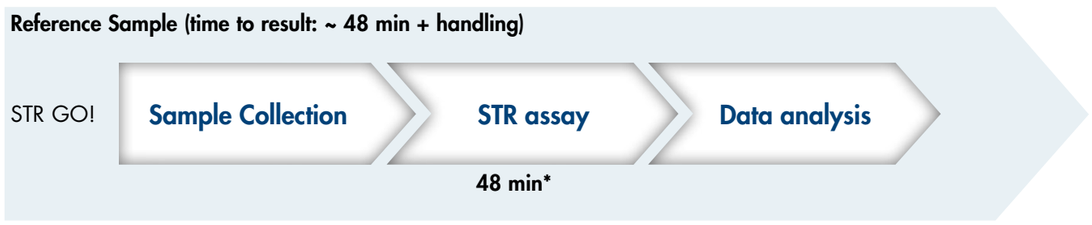
Development of the Investigator® STR GO! Kits for direct amplification of reference samples

M. Scherer, B. Alsdorf, L. Bochmann, A. Prochnow, H. Engel
QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

Introduction

Reference samples are typically of high quality and quantity, and the outcome of STR analysis of such samples is more predictable than that for unknown casework samples. Therefore, a streamlined process is possible: STR PCR can be performed directly from an FTA® punch or crude lysate of a buccal swab, with no need for sample extraction or quantification.

The Investigator STR GO! Kits were developed for such direct amplification. The two assay formats cover the CODIS set of markers (Investigator IDplex GO! Kit) and the new European Standard Set (ESS) including SE33 (Investigator ESSplex SE GO! Kit). Samples can be blood or buccal cells on FTA punches and other paper or buccal swabs. Direct PCR amplification takes just 48–55 min, depending on the sample type. The analysis gives balanced profiles and allows high first pass rates for all common reference samples. To overcome unbalanced amplifications due to rare mutations in the primer binding sites of vWA, D16S539, and SE33, new SNP primers have been introduced.



Workflow for processing reference samples using the Investigator GO! kits.

* For Blood on FTA samples

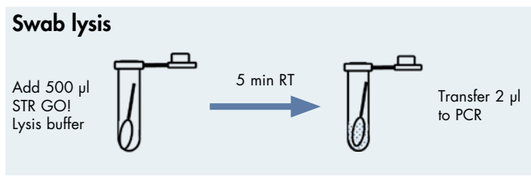
Method: Direct amplification

Investigator STR GO! Kits use the basic features of the Investigator STR Plus kits:

- Fast PCR cycling technology
- High inhibitor tolerance

The assays have been further optimized for direct amplification:

- Shorter PCR cycling protocols
- Streamlined reaction setup (only primer mix, reaction mix, sample)
- Fast and convenient lysis protocol for buccal swabs



For buccal swabs, 5 min lysis at room temperature yields a crude lysate for direct amplification.

Investigator STR GO! PCR Protocol

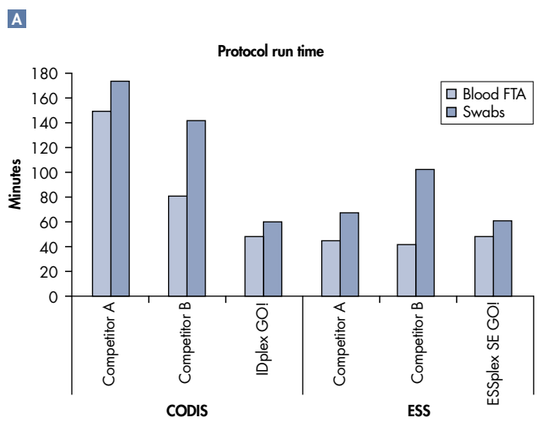
Temperature	Time	Cycles
95°C (Hot-start)	8 min	–
96°C	10 s	25–30*
61°C	38 s	
68°C	1 min	–
10°C	∞	

ABI GeneAmp 9700 (Max mode)

*Recommended: 25 cycles for blood on FTA paper; 28 cycles for buccal cells on FTA paper and buccal swab lysates

Results: Fast and efficient reference workflows

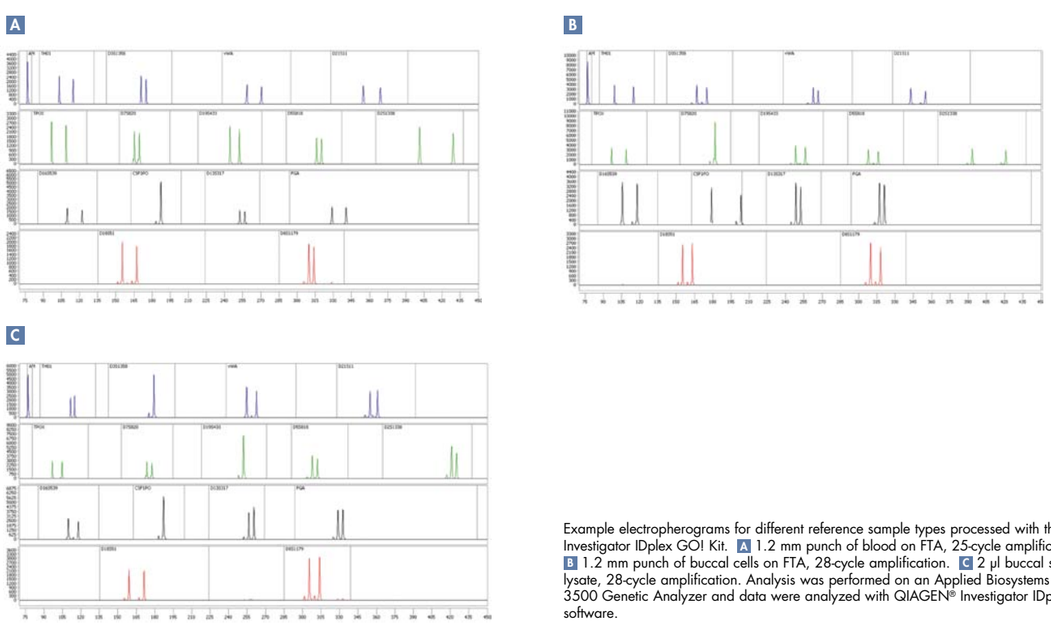
Investigator STR GO! Kits use optimized PCR cycling times. Reaction setup consists of preparing a two-component master mix (reaction mix + primer mix) and adding the sample. FTA punches can be added directly. Swabs only require a 5-min lysis at room temperature. For high-throughput analysis of blood or buccal cells on FTA paper, protocols for automated punching and reaction setup using the Hamilton® easyPunchTM system were developed.



A Protocol run time comparison for blood on FTA paper and swabs. Effective run time is shown for Investigator IDplex GO! and ESSplex SE GO! kits against the times for products from other suppliers. Analyses run on an Applied Biosystems® GeneAmp® PCR System 9700 Thermal Cycler with Gold-plated Silver Block. All assays were run according to manufacturer recommendations. Run times for swabs include pre-treatments to obtain a crude lysate according to corresponding handbook instructions.
B Fully automated punching of FTA cards using the Hamilton easyPunch system.

Results: EPGs for typical reference sample types

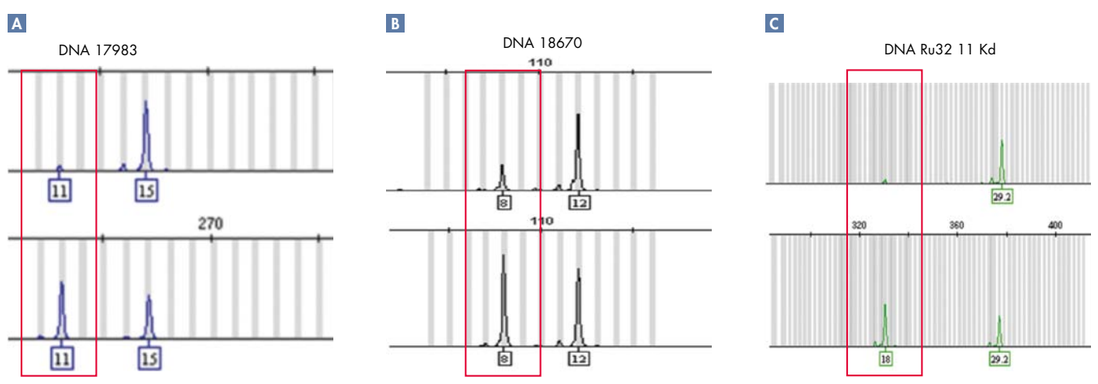
Direct amplification protocols are available for the three main reference sample types (blood or buccal cells on FTA or similar paper, and buccal swab crude lysates). All of the protocols show robust and balanced amplification and provide high first pass rates.



Example electropherograms for different reference sample types processed with the Investigator IDplex GO! Kit. A 1.2 mm punch of blood on FTA, 25-cycle amplification. B 1.2 mm punch of buccal cells on FTA, 28-cycle amplification. C 2 µl buccal swab lysate, 28-cycle amplification. Analysis was performed on an Applied Biosystems 3500 Genetic Analyzer and data were analyzed with QIAGEN® Investigator IDproof software.

Results: SNP primers

Three known mutations present at elevated frequencies in populations of African origin affect the primer binding sites of vWA, D16S539, and SE33. An 8 bp deletion in the vWA flanking region due to repeat sequence structure provides an almost perfect match, differing in just a single base. An additional SNP primer has been introduced to recover amplification of the affected allleles (Fig. A). Similarly, a 4 bp deletion in the SE33 flanking region leads to a binding site with one base changed. This was fixed with another SNP primer (Fig. C). A true SNP mutation is present in the binding region of the D16S539 marker (Fig. B).



Amplification of alleles carrying primer binding site mutations in markers vWA A, D16S539 B, or SE33 C in the presence or absence of corresponding SNP primers. Affected alleles are marked. Samples were amplified on an Applied Biosystems GeneAmp PCR System 9700 Thermal Cycler with gold-plated silver block. Analysis was performed on an Applied Biosystems Genetic Analyzer 3500, equipped with a 36 cm 8-capillary array and POP-4 polymer.

Conclusion

Using robust direct amplification technology, the Investigator GO! Kits were developed specifically for reference samples: punches from FTA or similar paper or in crude lysates from swabs. Both the Investigator IDplex GO! And ESSplex SE GO! kits are suitable for forensic applications and paternity testing.

Key features include:

- Fast direct amplification of all typical reference sample types (48–55 min protocol time)
- Fast and convenient processing of swabs (5 min lysis at room temperature)
- Easy two-component reaction setup
- Validated automated solutions for high-throughput work
- Innovative and extremely inhibitor-resistant chemistry for reliable results
- Minimized allelic overlap for unambiguous results
- Validated for forensic use according to SWGDAM and ENFSI guidelines

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 GO! kits and Investigator STR PCR kits not available in the US and certain other countries.

1077864 08/2013

Sample & Assay Technologies