Supplementary Protocol June 2017

Direct amplification of DNA from buccal cells on BODE Buccal DNA Collectors using the Investigator® ESSplex SE QS Kit

This protocol describes how to perform STR analysis by directly amplifying DNA from human buccal cells that have been transferred to BODE Buccal DNA Collectors.

The experimental conditions outlined in this protocol have been found to give the best results. However, depending on the sample material, PCR cycle numbers may be adapted to ensure the highest possible first-round success rates. We recommend running a representative batch of samples in order to confirm that the cycle numbers given in this protocol are optimal. Increase the cycle number by one if the signals in the resulting electropherograms are too low. Decrease the cycle number by one if the signals in the resulting electropherograms are too high.

IMPORTANT: Please read the *Investigator ESSplex SE QS Kit Handbook* for general information on safety, handling and storage.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Investigator ESSplex SE QS Kit (cat. nos. 381575 and 381577)
- Investigator STR GO! Punch Buffer (1000) or (200) (QIAGEN, cat. no. 386528 or 386526)
- Investigator STR GO! Lysis Buffer (cat. no. 386516)
- Uni-Core™ Punch 1.2 mm (GE Healthcare, cat. no. WB100028) or Whatman™ Harris Uni-Core1.2 mm Micro-Punch with Cutting Mat (Whatman/GE Healthcare, cat. no. WB100005)
- Pipets and pipet tips
- One of the following thermal cyclers:

  QIAGEN Rotor-Gene® Q

  Applied Biosystems® GeneAmp® PCR System 9700

  Bio-Rad® PTC-200

Biometra® UNO-Thermoblock

Eppendorf® Mastercycler® ep

- PCR tubes or plates
- Vortex

Important points before starting

- The best results for buccal cells on BODE Buccal DNA Collectors have been obtained with one punch of 1.2 mm diameter and the cycling conditions given in Table 2.
- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination risks.

Things to do before starting

- Before opening the tubes containing PCR components, vortex and then centrifuge briefly to collect the contents at the bottom of the tubes.

Procedure

1. Take a 1.2 mm punch from the center of the sample spot with a suitable tool (e.g., Harris Uni-Core Punch).

   Important: Do not use more than one punch at a time.
2. Transfer the punch to the bottom of an empty PCR tube.
3. Add 5 µl of Investigator STR GO! Lysis Buffer to each sample
4. Incubate for 5 mins at 95°C, leaving tubes open.
5. Prepare a master mix according to Table 1.

   The master mix contains all of the components needed for PCR. Prepare a volume of reaction mix 10% greater than that required for the total number of PCR assays to be performed. This should include positive and negative control reactions.
6. Dispense the master mix into final reaction plate/tubes.
7. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 2.

   Note: If using the GeneAmp 9700 thermal cycler with an Aluminum block, use "Std Mode", or with a Silver block or Gold-plated Silver block, use "Max Mode". Do not use "9600 Emulation Mode".

8. After the completion of the protocol, add 1 µl of the PCR product directly to 12 µl Hi-Di™ Formamide plus the Size Standard. Start the analyzer run as described in the corresponding kit handbook.

Table 1. Master mix setup

Component	Volume per reaction
Fast Reaction Mix 2.0	7.5 µl
Primer Mix	2.5 µl
Investigator STR GO! Punch Buffer	2.0 µl
Nuclease-free water (added in set 4)	14.0 µl*
Total volume	**26 µl**

* Note that if reduced PCR volumes are used, reagents should be scaled proportionally. Any changes to the recommended protocol must be validated by the testing laboratory.

Table 2. Recommended cycling protocol

Component	Time	Number of cycles
98°C*	30 s	3 cycles
64°C	55 s	
72°C	5 s	
96°C	10 s	23 cycles
61°C	55 s	
72°C	5 s	
68°C	2 min	
10°C	∞	–

* Hot-start to activate DNA polymerase.

Troubleshooting

For general troubleshooting, please consult the "Troubleshooting Guide" in the *Investigator ESSplex SE QS Handbook*.

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.