User-Developed Protocol

Direct PCR amplification of STR loci from Bode Buccal DNA Collectors™

This protocol is for direct PCR amplification of STR loci from punches of buccal cells on Bode Buccal DNA Collectors, using the Investigator® 24plex GO! Kit.

This protocol has not been thoroughly tested and optimized by QIAGEN.

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *Investigator 24plex GO! Kit Handbook*, before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate Safety Data Sheets (SDSs), available from the product supplier. The Investigator 24plex GO! Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Important points before starting

- 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 risk.

Things to do before starting

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

Procedure

1. Collect a 1.2 mm punch from the tip (rounded end) of the Bode Buccal DNA Collector with a suitable tool (e.g., Uni-Core™ punch, GE Healthcare) into a 0.2 ml PCR-grade plate or 0.2 ml PCR-grade tube.
   Important: Do not use more than one punch at a time, per well or per tube.

2. Add 2 µl of QIAGEN Investigator STR GO! Kit Lysis Buffer directly onto the 1.2 mm punch. Centrifuge briefly if necessary to collect the punch and buffer at the bottom of the plate or tube.

3. Incubate the sample at 95°C for 5 minutes. Do not seal the plate.
   Note: The Lysis Buffer will evaporate

4. Prepare a master mix according to Table 1. Mix the reaction mix thoroughly.
   Note: As some loss of reagents can occur during transfers, prepare excess master mix. Also, include positive and negative control reactions. The master mix contains all of the components required for the PCR, besides the template (sample) DNA.

5. After incubation, dispense 20 µl of the master mix into each well of the PCR plate or the PCR tubes, containing the 1.2mm punch.
   Note: Do not mix the reaction after distributing the master mix.

6. Prepare the positive and negative controls.
   Positive control: Use 2 µl Control DNA (i.e., 10 ng).

Note: The amount of Control DNA may need to be adapted after setting the optimal PCR cycle number in your laboratory, if signals are too low or too high. Do not add a blank disc to the positive control well.

Negative control: Do not add any template DNA. Do not add a blank disc or water to the negative control PCR tube or well.

7. Briefly centrifuge reactions to ensure discs are fully submerged.

8. Program the thermal cycler according to the manufacturer's instructions, using the conditions listed in Table 2.
   Note: If using the GeneAmp® PCR system 9700 with an Aluminum Sample Block Module, use "Std Mode". If using a Silver Block or Gold-Plated Silver Block Module, use "Max Mode". Do not use "9600 Emulation Mode".

9. After the cycling protocol is complete, store samples at –30°C to –15°C protected from light, or proceed directly with electrophoresis.

Table 1: Master mix setup

Component	Volume per reaction
Fast Reaction Mix 2.0	7.5 µl
Primer Mix	12.5 µl
Total volume	20.0 µl

Table 2: Cycling protocol for Bode Buccal Collectors

Temperature	Time	Number of cycles
98°C*	30 s	
64°C	40 s	3 cycles
72°C	5 s	
96°C	10 s	
61°C	40 s	24 cycles
72°C	5 s	
68°C	2 min	–
60°C	2 min	–
10°C	∞	–

* Hot start to activate DNA polymerase.

Ordering Information

Product	Contents	Cat. no.
Investigator 24plex GO! Kit (200)	Primer Mix, Fast Reaction Mix 2.0 including *Taq* DNA Polymerase, Control DNA, Allelic Ladder 24plex, DNA Size Standard 550 (BTO)	382426
Investigator 24plex GO! Kit (1000)	Primer Mix, Fast Reaction Mix 2.0 including *Taq* DNA Polymerase, Control DNA, Allelic Ladder 24plex, DNA Size Standard 550 (BTO)	382428
Related product		
Investigator STR GO! Lysis Buffer (200)	Lysis Buffer for 200 swab samples	386516

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