June 2021

Supplementary Protocol

Direct amplification of DNA using the Investigator® 26plex QS Kit

This protocol describes how to perform STR analysis by direct amplification using the Investigator 26plex QS Kit (cat. nos. 382615, 382617).

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 to confirm that the cycle numbers given in this protocol are optimal. Increase the cycle number by 1 if the signals in the resulting electropherograms are too low. Decrease the cycle number by 1 if the signals in the resulting electropherograms are too high.

IMPORTANT: Consult the "Safety Information" and "Important Notes" sections in the *Investigator 26plex QS Handbook*, **www.qiagen.com/HB-2681**, before beginning this procedure. For safety information about the additional chemicals mentioned in this protocol, consult the appropriate safety data sheets (SDSs) available from the product supplier.

Equipment and reagents to be supplied by the user

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.

For all protocols

- Control DNA 9948 (5 ng/µl) (cat. no. 386041)
- DNA Size Standard 24plex (BTO) (100) (cat. no. 386035)
- DNA Size Standard 450 (BTO) (100) (cat. no. 386045)
- Hi-Di™ Formamide, 25 ml (Applied Biosystems, cat. no. 4311320)
- Matrix Standards BT6 for multicapillary instruments (cat. no. 386224)
- Pipettes and pipette tips

- DNA Analyzer*
  - 3500 Genetic Analyzer (Applied Biosystems, cat. no. 4405673)
  - 3500xL Genetic Analyzer (Applied Biosystems, cat. no. 4405633)
- PCR thermal cycler*
  - QIAamplifier® 96
  - GeneAmp PCR System 9700
  - Veriti™ 96-Well Thermal Cycler
  - ProFlex™ 96-well PCR System
  - Bio-Rad® PTC-200
  - Biometra UNO-Thermobloc
  - Eppendorf® Mastercycler® ep
- PCR tubes or plates
- Microcentrifuge for PCR tubes or plates

For protocols based on blood or buccal cells on paper

- UniCore Punch 1.2 mm (cat. no. WB100028) and Harris Micro Punch Replacement Cutting Mat, 6.0 x 8.0 inches (cat. no. WB100020)
- Investigator STR GO! Punch Buffer (1000) or (200) (cat. no. 386528 or 386526)
- Investigator STR GO! Lysis Buffer (cat. no. 386516)

  Note: This is only for the BODE Buccal DNA Collector.

For protocols based on buccal swab lysates

- Investigator STR GO! Lysis Buffer (cat. no. 386516)
- 2 ml microcentrifuge tubes
- Shaker for 2 ml microcentrifuge tubes

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Protocol: PCR amplification from blood on FTA and other paper

This protocol is for direct PCR amplification of STR loci from punches of blood on FTA and other paper using the Investigator 26plex QS Kit.

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 risks.

Things to do before starting

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

Procedure

1. Prepare a master mix according to Table 1 (for full volume PCR) or Table 2 (for reduced volume PCR).

   The master mix contains all the components needed for PCR. Prepare a volume of reaction mix 10% greater than what is required for the total number of PCR assays to be performed. This should include both positive and negative control reactions.

Table 1. Recommended master mix setup for full volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	7.5 µl
Primer Mix	2.5 µl
Investigator STR GO! Punch Buffer	2.0 µl
Nuclease-free water	8.0 µl
Total reaction volume per sample	20.0 µl

Table 2. Recommended master mix setup for reduced volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	3.75 µl
Primer Mix	1.25 µl
Investigator STR GO! Punch Buffer*	2.0 µl
Nuclease-free water	3.0 µl
Total reaction volume per sample	10.0 µl

* If reduced PCR volumes are used, it is important to always use 2 µl of the STR GO! Punch Buffer, regardless of the master mix volume. All other reagents should be scaled proportionally. Any changes to the recommended protocol must be validated by the testing laboratory.

2. Vortex the reaction mix thoroughly and dispense the total required reaction volume per sample into PCR tubes or the wells of a PCR plate.
3. Take a 1.2 mm punch from the center of the sample spot with a suitable tool (e.g., UniCore Punch 1.2 mm).

   Important: Do not use more than one punch at a time.
4. Transfer one 1.2 mm disc to each reaction. Do not mix the reaction after disc transfer.
5. Prepare the positive and negative controls.

   Positive control: Use 2 µl Control DNA (5 ng/µl).

   Note: If signals are too low or too high in the subsequent PCR, you may need to adapt the amount of control DNA for your laboratory after setting the optimal PCR cycle number. Do not add a blank disc to the positive control well.

   Negative control: Do not add template DNA to the negative control. Do not add a blank disc or water to the negative control PCR tube or well.
6. Briefly centrifuge reactions to ensure that discs are fully submerged.
7. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 3.

   Note: If using the GeneAmp 9700 thermal cycler with an aluminum block, use "Std Mode"; with a silver block or a gold-plated silver block, use "Max Mode". Do not use "9600 Emulation Mode".

   After completion of the protocol, store samples at –30 to –15°C protected from light, or proceed directly with electrophoresis. 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 *Investigator 26plex QS Handbook*.

Table 3. Recommended cycling protocol for blood on FTA or other paper

Temperature	Time	Number of cycles
96°C*	12 min	–
96°C 64°C 72°C	10 s 55 s 5 s	3 cycles
96°C 61°C 72°C	10 s 55 s 5 s	22 cycles
68°C	2 min	–
60°C	2 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Protocol: PCR amplification from buccal cells on FTA and other paper

This protocol is for direct PCR amplification of STR loci from punches of buccal cells on FTA and other paper using the Investigator 26plex QS Kit.

Important points before starting

- For buccal cells collected using the UniCore Punch 1.2 mm, take the punch from a white area. This color indicates successful sample transfer.
- 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 the tubes briefly to collect the contents at the bottom.

Procedure

1. Prepare a master mix according to Table 4 (for full volume PCR) or Table 5 (for reduced volume PCR).

   The master mix contains all the components needed for PCR. Prepare a volume of reaction mix 10% greater than what is required for the total number of PCR assays to be performed. This should include both positive and negative control reactions.

Table 4. Recommended master mix setup for full volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	7.5 µl
Primer Mix	2.5 µl
Investigator STR GO! Punch Buffer	2.0 µl*
Nuclease-free water	8.0 µl
Total reaction volume per sample	20.0 µl

* If reduced PCR volumes are used, it is important to always use 2 µl of the STR GO! Punch Buffer, regardless of the master mix volume. All other reagents should be scaled proportionally. Any changes to the recommended protocol must be validated by the testing laboratory.

Table 5. Recommended master mix setup for reduced volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	3.75 µl
Primer Mix	1.25 µl
Investigator STR GO! Punch Buffer	2.0 µl*
Nuclease-free water	5.0 µl
Total reaction volume per sample	12 µl

* If reduced PCR volumes are used, it is important to always use 2 µl of the STR GO! Punch Buffer, regardless of the master mix volume. All other reagents should be scaled proportionally. Any changes to the recommended protocol must be validated by the testing laboratory.

2. Vortex the reaction mix thoroughly and dispense the total required reaction volume per sample into PCR tubes or the wells of a PCR plate.
3. Take a 1.2 mm punch from the center of the sample spot with a suitable tool (e.g., UniCore Punch 1.2 mm).

   Important: Do not use more than one punch at a time.
4. Transfer one 1.2 mm disc to each reaction. Do not mix the reaction after disc transfer.
5. Prepare the positive and negative controls.

   Positive control: Use 1 µl Control DNA (5 ng/µl).

   Note: If signals are too low or too high in the subsequent PCR, you may need to adapt the amount of control DNA for your laboratory after setting the optimal PCR cycle number. Do not add a blank disc to the positive control well.

   Negative control: Do not add template DNA to the negative control. Do not add a blank disc or water to the negative control PCR tube or well.
6. Briefly centrifuge reactions to ensure discs are fully submerged.
7. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 6.

   Note: If using the GeneAmp 9700 thermal cycler with an aluminum block, use "Std Mode"; with a silver block or a gold-plated silver block, use "Max Mode". Do not use "9600 Emulation Mode".

   After completion of the protocol, store samples at –30 to –15°C protected from light, or proceed directly with electrophoresis. 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 *Investigator 26plex QS Handbook*.

Table 6. Recommended cycling protocol for buccal cells on FTA or other paper

Temperature	Time	Number of cycles
96°C*	12 min	–
96°C	10 s	
64°C	55 s	3 cycles
72°C	5 s	
96°C	10 s	
61°C	55 s	24 cycles
72°C	5 s	
68°C	2 min	–
60°C	2 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Protocol: PCR amplification from buccal cells on BODE Buccal DNA Collectors

This protocol is for direct PCR amplification of STR loci from punches of buccal cells on BODE Buccal DNA Collector using the Investigator 26plex QS Kit.

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 risks.

Things to do before starting

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

Procedure

1. Prepare a master mix according to Table 7 (for full volume PCR reactions) or Table 8 (for reduced volume PCR reactions).

   The master mix contains all of the components needed for PCR. Prepare a volume of reaction mix 10% greater than what is required for the total number of PCR assays to be performed. This should include both positive and negative control reactions.

Table 7. Recommended master mix setup for full volume PCR reactions

Component	Volume per reaction
Fast Reaction Mix 3.0	7.5 µl
Primer Mix	2.5 µl
Investigator STR GO! Punch Buffer	2 µl*
Nuclease-free water	8 µl
Total reaction volume per sample	20 µl

* If reduced PCR volumes are used, it is important to always use 2 µl of the STR GO! Punch Buffer, regardless of the master mix volume. All other reagents should be scaled proportionally. Any changes to the recommended protocol must be validated by the testing laboratory.

Table 8. Recommended master mix setup for reduced volume PCR reactions

Component	Volume per reaction
Fast Reaction Mix 3.0	3.75 µl
Primer Mix	1.25 µl
Investigator STR GO! Punch Buffer	2 µl*
Nuclease-free water	3 µl
Total reaction volume per sample	10 µl

* If reduced PCR volumes are used, it is important to always use 2 µl of the STR GO! Punch Buffer, regardless of the master mix volume. All other reagents should be scaled proportionally. Any changes to the recommended protocol must be validated by the testing laboratory.

2. Vortex the reaction mix thoroughly.
3. Take a 1.2 mm punch from the center of the sample spot with a suitable tool (e.g., UniCore Punch 1.2 mm).

   Important: Do not use more than one punch at a time.
4. Transfer the punch to the bottom of an empty PCR tube.
5. Add 5 µl of Investigator STR GO! Lysis Buffer to each sample.
6. Incubate for 5 min at 95°C, leaving tubes open.
7. Dispense the master mix into final reaction plate/tubes.
8. Prepare the positive and negative controls.

   Positive control: Use 1 µl Control DNA (5 ng/µl).

   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.
9. Briefly centrifuge reactions to ensure discs are fully submerged.
10. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 9.

    Note: If using the GeneAmp PCR System 9700 thermal cycler with an aluminum block, use "Std Mode"; with a 96-Well Silver Sample Block or 96-Well Gold-Plated Silver Sample Block, use "Max Mode". Do not use "9600 Emulation Mode".

    After completion of the protocol, store samples at –30 to –15°C protected from light or proceed directly with electrophoresis. 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 *Investigator 26plex QS Handbook*.

Table 9. Recommended cycling protocol for buccal cells on BODE Buccal DNA Collectors

Temperature	Time	Number of cycles
96°C*	12 min	–
96°C 64°C 72°C	10 s 55 s 5 s	3 cycles
96°C 61°C 72°C	10 s 55 s 5 s	23 cycles
68°C	2 min	–
60°C	2 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Protocol: PCR amplification from buccal swab lysates

This protocol is for direct PCR amplification of STR loci from crude lysates of buccal swabs using the Investigator 26plex QS Kit.

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 risks.

Things to do before starting

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

Procedure

1. Place the swab in a 2 ml microcentrifuge tube.

   Carefully cut, break off, or eject the end part of the swab.
2. Add 500 µl STR GO! Lysis Buffer to the sample.
3. Incubate the sample at 95°C for 5 min, shaking at 1200 rpm in a thermomixer.
4. Prepare a master mix according to Table 10 (for full volume PCR) or Table 11 (for reduced volume PCR).

   The master mix contains all the components needed for PCR. Prepare a volume of reaction mix 10% greater than what is required for the total number of PCR assays to be performed. This should include both positive and negative control reactions.

Table 10. Recommended master mix setup for full volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	7.5 µl
Primer Mix	2.5 µl
Nuclease-free water	10.0 µl
Total reaction volume per sample	20.0 µl

Table 11. Recommended master mix setup for reduced volume PCR

Component	Volume per reaction
Fast Reaction Mix 3.0	3.75 µl
Primer Mix	1.25 µl
Nuclease-free water	5.0 µl
Total reaction volume per sample	10.0 µl

5. Vortex the reaction mix thoroughly and dispense the total required reaction volume per sample into PCR tubes or the wells of a PCR plate.
6. Mix the swab lysate thoroughly. Transfer 2 µl (for full reaction volume) or 1 µl (for reduced reaction volume) of swab lysate directly to each reaction.
7. Prepare the positive and negative controls.

   Positive control: Use 1 µl Control DNA (5 ng/µl).

   Note: If signals are too low or too high in the subsequent PCR, you may need to adapt the amount of control DNA for your laboratory after setting the optimal PCR cycle number.

   Negative control: Use a blank swab lysate.
8. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 12.

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

   After completion of the protocol, store samples at –30 to –15°C protected from light, or proceed directly with electrophoresis. 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 *Investigator 26plex QS Handbook*.

Table 12. Recommended cycling protocol for buccal swab lysates

Temperature	Time	Number of cycles
96°C*	12 min	–
96°C 64°C 72°C	10 s 55 s 5 s	3 cycles
96°C 61°C 72°C	10 s 55 s 5 s	23 cycles
68°C	2 min	–
60°C	2 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Troubleshooting

For general troubleshooting, please consult the "Troubleshooting Guide" in the *Investigator 26plex QS Handbook*.

Document Revision History

Date	Changes
02/2020	Initial release
06/2021	Revised cycling conditions in Table 3, Table 6, and Table 12. Changed the volume per reaction of nuclease-free water in Table 2. Added "Veriti 96-Well Thermal Cycler", "ProFlex 96-well PCR System", and "QIAamplifier 96" in the Equipment and reagents to be supplied by the user section. Added "Protocol: PCR amplification from buccal cells on BODE Buccal DNA Collectors". Editorial and layout changes.

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.