Supplementary Protocol May 2026

Direct Amplification of DNA Using Investigator® 24plex QS Kit

This protocol describes how to perform STR analysis by direct amplification using Investigator 24plex QS Kit (cat. nos. 382415 and 382417).

The experimental conditions outlined in this protocol have been found to yield 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. Note that the Quality Sensor will show reduced signal heights at lower cycle numbers.

Important: Consult the "Safety Information" and "Important Notes" sections in the *Investigator 24plex QS Handbook* (**www.qiagen.com/HB-1860**) before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, refer to the appropriate safety data sheets (SDSs) available from the product supplier.

Note: Protocols require the use of a higher concentration (5 ng/µL) of Control DNA 9948.

Equipment and reagents to be supplied by 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)
- Hi-Di™ Formamide, 25 mL (Applied Biosystems®, cat. no. 4311320)
- Matrix Standards BT6 (cat. no. 386244) for multi-capillary instruments
- Pipettes and pipette tips
- One of the following DNA analyzers*:
  - Applied Biosystems 3500 Genetic Analyzer (cat. no. 4405673)
  - Applied Biosystems 3500xL Genetic Analyzer (cat. no. 4405633)
- One of the following PCR thermal cyclers*:
  - GeneAmp® PCR System 9700
  - Veriti™ 96-Well Thermal Cycler
  - VeritiPro™ Thermal Cycler
  - ProFlex™ 96-well PCR System

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

- Bio-Rad® PTC-200
- Biometra™ UNO-Thermoblock
- 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 Kit 1.2 mm (cat. no. WB100028) and Cutting Mat, 6.0 × 8.0 inches (cat. no. WB100020)
- Investigator STR GO! Punch Buffer (1000) or (200) (cat. no. 386528 or 386526, respectively)
- Investigator STR GO! Lysis Buffer (cat. no. 386516)

  Note: This is only for Bode Buccal DNA Collector™ and, optionally, for long-term stored buccal cells on FTA cards.

For protocols based on buccal swab lysates

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

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 24plex 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.

Thing 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. Prepare a master mix according to Table 1 (for full volume PCR reactions) or Table 2 (for reduced volume PCR reactions). The master mix contains all of the components needed for PCR except the template (sample) DNA.

   As some loss of reagents can occur during transfers, prepare the mix with additional reactions included. Also include positive and negative control reactions.

Table 1. Recommended master mix setup for full volume PCR

Component	Volume (µL) per reaction
Fast Reaction Mix 2.0	7.5
Primer Mix	2.5
Investigator STR GO! Punch Buffer	2.0
Nuclease-free Water	10.0
Total reaction volume per sample	**22.0**

Table 2. Recommended master mix setup for reduced volume PCR

Component	Volume (µL) per reaction
Fast Reaction Mix 2.0	3.75
Primer Mix	1.25
Investigator STR GO! Punch Buffer*	2.0
Nuclease-free Water	5.0
Total reaction volume per sample	**12.0**

* 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 the 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 Kit 1.2 mm).

   Important: Use only than one punch at a time.

4. Transfer one 1.2 mm disc to each reaction.

   Note: 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: 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.

6. Briefly centrifuge the reactions to ensure that the discs are fully submerged.

7. Program the thermal cycler according to the manufacturer's instructions using the conditions given in Table 3a below or Table 3b on the facing page.

   Note: When using the GeneAmp PCR System 9700 with an Aluminum Block, select **Std Mode**; when using a 96-Well Silver Block or 96-Well Gold-plated Silver Block, select **Max Mode**. Do not select **9600 Emulation Mode**.

   Note: For cyclers with higher ramp rates (e.g., VeritiPro Thermal Cycler), adjust the ramp rates to 4°C/s. Alternatively, if the cycler offers an emulation mode, enable it and select a cycler that is included in the validation report.

After the cycling protocol is completed, store samples at –30°C to –15°C protected from light, or proceed directly with running the 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 24plex QS Kit Handbook*.

Table 3a. Standard cycling protocol for blood on FTA and other paper

Temperature (°C)	Time	Number of cycles
98*	30 s	
64	55 s	3
72	5 s	
96	10 s	
61	55 s	22
72	5 s	
68	5 min	–
60	5 min	–
10	∞	–

* Hot-start to activate DNA polymerase.

Table 3b. Optional cycling protocol for blood on FTA and other paper

Temperature (°C)	Time	Number of cycles
98*	30 s	
64	55 s	3
72	5 s	
96	10 s	
61	55 s	22
72	5 s	
68	2 min	–
60	2 min	–
10	∞	–

* Hot-start to activate DNA polymerase.

Table 3b details previously published cycling conditions that may continue to be used if incomplete adenylation is not visible within the electropherograms.

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 24plex QS Kit.

Important points before starting

- For buccal cells collected using UniCore Punch Kit 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.

Thing 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. Prepare a master mix according to Table 4 (for full volume PCR) or Table 5 (for reduced volume PCR). The master mix contains all of the components needed for PCR except the template (sample) DNA.

   As some loss of reagents can occur during transfers, prepare the mix with additional reactions included. Also include positive and negative control reactions.

Table 4. Recommended master mix setup for full volume PCR

Component	Volume (µL) per reaction
Fast Reaction Mix 2.0	7.5
Primer Mix	2.5
Investigator STR GO! Punch Buffer	2.0*
Nuclease-free Water	10.0
Total reaction volume per sample	**22.0**

* 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 (µL) per reaction
Fast Reaction Mix 2.0	3.75
Primer Mix	1.25
Investigator STR GO! Punch Buffer	2.0*
Nuclease-free Water	5.0
Total reaction volume per sample	**12.0**

* 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 Kit 1.2 mm).

   Note: For buccal cells collected using Indicating FTA Cards, take the punch from a white area. This color indicates successful sample transfer.

   Important: Use only one punch at a time.
4. Prepare PCR with sample punches (standard or optional).

   Standard protocol

   a. Dispense the total required reaction volume per sample into the PCR tubes or the wells of a PCR plate.

   b. Transfer one 1.2 mm disc to each reaction.

      Note: Do not mix the reaction after disc transfer.

   Optional protocol (e.g., for long-term stored cards or for cards that had not previously produced a loadable STR result)

   a. Add 5–10 µL of diluted Investigator STR GO! Lysis Buffer into each PCR well or tube.

      Note: Dilute Investigator STR GO! Kit Lysis Buffer 1:5 with PCR-grade water. The final volume of undiluted Investigator STR GO! Kit Lysis Buffer per PCR should not exceed 2 µL.

   b. Transfer one punch per sample into the well or tube containing the diluted lysis buffer.

   c. Incubate for 15 min at 95°C, leaving tubes open or PCR plate uncovered.

      Note: The Lysis Buffer will evaporate.

      Note: Cool down the PCR plate or tube for 5 min at room temperature (15–25°C) before dispensing the master mix.

   d. After incubation, dispense the total required reaction volume of the master mix per sample into each well of the PCR plate or the PCR tubes containing the 1.2 mm punch.

      Note: Do not mix the reaction after distributing the master mix.
5. Prepare positive and negative controls.

   - **Positive control**: Use 1 µL of 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.
6. Briefly centrifuge the reactions to ensure that the discs are fully submerged.
7. Program the thermal cycler according to the manufacturer's instructions using the conditions given in Table 6a or Table 6b.

   Note: When using the GeneAmp PCR System 9700 with an Aluminum Block, select **Std Mode**; when using a 96-Well Silver Block or 96-Well Gold-plated Silver Block, select **Max Mode**. Do not select **9600 Emulation Mode**.

   Note: For cyclers with higher ramp rates (e.g., VeritiPro Thermal Cycler), adjust the ramp rates to 4°C/s. Alternatively, if the cycler offers an emulation mode, enable it and select a cycler that is included in the validation report.

After the cycling protocol is completed, store samples at –30°C to –15°C protected from light, or proceed directly with running the 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 24plex QS Handbook*.

Table 6a. Standard cycling protocol for buccal cells on FTA and other paper

Temperature (°C)	Time	Number of cycles
98*	30 s	
64	55 s	3
72	5 s	
96	10 s	
61	55 s	24
72	5 s	
68	5 min	–
60	5 min	–
10	∞	–

* Hot-start to activate DNA polymerase.

Table 6b. Optional cycling protocol for buccal cells on FTA and other paper

Temperature (°C)	Time	Number of cycles
98*	30 s	
64	55 s	3
72	5 s	
96	10 s	
61	55 s	24
72	5 s	
68	2 min	–
60	2 min	–
10	∞	–

* Hot-start to activate DNA polymerase.

Table 6b details previously published cycling conditions that may continue to be used if incomplete adenylation is not visible within the electropherograms.

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 Collectors using the Investigator 24plex 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.

Thing 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. Collect a 1.2 mm punch from the tip (rounded end) of the Bode Buccal DNA Collector with a suitable tool (e.g., UniCore Punch Kit 1.2 mm, cat. no. WB100028) into a 0.2 mL PCR-grade plate or 0.2 mL PCR-grade tube.

   Important: Use only one punch at a time.

2. Add 2 µL of the 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 min. Do not seal the plate or close the tube.

   Note: The Lysis Buffer will evaporate.

   Note: Cool down the PCR plate or tube for 5 min at room temperature (15–25°C) before dispensing the master mix (step 5).

   Alternatively, or for an automated reaction setup, add 5–10 µL of diluted Investigator STR GO! Lysis Buffer into each empty PCR well or tube. Transfer one 1.2 mm punch into the well or tube containing the diluted lysis buffer. Increase the incubation time at 95°C for up to 15 min.

   Note: Dilute the Investigator STR GO! Kit Lysis Buffer 1:5 with PCR-grade water. The final volume of undiluted Investigator STR GO! Kit Lysis Buffer per PCR reaction should not exceed 2 µL.

4. Prepare a master mix according to Table 7 (for full volume PCR) or Table 8 (for reduced volume PCR). Vortex the reaction mix thoroughly. The master mix contains all of the components needed for PCR except the template (sample) DNA.

   As some loss of reagents can occur during transfers, prepare the mix with additional reactions included. Also include positive and negative control reactions.

Table 7. Recommended master mix setup for full volume PCR

Component	Volume (µL) per reaction
Fast Reaction Mix 2.0	7.5
Primer Mix	2.5
Nuclease-free Water	16.0
Total reaction volume per sample	**26.0**

Table 8. Recommended master mix setup for reduced volume PCR

Component	Volume (µL) per reaction
Fast Reaction Mix 2.0	3.75
Primer Mix	1.25
Nuclease-free Water	8.0
Total reaction volume per sample	**13.0**

5. After incubation, dispense the total required reaction volume of the master mix per sample into each well of the PCR plate or the PCR tubes containing the 1.2 mm punch.

   Note: Do not mix the reaction after distributing the master mix.

6. Prepare 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.

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

8. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 9a or Table 9b.

   Note: When using the GeneAmp PCR System 9700 with an Aluminum Block, select **Std Mode**; when using a 96-Well Silver Block or 96-Well Gold-plated Silver Block, select **Max Mode**. Do not select **9600 Emulation Mode**.

   Note: For cyclers with higher ramp rates (e.g., VeritiPro Thermal Cycler), adjust the ramp rates to 4°C/s. Alternatively, if the cycler offers an emulation mode, enable it and select a cycler that is included in the validation report.

After the cycling protocol is completed, store samples at –30°C to –15°C protected from light, or proceed directly with running the 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 24plex QS Handbook*.

Table 9a. Standard cycling protocol for buccal cells on Bode Buccal DNA Collectors

Temperature (°C)	Time	Number of cycles
98*	30 s	
64	55 s	3
72	5 s	
96	10 s	
61	55 s	23
72	5 s	
68	5 min	–
60	5 min	–
10	∞	–

* Hot-start to activate DNA polymerase.

Table 9b. Optional cycling protocol for buccal cells on Bode Buccal DNA Collectors

Temperature (°C)	Time	Number of cycles
98*	30 s	
64	55 s	3
72	5 s	
96	10 s	
61	55 s	23
72	5 s	
68	2 min	–
60	2 min	–
10	∞	–

* Hot-start to activate DNA polymerase.

Table 9b details previously published cycling conditions that may continue to be used if incomplete adenylation is not visible within the electropherograms.

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 24plex 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.

Thing to do before starting

- Before opening the tubes containing PCR components, vortex and then centrifuge 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 reactions) or Table 11 (for reduced volume PCR reactions). The master mix contains all of the components needed for PCR except the template (sample) DNA.

   As some loss of reagents can occur during transfers, prepare the mix with additional reactions included. Also include positive and negative control reactions.

Table 10. Recommended master mix setup for full volume PCR

Component	Volume (µL) per reaction
Fast Reaction Mix 2.0	7.5
Primer Mix	2.5
Nuclease-free Water	10.0
Total reaction volume per sample	**20.0**

Table 11. Recommended master mix setup for reduced volume PCR

Component	Volume (µL) per reaction
Fast Reaction Mix 2.0	3.75
Primer Mix	1.25
Nuclease-free Water	5.0
Total reaction volume per sample	**10.0**

5. Vortex the reaction mix thoroughly and dispense the total required reaction volume per sample into the PCR tubes or the wells of a PCR plate.

6. Mix the swab lysate thoroughly and transfer 2 µL (for full reaction volume) or 1 µL (for reduced reaction volume) of swab lysate directly to each reaction.
7. Prepare 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.
   - **Negative control**: Use a blank swab lysate.
8. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 12a or Table 12b.

   Note: When using the GeneAmp PCR System 9700 with an Aluminum Block, select **Std Mode**; when using a 96-Well Silver Block or 96-Well Gold-plated Silver Block, select **Max Mode**. Do not select **9600 Emulation Mode**.

   Note: For cyclers with higher ramp rates (e.g., VeritiPro Thermal Cycler), adjust the ramp rates to 4°C/s. Alternatively, if the cycler offers an emulation mode, enable it and select a cycler that is included in the validation report.

After the cycling protocol is completed, store samples at –30°C to –15°C protected from light, or proceed directly with running the 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 24plex QS Handbook*.

Table 12a. Standard cycling protocol for buccal swab lysates

Temperature (°C)	Time	Number of cycles
98*	30 s	
64	55 s	3
72	5 s	
96	10 s	
61	55 s	23
72	5 s	
68	5 min	–
60	5 min	–
10	∞	–

* Hot-start to activate DNA polymerase.

Table 12b. Optional cycling protocol for buccal swab lysates

Temperature (°C)	Time	Number of cycles
98*	30 s	
64	55 s	3
72	5 s	
96	10 s	
61	55 s	23
72	5 s	
68	2 min	–
60	2 min	–
10	∞	–

* Hot-start to activate DNA polymerase.

Table 12b details previously published cycling conditions that may continue to be used if incomplete adenylation is not visible within the electropherograms.

Troubleshooting

For general troubleshooting, please consult the "Troubleshooting Guide" in the *Investigator 24plex QS Handbook*.

Document Revision History

Date	Description
02/2021	Initial release
05/2026	Added note on reduced signal heights at lower cycle numbers for Quality Sensor. Added "VeritiPro™ Thermal Cycler" in "Equipment and Reagents to Be Supplied by User" section. Added note on ramp rates for fast cyclers. Added optional protocol for buccal cells on FTA and other paper. Removed Investigator STR GO! Punch Buffer from Master Mix for PCR amplification from buccal cells on Bode Buccal DNA Collectors.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.