Supplementary Protocol July 2022

Direct amplification of FTA® and swab samples using the Investigator® Argus X-12 QS Kit

This protocol is designed to show how to perform STR analysis by direct amplification of common reference sample materials using the Investigator Argus X-12 QS Kit (cat. No. 383223 and 383225). The protocol describes the preparation of samples and PCR conditions.

IMPORTANT: Please read the Investigator Argus X-12 QS Kit Handbook, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

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

- Pipets and pipet tips
- One of the following PCR thermal cyclers: QIAamplifier®, GeneAmp® PCR System 9700, Applied Biosystems® Veriti® Thermal Cycler, Bio-Rad® PTC-200 or Eppendorf® Mastercycler® ep

  Note: This is not a complete list of suppliers and does not include many important vendors of biological supplies.
- PCR tubes or plates
- Microcentrifuge for PCR tubes or plates
- Control DNA 9948 (5ng/µl) (cat. no. 386041), or other DNAs provided by the user that allow 5–10 ng input per reaction. Note that GeneMapper template files contain female Control DNA 9947 as positive control and must be adapted for controls to pass.

For protocols based on blood or buccal cells on paper

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

For protocols based on buccal swab lysates

- Investigator STR GO! Lysis Buffer (QIAGEN, cat. no. 386516)
- 2 ml microcentrifuge tubes
- Heater shaker for 2 ml microcentrifuge tubes

Important points before starting

The experimental conditions outlined in the protocols 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. Note that the Quality Sensor™ will show reduced signal heights at lower cycle numbers.

For all direct PCR amplification protocols, a separate control DNA has to be used and must be supplied by the user. The Control DNA 9947A included in the Investigator Argus X-12 QS Kit cannot be used. A control DNA with 5 ng/µl is recommended.

- 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: PCR Amplification from blood on FTA and other paper

1. Prepare a master mix according to Table 1.

   Note: 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.
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 blood spot with a suitable tool (e.g., Uni-Core Punch Kit 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 10 ng Control DNA, i.e. 2 µl of 9948 (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 reactions to ensure discs are fully submerged.
7. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 2.
   Note: If using the GeneAmp PCR System 9700 with an Aluminum block, use **Std Mode** or with a Silver 96-Well Block or Gold-plated Silver 96-Well Block, use **Max Mode**. Do not use **9600 Emulation Mode**.
8. After the cycling protocol is completed, store samples at –30 to –15°Cprotected 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	2.5 µl
Nuclease-free water	10 µl
Investigator STR GO! Punch Buffer	2.0 µl
Total volume	**22 µl**

Table 2. Cycling protocol for blood on FTA or other paper

Temperature	Time	Number of cycles
98°C*	60 s	
61°C	100 s	3 cycles
72°C	5 s	
96°C	10 s	
61°C	100 s	22 cycles
72°C	5 s	
68°C	2 min	
10°C	Hold	–

* Hot-start to activate DNA polymerase.

Procedure: PCR Amplification from buccal cells on FTA and other Paper

1. Prepare a master mix according to Table 3.

   Note: 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.
2. Vortex the reaction mix thoroughly, and dispense 22 µl into PCR tubes or the wells of a PCR plate.
3. Take a 1.2 mm punch from the center of the blood spot with a suitable tool (e.g., Uni-Core Punch Kit 1.2 mm).
   Important: Do not use more than one punch at a time.
   Note: For buccal cells collected on indicator cards (e.g., EasiCollect™ device), take the punch from a white area. This color indicates successful sample transfer.
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 10 ng Control DNA, i.e., 2 µl of 9948 (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 reactions to ensure discs are fully submerged.
7. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 4.

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

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

Table 3. Master Mix setup

Component	Volume per reaction
Fast Reaction Mix 2.0	7.5 µl
Primer Mix	2.5 µl
Nuclease-free water	10 µl
Investigator STR GO! Punch Buffer	2 µl
Total volume	**22 µl**

Table 4. Cycling protocol for Buccal cells on FTA or other paper

Temperature	Time	Number of cycles
98°C*	60 s	
61°C	100 s	3 cycles
72°C	5 s	
96°C	10 s	
61°C	100 s	24 cycles
72°C	5 s	
68°C	2 min	
10°C	Hold	–

* Hot-start to activate DNA polymerase.

Procedure: PCR Amplification from buccal swab lysates

1. Place the swab in a 2 ml microcentrifuge tube.
2. Carefully cut, break off, or eject the end part of the swab.
   Note: Prepare a blank swab as negative control.
3. Add 500 µl STR GO! Lysis Buffer to the sample.
4. Incubate at 95°C for 5 min, shaking at 1200 rpm in a heated mixer.
5. Prepare a master mix according to Table 5.
   Note: 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.
6. Vortex the reaction mix thoroughly, and dispense the total required reaction volume per sampleinto PCR tubes or the wells of a PCR plate.
7. Mix the swab lysate thoroughly and transfer 2 µl of swab lysate directly to each reaction.
8. Prepare the positive and negative controls.
   Positive control: Use 1 µl Control DNA (i.e., 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.
9. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 6.
   Note: If using the GeneAmp PCR System 9700 with an Aluminum block, use **Std Mode**, or with a Silver 96-Well Block or Gold-plated Silver 96 Well Block, use **Max Mode**. Do not use **9600 Emulation Mode**.
10. After the cycling protocol is completed, store samples at –30 to –15°C protected from light, or proceed directly with electrophoresis.

Table 5. Master Mix setup

Component	Volume per reaction
Fast Reaction Mix 2.0	7.5 µl
Primer Mix	2.5 µl
Nuclease-free water	13 µl
Total volume	**23 µl**

Table 6. Cycling protocol for Buccal Swab Lysates.

Temperature	Time	Number of cycles
98°C*	60 s	
61°C	100 s	3 cycles
72°C	5 s	
96°C	10 s	
61°C	100 s	25 cycles
72°C	5 s	
68°C	2 min	
10°C	Hold	–

* Hot-start to activate DNA polymerase.

Troubleshooting

For general troubleshooting, please consult the "Troubleshooting Guide" in the *Investigator Argus X-12 QS Kit Handbook*.

Document Revision History

Revision	Description
March 2016	Initial release
July 2022	Updated Equipment and reagents to be supplied by user section Updated protocols for buccal and blood samples on FTA or other paper Updated Table 1 to add Investigator STR GO! Punch buffer as component

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