

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

This protocol is designed for direct amplification of common reference sample materials. 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:

- Investigator Argus X-12 QS Kit (cat. no. 383223 or 383225)
- Pipets and pipet tips
- One of the following PCR thermal cyclers: GeneAmp[®] PCR System 9700, Applied Biosystems[®] Veriti[®] Thermal Cycler, Bio-Rad[®] PTC-200 or Eppendorf[®] Mastercycler[®] ep
- PCR tubes or plates
- Microcentrifuge for PCR tubes or plates
- Control DNA (5ng/μl)

For protocols based on buccal cells on paper:

- 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.

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. Take a 1.2 mm punch from the center of the blood spot with a suitable tool (e.g., Uni-Core Punch, GE Healthcare).

Note: Do not use more than one punch at a time.

2. Prepare a master mix according to Table 1.
3. Mix the reaction mix thoroughly, and dispense 25 μl into PCR tubes or the wells of a PCR plate.
4. Transfer one 1.2 mm disc to each reaction.
5. 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.

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.

8. After the cycling protocol is completed, store samples at -15°C to -30°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	2.5 μl
Nuclease-free water	15 μl
Total volume	25 μl

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

Temperature	Time	Number of cycles
98 $^{\circ}\text{C}$ *	60 s	
61 $^{\circ}\text{C}$	100 s	3 cycles
72 $^{\circ}\text{C}$	5 s	
96 $^{\circ}\text{C}$	10 s	
61 $^{\circ}\text{C}$	100 s	22 cycles
72 $^{\circ}\text{C}$	5 s	
68 $^{\circ}\text{C}$	2 min	
10 $^{\circ}\text{C}$	Hold	–

* Hot-start to activate DNA polymerase.

Procedure: PCR Amplification from Buccal cells on FTA and Other Paper

1. Take a 1.2 mm punch from the center of the blood spot with a suitable tool (e.g., Uni-Core Punch, GE Healthcare).
Note: For buccal cells collected on indicator cards (e.g., Whatman™ EasiCollect™ device), take the punch from a white area. This color indicates successful sample transfer.
2. Prepare a master mix according to Table 3.
3. Mix the reaction mix thoroughly, and dispense 25 µl into PCR tubes or the wells of a PCR plate.
4. Transfer one 1.2 mm disc to each reaction.
5. Prepare the positive and negative controls.
Positive control: Use 1 µl Control DNA (i.e., 5 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.
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.
8. After the cycling protocol is completed, store samples at –15°C to –30°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	13 µl
Investigator STR GO! Punch Buffer	2 µl
Total volume	25 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 μ l 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.
6. Mix the reaction mix thoroughly, and dispense 23 μ l into PCR tubes or the wells of a PCR plate.
7. Mix the swab lysate thoroughly and transfer 2 μ l of swab lysate directly to each reaction.
8. Prepare the positive and negative controls.
Positive control: Use 2 μ l 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: Use a blank swab lysate.
9. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 6.
10. After the cycling protocol is completed, store samples at -15°C to -30°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 μ l
Primer Mix	2.5 μ l
Nuclease-free water	13 μ l
Total volume	23 μ l

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*.

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