

9 DNA Amplification

Safety

Body fluids, tissues, and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves should be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

IMPORTANT! The fluorescent dyes attached to the primers are light sensitive. Protect the primer set from light when not in use. Amplified DNA, AmpF/STR Allelic Ladders, and Gene Scan-500 LIZ Size Standard should also be protected from light. Keep freeze-thaw cycles to a minimum.

Equipment, Materials, and Reagents

- calculator
- microcentrifuge
- microcentrifuge tubes, 1.5 ml
- microcentrifuge tube rack
- pipet tips
- pipettors, adjustable
- vortex
- 96-Well GeneAmp® PCR System 9700
- 0.2 mL reaction tube strips or 0.2 ml 96-well Optical Reaction plate
- AmpF/STR® Identifiler® PCR Amplification Kit, AmpF/STR® Identifiler® Plus PCR Amplification Kit or AmpF/STR® Yfiler™ PCR Amplification Kit
- TE Buffer
- MicroAmp® 8-Cap Strip

Standards, Controls, and Calibration

- An amplification positive control, consisting of kit Control DNA 9947A (autosomal) or Control DNA 007 (Y-STR), must be included with in each amplification.
- An amplification negative control must be included with in each amplification. This negative control will consist of all amplification reagents with TE Buffer added in place of sample DNA.

AmpF/STR® Identifiler® PCR Amplification

The AmpF/STR® Identifiler® PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 15 autosomal STR loci: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, and CSF1PO, D2S1338 and D19S433, and Amelogenin (a sex marker) in a single PCR reaction.

Reaction and Plate Setup Procedure

| PCR Instrument | Times and Temperatures for Identifiler kits | | | | | |
|----------------|---|----------------|----------------|----------------|-------------------------|-----------------------------|
| | Initial Incubation Step | 28 cycles each | | | Final Extension | Final Step |
| | | Denature | Anneal | Extend | | |
| 9700 | 95°C 11 min. hold | 94°C 1 min. | 59°C 1 min. | 72°C 1 min. | 60°C 60 min. hold | 4-25°C hold (forever) |

1. The thermal cycler should be programmed according to the chart above.
2. Label the reaction plate directly with the appropriate information (e.g., date and initials).
3. Vortex PCR reaction mix, primer set, and AmpliTaq Gold™ and spin tubes briefly to remove any liquid from the caps.
4. Prepare a Master Mix by adding the following volumes to **an appropriately sized tube**:
 - a. 10.5 µl Reaction Mix X # of samples
 - b. 0.5 µl AmpliTaq Gold X # of samples
 - c. 5.5 µl Primer Set X # of samples
5. Mix by vortexing for approximately 5 seconds
6. Spin briefly to remove liquid from cap.
7. Dispense 15 µl of Master Mix into each amplification well.
8. Add approximately 1 ng of sample DNA to the appropriate amplification wells, not to exceed 10 µl in total sample volume. Addition of more or less sample DNA is acceptable to obtain optimum results. For samples with high concentrations of DNA (i.e. >2 ng/µl), dilute these samples with TE buffer to attain an appropriate concentration. Enough TE should be added to template volume to ensure a total reaction volume of 25 µl.
9. Set up a positive control (5-10 µl of the Control DNA (**9947A**)) and a negative control (10 µl of TE buffer) to wells containing the Master Mix.
10. Place the tray into the Thermal Cycler and start the appropriate program.
11. After amplification, remove the samples from the Thermal Cycler and store away from light. Store samples refrigerated short periods, or frozen for longer periods.

AmpF/STR® Identifiler® Plus PCR Amplification

The AmpF/STR® Identifiler® Plus PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 15 autosomal STR loci: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, and CSF1PO, D2S1338 and D19S433, and Amelogenin (a sex marker) in a single PCR reaction.

Reaction and Plate Setup Procedure

| PCR Instrument | Times and Temperatures for Identifiler kits | | | | |
|----------------|---|-----------------|----------------|-------------------------|-----------------------------|
| | Initial Incubation Step | 28 cycles each | | Final Extension | Final Step |
| | | Denature | Anneal/Extend | | |
| 9700 | 95°C 11 min. hold | 94°C 20 sec. | 59°C 3 min. | 60°C 10 min. hold | 4-25°C hold (forever) |

1. The thermal cycler should be programmed according to the chart above.
2. Label the reaction plate directly with the appropriate information (e.g., date and initials).
3. Vortex PCR reaction mix and primer set for 3 seconds and spin tubes briefly to remove any liquid from the caps.
4. Prepare a Master Mix by adding the following volumes to an appropriately sized tube:
 - a. 10.5 µl Reaction Mix X # of samples
 - b. 5.5 µl Primer Set X # of samples
5. Mix by vortexing for approximately 5 seconds
6. Spin briefly to remove liquid from cap.
7. Dispense 15 µl of Master Mix into each amplification well.
8. Add approximately 0.5 – 1.0 ng of sample DNA to the appropriate amplification wells, not to exceed 10 µl in total sample volume. Addition of more or less sample DNA is acceptable to obtain optimum results. For samples with high concentrations of DNA (i.e. >2 ng/µl), dilute these samples with TE buffer to attain an appropriate concentration. Enough TE should be added to template volume to ensure a total reaction volume of 25 µl.
9. Set up a positive control (5-10 µl of the Control DNA (9947A)) and a negative control (10 µl of TE buffer) to wells containing the Master Mix.
10. Centrifuge the plate at about 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders to remove any bubbles.
11. Place the tray into the Thermal Cycler and start the appropriate program.
12. After amplification, remove the samples from the Thermal Cycler and store away from light. Store samples refrigerated short periods (less than 2 weeks), or frozen for longer periods.

AmpF/STR® Yfiler™ PCR Amplification

The AmpF/STR® Yfiler™ PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 17 Y-STR loci in a single PCR reaction. The kit amplifies the loci in the:

- "European minimal haplotype" (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393)
- Scientific Working Group-DNA Analysis Methods (SWGDM)-recommended Y-STR panel (European minimal haplotype plus DYS438 and DYS 439)
- Additional highly polymorphic loci (DYS437, DYS448, DYS456, DYS458, DYS635 (Y GATA C4), and Y GATA H4

Reaction and Plate Setup Procedure

| PCR Instrument | Times and Temperatures for Identifier kits | | | | | |
|----------------|--|----------------|----------------|----------------|-------------------------|--------------------------|
| | Initial Incubation Step | 30 cycles each | | | Final Extension | Final Step |
| | | Denature | Anneal | Extend | | |
| 9700 | 95°C 11 min. hold | 94°C 1 min. | 61°C 1 min. | 72°C 1 min. | 60°C 80 min. hold | 4°C hold (forever) |

1. The thermal cycler should be programmed according to the chart above.
2. Label the reaction plate directly with the appropriate information (e.g., date and initials).
3. Vortex PCR reaction mix, primer set, and AmpliTaq Gold™ and spin tubes briefly to remove any liquid from the caps.
4. Prepare a Master Mix by adding the following volumes to **an appropriately sized tube**:
 - a. 10.5 µl Reaction Mix X # of samples
 - b. 0.5 µl AmpliTaq Gold X # of samples
 - c. 5.5 µl Primer Set X # of samples
5. Mix by vortexing for approximately 5 seconds
6. Spin briefly to remove liquid from cap.
7. Dispense 15 µl of Master Mix into each amplification well.
8. Add approximately 0.5-1.0 ng of sample DNA to the appropriate amplification wells, not to exceed 10 µl in total sample volume. Addition of more or less sample DNA is acceptable to obtain optimum results. For samples with high concentrations of DNA (i.e. >2 ng/µl), dilute these samples with TE buffer to attain an appropriate concentration. Enough TE should be added to template volume to ensure a total reaction volume of 25 µl.
9. Set up a positive control (5-10 µl of the Control DNA (007)) and a negative control (10 µl of TE buffer) to wells containing the Master Mix. **The developmental and internal validations demonstrate that female DNA does not interfere with the amplification of male DNA, and thusly, a female positive control is not required for each amplification. However, the current TECAN Freedom EVO®150 Workstation**

script incorporates this female control in Y-STR amplifications, so this control will be present on the automated amplification set-ups; manual set-ups do not require this additional control.

10. Centrifuge the plate at about 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders to remove any bubbles.
11. Place the tray into the Thermal Cycler and start the appropriate program.
12. After amplification, remove the samples from the Thermal Cycler and store away from light. Store samples refrigerated short periods (**less than 2 weeks**), or frozen for longer periods.

Instrument Overview

96-Well GeneAmp® PCR System 9700

1. An automated heated sample block applies heating or cooling to samples in the 96-well plate block
2. The enclosed environment provides the best control over the ambient temperature
3. At the end of the run, the 9700 may ramp down to 4°C to preserve sample life.



Powering on the Instrument

1. Press the power button on the lower left front of the 9700 instrument.
2. The instrument will beep and cycle through a power on sequence.
3. When the screen prompts the user with potential commands, the instrument is ready for use.