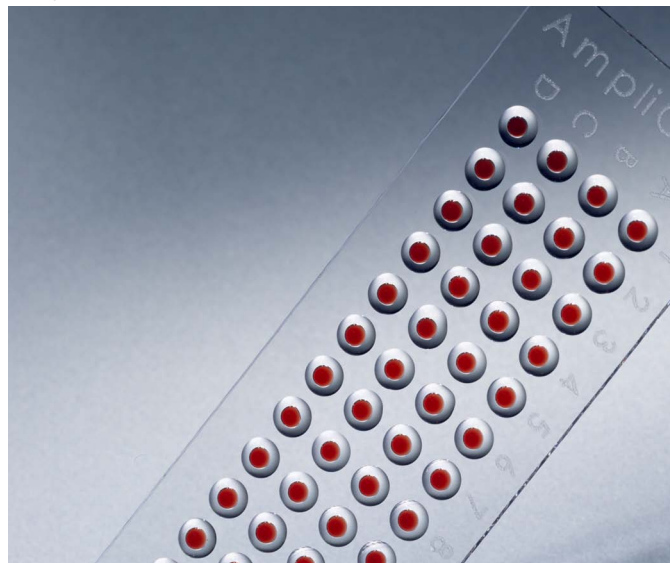


Genetic Profiling with Applied Biosystems AmpFISTR® Identifiler® on AmpliGrid AG480F

In this PCR system, 15 specific STR loci and the gender-determining Amelogenin are co-amplified using human DNA or cells as template and the AmpFISTR® Identifiler® kit from Applied Biosystems together with the AmpliGrid slides.

1 Figure 1: AmpliGrid AG480F



Material

PCR

Template: male DNA (Promega, 9948 Male DNA 10 ng/μL), female DNA (Promega, 9947A Female DNA 10 ng/μL) or Hoechst-stained single cells deposited on the reaction sites of an AmpliGrid 480F

Aliquot preparation

- DNA positive control, 250 pg/μL, diluted in Nuclease-free water

NOTE: Avoid frequent freeze / thaw cycles!

- Applied Biosystems AmpFISTR® Identifiler® Kit (Applied Biosystems, Cat. # 4322288)
 - AmpFISTR® PCR Reaction Mix
 - AmpliTaq Gold® DNA Polymerase 5 U/μL (Applied Biosystems, Cat. #4311814)
 - AmpFISTR® Identifiler® Primer Set
- Nuclease-free water
- AmpliGrid AG480F incl. sealing solution (Advalytix, e.g. OAX04503)
- AmpliSpeed slide cycler (Advalytix, e.g. OAX04101)
- Electronic multistep pipette

Protocol:

DNA TEMPLATE

- Deposit 1 μL DNA solution (250 pg/μL) on reaction sites and let air-dry at room temperature or at 37°C

SINGLE CELL TEMPLATE

- Check cells with cell detecting system at the fluorescent microscope in order to verify their presence on the reaction sites

MASTER MIX

- Prepare master mix in a fresh PCR-clean tube according to table A. Make sure to prepare enough master mix for all the reactions, taking pipetting errors into account (dead volume of electronic multistep pipette around 10 μL)

PCR Applied Biosystems

A Table A: PCR setup

| Component | Volume (1 reaction) |
|--------------------------------------|---------------------|
| AmpFISTR® PCR Reaction Mix | 0.4 μL |
| AmpFISTR® Identifiler® Primer Set | 0.2 μL |
| AmpliTaq Gold® DNA Polymerase 5 U/μL | 0.03 μL |
| Nuclease-free water | 0.37 μL |
| Total volume | 1 μL |

- Mix gently and spin down shortly
- Distribute 1 μL of master mix on each reaction site previously spotted with DNA or single cells
- Immediately cover with 5 μL sealing solution
- Transfer AmpliGrid onto the thermal cycler
- Run PCR program (table B)

B Table B: PCR program

| Temperature | Time | Setting |
|-------------|--------|------------------------|
| 60°C | 1 sec | Heating rate 1°C / min |
| 95°C | 1 sec | |
| 95°C | 11 min | |
| 94°C | 60 sec | |
| 59°C | 60 sec | 28 cycles |
| 72°C | 60 sec | |
| 60°C | 59 min | |
| Ambient | hold | |

Analysis

- Transfer samples to MTP either by pipetting the 1 µL from underneath the sealing solution or by recovering with 5 µL of PCR-clean water
- Optionally dilute sample (250 pg human genomic DNA should be diluted 1:35 using TBE buffer in case RFU values are higher than 1:500)
- Analyse samples with capillary electrophoresis (ABI PRISM®) according to manufacturer manual. We recommend to increase time for sample take-up in ABI software to maximum

2 Figure 2: AmpliSpeed slide cycler ASC200D

AmpFISTR®, AmpliTaq®, ABI PRISM® are registered trademarks of Applied Biosystems, USA

For research use only. Not for use in diagnostic procedures.