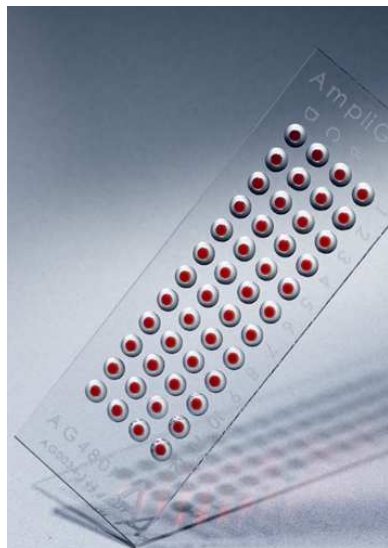


# Genetic Profiling with Applied Biosystems AmpFISTR® SEfiler™ on AmpliGrid AG480F

In this PCR system, 11 specific STR loci are amplified using human DNA as template and the AmpFISTR® SEfiler™ kit from Applied Biosystems together with AmpliGrid slides.

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, 100 pg/μl
- Aliquot dilution for storage: 1 μl (10 ng/μl stock DNA + 99 μl PCR-clean water)
- Applied Biosystems AmpFISTR® SEfiler™ Kit (Applied Biosystems, Cat. # 4335129)
  - AmpFISTR® PCR Reaction Mix
  - **either:** AmpliTaq Gold® DNA Polymerase 5 U/μl (Applied Biosystems, Cat. #4311814)
  - **or:** Promega GoTaq® DNA Polymerase 5 U/μl
  - AmpFISTR® SEfiler™ Primer Set
- PCR clean water
- AmpliGrid AG480F incl. sealing solution (Advalytix)
- AmpliSpeed slide cycler (Advalytix)
- Electronic multistep pipette

## Protocol:

### DNA TEMPLATE

- Deposit 1 μl DNA solution (e.g., 100 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 1 or 2

Make sure to prepare enough master mix for all the reactions, taking pipetting errors into account (dead volume of electronic multipipet around 10 μl)

### PCR Applied Biosystems:

A Table 1: PCR setup with Applied Biosystems reagents

Reagent	1 Spot
AmpFISTR® PCR Reaction Mix	0.318 μl
AmpFISTR® SEfiler™ Primer Set	0.167 μl
AmpliTaq Gold® DNA Polymerase 5 U/μl	0.015 μl
ddH <sub>2</sub> O	0.5 μl
<b>Total volume:</b>	<b>1 μl</b>

### PCR Promega:

B Table 2: PCR setup with Promega GoTaq® reagents

Reagent	1 Spot
AmpFISTR® PCR Reaction Mix	0.318 μl
AmpFISTR® SEfiler™ Primer Set	0.167 μl
GoTaq® DNA Polymerase 5 U/μl	0.015 μl
ddH <sub>2</sub> O	0.5 μl
<b>Total volume:</b>	<b>1 μl</b>

- 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 programme (table 3)

2 *AmpliSpeed  
ASC200D*



C *Table 3: PCR programme*

Temperature	Time	Cycles
93 °C	11 min	
92 °C	60 sec	
58°C	80 sec	28
71°C	60 sec	
59°C	45 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
- Clean-up sample using Sephadex G-50 column or any other Sephadex based clean-up kit.
- Analyse samples with capillary electrophoresis (ABI PRISM®) according to manufacturer manual. If CE signals are too low, increase time for sample take-up in ABI software

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