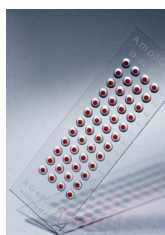


# Genetic Profiling with Promega PowerPlex® 16 on AmpliGrid AG480F

This protocol describes the process of amplifying STR DNA fragments using the Promega PowerPlex® 16 System.

- 1 AmpliGrid AG480F      2 AmpliSpeed ASC200D



## Material:

- Template: male DNA (Promega, Cat. # DD2061, 9948 Male DNA 10 ng/μl), or Hoechst-stained cells deposited on the reaction sites of an AmpliGrid AG480F

Aliquot preparation:

DNA positive control, 100 pg/μl

Aliquot dilution for storage: 1 μl (10 ng/μl stock DNA + 99 μl PCR-clean water)

- PowerPlex® 16 System PCR Kit (Promega, Cat. #DC6531)
  - GoldSTAR® 10x Buffer
  - PowerPlex® 16 10x Primer Pair Mix
- PCR clean water
- AmpliTaq Gold® DNA Polymerase 5 U/μl (Applied Biosystems, Cat. #4311814)
- Sealing Solution (Advalytix)
- AmpliSpeed slide cycler (Advalytix)
- Electronic multistep pipette

## Protocol:

### DNA TEMPLATE

- 1 μl DNA solution (100 pg/μl) is deposited on reaction sites and let air-dry at room temperature or at 37°C on the thermal cycler

### 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

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

**A** Table 1: Master Mix

Reagent	1 Spot
GoldSTAR 10x Buffer	0.1 μl
PowerPlex® 16 10x Primer Pair Mix	0.1 μl
AmpliTaq Gold® DNA Polymerase 5 U/μl	0.032 μl
ddH <sub>2</sub> O	0.768 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 prespotted with DNA or single cells and immediately cover with 5 μl sealing solution
- Transfer AmpliGrid onto the thermal cycler
- Run PCR programme as shown in table 2
- Note: samples are sensitive to light

**B** Table 2: PCR programme

Temperature	Time	Cycles
97 °C (cells) / 95°C (DNA)	11 min	
96 °C	60 sec	
94°C	30 sec	
59°C ramp 0.5°C/sec	45 sec	10
69°C ramp 0.3°C/sec	50 sec	
90°C	30 sec	
59°C ramp 0.5°C/sec	45 sec	20
69°C ramp 0.3°C/sec	50 sec	
59°C	30 min	
Ambient	hold	

## Analysis:

- Transfer samples to MTP either by pipetting the 1μl from underneath the sealing solution or by recovering with 5 μl PCR-clean H<sub>2</sub>O; add Formamide
- Put MTP into CE

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