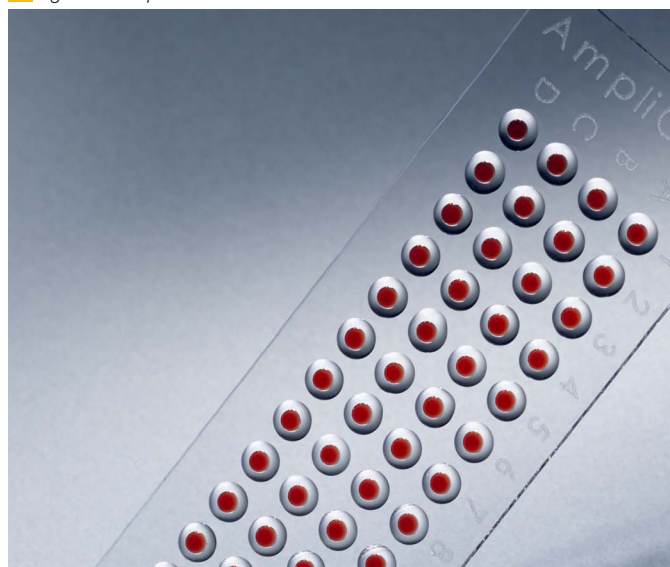


Genetic Profiling with Promega PowerPlex® 16 on AmpliGrid AG480F

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

1 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, 100 pg/μL
- Aliquot dilution for storage: 1 μL (10 ng/μL stock DNA + 99 μL Nuclease-free water) - NOTE: Avoid frequent freeze / thaw cycles!
- PowerPlex® 16 System PCR Kit (Promega, Cat. #DC6531)
 - GoldSTAR® 10x Buffer
 - PowerPlex® 16 10x Primer Pair Mix
- Nuclease-free water
- AmpliTaq Gold® DNA Polymerase 5 U/μL (Applied Biosystems, Cat. #4311814)
- AmpliGrid AG480F incl. sealing solution (Advantix, e.g. OAX04503)
- AmpliSpeed slide cycler (Advantix, e.g. OAX04101)
- Electronic multistep pipette
- Cell Extraction Kit (Advantix, OAX04523)

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

CELL EXTRACTION

- All reagents of the Cell Extraction kit are thawed completely, shortly vortexed and the components are combined in a sterile, nuclease free tube according to the following table:

A Table A: Cell Extraction working solution

Component	Volume (1 slide)	Volume (5 slides)
Lysis Enzyme	1 μL	5 μL
10x Lysis Buffer	6 μL	30 μL
Nuclease-free water	53 μL	265 μL
Total Volume	60 μL	300 μL

- The mix is gently vortexed and spinned down shortly.

Note: After preparing the working dilution of the cell extraction kit keep it on ice and use within 6 hours. Do not store the diluted enzyme as this will rapidly decrease activity.

- The AmpliGrid slide is positioned on a dark surface to ensure good visualization of the engraved markings
- 0.75 μL of the cell extraction working solution is pipetted on each of the AmpliGrid reaction sites and immediately covered with 5 μL of sealing solution
- The AmpliGrid slide is placed on the AmpliSpeed slide cycler and the following program is started: 5 - 10 min 75°C, 2 min 95°C

AMPLIFICATION

- Reagents of the Promega PowerPlex® 16 kit are thawed completely, shortly vortexed and the components are combined in a sterile, nuclease free tube according to the following table:

Note: protect samples from light

B Table B: Promega PowerPlex® 16 amplification mix

Component	Volume (1 reaction)	Volume (48 reaction)
10x GoldSTAR® Buffer	0.15 µL	9 µL
PowerPlex® 16 10x Primer Pair Mix	0.15 µL	9 µL
AmpliAq Gold® DNA Polymerase 5 U/µL	0.048 µL	2.88 µL
Nuclease-free water	0.402 µL	24.12 µL
Total Volume	0.75 µL	45 µL

- The master mix is gently vortexed and spinned down shortly
- 0.75 µL of the master mix is pipetted on top of each of the AmpliGrid reaction sites

NOTE: Aqueous solution that is pipetted on top of each reaction site will move through the sealing solution due to physical reasons and will automatically merge with the sample

- The following program on the AmpliSpeed slide cycler is started:

C Table C: amplification program

Temperature	Time	Cycle
97°C (cells) / 95°C (DNA)	11 min	
96°C	60 sec	
94°C	30 sec	
60°C	45 sec	10
70°C ramp 0.3°C/sec	50 sec	
90°C	30 sec	
60°C	45 sec	20
70°C ramp 0.3°C/sec	50 sec	
60°C	30 min	
Ambient	hold	

Analysis

- Transfer complete sample (incl. sealing solution) to a microtiter plate
- Add analysis buffer to a volume of 10 µL aqueous solution
- 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

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