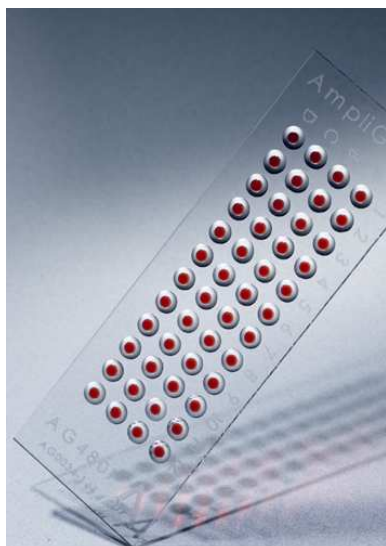


Plasmid Amplification and Sequencing on AmpliGrid AG480F

1 AmpliGrid AG480F



Material:

- AmpliSpeed slide cycler (Advalytix)
- AmpliGrid AG480F (Advalytix) incl. sealing solution
- Primer M13 f/rev (5 pmol/μl each)
- DNA pGEM[®] (1 pg – 1 ng) (Promega)
- PCR-clean water
- Electronic multistep pipette

PCR Promega:

- 10x GoldSTAR[®] reaction buffer
- GoTaq[®] DNA Polymerase (5 U/μL)

PCR Invitrogen:

- 10x reaction buffer without MgCl₂ (Platinum[®] Taq)
- Platinum[®] Taq (5 U/μL)
- MgCl₂ (50 mM)
- dNTP mix (10 mM each)

PCR Qiagen:

- 10x Qiagen PCR buffer
- Qiagen Taq Polymerase (5 U/μL)
- dNTP mix (10 mM each)

Exonuclease:

- ExoSAP-IT[®] (USB)

Sequencing:

- BigDye[®] v1.1 Terminator Ready Reaction Mix (Applied Biosystems)
- Sequencing Primer, 3 pmol/μL (metabion)
- PCR-clean water

Protocol:

Three different suppliers of PCR reagents have been tested successfully with the AmpliGrid system.

PCR Promega:

A Table 1: PCR setup with Promega GoTaq[®] reagents

Reagent	1 Spot
10x GoldStar [®] PCR buffer	0.1 μl
M13-Primer Mix	0.2 μl
GoTaq [®] DNA Polymerase	0.015 μl
pGEM [®] DNA	0.1 μl
PCR-clean water	0.585 μl
Σ	1 μl

PCR Invitrogen:

B Table 2: PCR setup with Invitrogen[®] Platinum Taq reagents

Reagent	1 Spot
10x Platinum [®] Taq reaction buffer	0.1 μl
M13 Primer Mix	0.2 μl
dNTP-Mix	0.01 μl
Platinum [®] Taq DNA Polymerase	0.015 μl
MgCl ₂	0.1 μl
PCR-clean water	0.475 μl
pGEM [®] DNA	0.1 μl
Σ	1 μl

PCR Qiagen:

C Table 3: PCR setup with Qiagen Taq reagents

Reagent	1 Spot
10x Qiagen PCR buffer	0.1 μl
M13-Primer Mix	0.2 μl
dNTP-Mix	0.01 μl
Qiagen Taq Polymerase	0.015 μl
PCR-clean water	0.575 μl
pGEM [®] DNA	0.1 μl
Σ	1 μl

- Transfer 1 µl of the selected master mix to each reaction site of the AmpliGrid AG480F slide
- Cover immediately with 5 µl sealing solution
- Place AmpliGrid slide on AmpliSpeed slide cycler
- Run PCR programme (see table 4)

D Table 4: PCR programme for plasmid amplification on AmpliSpeed slide cycler

Temperature	Time	Cycles
95°C	10 min	
95°C	40 sec	
53 °C	30 sec	30 - 35
71°C	30 sec	
71°C	10 min	

On-slide Exonuclease clean-up of PCR products

The amplification primers and unconsumed dNTPs are removed from the PCR reaction by an exonuclease step without removing the PCR solution from the AmpliGrid slide. The enzyme is pipetted through the sealing solution directly in the aqueous phase.

- Add 0.4 µl of ExoSAP-IT® to the PCR reaction by penetrating the sealing solution with a 10 µl pipette tip
- Run the temperature protocol on the AmpliSpeed slide cycler as shown in table 5

E Table 5: Exonuclease step temperature profile

Temperature	Time
37°C	15 min
80°C	15 min

On-slide BigDye® Sequencing

The BigDye® sequencing reaction is carried out directly on the AmpliGrid slide without removing of the aqueous phase.

- For each AmpliGrid reaction site combine the reagents as shown in table 6. After the exonuclease step the volume per reaction site is 1.4 µl.
- For the sequencing reaction add 0.6 µl of the BigDye®/primer mixture to each reaction site to a final volume of 2 µl

AmpliGrid slides are designed to hold 1 µl. Anyway it is possible to increase the volume of the aqueous phase. Droplet merging as a result may occur if not handled carefully. We do recommend to leave the slides on the cycler during pipetting.

2 AmpliSpeed ASC200D



F Table 6: Big Dye reaction mix setup

Component	Volume
BigDye® mix	0.5 µl
Sequencing primer, 3 pmol/µl	0.1 µl
Total volume	0.6 µl

- Run the sequencing protocol on the AmpliGrid slide cycler as shown in table 7

G Table 7: Sequencing reaction on AmpliSpeed slide cycler

Temperature	Time	Cycles
95°C	2 min	
95°C	30 sec	
50 - 52°C	30 sec	25 - 30
59°C	4 min	

- Recover sample with 5 µl PCR-clean water pipetted on top of sealing solution or recovering 2 µl from underneath the sealing solution.

Analysis:

- Transfer samples to MTP
- Clean-up sample using Sephadex G-50 column or any other Sephadex based clean-up kit.
- Analyse sample 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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