

Contamination-free ultra low volume pipetting on AmpliGrid AG480F slides

Based on AmpliGrid technology.

Introduction

In the R&D and diagnostic field the need for low volume applications is continuously increasing. Microplates or microtubes, commonly used in laboratory practice, show limitations and lead to unsatisfying results in applications requiring further volume reduction, e.g., low copy number templates, high price reagents or limited sample specimen as in forensics. Advalytix has developed the AmpliGrid system, an innovative 1 μ L reaction platform for amplification assays based on a microscope slide.

Here we demonstrate (using the Eppendorf epMotion 5070 automated pipetting station) contamination free and reliable sample treatment in the low volume range. The whole PCR set-up of up to 48 independent samples starting from the sample preparation to the downstream processing can be done in one lab automation system with a dramatic reduction of the manual handling procedure.

Experimental Conditions

Materials

- epMotion 5070 (Eppendorf, Germany)
- SlideHolder SBS (Advalytix AG, Germany)
- AmpliGrid AG480F (Advalytix AG, Germany)
- 200 bp rat ferritin gene fragment in pCR[®]2.1 TOPO[®] cloning vector (Invitrogen, US)
- M13 primer mix (M13 forward(-20) / M13 reverse) 10 pmol/ μ L each
- Taq DNA Polymerase (1000) (Qiagen GmbH, Germany)
- AmpliSpeed ASC100D slide cycler (Advalytix, Germany)
- FlashGel[™] System 1.2% agarose incl. loading buffer (Cambrex, US)

Methods

1. Defining the detection limit of the system

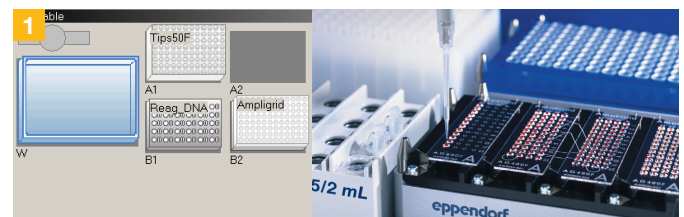
To ensure that even lowest amounts of template carry-over can be detected by the experiment described here, a dilution series of template DNA was analysed in a pre-experiment, starting from 5 template copies and ending up at 200.000.

In the set-up of this experiment identical amplification master mixes and protocols for running the process and analysing the results were used.

2. Detection of potential carry-over of the system

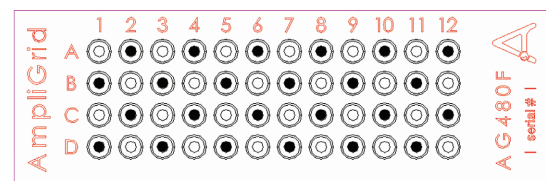
Automation set-up: epMotion 5070 Worktable and Program

The worktable layout of the epMotion 5070 is shown in figure 1. In position A1 50 μ L filtertips were placed. The rack in position B1 contained 1.5 mL Eppendorf tubes filled with the PCR master mix, the DNA template and the sealing solution respectively. In position B2 the AmpliGrid SBS slide holder was positioned on a 45 mm height adapter. The DNA solution and negative controls were deposited on the AmpliGrid slide in a checkerboard pattern (fig. 2). The master mix and sealing solution were deposited in the multidispensing mode closely to the surface of the AmpliGrid slide. A new liquid class "spotting" was created that significantly improved this multi-dispensing step by removing the trailing air gap during dispensing (fig. 3). The liquid class settings are available for the TS50 tool in multi-dispensing mode.



Worktable set-up epMotion 5070

- 2 Pipetting pattern on the AmpliGrid slide (black spots with DNA, white spots without DNA)



DNA Template

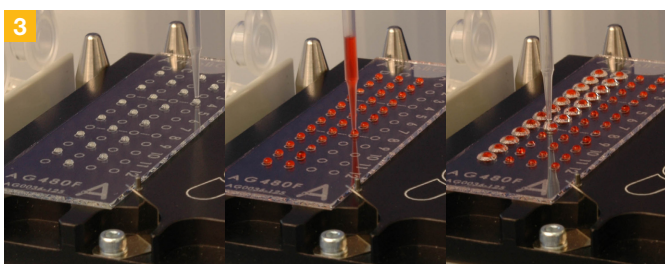
A pCR[®]2.1 TOPO[®] vector containing a 200 bp rat ferritin gene insert, in a DNA concentration of 1 pg/ μ L representing 200.000 copies was used. 1 μ L of template material was deposited alternating with 1 μ L negative controls as described above (fig 3). The samples were air-dried at room temperature for 15 minutes.

Master mix & slide loading

The AmpliGrid reaction sites were loaded with 1 μ L of amplification master mix each (see tab. A and fig. 3).

A Composition of master mix

Component	Volume
10x Qiagen PCR buffer	20 µL
M13 Primer Mix (10pmol/µL each)	8µL
dNTP-Mix (10 mM each)	2 µL
Qiagen Taq Polmerase (5U/µL)	3 µL
ddH ₂ O	147 µL
AdvaGold 0.1%	20 µL
Σ	200 µL



Loading DNA templates, amplification master mix and sealing solution

B Amplification programme

Temperature	Duration	
96°C	3 min	
95°C	30 sec	
53°C	30 sec	30 cycles
72°C	45 sec	
72°C	10 min	
ambient	∞	

To prevent evaporation during the amplification process each master mix droplet was covered with 5 µL of sealing solution (fig. 3). Both procedures were performed without changing the pipet tips.

Amplification

The AmpliGrid slide was transferred to an AmpliSpeed slide cycler for thermal cycling. The amplification programme is shown in table B.

Analysis

Sample analysis was done using the FlashGel™ agarose electrophoresis system (Cambrex, US). To transfer the samples to the agarose gel, 4 µL of loading buffer were pipetted on top of the sealing solution on the AmpliGrid slide. Consequently, the loading buffer moves through the sealing solution by gravity and merges with the PCR reaction without any further manipulation. The complete aqueous phase of 5µL (4µL loading buffer + 1µL sample) was then loaded to the FlashGel™. This way, the aqueous phase can be separated more easily from the sealing solution, as the sealing solution remains on the AmpliGrid slide. Run time was 5 min at 275V/ 50 mA.

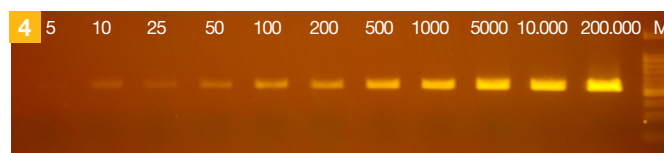
Results**1. Detection limit of the system**

The experimental system used for the analysis of carry over is sensitive enough to detect as less as to 5 DNA copies in the PCR. This is a result of the highly efficient reaction conditions in the 1µL volume geometry of the AmpliGrid slide.

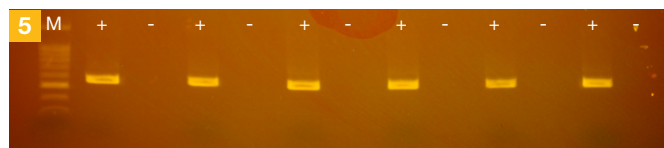
Figure 4 shows the result of the DNA titration series.

2. “Carry-over” experiment on the AmpliGrid slide

Primer sequences were chosen to amplify a 550 bp DNA fragment, consisting of 200 bp of inserted sequence and 350 bp vector sequence. In all positive samples the expected 550 bp fragment was successfully amplified (fig. 5, lanes “+”). The negative controls positioned in between remained without a detectable signal. Figure 4 shows the results of positions A 1-12 of the AmpliGrid. Rows B1-12, C1-12 and D1-12 exhibit the same result pattern (not shown).



Agarose gel analysis of PCR products from 5 to 200.000 template starting copies



Agarose gel analysis of “carry-over” experiment (alternating positive & negative controls)

Discussion

Cross-contamination free working with the AmpliGrid system was demonstrated even when starting with a very high amount of template material. Due to the high sensitivity of the AmpliGrid system even a low number of starting copies (in the case of cross-contaminations) would have lead to a positive result.

The total pipetting time for one slide or 48 reaction sites respectively is in the range of 3 minutes. The epMotion 5070 programme can be adapted quickly to different sample patterns, however, once programmed all subsequent set-ups are completely standardised and independent of the user. The enormous reduction of reagent stock solutions in combination with the ultra low volume needed for reactions on the AmpliGrid slide makes the process very cost efficient .

In summary, it can be stated that the combination of the AmpliGrid system with the epMotion 5070 automated set-up is a fast and reliable system for generating reproducible results in PCR applications.