

# Contamination-free ultra low volume pipetting on AmpliGrid AG480F slides

## Integration of 1 $\mu\text{L}$ assays in a commercial liquid handling platform.

### Introduction

In the life science research, 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 (as is common in forensics). Advalytix has developed the AmpliGrid system, an innovative 1  $\mu\text{L}$  reaction platform for amplification assays based on a microscope slide.

Here we demonstrate contamination-free and reliable sample treatment in the low volume range, using the Eppendorf epMotion 5070 automated pipetting station. 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 equipped with TS50 (Eppendorf, Germany)
- SlideHolder SBS (Advalytix, Germany)
- AmpliGrid AG480F incl. sealing solution (Advalytix, Germany)
- 200 bp rat ferritin gene fragment in pCR<sup>®</sup>2.1 TOPO<sup>®</sup> cloning vector (Invitrogen, US)
- M13 primer mix (M13 forward(-20) / M13 reverse) 10  $\mu\text{M}$  each
- Taq DNA Polymerase (1000) (Qiagen GmbH, Germany)
- AmpliSpeed ASC100D slide cycler (Advalytix, Germany)
- FlashGel<sup>®</sup> System 1.2% agarose incl. loading buffer (Lonza, US)

#### Methods

##### 1. Defining the detection limit of the system

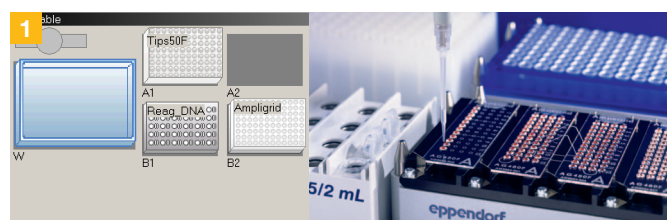
To ensure that even the smallest quantities of template carry-over can be detected by the experiment described here, a dilution series of template DNA ranging from 5 to 200,000 copies was analysed in a pre-experiment.

To ensure consistency, the experiment was designed to use identical amplification master mixes; protocols for running the process and analysing the results.

##### 2. Detection of potential system carry-over

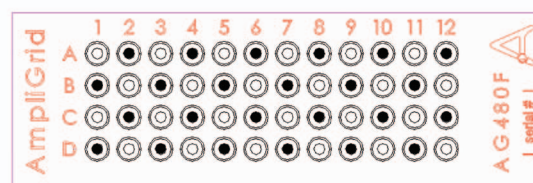
Automation set-up: epMotion 5070 Worktable and Program

The worktable layout of the epMotion 5070 is shown in figure 1 with 50  $\mu\text{L}$  filtertips in position A1. 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 SlideHolder SBS was fixed with a 40 mm height adapter (Eppendorf, Germany). The DNA solution and negative controls were dispensed on the AmpliGrid slide in a checkerboard pattern (fig. 2). The master mix and sealing solution were then dispensed in the multidispensing mode close 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. 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

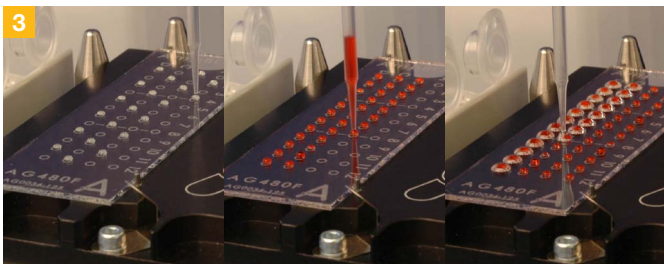
A pCR<sup>®</sup>2.1 TOPO<sup>®</sup> vector containing a 200 bp rat ferritin gene insert, was used at a DNA concentration of 1  $\mu\text{g}/\mu\text{L}$ , representing 200,000 starting copies. DNA amounts were measured using the Nanophotometer<sup>™</sup> with Labelguard<sup>™</sup> for ultravolume spectrophotometric analysis. 1  $\mu\text{L}$  of template material was dispensed alternating with 1  $\mu\text{L}$  of negative controls as described above (fig. 2). The samples were air-dried at room temperature for 15 minutes.

#### Master mix & slide loading

All the AmpliGrid reaction sites were loaded with 1  $\mu\text{L}$  amplification master mix (see tab. A and fig. 3).

**A** Composition of master mix

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



Loading DNA templates, amplification master mix and sealing solution

**B** Amplification program

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 in multi-dispensing mode (without changing the pipette tips).

**Amplification**

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

**Analysis**

Sample analysis was completed using the FlashGel™ agarose electrophoresis system (Cambrex, US). To enable transfer of the samples to the agarose gel, 4 µL loading buffer was pipetted on top of the sealing solution on each AmpliGrid reaction site. This loading buffer moves through the sealing solution by gravity since they are immiscible, where it merges with the PCR reaction without any further manipulation. The complete 5 µL aqueous phase (4µL loading buffer + 1µL sample) was then loaded on 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 approx. 5 min at 275V/ 50 mA.

For research use only. Not for use in diagnostic procedures Nanophotometer™ and Labelguard™ are trademarks of Implen GmbH, Germany

**Advalytix**  
Part of Beckman Coulter

**BECKMAN COULTER BIOMEDICAL GMBH**  
Sauerbruchstraße 50, 81377 Munich, Germany  
Tel: +49 (0)89 579589-0, Fax: +49 (0)89 579589-3503  
E-Mail: info@advalytix.com  
www.advalytix.com

US office  
58 Elsinore St., Concord, MA 01742, USA  
Tel: +1 (978) 405-2533, Fax: +1 (978) 405-2534  
Mobile: +1 (978) 979-7899  
E-Mail: info@advalytix.com

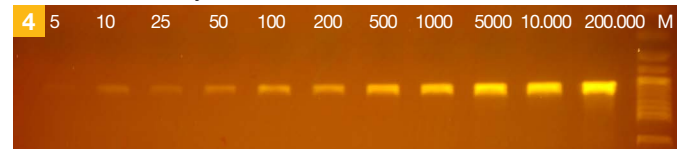
**Results****1. Detection limit of the system**

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

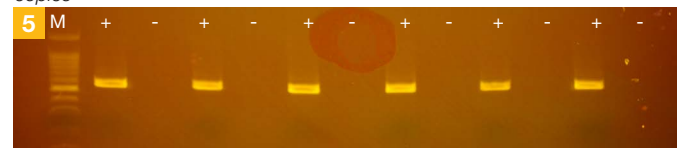
Figure 4 shows the result of the DNA titration series.

**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 of vector sequence. For all template-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 A1-12 of the AmpliGrid. Rows B1-12, C1-12 and D1-12 exhibit the same result pattern (not shown). In total, 30 slides (representing 1440 reactions) have been tested without any cross-contamination observed.



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



Agarose gel analysis of "carry-over" experiment (alternating positive &amp; 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-contamination) would have led to a positive result.

The total pipetting time for one slide or 48 reaction sites, is in the range of 3 minutes. The epMotion 5070 program 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 PCR reactions on the AmpliGrid slide makes the process highly 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.