

# Contamination-free pipetting of ultra-low volume PCR reactions on structured microscope slides

Petra Hartmann & Andreas Tögl, Advalytix AG, Brunthal, Germany  
Daniel Wehrhahn, Eppendorf AG, Hamburg, Germany

## Introduction

In R&D and diagnostics 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, i.e. low copy number templates, high price reagents or limited sample specimen as in forensics. Advalytix AG (Brunthal, Germany) has developed the AmpliGrid system, an innovative 1 µl reaction platform for amplification assays based on a microscope slide. The AmpliGrid system is an advanced platform for ultra-low volume applications in the 1 µl range. High quality microscope slides have been modified by semiconductor based technology to offer 48 hydrophilic reaction sites surrounded by a hydrophobic circle holding 1 µl of aqueous reaction solutions in place. Evaporation is prevented by covering the aqueous solution with a special sealing solution.

Typical applications for AmpliGrid are low volume PCR reactions in the forensic or DNA identity testing field, enabling single cell assays in the analysis of copy number variations in IVF or in combination with immunophenotyping in cancer diagnostics. It was shown that on-chip DNA amplification reproducibly yielded full allelic profiles from template DNA in the low pg range in forensic sample testing [1, 2]. Within this high sensitivity range, an error- and cross-contamination-free set-up of the PCR reactions is mandatory to ensure reliable results.

Using the Eppendorf epMotion® 5070 automated pipetting station, contamination-free and reliable sample treatment in the low volume range is demonstrated in this application note. 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 automated pipetting system with a dramatic reduction of manual handling.

## Materials

- epMotion® 5070 (Eppendorf)
- SlideHolder SBS (Advalytix)
- AmpliGrid AG480F (Advalytix)
- 200 bp rat ferritin gene fragment in pCR® 2.1 TOPO® cloning vector (Invitrogen, Carlsbad, CA, USA)
- M13 primer mix (M13 forward(-20) / M13 reverse) 10 pmol/µl each
- Taq DNA Polymerase (1000) (Qiagen GmbH, Valencia, CA, USA)
- Eppendorf Mastercycler® equipped with *in situ* Adapter (Eppendorf)
- FlashGel™ System 1.2% agarose incl. loading buffer (Cambrex, Charles City, IA, USA)

## Methods

*Automation set-up: epMotion 5070 worktable and program*

The worktable layout of the epMotion 5070 is shown in Fig. 1. In position A1 50 µl filter tips 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 Ampli-Grid 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 multi-dispensing mode closely to

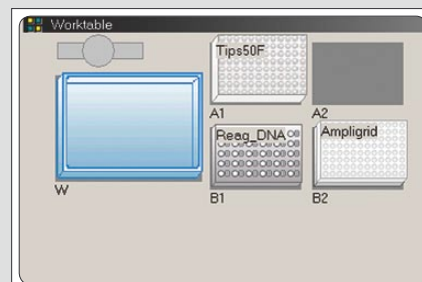


Fig. 1: Worktable set-up epMotion 5070

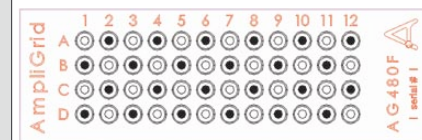


Fig. 2: Pipetting pattern on the AmpliGrid slide (black spots with DNA, white without DNA)

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. 3a and b).

The liquid class settings are applicable for the TS50 tool in the multi-dispensing mode.

## DNA template

A pCR® 2.1 TOPO® vector containing a 200 bp rat ferritin gene insert in a DNA concentration of 1 pg/µl representing 200,000 copies was used. 1 µl of template material was deposited alternately with 1 µl negative controls (water) as described above. The samples were air-dried at room temperature for 15 min.

## Master mix and slide loading

The AmpliGrid reaction sites were loaded with 1 µl of amplification master mix each (see Table 1 and Fig. 3b). This was performed without changing the tip.

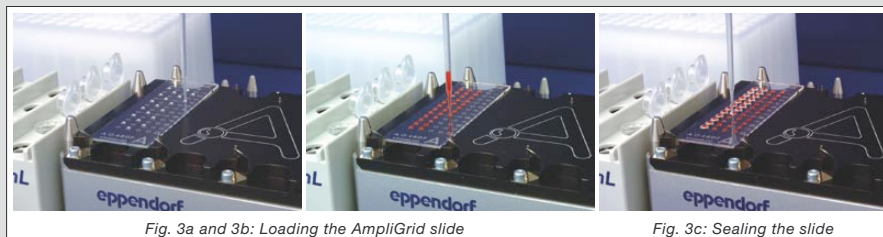


Fig. 3a and 3b: Loading the AmpliGrid slide

Fig. 3c: Sealing the slide

## Contamination-free pipetting of ultra-low volume PCR reactions on structured microscope slides

10x Qiagen PCR Buffer	20 µl
M13 primer mix (10 pmol/µl each)	8 µl
dNTP mix (10 mM each)	2 µl
Qiagen <i>Taq</i> Polymerase (5 U/µl)	3 µl
ddH <sub>2</sub> O	147 µl
AdvaGold 0.1%	20 µl
	Σ 200 µl

Table 1: Contents of amplification master mix

To prevent evaporation and cross-contamination during the amplification process each master mix droplet was covered with 5 µl of sealing solution (Fig. 3c). This procedure was also performed without changing the pipette tips.

**Amplification**

The AmpliGrid slide was transferred to an Eppendorf Mastercycler® for thermal cycling. The amplification program is shown in table 2.

**Analysis**

Sample analysis was done using the FlashGel™ agarose electrophoresis system (Cambrex). 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 5 µl (4 µl loading buffer + 1 µl sample) was then loaded to the FlashGel™. This way, the aqueous phase can be separated

Temperature	Time	Cycles
95 °C	3 min	30
95 °C	30 s	
53 °C	30 s	
72 °C	45 s	
72 °C	10 min	
ambient	∞	

Table 2: Amplification program

more easily from the sealing solution, as the sealing solution remains on the AmpliGrid slide. Run time was 5 min at 275 V/50 mA.

**Detection limit of the system**

To ensure that even lowest amounts of template carryover would have been detected by the experiment as described, a dilution series of template DNA was analysed in a second experiment, starting from 5 template copies and ending up at 200,000. The set-up to this “carry-over” experiment was carried out as described in the experiment before.

**Results**

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. 4, lanes “+”). The negative controls positioned in between remained without a detectable signal.

Fig. 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).

Fig. 5 shows the result of the DNA titration series used in the second experiment. The experimental system is sensitive enough to detect down to 5 DNA copies in the PCR.

**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 even a low number of starting copies would have led to a positive result in the case of cross-contaminations.

The total pipetting time for one slide or 48 reaction sites, respectively, is in the range of 3 min. The complete system can easily be integrated into an established laboratory and requires very little training and set-up time. 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 makes the PCR process very cost efficient.

In summary, the combination of the AmpliGrid system with the *epMotion* 5070 automated set-up is a fast and reliable system for generating reproducible results in ultra-low volume PCR applications.

**Literature**

- [1] U. Schmidt *et al.*, (2006): Low-volume amplification on chemically structured chips using the PowerPlex16 DNA amplification kit  
*Int J Legal Med*, 120(1): pp. 42-48
- [2] Advantix AG (2006): Getting full profiles from highly redundant low-volume amplifications (LV-PCR), *Application Note AmpliGrid AG480F*

**Readers' service**  
*epMotion*® 5070 • Ref. no. 178

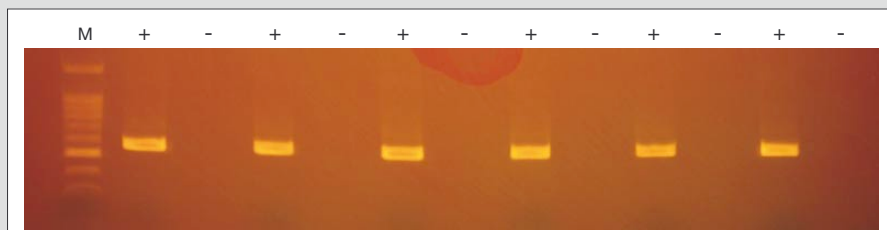


Fig. 4: Agarose gel analysis of “carryover” experiment (alternating positive and negative controls)

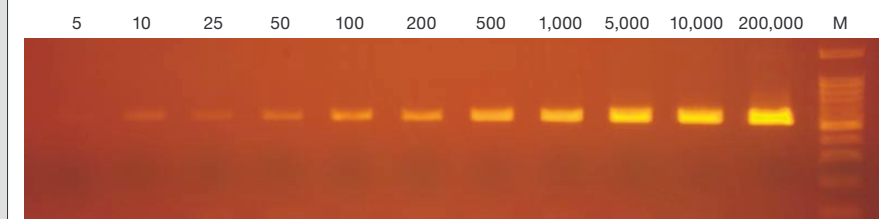


Fig. 5: Agarose gel analysis of a dilution series from 5 to 200,000 template starting copies