

High efficiency one-step lysis and PCR from single bacteria using the AmpliGrid System

Enabling single cell microbiology.

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The amplification of genetic material of single bacteria is an important topic in microbiology. Using the AmpliGrid technology, high efficiency and sensitivity are combined in a simple and seamless workflow. Single bacteria deposition can be visually controlled. Additionally the lysis step and the amplification step are combined to ensure a convenient and fully controlled workflow.

Introduction

This application report shows the amplification of a 16S rDNA fragment in single *Staphylococcus* and *Pseudomonas* bacteria. Single cell deposition is described using flow cytometry. Using the superior AmpliGrid technology single bacteria deposition can be visually controlled using a standard microscope. The lysis step using a commercially available product and the amplification are combined that no buffer change is necessary.

Experiment

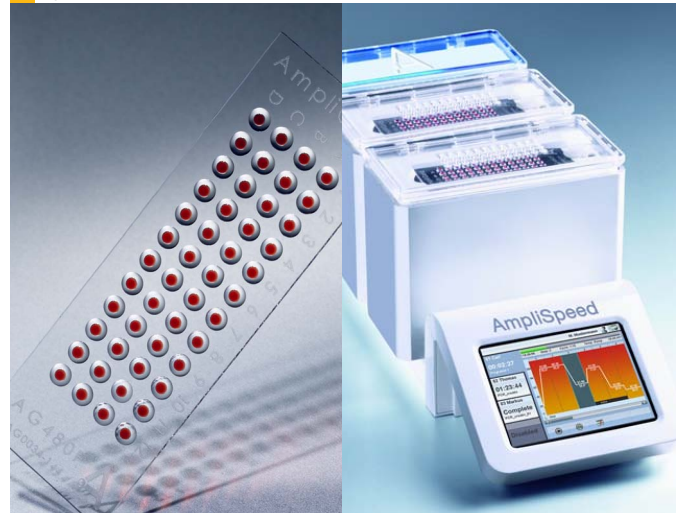
Sorting of single bacteria using flow cytometry

Stain bacteria of the gram positive strain *Staphylococcus warneri* and of the gram negative strain *Pseudomonas alcaliphila* with Hoechst H33342 (3.33 µg/ml) in PBS for 10-15 min at room temperature. Wash afterwards in 1x PBS (pH 7.4). Set up the flow cytometer BD FACSAria™ as follows:

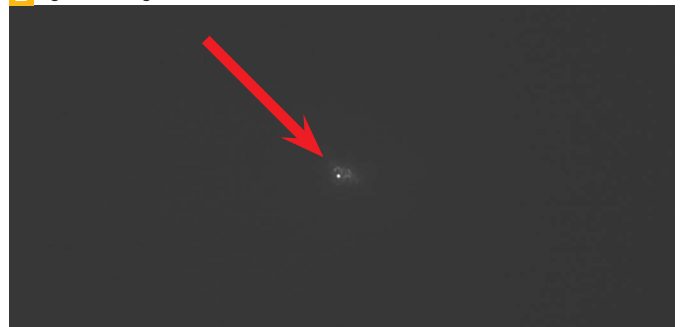
- 70 µm nozzle
- open FSC-A/SSC-A, FSC-A/FSC-W, FSC-A/Hoechst dot plots (log scale)
- gate on Hoechst positive, small population with low granularity, exclude doublets

Spot single bacteria on the reaction sites of the AmpliGrid AG480F slide (figure 1). Visually control the single deposited bacteria using an Olympus fluorescence microscope with 400x or 600x magnification (figure 2).

1 Figure 1: AmpliGrid AG480F and AmpliSpeed slide cycycler ASC200D



2 Figure 2: single bacteria detection



Lysis of single bacteria on the AG480F

Pipette 0.75 µl Lyse-N-Go™ mix to each reaction site and immediately cover with 5 µl of sealing solution. Transfer the AmpliGrid slide to the AmpliSpeed slide cycycler (figure 1) and run the program described in table A.

A Table A: Lysis programme

Temperature	Time	Cycle
65°C	30 sec	
37°C	30 sec	
65°C	90 sec	
97°C	3 min	
37°C	1 min	3 x
65°C	3 min	
97°C	1 min	
65°C	1 min	

Amplification of 16S ribosomal DNA in single bacteria

Prepare the master mix for PCR as described in Table B:

B Table B: Preparation of master mix

Component	Volume per reaction
2x Qiagen Multiplex Master Mix	0.75 µL
5x Q-Solution	0.045 µL
Primer mix (forward and reverse; 3 µM each)	0.1 µL

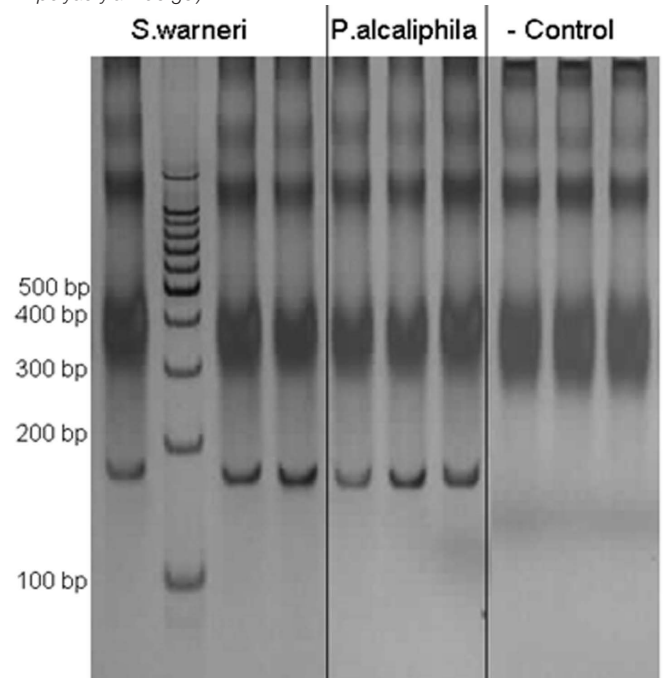
Add 0.75 µL of the amplification master mix to the reaction sites by pipetting through the sealing solution and transfer the AmpliGrid slide on the AmpliSpeed slide cycler. Start the program like mentioned in table C.

C Table C: PCR program

Temperature	Time	Cycle
95° C	10 min	
94° C	30 sec	
63°C	75 sec	45 x
72°C	75 sec	
72°C	10 min	

Results:

After amplification add 4 µl of gel loading dye to each reaction site of the AmpliGrid and transfer the total volume of amplicon and loading dye to a 8% polyacrylamide gel. Run the polyacrylamide gel electrophoresis and stain the polyacrylamide gel with silver reagents afterwards. The results in figure 4 show the amplification of 16S ribosomal DNA in both gram positive and gram negative single bacteria. The negative control shows that there is no contamination occurring on the AmpliGrid slide.

3 Figure 3: Detection of the 175 bp 16S rDNA fragment amplified in single *Staphylococcus* and *Pseudomonas* bacteria (silver stained polyacrylamide gel)

Discussion:

The AmpliGrid single cell analysis platform opens the possibility for the investigation of single gram positive and gram negative bacteria. Single bacteria deposition can be visually controlled using standard microscopy ensuring a quality control. In single cell microbiology a broad range of applications is now open to the researcher using a simple, seamless and sensitive platform.

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