

High sensitivity and cost effective STR profiling on AmpliGrid AG480F using limited sperm material

With the AmpliGrid system it is possible to achieve full profiles out of only 10 sperm cells using standard STR profiling kits in a convenient and user-friendly workflow. These results show the superior sensitivity of the AmpliGrid system. Additionally cost saving aspects make the AmpliGrid system the platform of choice for all assays requiring high sensitivity.

Dr. Diana Hops, Katja Dietz and Dr. Karin Görner, Beckman Coulter Biomedical GmbH, Germany

Summary

We deposit 10 sperm cells on reaction sites of the AmpliGrid slide. After sperm deposition quality control is performed using a standard fluorescent microscope after staining the sperm DNA with an intercalating dye such as Hoechst. We perform the lysis of the spermatozoa using a very low volume of a special lysis mixture. Directly after lysis, without any further manipulation therefore without any risk for template loss, we perform the STR amplification with the Applied Biosystems AmpFISTR® SEfiler™ kit or the Promega PowerPlex® 16 kit (data not shown). The limited manipulation of the samples avoids error-prone re-pipetting steps derived from the very little reagent volume. Capillary electrophoresis results and the corresponding success rate clearly underline AmpliGrid's high sensitivity and make the AmpliGrid system the platform of choice for sperm STR analysis, especially when only limited sample material is available.

Sperm deposition and lysis

Deposit 10 sperm cells from a 0.05x PBS buffer solution to each reaction site on the AmpliGrid slide (figure 1) using a standard pipette. Nuclear staining with Hoechst dye is performed immediately before the deposition according to standard protocols for easy verification of the cell deposition on the AmpliGrid reaction sites with a standard fluorescent microscope (Olympus).

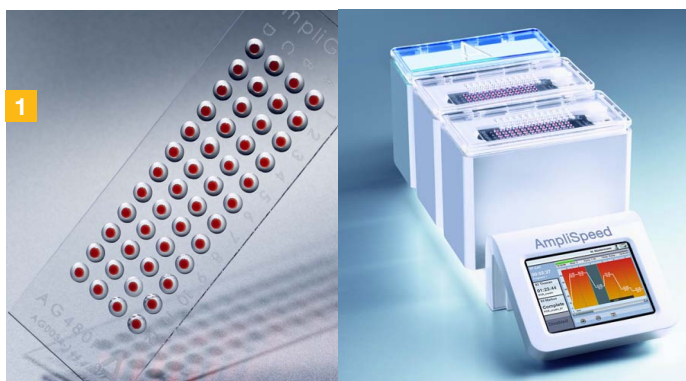


Fig. 1: AmpliGrid AG480F slide and AmpliSpeed ASC200D slide cycler

Lyse sperm cells directly on the reaction sites applying 0.75 μ L of the sperm lysis mix and cover it with 5 μ L of sealing solution in order to prevent evaporation.

Transfer the AmpliGrid slide to the AmpliSpeed slide cycler (figure 1) and run the sperm lysis program described in table A.

A Table A: Sperm lysis program

Temperature	Time	Heating Rate
55°C	120 min	
95°C	10 min	0.5°C/sec
Ambient	16 h	

STR Amplification

Prepare the STR amplification master mix according to table B, mix gently and spin down shortly.

B Table B: STR amplification master mix

Component	1 reaction
AmpFISTR® PCR Reaction Mix	0.477 μ L
AmpFISTR® SEfiler™ Primer Set	0.250 μ L
AmpliTaQ Gold® DNA Polymerase 5 U/ μ L	0.023 μ L
Total Volume	0.750 μL

Pipette 0.75 μ L of the STR amplification master mix on the AmpliGrid reaction sites holding the lysed sperm samples by piercing with the pipet tip through the sealing solution using a standard manual pipette. Protect the samples from direct light to avoid bleaching of the primer's fluorescent label. Place the AmpliGrid on the AmpliSpeed slide cycler and run the amplification program described in table C.

C Table C: Amplification program

Temperature	Time	Cycle
95°C	11 min	
94°C	60 sec	
59°C	60 sec	35
72°C	60 sec	
60°C	45 min	

For capillary electrophoresis (ABI PRISM®) analysis use the whole sample including the 1.5 µL aqueous reaction mix plus 5 µL sealing solution. Fill the aqueous phase up to 10 µL using loading buffer and formamide. Analyse the samples according to the manufacturer instructions. Adapt sample aspiration time to maximum.

Results

Sixteen samples out of two independent experiments are analysed following the described protocol. The results in figure 2 show that it is possible to achieve the full profile in eleven out of sixteen samples, which corresponds to approx. 69% of the samples having 10 sperm cells as template (figure 3). Of the remaining samples, 25% showed an amplification success rate higher than 95% (figure 3). All in all nearly 95% of the samples are amplified with a superior amplification success rate.

Comparable results are obtained using the Promega PowerPlex® 16 kit for STR analysis (data not shown).

Discussion

Genetic profiling of sperm samples based on STR analysis has become a very important application in forensic casework and the databases are continuously updated in many countries worldwide. The analysis of little amounts of sperm samples obtained for example during the crime scene investigation, is generally difficult because of a lack in sensitivity in standard platforms. High price sample analysis and labor intensive workflows discourage a routine use of these technologies for low priority cases.

In the present report we show that even when sperm template material is very limited, the AmpliGrid platform is the ideal tool for STR profiling due to its high sensitivity performance. In addition, the low PCR reaction volume contributes to an enormous cost saving. Especially when other standard methods are not suitable, the described protocol is a valid alternative for sample analysis, contributing to increase the STR database coverage.

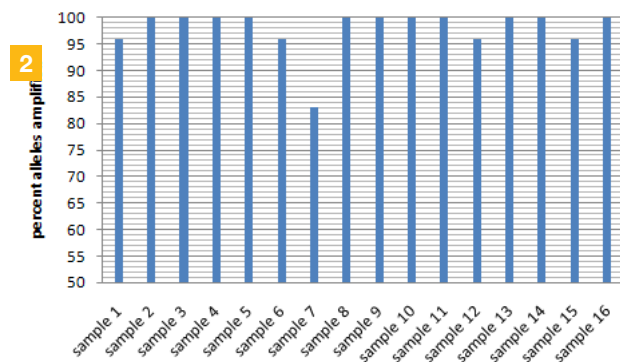


Figure 2: success rate of STR amplification using the Applied Biosystems AmpFISTR® SEfiler™ on sixteen samples having ten sperm cells deposited (two independent experiments); amplification is performed on the AmpliGrid platform (success rate is given in percent)

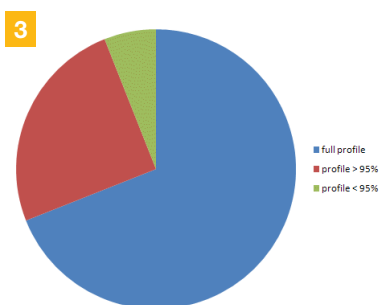


Figure 3: Distribution of partial and full profiles after STR amplification with an initial template amount of ten spermatozoa

For research use only. Not for use in diagnostic procedure