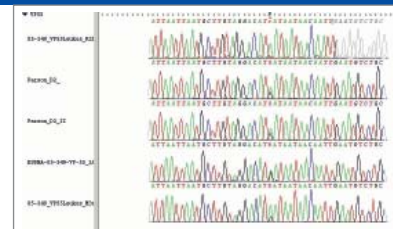


# The Evidence Points to Single Cell Analysis for Forensic Applications

**Interview with Dr. Sabine Lutz-Bonengel, Senior Scientist at the Institute for Legal Medicine, Albert-Ludwig University, in Freiburg, Germany.**



The goal in forensic science is to eliminate uncertainty, using technology to precisely determine identity. There is no doubt that technology has improved the ability to determine “who-dunit” – or who is it, yet researchers continue to refine and improve forensic methods. Single cell analysis is one technique being used with success for both increased sensitivity and cost savings.

Dr. Sabine Lutz-Bonengel, Senior Scientist at the Institute for Legal Medicine, Albert-Ludwig University, in Freiburg, Germany, is developing methods for forensic research using the AmpliGrid single-cell analysis platform from Advalytix, a division of Olympus. Dr. Lutz-Bonengel has analyzed approximately 5,000 PCR reactions using the AmpliGrid slide. This platform delivers improved DNA amplification in a low-volume (1  $\mu$ l) reaction format using single cells as a template source.

“In my working group, we have focused on the analysis of mitochondrial DNA (mtDNA) as a second source of information, besides genomic profiling, as well as on integrated workflows on the AmpliGrid platform,” said Dr. Lutz-Bonengel. “In low-copy number research, we’re starting with single cells for amplification. After the amplification, we just add the sequencing reagent without having to re-format the assay.”

Degradation of DNA and loss of template during the extraction process are two challenges for forensic scientists. Dr. Lutz-Bonengel has been able to successfully amplify scarce or degraded samples and obtain full profiles from very small amounts of nucleic acids.

“The AmpliGrid enables us to start with defined numbers of genome equivalents, based on single cells,” she reports. “By systematic analysis, one can validate the existing forensic test kits. It’s been shown by Carsten Proff that you can get full profiles from DNA from as little as four cells.”

While Dr. Lutz-Bonengel can only speculate on why the AmpliGrid shows increased sensitivity over 1  $\mu$ l tube reactions, she attributes the difference to the inert nature of a glass slide.

“Absorption of template molecules into the plastic material of tubes or microtiter plates has been shown to affect sensitivity,” she said. “The modified glass surface of the AmpliGrid does not absorb nucleic acids, increasing sensitivity.”

The results using the AmpliGrid have already been proven by Proff and colleagues. This group published a method in which samples are analyzed in a redundant way in 1  $\mu$ l reactions on the AmpliGrid. Pooling the information, or

pooling the reaction volumes of low copy number (LCN) samples, before the Capillary Electrophoresis run improved the results significantly, Proff reported. Dr. Lutz-Bonengel is taking a different approach with her research and is working to develop protocols for less complex multiplex PCR systems. She is experimenting with 5 loci, compared with the conventional 13 or 16, to improve sensitivity and reduce cost. The 5-plex system, because of its sensitivity and lower cost, can serve as a screening system in LCN and, if necessary, follow up with a 16-plex system.

“This will probably also increase sensitivity in the LCN field based on direct, single 1  $\mu$ l reactions,” she said.

## A special interest in mtDNA

mtDNA can be used to examine samples that can’t be analyzed by methods concerning genomic DNA. Compared to the low copy number in chromosomal DNA, mtDNA provides a couple hundred copy numbers – depending on the cell being analyzed – and therefore more abundant, viable template. It was found that analyzing mtDNA, in addition to genomic profiling, yields additional information – an important contribution to forensic scientists’ goal of increasing sensitivity, especially when working with challenged samples. mtDNA occurs in a number of copies per genome equivalent and enables researchers to retrieve genetic information below the genome equivalent.

“We wanted to see how sensitive the AmpliGrid could be for mtDNA analysis and found the 1  $\mu$ l reaction to be superior to conventional assays,” Dr. Lutz-Bonengel said. “Typically, we sequence the amplified fragments of the complete control region of the mtDNA. In this context, the AmpliGrid chip enables us to combine the amplification and sequencing in the identical reaction droplet. We have developed a protocol that even works starting with single cells as a target for PCR. Suddenly, we recognized that we could study the phenomenon of heteroplasmy on the single-cell level systematically. The results are not only of academic interest, but could influence forensics case work because the results could elucidate the phenomenon of heteroplasmy and its inheritance.”

Dr. Lutz-Bonengel said future work on mtDNA may enable the DNA sequencing of single mitochondria, instead of single cells.

“The only chance to be successful with this approach would be to make use of the AmpliGrid and its optical properties

*Continued on reverse.*

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in order to inspect the biological sample directly under the microscope prior to amplification,” she noted.

A colleague, Dr. Marielle Heinrich, also at the Institute, is working with the AmpliGrid to analyze single nucleotide polymorphisms (SNP) on the Y chromosome. These efforts, combined with mtDNA analysis and reduced complexity assays, are at the forefront of forensic science. In addition to sensitivity, low-volume analysis is attractive because of its lower cost and low contamination rates.

Dr. Lutz-Bonengel notes that, while one might suspect the open system of a chip would lead to higher contamination rates, she has experienced less than 0.5% contamination using the AmpliGrid.

“There is always risk for contamination with human DNA, since a person is handling the chip,” she said. “We have found the AmpliGrid slide to be extremely safe when properly handled. In addition, the general handling of the slide has some advantages when compared to conventional plates and tubes, because there is no need to handle the containment individually.”

Cost is lower than conventional plates and tubes. Because the AmpliGrid relies on 1 µl volume, compared to 12.5 µl or 25 µl requirements in a standard typing protocol, the savings on reagents is considerable. Advalytix estimates the cost of reagents at 1EUR/1 µl. According to Dr. Lutz-Bonengel, law enforcement agencies would run more tests on samples from crime scenes if they could afford it, but budget constraints limit the amount of testing. By reducing the cost per test, while increasing sensitivity, forensic researchers like Dr. Lutz-Bonengel are working to ensure the availability of the most accurate scientific methods to determine identity.

#### About Advalytix AG

Advalytix AG (Munich) was founded in 2000 and has been a wholly owned subsidiary of Olympus Life and Material Science Europa GmbH since 2005. Within the Olympus Group, Advalytix is the world wide centre of expertise for cell-based molecular diagnostics. One of the company’s main focuses is the miniaturisation of diagnostic and biological tests for research and development. Advalytix products uniquely link imaging technologies such as microscopy with nucleic acid analysis, enabling Advalytix to offer turnkey system solutions consisting of both instruments and reagents. Advalytix has developed a portfolio of products for these applications, based on proprietary microelectronics technologies, such as surface acoustic waves (SAW).



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