

# How Next Generation Sequencing Resolved a Difficult Case, Leading to the First Criminal Conviction of its Kind

Peter de Knijff explains how his laboratory used NGS methods to resolve a landmark case in the Netherlands.

## Introduction

Peter de Knijff is not unaccustomed to the role of pioneer in the field of forensic genomics. Twenty years ago, he and his forensic laboratory staff at Leiden University Medical Center (LUMC) in Holland were among the first to demonstrate the forensic utility of male specific Y-STR markers<sup>1</sup>—a method that is widely used today. They went on to develop a sensitive method to isolate DNA from fired ammunition casings<sup>2</sup> (a sample type that was widely considered too challenging for practical use), which they have now used to analyze over 15,000 items of firearms evidence.

Therefore, it should come as no surprise that Dr. de Knijff was one of the very first to see the potential of next generation sequencing (NGS), also known as massively parallel sequencing (MPS), for forensic applications. His laboratory acquired an Illumina MiSeq system soon after it was introduced in 2011, and became the first forensic laboratory to receive an ISO-17025 accreditation to perform casework analysis using this platform in 2015.

Since then, they have used MPS on a wide range of challenging cases, recovering critical data that could not be obtained using standard capillary electrophoresis (CE) methods, to support claims of innocence or guilt and generate “hits” (investigative leads) using the Dutch criminal offender database.

In one recent case, they used MPS to decipher highly discriminating data from a challenging mixed sample in a sexual assault case, which ultimately led to the first and only criminal conviction based on MPS data to be reported globally to date.

Verogen spoke with Dr. de Knijff recently regarding this interesting case, and its potential ramifications for MPS utilization moving forward.



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**Q: Can you describe the case that led to this first criminal conviction using MPS data?**

**Peter de Knijff (PDK):** It involves a sexual assault on a 28-year-old woman that took place in 2015. She preserved her clothes after the assault, and also took intimate samples from herself, and waited for three days until she was courageous enough to go to the police. The police sent all the samples to another forensic laboratory in the Netherlands, and they started as usual with capillary electrophoresis (CE) analysis. A year later in 2016, the CE results in one particular sample led to a database search in the Dutch convicted criminal database, which resulted in a hit. Based on the database hit, the police arrested a possible suspect.



In other cases, we were able to exclude suspects, which were suggestively included based on CE... That is as important as getting a conviction.

Soon after that, the method which led to the database hit was challenged by the defense lawyer because he thought that there were some irregularities with the interpretation of the CE results. In Holland, suspects of a crime have the right to access



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## Case Study

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contra-expertise performed by an independent laboratory, which is also paid for by the Dutch government. So, the lawyer contacted me for the possibility of performing the contra-expertise. He explained that the samples previously analyzed by the laboratory were mixed DNA samples with a major contributor consistent with the victim and a minor contribution less than 10%.

I then suggested to the lawyer a contra-expertise using massively parallel sequencing. Considering this particular case, I was convinced that MPS would be able to outperform CE, with the potential to exonerate his client; however, of course, we could also include his client and support the prosecution's hypothesis further. Much to my surprise, the defense lawyer declined the offer for MPS, and strictly wanted me to use CE as the method of contra-expertise.

According to Dutch law, we have to follow the request of the lawyer, so we did a contra-expertise on all the samples using CE. Not very surprisingly, we found that indeed there were mixed DNA samples with a very minor contributor, which could possibly belong to someone with a DNA profile identical to the suspect. However, we also noticed that there were many alleles in the CE profile which were in the stutter position of a major allele belonging to the victim. For those variants, we could not decide whether or not those alleles belonged to a stutter associated with the victim or to a minor contribution from the perpetrator. And that's what we wrote down in our report, that we had predominantly inconclusive results as to whether the suspect did or did not contribute to the mixed DNA samples in this particular case.

In April 2017, the judge, confronted with my report, found that there was not enough convincing evidence and the suspect was released.

Immediately thereafter, the prosecutor filed for an appeal, but it took more than a year to get permission from the appeal judges to use MPS. It was not that they didn't trust the technology, but there was an enormous administrative backlog at the court. Finally, in August 2018, our laboratory was given permission to use MPS in an effort to get the final answer in this case.



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**Q: What did the MPS testing reveal?**

**PDK:** We analyzed all the leftovers of the remaining samples

for a single MPS reaction, and we got, as I expected, really good results. Meaning that, in all the mixed DNA samples, we got much more clear results, predominantly implicating the suspect, because we could decipher whether the minor alleles belonged to a stutter or not. We used likelihood ratio statistics to express the evidentiary value of each individual stain, and that was very, very conclusive.

**Q: How did this affect the verdict?**

**PDK:** The results were reported in December 2018, and the appeal court convened and extensively discussed the MPS results. I was not asked to come and give evidence because, according to the judges, the prosecutor, and the defense lawyer, they had nothing further to ask in addition to what was written in our report. On January 17th, the appeal court ruled the suspect guilty for the crime. In the verdict, they clearly stated it was the MPS evidence combined with the likelihood ratio statistics which led them to the conviction of this particular suspect, who was sentenced for three years.



**I expect there will be more court cases using MPS data by my own lab or other labs that are starting to use this technology. It's just a matter of time.**

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**Q: The ability of MPS to distinguish true minor contributor alleles from stutter clearly played a large role here, but what about the ability to detect other alleles that were "masked" or undetectable with CE?**

**PDK:** In this particular case, we could only unmask two alleles with MPS where the victim and the suspect had the same fragment length but a different sequence composition. But at least that was two more which still helps. And we were able to identify more of the minor alleles which were unique to the suspect. That led to the total package of much more alleles present in the analysis that we could use for the likelihood statistics. But the most power came from unmasking the stutters in this case.

**Q: What accounted for being able to detect the additional minor peaks that were unique to the suspect?**

**PDK:** It's our experience that if you have a minor contribution in a mixed sample, anywhere in the level of around 5-10%, which is on the border of detection with CE, you almost always find all the minor alleles with MPS.

**Q: And this is due to increased sensitivity?**

**PDK:** Yes, this is due to the fact that most of the loci have a

more similar PCR fragment length (minimizing preferential amplification), and you no longer depend on the sensitivity of a fluorescent label. You can simply count the molecules which have been sequenced. That combined effect in many cases leads to higher sensitivity.

**Q: Do you believe you could have achieved these results with any different method or platform?**

**PKD:** No. I know there are other platforms which allow sequencing of micro-satellites, but, to my knowledge, those platforms use different PCR kits and software to call the variants, and they do not allow you to download the raw data, so you can't analyze the full sequence reads which the machine produces. For me, that is unacceptable, because I need to have access to the raw data that allows me to understand what the machine has been doing.

**Q: Were you worried or concerned with how these MPS data would be accepted in Dutch criminal courts?**

**PKD:** No, not for a second. I know a little bit how the Dutch legal system works. First, the fact that a method can only be used if it is accredited by the Dutch Board of Accreditation is a major quality assurance. Second, they know my own laboratory is a driving force behind forensic innovation, at least in the Dutch legal system. They know that if we introduce an accredited method, it is reliable, and that I'm always available for further questions. But it is quite rare in Holland that a DNA expert is asked to answer questions in court for a criminal case. I only go to court here, at most, once or twice a year. In this particular case, I expected that they may have some questions because it is a completely new technology, but they were just very impressed by the good results, and the defense lawyer had no further questions. It turned out to be a very simple case. But it was exciting that it came to this particular verdict for me, of course.



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**Q: Why was this so exciting for you?**

**PKD:** Well, I start a lot of forensic research projects and... many research ideas never make it to this particular stage,

because in the long term they do not seem viable or practical, or they simply are not picked up by the community. It's always exciting to see that something you've been working on—and in this particular case, working on for close to 11 years—finally reaches the ultimate stage, and that is being accepted in court as evidence either to exonerate or convict a suspect. You do not see that very frequently in a forensic genetics career.

I'm a very strong believer in MPS as a technology on its own, and this is a very simple application of MPS. I'm convinced that MPS can add a lot of value in court. This is only the beginning, and with the beginning, you always run the risk that a court doesn't accept it. If a court doesn't accept it, then you have to work very hard to get it accepted, which might take years. Now that we have the acceptance, which is an important hurdle, it will make it more acceptable in other courts of law also, I think.

**Q: Do you think this first court case is indicative of how other cases could be helped as well?**

**PKD:** Yes. My laboratory has already now used this technology in 35 different cases. Many of them were cold cases where we generated profiles, which went into the database, but we simply do not have a hit yet. In other cases, we were able to exclude suspects, which were suggestively included based on capillary electrophoresis. Those cases were not sent to court simply because we found that a potential suspect could be excluded. That is as important as getting a conviction; however, because that never reaches the phase of a court appearance, it doesn't count in terms of publicity, but it still counts for me.

I expect there will be more court cases using MPS data by my own lab or other labs that are starting to use this technology. It's just a matter of time.

**Q: Do you think more forensic laboratories will start using MPS for casework now?**

**PKD:** I think this particular case might help to persuade labs that there are situations where this is absolutely worth considering. That perhaps MPS is much better than just exhausting your DNA extract with different CE attempts, and that you should reconsider that strategy. If you see that you have a complex CE profile, it's better to move to another technology to robustly get answers.

And I think the fact that there are now SWGDAM Guidelines for MPS<sup>3</sup> initiated in the United States will certainly help the labs in the United States to reconsider their initial reluctance... and in Europe I see labs gradually are willing to introduce this. I'm just leading the pack, and I hope that the pack will be big in a few years, but I don't have any idea how fast it will grow.

### References

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