

## Australian and New Zealand Forensic Science Society

### Allan Hodda Memorial Award 2017

#### Report Dr SallyAnn Harbison

#### Introduction

The purpose of my travel was to gain an understanding of the current state of implementation of new technologies, particularly massively parallel sequencing (MPS), in forensic biology and to learn more about single molecule sequencing from Oxford Nanopore Technologies, and its application to forensic science. Along the way and through a series of coincidences and fortuitous timing I was also able to attend a mini Symposium organised to celebrate the retirement of Professor Ate Kloosterman and a European Network Forensic Science Institutes (ENFSI) Rapid DNA workshop.

My final itinerary was:

- 15-16<sup>th</sup> May 2018. The Netherlands Forensic Institute: mRNA typing for body fluid identification, implementation of MPS in casework, advancements in robotics for casework sample processing.
- 17<sup>th</sup> May 2018. Frontiers of Forensic Science Symposium: Forensic DNA Science from Current to Next Generation, University of Amsterdam, The Netherlands.
- 21-22<sup>nd</sup> May 2018. ENFSI Expert Working Group DNA: The Automation and Lims Sub group Rapid DNA workshop, London. A workshop to assist in the development and circulate guidance on the use & development of Rapid DNA devices in an operational setting.
- 23<sup>rd</sup> May 2018. Forensic Science Department, Kings College London: the development and implementation of massively parallel sequencing on the Illumina platform.
- 24<sup>th</sup> and 25<sup>th</sup> May 2018. Attendance at the Oxford Nanopore Technology annual meeting in London, London Calling 2018.

#### Executive Summary

Massively parallel sequencing is accredited and implemented in a small but growing number of laboratories currently for specific case types and situations. As a community we need to act to make sure we are not left behind.

Integration of Rapid DNA testing into forensic practise is underway but there are many processes and procedures to be considered for any laboratory or police force prior to taking this step. Laboratories have existing in house rapid methods that may be better used for urgent work but these may not be sufficiently scalable in all jurisdictions.

The proper interpretation of evidence remains an area both of concern and of active research.

The next generation of sequencing technology is upon us with some promising advantages for forensic applications. Whilst there are still some limitations, this should not detract us from the range of opportunities available to us for rapid on site work in particular.

An active research community is crucial to maintain and improve technology in all aspects of forensic science.

I have included links and references where appropriate for further reading.

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## The Netherlands Forensic Institute

Based in The Hague, more than 600 forensic scientists provide a wide range of forensic science services from a modern facility including the famous illuminated rabbits, DNA profiles on the forecourt of the building, vast function hall and rooftop cafe. The NFI is the main provider of forensic services and is part of the Ministry of Science and Justice.

The focus of the far reaching research programme is to adopt technological developments that allow scientists to gain more information, from smaller and smaller sample sizes and in an increasingly rapid manner. The NFI is very active in digital forensics, e-crime research and big data analytics. Interpretation of evidence is a key area of research at the NFI and includes probabilistic methods for fingerprint comparisons, including partial and poor quality prints previously discarded. "Beyond the Source" is a research program focused on providing activity level information in Bayesian frameworks to aid in the investigation and reconstruction of crimes.

The main purpose of my visit to the NFI was to discuss progress in mRNA typing for body fluid identification, implementation of MPS in casework and advancements in robotics for casework sample processing. For this I visited the research team in the Biological Traces Division.

### Research Team, Biological Traces Division

I was hosted by Dr Titia Sijen, the Research Team Leader in the Biological Traces Division.

Massively parallel sequencing is accredited and has been implemented in the Netherlands for casework on the Illumina MiSeq sequencer. The NFI have been accredited and are sequencing mitochondrial DNA control region in a 2 step triaged process on mainly hair samples. Professor Peter de Knijff's laboratory at the University of Leiden undertakes STR sequencing using the Promega Powerseq™ Auto kit for the NFI when required, mainly for the discrimination achieved with extra loci and sequencing variation.

### Mitochondrial sequencing

Natalie Weiler, Jerry Hoogenboom, Kristiaan J. van der Gaag.

A SNAPshot assay of 18 single nucleotide polymorphisms (SNPs), appropriate to the local population in the Netherlands, is used to screen hairs prior to sequencing, reducing the sample numbers and reducing costs. The SNAPshot assay works well on compromised samples of poor quality because of the small amplicon size. Not all cases progress to sequencing if no hairs of interest are found.

[Weiler, N.E.C., de Vries, G., Sijen, T. *Development of a control region-based mtDNA SNaPshot selection tool, integrated into a mini amplicon sequencing method*. *Sci. Justice*. 2016;56:96–103] DOI: <https://doi.org/10.1016/j.scijus.2015.11.003>.

The sequencing process is based on a mini-mito method that amplifies 10 fragments in the control region only and uses FDStools (see below) for the bioinformatic steps and EMPOP for interpretation. This sequencing process is cheaper to do than sanger sequencing is. The NFI are also investigating but not yet using whole genome mtDNA sequencing. An epimotion robot is being validated for some steps including the AMPure clean-up steps and they have developed a real time quantitation method that targets 70 and 200 base pair fragments.

Index hopping is the term used to describe the unwanted movement of indexes (or barcodes) between DNA sequences in the sequencing library. You can read more about this here:

<https://www.illumina.com/content/dam/illumina-marketing/documents/products/whitepapers/index-hopping-white-paper-770-2017-004.pdf>. It is not clear at which stage in the process this occurs but is believed to be during the library enrichment step. One way to detect this is to use unique combinations of barcodes for all sample libraries pooled in a sequencing run and this is done by some in the research community using custom barcodes and adaptors. Index hopping is difficult to detect as it only occurs in a very small proportion of samples (perhaps less than 1/10,000 sequences) and is removed by bioinformatic pipelines used. However, the NFI did detect it using a mtDNA primer-extension based capture method at detectable levels and overcame it by changing capture methods and using 8mer barcodes instead which offers 384 barcode combinations.

### Bioinformatics

The laboratory uses FDStools for bioinformatic analysis using threshold based calling methods.

[Hoogenboom J, et al. *FDStools: A software package for analysis of massively parallel sequencing data with the ability to recognise and correct STR stutter and other PCR or sequencing noise*.

*Forensic Science International: Genetics* 27 (2017) 27–40]. The input is a fastQC file. The software models stutter by repeat motif, not necessarily by locus. For complex STRs, each repeat motif is

modelled separately within the sequence. The end point is a graph that can be used to predict the stutter value for any allele encountered. Minimum coverage is 5 reads or 0.5% of the highest read count observed per locus. A useful feature is that stutter is identified and added to the parent peak by sequence. This gives a combined read depth, the consequence of which is that heterozygote imbalance is minimised. Hybrid STR alleles have been observed and detected when a SNP is present in the flanking region, likely caused during the target amplification process. The proportion of these and how they are identified will be dependent on how early in the process the hybrid is formed and the specific library preparation methods employed.

#### RNA methods- Margreet van den Berge

The NFI are working on methods to associate DNA and RNA profiles. They are also exploring machine learning methods for interpretation of RNA results and improved extraction processes, particularly for differential extractions. mRNA is only used in a small proportion of cases

#### Operational laboratories

The DNA laboratories are very well equipped with lots of automation, processing approximately 200 case samples per day.

The Bio Storage storage system from Hamilton for sample extracts is something to behold.

<https://www.hamiltoncompany.com/products/automated-sample-storage/platforms/hamilton-bios>.

This system provides long term storage at -80C for all samples with a fully automated picker system. For short term storage a 4C option is used. Each system is housed in a large laboratory space of its own. Samples are retrieved and re-stored by computer command.

The casework lab has Hamilton Star robots for extraction including AutoLys on Hamilton Star Plus robots. Their robots have both heater and centrifuge capability on board. They are moving from column based to magnetic bead based extraction methods, both from Qiagen. To date differential lysis is carried out manually and NFI are working on finding a solution to this. PCR set up and quant set up are each done on 2 Tecan evo's and they have four 3500s.

I was intrigued and impressed by the person sample collection process which is a Copan indicating nucleic acid card and foam swab custom device which delivers each card into an automated system for punching including a punch cleaning area on the cards. They can process 360 samples per ½ day using Qiagen extraction chemistry and a Pico green quantitation.

<https://www.hamiltoncompany.com/products/automated-liquid-handling/standard-solutions/easypunch-starlet>

A manual back up process is in place for cards that are overloaded or from a different laboratory. For this they use a CPA200 <https://www.thermofisher.com/nz/en/home/industrial/forensics/human-identification/forensic-dna-analysis/sample-preparation-extraction/card-processing-automation-instruments.html> for punching and an EZ1 extraction robot from Qiagen. The swabs can be extracted if insufficient material has transferred to the card. Cards are stored at 4C, DNA is stored in a Hamilton Verso storage system.

Whilst at the NFI I was also introduced to Sander Kneppers who is the chair of the ENSFI DNA Working Group. We discussed how rapid DNA is used in the Netherlands. He explained that under Dutch Law, forensic work needs to be accredited, so because the RapidHit instrument the Dutch police have is not under an accreditation system it is not being used operationally. There was the possibility that in extreme circumstances, such as a DVI situation, that it could be used. The NFI

themselves have a rapid laboratory based protocol of less than 6 hours and that is more likely to be used currently.

## Forensic DNA Science from current to next generation Symposium

I was honoured to be invited to attend a Frontiers of Forensic Science Symposium called “Forensic DNA Science from current to next generation” held in honour of Ate Kloosterman’s retirement. Ate has been in Forensic Science for more than 30 years and has been an active researcher and caseworker all through that time. The assembled programme of speakers included eminent forensic scientists, such as Peter Gill and Manfred Kayser and an array for former students, now forensic scientists and researchers in their own right. Here are some highlights from the day.

Peter Gill spoke on the need for evaluative frameworks for evidence evaluation and the potential for miscarriages of justice if these were not used and/or not used properly. He stressed that DNA evidence is not used in isolation from other forms of evidence and that we must all be alert to contextual bias. He stressed the need for being alert to transposing the conditional when presenting evidence and answering questions in court and that assessment of evidence should be on based on data. In his talk he referred to the Jama Case from Victoria, the Amanda Knox case from Italy, both of which are well described in the literature, a case where a plastic tray was reused during a laboratory process leading to contamination and an erroneous link, and the Easton case amongst others. You can find an article about this and these cases here [http://www.thejusticegap.com/2017/01/misuse-dna-evidence-led-miscarriages-justice/#\\_ftn2](http://www.thejusticegap.com/2017/01/misuse-dna-evidence-led-miscarriages-justice/#_ftn2).

Manfred Kayser described research into methods that would provide forensic intelligence in the absence of a DNA bank link and in missing persons’ cases. He described the development of the HiRIS plex S system and the freely available prediction tool for forensic scientists to use all of which is detailed in the paper. The method and system being shown to be a robust, reliable method and tool for the prediction of hair, skin and eye colour from forensic samples. [*Chaitanya L et al The HiRISplex-S system for eye, hair and skin colour prediction from DNA: Introduction and forensic developmental validation. FSI: Genetics 35 (2018) 123–135.* <https://doi.org/10.1016/j.fsigen.2018.04.004>]

Manfred also indicated that ongoing research was in progress in hair structure, hair loss, height, face, eyebrow, ear morphology and handedness. An example was given of a study on male pattern baldness from the UK where over 250 SNPs were needed to provide a good prediction of male pattern baldness using a study of 52,000 male participants. [*Hagenaars et al 2017 Genetic prediction of male pattern baldness. PLoS Genetics. Published: February 14, 2017* <https://doi.org/10.1371/journal.pgen.1006594>]. Manfred went on to talk about the case of Richard III as an example of the use of this technology in a forensic/ historical context. [*King et al Identification of the remains of King Richard III. Nature Communications Volume 5, Article number: 5631 (2014)*].

Peter deKnijff gave a passionate presentation in support of MPS for forensic science. He argued for the point that as forensic samples are very small we should make the most of what we have and not carry out several sequential tests. He suggests shot gun metagenomics to identify every DNA in a sample (animal, plant, microbial) as a forensic tool. His laboratory was an early adopter of the technology and the method they have validated and implemented for casework is PowerSeq™ and FDStools. In a future prediction he believes that whole genome sequencing will be common place within 10 years and as a discipline we should be considering single cell metagenomics (DNA, RNA and protein genomes) as is now being used in clinical research. I have to say I agree.

Bas Kokshoorn discussed the work the NFI did on the Louise Bell case from South Australia as an example of the importance of answering the question how and when did the DNA get there and the use of Bayesian networks. He stressed the importance of standardizing the collection and analysis of the transfer and persistence data between laboratories, otherwise there may be compatibility issues between sets of data used to inform these questions.

The proceedings were followed by the valedictory speech of Ate which was conducted in Dutch and then a presentation to him of a King's medal, for outstanding service to forensic science.

## ENSFI Rapid DNA workshop

The purpose of the workshop was to explore issues related to the implementation of Rapid devices and was led by Shazia Khan from the Met Police. Some of the information was confidential and is not able to be included here.

Kirsty Faulkner, Forensic Information Database Services, described the governance and key legislation including the Data Protection Act of 2018. A key priority for the Forensic Science Strategy [ <https://www.gov.uk/government/publications/forensic-science-strategy>] is timeliness of delivery of services. The Governance of the NDNAD is given in this document [ [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\\_data/file/320005/9781474106412\\_WEB.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/320005/9781474106412_WEB.pdf)]. An annual report is published and the one for 2017 can be found here <https://www.gov.uk/government/publications/national-dna-database-annual-report-2016-to-2017>. Data protection is assured through accreditation to relevant codes, change management processes around new technology, performance monitoring and internal operational and system quality assurance processes. She went onto explain the requirements that NDNAD would have regarding rapid DNA including accreditation to ISO 17025.

Two vendors were present, ThermoFisher with the Rapid Hit instruments and Ande from ANDE.

Short presentations included:

- Anna Mapes (Dutch Police) who commented that the sensitivity of Rapid instruments does not yet match lab based methods and operationally it might be difficult to decide which samples to commit to Rapid testing and which to send to the laboratory.
- Douglas Hares, CODIS: talked briefly about the Rapid DNA Act 2017. A working group has been established to explore the issues for case samples. CODIS is being developed to accept the results, with a pilot expected in 2019. The accreditation agencies in the US are also exploring the quality issues.
- Chanda Lowther Harris- Met Police discussed a number of matters relating to quality including the physical location of instruments, requirements for operator elimination databases, options for remote audits as not all locations will be suitable for or accessible to auditors.
- June Guinness, UK regulator's office, summarised issues relating to the Forensic Regulator's office including accreditation to ISO17025, with Rapid DNA added to the scope as a technology, validation requirements as laid out by the Regulator and a re-processing capability.

Key considerations discussed included:

- fit for purpose quality assurance and quality management according to national requirements

- contamination risk management including elimination databases, environmental monitoring, separation of suspect, complainant and scene samples
- procedures to address sample failures, collection of secondary samples
- communication between operators, databases, scientists and investigators
- security of data and its transfer between device, laboratory, database and investigators
- country specific requirements for data protection, destruction and retention and data sharing
- user training and ongoing competence assessment, proficiency tests
- stability, traceability and shelf life of consumables, equipment maintenance
- retrievable of sample extracts for alternative testing
- equivalent validation and technical performance for intel and evidential use
- if used for casework, then expert knowledge of profile interpretation required.

## Kings College London

A number of laboratories have implemented or are in the process of implementing MPS and some have automated some or most steps of the ForenSeq DNA signature prep kit process including post PCR library preparation where the bead clean up step has been modified to avoid centrifugation.

For example:

- Kings College use MPS for kinship, mainly for immigration cases where more complex relationships benefit from additional markers. They will use mtDNA for DVI cases and will seek accreditation for mtDNA sequencing via MPS first.
- the French National Forensic Police Institute in Lyon have fully automated the workflow and are using the technology for cold cases, reference samples and for increasing numbers of routine cases. For DNA mixtures they are developing software based on the text mining programme Piranha.
- the Department of Forensic Medicine, University of Copenhagen, have implemented the Precision ID identity panel (ThermoFisher Scientific ) for kinship testing.

## London Calling 2018: Oxford Nanopore Technology, 24-25th May 2018

The main objective of my attendance at this conference was to learn more about recent progress made in long read sequencing using the MinION and the associated GridION and PromethION sequencers and to better understand the range of applications of this technology for forensic science in particular. The attendance at London Calling 2018 (approximately 600) demonstrates that the MinION is maturing as a technology with a wide range of talks covering many applications. There are a reported 7000 MinION sequencers, 140 GridIONs and 40 Promethions currently placed in laboratories. I noted a change of emphasis at this meeting from more technical and bioinformatics heavy presentations demonstrating the use of the sequencers to more application based talks describing how MinIONs in particular are actually being used in research and testing. Many of these were focused on rapid diagnosis of pathogens in hospitals and in the field. Many of the presentations demonstrated how the MinION was able to provide information not possible using short read sequencing, for example mapping structural variants.

It is still true that sequencing errors are more prevalent than with other technologies, but many in the community are working on overcoming these both technically and bioinformatically. For areas of forensic work where long sequence reads would be helpful (eg metagenomics) or multiple analysis types are desirable for example epigenetic modifications and STR typing simultaneously, this is the way of the future.

### Introduction to Oxford Nanopore Technology:

ONT sequencing is single molecule sequencing and is capable of sequencing extremely long lengths of DNA, hundreds of kilobases in length under the right conditions. The essence of the technology is the device called the MinION, a chocolate bar sized device, in which a “disposable” flow cell is placed. Each flowcell contains a synthetic membrane in which nanopores are placed.

Each nanopore is an engineered protein that sits in a membrane that is a synthetic polymer. In its resting state, ions flow through each pore when a current is applied, analogous to the way molecules pass through the pores in a cell membrane. When an analyte is introduced (such as a DNA sequence) and passes through or near to the pore, this changes the flow of ions through the pore. The flow of ions through each pore is measured separately. Because the flow of ions is determined by the DNA sequence that is passing through each pore, any modification to the sequence such as a base or a methylated base causes a small change in the flow of ions. An enzyme (motor protein) added to the DNA controls the unzipping of ds DNA and controls the speed of the transfer through the pore. The motor protein attaches to the DNA first and then both to the Nanopore. The base calling algorithm is currently a recurrent neural network. Flow cells have approx. 2000 pores per cell but only 512 channels. This allows for redundancy in case of pore failure.

Flow cells can be run for minutes or days (up to 3 days at most), sequencing is in real time, so the operator can stop the run if sufficient sequence data has been obtained. Barcodes are available for indexing. ONT say flow cells last for about 70 hours and you can multiplex and multiwash flow cells.

You can watch an introductory video on the ONT website.

<https://nanoporetech.com/applications/dna-nanopore-sequencing>

Nanopore sequencing is portable as long as you have a computer, power and a decent internet connection. The device is controlled by software called MinKNOW. Software is also available (Metrichor) from ONT that enables the analysis of the MinION data specifically. Other researchers are developing software all the time that is freely available.

The MinION has been tested all over the place and for all sorts of applications for example goats, plants, planes, in space, on boats and in trains, down a mine and at both poles. I have divided this report into sections that reflect the use of the technology rather than on individual papers, although some application examples are given.

### Limitations of the current technology:

Let's start with the limitations. For forensic science the main one is currently sample size. Sequencing error can be overcome with read depth. The main design objective for the MinION was rapid on site testing. This means avoiding a pre PCR step other than a step to attach the motor protein. Consequently, there is a trade off in sample size versus speed. Microgram quantities are needed to generate good quality data of sufficient coverage, otherwise an amplification process is needed which slows the overall process down.



Sequencing error rate is still an issue although with every chemistry update and software improvement this is getting better. The company is actively working to solve homopolymer (repeated runs of the same base) issues, that most sequencers share.

Currently “in the field” realistically means with some sort of lab space to do library prep, an internet connection for data analysis, and in hot climates some sort of cooling as MinIONs have been known to overheat in Africa for example. It is possible to achieve though and there are some good examples, for example the cassava project referenced below.

#### Technical information: DNA/RNA Extraction

Success is very dependent on the quality of the DNA extracted and the presence of any inhibitors. Kit based extraction methods such as DNeasy give shorter read lengths than older methods such as phenol/chloroform due to more shearing of the DNA during the process, consequently many researchers have returned to the older methods. Several presenters lysed cells in a simple buffer and then used Qiagen genomic tips (100 protocol) to recover DNA from the sample. It was noted that some low bind tubes can have contaminants in them that kill the flow cells within seconds of loading. The Eppendorf brand are the only ones that work reliably and consistently. A big aim of the company is ambient shipping of reagents and flow cells.

#### Technical Information: Sequencing library preparation

Library preparation includes the ligation of the motor protein and any barcodes to the DNA/RNA prior to sequencing. The ONT kit selector tool helps the user determine what library prep method is best for each/any application. In development is an end to end work flow predictor which will include sequencing purpose, user priorities (speed, length etc), library preparation options, sample and extraction type and bioinformatics choices.

The Voltrax is an automated library preparation system which is soon to be released for general sales. A starter pack will contain 1 device, 1 configuration kit, 12 cartridges and 12 sequencing kits all for \$8000.

#### Technical Information: Sequencers

The most famous sequencer, the MinION belongs to a family of sequencers including the Flongle, a “disposable” MinION flow cell which has lower through-put and is considerably cheaper than the normal MinION flow cell, it producing between 1Gb and 3 Gb of sequence. Other options include the larger capacity GridION and PromethION which have not yet been investigated for forensic purposes.

#### Technical Information: Software

Software solutions for the ONT technology are many and varied and frequently updated. Here are some issues identified and some examples of some newer developments.

- minKnow 2 is released
- “read until” software is being re-introduced.
- MinIT is a device, essentially a mini laptop with almost no configuration that can be used to run a minION and that can use a phone or other blue tooth device for connectivity. Some analysis software is available on the MinIT, the purpose is to allow in field analysis.
- It was acknowledged that most users assemble the sequence and then polish it and that this shouldn't be needed. Modified and damaged bases need some work and difficulties with homopolymers are being worked on. This being investigated in two ways: 1) by

lengthening the read length passing through the pore and 2) by looking at 2 very short lengths with a space in between. The idea is that you would use both and then combine.

- Modifications: because the MinION detects changes in the charge of bases, with the appropriate software any modification can be detected. For example, TOMBO software can detect methylated bases m<sup>5</sup>C in eukaryotes and m<sup>6</sup>A in prokaryotes in RNA and DNA (with an AUC of 0.99/0.98).
- Minimap 2 is the fastest aligner, but not necessarily the best. An alternative called LAST has been used which is better at capturing breakpoints in the data and is good for phasing reads based on SNP patterns such as might be found in mitochondrial DNA.

### Examples of applications described of relevance to forensic science

Oxford Nanopore Technology is now being used for a wide range of applications in number of varied disciplines and some of these have forensic applications. For example, the direct detection of DNA methylation, direct sequencing of RNA, metagenomics and sequencing of whole microbial genomes and mtDNA. Here are some highlights from the conference.

Angela Brookes, Nanopore RNA consortium. Traditional short read sequencing requires a cDNA synthesis step which introduces PCR artefacts and loss of some modifications. Using the MinION the consortium of 6 labs has sequenced whole human polyadenylated transcriptomes with the objective of developing better technical methods. About 45% of sequences were full length transcripts, the remainder being partial transcripts some of which appear to be technical artefacts. The longest full transcript found was for the SORL1 gene at 10,313 nucleotides, including 48 exons. Further work is underway on bioinformatic developments.

Jennifer Idol, The Jackson Laboratory, University of Iowa: High Coverage ultra-long sequencing of the human genome. Three samples from the 1000 genomes project were sequenced with 30x coverage on the GridION sequencer. Methods used were phenol chloroform extraction, needle shearing, damage repair, end repair and dA tailing followed by the 1d kit. For 30 x coverage they needed 460ug – 600 ugDNA and it took 2 weeks to get to 30x coverage with a maximum read length of about 200kb and an average of 30-45kb.

The Cassava Virus Action Project led by Titus Alicai and Laura Boykin, University of Western Australia. <https://cassavavirusactionproject.com/> and associated paper <https://www.biorxiv.org/content/early/2018/05/31/314526>. This research team really are sequencing in the field and for social good, you can read all about their research on the website. In summary, conventional virology can take between 3-12 months to identify a virus from a crop sample. This is too long to intervene in real life. The aims of this project are to carry out sequencing one farmer at a time and provide results within 48 hours, so that the farmer can know whether the crop can be safely harvested and sold or has to be destroyed. The team also provide plants resistant to any virus found locally. Farmers are involved in the testing which is done with them and for them. Technically the team were able to detect viral sequences from samples where qPCR failed and from plants with no visible infection and from mixed viral strains. This is a good example of the potential for field testing in difficult locations.

Arne de Roeck, University of Antwerp spoke about sequencing tandem repeats (24 bp mini-satellites) associated with dementia in patients. The ability of the ONT technology to sequence longer lengths allows the repeat regions to be sequenced in one read, which is of interest in forensic applications. Difficulties were found with the accuracy of the current base callers although repeat number could be observed and estimated from the squiggle plot. This was also mentioned by Franz-Josef Mueller who described his work on sequencing STR expansions such as those found in fragile X

and Huntingtons disease which are difficult to amplify and sequence. He has developed an analysis tool called nanoSTRique which identifies, quantitates and evaluates STRs using a hidden markov model based on the work of Liu et al 2017 (<https://doi.org/10.1186/s13073-017-0456-7>).

Timothy Gilpatrick, John Hopkins University, spoke about detecting 5<sup>m</sup>C methylation directly, but more interestingly described using a cas9 enrichment strategy for enriching methylated sites prior to sequencing. ONT are developing his methods further. The assay is based on the ability of the ONT motor protein to evict the Cas protein as it goes through the pore. Using this method, long range methylation patterns were able to be detected and SNPs were used to phase reads. The ability to capture specific methylated sites makes sense for forensic applications where known CpG sites are associated with age and body fluid identification and identification of these without PCR amplification or bisulphite conversion would be useful.

A poster by Thomas Krahn and colleagues from the YSEQ group in Berlin described the development of methods to sequence the mitochondrial genome and selected parts of the Y chromosome allowing haplo-group assignment from 96 barcoded samples at a time on a single flow cell. This was used to study population genetics in a native population on the Kamchatka peninsula.

Of interest: The Chief Medical Officer of Health in the UK has said that sequencing should be the standard practice in the NHS. They have investigated an MRSA outbreak as a proof of principle for the MinION. In 5 days they were able to sequence samples from 9 patients including a 24-hour broth enrichment step, DNA extraction, sequencing and data analysis.

## Acknowledgements

I would like to conclude by thanking those who made my travel possible and those who gave freely of their time to meet with me and share their work. In particular I would like to thank:

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