Institution: University of Leicester		
Unit of Assessment: 5		
Title of case study: Expanding the toolbox of forensic genetics		
Period when the underpinning research was undertaken: 2000–2019		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Periods employed by submitting HEI:**
(1) Prof Mark Jobling	(1) Professor of Genetics	(1) 1992–Present
(2) Dr Jon Wetton	(2) Senior Post-Doctoral Fellow	(2) 2012–Present
(3) Dr Celia May	(3) Lecturer in Genetics	(3) 1993–Present
Period when the claimed impact occurred: 2014–2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

University of Leicester research into novel tools for forensic DNA analysis has developed and characterized male-specific Y-chromosome DNA markers for the investigation of crime. This has contributed to national and international guidelines for their use, facilitating their application in sexual assault cases where male and female DNAs are mixed, thus enabling prosecutions and exonerations globally, and benefiting companies that manufacture testing kits. Additionally, University of Leicester researchers have resurrected and applied 'analogue' DNA fingerprinting technology, supporting the conviction of a violent rapist in a forensic 'cold case' 30 years after the offence.

2. Underpinning research

Since the revolutionary 1984 invention of genetic fingerprinting by Alec Jeffreys at the University of Leicester (UoL), DNA analysis has been central to forensic science and criminal justice worldwide. Standard DNA profiling targets variable short-tandem repeats (STRs) on the autosomes (chromosomes shared by males and females). Because these STRs have many different versions (alleles) and are inherited independently, as few as ten give a uniquely identifying DNA profile. However, standard profiles cannot resolve all criminal cases.

For over 25 years, Jobling has studied the genetic diversity of the human **Y chromosome [G1–G3]**. This is male specific, so targeted profiling of Y-DNA provides valuable information on the male component in a mixed DNA sample, often encountered in sexual assault cases. Jobling's research **[R1]** described the fundamental properties of Y-DNA profiles that support their forensic application: (i) low diversity compared to autosomal profiles because Y-STRs are not independently inherited; (ii) sharing among male-line relatives, so not individually identifying, but powerful for exclusions; and (iii) high geographical differentiation. Many highly variable Y-STRs are therefore needed to maximize discriminatory power and extensive population surveys are required to interpret the significance of matching profiles, and to predict where in the world a man is likely to originate from (**biogeographic ancestry**). Profiling based on nine Y-STRs began in the 1990s, and in 2001 formed the basis for the first open online Y Haplotype Reference Database (yhrd.org; led by Lutz Roewer [Charité, Berlin]), in which Jobling's team was a partner **[R2]**. YHRD is now at release 63 and contains 321,472 reference profiles from >1000 worldwide populations, including profiles from 21 diverse populations from Leicester's research projects **[**e.g. **R3]**.

The Y chromosome sequence was published in 2003, allowing systematic searches for novel Y-

STRs. UoL was a key partner **[R4]** in a collaboration (with Manfred Kayser [Max Planck Institute for Evolutionary Anthropology], and Chris Tyler-Smith [Wellcome Sanger Institute]) that discovered 166 new STRs (a 313% increase on what was previously known). This innovation catalyzed development of new highly discriminating commercial Y-STR kits by several manufacturers, allowing simultaneous typing of up to 37 STRs. One kit, PowerPlex Y23 (PPY23, Promega Corp.) was used in a multi-centre research collaboration, including Leicester, to analyse 19,630 Y-chromosomes from 129 different populations **[R5]**. Jobling's team worked with Prof Lisa Smith (UoL Criminology) to demonstrate that a novel intimate self-examination swab could collect DNA evidence for Y-STR profiling following sexual assault, opening the technology to low-resource environments **[R6, G4]**.

Massively parallel sequencing (MPS) has revolutionized DNA analysis by increasing throughput and reducing cost. In forensics, it offers higher resolution via STR sequences, rather than lengths, and increases the number analysable in a single test. Jobling and Wetton's team analysed Y-STRs via MPS in a global sample **[G5; R7]** using Promega's pre-released PowerSeq Auto/Mito/Y kit.

Forensic 'cold cases' often remain unsolved until new technology arrives. When archived DNA fingerprints are key evidence in a case, it is necessary to re-establish old technologies to provide a critical link to support current prosecutions. Supported by funding from the East Midlands Special Operations Unit **[G6]**, May and Wetton carried out research to re-establish and validate Alec Jeffreys' original DNA fingerprinting methods, allowing them to be applied to cold cases.

3. References to the research

R1. Jobling, M.A. (2001). Y-chromosomal SNP haplotype diversity in forensic analysis. *Forensic Sci. Int.* **118**, 162-172.
R2. Roewer L, 42 others inc. Bosch E, Jobling MA, Kayser M (2001). Online reference database of European Y-chromosomal STR haplotypes. *Forensic Sci Int* **118**, 103-111.
R3. Khubrani YM, Wetton JH, Jobling MA (2017). Extensive geographical and social structure in the paternal lineages of Saudi Arabia revealed by analysis of 27 Y-STRs. *Forensic Sci Int Genet* **33**, 98–105.
R4. Kayser M, Kittler R, Erler A, Hedman M, Lee AC, Mohyuddin A, Mehdi SQ, Rosser Z, Stoneking M, Jobling MA, Sajantila A, Tyler-Smith C (2004). A comprehensive survey of human Y-chromosomal microsatellites. *Am J Hum Genet* **74**, 1183-1197.
R5. Purps J, 161 others, inc. Wetton JH, Gwynne GM, Jobling MA, Roewer L (2014). A global analysis of Y-chromosomal haplotype diversity for 23 STR loci. *Forensic Sci Int Genet* **12**, 12-23.
R6. Smith L, Wetton JH, Lall GKM, Flowe HD, Jobling MA (2017). Testing the efficacy of self-examination intimate DNA swabs to enhance victim-centred responses to sexual violence in low-resource environments. *Sci Justice*; **57,** 331-335.
R7. Huszar TI, Jobling MA, Wetton JH (2018). A phylogenetic framework facilitates Y-STR variant discovery and classification via massively parallel sequencing. *Forensic Sci Int Genet* **35**, 97-106.

Research Grants

G1. Jobling: *The Y chromosome as a marker for the history and structure of human populations,* GBP819,610; 1999–2004**; Wellcome Trust Senior Research Fellowship**.
G2. Jobling: *Pattern and process in human genetic diversity: from genomes to populations*, GBP1,173,604; 2004–2009: **WT Senior Research Fellowship**.

G3. Jobling: *Sex, genomes, history: molecular, evolutionary and cultural effects on human genetic diversity,* GBP1,700,211; 2009–2015; **WT Senior Research Fellowship**.
G4. Lisa Smith (Criminology), Jobling & Wetton: *Self-examination intimate DNA swabs to enhance victim-centred responses to sexual violence in humanitarian contexts,* GBP49,784; 2018: **Humanitarian Innovation Fund**.
G5. Jobling: *Next-generation sequencing approaches to short-tandem repeat sequence variation*, GBP95,042; 2015–2019: **BBSRC-iCASE studentship, with Key Forensic Services**.
G6. May & Wetton: *Reactivating DNA Fingerprinting as a Casework Tool* GBP13,000; 2017–2018; **East Midlands Special Operations Unit - Forensic Service**.

4. Details of the impact

University of Leicester (UoL) research has underpinned developments benefiting victims of violent crime and their families, wrongfully convicted individuals, police, forensic practitioners and forensic service providers, legal teams, government and policy makers, and companies manufacturing and selling DNA testing kits.

Increasing the power of Y-STR typing: The UoL team's research contributed to the 2001 origin and subsequent growth of **YHRD [R2, R5]**, the only global and open database of Y-DNA reference profiles. Originally the USA maintained its own database (www.usystrdatabase.org), but in 2018 this was decommissioned, and its 35,295 profiles were transferred into YHRD, recognising its primacy and the need for a single global standard. YHRD now allows forensic scientists, police, prosecutors, and defence teams anywhere in the world to search >320,000 profiles and determine the significance of a crime-scene match: without it, Y-STR profiling simply could not be applied in forensic casework. For example, YHRD underpins the use of Y-STR profiling in the UK as set out in the Forensic Science Regulator's Guidelines **[E1]**. Because it is classified into populations, the database also provides information about the likely **biogeographic ancestry** of a profile. YHRD users typically made around 4,000 visits/month up to 2018, and, following the transfer of US profiles into YHRD, 8,600 visits/month.

Guidelines and evidence interpretation: UoL contributed to internationally agreed principles for the application of Y-STR profiling, vital given the differences among criminal justice systems worldwide. The *International Society for Forensic Genetics* (including Jobling) published its first recommendations for the use and interpretation of Y-STR profiling in 2001 **[E2]**, and these form the basis of current recommendations that reflect new technologies. In the UK, Jobling and Wetton were invited to join the Y-STR Working Group of the Government's Forensic Science Regulator, and the Association of Forensic Science Providers DNA Working Group, to develop the UK's guidelines **[E3]**. In interpreting evidence, naming STRs and (since the implementation of MPS) STR sequences in a consistent way is essential. Work carried out in Leicester **[R7]** proposed allele nomenclatures for Y-STRs, leading to an invitation to a 2019 STRAND Working Group meeting, contributing to key decisions on nomenclature **[E3]** and feeding into forensic and manufacturer practice.

New Y-STRs developed in UoL research **[R4]** drove a step-change in the discriminatory power of commercial profiling kits. As well as publications, Jobling gave invited keynote talks on these new developments at international conferences that included company representatives (e.g. International Society for Human Identification meeting, National Harbor, MD, USA, 2011; 27th Congress of the International Society for Forensic Genetics, Seoul, 2017). The current generation of kits produced by the US-based global companies Promega and Thermo Fisher

Scientific are respectively PPY23 (**[E4]**; 23 STRs, including seven from **[R4]**, developed 2012) and YFiler Plus (**[E5]**; 27 STRs, including nine from **[R4]**, 2014). Three Chinese companies, PeopleSpot, AGCU ScienTech, and Microread Genetics also developed kits using STRs from **[R4]**, which are used mostly in China. The UK's forensic service providers (LGC, Cellmark, Key Forensic Services, Scottish Police Authority and Forensic Science Ireland) chose PPY23 as their Y-profiling tool in 2014 **[E1]**.

Supporting convictions & exonerations is the goal of Y-STR profiling and is enabled by UoL's contribution to the development and application of this technology. In the UK ONS Crime Survey (2018) ~700,000 people reported sexual assault (10,127 prosecutions in 2018-19). Since 99% of cases involve male assailants, Y-STR testing is invaluable: without this technology, 10% of cases could not be prosecuted, and detecting multiple male assailants is three times more likely when using Y- compared to autosomal STR profiling **[E6]**. In the UK, Y profiling is increasing (80,550 PPY23 tests since 2014). Biogeographic ancestry prediction from Y-STR profiles is valuable: for example, in Operation Pettyridge (2015), a Leeds rapist thought to be Middle Eastern from witness testimony was identified via the demonstration that his Y-STR profile was SE European: this led to the identification and extradition of a Slovakian man now serving a 20-year sentence **[E7]**. Y-STR profiling is used in the exonerations of prisoners via testing of archived case materials. Although Y-STR profiles cannot individually identify, a single Y-STR mismatch is sufficient for an exoneration. In the US-based Innocence Project, Y-STR profiling was the key evidence in 16% of 194 exonerations **[E8]**. More discriminating profiling kits have helped: for example, a man convicted of rape in Taiwan based on a 17 Y-STR test was exonerated in 2014 when the PPY23 kit was applied **[E9]**.

Sale of kits for forensic DNA analysis has been enabled by the research. Generating reference data for YHRD brought sales to Thermo Fisher and Promega of GBP10,800,000 and GBP9,800,000 respectively. In the UK alone, the 80,550 PPY23 (Promega) tests done by forensic service providers in sexual assault and other criminal casework to date represent GBP1,980,000 sales, with an upward trend **[E10]**.

Solving cold cases: 2018 work by Wetton and May brought UoL's involvement with forensic DNA analysis full circle by recreating and validating legacy techniques in DNA fingerprinting. This was key evidence in securing the conviction of a suspect linked to violent rape and burglary committed in 1988, for which a contemporaneous DNA fingerprint was the key evidence **[E11]**. Other cold cases can now be investigated in the same way.

5. Sources to corroborate the impact

E1. Forensic Science Regulator Guidance on Y-STR Profiling (FSR-G-227) www.gov.uk/government/organisations/forensic-science-regulator (February 2021).
E2. Gill P, 16 others inc. Jobling MA (2001). DNA Commission of the International Society of Forensic Genetics: recommendations on forensic analysis using Y-chromosome STRs. *Forensic Sci Int* 124, 5-10. Also published in *Int J Legal Med* 114, 305-309.
E3. Gettings KB, Ballard D, Bodner M, Borsuk LA, King JL, Parson W, Phillips C (2019) Report from the STRAND Working Group on the 2019 STR sequence nomenclature meeting. *Forensic Sci Int Genet* **43,** 102165.
E4. PPY23 Y-STR kit: www.promega.co.uk/products/genetic-identity/genetic-identity-workflow/str-amplification/powerplex-y23-system/?catNum=DC2305
E5. YFiler Plus Y-STR kit: www.thermofisher.com/order/catalog/product/4484678

E6. Purps J, Geppert M, Nagy M, Roewer L (2015). Validation of a combined autosomal/Y-chromosomal STR approach for analyzing typical biological stains in sexual-assault cases. *Forensic Sci Int Genet* 19, 238-242.
E7. Evidence on request from Cellmark Forensic Services, Abingdon, UK.
E8. Hampikian G, West A, Akselrod O (2011). The genetics of innocence: analysis of 194 U.S. DNA exonerations. *Annu Rev Genomics Hum Genet* **12**: 97–120.
E9. Hampikian G, Peri G, Lo SS, Chin MH, Liu KL (2017). Case report: coincidental inclusion in a 17-locus Y-STR mixture, wrongful conviction and exoneration. *Forensic Sci Int Genet* **31**, 1-4.
E10. Testimonial, Promega UK.
E11. https://www.bbc.co.uk/news/uk-england-nottinghamshire-46361749