Institution: University of Cambridge		
Unit of Assessment: 8		
Title of case study: Cambridge Epigenetix		
Period when the underpinning research was undertaken: 2009–2013		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s): Professor Sir Shankar Balasubramanian	**Role(s) (e.g. job title):** Herchel Smith Professor of Medicinal Chemistry	**Period(s) employed by submitting HEI:** 1993–present
Period when the claimed impact occurred: 01/08/2013–31/07/2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Research carried out in the Department of Chemistry led to the first quantitative, single-base resolution sequencing of the two most important epigenetic modifications of DNA, 5-methylcytosine and 5-hydroxymethylcytosine. The discovery of oxidative bisulfite sequencing (OxBS-seq) led to the spin-out company, Cambridge Epigenetix, which has made the technology commercially available worldwide. Cambridge Epigenetix as of early 2020 had raised GBP40,000,000 (USD56,500,000) in pre-revenue funding and currently employs around 35 staff. The OxBS-seq technology is transforming our understanding of the role of the epigenome in disease and has led to a new non-invasive diagnostic kit for widespread cancer screening.

2. Underpinning research

Epigenetics. In the early 21st century, there was growing realisation of the role of DNA methylation in physiological and pathological conditions, and the mechanisms by which DNA methylation profiles were inherited through generations. However, the study of these epigenetic modifications in health and disease was hindered by the lack of any high-throughput or quantitatively accurate approaches for characterizing them. It had been shown that the epigenetic mark 5-methylcytosine (5mC) plays an important role in genome stability and gene silencing. Furthermore, it was known that 5mC could be oxidized to 5-hydroxymethylcytosine (5hmC) by the ten eleven translocation enzymes, raising the possibility that 5hmC could be an important intermediate in DNA demethylation or an epigenetic mark itself. Although 5hmC in mammals had been detected, the state-of-the-art sequencing technologies of the time were limited in that they could neither distinguish between 5hmC and 5mC, nor give quantitative information.

Oxidative bisulfite sequencing. Professor Balasubramanian had already developed the world's most powerful next generation sequencing technology, Solexa Sequencing, which has revolutionised the field of genomics, and he now turned his attention to epigenetics. He teamed up with a world leader in the field, Professor Wolf Reik, who was working at the Babraham Institute in Cambridge and had discovered key epigenetic mechanisms important for mammalian development, physiology, genome reprogramming, and human diseases. Together they set about developing sequencing techniques that could detect the two most common epigenetic modifications, 5mC and 5hmC, at single-base resolution. The exploratory research carried out in the laboratory of Professor Balasubramanian in the Department of Chemistry at the University of Cambridge led to the development of the technique called oxidative bisulfite sequencing (OxBS-seq), which allowed for the first time the detection of both 5mC and 5hmC to single-base resolution. The initial results were obtained with synthetic DNA, then in collaboration with Reik, with genomic DNA from mouse embryonic stem cells.[R1] This 2012 study demonstrated a high level of 5hmC within specific areas of the genome associated with transcriptional regulators, suggesting these regions may play a role in epigenetic reprogramming in embryonic stem cells.

Exploitation. Balasubramanian went on to optimize the sequencing methodology, improving the purification steps and the formulation of oxidant, giving greater stability and reproducibility.[R2] In 2014, he developed a new method to quantitatively sequence a third epigenetic modification, 5-formylcytosine, in both synthetic DNA and that of mouse embryonic stem cells at single-base

resolution.[R3] These new sequencing methods allowed the epigenetic code of DNA to be mapped at high resolution for the first time, at a crucial period when the significance of epigenetics in health and disease was becoming apparent. The next step was to commercialise the technology and make it available for the wider genomics community in academia and the healthcare industries. The implication being that better understanding of the epigenome enables its role in disease mechanisms to be elucidated, and that specific markers could be exploited in novel diagnostic, prognostic and therapeutic tools. Balasubramanian protected the OxBS-seq technology with a series of patents and founded a company, Cambridge Epigenetix, to exploit his discoveries.[R4,R5]

3. References to the research

R1. Booth, M. J.; Branco, M. R.; Ficz, G.; Oxley, D.; Krueger, F.; Reik, W.; Balasubramanian, S. Quantitative Sequencing of 5-Methylcytosine and 5-Hydroxymethylcytosine at Single-Base Resolution. *Science* **2012**, *336*, 934-937.

R2. Booth, M. J.; Ost, T. W.; Beraldi, D.; Bell, N. M.; Branco, M. R.; Reik, W.; Balasubramanian, S. Oxidative Bisulfite Sequencing of 5-Methylcytosine and 5-Hydroxymethylcytosine. *Nat Protoc.* **2013**, *8*, 1841-1851.

R3. Booth, M. J.; Marsico, G.; Bachman, M.; Beraldi, D.; Balasubramanian, S. Quantitative Sequencing of 5-Formylcytosine in DNA at Single-Base Resolution. *Nat. Chem.* **2014**, *6*, 435-440.

R4. Booth, M. J.; Balasubramanian, S. Methods for Detection of Nucleotide Modification. WO/2013/017853, 2013.

R5. Booth, M. J.; Balasubramanian, S. Methods for Detection of Cytosine Modification. GB2507231, 2014.

Research outputs are published in peer-reviewed journals.

4. Details of the impact

Cambridge Epigenetix. In 2012, Balasubramanian co-founded Cambridge Epigenetix to commercially develop OxBS-seq. In 2013, the company launched their first product, TrueMethyl™ OxBS kit, which provides reproducible and accurate analysis of 5hmC and 5mC.[E1] Since DNA from cancer cells has a distinct epigenetic signature, this technology has formed the basis of epigenetic tests in cancer diagnosis. With their first kit on the market, Cambridge Epigenetix underwent three successful rounds of venture capital funding, raising USD5,500,000 in 2014, USD21,000,000 in 2016, and USD30,000,000 in 2018.[E2–E4] This investment has allowed the company to further develop a range of tools for epigenetic analysis. The company is currently located at Chesterford Research Park and employs around 35 staff.[E5] Balasubramanian is chair of the Scientific Advisory Board and a member of the commercial board.[E1]

Products on the market. In 2016, Cambridge Epigenetix launched their New TrueMethyl™ Whole Genome kit.[E6] This system includes all-in-one reagents for sample conversion, library creation and indexing, combined with bioinformatic tools to enable accurate DNA modification analysis, reducing costs and improving accessibility of bisulfite sequencing for researchers. In 2017, Cambridge Epigenetix and NuGEN Technologies entered into a partnership agreement to integrate the oxBS-Seq technology with next generation sequencing library preparation kits.[E7] The signing of the agreement enabled Cambridge Epigenetix to focus on its epigenetic biomarker discovery programmes, both in-house and in partnership with leading biopharma companies.

OxBS-Seq is being used by researchers in academia and industry to study the importance of DNA (hydroxy)methylation in biological mechanisms and in disease for various fields that include cancer biology, development, stem cell research, and neuroscience. The technology being developed at Cambridge Epigenetix is advancing the possibilities for medicine well beyond the realms of the genome, bringing a new generation of diagnostic and therapeutic innovations that offer hope for many patients living with life-threatening diseases. Abnormal patterns of DNA methylation have been identified in a number of aggressive cancers, as well as inflammatory, metabolic and neurodegenerative disorders.

Cancer diagnostics. In 2019, Cambridge Epigenetix launched a discovery and development programme for an epigenetic test to detect colorectal and other cancers from a simple liquid biopsy to analyse circulating free DNA in the blood stream.[E8] These new diagnostics kits provide a significantly less invasive procedure for cancer screening that does not require tissue samples. The company was granted broad and exclusive patent rights for the use of 5hmC as a diagnostic biomarker for cancer.[E8] The first studies analysed over 2,000 patient samples, including healthy volunteers, and individuals with adenomas and all stages of colorectal cancer. Colorectal cancer is expected to increase by 60% to more than 2.2 million new cases and 1.1 million deaths by 2030, and its diagnosis remains a challenge.[E8] As a Cambridge Epigenetix Clinical Advisory board member states 'the availability of a non-invasive, easy-to-administer and affordable screening test as our first initiative could transform the diagnosis, detection and treatment of pre-cancerous lesions and CRC.[E8]

Cambridge Epigenetix CEO claims "*Our ability to detect 5hmC in circulating, cell-free DNA, and the discovery and development of a liquid biopsy signature for tumours, could revolutionise cancer care and decrease cancer mortality through widespread screening, early detection and timely intervention. I am excited to lead the talented team at Cambridge Epigenetix for the development and launch of the first of many tests to detect cancer*".[E8]

5. Sources to corroborate the impact

E1. Cambridge Epigenetix - Company website 29.07.2019

E2. BusinessWeekly - Series A Funding $5.5M 29.10.2014

"New CEO as Cambridge Epigenetix raises $5.5m"

E3. BusinessWeekly - Google funding $21m 14.03.2016

"Google leads $21m Cambridge Epigenetix funding round"

E4. Ahren Innovation Capital - Series C Funding Round $30M 02.04.2018

"Ahren Leads $30mn Funding Round in Cambridge Epigenetix"

E5. Letter from Executive Chair of Cambridge Epigenetix 27.01.2021

E6. TrueMethyl® oxBS-Seq Module - NuGEN Technologies exclusive partner of Cambridge Epigenetix 29.07.2019

E7. Businesswire - NuGEN Technologies Partnership Agreement 15.08.2017

"Cambridge Epigenetix and NuGEN Technologies Sign Partnership Agreement to Combine Leading Technologies in Epigenetics Research"

E8. MarketWatch - Routine Screening and Detection of Cancer 08.01.2019

"Cambridge Epigenetix announces programme for routine screening and detection of colorectal cancer and other common tumours"