Institution: University of Cambridge		
Unit of Assessment: 8		
Title of case study: Next Generation Sequencing		
Period when the underpinning research was undertaken: 01/01/2000-30/09/2000		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Professor Sir Shankar Balasubramanian	Herchel Smith Professor of Medicinal Chemistry	1993-present
Professor Sir David Klenerman	Royal Society Research Professor	1994-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? Y		

1. Summary of the impact

Research in the Department of Chemistry into the action of DNA polymerase on immobilised DNA substrates using single molecule detection led to the spin-out company Solexa. Solexa Sequencing is currently used to sequence one million genomes per year. Since their acquisition of Solexa, Illumina has dominated the next generation sequencing market. Illumina has developed technologies and platforms that radically reduce the run time and cost of Solexa Sequencing. Use of the technology developed in the Balasubramanian and Klenerman laboratories has enabled a profound increase in the rate of sequencing and has had a major impact on human health and our understanding of the genetic basis of disease. Illumina currently has over 7000 employees worldwide and a revenue exceeding USD3,000,000,000 per annum, a business which is almost entirely based on Solexa Sequencing.

2. Underpinning research

The founding principle and early proof-of-concept experiments that gave rise to the Solexa sequencing technology took place in the Department of Chemistry at the University of Cambridge in laboratories led by Professor Shankar Balasubramanian and Professor David Klenerman. The Balasubramanian group has a long-standing interest in chemical and biophysical methods to study nucleic acids. The Klenerman group is interested in developing and applying a range of new biophysical methods, based on laser fluorescence spectroscopy and scanning probe microscopy.

Balasubramanian and Klenerman had previously collaborated on a project studying the action of a DNA polymerase on a DNA substrate, to explore what new insights could be gained using newly available single molecule fluorescence detection methods. They quickly realised the potential of this approach for DNA sequencing and set up a team of post-doctoral researchers (Colin Barnes, Mark Osborne, Xiaohai Liu, David Earnshaw and Scott Furey) to develop their ideas. In the initial experiments, discrete single molecules of DNA were immobilised on a surface, and the incorporation of fluorescently labelled deoxynucleotide monophosphates by a DNA polymerase was monitored using labelled 2'-deoxynucleoside triphosphates as co-substrates.[R1,R2] These key studies using microscopy to observe the interaction of DNA with DNA polymerase and the extension of a single strand of DNA using combinations of labelled enzymes and labelled molecules were adapted to enable solid phase DNA sequencing. By July 2000, the proof of principle experiments were in place, and the technology was transferred to Solexa, the company that Balasubramanian and Klenerman founded to investigate the potential of this new approach to sequencing. The first Solexa laboratory opened the following year in 2001, and the first Solexa sequencer, the Genome Analyzer, was launched in 2006.[R4-R6]

A key feature of solid phase sequencing is that it provides the means to achieve a massively parallel process by having many different DNA fragments immobilised on a surface that can be sequenced simultaneously. This approach that enabled each cycle to be driven to completion while minimizing misincorporation generated accurate data at a very high throughput rate and low

cost (determining an accurate whole human genome sequence in just 8 weeks for the initial sequencing systems).[R3] Previously, it had required years of effort and billions of pounds to sequence the first human genome. Illumina Inc. acquired Solexa in early 2007, and all subsequent technology improvements were carried out within the company. Balasubramanian and Klenerman contributed throughout these developments as consultants. Balasubramanian remained an Illumina consultant and a member of Illumina's scientific advisory board until April 2017, and Xiaohai Liu, one of the original post-doc team from the Department of Chemistry, works for Illumina to this day.

3. References to the research

[R1] Osborne, M. A.; Furey, W. S.; Klenerman, D.; Balasubramanian, S. Single Molecule Analysis of DNA Immobilised on Microspheres. *Anal. Chem.* **2000**, *72*, 3678-3681.

[R2] Osborne, M. A.; Barnes, C. L.; Balasubramanian, S.; Klenerman, D. Probing DNA Surface Attachment and Local Environment Using Single Molecule Spectroscopy. *J. Phys. Chem. B* **2001**, *105*, 3120-3126.

[R3] Bentley, D. R.; Balasubramanian, S.; […]; Smith, A. J. Accurate Whole Human Genome Sequencing Using Reversible Terminator Chemistry. *Nature* **2008**, *456*, 53-59.

[R4] Balasubramanian, S.; Klenerman, D.; Barnes, C.; Osborne, M. Arrayed Biomolecules and Their Use in Sequencing. US 6787308, 2004.

[R5] Balasubramanian, S. Polynucleotide Sequencing. US 6833246B2, 2004.

[R6] Barnes, C.; Balasubramanian, S.; Liu, X.; Swerdlow, H.; Milton, J. Labelled nucleotides. US 7057026B2, 2006.

Research outputs are published in peer-reviewed journals.

4. Details of the impact

It is estimated that one million genomes per year are sequenced using Solexa sequencing technology with instruments placed in smaller academic and industrial laboratories and new businesses, as well as large genome centres. This democratisation of sequencing capability is changing the nature and culture of life sciences and rapidly broadening the impact of sequencing across a number of fields such as plant sciences, environmental sciences, bioenergy and the study of all organisms.

Illumina Inc. The next generation sequencing (NGS) technology, invented by Balasubramanian and Klenerman, is at the heart of all of Illumina's sequencing products. In the last few years, Illumina has improved the technology further, dramatically reducing the costs and run time of DNA sequencing. In 2017, they launched their first production-scale sequencing system, **Novaseq**, currently the highest capacity instrument on the planet (six trillion bases per run),[E1] and it is expected to enable the landmark of the USD100 genome.[E2] Three years after launch, in June 2020, Illumina installed its 1000th Novaseq system.[E3] In 2018, Illumina launched a new bench top sequencer, **iSeq**. This easy to use instrument costs approximately USD20,000 and is capable of sequencing one billion bases per run, opening up the technology to a much wider community.[E4] Illumina currently employs over 7000 people and in 2018 its revenue was over USD3,000,000,000, mostly due to sales of NGS technologies (hardware, reagents, and services),[E5] all of which are based on the sequencing technology developed in the Balasubramanian and Klenerman laboratories.

Human genome sequencing. Solexa sequencing technology has made very large-scale projects possible, and there has been an exponential growth in the acquisition of genome sequence data. The use of high capacity sequencing to comprehensively characterise human genomes (DNA), transcriptomes (RNA) and the epigenomes (epigenetic changes) continues to have a demonstrable impact on our understanding of the genetic basis of disease, disease mechanisms, disease pre-disposition (especially greater understanding of rare diseases), as well as spread of infectious disease (genetics of pathogens and antimicrobial resistance mechanisms). Solexa-Illumina sequencers are the technology that underpins major projects such as the 100,000 Genomes Project, the International Cancer Genome Project, and Genome

Asia 100k.[E6] In 2018, the UK government secretary of health announced an expansion of the NHS Genomics England project to sequence one million NHS patients with a goal of sequencing five million patients over the next five years.[E7]

Oncology diagnostics. Many oncology clinics in the UK have now incorporated sequencing of cancer patients using Solexa-Illumina technology as routine in their diagnostics. Illumina have developed a number of clinically-directed products, including NGS Companion Diagnostics, which have been adopted by a number of pharmaceutical partners and clinical testing laboratories. In 2014, Illumina entered into a strategic partnership with AstraZeneca, Janssen, and Sanofi to develop a universal NGS-based oncology test system.[E8] In 2017, the Illumina MiSeqDx System was the first FDA-regulated and CE-IVD-marked NGS instrument for use *in vitro* diagnostic procedures.[E9] In 2018, Foundation Medicine launched FoundationOne CDx, the first NDA-approved companion diagnostic for cancer, whose cancer-sequencing panel uses Illumina sequencing technology.[E10] One major advance in the field is that of applying Solexa sequencing technology to detect mutations/signatures in pieces of DNA floating around freely (Circulating Tumour DNA) in the blood. This Liquid Biopsy is less invasive than a tissue biopsy and evidence suggests it may detect cancers much earlier (i.e. than by imaging). A whole new sector of the pharmaceutical industry has formed around this, including an Illumina spin-out company, Grail which was established in 2016, raising USD1,500,000,000 by 2018 in private investment.[E11]

Rare diseases. Solexa sequencing is being applied to elucidate the epidemiology of rare diseases. Illumina's product TruSight One is a targeted gene panel that uses NGS to simultaneously analyze up to 4813 genes associated with rare diseases. In a landmark study in 2016, Stephen Kingsmore from the Rady Children's Institute for Genomic Medicine (RCIGM) reached the Guinness book of records for diagnosing a rare genetic disorder in an infant in 26 hours by sequencing both of the parents and the child, a feat that was made possible by using one of Illumina's high-throughput sequencing instruments. Between July 2016 and January 2018, the RCIGM's team had completed testing and interpretation of the genomes of more than 335 children enrolled in its research studies. One-third of the patients have received a genomic diagnosis with 69% of those benefitting from an immediate change in clinical care.[E12]

Widespread adoption of the technology. Solexa sequencing has been applied to the detection and early diagnosis of pathogen related outbreaks, such as *Salmonella* using rapid MiSeq sequencing, and *Ebola* using the MiSeq System and a custom version of the TruSeq RNA Access Kit.[E13] Most recently, Illumina has provided sequencing of 35,000 genomes to further our understanding of the COVID-19 pandemic through genomics.[E14] NGS has been widely adopted by a number of fields beyond that of medicine. In anthropology, the technology has been applied to better understanding human migration. In archaeology, the complete genome sequence of a Neanderthal was made possible via sequences generated on the Illumina HiSeq 2500. The democratisation of sequencing capability made possible by the technology developed by Balsubramanian and Klenerman is changing the nature and culture of life sciences and rapidly broadening the impact of sequencing across a number of fields such as plant sciences, environmental sciences, bioenergy and the study of all organisms.

5. Sources to corroborate the impact

[E1] Novaseq tech specs

[E2] Business Wire - Novaseq $100 Genome 09.01.2017

"Illumina Introduces the NovaSeq Series - A New Architecture Designed to Usher in the $100 Genome"

[E3] Illumina website: "Illumina Reaches Milestone with 1,000 NovaSeq 6000"

[E4] Business Wire - Illumina Launches iSeq 100 08.01.2018

"Illumina Launches iSeq 100 Sequencing System - Industry-Leading Sequencing Technology for Under $20K"

[E5] Evidence for no. sequencers sold, revenues and staff numbers.

[E6] BBC News - Genomics England 100,000 Genomes Project 05.12.2018. “Faster diagnosis from 'transformational' gene project”. International Cancer genome project. Genome Asia 100k.

[E7] GOV.UK - NHS Genomic Medicine Service 5 million genomes project 02.10.2018

“Matt Hancock announces ambition to map 5 million genomes”

[E8] Business Wire - Companion Diagnostics for Oncology 21.08.2014. “Illumina Announces Strategic Partnerships with AstraZeneca, Janssen and Sanofi to Redefine Companion Diagnostics for Oncology”

[E9] Nasdaq - MiSeqDx instrument, the first FDA regulated NGS sequencer 16.11.2017

“Illumina Introduces NextSeq 550Dx, Updates Use of MiSeqDx”

[E10] FoundationOne CDx - Summary Of Safety And Effectiveness Data 30.11.2017

“Summary of Safety And Effectiveness Data”

[E11] Wired - Liquid Biopsy 06.03.2018

“A Blood-Based Cancer Test Gets Its First Results”

[E12] Rady Children's Hospital -Guinness World Records 12.02.2018

“New GUINNESS WORLD RECORDS™ Title Set for Fastest Genetic Diagnosis”

[E13] Reuters - Ebola Outbreak 19.11.2014

“Illumina teams with U.S. government, researchers to sequence Ebola”

[E14] Illumina COVID-19 Study 13.05.2020

“Illumina to Sequence Genomes for New UK-wide COVID-19 Study”