Institution: University of Oxford

Unit of Assessment: UOA5

Title of case study:

Oxford Gene Technology: the detection of genetic variation using microarrays

1. Summary of the impact

High-throughput genotyping has revolutionised the genome-wide search for associations between genetic variants and disease. Professor Sir Edwin Southern of the University of Oxford's Biochemistry Department invented the highly cost-effective array-based method of analysing genetic variation based on hybridisation between probes and samples on glass slides or 'chips'. The spin-out company Oxford Gene Technology (OGT) founded by Southern in 1995 licenses the patent to manufacturers of 'single nucleotide polymorphism (SNP) chips', including Illumina and Agilent, a global business exceeding $500M per year. Southern has continued to refine and extend this technology to increase its speed, efficiency and cost-effectiveness. This revolutionary technology has widespread applications such as prediction of individual risk, development of new drugs, provision of personalised treatments, and increased cost-effectiveness of clinical trials. Licence revenues fund R&D within OGT, and endow charitable trusts supporting primary school science education in the UK and crop improvement in the developing world.

2. Underpinning research

Over the past two decades the number of known genetic variants, in the form of SNPs, has increased from a few dozen to several million. Detection of genetic variation is the starting point for new therapeutic approaches to a very wide range of conditions, including cardiovascular disease and cancer. The promise of 'personalised' or 'stratified' medicine depends on treating the patient population not as a homogeneous group, but as individuals with differing therapeutic needs. For this promise to be fulfilled, pharmaceutical companies involved in drug discovery and clinical practitioners interested in accurate diagnosis need access to fast, high-resolution and reliable assay methods that deliver personalised SNP profiles.

Professor Southern developed rapid, cost-effective, high-throughput methods for identifying target DNA sequences as a logical extension to his Southern blotting technique. Work he published in 1993 showed how his patented method of synthesising and fixing an array of short lengths of DNA on a glass surface, could be used to probe mixtures of DNA or RNA molecules and to identify multiple targets in parallel through complementary base pairing[1]. This powerful technology is known as an oligonucleotide microarray.

In a series of studies from 1997–2003, with post-doctoral researchers Stephen Case-Green, Clare Pritchard and Nicholas Housby, Southern showed that DNA ligases, enzymes that catalyse the repair of DNA strands, could be used to detect SNPs. DNA polymerases, enzymes that catalyse the extension of a DNA strand by adding nucleotide bases or oligonucleotides, could be used in a similar fashion[2, 3]. They incorporated this new discovery into their existing microarray technology. As well as establishing the basic principle of using enzyme reactions to detect variation in genetic sequences, the Southern group explored the ligation efficiency of a number of different ligases and polymerases, derived from thermophilic bacteria[4].

Ligases and polymerases can make repairs or extensions respectively, only on a strand that has formed a duplex with its complementary strand in that region. The Southern group exploited this requirement for a perfect match. They provided radioactively-labelled single bases or oligonucleotides as substrates for the enzymes and incubated them with an oligonucleotide microarray that had previously been hybridised with the target DNA sample. If the target matched

the tethered probe perfectly then a bright spot on the array would signal the successful reaction. If the target carried a mutation, then the enzyme reaction would fail, and no signal would be transmitted. The Southern group demonstrated that this simple but powerful technology could analyse multiple mutations in many sequences in a single DNA sample, greatly increasing the speed, efficiency and cost-effectiveness of this essential laboratory task[5]. In 2000, Professor Southern, Dr Pritchard and Dr Case-Green were awarded US Patent No. 6150095, 'Method for analysing a polynucleotide containing a variable sequence'[6].

3. References to the research

1. Maskos U, Southern EM. (1993) A novel method for the parallel analysis of multiple mutations in multiple samples. Nucleic Acids Research 21: 2269-2270. doi: 10.1093/nar/21.9.2269 ***Shows how orthogonal stripes of oligonucleotide probes and DNA samples can analyse many samples simultaneously.***

2. Pritchard CE, Southern EM. (1997) Effects of base mismatches on joining of short oligodeoxynucleotides by DNA ligases. Nucleic Acids Research 25: 3403-3407. doi: 10.1093/nar/25.17.3403 ***Shows how mismatches in complementary pairing prevent DNA repair by DNA ligases, providing the basis for positive/negative signalling on an oligonucleotide microarray.***

3. Housby JN, Southern EM. (1998) Fidelity of DNA ligation: a novel experimental approach based on the polymerisation of libraries of oligonucleotides. Nucleic Acids Research 26: 4259-4266. doi: 10.1093/nar/26.18.4259 ***Shows how libraries of oligonucleotides can be polymerised to tethered oligonucleotides, using DNA polymerases, and how successful polymerisation depends on complementary pairing between the tethered oligonucleotide and the target sample.***

4. Housby JN, Thorbjarnardottir SH, Jonsson ZO, Southern EM. (2000) Optimised ligation of oligonucleotides by thermal ligases: comparison of *Thermus scotoductus* and *Rhodothermus marinus* DNA ligases to other thermophilic ligases. Nucleic Acids Research 28: E10. doi: 10.1093/nar/28.3.e10 ***Demonstrates superior efficiency of named thermophilic ligases in catalysing DNA repair and providing accurate detection of mutations.***

5. Case-Green S, Pritchard C, Southern E. (2003) Oligonucleotide arrays for genotyping: enzymatic methods for typing single nucleotide polymorphisms and short tandem repeats. In: Bartlett JMS, Stirling D, editors. PCR Protocols. 2nd. ed. Totowa, NJ: Humana Press. p. 255-270. Methods Molecular Biology 226. doi: 10.1385/1-59259-384-4:255 ***Demonstrates the use of oligonucleotide arrays in combination with enzyme chemistry – DNA ligases and polymerases – to identify genotypes on the basis both of single nucleotide polymorphisms (SNPs) and short repeated sequences.***

6. Southern EM, Pritchard CE, Case-Green SC. (2000) Method for analyzing a polynucleotide containing a variable sequence. United States patent 6150095. Available from: http://patentscope.wipo.int/search/en/detail.jsf?docId=US39194617 ***Confirms details of US patent no. 6150095, 'Method for analyzing a polynucleotide containing a variable sequence'.***

Funding for research: Funding in excess of £7.8M was awarded to the University of Oxford for the period 1993 – 2005, principally from the MRC, Oxford Gene Technology, BBSRC, the European Commission, CRC and the Wellcome Trust.

4. Details of the impact

In 1995 Professor Southern founded a spin-out company, Oxford Gene Technology (OGT), to handle the licensing of his microarray patents. OGT is now one of the leading providers of genetics research and biomarker solutions internationally[7]. Based at the Begbroke Science Park outside Oxford, OGT currently employs over 60 staff having doubled the number in the last two years, and has an annual turnover of around £10M. Professor Southern retired from the Whitley Chair of Biochemistry at Oxford in 2005, but continues to head the research team at OGT.

OGT operates an open licencing policy that makes its technology very widely available and stimulates innovation. Non-exclusive licences are held by all the major companies in the microarray business. The largest player, Illumina Inc., is a $6 billion company, about half of whose business is in analysis of variable sequences. Affymetrix and Agilent are also licence-holders with major interests in DNA testing. More specialised licence-holders include Immucor[8], which uses genotyping to provide a genetic matching service to enhance the specificity of blood transfusions. The global market for DNA microarrays was estimated to be $760M in 2010: about $500M of this business related to 'SNP chips', or microarrays incorporating analysis of variable sequences using the enzyme chemistry described above. Analysts estimate that this market will have almost doubled in size by 2015[9].

Commercially-produced microarrays have been used to analyse genetic variation on a very large scale. For example, the Wellcome Trust Case Control Consortium is now in its third round of genome-wide association studies looking for SNPs and other genetic variants in more than 40 diseases or conditions. They are comparing genetic profiles of at least 2000 people with each condition, with those of a control sample of 6000 people drawn from the general population. The sample analysis is conducted using Affymetrix, Illumina and Agilent chips based on Southern's work. This award-winning study has already discovered many new associations between genes and disease, information that is being made available to pharmaceutical companies and researchers through an open-access database[10].

The technology developed by the Southern group to detect variation exactly meets the need of the emerging field of 'personalised' or 'stratified' medicine. For example, in 2013 OGT launched an assay using this technology that will speedily and reliably provide individual profiles of mutations in tissue taken from cancerous tumours. The company received £1.16M from the UK Technology Strategy Board's Stratified Medicine Innovation Platform to develop the assay, which will support doctors in providing targeted therapies and improve the experience of patients who are spared aggressive and unnecessary treatment[11].

Royalties received on the Southern patents have been used to establish two charitable trusts. The Kirkhouse Trust[12] supports crop development in India and Africa using marker-assisted selection. The Edina Trust[13] has to date given individual grants of up to £1500 to over 600 primary schools in the UK to support science education.

5. Sources to corroborate the impact

7. Oxford Gene Technology (OGT). Available from: http://www.ogt.co.uk/about/company
   Website detailing the history of Oxford Gene Technology.

8. Immunocor receives from OGT worldwide rights in the field of molecular immunohaematology. Available from:
   http://www.ogt.co.uk/news_events/news/296_ogt_settles_legal_action_with_bioarray_solutions
   BioArray, now part of Immucor, is given worldwide rights to OGT technology.

9. Market Wire. Global DNA & Microarray Market to Grow 13% Annually Through 2015. 25 May 2011. Available from: www.marketwire.com/press-release/global-dna-microarray-market-to-grow-13-annually-through-2015-1518723.htm ***News item confirming the size and growth of the microarray market from report by MarketResearch.com, which includes company***

profile of Oxford Gene Technology.

10. Craddock N, Hurles ME, Cardin N, Pearson RD, Plagnol V, Robson S, et al. (2010) Genome-wide association study of copy number variation in 16,000 cases of eight common diseases and 3,000 shared controls. Nature 464: 713-720. doi: 10.1038/nature08979 ***Study by the Wellcome Trust Case Consortium. OGT ran arrays and produced primary data and low-level summary statistics (see Methods Summary).***

11. Oxford Gene Technology. OGT launches ground-breaking 58-gene tumour profiling service. 28 May 2013. Available from: www.ogt.co.uk/news_events/news/806_ogt_launches_ground-breaking_58-gene_tumour_profiling_service ***News item confirming the launch by OGT of its 58-gene tumour profiling service.***

12. The Kirkhouse Trust. Available from: www.kirkhousetrust.org ***Web-site describing the work of the Charitable Trust funded by OGT which has agricultural crop improvement research for the developing world (specifically legumes) as its current focus.***

13. The Edina Trust. Available from: www.edinatrust.org.uk ***Website describing the work of the Trust, which promotes the teaching of science and gardening.***