Institution: Oxford Brookes University

Unit of Assessment: 5 - Biological Sciences

Title of case study: Oxford Expression Technologies: making baculovirus expression accessible for protein production and vaccine development

1. Summary of the impact (indicative maximum 100 words)

Oxford Expression Technologies (OET) is a spin out company launched jointly by Oxford Brookes University (Brookes) and the Natural Environment Research Council (NERC) to exploit Intellectual Property (IP) in the field of protein expression using novel insect virus vectors. OET generates revenue through sale of kits, services & licences to a range of global customers including academia, research institutes, pharmaceutical and biotechnology companies. OET provides employment, invests in in-house Research and Development including funding collaborative PhD students, and generates royalty income streams for Brookes and NERC. Customers are able to produce multiple recombinant proteins to higher yields and quality than was otherwise possible and a number of companies are using the developments for the commercial production of vaccines and other uses.

2. Underpinning research (indicative maximum 500 words)

The research that led to the creation of the IP and spin out of OET was the result of a long standing collaboration between Professor Linda King at Brookes and Professor Robert Possee at NERC's Centre for Ecology & Hydrology (CEH), who has been a visiting Professor at Brookes since 2000. Since 1995, joint research funded through a series of grants and PhD studentships, has been undertaken to study the basic biology and replication of baculoviruses, which are insect-specific viruses (for example, reference 1).

During the 1990s baculoviruses began to be exploited as expression vectors for recombinant protein production in insect cells but the processes to make the recombinant viruses were time-consuming and technically demanding. This led King and Possee to undertake research throughout the period 1995-2006, to make the production and selection of recombinant viruses much easier and quicker. The outcome of the work leading up to 2000 led an equally split joint Brookes-NERC exploitation agreement, and patent application in 2000; patents have since been approved world-wide (see reference 2), with Brookes taking the lead role in the exploitation process and King and Possee being jointly named as inventors.

Research subsequent to the patent filing sought to develop the patented technology for the high throughput production of recombinant baculovirus expression vectors, which culminated in the award of a 3-year Biotechnology and Biological Sciences Research Council (BBSRC) grant to Brookes, with King as principle investigator and Possee as co-PI (Reference 1c). Dr Richard Hitchman was the post-doctoral research assistant, who later transferred to OET as Director of Research. These studies led to the development of a new baculovirus expression system (commercially known as flashBAC) that made it possible to produce recombinant viruses in a one-step process without a tedious and demanding selection step to separate recombinant from non-recombinant virus, which was a feature of all other baculovirus expression systems available at the time. The work on the grant was focussed on developing the technology described in the patent application for use in high through put expression systems. A number of PhD students and external collaborators contributed to in-house and beta testing (references 3-6).

At the same time, other collaborative research projects have continued to improve the original flashBAC virus, making use of basic studies by Possee and King that identified the role of various non-essential genes that encode viral enzymes important in virus replication: chitinase, cathepsin and P10 (for example, reference 1a,b). Once the role of these proteins was established, King and Possee used this knowledge to make genetic modifications to the virus genome to delete chitinase (flashBAC), chitinase & cathepsin (flashBACGOLD) or all three genes (flashBACULTRA) and produced a series of papers to describe the improvements to recombinant protein production (references 3-6). Namely, deletion of chitinase enables secreted and membrane targeted protein to be processed through the insect cell secretory pathway more effectively (4-6). Cathepsin is a virus protease and so its deletion ensures that recombinant proteins are less likely to be degraded (5,6). P10 is a protein that forms an intricate cage like structure around and through the nucleus of

infected cells and facilitates cell lysis, deletion of P10 improves cell viability and prolongs the period of recombinant protein production leading to greater yields (3,5).

3. References to the research (indicative maximum of six references)

1) Grants:

a) *Defining the genetic and environmental parameters affecting virus transmission between insect larvae,* PI Linda King, NERC Research Grant, Reference NER/A/S/2001/01069, £255,801 awarded, 01/09/2002 to 31/08/2005. http://gotw.nerc.ac.uk/list_full.asp?pcode=NER%2FA%2FS%2F2001%2F01069

b) *The role of p10 in baculovirus morphogenesis and cellular pathogenesis*, PI Linda King, BBSRC Research Committee Studentship, £41,845 awarded, 31/10/2002 to 31/10/2005, Grant reference: 02/B1/C/08354 http://www.bbsrc.ac.uk/pa/grants/AwardDetails.aspx?FundingReference=02/B1/C/08354

c) *Automated high throughput systems for production of recombinant baculovirus expression vectors,* BBSRC, Grant Reference 332/B19427, 01/06/2003-30/092006, £192,144 awarded jointly to Brookes and CEH. http://www.bbsrc.ac.uk/pa/grants/AwardDetails.aspx?FundingReference=B19427

2) Patents:

a) US Patents;
   i) US 7413732, http://www.patentlens.net/patentlens/patent/US_7413732/en/
   ii) US 8252278, http://www.patentlens.net/patentlens/patent/US_8252278/en/

b) European Patent; EP 1144666 B1, http://www.patentlens.net/patentlens/patent/EP_1144666_B1/en/

c) Australian Patent; AU 782205 B2 http://www.patentlens.net/patentlens/patent/AU_782205_B2/en/

3) Carpentier, D, Griffiths, C and King, LA (2008). The baculovirus P10 protein of *Autographa californica* nucleopolyhedrovirus forms two distinct cytoskeletal-like structures and associates with polyhedral occlusion bodies during infection, *Virology*, 371 278-91. DOI: 10.1016/j.virol.2007.09.043

4) Possee, RD, Hitchman, RB, Richards, KS, Mann, SG, Siaterli, E, Nixon, CP, Irving, CH, Assenberg, R, Alderton, D, Owens, RJ & King, LA (2008) Generation of baculovirus vectors for the high throughput production of proteins in insect cells. *Biotechnol. Bioeng.* 101 (6) 1115-22. DOI: 10.1002/bit.22002 *Submitted to REF2014, Oxford Brookes University, UoA5 - Biological Sciences, REF2, LA King, Output identifier 8128.*

5) Hitchman, RB, Possee, RD, Crombie, AT, Chambers, A, Ho, K, Siaterli, E, Lissina, O, Sternard, H, Novy, R, Loomis, K, Bird, LE, Owens, RJ & King, LA (2010) Genetic modification of a baculovirus vector for increased expression in insect cells. *Cell Biol. Toxicol* 26(1) 57-68 (epub 2009). DOI: 10.1007/s10565-009-9133-y

6) Hitchman, RB, Possee, RD, Siarterli, E, Richards, KS, Clayton, AJ, Bird, LE, Owens, RJ, Carpentier, DC, King, FL, Danquah, JO, Spink, KG and King, LA (2010) Improved expression of secreted and membrane-targeted proteins in insect cells. *Biotechnol Appl Biochem* 56 85-93. DOI: 10.1042/BA20090130

Brookes staff:

Prof. Linda King (PI), Dr Richard Hitchman (PDRA, then Director of Research at OET from 2007), Dr Caroline Griffiths (Senior Lecturer), SG Mann (Technician), CH Irving (Research Assistant, transferred to OET staff in 2007)

NERC CEH staff:

Prof.Robert D Possee (Co-PI)

Brookes Students (PhD unless otherwise specified): (undertaking in-house testing)

KS Richards, E Siaterli (MPhil; transferred to OET 2007), CP Nixon, AT Crombie (Undergraduate), A Chambers (OET funded), JO Danquah (employed by OET as PDRA in 2011-12), D Carpentier, K. Ho, FL King

External Collaborators: (undertaking Beta-testing of the expression systems)

Alterton, Sternard, Novy & Loomis (EMD Biotech, was Novagen),
Owens & Bird (Oxford Protein Production Facility),
Clayton, Spink & Assenberg (Novartis, Basle)

4. Details of the impact (indicative maximum 750 words)

Pathway to impact

OET Ltd was founded in November 2007 as a spin-out business as a direct result of the successful Exploitation Agreement between Oxford Brookes University and the Natural Environment Research Council. The patents granted to Brookes and NERC CEH were licenced to the new company in return for shares and a royalty income stream. From the beginning, OET's ethos has been to generate revenue and profit to maintain and grow its activities, following an initial private equity investment of £330K. A number of staff working in the Brookes insect virus research group transferred into the company including Dr Richard Hitchman as Research Director, Ms Evi Siaterli as Scientific Officer and Ms Helen Irving as Business Development Manager. Professors King and Possee took on the roles of Scientific Advisor and were appointed to the Board of Directors.

Impacts 2008 to present

The innovative technology (*flash*BAC^{TM}) developed by King and Possee in the academic sector has resulted in a range of easy to use kits that enable users to make recombinant proteins in a rapid and convenient one step process. Its unique properties comprise (1) its capacity for high through-put production of multiple recombinant viruses and (2) the improvements to the genetic backbone of the virus to generate higher yields of good quality 'difficult to express' proteins. While competitor baculovirus expression products exist (for example, Invitrogen's, *BAC2BAC* and BD Biosciences' *BACULOGOLD*), the genetic backbone of these vectors has not been further developed since launching in the mid-1990s, and therefore *flash*BAC is the only product that has been genetically modified to improve the yield and quality of 'difficult to express' proteins and at the same time enable high throughput, simultaneous production of virus vectors.

First commercial sales of *flash*BAC kits began in 2008 and they are sold world-wide either directly from OET's Oxford base or through 19 international distributors to 21 different countries in Europe, Asia and North America. To access the American markets, the technology was licenced under Original Equipment Manufacturer agreements to EMD Millipore in 2008 and is sold under the brand name *BacMagic*. Clients are a mixture of commercial and academic laboratories. Sales during the period January 2008 to July 2013 have amounted to over £1.8M and royalty payments to Brookes/NERC has totalled £50K in the same period. About 45% of OET's sales derive from kit sales and of this about 60% from distributors and 40% through direct sales. This is evidence that the biotechnology sector has adopted a new technology as a result of the research undertaken at Brookes.

The *flash*BAC technology is also used in-house by OET to produce recombinant proteins for customers unable to make them in their own laboratories and this accounts for about 50% of annual turnover. OET has thus established itself in the rapidly expanding market for "off the shelf" research services that enable companies to avoid setting up their own dedicated facilities or enable them to outsource when in-house facilities are at capacity. OET has worked with companies such as Merck, Novartis, Epistem, Serotec, Medigene, Protein Sciences, Sanofi Pasteur, Sanofi Aventis to name a few, plus industry-related entities such as Oxford Structural Genomics Consortium, the Oxford Protein Production Facility and government laboratories such as the Health Protection Agency and The Pirbright Laboratory. We have enabled these companies and institutions to advance their drug discovery and/or vaccine development programmes by producing proteins more quickly and to higher yields and better quality than was possible previously. Companies utilizing OET technology are based in Europe, North and South America, South East Asia, Australia, Middle East and China, as well as in the UK.

A specific example of the licensed use of the technology has been in the development of a point-of-care serology assay for the detection of Crimean-Congo Haemorrhagic Fever by the Health Protection Agency, which involved the expression of the CCHF virus nucleoprotein using the *flash*BAC system.

In the time of its existence, OET has provided employment for 5-8 members of staff at any one

time. A number of OET R&D staff were trained at Brookes, including E Siaterli, CH Irving, JO Danquah and A Chambers.

OET invests in R&D to ensure its platform technologies remain competitive and at the forefront of new expression vector development through in-house and collaborative R&D with a range of UK and International, commercial and academic partners, such as Proteonics in The Netherlands, The BBSRC Pirbright Laboratory and Paratech Inc., USA. Most recently, OET has produced a new *flash*BAC variant that is enabling increased production and yield of Virus Like Particles (VLP) for the vaccine markets, which was released in October 2012 under the retail name *flashBACPRIME*. Through this in-house and collaborative product development pipeline, OET also provides a range of reagents to complement its flagship *flash*BAC kits including BaculoQUANT™, an innovative quantitative PCR method for fast and accurate titration of viruses, an effective transfection reagent for insect cells (BaculoFECTIN) and a range of novel transfer vectors for the expression of genes at various points in the virus replication cycle, for dual expression of proteins and for production of proteins in mammalian cells (BacMAM vectors).

OET has also funded or co-funded 3 PhD students at Brookes, which are directly related to further improving the Baculovirus expression system and most recently a studentship which aims to develop novel vaccines for African Horse Sickness, co-funded with BBSRC Pirbright Laboratory. A strategic partnership has been established with Proteonics and Paratechs and licences have been granted to a number of commercial users and the Health Protection Agency (HPA). OET also provides training courses to enable R&D staff within industry to gain high-level skills in using the bacculovirus expression system in their own laboratories.

In recognition of it's early impact in the local biotechnology sector, OET was awarded 'Best new start-up' in Oxfordshire and SE 2009 at the annual Oxfordshire Bioscience Network (OBN) awards.

5. Sources to corroborate the impact (indicative maximum of 10 references)
Sources b,c,d,e,g,h, can be confirmed by Corroborating Contact 1: CEO, Oxford Expression Technologies Ltd.

a) Website OET Ltd: http://oetltd.com
b) Sales figures from OET Ltd to show global impact and revenues available on request
c) List of clients and customers available on request
d) Details on number of Licence deals available on request
e) Royalty statements available on request
f) Number of distributors worldwide/OEM deals, inc. details of product on their catalogue accessed through OET website: http://oetltd.com
g) Employment statistics available on request
h) Example R&D tax credit statement from OET available on request
i) OBN award http://www.obn.org.uk/obn_/news_item.php?r=NTWLEX454771
j) Proteonics press release: http://www.proteonic.nl/38/company/news/proteonic-and-oxford-et-join-forces-combining-unic-with-flashbac-protein-expression-platform/?id=16#.US91VzBA3VU#