Institution: Oxford Brookes University

Unit of Assessment: 5, Biological Sciences

Title of case study: Oxford Expression Technologies Ltd: making the baculovirus expression system accessible for protein production, diagnostic assays and vaccine development

Period when the underpinning research was undertaken: 2000–2020

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:
Linda A. King	Professor of Virology	[text removed for publication]
Robert Possee	Visiting Professor	
Evi Siaterli	PGRA	
Richard Hitchman	PDRA	
Leo Graves	PDRA	

Period when the claimed impact occurred: August 2013 to December 2020

Is this case study continued from a case study submitted in 2014? Y

1. Summary of the impact

Oxford Expression Technologies Ltd (OET) is a spin-out company of Oxford Brookes University (OBU) and the Natural Environment Research Council (NERC) that exploits Intellectual Property in the area of gene expression using novel insect virus vectors. Initially time-consuming and requiring a high level of user skill, OET's *flash*BAC™ technology has enabled the production of recombinant proteins in a simplified, one-step process. OET sells kits, services and licences to pharmaceutical, biotechnological, diagnostic and vaccine companies in all continents as well as world-leading academic institutions, enabling them to increase the yields and quality of recombinant proteins, accelerating product development. This has resulted in diagnostic products reaching market for pig and poultry viral diseases, and viral vaccines entering clinical trials, including for [text removed for publication] and Covid-19. OET reinvests net profit and provides employment in collaborative research and development towards novel products, gene therapy and vaccines.

2. Underpinning research

Baculoviruses infect a wide range of insects and other invertebrates, but their inability to replicate in human cells makes them a very safe tool for biomedical researchers. OBU's research (led by King) has played an important role in establishing the baculovirus as a workhorse for the development of new biologics and led to the creation of a successful spin-out company: OET.

The production of recombinant proteins plays an increasingly important role in the development of medical therapeutics, diagnostics and vaccines. The insect-derived baculovirus system for gene expression has the advantage of being very safe to use and of producing complex proteins in a much shorter timescale than other systems. However, its widespread adoption was initially hindered because of technological limitations; making recombinant viruses was time-consuming and a high level of user skill was required.

King (OBU) and Possee (NERC's Centre for Ecology & Hydrology, CEH) have studied the basic biology and replication of baculoviruses since 1995. Their work led to the invention of a new, patented, baculovirus expression system that enabled the production of recombinant proteins in a simplified, one-step process. Since 2000, the research collaboration has been funded through a series of grants and PhD studentships (1) under the umbrella of a joint NERC-OBU exploitation agreement that led to the spin-out of OET (2007).

Since 2000, four patents have been approved worldwide (2), with OBU taking the lead role in exploitation and King and Possee named joint inventors. Research subsequent to patent filing has developed the technology further, enabling high-throughput production of baculovirus expression

vectors (1a). These studies have led to the development of a new baculovirus expression system (commercially known as *flash*BAC™) that makes it possible to produce recombinant viruses in a one-step process, without the tedious and demanding selection step that separates recombinant from non-recombinant virus that is a feature of all other baculovirus expression systems.

Other collaborative research projects have continued to improve the original *flash*BAC™ virus, making use of basic studies by King and Possee that identified the roles of various non-essential genes that encode viral enzymes important in virus replication: chitinase, cathepsin and P10 (1b, 1c). King and Possee have also made genetic modifications to the virus genome to delete chitinase and cathepsin (*flash*BACGOLD™), or all three genes (*flash*BACULTRA™), and described the resulting improvements in recombinant protein production (3, 4). Deletion of chitinase significantly improves yields of secreted and membrane-targeted proteins (3, 4). Cathepsin is a viral protease and its deletion ensures that recombinant proteins are less likely to be degraded (4). P10 is a protein that forms an intricate cage-like structure in infected cells and facilitates nuclear disintegration (5, 6); its deletion improves cell viability and prolongs the period of recombinant protein production, leading to greater yields (3, 4). This research was exploited in the development of a *flash*BAC^{maxtransduction} version with increased budded virus titres aimed at improving the efficacy of applications involving the transduction of human cells for gene therapy (1d, 5) (PDRA Graves moved to OET at the end of the grant).

Between 2013 and 2020, OET funded or co-funded five PhD students at OBU to further improve the baculovirus expression system, including a BBSRC iCASE studentship to develop novel approaches to vaccine production in insect cells. In 2018, OET was awarded a GBP2,000,000 Innovate UK/Small Business Research Initiative contract (Vaccines for Global Epidemics initiative) to develop a vaccine for Crimean Congo Haemorrhagic Fever (CCHF) Virus with Public Health England at Porton Down (PHE), University of Oxford Jenner Institute and OBU (King co-PI) as collaborators (1e). In May 2020, the remaining contract was repurposed to work on an insect cell-derived vaccine for Covid-19 (with Vaxine Pty, an Australian vaccine company), and in August 2020 Innovate UK provided a further GBP681,000 to progress the Covid-19 vaccine towards a Phase 1 clinical trial.

3. References to the research

1. **Grant and studentship examples:**
   a) *Automated high throughput systems for production of recombinant baculovirus expression vectors,* BBSRC, 2003-2006, GBP230,000 awarded jointly to Brookes and CEH, Grant Ref: 332/B19427
   b) *Defining the genetic and environmental parameters affecting virus transmission between insect larvae,* PI King, NERC Research Grant, 01/07/2002 to 30/06/2005, GBP255,801, Grant Ref: NER/A/S/2001/01069
   c) *The role of p10 in baculovirus morphogenesis and cellular pathogenesis*, PI King, BBSRC Research Committee Studentship, 31/10/2002 to 31/10/2005, GBP27,000, Grant Ref: 02/B1/C/08354
   d) *Pretransplant gene therapy of pancreatic islet tissue; towards a therapy for Diabetes type 1 in Mexico*. PI Possee, co-PI King, PDRA Graves, Innovate UK Newton (Mexico), 2016-2018, awarded jointly to OET (GBP180,000) and Brookes (GBP91,000), Grant Ref: 102731
   e) *Development of an economically viable vaccine for CCHF virus*, co-PI King, Innovate UK/SBRI awarded to Possee (PI), OET Ltd 2018 (GBP2,000,000); May 2020 repurposed to develop a vaccine for Covid-19, Grant Ref: 972237
   f) *COVID-19: a rapidly scalable SARS-CoV-2 vaccine platform based on recombinant spike protein manufactured in insect cells using flashBAC to maximise yield and quality*, co-PI King; Innovate UK awarded to Possee (PI), OET Ltd 2020 (GBP681,000), Grant Ref: 73370
2. **Patents:**
   a) US Patents
      i. US 7413732, https://patents.google.com/patent/US7413732B1/en
      ii. US 8252278, https://patents.google.com/patent/US8252278B2/en?oq=US+8252278
   b) European Patent; EP 1144666 B1, https://patents.google.com/patent/EP1144666B1/en?oq=+EP+1144666+B1

c) Australian Patent; AU 782205 B2, https://patents.google.com/patent/AU782205B2/en?oq=AU+782205+B2+

3. Possee, R. D., Hitchman, R. B., Richards, K. S., Mann, S. G., Siaterli, E., Nixon, C. P., Irving, C. H., Assenberg, R., Alderton, D., Owens, R. J. & King, L. A. (2008). Generation of baculovirus vectors for the high throughput production of proteins in insect cells. *Biotechnology and Bioengineering, 101*(6), 1115-1122. DOI: 10.1002/bit.22002
4. Hitchman, R. B., Possee, R. D., Siarterli, E., Richards, K. S., Clayton, A. J., Bird, L. E., Owens, R. J., Carpentier, D. C., King, F. L., Danquah, J. O., Spink, K. G. & King, L. A. (2010). Improved expression of secreted and membrane-targeted proteins in insect cells. *Biotechnology and Applied Biochemistry, 56*, 85-93. DOI: 10.1042/BA20090130
5. Graves, L. P., Aksular, M., Alakeely, R. A., Ruiz Bucks, D., Chambers, A. C., Murguia-Meca, F., Plata-Munoz, J. J., Hughes, S., Johnson, P. R. V., Possee, R. D. & King, L. A. (2018). Improved baculovirus vectors for transduction and gene expression in human pancreatic islet cells. *Viruses 20, 10*(10), 574. DOI: 10.3390/v10100574
6. Graves, L. P., Hughes, L., Irons, S. L., Possee, R. D. & King, L. A. (2019). In cultured cells the baculovirus P10 protein forms two independent intracellular structures that play separate roles in occlusion body maturation and their release by nuclear disintegration. *PLoS Pathogens* DOI: 10.1371/journal.ppat.1007827

4. Details of the impact

Pathway to impact

In 2007, with support from OBU's Research & Business Development Office and investment of approximately GBP375,000, OET was founded to exploit the new baculovirus technology developed by King and Possee through marketing a range of kits, and by providing specialist services to commercial and academic users worldwide (S1). The patents granted to OBU and NERC's CEH were licenced to the new company in return for shares and a royalty income stream. Since 2007, OET's ethos has been to invest in research & development (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 (S1). Since 2013, OET has launched four improved versions of its core *flash*BAC™ expression platform, including one that increases transduction of mammalian cells to enhance gene therapy applications, and has sub-licensed the core technology for use in both veterinary and clinical applications in biotech, diagnostic and vaccine companies including [text removed for publication]. Since 2015, OET has also been successful in gaining GBP2,421,953 Innovate UK contracts and grants and co-invested c.GBP750,000 own funds to enable it to capitalise on its own innovative platform technologies to develop diagnostics and vaccines for emerging viral diseases in a strategic partnership with PHE Porton Down and others. Between 2014 and 2020, OET has more than doubled its scientific staff from 7 to 16 with 50% at PhD level (S2), and increased annual turnover 4.5-fold to [text removed for publication] and profit by six-fold (S3), much of which has been reinvested in R&D and growing the company. Today OET is acknowledged as a world-renowned centre of excellence for baculovirus expression technologies, benefitting global academic and commercial end users for research, diagnostic and vaccine purposes (S1, S4).

Impact August 2013 to present

The innovative technology (*flash*BAC™) developed by King and Possee 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 (S5). The benefits of the technology for end users relate partly to the simplification of the process, which enables a wide variety of academic and commercial laboratories to access the technology to make recombinant proteins without the need for a high level of virology-related skills. The simplified process is also quicker, thus saving several days' time and resources in producing proteins at research scale through to bioreactors for commercial production. *flash*BAC™ technology also has unique properties, which comprise: (1) its capacity for high-throughput production of multiple recombinant viruses, (2) improvements to the genetic backbone of the virus to generate higher yields of good-quality 'difficult-to-express' proteins and (3) avoidance of sequences that can lead to long-term genetic instability of the viral vector backbone.

While competitor baculovirus expression products exist (for example, Thermo Fisher's *BAC2BAC* and BD Biosciences' *BACULOGOLD*), the genetic backbones of these vectors have not been further developed since launching in the mid-1990s, and *BAC2BAC* retains bacterial sequences that can cause long-term genetic instability, often decreasing expression yields to almost zero over six or more passages of the virus and thus making it difficult to amplify virus for use at scale in bioreactors;. *flash*BAC™ is the only baculovirus expression platform that has been genetically modified to improve the yield and quality of 'difficult-to-express' proteins (by deleting non-essential virus genes including chitinase, cathepsin and P10) and, at the same time, enabling high-throughput, simultaneous production of virus vectors in a simple one-step process. During the process of homologous recombination to insert foreign genes into the *flash*BAC™ genome, bacterial artificial chromosome sequences (used to produce stocks of *flash*BAC™ DNA) are deleted from the final vector sequences and hence recombinant *flash*BAC™ vectors are genetically stable over the long-term, which ensures yields do not diminish during scale-up, for example, when providing a vaccine.

OET's technology is now being used by multinational pharmaceutical (e.g. [text removed for publication]), biotech (e.g. [text removed for publication]), animal health companies (e.g. [text removed for publication]) and leading research institutions (e.g. PHE, the Francis Crick Institute and Universities of Oxford, Cambridge, Bristol, Manchester, Leicester, Reading, Gdansk, Aarhus, Wageningen, Harvard, Yale, Berlin, Tokyo, German Diabetes Centre, Animal & Plant Health Agency, APHA) worldwide to advance their drug discovery, biotherapeutic, diagnostic or vaccine development programmes. In addition, >100 companies and universities (many listed above) have also outsourced expression vector and protein production in insect cells to OET for 'proteins to sell' (e.g. [text removed for publication]) or incorporation into diagnostic assays or vaccines for veterinary (e.g. [text removed for publication]) and clinical purposes (e.g. [text removed for publication]); a number (>20) of larger academic research groups outsource construction of complex or challenging gene expression projects to OET's experts, e.g. membrane proteins, virus-like particles and multiprotein complexes (e.g. Universities of [text removed for publication]).

Over the last few years, OET has completed increasing numbers of licence deals (>15) to enable companies to progress target vaccines or biotherapeutics through to clinical trials, or to commercialise products, (e.g. diagnostic assays) prepared using OET's technology. For example, under a sub-licence agreement, OET produces many different proteins for [text removed for publication] for incorporation into its veterinary diagnostic assays for major avian (e.g. [text removed for publication]) and porcine (e.g. [[text removed for publication]) infectious diseases. In 2018, OET sub-licenced *flash*BAC™ to [text removed for publication] for [text removed for publication] vaccine targets, two of which have successfully completed Phase 1 and 2 clinical trials for [text removed for publication]. In November 2020, [text removed for publication] vaccine for SARS-CoV-2 (the virus responsible for the Covid-19 global pandemic) (S6). The [text removed for publication] (S7). In August 2020, the UK Government [text removed for publication] (S8), and more recently [text removed for publication] (S9). In May 2020, OET agreed terms to licence its technology to Vaxine Pty, Australia for a vaccine for Covid-19, and in August 2020 Innovate UK provided GBP681,000 funding to OET to help Vaxine progress to a Phase 1 clinical trial.

The first commercial sales of *flash*BAC kits began in 2008 and the new technology has been widely adopted by the biotechnology sector (S4). Clients are both commercial and academic from across the globe, including all continents, either directly from OET's Oxford base or through a number of international distributors (e.g. BioNovus for Australia, Cell Concepts for Germany, MirusBio for the US, Cosmo for Japan and Dakewe for Hong Kong and China) (S10), with non-UK kit sales comprising 80%. Approximately 43% of OET's sales income ([text removed for publication]; 27% turnover, S3) over the assessment period derives from kit & product sales and, of this, approximately 40% through distributors and 60% direct sales. To access the American markets, the technology was licenced under Original Equipment Manufacturer agreements to [text removed for publication] and continues to be sold under the brand name [text removed for publication].

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 approximately 43% [text removed for publication] of sales income (27% 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, enable them to outsource when in-house facilities are at capacity, or use OET's expertise for complex or challenging projects. Since August 2014, OET has worked with >100 companies including [text removed for publication] plus government laboratories such as [text removed for publication] and many UK and global universities. We have enabled these companies and institutions to advance their drug discovery, diagnostic kit and/or vaccine development programmes by producing proteins more quickly and efficiently, and to increased yields and quality more than was possible previously. Companies utilizing OET technology are based in Europe, North and South America, South East Asia, Australia, the Middle East and China, as well as in the UK.

An increasing proportion of OET's income comes from license deals to enable companies or organisations to use OET's technology for commercial use including clinical trials for vaccine development (e.g. [text removed for publication]), Adeno-associated virus gene therapy vector production (e.g. [text removed for publication]) and multiple licences for commercial protein production (e.g. [text removed for publication]). A specific example of the licensed use of the technology has been in the development of vaccines for [text removed for publication], which have progressed to Phase 1, 2 and 3 clinical trials, respectively. Commercial protein production varies from simple preparation of proteins for sale to use of proteins in *in vitro* diagnostic assays (e.g. [text removed for publication]). Licence sales income accounted for 14% of OET's sales income (8% of turnover; S4) in the designated period with individual licences ranging from [text removed for publication].

5. Sources to corroborate the impact

S1. OET website – www.oetltd.com

S2. OET staff organograms 2013 and 2020 to show increase in staff over the period

S3. OET finance statement to show income by kits, services, licence and R&D income and relative proportions over time per year 2013-2020 and in total over qualifying period

S4. Exemplar list of publications citing use of flashBAC to make proteins 2014-2020; Google Scholar = 359, 2014-2020

S5. List of baculovirus products in kit format; also https://oetltd.com/shop-baculovirus-expression/

S6. [text removed for publication] who licenced OET's technology to make a vaccine to Covid-19

S7. [text removed for publication]

S8. [text removed for publication]

S9. [text removed for publication]

S10. Map to show distributors of flashBAC worldwide; also https://oetltd.com/about/distributors/