Institution:
University of Cambridge

Unit of Assessment:
UoA5

Title of case study:
Improved efficiency for derivation of mouse embryonic stem cells: reducing use of animals and saving costs in life sciences

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

Mouse disease models provide an invaluable tool to the medical sciences, underpinning the understanding of disease mechanisms and the development of therapeutic interventions. A new cultivation protocol for deriving mouse embryonic stem (ES) cells was developed by Dr Nichols between 2006 and 2009. This has facilitated the production of ES cells from disease model mice that can be manipulated in vitro and used to establish modified transgenic mice with the required genetic profile, in a single generation. This method reduces the number of mice needed, as well as associated costs and staff time, by 90%. Dr Nichols has trained industry delegates from international transgenics companies and transgenic facility managers in the new technology. As a consequence, a minimum of 26820 fewer mice have been used in experiments, and a minimum of £536k have been saved since 2009.

2. Underpinning research (indicative maximum 500 words)

Dr Jennifer Nichols, Assistant Director of Research at the Wellcome Trust Centre for Stem Cell Research (CSCR) since its foundation in 2006 (previously at the University of Edinburgh) heads a laboratory investigating embryonic pluripotency (the ability of stem cells to differentiate into all cell types in the body). While in Edinburgh, Dr Nichols started to investigate how pluripotent cells in the embryo can be captured and propagated efficiently in culture as embryonic stem cell lines.

On arriving at Cambridge, between 2006 and 2008, Dr Nichols and co-workers at CSCR (Prof. Austin Smith, Director of CSCR since 2006, and team) investigated potential differences in lineage allocation and response to culture regimes between embryos from permissive and recalcitrant strains of mice during diapause and ES cell derivation (Ref. 1, Section 3). The research suggested that strain-specific recalcitrance, for ES cell derivation could be reduced by blocking the Erk pathway.

Work by Prof Smith, Dr Nichols and co-workers exploited this observation: In 2008, they combined Erk inhibition with GSK3 inhibition to develop the medium known as ‘2i’ for ‘2 inhibitors’ which allowed stable propagation of ES cells (Ref. 2, Section 3).

In 2009, Dr Nichols lead research at CSCR, which demonstrated that blocking the Erk pathway in cleavage stage mouse embryos causes an expansion of the epiblast population (the founder of the foetus and ES cells) at the expense of the hypoblast (an extra-embryonic tissue required for patterning and differentiation of the epiblast), and dramatically increases the efficiency of ES cell derivation (Ref. 3, Section 3).

The knowledge that strain-specific recalcitrance for ES cell derivation can be overcome by blocking the Erk pathway (Ref. 1, Section 3), and that a combination of Erk and GSK3 inhibition enables efficient and stable propagation of ES cells (Refs 2 and 3, Section 3) provided the foundation for the development of the optimised culture regime for deriving ES cells from embryos of the non-obese diabetic (NOD) mouse model for Type1 diabetes in 2009 (in a project jointly led from 2008 to 2012 by Dr Nichols and Prof Anne Cooke, Professor of Immunobiology from 2000, Department of Pathology; Ref. 4, Section 3). The technique developed by Dr Nichols and Prof Cooke allowed genes to be manipulated directly, enabling mice with the required genetic profile to be bred within a single generation, dramatically reducing the number of mice used from ~200 to 20 per gene and the time taken from ~2 years to 10 weeks to assess the result, as well as the associated research cost by a factor of 10.
The alternative method, backcrossing, even at 10 generations never eradicates the initial genetics entirely; this applies not only to the region neighbouring the allele of interest. Further functional advantages of the 2i method include that the ES cells are derived in the ground pluripotent state and therefore compared to ES cells grown in serum conditions; the ES cells grown in 2i (which is

serum-free) have characteristics that more closely resemble the developing preimplantation embryo. The serum-free 2i recipe also reduces the risk of batch-to-batch variation compared to medium with serum.

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

1. Batlle-Morera L, Smith AG, Nichols, J (2008). Parameters influencing derivation of embryonic stem cells from murine embryos. Genesis 46, 758-767. http://onlinelibrary.wiley.com/doi/10.1002/dvg.20442/abstract;jsessionid=5AD7985126BD69C9266EF0FBFE106872.d02t01 DOI: 10.1002/dvg.20442
2. Ying, Q-L., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., Cohen, P. and Smith, A. (2008). The ground state of embryonic stem cell self-renewal *Nature*, 453, 519-523 http://www.nature.com/nature/journal/v453/n7194/abs/nature06968.html DOI:10.1038/nature0696
3. Nichols J, Silva J, Roode M, Smith A (2009). Suppression of Erk signalling promotes ground state pluripotency in the mouse embryo. Development 136, 3215-3222. http://dev.biologists.org/content/136/19/3215 DOI:10.1242/dev.038893
4. Nichols J, Jones K, Phillips JM, Newland SA, Roode M, Mansfield W, Smith A, Cooke A. (2009) Validated germline-competent embryonic stem cell lines from nonobese diabetic mice. Nat Med 15(7):814-818. http://www.nature.com/nm/journal/v15/n7/full/nm.1996.html DOI:10.1038/nm.1996

Funding

5. MRC grant awarded to Prof A Smith. Molecular basis of stem cell self-renewal. Amount: £924,232. Period: 2006-2010
6. Wellcome Trust grant awarded to Dr J Nichols & Prof A Cooke. Strategies for replacing the beta cell mass in type 1 diabetes. Amount: £794,306. Period: 2009 - 2012
7. Wellcome Trust grant awarded to Prof A Smith & Dr J Nichols. The road from pluripotency to lineage determination. 1/6/10-31/5/15, Amount: £1,736,222. Period: 2010-2015

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

Impact on Animal Welfare / 3Rs:

- The number of animals used in biomedical research relating to polygenic diseases has been substantially reduced as a result of Nichols' work. In order to calculate the impact of this new method on the number of animal lives saved, Nichols contacted all those whom she has trained in the technique, and/or who have received relevant ES cell lines and protocols from her lab. Of 80 people contacted, 36 responded (45% response rate). Since the protocol's release, 149 transgenic modifications have been developed *in vitro* by those who responded to the survey, using ES cells derived directly from the mouse strain/genotype of interest (using only 20 mice per gene), rather than through backcrossing to the required strain (requiring 10 generations, and around 200 mice per gene).
- Hence per gene, 180 fewer mice needed to be used: so in total an estimated 26820 (149x180) mice were saved due to Dr Nichols' methodology. In reality the number is likely to be greater, when taking into consideration the research of those who did not respond.

Ref. 1 in Section 5 provides evidence of where the technology has been taken up.

- In 2009, in recognition of her original contribution to scientific and technological advances in the 3Rs, Dr Nichols was awarded the annual prize of the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs; an independent scientific organisation, tasked by Government to encourage and reward high quality research which has a positive impact on the use of animals in the life sciences) (Ref. 2, Section 5).

Impact on Practitioners:

Dr Nichols has since 2009 trained practitioners from biomedical companies, transgenic facilities and research institutions, teaching the new methodology both to individual external visitors to her lab, and through bespoke courses (Refs 3-5, Section 5). Training activities with global reach

(Europe, North and Latin America) included:

1. 'Derivation of ES cells in 2i medium' workshop, Cambridge 5-7 July 2010 (Ref. 3, Section 5): Dr Nichols' NC3Rs prize included a grant of £10k, and she used these funds to run a workshop at the Department of Pathology and Clare College to train 28 delegates (7 from industry, 21 from research institutes) across the world in this ground-breaking technique. The participants comprised:
   Managers who provide transgenic expertise in international companies: Polygene (Switzerland; 1 participant), Stem Cell Sciences (US and Europe; 2 participants), Regeneron (US; 1 participant) other industry representatives at the workshop included Leica (1 participant), Millipore (1 participant) and HamiltonThorne (1 participant)
   Transgenic Facility Managers from Spain, the UK, the US, Switzerland, Hungary, Italy, the Netherlands, Sweden, Finland, Germany, Denmark and Austria
2. Latin American "ES cells as a model system for embryonic development" courses, Brazil 6-21 February 2009 & Mexico 27 February-17 March 2011 (Refs 4-5, Section 5): a total of 44 delegates from research institutions were trained by Dr Nichols during these two-week long courses.
3. 8 visitors to CSCR have been trained in the technique by Dr Nichols since 2009 (Ref. 1, Section 5).

Dr Nichols contacted all those who have participated in the above training activities (80 in total); of the 36 respondents 30 indicated that they are now using the 2i protocol (83%), and one has included the methods taught by Dr Nichols into their own teaching programmes (Ref. 1, Section 5).

Commerce and economy

A business has adopted a new technology; performance and operation have been improved

The company Polygene have been trained by Nichols in the 2i methodology, and now use it routinely. They testify that "this has saved the use of ca 16200 mice for us […] 2i helped to improve the success rate of ES cell derivation […] we were able to reduce injection days per project […], since using 2i we are able to give guarantees on [..] germline transmission." (Ref. 6, Section 5)

New products commercialised; positive impact on performance and employment

The work of Dr Nichols and colleagues on the 2i methodology enabled the company Stem Cell Science in 2009 to bring two new 2i-based culture media products to market (GS1-R and GS2-M). They testify that this has had a positive effect on business performance and employment in their company (Ref. 7, Section 5).

A business has invested into R&D

The biotech company Crescendo Biologics Ltd have generated ES cells using the 2i method, and are evaluating their use for screening and for cryostorage of transgenic lines, as an addition to embryo cryopreservation (Ref. 8, Section 5).

Cost savings in medical R&D

Because mice with the required genetic profile for studying the link between genotypes and disease mechanisms can now be bred within a single generation (ie in 10 weeks, compared to ~100 weeks previously for backcrossing, needing 10 generations), the associated research costs have also been reduced by a factor of 10. Based on the data available from the responses to our survey (Ref. 1, Section 5), and on cage costs in Cambridge, a minimum of £529k within the eligible period is estimated to have been saved. This was calculated as follows:

Previously, using back-crossing, for each gene 20 mice had to be housed for 100 weeks, now only for 10 weeks. This **saves 90 weeks** of cage costs for 20 mice per gene. To provide a conservative estimate, full occupancy is assumed (5 mice/cage), ie **4 cages** for 20 mice. Ie the cost of 90*4=**360 cage-weeks** has been saved per gene. A cage-week in Cambridge costs £10 (this number will vary between institutions and countries, but serves as an indicative value), ie **£3600** have been saved **per gene**.

As indicated above, our survey has identified that so far **149 genes** have been bred into mice models using Nichols' 2i methodology. This equals £3600*149=**£536400** in cage costs alone that have been saved.

5. Sources to corroborate the impact (indicative maximum of 10 references)

1. Spreadsheet of 2i-trainees / users contacted, and their responses
2. Press release from NC3Rs on Nichols award, http://www.nc3rs.org.uk/news.asp?id=1203
3. "ES cell derivation" 2010 workshop: programme

4. "Embryonic stem cells as a model system for mammalian development " course: Brazil 2009 course report
5. "Embryonic stem cells as a model system for embryonic development" course (www.escellslatinamerica.org): Mexico 2011 course report
6. Letter from Executive Director Business & Development at PolyGene AG
7. Letter from Executive Vice President at Stem Cell Sciences PLC
8. Letter from Head of Transgenic Platforms at Crescendo Biologics Ltd