Refinement of Genetic Engineering Procedures

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Summary

During the development of the new Canadian Council on Animal Care (CCAC) guidelines five main concerns were raised: the invasiveness of the procedure, the high number of animals used, limits to genetic engineering, unanticipated welfare concerns, and accurate reporting of animal numbers. As transgenic laboratories around the world continue to develop and implement new technologies, it is important that we look to these new technologies to address the concerns raised in the CCAC guidelines, as well as to help refine our procedures for implementing the 3Rs.

Keywords: transgenic, genetic engineering, refinement

The creation of new genetically modified animals has increased exponentially over the last ten years. This is due, in large part, to the continued development and advancement in the technology involved in creating genetically modified animals. Although the continued development of new technologies to create genetically modified animals is useful in creating more sophisticated experimental animal models, it also is important to look at and develop new technologies that will help us implement the 3Rs.

Transgenic laboratories often are at the cutting edge of new technology when it comes to creating genetically modified animals. Since this is the case, they also should be among the first groups to look at the technology available that allows them to modify and refine their procedures in the implementation of the 3Rs. The 3Rs transgenic laboratories can have the biggest impact when working either to reduce their animal numbers or refine their procedures to become less invasive. Although a large number of developing technologies may be implemented, this discussion will address only five of the newest ones available, with a brief discussion of their advantages and disadvantages. The five technologies I would like to discuss are: zinc finger nucleases and transcription activator-like effector nucleases (TALENs) to create gene targeted rodents, NSET – non surgical embryo transfer, deathless transgenesis, Internet database searches, and genotyping genetically engineered animals.

Zinc finger nucleases and TALENs belong to a class of DNA-binding proteins or artificial restriction enzymes that facilitate targeted editing of the genome by creating double-strand breaks in DNA at user-specific locations. There are several advantages to using this technology. The first is unlimited species possibilities for creating genetically modified animals. Until now, only animals that had established ES cell methods and cell lines could be targeted to create disease-representative animal models. However, zinc finger nucleases and TALENs do not depend on creating new ES stem cell lines but instead use the host genome information for targeting, and once the sequence information for a species is known it can then be targeted. The mutation rate is also very robust. Mutation rates at around 10-15% have been reported, while traditional ES cell technology often runs only 1-4%. This improved efficiency in mutation targeting rates will help reduce animal numbers. Lastly and most importantly, this technology has been demonstrated to be heritable through the germ line. Once targeted animals have been created and identified, they can transmit the desired mutation with standard Mendelian genetics to their offspring. Some disadvantages must be taken into consideration, however. First, this is a new technology that is still in development. Second, future developers likely will be faced with patent and governmental regulation issues.

NSET is a simple method of transferring embryos non-surgically to the uterine horns of the mouse (Fath-Goodin et al., 2006). Briefly, embryos are loaded using a modified capillary tube and transferred through the cervix into the uterine horn of an unanesthetized mouse. The advantages to this technique are several, including, elimination of the pain and distress of surgery, thus completely removing the need for anesthetics and analgesics. It also eliminates the need for post-surgical monitoring and reduces the regulatory burden on laboratories and institutes. This is a valuable savings in staff time, which translates to a decrease in costs. This is a significant savings, since staff salaries are often are cited as the most costly expense to laboratory institutes. The only drawback to this technology is that the technology itself is expensive, since each individual unit costs $ 25. Hopefully, as this technique is established in a larger number of labs the increased demand could reduce the costs to purchase this product.

Deathless transgenics is a common name for a new spermatogonia-mediated *in vivo* approach for the generation of transgenic mice (Majumdar et al., 2008). It is an alternative method to generating transgenic animals. The technique in-

volves transfecting genes into repopulating undifferentiated spermatogonial cells through *in vivo* electroporation of the testis in male mice. With this technology more than 90% of electroporated male mice reportedly sired transgenic pups. The clear advantage of this technology is that it would dramatically reduce the number of animals used to generate transgenic founders. Traditional microinjection requires the use of donor females, stud males, vasectomized males, and pseudopregnant females. Using electroporation to transfer DNA directly to male mice would eliminate the need for these other colonies and dramatically reduce the number of animals used to generate transgenic founders. It also could offer huge cost savings after the initial investment in the equipment, since the cost of breeding or purchasing these mice would be unnecessary. The major disadvantage is that the protocol is still in development and has not been widely established.

Internet resource sites are among the most valuable tools available to us, but they remain seriously underutilized. Several sites can be very easily searched to see whether the animal model of interest has already been targeted or even created. The obvious advantage, of course, is that the targeted gene may already exist, and the animal model doesn't need to be duplicated or recreated. A search of this sort can be done very quickly with only a minimum of time and no real costs. Often the institutional transgenic laboratories at the research centers are very familiar with how to search these sites and can easily help researchers navigate these websites successfully. Most of these organizations have been funded by governmental organizations, so they are constantly expanding their stock of reagents and mouse lines. Therefore, if the animal model of interest does not exist at the time of the initial search, the sites should be rechecked on a regular basis. Some of the sites that can be searched are: www.knockoutmouse.org (IKMC), www.informatics.jax.org (MGI), www.mmrc.org (MMRC), www.findmice.org (IMRS).

Genetically engineered animals are now being used in ever increasing numbers. Developing efficient methods to manage these colonies is important in light of the increasing complexity and variety of genetically engineered rodents produced. One of the most important questions we can ask is: how can we accurately and efficiently genotype mice in order to maintain reasonable-sized breeding colonies and generate the correct rodents for specific experiments? The two tools we have available to us to determine the genotype of a mouse are genomic Southern blots and genomic PCRs. A genomic Southern blot refers to the use of probe-digested genomic DNA with a specific sequence of DNA that has been labeled with radioactive nucleotides. A genomic PCR (polymerase chain reaction) occurs when we amplify a small piece of genomic DNA using gene-specific oligonucleotides. Genomic PCRs have many advantages. First, well optimized PCRs are very accurate, and results can be obtained very quickly, often in 1-2 days. Second, they are very cost-efficient and not very labor intensive. The one disadvantage is the risk of false positives. This can be verified with well-designed experimental controls, however. A genomic Southern blot is advantageous in that it is the most accurate test we have. The Southern blot's very high labor and reagent costs, together with the long turn-around for results, however, make the PCR the screening the method of choice, given the increasing size and complexity of our animal colonies. One disadvantage of the need to genotype our animal colonies is that all the techniques used to isolate DNA include some form of amputation from the animal. An important refinement to our techniques would be to explore novel and non-invasive techniques to genotype our animal colonies (Hamann et al., 2010). Some potential new techniques involve either collecting fecal samples or using buccal swabs to isolate genomic DNA. These techniques are still in early development, and some optimization is still needed. They present many advantages, however, such as, the possibility of isolating the DNA solution in an hour, taking multiple samples at different times from the same animal, and a huge reduction in animal discomfort and distress.

In this presentation on refinement of genetic procedures in the creation of genetically modified rodents I have discussed several emerging technologies that can help us implement the goals of the 3Rs. As we increase the numbers and complexity of our use of genetically modified rodents, it is important that we continue to search for new ways to use technology to implement the CCAC guidelines and to develop and promote the 3R concept: Replace, Reduce, and Refine.

References

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