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This edition of the *BRL Bulletin* is dedicated to rodent genetic monitoring. Genetic monitoring is essential when breeding inbred and transgenic animals to verify that there is no genetic contamination that can adversely affect research outcomes. It also will introduce Transnetyx Inc., a company that provides genotyping services with drop-off stations in the BRL and COMRB facilities. More information about Transnetyx Inc., will be provided below.

Genetic Monitoring

Genetic monitoring is the process of examining molecular markers to identify an animal's genetic make-up. This technique is commonly performed in laboratory rodents, especially mice. Genetic monitoring is essential for quality control when breeding inbred and transgenic animals. Any genetic contamination or genetic drift over time can alter research results and interpretation. Therefore, accurate genotyping is necessary for obtaining quality research data.

Commercial rodent vendors adhere to strict protocols and maintain detailed records to prevent genetic contamination in their founder, expansion and production lines. Pedigrees are used to document the genotypes of the foundation mating pairs as well as their progeny. Relatives can be easily identified and traced back to their origin. Vendors use pedigrees to document the breeders and maintain all lines. Commercial vendors perform routine quality control by systematically screening genotypes, verifying the genetic backgrounds of the animals, verifying mutant alleles in mutant stocks, and culling of any animals with phenotypic changes.

The goals of genetic monitoring vary depending on whether the animals are outbred stocks, inbred strains, and genetically manipulated rodents. Outbred stocks are genetically diverse and are maintained by mating non-related animals to retain maximum heterozygosity. Therefore, the goal with genetic monitoring of outbred stocks is to preserve the heterogeneity and prevent formation of sublines. Strains are considered inbred if they have been maintained by 20 or more sibling matings. The goals of monitoring inbred strains are to ensure a consistent genetic profile and to detect or eliminate mutations. The purpose of monitoring genetically manipulated rodents is to identify the presence or absence of the gene of interest as well as to verify the background strain, which may influence gene expression.

Sources of Genetic Contamination

Genetic contamination can occur in several ways. The most obvious source of contamination is the introduction of new genes by inadvertent mixing and breeding of animals. This can happen if animals or cages are not properly identified, if animals are mixed during transport or housing, or by false genotyping results. Other sources of genetic contamination include residual heterozygosity and substrain divergence leading to genetic drift over time. Although inbred strains are considered to be genetically stable, random gene mutations can still occur. Even a small source of contamination can affect research data and limit the ability to replicate the study. Routine genetic screening can help to identify and prevent contamination, which in turn can help save time and money in the future.

Animal Identification

In order to perform genotyping, it is important that animals are first properly identified. There are several types of identification methods that have both advantages and disadvantages. Identification is often performed with ear punches or ear tags, while more permanent methods of identification can be accomplished

with tattoos or microchips.

Ear punches or notches are a common method of identification in rodents. This technique is relatively easy to perform and is inexpensive. A universal numbering system can be used to achieve a unique number for each animal in the colony. Using this universal numbering system requires skill both in performing the ear punch as well as reading the ear punches. An alternative method is to use a combination of punches only to identify the animal at the cage level. It is important to consider that ears can be torn and punches can heal closed making identification difficult if using this method.

Another common method of identification is to apply a metal tag to the animal's ear. Like ear punches, this is relatively easy to perform and inexpensive. This method can provide a unique number for each animal in the colony. Disadvantages of this method include ear tags falling out, localized inflammation associated with the ear tag, or being torn out by the animal or its cage mates. One helpful tip is to place even number tags in the right ear and odd number tags in the left ear so that in the event that two animals in the same cage lose tags, there is a better chance of identifying the correct animal.

A permanent method of identification is to tattoo a unique identifier on the tail of the animal. This is often time consuming and more expensive than ear punches or ear tags. There are automated commercial tattoo machines available that can save time and produce reliably readable results. Tattoo ink is available in a variety of colors as well as fluorescent ink that can be visualized with a black light on pigmented skin. The BRL has a tattoo device that is available for rental and training can be provided by BRL staff.

Microchips are small devices that contain a unique identification code and are implanted under the skin. This technology is commonly used in larger species, but it is also available for rodents. A reader is placed over the location of the microchip which displays the unique identification code. These systems tend to be more expensive due to the initial cost of the reader, software, and the cost of the microchips. Newer transponders can also measure subcutaneous body temperature, which may be useful for certain studies.

The Jackson Laboratory has recently released a new identification system (JAXTag) that uses a modified ear tag. The tag contains a barcode that is read by commercially available scanners. Like microchips, they require larger initial costs associated with the scanner and software. The advantages of this new system include that the tags are light-weight, non-irritating and secure for more than 18 months.

Monitoring Methods

There are several methods used to monitor genetic contamination including physical characteristics, biochemical and immunological techniques, and DNA-based techniques. Commercial vendors such as Charles River, Harlan, Jackson Labs, and Taconic offer genetic monitoring services. Below is a brief description of available methods utilized to screen for genetic variation.

Physical Characteristics

It is important to be familiar with the physical characteristics of rodent research models in order to identify changes over time. The most obvious change that can occur with genetic contamination is coat color. Other indications of genetic contamination may include an increased rate of abnormalities such as tail defects, eye defects, and seizures as well as changes in body size, weight, level of aggression, reproductive performance and lifespan. Being in tune with the normal variation among the strains used can help quickly identify a problem if one arises.

Biochemical Markers and Immunological Techniques

Inbred mice and rats may have variations in

specific biochemical and immunological markers that can be used to monitor genetic contamination. There are panels available to evaluate strain-specific distribution patterns of particular markers. Examples of biochemical and immunological markers include erythrocytic antigens, hemolytic complement, isoenzymes, and the major histocompatibility complex. Most of these methods can be performed using blood or tissue samples. Another immunological technique to determine genetic contamination is by performing skin grafts. This technique detects any genetic changes in histocompatibility. Animals that are genetically identical will not reject a graft. A major change in histocompatibility causes rejection within 10 days; however, minor changes can take months to detect. It is also important to keep in mind that graft failure may occur for technical reasons.

DNA-Based Molecular Techniques

There are several types of DNA-based molecular techniques available to perform genetic monitoring. The most common techniques include standard polymerase chain reaction (PCR) or real time PCR. Each technique has a specific use so it is important to have an understanding of the different tests that are available.

PCR is one of the most common methods for determining genetic variability. PCR has now replaced most of the biochemical and immunological tests described above. Current PCR technology uses amplification of single nucleotide polymorphism (SNPs) markers. A SNP is a variation of a single nucleotide in the genome that differs between members of a species. Typically, SNPs occur in the non-coding regions of a DNA sequence. This method is helpful to evaluate multiple markers on different chromosomes, which can be used to monitor for changes in genetic background. This technique can also be used to verify that an induced point mutation is still present.

Real time PCR is a method that quantifies the reaction, unlike standard PCR. There are two methods to quantify the amount of nucleic acid present. Absolute quantification determines an exact number by identifying specific target DNA molecules while relative quantification is based on the amount of DNA compared to internal reference genes. Both of these methods are helpful for differentiating a hemizygote from a homozygote and in determining transgene copy number, which can dramatically affect the phenotype.

Another PCR technique involves the use of microsatellites, which are short repetitive DNA sequences that contain unique flanking regions. The number of repeats is highly polymorphic and there are thousands contained within the mouse and rat genomes. Analysis of microsatellite locations can be used to characterize background strains as well as estimate genetic variation among animals. Panels have been developed for the most common inbred strains, but these panels can also be customized for other strains.

Tissue Collection for DNA-Based Methods

Tail snipping is a common method to collect tissue for genotyping. Using this technique, 5mm of the distal tail tip is removed and bleeding controlled by pressure, electric or chemical cautery methods. Tail snips can be performed in mice less than 28 days of age without anesthesia. Mice older than 28 days require general or local anesthesia. Repeated tail snips on the same animal is discouraged. Please see *UIC Guidelines on Tail Snipping in Rodents* for further details. Other samples that can be used for DNA-based methods include ear punches and oral swabs. These are often used as alternatives to tail snips in older rodents.

Genetic Contamination Prevention Strategies

Accidental mismatings can lead to the introduction of undesirable genetic material and can confound research. There are several strategies that can be instituted to prevent genetic contamination. First, it is important to

examine animals routinely for physical changes. Observation of any phenotypic or behavioral changes warrants investigation to identify accidental genetic contamination. Ideally, all animals undergoing experimentation should be genotyped. Animals should be properly identified with appropriate genotypes on cage cards and any other records. Pedigrees are useful in documenting and maintaining breeding lines. Other management strategies include organizing colony animals by genotype and separating cages with similar coat colors and genotypes to prevent inadvertent mixing of animals.

All laboratories breeding rodent strains should have a genetic monitoring program in place. There are several items to consider prior to starting a program. First, it is important to develop baseline genetic information about each strain that is being used. The availability of certain tests along with the goals of the specific laboratory will determine the type of test best suited for routine genetic monitoring. Once the appropriate tests are identified, the next step is to determine how many animals should be sampled, which depends on the size and scope of the animal colony. If breeding heterozygotes, testing is typically performed on every litter but if breeders are homozygotes or inbred strains, a minimum of testing one to two times per year is recommended. Also, large breeding colonies may require more intense screening of the foundation stock than the production lines, which can be randomly sampled. A well-devised genetic quality control program should not only encompass genotyping animals, but also comprehensive training of staff and application of good colony management strategies.

Transnetyx Automated Genotyping (TAG)

Transnetyx Inc. is a company that provides genotyping services with two drop-off stations. There is a TAG center located in the BRL (between rooms 123 and 125) and another TAG center located in COMRB (outside entrance) where samples are submitted and sent out for testing. The online ordering system allows you to place orders, manage your genetic strain information and assays, as well as view and manage your genotyping results. There are two pick up times: Wednesday by 2pm (results the following Tuesday AM) and Friday by 2pm (results the following Thursday AM). Transnetyx provides free sample submission supplies as well as free shipping. Charges are billed through the BRL billing system. Before getting started, it may be helpful to view the online ordering system and fee structure at http://www.mailyourtail.com/UIC/Default.aspx. If you have any questions or would like to consult a Transnetyx representative, please use the following contact information:

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