Introduction to genetic monitoring

by *Michael F. W. Festing,*
MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey, UK.

Why is genetic monitoring necessary?

There have been numerous examples of non-authentic or genetically-contaminated laboratory rats and mice being supplied for research. Some of these were reviewed by *Festing* (1982). Since then there have been several other examples. *Kahan et al* (1982) noted genetic contamination of "BALB/c" mice supplied by a commercial breeder in the USA. *Lovell et al.* (1984) found that two strains which were supposed to be congenic with C57BL/6 and DBA/2 were genetically segregating at several loci, Pennline et al. (1982) found genetic contamination in two rat colonies, and *Gubbels et al.* (1985) found that nude mice which were supposed to be on an inbred BALB/c genetic background had become genetically contaminated. A survey of genetic markers in 93 colonies of inbred rats summarized by *Festing & Bender* (1984) found at least two cases of apparent genetic contamination, and several more cases where nominally identical inbred strains differed. There was also some confusion over nomenclature, as none of the four inbred SD rats were the same, presumably because they had been derived from different outbred Sprague-Dawley colonies. Thus, it is clear that genetic quality control of some sort is essential if there is to be any hope of comparing different studies.

How can genetic monitoring be done?

1. *Technical methods*

Many of the technical methods have been described by *Nomura et al.* (1984), and will not be discussed in detail here. The polyvalent alloantisera method has been described by *Festing & Totman* (1980). Technical methods for DNA fingerprinting, which may also be suitable for single-locus DNA probes are described in detail by *Wells* (1988). More than 200 DNA probes associated with restriction fragment polymorphisms in the mouse are listed by *Elliott* (1989). These provide good markers on all chromosomes, though it is not always clear whether all probes are available. DNA methods will become considerably more convenient when non-isotopic methods are fully developed. Already one DNA fingerprinting kit based on probe 33.6 (*Jeffreys et al.* 1987), and using non-isotopic labeling is available ("Snap DNA fingerprinting kit", Molecular Biosystems Inc, 10030 Barnes Canyon Road, San Diego, CA 92121), and has been used to monitor inbred rat and mouse colonies (*Kurtz et al.* 1989).

Current monitoring methods include:

a) Methods which monitor individual loci.
   These methods are likely to be most accurate and flexible. Critical sub-sets of loci can be chosen, depending on circumstances, but the methods are expensive and labour intensive. These methods include:
   1) Biochemical markers
   2) Immunological markers
   3) DNA restriction-length polymorphisms
   4) Coat colour markers
b) Methods which monitor several loci simultaneously.
   These methods appeal as several loci are compared simultaneously. However, if a genetic contamination is observed, it may be impossible to discover the source of the contamination as individual loci can not be studied. Morphology and breeding performance do not really have the accuracy that can be achieved by the other methods. The methods include:
   1) Skin grafting

2) Polyvalent alloantisera
3) DNA finger-printing
4) Morphology
5) Breeding performance

2. *Operational requirements*

a) Cost
The cheapest methods are usually the least accurate. There may be a case for using a relatively quick and economical method such as polyvalent alloantisera for routine monitoring in cases where contamination is unlikely, backed up by the more expensive single locus methods for when some problem is suspected.

b) Speed
Most laboratory techniques take a few hours to a few days. Skin grafting, and litter size and coat colour studies which involve breeding can take a few weeks. The ideal technique would take 2–3 hours, and only some biochemical and immunological markers (including polyvalent immune serum) can be done so quickly. DNA methods have the potential to be speeded up to take about one day.

c) Accuracy
Biochemical, immunological and DNA methods are of comparable accuracy. With rigorous laboratory technique none of these should give false positive, and few should give false negative results under most operational conditions.

d) Live animal
DNA-based methods can conveniently be done on live animals, as only a few cm of tail provides ample DNA. Some biochemical methods can be done on blood, but many require kidney, liver or other vital organ. Most immunological methods require lymphocytes which can be obtained in small quantities from a live animal, though it is inconvenient.

e) Technical simplicity
The most accurate methods (biochemical markers, DNA methods) are usually technically difficult. Skin grafting is technically simple, and highly accurate at showing whether a strain is inbred, but has other disadvantages such as the time needed to obtain the answer.

f) Type of strain
Most methods are suitable for inbred strains and their derivatives. Single-locus methods are almost essential for monitoring outbred stocks, where genetic drift (*Papaioannou & Festing* 1980) and genetic differences between nominally-identical stocks can sometimes be demonstrated (*Yamada et al.* 1979).

g) Need for confirmatory tests
Some methods such as routine monitoring of litter size may give an indication of genetic contamination (e.g. a sudden increase in breeding performance in an inbred strain), but would require other methods to confirm genetic contamination. Most methods are sufficiently accurate to avoid the need for such confirmation, provided laboratory errors can be ruled out.

Who should do the monitoring?

At present, genetic quality control is mostly carried out in-house by laboratory animal breeders. They can only test a sample of their animals, and quality assurance programs are costly and require considerable skill. There may be a good commercial opportunity for independent laboratories to offer genetic monitoring to both breeders and users. This would be most practical if the monitoring could be done without having to transport live animals. DNA based methods have an obvious advantage in this respect, since mouse and rat tails could easily be sent through the post.

There may be a case for users to monitor batches of animals used in long-term studies such as aging or carcinogenesis trials where the investment in the animals at the end of the study may be very great, particularly if genetic authenticity is critical.

Which strains should be monitored?

Most monitoring methods can be applied efficiently to inbred strains. Immunological methods, and possibly specific DNA probes may be needed to distinguish among sets of congenic strains which only differ at a few loci. Morphological methods based on bone shape are often able to distinguish between sublines more efficiently than other methods (*Festing* 1973), though hyper-mutable mini-satellite loci may now be more useful for such studies (*Kelly* 1990).

Whether or not it is useful to monitor outbred stocks is open to debate. With larger species, where inbred strains are not available, genetic information could be of value in maintaining the stock, particularly in assessing and avoiding inbreeding. Such methods are likely to be of particular importance with zoo animals. With outbred rats and mice, monitoring may be counter-productive in that it may imply a degree of respectability that some would dispute (e.g. *Festing* 1987, 1990).

When should colonies/batches of animals be monitored?

When new colonies are established, or when genetic contamination is suspected, some sort of genetic monitoring will usually be needed. However, the need for routine monitoring will usually depend on the circumstances of the individual colony, and in particular on the risk of contamination. A single inbred strain maintained in an isolator has virtually zero risk of getting contaminated, whereas several albino inbred strains maintained in the same animal room may be a great risk.

Sample sizes also pose problems. Clearly, at present it is impossible to monitor every animal, and in most cases, because of cost, it is only possible to monitor a minute fraction of the animals. It is difficult to devise criteria for deciding on optimum sample sizes and frequencies, but these again will largely depend on the risk of contamination of an individual colony.

What technical advances can be expected?

DNA-based methods are only beginning to be used, and substantial technical advances can be expected. Use of the polymerase chain reaction (*Saiki et al.* 1988) may mean that monitoring could be carried out on just a few cells within a single day, without the need for blotting and probing of membranes. Such methods have already been developed for screening humans for deleterious genes such as that causing cystic fibrosis. Whether other techniques based on entirely different principles will emerge remains to be seen, or whether DNA-based methods will make all other methods obsolete remains to be seen.

References

Elliott, R.: DNA restriction fragment variants. Mouse News Letter 1989, *83,* 126–148.

Festing, M. F. W.: Contemporary issues in Toxicology: Use of genetically heterogenous rats and mice in toxicological research. A personal perspective. Toxi. Appl. Pharmacol. 1990, *102,* 197–204.

Festing, M. F. W.: A multivariate analysis of subline divergence in the shape of the mandible in C57BL/Gr mice. Genetic Research Camb. 1973, *21,* 121–132.

Festing, M. F. W.: Genetic contamination of laboratory animal colonies: an increasingly serious problem. ILAR News 1982, *25,* 6–10. National Academy of Sciences Press, Washington DC.

Festing, M. F. W.: Genetic factors in toxicology: implications for toxicological screening. CRC Crit. Rewiews in Toxicology 1987, *18,* 1–26.

Festing, M. F. W. & Bender, K.: Genetic relationships between inbred strains of rats. An analysis based on genetic markers at 28 biochemical loci. Genetic Research., Camb. 1984, *44,* 271–281.

Festing, M. F. W. & Totman, P.: Polyvalent strain-specific alloantisera as tools for routine genetic quality control of inbred and congenic strains of rats and mice. Laboratory Animals 1980, *14,* 173–177.

Gubbels, E., Poort-Keesom, R. & Hilgers, J.: Genetically-contaminated BALB/c nude mice. Current Topics in Microbiol. Immunol. 1985, *122,* 86–88.

Jeffrey, A. J., Wilson, V., Kelly, R., Taylor B. A. & Bulfield, G.: Mouse DNA 'fingerprints': analysis of chromosome localization and germ-line stability of hypervariable loci in recombinant inbred strains. Nucleic Acids Research 1987, *15,* 2823–2836.

Kahan, B., Auerbach, R., Alter, B. J. & Bach, F. H.: Histocompatibility and isoenzyme differences in commercially supplied "BALB/c" mice. Science 1982, *217*, 379–381.
Kelly, R.: Personal communication 1990.
Kurtz, T. W., Montano, M., Chan, L. & Kabra, P.: Molecular evidence of genetic heterogeneity in Wistar-Kyoto rats: implications for research with spontaneously hypertensive rats. Hypertension 1989, *13*, 1.
Lovell, D. P., Totman, P., Bigelow, S. W., Nebert, D. W., Hoffman, H. A., Greig, J. B. & Festing, M. F. W.: An investigation of genetic variation within a series of congenic strains of mice. Laboratory Animals 1984, *18*, 291–297.
Nomura, T., Esaki, K. & Tomita, T.: ICLAS Manual for genetic monitoring of inbred mice. University of Tokyo Press 1984.
Papaioannou, V. E. & Festing, M. F. W.: Genetic drift in a stock of laboratory mice. Laboratory Animals 1980, *14*, 11–13.
Pennline, K., Smith, J. P. & Bitter-Suermann, H.: My Kingdom for an Inbred Rat. Transplantation 1982, *34*, 70.
Saiki, R. K., Gyllensten, U. B. & Erlich, H. A.: The Polymerase Chain Reaction. pp 141–152 in K. E. Davies (ed.) Genome Analysis. Oxford, Washington DC, IRL Press 1988.
Wells, R. A.: DNA Fingerprinting. pp 153–170 in K. E. Davies (ed.) Genome Analysis. Oxford, Washington DC, IRL Press 1988.
Yamada, J., Nikaido, H. & Matsumoto, S.: Genetic variability within and between outbred Wistar strains of rats. Experimental Animals 1979, *28*, 259–269.

Summary of discussion

Why should we do genetic monitoring?

There is a long history of both documented and anecdotal reports of genetic contamination of strains of laboratory animals. In all cases the results of contamination have resulted in serious damage to research projects. There have been reports of commercial breeders attempting to hide known cases of genetic contamination.

How do we do genetic monitoring?

There are several methods available. In general the principles of genetic monitoring resemble those seen in microbiological monitoring with respect to sample size.

In general methods are based on 2 main test principles:

1. Monitor individual loci (accurate, flexible – expensive and labour intensive).

a. Biochemical markers
b. Immunological markers
c. DNA restriction-length polymorphisms
d. Coat colour markers

2. Monitor several loci simultaneously (of value since several loci can be compared simultaneously – may be impossible to discover the source of contamination since individual loci can not be studied).

a. Skin graft
b. Polyvalent alloantisera
c. DNA finger-printing
d. Morphology
e. Breeding performance

Operational requirements are based on an evaluation of accuracy and cost. Several methods may be necessary. The cheapest methods are often not the most accurate. Most breeders do in-house monitoring, whereas users do not usually have the resources to monitor breeders. Users often can make use of specific research activities (immunology, genetics) that will often pick up a genetic contamination.

DNA probe technology appears to be the method of the future (long term).

Who should do genetic monitoring?

The breeder.

Most breeders do in house or contract genetic monitoring of commercially available strains. Their test programmes are limited by the same restraints encountered in microbiological monitoring (sample size, frequency).

The user.

Users who breed should do some form of monitoring. More important however is that strains should not become contaminated in the first place (management).

When should genetic monitoring be done?

An ideal opportunity is when a colony changes location. The nature of the management

of the breeding programme will also determine the need for monitoring (a breeding nucleus held in an isolator will not need monitoring). Specific projects will require repeated monitoring.

What technical advances can be expected?
Cheap and inexpensive methods will inevitably evolve. DNA based methods are beginning to play a role. Polymerase chain reaction methods may play a significant role in the future.

What are the consequences of genetic monitoring?
In general a contamination usually only influences a single strain (compare to a microbiological contaminant in which the disease will affect several or all of the animals).

How do you safeguard against genetic contamination/drift?
Genetic drift occurs at a much higher rate than expected – mutation of mini satellites (lengths of highly repeated DNA). To all intents and purposes, the only way to reduce genetic drift to a minimum is by frozen embryo technology. The cost of freezing strains is high and there are problems associated with the technology. If priority is given to embryo freezing, initial costs may be high but future maintenance costs will be low.

Should outbred stocks be monitored?
YES – we can BUT NO – we shouldn't.
Of importance is that commercial breeders should quote degree of heterozygosity associated with an given outbred stock.
For most purposes we should not be using outbred animals. It is scientifically more correct to use inbred strains when doing most forms of biomedical research. It is however important to collect hard data that supports this concept.
When using inbred strains instead of outbred stocks you should use general purpose strains. Special purpose strains should be used for specialized questions that would be best answered by use of an inbred strain.
The use of inbred strains raises another question: Why do strains differ? The answers to this question may induce users to narrow their choice of strain when starting an experiment.
In general however, it may be argued that one is able to reduce the number of animals used in an experiment by using an inbred strain rather than an outbred stock.
If outbred stocks are to be used, it is necessary to monitor the stock colony if one can't get a suitable inbred strain for the purpose of the experiment.

What about transgenic animals?
The researcher will monitor the transgene. The laboratory animal facility should monitor the background. Transgenics are often backcrossed to an inbred strain.

The defined animal – a definition.
The defined animal concept should be extended to include a definition of the genetic status.