Guidelines for the production and nomenclature of transgenic rodents

Report of the FELASA Working Group:

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Introduction

There has been a substantial increase in the use of transgenic rodents in recent years. One reason for this is that the genomes of man, mouse and rat have now been completely sequenced and this has stimulated the development of models for investigating the functional roles of newly discovered genes and prompted basic studies of gene function and regulation. Some mutant mice and rats also serve as models for the study of genetically determined human and animal diseases.

In comparison with standard strains of laboratory animals, several additional matters need to be addressed before the development and maintenance of a transgenic mouse or rat strain is contemplated. Not only is it important to thoroughly understand the general biology, genetics and methods appropriate for breeding transgenic rodents, but also to assess the phenotypic changes likely to arise from the genetic modification. In particular, careful consideration needs to be given to special needs for housing, husbandry, handling and the maintenance of records.

In this context, it is essential to use an effective, standardized nomenclature system. Rigorous identification of genetically engineered rodent lines is indispensable if investigators are to understand and accurately interpret findings in the animals they are working with. It enables precise communication of scientific findings arising from research involving transgenic animals. Additionally, it facilitates incorporation of information concerning the animal model into databases and its subsequent retrieval to avoid unnecessary repetitions of models already available.

In making the following recommendations we wish to raise awareness of specific features of production and of current nomenclature systems used for transgenic rodents. In addition, we

highlight the limitations of current nomenclature systems in order to encourage the development of a more robust classification scheme.

A – Different types of mutations

Mutations can occur spontaneously or they can be induced experimentally by a wide range of manipulations. Experimentally induced genetic alterations may as arise from phenotype- or genotype-driven mutagenesis, depending on experimental purpose. Genotype-driven approaches are sequence-driven and focus on the role of a pre-selected and *in vitro* recombined genetic sequence. The phenotype-driven route focuses on screening randomly induced mutations to identify animals with novel phenotypes and the altered genes are identified subsequently.

A.1 – Spontaneous mutations

Spontaneous mutations are genetic events that occur randomly within nature. In the past they were the only source of new genetic variants in rodents and many have given rise to valuable research models. The frequency per locus of spontaneous mutations is in the order of 10^{-4} to 10^{-7} per gamete in rodents; whilst the nature of the mutation can vary immensely, it is most often a point mutation, insertion or deletion in chromosomal DNA.

Most spontaneous mutations go undetected because they do not induce directly observable phenotypes. In comparison with outbred stock, brother-sister mating of inbred strains enables recessive mutations to be more quickly identified.

Spontaneous mutations, unaccompanied by a visible phenotype, have been recognised during experimental work because of significant deviations from strain-specific norms of an analytical parameter under investigation. One well-described example of this is the severe

combined immunodeficiency (SCID) mutation (*Prkdc*^{scid}) (Bosma *et al.* 1983). After an unexpected experimental observation, researchers should always look for the cause, instead of simply excluding data from the animals concerned.

A.2 – Chemically and physically induced mutations

Several physical and chemical agents have proven very effective at increasing the mutation rate of DNA in rodents; each has a distinct mode of action and induces a particular type of mutation. Although it can be logistically challenging to characterise a great number of induced mutants many researchers have come to see random mutagenesis as a very attractive and practicable way of creating vast numbers of novel mutants very quickly.

In particular *N*-ethyl-*N*-nitrosourea (ENU) is a powerful chemical mutagen (producing a per locus mutation rate of $>10^{-3}$ per gamete) that induces discrete lesions (often point mutations) by DNA ethylation; it acts predominantly on premeiotic spermatogonia (Russell *et al.* 1979). Several large-scale mutagenesis programs conducted in different countries have utilised ENU to produce large numbers of dominant and recessive mutations in the mouse genome (Rastan *et al.* 2004; Clark *et al.* 2004) (http://www.ingenium-ag.com; INGENOtyping archive).

A.3 – Transgenic animals

Methods to generate transgenic rodents

Methods of genetically modifying the germline of rodents, i.e. producing transgenic animals, have been available since about 1980. Transgenic animals are characterized by the presence of a stably introduced foreign (*in vitro* recombined) DNA sequence into their germline. Although there are important differences between their application in mice and rats,

engineered genetic modifications can generally be categorized as 'gain' or 'loss' of function mutations. Transgenes used for the mutation are classified into sequences constructed either for non-homologous (random insertion) or for homologous (gene targeting) DNA recombination.

Techniques for random insertion of foreign DNA include pronucleus injection (Gordon *et al.* 1980) and this has become the most popular approach for generating transgenic rodents, successfully applied to the rat, rabbit and several other laboratory and farm animal species. Alternatively, viral vectors can be used to introduce short DNA sequences (a few kilobases) via *in vitro* or *in vivo* infection of rodent germ cells, zygotes or early embryos. Lentiviral vectors recently derived from a modified human immunodeficiency virus (HIV) have proved to be useful tools for the production of transgenic mice (Miyoshi *et al.* 1998) as well as other species (Lois *et al.* 2002; Hofmann *et al.* 2003). A third method, sperm mediated gene transfer (SMGT), is based on the use of spermatozoa to transport foreign DNA into the genome of the zygote. SMGT has been successfully used in combination with intra cytoplasmatic sperm injection (ICSI) of modified spermatozoa; integration frequencies of the mediated transgene are comparable to those for pronuclear injection in mouse zygotes (Perry *et al.* 1999).

Fourthly, transgenesis can be achieved by non-homologous DNA recombination via transfecting pluripotent embryonic stem (ES) cells. The approach has been used as an alternative way of producing transgenic animals of demanding inbred strains of mice (Dinkel *et al.* 1999).

Another approach which induces random insertional mutations across the mouse genome, is gene trapping in ES-cells (Gossler *et al.* 1989), one of the core technologies used by EUCOMM (European Conditional Mouse Mutagenis Program) (Auwerx *et al.* 2004). A

large library of trapped ES cell lines with constitutive and conditional mutations in single genes is freely available to the scientific community from the International Gene Trap Consortium (http://www.genetrap.org) (De Zolt *et al.* 2006).

The main application of ES cell technology is, however, to create targeted mutations by homologous DNA recombination. Unfortunately, at present only mice have yielded ES cells which can be successfully isolated, cultured, modified and shown to contribute to germ line, and these from only a very few inbred backgrounds, mostly of 129 origin. There are large differences between the suitability of different inbred strains of mice for establishing ES cell lines using current protocols.

No functional embryonic stem cells have yet been generated in rats. It is possible that this constraint could be circumvented by techniques such as ENU mutagenesis (Zan *et al.* 2003) or gene targeting of differentiated cells combined with reproductive cloning. Cultured somatic cells can be used as nuclear donors and genetically modified beforehand, in ways similar to the genetic modification of ES cells. This technique is not restricted to mice and its application to the rat was recently reviewed (Tesson *et al.* 2005).

Homologous recombination can be used to create many different types of mutations in ES cells. Best known are the inactivation of an endogenous locus (knockout) and the replacement (knockin) of an endogenous gene with a homologous sequence carrying a subtle mutation in order to create a neomorph allele of the gene. Eventually, it is possible that most loci of the mouse genome will have been modified by gene targeting in ES cells (Austin *et al.* 2004).

Conditional mutagenesis

Constitutive inactivation of many endogenous genes is lethal, and the permanent or ubiquitous expression of specific transgenes may by harmful for the animals. It is very useful therefore, to be able to modify the expression of integrated transgenes in a conditional, i.e. time controlled and/or tissue specific manner. This approach also makes it possible to 'refine' procedures in cases where a severe phenotype may be expected, because neither breeder animals nor their offspring express the mutation in the absence of transgene activation. Conditional mutagenesis is mostly used for the accurate analysis of gene function and regulation, and hence is one of the strategies in current international Mouse Mutagenesis Programs (Auwerx *et al.* 2004).

The complexity and variety of procedures used for conditional gene targeting and transgene expression makes it important to take great care with the nomenclature of the genotypes produced. This is especially true if site-specific recombination systems like Cre-*loxP* (Schnutgen *et al.* 2003) have been used, because the genetic constitution before recombination of the target gene differs from that afterwards (see para. C.2, specific roles for conditional transgenes).

B – Genetic background

It has been known for decades that a given mutation does not always produce the same phenotype when it occurs in different inbred strains. The phenotypic variability of a mutation can often be attributed to the presence of background effects, i.e. of independent elements of the genome with allelic variations. The only way to establish whether or not a phenotype of a transgenic animal is affected by such polymorphic modifiers is to investigate the mutation on different genetic backgrounds (Linder 2001; Nadeau 2001).

B.1 – The genetic background of transgenic rodents

It is now widely acknowledged that it is too simplistic to view a spontaneous or engineered mutation as an autonomous functional entity. At a genetic level, the vast majority of expressed phenotypic traits and diseases are of a complex multifactorial origin and their inheritance does not follow simple single locus Mendelian patterns. Despite this, the phenotypes of transgenic animals are still frequently attributed exclusively to the induced mutation, without reference to possible effects of genetic background.

Which background is most appropriate with respect to the trait to be studied?

There is no universal ideal genetic background, onto which it would always be appropriate to introduce and study new mutations. Outbred stocks are often regarded as representative of human genetic diversity. Unfortunately, genetic complexity makes them less suitable as background for transgenic animals, because random genetic variation increases the sample size necessary to detect differences in the characteristics of interest or responses to experimental treatments (Festing 2004). Inbred strains provide a better way of controlling genetic variation over time. However, nearly every aspect of producing transgenic strains on commonly used inbred backgrounds such as C57BL/6 or BALB/c is less efficient than when hybrids or outbreds are used (Brinster *et al.* 1985; Ledermann 2000).

When selecting a suitable genetic background, it is important to consider the characteristics of different strains (Taft *et al.* 2006). For example, some inbred rodent strains are amaurotic, some become deaf early, others have poor learning abilities, etc. Thus, inbred animals often reveal extreme phenotypic characteristics that render them inappropriate models for particular studies, or may unnecessarily impair the welfare of a newly developed mutant. In recent years there has been a tendency to use C57BL/6J mice as the standard genetic

background whenever possible because many resources, including the current sequence of the mouse genome and molecular libraries, are derived from this strain, which as any other inbred strain reflects, however, only a single haplotype of the mouse species.

It is also very important to recognise that commercial vendors may breed and supply different sub-strains of an inbred strain, as a consequence of residual heterozygosity and differential fixation of still segregating loci at the time of separation (genetic drift) or undetected spontaneous mutations in a closed colony. For example, C57BL/6J inbred mice are not identical at all loci to C57BL/6N, or C57BL/6JOlaHsd mice (Specht and Schoepfer 2001; Specht and Schoepfer 2004; Wotjak 2003) and F344/DuCrj differ genetically from F344/Ztm rats (Bender *et al.* 1994).

Is it preferable to use an inbred background or a hybrid F1 background?

F1 hybrid strains consist of genetically identical individuals, which are more robust than individuals of inbred strains and do not suffer adverse effects associated with inbreeding depression; sometimes they are the preferred choice. To produce an F1-hybrid that is homozygous for a genetic modification it is necessary to maintain the mutation on two different defined parental inbred strains, and this usually requires the time consuming generation of a congenic strain (see para. B.2, changing the genetic background).

In either case, inbred or F1 hybrid strains should be selected, which are in common use and can be easily sourced worldwide as this facilitates comparison of experimental results between different laboratories (Silva *et al.* 1997). Whilst generating and breeding a transgenic strain we recommend the use of animals obtained from a single supplier, avoiding any changes.

B.2 – Changing the genetic background

Many mutant strains are still kept on a mixed genetic background. It should be appreciated that over time small, isolated colonies may undergo genetic drift and uncontrolled inbreeding. Mutations on undefined or inappropriate genetic backgrounds should be transferred into a more suitable inbred background by developing a "congenic strain". Congenic strains are obtained after a minimum of ten generations of backcrosses to the recipient strain, counting the first hybrid generation as F1 and the following backcrosses as N2-N10 (Flaherty L. 1981). Theoretically, approximately 0.2% of the donor strain's unlinked loci are retained. After the tenth generation two carriers of the mutation are intercrossed, offspring homozygous for the mutation are selected and the congenic strain is maintained by brother-sister mating.

Congenic strains as well as their genetic background strains may accumulate genetic differences over time due to unnoticed mutations that became fixed. Therefore, they should be backcrossed about four generations every 15-20 generations to mice of the parental inbred background and then sibling mating is resumed (pers. Comm.. G.D. Snell). If a mutant strain is bred for more than 10 generations, its individuals will be about 20 generations separated from the parent strain, which presumably was also bred for 10 generations during this same time period.

One way of accelerating the development of congenic strains is to apply marker assisted selective breeding**,** which involves genotyping all progeny for polymorphic markers such as simple sequence length polymorphisms (SSLPs, also known as microsatellite DNA) (Markel *et al.* 1997; Wakeland *et al.* 1997; Weil *et al.* 1997) or single nucleotide polymorphisms (SNPs) (Petkov *et al.* 2004). This enables selection of animals in each generation of

backcrossing that have retained the mutation and the least alleles of donor origin. The process is called “speed congenics” and reduces the number of backcross generations required to approximately five, by allowing selective elimination of unlinked sections of the donor strain’s genome. This strategy does not influence the size of the ‘differential segment’ (the residual chromosomal sequence which encompasses the mutation and may contain as many as 300 genes or still about 1% of the donor’s genome), unless specific markers, closely linked to the mutation are used. However, this requires previous identification of the candidate region (marker assisted introgression) and the availability of suitable markers.

An additional way of reducing the time necessary to produce a congenic strain is to reduce the interval between backcross generations. For this female donors of the mutation are superovulated long before the sexual maturity to produce fertilized oozytes for embryo transfer (Behringer 1998).

B.3 – Monitoring the background

As with inbred strains, mutant lines maintained on an inbred background should be monitored regularly for evidence of genetic contamination. Such genetic monitoring (e.g. using polymorphic genetic markers) can detect massive genetic contamination, but will not reveal gradual changes to the selected genetic background of a transgenic strain. If several mutants on the same genetic background and with no obvious phenotype are maintained in the same animal room, it is recommended that each colony be checked periodically to confirm that it carries exclusively the appropriate mutation and has not been genetically contaminated by another transgenic line.

For reliable genetic monitoring, all animals typed must be identified uniquely and permanently, and any tissue samples must be referenced to the unique animal ID. If the

breeding colony is of substantial size and complexity, it is strongly recommended that a centralized animal database be maintained, incorporating all genotypes present (a record for each animal including its pedigree or source and other essential data).

C – Nomenclature of transgenic rodents

For reasons presented earlier, a widely understood and rigorous nomenclature is essential when naming a new transgenic rodent line. The name given should provide as much detailed and precise information about the line as possible, whilst being of a manageable size and conforming to rules that are easy-to-follow. Genetic nomenclature for transgenic animals follows the pattern used for decades for spontaneous mutations and strains of various types. Unfortunately, many publications especially those reporting on transgenic rodents, use vague or inappropriate strain designations. This situation needs to be resolved for several reasons:

Firstly, new transgenic rodents are being reported at a rapid pace. Secondly, their correct assimilation into databases is dependent on unambiguous nomenclature, and thirdly, there is increased emphasis on the genetic background with respect to the phenotype of mutants.

C.1 – Reference sources for nomenclature guidelines

Nomenclature rules for rodent strains and genes are set by international committees and published on the worldwide web. Such committees include the International Committee on Standardized Genetic Nomenclature for Mice and the Rat Genome and Nomenclature Committee, which agreed in 2003 to unify the rules and guidelines for gene, allele and mutation nomenclature in mice and rats; the two committees update nomenclature for mice and rats annually. Current guidelines are published on the web sites of the respective genome databases (see Table 1). One official web site is hosted by The Jackson Laboratory.

Researchers should always follow the rules of nomenclature so as to facilitate communication and help avoid errors which might arise from use of the same name for different genes or using multiple names for the same gene. To help with this, the Mouse Genome Database (MGD) project should be used as a central repository of mouse gene names and symbols. Similarly, the Rat Genome Databases RATMAP and RGD integrate rat genetic and genomic information and allow search strategies under a variety of headings. Both databases provide advice and assistance in assigning new names and symbols.

C.2 – Basic principles in nomenclature of transgenic rodents

Attention has been drawn to the importance of consulting with the most recent published guidance for the designation of strains and mutations, for example by visiting official web sites (see Table 1). However, we wish to emphasise several principles that are often ignored.

Nomenclature of standard mice and rat strains

Inbred strains are commonly designated by a unique symbol made up of upper case roman letters, or a combination of letters and numbers beginning with a letter. Approved abbreviations of common strains can be used in place of the full name of an inbred strain (e.g. C = BALB/c; B6 = C57BL/6; OM = Osborne Mendel). Exceptions are allowed for strains already widely known by a designation that does not conform to these rules, such as 129 strains. Moreover, because of the importance of 129 as the origin for most existing ES cell lines, a revised nomenclature has been introduced to distinguish parental 129 and related 129 substrains (see Table 1).

Substrains are designated by the name of the parent strain followed by a forward slash and a substrain symbol, which may be a number and/or the Laboratory Registration Code of the

individual or the institution that maintains or has generated the substrain (e.g. A/J; IS/Kyo); the Laboratory Registration Code is assigned by and/or can be obtained from the ILAR website (see Table 1).

Designation of engineered mutations, genotypes and transgenic strains

The sum of the hereditary information stored in the chromosomes of an organism is referred to as its genome. The genotype of an individual is its genetic make-up, which can be defined as the combination of alleles throughout its genome. Although the terms ´genetic background´ and ´genotype´ are frequently used interchangeably when discussing their impact on the phenotype of a mutant strain, the term 'genotype' should be reserved to refer to the alleles at a specific locus and 'genetic background' to the entire genetic makeup of an animal.

Central to nomenclature of transgenic rodents is the name and symbol of the (trans-)gene or the modified endogenous locus. These designate the unit of inheritance. Other features such as alleles, variants and mutations are secondary to the gene name and so are coupled to it. Names of genes and loci may differ between species. Therefore, it is important always to refer to the official classification applicable to the species used. Generally, gene names should not change over time, although a change may become necessary if, for example, the mutated gene responsible for a named phenotypic change is identified. A widely known example is the Nude mouse; the spontaneous mutation was described for the first time according to its hairless phenotype in 1966 (Flanagan 1966). However, the gene for the *Whn* (Foxn1) transcription factor that is disrupted in rat (Whn^{rnu-N}) and mouse ($Foxn1^{nu}$) alleles has been discovered decades later (Nehls *et al.* 1994).

Mutations that occur in structural genes are named as alleles of the structural gene with a superscripted allele symbol, e.g. the symbol, of the spontaneous mutation shiverer (*shi*) in the myelin basic protein (*Mbp*) gene is written Mbp^{shi}. A new allele resulting from the targeted integration of a transgene by homologous recombination (knockout and knockin) additionally gets the symbol *tm* (targeted mutation) in the superscript to discriminate it from spontaneous and random insertional mutations (e.g. Mbp^{tm}). To specify the genotype of a targeted mutation we do not recommend the use of '–' and '0' instead *tm* to refer to a knockout allele.

In contrast, random integration of an additional transgene, independent of the insertion route, is denoted by the symbol *Tg* followed by the official gene symbol of the inserted DNA in parentheses, e.g. *Tg(MBP)*. Those transgenic rodents are commonly produced by pronuclear injection of DNA, with retroviral vectors or through random DNA insertion in transfected ES cells. The genetic background, a serial number from the laboratory of origin and the laboratory code where the mutation was produced is also included in the strain name. For daily use, a combination of the serial number and laboratory code can be used as an unique abbreviation - for example *Tg758Zbz* instead of the full name FVB/N-*Tg(L7-Cre)758Zbz* (designates the *Cre* transgene driven by a *L7* promoter, stably integrated in a FVB/N background, the 758 transgenic mouse line created at the Biological Central Laboratory, University Hospital Zurich).

Mutations caused by random integration of the transgene into an endogenous gene (insertional mutation) are given allele symbols in which the abbreviated symbol is superscripted to the structural locus symbol (e.g. $Col14^{Tg273Zbz}$, mutation in *Col14* caused by random integration of a transgene, mouse line 273, produced at the Zbz).

For practical breeding purposes it is absolutely essential to discriminate the different genotypes of the transgenic strain, i.e. the allelic combinations of the locus with a genetic

modification. In heterozygous mutants, the corresponding wild type allele is usually indicated by a '+' sign. Although a randomly inserted transgene has no endogenous wild type allele in hemizygous individuals, we still recommend use of the + symbol for the unaffected (wild type) insertion locus of the partner chromosome. The possible allelic combinations of a genetic modification generated by homologous or non-homologous DNA recombination are summarized in table 2.

A targeted mutation engineered in an inbred background results in a coisogenic strain that differs from its progenitor inbred strain by a single locus. However, the inbred background of the mutation must be retained by appropriate mating of the founder. In the case of ES cells this is the background strain from which the ES cell line was derived. For example, a targeted disruption of the adenosine kinase (*Adk*) locus generated in ES cells of 129P3/J origin (line mEMS32) and maintained in the same background by crossing the chimeric founder with the same strain will be designated as 129P3/J-Adk^{tm1Zbz} (the first targeted mutation of the *Adk* gene originating from Zbz laboratory).

However, the commonest way of establishing a knockout strain is to cross the chimeric founder exclusively with another inbred strain so that coat colour can be used to detect germ line transmission. This results initially in an F1 hybrid, and subsequently a mixed genetic background for the targeted locus. The nomenclature for transgenic animals on a mixed background (including animals of an incipient congenic strain) derived from 129P3/J and C57BL/6, would incorporate the abbreviation of the two parental strains separated by a semicolon (B6;129P3/J-Adk^{tm1Zbz}). If the genetic modification is maintained in a homozygous condition so that wild-type littermates are not available as control animals, the genetically closest controls are then represented by the F2 offspring of a B6129P3F1 intercross. Currently, many transgenic strains are continuously bred on a mixed genetic background. Although each individual of such a strain is unique and not reproducible it may still be

appropriate to give them designations reflecting their original parentage.

In contrast to coisogenic strains, congenic inbred strains are easily recognized by the full or abbreviated symbol of the recipient strain that is separated by a full-stop from an abbreviated symbol of the donor strain. For example, the nomenclature for the *Adk* knockout in an ES cell of 129P3/J background, fully backcrossed onto C57BL/6 to produce a congenic strain, will be B6.129P3-Adk^{tm1Zbz}. If the mutant locus originated from an outbred, mixed or unknown genetic background, the donor would be designated as Cg = congenic, and the resulting strain as B6.Cg-Adk^{tm1Zbz}.

Specific rules for conditional transgenes

Rules for nomenclature apply to changes to the genotype but not to (trans-) gene activity. This is an important issue when using transgenic rodents bearing conditional systems. In essence, somatic events, including the activation of transgenes by recombination in selective tissues, are not identified by the nomenclature. However, if the recombination occurs in germ cells, then the new transmissible allele has to be designated. This is simply done using the serial number of the transgenic strain and an allele number separated by a period (for example *tm1* for the original targeted mutation and *tm1.1* for the recombined allele).

D – Strain data sheet of transgenic strains

Spontaneous or induced genetic changes can cause new and unpredictable phenotypes and may impact the health and welfare of the affected animal. Impairments may be apparent, even within normal husbandry regimes and without the application of experimental procedures. In order to make best use of transgenic animals for scientific purposes, it is

essential to gather all available information on the strain so that the animals can be bred, housed and used so that high welfare standards are maintained and experiments are designed correctly.

At present, few transgenic rodents are bred and supplied by commercial companies; most are generated and exchanged directly within the scientific community. The transfer of information about the animals is particularly important for novel strains which have not yet been recorded in the Mouse Genome Database or the Phenome Database. Moreover, published experimental results derived from new transgenic animals do not usually include detailed information about the requirements for breeding and maintenance of the strain. It is recommended that for every transgenic strain, a data sheet should be prepared at the first opportunity, which collates all available information relating to its breeding, husbandry and care. This data sheet or a pointer to enable the information to be accessed via the web, should be sent in advance to the receiving laboratory or central repository, and a further copy should accompany the animals. It is recommended that such a data sheet should include as a minimum, the information listed in table 3. Several recommendations for such data record forms have been published and include detailed phenotype characterization (Mertens and Rulicke 1999; Mertens and Rulicke 2000; van der Meer *et al.* 2001; www.gv-solas.de; www.bvet.admin.ch) or a 'mouse passport' system (Wells *et al.* 2006).

Concluding remarks

Genetically modified rodents have made major contributions to recent advances in our understanding of gene function, gene regulation and the role of genetic defects in human and animal diseases. Many thousands of mutant mouse and rat strains are generated each year, and bred in laboratories around the world. Currently only 10% of the mouse's genes have been modified experimentally and incorporated into genetically engineered organisms. This

number will increase in the future, and it is therefore important to ensure that all characterized mutant strains are stored as frozen germ cells or embryos in central archives and repositories around the world for use within the wider scientific community. For such gene-banks to have scientific value, they must be accurately and comprehensively archived and documented. The consistent application of the rules of nomenclature for genes, alleles and strains is fundamental to the administration of both archived and breeding transgenic rodents.

In order to understand the complexity of genetic and epigenetic processes which underpin phenotypic traits and diseases, it is often necessary also to combine specific alleles of different loci or transgenes so as to create a multiple mutant strain. At present the nomenclature of very complex genetic modifications, such as occur in double, or multiple mutants, is still challenging and sometimes not compatible with line management databases. Similarly, the nomenclature of transgenic rodents developed by reproductive cloning, i.e. transfer of the nucleus of *in vitro* mutated somatic cells into enucleated oocytes, remains to be defined. Nevertheless, in all such cases, the information necessary to describe such complex genotypes should be documented in a way which is available and understandable by scientific colleagues. In addition, all new strains should be carefully monitored and a dossier compiled, incorporating all known information relevant to their breeding, maintenance and care, and reference to this should be included in publications and relevant databases.

Scientists and animal care staff working with and responsible for transgenic rodents are often confronted with subtle (or sometimes gross) impairments to fitness of mutant animals. By making use of existing knowledge in developing an efficient breeding and husbandry program, it will be possible to reduce the overall number of animals used and to minimise the potential for affected animals to experience pain, suffering or distress. Rapid and

unrestricted access to unambiguous, comprehensive information is a prerequisite to ensure that best practise prevails and welfare standards are not compromised, both of which are principles of good science.

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Table 1: Important web-site References for transgenic rodents

Inbred Strains of Mice and Rats	http://www.informatics.jax.org/external/festing/search_form.cgi
International Mouse Strain Resource (IMSR)	http://www.informatics.jax.org/imsr/index.jsp
The Laboratory Registration Code	http://dels.nas.edu/ilar_n/ilarhome/labcode.shtml]
Mouse Phenome Database (MPD)	http://www.jax.org/phenome
The Mouse Genome Database (MGD) at TJL	http://www.informatics.jax.org
RATMAP Rat Genome Database	http://ratmap.gen.gu.se/
The Rat Genome Databases (RGD)	http://rgd.mcw.edu/
Rules for Nomenclature of Mouse and Rat Strains	http://www.informatics.jax.org/mgihome/nomen/strains.shtml
NBRP rat phenome database	http://www.anim.med.kyoto-u.ac.jp/nbr/Phenotyping.htm
New Nomenclature for Strain 129 Mice	http://www.informatics.jax.org/nomen/strain_129.shtml http://jaxmice.jax.org/info/bulletin/bulletin01.html
Mouse SNP Query Form	http://www.informatics.jax.org/javawi2/servlet/WIFetch?page=snpQF

Table 2: Variants of the genotype of transgenic animals

Method of transgenesis	Genotype	
	homozygous	heterozygous/ hemizygous
non-homologous recombination	Tg/Tg	Tg/+
Homologous recombination / gene targeting	tm/tm	tm/+

Table 3: Strain data sheet of transgenic rodents

1. The name of the strain, including strain background and mutation symbol where applicable (according to the rules of nomenclature).
2. References to literature or web sites describing the animal
3. Reference to permission from authorities (in countries where it is mandatory) and to biosafety levels.
4. The nature and origin of the mutation.
5. Individual marking of the animals (method and number).
6. The genotype of the animal and methods for monitoring (protocols and other information).
7. The protocol for activation or inactivation of a conditional mutation.
8. Information about the expression pattern of mutant loci.
9. The relevant results of a thorough phenotypic characterization.
10. Guidelines on maintaining and breeding the animals.
11. The health status of the animals (according to FELASA recommendations).
12. The generator and the breeder of the strain (contact details of a person with first hand experience).