

Rodent Genetics and Genetic Quality Control for Inbred and F₁ Hybrid Strains, Part I

The best animals for research are those free of adventitious infections shown to cause disease or alter research findings. For certain types of research, animals with stable, identifiable, genetically determined characteristics are also essential.

This document reviews the development of such animals by selection and inbreeding, defines basic genetic terms and concepts, and describes how breeders prevent and detect genetic contamination. It ends with an introduction to genetic quality control, including a brief discussion of colony management. Genetic monitoring, the main component of quality control, is detailed in the 1992 document, Part II of this series. The subject of transgenics is covered in other issues.

Selection

Identifiable changes from the norm arise spontaneously. Visible changes (e.g., alopecia, a change in coat color, or obesity) may or may not be associated with invisible changes. For example, two years after Flanagan discovered the nude mouse in 1966 (*Genet. Res.* 8:295), Pantelouris demonstrated that besides being hairless and susceptible to severe infections, the animal is athymic (*Nature* 217:370). As the thymus produces T lymphocytes responsible for graft rejection, nude mice do not reject tumor grafts and are therefore crucial for cancer research.

Biologically significant changes may have no visible manifestation. In 1981, Bosma and coworkers at Fox Chase were doing radioimmunoassays to determine immunoglobulin levels in the serum of normal-looking, albino CB-17 mice. They noticed that some of the mice had little or no serum immunoglobulin. They maintained this trait by selective breeding and found it was associated with the absence of both T and B lymphocytes resulting in a severe combined immunodeficiency (*Nature* 301:527, 1983). This SCID mouse has been engrafted with human lymphocytes to create a chimeric animal with potential for studying HIV. (In Greek mythology, the chimera was a monster that combined parts of more than one animal.)

Unique animals have been developed by selecting for a change, as above, or for an existing trait. An example of the latter is the spontaneously hypertensive rat (SHR) developed from a Wistar colony in Kyoto, Japan, by Okamoto and Aoki (*ILAR News* 19(3), 1976).

Inbreeding

To stabilize the trait of high blood pressure, the SHR was established as an inbred strain at NIH in 1969. Inbred strains are produced by 20 or more generations of brother-sister (full-sib) mating, while outbred stocks are derived from matings of unrelated individuals. When inbred animals of the same strain are mated to one another, they "breed true," i.e., offspring are genetically uniform and have the same characteristics.

The vast majority of inbred strains used in biomedical research are murine because mice and rats have a short life cycle, produce large numbers of offspring, and show variation in inherited characteristics. They are also small and need little space and cost to maintain.

The first genetically defined inbred rodent strains were produced by Clarence C. Little in 1909. He began selecting and inbreeding mice with specific coat colors to demonstrate that mammals, like plants, could be inbred to fix certain traits. He developed the progenitor of today's DBA strains mainly to study the effect of hereditary factors on the development of cancer. Selection and inbreeding have since produced many unique strains with stable traits, including immunologic abnormalities, susceptibility to certain diseases, high rates of cancer and high blood pressure.

Genetic Terms and Concepts

To protect the integrity and authenticity of such unique strains requires a genetic quality control program. As health breaks can occur and must be detected by routine health monitoring, so genetic contamination can occur and must be detected by genetic monitoring. Contamination and control are best understood with the following terms and concepts in mind:

The **gene**, or basic unit of genetic inheritance, was discovered by Gregor Mendel and named by the Danish plant geneticist Wilhelm Johannsen. Genes usually code for proteins that act as enzymes, determine cell structure, and perform other functions (as in the case of hemoglobin, albumin and immunoglobulin). Genes are made of deoxyribonucleic acid (DNA) base pairs joined by hydrogen bonds and arranged in the double helix. The nucleotide bases include the purines adenine and guanine and pyrimidines thymine and cytosine. Adenine always pairs with thymine, and cytosine with guanine, but genes vary in the sequences of these pairs. As base sequences are distinct for different genes while base pairing is specific, the *in vitro* technique of **hybridization** can identify or isolate specific DNA sequences. (Hybridization is used to detect restriction fragment length polymorphisms. RFLP analysis is discussed in Part II of this series.)

Visible or measurable properties of a cell or organism are called the **phenotype**, while genetic factors that create the phenotype are called the **genotype**. Genes, though usually stable, may undergo a change or **mutation**, as discussed under "Selection." An organism carrying an altered gene (e.g., the nude mouse or SCID mouse) is a **mutant**. An organism carrying a normal gene is a **wild type**.

Alternative forms of a gene that arise in mutation are called **alleles**. Somatic or non-reproductive cells in animals and plants are **diploid**, with two copies of each gene. In contrast, gametes (i.e., eggs and sperm) generated during meiosis are **haploid**, containing only one copy of each gene. When both gene copies are identical alleles, the organism is said to be a **homozygote**. If the two copies of a gene are different, the organism is a **heterozygote**.

Genes are found on **chromosomes**, the strands of DNA. Each gene has a specific **locus**, or place, on its chromosome. As there are two copies of each gene in somatic cells, there are also two copies of each chromosome--and identical loci for the two genes. As mentioned, gametes are haploid. Only one chromosome of each pair is passed into a gamete during meiosis, to be inherited by the offspring. A homozygote, in which both copies of a gene are the same, produces only one type of gamete with respect to that gene. In a heterozygote, chances are that half the gametes will contain one allele while the rest will contain the other. The egg and sperm fuse during fertilization to form a diploid zygote containing equal numbers of chromosomes from each parents.

The phenotype of an organism with respect to a particular trait is determined by one of three allelic relationships:

- dominant/recessive, where the dominant phenotype is expressed in the heterozygote;
- codominant, where the phenotypes of both alleles are expressed;
- partial dominant, where the phenotype of the heterozygote is a mixture of the phenotype of each allele.

Mutant alleles, such as those that code for the obese, nude and SCID phenotypes, are usually inherited as **autosomal recessive**. Autosomal indicates that the genes that determine these traits are not on the X or Y sex chromosomes. Because the mutant alleles are recessive, the mutant phenotype is expressed only in animals homozygous for the mutant allele. By convention, the wild type allele is designated by a plus sign or uppercase letter, while the mutant allele is designated by a lower case letter (*Table 1*).

TABLE I. *Autosomal recessive mutant alleles*

Mutant Phenotype	Chromosome	Wild Type Allele	Mutant Allele
OBESE	6	+	ob
NUDE	11	+	nu
SCID	16	+	scid
ALBINO	7	C or +	c

Mendelian Inheritance: Mouse Coat Color Genetics. In mice, the mutant allele for albinism is a recessive

gene designated *c*. The dominant wild type allele for color (or pigment) is designated + or *C*. If an albino mouse is mated to a pigmented, homozygous *C/C* mouse, the F_1 offspring are all pigmented. As shown in *Figure 1*, the albino and pigmented parents each produce only one type of gamete, so all their offspring are heterozygotes that express the dominant phenotype of color. (Note: the *F* in F_1 stands for Latin *filius*, or offspring. F_1 is the first generation, F_2 the second generation, etc.)

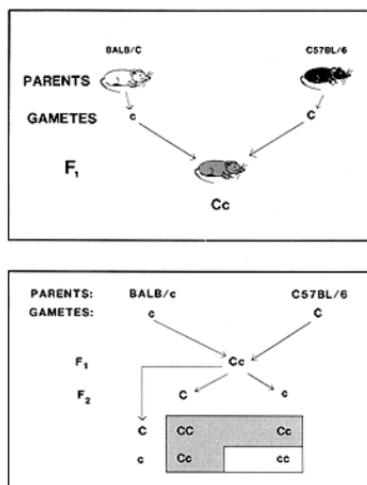


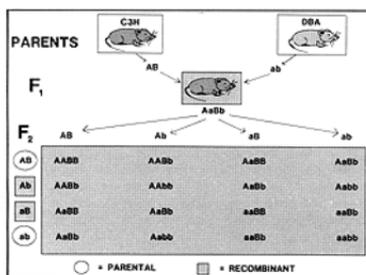
FIGURE 1. *Albino x pigmented cross.*

When the F_1 hybrids are crossed, most of their offspring are pigmented, but the albino phenotype reappears because the heterozygous parents produce *C* and *c* gametes in equal numbers. There is a 25 percent chance that an F_2 animal will be homozygous *c/c* or albino (*Figure 1*). The way different alleles for a trait segregate into separate gametes is described by Mendel's First Law, the Principle of Segregation.

The two genes of a pair can be unlinked (i.e., each located on a different chromosome) or linked on the same chromosome. Unlinked genes assort independently during meiosis (Mendel's Second Law), whereas linked genes tend to remain together. The major genes that determine coat color in laboratory mice are unlinked. These include:

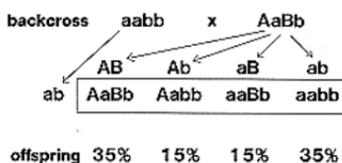
- agouti (*A*) and non-agouti (*a*) on chromosome 2 (agouti is a grizzled color of the fur resulting from yellow banding);
- black (*B*) and brown (*b*) on chromosome 4;
- pigmented (*C*) and albino (*c*) on chromosome 7.

Figure 2 shows a mating between a C3H black (or dark) agouti mouse, to a DBA dilute brown non-agouti mouse. As C3H coat color genes are dominant to those of the DBA, the F_1 **dihybrid** (the offspring of parents that differ in two characteristics) is phenotypically the same as the C3H. When the F_1 hybrids are crossed, the recessive phenotype of the DBA reappears in the F_2 offspring along with the dominant phenotype of the C3H. In addition, **recombinant** phenotypes are observed, including mice non-agouti but black and agouti but brown. The appearance of a substantial number of recombinant phenotypes in the F_2 generation indicates that *a* and *b* genes are segregating independently of one another during gametogenesis, i.e., they are unlinked. This independent assortment of genes during meiosis results in equal numbers of the parental gametes (*AB* and *ab*) and recombinant gametes (*Ab* and *aB*). The ratio of phenotypes for the F_2 offspring, predicted for these unlinked genes with Punnett's square, is 9:3:3:1. The 9 and 1 represent the parental dominant and recessive phenotypes, with 3 and 3 representing the recombinant phenotypes.

FIGURE 2. Intercross of C3H x DBA F₁ hybrids.

Test crosses can be performed to demonstrate whether two genes are linked. Rather than intercrossing two dihybrids as just described, it is more convenient to backcross a dihybrid to the double recessive parental strain. This produces offspring with phenotypes dependent on genotypes of gametes contributed by the F₁ hybrid. As shown in Table 2, the Punnett's square is then simpler because the double recessive parental strain contributes only one type of gamete. For unlinked genes, the expected phenotypic ratio is 1:1:1:1, but linkage is indicated when less than half the offspring express recombinant phenotypes. In Table 2, only 30 percent of the offspring are recombinant. This indicates that genes A and B are linked on the same chromosome and separated by 30 units on the chromosome map. In the case of linked genes, recombinant genotypes are formed by "crossing over" between homologous chromosomes during meiosis.

TABLE 2. Hypothetical test cross to demonstrate genetic linkage.



Types of Genetically Defined Rodent Strains. As noted, inbred strains are produced by 20 or more generations of full-sib or brother-sister mating. This inbreeding results in a high degree of homozygosity, which is measured as the **inbreeding coefficient (F)**. F is the likelihood that both copies of a gene were made identical by inbreeding. After 20 generations F equals 98.6 percent, leaving 1.4 percent **residual heterozygosity**, that can contribute to **subline divergence**, or differences between colonies of the same strain.

An F₁ hybrid strain is the first generation product of a mating between two different inbred strains. F₁ hybrids are heterozygous at genes for which the parental inbred strains have different alleles. The resulting hybrid vigor or **heterosis** makes such a strain more robust than its inbred parents. It is phenotypically more stable, being less susceptible to environmental influences or recessive genetic mutations. Compared to outbred stocks, F₁ hybrids have the major advantage of **isogenicity** (i.e., they are genetically identical), which leads to phenotypic uniformity, indentifiability, and genetic stability. An F₁ hybrid will accept tissue grafts from either parent.

Coisogenic strains are two inbred strains that differ by a single mutant gene but are otherwise genetically the same. **Congenic strains** are similar in that they primarily differ at a single gene. However, while coisogenic strains arise spontaneously as a result of mutation, congenic strains are produced by introducing a gene from a donor strain or stock onto an inbred background. This is done by crossing the background inbred strain with the gene donor. Subsequent generations of offspring with the selected gene are then backcrossed repeatedly to the background strain. The resulting animals have the genetic constitution of the background strain except for the selected allele(s) and those closely linked to it. After 8 generations, the likelihood that an unselected, unlinked gene will be homozygous for the allele carried by the background strain is 99.3 percent. (Genes remaining from the donor are known as **passenger genes**.) After 12

heterozygosity is difficult to detect and control and has minimal impact on most research. By far, the cause of subline divergence that most profoundly affects the usefulness of inbred strains is genetic contamination. Genetic quality control therefore seeks to prevent and detect genetic contamination, mainly by strict colony management and routine genetic monitoring.

Colony Management

Genetic contamination can be minimized by control of colony structure and maintenance of detailed breeding records. Breeders avoid keeping strains or stocks with the same coat color in the same room. They euthanize any escaped animals and commonly employ a pyramidal colony structure (Figure 4). At the apex is the nucleus or foundation colony, housed in gnotobiotic isolators or barrier rooms, maintained by full-sib mating (B x S) and pedigreed. The non-pedigreed expansion colony is also maintained by full-sib mating, but the production colony is random-mated. (Note: To reduce genetic drift caused by mutation, all nucleus animals should be traced back to a common breeding pair within 5-7 generations.)

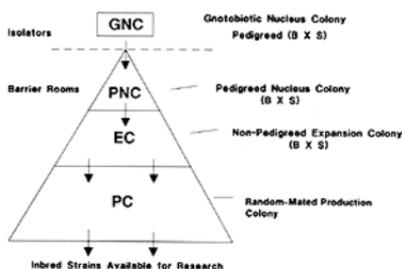


FIGURE 4. *Inbred rodent production.*

Careful colony management also helps to detect genetic contamination. For example, a substantial increase in litter size can signal heterosis due to genetic contamination. Inappropriate coat color or behavioral changes are other clues. Very experienced animal technicians can sometimes identify genetic contamination simply by observing or handling a strain.

Genetic Monitoring

However, the experience of breeders shows that colony management alone cannot prevent or detect genetic contamination. A complete genetic quality control program also requires routine monitoring of genetically determined phenotypic traits or markers. Ideal markers display simple Mendelian inheritance and have a phenotype not subject to environmental factors but corresponding to the genotype. Markers should be monitored on chromosomes or linkage groups found throughout the genome (i.e., all the genetic material in a cell). Preferably, alleles are codominant so that homozygotes and heterozygotes can be distinguished.

Techniques used by breeders include skin grafting; test matings to uncover hidden coat color genes; identification of biochemical and immunological markers to develop allelic profiles; and detection of restriction fragment length polymorphisms (RFLPs) by the Southern blot method of hybridization. These and other aspects of genetic monitoring are detailed in Part II.

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