# The Development and Maintenance of the Crl:CD®(SD)IGS BR Rat Breeding System

William J. WHITE, V.M.D., M.S. and Charn S. LEE, D.V.M., M.S.

*Charles River Laboratories 251 Ballardvalle Street Wilmington, MA 01887, USA Fax # (978) 658-7132 Email:wjw@criver.com or csl@vip.criver.com*

ABSTRACT. Systems for genetically standardizing inbred strains of rodents have been well documented. With the internationalization of biomedical research, it has become necessary for multi-national laboratory animal suppliers to globally standardize more commonly used non-inbred toxicology models such as the Sprague Dawley and Hanover Wistar rat stocks. This paper describes one such breeding system, the International Genetic Standard (IGS®) system to produce the Crl:CD®(SD) IGS BR and Crl:WI(Glx/BRL/Han) IGS BR rats for the international biomedical research community.—Key words: Outbred, breeder, breeding system, International Genetic Standard, IGS rats, SD rats, reproduction, inbred, random system

*CD (SD) IGS-1998: 8-14*

## BACKGROUND

The CD rat has a long history of use in toxicology research. This stock of rats has been maintained for research purposes for over 50 years and traces its origins back to a stock developed by Robert S. Dawley in the 1920s. The original Sprague-Dawley stock was developed from Wistar stock and a hybrid stock produced from laboratory and wild populations. Founder animals were obtained from Sprague-Dawley in the 1950s and caesarean rederived by Charles River Laboratories (CRL) to achieve an improved microbiological status. This stock was maintained using a random mating system and by the early 1990s was produced by the company in 23 separate production colonies in 8 different countries.

Late in the 1980s a disturbing trend towards a decrease in longevity was detected in the CD rat as well as other stocks of rats produced commercially including some inbred strains. Even though the cause of these changes still remains unknown, a genetic component resulting from unconscious selection pressures associated with the use of a random mating system may have resulted in inadvertent loss of heterozygosity that contributed to these changes. While other avenues have been sought to address problems associated with decreased longevity, Charles River Laboratories decided to reexamine its breeding practices and to take steps to minimize selection pressures on this heterogeneous population by instituting a comprehensive outbreeding system (for reviews of longevity related issues see Reference 1, 4, 5). At the same time, with the globalization of biomedical research, it became clear that steps also need to be taken to harmonize the breeding populations of CD rats throughout the world in order to minimize the degree of variation associated with genetic drift between these populations. Hence, in February of 1992, the company launched a comprehensive restructuring of its breeding programs for outbred stocks including the CD rat. This program resulted in the repopulation of all CRL production colonies of CD rats with a genetically harmonized stock of animals referred to as Crl:CD®(SD)IGS BR rats. Conversion of production colonies to CRL:CD®(SD)IGS BR colonies was gradual with the first animals available for commercial sale in 1994 with complete conversion and world-wide availability achieved by the beginning of 1998.

## GENETIC BASIS FOR THE INTERNATIONAL GENETIC STANDARD BREEDING PROGRAM

In general, there are three broad genetic classifications of laboratory rodents commonly used in research. These are inbreds, F1 hybrids, and non-inbreds. Transgenic animals which are becoming increasingly more common in research can be created on any of these backgrounds (for reviews of genetic management concepts see Reference 2, 3).

Inbred animals are the result of 20 generations of brother-sister mating resulting in over 98% genetic uniformity (homozygosity). In order to maintain this level of homozygosity, a rigorous program of brother-sister mating must be maintained using a very structured colony set up (see Figure 1).

A gnotobiotic foundation colony is usually maintained by commercial breeders in isolators and is extensively monitored genetically and microbiologically. This colony serves as the source of stock for individual pedigreed nucleus colonies at various production sites. Since spontaneous mutations can occur and may become fixed in a population that is separated from another, it is necessary every 5 to 10 generations (an arbitrary figure) to restart the pedigreed nucleus colony in each production room with founder animals from the gnotobiotic foundation colony. This assures that the inbred animals produced do not differ significantly from the foundation stock.

Hybrid animals are the product of the crossing of two inbred strains. These animals are heterozygous at all gene loci at which their parental strains differ. F1 hybrids are not self-perpetuating; therefore, it is necessary to maintain colonies of both parental inbred strains. F1 hybrids do provide advantages in terms of uniformity while not being homozygous as are their parental strains. The consistency of F1 hybrids is largely dependent upon (1) the breeding program for the inbred parental stains and (2) a carefully structured breeding program for the hybrids that avoids mismatings.

Non-inbred animals which include the designations random bred and outbred are animals derived from mating unrelated individuals. Presumably, they are desirable to the biomedical research community because of their great degree of individual

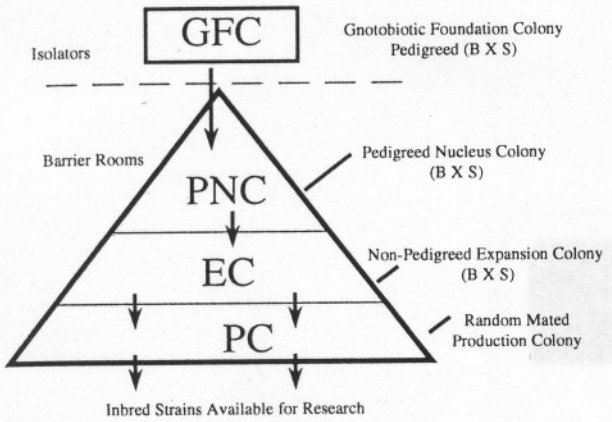


Fig. 1. International Standard Breeding Program for Inbred Strains

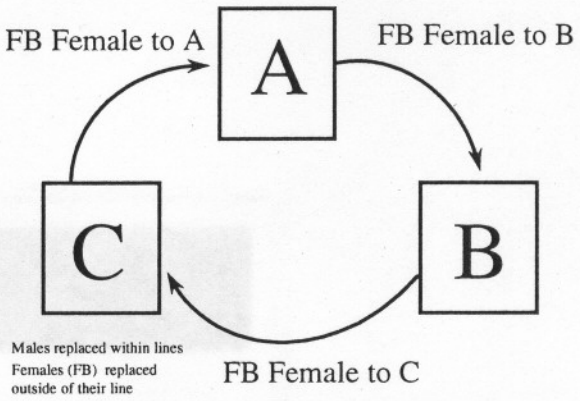


Fig. 2. Fixed 3-Line Outbreeding System - Single Sex Rotation

diversity. Like man, significant sample sizes must be used in order to acquire a representation of the entire population. It is also critical that concurrent and historical controls be acquired when conducting studies in order to characterize the population both at the time of the study as well as changes in the population overtime.

In order to produce non-inbred animals, inbreeding needs to be avoided. While the goal of any breeding program producing these animals is genetically heterogeneity, absolute heterogeneity is never achieved since there is always some unconscious selection imposed on the population which results in a tendency towards inbreeding. Some of these selection pressures are unavoidable if a constant supply of animals are to be produced by the colony.

Random breeding systems have been used to produce non-inbred animals in the past but depend upon a number of assumptions including (1) an infinite population size, (2) that every reproductively fit animals has an equal chance of participating in the breeding program, and (3) that there is no structured breeding program or selection criteria that would inhibit randomization. Unfortunately, these criteria are probably never met even in wild populations. In production colonies, a significant proportion of the colony is used for research and does not have an opportunity to participate in a breeding program. Limits on the numbers of animals to be produced, as well as on the sex of animals required for research, coupled with the physical limitations for housing the colony prohibit true random breeding from occurring. The fact that animals must be housed in cages and in groups automatically divides the population based on criteria that may not be completely random. In addition, unconscious selection criteria, as well as some conscious selection criteria for factors such as large litter size, fecundity, ability to bring a litter to term, aggressiveness, and perhaps even morphologic characteristics, may result in a tendency toward inbreeding. Failure of replacement breeders to accurately reflect the genetic make up of the existing colony as a result of such selection pressures or sampling error can result in long term colony alterations in genotype and phenotype which may compound similar changes resulting from spontaneous mutations that become fixed in the population.

In order to overcome some of the difficulties associated with random mating, purposeful mating systems that attempt to maximize heterozygosity (individual variation), referred to as outbreeding schemes, have been developed. These systems minimize the chance of inbreeding, ensure that a large percent of the population can participate in the breeding system, and reduce selection criteria, therefore, decreasing tendency towards inbreeding.

Generally, outbreeding systems divide the population into a series of groups that are referred to as families or lines. Replacement breeding pairs within lines are selected partially or in total from outside of that line using a fixed system of crosses (Figure 2). As an alternative, when the population is small enough, kinship (relatedness) can be calculated mathematically provided that all animals are pedigreed and new pairs can be constructed based upon mating least related animals.

As a general rule, the greater the number of lines and the greater the numbers of individuals per line, the less inbreeding that will occur. From a practical standpoint, however, the more lines and the more complex the migration patterns used to construct new pairs for replacement breeders, the more logistically difficult the system, the more costly and space intensive the system, and the more prone the system will be to error. Maintaining pedigree information on populations over 1000 breeders is impractical. For example, production colonies in academic, government, and pharmaceutical companies usually number no more than several 1000 individuals of any given species and stock whereas individual production colonies maintained at commercially breeders usually range between 60,000 and 300,000 individuals.

In addition to minimizing kinship, outbreeding systems must seek to reduce breeder selection criteria to an absolute minimum number of factors; and they must resist the temptation to utilize unjustified phenotypic characteristics for selection. Hence, while large litter size might be desirable from a commercial standpoint, the mature body size of breeders required to maintain such large litters may also select for obesity and decreased life span. On the other hand, very small litters may not be commercially viable and may have undesirable research

consequences. The number of siblings or related animals in the population used for breeding should be minimized, and steps such as selecting one pup per litter and selecting males and females from litters born on separate days of the week for replacement breeders ensures that inadvertent brother-sister matings do not occur. Similarly, selecting replacement breeders from the third through fifth litters of rodent breeders in a polygamous system, eliminates the possibility that male replacement breeders could be sexually mature at the same time that their mothers were still reproductively active members of the breeding colony.

Even though a purposeful outbreeding system can minimize inbreeding within a single non-inbred population, the random genetic drift that occurs both over time and geographically between different colonies can still occur if steps are not taken to somehow genetically link the colonies. There are three interrelated causes that can result in genetic divergence and loss of heterozygosity. The first of these is referred to as a genetic bottleneck or the founder effect. This occurs when only a small number of animals are used to start a non-inbred colony. This frequently results from the need to improve the health status of animals through a process called rederivation which can be done by caesarean section or embryo transfer of animals to produce founder animals of the appropriate microbiological profile for a new colony. As can be seen in Figure 3, the number of breeding pairs of unrelated animals greatly influences the rate of inbreeding produced by random mating as evidenced by a measure of kinship such as the coefficient of inbreeding. Clearly, the larger the number of founder animals, the slower the lost of heterozygosity and the greater the likelihood that the degree of heterozygosity will reflect the parent population.

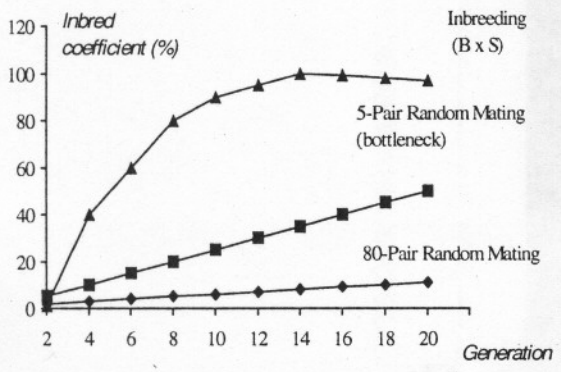


Fig. 3. Coefficient of Inbreeding with Different Colony Size and Mating Systems

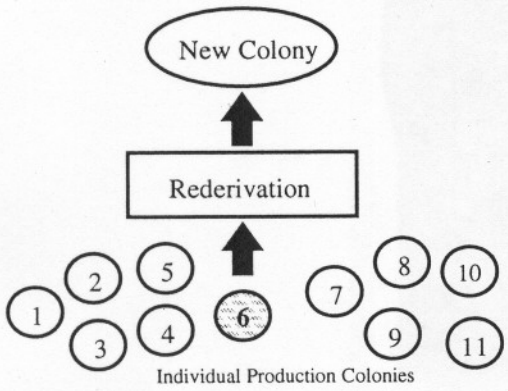


Fig. 4. Historical Process for New Colony Set-up

The second cause of genetic divergence or loss of heterozygosity is sampling error. This occurs when the breeders chosen to start a new colony are not an exact genetic representation of the colony from which they were derived. This can be magnified by the process of genetic bottlenecking which was previously described but can also occur in a broader sense if only one colony out of a series of several colonies is used to start a new colony. As illustrated in Figure 4, if only a single colony is chosen, the genotypic frequency of the rest of the population maintained in the other colonies is lost. The only way to overcome this is to take a large enough number of breeders from every population to start a new colony so that the frequency of genotypes of all the colonies taken as a whole is represented in the new one.

The third process that results in genetic divergence and lost of heterozygosity is mutation. As mutations occur within a colony, they may either become fixed or may not persist in the colony especially if the mutation occurs in an animal that is not a breeder. Some mutations are not advantageous and may be selected against such as a mutation that causes infertility.

The prevalence of various phenotypes/genotypes within a non-inbred population is constantly changing due to random assortment and mutation. For example, as illustrated in Table 1, a non-inbred population of CD-1 mice was established at four separate locations simultaneously using the same numbers of breeders for each population. Other parameters including colony size, breeding scheme and environmental conditions were similar between colonies. Three years after initial colony set up, the populations were surveyed by sampling 100 animals selected at random and assaying for a number of isoenzymes in blood and tissues that are known to be polymorphic (existing in more than one form).

Table 1. Distribution of Allelic Forms of Biochemical Markers Between Various Colonies of CD-1 Mice

| Biochemical Marker (allele) | A | B | C | D |
|---|---|---|---|---|
| Hbb (d) | 23 | 14 | 8 | 11 |
| Hbb (sd) | 60 | 42 | 40 | 50 |
| Hbb (s) | 17 | 44 | 52 | 39 |
| Gpi-1 (a) | 70 | 46 | 34 | 46 |
| Gpi-1 (ab) | 26 | 46 | 58 | 49 |
| GPi-1 (b) | 4 | 8 | 8 | 5 |
| Gpd-1 (a) | 3 | 12 | 34 | 28 |
| Gdp-1 (ab) | 46 | 38 | 34 | 47 |
| Gpd-1 (b) | 51 | 50 | 32 | 25 |
| Pgm-1 (a) | 27 | 38 | 34 | 31 |
| Pgm-1 (ab) | 50 | 46 | 52 | 50 |
| Pgm-1 (b) | 23 | 16 | 14 | 19 |
| Mod-1 (a) | 7 | 10 | 2 | 1 |
| Mod-1 (ab) | 21 | 14 | 10 | 6 |
| Mod-1 (b) | 72 | 76 | 88 | 93 |

As can be seen in Table 1, the distribution of allelic forms of certain isoenzymes was very similar between two or more of the colonies. However, with other isoenzymes, genetic divergence had clearly occurred between the populations suggesting that genotypic and hence phenotypic manifestations of this divergence might be able to be demonstrated depending upon the types of studies in which animals were used. Undoubtedly, many of the differences seen between non-inbred colonies sampled contemporaneously for use in similar studies, as well as those conducted on different populations or even the same population over the course of several years, can be attributed to this phenomenon.

In developing the IGS system, the challenge was to develop a mechanism to minimize the divergence that occurs between geographically separate colonies. One way to do this, is to migrate animals between colonies. Migration can be viewed as a form of genetic glue that holds colonies together and sets a limit on the amount of genetic divergence that occurs. Migration of animals is not without its difficulties since other factors such as the potential for microbiological contamination of existing colonies must be considered in the migration process. By trading breed stock between colonies or as with inbreds by forward migrating breed stock from a central foundation colony, the replacement of a portion of the breed stock in each production colony introduces a representative sampling of the genetics of other production or foundation colonies into each production colony. The size of the infusion and the frequency of this migration of breed stock determine how quickly and completely the genetic divergence within the production population is altered to more closely resemble the foundation colony as well as other colonies in the production program. Large or frequent infusions cause rapid corrections and potentially major shifts in the allele frequency of the production population. Smaller or infrequent migrations may make smaller or less significant changes in the populations. While the appropriate size and frequency of migrations are clearly a matter of professional judgment, measures of the heterogeneity of the population prior to migration using population genetics parameters calculated from assays of allele frequency can assist in making such determinations.

Backward migration of animals from each production colony (transfer of animals to the foundation colony) on a regular basis ensures that new phenotypes as well as representation of predominant genotypes within an given production colony is represented in the foundation colony. Given the microbiological risk associated with this process, rederivation is required. No single colony should unduly influence a production colony especially if newly formed genotypes resulting from mutations may have undesirable effects. Bringing them back to the foundation colony does not guarantee the fixation of these genotypes within the foundation colony but does allow that possibility to occur. Since the need for refocusing of the foundation colony by such infusions is much less than the need for forward migrations to production colonies, the interval for backward migrations can be greater than for forward migrations. Given the small size of the foundation colony relative to the production colonies and the relatively large number of production colonies, replacement of 1% of the foundation breeders with each backward migration from each production colony is an arbitrary but an appropriate level of replacement. Both the forward and backward migration processes favor gradual change in the colonies over time.

## THE IGS SYSTEM

Given the large number of CD rat production colonies that existed in 1992, migration of breed stock between production colonies in order to minimize the effects of genetic divergence would have been a very risky, time consuming and cumbersome task. In order to effect such an exchange process, regular forward and backward migration from each colony would be required as depicted in Figure 5. New colony start-up would require contributions from all existing colonies as depicted in Figure 6. As an alternative, a reference colony developed from existing genetic material in the various production colonies could be used as a means for maintaining a genetically diverse population that could be used for forward migration purposes as well as start-ups of new colonies as depicted in Figure 7. Since the size of the foundation colony can be kept within manageable limits, more complex outbreeding schemes could be applied in order to ensure that the tendency towards inbreeding is greatly reduced.

In selecting the CD rat breeding population that comprised the Crl:CD®(SD)IGS BR foundation colony, it was necessary to balance two competing concerns. The first was to select as large a number of breeders as possible from all of the colonies in order to accurately represent the genetic diversity of the entire production population. On the other hand, colonies that had recently been set up from other existing colonies either by rederivation or direct transfer of animals between colonies had not developed significant genetic drift from the parent population as compared to that which might be obtained by colonies that had been in production with no transfer of animals for at least five to ten years.

In reviewing all of the existing colonies in 1992, a total of 8 colonies (lines) were found that had been separated for a

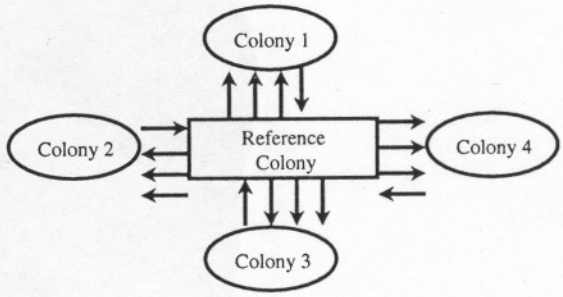


Fig. 5. Regular Forward and Backward Migration Using a Reference Colony System

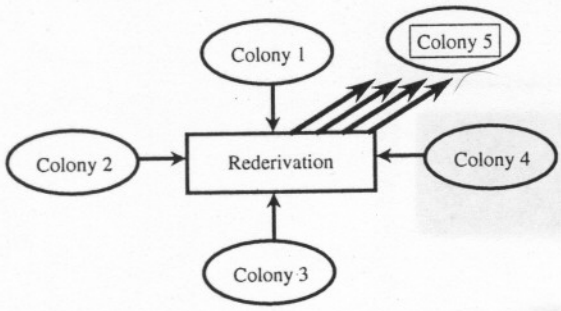


Fig. 6. New Colony Start-Up Using Stock Migration from Production Colonies

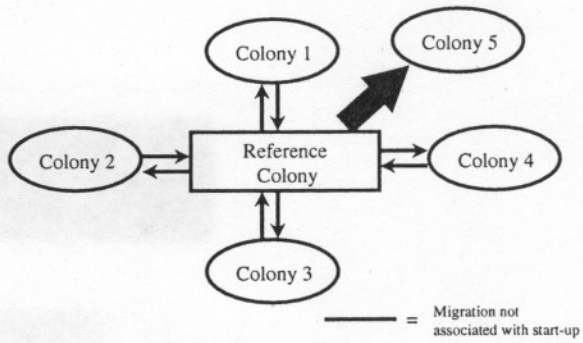


Fig. 7. New Colony Start Up Using the Reference Colony System

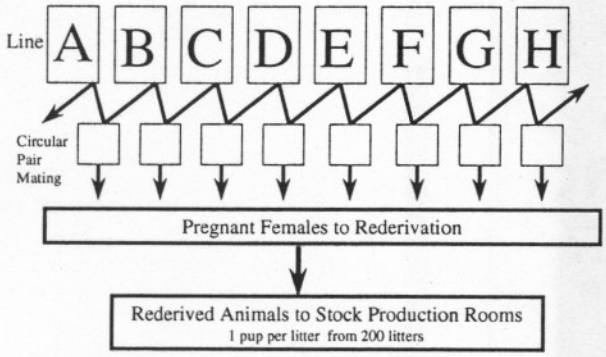


Fig. 8. Crl:CD,(SD)IGS BR Foundation ColonySystem for Producing Stock for Forward Migration

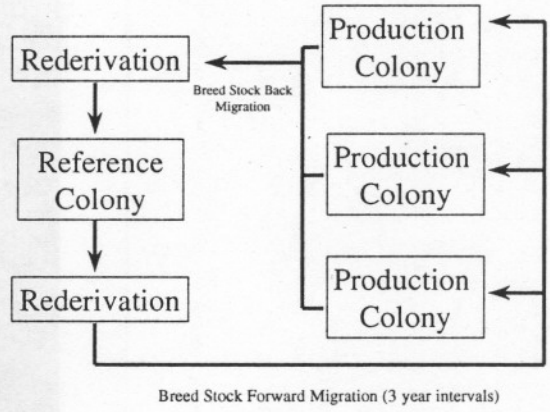


Fig. 9. Crl:CD,(SD)IGS BR Rat Forward and Backward Migration System

minimum of 12 years. A total of 100 breeding pairs were selected at random from each of these colonies and used to form a reference colony maintained within a barrier room at Charles River Laboratories' corporate headquarters in Wilmington, Massachusetts, USA. Initially, the foundation colony was maintained using a circular paired-mating system whereby the 8 lines were crossed in a systematic fashion to develop mating pairs that produced pregnant females for rederivation purposes. Once the pregnant females had undergone caesarean section and their pups transferred to foster mothers maintained in flora-defined isolators under the appropriate microbiological conditions, one pup per litter was used with a stocking number of 200 animals to be sent to start new Crl:CD®(SD)IGS BR colonies in existing barrier rooms (see Figure 8). The first Crl:CD®(SD)IGS BR colony to be stocked was in Hollister, California, USA in 1993. It was followed in short order by colonies in Raleigh, North Carolina, USA; several countries in Europe; and Japan.

The migration scheme utilizing the reference colony is depicted in Figure 9 At three year intervals, forward migration of breed stock to each production colony is made from the foundation colony. Since a polygamous mating system is used for rat production in order to efficiently produce large numbers of animals, the most effective method for incorporation of migrated breed stock is the transfer of males. To that end, sufficient male animals representing one pup per litter from foundation colony breeding pairs replace 25% of the male breeding population in each production colony.

At five year intervals, each production colony selects at random sufficient breeders to replace 1% of the foundation colony. These animals undergo rederivation such that one pup per litter is selected as a replacement breeder. Animals are introduced into the foundation colony only after their health status has been assured. Assignment of replacements by this process in the foundation colony is done using a table of random numbers. Breeding pairs so replaced cannot be replaced again with backward migrated animals from production colonies for at least six months. Backward migrations are staggered so these infusions do not occur all at the same time. Similarly, forward migrations are spread out over the three-year period so as not to unduly represent any given time period in the foundation colony's existence.

While this initial foundation colony set-up proved workable for several years, a number of disadvantages become evident.

The first of these was the inherent risk associated with trying to maintain a defined microbiological profile suitable for animal transfer to many colonies throughout the Charles River Laboratories system while housing animals in a barrier production room. Barrier production rooms provide a reasonable means for excluding rodent specific pathogens; however, the regular need for rederivation of animals from this large breeding population in the foundation colony in order to allow forward migrations introduces substantial risk of microbiological contamination through mishaps in the rederivation process. Moreover, such problems could have significant impact on the whole Crl:CD (SD)IGS BR production system if they went undetected for a very prolonged period of time.

Second, the relatively large colony size of 800 breeding pairs limits the ability to use more precise kinship mating systems effectively. Ideally, if each animal within the colony were pedigreed (breeders as well as replacement breeders), then all breeding could be done based upon mating least related animals using the coefficient of inbreeding as a basis for making such comparisons. This has the effect of making each breeding pair its own line thereby increasing the number of lines by hundreds as compared to the existing eight. This would provide a substantial improvement in the maintenance of individual heterozygosity.

Finally, since the entire population was maintained within a single barrier room, a disastrous incident affecting that barrier room such as the introduction of a pathogenic organism or a breach in the barrier caused by some natural or manmade disaster would result in the entire loss of the foundation colony. While reconstruction of such a colony would be possible using existing production colonies, the risk was deemed unacceptable.

Hence, starting in the fall of 1997 and completed in 1998, the Crl:CD(SD)IGS BR foundation colony was rederived and placed in 20 large semirigid isolators each holding 27 cages of animals. The foundation colony size was reduced to 250 breeding pairs based upon a change from a line breeding system to a computer assisted coefficient of inbreeding system. Prior to starting the rederivation process, kinship relationships and pedigrees were maintained manually in preparation for setting up the computer assisted program.

All breeding pairs are individually identified with ear tags as are replacement breeders from each pair. One male and one female is selected from the progeny of each breeding pair and is maintained as individually identified animals for future breed replacement. These animals are regularly replaced in the future breed section to ensure that animals that are young enough for breeding purposes are always available. The remainder of the animals produced from the matings are available for migration purposes but are not maintained beyond 4 weeks of age.

In the initial line breeding system for the foundation colony within the barrier room, breeders within a line were replaced with breed stock generated by males selected within the line and females selected from another line. In the case of the isolator maintained foundation colony, animals are retired from the breeding program at six months after being set up as a breeding pair or when replaced by backward migration. When replacing a breeding pair, the male future breeder from that breeding pair is set up with a new female selected by the computer based upon least relatedness. That female may exist within that isolator or within another isolator. A female in another isolator is transferred into the appropriate isolator to set up the new breeding pair using aseptic transfer techniques.

All materials introduced into the isolators are sterilized or suitably decontaminated. The microbiological status of the isolators is monitored by environmental bacteriological culturing and regular whole animal health monitoring conducted on each isolator. In addition, prior to any forward migration or new colony set-up, additional health monitoring and environmental culturing is done to ensure the microbiological status of each isolator.

The foundation colony is surveyed for a number of polymorphic microsatellite markers using a large sampling of animals distributed over all of the isolators in the foundation colony. Similarly, prior to each forward migration, the production colonies are also sampled for polymorphic markers. The results of this sampling are compiled and the distribution of markers is used to calculate a number of population genetic statistics. These serve as a guide to analyzing the degree of heterozygosity present in each population and to compare the amount of genetic divergence between the foundation and individual production colonies. An IGS advisory panel that includes population geneticists and veterinarians meets on a regular basis to review these findings as well as the details of the genetic monitoring, health and production programs. At three-year intervals, embryos from the foundation colony will be cryopreserved in order to guard against disastrous loss of the colony and to allow the possibility of genetic infusions reflective of the distribution of genotypes in past foundation colony samplings should that be warranted.

Within the individual production colonies, a purposeful outbreeding scheme has been put in place. Each production colony of Crl:CD®(SD)IGS BR animals utilizes a line breeding system with three lines. Each breeder male and female is ear punched to identify it as to which line it belongs to. A rotational system is used to set up replacement breeders in each line as illustrated in Figure 2. Males are replaced within their own line whereas females are rotated between lines in a fixed pattern. More complex biorotational systems were not considered necessary given the forward migration process. Certain basic selection criteria have been imposed on the line breeding system within each production room. These included the use of only one pup per litter for replacement breeders; the selection of male and female replacement breeders on separate days of birth; the selection of males only from the mothers' third through fifth litters; and selection criteria on litter sizes designed to reduce the tendency to select for very large litters.

A cap was placed on litter size for future breed selection such that future breeders could only be selected from animals having between 4 and 16 pups. Given the average litter size of CD rats, a lower cap of four pups was arbitrary but deemed appropriate given practical and economic production concerns. The use of litters with less than four pups would make production economically difficult. The cap on the upper end of 16 pups

was designed to reverse the trend which was presumed to have occurred in years past of unconsciously selecting replacement breeders from large litters. Animals produced from each line not only are used for replacement breeders, but also are commingled in stock cages to be used for sale in biomedical research (Figure 10).

## SUMMARY

The system of mating used to manage the Crl:CD(SD)IGS BR rat colonies has been purposely designed to produce non-inbred animals that possess a great degree of individual heterozygosity while harmonizing the range and distribution of genotypes/phenotypes within production colonies throughout the world. This has been accomplished utilizing well-established genetic management techniques. The same principals have been applied to the management of certain other non-inbred strains produced by Charles River Laboratories and bear some correlation to well-established concepts for linking geographically separated inbred colonies.

The Crl:CD®(SD)IGS BR rat is not a new stock. No foreign genetic material has been added. No selection for specific traits has been conducted. Rather, a process that retrieved the full range of diversity found within the existing CD populations was employed. The resulting foundation colony on which all production of Crl:CD®(SD)IGS BR animals is based reflects this diversity and, through the forward migration process, regularly links all production colonies to it in a way that the range of variation in phenotypes within individual production colonies becomes similar and is directly related to the degree of variation within the foundation colony. Loss of heterozygosity through inbreeding has been minimized hence the fixation of detrimental phenotypes within the population should be less likely. Overall, the global biomedical research community now has available to it a fully harmonized animal model that should react in a similar fashion no matter where in the world it is obtained.

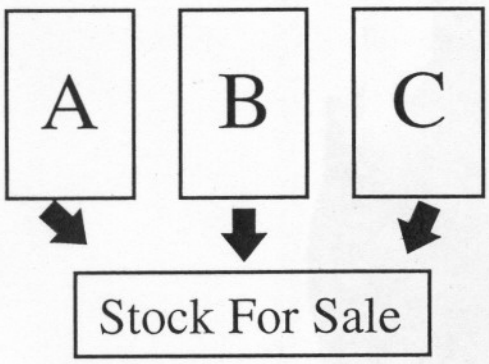


Fig. 10. Production of Crl:CD,(SD)IGS BR Rats for Sale Using a 3-Line Outbreeding System

## TERMS

Crl:CD®(SD)IGS BR - Stock designation of CD rats produced by Charles River Laboratories that have been produced using the IGS genetic management system.

Crl:CD®(SD) BR - Stock designation of CD rats produced by Charles River Laboratories that were not produced using the IGS genetic management system.

International Genetic Standard (IGS) - A globally integrated genetic management system using pedigreed gnotobiotic foundation colonies, a program of regular breed stock migration, and in the case of non-inbreds an avoidance of inbreeding production system.

Gold Standard - A term formerly used to describe an intensively managed gnotobiotic foundation colony used to standardize geographically separated production colonies by breed stock migrations.

CD Rat - A non-inbred stock of rats acquired by official transfer of breed stock from Sprague Dawley Inc. in 1950 often referred to as Sprague Dawley (SD) rats. The stock was caesarean derived ("CD") in 1955 from original Charles River SD(tm) colonies to form the nucleus of the current CD stock. The term is a contraction of the official designation Crl:CD®(SD)IGS BR or Crl:CD®(SD) BR.

New Generation or International Standard - Terms replaced by the term International Genetic Standard.

## REFERENCES

1. Hart, R.W; Neumann, D.A.; Robertson, R.T.(eds). 1995. Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies. ILSI Press, Washington, DC
2. Hartl, D.L. 1988. pp. 69-139 in A Primer of Population Genetics, 2nd ed, Sinauer Associates, Inc., Sunderland, MA
3. Green, E.L. 1981. Breeding Systems in the Mouse in Biomedical Research. pp.91-104. in History, Genetics, and Wild Mice, Vol. 1 (Foster, H.L.; Small, J.D.; and Fox, J.G. eds), Academic Press, Inc., San Diego, CA.
4. Keenan, K.P. 1996. The uncontrolled Variable in Risk Assessment: ad libitum Overfeed Rodents-Fat, Facts and Fiction. Tox. Path. 24:376-383.
5. Weindruch, R. and Walford, R.L. 1988. The Retardation of Aging and Disease by Dietary Restriction. Charles C. Thomas, Springfield, IL.