Inbred strains of animals, transgenic and gene knockout animals, cloning
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1. Introduction

In biomedical research, appropriate experimental models are essential for the study of biological properties and phenomena, and to answer correctly the scientific questions raised. Besides the selection of the optimal animal species, it is crucial that the genetic characteristics of the experimental animals should correspond to the aims of the experiment. Genetic factors determine the properties of the animals, including their sensitivities to different physical effects (e.g. light, heat, ionizing radiation, electric current, etc.), chemical substances (e.g. mediators, hormones playing a role in the functions, regulation and reactions of the organism, toxic substances, etc.), pathogenic microbes (bacteria, viruses, fungi, etc.) and foreign or self-antigens. Genetic factors may influence the susceptibility to pathological alterations and diseases, as well as the effectiveness and side-effects of treatments (e.g. drugs). Not only the induction, but also the intensity of the reaction is influenced by genes. Consequently, the responses of the individuals of a genetically diverse population can differ both qualitatively (a positive response or no response) and quantitatively (a low, moderate or high response). Therefore, if a disease caused by a pathogenic microbe is studied, a population (strain) of animals must be chosen in which all of the individuals carry the genes of susceptibility to the microbe. In contrast, a population resistant to the microbe should be selected to study resistance to the disease. If the incidence of a disease caused by a microbe is examined in a species, genetically diverse, nonselected experimental animals should be used.

The genetic characteristics of the experimental animals and the uniformity or variability of the population can be influenced by selecting an appropriate breeding method. The genetic constitution of the animals can be modified by genetic engineering (transgenic and gene knockout animals, etc.; see later).

2. Basic genetic terms and definitions

In the event of sexual reproduction, the progeny inherits a set of chromosomes from both parents, which are present in all body cells, but not in germ cells. As concerns the genes of the body chromosomes, every feature is encoded by a pair of paternal and maternal genes. The two genes with identical functions are located at the same site of each member of the chromosome pair [gene(tic) locus]. Polymorphic genes have two or more alleles. In the different alleles, the sequences of bases building up DNA differ from one another. If the progeny inherits a gene with the same DNA sequence, i.e. the same gene allele from both parents, which originates from a common ancestor of both parents, it can be regarded as a homozygote for the gene locus; conversely, it is a heterozygote. If the gene has both a dominant and a recessive allele, the property determined by the dominant allele is expressed in the heterozygotes, while the feature encoded by the recessive allele appears only if the individual is homozygotic for that gene, i.e. each chromosome of the pair carries the same recessive gene allele. In the event of codominant inheritance, the properties inherited from both parents are expressed in the heterozygotes.

2.1. Outbreeding

In this case, the breeding of related individuals must be avoided to the highest possible degree. Breeding of relatives is considered to occur if there is a common ancestor within four successive generations in the pedigree (family tree, or lineage). If the number of breeding pairs is less than 100, the possibility of breeding relatives must be totally excluded; if it is more than 100, rotation or random breeding can be used. In rotation breeding, to avoid the mating of littermates, males and females from the same litter are moved and mated among the cages consistently opposite each other, and this method is used from generation to generation. Outbreeding results in a genetically variable population, where the increase of inbreeding (the increase of homozygous gene loci) from generation to generation is less than 1%. This is named the inbreeding coefficient (F). In the overwhelming majority of genetic loci, the
individuals of an outbred population are heterozygous and the alleles of the paternal (A) and maternal (B) genes are different (AB). The population is *anisogeneic*, which means that the individuals have different genotypes.

### 2.2. Inbreeding

In this case, the strict breeding of relatives is applied. Most frequently, serial brother by sister matings are used (the members of the breeding pair are littermates, *i.e.* siblings), or the younger parent is mated with an offspring of the opposite sex. The chance of the development of a mutation in the younger parent and, due to this, the chance of the development of a genetic difference between the parent and the offspring is less than in the case of the older parent. Inbreeding results in increasing homozygosity (genetic drift): the genotype consists of identical gene alleles at more and more genetic loci (AA or BB, instead of AB). The homozigosity also increases in a closed breed. The breed is closed if no breeding animals are introduced from outside. In a balanced closed breed, half of the individuals are heterozygous (AB), while the other half are homozygous (AA and BB).

### 2.3. Inbred strain

If inbreeding (brother by sister or parent by offspring mating) is continued for more than 20 consecutive generations, an *inbred strain* develops, in which 98.6% of genetic loci are homozygous. In spite of the remaining genetic differences, this strain can be regarded as genetically identical, *i.e.* *isogeneic*. Animals from the same inbred strain are called *syngeneic*.

In isogeneic strains, any member of the strain accepts tissue grafts transplanted from any other member of the same sex of the strain without any sign of rejection, because the histocompatibility antigens of all individuals of the strain are identical. Due to a weak histocompatibility antigen encoded by a gene locus on the Y chromosome, grafts from male donors can be rejected in female recipients. The skin grafting method of Silvers has proved to be excellent for the testing of isogeneity.

The development of complete homozygosity can be delayed by spontaneous mutations which occur rarely. Because of the possible spontaneous mutations, inbred strains must be maintained by brother by sister matings. Inbred strains can be most easily produced from small laboratory mammals (mouse, rat, guinea pig, hamster or rabbit), because these, especially mice and rats, are prolific and their gestation time is short.

### 3. History of inbred strains

The history of inbred strains is closely related to the history of tumor research (mice) and studies of multifactorial inheritance (guinea pigs). The rediscovery of Mendel’s work led to the investigations of tumors as inherited diseases. In the initial experiments, spontaneous tumors developed in breeds of mice were studied. Jensen, Loeb, Ehrlich and Tyzzer attempted to transplant these tumors to other mice, but the grafts usually did not grow or regressed after transient growth. However, Jensen (1903) and Loeb (1908) successfully maintained two different mouse tumors by serial transplantations in relatively inbred mice. Little (1914) found that the rejection of tumors or the susceptibility to tumor growth is based on a number of dominantly inherited genes. This led to the discovery of histocompatibility genes and studies on cell-mediated immune responses.

In the meantime, Rommel (1906) started an inbreeding experiment involving guinea pigs. The descendants of these animals are still widely used as Strain 2 and 13 guinea pigs. His work was continued by Wright, who developed the mathematical theory of inbreeding. The introduction of the inbreeding coefficient is also linked to his name (1922).

Helen King started the inbreeding of rats in 1909 (PA and WKA strains). Little began the inbreeding of mice in the same year, which led to the development of the DBA strain. From his albino mice, Bagg produced the BALB strain in 1913, from which the BALB/c strain was developed in Snell’s laboratory in the 1930s. In 1920, Strong developed the CBA and C3H strains, from a cross of a Bagg albino male with a DBA female, and, in 1921, the A strain from a cross between Bagg albino and Cold Spring Harbor albino stocks. Little founded the family of C57 mice (C57BL, C57BR, C57L and C58). The C57BL strain was split into the 6 and 10 sublines before 1937. Many of the most widely used mouse and rat strains were developed from 1920-1930. In 1929, Little founded the Jackson Laboratory in Bar Harbor (Maine), which continuously developed the inbred strains,
isolated new mutations and studied the characteristics of the strains. The Jackson Laboratory has the largest collection and is one of the main supplier of inbred mouse strains.

4. Nomenclature of inbred strains

The rules for the nomenclature of inbred strains of mice were created by the committee on nomenclature in 1952. The name of the strains should be denoted by 1-4 capital letters (e.g. mouse strains: A, AKR, CBA, DBA, etc.; rat strains: LEW, WAG, BN, PVG, etc.). Numbers are permitted only in the names of strains already known and named at that time (e.g. mouse: C3H, C57BL, etc.; rat: F344, AS2, M520, etc.). Substrains are formed when a strain is separated into two or more branches after 8-19 generations of brother by sister matings; if parallel substrains within the same colony are found to be genetically different. This is denoted by a symbol or number which is separated from the strain name with a slash (e.g. C57BL/6 and C57BL/10). A new subline is formed when a new colony is set up in a different laboratory, where it is bred for a long time. During this, genetic changes can occur. The name of the subline is indicated by the abbreviated name of the breeder or the laboratory (e.g. Sc = Scott, Sn = Snell, He = Heston, J = Jackson Laboratory, Ca = Cambridge). The symbols are added in historical sequence (e.g. C57BL/10ScSn). A new subline develops when the strain has been manipulated (e.g. the offspring has been fostered onto animals of another strain). The manipulations can cause changes in the strain (e.g. fostering can influence the carrying of viruses). Therefore, the different manipulations performed during breeding must be indicated in the name. The most frequent strain manipulations and their symbols are as follows:

- f (foster nursing): This indicates the fostering of the progeny by another strain, e.g. C57BL/10fC3H. This procedure can be applied when specified-pathogen-free (SPF) strains are developed. The fetuses are removed with the pregnant uterus under aseptic conditions before birth and transferred to a mother of an SPF strain.
- e: This indicates that the strain has been developed by the transfer of embryos to another strain.
- h: This indicates hand rearing.
- o: This indicates an ovary transplant.
- p: This indicates the preservation of ova by freezing in liquid nitrogen.

The subline symbols follow one another in chronological sequence (e.g. C57BL/10ScSnfC3H: the C57BL/10 strain was passed for breeding to J. P. Scott (Sc), who then passed it to G. D. Snell (Sn), and thereafter the offspring of the strain were fostered onto the mice of the C3H strain.

4.1. Abbreviated strain names

If the name of the strain is too long, it is reasonable to abbreviate it, but the full name must also be given in the text. The following abbreviated names of mouse strains are recommended: AKR = AK, BALB/c = C, C3H = C3, C57BL = B, C57BL/6 = B6, C57BL/10 = B10, etc.

4.2. Inbreeding depression

An inbreeding depression involves a decline in the reproductive performance, in the ability to survive, and in health in the first few generations of inbreeding. It is caused by the homozygosity of deleterious recessive genes which occurs at the beginning of inbreeding. Later, these are eliminated, and accordingly, no further inbreeding depression will occur in the established inbred strain.
4.3. F₁ hybrids

F₁ hybrids are the first generation of crosses between two inbred strains. All individuals are isogenic and heterozygous for all genetic loci at which the two parental strains differ (e.g. histocompatibility genes). The hybrid vigor or heterosis which can be observed in the F₁ hybrids is the opposite of the inbreeding depression: it results from the covering up of deleterious recessive genes. The designation of F₁ hybrids is: (name of the maternal strain x name of the paternal strain) F₁, e.g. (BALB/c x C57BL/6)F₁; the abbreviated name is CB6F1.

5. Use of inbred strains in biological and medical research:

- Investigation of the properties of the species and genetic factors
- Examination of biological effects and responses
- Studies of physiological functions and mechanisms
- Investigation of immunological phenomena, immunogenetic factors, the function of the immune system, transplantation and tumor immunity
- Analysis of effects and mechanisms of action of drugs
- Creation of models of pathological conditions and diseases: studies on the pathomechanism and therapeutic possibilities

A wide range of species (mouse, rat, guinea pig, hamster, rabbit, chicken, etc.) and strains (mostly mice and rats) are available.

5.1. Coisogeneic strains

If a single-gene mutation occurs in an inbred strain, it can subsequently be maintained by establishing a new inbred strain. The new strain, which differs from the original strain at a single gene locus, is called a coisogeneic strain. The nomenclature of coisogeneic strains is indicated by the strain (substrain, subline) symbol, followed by a hyphen and the gene symbol in italics. For example, the CBA/Ca-nu mouse strain is a coisogeneic strain of the CBA/Ca strain, which carries the nu (nude) gene mutations determining hairless and athymic phenotype instead of the ‘normal’ alleles of the appropriate genes. The mutation may form the basis of a disease (see the following examples):

1. Mouse
   - Sex-linked anemia: sla gene
   - Diabetes: db gene
   - Obesity: ob gene
   - Amino acid metabolism disorders: his (histidinemia), pro (prolinemia) gene
   - Kidney disease: kd gene
   - Muscular dystrophy: dy gene
   - Embryonic disorder: t allele
   - Hairless and athymic: nu gene

2. Rat
   - Diabetes insipidus: di gene
   - Hairless and athymic: rnu gene
   - Bilirubinemia (jaundice): j gene

Coisogeneic strains make it possible to study the role of genes in determining the phenotype. Strains with disease-causing genes are suitable for the creation of experimental models of diseases.
5.2. Congenic strains, congenic resistant strains

In this case, the selected gene or genetic region of the donor strain (not necessarily an inbred strain) is introduced into an inbred recipient strain. The choice of the breeding method depends on whether the gene is dominant, codominant or recessive. Usually backcrossing is applied. In the cases of dominant or codominant genes, F1 hybrids are first produced by crossing the two strains, and these are then backcrossed with the recipient strain. The offspring carrying the donor gene are selected, and backcrossed with the recipient strain. This must be repeated at least 12 times (the selection for the presence of the donor gene must be performed on each occasion). After the last backcross, a male and a female animal, which are heterozygotes for the donor gene, should be selected and mated, and from the offspring two homozygotic twins must be mated (intercross). The new congenic strain carries the donor gene (but not the appropriate gene allele of the recipient), and, due to the serial backcrosses, all the other genes (background genes) are identical with the background genes of the recipient.

In 1946, Snell applied this method to develop the congenic resistant mouse strains. Selection was based on the resistance to tumors (the tumor did not begin to grow or was rejected in the recipients) or the rejection of skin grafts. Using these strains, he proved that a polymorphic genetic region (major histocompatibility complex = MHC) is responsible for the rejection of allografts. In mice, this corresponded to the polymorphic gene described by Gorer, which encodes for blood group antigen II and was named H-2.

The usual nomenclature for congenic strains is as follows: the name of the recipient strain, the name of the donor strain. Most often, the abbreviated names are used, e.g. B10.D2. In this case, C57BL/10 (B10) is the recipient and DBA/2 (D2) is the donor strain. The background genes of the B10.D2 strain are identical with those of the B10 strain, while its H-2 genes are identical with those of the D2 strain (H-2<sup>d</sup>), i.e. the B10.D2 strain differs from the B10, which carries the H-2<sup>b</sup> haplotype, only in the H-2 gene complex. H-2 congenic mice are very suitable for studies on transplantation immunology and immunogenetic research.

5.3. Recombinant inbred strains

Recombinant inbred strains are developed from the F<sub>2</sub> generation from the cross of two unrelated inbred strains with strict brother by sister matings through at least 20 generations. The first widely used recombinant inbred strains were developed from a cross between the BALB/cBy (C) and C57BL/6By (B6) strains and were designated CXBD, CXBE, CXBG, CXBH, CXBI, CXBJ and CXBK (D.W. Bailey, 1971). In the designation of these strains, the abbreviated names of the two parental strains are separated by a capital X, which is followed by a dash and a number (e.g. the names of strains developed from a cross between the AKR/J and C57L strains are AKXL-1, -2, -3, etc.). This rule is not used for the long-established, widely-known CXB strains. The recombinant inbred strains carry the mixed-up genes of the two parental strains. Because they are inbred, the genotype of all individuals of a strain is identical, i.e. they are isogeneic. They can be used for the identification and mapping of new polymorphic genetic loci and studies of the polygenic characteristics, which are determined by multiple genes (e.g. the life span, or the susceptibility to spontaneous and induced diseases), and also the biometric analysis of morphological, physiological, biochemical and behavior characteristics, and the investigation of drug effects.

5.4. Transgenic animals

Animals are considered to be transgenic animals if the genome contains foreign gene(s) introduced from outside. The term transgene originates from the expression transferred genetic material. Gordon and his coworkers reported in 1981 that they could successfully transfer foreign DNA into mammals, thereby creating transgenic mice. Later,
transgenic rats, sheep, goats, cattle, rabbits and chickens were also produced. In biological and medical studies, transgenic animals make possible the *in vivo* investigation of functional effect of genes or their products, the development of experimental models of diseases, in agriculture, the development of animals with favorable characteristics (*e.g.* a higher yield of milk or meat, or higher resistance to infectious diseases), and in biotechnology and the pharmaceutical industry, the large-scale production and excretion of specific human proteins by milk (*e.g.* hormones, blood coagulation factors, enzymes, cytokines, *etc.*). The human proteins can be extracted from the milk with high purity.

The gene construction is prepared *in vitro*, using recombinant DNA technology. Besides the structural gene (*e.g.* insulin gene), which is responsible for the function, it can contain a vector gene targeting the gene construction to the appropriate site of the genome, as well as promoter and enhancer DNA sequences regulating the function of the gene. They can be used to express genes only in particular tissues. For instance, lymphoid promoters and enhancers can be used to overexpress rearranged antigen genes in lymphocytes, and insulin promoters to express insulin genes in the β cells of pancreatic islets. Promoter elements can also be included that respond to drugs (*e.g.* tetracyclin or dexamethasone), hormones (*e.g.* insulin) or cytokines, making it possible that the transcription of the transgene can be controlled at will. Genes rendering the cells resistant (*e.g.* the neomycin resistance gene) or susceptible to particular cell-killing drugs (*e.g.* the thymidine kinase gene, which makes the cells sensitive to gancyclovir) can also be inserted into the gene construction. Following transfection, they make possible the *in vitro* selection of the successfully transfected cells: neomycin kills the nontransfected normal mammalian cells, while gancyclovir kills those transfected cells which express thymidine kinase (see later). The gene construction can be introduced by three different methods: 1. microinjection; 2. transfection and transfer of embryonic stem cells; and 3. virus (adenovirus, retrovirus)-mediated gene transfer.

*Creation of transgenic mice by microinjection*

Following superovulation induced by gonadotropic hormone treatment, females are mated with a male. A few hundred copies of the transgene are injected into the male pronuclei of fertilized eggs obtained from the oviducts. The two-cell embryos are implanted into the oviducts of pseudopregnant females. Pseudopregnancy is induced by mating females with vasectomized males. One to 50 copies of the transgene insert in tandem into a random site of breakage in a chromosome and are subsequently inherited as a single mendelian trait. Since integration usually occurs before DNA replication, about 75% of the transgenic pups carry the transgene in all their cells, including germ cells. In most cases, integration of the foreign DNA does not disrupt the endogenous gene function. Each animal carrying the transgene is a heterozygote, from which homozygous lines can be bred. The microinjection method can be applied in every mammalian species. In mice, about 2.6% of the born offspring are transgenic; in other species, this proportion is 0.7-1.6%.

The nomenclature of the transgenic animals is as follows: The name of the recipient is followed by a dash and the symbol of the transgene, *e.g.* C57BL/10J-TgN(XXXX)Y, where *Tg* = transgenic, *N* = nonhomologous (or *H* = homologous) insertion, XXXX = the name of the gene, and Yyy = the code of the laboratory.

*5.5. Gene knockout animals*

The targeted insertion of a transgene in the middle of an endogenous gene usually disrupts the coding sequences and ablates the expression or function of that gene. This is commonly achieved by homologous recombination. In this case, a gene homologous to the targeted endogenous gene is constructed. The DNA sequence of the construct is almost identical with that of the endogenous gene. If this is inserted into a cell, it will preferentially
recombine and and replace endogenous sequences. The targeting vector can be designed such
that homologous recombination will lead to the deletion of one or more exons of the
endogenous gene. From the changes caused by the ablation of the gene function in the gene
knockout animals, conclusions can be drawn concerning the physiological roles of the gene
and gene products. This method can also be used to develop animal models of single-gene
disorders.

Generation of gene knockout animals with the embryonic stem cell method. A targeting vector
is used to first disrupt the gene in an embryonic stem cell line. This contains the neomycin
resistance gene (\textit{neo}) between the two homologous exons and, flanking one of them, also the
thymidine kinase gene (\textit{tk}). Embryonic stem cells are obtained from the inner cells of the
blastocyst. These are pluripotent stem cells which can be propagated in tissue culture \textit{in vitro}.
They are also capable of differentiating, and thus they can produce all the cells of the mature
organism, including the germ cells. The targeting vector gene added to the embryonic stem
cell culture is inserted into some of the cells. In the event of homologous recombination, the
exogenous gene inserted into the genome contains the \textit{neo} gene, but not the \textit{tk} gene.
Accordingly, the transfected cells will be resistant to both neomycin and gancyclovir. During
random, nonhomologous insertion, not only the \textit{neo}, but also the \textit{tk} gene is inserted. Such
cells are resistant to neomycin, but sensitive to gancyclovir. If neomycin and ganciclovir is
added to the cell culture following transfection, neomycin will kill the nontransfected cells,
while gancyclovir will kill those transfected cells into which the vector has been integrated
with nonhomologous recombination. Thus, only the gene knockout embryonic stem cells
survive which have undergone homologous recombination. The presence of the desired
recombination can be verified by DNA analysis [with Southern blot hybridization or the
polymerase chain reaction (PCR)]. The selected cells are injected into blastocysts \textit{in vitro} and
implanted into pseudopregnant females. Animals that develop will be chimeric, \textit{i.e.} some of
the tissues will be derived from the gene knockout embryonic stem cells and others from the
remainder of the normal blastocyst. The germ cells are also chimeric, but because these cells
are haploid (they contain only one set of chromosomes), only some cells will contain the
chromosome copy with the disrupted gene. If chimeric animals are mated with normal
animals and either sperm or eggs containing the gene knockout chromosome fuse with germ
cells of the normal partner, all cells in the offspring will be heterozygous for the mutation. If
such heterozygous animals are mated, homozygous gene knockout mice, which do not
express the gene, will be obtained with a frequency that is predictable by a simple mendelian
segregation.

Homologous recombination can also be used to replace a normal gene sequence with
another functioning exogenous gene. The \textit{knockin animals} contain the transgene at a defined
site in the genome, in contrast with random insertion observed in conventional transgenic
animals. This method is used when the expression of the transgene should be regulated by
endogenous promoter or enhancer regions.

The above conventional gene-manipulating techniques have proved to be of great
usefulness, but they have some drawbacks. 1. The mutation of one gene during development
may be compensated for by the altered expression of other gene products, and therefore the
function of the modified gene may be obscured. 2. In a conventional gene knockout animal, the
importance of a gene in only one tissue or at only one time during development cannot be
easily assessed. 3. A functional selection marker gene, such as the \textit{neo} gene, is permanently
introduced into the genome, which can modify the phenotype of the animal. A refinement of
the gene knockout method is made possible by application of the bacteriophage-derived
\textit{Cre/loxp} recombination system, which eliminates some of the above drawbacks. The \textit{Cre}
enzyme is a DNA recombinase that recognizes the \textit{loxp} DNA sequences, and mediates the
deletion of such gene segments flanked by two \textit{loxp} sites. When a recombinant system based
on this principle is used, the neo gene can be deleted together with the linked homologous sequences from the gene knockout mice. The expression of the cre gene, i.e. the synthesis of the Cre enzyme and the gene deletion, can be restricted to certain tissues. A steroid-inducible promoter can also be used, so that Cre expression and subsequent gene deletion occur only after the animals are given an appropriate dose of dexamethasone. The Cre/loxP technology can also be used to create knockin animals. In this case, the loxP sites are placed to flank the neo gene and the targeting homologous sequences, but they do not flank the replacement (knockin) gene sequences. Therefore, after Cre-mediated deletion, the exogenous gene remains in the genome at the targeted site.

5.7. Cloned animals

The essential characteristic of the clone is that the genome of the offspring is fully identical with that of the starting organism. During cell divisions (e.g. the division of fertilized eggs and body cells), the clones of the same starting cell develop. The methods of cloning multicellular organisms are as follows:

1. **The embryo splitting technique**

   This is a routine method for cloning domestic animals with good properties (e.g. cattle), whereby identical twins are produced artificially. An egg is fertilized artificially with a sperm, and the zygote is allowed to divide into an eight-cell embryo. The embryo is then divided into two four-cell, or most often into four two-cell pieces. No differentiation of the cells occurs for the eight–cell embryos, and thus the split embryos will be genetically identical as identical twins. Therefore, this method is also called embryo twinning. The offspring do not resemble their parents, but rather one another. The separated embryos are allowed to develop to a state when they can be implanted into the uterus of a pseudopregnant female animal. Thereafter, a normal pregnancy and delivery can occur.

2. **Somatic cell nuclear transfer (SCNT)**

   This method was used by Ian Wilmut and Keith Campbell in the Roslin Institute in Scotland in 1997, when the sheep Dolly was created. SCNT requires two cells: a nucleus donor cell and an unfertilized oocyte or egg cell. The unfertilized egg cell is more likely than the fertilized egg cell to accept the implanted nucleus as its own. The egg cell is a more appropriate recipient cell than other cells, because it can be forced to be divided more easily. The steps of the nuclear transfer are as follows:

   1. A body cell serves as a donor cell. In the case of Dolly, it was a mammary gland cell. The donor cell is placed in tissue culture and allowed to divide in vitro. The donor cell is then placed into tissue culture medium without fetal calf serum, in which cell divisions are stopped, and the cells enter the G₀ or dormant state. This is necessary for the recipient cell to accept the nucleus from the donor cell.

   2. The nucleus must be removed from the egg cell. The nucleus contains most of the genetic information, but the mitochondrial DNA also stores genetic codes. The donor cell is placed next to the enucleated recipient cell, and 1-8 h after the removal of the nucleus from the egg cell, an electric stimulus is used to fuse the donor cell (or its isolated nucleus) with the enucleated egg cell and, at the same time, activate cell divisions and the development of an embryo. The genetic program of the differentiated donor cell is zeroed in the egg cell, and it returns to an undifferentiated state: it becomes pluripotent. If the embryo survives, it is allowed to grow in an oviduct of a sheep for 5-6 days (here the embryos survive better than in a tissue culture).

   3. Once the cloned embryo has reached the blastomere stage of development, consisting of about 100 cells, the embryo is implanted into the uterus of a surrogate mother. The mother carries the pregnancy. The progeny is a genetically identical copy of the donor. This is
called reproductive cloning. Dolly was the only lamb born from 277 attempts. Blastomeres can also be used as a source of stem cells.

In 1998, Teruhiko Wakayama and Ryuzo Yanagimachi modified the method of Ian Wilmut (the Roslin technique) and elaborated the Honolulu technique at the University of Hawai, whereby they successfully cloned mice. The cloned offspring were able to reproduce normally and maintain the clone by sexual reproduction.

Incomplete reprogramming of the donor cell is thought to be a leading factor in the low rate of success of animal cloning. Chromatin transfer is a new cloning technique aimed at reducing these problems. It involves treatment of the cell of the animal to be cloned to remove molecules associated with cell differentiation before the nucleus is removed.30, 72-74

References