Genetically Modified Animals

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In Press – Encyclopedia of Neuropsychopharmacology

Synonyms

Transgenic Animal; Mutant Animal; Genetically Engineered Animal; Genetically Modified Organism (GMO)

Definition

An animal whose genetic material has been altered by the use of genetic engineering or recombinant DNA technology. In biomedical sciences, genetically modified animals are typically generated for the purpose of studying the function of a particular gene.

Principles and Role in Psychopharmacology

One of the main goals of the field of genetics is to classify and functionally characterize individual genes. The investigative approach to studying genes in living organisms has principally been divided into three strategies: 1) analysis of natural variation, 2) random mutagenesis and, 3) targeted mutagenesis and transgenesis (Rudolph and Mohler 1999). Analysis of natural variation (e.g. spontaneous mutations) and random mutagenesis (e.g. chemical or irradiation) are the primary approaches of 'forward genetics' where the genetic cause (genotype) of an altered or abnormal phenotype is investigated. However, with random mutagenesis, many chromosomal loci are often targeted and it is difficult to trace any phenotype back to a specific genetic origin. The development of 'reverse genetic' approaches, where a particular gene is altered and the phenotype is investigated, provided tools to investigate specific gene function in a more targeted manner (Brusa 1999). Since the development of these tools in the 1980s and 1990s, their use in the field of biomedical research and pharmacology has been substantial due to the ability to develop suitable animal models of specific diseases, the ability to genetically dissect the underlying mechanisms of disease, and the ability to identify and verify molecular targets of pharmacological agents.

In the past two decades, a variety of techniques have been developed to introduce genetic modifications in various species for specific research purposes. Among the most targeted species are *Drosophila melanogaster*, *Caenorhabditis elegans* and mice, which have each played integral roles in identifying genes involved in development, aging, cell differentiation, and other major biological

functions. Other genetically modified animals that have been developed include xenopus, zebrafish, rabbits, pigs, and cows. More recently, transgenic and knockout rats have been developed which will allow more extensive research in the neurosciences due to their extensive use in behavioral paradigms (Abbott 2004). In addition, the first transgenic primate disease model (for Huntington's disease) was recently created (Yang et al. 2008). While a wide variety of genetically modified organisms have been created to date for numerous research purposes, techniques for genetically modifying mice are the most advanced and the most applicable to the field of psychopharmacology, which represent the main focus of the remainder of this article.

Transgenic Technology

While the term "transgenic" has grown to include any type of genetically modified animal, the traditional definition of a transgenic organism is one containing foreign DNA, whether from the same species or a different one. The expression of foreign DNA in a mouse is a valuable technique since it allows for the investigation of the functional role of this gene in a living organism. For instance, transgenic mice overexpressing a particular gene are often generated to analyze exaggerated phenotypes. The expression of a human gene or a mutated gene in mice is also often used to explore gene function, particularly in the context of a specific disease.

There are several ways to create a transgenic mouse, however, all methods consist of first designing a DNA fragment, or "genetic construct", which contains the gene of interest (GOI) and other features necessary for the expression of this gene in a mammalian system (e.g. gene promoter, enhancer, polyA signal, etc). The traditional transgenic method consists of physically injecting the transgenic construct into the nucleus of a fertilized egg (pronuclear microinjection), allowing it to develop in vitro to the blastocyst stage, and then implanting the egg into a pseudopregnant female **(Fig 1a).** The embryos must then be screened for the presence of the transgene and unlike the production of knockouts, the transgene typically occurs in an all or none fashion, with the embryo either containing the transgene in every cell or in no cells at all. Alternatively, transgenic animals can be produced by viral infection of the fertilized embryo (see below) or transfection of embryonic stem cells (ES) with the gene of interest (Dale 2002). While the transgenic approach is fast and efficient, limitations of the technology include: 1) The GOI may randomly integrate into the genome which can result in expression in ecotopic sites, interference with the endogenous gene, or severe disruption of the homeostasis of the cells and organism; 2) The level of gene expression is unable to be controlled and is dependent upon where the gene inserts into the host genome and on the number of copies inserted. Overexpression of the gene could have unexpected detrimental effects, including lethality; 3) Mosaic or chimeric animals are sometimes produced, particularly when the transgenic animal is generated with the viral method, due to infection of only a subset of cells within the blastocyst; 4) The

genetic background of mice can considerably influence the ability of the manipulated egg to survive microinjection, implant in the uterus, and develop to term (Brusa 1999; Dale 2002)

Gene Knockout Technology

The creation of a traditional knockout (i.e. removal of a gene) mouse consists of disrupting all or part of the coding sequence of the GOI, with the purpose of exploring the phenotype in the absence of the gene. The exact locus of the GOI can be targeted by creating a genetic construct that is homologous to the region of the GOI on a particular chromosome. The genetic construct is injected into embryonic stem cells where rare homologous recombination events can occur with the endogeneous GOI at the intended chromosomal locus. In the knockout approach, the GOI is often replaced with neomycin (Neo) or another selectable marker which allows for in vitro selection and identification of the stem cell colonies that have undergone appropriate recombination **(Fig. 1b)**. Selection against random integration into the genome is often attained by using a second selectable marker outside the inserted region. Once the mutated stem cells are identified and verified, they are microinjected into a blastocyst and implanted into a pseudopregnant female as in a transgenic mouse. Most of the offspring produced using this technique are chimeras (or mosaics) due to the presence of both mutated and non-mutated stem cells within the blastocyst. Embryonic stem cells from a different color strain of mice than the fertilized egg are often used because the chimeric mice are then easily identifiable due to bi-colored fur. The next step consists of breeding chimeric mice to identify individuals with germ cells that have undergone homologous recombination. These founder mice will then be bred to produce homozygous mutant animals (Dale 2002). Similar to knockout mice, knockin technology, where one or more exons of a certain gene are replaced with an altered version **(Fig. 1b)**, is often used to study specific polymorphisms, or the human equivalent of the GOI.

While knockout organisms have been paramount in the goal to elucidate the function of specific genes, problems associated with knockout technology include that removal of a gene is often lethal or that the absence of the gene product during development leads to compensatory events that can obscure the analysis of the function of the missing gene. Specifically, these compensatory developments likely differ from the disease mechanisms that the knockout animal is intended to model, as disease processes rarely include full loss of gene function.

Conditional Knockout Technology

The desire for greater specificity and the need to bypass potential developmental compensations and occasional lethality has lead to the development of conditional or inducible knockouts, where the experimenter has either temporal or regional control over expression of the GOI. Several systems have been developed for this purpose including the Cre-LoxP system, Flp-FRT

system, and the Tetracycline-inducible system. The Cre-LoxP system of bacterial origin, employs Cre recombinase, which mediates site-specific recombination by targeting a DNA sequence called LoxP (Houdebine 2007). To implement this system, one strain of mice is engineered to express Cre under a tissue- or developmental stage-specific promoter and these mice are bred to a second strain of mice in which the GOI has been flanked with LoxP sites. The resulting offspring then have the GOI removed only in the tissues (or at the developmental timepoint) where/when Cre is expressed **(Fig. 1c)**. The Flp-FRT system originates in yeast and is used in the same manner as the Cre-LoxP system with Flp recombinase excising the GOI flanked by FRT DNA sequences (Houdebine 2007). Importantly, these two systems can be used together to allow for a range of additional approaches.

The tetracycline-inducible system is based on the tetracycline resistant gene from bacteria and has been modified for use in mammals to essentially act as a switch to initiate or terminate gene expression. In bacteria, the tetracycline resistance gene is typically kept in the off position by a repressor bound to a specialized DNA sequence in the promoter of the gene, the tetracycline operator (*tetO*) sequence. For use in mammalian transgenics, the repressor protein was modified into a transactivator (tTA) which allows constitutive expression of genes bound to *tetO*. Thus, in the presence of tetracycline, gene expression is terminated when the drug binds to tTA, removing it or preventing its binding to *tet*O. A modified version of tTA (rtTA) requires the presence of tetracycline to bind to *tetO* thereby allowing activation of a gene in the presence of tetracycline. For these systems to work, two transgenes in a single animal are needed; one expressing tTA under the control of a site- or temporal-specific promoter and the GOI under control of the *tetO* **(Fig. 1d)** (Brusa 1999).

RNAi and Gene Transfer in vivo

The recent discovery and exploitation of the endogenous RNA interference (RNAi) mechanism has aided in the development of loss of function models. The endogenous mechanism consists of short sequences of double stranded RNA which bind and cleave complementary mRNA sequences thereby silencing or inducing downregulation of the specific gene (Dunn et al. 2005). Short sequences of double stranded RNA (~22 nucleotides), termed small interfering RNA (siRNA), are easily synthesized and can be delivered *in vivo* using a variety of gene transfer techniques (see below and **Fig. 1e**). Similar to RNAi, antisense technology is the expression of the reverse complement of mRNA which interferes with normal translation thereby reducing protein synthesis (Dale 2002).

While conditional and inducible knockouts provide certain level of site and temporal specificity, they are dependent upon the availability of promoters that provide the desired specificity. The development of viral-mediated gene transfer has allowed more flexibility in producing the desired manipulations. In addition to an alternative method of producing transgenic animals (discussed above), viral vectors can be used to transfer genetic material in a temporal and site specific manner in

both neonatal and adult mice. Modified virons such as herpes, lenti-, adeno-, and adeno-associated viruses can be engineered to carry a transgene, siRNA, or other genetic material. These viral vectors are then able to infect cells and transmit the desired genetic material. Viral-mediated gene transfer has the additional advantage over conditional mutants that the genetic manipulation occurs only at the site of infection and cell-type specific promoters can be used for additional specificity. Depending upon the type of virus used, the genetic material may be integrated into the genome or may remain epichromosomal. The type of virus also influences the infection rate, type of cells infected, and the size of DNA insert (Dunn et al. 2005). While viral-mediatd gene transfer technology is widespread, non-viral gene transfer to the central nervous system has also been achieved using in vivo electroporation and both intracerebroventricular and intrathecal infusion (Gilmore et al. 2006).

Roles of Genetically Modified Animals in Psychopharmacology

The creation of the first knockout mouse in 1989 let to a noble prize for Sir Martin Evans (Cardiff University in Wales), Oliver Smithies (University of North Carolina at Chapel Hill), and Mario Capecchi (University of Utah in Salt Lake City) in 2007. Since the development of the technology, using knockout mice along with other types of mutants to investigate critical questions in psychopharmacology has become standard practice. In particular, genetically modified mice have been essential in psychopharmacology for 1) Elucidating both the function of a gene and the molecular elements associated with a gene; 2) Creating animal models of human disease; 3) Identifying and validating drug targets and drug specificity and; 4) Examining temporal aspects of gene function.

One of the first major breakthroughs in the field of neuroscience using targeted mutagenesis came from Eric Kandel's group at Columbia University. In a series of experiments using the tetracycline inducible system, they were able to express a calcium-independent form of the forebrain specific calcium dependent kinase, calcium-calmodulin kinase II (CaMKII) and found deficits in spatial memory and hippocampal long-term potentiation (LTP) (Mayford et al. 1996). These groundbreaking experiments provide a classic example of the power of how spatial and temporal control over molecular elements can aid in elucidating the function of specific genes and their role in higher brain function. The CaMKII promoter still remains one of the most popular promoters to express numerous genetic constructs in the mouse forebrain.

Another critical role for transgenic animals is the development of animal models for human disease, by either the introduction of a mutated gene or the elimination of a gene putatively involved in the illness. In the field of neuroscience this has been particularly useful in modeling a wide variety of disorders, including Alzheimers disease, Huntington's disease, neuropsychiatric disorders, and cerebral ischemia. In particular, Alzheimers disease (AD) is characterized by the formation of neurofibrillary tangles of hyper-phosphorylated tau protein and by amyloid β-peptide (Aβ) plaques.

Mutations in the amyloid precursor protein (APP), presenilin 1 and 2 (PS1, PS2) and apolipoprotein E (APOE) are all implicated in the disease. Studies now show that mice overexpressing APP and PS1 form Aβ plaques and display memory deficits, both characteristic symptoms of Alzheimers disease (Brusa 1999), consequently highlighting the important role of genetically modified animals in testing potentially causal mechanisms involved in human disease. In some cases it is necessary to replace the murine gene with the human gene due to distinct structural differences between the human gene and mouse homologue at the molecular level (Rudolph and Mohler 1999).

The development of genetic animal models for human diseases has provided a solid foundation for drug discovery and for the identification of drug targets. The specificity of the genetic manipulation (i.e. removing a gene coding for a particular receptor subtype) ensures virtually absolute selectivity thereby offering a great advantage over classical pharmacological approaches. For instance, the function of receptor subtypes can be examined using both knock-in and knockout approaches. A particularly relevant example is that of the utilization of transgenic mice in determining the role of specific GABA receptor subunits in distinct actions of the benzodiazepine, diazepam. Diazepam is known to act on $GABA_A$ receptors containing the α1-, α2-, α3-, or α5- subunits. By examining mice carrying point mutations in the benzodiazepine binding sites of each subunit, investigators were able to genetically dissect the different functions of diazepam (e.g. sedative vs. anxiolytic) acting at otherwise similar $GABA_A$ receptors (Rudolph and Mohler 1999).

Other critical uses of genetically modified animals are the dissection of second messenger signaling pathways and the determination of critical developmental time-windows for gene function. The latter was elegantly demonstrated by the use of a tetracycline inducible knockout of the serotonin 1A receptor. Gross et al. 2002, showed that when the 1A receptor was knocked out during development, it resulted in behavior similar to the knockout mouse (increased anxiety). However, when the receptor was knocked out in adults, the phenotype was absent (anxiety levels were normal) thereby implicating the 1A receptor as a critical developmental factor for normal emotional behavior (Gross et al. 2002).

In summary, the primary goal of reverse genetics is to create a targeted mutation and then investigate the resulting phenotype. We briefly discussed several methods of targeted mutagenesis including the development of transgenic animals and the techniques for developing global, conditional and inducible knockouts. The values of this technology have been far-reaching and have played a considerable role in psychopharmacology. We have highlighted some of the most common uses for genetically modified animals in this field including the dissection of molecular mechanisms, modeling human disease, drug discovery and validation, and the investigation of critical time windows in gene function. While several other approaches for creating genetically modified animals exist (e.g. the use of modified male gametes) along with other applications for these animals (e.g. pharming), we focused on the role of genetically modified mice in psychopharmacology, as their impact on this field

has been substantial. New approaches to developing and using genetically modified organisms are quickly evolving, including modifications and combinations of the discussed systems, which will likely further impact psychopharmacology.

Cross-References

→Epigenetics

→Ethopharmacology

→Gene Expression and Transcription

→Pharmacogenetics

→Phenotyping of Behavioural Characteristics

→Translational Research

References

Abbott A (2004) Laboratory animals: the Renaissance rat. Nature 428: 464-6

Brusa R (1999) Genetically modified mice in neuropharmacology. Pharmacol Res 39: 405-19

Dale JWavS, Malcom (2002) Transgenics From Genes to Genomes: Concepts and Applications of DNA Technology. John Wiley & Sons, Ltd., pp 325-338

Dunn DA, Pinkert CA, Kooyman DL (2005) Foundation Review: Transgenic animals and their impact on the drug discovery industry. Drug Discov Today 10: 757-67

Gilmore IR, Fox SP, Hollins AJ, Akhtar S (2006) Delivery strategies for siRNA-mediated gene silencing. Curr Drug Deliv 3: 147-5

Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S, Hen R (2002) Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. Nature 416: 396-400

Houdebine LM (2007) Transgenic animal models in biomedical research. Methods Mol Biol 360: 163-202

Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER (1996) Control of memory formation through regulated expression of a CaMKII transgene. Science 274: 1678-83

Rudolph U, Mohler H (1999) Genetically modified animals in pharmacological research: future trends. Eur J Pharmacol 375: 327-37

Yang SH, Cheng PH, Banta H, Piotrowska-Nitsche K, Yang JJ, Cheng EC, Snyder B, Larkin K, Liu J, Orkin J, Fang ZH, Smith Y, Bachevalier J, Zola SM, Li SH, Li XJ, Chan AW (2008) Towards a transgenic model of Huntington's disease in a non-human primate. Nature 453: 921-4

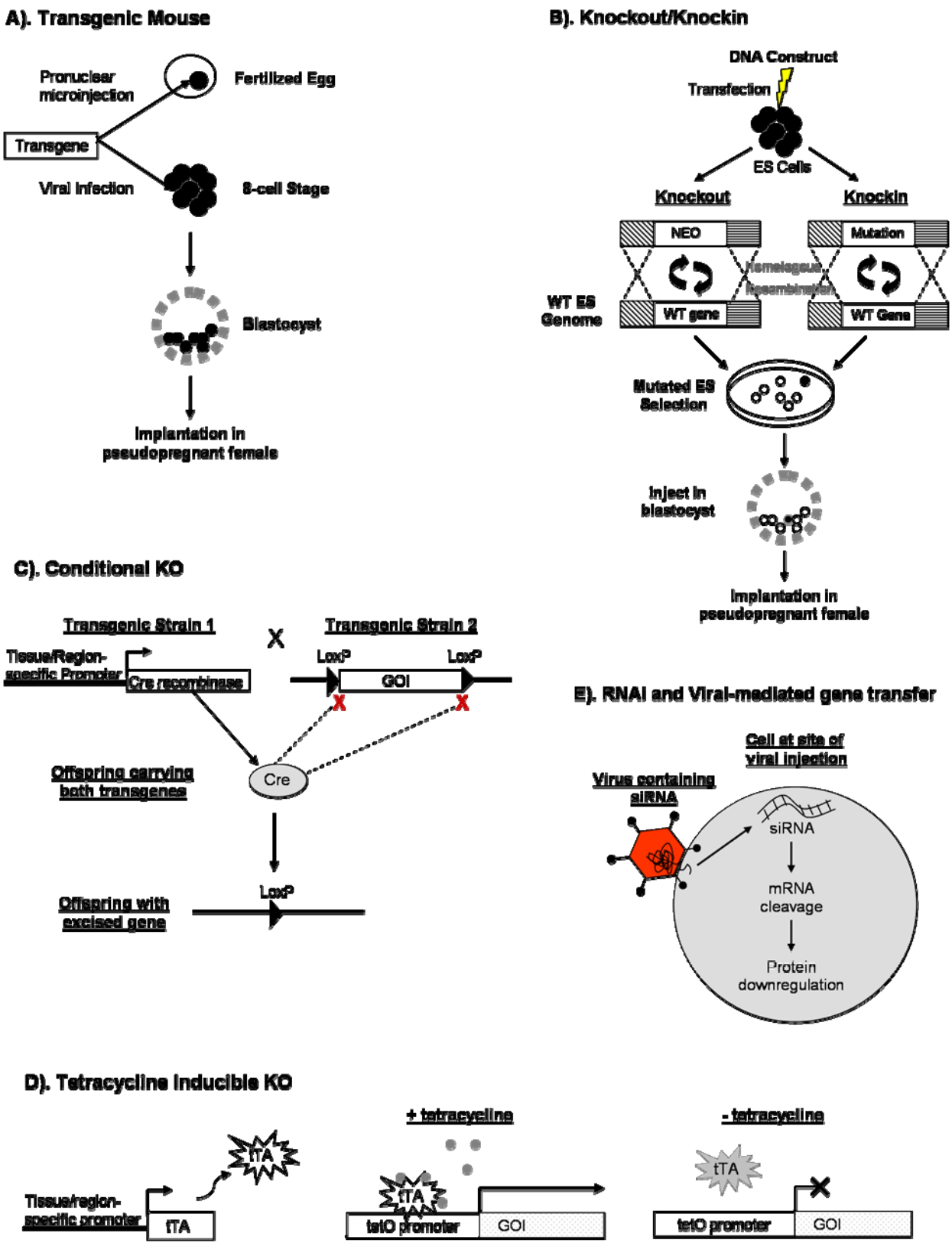

Figure 1: Diagrams of methods for producing genetically modified animals.

[Short Definitions]

Transgenic Organism

Synonyms

Mutant

Definition

Traditionally, an organism with the addition of foreign DNA, whether from the same species or a different one. More recently the term transgenic has been used to refer to any genetically modified organism.

Cross-References

→Genetically Modified Animals

→Ethopharmacology

→Phenotyping of Behavioural Characteristics

Knockout / Knockin

Synonyms

Global knockout; Constitutive knockout

Definition

Knockout - The removal or complete disruption of a specific gene in an animal from the blastocyst stage through adulthood. Knockin – The introduction of a mutated version of a specific gene in place of the wild type version.

Cross-References

→Genetically Modified Animals

→Ethopharmacology

→Phenotyping of Behavioural Characteristics

Conditional knockout

Synonyms

Site-specific knockout, region-specific knockout, cell type-specific knockout

Definition

The removal or complete disruption of a specific gene in a manner that controls the cell types and brain region or site where the disruption occurs. The Cre/loxP system is frequently used

to produce conditional knockouts and in this system, the promoter expressing Cre recombinase will give rise to the specificity of the excised gene.

Cross-References

→Genetically Modified Animals

→Ethopharmacology

→Phenotyping of Behavioural Characteristics

Inducible knockout

Synonyms

Time-specific knockout, temporal knockout

Definition

The use of a system where the experimenter controls the timing of gene removal. The tetracycline inducible system is frequently used to produce animals where a simple injection of tetracycline will either terminate or initiate gene transcription.

Cross-References

→Genetically Modified Animals

→Ethopharmacology

→Phenotyping of Behavioural Characteristics

RNAi (RNA interference)

Synonyms

siRNA (small interfering RNA)

Definition

An endogenous system where short sequences of double stranded RNA molecules induce the cleavage of matching mRNA resulting in downregulation of a particular gene. This system can be manipulated for experimental by using exogenous siRNA to downregulate a GOI.

Cross-References

→Genetically Modified Animals

→Gene Expression and Transcription

Chimera

Synonyms

Mosaic

Definition

An animal where individual cells contain genetic material from only one of two potential lineages. These animals are often produced in the creation of knockout mice where a mutated ES cell is introduced into the blastocyst containing wild type ES cells.

Cross-References

→Genetically Modified Animals

Forward Genetics / Reverse Genetics

Synonyms

Forward genetics – Random mutagenesis; Reverse genetics – Targeted mutagenesis

Definition

Forward genetics is the examination of the genetic cause of an altered or abnormal phenotype introduced by a chemical mutagenesis or mutation by irradiation (e.g. phenotype → genotype). In reverse genetics, a particular gene is altered and the phenotype is investigated (e.g. genotype → phenotype).

Cross-References

→Genetically Modified Animals

→Phenotyping of Behavioural Characteristics

Phenotype/Genotype

Synonyms

Definition

Genotype is the specific genetic constitution of an organism including the gene allelic make-up. Phenotype is the physical trait or characteristic arising from the genotype.

Cross-References

→Genetically Modified Animals

→Phenotyping of Behavioural Characteristics

Pseudopregnant

Synonyms

Definition

A hormonal state similar to pregnancy that is induced in mice by mating a female with a vasectomized male. In this state, the uterus is receptive to an implanted embryo.

Cross-References

→Genetically Modified Animals