Manipulating The Mouse Embryo A Laboratory Manual

Since 1998, the volume of research being conducted using human embryonic stem (hES) cells has expanded primarily using private funds because of restrictions on the use of federal funds for such research. Given limited federal involvement, privately funded hES cell research has thus far been carried out under a patchwork of existing regulations, many of which were not designed with this research specifically in mind. In addition, hES cell research touches on many ethical, legal, scientific, and policy issues that are of concern to the public. This report provides guidelines for the conduct of hES cell research to address both ethical and scientific concerns. The guidelines are intended to enhance the integrity of privately funded hES cell research by encouraging responsible practices in the conduct of that research.

With the advent of transgenic technology, which allows the identification of specific gene activities in developing mammalian organisms, the house mouse has once again taken a very important place in experimental research as one of the genetically best understood mammals. More than ever, molecular biologists are in need of a detailed, standardized description of the anatomy of the developing mouse embryo. In this classic compendium, now brought up to date and corrected, the author presents each stage of mouse development in photographs and micrographs using hybrids of two inbred strains as a standard. Organ systems are systematically reconstructed from fertilization until after birth. Molecular biologists tracing the effects of genetic manipulations, as well as students and researchers of developmental biology, will appreciate the renewed availability of this standard reference work for its unparalleled accuracy, its attention to anatomical detail, and the extent of its documentation.

The Atlas of the Prenatal Mouse Brain is the latest addition to Academic Press' list of atlases for neuroscientists and neuroscience students. It fills an urgent need for a comprehensive atlas of the developing mouse brain for use in studies of both normal and abnormal development. High-quality photomicrographs of brain sections are depicted in sagittal, coronal, and horizontal planes for four gestational age groups. Each photomicrograph is accompanied by a fully labeled, precision-drawn diagram for easy identification of brain structures. Researchers and students using normal, transgenic, or mutant mouse preparations in developmental neurobiology, neurotoxicology, and biotechnology will welcome this meticulously assembled and accessible guide. Presents 153 photomicrographs of serial brain sections Represents four gestational ages (GD 12 and 14 embryos; GD 16 and 18 fetuses), each depicted in sigittal, coronal, and horizontal planes Includes fully labeled diagrams identifying brain structures for each photomicrograph Provides complete alphabetical lists of brain structures and abbreviations Presents a full description of tissue preparation method Large format, 8-1/2 x 11" pages in a sturdy hardcover case

Of mouse development -- Setting up a colony for the production of transgenic

mice -- Recovery, culture, and transfer of embryos -- Introduction of new genetic information into the developing mouse embryo -- Iolation of pluripotential stem cell lines -- Techhniques for visualizing genes and gene products -- In vitro culture of eggs, embryos, and teratocarcinoma cels -- Chemicals, supplies, and solutions.

The generation of mutant mice raises many questions about the best means of phenotypic analysis, breeding, and maintenance. The answers are now available from two experts with a wealth of detailed knowledge never previously assembled in one volume. Informal and highly practical, this handbook provides stepâ€“byâ€“step methods for troubleshooting experiments, from the basics of gene targeting through the analysis of postnatal effects.

Introduction to immunochemistry for molecular biologists and other nonspecialists. Spiral.

In Mouse Molecular Embryology: Methods and Protocols, expert researchers in the field detail many of the protocols used to study mouse embryology. These include protocols and techniques that are "close to the embryo": such as, manipulating embryonic gene expression, culturing explanted embryonic tissue and harvesting embryonic RNA. With additional chapters on fluorescence imaging, lineage tracing, and genetic ablation. Written in the highly successful Methods in Molecular Biology series format, chapters include introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, readily reproducible laboratory protocols, and key tips on troubleshooting and avoiding known pitfalls. Authoritative and practical, Mouse Molecular Embryology: Methods and Protocols seeks to aid scientist in the further study of mouse embryo and its relation to other aspects of biological research.

This dissertation, "Development and Application of a Single Mouse Embryo DNA Methylation-detection Assay" by Chun-kit, Peter, Kwan, ???, was obtained from The University of Hong Kong (Pokfulam, Hong Kong) and is being sold pursuant to Creative Commons: Attribution 3.0 Hong Kong License. The content of this dissertation has not been altered in any way. We have altered the formatting in order to facilitate the ease of printing and reading of the dissertation. All rights not granted by the above license are retained by the author. Abstract: During preimplantation embryonic development, imprinting genes are susceptible to methylation changes by artificial manipulation, which may lead to developmental abnormalities. In addition, environmental endocrine disruptors (EDs) in everyday household products are also found to perturb fertility development and cause epigenetic aberrations. While embryo supply is scarce and conventional epigenetic studies require embryos in vast amount, an assay was developed in this study to examine the methylation statuses of imprinting genes using DNA from single mouse blastocysts cultured in-vitro or exposed to EDs. Promoter CpG methylation patterns of three imprinting genes, small nuclear ribonucleoprotein polypeptide N (SNRPN), paternally expressed 3 (Peg3), and potassium voltage-gated channel 1 overlapping transcript 1 (Kcnq1ot1), were examined from

genomic DNA of a single mouse blastocyst. The genomic DNA was isolated and treated with bisulfite modification to preserve the methylation statuses. Afterwards, the DNA was subjected to whole genome amplification (WGA). Methylation-specific polymerase chain reaction (methyl-PCR) was performed with allele-specific primers; the amplicons were cloned and sequenced. CpG methylations in SNRPN, Peg3 and Kcnq1ot1 showed no statistical significant difference (P>0.05; Mann Whitney U test) in both parental alleles between a single genomic-amplified blastocyst and 20 non-amplified blastocysts, indicating no artifact was being introduced during the WGA procedure. Using the assay, it was revealed that blastocysts cultured in-vitro expressed slight but nonsignificant deviation in methylation rates to both parental alleles of SNRPN and Kcnq1ot1 except in single blastocysts, which displayed significant loss in maternal methylation on SNRPN upon culturing. On the other hand, paternal methylation profile of Peg3 appeared unaffected, suggesting resistance to methylation perturbations induced by in-vitro culturing. Despite that there was no significant difference in overall methylation rates between in-vivo or in-vitro developed blastocysts, certain CpG residues appeared to displayed significant loss of methylation (LOM) or gain of methylation (GOM) induced by in-vitro culture in all three genes being studied. Furthermore, using the developed, assay the epigenetic effects of three endocrine disruptors, simazine, propiconazole, and cadmium chloride (CdCl2) on in-vitro cultured single blastocysts were revealed. When compared to blastocysts cultured with KSOM+AA medium as controls, CdCl2-treated blastocysts displayed the most methylation aberrations in both alleles and within particular CpG residues, possibly due to its dual effect in both hypermethylation and hypomethylation across the methylome. Both simazine- and propiconazole -treated blastocysts displayed overall methylation significant defects were observed within particular CpG residues. Overall, the assay used in this study allowed the comprehensive investigation of methylome from the DNA extracted from a single blastocyst.defects resembled to those blastocysts cultured with KSOM+AA medium alone but DOI: 10.5353/th_b5194756 Subjects: DNA - Methylation

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This book covers a variety of topics on animal reproduction and reproductive medicine. With evolving technology and a continual increase in knowledge, regarding domestic pets or agricultural animals, new information is available on diverse topics in this broad field. The book contents reflect the individual experience of authors, who developed a number of themes identified as attracting interest in the field. As it is, new opportunities were opened for productive collaboration. We have tried to provide you with current, specialised information that may be useful to students, clinicians and researchers. We hope this book inspires you to embrace these themes, foster the debate on particular topics and may be used as a start-up source for exploring the theriogenology field.

Not since the early 1970s has there been an attempt to describe and illustrate the anatomy of the developing mouse embryo. More than ever such material is needed by biologists as they begin to unravel the molecular mechanisms underlying development and differentiation. After more than ten years of painstaking work, Matt Kaufman has completed The Atlas of Mouse Development--the definitive account of mouse embryology and development. For all those researching or studying mammalian development, The Atlas of Mouse Development will be the standard reference work for many years to come. Provides a comprehensive sequential account of the development of the mouse from pre-implantation to term Contains clear and concise descriptions of the anatomical features relevant to each stage of development Large format for easy use Contains explanatory notes and legends, and more than 180 meticulously labeled plates, 1,300 photographs of individual histological sections, and 200 electron micrographs, illustrating: Intermittent serial histological sections through embryos throughout embryogenesis and organogenesis Differentiation of specific organs and organ systems, including the spinal cord, eyes, gonads, kidneys, lungs and skeletal system External appearance of intact embryos throughout development

Mice have long been recognized as a valuable tool for investigating the genetic and physiological bases of human diseases such as diabetes, infectious disease, cancer, heart disease, and a wide array of neurological disorders. With the advent of transgenic and other genetic engineering technologies, the versatility and usefulness of the mouse as a

"A subject collection from Cold Spring Harbor perspectives in biology."

This volume describes culture media and solutions used in human ART; how they have been developed for in vitro human pre-implantation embryo development, the function and importance of the various components in media and solutions and how they interact, and how the systems in which these are used can influence outcomes. Chapters discuss inorganic solutes, energy substrates, amino acids, macromolecules, cytokines, growth factors, buffers, pH, osmolality, and the interaction of these parameters. The role of incubators and other physical factors are reviewed, along with the relevance and prospects of emerging technologies: morphokinetic analysis using time-lapse imaging and dynamic fluid incubation systems. Results of prospective randomized trials are emphasized to ascertain the added value of these techniques for selecting viable embryos. This comprehensive guide will be invaluable for embryologists, physicians and all personnel involved in the fluid products used in human ART seeking to optimize their successful use of these components.

Never before has there been such a comprehensive book of protocols. This compendium offers a full range of research techniques-from cell culture, to biochemical, to microscopic and genetic. More focused books, like Cold Spring Harbor's Manipulating the Mouse Embryo, are similar though more narrow in scope. This book will appeal to a broad range of researchers, from basic experimental scientists to clinical and animal scientists.

Guide to Techniques in Mouse Development, Part A comprehensively covers new

technologies and methodologies that have appeared for the study of mouse development. Update of volume 225 of Methods in Enzymology, Guide to Techniques in Mouse Development, edited by P.M. Wassarman and M.L. DePamphilis and published in 1993 Covers new technologies and methodologies, including: new techniques for the cryopreservation of gametes and embryos production of transgenic and null (knockout) animals (use of ES cells) generation of conditional/inducible mutant animals use of gene-trap mutagenesis analysis of allele-specific expresion use of new reporter constructs humanizing of transgenic animals transcript profiling of mouse development imaging of mouse development rederivation of animals and use of mouse genomics

Provides information and guidelines for developing a mouse colony and conducting experiments, including proper protocols, step-by-step procedures, and analysis strategies.

This book is an essential anatomical resource for developmental biologists who need to know about any aspect of mouse developmental anatomy, as well as for geneticists using the mouse embryo as a model. The book is a companion to Kaufman's The Atlas of Mouse Development, and details the developmental anatomy of the early embryo, the transitional tissues, and all the major organ systems. It also provides extensive comparisons with human developmental anatomy, both normal and abnormal. The book has extensive reference indexes detailing developmental stage criteria. The Anatomical Basis of Mouse Development will be a key reference work for anyone who needs to understand developmental anatomy in normal and mutant mice. Key Features * Complements Kaufman's The Atlas of Mouse Development * Gives anatomical descriptions from oogenesis to birth, at a level of detail that goes beyond that found in most literature * Provides detailed explanations for geneticists and molecular biologists with limited anatomical background to help them understand the emergence of all the major structures in the mouse embryo * Contains comprehensive indexes detailing the appearance of over 1000 organs, tissues, and their components at different stages of mouse embryogenesis * Includes comparisons with normal and abnormal human development * Contains over 100 clear line diagrams showing mouse developmental anatomy as well as lineage relationships for the major organ systems

Live imaging of mammalian embryos can elucidate human embryonic development, which is governed by several genetic and environmental factors. Improvements in the acquisition and quality of imaging modalities can potentially contribute to understanding, prevention, and, eventually, treatment of congenital birth defects. This dissertation is devoted to investigate the morphological changes which are associated with mouse embryonic development, using optical coherence tomography (OCT). Firstly, the remodeling of the yolk sac vasculature in a mouse embryo is analyzed. Detection of 3D vasculature using Doppler OCT and speckle variance (SV) OCT were compared. The results demonstrate that SVOCT provides more accurate representation of the vascular structure, as it is not sensitive to the blood flow direction. Secondly, the development of ocular tissues from E13.5 to E18.5 was monitored in utero. The volumes of the eye lens and eye globe was used as the parameter to monitor the development of ocular structures. Results demonstrated the capability of OCT for high-resolution, high-contrast imaging of ocular development in mouse embryos in utero. Thirdly, OCT was compared with high-resolution ultrasound (US) to study the effects of prenatal exposure to ethanol on brain development. Volume of the lateral ventricles was used to assess the effect of ethanol exposure between the control and ethanol-exposed fetuses. The results demonstrated that the volume of lateral ventricles was twice as high in ethanol-exposed fetuses compared to the control ones. The results also demonstrated clear advantages of using OCT for quantitative assessment of embryonic brain development compared to US imaging.

An easy to read, practical description of the human IVF laboratory, from laboratory start-up and training to complex, specialized procedures.

History, Wild Mice, and Genetics, the first volume in the four volume set, The Mouse in Biomedical Research, provides information about the history, biology and genomics of the laboratory mouse (Mus musculus), as well as basic information on maintenance and use of mouse stocks. Mouse origins and relationships are covered in chapters on history, evolutionary taxonomy and wild mice. Genetics and genomics of the mouse are covered in chapters on genetic nomenclature, gene mapping, cytogenetics and the molecular organization of the mouse genome. Maintenance of laboratory mice is described in chapters on breeding systems for various types of strains and stocks and genetic monitoring. Use of the mouse as a model system for basic biomedical research is described in chapters on chemical mutagenesis, gene trapping, pharmacogenetics and embryo manipulation. The information in Volume 1 serves as a primer for scientists new to the field of mouse research.

Manipulating the Mouse EmbryoA Laboratory ManualCold Spring Harbor, N.Y. : Cold Spring Harbor Laboratory Press

The International Symposium on Experimental Robotics (ISER) is a series of bi-annual meetings which are organized in a rotating fashion around North America, Europe and Asia/Oceania. The goal of ISER is to provide a forum for research in robotics that focuses on the novelty of theoretical contributions validated by experimental results. This unique reference presents the latest advances in robotics, with ideas that are conceived conceptually and have been explored experimentally.

Kaufman's Atlas of Mouse Development: With Coronal Sections continues the stellar reputation of the original Atlas by providing updated, in-depth anatomical content and morphological views of organ systems.The publication offers written descriptions of the developmental origins of the organ systems alongside high-resolution images for needed visualization of developmental processes. Matt Kaufman himself has annotated the coronal images in the same clear, meticulous style of the original Atlas. Kaufman's Atlas of Mouse Development: With Coronal Sections follows the original Atlas as a continuation of the standard in the field for developmental biologists and researchers across biological and biomedical sciences studying mouse development. Provides high-resolution images for best visualization of key developmental processes and structures Offers in-depth anatomy and morphological views of organ systems Written descriptions convey developmental origins of the organ systems

With this valuable practical guide, three members of the Harvard Stem Cell Institute have compiled and edited the definite handbook for the exciting new field of human embryonic stem cell research. The editors have gathered protocols from scientists with extensive reputation and expertise, describing and comparing currently used techniques for the culture of human stem cells and discussing the strengths and weaknesses of the different approaches. Human Embryonic Stem Cells: The Practical Handbook contains the first centralised collection of methods used in human embryonic stem cell biology. The book covers the derivation of human stem cell lines, the obtaining of cells from human stem cell banks, the culturing and characterisation of the cells, and the differentiation of the cells in vitro and in vivo. Lastly, almost all of these protocols can also be used for

analyzing and manipulating induced pluripotency iPS stem cells. This allows an even greater number of opportunities for those interested in purusing work in pluripotent stem cells, disease modelling, and other aspects of basic regenerative medicine research. The novel and useful focus of this book sets it apart from other available books: Compares and evaluates the protocols used in leading laboratories working on human embryonic stem cells Centred solely on practical protocols for human (not mouse) embryonic stem cell research Includes extensive troubleshooting sections Addresses the different proclivities and behaviours of individual human embryonic cell lines Contains techniques currently known only to a small number of specialised laboratories worldwide This handbook represents an essential source of up-to-date practical information for all cell and developmental biologists working with human embryonic stem cells or wishing to enter the field. It is also essential reading for clinical researchers in areas such as diabetes, cardiovascular disease, and neurological diseases. Praise from the reviews: "...a highly readable and useful book... A notable feature of the book is its air of openness and honesty... This book... will help many to navigate the uncharted waters of human embryonic stem cell biology." BRITISH SOCIETY FOR CELL BIOLOGY "... the imaginative solutions in this book can inspire us to get past our most frustrating limitations." CELL STEM CELL "... the richness in the details of each protocol presented will certainly encourage more scientists to begin studies of Human pluripotent stem cells..." REGENERATIVE MEDICINE "In this fast-moving field, this [handbook] will help drive advances of more and more researchers." DIFFERENTIATION "...a valuable resource for seasoned and novice researchers... an excellent addition to the reference collection of any medical library or research laboratory." THE AMERICAN MEDICAL ASSOCIATION

This manual is a comprehensive compilation of "methods that work" for deriving, characterizing, and differentiating hPSCs, written by the researchers who developed and tested the methods and use them every day in their laboratories. The manual is much more than a collection of recipes; it is intended to spark the interest of scientists in areas of stem cell biology that they may not have considered to be important to their work. The second edition of the Human Stem Cell Manual is an extraordinary laboratory guide for both experienced stem cell researchers and those just beginning to use stem cells in their work. Offers a comprehensive guide for medical and biology researchers who want to use stem cells for basic research, disease modeling, drug development, and cell therapy applications. Provides a cohesive global view of the current state of stem cell research, with chapters written by pioneering stem cell researchers in Asia, Europe, and North America. Includes new chapters devoted to recently developed methods, such as iPSC technology, written by the scientists who made these breakthroughs.

A discussion of all the key issues in the use of human pluripotent stem cells for treating degenerative diseases or for replacing tissues lost from trauma. On the

practical side, the topics range from the problems of deriving human embryonic stem cells and driving their differentiation along specific lineages, regulating their development into mature cells, and bringing stem cell therapy to clinical trials. Regulatory issues are addressed in discussions of the ethical debate surrounding the derivation of human embryonic stem cells and the current policies governing their use in the United States and abroad, including the rules and conditions regulating federal funding and questions of intellectual property.

During the past 20 years, transgenesis has become a popular technique and a crucial tool for molecular geneticists and biologists. Transgene expression is now better-controlled and even specifically inducible by exogenous factors. While these techniques have quite significantly transformed the experimental approaches taken by biologists, the applications are more limited than expected and concerns have arisen regarding biosafety as well as physiological, social, and philosophical issues. Transgenic Animals: Generation and Use contains articles on the techniques used to generate transgenic animals and a section on the preparation of vectors for the optimally controlled expression of transgenes. It also examines the use of transgenic animals in the study of gene function and human diseases, the preparation of recombinant proteins and organs for pharmaceutical and medical use, and the improvement of genetic characteristics of farm animals. Finally, it discusses more recent problems generated by transgenic animals including conservation of transgenic lines, specific database patenting, biosafety, and bioethics. Drawn from both academia and industry, the contributors to this monograph present in one concise volume all the relevant information on the different aspects of transgenesis. This book can be used as both a reference book and a textbook for specialized university courses and will be of interest to everyone involved in basic research in animal biology, molecular genetics, animal biotechnology, pharmaceutical science, and medicine.

Scientific Frontiers in Developmental Toxicology and Risk Assessment reviews advances made during the last 10-15 years in fields such as developmental biology, molecular biology, and genetics. It describes a novel approach for how these advances might be used in combination with existing methodologies to further the understanding of mechanisms of developmental toxicity, to improve the assessment of chemicals for their ability to cause developmental toxicity, and to improve risk assessment for developmental defects. For example, based on the recent advances, even the smallest, simplest laboratory animals such as the fruit fly, roundworm, and zebrafish might be able to serve as developmental toxicological models for human biological systems. Use of such organisms might allow for rapid and inexpensive testing of large numbers of chemicals for their potential to cause developmental toxicity; presently, there are little or no developmental toxicity data available for the majority of natural and manufactured chemicals in use. This new approach to developmental toxicology and risk assessment will require simultaneous research on several fronts by experts from multiple scientific disciplines, including developmental toxicologists, developmental biologists, geneticists, epidemiologists, and biostatisticians.

This volume comprehensively covers new technologies and methodologies that have

appeared for the study of mouse development. This volume is an update of volume 225 of MIE, "Guide to Techniques in Mouse Development", edited by P.M. Wassarman and M.L. DePamphilis and published in 1993. During the past 17 years many new technologies or methodologies have appeared for the study of mouse development and this volume comprehensively covers these, including: new techniques for the cryopreservation of gametes and embryos, production of transgenic and null (knockout) animals (use of ES cells), generation of conditional/inducible mutant animals, use of gene-trap mutagenesis, analysis of allele-specific expresion, use of new reporter constructs, humanizing of transgenic animals, transcript profiling of mouse development, imaging of mouse development, rederivation of animals and use of mouse genomics.

In viviparous mammals, preimplantation embryo development occurs post fertilization and prior to implantation in the uterus. During this period of time the egg develops into a zygote which activates its own genome, and goes through a series of cell divisions called cleavage divisions to ultimately become a blastocyst ready to implant in the uterus and develop into a new organism. The preimplantation embryo development (Ped) gene regulates the rate of preimplantation embryonic cleavage division and subsequent embryo survival in mice. In the mouse, the Ped gene product is Qa-2 protein, a non-classical major histocompatibility complex (MHC) class Ib molecule encoded by four tandem genes, Q6/Q7/Q8/Q9. Most inbred strains of mice have all four genes on each allelic chromosome, making a total of eight Qa-2 encoding genes, but there are a few strains that are missing all eight genes, defining a null allele. Mouse embryos possessing the Qa-2 encoding genes express Qa-2 protein on their surface and develop at a faster rate and have a greater chance of survival compared to mouse embryos expressing the null allele. The B6.K1 and B6.K2 strains of mice are congenic strains that have identical genetic loci with the exception of the genes that encode Qa-2. The B6.K1 mice have a deletion of the four Qa-2 encoding genes whereas the B6.K2 mice do possess all four Qa-2 encoding genes. Using these mice as either controls or subjects I have done research towards three specific aims: (1) Test the hypothesis that the number of Qa-2 encoding genes varies in the wild mouse population; (2) Test the hypothesis that male preimplantation embryos develop faster than female preimplantation embryos from the B6.K1 and B6.K2 strains of mice; (3) Test the hypothesis that the Ped gene is influenced by the expression of two microRNAs, miR-125a and miR-125b. The results from the first specific aim showed that there was great variability in the number of Qa-2 encoding genes in 32 wild mice tested. The wild mouse with the highest number of Qa-2 encoding genes had 85 such genes, whereas one wild mouse was discovered without any Qa-2 encoding genes. The results from the second specific aim showed that there was no statistically significant difference in the ratio of male to female preimplantation embryos in either strain. Therefore, the Ped gene is entirely responsible for mediating the faster development of B6.K2 embryos compared to B6.K1 embryos. Finally, the results from the third specific aim showed that the absence of one of the Qa-2 encoding genes, Q9, resulted in a 10-fold increase in expression of the developmental timing miRNA miR-125a, but not of its close family member miR-125b. This finding is significant because miR-125a and its homolog lin-4 have been found to play important roles in development in many species. Our results suggest that similar to lower organisms,

miRNAs play an important role in developmental timing in mice. Taken together, the findings presented in this dissertation have answered important basic research questions regarding Ped gene control of the rate of preimplantation embryo development and have led to the discovery of an entirely new way of thinking about gene regulation during mouse embryo development, namely the involvement of miRNAs.