Chapter 3 Gene Transfer Methods Rd Springer

BRANSON HERNANDEZ

Improvements in Codon Usage Analysis for a More Detailed Understanding of Genome Content and Horizontal Gene Transfer Leuven University Press
In vivo transfer of DNA to mammalian cells is now a viable therapeutic strategy. Non-viral gene therapy strategies, utilising plasmids, are an attractive, potentially safer alternative to viral delivery. This thesis investigates non-viral plasmid gene delivery in vivo. Bacterial-mediated transfer of plasmid DNA into mammalian cells has significant clinical potential. Other species of bacteria appear to possess natural tumour specificity. Parameters influencing transgene expression from delivered plasmid are also examined. Furthermore, the combined use of physical methods of delivery in the absence of therapeutic agent was assessed as an anti-tumour treatment. Chapter 2 demonstrates that Listeria monocytogenes can invade and spread within tumours, and establishes for the first time the use of Listeria to deliver genes intracellularly to growing tumours. Chapter 3 shows that oral administration of Bifidobacteria to mice resulted in gastro-intestinal translocation with replication specifically in tumours. These findings indicate potential for safe and efficient treatment/detection of tumours via ingestion of non-pathogenic engineered bacteria. Chapter 4 assessed plasmid transgene expression variables. Gene expression associated with viral promoters, silenced in tumour and liver within one week of administration, unlike that of a mammalian promoter, which persisted up to 25 days. No reduction in expression was evident with either promoter in skeletal muscle. The potential for plasmid delivery to muscle in the context of tissue healing was further investigated in chapter 5. Employment of an inducible promoter cassette permitted regulation of gene expression on a temporal basis. An ex vivo patient tissue culture system was developed and used to demonstrate luciferase expression in human muscle, tendon, ligament and periosteal tissue. Chapter 6 of this thesis describes the use of a combination of physical delivery methods to directly induce tumour cell killing, in the context of human basal cell carcinomas, with objective favourable responses noted in the nodular histological subtype.

New Methods for Lentiviral-Based Hematopoietic Stem Cell Gene Therapy Springer Science & Business Media
Genetically engineered (GE) crops were first introduced commercially in the 1990s. After two decades of production, some groups and individuals remain critical of the technology based on their concerns about possible adverse effects on human health, the environment, and ethical considerations. At the same time, others are concerned that the technology is not reaching its potential to improve human health and the environment because of stringent regulations and reduced public funding to develop products offering more benefits to society. While the debate about these and other questions related to the genetic engineering techniques of the first 20 years goes on, emerging genetic-engineering technologies are adding new complexities to the conversation. Genetically Engineered Crops builds on previous related Academies reports published between 1987 and 2010 by undertaking a retrospective examination of the purported positive and adverse effects of GE crops and to anticipate what emerging genetic-engineering technologies hold for the future. This report indicates where there are uncertainties about the economic, agronomic, health, safety, or other impacts of GE crops and food, and makes recommendations to fill gaps in safety assessments, increase regulatory clarity, and improve innovations in and access to GE technology.

Receptor-mediated DNA-based Therapeutics Delivery Springer Science & Business Media
Gene transfer to animal cells was first achieved more than thirty years ago. Since then, transformation technology has developed rapidly, resulting in a multitude of techniques for cell transformation and the creation of transgenic animals. As with any expanding technology, it becomes difficult to keep track of all the developments and to find a concise and comprehensive source of information that explains all the underlying principles. Gene Transfer to Animals Cells addresses this problem by describing the principles behind gene transfer technologies, how gene expression is controlled in animal cells and how advanced strategies can be used to add, exchange or delete sequences from animal genomes in a conditional manner. A final chapter provides an overview of all the applications of animal cell transformation in farming, medicine and research.
Guide to Research Techniques in Neuroscience Academic Press
Cancer is the most common cause of death in developed countries, and as such is a massive burden on society. As new techniques and knowledge became available, a shift from the use of gene therapy solely to target monogenetic disorders towards its additional use as a cancer treatment was observed. The culmination being that cancer gene therapy is now the most studied application of the gene therapy field with a significant portion of these studies focused on immune-based therapies for various cancer types. While Adeno-associated virus (AAV) vectors have shown great promise in the course of research into treatment of numerous indications ranging from cystic fibrosis to haemophilia B, only in recent years have they begun to be investigated in a cancer setting. This thesis seeks to examine the use of AAV2 as a vector in a cancer gene therapy setting, from initial vector characterisation and optimisation through to the use of AAV2 to deliver therapeutics in preclinical tumour trials. Initial work focused on the identification of the optimal a) parameters for AAV2 titration, b) in vitro and in vivo models and c) in vivo vector administration regimen. Chapter 2 deals with a broad range of parameters relating to AAV2 mediated gene transfer and expression compared with other commonly used delivery methods. This study demonstrated that AAV2-mediated delivery and expression was generally superior to other methods examined. Chapter 3 deals with the efficacy of AAV2-mediated cancer therapeutic strategies, specifically an immune based strategy, an anti-angiogenic/anti-metastatic strategy or a combination of both strategies. AAV2 mediated immune therapy focused on the delivery of the cytokine granulocyte macrophage-colony stimulating factor (GM-CSF) and the co-stimulatory molecule B7-1 to growing tumours in vivo. AAV2 mediated anti-angiogenic/anti-metastatic therapy focused on the use of the bifunctional molecule Nk4 for the local or systemic treatment of growing tumours in vivo. Significant anti-tumour effects were observed, with decreases in tumour burden and increased survival. Chapter 4 assessed the influence of a mouse parvovirus on AAV2 vector related expression in murine models. An interaction between mouse parvovirus-1 (MPV-1) and AAV2 vectors was demonstrated both in vivo and in vitro resulting in increased gene expression featuring replication of vector DNA. Specific AAV2 and MPV-1 sequences were identified to be involved in the interaction Overall, the data presented here advance the field of exploration of AAV2-mediated cancer gene therapy strategies as well as demonstrate pre-clinically the potential for novel anti-cancer therapies.
Adenoviral Vectors for Gene Therapy Daya Books
The goal of gene transfer is protein expression. a process brought about by the insertion of a gene coding for a foreign protein into target cells resulting in the synthesis of the foreign protein For gene therapy, a tmnsferred therapeutic gene must be expressed at a level beneficial for the patient. This chapter provides an introductory overview of the rapidly evolving field of non-viral approaches for gene delivery to rnarnrnalian cells. Although currently there are fewer ongoing clinical trials using non-viral approaches than those using viral based systems, the number of non-viral trials is increasing. The long range goal of some research groups is the development of a genetically engineered artificial virus targeted to specific cells in the human body. An arurual conference, organized by Cambridge Healthtech Institute entitled "Artificial Self-Assembling Systems for Gene Transfer", brings together researchers interested in this field [1]. Assembly of an artificial virus is very complex; other research groups aim to develop simpler delivery systems consisting of a plasmid combined with delivery agents. Viral-based systems are very successful for gene delivery, but despite their successes, viral-based systems have some geneml limitations and system-specific limitations. When employing a viml-based system, the following limitations should be considered: • size limitation of the inserted gene due to packaging constraints (e. g. adenovirus, retrovirus) . • potential tumorigenesis (e. g. retrovirus) • potential for insertional mutagenesis (greater than plasmid based systems) • potential imrnunogenicity (e. g.
Development of Non-viral Gene Delivery Strategies Elsevier
First published in 1996, liposomes have become an important model in fundamental biomembrane research, including biophysical, biochemical, and cell biological studies of membranes and cell function. They are thoroughly studied in several applications, such as drug delivery systems in medical applications and as controlled release systems, microencapsulating media, signal carriers, support matrices, and solubilizers in other applications. While medical applications have been extensively reviewed in recent literature, there is a need for easily accessible information on applications for liposomes beyond pharmacology and medicine. The Handbook of Nonmedical Applications of Liposomes fills this void.This unique new handbook series presents recent developments in the use of liposomes in many scientific disciplines, from studies on the origin of life, protein function, and vesicle shapes, to applications in cosmetics, diagnostics, ecology, bioreclamation, and the food industry. In these volumes many of the top experts contribute extensive reviews of their work.
Lost in Translation CRC Press
Recent years have witnessed an explosion of activity in the field of gene therapy. Following advances in our understanding of the molecular basis of disease, hopes are high that the tremendous potential market for drugs employing antisense nucleotides and genes will one day be fulfilled. However, many obstacles remain, not least problems in the technology of gene delivery systems. Much of today's research focuses on non-viral approaches to gene delivery. Of particular importance are supramolecular complexes formed between DNA and various natural and synthetic polymers and lipids, otherwise known as 'self-assembling complexes for gene delivery'. In view of this fact, the editors of this volume have assembled an international team of contributors to present up-to-date reviews of the major chemical, biological and clinical aspects of such gene delivery vectors. Reflecting the diversity of research in this field, this book comprehensively covers: * the principles of self-assembly * natural mechanisms for gene delivery to cells * cationic lipids and liposomes * polyelectrolyte DNA complexes * systemic biodistribution of drug delivery systems * targeting of conjugates for gene delivery * new approaches to gene delivery * clinical evaluation Self-assembling Complexes for Gene Delivery is an essential reference for all professionals with an interest in gene and antisense therapy or novel drug delivery systems, including medicinal and pharmaceutical chemists, clinicians, human geneticists, molecular biologists and pharmacologists.
Cardioselective Nitric Oxide Synthase Gene Transfer to Target Myocardial Ischemia Springer Science & Business Media
Liposomes have become an important model in fundamental biomembrane research, including biophysical, biochemical, and cell biological studies of membranes and cell function. They are thoroughly studied in applications, such as drug delivery systems in medical applications and as controlled release systems, microencapsulating media, signal carriers, support matrices, and solubilizers in other applications. While medical applications have been extensively reviewed in recent literature, there is a need for easily accessible information on applications for liposomes beyond pharmacology and medicine.
Adeno-Associated Virus Vectors for Cancer Gene Therapy Springer Science & Business Media
Animal biotechnology is a broad field including polarities of fundamental and applied research, as well as DNA science, covering key topics of DNA studies and its recent applications. In Introduction to Pharmaceutical Biotechnology, DNA isolation procedures followed by molecular markers and screening methods of the genomic library are explained in detail. Interesting areas such as isolation, sequencing and synthesis of genes, with broader coverage of the latter, are also described. The book begins with an introduction to biotechnology and its main branches, explaining both the basic science and the applications of biotechnology-derived pharmaceuticals, with special emphasis on their clinical use. It then moves on to the historical development and scope of biotechnology with an overall review of early applications that scientists employed long before the field was defined. Additionally, this book offers first-hand accounts of the use of biotechnology tools in the area of genetic engineering and provides comprehensive information related to current developments in the following parameters: plasmids, basic techniques used in gene transfer, and basic principles used in transgenesis. The text also provides the fundamental understanding of stem cell and gene therapy, and offers a short description of current information on these topics as well as their clinical associations and related therapeutic options.
Gene Transfer in the Cardiovascular System CRC Press
Biology is the study of living things. The classical approach might be described as holistic and descriptive, whereas the modern molecular - proach aims to be investigative, reductionist, and mechanistic . Genes contain all the information for the structure of all living things ; thus, the understanding of how genes are regulated is an important step toward understanding the nature of living things. The study of gene regulation has been made more tractable by the design of simple expe- mental models in which a single gene can be isolated from the milieu of the organism. The new science of molecular biology has introduced techniques that permit the design of such experimental models. In - sence, the genome of the organism is dissected in such a manner that specific genes may now be introduced into an appropriate cell line . Subsequent analysis of the proteins expressed from the genes under study results in the identification of the regulatory DNA sequences .
CRISPR Gene Editing Wiley-Blackwell
Developmental biology has been transformed recently by discoveries in the fields of molecular biology, cell biology, and immunology. New ways of manip ulating mammalian development are uncovering control mechanisms and ena bling us to apply them in solving practical problems in animal production and human health. This book outlines some of these new manipulations and how they have contributed to the present state of developmental biology. Chapter 1 describes gene transfer by micro injection of cloned recombinant DNA into zygotes. Although the factors that affect transformation frequencies and integration sites are still unknown, such techniques offer a number of exciting prospects. Research models for human disease coula be artificially created and desirable characteristics in agricultural animals could be - hanced. . The theme of cell-to-cell transfer is continued in Chapters 2 and 3. Chapter 2 describes pronuclear transplantation by Sendai virus-induced fusion of the karyoplast with the enucleated embryo. Using this procedure, it has been dem onstrated that both male and female genomes are essential for normal develop ment, although the reason for this is not yet understood. Chapter 3 describes studies on the fusion of whole oocytes. .
Molecular Biology and Genetic Engineering National Academies Press
Hematopoietic stem cell (HSC) transplant with gene therapy has recently emerged as a successful clinical treatment of a number of previously incurable genetic blood diseases. This approach aims to permanently fix genetic defects in HSCs, a rare and specialized type of cell with the unique ability to regenerate the entire blood system throughout a patient's lifetime. In this approach, bone marrow (BM) or mobilized peripheral blood (mPB) is collected from a patient, enriched for HSCs, transduced with an engineered lentiviral vector (LV) encoding the correct genetic sequence, and transplanted back into the patient. After transplant, modified HSCs engraft in the BM and produce healthy blood cells throughout the patient's lifetime. While the last decade of research has yielded major advances including successful Phase I/II gene therapy clinical trials, clinical and commercial scaling of this technology to a broader range of patients and diseases has revealed a number of hurdles. One major limitation is the great expense and difficulty of producing clinical-grade LV, which I address in Chapters 2 and 3 by presenting two methods that improve the efficiency of LV transduction of HSC. In Chapter 4, I demonstrate the successful application of a new LV gene therapy for an autoimmune blood disease. Chapter 2 presents a method to enhance the enrichment of HSCs from the heterogeneous cell population obtained from the collection of bone marrow cells, addressing a critical limitation in creating cost-effective, clinical-grade LV vector. This method utilizes immunomagnetic beads to purify CD34+CD38- cells, a population highly enriched for HSCs beyond standard CD34+ selection. Using immune-deficient xenograft models, we demonstrate that enrichment of CD34+CD38- cells reduces gene therapy culture scale and lentiviral vector requirements by ~10-fold while still maintaining the long-term gene-marked engraftment required for clinical benefit. Therefore, this strategy represents an easily translatable method which can conserve valuable clinical grade LV preparations and could lower the cost per patient, or allow for the treatment of a greater number of patients. Chapter 3 presents a method to further improve HSC transduction efficiency with the use of two compounds: Prostaglandin E2 (PGE2) and poloxamer synperonic F108 (PS-F108). While transduction enhancement with each individual compound has previously been reported, the combination of these compounds leads to a synergistic and marked improvement in LV transduction of HSCs using a globin LV. Remarkably, this synergistic combination achieved a 6-fold improvement in gene transfer to long-term engrafting HSCs while using a LV dose 10-fold lower than the dose in our current clinical protocol. Thus this strategy has two major advantages: it reduces the amount of viral particles required to transduce HSCs, and also allows for better gene transfer and ultimate globin transgene expression, which is critical to improving clinical efficacy. Finally, chapter 4 demonstrates the effectiveness of

a newly engineered LV for the treatment of a severe form of genetic autoimmunity called IPEX syndrome. IPEX is caused by mutations in FoxP3, the key lineage-determining transcription factor required for the development and function of regulatory T cells (Treg cells). We developed a new LV using endogenous human FOXP3 regulatory elements to restore FoxP3 expression in a developmentally appropriate manner. We use this LV to transduce HSCs and restore functional Treg development in a mouse model of FoxP3 deficiency and successfully rescue autoimmune defects associated with this phenotype. These findings demonstrate preclinical efficacy for the treatment of IPEX patients by autologous HSC transplant and may provide further insight into new cell therapies for autoimmunity. Collectively, the work described here advances the field of gene therapy by improving the efficiency of the manufacturing process and expanding the range of diseases which can be treated by this method.

Pharmaceutical Gene Delivery Systems Garland Science

Adenoviral Vectors for Gene Therapy, Second Edition provides detailed, comprehensive coverage of the gene delivery vehicles that are based on the adenovirus that is emerging as an important tool in gene therapy. These exciting new therapeutic agents have great potential for the treatment of disease, making gene therapy a fast-growing field for research. This book presents topics ranging from the basic biology of adenoviruses, through the construction and purification of adenoviral vectors, cutting-edge vectorology, and the use of adenoviral vectors in preclinical animal models, with final consideration of the regulatory issues surrounding human clinical gene therapy trials. This broad scope of information provides a solid overview of the field, allowing the reader to gain a complete understanding of the development and use of adenoviral vectors. Provides complete coverage of the basic biology of adenoviruses, as well as their construction, propagation, and purification of adenoviral vectors Introduces common strategies for the development of adenoviral vectors, along with cutting-edge methods for their improvement Demonstrates noninvasive imaging of adenovirus-mediated gene transfer Discusses utility of adenoviral vectors in animal disease models Considers Federal Drug Administration regulations for human clinical trials

Challenges in Delivery of Therapeutic Genomics and Proteomics Springer Science & Business Media

This special issue of the Advances in Experimental Medicine and Biology presents much of the research described at the recent 2nd International Tissue Engineering Conference held in Crete in May 2005. The conference brought together over 150 researchers from around the world to examine the emerging and most advanced aspects of their particular field. The chapters reflect a diverse group of authors, including both clinicians and academicians.

Strategies for National Competitiveness Humana Press

Abstract: The objective of this dissertation was to develop and evaluate receptor-mediated non-viral delivery systems for DNA-based therapeutics. Novel strategies might prove critical for the in-vivo performance of receptor-targeted vectors. Continued efforts in optimization of receptor-mediated delivery systems may lead to the development of tumor-specific vehicles for DNA-based therapeutics delivery and promote the advancement of clinical translation of cancer gene therapy. In Chapter 2, a non-viral, PEI-based, HER2-targeted gene transfer vector was developed. The anti-HER2 antibody (Herceptin®) was conjugated to PEI and polyplexes were shown to selectively deliver plasmids to HER2-overexpressing cells with high resistance to serum. Herceptin/PEI polyplexes exhibited promising HER2-receptor-specific gene transfer properties. In Chapter 3, an ethanol dilution method for the preparation of ODN was developed. This method provides a suitable platform to prepare receptor-targeted-ODN-containing liposomes. The small size, low toxicity, and, more importantly, high encapsulation efficiency of ODNs at optimized conditions are important characteristics for the development of DNA-based therapeutics delivery systems. In the next two chapters, similar method was applied to other systems including ODNs and siRNAs with high molecular weight target-ligands. The aim of Chapter 4 was to develop a targeted ODN(G3139)-containing liposome formulation that can efficiently and specifically delivery ODNs to leukemias. Transferrin receptors were overexpressed in many tumor and leukemia cells. A Tf-targeted liposomal formulation of antisense G3139 was evaluated in K562 leukemia cells, which exhibited excellent characteristics in terms of particle size, loading efficiency, colloidal stability, and vehicle toxicity. Furthermore, this formulation was very efficient in antisense delivery, showing excellent bcl2 down-regulation efficiency and TfR specificity. In Chapter 5, similar strategy was applied to siRNA delivery. Desferrioxamine(DFO) was used to up-regulate TfR in K562 cells. The data demonstrated that DFO pretreatment increased the uptake of TfR-targeted siRNA in K562 cells and exhibited higher luciferase downregulation effect. Tf-targeted siRNA formulation with DFO pretreatment was a highly efficient delivery vehicle for siRNA for leukemias that express TfR. This formulation provides the prospect of more selective targeting effect in association with increased intracellular concentrations in target cells. More future studies such as optimization and in-vivo studies are needed for this formulation to work clinically.

Handbook of Nonmedical Applications of Liposomes, Vol IV From Gene Delivery and Diagnosis to Ecology Improvements in Codon Usage Analysis for a More Detailed Understanding of Genome Content and Horizontal Gene TransferThe genetic code has evolved with considerable elasticity, enabling most amino acids to be encoded by multiple synonymous codons. Genes can vary in their utilization of synonymous codons, and this provides a basis of comparison for studying the compositional histories and evolution of genomes. The original goal of this dissertation work was to study the effects of horizontal gene transfer in diverse genomes; however, these efforts were quickly encumbered by limitations in the current methods of codon usage analysis. In this dissertation, we describe the limitations of these methods, and challenge the fundamental assumptions that they are based upon. In order to evaluate horizontal gene transfer (or any other source of variation within a genome) it is first necessary to define what is ó−typicalò+. Many previous studies have considered the typical codon usage of a genome to be the genome-wide average. In Chapter 2, we establish a method for calculating the modal codon usage of a genome and demonstrate that it is more resistant to the effects of aberrant genes than the average. In Chapter 3, we use the mode algorithm to study the evolution of Agrobacterium tumefaciens and Borrelia burgdorferiÓ4two bacterial genomes that contain multiple replicons. In A. tumefaciens we discover that the two plasmids are closely related, despite being independently conjugative. By using the mode algorithm on the B. burgdorferi genome, we are able to demonstrate a higher resolution of codon usage relationships than had been previously shownÓ4we observe a close similarity between the linear plasmid lp38 and the chromosome, and a close similarity between the members of the cp32 family of plasmids. We observe that these codon usage similarities also appear to be independent of replicon topology. In Chapter 3, we also identify the bacterial and archaeal genomes that are the most heterogeneous and homogeneous in codon usageÓ4a characteristic that can be assessed by determining the number of genes that are significantly different from the modal codon usage of the genome. We find that the genomes with the most homogeneous codon usage are predominantly from organisms with reduced genomes including endosymbionts, parasites, and free-living marine bacteria. The most heterogeneous genomes include members of the genera Bacteroides, Corynebacterium, Xylella, Neisseria, Bifidobacterium, and Desulfotaela. In these latter organisms, greater than 2/3 of the genes in the genome differ significantly from the mode. In Chapter 4, we provide a method for evaluating expression-related codon usage bias (a major source of heterogeneity within genomes). This method is based upon the calculation of an axis that intersects the modal codon usage of a genome and the mode of a set of highly expressed genes. We show that this method is well suited for evaluating expression-related codon usage bias in genomes with extreme base compositions, such as Pseudomonas aeruginosa (66% G+C for the genome), a problem that has plagued previous methods. This method also provides a criterion for identifying foreign genes that have been recently acquired by the genome via horizontal gene transfer. In Chapter 5, we use the mode to characterize the major codon usage groups within the genomes of Escherichia coli K-12 and Salmonella enterica subsp. enterica serovar Typhimurium LT2. When we compare the codon usages in these genomes, we find that the genes that have been recently acquired via horizontal gene transfer are more similar in codon usage than are the genes that have been vertically inherited. To explore the generality of this observation, we compare genomes of three Agrobacterium species and find that the modal codon usages of the plasmids from different species are more similar than the modal codon usages of the corresponding chromosomes. Implications of the methods and data presented in this dissertation, particularly their implications for the study of horizontal gene transfer, are discussed.Plant Genetic Transformation Technology

Modern neuroscience research is inherently multidisciplinary, with a wide variety of cutting edge new techniques to explore multiple levels of investigation. This Third Edition of Guide to Research Techniques in Neuroscience provides a comprehensive overview of classical and cutting edge methods including their utility, limitations, and how data are presented in the literature. This book can be used as an introduction to neuroscience techniques for anyone new to the field or as a reference for any neuroscientist while reading papers or attending talks. • Nearly 200 updated full-color illustrations to clearly convey the theory and practice of neuroscience methods • Expands on techniques from previous editions and covers many new techniques including in vivo calcium imaging, fiber photometry, RNA-Seq, brain spheroids, CRISPR-Cas9 genome editing, and more • Clear, straightforward explanations of each technique for anyone new to the field • A broad scope of methods, from noninvasive brain imaging in human subjects, to electrophysiology in animal models, to recombinant DNA technology in test tubes, to transfection of neurons in cell culture • Detailed recommendations on where to find protocols and other resources for specific techniques • “Walk-through boxes that guide readers through experiments step-by-step

Volume 1: Principles and Development Leuven University Press

Delivery of therapeutic proteomics and genomics represent an important area of drug delivery research. Genomics and proteomics approaches could be used to direct drug development processes by unearthing pathways involved in disease pathogenesis where intervention may be most successful. This book describes the basics of genomics and proteomics and highlights the various chemical, physical and biological approaches to protein and gene delivery. Covers a diverse array of topics from basic sciences to therapeutic applications of proteomics and genomics delivery Of interest to researchers in both academia and industry Highlights what’s currently known and where further research is needed

Experiences and Prospects National Academies Press

The introduction of foreign genetic material into host cells is a vital step in genetic engineering. It is especially important when one considers the potential application of gene transfer systems to crop improvement with the aim of engineering specific traits into a wide variety of plants. The book is an overview of the current research into gene transfer technology and will be valuable for those, who are involved in the field of plant molecular biology, genetics, biochemistry, physiology and biotechnology. Contents Chapter 1: Genetic Transformation; History & Definition, Gene transfer systems, Natural transformation system (vector system), Direct gene transfer (vector-free systems), Genetic transformation strategy, Biological parameters, Requirements for genetic transformation, Arrangement of foreign DNA in the plant genome, Stability of the foreign gene, Modes of genetic recombination, Genetic transformation approaches, Classes of transformants, Inter-transformant variability; Chapter 2: Gene Delivery systems; Polycation-mediated transformation, Particle gun, Electroporation, Microinjection, U V laser microbeam, Electroinjection, Electrophoresis, Protoplast fusion, Macroinjection, Liposome system, Ca-DNA co-precipitation method, Silicon carbide fiber-vortex, Sonication; Chapter 3: Strategies for Improving Transformation Efficiency; Plasmid DNA, Carrier DNA, DNA repair, Transformation of synchronized protoplasts, Restriction-enzyme mediated event, Transformation booster sequence; Chapter 4: Organelle Transformation; Chapter 5: Shotgun Transformation; Plasmid rescue, Gene rescue, Promoter & enhancer rescue.

Genetically Engineered Crops Rastogi Publications

From the pre-historic era to modern times, cereal grains have been the most important source of human nutrition, and have helped sustain the increasing population and the development of human civilization. In order to meet the food needs of the 21st century, food production must be doubled by the year 2025, and nearly tripled by 2050. Such enormous increases in food productivity cannot be brought about by relying entirely on conventional breeding methods, especially on less land per capita, with poor quality and quantity of water, and under rapidly deteriorating environmental conditions. Complementing and supplementing the breeding of major food crops, such as the cereals, which together account for 66% of the world food supply, with molecular breeding and genetic manipulation may well provide a grace period of about 50 years in which to control population growth and achieve sustainable development. In this volume, leading world experts on cereal biotechnology describe the production and commercialization of the first generation of transgenic cereals designed to substantially reduce or prevent the enormous losses to cereal productivity caused by competition with weeds, and by various pests and pathogens, which is an important first step in that direction.

Genetic Manipulation of the Nervous System National Academies Press

PART I Molecular Biology 1. Molecular Biology and Genetic Engineering Definition, History and Scope 2. Chemistry of the Cell: 1. Micromolecules (Sugars, Fatty Acids, Amino Acids, Nucleotides and Lipids) Sugars (Carbohydrates) 3. Chemistry of the Cell . 2. Macromolecules (Nucleic Acids; Proteins and Polysaccharides) Covalent and Weak Non-covalent Bonds 4. Chemistry of the Gene: Synthesis, Modification and Repair of DNA DNA Replication: General Features 5. Organisation of Genetic Material 1. Packaging of DNA as Nucleosomes in Eukaryotes Techniques Leading to Nucleosome Discovery 6. Organization of Genetic Material 2. Repetitive and Unique DNA

Sequences 7. Organization of Genetic Material: 3. Split Genes, Overlapping Genes, Pseudogenes and Cryptic Genes Split Genes or .Interrupted Genes 8. Multigene Families in Eukaryotes 9. Organization of Mitochondrial and Chloroplast Genomes 10. The Genetic Code 11. Protein Synthesis Apparatus Ribosome, Transfer RNA and Aminoacyl-tRNA Synthetases Ribosome 12. Expression of Gene . Protein Synthesis 1. Transcription in Prokaryotes and Eukaryotes 13. Expression of Gene: Protein Synthesis: 2. RNA Processing (RNA Splicing, RNA Editing and Ribozymes) Polyadenylation of mRNA in Prokaryotes Addition of Cap (m7G) and Tail (Poly A) for mRNA in Eukaryotes 14. Expression of Gene: Protein Synthesis: 3. Synthesis and Transport of Proteins (Prokaryotes and Eukaryotes) Formation of Aminoacyl tRNA 15. Regulation of Gene Expression: 1. Operon Circuits in Bacteria and Other Prokaryotes 16. Regulation of Gene Expression . 2. Circuits for Lytic Cycle and Lysogeny in Bacteriophages 17. Regulation of Gene Expression 3. A Variety of Mechanisms in Eukaryotes (Including Cell Receptors and Cell Signalling) PART II Genetic Engineering 18. Recombinant DNA and Gene Cloning 1. Cloning and Expression Vectors 19. Recombinant DNA and Gene Cloning 2. Chimeric DNA, Molecular Probes and Gene Libraries 20. Polymerase Chain Reaction (PCR) and Gene Amplification 21. Isolation, Sequencing and Synthesis of Genes 22. Proteins: Separation, Purification and Identification 23. Immunotechnology 1. B-Cells, Antibodies, Interferons and Vaccines 24. Immunotechnology 2. T-Cell Receptors and MHC Restriction 25. Immunotechnology 3. Hybridoma and Monoclonal Antibodies (mAbs) Hybridoma Technology and the Production of Monoclonal Antibodies 26. Transfection Methods and Transgenic Animals 27. Animal and Human Genomics: Molecular Maps and Genome Sequences Molecular Markers 28. Biotechnology in Medicine: I.Vaccines, Diagnostics and Forensics Animal and Human Health Care 29. Biotechnology in Medicine 2. Gene Therapy Human Diseases Targeted for Gene Therapy Vectors and Other Delivery Systems for Gene Therapy 30. Biotechnology in Medicine: 3. Pharmacogenetics / Pharmacogenomics and Personalized Medicine Phannacogenetics and Personalized 31. Plant Cell and Tissue Culture' Production and Uses of Haploids 32. Gene Transfer Methods in Plants 33. Transgenic Plants . Genetically Modified (GM) Crops and Floricultural Plants 34. Plant Genomics: 35. Genetically Engineered Microbes (GEMs) and Microbial Genomics References