Gene Ther Mol Biol Vol 12, 77-82, 2008

Transgenomics

Review Article

Virendra S Gomase*, Somnath Tagore
Department of Bioinformatics, Padmashree Dr. D.Y. Patil University, Navi Mumbai, 400614, India

---

***Correspondence**: Virendra S. Gomase, Department of Bioinformatics, Padmashree Dr. D.Y. Patil University, Plot No-50, Sector-15, CBD Belapur, Navi Mumbai, 400614, India; Mobile- +91-9226960668; e-mail: virusgene1@yahoo.co.in
Key words: Blastocyte, genetically modified organisms, germ line, oocyte, transgene
Abbreviations: Alzheimer's disease, (AD); Birt-Hogg-Dube, (BHD); cell wall, (CW); denaturing high-performance liquid chromatography, (dHPLC); doxycycline, (Dox); Enzyme Linked Immunosorbent Assay, (ELISA); Gas chromatography, (GC); genetically modified organisms, (GMO); genetically modified, (GM); Green Fluorescence Protein, (GFP); Intracytoplasmic sperm injection, (ICSI); long QT syndrome, (LQTS); magnetic resonance imaging, (MRI); Mass spectrometry, (MS); mprinting control regions, (ICRs); poly(hydroxyalkanoate)s, (PHA); positron emission tomography, (PET); RNA interference, (RNAi); single photon emission tomography, (SPECT); Tet Responsive Element, (TRE); variable-temperature high performance liquid chromatography, (VT-HPLC); vitreoscilla hemoglobin protein, (VHb)

Received: 6 June 2008; Revised: 9 June 2008
Accepted: 10 June 2008; electronically published: July 2008

Summary

Papaya ringspot is a destructive disease characterized by a yellowing and stunting of the crown of papaya trees and assay was designed to help assign putative genome polyprotein analysis of Papaya ringspot virus strain W. We used different methods for the prediction of linear epitopes using a combination of a hidden Markov model and a propensity scale method. Data set was collected from the literature, and data sets of epitopes in the genome polyprotein having twenty four antigenic determinants in 675 residues long sequence. The structural homology modeling method is allows potential drug targets to identify active sites i.e. linear epitopes, which form antibodies in host cells. The method integrates prediction of peptide MHC class I binding; proteasomal C terminal cleavage and TAP transport efficiency. The challenges for the future are to establish the function of all of protein structures. In this assay we use of multiple methods towards the accurate identification of antigenic epitopes. The proposed approach is useful not only for plant and viral biology but it covers the wide area of vaccines and antibodies for therapeutic purposes in humans.

I. Introduction

Transgenomics is a branch of omics that deals with developing novel traits and agriculturally relevant characteristics where the novel phenotypes are generated through changes in gene regulation. It also provides various techniques and methodologies for automated high sensitivity genetic variation and mutation analysis. Transgene describes a segment of DNA containing a gene sequence that are isolated from one organism and is introduced into another organism. This can be done using various genetic engineering techniques. This non-native segment of DNA may retain the ability to produce RNA or protein in the transgenic organism. It may also alter the normal function of the transgenic organism's genetic code and the DNA is incorporated into the organism's germ line. For example, in higher vertebrates this can accomplish by injecting the foreign DNA into the nucleus of a fertilized ovum. This technique is routinely used to introduce human disease genes or other genes of interest into strains of laboratory mice to study the function or pathology involved with that particular gene. Transgenome is the genome that is created by chromosomal, gene and partial genome mixture of the same or different species. Thus, transgenomics deals with the study of transgene and transgenome in higher organisms as well as in plants. Its various offerings include generation of systems, products and some novel discovery in the fields of life science and personalized medicine (Barhtolmes et al, 2008; Gascon et al, 2008; Gomase et al, 2008a,b; Zhang et al, 2008).

II. History of Transgenomics

The history of transgenomics is traced in terms of studying the development of Genetically Modified Organisms (GMO) as well as producing various transgenic plants. The general principle of producing a GMO is to insert genetic material into an organism's genome to generate new traits. This methodology is termed as

Genetic engineering and was made possible through a series of scientific advances including the discovery of DNA and the creation of the first recombinant bacteria, i.e., *E .coli* expressing a salmonella gene in 1973. But, this led to concerns in the scientific community about potential risks from genetic engineering, for which several recommendations were laid out for conducting this type of research. Herbert Boyer founded the first company to use recombinant DNA technology, Genentech in 1978. The company announced the creation of an *E. coli* strain producing the human protein insulin. In 1986, field tests of bacteria genetically engineered to protect plants from frost damage at a small biotechnology company called Advanced Genetic Sciences of Oakland, California, are repeatedly delayed by opponents of biotechnology. Examples of GMOs include transgenic animals, viz., mice, fish, transgenic plants, microbes. Transgenic plants are developed for various purposes, for example, resistance to pests, herbicides, combating harsh environmental conditions, also included improving shelflife and nutritional value. The first commercial cultivation of Genetically Modified (GM) plants was in 1996. GM plants tolerant to the herbicides glufosinate and producing the Bt toxin was discovered (Canastar et al, 2008; Dawe et al, 2008; Kissa et al, 2008).

III. The transgenome project

Development of improved molecular detection methods for Bacillus cereus toxins was undertaken by the Department of Microbiology and Immunology, James Cook University. The various objectives of this project were to determine the prevalence of Bacillus cereus in food and other environmental sources, to develop improved toxin detection methods for toxigenic strains of Bacillus cereus and to make the developed toxin assays available to the rice industry and to relevant Australian health and food laboratories. Research shows that *Bacillus cereus* is a heat resistant bacterium that is capable of surviving the cooking process. It is a common soil born contaminant of food products. This organism is capable of prolific growth in cooked foods and it is capable of producing a variety of toxins that results in food poisoning. Methods for detecting toxin production are relatively limited and the emetic toxin that is most commonly found in rice-based foods is particularly elusive. Now, commercial kits for the demonstration of some of the toxins were evaluated. A method for detecting the emetic toxin that is capable of producing vomiting was developed and this was shown to be more sensitive than any method published to date. The genes responsible for the elusive emetic toxin were identified. A simple, sensitive and specific technique was developed for detecting strains of the organism carrying the genes required for the production of the emetic toxin were developed and standardized. This project showed that most of the isolates of *B. cereus* isolated from food in Australia were capable of producing at least one of the important toxins that induce food poisoning with diarrhoea. A technique for the detection of the emetic toxin was used to determine whether organisms isolated from food is capable of producing the emetic toxin. The identification of the genes responsible for the production of this toxin has allowed the diagnostic tests to be developed and will pave the way for new research into the production of this toxin (Bajaj et al, 2005; Zeyaullah et al, 2007).

IV. Transgenomics strategies

Intracytoplasmic sperm injection (ICSI)-mediated gene transfer was shown to be an effective technique for producing transgenic pigs. A performed ICSI-mediated gene transfer using pig sperm subjected to various pretreatments and determined the developmental potential of sperm-injected oocytes and introduction efficiency of exogenous DNA. It was found that when ICSI was performed using unfrozen sperm heads with tails removed by piezo-pulse, the rates of blastocyst formation and transgene (EGFP) expression were both low. Use of heads of sperm frozen-thawed with or without a cryoprotective agent resulted in rates of blastocyst formation and EGFP expression that tended to be generally high. It was seen that use of unfrozen sperm confered no advantages on ICSI-mediated gene transfer (Kurome et al, 2008). For studying gene function in Germline transformation, *Capsella bursa-pastoris* act as model system. Here, scientists adopted the floral dip method for achieving germline transformation of *C. bursa-pastoris*. The Green Fluorescence Protein (GFP) genes were used as markers for screening or selecting. Testing of two *Agrobacterium* strains, LBA4404 and GV3101, for their ability to transform C. bursa-pastoris was done. They also evaluated the effects of different concentrations of sucrose and the surfactant Silwet L-77 on the efficiency to generate transgenic C. bursa-pastoris plants and identified an efficient medium containing 10% (w/v) sucrose and 0.02-0.05% (v/v) Silwet L-77 (Savage et al, 2008).

V. Transgenomics Technology

A. Analytical technologies: Separation techniques

1. Gas chromatography (GC)

Pseudomonas putida KT2442 is able to accumulate medium-chain-length poly(hydroxyalkanoate)s (PHA) consisting of 3-hydroxyhexanoate, 3-hydroxyoctanoate, 3-hydroxydecanoate, and 3-hydroxydodecanoate from a wide range of carbon sources. Study has analyzed the PHA synthase pha operon (phaC1-phaZ-phaC2) using knock out technique and the vgb gene encoding vitreoscilla hemoglobin protein (VHb) that could enhance oxygen uptake rate especially at low oxygen concentration. They were integrated into the *P. putida* KT2442 genome to replace the deleted fragment. The resulting mutant P. putida KTOY01 or gene-replaced mutant KTOY02 was used as the host to study PHA synthase properties and PHA production (Kumar et al, 2006). For the analysis of production of novel fatty acids in oilseeds, the genetic and molecular techniques available for *Arabidopsis* to characterize modifying mutations affecting the accumulation of hydroxy fatty acids in the seeds of *Arabidopsis* plants that express a transgene for the castor bean fatty acid hydroxylase, FAH12. A high-

throughput analytical system for identifying three complementation classes of mutations with reduced hydroxy fatty acid accumulation from among Arabidopsis M3 seed samples derived from chemical mutagenesis. Also identified one of the mutations by positional cloning as a single base pair change in a gene encoding NADH:cytochrome b5 reductase. Characterization of homozygous mutant lines with and without the FAH12 transgene indicated that the only detectable consequence of the cbr1-1 mutation was on desaturase and hydroxylase reactions in the developing seed. The leaf and root fatty compositions, along with growth, development and seed production of mutant plants were found to be indistinguishable from wild type (Allen et al, 2008).

2. High performance liquid chromatography (HPLC)

The patients with Birt-Hogg-Dube (BHD) syndrome harbor germline mutations in the BHD tumor suppressor gene that are associated with an increased risk for kidney cancer. BHD encodes a protein called folliculin that interacts with the energy- and nutrient-sensing 5'-AMP-activated protein kinase-mammalian target of rapamycin (AMPK-mTOR) signaling pathways. In which recombineering methods was used to generate mice with a conditional BHD allele and introduced the cadherin 16 (KSP)-Cre transgene to target BHD inactivation to the kidney. BHD knockout mice and kidney cells isolated from BHD knockout and control mice were treated with the mTOR inhibitor rapamycin. Mouse survival was evaluated by Kaplan-Meier analyses. All statistical tests were two-sided. The homozygous loss of BHD may initiate renal tumorigenesis in the mouse; also, the conditional BHD knockout mouse was found to be a good model for dissecting multistep kidney carcinogenesis and rapamycin may considered as a potential treatment for Birt-Hogg-Dubé syndrome (Baba et al, 2008). A sensitive, ubiquitously expressed tetracycline inducible system could act as an essential tool in mouse transgenesis. Tested utility of a mammalian methylation-free CpG island was to drive ubiquitous expression of the sensitive doxycycline (Dox) inducible rtTA2S-M2 Tet-transactivator in transgenic mice. An 8 kb genomic fragment from the methylation-free CpG island of the human hnRNPA2B1-CBX3 housekeeping gene locus was tested. The characterization of the highest expressing rtTA2S-M2 transgenic mouse line demonstrated Dox-inducible GFP transgene expression in many tissues. Under the control of a Tet Responsive Element (TRE), line to show the highly sensitive quantitative induction with low doses of Dox of an assayable plasma protein transgene. The ubiquitously expressing rtTA2S-M2 transgenic mouse line is a very useful tool for studying the effects of the widespread, inducible over-expression of genes during embryonic development and in adult mice (Katsantoni et al, 2007).

B. Analytical technologies: Detection techniques

1. Magnetic resonance imaging

Currently, various methods are available for non-invasive imaging of gene delivery and transgene expression. This includes magnetic resonance imaging (MRI), single photon emission tomography (SPECT)/positron emission tomography (PET), and fluorescence and bioluminescence imaging. MRI produces a resolution of approximately 50 mum. Similarly, SPECT and PET give a resolution of only 1-2 mm but provide for relatively easy quantitation of the signal and need only nanograms of probe, compared with the microgram or milligram levels required for MRI and optical imaging. For quantification of the transgene expression profile MRI are actively used and the difference between modalities have a significant effect on the resultant imaging resolution for gene therapy (Kristian Raty et al, 2007). Advances in transgene technology have made it possible to create cancer cells, or mice with specific genetic alterations. Also, the application of an array of both functional and molecular non-invasive MR methods to these transgenic cancer cells has revolutionized the understanding of cancer. With the establishment of multi-modality molecular imaging centers within barrier or pathogen-free facilities, multi-parametric and multi-modality imaging of transgenic mouse models of human cancer are becoming increasingly prevalent. Currently there are several methods that are available for generating transgenic mice and cancer cell lines, including MRI (Raman et al, 2007).

2. Mass spectrometry (MS)

Observed pleiotropic effects are one of the main concerns regarding GMOs. Also flavonoids represented one of the most prominent groups of secondary metabolites in wheat. Many flavonoids function as signaling or defense molecules. Researchers used a robust and reproducible analytical method to compare the flavonoid content of GM wheat expressing genes that confer increased fungal resistance with their non-GM siblings. The transgenes provide either a broad-spectrum fungal defense or bunt-specific resistance by a viral gene (KP4). The results were shown in agreement with the hypothesis that the transgenes used to increase wheat defence to fungal pathogens do not interfere with the flavonoid biosynthesis pathway (Ioset et al, 2007).

C. Transgenomics in practice

A report the spectrum of long QT syndrome (LQTS) and Brugada mutations identified by a pilot LQTS gene testing program in New Zealand. They evaluated eighty-four consecutive index cases referred for LQT gene testing, from New Zealand and Australia. The coding sequence and splice sites of 5 LQTS genes were screened for genomic variants by transgenomics denaturing high-performance liquid chromatography (dHPLC) system and automated DNA sequencing. They found that the spectrum of New Zealand LQTS and Brugada mutations was similar to previous studies. The high proportion of novel

mutations (40%) dictates a need to confirm pathogenicity for locally prevalent mutations (Chung et al, 2007). In another study, the potential of variable-temperature high performance liquid chromatography (VT-HPLC) is a tool for dissecting and modulating nucleic acid structural transitions and used as a model the duplex-hairpin-coil transitions of d(CGCGAATTCGCG). Demonstration shows VT-HPLC, combined with diode-array detection of the ultraviolet signal, enables a physical separation of spectroscopically distinct species that can be assigned to the duplex, hairpin, and coil forms of d(CGCGAATTCGCG). If fractions from the peaks for hairpin or duplex forms are collected and subsequently reinjected onto the cartridge, re-equilibration occurs, and both hairpin and duplex peaks are observed. Concentration-dependent equilibrium constants, melting temperatures, and standard state enthalpies extracted from the measurements compare very well with previous literature results (Braunlin et al, 2004).

D. Technology development in transgenomics and approaches

1. Knockdown strategies

To test this possibility that the YY1 transcription factor controls several imprinted domains, researchers are used RNA interference (RNAi) strategies for generating transgenic mouse lines that express reduced levels of the cellular YY1 protein. In neonatal brains, most imprinted genes of the Peg3 domain are found to be up-regulated. In the Gnas domain, Nespas was down-regulated, whereas three other imprinted transcripts were up-regulated, including Nesp, Gnasxl and Exon1A. YY1 knockdown also changed the methylation levels at the imprinting control regions (ICRs) of these domains in a target-specific manner. The certain gender-specific outcome is caused by the YY1 knockdown effect on the Xist locus of females; also these results demonstrated that YY1 indeed functions as a trans factor for transcriptional regulation and DNA methylation of these imprinted domains in vivo (Kim et al, 2008).

2. Navigational strategy

Navigation deficits are prominent in Alzheimer's disease (AD) patients and transgenic mice expressing familial AD-mutant hAPP and A β peptides. For determining the impact of strategy use on these deficits, the scientists assessed hAPP and non-transgenic mice in a cross maze that can solved by allocentric and egocentric strategies. The most non-transgenic mice used allocentric strategies, whereas half of hAPP mice were egocentric and striatal pCREB expression was unaltered in hAPP mice, suggesting striatal sparing. These egocentric strategy use for earlier indicator of hAPP/A β-induced hippocampal impairment than spatial learning deficits. If there is hippocampal damage, then egocentric strategies are available is maladaptive, such cases persistent use of allocentric strategies are applicable (Deipolyi et al, 2008).

3. RNA interference

Peanut allergy are found to one of the most life-threatening food allergies and one of the serious challenges facing the peanut and food industries. With the advent of genetic engineering novel strategies proposed to solve the problem of peanut allergy from the source. One such methods was to eliminate the immunodominant Ara h 2 protein from transgenic peanut using RNAi, and to evaluate the allergenicity of resulting transgenic peanut seeds. For this, a 265-bp-long PCR product was generated from the coding region of Ara h 2 genomic DNA, and cloned as inverted repeats in pHANNIBAL, an RNAi-inducing plant transformation vector. Transgenic peanuts were produced by infecting peanut hypocotyl explants with Agrobacterium tumefaciens EHA 105 harbouring the pDK28 construct. A total of 59 kanamycin-resistant peanut plants were regenerated with phenotype and growth rates comparable to wild type. PCR and Southern analyses revealed that 44% of plants stably integrated the transgene. The allergenicity of transgenic peanut seeds expressed as IgE binding capacity was evaluated by Enzyme Linked Immunosorbent Assay (ELISA) using sera of patients allergic to peanut. The data showed a significant decrease in the IgE binding capacity of selected transgenic seeds compared to wild type, thus, demonstrating the feasibility of alleviating peanut allergy using the RNAi technology (Dodo et al, 2008).

VI. Applications of transgenomics

Production of biopharmaceuticals - In which transgenic animals are used to produce drugs, vaccines, hormones, and other substances of value to the pharmaceutical industry, in their milk. Animals are treated as living factories that offer a low-cost alternative for the large-scale production of drugs and proteins. Factory farming - In which genetic manipulation is aimed at increasing the growth efficiency and productivity of animals that are farmed for their flesh, milk, and eggs. Despite the fact that traditional, selective breeding has already resulted in a wide range of serious animal welfare problems, viz., turkeys that are bred with such large chest muscles that they can no longer mate naturally; the profit-driven food industry is forever seeking to create more with less, to the detriment of the animals involved. Disease models - In which animals are actually bred to suffer as “models” for human disorders. For example, the Harvard Oncomouse and other “animal models” of painful and distressing human illnesses such as cystic fibrosis, arthritis and psychological disorders. Organ transplants - In which some transgenic pigs were imported into Canada and bred for use as a source of organs for transplantation. However, there are serious concerns that transplanting pigs’ organs into humans may allow an animal virus to pass into the human population, in much the same way that AIDS is now thought to have come from monkeys and somehow crossed the species barrier. Transgenic plants - In which possess genes that are transferred from a different species. The aim is to design plants with specific characteristics by artificial insertion of genes from other species or sometimes entirely different kingdoms. For example, Leaf rust resistant plants, viz., Lr9 from *Aegilops umbellulata*

and Lr18 from *Triticum timopheevi* (Hochstein et al, 2007; Lohning et al, 2008; Swanson et al, 2008).

VII. Current research

The fruit fly Drosophila is a leading model system for studying the transcriptional control by cis-regulatory elements. There are some high-efficiency systems for directionally cloning PCR-amplified, or PCR-mutated, or synthetic enhancer sequences into the GANESH family of P element reporter constructs, which contain reporter genes encoding nuclear-localized eGFP, DsRed, or β-galactosidase. This system is scalable for either small projects or high-throughput approaches and makes use of Gateway cloning technologies for directional, efficient cloning, without the need for restriction digestion or ligation reactions. It is especially useful for those researchers who wish to test large numbers of putative enhancers (Streetz et al, 2008). One of the important processes, viz., fruit ripening were found to be characterized by processes that modify texture and flavor but also by a dramatic increase in susceptibility to necrotrophic pathogens for example *Botrytis cinerea*. For determining whether endogenous cell wall (CW) disassembly influences the ripening-regulated increase in necrotropic pathogen susceptibility, *B. cinerea* susceptibility was assessed in transgenic fruit with suppressed polygalacturonase (LePG) and expansin (LeExp1) expression. The results demonstrated that altering endogenous plant CW disassembly during ripening influenced the course of infection by B. cinerea mainly by changing the structure or the accessibility of CW substrates to pathogen CW-degrading enzymes (Cantu et al, 2008).

VIII. Conclusion

One of the greatest concerns associated with transgenic plants and transgenomics is the potential impact on nearby ecosystems and transgenes is the potential for significant ecological impact if the plants can increase in frequency and persist in natural populations. These concerns are similar to those surrounding conventionally bred plant breeds. Several risk factors could be considered, viz., the capability of the transgenic plant of growing outside a cultivated area; the fertility of the offspring. Many domesticated plants were found to mate and hybridize with wild relatives when they are grown in proximity. This applies equally to transgenic plants and conventionally bred plants, as in either case there are advantageous genes that may negative consequences to an ecosystem upon release. This is normally not a significant concern, despite fears over mutant super-weeds overgrowing local wildlife. The use of GMO are also sparked significant controversy in many areas. While some groups advocate the complete prohibition of GMOs, others call for mandatory labeling of genetically modified food or other products. Other controversies include the definition of patent and property pertaining to products of genetic engineering and the possibility of unforeseen local and global effects as a result of transgenic organisms proliferating. Some critics are also raised the concern that conventionally bred crop plants can cross-pollinated from the pollen of modified plants. Defenders of genetic engineering technology point out that each crop is assessed on a case by case basis to determine if there is any risk associated with the outcrossing of the genetically modified trait into wild plant populations. The fact that a genetically modified plant may outcross with a related wild relative is not, in itself, a risk unless such an occurrence are consequences. Thus, transgenomics is a field where there is a controversy between scientists over using this methodology and despite of some oppose, methodology whereas some accept these are the new challenge for studying various branches of life science.

References

Allen RS, Miller JA, Chitty JA, Fist AJ, Gerlach WL, Larkin PJ (**2008**) Metabolic engineering of morphinan alkaloids by over-expression and RNAi suppression of salutaridinol 7-O-acetyltransferase in opium poppy. **Plant Biotechnol J** 6, 22-30.

Baba M, Furihata M, Hong SB, Tessarollo L, Haines DC, Southon E, Patel V, Igarashi P, Alvord WG, Leighty R, Yao M, Bernardo M, Ileva L, Choyke P, Warren MB, Zbar B, Linehan WM, Schmidt LS (**2008**) Kidney-targeted Birt-Hogg-Dube gene inactivation in a mouse model: Erk1/2 and Akt-mTOR activation, cell hyperproliferation, and polycystic kidneys. **J Natl Cancer Inst** 100, 140-154.

Bajaj S, Mohanty A (**2005**) Recent advances in rice biotechnology--towards genetically superior transgenic rice. **Plant Biotechnol J** 3, 275-307.

Bartholmes C, Nutt P, Theiben G (**2008**) Germline transformation of Shepherd's purse (Capsella bursa-pastoris) by the 'floral dip' method as a tool for evolutionary and developmental biology. **Gene** 409, 11-19.

Braunlin WH, Giri I, Beadling L, Breslauer KJ (**2004**) Conformational screening of oligonucleotides by variable-temperature high performance liquid chromatography: dissecting the duplex-hairpin-coil equilibria of d(CGCGAATTCGCG). **Biopolymers** 74, 221-231.

Canastar A, Maxson SC, Bishop CE (**2008**) Aggressive and mating behaviors in two types of sex reversed mice: XY females and XX males. **Arch Sex Behav** 37, 2-8.

Cantu D, Vicente AR, Greve LC, Dewey FM, Bennett AB, Labavitch JM, Powell AL (**2008**) The intersection between cell wall disassembly, ripening, and fruit susceptibility to Botrytis cinerea. **Proc Natl Acad Sci U S A** 105, 859-864.

Chung SK, MacCormick JM, McCulley CH, Crawford J, Eddy CA, Mitchell EA, Shelling AN, French JK, Skinner JR, Rees, MI (**2007**) Long QT and Brugada syndrome gene mutations in New Zealand. **Heart Rhythm** 4, 1306-1314.

Dawe AS, Nylund R, Leszczynski D, Kuster N, Reader T, De Pomerai DI (**2008**) Continuous wave and simulated GSM exposure at 1.8 W/kg and 1.8 GHz do not induce hsp16-1 heat-shock gene expression in Caenorhabditis elegans. **Bioelectromagnetics** 29, 92-99.

Deipolyi AR, Fang S, Palop JJ, Yu GQ, Wang X, Mucke L (**2008**) Altered navigational strategy use and visuospatial deficits in hAPP transgenic mice. **Neurobiol Aging** 29, 253-266.

Dodo HW, Konan KN, Chen FC, Egnin M, Viquez OM (**2008**) Alleviating peanut allergy using genetic engineering: the silencing of the immunodominant allergen Ara h 2 leads to its significant reduction and a decrease in peanut allergenicity. **Plant Biotechnol J** 6, 135-145.

Gascon S, Paez-Gomez JA, Díaz-Guerra M, Scheiffele P, Scholl FG (**2008**) Dual-promoter lentiviral vectors for constitutive

and regulated gene expression in neurons. **J. Neurosci. Methods** 168, 104-112.

Gomase VS, Changbhale SS, Patil SA, Kale KV (**2008**) Metabolomics. **Curr Drug Metab** 9, 89-98.

Gomase VS, Kale KV, Tagore S, Hatture SR (**2008**) Proteomics: technologies for protein analysis. **Curr Drug Metab** 9, 213-220.

Hochstein N, Muiznieks I, Mangel L, Brondke H, Doerfler W (**2007**) Epigenetic status of an adenovirus type 12 transgenome upon long-term cultivation in hamster cells. **J Virol** 81, 5349-5361.

Ioset JR, Urbaniak B, Ndjoko-Ioset K, Wirth J, Martin F, Gruissem W, Hostettmann K, Sautter C (**2007**) Flavonoid profiling among wild type and related GM wheat varieties. **Plant Mol Biol** 65, 645-654.

Katsantoni EZ, Anghelescu NE, Rottier R, Moerland M, Antoniou M, de Crom R, Grosveld F, Strouboulis J (**2007**) Ubiquitous expression of the rtTA2S-M2 inducible system in transgenic mice driven by the human hnRNPA2B1/CBX3 CpG island. **BMC Dev Biol** 7, 108.

Kim J, Kim JD (**2008**) In vivo YY1 knockdown effects on genomic imprinting. **Hum Mol Genet** 17, 391-401.

Kissa K, Murayama E, Zapata A, Cortes A, Perret E, Machu C, Herbomel P (**2008**) Live imaging of emerging hematopoietic stem cells and early thymus colonization. **Blood** 111, 1147-1156.

Kristian Raty J, Liimatainen T, Unelma Kaikkonen M, Grohn O, Jumani Airenne K, Yla-Herttuala S (**2007**) Non-invasive Imaging in Gene Therapy. **Mol. Ther.** 15(9), 1579-1586.

Kumar R, Wallis JG, Skidmore C, Browse J (**2006**) A mutation in Arabidopsis cytochrome b5 reductase identified by high-throughput screening differentially affects hydroxylation and desaturation. **Plant J** 48, 920-932.

Kurome M, Saito H, Tomii R, Ueno S, Hiruma K, Nagashima H (**2008**) Effects of sperm pretreatment on efficiency of ICSI-mediated gene transfer in pigs. **J Reprod Dev** 53, 1217-1226.

Lohning M, Hegazy AN, Pinschewer DD, Busse D, Lang KS, Hofer T, Radbruch A, Zinkernagel RM, Hengartner H (**2008**) Long-lived virus-reactive memory T cells generated from purified cytokine-secreting T helper type 1 and type 2 effectors. **J Exp Med** 205, 53-61.

Raman V, Pathak AP, Glunde K, Artemov D, Bhujwalla ZM (**2007**) Magnetic resonance imaging and spectroscopy of transgenic models of cancer. **NMR Biomed** 20, 186-199.

Savage PA, Vosseller K, Kang C, Larimore K, Riedel E, Wojnoonski K, Jungbluth AA, Allison JP (**2008**) Recognition of a ubiquitous self antigen by prostate cancer-infiltrating CD8+ T lymphocytes. **Science** 319, 215-220.

Streetz KL, Doyonnas R, Grimm D, Jenkins DD, Fuess S, Perryman S, Lin J, Trautwein C, Shizuru J, Blau H, Sylvester KG, Kay MA (**2008**) Hepatic parenchymal replacement in mice by transplanted allogeneic hepatocytes is facilitated by bone marrow transplantation and mediated by CD4 cells. **Hepatology** 47, 706-718.

Swanson CI, Hinrichs T, Johnson LA, Zhao Y, Barolo SA (**2008**) A directional recombination cloning system for restriction- and ligation-free construction of GFP, DsRed, and lacZ transgenic Drosophila reporters. **Gene** 408, 180-186.

Zeyaullah M, Nabi G, Malla R, Ali A (**2007**) Molecular studies of E. coli mercuric reductase gene (merA) and its impact on human health. **Nepal Med Coll J** 9, 182-185.

Zhang F, Wu S, Lu X, Wang M, Liu M (**2008**) Gene transfer of endothelial nitric oxide synthase attenuates flow-induced pulmonary hypertension in rabbits. **Ann Thorac Surg** 85, 581-585.