# 28th ISAG Conference

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# **Guest Speakers**

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# Topic

The immunobiology of prion diseases

Biotechnology and Transgenesis in fish

Comprehensive DNA analysis using Pyrosequencing<sup>TM</sup> techn.

Ethical Issues of Genetic Manipulation of Lifestock

Can we use genomics to select for healthier swine?

Biodiversity and Conservation in Amazon

Proteomics

SNP technology

# 28<sup>th</sup> ISAG Conference





Adriano Aguzzi studied medicine at the University of Freiburg (Germany) form 1980-1986. He received his Ph.D. in 1986. From 1986 to 1989 he was resident in Neuropathology at the University Hospital of Zürich (Switzerland). Since 1997 he is full professor of Neuropathology and Director of the Institute of Neuropathology at the University of Zürich. He is president of the Swiss Society of Neuropathology and member of several

international Societies. His research interests are in the area of prion diseases with special focus on their pathogenesis.



Choy Leong Hew is Head of the Department of Biological Sciences at the University of Singapore. He received his Ph.D. in 1970 at the University of British Columbia. From 1972 to 1974 he was a C.H. Best fellow at the Banting & Best Dept. of Medical Research of the University of Toronto (Canada). He was then working at the Memorial University of Newfoundland as assistant professor until 1983. From 1983 to 1999 he was a visting professor at a variety of Universities in Canada and China. His

main research areas are biology and biotechnology of antifreeze proteins, transgenic fish, molecular endocrinology and proteomics.



**Björn Ingemarsson** is Director of Pyrosequencing AB's technical and scientific support function based in Uppsala. Prior to joining Pyrosequencing 3 years ago, he worked as a specialist in automated DNA analysis at Amersham Pharmacia Biotech. He aquired his Ph.D. in plant physiology at the Stockholm University, where he thereafter continued research on the physiology, biochemistry and molecular biology of nitrogen assimilation in plants for several years.



**Bernhard Irrgang** studied Philosophy, Theology and German Philolog<sup>\*</sup> at the University of Würzburg (Germany), Passau (Germany) and Munich (Germany). He received his Ph.D. in Philosophy in 1982 and Theology in 1991. From 1982 to 1992 he worked at different Universities in Germany. Currently, he is Director of the Institute for Philosophy and the Centre for Interdisciplinary Technical Research at the Technical University of Dresden

(Germany). Bernhard Irrgang main interests are in the area of medical and technical ethics, consequences of technology and philosophy of technology.



Joan K. Lunney studied Chemistry at the John Hopkins University (Baltimore, USA) and received her Ph.D. on studies on the regulation of serum glycoprotein homeostasis in 1976. From 1976 to 1979 she was guest postdoctoral research worker in the Immunology Branch (NCI, NIH, Bethesda, MD). From 1983 until 1995 she was working in different position in the Helminthic Diseases Laboratory at the USDA, ARS (Beltsville, MD). Since 1995 she is GM15 Research Leader and

Supervisory Research Immunogeneticist at the Immunology and Disease Resistance Laboratory, USDA, ARS (Beltsville, MD).



Maria-Paula Cruz Schneider is Director of the Laboratory of DNA Polymorphisms at the University of Belém (Para, Brasil). She received her Ph.D. 1988 at the Federal University of Rio Grande do Sul (Brasil) working on protein polymorphisms in primates of the Amazon region. Her main interests are in the area of genetics and molecular genetics in domestic species.



Hans-Jürgen Thiesen studied medicine at the University of Hamburg (Germany) from 1976 to 1982. He received his Ph.D. in 1983 for studying the physiology of parathyroid hormone. From 1985 to 1987 he was working at the EMBL followed by a fellowship the Basel Institute of Immunology. In 1996 he received a Chair in Immunology at the University of Rostock (Germany). His main interests are focussed on technologies of functional genomics in a clinically-oriented environment.

Hans-Jürgen Thiesen is member of the Proteome-Center at which complex human diseases are analysed by microarray analysis.



Alain Vignal received his B.Sc. in Agronomic Sciences in 1981. From 1982-1987 he studied biochemistry, cellular genetics and molecular biology at the University of Paris. In his Ph.D. thesis at the INSERM (Laboratory of Jean-Pierre Carton) Alain Vignal analysed the human glycophorin A and B gene family. Between 1991 and 1993 he had a postdoctoral fellowship at the Genethon (Laboratory of Jean Weissenbach). During this period he was involved in the human genome project, genotyping the CEPH reference families. Since 1994 he is at the

INRA in Toulouse (France). He is responsible for the development of structural genomics in chicken and expert in the field of QTL mapping.

# **Plenary Session:**

## **Invited Speakers**

### P001:

#### Comprehensive DNA analysis using Pyrosequencing<sup>™</sup> technology

Björn Ingemarsson Pyrosequencing AB, Uppsala, Sweden

Pyrosequencing<sup>™</sup> is a fast and accurate technology for analysis of short to medium length DNA sequences. It is a non-electrophoretic sequencing method based on luminometric real-time detection of pyrophosphate released upon nucleotide incorporation by DNA polymerase. Samples are sequenced and analyzed in a standard microtiter plate format, without any need for labeled primers or nucleotides.

The technique is well established for rapid genotyping of single nucleotide polymorphisms (SNPs). High accuracy is achived by polymerase-catalyzed incorporation of nucleotides at the polymorphic positions as well as adjacent nucleotides. The non-variant positions serve as internal controls and allow for automatic quality assessment of each analyzed sample. Single well multiplex genotyping on pooled simplex or multiplex PCR products can be accurately performed for SNPs and insertion/deletion polymorphisms.<sup>\*</sup>

The close correlation between nucleotide incorporation/pyrophosphate release and light detection, makes Pyrosequencing technology suitable for quantitative applications such as, allele frequency determination, assessment of gene copy number, analysis of splice mutations and CpG methylation, genotyping in mixed cell populations or polyploidic genomes, as well as for determination of viral load

The technology can also be used for sequence identification/verification and *de novo* sequencing of 20–50 nucleotides in less than one hour. Hence, it can be used for a number of applications including, analysis of mutation hot spot areas, cDNA re-sequencing, virus and bacteria identification/typing, as well as resistance typing.

Pyrosequencing AB has developed complete systems for low to high troughput demands, which include instrument, optimized reagent kits and dedicated software.

3.

#### P002:

#### SNP technology

Alain Vignal

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Although the highly informative, multi-allelic microsatellite markers have dominated for now over ten years the field of molecular genetic studies in human and in animals, either used as model organisms or studied for their interest in agriculture, an increasing importance is now given to SNPs (Single Nucleotide Polymorphisms), that are merely di-allelic base substitutions of lower heterozygosity. This is due to several reasons, amongst which the main are that SNPs can be found at very high densities throughout the genome (1 SNP every 1300 bp, when two random human chromosomes are compared), and that they can be found in exons of genes. The very high densities of SNPs in the genome make them ideal for association and short range haplotype studies, although this usually means that far more markers will have to be used than for microsatellites. The coding SNPs, sometimes referred to as cSNPs, are often studied as candidate polymorphisms when they imply an amino acid change, although it is not always easy to distinguish between the real implication of the polymorphism in the biological phenomenon studied and a simple allelic association.

However, contrariwise to microsatellites, for which the genotyping technology has only evolved from manual scoring of alleles, seen as PCR products of varying sizes on acrylamide gels, to a semi-automated method using sequencing machines, the situation is not so clear concerning the genotyping of SNPs, for which a broad range of methodologies are available. Amongst these are: direct hybridisation of allele-specific oligonucleotides (ASO) on membranes or glass arrays containing PCR products of individuals to genotype; hybridisation of PCR products on chips containing oligonucleotides; single base primer extensions (SBE) whose products can be separated by various means such as gel electrophoresis, tagged arrays, tagged beads or matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF); oligonucleotide ligation assay (OLA); monitoring the exonuclease degradation of an internal allele-specific oligonucleotide during PCR; real time sequencing...

For small scale studies, the major concerns when choosing an SNP genotyping technology, will be the possibilities of using existing platforms available at hand, such as an automatic sequencer or a DNA spotter. For larger scale studies, according to the number of SNPs and of individuals to be genotyped, the choices will vary. Indeed, some technologies, such as those involving glass arrays or chips, will enable to study high numbers of SNPs in parallel, but only on a low number of individuals, and others, such as MALDI-TOF separation of SBE products, will be more efficient with a high number of samples, but will need more effort put into the development of each individual SNP. Efforts are still underway, to develop a very high throughput genotyping system, capable of generating the very high numbers of genotypes needed for the genome-wide association studies, such as envisaged in human genetics.

#### P003:

#### **Biotechnology and Transgenesis in fish**

Choy L.Hew, Xiaobo Zhang, Canhua Huang, Qingsong Lin Department of Biological Sciences and Tropical Marine Science Institute National University of Singapore, Singapore and

#### Garth Fletcher

#### Ocean Science Center, Memorial University of Newfoundland, St John's, Canada

Food security will be a major challenge facing mankind as we enter the new millennium. The challenge for the agricultural sector is to double food production by year 2025 and triples it by year 2050. It is anticipated that aquaculture and mariculture will become a major driving force to increase food production. However, new technologies will needed to be employed to improve the efficiency and many bottleneck issues facing the industries.

Numerous platform biotechnologies that include molecular biology, genomics, cloning technologies, DNA chips and proteomics can improve the industry's output substantially. In this presentation, we will describe two specific approaches from our and other laboratories.

A.Transgenesis. Transgenic technology is the transfer of a foreign gene into a host organism enabling the host to acquire a new and inheritable trait. The technology is specific for the properties of the candidate gene and appears to be better than the traditional selective breeding methods. We have attempted to generate transgenic fish that are freeze-tolerant, faster growing and disease resistant using the antifreeze protein, growth hormone or the pleurocidin genes from different fish species respectively. We have demonstrated that these transgenes can be stably integrated, inherited and expressed. There is also a strong correlation between genotypes and phenotypes. The faster growing salmon (GH transgenic) is now being commercialized. Some of the issues dealing with food safety and ecology will be commented.

b. Proteomics. Proteomics provides a global and dynamic profiling of protein activities within a cell or organisms and is now a popular method in functional genomics to examine cellular activities as well as for biomarker identifications. We have used this approach successfully to characterize the viral proteins from shrimp white spot syndrome virus. More than 15 novel proteins have been isolated and these proteins are potential candidates for vaccine development to prevent this pandemic disease in the shrimp industry.

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#### P004:

# Biodiversity and Conservation in Amazon – Prospects of Habitat Fragmentation Studies

#### Maria Paula Cruz Schneider

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Brazil is the country with the largest undisturbed Tropical rain forest in the world, with almost half of the 6.5 million km<sup>2</sup> of the Amazon river basin being found in its territory. Tropical rain forests are also found in Central America, Africa and Southeast Asia, and they are supposed to play a central role in environment and climate stability worldwide. Besides the forest, the Amazon region is formed by a complex of ecosystems which includes cerrado and other types of vegetation totaling 5,029 millions km<sup>2</sup>, comprising around 10 to 20% of all animal and plant species of the planet. However, such biodiversity has been threatened by an accelerated rate of damaging and destruction of various habitats.

Official reports alerted to the high level of deforestation and decline of biodiversity due to commercial logging, burning, construction of dams for hydroelectric plants, cattle ranching, and agriculture expansion. Thus the Brazilian Government, through its Ministry of Environment, provided incentives, by way of grants and scientific meetings, in order to elaborate a national policy for biodiversity based on the intentions of the 1992 UN Conference on Environment and Development (Earth Summit). The granted proposals would identify environmental, social and economic conditions for conservation and sustainable use of animal and plant species, as well as partition of the benefits resulting from biodiversity usage. Priority areas for conservation and inventory of species were also planned to be defined.

The research conducted by our group in Belém was always related to biodiversity and conservation issues. One of our main interests during two decades was the study of genetic polymorphism and molecular phylogeny of Neotropical primates1- (3). The aim of assessment of the diversity of New World primates is to have at hand a list of Primates as a basis for conservation measures, and to stimulate further research into the systematics an taxonomy of the group. In fact, without the formal structure of names and an agreed system of usage, there can be no understanding of what exists to be conserved (4,5). More recently we focused our research on the effects of fragmentation of some Amazonian habitats on populations of silvery marmoset (Mico argentatus), howler monkey (Alouatta belzebul) and birds species (guará, Eudocimus ruber, and some species of migrating shorebirds). Studies of habitat fragmentation has clearly and easily evaluated effects on parameters such as species diversity, population density, and behavioral patterns, but there are as yet few data available on its influence on genetic variation in free-ranging primate populations (6,7). Inbreeding and loss ofgenetic variability are generally presumed to be the primary results of the fragmentation of continuous populations, although outbreeding depression may also be relevant in many cases (8,9).

Preliminary data on the variability of DNA microsatellite markers in silvery marmoset populations at four sites in central Amazonia (10), representative of different degrees of habitat fragmentation, found a surprisingly high genetic variability among populations even in the smallest ones. However, the level of heterozygosity indicates that all populations are subject to inbreeding or genetic drift. Despite the preliminary nature of the data, the results show that the remnant populations of M. argentatus are quite genetically distinct. Direct evidence of inbreeding depression has been recorded in fragmented populations of golden lion tamarins (Leontopithecus rosalia), a species belonging to the same family as the silvery marmoset (Callitrichidae) (11), although no clear indications of this phenomenon were found in the M. argentatus population from the right bank of the Tapajós river.

Whatever the exact effect on genetic parameters, habitat fragmentation, in particular the isolation of relatively small subsets of original populations, is likely to have highly deleterious implications for the long-term viability of remnant populations (12). A systematic analysis of complementary ecological factors will be required before definitive guidelines can be drawn up for the management of remnant populations of silvery marmosets (10).

Another research currently under way by our group aims to provide ecological and genetic data about the long-term effects fragmentation habitats created after building of a hydroelectric plant dam. For this purpose we use a wide range primate species, the howler monkey (Alouatta belzebul), whose habitat was radically altered through the formation of thousands of islands along the Tocantins river banks after the flooding of 2,500 km<sup>2</sup> due to the construction of the Tucuruí Hydroelectric plant (1984/1985). In order to obtain a more precise picture of how the genetic variability changed through time in this species, DNA microsatellite markers are being used in recent collected samples of the species, as well as in samples obtained 18 years ago, before the dam was built. Hence, the data gathered in that study may help to define viable population size and management in A. belzebul, as well as in other mammal populations, after habitat fragmentation.

#### Acknowledgements

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#### REFERENCES

1.Schneider& Rosenberger, 1996. Molecules, morphology, and Platyrrhini systematics. In: Adaptative Radiations of Neotropical Primates, M. A. Norkonk, A.L. Rosenberger and P.A. Garber (Eds.), pp.3-19. Plenum Press, N.Y

2.Schneider, H., Schneider, M. P. C., Sampaio, I., Harada, M. L., Stanhope, M., Czesłuniak, J. and Goodman, M. 1993. Molecular phylogenetics of the New World monkeys (Platyrrhini, Primates) Molecular Phylogenetics and Evolution 2 (3): 225-242.

3.Schneider, H., Sampaio, I., Harada, M. L., Barroso, C. M. L., Schneider., M. P. C., Czelusniak, J. and Goodman, M. 1996. Molecular phylogeny of the New World monkeys (Platyrrhini, Primates) based on two unlinked nuclear genes: IRBP Intron 1 and epsilon-globin sequences. Am. J. Phys. Anthropology. 100: 153-179

4. Collar (1997), N. J. 1997. Taxonomy and conservation: Chicken and egg. Bull. B. O. C. 117 (2):122-136.

5.Rylands, A. Schneider, H., Lannguth, A. Mittermeier, R. A., Groves, C., Rodrigues-Luna E. 2000. An assessment of the diversity of New World primates. Neotropical Primates 8 (2).

6.Pope, T. R. 1996, Socioecology, population fragmentation, and patterns of genetic loss in endangered primates, in: J. C. Avise and J. L. Hamrick, eds., Conservation Genetics: Case Histories from Nature, Chapman and Hall, New York, pp. 119-159.

7.Pope, T. R. 1998, Genetic variation in remnant populations of the woolly spider monkey (Brachyteles arachnoides). Int. J. Primatol. 19: 95-109.

8. Templeton, A. R., 1986, Coadaptation and outbreeding depression, in: M. E. Soulé, ed., Conservation Biology. The Science of Scarcity and Diversity, Sinauer Associates, Sunderland, pp. 105-116.

9.Dudash, M. R. and Fenster, C. B., 2000. Inbreeding and outbreeding depression in fragmented populations, in: A. G. Young and G. M. Clarke, eds., Genetics, Demography and Viability, of Fragmented Populations, Cambridge University Press, Cambridge, pp. 35-53.

10.Gonçalves, E. C., Ferrari, S. F., Silva, A., Coutinho, P., Menezes, E., Schneider, M. P. C. 2002.Effects of habitat fragmenttion on the genetic variability of silvery marmosets, Mico argentatus. In L. K. Marsh, ed., Primates in fragments, Plenum Press, New York, in Press.

11.Dietz, J. M., Baker, A. J. and Ballou, J. A. 2000, Demographic evidence of inbreeding depression in wild golden lion tamarins, in: A. G. Young and G. M. Clarke, eds., Genetics, Demography and Viability of Fragmented Populations, Cambridge University Press, Cambridge, pp. 203-211.

12.Lacy, R. C. 1997, Importance of genetic variation to the viability of mammalian populations. J. Mammal. 78: 320-335.

#### P005:

## Can we use genomics to select for healthier swine?

## Joan K. Lunney

## USDA, ARS, ANRI, Immunology and Disease Resistance Laboratory, Beltsville, MD USA

Genome mapping efforts in swine have been targeted mainly to production traits, reproductive issues and meat quality. Early efforts did result in identifying genes encoding resistance to specific bacterial infections, e.g., K88R. Recent work has identified swine with improved, not complete, resistance to specific viral infections. However, identifying genes encoding resistance for each of the large number of viral and bacterial infections that impact swine production is virtually impossible. Therefore our research has been aimed at defining immune properties that result in swine with improved production traits and survival capacity due to improved disease responses, targeting cells and genes associated with type 1 immunity. Edfors-Lilja, Andersson, et al. mapped QTL associated with 'stress' (mixing and transport) induced alterations of porcine immune functions. Mallard, Wilkie, et al. selected swine for high immune response. Delineation of traits most appropriate to targeting for "enhanced immune properties" associated with disease resistance as well as vaccine and immune responsiveness will be discussed. Functional genomic tools, real time PCR and microarray analyses, as well as SNP studies, will help identify relevant genes, their regulators and best alleles. Major collaborative studies that integrate analyses for enhanced immune properties along with production traits are required to confirm the potential of using these genomic approaches to select for healthier swine.

Nomenclature: Functional genomics, disease resistance, immune responsiveness.

#### P006:

#### Proteomics

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With the advent of complete genome sequences the analysis of gene functions is one of the scientific challenges in the post-genome-era. Great efforts have to be put forward on the analysis of complex gene and protein networks describing cellular as well as intercellular interactions. Corresponding research projects take advantage of novel enabling technologies i.e. chip technology and mass spectrometry.

#### **Comparative Genomics**

Research projects studying processes in developmental biology and cellular differentiation, demonstrated in the past as exemplified by the functional analysis of homeobox-genes how informative the comparative analysis of protein domains from drosophila to human sequences turned out to be (Comparative Genomics). The functional analysis of protein domains manifests that protein functions can be correlated with structural domains. Based on sequence information on evolutionarily conserved structural elements, proteins can be grouped, classified and assigned to functional groups enabling phenotype-genotype comparisons.

#### Functional genomics

Currently, multiple genomes have already been completely sequenced. Nowadays, the elucidation of individual gene functions becomes one of the prominent enterprises in research. Hereto, new research disciplines evolved such as transcriptomics, proteomics and metabolomics that are combined by integrative bioinformatic platforms (Figure I). Each of these disciplines is based on enabling technology platforms that requires specific instrumentation, expertise and the establishment of standard operating procedures (SOPs). How these technologies can be successfully integrated in one centre, has recently been realized by the Proteome Centre Rostock (www. proteome-alliance.de).

#### **Definition Proteome:**

The expression "Proteome" firstly coined by Marc Wilkins in 1994 describes the attempt of describing the composition of all proteins in a qualitative and quantitative manner present within one entity (tissue, body fluid, organism) at specified physiological states and time points.

#### **Comparison Genome and Proteome**

In contrast to a genome, the proteome is dynamic and presents specific physiological states under specified environmental conditions at specific time points. Furthermore, the proteome determines the functional state of living organisms. The genome just ensures that the proteome is functional. How the proteome of a cell operates, is based on environmental factors i.e. such as light, energy, nutrition and physical activities. In multicellular organisms, the interplay and turn-over of cells within one organism has to be taken into account as well. Obviously, the proteome determines how the genetic information is being used (Transcription factors regulate the retrieval of genetic information). Thus, one might even say the proteome determines under evolutionary aspects how the corresponding genome has been assembled. The genome probably ensures that the proteome remains operational in living organisms. In general, protein compositions have to be stabilized by replacing proteins according to their specific half-lives. Furthermore, perturbations of the proteome can then be introduced by changing the composition or the rate of protein renewal.

#### Proteome-analysis:

With the development of the 2-dimensional gel electrophoresis by Patrick O'Farell and Joachim Klose in 1975, the basis for proteome analysis had been established. This technology was then extended by the engineering of sophisticated mass spectrometry tools such as MALDI-TOF- (Matrix-Assisted-Laser-Desorption-Ionisation-Time-Of- Flight-) and electrospray mass spectrometry. In general, proteome analysis starts with the sampling of biological material under standardised conditions and ends with the computer analysis of mass spectrometric data that finally leads to the assignments of

identified proteins to pathways displaying functional activities. In particular, biological samples are collected under standardized conditions (SOPs) and prefractinated i.e. using gradient centrifugation, free-flow-electrophoresis, multidimensional protein identification technology (mudPIT) followed by 2-dimensional gel electrophoresis. Either chromatographicly separated protein fractions are directly subjected to liquid chromatographic mass spectrometry (Nano-electrospray-MS) or are further separated on 2D-gel-electrophoresis (2DE) using immobiline strips or ampholyte gel electrophoresis. Proteins identified in 2DE to be differentially expressed are isolated by picking robots and digested by enzyme digestion (i.e. Trypsin). The obtained peptide fragments are then subjected to MALDI-TOF-MS analysis for protein identification by mass finger printing.

#### **Proteome analysis**

Initially, global proteomic approaches were performed followed by the analysis of subproteomes ( Organelles and protein complexes). Hereto, Cellzome (Heidelberg) developed technologies for determining protein-protein interaction maps. MELTEC (Magdeburg) developed protocols to analyze the toponome of cells and tissues coined toponomics and Biovision in Hannover concentrates on studying the peptidome (peptidomics).

#### **New Technologies**

Recently, fluorescence dyes have been introduced to visualize differentially expressed proteins (Pharmacia), or to meet the sensitivity of silver stains (SyproRuby). Microfluid-Chips are expected in the future to replace 2DE gels, multidimensional protein identification technology (MudPIT) has been successfully developed to approach proteins that do not separate well in conventional 2DE gels., ICAT methods are used for quantification purposes.

#### **Bioinformatics**

Laboratory-Information-Management-Systems (LIMS) are essential to enable high-throughputanalysis. Hereto, the Proteome-Center Rostock has established the "Proteobase-Data-Management-System", in which clinical data, SOPs, RNA and protein expression data are going to be integrated.

#### The BMBF-Leitprojekt:

In the BMBF-Leitprojekt "Proteom-Analyse des Menschen" (www.proteome-alliance.de), genome-, transcriptome- and proteome analyses are combined to elucidate common and disease specific mechanisms involved in the pathophysiology of autoimmune diseases. For instance, only a correlation coefficient of 0.2 was obtained once RNA and protein expression levels of synovial tissues were compared. That RNA- and protein expression levels are not well correlated is not surprising since numerous kinds of transcriptional and translational regulation are known to exist in the cell. In addition, mRNA molecules and proteins vary in their propensities to become degraded. This comparative analysis underlines the importance of determining RNA and proteome expression levels in order to validate the relevance of RNA and protein expression profiling experiments.

#### Services

The Steinbeis-Transfer-Center for Proteome-Analysis offers services in protein and transcriptome analysis (<u>http://www.stw.de/stz/424.htm</u>) by making use of our Affymetrix Service / custom-made chip approach as well as the technology platform in proteomics run by Prof. Dr. M.O. Glocker.

#### Perspectives

By bringing experts of experimental research together with mathematicians and engineers, a new discipline is about to be born coined **system biology** with the aim of developing new theoretical approaches and computational tools in order to analyze, to model, and to predict network behaviours of proteomes.

#### Acknowledgement:

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URLs: http://dip.doe-mbi.ucla.edu/ http://www.cgr.harvard.edu/research/biological.html http://yeast.cellzome.com/browsec.php





#### P007:

#### The immunobiology of prion diseases

#### Adriano Aguzzi

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Mice deficient in the normal prion protein are resistant to exposure to prion infectivity, and expression of the normal prion protein by neurons is necessary for the development of histological damage<sup>1</sup>. But how do prions reach the brain after entering the body from peripheral sites? The first portal of entry in the gut may be represented by M-cells<sup>2</sup>. Neuroinvasion, i.e. the process by which prions march through the body of the host towards the brain, is dependent upon expression of the normal prior protein in a non-hematopoietic extracerebral site<sup>3</sup>. We therefore developed the hypothesis that neuroinvasion takes place in two distinct steps: first the lymphoreticular system is diffusely colonized by the agent, while at a later time infectivity progresses from lymphoreticular organs to the central nervous system<sup>4</sup>, probably via sympathetic nerves<sup>5,6</sup>. There is an absolute requirement for Blymphocytes in peripheral prion pathogenesis7. Surprisingly, the presence of the normal prion protein is not necessary on B-lymphocytes to enable them to support this process8. The mechanism of action of B lymphocytes may consist of presentation of lymphotoxin-ß to follicular dendritic cells9. This paves the way to post-exposure prophylaxis strategies<sup>10</sup> that exploit the anti-prion effect of soluble lymphotoxin-ß receptors. Why do follicular dendritic cells accumulate prions? We tested the hypothesis that prion uptake may be complement-mediated. Indeed, certain components of the complement system (C1q, CR1/2) proved to play an important role in pathogenesis<sup>11</sup>. Finally, we have found that transgenic expression of an anti-PrP antibody heavy chain suffices to confer to mice antiprion protection – a finding that may be relevant to the development of antiprion vaccines<sup>12</sup>.

- Brandner, S., Isenmann, S., Raeber, A., Fischer, M., Sailer, A., Kobayashi, Y., Marino, S., Weissmann, C. & Aguzzi, A. Normal host prion protein necessary for scrapie-induced neurotoxicity. *Nature* 379, 339-43 (1996).
- Heppner, F. L., Christ, A. D., Klein, M. A., Prinz, M., Fried, M., Kraehenbuhl, J. P. & Aguzzi, A. Transepithelial prion transport by M cells. *Nat Med* 7, 976-7 (2001).
- Blättler, T., Brandner, S., Raeber, A. J., Klein, M. A., Voigtländer, T., Weissmann, C. & Aguzzi, A. PrP-expressing tissue required for transfer of scrapie infectivity from spleen to brain. *Nature* 389, 69-73 (1997).
- 4. Aguzzi, A. & Weissmann, C. Prion research: the next frontiers. Nature 389, 795-798 (1997).
- Glatzel, M., Flechsig, E., Navarro, B., Klein, M. A., Paterna, J. C., Bueler, H. & Aguzzi, A. Adenoviral and adeno-associated viral transfer of genes to the peripheral nervous system. *Proc Natl Acad Sci U S A* 97, 442-7 (2000).
- Glatzel, M., Heppner, F. L., Albers, K. M. & Aguzzi, A. Sympathetic innervation of lymphoreticular organs is rate limiting for prion neuroinvasion. *Neuron* 31, 25-34. (2001).
- Klein, M. A., Frigg, R., Flechsig, E., Raeber, A. J., Kalinke, U., Bluethmann, H., Bootz, F., Suter, M., Zinkernagel, R. M. & Aguzzi, A. A crucial role for B cells in neuroinvasive scrapie. *Nature* 390, 687-90 (1997).
- Klein, M. A., Frigg, R., Raeber, A. J., Flechsig, E., Hegyi, I., Zinkernagel, R. M., Weissmann, C. & Aguzzi, A. PrP expression in B lymphocytes is not required for prion neuroinvasion. *Nat Med* 4, 1429-33 (1998).
- Montrasio, F., Frigg, R., Glatzel, M., Klein, M. A., Mackay, F., Aguzzi, A. & Weissmann, C. Impaired prion replication in spleens of mice lacking functional follicular dendritic cells. *Science* 288, 1257-9 (2000).
- Aguzzi, A. & Collinge, J. Post-exposure prophylaxis after accidental prion inoculation. *Lancet* 350, 1519-20 (1997).
- Klein, M. A., Kaeser, P. S., Schwarz, P., Weyd, H., Xenarios, I., Zinkernagel, R. M., Carroll, M. C., Verbeek, J. S., Botto, M., Walport, M. J., Molina, H., Kalinke, U., Acha-Orbea, H. & Aguzzi, A. Complement facilitates early prion pathogenesis. *Nat Med* 7, 488-92. (2001).
- Heppner, F. L., Musahl, C., Arrighi, I., Klein, M. A., Rulicke, T., Oesch, B., Zinkernagel, R. M., Kalinke, U. & Aguzzi, A. Prevention of Scrapie Pathogenesis by Transgenic Expression of Anti-Prion Protein Antibodies. *Science* 294, 178-182 (2001).

#### P008:

#### Ethical Issues of Genetic Manipulation of Lifestock

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I would like to answer the question of ethical general conditions for genetic manipulation of life in two steps:

 First it needs to be clarified why we have moral obligations towards live and how we can point them out;

(2) Then the question of necessary special regulations for genetic manipulation needs to be answered. These issues should be discussed concerning the positions of Biocentric and "Deep Ecology".

1) About the attempt of an Ethics of nature

Regarding the question of man's use of live in a morally acceptable way, various attitudes are possible: (1) Anthropocentric, (2) Pathocentric, and (3) Biocentric. From the ethical point of view it is important to know why we have the obligation of moral behaviour (for example consideration) towards living organisms. The classical Anthropocentric (I. Kant) considers cruel treatment of animals as an act against man's duties towards himself because through becoming insensible, morality will be weakened ore destroyed. For those who follow Pathocentric (P. Singer and M. St. Dawkins) in the tradition of Utilitarism, the ability of suffocation is the central criteria. Following Bentham's thesis they also include animals because of their ability of suffocation within the weighing of interests. But it is difficult to find out empirical criteria for animal suffocation for example the phenomenon of stress (measurement of heartbeat) ore the behaviour of an animal in painful situations. The scoop of interpretation is large. The members of Biocentric (A. Schweitzer, G. M. Teutsch, T. Regan) with their claim for an attitude of "respect for live" avoid this problems. Modern biocentrical positions rely on the consciousness of animals and their interests. Many philosophers of Biocentric consider the difference between the human and the animal consciousness as gradual and not essencial, a problematic premise. They demand an equality of man and animal, as extensive as possible. O. Höffe takes a mediate position with his though of a (regarding the organisation of the sense organs and the central nervous system) hierarchical solidarity between man and animal. Interpreted as a priority criteria this leads to the demand of equal treatment of man and animal in comparable situations. Based upon this urgency criteria concrete demands for the use of nature ore animals can be made (Irrgang 1997, 172-182).

Within the field of Philosophy especially since Descartes the animal-man-difference and since Darwin the animal-man-transition is being discussed. The question of man-animal-comparison (Teutsch 1985, 133-135) is of primary importance for the personal status but also difficult: Until today there has been no real success in the exact definition of difference between man and animal (Brockhaus 1975, 110). The consideration would be widely extended for those animals to which we attribute the personal status and the individual interest of survival. Peter Singer discusses such a personal status for chimpanzees, gorillas, dolphins and whales. Traditionally the animal-man-difference and the personal status is considered to be located in the rationality and morality of man. If personality is being connected to criteria like feelings of pleasure and reluctance ore consciousness ore individuality, it is allowed to ask the question weather there can be something like an animal-person.

In deed, some common ideas about animals and their consciousness will have to be corrected. Griffin could make this plausible in many cases (Griffin 1990, 28-30). But there is no reliable criteria for ascribing consciousness to animals (Griffin 1990, 68). Nevertheless it is possible to barn something about their thinking from their ability to learn, to adjust and their communication behaviour. Even though I do not consider a animal-person from the ethical point of view, ethically relevant criteria for the consideration of animals are pain, their repertoire of behaviour, especially their communication

behaviour and the possibility of consciousness. Therefore a close inspection is necessary. In this sense I do not know any position which claims that the acting of animals is morally responsible. Therefore it would have to be proofed that a least some animals do act morally responsible and give reasons for their objectives.

The most radical ethical duties towards nature and live are being claimed by the "deep ecology". Already in 1972 when the Norwegian analytical Philosopher Ame Naess introduced the expression "Deep Ecology", it was the central objective to make a revolutionary change in the anthropocentric orientation of western Ethics and Politics. The utopical character of many of the deep ecology objectives is obvious. The basic attitude of society should be overcome with the interpretation constructions "the nature" and especially the system as a whole like landscape, ecosystems and biological species all of which are nothing purely existing but constructions which include human perception, cultural interpretation and scientific conventions (Birnbacher 1997, 12). Deep Ecology does support many of the objectives of the Reformism but it is also revolutionary and seeks a new Metaphysics, Epistemology, Cosmology and Environmental Ethics for the man-earth-system (Dervall 1997, 17). Following the current paradigm, nature is only a supply of resources which has to be produced in order to meet the permanently growing needs of the permanently increasing number of people. Science and technology work hand in hand. Technology develops techniques to conduct natural processes. Changes become an end in itself. The new is higher regarded than the old, the currant generation higher than the future one i (Dervall 1997, 19f). Deep ecology puts it's main effort into finding and discussing alternatives to the conventional way of thinking of the modern West. That includes modern natural sciences and technology, especially genetic engineering and it's full technological handling of live. But did modern science bring such a big change into our relation to nature?

2) The claim for Biocentic and "Deep Ecology"

Because of the demoralisation of the definition of live in modern biology, modern biocentric positions ore the so called "deep ecology" rather follows the romantic – organic definition of live which is especially for Schopenhauer and in the philosophy of live connected to a (cultural-) pessimistic thinking. This background is also being established in the basic attitude of respect for every live as it has been formulated in the Ethics of Albert Schweitzer. But if one tries to live consequently following this attitude, huge problems come up. In it's close sense it is not possible to realise. Also, respect is a religious ore at least no specific moral attitude. One can have respect for virus but fight them with good reasons without guiltiness if they harm ore endanger man. Attitudes towards live which are similar to Biocentric can be found in Jainism (Religion of he Jaina, India), in Buddhism (Eastern Asia) and in a slightly different way in Hinduism (India) in the Ahimsa-Commandment (prohibition of harming any life).

Biocentric position demands protection of life by itself. Interventions in nature are actually prohibited and need to be justified. Günther Altner claims: "The possibility of a self-recreational evolution for nature has to be preserved because this is it's freedom" (Altner 1987, 216). Like in the definition of the animal-person, the living nature is being personalised in order to ascribe moral values and moral rights to it. The first claim of biocentric positions is the protection of species: "The self-value of nonhuman creatures shows itself in the unique developed nature, in it's natural interplay which again expresses itself in the typical community of species (biotopes) and in the relatively stable ecological balance" (Altner 1991, 217). A living nature is being imputed in which man does not exist. To give reasons for it's protection, nature is being personalised. But in the other hand, a more correct method would be the use of definition of species in the sense of ecological adjusting as an ethically relevant empirical criteria for the protection of species.

Further, biocentric positions ascribe consciousness to animals and often demand a general prohibition of killing animals. Tom Reagan regards the difference in consciousness of animal and man as only gradual and not essential. He ascribes a certain autonomy to animals because they can express preferences especially interests of wellbeing. The definition of interests is connected to the definitions: selfishness, use in general, survival, egoism, protection of benefits and avoiding of damages, though calculated benefits on the basis of reason and needs as action motivation can most probably only be

ascribed to man, natural efforts towards damage prevention and protection of ones own life can be found also with other organisms.

#### 3) Fairness towards every living being

The following critics against Biocentric and "Deep Ecology" have to be done: Although the fact of one origin of every living being, including man, gives a certain respects towards all forms of life although not all of them are fundamentally equal. The needs of man are more differentiated than the ones of animals. As well animals do not have the reflective consciousness which enables man to make experiences and act responsible, to make claims ore judgements (Frey 1980, 120). Moral acting cannot be ascribed to animals. From the ethical point of view the self-value of nature cannot be the same as the one of man (Irrgang 1997, 211f). This puts the personification of nature but not the use of biology in the sense of an ethically relevant empirical criteria into question. In the end, not the use of empirical results like certain proofs of live but the use of ethical principals like justice ore damage prevention gives reasons for claims like prohibition of direct harming of animals (animal prevention) and the protection of species (biodiversity).

That means that the principal of justice of life is fundamental for Bioethics of life. It applies to all living beings. Nobody seriously claims that animals can act morally responsible. At the most a morallike behaviour of animals is being described (Brockhaus 1975, 111). Although Bioethics does not justify the concept of a morally responsible acting animal-person it does develop a ethically relevant empirical criteria for the consideration of animals: Ability to feel pain, behaviour repertoire, communication behaviour and the possibility of consciousness.

In order to avoid the naturalistic fallacy from evolutionary justified "similarities" to moral obligations (Irrgang 1997, 163-182), a gradualism has been developed on the base of an ecological conception of justice. The gradual urgency criteria of consideration of the concerned acting persons (Irrgang 1997, 204-212), considers as commandment of fairness ore justice with growing grade of urgency the balance and cycles of nature which are fundamental for other general coherences of life, as well as issues of species protection not only for vertebrates, emotionality and ability to feel pain of organisms and the attempt of higher developed animals to lead a live without suffering, especially if we have a special responsibility for them as useful animals and that means not only to consider them as genus but as individuals and also the urgency criteria does ascribe a very high consideration to highly developed mammals like monkeys ore certain sea mammals (Irrgang 1997, 205).

This way a differentiated Ethics of protection of life on the basis of an ecological anthropocentric can be justified. Animals are to be protected in coherence to their communication ability and competence, pain and forms of consciousness. The unjustified harming of any parts of nature and especially highly developed animals does brake the ethical principal of justice. In order to measure weather we are harming animals, ethically relevant criteria are needed. Obligations towards animals ore towards nature cannot be shown apodictically but they need an argument of convergence which considers most of the mentioned criteria as possible. In general these are ethically relevant empirical criteria which help to assess in which cases the obligation of the protection of animals does ore does not apply.

## 4) Biodiversity - an absolutely obligatory criteria ?

Many people consider Biodiversity as diversity of life – in German mentioned as diversity of species – as an indicator which they suppose to know from direct [empirical] experience within a nature which is intact and meets the needs of humans. But it is unclear what diversity of life means and through which criteria and basis this diversity has to be seen. The problem already begins with the attempt to find a clear definition for the classical objects which are supposed to show the diversity of life, these are "species", "gene" and "ecosystem". The differences in the terminology are signs for divergences regarding the in biological sciences recognised research procedures and their corresponding research objects. All together, throughout the whole earth age, it turned out that the paleo-biodiversity has neither through background events nor through exogenous catastrophes been extinguished nor damaged with lasting effect. Although living beings have died out not only as individuals but also as families ore orders but this has always been an accompanying phenomena for the development of life

on earth in which the decreasing of diversity was always been followed by an increase. Even though some species, families ore orders have disappeared from earth forever, it did not lead to a long-term damage of the world of organisms in total.

The micro-biological diversity research showed that only a very small number of currently living micro-organisms have been isolated given a valid description. Regarding this it has to be mentioned that micro-biologists do not demand the protection of single species but the protection of locations for the taking of specimen. Procedures of molecular-biology put main emphasis on genetic information. This is especially suitable as a base for procedures of proving of biodiversity. Significant parts of the biodiversity debates like gene, species ore ecosystem are no purely natural ore scientific objects but are also determined by giving practical purposes (Janich et al. 2001, XXI till XXIX). The reasons for the widespread dying of species are shield tectonic, sea level and changing of magnetic fields as well as volcano and asteroids (Janich et al. 2001, 70-99). The value of species can be differentiated as a self-value and an instrumental value. Biodiversity does have a certain production value, although the problem of value assessment of biodiversity has to be regarded in relation to the problem of prevention of nature.

Biodiversity and prevention of species are ethical claims which can be justified, especially considering our non-knowledge about most of the biological species and considering the caution regulation, but it is no absolute commandment. Above all a prohibition of an inter-species gene transfer cannot be justified on this base. In order to answer this question it needs the reflection of the specification of genetic acting in comparison to all other technological acting. Special regulations are only obligatory, if the genetic praxis does fundamentally differ from all the other forms of technical praxis.

5) Genetic Engineering as Laboratory Praxis

The praxis of genetic engineering does basically not differ from other technological praxis. The structure of technological acting gives reasons for the unavoidable trying out. This trying out can never be fully calculated although certain possibilities can in advance be excluded as being practically irrelevant through calculation. Technological acting is no blind use of nature ore objects. It is no accidental process but it is lead by an organised ore at least a heuristic process of searching and finding. Useful animals and plants are no objects in the close sense but in order to deal with them, man first of all uses the natural processes of reproduction. Useful animals are no technological means, no tools ore machines in the average sense but they also are no classical objects. The expanded definition of technology as handling of both technological and natural processes can avoid a to close understanding of technology in the sense of tool and object. It constitutes a concept of technological acting both from the point of view of the participant and of the observer (about the concept of instrumental understanding see Irrgang 1998a, 75-120, about technological acting see Corona/Irrgang 1999, 166-212). The technological handling of a single and concrete technological problem takes place on the basis of a (implicit) technological regulation knowledge with the aid of means and / ore technological knowledge. This regulation knowledge depends on frameworks ore paradigms which often cannot be shown explicitly. But on the basis of a reflected handling knowledge they can be understood, put as subject and be criticised. I explained this more detailed in my book "Technische Kultur" (Irrgang 2001a).

In the praxis of breeding organisms of one and the same kind are being crossed with a combination of genetic marks without having an exact knowledge of the genetic information. The definition of species can be regarded as a human interpretation construction, which although it might give some kind of information about the biological reality it does give reasons for doubt.

The gene transfer changes one ore several genetic marks of an organism although it does normally not change the species which in theory would be possible. The differences between technical and technological praxis that is differences between breeding and genetic engineering are in general the inserted laboratory procedures. These lead to better information about details and causal paradigm of biological processes but not to information about general coherences. This is the difference between breeding praxis on farms and breeding research in labs, whereas the modern seed breeding companies are differing from university ore MPI- laboratories. The procedures of genetic engineering in the sense

of a technological praxis - a reflected strategy of attempts and errors, ethical evaluation included does correspond with a concept of technological acting which reduces technology to objects. Technological acting which opens new technological possibilities opens a new range of possibilities using them in praxis which often were regarded as technological imperatives (this is possible and is sometimes being done) but actually it is the opposite: only after trying out you know what is possible and you can decide which action options one wants ore wants not to take. Technology as technological acting can be seen under several aspects: objectives, technological means, consequences ore interventions in nature can also be relevant. Especially the last point is of interest. Technology is not to be regarded as practical experimental science. On one hand experimental science also needs to give things a trial but on the other hand a lot of things work out in theory but not in praxis. Asking for the possibilities of trying out ore making experiments - at the end there are no other ways to handle technological action than to give technologies a try in praxis. The meaning of technological experiences can never be reduced to the pure observer position. Possibilities of making mistake and of making innovation depend on each other. Organised and rational experiments also includes responsibility and not to give it a try no matter what happens. This also has consequences for ethical evaluation of genetic engineering. Nevertheless, every form of selection includes certain risks of failure. Risks in the releasing of trans-genetic organisms cannot be totally excluded. No form of technological acting is without risks. Although, they do not depend on the method of gene transfer but on the nature of the changed organism and ecosystems into which it is being released, which means they depend on the way of technological acting. A special Ethics for genetic engineering such as "Gene-ethics" is not necessary, but a differentiated ethical reflection within specified areas for a specialised form of technological practice is needed.

#### Literature:

Altner, Günther 1987: Die Nutzungsziele der Gentechnologie unter der Perspektive von Umwelt- und Sozialverträglichkeit; in: V. Braun, D. Mieth, K. Steigleder (ed.); Ethische und rechtliche Fragen der Gentechnologie und der Reproduktionsmedizin; Munich, 213-223

Altner, Günther 1992: Naturvergessenheit. Grundlagen einer umfassenden Bioethik; Darmstadt

Bayertz, Kurt 1989: Entmoralisierung des Lebendigen; in: Matthias Gatzemeier (ed.): Verantwortung in Wissenschaft und Technik; Mannheim, Vienna, Zurich, 220-238

Bimbacher, Dieter 1997: Ökophilosophie, Stuttgart

Brockhaus, Wilhelm 1975: (Ed.) Das Recht der Tiere in der Zivilisation. Eine Einführung in Naturwissenschaft, Philosophie und Einzelfragen des Vegetarismus, Munich

Corona, Nestor; Irrgang, Bernhard 1999: Technik als Geschick? Geschichtsphilosophie der Technik; Dettelbach

Dervall, Bill 1997: "Tiefenökologie" – Eine ökologische Ganzheitsphilosophie; in: D. Birnbacher, Ökophilosophie, Stuttgart, 17-59

Frey, R. G. 1980: Interests and Rights. The case Against Animals; Oxford

Griffin, Donald R. 1990: Wie Tiere denken. Ein Vorstoß ins Bewußtsein der Tiere; translation by Elisabeth M. Walther (1984); Munich

Irrgang, Bernhard 1991: - Transgene Tiermodelle im biomedizinischen Experiment. Forschungsethische Überlegungen; in: Forum für interdisziplinäre Forschung 4 (1991), issue 1, 54-66

Irrgang, Bernhard 1992: Ethische Aspekte der Biotechnik; in: H. Wilhelm Schaumann-Stiftung (ed.): Biologisch-technische Entwicklungen in der Tierproduktion (14. Hülsenberger Gespräche); Hamburg, 36-46

Irrgang, Bernhard 1996: Handbuch der angewandten Ethik; ed. by Julian Nida-Rümelin; article Gen-Ethik; Stuttgart 1996, 510-551

Irrgang, Bernhard 1997: Forschungsethik Gentechnik und neue Biotechnologie. Grundlegung unter besonderer Berücksichtigung von gentechnologischen Projekten an Pflanzen, Tieren und Mikroorganismen; Stuttgart

Irrgang, Bernhard 1998a: Praktische Ethik aus hermeneutischer Perspektive; Paderborn

Irrgang, Bernhard 1998b: Wozu können Klonierungsverfahren dienen: ethische Bewertungskriterien; in: Johannes S. Ach, Gerd Brudermüller, Christa Runtenberg (ed.): Hello Dolly? Über das Klonen; Frankfurt/M., 72-89

Irrgang, Bernhard 1998c: Lexikon der Bioethik; ed. by Wilhelm Korff, Lutwin Beck, Paul Mikat; Gütersloh 1998; art. Biozentrik (vol. I, 402-404); art, Pathozentrik (vol II. 2, 834-835); art. Physiozentrik (vol. III, 28-30); art. Theozentrik (vol. III, 526-528); art. Tierschutz (vol. III, 561-567)

Irrgang, Bernhard 1998d: Am Ende der Anthropozentrik? Wie lassen sich unsere Verpflichtungen gegenüber der Natur begründen; in. Gotthard Fuchs, Guido Knörzer (ed.): Tier, Gott, Mensch. Beschädigte Beziehungen; Frankfurt 1998, 13-32

Irrgang, Bernhard 1999a: Gentechnik in der Tierzüchtung; in: Thomas Hausmanninger, Scheule, Rupert (ed.): ... geklont am 8. Schöpfungstag. Gentechnologie im interdisziplinären Gespräch; Augsburg, 227-242

Irrgang, Bernhard 199b: - Nachhaltigkeit als Leitbild für Grüne Gentechnik? in: Dirk Harreus (ed.) Gentechnologie. Fakten und Meinungen zum Kernthema des 21. Jahrhunderts; Berlin 1999, 146, 195-210, 241f

Irrgang, Bernhard 2000: Ethische Betrachtungen der Anwendung bio- und gentechnologischer Verfahren in der Tierzucht; in: Deutsche Gesellschaft für Züchtungskunde e.V. (ed.) Bio- und Gentechnologie in der Rinderzucht – Risiko oder Chance? DGfZ-Schriftenreihe 17; Bonn, 56-66

Irrgang, Bernhard 2001a: Technische Kultur. Instrumentelles Verstehen und technisches Handeln; (Philosophie der Technik vol. 1); Paderborn

Irrgang, Bernhard 2001b: Gefangen in Sachzwängen? Zur ethischen Dimension der Gestaltbarkeit der Biotechnologie; in: St. Heiden et al. (ed.): Biotechnologie als interdisziplinäre Studie; Heidelberg / Berlin, 83-96

Irrgang, Bernhard 2002: Technische Praxis. Gestaltungsperspektiven technischer Entwicklung; (Philosophie der Technik vol. 2); Paderborn 2002

Irrgang, Bernhard et al. 2000: Gentechnik in der Pflanzenzucht. Eine interdisziplinäre Studie; Dettelbach

Janich, P. et al. 2000 (ed.): Biodiversität. Wissenschaftliche Grundlagen und gesetzliche Relevanz; Berlin et al.

Lenk, Hans; Maring, Matthias 2001: Advances and Problems in the Philosophie of Technology; Münster

Teutsch, Gotthard M. 1985: Lexikon der Umweltethik; Göttingen

Zimmerli, W. Ch. 1993: Die Bedeutung der empirischen Wissenschaften und der Technologie für die Ethik; in: A. Hertz, W. Korff, T. Rendtorff, H. Ringeling (ed.): Handbuch der christlichen Ethik; Freiburg, Basel, Vienna, Aktualisierte Neuausgabe vol. I, 297-316

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