An international genetic platform for the assessment of gene function in rice

Ronald L. Phillips[1], Hei Leung[2], and Ronald P. Cantrell[2]

[1]Department of Agronomy and Plant Genetics and Center for Microbial and Plant Genomics, University of Minnesota, St. Paul, MN 55108 Email: phill005@umn.edu
[2]International Rice Research Institute (IRRI), DAPO Box 7777, Metro Manila, Philippines
Email: h.leung@cgiar.org, r.cantrell@cgiar.org

Abstract
Rice is a food staple throughout much of the world, especially in less developed countries. Fortunately, rice has the lowest amount of DNA among the common cereals and has become a pivotal species for genomics investigations. With the complete DNA sequence virtually at hand, the time has come to have a consortium to develop an understanding of the functional genomics of this important species. With 50,000 or more genes predicted, the task ahead requires the development of many genetic resources to unravel the function and interaction of these genes and their relationship with important traits. The DNA sequence coupled with full-length cDNAs, insertional and chemical-/radiation-induced mutants, and gene arrays represent resources for examining functional genomics questions. An International Rice Functional Genomics Consortium has been established to share materials, integrate databases, elucidate gene function, and translate the information into benefits for society.

Media Summary
The application of science in agriculture is revolutionizing the production of food, changing the nutritional value of major crops for animals and humans, and allowing new methods for the development of pharmaceuticals, vaccines, and other products. Since the genetic improvement of crops has generally accounted for half of the increases in production over the last several decades, the new investigations in genomics will add to our genetic knowledge of crops and increase our ability to produce enough food for the ever-expanding population. The genetic code of rice is nearly elucidated. This paper summarizes the current status of genetic resources that will allow the understanding of the function of the estimated 50,000 genes of rice and reviews the development of a consortium to maximize progress.

Key Words
Genomics, *Oryza*, Consortium, DNA, Intellectual Properties

Introduction
Rice is the most important grain in the world. Three billion people in the developing world depend on it as the major component of their subsistence diet (Cantrell and Reeves, 2002). The nutritional value of rice is directly reflected in their quality of life. Rice has a rich history in terms of the production of genetic information. The entry of rice into the molecular genetics age was delayed until the Rockefeller Foundation astutely recognized that future improvements in the lives of a large fraction of humankind would depend, in part, on improving the genetic knowledge of rice. Knowing that 50% of the yield improvements of most major crops are due to genetic changes (Duvick 1984), the development of genetic technologies and information about rice is an important way to achieve better lives for people of the developing world. In addition, the recognition that rice had the lowest DNA content of the cereals coupled with other features led to rice becoming the second plant species—and the first crop species—to have its whole genome sequenced. Then, the finding that grass genomes were quite similar in terms of gene content and gene order made rice the focal species (Moore et al. 1995). Genes and DNA sequences of interest in many other species are compared to those in rice to gain further insight.
The ability to advance in functional genomics for rice depends on this rich history of genetic information. Figuring out the function of the 50,000 to 60,000 predicted genes in rice and translating that information into an understanding of the phenotype depend on many kinds of genetic resources. We are fortunate to have the benefit of years of careful research on the genetics of rice. The time has come to work together and form a consortium to generate the tools for developing the field of functional genomics for rice and making those tools and the derived information available to the public..

26 Sep – 1 Oct 2004, Brisbane, Australia. Published on CDROM. Web site www.cropscience.org.au

Definition and Other Considerations

Functional genomics includes essentially everything that can be learned beyond the basic structural sequence of the DNA. Determining the function of every gene in an organism is perhaps the most common definition of functional genomics today. However, genes do not function in singularity; genes interact within a pathway or between and among pathways. Epigenetic modification of the DNA is also related to the function of specific genes, either by affecting their expression or maintaining expression profiles once established. The assignment of function to individual genes is the next step, but all the possible complexities associated with networking of genes will be the subject of intense investigation as the field advances. The U.S. National Research Council Report on "The National Plant Genomics Initiative: Objectives for 2003-2008" defines functional genomics as "the analysis of genes, their resulting proteins, and the role played by the protein in an organism's biochemical processes." Clearly, functional genomics takes us beyond DNA to its RNA and protein products, and their interactions, and to the phenotype.

Recent DNA sequence information from a variety of species has revealed a surprisingly high degree of similarity. However, individual plant species do have their uniqueness. Only 50% of rice genes have homologous sequences in *Arabidopsis*, whereas 91% of maize genes hybridize to rice genes (Goff et al. 2002; Bennetzen 2002). This emerging information coupled with the availability of DNA sequence information for the two major subspecies of rice indicates that rice will play a major role in identifying genes in other crop species and in deciphering the function of those genes.

The ability to examine gene function depends on a robust set of genetic tools and technologies. Mutants of various kinds are essential for the genetic analysis of an organism. Knock-out mutants are especially useful for assigning function to particular DNA sequences and to relate that function to phenotype. Microarray or chip-based mapping tools will accelerate the localization of genes at unprecedented rates (Borevitz et al. 2003). Expression profiling under different genetic or environmental regimes will associate gene expression with phenotype. Techniques to find rare alleles in populations will facilitate the development of new germplasm products (Till et al. 2003).

High-throughput technologies will enable breeding applications in unprecedented ways. Small segments of every chromosome will likely be followed through multiple generations and selection will be performed based on knowledge of quantitative trait loci (QTLs), isolated genes, or known proteins. Large-sample-size experiments will be readily possible. High-density microarrays or chips will allow the characterization of gene expression in different generations, various genotypic backgrounds, and multiple environments. Characterization of genetic resources in advance of their use in crosses may allow better choices of which materials to introduce into a breeding program. Well-managed seed repositories are essential for keeping seed viable and having a large community of researchers use the available resources.

Bioinformatics is an essential component of a functional genomics program. Databases categorizing information on DNA sequence, cDNAs, proteins, genetic stocks, and many other important genomic parameters are available and expanding. Multiple software programs useful in genomic analysis also must be available, and these, too, are expanding constantly.

Coupled with all the above technologies are many more that need to be developed or improved for the full implementation of a functional genomics platform. These include enhanced transformation capabilities, methods to knock out complex traits, measurement of gene expression in specific organs and developmental stages, trait-diagnostic gene chips, microarray databases, cell-specific gene expression studies, spatial and temporal array protein data, *in situ* hybridization procedures, and gene-silencing methods.

Genetic background effects will be apparent as the functional genomics information is derived from most of the techniques. These effects can be minor to major, with phenotypes expressing from extremely high to extremely low (or absent) levels in various genetic backgrounds. Applications of functional genomics information will require a careful assessment of genetic background effects. Such consideration is essential for meeting the end goal of functional genomics, which is to produce adapted and nutritious rice.

Rice genomic resources
Parallel to the initiation of the International Rice Genome Sequencing Project in 1998,The International Rice Research Institute (IRRI) recognized the need to bring together diverse expertise to capitalize on the wealth of genome information for functional analysis. Informal meetings were held in 1999 and 2000 that led to the formation of an International Rice Functional Genomics Working Group (Fischer et al. 2000). The Working Group brought together research groups to discuss mutual interests and promoted collaboration in anticipation of the completion of the rice genome sequence. During this time, members of this research community developed useful materials to form a base for collaboration in functional genomics studies in rice (Table 1).

Forward and reverse genetics
Hirochika et al. (n.d.) summarizes the rice mutant resources produced by different laboratories around the world. At the National Institute of Agrobiological Sciences (NIAS) of Japan, about 50,000 lines are available for forward genetic analyses based on the retrotransposon *Tos17*, together with a database for *Tos 17* tagged sequences (Miyao et al. 2003). The website http://tos.nias.affrc.go.jp provides information on how to request seed of individual lines. In addition, there are more than 150,000 lines from *Ac/Ds*, enhancer traps, T-DNA, and activation tagging lines produced by laboratories in Korea, Australia, China, Taiwan, France, Singapore, Netherlands, and the United States.

Insertional mutants have been made with T-DNA (100,000 lines with about 1.5 to 2 insertion sites per line, Jeon et al. 2000), *Tos17* (50,000 lines with about 10 sites per line), and *Ac/Ds* (50,000 lines with at least one insert per line). An increasing number of activation-tagged mutations have also been produced that would be useful for studying gain-of-function mutations (Jeong et al. 2002). Together, about a million tagged sites may already be available in various lines of *japonica* rice. Despite these rapid advances, however, there is a deficiency in insertional mutants of *indica* rice because of an inherently low transformation efficiency.

At IRRI, the focus has been on *indica* rice and making the advanced generation materials available for screening a variety of agronomic traits. More than 40,000 chemical- (diepoxybutane and EMS) and radiation-induced (fast neutrons and gamma ray) mutants have been produced in the most popular rice variety, IR64. These are at the M_4 stage and seed is available upon request. Approximately 16,000 lines have been distributed worldwide for genetic and phenotypic screens. DNA pools for TILLING (Till et al 2003) and PCR deletion detection (Manosalva et al. 2003) have been prepared for more than 5,000 lines for reverse genetic screens.

Flanking sequence tags (FST) databases
Insertional mutagenesis allows the cloning and sequencing of regions flanking the insert as a means to identify the gene responsible for the mutation. *Ac/Ds*-based gene and enhancer trap systems are useful for this purpose. Databases of flanking sequences that reflect the disrupted gene have been developed by several laboratories and some of the FST databases are available to the public for searching for mutations in genes of interest. For example, of 260 *Ds* flanking sequences isolated by the CSIRO (Australia) Plant Industry Rice Functional Genomics project, 119 showed homology with known genes (www.pi.csiro.au/fgrttpub/home.htm). Figure 1 summarizes the current status of insertion mutants with FST. The FST databases of different laboratories provide a good measure of the progress toward saturation mutagenesis of the genome.

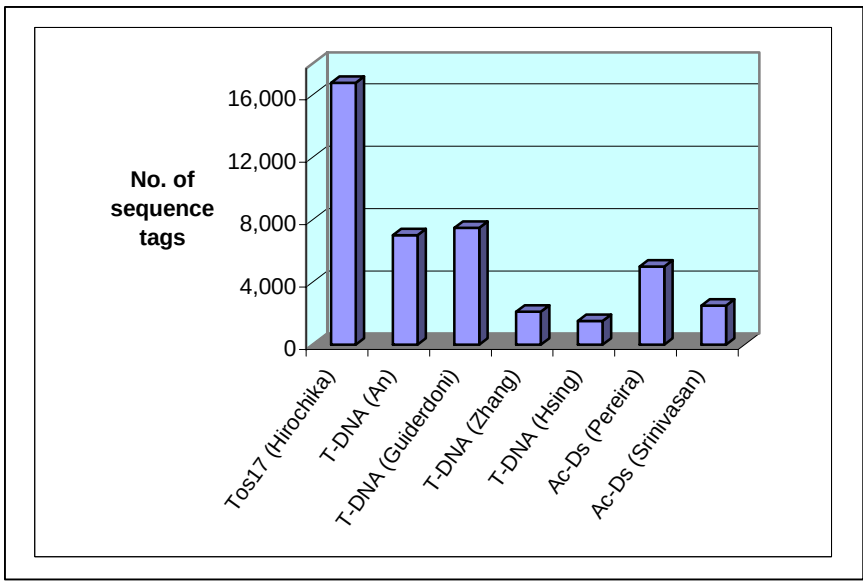

Figure 1. Flanking sequence tags using different insertional elements produced by different laboratories as of September 2003 (information derived from Hirochika et al. n.d.).

Arrays

NIAS produced the first-generation rice cDNA microarrays with >8,000 unigenes, and organized collaborative projects within Japan to develop a Rice Expression Database (RED) (Yazaki et al 2002). The first genome-wide oligo chip was produced by Syngenta using Affymetrix technology. The first-generation rice GeneChip contains 22,000 genes, with each gene represented by 16 ?25-oligomers. The oligo-design of the Affymetrix GeneChips in rice as well as in other species has proven to be a powerful tool for gene expression analysis as well as for the detection of genetic lesions in deletion mutants (Cheng et al. 2003; Borevitz et al. 2003). The new rice GeneChip has expanded to include 50,000 predicted genes. These chips are accessible for experimentation through a collaborative agreement with Syngenta (www.syngentabiotech.com/traitexpression.htm). Since then, additional rice gene chips in different formats have been produced through joint projects between the public and private sector.

NIAS collaborated with Agilent to produce an oligomeric rice chip corresponding to 22,000 transcriptional units based on the 28,000 full-length cDNA database (Kikuchi et al. 2003). Genome-wide oligomer chips have been produced by the Beijing Genomics Institute (BGI) and GreenGene BioTech Inc. (GGB, Dr. Baek Hie Nahm, Myongji University). In addition to these large-scale gene chips, microarrays of smaller collections of genes have also been produced for investigating specific traits. For example, IRRI has produced microarrays carrying 10,000 ESTs produced from rice panicles under drought stress. These subarrays can be useful for investigating specific traits or genetic materials. With the increasing accessibility of the high-density gene arrays, whole-genome transcript characterization of mutants and isogenic lines and segregating mapping populations could be commonplace in the next few years (Schadt et al. 2003).

Massively parallel signature sequencing (MPSS) technology

Although the rice genome sequence is practically complete, many computationally predicted genes are yet to be confirmed experimentally. A public project has been funded to apply MPSS technology (Brenner et al. 2000) to characterize the diversity and patterns of rice transcripts under different experimental conditions (Blake Meyer and Guoliang Wang, personal communication). The technology will reveal novel and rare transcripts and improve gene annotation of the rice genome. The data and analytical tools generated will be available at a public website to facilitate public use of the rice MPSS data and show the abundance and genomic locations of rice MPSS data, similar to the website developed for *Arabidopsis* (www.dbi.udel.edu/mpss).

Table 1. Rice genomic resources currently publicly available.

Resource	Description	Source or reference
Genome sequence	*Japonica* variety Nipponbare (with data from Monsanto and Syngenta integrated into the high-quality sequence)	IRGSP TIGR (www.tigr.org)
	Indica variety	Beijing Genomics Institute
Gene clone	22,000 full-length cDNA	Rice Genome Resource Center, Japan (www.rgrc.dna.affrc.go.jp/index.html.en)
Mutant	*Tos17* lines	Rice Genome Resource Center, Japan, or http://tos.nias.affrc.go.jp
	T-DNA lines	Pohang University of Science and Technology, Korea genean@postech.ac.kr
	IR64 chemical-irradiation-induced mutants	www.iris.irri.org/cgi-bin/mutantHome.pl
Gene array	22,000 rice oligo chip (based on 28,000 FL-cDNA dataset of NIAS)	? (not consistent with table heading) Commercially available from Agilent near end of 2003 or early 2004
	60,000 oligo chip based on EST and genomic sequences	? is this consistent with table heading GreenGene BioTech Inc., Korea www.ggbio.com
	60,000 oligo chip based on EST and genomic sequences	Beijing Genomics Institute
	1,100 defense-response genes microarray (rice and maize)	IRRI

Public functional genomics platform

Now that the sequencing of the rice genome is essentially complete—about five years ahead of schedule (Yu et al. 2002, Goff et al. 2002; Sasaki et al., 2002; Feng et al. 2002)—the development of a functional genomics platform seems timely to broaden access to the new science and to help nourish the poorest of the poor of the world (Cantrell and Reeves 2002). But this is a formidable task. Although rice has fewer repetitive sequences than other cereals, the observation that approximately 75% duplicate genes exist in each subspecies will present a challenge to functional genomics studies. On the other hand, knowledge of the sequence of two rice subspecies will provide powerful verification tools for assigning function to genes. Broad alliances with multiple institutions having different expertise will be needed.

To date, the accelerated completion of the rice genome has been an example of public investment and commitment leveraging contributions from the private sector. There is a general recognition that it is difficult to recover commercial benefits directly from rice. Continued public investment in functional genomics is therefore necessary if we are to reap the benefits of the investment in genome sequencing. Ironically, the completion of the rice sequence has occurred at a time when many rice-growing countries in Asia have reduced public funding for agricultural research. Fortunately, we also see a new commitment and renewal of interest in others, such as Korea, the U.S., India, and China, especially in the area of functional genomics (www.tigr.org/tdb/ezk1/osa1/link.shtml).

The idea of a rice functional genomics platform is built upon a commitment from several international institutions and national programs to pool their expertise and resources to accelerate gene discovery. This vision is shared by a diverse group of scientists and institutions with strong links to both basic research and crop improvement programs around the world. The targets of the group are the functional characterization of all rice genes through the use of mutants, allelic variation, expression profiling, over- and under-expression, integration of multiple data sources, and other methods.

Public- and private-sector goals are now similar. The application of genomics to rice still requires the development of a considerable amount of information. The private sector can contribute in significant ways, such as already demonstrated by making DNA sequence information available. Scientists in the private sector should be encouraged to participate as full members of the public consortium. Funding without encumbrances from the private sector can significantly advance the research. Product development will depend on these advances. The private sector can also influence policymakers in various countries to fund rice functional genomics research. The return to the private sector may be in the future but it could be quite significant, especially if hybrid rice creates the need for a fully functional seed industry.

International Rice Functional Genomics Consortium
After two years of discussion, the idea of creating a more structured consortium evolved at a Working Group meeting in Beijing in September 2002, and at the meeting "Towards building a global rice gene machine" organized by CSIRO in Canberra, Australia in November 2002. An interim steering committee for a consortium was formed at the Plant and Animal Genome Conference in San Diego in January 2003, leading to the formation of an International Rice Functional Genomics Consortium. Currently, the consortium is guided by a 21-member interim steering committee and coordinated by IRRI. Seventeen institutions from 10 countries and two Future Harvest Centers (www.iris.irri.org/IRFGC) participate in the steering committee. The collective goals are to "share materials, integrate databases, seek bilateral and multilateral partnerships, implement initiatives for the cooperative elucidation of gene function, and accelerate delivery of research results to benefit rice production."

While the IRFGC has as its model the Multinational Arabidopsis Consortium (www.*Arabidopsis*.org/info/2010_projects/MASC_info.html), it uniquely emphasizes the need to incorporate the practical aspects of plant improvement and at the same time make use of the expertise and experience from rice research and breeding institutions around the world. The consortium will initially focus on four areas: mutant stocks, gene arrays and expression profiling, bioinformatics, and functional verification. Subcommittees have been formed to develop a set of activities in each of these areas.

Although IRFGC is still in its infancy, there are positive signs that it will serve as an important link to genomic science for developing countries. Some national granting agencies have considered IRFGC as a "receptacle" for public goods generated by public research grants. For example, the USDA National Research Initiative Program encourages grant applicants to link their proposals with IRFGC. India has initiated a national consortium on rice functional genomics with interest in linking with IRFGC. We envision that the interaction of research projects supported by national and international programs will bring high quality outputs and synergy to the consortium.

To advance the agenda of the consortium, four subcommittees have been formed to coordinate activities related to mutants, gene chips, expression analysis, and functional verification.(repetition of para 2 above) Specifically, the consortium considers the following goals: 1) make genome-wide oligo chips publicly available by 2004-05, 2) tag 90% of the rice genes using a variety of mutant collections by 2005, 3) establish a global Internet network of rice functional genomics databases by the end of 2005, 4) develop and make available by the end of 2007 robust high-throughput gene functional verification systems, and 5) characterize the function of 50% of the rice genes by 2010.

These are ambitious goals, yet they are not without precedent. These goals are similar to those of the 2010 *Arabidopsis* Project (www.arabidopsis.org/workshop1.jsp and www.nsf.gov/od/lpa/news/media/2000/fsarabidopsis.htm). Given the rapid accumulation of rice genomics resources and the growing interest of researchers in rice, the goal of knowing the basic function of 50% of the rice genes by 2010 is not beyond our reach if we can harness the collective efforts of diverse groups (Table 1). Recent developments to build and expand rice genomics resources for gene discovery support this optimism.

In Japan, a Rice Genomics Resource Center was launched (www.rgrc.dna.affrc.go.jp/index.html.en) in April 2003, with the objective of providing access to a large amount of genomics resources produced over the many years of investment by the Japan Rice Genome Program. The main resources include the *Tos17* insertion lines, full-length cDNA library, and genetic mapping stocks. Similarly, the U.S. National Science Foundation (NSF) is interested in supporting a U.S. rice resource and stock center to facilitate the maintenance and distribution of valuable genetic resources to sustain gene discovery efforts in rice (Buell and Sundaresan 2003; www.gramene.org/; www.ars-grin.gov/ars/PacWest/Aberdeen/interestimage.htm). A consultation report on the Workshop on Establishing a U.S. Rice Resource Center listed an impressive array of potential materials that can be consolidated and distributed for public use. These include a large rice cDNA collection, 25 BAC libraries, 2,000 RFLP markers, 6,500 YAC-anchored EST markers, more than 1,000 characterized mutants, 400 rice activation-tagged starter lines, 200 kinase-knockout mutants, and deletion lines of various genotypes. Efforts are also being made to facilitate access to the large collection of mutants from the international community.

Intellectual property and access

Intellectual property (IP) issues will no doubt arise. Some researchers are willing to provide materials without restrictions as long as proper credit is given upon publication and in public presentations. Others will probably require material transfer agreements to satisfy institutional requirements for IP management, but we would hope that these would not prohibit or slow down the rapid exchange of information and materials. The Future Harvest Centers provide products of their research to national programs around the world as international public goods. Reach-through IP issues can severely hamper the introduction of new products to the developing world and we hope that this consortium will minimize these effects. Considering the functional genomics activities as basic research and not encumbering the information with IP will help to advance collaborative efforts.

An International Rice Functional Genomics Consortium is needed that enhances international collaboration on saturation mutagenesis of the whole rice genome, evaluation of genome-wide rice chips, the development of specialized diagnostic gene chips for important traits, ectopic gene expression of full-length cDNA for rapid gene functional analysis, reverse genetics for targeted genes, proteomics, database development, appropriate IP procedures, and training in genomics technologies and applications. We believe that the consortium would make all information accessible to the public.

Role of developing countries

Two key aspects of large-scale gene discovery often understated are the natural genetic variation in rice germplasm and the capacity to evaluate biological variation in complex traits (Leung and An, 2004). Developing countries are well endowed with genetic resources and scientists of the national agricultural research and extension systems (NARES) have years of experience in evaluating agronomic traits. Scientists in developing countries have much to offer and should be active participants in building the public research platform. Unlike traits in *Arabidopsis*, those in rice need to be evaluated in agronomic settings or under field conditions. The functional assignment of genes, particularly natural alleles in germplasm, will require a combination of molecular characterization of germplasm as well as high-quality phenotypic data generated in proper experimental or environmental contexts. Thus, engaging developing countries in gene discovery will bring together a full range of expertise in basic and applied research. This will ensure a fair and equitable distribution of products from advanced science to serve the needs of less-developed nations.

Conclusions

The most important grain for the world's developing countries has fortunately become the model crop for genomic analysis. The genome sequencing of this crop is nearly complete, which is true for both important subspecies (*japonica* and *indica*). This gigantic achievement is perhaps dwarfed by the magnitude of what remains to be accomplished to apply this valuable information to the benefit of poor people. Understanding the function of genes determining different phenotypes must now be a high priority to increase the adaptability and nutritive value of the crop. A public platform for rice functional genomics will greatly enhance the opportunity for modern molecular biology to contribute to poverty alleviation.

References:

Bennetzen J. (2002) Opening the door to comparative plant biology. Science 296: 60-63.

Borevitz JO, Liang D, Plouffe D, Chang H-S, Zhu T, Weigel D, Berry CC, Winzeler E, and Chory J (2003). Large-scale identification of single-feature polymorphisms in complex genomes. Genome Res. 13:513-523.

Brenner S, Williams SR, Vermaas EH, Storck T, Moon K, McCollum K, Mao JI, Luo S, Kirchner JJ, Eletr S, DuBridge RG, Burcham T, Albrecht G. (2000). ***In vitro*** **cloning of complex mixtures of DNA on microbeads: physical separation of differentially expressed cDNAs** Proc. Natl. Acad. Sci. USA 97:1665-1670.

Buell R, Sundaresan V (2003) A workshop on establishing a rice resource center in the United States. Plant Physiol. 132:1137.

Cantrell RP and Reeves TG (2002). The cereal of the world's poor takes center stage. Science 296: 53.

Chang HS, Wu C, Zeng L, Dunn M, Wang GL, Leung H, Goff S, Wang X, Zhu T and Leach JE (2003). Detection of deleted genes in rice mutants using the Rice GeneChip genome array. Page 100 in Abstracts of Plant and Animal Genomes XI. January 11-15, 2003, San Diego, California.

Duvick DN (1984). Genetic contributions to yield gains of U.S. hybrid maize, 1930 to 1980. In: Genetic contributions to yield gains of five major crop plants. (Ed. W.R. Fehr) pp. 15-47. Madison, Wisconsin, Crop Science Society of America, Special Publication No. 7.

Feng Q, Zhang Y, Hao P, et al. (2002). Sequence and analysis of rice chromosome 4. Nature 420:16-320

Fischer KS, Barton J, Khush GS, Leung H and Cantrell R. (2000). Collaboration in rice. Science 290:279-280.

*Goff SA, Ricke D, Tien-Hung L et al (2002). A draft sequence of the rice genome (*Oryza sativa *L. ssp. japonica). Science 296:92-100.*

Hirochika H, Guiderdoni E, An G, Hsing Y, Eun MY, Han CD, Upadhyaya N, Ramachandran S, Zhang Q, Pereira A, Sundaresan V, and Leung H. (n.d.) Rice mutant resources for gene discovery. Plant Physiol. (submitted).

Jeon JS, Lee S, Jung KH, Jun SH, Jeong DH, Lee J, Kim C, Jang S, Yang K, Nam J et al. (2000) T-DNA insertional mutagenesis for functional genomics in rice. Plant J. 22: 61-570.

Jeong DH, An S, Kang HG., Moon S, Han J, Park S, Lee HS, An K, An G. (2002). T-DNA insertional mutagenesis for activation tagging in rice. Plant Physiol. 130:1636-1644.

Kikuchi S, Satoh K, Nagata T, et al. (2003). Collection, mapping, and annotation of over 28,000 cDNA clones from *japonica* rice. Science 30: 376-379.

Leung H and An G (2004). Rice functional genomics: large-scale gene discovery and applications for crop improvement. Adv. Agron. 82:55-111.

Manosalva P, Ryba-White M, Wu C, Lei C, Baraoidan M, Leung H, Leach J (2003). A PCR-based screening strategy for detecting deletions in defense response genes in rice. Phytopathology 93:S57.

Miyao A, Tanaka K, Murata K, Sawaki H, Takeda S, Abe K, Shinozuka Y, Onosato K, Hirochika H (2003) Target site specificity of the *Tos17* retrotransposon shows a preference for insertion within genes and against insertion in retrotransposon-rich regions of the genome. Plant Cell 15:1771-1780.

Moore G, Devos KM, Wang Z and Gale MD (1995). Grasses, line up and form a circle. Curr. Biol. 5:737-739.

Till BJ, Reynolds SH, Greene EA, Codomo CA, Enns LC, Johnson JE, Burtner AR, Young K, Taylor NE, Henikoff JG, Comai L and Henikoff S (2003). Large-scale discovery of induced point-mutations with high-throughput TILLING. Genome Res. 13:524-530.

Sasaki T, Matsumoto T, Yamamoto K, Sakata K, Baba T, Katayose Y, Wu J, Niimura Y, Cheng Z, Nagamura Y et al. (2002) The genome sequence and structure of rice chromosome 1. Nature 420:312-316.

Schadt EC, Monks SA, Drake TA, Lusis AJ, Che N, Colinayo V, Ruff TG, Milligan SB, Lamb JR, Cavet G, Linsley PS, Mao M, Stoughton RB and Friend SH (2003) Genetics of gene expression surveyed in maize, mouse and man. Nature 422:297-302.

Yazaki J, Kishimoto W, Isikawa M, Endo D, Kojima K, MicroArray Center and Kikuchi S (2002). The Rice Expression Database (RED): gateway to rice functional genomics. Trends Plant Sci. 7:563-564.

Yu J, Hu S, Wang J et al (2002). A draft sequence of the rice genome *(Oryza sativa* L. ssp. *indica*). Science 296:79-92.