RICE BIOTECHNOLOGY QUARTERLY

VOLUME 1, NUMBER 1 JANUARY 1990

CONTENTS IN BRIEF

...IN THE NEWS...

Dr. Thomas Cech of the University of Colorado and Dr. Sidney Altman of Yale Univeraity received the Nobel Prize in Chemistry for independently discovering that RNA can act as highly specific enzymes, shattering the dogma that all enzymes must be proteins. Such catalytic RNA molecules or ribozymes have the potential of many practical applications in the fields of diagnostics, therapeutics and agriculture.

Dr. Lesley Sitch, presently at IRRI in the Philippines working on wide hybridizations, will join the staff of the Rockefeller Foundation in New York this coming summer.

New studies from the U.S. Department of Agriculture have found that rice bran could be more effective in reducing blood cholesterol levels than oat bran. The USDA noted that a diet which included 10% rice bran lowered the cholesterol level by more than 15% in laboratory animals. It also tastes sweeter and is less expensive than oat bran. Results on human testing should be available within the year.

The 2nd International Workshop on Molecular Biology of Rice will be held on January 8-9, 1990 in Japan. Topics to be covered include: gene organization, organelles, stress response, databases, transgenics, gene structure and expression, and enzymes. See page 16 for more information.

RICE BIOTECHNOLOGY QUARTERLY

Volume 1, Numer 1

January 1990

The *Rice Biotechnology Quarterly* begins its first year of publication with this issue. The idea for its conception arose from a suggestion at a workshop sponsored by the Rockefeller Foundation. The participants felt there was a real and vital need for a vehicle to speedily report on research of a biotechnological nature, that is, research based upon the tools and techniques of molecular biology applied to solving the problems associated with improving the rice plant.

The *Rice Biotechnology Quarterly* will furnish information designed to help rice scientists stay informed of each other's work, and also work done in related fields that may be applied to the study of rice. Some of the topics that may be included in each issue are: gene expression, RFLP mapping, protoplasts, transformation, cloning, *in situ* hybridization, stress, new techniques, viral diseases, somaclonal variation, molecular-cytogenetic studies, gene sequencing, chloroplast and mitochondria research, tissue culture and recent advances in instrumentation and methodology.

The *Rice Biotechnology Quarterly* accepts short manuscripts of recent or on-going research for consideration of publication. The submission should not exceed two typewritten, double-spaced pages. No more than one figure may be enclosed. A photo of the author(s) may also be included. All manuscripts are reviewed and accepted on the basis of scientific merit and suitability for the journal. Submit all manuscripts, subscription requests, and other correspondence to the Editor.

The *Rice Biotechnology Quarterly* is sponsored by the Rockefeller Foundation and is available free of charge to individuals, laboratories, and institutions involved in improving rice through biotechnological means. The liaison officers are Robert W. Herdt, Director of Agricultural Sciences, and Gary H. Toenniessen, Associate Director of Agricultural Sciences.

Editor
John E. Dille'
Winthrop-Biology
Rock Hill, SC 29733
Telephone: (803) 323-2111
FAX: (803) 328-2855

TABLE OF CONTENTS

Published by: *Winthrop University Press*

Dr. Ray Wu

Chloroplast b6 Gene Nucleotide Sequence

The nucleotide sequence of the rice chloroplast apocytochrome b6 (petB) gene has been determined by Cote *et al.* (1) using the dideoxynucleotide chain termination method.

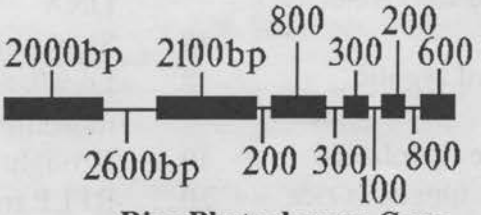
Chloroplast DNA from cv. Labelle was digested with HindIII, run on agarose, and transferred to Nytran. The probe consisted of a radioactive fragment (309 bp) containing a section of the spinach chloroplast apocytochrome b6 gene. It hybridized to a single 2.5 kb DNA band which was electroeluted and ligated to HindIII-digested pBR322 and transformed into HB101 competent cells. After colony hybridization, one clone was sequenced and found to contain an 813 bp class II-like intron between nucleotides 73 and 885.

```
GTGTGACTTG TTAGAATTTG CTCCTATTGA TAATACATAG
AAAGCACCTG TTATCTCTAT CAAGATGATT CTAATTCGTC
GGATATTATT TATTCTAGTA TCTGGAACAC GAAATAGATA
GAGTGGATCA AGAAAAAAAA ATGAAACTAT GATTCATATT
AACTATTCAG ACCTCGCAAC CAGACTGAAA AAAATTCAAG
TAGTTCTTAA TAAAAATAAA AAAAGAAAAT TTCTTCCTTC
CAATTTTGTT TGCCCAAAAA ACAACTTTTT TTCTCTCGAT
CATTACACCG ATTCAATAAA TGATCATCAA GCGGTTCTTA
TTCGAAGAAC CCTTGCCTTT TGTTTAGCTT GAGACTCAAT
CATCGTGGCT CTAGTATGAA TCTAAGGTTT TAATTGAACT
GATTCATAGG ATCGCAACAA GATAATTTCT ACCAGAAAAC
TACTCCAATT TTTG..... GGTTAGTTAC CTATCTCAAT
```

This rice intron shares 86% homology with maize and 63% with tobacco. The coding region shares 98% and 99% homology at the predicted amino acid level with maize and tobacco respectively.

Sequences of Rice Genes for Light Harvesting Chlorophyll a/b Binding Proteins

Luan and Bogorad (2) of Harvard University have determined the DNA sequences of two of the four genes that code for the light harvesting chlorophyll a/b binding protein (LHCP). The coding regions (about 1000 bp) of both these genes were very similar to each other and to the LHCP genes of wheat and pea. However, the flanking sequences and the transit sequences were very different.



Rice Phytochrome Gene Sequence

Kay *et al.* (3) have presented the entire sequence of *phy*18. This is a genomic subclone of a rice phytochrome gene consisting of almost 10,000 bp.

They found 5 intron sequences, the longest consisted of nearly 2500 bp and the shortest close to 200 bp.

Nucleotide Sequence and Expression of the Quinone-Binding Protein Gene

The gene (psbA) for the quinone-binding protein is encoded as a single copy in the chloroplast DNA of higher plants. The mRNA for this protein has been found to be as abundant as the mRNA of the larger subunit of RuBisCo. Kanno and Hirai (4) have determined the nucleotide sequence of this gene and located the initiation site for transcription.

The protein coding regions of psbA from rice, spinach and tobacco showed almost 92% homology in terms of nucleotide sequence. In terms of amino acid sequences, the percent homology was even higher at nearly 99% with the same plants.

> ...no evidence to indicate whether this inverted sequence acts as a terminator...

By using primer extension analysis, Kanno and Hirai were able to determine the location of the 5' end of the mRNA transcribed from the psbA gene. It was mapped 77 bp upstream from the ATG codon. This is identical to the site of this gene in tobacco but 3 bp behind the site in spinach.

Analysis of mtDNA of Different Subspecies of Rice

The rice genotypes used by Chowdhury *et al.* (5) were Calrose 76 (C76) and PI353705 (A5). C76 is a *japonica* subspecies while A5 is an *indica* subspecies and had been maintained in cell suspension for 7 years.

MtDNA was isolated by

grinding etiolated 10-day-old seedlings in a blender with a saline extraction buffer. The mitochondria were isolated by differential centrifugation and treated with DNase to remove extraneous DNA. The mtDNA was purified by three precipitations.

MtDNA was also prepared from cell suspensions by grinding in liquid N_2, differential centrifugation and, after lysis, purified by using a CsCl-bisbenzamide gradient.

Chloroplast DNAs were prepared from light-grown 12-day-old seedlings by grinding in liquid N_2 and centrifugation through a sucrose step gradient. After lysis, the DNA was purified by using phenol, phenol-chloroform, and chloroform extraction.

Restriction endonuclease digestions were made by using *Bam*HI, *Hin*dIII, *Pst*I, *Xho*lI, *Ava*I, *Bgl*I, *Eco*RI, *Sal*I, *Sac*I, *Pvu*I, and *Xba*I. Various maize gene clones were used in the preparation of the probes.

Comparison of the restriction endonuclease fragment patterns showed variation in the ctDNA of these two subspecies with only two (*Ava*I and *Bgl*I) of the 11 endonucleases. The mtDNA showed much more variation when restricted by 4 of the 11 enzymes. That is, the chloroplast DNA appeared more highly conserved than the mitochondrial DNA.

The differences observed in restriction and hybridization patterns between A5 and BL2 demonstrate that mtDNA changes have taken place during cell culture.

Hybridization data indicated that all the gene clones except two were able to distinguish these subspecies and may be useful for genetic analysis, screening cybrids, and monitoring protoplast fusion and plant regeneration.

Genome-Specific Repetitive Sequences

The genomes of most higher plants and animals contains large amount of repetitive DNA. Recent studies have shown that repetitive sequences change rapidly during evolution and this makes them useful in studying genome evolution at the molecular level. The rice genome contains about 50% repetitive DNA in six genome types: AA, BB, CC, DD, EE, and FF. Zhao *et al.* (6) reported the isolation and characterization of four genome-specific repetitive sequences from *O. australiensis, O. brachyantha, O. officianalis,* and *O. sativa*.

Thirty seven different rice entries were grown in pots in greenhouses with 14 h light at 26 C. At two months, leaves were harvested, chopped, frozen in liquid N_2, powdered, and ground in homogenization buffer. After incubation at 65 C for 1 h, an equal volume of phenol (buffered at pH 8.0) was added, mixed by inversion, and spun at 3,000xg for 10 min. The DNA was precipitated by a double volume of ethanol and again spun at 12,000xg for 10 min at 4 C. The resulting pellet was dissolved in TE buffer and digested with RNase A at 37 C for 1 h. The DNA was again extracted twice with buffered phenol, once with phenol:chloroform, and then again with chloroform. Finally, the DNA was precipitated with a double volume of ethanol, spooled with a glass hook, resuspended in TE buffer, and stored at 4 C.

To clone the repetitive DNA, the total rice DNA (above) was digested with *Eco*RV or *Hin*cII, fractionated on agarose, and stained with ethidium bromide. A 500 bp band was eluted from the gel and cloned into the *Hin*cII site of pUC13 by blunt-end ligation. This ligation mixture was used to transform *E. coli* JM101 cells. Four plasmids were obtained: pOa4, pOo2, pOb1, and pOs48. The first three were from wild rice species, the last from variety Labelle.

Slot blot hybridization was used to quantify the copy numbers of repetitive DNA sequences in the different rice genomes. For nick translation and genomic blot hybridization, repetitive sequence fragments were separated from recombinant plasmids after restriction endonuclease digestion and then fractionated by gel electrophoresis.

...Transformation of rice using cloned sequences is a promising alternative for introducing useful genes...

These researchers showed that all rice species containing the AA genome had the same repetitive sequence pOs48. This confirmed the close relationship between *O. sativa, O. glaberrima, O. meridionalis, O. rufipogon, O. glumaepatula, O. nivara,* and *O. longistaminata.* However, the other genomes showed no hybridization to the AA genome-specific repetitive sequence. This indicated that there is a clear molecular distinction between genomes AA

and BB, CC, DD, EE, and FF. In addition, differences in copy number, ranging from 2,000 for pOs48 in *O. sativa* to 184,000 for pOb1 in *O. brachyantha* were observed.

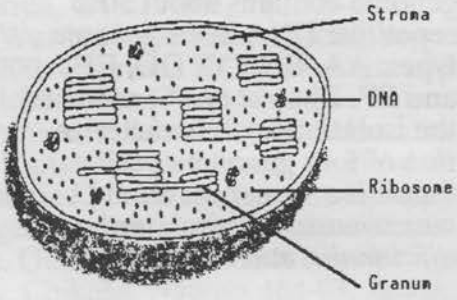
The Complete Chloroplast Genome Sequence

Chloroplasts and mitochondria both contain genomes separate from the nucleus of the cell. The chloroplast genome is composed of a single circular DNA molecule which codes for all the rRNA and tRNA and most of the mRNA needed for photosynthesis. This DNA molecule is about 120-160 kbp in length and appears divided into one large single copy, one small single copy, and two inverted repeats. In order to study the evolution of this genome, determine the coding potential, and compare the sequences of chloroplast DNA with the sequences of tobacco, liverwort, and other plants, Hiratsuka *et al.* (7) have sequenced the entire chloroplast genome of rice.

DNA sequencing was accomplished by dideoxy chain termination using a Klenow fragment or a modified T7 DNA polymerase. The cv. Nipponbare was used to produce a clone bank of overlapping fragments. Computer-assisted analysis treated introduced gaps as mismatches. This made gap penalties proportional to gap size. The percentage of amino acid residue identity was calculated for proteins appearing homologous.

Thirty tRNA genes and four rRNA genes were found identical in location and anticodon to those previously identified in tobacco. Of the 20 introns found in tobacco and liverwort chloroplast genomes, 17 were also present in rice and most of the ORFs (open reading frames) conserved in these two plants were also found in rice.

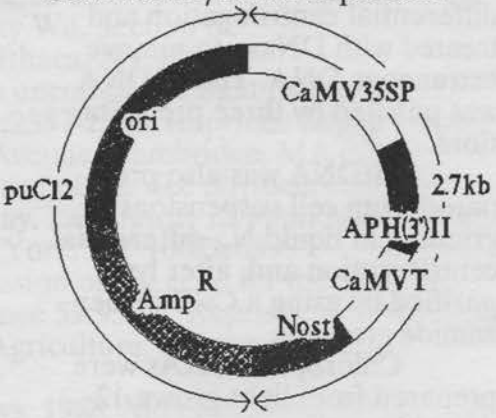
However, some rearrangements in the rice chloroplast genome was evident. The inverted sequences had expanded outward, the large single copy region had undergone a series of inversions, and a chimeric tRNA pseudogene had overlapped the endpoint of the largest inversion.



Transgenic Rice After Direct Gene Transfer

The research team of Toriyama *et al.* (8) have regenerated whole rice plants from protoplasts that had been electroporated with a plasmid gene coding for aminoglycoside phosphotransferase II (APH(3')II).

Fragments produced by *Bam*HI and *Bgl*II on pH1K1 which contained APH(3')II and CaMVT were inserted into the promoter terminator cassette of CaMV35SP/NosT of pUC12.



Protoplasts were isolated from anther-derived cell suspensions of cv. Yamahoushi, electroporated, and transferred to B5 medium supplemented with 2,4-D and mannitol. After two weeks, this medium was supplemented with B5 medium without ammonium sulfate but with G418 sulfate. One month later, microcalli were transferred to another modified B5 medium. Finally, two weeks later, growing calli were transferred to N6 medium containing IAA, kinetin, and 1% agarose to stimulate regeneration.

DNA was digested with *Hin*dIII and *Eco*RI, electrophoresed, blotted onto nitrocellulose, and hybridized with the DNA probe.

Five rice plants were regenerated which were not

To subscribe to the Genetics Manipulation In Plants Newsletter, write Professor Shao Qiquan, Managing Editor, Institute of Genetics, Academia Sinica, Bejing, China. Two issues per year at $12.00 (US) per subscription. This journal provides a forum where Chinese scientists may present their work in English.

albino and which did not bleach in the presence of 50 ug/ml kanamycin sulfate. APH(3')II activity was detected in leaf extracts. Similar results have been shown in maize protoplasts.

Dr. Tom Hodges

Genetic Analysis of Rice Regeneration

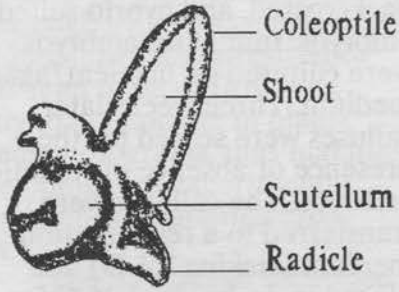
Peng and Hodges (9) used reciprocal crosses of four gentoypes (IR8, IR36, IR54, and IR64) in a diallel study in an attempt to understand the genetic mechanism of regeneration from immature embryo-calli.

Plants were grown in the greenhouse for 40 days and transferred to a short-day growth chamber to stimulate initiation of flowering. Crosses were made in all possible combinations of the four parents. At 7-10 days post-anthesis, immature seeds were collected from the parental varieties and at 6-9 days after pollination for the F1 hybrids. Immature seeds were sterilized, the embryos excised and plated in callus induction medium. After three weeks in darkness at 26 C, the calli were transferred to light and a regeneration medium similar to the induction medium but omitting the 2,4-D and with only 0.2 mg/liter 6BA.

The frequency of callus induction of the four parents varied from 90% in IR8, to 100% in IR54 and IR64. However, the regeneration frequency of the four parents was 0% (IR36), 46% (IR8), 47% (IR64), and 86% (IR54). The hybrids that showed the highest mean plant regeneration frequency were IR54/IR36 and its reciprocal.

The highly significant variances of the general combining ability and specific combining ability indicated that the nuclear genes involved in plant regeneration exhibit both dominant and additive effects. A similar situation has been noted on wheat regenerated from anther derived calli.

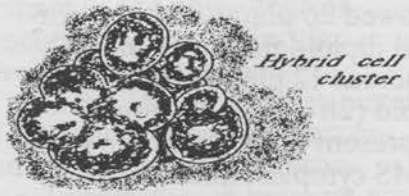
These results suggest that the genetic background of elite varieties with poor regeneration response could be incorporated into genotypes with high regeneration abilities. These researchers recommend that genetic effects and environmental conditions both be taken into account to improve and increase plant regeneration.



Typical somatic embryo

High-Frequency Embryogenesis in Rice

Long term suspension cultures (older than 18 months) were established from cv. Konansou by Ozawa and Lomanine (10). Subculturing occurred every three days with N6 medium supplemented with 2,4-D, sucrose, casein hydrolysate and proline. One millimeter cell clusters were transferred to solid N6 medium with kinetin, NAA, sucrose, and Gelrite. The frequency of embryogenesis was estimated at 90%. Smaller clusters produced lower rates (50%) of embryogenesis from long-term cell suspensions of rice.



Asymmetric Protoplast Fusion Produces Rice Cybrids

Cytoplasmic male sterility in rice may be determined by the mitochondrial genome as it is in other plants. The transfer of maternally inherited traits (including the mitochondrial genome) by protoplast fusion in monocots has been reported only once, between rice and barnyard grass. By using X-irradiation (to inactivate the nucleus) and iodoacetamide (IOA) (to inactivate the cytoplasm) Akagi *et al.* (11) were able to construct cybrid plants between cytoplasmic male sterile and fertile rice.

The cytoplasmic male sterile rice strain used in this work was MTC-9A. A mutant line, Norin 8, which lacks the aryl acylamidase I gene (which breaks down propanil, a herbicide) was used as a recessive marker.

Protoplasts were isolated,

treated with either X-irradiation or IOA, and placed in a fusion chamber for electrofusion. The cell clusters were cultured for 3 weeks and transferred to callus growth medium. Upon reaching 5 mm in diameter, they were transferred to plant regeneration medium and eventually grown in a greenhouse.

MtDNA from regenerated plants exhibited *Pst*I and *Bgl*I restriction patterns with fragments specific to both parents. Cytogenetic analysis showed 26 plants with the normal diploid number (2n=24) whereas 12 plants were tetraploid (2n=48). These may represent fusion between one CMS cytoplast and two N8 protoplasts. Only two of 560 regenerated plants set seed at levels similar to the N8 controls. About 80% were completely sterile.

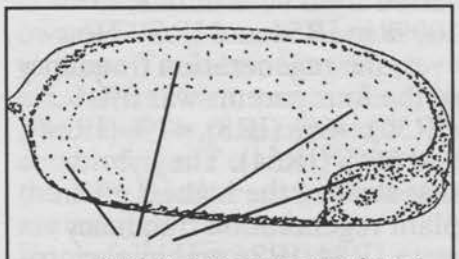
Such cybrids may provide crucial information that would allow analysis of mtDNA from recombinants and provide an understanding of the causes of male sterility in rice.

Fertile Rice from Transformed Protoplasts

Shimamoto *et al.* (12) have reported in Nature that they have produced fertile transgenic rice plants by transferring both the *hph* gene (bacterial gene encoding hygromycin B resistance) and the beta-glucoronidase (GUS) gene to protoplasts by electroporation.

Southern blot analysis and Southern hybridization confirmed the presence of the *hph* gene in the chromosomes of the stable transformants. GUS activity was also demonstrated in the seed and leaf extracts.

This work showed that it



GUS activity in seed co-transformed with pGL2.

was possible to co-transform using both a selectable marker and a non-selectable gene. Such a technique appears to be a simple alternative method for generating transgenic plants.

Inheritance of Somatic Embryogenesis and Plantlet Regeneration in Maize

Willman *et al.* (13) have investigated several of the genetic factors involved in somatic embryogenesis and plantlet regeneration using three inbreds of maize: A188, Mol7, and B73. Plants were selfed or crossed to produce various combinations of inbred, hybrid, backcrossed, and hybrid-selfed embryos. Immature embryos were cultured on nutrient/agar medium. Three weeks later, calluses were scored for the presence or absence of somatic embryos. The calluses were transferred to a regeneration medium (lacking 2,4-D) and placed in darkness at 25 C for one week. After exposure to 16 h light and 8 h dark for two weeks, plantlets were observed and removed to fresh medium.

...Progress from selection for embryogenesis or plant regeneration can be made to better understand these processes and improve the frequency in

Numbers of calli that produced embryos and numbers of embryos that regenerated were used to calculate frequencies of somatic embryogenesis and plantlet regeneration.

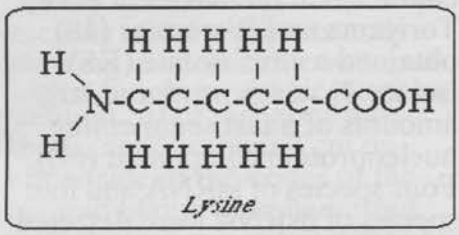
The results showed that somatic embryogenesis and plantlet regeneration were strongly influenced by genetic factors. Additive gene effects appeared more important than dominant gene effects for percent somatic embryogenesis and number of plantlets regenerated per embryo. These researchers suggested that at least one gene controls the expression of the frequency of somatic embryogenesis in addition to environmental, cytoplasmic, maternal, and paternal factors.

Endosperm Segregation of *In Vitro*-selected Rice

Of the 20 or so amino acids found in rice, lysine is the only one in such low amounts that the optimal nutritional quality of the rice grain is reduced. Schaeffer *et al.* (14) have reported on the genetics of lysine expression and determined the relationships between percent lysine, seed size, and chalkiness in progeny of crosses with high lysine mutants obtained by *in vitro* selection.

Anther-derived callus (Calrose 76) was treated with 1mM or higher levels of lysine plus threonine. After additional treatment with S-(2-aminoethyl)-cysteine, surviving calli were regenerated, selfed at least two times, and crossed to the male parent M-101. The female parent carried the gene for increased lysine

content, while the male contained a single recessive marker gene for glabrousness (smooth leaf). Two seeds were recovered from this cross. Subsequent generations were grown in a greenhouse.



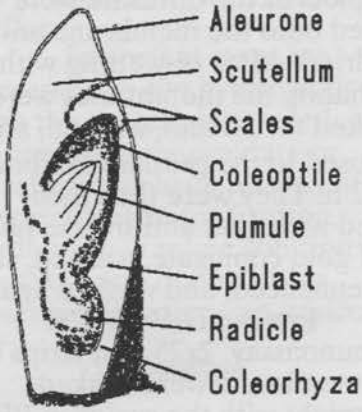
The results showed that high lysine phenotypes were recovered in the progeny of anther-derived callus, that this trait was inherited as a recessive, and that high lysine was associated with reduced seed weight. All the *in vitro* selections produced chlorophyll variants and exhibited extensive infertility. In the homozygous material, some flowers appeared abnormal with multiple stigmas which were completely sterile. However, the germplasm obtained from *in vitro* selection had lysine levels above the original Calrose 76 parent.

Ultrastructure of Somatic and Zygotic Embryogenesis

Jones and Rost have published two related papers dealing with the rice embryo. The first work (15) examined the development of the embryo from the time of fertilization to maturity. Rice grains of cv. L-202 were grown to flowering (about 75 days) and samples of flowers from the middle third of each spikelet were harvested at various times after anthesis. Samples were prepared for both light and electron microscopy.

They found that within two days after fertilization, the embryo was globular and within 6 days resembled a mature embryo but lacking the full complement of storage reserves.

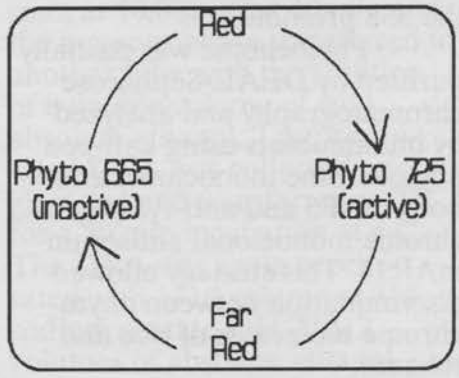
This paper contains a full description of zygotic embryogenesis along with many micrographs.



The second paper (35) investigated the development of somatic embryos derived from tissue cultures. They observed that the initiation of somatic embryos arose from single cells of the basal scutellar epithelium. Once initiated, the continued development of the somatic embryos parallels that of zygotic embyros.

Rice Phytochrome in Transgenic Tobacco

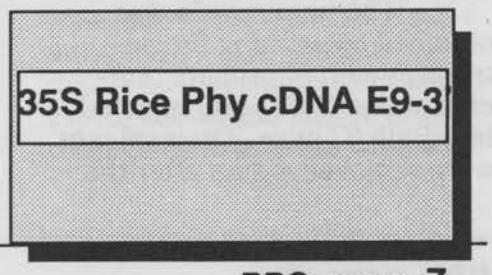
Many of the developmental processes that occur in green plants result from the combined action of chromoproteins called photoreceptors. The best known of these is the red light photoreceptor, phytochrome. It is composed of a large apoprotein (about 124 kD) and a linear



tetrapyrrole. It exists as a dimer in solution. Phytochrome can exist in two convertible forms: an inactive form produced by exposure to far red light and an active form produced by exposure to red light. It acts as an on and off switch to control many light-activated responses including the differentiation of plasmids, germination of seeds, elongation of stems, leaf initiation, and even flowering. Recently it has been shown that there are actually two distinct kinds of phytochrome: Type I and Type II. The main differences between the two is that Type I is depleted upon exposure to light whereas Type II is not.

To investigate the mechanisms of phytochrome action, *in vivo*, Kay *et al.* (16) have overexpressed Type I rice phytochrome in transgenic tobacco by using the cauliflower mosaic virus 35S promoter.

A full length cDNA of rice phytochrome was fused to the 35S promoter of cauliflower mosaic virus and transferred to tobacco. A 3' polyA addition site (from pea *rbc*S-E9) was placed at the 3' end and a 50 bp of 5'-untranslated leader was fused to



the 35S promoter.

Phytochome was partially purified by DEAE-Sepharose chromatography and analyzed by immunoblots using anti-pea phytochrome monoclonal antibody mAP5 and anti-rye phytochrome monoclonal antiserum mAR14. This strategy allowed discrimination between phytochrome molecules of rice and tobacco.

These workers showed that rice phytochrome mRNA, its polypeptide, and a photoreversible holoprotein was produced in the transgenic tobacco plants. They also observed that since *Cab* mRNA was cycled longer in the transgenic plants than in the controls, the rice phytochrome was therefore biologically active. This established a biological system for the analysis of phytochrome functioning and the interaction between the phytochromes and *Cab* gene expression.

Immunoassays of *Humicola* Fungus in Rice

Much post-harvest deterioration of rice is due to the presence of fungi which reduce nutritional value, change the color, flavor, and texture, and even pose a health hazard through exposure to mycotoxins. Early detection by classical procedures is difficult so Dewey *et al.* (17) developed several monoclonal antibody asssays that recognized *Humicola lanuginosa* in plant tissues.

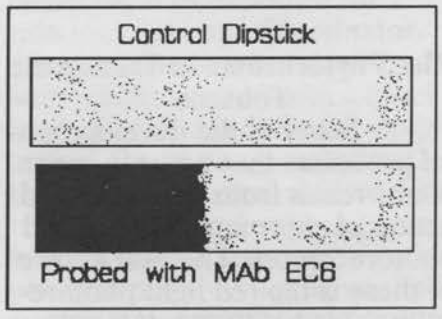
The immunogen was obtained by washing fungal cultures with phosphate buffered saline (PBS) and injected into Balb/C mice. Their spleens were removed 4 days after the last booster injection and the splenocytes fused with myeloma cell line SP2/O-Ag14 using PEG.

For the DOT-BLOT immunoassay, polyvinylidine difluoride membranes proved superior to nitrocellulose. Individual grains were soaked overnight at 4 C in PBS in Eppendorf tubes to allow fungal antigens to diffuse into solution. Droplets of the diffusate were placed onto the membrane and air-dried. After re-wetting with methanol, the membranes were blocked for 30 min, washed, and exposed to the primary antibody for 2 h. They were then incubated with goat anti-mouse IgG-IgM gold conjugate, washed, silver enhanced, and washed again.

For the DIP-STICK immunoassay, 2x25 mm strips of the membrane were soaked overnight with the grains in PBS, air-dried, and processed as above.

For the SQUASH-BLOT immunoassay, rice grains from the DIP-STICK assay were pressed onto the surface of the membrane and processed as above.

The IgM antibody EC6 worked best with the DIP-STICK assay and proved to be simple to perform and suitable for field use.



Single- and Double-stranded RNAs in Rice Stripe Virus

The rice stripe virus (RSV group) replicates in plants and in its planthopper vector. The mechanism of replication is still unknown. In an attempt to characterize the RNAs of RSV, Toriyama and Watanabe (18) obtained a virus isolate (RSV isolate T) which produced large amounts of a fast sedimenting nucleoprotein component (nB). Four species of ssRNA and four species of dsRNA were detected in extracts of isolate T.

To separate and purify the RNAs, these researchers electrophoresed the RNA extract in a 1.5% low melting temperature agarose gel at 4 C for 16 h at 40 V. The gel was stained with ethidium bromide, the bands removed with a surgical blade and placed in an Eppendorf tube. The agarose was melted with 3 volumes of 0.5 M ammonium acetate and SDS at 65 C with frequent vortexing. After phenol extraction and three washes in chloroform-isoamyl alcohol, the RNAs were precipitated with ethanol. They were again electrophoresed, but in 1.2% LGT agarose and purified as above.

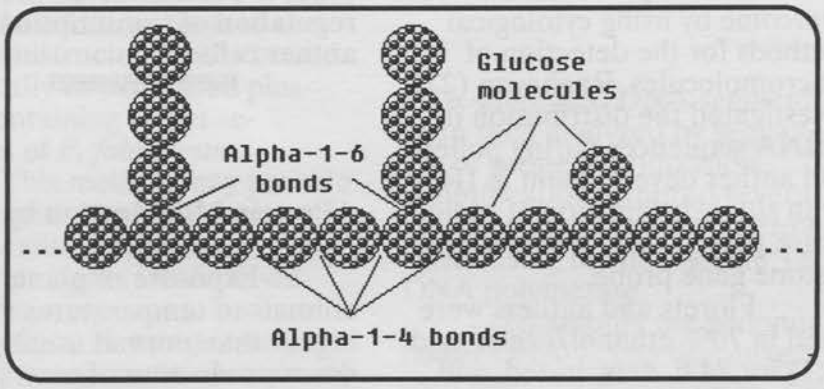
Two types of probe were used: one was viral RNA fragments end-labelled with ^{32}P and the other was a ^{32}P-labelled cDNA probe produced with random primers.

The results showed that the nB component contained the largest RNA and that it is the only one that is infective. The hybridization experiments indicated that the four ssRNA do not contain common sequences. The ssRNA sequence was also found in each dsDNA of the same length. These researchers suggested that the functions of ss- and dsRNA

during replication might be explained by work on RNA polymerase activity in this virus.

Branched Amyloses in Rice

Starches of most plants consist of two components: amylose and amylopectin. Amylopectin is generally regarded as a branched molecule with alpha 1-4 and alpha 1-6 linkages between glucose molecules. Amylose is generally represented as a linear polysaccharide with only alpha 1-4 linkages.



In their recent paper, Takeda (19) *et al.* have reported on the structures and amounts of branched amyloses in several varieties of rice including IR32, IR36, IR42, and several others.

All varieties had branched molecules of 7.5 to 9.7 chains except for IR36 which had 5.7 chains. The chain length range was from 90-155. This is similar to the lengths from other plants. The molar fractions of the branched molecules (0.3 to 0.49) were roughly similar to those of other cereals, but significantly different from that of sweet-potato (0.70). This suggested that the branched amyloses in rice starch were a minor component of the storage carbohydrates..

Cloning of mRNA for a Prolamin Polypeptide

Prolamin is an alcohol-soluble protein and along with glutelin is one of the major storage proteins in most cereals, . Although other genes of cereal prolamins (zein of maize, gliadin of wheat, hordein of barley) have been isolated, the rice prolamin gene had not been cloned.

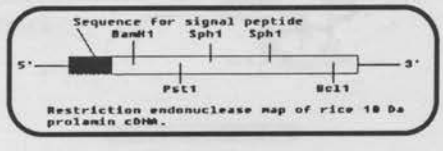
Masumura *et al.* (20) isolated a full-length cDNA encoding a prolamin (10 kDa) containing a large amount of sulfur-rich amino acids.

These researchers used cv. Nipponbare and harvested the seeds about 10 days after flowering. Messenger RNA was obtained by several phenol extractions and oligo(dT)-cellulose chromatography.

The cDNA was synthesized by a reaction mixture containing RNasin and AMV reverse transcriptase. Incubation was performed for 90 min at 42 C. This reaction mixture was diluted, *E. coli* DNA polymerase and *E. coli* RNaseH added, and incubated for 1 h at 12 C and 1 h at 22 C. The ds-cDNA was phenol extracted, twice precipitated with ethanol, and a library containing 30,000 recombinants constructed using pUC9. A second library was produced using \gtll as a vector.

To isolate the polypeptides and sequence the amino acids, 100 g of 10 day old rice seeds were homogenized in a Tris-HCl and EDTA buffer with 1% Triton X-100. The homogenate was passed through four layers of gauze and spun to remove starch grains. The supernatant was spun at 8,000xg for 20 min to obtain a crude protein fraction. This fraction was separated by SDS-PAGE, and the prolamin polypeptides in the gel were observed by precipitation with 1M KCl. The gel portion containing the 10 KDa prolamin was removed and dissolved in 1% SDS to extract the polypeptide which was then dialyzed and lyophylized. An automated amino acid sequencer was to used to characterize the prolamin.

The sequence of the amino acids in this 134 amino acid polypeptide was deduced from the nucleotide sequence and indicated high amounts of methionine (20%), glutamine (16%), cysteine (10%), and proline (6%). The protein accumulation mechanism in rice appeared similar to that of zein in maize: the prolamin is synthesized by polysomes attached to the protein body membrane and accumulates in the endosperm cells.



Restriction endonuclease map of rice 10 Da prolamin cDNA.

Transient Expression After Particle Bombardment

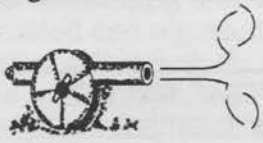
Wang *et al.* (21) have described a technique for transferring foreign genes directly into rice, wheat, and soybean cells. Callus of rice, cv. Taipei 309, was produced from immature embryos, developed into a cell suspension, and cultured in a medium using amino acids as the nitrogen source.

The cell suspensions were placed on a rotary shaker at 120 RPM in the dark at 26 C. The rice cell suspensions were subcultured twice a week. Recombinant plasmids were grown in *E. coli,* lysed with alkali, phenol extracted, and purified by CsCl/ethidium bromide density centrifugation.

Before bombardment with the DNA coated particles, the cell suspension was applied to 3 layers of filter paper and placed in a plastic Petri dish. After bombardment, the cells were incubated for 48 h in the dark at 26 C.

Intact cells of rice (and wheat) produced detectable levels of GUS activity. Rice cells (and wheat, maize, and soybean) that had been bombarded with particles coated with plasmid DNA containing the CAT gene also showed expression.

This method can avoid the difficulties encountered during the regeneration of plants from protoplasts. The frequency of foreign gene transfer was estimated as 1/1000. The next step would be to obtain a stable incorporation of the foreign genes.



...the first example of differential accumulation of a specific mRNA during anther development in an angiosperm...

Differential Expression in a Cloned Gene

There are many problems associated with the biochemical analysis of gene expression in the anthers of rice. Some of these problems may be overcome by using cytological methods for the detection of macromolecules. Raghavan (22) investigated the distribution of mRNA sequences during pollen and anther development in IR30 by in situ hybridization of radioactive poly(U) and a cloned rice histone gene probe.

Florets and anthers were fixed in 70% ethanol/acetic acid (3:1) for 24 h, dehydrated, and embedded in glycol methacrylate. Sections were attached to slides and dried overnight at 55 C. The slides were rinsed in hybridization buffer, treated with 80 ul of the hybridization mixture, sealed with a coverslip, and incubated at 47 C for 4 h. After incubation, the slides were rinsed in cold tap water, hybridization buffer, and RNase digestion buffer. Pancreatic RNase was used to remove unhybridized poly(U). The binding sites were detected by dipping the dry slides in diluted Kodak NTB-3 liquid emulsion. After 4 weeks in darkness, the slides were developed and stained with 0.1% eosin Y, dried, and mounted.

The histone gene was a 1.3 kb insert from an IR26 genomic library. This sequence showed 80% and 92% homology with the H3 histone genes of sea-urchin and wheat, respectively. After hybridization, the slides were developed and stained in Toluidine Blue, dried, and mounted.

Results show that poly(A)+RNA and the histone message are not localized in the same pattern, histone mRNA is not necessarily found in rapidly dividing cells. The lack of binding of the probe to the generative cell may indicate that histones are not involved in the regulation of transcription in anther cells.

Protein Modification by Heat Shock

Exposure of plants and animals to temperatures much higher than normal usually shuts down production of most proteins while inducing a new set of proteins referred to as heat shock proteins (hsps). A possible role of such proteins is as a thermoprotectant for the cellular membranes and cytoskeleton. Fourre and Lhoest (23) in Belgium have reported on their research with callus cells of *Oryza sativa* L. exposed to heat shock.

Seeds of cv. Romeo were surface sterilized and germinated for 16 h at 30 C. Callus cultures were initiated from the embryos and 2 months later cell suspensions were produced. The cells were radioactively labeled with tritiated leucine (40-70 Ci/mmol) for 1 h at various tem-

peratures. The cells were harvested and the proteins precipitated onto glass fiber discs and counted. After electrophoresis on a 10-20% sodium deodecyl sulfate gel gradient, the labeled proteins were detected on Kodak X-Omat film.

Cells were also exposed to radioactive S-adeonosylmethionine (a universal methyl group donor) to detect methylation of hsps.

Amino acid analysis was performed by high-voltage paper electrophoresis and descending paper chromatography.

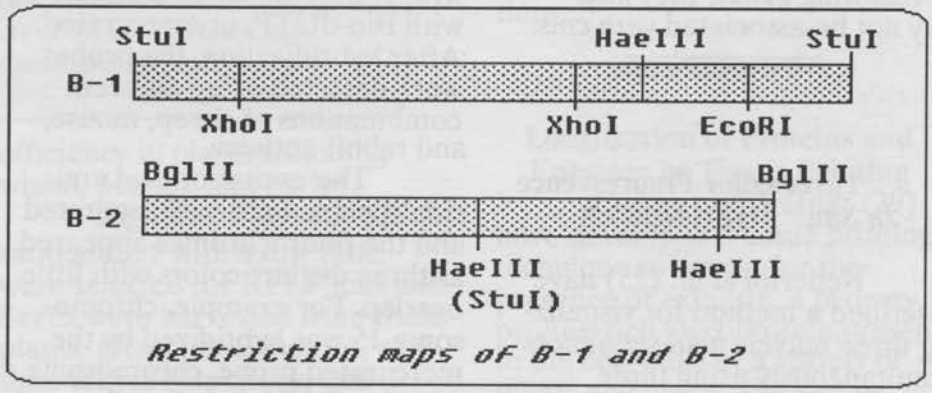
These researchers noted that rice cells of calli derived from seed embryos responded to heat shock differently depending upon whether the cells were subjected to a rapid heat shock or a progressive increase in temperature. At 45 C, the induction of hsps was greater when the temperature increase was gradual. After sudden heat shock, hsps synthesis was almost completely shut down.

Although a large part of the radioactivity was found incorporated in the nucleic acids, about 20% was located in the proteins, primarily associated with methionine. These results suggested that high temperatures (heat shock) causes methylation during protein synthesis. The role of methylation of heat shock proteins is still unknown.

Plasmid-Like Mitochondrial DNAs in Cytoplasmic Male Sterile Rice

Hybrid seeds of rice are often commercially produced using cytoplasmic male sterility (*cms*) which is maternally inherited and appears to be located within the mitochondrial genome. Earlier work indicated that there were two plasmid-like DNAs, B1 and B2, in the sterile rice strain known as BT line. These same plasmids were absent in the fertile line Taichung 65. To determine whether these plasmids were associated with *cms*, Sakamoto *et al.* (24) isolated mtDNAs from 5 rice strains and investigated the homology of B1 and B2 to other plasmid-like DNAs found in these strains.

The strains included Chinsurah boro II, BT line, Zhen Shan 97A, Zhen Shan 97, and Akihikari. All were maintained as calluses except for Akihikari, which was used as week- old seedlings. The calli or seedlings were homogenized, filtered through 4 layers of gauze and 2 layers of nylon mesh, and centrifuged twice at 1,500xg at 0 C for 10 min. The supernatants were spun at 10,000xg and the pellets suspended in a buffer containing 10mM $MgCl_2$. After treatment with DNaseI, the mixture was incubated at 4 C for 30 min, and washed 3 times. The DNA was extracted with phenol-chloroform and precipitated with ethanol and resuspended in TE buffer. The B1 and B2 DNAs (BT line) were separated by gel electrophoresis and cloned.



Restriction maps of B-1 and B-2

Probes were prepared and labeled with photobiotin.

B1 and B2 were analyzed by restriction endonuclease digestion. Digestion with *Stu*I showed B1 to be 2.1 kb long. It was cloned into the *Sma*I site of pUC13. B2 was digested with *Bgl*II and appeared to be 1.5 kb in length. It was cloned into the *Bgl*II site of pNEO. When mapped, these two segments showed no common fragment.

Plasmid-like DNAs were also isolated from the mitochondria of Zhen Shan 97A and Zhen Shan 97 by agarose gel electrophoresis. Using the B1 and B2 probes, hybridization analysis was performed to determine if the mtDNAs were identical to the B1 and B2 DNAs.

The two plasmids of Zhen Shan 97A were found to be identical to B1 and B2 while only one of the plasmids of Zhen Shan 97 was identical to B2. The smaller of the two plasmids of Zhen Shan 97 was not homologous to either B1 or B2. It was suggested by these authors that B2 (by itself) may not be associated with cytoplasmic male sterility. And, since the

mtDNA plasmids exist independently of the nuclear fertility-restoring genes, they also may not be associated with *cms*.

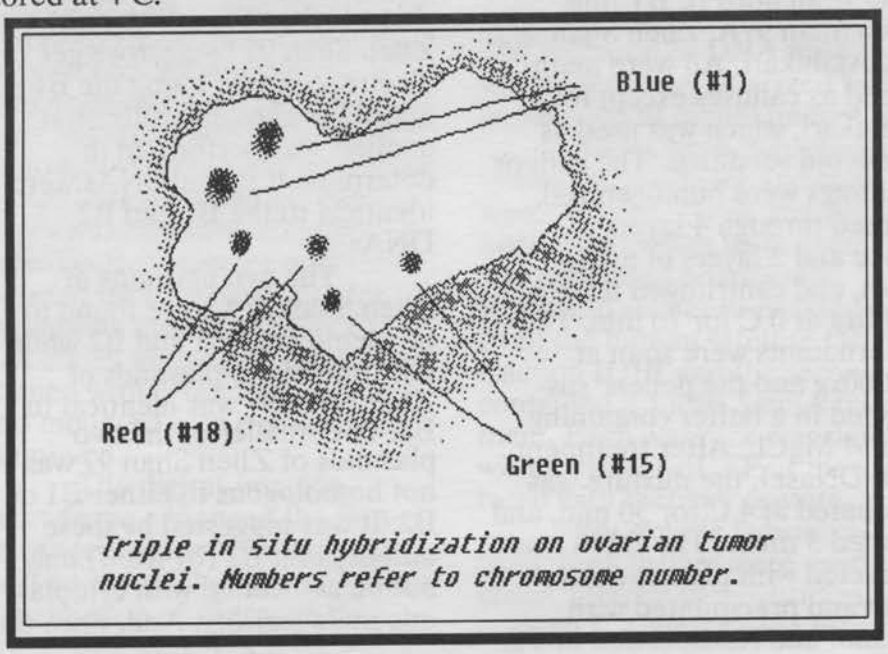
Three-color Fluorescence *In Situ* Hybridization

Nederlof *et al.* (25) have described a method for visualizing three nucleic acid sequences simultaneously using three different haptenized probes combined with three fluorochromes. Although previous workers have used double hybridizations, this is the first time for triple hybridizations. The success of this technique was the result of the use of a newly developed blue immunofluorochrome, aminomethyl coumarin acetic acid (AMCA). Metaphase preparations made from human lymphocytes and ovarian tumor material were air dried, washed in PBS, dehydrated in ethanol, and stored at 4 C.

Probes of four different chromosomes were modified with N-acetoxy-AFF, biotinated with Bio-dUTP, or mercurated. After hybridization, the probes were detected using different combinations of sheep, mouse, and rabbit antisera.

The excitation and emission spectra were well separated and the fluorochromes appeared as three distinct colors with little overlap. For example, chromosome 15 was hybridized by the mercurated probe, chromosome 1 by the AAF labeled probe, and chromosome 18 by the biotinated probe. In triple-exposed color photomicrographs, three colors were observed: the centromere regions of chromosome 1 appeared as two blue dots, chromosome 18 was labeled with red dots, and chromosome 15 was labeled with green dots.

The interphase nuclei also exhibited labeling. Such simultaneous labeling by multi-colored probes may provide more information than would be observed by separate hybridizations. An obvious application of multiple in situ hybridization would be in those cytogenetic studies where rapid analysis of material precludes detailed karyotypic analysis. In such cases, the use of colored-coded probes for common chromosomes involved in aberrations would greatly facilitate the analyses.



Triple in situ hybridization on ovarian tumor nuclei. Numbers refer to chromosome number.

E Genome and Salt-stress Response

In Plant, Cell and Environment, Schachtman *et al.* (26) have given the results of a study that examined changes in salt-stress responses due to the addition of the E genome (Lophopyrum) to the A, B, and D genomes of hexaploid bread wheat. They compared parents and derivatives of two crosses: wheat cv. Chinese Spring X L. elongatum (a close relative of wheat with a high degree of salt tolerance) and PI178704 (fairly salt tolerant wheat accession) X L. ponticum.

Chromosome analyses were performed on root tips of seeds of the four lines and the plants transferred to hydroponic tanks containing half-strength aerated Hoagland solution. Salt stress was imposed by increasing the sodium chloride concentration in one tank.

Plants were harvested and divided into components; roots, shoots, oldest leaves, and newest leaves. The tissue was dried and analyzed for ion content. The wheat lines with chromosomes from the salt tolerant Lophopyrum showed lower concentrations of sodium

...This study demonstrates that the introduction of chromosomes from salt-tolerant Lophopyrum into wheat resulted in lower accumulation of Na+ and Cl- in the shoots of the derived lines...also showed a greater salt -tolerance ...accumulated more dry weight.

and chloride and accumulated more dry weight under salt stress than the wheat parents. This may indicate a causal association between low shoot sodium and chloride accumulation and salt tolerance. These results are similar to those found in other halophytes.

RFLPs Associated with Water Use Efficiency

Working with a large number of F3 and backcross families obtained by crossing the drought-sensitive tomato cv. UC828 and UC204B, with the wild drought-tolerant species *Lycopersicon pennellii,* Martin *et al.* (27) reported they had found three RFLPs strongly associated with water use efficiency. Evaluation for this component of drought-resistence had been unreliable in field grown plants. RFLPs had previously been associated with insect resistance and soluble solids in tomato.

Plants were grown in the field and thinned to 20 plants spaced 30 cm apart within rows. The soil was kept moist during seedling growth. About two months later, irrigations were reduced to evaluate the performance of plants under drought. Leaves were sampled and combusted under vacuum and the carbon dioxide formed was released into a mass spectrometer to determine isotopic carbon ratios. This ratio had earlier been shown to be an excellent predictor of water use efficiency in plants including wheat, peanut, and barley.

Eight families with a high ratio and 11 with a low ratio were selected for RFLP analysis. Seeds were harvested from these plants, grown for 2 months, and their leaves lyophilized and ground for DNA extraction. The DNA was digested with Bg1II or HindIII, subjected to electrophoresis, and blotted onto nylon membranes. Seventeen probes were hybridized to the blotted DNAs which covered 12 of the 19 known linkage groups in tomato. Analysis of the data indicated significance for all three RFLP markers.

Each of these markers could be associated with several or more genes but such work should open new ways of studying water use efficiency and other traits that are difficult to analyze.

Ultrastructural Resolution with *In Situ* Hybridization

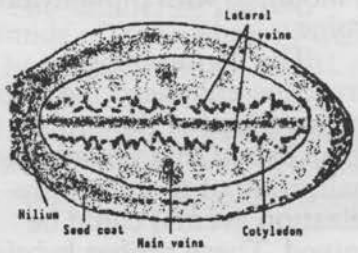
McFadden (28) compared several *in situ* methods for localization of RNAs in plant tissues. Of the isotopic probes used on various plant tissues (pea, barley, and tobacco), the finest resolution was achieved with probes labelled with tritium which has a low beta particle energy level causing a short path length (0.5 um). However, it was found that non-radioactive probes (biotinylated) produced even finer resolution than isotopic labels, although with reduced sensitivity in thin sections.

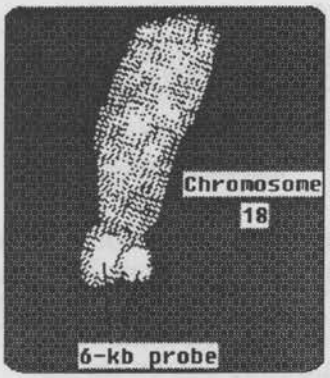
Localization of Proteins and Enzymes by Tissue Printing

Cassab and Varner (29) have developed a tissue printing technique to screen for the presence of extensin, a hydroxy proline-rich glycoprotein present in the cell walls of a variety of plants and algae. The simple process consisted of slicing through the tissue of interest with a razor blade and pressing the freshly cut surface onto a piece of nitrocellulose paper for 15-20 sec which had been presoaked in 0.2 M calcium chloride.

The paper was dried and treated with extensin primary antibody for detection of AP-conjugated second antibody with 5-bromo-4-chloroindoxylphosphate and nitroblue-tetrazolium.

Tissue prints of soybean seeds showed that the distribution of extensin resembled that shown by the traditional immunocytological localization of proteins. Tissue printing has also been used to follow peroxidase activity in ethylene treated etiolated pea epicotyls. It appears to be a simple technique suitable for studies of proteins, enzymes, and anatomy of imprinted tissues.





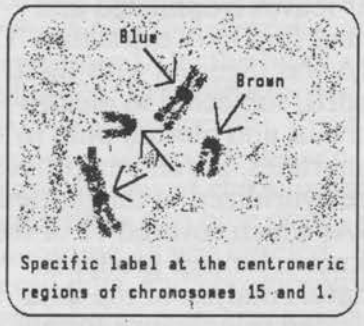
Enhancement of Mapping by Digital-Imaging

In situ hybridization of single-copy genes or DNA sequences shorter than 10 kb is often a matter of percentages, that is, the hybridization on any particular chromosome occurs a certain percentage of the time. By using intensified-fluorescence digital-imaging microscopy, Viegas-Pequignot *et al.* (30) showed that such statistical analysis of spot distribution was unnecessary.

Slides of human lymphocytes were produced and stored at -20 C until use. Two probes (6 and 2.3 kb) were labeled with bio-11-dUTP. Slides were treated with RNase and the chromosomes were denatured with 70% formamide in 2xSSC at 70 C for 2 or 3 min. After rinsing and dehydration, the slides were flooded with the hybridization mixture and covered with a plastic film for incubation overnight at 42 C. The slides were again rinsed and stained with propidium iodide and mounted with p-phenylenediamine.

The digital-imaging microscope enhanced the fluorescence signal of the probes to such an extent that unambiguous visualization of the spots (hybridization events) could be observed. These probes hybridized to chromosomes 18p and 22q. In every metaphase plate observed, no spots were found on both chromatids of a chromosome other than 18 and 22.

These researchers claimed that the best results were obtained when probes were denatured separately from the chromosomes and that the percentage of cells with labeled chromatids increased with the concentration of the probe.



Double *In Situ* Hybridization

In Experimental Cell Research, Emmerich *et al.* (31) have described their work dealing with double *in situ* hybridization using biotinylated and mercurated DNA probes which were later analyzed by the production of digital images.

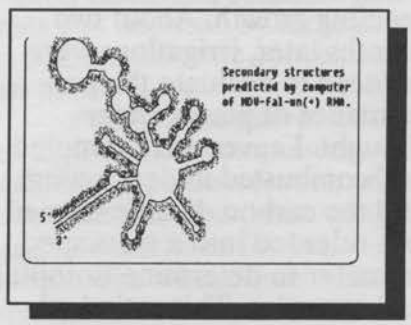
Slides were prepared of human lymphocytes and cells from amniotic fluids and fibroblasts. Two probes were used: one contained a tandemly organized sequence isolated from the human satellite DNA fraction II/III and the second contained a 1.8 kb *Sau*3A fragment. Each was nick-translated with biotin-11-dUTP or mercurated with mercury acetate.

For double hybridizations, both probes were used at about 1 ug/ml in a hybridization mixture containing 65% formamide and 2xSSC.

Camera lucida drawings were digitized and stored in a VAX 11-780 computer to produce 2D images. Appropriate analyses of these images indicated that, at least in this material and with chromosomes 1 and 15, there is no distinct association of homologous chromosomes in somatic cells. This technique enabled the researchers to simultaneously determine the positions of heterochromatin of two different chromosomes during interphase.

Billion-Fold Amplification of Probes

In Bio/Technology, Lizardi et al. (32) reported the synthesis of recombinant RNA molecules that function both as probes and as templates for exponential amplification by QB replicase. This enzyme initiates the synthesis of a large number of RNA strands, greater than a million-fold increase as a result of the exponential reaction mechanism. That is, single-stranded RNAs are copied into complementary single stranded products which in turn serve as templates in the next round of synthesis. As long as there is an excess of the enzyme QB repli-



case, the number of RNAs increases exponentially.

Working with a repetitive DNA sequence from *Plasmodium falciparum,* these researchers constructed an RNA molecule that served as a probe and template. Four different RNAs were produced: MDV-1, MDV-fal-un, MDV-fal-st, and MDV-poly.

Using decreasing amounts of MDV-fal-un with a fixed amount of QB replicase, it was shown that by starting the reaction mixture with as few as 1000 molecules, in just 30 min there was a one billion-fold increase in the amount of RNA. Similar results were obtained with two other probes.

In addition, each of the newly constructed RNAs bound specifically to denatured plasmids containing target sequences of *P. falciparum.*

This method may provide an alternative to the exponential amplification of targets by the PCR technique (DNA polymerase chain reaction). Such amplification should permit detection at extremely low levels of target, increase speed of detection, and provide for simultaneous detection by using a mixture of different probes.

Mutation Analysis Using PCR

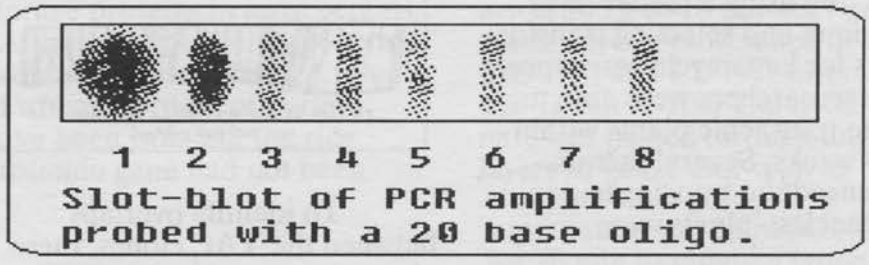
In the technical newsletter Clontech Cruiser, Frye et al. (33) described the methods they used to detect ras mutations in human tissues. There are three ras genes that code for three similar 21 kD proteins located in the cell membrane and serve in a regulatory capacity. All three display intrinsic GTPase activity, converting GTP to GDP. The ras gene was shown in 1982 to be responsible for induced transformation of mouse cells to tumor cells.

The polymerase chain reaction (PCR) was used to amplify DNA segments in regions of the three ras genes where mutations occur. The PCR-amplified DNA segments were then analyzed by sequencing or probe hybridization. Amplification required several hours on an automated thermal cycler using the heat stable Taq DNA polymerase.

To amplify the genomic target DNA, 0.5 ug was mixed with amplification primers, Taq polymerase, PCR buffer, 10X dNTP stock solution, and water. Mineral oil was layered over the solution to prevent evaporation. The first cycle of amplification included denaturation at 94 C for 3 min, annealing at 50 C for 30 sec, and primer extension for another 30 sec. All forty subsequent cycles were run for 30 sec at each temperature. These researchers found the concentration of Mg++ to be critical: less than 1.5 mM gives almost no amplification and more than 5 mM causes increased random priming.

After PCR amplification, the mineral oil was removed by extraction with chloroform. About 80 ul of the amplified sample was labeled and hybridized onto filters. Using these techniques, it was possible to analyze the DNA from small numbers of cells, as few as 30 cells.

Depending on the GC content, the temperature of denaturation and annealing was adjusted. The higher the GC content, the higher the temperature needed to reduce nonspecific binding. Also, the wash conditions were monitored carefully by measuring the temperature in the wash container, not the water bath, and keeping the wash container covered.

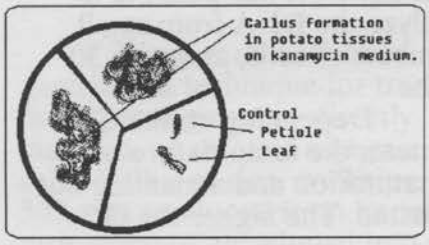


Slot-blot of PCR amplifications probed with a 20 base oligo.

Efficient Transformation in Potato

An efficient method of obtaining transgenic potato plants has been reported in Plant Science by Tavazza, *et al.* (34).

Leaf discs were inoculated with *Agrobacterium tumefaciens* LBA4404 containing a binary vector, pGA492, in which the tumor inducing genes had been replaced by Neomycin phosphotransferase, a selectable marker.

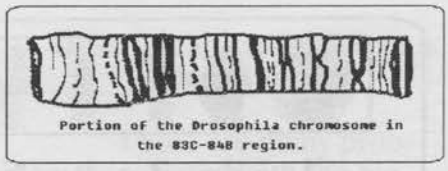


By using a feeder layer technique and selecting transformants for kanamycin resistance, these researchers were able to isolate transgenic plants within just 4 weeks. Several factors influenced the transformation frequencies: plants were younger than 3 weeks and temperatures for growth were kept below 19 C.

Since the adventitious shoots grew rapidly with little callus formation, it was expected that such plants should exhibit greater genetic stability. Other workers have also reported minimum variation in plants produced by this technique.

Inverse and Microdissection PCR

Garza *et al.* (35) have developed two variations of the polymerase chain reaction (PCR). During microdissection PCR, the salivary chromosomes of *Drosophila melanogaster* were squashed and a small protein, corresponding to a specific region, was dissected from one of the chromosomes. The DNA was extracted and digested to completion with *Mbo*I. By using a 20-mer as a primer, the microdissected DNA fragments were amplified with 35-40 cycles of PCR which yielded about 100 ng of DNA. The amplified DNA was used to probe yeast colonies containing YACs (yeast artificial chromosomes) of *Drosophila* DNA. *In situ* hybridizations with salivary gland chromosomes and the largest YACs showed seven clones that hybridized to the microdissection probes.


Portion of the Drosophila chromosome in the 83C-84B region.

To identify overlaps between the YAC clones, these researchers used inverse PCR to create probes that corresponded to the junctions between the YAC vector arms and the inserted *Drosophila* DNA. The YAC DNA was first isolated by agarose and then digested with *Eco*RV and/or *Hinc*II. The *Drosophila* DNA in the resulting junction fragments was amplified by PCR. Probes were produced which had several uses: end points of the inserts were cytologically detected by *in situ* hybridization, Southern blots allowed overlaps between YAC inserts to be identified at the molecular level, and probing the entire YAC library allowed chromosome walking by the identification of additional overlapping clones.

2nd International Workshop on Molecular Biology of Rice: Jan. 8-9, 1990. For more information write: Dr. Ken-ichi Higo, Secretary Organizing Committee. National Institute Of Agrobiological Resources, 2-1-2, Kannondai, Tsukuba, Ibaraki, 305 Japan.

In Situ Removal of RNA

Wagner *et al.*(36) have developed an effective method for the removal of RNA from plasmid samples when screening by electrophoresis. They report in BioTechniques that by simply adding RNase directly to the agarose gel during its preparation, RNA was removed making DNA visualization much clearer and the bands sharper.

RNase was added, prior to pouring, at a concentration of 10 ul/ml. It appeared that RNA removal is a function of time and the migration of tracking dye of less than 2.5 cm resulted in the removal of almost all the RNA.

Dry Iceless Nucleic Acid Precipitation

For laboratories with short supplies of dry ice but access to a -70 to -80 C freezer, Bird and Wu (37) suggested the following. Fill an empty micropipet box (rack removed) to a depth of 4 cm with 80% isopropanol and place in the freezer till equilibrated. The solution forms a slush into which the microtubes can be inserted. Return the box to the freezer for precipitation. This technique has been successful on both DNA and RNA and it eliminates the need to use pre-chilled (-20 C) ethanol.

Chromosome Doubling in Haploid Rice

A new approach to doubling the chromosome number in rice plants was reported this year by Wong (38). Haploids produced by anther culture were mass produced by excising nodal segments and planting in vermiculite watered with a simple inorganic nutrient solution. At or just after anthesis, nodes (about 3 cm on either side of the node) of the haploid plants were harvested into plastic bags and incubated 12-18 h at 30 C.

The nodal segments were then treated with either 0.3% or 0.4% colchicine containing 2% DMSO at 30 C for 6-12 h. After rinsing for 12-18 h, the cut ends were coated with 50% Benlate powder (DuPont) and planted in vermiculite moistened with nutrient solution.

Although the treated plants were slower to sprout (10-20 days later) and appeared stunted, 42% survived the doubling treatment. And 12% of the plants that went on to produce seeds were shown to be diploid or diploid-like.

Improved Detection of mRNA with Sulfonated cDNA Probes

Researchers at the University of Paris have reported an efficient *in situ* hybridization method for detection of specific mRNAs. Perrot-Rechenmann *et al.* (39) have based the system on the use of sulfonated cDNA probes which exhibited a sensitivity similar to radiolabeled probes and were more sensitive than biotinylated probes.

Background was almost completely eliminated by incubating the slides in a blocking solution for 1 h at room temperature. This solution contained: 35% dry skimmed milk, 3.5% bovine serum albumin, 0.35% heparin (w/v), and 0.3% Tween 20 (w/w) in TEN buffer.

Important steps for successful *in situ* hybridization with this material included: use of dimethylsuberimidate or paraformaldehyde instead of glutaraldehyde for fixation, incubation in saline solution (2xSSC), and drying slides overnight in a vacuum desiccator.

...in situ hybridization offers many possibilities of application and should provide a reliable and efficient approach to study cell specificity and plant tissue differentiation.

Extraction with Sodium Perchlorate

Johns and Paulus-Thomas (40) have developed a new method for the isolation of high molecular weight human genomic DNA from whole blood samples. The final product was suitable for restriction endonuclease digestion and Southern hybridization analysis.

After lysis of the blood cells with SDS, removal of RNA with RNase A, and heat denaturation of the proteins, 5M sodium perchlorate was added and gently inverted to mix. An equal volume of chloroform/isoamyl alcohol (24:1) was added and extracted for 30 min at room temperature. The mixture was spun at 10,000xg for 10 min and the aqueous phase transferred to another tube with the addition of a double volume of cold absolute ethanol. The precipitated DNA was collected on a glass rod and transferred to TE for a 30 min incubation at 4 C. The DNA was again precipitated with 1/10 volume of 3M sodium acetate (pH 7.0), 2 volumes of absolute ethanol, and washed several times with 70% ethanol. After air-drying, the DNA was dissolved in TE at 4 C overnight.

The amount of DNA (150-250 ug/10 ml whole blood) obtained by this method was equivalent to that obtained with proteinase K/phenol extraction. The 260/280 ratio was 1.7-1.8 and indicated little RNA and/or protein contamination. Agarose gel electrophoresis showed that the DNA was less degraded by this method.

Protein-DNA Conjugates Produced by UV Irradiation

Czichos et al. (41) have presented a simple procedure for joining together DNA and proteins by low energy UV irradiation to produce hybridization probes. SP64 plasmid DNA (3 kbp) was digested by EcoRI and end-labeled with radioactive dATP and dTTP using the Klenow fragment. This linearized DNA was mixed with various proteins in 50 mM Na-citrate (pH 2.0) and irradiated for 10 min with a low-pressure mercury lamp (254 nm-filter) at a distance of 7 cm. The mixture was neutralized with 1N NaOH and run on a 1% agarose gel to detect DNA-protein coupling expressed by the failure of the conjugates to migrate from the

wells.

The SP64 plasmid DNA was sonicated to yield 300-500 bp fragments and used as the DNA component in preparations of dot blots. After hybridization, the filters were washed and blocked for 1 h at 20 C in 10% non-fat dry milk, 2% BSA, 25mM NaCl, 50mM Tris-HCl (pH 7.4), 1mM EDTA, and 0.3% Tween 20.

The results showed that DNA retardation (failure to migrate from the wells) was observed only if the irradiated mixture was between pH 1.5 and 2.5. That is, the protein conjugated to the DNA molecule only under very acidic conditions. The protein/DNA ratio in the irradiation mixture varied with different proteins, ranging from 1:1 (histone H1) to 30:1 (SSB). Ion exchange chromatography indicated that one molecule of protein (protein A, 42 kD) bound to the DNA molecule every 200 nucleotides.

Such probes were detected immunologically, were resistant to temperatures of 100 C, stable at pH 1-12, and could be stored for long periods of time.

...biotin is a universal non-radioactive label for DNA sequencing...

Chemical Sequencing of Biotin Labelled DNA

Richterich (42) has described a method for nonradioactive chemical sequencing of DNA using a biotin marker attached to an oligonucleotide primer. The sequencing reactions were performed in siliconized 1.5 ml Eppendorf tubes with 250-400 ng of DNA and adjusted to 5 ul volume with water. Base modification reactions were: G (2mM dimethyl sulfate for 50 min at room temperature), A+G (15mM acetic acid for 2 min at 65 C), C (10 parts hydrazin to 5 parts sodium acetate for 10 min at room temperature), and C+T (9 parts hydrazin to 6 parts water for 10 min at room temperature). After treatment with piperidine, the DNA was lyophilized three times and dissolved in a 10 ul formamide dye mixture.

DNA was denatured at 90 C for 3 min and chilled before loading on a direct blotting apparatus which employed a conveyor belt moved by a stepping motor and gear box. Gel electrophoresis was run at 1800 V and 15 mA using a nylon membrane as the blotting matrix.

The sequence patterns were visualized by streptavidin-alkaline phosphatase. Briefly, the nylon membrane was dried at 80 C for 10 min and the DNA crosslinked by exposure for 2 min on a UV transilluminator. The membrane was rehydrated with water, washed with a blocking buffer, incubated with streptavidin for 10 min, washed with the blocking buffer, washed with triton buffer for 3 min, incubated with biotinylated phosphatase for 10 min, washed several times with triton buffer and reaction buffer and incubated with BCIP and NBT for 1-4 h until the bands were visible.

Signal Intensity is Linear

Lee and McGee (43) have shown that the calorimetric signal on Southern blots, with biotinylated probes, increased linearly with time from about 2 h to 22 h. The signal was also directly proportional to the quantity of DNA loaded on each track from 12.5 pg to 100 pg.

Localization of rRNA Genes on Rice Chromosomes

K. Fukui
Department of Molecular Biology
National Institute of Agrobiological Resources
Kannondai 2-1-2, Tsukuba 305, Japan

In situ mapping of DNA fragments is an effective method used to localize them on the chromosome. Combining the cell wall-free method for the preparation of chromosome samples and the biotin-labeled *in situ* method, we obtained clear signals on rice chromosomes. As a preliminary experiment, we used an rRNA sequence as proof. As shown in Fig. 1, all the rice chromosomes demonstrate uneven staining which is useful for their identification. According to the cyto-

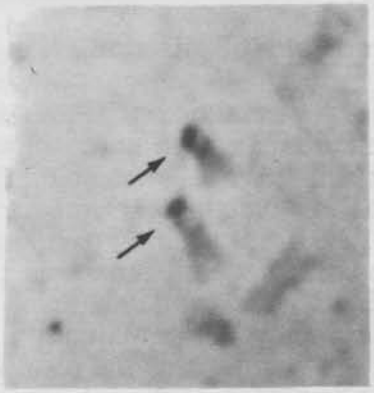


Figure 1.

logical results, it is known that the rRNA sites are located at the end of the short arm of chromosome No. 12 (K12). The signal location and condensation pattern (Fukui and Mukai, 1988) matched the NOR site and characteristics of chromosome No. 12.

Fig. 1. rRNA regions (NOR) detected by the in situ hybridization method. Arrows show the signals.

Fukui, K., and Y. Mukai. 1988. Condensation pattern as a new image parameter for identification of small chromosomes in plants. Jpn. J. Genet. 63:359-366.

Drought Resistance in Rice

Gary Toeniessen has provided the following conclusions of the recent meeting on Drought Resistance in Rice: Problems and Opportunities.

There is a misconception that rice, being grown largely in irrigated or wet environments, is not limited by lack of water. In reality, only about half of the 148 million hectares on which rice is grown is irrigated. In the remaining half, which accounts for one-quarter of total rice grain production, yields are often depressed by drought. The area of this drought prone rainfed rice is expanding as populations relying on these crops, many of them among the poorest in the world, continue to increase. Moreover, in many irrigated rice areas, hard pans develop in clay soils and prevent water extraction by blocking root growth.

The impact of drought on rice production could be reduced by developing varieties better adapted to water deficits. Despite this, drought resistance in rice has historically had a low priority in breeding programs. Although this is due partly to competition for breeders' resources from other pressing problems (disease and insect resistance, for example), two other factors are at work. The first is lack of understanding of the mechanisms of drought injury and resistance in rice, itself a function of lack of basic research. The second is the want of ready means to connect physiological and biochemical knowledge with rice genetics and breeding. Stress-resistance traits tend to be difficult and costly to manipulate in plant-breeding programs, thus making development of experimental genetic lines a daunting prospect.

The limitations identified above might be restated as questions: What to breed for? How best to do it? Advances in plant biotechnology--genetic transformation--could provide timely new approaches to answers, as emerged during a recent workshop organized by Andrew Hanson of the University of Montreal and held at the Rockefeller Foundation's Bellagio Conference Center. The workshop reviewed major areas of plant water research in which progress is currently being made, emphasizing the particular problems of rice under drought, and the opportunities for building on the International Program on Rice Biotechnology. The workshop discussion highlighted the following points:

(a) Especially for tropical rices, there is an urgent need to understand the plant better, in relation to drought at various stages of the life cycle and with respect to the physiological, biochemical and molecular levels of organization.

(b) The genetic resources of rice and the research expertise available in the international rice research network are a great resource. Biotechnology could build upon this resource in two mutually-reinforcing ways: by providing new tools for breeding programs for drought resistance, and by andvancing basic knowledge of water stress responses in rice.

(c) New tools for breeding programs would be provided primarily by incorporating selected physiological traits into the RFLP program. An example here would be the major genes governing deep rooting/water extraction and penetration of compacted soil layers. Once identified and mapped, these genes (which are extremely difficult to score in the field) could be readily tracked in a breeding program via the associated molecular markers.

(d) Advances in basic knowledge of stress responses could come first from using the RFLP map in an analytical way to dissect major components of drought resistance, and second, by creating novel genetic variation by molecular gene transfer. An example of the former would be the identification of

independent sets of genes controlling the same complex trait, such as genes regulating embryo abortion or pollen infertility, both of which cause drought stress-induced sterility. Gene transfer provides the power to manipulate individual genes, and so to probe their biochemical and physiological function. Conceptually akin to the classical approach of comparing isogenic lines, it is in principle faster and more precise. Although more knowledge is needed to design and produce agronomically useful genotypes by gene transfer techniques, some valuable experimental types can be envisioned. One case would be the transfer of genes for betaine synthesis into rice, as rice seems to have lost the capacity to accumulate this osmoprotective compound.

RFLP Training Course Held at Cornell

Twenty-five scientists from national rice research institutions in developing countries and international agricultural research centers participated in a training course at Cornell University on RFLP markers and genetic mapping. The course included lecture and laboratory sessions on plant genomes and restriction fragment length polymorphisms (RFLPs), development of an RFLP linkage map, application of RFLPs in plant breeding, physical mapping and map-based gene cloning, and the setting up of an RFLP laboratory. The particpants were provided with a set of rice RFLP probes to take back to their home institutions to begin their own RFLP research. Thanks go to Steve Tanksley, Gary Kochert, James Price, and Susan McCouch for preparing and conducting the course. Copies of the RFLP Laboratory Manual are available from the Rockefeller Foundation.

Repository for Rice DNA Clones

A note from Steven Tanksley and Thresa Fulton indicates that their lab is maintaining DNA clones derived from research funded by the Rockefeller Foundation. Included in the collection are clones corresponding to the rice RFLP map (see attached map) as well as clones for repetitive elements and known genes isolated by other researchers in the program. They invite researchers to deposit clones by sending a small amount of plasmid along with information about the vector, cloning site, insert size and any other relevant information about the clone along with any references which describe the clone.

Those wishing to obtain rice clones should write to the address below. Please indicate which clones you need. A kit is available which covers the genome at fairly regular intervals. Clones are sent out as a plasmid along with relevant technical information for transferring the clones back into bacterial stocks. Also included will be general information on RFLP techniques. Address:

Dr. Steven D. Tanksley
Department of Plant
Breeding and Biometry
Cornell University
Ithaca, New York 14853

Patent Protection

A workshop on "Equitable Patent Protection for the Developing World" was held at Cornell University on October 2-3, 1989. Four position papers were presented and generated much discussion and debate. Workshop organizer William Lesser is synthesizing the results into a "primer" on the subject. The position papers listed below are available as Cornell Agricultural Economics Staff Paper 89-36.

1) "An Economic Assessment of Patent Law Applied to Developing Countries" by William Lesser, Agricultural Economics, Cornell University.

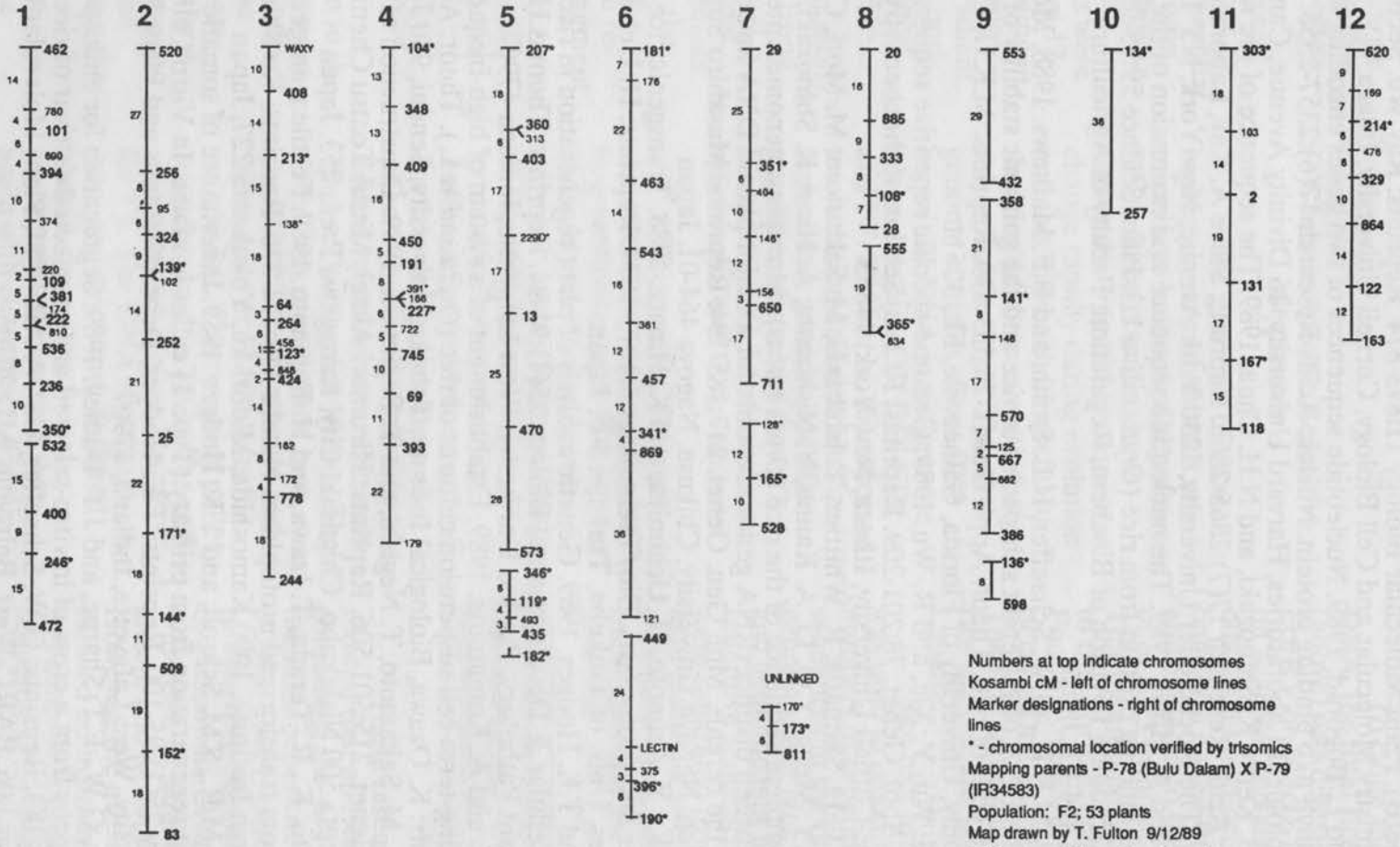
2) "Patent Protection in Developing Countries-An Overview" by Joseph Straus, Max Planck Institute for Foreign and International Patent, Copyright, and Competition Law, Munich.

3) "Intellectual Property Needs of Multinationals" by William H. Duffy, General Patent Counsel, Monsanto Co.

4) "An Overview of Concerns Regarding the Impacts of Patenting Life Forms in the Third World" by Renee Vellve, ICDA Seeds Campaign, Barclona.

SEPTEMBER 1989

RICE RFLP FRAMEWORK MAP-CORNELL UNIVERSITY



Numbers at top indicate chromosomes
Kosambi cM - left of chromosome lines
Marker designations - right of chromosome lines
* - chromosomal location verified by trisomics
Mapping parents - P-78 (Bulu Dalam) X P-79 (IR34583)
Population: F2; 53 plants
Map drawn by T. Fulton 9/12/89
LOD score = 2, based on Mapmaker

References and Reprints

(1)Cote, J.C., N.H. Wu, and R. Wu. 1988. Nucleotide sequence of the rice chloroplast apocytochrome b6 gene (petB). Plant Molecular Biology 11:873-874. Reprints: Ray Wu, Section of Biochemistry,Molecular and Cell Biology, Cornell University, Ithaca, NY 14853

(2)Luan, S., and L. Bogorad. 1989. Nucleotide sequences of two genes encoding the light harvesting chlorophyll a/b binding protein. Nucleic Acids Research 17(6):2357-2358. Reprints: Sheng Luan, The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138

(3)Kay, S.A., B. Keith, K. Shinozaki, and N.H. Chua. 1989. The sequence of the rice phytochrome gene. Nucleic Acids Research 17(7):2865-2866. Reprints: Steve A. Kay, Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399

(4)Kanno, A., and A. Hirai. 1989. The nucleotide sequence and expression of the gene for the 32-kDa quinone-binding protein from rice (*Oryza sativa* L.). Plant Science 59:95-99. Reprints: Atsushi Hirai, Graduate Division of Biochem. Regulation, Faculty of Agriculture, Nagoya University, Nagoya 464-01, Japan

(5)Chowdhury, M.K.U., G.W. Schaeffer, R.L. Smith, and B.F. Mathews. 1988. Molecular analysis of organelle DNA of different subspecies of rice and the genomic stability of mtDNA in tissue cultured cells of rice. Theor. Appl. Genet. 76:533-539. Reprints: M.K.U. Chowdhury, Dept. of Agronomy, University of Florida, Gainesville, FL, 32611

(6)Zhao, X., T. Wu, Y. Xie, and R. Wu. 1989. Genome-specific repetitive sequences in the genus *Oryza*. Theor. Appl. Genet. 78:201-209. Reprints: R. Wu, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York, 14853

(7)Hiratsuka, J., H. Shimada, R. Whittier, T. Ishibashi, M. Sakamoto, M. Mori, C. Kondo, Y. Honji, C.R. Sun, B.Y. Meng, Y.Q. Li, A. Kanno, Y. Nishizawa, A. Hirai, K. Shinozaki, and M. Sugiura. 1989. The complete sequence of the rice (*Oryza sativa*) chloroplast genome: intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. Mol. Gen. Genet. 217:185-194. Reprints: Masahiro Sugiura, Center for Gene Research, Nagoya University, Chikusa, Nagoya 464-01, Japan

(8)Toriyama, K., Y. Arimoto, H. Uchimiya, and K. Hinata. 1988. Transgenic rice plants after direct gene transfer into protoplasts. Bio/Technology 6:1072-1074. Reprints: H. Uchimiya, Institute of Bio. Sciences, Univ. of Tsukuba, Tsukuba 305, Japan.

(9)Peng, J., and T.K. Hodges. 1989. Genetic analysis of plant regeneration in rice (*Oryza sativa* L.). *In Vitro* Cellular & Developmental Biology 25(1):91-94. Reprints: Thomas Hodges, Dept. Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907

(10)Osawa, K. and A. Komamine. 1989. Establishment of a system of high-frequency embryogenesis from long-term cell suspension cultures of rice (*Oryza sativa* L.). Theor. Appl. Genet. 77:205-211. Reprints: K. Ozawa, Biological Insitute, Tohoku University, Sendai, 980 Japan.

(11)Akagi, H., M. Sakamoto, T. Negishi, and T. Fugimura. 1989. Construction of rice cybrid plants. Mol. Gen. Genet. 215:501-506. Reprints: Hiromori Akagi, Mitsui Toatsu Chemicals, Plant Biotechnology Dept., 500 Nishikubo, Chigasaki City, Kanagawa Pref., 253, Japan

(12)Shimamoto, K., R. Terada, T. Izawa, and H. Fujimoto. 1989. Fertile transgenic rice plants regenerated from transformed protoplasts. Nature 338:274-276. Reprints: Ko Shimamoto, Plantech Research Institute, 1000 Kamoshida, Midori-ku, Yokohama 227, Japan

(13)Willman, M.R., S.M. Schroll, and T.K. Hodges. 1989. Inheritance of somatic embryogenesis and plantlet regeneration from primary (Type 1) callus in maize. In Vitro Cellular & Developmental Biology 25(1):95-100. Reprints: T.K. Hodges, Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, 47907

(14)Schaeffer, G.W., F.T. Sharpe, and J.T. Dudley. 1989. Segregation for endosperm lysine in F_2, F_3, and F_4 progeny from a cross of in vitro-selected and unselected cultivar of rice. Theor. Appl. Genet. 77:176-183. Reprints: G.W. Schaeffer, USDA-ARS-PSI, Plant Molecular Biology Laboratory, Building 006, BARC-West, Beltsville, MD 20705

(15a)Jones, T.J., and Thomas L. Rost. 1989. Histochemistry and ultrastructure of rice (*Oryza sativa*) zygotic embryogenesis. Amer. J. Bot. 76(4):504-520. Reprints: Todd J. Jones, Dept. of Genetics, University of California, Berkeley, CA 94720

(15b)Jones, T.J., and T.L. Rost. 1989. The developmental anatomy and ultrastructure of somatic embryos from rice (*Oryza sativa* L.) scutellum epithelial cells. Bot. Gaz. 150(1):41-49. Reprints: Todd Jones, Dept. of Genetics, University of California, Berkeley, CA 94720

(16)Kay, S.A., A. Nagatani, B. Keith, M. Deak, M. Furuya, and N.H. Chua. 1989. Rice phytochrome is biologically active in transgenic tobacco. The Plant Cell 1:775-782. Reprints: Steve A. Kay, The Laboratory of Plant Molecular Biology, The Rockefeller University, New York, New York, 10021

(17)Dewey, F.M., M.M. MacDonald, and S.I. Phillips. 1989. Development of monoclonal-antibody-ELISA, -DOT-BLOT AND -DIP-STICK immunoassays for *Humicola lanuginosa* in rice. Jr. General Microbiology 135(2):361-374. Reprints: F.M. Dewey, Dept. Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RA, UK

(18)Toriyama, S., and Y. Watanabe. 1989. Characterization of single- and double-stranded RNAs in particles of rice stripe virus. J. Gen. Virol. 70:505-511. Reprints: Shigemitsu Toriyama, Division of Microbiology, National Institute of Agro-Environmental Sci., 1-1 Kannondai 3-chome, Tsukuba-shi, Ibaraki 305, Japan

(19)Takeda, Y., S. Hizukuri, and B.O. Juliano. 1989. Structures and amounts of branched molecules in rice amyloses. Carbohydrate Research 186:163-166. Reprints: Yasuhito Takeda, Dept. Agri. Chemistry, Faculty of Agriculture, Kagoshima University, Korimoto-1, Kogoshima 890, Japan

(20)Masumura, T., D. Shibata, T. Hibino, T. Kato, K. Kawabe, G. Takeba, K. Tanaka, and S Fujii. 1989. cDNA cloning of an mRNA encoding a sulfur-rich 10 kDa prolamin polypeptide in rice seeds. Plant Molecular Biology 12:123-130. Reprints: Takehiro Masumura, Dept. Biochemistry, College of Agricultural Chemistry, Kyoto Predectural University, Shimogamo, Kyoto 606, Japan

(21)Wang, Y.C., T.M. Klein, M. Fromm, J. Cao, J.C. Sanford, and R. Wu. 1988. Transient expression of foreign genes in rice, wheat and soybean cells following particle bombardment. Plant Molecular Biology 11:433-439. Reprints: Ray Wu, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853.

(22)Raghavan, V. 1989. mRNAs and a cloned histone gene are differentially expressed during anther and pollen development in rice (*Oryza sativa* L.). Journal of Cell Science 92:217-229. Reprints: Raghavan, Dept. of Botany, The Ohio State University, Columbus, OH 43210

(23)Fourre, J.L., and J. Lhoest. 1989. Protein synthesis and modification by heat in rice cell culture. Plant Science 61:69-74. Reprints: J. Lhoest, Catholic University of Louvain, Cytogenetics Laboratory, Institute Carnoy, Croix du Sud, 4, B-1348 Louvain-la-Neuve, Belgium

(24)Sakamoto, W., M. Momose, N. Tsutsumi, S. Tano, and H. Yamaguchi. 1989. Analysis of homology of small plasmid-like mitochondrial DNAs in the different cytoplasmic male sterile strains in rice. Jpn. J. Genet. 64:49-56. Reprints: Wataru Sakamoto, Faculty of Agriculture, Univ. of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

(25)Nederlof, P.M., D. Robinson, R. Abuknesha, J. Weigant, A.H.N. Hopman, H.J. Tanke, and A.K. Raap. 1989. Three- color fluorescence *in situ* hybridization for the simultaneous detection of multiple nucleic acid sequences. Cytometry 10:20-27. Reprints: P.M. Nederlof, Department of Cytochemistry and Cytometry, University of Leiden, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands.

(26)Schachtman, D.P., A.J. Bloom, and J. Dvorak. 1989. Salt-tolerant *Triticum* X *Lophopyrum* derivatives limit the accumulation of sodium and chloride ions under saline-stress. Plant, Cell and Environment 12:47-55. Reprints: Bloom, Dept. Veg. Crops, Univ. of Calif., Davis, CA 95616.

(27)Martin, B., J. Nienhuis, G. King, and A. Schaefer. 1989. Restriction fragment length polymorphisms associated with water use efficiency in tomato. Science 243:1725-1728. Reprints: Bjorn Martin, Native Plants, Inc., 417 Wakara Way, Salt Lake City, UT 84108

(28)McFadden, G.I. 1989. *In Situ* Hybridisation in Plants: From Macroscopic to Ultrastructural Resolution. Cell Biology International Reports 13(1):3-21. Reprints: McFadden, Plant Cell Bio. Ctr., School of Botany, University of Melbourne, Parkville, Victoria 3052, Australia.

(29)Cassab, G.I., and J.E. Varner. 1989. Tissue printing on nitrocellulose paper: a new method for immunolocalization of proteins, localization of enzyme activities and anatomical analysis. Cell Biology International Reports 13(1):147-152. Reprints: Gladys Cassab, Plant Biology Program, Dept. of Biology, Washington University, St. Louis, MO 63130

(30)Viegas-Pequignot, E., B. Dutrillaux, H. Magdelenat, and M. Coppey-Moisan. 1989. Mapping of single-copy DNA sequences on human chromosomes by *in situ* hybridization with biotinylated probes: enhancement of detection sensitivity by intensified-fluorescence digital-imaging microscopy. Proc. National Academy of Science USA 86:582-586. Reprints: Evani Viegas-Pequignot, U.R. 620, Structure et Mutagenese Chromosomiques, Section de Biologie, Institut Curie, 26 rue d'Ulm, 75231 Paris Cedex 05, France.

(31)Emmerich, P., P. Loos, A. Jauch, A.H.N. Hopman, J. Wiegant, M.J. Higgins, B.N. White, M. Van der Ploeg, C. Cremer, and T. Cremer. 1989. Double *in situ* hybridization in combination with digital image analysis: a new approach to study interphase chromosome topography. Reprints: Patricia Emmerich, Institute of Anthropology and Human Genetics, University of Heidelberg, INF 328, D-6900 Heidelberg, Federal Republic of Germany

(32)Lizardi, P.M., C.E. Guerra, H. Lomeli, I. Tussie-Luna, and F.R. Kramer. 1988. Exponential amplification of recombinant-RNA hybridization probes. Bio/Technology 6:1197-1202. Reprints: Fred Russell Kramer, Dept. of Molecular Genetics, Public Health Research Institute, 455 First Avenue, New York, NY 10016

(33)Frye, R., P. Cogswell, and E. Liu. 1989. The analysis of mutations in the ras gene family using the polymerase chain reaction (PCR). Clontech Cruiser 1(1):1-6. Reprints: Roy Frye, 227 Lineberger Cancer Research Center, Univ. of North Carolina, Chapel Hill, NC 27599

(34)Tavazza, R., M. Tavazza, R.J. Ordas, G. Ancora, and E. Benvenuto. 1989. Genetic transformation of potato (*Solanum tuberosum*): an efficient method to obtain transgenic plants. Plant Science 59:175-181. Reprints: Eugenio Benvenuto, ENEA, Dept. Agrobiotechnology, Divisione Ingegneria Genetica, C.P. 2400, Roma A.D., Italy

(35)Garza, D., J.W. Ajioka, J.P. Carulli, R.W. Jones, D.H. Johnson, and D.L. Hartl. 1989. Physical mapping of complex genomes. Nature 340:577-578. Reprints: Dan Garza, Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110-1095

(36)Wagner, P.M., B. Oskouian, and G.C. Stewart. 1989. *In situ* removal of RNA during agarose gel electrophoresis. BioTechniques 7(4):338-339. Reprints: Patricia Wagner, Dept. of Microbioloby, University of Kansas, Lawrence, KS 66045

(37)Bird, R.C., and G. Wu. 1989. An alternative to the dry ice/ethanol bath for the precipitation of nucleic acids. BioTechniques 7(4):337-338. Reprints: R. Curtis Bird, Dept. of Pathology, College of Veterinary Medicine, Auburn University, AL 36849

(38)Wong, C.K. 1989. A new approach to chromosome doubling for haploid rice plants. Theor. Appl. Genet. 77:149-151. Reprints: C.K. Wong, 618 Harris Place NW, Calgary, Alberta T3B 2V4, Canada

(39)Perrot-Rechenmann, C., M. Joannes, D. Squalli, and P. LeBacq. 1989. Detection of phosphoenolpyruvate and ribulose 1,5-bisphosphate carboxylase transcripts in maize leaves by *in situ* hybridization with sulfonated cDNA probes. The Journal of Histochemistry and Cytohistochemistry 37(4):423-428. Reprints: Catherine Perrot-Rechenmann, Unite de Genetique de la Differenciation, Institut Pasteur, 25, Rue du Dr Roux, 75724 Paris Cedex 15, France

(40)Johns, M.B., and J.E. Paulus-Thomas. 1989. Purification of human genomic DNA from whole blood using sodium perchlorate in place of phenol. Analytical Biochemistry 180:276-278. Reprints: Jeanne E. Paulus-Thomas, Dept. Medical Genetics, Western Pennsylvania Hospital, Pittsburgh, Penn. 15224

(41)Czichos, J., M. Kohler, B. Reckmann, and M. Renz. 1989. Protein-DNA conjugates produced by UV irradiation and their use as probes for hybridization. Nuclei Acids Research 17(4):1563-1572. Reprints: Bernd Reckmann, Medizinisch-Technisches Laboratorium, Denagen, Im Neuenheimer Feld 517, D-6100 Darmstadt, Federal Republic of Germany.

(42)Richterich, P. 1989. Non-radioactive chemical sequencing of biotin labelled DNA. Nucleic Acids Research 17(6):2181-2186. Reprints: Peter Richterich, Fakultat fur Biologie, Universitat Konstanz, D-7750 Konstanz, Federal Republic of Germany

(43)Lee, A.S.G., and J.O'D. McGee. 1989. The signal intensity on Southern blots developed by nonisotopic methods is linear with time and quantity of DNA. Nucleic Acids Research 17(6):2364. Reprints: A. Lee, University of Oxford, Nuffield Dept. Path. and Bact., John Radcliffe Hospital, Headington, Oxford OX3 9DU, England

IN THE NEXT ISSUE

Visualizing mRNA expression in plant protoplasts: factors influencing efficient mRNA uptake and translation. Gallie, Lucas, and Walbot. The Plant Cell 1:301-311, 1989.

Stable transformation of maize: the impact of feeder cells on protoplast growth and transformation efficiency. Lyznik, Kamo, Grimes, Ryan, Chang, and Hodges. Plant Cell Reports 8:292-295, 1989.

A small-scale procedure for the rapid isolation of plant RNAs. Verwoerd, Dekker, and Hoekema. Nucleic Accids Research 17(6):2362, 1989.

A study on isolation and culture of pollen protoplasts. Zhou. Plant Science 59:101-108, 1989.

Effects of cold-treatment on protein synthesis and mRNA levels in rice leaves. Hahn and Walbot. Plant Physiology 91:930-938, 1989.

Sequence of the chloroplast *rps* 14 gene encoding the chloroplast ribosomal protein S14 from rice. Cote and Wu. Nucleic Acids Research 17(4):1780, 1989.

Regeneration of indica rice (*Oryza sativa* L.) from primary callus derived from immature embryos. Koetje, Grimes, Wang, and Hodges. J. Plant Physiology 135:184-190, 1989.

Magnetic separation of DNA. Uhlen. Nature 340:733-734, 1989.

Asymmetric polymerase chain reaction. McCabe. Amplifications 3:1-3, 1989.

Plant regeneration from indica rice (*Oryza sativa* L.) protoplasts. Lee, Schroll, Grimes, and Hodges. Planta 178:325-333, 1989.

Immunocytochemical detection of DNA synthesis in plant cells. Wang, Cutler, Saleem and Fowke. J. Plant Physiology 135:15-20, 1989.

Stable co-transformation of maize protoplasts with *gus*A and *neo* genes. Lyznik, Ryan, Ritchie, and Hodges. Plant Molecular Biology 13:151-161, 1989.

Quantitative fluorescence of DNA-intercalated ethidium bromide on agarose gels. Ribeiro, Larcom, and Miller. Analytical Biochemistry 181:197-208, 1989.

Colored silver-intensified gold technique for light microscopy. Bertsch, Bialecki, Emmons, and Korytko. BioTechniques 6(5):448-451, 1989.

Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Newton, Grahm, Heptinstall, Powell, Summers, Kalsheker, Smith, and Markham. Nucleic Acids Research 17(7):2503-2516, 1989.

An automated analysis method for *in situ* hybridization using high-resolution image analysis. Stolz, Scharffetter, Koditz, and Drieg. Arch. Dermatol. Res. 281:336-341, 1989.

DNA restriction fragment length polymorphisms: a strategy for genetic mapping of D genome of wheat. Kam-Morgan and Gill. Genome 32:724-732, 1989.