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Probe

Newsletter for the USDA Plant Genome Research Program

Volume 2, No. 3 Fall 1992

FDA's Policy on Foods Derived From New Plant Varieties

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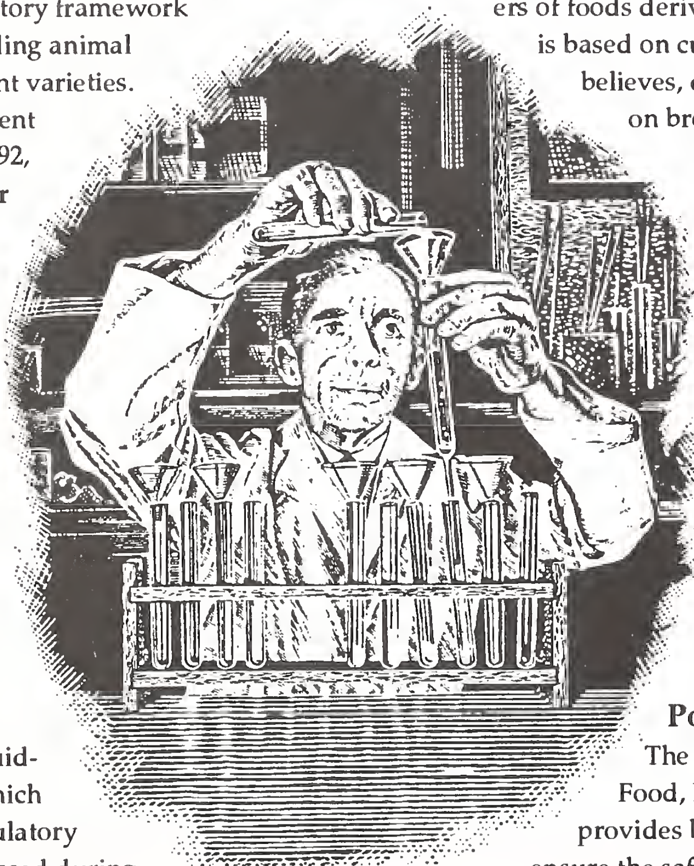
Dr. James H. Maryanski, Biotechnology Coordinator
Center for Food Safety and Applied Nutrition;
Dr. Eric L. Flamm, Office of Biotechnology; and
Dr. Linda S. Kahl, Division of Food and Color Additives
Center for Food Safety and Applied Nutrition;
Food and Drug Administration
Washington, DC

The Food and Drug Administration (FDA) recently clarified its legal and regulatory framework for oversight of food (including animal feed) derived from new plant varieties.

An FDA policy statement published in the May 29, 1992, issue of the **Federal Register** (57 FR 22984) addresses foods--such as fruits, vegetables, grains, and their byproducts--derived from any new plant variety, whether developed by traditional breeding techniques or cellular or molecular techniques.



Standard of Care Established

Of particular importance to producers is the policy's Guidance to Industry section, which describes scientific and regulatory issues that should be addressed during the development of new crop varieties. This section establishes a "standard of care" for producers of foods derived from new plant varieties. It is based on current practices and, FDA believes, does not impose new burdens on breeders.

This policy is based on FDA's understanding of current developments in agricultural research and is intended to be sufficiently flexible to accommodate rapid advances in this field. The scientific principles that underpin FDA's policy were discussed in an article in *Science* magazine (see Kessler, et al., 256:1747, June 26, 1992). FDA invites comments on the policy.

Postmarket Authority

The policy explains that the Federal Food, Drug, and Cosmetic Act provides broad authority to FDA to ensure the safety and wholesomeness of foods and food ingredients. FDA regulates the safety of

foods primarily under the agency's postmarket authority under the adulteration provisions of Section 402(a)(1) of the act. This section provides FDA with authority to remove unsafe foods from the market and places a legal duty upon producers to ensure that the foods they market are safe and wholesome.

Food Additives

FDA also has pre-market approval authority for food additives, stemming from Section 409 of the act. Under the food additive provisions, all substances intentionally introduced into food must undergo pre-market approval unless they are generally recognized as safe (GRAS). The policy statement makes clear that substances introduced by breeding are subject to these provisions just as are chemical additives introduced during processing.

Labeling Requirements

The act also contains labeling requirements. Section 403 of the act requires that a producer of a food product describe the product by its common name, and reveal all facts that are material in light of representations made or suggested by labeling or with respect to consequences that may result from its use.

FDA's policy does not contemplate special "genetically engineered" labeling for foods derived from plants developed via recombinant DNA methods. Historically, FDA has not considered plant breeding techniques to be material information subject to labeling. Such labeling would not provide information about the composition of the food and often would be impractical.

For example, wheat varieties developed with different techniques would have to be segregated during production, storage, distribution, and processing. The difficulties would be magnified as varieties developed with different techniques are crossed in breeding programs.

Rather, FDA believes that food labeling should identify significant changes in composition or safety or usage issues. So, for example, if wheat gluten were introduced into potatoes, labeling would be required so that consumers sensitive to gluten, such as those with celiac sprue, would be able to avoid those potatoes and products containing those potatoes.

Industry Guidance

The Guidance to Industry section addresses principal safety and regulatory issues through a series of flowcharts and accompanying text. Briefly, this section points out that developers should initially consider the characteristics of the host plant that is being modified, the donor organism that is contributing genetic information, and the genetic material and substances being introduced or modified.

Based on this information, the developer can then decide what other information may be needed to evaluate the safety and regulatory status of food derived from the new plant variety.

For example, the developer should evaluate whether or not existing varieties of the host plant are known to produce toxicants at unsafe levels and, if so, ensure that the levels of these toxicants in the new variety are within acceptable limits. Similarly, if the donor organism produces undesirable toxicants, the developer should ensure that the genes for these toxicants were not introduced or that the new variety does not produce unacceptable levels of such toxicants.

The guidance section provides criteria by which developers can determine whether a substance

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intentionally introduced or altered by genetic modification will require pre-market approval as a food additive. Presently, these substances are primarily proteins, fats and oils, and carbohydrates since they are the focus of plant breeding programs using the newer molecular techniques.

In general, the policy states that newly introduced or modified proteins of known function would not require FDA review if they are derived from food sources or are substantially the same as food substances; are not known to be toxic or raise food safety concerns; and will not be a major constituent of the diet.

New carbohydrates with unusual structural or functional groups or oils that contain new, unusual fatty acids may also require pre-market approval as food additives.

The guidance section also identifies other instances where FDA consultation is required. For example, producers may need to consult FDA about test protocols for allergens when genetic material from a commonly allergenic food has been introduced into a new variety. Generally, modifications to carbohydrates do not raise safety questions that would warrant consultation with FDA, unless the digestibility or nutritional value of a carbohydrate has been altered and the substance is likely to be a major constituent of the diet.

When producers modify fats or oils to the extent that they are likely to be a major constituent of the diet, they should contact FDA to determine if a safety issue exists (for example, if the modification results in a change in digestibility) or if labeling will be required (for example, if the composition is no longer representative of fats and oils ordinarily obtained from that crop).

Consultations Encouraged

These are some of the safety and regulatory issues discussed in the policy statement. Because of the potential consequences that may arise if FDA challenges a product on safety or legal grounds, producers routinely consult with the agency before introducing new products. FDA encourages such consultations, especially when new scientific techniques are under development.

For FDA contact see page 18

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Managing Editor
Susan McCarthy, Ph.D.

Editor
Carolyn Bigwood

Production Manager
Terrance Henrichs

Layout and Design
Terrance Henrichs

Special Thanks to:
Bruce Morrissey
Joe Lapovski
Barbara Buchanan
Joanne Mell
Lori Star

Articles, announcements, and suggestions are welcome.

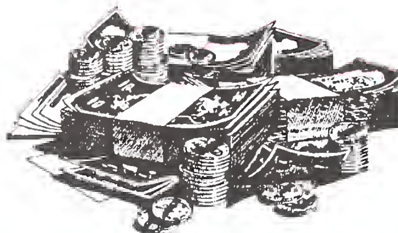
Correspondence Address
Susan McCarthy, Ph.D.
NAL, Room 1402
10301 Baltimore Blvd
Beltsville, MD 20705
Phone:(301)504-6875
FAX: (301)504-7098
email: smccarthy@asrr.arsusda.gov

USDA Program Office
Dr. Jerome Miksche
USDA/ARS/NPS/PNRS
Room 331C, Bldg 005
BARC-WEST
Beltsville, MD 20705
Phone:(301)504-6029
FAX: (301)504-6231

Competitive Edge

NRICGP: FY 1993 Applications Invited

Anne Datko
Program Director
National Research Initiative Competitive Grants Program
Cooperative State Research Service, USDA
Washington, DC



The National Research Initiative Competitive Grants Program (NRICGP) invites applications for competitive grant awards in agricultural, forestry, and related environmental sciences in fiscal year 1993. Specific program areas, postmark deadlines, and telephone contacts are given in the accompanying table* on page 5.

Copies of the 1993 Program Description and Guidelines for Proposal Preparation, and Grant Application Kit may be requested from the Proposal Services Branch, Awards Management Division, Cooperative State Research Service, U.S. Department of Agriculture, Room 303, Aerospace Center, Washington, DC 20250-2200; Phone (202) 401-5048.

*(Please note that the postmark deadline for applications in the Plant Genome program area is December 14, 1992.) ◆

FYI

Participants "Survive" Plant Biochemistry Course

Dr. J. E. Varner
Department of Biology
Washington University
St. Louis, MO

Graduate students, postdoctoral fellows, and other interested individuals had the opportunity to test their endurance while increasing their knowledge of plant biochemistry as participants in a nonstop, 3-week course, Plant Biochemistry 1992.

Held in La Jolla, CA, June 28 through July 19, the comprehensive course was organized in response to the belief that full exploitation of the ongoing activities in plant genetics and molecular biology requires further training and research in plant biochemistry.

Some students proclaimed themselves "survivors" after completing the course, which included 120 hours of lectures given by top plant biochemists from universities and various industries.

Lecturers

Dr. James Bonner presented the first lecture. His book, "Plant Biochemistry" (1950, 1965, and 1976, Academic Press, New York), provided inspiration for many of the subsequent speakers.

Cont. on page 22 ➤

Table 1

National Research Initiative Competitive Grants Program
Submission Deadlines

Postmarked Dates	Program Codes	Program Areas	Contacts (202)
December 7, 1992	23.0	Forest/Rangeland/Crop Ecosystems	401-5114
	51.1	Pathology	401-4310
	51.4	Weed Science	401-4310
December 14, 1992	**52.1**	**Plant Genome**	**401-4871**
December 21, 1992	31.0	Human Nutrient Requirements for Optimal Health	205-0250
	52.2	Plant Genetic Mechanisms and Molecular Biology	401-5042
January 11, 1993	43.0	Animal Molecular Genetics and Gene Mapping	401-4399
	54.1	Photosynthesis and Respiration	401-6030
January 19, 1993	41.0	Reproductive Biology of Animals	401-6234
	51.2	Entomology	401-5114
	51.3	Nematology	401-5114
January 25, 1993	22.1	Plant Responses to the Environment	401-4871
	55.0	Alcohol Fuels	401-4310
February 1, 1993	21.0	Water Quality	401-4082
	24.0	Improved Utilization of Wood and Wood Fiber	401-1952
February 8, 1993	61.0	Market Assessments, Competitiveness, and Technology Assessment	401-4772
	62.0	Rural Development	401-4425
February 16, 1993	53.0	Plant Growth and Development	401-5042
February 22, 1993	42.0	Cellular Growth and Developmental Biology of Animals	205-0250
	44.0	Mechanisms of Animal Disease	401-4399
March 1, 1993	71.0	Processing for Value-Added Products	401-1952
March 15, 1993	54.2	Nitrogen Fixation/Metabolism	401-6030
	32.0	Food Safety	401-4399
March 22, 1993	80.1	Research Career Enhancement Awards	401-6234
	80.2	Equipment Grants	401-6234
	80.3	Seed Grants	401-6234

Programmatic questions regarding the Special Research Grants Water Quality Program should be directed to (202) 401-4504.

20th *Annual Meeting of the*

Plant Growth Regulator Society of America

Featuring:

Symposia and research reports on a variety of topics related to plant growth regulation.

When:

August 6-9, 1993

Where:

Clarion Hotel
St. Louis, Missouri

Symposium Topics:

Plant Growth Regulators in Post-harvest Biology and Technology of Horticultural Crops. Speaker: Dr. Adel Kader, University of California, Davis, CA

The Role of Plant Growth Regulators in the Development and Reproduction of Cereal Plants. Speaker: Dr. David Ho, Washington University, St. Louis, MO

Research Reports:

Original research reports are invited in all areas of plant growth regulation. The Society will award prizes of $300 and $100 for the two best student papers. All reports will be published in the Society's proceedings.

For futher information, contact Dr. Louise Ferguson, Program Chair, University of California, Kearny Agricultural Research Center, 9240 S. Riverbend Avenue, Parlier, CA 93648, telephone (209) 891-2500

Vocabulary Control in the Plant Genome Database

Dr. C. Rose Broome, Database Specialist
Plant Genome Data and Information Center
National Agricultural Library, USDA
Beltsville, MD

A filing system is a successful one only if its users can easily find the information they need within it.

The most natural and direct way of accessing a document or other information source is by subject matter. Folders, library card catalog entries, or database records can be filed (physically or logically) according to some kind of subject classification. As long as the classification scheme used by the "filer" is the same as that employed by the "retriever," what goes in can be extracted "out" selectively and usefully.

Computerized databases have proved to be superior filing systems, allowing fast access to information in many ways, no matter how the records are physically stored. A record may have several subject terms assigned to it by the "filer" (or more correctly, the *indexer*), who places the terms in a special database field that is often labeled "Descriptor," "Keyword," or "Subject Heading." This field is analogous to the subject index that is found in the back of a book, and it may be used by the "retriever" (or *searcher*) to locate the precise kind of information desired.

This system of indexing works well only if the searcher asks for the record using one or more of the exact terms the indexer chose to classify the information in the record. But even two experts in the same subject matter may not use exactly the same word or phrase to describe the contents of a document. One geneticist may use the term "b chromosomes" whereas another might choose "supernumerary chromosomes" as a subject term. One may think of a term in the singular form (e.g., "leaf"); another may use the plural form ("leaves"). Regional spellings may differ ("color" vs. "colour"; "aluminum" vs. "aluminium"), as may regional usage ("lucerne" in Great Britain vs. "alfalfa" in the United States).

Controlled Vocabularies

The library and information science community has addressed the problems inherent in subject access by adopting the use of "controlled vocabularies," lists of acceptable subject terms that must be used by indexer and searcher alike. The indexer, armed with a knowledge of the subject matter and with a list of subject terms, selects the most appropriate terms with which to classify the item and enters them into the subject field of the database record. The searcher, armed with some degree of knowledge about the subject and with a copy of the same controlled vocabulary list, then selects from the subject field those terms that most closely match the precise subject matter of interest. Precision in retrieval is greatly aided if one knows the exact form and spelling of a subject term.

In a well-constructed vocabulary list, one term should represent one concept. The term chosen to characterize a data record may consist of one word (e.g., "aneuploidy"), or the concept may be represented by a multi-word phrase (e.g., "single-seed descent" or "ornamental woody plants"). The thing represented is conceptualized by the indexer and the searcher alike as a unitary class within the context of the subject matter.

Plant Genome Database

Controlled vocabularies may consist of lists containing a few well-defined categories. For example, in the Plant Genome Database (PGD) being

developed at NAL, a field called "genome type" has a controlled vocabulary of only three terms: "nuclear," "chloroplast," and "mitochondrial." Fields such as "linkage-group type," "map type," and "stock type" (for genetic stock collections) have longer but fairly brief, stable lists of acceptable terms. But such fields as "phenotypic trait" (containing such values as "flower color," "yield," "chromosome number," and "seed weight") may eventually have hundreds or thousands of acceptable terms--some of them common to more than one species of organism, but others being unique characteristics of but one species.

In its initial phase, PGD will include data on only a small number of vascular plant species, so vocabulary control will, at first, be relatively simple. But over the next few years the database is expected to expand to include data on the genetics and biology of an, as yet, undetermined number of agriculturally significant plant, animal, and microbial species. The more species (and their specialized terminologies) that are added, the more challenging becomes the work of controlling the terminology to permit selective retrieval of information.

Problems

Several problems arise when one attempts to merge several precise, highly technical vocabularies into a general-purpose vocabulary of biological terms.

The English language is rich and complex, and filled with such words as homographs (words spelled the same way, but with multiple meanings) and synonyms or near-synonyms. In building a controlled vocabulary of terms that describe the morphology and anatomy of plants, animals, and microorganisms, some ambiguities will need to be resolved such as the word "ear" (the infructescence of the corn plant or the auditory organ of a mammal?) and "cob" (part of that "ear" on the corn plant or a kind of small horse?).

Large and complex controlled vocabularies, including lists of gene symbols, names of chemical components, metabolic processes/pathways, and lists of accepted scientific names for organisms, will be required for other data elements in PGD. These vocabularies will of necessity be developed by the collaborative efforts of many biologists over a considerable time. Fortunately these will not all have to be created *de novo*, as many published thesauri, glossaries, and dictionaries exist that cover the disciplines to be represented in PGD. These authority lists will be carefully examined to see if they may be incorporated into what may ultimately become a full-blown thesaurus for agricultural genomics.

CAB Thesaurus

NAL uses the CAB Thesaurus, published in England by CAB International, to index journal articles for the AGRICOLA database. To search AGRICOLA for articles on a particular subject, one may obtain a copy of the CAB Thesaurus [from CAB International, 845 N. Park Avenue, Tucson AZ 85719, telephone 800-528-4841] and find out exactly what terms are used in the "Descriptor" field to describe subjects of interest to the searcher. By using precise terminology, the searcher can attain great precision in retrieval and avoid "false drops."

The scope of the CAB Thesaurus covers all of agriculture. Because of its breadth, it is presently inadequate as a single source of controlled vocabulary terms for a database so detailed as PGD. However, it may serve as a starting point. More detailed hierarchies of terms may be added to PGD vocabularies by the collaboration of scientists contributing data to the database.

Nomenclatural Lists

Standardized vocabulary and nomenclature lists are being developed by several international organizations for such data types as gene names (International Society of Plant Molecular Biology), plant names in common use (International Union of Biological Sciences), and enzyme nomenclature (International Union of Biochemistry). PGD will utilize pertinent nomenclatural lists sanctioned by the International Unions for use as authority files for the appropriate database fields.

Vocabulary Development

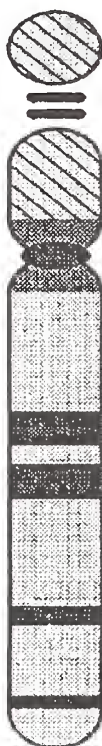
As more different species of life forms are accommodated in PGD, more problems will be inevitable in vocabulary control. However, to enable geneticists to search across species for common genetic factors, mechanisms, and expressions, the terms chosen for description must allow them, whenever possible, to

Cont. on page 17➤

Call for Papers

The First International Conference on

Intelligent Systems for Molecular Biology



July 7-9, 1993
Washington DC

Organizing Committee

Lawrence Hunter,
National Library of Medicine
David Searls,
University of Pennsylvania
Jude Shavlik,
University of Wisconsin

Schedule

Papers and Tutorial Proposals Due:
February 15, 1993
Replies to Authors:
March 29, 1993
Revised Papers Due:
April 26, 1993
Conference: *July 7-9, 1993, National Library of Medicine, Bethesda, Maryland*

Program Committee

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F. Cohen, *UC-SF*
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K. Nitta, *ICOT*
M. Noordewier, *Rutgers*
R. Overbeek, *Argonne*
C. Rawlings, *ICRF*
D. Sleeman, *Aberdeen*
D. States, *Wash. U.*
G. Stormo, *U. of Colorado*
E. Uberbacher, *Oak Ridge*
D. Waltz, *Thinking Machines*

The First International Conference on Intelligent Systems for Molecular Biology will take place in Washington, DC, July 7-9, 1993 . The conference will bring together scientists who are applying the technologies of artificial intelligence, robotics, neural networks, massively parallel computing, advanced data modelling, and related methods to problems in molecular biology. Participation is invited from both producers and consumers of any novel computational or robotic system, provided it supports a task in molecular biology that is cognitively challenging, involves a synthesis of information from multiple sources at multiple levels, or in some other way exhibits the abstraction and emergent properties of an "intelligent system." The three-day conference, to be held in the attractive conference facilities of the Lister Hill Center, National Library of Medicine, National Institutes of Health, will feature both introductory tutorials and original, refereed papers, to be published in an archival Proceedings. The conference will immediately precede the Eleventh National Conference of the American Association for Artificial Intelligence, also in Washington.

Papers should be 12 pages, single-spaced and set in 12 point type, including title, abstract, figures, tables, and bibliography. The first page should give keywords, postal and electronic mailing addresses, telephone, and FAX numbers. Submit 6 copies to the address shown. For more information, contact ISMB@nlm.nih.gov.

Jude Shavlik
Computer Sciences Dept.
University of Wisconsin
1210 W. Dayton Street
Madison, WI 53706

Sponsored by AAAI, NLM, and the BioMatrix Society

Touching Base with J. Michael Cherry

Codon Usage and Splice-Site Tables Available From AAtDB

Dr. J. Michael Cherry and Dr. Sam Cartinhour, Department of Molecular Biology Massachusetts General Hospital and Department of Genetics, Harvard Medical School Boston, MA

The ACeDB software used by the *Arabidopsis thaliana* database, AAtDB, includes several DNA and protein analysis utilities. Shown here are two examples, which were produced by the ACeDB software.

The utilities make use of the GenBank/EMBL features to identify coding regions and splice-site junctions. The software does not search sequences for these features. Both the splice-site table and the codon usage table can be determined either for all *Arabidopsis* sequences in the database or any user defined subset. As new sequences are added, it becomes an easy matter to re-calculate the tables.

The RNA splice-site consensus utility uses the GenBank/EMBL features table entries to identify exon-intron junctions. The utility tabulates the results. The example provided in table 1 was produced using all the *Arabidopsis* sequences currently found in AAtDB. The table is a frequency distribution. To find the most probable 5' exon-intron sequence, for example, simply find the highest frequency nucleotide for each position. In this example, the consensus sequence is: AAAG | GTAAGTT.

The codon usage table shown in table 2 was also produced using all *Arabidopsis* sequences currently in AAtDB. The ACeDB utility relies in this case on the GenBank/EMBL feature tables to identify the protein coding regions. The results are then tabulated. (See table 2.) ◆

Table 1. Exon-Intron Juctions

I scanned 209 sequences, containing 341 introns

5'consensus

					--- intron --->						
A	91	130	190	41	12	13	217	190	84	83	108
C	85	102	48	13	9	6	19	54	30	53	66
G	74	45	35	244	306	14	37	24	164	43	44
T	89	62	66	41	12	306	66	71	61	160	121
X	0	0	0	0	0	0	0	0	0	0	0

3'consensus

	--- intron --->										
A	59	69	61	111	31	304	21	76	76	95	86
C	52	49	35	29	174	11	8	55	61	53	52
G	72	55	36	114	11	12	301	159	66	80	114
T	157	167	208	86	124	13	10	50	136	111	87
X	0	0	0	0	0	0	0	0	0	0	0

Table 2. Codon Usage

Codon Usage

```
 I scanned 313 sequences, containing 239 coding sequences

 Amino acids usage:

Hydrophilic 49.8%
   Basic 13.4%
       Lys  6.4%  Arg  5.1%  His  1.9%
   Acidic 11.4%
       Asp  5.2%  Glu  6.2%
   Neutral 25.0%
       Asn  4.0%  Gln  3.5%  Cys  1.7%  Met  2.6%  Ser  7.7%  Thr  5.5%

Hydrophobic 49.6%
   Aliphatic 41.4%
       Gly  7.8%  Ala  7.6%  Val  6.9%  Pro  4.8%  Leu  8.9%  Ile  5.6%
   Aromatic  8.1%
       Phe  4.1%  Tyr  2.9%  Trp  1.2%

 Codon usage.                            Total 81166 codons, 352 stops
         133 ambiguous codons.           15 uncomplete codons.
```

	U	C	A	G
U	UUU Phe 42.3% UUC Phe 57.6% UUA Leu 10.2% UUG Leu 23.0%	UCU Ser 26.6% UCC Ser 14.7% UCA Ser 18.1% UCG Ser 9.5%	UAU Tyr 38.4% UAC Tyr 61.5% UAA *** 30.3% UAG *** 18.7%	UGU Cys 53.9% UGC Cys 46.0% UGA *** 50.8% UGG Trp100.0%
C	CUU Leu 26.7% CUC Leu 20.4% CUA Leu 9.7% CUG Leu 9.7%	CCU Pro 35.5% CCC Pro 13.8% CCA Pro 34.6% CCG Pro 15.9%	CAU His 50.1% CAC His 49.8% CAA Gln 49.6% CAG Gln 50.3%	CGU Arg 19.9% CGC Arg 6.7% CGA Arg 9.7% CGG Arg 6.7%
A	AUU Ile 41.1% AUC Ile 42.7% AUA Ile 16.1% AUG Met100.0%	ACU Thr 34.6% ACC Thr 26.0% ACA Thr 26.6% ACG Thr 12.6%	AAU Asn 40.2% AAC Asn 59.7% AAA Lys 38.7% AAG Lys 61.2%	AGU Ser 15.5% AGC Ser 15.3% AGA Arg 31.7% AGG Arg 24.9%
G	GUU Val 39.6% GUC Val 23.6% GUA Val 10.2% GUG Val 26.3%	GCU Ala 47.3% GCC Ala 19.7% GCA Ala 21.9% GCG Ala 10.9%	GAU Asp 59.5% GAC Asp 40.4% GAA Glu 46.4% GAG Glu 53.5%	GGU Gly 36.3% GGC Gly 13.5% GGA Gly 38.2% GGG Gly 11.8%

A Question of Ownership-- Patent Rights on Genome Maps Clarified

Howard Silverstein, Deputy Assistant General Counsel for Patents
Office of the General Counsel, USDA
and
William Tallent, Assistant Administrator
Agricultural Research Service, USDA
Washington, DC

The U.S. Department of Agriculture (USDA) awards plant genome grants to universities and other organizations in support of the Department's Plant Genome Program. In many of the genome mapping projects funded by the grants, DNA sequences and maps for economically important genes will likely be generated.

These genome mapping efforts raise some important patent-related questions: (1) Who is the owner of the intellectual property rights in these discoveries? (2) Can USDA release the resultant sequence data or map to the scientific community? and (3) If yes, when can the data be released?

Ownership

By law (35 U.S.C. §§200-204), a grantee would be entitled to ownership of the patent rights in such discoveries. However, the recipient must promptly disclose the sequences or maps to the Department and, within 2 years thereafter, apply for patent protection. Otherwise, the Government is entitled to own the patent rights.

Public Disclosure

During these option periods, the Government's disclosure of the maps or sequences to the public would be inappropriate since the grantee's patent rights may be adversely affected. Public disclosure includes printed publications or a public-accessible database. After the option periods have expired, or after a grantee elects not to file for patents, the information may be disclosed by the Government to the public without adversely affecting a grantee's patent rights.

Thus, the Government, in effect, is required to refrain from publicly disclosing a map or sequence for a variable period. In most instances, the period of confidentiality will be short due to (a) a grantee's interest in early public disclosure, (b) early filing for a patent, or (c) no interest in patenting.

Gene Fragment Question

Still to be answered is the legal question of whether or not patents may be obtained on gene fragments. The recent attempt by the National Institutes of Health (NIH) to patent gene fragments in the United States was met with a "first-round" rejection by the U.S. Patent and Trademark Office.

According to an article in *The Washington Post* (September 24, 1992), NIH Director Bernadine Healy has asked Congress to clarify this issue through appropriate legislative amendment of the patent laws. ◆

Overview Available on Genome Patenting Issues

On May 21, 1992, an open forum on genome patenting issues was convened by the Genome Patent Working Group, a subcommittee of the Federal Coordinating Council for Science, Engineering, and Technology (FCCSET) in Washington, DC. A proceedings of the meeting is now available. (Ordering information is provided below.) If you are interested in following this complex issue, this publication will provide an excellent balanced overview.

Presenters at the forum provided background information on the following topics:

- Daniel Nathans reviewed genome research biology;
- Robert Merges reviewed patenting and licensing laws as related to genome research; and
- Lawrence Rudolf reviewed the Federal technology transfer law.

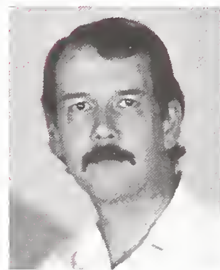
Agency perspectives were aired for the U.S. Department of Health and Human Services, the U.S. Department of Energy, the U.S. Department of Agriculture, and the U.S. Department of Commerce.

Oral and written comments were solicited. FCCSET will carefully consider the input from this meeting in formulating recommendations for Federal policy and practices related to intellectual property protection and genome research.

To obtain a copy of the report, please call or write:

Sandra Beaulieu
Science/Engineering Education Division
Oak Ridge Associated Universities
P.O. Box 117
200 Badger Avenue
Oak Ridge, TN 37831-0117
Ph. (615) 576-7393

Introducing Dr. Randy Shoemaker



Dr. Randy Shoemaker is associate professor of the Departments of Agronomy and Genetics at Iowa State University (ISU). The position is in USDA's Agricultural Research Service (ARS).

In this position Dr. Shoemaker conducts research in the areas of soybean genome mapping, genome organization and structure, cytoplasmic diversity, somaclonal variation, soybean transformation, and gene regulation.

He also serves as coordinator for the soybean genome database--a component of USDA's plant genome database project. He has taken a lead role in developing the soybean database, including organizing the Soybean Genome Database Conference held last year and coordinating the working committees.

Dr. Shoemaker began his USDA career in 1985 as a research geneticist and assistant professor at ISU. He became a full member of the graduate faculty in 1987. In 1991, Dr. Shoemaker assumed his current status as associate professor.

Prior to coming to USDA, Dr. Shoemaker was a postdoctoral research associate at the University of Nebraska, Lincoln, College of Life Sciences.

He holds a Ph.D. in genetics from ISU. He earned his M.S. degree in agricultural genetics from the University of Wisconsin, Green Bay; a B.S. degree in natural resource management from the University of Wisconsin, Stevens Point; and an A.A. degree in conservation technology from Fox Valley Technical Institute in Appleton, WI.

Dr. Shoemaker has authored and co-authored numerous technical publications. He has presented papers and has been an invited speaker at conferences and workshops throughout the United States and abroad. In addition, Dr. Shoemaker has organized various conferences, including the 1st, 2nd, and 3rd Biennial Conferences on Molecular and Cellular Biology of the Soybean; and he has served as chair for several conference sections, including the Biotechnology Section of the World Soybean Research Conference III.

Dr. Shoemaker is a member of the American Society of Agronomy. He also serves as editor and associate editor of two journals. ◆

Other Pursuits

Completing and Using the Barley Genome Map

Tom Blake, Corresponding Secretary
North American Barley Genome Mapping Project
Montana State University
Bozeman, MT

Participants in the North American Barley Genome Mapping Project (NABGMP) convened at Bozeman, MT, August 10-11 to evaluate the project's progress and determine future direction.

NABGMP is an organization of U.S. and Canadian scientists with the shared objective of understanding the relationship between barley's genotype and its phenotype. The original objective of the project was to construct a 10cM map of the barley genome and to use the map to identify and locate gene-controlling traits of economic importance, including yield, adaptation, malting and nutritional quality, and pest and disease resistance.

NABGMP is unique in that it is a multi-institutional, mission-oriented project that involves scientists from numerous institutions and disciplines. The project has received support from Federal grants; commodity boards; and malting, brewing, and feeding organizations in both the United States and Canada. In the United States, Federal funding has been garnered through a USDA, Cooperative State Research Service "Special Grant"; in Canada, Federal funding has been provided through the Natural Sciences and Engineering Research Council.

Accomplishments

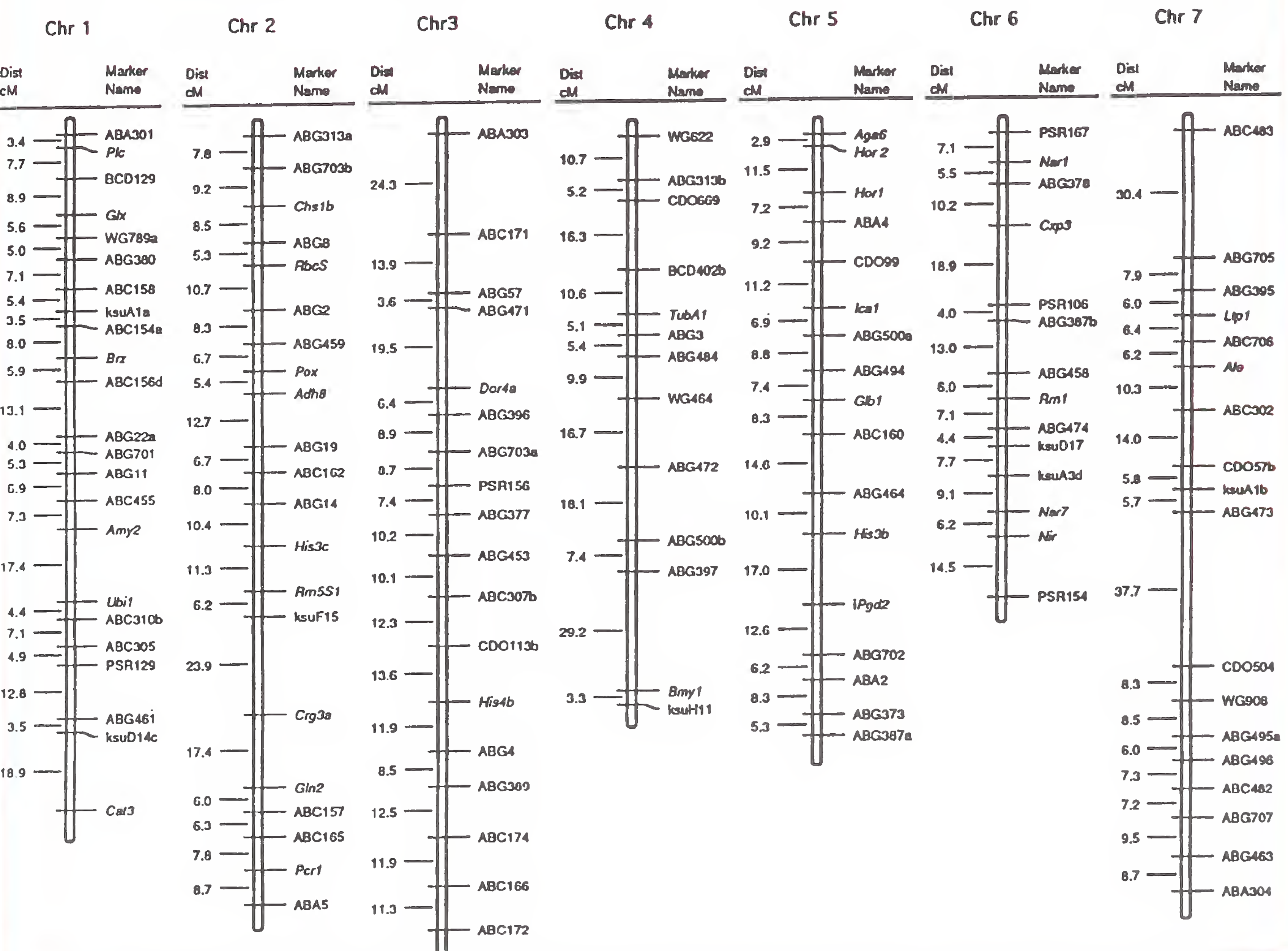
Thus far, objectives of the project have been pursued through the use of doubled haploid lines derived from a cross between Steptoe and Morex, representatives of the two major spring six-rowed barley germplasm groups. Doubled haploids from a second cross (Harrington x TR306) between two-rowed parents are now in evaluation. Steptoe and TR306 are varieties that lack useful malting quality; however, they do have agronomic properties that Morex and Harrington lack. In the next year, doubled haploids from a cross between Morex and Harrington will permit a comparison of the genetic underlying malting quality in two-rowed and six-rowed germplasm bases.

At the Bozeman meeting, Dr. Andy Kleinhofs presented an overview of the mapping progress in the Steptoe/Morex cross. In this cross, 295 markers have been mapped (fig. 1 shows a skeletal map). Complete maps have been submitted to Theor. Appl. Genetics. The average distance between markers is 4.2cM. Six centromeres and five telomeres have been located on the map. While the average map density exceeds the objectives, six gaps greater than 20cM remain. The largest gaps remain on chromosome 7 (2 gaps) and chromosome 4 in the region of the ml-o locus.

The gaps may represent physically small regions of high recombination. Physically large regions of low-frequency recombination were also observed. The nucleolar organizers, the sites for the 26s and 18s ribosomal RNA genes, have long been known to reside at the secondary constriction on chromosomes 6 and 7. These are located approximately a chromosome arms' length from the centromere on each chromosome. Nonetheless, the recombinational distance between the centromere of chromosome 6 and NOR is 12cM. For chromosome 7, the distance between centromere and NOR is 2cM.

The quality and utility of the Steptoe/Morex map will be tested by transferring well-spaced markers to the Harrington x TR306 map. NABGMP barley maps will be

Figure 1. A skeletal map of the barley genome.



merged with barley maps constructed in Germany by adding selected markers from our maps to the German maps and vise versa.

Nomenclature remains a problem. Due to our collaborative effort, the nomenclature used by NABGMP is uniform, but, in several cases, it differs from that being used in Europe. In the hope of reducing future confusion, an international committee has been organized to attempt to standardize barley gene map nomenclature.

Drs. Pat Hayes and Ben Liu reported on quantitative trait analyses performed on replicated field and malt quality data gathered from testing sites in Oregon, Washington, Idaho, and Montana, using software designed by Drs. Ben Liu and Steve Knapp at Oregon State University (fig. 2). GMENDEL and QTL-STAT provided an integrated software package, which developed linkage maps identical to MAPMAKER, and QTL analyses, which estimated both genotype effects and genotype-by-environment interaction. The ability of QTL-STAT to handle data from multiple environments and to provide nonlinear estimates of gene effects appeared to be a significant improvement over its predecessors. Major genes that modify yield, quality, and related traits were identified. When a "best possible" progeny genotype was synthesized from the data, it showed an estimated agronomic profile dramatically better than anything grown in the Western United State and of slightly better quality than Morex.

Future Efforts

While all well-designed genome mapping projects will produce both basic information and information of direct commercial value, maintenance of the tie between application and basic research was deemed critical to future success. Over the next 5 years, NABGMP will emphasize both basic (map construction and saturation of regions, genome location, and map-based gene cloning) and applied (QTL analysis,

Chromosome 3

		Agronomic Characters					Quality Characters				
Marker1	Marker2	Recombination (%)	Yield (kg/ha)	Heading Date (days)	Height (cm)	Lodging (%)	Malt Extract (%)	alpha Amylase (20 Deg units)	Diastatic Power (%)	Plump Barley (% on 6/64)	Grain Protein (%)
ABR340	ABC171	23.01									
ABC171	ABG57	13.48			3M						
ABG57	ABG460	0.7	453S		4M	13M					0.3M
ABG460	ABG10	0	387S		4M	14M				5.8S	
ABG10	ABG471	2.00	322S		5M	14M				4.5S	
ABG471	ABG399	18.98	290S		5M	16M				6.8S	0.4M
ABG339	ABR334	2.34	842S		7M	29M	0.7S			8.8S	0.4M
BR334	ABC156C	1.52	881S		7M	29M	0.6S		6M	9.0S	0.4M
ABC156C	ABG462	0.7	833S		7M	27M			6M	8.0S	0.4M
ABG462	ksuF2B	0	860S		8M	28M			9M	8.1S	0.4M
ksuF2B	Adh5	0	832S		8M	29M			8M	7.8S	
Adh5	ksuA3C	0	804S		8M	28M		1.7M	6M	8.2S	0.4M
suA3C	Dor4A	0.83	818S		8M	29M		1.7M	6M	9.5S	0.4M
Dor4A	BCD828	1.65	790S		8M	29M		1.4M		9.5S	0.4M
BCD828	ABG396	4.47	807S		7M	26M				8.5S	0.3M
BG396	ABG398	3.10	729S	1S	6M	26M	0.4S			7.5S	
ABG398	ABG703A	4.88	781S		8M	26M			6M	7.7S	0.4M
ABG703A	PSR156	9.29	720S		6M	22M				7.5S	0.3M
PSR156	ABC176	2.02	515S		4M	18M				7.5S	
BC176	ABG377	6.29	472S	1S	4M	18M				6.5S	
ABG377	Rrn5S2	0.83	399S		3M	11M				7.0S	
rn5S2	ABG453	8.79	414S		4M	12M				7.7S	0.3M
ABG453	ABR320	7.4	367S		2M					5.4S	0.3M
ABR320	ABC307B	3.17	308S							6.3S	
BC307B	Crg3B	1.87	243S	1S						7.8S	0.3M

Figure 2. *Quantitative Trait Loci identified on barley chromosome 3. Units are listed for each character. Data represent mean differences between allele classes, with the parent providing the allele contributing to the larger mean value indicated beside the value (i.e., Steptoe (S) or Morex (M)). Obviously, a gene or linkage block affecting plant height, lodging, and yield is located on chromosome 3. The Morex allele contributes to a taller plant, which lodges more and yields less than the contrasting allele from Steptoe.*

selection experiments, and technology simplification) research.

Future work on mapping includes expanding the Steptoe/Morex map to reduce gaps, adding morphological markers, and merging maps with maps produced by other projects. Maps suitable for QTL analysis will be developed with the Harrington/TR306 and Morex/Harrington crosses. Expansion of the mapped germplasm pool will take place along with development and utilization of YAC libraries, fine structure analyses of disease resistance loci, and QTL-based selection experiments.

The work already accomplished with barley demonstrates the value of this species as a model genetic system for the cool season grasses. Participants in the project look forward to transferring these technologies to other economically important grasses.◆

Vocabulary-Cont. from page 8

detect genetic commonalities not only when comparing apples with apples, but also when comparing apples with oranges or orangutans.

Vocabulary development must be considered an important adjunct to data collection and input throughout the life cycle of the PGD project to ensure that valuable information in the database is found and put to good use.

For further reading on this subject refer to *Vocabulary Control for Information Retrieval,* (1986) 2nd ed., by F. W. Lancaster, Information Resources Press, Arlington, VA. ◆

Connections

Biodiversity Information Network Initiated

Dora Ann Lange Canhos, Project Manager
Tropical DataBase, Campinas, Brazil

A Biodiversity Information Network is currently under development to help solve the increasing problem of managing global diversity information. The network, known as BIN/21, will disseminate and facilitate access to biodiversity information worldwide.

An international group of scientists and other interested persons initiated BIN/21 in support of recommendations from the United Nations Conference on Environment and Development (UNCED) held in Rio de Janeiro in June. Providing guidance for the network developers are two chapters of UNCED's official document, *Agenda 21,* "Conservation of Biological Diversity" and "Information for Decision-making."

The first mission of the initiative is to ensure participation of the entire biodiversity community. Active involvement of all regions of the world is encouraged.

Planning Workshop

To initiate the effort, approximately 35 scientists and others in government and nongovernment organizations participated in a Workshop on the Needs and Specifications for a Biodiversity Information Network, which was held at the Tropical Data Base, in Campinas Brazil, July 26-31.

Sponsors for the workshop were the International Union of Biological Sciences, the International Union of Microbiological Societies, and the World Federation for Culture Collections. The workshop was also made available online through a variety of electronic networks to approximately 200 additional individuals. Funding for the workshop came from various sources, including the United Nations Environment Program, the

Brazilian Institute for Environment and Renewable Natural Resources, the National Council for Scientific and Technological Development, the Projects and Studies Financing Agency, and the British Council.

Need for Network

The sustainable management of the environment and conservation of the biodiversity of plants, animals, microorganisms, and all living things depend on reliable and readily accessible information. Without information on the names, the locations, the activities, and the interactions of organisms in the ecosystem, appropriate policies, conservation strategies, and remedial actions cannot succeed.

The amount of information currently in existence and soon to be developed is vast. Since information is scattered around the world and not easily obtainable, a clear need exists for a network to link these resources and to make them readily available.

The network will consist primarily of electronically linking databases and providing a communications system. However, other means will also be used for distributing information. BIN/21 will encourage the exchange of data and ensure that the needs of developing countries are met. The information resource will be global; participation of the developing world will be actively sought.

An interim steering group is to provide support for the initiative and seek funding and sponsorship. Working groups already set up will give technical, educational, and administrative support to initiate the establishment of the network.

Worldwide Interest

From the interest shown by individuals who attended the workshop and those who participated through electronic means, BIN/21 is attracting worldwide interest, reflecting the general recognition that information is an essential element in the underpinning of the Rio Convention.

BIN/21's participants invite the active involvement of all individuals and organizations with an interest in the aims of the network. If you wish to subscribe to the Biodiversity Bulletin Board, send your message to listserv@bdt.ftpt.ansp.br. Within the text, type: subscribe biodiv-1 (add your name).

Contacts

For further information, contact the following individuals:

Vanderlei Canhos, Base de Dados Tropical, Fundacao Tropical de Pesquisas e Tecnologia "Andre Tosello", Rua Latino Coehlo, 1301 - Parque Taquaral, 13087-010 Campinas, SP, Brasil, Tel:+55 192 42-7022, Email: dora@bdt.ftpt.ansp.br

John McComb, World Conservation Monitoring Centre, 201 Huntingdon Road, Cambridge, CB3 ODL, UK; Tel: +44 223 277314, Email:johnm@wcmc.co.uk, BT Gold 75:DBI0710

Barbara Kirsop, Microbial Strain Data Network, 307 Huntingdon Road, Cambridge, CB3 OJX, UK, Tel: +44 223 276622, Email: msdn@cgnet.com or BT Gold 75:DBI0005

Anthony Whitworth, Association for Progressive Communications, 2284 Railroad Drive, Fairbanks, AK 99709, Tel: +1 907 4798129, Email: anthony@igc.apc.org or anthony@gis.lter.alaska.edu

Hideaki Sugawara, World Data Center for Microorganisms, The Institute of Physical and Chemical Research, RIKEN, Saitama, Japan, Tel: +81 48 4621111, Email: r35118@rkna50.riken.go.jp ◆

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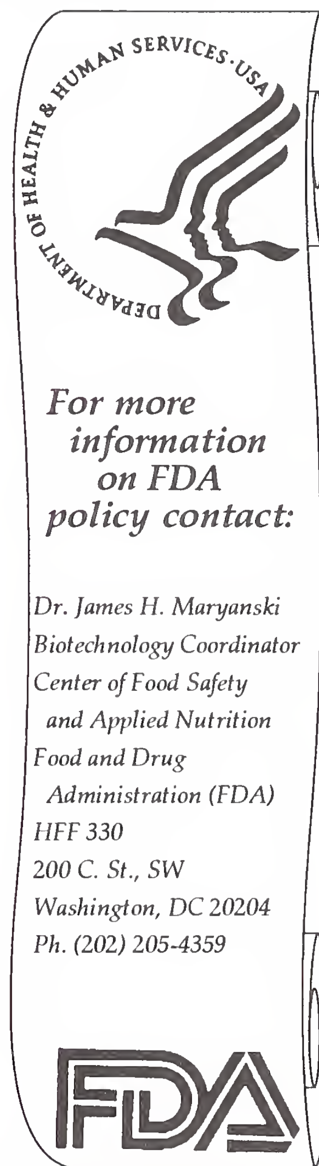

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Legend of the Lamb-Plant

*Judith J. Ho, A curator for
Special Collections
National Agricultural Library, USDA
Beltsville, MD*

Figure 1. *"The Vegetable Lamb of Tartary," The Sythian Lamb," and "The Borometz"*

Through history, science has crystallized from many divergent paths. From Roger Bacon (1214-94) until well into the present century, discoveries were made and lost and made again.[1] The word "biology" was not even coined until 1802. It has been said that if there is a moment at which biology began, it must have been in 1615, when William Harvey, then the Court physician of Charles I of England, conceived of the heart as a pump circulating the blood.

The idea that a living body could be analyzed in purely mechanical terms was one of the greatest milestones in man's intellectual history. Until that discovery, life in all its forms had been a quasi-magical phenomenon, intertwined with religion and emotions that ordinary men were not expected to understand. In fact, such individual expectations were considered impious, perhaps even sacrilegious.[2]

During the Middle Ages, medieval men craved order in science as well as in life. When they were halted in finding true laws, they took recourse in symbolism to explain life's mysteries. To the thinkers of that time, ideas were more real than material things, and myths were very much a part of the age of pre-scientific thought.

Trees as Symbols

Trees were among the first plants worshipped by man and were also among the first symbols, representing the ideas of reproduction and eternity. Similar ideas were represented by bushes and flowering plants, sometimes by combining more than one plant or species on the same stylized plant drawing, sometimes the drawing or figure would be stylized into animal or human shapes, such as the tree of life and the tree of knowledge.

These symbols were taken up by all beliefs and religions in both the western and eastern worlds.[2b] The Greek Historian Heroditus (484-425 B.C.), whose travels took him to northern Africa, Egypt, Assyria, and Persia, was one of the earliest explorers responsible for the discovery of

many plants, for bringing them from one continent to another, and also for bringing with him knowledge of their properties and cultivation. Heroditus mentions the Barametz as early as 442 B.C. Mentioned again in the Mishna Kilain portions of the Talmud, this passage occurs referring to the Borametz zoophyte, the famous Lamb of Tartary or lamb-plant:

> Creatures called Adne Hasadeh (literally, "Lords of the Field") are regarded as beasts.[3]

In 1235, Talmudic mention is again made: "It is stated in the Jerusalem Talmud that is a human being of the mountains: it lives by means of its navel: if its navel be cut, it cannot live. ...this is the animal called Jeduah."

This is also the Jedoui mentioned in the Christian Bible in the book of Leviticus (xix, 31). Called Jedua, this animal is human in all respects, except that by its navel it is joined to the stem that issues from the root. No creature can approach within the tether for it seizes and kills them. Within the tether of the stem, it devours the herbage all around it. To kill it, men must tear at it or aim arrows at its stem until it is ruptured, whereupon the animal dies.[4] It is little wonder then that medieval thinkers strongly believed in and hotly debated the existence of such things as the mysterious plant animals embodied in the myth of "the Lamb of Tartary" (fig. 1) and in other myths of that time.

Curious Fable

The fable of the Lamb of Tartary, variously entitled "The Vegetable Lamb of Tartary," "The Sythian Lamb," and "The Borometz," or "Barametz," is a curious one. This "lamb-plant" is represented as springing from a seed like that of a melon, but rounder, and supposedly cultivated by natives of the country where it grew. The lamb was contained within the fruit or seed-capsule of the plant, which would burst open when ripe to reveal the little lamb within it. The wool of this little lamb was described as being "very white."[3]

When planted, it grew to a height of 2 1/2 feet and had a head, eyes, ears, and all the parts of the body of a newly born lamb. It was rooted by the navel in the middle of the belly, and devoured the surrounding herbage and grass.[4]

This particular story of the mythical Scythian Lamb captured the imaginations of men everywhere during this early period. In the 16th and 17th centuries, the Scythian Lamb was again made the subject of investigation and argument by the most celebrated writers, philosophers, and scientific men of that time. Theophrastus (306 B.C.), the disciple of Aristotle, had earlier described wool-bearing trees with a pod the size of a spring apple, leaves like those of the black mulberry, but the whole plant resembled the dog-rose.[5] This was a very correct description of the cotton plant. Pliny the Elder (A.D. 77) also mentioned "wool-bearing trees," but seemed to confuse cotton and flax in his writings.[6]

Sigismund, Baron von Herberstein, who in 1517 and 1526 was the Ambassador to the Emperors Maximilian I and Charles V and to the "Grand Czard or Duke of Muscovy," spoke for many of his time when he said in his "Notes on Russia" (*Rerum Muscoviticarum Commentarii*, 1549) of the "Vegetable lamb":

> It had a head, yes, ears, and all other parts a newly born lamb. ...For myself, although I had previously regarded these Barametz as fabulous, the accounts of it were confirmed to me by so many persons of credence that I thought it right to describe it.

The numerous descriptions differed so little that he accepted them as truth.[5]

Claude Duret (1605) of Moulins devoted an entire chapter to the "Borametz of Scythia or Tartary" in his work entitled *Histoire Admirable des Plantes*. His imaginative illustration from the book appears in Figure 1 of this article. John Parkinson (1656) figured the lamb-plant in the frontispiece of his *Paridisi in Sole*--in the center just to the left is a tiny Borametz.

All of these men were well-known and respected in their time. They either figured the lamb-plant in their respective works or reported in their writings that they had seen the mysterious Borametz, thus enhancing and perpetuating the authenticity of this strange story.

Search Continued

Explorers continued to go in search of it, and collectors examined what they thought were specimens of it. Engelbrecht Kaempfer went to Persia in 1683 to search for the "zoophyte feeding on grass," but could not find

it and reported that in his writings, entitled *Amoentitatum Exoxticarum politico-physico-medicarum fasciculi*, 1712. John Bell of Autermony made a diplomatic journey to Persia in 1715-1722 and tried to obtain authentic information on the vegetable lamb, but he was not successful. He reported as much in his writings, entitled *Travels from St. Petersburg in Russia to Various Parts of Asia, in 1716, 1719, 1722, &c*: Dedicated to the Governor, Court Assistants, and Freemen of the Russia Company, London, 1764.

Kaempfer's manuscripts and collections were acquired by Sir Hans Sloane, wealthy British patron, collector, and eventually founder of the British Museum, who in 1698 received a specimen that was supposed to be the mysterious Borametz or Lamb of Tartary. His description was printed in the Royal Society's *Transactions*. Dr. Philip Breyn, a colleague of Sloane's, also debunked the borametz from a specimen he also received, examined, and reported in his work, entitled "Dissertiuncula de Agno Vegetabili Scythico, Borametz Vulgo Dicto," which appeared in the British *Philosophical Transactions* (vol. xxxiii, p. 353, 1725).

Sloane identified his specimen as being constructed of a portion of one of the arborescent ferns (Dicksonia) of which there are about 35 species, some of which grow in the United States and one of which bears the name to this day of Dicksonia borametz. Sloane exposed his specimen as the stem or rootlet of a fern, artificially and cleverly manipulated to look like a lamb, thus dealing what appeared to be a crushing blow to this fable.

But the story would not die. Half a century later in 1768, the Abbe Chappe-Auteroche made a visit to Tartary searching for information on the elusive Scythian Lamb, but again to no avail. Then, in 1778, Hohn and Andrew Rymsdyck in their work, entitled *Museum Britannicum*, figured it in Plate XV.

Poetry Subject

Toward the end of the 18th century, eminent botanists, who were well acquainted with the specimens described earlier by Sloane, Breyn, and others, again made the legendary Borametz their theme. This time it was also picked up by the literary men of the time. In 1781, Dr. Erasmus Darwin made it the subject of his poem, *The Botanic Garden* (London, 1781):

> E'en round the Pole the flames of love aspire,
> And icy bosoms feel the secret fire,
> Cradled in snow, and fanned by Arctic air,
> Shines, gentle borametz, thy golden hair;
> Rooted in earth, each cloven foot descends,
> And round and round her flexile neck she bends,
> Crops the grey coral moss, and hoary thyme,
> Or laps with rosy tongue the melting rime;
> Eyes with mute tenderness her distant dam,
> And seems to bleat - a vegetable lamb.

Later, in 1791, Dr. De la Croix, in his *Connubia Florum, Latino Carmine Demonstrata* (Bath, 1791), extolled the fabulous plant-animal in a Latin poem, which critics at the time hailed as approaching the quality of Virgil's "Georgics." The poem says, in part (translated):

> For in his path he sees a monstrous birth,
> The Borametz arises from the earth
> Upon a stalk is fixed a living brute,
> A rooted plant bears quadruped for fruit,
> ...It is an animal that sleeps by day
> and wakes at night, though rooted in the ground,
> to feed on grass within its reach around.[6]

Cotton Plant

Henry Lee in his work, *The Vegetable Lamb of Tartary; A Curious Fable of the Cotton Plant* (London, 1887), claims that this curious myth actually originated in the early descriptions of the cotton plant. Lee stated it thus: Tracing the growth and transition of this story of the lamb-plant from a rumour of a curious fact into a detailed history of an absurd fiction, there can be no doubt that it originated in early descriptions of the cotton plant, and the introduction of cotton from India into Western Asia and the adjoining parts of Eastern Europe.

Interest Continued

The lamb-plant was discussed by philosophers, sought after by travellers and explorers of that time,

written about in the literature, and talked about all over Europe. In spite of some confusion of facts, and both accidental and purposeful misrepresentation, there was just enough basis in observed fact, coupled with reports and assertions of truth by respected scientific men of the time, to perpetuate interest in the lamb-plant story from generation to generation.

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2. Facts on File, p. 12.

2b. Lehner, Ernst and Johanna. Folklore and Symbolism of Flower, Plants and Trees, New York, Tudor Publishing Co., 1960, p. 16-19.

3. Lee, Henry. The Vegetable Lamb of Tartary; A Curious Fable of the Cotton Plant. to Which is added A Sketch of the History of Cotton and the Cotton Trade. Sampson Low, Marston, Searle & Rivington, London, 1887, p. 45.
4. Ibid., p. 12.
5. Op. Cit., Lee, p. 11.
6. Op. Cit., Lehner, p. 86. ◆

"Survive"--Cont. from page 4

Additional lecturers included G. Kishore, G. Coruzzi, S. F. Yang, J. Zeevaart, D. Ho, N. Crawford, D. Phillips, D. Soll, N. Amrhein, P. Kolattukudy, J. Siedow, D. Randall, C. Yocum, J. Whitmarsh, R. Malkin, H. Pakrasi, R. McCarty, G. Lorimer, W. Ogren, A. Huang, R. Buchanan, I. Ting, R. Vierstra, M. Chrispeels, T. Farmer, C. Somerville, B. Mudd, W. Briggs, J. Varner, C. Lamb, C. West, A. Darvill, D. Delmar, and E. Conn.

(See the shaded box for lecture topics presented.)

Sponsors for the course were the American Society of Plant Physiologists (ASPP); University of California, San Diego (UCSD); the Salk Institute; and the Scripps Research Institute. The 1992 course was supported by grants from USDA, the Department of Energy (DOE), and the National Science Foundation (NSF).

Plans for 1993

Planning is underway for Plant Biochemistry 1993, which is to be held next summer at the University of Wisconsin, Madison. Course information will be available around January. The deadline to receive applications will be either March or April. A general biochemistry background is required for participants.

Students are requested to pay one-half of their room and board costs. Other expenses will be covered by the granting agencies through ASPP. Sponsors for the course expect to have 40 to 50 registered participants. Locals in the area are free to attend the course.

For more information, contact the following individuals:

Dr. Himadri Pakrasi, Washington University, One Brookings Dr., Campus Box 1137, St. Louis, MO 63130, Ph. (314) 935-6853, FAX (314) 935-4432

Dr. Kenneth Keegstra, Botany Department, University of Wisconsin, 430 Lincoln Drive, Madison, WI 53706, Ph. (608) 262-9997, FAX (608) 262-7509 ◆

Lecture Topics

Amino Acid Metabolism
Ethylene Biosynthesis and Action
Plant Hormone Analysis
Biosynthesis and Further Metabolism
Hormonal Regulation of Gene Expression
Nitrate Assimilation
Nodulation and Nitrogen Fixation
Chlorophyll Biosynthesis
Cyanide Resistant Respiration
Cutin, Suberin, and Peroxidase
Mitochondrial Physiology and Biochemistry
Protein Kinases and Related Metabolism
Photosystem II and I Thylakoid Structure
Cytochrome bf Complex
Use of Molecular Biology in the Study of Photosynthesis
ATP Synthase
Metabolite Transport Across Chloroplast Membranes
Targeting Proteins to Chloroplasts
RUBISCO Assembly
CO_2-Fixation
C_3/C_4 Photosynthesis
Assembly of Oleosomes
Redox Metabolism in Chloroplasts and in Seeds
C_4 and CAM, Protein Turnover Glycoproteins
Secretory Pathway and Vacuolar Targeting
Wound Signaling, Systemin and Jasmonate, Lipids
Sulfur Metabolism, Phospho- and Sulfo-lipids
Blue Light/Photocontrol
Cell Wall Polysaccharides, Cellulose
Phenolic Acids and Cyanogenic Glycosides

Pulsed Field Electrophoresis for Separation of Large DNA

Barbara Joppa, Research Technician
Samantha Li, Research Technician
Scott Cole, Product Specialist
Sean Gallagher, Director of Laboratory
HSI Laboratories, Hoefer Scientific Instruments
San Francisco, CA

Manipulating and analyzing DNA are fundamentals in the field of molecular biology. Indeed, separating complex mixtures of DNA into different-sized fragments by electrophoresis was a well-established technique by the early 1970's.

Typically, DNA was isolated intact and then treated with restriction enzymes to generate pieces small enough to resolve by electrophoresis in agarose or acrylamide. Although this procedure still forms the core of DNA separation and analysis in today's laboratories, the rules of the separation have changed.

In 1984, Schwartz and Cantor described pulsed field gel electrophoresis (PFGE), introducing a new way to separate DNA. In particular, PFGE resolved extremely large DNA for the first time, raising the upper size limit of DNA separation in agarose from 30-50 kb to well over 10 Mb (10,000 kb).

After this initial report, a succession of papers described new and improved instrumentation and methods. As a result, routine procedures and several commercial pulsed field units are currently available. Now, instead of cloning a large number of small fragments of DNA, PFGE permits cloning and analysis of a smaller number of very large pieces of a genome.

Applications

Applications of PFGE are numerous and diverse (Gemmill, 1991; Birren and Lai, 1990, 1993; and Van Daelen and Zabel, 1991). These include cloning large plant DNA using yeast artificial chromosomes (YAC's) (Ecker, 1990; see also *Probe*, Vol. 1, No. 1/2; and Butler, et al., 1992) and P1 cloning vectors (see *Probe*, Vol. 1, No. 3/4); identifying restriction fragment length polymorphisms (RFLP's) and construction of physical maps; detecting *in vivo* chromosome breakage and degradation (Elia, et al., 1991); and determining the number and size of chromosomes ("electrophoretic karyotype") from yeasts, fungi, and parasites such as Leishmania, Plasmodium, and Trypanosoma.

Theory

Although the theory of pulsed field electrophoresis is a matter of debate, qualitative statements can be made about the movement of DNA in agarose gels during PFGE. During continuous field electrophoresis, DNA above 30-50 kb migrates with the same mobility regardless of size. This is seen in a gel as a single large diffuse band. If, however, the DNA is forced to change direction during electrophoresis, different sized fragments within this diffuse band begin to separate from each other.

With each reorientation of the electric field relative to the gel, smaller sized DNA will begin moving in the new direction more quickly than the larger DNA. Thus, the larger DNA lags behind, providing a separation from the smaller DNA.

Currently, there are three models that attempt to describe the behavior of DNA during PFGE (reviewed by Chu, 1990), the biased

reptation model (BRM), the chain model, and, most recently, the bag model (Chu, 1990, 1991).

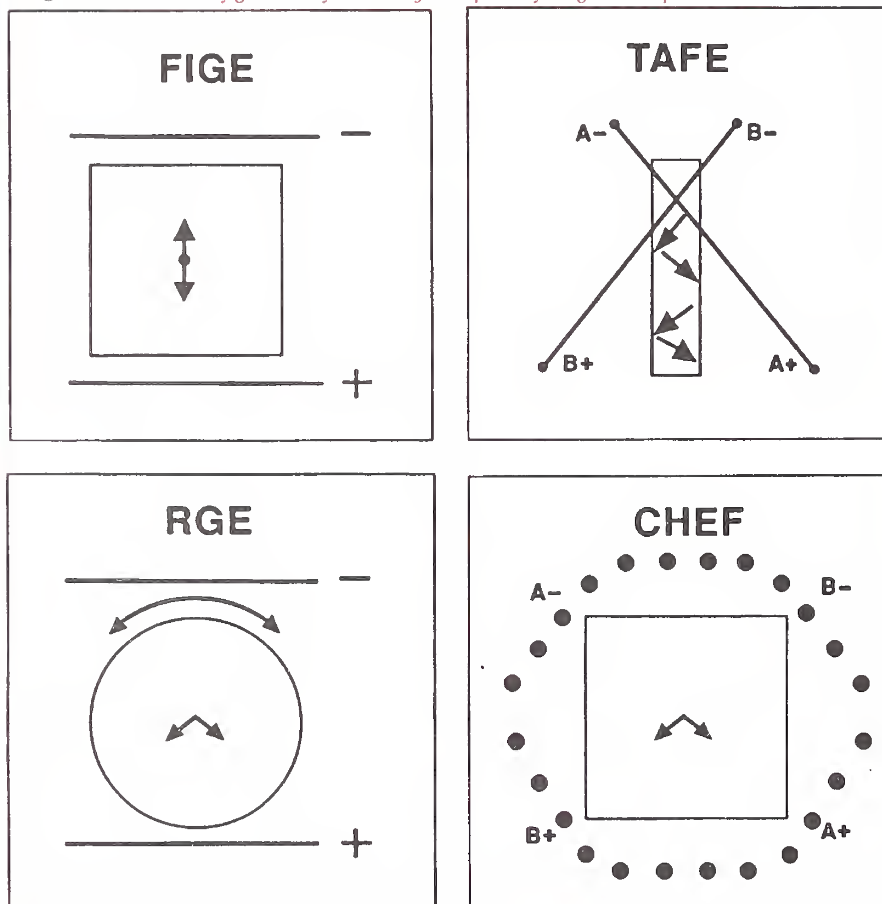
Instrumentation

Although many types of PFGE instrumentation are available (fig. 1), they generally fall into two categories. The simplest equipment is designed for field inversion gel electrophoresis (FIGE) (Carle, et al., 1986). FIGE works by periodically inverting the polarity of the electrodes during electrophoresis. Because FIGE subjects DNA to a 180° reorientation, the DNA spends a certain amount of time moving backwards. Only an electrical field switching module is needed; any standard vertical or horizontal gel box that has temperature control can be used to run the gel.

Although more complex in its approach, zero integrated field electrophoresis (ZIFE) (Turmel, et. al, 1990) also falls into this first category. Compared with simple FIGE, ZIFE is very slow. However, ZIFE is capable of resolving larger DNA and giving a larger useful portion of the gel.

The other category contains instruments that reorient the DNA at smaller oblique angle, generally between 96 and 120°. This causes DNA to always move forward in a zigzag pattern down the gel. For a similar size range under optimal conditions, these separations are faster, resolve a wider size range, and give a larger useful portion of the gel compared to FIGE.

Figure 1. *Electrode configuration of commonly used pulsed field gel electrophoresis units.*

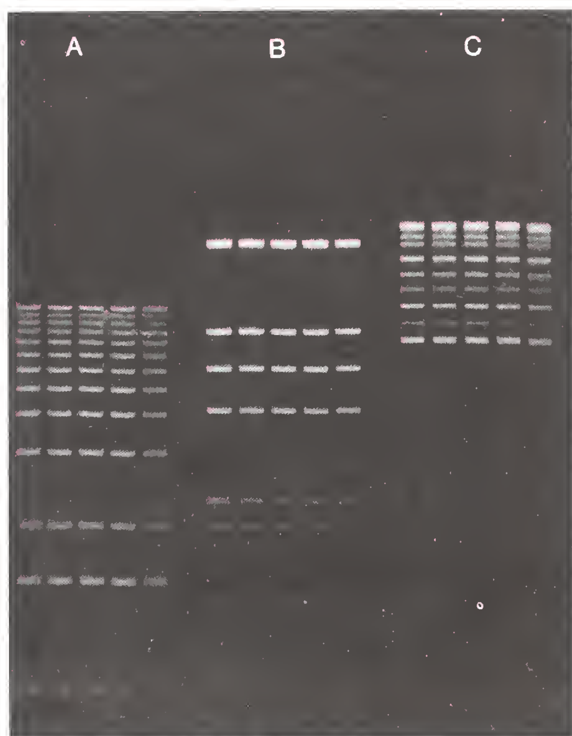


Contour-clamped homogeneous electric field (CHEF) (Chu, et al., 1986, 1990); transverse alternating field electrophoresis (TAFE) (Gardiner, et al., 1986) and its relative ST/RIDE™ (Stratagene); and rotating gel electrophoresis (RGE) (Southern, et al., 1987; Anand and Southern, 1990; Gemmill, 1991; and Serwer and Dunn, 1990) are all examples of commonly used transverse angle reorientation techniques for which instrumentation is available. In a further elaboration of the above procedures, Lai and coworkers developed the programmable autonomously controlled electrophoresis (PACE) unit which allows complete control over reorientation angle, voltage, and switch time (Clark, et al., 1988; and Birren, et al., 1989). In contrast with FIGE, these systems require both a special gel box with a specific electrode and gel geometry, and the associated electronic control for switching and programming the electrophoresis run.

Ideally, the DNA should separate in straight lanes to simplify lane-to-lane comparisons. The original pulsed-field systems used inhomogeneous electric fields that did not produce straight lanes, making interpretation of gels difficult (Schwartz and Cantor, 1984). Again, the simplest approach to straight lanes is FIGE, which uses parallel electrodes to assure a homogeneous electric field.

Although extremely useful for separating relatively small DNA, 4-1,000 kb (fig. 2), FIGE's reorientation

Firgure 2.*Increased separation of the 20-50 kb range with field inversion gel electrophoresis (FIGE). Run conditions: 230 V, 7.9 V/cm, 16 hrs., 50 msec. pulse, forward:reverse pulse ratio = 2.5:1, 1% GTG agarose, 0.5X TBE, 10°C. a) 1 kb ladder, 0.5-12 kb; b) Lambda/Hind III, 0.5-23 kb; and c) High molecular weight markers, 8.3-48.5 kb.*



angle of 180° results in a separation range most useful under 2,000 kb. Furthermore, like other PFGE techniques, FIGE has mobility inversions in which larger DNA can move ahead of smaller DNA during electrophoresis. Ramping, where the reorientation pulse length is constantly increased during separation, will minimize inversions. This capability is included in most commercial instrumentation.

Increasing both the separation range and the resolution of large DNA requires smaller reorientation angles, generally 96-140°, with 120° most common. Smaller angles (e.g., 100°) increase the mobility of the DNA generally without seriously affecting resolution. The lower limit is approximately 96°. Below this, separation is seriously compromised.

TAFE and ST/RIDE™ use a complicated geometry between the electrodes and a vertically placed gel to get straight lanes. CHEF and RGE maintain a homogeneous electric field in combination with a horizontal gel. CHEF changes the direction of the electric field electronically to reorient the DNA by changing the polarity of an electrode array. With RGE the electric field is fixed; to move the DNA in a new direction, the gel simply rotates.

Rotating Gel Electrophoresis

RGE is one of the most recent commercial introductions of pulsed field equipment and combines variable angles with a homogeneous electric field (figs. 3 and 4) (Southern, et al., 1987; Anand and Southern, 1990; Serwer and Dunn, 1990; and Gemmill, 1991). The electrodes are positioned along opposite sides of the buffer chamber with their polarity fixed. Briefly, the gel is cast on a circular running plate and then placed in the buffer chamber. The gel is coupled to a magnetic drive beneath the buffer chamber to eliminate the possibility of leakage that a direct connection might cause.

To force the migrating DNA to a new direction, the magnetic drive simply rotates the gel to the new angle. Because the reorientation angle of the DNA is determined by a straightforward mechanical coupling, RGE offers a lot of flexibility at a reduced cost. Voltage, angle, and pulse times are varied with the program stored into memory of the unit.

Sample Preparation

Along with the ability to separate large DNA came the need for new sample preparation and handling procedures. Large DNA (e.g., yeast chromosomes) is easily sheared and also difficult to pipet due to its high viscosity. The solution to this problem is to first embed the bacteria or yeast in agarose plugs and then treat the plugs with enzymes to digest away the cell wall and proteins, thus leaving the naked DNA undamaged in the agarose. The plugs then are cut to size, treated with restriction enzymes if necessary, loaded in the sample well, and sealed into place with agarose.

Separation Parameters

Several parameters act in concert during PFGE (Southern, et al., 1987; Anand and Southern, 1990; Birren, 1989; and Gemmill, 1991). These will be discussed briefly below as they relate to transverse field instruments such as RGE.

The minimum amount of information needed to repeat a separation should include a short description of the pulsed field instrumentation used; applied

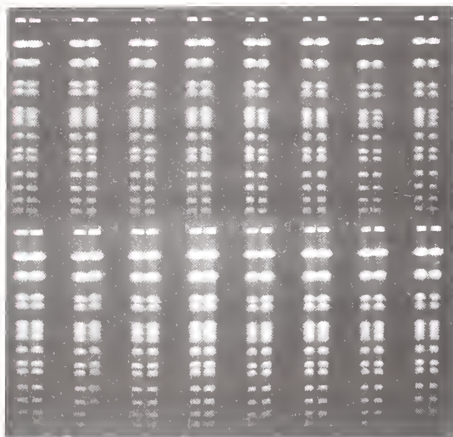


Figure 3. *Rotating gel electrophoresis (RGE) separation Saccharomyces cercevisiae chromosomes (245-2190 kb). Run conditions: 180 V, 5.1 V/cm, 34 hrs., 120° angle, 60-120 sec. pulse ramp, 0.5X TBE, 1.2% GTG agarose, 10°C. Two combs were used on the same gel to load 32 samples, a maximum of 72 are possible.*

voltage and field strength (e.g., 180 V at 5.3 V/cm); pulse length (e.g., 87 seconds); reorientation angle (e.g., 120°); the buffer (0.5X TBE); the agarose type and concentration (SeaKem Gold, 1.1%); the buffer chamber temperature (e.g., 10°); the type of standards (Clontech *S. cerevisiae*); and, if possible, the amount of DNA loaded. Although the data listed above is necessary to faithfully reproduce a separation, the information supplied in publications is rarely this complete.

Separation Area

Most PFGE systems separate DNA over a relatively small area, limiting the resolution of complex samples. RGE is an exception to this, with a useful separation distance up to 20 cm and a maximum gel size of 18 x 20 cm.

Field Strength

The field strength has a profound effect on pulsed field separations and is a compromise between separation time and resolution of a particular size class. Four to six volts/cm is generally required for resolving DNA up to 2000 kb (e.g., *S. cerevisiae* chromosomes) in a reasonable period of time (e.g., 1-2 days). However, these field strengths trap and immobilize even bigger DNA in the agarose matrix, and DNA > 3000 kb requires 2 V/cm or less for separation.

Pulse Time

Pulse time primarily changes the size range of separation. Longer pulse times lead to separation of larger DNA. For example, at 5.4 V/cm, the 1.6 Mb and 2.2 Mb chromosomes from *S. cerevisiae* separate as a single band with 90-second pulse length. Increasing the pulse length to 120 seconds resolves these into two bands (Gemmill, 1991).

Reorientation Angle

Any angle between 96 and 165° produces roughly equivalent separation (Birren, et al., 1988; and Gemmill, 1991). The smaller the angle, however, the faster the DNA mobility. And for separating extremely large DNA, 96 to 105° is almost a requirement to get a good separation in the shortest possible time.

Buffers

Two buffers are commonly employed for PFGE--TAE and TBE (1x TAE is 40 mM Tris acetate, 1 mM EDTA, pH 8.0; 1x TBE is 89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0). Both are used at a relatively low ionic strength to prevent heating and carry the designations of either 0.25 and 0.5x to indicate the dilution relative to the standard concentration. An added benefit to low ionic strength buffers is an increase in DNA mobility. For example, while using RGE to compare various buffers and agaroses, White (1992) found that lowering both TAE and TBE to 0.25 x gave the maximum mobility (40-50% faster than 1x). Below 0.25x, the DNA mobility dropped off.

Agarose

The type of agarose also affects DNA separation, with the fastest mobilities and best resolution achieved in gels made of low electroendosmosis (EEO) agarose (Birren, et al., 1989; and White, 1992). Although most standard electrophoresis grades of agarose are suitable for PFGE (e.g., SeaKem GTG), agarose with minimal EEO will provide a faster separation. Several low EEO "pulsed field grades" are available, including FastLane and Gold (FMC BioProducts), and Megarose (Clontech).

The concentration of agarose affects both the resolution and mobility of the DNA (Birren, et al., 1989; and White, 1992). Higher concentrations of agarose yield sharper, but slower moving bands. And the typical concentrations used (0.8-1.2%) represent a tradeoff between speed and resolution. High percentages of low EEO agarose may

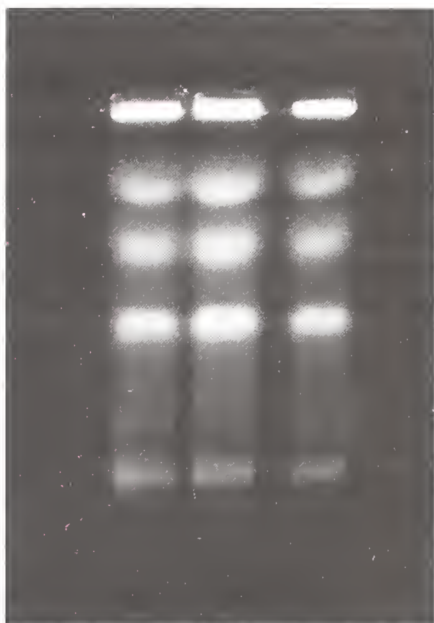


Figure 4. *Rotating gel electrophoresis (RGE) separation of 3000 to 6000 kb DNA Schizosaccharomyces pombe chromosomes. Run conditions: 50 V, 1.4 V/cm, 100 hrs., 100° angle, concatamated multiple runs: 2500 sec./50hrs, 3000 sec./50hrs, 0.5X TBE, 0.8% megarose (Clontech), 10°C.*

improve resolution without sacrificing the speed of separation (White, 1992).

Temperature

Because DNA mobility also depends on the separation temperature, the temperature must be constant both during and between runs. Although higher temperatures increase DNA mobility, it does so at the expense of resolution (Birren, et al., 1989; and Gemmill, 1991).

Conclusion

Since its introduction over 8 years ago, PFGE has evolved into a routine, pragmatic technique for molecular biologists. This is reflected in the present availability of methods chapters and manuals (e.g., Birren and Lai, 1990, 1993; Anand and Southern, 1990; Van Daelen and Zabel, 1991).

What does the future hold? Possibilities include using a new or improved separation material, and going beyond the current size limit of @ 10 Mb. Anecdotal reports suggest separations in the range of 20 Mb or larger are possible, which would further simplify the complex task of genome mapping.

For more information on Hoefer's HulaGel™ rotating gel electrophoresis unit, contact Technical Services at Hoefer Scientific Instruments at (415) 282-2307 or 800-227-4750.

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Off the Wire

APINMAP--An Asian Medicinal Plants Database

Dr. Susan McCarthy, Coordinator
Plant Genome Data and Information Center
National Agricultural Library, USDA
Beltsville, MD

Today's human population explosion is threatening not only native plants, animals, and their habitats, but also a loss in indigenous knowledge of medicinal plants. This loss is the result of demographic changes where populations are moving from rural to urban areas. These changes have left many populations with little or no medical coverage.

In response to this need for medicinal plant information, the United Nations Educational, Scientific, and Cultural Organization (UNESCO) launched the Asian Pacific Information Network on Medicinal and Aromatic Plants (APINMAP) in July 1987.

APINMAP is a decentralized information-gathering network of 13 member countries in Asia and the Pacific region. The primary objective of the voluntary cooperative program is to promote the exchange of information on medicinal and aromatic plants.

Members feed their data into the Network Center (The Agricultural Information Bank for Asia (AIBA), Philippines). AIBA then compiles the incoming information and redistributes the data to the member countries.

The Network has the following objectives:

- Provide access to information from regional and international sources, including scientific research results.
- Develop and improve specialized information services for member states.
- Assist in the development of information product and services for the targeted end-user communities.
- Establish linkages to regional and international networks or services in the fields of medicinal or aromatic plants and natural product chemistry.

APINMAP is developing a factual database, which will contain actual research data. It is currently

for each medicinal plant. The Plant Record Type form provides a complete physical and taxonomic description of the plant. The Indication/Preparation/Administration Record form records information on the ailment treated, the plant part used, method of preparation, and how the medicine is given. The Marketing Record Type form relays information on the commercial aspects of the plant such as tariff, patent, and availability information.

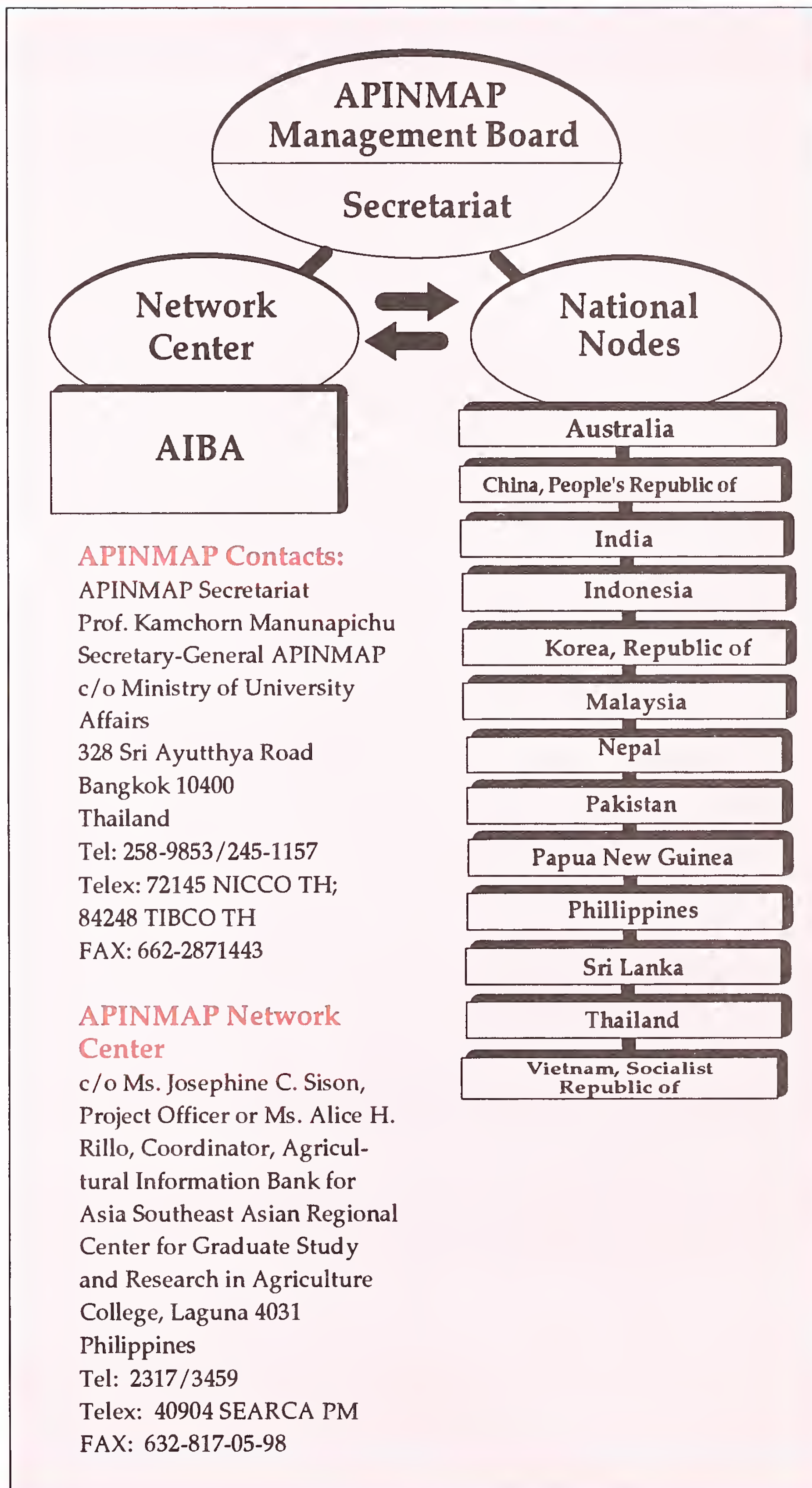
Data is stored and maintained using micro CDS/ISIS software. A separate retrieval software program was written to provide easy data access. The factual database can be queried in two ways:

1) Menu approach: For example, an ailment can be input; plant names that treat the ailment are then retrieved.

2) Query-By-Example: Developed by IBM for complex queries.

In addition to the medicinal plant specific information, APINMAP also has a database called the Integrated Database. The Integrated Database contains bibliographic and directory type information (i.e., research projects, research institutions, information centers, and researchers), all relevant to medicinal and aromatic plants. Boolean operators are used for searching this database. ◆

Mention of a trade name or brand does not constitute endorsement or recommendation by the Department over similiar products not named.



APINMAP Contacts:

APINMAP Secretariat
Prof. Kamchorn Manunapichu
Secretary-General APINMAP
c/o Ministry of University Affairs
328 Sri Ayutthya Road
Bangkok 10400
Thailand
Tel: 258-9853/245-1157
Telex: 72145 NICCO TH; 84248 TIBCO TH
FAX: 662-2871443

APINMAP Network Center

c/o Ms. Josephine C. Sison, Project Officer or Ms. Alice H. Rillo, Coordinator, Agricultural Information Bank for Asia Southeast Asian Regional Center for Graduate Study and Research in Agriculture
College, Laguna 4031
Philippines
Tel: 2317/3459
Telex: 40904 SEARCA PM
FAX: 632-817-05-98

Calendar of Upcoming Genome Events

MEETINGS

January 9-15: **Keystone Symposia on Molecular & Cellular Biology: The Extracellular Matrix of Plants: Molecular, Cellular and Developmental Biology,** Santa Fe, NM. Contact: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498. Telephone: (303) 262-1230.

January 17-22: **Miami Bio/Technology Winter Symposia, Advances in Gene Technology: Protein Engineering and Beyond,** Miami, FL. Contact: Sandra Black, P.O. Box 016129, Miami, FL 33101. Telephone: (800) 642-4363, FAX: (305) 324-5665.

January 24-27: **BIOEAST '93,** Washington, D.C. Telephone: (301) 762-2957.

January 26-February 1: **Keystone Symposia on Molecular & Cellular Biology: Evolution and Plant Development,** Taos, NM. Contact: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498. Telephone: (303) 262-1230.

January 31-February 5: **Recombinant DNA Technology II,** Palm Coast, FL. Contact: C.V. Freiman, Director, Engineering Foundation, 345 East 47th St., New York, NY 10017.

February 8-14: **Keystone Symposia on Molecular & Cellular Biology: Genetic and In Vitro Analysis of Cell Compartmentalization,** Taos, NM. Contact: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498. Telephone: (303) 262-1230.

February 22-26: **Recombinant DNA: Techniques and Applications,** Rockville, MD. Contact: Workshop Coordinator, American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852. Telephone: (301) 231-5566, FAX: (301) 770-1805.

February 23-March 1: **Keystone Symposia on Molecular & Cellular Biology: Nucleases: Structure, Function and Biological Roles,** Tamarron, CO. Contact: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498. Telephone: (303) 262-1230.

March 7-14: **Keystone Symposia on Molecular & Cellular Biology: Frontiers of NMR in Molecular Biology-III,** Taos, NM. Contact: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498. Telephone: (303) 262-1230.

March 31-April 3: **Twelfth Annual Symposium: Current Topics in Plant Biochemistry, Molecular Biology and Physiology,** Columbia, MO. Contact: Doug Randall, 117 Schweitzer Hall, University of Missouri-Columbia, Columbia, MO 65211. Telephone: (314) 882-7796, FAX: (314) 882-5635.

April 13-16: **ABC 7th International Biotech Meeting set at R.T.P.,** Research Triangle Park, NC. Contact: R. Okiuye, Association of Biotechnology Companies, 1666 Connecticut Ave., NW, Suite 330, Washington, DC 20009-1039. Telephone: (202) 234-3330.

April 18-25: **Keystone Symposia on Molecular & Cellular Biology: Transposition and Site-Specific Recombination: Mechanism and Biology,** Keystone, CO. Contact: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498. Telephone: (303) 262-1230.

June 5-9: **Congress on Cell and Tissue Culture: 1993 Meeting of the Tissue Culture Association. Growth Control: From the Receptor to the Nucleus,** San Diego, CA. Contact: Congress on Cell and Tissue Culture, 8815 Center Park Drive, Suite 210, Columbia, MD 21045. Telephone: (410) 992-0946.

July 7-9: **The First International Conference on Intelligent Systems for Molecular Biology,** Washington, DC. Contact: J. Shavlik, Computer Sciences Dept., University of Wiscon-

sin, 1210 W. Dayton St., Madison, WI 53706. Email: ISMB@nlm.nih.gov.

August 2-4: **44th American Institute of Biological Sciences Annual Meeting,** Ames, IA. Contact: AIBS, 730 11th Street NW, Washington, DC 20001-4521. Telephone: (202) 628-1500, FAX: (202) 628-1509.

August 6-10: **Science Innovation '93: New Techniques in Bimolecular Research,** Boston, MA. Contact: AAAS Meetings Office, 1333 H St., NW, Washington, DC 20005. Telephone: (202) 326-6450, FAX: (202) 289-4021.

August 6-9: **Plant Growth Regulator Society of America 20th Annual Meeting,** St. Louis, MO. Contact: Dr. L. Ferguson, Program Chair, University of California, Kearny Agricultural Research Center, 9240 S. Riverbend Ave., Parlier, CA 93648. Telephone: (209) 891-2500.

WORKSHOPS AND COURSES

December 8-11: **DNA Fingerprinting,** Rockville, MD. Contact: Workshop Coordinator, American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852. Telephone: (301) 231-5566, FAX: (301) 770-1805.

January 4-8 OR March 8-12: **Recombinant DNA Methodology,** Washington, DC. Contact: Office Manager, Center for Advanced Training in Cell and Molecular Biology, 103 McCort-Ward Bldg., The Catholic University of America, Washington, DC 20064. Telephone: (202) 319-6161, FAX: (202) 319-4467.

January 9-11: **Polymerase Chain Reaction in Molecular Biology,** Washington, DC. Contact: Office Manager, Center for Advanced Training in Cell and Molecular Biology, 103 McCort-Ward Bldg., The Catholic University of America, Washington, DC 20064. Telephone: (202) 319-6161, FAX: (202) 319-4467.

January 11-15: **Basic Cell and Tissue Culture,** Washington, DC. Contact: Office Manager, Center for Advanced Training in Cell and Molecular Biology, 103 McCort-Ward Bldg., The Catholic University of America, Washington, DC 20064. Telephone: (202) 319-6161, FAX: (202) 319-4467.

February 8-12: **Molecular & Cellular Biology of Macrophage Activation for Cytotoxicity,** Washington, DC. Contact: Office Manager, Center for Advanced Training in Cell and Molecular Biology, 103 McCort-Ward Bldg., The Catholic University of America, Washington, DC 20064. Telephone: (202) 319-6161, FAX: (202) 319-4467.

March 2-5: **Polymerase Chain Reaction (PCR) Applications/ Cycle DNA Sequencing,** Rockville, MD. Contact: Workshop Coordinator, American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852. Telephone: (301) 231-5566, FAX: (301) 770-1805.

March 16-19: **Insect Cell Culture,** Rockville, MD. Contact: Workshop Coordinator, American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852. Telephone: (301) 231-5566, FAX: (301) 770-1805.

April 21-25: **Molecular Genetics of Plant-Microbe Interactions (Workshop and Symposia),** East Brunswick, NJ. Contact: Rutgers, State University of New Jersey, Registration Desk, Office of Continuing Professional Education, Cook College, P.O. Box 231, New Brunswick, NJ 08903. Telephone: (908) 932-9271, FAX: (908) 932-8726.

FUTURE EVENTS

May 8-13, 1994: **HPLC'94, Eighteenth International Symposium on High Performance Liquid Chromatography,** Minneapolis, MN. Contact: Barr Enterprises, P.O. Box 279, Walkerville, MD. Telephone: (301) 898-3772, FAX: (301) 898-5596.

August 4-6, 1994: **Plant Growth Regulator Society of America 21st Annual Meeting,** Portland, OR. Contact: Dr. L. Ferguson, Program Chair, University of California, Kearny Agricultural Research Center, 9240 S. Riverbend Ave., Parlier, CA 93648. Telephone: (209) 891-2500.

June 16-21, 1996: **HPLC'96, Twentieth International Symposium on High Performance Liquid Chromatography,** San Francisco, CA. Contact: Barr Enterprises, P.O. Box 279, Walkerville, MD. Telephone: (301) 898-3772, FAX: (301) 898-5596.

Plant Genome Publications

The following publications are available. If you would like to receive a copy, check off the title and mail your request to:

Plant Genome Data and Information Center
National Agricultural Library, Rm 1402
10301 Baltimore Blvd.
Beltsville, MD 20705-2351

Nucleotide Sequence Listings:

GenBank® nucleotide sequence databank was searched by species. A list was compiled giving the GenBank® locus name and a brief description of the sequence.

295 ***Algal*** **Nucleic acid sequences.** Susan McCarthy and Terrance Henrichs. A GenBank® search. 10 pp.

270 ***Oryza sativa*** **Nucleic acid sequences.** Susan McCarthy and Terrance Henrichs. A GenBank® search. 6 pp.

Quick Bibliographies:

The AGRICOLA bibliographic database was searched by topic. A bibliography was compiled of the relevant citations; abstracts are included when available.

Medical Botany and Herbal Medicine. January 1988 - December 1989. 400 citations. 35pp. Prepared by Jane Potter Gates. QB 90-44

Ethnobotany and Medicinal Plants. January 1990 - June 1991. 591 citations, 107 pp. Prepared by Susan McCarthy. QB 92-66

Ethnobotany and Medicinal Plants. July 1991 - July 1992. 546 citations, 134 pp. Prepared by Susan McCarthy. QB 93-02

Biotechnology: Patenting Issues. January 1979 - March 1992. 168 citations. 27 pp. Prepared by Robert D. Warmbrodt and Laura Wiggert. QB 92-42

Biotechnology: Public Perception. January 1979 - August 1991. 70 citations. 9 pp. Prepared by Robert D. Warmbrodt and Diana Airozo. QB 91-151

Special Reference Briefs:

Science and Technology Policy. April 1992. 454 citations. 56 pp. Prepared by Takefumi Tanaka and Kathleen C. Hayes. SRB 92-09

Biotechnology: Education. February 1992. 125 citations. 22 pp. Prepared by Diana Airozo and Robert D. Warmbrodt. SRB 92-05

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