RGT/CGT

STUDY GUIDE

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INTRODUCTION

MISSION STATEMENT

"SCST promotes professionalism and ensures proficiency by examining and continuing to educate seed analysts. This provides accurate and timely information to the seed industry. The SCST will build upon these strengths by broadening the membership base to include emerging technologies. SCST will continue to promote research and develop publications which enhance seed technology."

The Society of Commercial Seed Technologists is a seed testing organization comprised of commercial, independent and government seed technologists. Formed in 1922, the SCST functioned as a liaison between the Association of Official Seed Analysts (AOSA) and the American Seed Trade (ASTA). The SCST has developed over the years into a progressive organization that trains and provides accreditation of technologists, researches and develops rule changes, publishes training and education materials, and serves as an important resource to the seed industry.

MEMBERSHIP CATEGORES

There are eight membership categories in the SCST. Five of the membership categories (RST, RGT, CGT, CVT, CPT) require qualifying for and passing an examination. Research members have to meet certain qualifications related to access to research facilities and research history in order to become members. Association membership is open to all individuals with an interest in seed testing

Divided into eight categories:

1. Registered Seed Technologist
2. Registered Genetic Technologist
3. Certified Genetic Technologist
4. Certified Viability Technologist
5. Certified Purity Technologist
6. Research Member
7. Associate Member
8. Honorary Member

REGISTERED GENETIC TECHNOLOGIST (RGT)

Prior to taking any of the RGT written exams, the candidate must first be an Associate Member of the Society for two years. An RGT has qualified for and passed the Molecular Genetics exam as well as all three genetic technology exams currently available: Trait Purity, Genetic Purity and Adventitious Presence (AP). The RGT exam includes a required written molecular genetics/biology exam and area specific written and practical exams in the three genetic technology areas. These examinations are

designed to establish the applicant's competency as a well-trained genetic technologist in the seed industry.

RGTs are required to complete continuing education in order to maintain membership and are required to pay annual membership dues. They must sign a Membership Contract for Privilege of Use of the Societies name, logo, RGT seal, and the title Registered Genetics Technologist.

RGTs have one vote on all Society business and can vote on the amendments to the AOSA for Rules Testing Seeds. RGTs are eligible to run for elected office and can chair or participate on committees.

CERTIFIED GENETIC TECHNOLOGIST (CGT)

Prior to taking any of the CGT written exams, the candidate must first be an Associate Member of the Society for two years. A CGT has qualified for and passed the Molecular Genetics exam in addition to one or two of the three genetic technology exams currently available: Trait Purity, Genetic Purity and Adventitious Presence (AP). The CGT exam includes a required written molecular genetics exam and area specific written and practical exams in the three genetic technology areas. These examinations are designed to establish the applicant's competency as a well-trained genetic technologist in the seed industry.

CGTs are required to complete continuing education in order to maintain membership and are required to pay annual dues. They must sign a Membership Contract for Privilege of Use of the Societies name, logo, and the title Certified Genetic Technologist.

CGTs have one vote on all Society business and can vote on the amendments to the AOSA for Rules Testing Seeds. CGTs are eligible to run for elected office and can chair or participate on committees.

RGT/CGT MEMBERSHIP

MEMBERSHIP PROCESS

Membership as a Registered Genetic Technologist (RGT) or Certified Genetic Technologist (CGT) shall be attained in the following order:

1. Complete and return the membership application by April 1st for first exams scheduled for the SCST annual meeting, and October 1st for the December exams. Applications are available from the SCST website: http://www.seedtechnology.net/Membership.htm
2. Fulfill the qualifications for membership as prescribed (accumulation of 100 points) at least 14 days prior to written examination day. The first written examination will be given during Society of Commercial Seed Technologist (SCST) annual meeting and the second examination will be scheduled in December. The examination site for December will be determined by the RGT BOE committee and communicated back to interested candidates. Be actively involved in genetic testing for a minimum of two years.

3. Obtain unanimous approval of the RGT Board of Examiners (BOE). If a unanimous vote of said Board cannot be obtained, the SCST Executive Board will act as a Board of Review.
4. Attain passing grades in the prescribed written and practical examinations. The examinations consist of demonstrated written and practical competency in each of the three areas of genetic testing: Trait Purity, Genetic Purity and Adventitious Presence (AP).
    a. It is a requirement that the candidate passes the Molecular Genetics written exam. The MG exam score must be ≥ 70 points and this score is then averaged for each of the three genetic exams and the final score has to be ≥ 80 points. (Example: MG, Trait Purity written exam and Trait Purity practical exam averaged must be ≥ 80 points)
    b. An individual passing the written and practical portions for any of the three areas of the examination will be conferred the title of Certified Genetic Technologist in that area(s) of competency.
    c. An individual passing the written and practical portions for all three areas of the examination will be conferred the title Registered Genetic Technologist.
5. Pay any dues or assessments.
6. Sign and return RGT or CGT Membership Contract

APPLICATIONS FOR MEMBERSHIP

Membership applications can be downloaded from the SCST website or are available from the Executive Director. Please check the SCST website or contact the Executive Director to ensure that you are submitting a current version of the membership application. It is recommended that you submit a trial application to the Executive Director before the April 1st deadline to ensure that you have fulfilled the requirements for membership. Please contact the Executive Director if you have any questions or need help completing the application for membership

MEMBERSHIP QUALIFICATIONS

Applicants applying for membership, as a RGT or CGT shall meet the following qualifications:

POINTS REQUIRED TO QUALIFY FOR EXAMS

In order to qualify to take the RGT/CGT exam the candidate must accumulate **a minimum of 100 points** from work experience, workshops/meetings, and college courses.

1. **College courses:** Accepted accredited courses in Biological and Molecular Sciences – 3 points for each earned semester hour (2 points for each earned quarter hour). Maximum of 50 points allowed.

Examples of accredited courses which will be accepted:

Plant Physiology	Plant Taxonomy	Plant Genetics	Plant Breeding
Molecular Genetics	Biotechnology	Biochemistry	Chemosystematics
Research Methods	Gene Expression	Plant Genome	Cell Biology
Biometrics	Bacteriology	Microbiology	Horticulture
Biology	Immunology		

2. **Approved Genetic Purity Workshops** - Maximum of 20 points.
   Note: An additional 5 points will be allowed in this category for full attendance at an SCST Annual Conference. (Prior to taking the examination)

   Workshop points will be credited at 2 points/full day and 1 point/half day when they are directly related to genetic purity testing comprising a minimum of 50% of time toward "hands-on" type program. Workshops comprising less than 50% of time towards hands-on experience will be credited at 1 point/full day.

7. **Work Experience.** Candidate must be actively involved in genetic testing for a minimum of two years. Training under the supervision of a qualified supervisor (RGT, CGT, or other RGT BOE approved individual). 1 point for each 40 hours training. **Unsupervised** genetic purity testing experience: 1 point for each 80 hours experience. Combination of these which meet the requirement of a minimum of one year of experience in hands-on genetic purity testing.

   If hands-on genetic purity testing experience was obtained earlier than the immediate one year prior to submitting application for RGT or CGT examination, applicant shall complete the following additional requirement: **Note:** Proof of five points of hands-on continuing education between the time of original training and applying for the RGT or CGT examination.

EXAMINATION PROCESS

1. Submit a written application form to the SCST Executive Director including application fee by April 1st for the SCST annual meeting exams and October 1st for the December exams.
2. Applicants are approved by the RGT BOE, if a unanimous vote of the RGT BOE cannot be obtained, the Executive Board will act as a Board of Review.
3. Candidates take the written examination at the SCST annual meeting or at the scheduled time in December.
4. RGT BOE chair will provide test scores to the candidate at the annual meeting. Written exams taken in December, candidate will be notified by the end of the year.
5. Candidates must pass the written examination portion(s) before applying for the practical examination portion(s). Written exams will not be returned to the examinee.
6. Apply to the SCST Executive Director using the Genetic Technologist Practical Exam Application form. On this form the candidate can select which of the four areas (herbicide bioassay, Immunoassay, PCR and Electrophoresis they are requesting practical samples for and the respective species/methods. The candidate must also designate a proctor and must be approved by the RGT BOE Committee.
7. The approved proctor will receive the samples from the RGT BOE chair and inform the candidate that samples are onsite. The proctor will use the proctor forms to record receipt of samples, date candidate starts practical, and verify required steps or points of inspection in the practical examination process. Sample results must be returned to SCST within 30 days from the samples

being transferred to the examinee. Control samples may be given to examinee prior to test samples

8. Once the examination(s) are completed the proctor will mail/fax/email the results and proctor forms to the RGT Board of Examiners (Chair). The RGT BOE chair will then forward the coded practical exams to the respective BOE members for grading.
9. Practical exam scores will be returned to the candidate with 30 days of receipt by the RGT BOE chair. Practical exams will not be returned to the examinee.
10. An individual passing the Molecular Genetics exam and one or two of the written and practical portions for any of the three additional areas of the examination will be conferred the title of Certified Genetic Technologist in that area(s) of competency. An individual passing the Molecular Genetics exam and the written and practical portions for all three additional areas of the examination will be conferred the title Registered Genetic Technologist.

PASSING GRADES

1. All grading of the examination is done by RGT BOE Committee. All tests are identified by a candidate number which is assigned by the RGT BOE chair and exams are then sent out to RGT board members for grading. Candidates name is not shared in this grading process. Passing Grades: An applicant shall fulfill the following qualifications
    a. Achieve a grade of 70% or better on each part of the written and practical examination.
    b. Achieve an average grade of 80% or better for the written and practical exams in each area in which certification is sought.

    Example: in order to become certified in Trait purity the molecular genetics written exam score will be averaged with the Trait purity written and practical exam scores. Each individual score must be 70% or higher and the total score must be 80% or higher.

EXAMINATION FORMAT

WRITTEN EXAMINATIONS

2. Written examinations are proctored by the RGT BOE at the SCST annual meeting and a second examination will be scheduled six months after the annual meeting (December timeframe). The examination site will be determined by the RGT BOE Committee written portion of the examination will consist of:
    a. A general molecular genetics/biology exam- taken by all candidates
    b. Three area specific exams emphasizing Trait Purity, Genetic Purity and Adventitious Presence (AP). One hour and thirty minutes is given for each of the four exams. Breaks are given between each exam.
3. Questions will be a combination of definitions, multiple choice, true/false, short answer, and problem solving. Candidates will complete the exams and be informed of their scores at the SCST annual meeting and/or by email from the RGT BOE chair.

PRACTICAL EXAMINATIONS

1. Practical exams can only be taken after the written exams have been passed.
2. The practical portion of the examination will consist of four parts emphasizing Herbicide Bioassay, ELISA, Electrophoresis and PCR-based techniques. Examinations may be taken within the candidate's laboratory, or at an approved location.
3. Once proctored exams are complete, proctor will forward test results back to RGT BOE chair for grading. Chair will then forward practical results to RGT BOE members for grading. Exams will then be sent back to Chair and test scores will be communicated back to candidate by email and a Record of Examination form will be sent to examinee
4. Practical examinations must be completed within one year from the time the candidate passes the written examination. The RGT BOE chair will send quarterly reminders to candidates.
5. If a candidate fails the first practical examination they must complete eight hours of continuing education. Acceptable continuing education includes:
    a. Individualized study with a BOE approved tutor. An agenda must be submitted to the Executive Director for approval prior to the training session.
    b. Attendance at a genetic technology workshop.
    c. Other independent study approved by the RGT BOE.
6. Candidates must wait a minimum of two months before re-taking the exam. Once approved to re-take the practical exam it must be completed and returned within four months. If the candidate fails the exam again they will be required to re-take both the written and practical exam.

PRACTICAL EXAMINATION PROCTORS

1. Exam proctors are used to monitor candidates taking one or more of the practical exams.
2. An acceptable proctor may be an RST, RGT, or CGT. The RGT BOE will approve other proctors on a case by case basis when needed.
3. The role of proctor is to make sure the candidate is doing the work but will not grade or assist the candidate in taking the exam. Proctors receive the seed samples and test forms, deliver them to the candidate, and monitor testing of critical steps for each section. When testing is complete, the proctor sends the test materials to the RGT BOE chair.

MAINTENANCE OF MEMBERSHIP

CONTINUING EDUCATION

All RGT and CGT members are required to complete 5 points of continuing education every three years:

1. Attend a minimum of three (3) full days at the Annual Meeting of the Society, which shall include attendance at the SCST business meeting, Registered or Certified Members present at the meeting but not in attendance during Roll Call are responsible for having their name recorded by the Executive Director.

2. Attain five (5) points for attendance at workshops or seed schools directly related to seed testing that comprise a "hands-on" type program and have been approved prior to attendance by the Executive Director.
3. Attend individualized seed technology training that receives prior approval by the Executive Director. Points are credited on the basis of one (1) point for every three (3) hours, maximum two points per day. A certificate of attendance must be submitted to the Executive Director to receive proper point credits. College credits from approved seed related courses would be acceptable for up to half (1/2) of required points based on three points for each semester hour or two (2) points for each quarter hour.

PREPARING FOR THE RGT/CGT EXAMINATIONS

In addition to the references listed in this section there are a number of resources available on the SCST website at http://www.seedtechnology.net/meetings_workshops Webpage includes presentations from past Genetic Technology Workshops, The Library of Crop Technology Lesson Modules from the University of Nebraska Lincoln, and other helpful websites. Workshops and training opportunities are also listed on the website. Other RGT/CGT study material such as Chapter 14 can be purchased off the SCST website at http://www.analyzeseeds.com/publications/

It is highly recommended that candidates review the presentations from past genetic Technology Workshops; these workshops are specifically focused on preparation for the RGT/CGT exams.

GENERAL REFERENCES:

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3. *Handbook of Variety Testing. Growth Chamber – Greenhouse Testing Procedures: Variety Identification*. ISTA 1993
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11. Glossary of Biotechnology Terms http://biotechterms.org
12. ISTA Statistical Tool Box http://www.seedtest.org/en/stats_tool_box_content---1--1143.html

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http://www.ndsu.nodak.edu/instruct/mcclean/plsc731/dna/dna4.htm

GLOSSARY

ACCURACY
Agreement between a measured value and accepted reference sample.

ADJUVANT (to a herbicide)
From the Latin word for "aid", it refers to any compound that enhances the effectiveness (i.e., weed-killing ability) of a given herbicide. For example, adjuvants such as surfactants can be mixed (prior to application to weeds) with herbicide (in water), in order to hasten transport of the herbicide's active ingredient into the weed plant. That is because the herbicide must move from an aqueous (water) environment into one (i.e., the weed plant's cuticle or "skin") comprised of lipids/lipophilic molecules, before it can accomplish its task.

AGROBACTERIUM TUMEFACIENS
A naturally occurring bacterium that is capable of inserting its DNA (genetic information) into plants, resulting in a type of injury to the plant known as crown gall. In 1980, Marc van Montagu showed that *Agrobacterium tumefaciens* could alter the DNA of its host plant(s) by inserting its own ("foreign") DNA into the genome of the host plants (thereby opening the way for scientists to insert virtually any foreign genes into plants via use of *Agrobacterium tumefaciens*). Among others, Monsanto Company has developed a way to stop *Agrobacterium tumefaciens* from causing crown gall, while maintaining its ability to insert DNA into plant cells, and now uses *Agrobacterium tumefaciens* as a vehicle to insert desired genes into plants (e.g., the gene causing overproduction of CP4 EPSP synthase, thus conferring resistance to glyphosate-containing herbicide).

ALLELE
From the Greek *allelon* = "mutually each other", the term refers to one of several alternate forms of a gene occupying a given locus on the chromosome, which controls expression (of product) in different ways.

ANTIBODY
Also called immunoglobulin, Ig. A large defense protein that consists of two classes of polypeptide chains, light (L) chains and heavy (H) chains. A single antibody molecule consists of two identical copies of the L chain and two of the H chain. They are synthesized (i.e., made) by the immune system (B lymphocytes) of the organism. The antibody is composed of four proteins linked together to form a Y-shaped bundle of proteins (looks somewhat like a slingshot or two hockey sticks taped together at the handles). The amino acid sequence that makes up the stem (heavy chains) of the Y (i.e., the handles of the taped together hockey sticks) is similar for all antibodies. The stem is known as the Fc region of the antibody and it does not bind to antigen, but does have other regulatory functions.

The two arms of the Y are each made up of two side-by-side proteins called light chains and heavy chains (i.e., proteins are chains of amino acids), with identical antigen-binding (ab) sites on the tips of each "arm." The antibody is thus bivalent in that it has two binding sites for antigen. Taken together, the two arms of the Y are known as the Fab portions of the antibody molecule. The Fab portions can be cleaved from the antibody molecule with papain (an enzyme that is also used as a meat tenderizer) or the Fab portions can be produced via genetically engineered *Escherichia coli* bacteria.

When a foreign molecule (e.g., a bacterium, virus, etc.) enters the body, B lymphocytes are stimulated into becoming rapidly dividing blast cells, which mature into antibody-producing plasma cells. The plasma cells are triggered by the foreign molecule's epitope(s) [i.e., group or groups of specific atoms (also known as a hapten), that are recognized to be foreign by the body's immune system] into producing antibody molecules possessing antigen-binding (ab) sites (also called combining sites or determinants).

These fit into the foreign molecule's epitope. Thus, via the tips of its arms, the antibody molecule binds specifically to the foreign entity (antigen) that has entered the body. By this process it inactivates that foreign molecule or marks it for eventual destruction by other immune system cells.

System marking of the foreign molecule (e.g., pathogen or toxin) for destruction is accomplished by the fact that the stem of the Y (i.e., the Fc) fragment hangs free from the combined antibody-antigen clump, thereby providing a receptor for phagocytes, which roam throughout the body ingesting and subsequently destroying such "marked" foreign molecules. This system is called antibody-dependent cellular cytotoxicity.

Research published during 2001 indicates that antibodies may also **kill some pathogens themselves** by catalyzing the formation of hydrogen peroxide from **oxygen free radicals (singlet oxygen)** and water. Hydrogen peroxide is highly reactive, and could potentially kill pathogens when generated by an (attached) antibody.

There are five classes of immunoglobulin: IgG, IgM, IgD, IgA, and IgE.

ANTIGEN

Also called an immunogen. Any large molecule or small organism whose entry into the body provokes synthesis of an antibody or immunoglobin (i.e., an immune system response).

ANTISENSE (DNA SEQUENCE)

A strand of DNA that produces a messenger RNA (mRNA) molecule which (when reversed end-for-end) has the same sequence as (i.e., is complementary to) the unwanted ("bad")messenger RNA. The SENSE (i.e., forward) and ANTISENSE (i.e., backward) mRNA strands hybridize (i.e., tightly bond to each other), which prevents the bonded-pair from leaving the cell's nucleus, so that boded-pair is rapidly degraded (destroyed) by nucleases within the cell nucleus.

In genetic targeting using antisense molecules (to block "bad" genes), antisense molecules are used to bind to a "bad" gene's (e.g., an oncogene) messenger RNA (mRNA), thus cancelling the (cancer-causing) message of the gene and preventing cells from following its (tumor growth) instructions. Another example would be the use of antisense DNA to block the gene that codes for production of polygalacturonase (an enzyme that causes ripe fruit to soften).

Physically, "antisense" is accomplished by removing a given gene from an organism's genome, reversing it (end for end), and reinserting it back into the organism's genome.

ARABIDOPSIS THALIANA

A small weed plant possessing 70,000 kilobase pairs in its genome, with very little repetitive DNA. This makes it an ideal model for studying plant genetics. At least two genetic maps have been created for *Arabidopsis thaliana* (one using yeast artificial chromosomes). Because of this a large base of knowledge about it has been accumulated by the scientific community.

Arabidopsis thaliana was first genetically engineered in 1986. In 1994, researchers succeeded in transferring genes for polyhydroxylbutylate ("biodegradable plastic") production into *Arabidopsis thaliana.* Because production of polyhydroxylbutylate (PHB) requires simultaneous expression of three genes (i.e., the PHB production process is "polygenic") - yet researchers have only been able to insert a maximum of two genes -they have to insert two genes into one plant and one gene into a second plant, then finally get the (total) three genes into (offspring) plants via traditional breeding.

ASSAY

A test (specific technique) that measures a response to a test substance or the efficacy (effectiveness) of the test substance.

BACILLUS THURINGIENSIS (B.T.)

Discovered by bacteriologist Ishiwata Shigetane on a diseased silkworm in 1901. Later discovered on a dead Mediterranean flour moth, and first named *Bacillus thuringiensis,* by Ernst Berliner in 1915.

Today, *Bacillus thuringiensis* refers to a group of rod- shaped soil bacteria found all over the earth, that produce "cry" proteins which are indigestible by - yet still "bind" to -specific insects' gut (i.e., stomach) lining receptors, so those "cry" proteins are toxic to certain classes of insects (corn borers, corn rootworms, mosquitoes, black flies, some types of beetles, etc.), but which are harmless to all mammals. At least 20,000 strains of *Bacillus thuringiensis* are known.

Genes that code for the production of these "cry" proteins that are toxic to insects have been inserted by scientists since 1989 into vectors (i.e., viruses, other bacteria, and other microorganisms) in order to confer insect resistance to certain agricultural plants (e.g., via expression of those *B.t.* proteins by one or more tissues of the transgenic plant). For example, the *B.t.* strain known as *B.t. kurstaki,* which is fatal when ingested by the European corn borer was first (genetically) inserted into a corn plant (via vector) in 1991. *B.t. kurstaki* kills borers via perforation of that insect's gut by proteins that are coded for by the *B.t. kurstaki* gene. The vectors as listed above are entities that can take up and carry the DNA into plant or other cells. Vectors are DNA-carrying vehicles.

BASE PAIR (BP)

Two nucleotides that are in different nucleic acid chains and whose bases pair (interact) by hydrogen bonding. In DNA, the nucleotide bases are adenine (which pairs with thymine) and guanine (which pairs with cytosine). In RNA, the nucleotide bases are adenine (which pairs with uracil) and guanine (which pairs with cytosine).

BIOASSAY

Determination of the relative strength or bioactivity of a substance (e.g., a drug). A biological system (such as living cells, organs, tissues, or whole animals) is exposed to the substance in question and the effect on the living test system is measured.

BIOCHEMISTRY

The study of chemical processes that comprise living things (systems). The chemistry of life and living matter. Despite the dramatic differences in the appearances of living things, the basic chemistry of all organisms is strikingly similar. Even tiny one-celled creatures carry out essentially the same chemical reactions that each cell of a complex organism (such as man) carries out.

BIOTECHNOLOGY

The means or way of manipulating life forms (organisms) to provide desirable products for man's use. For example, beekeeping and cattle breeding could be considered to be biotechnology-related endeavors. The word biotechnology was coined in 1919 by Karl Ereky, to apply to the interaction of biology with human technology.

However, usage of the word biotechnology in the United States has come to mean all parts of an industry that knowingly create, develop, and market a variety of products through the willful manipulation, on a molecular level, of life forms or utilization of knowledge pertaining to living systems.

A common misconception is that biotechnology refers only to recombinant DNA (rDNA) work. However, recombinant DNA is only one of the many techniques used to derive products from organisms, plants, and parts of both for the biotechnology industry. A list of areas covered by the term biotechnology would more properly include: recombinant DNA, plant tissue culture, rDNA or gene splicing, enzyme systems, plant breeding, meristem culture, mammalian cell culture, immunology, molecular biology, fermentation, and others.

BLANK (in an ELISA plate)

Measures the optical density associated with the regents used in the test and plate. A well with no sample that is used as a baseline for the plate reader, removes any background color.

Btk

Transgenic protein Bacillus kurstakii, resistant to the corn borer.

C-DNA

Also known as copy DNA. A helical form of DNA. It occurs when DNA fibers are maintained in 66 percent relative humidity in the presence of lithium ions. It has fewer base pairs per turn than B-DNA.

CARBOHYDRATES

(saccharides) A large class of carbon-hydrogen-oxygen compounds. Monosaccharides are called simple sugars, of which the most abundant is D-glucose. It is both the major fuel for most organisms and constitutes the basic building block of the most abundant polysaccharides, such as starch and cellulose. While starch is a fuel source, cellulose is the primary structural material of plants. Carbohydrates are produced by photosynthesis in plants. Most, but not all, carbohydrates are represented chemically by the formula $Cx(H_20)n$, where n is three or higher. On the basis of their chemical structures, carbohydrates are classified as polyhydroxy aldehydes, polyhydroxy ketones, and their derivatives.

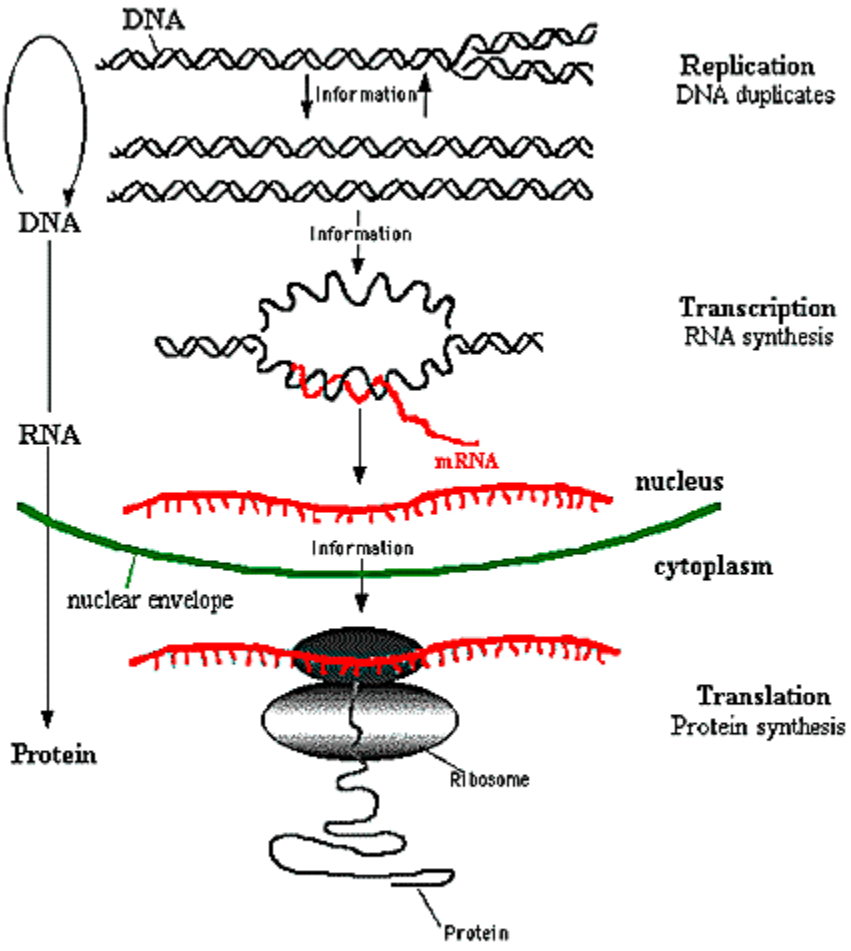
CATALYST

Any substance (entity), either of protein or of nonproteinaceous nature, that increases the rate of a chemical reaction, without being consumed itself in the reaction. In the biosciences, the term "enzyme" is used for a proteinaceous catalyst. Enzymes catalyze biological reactions.

CAULIFLOWER MOSAIC VIRUS 35S PROMOTER (CaMV 35S)

A promoter (sequence of DNA) that is often utilized in genetic engineering to control expression of (inserted) gene; i.e., synthesis of desired protein in a plant.

CENTRAL DOGMA



The Central Dogma of Molecular Biology

Legend:

Transcription of DNA to RNA to protein: This dogma forms the backbone of molecular biology and is represented by four major stages.

1. The DNA replicates its information in a process that involves many enzymes: **replication.**
2. The DNA codes for the production of messenger RNA (mRNA) during **transcription.**
3. In eucaryotic cells, the mRNA is **processed** (essentially by splicing) and migrates from the nucleus to the cytoplasm.

4. Messenger RNA carries coded information to ribosomes. The ribosomes "read" this information and use it for protein synthesis. This process is called **translation**.
Proteins do not code for the production of protein, RNA or DNA.
They are involved in almost all biological activities, structural or enzymatic.

CHROMATOGRAPHY

A process by which complex mixtures of different molecules may be separated from each other. This is accomplished by subjecting the mixture to many repeated partitionings between a flowing phase and a stationary phase. Chromatography constitutes one of, if not *the* most fundamental separation techniques used in the biochemistry/biotechnology arena to date.

CHROMOSOMES

Discrete units of the genome carrying many genes, consisting of (histone) proteins and a very long molecule of DNA. Found in the nucleus of every plant and animal cell.

CLONE (AN ORGANISM)

A group of individual organisms (or cells) produced from one individual cell through asexual processes that do not involve the interchange or combination of genetic material. As a result, members of a clone have identical genetic compositions. For example, protozoa and bacteria frequently reproduce asexually (i.e., without sex) by a process called binary fission. In binary fission a single-celled organism undergoes cell division. The result is two cells with identical genetic composition. When these two identical cells undergo division, the result is four cells with identical genetic composition. These identical offspring are all members of a clone. The word "clone" may be used either as a noun or a verb.

CODING SEQUENCE

The region within a DNA molecule (i.e., between the start and stop codons) that encodes the amino acid sequence of a protein, or for a specific micro-RNA.

COMPLEMENTARY DNA (c-DNA)

A single-stranded DNA that is complementary to a strand of mRNA. The DNA is synthesized *in vitro* by an enzyme known as reverse transcriptase. Then, a second DNA strand is synthesized via the enzyme known as DNA polymerase.

Complementary DNA is often utilized in hybridization studies and in microarrays (e.g., to detect/identify genes) because cDNAs usually don't contain regulatory sequences of DNA; since the cDNA was copied from mRNA. Because cDNA is a DNA copy of mRNA (messenger RNA), it is an exception to the (old) Central Dogma.

CONJUGATE

A molecule created by fusing together (e.g., via recombination or chemically) two unlike (different) molecules. The purpose of this is to create a molecule in which one of the original molecules has one function, for example, a toxic, cell-killing function, while the other original molecule has another function, such as targeting the toxin to a specific site in the body, which might be cancerous cells.

CONTAMINANT

By definition, any unwanted or undesired organism, compound, or molecule present in a controlled environment. Unwanted presence of an entity in an otherwise clean or pure environment.

CP4 EPSPS

The enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase, which is naturally produced by an *Agrobacterium* species (strain CP4) of soil bacteria. CP4 EPSPS is essential for the functioning of that bacterium's metabolism biochemical pathway. CP4 EPSPS happens to be unaffected by glyphosate-containing herbicides, so introduction of the CP4 EPSPS gene into crop plants (e.g., soybeans) makes those plants essentially impervious to glyphosate-containing herbicides.

CROSS REACTION

When an antibody molecule (against one antigen) can combine with (bind to) a different (second) antigen. This sometimes occurs because the second antigen's molecular structure (shape) is very similar to that of the first antigen.

CRY PROTEINS

A class of proteins produced by *Bacillus thuringiensis (B.t.)* bacteria (or plants into which a *B.t.* gene has been inserted). Cry (i.e., "crystal like") proteins are toxic to certain categories of insects such as corn borers, corn rootworms, armyworms, black cutworms, and velvetbean caterpillar.

DENATURATION

The loss of the native conformation of a macromolecule resulting, for instance, from heat, extreme pH (i.e., by acidity of basicity) changes, chemical treatment, etc. It is accompanied by loss of biological activity.

DEOXYRIBONUCLEIC ACID (DNA)

Discovered by Frederick Miescher in 1869, it is the chemical basis for genes. The chemical building blocks (molecules) of which genes (i.e., paired nucleotide units that code for a protein to be produced by a cell's machinery, such as its ribosomes) are constructed. Every inherited characteristic has its origin somewhere in the code of the organism's complement of DNA. The code is made up of subunits, called nucleic acids. The sequence of the four nucleic acids is interpreted by certain molecular machines (systems) to produce the required proteins of which the organism is composed. The structure of the DNA molecule was elucidated in 1953 by James Watson, Francis Crick, and Maurice Wilkins. The DNA molecule is a linear polymer made up of deoxyribonucleotide repeating units (composed of the sugar 2-deoxyribose, phosphate, and a purine or pyrimidine base). The bases are linked by a phosphate group, joining the 3' position of one sugar to the 5' position of the next sugar. Most molecules are double-stranded and anti-parallel, resulting in a right-handed helix structure that is held together by hydrogen bonds between a purine on one chain and pyrimidine on the other chain. DNA is the carrier of genetic information, which is encoded in the sequence of bases; it is present in chromosomes and chromosomal material of cell organelles such as mitochondria and chloroplasts, and also present in some viruses.

DNA PROFILING

Invented in 1985 by Alec Jeffreys, it is a technique used by forensic (i.e., crime-solving) chemists to match biological evidence (e.g., a blood stain) from a crime scene to the person (e.g., the assailant) involved in that particular crime. DNA profiling involves the use of RFLP (restriction fragment length polymorphism) analysis or ASO/PCR (allele-specific oligonucleotide/polymerase chain reaction) analysis to analyze the specific sequence of bases (i.e., nucleotides) in a piece of DNA taken from the biological evidence. Since the specific sequence of bases in DNA molecules is different for each individual (due to DNA polymorphism), a criminal's DNA can be matched to that of the evidence to prove guilt or innocence. Biological evidence may include among other things blood, hair, nail fragments, skin, and sperm.

DOMINANT ALLELE

Discovered by Gregor Mendel in the 1860s, it is a gene that produces the same phenotype when it is heterozygous as it does when it is homozygous (i.e., trait, or protein, is expressed even if only one copy of the gene is present in the genome).

ELECTROPHORESIS

A technique for separating molecules based on the differential movement of charged particles through a matrix when subjected to an electric field. The term is usually applied to large ions of colloidal particles dispersed in water. The most important use of electrophoresis (currently) is in the analysis of proteins, and then a technique known as gel electrophoresis is used. Since the proportion of proteins varies widely in different diseases, electrophoresis can be used for diagnostic purposes.

Electrophoresis, through agarose or other gel matrices, is a common way to separate, identify, and purify plasmid DNA, DNA fragments resulting from digestion (of DNA) with restriction endonucleases, and RNA. Electrophoresis is also used to study bacteria and viruses, nucleic acids, and some types of molecules, including amino acids.

ELISA (test for proteins).

An enzyme-linked immunosorbent assay which can readily measure less than a nanogram (10^{-9}g) of a protein. This assay is more sensitive than simple immunoassay (tests) because one of the two antibodies used to bind and quantitate (measure) the protein % antigen, based on two concurrent epitopes within the protein, is attached to an enzyme. The enzyme can rapidly convert an added colorless substrate into a colored product, or a nonfluorescent substrate into an intensely fluorescent product (thus enabling finer quantitation).

ENZYME

An organic, protein-based catalyst that is not itself used up in the reaction. It is naturally produced by living cells to catalyze biochemical reactions. Each enzyme is highly specific with regard to the type of chemical reaction that it catalyzes, and to the substances (called substrates) upon which it acts. This specific catalytic activity and its control by other biochemical constituents are of primary importance in the physiological functions of all organisms. Although all enzymes are proteins, they may, and usually do, contain additional nonprotein components called coenzymes that are essential for catalytic activity.

EPITOPE

Also called antigenic determinant. The specific group of atoms (on an antigen molecule) that is recognized by (that antigen's) antibodies.

ERROR RATE

The percentage of times that the test will produce a false reading.

EVENT

Refers to each instance of a genetically engineered organism. For example, the same gene inserted by man into a given plant genome at two different locations (i.e., loci) along that plant's DNA would be considered two different "events." Alternatively, two different genes inserted into the same locus of two same-species plants would also be considered two different "events."

Generally speaking, the world's regulatory agencies confer new biotech-derived product approvals in terms of events.

EXPRESS

To translate the cell's genetic information stored in the DNA (gene) into a specific protein (synthesized by the cell's ribosome system).

F1 HYBRIDS

The first-generation offspring of crossbreeding; also known as first filial hybrids. They tend to be more healthy, productive, and uniform than their parents.

FEMALE SELF

Protein banding patterns that are identical to the seed parent of a hybrid.

GENE

A natural unit of the hereditary material, which is the physical basis for the transmission of the characteristics of living organisms from one generation to another. The basic genetic material is fundamentally the same in all living organisms: it consists of chain-like molecules of nucleic acids- deoxyribonucleic acid (DNA) in most organisms and ribonucleic acid (RNA) in certain viruses- and is usually associated in a linear arrangement that (in part) constitutes a chromosome.

The segment of DNA that is involved in producing a polypeptide chain. It includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

GENETIC CODE

The set of triplet code words in DNA coding for all of the amino acids. There are more than 20 different amino acids and only four bases (adenine, thymine, cytosine, and guanine). The mRNA code is a triplet code, that is, each successive "frame" of three nucleotides (sometimes called a codon) of the mRNA corresponds to one amino acid of the protein. This rule of correspondence is the genetic code. The genetic code consists of 64 entries- the 64 triplets possible when there are four possible nucleotides, each of which can be at any of three places (4 x 4 x 4 = 64). A triplet code was required because a doublet code would have only been able to code for (4 x 4 = 16) sixteen amino acids. A triplet code allows for the coding of 64 theoretical amino acids. Since only a little over 20 exist, there is some redundancy in the system. Hence some certain amino acids ate coded for by two or three different triplets.

GENETICS

The branch of biology concerned with heredity, it was literally invented by Gregor Mendel in the 19th century. It is a study of the manner in which genes operate and are transmitted from parents to offspring. It involves the study of the mechanism of gene action- the manner in which the genetic material (DNA) affects physiological reactions within the cell.

GENOME

One complete set of genetic information from a genetic system; e.g., the single strand, circular chromosome of a bacterium is its genome.

GENOMICS

The scientific study of genes and their role in an organism's structure, growth, health, disease (and/or resistance to disease, etc.).

GENOTYPE

The total genetic, or hereditary, constitution that an individual receives from its parents. An individual organism's genotype is distinguished from its phenotype, which is its appearance or observable character.

GMO

Genetically manipulated organism, or genetically modified organism.

GOOD LABORATORY PRACTICES (GLP)

A set of rules and regulations issued by the Food and Drug Administration (FDA) that establishes broad methodological guidelines for procedures and record keeping. They are to be followed in laboratories involved in the testing and/or preparation of pharmaceuticals. GLPs also apply to the Environmental Protection Agency (EPA) (e.g., toxicity testing of new herbicides).

HELIX

A spiral, staircase-like structure with a repeating pattern described by two simultaneous operations (rotation and translation). It is one of the natural conformations exhibited by biological polymers.

HERBICIDE-TOLERANT CROP

Crop plants, cultivated by man, which have been altered to be able to survive application(s) of one or more herbicides by the incorporation of certain gene(s), via either genetic engineering or traditional breeding techniques. For example, crops (e.g., soybean, canola, cotton, corn/maize, etc.) are made tolerant to glyphosate-containing herbicides by insertion (via genetic engineering techniques) of the transgene for CP4 EPSPS. Corn (maize) is made tolerant to imidazolinone-containing herbicides by adding (via traditional breeding techniques) the imidazolinone-tolerant trait. That trait is imparted by the T-Gene, IT-Gene, or the IR-Gene.

HETEROTROPH

An organism that obtains nourishment from the ingestion and breakdown of organic matter.

HETEROZYGOTE

An individual organism with different alleles at one or more particular loci.

HOOK EFFECT

When an ELISA system is overwhelmed with the target antigen resulting in lower than expected optical density readings for lower dilution samples than higher diluted samples.

HORMONE

A type of chemical messenger (peptide), occurring both in plants and animals, that acts to inhibit or excite metabolic activities (in that plant or animal) by binding to receptors on specific cells to deliver its "message." A hormone's site of production is distant from the site of biological activity (i.e., where the message is delivered).

HYBRIDIZATION (MOLECULAR GENETICS)

The pairing (tight physical bonding) of two complementary single strands of RNA and/or DNA to give a double-stranded molecule.

HYBRIDIZATION (PLANT GENETICS)

The mating of two plants from different species or genetically very different members of the same species to yield hybrids (first filial hybrids) possessing some of the characteristics of each parent. Those (hybrid) offspring tend to be more healthy, productive, and uniform than their parents-- a phenomenon known as "hybrid vigor". Hybrids can also arise from more than two ("parent") species.

Hybrid com/maize seed was first commercialized (in the United States) in 1922. Other recently-created crop hybrids include tangelos (produced by crossing grapefruit with tangerines), nectarines (bred from peaches), etc.

Some hybrids have occurred spontaneously in nature. For example, wheat *(Triticum aestivum)* arose centuries ago from a naturally-occurring interbreeding of three Middle East grasses. In the 1980s, sugar beet *(Beta vulgaris subspecies vulgaris)* naturally interbred with the wild native weed known as sea beet *(Beta vulgaris subsp, maritima)* in Europe; resulting in an annual weed (in contrast to sugar beet, which is a biannual). Because that (new hybrid weed) is closely related to sugar beet, any herbicide that kills the (new hybrid weed) is likely to harm the sugar beet crop (unless the sugar beet crop is made herbicide-tolerant).

IMMUNOASSAY

The use of antibodies to identify and quantify (measure) substances by a variety of methods. The binding of antibodies to antigen (substance being measured) is often followed by tracers, such as fluorescence or (radioactive) radioisotopes, to enable measurement of the substance.

ISOELECTRIC POINT

The pH at which a particular molecule or surface carries no net electrical charge.

ISOZYMES

(isoenzymes) Multiple forms of an enzyme that differ from each other in their substrate (substance acted upon) affinity, in their maximum activity, or in their regulatory properties.

KB

An abbreviation for 1,000 (kilo) base pairs of deoxyribonucleic acid (DNA).

KILODALTON (KD)

A unit of mass equal to 1,000 Daltons.

LIMIT OF DETECTION (LOD)

The lowest analyte concentration that can be detected. The LOD is not necessarily the lowest amount that is quantified to an exact value.

LINKED GENES

Two or more Genes that are inserted within the same construct, into the same chromosome location, and are always present together. If a seed or plant has the DNA for one gene it also has the other present. It can be a marker gene linked to a gene of interest or 2 or more genes of interest linked together.

MALE SELF

Protein banding patterns that are identical to the pollen parent of a hybrid.

MARKER (DNA MARKER)

A DNA fragment of known size used to calibrate an electrophoretic gel.

MARKER (DNA SEQUENCE)

A specific sequence of DNA that is virtually always associated with a specified trait, because of "linkage" between that DNA sequence (the "marker") and the gene(s) that cause that particular trait.

MARKER (GENETIC MARKER)

A trait that can be observed to occur or not to occur in an organism such as, for example, bacteria or plant(s). Genetic markers include such traits as: expression of luciferase in leaf cells (causing leaves to glow), resistance to specific antibiotics, the nature of the cell wall and capsule characteristics, requirements for a particular growth factor, and carbohydrate utilization, to mention a few. For example, if a culture of dividing (growing) bacteria that is not resistant to a particular antibiotic (i.e., lacks the trait of antibiotic resistance) is exposed to only the DNA isolated from bacteria that are resistant to the antibiotic, then a fraction of the cells exposed will directly incorporate this trait (some DNA) into their genome, hence acquiring the trait. The first genetically engineered plants bearing a marker gene were field tested in 1986.

MESSENGER RNA (MRNA)

Messenger ribonucleic acid. The intermediary molecule between DNA and ribosomes (in a cell) which synthesize (i.e., make) those proteins coded for by the cell's DNA. Upon receiving the "message" encoded in the DNA, the messenger RNA passes through the ribosomes like a reel of punched paper passes through an old player piano (pianola) giving the ribosomes the specifications for making the coded-for proteins.

This process is aided by transfer RNA (tRNA) molecules, which forage for amino acids that float around in the cell (outside of the cell's nucleus and ribosomes). The transfer RNA (tRNA) molecules attach to, and escort individual amino acids to the ribosome, as and when the messenger RNA (mRNA) directs. Each of the 20 different amino acids has at least one of its own purpose-built tRNA molecules, which possess a three-letter code of nucleotides at the stem of the cloverleaf-shaped rRNA molecule.

The ribosome has room for only two tRNA molecules at a time. The messenger RNA (mRNA) molecule (which itself is passing through the ribosome) calls over the first tRNA molecule, which brings with it the specified amino acid. Short sections of the messenger RNA (mRNA) and transfer RNA (tRNA) molecules lock together inside the ribosome (because where these two molecules meet, their three nucleotides are complementary), the whole (locked together) apparatus shifts along by three notches (i.e., nucleotides), and a second tRNA molecule (bearing another amino acid) slips in next to the first tRNA molecule.

Next, the first amino acid (brought in by the first tRNA molecule) jumps over to the second tRNA molecule; joining to the amino acid that was brought in by the second tRNA molecule, thus making the start of a protein (i.e., a poly-amino acid molecule, also known as polypeptide or protein molecule). The empty (first) tRNA molecule falls out of the ribosome, and the whole (locked together) apparatus (i.e., mRNA plus second tRNA molecule) moves three more notches (i.e., nucleotides) along the mRNA molecule to make room for a third tRNA molecule bearing another amino acid, and so on.

This process of creating ever-longer chains of amino acids continues to repeat itself inside the ribosome until the protein (coded for by the DNA, which code was transferred to mRNA, which transferred it to the ribosome) is completed.

MOLECULAR BIOLOGY

A term coined by Vannevar Bush during the 1940s that eventually came to mean the study and manipulation of molecules that constitute, or interact with, cells. Molecular biology as a distinct scientific discipline originated largely as a result of a decision to provide "support for the application of new physical and chemical techniques to biology" during the 1930s by Warren Weaver, director of the biology (funding) program at America's Rockefeller Foundation (a philanthropic organization).

MONOCLONAL ANTIBODIES (MAB)

Are produced by selecting antibody producing cells from an immunized mouse and hydridizing them with cells derived from myelomas creating hybridoma cells. The antibodies produced from such hybrid cells are identical to one another.

Discovered and developed in the 1970s by Cesar Milstein and Georges Kohler, monoclonal antibodies are the name for antibodies derived from a single source or clone of cells that recognize only one kind of antigen. Made by fusing myeloma cancer cells (which multiply very fast) with antibody-producing cells, then spreading the resulting conjugate colony so thin that each cell can be grown into a whole, separate colony (i.e., cloning). In this way, one gets whole batches of the same (monoclonal) antibody, which are all specific to the same antigen. Monoclonal antibodies have found markets in diagnostic kits and show potential for use in drugs, imaging agents, and purification processes. One example of diagnostic use is the invention in 1997 by Bruno Oesch of a monoclonal antibody-based rapid test to detect the prion (PrP 5c) that causes bovine spongiform encephalopathy (BSE) in cattle.

NUCLEIC ACIDS

A nucleotide polymer. A large, chain-like molecule containing phosphate groups, sugar groups, and purine and pyrimidine bases; two types are ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The bases involved are adenine, guanine, cytosine, and thymine (uracil in RNA).

OFFTYPE
Isozyme patterns that do not match the hybrid or inbred in two or more loci.

OPTICAL DENSITY (OD)
Numerical value obtained for color intensity on a plate reader.
The absorbance of light of a specific wavelength by molecules normally dissolved in a solution. Light absorption depends upon the concentration of the absorbing compound (chemical entity) in the solution, the thickness of the sample being illuminated, and the chemical nature of the absorbing compound. An analytical instrument known as a spectrophotometer is used to (quantitatively) express the amount of a substance (dissolved) in a solution. Mathematically, this is accomplished using the Beer-Lambert Law.

ORGANIZATION FOR ECONOMIC COOPERATION AND DEVELOPMENT (OECD)
An international organization comprised of the world's wealthiest (most developed) nations. In 1991, the OECD's Group of National Experts on Safety in Biotechnology (GNE) completed a document entitled Report on the Concepts and Principles Underpinning Safety Evaluations of Food Derived from Modern Biotechnology. The "aim of that document was to elaborate the scientific principles to be considered (i.e., by OECD member nations' regulatory agencies) in evaluating the safety of new foods and food components" (e.g., genetically modified soybeans, corn/maize, potatoes, etc.)

OUTCROSSING
The transfer of a given gene or genes (e.g., one synthesized by man and inserted into a plant via genetic engineering) from a domesticated organism (e.g., crop plant) to wild type (relative of plant). In crop production, outcrossing is pollination from an undesired pollen source

PAT GENE
A dominant gene which, when inserted into a plant's genome, imparts resistance to glufosinate-ammonium containing herbicides. Because the glufosinate- ammonium herbicides act via inhibition of glutamine synthetase (an enzyme that catalyzes the synthesis of glutamine), this inhibition of enzyme kills plants (e.g., weeds). That is because glutamine is crucial for plants to synthesize critically needed amino acids. The PAT gene is often used by genetic engineers as a marker gene.

PHENOTYPIC MARKER
The outward physical appearance of a particular trait.

PHYSIOLOGY
The branch of biology dealing with the study of the functioning of living things. The materials of physiology include all life: animals, plants, microorganisms, and viruses.

Plant Variety Protection Act (PVP)
A law passed by the United States' Congress in 1970 that enables intellectual property protection (analogous to patent protection) for new seed plants and seeds in America.

POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)
A form of chromatography in which molecules are separated on the basis of size and charge. The stationary phase (the polyacrylamide gel) is a polymerized version of acrylamide monomers. The gel

looks and feels like Jello™. On a molecular basis it consists of an intertwined and cross-linked mesh of polyacrylamide strings. As can be imaged, there are holes in the gel (like in a plastic mesh bag) and with enough cross-linking the size of the holes begins to approach the size of the molecules which are to be separated. Since some molecules will be larger and some smaller, some of them will be able to pass through the gel matrix more easily than others. This is part of the basis for separation.

It should be noted at this point that if the gel is cross-linked enough and because of this the holes in that gel are smaller than the molecules to be separated, then the molecules will not be able to penetrate into the gel and no separation can occur. The charge on the molecule also plays a role in the separation. Functionally, the gel serves to hold and separate the molecules. Although details are not presented here, after the gel has been prepared (poured and cross- linked) a small amount of the solution containing the molecules to be separated is placed into wells (grooves to hold the liquid) on the gel and the system is subjected to an electric current. Over the course of minutes to hours molecules bearing different charge/mass separate.

POLYCLONAL ANTIBODIES

A mixture of antibody molecules (that are specific for a given antigen) that has been purified from an immunized (to that given antigen) animal's blood. Such antibodies are polyclonal in that they are the products of many different populations of antibody-producing cells (within the animal's body). Hence they differ somewhat in their precise specificity and affinity for the antigen. Years ago, antibodies (then called antitoxin) that were purified from an immunized animal's blood (e.g., a horse) were injected into humans suffering from certain diseases (e.g., diphtheria). In these cases the pathogen had caused disease by secreting large amounts of toxin into the victim's bloodstream. The antitoxin combined quantitatively (e.g., 1:1, 2:1, 1:2, 1:3, 3:1, etc.) with, and neutralized the toxin (for those few diseases for which it was applicable). Vaccines are now used instead, because of the adverse immune response caused by the horse's blood (antigens).

POLYMERASE CHAIN REACTION (PCR)

A reaction that uses the enzyme DNA polymerase to catalyze the formation of more DNA strands from an original one by the execution of repeated cycles of DNA synthesis. Functionally, this is accomplished by heating and melting double-stranded (hydrogen bonded) DNA into single-stranded (nonhydrogen bonded) DNA and producing an oligonucleotide primer complementary to each DNA strand. The primers bind to the DNA and mark it in such a way that the addition of DNA polymerase and deoxynucleoside triphosphates cause a new strand of DNA to form which is complementary to the target section of DNA. The process described previously is repeated (trait, product, etc.) again and again to produce millions of copies of the desired strand of DNA. PCR and its registered trademarks are the property of F. Hoffmann-LaRoche & Co. AG, Basel, Switzerland.

POLYMERASE CHAIN REACTION (PCR) TECHNIQUE

Developed in 1984 and 1985 by Kary B. Mullis, Randall K. Saiki, Stephen J. Scharf, Fred A. Faloona, Glenn Horn, Henry A. Erlich, and Norman Arnheim, the PCR technique is an *in vitro* method that greatly amplifies (makes millions of copies of) DNA sequences that otherwise could not be detected or studied. It can be utilized to amplify a given DNA sequence that constitutes less than one part per million of initial sample (e.g., a 100-base-pair target DNA sequence within the genome of one of the higher organisms, which can contain up to 500 million base pairs), The procedure alleviates the necessity of *in vivo* replication of a target DNA sequence, or of replication of one-of-a-kind tiny DNA samples (e.g., from a crime scene).

PRECISION

Measured by repeatability of results, one of the four criteria of method validation for purity testing.

PRIMER (DNA)

A short sequence deoxyribonucleic acid (DNA) that is paired with one strand of the template DNA. It is the growing end of the DNA chain and it simply provides a free 3'-OH end at which the enzyme DNA polymerase adds on deoxyribonucleotide units (monomers). Which deoxyribonucleotide is added is dictated by base pairing to the template DNA chain. Without a DNA primer sequence a new DNA chain cannot form since DNA polymerase is not able to initiate DNA chains.

PROMOTER

The region on DNA to which RNA polymerase binds and initiates transcription. The promoter "promotes" the transcription (expression) of that gene. A region of DNA (deoxyribonucleic acid) which lies "upstream" of the transcriptional initiation site of a gene. The promoter controls where (e.g., which portion of a plant, which organ within an animal, etc.) and when (e.g., which stage in the lifetime of an organism) that the gene is expressed. For example, the promoter named "Bce4" is "seed-specific" [i.e., it only "promotes" the expression of a given gene's product (e.g., protein, fatty acid, amino acids, etc.) within a plant's seed].

PROTEIN

From the Greek word *proteios*, which means "the first" or "the most important." Any of a class of high molecular weight polymer compounds composed of a variety of amino acids joined by peptide linkages. Via the synthesis (of this "chain") performed by ribosomes, each protein is the ultimate expression product of a gene. More than one protein can be expressed from a given gene (the particular protein expressed is determined by factors such as the cell's temperature or other environmental variable, presence of STATs-- some of which themselves are proteins, etc.).

During their synthesis (after emerging from cell's ribosome), proteins may also be phosphorylated (i.e., a "phosphate group" is added to the protein molecule), glycosylated (i.e., one or more oligosaccharides is added onto the protein molecule), acetylated (i.e., one or more "acetyl groups" is added to the protein molecule), farnesylated (i.e., a "farnesyl group" is added to the protein molecule), ubiquinated (i.e., a ubiquitin "tag" is added to the protein molecule), sulfated (i.e., a "sulfate group" is added to the protein molecule), or otherwise chemically modified. Proteins are the "workhorses" of living systems and include enzymes, antibodies, receptors, peptide hormones, etc. Proteins in living organisms respond to changing environmental and other conditions by changing their location within cells, by getting cut into (specific) pieces, by changing which (other) molecules they will bind (adhere) to, etc. All of the amino acids commonly found in (each and every one of the) proteins have an asymmetric carbon atom, except the amino acid glycine. Thus the protein is potentially chiral in nature.

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TECHNIQUE

A genetic mapping methodology that utilizes as its basis the fact that specific DNA sequences (polymorphic DNA) are "repeated" (i.e., appear in sequence) with gene of interest. Thus, the polymorphic DNA sequences are linked to that specific gene. Their linked presence serves to facilitate genetic mapping (i.e., "location" of specific gene(s) on an organism's genome).

RECESSIVE ALLELE

Discovered by Gregor Mendel in the 1860s, this refers to an allelic gene whose existence is obscured in the phenotype of a heterozygote by the dominant allele. In a heterozygote the recessive allele does not produce a polypeptide; it is switched off. In this case the dominant allele is the one producing the polypeptide chain.

RECOMBINANT DNA (RDNA)

DNA formed by the joining of genes (genetic material) into a new combination.

RENATURATION

The return to the natural structure of a protein or nucleic acid from a denatured (more random coil) state. For example, a protein may be denatured [lose its native (natural) structure] by exposure to surfactants such as SDS or to changes in the pH of the medium, etc. If the surfactant is slowly removed or the pH is slowly readjusted to the optimum for the protein, it will refold (snap) back into its original (native) form.

RIBONUELEIC ACID (RNA)

A long-chain, usually single-stranded nucleic acid consisting of repeating nucleotide units containing four kinds of heterocyclic, organic bases: adenine, cytosine, guanine, and uracil. These bases are conjugated to the pentose sugar ribose and held in sequence by phosphodiester (chemical) bonds. The primary function of RNA is protein synthesis within a cell. However, RNA is involved in various ways in the processes of expression and repression of hereditary information. The three main functionally distinct varieties of RNA molecules are: (1) messenger RNA (mRNA) which is involved in the transmission of DNA information, (2) ribosomal RNA (rRNA) which makes up the physical machinery of the synthetic process, and (3) transfer RNA (tRNA) which also constitutes another functional part of the machinery of protein synthesis.

RUGGEDNESS

Reproducibility of results obtained under varying conditions such as different labs and equipment.

SDS

Sodium dodecyl sulfate. Also known as sodium lauryl sulfate (SLS). A surfactant commonly used in biochemical and biotechnological applications for the solubilization of membrane components and hard-to- solubilize (dissolve) molecules. For example, it is often utilized at high concentration in water solution (e.g., along with potassium acetate) to dissolve plant DNA samples (e.g., when a scientist wants to sequence that sample of plant DNA). The SDS/PA in water solution helps the scientist to separate out contaminants that are commonly present in samples from plant tissues (i.e., polysaccharides, proteins, etc.) because DNA molecules are much more soluble in SDS/PA solution than are those contaminant molecules. Above a critical concentration (CMC), SDS forms micelles in water which are thought to be responsible for its solubilizing action. SDS is also used in such items as shampoo.

SEGREGATION

Segregation is the First Law of Mendelian genetics. Gametes from any heterozygous parent separate and propagate in a manner that is independent of the alleles of the same gene and all the other genes in the genome. Because of this, genetic purity can be determined for any given gene (Trait Purity) or any group of genes (Hybrid or Varietal Purity)

SELF
A pistol that is fertilized from pollen from the same plant that bears the pistil, the term also refers to the seed resulting from such fertilizations.

SEQUENCE (OF A DNA MOLECULE)
The specific nucleic acids that comprise a given segment of a DNA molecule.

SEQUENCE (OF A PROTEIN MOLECULE)
The specific amino acids (and the order in which they are coupled together) that comprise a given segment of a protein molecule.

SEQUENCING (OF DNA MOLECULES)
The process used to obtain the sequential arrangement of nucleotides in the DNA backbone. The cleavage into fragments (followed by separation of those fragments, which can then be sequenced individually) of DNA molecules by one of several methods: (1) a chemical cleavage method followed by polyacrylamide gel electrophoresis (PAGE), (2) a method consisting of controlled interruption of enzymatic replication methods followed by PAGE, (3) a didexyl method utilizing fluorescent "tag" atoms attached to the DNA fragments, followed by use of spectrophotometry to identify the respective DNA fragments by their differing "tags" (which fluoresce at different wavelengths). This (fluorescent tag) variant of the dideoxy method can be automated to "decipher" large DNA molecules (i.e., genomes). Such automated machines are sometimes called "gene machines."

"SHOTGUN" METHOD
[to introduce foreign (new) genes into plant cells] A technique for gene-into-cell introduction in which the gene is attached to tiny "bullets" made of tungsten or other metal. By means of a special device ("gene gun") the tiny particles are then literally "shot" through the plasma membrane into plant cells with:

(a) High-pressure gas (e.g., the GENEBOOSTER® gun developed at Hungary's Agricultural Biotechnology Center utilizes nitrogen).

(b) A rather conventional firearm (sometimes called a particle gun) which uses a .22 caliber shell minus the lead tip. The tiny particles are used in place of the lead tip. For example, the BIOLISTIC® Gene Gun invented at America's Cornell University utilizes "bullets" made of tungsten.

Some plant cells are destroyed in the process and the survivors heal (provided the "bullet" is small enough), and incorporate (some) of the new genetic material into their genetic complement, and produces whatever product (i.e., a protein) the newly introduced gene
codes for.

SPECIFICITY
The ability to accurately measure a target analyte in the presence of other components that may be present. Typically these might include proteins, nucleic acids, impurities, degradants, matrix and buffers

"STACKED" GENES

Refers to the independent insertion of two or more (synthetic) genes into the genome of an organism. One example of that would be a plant into which has been inserted a gene from *Bacillus thuringiensis (B.t.)* and a gene for resistance to a specific herbicide.

SUBSTRATE (CHEMICAL)

The substance acted upon, for example, by an enzyme. For example, the enzyme amylase breaks starch down into glucose molecules; starch is the substrate (of the enzyme amylase).

SURFACTANT

Acronym for surface active agent. Amphipathic molecules (i.e., molecules that contain both a polar and nonpolar domain) which, due to their unique properties, position themselves at interfacial regions (surfaces) such as ah oil/water interface. When surfactants are dissolved above a certain critical concentration in either water or nonpolar solvents they may form micelles or reverse micelles, respectively. Surfactants are commonly used to solubilize cell membrane components and other hard to solubilize molecules.

TAQ DNA POLYMERASE

A 94 kilodalton DNA polymerase, which was originally isolated from the thermophilic bacteria *Thermus aquaticus.* Commonly utilized to catalyze PCR reactions due to its heat resistance (needed for thermal cycles utilized in the PCR technique).

TERMINATION CODON

One of three triplet sequences (U-A-G, U-A-A, or U-G-A) found in DNA molecules (genes) that cause termination of protein synthesis; they are also called nonsense codons. The sequences cause the termination of the peptide chain and its release in free form.

TOXIN

A substance (e.g., produced in some cases by disease-causing microorganisms) which is poisonous to certain other living organisms.

TRAIT

A characteristic of an organism, which manifests itself in the phenotype (physically). Many traits are the result of the expression of a single gene, but some are polygenic (result from simultaneous expression of more than one gene). For example, the level of protein content in soybeans is controlled by five genes.

TRANSCRIPTION

The enzyme-catalyzed process whereby the genetic information contained in one strand of DNA (deoxyribonucleic acid) is used as a template to specify and produce a complementary mRNA strand. Transcription may be thought of as a rewriting of the information contained in DNA into RNA. The language, however, is the same - both are nucleic acid-based. This is in contrast to translation, in which the information is translated from one language (RNA, nucleic acid-based) into another language (protein, amino acid-based).

TRANSFER RNA (TRNA)

A class of relatively small RNA (ribonucleic acid) molecules of molecular weight 23,000 to about 30,000. tRNA molecules act as carriers of specific amino acids during the process of protein synthesis. Each of

the 20 amino acids found in proteins has at least one specific corresponding tRNA. The tRNA binds covalently with "its" specific amino acid and "leads" it to the ribosome for incorporation into the growing peptide chain.

TRANSGENE

A "package" of genetic material (i.e., DNA) that is inserted into the genome of a cell via gene splicing techniques. May include promoter(s), leader sequence, termination codon, etc.

TRANSGENIC

An organism whose gamete cells (sperm/egg) contain genetic material originally derived from an organism other than the parents or in addition to the parental genetic material.

VALUE-ENHANCED GRAINS

Those grains that possess novel traits that are economically valuable (e.g., higher-than-normal protein content, higher-than-normal oil content, etc.). For example, high-oil corn possesses a kernel oil content of 5.8% or greater, versus oil content of 3.5% or less for traditional No. 2 yellow com. High-amylose com possesses a kernel amylose (starch) content of 50% or greater, etc.

VARIANT

A variant is a plant that comes from a pure line that is not identical to the parent. It occurs at random and therefore, cannot be described using the segregation patterns identified in Mendelian genetics.

In both Isozyme Analysis and IEF, variants may be identified by a single band difference between the plant in question and the expected pattern of the hybrid or variety, as long as the difference is random and cannot be described with the common segregation patterns. With the sample sizes currently used is appears to be unstable

VIRUS

A simple, noncellular particle (entity) that can reproduce only inside living cells (of other organisms). The simple structure of viruses is their most important characteristic. Most viruses consist only of a genetic material - either DNA (deoxyribonucleic acid) or RNA (ribonucleic acid) – and a protein coating. This (combination) material is categorized as a nucleoprotein.

Some viruses also have membranous envelopes (coatings). Viruses are "alive" in that they can reproduce themselves - although only by taking over a cell's "synthetic genetic machinery" - but they have none of the other characteristics of living organisms. Viruses cause a large variety of significant diseases in plants and animals, including humans. They present a philosophical problem to those who would speak of living and nonliving systems because in and of it self a virus is not "alive" as we know life, but rather represents "life potential" or "symbiotic life."

WORLD TRADE ORGANIZATION (WTO)

The international organization composed of the more than 100 nations that signed the General Agreement on Tariffs and Trade (GATT), which was WTO's predecessor body. WTO permits signatory countries to ban specific imports from other countries in order to protect the health of humans, animals, or plants. Such import bans are allowed based on the (GATT/WTO) Agreement on Sanitary and Phytosanitary Measures, which was approved in 1994 by GATT.

The WTO's Agreement on Sanitary and Phytosanitary (SPS) Measures requires that such import bans must be based on sound internationally-agreed science. WTO recognizes only the following three international science organizations in order to resolve SPS disputes between member nations:

1. Codex Alimentarius Commission - for foods and food ingredients
2. Intemational Plant Protection Convention (IPPC) - for plants
3. International Office of Epizootics (OIE) - for animal diseases.