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In order to continue enjoying our site, we ask that you confirm your identity as a human. Thank you very much for your cooperation. In order to continue enjoying our site, we ask that you confirm your identity as a human. Thank you very much for your cooperation. This chapter provides a brief description of genetic modification methods used to develop new plant, animal, and microbial strains for use as human food. The next chapter (Chapter 3) presents a detailed analysis of the likelihood for these methods to result in unintentional compositional changes.Modification to produce desired traits in plants, animals, and microbes used for food began about 10,000 years ago. These changes, along with natural evolutionary changes, have resulted in common food species that are now genetically different from their ancestors.Advantageous outcomes of these genetic modifications include increased food production, reliability, and yields; enhanced taste and nutritional value; and decreased losses due to various biotic and abiotic stresses, such as fungal and bacterial pathogens. These objectives continue to motivate modern breeders and food scientists, who have designed newer genetic modification methods for identifying, selecting, and analyzing individual organisms that possess genetically enhanced features.For plant species, it can take up to 12 years to develop, evaluate, and release a new variety of crop in accordance with international requirements, which specify that any new variety must meet at least three criteria: it must be genetically distinct from all other varieties, it must be genetically uniform through the population, and it must be genetically stable (UPOV, 2002).While advances in modification methods hold the potential for reducing the time it takes to bring new foods to the marketplace, an important benefit of a long evaluation period is that it provides opportunities for greater assurance that deleterious features will be identified and potentially harmful new varieties can be eliminated before commercial release. As discussed more fully in Chapter 5, it is both prudent and preferable to identify potentially hazardous products before they are made commercially available, and with few exceptions standard plant breeding practices have been very successful in doing so.The easiest method of plant genetic modification (see Operational Definitions in Chapter 1), used by our nomadic ancestors and continuing today, is simple selection. That is, a genetically heterogeneous population of plants is inspected, and “superior” individuals—plants with the most desired traits, such as improved palatability and yield—are selected for continued propagation. The others are eaten or discarded. The seeds from the superior plants are sown to produce a new generation of plants, all or most of which will carry and express the desired traits. Over a period of several years, these plants or their seeds are saved and replanted, which increases the population of superior plants and shifts the genetic population so that it is dominated by the superior genotype. This very old method of breeding has been enhanced with modern technology.An example of modern methods of simple selection is marker-assisted selection, which uses molecular analysis to detect plants likely to express desired features, such as disease resistance to one or more specific pathogens in a population. Successfully applying marker-assisted selection allows a faster, more efficient mechanism for identifying candidate individuals that may have “superior traits.”Superior traits are those considered beneficial to humans, as well as to domesticated animals that consume a plant-based diet; they are not necessarily beneficial to the plant in an ecological or evolutionary context. Often traits considered beneficial to breeders are detrimental to the plant from the standpoint of environmental fitness. For example, the reduction of unpalatable chemicals in a plant makes it more appealing to human consumers but may also attract more feeding by insects and other pests, making it less likely to survive in an unmanaged environment. As a result, cultivated crop varieties rarely establish populations in the wild when they escape from the farm. Conversely, some traits that enhance a plant's resistance to disease may also be harmful to humans.Crossing occurs when a plant breeder takes pollen from one plant and brushes it onto the pistil of a sexually compatible plant, producing a hybrid that carries genes from both parents. When the hybrid progeny reaches flowering maturity, it also may be used as a parent.Plant breeders usually want to combine the useful features of two plants. For example, they might add a disease-resistance gene from one plant to another that is high-yielding but disease-susceptible, while leaving behind any undesirable genetic traits of the disease-resistant plant, such as poor fertility and seed yield, susceptibility to insects or other diseases, or the production of antinutritional metabolites.Because of the random nature of recombining genes and traits in crossed plants, breeders usually have to make hundreds or thousands of hybrid progeny to create and identify those few that possess useful features with a minimum of undesirable features. For example, the majority of progeny may show the desired disease resistance, but unwanted genetic features of the disease-resistant parent may also be present in some. Crossing is still the mainstay of modern plant breeding, but many other techniques have been added to the breeders' tool kit.Interspecies crossing can take place through various means. Closely related species, such as cultivated oat (Avena sativa) and its weedy relative wild oat (Avena fatua), may cross-pollinate for exchange of genetic information, although this is not generally the case. Genes from one species also can naturally integrate into the genomes of more distant relatives under certain conditions. Some food plants can carry genes that originate in different species, transferred both by nature and by human intervention. For example, common wheat varieties carry genes from rye. A common potato, Solanum tuberosum, can cross with relatives of other species, such as S. acaule (Kozukue et al., 1999) or S. chacoense (Sanford et al., 1998; Zimnoch-Guzowska et al., 2000).Chromosome engineering is the term given to nonrecombinant deoxyribonucleic acid (rDNA) cytogenetic manipulations, in which portions of chromosomes from near or distant species are recombined through a natural process called chromosomal translocation. Sears (1956, 1981) pioneered the human exploitation of this process, which proved valuable for transferring traits that were otherwise unattainable, such as pest or disease resistance, into crop species. However, because transferring large segments of chromosomes also transferred a number of neutral or detrimental genes, the utility of this technique was limited.Recent refinements allow plant breeders to restrict the transferred genetic material, focusing more on the gene of interest (Lukaszewski, 2004). As a result, chromosome engineering is becoming more competitive with rDNA technology in its ability to transfer relatively small pieces of DNA. Several crop species, such as corn, soybean, rice, barley, and potato, have been improved using chromosome engineering (Gupta and Tsuchiya, 1991).Sometimes human technical intervention is required to complete an interspecies gene transfer. Some plants will cross-pollinate and the resulting fertilized hybrid embryo develops but is unable to mature and sprout. Modern plant breeders work around this problem by pollinating naturally and then removing the plant embryo before it stops growing, placing it in a tissue-culture environment where it can complete its development. Such embryo rescue is not considered genetic engineering, and it is not commonly used to derive new varieties directly, but it is used instead as an intermediary step in transferring genes from distant, sexually incompatible relatives through intermediate, partially compatible relatives of both the donor and recipient species.Recent advances in tissue-culture technologies have provided new opportunities for recombining genes from different plant sources. In somatic hybridization, a process also known as cell fusion, cells growing in a culture medium are stripped of their protective walls, usually using pectinase, cellulase, and hemicellulase enzymes. These stripped cells, called protoplasts, are pooled from different sources and, through the use of varied techniques such as electrical shock, are fused with one another.When two protoplasts fuse, the resulting somatic hybrid contains the genetic material from both plant sources. This method overcomes physical barriers to pollen-based hybridization, but not basic chromosomal incompatibilities. If the somatic hybrid is compatible and healthy, it may grow a new cell wall, begin mitotic divisions, and ultimately grow into a hybrid plant that carries genetic features of both parents. While protoplast fusions are easily accomplished, as almost all plants (and animals) have cells suitable for this process, relatively few are capable of regenerating a whole organism, and fewer still are capable of sexual reproduction. This non-genetic engineering technique is not common in plant breeding as the resulting range of successful, fertile hybrids has not extended much beyond what is possible using other conventional technologies.Somaclonal variation is the name given to spontaneous mutations that occur when plant cells are grown in vitro. For many years plants regenerated from tis-sue culture sometimes had novel features. It was not until the 1980s that two Australian scientists thought this phenomenon might provide a new source of genetic variability, and that some of the variant plants might carry attributes of value to plant breeders (Larkin and Scowcroft, 1981).Through the 1980s plant breeders around the world grew plants in vitro and scored regenerants for potentially valuable variants in a range of different crops. New varieties of several crops, such as flax, were developed and commercially released (Rowland et al., 2002). Molecular analyses of these new varieties were not required by regulators at that time, nor were they conducted by developers to ascertain the nature of the underlying genetic changes driving the variant features. Somaclonal variation is still used by some breeders, particularly in developing countries, but this non-genetic engineering technique has largely been supplanted by more predictable genetic engineering technologies.Mutation breeding involves exposing plants or seeds to mutagenic agents (e.g., ionizing radiation) or chemical mutagens (e.g., ethyl methanesulfonate) to induce random changes in the DNA sequence. The breeder can adjust the dose of the mutagen so that it is enough to result in some mutations, but not enough to be lethal. Typically a large number of plants or seeds are mutagenized, grown to reproductive maturity, and progeny are derived. The progeny are assessed for phenotypic expression of potentially valuable new traits.As with somaclonal variation, the vast majority of mutations resulting from this technique are deleterious, and only chance determines if any genetic changes useful to humans will appear. Other than through varying the dosage, there is no means to control the effects of the mutagen or to target particular genes or traits. The mutagenic effects appear to be random throughout the genome and, even if a useful mutation occurs in a particular plant, deleterious mutations also will likely occur. Once a useful mutation is identified, breeders work to reduce the deleterious mutations or other undesirable features of the mutated plant. Nevertheless, crops derived from mutation breeding still are likely to carry DNA alterations beyond the specific mutation that provided the superior trait.Induced-mutation crops in most countries (including the United States) are not regulated for food or environmental safety, and breeders generally do not conduct molecular genetic analyses on such crops to characterize the mutations or determine their extent. Consequently, it is almost certain that mutations other than those resulting in identified useful traits also occur and may not be obvious, remaining uncharacterized with unknown effects.Worldwide, more than 2,300 different crop varieties have been developed using induced mutagenesis (FAO/IAEA, 2001), and about half of these have been developed during the past 15 years. In the United States, crop varieties ranging from wheat to grapefruit have been mutated since the technique was first used in the 1920s. There are no records of the molecular characterizations of these mutant crops and, in most cases, no records to retrace their subsequent use.Several commercial crop varieties have been developed using cell selection, including varieties of soybeans (Sebastian and Chaleff, 1987), canola (Swanson et al., 1988), and flax (Rowland et al., 1989). This process involves isolating a population of cells from a so-called “elite plant” with superior agricultural characteristics. The cells are then excised and grown in culture. Initially the population is genetically homogeneous, but changes can occur spontaneously (as in somaclonal variation) or be induced using mutagenic agents. Cells with a desired phenotypic variation may be selected and regenerated into a whole plant. For example, adding a suitable amount of the appropriate herbicide to the culture medium may identify cells expressing a novel variant phenotype of herbicide resistance. In theory, all of the normal, susceptible cells will succumb to the herbicide, but a newly resistant cell will survive and perhaps even continue to grow. An herbicide-resistant cell and its derived progeny cell line thus can be selected and regenerated into a whole plant, which is then tested to ensure that the phenotypic trait is stable and results from a heritable genetic alteration. In practice, many factors influence the success of the selection procedure, and the desired trait must have a biochemical basis that lends itself to selection in vitro and at a cellular level.Breeders cannot select for increased yield in cell cultures because the cellular mechanism for this trait is not known. The advantage of cell selection over conventional breeding is the ability to inexpensively screen large numbers of cells in a petri dish in a short time instead of breeding a similar number of plants in an expensive, large field trial conducted over an entire growing season.Like somaclonal variation, cell selection has largely been superceded by recombinant technologies because of their greater precision, higher rates of success, and fewer undocumented mutations.As noted in Chapter 1, this report defines genetic engineering specifically as one type of genetic modification that involves an intended targeted change in a plant or animal gene sequence to effect a specific result through the use of rDNA technology. A variety of genetic engineering techniques are described in the following text.Agrobacterium tumefaciens is a naturally occurring soil microbe best known for causing crown gall disease on susceptible plant species. It is an unusual pathogen because when it infects a host, it transfers a portion of its own DNA into the plant cell. The transferred DNA is stably integrated into the plant DNA, and the plant then reads and expresses the transferred genes as if they were its own. The transferred genes direct the production of several substances that mediate the development of a crown gall.Among these substances is one or more unusual nonprotein amino acids, called opines. Opines are translocated throughout the plant, so food developed from crown gall-infected plants will carry these opines. In the early 1980s strains of Agrobacterium were developed that lacked the disease-causing genes but maintained the ability to attach to susceptible plant cells and transfer DNA.By substituting the DNA of interest for the crown gall disease-causing DNA, scientists derived new strains of Agrobacterium that deliver and stably integrate specific new genetic material into the cells of target plant species. If the transformed cell then is regenerated into a whole fertile plant, all cells in the progeny also carry and may express the inserted genes. Agrobacterium is a naturally occurring genetic engineering agent and is responsible for the majority of GE plants in commercial production.Klein and colleagues (1987) discovered that naked DNA could be delivered to plant cells by “shooting” them with microscopic pellets to which DNA had been adhered. This is a crude but effective physical method of DNA delivery, especially in species such as corn, rice, and other cereal grains, which Agrobacterium does not naturally transform. Many GE plants in commercial production were initially transformed using microprojectile delivery.In electroporation, plant protoplasts take up macromolecules from their surrounding fluid, facilitated by an electrical impulse. Cells growing in a culture medium are stripped of their protective walls, resulting in protoplasts. Supplying known DNA to the protoplast culture medium and then applying the electrical pulse temporarily destabilizes the cell membrane, allowing the DNA to enter the cell. Transformed cells can then regenerate their cell walls and grow to whole, fertile transgenic plants. Electroporation is limited by the poor efficiency of most plant species to regenerate from protoplasts.DNA can be injected directly into anchored cells. Some proportion of these cells will survive and integrate the injected DNA. However, the process is labor intensive and inefficient compared with other methods.The genes of most plant and some animal (e.g., insects and fish) species carry transposons, which are short, naturally occurring pieces of DNA with the ability to move from one location to another in the genome. Barbara McClintock first described such transposable elements in corn plants during the 1950s (Cold Spring Harbor Laboratory, 1951). Transposons have been investigated extensively in research laboratories, especially to study mutagenesis and the mechanics of DNA recombination. However, they have not yet been harnessed to deliver novel genetic information to improve commercial crops.Genetic features can be added to plants and animals without inserting them into the recipient organism's native genome. DNA of interest may be delivered to a plant cell, expressing a new protein—and thereby a new trait—without becoming integrated into the host-cell DNA. For example, virus strains may be modified to carry genetic material into a plant cell, replicate, and thrive without integrating into the host genome. Without integration, however, new genetic material may be lost during meiosis, so that seed progeny may not carry or express the new trait.Many food plants are perennials or are propagated by vegetative means, such as grafting or from cuttings. In these cases the virus and new genes would be maintained in subsequent, nonsexually generated populations. Technically such plants are not products of rDNA because there is no recombination or insertion of introduced DNA into the host genome. Although these plants are not GE, they do carry new DNA and new traits. No such products are known to be currently on the market in the United States or elsewhere. (See McHughen [2000] for further information on genetic mechanisms used in plant improvement.)Modern breeds of livestock differ markedly from their ancestors as a result of breeding strategies. For example, milk production per cow has increased among Holstein dairy cattle. Similarly, breeding programs have resulted in lean, fast-growing pigs (Notter, 1999). Chickens from modern breeds each produce more than 250 eggs per year, approximately double that produced in 1950, again mainly due to genetic selection.Established and emerging biotechnologies in animal agriculture include assisted reproductive technologies; use of naturally occurring hormones, such as recombinant bovine somatotropin; marker-assisted selection; biotechnologies to enhance reproductive efficiency without affecting the genome; and biotechnologies to enhance expression of desirable genes.Modern breeds of livestock differ from their ancestors because the use of frozen semen for artificial insemination (AI), along with sire testing and sire selection, has markedly affected the genetic quality of livestock, especially dairy cattle. Select bulls are tested for fertility and judged on the basis of the milk that their daughters produce. A notable example is the milk from Holstein cows, which increased almost threefold between 1945 and 1995 (Majeskie, 1996) through a combination of AI using semen from select bulls and improved milk production management (Diamond, 1999; Hale, 1969). Using sophisticated statistical models to predict breeding values, sire testing and selection, crossbreeding, and marker-assisted selection, along with AI, have greatly advanced the production characteristics of livestock. It is expected that AI will continue to be an integral tool in animal production systems.(Assisted reproductive and recombinant hormone technologies are discussed in detail in the accompanying subreport, Methods and Mechanisms of Genetic Manipulation and Cloning of Animals.)Although the following are not methods to generate modifications per se, they are considered modern methods that support the overall breeding and selection system for propagating desired genotypes for animals expressing desired traits.Embryo recovery and transfer allow valuable animals to contribute more offspring to the gene pool (Seidel, 1984). Embryos that are frozen and stored before being used to initiate a pregnancy result in 40,000 to 50,000 beef calves per year (NAAB, 2000). Emerging technologies will allow the sexing of semen and embryos to control the gender of the offspring. The production of single-sex sperm, by cell sorting X and Y sperm, will greatly benefit the livestock industries (Johnson, 2000).Up to several thousand embryos can be produced using techniques for recovering and maturing immature eggs, or oocytes, in about one day in a medium containing hormones, and then fertilizing them with live sperm or injecting a single sperm or sperm head into their outer layers—either beneath the zona pellucida or directly into the cytoplasm. The resulting zygotes are cultured in vitro, usually to the blastocyst stage, before being transferred to recipient females (First, 1991). The commercial application of in vitro maturation and fertilization has resulted in as many as 4,000 calves being born in a single year (NAAB, 2000).Splitting or bisecting embryos yields zygotic twins, or non-GE clones, that are genetically identical in both their nuclear and mitochondrial genes (Heyman et al., 1998). Maternal twins exhibit greater variation in phenotype than paternal twins with only one X chromosome. Further, there is the potential for differences in mitochondrial DNA distribution to affect phenotype.These embryos are then placed in an empty zona pellucida and transferred to recipient females, which carry them to term. Through 2001, a total of 2,226 registered Holstein clones—754 males and 1,472 females—were produced from embryo splitting, with 1 to 2 percent of calves produced (NAAB, 2000).Cloning as a technique, and the implications for predicting and assessing adverse health effects that may be associated with this technique, are addressed in the committee's subreport that follows this report.Techniques employed to introduce novel genes into domestic animals are discussed in detail in the report Animal Biotechnology: Science Based Concerns (NRC, 2002). These transgenic approaches applicable to animals are summarized in the following text.Germline refers to the lineage of cells that can be genetically traced from parent to offspring. It is possible to access the germline of animals using one of five methods (NRC, 2002):1.directly manipulating the fertilized egg after it has been implanted in the uterus;2.manipulating the sperm that produces the zygote;3.manipulating early embryonic tissue in place;4.using embryonic stem cell lines in early embryos; and5.manipulating cultured somatic cells to transfer their nuclei into enucleated oocytes.Several of the methods used to transfect or introduce novel genes into animals are similar to those used for plants. Commonly used methods include:microinjection of DNA into the nucleus of anchored cells;electroporation, where DNA is introduced through cell membrane pores by pulsed electrical charges;polycationic neutralization of the cell membrane and the DNA to be introduced to improve passive uptake;lipofection, where DNA is; andsperm-mediated transfection, often used in conjunction with intracytoplasmic sperm injection or electroporation.As is the case with plants, microinjection is a highly inefficient means of creating transgenic animals. For example, an incredibly small percentage of livestock embryos that undergo microinjection yield transgenic animals (Rexroad, 1994). Moreover, successfully microinjected transgenic animals do not necessarily pass their transgenes on to their offspring (NRC, 2002).This method is similar to viral delivery methods used in plants in that virus strains are modified to carry genetic material into a cell. It differs in that after the novel DNA is delivered, the viral replication process integrates it into the host cell's genome.The use of transposable elements in animal cells has not been completely developed. Although no active naturally occurring transposable elements have been found in mammals, those found in insects and fish are under investigation for potential use in animals.Transgenic technology can also be used to create organisms that lack specific genes or those in which one existing gene has been replaced by another that has been engineered. The addition of (“knock-in”) or deletion (“knock-out”) of specific gene functions through introduced mutations or genetic engineering based on homologous recombination has become commonplace in animals used for experimentation, such as mice. Although at present this technology is not efficient and thus not practical for use in generating knock-in or knock-out domestic animals, there are examples of its use in domestic sheep and pigs. (NRC, 2002).Marker-assisted selection involves establishing a link between inheriting a desirable trait, such as milk yield, and segregating specific genetic markers that are coupled to that trait. Marker-assisted selection is important in animal breeding and selection strategies for studying complex traits governed by many genes (Georges, 2001). The use of this method is expected to increase exponentially as genome-sequencing projects identify greater numbers of useful, segregated markers for economically important traits.Initially animals will be screened for genes that control simple traits that may be undesirable, such as horns in cattle or metabolic stress syndrome in pigs. In time, easily identifiable markers that accompany multiple genes controlling more complex traits, such as meat tenderness and taste, growth, offspring size, and disease resistance, will become available to improve animal health and production traits (Dekkers and Hospital, 2002).Two notable examples can be found in sheep. One is the Booroola gene in which a single-nucleotide base change is responsible for the callipyge muscle hypertrophy phenotype—the only known example of polar over-dominance in a mammal (Freking et al., 2002). Another is introgression of the Booroola gene into the Awassi and the Assaf dairy breeds (Gootwine, 2001).Sequencing genomes of animals that are important to agriculture will identify genes that influence reproductive efficiency. For example, a growth-hormonereceptor variant on bovine chromosome 20 affects the yield and composition of milk, and is expected to increase milk production by 200 kg per lactation and decrease milk fat from 4.4 percent to 3.4 percent (Fletcher, 2003).Biotechnology can be used to modify endocrine function of domestic animals and affect reproduction, lactation, and growth. For example, in pigs and rats (Draghia-Akli et al., 2002) hypothalamic-specific expression of growth-hormone-releasing hormone is not essential since ectopic expression of a cloned DNA for this neuropeptide can be genetically driven by a synthetic muscle-specific transcriptional promoter to elicit increases in both growth hormone and insulin-like growth factor-I (Khan et al., 2002). This biotechnology has the potential, by using specific hormones and growth factors during critical developmental periods, to enhance uterine capacity and to increase milk production.Humans have used and genetically modified (GM) microbes for centuries to produce food. Wine, bread, and cheese are common examples of ancient foods, still popular today, that depend on microbial ingredients and activities. Endogenous populations of microbes, particularly bacteria and yeasts, are genetically varied enough to provide sufficiently different traits to allow the development of useful microbial strains through simple selection or induced mutation.Microorganisms play significant roles in food production. They serve primary and secondary roles in food fermentation and in food spoilage, and they can produce enzymes or other metabolites used in food production and processing. Fermentations can be initiated and conducted completely by the bacterial populations that are endogenous to the raw materials being fermented. However, it is more reliable in terms of uniformity and predictability to intentionally introduce starter cultures to initiate the fermentation and, in some instances, to perform the complete fermentation process. Most fermented products now are prepared this way in industrialized countries.The types of microorganisms that carry out food fermentations range from bacteria to molds and yeasts, but by far the most widely used organisms are lactic acid bacteria (LAB) and yeasts (Sacchromyces cerevisiae). Traditional genetic modification methods that have been employed—particularly for microbial starter cultures—include selection, mutagenesis, conjugation, and protoplast fusion, the last of which is analogous to somatic hybridization in plant systems.Before molecular genetics was developed and applied to LAB, the most widely used genetic modification method was chemical- or ultra-violet-induced mutagenesis, followed by an enrichment or selection process for mutants with superior characteristics.A second traditional approach, conjugation, relies on natural methods of genetic exchange whereby DNA is transferred from one strain to another. Conjugation can occur between LAB strains as well as between LAB and other bacteria (Steenson and Klaenhammer, 1987). Although the resulting strains could conceivably be labeled as recombinant, the fact that this process can occur naturally circumvents application of the GE organism's classification.A less common, but still useful, method has been to use protoplast fusion to facilitate recombination between two strains with superior but unique characteristics, producing a strain that possesses the desired characteristics of both parents. Protoplast fusion was classically used as a mapping method in bacteria and only recently has been used successfully to produce strains of LAB with desired characteristics (Patnaik et al., 2002). It has, however, been successfully used for some time to generate yeast strains that produce a greater number of biochemical substrates for use in the fermentation process (Pina et al., 1986).Given the number and combinations of desirable traits in starter culture organisms, producers have remained interested in developing improved starter cultures, using essentially two different approaches. The traditional approach has been to identify endogenous strains with desirable traits by conducting many small-scale fermentations. This type of trial-and-error approach is far from practical because, while productive, low throughput is a limiting factor in the success rate.The second approach is to produce the desired traits in the laboratory using molecular genetic and genetic engineering techniques. With the burgeoning field of genomics and the public availability of hundreds of fully sequenced bacterial genomes, this approach has become highly attractive and efficient and is favored by industry. Its primary advantage is the precision with which starter culture strains can be engineered.The most common method used to introduce recombinant DNA into microorganisms is transformation, whereby DNA of interest is introduced directly into recipient cells by making them permeable using chemical agents, enzymes, or electroporation. The first method developed for LAB was plasmid protoplast fusion, in which recipient cells are stripped of walls and subsequently fused with polyethylene glycol, trapping the newly introduced DNA between the cells (Kondo and McKay, 1984).Electroporation was developed for LAB during the late 1980s and employs electrical currents to create pores in the cell envelope, allowing DNA from other sources to enter (Luchansky et al., 1988). This method is probably the most widely used for research due to its simplicity. However, it lacks efficiency in many different species.Recombinant DNA also can be introduced into LAB using a technique called transduction, in which a bacteriophage is used to move DNA from one strain into another (Bierkland and Holo, 1993). Unlike transformation, transduction can be fraught with problems that cause deletions within the plasmid (known as transductional shortening that are typically of undefined length).Microbial transformation is usually simpler and more efficient than transformation in higher organisms, and has been in use longer for the development of commercial strains. Academic research also has been able to scrutinize the molecular genetic effects of transformation in microbes to a much greater extent than it has in higher organisms. Principles gleaned from studies of microbes have proven instrumental in understanding analogous events in the molecular genetics of higher organisms.

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