Polyethylene glycol (PEG) mediated transformation:

Plant protoplast can be transformed with naked DNA by treatment with PEG in the presence of divalent cations . e. g., Calcium.

PEG and divalent cations destabilize the plasma membrane of the plant protoplast and rendered it permeable to naked DNA. ü DNA enters the nucleus and integrates into the host genome. Calcium phosphate mediated DNA transfer

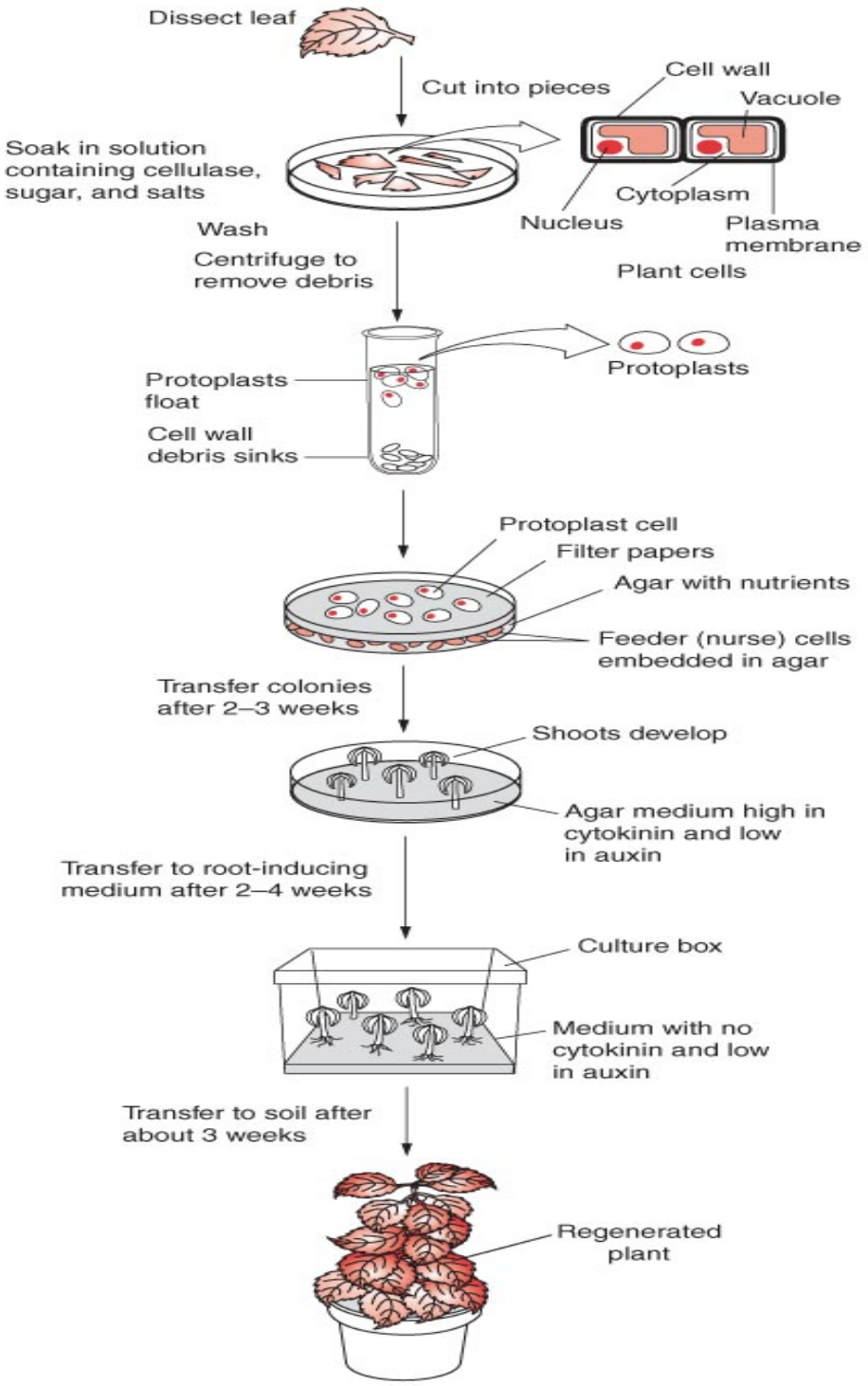
• The process of transfection involves the admixture of isolated DNA (10-100ug) with solution of calcium chloride and potassium phosphate so precipitate of calcium phosphate to be formed. Cells are then incubated with precipitated DNA either in solution or in tissue culture dish. A fraction of cells will take up the calcium phosphate DNA precipitate by endocytosis.

Protoplast fusion

▶ is the fusion of two protoplast cells from different species?

Cloning – growing plants from a single cell

- Protoplast cell is a callus cell whose cell wall has been dissolved by the enzyme cellulose.
- Fusion of the two protoplast cells creates a cell that can grow into a hybrid plant.


Dissect leaf
Cut into pieces
Cell wall
Vacuole
Soak in solution containing cellulase, sugar, and salts
Cytoplasm
Nucleus
Plasma membrane
Plant cells
Wash
Centrifuge to remove debris
Protoplasts
Protoplasts float
Cell wall debris sinks
Protoplast cell
Filter papers
Agar with nutrients
Feeder (nurse) cells embedded in agar
Transfer colonies after 2–3 weeks
Shoots develop
Agar medium high in cytokinin and low in auxin
Transfer to root-inducing medium after 2–4 weeks
Culture box
Medium with no cytokinin and low in auxin
Transfer to soil after about 3 weeks
Regenerated plant

Microinjection

Microinjection techniques for plant protoplasts utilize a holding pipette for immobilizing the protoplast. while an injection pipette is utilized to inject the macromolecule. In order to manipulate the protoplasts without damage, the protoplasts are cultured for about 1 to 5 days before the injection is performed to allow for partial regeneration of the cell wall.

- Advantage- Widespread use of transformation of cereal crops that initially proved difficult to transformation with Agro bacterium. ü Disadvantage-
- They tend to lead higher frequency of transgene rearrangement and higher copy number. This can lead to high frequency of gene silencing.

Viral Vectors

Since most plants can be infected by numerous viruses, viral vectors could potentially be used as another "natural" DNA introduction method for plants. Using their own transport mechanism, viruses can spread on their own throughout their host, so introduction of a virus into a single cell can eventually lead to the presence of virus genes in almost every cell of the inoculated plant. Although viral vectors can be used for extremely efficient introduction and transport of virus genes, these genes do not integrate into the genome of the host cell.

Therefore, they will not be transmitted to the next generation through the pollen and egg. However, inoculation of viruses into plant cells can be as simple as rubbing the leaf in the presence of the virus, and a single site of inoculation can lead to expression of viral genes in most cells of the plant (which is similar to production of a transgenic plant but is not quite the same). For successful introduction and expression, the gene of interest must be appropriately packaged in the viral genome, which tends to be less cooperative in accepting foreign DNA. Viral vectors are useful for very rapid production of proteins in plants without the need to generate a whole plant from a single, transformed cell.

Laser Micropuncture

For direct DNA introduction into plant cells, the use of microlasers continues with the theme of creating holes in the cell wall for DNA delivery. This is perhaps one of the more elegant and least often utilized methods for DNA introduction into plant cells. Lasers are very precise in targeting certain cells, but the instrumentation required for this method is quite involved, and the number of cells that are targeted is very small. In contrast, for particle bombardment, the number of cells that transiently express an introduced transgene will be 5000 (higher on occasion) per shot. Many more cells are actually targeted—this is the number of cells that receive the DNA close to or in the nucleus and transiently express the introduced DNA. For laser micropuncture (and protoplast microinjection, discussed above), cells are targeted one at a time. It is doubtful that the use of microlasers for DNA introduction will increase tremendously, but it is a noteworthy method for DNA introduction into plant cells.

Nanofiber Arrays

Successful use of nanofiber arrays for DNA introduction into plant cells has not yet been consistently obtained, but convincing results have been demonstrated using animal cells. Nanofiber arrays can best be described as a microscopic "bed of nails". Although not a new concept, the ability to precisely generate properly proportioned arrays is relatively new. Early attempts to generate nanofiber arrays resulted in the formation of nanoscale pyramid-shaped structures on a silicon chip. In this early work, the surface of the chips was precisely etched away, to leave the nanofiber pyramids. The newer arrays are composed of long, thin structures, and they hold much more promise for success with DNA introduction into plant cells.

Nanofiber arrays are actually grown on chips, with very precise composition, height, and spacing possible. DNA can be chemically bound to the fiber or simply precipitated onto it. For successful DNA introduction into animal cells, the arrays were stationary and the animal cells were propelled toward the chip. Cells were then allowed to grow, while still impregnated with fibers, on the chip. Although the cell wall is certainly much more of a barrier than the animal cell membrane, the fibers are sufficiently strong and rigid to penetrate the plant cell wall. Also, because the chip surface is covered with fibers, many cells can be targeted using a single chip. Early results with onion epidermal cells show the utility of this approach but the high-efficiency delivery of DNA-coated nanofibers directly to the nucleus of multiple plant cells remains a challenge.

PCR

It is much easier to transform a plant or other organism if you have many copies of a gene to work with. Bacteria can be used to clone or make multiple copies of genes, but now it is more common to use a method called the polymerase chain reaction (PCR). A PCR machine is sort of like a photocopier for genes.

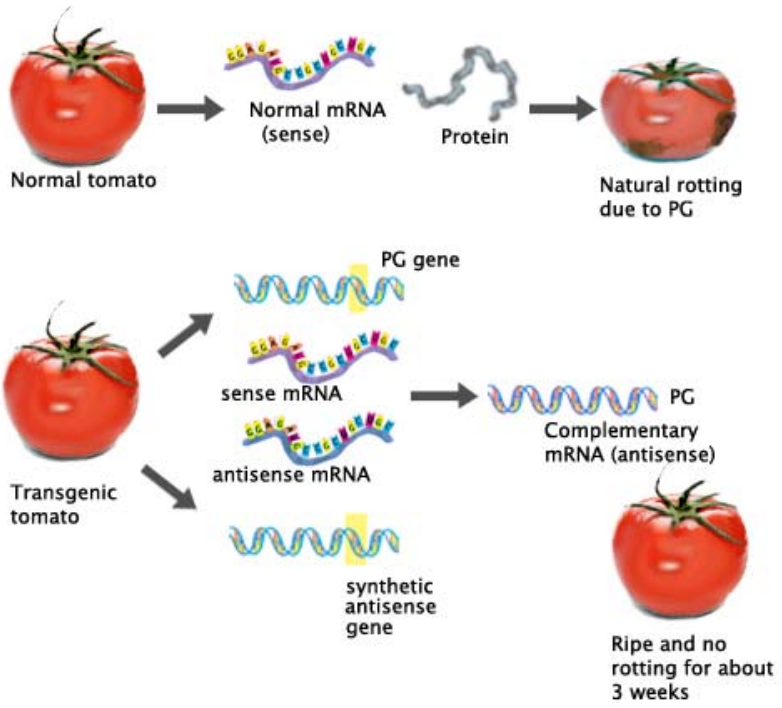
The basis for a PCR machine is a temperature-regulated block that can both heat and cool very rapidly. The sample chamber in the block is loaded with a sample of DNA that contains the gene, some enzymes (DNA polymerase and ligase), and a supply of each of the four nucleotides that make up DNA. Also in the mix are primers, short pieces of DNA that have been constructed to bind with the DNA at either end of the gene. Primers select the target gene to be amplified and help to avoid copying the entire genome.

The reaction is started by heating the sample to about 95°C (200°F), which separates the double-stranded DNA into single strands. The sample is then cooled to about 60°C (140°F), but before the two strands can reunite, the primers get in the way by binding to the DNA. DNA polymerase then fills in the gaps between the two primers and DNA ligase the newly inserted nucleotides together to form a new DNA strand. There are now two exact copies of the original DNA. The heating-cooling cycle is repeated to produce 4 copies, then 8, then 16, and so forth. Every time the cycle is repeated, the number of copies is doubled; 20 cycles will produce approximately 220 or more than 1 million copies from a single starting molecule.

Most proteins are denatured or inactivated at temperatures near 95°C (200°F), but the DNA polymerase used in PCR is a special form obtained from bacteria that are adapted to life in hot springs. You may also have wondered how forensic scientists can do DNA fingerprinting with DNA from a single hair, spot of blood, or other microscopic sample. The answer is that they use PCR to amplify the DNA. and help to avoid copying the entire genome.

Antisense Technology

- Flavr Savr™ tomato introduced in 1994
- Ripe tomatoes normally produce the enzyme, polyglacturonase (PG) which digests pectin
- Scientists isolated the PG gene, produced a complementary gene which produces a complementary mRNA that binds to the normal mRNA inactivating the normal mRNA for this enzyme.



CRISPR

New technology (Clustered Regularly Interspaced Short Palindromic Repeats), efficient and reliable way to make precise, targeted changes to the genome of living cells.