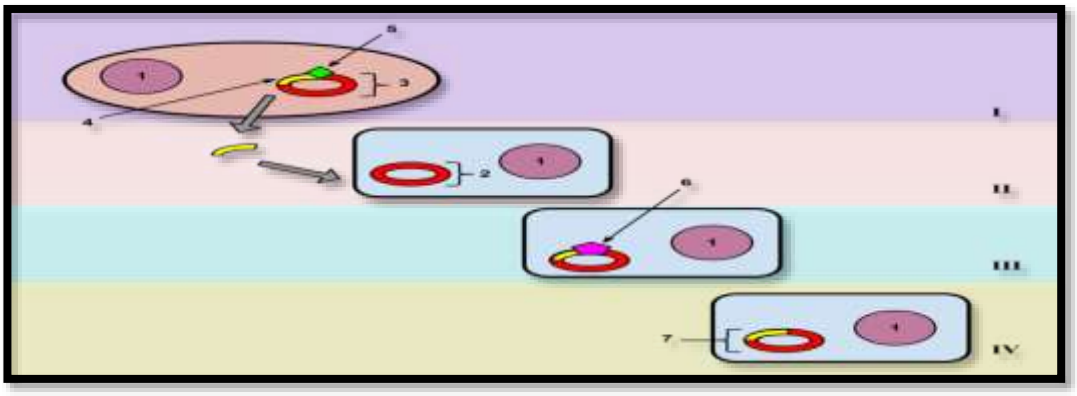
Genetic transformation: is a process that involves the insertion and expression of foreign genes in a genome of host organism.so the DNA becomes a permanent addition to the genome. so that it is inherited in subsequent generations.

Transformation systems have been developed for only a handful of fungi that are pathogenic to humans, including several species of filamentous fungi, and for a lot of filamentous fungi pathogenic to plants. Development of transformation systems in nearly all filamentous fungi has been delayed considerably and has only been possible recently with the introduction of electroporation and biolistic methods. The fate of incoming DNA varies greatly among the species, regardless of their phylogenetic relationships. Understanding the fate of incoming DNA is critical for the construction of transforming vectors and the molecular manipulation of the fungi.



Fungi Genetic Transformation methods

The establishment of genetic transformation systems has enabled scientists to transform foreign DNA into filamentous fungi and thus obtained the desired strains for industrial purposes. We now can take full advantage of the superior secretary power of fungi and their excellent efficiency in manufacturing valuable metabolites. Metabolite: is an intermediate or end product of metabolism.

Four techniques suitable for the genetic transformation of filamentous fungi:

1- Protoplast-mediated transformation (PMT).
2- *Agrobacterium* -mediated transformation (AMT).
3- Electroporation.
4- Biolistic methods.

1- Protoplast-mediated transformation (PMT)

PMT is the most commonly used fungal transformation method, which relies on a large number of competent fungal protoplasts. The principle is to use some commercially available enzymes to remove fungal complex cell wall components for generating protoplasts. Subsequently, some chemical reagents (such as Polyethylene glycol PEG) are used to promote the fusion of exogenous nucleic acids and protoplasts. The components of the fungal cell wall are highly variable among different strains. Even components of the spore coat are significantly different from that of hyphae from the same strain. Thus, there is no universal transformation method that can be applied to different fungal strains. Preparation of protoplast can hardly be standardized. Part of the difficulties comes from our limited knowledge of cell wall hydrolases. Development of an optimized PMT method for fungi still requires significant effort.

Basic steps of the PMT method:

1- Preparation of the protoplasts

- The first step in protoplast preparation is the removal of cell wall through enzymatic digestion.
- The fungal cell wall is comprised of glucan, and chitin.
- The cell wall varies during the cell division and growth of fungi, as well as in spore germination, hyphal branching .
- The cell wall components are also different in different fungal species, therefore, various enzymes should be used in combination. It has been reported that the selection of an appropriate enzyme mix is a key factor in protoplast preparation.
- In general, the hyphae are sensitive to a suitable enzyme which hydrolyzes its cell wall during the logarithmic phase.
- In the PMT procedure of Neurospora, the protoplasts are prepared by hydrolyzing the newly born hyphae (culture for 4–6 h under 25–30 °C)

- protoplasts can also be prepared with conidiospores. For example, for Aspergillus and Penicillium.

- Protoplasts are sensitive to osmotic pressure, care should be taken to maintain a stable osmotic pressure to keep the protoplasts intact during the enzymolysis of cell walls. Thus, osmotic stabilizers (such as sorbitol, sodium chloride, and potassium chloride) should be included in all of the buffers for protoplast preparation to avoid rupture of cells.

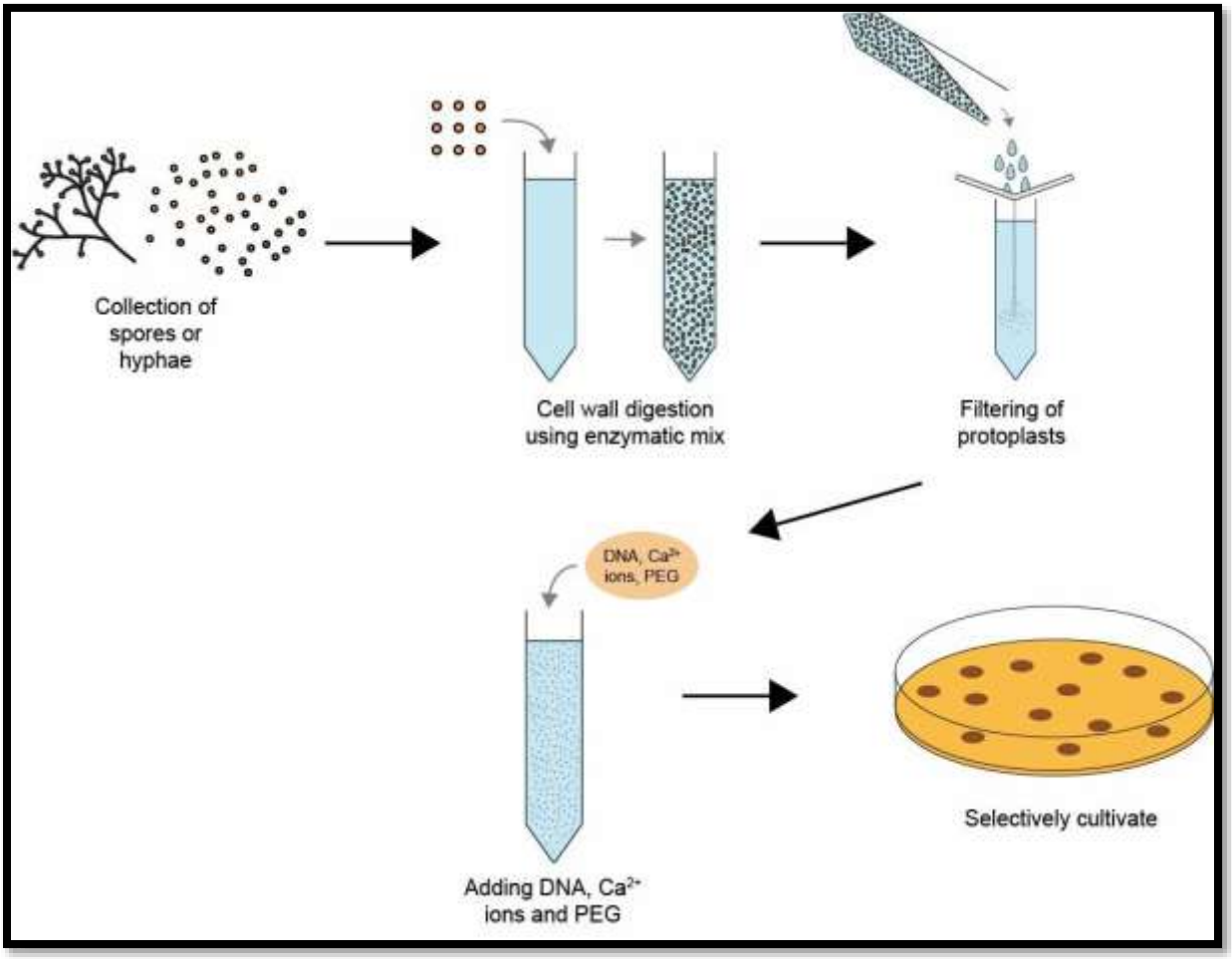


Figure (1) Basic steps of the PMT method

2- Uptake of exogenous DNA

- The solution used to suspend protoplasts usually contains calcium ions and osmotic stabilizers.
- Calcium is thought to open channels in the cytomembrane, which facilitates entry of exogenous DNA into the cell,
- osmotic stabilizer are necessary for maintaining the morphology of the protoplasts.
- certain amount of polyethylene glycol (PEG) is added together with purified DNA (which can either be the circular double-stranded DNA or the linearized DNA).
- PEG is a commonly used cell fusion promoter [38].
- PEG can form the molecular bridge between cells or between cytomembrane and DNA, and thus promotes adhesion.
- PEG can also induce disordered charges on the cytomembrane surface, alter the membrane permeability, and facilitate entry of exogenous nucleic acids into cells
- PEG is a crucial agent enhancing transformation efficiency. Low transformation efficiency in most cases can be improved by adding more PEG.
- the performance of low-molecular-weight PEG (like PEG3000) is superior to that of high-molecular-weight PEG (like PEG8000). However, this needs to be optimized for various species [40].
- Transformation efficiency is also influenced by temperature. Generally, the DNA and protoplast mixture should be placed on ice for 15–30 min, so that the DNA can adhere to the surface of protoplasts.

3- Regeneration of protoplasts

In order to guarantee good recovery of viable protoplasts, transferred to a selective plate. An osmotic stabilizer should be included in the regeneration culture. Stable osmotic pressure is the key factor for protoplast to regenerate cell wall. Only the protoplasts that carry exogenous nucleic acids can grow on the selective medium.