

# TRANSIENT GENE EXPRESSION SYSTEM UTILISING OIL PALM PROTOPLASTS

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**A** protoplast is a single cell without cell wall and only has plasma membrane to protect the cell. This property helps the protoplast to easily take up foreign macromolecules such as DNA and has been used since 1974 for transient and stable transformation of many plant species. Protoplast transient gene expression system is well-known as an easy, rapid, safe and efficient system, particularly via polyethylene glycol (PEG)-mediated transformation. Even though the transient gene expression system utilising tobacco and *Arabidopsis* protoplasts could be adopted for oil palm, these heterologous systems may exhibit aberrant results. For example, the oil palm mesocarp-specific promoter-driven reporter genes,  $\beta$ -glucuronidase (GUS) and green fluorescent protein (GFP), introduced into *Arabidopsis* plants have been shown to be expressed in the leaf even though the promoter was reported to be specific to the mesocarp tissue (Zubaidah and Siti Nor Akmar, 2013). Meanwhile, the PIPP (a chimeric antibody against human chorionic gonadotropin; hCG) gene under the control of an oil palm kernel-specific promoter was expressed in tobacco protoplasts isolated from leaf tissue (Masani, 2013). Although protoplast isolation from oil palm callus has been reported before (Sambanthamurthi *et al.*, 1996), only recently a successful protocol for the regeneration of oil palm plants from protoplasts originating from cell suspension cultures, has been developed (Masani *et al.*, 2013). In addition, the first successful protocol for the transformation of oil palm protoplasts by PEG-mediated transfection has also been established (Masani *et al.*, 2014). The protocol subsequently provides an extraordinarily valuable tool for oil palm gene functional analysis.

## OBJECTIVE

To determine the expression of oil palm genes or promoters, and the viability of plant expression vectors using protoplasts.

## METHODOLOGY

### Protoplast Isolation

Protoplasts are isolated from three-month old oil palm cell suspension cultures (*Figure 1A*). The cells are collected by filtration through a 300- $\mu$ m nylon mesh (*Figure 1B*), and 0.5 g fresh weight (fwt) of cells (*Figure 1C*) transferred to a 50-ml centrifuge tube containing 15 ml filter-sterilised enzyme solution (*Figure 1D*). The cells are incubated in the dark at 26°C for 14 hr. The mixture is diluted with 15 ml filter-sterilised washing solution (*Figure 1E*), filtered through a sterilised double layer of miracloth (*Figure 1F*) and collected in a 50-ml centrifuge tube. Subsequently, clean and viable protoplasts (*Figure 1G*) are obtained by optimal purification procedure (Masani *et al.*, 2013).

### PEG-mediated Transfection

PEG-mediated transfection is performed based on the protocol developed by Masani *et al.* (2014). Generally, a 500- $\mu$ l aliquot of the protoplast suspension is placed as a single droplet in the middle of a petri dish and five drops of 100  $\mu$ l PEG-MgCl<sub>2</sub> solution added in an adjacent but separate position (*Figure 1H*). A 50  $\mu$ g plasmid DNA (gene of interest) is added to the protoplasts and mixed gently by stirring with a 200- $\mu$ l pipette tip. The mixture is incubated at room temperature in the dark for 10 min and then the protoplasts + DNA



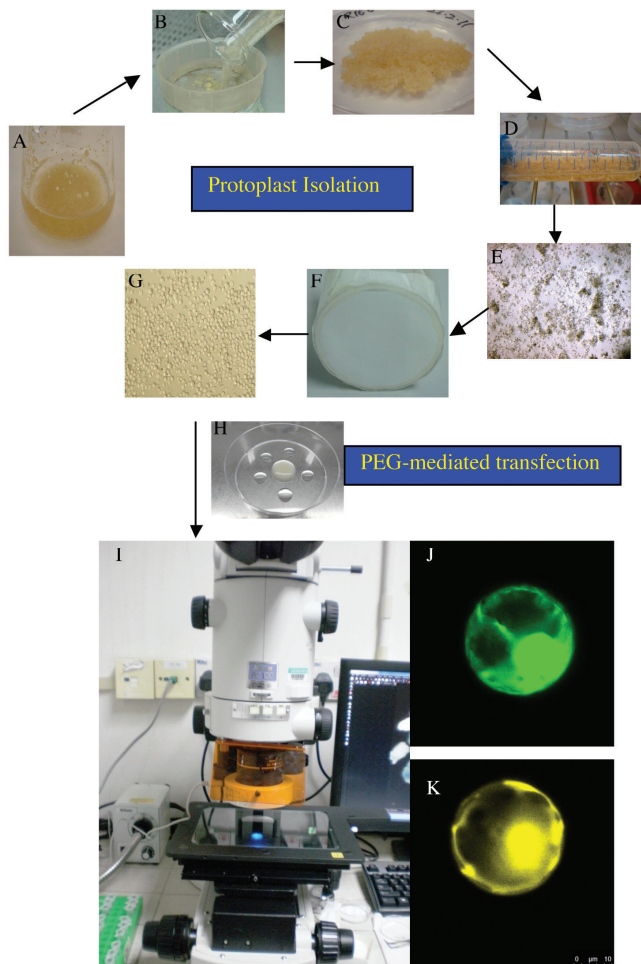


Figure 1. Oil palm protoplast transient expression system: (A) three-month old suspension callus, (B) callus are selected by filtration with 300  $\mu\text{m}$  nylon mesh, (C) callus on solid medium, (D) callus in enzyme solution, (E) protoplasts before filtration, (F) miracloth, (G) freshly isolated protoplasts, (H) protoplast suspension surrounding by PEG-MgCl solutions (I) fluorescence microscope, (J) protoplast expressing GFP and (K) protoplast expressing YFP.

are mixed with the adjacent PEG-MgCl<sub>2</sub> drops by stirring with the 200- $\mu\text{l}$  pipette tip. After a further 30 min incubation, 4 ml of washing solution are added drop by drop and the mixture incubated in the dark at 26°C for 24 hr. Finally, the transfected protoplasts are observed under a fluorescence microscope (Figure 1I) to determine the expression of the genes (Figures 1J and 1K).

### BENEFITS AND COST

Members of the oil palm industry and research scientists who are interested to analyse oil palm genes can use this transient expression system. Those who want to determine the function and expression of foreign genes in oil palm cells

should also benefit. This service requires minimal cost (enzymes and service costs) – RM 3000 to RM 5000, depending on the complexity and nature of the gene or promoter.

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