SERVICES FOR GENETIC TRANSFORMATION OF PLANT SPECIES

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Genetic engineering has been identified as a promising technology to further enhance the value of oil palm via the production of high value traits. Similarly, value can be generated for other plant species via the same technology. The establishment of a reliable transformation and regeneration system is essential for genetic engineering of any given species. This requires a number of services to ensure that the transfer of foreign gene(s) has taken place. Among the services are gene/DNA delivery, promoter analysis, and transient and stable gene integration analyses.

GENETIC TRANSFORMATION

Genetic transformation involves the uptake of naked DNA (gene/s of interest) by competent cells, followed by integration into the chromosome, and subsequent expression to produce the gene product. The process starts with the penetration of DNA into a cell through the cell wall and plasma membrane. The DNA eventually penetrates into the nucleus. Among the methods for delivering foreign genes into plant cells are microprojectile bombardment and *Agrobacterium*-mediated transformation. After the transformation has taken place, analyses to determine the effectiveness of the gene delivery method, promoter used, gene integration and functionality are required. Transient gene analysis using reporters such as green fluorescent protein (GFP) or glucuronidase (GUS) is the most common method to determine the integration and functionality of gene transferred. This requires the use of either a normal stereoscope or a fluorescent stereoscope with a special filter for GFP. Finally, quantification of the fluorescence activity using an appropriate software and documenting the reporter activity using a built-in digital camera system are essential.

SERVICES PROVIDED

Microprojectile Bombardment – Biolistics PDS-1000He

MPOB has a Biolistics PDS-1000He device (*Figure 1*) the use of which is offered to companies, research institutions and universities at a minimal cost to cover consumables and a small service charge. Optimization of DNA delivery conditions for a particular tissue type or plant species is also provided. Clients have to bring their own plasmid DNA for transformation, and MPOB will provide the service of binding the DNA to gold microcarriers and will perform the transformation experiments. [A service to custom-make a client's own plasmid DNA is also available through our previous *MPOB Information Series No. 309 (2005)* and *447 (2008)*. Clients have also to provide their own petri dishes containing their preferred tissue culture media.

Figure 1. Biolistics PDS-1000He device.

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Microprojectile Bombardment – Helios Hand-held Biolistics

MPOB also has a Biolistics Helios device (*Figure 2*), the use of which is offered to companies, research institutions and universities at a minimal cost to cover consumables and a small service charge. This device is special in that it can be used to transfer DNA or microparticles directly into plant organs, tissues or cells without having to place them into a sterile container, or to cut the parts from the main plant. Direct delivery into parts such as leaves or roots can be done easily. Clients have to provide their own plasmid DNA for transformation, and MPOB will provide the service of binding the DNA to gold microcarriers and perform the transformation experiments.

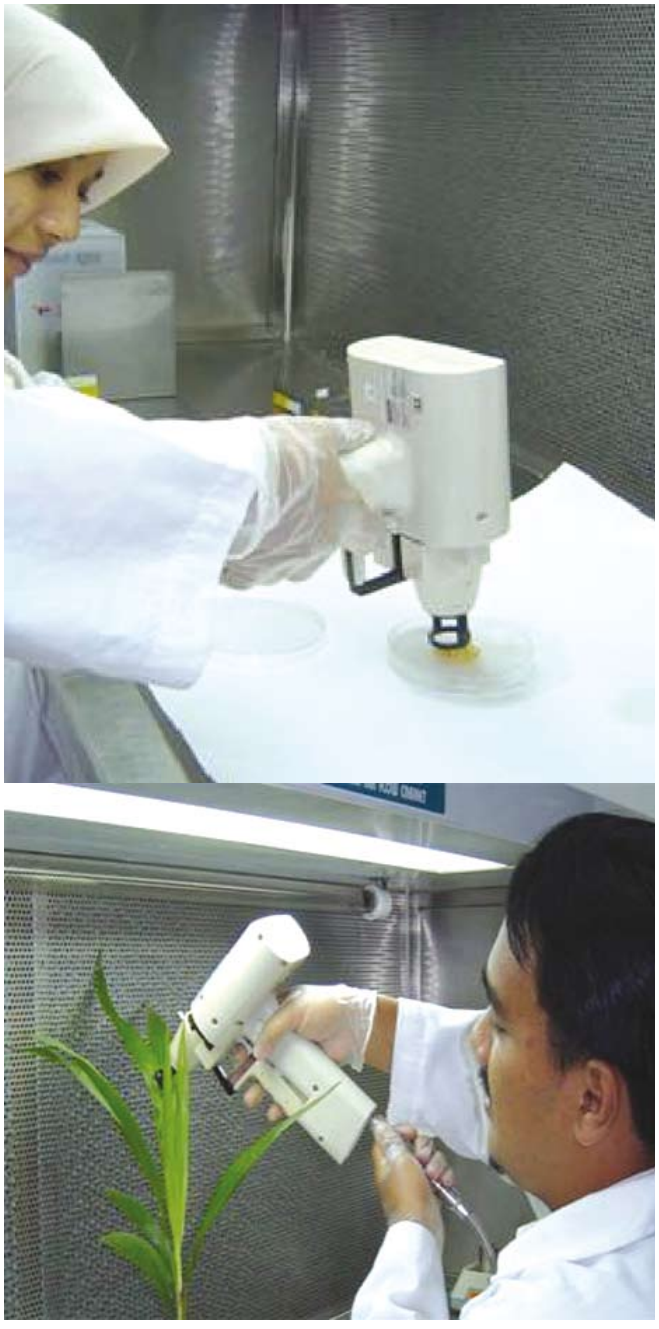

Figure 2. Biolistics Helios device.

Agrobacterium-mediated Transformation

MPOB has a facility to transform plants using *Agrobacterium* which is offered to companies, research institutions and universities at a minimal cost to cover consumables and a small service charge. Clients have to bring their own *Agrobacterium* strains with the desired genes, or MPOB can provide its available strains and transform the strain with the client's plasmid DNA. MPOB will provide the service of carrying out the transformation experiments.

Transient and Stable Gene Analysis – GUS

MPOB has a Nikon SMZ-U stereomicroscope (*Figure 3*) attached to a Nikon digital camera for analysis of β-GUS. Clients can request for this analysis after microprojectile bombardment carried out by MPOB or other parties. MPOB will use its own GUS assay buffer, incubate the sample, analyse the sample, and take the necessary photographs for the clients. Clients have to pay for the GUS buffer, printing and soft copies of the photographs as well as a minimal service charge.

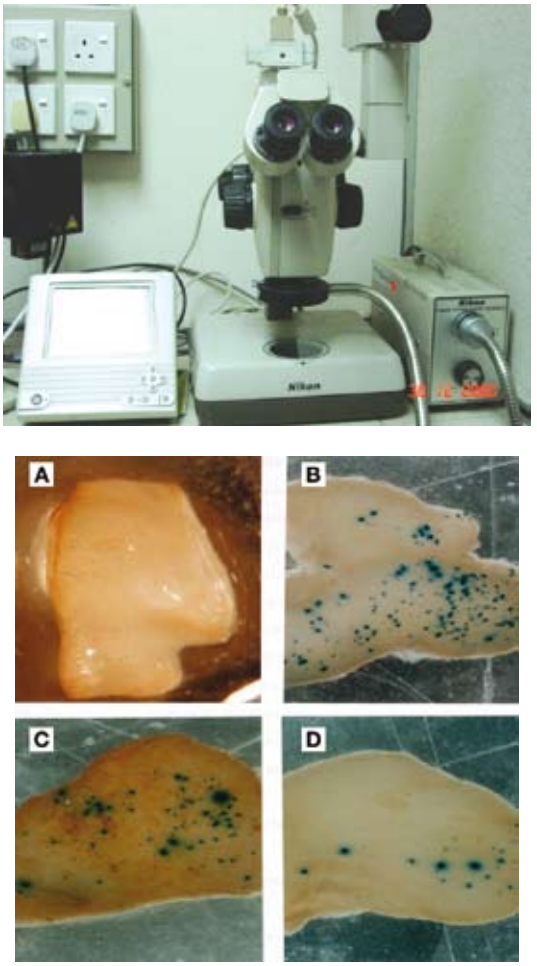

Figure 3. Nikon SMZ-U stereomicroscope with camera (top), and GUS expression in oil palm embryogenic calli (bottom).

Transient and Stable Gene Analysis – Green Fluorescent Protein (GFP)

MPOB has a Leica MZ 12_5 stereomicroscope (*Figure 4*) attached to a DC200 camera, together with its software for analysis of Green Fluorescent Protein (GFP). A GFP Plus Fluorescence filter is also attached to optimize GFP as well as to remove the fluorescence from chlorophyll. Clients can request for this analysis after microprojectile bombardment by MPOB or other parties. MPOB will analyse the sample and take the necessary photographs for the clients. Clients have to pay for use of the software, printing and for soft copies of the photographs as well as a minimal service charge.

WHO SHOULD BENEFIT

Molecular biologists or biotechnologists from the oil palm industry can benefit from these services to test any of their genes or promoters, or to develop their own transformation method. Similarly, molecular biologists and biotechnologists from local universities, research institutions and research-based companies can benefit from these services for their research, either to study gene expression or to regenerate transgenic plants. As mentioned earlier, MPOB provides services to make transformation vectors and RNAi constructs as well as to regenerate transgenic plants using both microprojectile bombardment and *Agrobacterium*-mediated transformation approaches.

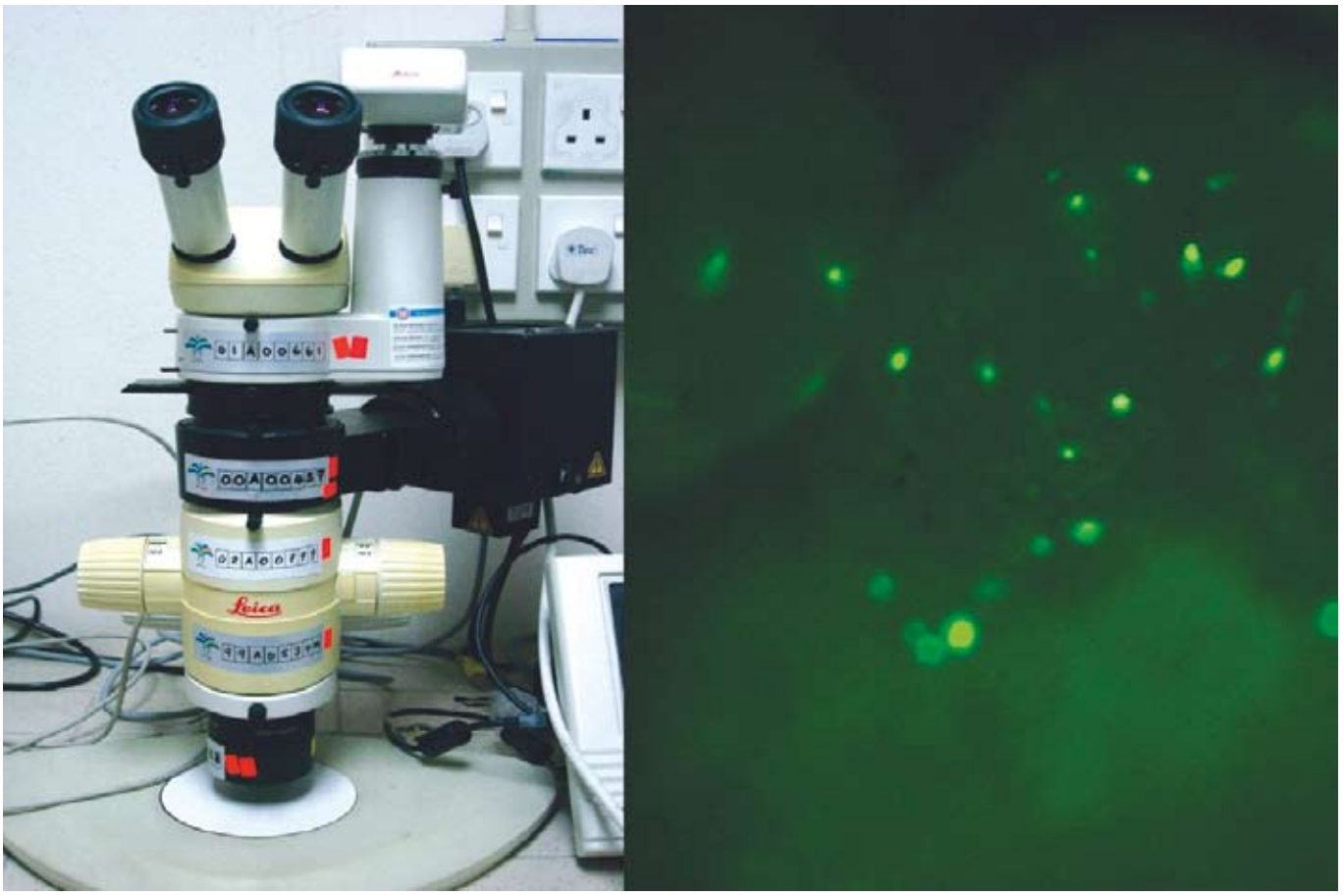

Figure 4. Leica MZ 12_5 fluorescence stereomicroscope (left), and GFP expression in oil palm embryogenic calli (right).

For more information kindly contact:

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