

# CONSTRUCTION OF TRANSFORMATION VECTORS FOR GENETICALLY MODIFYING PLANTS

by: ABDUL MASANI MAT YUNUS and AHMAD PARVEEZ GHULAM KADIR

309

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**A**dvances in MPOB's genetic engineering programme have made it possible to genetically engineer oil palm for producing valuable commercial products such as high oleate, high stearate and biodegradable plastics. New approaches have been invented to produce transgenic palms such as establishment of a transformation system, isolation of important genes and promoters, construction of transformation vectors with the required combination of genes to achieve the primary target of this programme. To date, a number of transformation vectors have been constructed for many important genes, as well as for a number of genes interacting within complex metabolite pathways. The constructs have been transformed into oil palm cultures which have been regenerated into hundreds of transgenic oil palm (Figure 1). □

## TRANSFORMATION VECTORS

Genetic elements typically considered in preparation of transformation vectors for oil palm genetic engineering include: (a) promoters for driving transgenes; (b) target genes; (c) polyadenylation

signals for termination of transgene expression; (d) selectable marker for selection of transformants; (e) transit sequence for targeting transgenes into appropriate subcellular compartments; (f) specific sequences such as matrix attachment region for stabilizing transgene expression; (g) backbone vectors which facilitate cloning, replication to high copy numbers in *E. coli*, and are stable in *Agrobacterium tumefaciens* (Figure 2). □

## TRANSFORMATION VECTORS FOR OIL PALM GENETIC ENGINEERING

A transformation vector designated pLSP13 for synthesizing PHB in oil palm leaf was constructed. The vector contained plastid-targeted *R. eutropha* genes encoding 3-ketothiolase (*phbA*), acetoacetyl-CoA reductase (*phbB*), and PHA synthase (*phbC*) driven by leaf-specific promoters (LSP1) (Figure 3). Transformation of oil palm embryogenic calli with the constructed transformation vectors has successfully produced transgenic plantlets. The efficiency and functionality of the constructed transformation vectors have been confirmed through molecular analysis such a PCR (Figure 4).

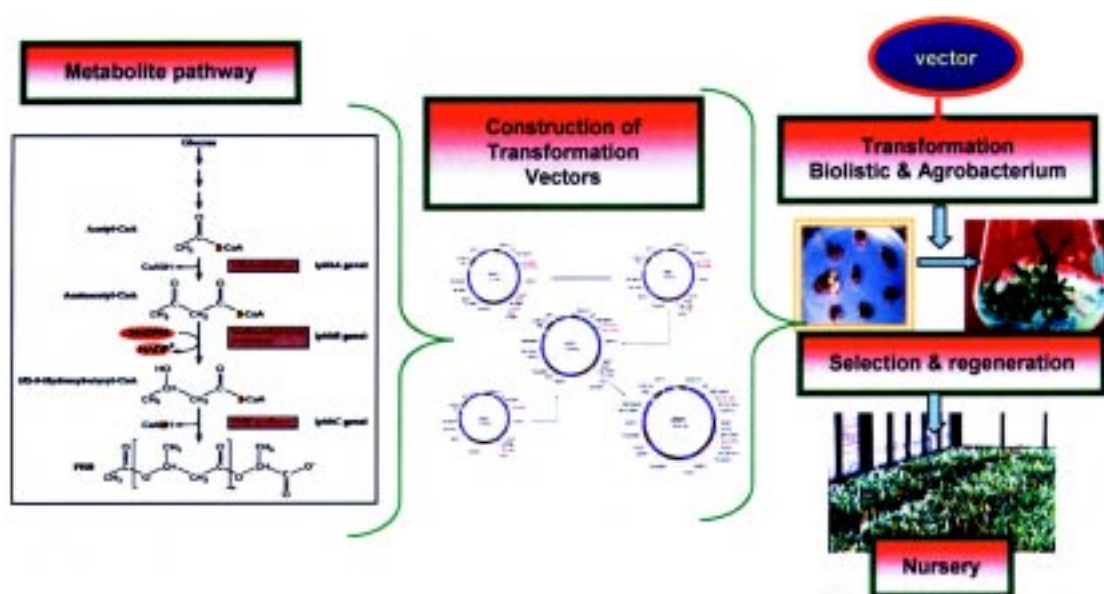


Figure 1. Oil palm genetic engineering.

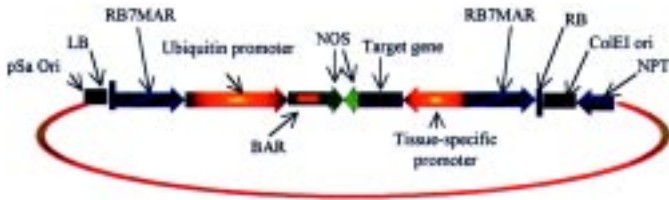


Figure 2. Basic transformation vector for oil palm genetic engineering.

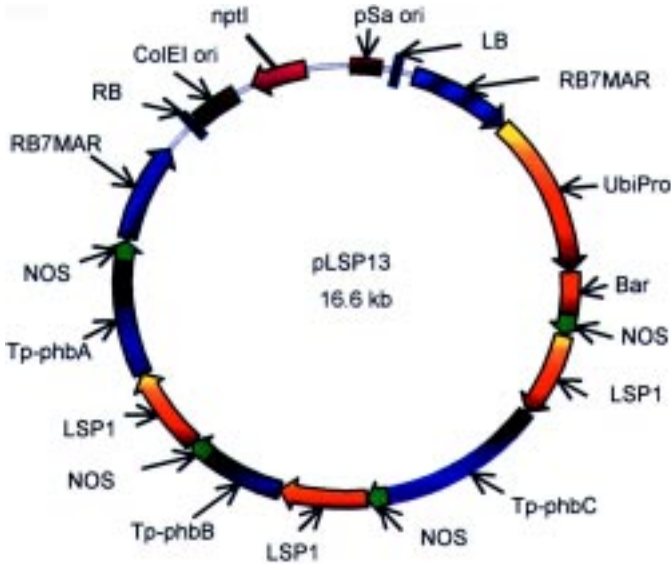


Figure 3. PHB transformation vectors.

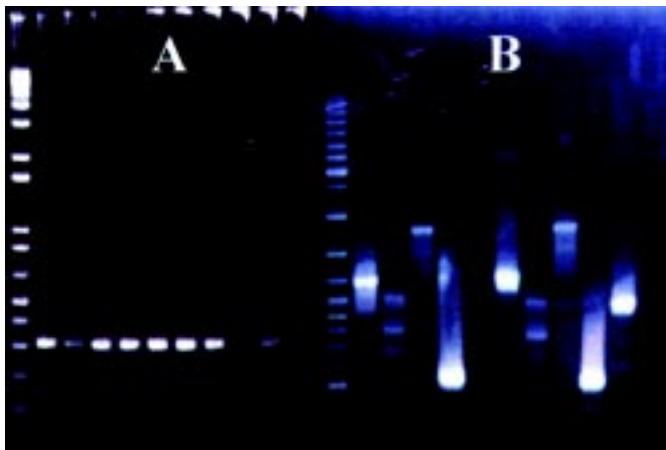


Figure 4. PCR analysis of transgenic oil palm (a) bar gene (b) PHB genes.

### CONFIRMATION OF THE CONSTRUCTED TRANSFORMATION VECTORS

All genetic materials amplified by PCR, intermediate plasmids and transformation vectors were sequenced to confirm their integrity. Restriction enzyme and PCR analyses were also performed to confirm that all the genes were inserted in the expected orientation (Figure 5). □

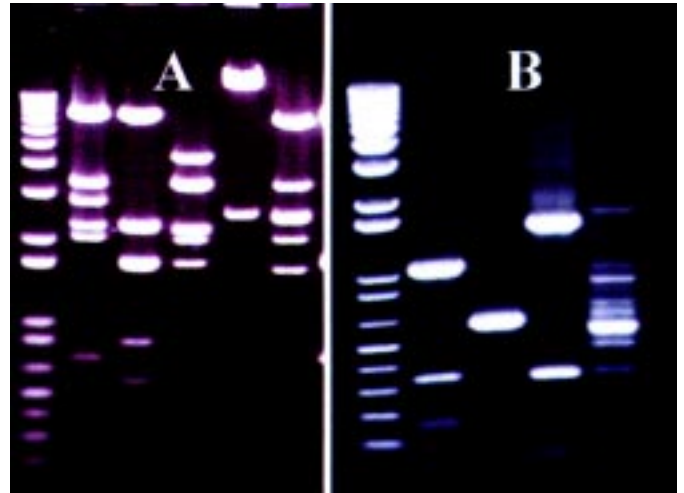


Figure 5. Confirmation of constructed transformation vector (a) restriction analysis (b) PCR analysis.

### BENEFITS OF TECHNIQUES FOR CONSTRUCTION OF TRANSFORMATION VECTORS

Construction of transformation vectors is very difficult due to technical limitations of existing methods. Here, we have constructed a number of transformation vectors allowing for the assembly of multiple genes for genetically modifying oil palm. Our approach is ideally suited for the construction of any plant transformation vector to produce any desired product. We are able to provide a service to construct transformation vectors for any genes of interest for modification of any plants.

### WHO SHOULD BENEFIT

Member of the oil palm industry interested in genetically modifying oil palm for novel fatty acids or novel products, should benefit from this technology. Other scientific, research or academic institutions which would like to genetically modify any plant for any targets should also benefit from this vector transformation technology.

For more information kindly contact:

Director-General  
MPOB  
P. O. Box 10620  
50720 Kuala Lumpur, Malaysia.  
Tel: 03-89259155, 89259775  
Website: <http://mpob.gov.my>  
Telefax: 03-89259446