

QUALITY CONTROL PROCESS FOR OIL PALM TISSUE CULTURE USING DNA PROBES

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INTRODUCTION

Despite the possible occurrence of clonal abnormalities, the prospect of commercializing oil palm tissue culture is still attractive. The move towards commercial production will entail scaling up plantlet production. In such a situation, culture inspection by visual means becomes impractical (Wooi, 1995). In addition, the handling of large numbers of cultures could lead to higher probability of cultures being mixed up. A procedure for quality control is thus necessary.

What is quality control?

The term "quality" can be broadly defined as satisfying customers' needs (Nadkarni, 1991). Quality control (QC) refers to the operational techniques and activities implemented during relevant stages of production in order to ensure that the quality of the product meets the needs of the users.

Applicability of a QC process in tissue culture

In the production of tissue culture plantlets, the customer requires plants which are homogeneous and "true-to-type". Measures must then be taken to provide such assurance. For this purpose, a procedure is proposed for monitoring uniformity of product through the use of deoxyribonucleic acid (DNA) probes. The proposed procedure, however, does not address the issue of somaclonal variation.

RFLP TECHNOLOGY

DNA probes developed at PORIM have been found to be useful for detecting genetic variations in oil palm (Cheah *et al.*, 1993) as well as for monitoring uniformity in tissue culture (Cheah and Wooi, 1995). The probes

detect genetic variation via restriction fragment length polymorphism (RFLP). In simple terms, RFLP refers to variation between genomes manifested by differences in the size of the DNA fragments detected by a probe. A DNA probe, on the other hand, is a piece or sequence of DNA that can be used to detect genetic differences. *Figure 1* shows how probes work.

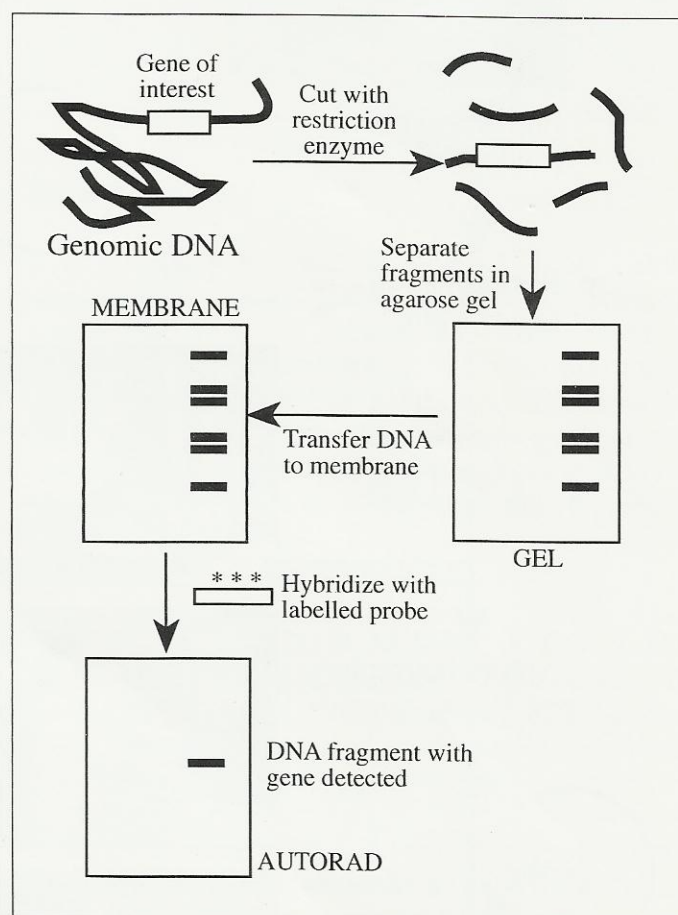


Figure 1. How gene probes work.

The RFLP Procedure

RFLP analysis is a multi-step procedure. *Figure 2* shows the steps involved and the elapsed time for each step.

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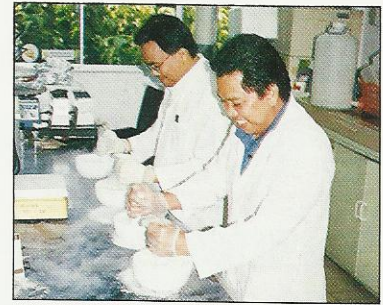


Field/laboratory sampling

Freezing of samples

1 day

DNA preparation and quantitation



5 days

Restriction enzyme digestion of DNA

Cut with enzyme

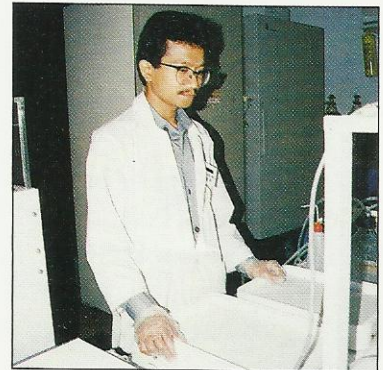
1 day

Gel electrophoresis



1 day

Blotting onto nylon membrane



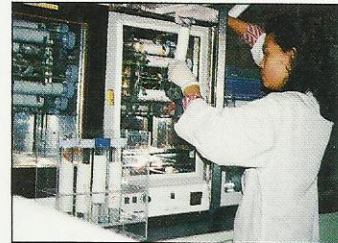
Preparation of probe and quantitation

Hybridization and washing

1 day

1 day

Autoradiography



Reprobe

3 -7 days

Data analysis



Figure 2. The RFLP procedure.

Where can QC be implemented in the tissue culture process?

A simplified form of the process of plantlet production is illustrated in *Figure 3*.

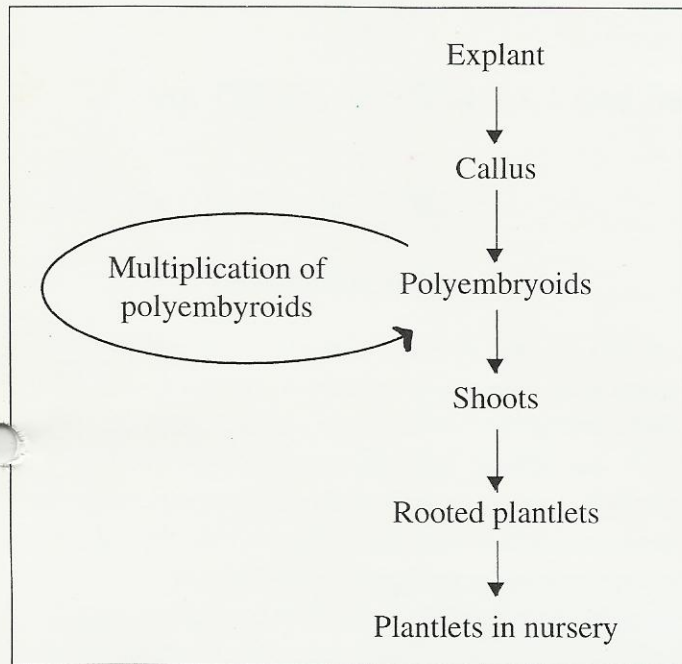


Figure 3. Oil palm tissue culture process.

RFLP analysis can be implemented at any stage of the process. Ideally, DNA profiling should be conducted for ortet palms at the time of sampling. Records will then be available for future reference. Line uniformity can be monitored in polyembryogenic cultures and spot checks made during shoot/root establishment in the test tube or at the polybag stage.

What can RFLP probes be used for?

- Clonal identification
- Monitoring line uniformity
- Detecting culture mix-up
- Confirmation of ramet identity for recloning

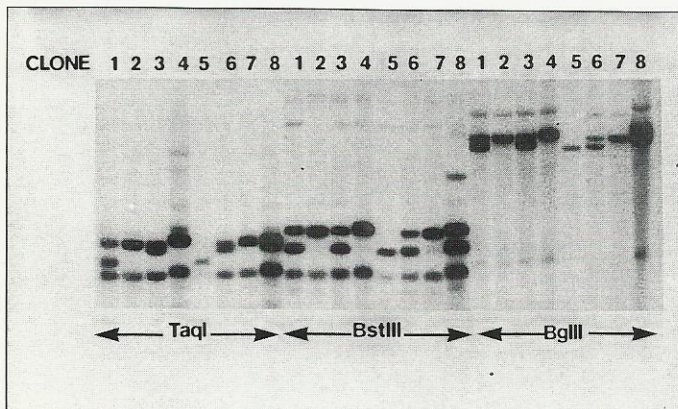


Figure 4. Clonal identification.

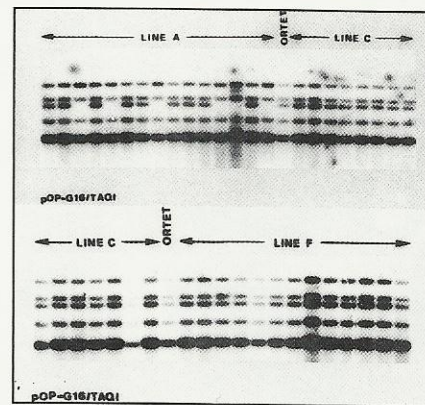


Figure 5. Monitoring line uniformity.

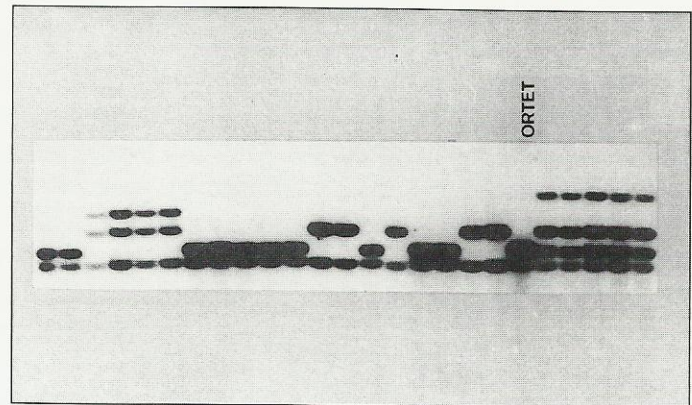


Figure 6. Detecting culture mix-up.

What is the cost?

It is estimated that for an existing tissue culture laboratory to set up a facility for RFLP analysis, the minimum cost of additional equipment is RM250 000.00. For genotyping 50 samples, the supplies cost ranges from RM20.00 per data point (one sample cut with one enzyme hybridized with one probe) for the protocol using one enzyme and one probe to RM1.60 per data point for use of 10 enzymes and 10 probes.

REFERENCES

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