SureSawit™ KARMA – A DIAGNOSTIC ASSAY FOR CLONAL CONFORMITY

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When micropropagation technology was first introduced and found to be viable for the oil palm industry, its adoption was quick with full of enthusiasm. The expectation of dramatically boosting oil yield productivity by 20% - 30% was enough to incentivise the industry to intensify their efforts amidst the current pressure of limited land bank and the need for sustainability.

THE PROBLEM

The positive outlook of large scale tissue culture to meet the demands for uniformed high yielding planting materials was however, short-lived when abnormal fruits, namely the mantled phenotype, were detected and first reported in 1986 amongst clones planted in the field. This phenomenon drastically reduced yields, and has largely halted the commercial production of clonal elite hybrids (*Figure 1*). In addition, the randomness of its occurrence made early culling of these materials difficult and generally dependent on visual identification of the affected palms, two to three years after planting when the fruits are formed and a substantial cost incurred.

THE DISCOVERY

The *MANTLED* locus was identified using epigenome-wide association studies of sets of ortets and ramets from various genetic backgrounds of *Elaeis guineensis*. DNA hypomethylation of a LINE retrotransposon related to rice *Karma*, in the intron of the homeotic gene DEFICIENS (*DEF1*), is common to all mantled clones and is associated with alternative splicing and premature termination (*Figure 2a*). Dense methylation near the *Karma* splice site (termed the *Good Karma* epiallele) predicts normal fruit set, whereas hypomethylation (the *Bad Karma* epiallele) predicts homeotic transformation, parthenocarpy and marked loss of yield (*Figure 2b*). Generally, loss of *Karma* methylation and of small RNA in tissue culture contributes to the origin of mantled.

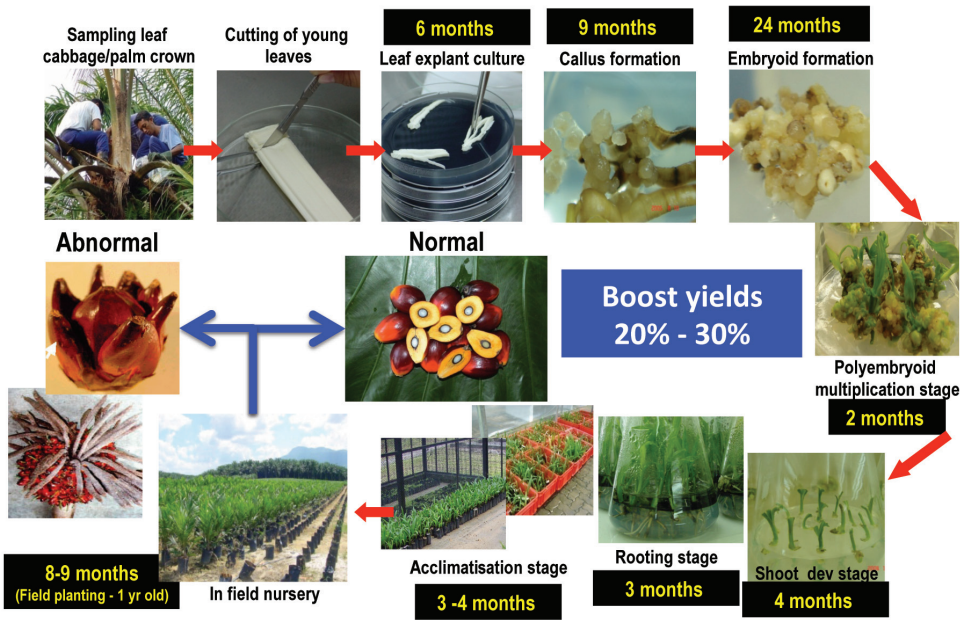

Figure 1. The oil palm tissue culture process.

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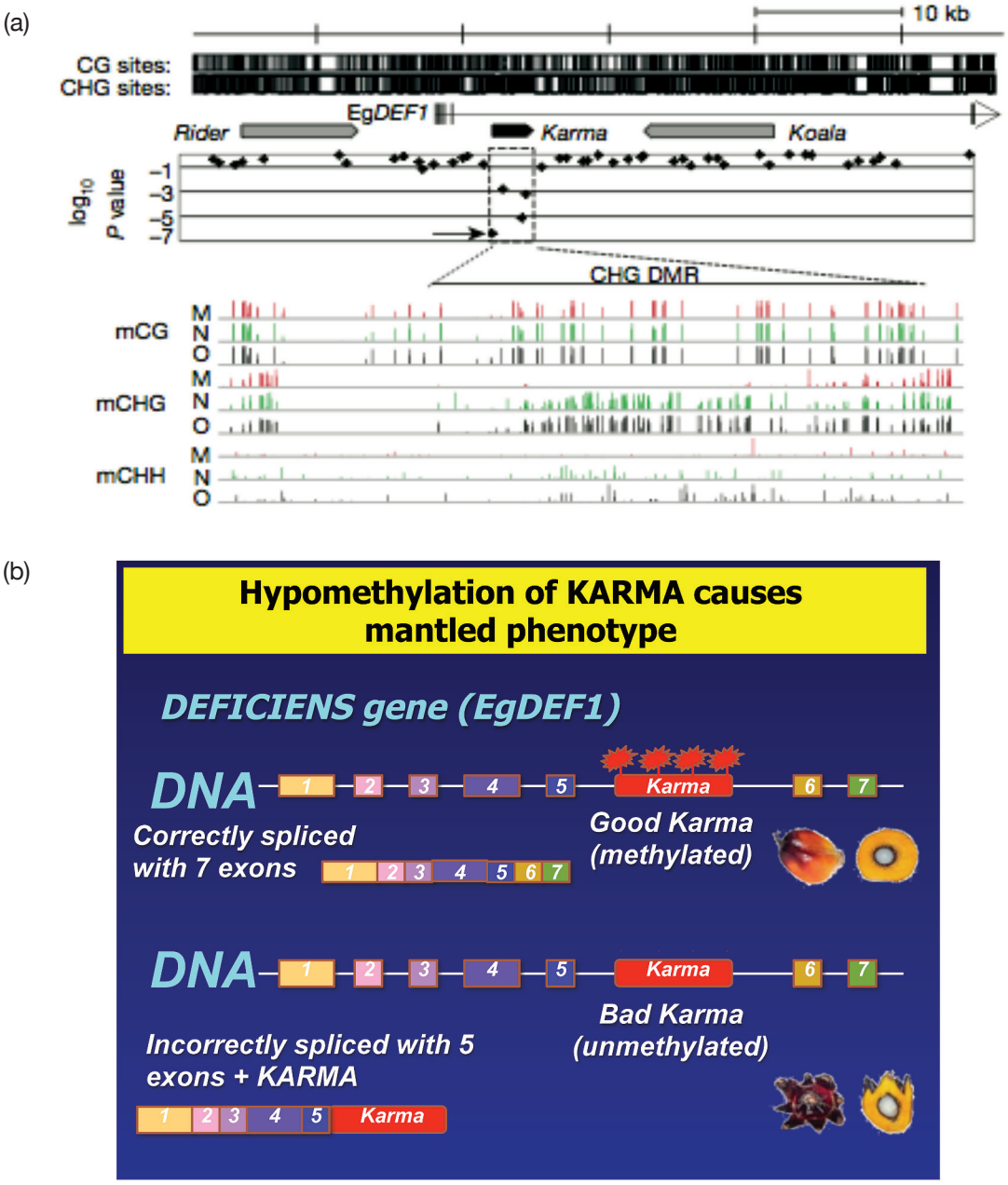

Figure 2. (a) Epigenetic profile of the EgDEF1/MANTLED gene on chromosome 12 and (b) a mechanism for mantling.

THE ASSAY

A PCR-based assay is developed to discriminate mantled (*Bad Karma*) clones from the normal (*Good Karma*). The detection takes advantage of the differential methylation level at the *Karma* region as well as the alternative splice variant present in mantled palms (*Figure 2b*). The general assay process is depicted in (*Figures 3a* to *3d*).

IMPACT

Clonal palms with its potential to increase yields by 20% to 30% have not been fully exploited to date. Only about 2% of total area planted with oil palm comprises tissue culture-derived materials. Prior to this breakthrough, there was no way of ensuring that all clones planted would turn out normal. But with the discovery of *KARMA* and the development of the assay, we now have a means to cull mantled palms early before committing limited plantation resources to planting clonal materials. We envision this discovery will positively transform the oil palm industry.

WHO WILL BENEFIT

- Industry can now pre-screen materials to ensure only mantled-free clones are planted.
- Smallholders can now have access to alternative high yielding planting materials.
- The gain in confidence in clonal planting materials will see larger areas planted with clones, thus boosting yields nationwide.

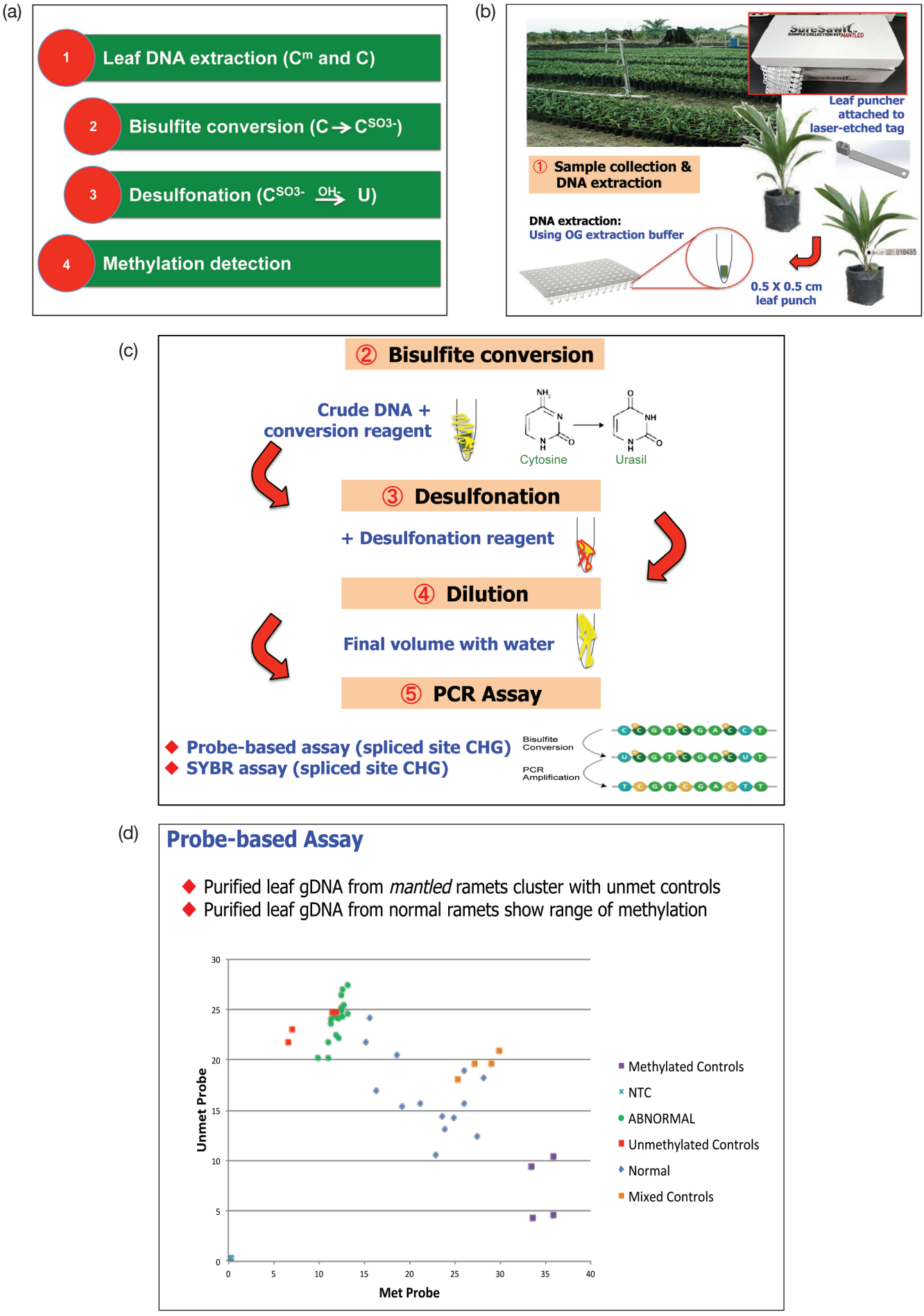

Figure 3. (a) The general process flow of the KARMA *assay. (b) Sampling procedure made-easy with the* SureSawit™ KARMA *collection kit and OG DNA extraction buffer. (c) Steps involved in preparation of DNA for the PCR assay and (d) method of methylation detection.*

PUBLICATION

The publication (*Figure 4*), *Loss of* Karma *Transposon Methylation underlies the Mantled Somaclonal Variant of Oil Palm* by Ong-Abdullah *et al.*, received international recognition by being featured on the cover of the September 2015 issue of *Nature*.

Figure 4. The publication by Ong-Abdullah et al. *(2015) featured on the cover of the prestigious scientific journal,* Nature.

PATENTS

Title: Mantle Phenotype Detection in Palm
Application Numbers: P00201502630 (Malaysia and Indonesia); 61/988,132; 62/091,471 (USA).

COMMERCIALISATION

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