SureSawit™ TRUE-TO-TYPE - A HIGH THROUGHPUT UNIVERSAL SINGLE NUCLEOTIDE POLYMORPHISM (SNP) PANEL FOR DNA FINGERPRINTING, PURITY TESTING AND ORIGIN VERIFICATION IN OIL PALM

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Marker assisted selection (MAS), is now seen as a viable option in oil palm breeding programmes. Increase in the number of trained staff, availability of appropriate genomics tools and infrastructure in many commercial plantation companies have provided the incentive to implement MAS. The most basic application of markers in oil palm breeding is probably testing for legitimacy within the progeny of controlled crosses in order to ensure that the palms within a family are of the correct parentage. This is very important information for breeding programmes, as parents are selected based on family/progeny performance, and if illegitimates are present, wrong conclusions can be drawn (Corley, 2005). Illegitimacy in oil palm breeding, a well-known phenomenon, is intrinsic to the breeding process (Corley, 2005; Kwan *et al*., 2017). Complicating this is the fact that illegitimacy rates can vary greatly from one cross to another. Use of an illegitimate palm in a breeding trial can have deleterious effects, hindering the ability to accurately score the combinability of two parental palms, and thereby limiting the ability to rank and select the best parental combinations.

Currently, simple sequence repeat (SSR) markers are the most popular choice for determining legitimacy of crosses (Thongthawee *et al.*, 2010; Hama-Ali *et al.*, 2015). Almost all publications on SSR define a different set of markers (panel) for DNA fingerprinting that are appropriate within constraints of the genetic background tested. Furthermore, SSR markers have limitations in terms of the number of samples that can be analysed at any one time, the cost of analysing SSRs, and the difficulty of data capture and analyses. As such, there is a need for a high throughput and high resolution DNA marker panel which can be adopted broadly by industry in a manner that overcomes the training, labour, throughput and cost limitations. Single Nucleotide Polymorphism (SNP) based DNA fingerprinting technology promises to achieve these aims. SNP fingerprinting technology is universally being adopted in forensic science for its superior genetic resolution over older DNA marker technologies, and its amenability to high throughput processing. MPOB has developed a high performing True-to-Type SNP panel which is effective in discriminating individual palms from unrelated pedigrees from a wide range of genetic backgrounds for use in breeding, tissue culture and supply chain quality control.

NOVELTY OF TECHNOLOGY

A high performing True-to-Type SNP Assay, consisting of a minimum set of SNP markers which are effective at:

- Uniquely fingerprinting and discriminating individual palms from unrelated pedigrees;
- Uniquely fingerprinting and discriminating between individual progeny in half-sibling, full sibling and selfed crosses;
- Identifying illegitimate palms present in half-sibling, full sibling and selfed crosses; and
- Grouping clonal material by their unique clonal lineage, while also discriminating between ramets of different clonal lines.

VERIFICATION OF THE TRUE-TO-TYPE SNP PANEL

Uniquely Fingerprint and Discriminate between Individual Palms from Unrelated Oil Palm Pedigrees

A total of 1520 palms from 10 categories were first genotyped with high performing assays designed to measure 768 SNPs that were discovered in MPOB's germplasm sequencing project. The top performing 135 SNP assays, and a minimal set of assays comprising the final True-to-Type SNP panel were selected from the 768 SNP set. Analysis of genotype data from the 135 SNP panel resulted in unique fingerprints from each of the

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1520 palms, giving 100% fingerprint resolution (*Figure 1*). Analysis of genotype data from the True-to-Type SNP panel resulted in unique fingerprints from 1519 of the 1520 palms, resulting in a 99.93% fingerprint resolution (*Figure 2*).

Palms	Category
821	Linked Half-Sibs
192	Germplasm
104	Bi-Parental Family 1 & 2
117	DxP_1
84	T128
78	DxP_2
77	DxP_3
22	TxP
22	DxP_4
2	Ortet - Tissue Culture
1.520	**Total**

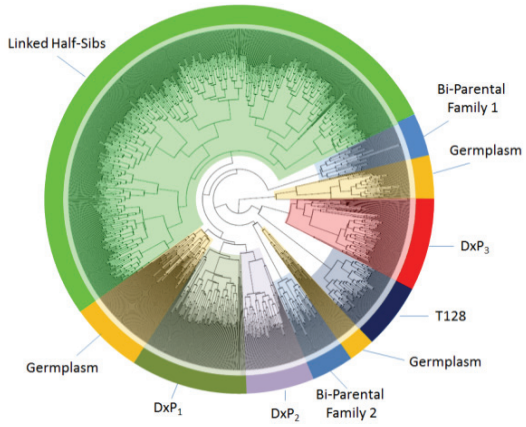

Figure 1. 135 SNP Panel – Genetic resolution across diverse material. Dendrogram of 135 SNP panel on 1520 palms from multiple sources. Produced 1520 unique fingerprints. 100% resolved – 1520/1520 palms.

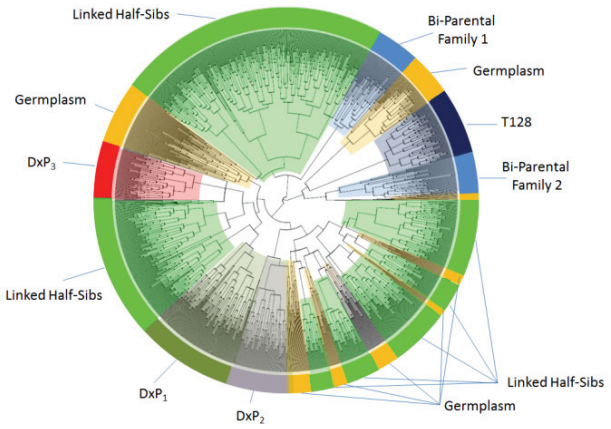

Figure 2. True-to-Type Panel – genetic resolution across diverse material. Dendrogram of True-to-Type SNP panel on 1520 palms from multiple sources. Produced 1519 unique fingerprints. 99.93% resolved – 1519/1520 palms.

Discriminate between Individual Progeny in Full Sibling Families Involving Two Parents

A bi-parental DxP family of 117 F_1 palms was genotyped with the 135 SNP panel, and with the minimal True-to-Type Assay, generating 117 unique fingerprints. Dendrogram analysis of the fingerprints in the 135 SNP panel (*Figure 3*) and the True-to-Type SNP panel (*Figure 4*) both resulted in 117 unique nodes, demonstrating that the True-to-Type Assay is able to discriminate between individual palms in a bi-parental DxP family with 100% resolution. Cluster analysis also identified the 109 palms which were true descendants of the intended bi-parental *dura* and *pisifera* parents, and 8 off-type palms were the result of an unintended selfing of the mother.

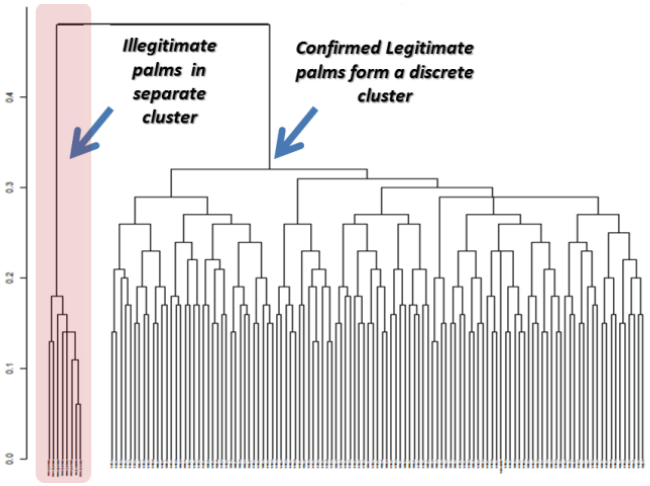

Figure 3. DxP_1 commercial cross. Dendrogram of DNA fingerprints of 117 palm Bi-Parental family using 135 SNP panel.

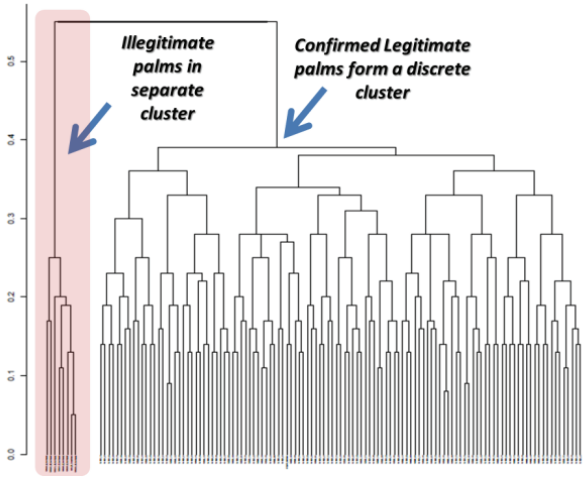

Figure 4. DxP_1 commercial cross. Dendrogram of DNA fingerprints of 117 palm Bi-Parental family using the True-to-Type SNP panel.

Discriminate between Individual Progeny in Full Sibling Families Involving a Single Selfed Parent

A selfed Nigerian *tenera* family of 84 F_1 palms was genotyped with the 135 SNP panel and the True-to-Type SNP panel, resulting in 84 unique fingerprints for both panels (*Figures 5* and *6*). This demonstrates that the True-to-Type panel is able to discriminate between individual palms in a selfed family with 100% resolution. In both cases, cluster analysis also identified which 80 F_1 progenies were descendants of the selfed *tenera*, and which four palms were the progeny of an unknown paternal palm (*Figures 5* and *6*).

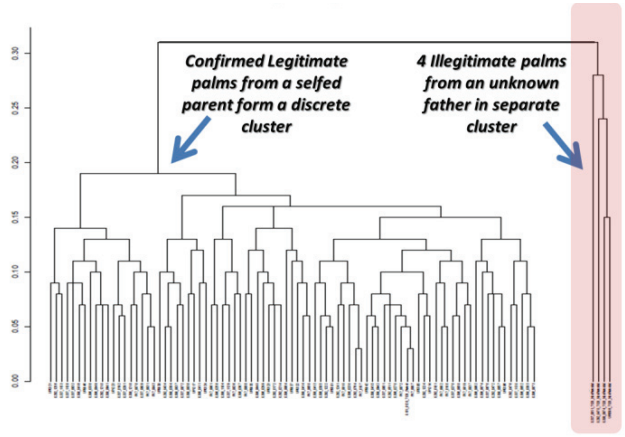

Figure 5. T128 selfed mapping cross. Dendrogram of DNA fingerprints of 84 palms in a selfed family using a 135 SNP panel.

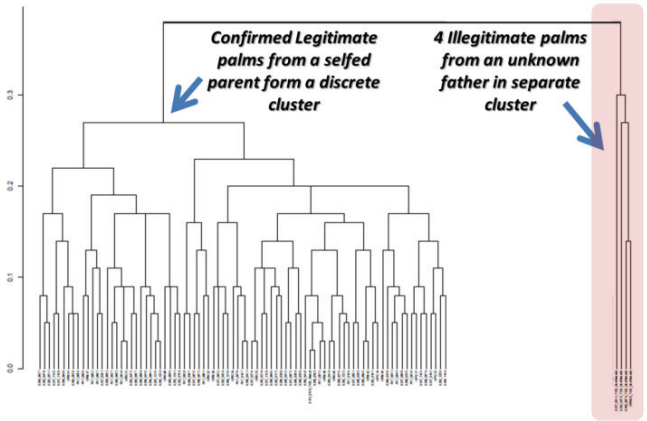

Figure 6. T128 Selfed mapping cross. Dendrogram of DNA fingerprints of 84 palms in a selfed family using the True-to-Type SNP panel.

Discriminate between Individual Progeny in Half-Sibling Families Involving more than Two Parents

Five related *pisifera* palms were crossed with 22 related Deli *dura* palms to generate 22 linked, highly related half-sibling families. 821 palms from these families were genotyped with the 135 SNP panel and the True-to-Type SNP panel, where 821 unique fingerprints were generated in both cases, (*Figures 7* and *8*). This demonstrates that the True-to-Type SNP panel is able to discriminate between individual palms in highly related linked half sibling populations with 100% resolution.

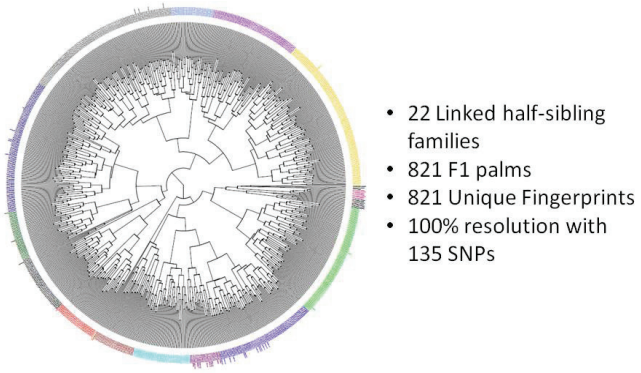

Figure 7. 22 Linked half-sib families. Dendrogram of DNA fingerprints of 821 palms in 22 linked half sibling families using the 135 SNP panel.

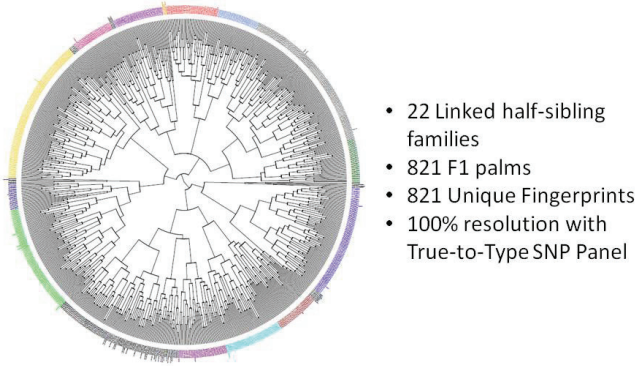

Figure 8. 22 Linked half-sib families. Dendrogram of DNA fingerprints of 821 palms in 22 linked half sibling families using the True-to-Type SNP panel.

Discriminate between a Set of Ramets Having a First Clonal Lineage, from a Second Set of Ramets Having a Second Clonal Lineage

Sixteen ramets from five clonal lines plus five originating ortets were genotyped with the True-to-Type Assay. Analysis of resulting genotype data generated five unique fingerprints, and dendrogram analysis of the fingerprints resulted in five unique nodes. In all cases, the ortet and derived ramets in each line clustered together with identical True-to-Type SNP fingerprints, yet each line was genetically distinguishable. This demonstrates that the True-to-Type Assay is able to discriminate between ramets of different clonal lineages, as well as to precisely identify the clonal lineage of an unknown ramet.

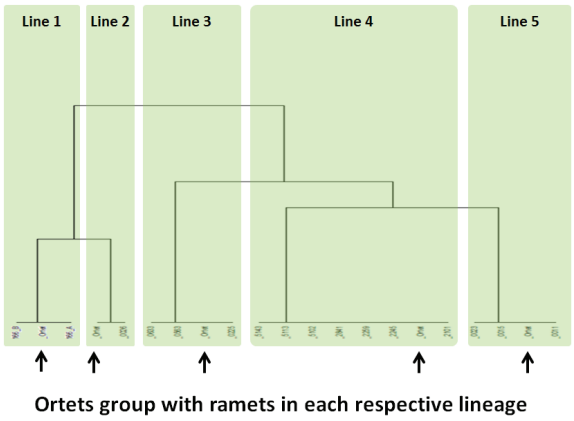

Figure 9. True-to-Type identification of clonal lineage. (16 ramets and 5 ortets in 5 clonal lines).

BENEFITS

The availability of the SureSawit™ True-to-Type test will:

- Enable breeders to identify and exclude illegitimate palms from their breeding populations;
- Increase the ability of industry members to more accurately rank the desirability of parental pairs of palms;
- Accelerate the development of new oil palm varieties;
- Improve the management of breeding, seed production, and nursery practices; and
- Serve as a tool for enforcement to track lineage in cases of dispute.

ECONOMIC ANALYSES

The SNP panel has good economic potential especially for an entity already having basic molecular biology facilities. The additional capital cost required is estimated at RM 100 000. The economic perspectives are feasible with a Net Present Value (NPV) of RM 1.89 million and Internal Rate of Return (IRR) estimated at 61%. The expected payback period is three years.

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