

## Contribution to a white paper on “Enforcement of Plant Breeders Rights”

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I will focus my contribution on section **4.2.9**, and more specifically on **Question 20**.

*Do molecular technologies have significant utility for reducing low compliance rates in variety identification?*

In my opinion the DNA-based test is likely to be the best way to ensure the increase compliance rates. This comment is founded mostly on the fact, that DNA-based genetic identification has become a standard in many areas of criminal law and gains increasing acceptance as reliable evidence in forensics. However, DNA evidence is still scientific expert evidence and therefore requires interpretation. This leaves the possibility of error, mainly due to a human mistake, and improper interpretation of the results (e.g. incorrect assessment of the probability of the two samples being a “match”). The techniques of DNA testing are increasing in the sophistication and accuracy, reducing the probability of error. The choices in the technology issue will be described below, but it is important to stress that the potential problems with genetic ID utilisation are well beyond the reliability of past and current testing techniques: the possibility of contamination of samples, sample tracking issues and the qualifications of the person interpreting the results of the tests.

It is important to stress that an efficient and dependable genetic identification system requires seamless integration of the whole process: from sample collection in the field (or silo) to final reporting of genetic information to the service ordering organisation. Substantial sample tracking and delivery challenges remain upstream from the genotyping facility and are practically independent from the choice of DNA analysis technology. Retaining sample integrity from the time of sampling to the time of performing an assay is a critical issue. Fortunately, basic technology for safe sample collection and transport has been already developed by Whatman in the form of FTA system (<http://www.whatman.com/products/?pageID=7.31.31>), primarily for forensic applications. DNA samples can be collected on FTA cards by untrained personnel in the field. Once bound to the card the DNA can be held at ambient temperature for transport to a laboratory or stored for later analysis. A number of applications of the FTA system are already fully functional, but the need for optimisation remains for more difficult plant tissues and for some DNA technologies. In my view setting up the sample collection system in “forensics-tested” format would be already a step towards reducing the risk of non-compliance. The compliance rate would be further increased by availability of genotyping technology(s) with high reliability which can work with any crop and which can resolve even genetically closely related varieties.

With full recognition of the biased position on the technology choice issue I would like to propose that Diversity Arrays Technology (DArT) offers a genotyping platform which could

be utilised very broadly for the genetic ID applications. This Australian technology has been developed and is commercially delivered in Australia and internationally already for over 25 crops, with a portfolio of crops increasing at the rate of one crop per every 1-2 months. The technology (described in detail at [www.diversityarrays.com](http://www.diversityarrays.com)) offers at least an order of magnitude of assay development cost reduction compared to other technologies. It offers also similar cost advantage in the assay performance area, especially if a number of samples can be submitted for the analysis at the same time. Significant reduction of the development and marker assay costs is an important element, especially if discrimination among highly related varieties is required. In such cases the number of markers assayed PER SAMPLE has to be increased to several hundreds to ensure highly robust/statistically significant identity determination. Fortunately, DArT detects variation in hundreds or even thousands of genetic loci (positions in chromosomes) in a single assay, therefore increasing resolution power of analysis without significant increase in per sample cost. It can therefore provide unambiguous ID determination for a manageable fee (in the range of 20-50 Au\$ per sample for hundreds of markers). The analysis is done completely automatically by the proprietary software (DArTsoft) and each marker score is reported with its own probability of error estimate, which is a useful and quite unique feature, at least compared to gel-based technologies. Importantly, it can also work with samples imbedded into FTA membranes, utilising only nonogram quantities of DNA for a complete assay (several hundreds of markers). This will significantly increase flexibility and security of sample processing, as many other technologies perform unreliably using FTA templates.

As I mentioned above, it is critical to have completely seamless integration of sample tracking and automated data capture/analysis in order to remove human error component. Diversity Arrays Technology Pty Ltd has established a good sample tracking system based on its proprietary DArTdb Laboratory Information Managements System. DArTdb is already commercially implemented by Diversity Arrays Technology Pty Ltd and by Triticarte Pty Ltd, its service provision subsidiary for wheat and barley. DArTdb is designed to work “from DNA to data” and can operate in “sample to data” format. DArTdb can offer “sample matching” functionality to facilitate genetic ID testing.

The other technologies with similar sample processing capacity as DArT are proprietary Single Nucleotide Polymorphism (SNP) typing technologies, led in the biomedical market by Illumina Inc. ([www.illumina.com](http://www.illumina.com)). However, all SNP applications in agriculture are still constrained by cost of developing marker information and assay development cost. All the SNP-typing platforms are also negatively affected by the polyploidy, a feature of genomes of many important crop plants (wheat, sugarcane, potato, cotton etc). The positive aspect of commercial SNP platforms is their high level of assay integration and automatic data extraction in diploid species.

In my opinion all gel-based technologies (e.g. Simple Sequence Repeats -SSRs) will be used less often in genetic ID area, due to the cost of their development and typing (high) and some technical issues linked with their performance (often very complex patterns to score). However, there are ways of managing these problems and with proper attitude and sufficient investment, therefore the SSR-based genetic ID is a viable option.

***Do these technologies require additional development to improve their utility?***

The situation varies significantly among the crops. For high value crops (barley, rice and maize, for example), all options described above (DArT, SNP and SSR) are already available and genetic ID test could be offered with any of the technology options. For the polyploid crops SNP tests are not a viable option at the moment and it remains to be seen if the improvement in performance for these crops can be achieved soon. SSRs are currently available for a larger number of crops (possibly >100 crops) compared to DArT (>25 crops), but both the number of crops and marker numbers increase rapidly for DArT. The investment

in SSR development appears to be significantly reduced in the recent years, as the attention shifts substantially towards non-gel based systems (SNP and DArT). Based on my personal experience the resources needed to have an equivalent resolution power for genetic ID based on DArT is 1-2 orders of magnitude lower compared to SNP-based assays. However, it is also my experience that the technology choices are not always based on rational and financial principles.