



The human genome shapes rice breeding

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in a rice hull

- A laboratory machine developed for the efficient analysis of the human genome, the Sequenom® MassARRAY®, is also being used to gather information about the rice genome
- Five important genes are tracked in the breeding of rice varieties – to date, up to eight different individual tests need to be carried out to find out if the breeding line carries the desirable trait
- The Sequenom® MassARRAY® can gather information on all five important rice genes in a single test

The human genome project, which endeavours to identify all the genes and their importance in human beings, has opened the way to personalised medicine – and more efficient rice breeding.

The ultimate goal of the human genome project has been to uncover all of the genetic variation within the human population and then to work out how this affects human health and disease. In order to do this, very efficient analysis tools needed to be developed which would allow genetic data to be gathered from many places within the genomes of many individual people. This search for efficient genetic information gathering in humans has resulted in the production of sophisticated analytical machines which can analyse DNA from not only humans but plants as well, including rice.

One of these machines, the Sequenom® MassARRAY®, is resident at the Centre for Plant Conservation Genetics at Southern Cross University in Lismore, NSW. The Sequenom® MassARRAY® at the Centre for Plant Conservation Genetics is being used to analyse many different types of plants, from relatively simple rice to more complex wheat to very complex sugarcane.

Finding genes of interest in rice

The Centre for Plant Conservation Genetics and NSW DPI recently commenced a project to develop tests for genes of interest to the rice breeding program and to further our understanding of rice grain quality. The Sequenom® MassARRAY® genotyping platform will be a valuable addition to our capacity to analyse the rice genome.

There are over 1.5 million known DNA differences within the rice genome, each of which can be analysed using the Sequenom® MassARRAY® platform. When operating at its most efficient the Sequenom® MassARRAY® can capture a huge quantity of data in a very short period of time – up to 40 different genes within 384 different plants can be analysed in one hour. This capacity is being exploited by the

Centre for Plant Conservation Genetics to construct a single test tube Sequenom® MassARRAY® test for all of the genes of interest to the Australian rice breeding program.

Genes are made up of DNA and the Mass ARRAY® measures the mass of DNA molecules very quickly and accurately. DNA is composed of long strings of four molecules called nucleotides which are represented by the letters, G, A, T and C. The MassARRAY® is so sensitive that it can measure the difference in the mass of two strings of 15–20 nucleotides of identical length which differ by only one nucleotide (or a single base) on the end of the string in a fraction of a second.

Using the Waxy gene which determines amylose content as an example, one version of the gene produces a string of DNA which has the sequence GGAAGAACATCTGCAAGG and is 5540 mass units while the other is GGAAGAACATCTGCAAGT and is 5580 mass units. Following a test tube reaction, the mass of these strings of DNA are measured by the MassARRAY® which appears as a peak on the machine readout which corresponds to the nucleotide by which they differ.

Until recently, the most popular and perhaps most efficient genotyping technologies used tests which separated pieces of DNA which differed by size. Although these pieces of DNA are useful tags for different parts of the genome, they generally do not explain how different parts of the genome control particular traits. In contrast, changes in nucleotide sequences or 'single base changes' are the very smallest DNA differences and are responsible for much of the important and useful variation within a genome.

The Sequenom® MassARRAY® finds 'single base changes' so it can look directly at the actual DNA difference which is responsible for many traits. This is invaluable when trying to work out which of the many DNA differences within a gene controls a trait. Once the important DNA differences have been identified, the same machine can be used for routine molecular marker work within the breeding program. This



is particularly useful because the molecular tag and the important DNA difference are one and the same, and so they can never become separated during the course of the breeding program.

A multiplex assay for important genes

A multiplex assay is a test that has been developed that looks for several important genes in the one analysis or test run. Many of the genes which control important traits in rice have been identified and characterised at the molecular level in recent years.

Genes included in this list are the semi-dwarf gene, which is responsible for the greatest yield gains of any one gene, the *Waxy* gene which controls amylose content, the *alk* gene which controls starch gelatinisation temperature and the fragrance gene. Although blast disease is not currently a problem for the Australian rice industry, genes which protect against blast that have been characterised at the molecular level are being introduced into Australian rice lines in order to protect against blast if it does become a problem.

Each of these genes can be tracked within a breeding program using molecular markers. Currently, these tests are all performed individually and so if the genotype of a rice

plant is to be determined for any of these five genes, up to eight different individual tests would need to be carried out. A Sequenom® MassARRAY® multiplex assay will allow the data on all five genes to be gathered in a single test.

Application of the new technology

Different varieties of rice retrograde to a different extent and this means retrogradation is genetically controlled. Retrogradation is the process in which the starch re-hardens when cooked rice cools. The extent of retrogradation that occurs is important for determining a variety's suitability to different types of cuisine or end uses. We will use the Sequenom® MassARRAY® to analyse the genetics of retrogradation. Because the Sequenom® MassARRAY® can gather molecular genetic data for low cost, it will be possible to assay more individuals to a deeper level of genome coverage, increasing the power of the genetic analysis.

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