Marker development
using Genotype-by-Sequencing (GBS)

The use of genetic markers for breeding becomes more mainstream due to the availability to genomic data and new sequencing techniques. At Iribov we develop markers, often in collaboration with costumers, with the use of *Genotyping-by-Sequencing (GBS)*. With this technique we can detect point-mutations throughout the population. By linking the phenotype with the mutations, we can link a specific mutation to a certain phenotype. Based on this mutation a marker can be developed for quick screening of the progeny.

Methodology - Markers for selection

To develop a marker, it is essential for the breeder to gather some material and data. First of all the breeder needs a source which contains the trait of interest (e.g. resistance or flower trait) of which the inheritance of the trait is known. Preferably the trait should follow the *3:1 ratio* correlated to a trait determined by a single gene. For traits based on more genes it will be more difficult to link with mutations.

When inheritance is known, the breeder should create a population from one cross which shows different phenotypes. The phenotyping must be reliable and consistent, otherwise there will be no good link with the genetics. Iribov can assist with phenotyping of traits such as a bioassay for resistance. Please contact us for more information.

Samples will be taken from the population for DNA extraction. The basic set-up uses up to 48 lines. Extracted DNA will be sent to an external company. They will digest the DNA, sequence small reads and analyse the point-mutations between the lines. It should be noted that the sequencing will not result in a full genome sequence.

We will further analyse the point-mutations detected and link them to the phenotype. If the phenotyping is consistent and inheritance is by a single locus, then there should be a clear link with between certain point-mutations and the phenotypic data. The linked mutations can be in the gene or close to the gene. It is possible that several mutations give a significant link to the data, these should be mapped close together on a chromosome. The significant mutations will be further developed into markers and validated on a broader progeny. It should also be tested if the markers work properly in all genetic backgrounds.

The research data will be compiled into a report for the costumer. The best marker will then go into production and can be used for breeding. Depending on the trait, the result of the marker for different lines will be reported as;

Table 1 | Reported marker output

	Explanation / verklaring
11	Homozygous resistant
12	Heterozygous
22	Homozygous susceptible
19	Homo or heterozygous resistant
99	Missing data or susceptible

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Methodology - Markers for (seed) purity

SNPs can also be used to distinguish varieties, which can be used as a quality control in seed production or vegetative propagation. To set up a quality control marker set it is important to use a wide range of genetic resources. This gives us a good idea of the mutations that provide most information to distinguish lines from each other. Based on the data, a subset of 20-30 SNPs will be converted to SNP markers. By analyzing multiple markers and linking the results (barcoding / haplotype), a system can be set up to distinguish varieties.

In Table 2, three cultivars are used as an example on which 5 SNP markers have been analyzed. As with a breeding marker, three results are possible (i.e. 11, 12, or 22). Table 2 shows that SNP1 has no distinctive information. SNP1 is therefore not usable to distinguish the varieties. Markers SNP2 - SNP5 can be used, because 1 or more lines give a different result. In this example, the combination of SNP2 and SNP3 contains enough information to distinguish the three cultivars. This distinctive information is called a haplotype.

Table 2 | SNP-barcoding (haplotype). Three cultivars with the result of five different SNP-loci.

	Cultivar 1	Cultivar 2	Cultivar 3
SNP1	11	11	11
SNP2	11	12	22
SNP3	11	22	22
SNP4	12	11	12
SNP5	22	11	11
Haplotype SNP2 + 3	22 - 11	12 - 22	22 - 22

To create a robust method, we advise to use varieties and parental lines to setup this system. In this way i twill be easier to add new varieties in the future. For some crops there is sequence- and/or SNP-data available in online databases. These data can be used to develop the variety identification system. The initial cost for GBS are not needed. It will however e needed to develop more SNP's at the start to distinguish all lines. Next to that, it might be more difficult to add new varieties to the system.

For more information about such a project or other questions, please contact our Analytical Lab research team at analysis@iribov.com

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