The next generation of molecular markers in plant science

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Introduction

A **molecular marker** is a DNA fragment originating from a certain position in the genome of an organism. Polymorphisms in molecular markers (insertions, deletions, Single Nucleotide Polymorphisms (SNPs)) can provide valuable information to distinguish individuals or species. During the last decade, **Next Generation Sequencing (NGS)** has caused a revolution in many disciplines of life sciences, leading to a new generation of molecular markers. At ILVO, we implemented one of these new fingerprinting techniques (**Genotyping-by-Sequencing or GBS**) in different ongoing research projects covering different disciplines of **plant science**, as illustrated below.

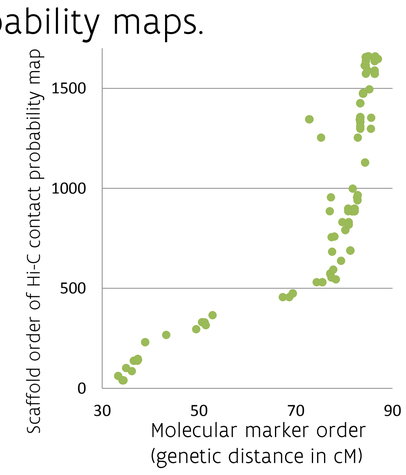
A high quality **genetic map** is an invaluable tool in **rose breeding**. We made an interspecific cross between the cultivar "Yesterday" and the wild species *Rosa wichurana* and we applied GBS to 180 individuals of the F1 progeny. Over 4500 high quality GBS SNP markers were selected to create a genetic map. This map can now be integrated with additional physical, cytogenetic and contact probability maps.

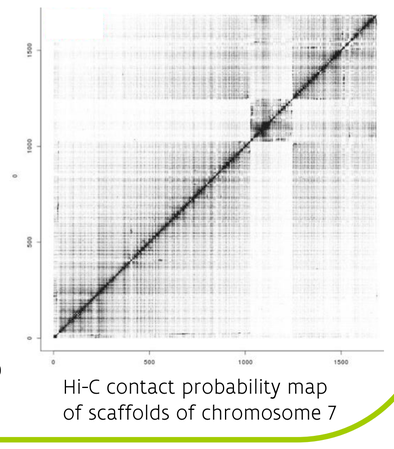
Yesterday × *R. wichurana*

interspecific cross

180 F1

Double-digest GBS resulting in >4500 high quality SNPs, 3300 of which can be positioned on scaffolds of a Hi-C contact probability map



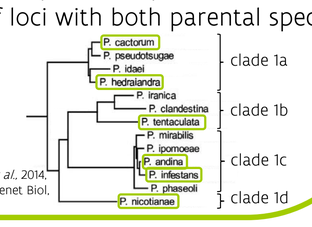


Hi-C contact probability map of scaffolds of chromosome 7

Oomycetes of the genus ***Phytophthora*** are important plant pathogens causing considerable yield losses in several crops as well as damage in natural forests. GBS-based markers are called in a reference-free approach and are used to identify *Phytophthora* **hybrid strains** (which may be more aggressive) and their potential ancestral species, based on **presence/absence** of genomic loci as well as **SNP** profiles.

	phylogenetic clade	*P. cactorum*	*P. hedraiandra*	*P. cactorum* x *P. hedraiandra*	*P. tentaculata*	*P. andina* (*P. infestans* hybrid)	*P. infestans*	*P. nicotianae*	*P. cactorum* x *P. nicotianae*
		1a	1a	1a	1b	1c	1c	1d	1d
P. cactorum	1a	18356	13888	17548	1671	1231	1128	1664	17517
P. hedraiandra	1a	13888	18609	18193	1692	1254	1148	1677	13809
P. cactorum x *P. hedraiandra*	1a	17548	18193	22225	1778	1313	1204	1761	17470
P. tentaculata	1b	1671	1692	1778	26926	1176	1092	1349	2328
P. andina	1c	1231	1254	1313	1176	24685	16786	1078	1805
P. infestans	1c	1128	1148	1204	1092	16786	20738	1007	1670
P. nicotianae	1d	1664	1677	1761	1349	1078	1007	16847	15306
P. cactorum x *P. nicotianae*	1d	17517	13809	17470	2328	1805	1670	15306	32363

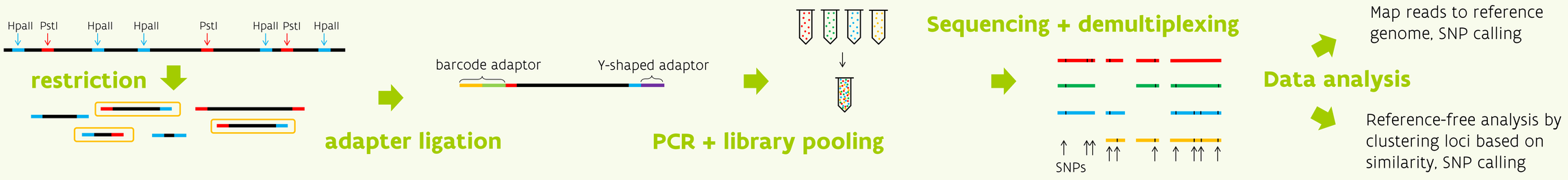
Similarity table showing the number of loci in common between strains of different *Phytophthora* species. Closely related species have many loci in common (ca. 80%). Hybrids typically have more loci than their putative parental species and share a lot of loci with both parental species.



Martin *et al.*, 2014. Fungal Genet Biol. 66, 19-32.

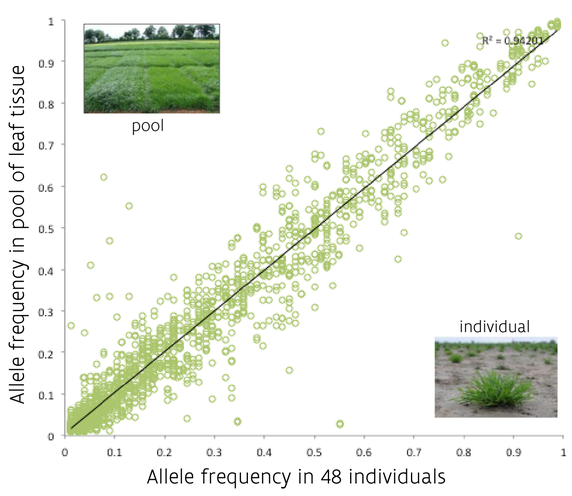
Genotyping-by-sequencing (GBS)

The principle of GBS is based on **restriction and amplification**, resulting in **genome-wide** molecular markers (Poland *et al.*, 2012, PLoS One, 7, e32253).

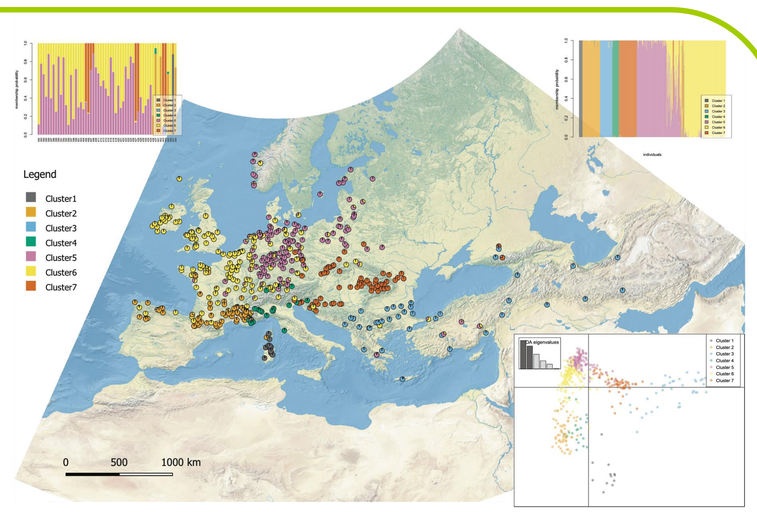


At ILVO, we choose to perform all steps (except the sequencing itself) **in-house**, to be able to **customize each protocol** (for DNA extraction, restriction enzyme(s), amplification cycles, etc.) **and each data analysis workflow** (reference based or without a reference) to the species of interest and relevant research questions.

Perennial ryegrass (*Lolium perenne*) is an important feed crop. It is an outbreeding species and hence genetically very diverse. We apply GBS on **pools** of individuals of perennial ryegrass for **allele frequency profiling**. We can use this data to study the temporal evolution of the genetic composition of a field under different circumstances: which alleles are lost over time and which alleles become more abundant in the population? We also apply this in **landscape genomics** where natural grass populations are sampled across Europe. This can provide insights in the **mechanisms underlying selection and adaptation** of a genotype or cultivar to specific environmental conditions.



Correlation of allele frequencies of ca. 50,000 SNPs in plants genotyped individually vs. in a pool



Landscape genomics in natural *L. perenne* accessions across Europe shows that genomic diversity is correlated with geographical gradients

Conclusion

GBS is a useful strategy to develop molecular markers of an unprecedented resolution. Because the technique is relatively cheap, it can be used on large numbers of individuals, maximizing the amount of gathered information. By adopting and applying GBS in different fields of plant sciences, we enable plant breeders, plant pathologists and evolutionary biologists to elevate their research to a new level.

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