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Genotype phasing and methylome profiling using >1kb reads in diverse barley populations with a single flow cell

Utilizing heterogeneous material (HM) instead of homozygote and uniform varieties can have substantial advantages when buffering unforeseeable weather conditions in a cropping season and adapting to local environments. Its increased diversity allows a more flexible adaptation to those varying scenarios. Besides the benefits of higher yield stability, maintaining or just describing an ever-changing population of HM by commonly applied methods is highly problematic. Farmers who used to buy uniform varieties with a precise description of their characteristics will raise doubts about the suitability of the OM for their purpose.

The FiBL plant breeding group is working on a method to describe the genotype composition of such HM populations using modern genomic approaches like Oxford Nanopore sequencing. The application of a long-read-ready sequencing method allows for phase and haplotype heterogeneous and heterozygote populations, even when multiple parents were used to establish the HM.

The first results indicate that a single MinIon flow cell can produce sufficient genomic coverage (3-4x) of the desired fragment length (avg. 4 kb) to extract the parental haplotypes from a pooled DNA sample of the entire population in a barley population. Combining a reference genome, SNP database, and Oxford Nanopore's latest Q20 improvements makes an accurate phasing possible.

This method has multiple application areas, like:

- Describing HM across generations and (in combination with phenotype information) allows us to predict the population's performance in different climatic scenarios
- Tracing beneficial alleles in complex crossing populations in a bulk segregant analysis
- Methylation profiling and mapping changes
- Monitoring the selection progress in breeding activities