

FLUO-TILL: A HIGH THROUGHPUT PLATFORM FOR TILLING BARLEY MUTANTS

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A large NaN₃-induced mutant population of barley (variety “Morex”) consisting of ca. 5,000 M₃ families has been developed at DiSTA-University of Bologna for identifying sets of induced point mutations in specific genes of agronomic interest using the TILLING (Targeting Induced Local Lesions IN Genomes, McCallum *et al.* 2000) procedure. Until now the mutant resource has been screened using Licor automatic sequencer detection, which, although efficient, is not amenable to very high-throughput and automated analyses.

The Parco Tecnologico Padano (PTP) and DiSTA have joined forces to increase the automation and throughput of the TILLING screening process. The entire workflow -DNA extraction, pooling, amplification, digestion and purification- has been automated using Tecan liquid handlers. The automated FLUO-TILL platform coupled with the use of the PGP-LIMS (Laboratory Information Management System) and of a barcode system ensures thorough quality controls and full traceability of each processed DNA sample. The final capillary electrophoresis step is performed using an Applied Biosystems' 3730 DNA Analyzer. The two primers used during the amplification step are both labelled using a different fluorophore, allowing to unambiguously distinguishing the two digestion fragments. In addition, a Perl-CGI script was developed, enabling mutant detection automatically.

Preliminary data confirm a significant increase in sensitivity and accuracy of our approach compared to the Licor detection system. The digestion fragment peaks can be precisely sized and clearly distinguished from the background peaks ensuring a highly efficient TILLING screening procedure.

A web-based interface for easy external access to the mutant resource and submission requests of TILLING screening projects is being implemented.