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Fragmentation of pooled PCR products for deep amplicon sequencing

Improvements to massively parallel sequencing have allowed the routine recovery of natural and induced variations in genomic DNA sequence. Thus, such approaches can be considered for routine application in plant mutation breeding projects. The need for high sequence coverage to accurately recover single nucleotide variants and small insertions and deletions limits the applicability of whole genome approaches. This is especially true for the many important crops with a large genome size or for applications requiring the screening of thousands of individuals, such as the reverse-genetic technique known as TILLING. Using PCR to target and sequence chosen genomic regions provides an attractive alternative as the vast reduction in interrogated bases means that sample size can be dramatically increased while maintaining suitable coverage for recovery of small mutations. Direct sequencing of PCR products is limited, however, due to limitations in read lengths of many next generation sequencers. Here we show the use of ultrasonication for the simultaneous fragmentation of 32 PCR products produced from large pools of barley mutant lines. Analysis of these fragmented PCR products using Illumina 2x300PE sequencing showed consistently high coverage and quality across the amplicons suitable for single nucleotide variant (SNV) calling.

Country or International Organization

PBGL

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