

## GeneThresher<sup>®</sup> Methylation Filtering Technology – A Path to Rapid Gene and Marker Discovery in Plants

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**Abstract:** The genomes of many plants are known to be composed of a large fraction of repetitive DNA, while a small portion is dedicated to genes. The bulk of the repetitive DNA constitutes transposable elements and is heavily methylated. GeneThresher<sup>®</sup> methylation filtering technology takes advantage of these differential methylation patterns by filtering genomic shotgun libraries to exclude methylated sequences. The result is a gene-enriched library. Random shotgun sequencing of plant gene space, enabled by GeneThresher technology, is a rapid and cost-effective strategy for comprehensive gene discovery in crops. We have applied GeneThresher to plants that span the major branches of the plant kingdom including species representing monocots, dicots, gymnosperms, and non-vascular plants with a last common ancestor estimated at 500 million years ago. Gene enrichment was achieved in all plants tested suggesting that GeneThresher will be effective across the whole plant kingdom. GeneThresher subclone libraries appear to be a random unbiased representation of the gene set and represent the 5', internal, and 3' portions of genes with equal frequency. Exons, introns, promoters, non-coding RNAs, and simple sequence repeats are preferentially represented while representation of interspersed repeats is minimized in the GeneThresher libraries. Using GeneThresher methylation filtering technology, we have tagged more than three quarters of the *Sorghum* gene set after only 0.3 × coverage of the *Sorghum bicolor* genome. DNA sequence obtained from these libraries provides a robust view of the functional parts of the genome and enables the design of DNA microarrays that can rapidly catalog complete gene sets of large plant genomes. In addition, GeneThresher data can help in developing research tools that achieve more rapid and precise plant improvements.