

DEVELOPMENT AND APPLICATION OF SINGLE PRIMER ENRICHMENT TECHNOLOGY (SPET) SNP ASSAY FOR POPULATION GENOMICS ANALYSIS AND CANDIDATE GENE DISCOVERY IN LETTUCE

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Cultivated lettuce (*Lactuca sativa* L.) is a main leafy vegetable crop widely appreciated by consumers for the content of fibres and the low-calorie intake being furthermore a rich source of vitamin C, iron, folate as well as different health-beneficial bioactive compounds. Recent years witnessed astonishing advancements in the development of cutting-edge technologies for next-generation sequencing opening new frontiers for dissecting the genetic basis of crops and discovery novel genes of interest. Among these, SPET (single primer enrichment technology) is a novel high-throughput genotyping method based on short-read sequencing of specific genomic regions harboring polymorphisms. SPET provides an

efficient and reproducible method for genotyping target loci, overcoming the limits associated with other reduced representation library sequencing methods that are based on a random sampling of genomic loci. The possibility to sequence regions surrounding a target SNP allows the discovery of thousands of closely linked, novel SNPs. Here we report the design and application of the first SPET panel in lettuce, consisting of 41,547 probes spanning the whole genome and designed to target both coding (~96%) and intergenic (~4%) regions. A total of 81,531 SNPs were surveyed in 160 lettuce accessions originating from a total of 10 countries in Europe, America and Asia and representing ten different horticultural types. Model ancestry population structure clearly separated the cultivated accessions (*Lactuca sativa*) from accessions of its presumed wild progenitor (*L. serriola*), revealing a total of six genetic subgroups, that reflected a differentiation based on cultivar typology. Phylogenetic relationships and principal component analysis revealed a clustering of butterhead types and a general differentiation between germplasm originating from Western and Eastern Europe. To determine the potentiality of SPET for gene discovery, we performed genome wide association analysis for main agricultural traits in *L. sativa* using eight models (GLM naive, GLM-Q, GLM-PCA, MLM, MLMM, CMLM, FarmCPU and BLINK) to compare their strength and power for association detection. Robust associations were detected for seeds colour on chromosome 7 at 50 Mbp. Colocalization of association signals was found for outer leaf colour and leaf anthocyanin content on the bottom chromosome 9 at 152 Mbp and on chromosome 5 at 96 Mbp. The association for bolting time was detected with the BLINK, farm CPU and MLM model on chromosome 7 at 164 Mbp. Detected associations fall in chromosomal regions previously reported to hold candidate genes of interest for these traits, thus confirming the effectiveness for GWAS. Our research demonstrates the reliability of SPET for detecting thousands of variable sites that help to dissect the genetic diversity of germplasm collections and to identify candidate genes for traits of agricultural interest, enabling a better use of lettuce germplasm.