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A NEW APPROACH TO GENOTYPING: SINGLE PRIMER ENRICHMENT TECHNOLOGY (SPET), AN INTEGRATED SYSTEM FOR BOTH TARGETED AND *DE NOVO* GENOTYPING

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Genotyping-by-sequencing (GBS) is a robust approach for enabling large scale, whole genome studies of genetic variation, such as genome wide association studies (GWAS).

In this study, two replicates of five *Zea mays* inbred lines (F7, H99, HP301, Mo17 and W153R) and five F1 crosses (A632 x B73, B73 x B96, B73 x F7, B73 x Mo17, W153 x HP301) were genotyped using the Single Primer Enrichment Technology (SPET). This technique combines the high-throughput of classic GBS techniques, such as the RAD method, and the targeting of desired loci throughout a scalable probe design.

We designed 55,198 probes based on the Illumina MaizeSNP50 genotyping array, obtained from 529 lines of a MAGIC maize population and their inbred founder lines. Genotypes called by the SNP array were used as a gold standard to measure the accuracy of SPET genotyping in 27,236 polymorphic target positions in three inbred lines and in the five F1 crosses.

The accuracy of SPET was 98.2% at a coverage of 20x and reached 98.5% considering only positions targeted by two probes. The reproducibility of SPET, obtained comparing the genotype calls for the two replicates, ranged from 97.87 to 99.73%. We observed an increase of both accuracy and reproducibility by increasing the minimum required coverage, with a plateau at about 50x. The accuracy of SPET genotyping was higher in homozygous positions with respect to heterozygous ones. Our results validated a high fraction of SNPs previously found using the SNP array.

By analyzing the whole region captured by the probes designed to target the set of polymorphic loci of the current study, SPET enabled the genotyping of 49,443 additional polymorphic sites in at least one sample of the cohort.

Our experiment showed that SPET represents a powerful tool to perform both targeted and *de novo* genotyping. SPET is a cost-effective alternative to SNP array to conduct genotyping experiments in large number of samples and it allows circumventing the ascertainment bias that is a limitation of the former technology.