

Pepper Consortium Array

Delivering genotyping tools to advance pepper breeding programs.

The Pepper Consortium Array is designed to enable cost-effective genotyping of multiple pepper species. Developed by a consortium formed by TraitGenetics GmbH, the University of California, Davis (UC Davis), and Illumina, the array contains expertly selected content derived from high-quality sequencing data.

How many single nucleotide polymorphisms (SNPs) are on the array?

The array contains 16,405 SNPs.

What methods did the consortium use to discover and select SNP content for the array?

The consortium derived the content for the array by sequencing 22 pepper lines and then filtering SNPs for quality. The following sections detail the methods.

Sequencing

Allen Van Deynze, Ph.D. (UC Davis) and Doil Choi, Ph.D. (Seoul National University) prepared and sequenced genomic libraries for all 22 pepper lines, including the public lines Maor, Early Jalapeño, Dempsey, and Perennial. Libraries were sequenced during a 2 × 100 bp run using 2 flow cell lanes on HiSeq® 2000 and HiSeq 2500 Systems to 19–30× coverage.

Read Mapping

After trimming the sequencing reads to 82 bp, Hamid Ashrafi, Ph.D. (UC Davis) mapped the reads to the publicly available pepper genome sequence (v1.5)² using CLC bio software.

SNP Calling and Filtering

SNPs were called using SAMtools and filtered using proprietary Perl scripts. Called genotypes were filtered to include SNPs for which at least 25% of the pepper lines were homozygous, with a minimum allele depth of 3 for each genotype. As a result, the minimum depth is 6 (3×2 samples) for a minor allele. When a base pair position met these criteria, the genotypes of the remaining lines were called regardless of the depth of coverage in those lines. If a sample did not cover a given position, the position was not counted toward the total number of genotypes. SNPs were filtered further to be \geq 50 bp from adjacent SNPs or predicted introns. The depth of coverage is reported for all lines.

SNP Annotation and Assignment of Chromosomal Locations

SNPs occurring between start and end positions of an annotated gene (Pepper.v.15.Assigned.Chr.gff3.gz and Pepper.v.1.5.total.gff3.gz)² were annotated accordingly. Afterwards, the 50 bases on both sides of each SNP were extracted from pepper genome FASTA files.

Filtering and Designing SNPs for Infinium® Assays

To choose SNPs for the array, Martin Ganal, Ph.D. (TraitGenetics) and Dr. Ashrafi selected only annotated SNPs within genes and assigned them to 3 categories. Category I is composed of SNPs that are polymorphic in sweet blocky peppers and hot peppers. Category II includes SNPs that are polymorphic in blocky peppers only. Category III contains SNPs that are polymorphic in hot peppers only. Within each category, 1 SNP was selected per gene and filtered against transposable elements, as well as A/T and C/G SNPs that would occupy 2 locations on the array. Finally, the filtered SNPs were searched against the Basic Local Alignment Search Tool (BLAST) to remove duplications. As a result, 14,708, 3,192, and 3,934 SNPs were selected from category I, category II, and category III, respectively. The final lists of SNPs were submitted to Illumina to determine whether a given SNP met the minimum requirements for primer design. From this list, 19,000 high-quality SNPs were selected, and 16,405 of these SNPs were included on the array.

What reagents and other consumables will I need?

You will receive all necessary reagents to run the arrays included in your order. The assay requires at least 200 ng of DNA at a minimum concentration of 50 ng/µl as measured by fluorescence quantification. You will also need access to an Illumina BeadArray[™] Reader or iScan® System to process the array. Illumina recommends emailing TraitGenetics (contact@traitgenetics.de) with analysis questions, as TraitGenetics will analyze the first set of arrays on pepper germplasm and generate genetic maps.

What is the minimum number of samples that I can order?

Illumina recommends a minimum order of 288 samples.

Will UC Davis or TraitGenetics provide a cluster file for faster genotype calling with this array?

Yes, a cluster file for the array will be provided as soon as possible after the array is available.

How do I order the array?

Email consortiamanager@illumina.com and an Illumina representative will contact you to answer any questions and help you place your order.

References

- 1. Ashrafi H, Hill T, Stoffel K, et al. *De novo* assembly of the pepper transcriptome (*Capsicum annuum*): a benchmark for *in silico* discovery of SNPs, SSRs and candidate genes. *BMC Genomics*. 2012;13:571.
- 2. Pepper Genome Platform (passport.pepper.snu.ac.kr/?t=PGP) Accessed 15 December 2014.





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