illumina®

MaizeLD BeadChip Kit

Cost-effective and scalable content for EDV assessment and other maize breeding applications.

Highlights

- **Comprehensive Solution for EDV Testing**
  High-discrimination coverage with 3047 evenly spaced markers designed for EDV assessment
- **Add-On Content for Multiple Applications**
  Customizable to support varietal identification, genetic purity testing, marker-assisted selection, and other applications
- **Affordable, Standard Platform**
  Single platform for various maize applications reduces costs and eliminates manual processing errors
- **Unrivaled Call Rates and Accuracy**
  > 99% average call rates and > 99.9% reproducibility

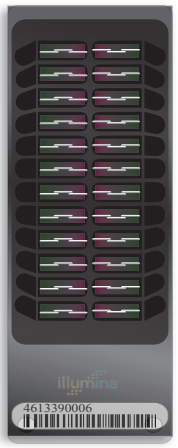

Figure 1: MaizeLD BeadChip—Designed for EDV assessment, the MaizeLD BeadChip features 3047 evenly spaced SNPs.

Introduction

Maize (*Zea mays* L. ssp. *mays*) was domesticated from the grass teosinte in Central America[1] over the last ~10,000 years, resulting in dramatic expansion of the maize genome through proliferation of long terminal repeat retrotransposons.[2] It is an important model organism for fundamental research into gene inheritance and function,[3] and a staple crop.

In 2009, researchers completed an improved draft sequence of the 2.3-gigabase genome of the B73 maize line.[4] This assembly was used to compare sequence data of other key maize lines. From these efforts, over 1 million SNPs have been identified.[5]

Maize exhibits high levels of both phenotypic and genetic diversity and has been subject to high selective breeding pressure for yield improvement, particularly among large companies supporting growers with regionally appropriate seed. To create standards for recognizing varietal development efforts, the International Union for the Protection of New Varieties of Plants outlined definitions for essentially derived varieties (EDV) in 1991.[6] EDVs are obtained, either directly or indirectly, from an initial variety (by selection of a natural or induced mutant), a somaclonal variant, a selection of variant plants from the initial variety, backcrossing, or transformation by genetic engineering. If they fulfill these conditions, EDVs are eligible for plant breeders' rights.

Under the guidance of the International Seed Federation, the American Seed Trade Association and the French Breeders Association identified SNPs that are equivalent to the simple sequence repeat (SSR) markers traditionally used to determine EDV status.[7] To provide the seed industry with a standard tool for EDV assessment, the MaizeLD BeadChip (Figure 1) contains 3047 internationally recognized EDV markers based on genetic and physical maps. This array also offers the capability to add custom markers, enabling other maize breeding applications to be run on the same platform.

SNP Selection Strategy

SNP selection was based on a comparison of pairwise distance data for inbred maize lines generated using SNP markers from the MaizeSNP50 BeadChip and SSR markers previously shown to discern EDV status.[7] In a collaboration between Illumina, Syngenta, and Pioneer, the markers were initially selected based on quality assurance analysis criteria from genotyping of US and European public and commercially relevant samples. SNP data were eliminated from subsequent analyses if they exceeded the following thresholds:

- > 10% of missing SNPs per genotype
- > 10% of missing genotypes per SNP
- > 5% heterozygous SNPs per genotype
- > 10% heterozygous genotypes per SNP

After discarding 43 SNPs with insufficient data quality or vulnerability to ascertainment bias, final comparisons were made using 26,874 SNPs. Also, 3047 SNPs were selected to provide even genomic coverage to facilitate SNP-based breeding applications.

Comprehensive Solution for EDV Testing

The MaizeLD BeadChip offers a comprehensive genotyping solution for EDV testing in maize, providing the high discrimination necessary to assess EDV status. Compared to traditional SSR markers, SNP genotyping offers higher throughput, resolution, and reproducibility, resulting in high accuracy. Many agricultural researchers today are transitioning from SSR analysis to SNP genotyping. With the MaizeLD BeadChip, researchers can assess all EDV markers at the same time across many samples rather than running separate assays for each gene of interest.

For Research Use Only. Not for use in diagnostic procedures.

AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
TCAACGTACCGTAACGAACGTATCATTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTT
CGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACC
AACGTACCATTAAGAGCTACCGTGCAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAA
AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
GATTACTTGATCCACTGATTCAACGTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAGCAACGAGC
CGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGAAAAGAATGATAACAGTA

High-Quality Data

The proven Infinium® HD Assay powers the MaizeLD BeadChip, providing the highest call rates in the industry. SNPs on the MaizeLD BeadChip were subjected to rigorous functional testing across multiple lines to ensure strong performance (Table 1). Samples used for marker validation include European and North American mapping populations, and the Plant Variety Protection Act panel used to establish the EDV marker set.[8] Validation of the MaizeLD BeadChip demonstrated a call rate of 99.4% across 280 samples. Reproducibility and Mendelian consistencies were 99.9%.

Table 1: MaizeLD BeadChip Performance Data and Specifications

Parameter	Percent	Product Specification
Average Call Rate	99.4%	> 99%
Reproducibility	99.9%	> 99.9%
Mendelian Consistencies	99.9%	< 0.1%

A standard cluster file is provided with the product to use as a starting point for calling genotypes. Given the complexity and diversity of maize lines, it is important to evaluate cluster positions for each data set. It is a best practice to include parental control inbred lines and their crosses for reference.

Flexibility for Multiple Applications

The MaizeLD BeadChip provides an ideal foundation for maize breeding applications, with the ability to impute additional SNPs for custom projects. Researchers can add up to 70,000 custom markers onto the MaizeLD BeadChip to support marker-assisted selection, varietal identification, genetic purity assessment, and other applications. By adding markers of interest to the base SNP content, researchers can perform all maize genotyping applications on the same platform, reducing costs.

Affordable, Standard Platform

With a focused set of SNP content containing all relevant markers, the MaizeLD BeadChip minimizes per-sample costs for EDV testing. Focused analysis produces a smaller data set compared to broader approaches, enabling seed industry researchers to make accurate EDV decisions with minimal amounts of data.

Complete Genotyping Solution

The MaizeLD BeadChip is compatible with the iScan® System. This array scanner features high-performance lasers and powerful optics systems that enable rapid scan times and precise assay detection. In addition, optional automation and the Laboratory Information Management System (LIMS) lower costs by eliminating errors associated with manual processing. Illumina genotyping products can be accessed via Illumina FastTrack™ Genotyping Services or any Illumina CSPro® Service.

Summary

Developed through a collaboration between Illumina scientists and the seed industry, the MaizeLD BeadChip features 3047 evenly spaced SNPs for comprehensive EDV assessment. In addition, this 24-sample BeadChip has the capacity to add thousands of custom markers, presenting a standard platform for multiple maize breeding applications.

Ordering Information

Product	Catalog No.
MaizeLD BeadChip Kit (48 samples) Each package contains 2 BeadChips and reagents for processing 48 samples.	WG-455-1001
MaizeLD BeadChip Kit (288 samples) Each package contains 12 BeadChips and reagents for processing 288 samples.	WG-455-1002
MaizeLD BeadChip Kit (1152 samples) Each package contains 48 BeadChips and reagents for processing 1152 samples.	WG-455-1003
MaizeLD+ BeadChip Kit (48 samples) Each package contains 2 BeadChips with custom add-on content and reagents for processing 48 samples.	WG-455-1011
MaizeLD+ BeadChip Kit (288 samples) Each package contains 12 BeadChips with custom add-on content and reagents for processing 288 samples.	WG-455-1012
MaizeLD+ BeadChip Kit (1152 samples) Each package contains 48 BeadChips with custom add-on content and reagents for processing 1152 samples.	WG-455-1013

Each MaizeLD BeadChip can process 24 samples in parallel and assay 3047 markers per sample.

References

1. Doebley JF, Gaut BS, Smith BD. The molecular genetics of crop domestication. *Cell.* 2006;127:1309-1321.
2. SanMiguel P, Gaut BS, Tikhonov A, Nakajima Y, Bennetzen JL. The paleontology of intergene retrotransposons of maize. *Nat Genet.* 1998;20:43-54.
3. Bennetzen JL, Hake S. Handbook of Maize: Genetics and Genomics (Springer, New York, 2009).
4. Schnable PS, Ware D, Fulton RS, et al. The B73 maize genome: complexity, diversity, and dynamics. *Science.* 2009;326:1112-1115.
5. Gore MA, Chia JM, Elshire RJ, et al. A first-generation haplotype map of maize. *Science.* 2009;326:1115-1117.
6. International Union for the Protection of New Varieties of Plants (2009) Explanatory Notes on Essentially Derived Varieties Under the 1991 Act of the UPOV Convention. (www.upov.int/edocs/expndocs/en/upov_exn_edv_1.pdf) Accessed 05 May 2015.
7. Rousselle Y, Jones E, Charcosset A, et al. Study on essential derivation in maize: III. Selection and evaluation of a panel of single nucleotide polymorphism loci for use in European and North American germplasm. *Crop Sci.* 2015;55:1170-1180.
8. Kahler AL, Kahler JL, Thompson SA, et al. North American study on essential derivation in maize: II. Selection and evaluation of a panel of simple sequence repeat loci. *Crop Sci.* 2010;50:486-503.

Illumina • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

For Research Use Only. Not for use in diagnostic procedures.

© 2015 Illumina, Inc. All rights reserved. Illumina, CSPro, FastTrack, Infinium, iScan, and the pumpkin orange color are trademarks of Illumina, Inc. and/or its affiliate(s) in the U.S. and/or other countries. Pub. No. 1370-2015-007 Current as of 25 June 2015

illumina®

AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
TCAACGTACCGTAACGAACGTATCATTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTT
CGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACC
AACGTACCATTAAGAGCTACCGTGCAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAA
AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
GATTACTTGATCCACTGATTCAACGTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAGCAACGAGC
CGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGAAAAGAATGATAACAGTA