GoldenGate® Genotyping with VeraCode® Technology

Custom 48-, 96-, 144-, 192-, and 384-plex SNP genotyping assays for high-throughput screening, biomarker validation, and routine testing at the low- to mid-plex level.

INTRODUCTION

Combining the proven GoldenGate Genotyping Assay with VeraCode technology provides one of the most robust and flexible platforms for SNP genotyping in the industry. Users benefit from the GoldenGate Assay's accuracy, sensitivity, and high signal-to-noise ratio. Coupled with the unique VeraCode technology, this assay provides superior data quality and high sample throughput at low per-sample costs.

The ease of the assay's workflow and the solution-based kinetics of VeraCode technology allow users to rapidly and easily interrogate 48, 96, 144, 192, and 384 SNP loci within a single well of a standard microplate. This platform delivers consistent performance, supports flexible assay content, and provides fast data turnaround, making it ideally suited for biomarker validation, studies involving large volumes of samples, and routine testing.

PROVEN GOLDENGATE GENOTYPING ASSAY

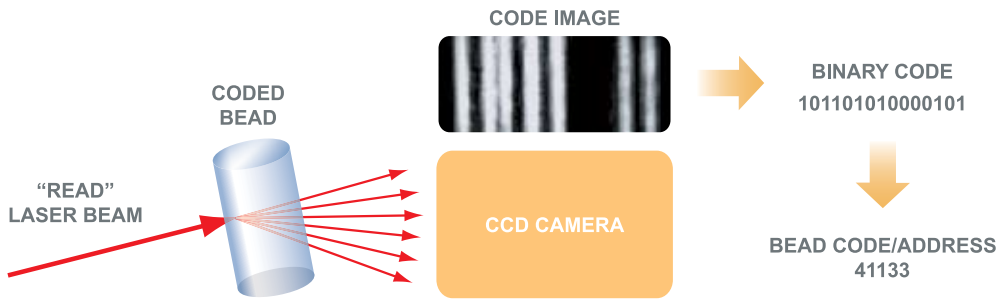
Illumina's GoldenGate Assay[1] has been shown over the years to be a highly robust SNP genotyping assay. Notably, it was used to generate approximately 250 million genotypes for the International HapMap Project[2]. Adapted for VeraCode technology, this assay is a powerful solution for lower plex genotyping applications including high-throughput screening, pharmaceutical development, pre-clinical, and clinical research[3–7].

ASSAY OVERVIEW

The first step in the GoldenGate Assay is DNA activation, which enables genomic DNA samples to bind to paramagnetic particles *(Figure 2)*. This activation process is highly robust and requires as little as 250 ng of genomic DNA. Assay oligonucleotides, hybridization buffer, and paramagnetic particles are then combined with the activated DNA in the hybridization step.

Three oligonucleotides are designed for each SNP locus. For each SNP site there are two allele-specific oligos (ASO). A third oligo, the locus-specific oligo (LSO), hybridizes several bases downstream from the SNP site.

FIGURE 1: THE VERACODE BEAD INSCRIBING PROCESS



The VeraCode digital code is formed using a holographic inscribing process. The holographic image in each bead diffracts a laser beam into multiple components. These components make up the optical signature of the bead code, which the BeadXpress Reader analyzes.

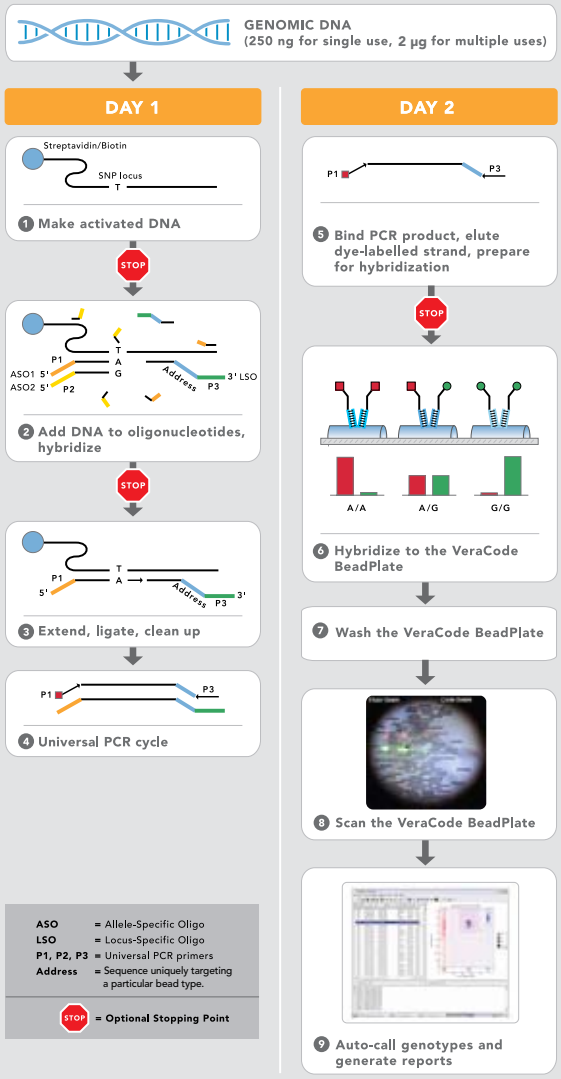
All three oligonucleotide sequences contain universal PCR primer sites; the LSO contains a unique address sequence that targets a particular VeraCode microbead type. During the primer hybridization process, the ASOs and LSOs hybridize to the genomic DNA sample bound to paramagnetic particles. Because hybridization occurs prior to any amplification steps, no amplification bias is introduced into the assay.

Following hybridization, several wash steps are performed, removing excess and mis-hybridized oligonucleotides. Extension of the appropriate ASO and ligation of the extended product to the LSO joins information about the genotype present at the SNP site to the address sequence on the LSO.

The ligation products serve as the PCR templates for universal PCR primers P1, P2, and P3. Primers P1 and P2 are Cy3- and Cy5-labeled, respectively. After downstream processing, the single-stranded, dye-labeled PCR products are hybridized to their complementary bead type through their unique address sequences. Hybridization of the GoldenGate Assay products onto the VeraCode beads separates the assay products for individual SNP genotype readout.

After hybridization, the BeadXpress® Reader is used for microbead code identification and fluorescent signal detection. During scanning, a laser beam penetrates the digitally inscribed VeraCode microbead to generate a unique code image, which allows for rapid and highly specific detection. Data generated using the BeadXpress Reader can be analyzed with Illumina's GenomeStudio™ data analysis software, which performs automated genotype clustering and calling.

FIGURE 2: THE GOLDENGATE ASSAY WITH VERACODE

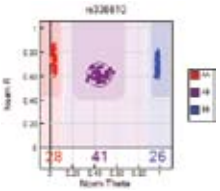


TYPICAL RESULTS

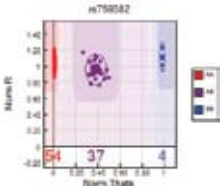
The GoldenGate Assay and the highly specific VeraCode technology exhibit superior consistency, reproducibility, and success rate. Results of laboratory testing of multiple plates at 96-plex and 384-plex scale are shown in Table 1.

TABLE 1: TYPICAL GOLDENGATE ASSAY PERFORMANCE

96-PLEX	
Call Rate	> 99.9%
Heritability	> 99.9%
DNA Success Rate	> 99%
Locus Success Rate	> 99%



384-PLEX	
Call Rate	> 99.9%
Reproducibility	> 99.9%
Heritability	> 99.9%
DNA Success Rate	> 99%
Locus Success Rate	> 98%



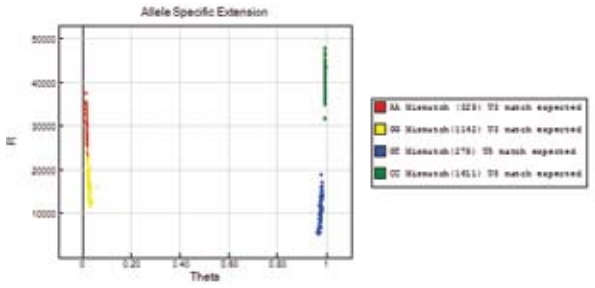
GoldenGate Assays at 48-, 144-,192-, and 384-plex meet the same performance specifications.

GOLDENGATE ASSAY CONTROLS

The GoldenGate Genotyping Assay includes 48 assay controls, lending a high level of confidence and the ability to troubleshoot errors such as PCR and hybridization failures *(Figure 3)*. VeraCode microbead digital coding serves a dual role of enabling built-in assay controls as well as internal tracking controls. Illumina's GenomeStudio data analysis software provides a dashboard for simple viewing of controls performance.

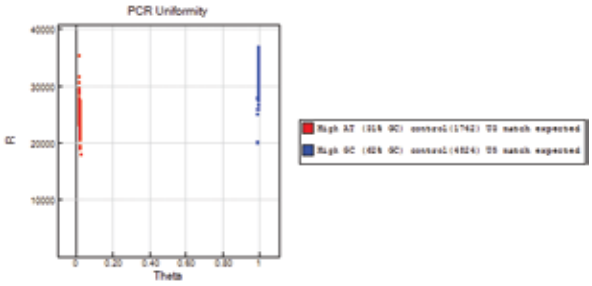
FIGURE 3: GOLDENGATE ASSAY CONTROLS DISPLAYED IN THE GENOMESTUDIO GENOTYPING MODULE CONTROLS DASHBOARD

ALLELE-SPECIFIC EXTENSION



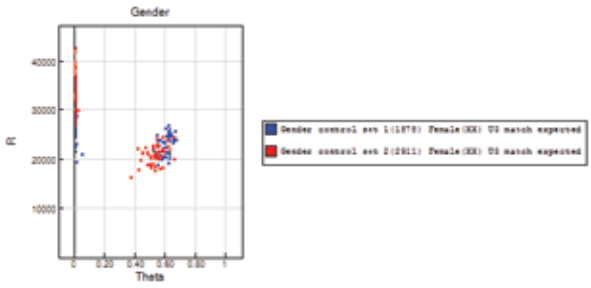
Tests extension efficiency of matched vs. mismatched ASOs

PCR UNIFORMITY



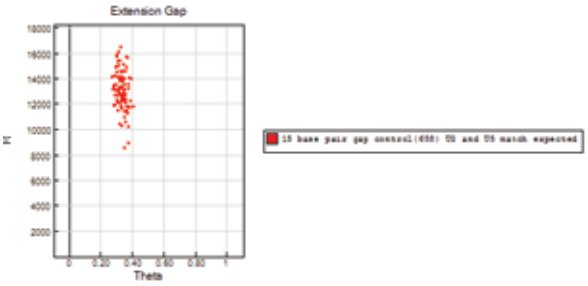
Tests PCR amplification efficiency

GENDER



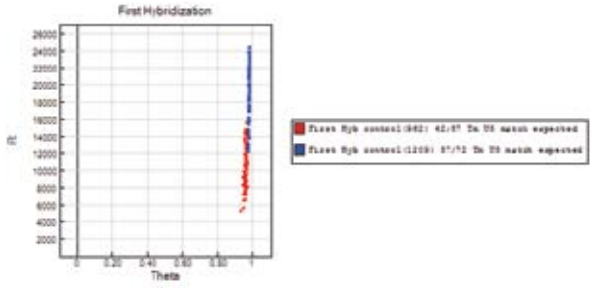
Performs sample sex verification

GAP



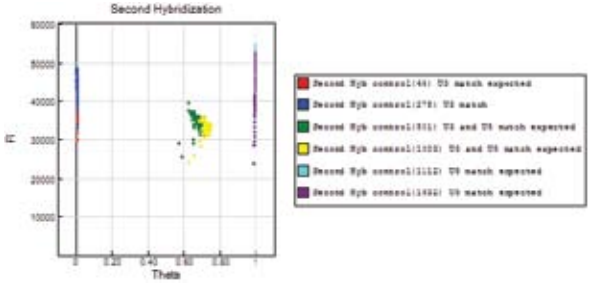
Tests the efficiency of gap extension

FIRST HYBRIDIZATION



Tests annealing specificity of ASOs

SECOND HYBRIDIZATION



Tests hybridization to array (sample independent)

REFERENCES

(1) Fan JB, Gunderson KL, Bibikova M, Yeakley JM, Chen J, et al. (2006) Illumina universal bead arrays. Methods Enzymol, 410: 57–73.

(2) The International HapMap Consortium. (2003) The International HapMap Project. Nature 426: 789-796.

(3) Lin CH, Yeakley JM, McDaniel TK, Shen R. (2009) Medium- to High-Throughput SNP Genotyping Using VeraCode Microbeads. Methods Mol Biol. 496:129–42.

(4) Kulathinal RJ, Bennett SM, Fitzpatrick CL, Noor MAF. (2008) Fine-scale mapping of recombination rate in Drosophila refines its correlation to diversity and divergence. PNAS 105(29): 10051–10056.

(5) Cooper GM, Johnson JA, Langaee TY, Feng H, Stanaway IB, et al. (2008) A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. Blood 112(4): 1022-7.

(6) Is Genetic Testing the Next Revolution in Agriculture? http://www.illumina.com/emailers/icommunitySep08/iC_Sep08_GeneticTesting.pdf.

(7) Using Genetics to Uncover Cat Secrets. http://www.illumina.com/emailers/icommunitySep08/iC_Sep08_CatSecrets.pdf.

ADDITIONAL INFORMATION

For more information about GoldenGate genotyping, VeraCode technology, and the BeadXpress System, visit www.illumina.com or contact us at the address below.

Illumina, Inc.
Customer Solutions
9885 Towne Centre Drive
San Diego, CA 92121-1975
1.800.809.4566 (toll free)
1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com

FOR RESEARCH USE ONLY

Pub. No. 170-2007-001 Current as of 10 December 2008