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GoldenGate® Indexing Assay Increases Sample Throughput

By combining sample indexing within the robust GoldenGate Assay, automation capabilities, and positive sample tracking with LIMS support, GoldenGate Indexing provides the highest level of throughput at the most affordable cost for low- to mid-plex custom genotyping screening.

Highlights

- **Highest Throughput:**
  Greater than 2,000 samples per day
- **Highly Flexible:**
  Advanced multiplexing enables analysis of 96, 192, or 384 loci per sample
- **Fully Integrated:**
  Automated platform incorporating LIMS
- **Proven Technology:**
  Robust assay used in genotyping centers worldwide with average call rates > 99%

Introduction

The GoldenGate Genotyping Assay is a highly successful genotyping technology proven in labs worldwide. In fact, it was used to make major contributions in the HapMap Project. Building on this strong foundation, the GoldenGate Indexing Assay allows researchers to pool multiple samples, increasing the number of samples that can be analyzed in a single run. With advanced automation and updates to Illumina LIMS (Laboratory Information Management System) to accommodate this new step, along with positive sample tracking, researchers now have the ability to screen up to 16 times as many samples per reaction as they could with the standard GoldenGate Assay. This dramatically increases throughput from 288 samples per day to greater than 2,000. Overall, researchers will realize a significant decrease in cost while maximizing throughput for low-complexity sample screening.

How GoldenGate Indexing works

GoldenGate Indexing, based on Illumina's BeadArray™ technology, maximizes the throughput of the original GoldenGate Assay (Figures 1 and 2). BeadArray technology uses illumiCodes, unique 23-bp single-stranded DNA oligos, to correctly identify each DNA sample as well as the loci being interrogated. Because each illumiCode is distinctive, multiplexing is possible. Current plexity ranges for GoldenGate Indexing include 96-plex, 192-plex, and 384-plex.

IllumiCodes Enable Pooling

During sample preparation, primers containing illumiCodes and universal primer sites are hybridized to the DNA. Individual samples can be processed using oligonucleotide assay pools containing non-overlapping illumiCodes. This enables pooling of multiple samples into a single well. Since the illumiCodes are discreet within the well, each sample can be independently examined during downstream analysis.

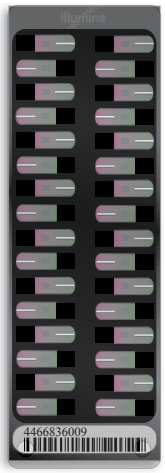
Amplification and Signal Reading

Prepared samples are amplified using universal PCR primers labeled with Cy3 and Cy5 fluorescent dyes. The resulting fluorescently labeled PCR products are hybridized to a Universal BeadChip. The BeadChip contains randomly assembled universal beads, each displaying an illumiCode corresponding to a specific loci. DNA will bind to the bead containing the complementary illumiCode. Unbound DNA is removed and the remaining fluorescence signal levels read on the iScan system or BeadArray Reader for individual SNP genotype readout. This information is then analyzed for automated genotype clustering and calling. The entire assay can be completed in as few as three days.

Illumina LIMS and Automation Control

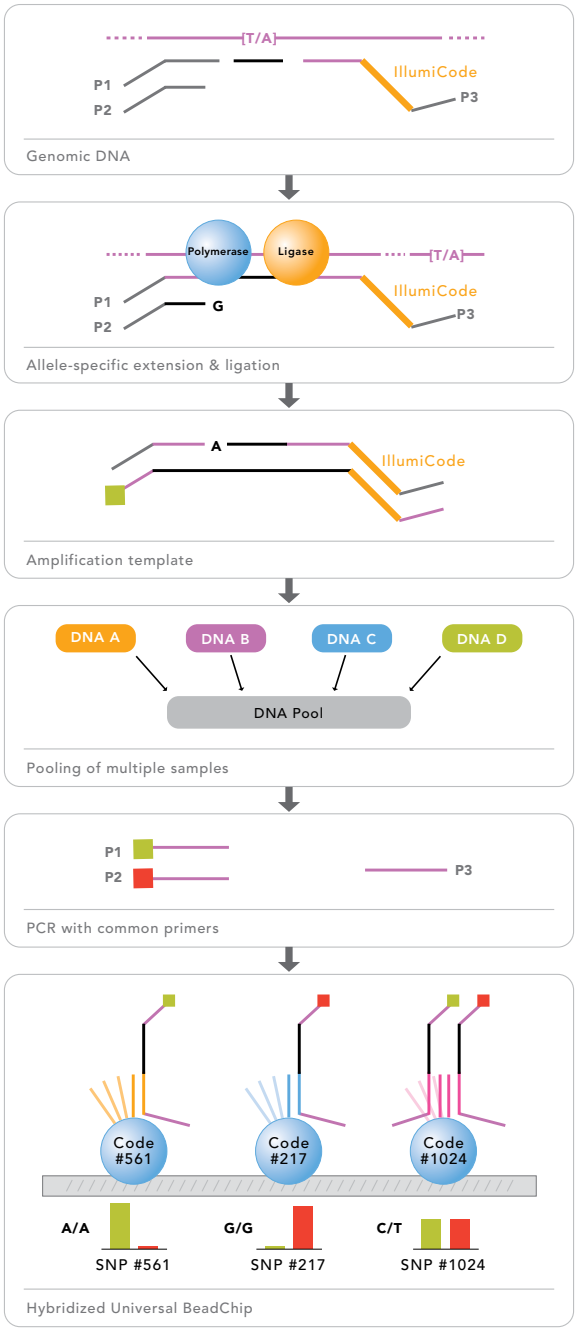
The GoldenGate Indexing assay is highly automated, maximizing throughput. Robotic liquid handlers automatically process samples through each step of the assay, enabling the assay to run with minimal hands-on operation. Pre-amplification steps are performed via a larger 96-tip–based robot, and post-amplification steps via a standard 8-tip robot.

Figure 1: Universal-32 beadchip



GoldenGate Indexing Assay products are hybridized onto the Universal-32 BeadChip for individual SNP genotype readout.

Figure2: Goldengate Indexing Assay Workflow



In the GoldenGate Indexing assay, a unique set of illumiCodes is used to identify each sample, allowing multiple samples to be pooled prior to amplification. This, along with automation capabilities, greatly increases the assay throughput.

Illumina's integrated LIMS delivers state-of-the-art management and tracking to ensure the highest quality data, efficient data acquisition, and significant savings in time and lab resources. As a ready-to-use solution, Illumina LIMS includes the server hardware and software needed to accurately manage and enforce assay workflow. Illumina LIMS provides the excellent project management capabilities needed to effectively manage samples from receipt through analysis.

Positive sample tracking by Illumina LIMS is achieved by direct control of the automated liquid handling robots, ensuring samples are automatically processed and queued to the proper step and eliminating error due to manual mishandling. LIMS tracks time-stamped lab transactions with associated user information by offering user authentication either through Illumina LIMS or through existing Windows password authentication. In addition, LIMS uses a barcode system for accurate sample identification in downstream analysis. Illumina provides software updates to accommodate new product formats and workflows, saving the time and cost of in-house software development. By managing time-consuming and error-prone sample/data handling from beginning to end, the LIMS environment greatly increases confidence and efficiency in genotyping studies.

Reliable Analysis

GoldenGate Indexing Assay results can be analyzed in the genotyping module of GenomeStudio™ data analysis software. This module recognizes each illumiCode and displays individual genotyping data for the pooled samples. In addition, GenomeStudio software features the ability to normalize raw data and perform clustering and automated genotyping calling.

Data Quality

GoldenGate Indexing Assays produce the same high-quality data as the original GoldenGate Assay. This ensures that important SNPs are captured and a high call accuracy is achieved (Table 1).

Summary

GoldenGate Indexing provides a fully automated, affordable assay for high-throughput low- to mid-plex genotype screening. Using a proven assay, researchers can now screen thousands of samples in just a few days, while still obtaining the high-quality data they require.

Additional Information

To learn how you can access the power of the GoldenGate Indexing Assay, visit www.illumina.com or contact us at the address below.

Figure 1: Specifications for the Goldengate Indexing Assay

Parameter	Specification
Average Call Rate	> 99%
Reproducibility	> 99.9%
Mendialian Inconsistencies	< 0.1%

Ordering Information

Product	Plexity	No. of Samples Indexed	No. of Samples Processed per Kit	Catalog No.
GoldenGate Indexing Assay Kit, Custom	96	16	768	GT-222-1003
	192	8	768	GT-222-1004
	384	4	768	GT-222-1005

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