

Infinium® XT Production-Scale Genotyping Solution

The accuracy and robustness of proven Infinium chemistry taken to the next level of scalability.

Highlights

- **Production-Scale Genotyping**
New assay and a 96-sample BeadChip enable production-scale genotyping of 100,000 to > 1,000,000 samples/year
- **Flexible Content**
Targeted single or multispecies assays of 100s to 50,000 single nucleotide polymorphisms (SNPs) with at least 95% conversion guaranteed for custom panels
- **High Efficiency Workflow**
Reduced total assay turnaround time from three days to two days with user-friendly enhancements and less hands-on time



Introduction

The Infinium family of genotyping assays harnesses proven chemistry and a robust BeadChip platform to produce high data quality, call rates, and reproducibility. Infinium XT technology builds on this success by updating to a 96-sample BeadChip and optimizing the Infinium workflow to provide the highest throughput genotyping solution to date from Illumina. The product was developed for customers who require up to 50,000 SNPs to perform large-scale screening applications on any species with user-defined content. The Infinium XT solution optimizes the user experience as labs take production-scale genotyping studies to the next level of throughput. It simplifies the custom assay design process, reduces overall hands-on time, and enhances automation robot performance and utilization. The workflow now offers an option to reduce the overall turnaround time, if the time to answer is more important than maximum weekly output. Also, the Infinium XT solution introduces an enhanced data analysis software solution for real-time data generation and on-demand quality control (QC) report functionality so production issues can be identified and corrected earlier.

Production-Scale Genotyping Technology

The Infinium XT solution features a 96-sample BeadChip (Figure 1) for genotyping large numbers of samples efficiently, scaling from hundreds of thousands to more than one million samples per year. It supports as few as several hundred SNPs, providing a production-scale solution for targeted genotyping applications, including animal parentage, sample traceability, or sample QC. This product also supports up to 50,000 SNPs, which are required for key agricultural applications like genomic selection for both diploid and polyploid organisms.

Figure 1: The Infinium XT 96-Sample BeadChip—The Infinium XT BeadChip offers the proven power and flexibility of the Infinium assay in the highest throughput available from Illumina. It supports targeted panels of 100s to 50,000 SNPs of catalog or custom content.

Human applications, such as biobanking, population screening, and personalized medicine initiatives can also benefit from this technology. The low- to mid-range number of SNPs and low cost are ideal for labs that require simple QC checks on large sample sets or need to perform a more thorough population stratification before proceeding with deeper genomic testing. The versatility of the Infinium XT BeadChip makes it a cost-effective, reliable solution for sample QC, tracking, and validation throughout an entire workflow.

Flexible, Application-Specific Content

The Infinium XT BeadChip fully supports custom iSelect® arrays, consortia, or commercial product opportunities within human or agrigenomics applications that meet the content and throughput requirements ($\leq 50,000$ markers, $\geq 100,000$ samples per year). To aid in the design of custom content for Infinium XT BeadChips, the online DesignStudio™ Software now includes a custom genotyping microarray probe designer, which replaces the older Assay Design Tool (ADT) software. With DesignStudio Software, custom content can be developed and ordered through a guided, easy-to-use interface with a guarantee of at least 95% custom content conversion rate. This is a significant increase over the 80% conversion rate guarantee for standard Infinium BeadChips and was enabled to support screening many samples for a focused number of critical SNPs.

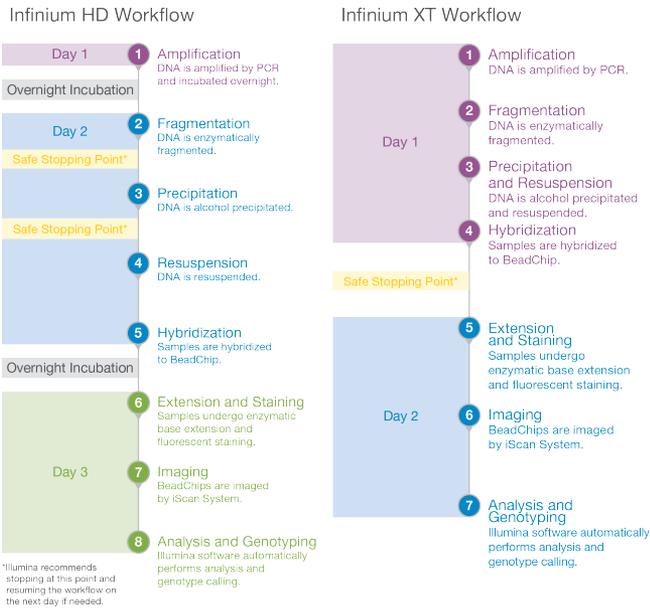


Figure 2: Infinium XT Workflow Improvements—Each step of the Infinium workflow is optimized in the Infinium XT workflow, reducing the turnaround time from three days to two days.

High-Efficiency Infinium Workflow

Each step of the fully automated Infinium HD workflow is optimized in the Infinium XT workflow, reducing the turnaround time (TAT) from three days to two days (Figure 2). On day one, DNA is amplified, enzymatically fragmented, precipitated, and resuspended. Samples are then hybridized to BeadChips during an overnight incubation, where DNA anneals to locus-specific 50-mer probes covalently linked to one of the Infinium bead types. On day two, the Infinium XT workflow continues with enzymatic base extension to confer allelic specificity, followed by fluorescent staining. The iScan® System detects the fluorescence intensities of the beads, and Illumina software automatically performs analysis and genotype calling. New efficiencies introduced to the Infinium workflow accommodate greater scalability with the Infinium XT BeadChip (Table 1).

- **DNA amplification:** increased batch size three-fold and reduced incubation time from overnight to three hours.
- **Enzymatic fragmentation:** decreased time by 50%.
- **Alcohol precipitation:** removed a 30-minute incubation step and reduced drying time by 75% (one hour to 15 minutes).
- **DNA resuspension:** reduced overall incubation time by 75% (one hour to 15 minutes).
- **BeadChip hybridization:** increased sample capacity three-fold using the same Tecan robotic system.
- **Sample extension/staining:** increased capacity two-fold; up to 48 BeadChips (4608 samples) per run.

Flexibility built into the Infinium XT workflow accommodates the needs of different users. Maximum weekly outputs can be balanced with TAT by adjusting workflow configurations that support alternating priorities. The Infinium XT assay maintains the input requirement of 200 ng of sample DNA with the same high-quality performance users have come to expect from Infinium technology.

Optimal Laboratory Layout

The Infinium XT BeadChip was optimized and designed for factory-scale laboratories to remove the complexities associated with process scaling and large sample number batching. Labs are encouraged to engage with Illumina for consultation about new efficiency gains. For example, a lab processing 1,000,000+ samples annually will need eight (8-tip) Tecan robots, three iScan Systems, two AutoLoader 2.x units, and ancillary lab equipment (Figure 3).

Updated Automation and LIMS

The ability to process an increasing number of samples efficiently requires scalable turnkey solutions. These include integrated systems that simplify sample preparation and provide the confidence to continue to scale operations. The Illumina Automation Control (IAC) software for the Tecan liquid handling robot has increased sample processing efficiency. This includes updates to Pre-PCR reagent transfer steps and doubling throughput capabilities at the X-stain task within the Infinium workflow.

The Illumina Laboratory Information Management System (LIMS) includes a redesigned database and updated server options using state-of-the-art hard drives to ensure maximum speed and performance during peak processing periods. Important features, such as the ability to create and run projects with multispecies BeadChip formats, improve efficiency.

Improved Data Analysis

With the high-throughput Infinium XT solution, there is a natural increase in data analysis. With this in mind, several improvements have been made to GenomeStudio® Software and Beeline™ Software.

GenomeStudio Software is the Illumina visualization and analysis program for microarray-based genotyping data. It provides a tabular view for quickly accessing all the data in an experiment and allows data to be exported for use by various third-party applications. The GenomeStudio Genotyping Module supports the analysis of Infinium array genotyping data with normalization, genotype calling, clustering, data intensity analysis, and more. In addition, GenomeStudio Software is necessary for creating and modifying clusters used for calling genotypes from scanned microarray signal intensities.

The updated GenomeStudio 2.0 Software speeds up genotype cluster generation, reducing the overall analysis turnaround time. Also, a new Polyploid Genotyping Module is now included,

appropriate for agricultural and other applications involving polyploid organisms (Figure 4). When used in tandem with Illumina LIMS, GenomeStudio Software provides an integrated experience to view and analyze data from processed samples in real time.

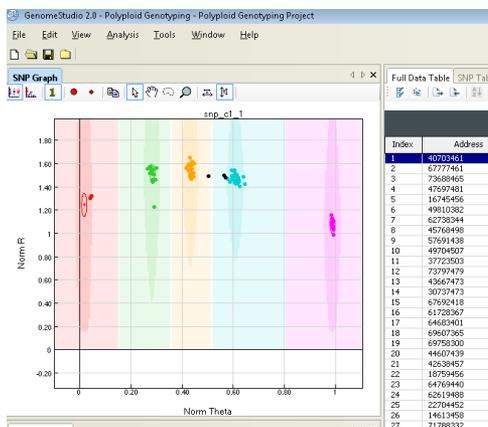


Figure 3: GenomeStudio 2.0 Software—GenomeStudio 2.0 Software includes a new Polyploid Genotyping Module to support applications involving polyploid organisms.

After a cluster file is created, production-level analysis can be transitioned to Beeline Software. Beeline Software is the Illumina microarray data analysis tool for prefiltering and reporting data from high-throughput processing in an automated fashion. This makes it ideal for routine use with Infinium XT data and beneficial for any large-scale Infinium genotyping projects. Beeline Software offers flexible filtering capabilities, reduces experimental array data size, and

identifies any samples or markers that do not meet user-defined performance specifications. Filtered data can also be imported directly into GenomeStudio Software for a more interactive analysis, if desired.

Beeline 2.0 Software optimizes its analysis and reporting capabilities for processing high-throughput genotyping studies. New features include QC on-demand, polyploid genotype calling, and faster genotype report generation. Transitioning from GenomeStudio Software to Beeline Software significantly reduces the time needed to observe quality performance specifications, generate genotyping reports, and analyze polyploid data without removing the flexibility for manual intervention.

Summary

The Infinium XT BeadChip and workflow provide a significant increase in sample throughput capabilities for microarray-based genotyping. This comprehensive solution enables large-scale genetic improvement programs in agrigenomics and supports large-scale screening for biobanks and personalized medicine initiatives. The Infinium XT workflow incorporates new design software, a four-fold increase in BeadChip capacity, a 33% reduction in sample turnaround time, increased conversion rate of custom content, multispecies design capabilities, and enhanced data analysis for both diploid and polyploid organisms. The high-throughput workflow combined with low cost per sample makes the Infinium XT solution the ideal choice for commercial genotyping labs that want to scale to a factory level of throughput and efficiency without sacrificing performance and reliability.



Figure 4: Example Lab Layout for Infinium XT Production-Scale Genotyping—An example lab layout is provided for processing 1,000,000+ samples per year and includes: three (8-tip) Tecan robots, three iScan Systems, two AutoLoader 2.x units, and ancillary lab equipment. This example layout requires approximately 1200 square feet. Layout is not to scale.

Table 1: Infinium Workflow Comparison

Workflow Step	Process Step	Infinium HD Workflow	Infinium XT High-Throughput Automated Workflow
Amplify DNA	Layering oil at first MA step	Present	Removed
	Incubation time	Overnight	Three hours
	Batch size	One plate (96 samples)	Three plates (288 samples)
Fragment DNA	Incubation time	One hour	30 minutes
	Batch size	Six plates (30 minutes) (576 samples)	Six plates (18 minutes) (576 samples)
Precipitate DNA	Vortex before adding 2-propanol	Included	Removed
	Incubation time	30 minutes	Removed
	Drying time	One hour	15 minutes
	Batch size	Six plates (75+ minutes) (576 samples)	Six plates (65 minutes) (576 samples)
Resuspend DNA	Incubation time	One hour	15 minutes
	Batch size	Six plates (90+ minutes) (576 samples)	Six plates (65 minutes) (576 samples)
Hybridize to BeadChip	Tip guides	Single tip guide	Three tip guides
	Hybridization chamber	Four BeadChips per chamber	Six BeadChips per chamber (new design)
	Batch size	288 samples	576 samples
Wash and Stain BeadChip	Minimum batch (no reagent waste)	Four BeadChips	24 BeadChips
	X-stain reagents	Tubes	Plate-based
	Glass back plates	Mylar spacers	Integrated spacers
	New assembly fixture	Four BeadChip capacity	Six BeadChip capacity
	Reagent used to prepare BeadChips for hybridization	Provided at 1x concentration	Provided at 20x concentration
	Batch size	24 BeadChips	48 BeadChips
Scan BeadChip	Scanners supported	HiScan® and iScan Systems	iScan System only
	SDF	HD	XT
	ICS	v 3.3.28	v 3.4
Software Updates	Tecan IAC	v 5.2.0	v 6.1
	Illumina LIMS	v 4.6.12	v 5.0

Abbreviations: MA, Multi-Sample Amplification; SDF, Sentrix Descriptor File; ICS, iScan Control Software; IAC, Illumina Automation Control; LIMS, Laboratory Information Management System.

Ordering Information

Product Name	Catalog No.	Product Name	Catalog No.
Infinium XT Starter Kit (48 BeadChip batches)	20011069	iScan System, 110/220 V	SY-101-1001
Infinium XT Starter Kit (24 BeadChip batches)	20011100	AutoLoader 2.x, Single-Scanner Configuration, 110/220 V	SY-202-1001
Infinium XT Upgrade Kit (24 BeadChip batches)	20011101	AutoLoader 2.x, Single-Dual Configuration, 110/220 V	SY-202-1002
Infinium XT Upgrade Kit (12 BeadChip batches)	20011102	Infinium Automation Kit 8-tip Tecan LIMS ready, 110/220 V	SC-30-403/404
Illumina LIMS 5.0 Package (server and software)	20018976	Infinium Automation Kit 8-tip Tecan non-LIMS, 110/220 V	SC-30-401/402
Illumina LIMS High Throughput Server Upgrade	20015563	Infinium XT iSelect-96 Kit (1152 samples)	20006613
Illumina LIMS Standard Throughput Server Upgrade	20018977	Infinium XT iSelect-96 Kit (4608 samples) ^a	20006614
Infinium XT PB20 Reagent Kit	20007420	Infinium XT iSelect-96 Kit (23,040 samples) ^a	20006615

a. For high-throughput, automated processing only. Contact your local sales representative for more information.

Learn More

To learn more about Infinium XT production-scale genotyping, visit www.illumina.com/InfiniumXT.