HumanLinkage Panels for Infinium® and GoldenGate® SNP Genotyping

Infinium HumanLinkage-12 and GoldenGate Linkage V SNP-based linkage panels from Illumina deliver high-confidence results and the statistical power required for genetic mapping.

Highlights Of the HumanLinkage Panels

- **Superior information content and call rates**
- **High accuracy with BeadArray™ technology**
- **Uniform, genome-wide distribution**
- **Multi-ethnic heritability information**
- **Easy sample management and multi-sample processing**

Introduction

Linkage analysis is a powerful approach for mapping the location of disease-causing loci by identifying genetic markers that are co-inherited with the phenotype of interest. Illumina's Linkage Panels present researchers with the optimal solution for identifying regions of statistically unequivocal linkage by delivering the information content, call rates, and accuracy that enable discovery of links between familial genotype and phenotype in both monogenic and polygenic disorders[1].

For linkage studies in humans, Illumina offers two linkage analysis platforms. The HumanLinkage-12 Genotyping BeadChip was designed for Illumina's revolutionary and powerful Infinium Assay. With the ability to analyze 12 samples on a single BeadChip, the HumanLinkage-12 Panel dramatically reduces the cost per sample without compromising data quality. Additionally, the PCR-free Infinium Assay protocol increases the ease and speed of studies requiring the analysis of a large number of samples. While the HumanLinkage-12 BeadChip offers a high-throughput solution for most linkage study designs at industry-leading prices, customers with formalin-fixed paraffin-embedded [FFPE] and whole-genome amplified samples, or without the Infinium upgrade may prefer the GoldenGate Linkage V Panel platform.

The Power of Confident Discovery

High information content improves the likelihood of detecting a recombinant event. Evenly-spaced loci and loci with high minor allele frequencies (MAF) were selected to maximize information content on both panels. The average genetic distance between uniquely mapped SNPs is 0.58 cM and 0.62 cM for HumanLinkage-12 and Linkage V panels, respectively. The high information content throughout the genome can be attributed to the appropriate level of marker density and the high heterozygosity of SNPs used in the panels. Compared to other SNP and STR (short tandem repeat) maps, the Linkage-12 and Linkage V panels are superior in uniform spacing and statistical power for detecting linkage to a disease or trait and for defining the linkage interval4. The HumanLinkage-12 and Linkage V Panels contain identical average minor allele frequencies (MAF) for the three HapMap populations (Table 1) with 36% for Caucasians (CEU), 26% for Yoruba (YRI), and 28% for Han Chinese/Japanese (CHB+JPT).

Figure 1: HumanLinkage-12 Genotyping BeadChip

The HumanLinkage-12 allows 12 samples to be interrogated on a single microarray.

In simulation studies, it has been suggested that a 1–2 cM bi-allelic map of polymorphic markers (MAF 20–50%) will extract most of the inheritance information. For linkage study designs, adding more markers provides diminishing returns[2]. Therefore, any markers that did not meet the performance or power criteria were removed from the panel to ensure optimal genetic power without the extra work, expense, and noise from extraneous, inferior markers.

Since linkage analysis relies on informative matings in pedigrees, heterozygosity is a good metric for the relative utility of each marker. Heterozygosity in this context is defined as the probability that an individual is polymorphic at a particular locus. The HumanLinkage-12 and the Linkage V Panels feature identical average observed heterozygosities with 43% in the Caucasian population, 33% in the Yoruba population, and 36% in the Han Chinese/Japanese population.

Importance Of Marker Distrobution

The HumanLinkage-12 and Linkage V Panels are optimized to detect recombination events. The likelihood of a recombination event occurring between two markers is related to the distance between them. Markers closest to a disease gene will co-segregate most strongly with the disease phenotype. On the HumanLinkage-12 Genotyping BeadChip, SNPs are distributed on every chromosome with an average gap of 441 kb and 0.58 cM (Figure 2).The fifth-generation GoldenGate Linkage Panel has been upgraded to reflect a 93% SNP overlap with the Infinium Assay, enabling data compatibility between formats. HumanLinkage-12 and Linkage V Panel SNP markers (6,090 and 6,056, respectively) were chosen from highly validated HapMap DNA

Table 1: HumanLinkage-12 BeadChip And Linkage V Panel Specifications

6,090 HumanLinkage-12 and 6,056 Linkage V Evenly Distributed SNP Markers*		
	Average Infinium/GoldenGate	Median Infinium/GoldenGate
Genetic (cM)	0.58/0.62	0.35/0.38
Physical (kb)	441/470	319/341
High Marker Heterozygosity And Minor Allele Frequency (MAF)		
	Average Heterozygosity† Infinium/GoldenGate	Average MAF† Infinium/GoldenGate
CEU	43%/43%	36%/36%
YRI	33%/33%	26%/26%
CHB+JPT	36%/36%	28%/28%

DNA required per sample: 1 ug for GoldenGate Linkage and 200 ng for Infinium Linkage

*SNP markers were selected from highly validated International HapMap Project DNA samples

†Results for both panels are based on 60, 60, and 90 unrelated DNA samples for the CEU, YRI, and CHB+JPT populations, respectively.

assays to optimize information content, enabling researchers to extract the greatest amount of data from the least number of markers at the lowest cost per sample. With 5,678 SNPs in common, the two panels have an almost identical physical and genetic distribution of markers across the genome. Every SNP marker has been robustly validated for physical and genetic map positions, distances, and unique sequence identification.

Illumina Linkage Panels are designed with uniform, genome-wide SNP distribution, ensuring outstanding statistical power for genetic mapping with high confidence. Both Infinium and GoldenGate Linkage panels provide the industry-leading call rates and reproducibility standard in all Illumina genotyping products.

Table 2: Infinium HumanLinkage-12 Panel And GoldenGate Linkage V Performance Comparison*

	Infinium HumanLinkage-12	**GoldenGate Linkage V**
Number of SNPs	6,090	6,056
Overlap of SNPs	93.2%	93.7%
Call Rate (average)	99.85%	99.86%
Reproducibility	100%	100%
Mendelian Inconsistencies	0.0058%	0.0048%

*Based upon analysis of 288 HapMap samples

Format Compatibility

The 93% SNP marker overlap between the HumanLinkage-12 and Linkage V Panels allows researchers to confidently combine genotypes interrogated by both platforms. This compatibility offers investigators the flexibility to take advantage of the high-throughput and cost-effective Infinium platform and also include FFPE and whole genome amplified DNAs using the GoldenGate HumanLinkage Panel in the same study.

Proven Infinium And GoldenGate Assay Performance

The Infinium Assay is an array-based, PCR-free, SNP genotyping assay that enables effectively unlimited multiplexing. This robust assay uses a single-base extension protocol that achieves a high signal-to-noise ratio by combining specific hybridization of probes with allele-specific primer extension and signal amplification. The conversion rate, call rate, and accuracy are comparable to those of high-performance PCR-based SNP assays[3].

The well-proven GoldenGate Assay has been subjected to rigorous functional testing to ensure a sufficiently high PCR multiplex (1,536), analytical detection, and suitability for linkage studies.

Maximizing Accuracy, Minimzing Effort

Past linkage studies have been hindered by a lack of automation, sample handling errors, and the need for specialized technician skills. The automated Illumina BeadStation and BeadArray Reader provide the solution to these issues.

Sample tracking is of particular concern in linkage analysis since a one-sample mixup causes at least two sample errors in heritability. Studies have shown that error rates of just 1% can result in a 50% decrease in LOD score[5].

The Illumina Laboratory Information Management System (LIMS) offers positive sample tracking, eliminating sample handling errors for both assay platforms.

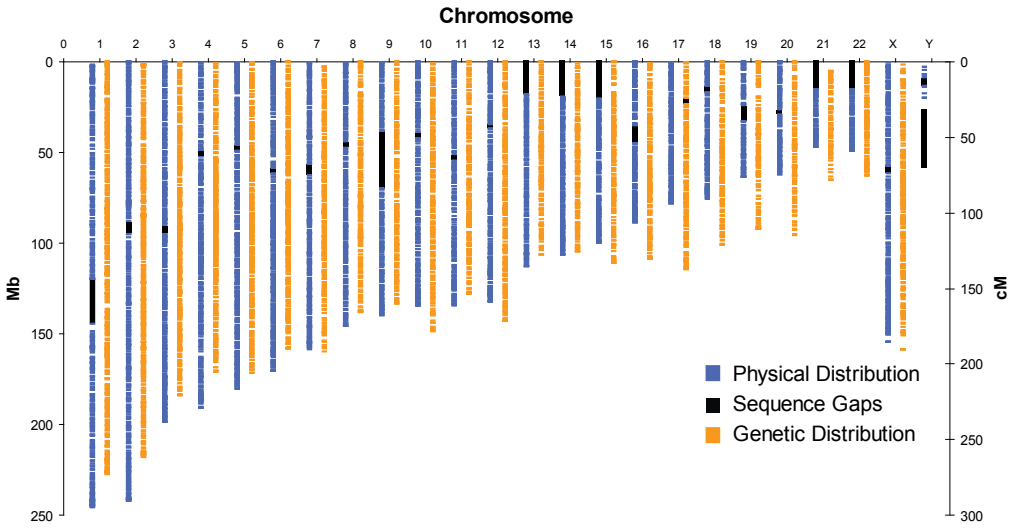
Summary

The high-quality data and low per-sample cost of the HumanLinkage-12 Genotyping BeadChip, and the GoldenGate Linkage V Panel, which generates excellent results with FFPE, whole-genome amplified, and degraded DNAs, are part of the power-ful Illumina SNP linkage analysis solution. The combination of Illumina's proprietary assays and high information content optimization delivers the highest quality data for linkage analysis. In addition, optional automation and LIMS lower

Table 3: Product Specifications

	HumanLinkage-12 BeadChip
Call Rate (average)	>99%
Reproducibility	>99.9%
Mendelian Inconsistencies	<0.1%

Figure 2: Physical And Genetic Distrobution Of HumanLinkage-12 BeadChip Markers



6,090 SNP markers distributed uniformly across the human genome. The GoldenGate Linkage V and Infinium HumanLinkage-12 panels share 5,678 SNPs.

costs by eliminating errors associated with manual processing. Illumina linkage products (standard and custom content) can be accessed via Illumina Fast Track Genotyping Services, the Illumina Customer Sample Evaluation (CSE) Program, or one's own Illumina BeadArray Reader or BeadStation. Illumina DNA Analysis solutions continue to provide industry-leading levels of accuracy, flexibility, and affordability.

References

1. International Multiple Sclerosis Consortium (2002) Enhancing linkage analysis of complex disorders: an evaluation of high-density genotyping. Hum Mol Genet 13: 1943-1949.
2. Kruglyak L, (1997) The use of a genetic map of biallelic markers in linkage studies. Nat Genetics 17: (21-24).
3. Gunderson KL, Steemers FJ, Lee G, Mendoza LG, Chee MS (2005) A genome-wide scalable SNP genotyping assay using microarray technology. Nat Genet 37: 549-554.
4. Shaw Murray S, Oliphant A, Shen R, McBride C, Steeke R J, et al. (2004) A Highly Informative SNP Linkage Panel for Human Genetic Studies. Nat Methods, 1(2): 113-117.
5. Abecasis G R, Cherny SS and Cardon LR (2001) The impact of genotyping error on family-based analysis of quantitative traits. Eur J Hum Genet 9: 130-134.

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Ordering Information

GoldenGate Linkage V Panel

CATALOG NO.	PRODUCT	DESCRIPTION
WG-31-140	Infinium HumanLinkage-12 Panel (48 samples)	Contains four BeadChips, each capable of analyzing 12 samples, along with reagents for amplifying, fragmenting, hybridizing, and detecting 48 human linkage genotyping samples.
WG-31-141	Infinium HumanLinkage-12 Panel (96 samples)	Contains eight BeadChips, each capable of analyzing 12 samples, along with reagents for amplifying, fragmenting, hybridizing, and detecting 96 human linkage genotyping samples.
WG-31-142	Infinium HumanLinkage-12 Panel (288 samples)	Contains 24 BeadChips, each capable of analyzing 12 samples, along with reagents for amplifying, fragmenting, hybridizing, and detecting 288 human linkage genotyping samples.

GoldenGate Linkage V Panel

Catalog No.	Product	Description
GT-17-240	GoldenGate Linkage V Panel OPA Set	A set contains four OPA tubes each capable of analyzing 96 samples.
FA-12-112	Universal-96 Array Matrix (1536-plex)	Contains a Universal-96 Sentrix Array Matrix (SAM) capable of processing 24 samples. Linkage V Panel set requires four Universal-96 Arrays to process 96 samples.
GT-95-202	Multiple-Use Activation Kit	Contains reagents for six 96-well plates of samples. Used in combination with the GoldenGate Assay Kit.
GT-95-205	GoldenGate Assay Kit with UDG	Contains reagents for genotyping 96 DNA samples. Includes UDG enzyme for contamination control.
GT-95-206	GoldenGate Assay Kit with UDG	Contains reagents for genotyping 576 DNA samples. Includes UDG enzyme for contamination control.

Illumina, Inc. • 9885 Towne Centre Drive, San Diego, CA 92121 USA • 1.800.809.4566 toll-free • 1.858.202.4566 tel • techsupport@illumina.com • illumina.com

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