

Linkage IV Panel

illumina's SNP-based linkage panel delivers the high-confidence results and the statistical power required for genetic mapping.

HIGHLIGHTS: LINKAGE IV PANEL

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

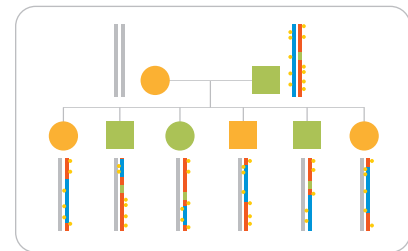
INTRODUCTION

illumina's Linkage IV Panel is the optimum solution for identifying regions of statistically unequivocal linkage, thereby accelerating your research. Studies have shown that genome-wide SNP (single nucleotide polymorphism) scans can be effective in isolating areas of interest in both Mendelian (monogenic) and complex disorder (polygenic) studies. The key components that make SNP linkage powerful enough for the more difficult polygenic disorders are the combination of genotyping success (call rate), information content, and genotyping accuracy (*Int'l. MS Consortium, 2004*). illumina's Linkage IV Panel and BeadArray technology provide the information content, call rate, and accuracy to enable this revolutionary step forward in the discovery of links between familial genotype and phenotype in both monogenic and polygenic disorders.

Linkage IV Panel SNP markers (5,861) were selected to extract the greatest amount of information from the least numbers of markers at the lowest cost per sample.

THE POWER OF CONFIDENT DISCOVERY

The genome-wide average information content for the Linkage IV Panel is over 97.1% and never drops below 81% in any region along the information-content curves for individual chromosomes when tested in eight CEPH pedigrees. The average genetic distance between uniquely mapped SNPs is 0.64 cM. This high information content throughout the genome can be attributed to both the appropriate level of marker density and the high heterozygosity of SNPs used in the panel. Compared to other SNP and STR (short tandem repeats) maps, the Linkage IV Panel is superior in uniform spacing and statistical power for detecting linkage to a disease or trait and for defining the linkage interval. A genetic and physical map for the Linkage IV Panel is available at illumina.com.



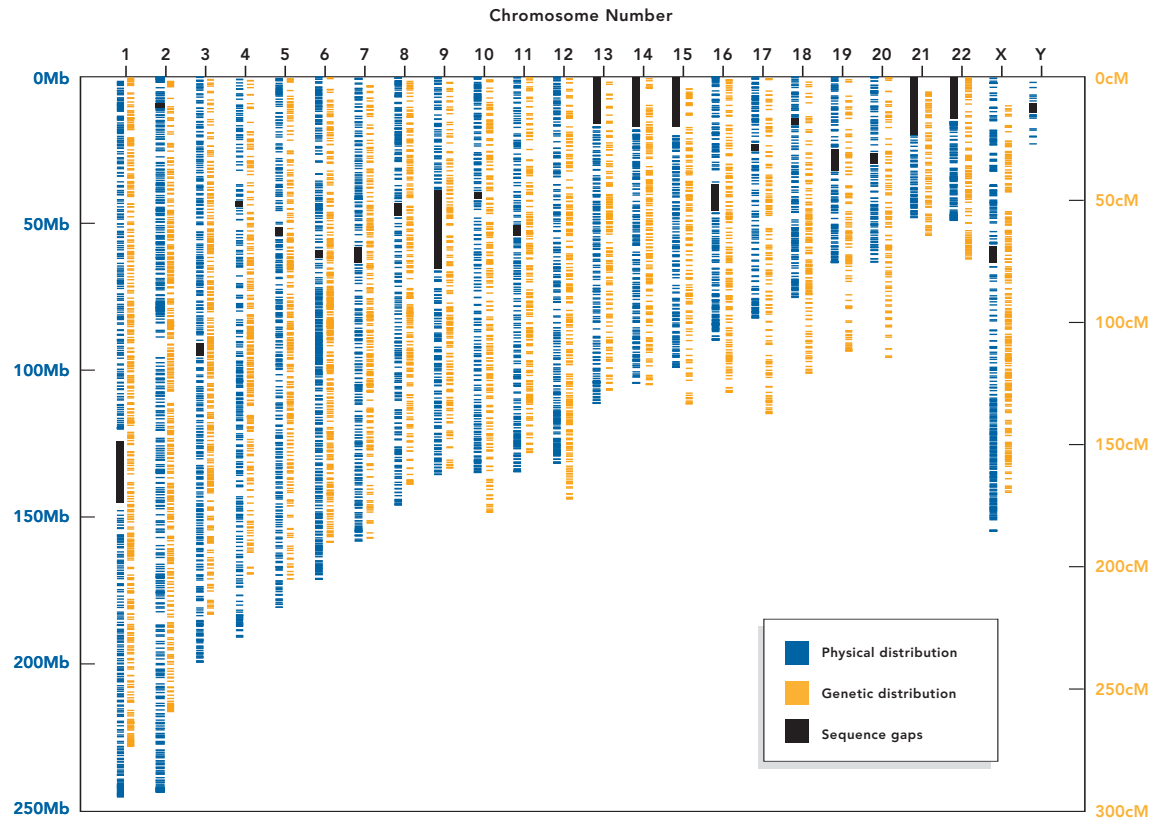
Linkage Mapping pedigree

The average minor allele frequency is 37% for the Caucasian population, 30% for African-American, and 29% for the Asian population (*Table 1*). By 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 (*Kruglyak, 1999*). Therefore, any markers that did not meet the performance or power criteria were

TABLE 1: LINKAGE IV PANEL SPECIFICATIONS

5,861 Evenly Distributed SNP Markers			
	AVERAGE	MEDIAN	MAXIMUM
GENETIC cM	0.64	0.35	8.8
PHYSICAL Kb	482	298	4,954
High Marker Heterozygosity and Minor Allele Frequency (MAF)			
	AVERAGE HETEROZYGOSITY	AVERAGE MAF	NUMBER OF DNAs
CAUCASIANS	43%	37%	192
AFRICAN AMERICAN	38%	30%	60
ASIAN	36%	29%	132
Reproducibility	99.961%	Genotype Call Rate	99.810%
Mendelian Inconsistencies	0.006%	DNA Required	250ng

FIGURE 1: PHYSICAL AND GENETIC DISTRIBUTION OF LINKAGE IV MARKERS



5,861 SNP markers distributed uniformly across the human genome. Black areas represent gaps in the genome sequence.

removed from the panel to ensure optimal genetic power without the extra work, expense, and noise from extraneous, inferior markers.

Heterozygosity is the probability an individual is polymorphic at a particular locus. Since linkage analysis relies on informative matings in pedigrees, heterozygosity is a good metric for the relative utility of each marker. The average observed heterozygosity was 43% in the Caucasian population, 38% in the African-American population, and 36% in the Asian population.

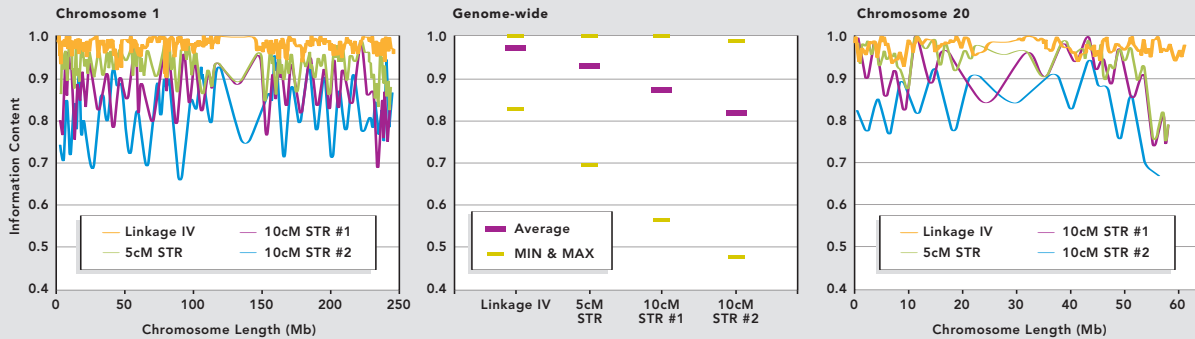
IMPORTANCE OF MARKER DISTRIBUTION

Illumina's Linkage IV Panel is a fourth generation SNP map designed to optimize the detection of recombination events. The likelihood of a recombination event occurring between two markers is related to the distance between them. Markers that are closest to a disease gene will co-segregate most strongly with the disease phenotype. In the Linkage IV Panel SNPs are distributed on every chromosome with an average gap of 482 Kb and 0.64 cM (Figure 1). Each SNP has been validated for physical and genetic map positions, distances, and unique sequence identification.

PROVEN PERFORMANCE

The SNPs in the Linkage IV Panel were subjected to rigorous functional testing to ensure a sufficiently high PCR multiplex (1,536), analytical detection and suitability for linkage studies. In addition, Illumina's Genotyping Services Group has been using the Linkage Panel on customer samples for over 2 years. The table to the right is a subset of the studies run by Genotyping Services as well as the validation studies for Linkage IV map development and testing.

FIGURE 2: INFORMATION POWER OF LINKAGE IV VS. STR MARKER MAPS



The figure shows the information content derived from 188 meioses from two representative chromosomes and a genome-wide summary when the Linkage IV Panel is compared to standard 5cM and 10cM STR marker maps.

**MAXIMIZING ACCURACY,
MINIMIZING EFFORT**

Past linkage studies have been hindered by a lack of automation, sample handling errors, and the need for specialized technician skills. A BeadLab or BeadStation system provides the solution to these issues, which have frustrated researchers for years.

Sample tracking is of particular concern in Linkage analysis since a one sample mix-up causes a minimum of 2 sample errors in heritability. Studies have shown that error rates of just 1% can result in a 50% decrease in LOD score (Abecasis, 2001). The Illumina BeadLab LIMS system offers positive sample track-

ing that virtually eliminates sample handling errors. Additionally, samples are handled in 96- or 384-sample microtiter plates which are directly compatible with the 96-sample Sentrix® Array Matrix, further reducing any sample handling errors.

TABLE 2: RESULTS FROM ILLUMINA GENOTYPING SERVICES AND VALIDATION STUDIES

Functional Samples*	Total Samples	Linkage Panel Version	Genetic Measures		Call Rate (functional DNA's)	Call Rate (all DNA's)	Reproducibility	Mendelian Inconsistencies
			REPLICATES	TRIOS				
1,376	1,398	III	192	140	99.95%	98.38%	99.950%	0.000%
384	384	IV	30	70	99.95%	99.95%	100.00%	0.010%
368	368	III	4	34	99.88%	99.88%	100.00%	0.009%
244	246	III	22	147	99.98%	99.16%	99.995%	0.010%
638	644	IV	15	99	100.00%	99.07%	99.970%	0.006%
45	46	IV	N/A	10	99.80%	97.63%	N/A	0.016%
46	46	IV	N/A	15	99.96%	99.96%	N/A	0.001%
89	92	IV	N/A	N/A	99.91%	96.65%	N/A	N/A
47**	48	IV	8	18	99.90%	97.82%	99.980%	0.003%
3,237	3,272		Weighted Averages:		99.810%	98.749%	99.621%	0.006%

*Samples (gDNA) were prepared by the customer, following Illumina's standard protocol. Samples which showed sporadic, random behavior (assumed to have low DNA quantity or contamination) were not included in study results. **DNA was Whole Genome Amplified before being genotyped. N/A - indicates information not disclosed to Illumina

ORDERING INFORMATION

PART NO.	PRODUCT	DESCRIPTION
GT-17-140	Linkage IV Panel OPA Set	Four OPA tubes each capable of analyzing 96 samples.

RELATED PRODUCTS

GT-95-202	Multiple-Use Activation Kit	Used in combination with the GoldenGate Assay Kit. Contains reagents for six 96-well plates of samples.
GT-95-205	GoldenGate Assay Kit with UDG	Prepares genotyping reactions for 96 DNA samples. Contains UDG enzyme for contamination control.
GT-95-206	GoldenGate Assay Kit with UDG	Prepares genotyping reactions for 576 DNA samples. Contains UDG enzyme for contamination control.
FA-12-103	Sentrix Universal-96 Array Matrix	Linkage IV Panel requires 4 Universal-96 Arrays per 96 samples.

ADVANTAGES OF BEADARRAY TECHNOLOGY

The reproducibility, turnaround time, and low cost of studies conducted with the Linkage IV Panel are made possible by powerful Illumina technologies that include the GoldenGate™ assay and multi-sample Sentrix formats. The BeadStation and BeadLab platforms offer end-to-end (from genomic DNA to called genotypes) solutions that integrate a multiplexed assay (Linkage IV is at 1,536-plex), bead-based microarrays, a submicron-resolution scanner, and automated scoring software.

Illumina’s genotyping systems can be used to perform linkage, fine mapping and disease-association studies as well as high-resolution, genome-wide scans (>100K markers) with fixed panels of SNPs or customer designed SNP sets. The same systems can also be deployed to study gene expression. Illumina systems enable investigators to genotype from 46 to 576 samples per day with industry-leading levels of accuracy, flexibility and affordability.

MULTIPLE WAYS TO ACCESS THE LINKAGE IV PANEL

Illumina’s Genotyping Service offers an efficient, hands-off solutions for genotyping. You provide Illumina with 4ug of DNA per sample, and in 45-90 days or less you will receive genotypes with confidence scores ready for analysis by linkage analysis programs.

Alternatively, the Linkage IV Panel can be run on a BeadStation or BeadLab at your facility. An individual researcher, genotyping samples manually on a BeadStation, can complete 1,000 samples (gDNA to genotype) in 3-7 work days—while utilizing only 40 arrays. Much higher throughput can be achieved using the BeadLab system.

REFERENCES

International Multiple Sclerosis Consortium. Enhancing linkage analysis of complex disorders: an evaluation of high-density genotyping. *Human Molecular Genetics* 13, 1943-1949, 2004.

Kruglyak, L. The use of a genetic map of biallelic markers in linkage studies. *Nature Genetics* 17:21-24, 1997.

Abecasis, G.R., Cherny, S.S. and Cardon, L.R. The impact of genotyping error on family-based analysis of quantitative traits. *European Journal of Human Genetics* 9, 130-134, 2001.

ADDITIONAL INFORMATION

To learn more about the Linkage IV Panel, to find an Illumina service provider, or to find out more about any of Illumina’s products and services, visit our website or contact us at the address below:

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