

MHC Panel Set

Illumina's Single Nucleotide Polymorphism (SNP)-based MHC Panel Set, comprised of the MHC Mapping Panel and the MHC Exon-Centric Panel, delivers high-quality data and provides comprehensive coverage for association mapping the MHC region.

INTRODUCTION

Illumina's Major Histocompatibility Complex (MHC) Panel Set consists of two assay pools of oligonucleotides (i.e., Panels), the MHC Mapping Panel and the MHC Exon-Centric Panel. These two panels can be used independently or combined for more comprehensive coverage. Each Panel offers a cost-effective and flexible method for mapping disease-associated variants in the MHC region.

The MHC region is one of the most difficult areas of the genome to study due to its inherent sequence characteristics. This gene-dense region spans ~4 Mb and encodes over 160 genes. Of these genes, approximately 40 per-cent encode proteins involved in the immune system, including the human leukocyte antigen (HLA) membrane glycoproteins that mediate T-lymphocyte signalling. The MHC region has been widely studied and is proposed to contain genomic sequences that contribute to the majority of autoimmune and inflammatory disorders. Association mapping this region can

HIGHLIGHTS OF THE MHC PANEL SET

- High-Quality Data: proven GoldenGate® technology
- MHC Access: dense SNP Panels across this challenging genomic region
- Flexible Approach: even spacing, exon-centric or both

TABLE 1: MHC PANEL SET DATA QUALITY

Population	Total Samples	Replicates	Trios	Call Rate	Reproducibility	Mendelian Inconsistencies
CEU	95	5	30	99.95%	99.99%	0.0085%
HCB/JPT	94	5	N/A*	99.58%	100.00%	N/A*
YRI	95	5	30	99.63%	100.00%	0.017%

CEU = Utah residents with ancestry from Northern and Western Europe

HCB/JPT = Han Chinese in Beijing, China/Japanese in Tokyo, Japan

YRI = Yoruba in Ibadan, Nigeria

*No parental DNA samples available

provide information to researchers to determine the gene associations that lead to disease susceptibility.

The HLA genes represent a minority of genes found in the MHC region. It is likely that many disease-associated genetic variants may actually reside outside of the HLA genes within the MHC region. As a result, it may be important to investigate sites other than those mapped to HLA genes. The MHC Mapping Panel used in combination with the MHC Exon-Centric Panel (MHC Panel Set) can interrogate this complex genomic area with one genotyping solution.

Two approaches are commonly used for association mapping genetic regions of interest. One approach utilizes interrogation of evenly-spaced SNPs. This approach is addressed by the MHC Mapping Panel, with emphasis on tag SNPs. A second approach focuses on SNPs near and within coding exons. The MHC Exon-Centric Panel provides this approach. Each panel is used to assay >1,200 SNPs with Illumina's proven

GoldenGate® assay. When combined, the two panels provide a more comprehensive approach for association mapping the region.

MHC PANEL SET: COMBINED COVERAGE

The 2,360 SNPs in the MHC Panel Set were subjected to rigorous functional testing to ensure high-quality data and suitability for fine mapping using Illumina's GoldenGate Assay. Three populations were studied to determine call rate, reproducibility, and Mendelian inconsistencies (Table 1). All loci in each population were in Hardy-Weinberg equilibrium ($P > 0.001$), suggesting that the loci were of high quality and segregated into expected genotype proportions.

The MHC Panel Set showed strong concordance (>99.5% for 527 loci) with the International HapMap Project.¹ Minor allele frequency (MAF) for loci in three major ethnic groups was

determined using the MHC Panel Set. The mean MAF was 0.26, 0.24, and 0.21 for the CEU, HCB/JPT, and YRI populations, respectively. All loci had a MAF >0.01 in at least one of the populations studied. The MHC Panel Set's loci have MAF within range to accurately assess disease associations.

MHC MAPPING PANEL: EVEN SPACING

The Mapping Panel consists of 1,293 SNPs evenly spaced across the MHC region. Approximately half of the SNPs in the Mapping Panel are tag SNPs identified by the program *IdSelect*.² The remaining SNPs were chosen based on linear spacing across the MHC region. *Figure 1* illustrates the proportion of loci by distance (kb) spanned between SNPs. The Mapping Panel has the following attributes:

- Average 3.8 kb between each SNP
- 82% loci with <5 kb spacing
- 97% loci with <10 kb spacing

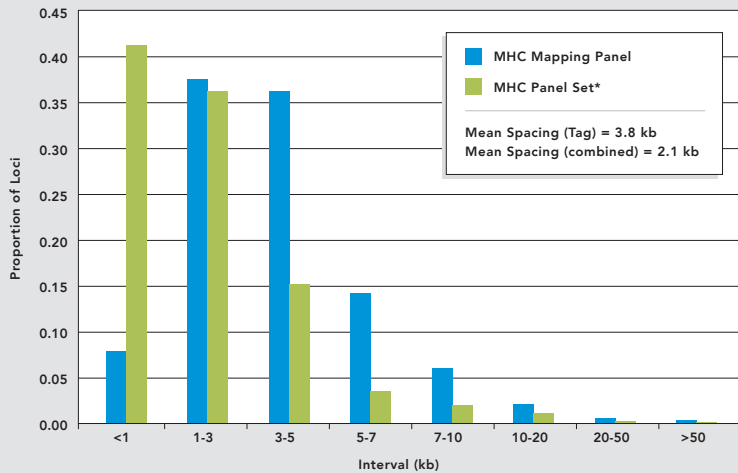
A higher density of SNP coverage can be obtained by using the MHC Panel Set. This 2,360-loci set has the following attributes:

- Average 2 kb between each SNP
- 93% loci, <5 kb spacing
- 98% loci, <10 kb spacing

MHC MAPPING PANEL: ROBUST LD COVERAGE

In addition to even spacing across the MHC region, the MHC Mapping Panel also has excellent coverage with respect to linkage disequilibrium (LD). The majority of markers are in strong LD, measured as r^2 , to at least one other marker (*Figure 2*).³

FIGURE 1: PROPORTION OF LOCI BY DISTANCE BETWEEN SNPS (KB)



*MHC Panel Set includes MHC Mapping Panel + MHC Exon-Centric Panel

TABLE 2: HAPLOTYPE BLOCK STRUCTURES

Haplotype Blocks	MHC MAPPING PANEL			MHC PANEL SET*		
	CEU	HCB/JPT	YRI	CEU	HCB/JPT	YRI
Mean block size (kb)	24.10	23.64	17.36	16.19	15.79	13.93
Number of blocks	134	129	139	223	215	207

*MHC Panel Set includes MHC Mapping Panel + MHC Exon-Centric Panel

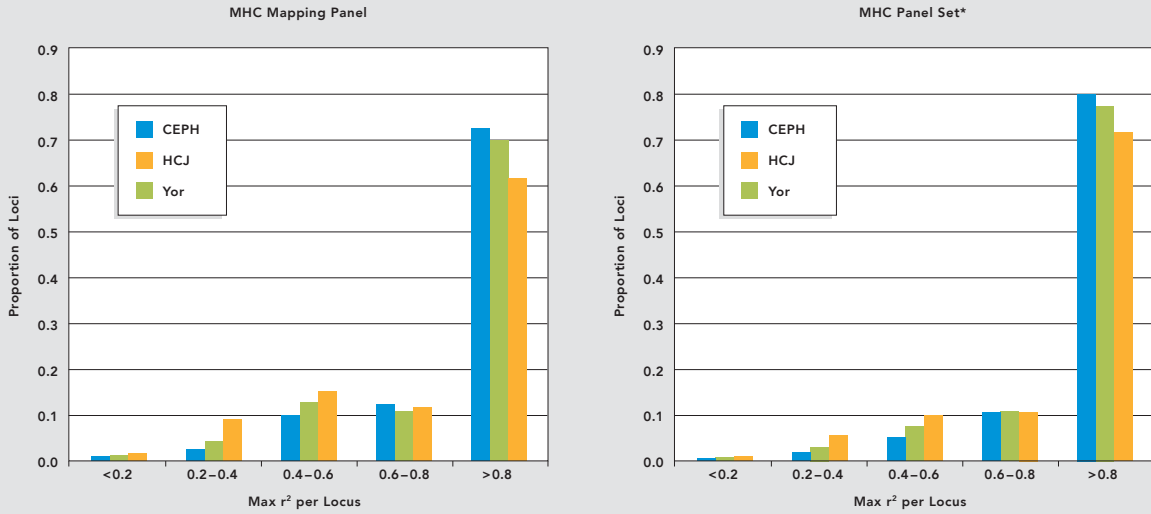
Haplotype structure and diversity were investigated in three populations (*Table 2*) using the program *Haploview*⁴ and the methods described in Gabriel et al.⁵ In the CEU population using the Mapping Panel, there were 134 haplotype blocks, with an average block size of 24.10 kb. When the SNP density was increased by using the MHC Panel Set, the number of haplotype blocks increased to 223 blocks, and the average block size decreased to 16.2 kb. The haplotype block structures across the MHC region in the CEU population are shown in *Figure 3*. These data demonstrate the dense coverage of the MHC

Mapping Panel and increased coverage of the MHC Panel Set, a benefit when searching for associations to disease phenotypes.

MHC EXON-CENTRIC PANEL: GENE-DENSE COVERAGE

The MHC Exon-Centric Panel consists of assays for 1,228 SNPs in and near coding regions in the MHC region. Assays for SNPs within 10 kb of exons in the MHC region were selected for the MHC Exon-Centric Panel to ensure complete coverage of coding sequences in the MHC region. The proportion of SNPs in various gene regions are shown (*Figure 4*,

FIGURE 2: LINKAGE DISEQUILIBRIUM COVERAGE



All pairwise combinations of r^2 were calculated and the maximum r^2 value for each marker is shown.
 *MHC Panel Set includes MHC Mapping Panel + MHC Exon-Centric Panel

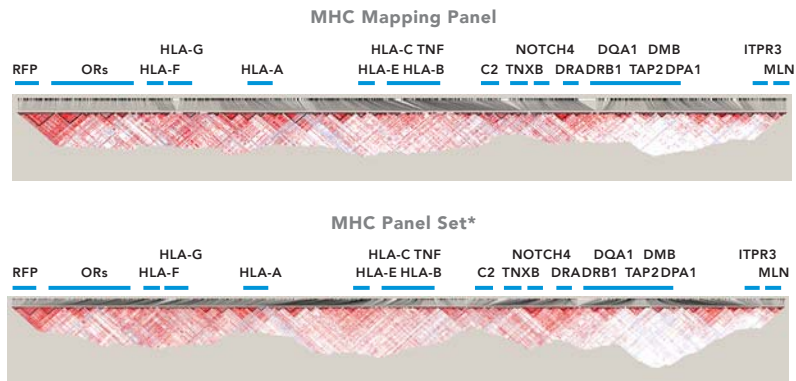
Table 3). The MHC Exon-Centric Panel provides an ideal solution for research questions related specifically to exon-centric SNP content, or combine with the MHC Mapping Panel for more comprehensive coverage.

ILLUMINA SOLUTIONS FOR GENOTYPING

The high-quality data and low cost-per-genotype of the MHC Panel Set are made possible by powerful Illumina technologies that include the GoldenGate Assay with multi-sample Sentrix® Array Matrix and BeadChip formats. Illumina’s genotyping solutions enable linkage and association mapping, as well as high-resolution, genome-wide scans (>100K markers). Whether utilizing standard or custom content, Illumina genotyping panels can be accessed via FastTrack Genotyping

Services or with an Illumina System. Illumina solutions provide industry-leading levels of accuracy, flexibility and affordability.

FIGURE 3: HAPLOTYPE BLOCK STRUCTURE ACROSS THE MHC REGION



General location of various genes in the MHC region are shown at the top, placement of SNPs is shown below gene names. Haplotype blocks are shown below placement of SNPs. LD strength in pairwise comparisons are shaded. Red indicates regions of high LD ($D' = 1$; $LOD \geq 2$), pink indicates regions of weaker LD ($D' < 1$; $LOD \geq 2$), and white blocks indicate weak to no LD ($D' < 1$; $LOD \leq 2$). Blue blocks indicate $D' = 1$ with $LOD < 2$.

*MHC Panel Set includes MHC Mapping Panel + MHC Exon-Centric Panel

FIGURE 4: DISTRIBUTION OF SNPs BY GENE REGION

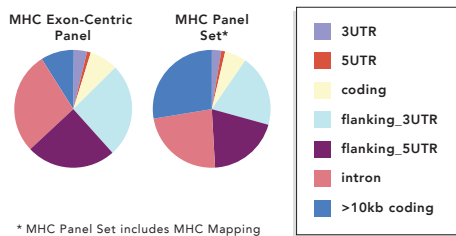


TABLE 3: SNP COVERAGE IN MHC GENES

	MHC Exon-Centric Panel	MHC Panel Set*
Mean SNPs/gene	7.7	10.7
Median SNPs/gene	4.0	7.0
SNPs within 10kb coding	1119	1708
SNPs within 10kb coding	91.12%	72.37%
RefSeq Genes with at least 1 SNP	146	159
RefSeq Genes with at least 1 SNP	86.39%	94.08%

* MHC Panel Set includes MHC Mapping Panel + MHC Exon-Centric Panel

ORDERING INFORMATION

PART NO.	PRODUCT	DESCRIPTION
GT-17-170	MHC Panel Set	Optimized set of two oligo pools (OPAs) for 2,360 SNP loci comprised of the Exon-Centric Panel and Mapping Panel capable of analyzing 96 samples.
GT-17-180	MHC Mapping Panel	One oligo pool (OPA) for 1,293 SNP loci that are evenly spaced across the MHC region with emphasis on tag SNPs sufficient for 96 samples.
GT-17-190	MHC Exon-Centric Panel	One oligo pool (OPA) for 1,228 SNP loci near coding sequences of genes in the MHC region sufficient for 96 samples.
GT-95-202	Multiple-Use Activation Kit (576 Samples)	Used in combination with the GoldenGate Assay Kit. Contains reagents for six, 96-well plates of samples.
GT-95-206	GoldenGate Assay Kit with UDG (576 Samples)	Prepares genotyping reactions for 576 DNA samples. Contains UDG enzyme for contamination control.
FA-12-104	Sentrix Universal-96 Array Matrix	One Sentrix Universal-96 Array Matrix can process 96 samples and up to 1536 assays/sample.

REFERENCES

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- (2) Carlson, C.S., Eberle, M.A., Rieder M.J., Yi, Q., Kruglyak, L., Nickerson, D.A. Selecting a maximally informative set of single-nucleotide polymorphisms for association analysis using linkage disequilibrium. *Am J Hum Genet*. (2004) 74:106-20.
- (3) Devlin, B., Risch, N. A. comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics* (1995) 29:311-322.
- (4) Barrett, J.C., Fry, B., Maller, J., Daly, M.J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. (2005) 21:263-265.
- (5) Gabriel, S.B., Schaffner, S.F., Nguyen, H., Moore, J.M., Roy, J., Blumenstiel, B., Higgins, J., DeFelice, M., Lochner, A., Faggart, M., Liu-Cordero, S.N., Rotimi, C., Adeyemo, A., Cooper, R., Ward, R., Lander, E.S., Daly M.J., Altshuler, D. The structure of haplotype blocks in the human genome. *Science*. (2002) 296:2225-2229.

ADDITIONAL INFORMATION

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