Multiplexed Haplotype-Specific Extraction (HSE) of Genomic DNA

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INTRODUCTION

Haplotype-Specific Extraction, HSE, physically separates a diploid sample into its haploid components using magnetic bead technology without knowledge of familial information.

HSE improves the accuracy of tissue typing: It enables unambiguous HLA typing of diploid allele combinations using current DNA-typing methods that may otherwise fail to be resolved.

Haplotyping can improve the power of Association Studies: The conversion of individual diploid samples into more informative haploid components can increase the power to find correlations between traits and shared variants in case-control studies.

HSE can increase the resolution and quality of genetic analysis in other diagnostic applications such as oncology testing, metabolic profiling and should improve our understanding of multi-factorial diseases and disease classification.

Probes for the HLA loci A, B and DRB1 were used to separate DNA fragments from a single tube containing 400ng genomic DNA of known HLA type on the GenoM-6/EZ-1 HSE robotic system. The haploseparated DNA was amplified in three independent PCRs and confirmed as haploid by HLA-typing with the InnoLiPA reverse SSOP (sequence specific oligonucleotide probe) system.

Multiplexed haploseparations provide DNA fragments that can allow the derivation of extended molecular haplotypes. Routine haplo-separation of large genomic regions can simplify the fine mapping of chromosomal regions for susceptibility genes and validate statistically derived haplotypes.

PRINCIPLE - HOW HSE WORKS

Fig. 1

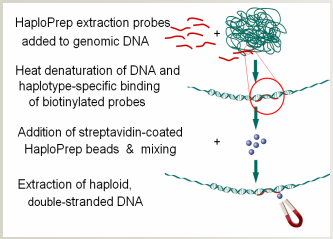


Fig. 2

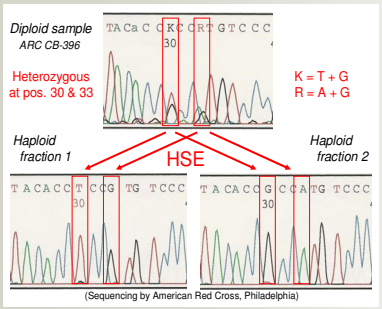
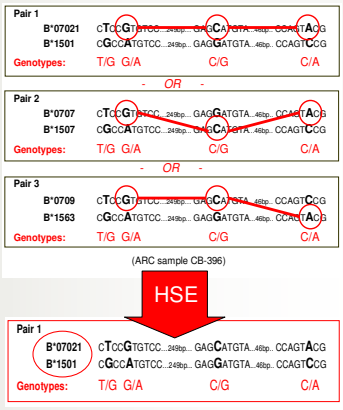


Fig. 3



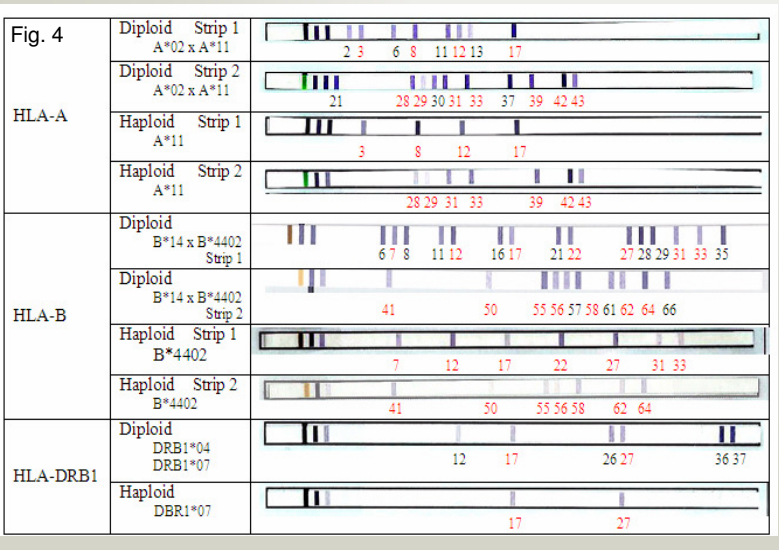
Ambiguities are resolved and haplotypes are determined when the linkage between multiple polymorphic sites is established by HSE:

The sample tissue type is resolved as Pair 1 (B*07021 & B*1501; **Fig. 3**).

RESULTS

Probes for the HLA loci A, B and DRB1 were used to separate DNA fragments from a single tube containing 400 ng genomic DNA of known HLA type on the GenoM-6 / EZ-1 HSE robotic system. The haploseparated DNA was amplified in three independent PCRs and confirmed as haploid by HLA-typing with the InnoLiPA reverse SSOP (sequence specific oligonucleotide probe) system (Fig. 4). The pattern of red numbers of the haploid DNA corresponds exactly to the alleles targeted during HSE. Note that the haploid DNA does not hybridize with any probe (black numbers) that would indicate the presence of contaminant DNA from the other allele.

Fig. 4



DISCUSSION

HSE can extract several genomic regions in parallel. Multiplexed haploseparations can provide DNA fragments that allow the derivation of the molecular haplotype over extended distances.

Based on publicly available SNP and other marker information it should be possible to separate the entire MHC for one person in one run on the GenoM-6 / EZ-1 HSE robot.

A 96-well robot such as the BioSprint96 would allow for the multiplexed separation and tiling of regions of about 60-90Mb size.

The approach is:

1) **SNP-genotype candidate regions**
2) **Haploseparate based on this information**
3) **Assemble contiguous molecular haplotypes**

Fig. 5

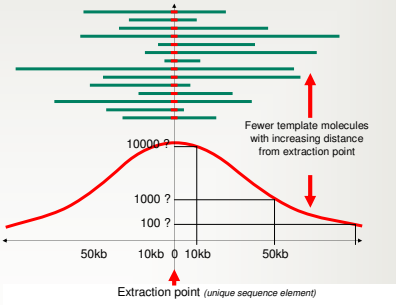
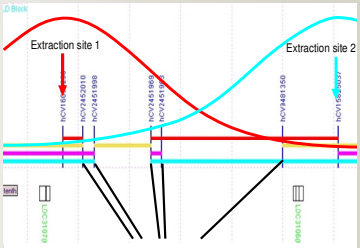
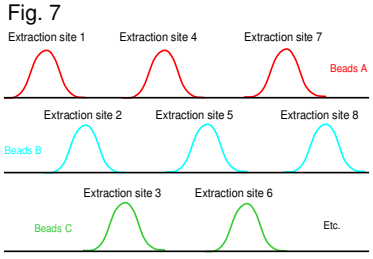


Fig. 6



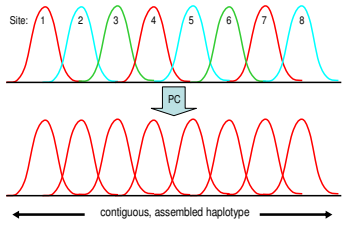
Overlapping SNPs are available to assemble extended, contiguous haplotype spanning across known block structure (Fig.6)

Fig. 7



Fragments from multiple loci are bound to each set of beads during a multiplexed extraction, but it is assured that each captured locus has been extracted by only one HSE-probe (Fig 7).

Fig. 8



Overlapping SNPs are then typed for each batch of multiplexed beads and the contiguous haplotype is assembled based on the information from consecutive, multiplexed extractions (Fig. 8).

(Discussion continued)

HSE is used for SNP-based separation and typing of chromosomal regions relevant for transplantation (Major Histocompatibility Complex, MHC and Killer Immunoglobulin-like Receptor, KIR) and for disease association studies of the MHC. HSE is in routine use with HLA (Human Leukocyte Antigen) typing labs in North America and Europe.

HSE works with common downstream assays such as DNA sequencing or realtime PCR and can be used in studies that rely on existing collections of samples for which access to the original donors may no longer be possible, and in retrospective studies on anonymous, banked or forensic specimens.

CONCLUSION

Routine haploseparation of large genomic regions can simplify the fine mapping of chromosomal regions for susceptibility genes and validate statistically derived haplotypes. HSE can increase the resolution and quality of genetic analysis in other diagnostic applications such as oncology testing, metabolic profiling and disease classification, and should have an impact on our understanding of multi-factorial diseases.

LITERATURE

A Rapid, Automatable Method For Molecular Haplotyping. J. Dapprich, M.A. Cleary, H.W. Gabel, A. Akkapeddi, B. Iglehart, C. Turino, L. Beaudet, J. Lian, N.B. Murphy. HLA 2004: Immunobiology of the Human MHC. Proceedings of the 13th International Histocompatibility Workshop and Congress. (Hansen JA and Dupont B, eds) Volume I & II, IHWG Press, Seattle, WA, 2004. ISBN: 0-945278-03-9

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