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Technology Transition Workshop| *Ranajit Chakraborty, Ph.D.*

Evaluation of Genome-wide SNP Haplotype Blocks for Human Identification Applications

Overview

- Some brief remarks about SNPs
- Haploblock structure of SNPs in the human genome
- Criteria for selection of optimal SNP haploblocks for forensic applications
- Preliminary results of optimal parameter combinations from HapMap Data (Phase I and Phase II)
- Feasibility of SNP haploblock selection from human genome
- Strategies of interpretation of SNP haploblock-based forensic evidence
- Preliminary conclusions and future directions

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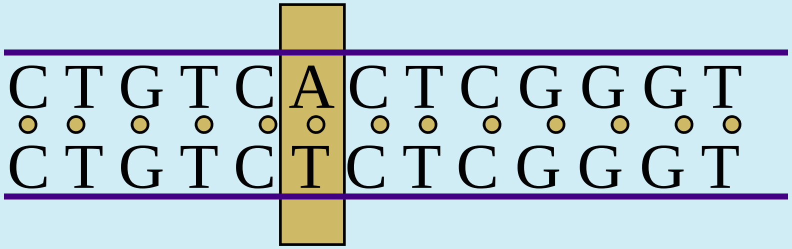
Single Nucleotide Polymorphism (SNP)



- Most SNPs are biallelic
- About three million SNPs in human genome (characterized)
- Provide more results from low quantity template DNA or degraded samples than STR typing
- Complete automation feasible
- Low mutation rates (10^{-8}/site/generation)
- Use of SNPs in forensics is not new (e.g., HLA-DQα)

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How Many SNPs Would be Needed for Forensic Applications?

- **Answer depends upon allele frequencies at SNP sites, efficiency in different types of applications**
  - **For example, power of discrimination in identity testing; PE or PI in parentage analyses; LR in kinship assessment, etc.**
- **Chakraborty, et al. (1999, *Electrophoresis* 20: 1682-96) showed nomograms suggesting that the number of SNPs needed to equal the power of the current battery of STR loci would necessitate the use of several sets of syntenic SNPs**
  - **For example, SNPs residing on the same arm of several chromosomes**

Strategies for Improving Power of SNPs for Forensic Applications

- **Translate sets of SNPs into multiallelic markers**
- **Select a panel of SNP sets that satisfy conditions of the product rule**
  - **For example, statistically independent sets of SNPs**
- **Search for genome-wide availability of desired SNPs for feasibility of detection of such panels of SNPs**
- **Test the robustness of typing selected SNPs in forensic samples of compromised DNA quality**

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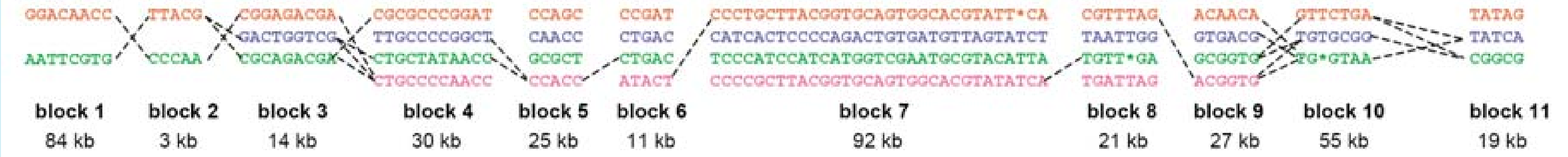
Haplotype Block (Haploblock)



Haplotype structure across 500 kb on 5q31 (Daly, M.J., et al. 2001, *Nat. Genet.* 29: 229-232)

- **Linkage disequilibrium (LD): allelic association between two loci (for example, SNP sites)**
- **Closely linked SNPs with high LD → haplotype blocks**
- **Human genome is composed of block-like structures of low haplotype diversity (strong LD within block) separated by recombination hot spots**
- **Complete LD among *n* linked SNPs →(*n* + 1) haplotypes**

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Advantages of Haploblock as Forensic Marker

- Can be typed in highly degraded samples
  - Where no results from STR analysis may be obtained
  - Improves the limited discrimination power of individual SNPs
- Haploblock can be considered as “pseudo STRs”
  - One haploblock → one “STR” locus
  - Different haplotypes → different “alleles”
- Each haplotype treated as a lineage marker like Y-chromosome and mtDNA
  - Exception – possible transmission from both parents following standard Mendelian principles

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HapMap Project (www.hapmap.org)

- **Three major populations (90 Caucasian, 90 African, 45 Chinese and 45 Japanese)**
- **Phase II data: > 3,000,000, SNPs**
  - **LD information: D', r2**
  - **Phase information**
  - **Genotype information**

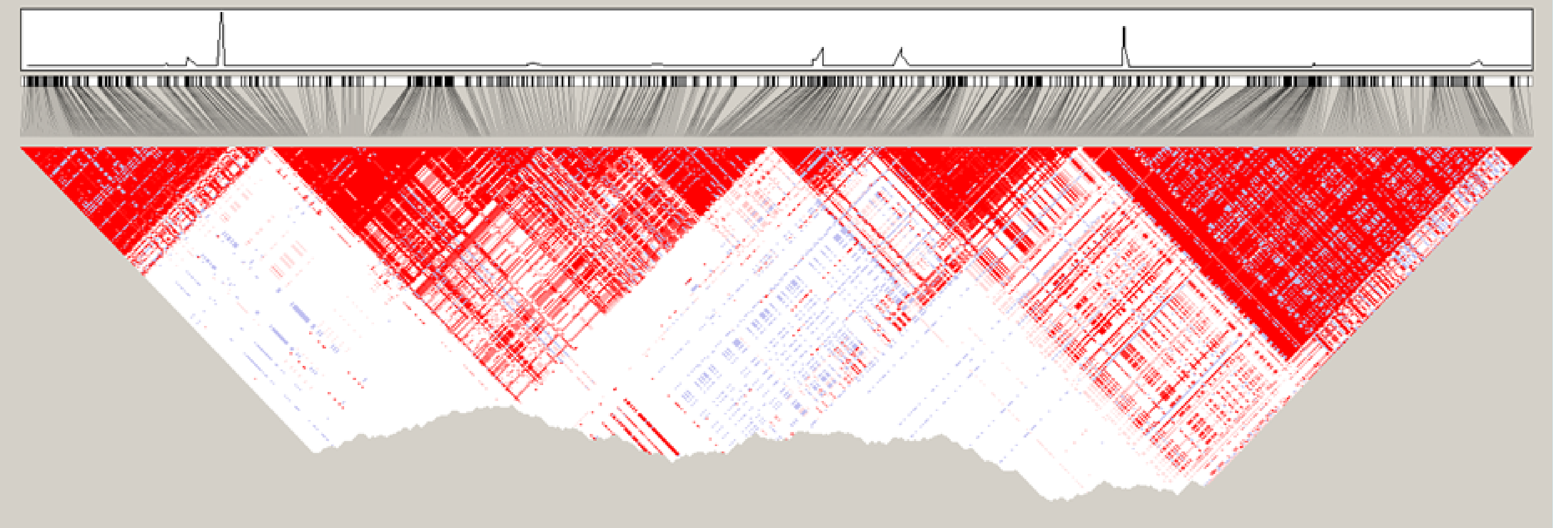

Image courtesy of http://hapmap.ncbi.nlm.nih.gov/

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Haploblock Selection Criteria

- Exist in all major populations (Caucasian, East Asian, African)
- Higher discrimination power (for example, lower match probability) than that of the individual SNPs within the block
- Hardy-Weinberg Equilibrium for each block
- No significant LD between blocks
- Sufficient number of candidate haploblocks in the whole genome

Parameters Used in Selection

- **Maximum match probability reduction per haploblock (mmpr)**
- **Minimum LD between SNPs: r^2**
- **Population substructure: maximum F_{st}**
- **Minimum heterozygosity (MinHet)**
- **Minimum number of haplotypes in each population (MinHap)**
- **Minimum number of SNPs per haploblock (MinSNP)**

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Best Parameter Set

- mmpr = 0.85
- r^2 = 0.7
  - No haploblock found with $r^2 \geq 0.8$
- F_{st} = 0.06
- MinHet = 0.2
- MinHap = 3
- MinSNP = 3

The best thresholds of parameters other than r^2 found on Chr1

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Haploblocks with Best Parameter Set

Chromo -some	Num. blocks with PS	Num. blocks with PS & HWE	Num. blocks with PS & HWE & LD filters (*n*)	Avg. Cum. MP of blocks (*b*)	Cum. Min. MP of SNPs (*s*)	MP reduction per block (*mpr*)	Num. Of SNPs
1	9	9	0				
2	23	14	1	0.3287	0.4050	0.8117	6
3	12	10	2	0.1144	0.1617	0.8412	9
4	21	15	1	0.2926	0.3765	0.7773	6
5	16	12	3	0.02633	0.05480	0.7833	25
6	15	10	0				
7	16	9	2	0.1035	0.1465	0.8403	30
8	18	12	2	0.1025	0.1518	0.8215	7
9	8	6	0				
10	15	8	1	0.3527	0.4169	0.8460	4
11	14	12	3	0.03872	0.06700	0.8209	13
12	12	5	1	0.3036	0.3890	0.7806	5
13	17	14	3	0.0344	0.06409	0.8123	14
14	10	6	3	0.02339	0.04789	0.7876	11
15	9	4	0				
16	7	4	1	0.3310	0.4053	0.8167	3
17	5	4	0				
18	8	7	1	0.3123	0.3689	0.8465	5
19	5	4	0				
20	6	1	0				
21	6	3	0				
22	1	1	0				
Total	253	170	24	1.059E-12	1.566E-10	0.8121	138

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Haploblock Example – Chr2

Haplotype Frequencies

Haplotype	CEU	JPT+CHB	YRI
010011	0	0	0.0417
011000	0.0083	0	0.0417
001000	0.3417	0.5167	0.4833
000111	0	0.0056	0
110010	0	0.0056	0
111000	0	0	0.0167
110111	0.5833	0.4611	0.4167
101000	0.0083	0	0
110110	0.05	0.0056	0
110100	0.0083	0.0056	0

Num. SNPs = 6
Num. haplotypes = 10
Avg. Het. = 0.5499
MP of block = 0.3287
Min. MP of SNPs = 0.4050
MP reduction = 0.8117
F_{st} = 0.024

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Different Haploblock Structure Among Populations

- $r^2 = 0.7$ and MinSNP = 3
  - 11,741 haploblocks in Caucasian
  - 12,456 haploblocks in Chinese
  - 12,237 haploblocks in Japanese
  - 7,318 haploblocks in African
- Population-specific haploblock selection criteria may be necessary to obtain best performing systems

Evidence Interpretation Based on Haploblocks

- Transfer evidence
- Mixture interpretation
- Kinship analysis

One genotype → …(A/T)(A/T)…

Two possible haplotype combinations → TT+AA
TA+AT

Transfer Evidence

- Compared a single source profile from crime scene evidence with profile of the suspect
- Exclusion or inclusion → compare the genotypes
- If inclusion, random match probability is:

$$\Pr(G) = \sum_{\substack{\text{Haplotype combination} \\ (H_i, H_j) \text{ composes } G}} p_i p_j$$

- Mixture versus single source sample

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Transfer Evidence – Example

Haplotype	Frequency
TT	0.4
TA	0.3
AA	0.2
AT	0.1

Genotype **. . . (A/T)(A/T) . . .**

$$\textbf{Match Probability} = \begin{cases} \textbf{TT/AA:} & 2 \times 0.4 \times 0.2 = 0.16 \\ \textbf{TA/AT:} & 2 \times 0.3 \times 0.1 = 0.06 \end{cases}$$

= 0.22

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Mixture Detection

- **Multiple contributors → at least four haplotypes**
- **The probability of a genotype (G):**

$$\Pr(G) = \sum_{\substack{Haplotype\ combination \\ (H_k,\ \ldots\ ,\ H_l)\ composes\ G}} \prod_{i=k}^{l} p_i$$

- **The probability of a genotype (G) given number of contributors (N)**

$$\Pr(G \mid N = 1) = \sum_{\substack{Haplotype\ combination \\ (H_i,\ H_j)\ composes\ G}} p_i p_j$$

$$\Pr(G \mid N = 2) = \sum_{\substack{Haplotype\ combination \\ (H_i,\ H_j,\ H_k,\ H_l)\ composes\ G}} p_i p_j p_k p_l$$

$$\Pr(G \mid N = 3) = \sum_{\substack{Haplotype\ combination \\ (H_i,\ H_j,\ H_k,\ H_l,\ Hm,\ H_n)\ composes\ G}} p_i p_j p_k p_l p_m p_n$$

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Exclusion Probability and Likelihood Ratio for Mixture Analysis

- **Probability of exclusion (PE)**

$$PE = 1 - \left(\sum_{H_i} p_i \right)^2 ,$$ **where Σ is over all H_i's that are contributors to *G***

- **Likelihood ratio (LR): S is suspect; V is victim; UN is an unknown contributor**

$$LR = \frac{\Pr(V + S)}{\Pr(V + UN)}$$

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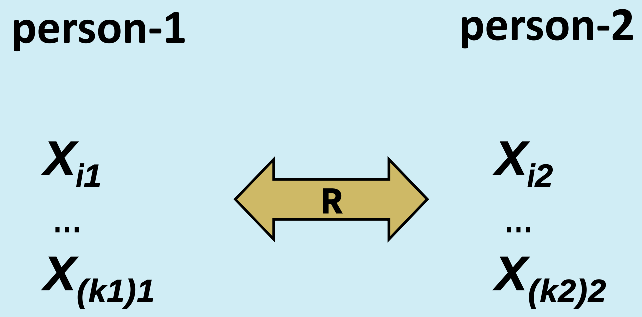
Pairwise Kinship Analysis

- **One genotype (G) has *k* haplotype combinations; $X_i = (H_{i1}, H_{i2})$ is i-th combination, with likelihood $P(X_i)$; w_i as the weight of X_i**

$$w_i = P(X_i) / \sum_{i=1}^{K} P(X_i)$$



Likelihood of these two persons given relationship (*R*):

$$L_{Block} = \sum_{i=1}^{k_1} \sum_{j=1}^{k_2} w_{i1} w_{j2} L(X_{i1}, X_{j2} \mid R)$$

Conclusions

- This is the first effort to assess the feasibility of genome-wide SNP haploblock structures for human identity testing encompassing all major forensic applications
- SNP haploblocks provide an alternative approach for forensic investigations, especially for highly degraded samples
- Haploblock selection depends on multiple criteria
- Consideration is needed for evidence interpretation based on haploblock results, because of multiple haplotype combinations that are possible for observed genotypes

Future Directions

- Portability/universality of efficient haploblocks to be tested with wider sets of genome data
- Alternatively, population-group specific panels of haploblocks have to be determined with validation data from anthropologically defined populations
- Robustness of genotyping in samples with compromised DNA quality (mimicking forensic samples) has to be tested

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Questions?

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