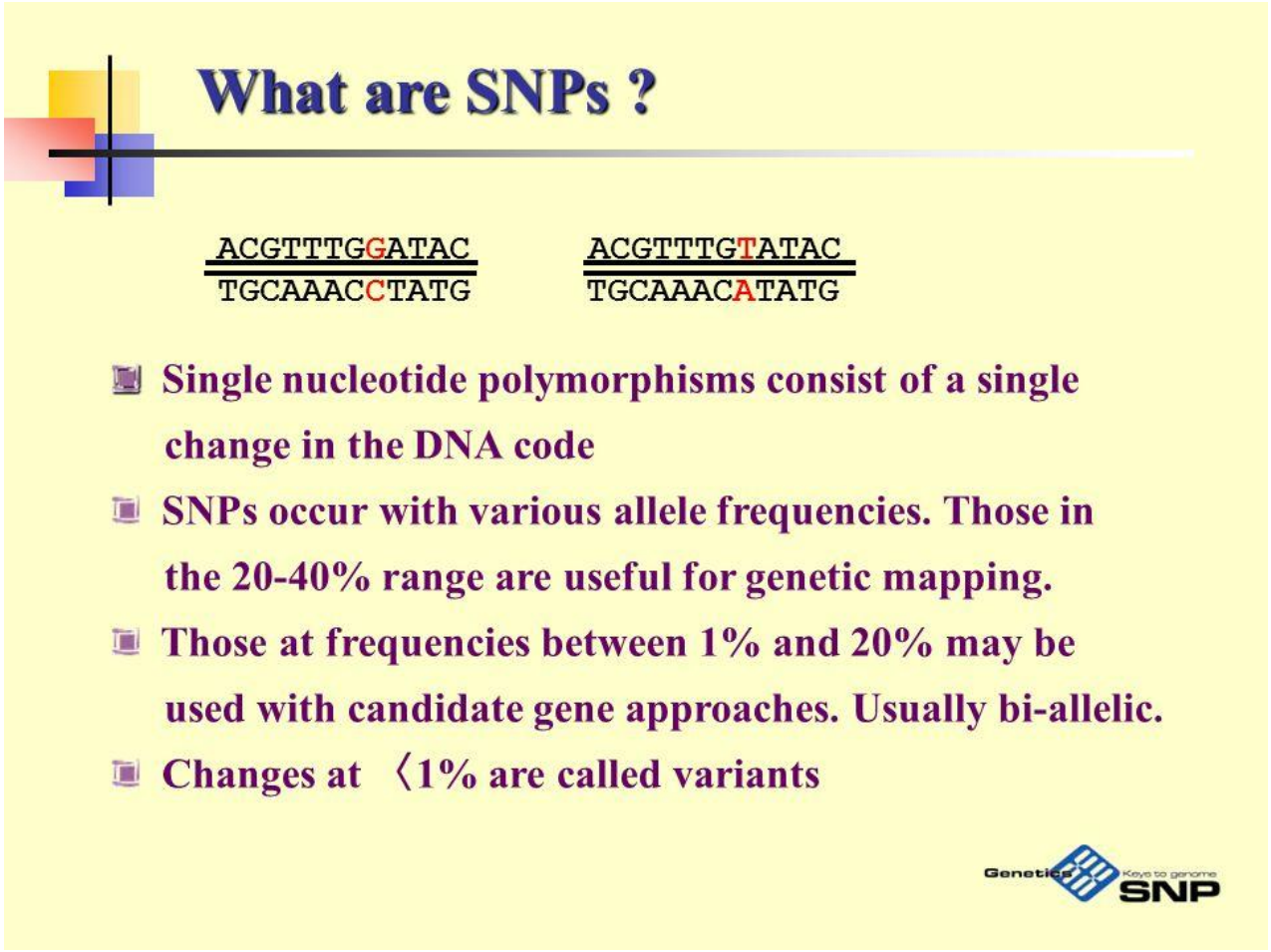
Genotyping

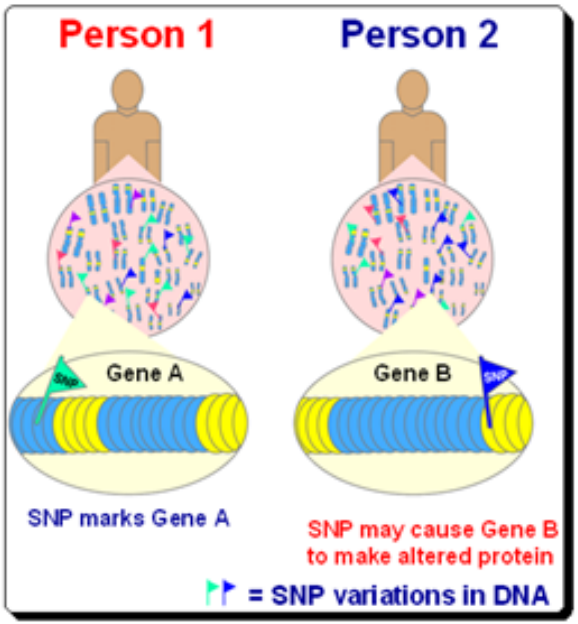
Genotyping

- Members of the same species have genetic variations. The process of determining differences in the genetic make-up (genotype) of an individual is called genotyping
- Genotyping is used for medical and forensic purposes

Single-nucleotide polymorphisms (SNPs)



WHY SNPs ARE SO IMPORTANT ?



1. SNPs can cause silent, harmless, harmful, or latent effects.
2. Most SNPs occur in noncoding regions and do not alter genes.
3. The remaining SNPs occur in coding regions. They could alter the protein made by that coding region, which in turn could influence a person's health.

"SNP is the key enabler in the realization of the concept of personalized medicine".

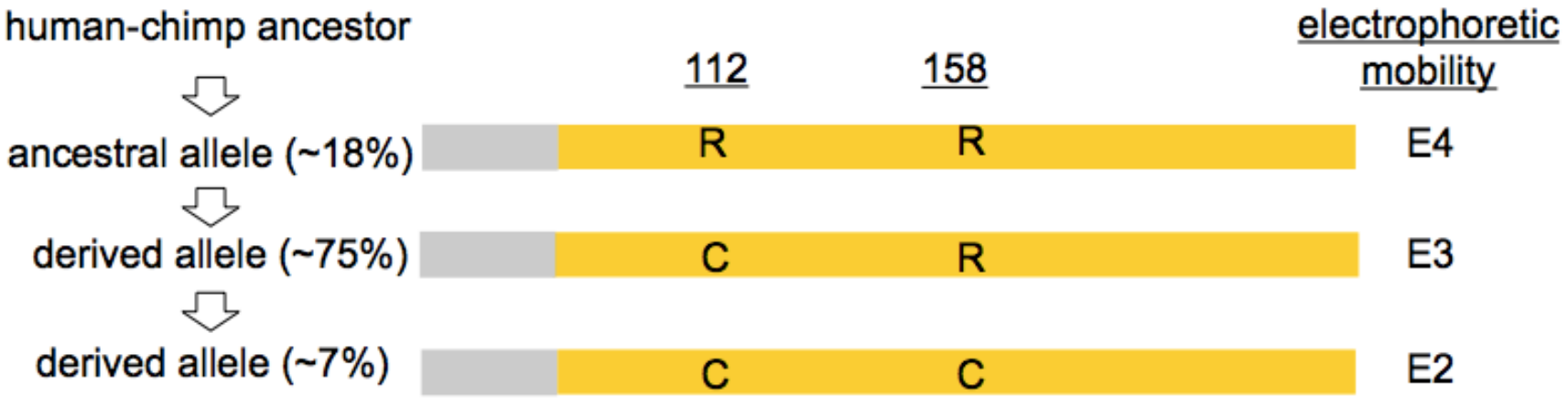
So, let us have a look how proteins are changed in presence of SNPs

Example of Genotyping: APOE gene

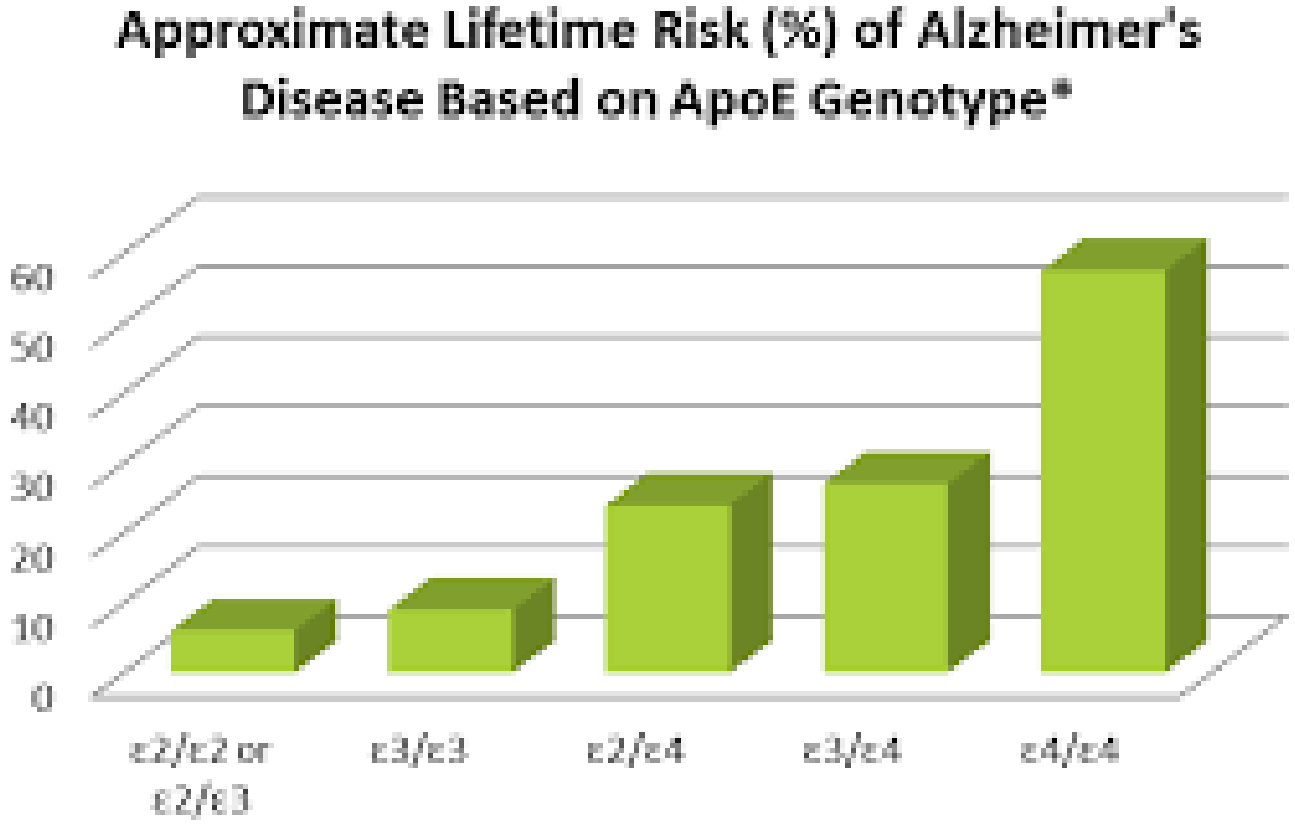
APOE

- Apolipoprotein E (ApoE) is a class of proteins involved in the metabolism of fats in the body.
- There are 3 variant alleles of APOE gene in human populations: APOE-ε2 (cys112, cys158), APOE-ε3 (cys112, arg158), and APOE-ε4 (arg112, arg158)

evolution of ApoE alleles



Estimated worldwide human allele frequencies of ApoE * in Caucasian population[57]			
Allele	**ε2**	**ε3**	**ε4**
General Frequency	8.4%	77.9%	13.7%
AD Frequency	3.9%	59.4%	36.7%


Approximate Lifetime Risk (%) of Alzheimer's Disease Based on ApoE Genotype*
60
50
40
30
20
10
0
ε2/ε2 or ε2/ε3
ε3/ε3
ε2/ε4
ε3/ε4
ε4/ε4

rs429358

- Rs429358 is SNP in the APOE gene that distinguishes allele APOE-ε4 from APOE-ε3 and APOE-ε2

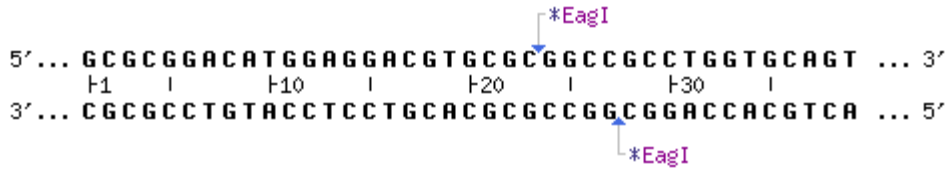
GCGCGGACATGGAGGACGTGTGCGGCCGCCTGGTGCAGT – 2 and 3

GCGCGGACATGGAGGACGTGCGCGGCCGCCTGGTGCAGT – 4

AflIII restriction site at rs429358

In ApoE2 and apoE3 alleles:

In ApoE4 allele:



Genotyping procedure:

- Amplify by PCR human DNA fragment containing rs429358.
- Digest the PCR product with AflIII.
- Analyze the digest on agarose gel electrophoresis

- There are several AflIII sites close to rs429358.
- We chose to amplify a 491 bp fragment of Chr19 that will contain **only one** AflIII site in **APOE2(3)** alleles and **no** AflIII sites in **APOE4** alleles.

We chose to amplify a 491 bp fragment of Chr19

APOE2 and APOE3 alleles:

GCCTACAAATCGGAACTGGAggaacaactgaccccggtggcggaggagac
gcgggcacggctgtccaaggagctgcaggcggcgcaggcccggctgggcg
cggacatggagg**ACGTGT**gcggccgcctggtgcagtaccgcggcgaggtg
AflIII
caggccatgctcggccagagcaccgaggagctgcgggtgcgcctcgcctc
ccacctgcgcaagctgcgtaagcggctcctccgcgatgccgatgacctgc
agaagcgcctggcagtgtaccaggccggggcccgcgagggcgccgagcgc
ggcctcagcgccatccgcgagcgcctggggcccctggtggaacagggccg
cgtgcgggccgccactgtgggctccctggccggccagccgctacaggagc
gggcccaggcctggggcgagcggctgcgcgcgcggatggaggagatgggc
agccggacccgcgaccgcctgg**ACGAGGTGAAGGAGCAGGT**

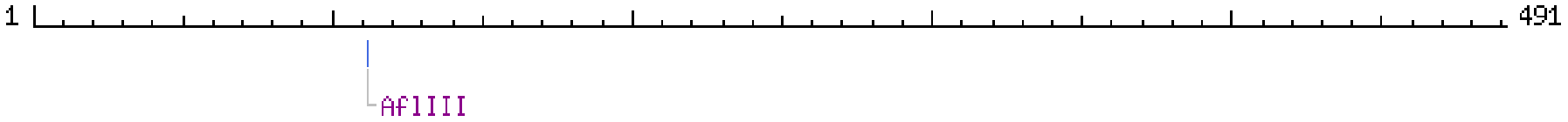
We chose to amplify a 491 bp fragment of Chr19

APOE4 alleles:

GCCTACAAATCGGAACTGGAggaacaactgaccccggtggcggaggagac
gcgggcacggctgtccaaggagctgcaggcggcgcaggcccggctgggcg
cggacatggagg**ACGTG**C**gcggccgcctggtgcagtaccgcggcgaggtg**
no *AflIII*
caggccatgctcggccagagcaccgaggagctgcgggtgcgcctcgcctc
ccacctgcgcaagctgcgtaagcggctcctccgcgatgccgatgacctgc
agaagcgcctggcagtgtaccaggccggggcccgcgagggcgccgagcgc
ggcctcagcgccatccgcgagcgcctggggcccctggtggaacagggccg
cgtgcgggccgccactgtgggctccctggccggccagccgctacaggagc
gggcccaggcctggggcgagcggctgcgcgcgcggatggaggagatgggc
agccggacccgcgaccgcctgg**ACGAGGTGAAGGAGCAGGT**

APOE fragment digestion with AflIII

APOE3 and ApoE2



#	Ends	Coordinates	Length (bp)
1	AflIII-(RightEnd)	114-491	378
2	(LeftEnd)-AflIII	1-113	113

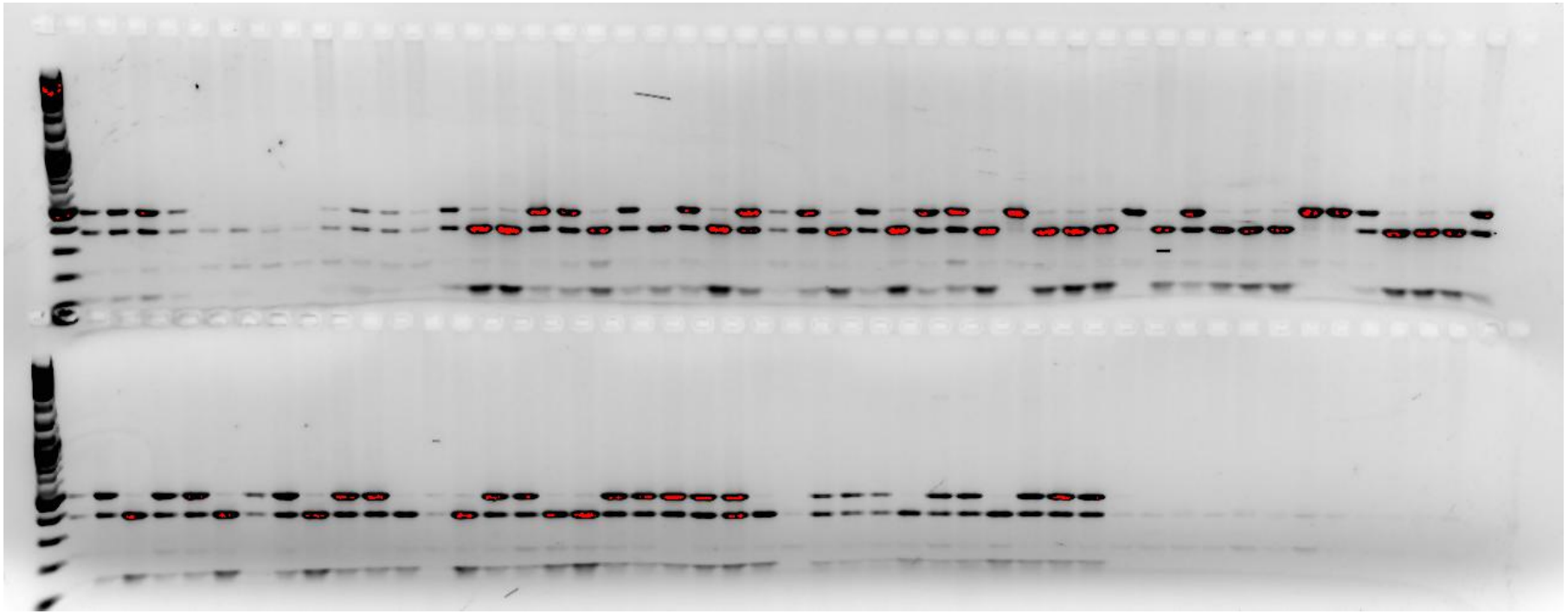
APOE4 fragment will not be cut with AflIII

Each person has two alleles of APOE gene. Let's consider all possible combinations of two alleles.

After amplification and digestion with AflIII:

- APOE2(3)/APOE2(3) will yield 491bp fragments
- APOE4/APOE4 will yield 378bp and 113bp fragments
- APOE2(3)/APOE4 will yield 491bp, 378bp and 113bp fragments

APOE4 genotyping of a panel of human DNA



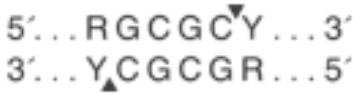
rs7412

- rs7412 is SNP in the APOE gene that distinguishes allele APOE-ε2 from APOE-ε3 and APOE-ε4

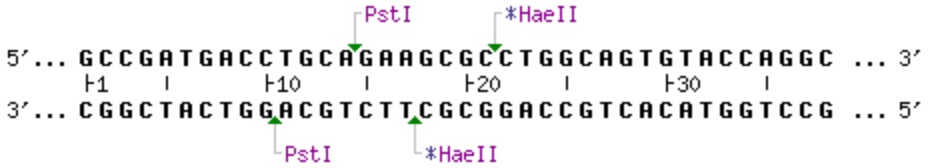
GCCGATGACCTGCAGAAGCGCCTGGCAGTGTACCAGGC – 3 and 4

GCCGATGACCTGCAGAAGTGCCTGGCAGTGTACCAGGC – 2

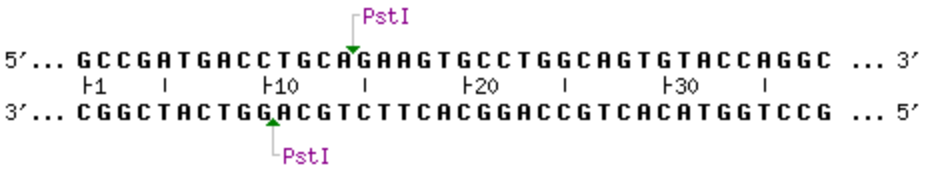
HaeII restriction site at rs7412



In ApoE3 and apoE4 alleles:

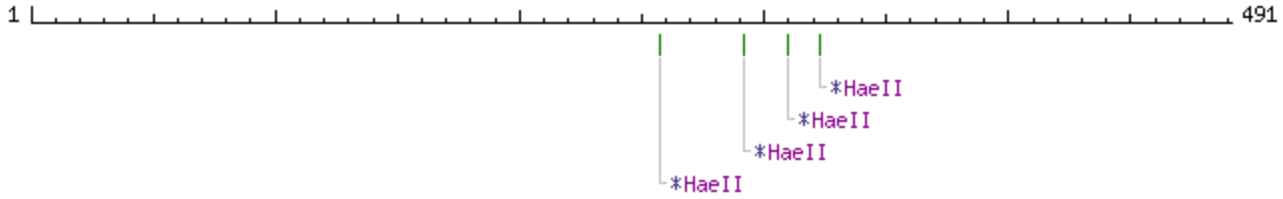


In ApoE2 allele:

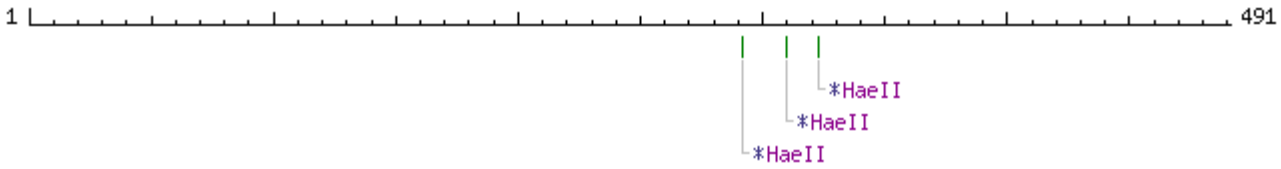


APOE fragment digestion with HaeII

APOE3 and ApoE4



APOE2



List of fragments in the order on the chromosome:

APOE3 and ApoE4

#	Ends	Coordinates	Length (bp)
1	(LeftEnd)-HaeII	1-258	258
2	HaeII-HaeII	259-293	35
3	HaeII-HaeII	294-311	18
4	HaeII-HaeII	312-324	13
5	HaeII-(RightEnd)	325-491	167

APOE2

#	Ends	Coordinates	Length (bp)
1	(LeftEnd)-HaeII	1-293	293
2	HaeII-HaeII	294-311	18
3	HaeII-HaeII	312-324	13
4	HaeII-(RightEnd)	325-491	167

List of fragments sorted by size:

APOE3 and ApoE4

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1	(LeftEnd)-HaeII	1-258	258
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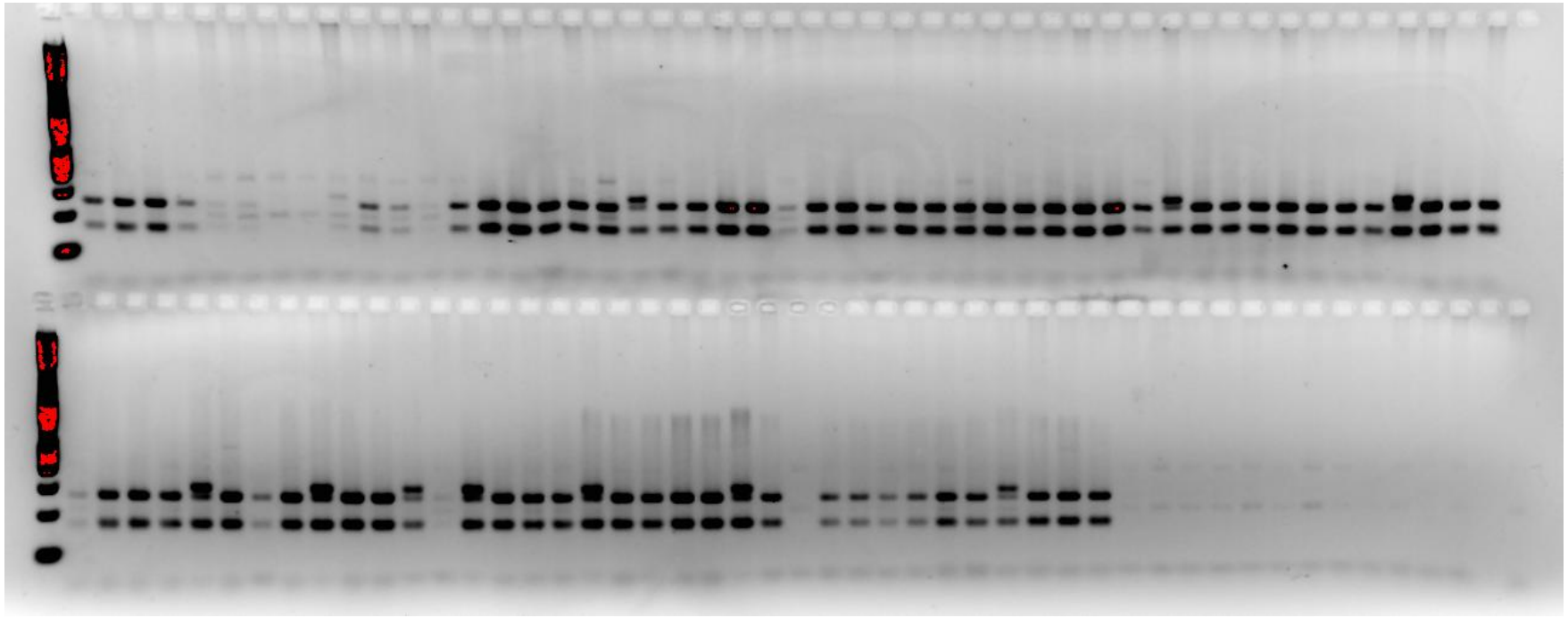

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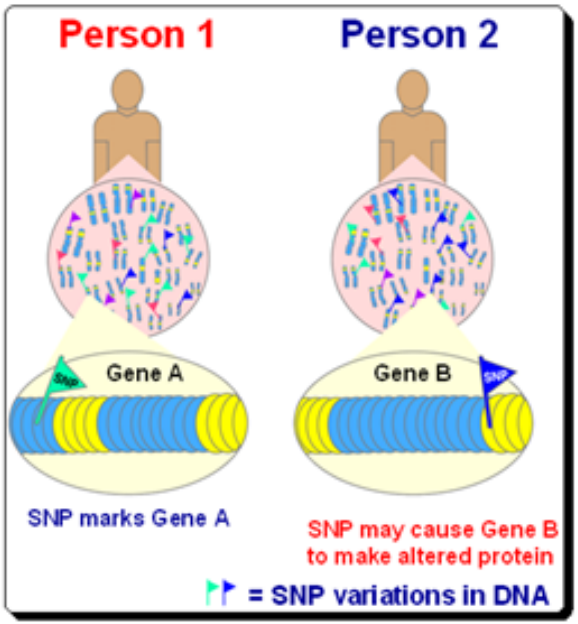

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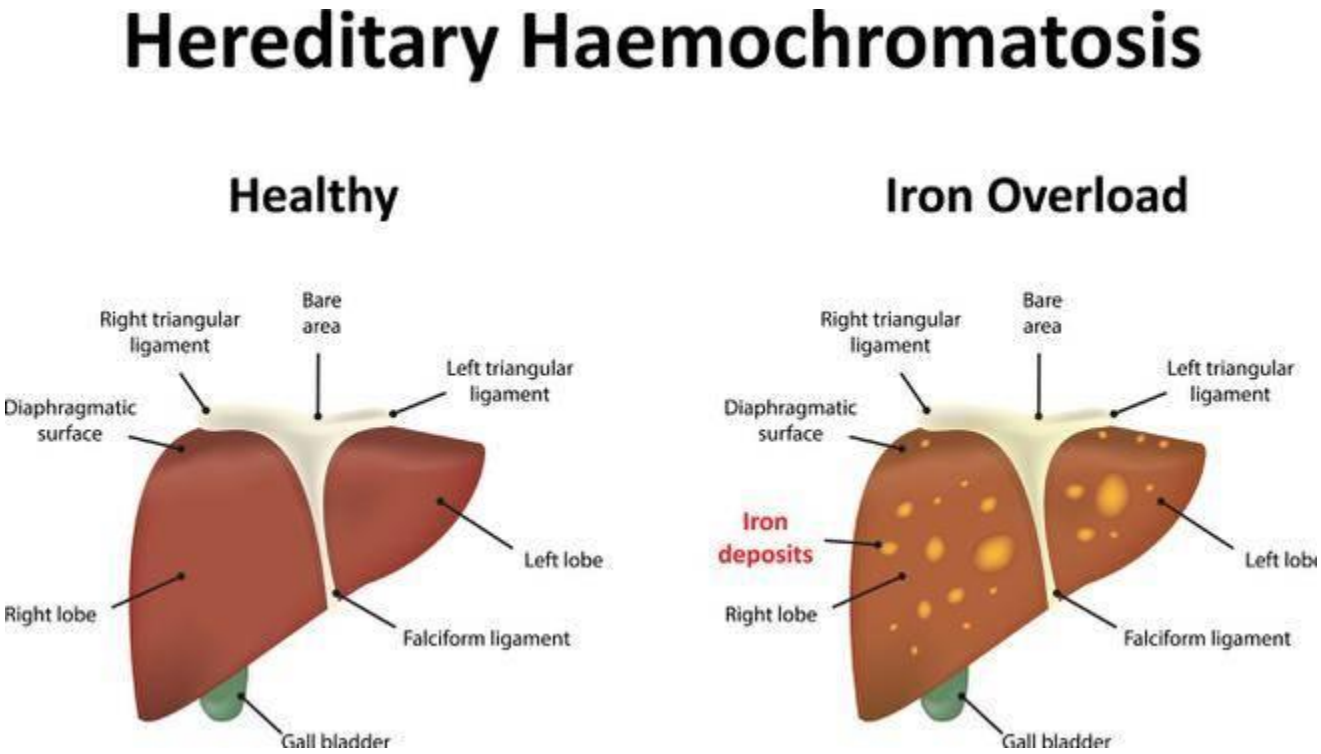
So, let us have a look how proteins are changed in presence of SNPs

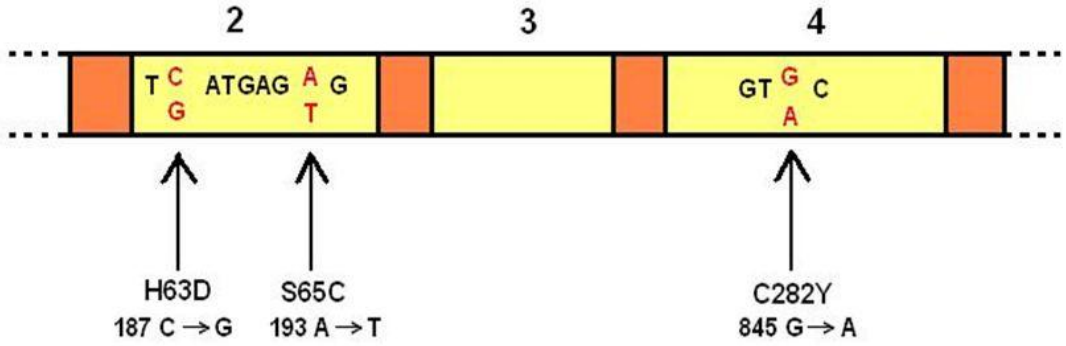
Example – hereditary hemochromatosis



Genetic basis

- Principal HFE gene defect was first described in 1996,
- Tightly linked to the HLA-A locus on chromosome 6p
- **G-to-a missense mutation** ;
- **Cysteine → tyrosine at 282 (C282Y)**
  - **C282Y homozygotes account for 80%-85%**
- Histidine → aspartate at 63 (h63d)
- Serine → cysteine at 65 (s65c)

HFE gene mutations



- Methodology: DNA is isolated from peripheral blood and is analyzed for two (2) mutations in the HFE gene, which have been associated with hereditary hemochromatosis. The analysis is performed by restrictionanalysis of PCR-amplified segments of the HFE gene:
- C282Y mutation is detected by restriction with Rsa1, and
- the H63D mutation is detected with Mbo1.
- the S65C mutation is relatively rare. There is no convenient restriction site to detect it.