Practical applications of modern methods of DNA analysis

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)

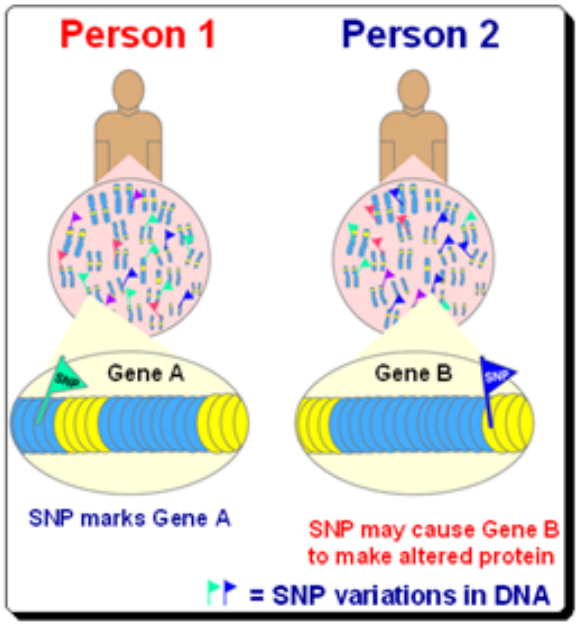
What are SNPs ?

```
ACGTTTGGATAC        ACGTTTGTATAC
TGCAAACCTATG        TGCAAACATATG
```

- Single nucleotide polymorphisms consist of a single change in the DNA code
- SNPs occur with various allele frequencies. Those in the 20-40% range are useful for genetic mapping.
- Those at frequencies between 1% and 20% may be used with candidate gene approaches. Usually bi-allelic.
- Changes at 〈1% are called variants

Genetics Keys to genome SNP

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".

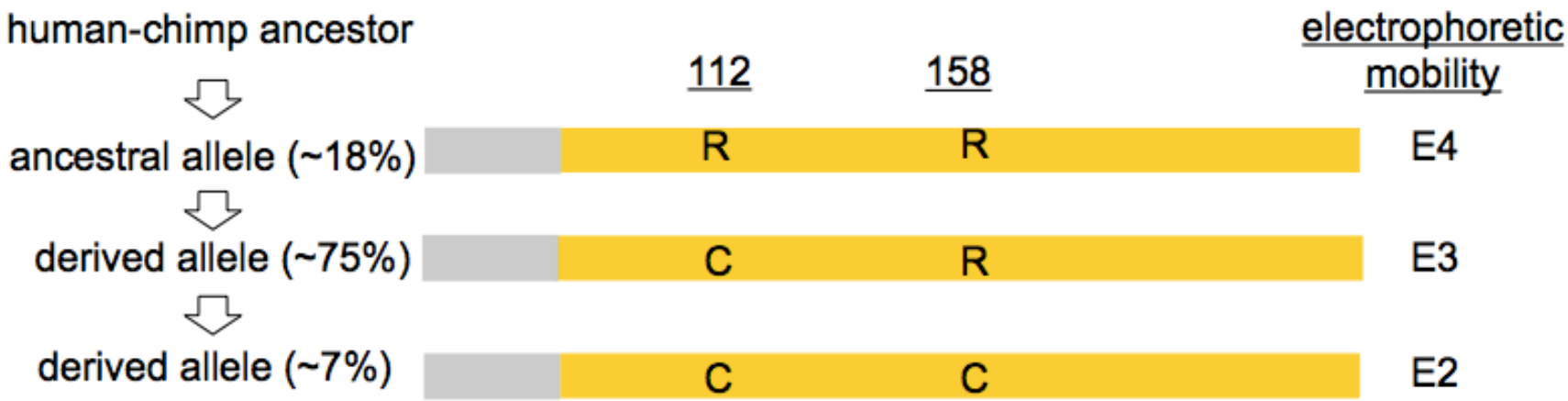
So, let us have a look how proteins are changed in presence of SNPs

Example of Genotyping: APOE gene

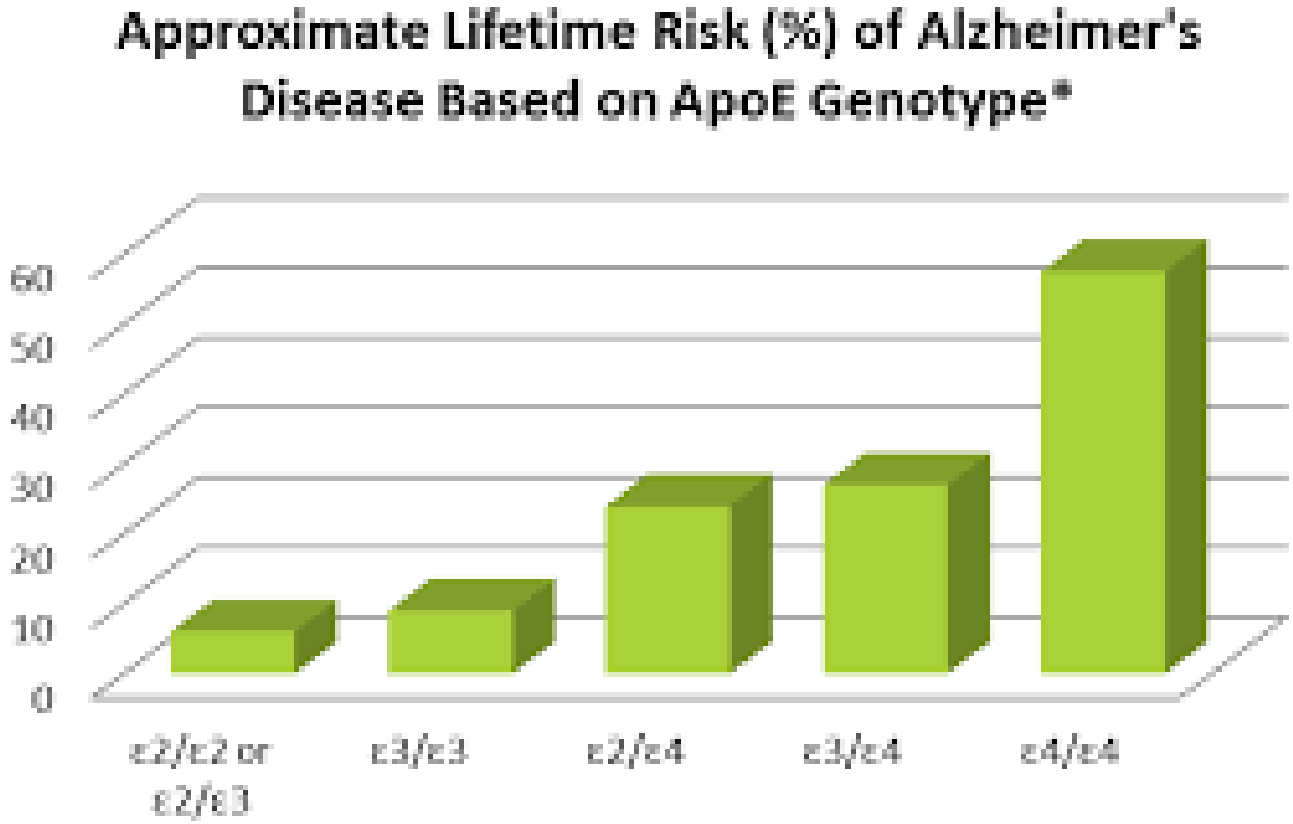
APOE

- 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

AflII restriction site at rs429358

5′...ACRYGT...3′
3′...TGYRCA...5′

In ApoE2 and apoE3 alleles:

```
                                AflIII     *EagI
5′... GCGCGGACATGGAGGACGTGTGCGGCCGCCTGGTGCAGT ... 3′
      ⊢1    |      ⊢10    |      ⊢20    |      ⊢30    |
3′... CGCGCCTGTACCTCCTGCACACGCCGGCGGACCACGTCA ... 5′
                                  AflIII     *EagI
```

In ApoE4 allele:

```
                                        *EagI
5′... GCGCGGACATGGAGGACGTGCGCGGCCGCCTGGTGCAGT ... 3′
      ⊢1    |      ⊢10    |      ⊢20    |      ⊢30    |
3′... CGCGCCTGTACCTCCTGCACGCGCCGGCGGACCACGTCA ... 5′
                                        *EagI
```

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

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GCCTACAAATCGGAACTGGAggaacaactgaccccggtggcggaggagac
gcgggcacggctgtccaaggagctgcaggcggcgcaggcccggctgggcg
cggacatggaggacgtgtgcggccgcctggtgcagtaccgcggcgaggtg
caggccatgctcggccagagcaccgaggagctgcgggtgcgcctcgcctc
ccacctgcgcaagctgcgtaagcggctcctccgcgatgccgatgacctgc
agaagcgcctggcagtgtaccaggccggggcccgcgagggcgccgagcgc
ggcctcagcgccatccgcgagcgcctggggcccctggtggaacagggccg
cgtgcgggccgccactgtgggctccctggccggccagccgctacaggagc
gggcccaggcctggggcgagcggctgcgcgcgcggatggaggagatgggc
agccggacccgcgaccgcctgg**ACGAGGTGAAGGAGCAGGT**

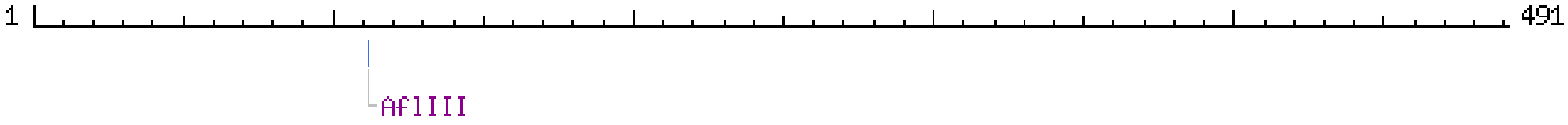
We chose to amplify a 491 bp fragment of Chr19

GCCTACAAATCGGAACTGGAggaacaactgaccccggtggcggaggagac
gcgggcacggctgtccaaggagctgcaggcggcgcaggcccggctgggcg
cggacatggaggacgtgtgcggccgcctggtgcagtaccgcggcgaggtg
caggccatgctcggccagagcaccgaggagctgcgggtgcgcctcgcctc
ccacctgcgcaagctgcgtaagcggctcctccgcgatgccgatgacctgc
agaagtgcctggcagtgtaccaggccggggcccgcgagggcgccgagcgc
ggcctcagcgccatccgcgagcgcctggggcccctggtggaacagggccg
cgtgcgggccgccactgtgggctccctggccggccagccgctacaggagc
gggcccaggcctggggcgagcggctgcgcgcgcggatggaggagatgggc
agccggacccgcgaccgcctgg**ACGAGGTGAAGGAGCAGGT**

dubel

APOE fragment digestion with AflIII

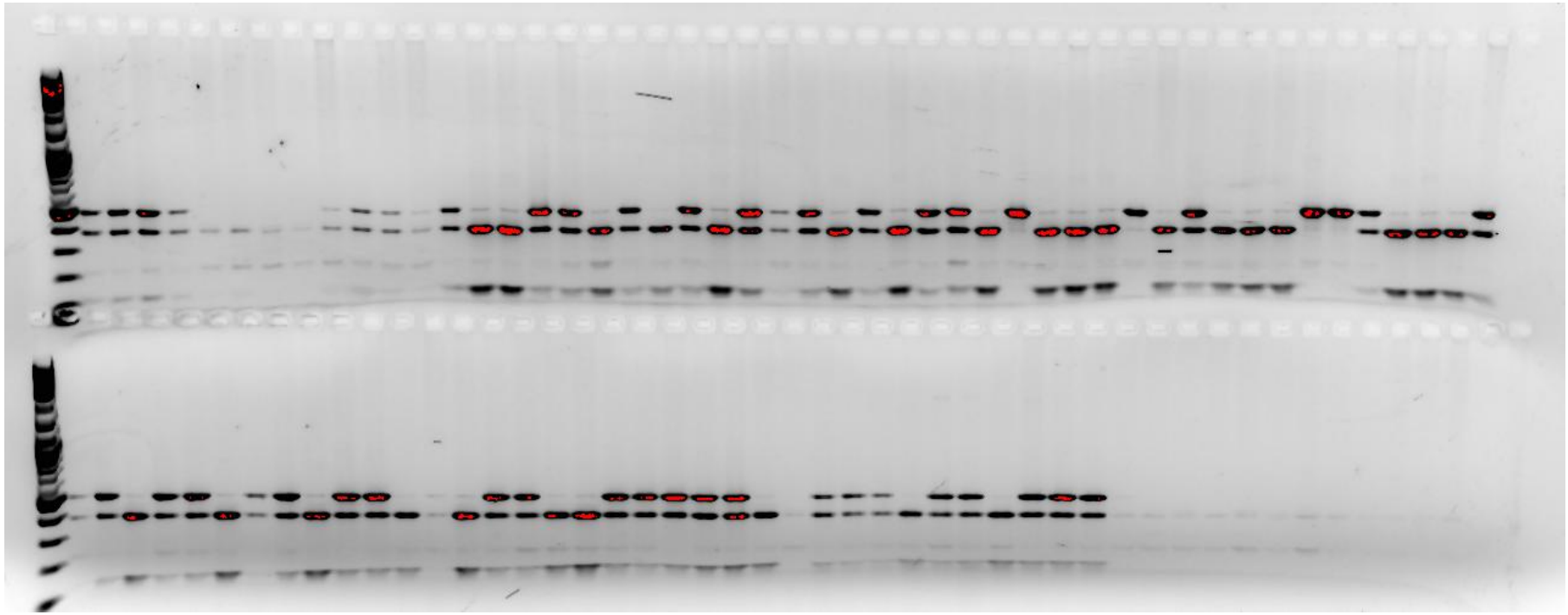
APOE3 and ApoE2



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

APOE4 fragment will not be cut with AflIII

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

```
5′...RGCGCY...3′
3′...YCGCGR...5′
```

In ApoE3 and apoE4 alleles:

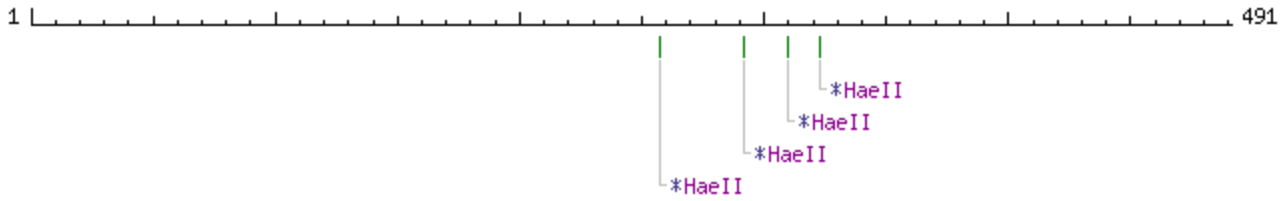
```
                        PstI        *HaeII
5′... GCCGATGACCTGCAGAAGCGCCTGGCAGTGTACCAGGC ... 3′
      1    |      10    |      20    |      30    |
3′... CGGCTACTGGACGTCTTCGCGGACCGTCACATGGTCCG ... 5′
                 PstI        *HaeII
```

In ApoE2 allele:

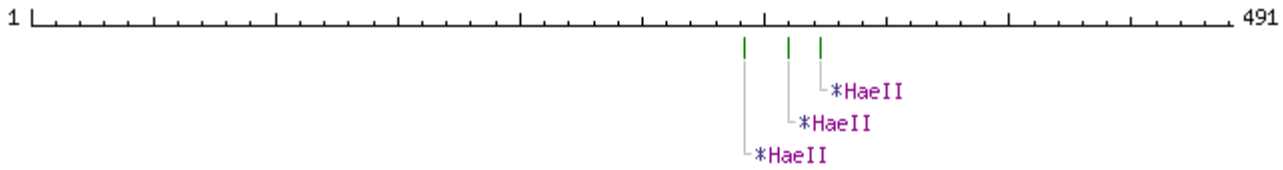
```
                        PstI
5′... GCCGATGACCTGCAGAAGTGCCTGGCAGTGTACCAGGC ... 3′
      1    |      10    |      20    |      30    |
3′... CGGCTACTGGACGTCTTCACGGACCGTCACATGGTCCG ... 5′
                 PstI
```

APOE fragment digestion with HaeII

APOE3 and ApoE4



APOE2



List of fragments:

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

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

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

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

APOE2 genotyping of a panel of human DNA