Restriction enzymes and their practical usage

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Presentation Plan

- Description
- General information
- Types
- Usage in general medicine
- Usage in molecular pathology
- A practical sample for usage of restriction enzymes in Molecular Pathology

Description

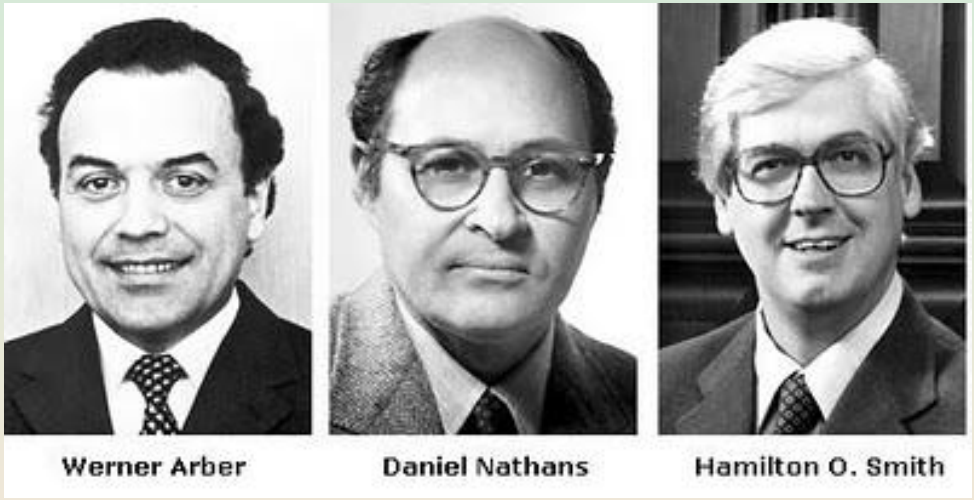
- **Restriction enzymes (RE);**

  - REs are **DNA-cutting enzymes**.

  - Because of this feature, they are often called **restriction endonucleases**.

General information

- Daniel Nathans, Verner Arber and Hamilton Smith have been awarded with Nobel prize in 1978.



General information

- These enzymes are found in bacteria and archaea and provide a defense mechanism against invading viruses
- Almost all bacteria has at least one restriction enzyme

General information

- Over 3000 restriction enzymes have been studied in detail, and more than 600 of these are available commercially

  - EcoRI
  - HaeIII
  - DdeI
  - BglI
  - HphI
  - BsaHI
  - Mae III
  - OpnII
  - MspI
  - BbsI

Denomination

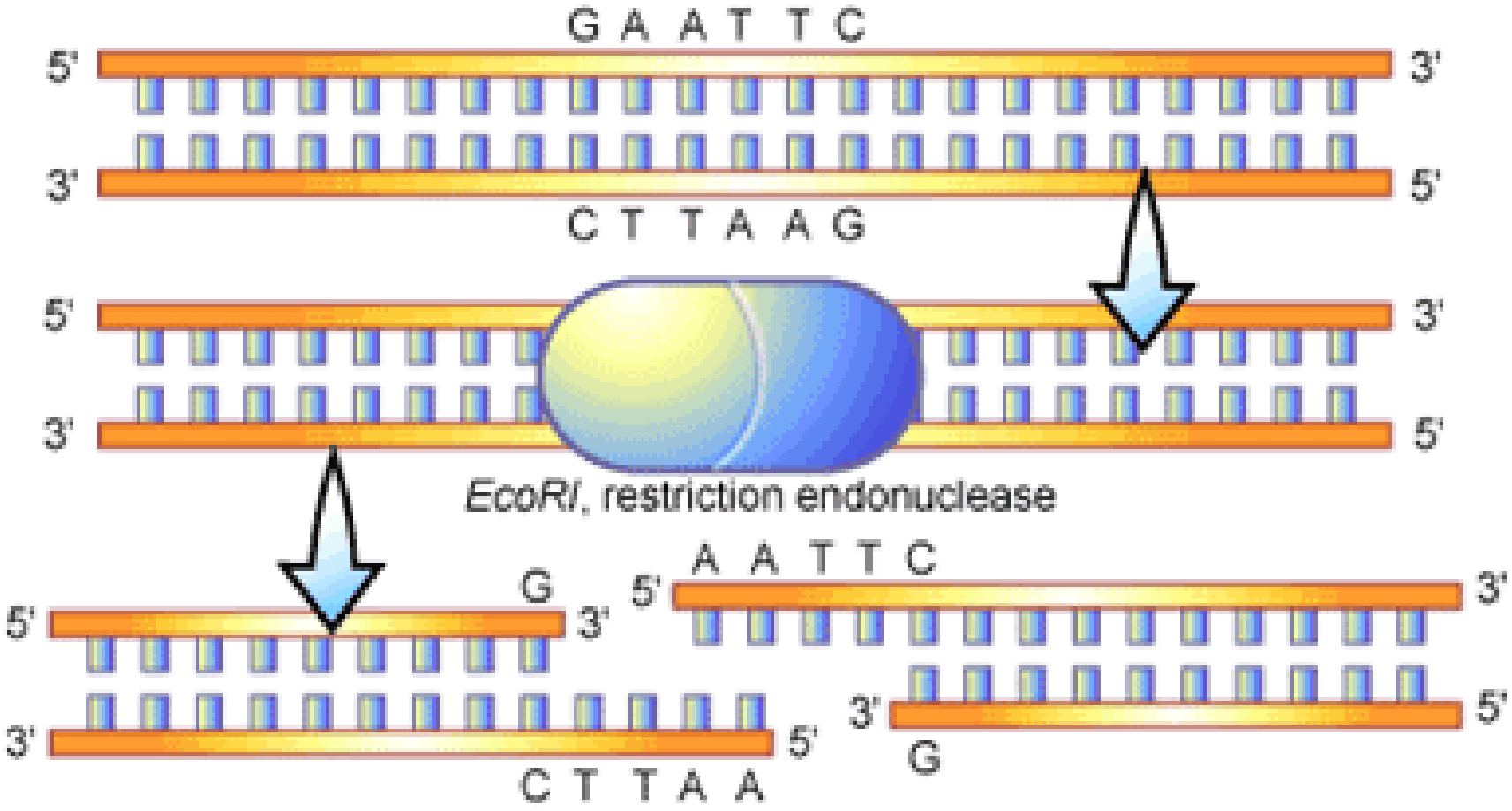
- ***EcoRI***
  - ***E = Escherichia (genus)***
  - ***co= coli (species)***
  - **R = RY 13 (strain)**
  - **I = the first RE, derivated from this strain**

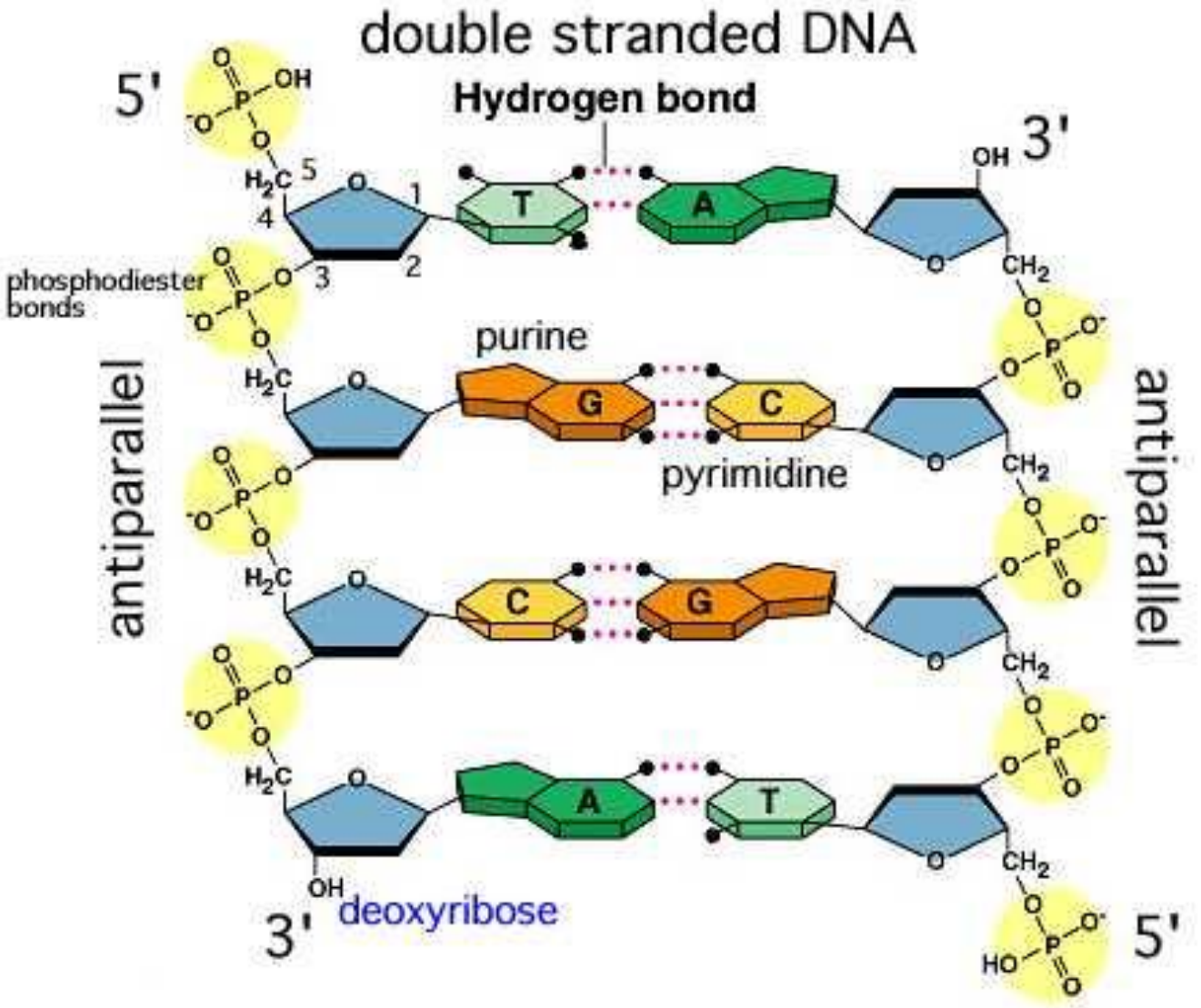
- ***HindIII***
  - ***H = Haemophilus (genus)***
  - **in = influenzae *(species)***
  - **d = Rd (strain)**
  - **III = the third RE, derivated from this strain**

DNA identification and cutting mechanism of RE

- REs have 2 functional subunit:
  - DNA identification region and
  - Catalytic region

EcoRI RE


G A A T T C
5'
3'
3'
5'
C T T A A G
5'
3'
3'
5'
EcoRI, restriction endonuclease
G
5'
3'
3'
5'
C T T A A
A A T T C
5'
3'
3'
5'
G


double stranded DNA
Hydrogen bond
5'
3'
phosphodiester bonds
purine
pyrimidine
antiparallel
antiparallel
T
A
G
C
C
G
A
T
3'
deoxyribose
5'

Classification

- REs
  - are categorized into four groups (Types I, II III, and IV) based on
    - their composition and cofactor requirements,
    - the nature of their target sequence,
    - the position of their DNA cleavage site relative to the target sequence.

Usage in general medicine

- Recombinant DNA technoligies
- Confirmation of mutations
- Haplotyping
- To detect known point mutations
- DNA mapping
- Preparation of probes
- .
- .

Recombinant DNA's

- Recombinant DNA's;
  - **Recombinant DNA** (**rDNA**) molecules are DNA sequences that result from the use of laboratory methods (molecular clonning). They are not exist in nature,
  - DNA fragments are cut out from multiple sources, and brought together to create new DNA sequences

Usage in medicine

Applications of recombinant DNA technology

- Recombinant DNA is widely used in biotechnology, medicine or research.
- Recombinant DNA is used to identify, map and sequence genes, and to determine their function.
  - Recombinant human **insulin**
  - Recombinant human **growth hormone**
  - Recombinant blood **clotting factor VIII**
  - Recombinant **hepatitis B vaccine**

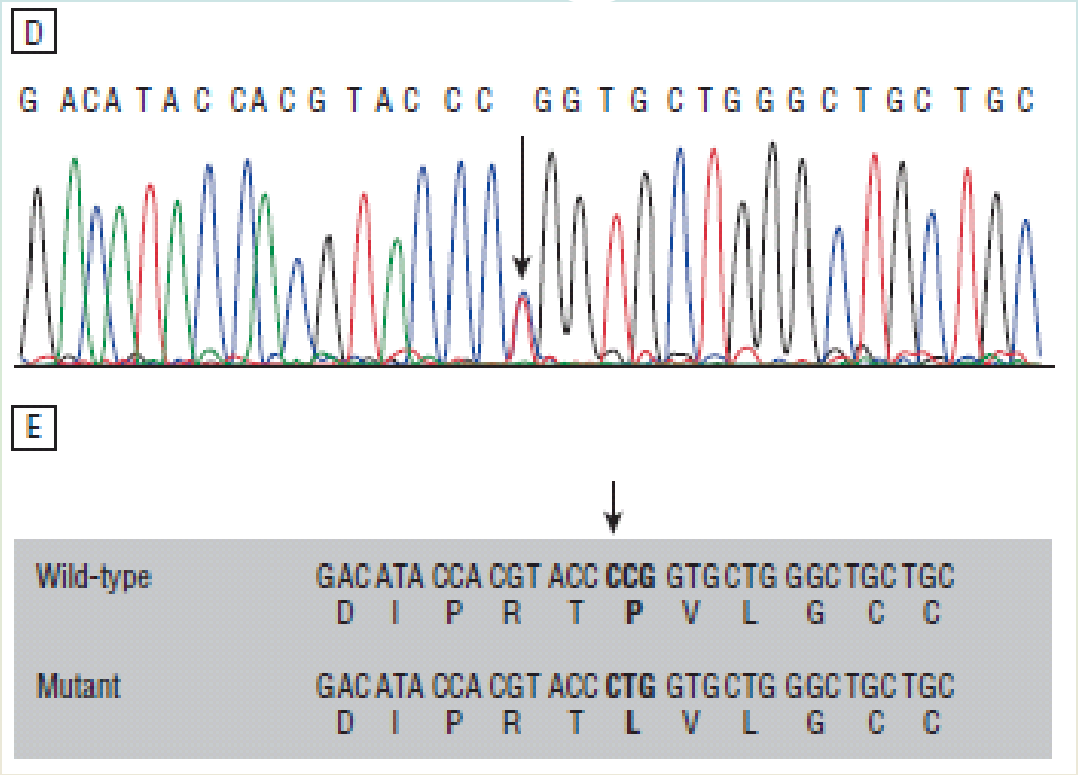
Usage in medicine

Confirmation of point mutation

- 9 year-old girl
- Hypotonia at birth
- Liver disfunction, weakness and hearing loss

- Muscle biopsy is diagnosed as Mitochondrial Disease

Confirmation for point mutation



PolG1 gene, 20. exon, C3218T substitution, P1073L (prolin to leucin)

Confirmation for point mutation

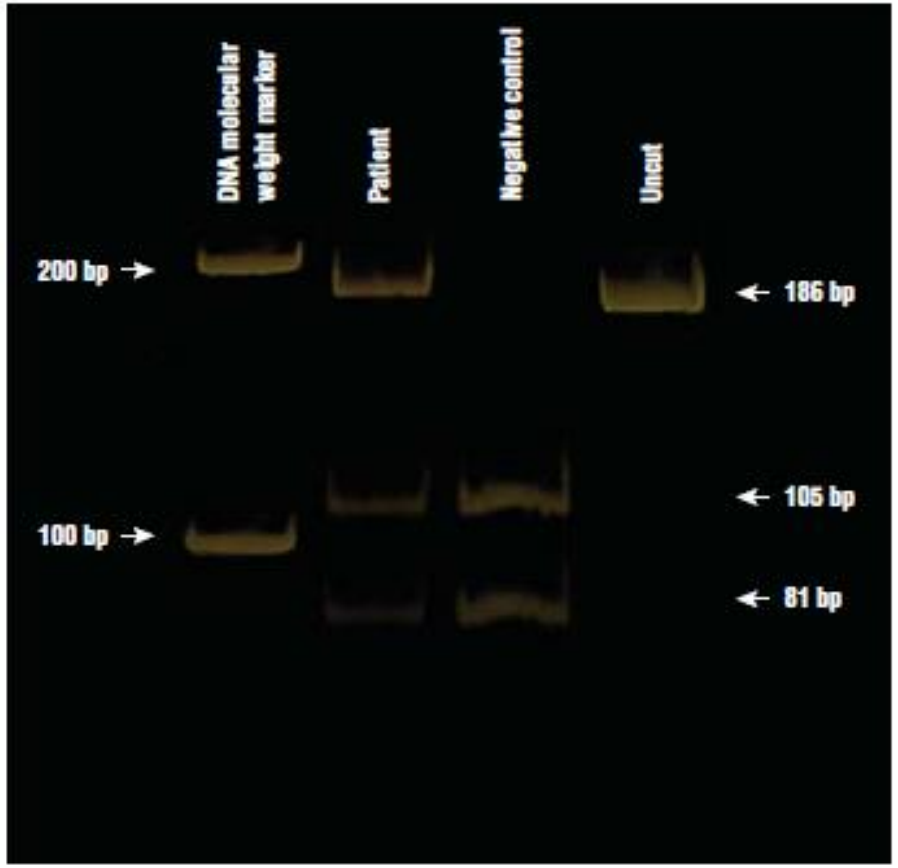
Primers;
F=5'-GGAAGAAGTGGGAGGTGGTT-3'
R=5'-CCATGCTCCAAAGGTAGCAA-3'

Restriction enzyme;
*Msp*I

Mutant fragment;
186*bp* (no cutting point)

Wild type fragment:
105 ve 81 (there is one cutting point)

Patient is heterozygous for C3218T in PolG



A novel PolG gen mutation in 4 children with Alpers-like hepatocerebral syndromes. Kurt et. al. *Arch Neurol. 2010;67(2):239-244*

Haplotyping

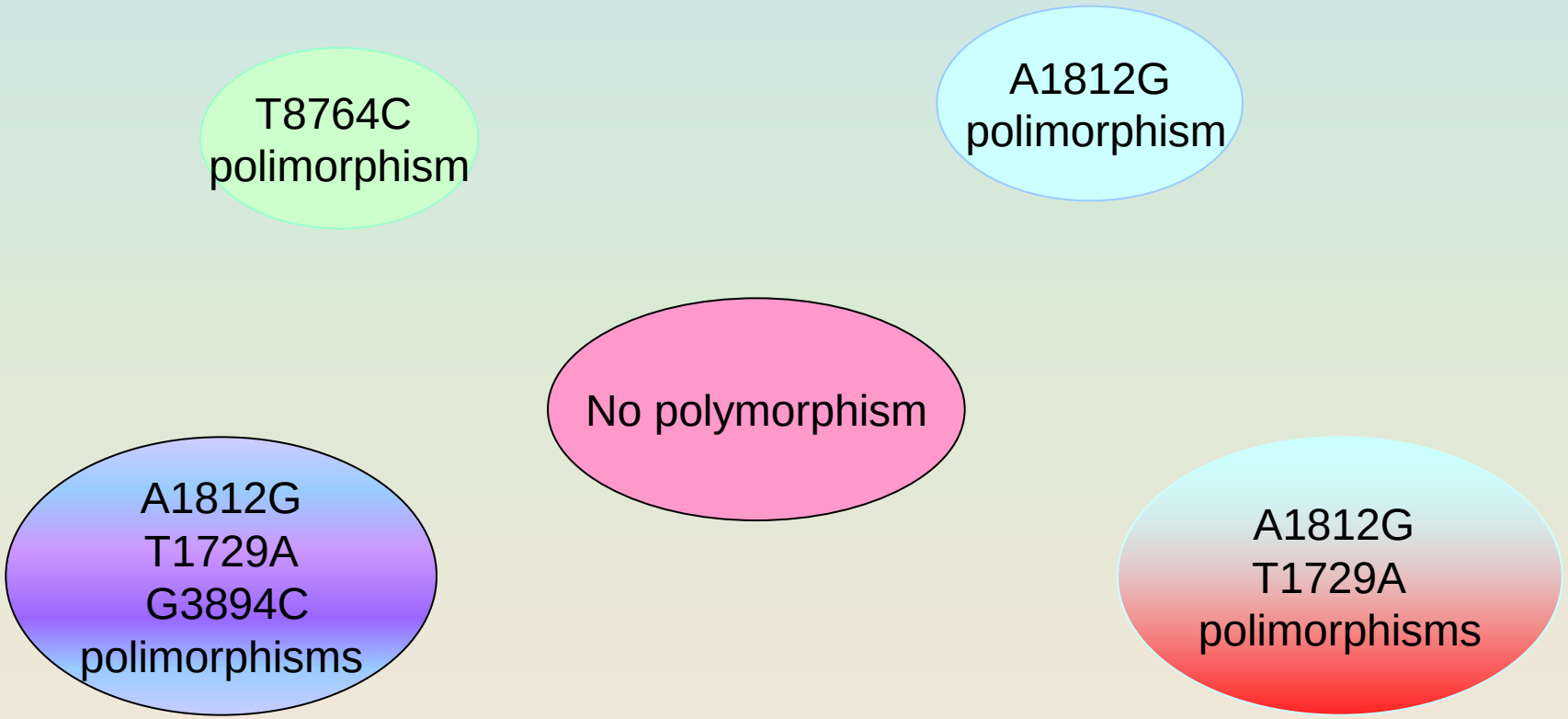
- Nucleotide substitutions might be pathogenic or non-pathogenic.
- Humanbody can live with non-pathogenic DNA mutations
- Non-pathogenic mutations accumulate in years and transfered to next generation
- All individuals has a set of polymorphism spesific to their population.

Usage in medicine

Haplotyping

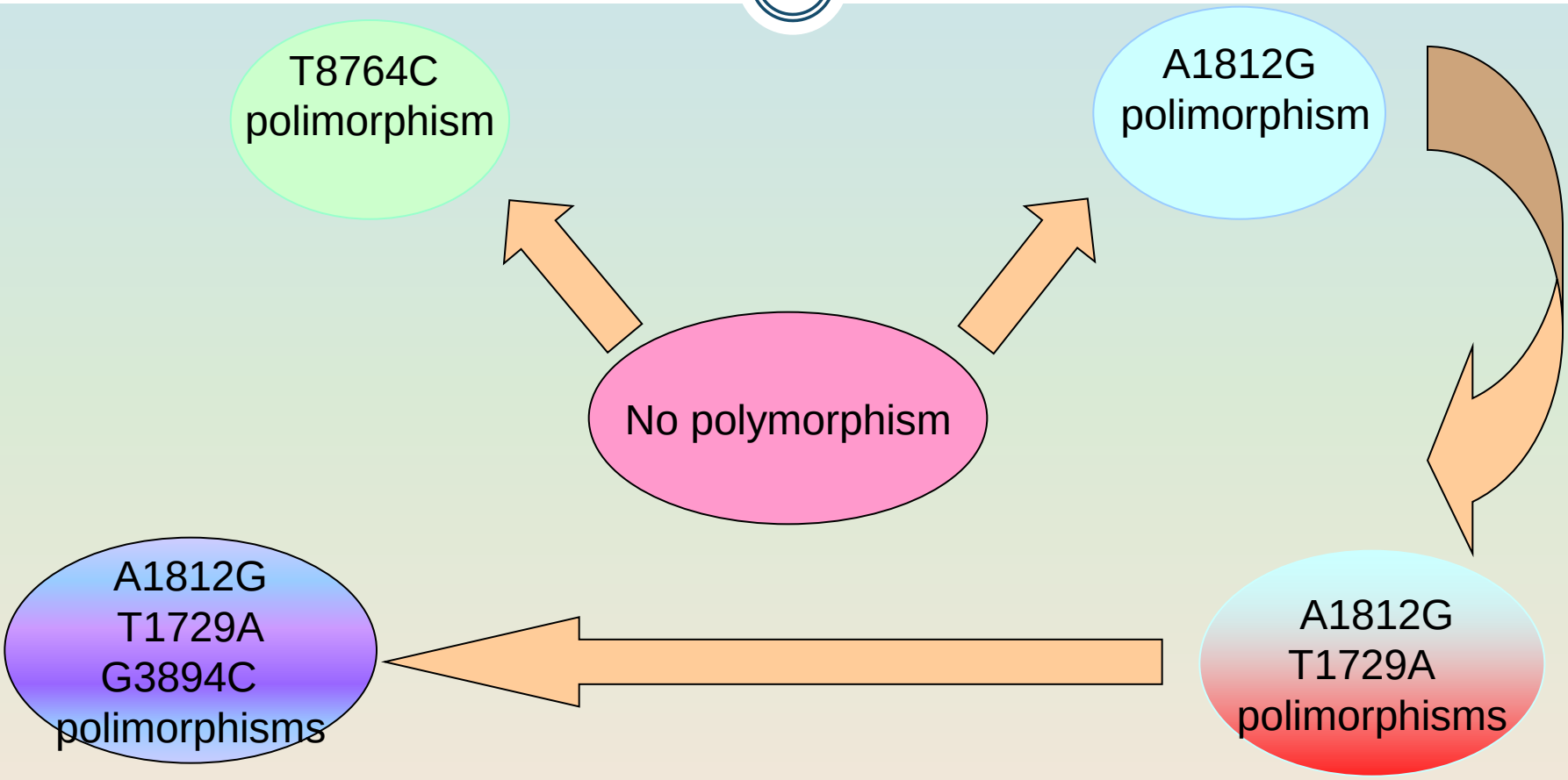
- With this «set of polymorphism»;
  - Races might be expected
  - Parents might be detected
  - Migration maps might be constructed

Haplotyping



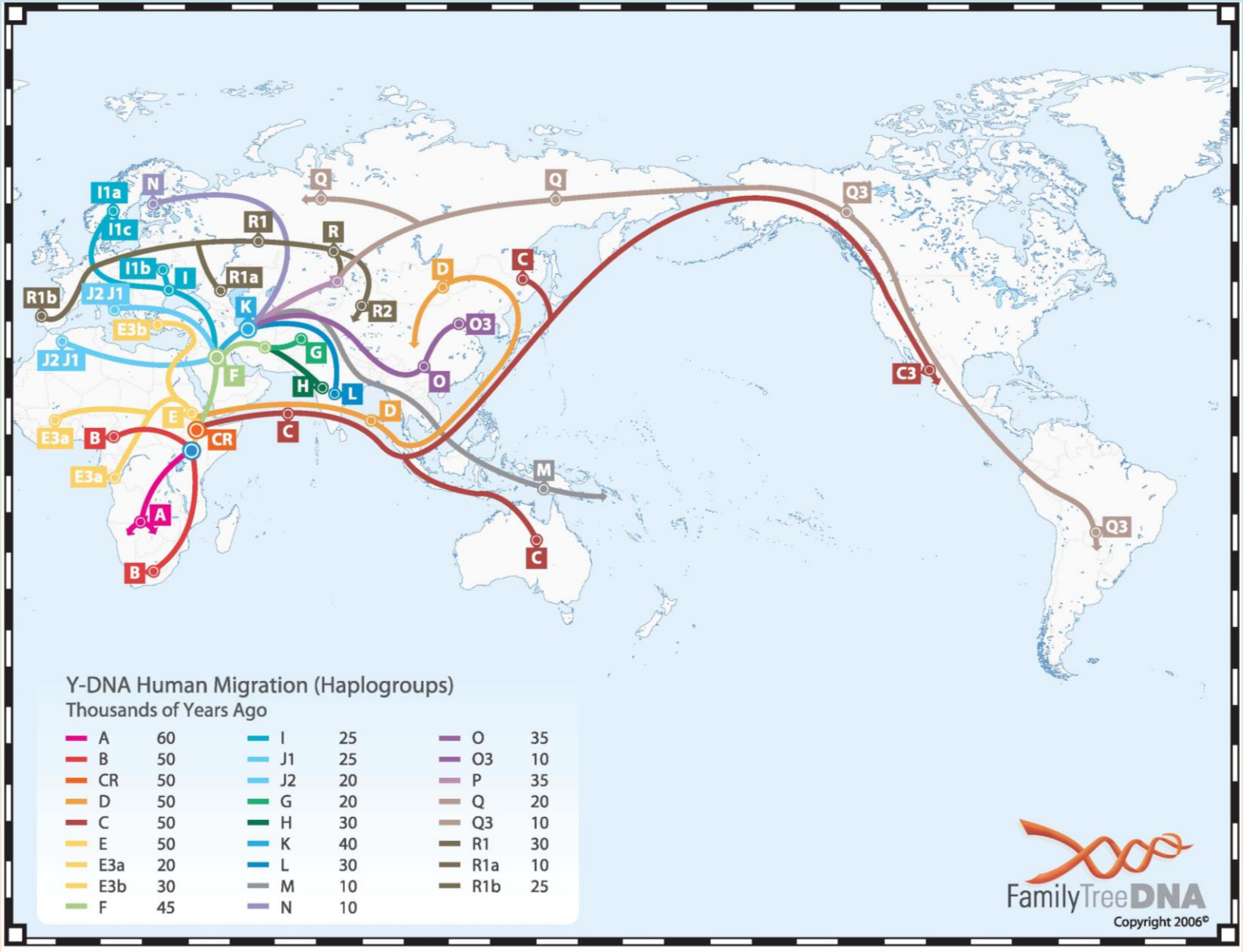
Usage in medicine

Haplotyping



Usage in medicine

World migration map



Usage in medicine

- Recombinant DNA technologies
- Confirmation of mutations
- Haplotyping
- DNA mapping
- Probe preparations
- .
- .
- .

In pathology practice

- Translocations
- Deletions
- **Point mutations**
- Repeating trinucleotides
- DNA metilation
- …

Detection of known point mutations with RE

- Restriction Fragment Length Polymorphism (RFLP)

RFLP Designing

- BRAF V600E mutation

(In BRAF protein, at 600th position, valin (V) substitute to glutamic acid (E)

- T1799A

In cDNA of BRAF gene, at 1799th position, T (timin) substitute to A (adenin)

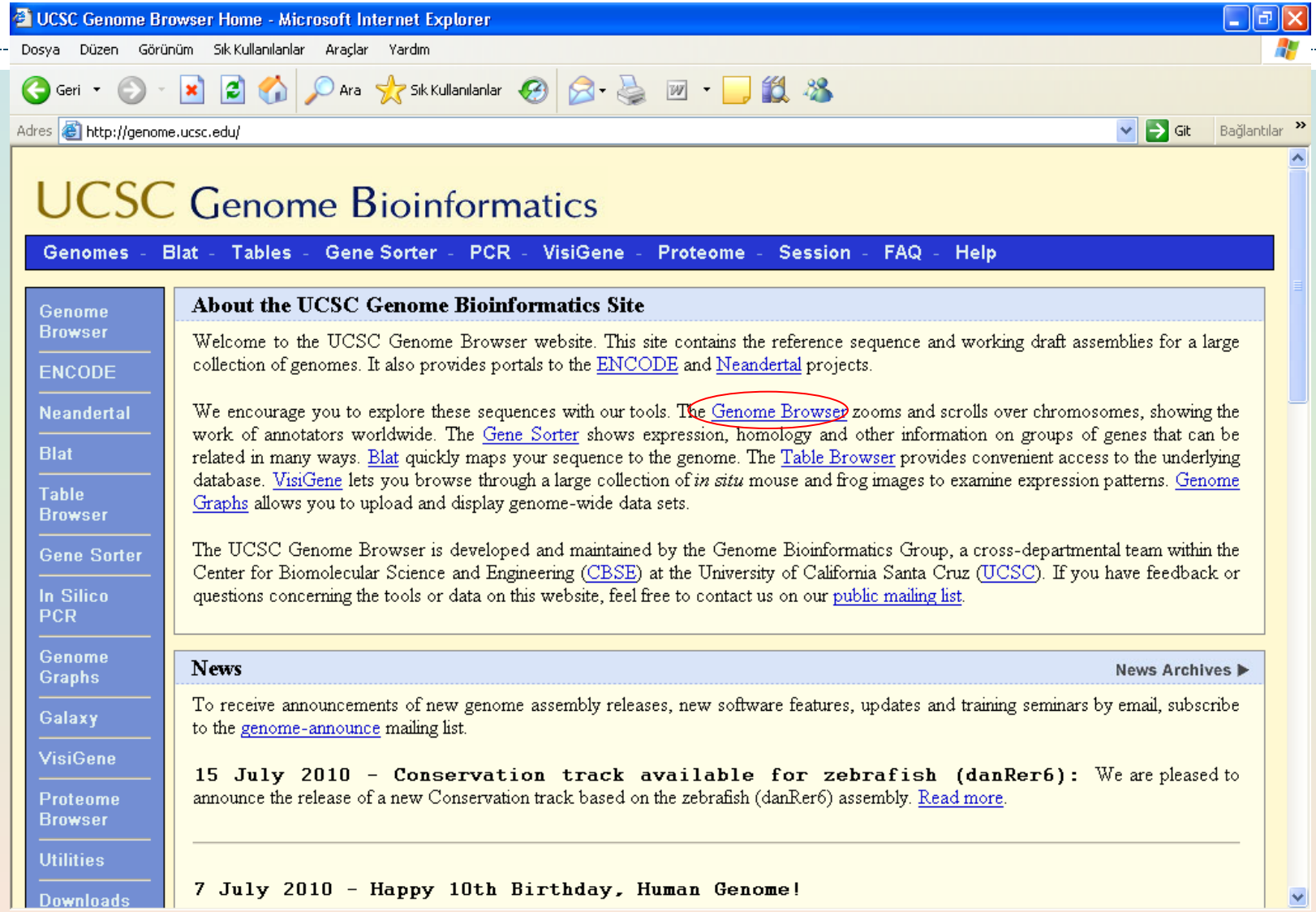
RFLP Designing forBRAF V600E (T1799A) mutation

1. Find BRAF Gene sequence
2. Extract an about 200 or 300 bp fragment, includes mutant point, from BRAF gene

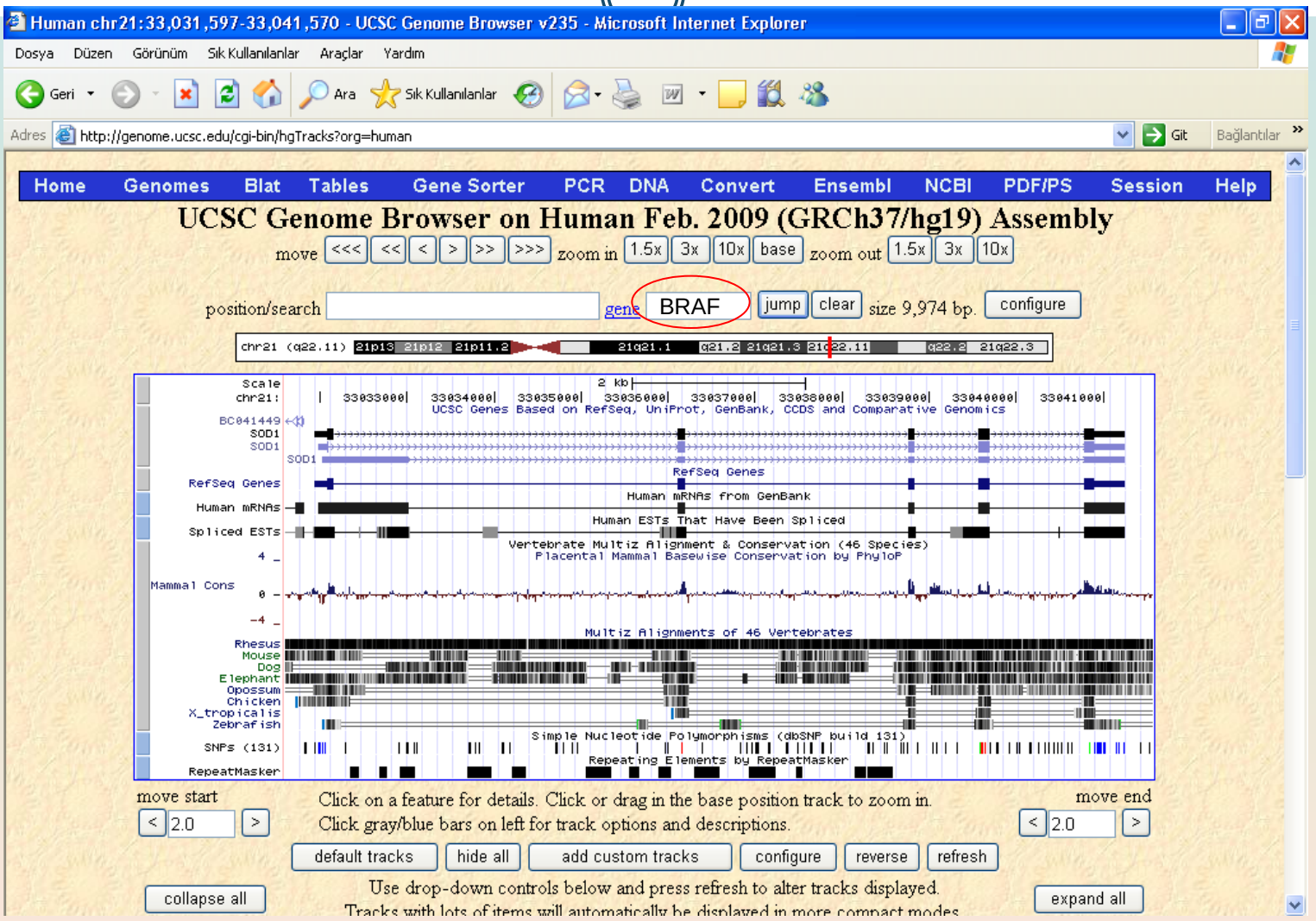
   the extracted fragment must include 1799th nucleotide
3. Detect RE, you will use

   RE must cut wild and mutant fragment differently
4. Design primers
5. Amplify fragment
6. Keep fragment with RE (at suitable degree and time)
7. Run your fragment on gel
8. Interpret the fragments

How can I find a gene's sequence?



1-BRAF gene sequence



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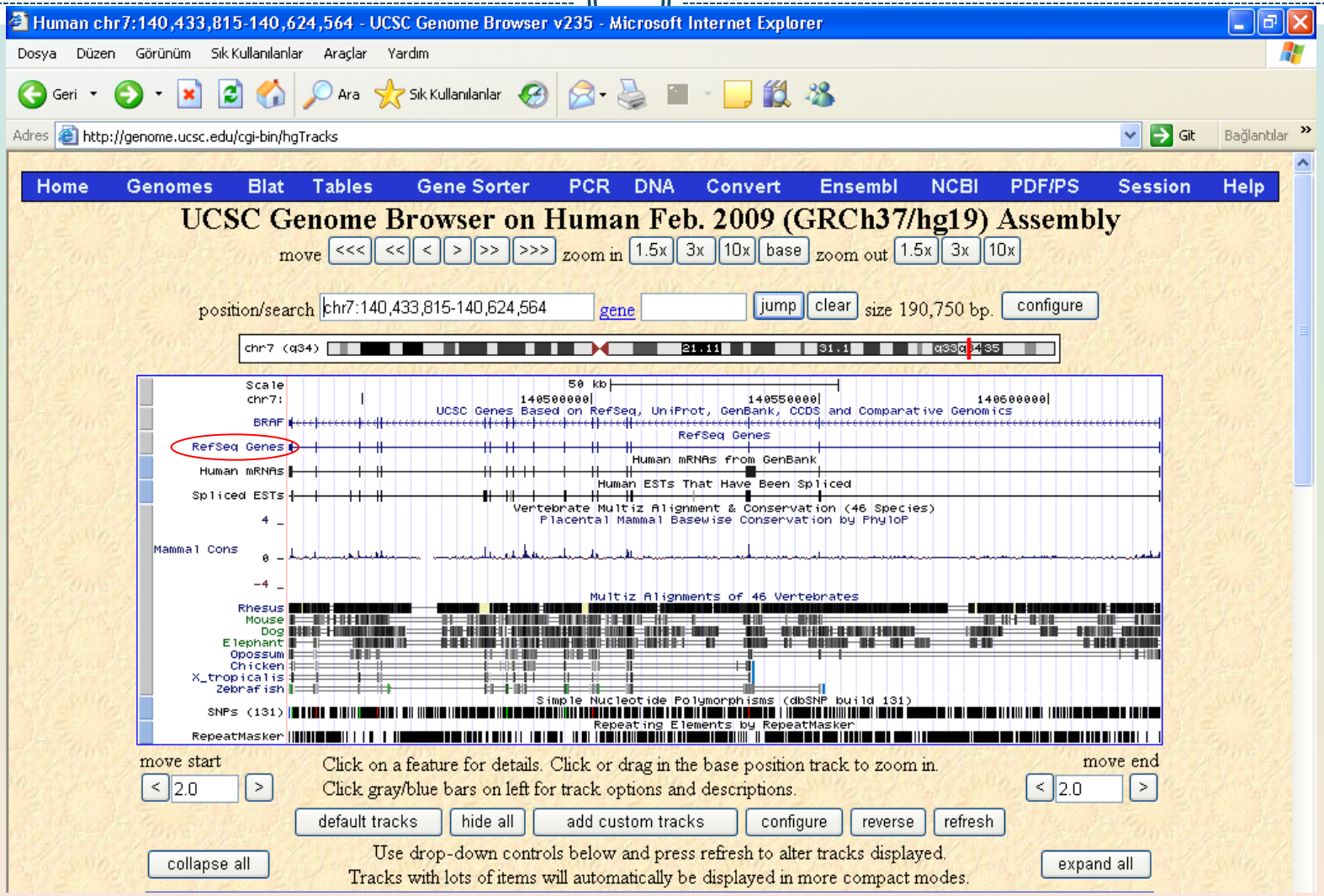

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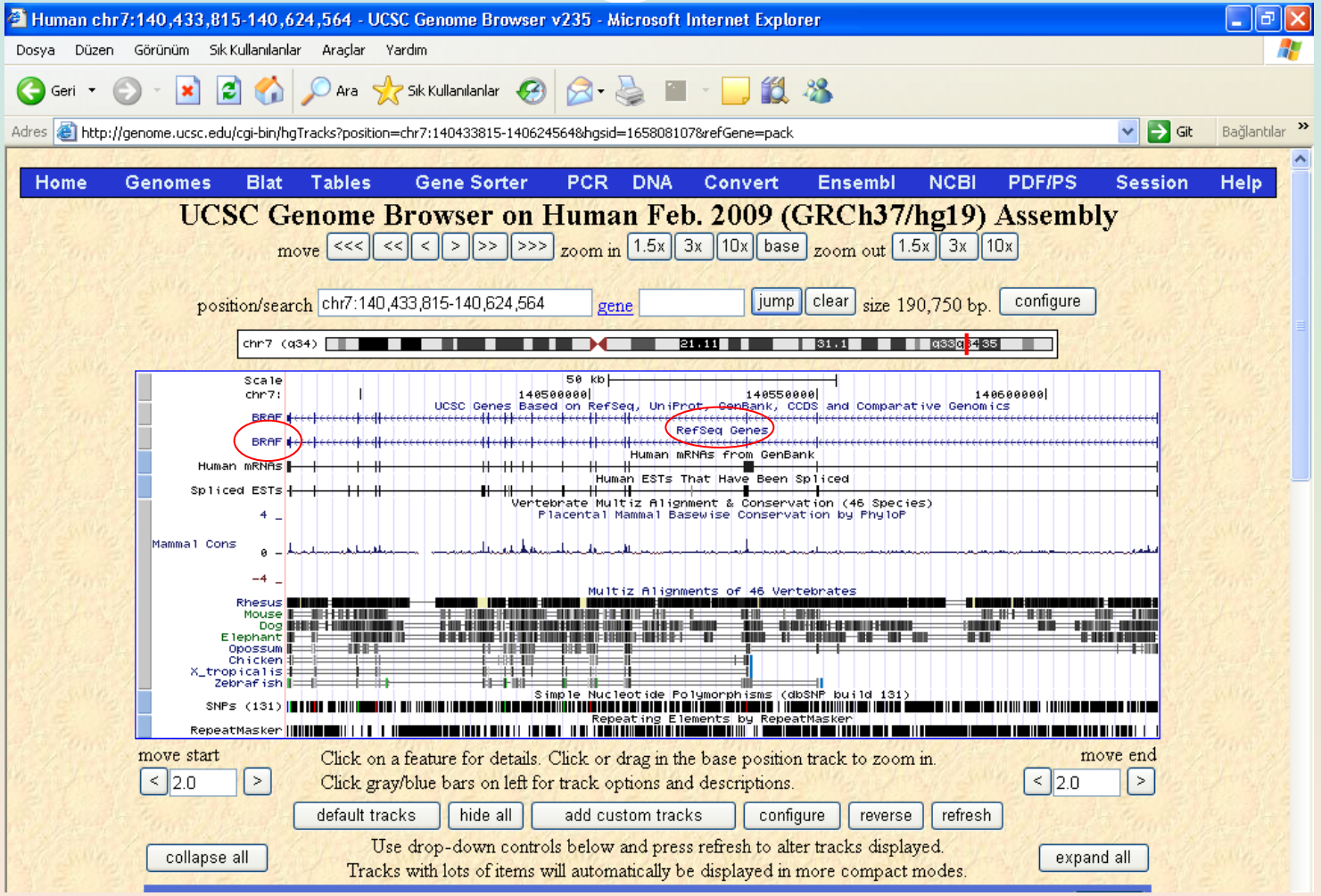

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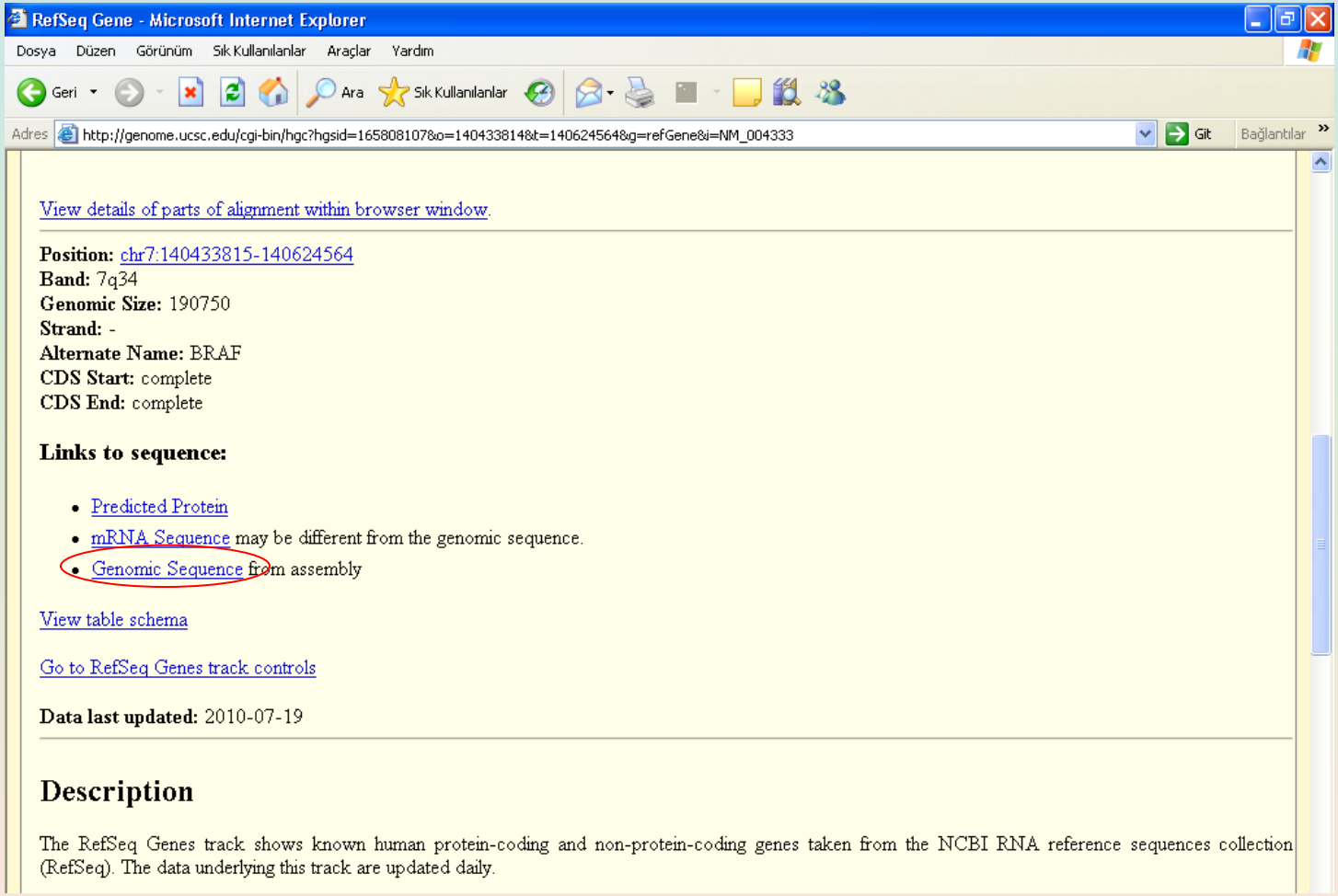

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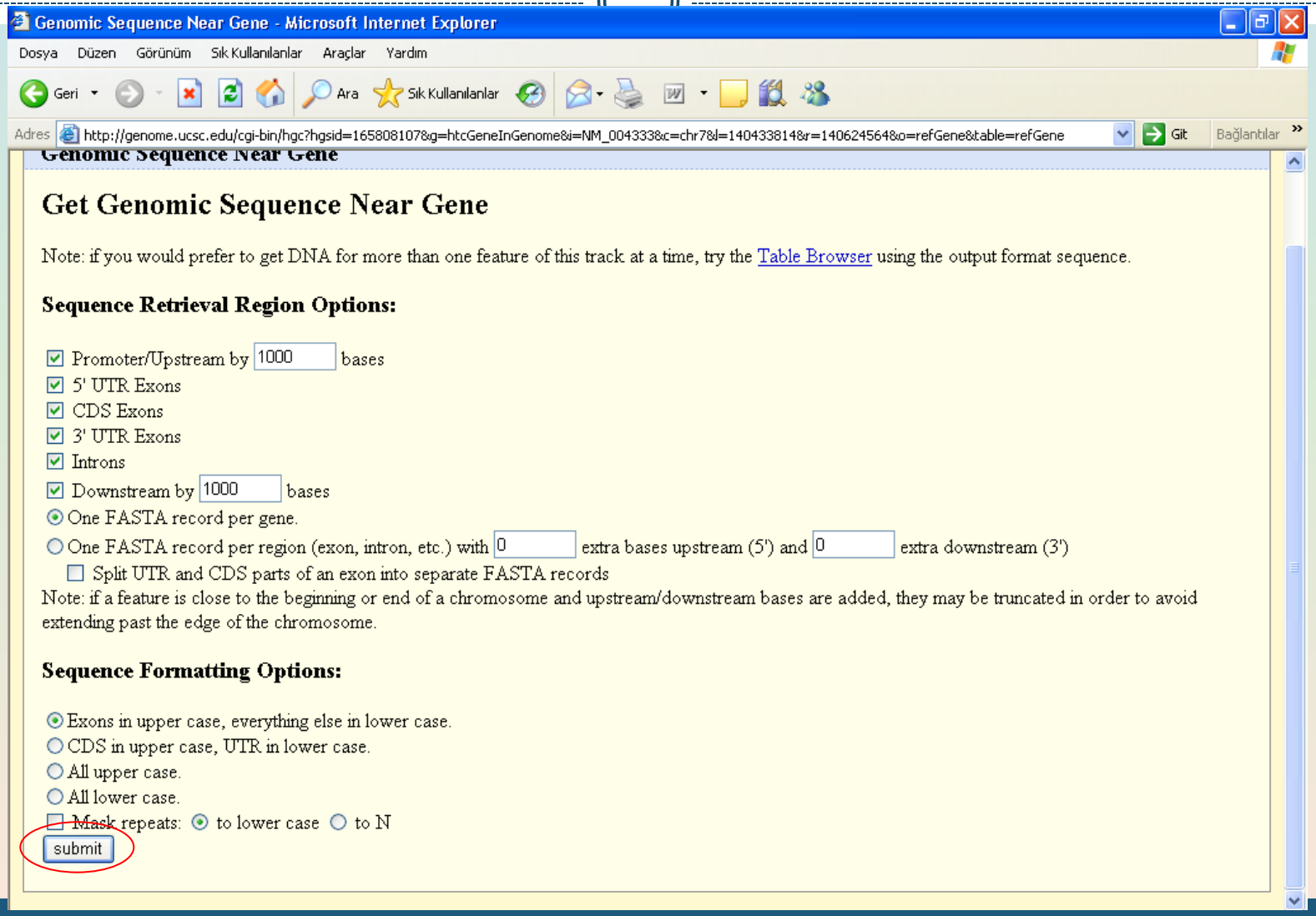

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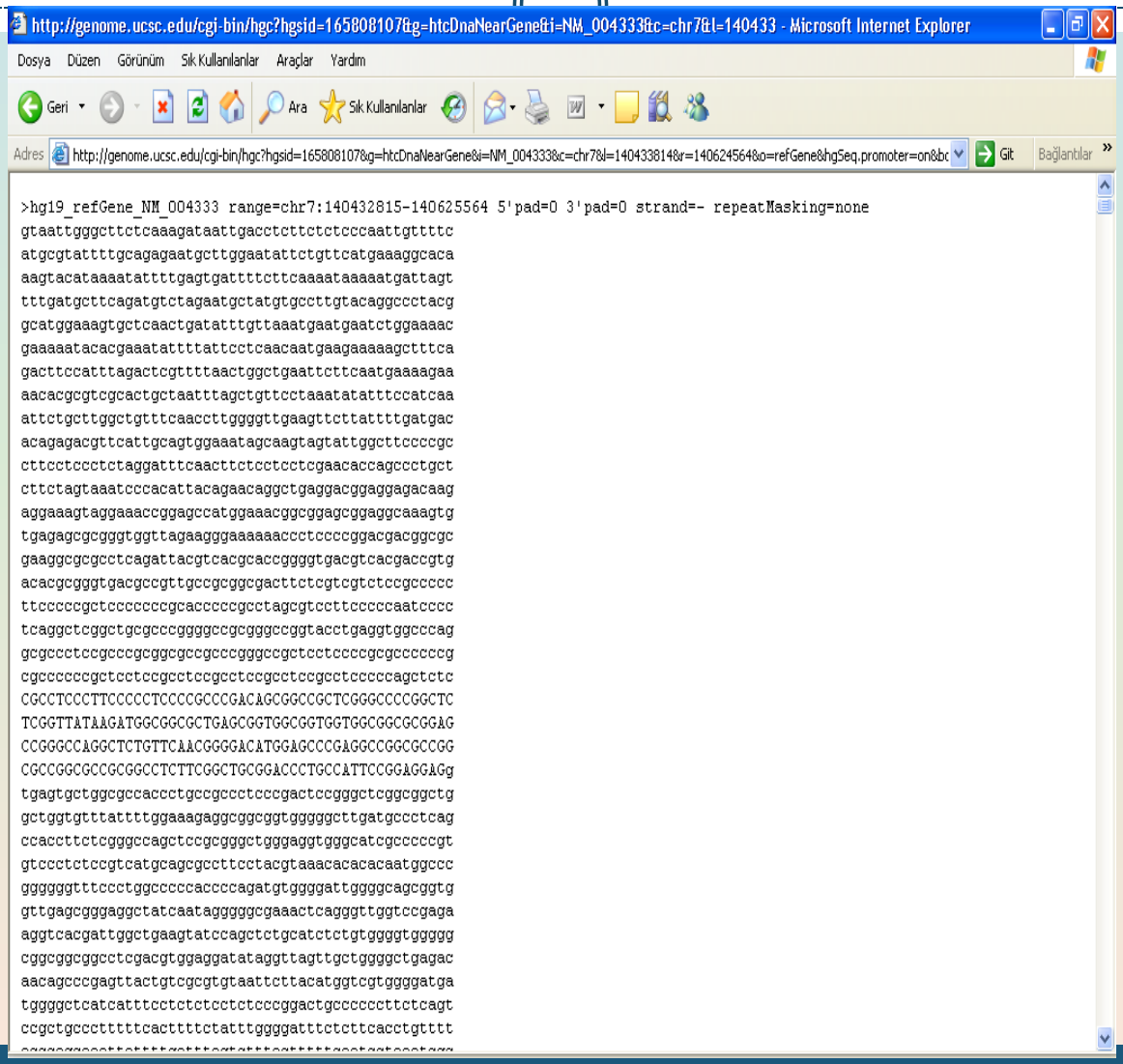
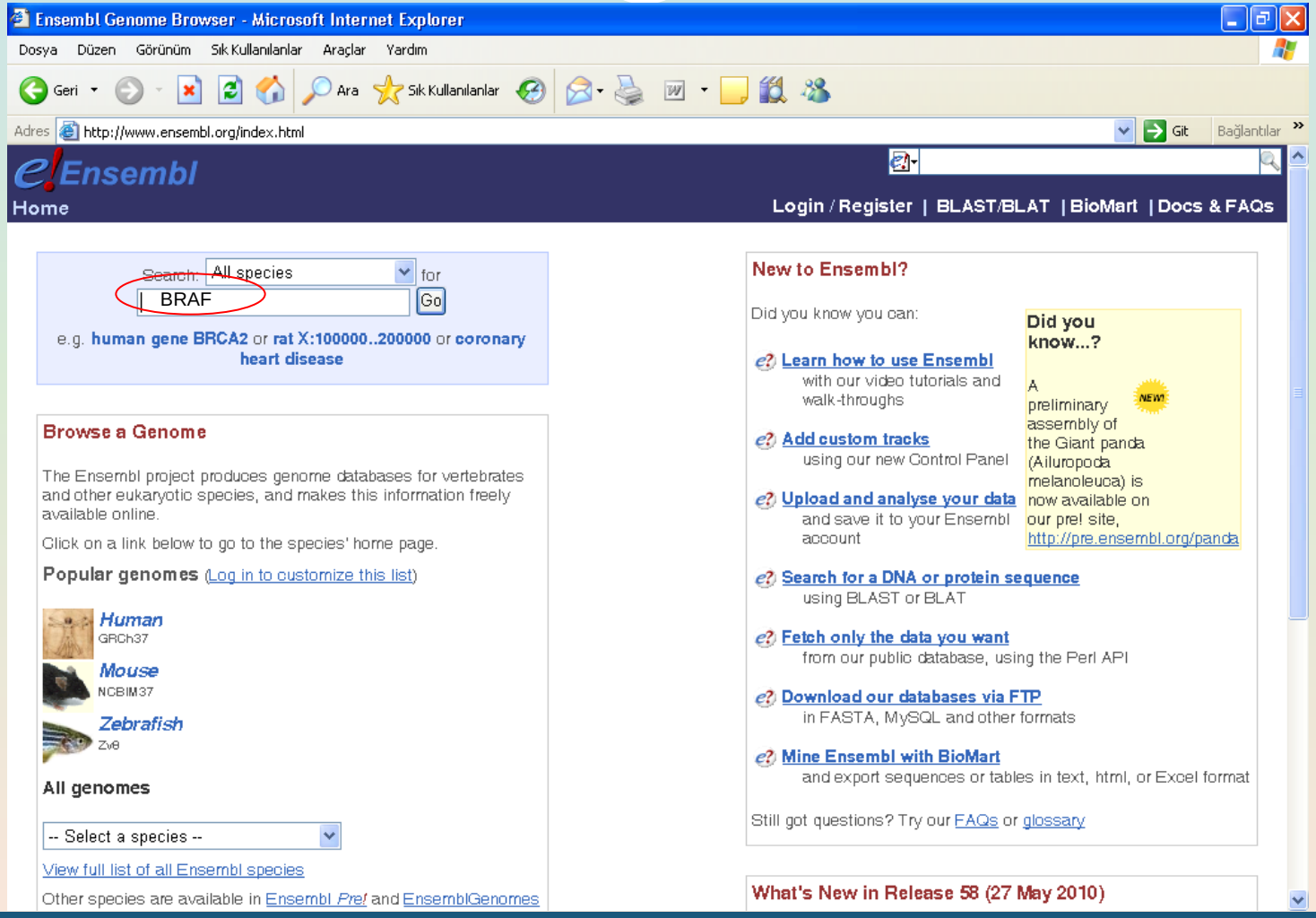


2-Detection of fragment

- Fragment must include 1799th nucleotide.
- It should be about 200-300 bp length
- 1799th nucleotide means the nucleotide at 1799th nucleotide on cDNA of BRAF gene.

- http://www.ensembl.org/index.html

2-Detection of fragment



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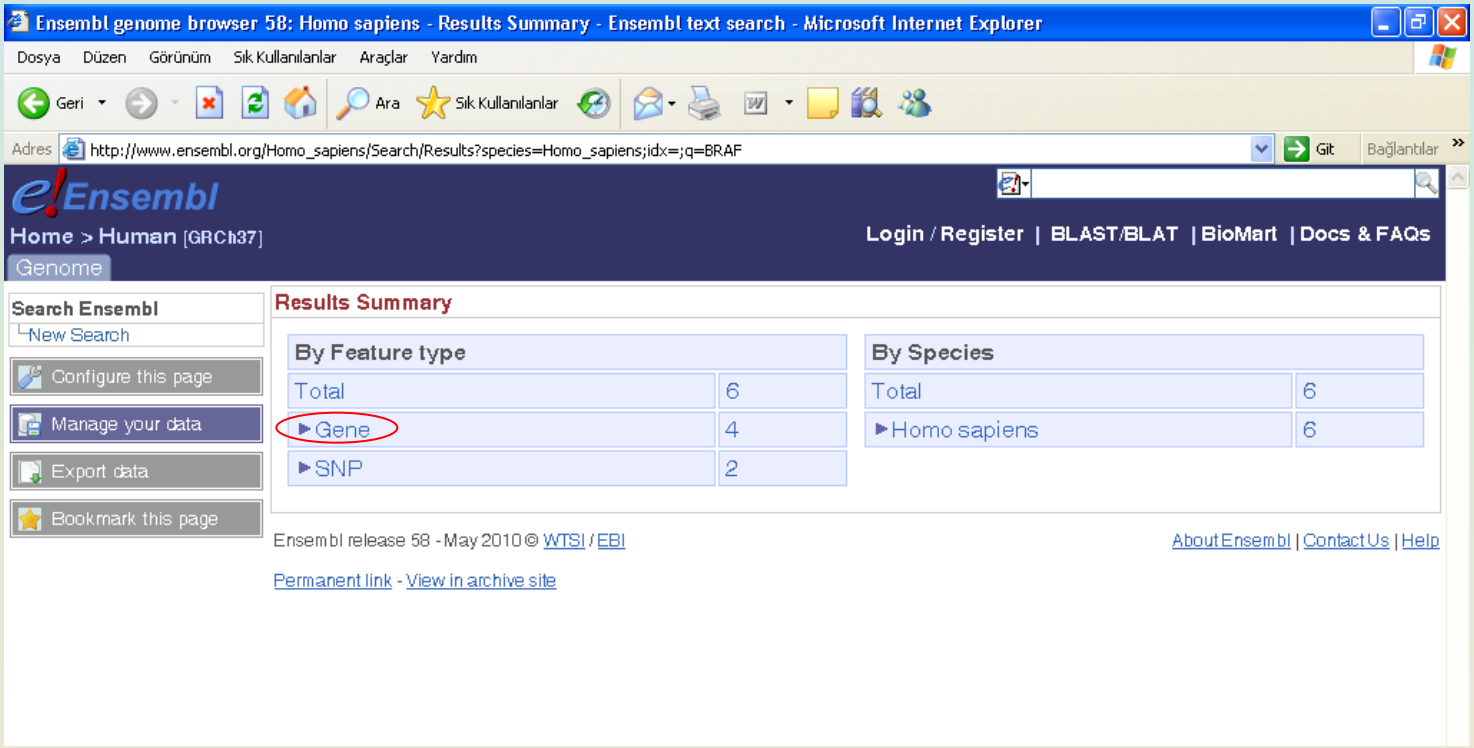

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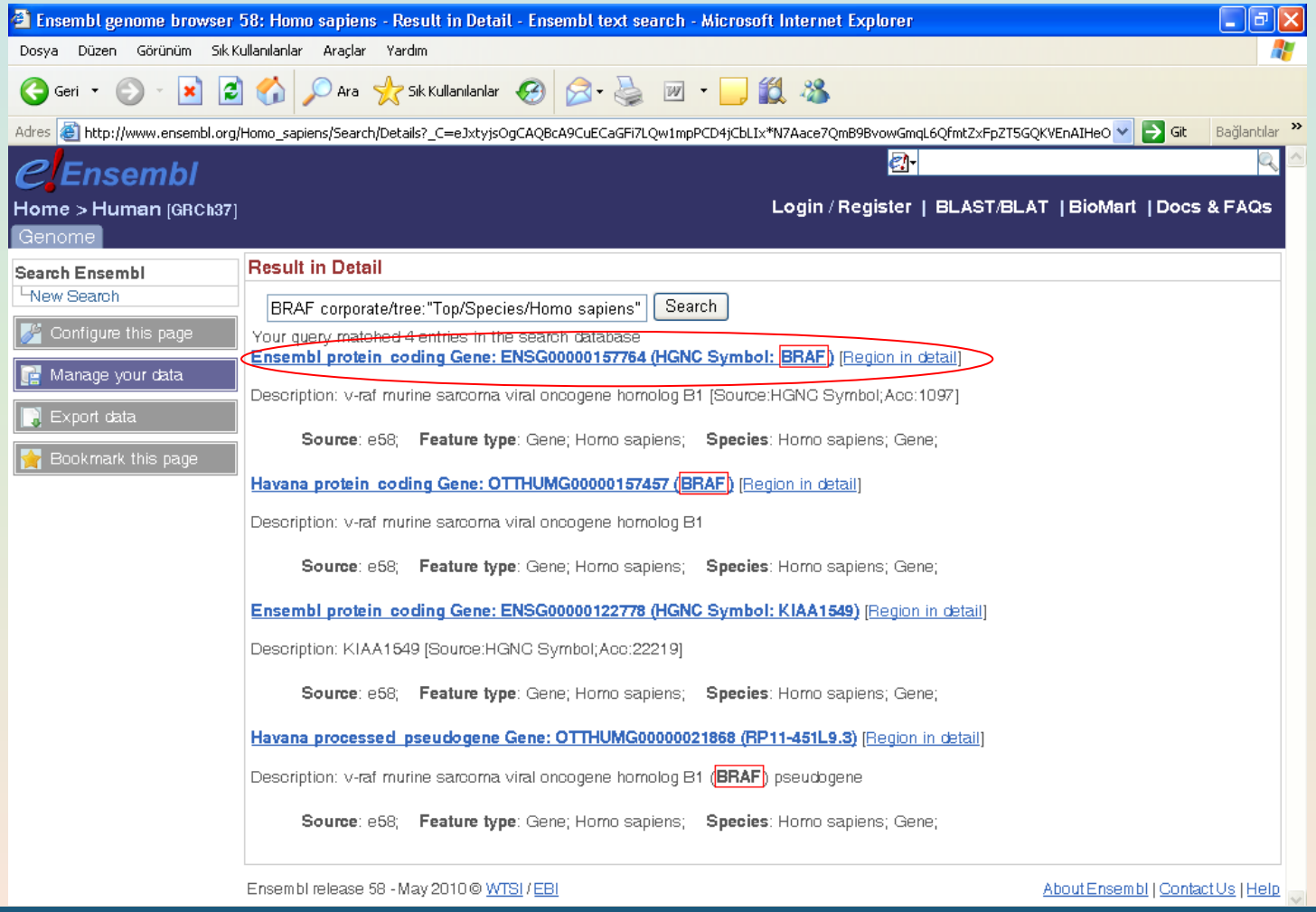

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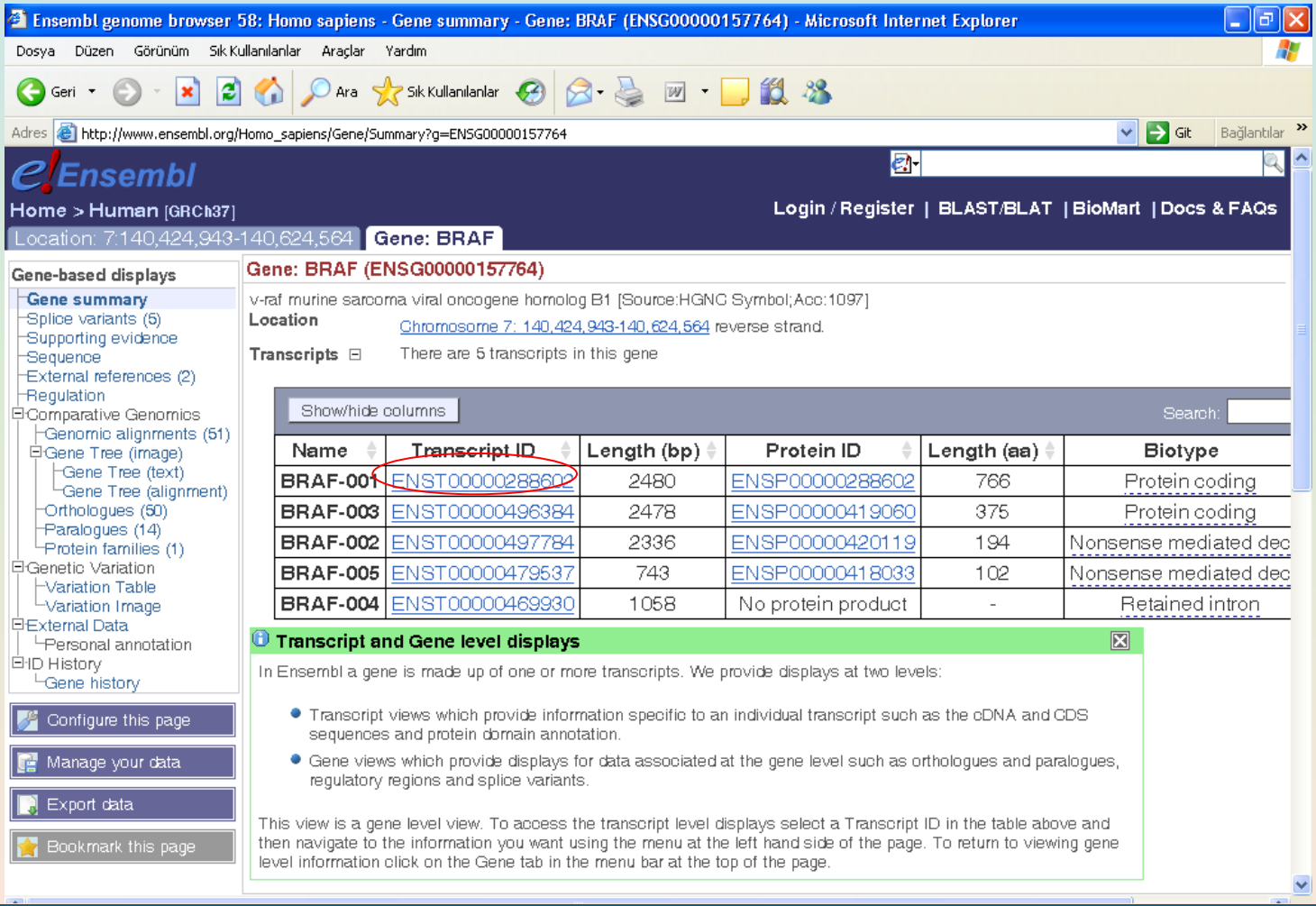

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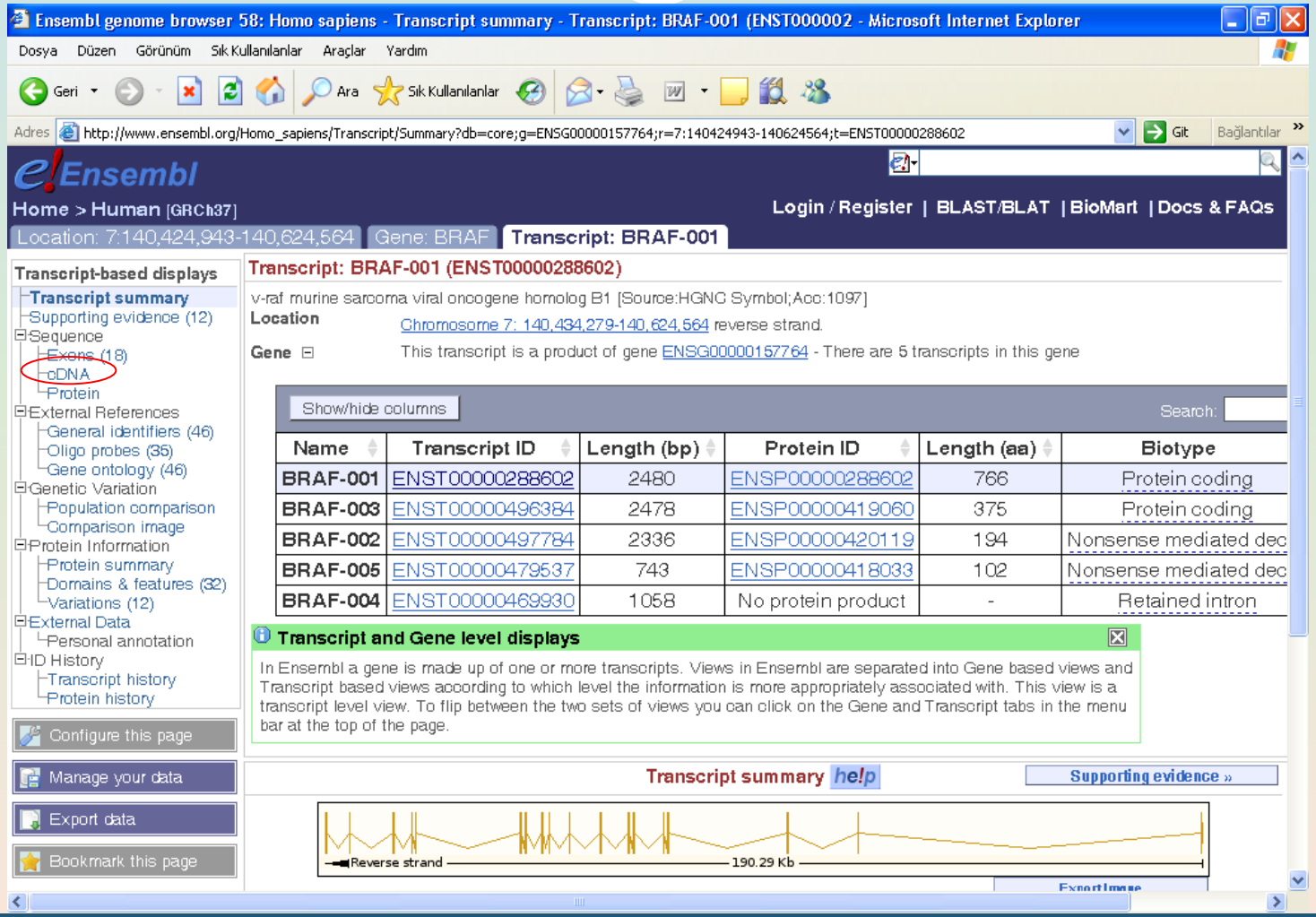

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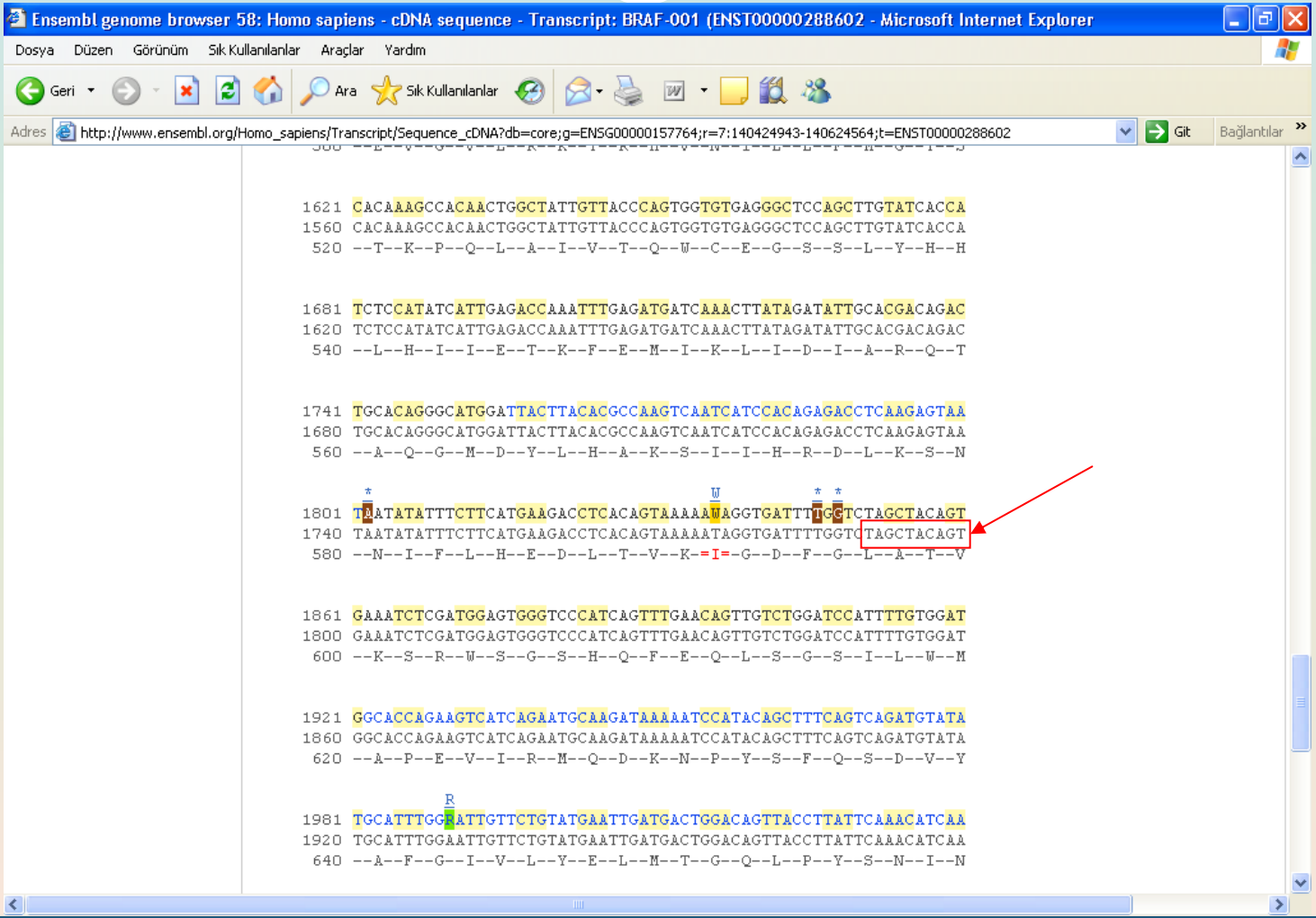

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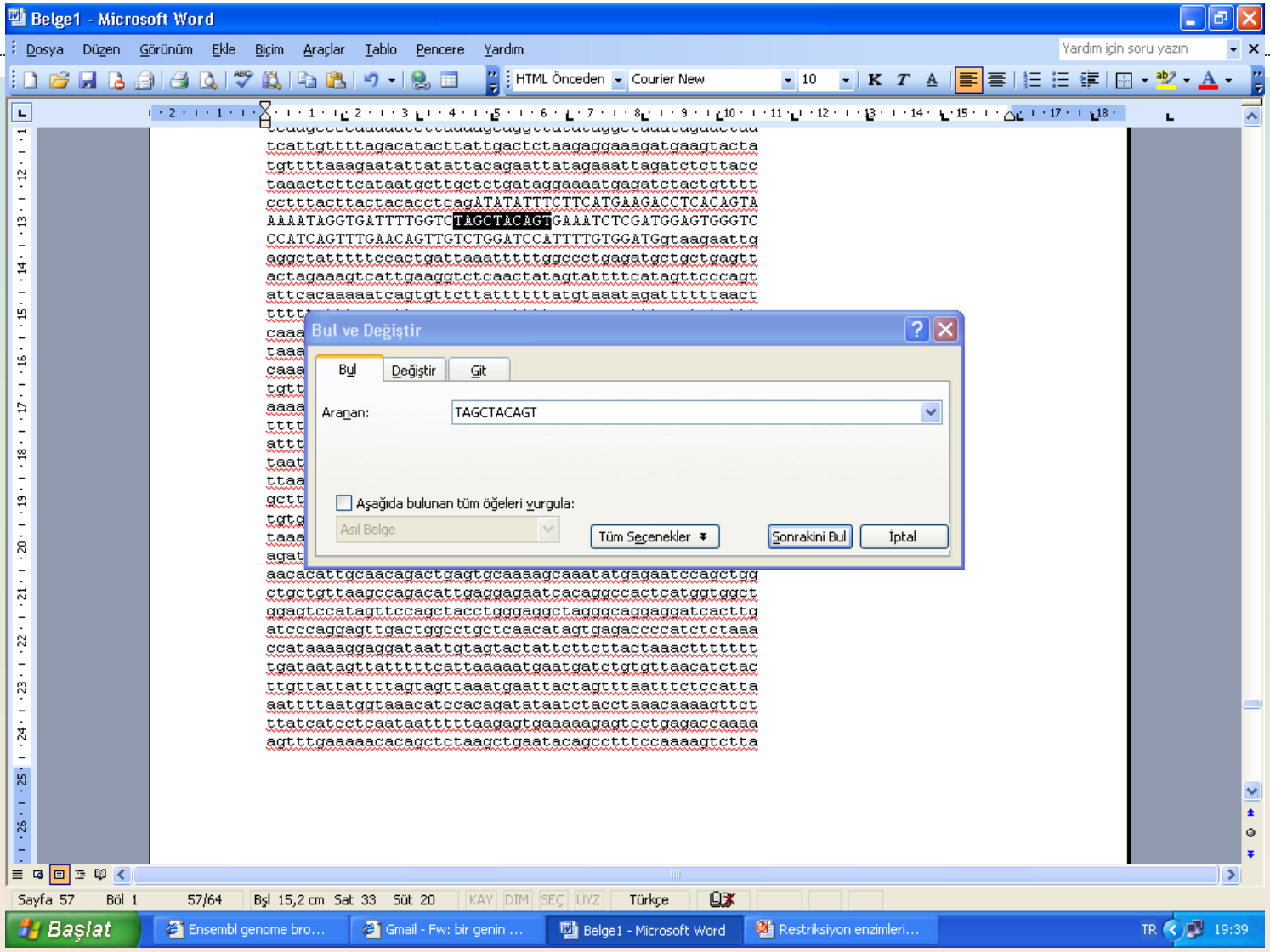

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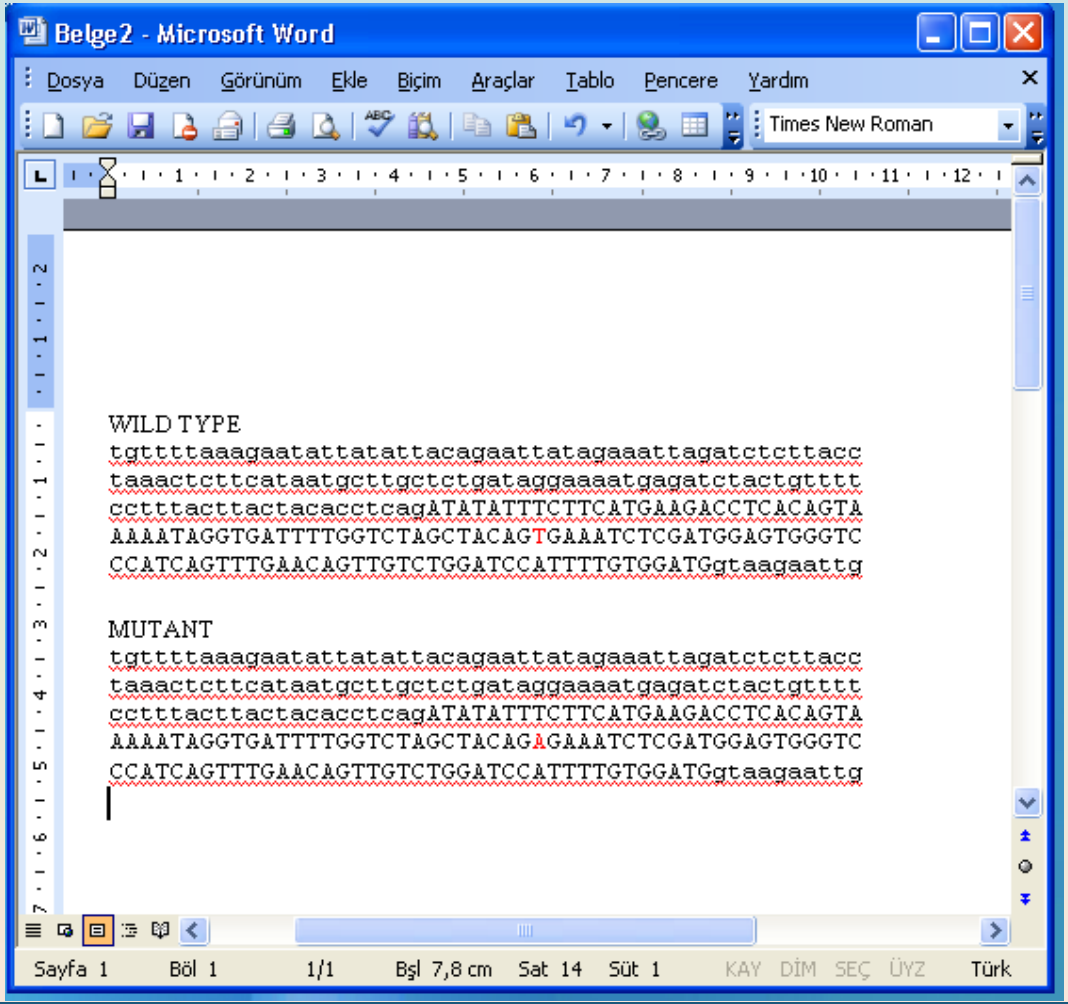
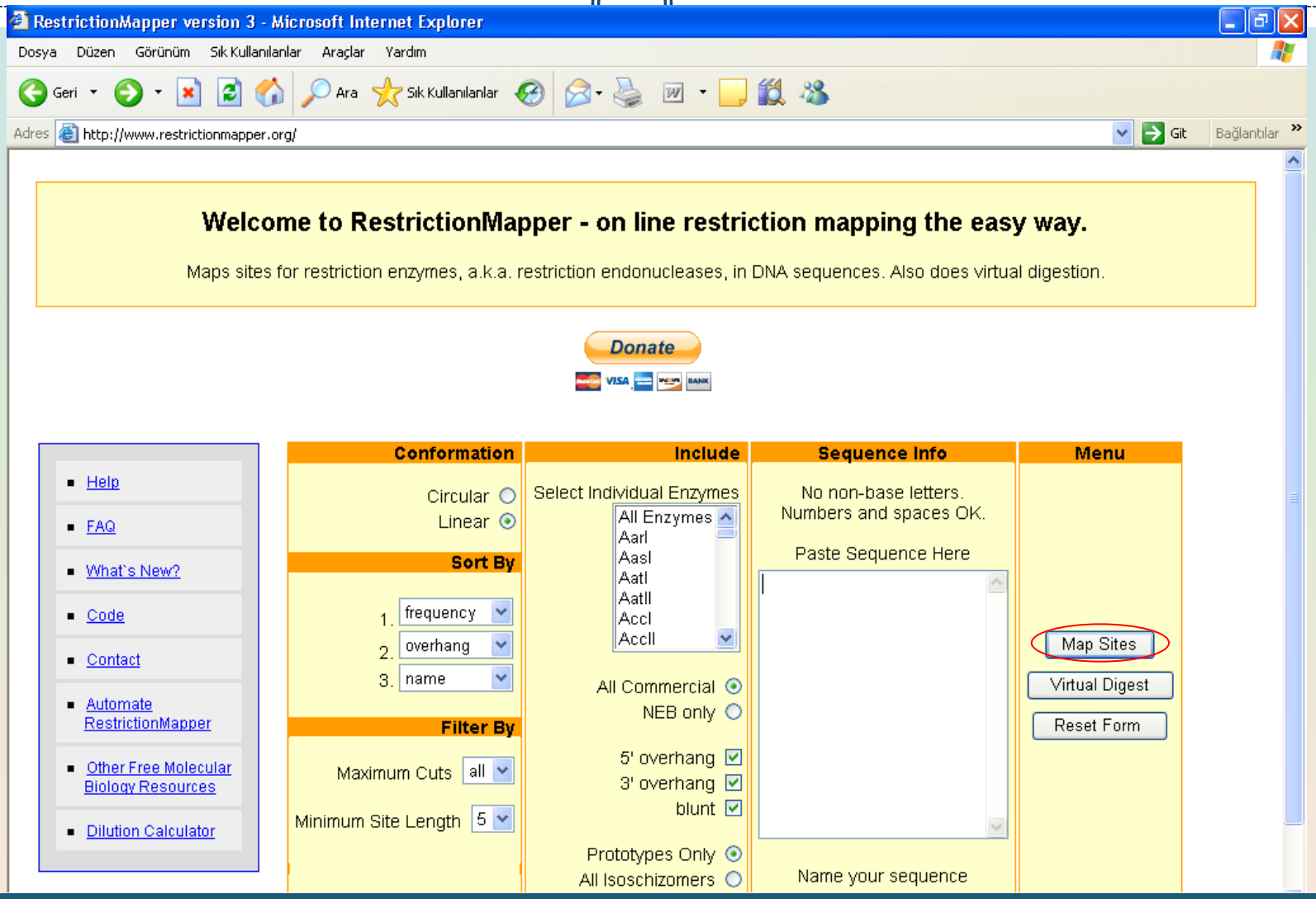


3-Selection of restriction enzyme



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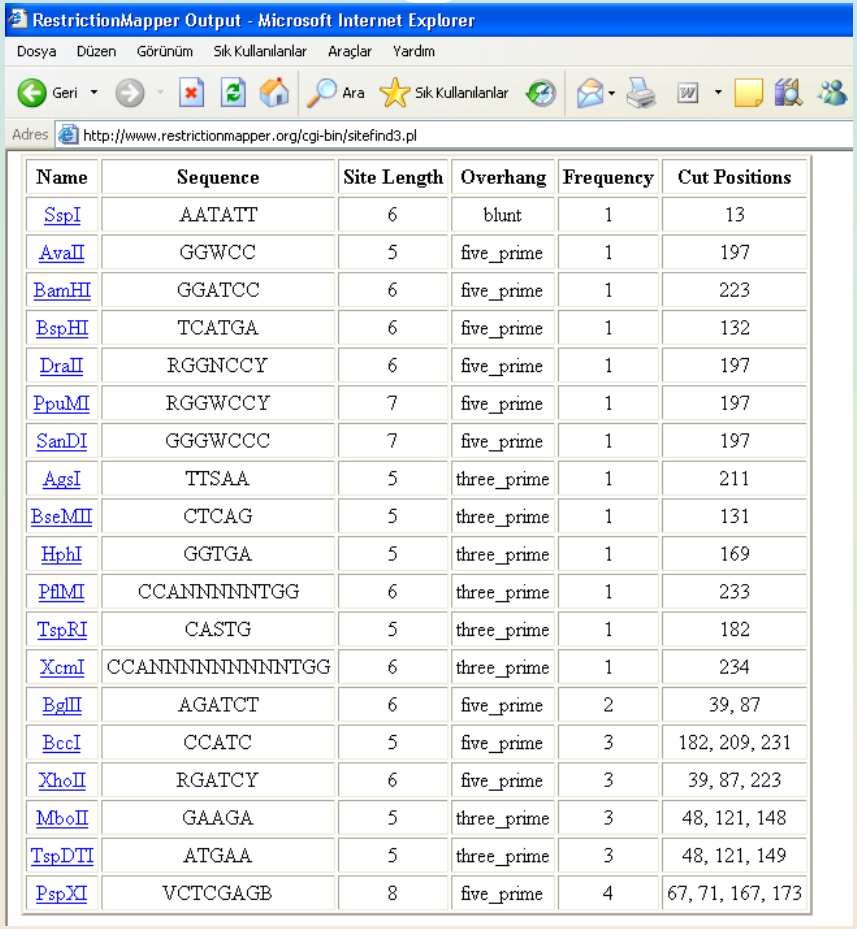
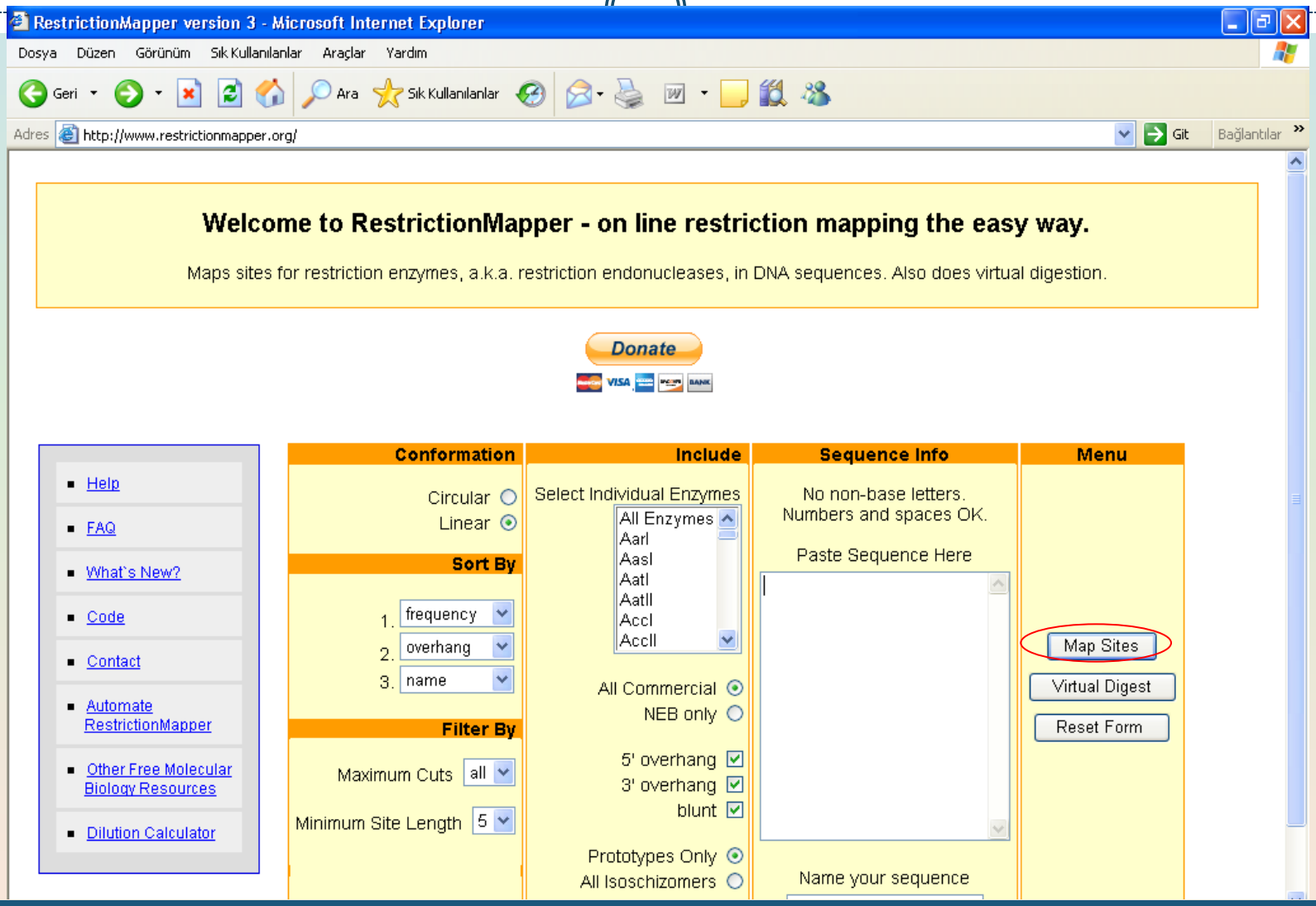


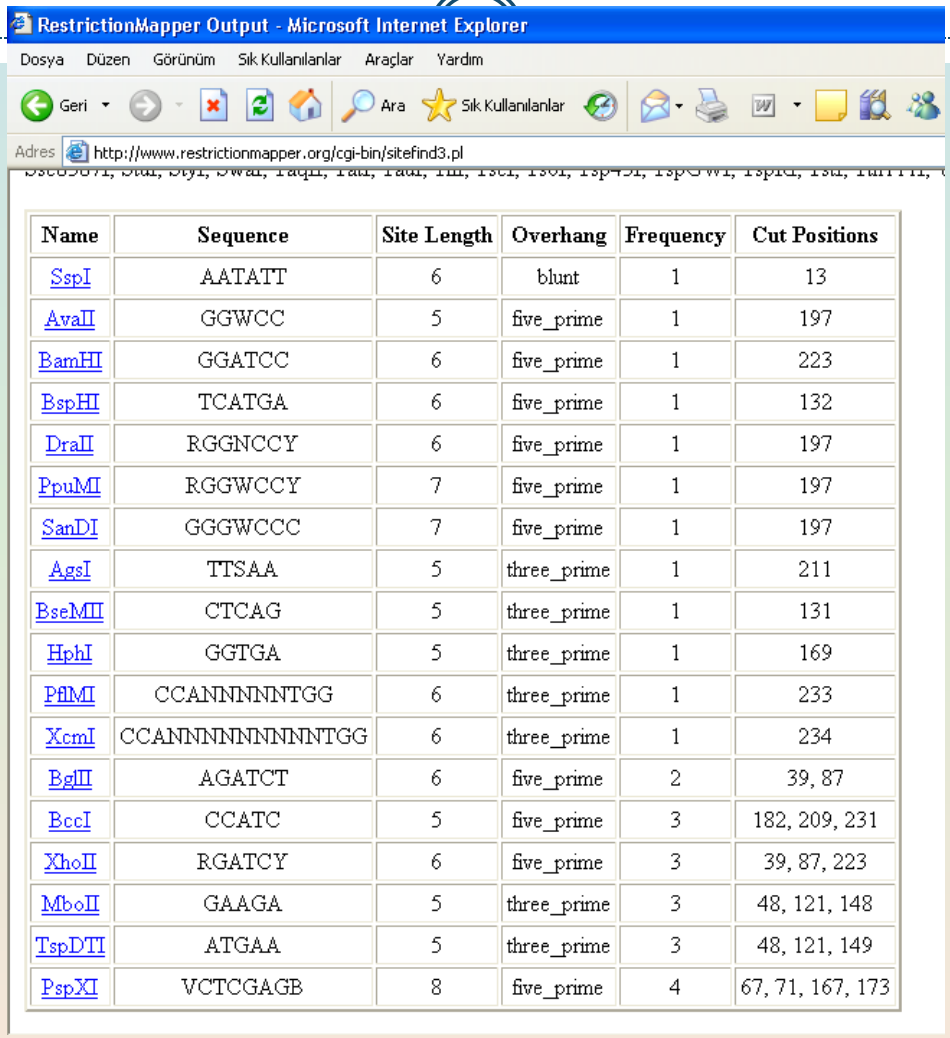
Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
SspI	AATATT	6	blunt	1	13
AvaII	GGWCC	5	five_prime	1	197
BamHI	GGATCC	6	five_prime	1	223
BspHI	TCATGA	6	five_prime	1	132
DraII	RGGNCCY	6	five_prime	1	197
PpuMI	RGGWCCY	7	five_prime	1	197
SanDI	GGGWCCC	7	five_prime	1	197
AgsI	TTSAA	5	three_prime	1	211
BseMII	CTCAG	5	three_prime	1	131
HphI	GGTGA	5	three_prime	1	169
PflMI	CCANNNNNTGG	6	three_prime	1	233
TspRI	CASTG	5	three_prime	1	182
XcmI	CCANNNNNNNNNTGG	6	three_prime	1	234
BglII	AGATCT	6	five_prime	2	39, 87
BccI	CCATC	5	five_prime	3	182, 209, 231
XhoII	RGATCY	6	five_prime	3	39, 87, 223
MboII	GAAGA	5	three_prime	3	48, 121, 148
TspDTI	ATGAA	5	three_prime	3	48, 121, 149
PspXI	VCTCGAGB	8	five_prime	4	67, 71, 167, 173

Restriction enzymes for WILD Type fragment

3-Selection of restriction enzyme



3-Selection of restriction enzyme



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BseMII	CTCAG	5	three_prime	1	131
HphI	GGTGA	5	three_prime	1	169
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XcmI	CCANNNNNNNNNTGG	6	three_prime	1	234
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PspXI	VCTCGAGB	8	five_prime	4	67, 71, 167, 173

Restriction enzymes for Mutant fragment

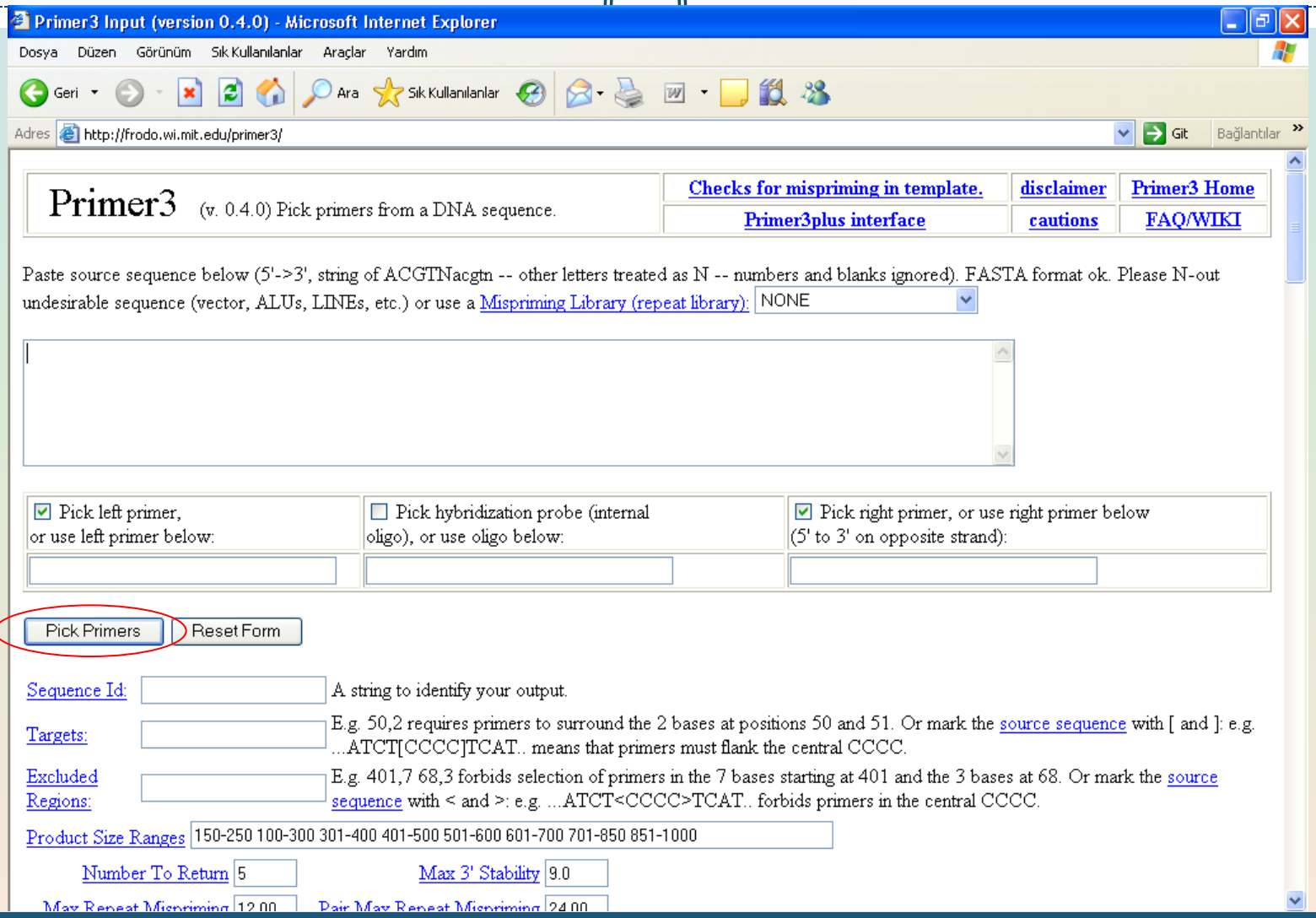
3-Selection of restriction enzyme

MUTANT		WILD TYPE	
Name	**Frequency**	**Name**	**Frequency**
SspI	1	SspI	1
AvaII	1	AvaII	1
BamHI	1	BamHI	1
BspHI	1	BspHI	1
DraII	1	DraII	1
PpuMI	1	PpuMI	1
SanDI	1	SanDI	1
AgsI	1	AgsI	1
BseMII	1	BseMII	1
HphI	1	HphI	1
PflMI	1	PflMI	1
TspRI	0	TspRI	1
XcmI	1	XcmI	1
BglII	2	BglII	2
BccI	3	BccI	3
XhoII	3	XhoII	3

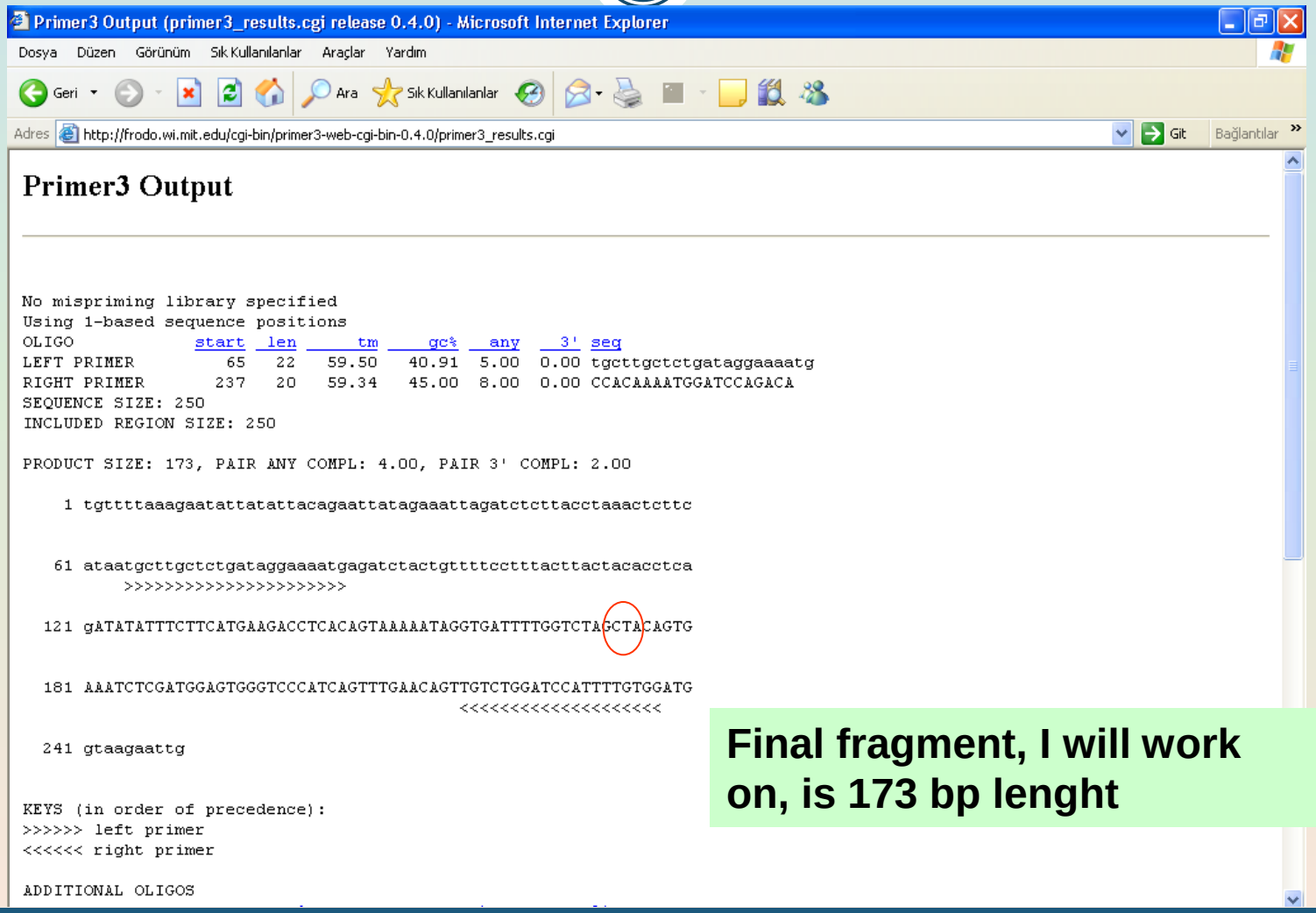
3-Selection of restriction enzyme

- TspRI

4-Primer designing



4-Primer designing



Final fragment, I will work on, is 173 bp lenght

4. .
5. Amplify fragment
6. Keep fragment with RE (at suitable degree and time)
7. Run your fragment on gel

8-Interpretation

Mutant fragment

- There is no cutting point with TspRI
- If the 1799th nucteotide is A, I will see only one band,173 bp.

Wild type fragment

- There is one cutting point in wild type fragment
- If the 1799th nucteotide is T, I will see two bands,118 ve 55 bp.

8-Interpretation

1799th nucleotide is A in BRAF gene

MUTANT

1799th nucleotide is T in BRAF gene

WILD TYPE

In conclusion

- RE can be used to detect known point mutations.
- The method is reliable and cheap but;
  - time-consuming
  - needs experience
  - has low sensitivity.

Thanks