



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.



Werner Arber



Daniel Nathans



Hamilton O. Smith

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***

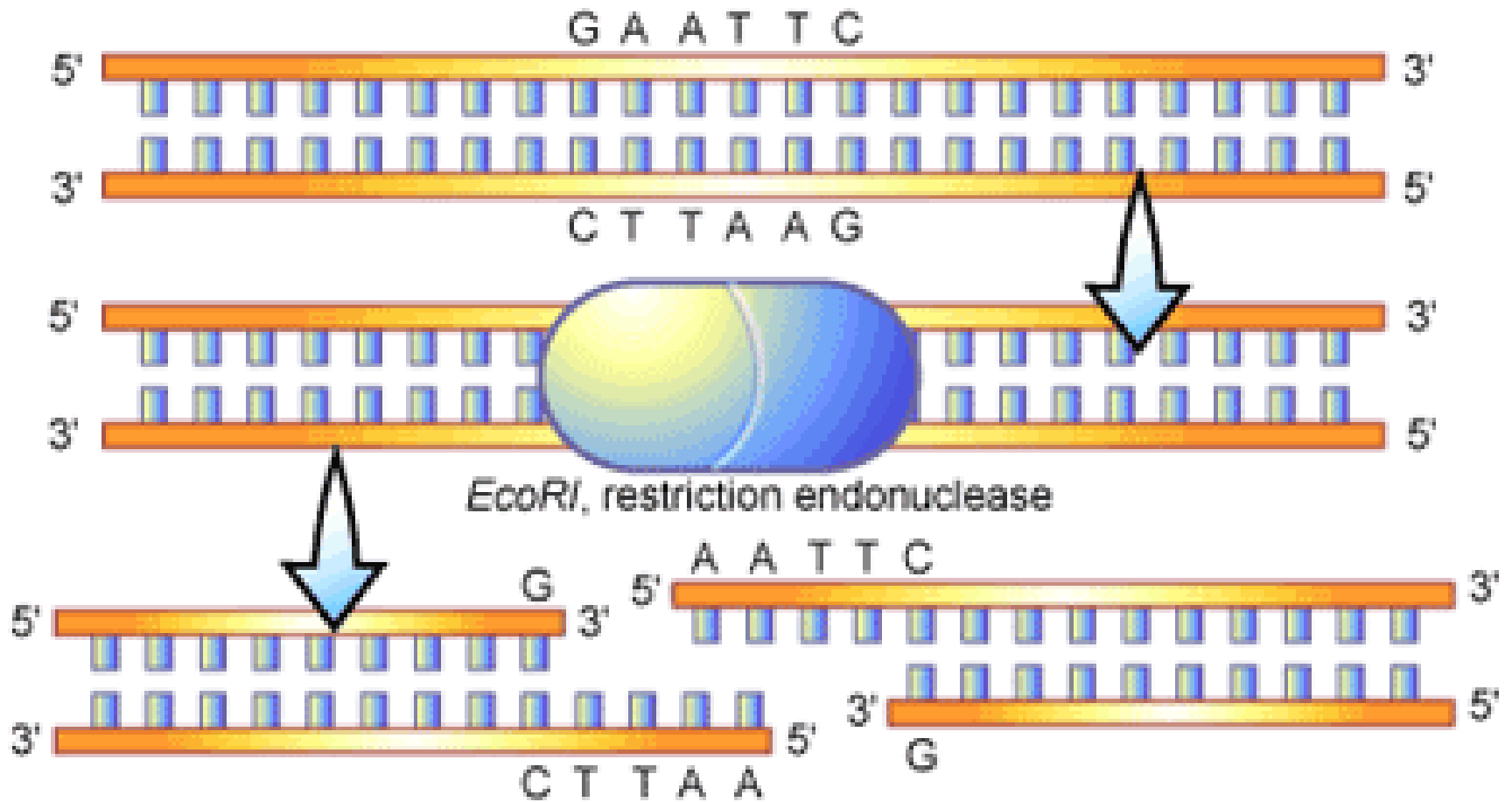
- ***HindIII***
 - ***H = Haemophilus (genus)***
 - ***in = influenzae (species)***
 - ***d = Rd (strain)***
 - ***III = the third RE, derivated from this strain***

DNA identification and cutting mechanism of RE

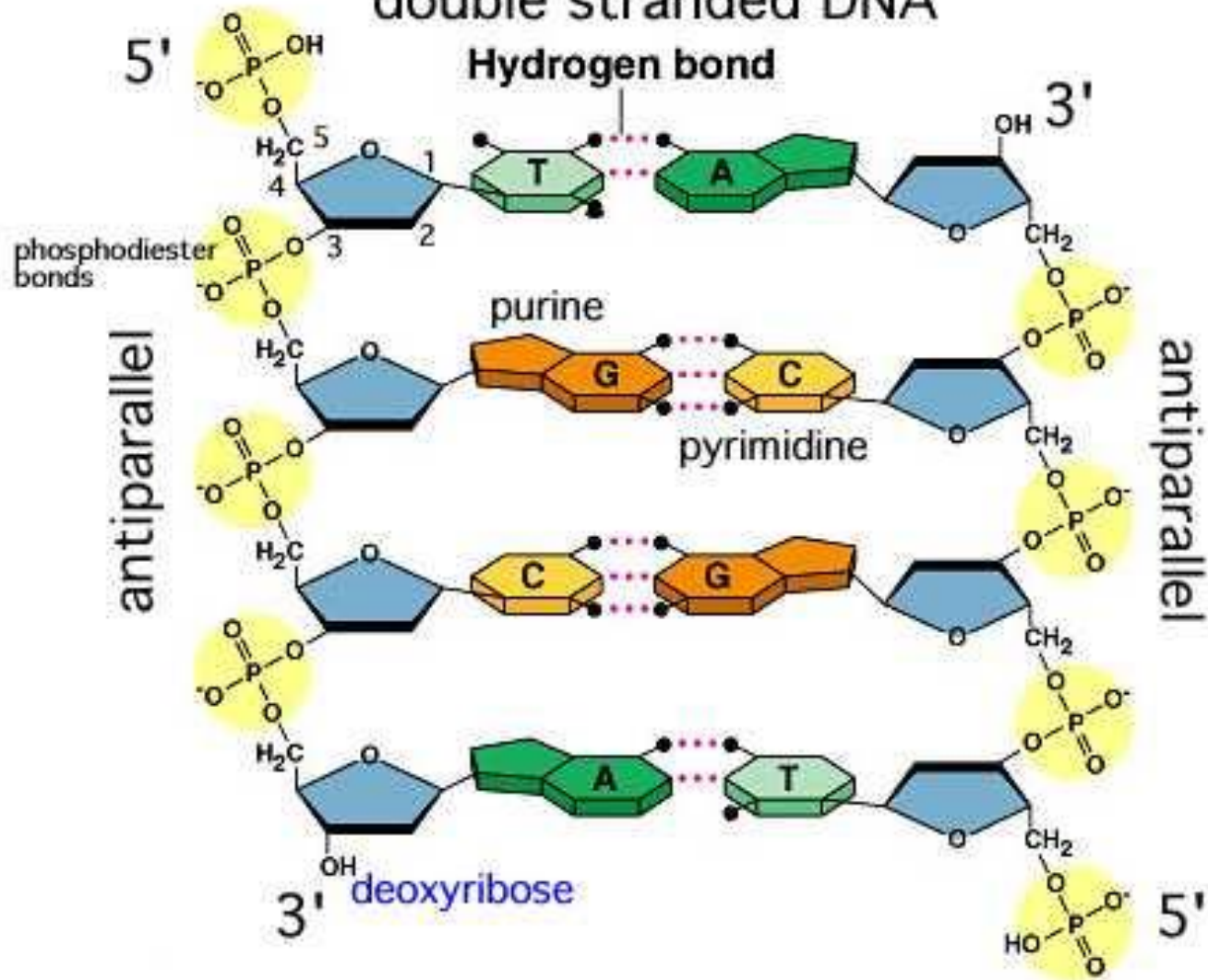


- REs have 2 functional subunit:
 - DNA identification region and
 - Catalytic region

EcoRI RE



double stranded DNA



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 technologies
- 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 cloning). They do 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**

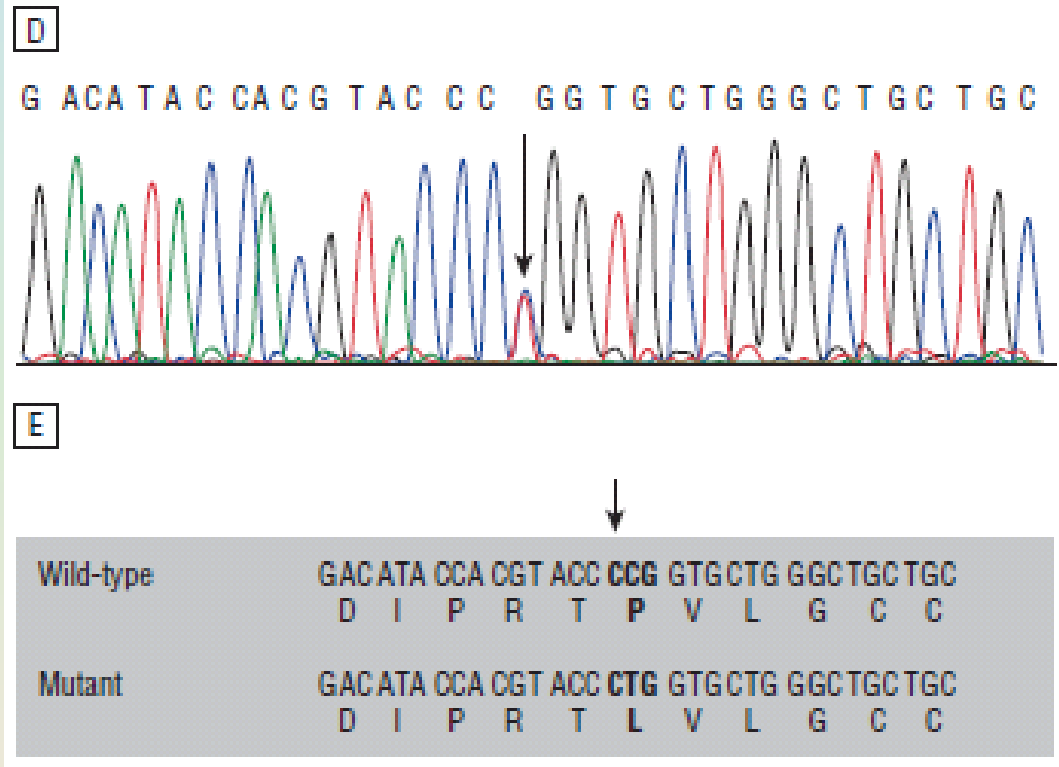
Usage in medicine

Confirmation of point mutation



- 9 year-old girl
- Hypotonia at birth
- Liver dysfunction, 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

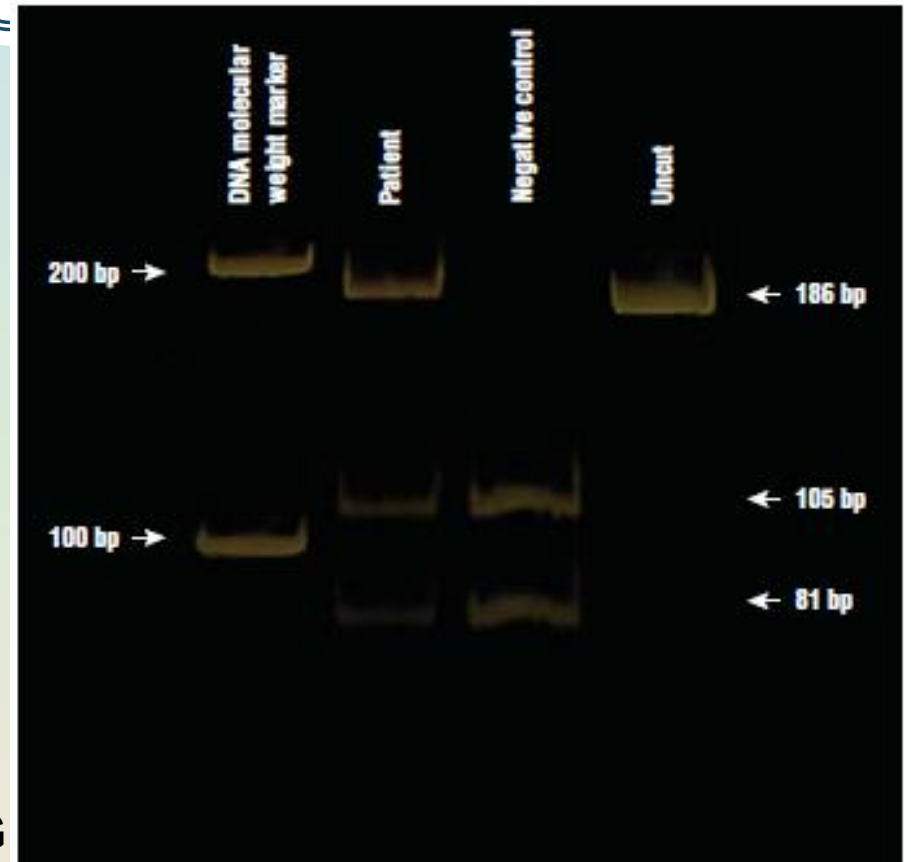
Primers;
F=5'-GGAAGAAGTGGGAGGTGGTT-3'
R=5'-CCATGCTCCAAAGGTAGCAA-3'

Restriction enzyme;
MspI

Mutant fragment;
186bp (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



T8764C
polimorphism

A1812G
polimorphism

No polymorphism

A1812G
T1729A
G3894C
polimorphisms

A1812G
T1729A
polimorphisms

Usage in medicine

Haplotyping



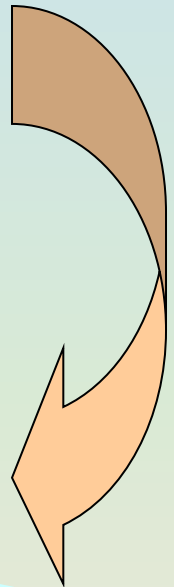
T8764C
polimorphism

A1812G
polimorphism

No polymorphism

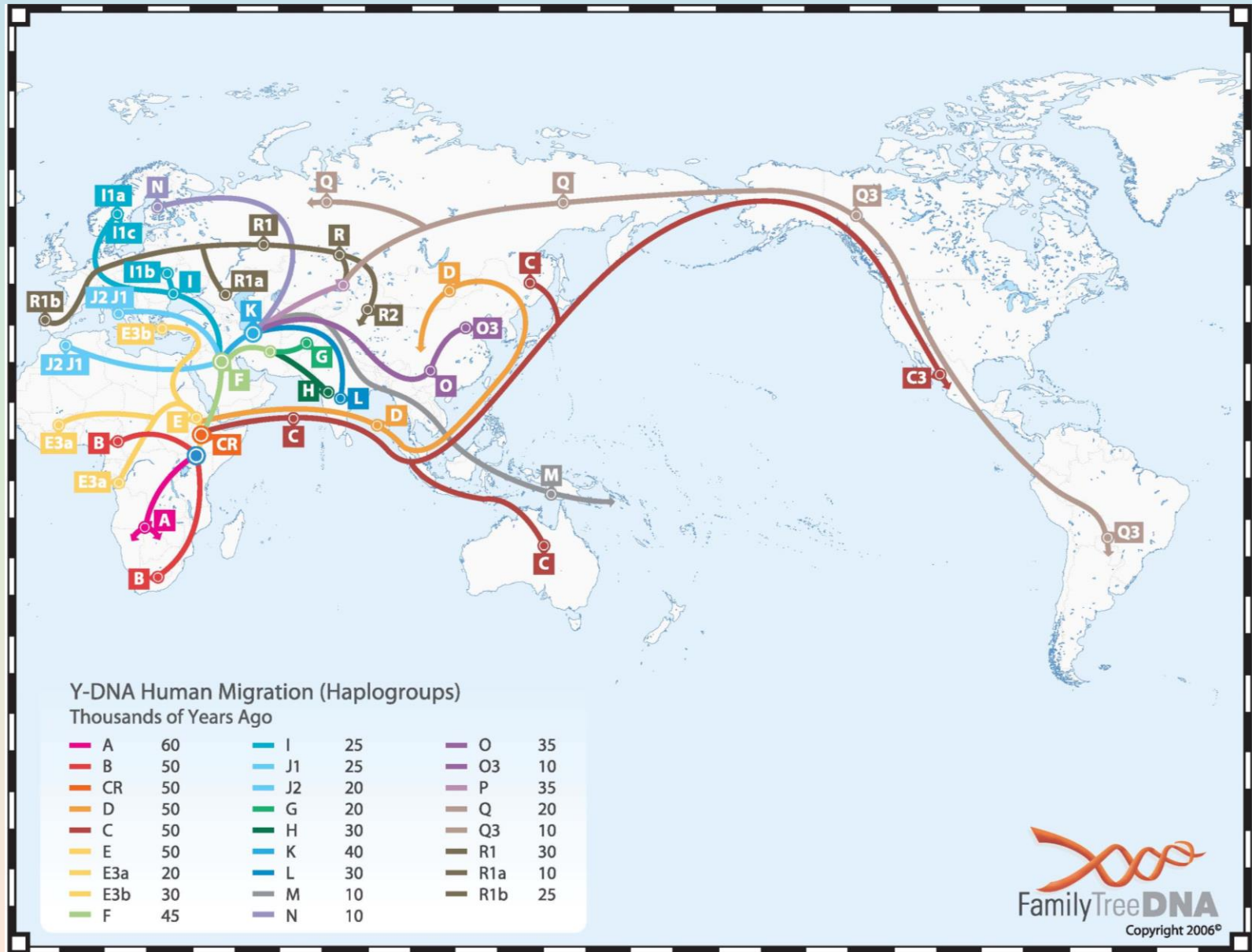
A1812G
T1729A
G3894C
polimorphisms

A1812G
T1729A
polimorphisms



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 for BRAF 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?

The screenshot shows the UCSC Genome Browser website in a Microsoft Internet Explorer browser window. The browser's address bar displays <http://genome.ucsc.edu/>. The website's main navigation bar includes links for Genomes, Blat, Tables, Gene Sorter, PCR, VisiGene, Proteome, Session, FAQ, and Help. A left-hand sidebar lists various tools and resources such as Genome Browser, ENCODE, Neandertal, Blat, Table Browser, Gene Sorter, In Silico PCR, Genome Graphs, Galaxy, VisiGene, Proteome Browser, Utilities, and Downloads.

About the UCSC Genome Bioinformatics Site

Welcome to the UCSC Genome Browser website. This site contains the reference sequence and working draft assemblies for a large collection of genomes. It also provides portals to the [ENCODE](#) and [Neandertal](#) projects.

We encourage you to explore these sequences with our tools. The [Genome Browser](#) zooms and scrolls over chromosomes, showing the work of annotators worldwide. The [Gene Sorter](#) shows expression, homology and other information on groups of genes that can be related in many ways. [Blat](#) quickly maps your sequence to the genome. The [Table Browser](#) provides convenient access to the underlying database. [VisiGene](#) lets you browse through a large collection of *in situ* mouse and frog images to examine expression patterns. [Genome Graphs](#) allows you to upload and display genome-wide data sets.

The UCSC Genome Browser is developed and maintained by the Genome Bioinformatics Group, a cross-departmental team within the Center for Biomolecular Science and Engineering ([CBSE](#)) at the University of California Santa Cruz ([UCSC](#)). If you have feedback or questions concerning the tools or data on this website, feel free to contact us on our [public mailing list](#).

News [News Archives](#) ►

To receive announcements of new genome assembly releases, new software features, updates and training seminars by email, subscribe to the [genome-announce](#) mailing list.

15 July 2010 - Conservation track available for zebrafish (danRer6): We are pleased to announce the release of a new Conservation track based on the zebrafish (danRer6) assembly. [Read more.](#)

7 July 2010 - Happy 10th Birthday, Human Genome!

1-BRAF gene sequence

Human chr21:33,031,597-33,041,570 - UCSC Genome Browser v235 - Microsoft Internet Explorer

Adres <http://genome.ucsc.edu/cgi-bin/hgTracks?org=human>

Home Genomes Blat Tables Gene Sorter PCR DNA Convert Ensembl NCBI PDF/PS Session Help

UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x

position/search **BRAF** size 9,974 bp.

chr21 (q22.11) 21p13 21p12 21p11.2 21q21.1 q21.2 21q21.3 21q22.11 q22.2 21q22.3

Scale 2 kb

chr21: 33033000| 33034000| 33035000| 33036000| 33037000| 33038000| 33039000| 33040000| 33041000|

UCSC Genes Based on RefSeq, UniProt, GenBank, CCDS and Comparative Genomics

BC041449 SOD1 SOD1 SOD1 RefSeq Genes Human mRNAs from GenBank Human ESTs That Have Been Spliced Spliced ESTs Vertebrate Multiz Alignment & Conservation (46 Species) Placental Mammal Basewise Conservation by PhyloP Mammal Cons 4 0 -4 Multiz Alignments of 46 Vertebrates Rhesus Mouse Dog Elephant Opossum Chicken X_tropicalis Zebrafish SNPs (131) Repeating Elements by RepeatMasker RepeatMasker

move start < 2.0 > Click on a feature for details. Click or drag in the base position track to zoom in. move end < 2.0 >

Click gray/blue bars on left for track options and descriptions.

Use drop-down controls below and press refresh to alter tracks displayed.

Tracks with lots of items will automatically be displayed in more compact modes.

1-BRAF gene sequence

Human chr7:140,433,815-140,624,564 - UCSC Genome Browser v235 - Microsoft Internet Explorer

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Adres <http://genome.ucsc.edu/cgi-bin/hgTracks>

Home Genomes Blat Tables Gene Sorter PCR DNA Convert Ensembl NCBI PDF/PS Session Help

UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x

position/search chr7:140,433,815-140,624,564 gene jump clear size 190,750 bp. configure

chr7 (q34) 21.11 31.1 q33c q35

Scale 50 kb

chr7: 140500000 140550000 140600000

UCSC Genes Based on RefSeq, UniProt, GenBank, CCDS and Comparative Genomics

RefSeq Genes

Human mRNAs from GenBank

Human ESTs That Have Been Spliced

Vertebrate Multiz Alignment & Conservation (46 Species)
Placental Mammal Basewise Conservation by PhyloP

Mammal Cons

Multiz Alignments of 46 Vertebrates

Rhesus
Mouse
Dog
Elephant
Opossum
Chicken
X_tropicalis
Zebrafish

Simple Nucleotide Polymorphisms (dbSNP build 131)

Repeating Elements by RepeatMasker

move start < 2.0 > move end < 2.0 >

Click on a feature for details. Click or drag in the base position track to zoom in.
Click gray/blue bars on left for track options and descriptions.

default tracks hide all add custom tracks configure reverse refresh

collapse all Use drop-down controls below and press refresh to alter tracks displayed.
Tracks with lots of items will automatically be displayed in more compact modes. expand all

1-BRAF gene sequence



Human chr7:140,433,815-140,624,564 - UCSC Genome Browser v235 - Microsoft Internet Explorer

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Adres <http://genome.ucsc.edu/cgi-bin/hgTracks?position=chr7:140433815-140624564&hgid=165808107&refGene=pack> Git Bağlantılar

Home Genomes Blat Tables Gene Sorter PCR DNA Convert Ensembl NCBI PDF/PS Session Help

UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x

position/search chr7:140,433,815-140,624,564 gene jump clear size 190,750 bp. configure

Scale 50 kb

chr7: 140500000 | 140550000 | 140600000

UCSC Genes Based on RefSeq, UniProt, GenBank, CCDS and Comparative Genomics

RefSeq Genes

Human mRNAs

Spliced ESTs

Human ESTs That Have Been Spliced

Vertebrate Multiz Alignment & Conservation (45 Species)
Placental Mammal Basewise Conservation by PhyloP

Mammal Cons

Multiz Alignments of 45 Vertebrates

Rhesus
Mouse
Dog
Elephant
Opossum
Chicken
X_tropicalis
Zebrafish

Simple Nucleotide Polymorphisms (dbSNP build 131)

RepeatMasker

move start Click on a feature for details. Click or drag in the base position track to zoom in. move end

< 2.0 > Click gray/blue bars on left for track options and descriptions. < 2.0 >

default tracks hide all add custom tracks configure reverse refresh

collapse all Use drop-down controls below and press refresh to alter tracks displayed. expand all

Tracks with lots of items will automatically be displayed in more compact modes.

1-BRAF gene sequence



RefSeq Gene - Microsoft Internet Explorer

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Geri Ara Sık Kullanılanlar

Adres http://genome.ucsc.edu/cgi-bin/hgc?hgsid=165808107&o=140433814&t=140624564&g=refGene&i=NM_004333 Git Bağlantılar

Home Genomes Genome Browser Blat Tables Gene Sorter PCR Session FAQ Help

RefSeq Gene

RefSeq Gene BRAF

RefSeq: [NM_004333.4](#) Status: Reviewed

Description: ~~Home~~-sapiens v-raf murine sarcoma viral oncogene homolog B1 (BRAF), mRNA.

CCDS: [CCDS5863.1](#)

CDS: 3'-complete

OMIM: [164757](#)

Entrez Gene: [673](#)

PubMed on Gene: [BRAF](#)

PubMed on Product: [serine/threonine-protein kinase B-raf](#)

GeneCards: [BRAF](#)

Ace View: [BRAF](#)

Stanford SOURCE: [NM_004333](#)

CDS FASTA alignment from multiple alignment: [NM_004333](#)

Summary of BRAF

This gene encodes a protein belonging to the raf/mil family of serine/threonine protein kinases. This protein plays a role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation, and secretion. Mutations in this gene are associated with cardiofaciocutaneous syndrome, a disease characterized by heart defects, mental retardation and a distinctive facial appearance. Mutations in this gene have also been associated with various cancers, including non-Hodgkin lymphoma, colorectal cancer, malignant melanoma, thyroid carcinoma, non-small cell lung carcinoma, and adenocarcinoma of lung. A pseudogene, which is located on chromosome X, has been identified for this gene. [provided by RefSeq]. Publication Note: This RefSeq record includes a subset of the publications that are available for this gene. Please see the Entrez Gene record to access additional publications.

1-BRAF gene sequence



RefSeq Gene - Microsoft Internet Explorer

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Adres http://genome.ucsc.edu/cgi-bin/hgc?hgsid=165808107&o=140433814&t=140624564&g=refGene&j=NM_004333 Git Bağlantılar

[View details of parts of alignment within browser window.](#)

Position: [chr7:140433815-140624564](#)
Band: 7q34
Genomic Size: 190750
Strand: -
Alternate Name: BRAF
CDS Start: complete
CDS End: complete

Links to sequence:

- [Predicted Protein](#)
- [mRNA Sequence](#) may be different from the genomic sequence.
- [Genomic Sequence](#) from assembly

[View table schema](#)

[Go to RefSeq Genes track controls](#)

Data last updated: 2010-07-19

Description

The RefSeq Genes track shows known human protein-coding and non-protein-coding genes taken from the NCBI RNA reference sequences collection (RefSeq). The data underlying this track are updated daily.

1-BRAF gene sequence

Genomic Sequence Near Gene - Microsoft Internet Explorer

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

← Geri → × ↻ 🏠 🔍 Ara ★ Sık Kullanılanlar 📧 🖨️ 📄 📁 📖 👤

Adres http://genome.ucsc.edu/cgi-bin/hgc?hgscid=165808107&g=htcGeneInGenome&i=NM_004333&c=chr7&l=140433814&r=140624564&o=refGene&table=refGene [Git](#) [Bağlantılar](#) >>

Genomic Sequence Near Gene

Get Genomic Sequence Near Gene

Note: if you would prefer to get DNA for more than one feature of this track at a time, try the [Table Browser](#) using the output format sequence.

Sequence Retrieval Region Options:

- Promoter/Upstream by bases
- 5' UTR Exons
- CDS Exons
- 3' UTR Exons
- Introns
- Downstream by bases
- One FASTA record per gene.
- One FASTA record per region (exon, intron, etc.) with extra bases upstream (5') and extra downstream (3')
- Split UTR and CDS parts of an exon into separate FASTA records

Note: if a feature is close to the beginning or end of a chromosome and upstream/downstream bases are added, they may be truncated in order to avoid extending past the edge of the chromosome.

Sequence Formatting Options:

- Exons in upper case, everything else in lower case.
- CDS in upper case, UTR in lower case.
- All upper case.
- All lower case.
- Mask repeats: to lower case to N

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



Ensembl Genome Browser - Microsoft Internet Explorer

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Adres <http://www.ensembl.org/index.html>

Ensembl Home Login / Register | BLAST/BLAT | BioMart | Docs & FAQs

Search: All species for
BRAF Go

e.g. [human gene BRCA2](#) or [rat X:100000..200000](#) or [coronary heart disease](#)

Browse a Genome

The Ensembl project produces genome databases for vertebrates and other eukaryotic species, and makes this information freely available online.

Click on a link below to go to the species' home page.

Popular genomes ([Log in to customize this list](#))

- Human**
GRCh37
- Mouse**
NCBIM37
- Zebrafish**
Zv8

All genomes

-- Select a species --

[View full list of all Ensembl species](#)

Other species are available in [Ensembl Pre!](#) and [EnsemblGenomes](#)

New to Ensembl?

Did you know you can:

- [Learn how to use Ensembl](#) with our video tutorials and walk-throughs
- [Add custom tracks](#) using our new Control Panel
- [Upload and analyse your data](#) and save it to your Ensembl account
- [Search for a DNA or protein sequence](#) using BLAST or BLAT
- [Fetch only the data you want](#) from our public database, using the Perl API
- [Download our databases via FTP](#) in FASTA, MySQL and other formats
- [Mine Ensembl with BioMart](#) and export sequences or tables in text, html, or Excel format

Still got questions? Try our [FAQs](#) or [glossary](#)

Did you know...?

A preliminary assembly of the Giant panda (*Ailuropoda melanoleuca*) is now available on our pre! site, <http://pre.ensembl.org/panda>

What's New in Release 58 (27 May 2010)

2-Detection of fragment



The screenshot shows the Ensembl genome browser interface in Microsoft Internet Explorer. The browser title is "Ensembl genome browser 58: Homo sapiens - Results Summary - Ensembl text search - Microsoft Internet Explorer". The address bar shows the URL: "http://www.ensembl.org/Homo_sapiens/Search/Results?species=Homo_sapiens;idx=;q=BRAF".

The main content area is titled "Results Summary" and contains two tables:

By Feature type	
Total	6
▶ Gene	4
▶ SNP	2

By Species	
Total	6
▶ Homo sapiens	6

At the bottom of the page, there is a footer with the text: "Ensembl release 58 - May 2010 © WTSI / EBI" and links for "About Ensembl", "Contact Us", and "Help". There is also a link for "Permanent link - View in archive site".

2-Detection of fragment



Ensembl genome browser 58: Homo sapiens - Result in Detail - Ensembl text search - Microsoft Internet Explorer

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Adres http://www.ensembl.org/Homo_sapiens/Search/Details?_C=eJxtyjsOgCAQBcA9CuECaGFI7LQw1mpPCD4jCbLIx*N7Aace7QmB9BvowGmQL6QfmtZxPpZT5GQKVENAIHeO Git Bağlantılar

e!Ensembl
Home > Human [GRCh37] Login / Register | BLAST/BLAT | BioMart | Docs & FAQs

Genome

Search Ensembl
New Search
Configure this page
Manage your data
Export data
Bookmark this page

Result in Detail

BRAF corporate/tree:"Top/Species/Homo sapiens" Search

Your query matched 4 entries in the search database

Ensembl protein coding Gene: ENSG00000157764 (HGNC Symbol: BRAF) [Region in detail]

Description: v-rf murine sarcoma viral oncogene homolog B1 [Source:HGNC Symbol;Acc:1097]

Source: e58; **Feature type:** Gene; Homo sapiens; **Species:** Homo sapiens; Gene;

Havana protein coding Gene: OTTHUMG00000157457 (BRAF) [Region in detail]

Description: v-rf murine sarcoma viral oncogene homolog B1

Source: e58; **Feature type:** Gene; Homo sapiens; **Species:** Homo sapiens; Gene;

Ensembl protein coding Gene: ENSG00000122778 (HGNC Symbol: KIAA1549) [Region in detail]

Description: KIAA1549 [Source:HGNC Symbol;Acc:22219]

Source: e58; **Feature type:** Gene; Homo sapiens; **Species:** Homo sapiens; Gene;

Havana processed pseudogene Gene: OTTHUMG00000021868 (RP11-451L9.3) [Region in detail]

Description: v-rf murine sarcoma viral oncogene homolog B1 (BRAF) pseudogene

Source: e58; **Feature type:** Gene; Homo sapiens; **Species:** Homo sapiens; Gene;

Ensembl release 58 - May 2010 © WTSI / EBI

[About Ensembl](#) | [Contact Us](#) | [Help](#)

2-Detection of fragment



Ensembl genome browser 58: Homo sapiens - Gene summary - Gene: BRAF (ENSG00000157764) - Microsoft Internet Explorer

Adres: http://www.ensembl.org/Homo_sapiens/Gene/Summary?g=ENSG00000157764

Gene: BRAF (ENSG00000157764)

v-raf murine sarcoma viral oncogene homolog B1 [Source:HGNC Symbol;Acc:1097]

Location [Chromosome 7: 140,424,943-140,624,564](#) reverse strand.

Transcripts There are 5 transcripts in this gene

Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype
BRAF-001	ENST00000288602	2480	ENSP00000288602	766	Protein coding
BRAF-003	ENST00000496384	2478	ENSP00000419060	375	Protein coding
BRAF-002	ENST00000497784	2336	ENSP00000420119	194	Nonsense mediated dec
BRAF-005	ENST00000479537	743	ENSP00000418033	102	Nonsense mediated dec
BRAF-004	ENST00000469930	1058	No protein product	-	Retained intron

Transcript and Gene level displays

In Ensembl a gene is made up of one or more transcripts. We provide displays at two levels:

- Transcript views which provide information specific to an individual transcript such as the cDNA and CDS sequences and protein domain annotation.
- Gene views which provide displays for data associated at the gene level such as orthologues and paralogues, regulatory regions and splice variants.

This view is a gene level view. To access the transcript level displays select a Transcript ID in the table above and then navigate to the information you want using the menu at the left hand side of the page. To return to viewing gene level information click on the Gene tab in the menu bar at the top of the page.

2-Detection of fragment



Ensembl genome browser 58: Homo sapiens - Transcript summary - Transcript: BRAF-001 (ENST00000288602 - Microsoft Internet Explorer)

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Adres http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000157764;r=7:140424943-140624564;t=ENST00000288602

e!Ensembl
Home > Human [GRCh37] Login / Register | BLAST/BLAT | BioMart | Docs & FAQs

Location: 7:140,424,943-140,624,564 Gene: BRAF Transcript: BRAF-001

Transcript-based displays

- Transcript summary
- Supporting evidence (12)
- Sequence
 - Exons (18)
 - cDNA
 - Protein
- External References
 - General identifiers (46)
 - Oligo probes (35)
 - Gene ontology (46)
- Genetic Variation
 - Population comparison
 - Comparison image
- Protein Information
 - Protein summary
 - Domains & features (32)
 - Variations (12)
- External Data
 - Personal annotation
- ID History
 - Transcript history
 - Protein history

Configure this page Manage your data Export data Bookmark this page

Transcript: BRAF-001 (ENST00000288602)
v-raf murine sarcoma viral oncogene homolog B1 [Source:HGNC Symbol;Acc:1097]
Location [Chromosome 7: 140,434,279-140,624,564](#) reverse strand.
Gene This transcript is a product of gene [ENSG00000157764](#) - There are 5 transcripts in this gene

Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype
BRAF-001	ENST00000288602	2480	ENSP00000288602	766	Protein coding
BRAF-003	ENST00000496384	2478	ENSP00000419060	375	Protein coding
BRAF-002	ENST00000497784	2336	ENSP00000420119	194	Nonsense mediated dec
BRAF-005	ENST00000479537	743	ENSP00000418033	102	Nonsense mediated dec
BRAF-004	ENST00000469930	1058	No protein product	-	Retained intron

Transcript and Gene level displays

In Ensembl a gene is made up of one or more transcripts. Views in Ensembl are separated into Gene based views and Transcript based views according to which level the information is more appropriately associated with. This view is a transcript level view. To flip between the two sets of views you can click on the Gene and Transcript tabs in the menu bar at the top of the page.

Transcript summary [help](#) [Supporting evidence »](#)

Reverse strand 190.29 Kb

2-Detection of fragment



Ensembl genome browser 58: Homo sapiens - cDNA sequence - Transcript: BRAF-001 (ENST00000288602 - Microsoft Internet Explorer)

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Adres http://www.ensembl.org/Homo_sapiens/Transcript/Sequence_cDNA?db=core;g=ENSG00000157764;r=7:140424943-140624564;t=ENST00000288602 Git Bağlantılar

```
1621 CACAAAGCCACAACCTGGCTATTGTTACCCAGTGGTGTGAGGGCTCCAGCTTGATCACCA
1560 CACAAAGCCACAACCTGGCTATTGTTACCCAGTGGTGTGAGGGCTCCAGCTTGATCACCA
520 --T--K--P--Q--L--A--I--V--T--Q--W--C--E--G--S--S--L--Y--H--H

1681 TCTCCATATCATTGAGACCAAATTTGAGATGATCAAACCTTATAGATATTGCACGACAGAC
1620 TCTCCATATCATTGAGACCAAATTTGAGATGATCAAACCTTATAGATATTGCACGACAGAC
540 --L--H--I--I--E--T--K--F--E--M--I--K--L--I--D--I--A--R--Q--T

1741 TGCACAGGGCATGGATTACTTACAGCCAAAGTCAATCATCCACAGAGACCTCAAGAGTAA
1680 TGCACAGGGCATGGATTACTTACAGCCAAAGTCAATCATCCACAGAGACCTCAAGAGTAA
560 --A--Q--G--M--D--Y--L--H--A--K--S--I--I--H--R--D--L--K--S--N

1801 TAATATATTTCTTCATGAAAGACCTCACAGTAAAAAAGGGTATTTCCTCTAGCTACAGT
1740 TAATATATTTCTTCATGAAAGACCTCACAGTAAAAAAGGGTATTTCCTCTAGCTACAGT
580 --N--I--F--L--H--E--D--L--T--V--K--I--G--D--F--G--L--A--T--V

1861 GAAATCTCGATGGAGTGGGTCCCATCAGTTTGAACAGTTGCTGGATCCATTTGTGGAT
1800 GAAATCTCGATGGAGTGGGTCCCATCAGTTTGAACAGTTGCTGGATCCATTTGTGGAT
600 --K--S--R--W--S--G--S--H--Q--F--E--Q--L--S--G--S--I--L--W--M

1921 GGCACCCAGAAGTCATCAGAATGCAAGATAAAAAATCCATACAGCTTTTCAGTCAGATGTATA
1860 GGCACCCAGAAGTCATCAGAATGCAAGATAAAAAATCCATACAGCTTTTCAGTCAGATGTATA
620 --A--P--E--V--I--R--M--Q--D--K--N--P--Y--S--F--Q--S--D--V--Y

1981 TGCATTTGGATTGTTCTGTATGAATTGATGACTGGACAGTTACCTTATCAAACATCAA
1920 TGCATTTGGATTGTTCTGTATGAATTGATGACTGGACAGTTACCTTATCAAACATCAA
640 --A--F--G--I--V--L--Y--E--L--M--T--G--Q--L--P--Y--S--N--I--N
```

2-Detection of fragment

The image shows a Microsoft Word window titled "Belge1 - Microsoft Word" with a DNA sequence document. A "Bul ve Değiştir" (Find and Replace) dialog box is open, showing the search term "TAGCTACAGT" in the "Aranan:" field. The search term is highlighted in the original DNA sequence. The dialog box has buttons for "Bul", "Değiştir", and "Git". Below the search field, there is a checkbox for "Aşağıda bulunan tüm öğeleri yargula:" (Match case) which is unchecked. At the bottom of the dialog, there are buttons for "Asıl Belge", "Tüm Seçenekler", "Sonrakini Bul", and "İptal".

The DNA sequence in the background is as follows:

```
tcattggttttagacatacttattgactcctaaagaggaaagatgaagtacta
tggtttaaagaatatatatcacagaattatagaattagatctcttacc
taaactcttcataatgcttgctctgataggaaaaatgagatctactgtttt
cctttacttactacacctcagATATATTTCTTCATGAAACCTCACAGTA
AAAATAGGTGATTTTGGTCTAGCTACAGTGAAAATCTCGATGGAGTGGGTC
CCATCAGTTTGAACAGTTGCTGGATCCATTTTGTGGATGgt aagaattg
aggctattttccactgattaaattttggccctgagatgctgctgagtt
actagaaagtcatgaaagtctcaactatagtttttcatagttccagtt
attcacaaaaatcagtttcttattttttagtaaatagattttttaact
tttt
caaa
taaa
caaa
tggt
aaaa
tttt
attt
taat
ttaa
gctt
tggt
taaa
agat
aacacattgcaacagactgagtgcaaaagcaaatatgagaatccagctgg
ctgctgttaagccagacattgaggagaatcacaggccactcatggtggct
ggagtcctatagttccagctacctgggaggctagggcaggaggatcacttg
atcccaggagtggactggcctgctcaacatagtgagaccccatctctaaa
ccataaaaaggaggataaatgtagtactattctcttactaaactttttt
tgataaatagttattttcataaaaatgaatgatctgtgttaacatctac
ttgttatttttagtagttaaataactactagtttaattctccatta
aattttaatggttaaacatccacagatataatctacctaaacaaaagtctt
ttatcctcaataaattttaagagtgaaaaaggtcctgagaccaaaa
agtttgaaaaacacagctcctaaagctgaatacagccttccaaaagtctta
```

2-Detection of fragment



Belge2 - Microsoft Word

Dosya Düzen Görünüm Ekle Biçim Araçlar Tablo Pencere Yardım

Times New Roman

1 2 3 4 5 6 7 8 9 10 11 12

WILD TYPE

tgttttaagaatattatattacagaattatagaaattagatctcttacc
taaactcttcataatgcttgctctgataggaaaatgagatctactgtttt
cctttacttactacacctcagATATATTTCTTCATGAAGACCTCACAGTA
AAAATAGGTGATTTTGGTCTAGCTACAGTAAAATCTCGATGGAGTGGGTC
CCATCAGTTTGAACAGTTGTCTGGATCCATTTGTGGATGgtaagaattg

MUTANT

tgttttaagaatattatattacagaattatagaaattagatctcttacc
taaactcttcataatgcttgctctgataggaaaatgagatctactgtttt
cctttacttactacacctcagATATATTTCTTCATGAAGACCTCACAGTA
AAAATAGGTGATTTTGGTCTAGCTACAGTAAAATCTCGATGGAGTGGGTC
CCATCAGTTTGAACAGTTGTCTGGATCCATTTGTGGATGgtaagaattg

Sayfa 1 Böl 1 1/1 Bşl 7,8 cm Sat 14 Süt 1 KAY DİM SEÇ ÜYZ Türk

3-Selection of restriction enzyme

RestrictionMapper version 3 - Microsoft Internet Explorer

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Adres <http://www.restrictionmapper.org/>

Welcome to RestrictionMapper - on line restriction mapping the easy way.

Maps sites for restriction enzymes, a.k.a. restriction endonucleases, in DNA sequences. Also does virtual digestion.

[Donate](#)

Conformation	Include	Sequence Info	Menu
<p>Circular <input type="radio"/></p> <p>Linear <input checked="" type="radio"/></p>	<p>Select Individual Enzymes</p> <p>All Enzymes ▲</p> <p>AarI</p> <p>AasI</p> <p>AatI</p> <p>AatII</p> <p>AccI</p> <p>AccII ▼</p>	<p>No non-base letters. Numbers and spaces OK.</p> <p>Paste Sequence Here</p> <p>_____</p>	<p>Map Sites</p> <p>Virtual Digest</p> <p>Reset Form</p>
<p>Sort By</p> <p>1. frequency ▼</p> <p>2. overhang ▼</p> <p>3. name ▼</p>	<p>All Commercial <input checked="" type="radio"/></p> <p>NEB only <input type="radio"/></p> <p>5' overhang <input checked="" type="checkbox"/></p> <p>3' overhang <input checked="" type="checkbox"/></p> <p>blunt <input checked="" type="checkbox"/></p> <p>Prototypes Only <input checked="" type="radio"/></p> <p>All Isoschizomers <input type="radio"/></p>	<p>Name your sequence</p>	
<p>Filter By</p> <p>Maximum Cuts all ▼</p> <p>Minimum Site Length 5 ▼</p>			

- [Help](#)
- [FAQ](#)
- [What's New?](#)
- [Code](#)
- [Contact](#)
- [Automate RestrictionMapper](#)
- [Other Free Molecular Biology Resources](#)
- [Dilution Calculator](#)

3-Selection of restriction enzyme



RestrictionMapper Output - Microsoft Internet Explorer

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Gerçekleştiren Ara Sık Kullanılanlar

Adres: <http://www.restrictionmapper.org/cgi-bin/sitefind3.pl>

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
SmaDI	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
PfiMI	CCANNNNTGG	6	three_prime	1	233
TspRI	CASTG	5	three_prime	1	182
XcmI	CCANNNNNNNNTGG	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	VCTCGAGE	8	five_prime	4	67, 71, 167, 173

Restriction enzymes for WILD Type fragment

3-Selection of restriction enzyme

RestrictionMapper version 3 - Microsoft Internet Explorer

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Adres <http://www.restrictionmapper.org/>

Welcome to RestrictionMapper - on line restriction mapping the easy way.

Maps sites for restriction enzymes, a.k.a. restriction endonucleases, in DNA sequences. Also does virtual digestion.

[Donate](#)

Conformation: Circular Linear

Sort By:

- frequency
- overhang
- name

Filter By:

Maximum Cuts: all

Minimum Site Length: 5

Include: Select Individual Enzymes

- All Enzymes
- AarI
- AasI
- AatI
- AatII
- AccI
- AccII

All Commercial NEB only

5' overhang 3' overhang blunt

Prototypes Only All Isoschizomers

Sequence Info: No non-base letters. Numbers and spaces OK.

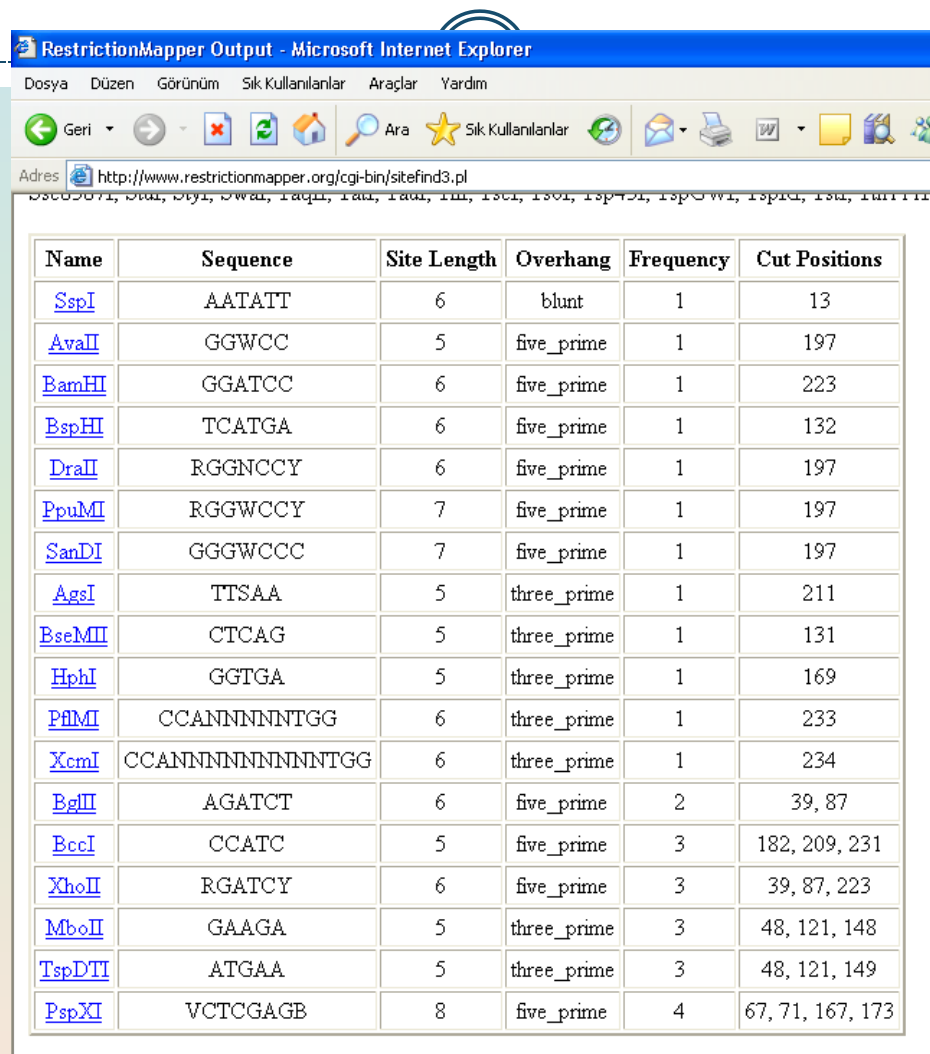
Paste Sequence Here

Name your sequence

Menu: [Map Sites](#) [Virtual Digest](#) [Reset Form](#)

- [Help](#)
- [FAQ](#)
- [What's New?](#)
- [Code](#)
- [Contact](#)
- [Automate RestrictionMapper](#)
- [Other Free Molecular Biology Resources](#)
- [Dilution Calculator](#)

3-Selection of restriction enzyme



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	CCANNNNNNTGG	6	three_prime	1	233
XcmI	CCANNNNNNNNTGG	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
TspDII	ATGAA	5	three_prime	3	48, 121, 149
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
<u>SspI</u>	1	<u>SspI</u>	1
<u>AvaII</u>	1	<u>AvaII</u>	1
<u>BamHI</u>	1	<u>BamHI</u>	1
<u>BspHI</u>	1	<u>BspHI</u>	1
<u>DraII</u>	1	<u>DraII</u>	1
<u>PpuMI</u>	1	<u>PpuMI</u>	1
<u>SanDI</u>	1	<u>SanDI</u>	1
<u>AgsI</u>	1	<u>AgsI</u>	1
<u>BseMII</u>	1	<u>BseMII</u>	1
<u>HphI</u>	1	<u>HphI</u>	1
<u>PflMI</u>	1	<u>PflMI</u>	1
<u>TspRI</u>	0	<u>TspRI</u>	1
<u>XcmI</u>	1	<u>XcmI</u>	1
<u>BglII</u>	2	<u>BglII</u>	2
<u>BccI</u>	3	<u>BccI</u>	3
<u>XhoII</u>	3	<u>XhoII</u>	3

3-Selection of restriction enzyme



- TspRI

4-Primer designing

Primer3 Input (version 0.4.0) - Microsoft Internet Explorer

Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım

Adres <http://frodo.wi.mit.edu/primer3/>

Primer3 (v. 0.4.0) Pick primers from a DNA sequence.

[Checks for mispriming in template.](#) [disclaimer](#) [Primer3 Home](#)
[Primer3plus interface](#) [cautions](#) [FAQ/WIKI](#)

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a [Mispriming Library \(repeat library\)](#):

<input checked="" type="checkbox"/> Pick left primer, or use left primer below: <input type="text"/>	<input type="checkbox"/> Pick hybridization probe (internal oligo), or use oligo below: <input type="text"/>	<input checked="" type="checkbox"/> Pick right primer, or use right primer below (5' to 3' on opposite strand): <input type="text"/>
---	---	---

Sequence Id: A string to identify your output.

Targets: E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the [source sequence](#) with [and]: e.g. ...ATCT[CCCC]TCAT.. means that primers must flank the central CCCC.

Excluded Regions: E.g. 401,7 68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the [source sequence](#) with < and >: e.g. ...ATCT<CCCC>TCAT.. forbids primers in the central CCCC.

Product Size Ranges

Number To Return **Max 3' Stability**

Max Repeat Mispriming **Pair Max Repeat Mispriming**



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 nucleotide 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 nucleotide is T, I will see two bands, 118 ve 55 bp.

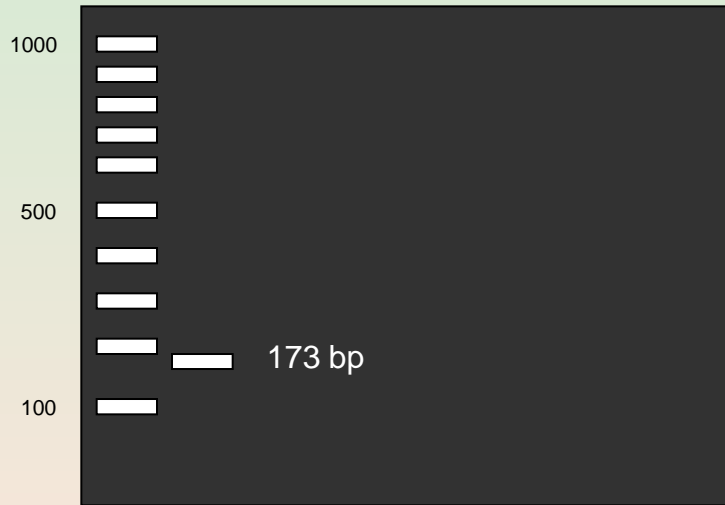
8-Interpretation



1799th nucleotide is A in
BRAF gene



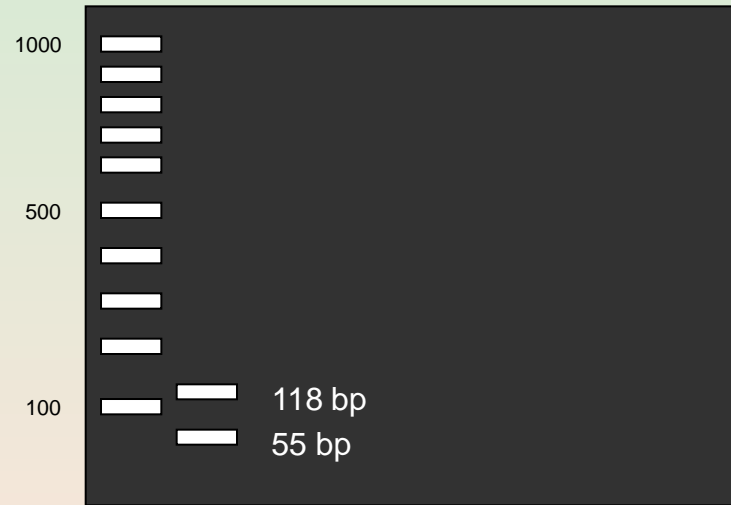
MUTANT



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