

Домашнее задание №2: подбор условий для обнаружения SNP методом RFLP

1. Найти по rs номеру полиморфизм в базе данных dbSNP NCBI.

Приложения Места Firefox ESR en Воскресенье, 20:45

rs5743810 - SNP - NCBI - Mozilla Firefox

Методы детекции SNP | rs5743810 - SNP - NCBI | Sci-Hub: устраняя прерпа | All: polymorphism detect | RestrictionMapper Output

https://www.ncbi.nlm.nih.gov/snp/?term=rs5743810 Поиск

NCBI Resources How To Sign in to NCBI

dbSNP SNP rs5743810 Search

Create alert Advanced Help

Variation Class snp Display Settings: Summary, Sorted by SNP_ID Send to: Filters: Manage Filters

Annotation Cited in PubMed PubMed nucleotide protein structure

Function Class missense

Global MAF Custom range...

Validation Status by-1000 Genomes by-cluster by-frequency

Clear all Show additional filters

Search results

Items: 4

rs5743810 [*Homo sapiens*]

1.

ATTTTATCAGAACTCACCAGAGG[C/T]CAACCTTACTGAATTTTACCCTCAA

Chromosome: 4:38828729

Gene: TLR6 (GeneView)

Functional Consequence: missense

Validated: by 1000G,by cluster,by frequency

Global MAF: A=0.1152/577

HGVS: CM000666.2:g.38828729A>G, NC_000004.11:g.38830350A>G, NC_000004.12:g.38828729A>G, NG_028087.1:g.33089T>C, NM_006068.2:c.745T>C, NM_006068.3:c.745T>C, NM_006068.4:c.745T>C, NP_006059.2:p.Ser249Pro, XP_005262693.1:p.Ser249Pro, XP_005262694.1:p.Ser249Pro, XP_011511914.1:p.Ser249Pro, XP_011511915.1:p.Ser249Pro, XP_011511916.1:p.Ser249Pro

PubMed

rs17496037 has merged into rs5743810 [*Homo sapiens*]

2.

ATTTTATCAGAACTCACCAGAGG[C/T]CAACCTTACTGAATTTTACCCTCAA

Chromosome: 4:38828729

Gene: TLR6 (GeneView)

Functional Consequence: missense

Validated: by 1000G,by cluster,by frequency

Global MAF: A=0.1152/577

HGVS: CM000666.2:g.38828729A>G, NC_000004.11:g.38830350A>G, NC_000004.12:g.38828729A>G, NG_028087.1:g.33089T>C, NM_006068.2:c.745T>C, NM_006068.3:c.745T>C, NM_006068.4:c.745T>C, NP_006059.2:p.Ser249Pro, XP_005262693.1:p.Ser249Pro, XP_005262694.1:p.Ser249Pro, XP_011511914.1:p.Ser249Pro, XP_011511915.1:p.Ser249Pro, XP_011511916.1:p.Ser249Pro

rs52796179 has merged into rs5743810 [*Homo sapiens*]

https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=5743810

Find related data Database: Select Find items

Search details rs5743810[All Fields] Search See more...

Recent activity Turn Off Clear

- rs5743810 (4) SNP
- SNP Links for Gene (Select 18) (8131) SNP
- ABAT 4-aminobutyrate aminotransferase [*Homo sapiens*] Gene
- transaminase AND (alive[prop]) (12000) Gene
- SNP Links for Nucleotide (Select 568802081) (1811567) SNP

Занятие №5-6 - Методы... Занятие 5-6. - Методы о... rs5743810 - SNP - NCBI ... [Занятие 2. - ПЦР,одр - ... [Без имени 1 - LibreOffic... Занятие №5.odt - LibreO... 1 / 4

2. Скопировать последовательность около полиморфизма SNP ±250 нуклеотидов (в примере SNP обозначен как Y, т.е. вариация пиримидинов C/T) в текстовый файл.

Приложения Места Firefox ESR en Воскресенье, 20:46

Reference SNP (refSNP) Cluster Report: rs5743810 - Mozilla Firefox

Методы детекции SNP | Reference SNP (refSNP) | Sci-Hub: устраняя прерыва... | All: polymorphism detecti... | RestrictionMapper Output

https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=5743810

ss3022364805 ILLUMINA|MEGA_Consortium_v2_15070954_A2_4:38830350-AG-0_T_F_2299716402 rev/ A/G ttgagggtaaaattcagtaagggtg acctctggtgagtctgataaa
 ss3024869313 BIOINF_KMB_FNS_UNIBA|4.38828729A>G rev/ A/G ttgagggtaaaattcagtaagggtg acctctggtgagtctgataaa
 ss3345650200 CSHL|rs5743810 rev/ A/G ttgagggtaaaattcagtaagggtg acctctggtgagtctgataaa
 ss3425757970 TOPMED|TOPMed_freeze_5?chr4:38,828,729 rev/ A/G ttgagggtaaaattcagtaagggtg acctctggtgagtctgataaa

Fasta sequence (Legend)

>gn1|dbSNP|rs5743810|allelePos=501|totalLen=1001|taxid=9606|snpclass=1|alleles='C/T'|mol=Genomic|build=151

```
GACTTTCCA TAACAGAATC CAGCTACTTG ATTTAAGTGT TTTCAAGTTC AACCAGGATT
TAGAATATTT GGATTATCTC CATAATCAGT TGCAAAAGAT ATCCTGCCAT CCTATTGTGA
GTTTCAGGCA TTTAGATCTC TCATTCAATG ATTTCAAGGC CCTGCCATC TGTAAGGAAT
TTGGCAACTT ATCACAACTG AATTCTTGG GATTGAGTGC TATGAAGCTG CAAAATTAG
ATTTGCTGCC AATTGCTCAC TTGCATCTAA GTTATATCTC TCTGGATTTA AGAAATATT
ATATAAAGA AAATGAGACA GAAAGTCTAC AAATTTCTGAA TGCAAAAACC CTTCACCTTG
TTTTTCACCC AACTAGTTTA TTCGCTATCC AAGTGAACAT ATCAGTTAAT ACTTTAGGGT
GCTTACAAC GACTAATATT AAATTGAATG ATGACAACCT TCAAGTTTTC ATTAATTTT
TATCAGAACT CACCAGAGGT
Y
CAACCTTACT GAATTTTACC CTCAACCACA TAGAAACGAC TTGAAATGC CTGGTCAGAG
TCTTTCAATT TCTTTGGCCC AAACCTGTGG AATATCTCAA TATTTACAAT TTAACAATAA
TTGAAAGCAT TCGTGAAGAA GATTTTACTT ATTCTAAAC GACATTGAAA GCATTGACAA
TAGAACATAT CACGAACCAA GTTTTTCTGT TTTACAGAC AGCTTTGTAC ACCGTGTTTT
CTGAGATGAA CATTATGATG TTAACCATTT CAGATACACC TTTTATACAC ATGCTGTGTC
CTCATGCACC AAGCACATTC AAGTTTTTGA ACTTTACCCA GAACGTTTTT ACAGATAGTA
TTTTTGAAAA ATGTTCCACG TTAGTTAAAT TGGAGACACT TATCTTACAA AAGAATGGAT
TAAAAGACCT TTTCAAAGTA GGTCTCATGA CGAAGGATAT GCCTTCTTTG GAAATACTGG
ATGTTAGCTG GAATTCCTTG
```

NCBI Resource Links

Submitter-Referenced dbSNP Blast Analysis UniGene Cluster ID 3D structure mapping

GenBank NT_016297.15 366986 NP_006059

Population Diversity (Alleles in RefSNP orientation) . See additional population frequency from 1000Genome [here] Note: rs5743810 allele is reverse to the genome

ss#	Sample Ascertainment			Genotype Detail				Alleles		
	Population	Individual Group	Chrom. Sample Cnt.	Source	C/C	C/T	T/T	HWP	C	T
ss116958760	YRI		2	IG	1.00000000				1.00000000	
ss1309284606	EAS		1008	AF					1.00000000	
	EUR		1006	AF					0.59149998	0.40849999
	AFR		1322	AF					0.98339999	0.01660000
	AMR		694	AF					0.81699997	0.18300000
	SAS		978	AF					0.98259997	0.01740000

Занятие №5-6 - Методы... Занятие 5-6. - Методы о... Reference SNP (refSNP) ... [Занятие 2. - ПЦП.odp - ... [Без имени 1 - LibreOffic... Занятие №5.odt - LibreO... 1 / 4

3. На сайте проекта RestrictionMapper вставить в окно формы последовательность SNP±250 нуклеотидов и нажать Map Sites для поиска сайтов рестрикции (на месте SNP указать какой-то его вариант)

Приложения Места Firefox ESR en Воскресенье, 21:23

RestrictionMapper version 3 - Mozilla Firefox

Методы детекции SNP | Reference SNP (refSNP) | Sci-Hub: устраняя прерп | All: polymorphism detecti | RestrictionMapper version 3

restrictionmapper.org Поиск

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.

Conformation	Include	Sequence Info	Menu
<p>Circular <input type="radio"/></p> <p>Linear <input checked="" type="radio"/></p> <p>Sort By</p> <p>1. frequency <input type="text"/></p> <p>2. overhang <input type="text"/></p> <p>3. name <input type="text"/></p> <p>Filter By</p> <p>Maximum Cuts <input type="text" value="all"/></p> <p>Minimum Site Length <input type="text" value="4"/></p>	<p>Select Individual Enzymes</p> <p>All Enzymes</p> <p>AarI</p> <p>AasI</p> <p>AatI</p> <p>AatII</p> <p>Accl</p> <p>AcclI</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 type="radio"/></p> <p>All Isoschizomers <input type="radio"/></p>	<p>No non-base letters. Numbers and spaces OK.</p> <p>Paste Sequence Here</p> <pre>GACAGAAAGTCTCAAATTCG AATGCAAAAACCCITCACCTTG TTTTCCACCAACTAGTTATT CGCTATCCAAGTGAACATCA GTTAATACTTTAGGGTGCTTAC AACTGACTAATATTAATTGAA TGATGACAACTGTCAAGTTTTC ATTAAATTTTATCAGAACTCA CCAGAGGT T CAACCTTACTGAATTTTACCCT CAACCACATAGAAACGACTTGG AAATGCCGTGTCAGAGCTTTTC AATTTCTTTGGCCAAACCTGT</pre> <p>Name your sequence</p> <input type="text" value="Untitled"/>	<p>Map Sites</p> <p>Virtual Digest</p> <p>Reset Form</p>

Занятие №5-6 - Методы обнару... Занятие 5-6. - Методы обнару... RestrictionMapper version 3 - M... Без имени 1 - LibreOffice Writer Занятие №5.odt - LibreOffice ... 1 / 4

4. В появившемся отчёте выбрать рестриктазы с единичным сайтом разрезания (Cut Position) недалеко от позиции SNP (251). Выполнить пп.3-4 с альтернативным вариантом SNP и выбрать рестриктазу, у которой сайт узнавания есть только для одного из вариантов.

Приложения Места Firefox ESR en Воскресенье, 21:24

RestrictionMapper Output - Mozilla Firefox

Методы детекции SNP | Reference SNP (refSNP) | Sci-Hub: устраняя прерпа | All: polymorphism detecti | RestrictionMapper Output

restrictionmapper.org/cgi-bin/sitefind3.pl

Name: Untitled

Conformation: linear

Overhang: five_prime, three_prime, blunt

Minimum Site Length: 4 bases

Maximum Number of Cuts: all

Included: all commercial, prototypes only

Noncutters: AarI, AatII, AbsI, AciI, AclI, AcyI, AflII, AflIII, AgeI, AjuI, AlfI, AloI, AlwNI, ApaI, ApaLI, ArsI, AscI, AsuII, AvaI, AvaII, AvrII, BaeI, Ball, BamHI, BarI, BbvI, BbvCI, BccI, BcgI, BciVI, BclI, BfiI, BglI, BglII, BsiI, BplI, Bpu10I, BsaAI, BsaBI, BsaXI, BsePI, BseRI, BseSI, BseYI, BsgI, BspHI, BspMI, BsrI, BsrBI, BsrDI, BstEII, BtgZI, BtrI, BtsI, Cac8I, CfrI, Cfr10I, ClaI, CspCI, DpnI, DraII, DraIII, DrdI, EciI, Eco31I, Eco47III, Eco57I, Eco57MI, EcoNI, EcoP15I, EcoRI, EcoRV, Esp3I, Fall, Faul, Fnu4HI, FokI, FseI, FspAI, GlaI, GsuI, HaeII, HgaI, HhaI, HindII, HindIII, HpaI, HpaII, Hpy99I, KpnI, MaeII, MaeIII, MauBI, MboI, MfeI, MluI, MmeI, MslI, MwoI, NaeI, NarI, NcoI, NdeI, NheI, NlaIII, NlaIV, NmeAIII, NotI, NruI, NspI, OliI, PacI, Pasi, PfiMI, PfoI, PmaCI, PmeI, PpiI, PpuMI, PsiI, PI-PspI, PspXI, PsrI, PstI, PvuI, PvuII, RsrII, SacI, SacII, SalI, SanDI, SapI, ScaI, PI-SceI, SduI, SexAI, SfiI, Sgfi, SgrAI, SgrDI, SmaI, SmlI, SnaBI, SphI, SrfI, Sse8387I, StuI, StyI, SwaI, TaqI, TaqII, TauI, TfiI, TseI, TsoI, Tsp45I, TspGWI, TspRI, TstI, Tth111I, VspI, XbaI, XcmI, XhoI, XhoII

Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
AluI	AGCT	4	blunt	1	473
HaeIII	GGCC	4	blunt	1	328
PshAI	GACNNNGTC	6	blunt	1	207
RsaI	GTAC	4	blunt	1	479
XmnI	GAANNNTTC	6	blunt	1	378
AccI	GTMKAC	6	five_prime	1	76
BsmAI	GTCTC	5	five_prime	1	59
Bsp1407I	TGTACA	6	five_prime	1	477
EcoRII	CCWGG	5	five_prime	1	300
HinfI	GANTC	4	five_prime	1	309
MaeI	CTAG	4	five_prime	1	123
PleI	GAGTC	5	five_prime	1	317
ScrFI	CCNGG	4	five_prime	1	302
SfaNI	GCATC	5	five_prime	1	22
SneI	ACTAGT	6	five_prime	1	122

Занятие №5-6 - Методы обнар... Занятие 5-6. - Методы обнар... RestrictionMapper Output - Mozi... Без имени 1 - LibreOffice Writer Занятие №5.odt - LibreOffice ... 1 / 4

5. Записать в файл название рестриктазы и место разрезания для неё (информация доступна, если нажать на название рестриктазы на предыдущем этапе).

The screenshot shows a web browser window displaying the REBASE website page for the AvaII restriction enzyme. The browser's address bar shows the URL `rebase.neb.com/rebase/enz/AvaII.html`. The page features the REBASE logo, a 'GOLD STANDARD' badge, and the enzyme name 'AvaII' in red. It identifies the enzyme as a 'Type II restriction enzyme' with a 'subtype: P'. The recognition sequence is shown as `G^GWCC` with arrows indicating the cleavage sites on the 5' and 3' strands. Below this, the enzyme's REBASE number is 166, and it lists various characteristics such as its organism (*Anabaena variabilis* ATCC 27893), growth temperature (26 °C), and molecular weight (26127). The page also includes a table of sites on various phages and a list of related enzymes and references. At the bottom, there is a footer with the date 10/13/2018 and the authors' names: Dr. Richard J. Roberts and Dana Macelis.

Приложения Места Firefox ESR en Воскресенье, 20:50

RJR REBASE Enz 166 - AvaII - Mozilla Firefox

Методы детекции SNP | Reference SNP (refSNP) | Sci-Hub: устраняя прерыва... | All: polymorphism detecti... | RJR REBASE Enz 166 - Av... +

← → ↻ 🏠 ⓘ rebase.neb.com/rebase/enz/AvaII.html 🔍 Поиск

REBASE®
home page...
rebase.neb.com

GOLD STANDARD

AvaII

Type II restriction enzyme
subtype: P

Different enzyme: Go

Recognition Sequence: [help?](#)

G[^]GWCC

5' .. G G W C C .. 3'
3' .. C C W G G .. 5'

REBASE Enz Num 166 entered Jan 1 1976 ... modified Oct 17 2016

Acronym: [Ava](#)
Prototype: AvaII
Org #: [155](#)
Organism: [Anabaena variabilis ATCC 27893](#)
DNA name: chromosome
Organism type: [bacteria](#)
Complete genome
Organism source: [ATCC 27893](#) ([ATCC LINK](#))
Growth Temperature: 26 °
Experimental Evidence: [biochemistry](#)
Exhibits star activity
Single-stranded cleavage: γ
Enzyme gene **cloned**
Enzyme gene **sequenced**
Molecular Weight: 26127

sites on
Adeno2: 73
Lambda: 35
pBR322: 8
PhiX174: 1
SV40: 6

Status of methylation sensitivity testing...
[Site frequency in sequenced genomes...](#)
[View Genome for AvaII...](#)
[DNA RNA hybrids...](#)
[RNA duplexes...](#)
[Single stranded DNA...](#)
[Combined report...](#)

Related Enzymes:
[M.AvaII](#)

Related References
[sorted by date](#) [in new window](#)
[sorted by authors](#) [in new window](#)

[Commercially Available...](#)

[Similar enzymes...](#)

Sequence Data:
[AvaII](#)

[Methylation Sensitivity...](#)

REBASE Enzymes

REBASE Lists

10/13/2018
Dr. Richard J. Roberts and Dana Macelis
<http://rebase.neb.com/rebase/enz/AvaII.html>

REBASE HELP ?

REBASE Tools

Занятие №5-6 - Методы... Занятие 5-6. - Методы о... RJR REBASE Enz 166 - Av... [Занятие 2. - ПЦР.odp - ... [Без имени 1 - LibreOffic... Занятие №5.odt - LibreO... 1 / 4

6. Подобрать к данной последовательности (501 нуклеотид) праймеры (см. Предыдущую работу) с таким расчётом, чтобы ампликон составил 300-400 нуклеотидов, а рестрикция давала два куска с соотношением 2:3 (приблизительно). Записать в файл последовательность ампликона, праймеры, их температуру плавления, температуру отжига для ПЦР (см. соответствующее занятие) и фрагменты после рестрикции

- SNP для ДЗ:
- 1). rs4833095
- 2). rs5744168
- 3). rs11096955
- 4). rs11096956
- 5). rs4129009