

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

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

The screenshot shows the NCBI dbSNP search results for the SNP rs5743810. The browser window title is "rs5743810 - SNP - NCBI - Mozilla Firefox". The address bar shows the URL "https://www.ncbi.nlm.nih.gov/snp/?term=rs5743810".

The search results are displayed in a table with the following columns: Variation Class, Annotation, Function Class, Global MAF, Validation Status, and Search results. The search results are sorted by SNP_ID.

The search results are as follows:

Variation Class	Annotation	Function Class	Global MAF	Validation Status	Search results
snp	Cited in PubMed PubMed nucleotide protein structure	missense	Custom range...	by-1000 Genomes by-cluster by-frequency	1. <input type="checkbox"/> rs5743810 [<i>Homo sapiens</i>] ATTTTTATCAGAACTCACCAGAGG[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
					2. <input type="checkbox"/> rs17496037 has merged into rs5743810 [<i>Homo sapiens</i>] ATTTTTATCAGAACTCACCAGAGG[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
					3. <input type="checkbox"/> rs52796179 has merged into rs5743810 [<i>Homo sapiens</i>]

The page also includes a sidebar with navigation options, a search bar, and a "Recent activity" section. The "Recent activity" section shows a list of recent searches, including rs5743810 (4), SNP Links for Gene (Select 18) (8131), ABAT 4-aminobutyrate aminotransferase [*Homo sapiens*] (Gene), transaminase AND (alive[prop]) (12000) (Gene), and SNP Links for Nucleotide (Select 568802081) (1811567) (SNP).

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 detect... | 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 AACCAAGATT
TAGAATATTT GGATTTATCT CATAATCAGT TGCAAAAGAT ATCCTGCCAT CCTATTGTGA
GTTTCAGGCA TTTAGATCTC TCATTCAATG ATTTCAAGGC CCTGCCATC TGTAAGGAAT
TTGGCAACTT ATCACAAC TG AATTCTTGG GATTGAGTGC TATGAAGCTG CAAAAATTAG
ATTTGCTGCC AATTGCTCAC TTGCATCTAA GTTATATCTC TCTGGATTTA AGAAATATT
ATATAAAGA AAATGAGACA GAAAGTCTAC AAATTTCTGAA TGCAAAAACC CTTCACCTTG
TTTTTCACCC AACTAGTTTA TTCGCTATCC AAGTGAACAT ATCAGTTAAT ACTTTAGGGT
GCTTACAAC GACTAATATT AAATTTGAATG ATGACAAC TG TCAAGTTTTC ATTAATTTT
TATCAGAAC 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

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 прерыва... | 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
Circular
Linear
Sort By
1. frequency
2. overhang
3. name
Filter By
Maximum Cuts
Minimum Site Length

Include
Select Individual Enzymes
All Enzymes
AarI
AasI
AatI
AatII
Accl
AcclI
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
GACAGAAAGTCTCAAATTCG
AATGCAAAAACCCITCACCTTG
TTTTCCACCAACTAGTTATT
CGCTATCCAAGTGAACATCA
GTTAATACTTTAGGGTGCTTCA
AACTGACTAATATTAATTGAA
TGATGACAACTGTCAAGTTTTCA
ATTAATTTTTATCAGAACTCA
CCAGAGGT
T
CAACCTTACTGAATTTACCTT
CAACCACATAGAAACGACTTGG
AAATGCCGTGTCAGAGCTTTTCA
AATTTCTTTGGCCAAACCTGT
Name your sequence
Untitled

Menu
Map Sites
Virtual Digest
Reset Form

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 restriction enzyme **AvaII**. 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. Below the name, it is identified as a **Type II restriction enzyme** with a **subtype: P**. The **Recognition Sequence** is shown as `G^GWCC`, with a diagram illustrating the cleavage sites on both strands of DNA. The top strand is `5'... G G W C C ... 3'` and the bottom strand is `3'... C C W G G ... 5'`. Arrows indicate the cleavage sites between the first and second 'G' on the top strand and between the second and third 'G' on the bottom strand. A metadata bar indicates the enzyme was entered on Jan 1 1976 and modified on Oct 17 2016. The page provides detailed information including the acronym (**Ava**), prototype (**AvaII**), organism (**Anabaena variabilis ATCC 27893**), DNA name (chromosome), and growth temperature (**26 °**). It also lists experimental evidence, such as **biochemistry**, and provides links for related enzymes, references, and sequence data. A table of sites on various genomes is also present, listing **Adeno2: 73**, **Lambda: 35**, **pBR322: 8**, **PhiX174: 1**, and **SV40: 6**. The footer includes the REBASE logo, a "REBASE Lists" icon, a copyright notice for Dr. Richard I. Roberts and Dana Macelis dated 10/13/2018, a "HELP" icon, and a "REBASE Tools" icon. The browser's taskbar at the bottom shows several open windows, including "Занятие №5-6 - Методы...", "Занятие 5-6. - Методы о...", "RJR REBASE Enz 166 - Av...", "[Занятие 2. - ПЦР.odp - ...", "[Без имени 1 - LibreOffic...", and "Занятие №5.odt - LibreO...". The system tray shows the time as 20:50 on a Sunday.

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