2021年12月16日（木）
AJACSオンライン９ 「疾患に関する多型データを解析する」

バリアントの機能を推定する

浜松医科大学医化学講座
才津　浩智

自己紹介

1998年　　九州大学　産婦人科入局
2001-2006年　京都大学　医学研究科（塩田浩平教授）-- 発生生物学
2006-2015年　横浜市立大学 遺伝学教室（松本直通教授）
2016年-現在　浜松医科大学 医化学講座
　日本遺伝子診療学会　ジェネティックエキスパート認定制度委員会委員長

浜松医科大学

第１７回臨床遺伝情報検索講習会（報告）

日　時：　2020年9月16日（水）　10:00 ～ 2020年10月15日（木）23:59
開催形式：　e-ラーニングシステムを利用したウェブ配信

プログラム：

- **次世代シークエンスの基礎知識とメンデル遺伝病におけるバリアントの解釈について**
  才津浩智（浜松医科大学医化学講座）
- **臨床遺伝情報検索のweb サイト**
  中山智祥（日本大学医学部病態病理学系臨床検査医学分野）
- **単一遺伝子疾患の情報検索（基礎）**
  足立香織（鳥取大学研究推進機構 研究基盤センター）

第１８回臨床遺伝情報検索講習会（報告）

日　時：　2021年3月8日（月）　0:00 ～ 2021年4月11日（日）23:59
開催形式：　e-ラーニングシステムを利用したウェブ配信

プログラム：

- **UCSC Genome Browser を用いたヒトゲノム構成の閲覧**
  佐藤謙一（国際医療福祉大学　福岡保健医療学部）
- **がんゲノム医療における基礎知識**
  雨宮健司（山梨県立中央病院　ゲノム解析センター）
- **がん遺伝子パネル検査に関する情報検索と検査結果解釈の実技演習**
  柿島裕樹（国立がん研究センター中央病院　臨床検査科）

第１９回臨床遺伝情報検索講習会（報告）

日　時：　2021年7月12日（月）　0:00 ～ 2021年8月11日（水）23:59
開催形式：　e-ラーニングシステムを利用したウェブ配信

プログラム：

- **臨床遺伝情報検索のための統合TVの活用法**
  小野浩雅（ライフサイエンス総合データベースセンター）
- **ACMG-AMPガイドラインによるバリアントの評価**
  才津浩智（浜松医科大学　医化学講座）

Contents

1. バリアントの種類

（シノニマス、ミスセンス、ナンセンス、インフレーム/フレームシフト、スプライスサイト、イントロンバリアント）

2. バリアントの機能推定（*in silico*解析）の役割

3. *in silico*解析プログラムの紹介

SIFT(ミスセンスバリアント)
PolyPhen-2 (ミスセンスバリアント)
M-CAP (ミスセンスバリアント)
PROVEAN (ミスセンスバリアント/インフレーム)
MutationTaster2021 (ミスセンスバリアント/インフレーム/フレームシフト)
CADD (ミスセンスバリアント/インフレーム/フレームシフト/イントロン)

SpliceAI（スプライシング予測）
ESEfinder（スプライシング予測）

バリアントの大まかな種類

1 bp

一塩基置換(SNV)

5′-CTTTGTGCC-3′
3′-GAAACACGG-5′

G>A

5′-CTTTATGCC-3′
3′-GAAATACGG-5′

アミノ酸の置換
早期終止コドンの出現
スプライシング異常

1～1000 bp

挿入欠失

5′-CTTTGTGCC-3′
3′-GAAACACGG-5′

Gdel

5′-CTTTTGCC-3′
3′-GAAAACGG-5′

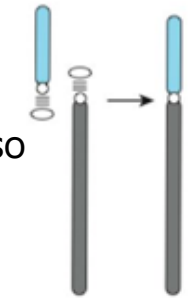
フレームシフトによる早期終止コドンの出現

～数100 Mb

染色体異数性

21トリソミー

染色体構造異常



Robertson（型）転座

トンプソン&トンプソン遺伝医学　2009

200 bp ～ 30 kb

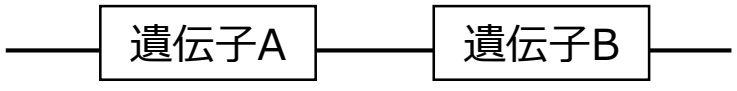
反復配列の伸長

正常　$(CAG)^{12-35}$
異常　$(CAG)^{36-121}$

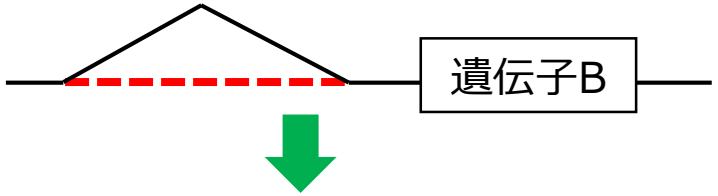
リピート配列の伸長

1 kb～ 数10 Mb

コピー数変化
(CNV, copy number variant)



遺伝子Aの欠失



通常2コピーある（常染色体は2本あります）遺伝子が1コピーになって発現量が変わる

シークエンスバリアント

4

シークエンスバリアントの分類

シノニマス(synonymous)バリアント

		Gly	Gly	Thr	
参照配列	5′-	GGT	GGA	ACT	-3′
バリアント	5′-	GTT	GGG	CTA	-3′
			Gly		

ミスセンス(nonsynonymous)バリアント

		Gly	Gly	Thr	
参照配列	5′-	GGT	GGA	ACT	-3′
バリアント	5′-	GTT	AGA	CTA	-3′
			Arg		

ナンセンスバリアント

		Gly	Gly	Thr	
参照配列	5′-	GGT	GGA	ACT	-3′
バリアント	5′-	GTT	TGA	CTA	-3′
			Stop		

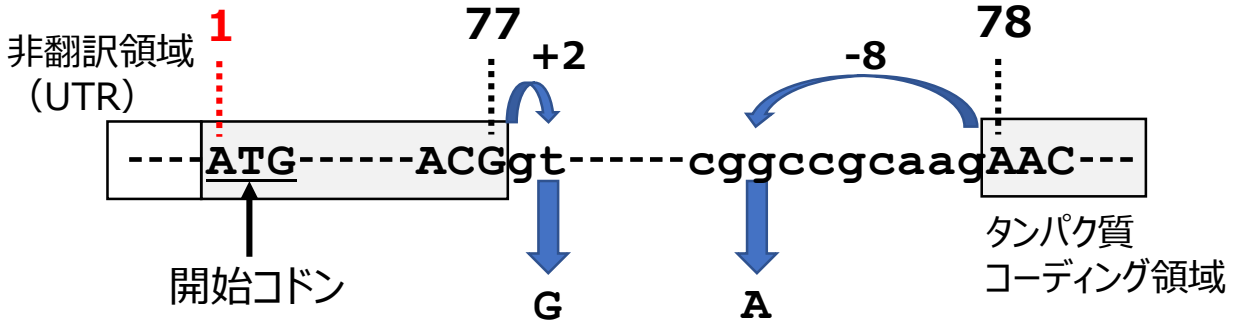
フレームシフト/インフレームバリアント(欠失・挿入)

		Thr	Ile	Lys	Ile	Gly	Leu	Thr	
参照配列	5′-	ACC	ATA	AAA	ATT	GGT	TTG	ACT	-3′
バリアント1	5′-	ACC	ATA	AAA	TTG	GTT	TGA	CT	-3′
					Leu	Val	Stop		

Leuを含めて3つ目のコドンでストップ

		Thr	Ile	Lys	Ile	Gly	Leu	Thr	
参照配列	5′-	ACC	ATA	AAA	ATT	GGT	TTG	ACT	-3′
バリアント2	5′-	ACC	ATA	ATT	GGT	TTG	ACT		-3′
		Thr	Ile	Ile	Gly	Leu	Thr		

1アミノ酸（Lys）の欠失



c.77+2T>G スプライスサイトバリアント

c.78-8G>A イントロンバリアント

タンパク質の機能に与える影響

ナンセンス、フレームシフト、スプライスサイト >> ミスセンス、インフレーム >> シノニマス、イントロン

バリアントの機能推定（*in silico*解析）の役割

1. 遺伝子診断で既知原因遺伝子に同定されたバリアントの病原性の評価

2. 網羅的遺伝子解析のバリアントのフィルタリングの一つの指標として用いる

＜メンデル遺伝病＞

米国臨床遺伝・ゲノム学会(ACMG)と分子病理学協会(AMP)が定めたシークエンス解析で同定された生殖細胞系列バリアント(遺伝する）に対する評価基準（ACMGガイドライン 2015)

バリアントを5つに分類

(1) pathogenic
(2) likely pathogenic
(3) uncertain significance
(4) likely benign
(5) Benign

エビデンス

ヌルバリアント（ナンセンス、フレームシフト、スプライス部位）	Very strong
***De novo*であること（要、親子鑑定）** **病的意義が確立されたアミノ酸置換と同一** **バリアントの機能解析で機能低下が証明**	Strong
バリアントのアレル頻度（稀である） **同じアミノ酸の違うミスセンス変化**	Moderate
表現型との分離 ***in silico*の病原性予測**	Supportive

病原性予測が高いから病的という使い方よりは、予測が低いので病的でない可能性が高いという使い方

バリアントの機能推定（*in silico*解析）の役割

イントロンバリアントに関しては、スプライス異常を示唆する所見として、
RNA解析をするかの決定に重要

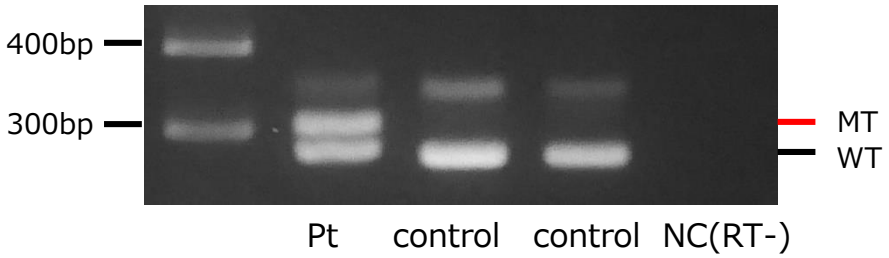
GNAO1 c.724-8G>A

↓

SpliceAIでスプライス異常を予測

↓

末梢血単核球からmRNA抽出



*in silico*解析プログラムの紹介

nonsynonymous SNV
スプライスサイト SNV

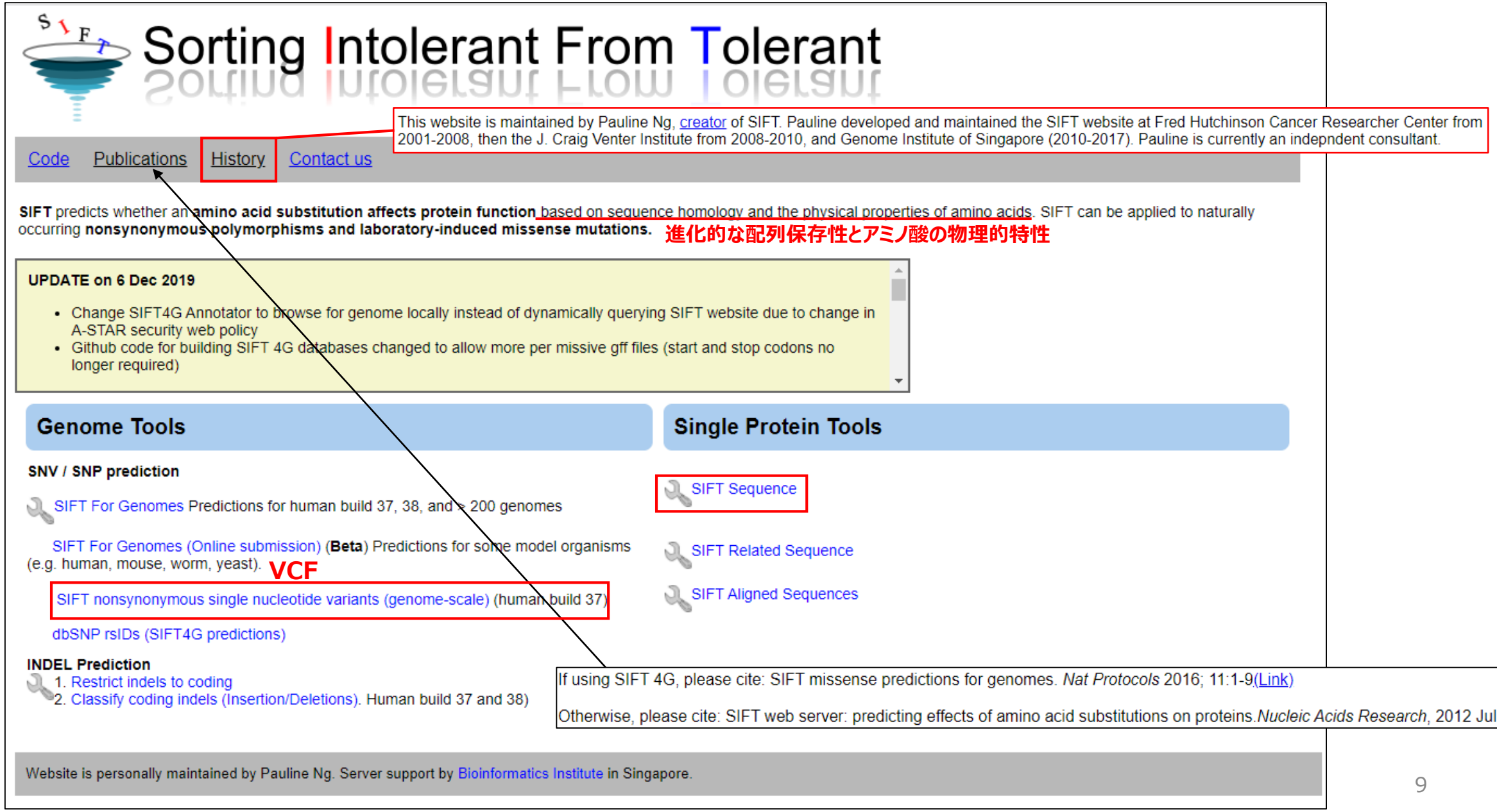
dbNSFP
http://database.liulab.science/dbNSFP

- SIFT(ミスセンスバリアント)
- PolyPhen-2 (ミスセンスバリアント)
- M-CAP (ミスセンスバリアント)
- PROVEAN (ミスセンスバリアント/インフレーム)
- MutationTaster (ミスセンスバリアント/インフレーム/フレームシフト)
- CADD (ミスセンスバリアント/インフレーム/フレームシフト/**イントロン**)

SpliceAI Lookup（スプライシング予測）
ESEfinder（スプライシング予測）

***BRCA2* NM_000059.3:c.53G>A, p.(Arg18His)**
NC_000013.11:g.32316513G>A (GRCh38)
NC_000013.10:g.32890650G>A (GRCh37)

SIFT (https://sift.bii.a-star.edu.sg/)



SIFT nonsynonymous single nucleotide variantsの使い方

***BRCA2* NM_000059.3:c.53G>A, p.(Arg18His)**
NC_000013.10:g.32890650G>A (GRCh37)

Score < 0.05でintorelant

SIFT nonsynonymous single nucleotide variants (genome-scale)

This page provides SIFT predictions for a list of **chromosome positions and alleles**. Click here to get more information

User Input

Homo sapiens (hg19) NCBI 37

Chromosome Coordinates
Paste in comma separated list of chromosome coordinates, orientation (1,-1) and alleles see [sample format]

13,32890650,1,G/A

-or-

Upload file containing chromosome coordinates and nucleotide substitutions (size limit: 1M)
ファイルを選択 選択されていません

Email:
Please enter your email address if you want your results via email.

Output Options

SIFT version 5.1.1, SIFT database release February 27, 2012

Coordinates	Codons	Ensembl Transcript ID	RefSeq Transcript ID	Known Transcript ID	CCDS Transcript ID	Ensembl Protein ID
13,32890650,1,G/A	CGC-CaC	ENST00000380152,ENST00000544455	NM_000059	uc001uub.1	CCDS9344.1	ENSP00000369497,ENSP00000439902

RefSeq Protein ID	Known Protein ID	Substitution	Region	dbSNP ID	SNP Type	Prediction	Score	Median Info	# Seqs at position
NP_000050	NP_000050	R18H	EXON CDS	rs80358762:A	Nonsynonymous	TOLERATED	0.23	2.91	18

000 Genome Allele Frequencies
- All available populations
- European population
- East Asian population
- African population
- South Asian population
- American population

Select All UnSelect All

送信 リセット

SIFT sequenceの使い方

SIFT Sequence

SIFT Sequence provides SIFT predictions for a given protein FASTA sequence. This will take 10-15 min because we must search your protein sequence against a database to pick the related sequences. You can also submit your protein sequence and related sequences or aligned sequences if you already have them.

Results are deleted after 24 hours, so please save them!
[Preventing connection failures]

Protein sequence

Name of file containing protein query sequence (fasta format).
ファイルを選択 選択されていません

-or-

Paste in your protein query sequence (Upload example) (fasta format).

BRCA2のFASTA配列が必要

Enter the substitutions of interest [format]:

R18H

-or-

Upload a file containing substitutions of interest [format]:
ファイルを選択 選択されていません

***BRCA2* NM_000059.3:c.53G>A, p.(Arg18His)**

Parameters

Select database to search UniProt-SwissProt 2010_09
Warnings for you predictions? Change this option

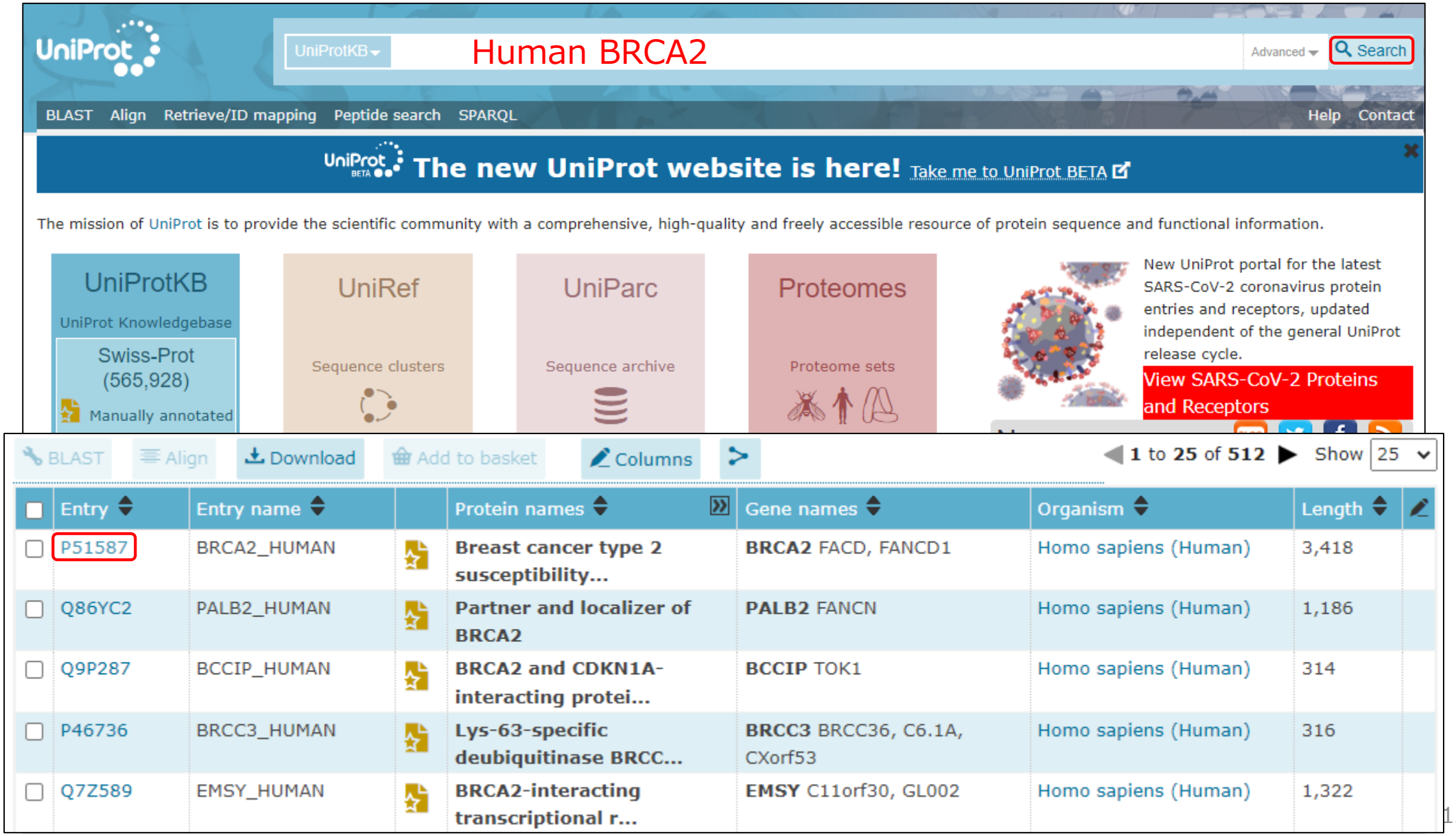
Median conservation of sequences 3.00
(between 0 and 4.32, we recommend between 2.75 and 3.25)

Remove sequences more than 90 percent identical to query
This cleans out any polymorphic alleles (sequences in the database that are nearly identical to your protein but already containing the substitutions of interest). The contaminating sequences may cause the substitution to be predicted as tolerated.

Enter your email address if you want the results through email : (Optional)

Submit リセット

UniProt (https://www.uniprot.org/)



UniProtKB - P51587 (BRCA2_HUMAN)

Display | Help video | BLAST | Align | Format | Add to basket | History | Community curation (1) | Add a publication

- Entry
- Publications
- Feature viewer
- Feature table

None

- Function
- Names & Taxonomy
- Subcellular location
- Pathology & Biotech
- PTM / Processing
- Expression
- Interaction
- Structure
- Family & Domains
- Sequences (1+)
- Similar proteins
- Cross-references
- Entry information
- Miscellaneous

Top

Protein **Breast cancer type 2 susceptibility protein**

Gene **BRCA2**

Organism *Homo sapiens (Human)*

Status Reviewed - Annotation score: ●●●●● - Experimental evidence at protein level

Function

Involved in double-strand break repair and/or homologous recombination. Binds RAD51 and potentiates recombinational DNA repair by promoting assembly of RAD51 onto single-stranded DNA (ssDNA). Acts by targeting RAD51 to ssDNA over double-stranded DNA, enabling RAD51 to displace replication protein-A (RPA) from ssDNA and stabilizing RAD51-ssDNA filaments by blocking ATP hydrolysis. Part of a PALB2-scaffolded HR complex containing RAD51C and which is thought to play a role in DNA repair by HR. May participate in S phase checkpoint activation. Binds selectively to ssDNA, and to ssDNA in tailed duplexes and replication fork structures. May play a role in the extension step after strand invasion at replication-dependent DNA double-strand breaks; together with PALB2 is involved in both POLH localization at collapsed replication forks and DNA polymerization activity. In concert with NPM1, regulates centrosome duplication. Interacts with the TREX-2 complex (transcription and export complex 2) subunits PCID2 and SEM1, and is required to prevent R-loop-associated DNA damage and thus transcription-associated genomic instability. Silencing of BRCA2 promotes R-loop accumulation at actively transcribed genes in replicating and non-replicating cells, suggesting that BRCA2 mediates the control of R-loop associated genomic instability, independently of its known role in homologous recombination (PubMed:24896180).

11 Publications

GO - Molecular function

- gamma-tubulin binding — Source: UniProtKB
- H3 histone acetyltransferase activity — Source: UniProtKB
- H4 histone acetyltransferase activity — Source: UniProtKB
- identical protein binding — Source: IntAct
- protease binding — Source: UniProtKB
- protein C-terminus binding — Source: MGI
- single-stranded DNA binding — Source: UniProtKB

Complete GO annotation on QuickGO ...

GO - Biological process

- brain development — Source: Ensembl
- cell aging — Source: Ensembl
- centrosome duplication — Source: UniProtKB
- DNA damage response, signal transduction by p53 class mediator resulting in transcription of p21 class mediator — Source: Ensembl
- double-strand break repair — Source: UniProtKB

BLAST | Align | Format | Add to basket | History

Protein **Breast c** ...otibility protein

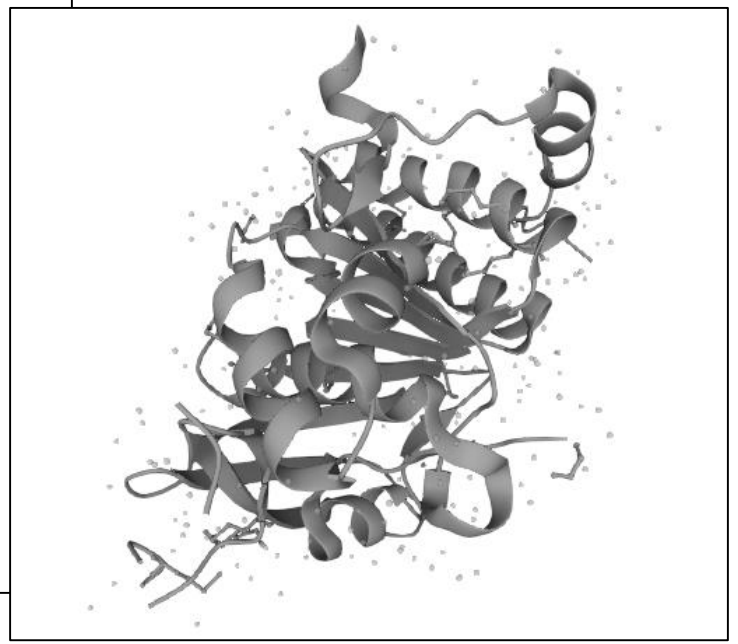
Gene **BRCA2**

Organism *Homo sap*

Status Reviev ●●●●● - Experiment

View format

- Text
- FASTA (canonical)
- XML
- RDF/XML
- GFF



>sp|P51587|BRCA2_HUMAN Breast cancer type 2 susceptibility protein OS=Homo sapiens OX=9606 GN=BRCA2 PE=1 SV=3
MPIGSKERPTFFEIFKTRCNKADLGPISLNWFEELSSEAPPYNSEPAEESEHKNNNYEPN
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KTKMDQADDVSCPLLNSCLSESPVVLQCTHVTPQRDKSVVCGSLFHTPKFVKGRQTPKHI
SESLGAEVDPDMSWSSSLATPPTLSSTVLIVRNEEASETVFPHDTTANVKSYFSNHDESL
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SSFGTILRKCSRNETCSNNTVISQDLDYKEAKCNKEKLQLFITPEADSLSCLQEGQCEND
PKSKKVSDIKEEVLAAACHPVQHSKVEYSDTDFQSQKSLLYDHENASTLILTPTSKDVLS
NLVMISRGKESYKMSDKLKGNNYESDVELTKNIPMEKNQDVCALNENYKNVELLPPEKYM
RVASPSRKVQFNQNTNLRVIQKNQEETTSISKITVNPDSEELFSDNENNFVFQVANERNN
LALGNTKELHETDLTCVNEPIFKNSTMVLYGDTGDKQATQVSIKKDLVYVLAEENKNSVK
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DNKYTAASRNSHNLEFDGSDSSKNDTVCIHKDETDLLFTDQHNICLKLSGQFMKEGNTQI
KEDLSDLTFLEVAKAQEACHGNTSNKEQLTATKTEQNIKDFETSDTFFQTASGKNISVAK
ESFNKIVNFFDQKPEELHNFSLNSELHSDIRKNKMDILSYEETDIVKHKILKESVPVGTG
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KVKDIFTDSFSKVIKENNENKSKICQTKIMAGCYEALDDSEDILHNSLDNDECSTHSHKV
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NVSKILPRVDKRNPEHCVNSEMEKTCSKEFKLSNNLNVEGGSSENNHSIKVSPYLSQFQQ
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SETSSNKTSSADTQKVAIIELTDGWYAVKAQLDPPLLAVLKNGRLTVGQKIILHGAELVG
SPDACTPLEAPESLMLKISANSTRPARWYTKLGFFPDPRPFPLPLSSLFSDGGNVGCVDV
IIQRAYPIQWMEKTSSGLYIFRNEREEEKEAAKYVEAQQKRLEALFTKIQEEFEEHEENT
TKPYLPSRALTRQQVRALQDGAELYEAVKNAADPAYLEGYFSEEQLRALNNHRQMLNDKK
QAQIQLEIRKAMESAEQKEQGLSRDVTTVWKLRIVSYSKKEKDSVILSIWRPSSDLYSLL
TEGKRYRIYHLATSKSKSKSERANIQLAATKKTQYQQLPVSDEILFQIYQPREPLHFSKF
LDPDFQPSCSEVDLIGFVVSVVKKTGLAPFVYLSDECYNLLAIKFWIDLNEDIIKPHMLI
AASNLQWRPESKSGLLTLFAGDFSVFSASPKEGHFQETFNKMKNTVENIDILCNEAENKL
MHILHANDPKWSTPTKDCTSGPYTAQIIPGTGNKLLMSSPNCEIYYQSPLSLCMAKRKSV
STPVSAQMTSKSCKGEKEIDDQKNCKKRRALDFLSRLPLPPPVSPICTFVSPAAQKAFQP
PRSCGTKYETPIKKKELNSPQMTPFKKFNEISLLESNSIADEELALINTQALLSGSTGEK
QFISVSESTRTAPTSSEDYLRLKRRCTTSLIKEQESSQASTEECEKNKQDTITTKKYI

3418aaあって大きい

SIFT Sequence

SIFT Sequence provides SIFT predictions for a given protein FASTA sequence. This will take 10-15 min because we must search your protein sequence against a database to pick the related sequences. You can also submit your protein sequence and related sequences or aligned sequences if you already have them.

Results are deleted after 24 hours, so please save them!
[Preventing connection failures]

Protein sequence

Name of file containing protein query sequence (fasta format).
ファイルを選択 選択されていません

-or-

Paste in your protein query sequence (Upload example) (fasta format).

コピペしてsubmit

Enter the substitutions of interest [format]:
R18H

-or-

Upload a file containing substitutions of interest [format]:
ファイルを選択 選択されていません

SIFT Results (Protein Sequences)

Your job id is abab675a9d and is currently running.

If your browser times out before results are shown, please go to https://sift.bii.a-star.edu.sg//sift-bin/format.pl?abab675a9d_sequences in **20 minutes**. Opening it up before 20 minutes will throw an error (in which case, just refresh).

Problems? Contact us with your job id.
Your results will be available here for the next 24 hours.
4 sequences were selected to be closely related to your query sequence.
PSIBLAST alignment of submitted sequences
Alignment in FASTA format (for modification)
The alignment taken from PSIBLAST is returned in msf format.
Note: Xes are placeholders at the beginning and end of sequences. While **-** means a gap in the alignment an X means a lack of information such as a partial alignment or incomplete sequence and do not contribute to the prediction.
Please check the sequences that have been chosen. If the sequences are too diverged from your query or the alignment is questionable, we suggest you modify the fasta-formatted file above and resubmit.

SIFT amino acid predictions for:
Positions 1 to 100
Positions 101 to 200
Positions 201 to 300
Positions 301 to 400
Positions 401 to 500
Positions 501 to 600
Positions 601 to 700
Positions 701 to 800
Positions 801 to 900
Positions 901 to 1000
Positions 1001 to 1100
Positions 1101 to 1200
Positions 1201 to 1300
Positions 1301 to 1400
Positions 1401 to 1500
Positions 1501 to 1600
Positions 1601 to 1700
Positions 1701 to 1800
Positions 1801 to 1900
Positions 1901 to 2000
Positions 2001 to 2100
Positions 2101 to 2200
Positions 2201 to 2300
Positions 2301 to 2400
Positions 2401 to 2500
Positions 2501 to 2600
Positions 2601 to 2700
Positions 2701 to 2800
Positions 2801 to 2900
Positions 2901 to 3000
Positions 3001 to 3100
Positions 3101 to 3200
Positions 3201 to 3300
Positions 3301 to 3400
Positions 3401 to 3500

Scaled Probabilities for Entire Protein
May take some time to load!! *Please be patient if you do not see the table immediately.*
Amino acids with probabilities < .05 are predicted to be deleterious.

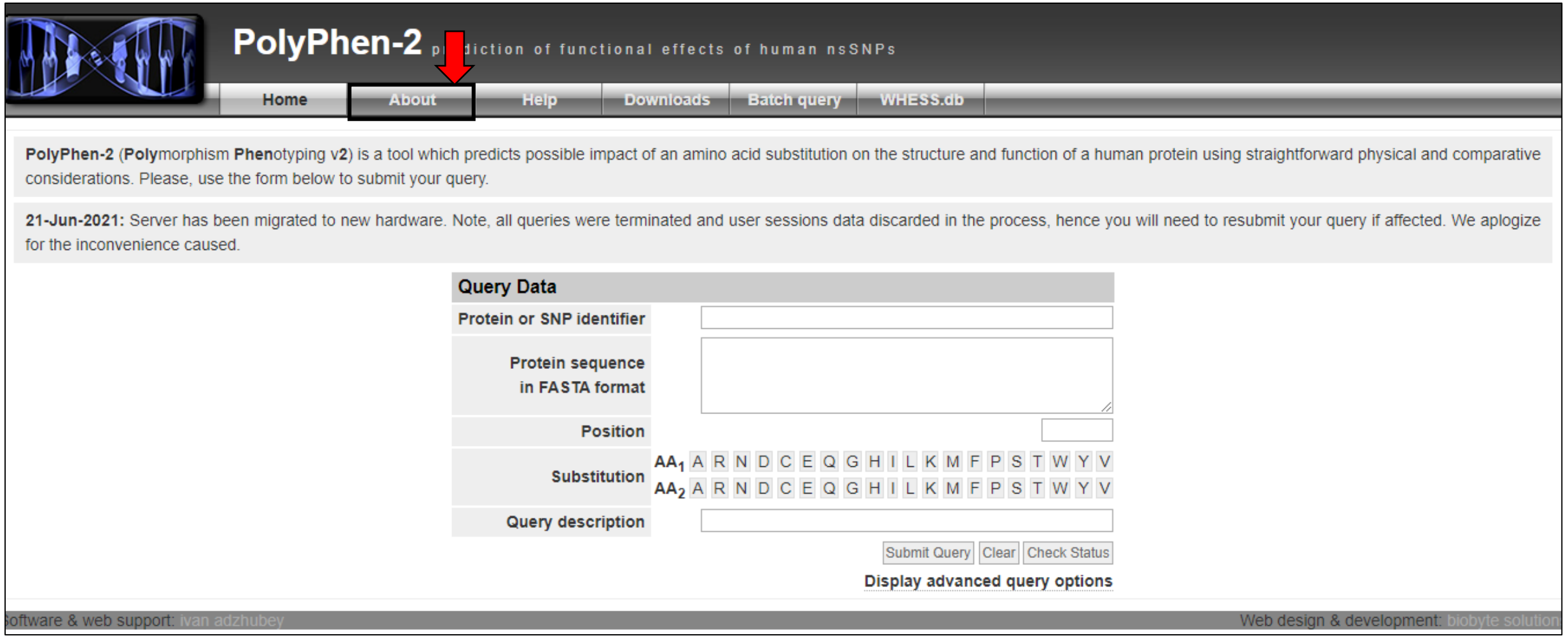
Predictions of substitutions entered

*** The following sequences have been removed because they were found to be over 90% identical with your protein query: P51587,.

Substitution at pos 18 from R to H is predicted to AFFECT PROTEIN FUNCTION with a score of 0.00.
Median sequence conservation: 3.63
Sequences represented at this position:4
WARNING!! This substitution may have been predicted to affect function just because the sequences used were not diverse enough. **There is LOW CONFIDENCE in this prediction.**

ゲノムでやった結果とアミノ酸配列からやったデータで矛盾がある

PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/)



PolyPhen-2の予測アルゴリズム

about

About

PolyPhen-2 is a new development of the **PolyPhen** tool for annotating coding nonsynonymous SNPs. Some of the highlights of the new version are:

- High quality multiple sequence alignment pipeline
- Probabilistic classifier based on machine-learning method
- Optimized for high-throughput analysis of the next-generation sequencing data

マルチプルアライメントによる進化的保存性とタンパク質構造を加味した機械学習によるアノテーション

- Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. Nat Methods 7(4):248-249 (2010). PubMed PDF Supplemental Information

Other

1.

2.

3.

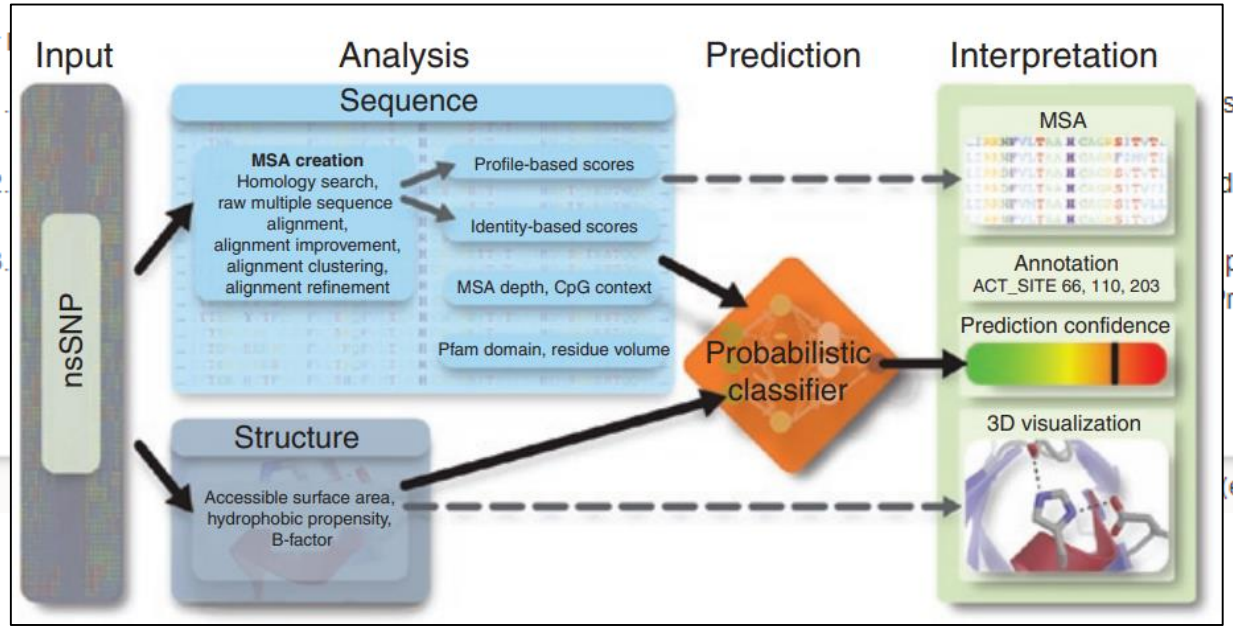
sing

ds Res

profile

rotein

(external edit)



PolyPhen-2の使い方

***BRCA2* NM_000059.3:c.53G>A, p.(Arg18His)**

Query Data

Protein or SNP identifier	BRCA2 **またはUniProt ID: P51587**
Protein sequence in FASTA format	
Position	18
Substitution	AA_1 A **R** N D C E Q G H I L K M F P S T W Y V AA_2 A R N D C E Q G **H** I L K M F P S T W Y V
Query description	

Submit Query | Clear | Check Status

Display advanced query options

ミスセンス(nonsynonymous)バリアントのみ

Grid Gateway Interface
v2.2.8

Help | Troubleshooting/FAQ

Sunyaev Lab

Service Name: PolyPhen-2
Session ID: 56a79c3ee69fda6181c4bc80dfc6a16ec80983b8 ☐ Overwrite default
Grid Status:

Load	Health	Jobs: Pending	Running
Light	100%	1	11

Jobs (1 total):

Pending/Running (1/0)

ID	Pos.	State	Date/Time	Delete	Description
8058022	1	qw	2021-11-30 07:00:09	☐	

Refresh All items with **Delete** boxes checked will be removed!

Service Name: PolyPhen-2
Session ID: 56a79c3ee69fda6181c4bc80dfc6a16ec80983b8 ☐ Overwrite default
Grid Status:

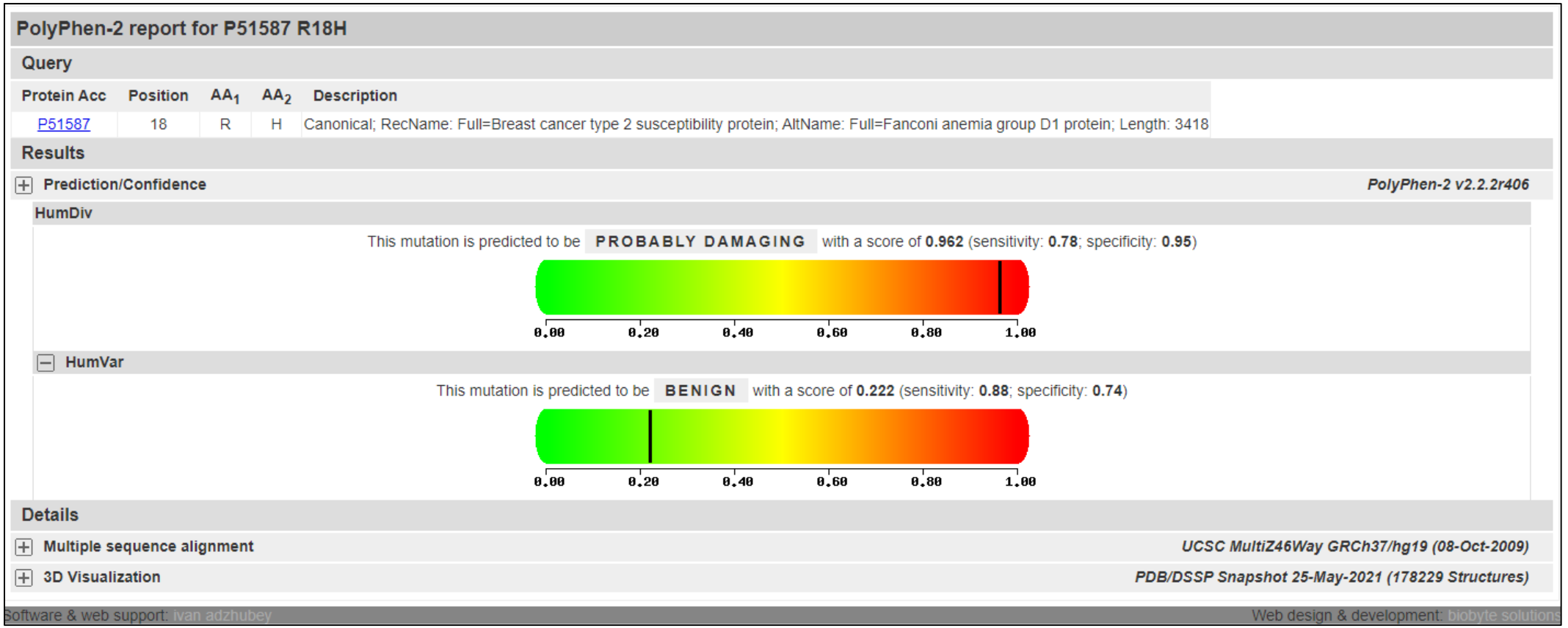
Load	Health	Jobs: Pending	Running
Light	100%	0	11

Jobs (1 total):

Completed (1)

ID	Results	Errors	Date/Time	Delete	Description
8058022	View	-	2021-11-30 07:00:16	☐	

Refresh All items with **Delete** boxes checked will be removed!



HumDiv- and HumVar-trained PolyPhen-2モデルを選ぶ必要ある

HumDiv - 3,155 damaging alleles in Mendelian diseases, 6,321 differences between human protein and mammalian homologs(non-damaging)
- Genome-wide association studies

HumVar – 13,032 human disease-causing mutations, 8,946 human nonsynonymous SNV without annotated involvement of disease(non-damaging)
– Diagnostic of Mendelian diseases

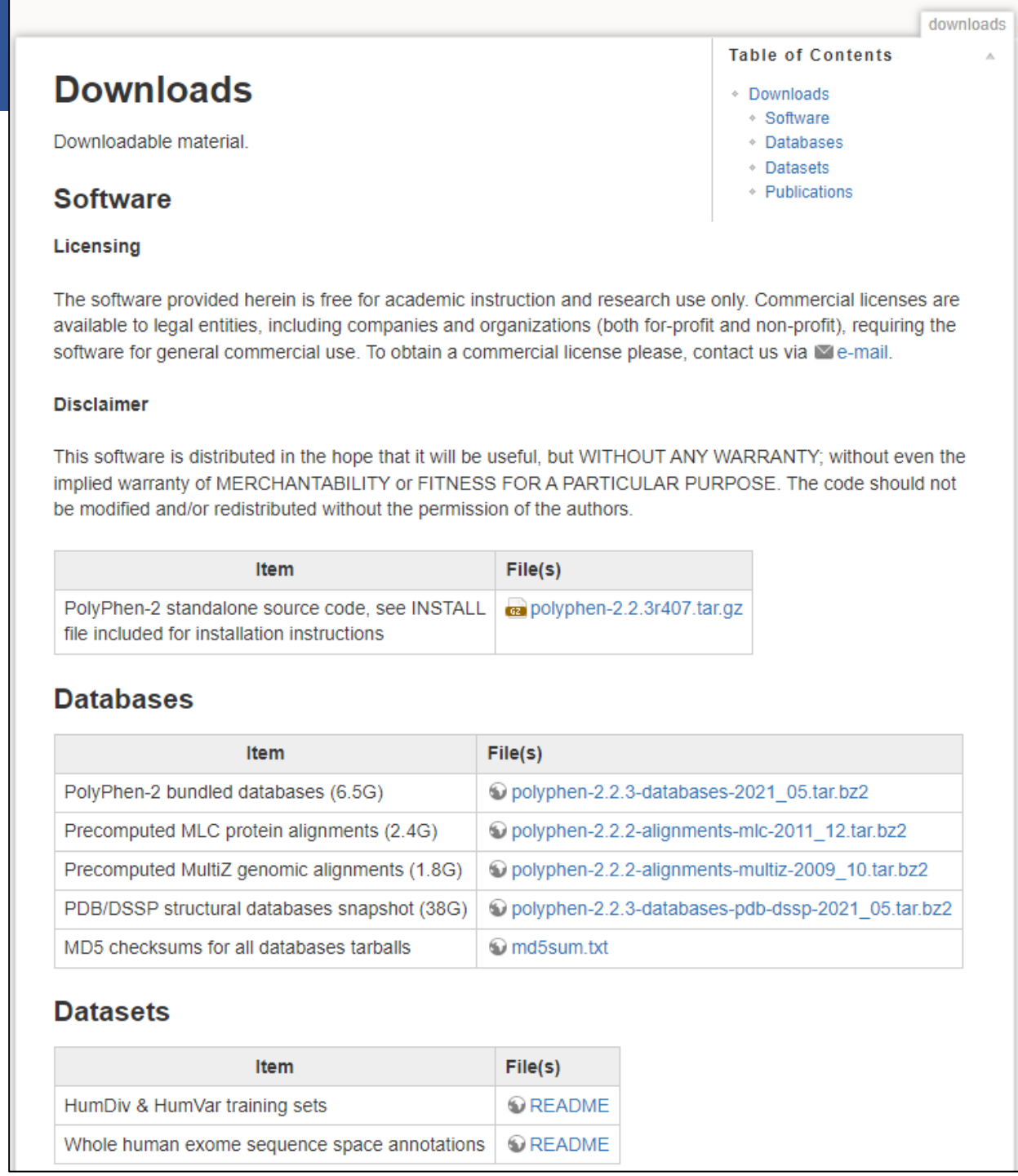
マルチプルアライメントの図と（利用可能な場合は）3次元構造の図が見られる

STXBP1のC180Y

Local serverでscoreをつける

1. 右のsource codeをダウンロードしてインストール（ハードル高い）

2. ミスセンスバリアントだけなので、
Annovar (https://annovar.openbioinformatics.org/en/latest/)
SnpEff (http://pcingola.github.io/SnpEff/)
で**dbNSFP**を利用する



downloads

Table of Contents
- Downloads
  - Software
  - Databases
  - Datasets
  - Publications

Downloads

Downloadable material.

Software

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Disclaimer

This software is distributed in the hope that it will be useful, but WITHOUT ANY WARRANTY; without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE. The code should not be modified and/or redistributed without the permission of the authors.

Item	File(s)
PolyPhen-2 standalone source code, see INSTALL file included for installation instructions	polyphen-2.2.3r407.tar.gz

Databases

Item	File(s)
PolyPhen-2 bundled databases (6.5G)	polyphen-2.2.3-databases-2021_05.tar.bz2
Precomputed MLC protein alignments (2.4G)	polyphen-2.2.2-alignments-mlc-2011_12.tar.bz2
Precomputed MultiZ genomic alignments (1.8G)	polyphen-2.2.2-alignments-multiz-2009_10.tar.bz2
PDB/DSSP structural databases snapshot (38G)	polyphen-2.2.3-databases-pdb-dssp-2021_05.tar.bz2
MD5 checksums for all databases tarballs	md5sum.txt

Datasets

Item	File(s)
HumDiv & HumVar training sets	README
Whole human exome sequence space annotations	README

M-CAP (http://bejerano.stanford.edu/mcap/)

Mendelian Clinically Applicable Pathogenicity (M-CAP) Score

M-CAP is the first pathogenicity classifier for rare missense variants in the human genome that is tuned to the high sensitivity required in the clinic (see Table). By combining previous pathogenicity scores (including SIFT, Polyphen-2 and CADD) with novel features and a powerful model, we attain the best classifier at all thresholds, reducing a typical exome/genome rare (<1%) missense variant (VUS) list from 300 to 120, while never mistaking 95% of known pathogenic variants as benign. Further details can be found here.

進化的保存性

Method	Authors' Recommended Pathogenicity threshold	Misclassified known pathogenic variants
SIFT	< 0.05	38%
Polyphen-2	> 0.8	31%
CADD	> 20	26%
MetaLR	> 0.5	27%
M-CAP	> 0.025	5%

3/25/2019: M-CAP score file with an interpretable sensitivity score can be found here! Sensitivity score ≤ 0.95 is possibly pathogenic.

Score a variant

機械学習

Enter the GRCh37/hg19 coordinate for a missense variant to retrieve its M-CAP score.

GRCh37/hg19 | e.g. 16:76,486,622 | Go! | Demo!

How to cite

Jagadeesh, K., Wenger, A., Berger, M., Guturu, H., Stenson, P., Cooper, D., Bernstein, J., and Bejerano, G. (2016). M-CAP eliminates a majority of variants with uncertain significance in clinical exomes at high sensitivity. Nature Genetics, 2016. 48 (12) 1581 DOI: 10.1038/ng.3703

稀 (≤ 1% allele frequency)
ミスセンス(nonsynonymous)バリアントのみ

Download M-CAP Scores

M-CAP only scores rare missense variants: hg19, ENSEMBL 75 missense, ExAC v0.3 where no super population has minor allele frequency above 1%. If a missense variant has no M-CAP score, the M-CAP prediction should be assumed to be likely benign.

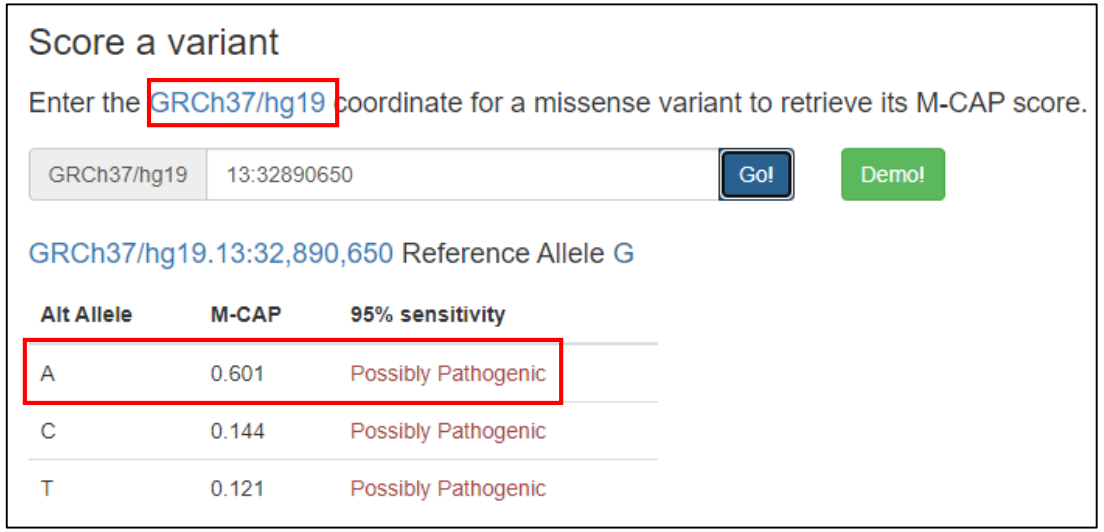
- ~~M-CAP v1.0 scores 10/24/2016~~ please do not use
- M-CAP v1.3 raw scores 10/30/2018
- M-CAP v1.4 raw and normalized scores 3/25/2019 (v1.3 raw score of 0.025 converted to sensitivity score 0.95, to facilitate interpretation)

スコアをダウンロード可能

M-CAPの使い方

WEBで一個ずつ

***BRCA2* NM_000059.3:c.53G>A, p.(Arg18His)**
NC_000013.10:g.32890650G>A (GRCh37)



Local serverで

1. **Annovar** (https://annovar.openbioinformatics.org/en/latest/)
   SnpEff (http://pcingola.github.io/SnpEff/)
   で**dbNSFP**を利用

2. **mcap_v1_4.txt**を使って、**Annovar**のgeneric databaseとしてアノテーション

#grch37_chrom	pos	ref	alt	mcapv1.4	mcap_sensitivityv1.4
1	69091	A	T	0.007082	0.99308
1	69091	A	C	0.007082	0.99308
1	69091	A	G	0.009964	0.98538
1	69092	T	A	0.003693	0.99846
1	69092	T	C	0.003264	0.99846
1	69092	T	G	0.003694	0.99846
1	69093	G	A	0.001453	1.00000
1	69093	G	T	0.001453	1.00000
1	69093	G	C	0.001447	1.00000
1	69094	G	A	0.005047	0.99846

PROVEAN
(http://provean.jcvi.org/index.php)

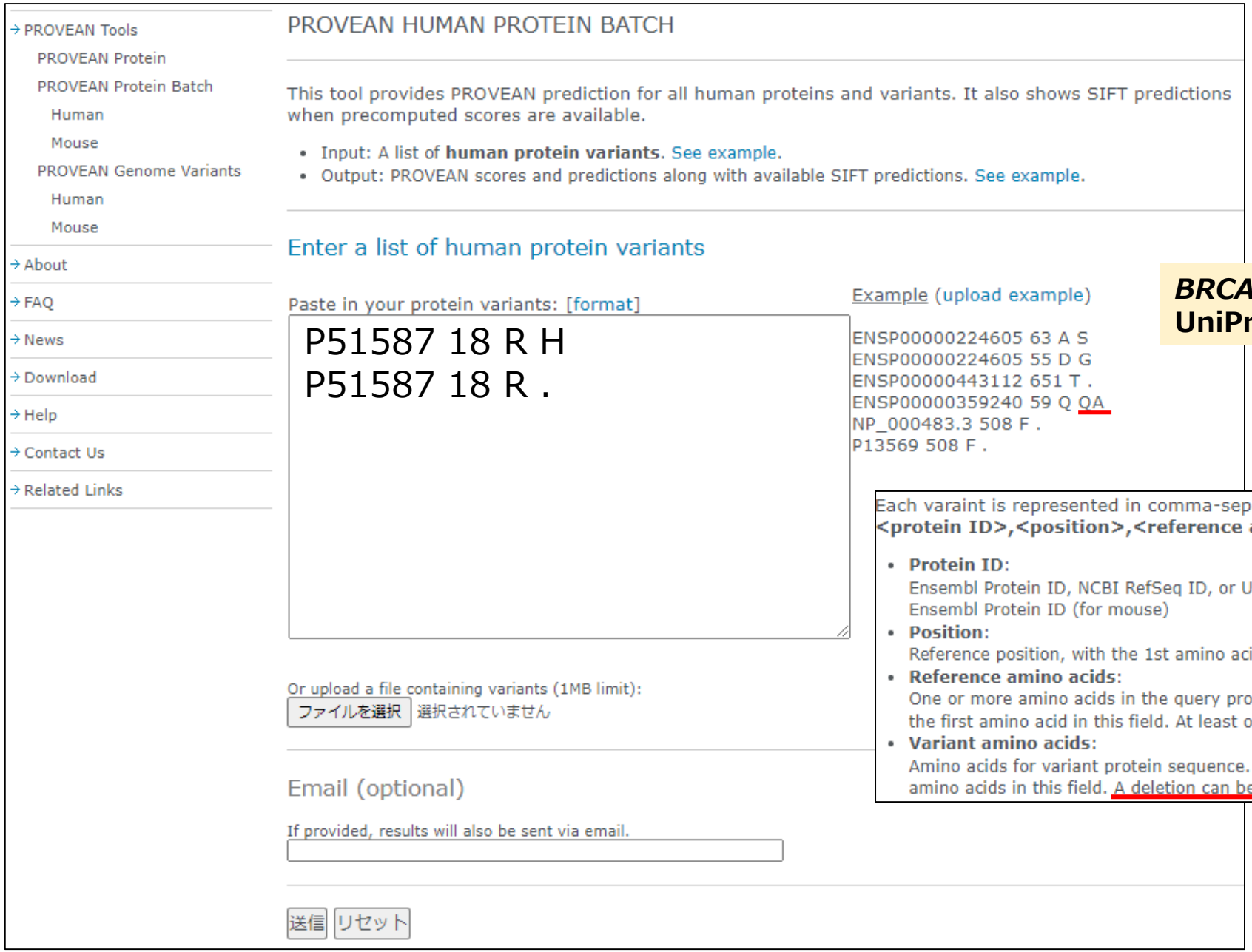
アミノ酸置換（ミスセンスバリアント）とインフレームの挿入や欠失も判断可能

バリアント配列とタンパク質配列ホモログ間の類似性

アミノ酸配列からでも、ゲノム配列からでもどちらでも解析可能

SIFTの開発者が2008-2010年にジョン・クレイグ・ヴェンター研究所に在籍していたためか、SIFTのスコアも出力される

PROVEAN Protein Batchの使い方

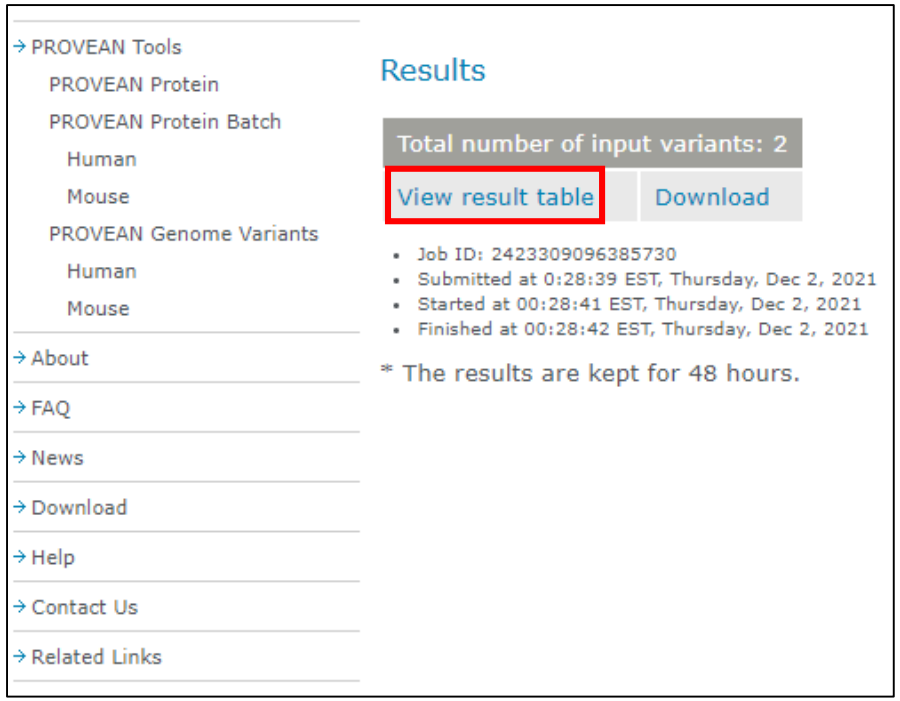


***BRCA2* NM_000059.3:c.53G>A, p.(Arg18His)**
UniProt P51587

Each varaint is represented in comma-separated (or space-separated) values as the following:
<protein ID>,<position>,<reference amino acids>,<variant amino acids>,<comment(optional)>

- **Protein ID**:
  Ensembl Protein ID, NCBI RefSeq ID, or UniProt Accession ID (for human)
  Ensembl Protein ID (for mouse)
- **Position**:
  Reference position, with the 1st amino acid having position 1.
- **Reference amino acids**:
  One or more amino acids in the query protein sequence. The value in the "Position" field refers to the position of the first amino acid in this field. At least one amino acid is required.
- **Variant amino acids**:
  Amino acids for variant protein sequence. The amino acids in the "Reference amino acid" field are replaced by the amino acids in this field. A deletion can be described by using a period ("."), which means no amino acids (empty).

PROVEAN Protein Batchの結果



Cutoff=-2.5 - deleterious or neutral

VARIATION		PROTEIN SEQUENCE CHANGE			
ROW_NO.	INPUT	PROTEIN_ID	POSITION	RESIDUE_REF	RESIDUE_ALT
1	P51587 18 R H	P51587	18	R	H
2	P51587 18 R .	P51587	18	R	.

PROVEAN PREDICTION				SIFT PREDICTION			
SCORE	PREDICTION (cutoff=-2.5)	#SEQ	#CLUSTER	SCORE	PREDICTION (cutoff=0.05)	MEDIAN_INFO	#SEQ
-1.17	Neutral	147	30	0.310	Tolerated	2.97	42
-3.63	Deleterious	147	30	NA	NA	NA	NA

SIFT nonsynonymous single nucleotide variants 0.23

PROVEAN Genome Variantsの使い方と結果

PROVEAN HUMAN GENOME VARIANTS

This tool provides PROVEAN and SIFT predictions for a list of human genome variants.

- Input: A list of **human genomic variants**. See example.
- Output: PROVEAN scores and predictions along with available SIFT predictions. See example.

Step 1. Enter a list of genomic coordinates and variants

Paste in your coordinates and variants: [format]

13,32890650,G,A

Example (upload example)

1,100382265,C,G,user comment 1
1,100380997,A,G,user comment 2
22,30163533,A,C
X,12905093,A,T
2,230633386,G,C
1,100382265,C,A
7,117199641,ATCA,.
7,117199647,TTT,.
10,50184923,TGG,.
12,121438957,ACC,.
1,43217995,G,GCCA
10,102762472,G,GGCG
9,117856130,T,G
9,117856135,C,G

Or upload a file containing variants (5MB limit):
ファイルを選択 選択されていません

Step 2. Select gene annotation (optional)

- ☐ Ensembl Gene ID
- ☐ Associated Gene Name
- ☐ Ensembl Transcript ID
- ☐ Transcript Status
- ☐ Gene Description
- ☐ % GC Content
- ☐ Chromosome band
- ☐ Ensembl Protein Family ID
- ☐ Ensembl Family Description
- ☐ UniProt/SwissProt ID
- ☐ RefSeq Protein ID
- ☐ MIM Disease Accession
- ☐ PFAM ID
- ☐ TIGRFam ID
- ☐ Interpro ID
- ☐ GO Term Accession
- ☐ GO Slim GOA Accession

Parameters

Assembly/Annotation: Human GRCh37 Ensembl 66

Email (optional)

If provided, results will be sent via email.

送信 リセット

***BRCA2* NM_000059.3:c.53G>A, p.(Arg18His)**
NC_000013.10:g.32890650G>A (GRCh37)

Results

Total number of input variants: 1	
View result table (full version)	Download
View result table (condensed version)	Download
View summary table	Download

- Job ID: 362859271449138
- Submitted at 2:45:13 EST, Thursday, Dec 2, 2021
- Started at 02:45:15 EST, Thursday, Dec 2, 2021
- Finished at 02:45:15 EST, Thursday, Dec 2, 2021

* The results are kept for 48 hours.

PROVEAN Genome Variants Result - Condensed version (Download)

Database: human37_66

Note: For each variant only the **longest** protein isoform is shown.

VARIATION		PROTEIN SEQUENCE CHANGE							
ROW_NO.	INPUT	PROTEIN_ID	LENGTH	STRAND	CODON_CHANGE	POS	RESIDUE_REF	RESIDUE_ALT	TYPE
1	13,32890650,G,A	ENSP00000369497	3418	1	ACA C[G/A]C TGC	18	R	H	Single AA Change

PROVEAN PREDICTION				SIFT PREDICTION			
SCORE	PREDICTION (cutoff=-2.5)	#SEQ	#CLUSTER	SCORE	PREDICTION (cutoff=0.05)	MEDIAN_INFO	#SEQ
-1.17	Neutral	147	30	0.256	Tolerated	2.95	40

SIFT scoreはツールで異なるので注意が必要

Local serverでPROVEAN scoreをつける

1. 右のsource codeをダウンロードしてインストール（ハードル高い）

2. ミスセンスバリアントだけを考えるなら
Annovar (https://annovar.openbioinformatics.org/en/latest/)
SnpEff (http://pcingola.github.io/SnpEff/)
で**dbNSFP**を利用する

DOWNLOADS

PROVEAN software

- PROVEAN source code
- README for installation and usage instructions
- LICENSE

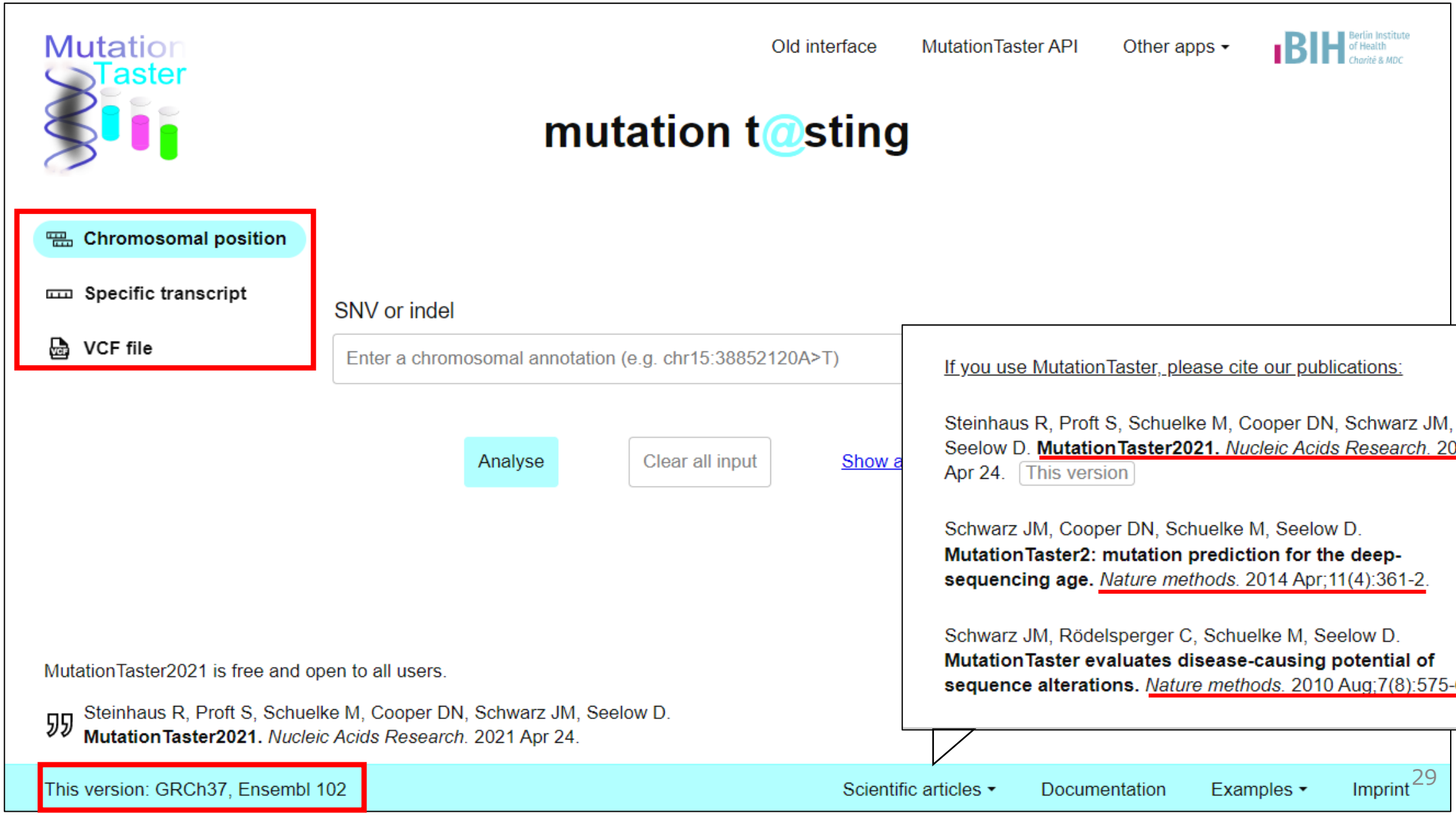
PROVEAN precomputed scores

- PROVEAN scores (v1.1) on all possible single AA substitutions and deletions in human proteins from Ensembl 66 (SIFT scores are also available for single AA substitutions)
- PROVEAN scores (v1.1) on *Human dbSNP 137* coding variants
- PROVEAN scores (v1.0) on *UniProt human polymorphisms and disease mutations* dataset (Release: 2011_09, 20821 disease variants and 36825 polymorphisms)
- PROVEAN scores (v1.0) on indel dataset collected from UniProt/Swiss-Prot database (Release: 2011_09)
- PROVEAN scores (v1.0) on indel dataset collected from 1000 Genomes Project (Release: August 2010)

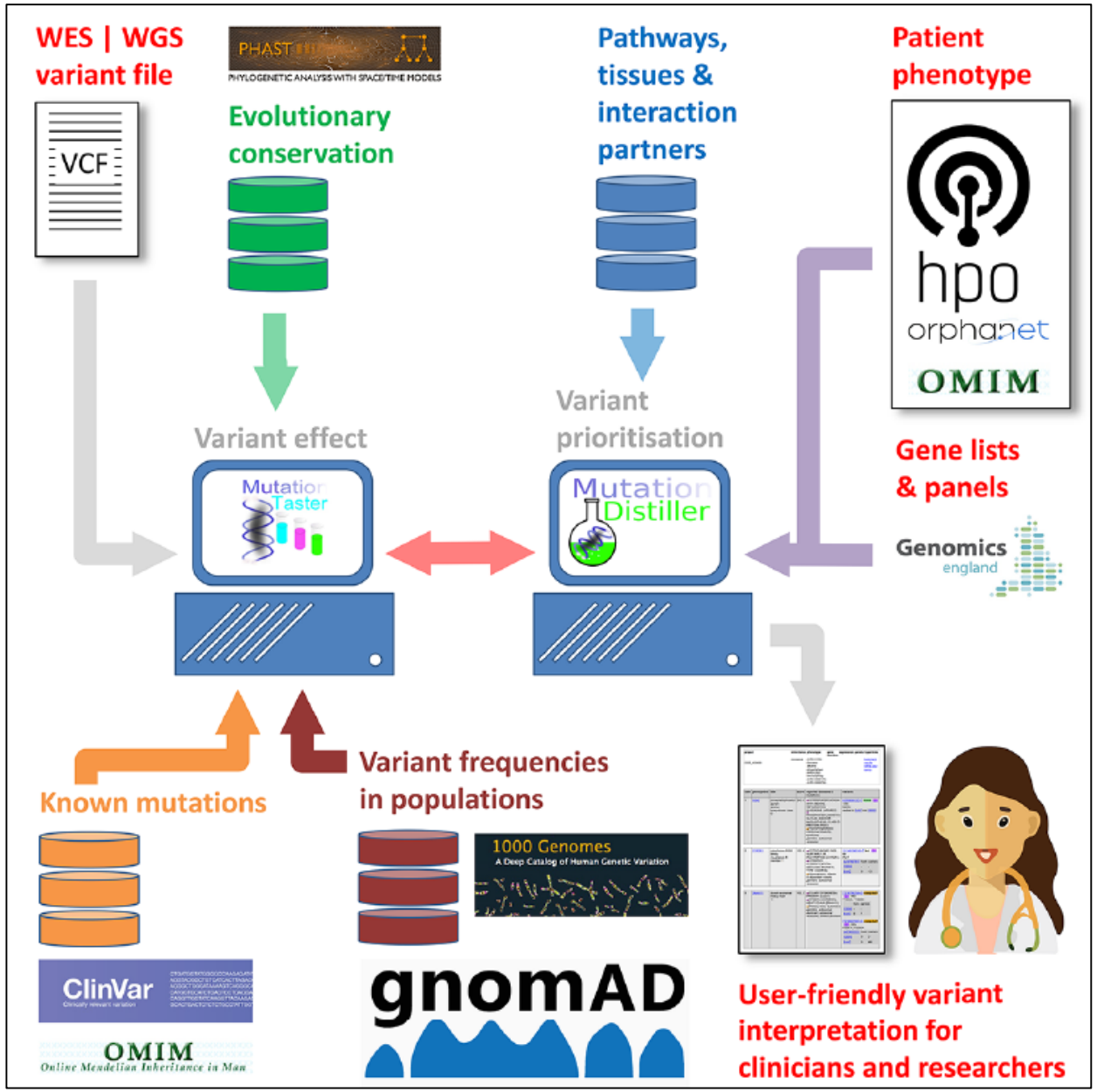
Prerequisite software and database

- NCBI BLAST (download)
- CD-HIT (download, we recommend not using v4.6 or v4.6.1 (known issue))
- NCBI nr protein database (download latest release or download August 2011 release used in our publication)

MutationTaster
(https://www.genecascade.org/MutationTaster2021/#transcript)



MutationTasterの特徴



W446–W451 Nucleic Acids Research, 2021, Vol. 49, Web Server issue

進化的保存性

CliVar, HGMD(Human Gene Mutation Database)で病的と判断されるバリアントを病的バリアントして、ホモ接合性バリアントを持つ人がいるバリアントを良性バリアントとして学習

スプライシング予測はMaxEntScan (2004)を使用

VCFファイルを取り扱える

Chromosomal positionでの解析

SNV or indel

chr13:32890650G>A

Analyse | Clear all input | Show an example

***BRCA2* NM_000059.3:c.53G>A, p.(Arg18His)**
NC_000013.10:g.32890650G>A (GRCh37)

Transcript summary: Permalink

Transcript	ENST00000544455	ENST00000380152
Gene symbol	BRCA2	BRCA2
Prediction	Benign	Benign
Tree vote	10|90	10|90
Model	simple_aae	simple_aae
Prediction problem		
Splice site change	No	No
Known ClinVar disease mutation		
Potential ClinVar disease mutation		
Amino acid changes	R18H	R18H
Variant type	Single base exchange	Single base exchange
dbSNP ID	80358762	80358762
Protein length	Normal	Normal
Features at a glance	Amino acid sequence changed	Amino acid sequence changed
	Known disease mutation at this position (HGMD CM065025)	Known disease mutation at this position (HGMD CM065025)
	Protein features (might be) affected	Protein features (might be) affected

Known variant

Reference ID: rs80358762

database	homozygous (A/A)	heterozygous	allele carriers
1000G	0	2	2
ExAC	0	5	5
gnomAD	0	6	6

Known disease mutation at this position, please check HGMD for details (HGMD ID CM065025)

HGMDでは乳癌でDM?で登録されている

Specific transcriptでの解析

***BRCA2* NM_000059.3:c.53G>A, p.(Arg18His)**
NC_000013.10:g.32890650G>A (GRCh37)

Gene symbol: BRCA2
Transcript: ENST00000544455

Hide transcripts (2)

Choose the transcript:

- ● ENST00000544455 (Protein coding, 10984 bases, Ensembl)
- ○ ENST00000380152 (Protein coding, 10930 bases, Ensembl)

Variant by sequence snippet A

Enter a few bases around your variant (e.g. ACTGTC[AG/T]GTGTF)

Variant by position B

SNV:	53	A
Indel:	Position of last wild-type base before variant	
	Position of first wild-type base after variant	
	Inserted bases (optional)	

Reference A B

- ● Coding sequence (c.)
- ○ Transcript (cDNA)
- ○ Gene (genetic sequence)

Hide snippet (SNV)

Sequence snippet: TTTTGAAATTTTTAAGACAC**G**CTGCAACAAAGCAGGTATTG

Analyse | Clear all input | Show an example

古い方のMutationTaster(https://www.mutationtaster.org/)

BRCA2 — HGNC gene symbol, NCBI Gene ID, Ensembl gene ID show available transcripts | clear input

Ensembl transcript ID

Choose the transcript:

- ○ ENST00000544455 (*protein_coding*, 10984 bases) NM_000059
- ○ ENST00000380152 (*protein_coding*, 10930 bases)
- ○ ENST00000470094 (*nonsense_mediated_decay*, 842 bases)
- ○ ENST00000533776 (*retained_intron*, 523 bases)
- ○ ENST00000528762 (*nonsense_mediated_decay*, 495 bases)

サンガーシークエンスのデータがあるときに、バリアントの記述が正しいかの確認にも使える

Gene symbol: BRCA2
Transcript: ENST00000544455

Show available transcripts

Variant by sequence snippet A

ACAC[G/A]CTGC

CADD
https://cadd.bihealth.org/

SNVだけでなくinsertion/deletionもスコアをつけることが可能

シュミレーションで生じるバリアントと自然選択を生き延びてきた実際に認められるバリアントを対比することで、複数のアノテーションを統合

コーディング領域だけでなく、ノンコーディング領域もスコアを付けられる

CADD v1.6ではDeep learningによるスプライスへの影響予測が改善

MMSplice (Cheng et al. Genome Biology, 2019)

SpliceAI (Jaganathan et al. Cell, 2019)

CADD - Combined Annotation Dependent Depletion

News Score SNV Downloads About (Alt. site:)

What is Combined Annotation Dependent Depletion (CADD)?

CADD is a tool for scoring the deleteriousness of single nucleotide variants as well as insertion/deletions variants in the human genome.

While many variant annotation and scoring tools are around, most annotations tend to exploit a single information type (e.g. conservation) and/or are restricted in scope (e.g. to missense changes). Thus, a broadly applicable metric that objectively weights and integrates diverse information is needed. Combined Annotation Dependent Depletion (CADD) is a framework that integrates multiple annotations into one metric by contrasting variants that survived natural selection with simulated mutations.

C-scores strongly correlate with allelic diversity, pathogenicity of both coding and non-coding variants, and experimentally measured regulatory effects, and also highly rank causal variants within individual genome sequences. Finally, C-scores of complex trait-associated variants from genome-wide association studies (GWAS) are significantly higher than matched controls and correlate with study sample size, likely reflecting the increased accuracy of larger GWAS.

CADD can quantitatively prioritize functional, deleterious, and disease causal variants across a wide range of functional categories, effect sizes and genetic architectures and can be used prioritize causal variation in both research and clinical settings.

In addition to this website, CADD has been described in three publications. The most recent manuscript describes CADD-Splice (CADD v1.6), the latest extension of CADD to improve its predictions of splicing effects:

> Rentzsch P, Schubach M, Shendure J, Kircher M.
> *CADD-Splice—improving genome-wide variant effect prediction using deep learning-derived splice scores.*
> Genome Med. 2021 Feb 22. doi: 10.1186/s13073-021-00835-9.
> PubMed PMID: 33618777.

Our second manuscript describes the updates between the initial publication and CADD v1.4, introduces CADD for GRCh38 and explains how we envision the use of CADD. It was published by Nucleic Acids Research in 2018:

> Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M.
> *CADD: predicting the deleteriousness of variants throughout the human genome.*
> Nucleic Acids Res. 2018 Oct 29. doi: 10.1093/nar/gky1016.
> PubMed PMID: 30371827.

The original manuscript describing the method was published by Nature Genetics in 2014:

> Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J.
> *A general framework for estimating the relative pathogenicity of human genetic variants.*
> Nat Genet. 2014 Feb 2. doi: 10.1038/ng.2892.
> PubMed PMID: 24487276.

まとめて解析

GRCh37/38
バージョンを指定可能

CADD - Combined Annotation Dependent Depletion

News | Score | SNV | Downloads | About | (Alt. site:)

CADD scores are freely available for all non-commercial applications. If you are planning on using them in a commercial application, please contact us.

Please upload a VCF file containing up to 100,000 variants

Please provide a (preferentially gzip-compressed) VCF file of your variants. For information on the VCF format see http://vcftools.sourceforge.net/specs.html. It is sufficient to provide the first 5 columns of a VCF file without header, as all other information than CHROM, POS, REF, ALT will be ignored anyway. The maximum accepted file size is set at 2MB (>100,000 variants for 5 column compressed VCF). If you try to upload files larger than 2MB, you will receive an error ("Connection reset"). You will be able to retrieve your variants faster, if you upload them in smaller sets. The file that will be provided for download is a gzip-compressed tab-separa
extension (.tsv.gz) during download; otherwise your operating system will not be
you need more variants, we suggest downloading the full set of variants.

ファイルを選択 CADD.txt

GRCh38-v1.6

Optional: Provide your email and you will be contacted when your job

#CHROM	POS	ID	REF	ALT
7	5999131	rs376258383	C	T
9	95479063	.	G	C
9	95508222	.	C	A
13	32316513	rs80358762	G	A
19	2111827	rs747848580	T	C
20	63419667	rs201750561	C	A
14	87939915	rs138577661	A	G
17	45983222	rs151115928	C	T
16	1474841	.	GCAGCCCCAGGCC	G
16	72788110	rs727502780	TGCTGCTGCTGCTGCTGTAGTTGCC	T
12	31084974	rs778497594	GGAA	G
	58364422	.	AAAC	A
	31228152	.	T	C
	65660221	rs746773448	C	T
	6623292	rs368018490	GA	G
	13919211	rs371243328	T	C
	70060049	rs141673274	T	C
	11802878	rs1413355	T	C
	58735336	.	T	C
	47828020	rs148954387	A	G
	01187781	rs768931544	G	A
	15238842	.	G	A
	58358303	.	A	G
	50350599	.	C	G
19	13208933	.	C	T
20	8788472	rs577076166	C	A

CADD - Combined Annotation Dependent Depletion

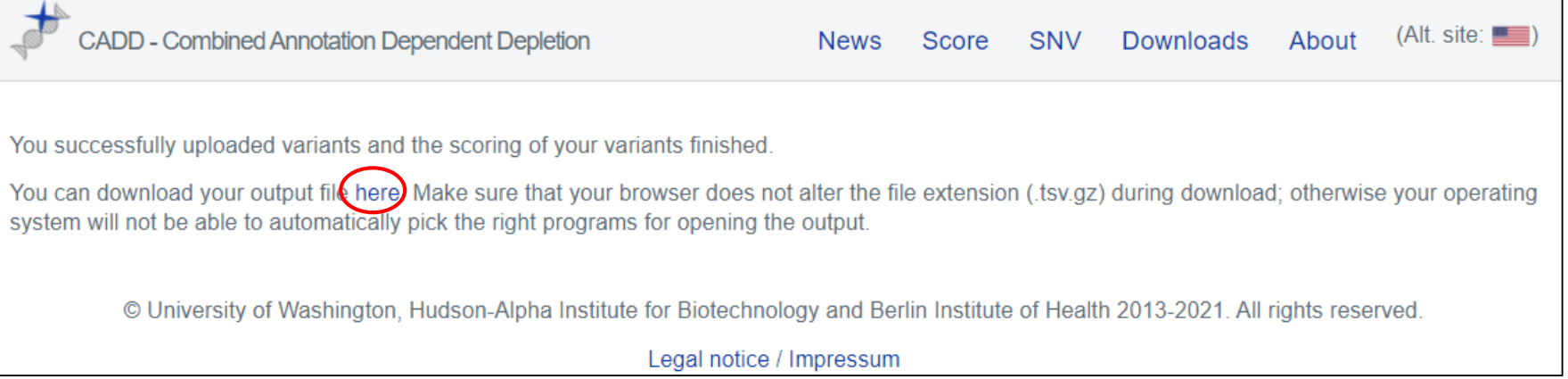
News | Score | SNV | Downloads | About | (Alt. site:)

Read 27 lines, 26 variants saved.

You successfully uploaded variants and the scoring of your variants is not yet finished.

Recheck in a few minutes to hours (depending on the number of variants submitted) by clicking here

UPLOAD VARIANTS



#CHROM	POS	ID	REF	ALT		RawScore	PHRED	
7	5999131	rs376258383	C	T		2.32179	21.7	PMS2:NM_001322008:exon4:c.364G>A:p.G122S
9	95479063	.	G	C		1.743024	17.32	PTCH1:NM_000264:exon8:c.1152C>G:p.H384Q
9	95508222	.	C	A		2.1063	20.2	PTCH1:NM_000264:exon1:c.140G>T:p.R47L
13	32316513	rs80358762	G	A	ミスセンス	3.466603	24.6	BRCA2:NM_000059:exon2:c.53G>A:p.R18H
19	2111827	rs747848580	T	C		2.529657	22.5	AP3D1:NM_003938:exon23:c.2603A>G:p.K868R
20	63419667	rs201750561	C	A	(nonsynonymous)	2.757074	22.9	KCNQ2:NM_172107:exon12:c.1253G>T:p.G418V
14	87939915	rs138577661	A	G		4.045992	27.3	GALC:NM_001201401:exon15:c.1832T>C:p.L611S
17	45983222	rs151115928	C	T		0.938738	10.86	MAPT:NM_001377265:exon5:c.643C>T:p.P215S
16	1474841	.	GCAGCCCCAGGCC	G		1.892505	18.43	CLCN7:NM_001114331:exon1:c.122_133del:p.G41_A44del
16	72788110	rs727502780	TGCTGCTGCTGCTGCTGTAGTTGCC	T	コーディングの	2.086341	19.91	ZFHX3:NM_001164766:exon9:c.7400_7423del:p.R2467_Q2474del
12	31084974	rs778497594	GGAA	G	インフレーム欠失	1.9976	19.24	DDX11:NM_001257144:exon5:c.487_489del:p.E166del
14	58364422	.	AAAC	A		0.494642	6.448	ARID4A:NM_002892:exon20:c.2334_2336del:p.T779del
9	131228152	.	T	C		1.975673	19.07	NM_005085:exon33:c.5903-8T>C
11	65660221	rs746773448	C	T		-0.216828	0.47	NM_001243985:exon5:c.336-6G>A
5	6623292	rs368018490	GA	G		0.147205	2.547	NM_001193455:exon4:c.361-8T>-
19	13919211	rs371243328	T	C	イントロン	-0.730796	0.037	NM_017721:exon11:c.1222+9T>C
18	70060049	rs141673274	T	C		2.649794	22.7	NM_001318520:exon35:c.2012-7A>G
1	11802878	rs1413355	T	C		2.089058	19.93	NM_005957:exon2:c.236+3A>G
18	58735336	.	T	C		0.969444	11.24	NM_006785:exon13:c.1603+7T>C
5	147828020	rs148954387	A	G		4.06441	27.5	NM_003122:exon4:c.194+2T>C
2	201187781	rs768931544	G	A		-0.456302	0.147	
19	15238842	.	G	A		-0.276793	0.349	
17	58358303	.	A	G	Synonymous	-0.36671	0.226	
3	50350599	.	C	G		1.282571	14.26	
19	13208933	.	C	T		0.641051	7.906	
20	8788472	rs577076166	C	A		0.205429	3.186	

CADDスコアの評価

RawScore	PHRED
2.32179	21.7
1.743024	17.32
2.1063	20.2
3.466603	24.6
2.529657	22.5
2.757074	22.9
4.045992	27.3
0.938738	10.86
1.892505	18.43
2.086341	19.91
1.9976	19.24
0.494642	6.448
1.975673	19.07
-0.216828	0.47
0.147205	2.547
-0.730796	0.037
2.649794	22.7
2.089058	19.93
0.969444	11.24
4.06441	27.5
-0.456302	0.147
-0.276793	0.349
-0.36671	0.226
1.282571	14.26
0.641051	7.906
0.205429	3.186

Raw scoreが高いほど、実際の集団中には見られず、シュミレーションで認められるバリアントである
⇒ “deleterious effects”を持つ可能性が大

総バリアントをランキング化し、スケール化されたCADDスコアが”PHRED”

Phred score	塩基の正確性
40	99.99 %
30	99.9 %
20	99 %
10	90 %

CADD PHREDの場合

Top 0.01% -- CADD-40
Top 0.1% -- CADD-30
Top 1% -- CADD-20
Top 10% -- CADD-10

Cut off値はカットオフを10から20の間のどこかに置くことを推奨
15 (v1.0でのスプライスサイトバリアントとミスセンスバリアントの中央値)

SNVは個別入力も可能

***BRCA2* NM_000059.3:c.53G>A, p.(Arg18His)**
NC_000013.11:g.32316513G>A (GRCh38)
NC_000013.10:g.32890650G>A (GRCh37)

CADD - Combined Annotation Dependent Depletion

News Score SNV Downloads About (Alt. site:)

Single
Range

Single nucleotide variant (SNV) lookup

This form allows you to quickly access the score (and annotation) of a single nucleotide variant (SNV) or all scores at a specific genomic position. If you are investigating multiple or even ranges of CADD SNV scores, please have a look at our Multi-SNV scoring form. Please note that copying and downloading of the annotation tables is not working in Internet Explorer.

CADD scores are freely available for all non-commercial applications. If you are planning on using them in a commercial application, please contact us.

Chromosome: 22 Position: 43451447

T Alt (optional): A

GRCh37-v1.6 INCLUDE ANNOTATIONS TRANSPOSE TABLE

Single nucleotide variant (SNV) lookup

This form allows you to quickly access the score (and annotation) of a single nucleotide variant (SNV) or all scores at a specific genomic position. If you are investigating multiple or even ranges of CADD SNV scores, please have a look at our Multi-SNV scoring form. Please note that copying and downloading of the annotation tables is not working in Internet Explorer.

CADD scores are freely available for all non-commercial applications. If you are planning on using them in a commercial application, please contact us.

Chromosome: 13 Position: 32316513

Ref (optional): T Alt (optional): A

CADD model: GRCh38-v1.6 INCLUDE ANNOTATIONS TRANSPOSE TABLE

LOOKUP VARIANT(S)

The (maximum) CADD score at this position is 24.8. (Request: Chromosome 13, Position 32316513, CADD GRCh38-v1.6)

Details: COPY TABLE SAVE TABLE

Chrom	Pos	Ref	Alt	RawScore	PHRED
13	32316513	G	A	3.466603	24.6
13	32316513	G	C	3.532795	24.8
13	32316513	G	T	2.795310	23.0

cess the score (and annotation) of multiple single nucleotide variants (SNV). If you are investigating longer ranges of interested in our UCSC Genome Browser Tracks for hg19/GRCh37 and hg38/GRCh38. Please note that copying and les is not working in Internet Explorer.

for all non-commercial applications. If you are planning on using them in a commercial application, please contact us.

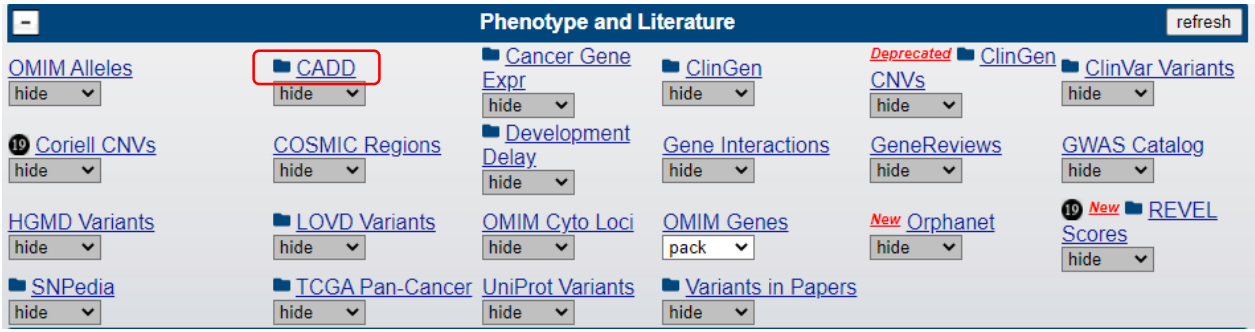
22

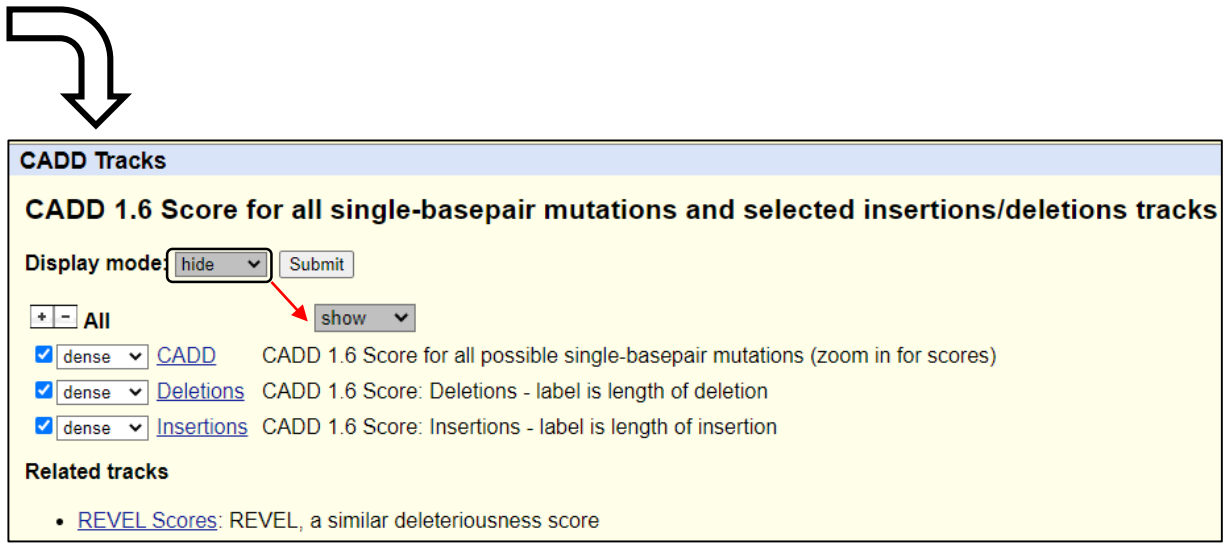
43451447 End: 43451450

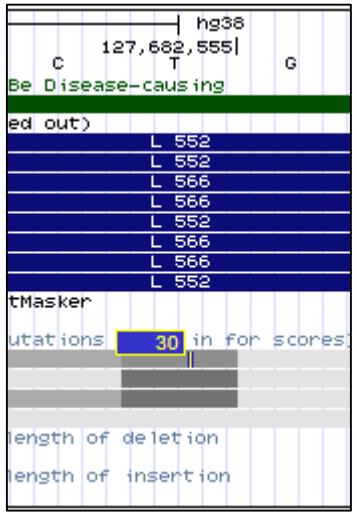
GRCh37-v1.6 INCLUDE ANNOTATIONS TRANSPOSE TABLE

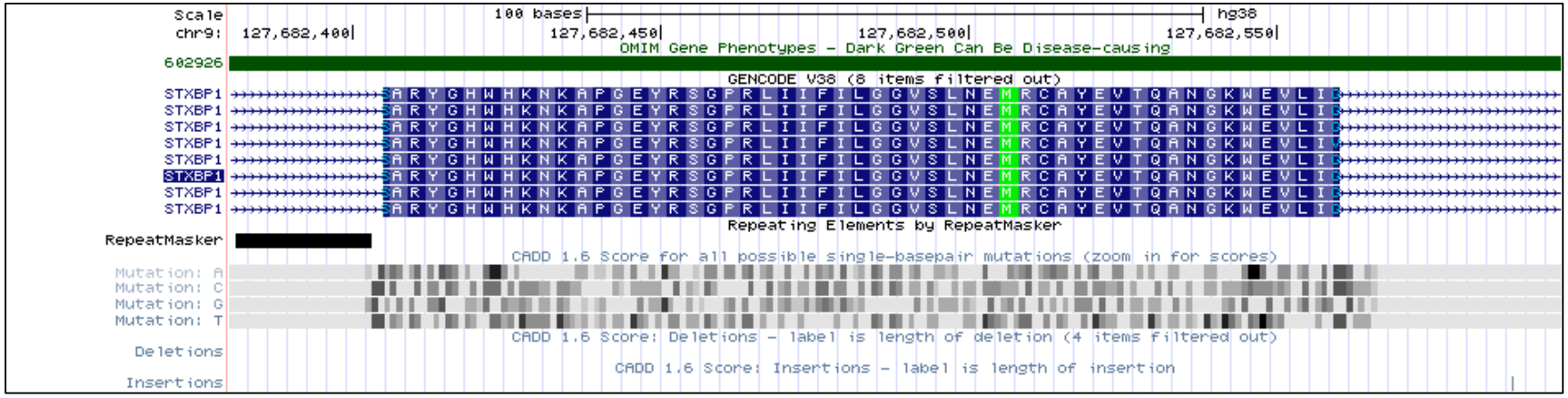
LOOKUP RANGE

UCSC Genome Browserでもスコアの確認が可能









Local serverで自分のVCFファイルにアノテーションすることも可能

conda環境とsnakemakeが必要

Developmental release: v1.6 [release notes]

Offline scoring scripts for v1.6

Scripts and README for offline installation	file (360K)

Genome build GRCh38 / hg38

Description	Link (Size)	Tabix Index (Size)
All possible SNVs of GRCh38/hg38	US | DE (81G)	US | DE (2.6M)
All possible SNVs of GRCh38/hg38 incl. all annotations	US | DE (313G)	US | DE (2.7M)
All gnomad release 3.0 InDels to initiate a local setup	US | DE (1.1G)	US | DE (1.8M)
All gnomad release 3.0 InDels incl. all annotations to initiate a local setup	US | DE (7.2G)	US | DE (2.5M)
All gnomad release 3.0 SNVs	US | DE (5.9G)	US | DE (2.4M)
MD5Sums	US | DE	
All GRCh38 annotations required for offline installation	US | DE (206G)	

Genome build GRCh37 / hg19

SpliceAI Lookup（https://spliceailookup.broadinstitute.org/）

Enter a variant below to see its SpliceAI scores. This can be any SNV or simple InDel within an exon or intron defined by GENCODE v38.

Translational Genomics Group

This web interface and the underlying SpliceAI server are being developed by the TGG at the Broad Institute. The SpliceAI server optionally uses pre-computed scores provided by Illumina for SNVs and small InDels, but resorts to running the SpliceAI model directly for scores not available in the pre-computed files - such as larger InDels or non-default "Max distance" values.

NOTE: The API currently only supports interactive use. For automated batch processing, please use the source code to set up your own local instance of the API server or just run the underlying SpliceAI tool.

For more information on the SpliceAI model, see Jaganathan et al. Cell 2019 and github.com/Illumina/SpliceAI, as well as in this recorded talk by Kishore Jaganathan.

Examples (on hg38):

chr8-140300616-T-G
chr8 140300616 T G
NM_000552.4(VWF):c.3797C>A (p.Pro1266Gln)

more examples...

8:140300616 T>G	'chr' *prefix is optional*
1:930130:C:G	*position with overlapping genes*
1-1042601-A-AGAGAG	*insertion of GAGAG*
1-1042466-GGGC-G	*deletion of GGC*
NM_000249.4(MLH1):c.116G>A	*another HGVS exapmle*

Enter a variant...

Genome version: ○ hg19 ◉ hg38

Submit

Score type: ○ masked ◉ raw ⓘ ← 正常のスプライシングが強くなり、新たなスプライシングが弱くなるような予測をマスクするか

Max distance: 50 ← バリアントによって影響を受ける（消失あるいは出現）スプライス部位をどの範囲で調べるか
Max 10,000

Use Illumina's pre-computed scores: ○ yes ◉ no ⓘ

What's New

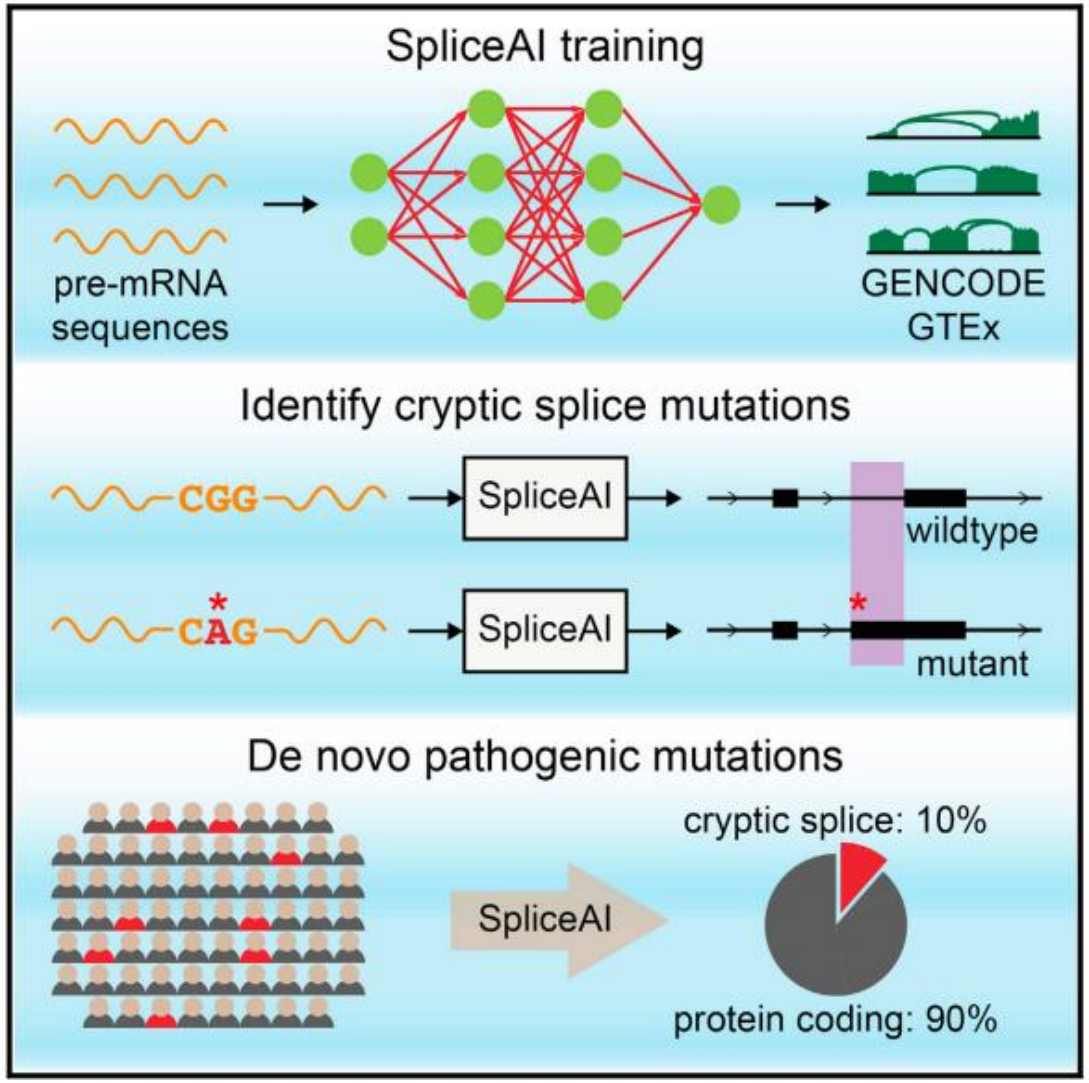
To post issues or feature requests, please use SpliceAI-lookup/issues

June 2, 2021
- show RefSeq ids for the subset of Ensembl ENST ids (~30%) that have one or more matching RefSeq NM ids according to Ensembl. See issue #8 for details.
- update to Gencode v38 (was previously on v37)

April 11, 2021
- show gray background for non-coding transcripts
- switch to showing all non-coding transcripts. (Previously, transcripts with Gencode biotypes like *lncRNA*, *processed_pseudogene*, *processed_transcript*, *retained_intron*, and *nonsense_mediated_decay* were filtered out if they overlapped *protein_coding* transcripts). See issue #6 for details.
- update to Gencode v37 (was previously on v36)

Related web tools:
liftover - simple hg19 <=> hg38 conversion for variants, positions, intervals
CMA search - search OMIM by genomic region, gene name, phenotype, etc.

SpliceAIの予測アルゴリズムとそれを用いた成果



Jaganathan et al, Cell. 2019 24;176(3):535-548.e24

- ✓ イルミナ社が作ったプログラム
- ✓ Deep learning (Deep neural network)で、pre-mRNA配列からスプライシングを予測
- ✓ GENCODE-annotated pre-mRNA配列を使ってトレーニング
- ✓ 予測された潜在的(cryptic)なスプライス部位を使う転写産物の75%を、RNA-seqで確認
- ✓ 潜在的なスプライス部位を使うような異常な転写は、神経発達障害の～10%を占めると考えられる

***GNAO1* NM_020988.3:c.724-8G>A**
NC_000016.10:g.56351376G>A(GRCh38)
NC_000016.9:g.56385288G>A(GRCh37)

16:56351376G>A

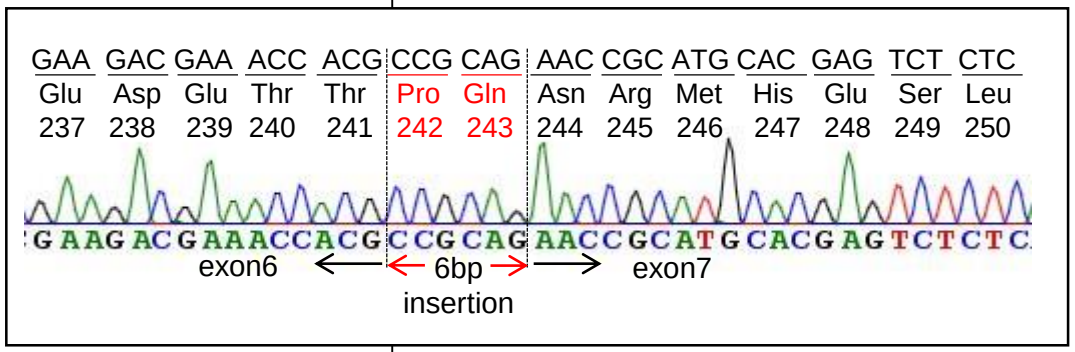
Genome version: hg19 hg38 Submit

Score type: masked raw

Max distance: 50

Use Illumina's pre-computed scores: yes no

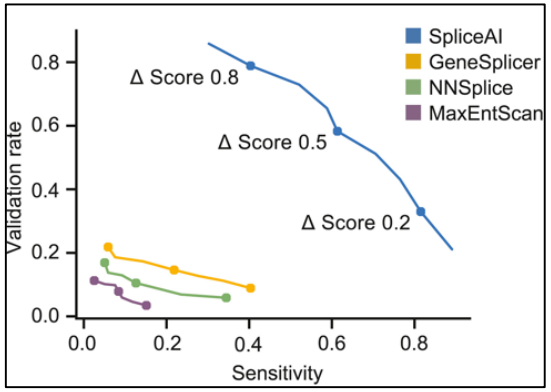
variant	gene	Δ type	Δ score	pre-mRNA position
16-56351376-G-A UCSC, gnomAD	GNAO1 (ENSG00000087258.16 / ENST00000262493.12 / NM_020988.3) biotype: protein coding OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards	Acceptor Loss	0.92	8 bp
		Donor Loss	0.00	
		Acceptor Gain	1.00	2 bp
		Donor Gain	0.00	



バリアント±50-bpでAcceptorあるいはDonorが消失・出現するかを計算し、最もスコアが高い位置を表示。

正の値が3’側、負の値が5’側の位置を示す。

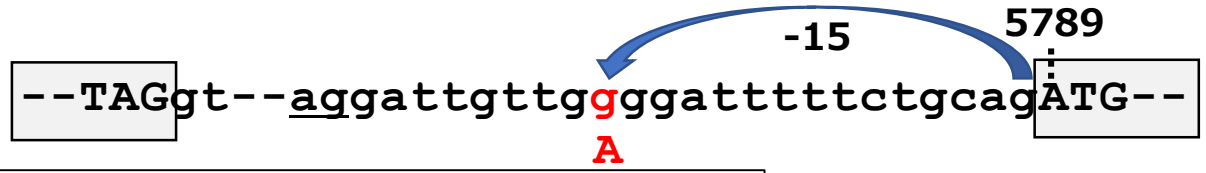
Δ scoreのカットオフ値



0.2 (感度、再現率が高い)
0.5 (推奨)
0.8 (適合度、精度が高い)

Jaganathan et al, Cell. 2019 24;176(3):535-548.e24

FBN1 **NM_000138.4:c.5789-15G>A**
NC_000015.10:g.48445519C>T(GRCh38)
NC_000015.9:g.48737716C>T(GRCh37)



イントロン保持

15:48445519C>T

Genome version: hg19 hg38 Submit

Score type: masked raw

Max distance: 50

Use Illumina's pre-computed scores: yes no

variant	gene	Δ type	Δ score	pre-mRNA position
15-48445519-C-T UCSC, gnomAD	FBN1 (ENSG00000166147.15 / ENST00000316623.10 / NM_000138.5) biotype: protein coding canonical transcript OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards	Acceptor Loss	0.24	-15 bp
		Donor Loss	0.00	
		Acceptor Gain	0.08	8 bp
		Donor Gain	0.00	

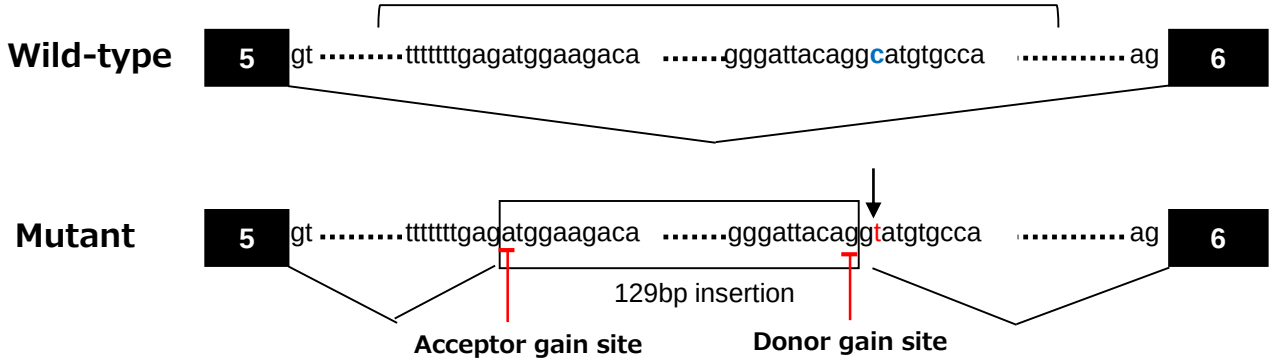
FBN1はアンチセンス鎖に位置しているので、ゲノムで上流15-bpが通常のアクセプター部位

Max distance: 1000

Max distanceは大きい方が良さそう

variant	gene	Δ type	Δ score	pre-mRNA position
15-48445519-C-T UCSC, gnomAD	FBN1 (ENSG00000166147.15 / ENST00000316623.10 / NM_000138.5) biotype: protein coding canonical transcript OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards	Acceptor Loss	0.24	-15 bp
		Donor Loss	0.00	
		Acceptor Gain	0.62	56 bp
		Donor Gain	0.00	

***POLR3A* NM_007055.3:c.645+312C>T**
NC_000010.11:g.78024237G>A(GRCh38)
NC_000010.10:g.79783995G>A(GRCh37)



10:78024237G>A

Genome version: ○ hg19 ● hg38 **Submit**

Score type: ● masked ○ raw ⓘ

Max distance: 1000 ⓘ

Use Illumina's pre-computed scores: ○ yes ● no ⓘ

variant	gene	Δ type	Δ score ⓘ	pre-mRNA position ⓘ
10-78024237-G-A UCSC, gnomAD	POLR3A (ENSG00000148606.13 / ENST00000372371.8 / NM_007055.4) biotype: protein coding canonical transcript OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards	Acceptor Loss	0.00	
		Donor Loss	0.00	
		Acceptor Gain	0.55	130 bp
		Donor Gain	0.77	2 bp

Local serverで自分のVCFファイルにアノテーションすることも可能

SpliceAI: A deep learning-based tool to identify splice variants

release v1.3.1 | license GPLv3 | downloads 115k

This package annotates genetic variants with their predicted effect on splicing, as described in Jaganathan *et al*, Cell 2019 in press. The annotations for all possible substitutions, 1 base insertions, and 1-4 base deletions within genes are available here for download. These annotations are free for academic and not-for-profit use; other use requires a commercial license from Illumina, Inc.

License

SpliceAI source code is provided under the GPLv3 license. SpliceAI includes several third party packages provided under other open source licenses, please see NOTICE for additional details. The trained models used by SpliceAI (located in this package at spliceai/models) are provided under the CC BY NC 4.0 license for academic and non-commercial use; other use requires a commercial license from Illumina, Inc.

Installation

The simplest way to install SpliceAI is through pip or conda:

```
pip install spliceai
# or
conda install -c bioconda spliceai
```

Alternately, SpliceAI can be installed from the github repository:

```
git clone https://github.com/Illumina/SpliceAI.git
cd SpliceAI
python setup.py install
```

SpliceAI requires `tensorflow>=1.2.0`, which is best installed separately via pip or conda (see the TensorFlow website for other installation options):

```
pip install tensorflow
# or
conda install tensorflow
```

- ✓ Python環境が必要
- ✓ pipやcondaで楽にインストール可能
- ✓ 機械学習用のtensorflowを使って動く
- ✓ インプットのVCFをアウトプットのVCFを指定するだけで動く（シンプル）
- ✓ 結果はINFO fieldに記載される
- ✓ MaxDistance(-D)を増やすと時間はかかる

ESEfinder

(http://krainer01.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home)

Exonic splicing enhancer (ESE)を見つけるプログラムで、ESEsに結合するSR proteinを見つけるのが主な目的

SRProteinとSpliceSiteの2つのマトリックスがある

スプライス部位の予測もやってくれる

<5000-bpで配列を張り付ける必要がある

last update: 02/27/2007

release 3.0

Search | background | matrices & thresholds | input & output | caveats

Go to ESEfinder2.0

Matrix infomration

Please choose a matrix library: SpliceSites

Choose the matrix and the threshold to be used:

	Matrices (select one or more)	Threshold
☑	**5SS_U2_human** (5' splice sites (donor) of human (U2 type))	6.67
☑	**3SS_U2_human** (3's splice sites (acceptor) of human (U2 type))	6.632
☑	**5SS_U2_mouse** (5' splice sites (donor) of mouse (U2 type))	6.679
☑	**3SS_U2_mouse** (3's splice sites (acceptor) of mouse (U2 type))	6.724
☑	**BranchSite** (Mammalian branch site (U2 type))	0
		Reset thresholds

Sequence infomration

Enter here your input data in FASTA or MULTI-FASTA format (**<5000nt, accept both 'T' and 'U' as being equivalent**)
(please read important information about search format description)

alternatively, upload a text file: ファイルを選択 選択されていません

Output information

Override the thresholds above and use one of the following two options:

☐ Report only the best hit in each sequence (instead of hits above the thresholds)

☐ Report all scores in each sequence (instead of hits above the thresholds)

(**Note**: if you check both, the program will report the best hit in each sequence.)

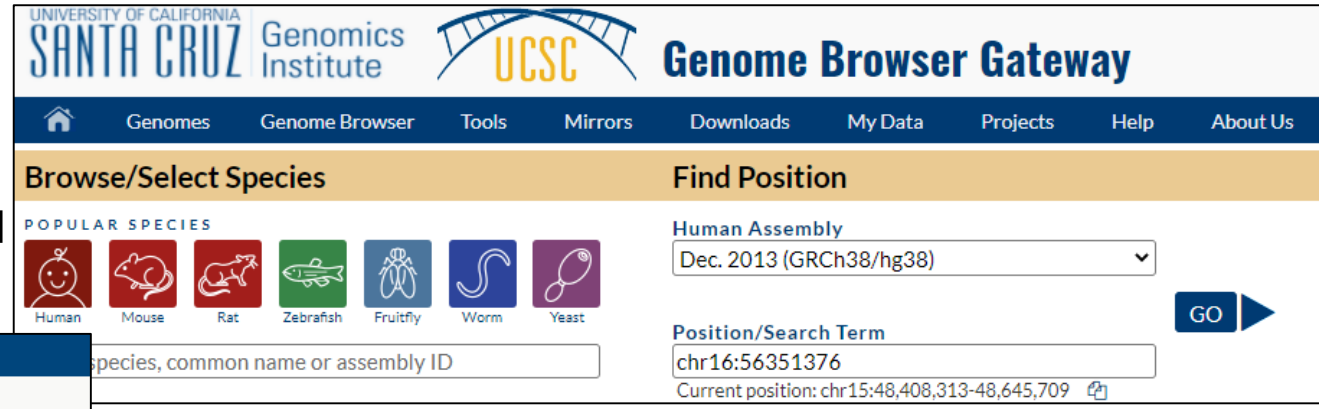
☐ Output as a plain text file

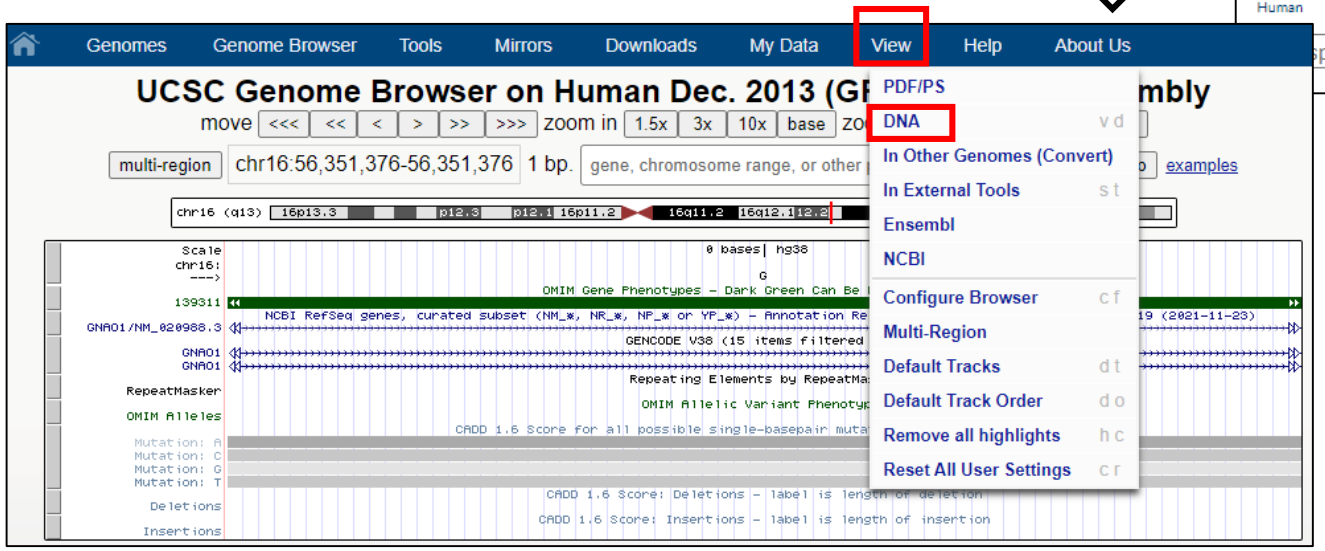
☐ please send the results to the following e-mail address:

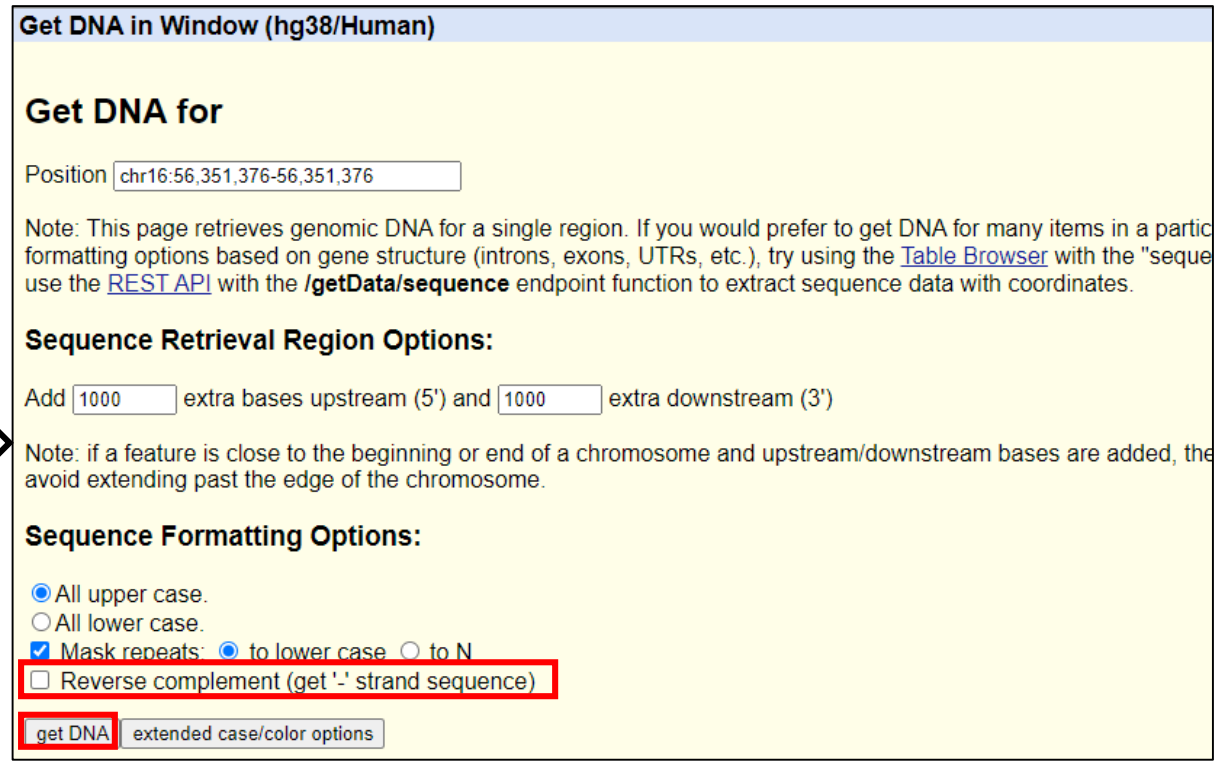
Send Restore defaults

UCSC genome browser(https://genome.ucsc.edu/)でバリアント前後の配列を取得

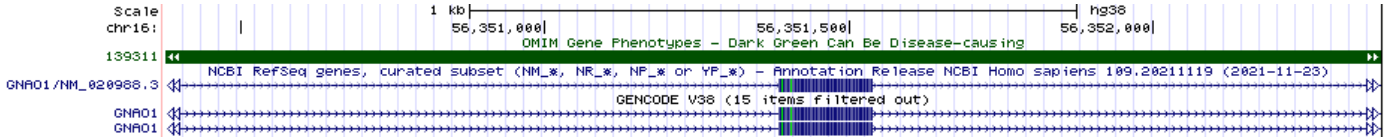
GNAO1 **NM_020988.3:c.724-8G>A**
NC_000016.10:g.56351376G>A(GRCh38)
NC_000016.9:g.56385288G>A(GRCh37)







当該遺伝子がアンチセンス鎖にある場合は
チェックを入れた方が分かりやすい



>hg38_dna range=chr16:56350376-56352376 5'pad=1000 3'pad=1000 strand=+ repeatMasking=lower

TTCCTCCCACTCCCTGTGCAGAAATGGATTCGTTCACAGAGAAAGATGCC
CTCTGTGCTGCTGAGAGACAGACTCTGCCCAAGTGAATGAGCTCAGCGCC
CCAAAGCAGAGTTTTTGCCCAGGGCAAGAGGCTGCCCCCGTTCGGCATCT
GTGGGGCGCCCATCACGTTCATCCATTTGCTAAACAGGCTCCTGTGTGCA
GGGCAGCCCTCCACATCTGCCTTGCTTAGAACATCATGCAGTGTCAGATG
CAGCTGAGTAGGACCCAGCCAGAGGAGGTGTGGCCAGTCCCCCAGGTGCT
CCTGGAGGTGGCCCTGCCAGGAGCCTCTCCCCTGAGTGGTGGCACCCAGG
ATGCTGGCAGGACCTGGCATGCCTTCCCCTGAGACCATAGGAAGGGCCCG
GAGTCTCTCACCACTCCCAGAGAACATCCCCCTACCCAGGATGGGCCCAG
GAGGAGAGGTCTAGGCAGGAAGGGCCAAAGCCCAGCTCTCCGGCTCCGGT
CCAGACTCCCCCAACGCCcacacacacaggcacacacacaggcacgcaca
cacactcaagcacatggatgcacacaggcatgcacacacaggtgcacaca
cataggcacacatgcacacgaacacacacaggcacacataacaggtgcat
gcacacacacacaggcacatgcacacagccacacacacGTGTGCTGGGAG
ACCCTCACATAGCTGGGAACTCTGCAGTGGGGAGAGCCCGGGTCACCCTG
TCCTCAGCCTCCCCAGAGAGCCATGGCTGGTGTCCAGTCCAGTCTCTGCC
TCTGGCCCCGACATGACGGGTTCTCCGGAAGCAGGGAATGAGATGTGCTG
TGAGGTGTAACTGACTCAGGTTCCATCTGGCAGCCTCTCGGAGGAGCTGC
CGAGTAGCCCAGTCCCTCTCTGTCAAGCCTAATTCtctccttctctttcc
ctgtctctgtgtctccctcccgctgtctgtcctctctcCTCCCTTCCTGC
GGCCGCAGAACCGCATGCACGAGTCTCTCATGCTCTTCGACTCCATCTGT
AACAACAAGTTCTTCATCGATACCTCCATCATTCTCTTCCTCAACAAGAA
AGATCTCTTTGGCGAGAAGATCAAGAAGTCACCTTTGACCATCTGCTTTC
CTGAATACACAGGTGGGTGCCAGGCAGTCCTGTGCAGGGGGAAGCCTGCC
CCTGACAGGGACCCATGGCTCAGAGAACAGGCTGCTCGGCCAGCACTGCT
GGGGCTAGCTGGCGAGCGGGACCCATTGCAGGAGACTGGTGGGGCGCAAA
AGAAAAACAGTGCTGTGCCACCAGGTTTGGGCTTCCCAGGACACAGCCCT
CAGCGTGGTGGAAATGGCCCCTCCTAAGATATATGTGTTAGGACCAAGTG
ACTCAGGAGTGGTGGGGAGCAGGGGGCAGGACAGGATCACAGGCTTCCCC
CTTCCTCCTGGTCACCCTCTAGAAGAGGTCTCAGGACAGCCTCCTGTAAT
CTCAGCAGTGGCCTCCCCTCACCCCTGCCCAGAGCCCCACAGCACCTTTG
CATGAAACCGAAAAGTGGACACGTGGCCTGTGAACAGGGCCTGGACTGCC
TCTTGGCCCCACCTGCTTTCCCTGCTCCCTGCTTTGGTGCCCGGAACATG
CTTCATCTCTGTGCAGCCGCCCAGCCAGACCACTCCACCCATCCCACATC
GTGCCTGCCCTCCCTCCCCAACCCCAGCCCCATTGCGCCTCCTCTCTCCC
CTTCTTCCTTCGTCCCTGGTCTTTGCCCCAAATCCAACCACTCCAGCTAT
CTGCAAGCCTCCTGTATCACTGCCTCCCTTTCTTCTGCCTTCTCTGGGGC
TTTCTTCTTGCTCTCAGGGGAATGTGTCACAGACCTTCCCCCCCACCCGG
CTCAATGACGCTCTTTCCAAATGGATGGATGAGGGATGGTGGCTTCCGTG
GAATGGGCCAACTCTGGGCTACTGCTAATGCCTAAAAGCAGCACTGATGG
G

Matrix infomration

Please choose a matrix library: SpliceSites

Choose the matrix and the threshold to be used:

バリアントによって予測スコアが低下すること を確認するために閾値は0とする

	Matrices (select one or more)	Threshold
☑	**5SS_U2_human** (5' splice sites (donor) of human (U2 type))	0
☑	**3SS_U2_human** (3's splice sites (acceptor) of human (U2 type))	0
☑	**5SS_U2_mouse** (5' splice sites (donor) of mouse (U2 type))	0
☑	**3SS_U2_mouse** (3's splice sites (acceptor) of mouse (U2 type))	0
☑	**BranchSite** (Mammalian branch site (U2 type))	0
		Reset thresholds

Sequence infomration

Enter here your input data in FASTA or MULTI-FASTA format (**<5000nt, accept both 'T' and 'U' as being**
(please read important information about search format description)

```
>WT
--gtctctgtgtctccctcccgctgtctgtcctctctcCTCCCTTCCTGC
GGCCGCAGAACCGCATGCACGAGTCTCTCATGCTCTTCGACTCCATCTGT
AACAACAAGTTCTTCATCGATACCTCCATCATTCTCTTCCTCAACAA----
>Variant
--gtctctgtgtctccctcccgctgtctgtcctctctcCTCCCTTCCTGC
aGCCGCAGAACCGCATGCACGAGTCTCTCATGCTCTTCGACTCCATCTGT
AACAACAAGTTCTTCATCGATACCTCCATCATTCTCTTCCTCAACAA---
```

ESEfinderの結果の解釈

5SS_U2_human threshold: 0			3SS_U2_human threshold: 0		
Position*/Site/Score			Position*/Site/Score		
1565 (-437)	GTGGACACGTGGCCTGTGAACAGGGCCTGG	3.10990	985 (-1017)	tctcCTCCCTTCCTGCGGCCGCAGAACCGC	2.42960
1622 (-380)	CTGCTCCCTGCTTTGGTGCCCGGAACATGC	0.56590	994 (-1008)	TTCCTGCGGCCGCAGAACCGCATGCACGAG	8.33480
1686 (-316)	CACCCATCCCACATCGTGCCTGCCCTCCCT	1.37990	1084 (-918)	CTCTTCCTCAACAAGAAAGATCTCTTTGGC	3.30600

WT

1565 (-437)	GTGGACACGTGGCCTGTGAACAGGGCCTGG	3.10990	985 (-1017)	tctcCTCCCTTCCTGCaGCCGCAGAACCGC	2.37420
1622 (-380)	CTGCTCCCTGCTTTGGTGCCCGGAACATGC	0.56590	988 (-1014)	cCTCCCTTCCTGCaGCCGCAGAACCGCATG	12.37800
1686 (-316)	CACCCATCCCACATCGTGCCTGCCCTCCCT	1.37990	994 (-1008)	TTCCTGCaGCCGCAGAACCGCATGCACGAG	8.04330
1754 (-248)	CTTCCTTCGTCCCTGGTCTTTGCCCCAAAT	0.27270	1084 (-918)	CTCTTCCTCAACAAGAAAGATCTCTTTGGC	3.30600

Variant

994が正常のスプライス部位のスコア　8.33480⇒8.04330 微減

988に12.37800と高いスコアで新しいアクセプター部位が出現

⇒　新しいアクセプター部位の出現を高い自信をもって予測できている

FBN1 **NM_000138.4:c.5789-15G>A**
NC_000015.10:g.48445519C>T(GRCh38)
NC_000015.9:g.48737716C>T(GRCh37)

イントロン保持

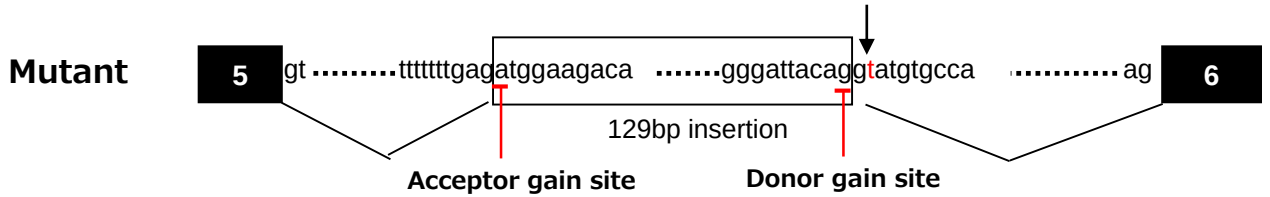
3SS_U2_human threshold: 0		
Position*/Site/Score		
1001 (-1001)	GGGATTTTTCTGCAGATGTTGATGAATGTG	7.20780
1001 (-1001)	aGGATTTTTCTGCAGATGTTGATGAATGTG	6.86040

WT

Variant

1001が正常のスプライス部位のスコア　7.20780 ⇒ 6.86040 微減
新たなスプライス部位の出現なし

POLR3A **NM_007055.3:c.645+312C>T**
NC_000010.11:g.78024237G>A(GRCh38)
NC_000010.10:g.79783995G>A(GRCh37)



5SS_U2_human threshold: 0			3SS_U2_human threshold: 0		
Position*/Site/Score			Position*/Site/Score		
985 (-1017)	tagctgggattacaggcatgtgccaccatg	3.94440	856 (-1146)	ttttttttttttgagatggaagacagagtc	7.06220
985 (-1017)	tagctgggattacaggTatgtgccaccatg	10.88050	856 (-1146)	ttttttttttttgagatggaagacagagtc	7.06220

WT

Variant

985が新たに出現するドナー部位　3.94440 ⇒ 10.88050 劇的にスコアが増加
856が新たに使われるアクセプター部位 7.06220で変化ないが、スコアはもともと高く潜在的なアクセプター部位

Take-home message

- ✓様々な*in silico*解析プログラムが開発されており、バリアントの解釈や絞り込みに利用可能
- ✓機械学習やDeep learningといった人工知能を利用するプログラムが最近では主流
- ✓ただ、*in silico*解析はあくまでsupportiveなエビデンスであることを認識する必要がある（ミスセンスバリアントの解釈のstrongエビデンスは機能解析で、ハードルが高い）
- ✓スプライス異常は、逆転写PCRで証明可能であり、機能解析を実行するハードルは低い。スプライス異常を起こしうるバリアントをピックアップするためには、*in silico*解析が重要