SmProt v2 Tutorial

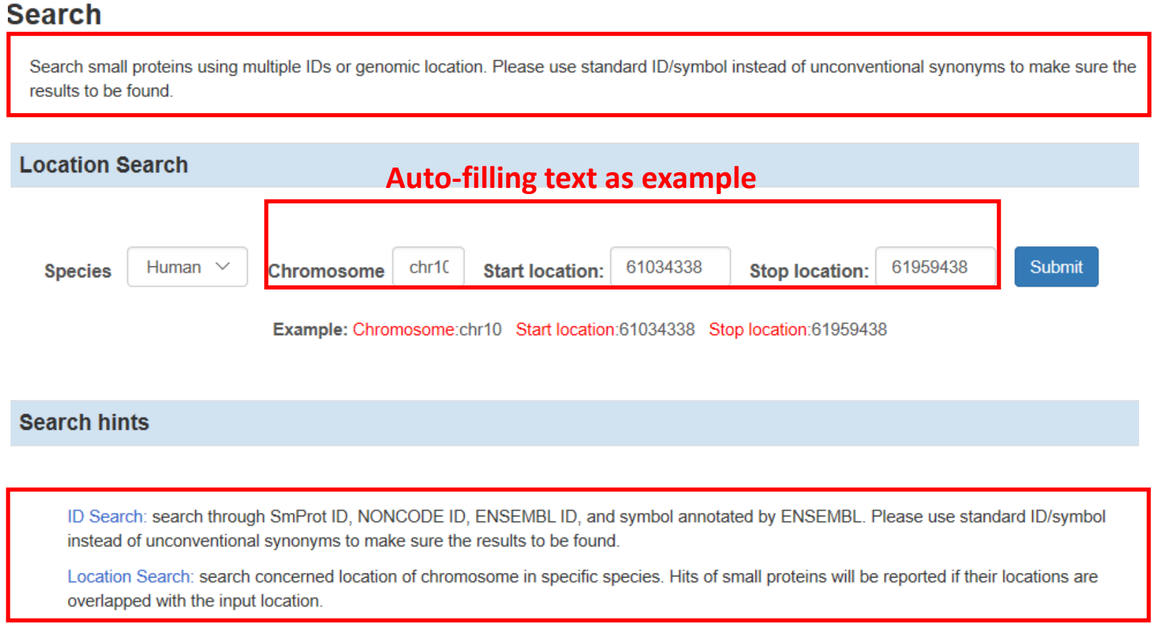
2020.09.08

1、Help page: introduction of SmProt and each page

3.How to use SmProt database?

Search	ID Search: search through SmProt ID, NONCODE ID, ENSEMBL ID. Location Search: search concerned location of chromosome in specific species. Hits of small proteins will be reported if their locations are overlapped with the input location.
Browse	On Browse webpage, users can choose species (human, mouse, etc.), start codon (ATG, non-ATG), data source (ribosome profiling, mass spectrum, etc.), predicted function (yes/no, means whether have function domain prediction). Click Browse button and the filtered results with brief information will be listed below. Click on one SmProt_ID to jump to the page with detailed information.
Variants	On Variants webpage, variants related to small ORFs in 5'UTR called from WGS data of multiple projects are provided, as well as their effects on downstream gene expressions and translated uORF in SmProt. Users can choose data source (WGS project) and variant type (uAUG_gained, uSTOP_lost, etc., means effects of variants). Click on one variant to jump to the page with detailed information.
Diseases	On Diseases webpage, disease-specific translation events and variants in small proteins predicted from ribosome profiling data are provided (confidence: predicted specific), as well as disease-related small proteins reported in literature (confidence: reported related). Users choose species, then diseases list will be attached to the chosen species. Users can further choose confidence and start codon of small proteins.
Human Microbio	On HumanMicroBio webpage, users can choose body site (skin, gut, etc.) to see small proteins identified from microorganism samples from the body site. The brief results show total number, length and representative sequence of each family. Click on the Family ID to jump to the page with corresponding detailed information.
Inner BLAST	On Blast webpage, users can assess sequence similarity of small proteins in multiple species. All small proteins in SmProt v2.0 were added to the blast database. Program blastp means from protein to protein, blastx means from translated nucleotide to protein. Users can enter fasta format sequence directly or load fasta files from disk. The results can be generated with default parameters or specified parameters.
Genome Browser	Users can click Genome Button on Navigation Bar, or location link in General Information table in any small protein page, or genome browser link on Dataset table in any small protein page, to jump to Genome browser webpage to check small proteins on a genomic region. Users can manually change tracks to be shown or hiden.
Terminology Explaination	PhyloCSF: conservation of genomic region which reflects the coding potential. RiboPvalue: One tailed rank sum test p-value for regular riboseq frame bias inside ORF (frame test). TISPvalue: One tailed negative binomial test p-value for TISCount (TIS test). MS evidence: translation evidence from mass spectrum experiments. TISCount: Number of reads with P-site at TIS site. Kozak sequence: (GCC)GCCA/GCCATGG, emerges as the consensus sequence for initiation of translation in vertebrates. Kozak Strength: the likelyhood of an AUG initiating translation. oORF: overlapping open reading frame (with downstream gene).

Beside help page, usage introduction can also be found in each page



2、Search page: Search small proteins

Search

Search small proteins using multiple IDs or genomic location. Please use standard ID/symbol instead of unconventional synonyms to make sure the results to be found.

ID Search

Choose species and ID type

Species: Human

small protein ID
ENSEMBL Gene
Gene Symbol
NONCODE Gene

Auto-filling text as example

SPROHSA197474

Submit

Example: SmProt ID:SPROHSA197474 ENSEMB 7877 Gene Symbol:FGF7P3 NONCODE gene ID:NONDMEG000001

Location Search

Species: Human Chromosome: chr1(Start location: 61034338 Stop location: 61959438 Submit

Example: Chromosome:chr10 Start location:61034338 Stop location:61959438

Search results of ID

Species: Human StartCodon: All

Data Sources: All predicted functions: All Browse

Change items showed per page

Display 20 items per page

Page 1 Total Page: 1 Total amount: 1

Change page

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SmProt_ID	Organism	SmProt_length	Protein Sequence	Start Codon
SPROHSA197474		77	MAGVLKKTTGLVGLAVCNTPHEEPDVKKLEDQLQG...	ATG

Users can further filter the search results:

Species: Human StartCodon: All

Data Sources: All predicted functions: All Browse

Search results of location

Display 20 items per page

Page 1 Total Page: 1 Total amount: 13

download the results

first page | previous page | next page | last page TXT Excel

SmProt_ID	Organism	SmProt_length	Protein Sequence	Start Codon
SPROHSA84741		68	RDHKQQQVSVLVIFLLTGGLRARPAGSWGRRQGDV...	AGG
SPROHSA226181		80	MFVGTAADILEFTSETLEEQNVRNSPALVYAILVI...	ATG
SPROHSA283407		71	LEFTSETLEEQNVRNSPALVYAILVIWTWSMLQFP...	CTG

Click result ID to see detailed information

3、Browse page: browse small proteins

Small Proteins List

Customize conditions for screening small proteins of interest.
All results derived from ribosome profiling are totally new!
The organization structure of all data is brand new!

Browse options

Species: Human | Start Codon: ATG | Data Sources: All | Mass Spectrum: All | predicted functions: All | Browse

Species: Human, Mouse, Zebrafish, Yeast, Fruitfly, Escherichia coli, Rat, C.elegans

Data Source: All, Literature Mining, Ribosome profiling, Known Database

Start Codon: All, ATG, non-ATG, AAG, ACG, AGG, ATA, ATC, ATT, CTG, GTG, TTG

Mass Spectrum: All, Has MS evidence, No MS evidence

Predicted function: All, Have predicted functions, No predicted functions

Display 20 items per page

Page 1 Total Page: 5447 Total amount: 108931

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Click the triangle symbol to sort by ID, length, sequence...

SmProt_ID	Organism	SmProt_length	Protein Sequence	Start Codon
SPROHSA136182		65	MKKKKKKSLRSLQFQFLFHSVSQTPTHHSLENGKK...	ATG
SPROHSA136183		88	MKKNNIPEPVVIEIVWSNVMSAVEWNKREEIVAEQ...	ATG

Click result ID to see detailed information

Datailed information of browse/search results in small protein page

General Information	
Small Protein ID	SPROHSA136197
Organism	human (Homo sapiens)
Small Protein Sequence	MKKMHYVDPDHVKTYTVPLKEAGPSLLKHSVSPGTSIFKPSLFSP*
RNA Sequence	ATGAAAAAAATGCATTATGTGGACCCTGACCATGTAAAGACCTACACCGTGCCTTTAAAGGAAGCAGGGCCCTCCCTGCTGAAGCATTCAGTGAGC
Protein Length	45
Start Codon	ATG
Location	chr2:85852901-85861219:-
Blocks	85852901-85853000,85861180-85861219
Mean PhyloCSF	-8.62465220258

4、Browse Variants page: variants related to small proteins

Human 5'UTR Variants List

Now you can browse variants in 5'UTRs called from WGS data of multiple projects such as gnomAD2, gnomAD3, NyuWa, 1KGP, etc., and small proteins related to the variants, as well as vatiants in sORFs called from Ribo-seq data. The data are being updated continuously.

Select variants effect on upstream ORF

Select variants detected in WGS project or ribo-seq datasets

Variant Type: All / uAUG_gained / uSTOP_lost

Data Source: All / WGS / 1KGP3 / gnomAD3 / TOPMed / GAsP / NyuWa / Ribosome profiling

Browse

Result for Variants: All All

Display 20 items per page

Page 1 Total Page: 2191 Total amount: 43815

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Variant	Effect	Gene	Distance to CDS	Variant Type	Clinvar
9-76394190-A-C	CDS_elongated	RFK	21	uAUG_gained	.
19-43934947-T-G	uORF_elongated	ZNF45	.	uSTOP_lost	.

Click result ID to see detailed information

Specific Information of Variant: 9-76394190-A-C

General Information	
Variant ID	9-76394190-A-C
Genome Position	chr9:76394190
ClinVar	.

Related Small Proteins

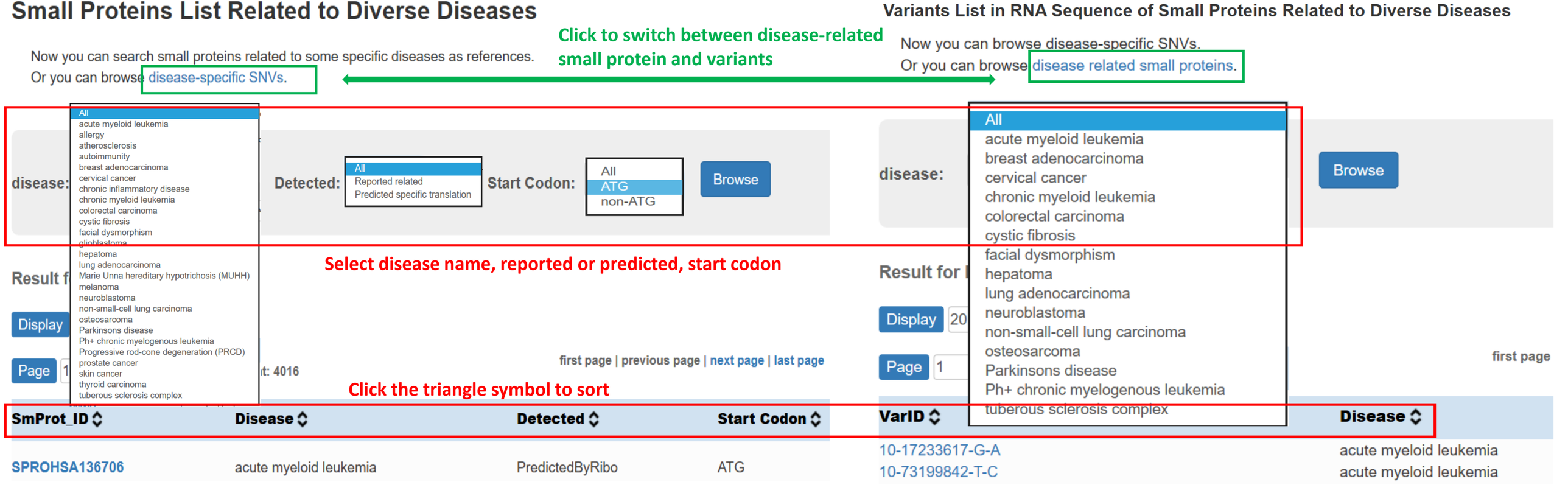
ID	Length	Start Codon	Strand	Blocks	Consequence
SPROHSA51610	35	ACG	-	76394167-76394275	Non-Synonymous p.F29C
SPROHSA67636	16	AGG	-	76394167-76394218	Non-Synonymous p.F10C

Data sources

Source	Allele Count	Allele Frequency
gnomAD3	143170	9.98730e-01
1KGP3	5005	0.999401
TOPMed	125408	0.998726

5、Disease page: browse small proteins or variants on small proteins related to diseases

Disease-specific translation events and variants in small proteins predicted from ribosome profiling data are provided (confidence: predicted specific), as well as disease-related small proteins reported in literature (confidence: reported related)



6、HumanMicroBio page: browse small proteins of Human Microbiomes

Small Proteins of Human Microbiomes (Body Site: All)

Now you can browse small proteins related to Human Microbiomes identified by
Large-Scale Analyses of Human Microbiomes Reveal Thousands of Small, Novel Genes. Cell. 2019 Aug 6.

Select to get small proteins of human microbiomes in corresponding body site

Body Site: All / mouth / skin / vagina / gut / non-human environments — Browse

Display 20 items per page

Page 1 Total Page: 227 Total amount: 4539

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Family ID	NO. Members	Length of peptide	AA sequence of clusters representative
0	25	50	MKTLYVYADFDWIEETELVGELGYESLRSSDCYCFTLNDGCFEKNTEICF
10	2455	50	MKRIKTIETRDLKKSVKTGGCGECQTSCQSACKTSCGVANQQCEKCMSEK

Click on family ID to see detailed information of small proteins in this family

Detail Information of small proteins related to Human Microbiomes for Family ID: 10

General Information	
Family ID	10
No. Members	2455
Small Protein Length	50
Representative AA Seq (detail)	MKRIKTIETRDLKKSVKTGGCGECQTSCQSACKTSCGVANQQCEKCMSEK
Representative DNA Seq (detail)	ATGAAGAGAATTAAAACTATCGAAACTCGTGACCTCAAGAAGAGCGTTAAGACAGGCGGTTGCGGCGAGTGCCAGA
Example of a Refseq homolog	WP_042682158.1
Associated HGT genes	HTH_MerR-trunc, Tn916-Xis, SR_IS607_transposase_like, INT_C_like_4, Y1_Tnp, PRK09871, INT_ICEBs1_C_like, R INT_StrepXerD_C_like, INT_RitA_C_like, SR_Res_par, INT_Lambda_C, RecA-like_NTPases, PHA02517, HTH_28, re T_den_put_tspse, INT_RitC_C_like, SR_ResInv, INT_C_like_5, int, MULE, INT_C_like_3, RecA, PRK09409, HTH_Hin Integrase_DNA, PinE, INT_Rci_Hp1_C, INT_C_like_6, INT_IntI_C, INT_C_like_1, PRK15417, Phage_integrase, integr
Number of non bacterial	1
Non bacterial classification_numbe of members that were classified to it	cellular organisms_3
RNA code p-value igned to family	5.73E-11
number of species	192

7、Blast page: BLAST small proteins in SmProt similar with the input sequence

Blast in SmProt database

Use this to assess sequence similarity of small proteins in multiple species.

All new small proteins were added to the blast database, including human microbiomes.

Choose program to use and database to search:

Program blastp means from protein to protein, blastx means from translated nucleotide to protein

Program: blastp Database: SmProt_All

Enter sequence below in FASTA format Example sequence

```
>example sequence
MSGKSWVLISTTSPQSLEDEILGRLLKILFVLFVDLMSIMYVVI
TS
```

Input sequence or upload sequence in text files

Or load it from disk Browse

Set subsequence: From To

Clear sequence Search

Click here to get BLAST results

Sequences producing significant alignments:

	Score (bits)	E Value
SPROHSA409509	90	9e-19
SPROMMU172247	90	9e-19
SPROMMU95127	90	9e-19
SPROHSA235457	79	1e-15
SPROHSA149855	79	1e-15
SPROMMU95126	57	5e-09
SPROCEL7466	27	5.3

Results with similar sequences to the input one, and respective scores of similarity

```
>SPROHSA409509
          Length = 46

 Score = 89.7 bits (221), Expect = 9e-19
 Identities = 46/46 (100%), Positives = 46/46 (100%)

Query: 1  MSGKSWVLISTTSPQSLEDEILGRLLKILFVLFVDLMSIMYVVITS 46
          MSGKSWVLISTTSPQSLEDEILGRLLKILFVLFVDLMSIMYVVITS
Sbjct: 1  MSGKSWVLISTTSPQSLEDEILGRLLKILFVLFVDLMSIMYVVITS 46
```

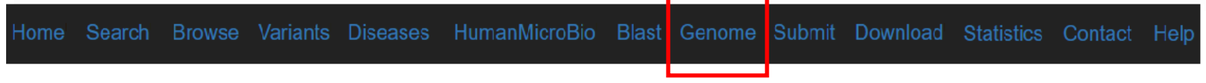
Detailed information of results

```
>SPROMMU172247
          Length = 60

 Score = 89.7 bits (221), Expect = 9e-19
 Identities = 46/46 (100%), Positives = 46/46 (100%)

Query: 1  MSGKSWVLISTTSPQSLEDEILGRLLKILFVLFVDLMSIMYVVITS 46
          MSGKSWVLISTTSPQSLEDEILGRLLKILFVLFVDLMSIMYVVITS
Sbjct: 15 MSGKSWVLISTTSPQSLEDEILGRLLKILFVLFVDLMSIMYVVITS 60
```

8、Browse page: browse small proteins



Click Genome Button on Navigation Bar, or location link in General Information table in any small protein page and variant page, to jump to Genome browser to check small proteins on a genomic region.

General Information	
Small Protein ID	SPROHSA136183
Organism	human (Homo sapiens)
Small Protein Sequence	MKKNNIPEPVVIEIVWSNVMSAVEWNKREEIVAEQAIKHLKQHSPLLAAFTTQSQSELTLLLKIQEYCYDNIHFMKAFRKIVVLFIKL*
RNA Sequence	ATGAAAAAAAACAACATCCCAGAACCAGTTGTCATCGAAATAGTCTGGTCAAATGTAATGAGCGCTGTGGAATGGAACAAAAG
Protein Length	88
Start Codon	ATG
Location	chr3:172425991-172426258:-
Blocks	172425991-172426258