北京大學
PEKING UNIVERSITY

Evolution and Function of SCN5A

Structural, Functional, and Evolutionary Comprehensive Analysis of Vertebrate Voltage-Gated Sodium Channel Sequences

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负责部分：进化树构建，Dotplot分析

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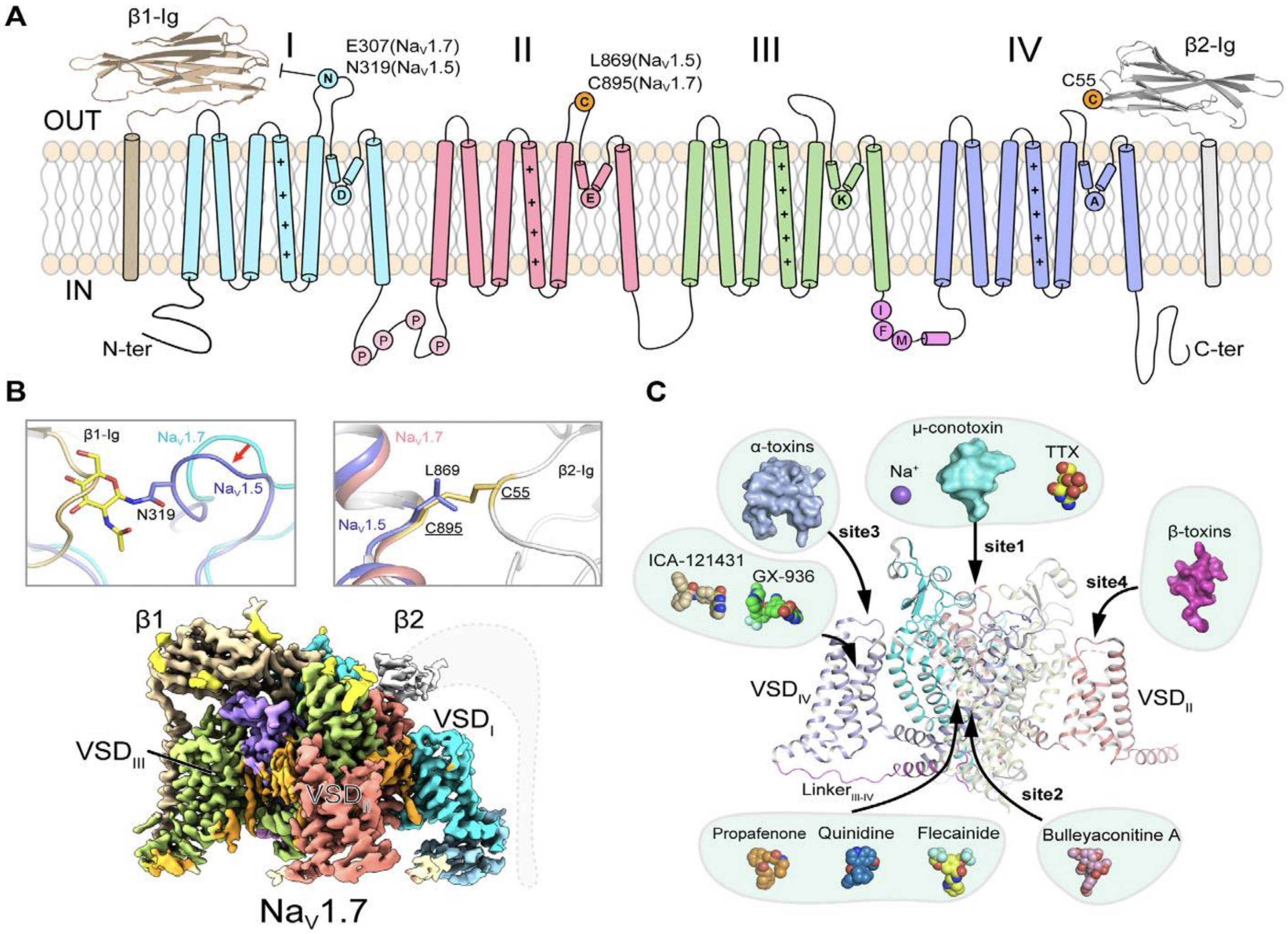
背景介绍

电压门控钠通道（Voltage-gated sodium channels, Nav）是一类整合膜蛋白，在响应去极化刺激时选择性地传导钠离子穿过细胞膜。自1984年Noda首次克隆Nav通道基因以来，人类已鉴定出九种高度保守的Nav通道亚型（**Nav1.1–Nav1.9**）。

后生动物的Nav通道由一个大的成孔**α**亚基和一到两个辅助**β**亚基（**β1–β4**）组成。**α**亚基是Nav通道的核心功能单元，由**24**个跨膜螺旋组成，分为四个同源结构域（**DI–DIV**）。



哺乳动物Nav通道的拓扑结构与三维构架

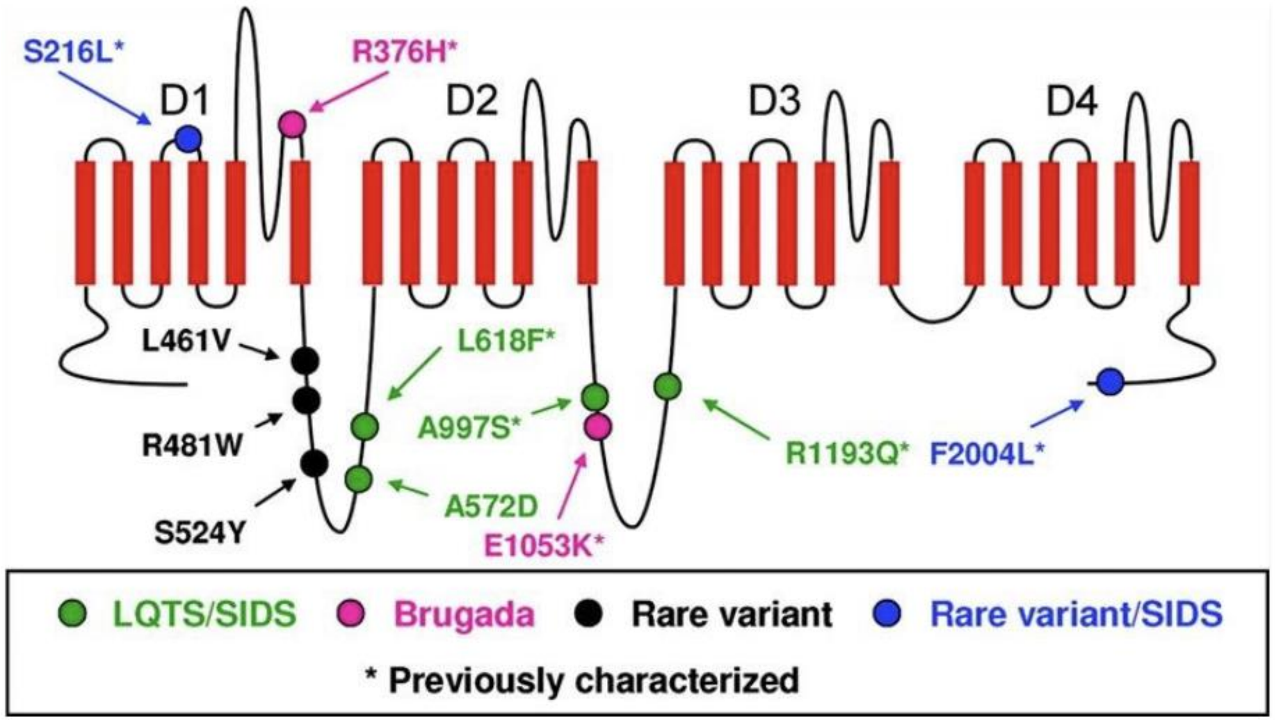
背景介绍

在Nav中，是否在不同物种中存在保守性？

比如，研究表明，Nav通道广泛分布于真核生物、原核生物以及海洋单细胞浮游植物中，凸显了Nav通道的进化保守性；

同时，钠通道与钾通道的氨基酸序列有显著差异，但钠通道的P-loop在不同物种和组织特异性亚型之间高度保守……

"Although the amino acid sequences of the Na and K channels are not highly homologous, hydrophobicity analysis suggests a topological analogy." — ***Balser, 1999***



罕见且与疾病相关的SCN5A变异在房颤患者中的分布

背景介绍

点突变研究也揭示了Nav通道钠离子选择性的分子基础——**DEKA**基序：

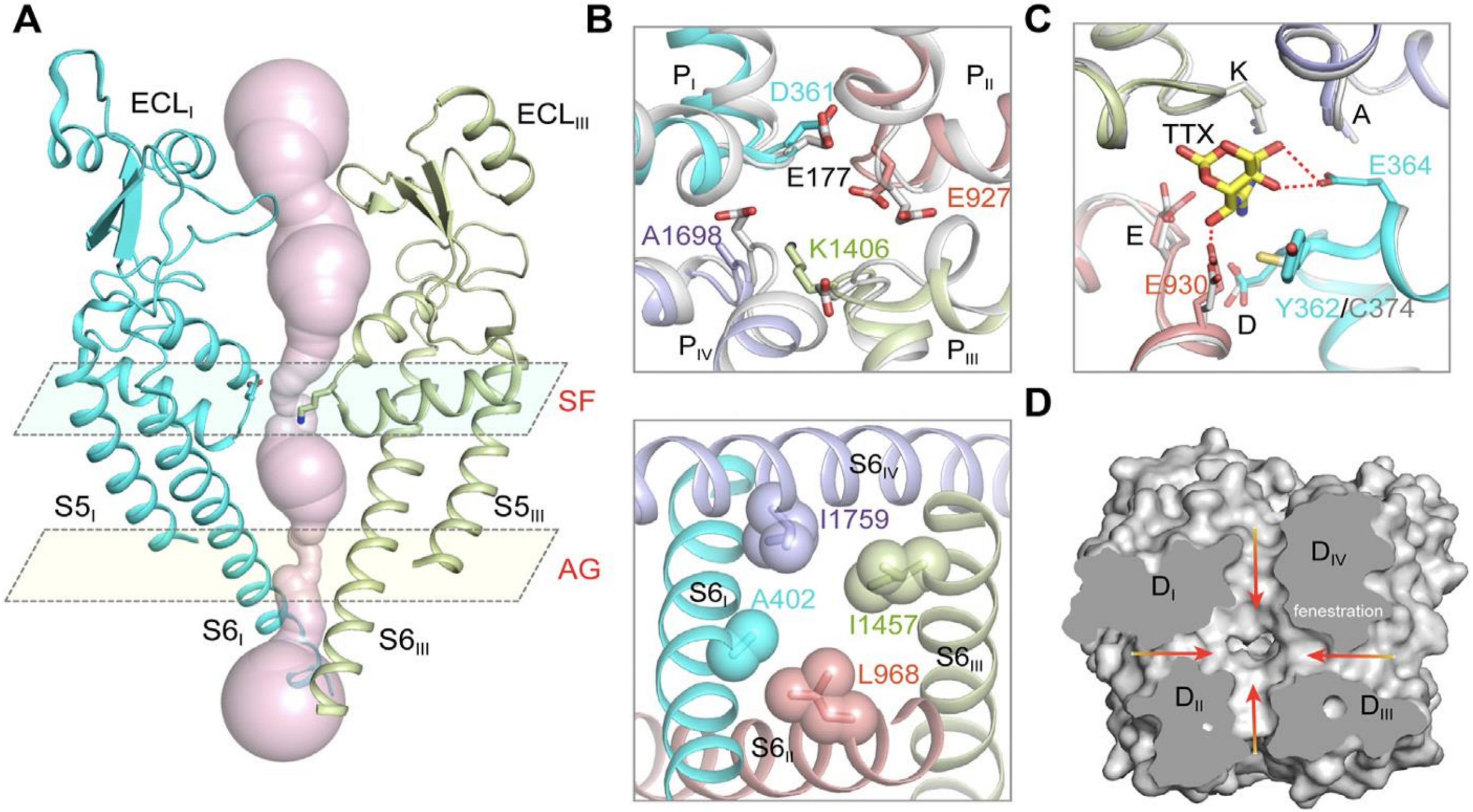
DI：天冬氨酸（Asp, D）

DII：谷氨酸（Glu, E）

DIII：赖氨酸（Lys, K）

DIV：丙氨酸（Ala, A）

DEKA基序中的四个氨基酸残基如何协同工作，形成一个结构上精确的钠离子选择性滤器，确保Nav通道的钠离子选择性和功能保守性。



哺乳动物Nav通道的离子通路

背景介绍

近年来，冷冻电镜（cryo-EM）技术的突破使得高分辨率解析真核Nav通道结构成为可能。2017年，颜宁实验室报道了美洲蟑螂Nav通道（NaVPas）的突破性高分辨率cryo-EM结构。随后，Nav1.1至Nav1.7等多个人类Nav亚型的结构相继被解析。

这些高分辨率结构一致表明：

整体结构保守：Nav通道的整体结构在不同亚型间高度保守

跨膜核心区保守：与细菌Nav通道高度相似，以结构域交换方式形成

功能基序保守：DEKA选择性滤器、IFM快速失活门、S4门控电荷等功能基序一致

配体结合位点保守：TTX结合位点、抗心律失常药物结合位点等在亚型间保守

探讨核心问题：**Nav/SCN 蛋白在不同脊椎动物中是否同源？其四重复结构域（DI–DIV）与跨膜拓扑是否高度保守？这种保守性如何在序列、进化和结构层面形成证据链？**

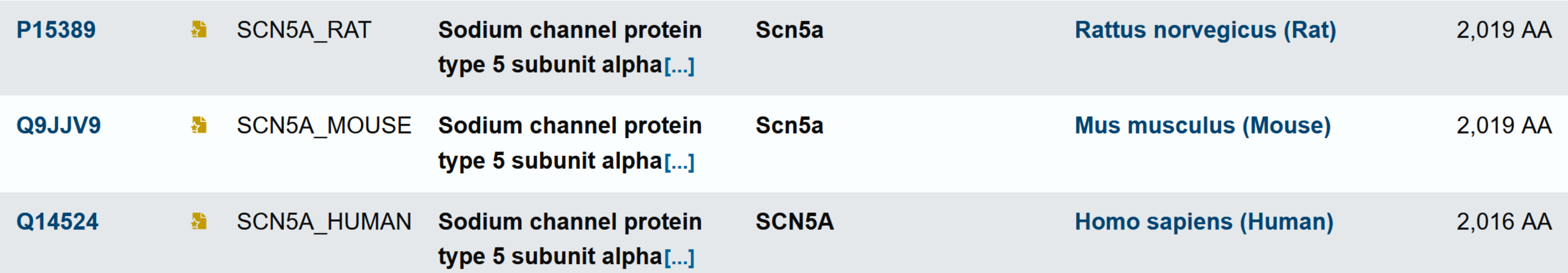
CONTENTS

进化分析

序列筛选——Reviewd序列，SCN5A序列，非近缘关系序列

只有三个物种的序列信息，序列数目不足

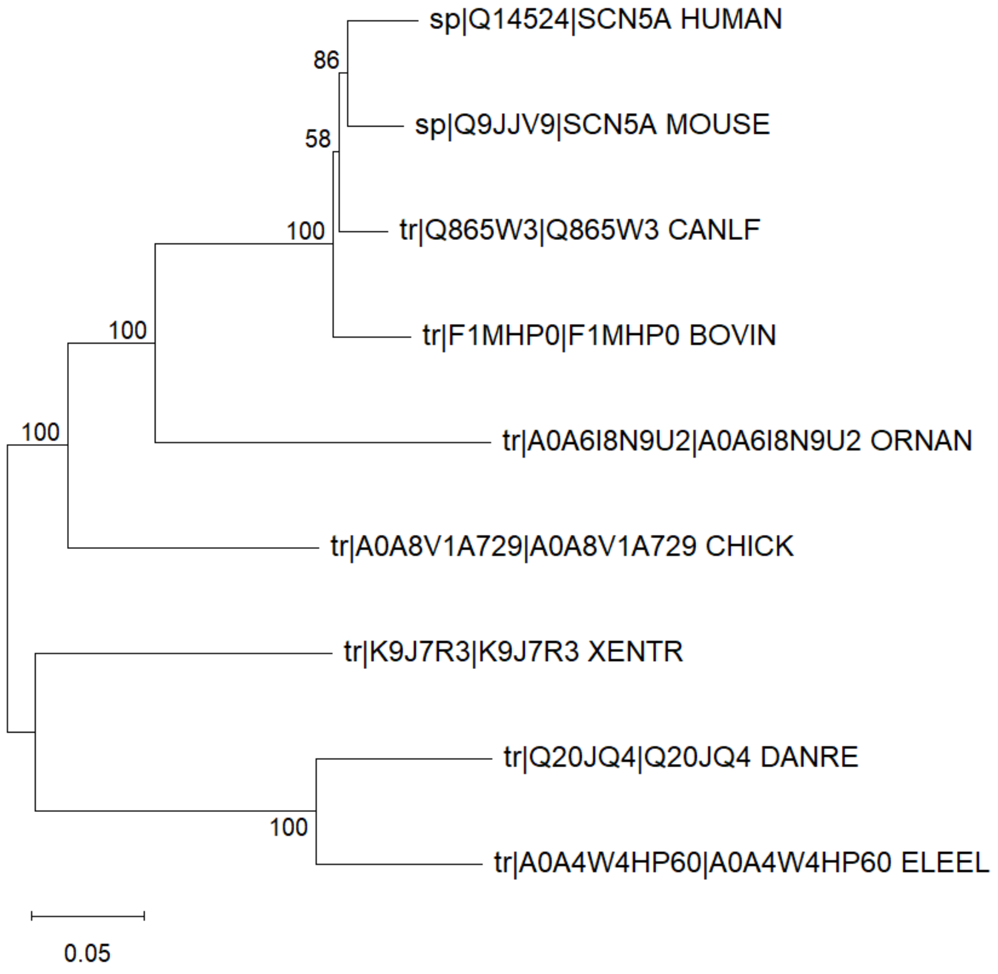
- 人类：Homo sapiens(Human)
- 小鼠：Mus musculus(Mouse)
- 大鼠：Rattus norvegicus(Rat)



P15389		SCN5A_RAT	**Sodium channel protein type 5 subunit alpha[...]**	**Scn5a**	Rattus norvegicus (Rat)	2,019 AA
Q9JJV9		SCN5A_MOUSE	**Sodium channel protein type 5 subunit alpha[...]**	**Scn5a**	Mus musculus (Mouse)	2,019 AA
Q14524		SCN5A_HUMAN	**Sodium channel protein type 5 subunit alpha[...]**	**SCN5A**	Homo sapiens (Human)	2,016 AA

进化分析

增添非Reviewed：物种被错误分类，序列质量不足



关键的不合理之处：

1. 鸭嘴兽位置错误（核心问题）

鸭嘴兽是单孔目哺乳动物，是现存最原始的哺乳动物，正确位置：应该在所有有胎盘哺乳动物（人、鼠、狗、牛）的基部，而实际位置插在有胎盘哺乳动物和鸟类之间，不正确。

2. 鱼类位置错误

象鲨（软骨鱼）应该是所有其他脊椎动物的外群，而实际位置与斑马鱼（硬骨鱼）聚类在一起，不正确

3. 鸟类位置可疑

鸡（鸟类）出现在奇怪的位置，不与任何类群紧密聚类

4. 哺乳动物内部关系可疑

狗和牛的位置可能不对

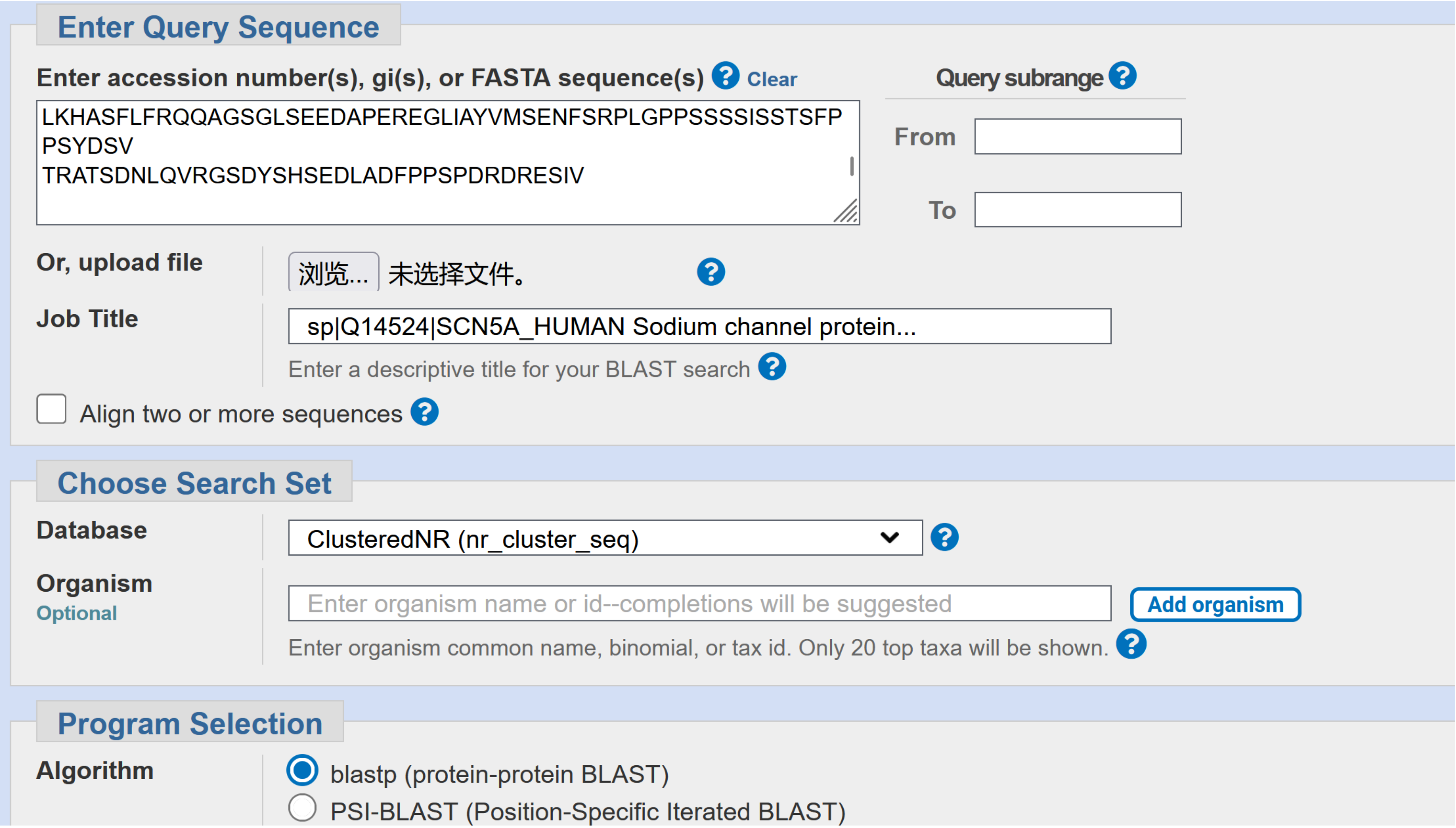
进化分析

序列筛选——Reviewd序列，Nav序列

物种	物种(英文)	UniProt ID	Entry Name	蛋白名称	选择理由
人	Homo sapiens	Q14524	SCN5A_HUMAN	Sodium channel protein type 5 subunit alpha	研究主对象SCN5A
小鼠	Mus musculus	Q9JJV9	SCN5A_MOUSE	Sodium channel protein type 5 subunit alpha	研究主对象SCN5A
大鼠	Rattus norvegicus	P15389	SCN5A_RAT	Sodium channel protein type 5 subunit alpha	研究主对象SCN5A
兔	Oryctolagus cuniculus	Q28644	SCN9A_RABIT	Sodium channel protein type 9 subunit alpha	该物种唯一可用序列
河豚	Takifugu rubripes	Q2XVR7	SC4AA_TAKRU	Sodium channel protein type 4 subunit alpha A	该物种唯一可用序列
绿河豚	Tetraodon nigroviridis	Q2XVR5	SC4AA_TETNG	Sodium channel protein type 4 subunit alpha A	该物种唯一可用序列
斑马鱼	Danio rerio	Q2XVR3	SC4AA_DANRE	Sodium channel protein type 4 subunit alpha A	选择A型（主要型）
马	Equus caballus	Q28371	SCN4A_HORSE	Sodium channel protein type 4 subunit alpha	该物种唯一可用序列

进化分析

BLAST：人源SCN5A序列设置



Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) ? Clear

LKHASFLFRQQAGSGLSEEDAPEREGLIAYVMSENFSRPLGPPSSSSISSTSFP
PSYDSV
TRATSDNLQVRGSDYSHSEDLADFPPSPDRDRESIV

Query subrange ?

From:

To:

Or, upload file: 浏览... 未选择文件。 ?

Job Title: sp|Q14524|SCN5A_HUMAN Sodium channel protein...

Enter a descriptive title for your BLAST search ?

☐ Align two or more sequences ?

Choose Search Set

Database: ClusteredNR (nr_cluster_seq) ?

Organism Optional: Enter organism name or id--completions will be suggested   Add organism

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown. ?

Program Selection

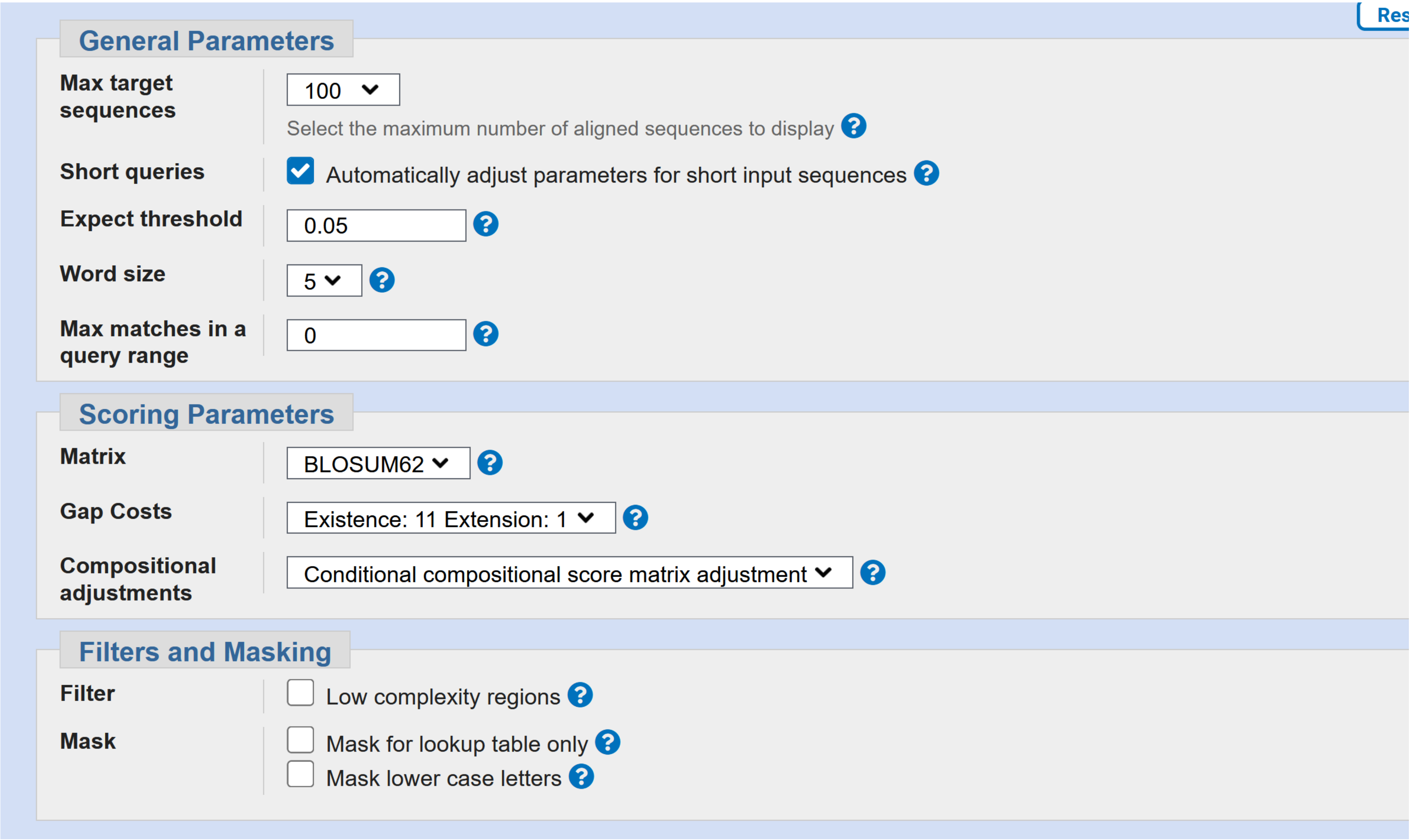
Algorithm:

● blastp (protein-protein BLAST)

○ PSI-BLAST (Position-Specific Iterated BLAST)

进化分析

BLAST：人源SCN5A序列设置



General Parameters

Max target sequences: 100
Select the maximum number of aligned sequences to display

Short queries: ☑ Automatically adjust parameters for short input sequences

Expect threshold: 0.05

Word size: 5

Max matches in a query range: 0

Scoring Parameters

Matrix: BLOSUM62

Gap Costs: Existence: 11 Extension: 1

Compositional adjustments: Conditional compositional score matrix adjustment

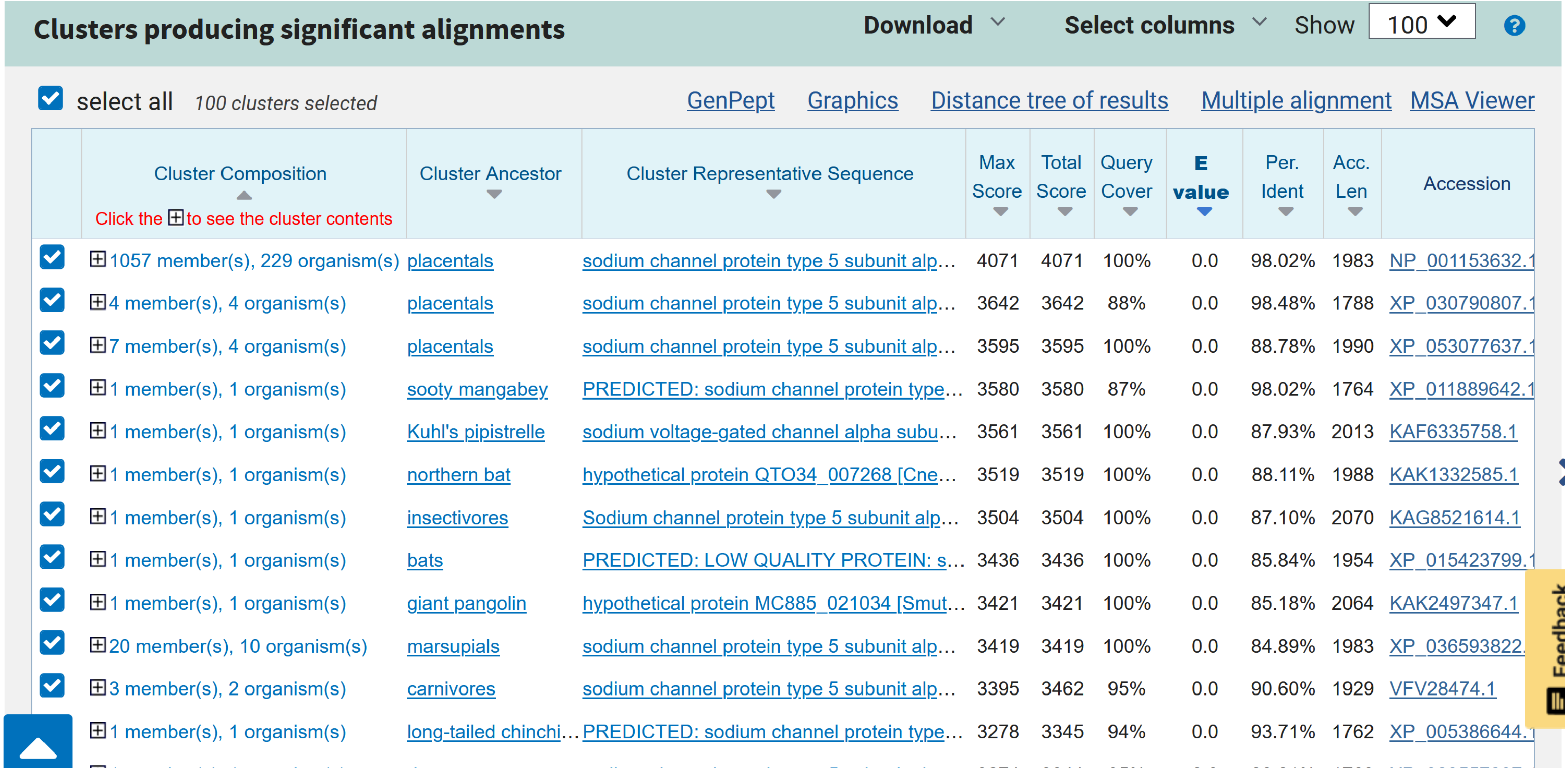
Filters and Masking

Filter: ☐ Low complexity regions

Mask: ☐ Mask for lookup table only
☐ Mask lower case letters

进化分析

BLAST结果



Clusters producing significant alignments

☑ select all *100 clusters selected*

	Cluster Composition Click the ⊞ to see the cluster contents	Cluster Ancestor	Cluster Representative Sequence	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	⊞1057 member(s), 229 organism(s)	placentals	sodium channel protein type 5 subunit alp…	4071	4071	100%	0.0	98.02%	1983	NP_001153632.1
☑	⊞4 member(s), 4 organism(s)	placentals	sodium channel protein type 5 subunit alp…	3642	3642	88%	0.0	98.48%	1788	XP_030790807.1
☑	⊞7 member(s), 4 organism(s)	placentals	sodium channel protein type 5 subunit alp…	3595	3595	100%	0.0	88.78%	1990	XP_053077637.1
☑	⊞1 member(s), 1 organism(s)	sooty mangabey	PREDICTED: sodium channel protein type…	3580	3580	87%	0.0	98.02%	1764	XP_011889642.1
☑	⊞1 member(s), 1 organism(s)	Kuhl's pipistrelle	sodium voltage-gated channel alpha subu…	3561	3561	100%	0.0	87.93%	2013	KAF6335758.1
☑	⊞1 member(s), 1 organism(s)	northern bat	hypothetical protein QTO34_007268 [Cne…	3519	3519	100%	0.0	88.11%	1988	KAK1332585.1
☑	⊞1 member(s), 1 organism(s)	insectivores	Sodium channel protein type 5 subunit alp…	3504	3504	100%	0.0	87.10%	2070	KAG8521614.1
☑	⊞1 member(s), 1 organism(s)	bats	PREDICTED: LOW QUALITY PROTEIN: s…	3436	3436	100%	0.0	85.84%	1954	XP_015423799.1
☑	⊞1 member(s), 1 organism(s)	giant pangolin	hypothetical protein MC885_021034 [Smut…	3421	3421	100%	0.0	85.18%	2064	KAK2497347.1
☑	⊞20 member(s), 10 organism(s)	marsupials	sodium channel protein type 5 subunit alp…	3419	3419	100%	0.0	84.89%	1983	XP_036593822.
☑	⊞3 member(s), 2 organism(s)	carnivores	sodium channel protein type 5 subunit alp…	3395	3462	95%	0.0	90.60%	1929	VFV28474.1
	⊞1 member(s), 1 organism(s)	long-tailed chinchi…	PREDICTED: sodium channel protein type…	3278	3345	94%	0.0	93.71%	1762	XP_005386644.

进化分析

结果分析

1. SCN5A蛋白高度保守

所有比对结果的E值都是**0.0**，表明这些匹配在统计学上极其显著，不是随机产生的。序列相似度普遍在**85%-98%**之间，说明该蛋白在哺乳动物中高度保守。

2. 进化关系的体现

从结果可以看到清晰的进化层次：有胎盘哺乳动物（placentals）相似度最高，约98%，有袋类动物（marsupials）相似度稍低，约84.89%，这符合哺乳动物的系统发育关系

3. 功能重要性的暗示

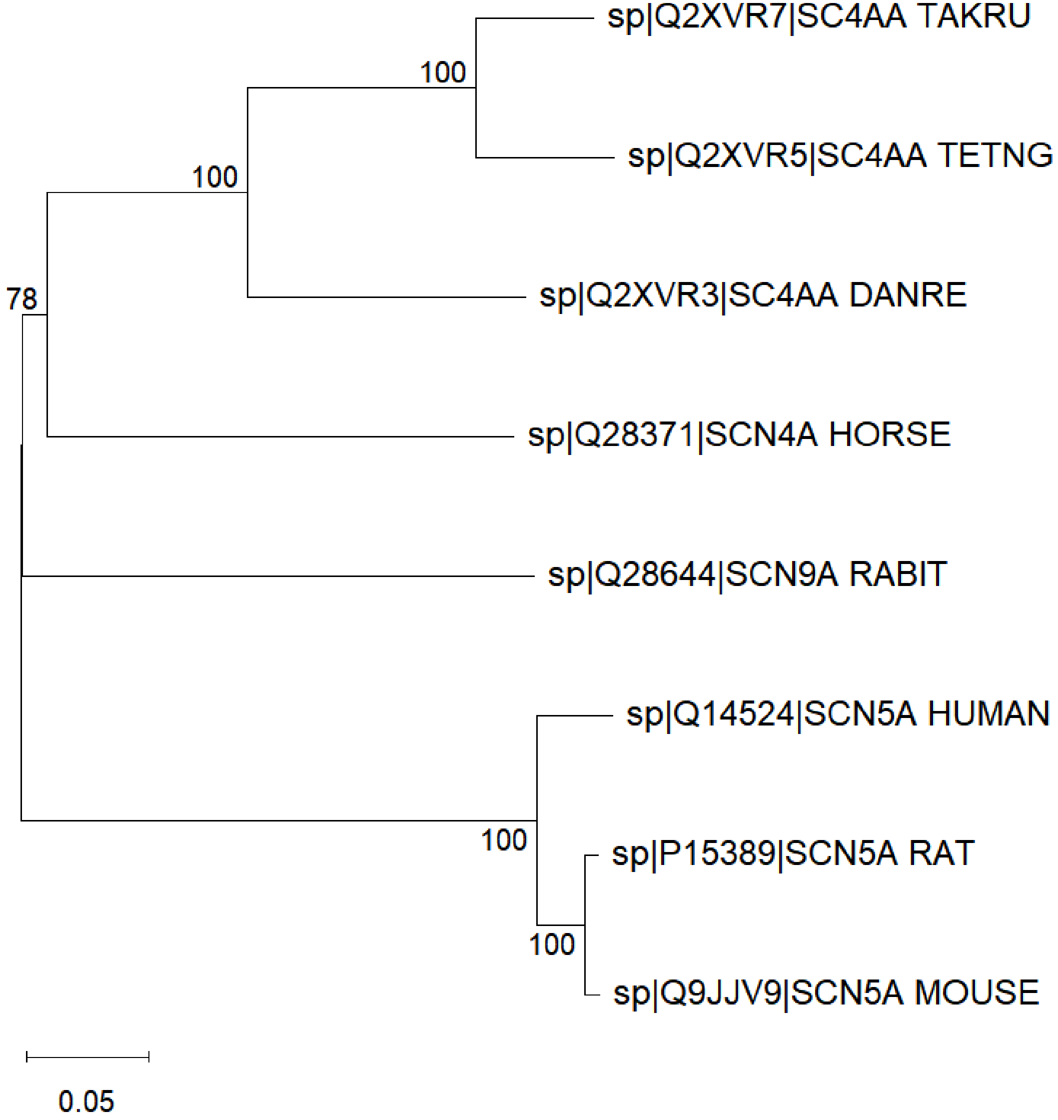
蛋白质越保守，通常意味着其功能越重要、越不能容忍突变。SCN5A是心肌和神经系统中关键的电压门控钠通道，突变会导致严重疾病（如Brugada综合征、长QT综合征），所以进化压力使其保持高度保守。

4. 物种覆盖广泛

结果涵盖了多种哺乳动物类群：灵长类、蝙蝠、食虫目、穿山甲、有袋类、食肉目等，说明这个蛋白在哺乳动物中普遍存在且功能一致。

进化分析

进化树



- 电压门控钠通道三个亚型（SCN4A、SCN5A、SCN9A）各自**独立形成进化支**
- 相同功能亚型的序列高度保守，聚类节点的支持率均为**100%**，证明其分类可靠。
- 不同亚型之间的遗传距离较远，反映了它们在脊椎动物中经历了早期的基因复制与功能分化。

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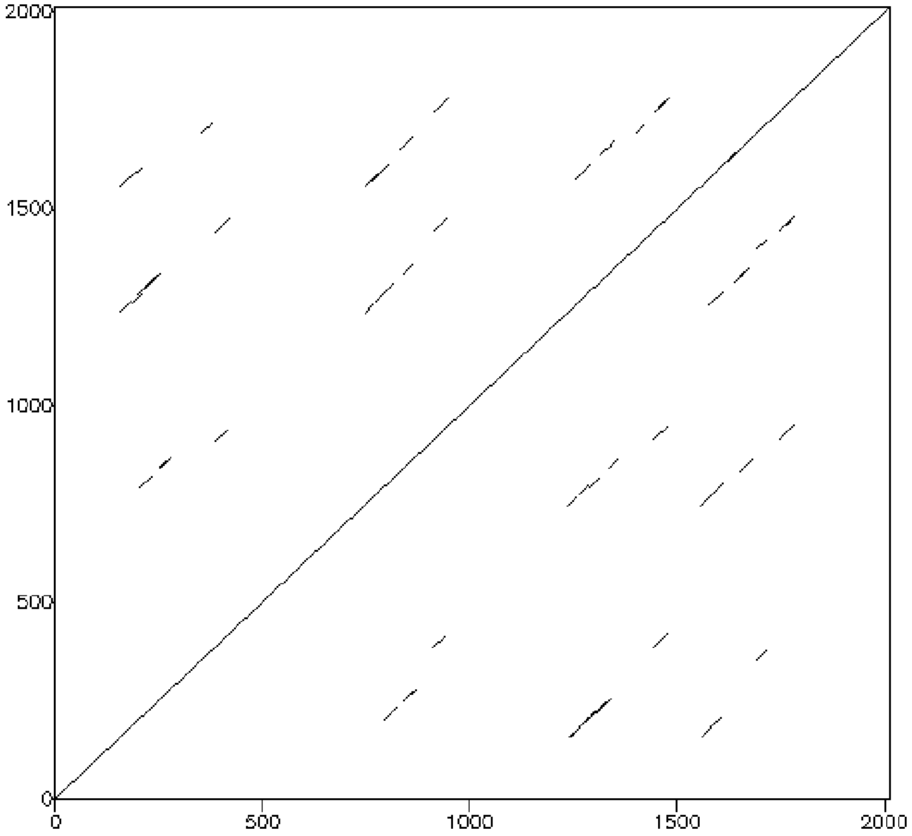
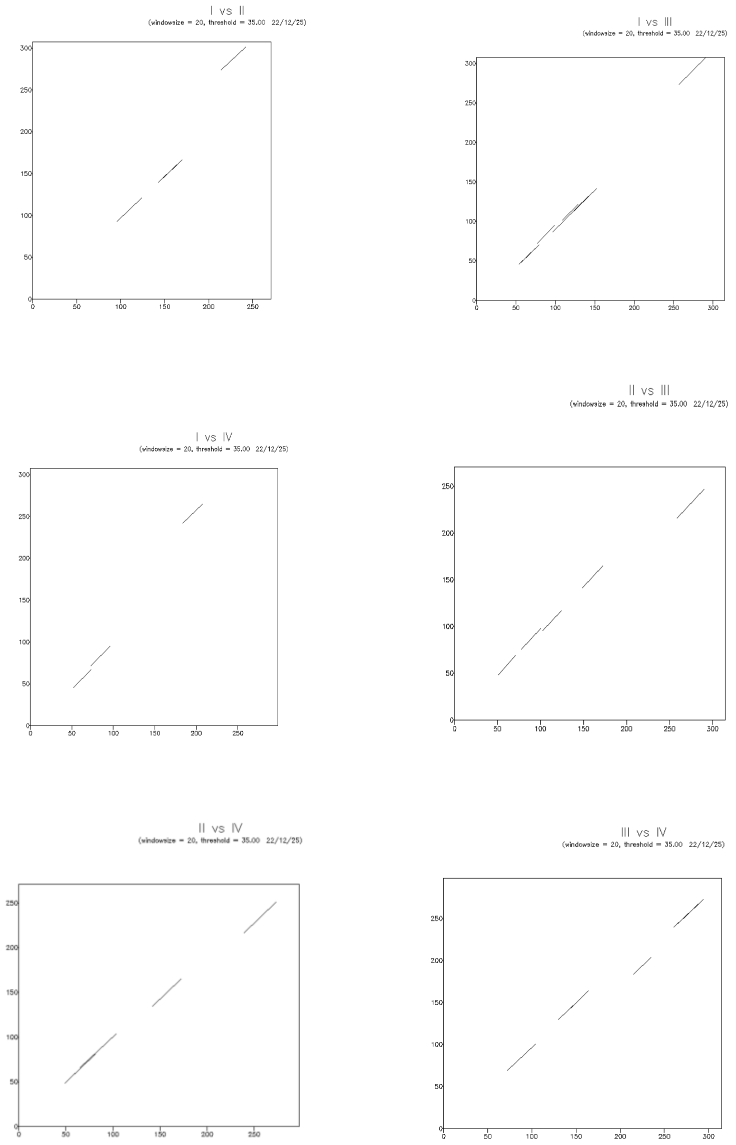
功能&结构分析

自比对/分段比对Dotplot示意图



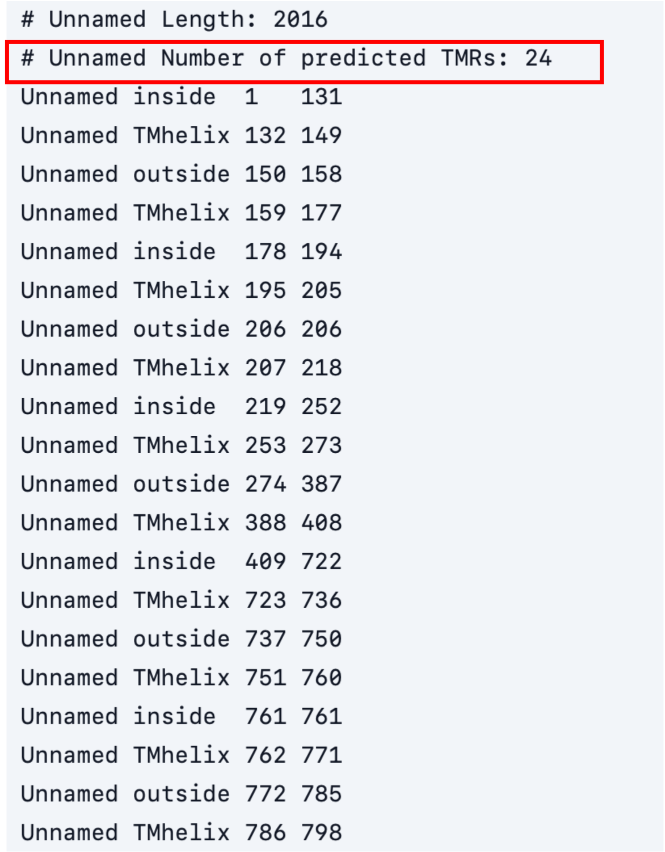
SCN5A_HUMAN 的自比对dotplot示意图



DI~DVI 的分段对比示意图

功能&结构分析

DeepTMHMM分析



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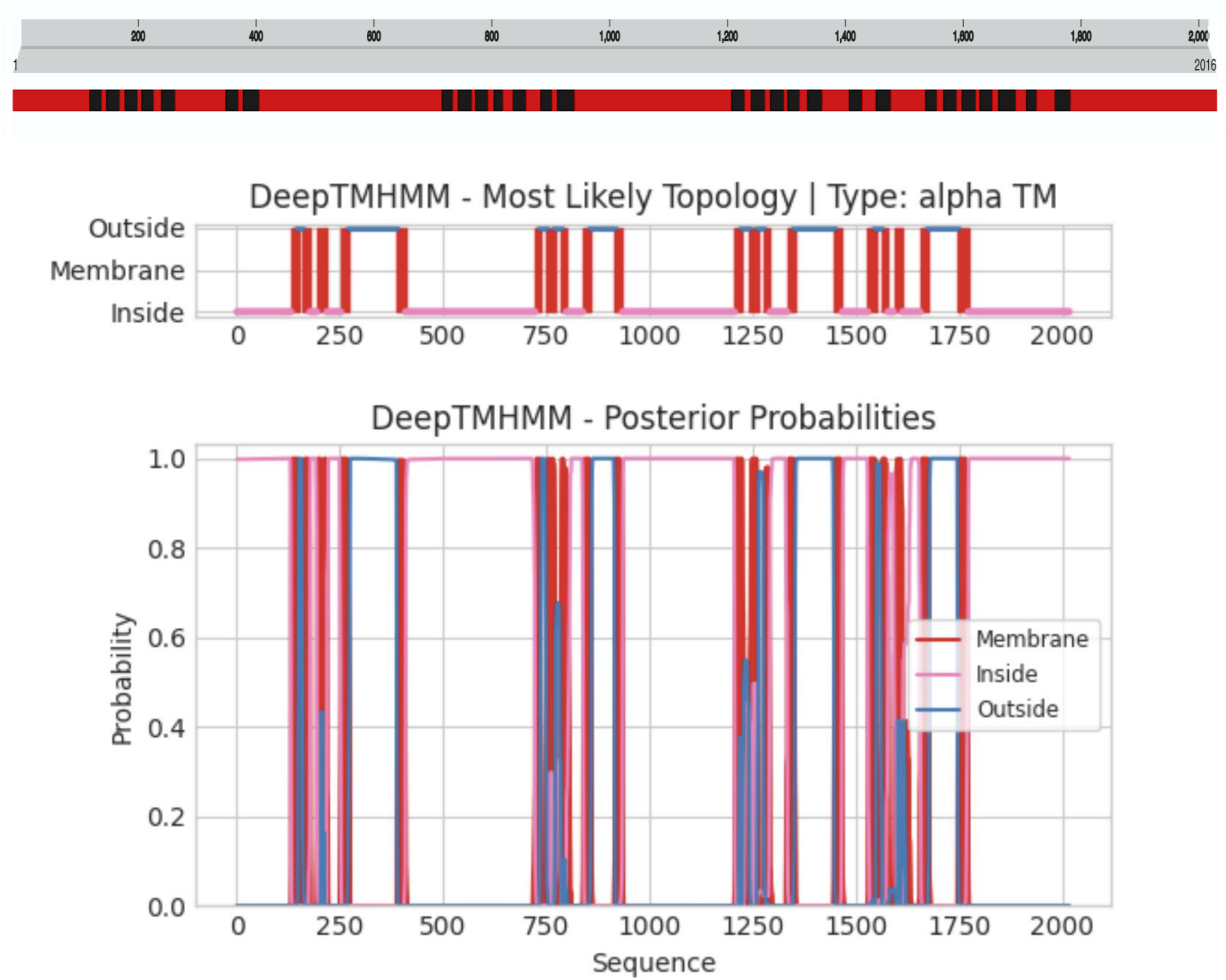
# Unnamed Length: 2016
# Unnamed Number of predicted TMRs: 24
Unnamed inside  1   131
Unnamed TMhelix 132 149
Unnamed outside 150 158
Unnamed TMhelix 159 177
Unnamed inside  178 194
Unnamed TMhelix 195 205
Unnamed outside 206 206
Unnamed TMhelix 207 218
Unnamed inside  219 252
Unnamed TMhelix 253 273
Unnamed outside 274 387
Unnamed TMhelix 388 408
Unnamed inside  409 722
Unnamed TMhelix 723 736
Unnamed outside 737 750
Unnamed TMhelix 751 760
Unnamed inside  761 761
Unnamed TMhelix 762 771
Unnamed outside 772 785
Unnamed TMhelix 786 798
```

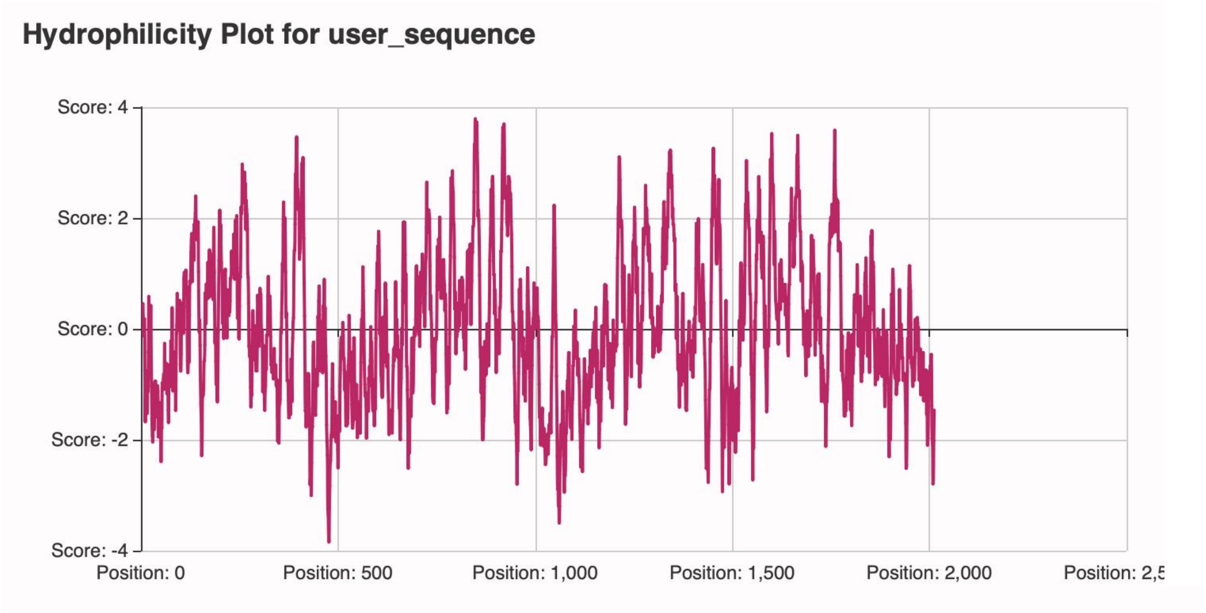


DeepTMHMM相关的跨膜螺旋数据

功能&结构分析

DeepTMHMM

Novo Pro分析得到跨膜蛋白亲疏水分析结果，与跨膜蛋白四簇结构相匹配



亲疏水得分图

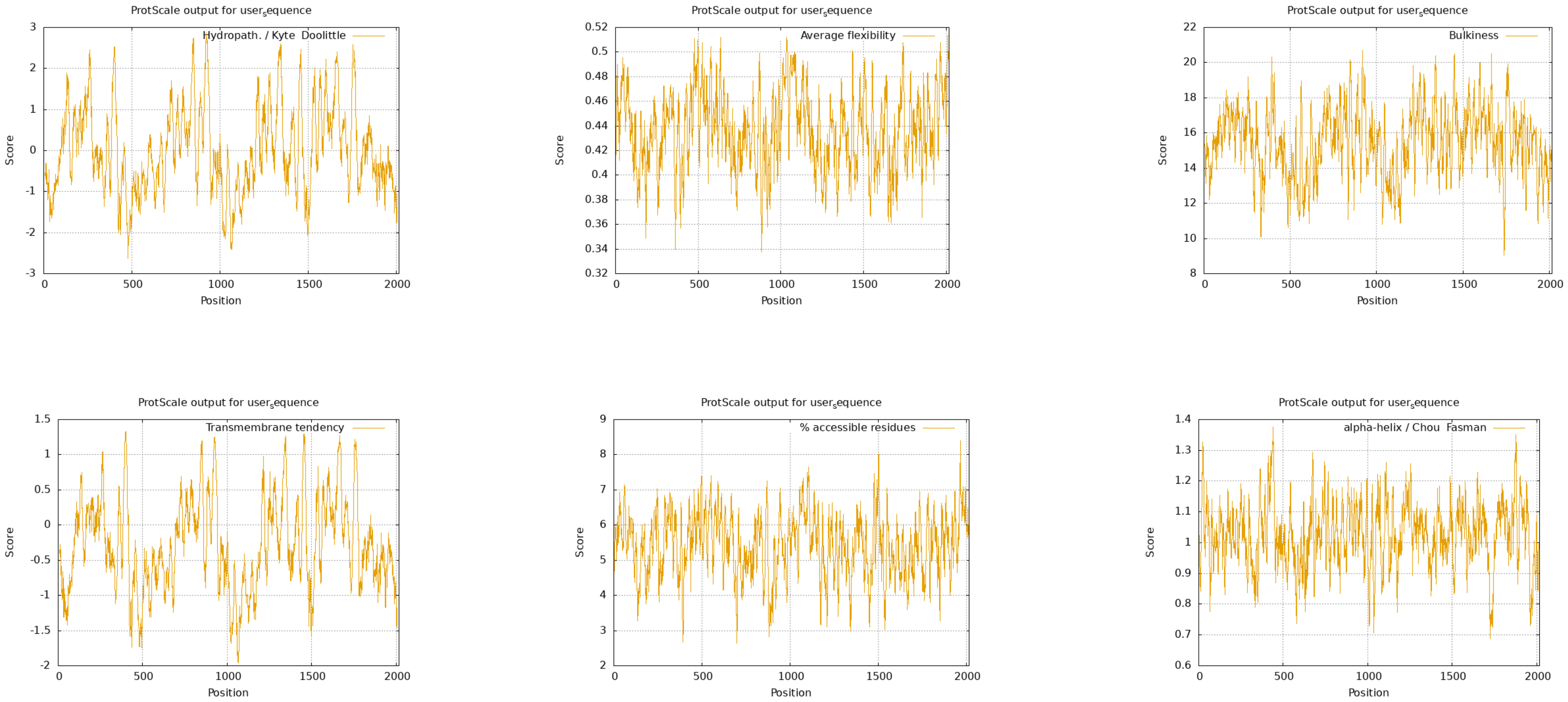

Amino Acid Hydropathy Scores

Amino Acid	One Letter Code	Hydropathy Score
Isoleucine	I	4.5
Valine	V	4.2
Leucine	L	3.8
Phenylalanine	F	2.8
Cysteine	C	2.5
Methionine	M	1.9
Alanine	A	1.8
Glycine	G	-0.4
Threonine	T	-0.7
Serine	S	-0.8
Tryptophan	W	-0.9
Tyrosine	Y	-1.3
Proline	P	-1.6
Histidine	H	-3.2
Glutamic acid	E	-3.5
Glutamine	Q	-3.5
Aspartic acid	D	-3.5
Asparagine	N	-3.5
Lysine	K	-3.9
Arginine	R	-4.5

亲疏水蛋白打分表

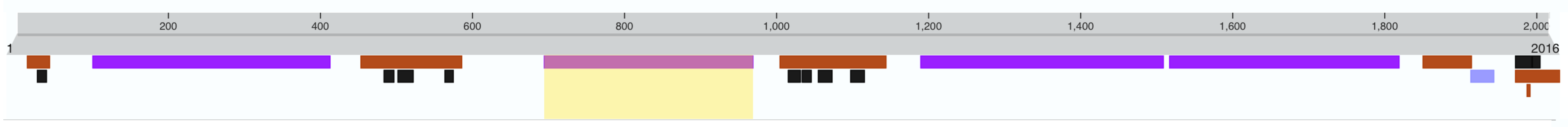
功能&结构分析

Protscale

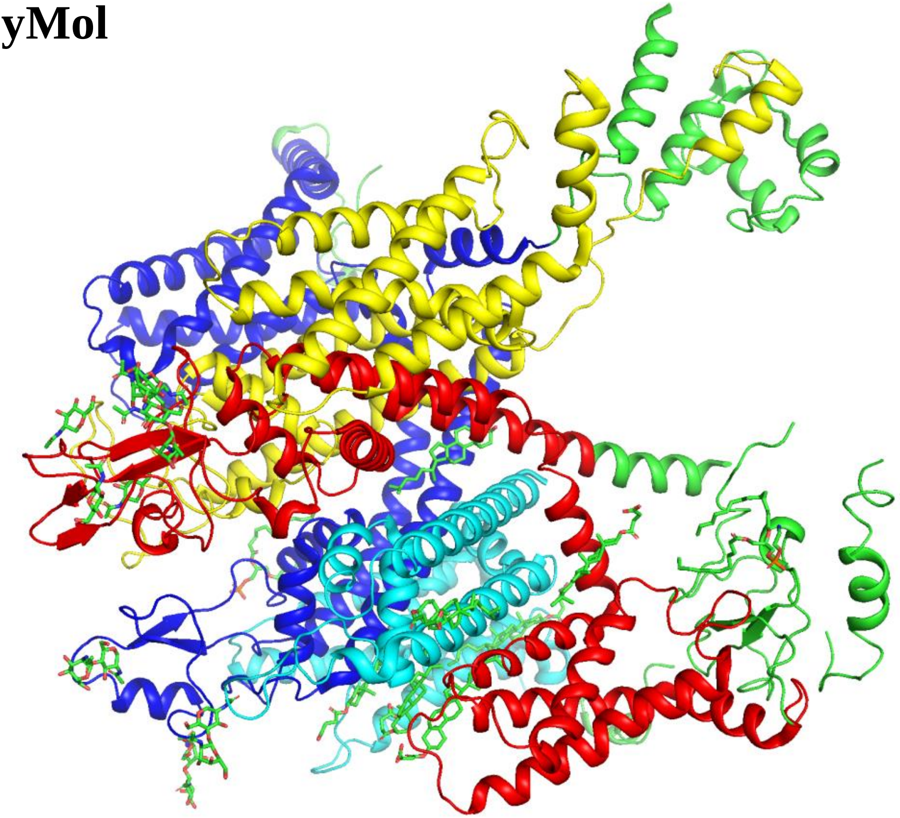


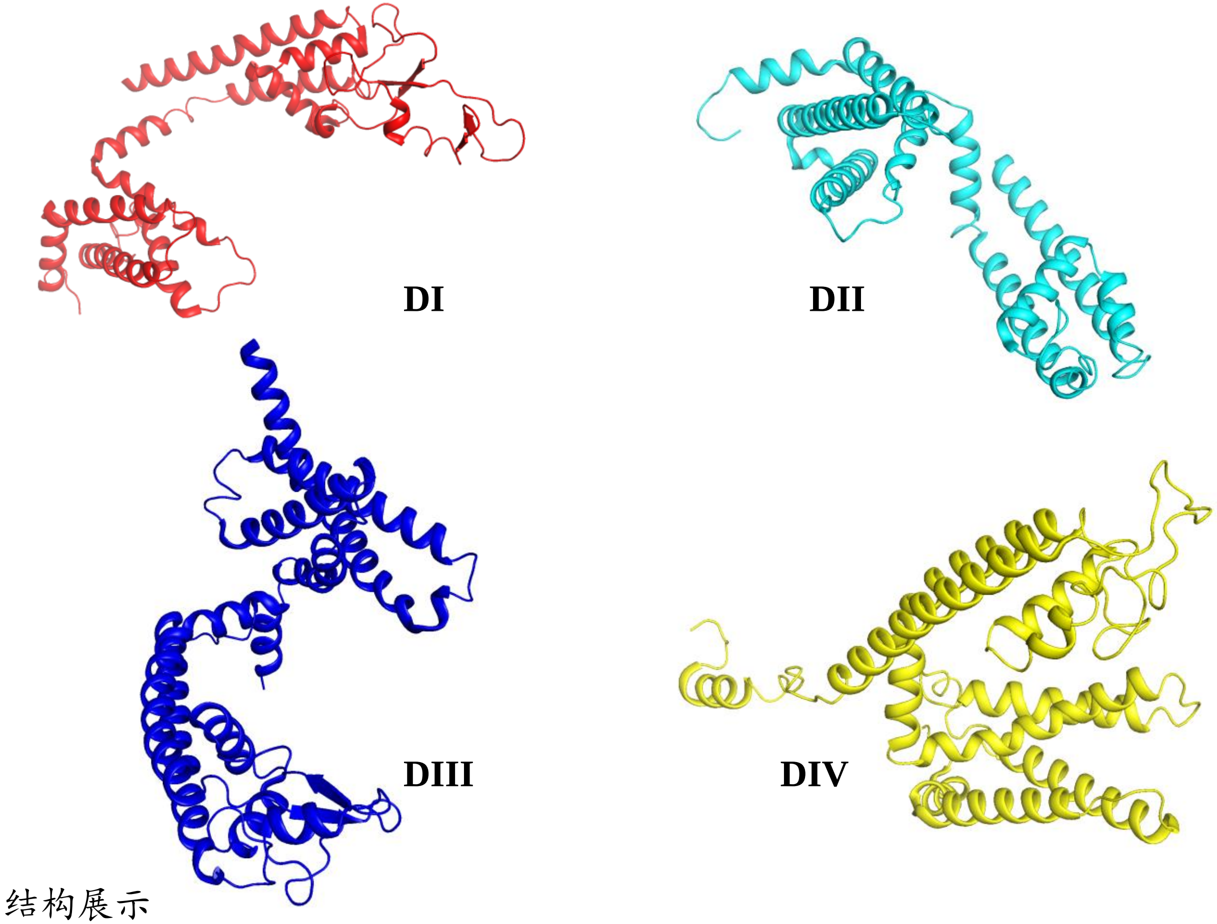
基于ProtScale的蛋白理化性质分析

功能&结构分析



PyMol



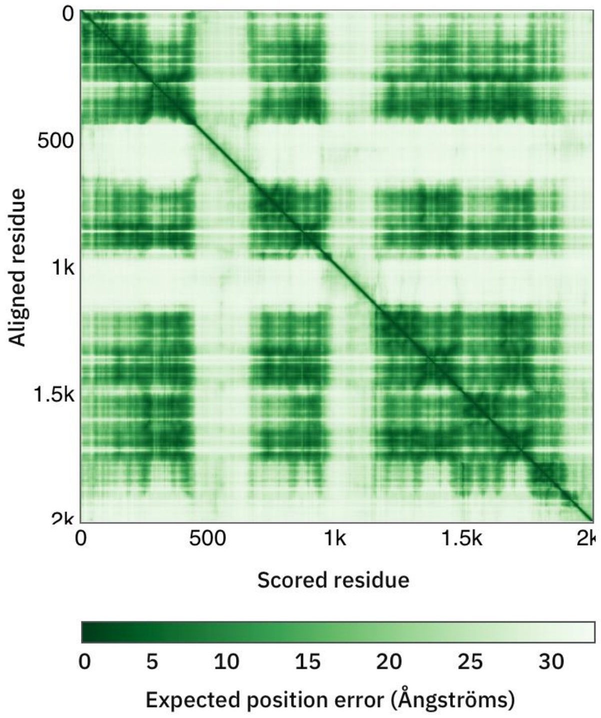


可重复区域在PyMol中的结构展示

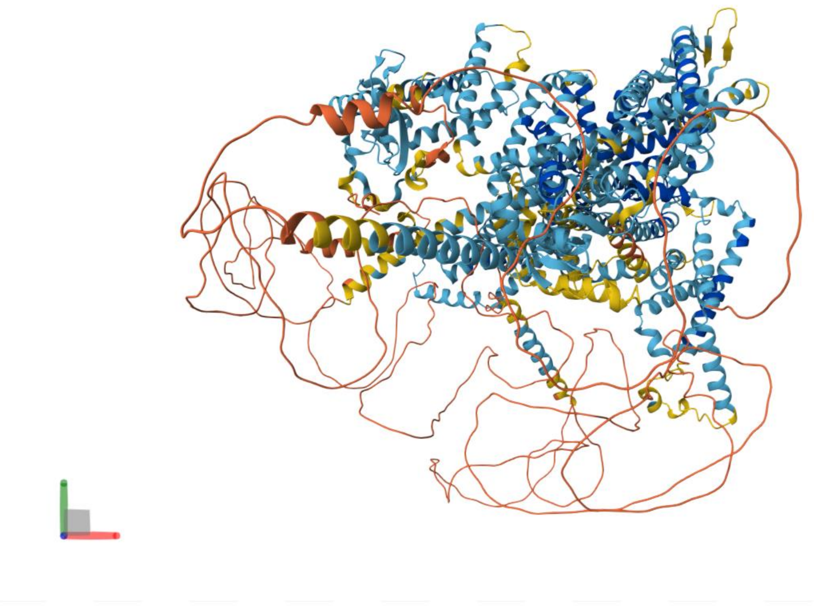
功能&结构分析

AlphaFold

采用**AlphaFold2**模型对**SCN5A**蛋白进行的**结构预测**结果，左侧为结构预测误差图，右侧为三维结构预测图



预测误差图



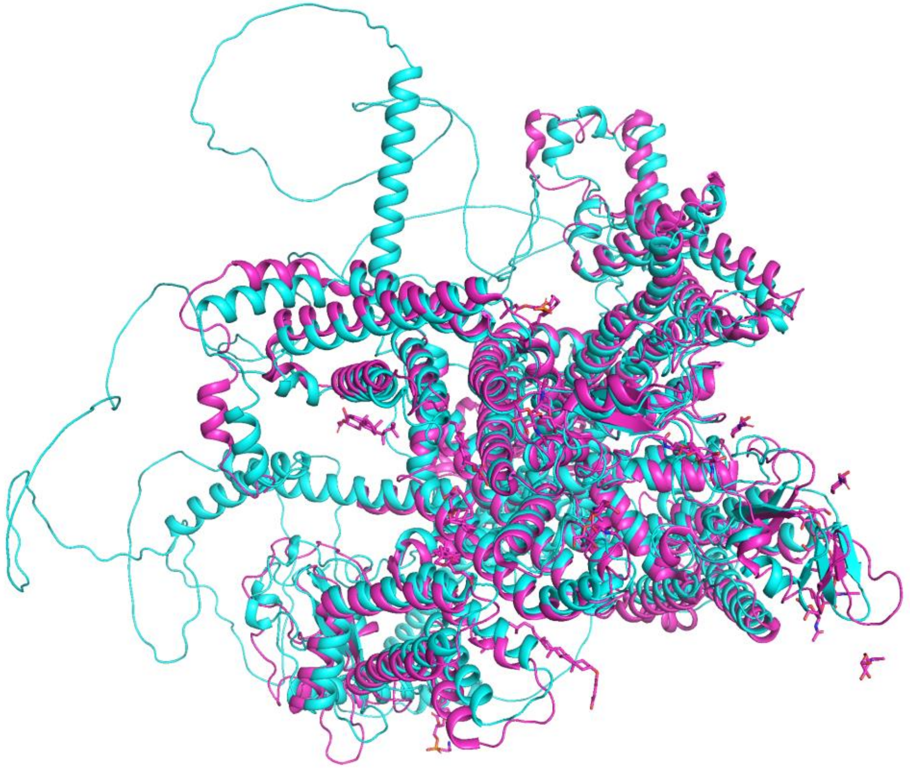
预测蛋白三维结构图

Model Confidence

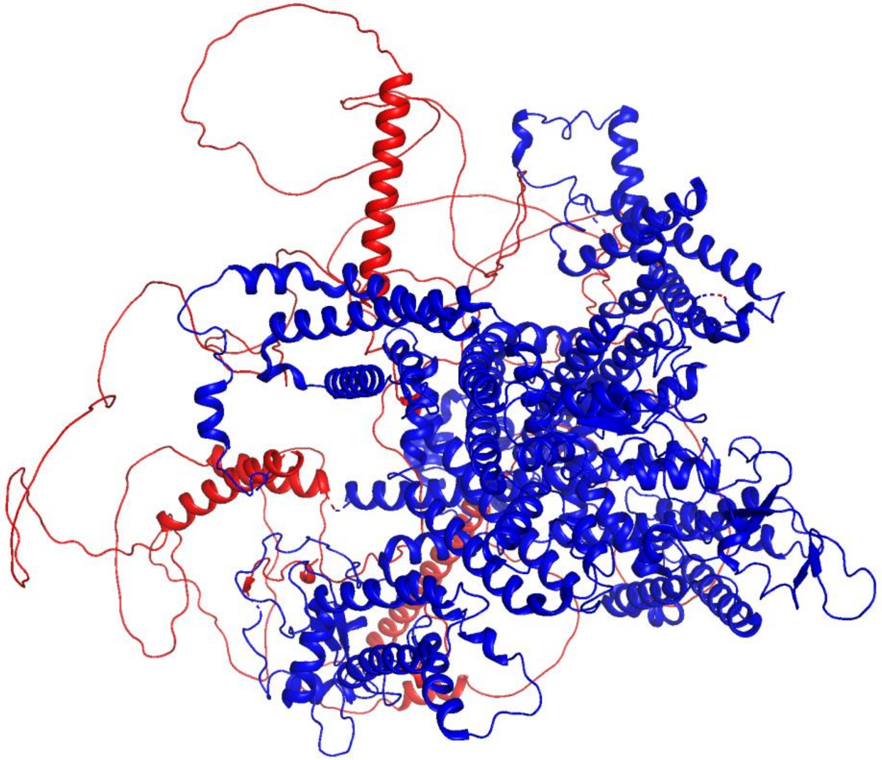
- 🟦 Very high (pLDDT > 90)
- 🟦 High (90 > pLDDT > 70)
- 🟨 Low (70 > pLDDT > 50)
- 🟧 Very low (pLDDT < 50)

功能&结构分析

PyMol对预测蛋白与普通蛋白重合结果



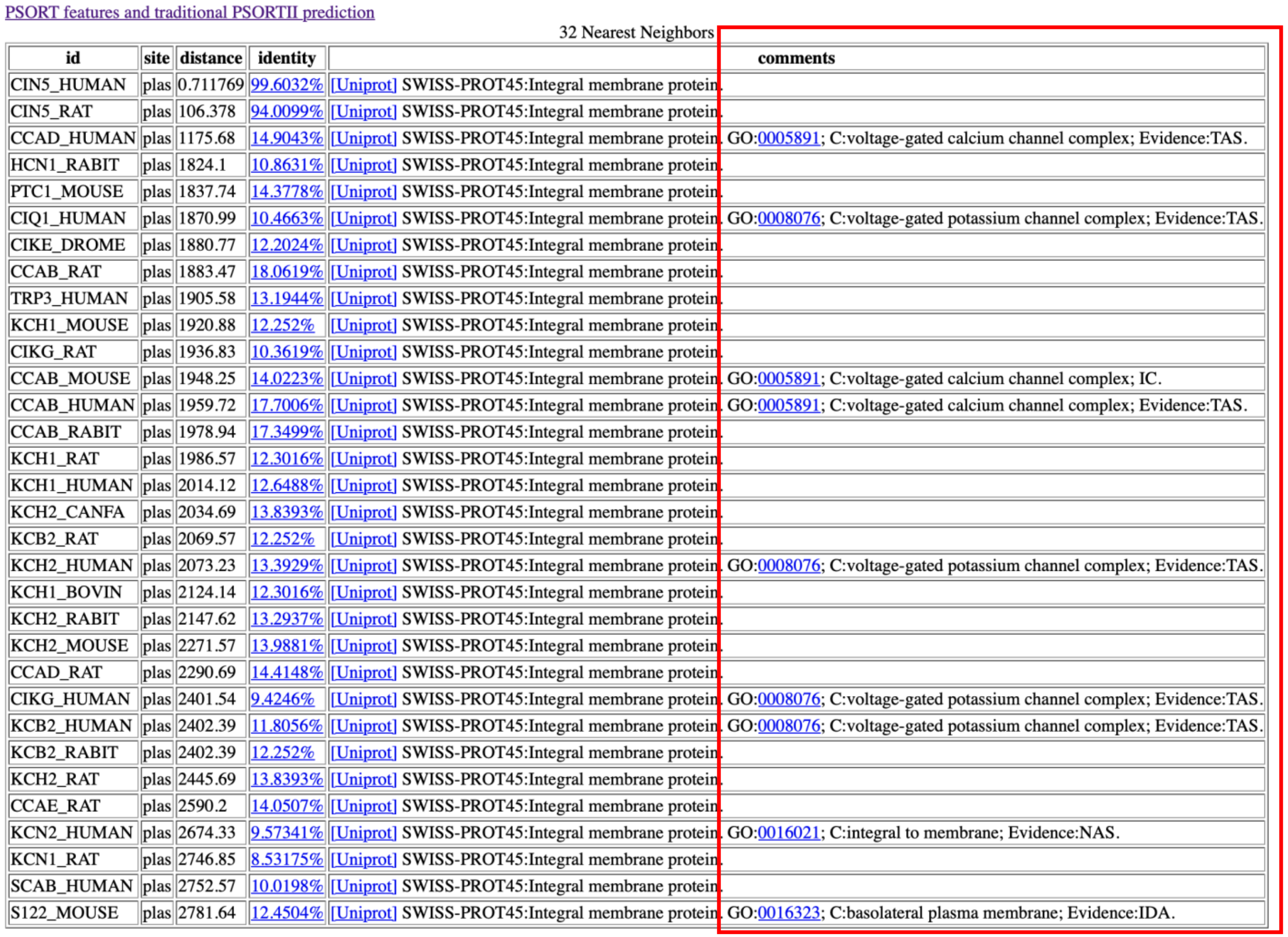
预测蛋白与PDB结构重合图



重合结构与非重合结构

功能&结构分析

WoLF PSORT进行蛋白结构亚细胞定位

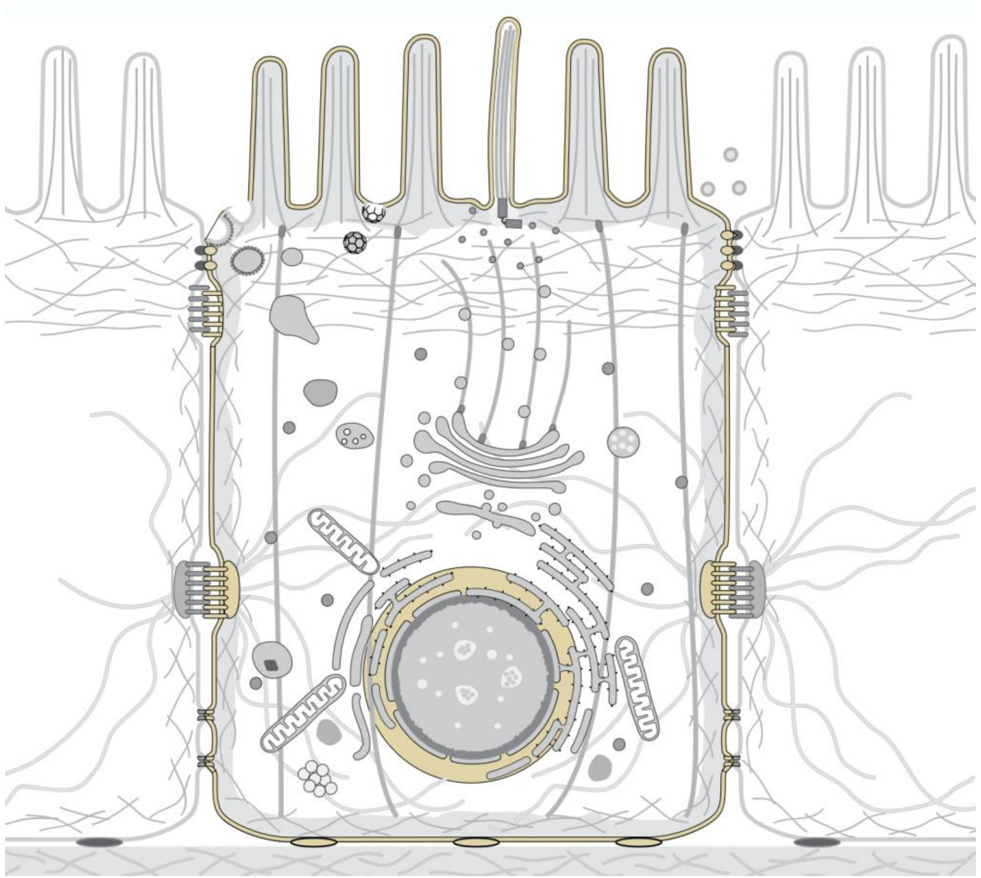


PSORT features and traditional PSORTII prediction

32 Nearest Neighbors

id	site	distance	identity	comments
CIN5_HUMAN	plas	0.711769	99.6032%	[Uniprot] SWISS-PROT45:Integral membrane protein.
CIN5_RAT	plas	106.378	94.0099%	[Uniprot] SWISS-PROT45:Integral membrane protein.
CCAD_HUMAN	plas	1175.68	14.9043%	[Uniprot] SWISS-PROT45:Integral membrane protein. GO:0005891; C:voltage-gated calcium channel complex; Evidence:TAS.
HCN1_RABIT	plas	1824.1	10.8631%	[Uniprot] SWISS-PROT45:Integral membrane protein.
PTC1_MOUSE	plas	1837.74	14.3778%	[Uniprot] SWISS-PROT45:Integral membrane protein.
CIQ1_HUMAN	plas	1870.99	10.4663%	[Uniprot] SWISS-PROT45:Integral membrane protein. GO:0008076; C:voltage-gated potassium channel complex; Evidence:TAS.
CIKE_DROME	plas	1880.77	12.2024%	[Uniprot] SWISS-PROT45:Integral membrane protein.
CCAB_RAT	plas	1883.47	18.0619%	[Uniprot] SWISS-PROT45:Integral membrane protein.
TRP3_HUMAN	plas	1905.58	13.1944%	[Uniprot] SWISS-PROT45:Integral membrane protein.
KCH1_MOUSE	plas	1920.88	12.252%	[Uniprot] SWISS-PROT45:Integral membrane protein.
CIKG_RAT	plas	1936.83	10.3619%	[Uniprot] SWISS-PROT45:Integral membrane protein.
CCAB_MOUSE	plas	1948.25	14.0223%	[Uniprot] SWISS-PROT45:Integral membrane protein. GO:0005891; C:voltage-gated calcium channel complex; IC.
CCAB_HUMAN	plas	1959.72	17.7006%	[Uniprot] SWISS-PROT45:Integral membrane protein. GO:0005891; C:voltage-gated calcium channel complex; Evidence:TAS.
CCAB_RABIT	plas	1978.94	17.3499%	[Uniprot] SWISS-PROT45:Integral membrane protein.
KCH1_RAT	plas	1986.57	12.3016%	[Uniprot] SWISS-PROT45:Integral membrane protein.
KCH1_HUMAN	plas	2014.12	12.6488%	[Uniprot] SWISS-PROT45:Integral membrane protein.
KCH2_CANFA	plas	2034.69	13.8393%	[Uniprot] SWISS-PROT45:Integral membrane protein.
KCB2_RAT	plas	2069.57	12.252%	[Uniprot] SWISS-PROT45:Integral membrane protein.
KCH2_HUMAN	plas	2073.23	13.3929%	[Uniprot] SWISS-PROT45:Integral membrane protein. GO:0008076; C:voltage-gated potassium channel complex; Evidence:TAS.
KCH1_BOVIN	plas	2124.14	12.3016%	[Uniprot] SWISS-PROT45:Integral membrane protein.
KCH2_RABIT	plas	2147.62	13.2937%	[Uniprot] SWISS-PROT45:Integral membrane protein.
KCH2_MOUSE	plas	2271.57	13.9881%	[Uniprot] SWISS-PROT45:Integral membrane protein.
CCAD_RAT	plas	2290.69	14.4148%	[Uniprot] SWISS-PROT45:Integral membrane protein.
CIKG_HUMAN	plas	2401.54	9.4246%	[Uniprot] SWISS-PROT45:Integral membrane protein. GO:0008076; C:voltage-gated potassium channel complex; Evidence:TAS.
KCB2_HUMAN	plas	2402.39	11.8056%	[Uniprot] SWISS-PROT45:Integral membrane protein. GO:0008076; C:voltage-gated potassium channel complex; Evidence:TAS.
KCB2_RABIT	plas	2402.39	12.252%	[Uniprot] SWISS-PROT45:Integral membrane protein.
KCH2_RAT	plas	2445.69	13.8393%	[Uniprot] SWISS-PROT45:Integral membrane protein.
CCAE_RAT	plas	2590.2	14.0507%	[Uniprot] SWISS-PROT45:Integral membrane protein.
KCN2_HUMAN	plas	2674.33	9.57341%	[Uniprot] SWISS-PROT45:Integral membrane protein. GO:0016021; C:integral to membrane; Evidence:NAS.
KCN1_RAT	plas	2746.85	8.53175%	[Uniprot] SWISS-PROT45:Integral membrane protein.
SCAB_HUMAN	plas	2752.57	10.0198%	[Uniprot] SWISS-PROT45:Integral membrane protein.
S122_MOUSE	plas	2781.64	12.4504%	[Uniprot] SWISS-PROT45:Integral membrane protein. GO:0016323; C:basolateral plasma membrane; Evidence:IDA.

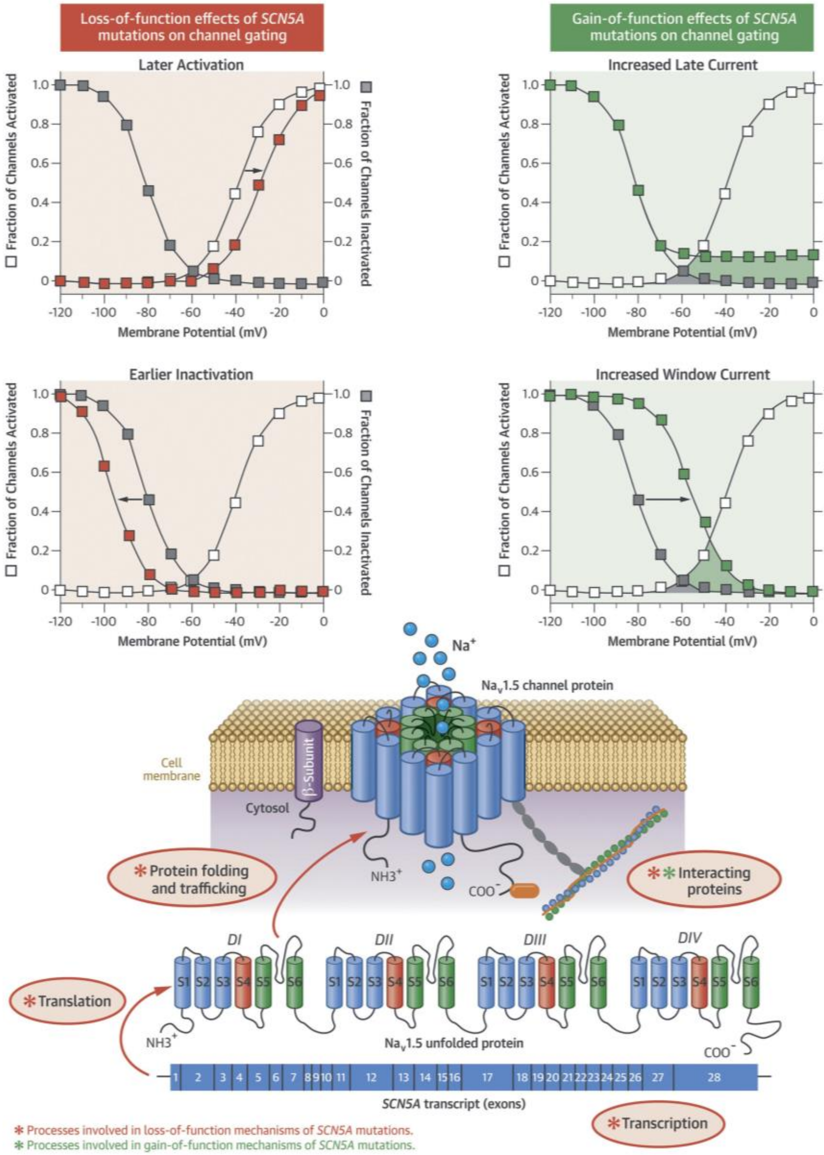
临近蛋白列表



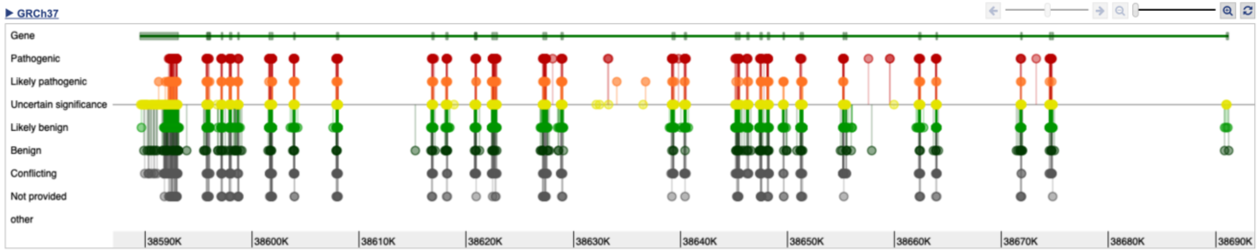
UniProt亚细胞定位

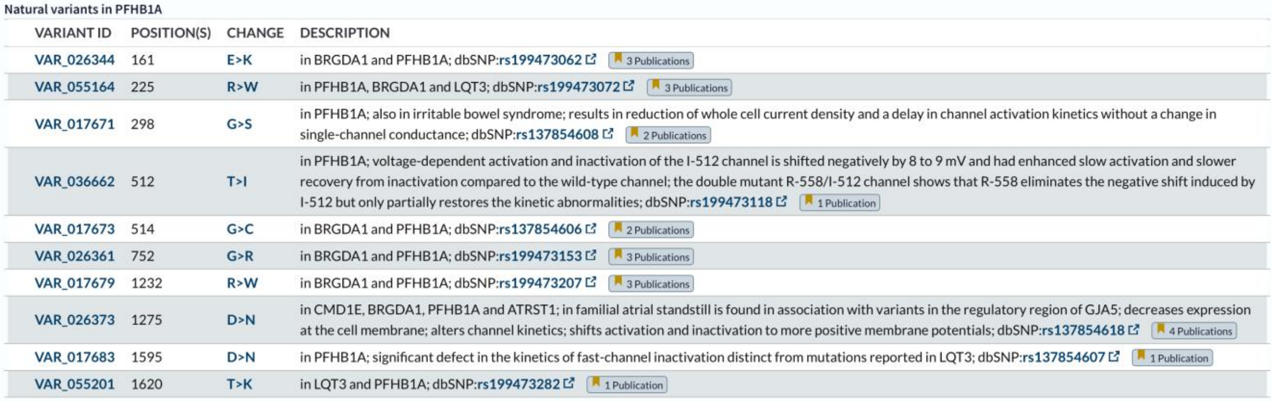
功能&结构分析

蛋白位点突变敏感性分析



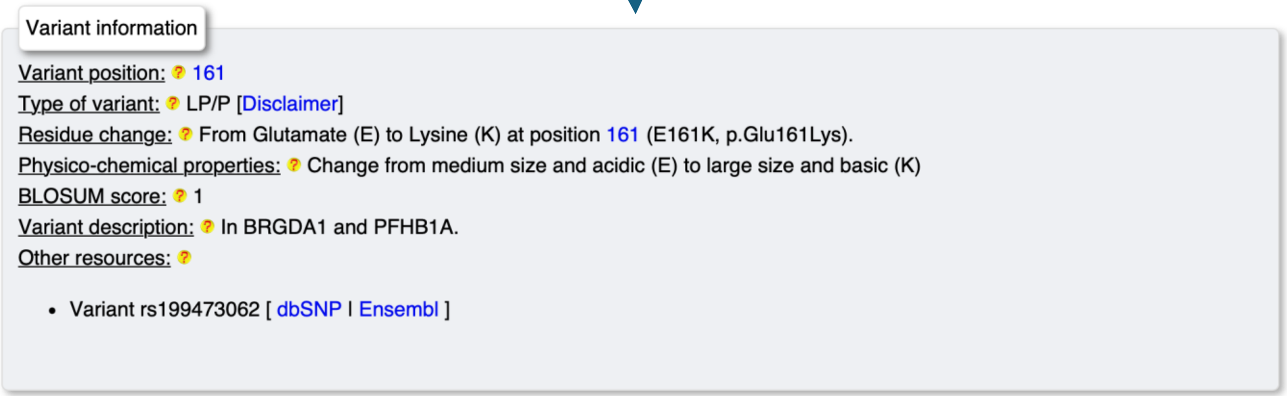
✱ Processes involved in loss-of-function mechanisms of *SCN5A* mutations.
✱ Processes involved in gain-of-function mechanisms of *SCN5A* mutations.





Natural variants in PFHB1A

VARIANT ID	POSITION(S)	CHANGE	DESCRIPTION
VAR_026344	161	E>K	in BRGDA1 and PFHB1A; dbSNP:rs199473062 3 Publications
VAR_055164	225	R>W	in PFHB1A, BRGDA1 and LQT3; dbSNP:rs199473072 3 Publications
VAR_017671	298	G>S	in PFHB1A; also in irritable bowel syndrome; results in reduction of whole cell current density and a delay in channel activation kinetics without a change in single-channel conductance; dbSNP:rs137854608 2 Publications
VAR_036662	512	T>I	in PFHB1A; voltage-dependent activation and inactivation of the I-512 channel is shifted negatively by 8 to 9 mV and had enhanced slow activation and slower recovery from inactivation compared to the wild-type channel; the double mutant R-558/I-512 channel shows that R-558 eliminates the negative shift induced by I-512 but only partially restores the kinetic abnormalities; dbSNP:rs199473118 1 Publication
VAR_017673	514	G>C	in BRGDA1 and PFHB1A; dbSNP:rs137854606 2 Publications
VAR_026361	752	G>R	in BRGDA1 and PFHB1A; dbSNP:rs199473153 3 Publications
VAR_017679	1232	R>W	in BRGDA1 and PFHB1A; dbSNP:rs199473207 3 Publications
VAR_026373	1275	D>N	in CMD1E, BRGDA1, PFHB1A and ATRST1; in familial atrial standstill is found in association with variants in the regulatory region of GJA5; decreases expression at the cell membrane; alters channel kinetics; shifts activation and inactivation to more positive membrane potentials; dbSNP:rs137854618 4 Publications
VAR_017683	1595	D>N	in PFHB1A; significant defect in the kinetics of fast-channel inactivation distinct from mutations reported in LQT3; dbSNP:rs137854607 1 Publication
VAR_055201	1620	T>K	in LQT3 and PFHB1A; dbSNP:rs199473282 1 Publication

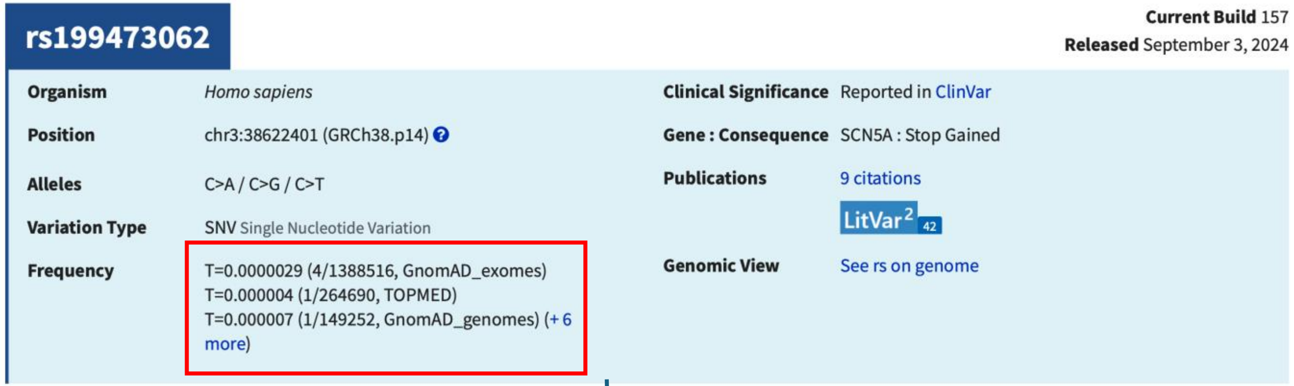


Variant information

Variant position: ? 161
Type of variant: ? LP/P [Disclaimer]
Residue change: ? From Glutamate (E) to Lysine (K) at position 161 (E161K, p.Glu161Lys).
Physico-chemical properties: ? Change from medium size and acidic (E) to large size and basic (K)
BLOSUM score: ? 1
Variant description: ? In BRGDA1 and PFHB1A.
Other resources: ?

- Variant rs199473062 [dbSNP I Ensembl]

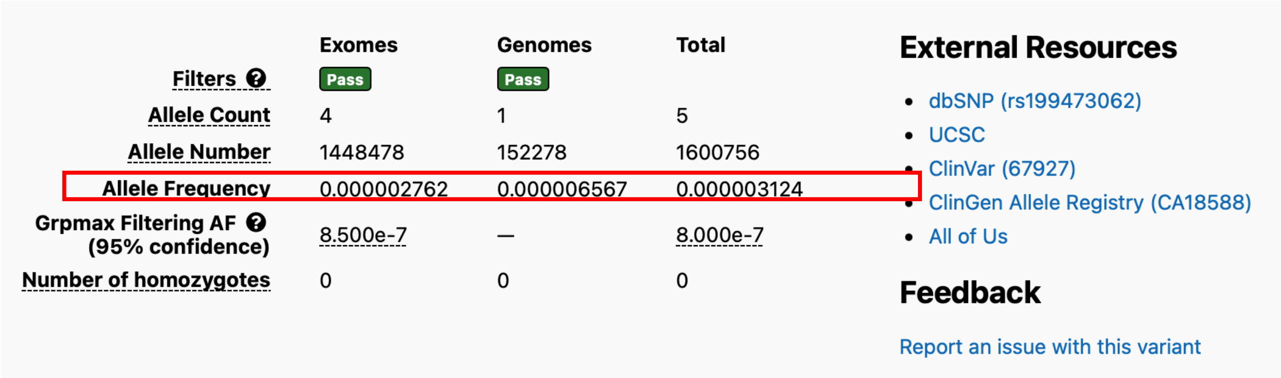
北京大學
PEKING UNIVERSITY



rs199473062

Current Build 157
Released September 3, 2024

Organism	*Homo sapiens*	**Clinical Significance**	Reported in ClinVar
Position	chr3:38622401 (GRCh38.p14)	**Gene : Consequence**	SCN5A : Stop Gained
Alleles	C>A / C>G / C>T	**Publications**	9 citations LitVar2 42
Variation Type	SNV Single Nucleotide Variation		
Frequency	T=0.0000029 (4/1388516, GnomAD_exomes) T=0.000004 (1/264690, TOPMED) T=0.000007 (1/149252, GnomAD_genomes) (+ 6 more)	**Genomic View**	See rs on genome



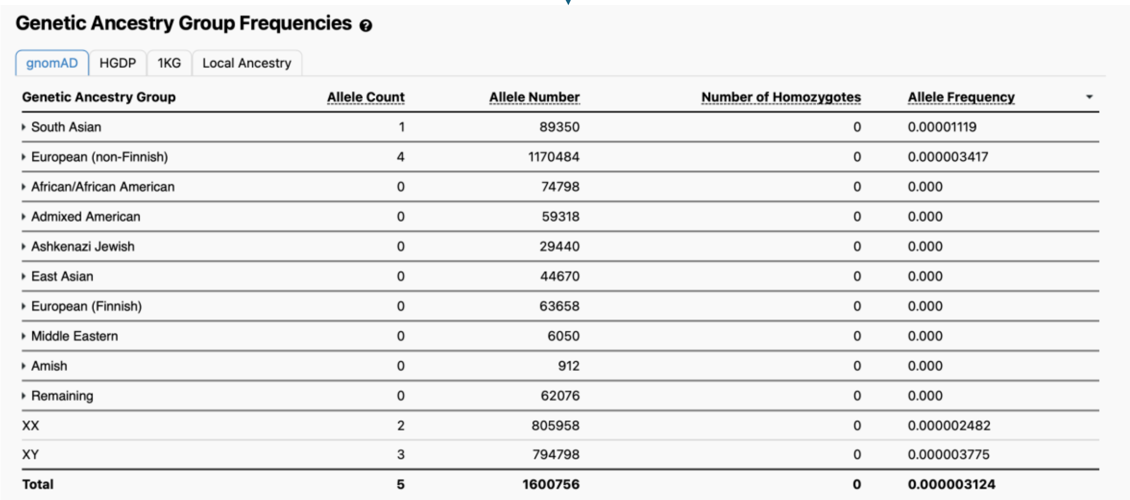
	Exomes	Genomes	Total
Filters	Pass	Pass	
Allele Count	4	1	5
Allele Number	1448478	152278	1600756
Allele Frequency	0.000002762	0.000006567	0.000003124
Grpmax Filtering AF (95% confidence)	8.500e-7	—	8.000e-7
Number of homozygotes	0	0	0

External Resources

- dbSNP (rs199473062)
- UCSC
- ClinVar (67927)
- ClinGen Allele Registry (CA18588)
- All of Us

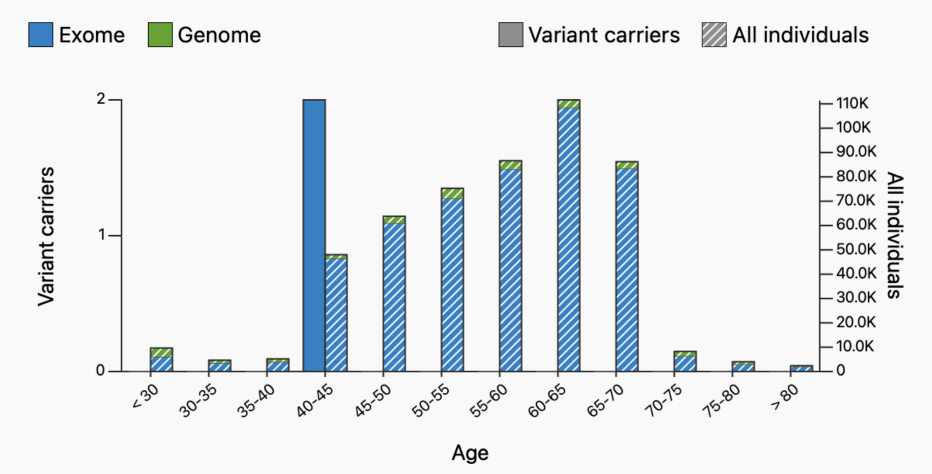
Feedback

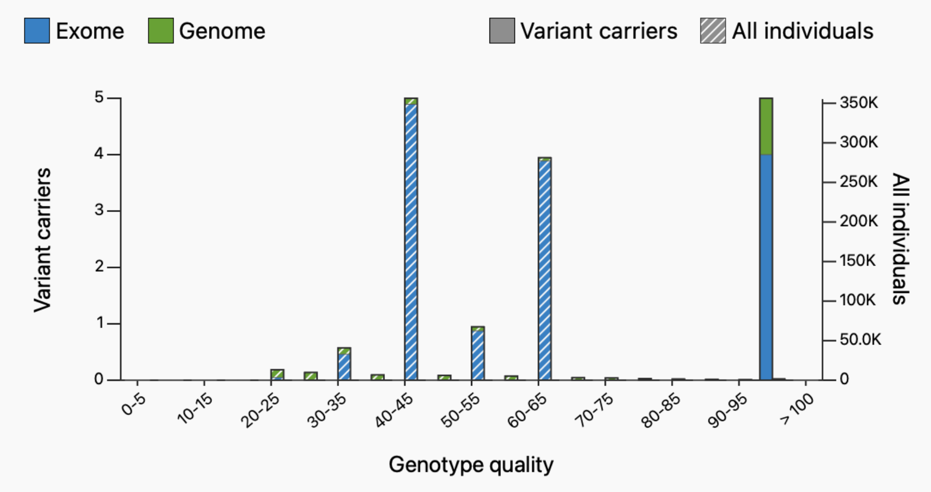
Report an issue with this variant



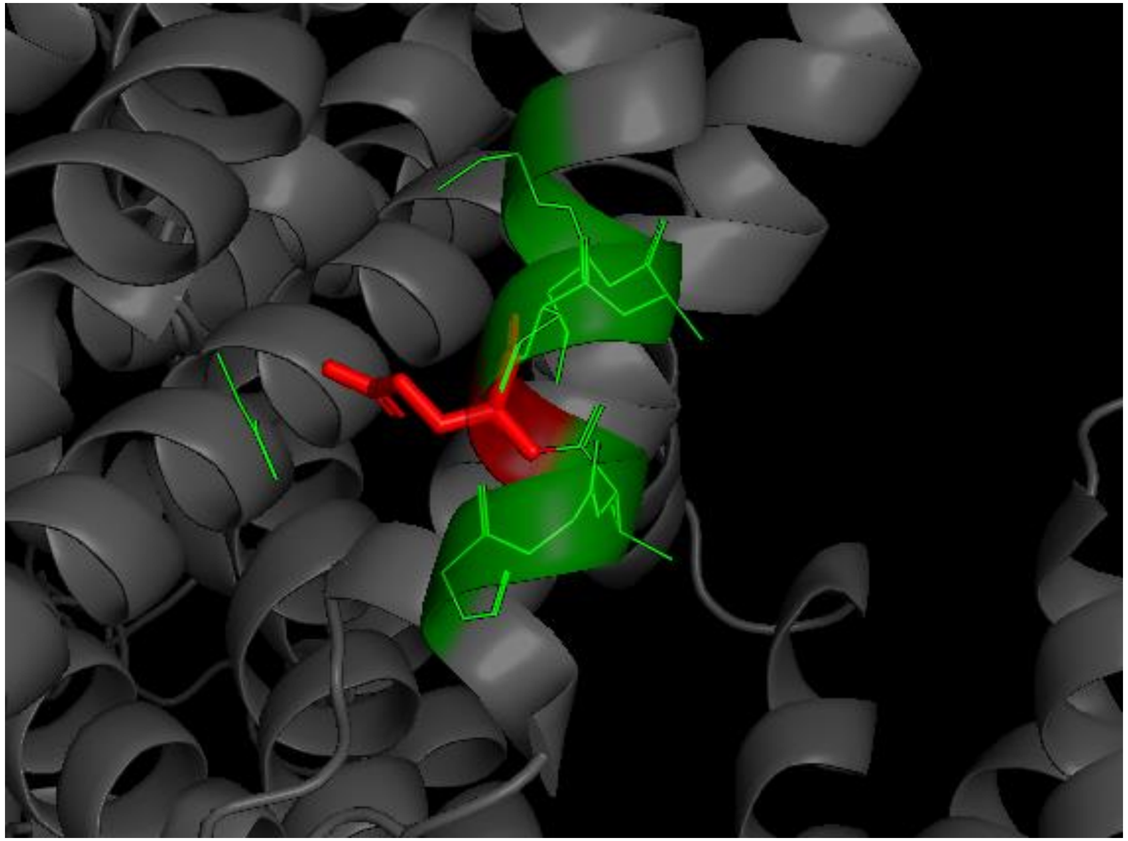
Genetic Ancestry Group Frequencies

Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
South Asian	1	89350	0	0.00001119
European (non-Finnish)	4	1170484	0	0.000003417
African/African American	0	74798	0	0.000
Admixed American	0	59318	0	0.000
Ashkenazi Jewish	0	29440	0	0.000
East Asian	0	44670	0	0.000
European (Finnish)	0	63658	0	0.000
Middle Eastern	0	6050	0	0.000
Amish	0	912	0	0.000
Remaining	0	62076	0	0.000
XX	2	805958	0	0.000002482
XY	3	794798	0	0.000003775
Total	**5**	**1600756**	**0**	**0.000003124**





后续计划



rs 199473062 突变位点示意图

从反应性，动力学的角度分析突变造成的影响

- 使用PyMol软件，是否可以“看到”目标位点可能造成的结构影响，如盐桥，氢键，紧密接触程度和溶剂暴露程度等？
- 是否可以用动力学方法拟合位点被替换后可能发生的结构变化？
- ……

THANKS FOR LISTENING!