

# 超氧化物歧化酶基因的克隆表达和结构的分析

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组员：4G01 4G03 4G11

22/06/2018



## 普丽普莱 天然SOD超氧化物/歧化酶营养片

- 包装规格:** 250mg\*50粒/瓶
- 贮藏方式:** 常温保存, 置于阴凉干燥处, 勿让儿童触及
- 原产地:** 美国
- 有效期:** 见瓶身
- 产品ID:** 2560
- 食用方法:** 成人一天一次, 一次1粒, 建议随餐服用

**特别声明:**本品只作为一种膳食补充剂, 不能代替药物用来诊断、治疗、治愈或预防任何疾病

# 作用



## 自由基引发人体多种疾病

# SOD

心脑血管疾病	恶性肿瘤	糖尿病
过度衰老	植物神经紊乱	肝炎/脂肪肝/肝硬化
消化系统疾病	内分泌失调	眼部疾病/白内障
各种炎症	亚健康症状	



### 什么是自由基?

自由基是带有一个单独不成对电子的分子基团。多余的自由基，会破坏细胞结构，造成组织器官的各种损伤，加速机体的衰老进程并诱发各种疾病。



# 超氧化物歧化酶SOD

SOD (superoxide dismutase) 是一种广泛存在于动-植物和微生物中的金属酶。它催化超氧化物阴离子自由基 ( $O_2^{\cdot-}$ ) 发生歧化反应, 从而清除 $O_2^{\cdot-}$ , 在维持生物体内超氧阴离子自由基产生与消除的动态平衡中起着重要的作用。

根据结合的**金属离子不同**, 超氧化物歧化酶分为三类:

一是Cu-SOD、Zn-SOD

二是能与 $Fe^{2+}$ 、 $Mn^{2+}$ 分别或同时结合, 即Fe-SOD、Mn-SOD或Fe/Mn-SOD

三是Ni-SOD

# 对SOD进行生物信息学分析

- 1 利用NCBI、Uniprot查找SOD序列信息
- 2 利用MEGA7进行多序列比对
- 3 利用MEGA7构建进化树
- 4 利用NCBI 对SOD预测ORF
- 5 利用EMBOSS EXPLORER plotorf分析其核酸序列及氨基酸序列的相似性
- 6 利用CBS、SMART、ProtScal进行跨膜域、信号肽、结构域预测及性质分析
- 7 利用Swiss-PdbViewer对SOD结构进行分析
- 8 利用Uniprot查找蛋白结构
- 9 利用NCBI 进行引物设计

# 1、利用NCBI、Uniprot查找SOD序列信息

Protein Protein Mn-SOD Search

Create alert Advanced

Help

## Species

Animals (157)  
Plants (41)  
Fungi (99)  
Protists (6)  
Bacteria (103)  
Archaea (1)  
Customize ...

## Source databases

PDB (31)  
RefSeq (30)  
UniProtKB / Swiss-Prot (5)  
Customize ...

## Genetic

compartments  
Mitochondrion (1)

## Sequence length

Custom range...

## Molecular weight

Custom range...

## Release date

Custom range...

## Revision date

Custom range...

Summary 20 per page Sort by Default order

Send to:

Filters: [Manage Filters](#)

See [MNSOD manganese superoxide dismutase](#) in the Gene database  
mnsod reference sequences [Transcript \(1\)](#) [Protein \(1\)](#)

See the [results of this search \(14 items\)](#) in our new [Identical Protein Groups](#) database.

Items: 1 to 20 of 407

<< First < Prev Page 1 of 21 Next > Last >>

- [Mn-sod \[Geobacillus sp. POT5\]](#)
  1. 204 aa protein  
Accession: ADU05414.1 GI: 315361444  
[Nucleotide](#) [Taxonomy](#) [Related Sequences](#)  
[GenPept](#) [Identical Proteins](#) [FASTA](#) [Graphics](#)
- [Mn-SOD \[Spirometra erinaceieuropaei\]](#)
  2. 222 aa protein  
Accession: AAT79388.1 GI: 50593188  
[Nucleotide](#) [Taxonomy](#) [Related Sequences](#)  
[GenPept](#) [Identical Proteins](#) [FASTA](#) [Graphics](#)
- [Mn-SOD \[Paraqonimus westermani\]](#)

## Results by taxon

Top Organisms [\[Tree\]](#)

Bursaphelenchus xylophilus (67)  
Glaesserella parasuis (32)  
Homo sapiens (21)  
Ganoderma lucidum (19)  
Ganoderma tsugae (12)  
All other taxa (256)

[More...](#)

## Find related data

Database:

## Search details

Mn-SOD[All Fields]

Nucleotide

Nucleotide Mn-SOD

Search

Create alert Advanced

Help

Species

- Animals (163)
- Plants (44)
- Fungi (102)
- Protists (4)
- Bacteria (81)
- Archaea (1)
- Customize ...

Molecule types

- genomic DNA/RNA (289)
- mRNA (112)
- Customize ...

Source databases

- INSDC (GenBank) (363)
- RefSeq (44)
- Customize ...

Sequence length

Custom range...

Release date

Custom range...

Revision date

Custom range...

Clear all

Summary 20 per page Sort by Default order

Send to:

Filters: Manage Filters

See [MNSOD manganese superoxide dismutase](#) in the Gene database  
 mnsod reference sequences [Transcript \(1\)](#) [Protein \(1\)](#)

Items: 1 to 20 of 407

<< First < Prev Page 1 of 21 Next > Last >>

Found 412 nucleotide sequences. Nucleotide (407) EST (5)

- [Geobacillus sp. POT5 Mn-sod gene, complete cds](#)  
 1. 615 bp linear DNA  
 Accession: HM992936.1 GI: 315361443  
[Protein](#) [Taxonomy](#)  
[GenBank](#) [FASTA](#) [Graphics](#)
- [Spirometra erinaceieuropaei Mn-SOD mRNA, complete cds](#)  
 2. 872 bp linear mRNA  
 Accession: AY675509.1 GI: 50593187  
[Protein](#) [Taxonomy](#)  
[GenBank](#) [FASTA](#) [Graphics](#)
- [Paragonimus westermani Mn-SOD mRNA, complete cds](#)  
 3. 911 bp linear mRNA  
 Accession: AY675508.1 GI: 50593185

Results by taxon

Top Organisms [Tree](#)

- Bursaphelenchus xylophilus (67)
- Glaesserella parasuis (32)
- Homo sapiens (24)
- Ganoderma lucidum (19)
- Ganoderma tsugae (12)
- All other taxa (253)

More...

Find related data

Database: Select

Find items

Search details

Mn-SOD[All Fields]

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# UniProtKB results

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[Basket](#)

## Filter by<sup>i</sup>

Unreviewed (1)  
TrEMBL

## Popular organisms

[Geobacillus sp. POT5 \(1\)](#)

## View by

[Results table](#)

[Taxonomy](#)

[Keywords](#)

We'd like to inform you that we have updated our [Privacy Notice](#) to comply with Europe's new General Data Protection Regulation (GDPR) that applies since 25 May 2018.

[Enzyme class](#)

[Pathway](#)

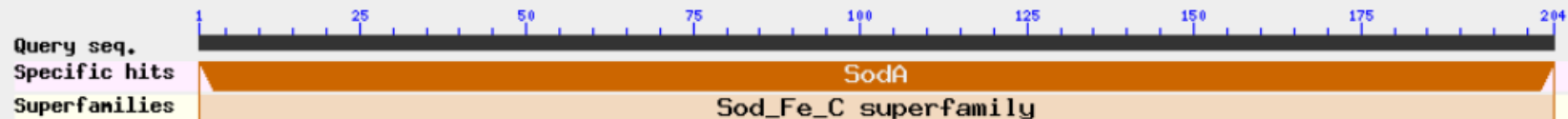
<input type="checkbox"/>	Entry	Entry name	Protein names	Gene names	Organism	Length	
<input type="checkbox"/>	E7D085	E7D085_9BACI	Fe/Mn-sod		Geobacillus sp. POT5	411	

1 to 1 of 1 Show 25

1 to 1 of 1 Show 25

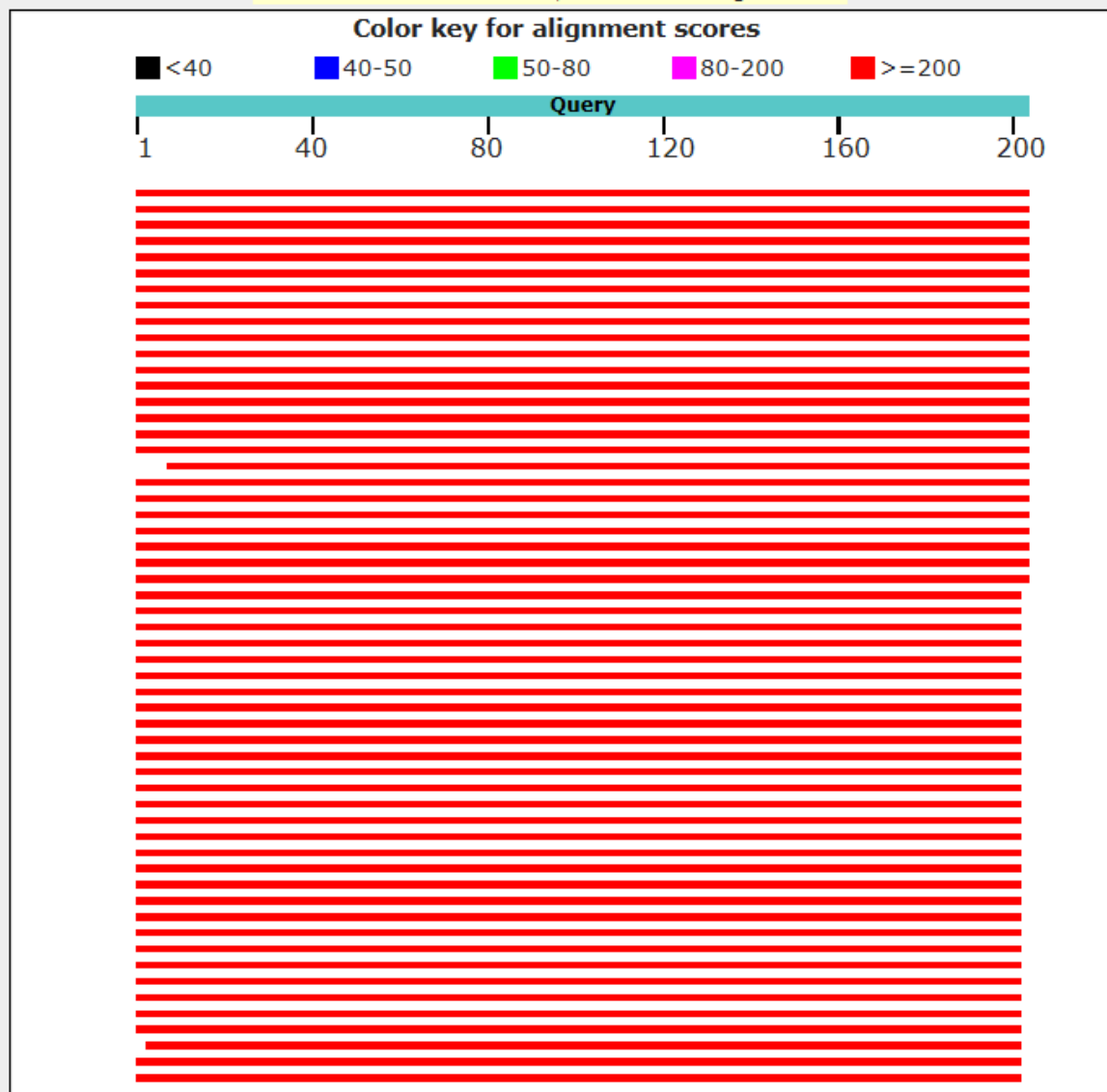
[Do not show this banner again](#)





### Distribution of the top 100 Blast Hits on 100 subject sequences

Mouse over to see the title, click to show alignments



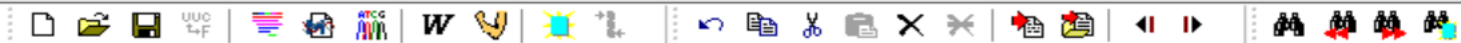


	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	<a href="#">MULTISPECIES: superoxide dismutase [Mn] [Geobacillus]</a>	421	421	100%	4e-149	100%	<a href="#">WP_011231937.1</a>
<input type="checkbox"/>	<a href="#">MULTISPECIES: superoxide dismutase [Geobacillus]</a>	419	419	100%	1e-148	99%	<a href="#">WP_047818706.1</a>
<input type="checkbox"/>	<a href="#">MULTISPECIES: superoxide dismutase [Mn] [Geobacillus]</a>	419	419	100%	2e-148	99%	<a href="#">WP_033010224.1</a>
<input type="checkbox"/>	<a href="#">MULTISPECIES: superoxide dismutase [Mn] [Geobacillus]</a>	419	419	100%	3e-148	99%	<a href="#">WP_044730821.1</a>
<input type="checkbox"/>	<a href="#">superoxide dismutase [Mn] [Anoxybacillus geothermalis]</a>	419	419	100%	3e-148	99%	<a href="#">WP_044743151.1</a>
<input type="checkbox"/>	<a href="#">superoxide dismutase [Geobacillus jurassicus]</a>	417	417	100%	8e-148	99%	<a href="#">WP_066230603.1</a>
<input type="checkbox"/>	<a href="#">MULTISPECIES: superoxide dismutase [Geobacillus]</a>	416	416	100%	4e-147	99%	<a href="#">WP_033018968.1</a>
<input type="checkbox"/>	<a href="#">superoxide dismutase [Geobacillus thermoleovorans]</a>	416	416	100%	4e-147	99%	<a href="#">BAA95631.1</a>
<input type="checkbox"/>	<a href="#">superoxide dismutase [Geobacillus vulcani]</a>	415	415	100%	8e-147	99%	<a href="#">WP_031408245.1</a>
<input type="checkbox"/>	<a href="#">superoxide dismutase</a>	414	414	100%	2e-146	99%	<a href="#">1918164A</a>
<input type="checkbox"/>	<a href="#">superoxide dismutase [Geobacillus stearothermophilus]</a>	413	413	100%	6e-146	98%	<a href="#">WP_053413784.1</a>
<input type="checkbox"/>	<a href="#">superoxide dismutase [Geobacillus sp. 46C-IIa]</a>	412	412	100%	1e-145	98%	<a href="#">WP_081209548.1</a>
<input type="checkbox"/>	<a href="#">MULTISPECIES: superoxide dismutase [Geobacillus]</a>	411	411	100%	2e-145	98%	<a href="#">WP_008879878.1</a>
<input type="checkbox"/>	<a href="#">superoxide dismutase [Geobacillus sp. PA-3]</a>	411	411	100%	3e-145	98%	<a href="#">WP_060476068.1</a>
<input type="checkbox"/>	<a href="#">MULTISPECIES: superoxide dismutase [Geobacillus]</a>	411	411	100%	4e-145	97%	<a href="#">WP_063165262.1</a>
<input type="checkbox"/>	<a href="#">superoxide dismutase [Geobacillus genomsp. 3]</a>	409	409	100%	2e-144	97%	<a href="#">WP_020960593.1</a>
<input type="checkbox"/>	<a href="#">MULTISPECIES: superoxide dismutase [Bacillaceae]</a>	405	405	100%	5e-143	96%	<a href="#">WP_017435080.1</a>
<input type="checkbox"/>	<a href="#">manganese superoxide dismutase [Geobacillus kaustophilus GBlys]</a>	405	405	96%	5e-143	99%	<a href="#">CAD12532.1</a>
<input type="checkbox"/>	<a href="#">superoxide dismutase [Parageobacillus thermantarcticus]</a>	400	400	100%	7e-141		

# 2、利用MEGA7进行多序列比对

MEGA7: Multiple Sequence Alignment Software

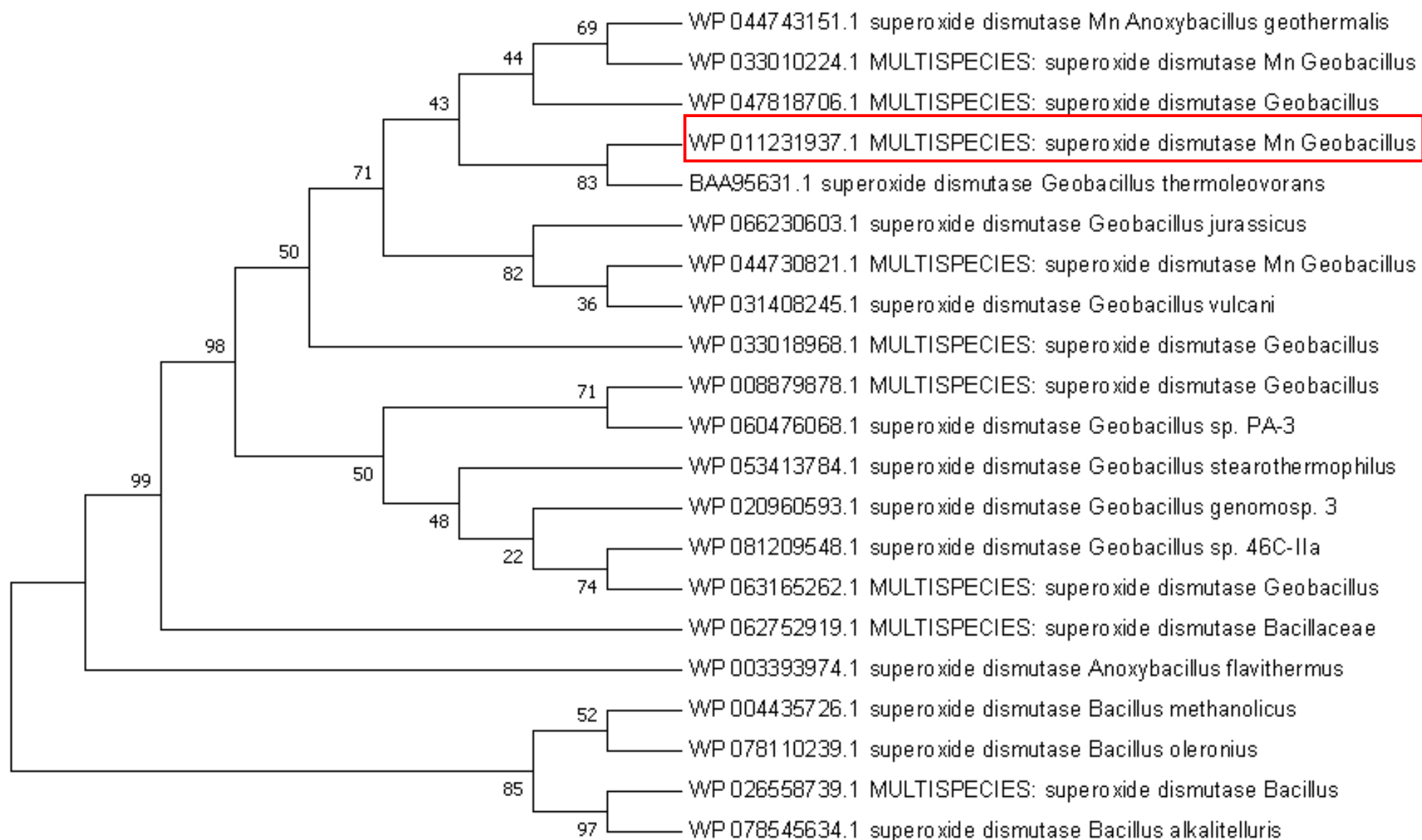
Data Edit Search Alignment Web Sequencer Display Help



Protein Sequences

Species/Abbrev	Group Name	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*																				
1. WP_011231937.1 MULTISPECIES: superoxide dismutase Mn Geobacillus		M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L	S	
2. BAA95631.1 superoxide dismutase Geobacillus thermoovorans		M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L	S	
3. WP_008879878.1 MULTISPECIES: superoxide dismutase Geobacillus		M	P	F	E	L	P	A	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	T	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	A	D	L	Q	N	K	S	L	E	E	L	L	S	
4. WP_047818706.1 MULTISPECIES: superoxide dismutase Geobacillus		M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L	S	
5. WP_044743151.1 superoxide dismutase Mn Anoxybacillus geothermalis		M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	M	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L	S	
6. WP_033010224.1 MULTISPECIES: superoxide dismutase Mn Geobacillus		M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L	S	
7. WP_044730821.1 MULTISPECIES: superoxide dismutase Mn Geobacillus		M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L	S	
8. WP_033018968.1 MULTISPECIES: superoxide dismutase Geobacillus		M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	A	D	L	Q	N	K	S	L	E	E	L	L	S	
9. WP_066230603.1 superoxide dismutase Geobacillus jurassicus		M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	S	K	S	L	E	E	L	L	S	
10. WP_031408245.1 superoxide dismutase Geobacillus vulcani		M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	Y	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L	S	
11. WP_053413784.1 superoxide dismutase Geobacillus stearothermophilus		M	P	F	E	L	P	A	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	A	D	L	Q	N	K	S	L	E	E	L	L	S	
12. WP_081209548.1 superoxide dismutase Geobacillus sp. 46C-IIa		M	P	F	E	L	P	A	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	A	D	L	Q	N	K	S	L	E	E	L	L	S	
13. WP_060476068.1 superoxide dismutase Geobacillus sp. PA-3		M	P	F	E	L	P	A	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	T	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	V	D	L	Q	N	K	S	L	E	E	L	L	S	
14. WP_020960593.1 superoxide dismutase Geobacillus genomosp. 3		M	P	F	E	L	P	A	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	Q	A	D	L	Q	N	K	S	L	E	E	L	L	S	
15. WP_063165262.1 MULTISPECIES: superoxide dismutase Geobacillus		M	P	F	E	L	P	A	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	A	D	L	Q	N	K	S	L	E	E	L	L	S	
16. WP_004435726.1 superoxide dismutase Bacillus methanolicus		M	A	F	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	L	E	G	N	E	E	L	L	S	K	T	V	E	E	V	I	S		
17. WP_026558739.1 MULTISPECIES: superoxide dismutase Bacillus		M	A	Y	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	I	T	N	V	N	A	A	L	E	G	N	A	D	L	A	G	K	S	V	E	E	L	V	I	S
18. WP_078110239.1 superoxide dismutase Bacillus oleronius		M	A	F	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	N	D	E	L	L	N	K	S	V	E	E	V	I	S	
19. WP_003393974.1 superoxide dismutase Anoxybacillus flavithermus		M	A	F	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	A	Y	V	T	N	L	N	A	A	L	E	G	H	P	E	L	Q	N	K	T	I	E	E	L	M	A	
20. WP_062752919.1 MULTISPECIES: superoxide dismutase Bacillaceae		M	A	F	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	A	D	L	Q	N	K	S	I	E	E	L	L	S	
21. WP_078545634.1 superoxide dismutase Bacillus alkalitelluris		M	A	Y	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	I	T	N	V	N	A	A	L	E	G	-	S	D	L	L	N	K	S	V	E	E	L	I	S	

### 3、利用MEGA7构建进化树



# 4、利用NCBI 对SOD预测ORF

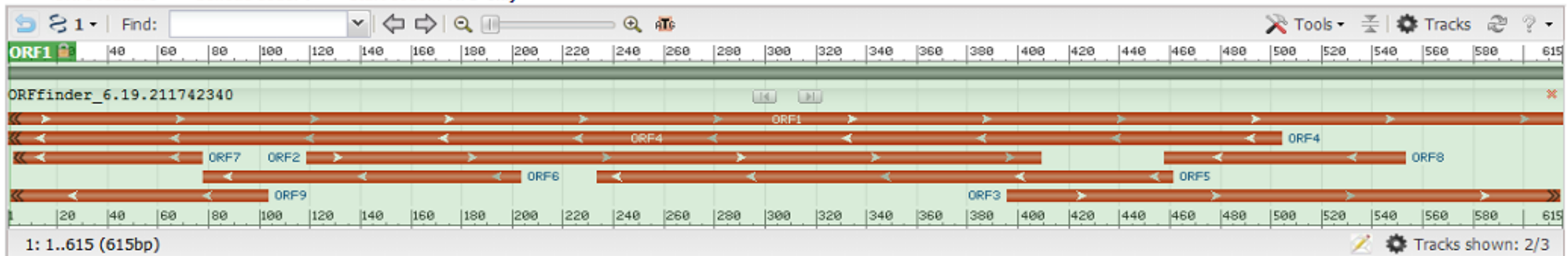
ORFfinder PubMed  Search

## Open Reading Frame Viewer

Help

### Sequence

ORFs found: 9 Genetic code: 1 Start codon: 'ATG' only



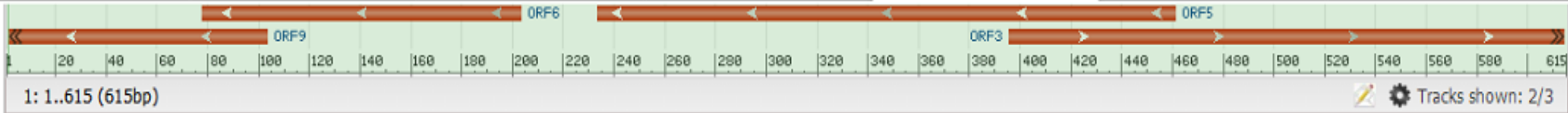
Six-frame translation...

ORF1 (204 aa) Display ORF as... Mark

```
>lcl|ORF1
MPFELPALPYDYDALEPHIDKETMNIHHTKGGHNTYVTVNLNAALEGHPDLQ
NKSLEELLSNLEALPESIRTAVRNMGGHHANMSLFWITILSPNGGGEPTGE
LAEAINKKKFGSFTAFKDEPSKAAAGRFGSGWAWLVVWNGELEITSTPNQD
SPINEGKTFILGLDWEHAYYLLKYNRRRPEYIAAFWNIWVWDEVAKRYSE
AKAK
```

Mark subset... Marked: 0 Download marked set as Protein FASTA

Label	Strand	Frame	Start	Stop	Length (nt   aa)
ORF1	+	1	<1	615	615   204
ORF4	-	1	504	>1	504   167
ORF2	+	2	119	409	291   96
ORF5	-	2	461	234	228   75
ORF3	+	3	396	>614	219   72



[Six-frame translation...](#)

**ORF1 (204 aa)**

[Display ORF as...](#)

[Mark](#)

```
>|e1|ORF1
MPPFELPALPYFDALPHIDKETMNIHNTKQHNYYVTNLNAALEGHPDLQ
NKSLELLSNLEALPESIRTAVRNNGGGHANHSLFWTILSPNGGGEPTGE
LAEAINKKFGSFTAFKDEFSKAAAGRFGSGWANLVVHNGELEITSIPNQD
SPIMEGKTPILGLDVWEHAYYLKYQRRPEYIAAFWNIWVWDEVAKRYSE
AKAK
```

[Mark subset...](#)

Marked: 0

[Download marked set](#)

as [Protein FASTA](#) ▾

Label	Strand	Frame	Start	Stop	Length (nt   aa)
<b>ORF1</b>	+	1	<1	615	615   204
ORF4	-	1	504	>1	504   167
ORF2	+	2	119	409	291   96
ORF5	-	2	461	234	228   75
ORF3	+	3	396	>614	219   72
ORF6	-	2	203	78	126   41
ORF9	-	3	103	>2	102   33
ORF8	-	3	553	458	96   31
ORF7	-	2	77	>3	75   24

**ORF1**

Marked set ( 0 )

[SmartBLAST](#)

SmartBLAST best hit titles... [?](#)

[BLAST](#)

[BLAST](#)

BLAST Database:

[UniProtKB/Swiss-Prot \(swissprot\)](#) ▾

# 5、利用EMBOSS EXPLORER 分析其核酸序列及氨基酸序列的相似性

## ALIGNMENT CONSENSUS

[cons](#)  
[consambig](#)  
[megamerger](#)  
[merger](#)

## ALIGNMENT DIFFERENCES

[diffseq](#)

## ALIGNMENT DOT PLOTS

[dotmatcher](#)  
[dotpath](#)  
[dottup](#)  
[polydot](#)

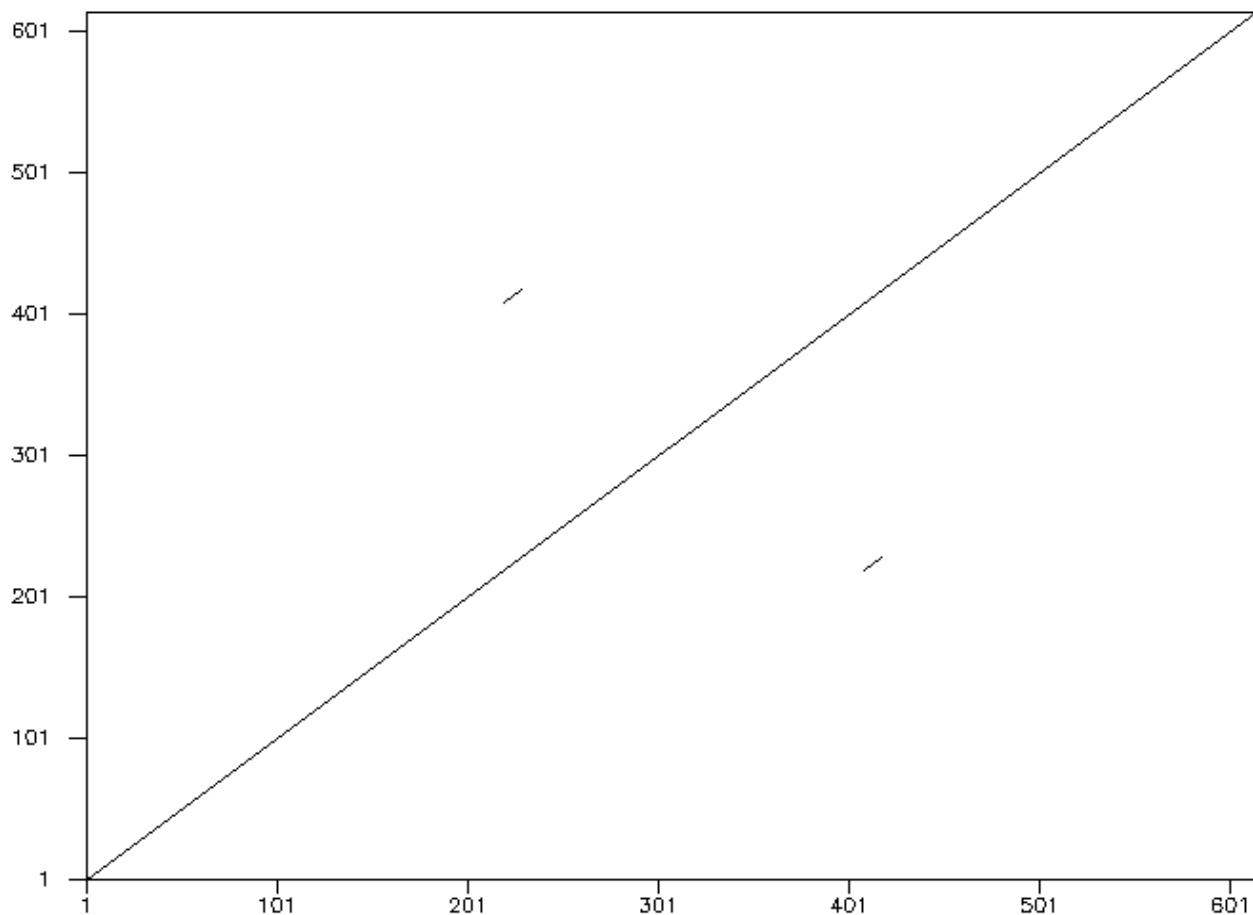
## ALIGNMENT GLOBAL

[est2genome](#)  
[needle](#)  
[needleall](#)  
[stretcher](#)

## ALIGNMENT LOCAL

[matcher](#)  
[segmatchall](#)  
[supermatcher](#)  
[water](#)

Dottup: raw::/var/lib/emboss-explorer/output/407255/.ase...  
Tue 19 Jun 2018 15:30:01



DNA序列相似性

**ALIGNMENT  
CONSENSUS**

- [cons](#)
- [consambig](#)
- [megamerger](#)
- [merger](#)

**ALIGNMENT  
DIFFERENCES**

- [diffseq](#)

**ALIGNMENT  
DOT PLOTS**

- [dotmatcher](#)
- [dotpath](#)
- [dottup](#)
- [polydot](#)

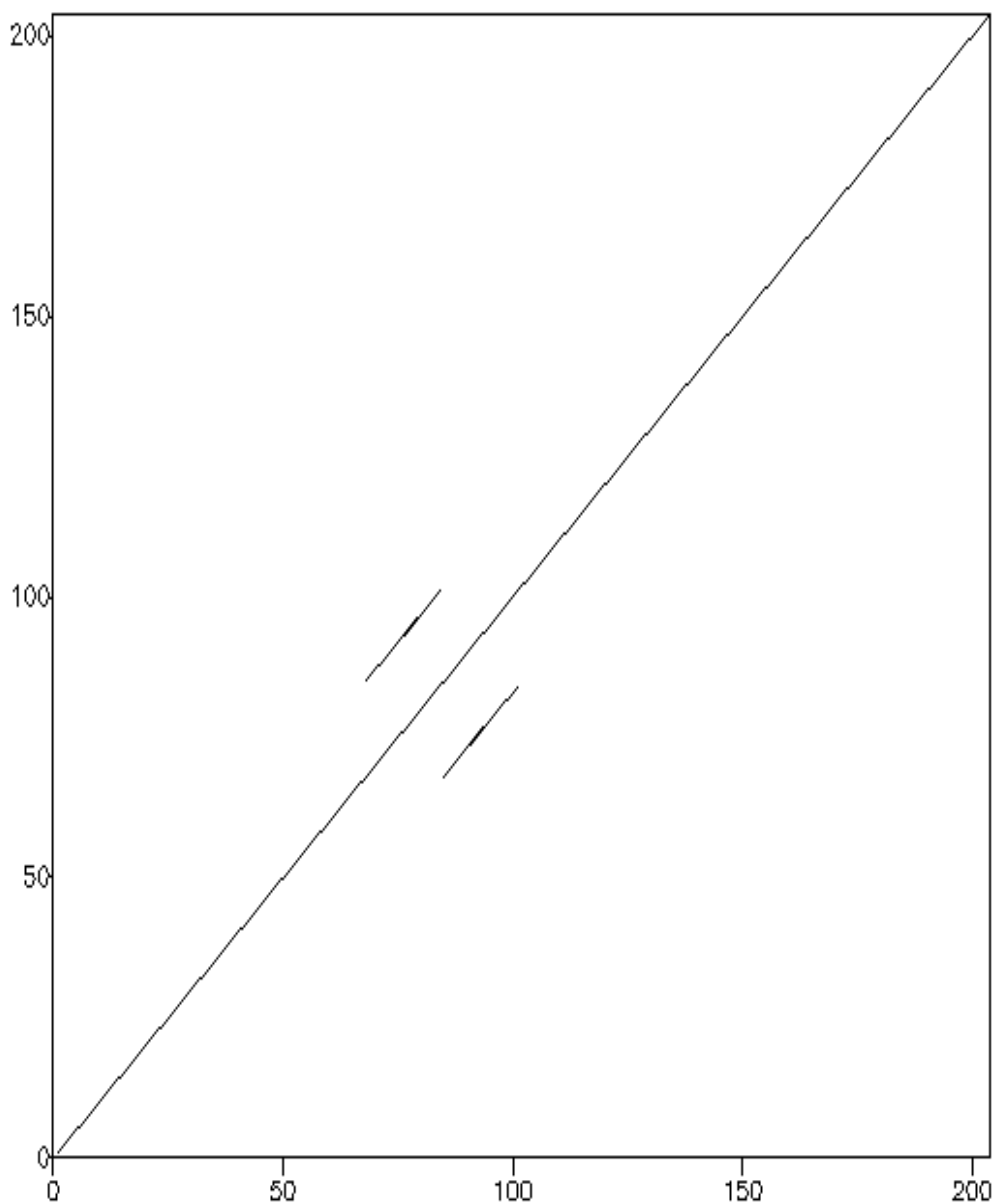
**ALIGNMENT  
GLOBAL**

- [est2genome](#)
- [needle](#)
- [needleall](#)
- [stretcher](#)

**ALIGNMENT  
LOCAL**

- [matcher](#)
- [seqmatchall](#)
- [supermatcher](#)
- [water](#)

Dotmatcher: raw::/var/lib/emboss-explorer/output/363167/...  
(window size = 10, threshold = 23.00 19/06/18)



# 氨基酸序列相似性



# 6、利用CBS对SOD进行结构预测

CBS:



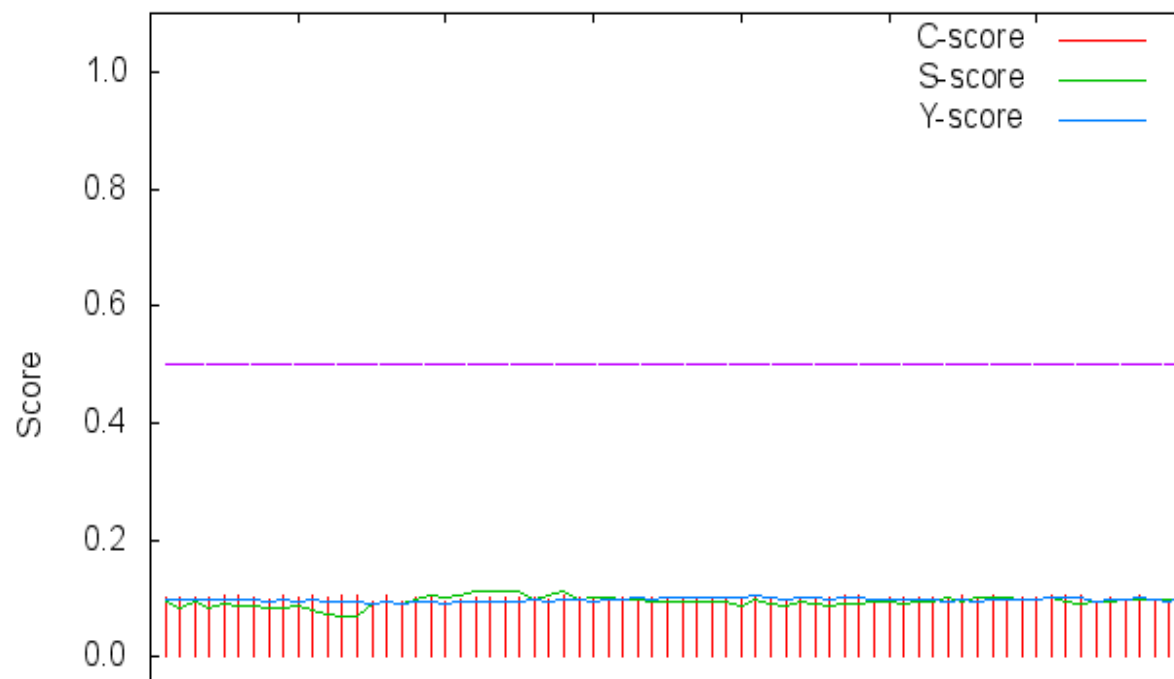
SignalP 4.1 Server - prediction results

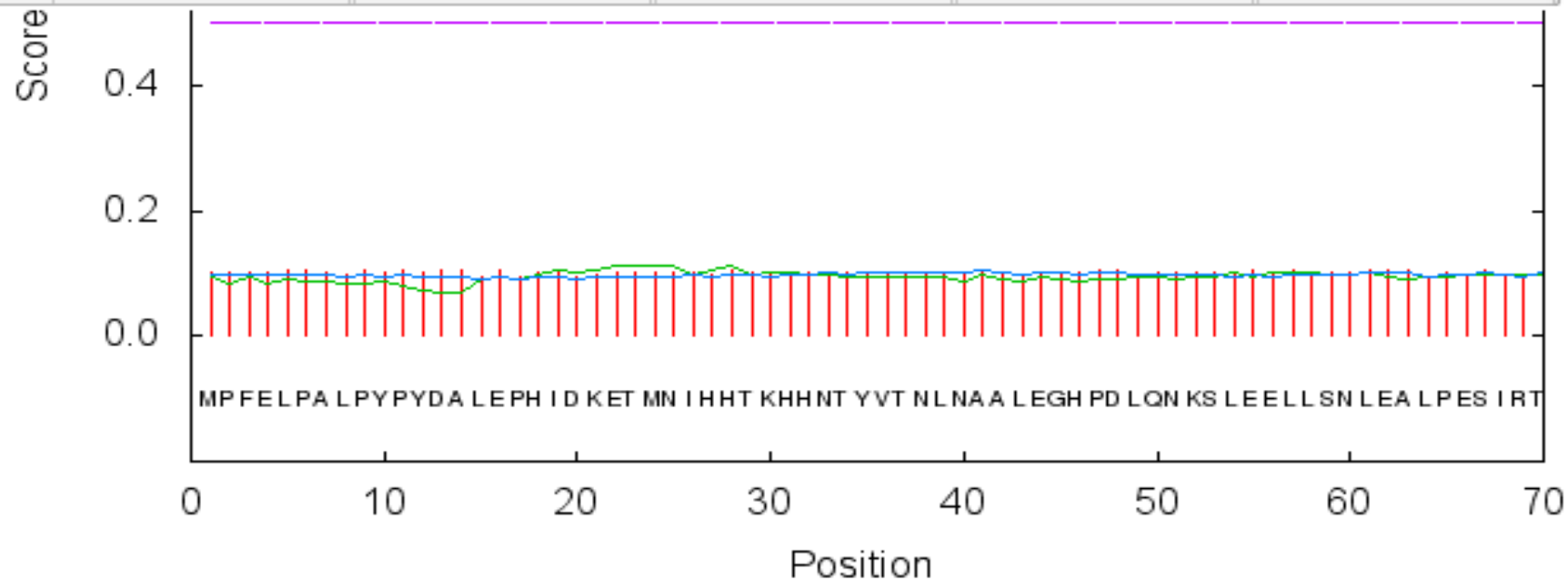
Technical University of Denmark

# SignalP-4.1 euk predictions

>tr\_E7D087\_E7D087\_9BACI Superoxide dismutase OS\_Geobacillus sp. POT5 OX\_415964 PE\_3 SV\_1

SignalP-4.1 prediction (euk networks): tr\_E7D087\_E7D087\_9BACI





```

# Measure  Position  Value  Cutoff  signal peptide?
max. C      41      0.107
max. Y      41      0.105
max. S      25      0.114
mean S     1-40     0.095
D           1-40     0.099  0.450  NO
Name=tr_E7D087_E7D087_9BACI      SP='NO' D=0.099 D-cutoff=0.450 Networks=SignalP-noTM
# data
# gnuplot script

```

Please cite:

SignalP 4.0: discriminating signal peptides from transmembrane regions  
 Petersen TN., Brunak S., von Heijne G. & Nielsen H.  
 Nature Methods, 8:785-786, 2011

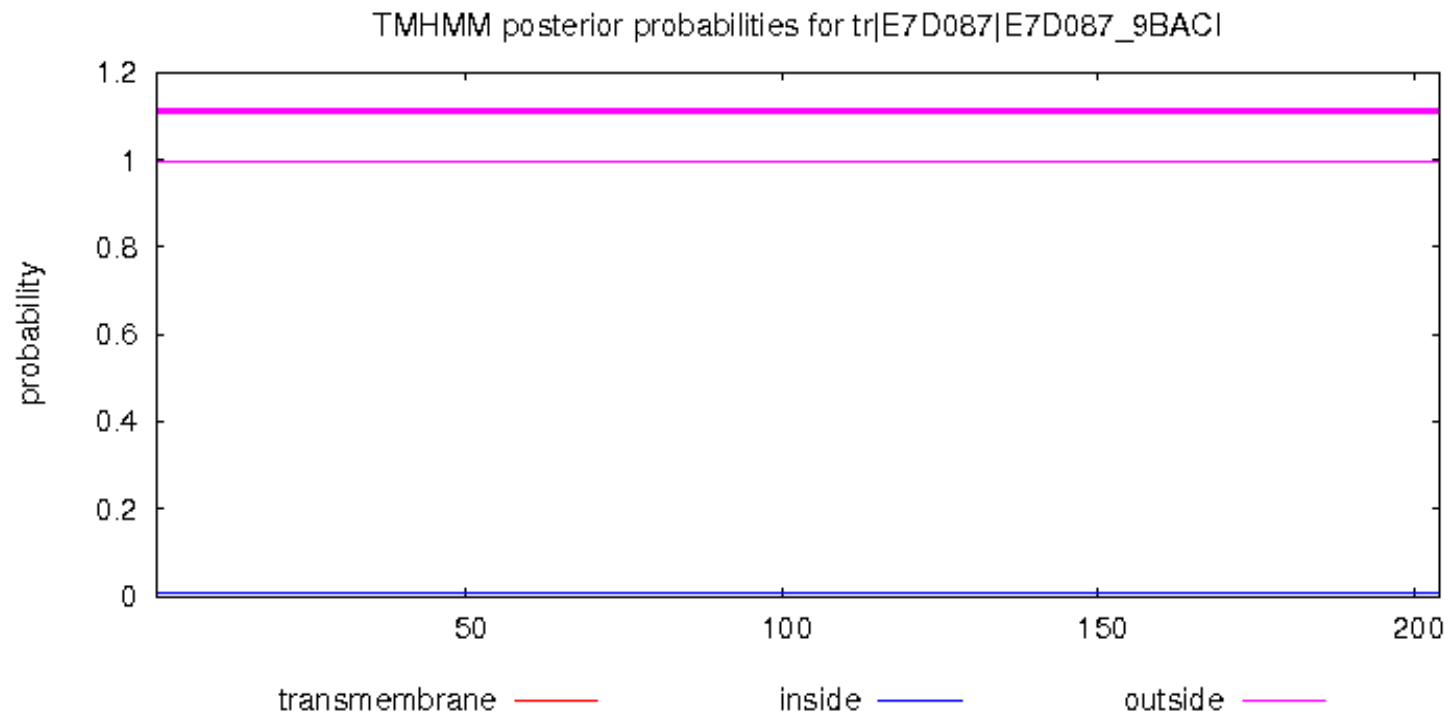
[Explain](#) the output. Go [back](#).

# CBS TMHMM 跨膜区域预测

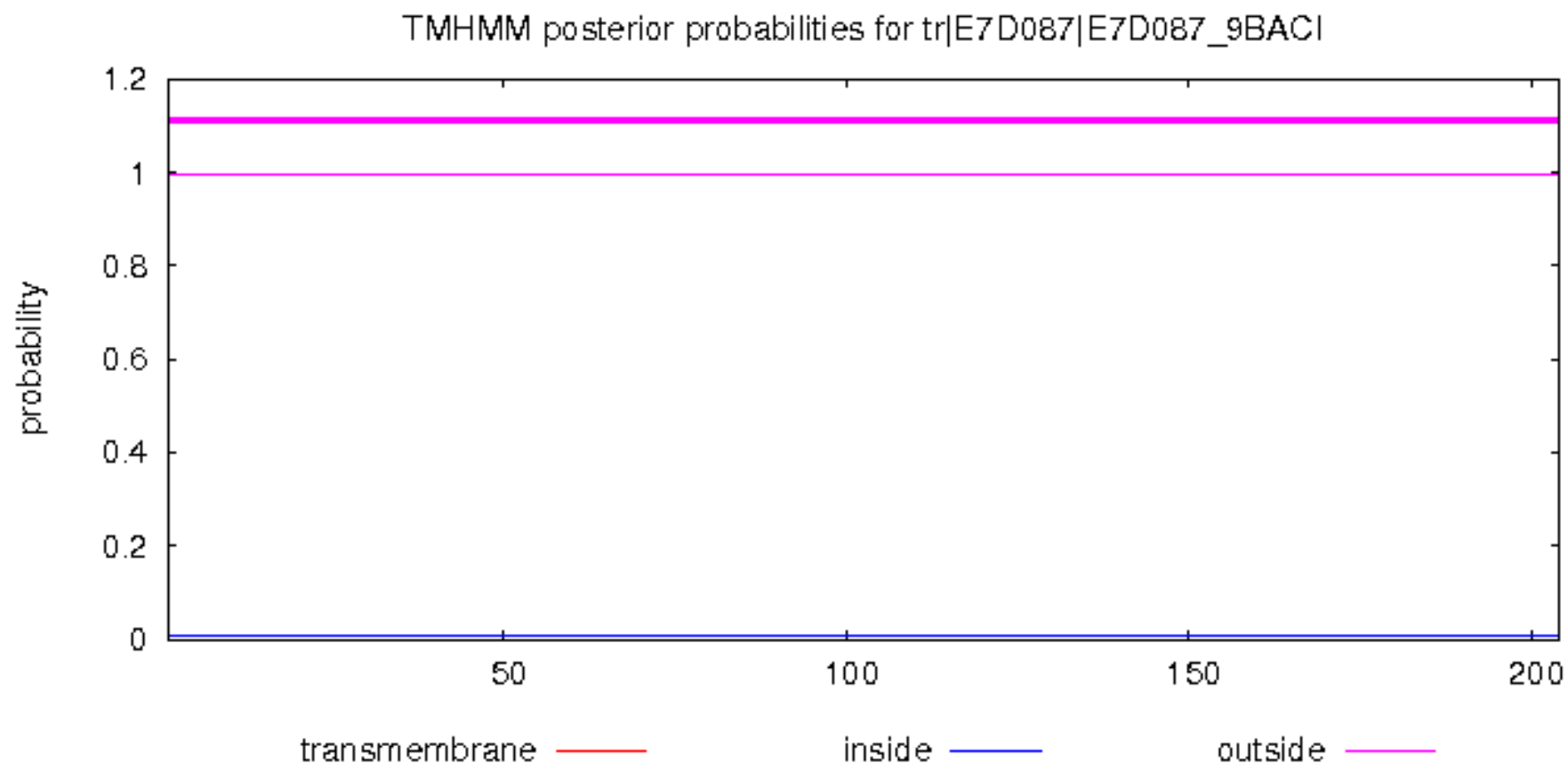
## TMHMM result

[HELP](#) with output formats

```
# tr|E7D087|E7D087_9BACI Length: 204
# tr|E7D087|E7D087_9BACI Number of predicted TMHs: 0
# tr|E7D087|E7D087_9BACI Exp number of AAs in TMHs: 0.00149
# tr|E7D087|E7D087_9BACI Exp number, first 60 AAs: 0
# tr|E7D087|E7D087_9BACI Total prob of N-in: 0.00655
tr|E7D087|E7D087_9BACI TMHMM2.0 outside 1 204
```



```
# tr|E7D087|E7D087_9BACI Length: 204
# tr|E7D087|E7D087_9BACI Number of predicted TMHs: 0
# tr|E7D087|E7D087_9BACI Exp number of AAs in TMHs: 0.00149
# tr|E7D087|E7D087_9BACI Exp number, first 60 AAs: 0
# tr|E7D087|E7D087_9BACI Total prob of N-in: 0.00655
tr|E7D087|E7D087_9BACI TMHMM2.0 outside 1 204
```



# [plot](#) in postscript, [script](#) for making the plot in gnuplot, [data](#) for plot

# ProtScale进行疏水性、亲水性分析

## ProtScale

### User-provided sequence:

```
10      20      30      40      50      60
MPFELPALPY PYDALEPHID KETMNIHHTK HHNTYVTNLN AALEGHPDLQ NKSLEELLSN

70      80      90      100     110     120
LEALPESIRT AVRNNGGGHA NHSLFWTILS PNGGGEPTGE LAEAINKKFG SFTAFKDEFS

130     140     150     160     170     180
KAAAGRFGSG WAWLVVWNGE LEITSTPNQD SPIMECKTPI LGLDVWEHAY YLKYQNRPE

190     200
YIAAFWNIVN WDEVAKRYSE AKAK
```

SEQUENCE LENGTH: 204

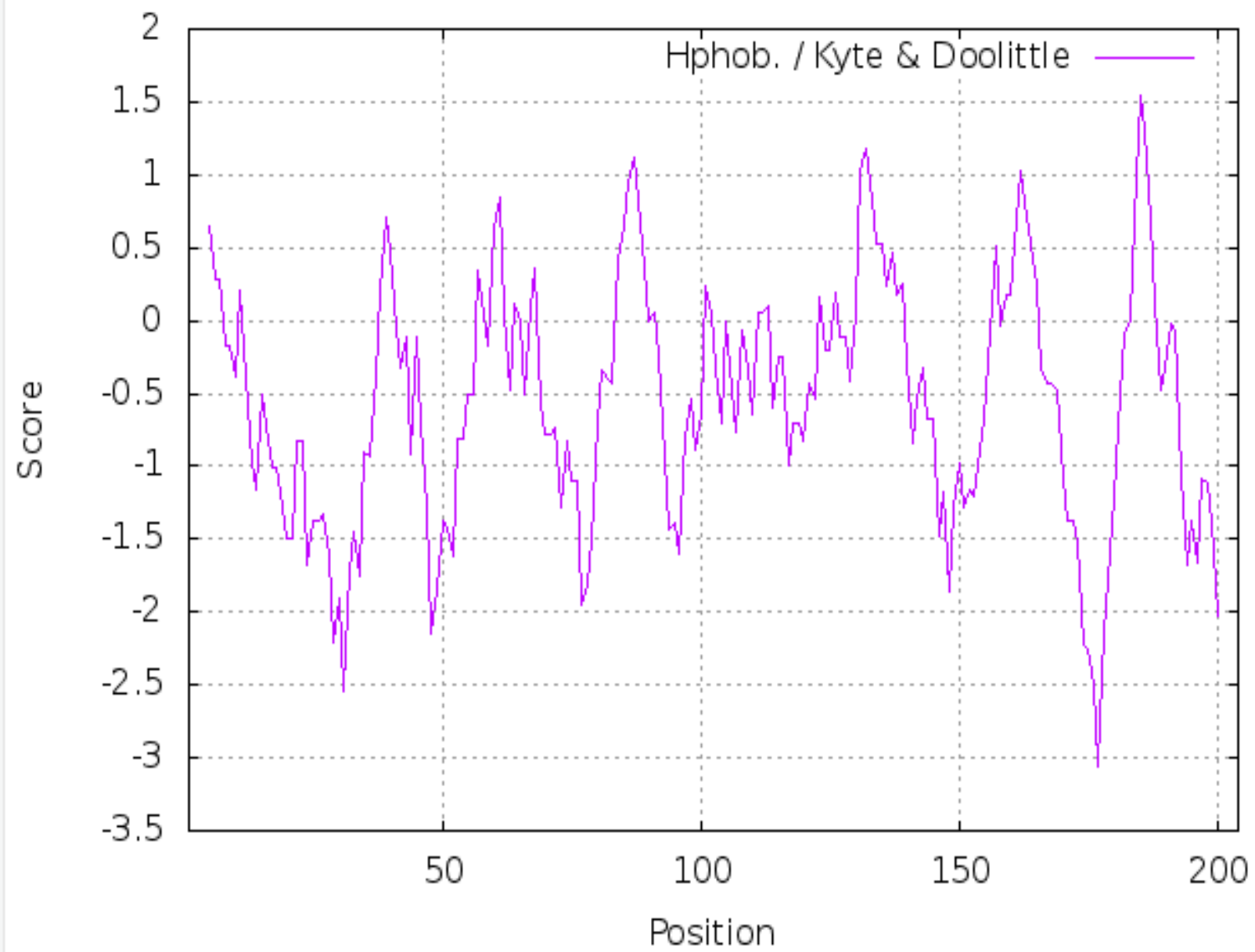
Using the scale [Hphob. / Kyte & Doolittle](#), the individual values for the 20 amino acids are:

```
Ala: 1.800  Arg: -4.500  Asn: -3.500  Asp: -3.500  Cys: 2.500  Gln: -3.500
Glu: -3.500  Gly: -0.400  His: -3.200  Ile: 4.500  Leu: 3.800  Lys: -3.900
Met: 1.900  Phe: 2.800  Pro: -1.600  Ser: -0.800  Thr: -0.700  Trp: -0.900
Tyr: -1.300  Val: 4.200   : -3.500   : -3.500   : -0.490
```

Weights for window positions 1,...,9, using [linear weight variation model](#):

```
1 2 3 4 5 6 7 8 9
1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00
edge center edge
```

ProtScale output for user\_sequence



# SMART结构域预测

## Domains within *Bacillus caldotenax* protein SODM\_BACCA (P28760)

Superoxide dismutase [Mn]

+ = - SAVE

Low complexity region

Position: 53 to 67

Information Interactions Orthology

Length 204 aa

Source database UniProt

Identifiers SODM\_BACCA, P28760

The SMART diagram above represents a summary of the results shown below. Domains with scores less significant than established cutoffs are not shown in the diagram. Features are also not shown when two or more occupy the same piece of sequence, the priority for display is given by SMART > PFAM > PRO-SPERO repeats > Signal peptide > Transmembrane > Coiled coil > Unstructured regions > Low complexity. In either case, features not shown in the above diagram are marked as 'overlap' in the right side table below.

### Confidently predicted domains, repeats, motifs and features:

Name	Start	End	E-value
<a href="#">low complexity</a>	53	67	N/A

Click on a row to highlight the feature in the diagram above. Click the feature name for more information.

### Features NOT shown in the diagram: [?](#)

There are no hidden domains or features present.

Click on a row to highlight the feature in the diagram above. Click the feature name for more information.





# 8、Uniprot的蛋白结构



Sequence Features



Metal binding

InterPro

Sequence Alignments ^

Superoxide dismutase [Mn]

Toggle Identical (AB)

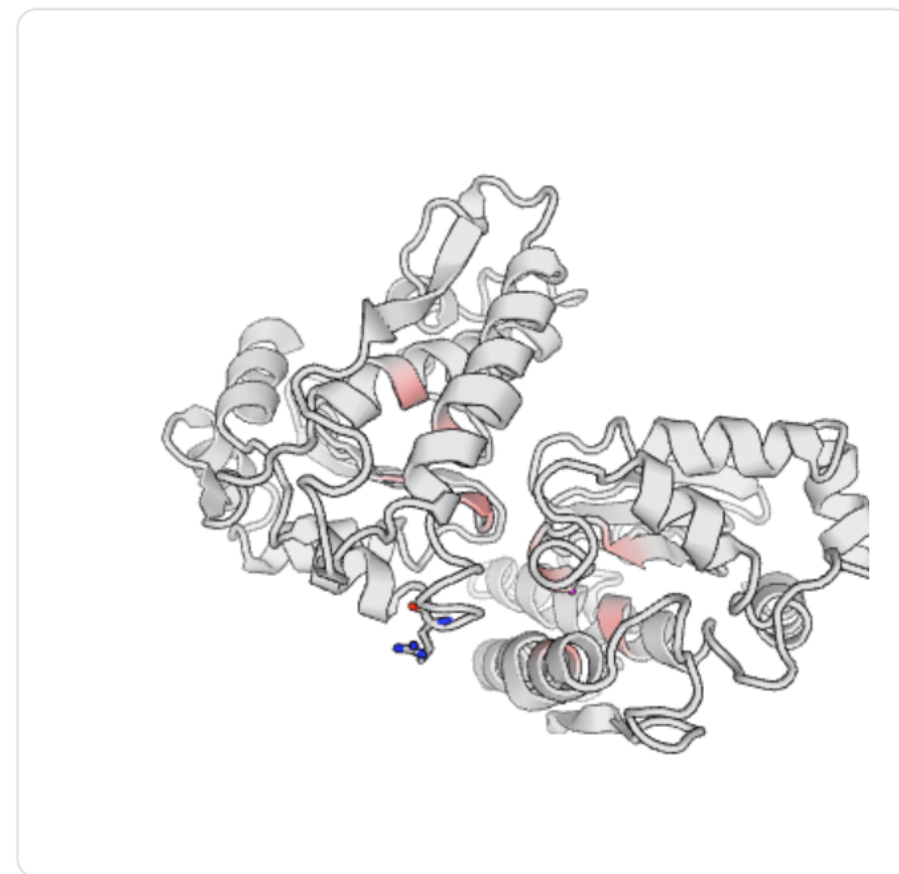
```
MODEL:A PFELPALPYPYDALEPHIDKETMNIHHTKHHNTYVTNLNAALEGHDPDLQNKSLLELLSNLEALPESIRTA 71
MODEL:B PFELPALPYPYDALEPHIDKETMNIHHTKHHNTYVTNLNAALEGHDPDLQNKSLLELLSNLEALPESIRTA 71
2rcv.1.A AYELPELPYAYDALEPHIDKETMTIHHTKHHNTYVTNLNKAVEGNTALANKSVEELVADLDSVPENIRTA 70

MODEL:A VRNNGGGHANHSLFWTILSPNGGGEPTGELAEAINKKFGSFTAFKDEFSKAAAGRFGSGWAWLVVNNGEL 141
MODEL:B VRNNGGGHANHSLFWTILSPNGGGEPTGELAEAINKKFGSFTAFKDEFSKAAAGRFGSGWAWLVVNNGEL 141
2rcv.1.A VRNNGGGHANHKLFWTLLSPNGGGEPTGALAEIINSVFGSFDKFKEQFAAAAAGRFGSGWAWLVVNNGKL 140

MODEL:A EITSTPNQDSPIMEGKTPILGLDVWEHAYYLKYQNRPEYIAAFWNIVNWDEVAKRYSEA 201
MODEL:B EITSTPNQDSPIMEGKTPILGLDVWEHAYYLKYQNRPEYIAAFWNIVNWDEVAKRYSEA 201
2rcv.1.A EITSTPNQDSPLSEGKTPILGLDVWEHAYYLNKYQNRDPDYISAFWNVVNWDEVARLYSER 200
```

Homology models

Oligo-state	Ligands	QMEAN	Template	Range	Seq id (%)	Report	Download
homo-2-mer	2×MN;	0.65	2rcv.1.A		79.70		



Colours ⚙

PV ▲

Cartoon ▲



# 9、利用NCBI 进行引物设计

PrimerSelect - [Primer Catalog]

File Edit Conditions Locate Log Report Options Net Search Window Help

✓	lgth	Tm	GC	Sequence	Note
✓	25	64.4 °C	44.0 %	ATGCCATTTGAATTGCCAGCATTGC	
✓	28	62.8 °C	50.0 %	TTACTTCGCTTCGCTTCGCTGTACC	

# 总结：

该蛋白无信号肽，无跨膜结构域；

是亲水性蛋白质；

与热坚芽孢杆菌分离出来的SOD亲缘关系最为相近；

其高级结构主要由两个同源亚基构成，每个亚基包括4个 $\alpha$ 螺旋和2个 $\beta$ 折叠；

每个亚基有四个金属结合位点，分别与三个组氨酸和一个天冬氨酸结合；

得到了一对引物，可以用于后续的克隆实验中。



*			*	*	*		*	*	*		*	*		*	*	*	*	*	*	*	*		*	*		*	*		*	*		*	*		*	*		*	*		*	*															
M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	T	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	A	D	L	Q	N	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	A	D	L	Q	N	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	S	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	P	Y	D	A	L	E	P	Y	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	D	L	Q	N	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	A	D	L	Q	N	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	T	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	V	D	L	Q	N	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	Q	A	D	L	Q	N	K	S	L	E	E	L	L
M	P	F	E	L	P	A	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	A	D	L	Q	N	K	S	L	E	E	L	L
M	A	F	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	N	A	L	E	G	N	E	E	L	L	S	K	T	V	E	E	V	I
M	A	Y	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	I	T	N	V	N	A	A	L	E	G	N	A	D	L	A	G	K	S	V	E	E	L	V
M	A	F	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	N	D	E	L	L	N	K	S	V	E	E	V	I
M	A	F	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	P	E	L	Q	N	K	T	I	E	E	L	M
M	A	F	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	V	T	N	L	N	A	A	L	E	G	H	A	D	L	Q	N	K	S	I	E	E	L	L
M	A	Y	E	L	P	Q	L	P	Y	A	Y	D	A	L	E	P	H	I	D	K	E	T	M	N	I	H	H	T	K	H	H	N	T	Y	I	T	N	V	N	A	A	L	E	G	-	S	D	L	L	N	K	S	V	E	E	L	I

氨基酸单位点突变

氨基酸双位点突变

分子克隆构建载体

原核表达

酶活测定以及SDS-PAGE

THANKS  
FOR  
LISTENING!

