

Analysis of gene and protein of hydroxylamine oxidase

G03: 张宇、许欣颖、秦超、孔垂正

技术路线

- 找已报道序列---Uniprot, Blast
- 多序列比对---MEGA6
- 建进化树---MEGA6
- 核苷酸序列分析---WebLab
- 蛋白序列分析---WebLab
- 引物设计扩增*hao*基因序列---Primer5
- 构建载体---Vector
- 蛋白模型---Phyre2
- 模型分析---Swiss-PdbViewer

找已报道序列---Uniprot

Filter byⁱ

 Reviewed (7)
Swiss-Prot ✕

Popular organisms

Human (2)

Mouse (2)

Bovine (1)

Rat (1)

NITEU (1)

Search terms


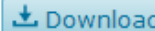

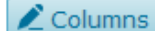

Filter "hao" as:

gene name ✕










View by

Taxonomy

Keywords

BLAST     

1 to 7 of 7 Show 25 ▾

<input type="checkbox"/>	Entry	Entry name		Protein names	Gene names	Organism	Length	
<input type="checkbox"/>	Q07523	HAOX2_RAT		Hydroxyacid oxidase 2	Hao2 Hao3,Haox2	Rattus norvegicus (Rat)	353	
<input type="checkbox"/>	Q50925	HAO_NITEU		Hydroxylamine oxidoreductase	hao1 NE2044 hao2 NE0962 hao3 NE2339	Nitrosomonas europaea (strain ATCC 19718 / NBRC 14298)	570	
<input type="checkbox"/>	Q9UJM8	HAOX1_HUMAN		Hydroxyacid oxidase 1	HAO1 GOX1,HAOX1	Homo sapiens (Human)	370	
<input type="checkbox"/>	Q9NYQ2	HAOX2_MOUSE		Hydroxyacid oxidase 2	Hao2 Hao3,Haox2	Mus musculus (Mouse)	353	
<input type="checkbox"/>	Q9NYQ3	HAOX2_HUMAN		Hydroxyacid oxidase 2	HAO2 HAOX2,GIG16	Homo sapiens (Human)	351	
<input type="checkbox"/>	Q9WU19	HAOX1_MOUSE		Hydroxyacid oxidase 1	Hao1 Gox1,Hao-1	Mus musculus (Mouse)	370	
<input type="checkbox"/>	Q3ZBW2	HAOX2_BOVIN		Hydroxyacid oxidase 2	HAO2	Bos taurus (Bovine)	353	

1 to 7 of 7 Show 25 ▾

蛋白序列

10	20	30	40	50
MRIGEWMRGL	LLCAGLMMCG	VVHADISTVP	DETYDALKLD	RGKATPKETY
60	70	80	90	100
EALVKRYKDP	AHGAGKGTMG	DYWEPIAISI	YMDPNTFYKP	PVSPKEVAER
110	120	130	140	150
KDCVECHSDE	TPVWVRAWKR	STHANLDKIR	NLKSDDPLYY	KKGKLEEVEN
160	170	180	190	200
NLRSMGKLGE	KETLKEVGC	DCHVDVNKKD	KADHTKDIRM	PTADTCGTCH
210	220	230	240	250
LREFAERESE	RDTMVWPNQ	WPAGRPSHAL	DYTANIETTV	WAAMPQREVA
260	270	280	290	300
EGCTMCHTNQ	NKCDNCHTRH	EFSAAESRKP	EACATCHSGV	DHNNWEAYTM
310	320	330	340	350
SKHGKLAEMN	RDKWNWEVRL	KDAFSKGGQN	APTCAACHME	YEGEYTHNIT
360	370	380	390	400
RKTRWANYPF	VPGIAENITS	DWSEARLDSW	VLCTQCHSE	RFARSYLDLM
410	420	430	440	450
DKGTLEGLAK	YQEANAIVHK	MYEDGTLTGQ	KTNRPNPPEP	EKPGFGIFTQ
460	470	480	490	500
LFWSKGNPA	SLELKVLEMA	ENNLAKMHVG	LAHVNPGGWT	YTEGWGPMNR
510	520	530	540	550
AYVEIQDEYT	KMQELSALQA	RVNKLEKQT	SLLDLKGTGE	KISLGGGG
560	570			
MLLAGALALI	GWRKRKQTRA			

blastp同源比对

blastn **blastp** blastx tblastn tblastx

BLASTP programs search protein databases

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#) [Query subrange](#) [?](#)

From
To

```
SKHGKLAEMNRDKWNWEVRLKDAFSKGGQNAPTCAACHMEYEGEYTHNITRKRTRWANYPF
VPGIAENITSDWSEARLDSWVLTCTQCHSERFARSYLDLMDKGTLEGLAKYQEAANAIVHK
MYEDGTLTGQRTNRPNPPEPEKPGFGIFTQLFWSKGNPASLELKVLEMAENNLAKMHVG
LAHVNPGGWYTYTEGWGPMNRAYVEIQDEYTKMQELSALQARVKNLEGGKQTSLLDLKGTGE
KISLGGGLGGMLLAGALALIGWRKRKQTRA
```

Or, upload file 未选择任何文件 [?](#)

Job Title
Enter a descriptive title for your BLAST search [?](#)

[Align two or more sequences](#) [?](#)

Choose Search Set

Database ?

Organism Optional

Exclude Optional

Entrez Query Optional

- Non-redundant protein sequences (nr)
- Reference proteins (refseq_protein)
- UniProtKB/Swiss-Prot (swissprot)
- Patented protein sequences (pat)
- Protein Data Bank proteins (pdb)
- Metagenomic proteins (env_nr)
- Transcriptome Shotgun Assembly proteins (tsa_nr)

Exclude
top taxa will be shown. [?](#)
sequences

[YouTube](#) [Create custom database](#)

Enter an Entrez query to limit search [?](#)

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

Alignments Download GenPept Graphics Distance tree of results Multiple alignment							
	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	hydroxylamine oxidoreductase [Nitrosomonas europaea]	1196	1196	100%	0.0	100%	WP_011111571.1
<input type="checkbox"/>	hydroxylamine oxidoreductase [Nitrosomonas europaea]	1193	1193	100%	0.0	99%	KXK48351.1
<input type="checkbox"/>	hydroxylamine oxydoreductase [Nitrosomonas sp. ENI-11]	1193	1193	100%	0.0	99%	BAA82703.1
<input type="checkbox"/>	hydroxylamine oxidoreductase [Nitrosomonas europaea]	1192	1192	100%	0.0	99%	AAC43216.1
<input type="checkbox"/>	hydroxylamine oxydoreductase [Nitrosomonas sp. ENI-11]	1191	1191	100%	0.0	99%	BAA82705.1
<input type="checkbox"/>	Chain A, Complex Crystal Structure Of Hydroxylamine Oxidoreductase And Ne1300 From Nitrosomonas Europaea	1144	1144	95%	0.0	100%	4FAS_A
<input type="checkbox"/>	Chain A, X-Ray Structure Of Hydroxylamine Oxidoreductase	1141	1141	95%	0.0	99%	1FGJ_A
<input type="checkbox"/>	hydroxylamine reductase [Nitrosomonas eutropha]	1100	1100	100%	0.0	93%	WP_011634719.1
<input type="checkbox"/>	hydroxylamine reductase [Nitrosomonas eutropha]	1100	1100	100%	0.0	93%	WP_011634833.1
<input type="checkbox"/>	hydroxylamine reductase [Nitrosomonas communis]	951	951	100%	0.0	80%	WP_046848853.1
<input type="checkbox"/>	hydroxylamine reductase [Nitrosomonas communis]	950	950	100%	0.0	80%	WP_046851043.1
<input type="checkbox"/>	hydroxylamine reductase [Nitrosomonas ureae]	932	932	100%	0.0	78%	WP_062557488.1
<input type="checkbox"/>	hydroxylamine reductase [Nitrosomonas sp. AL212]	930	930	100%	0.0	78%	WP_013647701.1
<input type="checkbox"/>	hydroxylamine oxidoreductase [Nitrosomonas cryotolerans ATCC 49181]	929	929	99%	0.0	79%	CCQ48705.1
<input type="checkbox"/>	hydroxylamine reductase [Nitrosomonas sp. Is79A3]	921	921	99%	0.0	78%	WP_013964980.1
<input type="checkbox"/>	hydroxylamine reductase [Nitrospira briensis]	858	858	99%	0.0	72%	WP_025042261.1

多序列比对---MEGA6

The screenshot displays the MEGA6 software interface. The main window, titled "M6: Alignment Explorer", shows a multiple sequence alignment of 10 protein sequences. The alignment is displayed in a table with columns for "Species/Abbrv" and "Group Name". The sequences are aligned, with gaps represented by dashes. The alignment is as follows:

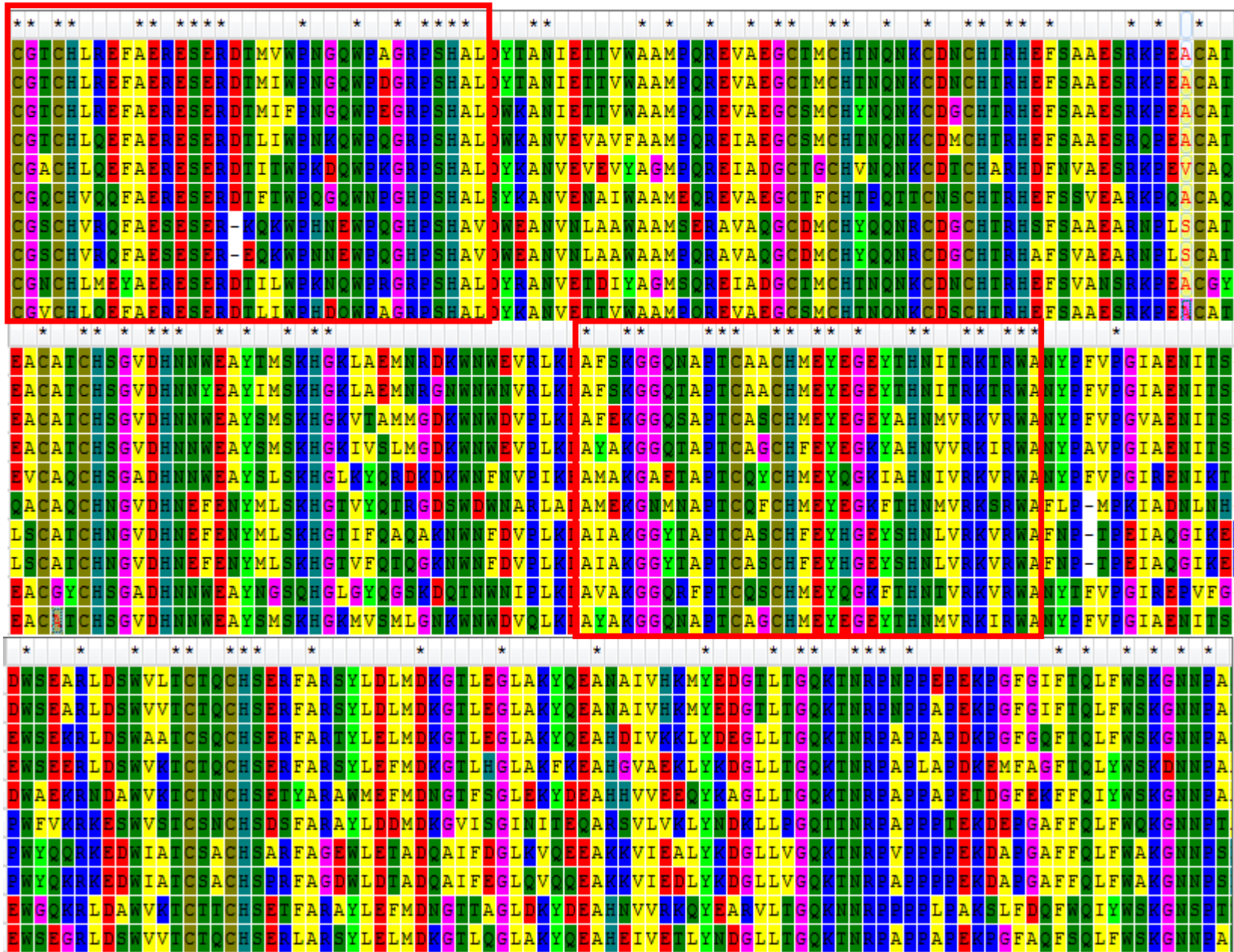
Species/Abbrv	Group Name	Sequence
1. gi 1000286802 gb KXK48351.1 hydroxylamine oxidoreductase N		MRIGEWMR
2. gi 499953985 ref WP_011634719.1 hydroxylamine reductase Ni		MRLGEYLR
3. gi 821529121 ref WP_046851043.1 hydroxylamine reductase Ni		MDARKWLK
4. gi 640614187 ref WP_025042261.1 hydroxylamine reductase Ni		MVVKPLK
5. gi 953252464 emb CUS31385.1 Hydroxylamine dehydrogenase Ca		MTVKHLVK
6. gi 738329532 ref WP_036282308.1 hydroxylamine reductase Me		MFKLKTIL
7. gi 748146778 ref WP_039721486.1 cytochrome c Methylacidiph		MRWAFGFL
8. gi 501441319 ref WP_012464768.1 cytochrome c Methylacidiph		MYKESYQP
9. gi 1028418878 gb OAI48919.1 hydroxylamine reductase Nitros		MTNKFWLR
10. gi 655013550 ref WP_028462535.1 hydroxylamine reductase p		HVDINAKK

The "ClustalW Parameters" dialog box is open on the right side of the screen. It contains the following settings:

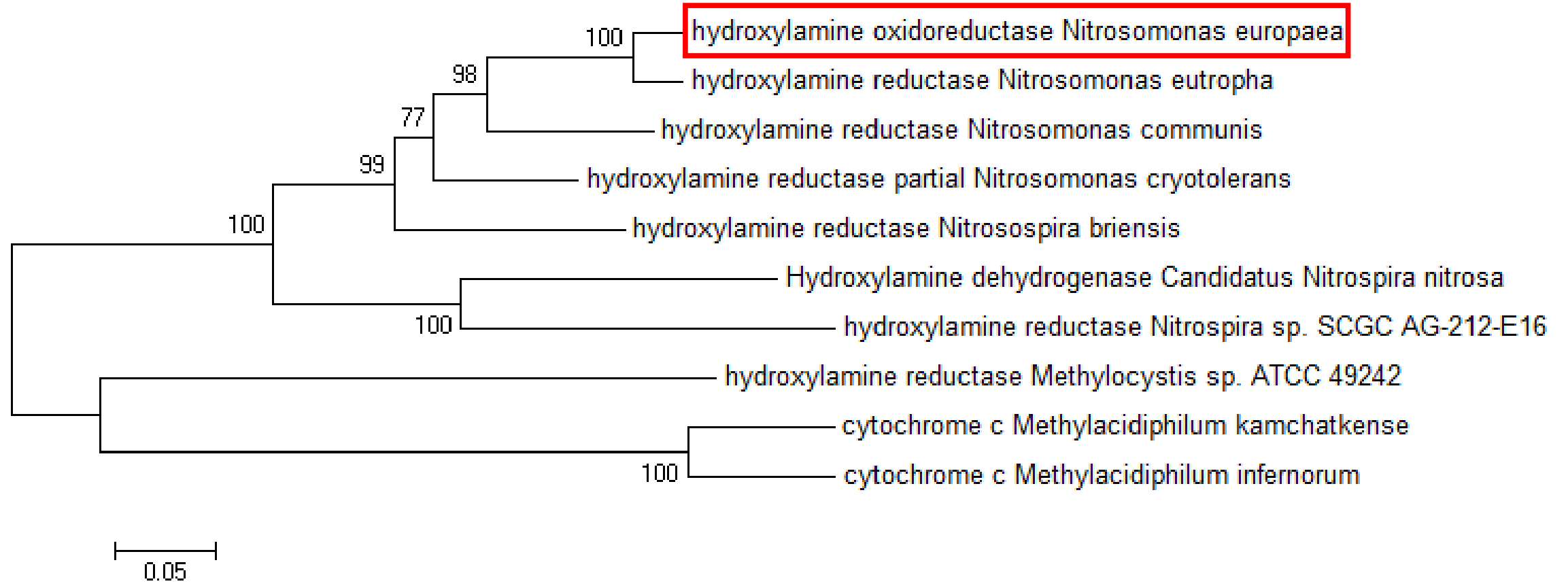
- Protein: Pairwise Alignment
 - Gap Opening Penalty: 10
 - Gap Extension Penalty: 0.1
- Multiple Alignment
 - Gap Opening Penalty: 10
 - Gap Extension Penalty: 0.2
- Protein Weight Matrix: Gonnet
- Residue-specific Penalties: ON
- Hydrophilic Penalties: ON
- Gap Separation Distance: 4
- End Gap Separation: OFF
- Use Negative Matrix: OFF
- Delay Divergent Cutoff (%): 30
- Keep Predefined Gaps:
- Specify Guide Tree: [Empty field]

At the bottom of the dialog box, there are three buttons: "Help", "OK", and "Cancel".

部分比对结果



建进化树---MEGA6



核苷酸序列分析---WebLab

密码子统计程序 Cusp

	HAO
Coding GC	55.59%
1st letter GC	54.06%
2nd letter GC	45.58%
3rd letter GC	67.14%

内切酶分析程序 Remap

共108 个酶及其酶切位点，1-60 位序列的酶切位点

```

                HindIII
                | AluI
                | AluBI
                \ \
AACCTGCGETCGATGGGCAAGCTTGGGGAAAAAGAAACCCTCAAGGAAGTAGGCTGTATC
      10      20      30      40      50      60
-----|-----|-----|-----|-----|-----|-----|
TTGGACGCGAGCTACCCGTTTGAACCCCTTTTTCTTTGGGAGTTCCTTCATCCGACATAG
                / /
                | HindIII
                AluBI
                AluI

```

蛋白序列分析---WebLab

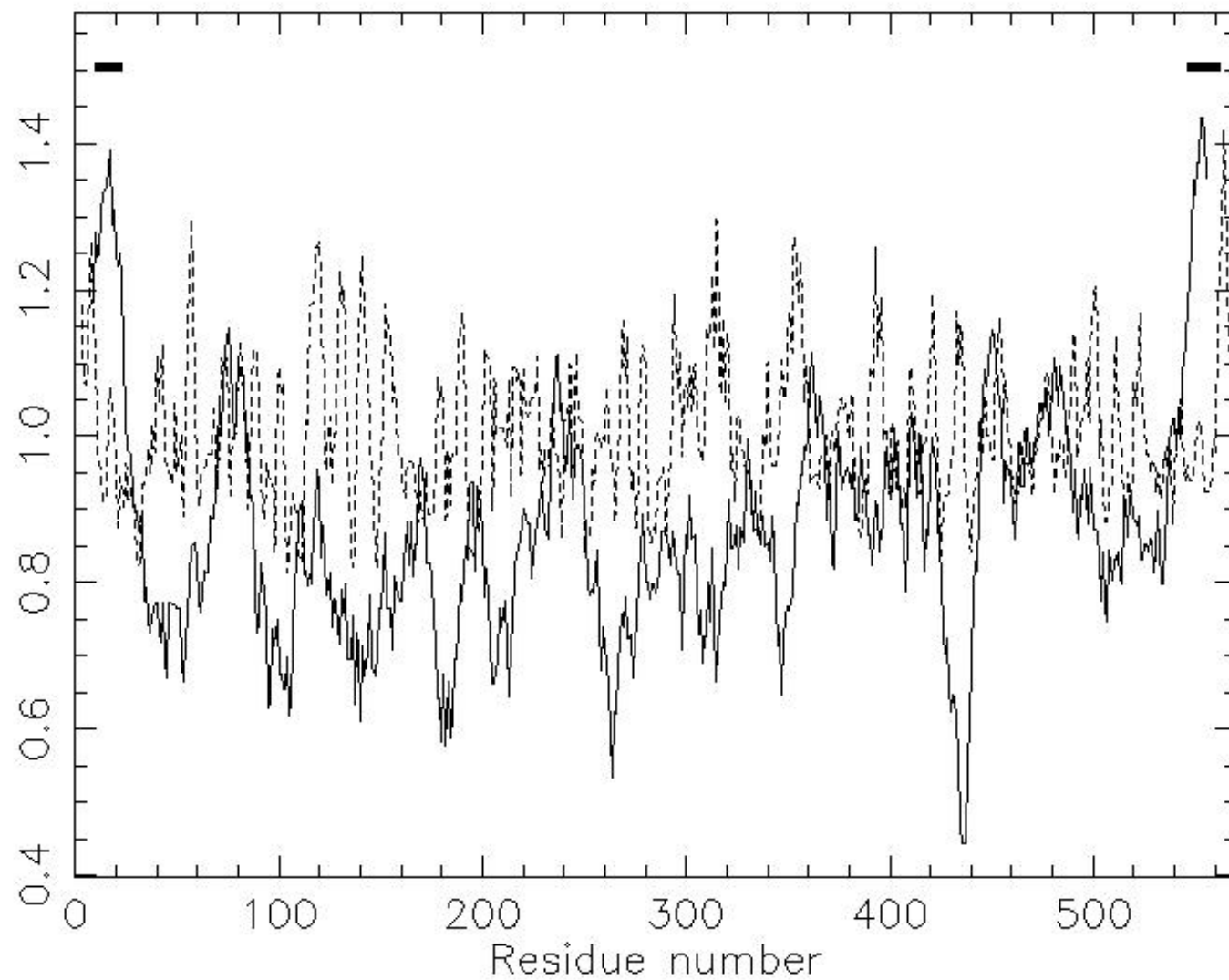
氨基酸组成分析程序 Pepstats

Property	Residues	Number	Mole%
Tiny	(A+C+G+S+T)	175	30.702
Small	(A+B+C+D+G+N+P+S+T+V)	288	50.526
Aliphatic	(A+I+L+V)	134	23.509
Aromatic	(F+H+W+Y)	65	11.404
Non-polar	(A+C+F+G+I+L+M+P+V+W+Y)	290	50.877
Polar	(D+E+H+K+N+Q+R+S+T+Z)	280	49.123
Charged	(B+D+E+H+K+R+Z)	174	30.526
Basic	(H+K+R)	95	16.667
Acidic	(B+D+E+Z)	79	13.860

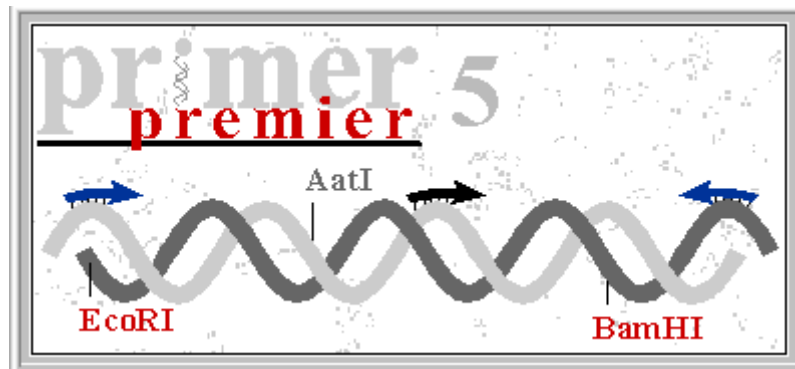
Residue	Number	Mole%	DayhoffStat
A = Ala	48	8.421	0.979
B = Asx	0	0.000	0.000
C = Cys	18	3.158	1.089
D = Asp	31	5.439	0.989
E = Glu	48	8.421	1.404
F = Phe	9	1.579	0.439
G = Gly	45	7.895	0.940
H = His	20	3.509	1.754
I = Ile	17	2.982	0.663
J = ---	0	0.000	0.000
K = Lys	45	7.895	1.196
L = Leu	43	7.544	1.019
M = Met	21	3.684	2.167
N = Asn	29	5.088	1.183
O = ---	0	0.000	0.000
P = Pro	27	4.737	0.911
Q = Gln	13	2.281	0.585
R = Arg	30	5.263	1.074
S = Ser	24	4.211	0.602
T = Thr	40	7.018	1.150
U = ---	0	0.000	0.000
V = Val	26	4.561	0.691
W = Trp	17	2.982	2.294
X = Xaa	0	0.000	0.000
Y = Tyr	19	3.333	0.980
Z = Glx	0	0.000	0.000

跨膜结构预测程序 Tmap

Tmap



引物设计扩增hao基因序列---Primer5



GeneTank - NewSequence

Function: **Primer** (highlighted in red) | Align

Enzyme | Motif

Translation: Active Sequence: NewSequence

Translations: Original Protein | Translated DNA

DNA | Protein

5' Seq No | Header | 3 | 10 | Find | Find Next | S | A | dsDNA | 🔊 | 🗣️

```
1 | TGY GGN ACN TGY CAY YTN MGN GAR TTY GCN GAR MGN GAR WSN GAR MGN
49 | GAY ACN ATG ATH TGG CCN AAY GGN CAR TGG CCN CAR GGN MGN CCN WSN
97 | CAY GCN YTN GCN TTY GCN AAR GGN GGN CAR ACN GCN CCN ACN TGY GCN
145 | WSN TGY CAY ATG GAR TAY GAR GGN GAR TAY ACN CAY AAY ATH GTN MGN
193 | AAR GTN MGN TGG GCN
```

Pos: 00001

各项参数

Primer Premier

Primer: S A **Search** Results Edit Primers

Direct Select:

3' ACRCNTGNACRGTRRANKCNCTYA 5'
 || || || || || || || || || ||
 5' TGYGGNACNTGYCAYYTNMGNARTTYGCNGARMGNARWSNGARMNGAYACNATGATHHTGGCCNAAAYGGNCARTGGCCNC 3'

10 20 30 40 50 60 70 80

C G T C H L R E F A E R E S E R D T M I W P N G Q W P Q

	Rating	Seq No	Length	Tm [°C]	GC%	Δ G [kcal/mol]	Activity [μg/OD]	Degeneracy	Ta Opt [°C]
Sense	100	1	25	0.0	52.0	-41.8	33.3	16384	--
Anti-sense	100	25	25	0.0	52.0	-41.3	32.7	16384	--
Product	100	--	25	70.4	52.0	--	--	--	24.3

	Hairpin	Dimer	False Priming	Cross Dimer
Sense	None	None	None	None
Anti-sense	None	None	None	

No Hairpins Found All

Search Criteria



Search For:

PCR Primers Sequencing Primers Hybridization Probes

Search Type:

Sense Primer Compatible with Sense Primer
 Anti-sense Primer Compatible with Anti-sense Primer
 Both Pairs

Search Ranges:

Sense Primer:

to

Anti-sense Primer:

to

PCR Product Size:

bp to bp

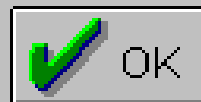
Primer Length:

bp ? bp

Search Mode:

Automatic Manual

Search Parameters



Search Progress

Search Complete.

Primer Search Results:

	Remaining/Rejected	
	Sense:	Anti-sense:
Total Possible	655	195
<input checked="" type="checkbox"/> Tm	8	43
<input checked="" type="checkbox"/> GC%	2	0
<input checked="" type="checkbox"/> Degeneracy	530	103
<input checked="" type="checkbox"/> 3' End Stability	2	0
<input checked="" type="checkbox"/> GC Clamp	15	16
<input checked="" type="checkbox"/> Redundancy	69	28
<input checked="" type="checkbox"/> Repeats/Runs	0	0
<input checked="" type="checkbox"/> Dimer/Hairpin	0	0
<input type="checkbox"/> False Priming		
<input checked="" type="checkbox"/> Optimal Primers	29	5

OK

Cancel

Stringency:


- Very High
- High
- Moderate
- Low
- Very Low
- Manual

Primer Pairs:

Pairs Found

32

结果

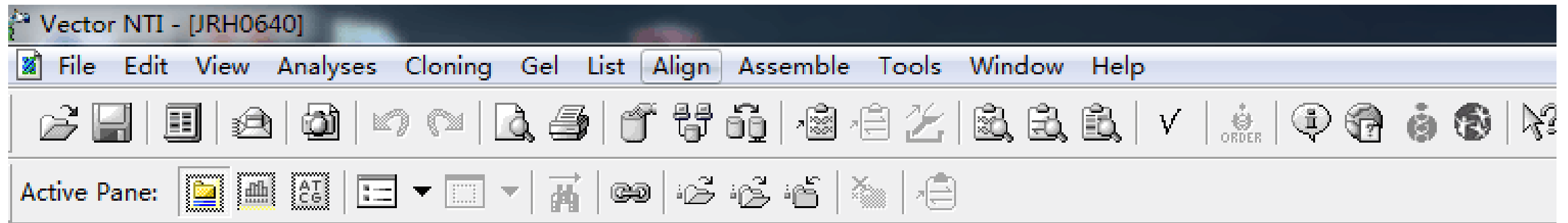
 Search Results

Sense Anti-sense Pairs

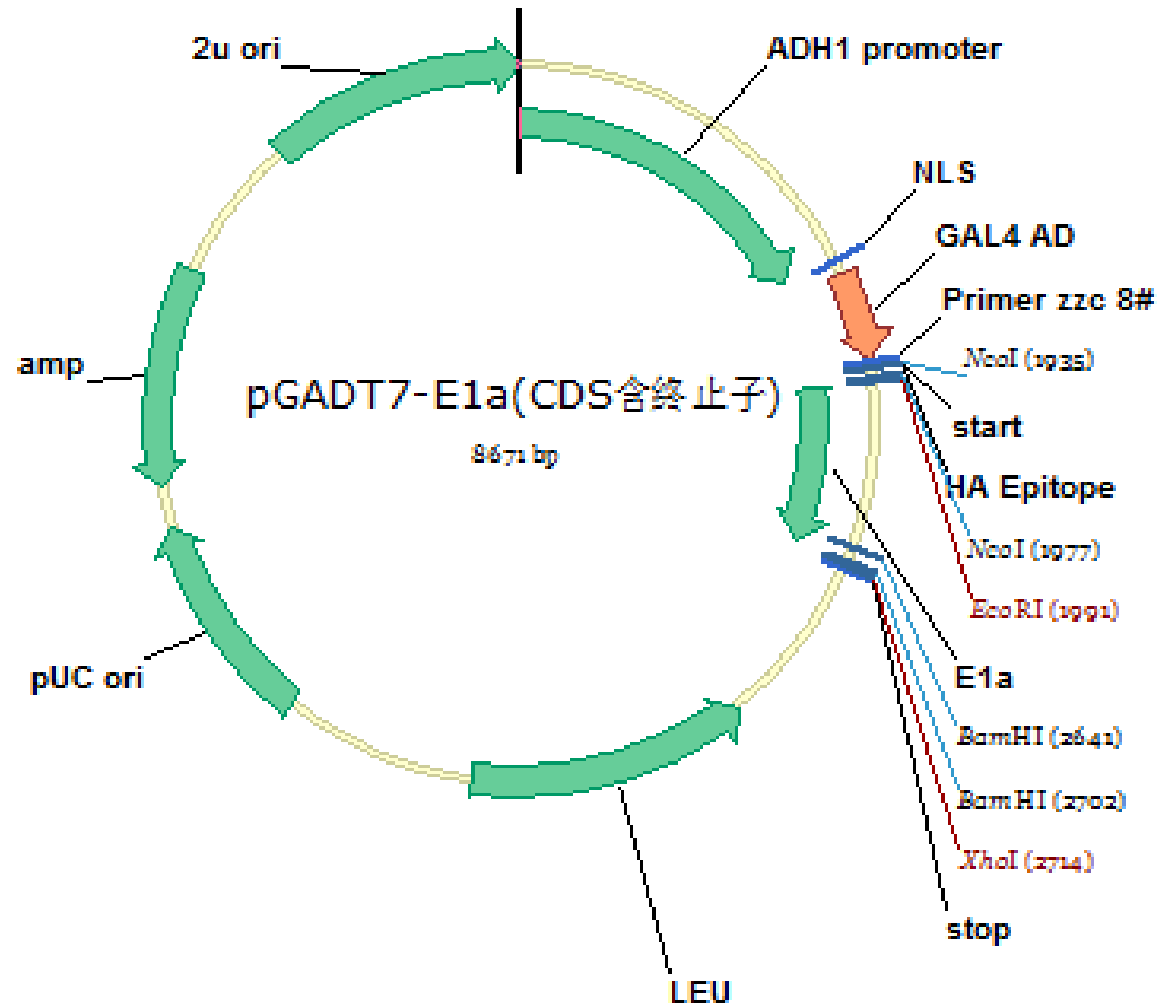
32 pairs found.

#	Rating	Tm [發]	Product Size	Ta Opt [發]	Mark
1	92	0.0 0.0	130	37.2	<input checked="" type="checkbox"/>
2	92	0.0 0.0	128	37.3	<input checked="" type="checkbox"/>
3	92	0.0 0.0	127	37.5	<input checked="" type="checkbox"/>
4	92	0.0 0.0	126	37.4	<input checked="" type="checkbox"/>
5	92	0.0 0.0	125	36.8	<input checked="" type="checkbox"/>
6	92	0.0 0.0	124	36.7	<input checked="" type="checkbox"/>
7	92	0.0 0.0	123	37.0	<input checked="" type="checkbox"/>
8	92	0.0 0.0	122	37.0	<input checked="" type="checkbox"/>
9	92	0.0 0.0	122	36.8	<input checked="" type="checkbox"/>
10	92	0.0 0.0	121	37.0	<input checked="" type="checkbox"/>
11	92	0.0 0.0	120	36.8	<input checked="" type="checkbox"/>
12	91	0.0 0.0	138	37.1	<input type="checkbox"/>
13	91	0.0 0.0	136	37.3	<input type="checkbox"/>
14	91	0.0 0.0	135	37.4	<input type="checkbox"/>
15	91	0.0 0.0	134	37.2	<input type="checkbox"/>
16	91	0.0 0.0	133	37.5	<input type="checkbox"/>

构建载体---Vector



构建载体图



酶切位点

Restriction/Methylation Map: (no more than 1 site)		
<input type="checkbox"/>	ApaI: 2 sites	G TGCAC CACG T
<input type="checkbox"/>	AvaI: 7 sites	C YCGRG GRG C Y C
<input checked="" type="checkbox"/>	BamHI: 1 site	G SATCC CCTAG G
<input type="checkbox"/>	ClaI: 2 sites	AT C GAT TAG C TA
<input checked="" type="checkbox"/>	EcoRI: 1 site	G AATTC CTTAA G
<input checked="" type="checkbox"/>	HindIII: 1 site	A AGCTT TTCGA A
<input checked="" type="checkbox"/>	NcoI: 1 site	C CATGG GGTAC C
<input checked="" type="checkbox"/>	PstI: 1 site	C TGCAG GACG T C
<input checked="" type="checkbox"/>	SmaI: 1 site	C CCGGG GGG C CC
<input checked="" type="checkbox"/>	SpeI: 1 site	A C TAGT TGAT C A
<input checked="" type="checkbox"/>	XmaI: 1 site	C CCGGG GGG C CC

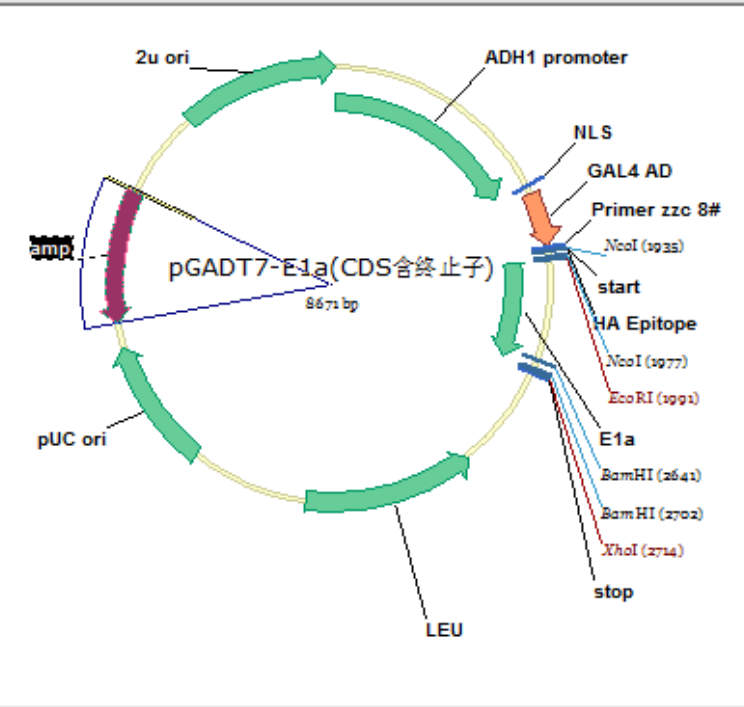


pGADT7-E1a(CDS含终止子)

- General Description
- Standard Fields
- Original Author
- Comments
- Annotations
- Feature Map

Restriction/Methylation Map: (no more than 2 sites)

- ApaI: 5 sites**
GTGCAC
CACGTG
- AvaI: 3 sites**
CTCGRG
GRCYTC
- BamHI: 2 sites**
GGATCC
CCTAGC
- ClaI: 0 sites**
ATCGAT
TAGCTA
- EcoRI: 1 site**
GAATTC
CTTAAG
- HindIII: 3 sites**
AAGCTT
TTCGAA
- NcoI: 2 sites**
CCATGG
GGTACC
- PstI: 6 sites**
CTGCAG
GACCTC



6801	ACTGGTGAGT ACTCAACCAA GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGGCGT CAATACGGGA TAATACCGCG CCACATAGCA TGACCACTCA TGAGTTGGTT CAGTAAGACT CTTATCACAT ACGCCGCTGG CTCAACGAGA ACGGGCCGCA GTTATGCCCT ATTATGGCGC GGTGTATCGT
amp	
6901	GAACITTTAAA AGTGCTCATC ATTGAAAAC GTTCTTCGGG GCGAAAACCTC TCAAGGATCT TACCGCTGTT GAGATCCAGT TCGATGTAAC CCACTCGTGC CTTGAAATTT TCACGAGTAG TAACCTTTTG CAAGAAGCCC CGCTTTTGAG AGTTCCTAGA ATGGCGACAA CTCTAGGTCA AGCTACATTG GGTGAGCAGC
amp	
7001	ACCCAAGTGA TCTTCAGCAT CTTTACTTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT GCCGCAAAA AGGGAATAAG GCGGACACGG TGGGTTGACT AGAAGTCGTA GAAAATGAAA GTGGTCGCAA AGACCCACTC GTTTTTGTCC TTCCGTTTTA CGGCGTTTTT TCCCTTATTC CCGCTGTGCC
amp	
7101	AAATGTTGAA TACTCATACT CTTCTTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCATATGA GCGGATACAT ATTTGAATGT ATTAGAAAA TTTACAACCTT ATGAGTATGA GAAGGAAAAA GTTATAATAA CTCGTAAAT AGTCCCAATA ACAGAGTACT CGCCTATGTA TAAACTTACA TAAATCTTTT
amp	

蛋白模型---Phyre2

Top model

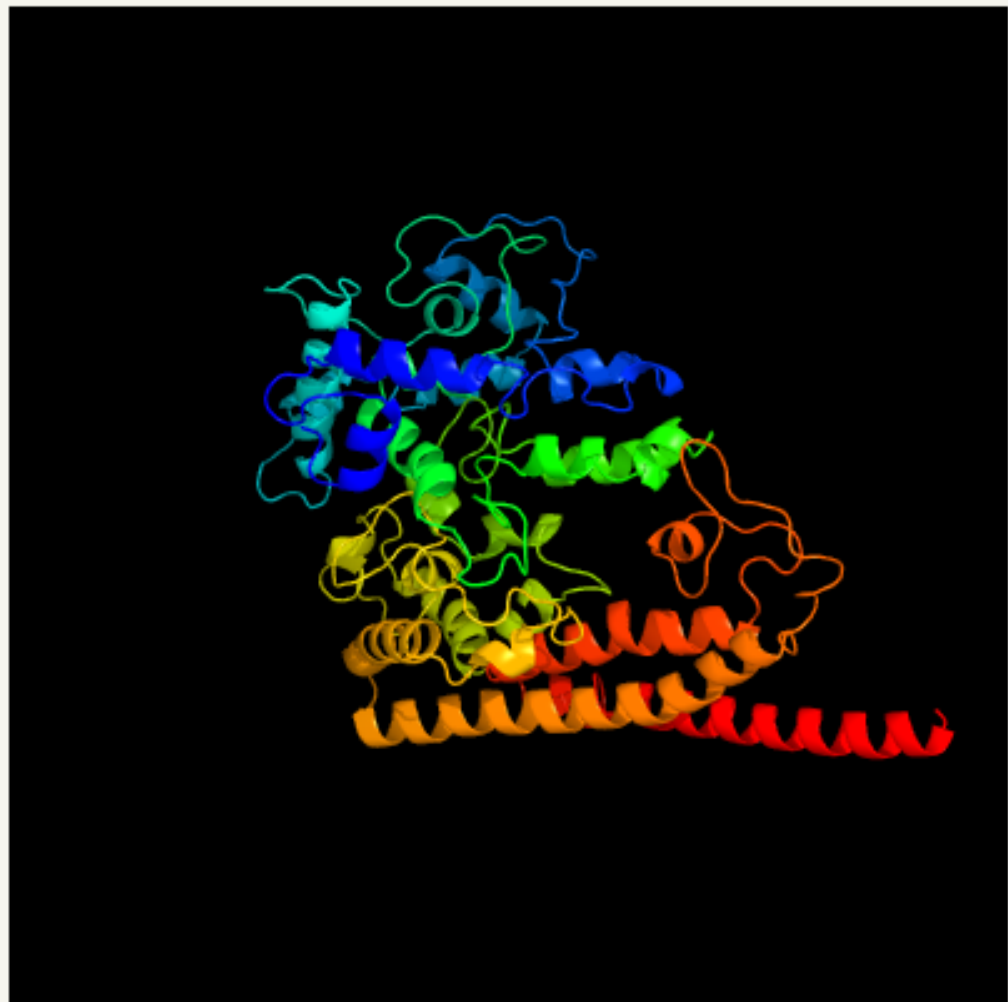


Image coloured by rainbow N → C terminus

Model dimensions (Å): **X**:66.455 **Y**:54.831 **Z**:94.066

Model (left) based on template [c1fgjA](#)

Top template information

PDB header:oxidoreductase

Chain: A: **PDB Molecule:**hydroxylamine
oxidoreductase;

PDBTitle: x-ray structure of hydroxylamine
oxidoreductase

Confidence and coverage

Confidence: **100.0%**

Coverage: **88%**

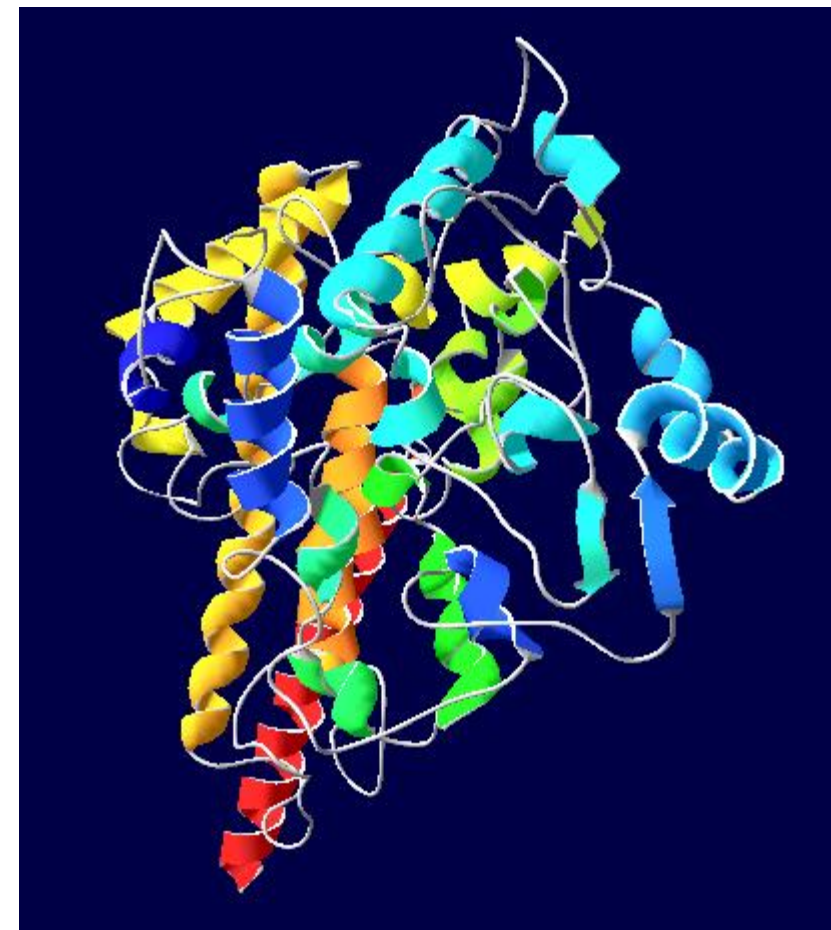
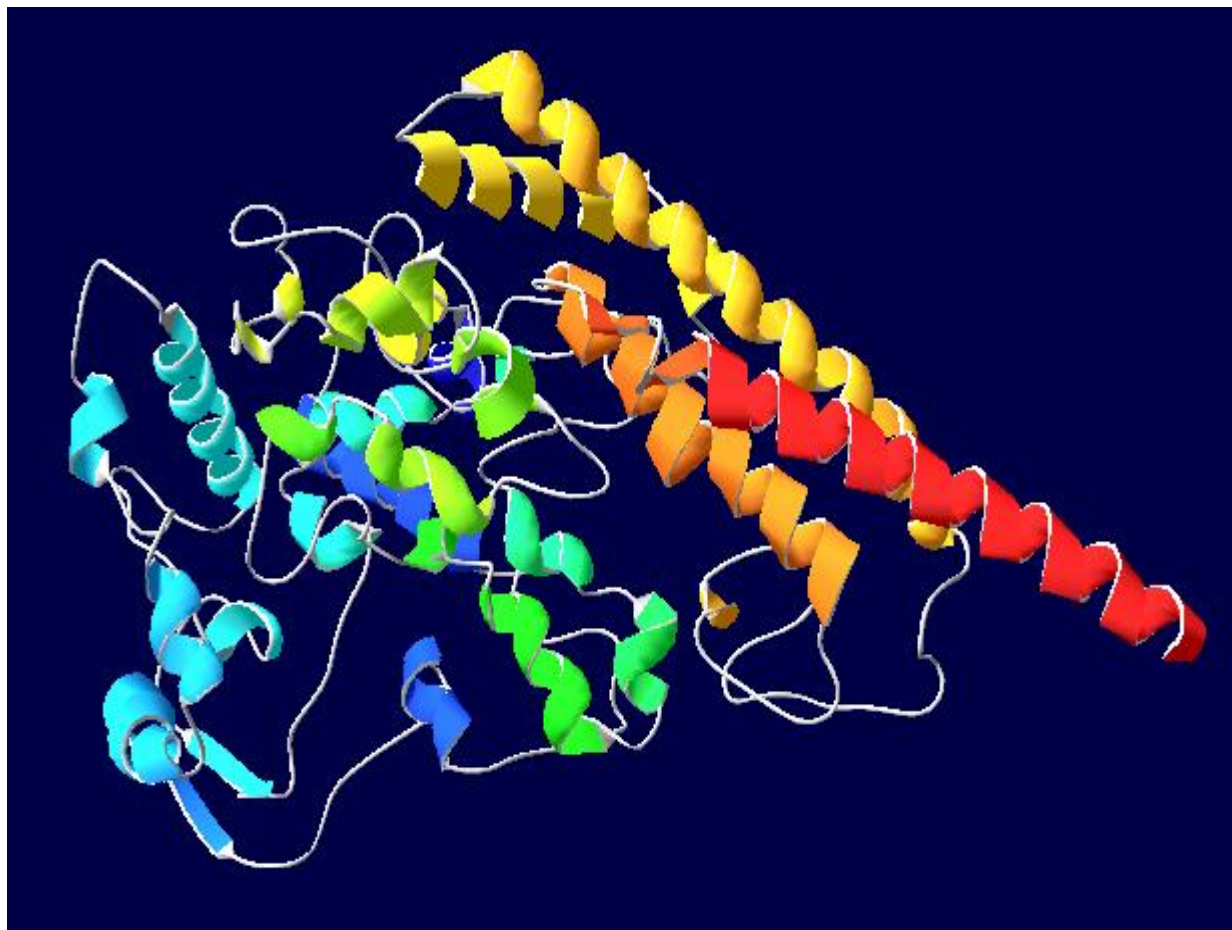
499 residues (88% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template.

3D viewing

[Interactive 3D view in JSmol](#)

For other options to view your downloaded structure offline see the [FAQ](#)

模型分析---Swiss-PdbViewer



Thank you !