Rip3 小分子抑制剂的预测

报告人：曾凡新

组员：谷俊中、曾凡新、冯慧慧、吴晶

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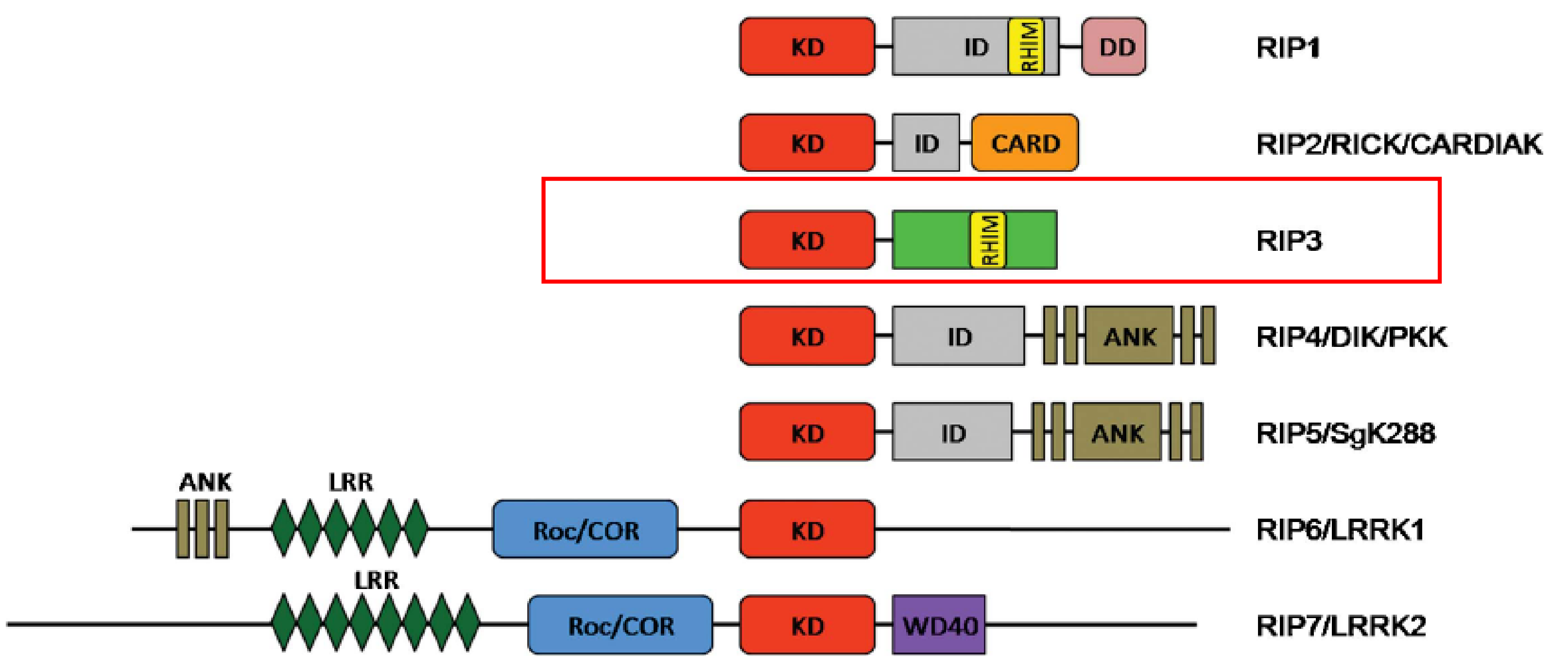
目录

1. Rip3 背景介绍

Rip3 (Receptor-Interacting Proteins 3)

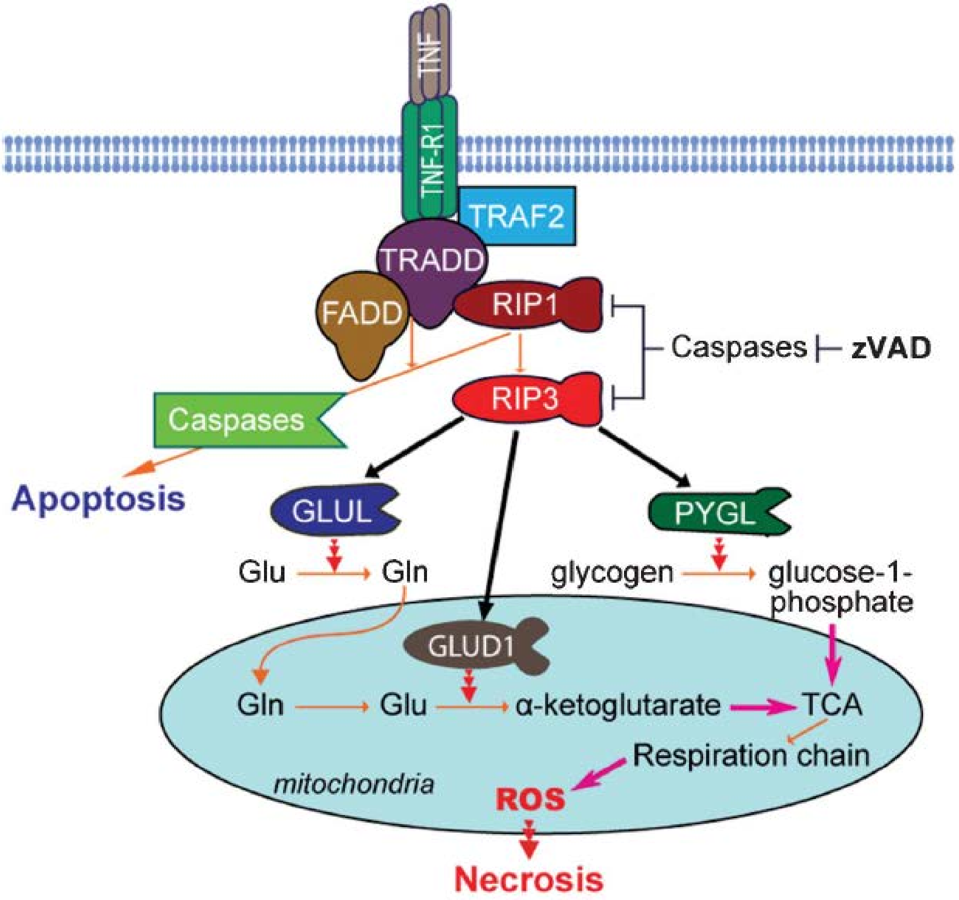


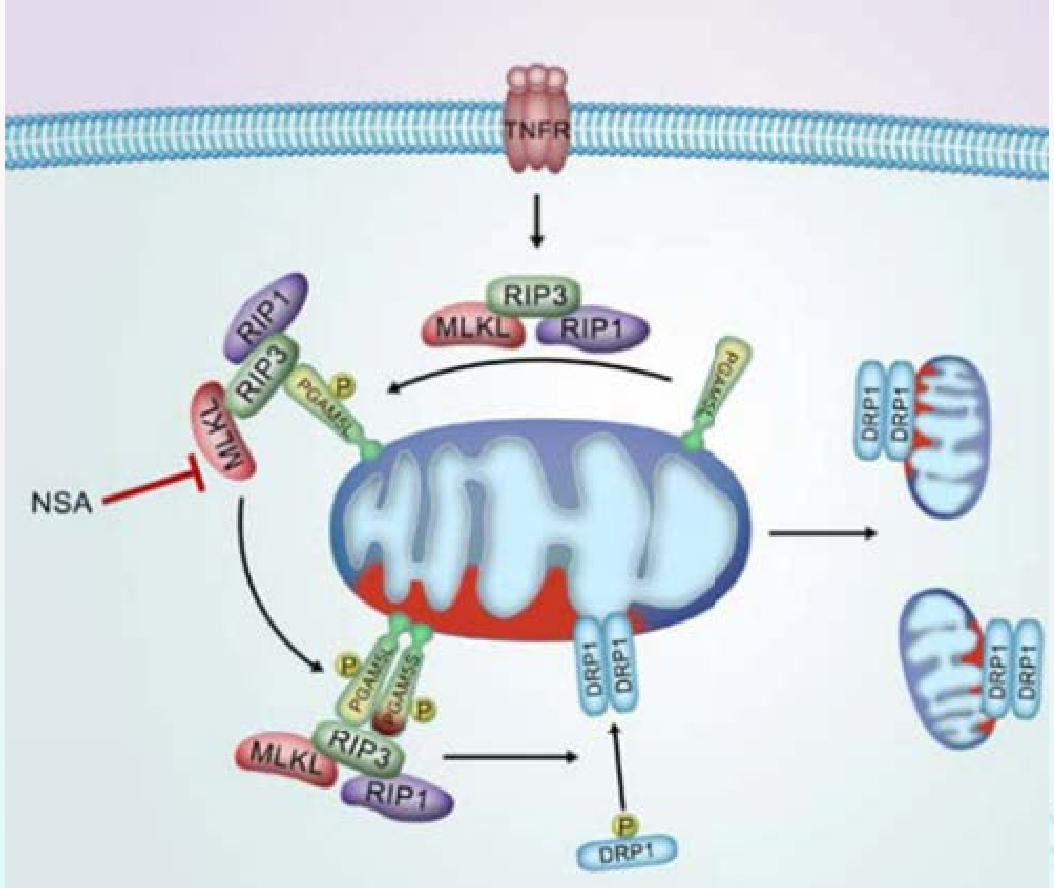
Duanwu Zhang et al, NAT REV MOL CELL BIO 2010 243-249

Rip3 蛋白

- Receptor-interacting protein 3
- 518 AA
- **亚细胞定位：** 多元化，可能位于膜上以及线粒体周围。
- **催化活性：** ATP + a protein = ADP + a phosphoprotein
- **功能：** Rip3是控制坏死信号通路的关键因子。在诱导细胞坏死时，Rip3与Rip1相互作用，并使其磷酸化。Rip3通过与一些代谢酶结合刺激三羧酸循环和氧化磷酸化，导致ROS的产生，从而诱发细胞坏死。

Rip3/Rip1介导细胞坏死





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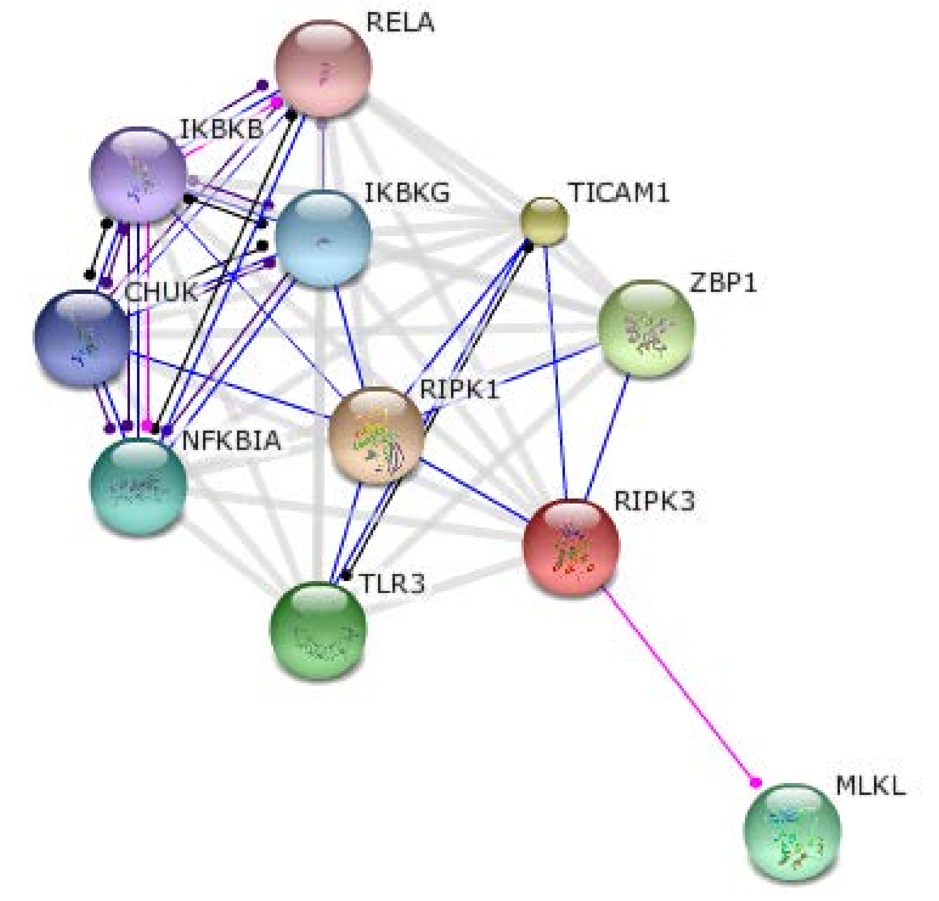
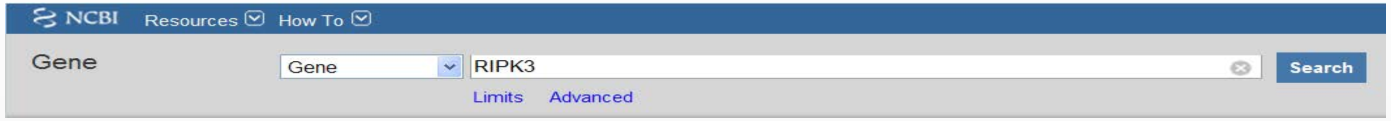
Rip3 蛋白与其他蛋白相互作用



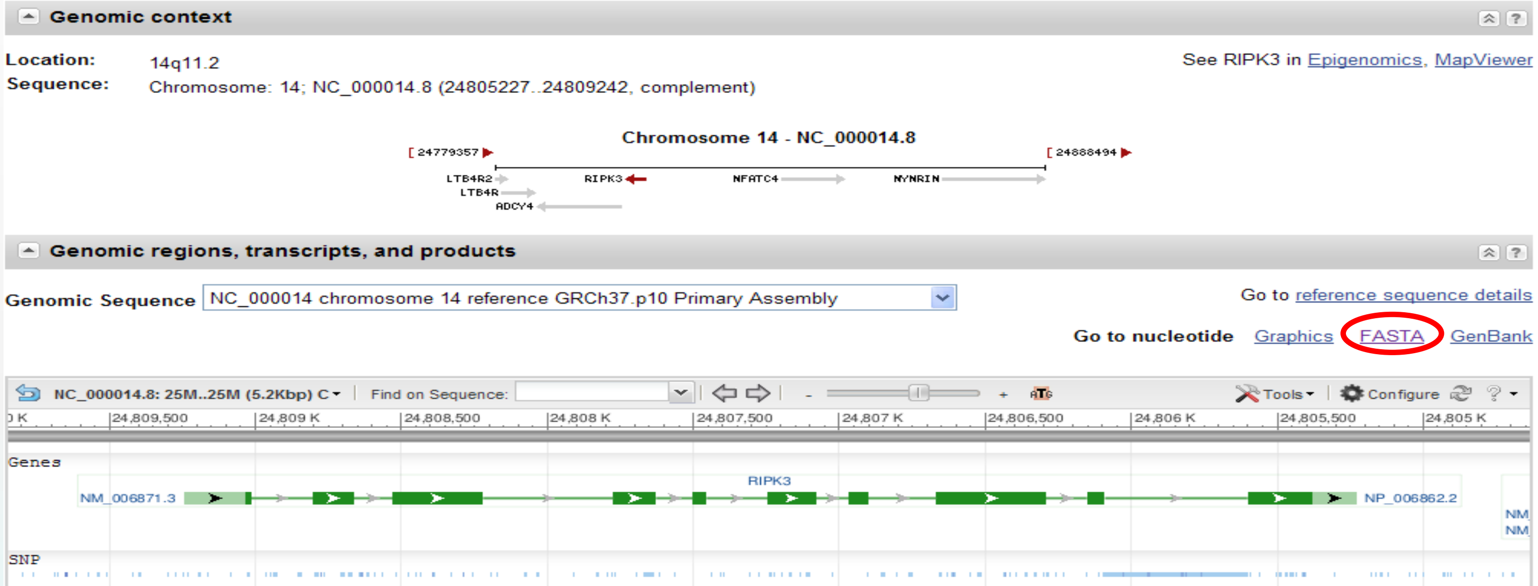
蓝线：蛋白相互结合　　　　红线：翻译后修饰

2. Rip3 基因结构与序列分析

Rip3 genome DNA——4044 bp



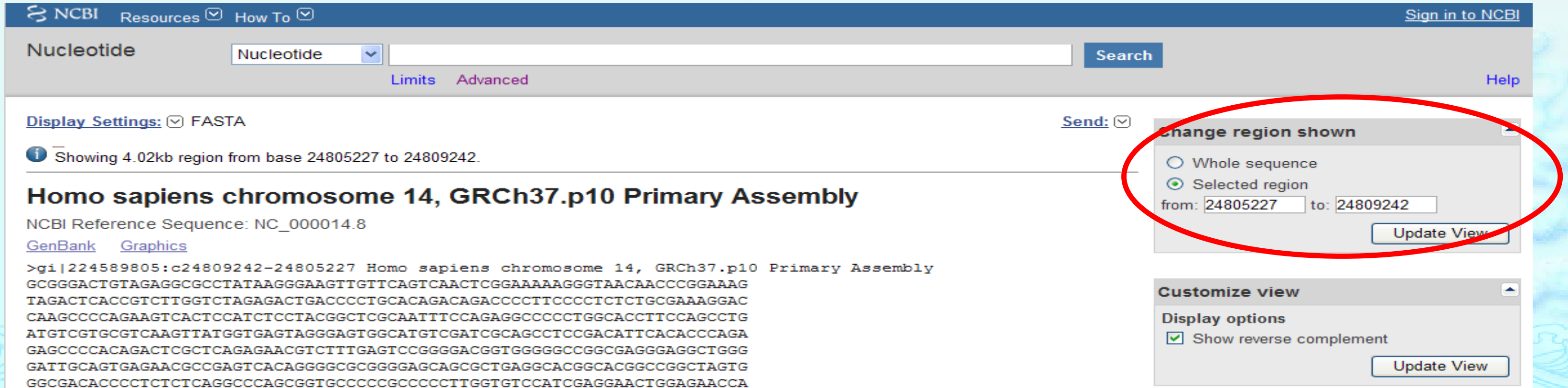
Gene: Gene RIPK3



Location: 14q11.2

Sequence: Chromosome: 14; NC_000014.8 (24805227..24809242, complement)

Genomic Sequence: NC_000014 chromosome 14 reference GRCh37.p10 Primary Assembly



Nucleotide: Nucleotide

Display Settings: FASTA

Showing 4.02kb region from base 24805227 to 24809242.

Homo sapiens chromosome 14, GRCh37.p10 Primary Assembly

NCBI Reference Sequence: NC_000014.8

```
>gi|224589805:c24809242-24805227 Homo sapiens chromosome 14, GRCh37.p10 Primary Assembly
GCGGGACTGTAGAGGCGCCTATAAGGGAAGTTGTTCAGTCAACTCGGAAAAAGGGTAACAACCCGGAAAG
TAGACTCACCGTCTTGGTCTAGAGACTGACCCCTGCACAGACAGACCCCTTCCCCTCTCTGCGAAAGGAC
CAAGCCCCAGAAGTCACTCCATCTCCTACGGCTCGCAATTTCCAGAGGCCCCCTGGCACCTTCCAGCCTG
ATGTCGTGCGTCAAGTTATGGTGAGTAGGGAGTGGCATGTCGATCGCAGCCTCCGACATTCACACCCAGA
GAGCCCCACAGACTCGCTCAGAGAACGTCTTTGAGTCCGGGGACGGTGGGGGCCGGCGAGGGAGGCTGGG
GATTGCAGTGAGAACGCCGAGTCACAGGGGCGCGGGGAGCAGCGCTGAGGCACGGCACGGCCGGCTAGTG
GGCGACACCCCTCTCTCAGGCCCAGCGGTGCCCCCGCCCCCTTGGTGTCCATCGAGGAACTGGAGAACCA
```

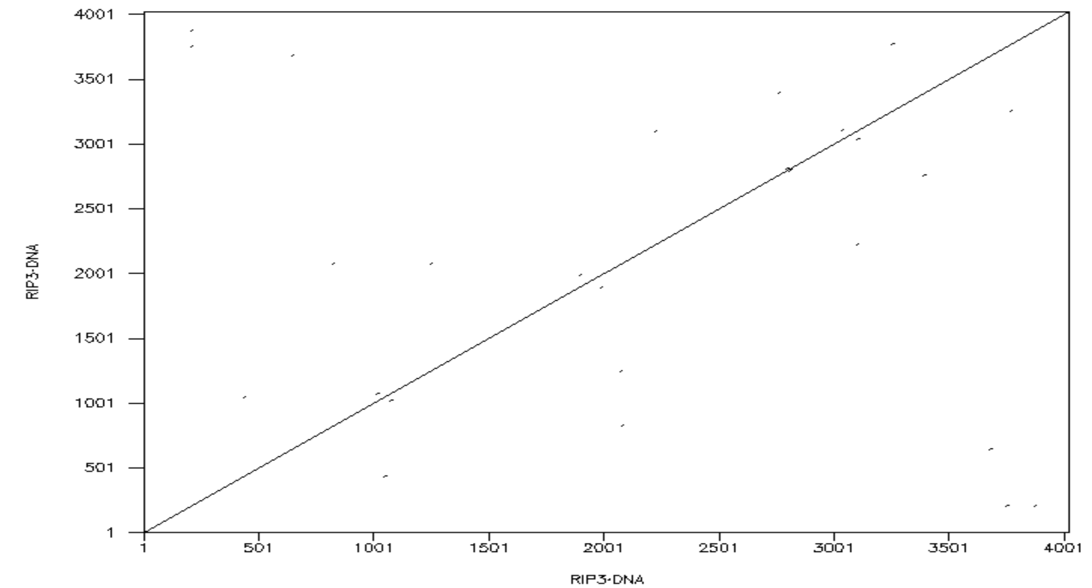
○ Whole sequence

● Selected region

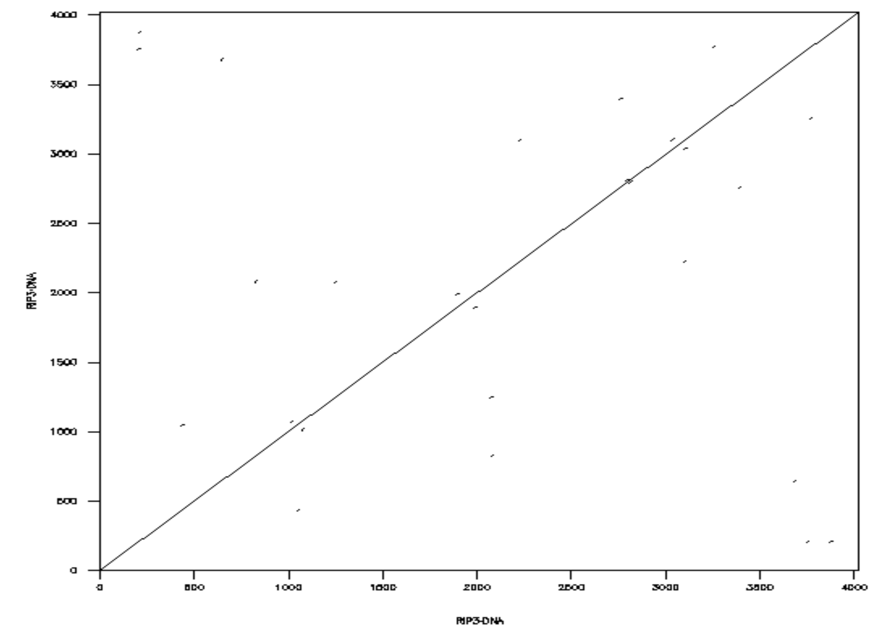
from: 24805227 to: 24809242

☑ Show reverse complement

Rip3 genome DNA——4044 bp

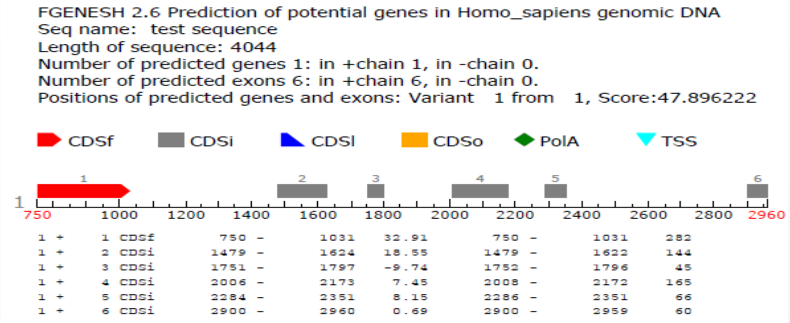


dottup



playdot

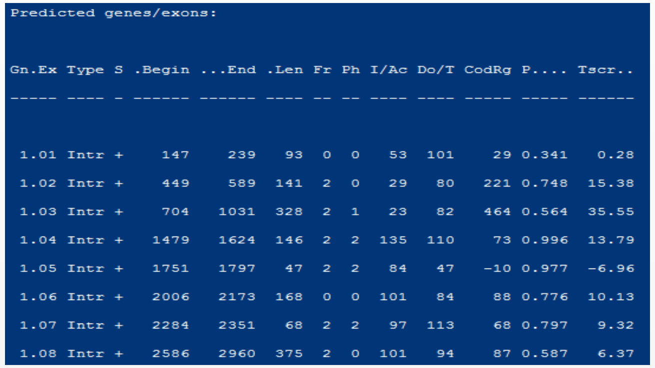
Rip3 gene structure



```
FGENESH 2.6 Prediction of potential genes in Homo_sapiens genomic DNA
Seq name:  test sequence
Length of sequence: 4044
Number of predicted genes 1: in +chain 1, in -chain 0.
Number of predicted exons 6: in +chain 6, in -chain 0.
Positions of predicted genes and exons: Variant   1 from   1, Score:47.896222
```

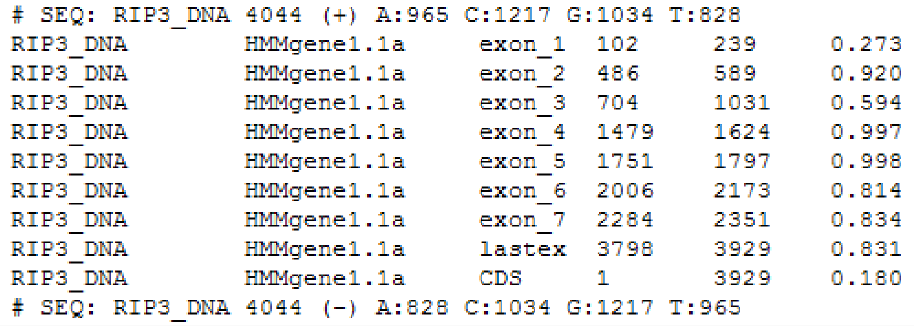
```
 1 +    1 CDSf        750 -      1031   32.91        750 -      1031     282
 1 +    2 CDSi       1479 -      1624   18.55       1479 -      1622     144
 1 +    3 CDSi       1751 -      1797   -9.74       1752 -      1796      45
 1 +    4 CDSi       2006 -      2173    7.45       2008 -      2172     165
 1 +    5 CDSi       2284 -      2351    8.15       2286 -      2351      66
 1 +    6 CDSi       2900 -      2960    0.69       2900 -      2959      60
```

Softberry



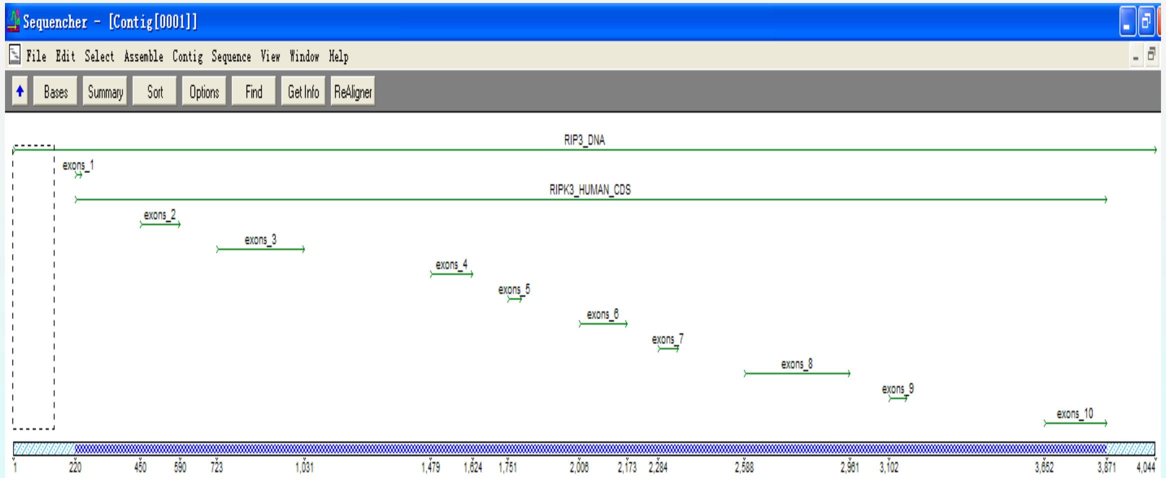
```
Predicted genes/exons:

Gn.Ex Type S .Begin ...End .Len Fr Ph I/Ac Do/T CodRg P.... Tscr..
----- ---- - ------ ------ ---- -- -- ---- ---- ----- ----- ------

 1.01 Intr +    147    239   93  0  0   53  101    29 0.341   0.28
 1.02 Intr +    449    589  141  2  0   29   80   221 0.748  15.38
 1.03 Intr +    704   1031  328  2  1   23   82   464 0.564  35.55
 1.04 Intr +   1479   1624  146  2  2  135  110    73 0.996  13.79
 1.05 Intr +   1751   1797   47  2  2   84   47   -10 0.977  -6.96
 1.06 Intr +   2006   2173  168  0  0  101   84    88 0.776  10.13
 1.07 Intr +   2284   2351   68  2  2   97  113    68 0.797   9.32
 1.08 Intr +   2586   2960  375  2  0  101   94    87 0.587   6.37
```

GenScan

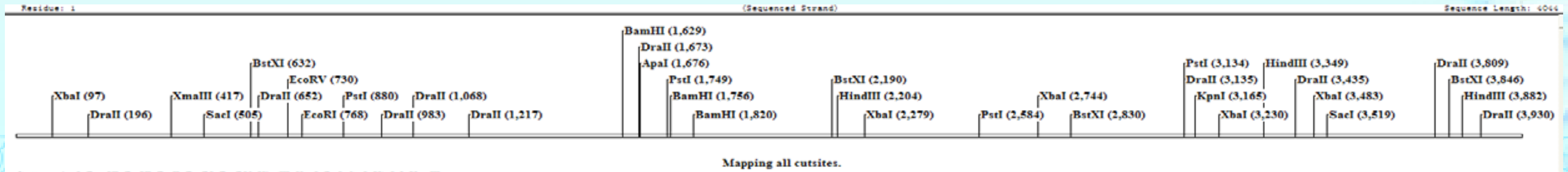


```
# SEQ: RIP3_DNA 4044 (+) A:965 C:1217 G:1034 T:828
RIP3_DNA        HMMgene1.1a     exon_1  102     239     0.273
RIP3_DNA        HMMgene1.1a     exon_2  486     589     0.920
RIP3_DNA        HMMgene1.1a     exon_3  704     1031    0.594
RIP3_DNA        HMMgene1.1a     exon_4  1479    1624    0.997
RIP3_DNA        HMMgene1.1a     exon_5  1751    1797    0.998
RIP3_DNA        HMMgene1.1a     exon_6  2006    2173    0.814
RIP3_DNA        HMMgene1.1a     exon_7  2284    2351    0.834
RIP3_DNA        HMMgene1.1a     lastex  3798    3929    0.831
RIP3_DNA        HMMgene1.1a     CDS     1       3929    0.180
# SEQ: RIP3_DNA 4044 (-) A:828 C:1034 G:1217 T:965
```

HMMgene



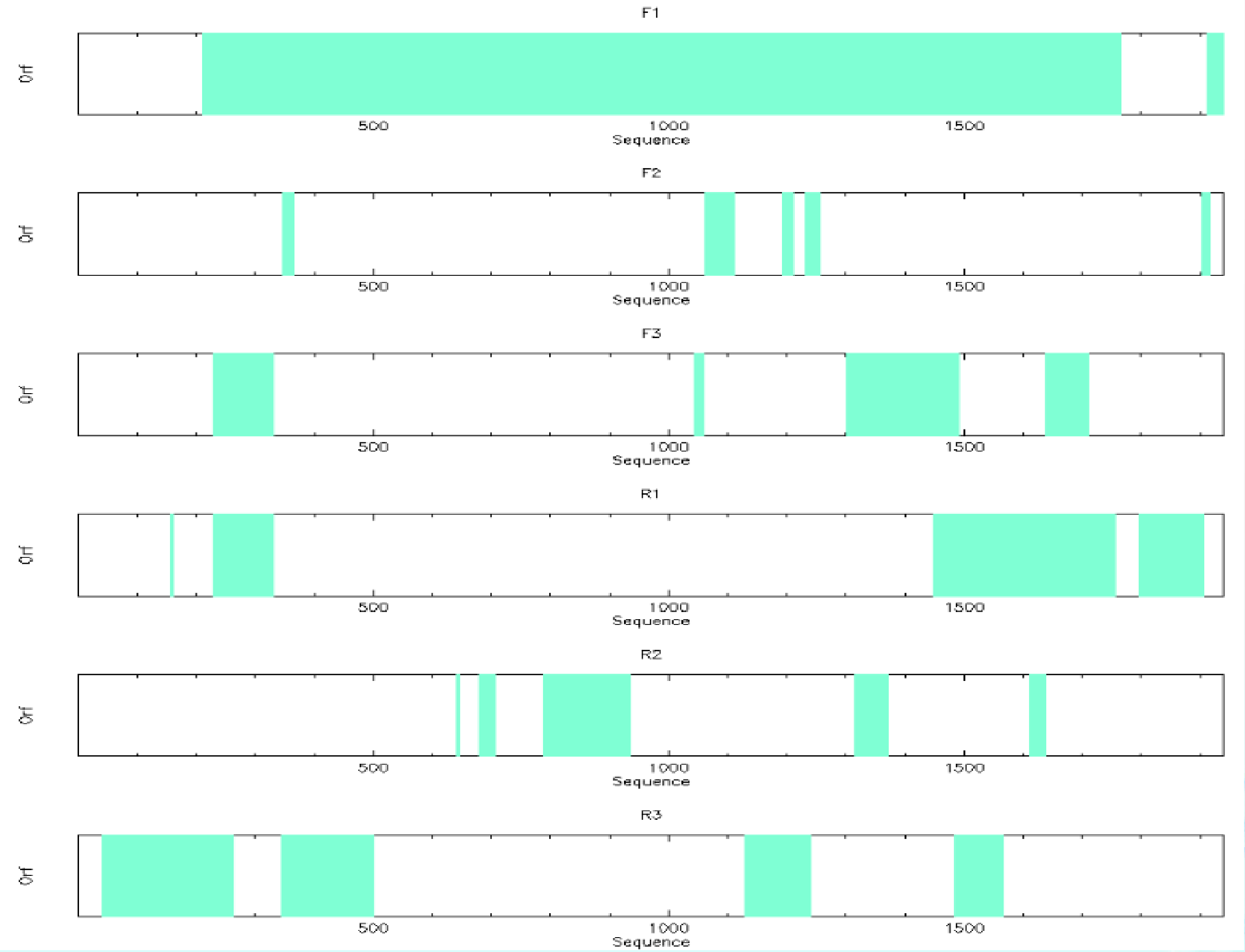
Sequencher



Mapping all cutsites.

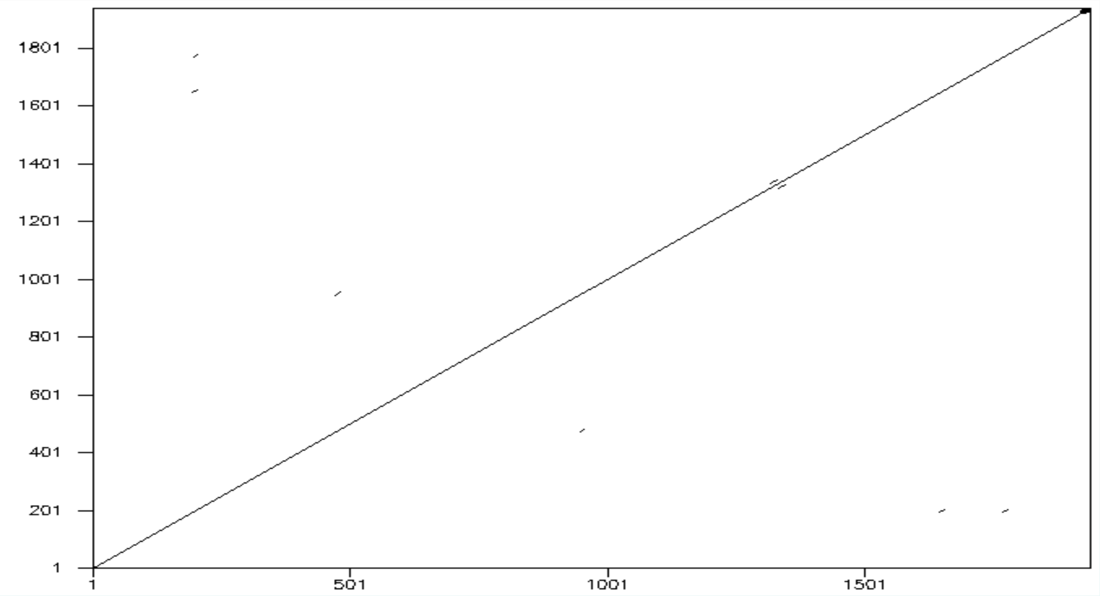
Cutters : ApaI, BamHI, BstXI, DraII, EcoRI, EcoRV, HindIII, KpnI, PstI, SacI, XbaI & XmaIII
Non-Cutters : Bsp106, NotI, SacII, SalI, SmaI, SpeI & XhoI

Rip3 mRNA——1940 bp

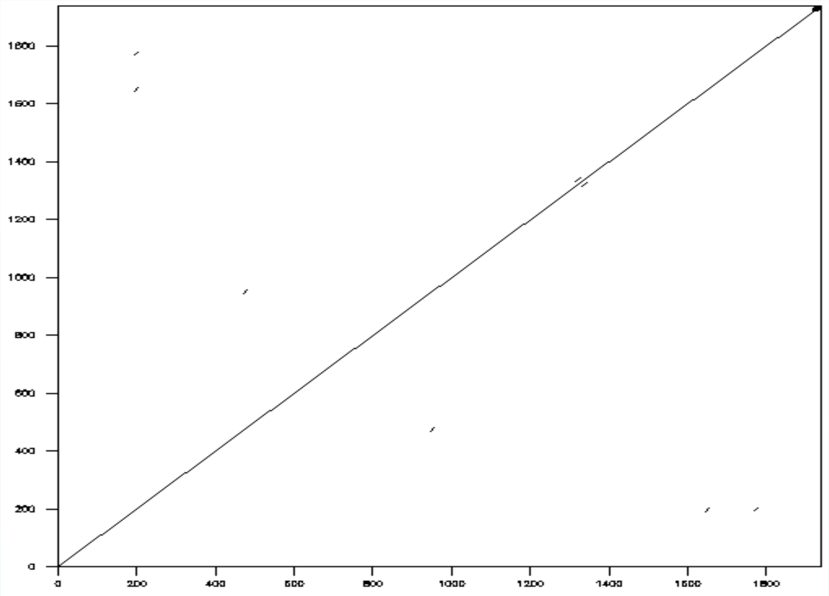


PlotORF

Rip3 mRNA——1940 bp

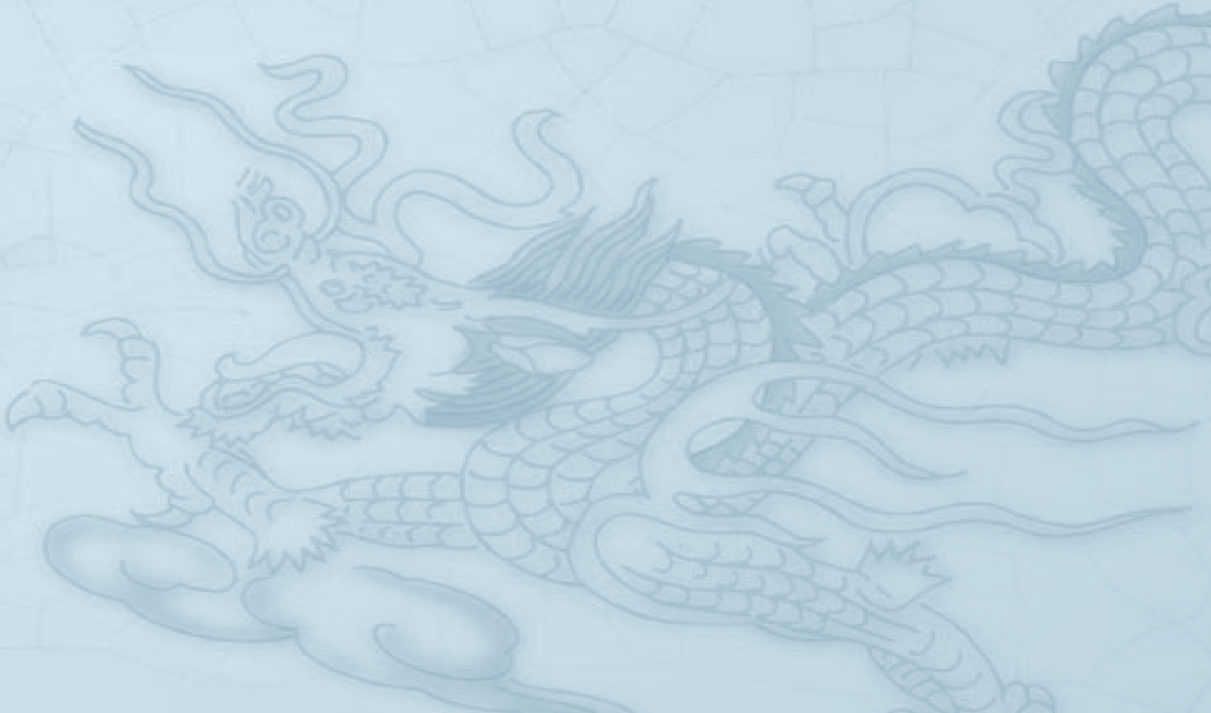


dottup



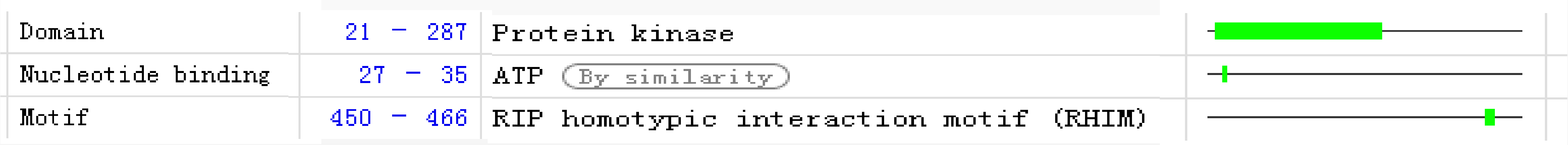
playdot

3. Rip3 蛋白序列与结构分析

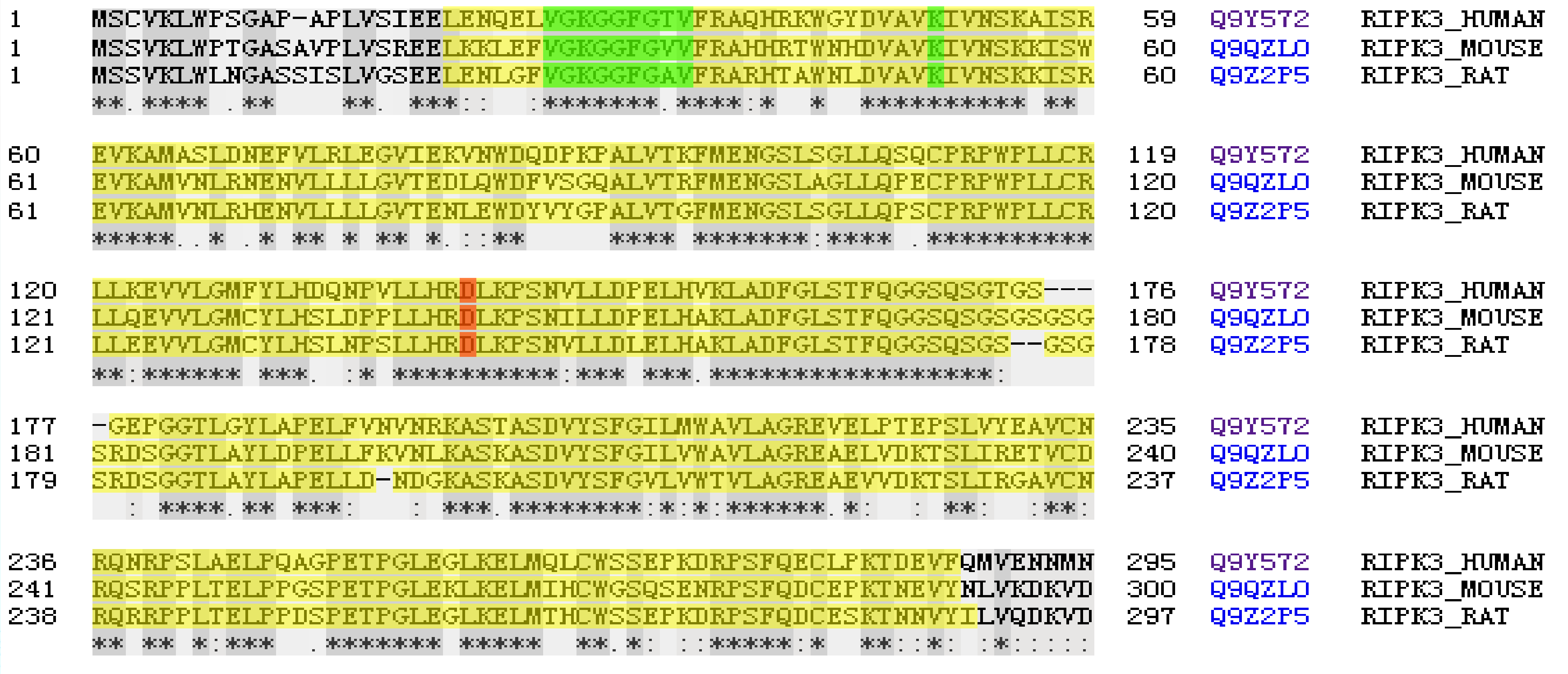


Rip3 蛋白序列特征

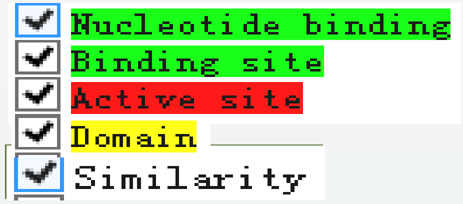
Regions



Domain	21 – 287	Protein kinase	
Nucleotide binding	27 – 35	ATP (By similarity)	
Motif	450 – 466	RIP homotypic interaction motif (RHIM)	



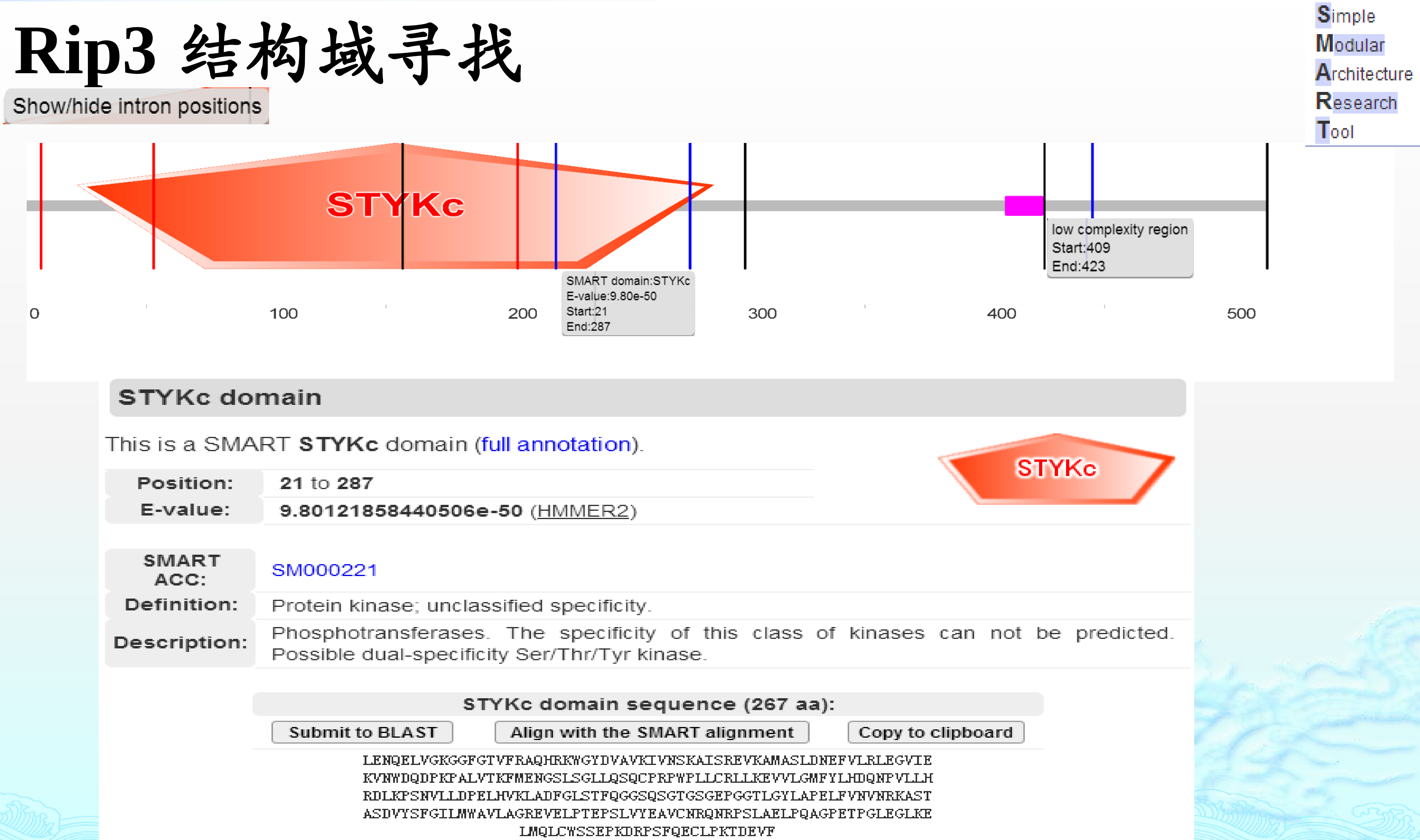
```
1      MSCVKLWPSGAP-APLVSIEELENQELVGKGGFGTVFRAQHRKWGYDVAVKIVNSKAISR   59  Q9Y572  RIPK3_HUMAN
1      MSSVKLWPTGASAVPLVSREELKKLEFVGKGGFGVVFRAHHRTWNHDVAVKIVNSKKISW   60  Q9QZL0  RIPK3_MOUSE
1      MSSVKLWLNGASSISLVGSEELENLGFVGKGGFGAVFRARHTAWNLDVAVKIVNSKKISR   60  Q9Z2P5  RIPK3_RAT
       **.**** .**    **. ***::  :*******.****:*  *  ********** **

60     EVKAMASLDNEFVLRLEGVIEKVNWDQDPKPALVTKFMENGSLSGLLQSQCPRPWPLLCR  119  Q9Y572  RIPK3_HUMAN
61     EVKAMVNLRNENVLLLLGVTEDLQWDFVSGQALVTRFMENGSLAGLLQPECPRPWPLLCR  120  Q9QZL0  RIPK3_MOUSE
61     EVKAMVNLRHENVLLLLGVTENLEWDYVYGPALVTGFMENGSLSGLLQPSCPRPWPLLCR  120  Q9Z2P5  RIPK3_RAT
       *****..* .* ** * ** *.::**     **** *******:**** .**********

120    LLKEVVLGMFYLHDQNPVLLHRDLKPSNVLLDPELHVKLADFGLSTFQGGSQSGTGS---  176  Q9Y572  RIPK3_HUMAN
121    LLQEVVLGMCYLHSLDPPLLHRDLKPSNILLDPELHAKLADFGLSTFQGGSQSGSGSGSG  180  Q9QZL0  RIPK3_MOUSE
121    LLEEVVLGMCYLHSLNPSLLHRDLKPSNVLLDLELHAKLADFGLSTFQGGSQSGS--GSG  178  Q9Z2P5  RIPK3_RAT
       **:****** ***. :* **********:*** ***.*****************:

177    -GEPGGTLGYLAPELFVNVNRKASTASDVYSFGILMWAVLAGREVELPTEPSLVYEAVCN  235  Q9Y572  RIPK3_HUMAN
181    SRDSGGTLAYLDPELLFKVNLKASKASDVYSFGILVWAVLAGREAELVDKTSLIRETVCD  240  Q9QZL0  RIPK3_MOUSE
179    SRDSGGTLAYLAPELLD-NDGKASKASDVYSFGVLVWTVLAGREAEVVDKTSLIRGAVCN  237  Q9Z2P5  RIPK3_RAT
         : ****.** ***:   : ***.********:*:*:******.*:  : **:  :**:

236    RQNRPSLAELPQAGPETPGLEGLKELMQLCWSSEPKDRPSFQECLPKTDEVFQMVENNMN  295  Q9Y572  RIPK3_HUMAN
241    RQSRPPLTELPPGSPETPGLEKLKELMIHCWGSQSENRPSFQDCEPKTNEVYNLVKDKVD  300  Q9QZL0  RIPK3_MOUSE
238    RQRRPPLTELPPDSPETPGLEGLKELMTHCWSSEPKDRPSFQDCESKTNNVYILVQDKVD  297  Q9Z2P5  RIPK3_RAT
       ** ** *:***  .******* *****  **.*: ::*****:*  **::*: :*:::::
```



☑ 🟩 Nucleotide binding
☑ 🟩 Binding site
☑ 🟥 Active site
☑ 🟨 Domain
☑ Similarity

Rip3 Sequence Alignment

Rip3 结构域寻找



STYKc domain

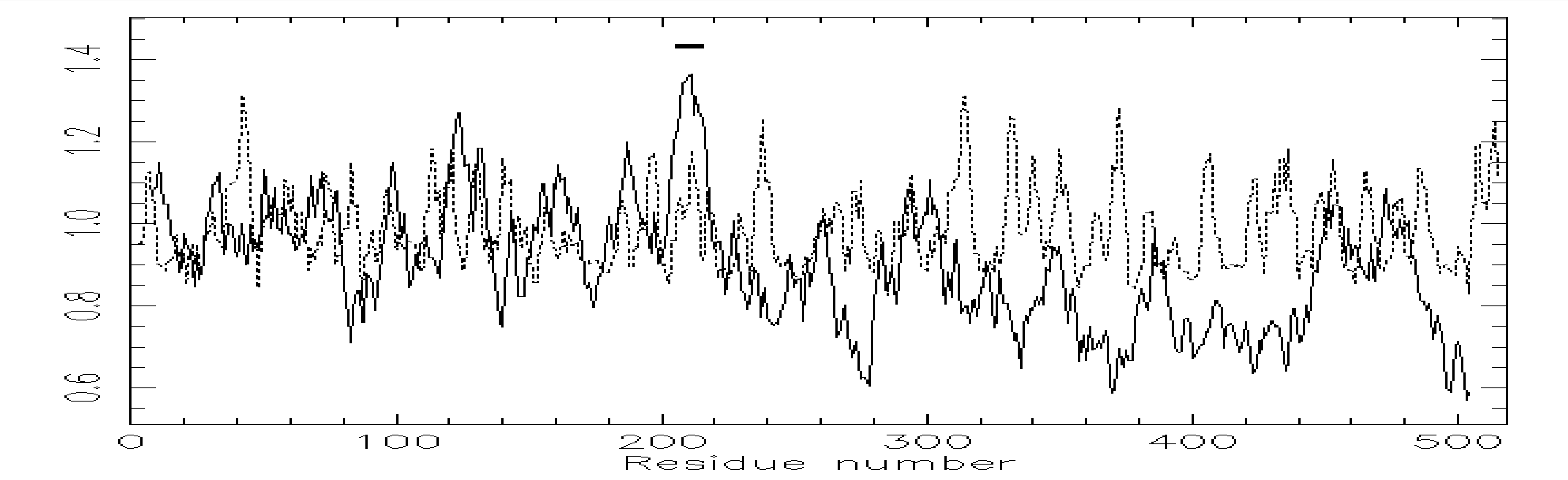
This is a SMART **STYKc** domain (full annotation).

Position:	**21** to **287**
E-value:	**9.80121858440506e-50** (HMMER2)
SMART ACC:	SM000221
Definition:	Protein kinase; unclassified specificity.
Description:	Phosphotransferases. The specificity of this class of kinases can not be predicted. Possible dual-specificity Ser/Thr/Tyr kinase.

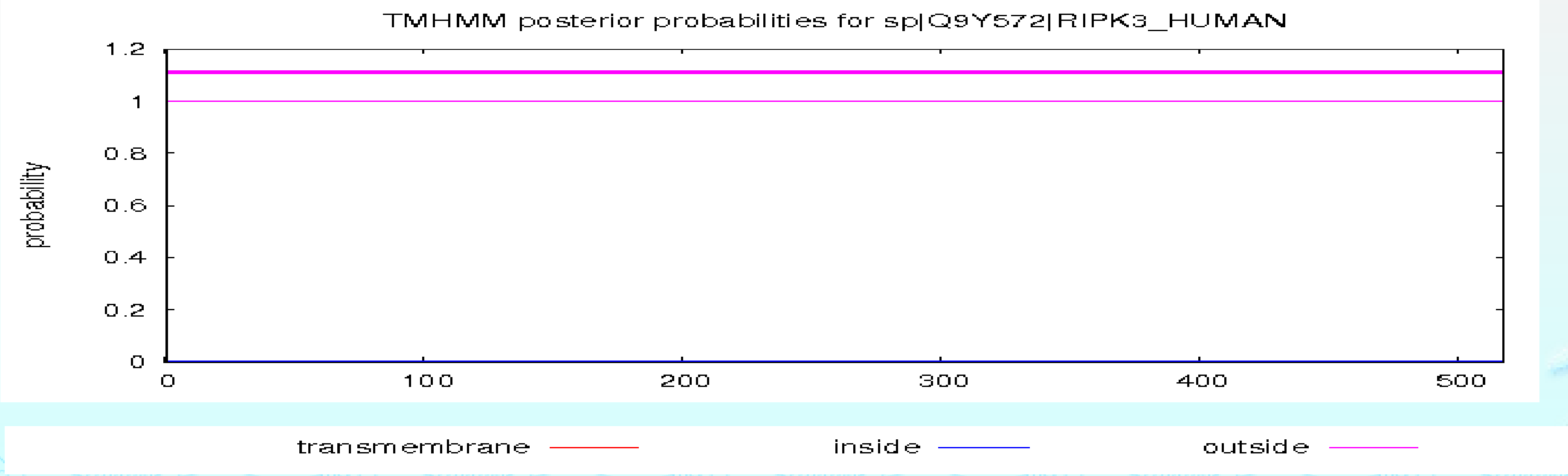
STYKc domain sequence (267 aa):

```
LENQELVGKGGFGTVFRAQHRKWGYDVAVKIVNSKAISREVKAMASLDNEFVLRLEGVIE
KVNWDQDPKPALVTKFMENGSLSGLLQSQCPRPWPLLCRLLKEVVLGMFYLHDQNPVLLH
RDLKPSNVLLDPELHVKLADFGLSTFQGGSQSGTGSGEPGGTLGYLAPELFVNVNRKAST
ASDVYSFGILMWAVLAGREVELPTEPSLVYEAVCNRQNRPSLAELPQAGPETPGLEGLKE
LMQLCWSSEPKDRPSFQECLPKTDEVF
```

跨膜结构预测结果

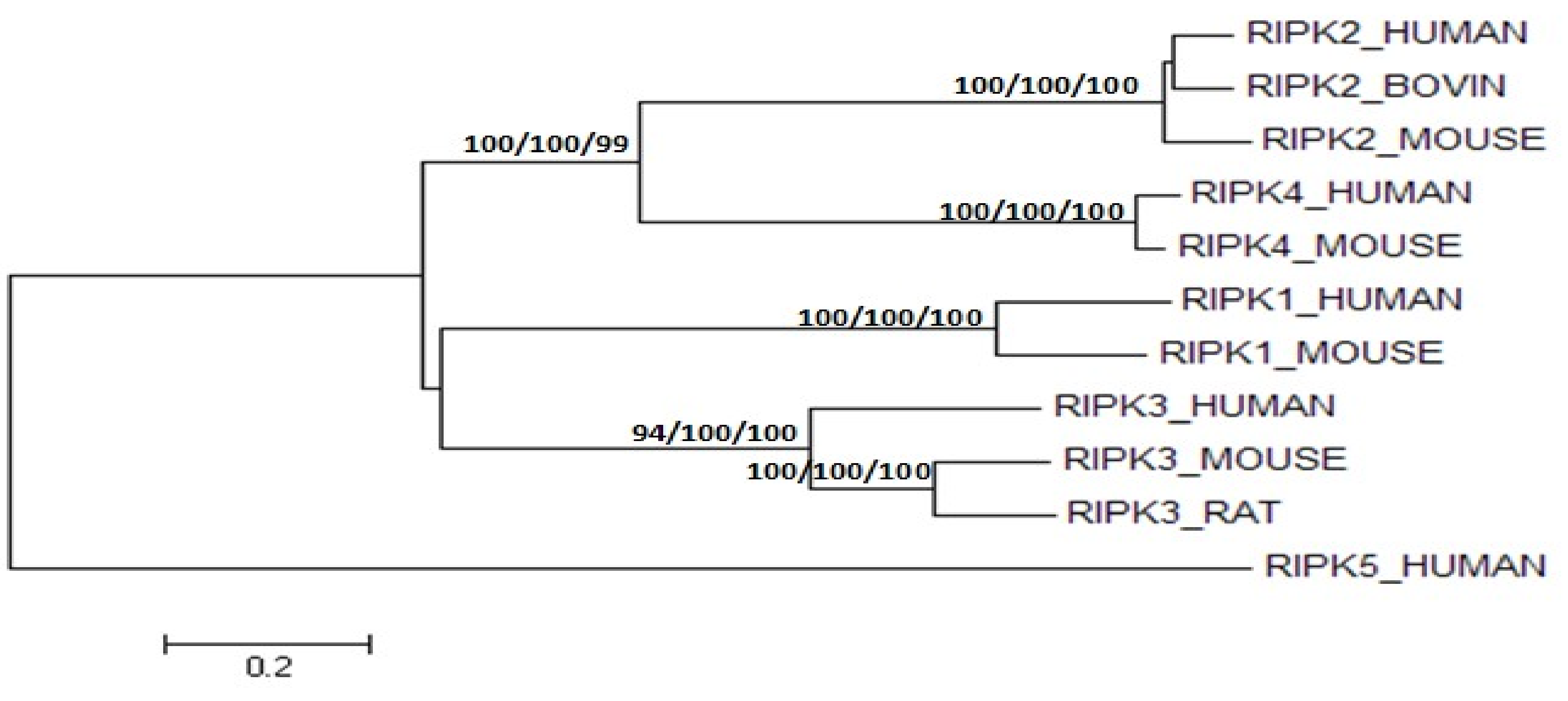


tmap



TMHMM

Rip家族亲缘关系分析



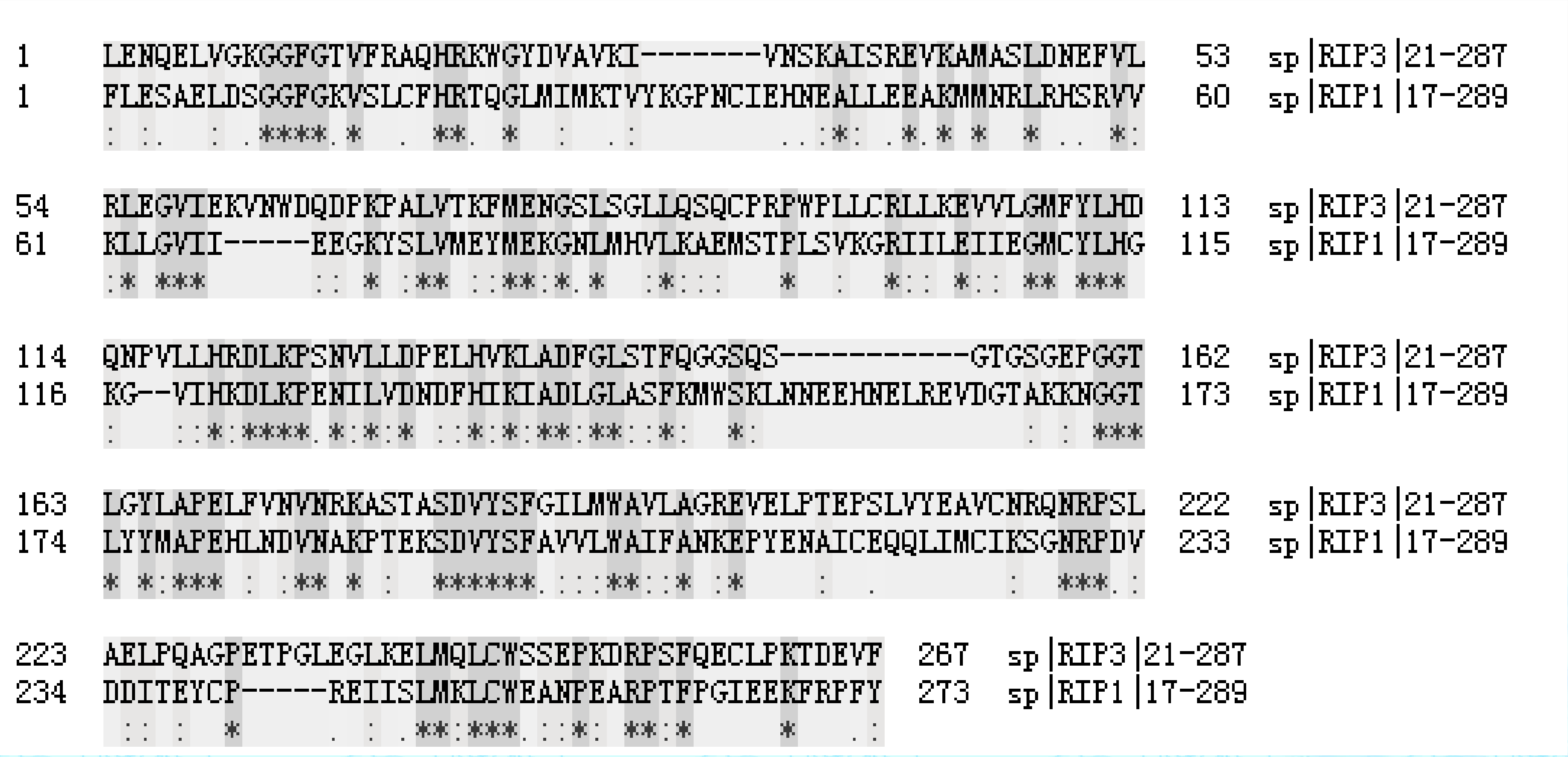
Rip1/Rip3序列比对结果

Rip3

21 – 287	267	Protein kinase

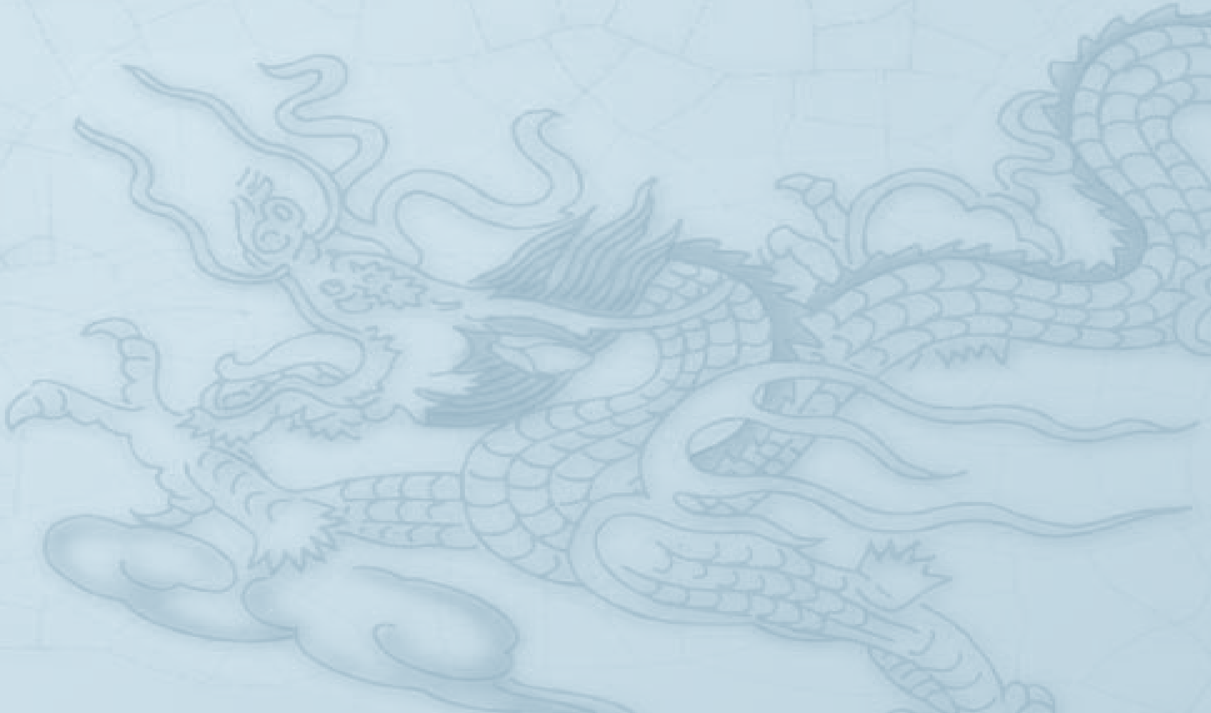
Rip1

17 – 289	273	Protein kinase



```
1      LENQELVGKGGFGTVFRAQHRKWGYDVAVKI-------VNSKAISREVKAMASLDNEFVL   53   sp|RIP3|21-287
1      FLESAELDSGGFGKVSLCFHRTQGLMIMKTVYKGPNCIEHNEALLEEAKMMNRLRHSRVV   60   sp|RIP1|17-289
       : :.  : .****.*  . **. *  :  .:        ..:*: .*.* *  * .. *:

54     RLEGVIEKVNWDQDPKPALVTKFMENGSLSGLLQSQCPRPWPLLCRLLKEVVLGMFYLHD   113  sp|RIP3|21-287
61     KLLGVII-----EEGKYSLVMEYMEKGNLMHVLKAEMSTPLSVKGRIILEIIEGMCYLHG   115  sp|RIP1|17-289
       :* ***      :: * :** ::**:*.*  :*:::   *  :  *:: *:: ** ***

114    QNPVLLHRDLKPSNVLLDPELHVKLADFGLSTFQGGSQS-----------GTGSGEPGGT   162  sp|RIP3|21-287
116    KG--VIHKDLKPENILVDNDFHIKIADLGLASFKMWSKLNNEEHNELREVDGTAKKNGGT   173  sp|RIP1|17-289
       :   ::*:****.*:*:* ::*:*:**:**::*:  *:               : : ***

163    LGYLAPELFVNVNRKASTASDVYSFGILMWAVLAGREVELPTEPSLVYEAVCNRQNRPSL   222  sp|RIP3|21-287
174    LYYMAPEHLNDVNAKPTEKSDVYSFAVVLWAIFANKEPYENAICEQQLIMCIKSGNRPDV   233  sp|RIP1|17-289
       * *:*** : :** * :  ******.:::**::* :*    :  .       :  ***.:

223    AELPQAGPETPGLEGLKELMQLCWSSEPKDRPSFQECLPKTDEVF   267  sp|RIP3|21-287
234    DDITEYCP-----REIISLMKLCWEANPEARPTFPGIEEKFRPFY   273  sp|RIP1|17-289
        :: :  *     . : .**:***.::*: **:*     *   .:
```

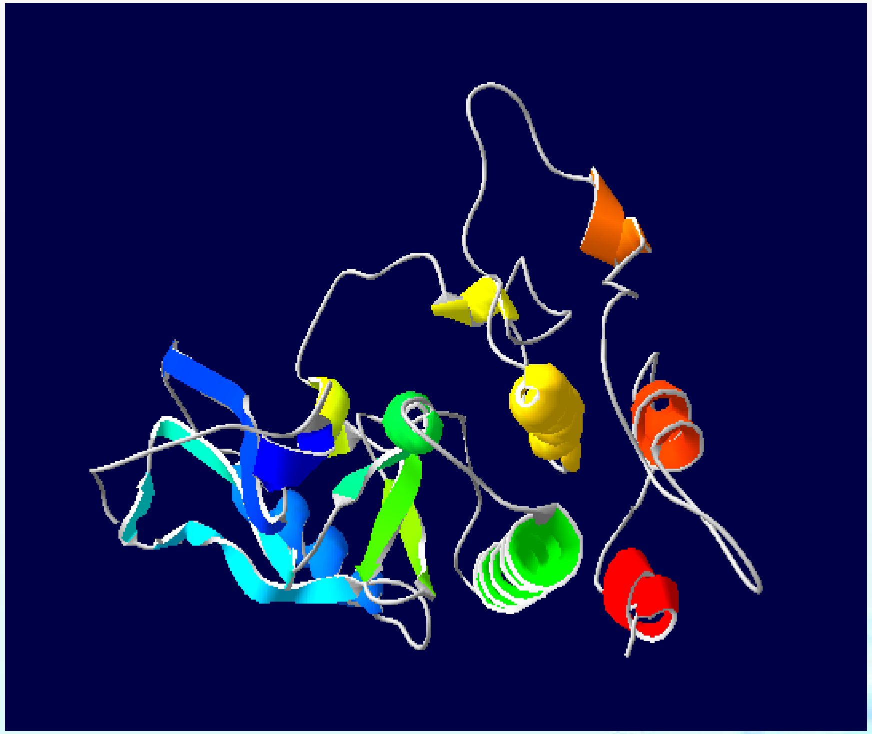
4. Rip3小分子抑制剂预测



Rip3激酶结构域同源模建结果

Modelled residue range: 21 to 288；
Based on template: 4ITI α
Sequence Identity [%]: 36%

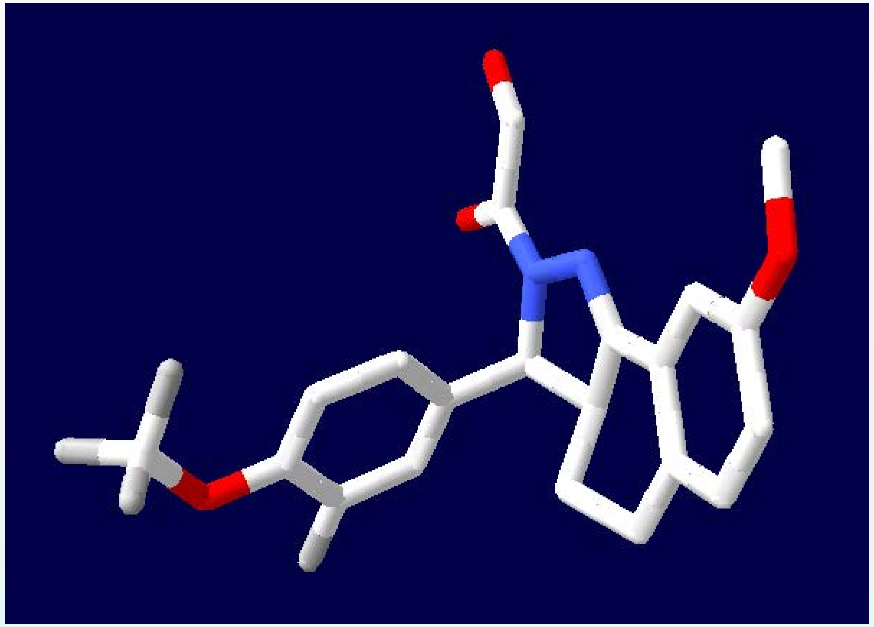
RIPK1_HUMAN α



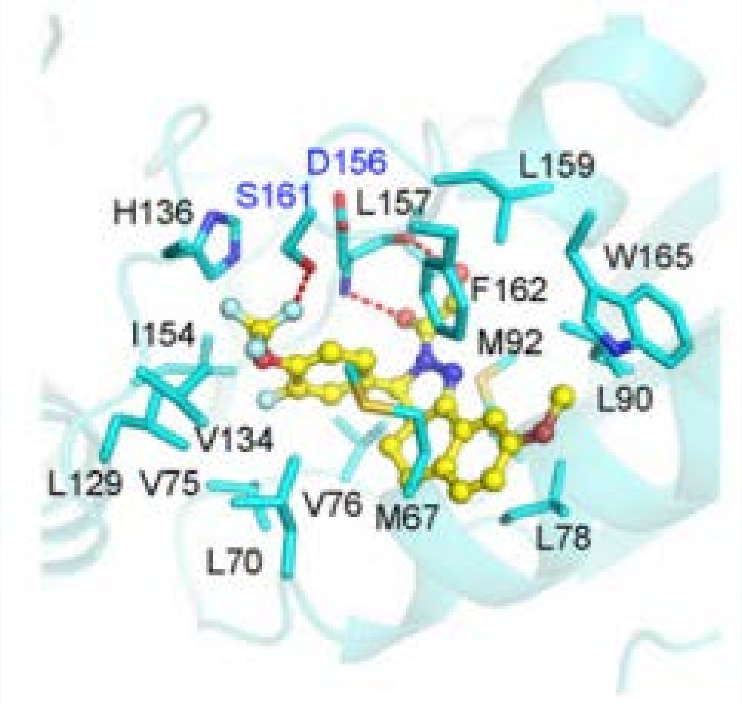
RIPK3_HUMAN_MODEL

RMS: 0.43

小分子抑制剂：Necrostatin



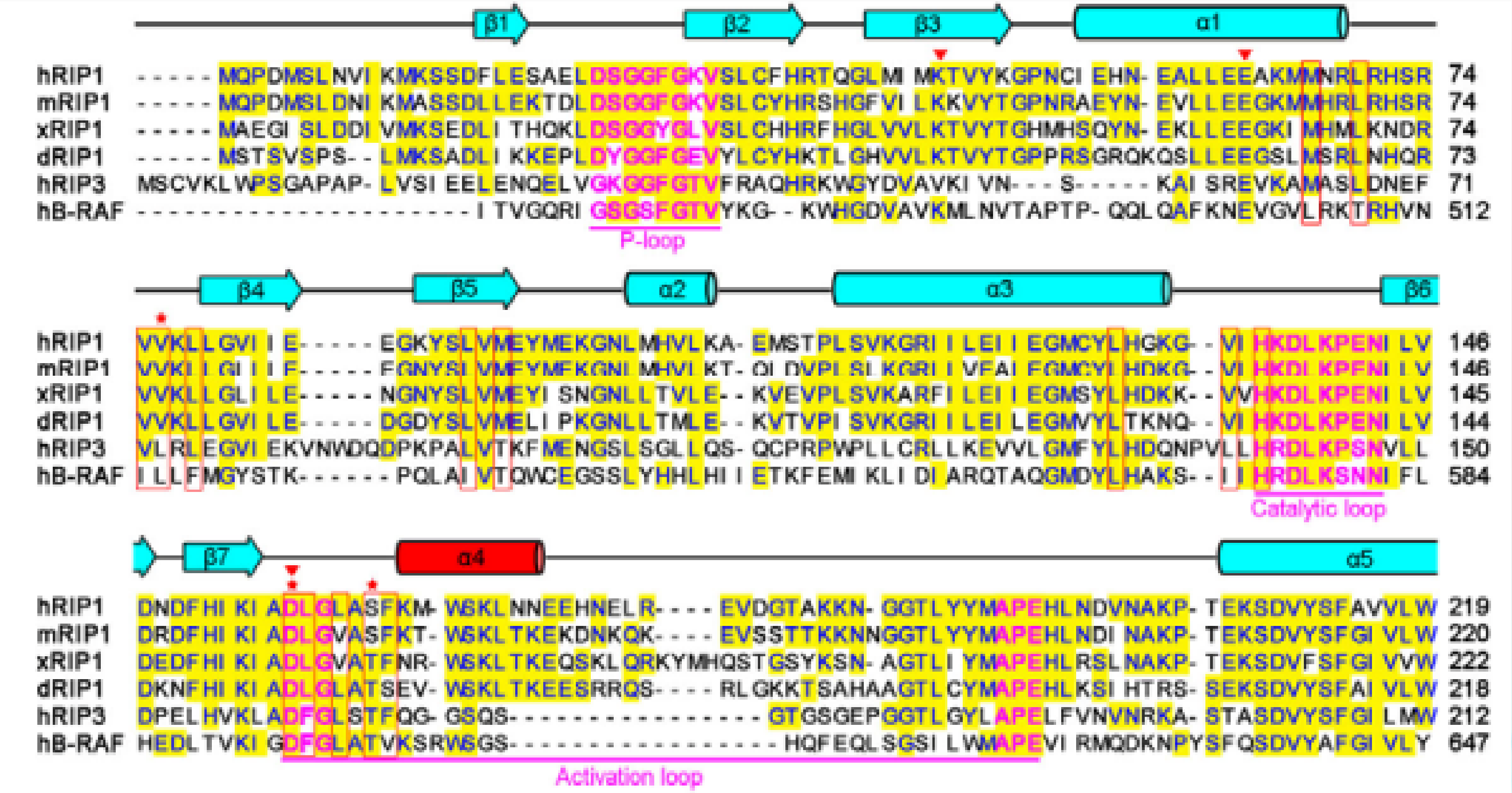
Necrostatin-3



Necrostatin-3的作用原理

Tian Xie etc. Structure. 2013 Mar 5;21(3):493-9.

活性位点

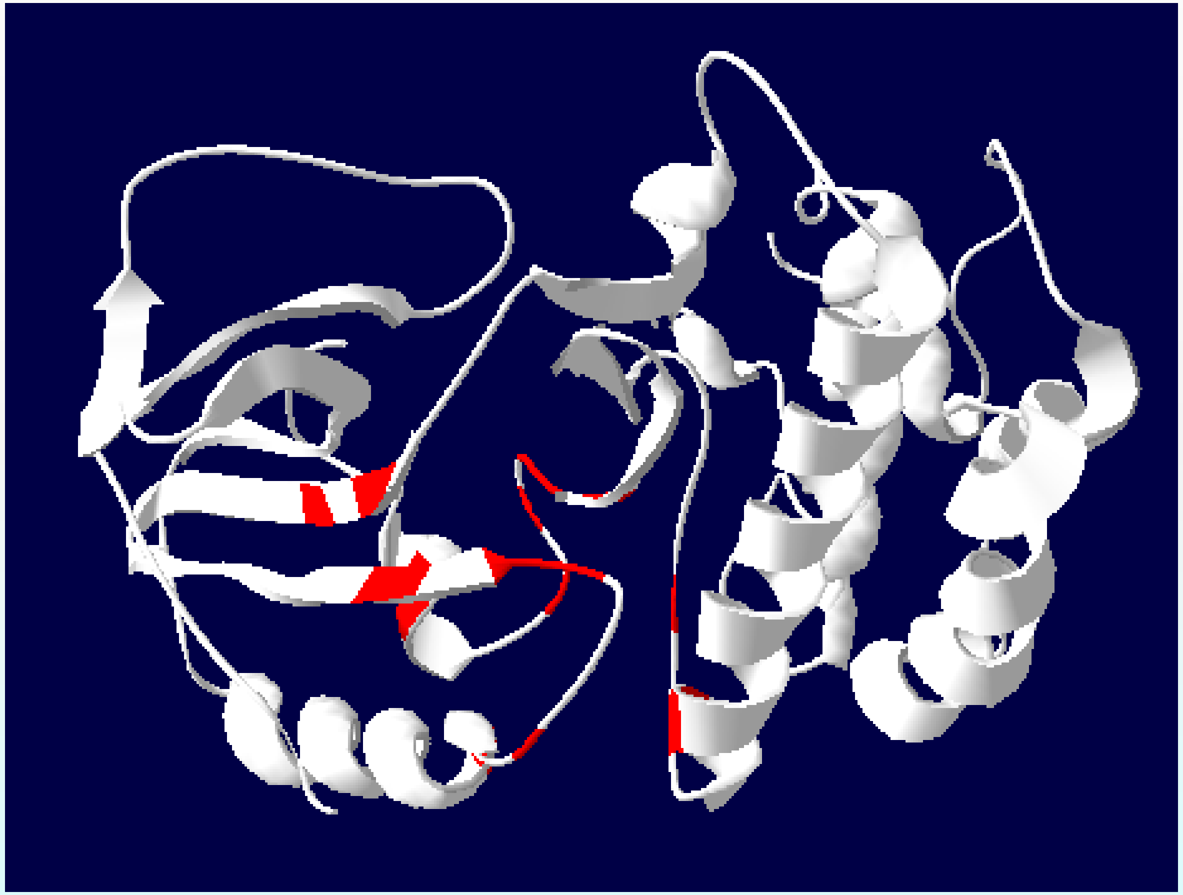


hRipK1 67 70 75 76 78 90 92 129 134 136 154 156 157 159 161 165

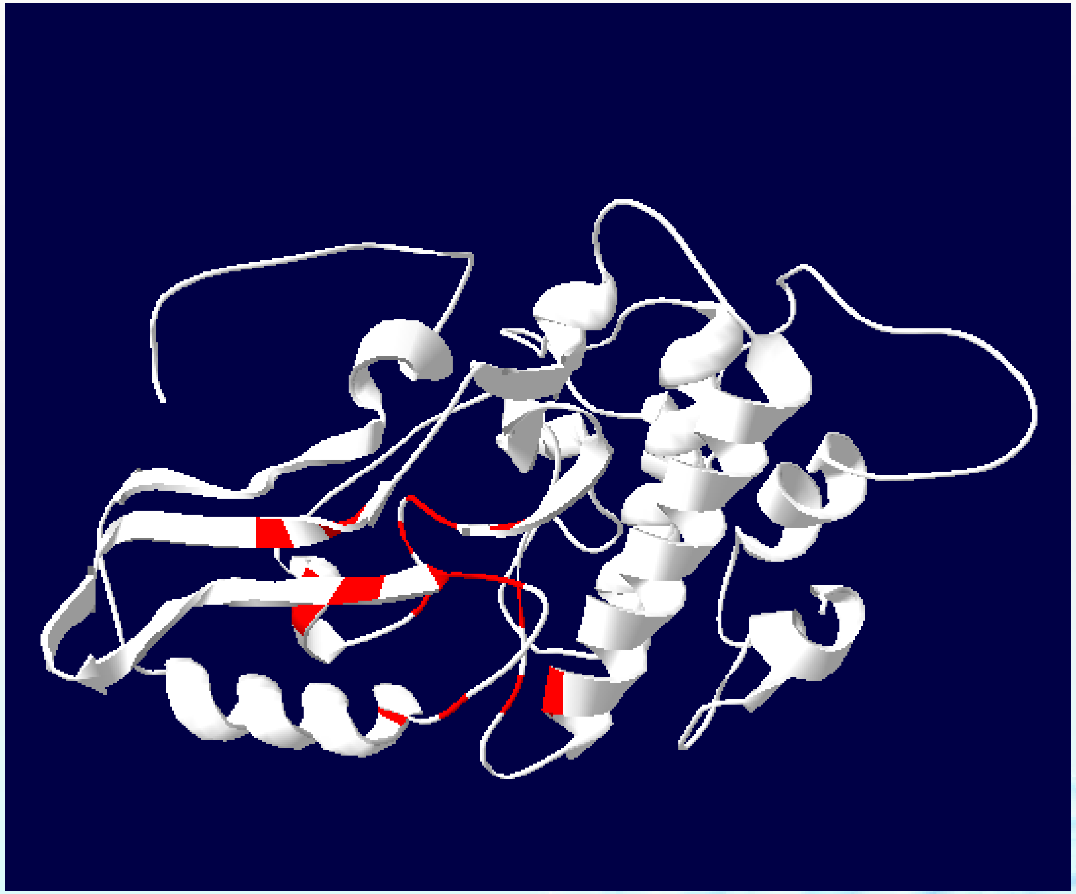
hRipK3 64 67 72 73 75 92 94 131 138 140 158 160 161 163 165 169

Tian Xie etc. Structure. 2013 Mar 5;21(3):493-9.

活性位点比较

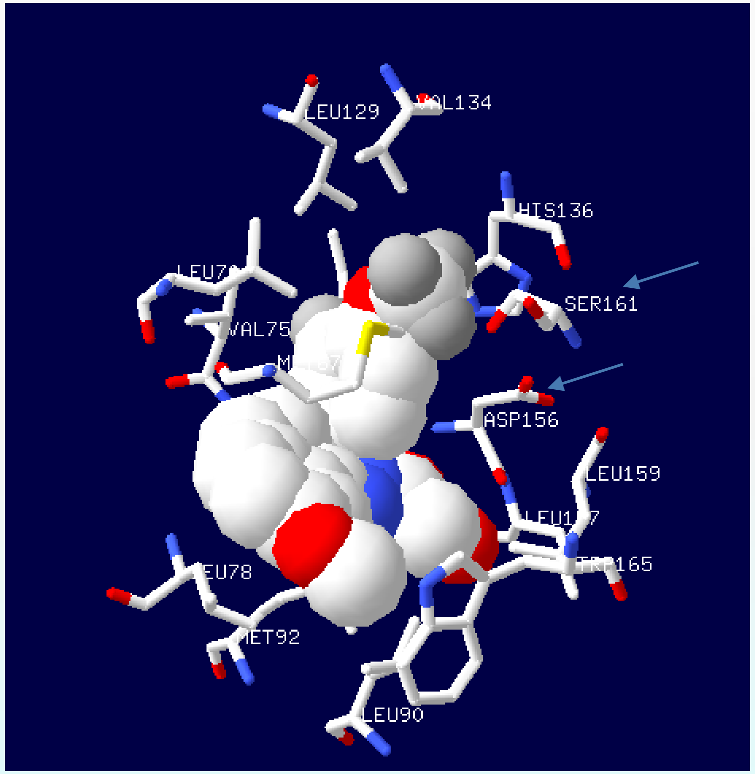


RIPK1_HUMAN (4ITIa)

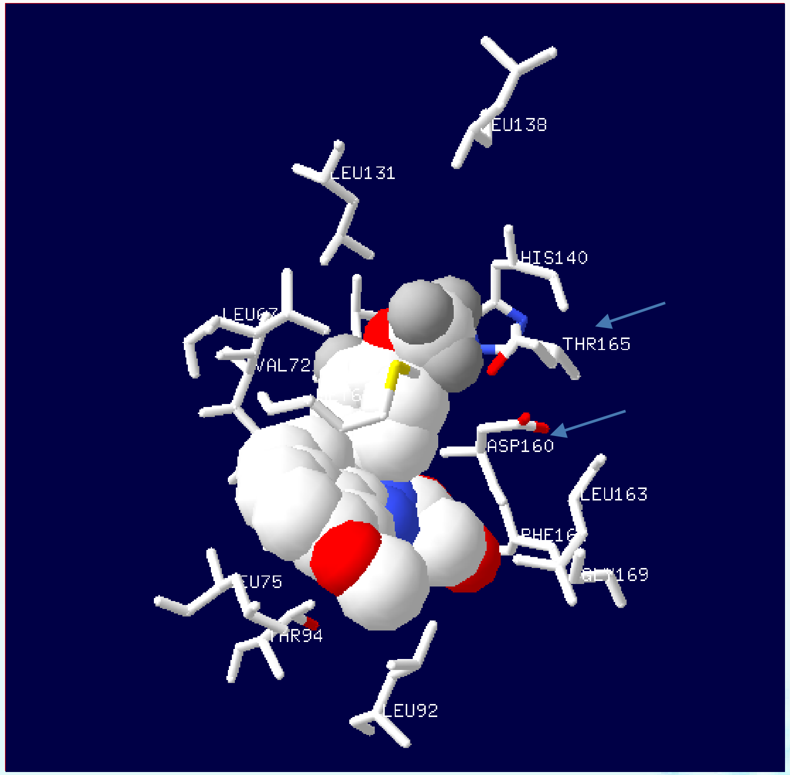


RIPK3_HUMAN_MODEL

Necrostatin-3与活性位点结合情况

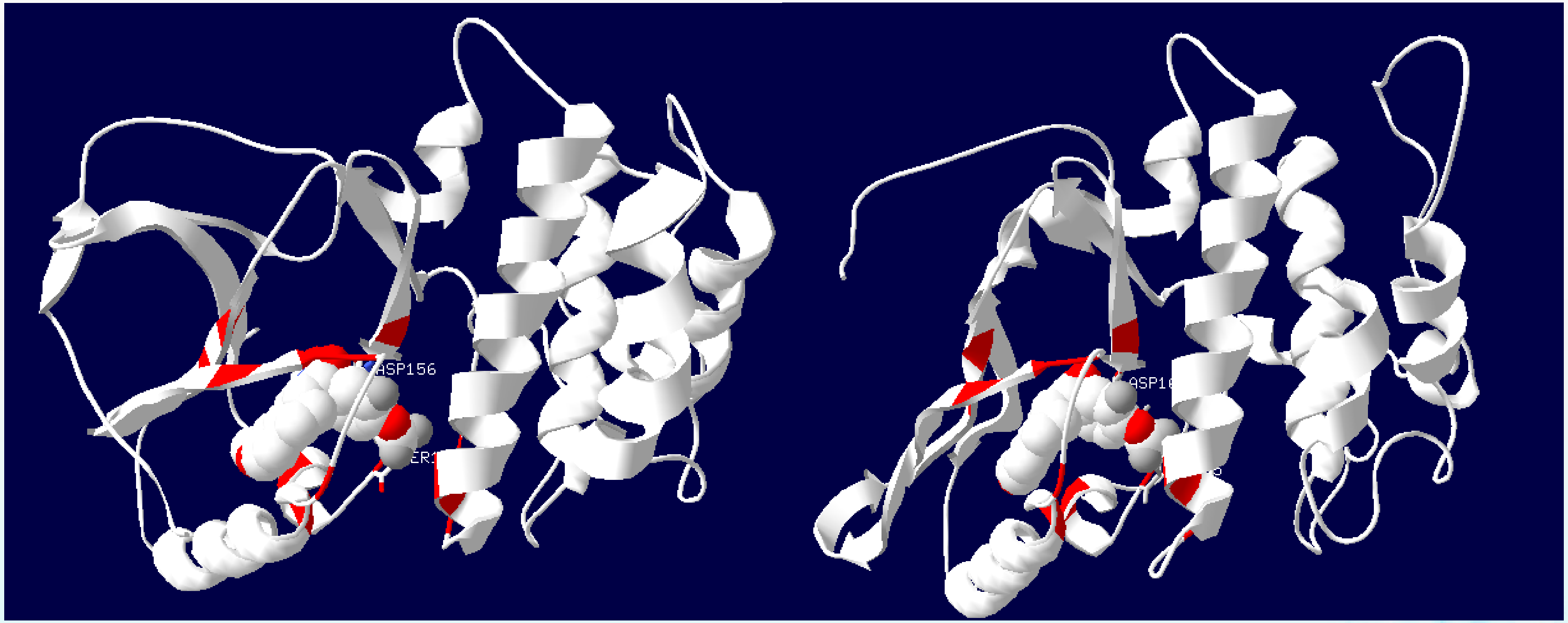


RipK1_HUMAN (4ITIa)



RipK3_HUMAN_MODEL

Necrostatin-3与活性位点结合情况



RipK1_HUMAN (4ITIa)

RipK3_HUMAN_MODEL

结论

1. 通过同源模建，以Rip1的结构为模板，构建了Rip3的蛋白结构；

2. Rip1的小分子抑制剂Necrostatin-3可能是Rip3抑制剂。

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致谢

感谢罗老师的悉心指导；

感谢ABC全班同学；

谢谢！！！