

马铃薯抗晚疫病基因的分 离及功能分析

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研究背景

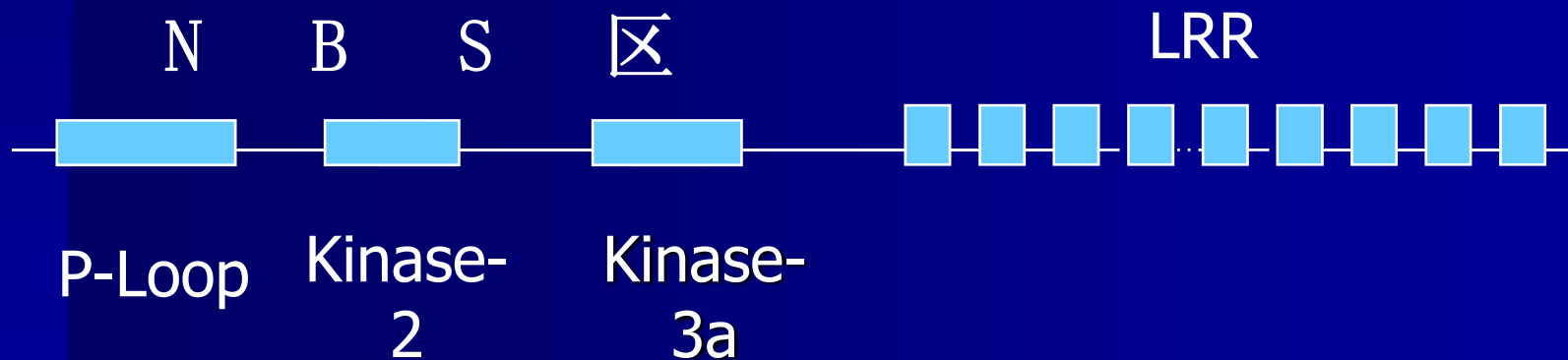
- 马铃薯作为世界上最重要的粮食和经济作物，在世界农业生产中有举足轻重的作用。晚疫病是危害马铃薯生产最主要的病害，加强晚疫病的防治工作是马铃薯生产须优先考虑的重要环节。19世纪中叶爱尔兰空前的“大饥荒”就是由晚疫病引起。



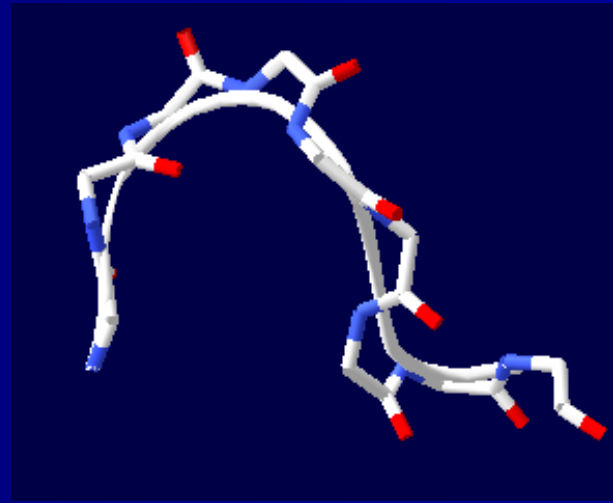
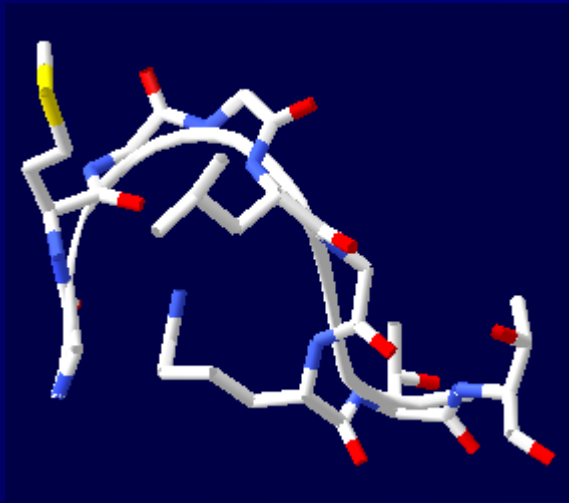
- 利用基因工程技术将高效的晚疫病抗病基因导入到马铃薯主栽品种，是培育抗病品种快捷有效的途径之一。
- 目前已从马铃薯抗性野生种 *S.demissum* 中鉴定出了十几个晚疫病抗病基因，其中4个抗病基因已经被克隆出来，*S.demissum* 中尚有大量的抗病基因资源有待开发。
- 我们的工作就是要从此野生种中继续分离晚疫病抗病基因

R基因的序列保守性

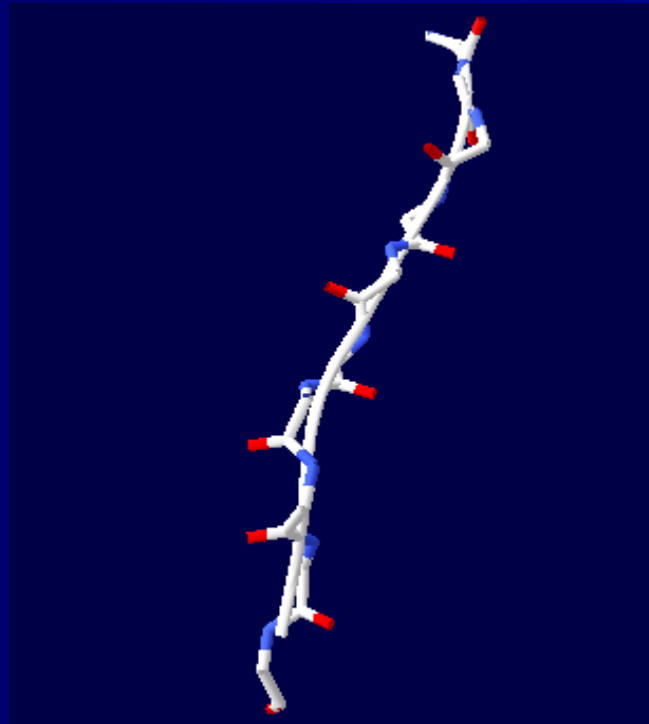
- 在已克隆并加以分析的70多个已知功能的R基因中它们似乎都编码植物防卫反应信号转导系统中的组分,并存在有特征性的保守结构域**NBS**（核酸结合位点）-**LRR**（亮氨酸重复区）



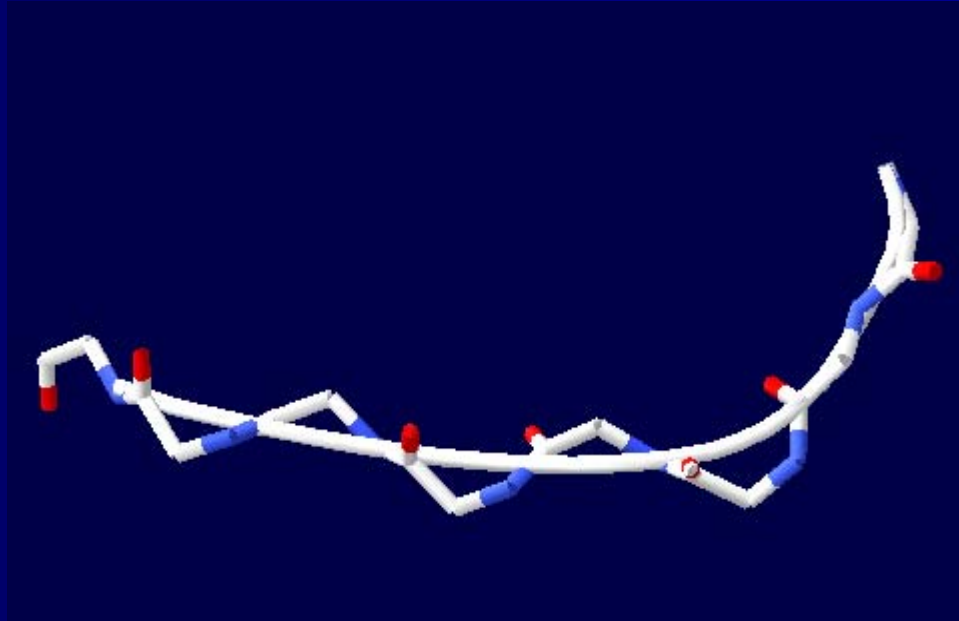
P-loop 作用是结合ATP/GTP的磷酸



Kinase-2, 4个疏水氨基酸残基后紧跟一个恒定的带负电荷的天冬氨酸, 其共有序列为
K(K/R)XaaaaDDV(W/D)



Kinase-3a 与嘌呤核苷酸的嘌呤或核糖结合有关，通常含有一个精氨酸残基

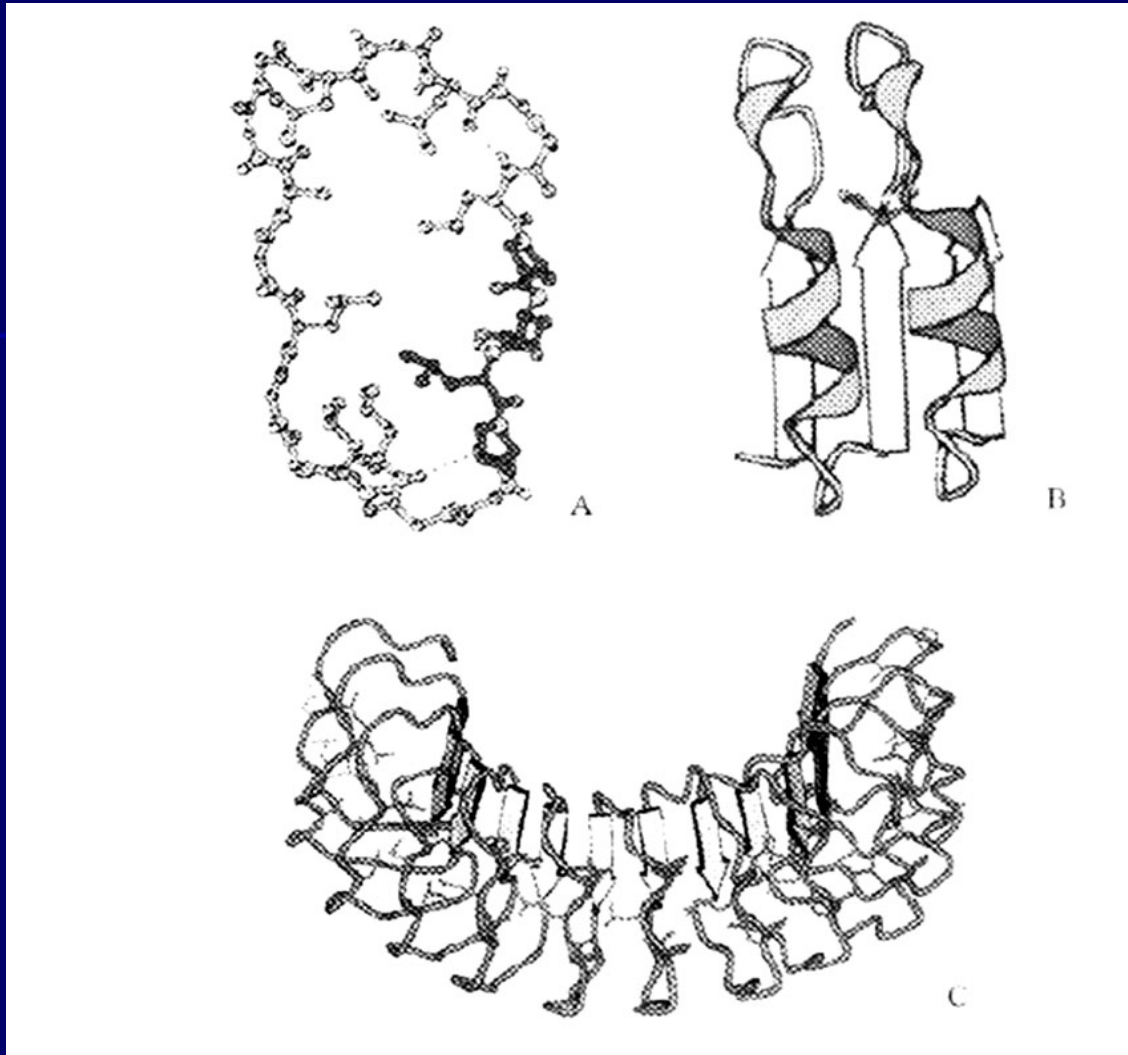


- 这三个单元共同组成R蛋白NBS区的ATP/GTP的结合位点，它在结构上与动物细胞凋亡的调节因子相似；被认为是在蛋白质与蛋白质相互作用中作为一个功能组件发挥作用。比如嘌呤核苷酸的结合被认为是改变了R蛋白和防御信号途径中其他成员间的相互作用。

LRR

- LRR是蛋白质与蛋白质相互作用的一种典型类型,具有LRR的蛋白质可以组成一个超家庭,生物中许多膜受体便属于这一家庭。
- 一般认为, LRR域是由大约每24个氨基酸残基作为一个重复单元而构成的一系列重复基序, 在每一个重复单元中, 亮氨酸与其他疏水氨基酸以有规则的间隔交错排布, 进而形成近似卷曲弹簧的三维结构。在这一结构中, 保守的亮氨酸残基形成疏水的核心, 而其它非保守的氨基酸残基则暴露于卷曲弹簧外周形成了亲水的配体结合表面。

LRR的三级结构

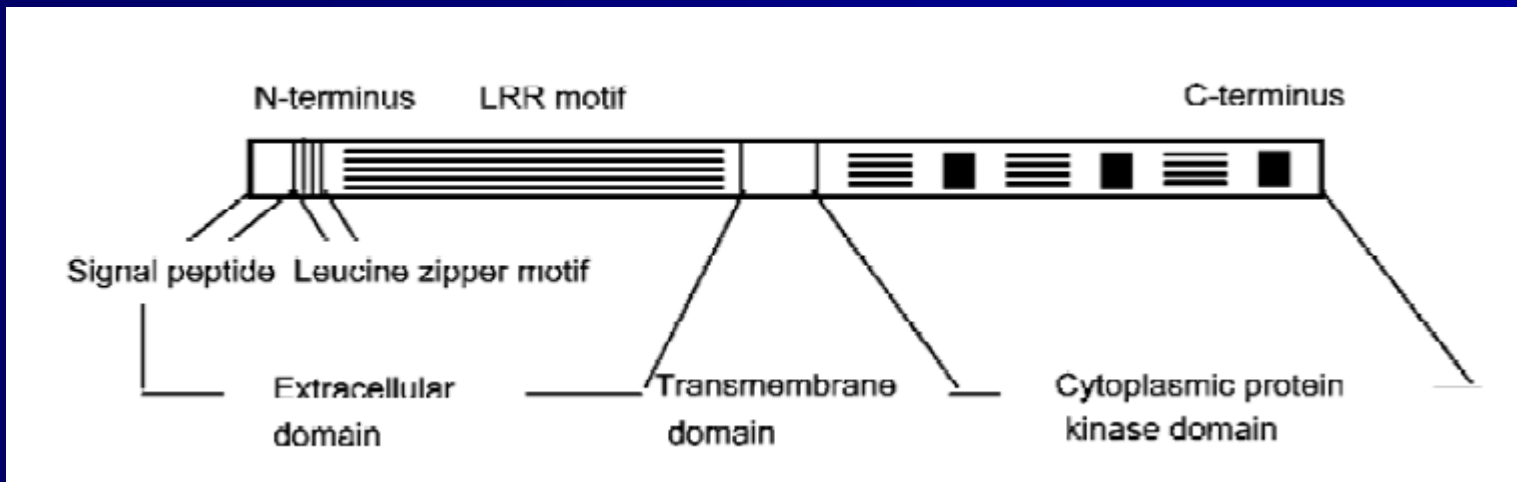


A 用球棍表示的1个LRR

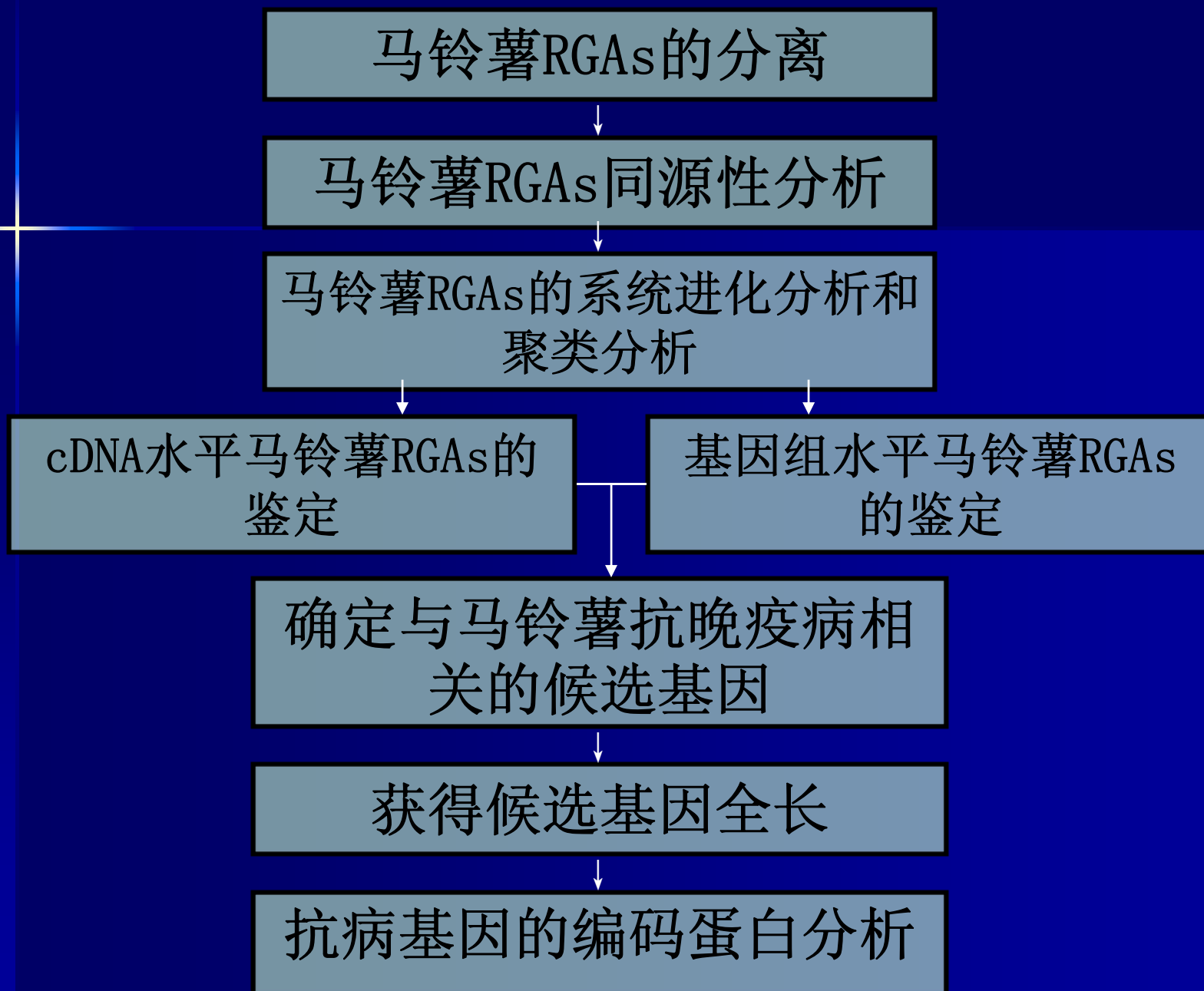
B 用 α 螺旋和 β 折叠表示的2个LRR

C 连续多个LRR形成的马蹄形

- 植物细胞对不同病原体的识别是植物抗病的基础，抗性基因编码蛋白的LRR区域的多样性较高则有利于植物细胞识别不同病原体无毒基因（Avr gene）编码的蛋白。
- 共有序列可概括为“XLXXLXLXXNXLXGXIPXXLXXLX”



技术路线



研究过程

材 料

- 高抗晚疫病的马铃薯野生近缘种 *S. demissum* 作为试验材料，六个马铃薯栽培品种垄3、尼勒克，大西洋、紫花白、*MC-4*、*Favorita* 作为对照材料

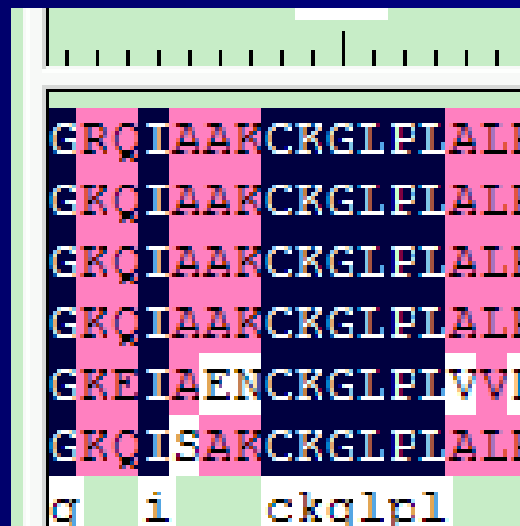
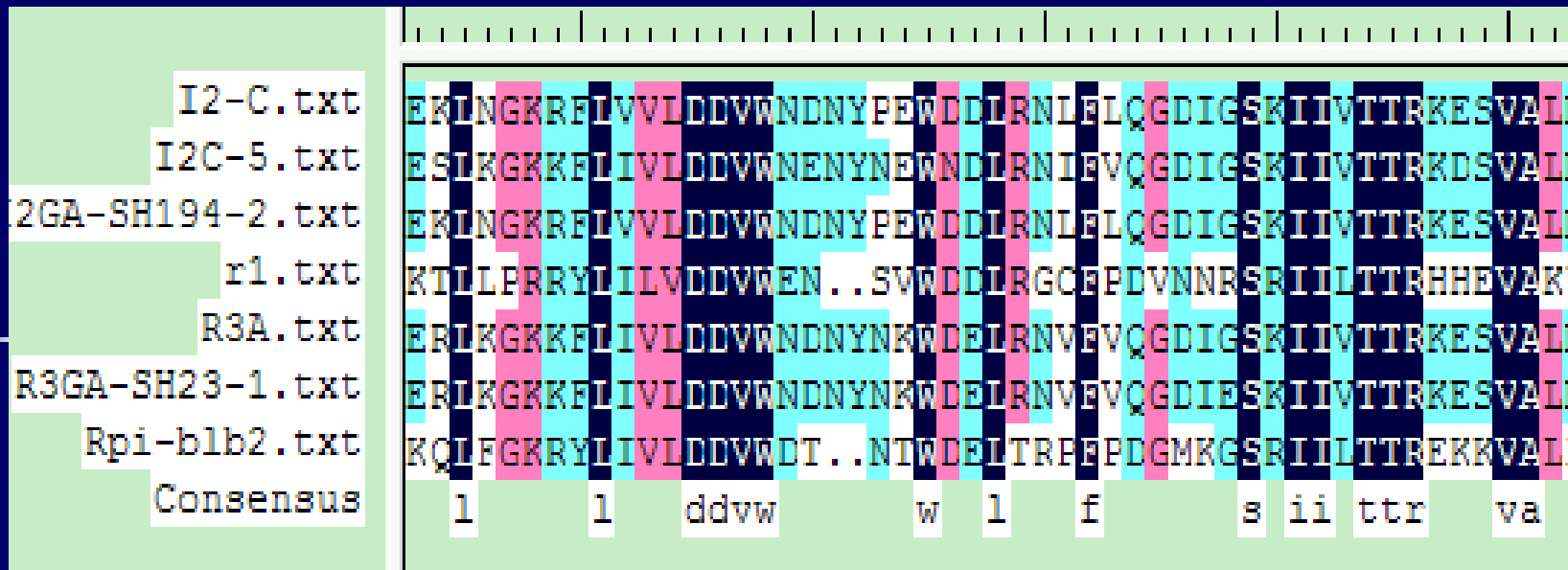
植物中已知抗病基因的序列分析

- 以马铃薯抗晚疫病野生品种 *Solanum dimissum* 为试材，根据GenBank中已登录的植物抗病基因 (*R1*、*R3a*、*Rpi-b1b2*、蕃茄中的抗镰刀霉枯萎病基因 *I2*、*I2C-5*、*I2-C*、*I2GA-SH23-1*、*I2GA-SH23-3*、*I2GA-SH194-2*等) 的NBS区的保守序列kinase-2 (DDVW) 和GLPL区设计简并引物。

利用NCBI的GeneBank上依次找到以上序列

名称	序列号	序列信息
<i>R1</i>	EU599037	4392 bp Solanum demissum
<i>R3a</i>	AY849382	3849 bp Solanum tuberosum
<i>Rpi-b1b2</i>	DQ122125	3804 bp Solanum bulbocastanum
<i>I2GA-SH23-1</i>	AY849383	3798 bp Solanum tuberosum
<i>I2GA-SH23-3</i>	AY849384	3984 bp Solanum tuberosum
<i>I2GA-SH194-2</i>	AY849385	3861 bp Solanum tuberosum
<i>I2C-5</i>	AF408704A	4491 BP Lycopersicon pimpinellifolium

KLEGILLGLQIVLSDAENKQASNRHVSRWFNKLQNAVDSAENLIEEVNYEVLRLKVEG
QHQNLAETGNQQVSDNLNCLSD EFFLNKDKLEDTIETLTKDLQE QIGLLGLKEHFVST
KQETRAPSTSLVDDAGIFGRQNEIENLIGRLLSKDTKGKNLAVVPIVSMGGGLGKTILA
KAVYNDERVQKHFGGLKAWFCVSEAYDALRITKGLLQEIDSFDLKVDDNLNLQVRLKE
KLNKGRFLVVLDDVWVNDNYPEWDDLRLNFLQGDIGSKIIVTTRKESVALMMGGGAIYM
GILSSEDSWALFKRHSLENMDPMGHPELEEVGKQIAAKCKGLPLALSTLAGMLRSKSE
VEEWKRILRSEIWELPHNDILPALILSYNDLPAHLKRCFSYCSI FPKDY PFRKEQVIH
LWIANGLVPPQDEI IEDSGNQYFLELRSRSLFQRVPNPSEGNTENLFFMHDLVNDLAQ
IASKLCIRLEESQGSHMLEQSRHLSYSKGYGGEFEKLTPLYKLEQLRTLLPICIDIN
CCFLSKRVQHNILPRLRSLRALSLSGYMIKELPNDLFIKLLRFLDLSEAWIEKLPD
SVCGLYNLDTLLLSSCYNLEELPLQMEKLINLRHLDISYTRLLKMPHLHLSKLISLQVL
VGAKFLVGGLRMEDLGEVYNLYGSLSVVELQNVVDSREAVKAKMREKNHVDKLSLEWS
ESSADNSQTERDILDEL RPHKNIKELQIIGYRGTKFPNWLADPLFLKLVQLSIDNCK
NCYSLPALGQLPFLKFLSIRGMHGITEVTEEFYGS CSSK KPFNSLVELRFEDMPEWKQ
WDLGSGEFPILEKLLIENCPELSLETVP IQLSSLKSFEVSGSPMVINFPFSILPTTL
KRIRIIDCQKLEQPVGEMSMFLEELTLQNCDCIDDISPPELLPRARHLCVYDCHNLT
RFLIPTASESLYICNCENVEVLSVACGGTQMTSLSIDGCLKLGKGLPERMQELFPSLNT



利用DNAMAN多序列比对设计简并引物

- 以 *S. demissum* 的 cDNA 作模板进行 RT-PCR 扩增, 产物经克隆测序, 得到 47 条序列
- 共获得 31 条具有开放阅读框的含 NBS 区保守结构域的序列。

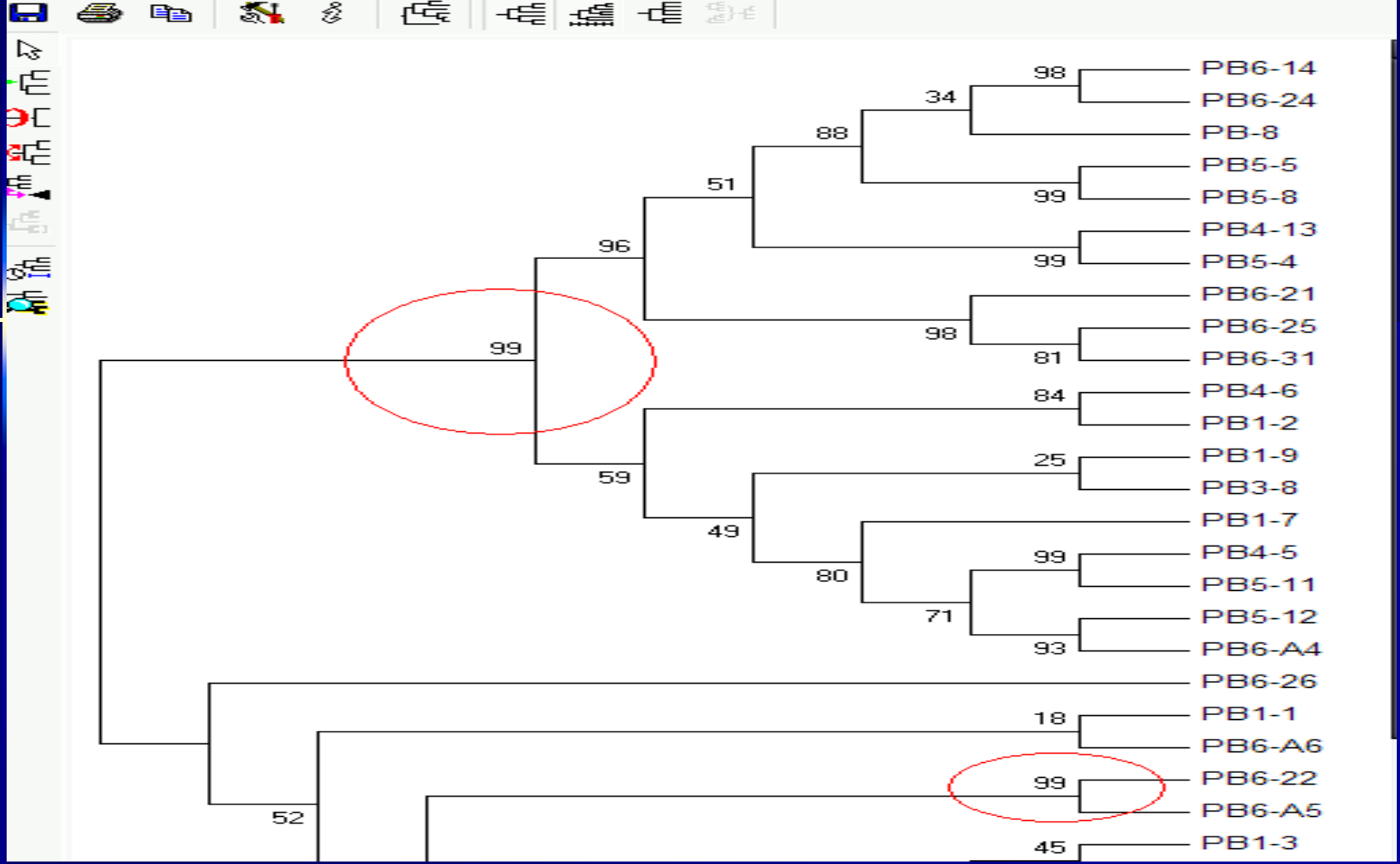
利用DNAMAN和ClustalX进行蛋白相似性比对，利用Mega对所有的NBS区序列构建系统进化树，并根据进化树结果对所有序列进行聚类分析。

Identity= 86.02%

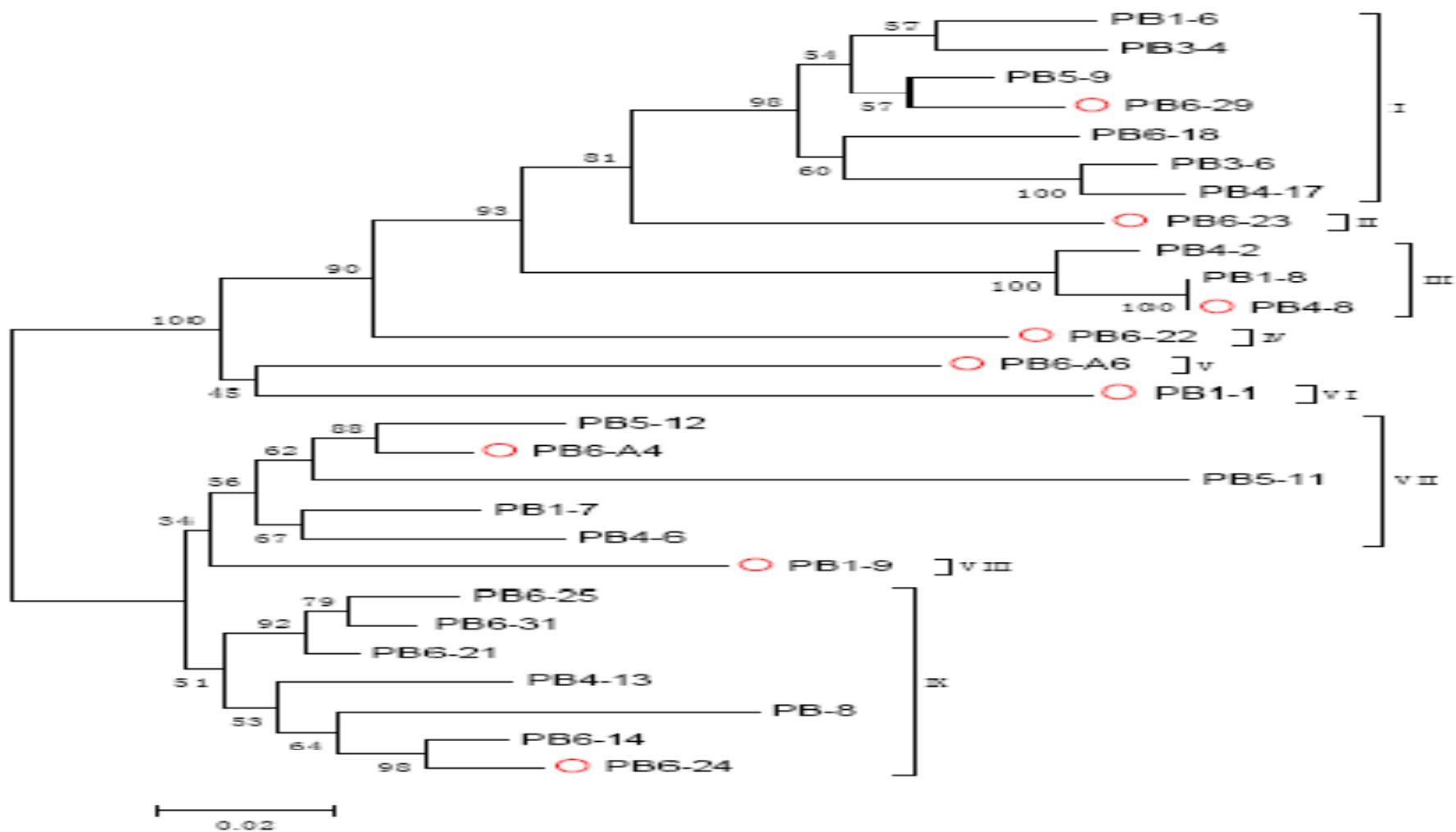
1

0 10 20 30 40 50 60 70 80 90 100 110

```
3LGKTTFAKAVYHDERVKNHFGLKAWYCVSEFYDALRITKGLLQEIGKFD SKDVHNNLNQLQVKLKE SLKRRKFLIVLDDVWVNDNYNEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTLAKAVYNDEKVNRRHFDLKAWFCVSEQYDAFRITAKGLLQEIG... LKVDDDNINQIQIKLKE SLIGKKFLIVLDDVWVNDNYNEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTFAKSVYNDEKVKKHFGLKAWICVSEFYDAVRITKELLQEIG... LTVDNN.LNQLQVKLKEGLKGGKFLIVLDDVWVNDNYKEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTFAKAVYNDEKVNHFGLKAWICVSEFYDILRITKELLQEFG... LMVDNN.LNQLQVKLKE ILKGGKFLIVLDDVWVNDNYKEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTFAKAVYNDEKVNDFHFDLKAWFCVSEQYDAFRITAKGLLQEIG... LQVDDN.MNQIQIKLKE SLKGGKFLIVLDDVWVNDNYNEWDDLRLNFVQGYLGSKIIVTTRKESVALM
3LGKTTFAKSIYHDERVKNHFGLKAWYCVSEFYDALRITKGLIQEI... DSQDVHNNLNQLQVKLKEKLGKGGKFLIVLDDVWVNDNYNEWDDLRLNFVQGDIGSKIIVTTRKERVALLM
3LGKTTLAKAVYNDEKVNRRHFDLKAWFCVSEQYDAFRITAKGLLQEIG... LKVDDDNINQIQIKLKE SLIGKKFLIVLDDVWVNDNYNEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTFAKEVYNDEKVKKHFGLKAWFCVSEAYDAFRITKGLLQEIGSTDVK.VDDNINQLQVKLKE SLKGGKFLIVLDDVWVNDNYNKWDELRLNIFAQGDIGSKIIVTTRKESVALM
3LGKTTFAKAVYNDEKVNHFGLKAWICVSEFYDILRITKELLQEFG... LMVDNN.LNQLQVKLKEGLKGGKFLIVLDDVWVNDNYKEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTLAKSVYKDEKVKKHFGLKAWICVSEFYDAVRITKELLQEIG... LKVDNN.LNQLQVKLKEGLKGGKFLIVLDDVWVNDNYKEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTFAKAVYNDEKVNHFGLKAWYFVSEGYDALRITKGLLQEIDKFDLKD VHNLNQLQVKLKE SLKGGKFLIVLDDVWVNDNYNEWDDLRLNSFVQGDIGSKFIVTTRKESVALM
3LGKTTFAKAVYNDEKVKKHFGLKAWFCVSEAYDAFRITKGLLQEIGSTDVK.VDDNINQLQVKLKEGLKGGKFLIVLDDVWVNDNYNEWDDLRLNFVQGDIGSKIIVTTRKESVALI
3LGKTTFAKAVYNDEKVNHFGLKAWICVSEFYDILRITKELLQEIG... LTVDNN.LNQLQVKLKE SLKGGKFLIVLDDVWVNDNYKEWDDLRLNFVQGDIGSKIIVTTRKESVALI
3LGKTTLAKAVYNDEKVNHFGLKAWLCVSESYDAFRITKGLLQEIGSFDLK.VDDNINQLQVKLKE SLKGGKFLIVLDDVWVNDNYNEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTFAKAVYNDEKVNHFGLKAWFCVSEAYDAFRILTRGLLQEIGSFDLKDDNN.MNQLQLKKE SLKGGKFLIVLDDVWVNDNYNEWDYLRNIFAQGDIGSKIIVTTRKESVALM
3LGKTTFAKAVYNDEKVKDFHGLKAWICVSEFYDAVRITKELLQEISSDCTGNSN.LNQLQIKLKE SLKGGKFLIVLDDVWVNDNYDEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTLAKAVYNDEKVKKHFGLKAWFCVSESYDAFRITKGLLQEIGSTDVK.VDDNINQLQVKLKEGLKGGKFLIVLDDVWVNDNYNEWDDLRLNFVQGDIGSKIIVTTRKESVALI
3LGKTTFAKAVYNDEKVNHFGLKAWFCVSESYDAFRITKGLLQEIGSFDLK.VDDNINQLQVKLKE SLKGGKFLIVLDDVWVNDNYNEWDDLRLNFVQGDIGSKIIVTTRKESVALI
3LGKTTFAKAVYNDEKVKKHFGLKAWICVSEFYDAVRITKELLQEIG... LKVDNN.LNQLQVKLRE SLEGKKFLIVLDDVWVNDNYKEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTFAKAVYNDEKVNHFGLRAWFCVSESYDAFRITKGLLQEIGSFDLK.VDDNINQLQVKLKE SLKGGKFLIVLDDVWVNDNYNEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTFAKAVYNNERVKNHFGLKAWYCVSEGYDALRITKGLLQEIGKFD SKDVYNNLNQLQVKLKE SLKGGKFLIVLDDVWVNDNYNEWDDLRLNFVQGDIGSKIIVTTRKESVALM
3LGKTTFAKAVYNDEKVNHFGLKAWFCVSENYDAFRILTRGLLQEIGSFDLKDDNN.MNQLQLKKE SLKGGKFLIVLDDVWVNDNYNEWDYLRNIFAQGDIGSKIIVTTRKESVALM
3LGKTTFAKAVYNDEKVKKHFGLKAWFCVSEAYDAFRITKGLLQEIG... LK.VDDNINQLQVKLKEKLNKGRFLVVLDDVWVNDNYPEWDDLRLNIFAQGDIGSKIIVTTRKESVALM
```

对所有测序所得的NBS区序列取核酸相似性在97%的进行进一步分析 (Neighbour-joining method, Bootstrap Reps = 1000) .



共有**27**条序列参与了分析，以核酸相似性**90%**分为**九大**类

这27条序列以核酸相似性90%为界 分为九类

类别	序列名称
I	PB1-6/PB3-4/PB5-9/ PB6-29 /PB6-18/PB3-6/PB4-17
II	PB6-23
III:	PN4-2/PB1-8/ PB4-8
IV	PB6-22
V	PB6-A6
VI	PB1-1
VII	PB5-12/ PB6-A4 /PB5-11/PB1-7/PB4-6
VIII	PB1-9
IX	PB6-25/PB6-31/PB6-21/PB4-13/PB-8/PB6-14/ PB6-24

检测

- 九类特异引物分别在*S. demissum*和六个栽培品种基因组中的进行PCR检测
- 结果:第IV类只在*S. demissum*中有扩增条带,而在其他的六个对照品种中没有扩增

确定**PB6-22**为马铃薯抗晚疫病
相关的候选基因

获得基因全长序列

- 产物用RACE方法找出目的基因PB6-22的核酸全长序列
- 得到编码蛋白序列

获得基因PB6-22的编码蛋白

MDICTVALLKNOEYYSLLGLKVEGQHQILAETSNQOVSDAFFRNIKDKLEDTI
ETLEDLEEHFLDLKKHFGPTNQETRTPSTSFVDESDFGROSETEDLIDRLLSE
DASGKNLTVVPIVGMGGLGKTTFAKAVYNDERVKNHFDLKAWFCVPENYDA
FRLTRGLLOEFGSFDLKDDNNMNQLQLKLKESLKGK**KFLVVLDDVW**NDDYN
EWDYLRNIFVQGDM**GSKIIVTTR**KESVALMMGSRPINVGTLSDEASWDLFK
RHSLENRDPKEHPELVEIGKQIADKCK**GLPLALK**ALAGVLRCKSEVDEWRDI
LRSEIWELPSCSNG**ILPALMSY**NDLPAHLKQCFAYCAIYPKDYQFCKDOVIH
LWIANGLVQOFHSGNOYFLELRSRLFEMVSESSEWNSEKFLMHDLVNDLA
QIAPSNLCIRLEENKGLHMLEQCRHISYSIGQDGDGDFDKLKSFLKSKQLRTLLA
INVHPHYDIIKLSKRVLHNVLPRLT**SLRALSLR**YEIVELPNDLFIKLLRFLD
ISRTKIKRVPDSIC**VLYNLETLLLSSCDYLEELPOMEKLINLRHLDISNSFRLK**
MPLHLSKLSLQVLVGAKFLLSGWRMEDLGEAONLYGSSVSVVELENVVDRRE
AVKAKMREKNHVDKLSLKWSSADNSQTERDILDELRPHTNIKEVEITGY
RGTTFPNWLADPLFL**KLVKLSLSN**CKDCY**SLPALGELPCKI**LSIKGMHGITEV
MEESYGSLSSKPYCLEELRFEDMAEWKQWHVLGSGEFPTVEKLFIIINCP
LETPIQLSSLKRFQVSGCPKVGVFDDTELFRSOLEGMKQIEELCIRYCNSV
TSLPFSILPTTLKRITISGCLKLEAPVGENSM**CLEELSLEE**CDCVSPELLPTA
RYLSVRRCDNLTRFLIPTATETLNIWGSSENVEKLSVACGEAQMILLSISDCSK
LKCLPELLPSLKTQLYECPEIESFPEGGLPFNLQLLOINNCTKLVNGRKEWCL
QRLPCLNWLEISHDGSDEEIVGGENWELPSSIQTLLIQNLKTLSSQHLKNLTS
LQSLYYGYLPQIQSMLEQVRLTSS**SLFDLILYGHHELHSLDLWHL**TSLSLSLQIM
NCPNLOSSESALPSSLSHLIISDCPNLQSLPVKGMPSLSKLSISNCPLLGPL
LEFNKGEYWPNI AHISTIKINGECL—

通过网站预测得到目的基因的蛋白结构

(<http://www.cbs.dtu.dk/services/CPHmodels/>)

The screenshot shows the homepage of the CPHmodels 2.0 Server. At the top, there is a navigation menu with various categories: EVENTS, NEWS, RESEARCH GROUPS, CBS PREDICTION SERVERS, CBS DATA SETS, PUBLICATIONS, and BIOINFORMATICS EDUCATION PROGRAM. Below the menu, there is a breadcrumb trail: CBS >> CBS Prediction Servers >> CPHmodels. The main heading is "CPHmodels 2.0 Server", followed by a brief description: "CPHmodels 2.0 is a homology modeling server." and a link to the "version history". Below this, there are three tabs: "Instructions", "Output format", and "Article abstract". The "SUBMISSION" section contains a text input field for pasting a sequence in FASTA format, a file upload button, and "Submit" and "Clear fields" buttons. At the bottom, there are "Restrictions" and "Confidentiality" sections.

CBS >> [CBS Prediction Servers](#) >> CPHmodels

CPHmodels 2.0 Server

CPHmodels 2.0 is a homology modeling server.

View the [version history](#) of this server.

[Instructions](#) | [Output format](#) | [Article abstract](#)

SUBMISSION

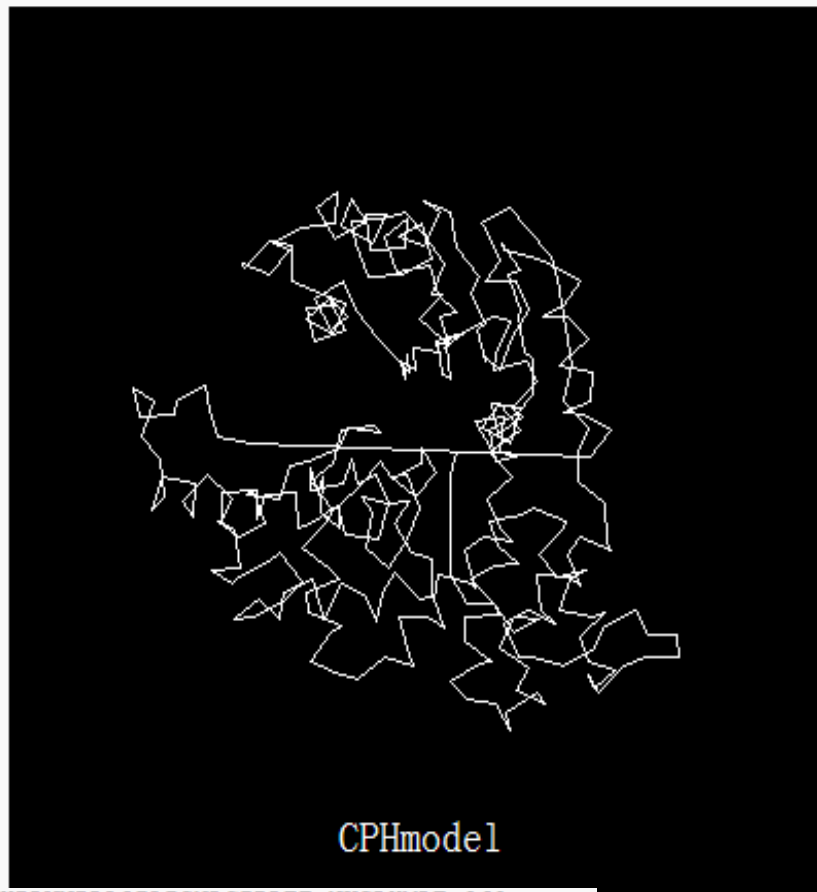
Paste a single sequence or several sequences in **FASTA** format into the field below:

Submit a file in **FASTA** format directly from your local disk:

Restrictions:
Only one sequence per submission with not less than 15 and not more than 4,000 amino acids.

Confidentiality:
The sequences are kept confidential and will be deleted after processing.

CENTER FOR BIOLOGICAL SEQUENCE ANALYSIS ■ TECHNICAL UNIVERSITY OF DENMARK DTU



```
Query: 301 GNQYFLELRSRLLFEMVSESSEWNSEKFLMHDLVNDLAQIAPSNLCIRLEE-NKGLHMLE 360
      +++++ E+  + L+  +   ++  + +HDL D+  + + C +L++ +K++  +
Templ: 301 VEDILQEFVNKSLFLCDRNGKSFR---YYLHDLQVDF---LTEKNCSQLQDLHKKIITQF 360
```

```
Query: 361 QCRHISYSIGQDGD 374
      Q H +++ D +
Templ: 361 QRYHQPHLSPDQE 374
```

Modeling ...

File with coordinates for model in pdb format: [model.pdb](#)

- 此编码蛋白在预测其结构时是以**1Z6T**蛋白A链为模型
- **1Z6T**是一种结合**ADP**的细胞凋亡蛋白激活因子
- **entry: 1Z6T chain: A score: 99 E: 2e-20**
- **E**值表示依此蛋白为模型预测目的蛋白结构的错误概率，证明此结果很可信

1Z6T和PB6-22蛋白序列比对

- 利用WebLab中的water程序进行局部序列比对

```
Length: 353
Identity:      80/353 (22.7%)
Similarity:   140/353 (39.7%)
Gaps:         89/353 (25.2%)
Score: 144.5
```

```

150 VTIHGMAGCGKSVLAA-----EAVRDHSLLEG--CFPGGVHVVSVGKQDK
   |.|.|.|.|.|.:|. . . . |.|.:|. . . . |.|. . . . . . . . . .
   1 VPIVGMGGLGKTTFKAVYNDERVKNHFDLKAWFCVPENYDAFRLTR---

193 SGLLMKLQNLCTRLDQDESFSQRLPLNIEEAKDRLRILMLRKHPRSLLIL
   |||. . . . . .|. . .|. . .|. |.|.:|. . . . . .|. . . .|. . .|.
   48 -GLLQEFGSF--DLKDDNNMNQ-LQLKLKES-----LKGKKFLVVL

243 DDVW----DSW-----VLKAFDSQCQILLTTRDKSVTDSVMGPKYVVPV-
   |||| . . . .|. . . . . .|. . . .|. . .|. . .|. . .|. . .|. . .|.
   85 DDWVNDYNEWDYLRNIFVQGMGSKIIVTTRKESVA-LMMGSR---PIN

283 -----ESS--LGKEKGLEILSLFVNMKKADLPEQAH---SIIKECKGSP
   .|. |.|. . .|| . . . . .|. . . .|. . . .|. . .|. . .|. . .|.
   131 VGTLSDEASWDLFKRHSLE-----NRDPKEHPELVEIGKQIADKCKGLP

322 LVVSLIGALLRDFPNRWEYYLKQLQNKQFKRIRKSSSYDYEALDE----A
   |. . . . . .|| . . . . . .|. . .|. . .|. . .|. . .|. . .|. . .|.
   175 LALKALAGVLR-----CKSEVDEWRDILRSEIWELPSCSNGILPA

368 MSISVEMLREDIKDYVTDLSILQKDVKVP TKVLCILWDMETEEVEDILQE
   . . .|. . .|. . .|. . .|. . .|. . .|. . .|. . .|. . .|. . .|. . .|.
   215 LMLSyndLPAHLKQCFAYCAIYPKDYQFCKDQVIHLW----IANGLVQQ

418 FVNKSLIFCDRNGK-----SFRYYLHDLQWDF--LTEKN-CS
   |. . . .|. . . . . . . . . . .|. . . .|. . .|. . .|. . .|. . .|.
   260 FHSGNOYFLELRSRLLFEMVSESSEWNSEKFLMHDLVNDLAQIAPSNLCI

```

DeepView / Swiss-PdbViewer

File Edit Select Build Tools

1.5 Å

Move All

model1 (701 x 502)

Find Sequence / PROSITE pattern

DDVW

? = any amino-acid

0 allowed mismatches

Highlight residues in structure

Cancel

OK

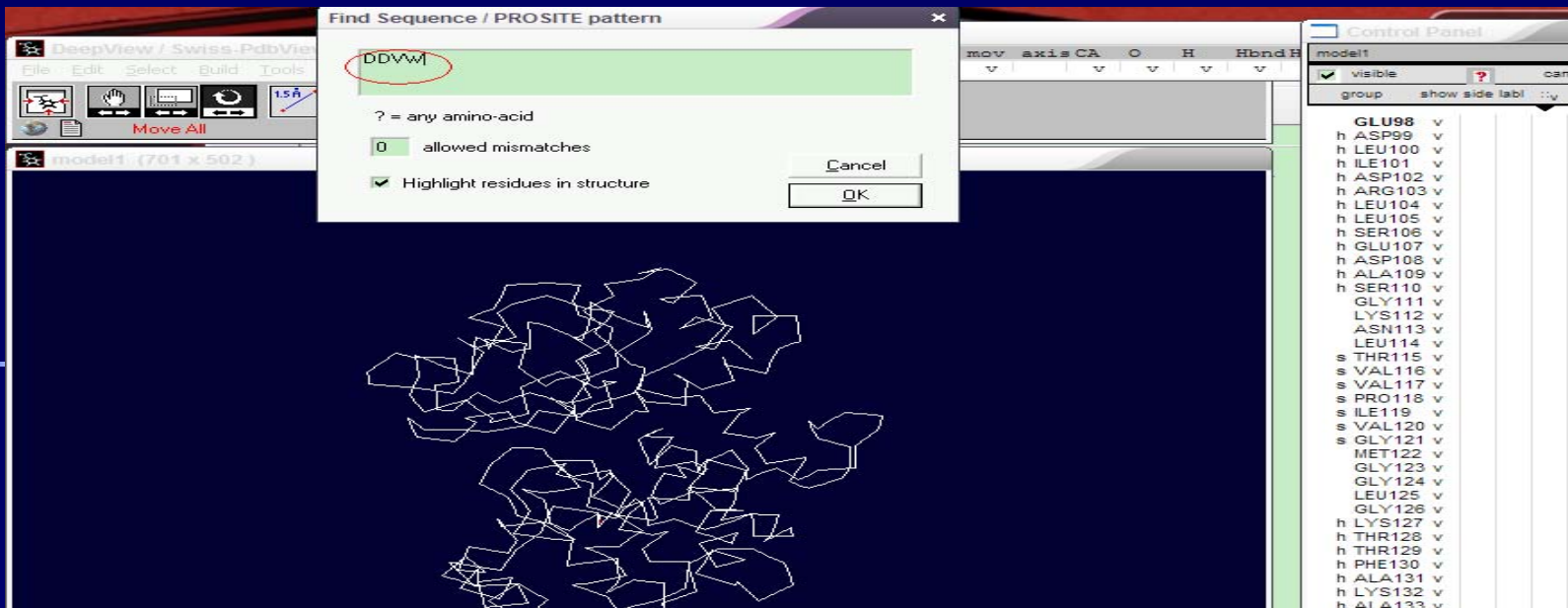
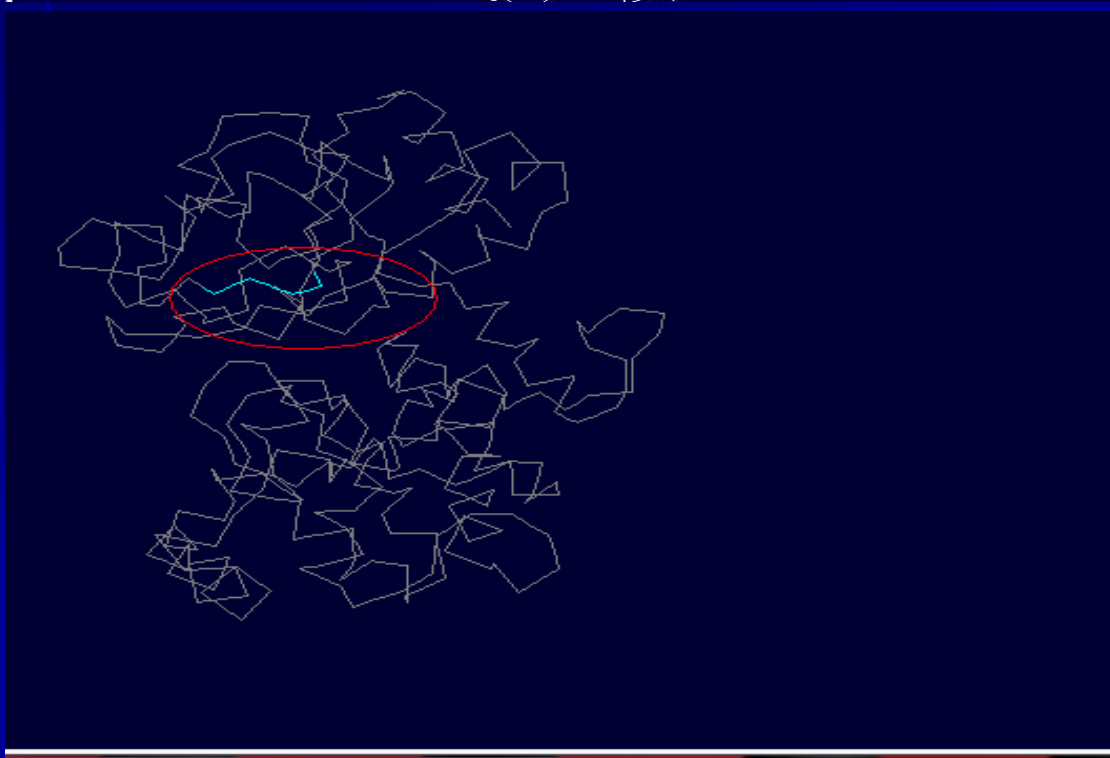
Control Panel

model1

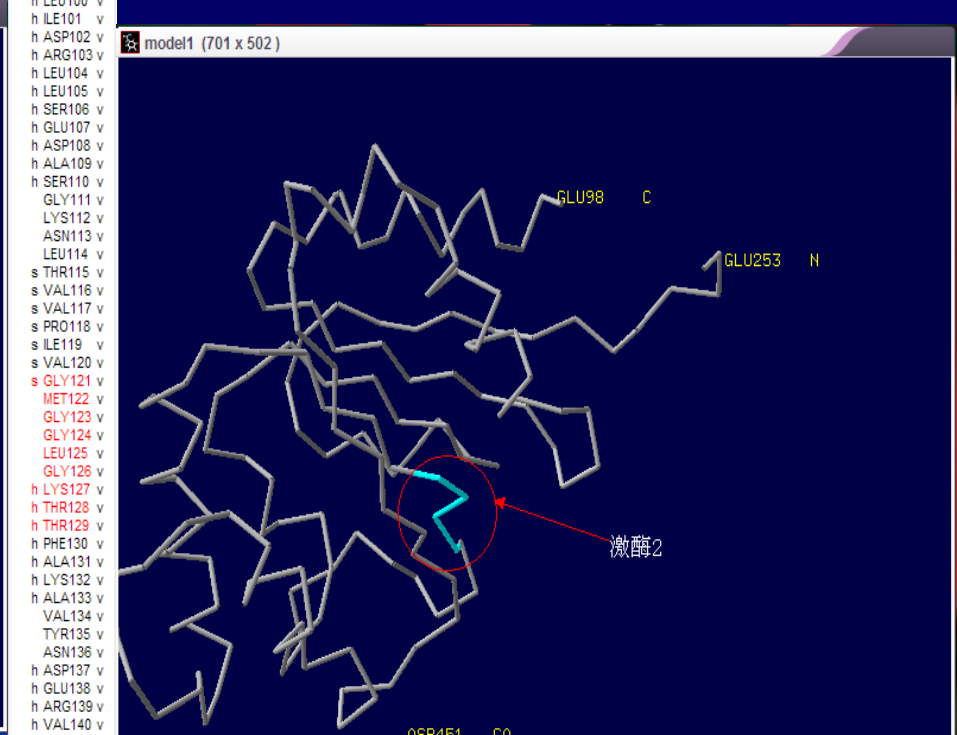
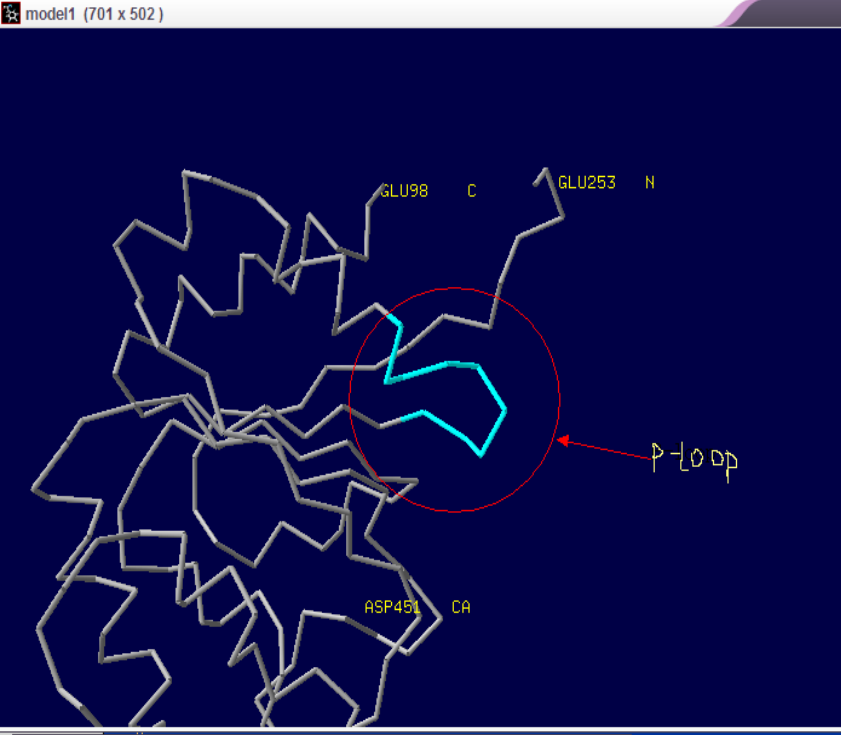
visible ? can

group show side labl ...

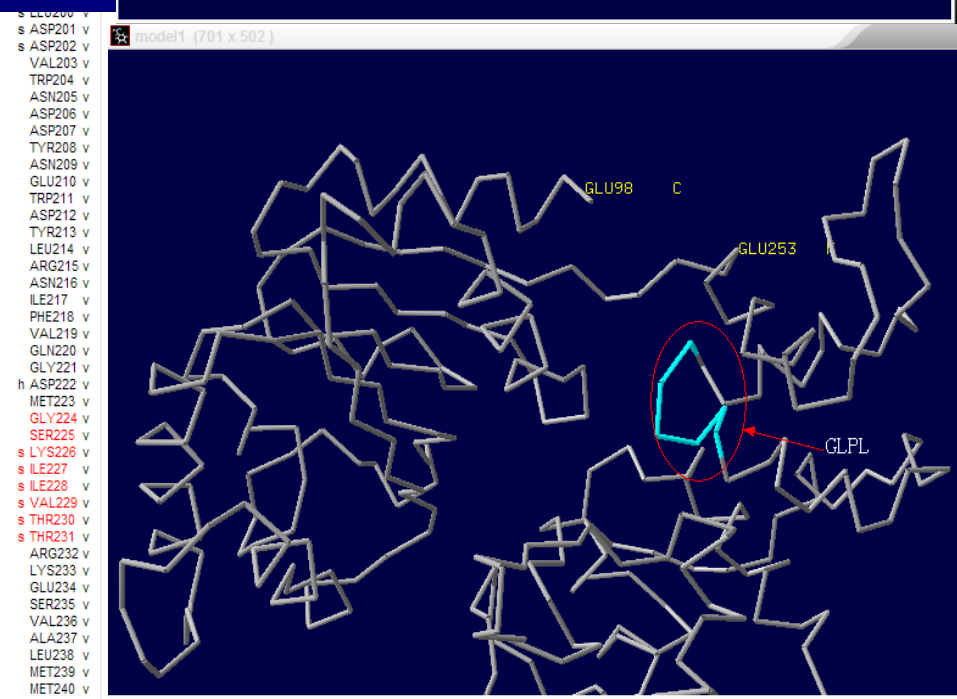
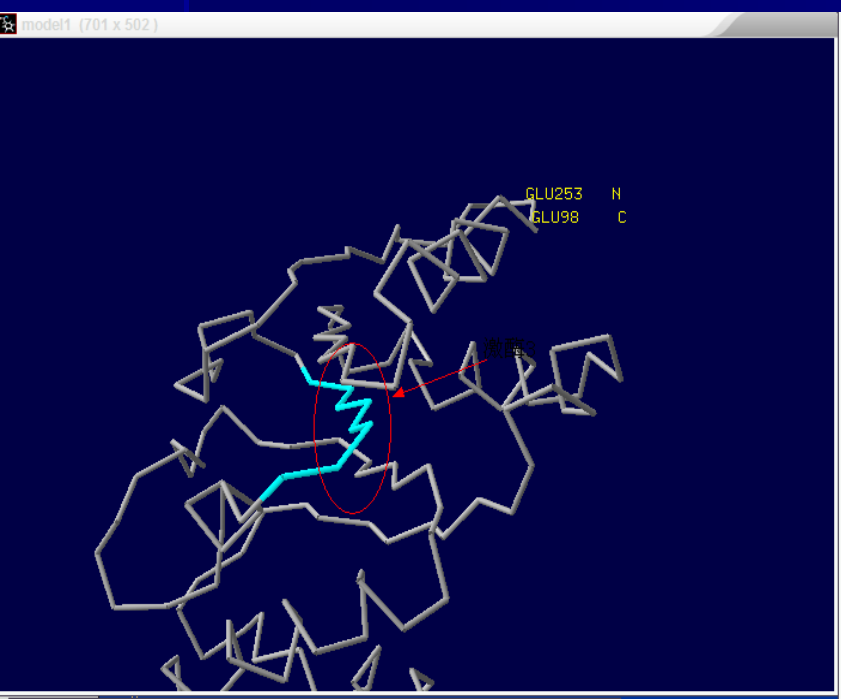
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- h LEU100 v
- h ILE101 v
- h ASP102 v
- h ARG103 v
- h LEU104 v
- h LEU105 v
- h SER106 v
- h GLU107 v
- h ASP108 v
- h ALA109 v
- h SER110 v
- GLY111 v
- LYS112 v
- ASN113 v
- LEU114 v
- s THR115 v
- s VAL116 v
- s VAL117 v
- s PRO118 v
- s ILE119 v
- s VAL120 v
- s GLY121 v
- MET122 v
- GLY123 v
- GLY124 v
- LEU125 v
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- h ALA133 v

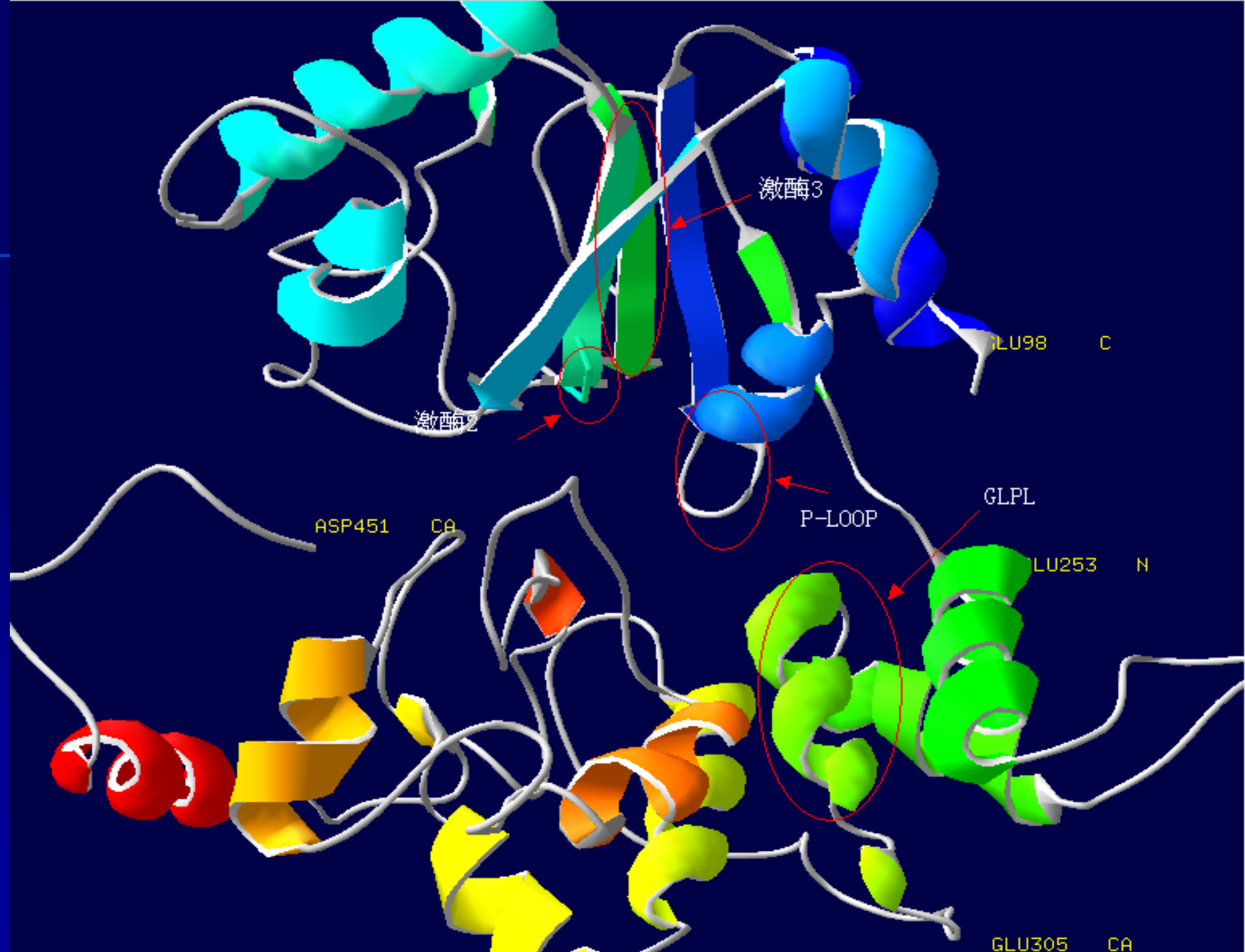
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- h SER190 v
- h LEU191 v
- h LYS192 v
- h GLY193 v
- h LYS194 v
- LYS195 v
- PHE196 v
- s LEU197 v
- s VAL198 v
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- s ASP202 v
- VAL203 v
- TRP204 v
- ASN205 v
- ASP206 v
- ASP207 v
- TYR208 v
- ASN209 v
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- ASN216 v
- ILE217 v



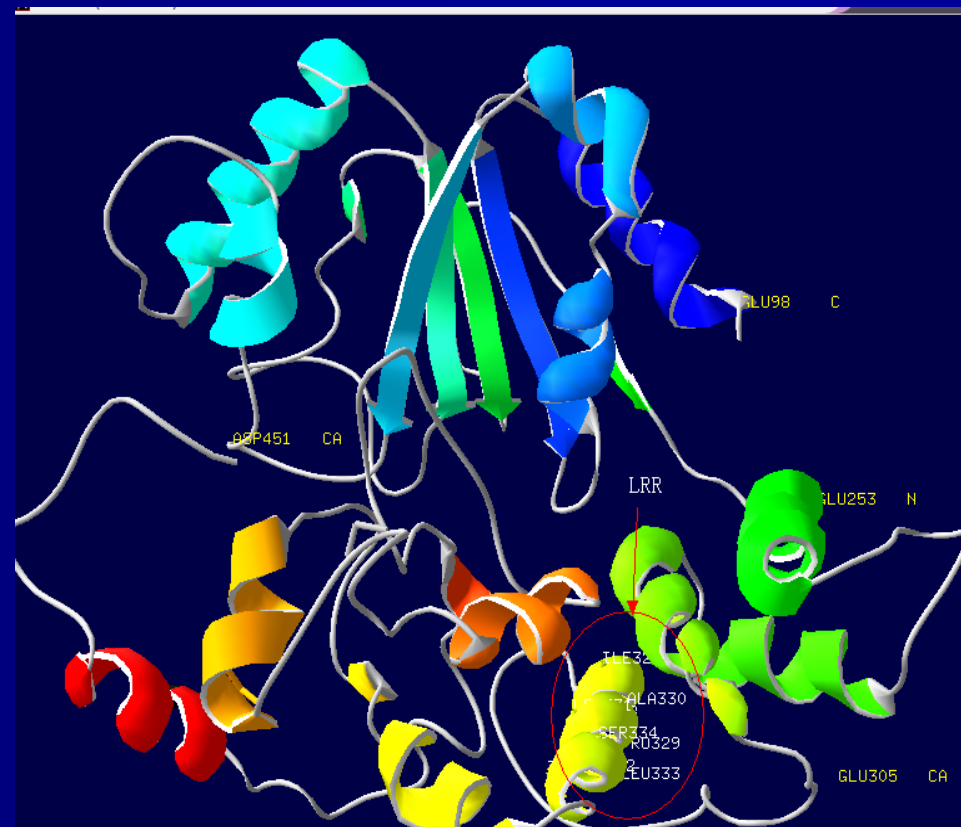
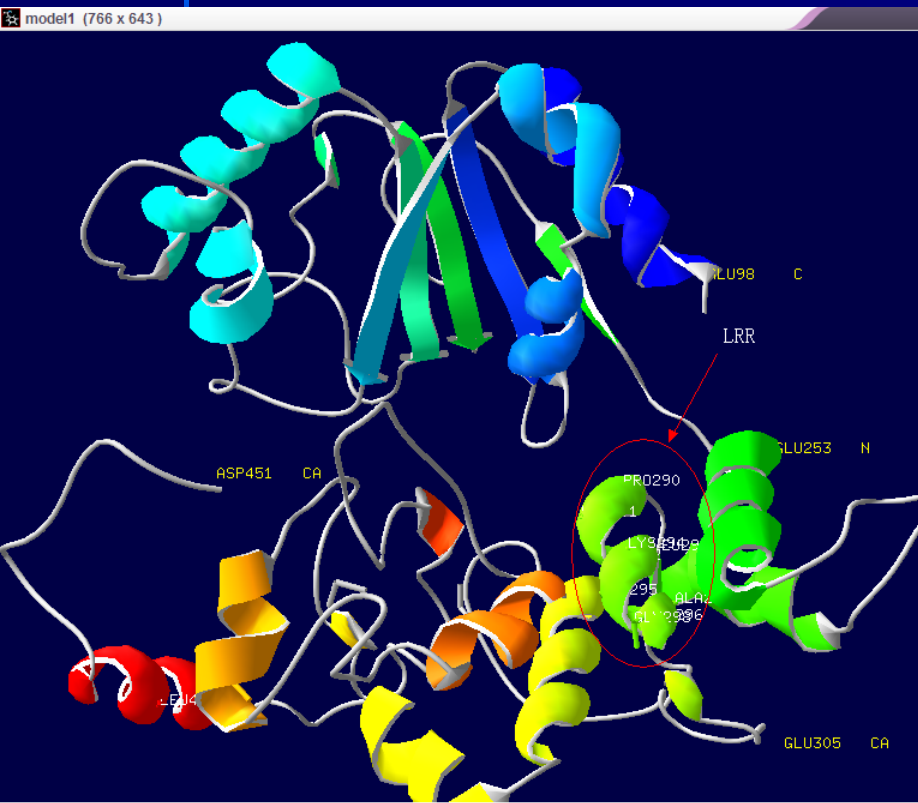
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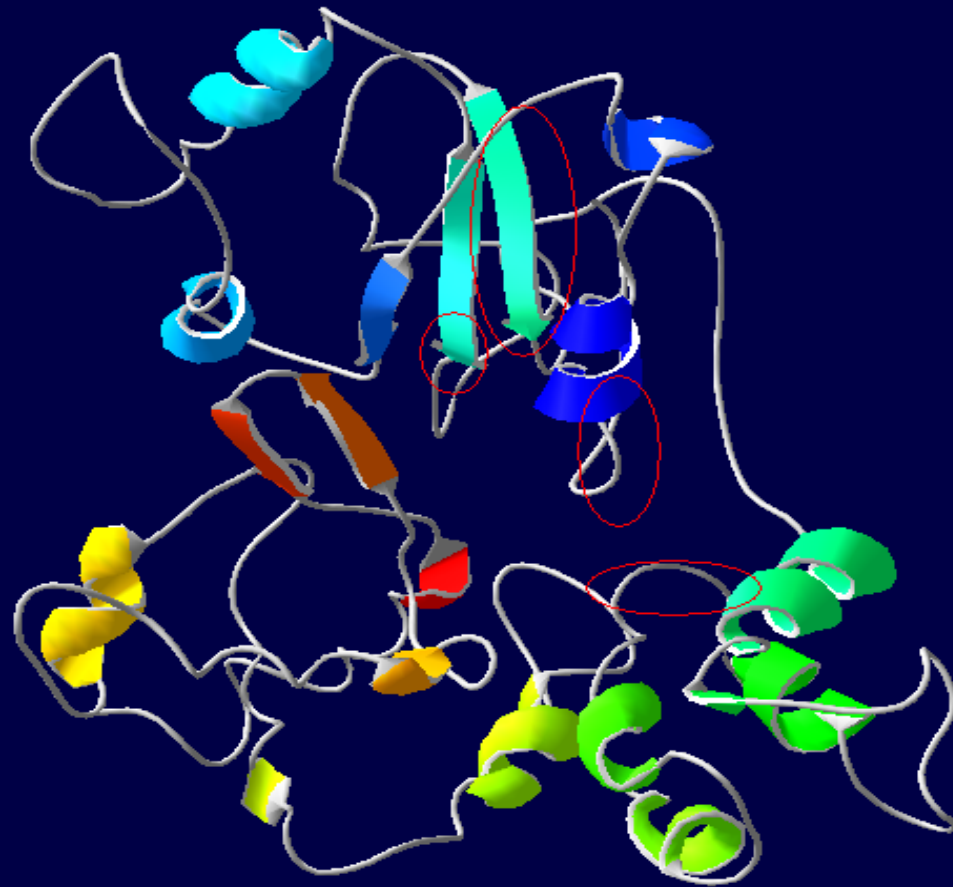
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亮氨酸重复区

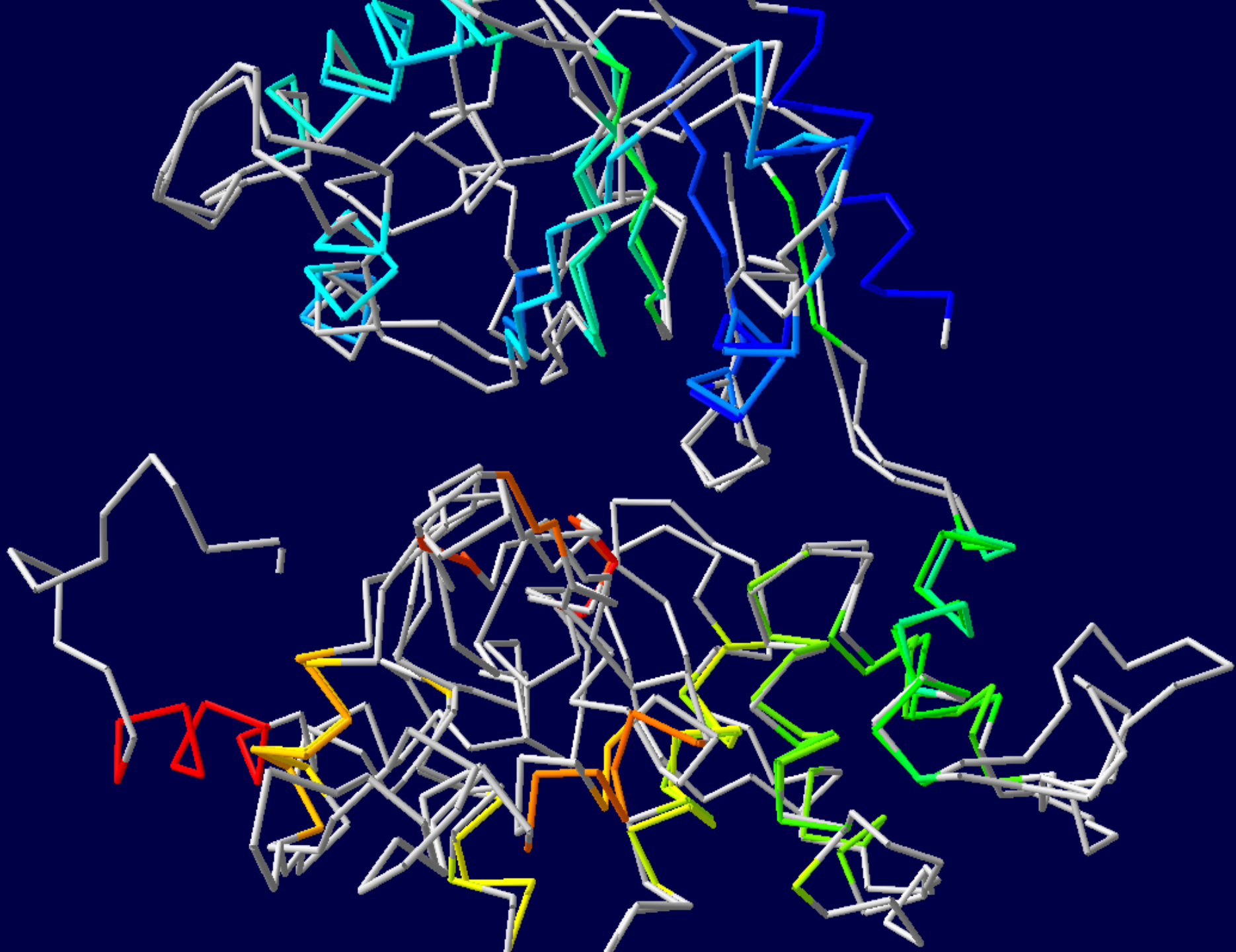


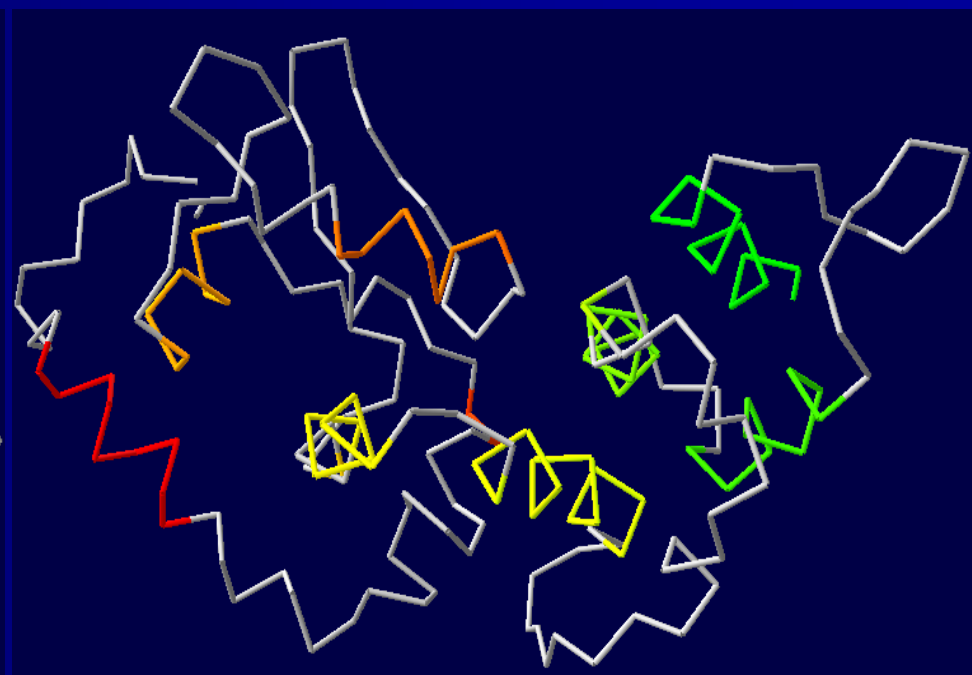
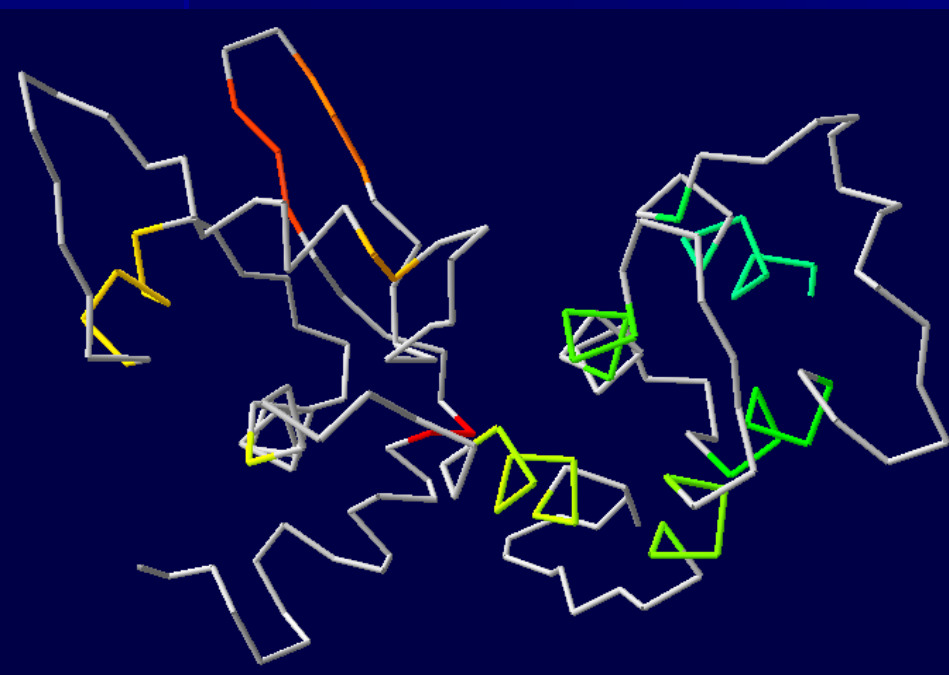
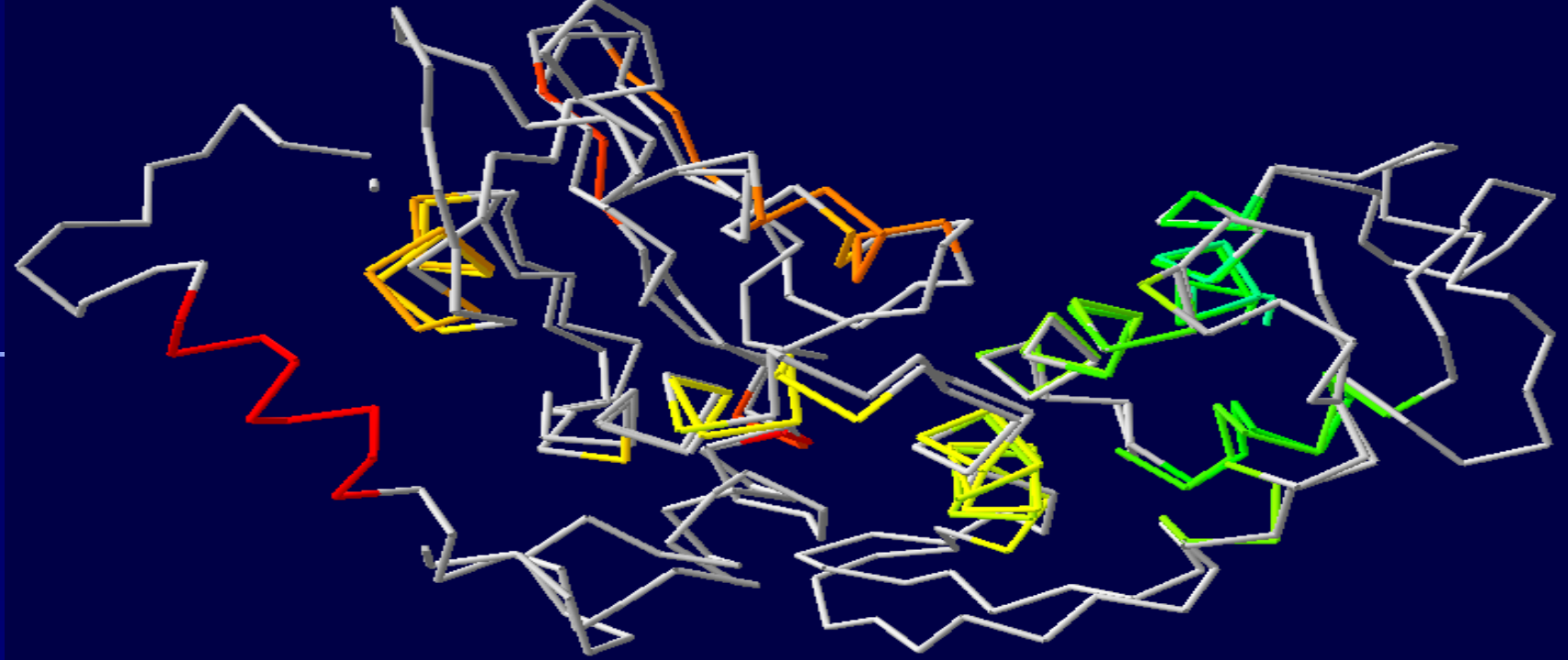
Cn3D



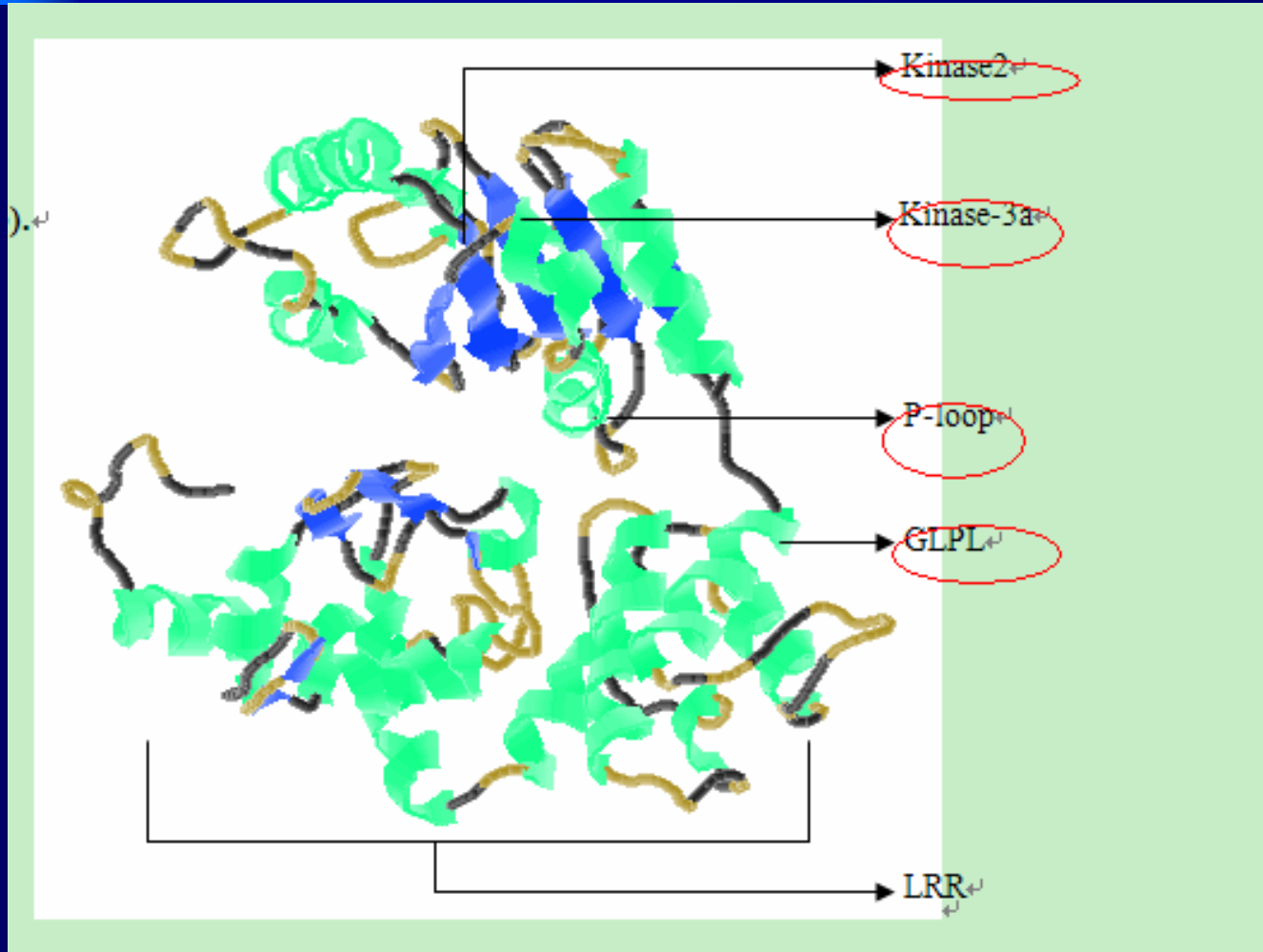
3D Jigsaw







raswin



结果分析

据文献报道，植物抗病基因的**NBS**区域被认为与传递信号有关，**NBS**中的基序如磷酸结合环(**P-loop**)为**ATP/GTP**结合位点，**NBS**能激活激酶或G蛋白，使细胞产生防御反应。**NBS-LRR**蛋白并不是仅仅由一些单独的功能单元构成，可能是由不同的共同进化的区域构成的，而且信号感应和传导需要分子间的相互作用。对抗性基因的克隆发现它们更象膜受体和信号转换分子。

- 利用RACE技术克隆出了第IV类（PB6-22）的基因，基因全长为3570bp，编码1189氨基酸，该基因含有抗病基因的NBS保守结构域（P-loop、Kinase2、Kinase-3a、GLPL等），并在此基因中鉴定出了至少8个LRR结构域。

- PB6-22编码蛋白与*Solanum demissum*中的抗病蛋白 I2C-5 (AAU90287.1) 相似性最高，达77%。

I2C-5与PB6-22:

- Length: 1198
- Identity: 923/1198 (77.0%)
- Similarity: 1001/1198 (83.6%)

```
Length: 1198
Identity:      923/1198 (77.0%)
Similarity:   1001/1198 (83.6%)
Gaps:         46/1198 ( 3.8%)
Score: 4565.0
```


存在的问题

虽然PB6-22基因编码的蛋白四个保守区与其它抗病基因的存在很高的序列相似性，但这些保守区的具体功能以及该基因与晚疫病抗性是否具有直接的相关关系还有待进一步的实验研究确定。基因表达及抗病性功能鉴定工作有待于进一步研究。

References

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- <http://www.cbs.dtu.dk/services/CPHmodels>
- <http://www.ncbi.nlm.nih.gov/>
- <http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml>
- <http://bmm.cancerresearchuk.org/~3djigsaw/>
- swiss-Pdb viewer
- DNAMAN
- clustalX
- MEGA

致 谢

感谢第一组全体成员的共同努力！
感谢周军会师兄的大力协助与指导！

感谢罗静初老师！