

1. Primary and Secondary Structure Analysis

Yuejian Mo,11510511

Introduction

Protein's primary structure is sequence of aminod acid. We can find some conservation of same protein which vary on different organs and different species. So it is possbile to find out domain from these conservative sequence. Comparing amino acid sequence and certain algorithm, we can print the phylogenetic tree.

The information of amino acid sequence is not enough to build up protein with certain biological activity, but the secondary structure and higher dimemnsion structure do. Based on the features of known experiment result between primary structure and secondary structure, we can predict the helix, strand and turn structure in unkown protein.

Methods

In the first part, we selected 10 homology sequences of human UNC5b, including both orthologues and paralogues from uniprot.org. There are

UNC5B_HUMAN,
K7DDTY1_PANTR,K7B3D8_PANTR,K7BVI5_PANTR,H2NAM6_PONAB,G7N281_MACMU,
F6UDU4_MACMU,F6UDV7_MACMU,F7A1Z5_CALJA,U3F339_CALJA

Then we aligned and generated a phylogenetic tree sequence with the Clustal Omega program in uniprot.org.

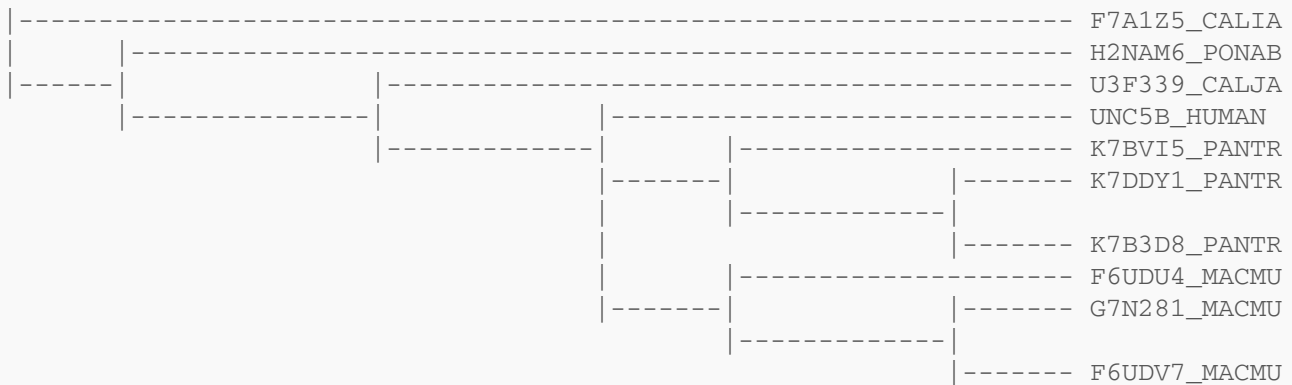
In the second part, we used JPred to predicte secondary structure of the cytoplasmic domain of human UNC5b. Then we compared the predicted result with X-ray structure(PDB:3G5B) to get error.

Last part, we design a truncation construct with minimal size, wheres retaining the overall fold. We keep all the E and H domain sequence and binding site for residue PO4A.

Results

Target Sequence and Alignment results

<u>081ZJ1</u>	UNC5B_HUMAN	359	TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFTDITDSSAALT	418
<u>K7DDY1</u>	K7DDY1_PANTR	359	TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFTDITDSSAALT	418
<u>K7B3D8</u>	K7B3D8_PANTR	359	TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFTDITDSSAALT	418
<u>K7BVI5</u>	K7BVI5_PANTR	359	TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRHNCRDFDITDSSAALT	418
<u>H2NAM6</u>	H2NAM6_PONAB	73	TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFTDITDSSAALT	132
<u>G7N281</u>	G7N281_MACMU	359	TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFTDITDSSAALT	418
<u>F6UDU4</u>	F6UDU4_MACMU	356	-----MLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFTDITDSSAALT	407
<u>F6UDV7</u>	F6UDV7_MACMU	359	TLSDPNSHLLEASGDAALYAGLVVAVFVVVAILMAVGVVVYRRNCRDFTDITDSSAALT	418
<u>F7A1Z5</u>	F7A1Z5_CALJA	335	TLSDPNSHLLEASGDAALYAGLVVAVFVVVAVLMAVGVVVYRRNCHDFDITDSSAALT	394
<u>U3F339</u>	U3F339_CALJA	359	TLSDPNSHLLEASGDAALYAGLVVAVFVVVAVLMAVGVVVYRRNCHDFDITDSSAALT	418
:*****:*****:*****:*****:*****:*****:				
<u>081ZJ1</u>	UNC5B_HUMAN	419	GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP	478
<u>K7DDY1</u>	K7DDY1_PANTR	419	GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP	478
<u>K7B3D8</u>	K7B3D8_PANTR	419	GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP	478
<u>K7BVI5</u>	K7BVI5_PANTR	419	GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP	478
<u>H2NAM6</u>	H2NAM6_PONAB	133	GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP	192
<u>G7N281</u>	G7N281_MACMU	419	GGFHPVNFKTARPNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP	478
<u>F6UDU4</u>	F6UDU4_MACMU	408	GGFHPVNFKTARPNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP	467
<u>F6UDV7</u>	F6UDV7_MACMU	419	GGFHPVNFKTARPNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP	478
<u>F7A1Z5</u>	F7A1Z5_CALJA	395	GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKILMTNSPLLDPLP	454
<u>U3F339</u>	U3F339_CALJA	419	GGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKILMTNSPLLDPLP	478
*****:*****:*****:*****:*****:*****:				
<u>081ZJ1</u>	UNC5B_HUMAN	479	SLKVKVYSSSTTGSGPGLADGADLLGVLPPGTYPSPDFARDTHFLHLRSASLGSQQLGLP	538
<u>K7DDY1</u>	K7DDY1_PANTR	479	SLKVKVYSSSTTGSGPGLADGADLLGVLPPGTYPSPDFARDTHFLHLRSASLGSQQLGLP	538
<u>K7B3D8</u>	K7B3D8_PANTR	479	SLKVKVYSSSTTGSGPGLADGADLLGVLPPGTYPSPDFARDTHFLHLRSASLGSQQLGLP	538



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Seq :
YRRNCRDFDTDITDSSAALTGGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP
Pre :-----EE-----
Exp :
Err :          12

SLKVKVYSSSTTGSGPGLADGADLLGVLP PGTYPSDFARDTHFLHLRSASLGSQQLLGLPRDPGSSVSGTFGCLGGRLSI
-----EEEEEE-----EEEE
                      EEEEE  EEEEE
                        3

PGTGVSLLVPNGAIPQGKFYEMYLLINKAESTLPLSEGTQTVLSPSVTCGP TGLLLCRPVILTMPHCAEVSARDWIFQLK
E--EEEEEE-----EEEEEE-----EEEEEEEE-----EEEEEE-----EEEE
  EEEEE  EEEEE  EEE  EEE  EEEEE  EEEEE
4         5         6  78910  11      12  13      14

TQAHQGHWEVVTLDEETLNTPCYQLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPALCTSLEYSLRVYCLE
E-----EEE-----EEEE--EEEE--HHHH--EEEEEE-----EEEE--
EE  EEEEE  EEEEE  EEEEE  EEEEEEE  EEEEEEEEE  EEEEEEEEE
15  16  17      1819  20      21      2223      2425  2627

DTPVALKEVLELERTLGGYLVEEPKPLMFKDSYHNLRLSLHDLPHAHWSKLLAKYQEIPFYHIWSGSQKALHCTFTLER
---HHHHHHHHHH---EEE---EEEE---EEEEEE---E---EEEE---HH-----EEEE--
  HHHHHHHHHHHHHH  EEE  EEEEE  EEEEE  EE  EEE HHHHH  EEEEEEE
2829      3031 32 33  34  35      36      37  383940      41  42

HSLASTELTCKICVRQVEGEGQIFQLHTTLAETPAGSLDTLCSAPGSTVTTQLGPYAFKIPLSIRQKICNSLDAPNSRGN
-----EEEEEE-----EEEE-----HHHHHHHHHH-----
      EEEEEEEEE  EEEEEEEEE  EEE  HHHHHHHHHH
      43      4445  464748 4950      515253      54

DWRMLAQKLSMDRYLNYFATKASPTGVILDLWEALQQDDGDLNSLASALEEMGKSEMLVAVATDGDC
HHHHHHHH-----HHHH--HHHHHHHHHHHH-----HHHHHHHHHH--HHHHHHHHHH---
  HHHHHHH  HHHH  HHHHHHHHHHHHH  HHHHHHHHH  HHHHHHH
55         56         57  58         59  60

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Sequence total length: 339 Error ratio= (mismatch of predition compare eith correct)/(total sequence length)=60/339=17%

Minisize sequence:

The minisize sequence are marked as *

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Seq      :
YRRNCRDFDITDITDSSAALTGGFHPVNFKTARPSNPQLLHPSVPPDLTASAGIYRGPVYALQDSTDKIPMTNSPLLDPLP
Known    :
Design   :

SLKVKVYSSSTTSGSGPLADGADLLGVLPPGTYPSPDFARDTHFLHLRSASLGSQQLLGLPRDPGSSVSGTFGCLGGRLSI
                                             EEEEEEE   EEEEE
                                             *****   *****

PGTGVSLLPNGAIPQGKFYEMYLLINKAESTLPLSEGTQTVLSPSVTCGP TG LLLCRPVILTMPHCAEVSARDWIFQLK
EEEEEE   EEEEEEE   EEE   EEE   EEEEE   EEEEE
*****   *****   ***   ***   *****   *****

TQAHQGHWEDEVVTLDEETLNTPCYQCLEPRACHILLDQLGTYVFTGESYSRSAVKRLQLAVFAPALCTSLEYSRLRVYCLE
EE      EEEEE   EEEEE   EEEEE   EEEEEEE   EEEEEEEEEE   EEEEEEEEEE
**      *****   *****   *****   *****   *****   *****

DTPVALKEVLELERTLGGYLVEEPKPLMFKDSYHNRLSLHDLPHAHWSKLLAKYQEIPFYHIWSGSQKALHCTFTLER
HHHHHHHHHHHHHHH   EEE   EEEEE   EEEEE   EE   EEE HHHHH   EEEEEEE
*****   *****   *****   *****   **   ***   *****   *****

HSLASTE LTCKICVRQVEGEGQIFQLHTTLAETPAGSLDTLCSAPGSTVTTQLGPYAFKIPLSIRQKICNSLDAPNSRGN
EEEEEEEEEE   EEEEEEE   EEE   HHHHHHHHHHHH
*****   *****   ***   *****

DWRMLAQKLSMDRYLNYFATKASPTGVILDLWEALQQDDGDLNSLASALEEMGKSEMLVAVATDGDC
HHHHHHH   HHHH   HHHHHHHHHHHHH   HHHHHHHHH   HHHHHHH
*****   ****   *****   *****   *****

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Conclusions

Here are conservation of protein in different organs and speices, which suggested the evolution of protein. Using cetain technique, secondary structure can be predicted in acceptable range. But we think that the different of our choose target is small and not enough to show the essential domain. And we risks to keep beta strand and alpha helix.