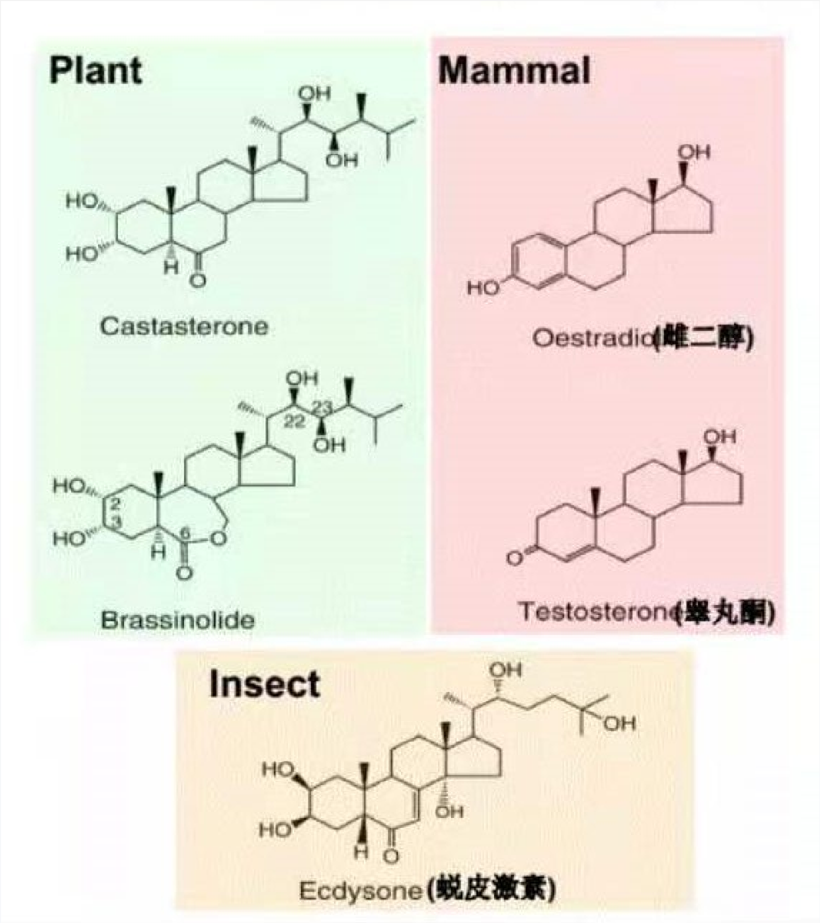
水稻油菜素内酯信号转导关键基因*OsGSK2*生物信息学分析

Bioinformatics Analysis of *OsGSK2*(*GLYCOGEN SYNTHASE KINASE2*), a Key Gene in Rice Brassinolide Signal Transduction

Repoter: Group 3G05
杨彦钊：建树分析与分子对接
郭冬冬：序列分析
武兰新：二级结构分析
朱梦晨：文献检索收集

What is Brassinolides(BR)?



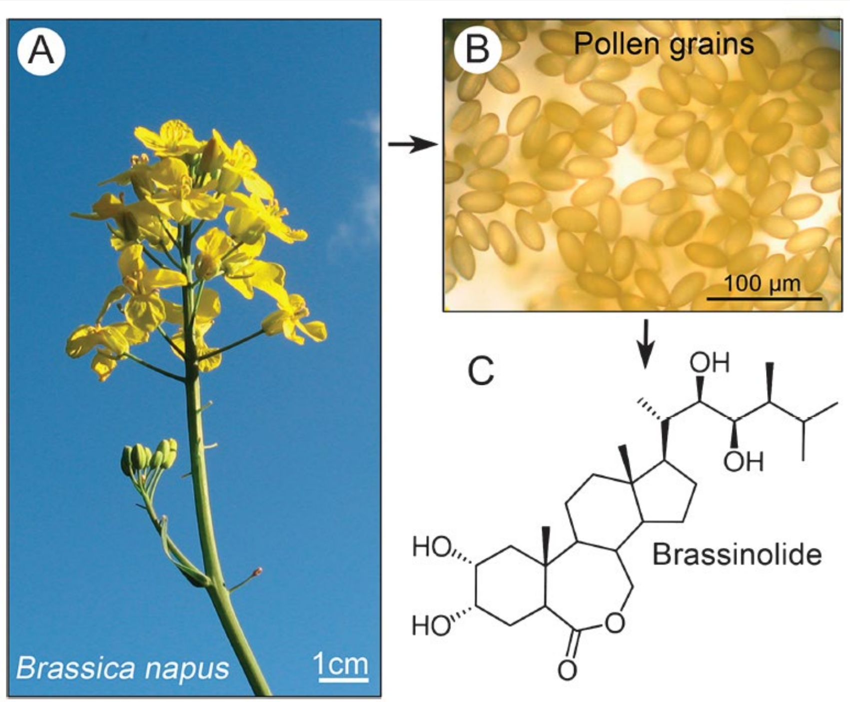
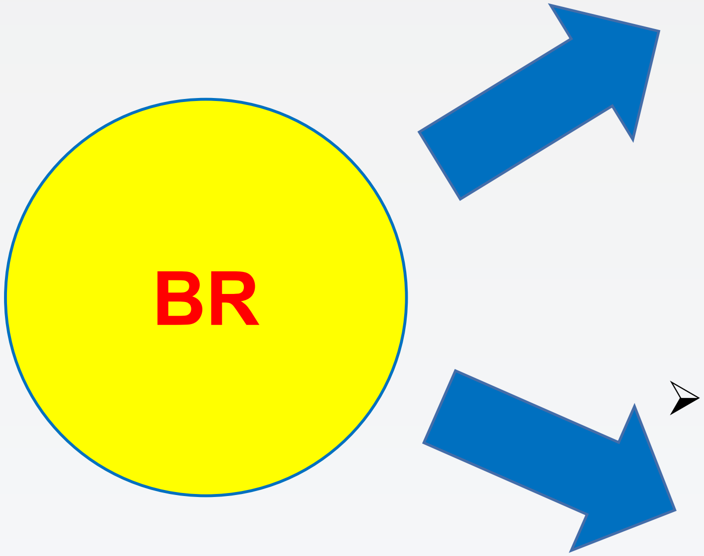


Fig. 3. Flowering stalk of a rapeseed (*Brassica napus*) plant (A), pollen grains isolated from the mature stamina (B) and the structure of the steroidal phytohormone brassinolide (C). In 1979, brassinolide was isolated from bee-collected rape pollen and its chemical structure determined by X-ray analysis.

Bishop, G.J.et al.2002

Mitchell et al., 1970

BRassinosteroids regulate most of the yield-related factors



BR

- **Increase yield:**

  Grain weight
  Grain number
  Tiller number
  Photosynthesis
  Nutrition utilization

- **Decrease loss:**

  Lodging resistance
  Stress resistance
  Disease resistance
  Nutrition utilization efficiency

BR has a great potential in agricultural improvement.

Morphology of severe BR-deficient mutants

Arabidopsis

Rice

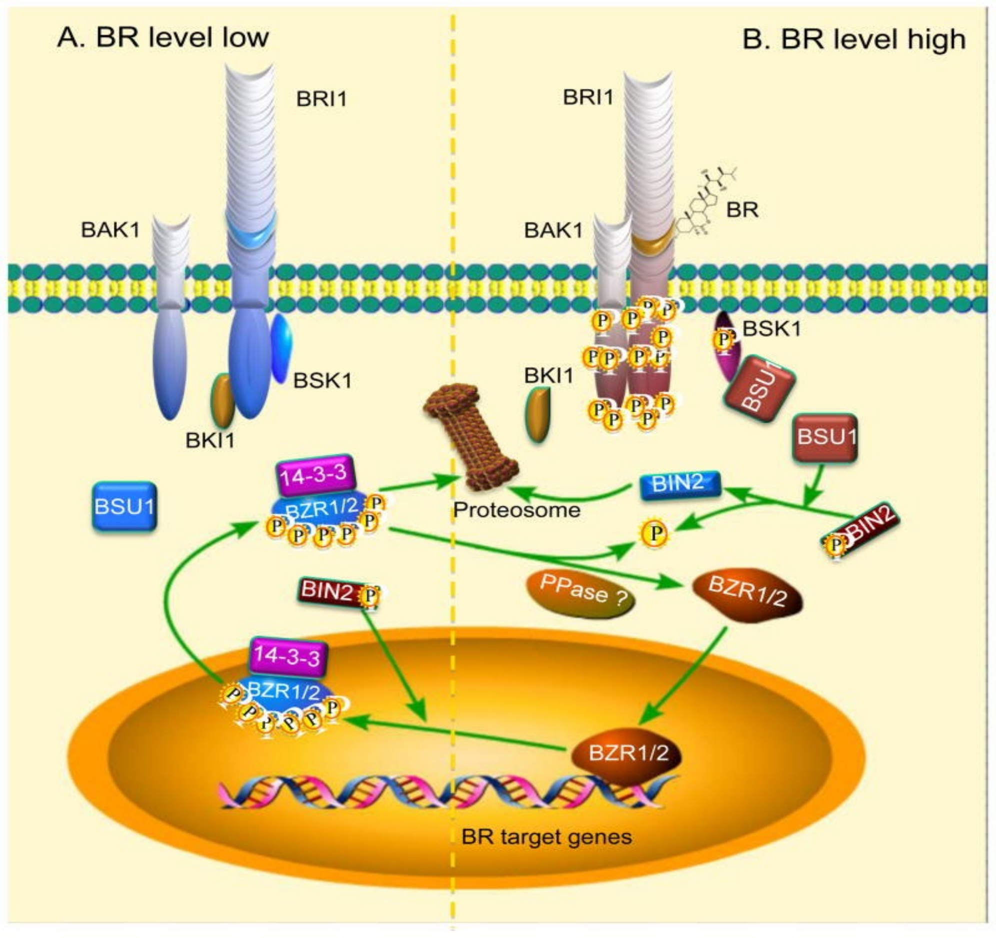
Tomato

Szekeres *et al.*, Cell, 1996; Mori *et al.*, Plant Physiol, 2002; Koda *et al.*, Plant Physiol, 2000

Learn more about BR signal transduction from NCBI

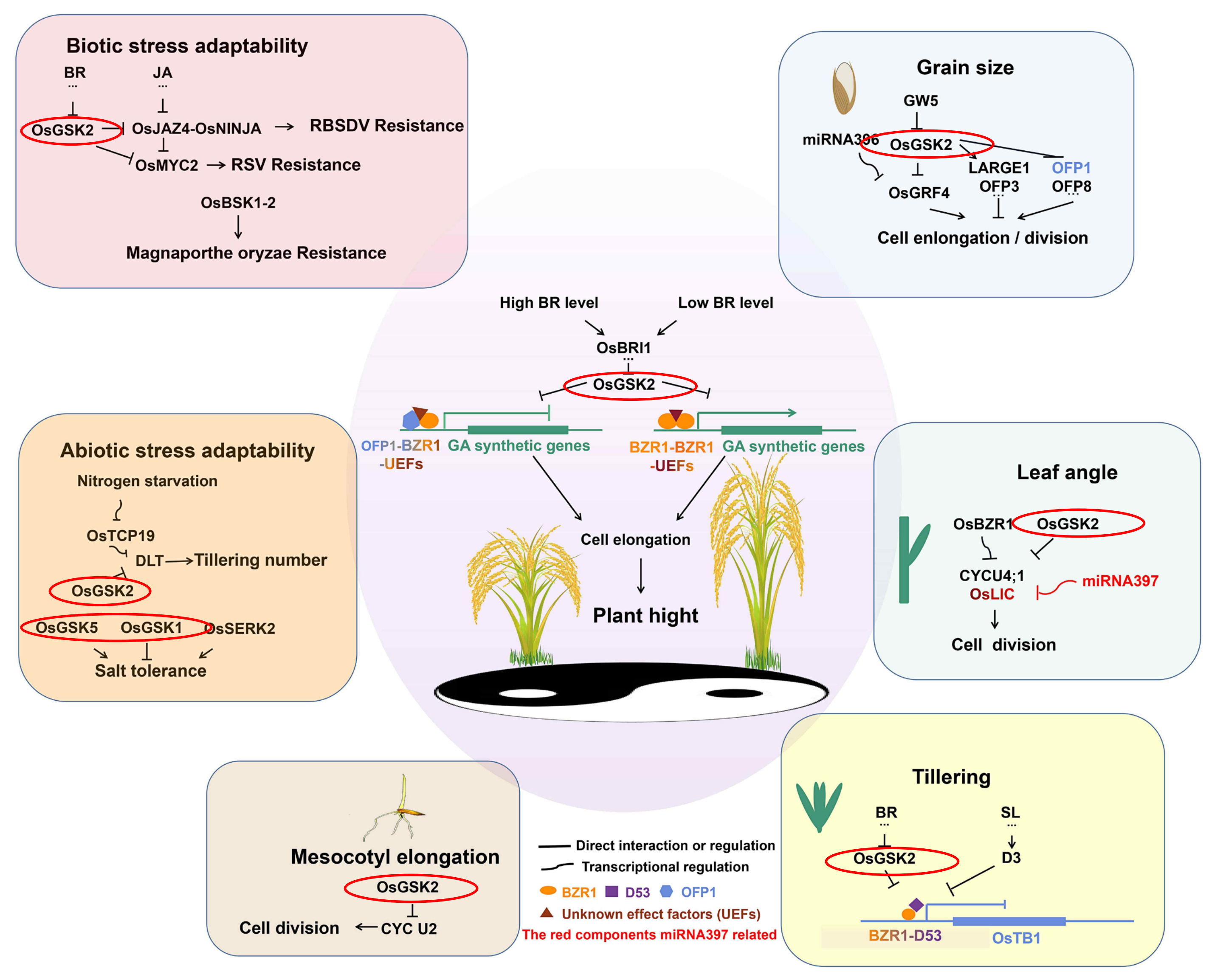
Key words: (brassinosteroids[Title]) AND ((pathway[Title/Abstract]) OR (signal[Title/Abstract])OR (function[Title/Abstract]))

Review

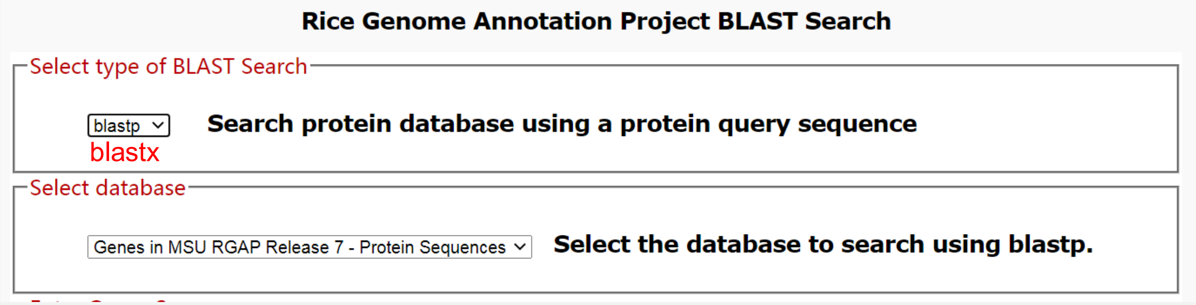


Yang CJ et al. The mechanisms of brassinosteroids' action: from signal transduction to plant development. Mol Plant. 2011 Jul;4(4):588-600.

OsGSK2, big player in rice BR pathway



Seacher for GSKs in plants



Rice Genome Annotation Project BLAST Search

Select type of BLAST Search

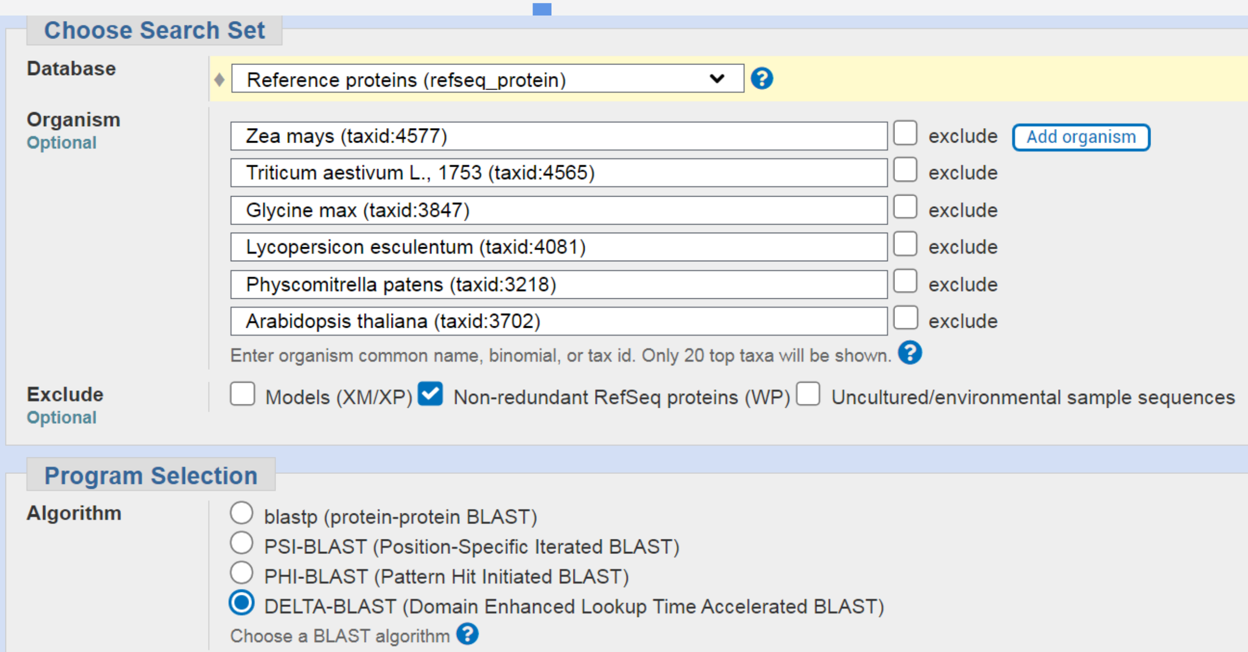
blastp — **Search protein database using a protein query sequence**

blastx

Select database

Genes in MSU RGAP Release 7 - Protein Sequences — **Select the database to search using blastp.**

Paralogs of OsGSK2



Choose Search Set

Database: Reference proteins (refseq_protein)

Organism Optional

Zea mays (taxid:4577) ☐ exclude
Triticum aestivum L., 1753 (taxid:4565) ☐ exclude
Glycine max (taxid:3847) ☐ exclude
Lycopersicon esculentum (taxid:4081) ☐ exclude
Physcomitrella patens (taxid:3218) ☐ exclude
Arabidopsis thaliana (taxid:3702) ☐ exclude

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Exclude Optional

☐ Models (XM/XP) ☑ Non-redundant RefSeq proteins (WP) ☐ Uncultured/environmental sample sequences

Program Selection

Algorithm

○ blastp (protein-protein BLAST)
○ PSI-BLAST (Position-Specific Iterated BLAST)
○ PHI-BLAST (Pattern Hit Initiated BLAST)
● DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)

Choose a BLAST algorithm

Ortholog of OsGSK2

Family analysis of GSKS based on sequence alignment

Clustal-w

```
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AT3G05840.1                YTTAIDVWSAGCVLAELLLGQPLFPGESGVDQLVEIIKVLGTPTREEIKC
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AT4G00720.1                NYLHRVVGVCHRDIKPQNLLVNPQTHQLKICDFGSAKMLVPGEPNISYIC
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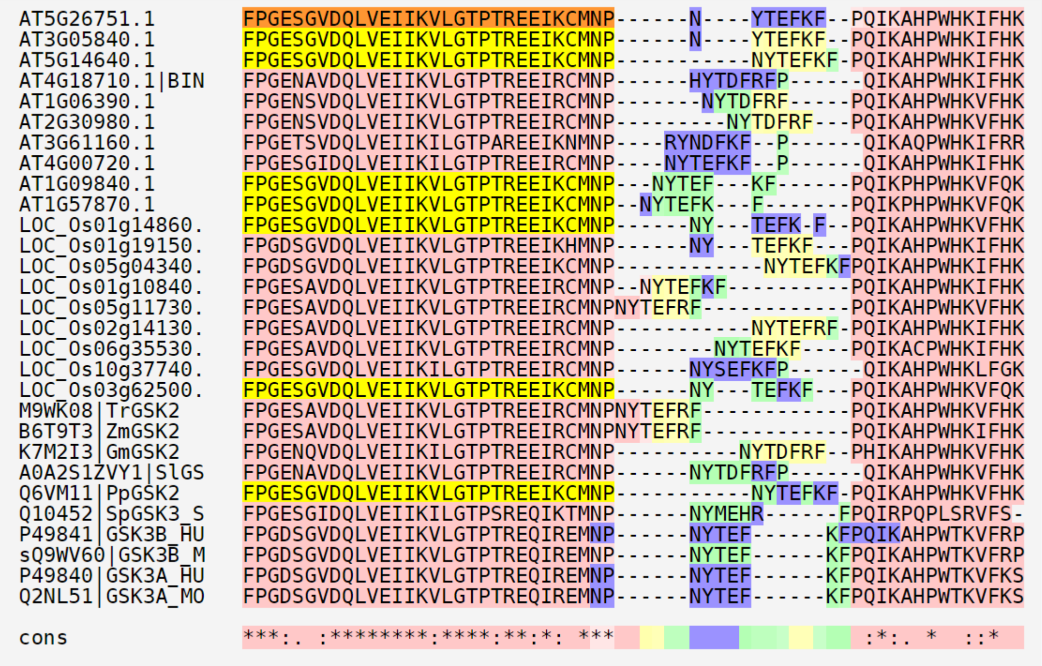
T-Coffee + AtBIN2.PBD



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cons             ***:. :********:****:**:*: ***                    :*:. *  ::*
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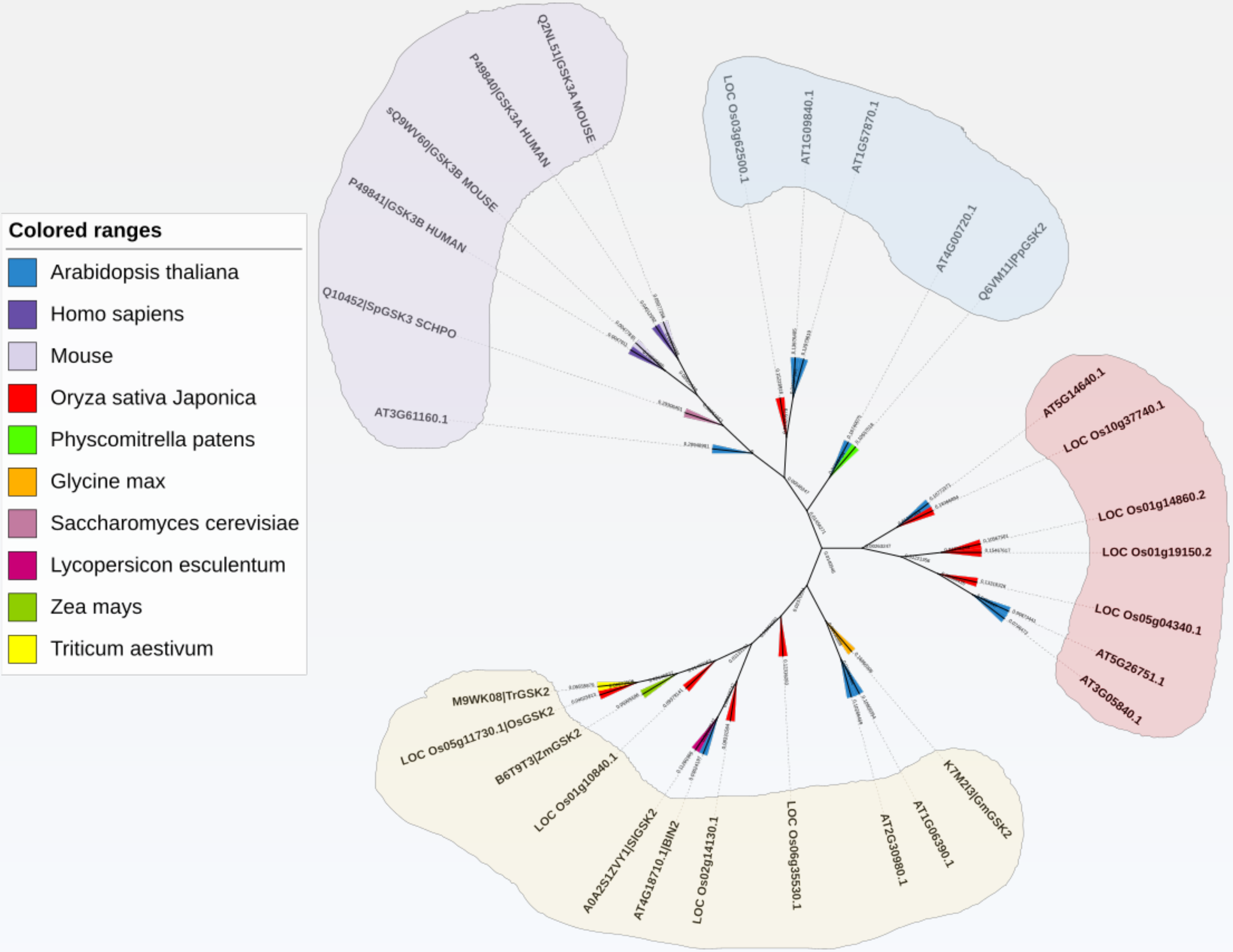
Seq:FPGESGVDQLVEIIKVLGTPTREEIKCMNP

GSKs are ancient conserved kinases in eukaryotes



Conserved motifs in GSKS

Name	*p* -value	Motif Locations
AT5G26751.1	2.51e-168	
AT3G05840.1	2.59e-168	
AT5G14640.1	3.11e-168	
AT4G18710.1|BIN2	7.25e-163	
AT1G06390.1	4.13e-162	
AT2G30980.1	4.98e-164	
AT3G61160.1	5.76e-148	
AT4G00720.1	2.19e-157	
AT1G09840.1	1.03e-156	
AT1G57870.1	2.37e-160	
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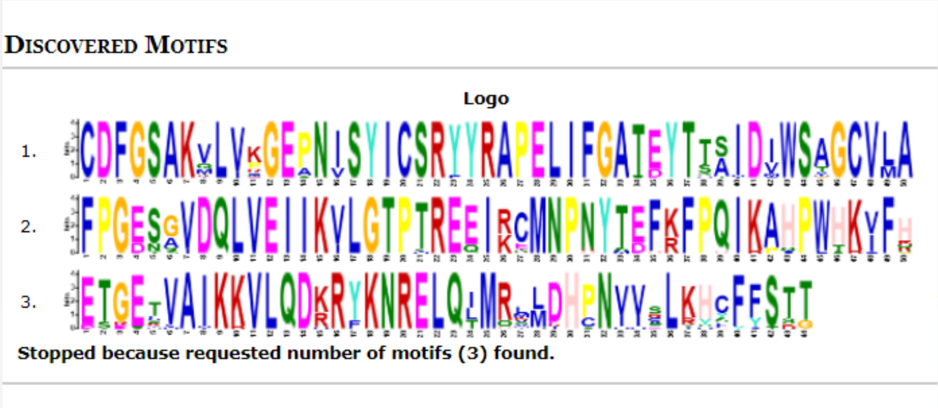
LOC_Os01g19150.2	1.57e-156	
LOC_Os05g04340.1	1.98e-165	
LOC_Os01g10840.1	6.25e-166	
LOC_Os05g11730.1|OsGSK2	9.58e-166	
LOC_Os02g14130.1	2.57e-168	
LOC_Os06g35530.1	5.54e-165	
LOC_Os10g37740.1	3.79e-156	
LOC_Os03g62500.1	5.01e-160	
M9WK08|TrGSK2	9.88e-163	
B6T9T3|ZmGSK2	7.86e-162	
K7M2I3|GmGSK2	2.57e-152	
A0A2S1ZVY1|SlGSK2	3.41e-164	
Q6VM11|PpGSK2	6.85e-166	

Motif	Symbol	Motif Consensus
1.	🟥	CDFGSAKVLVKGEPNISYICSRYYRAPELIFGATEYTTSIDIWSAGCVLA
2.	▭	FPGESGVDQLVEIIKVLGTPTREEIRCMNPNYTEFKFPQIKAHPWHKVFH
3.	🟩	ETGETVAIKKVLQDKRYKNRELQIMRLLDHPNVVSLKHCFFSTT



Discovered Motifs

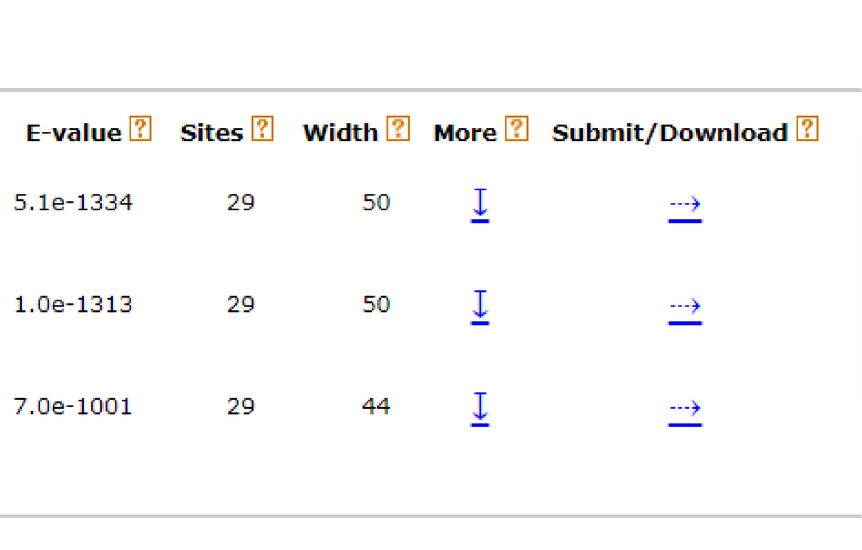
Logo

1\.

2\.

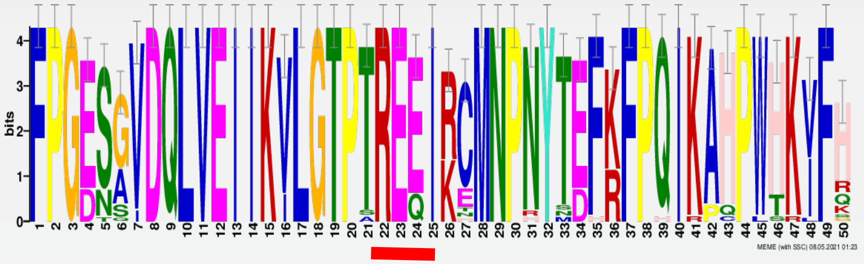
3\.

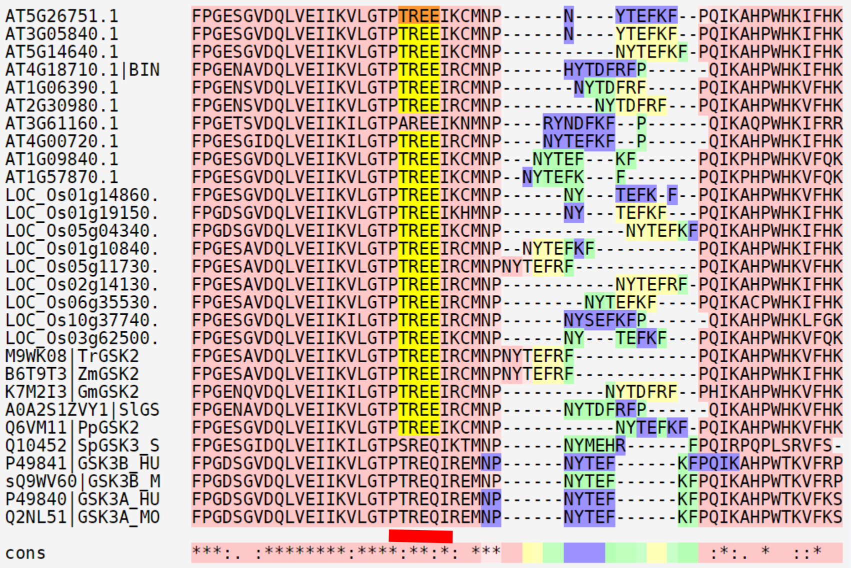
Stopped because requested number of motifs (3) found.



E-value ?	Sites ?	Width ?	More ?	Submit/Download ?
5.1e-1334	29	50	↧	--->
1.0e-1313	29	50	↧	--->
7.0e-1001	29	44	↧	--->

The “TREE” motif is important to the function of GSK2





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Q10452|SpGSK3_S    FPGESGIDQLVEIIKILGTPSREQIKTMNP------NYMEHR------FPQIRPQPLSRVFS-
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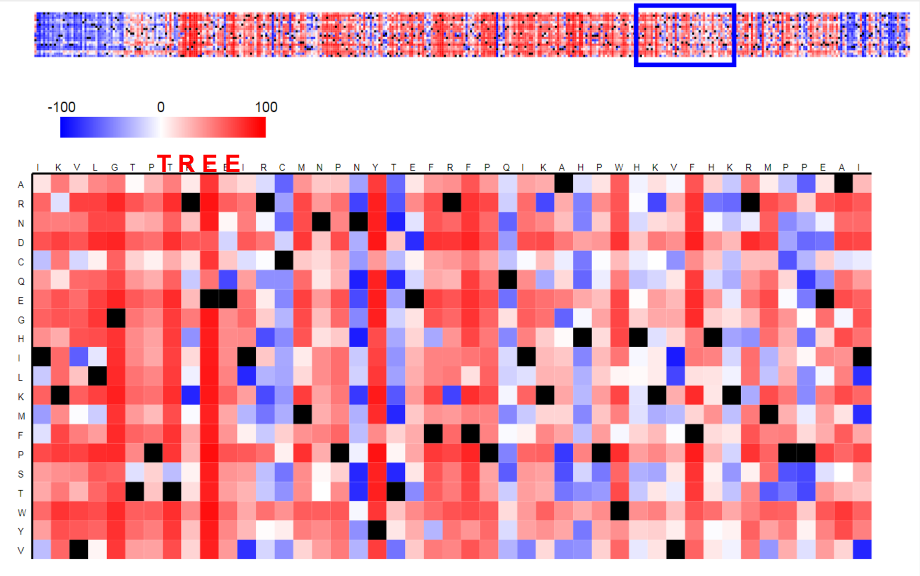
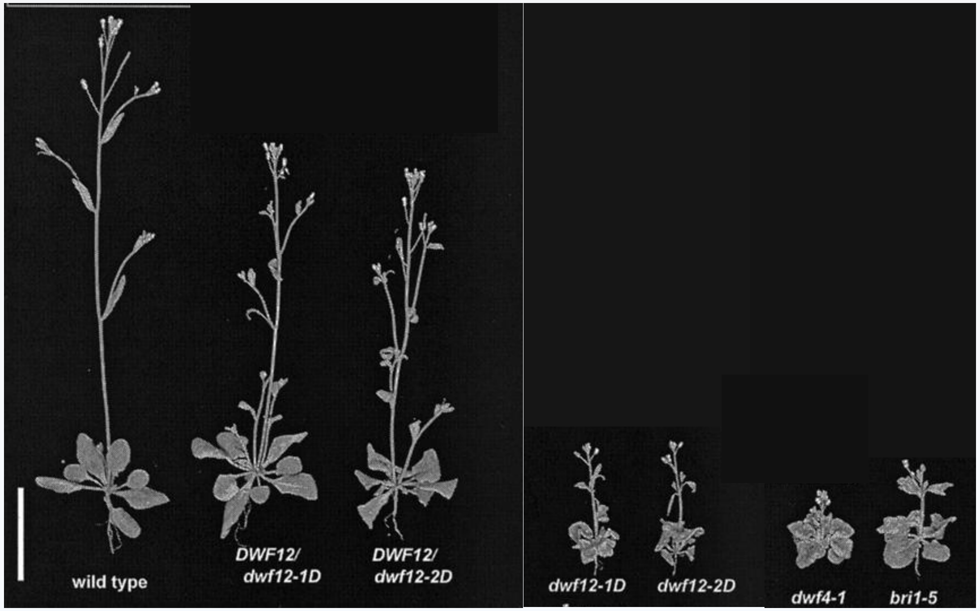
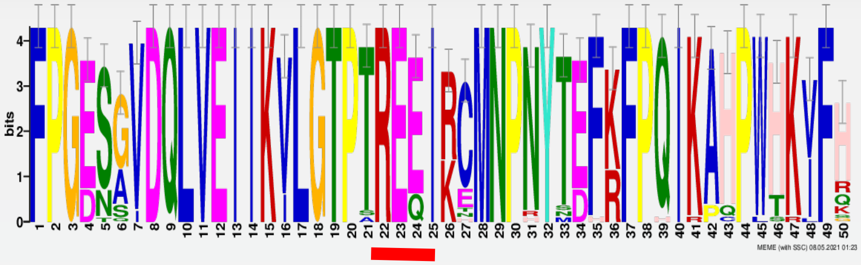




Choe S et al. 2002

The “TREE” motif is important to the function of GSK2



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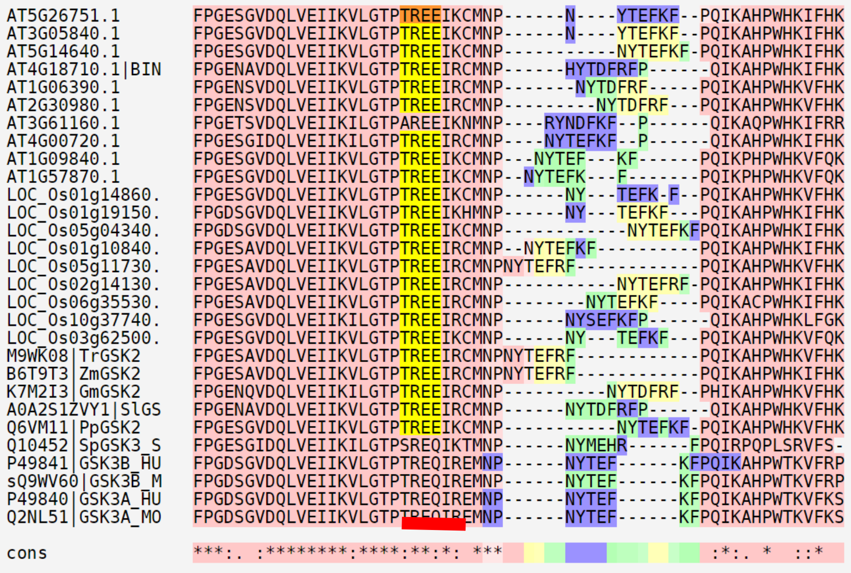
T. aestivum	A C C C G T G A G G A A	Ⅰ	TREE	656
T. spelta	A C C C G T G A G G A A	Ⅰ	TREE	121
T. sphaerococcum	A C C C G T G A G A A A	Ⅱ	TREK	10
T. sphaerococcum	A C C G G T G A G G A A	Ⅲ	TGEE	4



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AT5G14640.1        FPGESGVDQLVEIIKVLGTPTREEIKCMNP-----------NYTEFKF-PQIKAHPWHKIFHK
AT4G18710.1|BIN    FPGENAVDQLVEIIKVLGTPTREEIRCMNP------HYTDFRFP------QIKAHPWHKIFHK
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AT2G30980.1        FPGENSVDQLVEIIKVLGTPTREEIRCMNP---------NYTDFRF---PQIKAHPWHKVFHK
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M9WK08|TrGSK2      FPGESAVDQLVEIIKVLGTPTREEIRCMNPNYTEFRF------------PQIKAHPWHKVFHK
B6T9T3|ZmGSK2      FPGESAVDQLVEIIKVLGTPTREEIRCMNPNYTEFRF------------PQIKAHPWHKIFHK
K7M2I3|GmGSK2      FPGENQVDQLVEIIKILGTPTREEIRCMNP----------NYTDFRF--PHIKAHPWHKVFHK
A0A2S1ZVY1|SlGS    FPGENAVDQLVEIIKVLGTPTREEIRCMNP------NYTDFRFP------QIKAHPWHKVFHK
Q6VM11|PpGSK2      FPGESGVDQLVEIIKVLGTPTREEIKCMNP-----------NYTEFKF-PQIKAHPWHKVFHK
Q10452|SpGSK3_S    FPGESGIDQLVEIIKILGTPSREQIKTMNP------NYMEHR------FPQIRPQPLSRVFS-
P49841|GSK3B_HU    FPGDSGVDQLVEIIKVLGTPTREQIREMNP------NYTEF------KFPQIKAHPWTKVFRP
sQ9WV60|GSK3B_M    FPGDSGVDQLVEIIKVLGTPTREQIREMNP------NYTEF------KFPQIKAHPWTKVFRP
P49840|GSK3A_HU    FPGDSGVDQLVEIIKVLGTPTREQIREMNP------NYTEF------KFPQIKAHPWTKVFKS
Q2NL51|GSK3A_MO    FPGDSGVDQLVEIIKVLGTPTREQIREMNP------NYTEF------KFPQIKAHPWTKVFKS

cons               ***:. :********:****:**:*: ***                     :*:. *   ::*
```
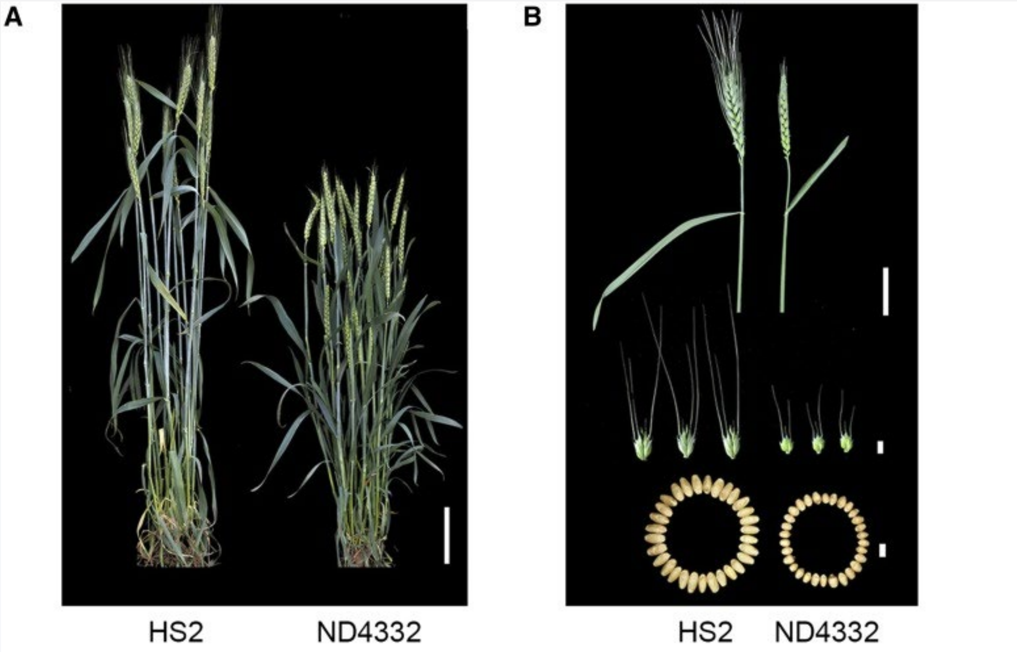
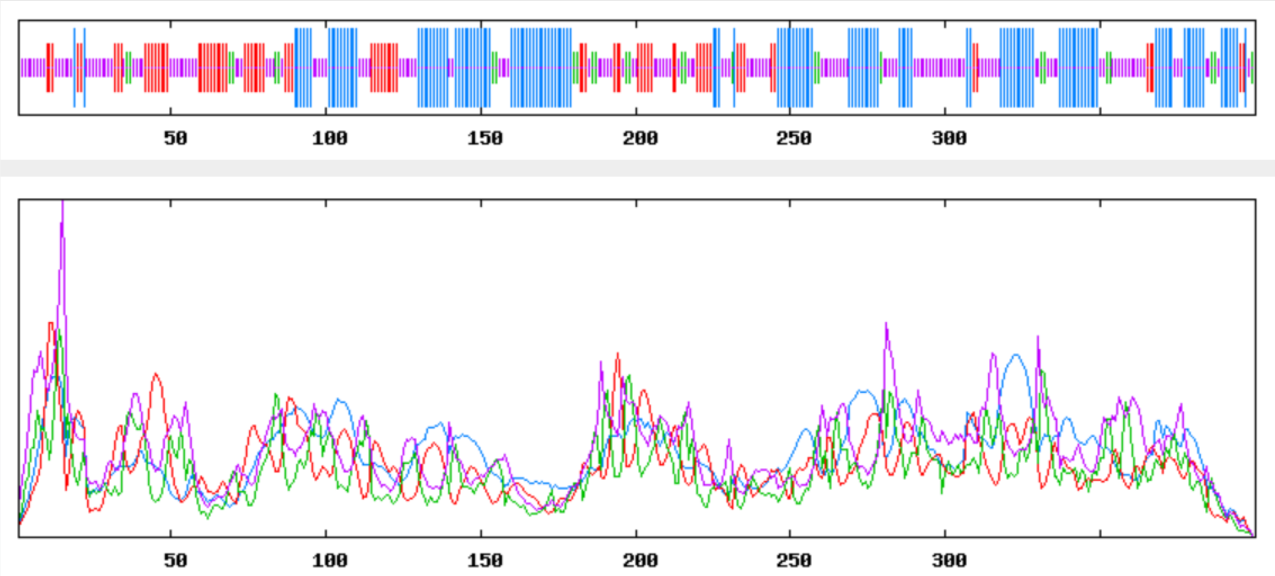
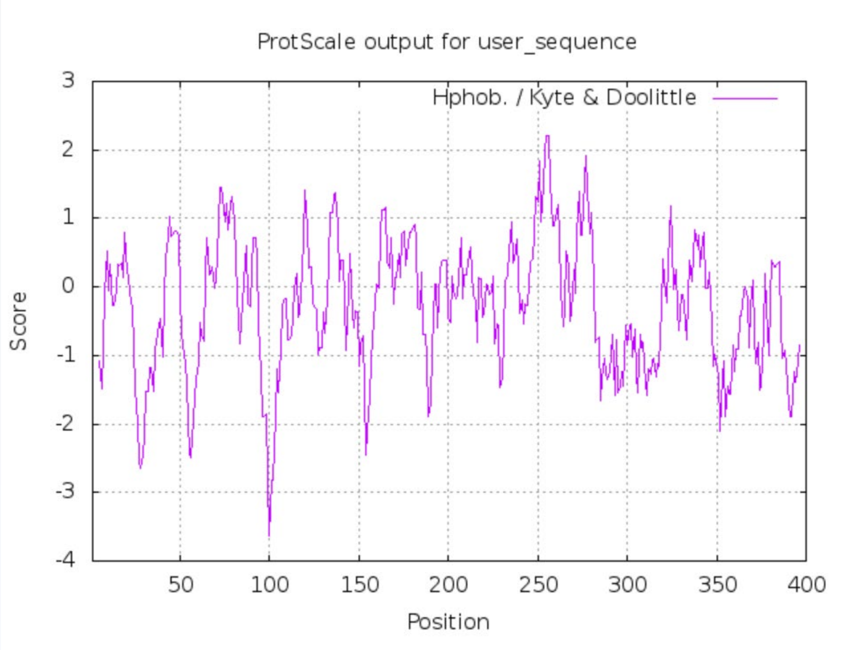



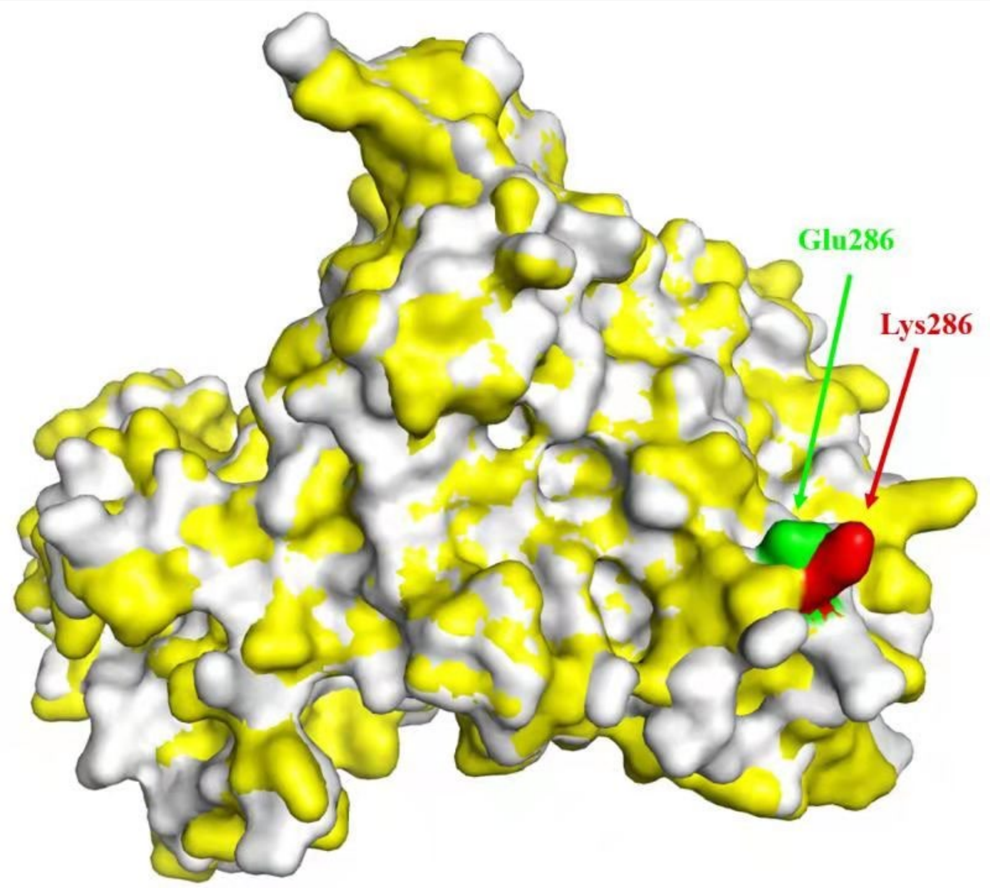
Cheng X, et al.2020

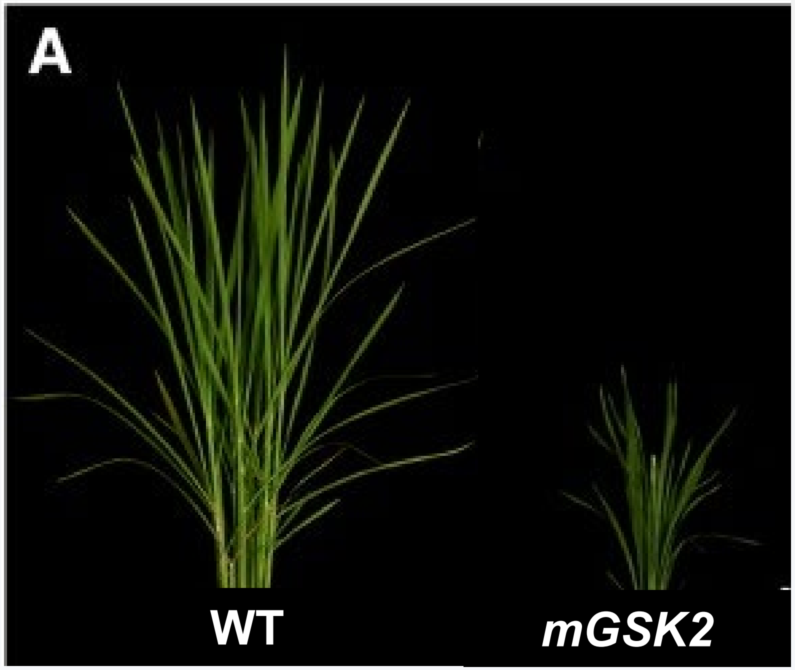
Insight into structure of OsGSK2





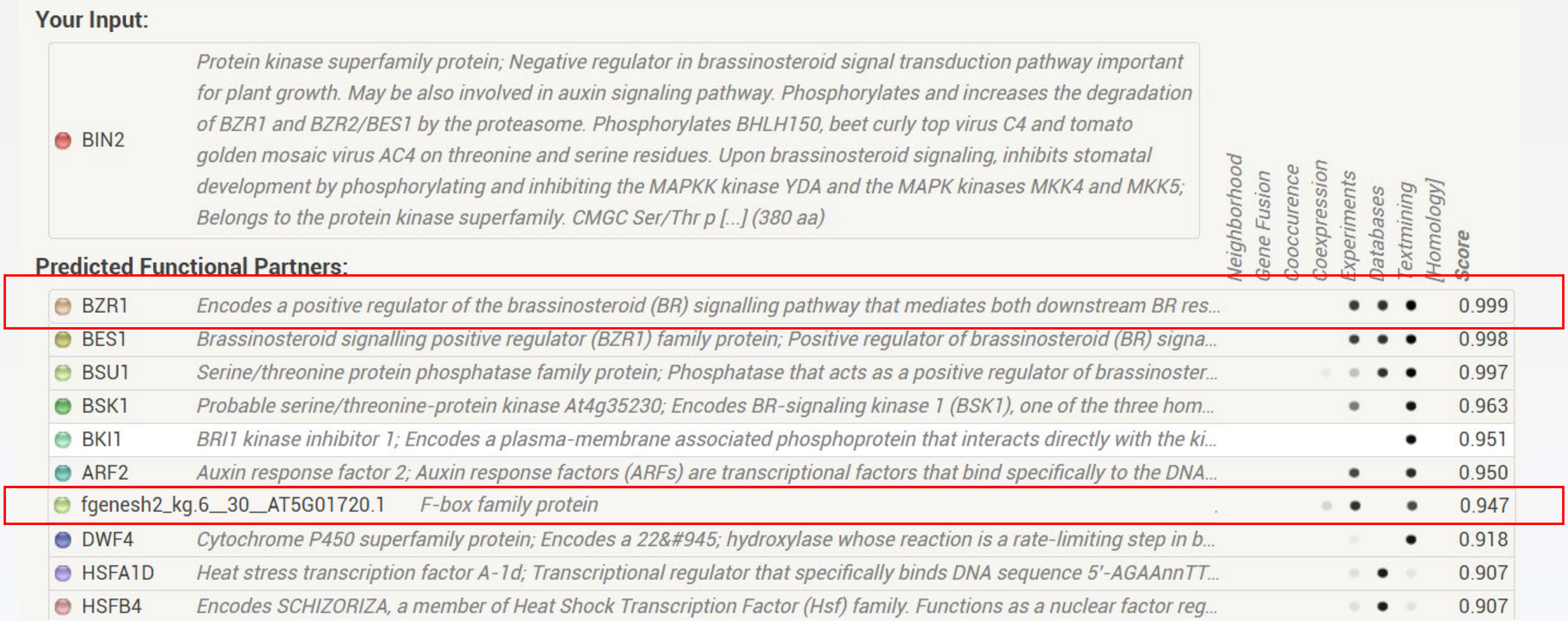
Mutations in OsGSK2 (268E-K) contributes to stronger functional translation of the protein





Tong et al. 2006

View BIN2 interactors in STRING



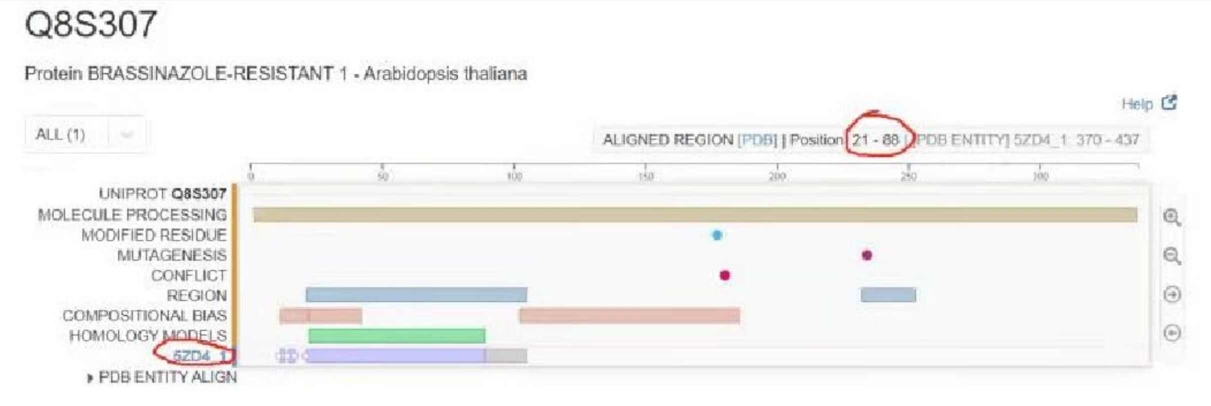
Your Input:

BIN2 *Protein kinase superfamily protein; Negative regulator in brassinosteroid signal transduction pathway important for plant growth. May be also involved in auxin signaling pathway. Phosphorylates and increases the degradation of BZR1 and BZR2/BES1 by the proteasome. Phosphorylates BHLH150, beet curly top virus C4 and tomato golden mosaic virus AC4 on threonine and serine residues. Upon brassinosteroid signaling, inhibits stomatal development by phosphorylating and inhibiting the MAPKK kinase YDA and the MAPK kinases MKK4 and MKK5; Belongs to the protein kinase superfamily. CMGC Ser/Thr p [...] (380 aa)*

Predicted Functional Partners:

		Neighborhood	*Gene Fusion*	*Cooccurence*	*Coexpression*	*Experiments*	*Databases*	*Textmining*	*[Homology]*	***Score***
BZR1	*Encodes a positive regulator of the brassinosteroid (BR) signalling pathway that mediates both downstream BR res...*					●	●	●		0.999
BES1	*Brassinosteroid signalling positive regulator (BZR1) family protein; Positive regulator of brassinosteroid (BR) signa...*					●	●	●		0.998
BSU1	*Serine/threonine protein phosphatase family protein; Phosphatase that acts as a positive regulator of brassinoster...*				●	●	●	●		0.997
BSK1	*Probable serine/threonine-protein kinase At4g35230; Encodes BR-signaling kinase 1 (BSK1), one of the three hom...*					●		●		0.963
BKI1	*BRI1 kinase inhibitor 1; Encodes a plasma-membrane associated phosphoprotein that interacts directly with the ki...*							●		0.951
ARF2	*Auxin response factor 2; Auxin response factors (ARFs) are transcriptional factors that bind specifically to the DNA...*					●		●		0.950
fgenesh2_kg.6__30__AT5G01720.1	*F-box family protein*				●	●		●		0.947
DWF4	*Cytochrome P450 superfamily protein; Encodes a 22α hydroxylase whose reaction is a rate-limiting step in b...*					●		●		0.918
HSFA1D	*Heat stress transcription factor A-1d; Transcriptional regulator that specifically binds DNA sequence 5'-AGAAnnTT...*					●	●	●		0.907
HSFB4	*Encodes SCHIZORIZA, a member of Heat Shock Transcription Factor (Hsf) family. Functions as a nuclear factor reg...*					●	●	●		0.907

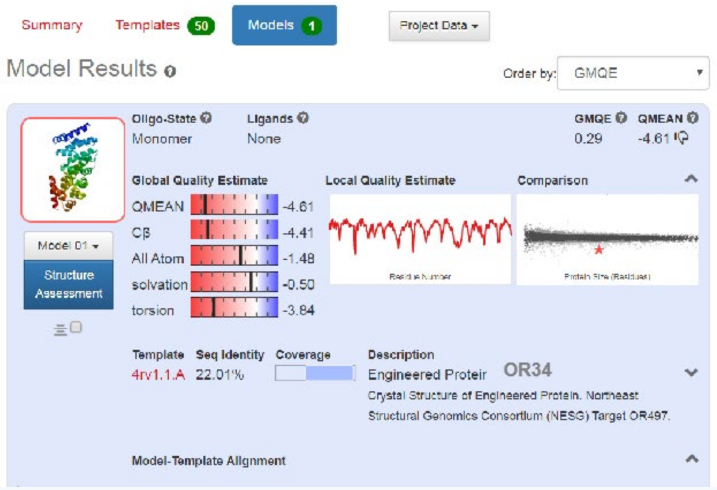
A U box protein is a more available candidate protein



Q8S307

Protein BRASSINAZOLE-RESISTANT 1 - Arabidopsis thaliana

ALIGNED REGION [PDB] | Position 21 - 88 | [PDB ENTITY] 5ZD4_1: 370 - 437



Model Results

Order by: GMQE

Oligo-State: Monomer

Ligands: None

GMQE: 0.29

QMEAN: -4.61

Global Quality Estimate

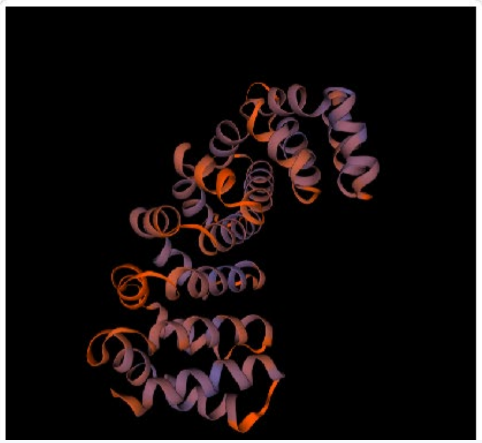
QMEAN	-4.61
Cβ	-4.41
All Atom	-1.48
solvation	-0.50
torsion	-3.84

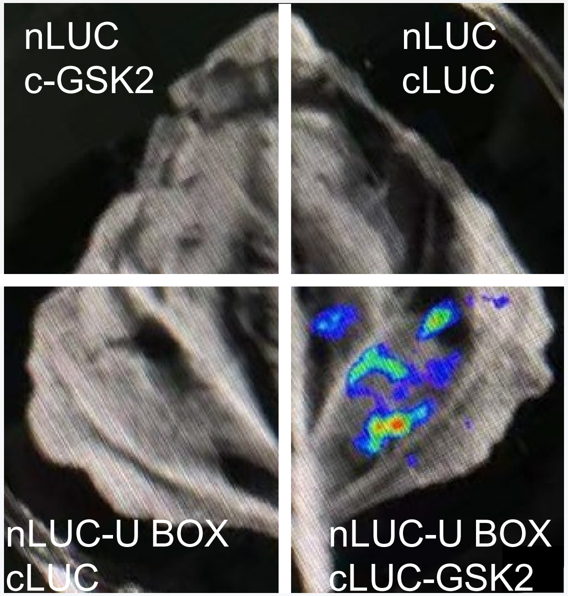
Local Quality Estimate

Comparison

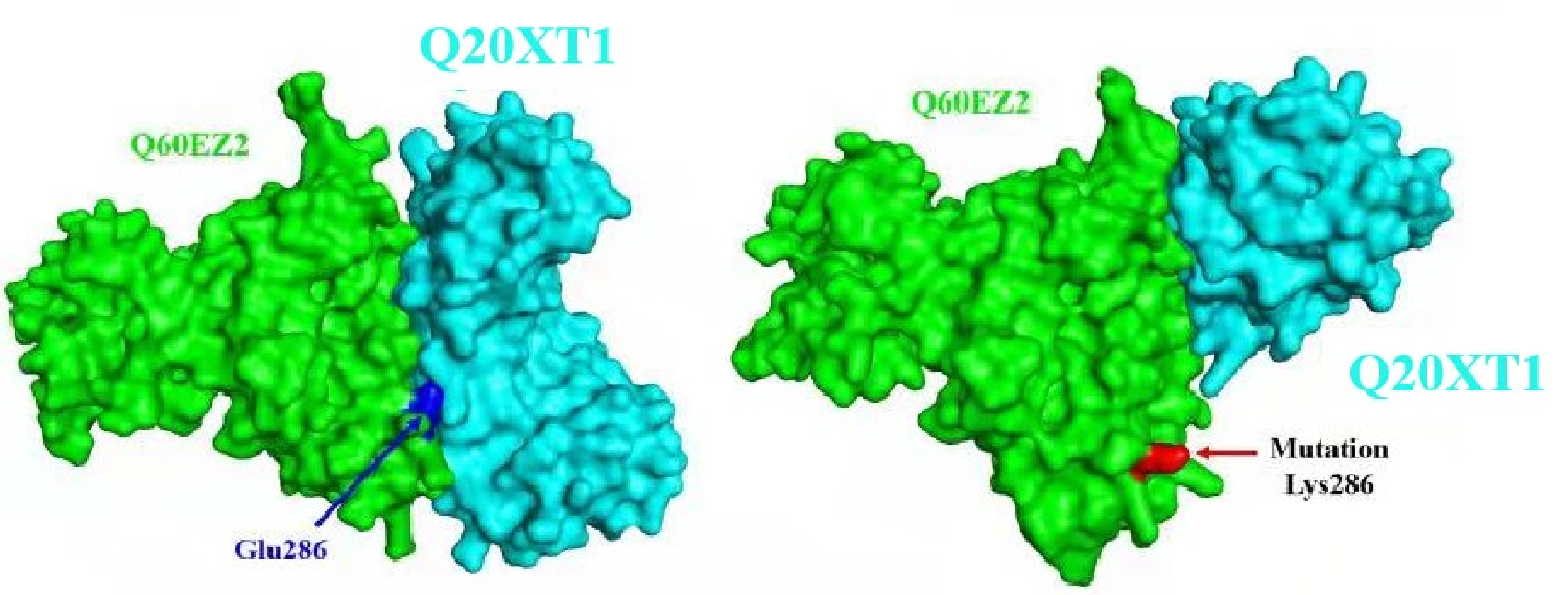
Template	Seq Identity	Coverage	Description
4rv1.1.A	22.01%		Engineered Proteir OR34 Crystal Structure of Engineered Protein. Northeast Structural Genomics Consortium (NESG) Target OR497.

Model-Template Alignment

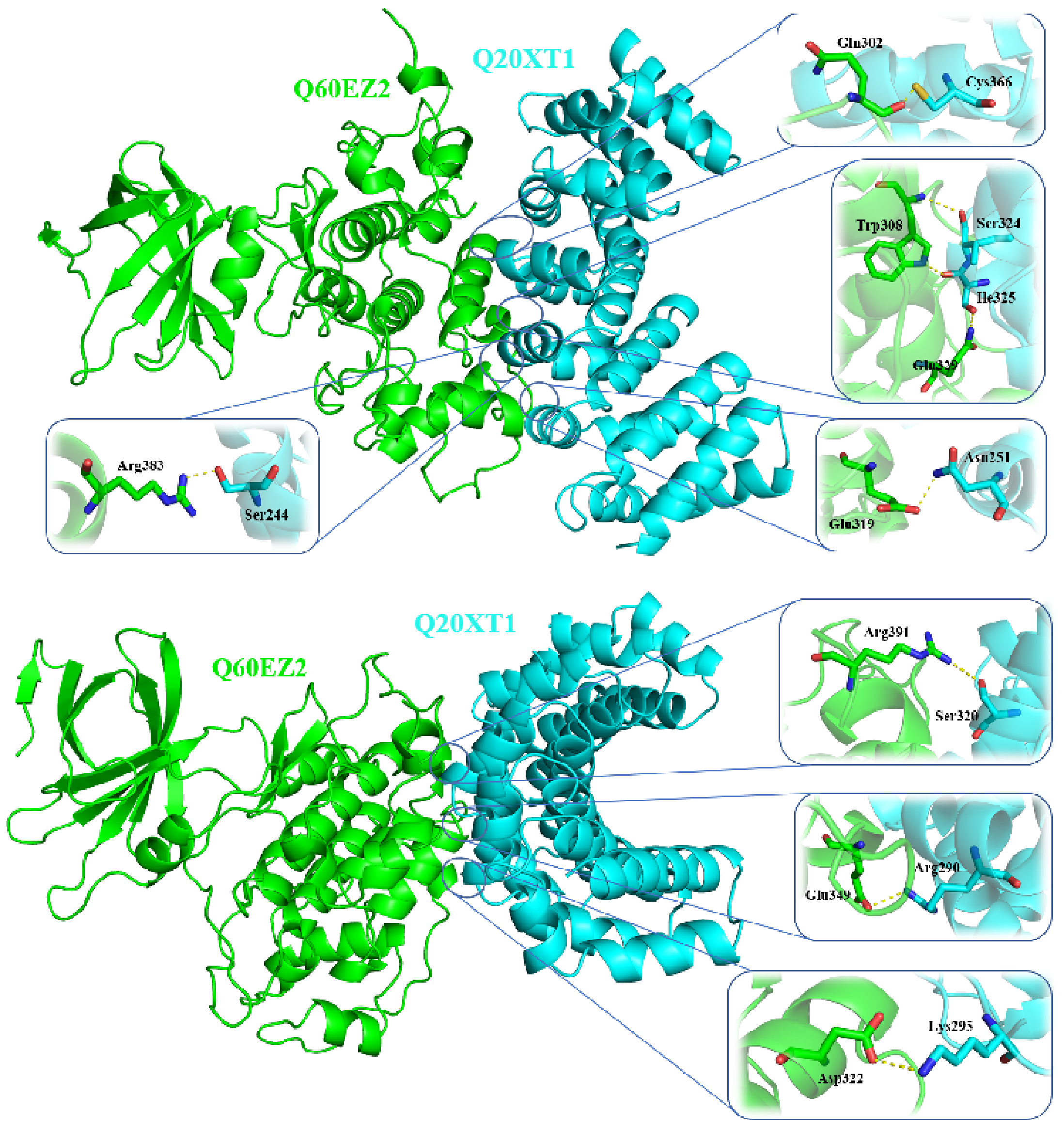




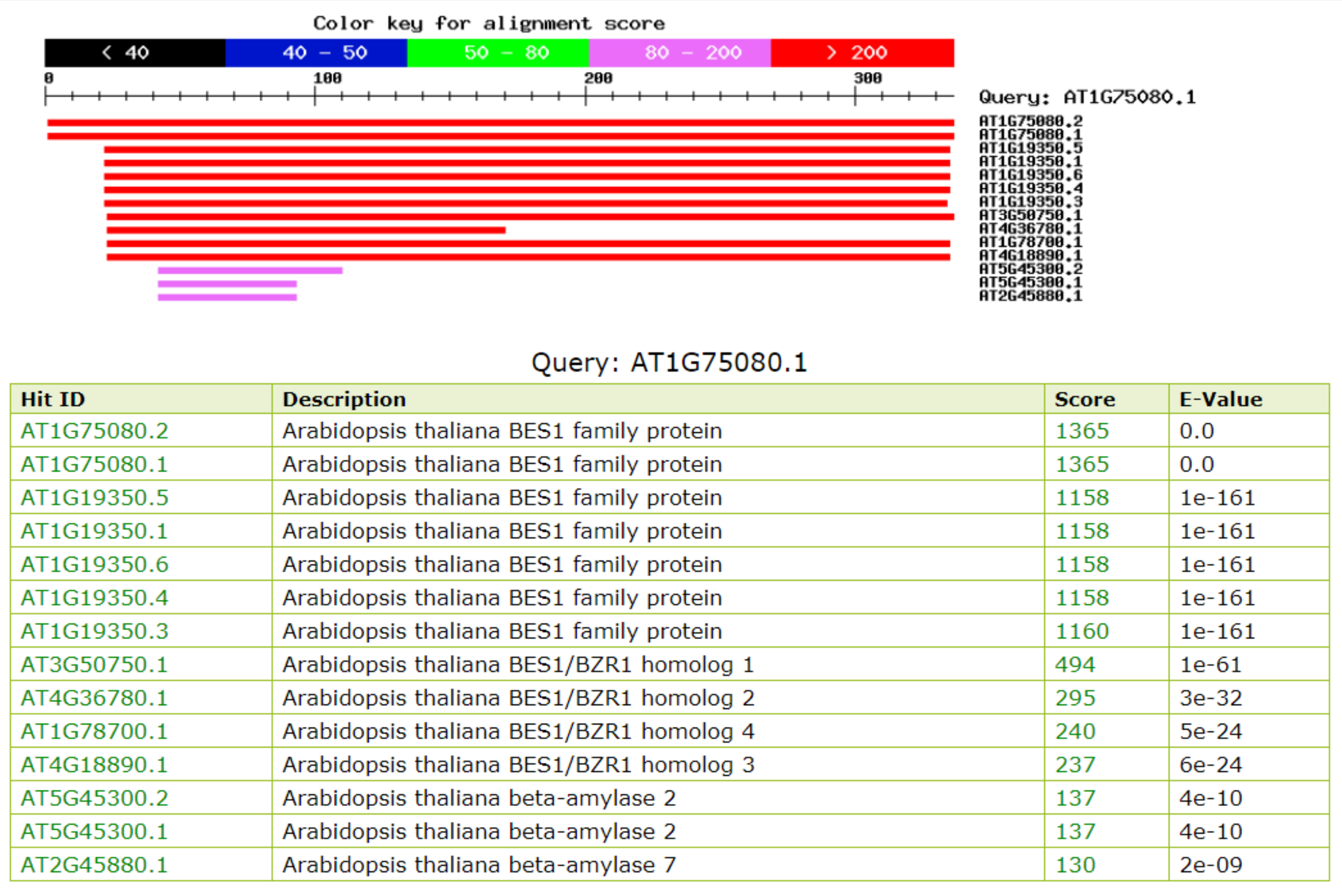
Mutation of OsGSK2 (268E-K) changes the interaction between GSK2 and U Box protein



Mutation of OsGSK2 (268E-K) weakens the GSK2-U Box nteraction



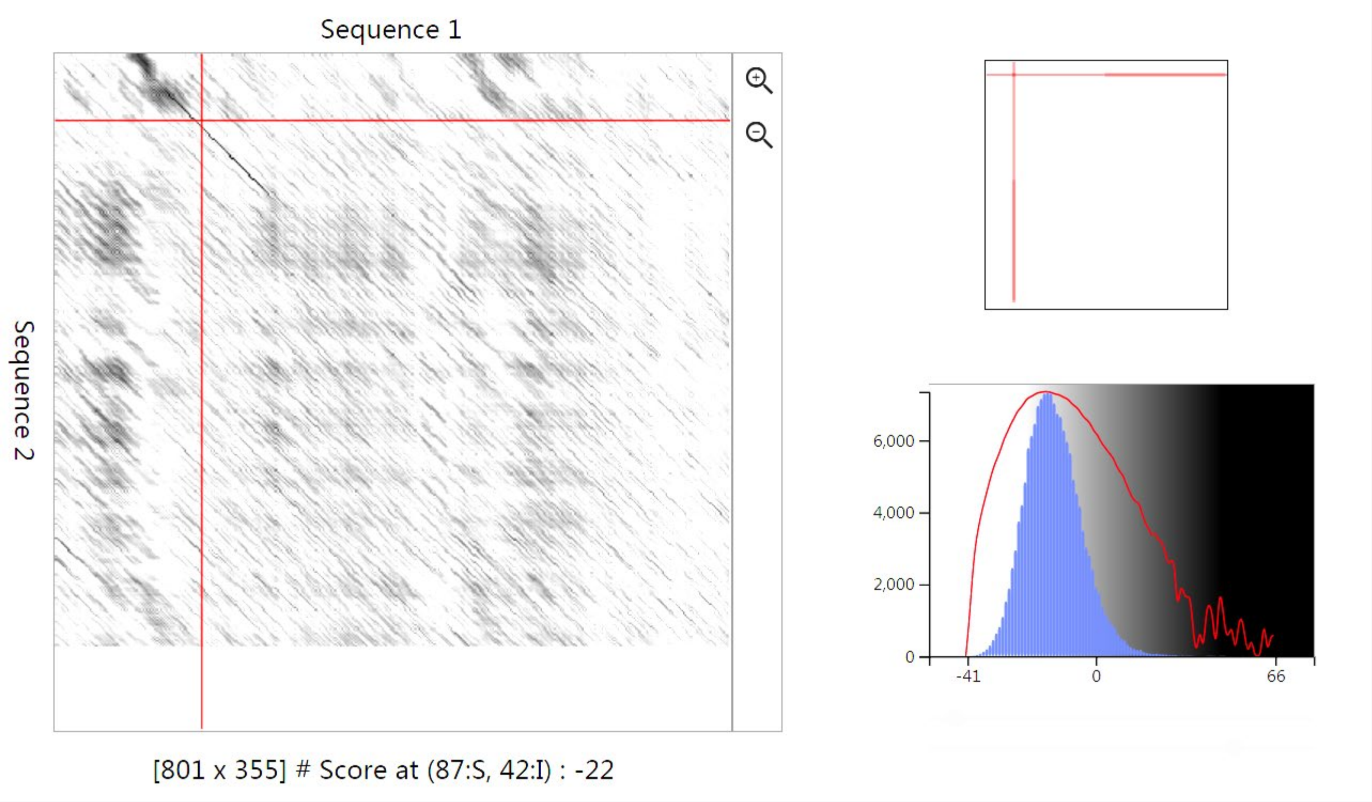
Some surprising findings



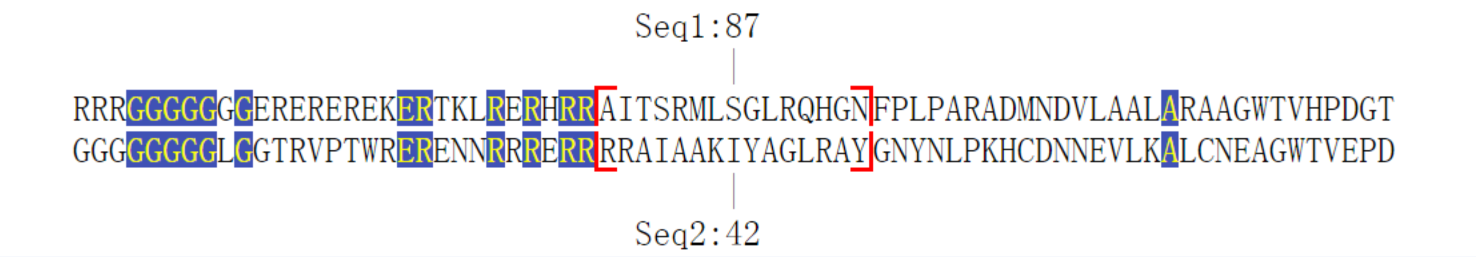
Query: AT1G75080.1

Hit ID	Description	Score	E-Value
AT1G75080.2	Arabidopsis thaliana BES1 family protein	1365	0.0
AT1G75080.1	Arabidopsis thaliana BES1 family protein	1365	0.0
AT1G19350.5	Arabidopsis thaliana BES1 family protein	1158	1e-161
AT1G19350.1	Arabidopsis thaliana BES1 family protein	1158	1e-161
AT1G19350.6	Arabidopsis thaliana BES1 family protein	1158	1e-161
AT1G19350.4	Arabidopsis thaliana BES1 family protein	1158	1e-161
AT1G19350.3	Arabidopsis thaliana BES1 family protein	1160	1e-161
AT3G50750.1	Arabidopsis thaliana BES1/BZR1 homolog 1	494	1e-61
AT4G36780.1	Arabidopsis thaliana BES1/BZR1 homolog 2	295	3e-32
AT1G78700.1	Arabidopsis thaliana BES1/BZR1 homolog 4	240	5e-24
AT4G18890.1	Arabidopsis thaliana BES1/BZR1 homolog 3	237	6e-24
AT5G45300.2	Arabidopsis thaliana beta-amylase 2	137	4e-10
AT5G45300.1	Arabidopsis thaliana beta-amylase 2	137	4e-10
AT2G45880.1	Arabidopsis thaliana beta-amylase 7	130	2e-09

Detect BZR1 in AT2G45888.1



[801 x 355] # Score at (87:S, 42:I) : -22



Seq1:87

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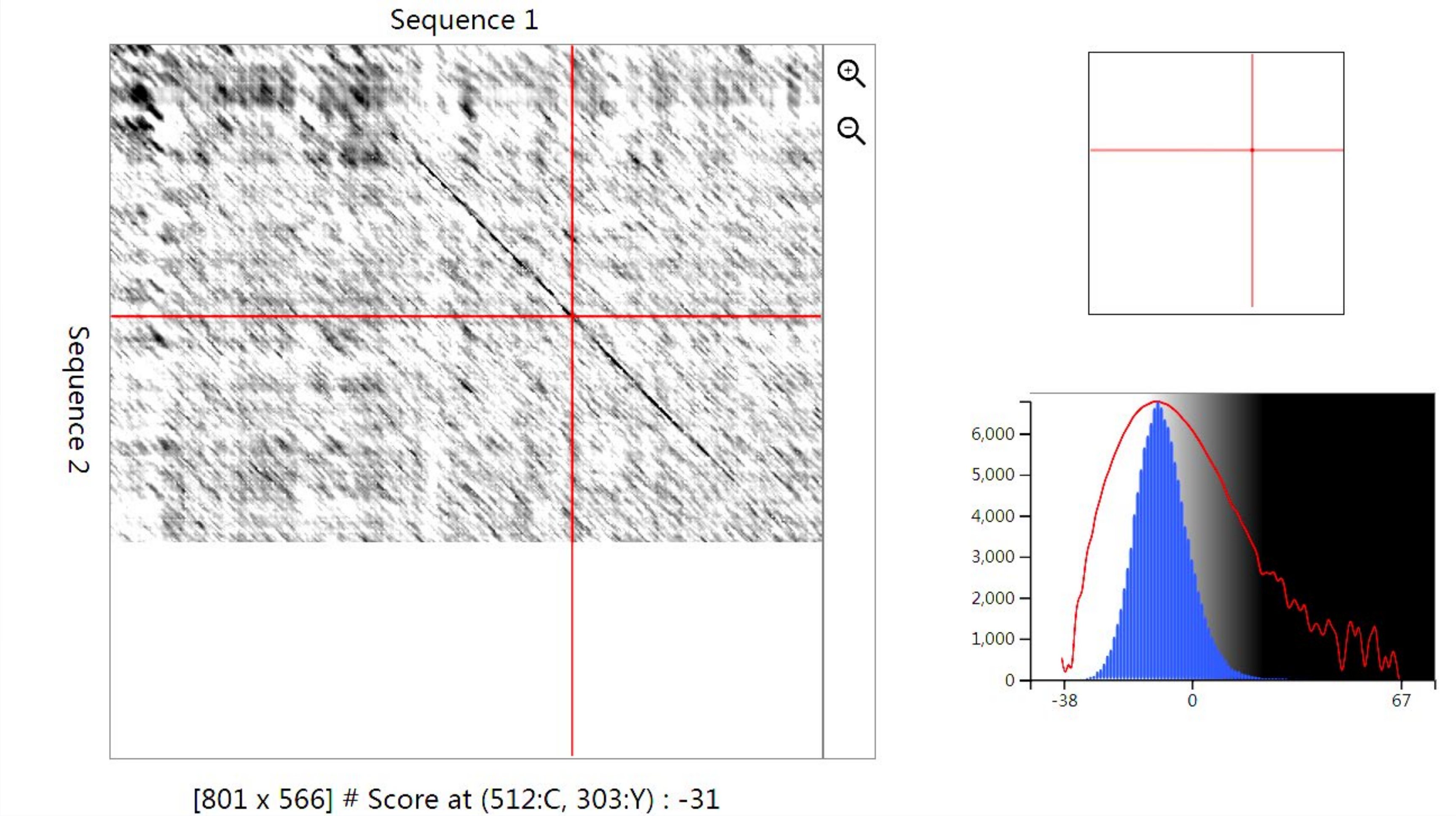
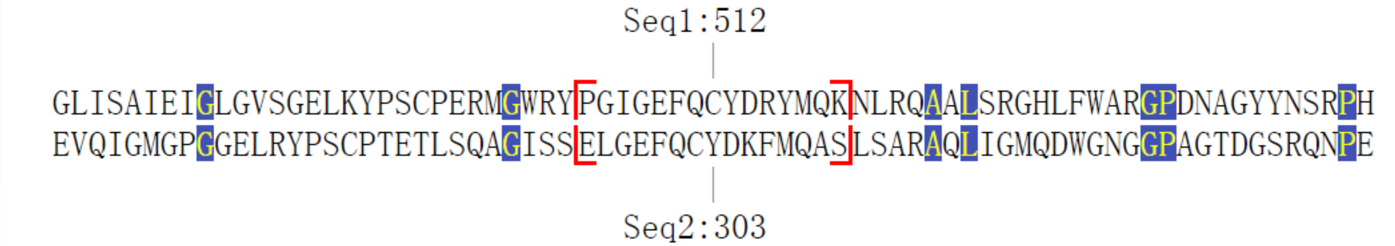
RRRGGGGGGGEREREREKERTKLRERHRRAITSRMLSGLRQHGNFPLPARADMNDVLAALARAAGWTVHPDGT
GGGGGGGGLGGTRVPTWRERENNRRRERRRRAIAAKIYAGLRAYGNYNLPKHCDNNEVLKALCNEAGWTVEPD
```

Seq2:42

Detect beta-amylase in AT2G45888.1



[801 x 566] # Score at (512:C, 303:Y) : -31



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Seq1:512
GLISAIEIGLGVSGELKYPSCPERMGWRY[PGIGEFQCYDRYMQK]NLRQAALSRGHLFWARGPDNAGYYNSRPH
EVQIGMGPGGELRYPSCPTETLSQAGISS[ELGEFQCYDKFMQAS]LSARAQLIGMQDWGNGGPAGTDGSRQNPE
Seq2:303
```

工作计划

1. “TREE”motif更多组合的突变模拟对接

2.FRET荧光共振能量转移、Western blot定量检测mGSK2、OsGSK2与U-BOX的互作强度

3.继续学习和收集相关知识

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Thank's for listening !