

山药 *HXKs* 鉴定及 DoHXK3 互作蛋白验证

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DOI: 10.13560/j.cnki.biotech.bull.1985.2025-1246

Identification of *HXKs* in Yam and Validation of Proteins Interacting with DoHXK3

附表 1 *DoHXKs* 生物信息学分析相关软件

Table1 Related software for *DoHXKs* bioinformatics analysis

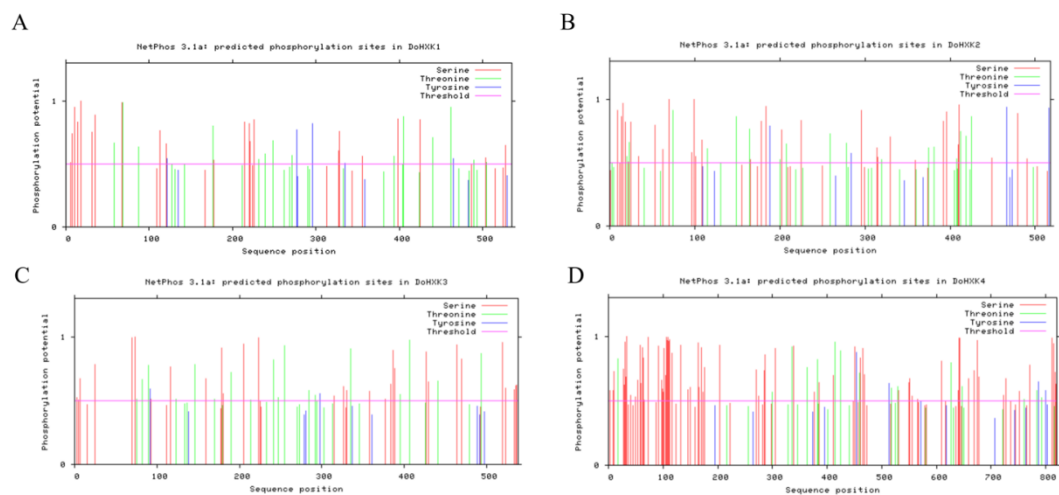
软件 Software	用途 Usage
ExPASy http://web.expasy.org/protparam/	蛋白理化性质分析 Analysis in physicochemical properties of coding protein
WoLF PSBRT https://wolfsbrt.hgc.jp/	亚细胞定位预测 Prediction of subcellular localization
MEGA11.0 http://www.megasoftware.net	氨基酸序列比对 Alignment of amino acid sequences
MEGA11.0 http://www.megasoftware.net	系统进化树的构建 Construction of phylogenetic tree
MEME http://meme-suite.org/tools/meme	保守结构域分析 Analysis of conservative domain
SOPMA https://npsa-prabi.ibcp.fr	蛋白结构预测 Prediction of protein structure
STRING11.5 http://string-db.org/	蛋白互作网络预测 Prediction of protein interaction network

附表 2 *DoHXKs* 相关的引物序列

Table2 Primer sequences related to *DoHXKs*

引物名称 Primer name	引物序列 Primer sequences (5'→3')	备注 Note
<i>DoHXK1</i> -Q-F	CCTTCGTTGATGCCCTTCCT	RT-qPCR
<i>DoHXK1</i> -Q-R	GTTGTTCAACCTGACGCCAC	RT-qPCR
<i>DoHXK2</i> -Q-F	GGTTGTGAGGGAGTTGGAGG	
<i>DoHXK2</i> -Q-R	ATCTCCACCGTCATCGCATC	
<i>DoHXK3</i> -Q-F	TCACAACCATTGCAATCGA	
<i>DoHXK3</i> -Q-R	CGTCGATAACCTCCCCACTG	

<i>DoHXK4-Q-F</i>	GAAGAAGCAGGGTCGTGACA	
<i>DoHXK4-Q-R</i>	TGAACACCGTGGATGCTACC	
<i>UBQ-F</i>	GGGCTTTCAAGGTCGTC	内参基因
<i>UBQ-R</i>	TGAAGGGTTTGCTCATCC	Internal genes
<i>DoHXK3-SF</i>	GGGGTACCCCATGGTGATTAACATGGAATGG	瞬时表达载体构建
<i>DoHXK3-SR</i>	GCTCTAGAGCTCGCTCCGACGAGAGTGATGC	Construction of transient expression vector
<i>DoHXK3-Y-F</i>	GAATTCATGGTGATTAACATGGAATGG	诱饵载体构建
<i>DoHXK3-Y-R</i>	GGATCCTCATCGCTCCGACGAGAGTGATGC	Construction of decoy vector
pPR3-N-F	CGGTAAAACCGGAACATTGGA	酵母菌液 PCR
pPR3-N-R	ACTTCAGGTTGTCTAACTCTCT	Yeast Liquid PCR
<i>DoA2C-F</i>	TACGATGTTCCAGATTACGCTGGATCCATGTTGATGTCAGA GATTGTT	AD 载体构建
<i>DoA2C-R</i>	GGTATCGATAAGCTTGATATCGAATTCTACTTTGGGCTGGT GGAAC	Construction of AD vector
<i>DoERF3A-F</i>	TACGATGTTCCAGATTACGCTGGATCCATGGACGAAGAAGC GAGAGTC	
<i>DoERF3A-R</i>	GGTATCGATAAGCTTGATATCGAATTCTTATGCTGAAAATG AGGAGCT	
<i>DoHXK3-B-F</i>	GGTACCATGGTGATTAACATGGAATGG	BiFc
<i>DoHXK3-B-R</i>	GAGCTCTCATCGCTCCGACGAGAGTGATGC	BiFc
<i>DoA2C-B-F</i>	GGACTAGTATGTTGATGTCAGAGATTGTT	
<i>DoA2C-B-R</i>	GAGCTCCTACTTTGGGCTGGTGGAAC	
<i>DoERF3A-B-F</i>	ACTAGTATGGACGAAGAAGCGAGAGTC	
<i>DoERF3A-B-R</i>	CTTAAGTTATGCTGAAAATGAGGAGCTTCC	

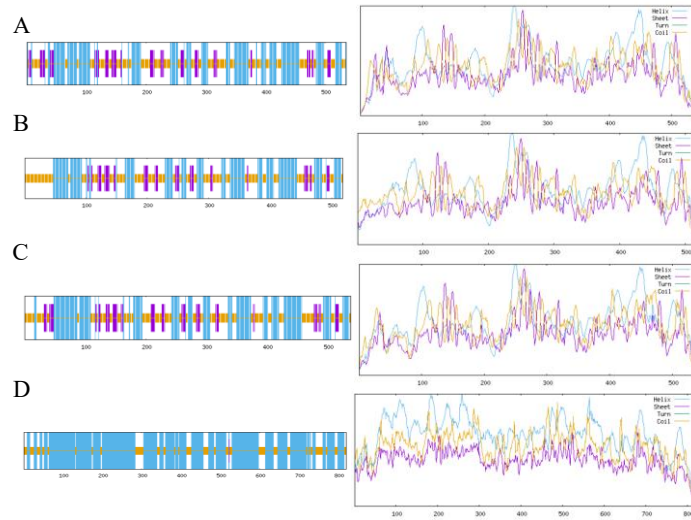


A, B, C, D: 分别为 DoHXK1, DoHXK2, DoHXK3, DoHXK4 磷酸化位点

A, B, C, D: Prediction of phosphorylation site of the DoHXK1, DoHXK2, DoHXK3, DoHXK4 protein

附图 1 DoHXKs 磷酸化位点预测

Fig.1 Prediction of phosphorylation site of the DoHXKs

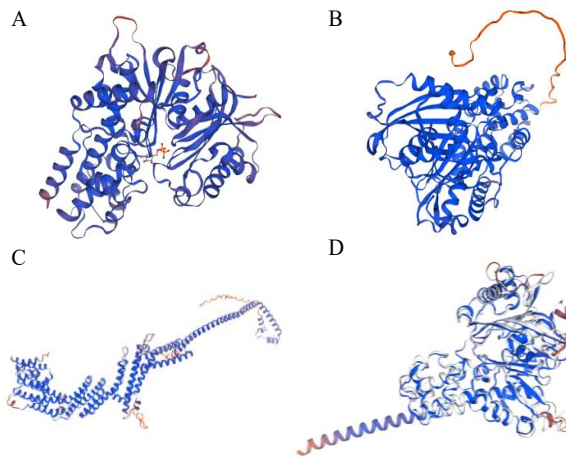


蓝色： α -螺旋；红色：延伸链；紫色：延伸链；A, B, C, D: 分别为 DoHXK1, DoHXK2, DoHXK3, DoHXK4 蛋白质二级结构预测

Blue: Alpha helix; Red: Extended strand; Purple: Random coil. A, B, C, D: Prediction of the secondary structure of the DoHXK1, DoHXK2, DoHXK3, DoHXK4 protein

附图 2 DoHXKs 蛋白质二级结构预测

Fig.2 Prediction of the secondary structure of the DoHXKs

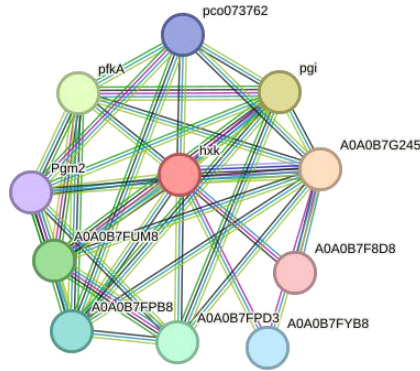


A, B, C, D: 分别为 DoHXK1, DoHXK2, DoHXK3, DoHXK4 蛋白质三级结构预测

A, B, C, D: Prediction of tertiary structure model the DoHXK1, DoHXK2, DoHXK3, DoHXK4 protein

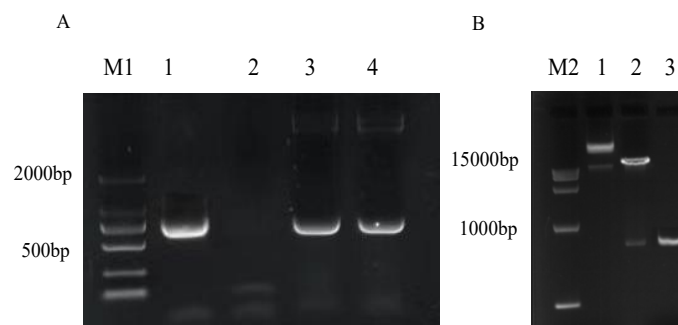
附图 3 DoHXKs 蛋白质三级结构模型预测

Fig.3 Prediction of tertiary structure model of the DoHXKs



附图 4 DoHXKs 蛋白互作预测

Fig.4 Prediction of protein-protein interaction of DoHXKs

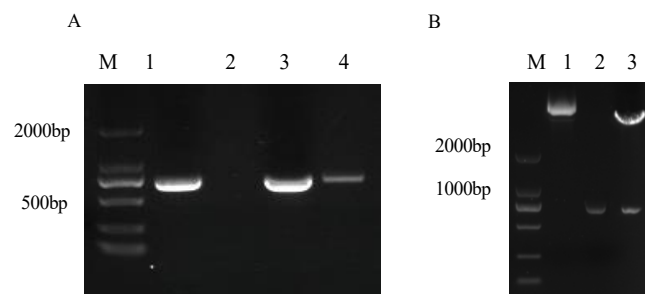


A: M1: DL2 000 DNA 标记; 1: 阳性对照; 2: 阴性对照; 3, 4: PCR 条带; B: M2: DL15 000 DNA 标记; 1: 重组质粒; 2: 酶切鉴定; 3: 阳性对照.

A: M1: DL2 000 DNA marker; 1: positive control; 2: Negative control; 3,4: PCR band; B: M2: DL15 000 DNA marker; 1: recombinant plasmid; 2: enzyme digestion identification; 3: positive control.

附图 5 PCR 检测和双酶切法检测瞬时表达载体 *CaMV35S-DoHXK3*

Fig.5 Detection of transient expression vector *CAMV35S-DoHXK3* by PCR and double digestion

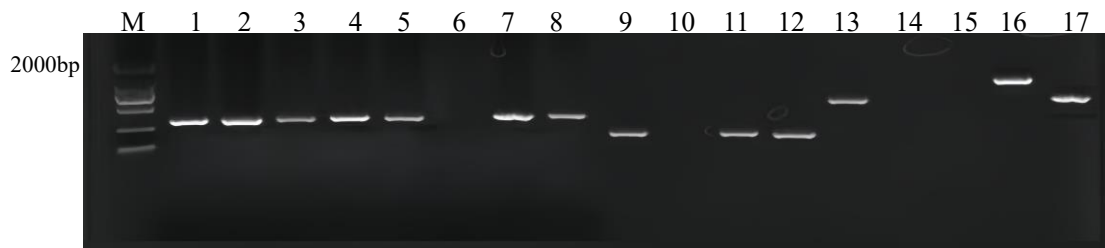


A: M: DL2 000 DNA 标记; 1: 阳性对照; 2: 阴性对照; 3, 4: PCR 条带; B: M: DL2 000 DNA 标记; 1: 重组质粒; 2: 阳性对照; 3: 酶切鉴定.

A: M: DL2 000 DNA marker; 1: positive control; 2: Negative control; 3,4: PCR band; B: M: DL2 000 DNA marker; 1: recombinant plasmid; 2: positive control; 3: enzyme digestion identification.

附图 6 PCR 检测和双酶切法检测诱饵载体

Fig.6 Construction of Bait Vector by PCR and digestion



M: DL2000 DNA 标记; 1-15: 菌液 PCR 结果.

M: DL2000 DNA marker; 1-15: PCR results of 1-15 bacterial solution.

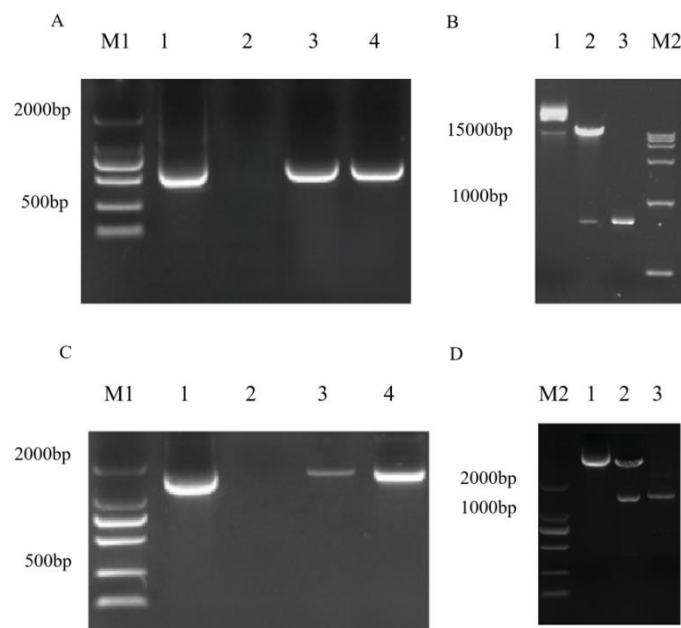
附图 7 阳性克隆菌落 PCR 检测

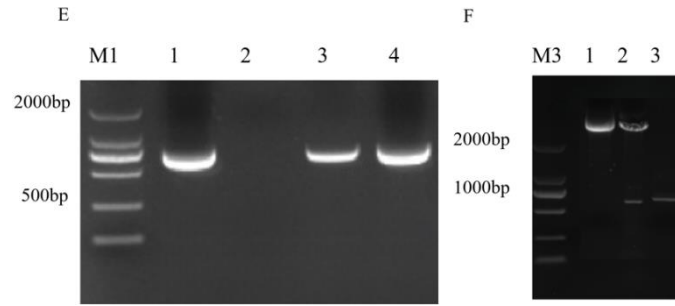
Fig.7 Positive clonal colonies were detected by PCR

附表 3 互作蛋白 Blast 分析

Table 2 Blast analysis of interacted proteins

编号 No.	功能注释 Feature annotations	序列号 Serial number
1	热应激转录因子 A-2c	XP_039114790.1
2	真核生物肽链释放因子 GTP 结合亚基 ERF3A	XP_039127275.1
3	非特异性丝氨酸/苏氨酸蛋白激酶蛋白	XP_060213580.1
4	假设蛋白质 PVL29_006712	KAJ9701478.1
5	S-腺苷甲硫氨酸合成酶	XM_039271379.1





A, C, E: 分别为 *DoHXX3/DoA2C/DoERF3A* 质粒 PCR; M1: DL2 000 DNA 标记; 1: 阳性对照; 2: 阴性对照; 3, 4: PCR 条带; B, D, F: 分别为 *DoHXX3/DoA2C/DoERF3A* 酶切鉴定; M2: DL15 000 DNA 标记; M3: DL2 000 DNA 标记; 1: 重组质粒; 2: 阳性对照; 3: 酶切鉴定.

A, C, E: PCR of *DoHXX3/DoA2C/DoERF3A* plasmids; M1: DL2 000 DNA marker; 1: positive control; 2: Negative control; 3,4: PCR band; B, D, F: Enzymatic digestion identification of *DoHXX3/DoA2C/DoERF3A*; M2: DL15 000 DNA marker; M3: DL2 000 DNA marker; 1: recombinant plasmid; 2: positive control; 3: enzyme digestion identification.

附图 8 PCR 检测和双酶切法检测载体 p1300-SCYNE-*DoHXX3*, p1300-SCYCE-*DoA2C/DoERF3A*

Fig.8 Detection of vector p1300-SCYNE-*DoHXX3*, p1300-SCYCE-*DoA2C/DoERF3A* by PCR and double