( ),2017,48(4):576-581 VOL.48 NO.4 2017
Journal of Shandong Agricultural University ( Natural Science Edition ) doi:10.3969/j.issn.1000-2324.2017.04.020

# 苹果 *MdAFS* 基因亚细胞定位表达载体的构建及分析

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**摘 要:** *Malus domestica* Borkh cv. White Winter Pearmain) PCR α- (*AFS*) *Rubisco* (*Ru*) (*Mit*) (*Pla*) plantCARE SignalP4.1Server *Ru* TATAbox CAATbox

*Ru* *AFS* PCR

**关键词:** ; *MdAFS* ; ;

**中图法分类号:** S661.1 **文献标识码:** A **文章编号:** 1000-2324(2017)04-0576-06

# Construction and Analysis of Apple *MdAFS* Gene Expression Vector into Different Subcellular Compartments

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**Abstract:** To study the change of terpene synthase subcellular localization influenced terpenoids metabolism and plant development, we used the White winter pearmain peels, Chrysanthemum morifolium, Yeast and Arabidopsis thalianas as experimental materials and cloned the strong promoter of alpha-*Farnesene synthase*, *Rubisco*, transit peptide of mitochondria(*mit*) and transit peptide of chloroplast(*pla*) by RT-PCR. Then the sequence of promoter and transit peptide were predicted by PlantCARE and SignalP4.1Server. The results showed that *Ru* had typical characteristics of strong promoter elements(TATAbox, CAATbox and hormone response elements) and both of *mit* and *pla* maybe have the function of transit peptide. We have successfully constructed the alpha-*Farnesene synthase* gene expression vector in different subcellular localization driven by *rubisco* strong promoter, and transformed them into tobacco. Quantitative real-time PCR showed that the gene had a higher expression and was tissue-specific in transgenic tobacco.

**Keywords**: Apple; *MdAFS* gene; subcellular; expression

[1,2]

C10

C15

[3]

[4]

/

[5]

*β*- *β*-

[6]

**收稿日期:** 2015-12-10 **修回日期:** 2016-01-11

**基金项目:** (31370359)

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*α*-

[7] [8]

— [9] α- *AFS* *α*-

*α*-

TPS

[4] *α*-

*Rubisco*

*α*-

## 1 材料与方法

### 1.1 材料及试剂

(*Malus domestica* Borkh. cv. white winter pearmain

(*Chrysanthemum morifolium* *Columbia* *Arabidopsis thaliana*

NC89 *E. coli* DH5α LBA4404

pMD18-T pBI121 SalI

TaqDNA DNAMarker

T4-DNA Fermentas

MS

AR

### 1.2 材料处理

T1 NC89 MS 25 ℃ 2

50~60% 145 μmol·m$^{-2}$·s$^{-1}$ 16 h /8 h

–80 ℃

### 1.3 基因的提取

Ultrapure RNAKit RNA CWBIO RNA cDNA

–20 ℃ PCR CTAB

DNA

### 1.4 引物设计

PCR 1

**表 1 基因克隆及引物序列**

**Table 1 Gene cloning and sequence of primers**

| Primer name | Genbank accesssion number | Primer sequence(5′–3′) | /bp Size of fragment |
|---|---|---|---|
| RuFS1 | AY563622 | GCCCATGGAATTCAGAGTTCAC | 1780 |
| RuFS2 | | GCGTCGACTAGTTTACAAGAGG | |
| Ru1 | AY163904.1 | CTGAAGCTTAAGCTTAGACAAACACCCCTTG | 1100 |
| Ru2 | | TCCCCCGGGGGTTTCTGATACTTAGGAGGAATGG | |
| MitP1 | X01418.1 | GGATCCATGTTGTCACTACGTCAATCTATAA | 87 |
| MitP2 | | CCATGGGTTTTTGCTGAAGCAGATATCTA | |
| PlasmidP1 | CP002685.1 | GGATCCATGGCGATGTCTTTCTCAGGAGCTG | 156 |
| PlasmidP2 | | GCCATGGCGTAGATCAACGACTTCTTGCG | |

### 1.5 构建载体

1 PCR :94 ℃ 5 min 94 ℃ 1 min 55 ℃ 1 min 72 ℃ 1 min 15 s 35 72 ℃ 10 min 20 μL 1% pMD18-T 3-10:1 4 ˚C *Pla*/*Mit*

PCR LB Amp+ DNA pMD18-T+*Pla*/*Mit*+*AFS* pBI121 35S *Rubisco* pBI121+*Ru* pBI121+*Ru* pMD18-T+*Pla*/*Mit*+*AFS* pBI121+*Ru*+*Pla*/*Mit*+*AFS*

### 1.6 农杆菌介导烟草转化

pBI121+*Ru*+*Pla*/*Mit*+*AFS* 100 mg·L$^{-1}$ Kan PCR

### 1.7 实时荧光定量 PCR 分析

$T_2$ 6 ( Ultrapure RNAKit RNA CWBIO RNA cDNA –20 ℃

18SF1 5′-TTCCTAGTAAGCGCGAGTCATCAGC-3′ 18SR1 5′-GCGACGGGCGGTGTGT-3′ *AFS* PaFSF 5′-AAAGCGACAATCTCGGCACAA-3′ PaFSR 5′-GCGCGAAATGGTGGTTCTCTAAT-3′ qPCR

## 2 结果与分析

### 2.1 *AFS*、*Ru* 强启动子、*Mit* 和 *Pla* 的克隆

Ultrapure RNAKit RNA CWBIO RNA CTAB DNA RuFS1 RuFS2 Ru1 Ru2 PlasmidP1 PlasmidP2 MitP1 MitP2 PCR *AFS* *Ru* *Pla* *Mit* PCR 1 PCR pMD-18T PCR *AFS* *Ru* *Mit* *Pla* 1780 bp 1100 bp 87 bp 156 bp

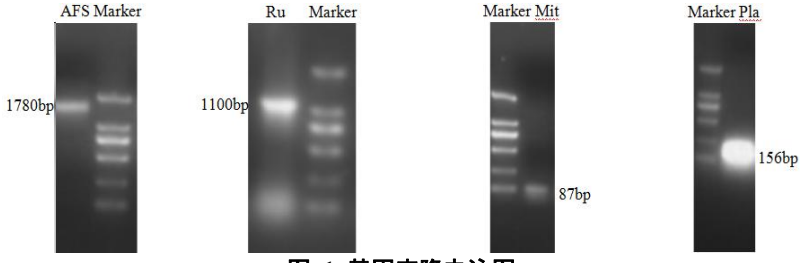


**图 1 基因克隆电泳图**

**Fig.1 Electrophoresis results of genes cloning**

Marker DL2000 ladder A *AFS* ;B *Ru* C *Mit* D *Pla*

Marker: DL2000 ladder; A: Amplified fragment of *AFS*; B: Amplified fragment of *Ru*; C: Amplified fragment of *Mit*; D: Amplified fragment of *Pla*

### 2.2 *Ru*、*Mit* 和 *Pla* 序列预测分析

DNAman GeneBank PlantCARE *Ru* *AFS* *Pla* *Mit* *Ru*

GeneBank PlantCARE

*Ru* TATAbox CAATbox

( 2 SignalP4.1Server(http://www.cbs.dtu.dk/services/SignalP/)

( 3

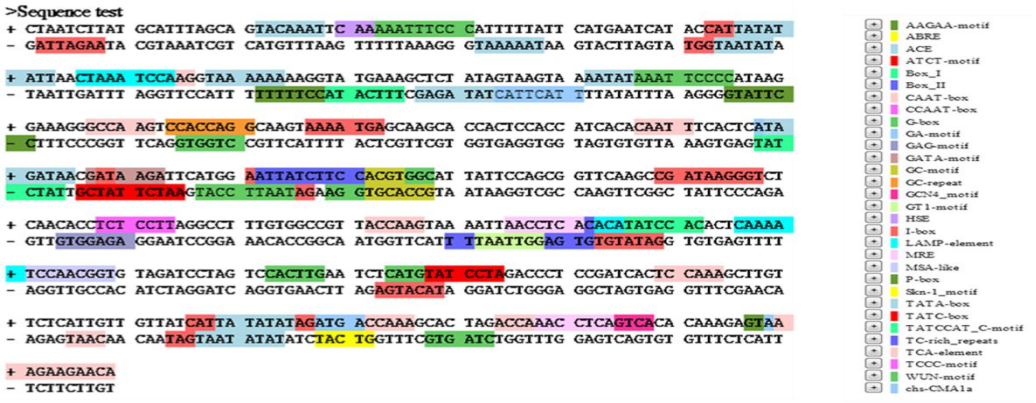


**图 2 *Ru* 启动子核苷酸序列预测**

**Fig.2 Deduced nucleotide sequences of *Ru* promoter**

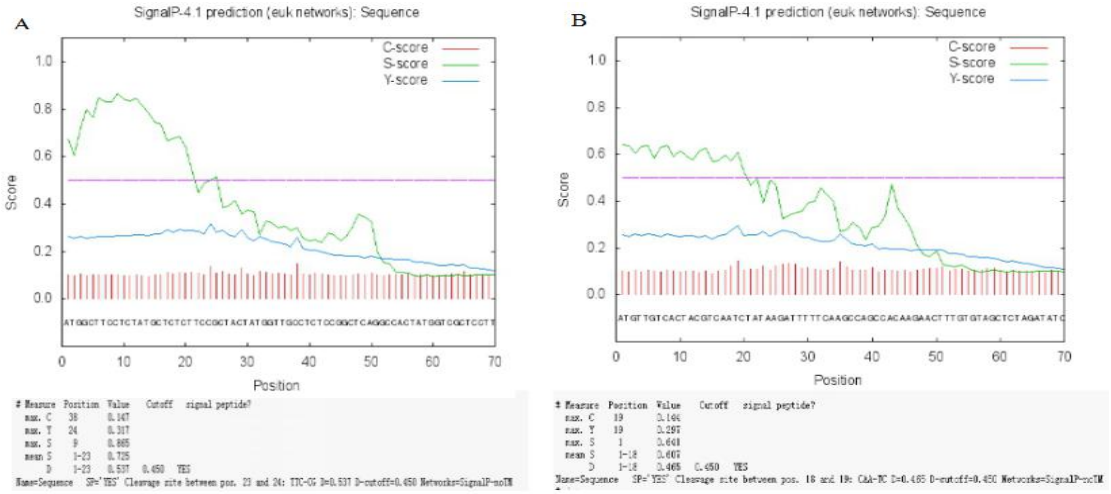


**图 3 线粒体和叶绿体转运肽序列预测**

**Fig.3 Deduced sequences of Mitochondria transit peptide and chloroplast signal peptide**

A B

A: Targeting peptide of chloroplast; B: Targeting peptide of mitochondria

## 2.3 真核表达载体构建

*AFS* *AFS*

*Rubisco* CaMV35S

( 4 *AFS*

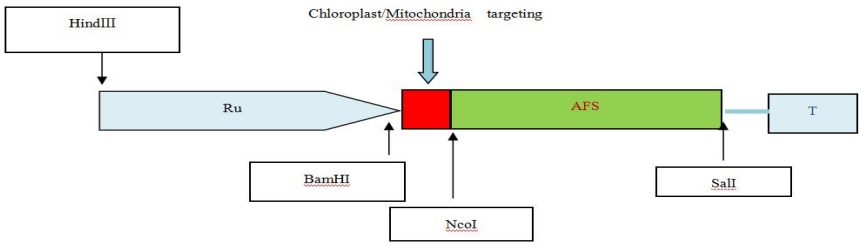


**图 4 表达载体的构建图**

**Fig.4 Construction of expression vector**

## 2.4 qPCR 分析不同转基因烟草株系中 *AFS* 的表达

$T_1$ 6 *AFS*

PCR ( 5 qPCR

P1 P2 P3 M3 M4 M5

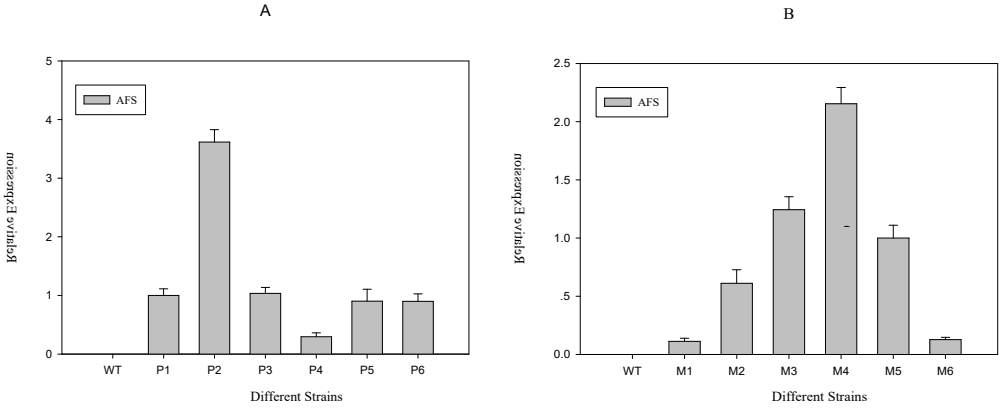


**图 5 qPCR 分析不同转基因烟草株系中 *AFS* 的表达**

**Fig.5 Expression of *AFS* in different transgenic tobacco strains by qPCR**

A B

A: Chloroplast over-expression plants; B: Mitochondria over-expression plants

## 2.5 转基因植株 *AFS* 表达的空间特异性

*AFS*

qPCR *AFS* *AFS*

*AFS*

*AFS* ( 6 A *AFS*

( 6 B *AFS*

*AFS*

*AFS*

*AFS* *AFS*

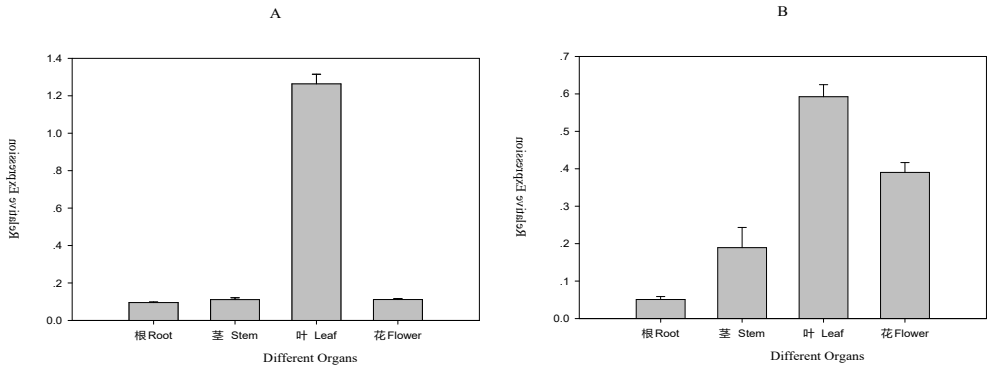


**图 6 *AFS* 在不同器官中的表达**

**Fig.6 Expression of *AFS* in different organs**

A B

A: Chloroplast over-expression plants; B: Mitochondria over-expression plants

# 3 讨 论

FPP C15 [10]

*FaNES1* *FaNES1*

FPP [11,12]

[4] *Rubisco* 35S 7~8 [13]

*Rubisco* 35S

[14] *Ru*

, PCR

*α*- *α*-

[15,16]

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