

Cloning and Ectopic Expression of *ScYCF*1 Gene from *Saccharomyces cerevisiae* in Cotton

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Abstract

Yeast cadmium factor 1 (YCF1), is a member of the ATP-binding cassette (ABC) transporter family. To explore the functions of YCF1 of Saccharomyces cerevisiae (ScYCF1) in the cotton, ScYCF1 was cloned from Saccharomyces cerevisiae As2.375, with the full-length of 4548 bp. The bioinformatics analysis revealed that the largest component of ScYCF1 protein is leucine (12%). ScYCF1 is alkaline and positive charged, stable, and hydrophilic protein. The predictive secondary structure is mainly composed of *a*-helix areas, random coils and β -sheets. We constructed the pBI121-ScYCF1:GFP infusion expression vector and verified it by enzyme ingestion. The transient expression results of cotton pollen showed that the green fluorescence phenomenon of three kinds of upland cotton pollen significantly increased after transforming ScYCF1. The salt sensitive material upland cotton CCRI12 was transformed in vivo simultaneously, and the germination ability of trans-ScYCF1-gene T₀ seeds was much better than the acceptor material CCRI12 under the stress of 100 mM NaCl saline solution. According to the gene nucleotide sequences, four pairs of primers were designed for molecular detection of T₀ generation, and the sequencing results of PCR products of four specific primers evidence that the transgene is successful. Salt tolerance analysis of leaf discs of identified transgenic cotton showed that the chlorophyll content of leaf discs of transgenic cotton was higher than the content of the control cotton under salt stress. ScYCF1 gene was cloned and introduced into cotton, showing that ScYCF1 plays an important role in improving the salt tolerance of cotton.

Keywords

Saccharomyces cerevisiae, *ScYCF*1, Upland Cotton, Pollen Instantaneous Expression, Molecular Detection

^{*}These authors contributed equally to this work.

1. Introduction

Soil salinization is a phenomenon that the water-soluble salts in soil and groundwater move on with capillary water to surface soil, and the water of the top soil evaporates resulting in accumulation of salts in the soil solum or regolith [1]. Salinization affects about 1/5 of the world's arable land and causes loss of 10 million hectares per year [2] [3] [4].

Numerous studies showed that related salt tolerant genes of halotolerant fungi can increase the salt tolerance of fungal microorganisms and plants [5] [6] [7]. *SOD*2 of yeast was transferred into rice, and the salt tolerance of transgenic plants was improved [8]. *Chaetomium thermophilic SOD* was cloned and transferred into tobacco, and found that it cannot only improve the salt tolerance of transgenic plants but also has certain resistant activity against tobacco brown spot disease and anthracnose [9]. Salt tolerant gene *RPL*44 isolated from *Aspergillus glaucus* and transferred into arabidopsis and tobacco, and showed that tolerance of transgenic plants to salinization was significantly higher than the control wild-types [10].

In 1996, genome sequencing of *Saccharomyces cerevisiae* was completed [11]. Nelson et al. [4] showed that there are more than 200 genes in the genome of Saccharomyces cerevisiae that are related to salt tolerance. It was found in the genome sequencing results that there are 31 ABC genes in the genome; phylogenetic analysis showed that the yeast ABC proteins can be divided into six different subfamilies, which are named MDR, PDR, MRP/CFTR, ALDp, YEF3 and RLI [12]. ScYCF1 is the member of the most studied MRP subfamily. Based on the correlation between YCF1 protein and liquid bubble detoxification, the salt tolerance experiment was conducted in arabidopsis under high salt conditions. Three weeks old arabidopsis wild type and transgenic seedlings were treated with 75,100,150 and 200 mM NaCl for 6 h and results showed that after being treated with 200 mM NaCl the accumulation amount of Na⁺ in Arabidopsis was 26.5% higher than that in wild type, but under stress of other salt concentrations there was no significant difference between them, indicating that Arabidopsis plants with transformed ScYCF1 gene has stronger stress tolerance to NaCl than the wild type, it is because of the transgenic plants can accumulate more Na⁺ in vacuoles [13].

Cotton is one of the ideal crops for development and utilization of saline land, and improving salinity tolerance of cotton through genetic engineering and transgenic technology become an urgent demand and important direction for current cotton breeding. This study took *Saccharomyces cerevisiae* As2.375 as the materials, cloned *ScYCF*1 gene and constructed expression vector, used a gene gun *in vivo* conversion technology to transform upland cotton (*Gossypium hirsutum* L.) CCRI12, aiming to explore whether the fungi salt-tolerant gene can successfully play its function in cotton and provide a theoretical basis for obtaining new salt tolerant cotton varieties.

2. Materials and Methods

2.1. Experiment Material

Saccharomyces cerevisiae As2.375 is purchased from Shanghai Industrial Institute of Microbiology. The pMD18-T Vector is purchased from TaKaRa. E. coli strain DH5*a*, expression vector pBI121:GFP are preserved by the research group. CCRI12, GK50, Y2067 and GZ-2 were conserved in the stress-resistance identification research group of Institute of Cotton Research of CAAS.

2.2. Cloning of Targeted Gene and Sequential Analysis

The RNA of *Saccharomyces cerevisiae* AS2.375 was extracted with the Ultra Clean Microbial RNA Isolation Kit (MO BIO) kit, whose concentration and purity was assessed by Nanodrop 2000 ultramicrospectrophotometer. The RNA was reversely transcribed into cDNA with ReverTra Ace qPCR RT Master Mix with gDNA Remover kit. According to the obtained cDNA as the template, the full-length gene primer was designed using the Primer Premier 5 software, 5'-end: *ScYCF*1-F and 3'-end: *ScYCF*1-R (**Table 1**). The full-length sequence of mRNA was amplified using $2 \times \text{TransTaq-T}$ PCR SuperMix. The amplification procedure was 94° C 5 min, 94° C 45 s, 55° C 45 s, 72° C 1 min, 40 cycles; 72° C 10 min. The connected pMD-18T vector was transformed into competent Escherichia coli cells DH5*a*, and the positive clone was picked for sequencing verification. The physicochemical property of *ScYCF*1 protein was analyzed using the online-software ProtParam (<u>http://web.expasy.org/protparam/</u>), and the hydrophilic-hydrophobic property of protein was predicted by ProtScale

Table	1. Primers	used in	the ex	periments.
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Primers name	Sequence (5 '- 3)
ScYCF1-F	ATGGCTGGTAATCTTGTTT
ScYCF1-R	TTAATTTTCATTGACCAAACCA
In <i>ScYCF</i> 1-F	CACGGGGGACTCTAGAATGGCTGGTAATCTTGTTT
In <i>ScYCF</i> 1-R	ACTCATACTAGTCCCGGGATTTTCATTGACCAAAC
ScYCF1-F1	ACGGTGTGATCCTAAATCTATCAG
ScYCF1-R1	AAGTTCTGCATATAGCCCATGA
ScYCF1-F2	GCTATGTTTCTGGTGGGCTT
ScYCF1-R2	CTTTTCCCTATAAGGCTTCTCCCA
ScYCF1-F3	GGCATAGATACGACGCGGAA
ScYCF1-R3	GCAGTTGTTCCAGCTCTCCT
ScYCF1-F4	CATCAAGGGAGTTGCGTCGT
ScYCF1-R4	GTGGTCTGTGGCCTTCAACT

(<u>http://web.expasy.org/protscale/</u>). The secondary structure of protein was analyzed with SOPMA

(http://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/MPSA/npsa_sopma.h tml).

2.3. Construct Fluorescence Expression Vector pBI121-ScYCF1:GFP

The In-Fusion primer was designed on line

(http://bioinfo.clontech.com/infusion) 5'-end In*ScYCF*1-F and 3'-end In*ScYCF*1-R (**Table 1**). The target sequence was obtained through amplification of the *ScYCF*1 plasmid as the template. The double cleavage sites Xba I and Sma I were chosen, and double enzyme digestion was performed on the expression vector pBI121:GFP. The fusion expression vector pBI121-*ScYCF*1:GFP was constructed with In-Fusion method, followed by recombinant clone verification and sequencing.

2.4. Transient Expression Analysis of *ScYCF*1 in the Pollen from Upland Cotton

The pollen from upland cotton Y-2067, ZA-23 and GZ-3 were bombarded with portable particle gun GDS-80, and the gene expression was observed under laser scanning confocal microscope FV1000 (Olympus, Japan).

2.5. In vivo Transformation of Cotton with Gene Gun

The powders and plasmids were treated as the way of Kong Jingjing [14] and the particle gun transformation *in vivo* was completed.

2.6. Molecular Detection of Transgenic Cotton

Four pairs of primers were designed for *ScYCF*1 gene sequence, 5'-end primers were *ScYCF*1-F1, *ScYCF*1-F2, *ScYCF*1-F3, *ScYCF*1-F4 and the 3'-end primers were *ScYCF*1-R1, *ScYCF*1-R2, *ScYCF*1-R3, *ScYCF*1-R4 (**Table 1**). DNA from transgenic plants was extracted with CTAB method for PCR amplification, and the program is 94°C, 5 min; 94°C 45 s, 58°C 45 s, 72°C 30 s, 40 cycle; 72°C 10 min.

2.7. Analysis of Salt Tolerance of Trans-ScYCF1 Cotton

The germination experiment was performed on obtained T_0 seeds with the double walled filter paper method [14], using 100 mM NaCl solution as treatment solution and water as control. Each group repeated for three times. The seeds were sent into the illumination incubator for incubation (condition of culture: 28°C, illumination for 14 h; 25°C, darkness for 10 h). The germination results were summarized after 7 d. Measured and compared the content of chlorophyll of the leaf discs, which obtained from the same position of 6 transgenic cotton and acceptor material CCRI12, were treated with 0, 400 and 600 mM

NaCl and cultured at 28°C under illumination conditions for 72 h to analyze salt tolerance of trans-*ScYCF*1 cotton.

3. Results

3.1. Cloning of *ScYCF*1 Gene and Construction of Expression Vector

The specific primer was designed according to the full-length sequence of *Saccharomyces cerevisiae ScYCF*1 gene retrieved from NCBI database (GenBank: NM_001180442.3). Gene amplification was performed using cDNA as template to obtain a nucleotide sequence with a length of 4584 bp (Figure 1), which was connected to vector for sequencing and turned out correct. *ScYCF*1 gene was inserted into a plant expression vector pBI121:GFP to construct green fluorescent fusion expression vector. Digestion by restriction endonuclease Bgl II was verified to be properly inserted (Figure 2), indicating that the expression vector was successfully constructed and was named as pBI121-*ScYCF*1:GFP.

3.2. Bioinformatics Analysis

The results of bioinformatics analysis indicated that ScYCF1 gene encodes a

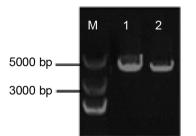


Figure 1. Gene amplification PCR product. M: DNA Marker DL5000; 1- 2: *ScYCF*1 gene.

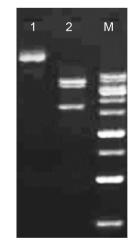


Figure 2. Enzyme digestion of expression vector pBI121-*ScYCF*1:GFP. M: 1 KB ladder; 1: vector plasmid; 2: digested with Bgl II.

protein ScYCF1 with 1515 amino acid, the molecular formula of ScYCF1 protein was C7777H12253N2031O2199S54, with the molecular weight of 120.34 kDa and the isoelectronic point of 8.64, and located on the cell membrane. Amino acid composition showed that ScYCF1 protein leucine (Leu) takes up the largest proportion, 12%; which is followed by valine (Ile) and serine (Ser) taking up 8% each (Figure 3). With 156 positively charged alkaline amino acids (Arg + Lys) and 143 negatively charged acidic amino acid (Asp + Glu), the protein is alkaline and positively charged, the instability index is 36.15, with a half-life over 20 h, which belonged stable protein. The hydrophilicity and hydrophobicity analysis of ScYCF1 protein revealed that the quantity of hydrophobic amino acids in the protein much larger than that of hydrophilic amino acids (Figure 4), of which the GRAVY was 0.064 namely the whole protein was hydrophobic. The secondary structure prediction result (Figure 5) showed that in the protein there were 700 amino acids in α helical region, which constituted the main structure of the protein; there were 309 β -pleated sheets, accounting for 20.4%, 375 randomly coiled amino acids, accounting for 24.75%, and β -turn contains 131 amino acids, accounting for 8.65%. Thus, we could speculate that the structural and functional domain might be composed of β -pleated sheets and randomly coils.

3.3. Transient Expression Analysis of Cotton Pollen

Cotton pollen has auto-fluorescence phenomenon. Under excitation light at a wavelength of 488 nm, the auto-fluorescence of cotton pollen is green [14]. According to previous findings, three varieties Y2067, ZA-23 and GZ-2 with weak auto-fluorescence [14] were selected to collect pollen, and gene gun in vivo conversion technology was used to bombard the cotton pollen. The study found that

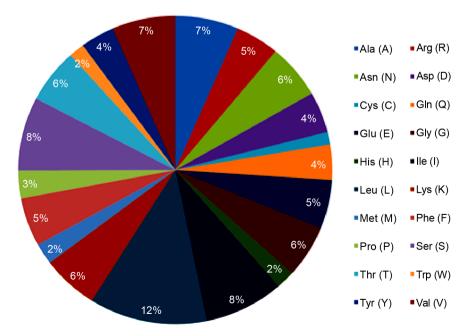


Figure 3. Amino acid composition of ScYCF1 protein.

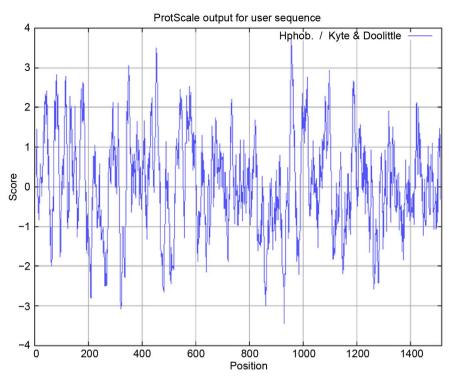


Figure 4. Hydrophilic and hydrophobic characteristics of ScYCF1 protein.

after the vector pBI121-*ScYCF*1:GFP was bombarded, the green fluorescence of pollens of three materials was significantly enhanced (**Figure 6**), indicating that *ScYCF*1 can be expressed in cotton pollen, which lay the foundation of using gene gun *in vivo* conversion technology to obtain transgenic salt-tolerant materials in the next step.

3.4. Salt Tolerance Analysis of T_0 Generation of Transgenic Cotton Seeds

Choosing 20 grains of plump and uniform transgenic *ScYCF*I seeds and CCRI12 inbreds was used for salt tolerance screening experiments. The seeds treated with 100 mM NaCl solution were taken as the experimental group, and those treated with water as the control group. The result (**Figure 7**) shown that under stress from 100 mM NaCl solution, the germination capacity of transgenic seeds was significantly higher than the control seeds, and the growth capacity of the root was significantly stronger than the control group. The root length of CCRI12 inbreds and transgenic seeds in water and under stress from 100 mM NaCl was measured respectively and it was found that under two kinds of treatment, the root length of transgenic seeds was longer than that of CCRI12 inbreds, indicating that the expression of salt tolerant gene *ScYCF*I can not only enhance salt tolerance of cotton seeds, but also can enhance the growing ability of them.

3.5. Molecular Detection of Transgenic Seeds

Four pairs of primers were designed based on mRNA of ScYCF1 gene for

10 I	20 	30 	40 	50 	60 I	70
MAGNLVSWACKLCRS		-				
IIVSRMALVLLEIAF	VSLASLNISK	EEAENFTIV	SQYASTMLSL	FVALALHWIEY	DRSVVANTVLI	LFYWL
FETFGNFAKLINILI	RHTYEGIWYS	GQTGFILTL	FQVITCASIL	LLEALPKKPLM	PHQHIHQTLTI	RRKPN
hhhhhhhhhhhhhhhh PYDSANIFSRITFSW						
CCCCChhhhhhhhhh RTFGSKMLLAAFFKA						
hhht chhhhhhhhhh	hhhhhhecct	hhhhhhhh	hhhccttccc	chhhhhhccc	cccccceehh	hhhhh
AMFLVGFTQTSVLHQ hheeetcccthhhhh	heeeecttc	chhhhhhhh	hhttheeet	ccttccctthh	eeeehhhhhh	hhhhh
QWLNLIWSGPFQIII hhhhhhcccccceee						
LNNIKSLKLYAWEKP						
TDLVFPALTLFNLLS	FPLMIIPMVL	NSFIEASVS	IGRLFTFFTN	EELQPDSVQRL	PKVKNIGDVA	INIGD
hheecchhhhhhhhc DATFLWQRKPEYKVA						
cheeeetcccceee WIMNGTVKENILFGH						
eeettccccheeecc ADTYLLDDPLAAVDE						
ccheeeccchhhhhh	hhhhhhhh <mark>e</mark>	ccttceecc	cheeeehcccl	hhhhhhhhee	etttcccccc	ehh h
TKDADSPLWKLLNNY hhcccchhhhhhhht						
TLGSIDFGDDENIAK	-					
RYGSNPNAARYLAIY	FALGIGSALA	TLIQTIVLW	VFCTIHASKY	LHNLMINSVLR	APMTFFETTP:	IGRIL
NRFSNDIYKVDALLG	RTFSQFFVNA	VKVTFTITV	ICATTWQFIF	IIIPLSVFYIY	YQQYYLRTSRI	ELRRL
hhhcccheehhhhht DSITRSPIYSHFQET						
hhccccccehhhhhh AATLSVFRLKQGTLT						
hhheeeeetttccc RPPKEWPSQGDIKFN	tthhhhhhhh	hhhhhhhh	hhheeeett	cceeehhhhhh	hhhhcttcce	eeecc
cccccccccccceec	cccccctth	heeeeeee	eccttceeee	eecccccchhh	hhhhhhhh	ttcce
VIDNIAINEIGLYDL eeeeeecteeeeh						
AQLTEGGGNLSVGQR heehtttcceeecch						
IMDSDRIIVLDNGKV	AEFDSPGQLL	SDNKSLFYS	LCMEAGLVNE	N		
				~		

Sequence length: 1515

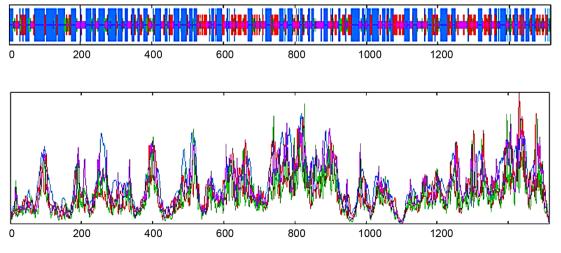


Figure 5. Prediction of secondary structure of *ScYCF*1 protein.

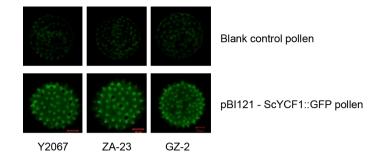


Figure 6. The transient expression analysis of cotton pollen.

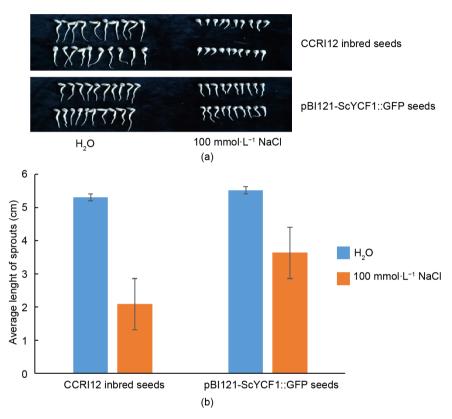


Figure 7. The test of transgenic cotton with (a) seed germination and (b) buds long statistics.

molecular detection of transgenic *ScYCF*1 plants. The length of amplified fragments of primers was 747 bp, 477 bp, 525 bp and 450 bp. The detection results were shown in **Figure 8**, expected band can be amplified from the transgenic plants and plasmid, while in the negative control CCRI12 inbreds there were no band amplified. Combining the sequencing results of PCR products (**Figure 9**), it was considered that the cotton was successfully transformed with *ScYCF*1 gene.

3.6. Salt Tolerance Analysis of Leaf Discs

Salt tolerance experiments of leaf discs were conducted for six transgenic plants that were detected with correct molecular. After being treated with stress from

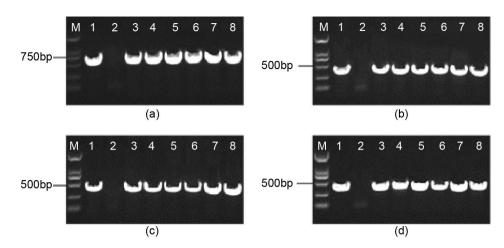


Figure 8. The transgenic plants molecular detection of *ScYCF*1 with (a) *ScYCF*1-F1 and *ScYCF*1-R1; (b) *ScYCF*1-F2 and *ScYCF*1-R2; (c) *ScYCF*1-F3 and *ScYCF*1-R3; (d) *ScYCF*1-F4 and *ScYCF*1-R4. M: DNA Marker DL2000; 1: Vector plasmid; 2: Receptor species of CCRI12; 3 - 8: Transgenic plant.

NM 001180442.3	ACGGIGIGAICCIAAA <mark>ICIAICAACAACHICAAGAAAAAAACACTICGGAACAAGAAAAAAACACTITIGCAAGAAAAAAACACTICIGGCAICAAATAIAG</mark>	200
1-1.txt		32
1-2.txt		82
1-3.TXT	TCTATCAGCAATITTCATGATAACCTTCGGTATCAGAGATITAGTTAACCTTTGCAAGAAAAAAACACTCTGGCATCAAATATAG	84
1-4.txt	AGCAATITICATGATAACCITCGGTAICAGAGATITAGITAACCTI	78
1-5.TXT		84
Consensus	tgcaagaaaaacactcjggcatcaaatatag	
NM 001180442.3	GCGGAATTGGATTATTGTCTCTAGGATGGCACTAGTTCTGTTGGAGATAGCGTTTGTTT	300
1-1.txt	GCGGAATTGGATTATTGTCTCTAGGATGGCACTAGTTCTGTTCGAGATAGCGTTGTTTCACTTGCGTCTTTAAATATTTCTAAAGAAGAAGCAGCGGAAAAC	132
1-2.txt	GCGGAAIIGGAIIATGICITAGGAIGGCACTAGIICIGIIGGAGAIAGCGIIGIICACTIGCGICIIIAAAIIIICIAAAGAAGAAGCAGCGGAAAAC	182
1-3.TXT	GCGGAATTGGATTATTGTCTCTAGGATGGCACTAGTTCTGTTGGAGATAGCGTTTGTTT	184
1-4.txt	GCGGAAIIGGAIIATIGTCTCIAGGAIGGCACIAGTICIGTIGGAGAIAGCGIIIGITTCACTIGCGICIIIAAAIATITCTAAAGAAGAAGAAGCAGGAAAAC	178
1-5.TXT	GCGGAATTGGATTATTGTCTCTAGGATGGCACTAGTTCTGTTGGAGATAGCGTTTGTTT	184
Consensus	gcggaattggattattgtctctaggatggcactagttctgttggagatagcgtttgttt	101
NM 001180442.3	TTTACCATTGTAAGTCAATATGCTTCTACAATGTTATCTTTATTTGTTGCTTTAGCCTTACACTGGATAGAATACGATAGATCAGTTGTAGCCAATACGG	400
1-1.txt	ITTACCATIGIAAGICAATAIGCTICIACAATGITAICTITAITGITGCTITAGCTIAGCTI	232
1-2.txt	TTTACCATTGTAAGTCAATATGCTTCTACAAGTTATCTTTATTGTGCTTTAGCCTTAGCCTGGATAGAATAGGATAGGTCAGTTGTAGATCAGTTGTAGCCAATACGG	282
1-3.txt	TTTACCATTGTAAGTCAATATGCTTCTACAATGTTACTTTATTGTTGCTTTAGCCTTACACTGGATAGAATACGATAGATCAGTTGTAGATCAGTTGTAACGG	284
1-4.txt	III TACCATIGIAAGICAATAIGCIICIACAAGIITATCIITATIGIGGIITAGCIIAGACIGGATAGATACGATAGACAGIIGGAGACAGIAGAICAGIIGIAGCAATACGG	278
1-5.txt	TTTACCATTGTAAGTCAATATGCTTCTACAATGTTATCTTTATTGTTGCTTTAGCCTTAGCCTTAGATGGATAGAATACGATAGATCAGTTGTAGCCAATAGGG	284
Consensus	tttaccattgtaagtcaatatgcttctacaatgttattttattgttgctttagccttacactggatagaatacgatagatcagttgtagccaatacgatagatcagttgtagccaatacgatagatcagttgtagccaatacgatagcaatagcaatagcaatagcaatacgatagcatagcaatacgatagcaatacgatagcatacgatagcatagcatagcatacgatagcatagcatacgatagcatagcatagcatagcatagcatag	201
NM 001180442.3	TACTITITATICTATIGGCTTTTTGAAACATICGGTAATTTTGCTAAACTAATAAATATTCTAATTAGACACACCTACGAAGGCATTTGGTATTCCGGACA	500
1-1.txt	TACTITIATICTATIGGCTITIGAACATIGGTAATITGGTAAATTACTAATAATTATICTAATTAGACACACCTACGAAGGCATTGGTATCCGGACA	332
1-1.txt	TACITITATICIALINGCITITIGAAAATICGGTAATITIGCTAAATAATAATAATAATAATAATAATAATAATAATAATA	382
1-3.txt	TACTITIATICTATIGGCTITICGAAAATICGGTAATITIGCTAAACTAATAAATITICTAATIGACAACACCTACGAAGGCATIGGCAATGCGACA	384
1-4.txt	TACTITIATICIATIGGCITITIGAAACATICGGTAATITIGCTAAACTAATAATAATATICIAATIAGAACAACCACCTACGAAGGCATIIGGTATICCGGACA	378
1-5.txt	TACTITITATICTATTGGCTITITGAAACATTCGGTAATTTGCTAAACTAATAAATATTCTAATTAGACACACCTACGAAGGCATTTGGTATTCCGGACA	384
Consensus	tactttattcattggctttttgaaacattcggtaattttgctaaactaataaata	
NM_001180442.3	AACGGGTTTCCATACTAACGTTATTCCAAGTAATAACATGTGCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA	600
1-1.txt	AACGGGTTTCATACCATAACGTTATTCCAAGTAATAACATGTGCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA	432
1-2.txt	AACGGGTTTCATACTAACGTTATTCCAAGTAATAACATGTGCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA	482
1-3.txt	AACGGGTTTCCATACCTAACGTTATTCCAAGTAATAACATGTGCCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA	484
1-4.txt	AACGGGTTTCATACTAACGTTATTCCAAGTAATAACATGTGCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA	478
1-5.txt	AACGGGTTTCCATACTAACGTTATTCCAAGTAATAACATGTGCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA	484
Consensus	a a cgggttt catacta a cgtt atteca a gta a ta a catgt g c cagta t c c t g t a c t t c c a a g a a g c c g c t a t g c c a c a c a c a c a c a c a c a c a	
NM_001180442.3	CATCAAACTITAACAAGAAGAAAACCAAAATCCATACGATAGCGCAAACATATTITCCAGGATTACCITCTCTGGATGICAGGTITGATGAAAACTGGCT	700
1-1.txt	CATCAAACTTTAACAAGAAGAAAAACCAAAATCCATACGATAGCGCAAACATATTTTCCAGGATTACCTTCTCTGGATGTCAGGTTTGATGAAAACTGGCT	532
1-2.txt	CATCAAACTTTAACAAGAAGAAAAACCAAAATCCATACGATAGCGCAAACATATTTTCCAGGATTACCTTCTCTGGATGTCAGGTTTGATGAAAACTGGCT	582
1-3.txt	CATCAAACTTTAACAAGAAGAAAACCAAATCCATACGATAGCGCAAACATATTTTCCAGGATTACCTTCTTGGATGTCAGGTTTGATGAAAACTGGCT	584
1-4.txt	CATCAAACTTTAACAAGAAGAAAACCAAATCCATACGATAGCGCAAACATATTTTCCAGGATTACCTTCTTGGATGTCAGGTTTGATGAAAACTGGCT	578
1-5.txt	CATCAAACTTTAACAAGAAGAAAACCAAAATCCATACGATAGCGCAAACATATTTTCCAGGATTACCTTCTTTGGATGTCAGGTTTGATGAAAACTGGCT	584
Consensus	catcaaactttaacaagaagaaaaccaaatccatacgatagcgcaaacatattttccaggattaccttctcttggatgtcaggtttgatgaaaactggct	
NM_001180442.3	ATGAAAAATACTTAGTGGAAGCAGATTTATATAAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAA <mark>AATTGGAGA</mark> A <mark>AAACTGGGAAAATGAGTT</mark>	800
1-1.txt	ATGAAAAATACTTAGTGGAAGCAGATTTATATAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAA	604
1-2.txt	ATGAAAAATACTTAGTGGAAGCAGATTTATATAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAA <mark>AATTGGAGA</mark> AAAC <mark>TGGGAAAATGAGTT</mark>	682
1-3.txt	ATGAAAAATACTTAGT6GAAGCAGATTTATATAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAA <mark>AATTGGAGAAAAACTGGGAAAAATGAGTT</mark>	684
1-4.txt	ATGAAAAATACTTAGTGGAAGCAGATTTATATAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAA <mark>AATTGGAGA</mark> G <mark>AAACTGGGAAAATGAGTT</mark>	678
1-5.txt	ATGAAAAATACTTAGTGGAAGCAGATTTATATAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAA <mark>AATTGGAGAAAAACTGGGAAAATGAGTT</mark>	684
Consensus	atgaaaaatacttagtggaagcagatttatataaattaccgaggaactttagtagtgaagaactctctcaaa	

NM_001180442.3	ATAACCACCCACAAAAATTACCCATTGTAAGAGGGTITTIGATIGCGTITGCTATGTITCIGG <mark>IGGGCTTTACTCAGACATCTGTCCTGCATCAATATTT</mark>	1100
2-1.txt 2-2.txt	GGGCTTTACTCAGACATCTGTCCTGCATCAATATTT TGGGCTTTACTCAGACATCTGTCCTGCATCAATATTT	36 37
2-3.txt	GIGGGCTITACICAGACATCIGICCGCAACAATATIT	38
2-4.txt	GGGCTTTACTCAGACATCTGCCTGCATCAATATTT	36
2-5.txt Consensus	GGGTTTACTCAGACATCTGTCCTGCATCAATATTT gggctttactcagacatctgtcctgcatcaatattt	36
consensus	gggettacteggeatetgetetgeateaatatt	
NM_001180442.3	CCIGANIGTCICANCACAGGCAIGIAIATIANGAGCGCCCIAACGGCIIIAAIAIAICAAAAAATCCIIAGIGCIAICIAAIGAGGCIICIGGACIIICC	1200
2-1.txt 2-2.txt	CCTGAATGTCTTCAACACAGGCATGTATATTAAGAGCGCCCCTAACGGCTTTAATATATCAAAAATCCTTAGTGCTATCTAATGAGGCTTCTGGACTTTCC CCTGAATGTCTTCAACACAGGCATGTATATTAAGAGCGCCCCTAACGGCTTTAATATATCAAAAATCCTTAGTGCTATCTAATGAGGCTTCTGGACTTTCC	136 137
2-3.txt	${\tt cctgaatgtcttcaacacaggcatgtatattaagagcgccctaacggctttaatatcaaaaatccttagtgctatctaatgaggcttctggactttcc$	138
2-4.txt	CCTGANTGTCTTCAACACAGGCATGTATATTAAGAGCGCCCTAACGGCTTTAATATATCAAAAAATCCTTAGTGCTATCTAATGAGGCTTGGACTTIGGACTTTCC	136
2-5.txt Consensus	CCTGANIGTCTTCAACACAGGCATGTATATTAAGAGCGCCCCTAACGGCTTTAATATATAT	136
NM_001180442.3 2-1.txt	TCTACCGGTGACATTGTCAATCTCATGAGTGTGGATGTTCAAAAATTACAAGATTTAACAACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAATCA TCTACCGGTGACATTGTCAATCTCATGAGTGTGGATGTTCAAAAATTACAAGATTTAACAACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAATCA	1300 236
2-2.txt	TCTACCGGTGACATTGTCAATCTCATGAGTGTGGATGTTCAAAAATTACAAGATTTAACACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAAATCA	237
2-3.txt 2-4.txt	TCTACCGGTGACATTGTCAATCTCATGAGTGTGGATGTTCAAAAATTACAAGATTTAACAACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAATCA TCTACCGGTGACATTGTCAATCTCATGAGTGTGGATGTTCAAAAATTACAAGATTTAACAACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAATCA	238 236
2-5.txt	ICTACC6GTGACATTGTCAATCTCATGAGTGTGGGATGTICAAAAATTACAAGATTTAACACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAATCA	236
Consensus	tctaccggtgacattgtcaatctcatgagtgtggatgttcaaaaattacaagatttaacacaatggctaaatttaatatggtcagggccttttcaaatca	
NM_001180442.3	TTATTIGCTTATATTCTCTGTATAAGTTGTIGGGAAATTCCATGTGGGGTTGGCGTGATTATACTAGTTATTATGATGCCATTGAACTCATTTTTGATGAG	1400
2-1.txt 2-2.txt	ITATITGCTTATATICTCTGTATAAGTTGTTGGGAAATTCCATGTGGGTTGGCGTGATTATACTAGTTATTATGATGCCATTGAACTCATTTTTGATGAG TTATTTGCTTATATTCTCTGTATAAGTTGTTGGGAAATTCCATGTGGGGTTGGCGTGATTATACTAGTTATTATGATGCCATTGAACTCATTTTTGATGAG	336 337
2-3.txt	TTATI TGCTTATATI TCTCTGTATAASTIGTTGGGAAATTCCATGTGGGTTGGGGTGGGTTATACTAGTTATTATGATGCCATTGAACTCATTTTGATGAG	338
2-4.txt	TTATTTGCTTATATTCTCTGTATAAGTIGTTGGGAAAATTCCATGTGGGTTGGCGTGATTATACTAGTTATTATGATGCCATTGAACTCATTTTTGATGAG	336
2-5.txt Consensus	ITATTIGCTTATATICICIGTATAAGTIGTIGGGAAATICCAIGIGGGTIGGCGIGATTATACTAGTIATTATGATGCCATIGAACICATTITIGATGAG ttatttgcttatattctctgtataagttgttgggaaattccatgtgggttggcgtgattatactagttattatgatgccattgaactcatttttgatgag	336
NM_001180442.3 2-1.txt	GATACAAAAGAAGTTGCAAAAATCCCAGATGAAGTACAAAGATGAAAGGACCCGTGTTATAAGTGAAATACTAAACAATATTAAATCTTTGAAGTTATAT GATACAAAAGGAGTTGCAAAAATCCCAGATGAAGTACAAAGATGAAAGGACCCGTGTTATAAGTGAAATACTAAACAATATTAAATCTTTGAAGTTATAT	1500 436
2-2.txt	датасалаладалдттеслалалатсссадателадтасаладателалделссебтеттаталдетелалатасталасалтатталатстттеладеттатат	437
2-3.txt 2-4.txt	GATACAAAAGAAGTTGCAAAAATCCCAGATGAAGTACAAAGATGAAAGGACCCGTGTTATAAGTGAAATACTAAACAATATTAAATCTTTGAAGTTATAT GATACAAAAGGAGTTGCAAAAAATCCCAGATGAAGTACAAAGATGAAAGGACCCGTGTTATAAGTGAAATACTAAACAATATTAAATCTTTGAAGTTATAT	438 436
2-5.txt	GA IACARAMGANG I ICCARAMANA ICCACATGANGI ICAMAGA IGMANGGACCGG IGI IATANG IGMANG IATANI ATANI ATANI ITI IAGA ITA GATACARAMGANGTI CCARAMATCCCACATGANGTACAAGATGANAGGACCCGG IGTI ATANG IGANATACTANACAATATTI AATI ITI AAGTI ATATI	436
Consensus	gatacaaaagaagttgcaaaaatcccagatgaagtacaaagatgaaaggacccgtgttataagtgaaatactaaacaatattaaatctttgaagttatat	
NM 001180442.3	CONTIGERIGATE ATTAGEGERARAGETAGARGARGTAGARGARGTARGARATARCARAGEGTTARARARATETTACARARCTAGGATGTTATAGEGTGTGACARGTT	1600
2-1.txt	GCATGGGAGAAG <mark>C</mark> .	449
2-2.txt 2-3.txt	GCATGGGAGAAGCCTT. GCATGGGAGAAGCCT.	453 453
2-4.txt	GCATEGGAGAAG	448
2-5.txt Consensus	GCATGGCAGAAG <mark>G</mark> gCatgggagaag	449
consensus	(b)	
NM_001180442.3 3-1.txt	TGGATAATGAATGGTACIGTAAAGGAAAACAIIITAITIGGGCATAGATACGA <mark>CGCG</mark> GAATTITACGAAAAAACGATCAAGGCCTGTGCGTTAACTAITG GCGGAATTITACGAAAAAACGATCAAGGCCTGCGCTTAACTAITG	2200 46
NM_001180442.3 3-1.txt 3-2.txt	GCCGAAAAAAACGATCAAGGCCTGCGCTTAACTATTG GA <mark>CGCC</mark> GAAAAAAACGATCAAGGCCTGTGCGTTAACTATTG	46 49
3-1.txt 3-2.txt 3-3.txt	GCCGAAAAAAACGATCAAGGCCTGTGCGTTAACTATTG GACGCCGAAAAAAACGATCAAGGCCTGTGCGTTAACTATTG CGCCGCGAAAAAAACGATCAAGGCCTGTGCGTTAACTATTG CGCCGCAAAAAAACGATCAAGGCCTGTGCGTTAACTATTG	46 49 47
3-1.txt 3-2.txt	GCCGAAAAAAACGATCAAGGCCTGCGCTTAACTATTG GA <mark>CGCC</mark> GAAAAAAACGATCAAGGCCTGTGCGTTAACTATTG	46 49
3-1.txt 3-2.txt 3-3.txt 3-4.txt	GCCGAAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTAITG CAACGC GAATTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG CGCCGAATTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG GAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG	46 49 47 44
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt	GCCCAAAAAAACGATCAAGGCCTGTGCGTTAACTAATG CACGCGGAAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG CGCCGAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG CGCGAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG GAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG	46 49 47 44
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM 001180442.3 3-1.txt	ATCTIGCAATTITGATGGATGGAGATAAGACATTAGTIGGCGAGAAAGGGGATCCCTTATCGGACAAAGACCGTTAGCATTAGCGATGAAGGCCTGTGCGTTAACTATTG	46 49 47 44 43 2300 146
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM_001180442.3	ATCTIGCAATTTIGATGGATGGAGATAAGACATTAGTIGGCCAGAAAAGGGCTCGTTGCCTTAGCAGAAAAGCCTCGTGCGTTAACTATIG	46 49 47 44 43 2300
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM 001180442.3 3-1.txt 3-2.txt 3-3.txt 3-4.txt	ATCTISCAATTITGATGGATGGAGATAAGACATTAGTIGGCGAGAAAGGGATCCCTTATCTGGAGGACAAAAAGCTCGTTGGCTTAAGCATG	46 49 47 44 43 2300 146 149 147 144
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM 001180442.3 3-1.txt 3-2.txt 3-3.txt	AICTIGCAATTITGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCCCTTATCTGGAGGACAAAAAGCTCGTTGTCTTTAGCAAGACATTAGTAGGCAGGACAAAGCACTAAGACATTAGTGGAGGACAAAAGCCCGTTGGCGTTAACTATTG	46 49 47 44 43 2300 146 149 147
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-2.txt 3-2.txt 3-3.txt 3-5.txt Consensus	GCC CANTITTACGANAAACGATCAAGGCCTGTGGGTTAACTATTG GACCG GAATITTACGANAAACGATCAAGGCCTGTGGGTTAACTATTG GCCG GAATITTACGANAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGANAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG ATCTTGCAATTTTGATGGATGGAGGATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTGGCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTGGCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTGGCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGACACGT ATCTTGCAATTTTGATGGATGGAGAAAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGACACGA ATCTTGCAATTTTGATGGATGGAGAATAAGACATTAGTTGGCGAGAAAAAGGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGACACG	46 49 47 43 2300 146 149 147 144 143
3-1.txt 3-2.txt 3-3.txt 3-4.txt 5-5.txt Consensus NM_001180442.3 3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM_001180442.3	ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCCCTTATCTGGAGGACAAAAAGCTCGTTGTCTTTAGCAAGACATTAGTGGCGGAGAAAGGGATCCCTTATCTGGAGGACAAAAAGCTCGTTGTCTTTAGCAAGACATTAGTGGCGAGAAAGGGATCCCTTATCTGGAGGACAAAAAGCTCGTTGTCTTTAGCAAGACATTAGTGGCGAGAAAGGGATCCCTTATCTGGAGGACAAAAAGCTCGTTGTCTTTAGCAAGACCATTAGTGGCGAGAAAGGGATCCCTTATCTGGAGGACAAAAAGCTCGTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTGGCGAGAAAGGGATCCCCTTATCTGGAGGACAAAAAGCTCGTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTGGCGAGAAAGGGATCCCCTTATCTGGAGGACAAAAAGCTCGTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGAATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGAATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGAATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGAAAGGAATTAGTTGGCGAGAAAAGGGATCCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAACAGT	46 49 47 44 43 2300 146 149 147 144 143 2400
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-2.txt 3-2.txt 3-3.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-1.txt 3-1.txt	GCC CAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GACCG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GCGC GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTTTGGCTTAGGTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGGATAAGACATTAGTTGGCGAGAAAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAACTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGGAAAGGGATCTCCTTATCTGGAGGACAAAAAACTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGGAAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGGAAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGGAGAGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGGGAGGAGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGGATGGAGAAAGGGATCCCTTGGCAGGAGACAAAAAAGCTCGTTTGTCTTAGCAAGAGCAGT ACCUCGACUCUGACUGGAGGAGATAAGACATTAGTTGGCGGGAGGAGGATCCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGAGCGACTGTTGGCAGGAGACGACGAGGACCCCTTAGCTGGACCAAAGGGACCCCTTATCTGGAGGACCACTTGGTCTTACTGGAGGACGACGACGTGGTGATGACACTGGTTGCCAAGGGGCCCAAATGGGTTGGCAGGACGTGGTGGAGGACCCCTTGGCCAGGACCTTGATCGAACATGGTGTGGGGCCAAATGGGCCCAAATGGGTTGGATGACCCTTTGGCAGCCACTTGGCCAGACCTTGGTGGGGCCAAAAGGCCTTTGGCGGCCCAAATGGGTTGGGGTCCAAATGGT TTATGGAAGACGGACCTTATTTACTTGATGATCCTTTGGCAGCCTGTTGGCAGCCACTTGGCCAGACCATGGTGGGGCCCAAATGGGTTGGATGACCCTTTGGCCAGCCA	46 49 47 44 43 2300 146 149 147 144 143 2400 246 249
3-1.txt 3-2.txt 3-3.txt 3-4.txt 2-5.txt Consensus NM_001180442.3 3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-2.txt 3-2.txt 3-2.txt 3-2.txt 3-2.txt 3-2.txt 3-2.txt	- GCC GAATITTACCAAAAAACCATCAAGGCCTGTGCGTTAACTATTG GACCG GAATITTACCAAAAAAACGATCAAGGCCTGTGGGTTAACTATTG CGCC GAATITTACCAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACCAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACCAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTACGAAAAAACGATCAAGGCCTGTGGGTTAACTATTG GAATITTGCGAAAAAACGATCAAGGCCTGTGGCTTAACTATTG GAATITTGCGAAGGATCAAGGCCTGTGGCTTAACTATTG GAATITTGCGAAGGATCAAGGCCTGTGGCGTTAACTATTG GAATITTGCGAAGGACGATCAAGGCCATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGACAGT ATCTTGCAATTTTGATGGATGGAGGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGACAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGACAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGACAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGACAGT ATCTTGCAATTTTGATGGATGGAGGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGACCAGT ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGGAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGCTTTAGCAAGACCAGT ATCTTGCAATTTTGATGGATGAGACATTAGTTGGCGAGAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGATGAGAGAAGACATTAGTTGGCGAGGAAGGGATCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGCTTTAGCAAGAGCAGT ATCTTGCAATTTTGATGGAGGAGAGAAGACATTAGTTGGCGAGGAGGAGGCTCTCCTTATCTGGAGGACAAAAAAGCTCGTTTGCTTTAGCAAGAGCAGT ATCTTGCAAGTTTGGATGGAGGAGGAGGAGCCGTTGGCGAGGAGGACCACTAGGACAGGGACCACTTGGACGACGAGGAGGCCAATTAGTTGGCAGGAGGGAG	46 49 47 44 43 2300 146 149 147 144 143 2400 246 249 247
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-2.txt 3-2.txt 3-3.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-1.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-4.txt 3-5.txt	ITAIGG AGAGCIGACACITATITACITGATGGATCCTITGGCAGCAGCAGGATGTCCTTATCGGAGGACAAAAACCAGGCTGTGGGCCAAAGGG TITAGG AGAGCIGACACITATITACITGATGATCCTITGGCAGCGGTGTGATGAAAGGGATCCCGTGGCAGCACITGGGTGAAGAGGG TATCTGCAATTITGATGGATGGAGATAAGACATTAGTGGCGGGGGGGGGG	46 49 47 44 43 2300 146 149 147 144 143 2400 246 249
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM 001180442.3 3-1.txt 3-2.txt 3-4.txt 3-4.txt Consensus NM 001180442.3 3-1.txt 3-2.txt 3-2.txt 3-3.txt 3-3.txt 3-4.txt	Internet to the second	46 49 47 41 43 2300 146 149 147 144 143 2400 246 249 247 244
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-2.txt 3-2.txt 3-3.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-3.txt 3-5.txt Consensus NM_001180442.3	International and the second s	46 49 47 44 43 23000 146 149 147 144 143 2400 246 249 247 244 243 2500
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM 001180442.3 3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-4.txt 3-4.txt 3-1.txt 3-1.txt 3-2.txt 3-1.txt 3-2.txt 3-1.txt 3-1.txt 3-3.txt 3-1	International and a second sec	46 49 47 41 43 2300 146 149 147 144 143 2400 246 249 247 244 243 2500 346
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-2.txt 3-2.txt 3-3.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-3.txt 3-5.txt Consensus NM_001180442.3	International and the second s	46 49 47 44 43 23000 146 149 147 144 143 2400 246 249 247 244 243 2500
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM 001180442.3 3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-4.txt 3-5.txt Consensus NM 001180442.3 3-1.txt 3-3.txt 3-3.txt 3-5.txt Consensus NM 001180442.3 3-1.txt 3-2.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt 3-3.txt	INTERCONTROLOGIES AND	46 49 47 41 43 2300 146 149 147 144 143 2400 246 249 247 244 243 2400 346 349 347 344
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-2.txt 3-2.txt 3-3.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-3.txt 3-3.txt 3-3.txt Consensus NM_001180442.3 3-1.txt 3-5.txt Consensus NM_001180442.3 3-1.txt 3-3.txt X_1.txt 3-3.txt X_2.txt 3-3.txt X_2.txt 3-3.txt	Internet the second sec	46 49 47 41 43 2300 146 149 147 147 147 147 244 249 247 244 243 2500 346 349 347
3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM 001180442.3 3-1.txt 3-2.txt 3-3.txt 3-4.txt 3-5.txt Consensus NM 001180442.3 3-1.txt 3-2.txt 3-3.txt 3-5.txt Consensus NM 001180442.3 3-1.txt 3-3.txt 3-4.txt 3-4.txt 3-4.txt 3-4.txt 3-5.txt Consensus	Integration and a second a second a second a	46 49 47 41 43 2300 146 149 147 144 143 2400 246 249 247 244 243 2500 346 349 347 344 343
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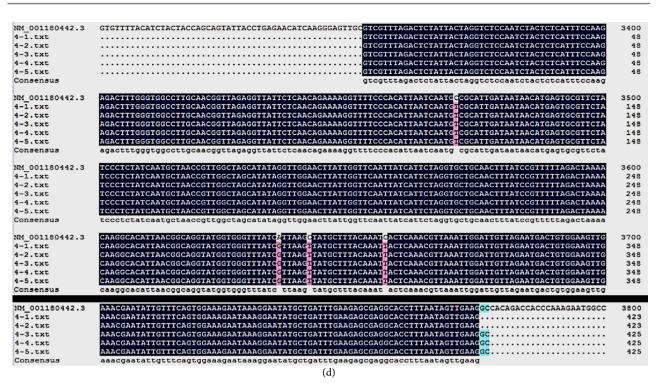


Figure 9. The gene sequencing result of trans-*ScYCF*1: (a) The sequencing results of *ScYCF*1-F1 and *ScYCF*1-R1; (b) The sequencing results of *ScYCF*1-F2 and *ScYCF*1-R2; (c) The sequencing results of *ScYCF*1-F3 and *ScYCF*1-R3; (d) The sequencing results of *ScYCF*1-F4 and *ScYCF*1-R4.

400 and 600 mM NaCl saline for 72 h, (**Figure 10**) the color of the cotton leaf discs gradually turned yellow, and the leaf margin turned white, while the leaf discs under 0 mM NaCl solution remained green; Under the same concentration of saline, the color and shape of leaf discs in *ScYCF*1 transgenic plants were more obvious than the control leaf discs. The chlorophyll content of the leaf discs processed was measured and results showed that under three kinds of treatment, the chlorophyll content of leaf discs in transgenic plants was significantly higher than that in CCRI12; but after treated with 400 mM NaCl, there wasn't much difference in chlorophyll content between the two groups, so it is speculated that *ScYCF*1 gene of *Saccharomyces cerevisiae* can increase the chlorophyll content of cotton and plays a significant role in the tolerance of the cotton to salt stress.

4. Discussion

In recent years, domestic and foreign experts have used transient expression technology to establish corresponding expression systems in wheat (*Triticum monococcum*), corn (*Zea mays*), rice (*Oryza sativa*), petunia (*Petunia hybrid*), periwinkle (*Catharanthus roseus*) and green algae (*Chlorophyta*) and other plants [15] [16] [17] [18] [19]. Kong Jingjing selected 26 cotton varieties to study the auto-fluorescence phenomenon of cotton pollen and results showed that the auto-fluorescence of cotton pollen is green under 288 nm excitation light, and

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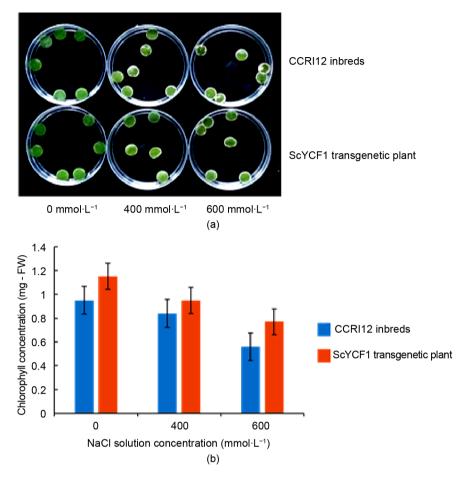


Figure 10. Transgenic cotton semal salt resistance analysis: (a) The color and form of the *ScYCF*1 transgenic plant and CCRI12 in different concentrations of salt-stress in solution after 96 hours; (b) The chlorophyll of semal (six slices).

the auto-fluorescence of pollens of three cotton varieties (Y2067, GZ-2 and ZA-23) is weaker than it of other cotton varieties [14]. This study chose the three cotton varieties with weak pollen auto-fluorescence, bombarded the target gene to the pollen by a gene gun and results showed that the fluorescence of three kinds of cotton pollens was strengthened. If to transform upland cotton with genes of *Saccharomyces cerevisiae*, the distant genetic relationship between the two may lead to that the genes of *Saccharomyces cerevisiae* cannot be expressed in the upland cotton, while in the transient expression assay of cotton pollen, the expression of yeast genes was observed in a short time, which laid a foundation for successful transformation of cotton with yeast genes.

 T_0 cotton seeds were harvested after transforming with a gene gun, but whether the yeast salt-tolerant gene can enter and be integrated into the genome of upland cotton, the size of integrated fragments, and functional performance of the target gene in the transgenic plants all needs to be further explored, therefore, detection of transgenic cotton materials is a critical step. Given that more T_0 generation of seeds ask for arduous testing work and the growing cycle is

quite long, this study started from verification of the gene function, coerced the transgenic seeds with 100 mM NaCl solution, used double-filter paper method to filter its salt tolerance, and combined with PCR method for amplification of target gene sequences, and directly sequenced the purified fragments after amplification, which greatly reduced the time of a series of experiments including molecular detection and function verification of target genes and sped up the progress of the experiment.

Given that ScYCF1 protein is related to vacuole detoxification, salt tolerance experiments were conducted for ScYCF1 transformed arabidopsis plants under high salt conditions. Three weeks old arabidopsis wild type and transgenic seedlings were treated with 75, 100, 150 and 200 mM NaCl for 6 h and results showed that after being treated with 200 mM NaCl the accumulation amount of Na+ in Arabidopsis was 26.5% higher than that in wild type, but under stress of other salt concentrations there was no significant difference between them, indicating that Arabidopsis with transformed ScYCF1 gene has stronger stress tolerance to NaCl than the wild type, which is because that the transgenic plants can accumulate more Na+ in vacuoles [13]. This study conducted salt tolerance germination experiment for transgenic cotton plants with 100 mM NaCl solution and results showed that the bud length of transgenic seeds under salt stress was significantly shorter than that in water of the control group, but was longer than that of non-transgenic seeds under salt stress. Salt tolerance analysis of leaf discs in transgenic plants showed that transforming the cotton with yeast ScYCF1 improves the salt tolerance of leaf discs and plays an important role in improving the salt tolerance under high salt concentrations. The transgenic plants will be further explored in subsequent trials.

5. Conclusion

The *ScYCF*1 gene of *Saccharomyces cerevisiae* were transformed and expressed in cotton and its pollen, which not only enhanced salt tolerance of cotton seeds and the growing ability of them, but also increased the chlorophyll content of cotton and played a significant role in the tolerance of the cotton to salt stress.

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