

Genes regulating gland development in the cotton plant

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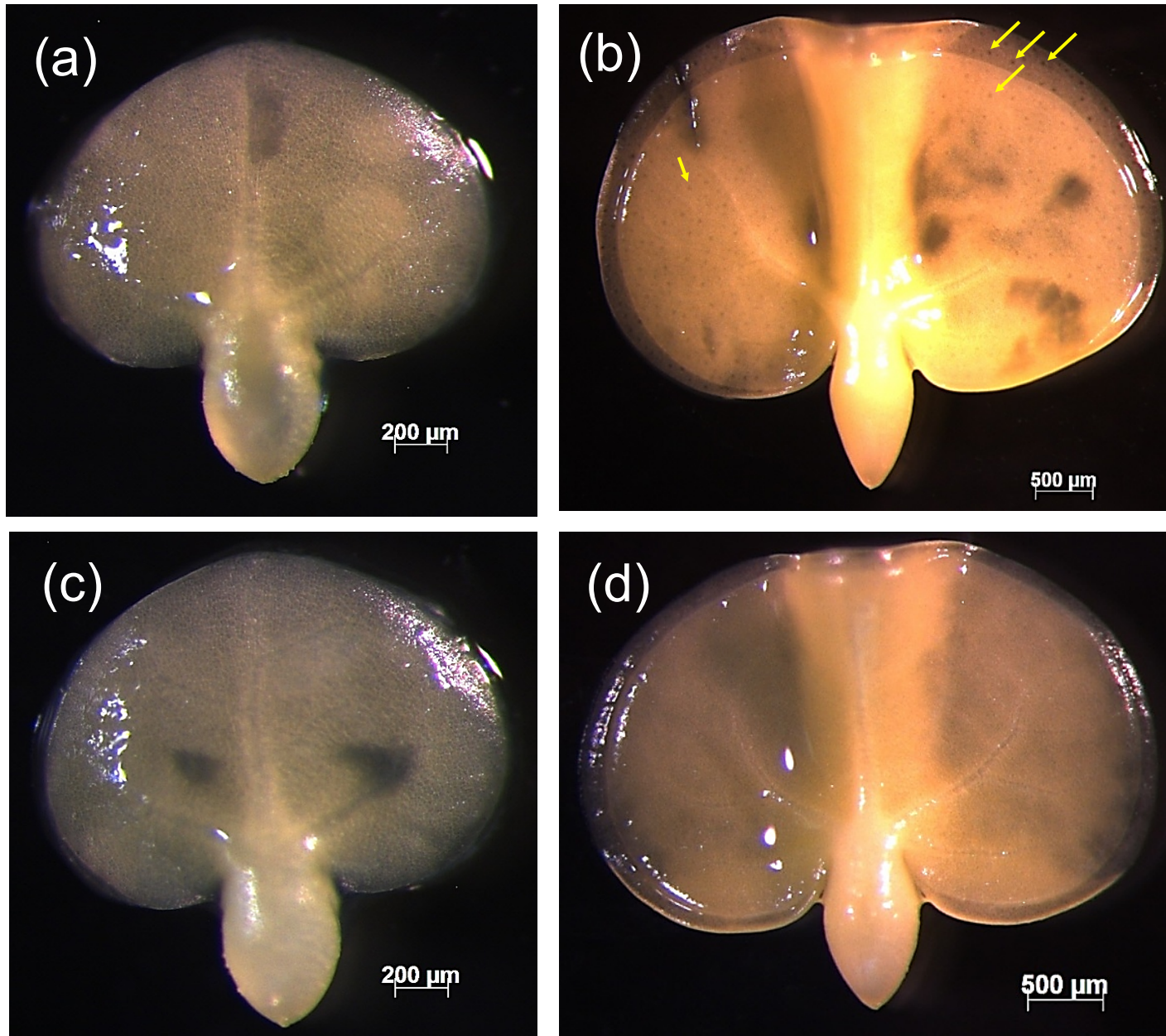


Figure S1 Microscopic images of developing embryos of Stoneville 7A glanded (GL; GVS4) and glandless (gl; GVS5), near-isogenic lines, used for comparative RNA-seq analysis. (a) 14-days post-anthesis (dpa) glanded embryo; (b) 16-dpa glanded embryo, the arrows indicate glands that are visible microscopically; (c) 14-dpa glandless embryo; (d) 16-dpa glandless embryo.

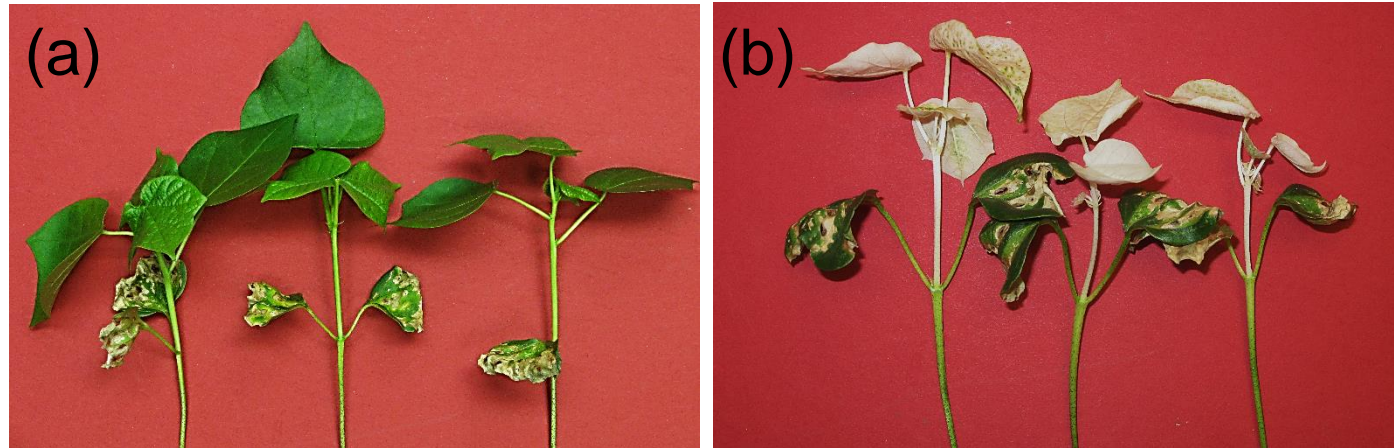


Figure S2 Virus Induced Gene Silencing (VIGS) in cotton. (a) empty vector (RNA1 and RNA2) infiltrated plants. (b) plants subjected to VIGS-mediated silencing of *GhCLA* gene. The leaves showing albino phenotype indicate the efficacy of the VIGS treatment. The photographs were taken four weeks after infiltration.

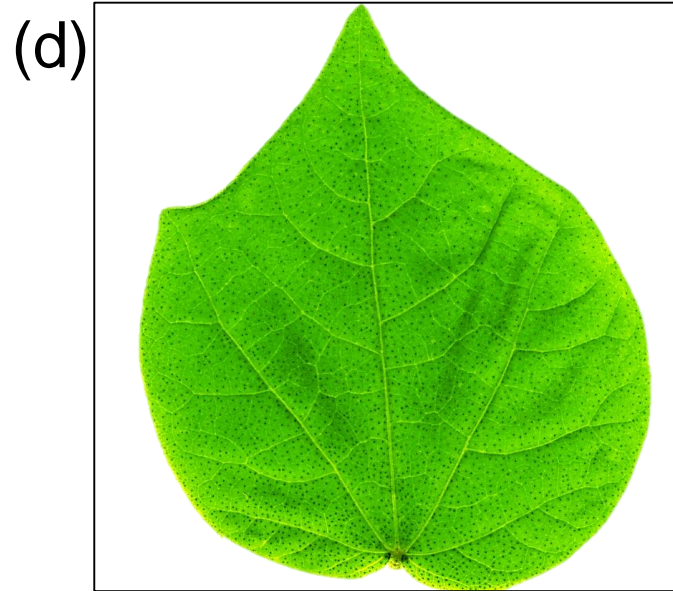
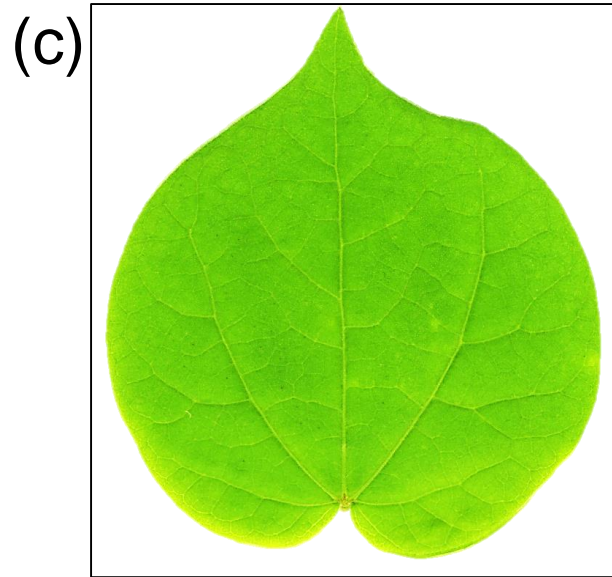
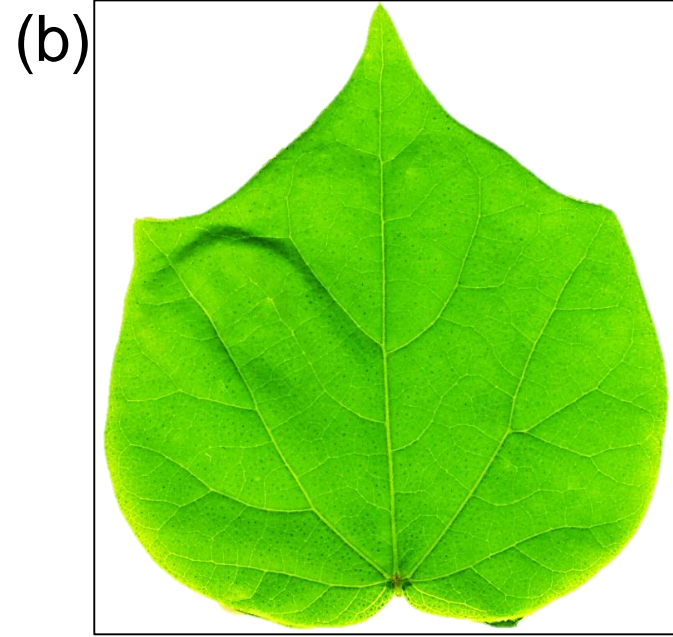
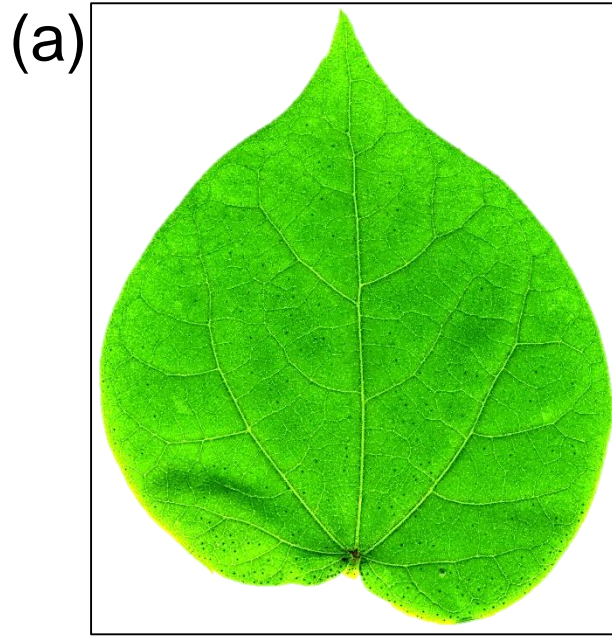


Figure S3 Leaves from plants that had undergone Virus-induced Gene Silencing against *CGF* genes showing the effects on gland formation. (a) silencing of *CGF1* gene, (b) silencing of *CGF2* gene, (c) silencing of *GoPGF* (synonym *CGF3*) gene, (d) empty vector control.

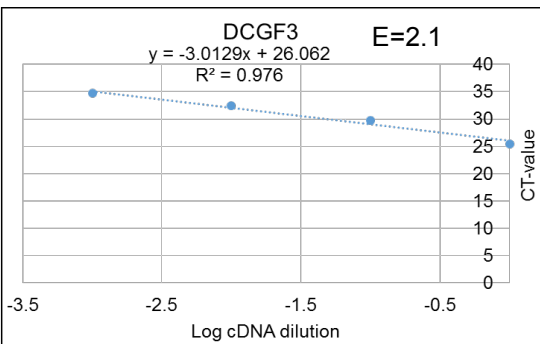
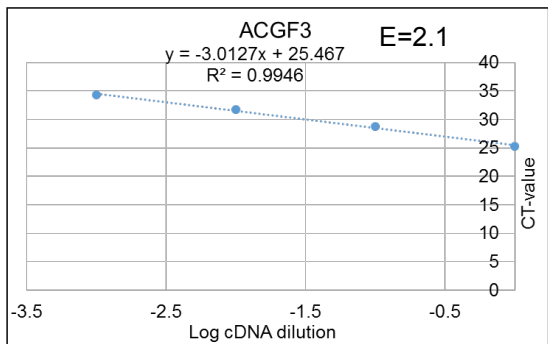
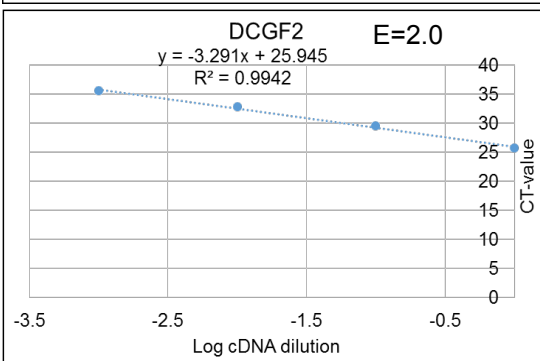
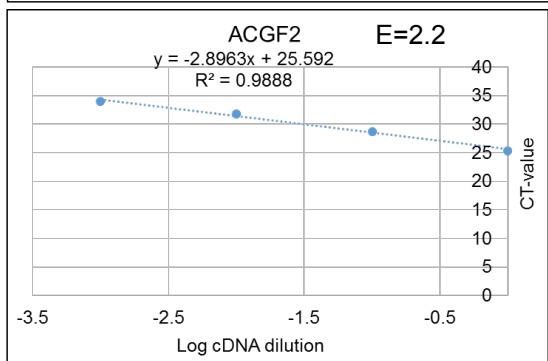
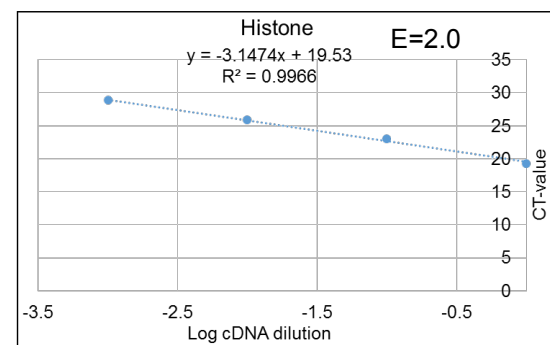
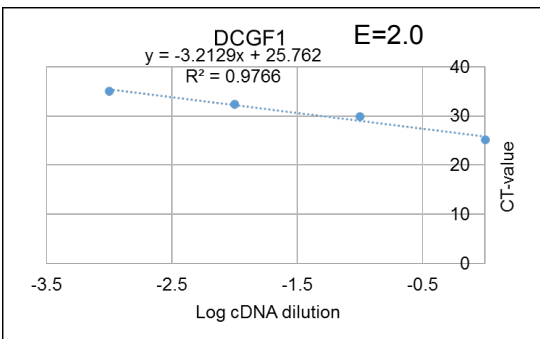
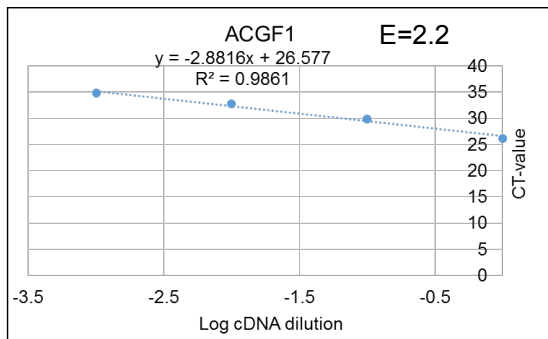


Figure S4 Real-time PCR standard curves representing PCR efficiency for each of the CGF homeologs and Histone gene.

ACGF1-GVS4 TCTCTCCAAAATCAACCATACTCACAATGCCACTTTTATTAATAAATGACATTTCTTCAAAAATAGTCTTCATTCTCTCTAATTAATAGGAGATTTGATGGAGGAATCACATTATTTTGAATGACAAGAAGGCATGATTATGAG 150

ACGF1-GVS5 150

ACGF1-TM1 150

ACGF1-GVS4 ATATATGTACGGGGCAACAATTTAATAATGACAAAGTTTATTATATTATTCGATGAATCTTTTCATTTCACATCAAAAGTAAATGTTCAATGAATTTGCTTCTACCCTTTGCTTCACGTACTTCAAAAATAATGAAAACCTCAATATAAA 300

ACGF1-GVS5 300

ACGF1-TM1 300

ACGF1-GVS4 TTGTGATAAGTTGGTGAATAATAGTGTAGTTTCATGATGTTGTGTAATAATTAATCTCGTTAAAAATTTTATTATATGATATTATAAAGTGGTGAATTTGAATTTTTTAATTTTTTTTATTATAATAATAACCTGAAATTAATGATGA 450

ACGF1-GVS5 450

ACGF1-TM1 450

ACGF1-GVS4 GTAGAATTAAGTAAAAATTCATAGTATCCATCTACTAAAGGTTATATTTGATTTTGTCAATTCATTTGAAAAAATGAATAAATTCATCCTTATAAGTTAGAGACCAGAAACAAAATGGCTATTTTGTAAAAATTTATTCATTAT 600

ACGF1-GVS5 600

ACGF1-TM1 600

ACGF1-GVS4 ACTTTTAAAGTATGATGTGACTATCGAAGTAACTAAATAGCAACATGTTGGTATGCCACATGTACCTCATGCTAATGTGACACATATTACCATATCAACAAATATTTAAAAATTAATAAAATCTAAAAATAAATAGACAAATAGTTGTA 750

ACGF1-GVS5 750

ACGF1-TM1 750

ACGF1-GVS4 CCCAAGTTCATCCTAGACGTTGTTTTAAAAATAATTATAAAAAATTAATATTTTTTTTAAAAATTAATGTTGTCCACATAAGCATAAGGTATACGTGGCACAATTAATGTTGTTATTTGATTGCTTCGTCAGTTACGTAATCATTAAAAAGC 900

ACGF1-GVS5 900

ACGF1-TM1 900

ACGF1-GVS4 ATAAATGGATGAATTTTTTAAACAGGACTTCCATTATTAAAGTAAAAAGAGTAAATTAACAATCCAACCTTAATTAAGGTATCTTTATGGCCTTTTACCTTAATTAATCCATGTCACCTAAAAATTCGAAATCATCACATATAAATAG 1050

ACGF1-GVS5 1050

ACGF1-TM1 1050

ACGF1-GVS4 AAATTTTAAACAAAATAATAATTTACATTTTTTTTCTAATTTACTATTTTTTAAATAAAAAACAAAATGCAATCCATATTTTAAACACTGTATCACAACATAAAAGGAAAGTAGATAAGTTTAAAAAGTAGGTTGAGACAAGAAATGT 1200

ACGF1-GVS5 1200

ACGF1-TM1 1200

ACGF1-GVS4 GTGATGAATTAATTAAGGGTAAAGATAAAAAATAATTTTTTATTTTGGTTCAACAGTTGCCCTCTTGAGTTTAGTATAGGAAACATTTAAAAATGGTTTAAATGCTATTCCTTGTAAAAATGGTTTAAATGATTTTGGTATTGATTGA 1350

ACGF1-GVS5 1350

ACGF1-TM1 1350

ACGF1-GVS4 GGTTTAAGATAAAAAGAAAGAAAAGAGAAGAAATTTGATGTAAACGCAACAAATGAGGGAGGAGGACCCACACGCATCAGTAGTCAGTCAGTCACTGGGCTTTCGCTATCACCTTCATGCTAGTCTGCTAATAGTCCCCCC 1500

ACGF1-GVS5 1500

ACGF1-TM1 1500

ACGF1-GVS4 CCCCCCCCCCAACCCCTCCATCTTCGTATCTGGCCCTTATAAACCCCAACGCCCCCTCCAACCTCTCATTCCATTCCACACCTCCCTGTTTTCTCCTTTCGTACCAGTGAAGACCTCATAAATCTCTCCCTCTTCATCTCTTCT 1650

ACGF1-GVS5 1650

ACGF1-TM1 N----- 1638

ACGF1-GVS4 CTGGTTTCCTTTCCAGGAACCTCCGTCCTCAATCTTCAGCAGAGGCTGCAGTTTGTAAATCAAAAGCCAGCAAGATTTGTGGGCATATGCTATATTATGGCAGACACTGAACGATGACCTGGGTCGCTGTCTTGGCTTGGGGAGAT 1800

ACGF1-GVS5 1800

ACGF1-TM1 1788

ACGF1-GVS4 GGTCATCTTCAATGCACATAAGATGCTTCTCCAAGTTGAGTTCCGGCTTCCACAGCGAACGAACTAAGGTGATTAAGGAATCCAAGCCCTCATTGGAGACCAACATGACATCGATTGCTATGATCGACGGAAACCGATATCACTGAT 1950

ACGF1-GVS5 1950

ACGF1-TM1 1938

ACGF1-GVS4 GTCGAATGGTCTATATGATGTCATGGCTCGATCCTTCTCTGCTGGCGAAGGGATTCTGGCAAGGCTCTTAGTACTGGGTCTTGGTTGGTTAACTGGTGCTCATGAGTTGCAATTTTACAATTGTGAAAGAGCTAGAGAAGCCCAA 2100

ACGF1-GVS5 2100

ACGF1-TM1 2088

ACGF1-GVS4 ATGCATGGCCTTGAACACTGGTTTGCATACCCACTTCTTGTGGTGTCTTGAACCTAGGATCCTCAGAGATTATCAGGAAAACCTGGGTTTAGTCCAGCAAGTGAATCCCTATTGAAATCCGATCTCATTGGTCTGGTTCCAAAACAA 2250

ACGF1-GVS5 2250

ACGF1-TM1 2238

ACGF1-GVS4 TCGACTCCTCCAAATTTAACCCAGCTTCGGTCCAGTTCCTTAAACAGAAATATCTCATTTCAGACATCGGCATAATAGCGGGTGTTCAGGAAGAAGCGGTGCAAGCCAGGATATTAACCAAAAGCAAGAGCATAACAAATAACCAAAAC 2400

ACGF1-GVS5 2400

ACGF1-TM1 2388

ACGF1-GVS4 AAGAAAGATTCCTCAAACTTGGGCAACCTCTTATGTGACTCAGAGCATTCGGATTCTGATTTTCCATTACTAGCCATGAATAACGTGGAGAGCGAACCCCAAGAAACGAGGAAGGAAACCCGGGCTCGGACGAGAGACCCGTTG 2550

ACGF1-GVS5 2550



Start codon

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ACGF1-TM1 ..... 2538
ACGF1-GVS4 AATCAATGTTGGAAGCGGAGAGGCAACGCCGTGAGAAGCTGAACCACAGATTCTACGCTCTCCGAGCAGTAGTCCCGAACGTTGTCGCGCATGGACAAAGCATCGCTCTTATCTGACGCTGTTCCCTACATCAATGATCTAAAGGCAGAAAT 2700
ACGF1-GVS5 ..... 2700
ACGF1-TM1 ..... 2688
ACGF1-GVS4 GATGAATTAGAGTCGCAGCTTCAGAGAGAGTGAAGAAAGTGAAGGTAGAAATGGTTGATACAATGGATAACCAAGCACCCTACTACTACCACATCTGAGGAAGAGCAACAGGCAGCCAGGCCAGCTATTCATCTCCTGGAACTGGG 2850
ACGF1-GVS5 ..... 2850
ACGF1-TM1 ..... 2838
ACGF1-GVS4 AGTGGCATTGAACTGGAGGTCAAGATTATGGTAAATGATGCAATGATAAGGGTTCACCTCAGAGAATGTGAACATATCCAGCAGCTAGGCTAATGGGTGCCCTTCGTGACTTGGAAATCCAGGTCCGTCATGCAAGCATGTCGCTCTGTTAAC 3000
ACGF1-GVS5 ..... 3000
ACGF1-TM1 ..... 2988
ACGF1-GVS4 GACCTCATGCTTCAAGACATAGTGGTCAGGCTTCCGTATGGGTTGAGAAGCTGAAGAAAGCCCTCAAATCTGCTCCTCAGGAGGCTAGATCTGCAGTAAAGGTTTATTATGGTTATCCAGAGTTTGTATTTTAAATGCTCTATTTCCTC 3150
ACGF1-GVS5 ..... 3150
ACGF1-TM1 ..... 3138
ACGF1-GVS4 CTACTTCTTCAGTTATTTGTTTAACTTTGCTTCATCTCAGGTTTCTTTAGCCGTTCTTTGCTTTCTGCTTGTACTATGTAACCTTTGGGAGAGGGTTCTGCACTGCTTCTTTTATCAAATGTTCTATATTTATCTAG 3300
ACGF1-GVS5 ..... 3300
ACGF1-TM1 ..... 3288
ACGF1-GVS4 TGCTGTTCCCCGAGTTAAGGACAAATAATTCCTTAATGGATTATACACATACAAAAAGAACGGAAACAATATTCGTGCATGTTTATCAACTAGAAACCAATCCATGCCAAAAACATGTATAATAATGTCTGCCCTATTTAAGGAC 3450
ACGF1-GVS5 ..... 3450
ACGF1-TM1 ..... 3438
ACGF1-GVS4 TCAAATATACATATTTT 3467
ACGF1-GVS5 ..... 3467
ACGF1-TM1 ..... 3455

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Stop codon

Figure S5 Alignment of *A* subgenome *CGF1* gene sequences from GVS4 (glanded) and GVS5 (glandless, recessive mutant) near-isogenic lines. Gene sequence of Texas Marker-1 (TM-1, a widely used genetic standard) is also provided for comparison purpose. The ~3.47 kb sequence of *A CGF1* representing the promoter region (~1.59 kb), 5'-UTR (italics), coding region, 3'-UTR (italics), and the terminator is identical between GVS4 and GVS5. Translation initiation codon and stop codon are indicated.

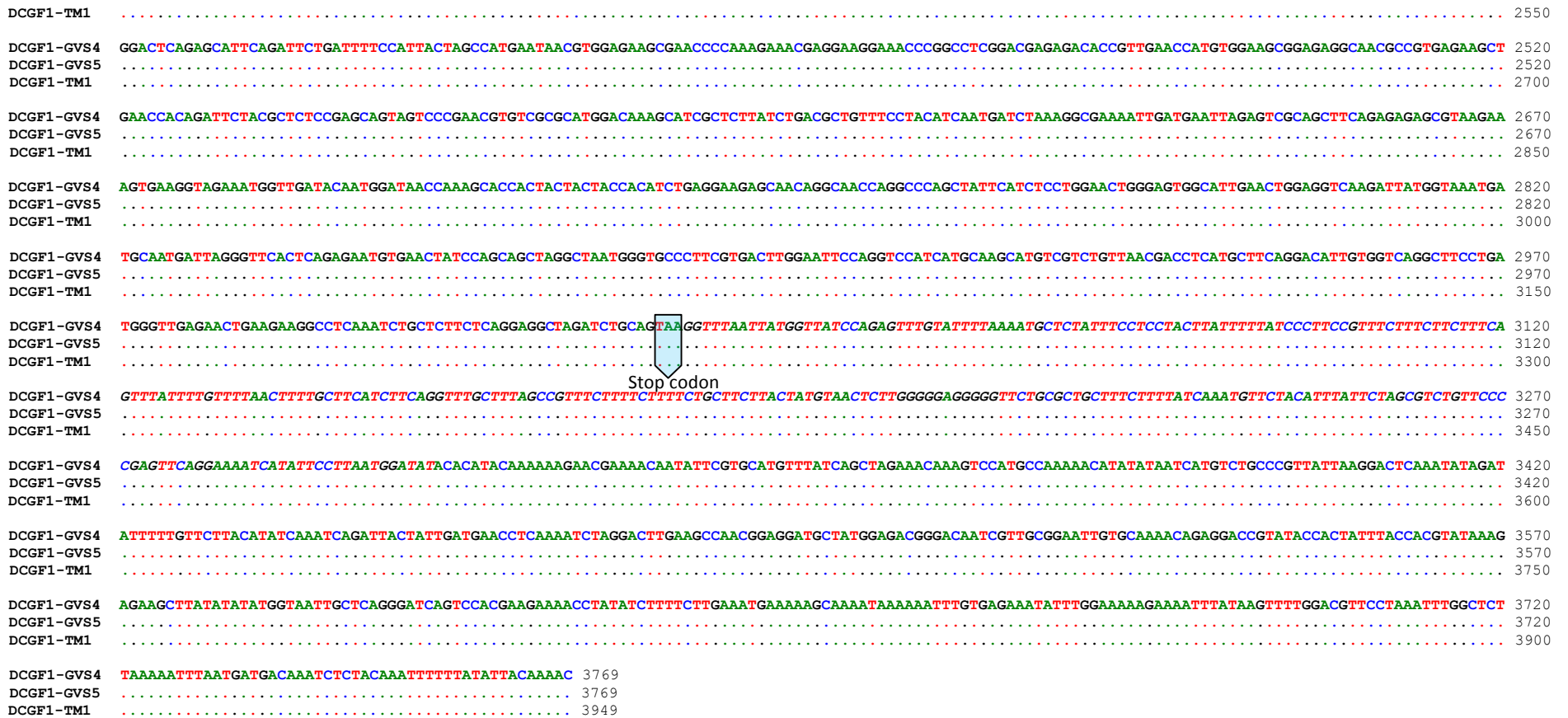


Figure S6 Alignment of D subgenome *CGF1* gene sequences from GVS4 (glanded) and GVS5 (glandless, recessive mutant) near-isogenic lines. Sequence of Texas Marker-1 (TM-1, a widely used genetic standard) is also provided for comparison purpose. The ~3.77 kb sequence of *D CGF1* representing the promoter region (~1.5 kb), 5'-UTR (italics), coding region, 3'-UTR (italics), and the terminator is identical between GVS4 and GVS5. Translation initiation codon and stop codon are indicated.

ACGF2-GVS4 CAATATTACCTGGCTAGTAATGAACCTAATTAGGGCTTCAATCTCTCTTCAGGCATAAAGAATGTTTAGGAATTGGTGGATTGTTCAACAAGTGTGACTTCAGCAATCACTTGTAGTAACATCCATAGCCTTCAATGAGGTTTCT 150
ACGF2-GVS5 150
ACGF2-TM1 150
CDS ----- 1

ACGF2-GVS4 CCCTCTACCTCAGTAGTAACATAAAAAATATCATTTCCTTTTCAATAACAGTCACCTTGATGACAATTTTTTTTAGGCAAAGATTAGAGCATCTTTGTAAACAGCTCTAAGTTTGAGTATCTTCCACCAGGCAAAAACCTAATTTT 300
ACGF2-GVS5 300
ACGF2-TM1 300
CDS ----- 1

ACGF2-GVS4 AAATGTCATAAAGCTTTCATAGAAATTTAGATAAGGCTTATTGTATACATTGTTAAAGTTTCAAATCTCGTAGTCAGCATTGAAAGTTTGGATTACTTCCAAATTTGGTCTTTATAAGTAGTTTCAAGAATAGTCTAAGCTTCTCTAG 450
ACGF2-GVS5 450
ACGF2-TM1 450
CDS ----- 1

ACGF2-GVS4 CAAATGATCATTAGATATTGACCTAAAACTTGAGGATTAACTCTAGAGAAAAATCCTTAAAGTCTTAGAGTTTGATTAGCCAAATTTCTCTTCATCATTAGTCTAAGTTAGTTTAGTTTGGAGTTTCTCACCCATATACACTAGT 600
ACGF2-GVS5 600
ACGF2-TM1 600
CDS ----- 1

ACGF2-GVS4 TATTAGTAGCTCCTAACCCAGTCAAAATGAGTGCCAAAGATTTTTTCATCCATGAACCTTGATAAAGCATCATCCTTACCTTCTACTAGGCATAGTTTGTACCATCCAACATGGGGGAACCTGGGGCTATTAGTCCCTCCATCTTAATTGCAA 750
ACGF2-GVS5 750
ACGF2-TM1 750
CDS ----- 1

ACGF2-GVS4 ATCAAAAATAGGATCACAACTTAGTACATTAAGTGTCTAGCTCTGATACCAAATATGGATTCTTTGAAATTTGCCTTGCAATTTTTCATCCAATTTGTAACTAGATGAAGTGAATTAATAGTTATGAAACGAGAGATGGAAAAAAGAGTTT 900
ACGF2-GVS5 900
ACGF2-TM1 900
CDS ----- 1

ACGF2-GVS4 GGTTCGCGAGTTCAAAAATTTCTACATTTGTAGAGCTTTACCCAAAGAGTAACTACTATAAAATCAATATGTATAAGACGCTTATTTAAGTTCAACTCACTCACTAAGTTGCAACCCCTACTCCATATACTTCAATCTAGATCACTTTCCAA 1050
ACGF2-GVS5 1050
ACGF2-TM1 1050
CDS ----- 1

ACGF2-GVS4 CTAAGTATAGCTTAGCTACAAATGATGTACAAAACAAATTTACGTACACAAGTATGCATTGGAAATTAAGTTCCCAATGAACAAGTTAAGTGCTCTCTAATGCTCTTAACCTTCAACCAAGTTGCCTTTAAATTAAGTAAATTAACG 1200
ACGF2-GVS5 1200
ACGF2-TM1 1200
CDS ----- 1

ACGF2-GVS4 TGTTAAAATACAATCAATGTAATATCTAATTTCTCAAAGGAGCCCTAGAAAATGCTTCAATAAAGAATATACTTGTAGAGATCTTCTCGATATTAAATTTATTTAGATCTAATCTCTCAAATAAGAGAATCTTAATCACATCGAG 1350
ACGF2-GVS5 1350
ACGF2-TM1 1350
CDS ----- 1

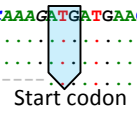
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ACGF2-GVS5 1500
ACGF2-TM1 1500
CDS ----- 1

ACGF2-GVS4 GTGGTGTGCCCTCACCCCTTAAGAGACCCTAACAATATATGCATAACATATCTTAAAGTATATATTTGACATTGGATTCCCAAAATTTTCTAAGGTGCTTATCATGAAATGTTTGGTGCTAGGTGTTTGAACATGAAGAATCAA 1650
ACGF2-GVS5 1650
ACGF2-TM1 1650
CDS ----- 1

ACGF2-GVS4 GACTCTTTCGAGCTGATTGGGGCTCTTTGTGCATAATAAGTCAATAATTTGATTTTGAACCGAGCAACCGGAAGTGGGACGACTCCCCCAAAACATTATTTAAGAAGGTTCCATAATGTTTCAAAACTAGCAGCTAGCTTCTCTCTG 1800
ACGF2-GVS5 1800
ACGF2-TM1 1800
CDS ----- 1

ACGF2-GVS4 TAAGTTTGATTGACGATTTCAATGCCACTGTACACACCAAAAATGAATGATTAACACTAATCTTTGATTTCTTTCCCGAGCTTGTATCCACACTTAGCCAGCTTCGTCAAAATGATGAACGTCGACGACGTCCAAGGTGTTAACGATAACA 1950
ACGF2-GVS5 1950
ACGF2-TM1 1950
CDS ----- 40

ACGF2-GVS4 AGGCCGATCACGAAGATATCAACTTCTGGGTTCCGGTTTCATCTACTGATGAAGAGCTCGTAGGGTTTTATCTTAAACGCAAGTTGAAAAGAAACCCCTAAGATTCGAGCTTATCAAACAGATTGATTTACAAATTTGATCCCT 2100




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ACGF2-TM1 ..... 4050
CDS ..... 960

ACGF2-GVS4 TTTAGAATTTATGATTTTAATTTTTATAGAATTTATGATTCTTTTTATTGGAATTAACAAAATTTTATTAATAATTACTCAAAATGAAGTCATTATATGGTTTTTTTCTAATGGTATACTAAAATTTATTATTAT 4200
ACGF2-GVS5 ..... 4200
ACGF2-TM1 ..... 4200
CDS ..... 960

ACGF2-GVS4 GATTCTATTTGCATGATGGATGTAATTCATCAAAATAATAATTATATTTTTCTACAAAG 4264
ACGF2-GVS5 ..... 4264
ACGF2-TM1 ..... 4264
CDS ..... 960

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Figure S7 Alignment of *A* subgenome *CGF2* gene sequences from GVS4 (glanded) and GVS5 (glandless, recessive mutant) near-isogenic lines. Sequence of Texas Marker-1 (TM-1, a widely used genetic standard) is also provided for comparison purpose. The ~4.26 kb sequence of *A CGF2* representing the promoter region (~1.8 kb), 5'-UTR (italics), coding region (including two introns, shown in small case), 3'-UTR (italics), and the terminator is identical between GVS4 and GVS5. Translation initiation codon and stop codon are indicated.

DCGF2-GVS4 ATGAGGTTTCTCCCCFACCTCAGTAGCAACTATAGAAAATATCATTGCTTCTCAATAACAGTCACCTTGATGACAAATTTTATAGGCAAAGATCGACGTATCTTGTAAACAAGCTTTAAGTTGAGTATTCCTCACCAAAGGCAAAA 150
 DCGF2-GVS5 150
 DCGF2-TM1 150
 CDS 1

DCGF2-GVS4 ACCTAATTTTAGATGTCATAAAGCTTTCATAGAAATTTAGATAAGGCTTATTGTCATGCATTGTTAAAGTCTCAAACTCTCGTAGTCAGCATTGAAATTTGGAATGCTTCAAAATGGTCCTTTATAAGTAGTTTCAAGAAATAGTCTAAG 300
 DCGF2-GVS5 300
 DCGF2-TM1 300
 CDS 1

DCGF2-GVS4 CTTCTCTAGCAAATGATCATTAAAGATATTGACCTAAAATCTTGAGGATCAACTCTAGAGAAAATACCTTAAAGCTTGGAGTTTGCATTGGCCAAATTTCTCATCATCAATTGGTCTAAGTTACTTTAGGTTTGAAGATTTCTCACACTAT 450
 DCGF2-GVS5 450
 DCGF2-TM1 450
 CDS 1

DCGF2-GVS4 ATACACTAGTTATTGGTAGCTCCTAACCAGTCAAAATGAGTGTAAAGATTTTTCATCCATGAACCTTGATAAAAGCATCATCCCTTACCTTCTAGTAGGCATAGTTTGTCCATCCAACATGGGGGGACTTGGGACTAATAGTCCCTCCAT 600
 DCGF2-GVS5 600
 DCGF2-TM1 600
 CDS 1

DCGF2-GVS4 CTC AATTGCAAATCAATAATAGGATCTCTACTTAGTACCCAAGTGCTAGCTCTGATACATAAATGGAATTTTTGAATTTGCTTGC AATTTTTTCATTCAATTGTAAC TAGATCAAGTGACTAATAGTTATAAAAATGAGAGATGGAC 750
 DCGF2-GVS5 750
 DCGF2-TM1 750
 CDS 1

DCGF2-GVS4 AAAAAGAGTTTGGTCTCGTAGTTTGAAAACTTTCTACATTTGCAGAGCTTTACCCAAAGAGTAATCTACAATAATCAATATGTATAAGCGTGATTTAAGTCAACTCACTCACTAAGTTGCAACCTACTCTATACTTCAATCTAGA 900
 DCGF2-GVS5 900
 DCGF2-TM1 900
 CDS 1

DCGF2-GVS4 TCACATTTCCCCTAAGTATAACTTAGCTATAAATGATGAATTAACAATTTACTTACACCAAGTATGCATTGGAATTAAGTTTCTCAATGAACAAAGTTAAGTGCTCTCTCATGCTCTTAACTTAAAACAAGTTGCCCTTTAAATTAAG 1050
 DCGF2-GVS5 1050
 DCGF2-TM1 1050
 CDS 1

DCGF2-GVS4 TGTAAATAGCGTGTAAAATACAATCAATGTAAATATCTAAATCTTCAAAAGTAGCCCTAGAAAATGCTTCAATAAAGAAATATACTTGCAGAAATCTTCTTGATATTAATTTATTGTAGATCTAATCTCTCAAATAAGAGAACTTCT 1200
 DCGF2-GVS5 1200
 DCGF2-TM1 1200
 CDS 1

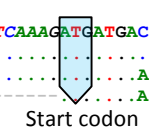
DCGF2-GVS4 GATCACATCAAGAGTATTCACAAAGTCCATGCTTGACTTGATTCTTGCACTATCTATCCTTACTAATGGATTGCTAAAACCTTCAAGGATATACATTTAAAAGTTCCAACAATTTAATTAAAATATGCTAATCACATAAGATAGCTTA 1350
 DCGF2-GVS5 1350
 DCGF2-TM1 1350
 CDS 1

DCGF2-GVS4 CATTAAATTTTTGCTAGATGTGGTGGTCCCTCACATCCTTAAAGAGCCCTAACAAATATGTAGAGCATACTTAAAGTATATATTTGACATTGGATTTCCAAAAATTTCTAAGGTGTCCATTATGATATGTTGGTGTAGGTGTTT 1500
 DCGF2-GVS5 1500
 DCGF2-TM1 1500
 CDS 1

DCGF2-GVS4 GAAACATGAAAGAAATCAAGACTTTTCAAGCTGATTGGGGCTTCTTTGTCATAATAAGTCAATAATTTGACTTTGACCCGAGCAACCGGCAAGTGGGACGACTCCCCCTAAACATTATTTAAGAAGGTTCCATAATGTTCAAAGG 1650
 DCGF2-GVS5 1650
 DCGF2-TM1 1650
 CDS 1

DCGF2-GVS4 TAGCAGCTAGCTTCTCTCTGTAAGTTTATTGACGATTTTCATGCCACTGTACACACCAAAAATGAATGATTAACACTAATCTTTGATTTCTTCCCGAGCTTGATCCACACTTAGCCAGATTGCTCAAAGATGATGACCGTCGACGACGT 1800
 DCGF2-GVS5 1800
 DCGF2-TM1 1800
 CDS 20

DCGF2-GVS4 CCAAGGTGTTAACGATAACAAGGCCGATCAGCAAGATATTC AACTTCTTGGGTTCCGGTTTCATCCTACTGATGAAGAGCTCGTAGGGTTTATCTTAAACGCAAGTTGAAAAGAAACCCCTAAGATTTCGAGCTTATCAAACAGATTGA 1950
 DCGF2-GVS5 1950
 DCGF2-TM1 1950
 CDS 170



DCGF2-GVS4	TATTTACAAATTTGATCCCTGGGATCTCCCAAGt	2100
DCGF2-GVS5	2100
DCGF2-TM1	2100
CDS	-----	202
DCGF2-GVS4	ttaaaaatggaaggataaccaaaaccaa	2250
DCGF2-GVS5	2250
DCGF2-TM1	2250
CDS	-----	225
DCGF2-GVS4	GGAGAGAGTGAATCGTACTTCTTCGCAAA	2400
DCGF2-GVS5	2400
DCGF2-TM1	2400
CDS	-----	375
DCGF2-GVS4	TGCATTGGGTTAAAGAAAACCTTAGTGT	2550
DCGF2-GVS5	2550
DCGF2-TM1	2550
CDS	-----	525
DCGF2-GVS4	GAAGCTGtaagtataaaatattgctcaag	2700
DCGF2-GVS5	2700
DCGF2-TM1	2700
CDS	-----	532
DCGF2-GVS4	gcctagcagtttaattagtggttaaat	2850
DCGF2-GVS5	2850
DCGF2-TM1	2850
CDS	-----	532
DCGF2-GVS4	aaatgtaagaggactatttgtttttaa	3000
DCGF2-GVS5	3000
DCGF2-TM1	3000
CDS	-----	532
DCGF2-GVS4	acatthtgaagtthtttgatcgattaga	3150
DCGF2-GVS5	3150
DCGF2-TM1	3150
CDS	-----	581
DCGF2-GVS4	ACACAACAAGATTGGAGACAAATCACAG	3300
DCGF2-GVS5	3300
DCGF2-TM1	3300
CDS	-----	731
DCGF2-GVS4	TCATAACGACGAGGAGAAGCCATCAAC	3450
DCGF2-GVS5	3450
DCGF2-TM1	3450
CDS	-----	881
DCGF2-GVS4	AAATGATGATTTCTTCACTGAGGCCAAT	3600
DCGF2-GVS5	3600
DCGF2-TM1	3600
CDS	-----	963
DCGF2-GVS4	ATTAATAATTAGTTGCTAATTTTATATTA	3750
DCGF2-GVS5	3750
DCGF2-TM1	3750
CDS	-----	963
DCGF2-GVS4	AAATCAATATTTATGAAATTTGAGAATCT	3900
DCGF2-GVS5	3900
DCGF2-TM1	3900
CDS	-----	963



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DCGF2-GVS4 AGATTCTTTGATTTCAATATTTTTTAGAATTTATGATTTTAGTTTTTTATAGAAATTTATGATTTCTTTTATTGAAATATAATGATACGTAAACATACGAAAACCATTTGTTACG 4023
DCGF2-GVS5 ..... 4023
DCGF2-TM1 ..... 4023
CDS ----- 963

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Figure S8 Alignment of D subgenome *CGF2* gene sequences from GVS4 (glanded) and GVS5 (glandless, recessive mutant) near-isogenic lines. Sequence of Texas Marker-1 (TM-1, a widely used genetic standard) is also provided for comparison purpose. The ~4.02 kb sequence of *DCGF2* representing the promoter region (~1.65 kb), 5'-UTR (italics), coding region (including two introns, shown in small case), 3'-UTR (italics), and the terminator is identical between GVS4 and GVS5. Translation initiation codon and stop codon are indicated.

ACGF3-GVS4 ACGAAATAGAAACAAATATTCCTTTTATGAGAGAGAACTGAGCAAATGACTTTGTAAATTAGAAGAAATAGTTGTTAAAGAGAGGTAAAGAAATGTATTGAAAACATTCCTCAAGATCCTCCTATTTATTGCATAAGTGTTCCT 150
ACGF3-GVS5 150
ACGF3-TM1 150

ACGF3-GVS4 TCTCCCTTCAATATTTGTGATGCCTTACAGAAAAATGATGAATTTCTTTTATGGGGTCAGACATAGATGGAAATAGGAAAAACACTAAGCATCATGGACAAATTTGTGCAAGCCATAGAAGATGTGTTGTATGAGTATCCAAAATTT 300
ACGF3-GVS5 300
ACGF3-TM1 300

ACGF3-GVS4 AAGATGCTTTAATCTGTTATGCTTGGCAAATAGAGTTGGTGGATTATAAAACAATCCCAATCCTCTTCAAGATCAAATATTCAAAGCTAAATAGTCCCGAATTGTGGGTTCTTGAATCTAGTGAAGGATGATTGTTGAAAACCTTAGA 450
ACGF3-GVS5 450
ACGF3-TM1 450

ACGF3-GVS4 CCATGTTTAAATGATGTATATCAAGCTCAATTTGTATTGCATATTGTTGGGTTAAGGCTATTGCAAGATTCAACCTTGGAGTTCTCTATTACCACCTTAATATGCAAGATGATAATGAGCTGAGTTGGTGGTTAGGTGTAGCATGGAG 600
ACGF3-GVS5 600
ACGF3-TM1 600

ACGF3-GVS4 TATATGGACACATAGGAACCAAGTTTATTTAGAATAAAAAAGATCATAACTAGTCCAAATTTGTTCAATTTGAAATTTCTTACATGGAGGCTTGGTAGGGAGCTCACACGACGATGACTCGTATTCAACCTTCCAGGTTAAGTTGTCTTA 750
ACGF3-GVS5 750
ACGF3-TM1 750

ACGF3-GVS4 CTTCTAAGTAGAGTTGGTGTAAATCGCTAGGTAATGGGTTCAAATTTAACATCGATATGGTTATTTTTATAGAAATGTTGCCACGAGCTGATCGACTATCATTATAATGAAAATGGTGAATTTTGACAAAGGTCGCACAGGTTTATCCA 900
ACGF3-GVS5 900
ACGF3-TM1 900

ACGF3-GVS4 AGCAACTATGGCTCCAAAAGTTACACATGATTTGTTATTGAAAGGCATTGCTTTAGTTGAAGTCGTTGCAAATTTGAGAGATGATCATTTGAGACGGATTGTTGATTGTACCGAAGATTTAACGAGTAAAAAAGGTTATCTCTATGAT 1050
ACGF3-GVS5 1050
ACGF3-TM1 1050

ACGF3-GVS4 GGGTTGATTATTGAAGGCTGCTCATGTTGAAAGCTTTTTTTTTTTCAATTTATGTCCTTAAAGTTGAATGAGTAGAAACATTGATTAAGATCGCTCATGCTCTTGCAGGGTAGCTTTATGTCATGCAAATCCTTATGTTTAGAATCT 1200
ACGF3-GVS5 1198
ACGF3-TM1 1200

ACGF3-GVS4 TCTCCTAGTTGCATGCTTCCATTCTTATTTAAGATGTTCTACTCTTTTATCTAATGAGGAGTCGAGTGTATAGGATAAGCCCAATTAGCTTAAAGAAAATAATTTCCAATCCTTATTTTGGTAAATTTTGTGTTTCATTCCGATTTG 1350
ACGF3-GVS5 1348
ACGF3-TM1 1350

ACGF3-GVS4 ATAAATACTATTTTCTTCTTCCCTTAAAAAAGGCTAGGAAGAGTTATTGAATAAATAACAAGTTGAAGAGCTAATTTAATAGATTAAACATTTGATCAAATATTAACAACAAGGATGTTAAATGGGAGTTGAGTTGGGGTT 1500
ACGF3-GVS5 1498
ACGF3-TM1 1500

ACGF3-GVS4 AAAGGTGACACGATAGCTGAATAATTAAACAAAATATGGATGGGAATGGTCTAATATCTAAAAATAGTTAAAAATCATGGGAAATAGAAAAAGACAGATAAAAGATGATCACGAAATCGCCCATAACTGGTGTGAATTAGTTA 1650
ACGF3-GVS5 1648
ACGF3-TM1 1650

ACGF3-GVS4 AGATCATGAGTGGAGGGGTTAAGACGCCAACAGAACCCGCAACTCCACATCAAATAGAGTAAACCATCCATGCCATAAAGAACAAAAAGAAAGAAAATTCAGACCAGGTCATCATCCCTTATTTGGAGAGAACTGGAACAGGAGAT 1800
ACGF3-GVS5 1798
ACGF3-TM1 1800

ACGF3-GVS4 TTCCACGTGATAATGATGTCAGAATTTGACTGACAATAGGACCATGAGGTTGATGGTCCCTTTATTTCCCGGAAAAATTACTTTGTTTTCTCCAAAGCACTTCACTTAATAATTATAGTTTTCTCTTTTTCTCATCTGCTCCATGGG 1950
ACGF3-GVS5 1948
ACGF3-TM1 1950

ACGF3-GVS4 GTTTATAGCCACCTAGTGACGACTTGTATCATCACTCTTTTCCCTTTTATTTATTCCTTCTGCTGCTGAGTCCGCTTGGTTAGTTTTCTTTTTCTCTTTGAAAAACGGTAGTACACACTATCATATTCAATAATGCTTCCTCTTC 2100
ACGF3-GVS5 2098
ACGF3-TM1 2100

ACGF3-GVS4 TTCGTTCTCTTATTAACCTTAGGTCGAAGATGCCTCACCTACTTTGCACACGCTCCATTTTCATCGTCCAAAGTAGGCCGTAATGGTGGGTATACCTCATTTTCTGGCAAGCTTCAAGGGATGTTGATGGTCGCGTTGTTTGTCTATG 2250
ACGF3-GVS5 2248
ACGF3-TM1 2250

ACGF3-GVS4 GGGCGATGGCTATTTTCGAGGGACCCGAGATGGTACGGGAAAAATCCATCAATAGGCTGAGCCCTCCAAAATTTGGGGTCCAGTTTCGAGAGGAAAAAGGTCGGGAAAAGATCAGGTGCAAGCTTATTTTAAATGAAGTGTGGACGTGGACCG 2400
ACGF3-GVS5 2398
ACGF3-TM1 2400



Start codon

ACGF3-GVS4	TATGGTAGATGGCGATGTGACTGATTATGAGTGGTACTATACCGTATC	2448
ACGF3-GVS5IGTTGCGGAAAG.....TCGATTTCGAGAACTCCGATATGACTACAAGTAAATTGACCGGAACGAAAAATAAAGTGAACACAAGGATTTACGTGGTTCGGCTTTAA	2548
ACGF3-TM1	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	TGCCACGTCCACGGGCAGAGGCCAAAAGAAATTTCACTATAACAAGATGAAGATTACAAGTFTTTCACTCAAGACACAACCCGAGAACCTCACAACTCTCAACCCCTATAGAAAACCCGAAAGCTAAAAATCCTAGCTTTCAAAGAAC	2698
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	AAGCTTTGCTCTCTCTTGGTTTGGGATGATGAATATGGCTAATGAACCTCTCTATTTATAAGAGAGTCAAGGACATAATTGTCCAAAACTTTGGCAAAGATTTGGTTGAAAAATGGACACCAACAACCATCCACTAATCCACTACCAC	2848
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	CAACAACCCACTACCAACAACAACATCCACTACCAATTTTAAAGCCATTTCTTAACAAATCTCCACCTTGGCTTGAAATTTACCAAGCTGAACATCATAGTGAATTCCTCGAAGCTGGATCACCTCTTCCAACGCCTCTCTGGCGCTAAT	2998
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	CAATCCCGAATACCTATCAAGTCCAAGCAATGCTTGAACCTTATATGCAGGGATCACCTTAGTTAACATATCTGCAGGATTCCTCTCGGTAGCAACCTTGCTGATAACAATGTCACTTGCCTAACATGTTCCCGAACAAAATGATATCTG	3148
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	AACATCAATGTTTGGTCCGTTCAAGCAATCTGATTTTTAGTCAAGTGTATGGCACTCTGGCTATCGAAAAACAACAGTGAATCCCTGTTGTAAACCCAACTGCTTACTAGACCTTTCACCAATGCTTCTTTCACCTGCTTCT	3298
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	GCTAACGCCATATATTCGCCCTCAGTCGTAGATAAGGCTACGGTAGACTGTAACACAGCTTCCAACTAATGGCTCCTCCAGAATAAGTGAACAATAACCCGTCAGAGATCTTCTTTTGTCCAGATCTCCAGCATAAATCAGAGTCCACA	3448
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	TAGCCCGTGACACTGCTAGTGCAGTCACTCTGATCATATACCAAGCATAAATCTGCAGAACCTCTCAAGTACCTGAGAAATCCATTTACGGCCTGCCAGTGTCTTCTGCCAGGACAACTCATGTATCTGCTGACCACACTAACTGCATGT	3598
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	GAAATGCTTGGACGAGTGCAAAACATTCATACATCAACGCTTCCAACCTGCACTCGAGTATGGAATGTGAGACATTTGCTGCTTTTCTTCATCAGATTGTGGTGACAACTCTGCAGAGAGTTTGAATGTGGTGCCAAACGGAGTACTCACA	3748
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	GTTTTCGCTTTATCCATGCCGAAGCGTTGAAGAACCTTTTCAATGTAGTTCTTCTGCACACACGAAGCTTGCCCGCTTTCGATCTCTGTGAATATCCATGCCAAAATTTTCTTGGCAGCACCCAGATCCTTCATCTCGAATCACCAC	3898
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	CTCAACTGCGACTTTAACTTGTGATTTCCGACATGTTTTTGAAGCAATTAACATGTCATCAACATACAGCAACAAATAAATGTGCGAACCATCTGAGAGCTTCGGATGATAGACACAAGCATCATAGTACATCTTGTGTAACCATGC	4048
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	TGAATCATGAAGCTATCAAAACCGCTTGTACCCTGCCCTGGGGATGTTTCAATCCATATAAAGACTTCTTTAATAGACAGACATGGTCTTCTTTACCAGGAAGTGTAAAACCTCTGGTTGACGCATGTAGATTGTTTCTCGAGCTCA	4198
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	CCATGCAAGAACCGCTTTTACGTCAAGCTGCTCAAGTCTAGATCAGACTTGGCCACCATGGCAAGTAATACACGAATGGAAGAAATGCTTTACGACTGGGGAGAAAACCTCATGTTAGTCAATCCCCTCTTTCTGAGTGAAGCCTTTA	4348
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	GCAACCAATCGTGCCTTGAATCTAGTTGCTTCAACCCCTAGGATGCCCTCTTTCTTGAAGACCCATTTGCAACCAACTATCTTCTGGTTACTTGGCGGTTAACCAACTCCCAAGTATGGTTCTTGTGAAGAGATTCATCTCCTCA	4498
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	CTCATAGCAATTGCCACTGTGCCATTATCACAGTGCAGCCCTATTATAACTGGAAGGTTCTATACCAATGGACTCCGCCACACTGAGCGCGAAAGACACCAGATTAGCGTAAACCGGATTTGGTTTGAATTTGCTCTTCGTTCTT	4648
ACGF3-TM1	-----	2448
ACGF3-GVS4	-----	2448
ACGF3-GVS5	CCAGTGGCAATGCTATATGGTTTTCTTGAAGTACTCATCTTCAATCGGAATCTGCACCTCAACTTGGATCATCTGGACTGAAGTACCTTCGGTAGGAAATGGAGCTCCACCTGACACTCCACCTGCTTCTCAACACCGTGAATCTCC	4798
ACGF3-TM1	-----	2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 ATTCATCTGACTCCTCCTTTCCCGGAAATTGTGGTGGATCGAAGCATGGATGACTCATCAAAGTCACATCTCTGCTGATGATGAAC TTGGACGAAACCGGATCAGGACACCACACCTGTATCCTTTACCCCTTGGCCGATCCA 4948
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 AGAAAATATGCATTTCTTCGCCCTCGGTTTGAGTTTCCCTCATTACATGAGCATACGCAGGGCAGCCAAACACTCTTAAACCAGAGTAAFCAGCAGGAGAACCCAGACCACTTCCCTCAGGAGTCTTCAGCTCAATAGCTGTTGATGGA 5098
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 GATCTGTAAACCAATAACAAGCAGTATAACAGCTTCAGCCAAAATTCCTCACCGAGCCAGCATTGATCGCATGCAACGAGCTCGTCCAAGAGAGTTCATTTATGCGTTCTGCAACTCCATTTTGTGTGGTGTTCGACGAACA 5248
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 GTGCGGTGCTCACTATTCCCTCATTTTTGCAGAACTCATTGAATTCACCTGAGCAAACTCCAAGCCATTATCCGTCGGAAATCGCTTGATCTTCTTTCCGTTTGAATTTTCGATCAAAGCTTTAAATTGCTTGAAGTTGATGAGAACC 5398
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 TCATACTTGTCTCTTCAGAAAATACCCCAAACCTTTCGAGTAATCATCGATAAAGGTAAGCAGATATCTGTAACCACCTTTAGAAATGTTCGGAGAAAGCCCCAAAGGTCAGAAATGGAAGTAATCCACTGTCCCTTTGTCTTGTA 5548
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 ATCCCAGTGTGAACCTTTACCCGAGTCTGCTTACCAGAGACGCAGTGTCTCAGAAAATCAACTTTCCCTGTACACTGCCAGACAATTAATCCTCGTTTGTCTTAGCACCAGATAAGCCTCTCTCGCTCATATGCCCGAGCCGATATGCCAT 5698
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 AAC TTCGTGGTGCAGAACTTAGATCATCTGATGATGACACTCCCGCAACACCTGTAACCGAGGAACCATCGAGGAAGTAAAGGCCCGCTCCAAATTACCAGTATCACAGTCAAAGCACCCGAGAAATACCTTGAGAACTCCACCTTCA 5848
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 GCAGAGTACCAGAAAGCCCTTCTTGTCACACTCAAGAGATCAAAATTTTCTTCATTTCTGGAATGTGCCAATCATCAGTTAGAGTTCTAAACAATACCGTCAAACATCTTTATACGAATGTGCCAATCCCCATGACTTGACATGCG 5998
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 TGGTCATTTCCCAATTAGTACTGAAACCAGAAATGCTTCTCGTATGTTGAGAAATGCATCTTTGGAAGTACTTATATGAAATGTAGCTCCCGTATCAAGAAATCCATCTCCCACCAGCGTAAGAGTCGGATACTGCAAGAACGATCTCGGCATCA 6148
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 CTTGAAGAACTGCATCAGCTACACTTCGCAGCATATTTTGTGCTCCTGTGATCTGATTTCTCCCTTTTCGGACAATCTACCTTCATGTGCCGTACTTCTTACAGTAGTAGCACTGTATTTCTTTCTTGGACTGCGATCTAGGA 6298
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 TGGCTTTTACTTGAACCTTCCACCCCTTGGCTTGGATCTCCCTCGAGCAACCAAGCCTTCGCCTTCAATGTTTTCGACCACCTTACCAGTGAATTTCTTCCCAACTCACTGGAACCTTAAGGCATTTTTACCTCCTCTAGAGTCAGGTCA 6448
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 TCACGACCGTACATCATTGTATCAACAAAATTCCTCATACGAGGGAGGCAAGAAACACAAAACAAATTAATGCTTGGTCCCTCATCATCGATTTTGTATCGATATTAATCAAATCCATGATAATGGAATGAATTAATCCAGGTGCTGGAA 6598
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 ACAGGTGTACCTTCTCCATCTTCAGGGCATAGAGTCTTTGCTTGAGGTAGAGCCGGTTCGTCAATGACTTCGTGATGACTTGTCTCTTAAACCAGGACCAAAACCAGGACCGGTTTCTCATCCGCTACTTCTCGTAGCACTTCATCT 6748
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 CCTAGACATAGCAGAAATAGCAGTATGTGCTCTTTAGCATGTCATCCTTTTGTCTTCCGAAAGCGTGTGGTAATTTATCTTTACCAGACAATGCTTTTAGCAATCCCTTGTGAACCAGCACTGCCCGCATCTTGATGCGCCATAAA 6898
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 CTGAAACTATTTTTCCCGGTAAATTTCTCGACATCATCTTAGTTCGATGAAACACTTGTAGCCATAAATTTGGAACCTTTGAATGAATGATAATAATTTCTTCTGATGTGAAGATCAGACAAAGCTGCAGTCAAGGGCAATTCAGAAA 7048
ACGF3-TM1 ----- 2448

ACGF3-GVS4 ----- 2448
ACGF3-GVS5 ATAATTATCACCTTCAACTACGCTCTGATACCAATTTGTTGCGGAAAGGATCGATTGAGAACTCCGATATGACTACAAGTAAATTTGACCAGGAAACGAAAAATAAAGTGAACACAGGATTTACGTGGTTCGGCTTTAATGCCTACGCTCC 7198
ACGF3-TM1 ----- 2448

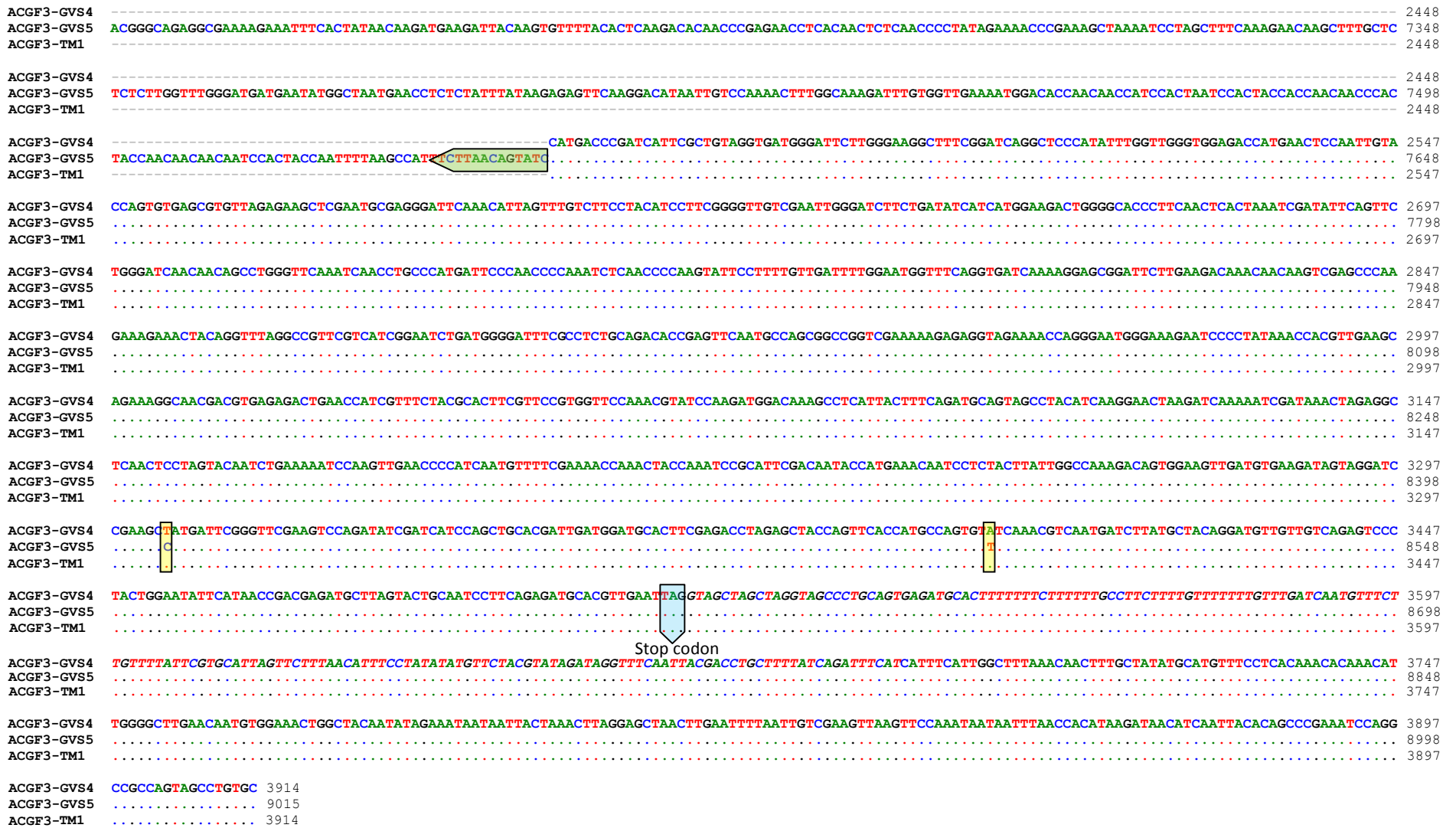


Figure S9 Alignment of A subgenome *GoPGF* (synonym *CGF3*) gene sequences from GVS4 (glanded) and GVS5 (glandless, recessive mutant) near-isogenic lines. Sequence of Texas Marker-1 (TM-1, a widely used genetic standard) is also provided for comparison purpose. The ~1.95 kb sequence upstream of the start codon, representing the promoter region of *A CGF3* in the glandless GVS5, has 2 SNPs (yellow box) and one deletion (2 bp long; red box) compared to the glanded GVS4. In addition, this homeolog in the GVS5 has two SNPs and a ~5.1 kb transposon insertion (indicated with two opposing green arrows) in the coding sequence. 5'-UTR and 3'-UTR are shown in italics. Translation initiation codon and stop codon are indicated.

DCGF3-GVS4 CTTCCCTATAACACCCCAATCCACGTAGGTCAACTGAAGTGTACACATATAGAAAAAGACATGCATAAATTTGATTTTTAGTTCAACTTAAATACATATTAACCTAACTAGGTGACCTCAGAAAACCTCTACAAAACACAGTTTATTT 150
 DCGF3-GVS5 150
 DCGF3-TM1 150

DCGF3-GVS4 AAAAAATATACCCATAGAAATAAAGTAAATGGATTAAATATTTTCGATGAAATACCTCCCTTGAATAAAATCTAATGGTAACTATTACTTTATAAAACATTCCTGGTGATACAAGTAGAAATAAATTTCTAAATGAGAACTTAACTAA 300
 DCGF3-GVS5 300
 DCGF3-TM1 300

DCGF3-GVS4 CTTTAAACAAATCCCTCAAGGGGTACATTCCTTATAAAGATGTAATTGAATGAGAAAGCTATTTATTATAGATTAATAATTTAGAAAATTTTAAACATGCCGCCCCAAAACATATGCATGTTACAAAAAAAACAAATGAAGCCCTACTGT 447
 DCGF3-GVS5 450
 DCGF3-TM1 447

DCGF3-GVS4 AGAGAATGCTTCCTTTGGCTTAGTCCCTTCAGAAAGCCCAATAACAAAGATCACCTGGAAGGAAGGAAAGAAAATGGGTAAAGTTTAAAAATAACTTAGTAACTCCACAAGGAAAACCAACACTTAAATAAGAACAGATTACACTACAA 597
 DCGF3-GVS5 600
 DCGF3-TM1 597

DCGF3-GVS4 GTTCATAACACAGACTAATACATTAGACTCTATACGCTGAGACATATGGGCGTATACCTTATCTCATTTGGCGAACACTTAACCTTGGCGTATAACCTTTGAGCATACCCACATATATAGAGCATACCCTTTTCATGAAATGGGCAT 747
 DCGF3-GVS5 750
 DCGF3-TM1 747

DCGF3-GVS4 ACACCTATATCCTTTCCCTACATAGCACTAAAACCTAAACAGAAATGATGCTTCTGCGTTAACATAACTTTTTTTCATAAGTACTTTTGATCACAACTCTCTTTAGGTGAAGCTACACTTACTTTCAAACCTTATTAGGGCGAGTTC 897
 DCGF3-GVS5 899
 DCGF3-TM1 897

DCGF3-GVS4 AATATATCCCTCATCTTTCTAATGAATACATTATGATCTCGATACGTCATAGGAATCAACATACTTCCCTAATTTATTAATCATCTCTTTTCATCTCAACTGATATATGCATCACACAAACATACACAATATCGTATTAGCTTATCC 1047
 DCGF3-GVS5 1049
 DCGF3-TM1 1047

DCGF3-GVS4 TTTCAATACAAAATATCTTTATAACAAATACAGAGACAAATGCTTACTACTTCAAAACCTTCCCTTAGGTTGACCTCGAATTTTACATATCAGAACAGATCTTTTGTATCATATCTTTCGAACACCTATGTATTCTTGTGAGTTTCA 1197
 DCGF3-GVS5 1199
 DCGF3-TM1 1197

DCGF3-GVS4 GGCTATTTGCTATGGTTAACAACTAACATGCTTATAGTAGGCAGCACCATAACGTTAGGCTAGTTAGCACTAGTATGCCCTACCAAACCTAACACCCACCAACTTGTAGTAACTAACATACCCCAACATGGTCGCCAACTA 1347
 DCGF3-GVS5 1349
 DCGF3-TM1 1347

DCGF3-GVS4 TTTAACAACTAACATGCTTACTAGTGGCCATCATATATCCCTATCTGGTTAGCAATTAACATGCCCTACCAGTGGTCACACATTTCTGCCATTTAGTTTGAATTAACATGCCCTACCAGTGGCTCATCTTATCCGGATATTGAGTT 1497
 DCGF3-GVS5 1450
 DCGF3-TM1 1497

DCGF3-GVS4 CATATCCGTAAGGAAGTCATTTCATATCATGCCACGATTATGCATATATAACACAGGCAATTAACATAAAATAGAACACAACTAGAAAAGAATCACATGGTCTGGATCCCTCATAGATGATTTTAAAGAAAATGTTATTTTATCACTACT 1647
 DCGF3-GVS5 1600
 DCGF3-TM1 1647

DCGF3-GVS4 ACCGCATGCACACCTCTTCTTTCTTTTGTAACTTGTACTTTTTTCCCAGAACTCCTCTTAAACAAGATTTCAAAGTATAACATAGCAAAAAGAAAGAGAAAGAAAACCCCACTACAAAGTTTTCACCTGATATTTATATCTCACA 1797
 DCGF3-GVS5 1750
 DCGF3-TM1 1797

DCGF3-GVS4 GCTTCCTCACCTTAGGATAAATATGGCTAACATGCAAGGCTAACTCAATCTATCCTAACCAACATAATTTATAGGTCATTATACATAAAAAAAATAATCATAAAACCTTTGGTATTTCTAATTTGGATCCCAATTAAGCACAACTAG 1946
 DCGF3-GVS5 1900
 DCGF3-TM1 1946

DCGF3-GVS4 AACTGAATAGAAATACATGTCACAAACAAACAAATTTTACATCCCAATCCCAACATCCTTTACTGGCTTACATAAAGAGAAGGCTTAAACACACAACTTTTCAAAGAAAACACTGATTTGGCGGTTCTTTAAAGTAACCCAGCCAAA 2096
 DCGF3-GVS5 2050
 DCGF3-TM1 2096

DCGF3-GVS4 ACAACTTAGACTTACCCAAAATACAAATCTTAAACAGGCTTACCTATAAATATACAGTCTGCCACACACAGCGTACAACATATACAATTAACCACTCTGACGAATAAACAATAAATCAGTCTTACCTTACCTTAAATCTTAGCTAATTTT 2246
 DCGF3-GVS5 2200
 DCGF3-TM1 2246

DCGF3-GVS4 AGCACATACAAACCATCAACAAAGACTACGTTGGACAGGGTATTACATCCCTCTTTATTGGACGAAATAAGAAAACAAATATCTCTTTTCTGAGAGAGAAATTTGGCAAATGACTTTGTAATTAAGGAATAAGTTGTTGAAAGAGGTA 2396
 DCGF3-GVS5 2350
 DCGF3-TM1 2396

DCGF3-GVS4 AAGAAATGTTATTGAAAACCTATTGCTCAGGATCTCCATTTTATTGTCATAAGTGTCTTCTTTTCTTTTCAACATTTGATGCCTTACAGAAAATGATGAATTTCTTTGATGGGTCAGACATAGATGGTAAATAAGAAAACACACTA 2546
 DCGF3-GVS5 2500
 DCGF3-TM1 2546

DCGF3-GVS4 AGCATCATGGGAAAATTGTGCAAGCCTTAGAAGATGTTGTATGAGTTCCAAAATTTAAGATGCTTTAATCTGTTATGCTTGGCAAATAGCGTTGATGGATTATAAACAAATCCCAATCCTCTTCCGAGTCAAATATTCAAAGCTAA 2696
 DCGF3-GVS5 2650
 DCGF3-TM1 2696

DCGF3-GVS4 ATAGTCCCGAATTGTAGGTTCTTGAATCTAGTGAAGGATTGATTTGTTGAGACTTAGACCATGTTTTAATGATGTATAGCAAAGCTTAATTTGTATGTCATGTTGTTGGGTTAAGGCCAATGCAAGATTCAACCCGTAGTTCTTTG 2846
 DCGF3-GVS5 2800
 DCGF3-TM1 2846

DCGF3-GVS4 CTACTACCTTAATATGCAAGACGCTAATGAGCTGAGTTGGTATTTAAGTGTAGCATGAAAGCATATGGACACATGSAACCGATTATTTGGAATAAAAAAGATGACAAATGAGTTTAAATGTTCAATTTCAATATTCTTACACGAGGC 2996
 DCGF3-GVS5 2950
 DCGF3-TM1 2996

DCGF3-GVS4 TTGGTAGGGAGCTCACACGACGATGACTCGTTCACCTACCAGGTTAAGTGTCTACTTCTAAGTAGTGTGGTGC AAAATTGCTAGGTAATAGGTTCAAATGTAACACCGATATGGTTATTTTTATAAGAAATGTTGTACCGAGCTAA 3146
 DCGF3-GVS5 3100
 DCGF3-TM1 3146

DCGF3-GVS4 TCGGCTATCATTCAATGAGAATGGTGATTTTGACAAAGGTCGCACAGGTTTTATCCAAGCAACTATGACTCTAGAAGTTATAAATGATTGTTGTTTGTAGAGGCATTGCTTTAGTTGAAGTCGTTGCAAATGAGAGAGTGATCATTGA 3296
 DCGF3-GVS5 3250
 DCGF3-TM1 3296

DCGF3-GVS4 GACGAATTGTTGATTTAGCGCAAGACTTAACGAGTAAAAGCAAGGTTATCTCTATGGTGGGTTTGATTATTGAAGACTGCTCATGTTGAATGAGTTGGATTAGTAGAAACATTGATAAGACCGCTCATGCTCCTGCTGTTGCAAGGG 3446
 DCGF3-GVS5 3400
 DCGF3-TM1 3446

DCGF3-GVS4 TAGCTTTATGTCATGCAAATCCTTATGTTTGAATCTTCTCTTAGTTGCATGCTTCCATTCTTATTAAAGATGTTCCACTTTTTTATCTAATGAGTGTATAGTATAAGCCATTAGCTTAAGAAATTAATTTTCCAAATCCTTATTTTG 3596
 DCGF3-GVS5 3550
 DCGF3-TM1 3596

DCGF3-GVS4 GGTGGCTTTGTTTGTTCCTTCGATTGGTAATAACTATTTTTCTTCTTCCTCGAAAAACAAGGCAGGGAGAGTTATTTGAATAAATTAACAAGTTGAAGAGCTAATTAATAGATTAACATTTGTACCAATATTAACA 3746
 DCGF3-GVS5 3700
 DCGF3-TM1 3746

DCGF3-GVS4 GGTAGTTAATTGGGAGTTGAGTTGGGTTAAGGTGACACGATAAGCAGAAATAATTAACAAAATATGGATGGGAATGGTCCATAATCTAAAAATAGTTAAGAATCGTGGGAAATAGAAAAAGACAAATAAAGATGATCAGAAATCGC 3896
 DCGF3-GVS5 3850
 DCGF3-TM1 3896

DCGF3-GVS4 CCCGTAATAACTGGTGTGAATTAAGTTAAATCATGAGTGGAGGGGTTAAGACGCCAACAGAAGCATCGCAACTCCACATCAAATAGAGTAAACCATCCATGCGATAAAGAACAAAAAGAAAGAAAATTCAGACCGGTCATCATCCT 4046
 DCGF3-GVS5 4000
 DCGF3-TM1 4046

DCGF3-GVS4 TATTTGGAGAGAACTGGAAACAGGAGATTTCCACGTGATAATGATGTCAAGATTTGACTGACAAATAGGACCATGAGGTTGATGGTCCCTTTATTTCCCGGAAAAATTATACTTTGTCTTCCAAAGCACTTCACTTATTAATTATGTTT 4196
 DCGF3-GVS5 4150
 DCGF3-TM1 4196

DCGF3-GVS4 TCCTCTTTTTCTCAGCT GCTCCATGGGGTTTATAGCCACCTAGTGACGACTTTGATCATCACTCTTTTT 4266
 DCGF3-GVS5 4220
 DCGF3-TM1 N. TTATTTCCCGGAAAAATTATACTTTGTCTTCCAAAGCACTTCACTTAATAATTATAGTTTTCTCTTTTTCTCATCT 4346

DCGF3-GVS4 CCTCTTATTTATCTTCTGCTGCTGAGTCCGCTTTGGTTACTTTTTCTTTTTCTCTTTGAAAAACAGTAGTACACACTATCATATTC AATGCTTCCCTCTTCTCGCTTCTCTTATTACCTTAGGTC AAGATGCCTCACCAC 4416
 DCGF3-GVS5 4370
 DCGF3-TM1 G. G. Start codon T. 4496

DCGF3-GVS4 TTTGCAACAACGCCATTCATCGTCCAAAGTAGGCTGAATGGTGGGTATACTCCATTTCTGGCAAGCTTCAAGGGATGTTGATGGTCCGCTTGTTTTGTATGGGCGATGGCTATTTTCGAGGGACCCGAGATGGTACGGGAAA 4566
 DCGF3-GVS5 4520
 DCGF3-TM1 4646

DCGF3-GVS4 ATCCATCAATAGGCTGAGCCCTTCCAAATGGGGTCCAGTTTCGAGAGGAAAAGGTCGGGAAAAGATCAGGTGCAAGCTTATTTTAATGAAGTATGGACGTGGACCGAATGGTAGATGGCGATGTGACTGATTATGAGTGGTACTATAC 4716
 DCGF3-GVS5 4670
 DCGF3-TM1 T. 4796

DCGF3-GVS4 CGTATCCATGACCCGATCATTGCTGTAGGTGACGGGATTTGGGAAGGCTTTCGGATCAGGCTCCATATTTGGTTGGTGGAGACCATGAACCTCAATGTTACCAATGTGAGCGTGTAGAGAAGCTCGAATCGAGGGATTC AAAC 4866
 DCGF3-GVS5 4820
 DCGF3-TM1 T. G. 4946

DCGF3-GVS4 ATTAGTTTGTCTCTACATCCTTCGGGGTTGTCAAATGGGATCTTCTGATATCATCATGGAAGACTGGGGCACCCCTCAACTCACTAAATCGATATTCAGTTCTGGGATCAACAACAGCCTGGGTTCAAATCAACCTGCCATGATTC 5016
 DCGF3-GVS5 4970
 DCGF3-TM1 5096

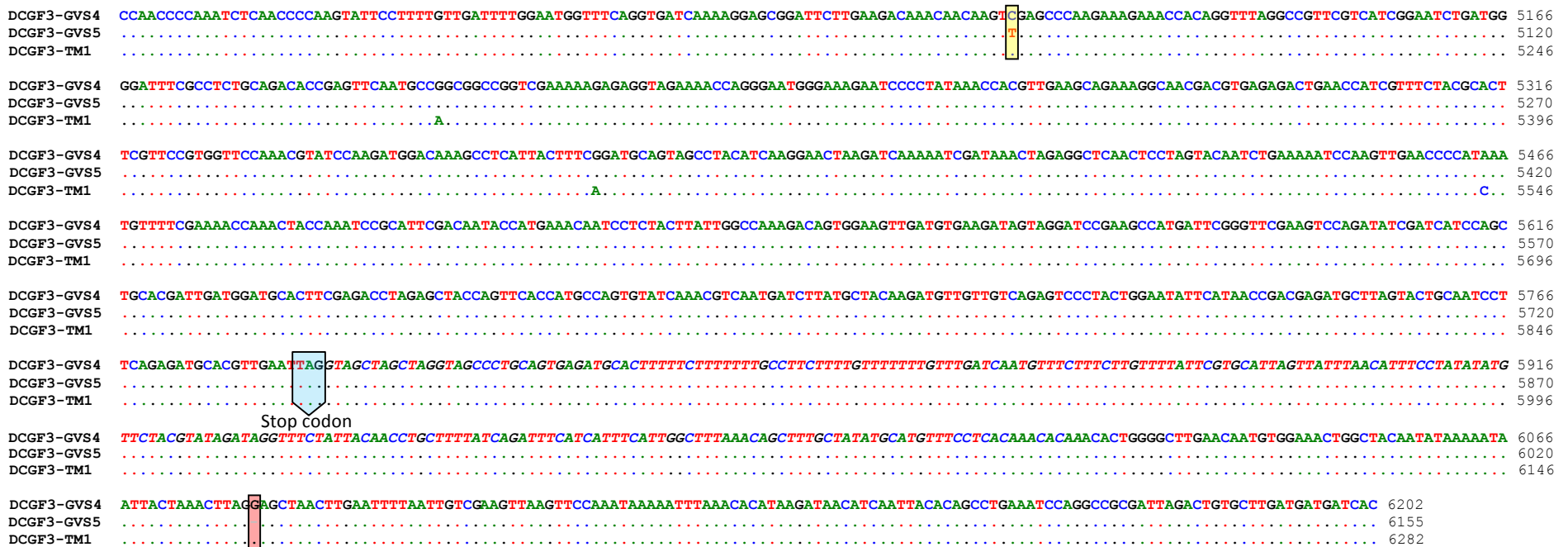


Figure S10 Alignment of D subgenome *GoPGF* (synonym *CGF3*) gene sequences from GVS4 (glanded) and GVS5 (glandless, recessive mutant) near-isogenic lines. Sequence of Texas Marker-1 (TM-1, a widely used genetic standard) is also provided for comparison purpose. The ~4.2 kb sequence upstream of the start codon, representing the promoter region of *D CGF3* in the glandless GVS5, has 15 SNPs (yellow box), two deletions (1 and 49 bp long; red box), and two insertions (1 and 3 bp; green box) compared to the glanded GVS4. In addition, the GVS5 has two SNPs in the coding sequence and one base pair deletion in the terminator. 5'-UTR and 3'-UTR are shown in italics. Translation initiation codon and stop codon are indicated.

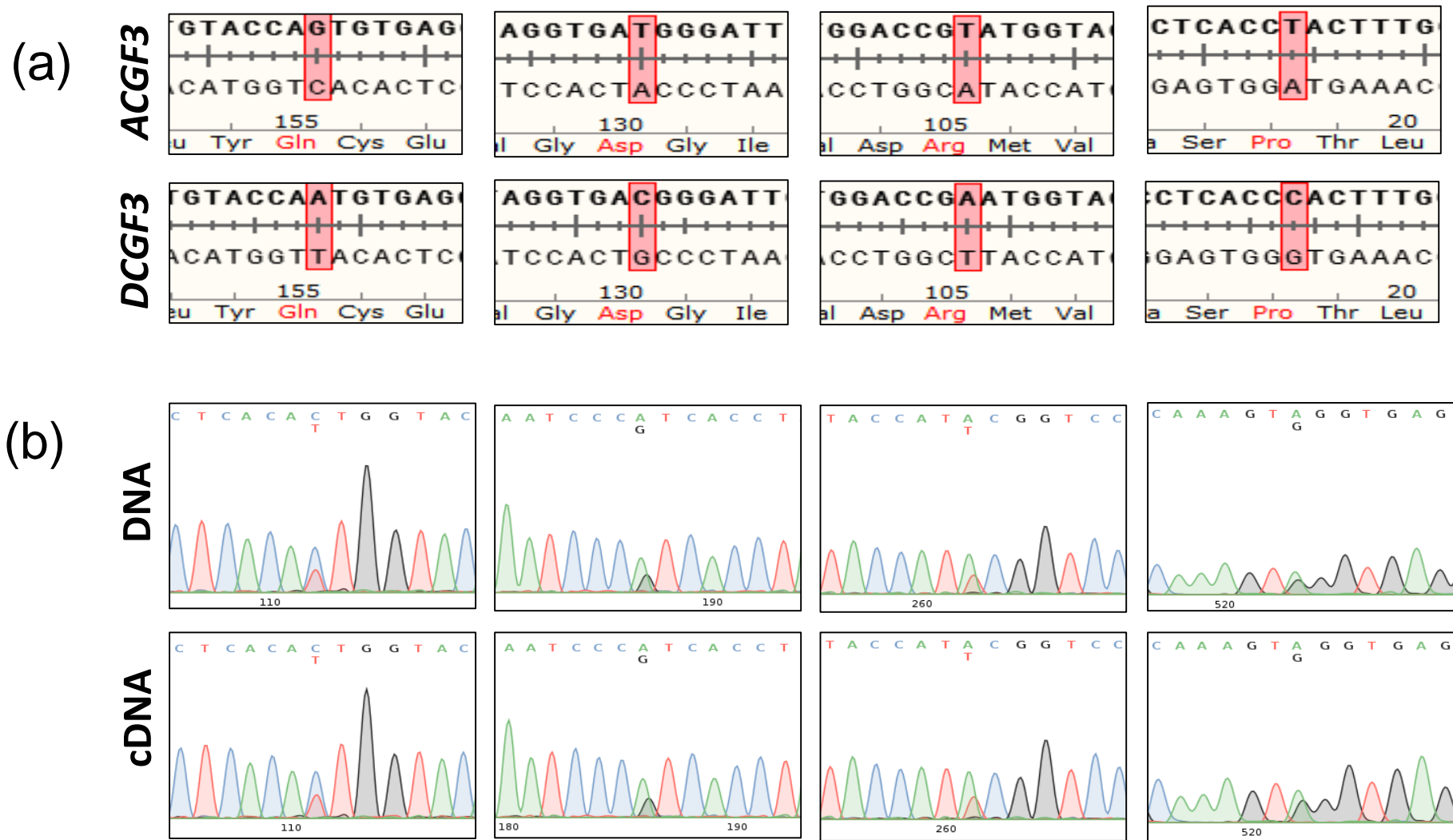


Figure S11 Sequencing results showing four different SNPs that differentiate A and D subgenome *GoPGF* (synonym *CGF3*) gene sequence showing four SNPs between A and D homeologs; (b) chromatograms showing the expected SNPs between the two homeologs of the *GoPGF* (synonym *CGF3*) gene in genomic DNA and cDNA samples

gene sequence showing four SNPs between A and D homeologs; (b) chromatograms showing the expected SNPs between the two homeologs of the *GoPGF* (synonym *CGF3*) gene in genomic DNA and cDNA samples

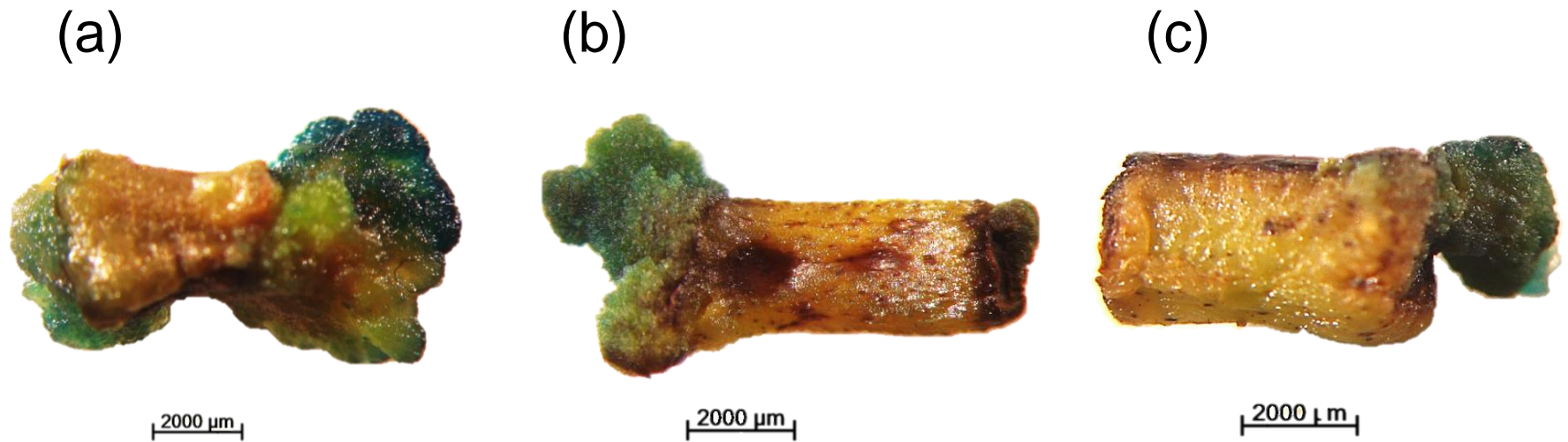


Figure S12 Promoter (2.05 kb) activity evaluation of the D subgenome *GoPGF* (synonym *CGF3*) gene from glanded and glandless cotton using *gusA* as the reporter gene. (a) CaMV 35S promoter (used as a positive control); (b) 2 kb promoter from glanded cotton; (c) 2 kb promoter from glandless cotton.

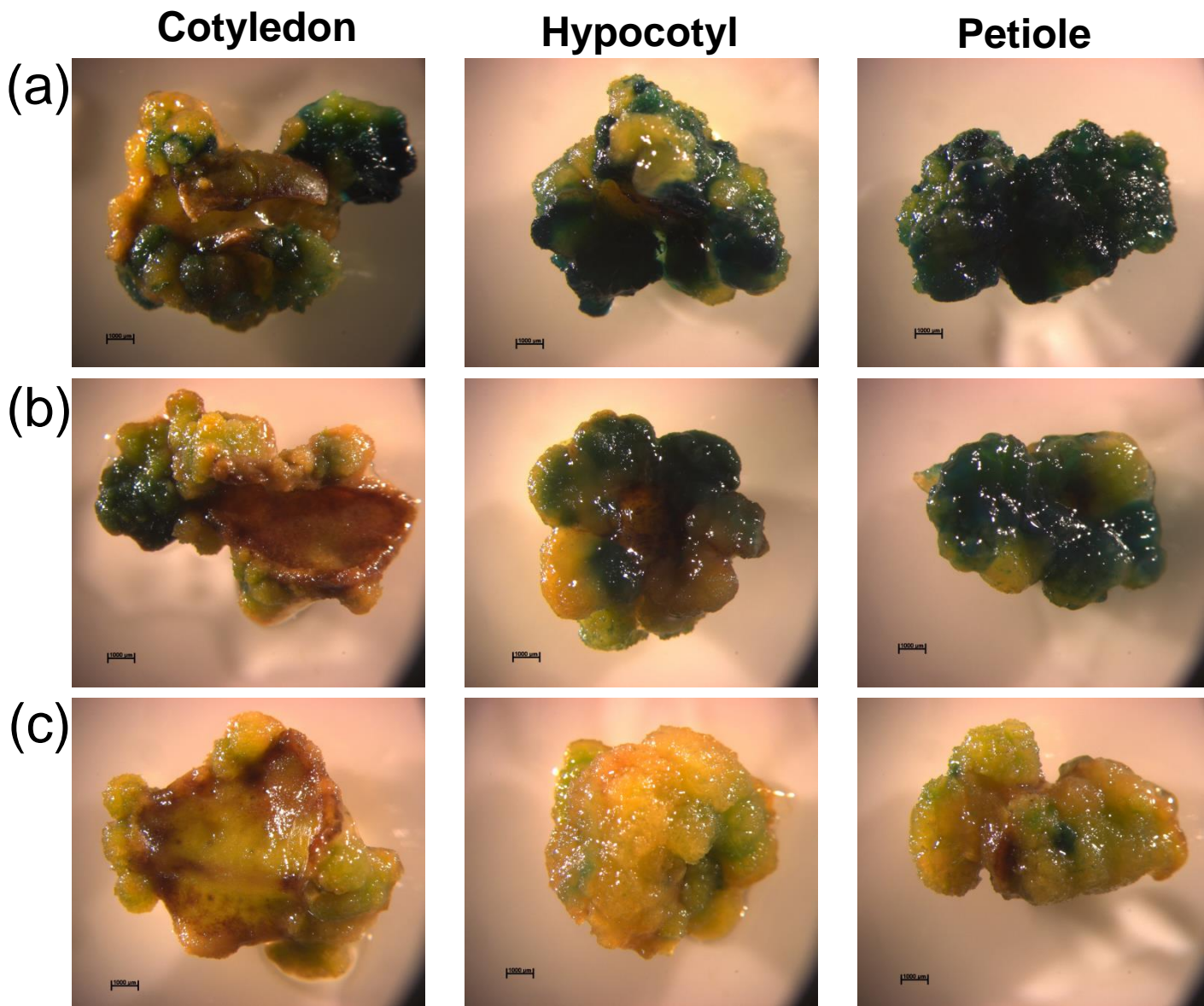


Figure S13 Promoter (~4.2 kb) activity evaluation of the D subgenome *GoPGF* (synonym *CGF3*) gene from glanded and glandless cotton using *gusA* as the reporter gene. (a) CaMV 35S promoter (used as a positive control); (b) ~4.2 kb promoter from glanded cotton; (c) ~4.2 kb promoter from glandless cotton.

Control

236

237

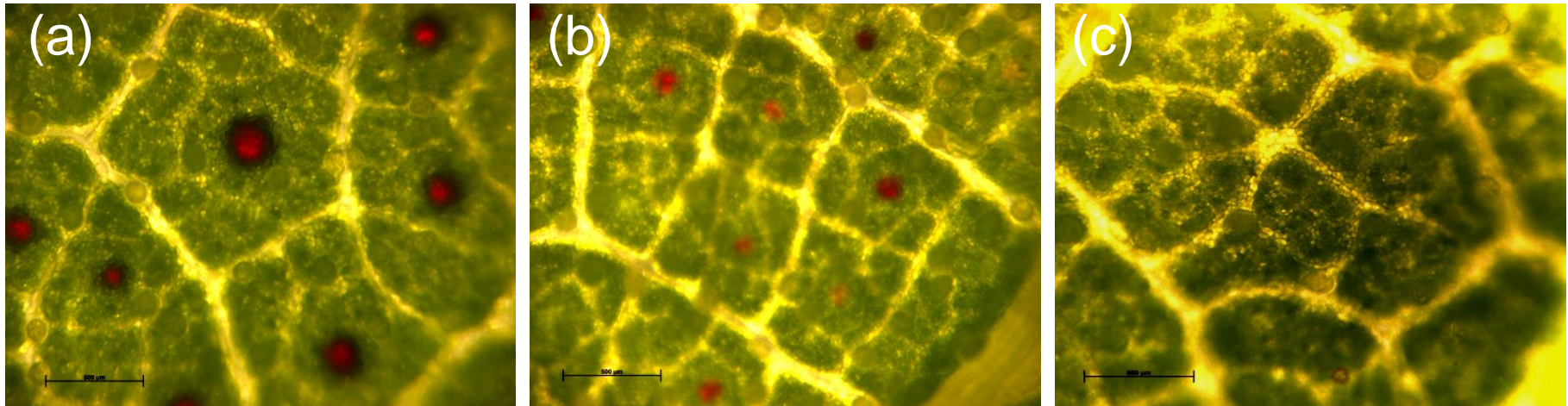


Figure S14 Effect of CRISPR/Cas9-mediated knockout of *CGF2* and *GoPGF* (synonym *CGF3*) genes on gland formation in T0 plants in comparison to glanding pattern seen in a wild-type, control cotton plant. Microscopic images of leaves undergoing expansion: (a) control plant showing well developed glands, (b) line 236-10 showing smaller and malformed glands, (c) line 237-3 showing complete absence of glands.

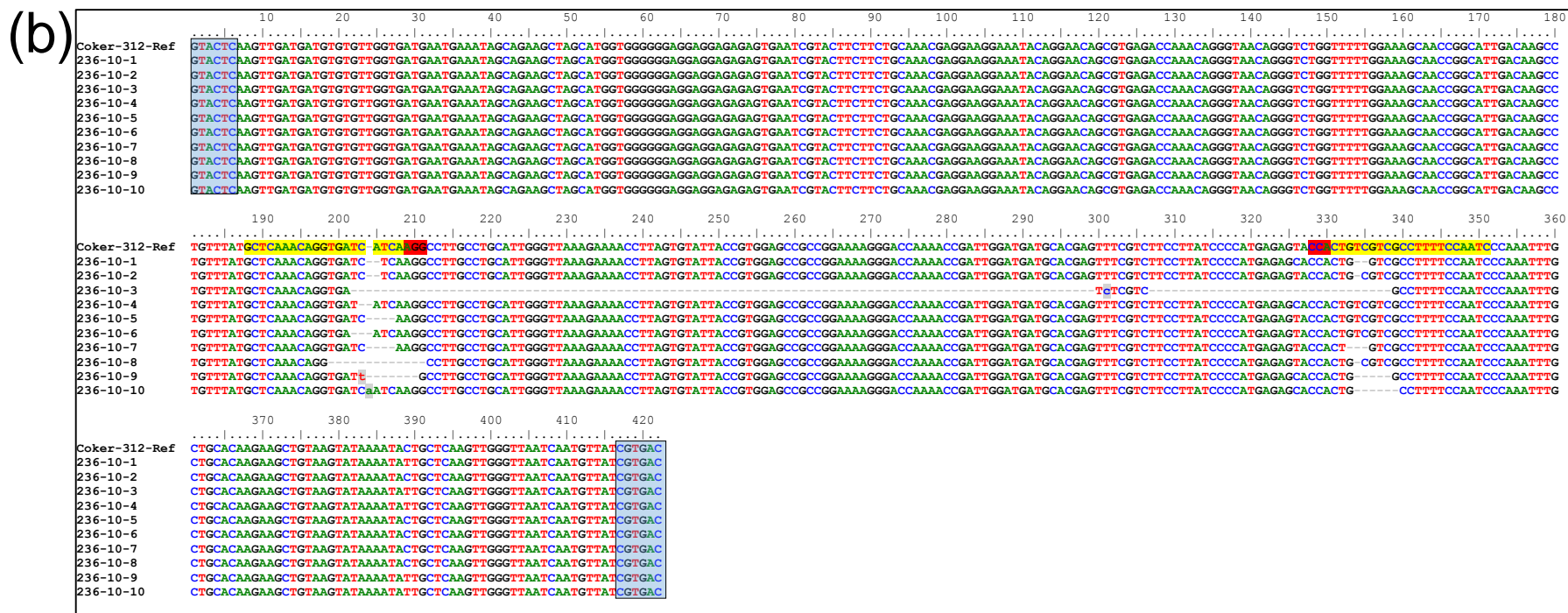
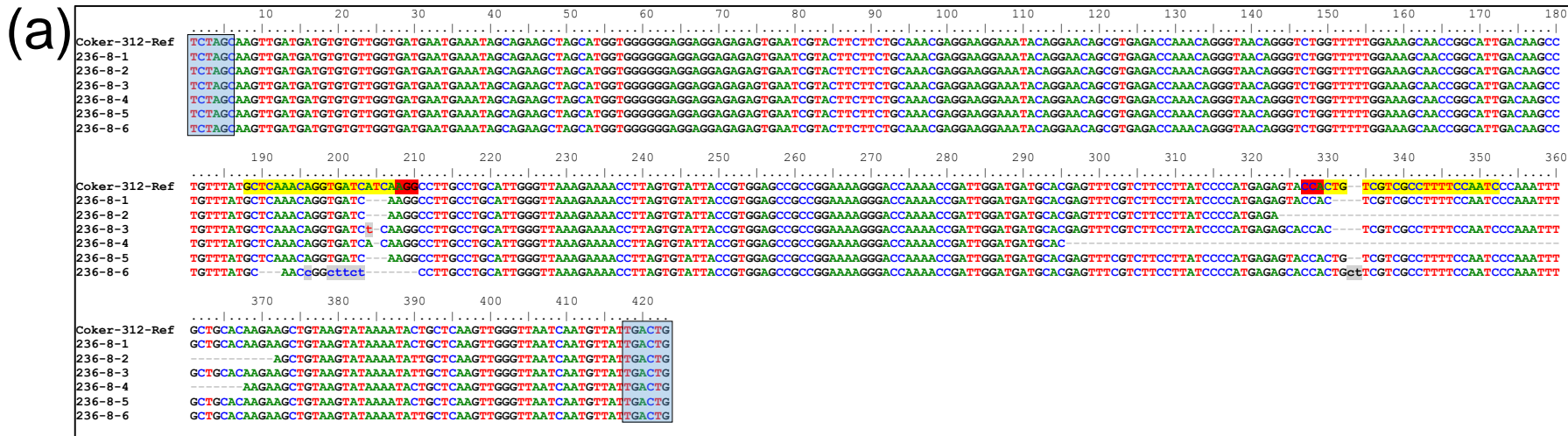
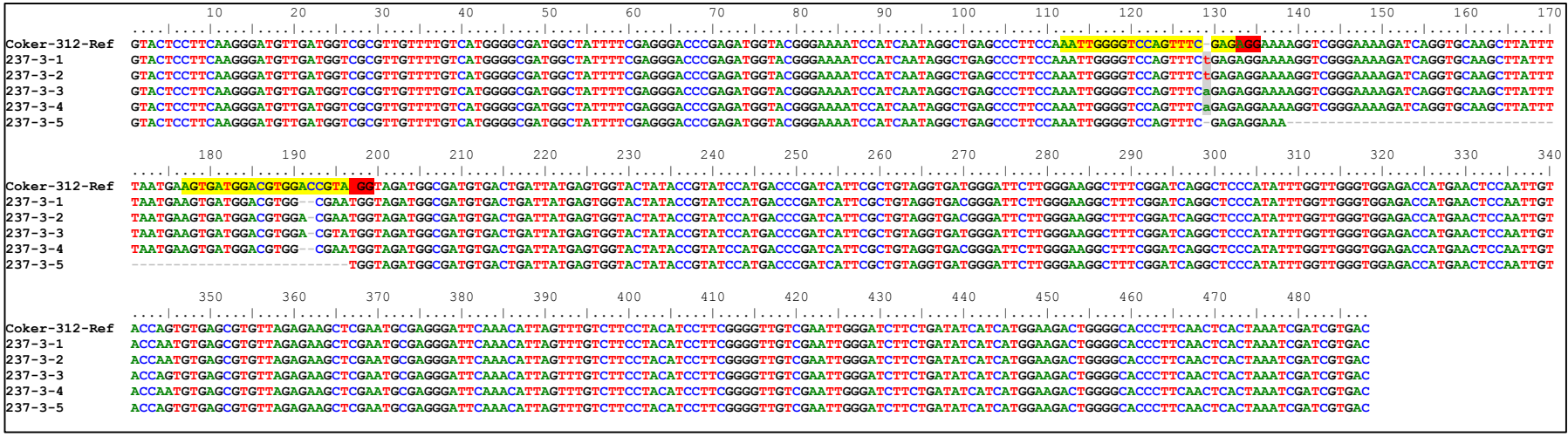


Figure S15 Mutations observed in two *CGF2* knockouts lines, (a) 236-8 and (b) 236-10. The two target sites are highlighted yellow and PAM sequences are red. Nucleotide insertions are shown in lower case and highlighted grey.

(a)



(b)

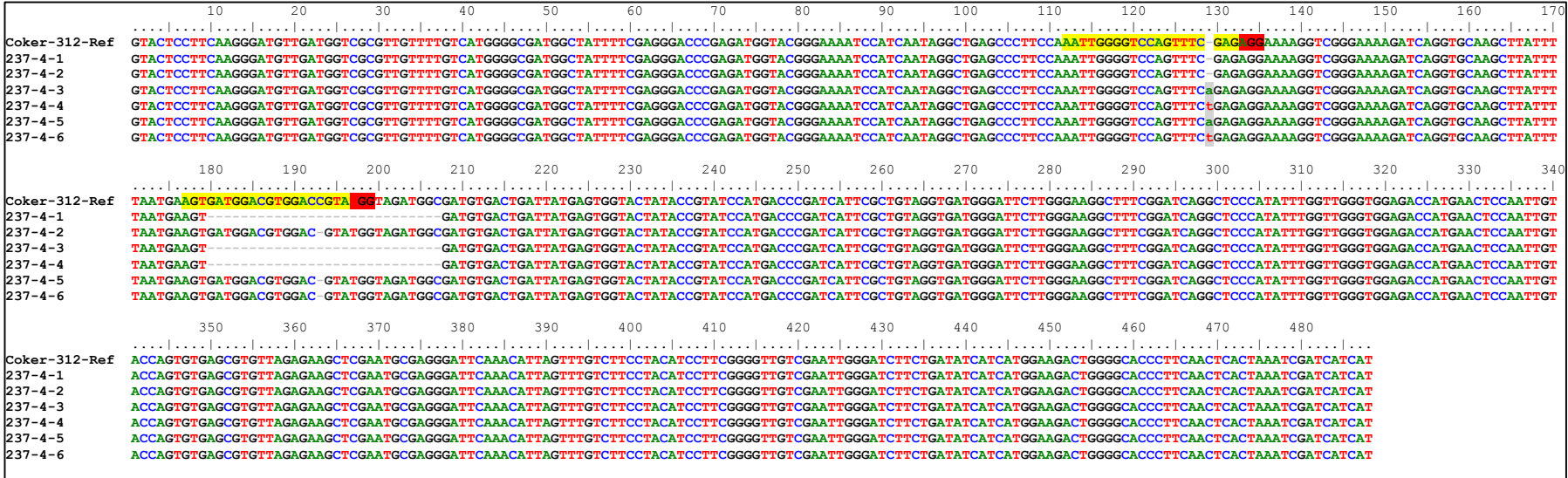


Figure S16 Mutations observed in two *GoPGF* (synonym *CGF3*) knockout lines, (a) 237-3 and (b) 237-4. The two target sites are highlighted yellow and PAM sequences red. Nucleotide insertions are shown in lower case and highlighted grey.

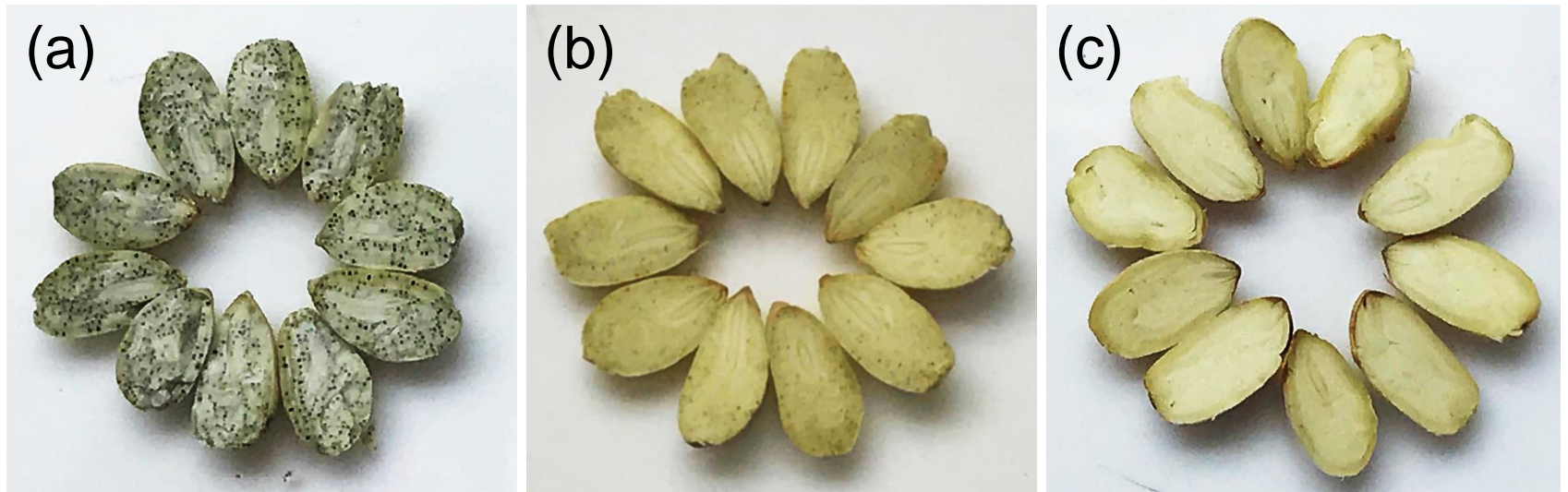


Figure S17 Effect of CRISPR/Cas9-mediated knockout of *CGF2* and *GoPGF* (synonym *CGF3*) genes on gland formation observed in cottonseed kernels. (a) Seeds of control plant showing well developed glands;(b) T1 seeds of line 236-10 showing smaller and malformed glands; (c) T1 seeds of line 237-3 showing complete absence of glands.

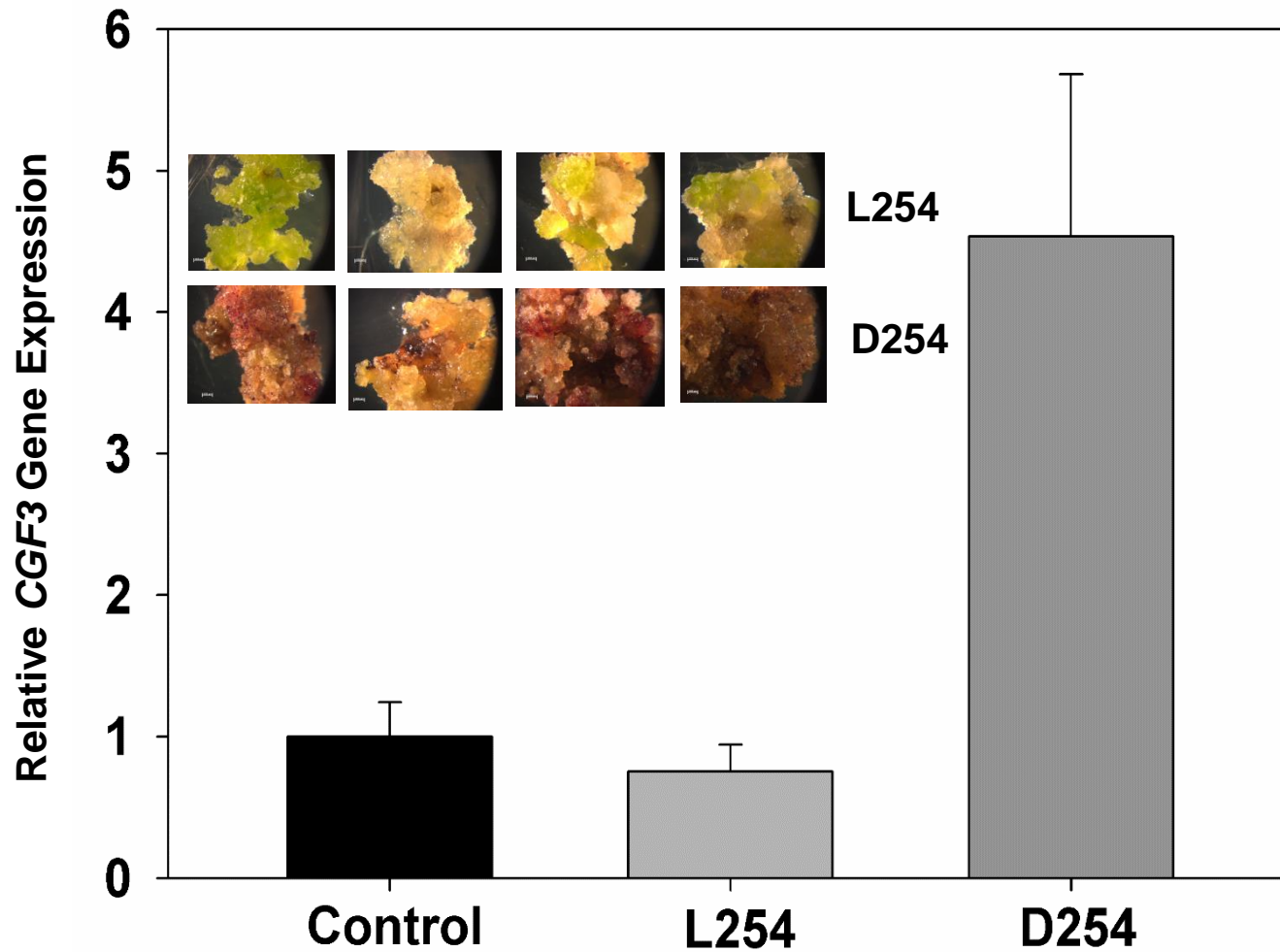


Figure S18 qRT-PCR analysis of *GoPGF* (synonym *CGF3*) transcripts in cotton callus cultures obtained following transformation with *ACGF3* overexpression construct. L254: light-colored callus lines; D254 dark-colored callus lines; Control: non transgenic callus.

Table S1 RNA-seq reads for glanded (GL; GVS4) and glandless (gl; GVS5) embryos at 14-, 16- and 32-days post-anthesis and their mapping to the reference genome.

Tissue	Quality filtered paired reads	Uniquely mapped reads	Reads mapped >1 time	Total mapped reads	Reads not mapped	% of reads not mapped	% of reads mapped one time	% of reads mapped >1 time	% total mapped
14GL	57,526,347	41,539,721	13,219,522	54,759,243	2,767,104	4.81	72.21	22.98	95.19
14gl	55,574,944	39,411,172	13,043,137	52,454,309	3,120,635	5.62	70.92	23.47	94.38
16GL	45,027,488	31,957,244	10,240,975	42,198,219	2,829,269	6.28	70.97	22.74	93.72
16gl	58,872,534	41,914,607	13,567,579	55,482,186	3,390,348	5.76	71.2	23.05	94.24
32GL	78,953,741	57,667,091	18,523,395	76,190,486	2,763,255	3.5	73.04	23.46	96.5
32gl	81,721,688	60,837,058	17,351,295	78,188,353	3,533,335	4.32	74.44	21.23	95.68
	377,676,742	273,326,893	85,945,903	359,272,796	18,403,946	5.05	72.13	22.82	94.95

Table S2 Genes that expressed at higher levels in the glanded embryos (STV GL; GVS4; *Gl₂Gl₂Gl₃Gl₃*) in comparison to those in the glandless (STV gl; GVS5; *gl₂gl₂gl₃gl₃*) embryos at 14-days post-anthesis stage of development based on RNA-seq analysis. Genes encoding putative transcription factors were tested for their role in gland formation using virus-induced gene silencing (VIGS). Note that because of a high degree of homology between the two homeologs, the same VIGS construct will silence both the copies in A and D subgenomes.

Gene	<i>G. hirsutum</i> Chrom- osome Number	Shrunk log fold change	FDR	Putative Function	Size bp	VIGS	Laboratory Designation
Gh_A11G0909	11 (A11)	3.459546	0.000000	Basic helix-loop-helix (bHLH) DNA-binding family protein	1488	Yes	<i>CGF1</i>
Gh_D11G1055	24 (D11)	6.412898	0.000000	Basic helix-loop-helix (bHLH) DNA-binding family protein	1488		<i>CGF1</i>
Gh_A01G0267	01 (A01)	4.992123	0.000001	NAC domain containing protein 42	960	Yes	<i>CGF2</i>
Gh_D01G0278	14 (D01)	4.656837	0.000013	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	963		<i>CGF2</i>
Gh_A12G2172	12 (A12)	6.231119	0.000000	Basic helix-loop-helix (bHLH) DNA-binding family protein	1428	Yes	<i>CGF3</i>
Gh_D12G2351	25 (D12)	4.284841	0.001531	Basic helix-loop-helix (bHLH) DNA-binding family protein	1428		<i>CGF3</i>
Gh_A10G0388		4.903984	0.000002	Jasmonate-zim-domain protein 8	363	Yes	
Gh_D10G0403		3.747897	0.003033	Jasmonate-zim-domain protein 8	363		
Gh_A12G1233		5.43426	0.000000	B-box type zinc finger family protein	393	Yes	
Gh_D12G1358		2.11097	0.000000	B-box type zinc finger family protein	393		
Gh_A01G0135		2.064435	0.000139	Zinc finger C-x8-C-x5-C-x3-H type family protein	1020	No	
Gh_A04G0546		4.370138	0.001173	HEAT repeat ;WD domain, G-beta repeat protein	192	No	
Gh_A05G0334		2.677935	0.000088	NAD(P)-binding Rossmann-fold superfamily protein	987	No	
Gh_A05G2973		2.888822	0.000000	Lysine histidine transporter 1	1077	No	
Gh_A06G0017		2.788916	0.000000	Thioredoxin superfamily protein	555	No	
Gh_A06G0018		2.25659	0.008180	Expansin 11	768	No	

Gh_A06G0213		2.775923	0.012662	Uncharacterized protein	957	No	
Gh_A06G1947		4.176235	0.000392	NAC domain containing protein 42	930	Yes	
Gh_A08G2056		2.912436	0.000230	NAC domain containing protein 42	867	Yes	
Gh_A10G0667		5.494345	0.000000	Pectin lyase-like superfamily protein	2202	No	
Gh_A12G1784		1.995549	0.000013	Integrase-type DNA-binding superfamily protein	711	No	
Gh_A12G2056		2.388568	0.000000	Uncharacterized protein	1251	No	
Gh_A13G0385		2.137374	0.023065	S-methyl-5-thioribose kinase	660	No	
Gh_D04G0529		3.720997	0.028702	Phosphoenolpyruvate (pep)/phosphate translocator 2	1251	No	
Gh_D05G0292		2.546437	0.031860	myb-like transcription factor family protein	768	Yes	
Gh_D05G0439		2.126081	0.007311	NAD(P)-binding Rossmann-fold superfamily protein	894	No	
Gh_D06G1859		2.756698	0.000000	P-loop containing nucleoside triphosphate hydrolases superfamily protein	987	No	
Gh_D07G2328		4.295568	0.000100	WRKY family transcription factor family protein	501	Yes	
Gh_D08G2336		3.448213	0.031860	Uncharacterized protein	345	No	
Gh_D11G0631		3.446236	0.007958	Uncharacterized protein	417	No	
Gh_D11G0996		4.052173	0.008242	Cytokinin response factor 6	894	No	
Gh_D12G1160		5.449322	0.000000	Basic helix-loop-helix (bHLH) DNA-binding family protein	1464	Yes	
Gh_Sca007330G01		4.131307	0.000000	Plant invertase/pectin methylesterase inhibitor superfamily protein	630	No	

Table S3 Primers used to amplify segments of the coding sequence of the target gene for cloning into TRV2 binary vector to conduct VIGS experiments.

Primers	Sequences (5' to 3')	size
GhA01G0267 EcoRI-F	CGgaattcCTGGGATCTCCCGAAAGCTAGC	634
GhA01G0267 SacI-R	ACGCgagctcCTCATTCTATCTGTAACATGCCATTGGC	
GhA10G0388 EcoRI-F	CGgaattcATGAGACGAAACTGCAACTTGGAG	357
GhA10G0388 SacI-R	ACGCgagctcGTAAGGAGAGGTAGCTTGGATTCCG	
GhA12G2172 EcoRI-F	CGgaattcATGTCTTCTCTTCTTCGTCTTCTC	600
GhA12G2172 SacI-R	ACGCgagctcCGATTTAGTGAGTTGAAGGGTGC	
GhA12G1233 XbaI-F	GCtctagaATGTGCAAAGGTTTACAACAAGGAAG	366
GhA12G1233 XmaI-R	TCCcccgggGGTTGTTGAAGACTCGGTTTCCGTG	
GhD07G2328 XbaI-F	GCtctagaTCAAATGTTCTTCCCTATCTCGG	491
GhD07G2328 XmaI-R	TCCcccgggTCAGAAGGGAGTGTAATCTGCA	
GhD11G1055 XbaI-F	GCtctagaATGGAAGTCCTCATAATGTCTCCCTC	628
GhD11G1055 XmaI-R	TCCcccgggCCAGACCAATGAGATCGGATTC	
GhA06G1947 XbaI-F	GCtctagaATGGAAGATGTGGAGATGGAGA	505
GhA06G1947 XmaI-R	TCCcccgggCTTCAAAGTTGTCTTTGGCATG	
GhD05G0292 XbaI-F	GCtctagaATGGGCAGGAAATGCTCACATTG	614
GhD05G0292 XmaI-R	TCCcccgggAATCAATGCATCCGTACTIONGCAAC	
GhD12G1160 XbaI-F	GCtctagaATGGAAGAACTAATCATCTCTCCATC	587
GhD12G1160 XmaI-R	TCCcccgggGATCCAAGTTCAAGAACACCACG	
GhA08G2056 XbaI-F	GCtctagaATGAGCATGGTCCATGGCACCA	630
GhA08G2056 XmaI-R	TCCcccgggTATCTTAACGATGGCTGCATGAACC	

Table S4 Primers used to amplify and isolate *CGF* genes from A and D subgenomes of glanded (GVS4) and glandless (GVS5) cotton plants.

Primer	Sequence 5' to 3'	Purpose
A11GhCGF-prom-3F	CTCTCCAAAATCAACCATACTCACAAATGCCTAC	To amplify CGF1 from A genome
A11GhCGF-term-R	CTCCATGGCATCCTCAAGTCACAG	
D11GhCGF-prom-F	ATCTTCTCACTCCGAAACCGACC	To amplify CGF1 from D genome
D11GhCGF-term-R	TGGAAGAAACAAGATCGGATGTGGC	
A01CGF2-P-F	GGCTGTCAGATGTAGTAAAATCAGTATTGGT	To amplify CGF2 from A genome
A01CGF2-T-R	CAAATATATATGGGTCTGATATGCATGTCTCC	
D01CGF2-P-F	CAAAGTGTTGATTTCAAGCAATAACTTGTAGC	To amplify CGF2 from D genome
D01CGF2-T-R	CGTAACAAAATGGTTTTTCGTATGTTACGTATC	
A12CGF-prom-F	CATCCATACAAACTATTAACAAGATTACGTCGGATG	To amplify CGF3 from A genome
A12CGF-term-R	GTGATCATCATCAAGCACAGGCTACTG	
D12CGF-Prom-F	CAAACCATCAACAAGACTACGTTGGACA	To amplify CGF3 from D genome
D12CGF-term-R	CTAATTTAAGTGATCATCATCAAGCACAGTCTAATC	

Table S5 Guide sequences used to target *CGF2* and *GoPGF* (synonym *CGF3*) genes.

Target name	Sequence 5' to 3'
CGF2-guide-1	GCTCAAACAGGTGATCATCA
CGF2-guide-2	GATTGGAAAAGGCGACGACAG
CGF3-guide-1	AATTGGGGTCCAGTTTCGAG
CGF3-guide-2	AGTGATGGACGTGGACCGTA
CGF3-guide-3	GCTTCTCTAACACGCTCACAC

Table S6 Primers used for amplicon sequencing of regenerated plants targeted with LCT236, LCT237 and LCT238 constructs. These primers contained barcodes to distinguish amplicons from different plants.

Primers	Sequences (5'-3')
A_CGF2.Ampseq-F	gtactcAAGTTGATGATGTGTGTTGGTGATG
B_CGF2.Ampseq-F	tctagcAAGTTGATGATGTGTGTTGGTGATG
C_CGF2.Ampseq-F	gagtcaAAGTTGATGATGTGTGTTGGTGATG
D_CGF2.Ampseq-F	gctagtAAGTTGATGATGTGTGTTGGTGATG
E_CGF2.Ampseq-F	atgctaAAGTTGATGATGTGTGTTGGTGATG
F_CGF2.Ampseq-F	ctgcgaAAGTTGATGATGTGTGTTGGTGATG
G_CGF2.Ampseq-R	cgactgATAACATTGATTAACCCAACTTGAGC
H_CGF2.Ampseq-R	tgatagATAACATTGATTAACCCAACTTGAGC
I_CGF2.Ampseq-R	gtcacgATAACATTGATTAACCCAACTTGAGC
J_CGF2.Ampseq-R	atgatgATAACATTGATTAACCCAACTTGAGC
K_CGF2.Ampseq-R	cagtcaATAACATTGATTAACCCAACTTGAGC
L_CGF2.Ampseq-R	acgtcaATAACATTGATTAACCCAACTTGAGC
A_CGF3.Ampseq-F	gtactcCTTCAAGGGATGTTGATGGTCG
B_CGF3.Ampseq-F	tctagcCTTCAAGGGATGTTGATGGTCG
C_CGF3.Ampseq-F	gagtcaCTTCAAGGGATGTTGATGGTCG
D_CGF3.Ampseq-F	gctagtCTTCAAGGGATGTTGATGGTCG
E_CGF3.Ampseq-F	atgctaCTTCAAGGGATGTTGATGGTCG
F_CGF3.Ampseq-F	ctgcgaCTTCAAGGGATGTTGATGGTCG
G_CGF3.Ampseq-R	cgactgATCGATTTAGTGAGTTGAAGGGTGC
H_CGF3.Ampseq-R	tgatagATCGATTTAGTGAGTTGAAGGGTGC
I_CGF3.Ampseq-R	gtcacgATCGATTTAGTGAGTTGAAGGGTGC
J_CGF3.Ampseq-R	atgatgATCGATTTAGTGAGTTGAAGGGTGC
K_CGF3.Ampseq-R	cagtcaATCGATTTAGTGAGTTGAAGGGTGC
L_CGF3.Ampseq-R	acgtcaATCGATTTAGTGAGTTGAAGGGTGC