1 **The sweet potato *RbcS* gene (*IbRbcS1*) promoter confers high-level and green**
2 **tissue-specific expression of the *GUS* reporter gene in transgenic Arabidopsis**

3

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19

1 **1. Introduction**

2 Because more than 90% of crop biomass is derived from photosynthetic
3 products, enhancement of the photosynthetic capacity of plants has been reported to
4 increase crop yields (Yamori, 2013). Plant transformations have been proposed to
5 increase the inherent yielding capability of plants (Sinclair et al., 2004). In the Calvin
6 cycle, sedoheptulose 1,7-bisphosphatase (SBPase), transketolase (TK), and fructose
7 1,6-bisphosphate aldolase (aldolase) are considered to have higher control coefficients
8 on photosynthesis than other Calvin cycle enzymes and, thus, are candidate targets of
9 engineering to improve photosynthetic carbon fixation (Raines, 2011). However,
10 significant improvements in plant yield by engineering carbon assimilation have only
11 been achieved in a few cases (Miyagawa et al., 2001; Yokota and Shigeoka, 2008;
12 Uematsu et al., 2012). We previously reported that transgenic tobacco and lettuce
13 plants overexpressing the fructose-1,6-/sedoheptulose-1,7-bisphosphatase
14 (FBP/SBPase) of *Synechococcus elongatus* PCC7942 (*S.7942*) in their chloroplasts
15 showed an enhanced CO₂ assimilation rate and increased biomass production
16 (Miyagawa et al., 2001; Yabuta et al., 2008; Ichikawa et al., 2010).

17 Promoters are regions of DNA upstream of the coding region that contain
18 specific sequences, which regulate gene transcription. Constitutive promoters such as
19 the Cauliflower mosaic virus 35S (CaMV 35S) are widely used in the genetic
20 engineering of plants. However, since the constitutive expression of foreign proteins
21 in transgenic plants can often have adverse effects, the utilization of an appropriate
22 promoter is important for the transgene expression (Bakhsh et al., 2011).
23 Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the initial

1 carbon dioxide fixation step in the Calvin cycle and also catalyzes the oxygenase
2 reaction leading to the photorespiration (Hartman and Harpel., 1994). In higher plants,
3 Rubisco comprises eight large subunits (RbcL) and eight small subunits (RbcS)
4 (Andersson and Backlund, 2008). RbcL is encoded by a single gene on the
5 chloroplast genome, while RbcS is encoded by a multigene family in the nuclear
6 genome. The RbcS multigene family consists of 2-22 members, depending on the
7 species (Sasanuma, 2001; Spreitzer, 2003). RbcS polypeptides are formed as
8 precursors that contain a transit peptide, which is removed during transport into
9 chloroplasts. Rubisco is the most abundant protein found in plant leaves and
10 comprises approximately 50-60% of soluble protein (Ellis, 1979). Thus, *RbcS*
11 promoters and their transit peptides are attractive candidates for the expression of
12 exogenous genes at high levels in green tissue and the targeting of exogenous proteins
13 into chloroplasts.

14 Sweet potato (*Ipomoea batatas*) is one of the most important crops in the
15 world and represents an attractive target for molecular breeding because of its clonal
16 propagation, ease of cultivation, and the high productivity of its storage roots and
17 leaves. However, sweet potato is a genetically hexaploid plant and its genomic
18 sequence has not yet been decoded; therefore, few studies have examined gene
19 promoters from sweet potato. The promoter activity of sweet potato *ADP-glucose*
20 *pyrophosphorylase* (*IbAGP1*) was previously shown to confer the high-level
21 expression of the *GUS* gene in the leaves and tubers of a transgenic potato (Kim et al.,
22 2009). Furthermore, the promoter activity of sweet potato *sporamin* was highly active

1 in the tubers of a transgenic potato (Hong et al., 2008). However, information
2 regarding green tissue-specific promoters from sweet potato is limited. In the present
3 study, we cloned two *RbcS* genes (DDBJ accession number LC036584 and LC036585)
4 from the sweet potato genome. We demonstrated the green tissue-specific expression
5 of the *IbRbcS1* gene and studied the promoter activity of the 5'-flanking sequences of
6 *IbRbcS1* using *GUS* as a reporter gene in Arabidopsis plants.

8 **2.# Materials and methods**

9 **2.1. Plant materials and growth conditions**

10 Sweet potato (*I. batatas* L. cv. Kokei 14) plants were grown in soil under
11 28°C/23°C day-night temperatures and 14-h photoperiod (light intensity of 500 μmol
12 $\text{m}^{-2} \text{s}^{-1}$). Arabidopsis (*Arabidopsis thaliana*) ecotype Columbia-0 plants were grown
13 on MS medium (Murashige and Skoog, 1962) containing 3% sucrose under 25°C/23°C
14 day-night temperatures and 14-h photoperiod (light intensity of 100-150 $\mu\text{mol m}^{-2} \text{s}^{-1}$).
15

16 **2.2. Isolation of IbRbcS genes**

17 All primers used for the isolation of *IbRbcS* genes are listed in Supplemental
18 Table S1. Genomic DNA was isolated from the leaves of sweet potato using DNA
19 suisui-VS (Rizo, Tsukuba, Japan). Isolated genomic DNA was treated with RNase A
20 (Thermo scientific, Waltham, MA) before being subjected to PCR. DNA fragments
21 of *IbRbcS1* and *IbRbcS2* were amplified by PCR using degenerated primers (Firon et
22 al., 2013). The PCR amplification was performed using Gflex polymerase (Takara,
23 Shiga, Japan) with an initial enzyme activation step of 2 min at 95°C, followed by 35

1 cycles of 10 s at 98°C, 15 s at 55°C, 15 s at 68°C, and a final extension of 5 min at 68°C.
2 The PCR products obtained were directly cloned into the pMD20-T vector using the
3 Mighty TA-cloning Reagent set for PrimeSTAR (Takara, Shiga, Japan).
4 Insert-containing clones were then grown and the plasmid DNAs were isolated.
5 Nucleotide sequences were analyzed using a Big-Dye terminator Cycle Sequencing
6 Ready Reaction Kit and ABI 3100-Avant Genetic Analyzer (Applied Biosystems, CA,
7 USA). The 5'- and 3'- flanking regions of *IbRbcS* genes were isolated using an LA
8 PCR *in vitro* cloning Kit (Takara, Shiga, Japan). Genomic DNA was digested for 5 h
9 using one of seven restriction enzymes (*EcoRI*, *XbaI*, *Sall*, *HindIII*, *BamHI*, *PstI*, or
10 *Sau3A*) and ligated with each restriction enzyme cassette, respectively. The first PCR
11 reaction was performed using the ligated genomic DNA as a template with a cassette
12 and gene-specific primer. The PCR amplification steps was performed using Gflex
13 polymerase with an initial enzyme-activation step of 2 min at 95°C, followed by 35
14 cycles of 10 s at 98°C, 15 s at 55°C, 1 min at 68°C, and a final extension of 5 min at
15 68°C. The PCR products were used as a template for second PCR with a cassette and
16 gene-specific primer following the PCR procedure described above. The PCR
17 products were cloned into the pMD20-T vector and sequenced as described above.

18

19 **2.3. Construction of plant expression vector and generation of transgenic** 20 ***Arabidopsis* plants**

21 All primers used for plasmid construction are listed in Supplemental Table
22 S1. To construct *IbRbcS1 pro:GUS* chimeric genes, the 5'-flanking region of

1 *IbRbcS1* (from -991 to -1, numbered from the ATG initiation site) was amplified with
2 primers containing *HindIII* and *NdeI* at the 5' and 3' ends of the *IbRbcS1* promoter
3 gene, respectively, and cloned into pMD20-T. The promoter fragment was ligated
4 upstream of *GUS* in the binary vector pRI201-AN *GUS* (Takara, Shiga, Japan).
5 Arabidopsis was transformed via *Agrobacterium tumefaciens*-mediated gene transfer.
6 Transformed seeds were selected in the T1 generation on MS plates with 50 µg/mL
7 kanamycin. Seed batches that contained single insert heterozygote seeds and showing
8 a 1/4 death ratio on the selection plates were used to obtain homozygote lines at T2
9 stage. Transgenic lines designated *IbRbcS1 pro:GUS* 4-1 and 6-3 at T3 generation,
10 were used in subsequent experiments.

11

12 **2.4. RT-PCR**

13 All primers used for the RT-PCR are listed in Supplemental Table S1.
14 Total RNA was extracted from the various tissues of sweet potato using RNA suisui-R
15 (Rizo Tsukuba, Japan) or Arabidopsis using Plant RNA Reagent RNazol (Life
16 technologies, Carlsbad, CA). Two micrograms of total RNA were treated with
17 RNase-free DNaseI (Thermo scientific, Waltham, MA). After extraction with a
18 phenol:chloroform (1:1) solution and ethanol precipitation, 1st strand cDNA was
19 synthesized from 1 µg of total RNA using the PrimeScript 1st strand cDNA Synthesis
20 Kit (Takara, Shiga, Japan). Semi-quantitative RT-PCR was performed using Gflex
21 polymerase and the primers listed in Supplemental Table S1. DNA polymerase was
22 first activated at 94°C for 2 min, and PCR was carried out for 23 to 26 cycles of 10 s at

98°C, 15 s at 55°C, and 15 s at 68°C, followed by a final extension step for 5 min at 68°C.

2.5. GUS staining

Histochemical GUS staining was performed according to the method described by Jefferson et al. (1987). Plants were incubated with GUS staining solution (50 mM HEPES-NaOH, 0.5 mM 5-bromo-4-chloro-3-indolyl- β -D-glucuronide, 0.5 mM K₃Fe(CN)₆, 0.5 mM K₄Fe(CN)₆, and 0.05% [v/v] Triton X-100, pH 7.5) for 8 to 12 h at 37°C. An ethanol wash was performed to stop the GUS reaction and remove chlorophyll from the plants.

2.6. qPCR

All primers used for the Quantitative Real-Time PCR (qPCR) are listed in Supplemental Table S1. qPCR was performed with a LightCycler 96 System (Roche, Basel, Switzerland), using the FastStart Universal SYBR Green Master (ROX) (Roche, Basel, Switzerland). Quantities were determined from a standard curve and were normalized to the amount of 18S rDNA. At least four experimental replicates were performed and standard errors were determined.

2.7. Accession number

Nucleotide sequences of *IbRbcS1* and *IbRbcS2* have been deposited at DNA Data Bank of Japan (DDBJ: <http://www.ddbj.nig.ac.jp>). Accession number of

IbRbcS1 and IbRbcS2 was LC036584 and LC036585, respectively.

3. Results and Discussion

3.1. Isolation and characterization of *IbRbcSs*

In order to isolate sweet potato *RbcS* genes, genomic DNA was isolated from the leaves and subjected to genomic PCR using degenerated *RbcS* primers. Two different DNA fragments were amplified and sequenced at least three independent clones. Sequence analysis revealed that those DNA fragments were identified with similarity to the known *RbcS* genes of other plants. Based on the identified partial sequence of the *RbcS* genes, the remaining 5' and 3'-sequences were obtained by cassette-ligation mediated PCR. We identified two *IbRbcS* genes (*IbRbcS1* and *IbRbcS2*) and the *IbRbcS* cDNA fragments were then obtained by RT-PCR. A sequence analysis of these cDNA fragments revealed that three exons and two introns were assigned to the coding region in both *IbRbcS* genes (Fig.1A and 1B). The consensus GT donor and AG acceptor sequences at the 5'- and 3'-termini of the introns, respectively, were found in all introns in the *IbRbcS1* and *IbRbcS2* genes. The numbers of introns of *RbcS* genes were divided into three groups, the *RbcS* ORFs of which contained one (*Oryza sativa* and *Zea mays*), two (*Arabidopsis thaliana* and *Solanum lycopersicum*), or three (*Solanum lycopersicum RbcS2*) introns, indicating that the exon/intron structure of the *IbRbcS* genes was more similar to those of the *Arabidopsis thaliana RbcS* genes (Lebrun et al., 1987; Sugita et al., 1987; Krebbers et al., 1988; Xie and Wu., 1998). However, intron 2 (526 bp) of *IbRbcS2* was longer

1 than that of other plant RbcS genes. The deduced amino acid sequences of the
2 IbRbcS1 and IbRbcS2 proteins contained 177 and 167 amino acid residues,
3 respectively, and showed higher identities with other mature RbcS proteins (69-80%
4 and 53-61%, respectively) (Fig. 2). The putative transit peptide of the IbRbcS1
5 protein was well conserved with other plant RbcS proteins, while that of the IbRbcS2
6 protein had a lower degree of identity.

8 **3.2. Tissue-specific expression of RbcSs in sweet potato**

9 The tissue-specific expression levels of *IbRbcS1* and *IbRbcS2* mRNA in
10 2-month-old leaves, stems, and roots and 4-month-old tubers were examined by a
11 semi-quantitative RT-PCR using *IbRbcS1*- or *IbRbcS2*-specific primers. The
12 expression of *IbRbcS1* was detected in green tissues such as leaves and stems. The
13 expression of *IbRbcS2* was not detected in leaves, but was in stems, roots, and tubers
14 (Fig. 3). Although number of *RbcS* genes in sweet potato was still unknown, these
15 results implied that IbRbcS1 was one of the main RbcS family member in the leaves of
16 sweet potato and also indicated that the promoter of *IbRbcS1* was eligible for the green
17 tissue expression of transgenes. Recently, a functional RbcS that is not expressed in
18 leaves was identified in rice (Morita et al., 2014). Detailed analyses of IbRbcS2 will
19 be interesting for understanding the function of RbcS family in plants. We then
20 cloned a -991 bp 5' flanking sequence of the *IbRbcS1* gene (Fig. 4). The putative
21 *cis*-acting regulatory DNA elements in the 5'-flanking region of the *IbRbcS1* gene were
22 identified by the PLACE program (PLACE:

1 <http://www.dna.affrc.go.jp/PLACE/signalscan.html>) (Higo et al., 1999). A TATA
2 box element was located -86 to -80 from the ATG initiation site. The presumptive
3 promoter sequence including AATCCAA, which was the same as the RbcS conserved
4 sequence, was located at -165 to -159. The regulatory *cis*-acting sequences, the
5 CCAAT element, GATA motif, G-box, and I-box, were present as observed in
6 promoters from light-regulated genes including the *RbcS* genes of many plants.

8 **3.3. Analysis of the *IbRbcS1* promoter in *Arabidopsis* plants**

9 The sequences located upstream of the *IbRbcS1* start codon were tested for
10 their capacity to express the *GUS* reporter gene in the leaves of transgenic *Arabidopsis*
11 plants. The -991 bp putative *IbRbcS1* promoter region was cloned in the binary
12 vector pRI201-AN GUS (Takara, Japan) to drive β -glucuronidase. Histochemical
13 staining revealed strong GUS signal in the leaves and stems of *IbRbcS1 pro:GUS*
14 plants, in contrast to the constitutive GUS expression driven by the CaMV 35S
15 promoter (Fig. 5A). To obtain further information on the expression patterns of the *Ib*
16 *rbcS1* promoter, semi-quantitative RT-PCR was performed using GUS-specific primers
17 on total RNA extracted from leaves and roots. In accordance with GUS staining data,
18 the semi-quantitative RT-PCR analysis revealed that the expression of *GUS* was only
19 detected in the leaves of *IbRbcS1 pro:GUS* plants, but was detected in the leaves and
20 roots of *CaMV 35S pro:GUS* plants (Fig. 5B).

22 **3.4. The *IbRbcS1* promoter conferred the light-response expression of transgenes**

1 We determined whether the expression of *IbRbcS1* was regulated by light in
2 the leaves of sweet potato by semi-quantitative RT-PCR using *IbRbcS1*-specific
3 primers. Sweet potato plants were grown under a 14-h/10-h light/dark photoperiod
4 and samples were harvested at 3-h intervals from 0-h (dark period) to 12-h. The
5 expression of *IbRbcS1* was induced at 3-h and increased during the photoperiod (Fig.
6 6). We examined the expression of the *GUS* gene in Arabidopsis in order to evaluate
7 the light-response capability of the *IbRbcS1* promoter. In accordance with the
8 expression pattern of *IbRbcS1* in sweet potato, the expression of the *GUS* gene was
9 induced at 3-h and increased during the photoperiod in *IbRbcS1 pro:GUS* plants (Fig.
10 6B). The expression of *RbcS* gene family members is known to be light-dependent,
11 and a large number of light-responsive *cis*-elements have been identified in *RbcS*
12 promoters. Two *cis*-acting elements, the I-box and G-box, were previously shown to
13 be important for tissue-specific expression (Donald and Cashmore 1990; Manzara et al.,
14 1991). The expression of *GUS* driven by the tomato *RbcS2* and *RbcS3A* promoters
15 was detected in specific organs of the transgenic tomato such as the leaves and young
16 tomato fruit (Iris et al., 1995). In these genes, the I-box and G-box were located -600
17 to -100 bp upstream of the transcription initiation site. In the *IbRbcS1* promoter
18 region, the I-box and G-box were located -550 to -100 bp upstream of the start codon
19 (Fig. 4), suggesting that these regulatory elements were involved in the
20 light-responsive and green tissue-specific gene expression of the *IbRbcS1* gene. In
21 order to evaluate the activity of the *IbRbcS1* promoter, we compared the expression
22 levels of the *GUS* gene in the leaves of *IbRbcS1 pro:GUS* and *CaMV 35S pro:GUS*

1 plants at 12-h light period after transition from the dark by qPCR. The qRT-PCR
2 analysis revealed that the expression levels of the *GUS* gene in *IbRbcS1 pro:GUS*
3 plants were same as those in *CaMV 35S pro:GUS* plants (Fig. 7). A previous study
4 reported that the targeting of GUS into plastids using *IbAGPI* transit peptides was
5 highly effective for elevating the accumulation of foreign protein in transgenic plants
6 (Kwak et al., 2007). Thus, it is likely that the application of *IbRbcS1* transit peptide
7 sequences for the accumulation of proteins in the chloroplasts of transgenic plants.

9 **4. Conclusion**

10 We herein obtained the complete cDNA sequences of *RbcS* genes from
11 sweet potato (*IbRbcS1* and *IbRbcS2*). The deduced amino acid sequences were well
12 conserved with plant RbcS proteins. The expression of *IbRbcS1* was green
13 tissue-specific and was also regulated by light, whereas that of *IbRbcS2* was specific to
14 non-photosynthetic tissues, suggesting that the IbRbcS protein functioned as one of the
15 main RbcS family member in sweet potato. The expression of GUS driven by the
16 5'-flanking sequence of *IbRbcS1* was green tissue-specific and was also regulated by
17 light in transgenic plants. Compared with other plants, information regarding gene
18 promoters in sweet potato is limited. The expression system using the *IbRbcS1*
19 promoter and its transit peptide sequences may offer promise as high expression of
20 transgene in green tissue-specific manner in sweet potato.

1

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8

1 **Figure legends**

2 **Fig. 1** Nucleotide sequences of (A) *IbRbcS1* and (B) *IbRbcS2* genes. The non-coding
3 regions including the protein coding regions and 150-bp of the flanking region are
4 shown. Uppercase and lowercase letters indicate the coding region and non-coding
5 region, respectively.

6

7 **Fig. 2** Multiple alignments of deduced amino acid sequences of RbcS among *Ipomoea*
8 *batatas* RbcS1 and S2, *Arabidopsis thaliana* (GenBank accession no. P10797),
9 *Nicotiana tabacum* (P69249), *Solanum tuberosum* (P26577), *Solanum lycopersicum*
10 (CAA29402), and *Zea mays* (NP_001105294). The amino acid residues common to
11 at least four among species with IbRbcS1 or IbRbcS2 are colored gray. The filled
12 arrowhead shows the cleavage site of the transit peptide for targeting to the chloroplast.
13 The open arrowheads show the positions of the intron in *IbRbcS1* and *IbRbcS2*.
14 Sequences were analyzed with GENETYX-MAC Version 17.

15

16 **Fig. 3** Expression of *IbRbcS1* and *IbRbcS2* mRNAs in various tissues of sweet potato.
17 Actin was used as a control for the experiments. Amplified products were
18 electrophoresed on 1.5% (w/v) agarose gels. L, leaves; S, Stems; R, roots; T, tubers; M,
19 100 bp Ladder DNA marker (Takara).

20

21 **Fig. 4** Nucleotide sequences of the 5'-flanking regions (from -991 to -1) of the *IbRbcS1*
22 gene determined in this study. Putative *cis*-acting elements including the TATA box,
23 RbcS conserved, GATA motif, G-box, and I-box were masked with gray. Numbers

are nucleotides relative to the 5'-flanking regions of the protein coding region.

Fig. 5 The expression of GUS in *IbRbcS1 pro:GUS* plants. (A) GUS histochemical staining of 2-week-old seedlings grown *in vitro* (*CaMV 35S pro:GUS* and *IbRbcS1 pro:GUS* plants). (B) Expression of *GUS* mRNA in the leaves and roots of transgenic Arabidopsis plants. *CaMV 35S pro:GUS* was used as a control. Amplified products were electrophoresed on 1.5% (w/v) agarose gels. L, leaves; R, roots.

Fig. 6 Effects of light on the expression of the *IbRbcS1* gene or *GUS* gene. (A) The expression levels of the *IbRbcS1* gene in the leaves of sweet potato grown under a 14-h/10-h light/dark photoperiod. *Actin* was used as a control for the experiments. (B) The expression of the *GUS* gene was driven by the *IbRbcS1 promoter* in the leaves of transgenic plants. *Actin8* was used as a control for the experiments. Amplified products were electrophoresed on 1.5% (w/v) agarose gels.

Fig. 7 Quantitative Real-Time PCR analysis of the *GUS* gene in transgenic Arabidopsis plants. *CaMV 35S pro:GUS* was used as a control. Values are the means $\pm$ SE of four replications for relative normalized expression.

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16

A *IbRbcS1*

cttcctcaaagattaggttgattaaacgtcgcccgtagattggactcatgtgacaccttatcgttatatataccaagcaatagcacactatcaccaccattacaaagtaaagtagtaag	120
taatacagagaagagcggcagctgctagcaATGGCTTCCTCAGTCTCTCTCCACCGCCATCGCCTCCCGCGCCACCGCTGCCCAGGCCAGCATGGTCGCCCCCTTCACCGGCCTCAAG	240
TCCACCGCCTCCTTCCCCGTACCAAGAAGTCCGCGACTTTACTTCCATCTCCACCAACGGCAGCCGAGTACAATGCATGCAGgtgtatatataataactataaccctcagttaact	360
gaggggtgtacactgggttaacccccgccttatatatatgatgtgcagGTGTGGCCCCCATACGGGAAGATGAAGTTCGAGACCCGTGTACACTGCCCCATTGAGCACCGAGCAATTGATC	480
AAACAAGTGGAGTACCTGCTGAACAACGGTTGGGTTCCTTGCTTGAATTTCGAGACCGAGgtcaaaatatagtcactagttaagttactaatatataaattagttatatgtgagtttaaa	600
cttttagttttgactggggtattatgtacagAAAGGATTCGTGTACCGTGAATGCCACAGGTCACCAGGGTACTATGACGGAAGATACTGGACCATGTGGAAGCTGCCCATGTTCGGGTG	720
CACCGACGCGACTCAGGTGATTGCTGAGATGCAGGAGTGCAAGAAGGCTTACCTGAAGCGTGGGTAGAAATCCTGGGATTCGACAACGTGCGCCAAGTGCAGTGCATCATGTTCTCTTGC	840
AGCCATGCCCCAACTAAtagattccatcacacatcatccattaattcctgatagatggatggattccattgcatgatgttttcccattaattaatcttgtcgtccttttgtttttgattt	960
caattcggtcacatgttaagttggttgcatcccatgccttggatc	1056

B *IbRbcS2*

ctcccccaaaccaacccaccattttcagtggtgccctcaaaaaataaccttttatcatctctacataacctccattttctcaaccttttatctccctaatttgcttctgtgatcatctgt	120
agctagctagctacctcaattttctcaaagATGTCTCTGTGTTTTCTTCGGCTCCGTTTGGTGTGGCGGATCAAGCTTTGTTGGCCTCAAACGCACCCTTCTTCCTTCCAACACTACC	240
GTTTCTTGGAGCCGCAAAACAGTTTCCAATGGCTCCAAAACCTCTGTGTATGAAGgtaaactagtatatgtttttatactttctcaactctgccaaaaaatctaataatgcccctgtttttttgtt	360
tttcataagtttattaatttgtagagttgatctgagatgatgtttgtatgttttggtgattaaatatttggttggtggggtgcagACTTGAATCCAATTGATAACAAAAGGTTTCGAGAC	480
ACTCTCCTACCTTCCCCACTCTCACAGGAATCTATTGCAAAGGAAGTTGATTACATTATTAACAAGGGATGGATTCCCTTGCCTTGAATTTGATCAGgttttgtatttgtaagatgaagc	600
acttatcactctctgccataaattatacggagtaactatttagtcttaattgagagcagatgtattatgatcggtatacaattgtatggaatttgaaataaaagtattatattctaaataaa	720
acaataaaaatatattttttggtatataaacaattaaaatatataaataatacaattatgctaatttttctgtttattttatttagaaaaatgaactaaatagttttttgtgtgcagcccaa	840
cactagaaaggaaaaatgttttctaaaaaatgattcattttttaaaagtcatctttcagctttccaaaccaatttttagtaaaatatttaactagtagtcaagacataaactttatttccctt	960
atactaccacttagttttgaaagtcaagtgtctggacttagtcttatctccccctaacaattctcttagccactggactatatatatatatatatatatatatatatattttgaa	1080
atgtgtgtggaattgaattgaagGTGGGACGACAGCACAGGAAAAATAGCCGAATGCCGGGATACTACGACGGCAGGTACTGGACGTTGTGAAACTGCCGATGTTTGGGTGCACAGATT	1200
CATCTCAGCTTCTCAAAGAAATGGAAGAGTGCAAAAACACATACCCAAATGCTTACATCCGTTGCCTTGCAATTTGACAATGTCAAAAAGGCTCAATGCATGTCTTTTATCATTCACAAAC	1320
CACGAATTTAGctcactggttcaatagatagtatttgagagatgagacaaaatttgaaacttgatagcgatagtggtgggattatgtctaaagtgggcataatcctttgtgcgcaccaac	1440
attagctctgtctgttaagcttttaagttatcgtgatttatatcctcataaatatt	1547

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Ib1	MASSVLSSATAIASRATAAQAS-MVAPFTGLKSTASFPVTKKSA-DFTSISTNGSRVQCMQ	58	
Ib2	M-SSVFSS-APFGV----GGSS----FVGLKRTL-LPSNYSVSWSRKTVS-NGSKTLCMK	48	
At	MASSMFSSTAVVT--SPAQAT-MVAPFTGLKSSASFPVTRKANNDITSITSNGGRVSCMK	57	
Nt	MASSVLSSAAVATRTNVAQAN-MVAPFTGLKSAASFVSRKQNLDTITSIASNGGRVQCMQ	59	
St	MASSVISSAAVATRTNVTQASSMVAPFTGLKSTATFPVSRKQNLDTITSIASNGGRVRCMQ	60	
Sl	MASSVMSSAAVATRGNGAQAS-MVAPFTGLKSTASFPVSRKQNLDTITSIASNGGRVSCMQ	59	
Zm	MAP-----TV-----MMASSATAVAPFQGLKSTASLPVARRSSRSLGNV-SNGGRIRCMQ	49	
		▽	
Ib1	VWPPYGKMKFETLSYLPPLSTEQLIKQVEYLLNNGWVPCLEFETEKGFVYRECHRSPGY	118	
Ib2	TWNPIDNKRKFETLSYLPPLSQESIAGEVDYIINKGWIPCLEFD-QVGRQHRENSRMPCGY	107	
At	VWPPIGKKKFETLSYLPDLSDVELAKEVDYLLRNKWIPCFEFELHGFVYREHGNTPGY	117	
Nt	VWPPYGKKKYETLSYLPDLSEEQLLSEIEYLLKNGWVPCLEFETERGFVYRENNKSPGY	119	
St	VWPPINMKKYETLSYLPDLTDEQLLKEVEYLLKNGWVPCLEFETEHGFVYRENNKSPGY	120	
Sl	VWPPINMKKYETLSYLPDLSDQLLSEIEYLLKNGWVPCLEFETEHGFVYRENHKSPGY	119	
Zm	VWPAYGNKKFETLSYLPPLSTDDLKQVDYLLRNGWIPCLEFS-KVGFBVYRENSTSPCY	108	
Ib1	DGRYWTMWKLPMFGCTDATQVIAEMQECKKAYPEAWVRIIGFDNVRQVQCIMFLAAMPN-	177	
Ib2	DGRYWTLWKLPFMFGCTDSSQLLKEMEECKNTYPNAYIRCLAFDNVKKACMSFIIHKPRI	167	
At	DGRYWTMWKLPLFGCTDSAQVLKEVEECKKEYPGAFIRIIGFDNTRQVQCISFIAYKPPS	177	
Nt	DGRYWTMWKLPMFGCTDATQVLAEVGEAKKAYPEAWIRIIGFDNVRQVQCISFIAYKPEG	179	
St	DGRYWTMWKLPMFGCTDATQVLAEVQECKNAYPQAWIRIIGFDNVRQVQCISFIAYKPEG	180	
Sl	DGRYWTMWKLPMFGCTDATQVLAEVQECKNAYPQAWIRIIGFDNVRQVQCISFIAYKPEG	179	
Zm	DGRYWTMWKLPMFGCNDATQVYKELQEAIKSYPDADFHRVIGFDNIQTQCVSFIAYKPPG	168	
Ib1	----	177	
Ib2	----	167	
At	FTEA	181	
Nt	Y---	180	
St	Y---	181	
Sl	Y---	180	
Zm	SD--	170

**Tanabe et al Fig. 3
(grayscale)**

**Tanabe et al Fig. 7
(grayscale)**