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## Cover page

Title: Unique peripheral antennas in the photosystems of the streptophyte alga Mesostigma viride.

Running Title: Unique Photosystems in Mesostigma viride

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Title: Unique peripheral antennas in the photosystems of the streptophyte alga

## Mesostigma viride.

Running Title: The Unique Photosystems in Mesostigma viride

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Abbreviations: CN, clear-native; $\alpha$-DDM, $\alpha$-dodecyl maltoside; Isoseq, isoform sequencing; LHC, light-harvesting complex; PAGE, polyacrylamide gel electrophoresis


#### Abstract

[Abstract]

Land plants evolved from a single group of streptophyte algae. One of the key factors needed for adaptation to a land environment is the modification of the peripheral antenna systems of photosystems. Here, the photosystems of Mesostigma viride, an earliest-branched streptophyte alga, were analyzed to gain insight into their evolution. Iso-seq and phylogenetic analyses of Light-Harvesting Complexes (LHCs) revealed that M. viride possesses three algae-specific LHCs, including algae-type LHCA2, LHCA9, and LHCP; while the streptophyte-specific LHCB6 was not identified. These data suggest that the acquisition of LHCB6 and the loss of algae-type LHCs occurred after the $M$. viride lineage branched off from other streptophytes. Clear-native (CN)-PAGE resolved the photosynthetic complexes, including the PSI-PSII megacomplex, PSIILHCII, two PSI-LHCI-LHCIIs, PSI-LHCI, and the LHCII trimer. Results indicated that the higher-molecular weight PSI-LHCI-LHCII likely had more LHCII than the lowermolecular weight one, a unique feature of $M$. viride photosystems. CN-PAGE coupled with mass spectrometry strongly suggested that the LHCP was bound to PSII-LHCII, while the algae-type LHCA2 and LHCA9 were bound to PSI-LHCI, both of which are different from those in land plants. Results of the present study strongly suggest that $M$.


viride photosystems possess unique features that were inherited from a common ancestor of streptophyte and chlorophyte algae.

## [Introduction]

Photosystems (PSs) in oxygenic photosynthetic organisms are composed of core and peripheral antenna complexes. While the peripheral antenna complex in these organisms is highly divergent, the core complex is highly conserved (Green and Durnford, 1996; Neilson and Durnford, 2010). The diversity of the peripheral antenna system contributes to the ability of photosynthetic organisms to adapt to different light environments due to the multiple roles it plays in photosynthesis, including the harvesting of light energy and transferring it to the core complex. The variety of light spectra available in different environments is one of the driving forces behind the evolution of different peripheral antenna systems in photosynthetic organisms. (Croce and Amerongen, 2014; Stomp et al., 2007). The thermal dissipation of excess light energy, which occurs mainly in peripheral antennas (Ruban, 2018), is also necessary to avoid photooxidative damage. Therefore, photosynthetic organisms have also evolved a variety of thermal dissipation mechanisms in response to different environments (Giovagnetti and Ruban, 2018; Goss and Lepetit, 2015; Niyogi and Truong, 2013; Wobbe et al., 2016).

Notable differences in peripheral antenna systems have been reported, even within the green plants of photosynthetic organisms, and especially for PSI (Pan et al., 2020; Suga and Shen, 2020). In vascular plants, PSI retains four LHCI (Lhca1-Lhca4) proteins as a peripheral antenna and transiently gains a few extra LHCII proteins (Mazor et al., 2017; Pan et al., 2018; Qin et al., 2015). PSI in chlorophyte algae typically have a greater number of LHCI proteins forming their peripheral antenna than vascular plants. For example, PSI in Chlamydomonas reinhardtii has ten LHCI (two Lhca1 and one Lhca2-Lhca9) proteins and one mobile LHCII trimer that can transiently bind to the antenna (Su et al., 2019; Suga et al., 2019). A similar antenna structure has also been reported in Bryopsis corticulans (Qin et al., 2019). Chlorophyte algae may typically have a larger size PSI antenna than vascular plants as an adaptation to aquatic environments where light intensity is weaker than in terrestrial environments (Suga and Shen, 2020). In contrast, Dunaliella salina has a "mini-PSI" that does not possess a second LHCI belt unlike other reported chlorophyte algae (Perez-Boerema et al. 2020). Since $D$. salina has the ability to survive high salinity and high light environments (Perez-Boerema et al. 2020), the unique PSI structure may represent an adaptation to an extreme habitat. In the model moss plant $P$. patens, PSI can possess five (Pinnola et al., 2018) or eight (Iwai et al., 2018) more LHCI proteins than occurs in PSI-LHCI in
vascular plants, plus one additional mobile LHCII trimer. The number of LHC proteins in P. patens PSI can change in response its photosynthetic status (Iwai et al., 2015; Pinnola et al., 2018). The changes in the PSI antenna size reflect the variable light environment that is typically experienced by mosses, which is characterized by low irradiance complemented by sunflecks (Pinnola et al., 2018). These reports reveal that the peripheral antenna system of PSI Hin green plants exhibit large species-specific differences that apparently reflect their adaptation to different light environments. However, to the best of our knowledge, detailed investigations on the peripheral antenna system of PSI in streptophyte algae have not been reported so far.

Significant diversity is also present in thermal dissipation mechanisms in green plants. Thus far, PsbS- and LHCSR-dependent thermal dissipation mechanisms have been reported (Niyogi and Truong, 2013). The LHCSR-dependent mechanism is the primary process used for thermal dissipation in chlorophyte algae, although they possess both PsbS and LHCSR proteins (Correa-Galvis et al., 2016; Tibiletti et al., 2016). In contrast, vascular plants only possess PsbS-dependent thermal dissipation, having lost the LHCSR protein during their evolution (Giovagnetti and Ruban, 2018; Niyogi and Truong, 2013; Pinnola, 2019). Notably, both PsbS and LHCSR play an essential role in thermal dissipation in the model moss plant, P. patens (Pinnola, 2019).

Phylogenetically, P. patens represents an early-branched land plant and possesses thermal dissipation characteristics that are intermediate between chlorophyte algae and vascular plants. In addition, the contribution of PsbS to the thermal dissipating capacity in land plants is much higher than it is in chlorophyte algae. How thermal dissipation mechanisms have changed over the course of evolution from ancestral green algae to land plants, however, has not been fully elucidated. This can be mainly attributed to the absence of a suitable streptophyte alga species that could serve as a model for photosynthesis research.
$M$. viride is one of the earliest-branched freshwater streptophyte algae, although its exact phylogenetic position in green plants is still under debate (Lemieux et al., 2007, 2000; Li et al., 2020; Wang et al., 2020). Nevertheless, it is worth testing whether this basally branching alga retains a feature present in a common ancestor of streptophytes and chlorophytes. Therefore, in the present study, the photosystems of the earliest-branched $M$. viride were characterized to gain insight into the structure of photosystems of a common ancestor of streptophytes and chlorophytes. Results revealed that considerable changes occurred in photosystems after the $M$. viride lineage branched off from other streptophyte algae.

## [Results]

## Separation of photosystems by ClearNative (CN)-PAGE

Clear-Native (CN)-PAGE is a powerful technique that enables one to separate protein complexes while retaining their structure. Here, the M. viride photosystems were separated using amphipol-based CN-PAGE (Furukawa et al., 2019) after solubilization with a mild detergent, dodecyl maltoside ( $\alpha$-DDM). As a result, the PSIPSII megacomplex, the PSII-LHCII supercomplexes, the PSI-LHCI-LHCII bands, the PSI-LHCI, and the LHCII trimer were resolved (Fig. 1A). The identification of the separated bands was accomplished using 2D-CN/SDS-PAGE followed by immunoblot analysis (Fig. 1B) and silver-staining (Fig. 1C), as described in previous studies (Järvi et al., 2011; Takabayashi et al., 2011). The identification of two PSI-LHCI-LHCII bands were confirmed by further analysis described in a later section of this report. The overall band profile (Fig. 1A) was similar to the profile for P. patens presented by Furukawa et al. (2019), however, a substantial difference was evident for PSI-LHCI-LHCII. Two PSI-LHCI-LHCII bands were found in M. viride (Fig. 1A), whereas only one PSI-LHCI-LHCII band was found in the profile of $P$. patens presented by Furukawa et al. (2019). The presence of two PSI-LHCI-LHCII bands appears to be a unique characteristic of the PSs of $M$. viride since land plants also exhibit one PSI-LHCI-

LHCII band in BN-PAGE and CN-PAGE gels (Järvi et al., 2011; Pesaresi et al., 2009; Pinnola et al., 2018).

## Iso-seq analysis to provide a protein database for identification of M. viride LHC

 proteins by MSNo information is available on whether commercially-available LHC antibodies react with M. viride LHCs. Therefore, mass spectrometry (MS) analysis of the protein complexes resolved in the CN-PAGE was conducted, as described in our previous papers (Takabayashi et al., 2017, 2013), to elucidate the composition of the LHC proteins in the resolved PSI and PSII supercomplexes. This approach can be used to estimate the positions of the bands of protein complexes separated by CN-PAGE, especially for the relatively high expressed proteins. The number of $M$. viride photosynthetic protein sequences available in public databases was limited at the time of our MS analysis. Therefore, Isoform sequencing (Iso-seq) analysis was used to obtain a transcriptome using PacBio sequencing, which provides longer and more complete sequence information relative to short-read sequencing platforms such as Illumina (Zhao et al., 2019). Full-length cDNA sequences enabled us to estimate full-length
amino acid sequences with high reliability, a feature that is advantageous for protein identification by MS.

The iso-seq analysis (see Materials and Methods) allowed us to identify nine PSI core proteins, 11 PSII core proteins, and 14 LHC proteins (Table S1). The small subunits of PSI and PSII were not identified because the TransDecoder software identified ORFs that are at least 100 amino acids long using the default settings. Notably, the rate of false positives drastically increased when a shorter minimum length was used. Therefore, default settings were used to predict ORFs in our analysis to obtain reliable results.

## Phylogenetic analysis of LHC proteins

A BLAST query identified 14 putative $M$. viride LHC proteins (Table S1). The annotation of the LHC proteins derived from the BLAST query, however, may not be reliable as considerable sequence similarity has been reported among different LHC proteins. Therefore, a phylogenetic tree of LHC sequences was constructed based on the alignment shown in Fig. S1 and each LHC sequence of $M$. viride was annotated based on the nomenclature of A. thaliana LHC proteins, except for LHCBMs (corresponding to major LHCII (LHCB1, LHCB2, and LHCB3)) and three algae-specific LHCs (algae-
type LHCA2, LHCA9, and LHCP). Algae-type LHCA2 is conserved among chlorophytes, however, it is not closely related to plant-type LHCA2 (Fig. 2). Therefore, we annotated it as algae-type LHCA2 in this study to avoid any misrepresentation. Based on the phylogenetic analysis, M. viride possesses LHCBMs, LHCB4, LHCB5, LHCA1, two LHCA2, LHCA3, and three algae-specific LHCs as peripheral antenna proteins. The M. viride LHC sequences identified by the Iso-seq analysis exhibited good correspondence with previously reported M. viride LHCs (Koziol et al., 2007) (Fig. 2). Some differences, however, were observed relative to the previous classification (Koziol et al., 2007). These differences are likely due to the considerable sequence similarities among LHCs, as the LHCs in green lineages rapidly diversified during the early evolution of green algae. LHCs that were classified differently in the previous report (Koziol et al., 2007) and our present study have been marked with an asterisk (Fig. 2).

The Iso-seq analysis did not detect an LHCB6 gene, even though LHCB6 is highly conserved among streptophyte algae and land plants (Kourril et al., 2016). This result is consistent, however, with a previous study by Koziol et al. (2007) in which LHCB6 sequences were also not identified in $M$. viride, and with the $M$. viride RNAseq data generated in the 1000 plant transcriptomes ( 1 kP ) project (Carpenter et al., 2019; Leebens-Mack et al., 2019). In contrast, LHCP proteins were found in M. viride
(Fig.2), which is also consistent with the previous report by Koziol et al. (2007). LHCP is a unique LHC protein found in prasinophyte algae, including Ostreococcus tauri and Micromonas pusilla. Prasinophyte algae is a group of early-diverging chlorophyte algae, which gave rise to core chlorophyte algae including $C$. reinhardtii. It is very notable that M. viride, an early-divergent streptophyte alga, also encodes LHCP. Previous studies of a model prasinophyte alga, O. tauri, reported that it contains LHCP as the peripheral antenna of PSI-LHCI (Six et al., 2005; Swingley et al., 2010), although no detailed studies have been conducted on LHCP-containing photosystems. A possible loss of LHCB6 and the presence of LHCP in M. viride suggest that the LHC composition in $M$. viride is likely inherited from a common ancestor of streptophyte and chlorophyte algae.

## The distribution of LHC proteins in PSI and PSII

No studies have been conducted on the distribution of M. viride LHCs in PSI and PSII, although they have been identified and classified (Koziol et al., 2007). Therefore, we determined the protein composition of the separated photosystems using MS. As expected, PSI, PSII, and LHC proteins were detected in the PSI-PSII megacomplex band (Table S2). Also as expected, PSI and LHC proteins were detected in the PSILHCI band (Table S3). Unexpected proteins were rarely detected in these two
photosystems, suggesting that contamination from the other protein complexes was limited. The PSII-LHCII band also contained PSII and LHC proteins, although some contamination from PSI subunits and the NDH-like complex subunits were observed to some extent (Table S4).

Importantly, the bands representing protein complexes on electrophoresis gels are often distorted by tailing at both ends. The protein complexes in the tailings are also detected by high-resolution of MS, which is the main source of contamination in a band of interest. To predict unknown protein complexes and to estimate the protein compositions of known protein complexes, a protein migration profile was used after the native-PAGE coupled with MS (Takabayashi et al., 2017, 2013). A protein migration profile is a plot where the $y$-axis represents the amount of protein estimated from MS data, while the x -axis represents the migration distance on a native-PAGE gel. A peak in the protein migration profile has been demonstrated to correspond to the position of the band (Helbig et al., 2009; Remmerie et al., 2011; Wessels et al., 2009). This approach allows one to determine if the proteins in a band detected by MS are contaminants from other protein complexes by verifying their peak positions in protein migration profiles (Helbig et al., 2009; Müller et al., 2016; Takabayashi et al., 2017; Wessels et al., 2009).

In the present study, migration profiles were constructed for PSI, PSII, and

LHC proteins based on MS data. A normalized spectral abundance factor (NSAF) method, which is a label-free quantification method (Zybailov et al., 2006), was used to estimate the amount of protein from MS data. First, the migration profiles of PSI and PSII were compared. The distance between PSII-LHCII and PSI-LHCI-LHCII on the CN-PAGE gel was relatively close, and PSI-LHCI-LHCII bands contained considerable amounts PSI-LHCI-LHCII proteins. Nevertheless, it was possible to isolate those bands using their migration profiles (Fig. 3A). The PSII-LHCII peak was observed in the position of gel slices 8 and 9 (Fig. 3A), where the band was distinctly visible in the CNPAGE gel (Fig. 1A). The PSI-LHCI-LHCII and the PSI-LHCII peaks were observed in the positions of gel slices 12 to 15 (Fig. 1A), where those bands were also visible in the CN-PAGE gel (Fig. 1A). These data indicate that it is possible to distinguish PSI-bound LHCs and PSII-bound LHCs by comparing their migration profiles with the migration profiles of PSI and PSII.

Comparing the migration profiles of LHCs with those of PSI and PSII resulted in the classification of LHCs into two groups. One group included LHCA1, LHCA2, LHCA3, algae LHCA2, and LHCA9 proteins, whose peaks overlapped with the peaks of PSI-LHCI and PSI-LHCI-LHCII (Fig. 3B). The other group included LHCB4,

LHCB5, and LHCP proteins, whose peaks overlapped with the peak of PSII-LHCII (Fig. 3C). These data strongly suggest that PSI-LHCI includes the former LHCs, whereas PSII-LHCII includes the latter LHCs. Notably, the migration profile of LHCBM proteins appeared to be different from the profiles of other LHCs. Its highest peak corresponded with LHCII trimers, suggesting that LHCBM is the major component of the LHCII trimer.

## Separation of PSI-LHCI-LHCII complexes by sucrose density gradient

Two PSI-LHCI-LHCII bands were observed on CN-PAGE gels. A "two-step" separation of those bands was performed to improve the purity of the bands observed on CN-PAGE using sucrose density gradient centrifugation as the first "rough" separation step prior to further separation by CN-PAGE. As a result, three bands (B1-B3) containing photosynthetic pigments were identified after separation by sucrose density gradient centrifugation (Fig. 4). After subsequent separation by CN-PAGE, PSI-LHCILHCII, in addition to PSI-LHCI, were found to be present in the middle band (B2) (Fig. 4), while the bottom band (B3) primarily contained PSII-LHCII, and the upper band (B3) contained LHCII trimer (Fig. 4).

The three PSI-containing bands present in the CN-PAGE gel were further separated by SDS-PAGE after they were cut out of the CN-gel to compare their composition. Two replicates for each PSI-LHCI-LHCII band were subjected to 2D-SDS-PAGE (Fig. 5). Silver-staining of the resulting gels revealed the PsaA/PsaB heterodimer, the PsaA and the PsaB monomers, LHC monomers (c.a. 20kDa), and the small PSI subunits. A portion of PsaA and PsaB remained as a heterodimer and migrated slowly in the gel despite the inclusion of SDS (Fig. 5). A comparison of the three CNPAGE bands containing PSI indicated that the stoichiometry of the PSI and LHCI subunits were similar, while the amounts of LHCII were greatest in the top band and lowest in the bottom band. These results indicate that variations in the size of the three CN-PAGE bands containing PSI were attributed to differences in the number of LHCII (mainly LHCBM) subunits bound to each PSI-LHCI complex. Importantly, the identification of the LHCII band was based on the separation pattern of the B1 and B3 bands containing LHCII trimers by the subsequent 2D-SDS-PAGE (Fig. S2). These data suggest that the larger PSI-LHCI-LHCII possesses more LHCII than the smaller one.

## [Discussion]

Unique peripheral antenna systems of $M$. viride

We classified the LHCs in the peripheral antenna of PSI and PSII. LHCB4 and LHCB5 were identified as the peripheral antenna of PSII-LHCII, similar to land plants. In contrast, LHCB6 was not identified in the Iso-seq analysis in this study. The absence of LHCB6 is consistent with the reported absence of LHCB6 sequences in $M$. viride LHC sequences (Koziol et al., 2007) and is also consistent with the M. viride RNA-seq data generated in the 1KP project (Carpenter et al., 2019; Leebens-Mack et al., 2019). Therefore, it is likely that $M$. viride does not possess LHCB6, although we do not exclude the possibility that the expression level of LHCB6 is relatively low compared to other LHCs, and thus was undetected. LHCB6 is widely distributed among streptophyte algae and land plants but has not been found in chlorophyte algae, including $C$. reinhardtii (Grebe et al., 2019; Kouřil et al., 2016). The putative loss of LHCB6 in M. viride suggests that LHCB6 was acquired during evolution after the divergence of $M$. viride.
M. viride PSII possesses LHCP (Koziol et al., 2007), the main peripheral antenna protein in the model prasinophyte alga, O. tauri (Six et al., 2005; Swingley et al., 2010), whereas LHCP has not been found in land plants. The peaks in the migration profile of LHCP corresponded with the PSI-PSII megacomplex and the PSII-LHCII (Fig. 3C). These data suggest that LHCP binds to PSII-LHCII in M. viride, although
further biochemical studies will be required to confirm this possibility. It should be noted that detection of the LHCP peak at the position of the PSI-PSII megacomplex could be explained by assuming co-migration of the PSI-PSII megacomplex and PSIILHCII at the top part of CN-PAGE (Fig. 3C). Such co-migration of these complexes was actually observed in A. thaliana (Yokono et al. 2019).

A previous report revealed that LHCP, which is conserved among algae in the Mamiellophyceae, including $O$. tauri and M. pusilla, is a major LHC antenna protein in PSI-LHCI in the model prasinophyte algae, O. tauri (Swingley et al., 2010). It has also been suggested that LHCP functions as the peripheral antenna of PSII-LHCII, although no direct evidence has been provided (Six et al., 2005; Swingley et al., 2010). Our data support the idea that LHCP can function as the peripheral antenna of PSII-LHCII (Fig. 3). Collectively, the data suggest that ancestral green algae possessed LHCP proteins as their peripheral antenna because the earlier-branched streptophyte M. viride and the prasinophyte $O$. tauri possess LHCP proteins in their photosystems. The LHCPcontaining photosystem in M. viride was likely inherited from a common ancestor of chlorophyte and streptophyte algae that has been lost during the evolution of streptophyte algae to land plants. Considering that the loss of LHCP and the acquisition of the LHCB6 seemed to occur concomitantly according to our phylogenetic analysis, it
is possible to hypothesize that LHCP in streptophyte PSII-LHCII have been replaced by LHCB6 in land plants. Further investigation will be required, however, to confirm this hypothesis.

In regards to the peripheral antenna of PSI, two PSI-LHCI-LHCII supercomplexes were stably observed after their separation by CN-PAGE. LHCII was more abundant in the higher molecular weight band than it was in the lower molecular weight band, suggesting that the higher molecular PSI-LHCI-LHCII possesses at least two LHCII trimers. It is not presently known if at least two PSI-bound LHCII trimers change their binding to PSI in response to different light environments, i.e., if they are involved in state transitions. Since the binding of LHCII trimer to PSI was involved in the state transition according to previous studies in green algae and land plants (Drop et al., 2014; Pesaresi et al., 2009; Pinnola et al., 2018, 2018; Pribil et al., 2010; Takahashi et al., 2006), we hypothesize that the additional $M$. viride LHCII trimers are also involved in the state transition. We do not exclude the possibility, however, that the smaller form of PSI-LHCI-LHCII represents an artifact caused by the dissociation of LHCII from the larger form. Further studies are required to confirm this supposition.

In addition, PSI-LHCI in M. viride possesses algae-type LHCA2 and LHCA9 proteins, which are conserved among chlorophytes including C. reinhardtii (Su et al.,

2019; Suga et al., 2019) and Bryposis corticulans (Qin et al., 2019), but not conserved among streptophytes (Fig. 2). The loss of these algae-specific LHCs must have occurred after the $M$. viride lineage branched off from the other streptophyte algae, although further studies will also be required to confirm this possibility.

We constructed a hypothetical model of the antenna structure of PSI-LHCILHCII in M. viride using the reported structure of PSI-LHCI in chlorophyte algae and land plants as references (Fig. 6). Based on their sequence similarities, the binding manner of LHCA1, two LHCA2s, and LHCA3 proteins to the PSI core in M. viride may be similar to the binding of LHCA1, LHCA2, LHCA3, and LHCA4 proteins to the PSI core in vascular plants. Likewise, the binding manner of algae-type LHCA2 and LHCA2 to PSI-LHCI may be similar to the binding mechanism that occurs in PSI-LHCI in C. reinhardtii (Su et al., 2019; Suga et al., 2019) and B. corticulans (Qin et al., 2019). The binding site of the second LHCII trimer to PSI-LHCI, however, is unknown, as a PSI-LHCI with two LHCII trimers has not, to our knowledge, been reported. Similarly, the binding site of LHCP to PSII-LHCII is also unknown as no structural studies on LHCP-bound PSI-LHCI have been reported. Given the phylogenetic position of $M$. viride and its unique antenna system in PSI and PSII, further structural analyses of $M$. viride PSI-LHCI-LHCII are warranted and would significantly contribute to our
understanding of the changes that occurred in photosystems during the evolution of a common ancestor of chlorophytes and streptophytes to land plants.

## [Materials and Methods]

## Algal strain and culture conditions

M. viride strain, NIES-296, was obtained from the National Institute for Environmental Studies (NIES) (Ibaraki, Japan). The strain was cultured in 500 mL of C medium in a 1L Erlenmeyer flasks at $22^{\circ} \mathrm{C}$ under a 14 -h photoperiod of $20 \mu \mathrm{~mol}$ photons $\mathrm{m}^{-2} \mathrm{~s}^{-1}$, as previously described by (Kunugi et al., 2016).

## RNA extraction

One liter of a $M$. viride culture was centrifuged at $10,000 \times g$ for 10 min at $4^{\circ} \mathrm{C}$. The pellet was resuspended in 0.5 mL RLT buffer (RNeasy Plant Mini Kit, Qiagen) supplemented with 1\% 2-mercaptoethanol. The suspension was then transferred to a 2ml vial containing 500 mg of glass beads ( 0.5 mm diameter). The vial was subjected to 10s disruption treatments using a Mini-Bead Beater (Merck, Germany) and the suspension was then centrifuged at $21,600 \times \mathrm{g}$ at $4^{\circ} \mathrm{C}$ for 5 min . Supernatants from the
vials were used for total RNA isolation with a RNeasy Plant Mini Kit (Qiagen), according to the manufacturer's instructions.

## Full-length isoform sequencing (Iso-seq) using PacBio data

The integrity of extracted total RNA was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc). The Iso-seq library was prepared according to the protocol described by Pacific Biosciences (PN 101-070-200) using a SMARTer PCR cDNA Synthesis Kit (Clontech) and SMRTbell Template Prep Kit 1.0 SPv3 (Pacific Biosciences). Sequencing was performed on a PacBio Sequel platform.

Raw sequence data were processed using SMRT Link v6.0 (Pacific Biosciences) software and then further processed using IsoSeq3 (version 3.1) software tools (https://github.com/PacificBiosciences/IsoSeq) with default parameters. Circular consistency sequence (CCS) reads were generated from subread sequences. Full-length cDNA reads were then selected by finding the 5 ' and 3 ' primers or polyA tail using the lima tool in IsoSeq3. After trimming of the polyA tail and removal of the concatemer sequences, 84,545 full-length cDNA reads were obtained. Finally, 7,209 high-quality consensus full-length cDNA sequences were obtained after isoform clustering and polishing.

## Estimation of full-length amino acid sequences using the Iso-seq data

TransDecoder (https://github.com/TransDecoder/TransDecoder/) software was used to identify full-length protein sequences in the 7,209 full-length cDNAs. Candidate open reading frames (ORFs) with a minimum length of 100 amino acids were identified using the TransDecoder.LongOrfs module. Candidate ORFs were validated by BLASTP queries using an e-value cutoff of $10^{-5}$ against data protein database comprising proteins of Arabidopsis thaliana, Chara braunii, Chlamydomonas reinhardtii, Coccomyхa subellipsoidea, Cyanidioschyzon merolae, Cyanophora paradoxa, Klebsormidium flaccidum, Oryza sativa, Ostreococcus lucimarinus, Physcomitrella patens, and Selaginella moellendorffii within the Phytozome database (v12.1)(Goodstein et al., 2012). After the blast queries, 5,829 protein sequences with significant blast hits were obtained using the TransDecoder.Predict module. CD-HIT (Fu et al., 2012) software was employed to remove redundant protein sequences using a cutoff value of 0.9 . A total of 3,198 protein sequences were obtained using CD-HIT software. These protein sequences were used as a $M$. viride protein database for MS queries.

Phylogenetic analysis of $M$. viride LHC proteins
M. viride LHC proteins were identified by NCBI-BLASTP homology queries against the $M$. viride protein sequence database described above. A. thaliana and C. reinhardtii LHC proteins were used as query sequences in the BLASTP searches. The LHC sequences of Arabidopsis thaliana, Marchantia polymorpha, Klebsormidium flaccidum, Ostreococcus lucimarinus, and Chlamydomonas reinhardtii were obtained from the Phytozome database (https://phytozome.jgi.doe.gov/). The LHCs of Cyanidioschyzon merolae and Pyropia yezoensis were used as the outgroups in the phylogenetic tree analysis. Amino acid sequences of LHCs were aligned using the MAFFT algorithm (Katoh and Standley, 2013). Trimming of the alignment was done by a ClipKIT program (Steenwyk et al. 2020) with default parameters. A maximum likelihood (ML) phylogenetic tree including $M$. viride LHCs was constructed using W-IQ-TREE (Trifinopoulos et al., 2016) software under the best-fitting model (LG+F+I+G4). Ultrafast bootstrap values (1,000 replicates) are shown below the branches.

## Thylakoid membrane preparation

A pellet obtained from the centrifugation $\left(10,000 \mathrm{x} g\right.$ for 10 min at $\left.4^{\circ} \mathrm{C}\right)$ of 1 L of a $M$. viride culture was suspended in 2 mL BN- solubilization buffer ( 50 mM imidazole/ HCl (pH 7.0), 20\% glycerol) with 1\% Protease Inhibitor Cocktail for plant cell lysate
(Merck, Germany). The suspension was transferred to a 2-ml vial containing 500 mg of glass beads ( 0.5 mm diameter). The vial was first immersed in liquid nitrogen and the cells were subsequently subjected to three 10s disruption treatments using a Mini-Bead Beater (Merck, Germany). The resulting sample was centrifuged at $21,600 \times$ g at $4^{\circ} \mathrm{C}$ for 5 min , and the supernatant was suspended in BN - solubilization buffer. The suspension was centrifuged at $200 \times$ g at $4^{\circ} \mathrm{C}$ for 1 min , and the supernatant was centrifuged at $21,600 \times \mathrm{g}$ at $4^{\circ} \mathrm{C}$ for 5 min to obtain the thylakoid membranes.

## Clear-Native (CN)-PAGE

CN-PAGE was performed as previously described (Furukawa et al. 2019). A linear 4$13 \%$ gradient polyacrylamide gel was used as the separation gel, and a 3.5\% polyacrylamide gel was used as the sample gel. Thylakoid membranes were solubilized in $1 \% \alpha$-DDM ( $\alpha$-dodecyl maltoside) at $4^{\circ} \mathrm{C}$ for 1 min . After centrifugation at $21,600 \times$ g at $4{ }^{\circ} \mathrm{C}$ for 5 min , the supernatant (roughly $5 \mu \mathrm{~g}$ chlorophyll equivalent) was supplemented with 1\% Amphipol A8-35 and loaded onto CN-PAGE gels. An anode buffer (25mM imidazole/ HCl ( pH 7.0 )) and cathode buffer ( 50 mM Tricine, 7.5 mM imidazole) were used for the electrophoresis.

## Two-dimensional (2D)-CN/SDS-PAGE

CN-gel strips were soaked in denaturation buffer ( $1 \%$ SDS, 50 mM DTT) for 30 min , and the 2D-SDS-PAGE was performed using a $14 \%$ polyacrylamide gel with 4M urea. The proteins on the 2D-CN/SDS-gel were visualized by silver-staining using a Pierce Silver Stain kit (ThermoFisher Scientific, USA), according to the manufacturer's instructions. Alternatively, the obtained 2D-gels were used in the immunoblot analyses.

## Immunoblot analysis

Proteins from the 2D-CN/SDS-gel were transferred to a polyvinylidene fluoride membrane (PolyScreen PVDF transfer membrane, PerkinElmer Life Sciences, MA, USA). Protein detection using specific antibodies was performed using Western Lightning Plus-ECL (PerkinElmer Life science, MA, USA) reagent. All antibodies, except anti-PsbB (AS04 038)), were purchased from Agrisera (Vännäs, Sweden).

## Sucrose density gradient

M. viride thylakoid membranes were solubilized in $1 \% \alpha$-DDM and then loaded onto a continuous sucrose density gradient ( 0.3 M sucrose to 1.3 M sucrose in a buffer containing 25 mM MES-KOH (pH 6.5) and 0.1\% GDN (GDN101, Anatrace)). The
samples were then subjected to ultracentrifugation at $72,000 \mathrm{x} g$ using a S-65T rotor (Hitachi-koki, Japan) for 15 h at $4^{\circ} \mathrm{C}$.

## LC-MS/MS analysis

CN-PAGE gel strips were cut horizontally as shown in Fig.1A. All gel pieces were subjected to in-gel digestion with trypsin and analyzed to identify their peptides by LCMS/MS using Orbitrap Elite mass spectrometry (Thermo Fisher Scientific, Waltham, MA, USA) coupled with Thermo Easy-nLC (Thermo Fisher Scientific, Waltham, MA, USA). Each sample was loaded onto a C18-reversed phase EASY-Column ( $0.1 \mathrm{~mm} \times$ $20 \mathrm{~mm}, 5 \mu \mathrm{~m}$ particle size, 120 Å pore size), before separation on a C18 Tip column ( $75 \mu \mathrm{~m} \times 120 \mathrm{~mm}$; Nikkyo Technos, Tokyo, Japan). The samples were separated by a gradient formed by solvent A ( $0.1 \%$ formic acid) and solvent B (acetonitrile in $0.1 \%$ formic acid) at a flow rate of $300 \mathrm{~nL} / \mathrm{min}$. The gradient separation setting was as follows: 0-1min, 0-5\% B; 1-12 min, 5-35\% B; 12-25 min, 35\%-90\% B; 90\% B; 25-45 min.

Proteins in each sample were identified using SearchGUI (version 3.3.15) software and an andromeda search engine (Barsnes and Vaudel, 2018). The 3,198 M. viride protein sequences identified in the Iso-seq analysis were used for protein
identification. PeptideShaker (version 1.16.40) software with default settings was used to validate the protein identifications (Vaudel et al., 2015).

The normalized spectral abundance factor (NASF) (Zybailov et al., 2006) calculated by the PeptideShaker program was used as a label-free quantification method based on the LC-MS/MS data to estimate the abundances of the identified proteins in each gel slice. As previously described, protein migration profiles were then generated by plotting the NASF values on the $y$-axis, and plotting the gel slice number on the x axis (Takabayashi et al., 2017, 2013).

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## Data availability

Raw data of the Iso-seq analysis have been deposited to the DDBJ Sequence Read Archive (DRA) (https://www.ddbj.nig.ac.jp/dra/index.html) under the accession number PRJDB10366. In addition, the assembled full-length cDNA sequences of PSI, PSII, and

LHC sequences have been deposited to DDBJ/EMBL/GenBank as Transcriptome Shotgun Assembly (TSA) data under the accession numbers from ICQU01000001 to ICQU01000034.

## Disclosures

Conflicts of interest: The authors declare no conflicts of interest.

## [References]

Barsnes, H., and Vaudel, M. (2018) SearchGUI: A Highly Adaptable Common Interface for Proteomics Search and de Novo Engines. J Proteome Res. 17: 2552-2555.

Carpenter, E.J., Matasci, N., Ayyampalayam, S., Wu, S., Sun, J., Yu, J., et al. (2019) Access to RNA-sequencing data from 1,173 plant species: The 1000 Plant transcriptomes initiative (1KP). Gigascience. 8.

Correa-Galvis, V., Redekop, P., Guan, K., Griess, A., Truong, T.B., Wakao, S., et al. (2016) Photosystem II Subunit PsbS Is Involved in the Induction of LHCSR Proteindependent Energy Dissipation in Chlamydomonas reinhardtii. J Biological Chem. 291: 17478-87.

Croce, R., and Amerongen, V.H. (2014) Natural strategies for photosynthetic light harvesting.

Drop, B., K.N., S.Y., Boekema, E.J., and Croce, R. (2014) Consequences of state transitions on the structural and functional organization of Photosystem I in the green alga Chlamydomonas reinhardtii. Plant J. 78: 181-191.

Fu, L., Niu, B., Zhu, Z., Wu, S., and Li, W. (2012) CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics. 28: 3150-3152.

Furukawa, R., Aso, M., Fujita, T., Akimoto, S., Tanaka, R., Tanaka, A., et al. (2019) Formation of a PSI-PSII megacomplex containing LHCSR and PsbS in the moss Physcomitrella patens. J Plant Res. 132: 867-880.

Giovagnetti, V., and Ruban, A.V. (2018) The evolution of the photoprotective antenna proteins in oxygenic photosynthetic eukaryotes. Biochem Soc T. 46: 1263-1277.

Goodstein, D.M., Shu, S., Howson, R., Neupane, R., Hayes, R.D., Fazo, J., et al. (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res. 40: D1178-D1186.

Goss, R., and Lepetit, B. (2015) Biodiversity of NPQ. Journal of plant physiology. 172: 13-32.

Grebe, S., Trotta, A., Bajwa, A.A., Suorsa, M., Gollan, P.J., Jansson, S., et al. (2019) The unique photosynthetic apparatus of Pinaceae: analysis of photosynthetic complexes in Picea abies. Journal of experimental botany. 70: 3211-3225.

Green, B.R., and Durnford, D.G. (1996) The chlorophyll-carotenoid proteins of oxygenic photosynthesis. Annu Rev Plant Phys. 47: 685-714.

Helbig, A.O., Groot, M.J.L. de, Gestel, R.A. van, Mohammed, S., Hulster, E.A.F. de, Luttik, M.A.H., et al. (2009) A three-way proteomics strategy allows differential analysis of yeast mitochondrial membrane protein complexes under anaerobic and aerobic conditions. Proteomics. 9: 4787-98.

Iwai, M., Grob, P., Iavarone, A.T., Nogales, E., and Niyogi, K.K. (2018) A unique supramolecular organization of photosystem I in the moss Physcomitrella patens. Nature plants. 4: 904-909.

Iwai, M., Yokono, M., Kono, M., Noguchi, K., Akimoto, S., and Nakano, A. (2015) Light-harvesting complex Lhcb9 confers a green alga-type photosystem I supercomplex to the moss Physcomitrella patens. Nature plants. 1: 14008.

Järvi, S., Suorsa, M., Paakkarinen, V., and Aro, E.-M. (2011) Optimized native gel systems for separation of thylakoid protein complexes: novel super- and megacomplexes. Biochemical Journal. 439: 207-214.

Kouřil, R., Nosek, L., Bartoš, J., Boekema, E.J., and Ilík, P. (2016) Evolutionary loss of light-harvesting proteins Lhcb6 and Lhcb3 in major land plant groups - break-up of current dogma. New Phytol. 210: 808-814.

Koziol, A.G., Borza, T., Ishida, K.-I., Keeling, P., Lee, R.W., and Durnford, D.G. (2007) Tracing the evolution of the light-harvesting antennae in chlorophyll $a / b$ containing organisms. Plant Physiol. 143: 1802-1816.

Kunugi, M., Satoh, S., Ihara, K., Shibata, K., Yamagishi, Y., Kogame, K., et al. (2016) Evolution of green plants accompanied changes in light-harvesting systems. Plant Cell Physiology. 57: 1231-43.

Leebens-Mack, J.H., Barker, M.S., Carpenter, E.J., Deyholos, M.K., Gitzendanner, M.A., Graham, S.W., et al. (2019) One thousand plant transcriptomes and the phylogenomics of green plants. Nature. 574: 679-685.

Lemieux, C., Otis, C., and Turmel, M. (2000) Ancestral chloroplast genome in Mesostigma viride reveals an early branch of green plant evolution. Nature. 403: 649652.

Lemieux, C., Otis, C., and Turmel, M. (2007) A clade uniting the green algae Mesostigma viride and Chlorokybus atmophyticus represents the deepest branch of the Streptophyta in chloroplast genome-based phylogenies. Bmc Biol. 5: 2.

Li, L., Wang, S., Wang, H., Sahu, S.K., Marin, B., Li, H., et al. (2020) The genome of Prasinoderma coloniale unveils the existence of a third phylum within green plants. Nat Ecol Evol. 1-12.

Mazor, Y., Borovikova, A., Caspy, I., and Nelson, N. (2017) Structure of the plant photosystem I supercomplex at 2.6 Å resolution. Nat Plants. 3: 17014.

Müller, C.S., Bildl, W., Haupt, A., Ellenrieder, L., Becker, T., Hunte, C., et al. (2016) Cryo-slicing blue bative-mass spectrometry (csBN-MS), a novel technology for high resolution complexome profiling. Mol Cell Proteomics. 15: 669-681.

Neilson, J.A.D., and Durnford, D.G. (2010) Structural and functional diversification of the light-harvesting complexes in photosynthetic eukaryotes. Photosynth Res. 106: 5771.

Niyogi, K.K., and Truong, T.B. (2013) Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. Current opinion in plant biology. 16: 307-14.

Pan, X., Cao, P., Su, X., Liu, Z., and Li, M. (2020) Structural analysis and comparison of light-harvesting complexes I and II. Biochimica Et Biophysica Acta Bba Bioenergetics. 1861: 148038.

Pan, X., Ma, J., Su, X., Cao, P., Chang, W., Liu, Z., et al. (2018) Structure of the maize photosystem I supercomplex with light-harvesting complexes I and II. Science. 360: 1109-1113.

Pesaresi, P., Hertle, A., Pribil, M., Kleine, T., Wagner, R., Strissel, H., et al. (2009) Arabidopsis STN7 kinase provides a link between short- and long-term photosynthetic acclimation. Plant Cell Online. 21: 2402-2423.

Perez-Boerema, A., Klaiman, D., Caspy, I., Netzer-EI, S., Amunts, A., and Nelson, N. (2020) Structure of a minimal photosystem I from the green alga Dunaliella salina. Nature plants. 6: 321-327.

Pinnola, A. (2019) The rise and fall of light-harvesting complex stress-related proteins as photoprotection agents during evolution. J Exp Bot. 70: 5527-5535.

Pinnola, A., Alboresi, A., Nosek, L., Semchonok, D., Rameez, A., Trotta, A., et al. (2018) A LHCB9-dependent photosystem I megacomplex induced under low light in Physcomitrella patens. Nature plants. 4: 910-919.

Pribil, M., Pesaresi, P., Hertle, A., Barbato, R., and Leister, D. (2010) Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow. Plos Biol. 8: e1000288.

Qin, X., Pi, X., Wang, W., Han, G., Zhu, L., Liu, M., et al. (2019) Structure of a green algal photosystem I in complex with a large number of light-harvesting complex I subunits. Nat Plants. 5: 263-272.

Qin, X., Suga, M., Kuang, T., and Shen, J.-R. (2015) Structural basis for energy transfer pathways in the plant PSI-LHCI supercomplex. Science. 348: 989-995.

Remmerie, N., Vijlder, T.D., Valkenborg, D., Laukens, K., Smets, K., Vreeken, J., et al. (2011) Unraveling tobacco BY-2 protein complexes with BN PAGE/LC-MS/MS and clustering methods. J Proteomics. 74: 1201-1217. Ruban, A.V. (2018) Light harvesting control in plants. Febs Lett. 592: 3030-3039. Six, C., Worden, A.Z., Rodríguez, F., Moreau, H., and Partensky, F. (2005) New insights into the nature and phylogeny of prasinophyte antenna proteins: Ostreococcus tauri, a Case Study. Mol Biol Evol. 22: 2217-2230.

Steenwyk, J.L., Buida 3rd, T.J., Li, Y., Shen, XX., Rokas, A. (2020) ClipKIT: A multiple sequence alignment trimming software for accurate phylogenomic inference. PLoS Biol. 18: e3001007.

Stomp, M., Huisman, J., Stal, L.J., and Matthijs, H.C.P. (2007) Colorful niches of phototrophic microorganisms shaped by vibrations of the water molecule. Isme J. 1: 271-282.

Su, X., Ma, J., Pan, X., Zhao, X., Chang, W., Liu, Z., et al. (2019) Antenna arrangement and energy transfer pathways of a green algal photosystem-I-LHCI supercomplex. Nature Plants. 5: 273-281.

Suga, M., Ozawa, S.-I., Yoshida-Motomura, K., Akita, F., Miyazaki, N., and Takahashi, Y. (2019) Structure of the green algal photosystem I supercomplex with a decameric light-harvesting complex I. Nature Plants. 5: 626-636.

[^0]Takabayashi, A., Kadoya, R., Kuwano, M., Kurihara, K., Ito, H., Tanaka, R., et al. (2013) Protein co-migration database (PCoM -DB) for Arabidopsis thylakoids and Synechocystis cells. Springerplus. 2: 148.

Takabayashi, A., Kurihara, K., Kuwano, M., Kasahara, Y., Tanaka, R., and Tanaka, A. (2011) The oligomeric states of the photosystems and the light-harvesting complexes in the Chl b-less mutant. Plant Cell Physiol. 52: 2103-2114.

Takabayashi, A., Takabayashi, S., Takahashi, K., Watanabe, M., Uchida, H., Murakami, A., et al. (2017) PCoM-DB update: a protein co-migration database for photosynthetic organisms. Plant Cell Physiol. 58: e10-e10.

Takahashi, H., Iwai, M., Takahashi, Y., and Minagawa, J. (2006) Identification of the mobile light-harvesting complex II polypeptides for state transitions in Chlamydomonas reinhardtii. Proc National Acad Sci. 103: 477-482.

Tibiletti, T., Auroy, P., Peltier, G., and Caffarri, S. (2016) Chlamydomonas reinhardtii PsbS protein is functional and accumulates rapidly and transiently under high light. Plant Physiol. 171: 2717-2730.

Vaudel, M., Burkhart, J.M., Zahedi, R.P., Oveland, E., Berven, F.S., Sickmann, A., et al. (2015) PeptideShaker enables reanalysis of MS-derived proteomics data sets. Nat Biotechnol. 33: 22-24.

Wang, S., Li, L., Li, H., Sahu, S.K., Wang, H., Xu, Y., et al. (2020) Genomes of earlydiverging streptophyte algae shed light on plant terrestrialization. Nat Plants. 6: 95-106.

Wessels, H.J.C.T., Vogel, R.O., Heuvel, L. van den, Smeitink, J.A., Rodenburg, R.J., Nijtmans, L.G., et al. (2009) LC-MS/MS as an alternative for SDS-PAGE in blue native analysis of protein complexes. Proteomics. 9: 4221-8.

Wobbe, L., Bassi, R., and Kruse, O. (2016) Multi-level light capture control in plants and green algae. Trends Plant Sci. 21: 55-68.

Yokono, M., Takabayashi, A., Kishimoto, J., Fujita, T., Iwai, M., Murakami, A., et al. (2019) The PSI-PSII megacomplex in green plants. Plant Cell Physiol. 60: 1098-1108.

Zhao, L., Zhang, H., Kohnen, M.V., Prasad, K.V.S.K., Gu, L., and Reddy, A.S.N. (2019) Analysis of transcriptome and epitranscriptome in plants using PacBio Iso-Seq and Nanopore-based direct RNA sequencing. Frontiers Genetics. 10: 253.

Zybailov, B., Mosley, A.L., Sardiu, M.E., Coleman, M.K., Florens, L., and Washburn, M.P. (2006) Statistical analysis of membrane proteome expression changes in Saccharomyces cerevisiae. J Proteome Res. 5: 2339-2347.

## [Figure legends]

## Figure 1. Separation of photosynthetic complexes by CN-PAGE

Separation of M. viride thylakoid membrane protein solubilized in $1 \% \alpha$-DDM by amphipol A8-35-based CN-PAGE (A). The number in parentheses represents the number of the corresponding gel slice which was further subjected to mass spectrometry analysis (see Fig. 1A). Immunoblot analysis of PsaB (PSI), and PsbB (PSII) proteins after their separation by 2D-CN/SDS-PAGE (B). Anti-PsaB and anti-PsbB antibodies were purchased from Agrisera. 2D- CN/SDS-PAGE of M. viride thylakoid membrane protein complexes visualized by silver-staining (C).

Figure 2. A phylogenetic tree of $M$. viride LHC proteins

A maximum likelihood phylogenetic tree of $M$. viride LHC proteins. Sequences highlighted in yellow are clades containing the LHC from M. viride. The M. viride LHC
genes that are surrounded by a box represent the LHC genes identified in the Iso-seq analysis within the present study. LHCs that were classified differently between the previous report (Koziol et al., 2007) and our present study are marked with an asterisk.

## Figure 3. Comparison of LHC migration profiles in PSI and PSII

 M. viride thylakoid membranes were solubilized in $\alpha$-DDM and separated by CNPAGE. After separation, gel regions containing the resolved photosystems were horizontally cut into approximately 1 mm slices. The number shown in parentheses in Fig. 1A represents the number of the corresponding gel slice. (A) Migration profiles of PSI and PSII. The X-axis indicates the number of the gel slice as indicated on the CNPAGE gel (Fig. 1A). The Y-axis indicates the relative protein abundance estimated using a label-free quantification method on the data obtained in the MS analysis. The PSII peak shown in gel slices 8 and 9 likely correspond to the PSII-LHCII band on the CNPAGE gel, while the PSI peaks shown in gel slices 11 to 15 likely correspond to the PSI-LHCI-LHCII band and the PSI-LHCI band. (B) Migration profiles of LHCA1, LHCA2, LHCA3, algae-type LHCA2, and LHCA9 proteins. Their highest peaks correspond to the PSI-LHCI-LHCII and PSI-LHCI bands. The protein data of the two algae-type LHCA2 protein were combined to construct the migration profile of thealgae-type LHCA2, as the two LHCA2 protein sequences were similar. (C) Migration profiles of LHCB4, LHCB5, and LHCBM proteins. The highest peaks in the LHCB4 and the LHCB5 migration profiles correspond with the PSII-LHCII band, while the highest peak in the LHCBM migration profile corresponds to the LHCII trimer. All of the MS data obtained for the LHCBM proteins were combined due their sequence similarities.

## Figure 4. Separation of PSI-LHCI-LHCII supercomplexes using sucrose density

 gradient centrifugation followed by CN-PAGE.(A) Separation of M. viride thylakoid membrane protein complexes solubilized in $1 \% \alpha$ DDM by sucrose density gradient centrifugation (0.3M-1.3M). The middle band (B2) contained the PSI-LHCI and the PSI-LHCI-LHCII, while the bottom band (B3) primarily contained PSII-LHCII and the upper band (B3) contained LHCII trimer. (B) Further separation of the PSI-enriched fraction (B2) in the sucrose gradient by CNPAGE.

Figure 5. Separation of two PSI-LHCI-LHCII bands and a PSI-LHCI band by 2D-SDS-PAGE

Two PSI-LHCI-LHCII bands and a PSI-LHCI band were separated by CN-PAGE, followed by sucrose density gradient centrifugation (Fig. 4), and were then subjected to the 2D-SDS-PAGE. Protein bands were visualized by silver-staining.

Figure 6. A hypothetical model of evolutionary changes in the PSI peripheral antenna system of green plants.

During evolution, green plants have diverged into two groups: streptophytes and chlorophytes. Land plants are thought to have diverged from one group of freshwater streptophytes, while core chlorophytes, including C. reinhardtii, diverged from seawater chlorophytes (prasinophytes). The structure of PSI and its peripheral antennas have been previously reported in vascular plants (Qin et al. 2015; Mazor et al. 2017; Pan et al., 2018), moss plants (Iwai et al., 2018; Pinnola et al., 2018), and core chlorophytes, including C. reinhardtii (Su et al., 2019; Suga et al., 2019) and D. salina (PerezBoerema et al. 2020). Notably, the "minimal" PSI-LHCI in D. salina lacks several core subunits in addition to the second LHCI belt. The PSI structure of streptophyte algae and prasinophyte algae, however, has not been reported. Based on the data obtained in the present study, M. viride PSI possesses LHCA1, two LHCA2s, LHCA3, algae-type LHCA2, and LHCA9. Since M. viride LHCA1, two LHCA2s, and LHCA3 exhibit
considerable sequence similarity with LHCA proteins in vascular plants, their binding site may be similar to the binding site of the LHCIs in vascular plants. Based on sequence similarities, the binding site of algae-type LHCA2 and LHA9 may be similar to the binding site of PSI-LHCI in chlorophytes. The binding site of LHCP to PSIILHCII is unclear. M. viride PSI can bind at least two LHCII trimers, however, the binding site of the additional LHCII trimer is unknown. Fig. S1 A multiple sequence alignment of LHC sequences. Below is the multiple sequence alignment used to construct a phylogenetic tree in Fig. 2. A MAFFT program (https://mafft.cbrc.jp/alignment/software/) was used to align LHC sequences and a ClipKIT program was used to do trimming of the alignment.

## Figure S2. 2D-SDS-PAGE of the high-molecular-weight PSI-LHCI-LHCII, PSI-

## LHCI, and LHCII trimer.

The high-molecular-weight PSI-LHCI-LHCII, PSI-LHCI, and LHCII trimer bands separated by CN-PAGE after sucrose density gradient centrifugation were subjected to 2D-SDS-PAGE. Protein bands were visualized by silver-staining.

Supplementary Table Legends:

Table S1. List of $M$. viride LHC proteins predicted in the Iso-seq analysis.

Table S2. Proteins identified in the M. viride PSI-PSII band by MS.

Table S3. Proteins identified in the M. viride PSII-LHCII band by MS.

Table S4. Proteins identified in the M. viride PSI-LHCI band by MS.

Fig. 1

A
B


C


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Fig. 2


Fig. 3
A


B


C


Fig. 4

A


B


Aso et al.

Fig. 5


Aso et al.


Fig. S1 A multiple sequence alignment of LHC sequences. Below is the multiple sequence alignment used to construct a phylogenetic tree in Fig. 2. A MAFFT program (https://mafft.cbrc.jp/alignment/software/) was used to align LHC sequences and a ClipKIT program was used to do trimming of the alignment.

ABD37880_MvLHCA3
Gene. 11536
AtLHCA3
Mapoly0066s0050
kfloo560_0090
CrLHCA3
Cs_37969
MpLHCA3
OL_K08909
ABD37881_MvLHCA4.1
DAA05923_MVLHCA2
Gene. 11715
ABD37884_MvLHCA5.2
DAA05927_MVLHCA9
Gene. 10884
CrLHCA9
Cs_ 44136
MpLHCA9
OL_K08911
CrLHCA2
Cs_ 7387
MpLHCA2
Ol_PTHR21649
ABD37882_MvLHCA4.2
DAA05926_MvLHCA5
Gene. 11585
DAA05920_MvLHCA4
Gene. 11349
AtLHCA2
Mapoly0083s0003
AtLHCA6
kfloo564_0030
MpLHCA4
Ol_K08910
Ol_K08908
AtLHCA4
Mapoly0006s0261
kfloo023_0070
CrLHCA4
Cs_52367
CrLHCA5
Cs_61250
CrLHCA6
Cs_46127
AtLHCA1
Mapoly0082s0040
kflool00_0170
CrLHCA1
Cs_25286
DAA05929_MvLHCA1
Gene. 11703
CrLHCA7
Cs_25284
CrLHCA8
Cs_48543
AtLHCA5
kfloo214_0160




MPLHCA1 Cs_66689 kfloooos_0500 kfloo517_0030 kfloooos_0510 kfloo517_0040 ABD37890_MvLHCB4 Gene. 10541
AtLHCB4. 1
AtLHCB4. 2
Mapoly0001s0025 AtLHCB4.3 kfloo258_0020 CrLHCB4
Cs_30169
Cs_67011
ABD37885_MvLHCBM1
Gene. 11306
Gene. 11314
AtLHCB1. 1
AtLHCB2. 1
Mapoly0057s0073
Mapoly0371s000
Mapoly0199s0017
Mapoly0199s0018
Mapoly0199s0019
Mapoly0199s0012
MapolyO199s0014
Mapoly0199s0016
Mapoly0199s0015
Mapoly0199s0013
Mapoly0199s0020
Mapoly0057s0082
Mapoly0057s0083
Mapoly0068s0071
Mapoly0139s0012
Cs_15915
Cs_21950
Cs_58975
Cs_27246
Cs_35121
kflooo98_0080
kflooos8_0090
kflo1434_0010
CrLHCBM1
CrLHCBM2
CrLHCBM7
CrLHCBM3
CrLHCBM4
CrLHCBM8
CrLHCBM6
CrLHCBM9
kfloos09_0010
CrLHCBM5
Cs_38026
Mapoly0026s002
AtLHCB3
Mapoly0068s0047
Mapoly0068s0087

RALL-- - IISAPIV -
ARAGSFAlGRK ATNATN
MNLPSTTALSPVT
MA.---MTACAVK
MAVS--TLACAIK
MAS - - STIAASS
MAA - - SSARAIP

VDSIHFLG ASALAGSLASVSSST--QSSFCRSF---NVVP G-MPALASGLATICRSR-QTSFVQKG-- - GAVYANA LSKLAGFAATTASTSAARSSFFAGR---ALVS PA ISGVM TRGAGSAVRTALAGCRLAPQSCVSTGR QAIFGTKKGGTVVK--KGTVKK AATAVKT MNTSTFMAQLQVAT--QAIFGTKKGGTVVK--KGTVKK----AATAVKT AATSAAAAAASSIMT-RVAP DSSNSSRFTA--RFGF GK KA MATAIATSLAASSFCSCREVGSAYSRVF-GPSNAARIVC--RSAIKK----AASAAKG MATT--TAAAASGIFI-.........-DRPGTGRVQA--RFGF-.....-. - SFGK KP MATSLSSLPSVSKLSL---GS-AARAL-GASNGAAVVA--RVGTKK----VATVKK

MVGSS - ALLQRQAFCSSSAIGRRLNALFKGGTKQLKKATAPKTPIKK-...--AVPKTSV

 MCQSFLGAS RSAVP-.....-TKG-QGNKQVT AA MAKTFLGAS AKAVP-.-.-.-TSG-KGTKQVT AA TVM RT
 LVSSFTGKVAAS - - - - - - - - - - - KKVSQVSN



- -----AAI--------MKSSV-RSS --------RSTVSS-RSARVVP AA

|  |  |
| :---: | :---: |


|  |  |
| :---: | :---: |







MAALASQPVAAST-......-SVSQSV-SQAPLAAAEP - - KQAIS-- TTDETLP RV



kfloo120_0160
Cs_28488
ABD37891_MvLHCB5
Gene. 11404
DAAO5928_MVLHCB5
Cs_ 64185
AtLHCB5
Mapoly0011s0076
kfloo104_0350
AtLHCB7
Mapoly0008s0015
kfloo104_0370
CrLHCB7
kfloo422_0020
AtLHCB6
Mapoly0052s0039
kfloo276_0010
DAAO5919_MvLHCP1. 2
Gene. 11156
DAA05930_MVLHCP1. 1
Gene. 11308
MpLHCAP2. 1
MpLHCP1. 1
CmLHC
PyLHC
CrLHCSR1
CrLHCSR3
Cs_65904
kfloo478_0030
DAA05932_MvLI818;2
Gene. 11245
Gene. 11340
MpLI818;1
MpLI818;2

MAlSSLATSGVA
 -mTSTFL SA EVKAAR--KAAKKAPVKTVKKAAA AT MVSSFMGTQ KQAVVASTK-...-. - GTAKATPK AA MVSSFMGTQ KQAVVASTK......-GTAKATPK AA STQTWLAPS SRPLLPRPK-.-.-GTAKATPK AA LRSEFLGAT KRTEISQTKKTAKKVEKTASKTAS AT LGVSEMLGTP NFRAVSRSKKKPAPAK......-S AV LGRSEILGQG VASSST--KKGAAPKK-...-.-A TA VASKAFLGQA TSAETVATQKAAGTAKPAAARTVK AA FSAIPTAVKAI-.....-.-ANSSI-GVS RRRLEE--KKKEAVEN-.-.SSRF SK fSALPSVVKAI------ANSSL-GEA QERMMK--TKAAAQLD---AEAA AA LSAFPLFVKYL-------AEGPT-GDR MENAQK--TKAEKEPQ---KQEF EK

MAA - - TVLSRTL-.....-AAGPSFTGSSAHLLQTPQCKMAEPTDESPDVRIDS RR
 MAAVGIAVATPAA--.-.--TSSSSLSSRA AAFPLAYLQNGSRTVM---RAGT KV




Gene. 11536
AtLHCA3
Mapoly0066s0050
kfloo560_0090
CrLHCA3
Cs_37969
MpLHCA3
OL_K08909 ABD37881_MLLHCA4.1 DAAO5923_MvLHCA2
Gene. 11715
ABD37884_MLLHCA5. 2
DAAO5927_MvLHCA9
Gene. 10884
CrLHCA9
Cs_44136
MpLHCA9
O_K08911
CrLHCA2
Cs_ 7387
MpLHCA2

O__PTHR21649

ABD37882_MvLHCA4.2 DAA05926_MVLHCA5
Gene. 11585
DAA05920_MVLHCA4
Gene. 11349
AtLHCA2
Mapoly0083s0003
AtLHCA6
kfloo564_0030
MpLHCA4
OL_K08910
OL_K08908
AtLHCA4
Mapoly0006s0261
kfl00023_0070
CrLHCA4
Cs_52367
CrLHCA5
Cs_61250
CrLHCA6
Cs_46127
AtLHCA1
Mapoly0082s0040
kfloo100_0170
CrLHCA1
Cs_25286
DAAO5929_MvLHCA1
Gene. 11703
CrLHCA7
Cs_25284
CrLHCA8
Cs_48543
AtLHCA5
kfloo214_0160
MpLHCA1
Cs_66689
kfloooog_0500
kfloo517_0030
kfloooog_0510
kfloo517_0040
ABD37890_MvLHCB4
Gene. 10541
AtLHCB4.1
AtLHCB4.2
Mapoly0001s0025
AtLHCB4.3
kfloo258_0020
CrLHCB4
Cs_30169
Cs_67011
ABD37885_MvLHCBM1
Gene. 11306
Gene. 11314
AtLHCB1.1 AtLHCB2.1 Mapoly0057s0073
Mapoly0371s000
Mapoly0199s0017

RARVAA - RAANA
P--QAKFAV-FAAKA P--QAKFAV-FAAKA P--QAKFAV-FAAKA A--QAKFVV--SASS A-- QAKFVV--SASS P--DASVRA - VAADP A-- SFSV-- RAQAT EVSSVC-EPLPP V-- ISAASQ-GQYTA A - - RASVRV - GAVAAARPN M - - - SAKTSS-FKVEAK GE S——..-QS-LKVEAK GE P--.-. NG - ARVFAREAP W-- KSKSGG-AKRDAALPS -- - AARSAR - VVVRA RAS R-- RAVV-- VRAAA RKL R-- STFQVS-AAASQGRRL R-- SARRSV - VAKASSRPL Q - ............. VVRA RAL L---PNAGN-VGRIRMAAH L---STVSN-GSRVSMSSE L-- CRSIAG-SAKVSMRAS R-- - VAAVSN-GSRVTM GN K - - - - - SN-GSRVVM GN K - - KTDS - -RVTCMA RKL K - - KTDS - - RVTCMA RKL R-----AV-RAQAAVRPV R-----FV-AVSAA RQV R--.--KS VVTCVARQS L-- PSNG-- SRVCMKAGN AG----IN-PTVAVRAT QFAYKNRAFR-VSASA RPV GT-VGKVSARAV - - - - - - LV - - - - V SRPC - - - NARV - - TMAAA RPV AR--GARV--SMTTD RPM VQ--NARVMS - - - S GV I RAGGSKTASRKTVGSRRAV A-D-KLSASE-RQSGPRPK $A-D-K L S A S E-R Q S G P$ A-- PKKSAK-KTVTT S--PKKA--KTVIS A-- VKKAAP-RPGSA A-PPPKKSRQ-VQDDG AAPRPAASRR-PASGP R-- SGGVGY-RKY-QGDAL S--ATRTKK-F--DGDAL G-- TQRVKR-KGSGGGDAL -KSATPDS - PFYGP - - KSATPDS - PFYGP - - KSATPDS - PFYGP V-AKPKGPGS-PWYGS - - VKSTPQS - IWYGP

-     - SKSASSS - IWYGE

G--SKAASSS-IWYGE G--SKAASSS - IWYGE $R \vee T$
$R V T$
$R V T$
$R V K$
$R P K$
$R P K$
$R P K$
$R P K$ ---GSASNS - IWYGE RPK

$\cdots-A_{A}$ ATDSS $\cdots \cdots$ DPLKLAEDP $\cdots \cdot$.
 $D P L N A V E G-\cdots-V$ D TLGLAAPK PGEVFS DILGLAAPK PGEVFS DPFGLGKPA AGEVFG DPFGLGKPA YGEVFG DPLGLGKPA FGEVFG DPFGLGKPA AGEVFG DPLGLAKPA AGEVFG DPLGL KPS KGEVFG DPLELKPS AGEVFG DPLGL SPQ AGEVFG DTGL ADP $\quad \cdots \cdot-T$ D T $\quad$ GL A DP $\quad \cdots \cdots \cdot T$

DTAGL ADP $\cdots \cdots$.
$T$ - K - K - K - v v S T

DPLNLGSDP $-\cdots-$ L
DPLGLGEDKV - . - - A
DPLELGKDP $-\cdots-$ -
DPLGLAEDP $-\ldots-N$
dplglaEdpa- - - A
DPLGLGADPA… - N
DPLYLGQDPV $-\ldots$.
DPLRLGEDPA… - A
dPLGLGADPT… - A
dplglgak $\cdots$....A
dplglga a $-\cdots-$ -
DPLGLGADP $\quad \cdots-r^{-\cdots}$
DPLGLGEVPA- - - N
dplglaevp $\cdots \cdots$ n
DPLGFGEVP $-\cdots-N^{-}$
DPLSLGK PA - - - - S
dplglakDpl - . . - s
DPLGLAKDPV - . . - S
DPLFLGQ]P $-\cdots-T$
DPLSLGSDP $\quad \cdots-$ L
DPLGLGKDPV - . - - A
DPLKLGEDPA - - - S
DPLGLGEDP - - - - S
dplglgtdpa-… - D
DpLGLGK PA - - - N
 .
 . -號 -號


$$
\begin{array}{|l|l|l|l|l|ll}
\mathrm{D} & \mathrm{~T} & \mathrm{G} & \mathrm{~L} & \text { Al| } \mathrm{D} & \cdots \cdots & \cdots \\
\hline
\end{array}
$$

$$
\text { DTAGL ADP } \mid \cdots \cdots
$$

T

Cs_ 27246
Cs_ 35121
kflooo98_0080 kfloooss_0090 kflo1434_0010
CrLHCBM1 CrLHCBM2 CrLHCBM7 CrLHCBM3 CrLHCBM4 CrLHCBM8 CrLHCBM6 CrLHCBM9 kfloos09_0010 CrLHCBM5 Cs_38026
Mapoly0026s002 AtLHCB3 Mapoly0068s0047 Mapoly0068s0087 kfloo120_0160 Cs_28488 ABD37891_MvLHCB5 Gene. 11404 DAAO5928_MVLHCB5 Cs_ 64185 AtLHCB5 Mapoly0011s0076 kfloo104_0350 AtLHCB7 Mapoly0008s0015 kfloo104_0370 CrLHCB7 kfloo422_0020 AtLHCB6 Mapoly0052s0039 kfloo276_0010 DAA05919_MvLHCP1. 2 Gene. 11156
DAA05930_MVLHCP1.1 Gene. 11308 MpLHCAP2.1 MpLHCP1. 1 CmLHC

 3 2.1 $\qquad$
$\square$
--- GSAPSS - I WY G E -- - GSAPSS - IWYGE - - VKSTPTS - IWYGE - VKSTPTS - IWYGE - VKSTPTS - IWYGE - VKSTPTS - IWYGE - VKSTPTS - IWYGE ---GSAPSS - IWYGE -- SSKSSSS - IWYGE - SSKSSSS - IWYGE S--KAAAPAS-IWYGE - - KTSTPES - IWYGP


V-KGKTTPTS-MWYGE -- -GARTSMV-EFYGP GARTSMV-EFYGP VKSAPSS-PWYGP V-SKASTPDS-FWYGP IEWYGP IEWYGP -- AKQAPASIEFYGP APKSVEFYGP

 --.--APKGIEFYGP V---SSKAS-LWYGP
A-NGGNEKLS-AFYGP V---SSKAS-LWYGP
A-NGGNEKLS-AFYGP I------ EWYGP P-TETAVDLT-LWYGP P-TETAVDLT-LWYGP
---KYTMGND-LWYGP - - KVSMDAD - YWYGP D-EEFGVDLS-LWYGAGRVK GGQPGAGGLG-KWYGP GGQPGAGGLG-KWYGP
KAAKSASGAS -FWYGA A-- PADGAA-KWYGP A---PADGAA-KWYGP A--PADGAA-KWYGP
A---PADGAA-KWYGP KQAGGAQGLD-KWYGPSRAL S--ETSDELA - KWYGP
P--PSNAELA - KWYGP S--ETSDELA-KWYGP
P--PSNAELA-KWYGP RKNASDDELS - KWYGP A--QEARNDS-KWYGK Q--ADARAKS - LWFGDSRPK Q--TEARQRS-RWYGP RPK A-HAEARRQS - KWYGPSRPR LTPKGVDATK-ALYGP RPK
A---AKPAF-KGGKGG LS T-.-...-.-RSTSGR LN


 LTPKGVDATK-ALYGP RP | RAK | L |
| :--- | :--- |
| RPG | L | RPK GAFT--RS

LTGPADI
PTG K $-P T$ G P A D I
$-P T$ G P A D I RRI

RRI | $\mathrm{L}-\mathrm{P}$ G G P S D V |
| :--- |
| L |
| PD G R S E I | L-PEGKSDIP L-PEGREEVP






-

$$
-\mathrm{T}
$$

EYPGDYG
EFAGGD G
EFAGDY
G



$$
\left\lvert\, \begin{array}{lll|l|l|}
\hline D & T & A & G & A \\
D & D & P \\
D & G & \text { A } & \text { D }
\end{array}\right.
$$



DTAGL AD D
DTAGL ADP $\quad \cdots \cdots \cdot$
DTAGL ADP $\cdots \cdots$.
DTAGL ADP $\cdots \cdots$.
DSAGL ADP $\cdots \cdots$.
DTAGL ADP $\cdots \cdots$.
DTAGL ADP $\cdots \cdots$.
DTAGL ADP $\cdots \cdots$ -
DTAGL ADP $\cdots \cdots-$
DTAGL ADP $\cdots \cdots-\cdot$
DSSGL ADP $\cdots \cdots-\cdot$

## DTAGL ADP $\cdots \cdots$.

DPLGLG-KG… G
DPLGLG - KG $\ldots-\cdot G$
DPLGLG-KG…-G
DPLGLGKDTA $\cdots \cdot . .$.
DPFGLGKKP $\cdots \cdots N$
DPEGL KKPA…
DPLGLGKKE $\cdots \cdot-$.
$D$ IAGLGKDRL…... T
DILGLGR TA $\cdots \cdots \mathrm{N}$
$D \| N L G V D P S \cdots \cdots$
DPLGLK QA… - S
IDVLGCKDE $\cdots \cdot-A$
DPLGLGKDPA…...
DPLGLGRDPA… $-A$
DPLGLGKDPA…...A
PNWTEGKTEA $-\cdots-$ Q PNWTEGKTEA - - - Q PNWTKGKSDA…-D PNWTKGKSDA- - - D

號

PyLHC
CrLHCSR1 CrLHCSR3 Cs_65904 kfloo478_0030 DAA05932_MvL/818;2 Gene. 11245
Gene. 11340
MpL/818;1
MpL/818;2ABD37880_MVLHCA3Gene. 11536
AtLHCA3
Mapoly0066s0050
kfloo560_0090
CrLHCA3
Cs_37969
MpLHCA3
Ol_K08909
ABD37881_MvLHCA4.1
DAA05923_MvLHCA2
Gene. 11715
ABD37884_MvLHCA5.2
DAA05927_MvLHCA9
Gene. 10884
CrLHCA9
Cs_ 44136
MpLHCA9
Ol_K08911
CrLHCA2
Cs_7387
MpLHCA2
Ol_PTHR21649
ABD37882_MvLHCA4.2
DAA05926_MvLHCA5
Gene. 11585
DAAO5920_MvLHCA4
Gene. 11349
AtLHCA2
Mapoly0083s0003
AtLHCA6
kfloo564_0030
MpLHCA4
OL_K08910
OL_K08908
AtLHCA4
Mapoly0006s0261
kfloo023_0070
CrLHCA4
Cs_52367
CrLHCA5
Cs_61250
CrLHCA6
Cs_46127
AtLHCA1



P

 | $1 H C R$ | $A$ |
| :---: | :---: |
| 1 | $H C R$ |
| 1 | $G R$ |
| 1 | $G$ |
| 1 | $G$ |
| 1 | $G R$ |$A_{A}$ AM

AM
$A M$
$A M$
AM
WA
WA
WA $M L G$
$M M L C$
$M M L C$
$M M L C$
$A M L C$ $\begin{array}{ll}\mathrm{G} & \mathrm{M} \\ \mathrm{G} & \mathrm{M} \\ \mathrm{G} & \mathrm{A} \\ \mathrm{G} & \mathrm{A} \\ \mathrm{G} & \mathrm{A} \\ \mathrm{G} & \mathrm{A} \\ \mathrm{G} & \mathrm{V} \\ \mathrm{G}\end{array}$

 |  | $A$ | $P$ |
| :--- | :--- | :--- |
| $M$ | $A$ | $P$ |
| $A$ | $A$ | $P$ |
| $A$ | $A$ | $P$ |
| $M$ | $A$ | $P$ |
| $C$ | $A$ | $P$ |
| $C$ | $A$ | $P$ |
| $V$ | $A$ | $P$ |
| $M$ | $A$ | $P$ | L

 $I P A$
$I P A$
$I P A$
$I P Q$
$I P A$
$I P Q$
$I P A D$
$I P Q$
$S D$
$S D$
$S D$ PAE
PAE
PQE
PAK
PQ
PAD
PQE
D-ETG
ETG
ETA
ATA
TDE
TDTG

-     - L
LVN

 GPW
FT A GL-........PK

FT A GL SV SV SV KWMV A KWMV A KWMV AE


$\begin{array}{ll}\text { KW } & \text { V } \\ \text { KW } & \text { A }\end{array}$
KW


kflool00_0170
CrLHCA1
Cs_25286
DAA05929_MvLHCA1
Gene. 11703
CrLHCA7
Cs_25284
CrLHCA8
Cs_48543
AtLHCA5
kfloo214_0160
MpLHCA1
Cs_66689
kfloooog_0500
kfloo517_0030
kfloooog_0510
kfloo517_0040
ABD37890_MvLHCB4
Gene. 10541
AtLHCB4.1
AtLHCB4.2
Mapoly0001s0025
AtLHCB4.3
kfloo258_0020
CrLHCB4
Cs_30169
Cs_67011
ABD37885_MvLHCBM1
Gene. 11306
Gene. 11314
AtLHCB1.1
AtLHCB2.1
Mapoly0057s0073
Mapoly0371s000
Mapoly0199s0017
Mapoly0199s0018
Mapoly0199s0019
Mapoly0199s0012
Mapoly0199s0014
Mapoly0199s0016
Mapoly0199s0015
Mapoly0199s0013
Mapoly0199s0020
Mapoly0057s0082
Mapoly0057s0083
Mapoly0068s0071 Mapoly0139s0012 Cs_15915 Cs_21950
Cs_58975
Cs_27246
Cs_35121
kflooogs_0080
kflooogs_0090 kflo1434_0010
CrLHCBM1 CrLHCBM2 CrLHCBM7 CrLHCBM3

A

KW V A E
kw RW kw v kw v AE aw v A ${ }^{\text {GWLL }}$ AE Kt MAEAE at VEAE RN LVEGE
RN VIEGE
E

FAA
FAA REI
FA NREL
FA NREL
FA NRELE
FA NRELE
FA NRELE
FA NRELE
FA NRELE
FA NRELE
FA NRELE
FA NRELE
FA NRELE
FA NRELE
FA NRELE
FA NRELE
FA NRELE
FA NRELE
FS NRELE
FA REI
FA REI
FA REI
FA REI
FA REI
FA NREL
FA NRELE
FA NRELE
FK RELE
FK
FK RELE
FK


 LGLGNWLN - - AQD/NA



GQGDWY Q - - - AP L $W$ GQGTWLS - - AQDN
$\qquad$ G VPE LGKGNFGDIA-ASAP

 SVEMLTG….....VTNQDAGKVE-LVDGSSYLGQ



A AVEALTG $\ldots . . . . . . . . . . V H N Q D A G K V E-L V D G A S$


VAEASTG ................ SSN
C F LPEALEK SG-INFG - ES VN
HARWAM LGGT
IHSRWAM LGA
I HSRWAM LGA
I HARWAM L GA
I HARWAM L GA
I HARWAMLGA
I HARWA A M L G GA
I HSRWAM LGA
I HARWAMLGA
I HARWAMLGA
I HARWAMLGA
I HARWAM L GA
I HSRWAMLGA
I HARWAM L GA
I HARWAMLGA
I HARWAMLGA
I HSRWAMLGT
I HARWAMLGA

## I HARWAMLGA

I HARWAM L GA
I HARWAM L GA
I HARWAM LGA
I HARWAM L GA

C F L


F L
$C \quad F$
$C T F$
F
T
T F
T
T

 $-I N$
$-I$
$-V$
$-V$
$-V$
$-V$
$V$

A G GQ I F Q E G G L D Y Y $\mathrm{L}|\mathrm{C}| \begin{aligned} & \mathrm{N}\end{aligned}$ A GGQ I F Q E G G L D Y LLG

A G Q I F F S D G G L D

## CrLHCBM8

## СгLHCBM6

## CrLHCBM9

kfloos09_0010
CrLHCBM5
Cs_38026
Mapoly0026s002
AtLHCB3
Mapoly0068s0047
Mapoly0068s0087
kfloo120_0160
Cs_ 28488
ABD37891_MVLHCB5
Gene. 11404
DAAO5928_MvLHCB5
Cs_ 64185
AtLHCB5
Mapoly0011s0076
kfloo104_0350
AtLHCB7
Mapoly0008s0015
kfloo104_0370
CrLHCB7
kfloo422_0020
AtLHCB6
Mapoly0052s0039
kfloo276_0010
DAAO5919_MVLHCP1. 2
Gene. 11156
DAA05930_MvLHCP1. 1
Gene. 11308
MpLHCAP2. 1
MpLHCP1.1
CmLHC
PyLHC
CrLHCSR1
CrLHCSR3
Cs_65904
kfl00478_0030
DAA05932_MvL1818;2
Gene. 11245
Gene. 11340
MpLI818;1
MpL1818;2

ABD37880_MVLHCA3
Gene. 11536
AtLHCA3
Mapoly0066s0050
kfloo560_0090
CrLHCA3
Cs_37969
MPLHCA3
Ol_KO8909
ABD37881_MLLHCA4. 1 DAAO5923_MvLHCA2

FK

\section*{| FK | RELL |
| :--- | :--- |
| FK | RELE |}


| FR | NRELE |
| :--- | :--- |
| FK | RELE |

$\begin{array}{lrl}\text { FS } & \text { REILE } \\ \text { FA } \\ \text { REEL }\end{array}$
FA NRALE
FA NRALE
FA NRELE
FR NRELE IHRWAMLGA

| FS |
| :---: |
| E |
| E |
| E |
| E |
| FA |
| FD |
| FD |
| FD |
| FD |
| FQ |
| FA |

FA LRAQE KW
KW
KEAE
REAE KW REAE AYNVEVE AYNVEVE tynvsve - - NARRE 1Hgrwamaav
I hgrwamaav
I Hg GRWA AT G 1 HGGWAMLGCAG
I HGRWAMLGCAG
1 HGRWAMLGCAG
I HGRWAML GVTGAWAAENGTG KGRVAMLAC HFFVTEFYQF-......-PF AGAPKLA - . - AHDYFVK NWL EGE


FVQEFYTL-....-.-PF SGGPALA - - - SHNYFVT vGEQLQD-.....-FPL FDGRVS-..-AIYFQQ VGEQLQD - .....-. FPL WDGRVS - .- AIY FQQ VGEF AD - .-....-KKLLSDGRIT-..-AID FQQ VGEQ ED--.-.--FPARFFPHVT-.-AIY FQQ

 AE FNPL--.-.-.--FNG IK-.-AIN FQQ VGES EG-.....-. SS LFDSQVT-..-AIN FQQ VGEQ EG-......-SA LFDANIT-..-AID FQQ

| VGES | E G - - - - - S S | LFDSQVT---AIN | QQ |
| :---: | :---: | :---: | :---: |
| VGEQ | E G - - - - - - S A | LFDANIT---AID | FQQ |



IFVG AWSG
$\qquad$
$\qquad$ WAAERGTG——..... INN
$\qquad$
$\qquad$ 1 NN
$\qquad$ 1 PN




| P - - - - L | FW | LMQFAE | LRRWQDYRHP | GSQSKQYFLGLEQFFGGSG P |
| :---: | :---: | :---: | :---: | :---: |
| P - - - - L | FW | LMQFAE | LRRWQDYRHP | GSQSKQYFLGLEQFFGGSG |
| N - - - - ${ }^{\text {P }}$ T | F V | ALMGFAE | HRRLQDWYNP | GSMGKQYFLGLEKGLAGSG |
| P - - - - Y T | FV | ALMGFAE | HRRAQDYYKP | GSMGKQYFLGFEKVLGGSG |
| S - - - - Y T | F G | LMAFAE | HKRLADYRKP | GSQGKVFFLGMEKFLGGSG |
| P - ....- Y T | FF | AMQFAE | LRRLQDFRYP | SMGQQYFLGLEAIFKGSG |
| P - - - - Y S | F F | LGQ F AE | LRRWQDFRNP | SQGKQYFLGLEEVLKGSG P |
| P - . . - - T T | FW | N LMNFAE | VKRGQDYWYP | SQGETPLMGWEKGFAGSGAP |
| P - - - - - ${ }^{\text {P }}$ | FW | NAALMNFAE | LRRAQD YWNP | SMGKQELIGWEKMLGGSG |
| S--SPF-D QT | A | F FAT E | GFRISTWKKT |  |
| S--SPF-D QT | A | F FAT E | G FRIS TWKKT\| |  |








240

Gene. 11715
ABD37884_MLLHCA5. 2 DAAO5927_MvLHCA9
Gene. 10884
CrLHCA9
Cs_44136
MpLHCA9
OL_K08911
CrLHCA2
Cs_ 7387
MpLHCA2
OI_PTHR21649
ABD37882_MLLHCA4.2
DAAO5926_MvLHCA5
Gene. 11585
DAAO5920_MvLHCA4
Gene. 11349
AtLHCA2
Mapoly0083s0003
AtLHCA6
kfloo564_0030
MpLHCA4
Ol_K08910
Ol_K08908
AtLHCA4
Mapoly0006s0261
kflooo23_0070
CrLHCA4
Cs_52367
CrLHCA5
Cs_61250
CrLHCA6
Cs_46127
AtLHCA1
Mapoly0082s0040
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CrLHCAI
Cs_25286
DAAO5929_MvLHCA1
Gene. 11703
CrLHCA7
Cs_25284
CrLHCAB
Cs_ 48543
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kfloo214_0160
MpLHCA1
Cs 66689
kflooo09_0500
kfloo517_0030
kfloooos_0510 kfloo517_0040 ABD37890_MVLHCB4
Gene. 10541
AtLHCB4. 1
AtLHCB4. 2
Mapoly0001s0025
AtLHCB4.3
kfloo258_0020

S-SPF-D QT A

 PFA PT---SP GLGIFH AIAEGARLFRETEAGYDSSID..................TGIPPGPFA PT-.-SY GIGLFHLAI ELSRLFREAKNGYDDDIE.................VGIYPG-

P- PW-T TQ I
P-- PW-T TQ I
P-- PF-S ST W
P-- PF-S ST W

P-- PF-S SA W
P-- PF-S TT W
P-- PF-D PT TI
L

Cs_30169
Cs_67011
ABD37885_MVLHCBM1
Gene. 11306
Gene. 11314
AtLHCB1. 1
AtLHCB2. 1
Mapoly0057s0073
Mapoly0371s000
Mapoly0199s0017
Mapoly019950018
Mapoly0199s0019
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MapolyO199s0014
Mapoly0199s0016
Mapoly0199s0015
Mapoly0199s0013
MapolyO199s0020
Mapoly0057s0082
Mapoly0057s0083
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Cs_15915
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Cs_58975
Cs_27246
Cs_35121
kflooo98_0080
kflooo98_0090
kflo1434_0010
CrLHCBM1
CrLHCBM2
CrLHCBM7
CrLHCBM3
CrLHCBM4
CrLHCBM8
CrLHCBM6
CrLHCBM9
kfloos09_0010
CrLHCBM5
Cs_38026
Mapoly0026s002
AtLHCB3
Mapoly0068s0047
Mapoly0068s0087
kfloo120_0160
Cs 28488
ABD37891_MVLHCB5
Gene. 11404
DAAO5928_MvLHCB5
Cs_ 64185
AtLHCB5
Mapoly0011s0076 kfloo104_0350 AtLHCB7
Mapoly0008s0015
kfloo104_0370 CrLHCB7

|  | S | TQ | w |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | -S | SQ | w |  |  | EIYR | RNRS |  | LQ |  | YPGG |
| S-- | PF-S | SQ | w | A |  | $1 Y R$ | RN |  | LEP | AR | YPGG |
| PSL | HAQS | IAT | F | A | LM |  | R |  | EAG | KM |  |
| PSL | HAQS | IAT | F | A | LMGL |  | RVG |  | A |  |  |
| P - - |  |  |  |  |  |  |  |  |  |  |  |
| PSL | HAQS | LA | WAT |  | MGA | Err | RVAGN | PPLG | EAE |  |  |
| PNL | HAQS | LA W | WA |  | MGF | E Gr | RIGG | P | EG | P L | GG |
| SGL | HAQS | LA | WAC |  | M ${ }^{\text {a }}$ |  | RV |  | EV | PI | YPG G |
| SGL | HAQS | LA | WAC |  | M ${ }^{\text {A }}$ | G | RVS G | GP | EV | PI | YPGG |
| SGL | HAQS | LA | WAC |  | M |  | RVAG | PLG | EV | PI | YPGG |
| SGL | HAQS | LA | WAC |  | GA |  | RV | G | V | PI | YPGG |
| SGL | HAQS | LA | WAC |  | MGA |  | RVAG |  | EV | PI | YPGG |
| SSL | HAQS | LA | WAC |  | MGA |  | RV | P | DVV | PI | YPG G |
| SSL | HAQS | LA | WAC |  | M ${ }^{\text {A }}$ | GYR | RVAG | dPLG | EV | PI | YPGG |
| SSL | HAQS | LA | WAC |  | M |  | RVAG | P | EV | PI | YPGG |
| SSL | HAQS | LA | WAC |  | mea |  | RVAG | d ${ }^{\text {P }}$ | - DVV | PI | YP G G |
| SSL | HAQS | LA | WAC |  | LMGA | GYR | RVA |  | EVS | PI | YPGG |
| SSL | HAQS | LA | WAC |  | MGA |  | RV | - | - DVV | PI | YPGG |
| SSL | HAQS | LA | WAC |  | LMGA | EGYR | RVAG | GP | - DV | PI | YPG G |
| SSL | HAQS | LA | WAC |  | MGA |  | R A | GP | V | PI | YPGG |
| SSL | HAQS | LA | WAC |  | A |  | RV | P | - DVV | PI | YPGG |
| ENL | HAQS | LA | G C |  | MGL | GYR | RVGG | GP | AD | PI | YPG G |
| PNL | HAQS | VAT | A |  | LMGS |  | RAAG | APG | DG | K L |  |
| SSL | HAQS | IAT | AC |  | MGG | A YR | RA | - | EGL | L | Y |
| PSL | HAQS | IAT | AC |  | MG |  | RAN | d | -EGL | SL |  |
| PSL | HAQS | IA | FS |  | MGL |  | RVN | P | EG | AL | E |
| PSL | HAQS | IA | A S |  | LMgA | G | RVYG | dPG G | EGL | Pr | Y |
| PSL | HAQS | LA | A S |  | L.MGA |  | RVN | - | Eve | PL |  |
| PSL | HAQS | LA | A S |  | M ${ }^{\text {A }}$ |  | RVNG | dP | EVE | L | Y |
| PSL | HAQS | LA | GS |  | LMGA |  | RV |  | EI | PL |  |
| PNL | HAQS | LA | G T |  | LMGA |  | RVNG | PL | EGL |  | Y |
| ENL | HAQS | IAT | AF |  | MGL |  | RANG | dP LG | EGL | PL |  |
| ENL | HAQS | IAT | AF |  |  |  |  | P | L |  |  |
| PSL | HAQN | VAT | A |  |  |  | RVN | G | EGL |  | GE |
| PSL | HAQN | VAT | A |  | L |  | RVN | G | E |  | YPGE |
| PSL | HAQN | vat | A |  | L |  | RV | P | EGL | L | Y P GE |
| PSL | HAQN | VAT | A |  | L |  | RVNG | dPag | -EGL | , | YPGE |
| PSL | HAQN | VAT | A |  |  |  |  | P | GL |  |  |
| PGL | HAQS | LAT | AT |  | A |  | RV | d | EVE |  |  |
| PGL | HAQS | LAT | A |  | M ${ }^{\text {a }}$ | GYR | RVNG | dPag | EGL |  |  |
| PSL | HAQS | IAT | AT |  |  |  |  | P | EIt |  |  |
| PNL | HAQS | LA | WAS |  | LMEL | GYR | RIGG | GPLG | DAG | GL | YPGG |
| PNL | HAQS | LA | G F |  | LMEL | G FR | RINGL | - ${ }^{\text {dV }}$ | E | D |  |
| PNL | HAQS | LA W | WA |  | A |  | RQNGL | PGIG |  | - | Y |
| PNL | HAQS | LA | WAC |  | LMGL | EGr | RSGG | -回 $\mathrm{LG}^{\text {g }}$ | KVT | PL |  |
| SSL | HAQS | LAT | AC |  | LMGA |  | RVNGL | EGFQ | ERD |  |  |
| PGL | NAKN | IAT | A |  | LMgA |  | RVNG | - ${ }^{\text {P }}$ | -EGL | V |  |
| P--N | NPV-P | ALA | IF |  | FFL | R | RYQQD | - 6 PWG | -TGL | PL | YPGG |
| P--N | NPV-P | ALA | 1 F |  | FFL |  | RYQQD | PWG | TGL | L | YPGG |
| P - - | NPV-P | ALA | IF |  | FFL |  | RYQQD | - 6 PWG | TGL | P L |  |
| PWGN | NNPLP |  |  |  | L GA |  | RQSGE | GPPGY | FSGL |  | YPGG |
| N -- | PI-N | VLA | va |  |  | R | RITNG |  | - DFE | KL |  |
| S - - | PV-N | AAA | IA |  |  |  | RStnk | SPLG | SDL | RL |  |
| T - - | PL-N | AAAT | TIA |  | L-G | R | RSANK | SPLG | SDL |  |  |
| PGLA | AGSQG | IV A | AIC |  | LMVGP |  | RYCGIE | LEPLGI | -PG |  |  |
| PGLA | AGAQG | LV A | AFC |  | LMVGP | $Y$ | RYCGI | LePLGV | -PG |  | G |
| PGL | AGGQG | LI | AFC | A | LMLGP |  | RATGIA | LePVGL | PG |  |  |
| EGFA | AGKQG | GL | AAC | A | LMGGP | EY | RYVGIR | LEPVGV | - PG | QN | PGG |

kflo0422_0020
AtLHCB6
Mapoly0052s0039 kfloo276_0010 DAA05919_MvLHCP1.2 Gene. 11156
DAA05930_MVLHCP1.1
Gene. 11308
MpLHCAP2.1
MpLHCP1.1
CmLHC
PyLHC
CrLHCSR1
CrLHCSR3
Cs_65904
kfloo478_0030
DAA05932_MvL/818;2
Gene. 11245
Gene. 11340
MpL/818;1
MpL/818;2

PNV NAHS IA AAF A
PDA APFSFGS GT PSA APFSFGT GT PGA APFTFGT GT
|LMG|GA|E|F ARLKAPK - - - - - - - - - - - - - EM G L|YPGG| LMGW ESKRWVDFFNPDSQSVEWATPWSKTFANTG QGYPGG LMGW EGKRWADYVNPNSQLVDWATPWSRTFGNTGLQGYPGG LMSW EGKRWVDFYNPSSQSVEWATPWSKTFANTGQQGYPGG PAGSGFPNFYIQ A ST PAGSGFPNFYIQ A ST AMGLAEGYRGGLIDSC FPETVGDLPGG PEGSGFPNFYIQ G S PEGSGFPNFYIQ G S PEGSGYPSFWA A PEGSGYPSFWA A FL GSAECYRTGLFENP - SGAMQ LAF GFLEFL HRG VLYSDMEW - $\qquad$ FPEEL-SVTPGG

## -- QGAMQ LLW CGLE

 GQG---FWEPL AGVPAVL M-MQG
$\qquad$- - - -GVAESYRVAVGWTPTGTGFNN-- FPETVGDL MGLAEGYRGGLIDNV FPEEVGDL

## MG

 FPEEVGDL FPEEL-SVTPGG

ABD37880_MvLHCA3
Gene. 11536
AtLHCA3
Mapoly0066s0050
kfloo560_0090
CrLHCA3
Cs_37969
MpLHCA3
Ol_K08909
ABD37881_MvLHCA4.1
DAAO5923_MvLHCA2
Gene. 11715
ABD37884_MvLHCA5.2
DAA05927_MVLHCA9
Gene. 10884
CrLHCA9
Cs_ 44136
MpLHCA9
Ol_K08911
CrLHCA2
Cs_ 7387
MpLHCA2
Ol_PTHR21649 ABD37882_MvLHCA4.2 DAA05926_MVLHCA5
Gene. 11585
DAA05920_MvLHCA4
Gene. 11349
AtLHCA2
Mapoly0083s0003
AtLHCA6
kfloo564_0030
MpLHCA4
Ol_K08910
O__K08908

260
270
280

 | - F L | I |  |
| :--- | :--- | :--- |
| - F L | D | I |
| - F L | I |  |



250
0
.

A
 KEIKNGRLAM

290
300


AtLHCA4

AtLHCB4．2
Mapoly0001s0025
AtLHCB4．3
kfloo258＿0020
CrLHCB4
Cs＿30169
Cs＿67011
ABD37885＿MvLHCBM1
Gene． 11306
Gene． 11314
AtLHCB1．1
AtLHCB2．1
Mapoly0057s0073
Mapoly0371s000
Mapoly0199s0017
Mapoly0199s0018
Mapoly0199s0019
Mapoly0199s0012
Mapoly0199s0014
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Mapoly0199s0015
Mapoly0199s0013
Mapoly0199s0020
Mapoly0057s0082
Mapoly0057s0083
Mapoly0068s0071
Mapoly0139s0012
Cs＿15915

QEAKEKE A ANGRLAM 1FPLNFPV $-\cdots$ KESKDKE CANGRLAM IFDPEGN KG－－DIKSLK KEIKNGRLAM IFDPLGY KG－－NMAELK KEIKNARLAM IFDPFGF KG－－NLKELTKEIKNGRLAM IFDPFGF KG－－NFKE TKEIKNGRLAM VFAPF－－－IP－GDLAELK KEIKNGRLAM IFAP－－－IP－GDLAELK KEIKNGRLAM AFDPLGY KDP－KKLEELK PFDPLGFAKDP－KKLEEYK
AFDPLGFKDP－AKLDELK
AFDPLGFAKDS－SKSGELK
AFDPLGF KDS－KVLEENK AFDPLGFGKDE——－VNKKE NGRLAM
AFDPLGEGKDE－．．－VNK KE JNGRLAM RFFDP GL RGDAAKYQEYKQKE KKGRLAM KLFDPEGL RGSEGQLQ Y ENEIKNGRLAM －PFDPLGL K A－DKWA WK KE KNGRLAM －VEDPLGLKKG－－GYEAK KIKNGRLAM PLLPLGLAKDV－QNAH WK KEIKNGRLAM PFFITGLGTGIKDDVF TKE KKNGLAM －AFDPAGF KGA－－DFETLKKKEIANGRDAM APFDP GLTTDY ．．．．N QAE $\square$ CRLAM －FDPLGLATGDDAQIK T EIKNGRLAM －FDPLGLTTGEADEIKI EKEIKNGRLAM －FDPLGFAKDDAKVK T EIKNGRLAM －FDPLGFLEDKEEEVNEL TKE KKNGRLAM PFDPFGFTKASESRVNLK AEIK ARLGM PFDPFGFTKASESRVN LK AEIK ARLGM KFFDPLGLAADP－EKTA L AEIK ARLAM KFFDPLGLASDP－VKKA L AEIK ARLAM KYFDPLGLASDP－EKKEILK AEIK ARLAM －YFDPLGLAADP－EKLDTLK YFDPLNLASSP－EKAA LK AE［K GRLAM VFDPLKLAS DEERAFRLKTAEIK ARLAM LFDPLKLASDDSQRTF L EAEIK GRLAM YFDPLGLATGDDARAF LKEAE KK GRLAM －FDPLGLGDDP－DTLAELK KEIKNGRLAMF A S
－FDPLGLGDDP－DTLAELK


















KEIKNGRLAMF


## M <br> M <br>  <br>  <br> のの の の の の の の の の の の の の の の の の <br> 

Cs_21950

Cs_58975
Cs_27246
Cs_35121
kflooog8_0080 kflooogs_0090 kflo1434_0010 CrLHCBM1 CrLHCBM2 CrLHCBM7 CrLHCBM3 CrLHCBM4
CrLHCBM8
CrLHCBM6
CrLHCBM9
kfloos09_0010
CrLHCBM5
Cs_38026
Mapoly0026s002
AtLHCB3
Mapoly0068s0047
Mapoly0068s0087
kfloo120_0160
Cs_28488
ABD37891_MvLHCB5
Gene. 11404
DAA05928_MvLHCB5
Cs_64185
AtLHCB5
Mapoly0011s0076
kfloo104_0350
AtLHCB7
Mapoly0008s0015
kfloo104_0370
CrLHCB7
kflo0422_0020
AtLHCB6
Mapoly0052s0039
kfloo276_0010
DAA05919_MvLHCP1.2
Gene. 11156
DAA05930_MVLHCP1.1
Gene. 11308
MpLHCAP2.1
MpLHCP1.1
CmLHC
PyLHC
CrLHCSR1
CrLHCSR3
Cs_65904
kfloo478_0030
DAA05932_MvL/818;2
Gene. 11245
Gene. 11340
MpL/818;1
MpL/818;2

P-|FDPLGLADDDP - DTFS|ELK KEIKNGRLAMF
P-FDPLGLADDP - DTFAELKTKEIKNGRLAMF A-FDPLGLADDP-DTFAEL KEIKNGRLAMF DYFDPLGLADDP - DTFAELK KEIKNGRLAMF Q-FDPLNLAEDP - DTFAELTKEIKNGRLAMF Q-FDPLNLAEDP - DTFAEL TKEIKNGRLAMF Q-FDPLNLAEDP - DTFAELK KEIKNGRLAMF S -FDPLGLADDP - DTFAELK A-FDPLGLADDP - DTFAELK A-FDPLGLADDP - DTFAELK S-FDPLGLADDP - DTFAELK S-FDPLGLADDP - DTFAELK S-FDPLGLADDP - DTFAELK S-FDPLGLADDP - DTFAELK S-FDPLGLADDP - DTFAELK Q-FDPLGLADDP - EEFAELK QFFDPLGLAEDP - DAFAELK A-FDPLGLADDP - DTFAELK A-FDP $\square \mathrm{GL} \mathrm{EDP}$-EAFAELK QYFDPLGLADDP - VTFAELK KYFDPLGLAEDP - ETFAELK DYFDPLNLAQDP - DTLAELK A-FDPLGLADDP - EAAAELK S-FDPLGLADDP - DTFAELK KYFDPLGLASDP - SKADELK KYFDPLGLASDP - SKADELK KYFDPLGLASDP - SKADELK P-FDPLGLADDP - ETFAELK P-FDPLGLAKDP - EQGALLK A-FDPLGLAKDP-DQFALLK A-FDPLGLADDP - DQFALLK TLFDPLNLEDP-VAFE LK ALFDPLGL KDP-STFE LK ALFDPLGLADDP - EAFE LK GPFDPLNYAADA - DGFVEQA KAFDPLGFTDP-ESLAELK RFFDPLGLAGKNFEKLERLK KFFDPLSLAGDVYDKLRL KFFDPGGAGKVFAKLERL EHFDPLGLAN--KLDLDR K EHFDPLGLAN--KLDLDR K EHFDPLGLAD--KLDLDR K EHFDPLGLAD - -KLDLDR K R-FDPLGLAESG--DLEELK $R-F D P L G A E A G--D L E E L K$
$L G F P N L P N D K-\cdots-A$ FGFDPLNLAKTN-AAFD F NAE LGFDPLGLKPDP - EELKTL TKE LGFDPLGLKPDP-EELKV TKE NNGRLAM I GFDPLGLTPDP - KEKY L TKE NNGRLAM LGEDPLGLLPDP-AAKK SKE NNGRLAM LG DPLGLKP S-KELDE ATKE NNGRLAM LG DPLGLKP S-KELDESATKE NNGRLAM LG DPLGLKPDP-AALAEL TKE NNGRLAM
 KEIKNGRLAMF
KEIKNGRLAMF
KEIKNGRLAMF
KEIKNGRLAMF
KEIKNGRLAMF
KEIKNGRLAMF
KEIKNGRLAMF
KEIKNGRLAMF
KEIKNGRLA A
KEIKNGRLAMF
KEIKNGRLAMF
KEIKNGRLAQFA
KE KKNGRLAMTAM
KEIKNGRLAMF
KEIKNGRLAMF
KE KNGRLAMTAM

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KEIKNGRLA
KEIKNGRLAMFAM
KEIKNGRLAMF M
KEIKNGRLA F
KEIKNGRLAM
KEIKNGRLAM
KE KNGRLAM
KELKNGRLAM
KEIKNGRLAM
AEIK SRLAM
AEIK SRLAM
AEIK ARLAM

NNGRLAM V NGRLTM
NNGRLAM

NNGRLAM
NNGRLAM

MF|GF|F|VQA|
MF GFFVQA MFGFFVQA MFGFFVQA MFGFYVQA MFGFYVQA

KEIKNGRLAMF CFGFFVQA
KEIKNGRLAMF NFGFFVQA

G I AGMVVQE
G I A GMVVOE
GIAGMVVOEFTQ PIF..
AI TAMVAOE NTGINL LADVLG KGGG VNGLNL PADVLE GKAG
ABD37880_MvLHCA3

Gene. 11536 AtLHCA3
Mapoly0066s0050 kfloo560_0090
CrLHCA3
Cs_37969
MPLHCA3
OL_K08909 ABD37881_MvLHCA4.1 DAAO5923_MVLHCA2 Gene. 11715 ABD37884_MLLHCA5. 2 DAAO5927_MVLHCA9
Gene. 10884
CILHCA9
Cs_44136
MpLHCA9
OL_K08911
CrLHCA2
Cs_ 7387
MpLHCA2
Ol_PTHR21649
ABD37882_MLLHCA4.2
DAAO5926_MvLHCA5
Gene. 11585
DAA05920_MvLHCA4
Gene. 11349
AtLHCA2
Mapoly0083s0003
AtLHCAG
kfloo564_0030
MpLHCA4
OL_K08910
Ol_K08908
AtLHCA4
Mapoly0006s0261
kflooo23_0070
CrLHCA4
Cs_52367
CrLHCA5
Cs_ 61250
CrLHCA6
Cs 46127
AtLHCA1
Mapoly0082s0040
kfloo100_0170
CrLHCAI
Cs_ 25286
DAA05929_MVLHCA1
Gene. 11703
CrLHCA7
Cs_25284
CrLHCAB
Cs_48543
AtLHCAS
kfloonl4_0160
MpLHCA1
Cs_66689

|  |  |
| :---: | :---: |
|  | ILT-TFGKIGGSF |
|  | VLT-SLKFH |
|  | VLT-NLKI |
|  | ILT-TWGTP |
| NNN | ILT-NFGKL |
|  | ILT-NFGHPA |
|  | MLA-GFGAIG |
|  | LLV-NFQNIGGVS |
| QNNV | $V \mathrm{~S}$-TEYAPLFFVATVWGL |
| QNNV | $V$ S-TEYAPLFFVATVWGLIFRP |
| QNNV | $V$ S-TEYAPLFFVATVWGLIFRP |
|  | FIT-SIANLPNVVGK |
|  | FIT-SIANLPNVVGK |
| NNNF | FIT-SIANLPNVVGK |
| GKNI | ITY-YLTHLPETLGSA |
| SNNI | IIG-SIARLPETIGATAPPA |
| GCNM | MAT - NIMHIGSTF |
| GCNM | MAT-NIMNIPVNL |
|  | I T-SSVGPETAVTVALPMIYFP |
| 1 | I A-SKVGPEVTLAVITPIV |
| QV | T-SSVGNEFVAAIIAPCYFRP |
| A VNL | L T-SSVGGEAVAFIAAPTFFR |
|  | vCALL |
|  | VCALLPF |
|  | VCALLPF |
| A | ALDKSG |
| HANI | 1 ALDKSGL |
| ATI | 1 A-AFT |
| HATV | $V$ A-ALDKLQ |
|  | $v$ S-AFTSH |
| TI | I A-QL |
| HNTI | I T-NFVPIQF |
| HNNV | $V \mathrm{~A}-\mathrm{AFIGF}$ |
| QVGV |  |
| HNTI | IVQ-TFN |
| HTTI | IVQ-TLAN |
| HVTV | VVS-SIEKFVGSS |
|  | $V$ QNDLARL |
| SNNV | $v$ GIEHARL |
| GNNI | ILK-NIGTCT----VPHSTIP |
| GNNI | ITK-NIGTCA ---- IPSSTIP |
| GTTI | I S-KAAVVPGQAVAPIPASEIPT |
|  | I S-KAVVIPGQAIVPIPSSTI |
| HNNI | IGD-- IVIPFN |
| ANNI | IAN-- IIIPRSVL |
| HNTV | VAD--VFIPRSI |
| GANF | FAT-NGISVPFF |
| $G A N F$ | FAT-NGVSIPYLT |
| HNNAAR | AAR - - - - - - - - - - - - L |
| NNA | AAR |
| HV | FAT-NGVSIPIA |
| AVTF | FAT-NGVSLPFVH |
| HVNr | YAT-NGVSLPFL |
| A NNF | FTT-NGTSLPAQAHPLKP |
| HKTI | IIQ-TLFTSTS |
| HVTV | VAE-TLG |
| $\begin{gathered} H V N A \\ G Q N i \end{gathered}$ | $\left\lvert\, \begin{aligned} & A A V-N-C- \\ & 1 \quad T-Q G E K G T \end{aligned}\right.$ |

kfl00009_0500
kfloo517_0030
kfloooog_0510

ABD37890_MVLHCB4
Gene. 10541
AtLHCB4. 1
AtLHCB4. 2
Mapoly0001s0025
AtLHCB4. 3
kfloo258_0020
CrLHCB4
Cs_30169
Cs 67011
ABD37885_MLLHCBM1
Gene. 11306
Gene. 11314
AtLHCB1. 1
AtLHCB2.1
Mapoly0057s0073
Mapoly0371s000
Mapoly0199s0017
Mapoly0199s0018
Mapoly0199s0019
Mapoly0199s0012
Mapoly0199s0014
Mapoly0199s0016
Mapoly0199s0015
Mapoly0199s0013
Mapoly0199s0020
Mapoly0057s0082
Mapoly0057s0083
Mapoly0068s0071
Mapoly0139s0012
Cs_15915
Cs_21950
Cs_ 58975
Cs_27246
Cs_35121
kflooo98_0080
kflooo98_0090
kflo1434_0010
CrLHCBM1
CrLHCBM2
CrLHCBM7
CrLHCBM3
CrLHCBM4
CrLHCBM8
CrLHCBM6
CrLHCBM9
kfloosog 0010
CrLHCBM5
Cs_38026
Mapoly0026s002
AtLHCB3
Mapoly0068s0047
Mapoly0068s0087
kflool20_0160
Cs_ 28488
HNNY
H
A
AN
Q
Q
Q
H
H
H
H
-
T
N

ABD37891_MvLHCB5

Gene. 11404 DAA05928_MvLHCB5 Cs_64185 AtLHCB5 Mapoly0011s0076 kfloo104_0350 AtLHCB7 Mapoly0008s0015 kfloo104_0370 CrLHCB7 kfloo422_0020 AtLHCB6 Mapoly0052s0039 kfloo276_0010 DAA05919_MvLHCP1.2 Gene. 11156 DAA05930_MvLHCP1.1 Gene. 11308 MpLHCAP2.1 MpLHCP1.1 CmLHC
PyLHC
CrLHCSR1
CrLHCSR3
Cs_65904
kfl00478_0030 DAA05932_MvLI818;2
Gene. 11245
Gene. 11340
MpL/818;1
MpL/818;2

GYNFLT-ILGSGSERVPTL
GYNFLT-ILGSGSERVPTL
GYNFLT-ILGSGSERVPTL
GYNLLT--IIGAEDRVPTL
GNNLLT-VIAGTAERAPTL
GNNLLT-VLQGSAERVPSL
ANNIIS-VIGGNIERSPVL
HNNLIA-MLQT
HQNL A-YATSS
HNNI T-AFNSS
HNNI - -NLAHLQ
----- - H
-------------------------




HANVLT-NAASGFGFY
HANVLT-NAASGFGFY-........

-----LSGKLFP
ILELEGLPLTPLPDNLKAI
ILELDGLPVTPLPDNLKSL
-- SP
GL


ALKAMSLDEAACSKAFEAATAVL
ALEAMATNEAACAKAFEAAVFAA

Fig. S2


Figure S2. 2D-SDS-PAGE of the high-molecular-weight PSI-LHCI-LHCII, PSI-LHCI, and LHCII trimer.
The high-molecular-weight PSI-LHCI-LHCII, PSI-LHCI, and LHCII trimer bands separated by CN-PAGE after sucrose density gradient centrifugation (Fig. 7) were subjected to 2D-SDS-PAGE. Protein bands were visualized by silver-staining.

## Table S1-1. The list of the PSI proteins predicted by the Iso-seq analysis in this study

|  | Gene annotation in this study | Accesson No. | The best-hit gene in A.thliana |  |  | The best-hit gene in C. reinhardtii |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Gene3244 | PSAA | ICQU01000001 | ATCG00350.1 | PSAA | Photosystem I, PsaA/PsaB protein | NP_958375.1 | PSAA | Photosystem I P700 chlorophyll a apoprotein A1 |
| Gene3504 | PSAB | ICQU01000002 | ATCG00340.1 | PSAB | Photosystem I, PsaA/PsaB protein | NP_958404.1 | PSAB | Photosystem I P700 chlorophyll a apoprotein A2 |
| Gene9022 | PSAD | ICQU01000006 | AT1G03130.1 | PSAD-2 | photosystem I subunit D-2 | Cre05.g238332.t1.1 | PSAD | Photosystem I reaction center subunit II, 20 kDa |
| Gene11867 | PSAE | ICQU01000029 | AT2G20260.1 | PSAE-2 | photosystem I subunit E-2 | Cre10.g420350.t1.2 | PSAE | Photosystem 18.1 kDa reaction center subunit IV |
| Gene11501 | PSAF | ICQU01000021 | AT1G31330.1 | PSAF | photosystem I subunit F | Cre09.g412100.t1.2 | PSAF | Photosystem I reaction center subunit III |
| Gene11916 | PSAG | ICQU01000031 | AT1G55670.1 | PSAG | photosystem I subunit G | Cre12.g560950.t1.2 | PSAG | Photosystem I reaction center subunit V |
| Gene11865 | PSAH | ICQU01000028 | AT1G52230.1 | PSAH-2 | photosystem I subunit H2 | Cre07.g330250.t1.2 | PSAH | Subunit H of photosystem I |
| Gene11650 | PSAL | ICQU01000024 | AT4G12800.1 | PSAL | photosystem I subunit I | Cre12.g486300.t1.2 | PSAL | Photosystem I reaction center subunit XI |
| Gene11942 | PSAO | ICQU01000034 | AT1G08380.1 | PSAO | photosystem I subunit 0 | Cre07.g334550.t1.2 | PSAO1 | Photosystem I subunit O |

Table S1-2. The list of the PSII proteins predicted by the Iso-seq analysis in this study

|  | Gene annotation in this study | Accesson No. | The best-hit gene in A.thliana |  |  | The best-hit gene in C. reinhardtif |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Gene10292 | PSBA | ICQU01000008 | ATCG00020.1 | PSBA | photosystem II reaction center protein A | NP_958377.1 | PSBA | photosystem II protein D1 |
| Gene4825 | PSBB | ICQU01000003 | ATCG00680.1 | PSBB | photosystem II reaction center protein B | NP_958388.1 | PSBB | photosystem II 47 kDa protein |
| Gene6781 | PSBC | ICQU01000005 | ATCG00280.1 | PSBC | photosystem II reaction center protein C | NP_958422.1 | PSBC | photosystem II 44 kDa protein |
| Gene4841 | PSBD | ICQU01000004 | ATCG00270.1 | PSBD | photosystem II reaction center protein D | NP_958420.1 | PSBD | photosystem II protein D2 |
| Gene10723 | PSBO | ICQU01000010 | AT3G50820.1 | PSBO-2 | photosystem II subunit 0-2 | Cre09.g396213.t1.1 | PSBO | Oxygen-evolving enhancer protein 1 of photosystem II |
| Gene11499 | PSBP | ICQU01000020 | AT1G06680.1 | PSBP-1 | photosystem II subunit P-1 | Cre12.g550850.t1.2 | PSBP1 | Oxygen-evolving enhancer protein 2 of photosystem II |
| Gene11808 | PSBQ | ICQU01000027 | AT4G05180.1 | PSBQ-2 | photosystem II subunit Q-2 | Cre08.g372450.t1.2 | PSBQ | Oxygen evolving enhancer protein 3 |
| Gene11938 | PSBR | ICQU01000033 | AT1G79040.1 | PSBR | photosystem II subunit R | Cre06.g261000.t1.2 | PSBR | 10 kDa photosystem II polypeptide |
| Gene11934 | PSBW | ICQU01000032 | AT2G30570.1 | PSBW | photosystem II reaction center W |  |  |  |
| Gene11880 | PSBX | ICQU01000030 | AT2G06520.1 | PSBX | photosystem II subunit X |  |  |  |
| Gene9164 | PSBY | ICQU01000007 | AT1G67740.1 | PSBY | photosystem II BY | Cre10.g452100.t1.1 | PSBY | Ycf32-related polyprotein of photosystem II |

Table S1-3. The list of the LHC proteins predicted by the Iso-seq analysis in this study

|  | Gene annotation in this study | Accesson No. |  |  | The best-hit gene in A.thliana |  |  | he best-hit gene in $C$. reinhardtii |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Gene11245 | LHCSR | ICQU01000013 | AT1G15820.1 | LHCB6 | light harvesting complex photosystem II subunit 6 | Cre08.g367500.t1.1 | LHCSR3.1 | Stress-related chlorophyll $\mathrm{a} / \mathrm{b}$ binding protein 2 |
| Genel1340 | LHCSR | ICQU01000017 | AT3G61470.1 | LHCA2 | photosystem I light harvesting complex gene 2 | Cre08.g367500.t1.1 | LHCSR3. 1 | Stress-related chlorophyll $\mathrm{a} / \mathrm{b}$ binding protein 2 |
| Gene11306 | LHCBM | ICQU01000014 | AT2G05100.1 | LHCB2.1 | photosystem II light harvesting complex gene 2.1 | Cre01.g066917.t1.1 | LHCBM1 | Chlorophyll $\mathrm{a} / \mathrm{b}$ binding protein of LHCII |
| Gene11314 | LHCBM | ICQU01000016 | AT2G05070.1 | LHCB2. 2 | photosystem II light harvesting complex gene 2.2 | Cre01.g066917.t1.1 | LHCBM1 | Chloroohyll $\mathrm{a} / \mathrm{b}$ binding protein of LHCII |
| Gene10541 | LHCB4 | ICQU01000009 | AT2G40100.1 | LHCB4.3 | light harvesting complex photosystem II | Cre17.g720250.t1.2 | LHCB4 | Chlorophyll $\mathrm{a} / \mathrm{b}$ binding protein of photosystem II |
| Gene11404 | LHCB5 | ICQU01000019 | AT4G10340.1 | LHCB5 | light harvesting complex of photosystem II 5 | Cre16.g673650.t1.1 | LHCB5 | Minor chlorophyll $\mathrm{a} / \mathrm{b}$ binding protein of photosystem II |
| Gene11156 | LHCP | ICQU01000012 | AT5G54270.1 | LHCB3 | light-harvesting chlorophyl\| B-binding protein 3 | Cre03.g156900.t1.2 | LHCBM5 | Chlorophyll $\mathrm{a} / \mathrm{b}$ binding protein of LHCII |
| Gene11308 | LHCP | ICQU01000015 | AT5G54270.1 | LHCB3 | light-harvesting chloroohyll B -binding protein 3 | Cre04.g232104.t1.1 | LHCBM3 | Light-harvesting complex II chlorophyll a/b binding protein M3 |
| Gene11703 | LHCA1 | ICQU01000025 | AT3G54890.1 | LHCA1 | photosystem l light harvesting complex gene 1 | Cre06.g283050.t1.2 | LHCA1 | Light-harvesting protein of photosystem I |
| Gene11349 | LHCA2 | ICQU01000018 | AT3G61470.1 | LHCA2 | photosystem 1 light harvesting complex gene 2 | Cre16.g687900.t1.2 | LHCA7 | Light-harvesting protein of photosystem I |
| Gene11585 | LHCA2 | ICQU01000023 | AT3G47470.1 | LHCA4 | light-harvesting chlorophyll-protein complex I subunit A4 | Cre10.g452050.t1.2 | LHCA4 | Light-harvesting protein of photosystem I |
| Gene11536 | LHCA3 | ICQU01000022 | AT1G61520.1 | LHCA3 | photosystem 1 light harvesting complex gene 3 | Cre11.g467573.t1.1 | LHCA3 | Chlorophyll $\mathrm{a} / \mathrm{b}$ binding protein of photosystem I, type III |
| Gene11715 | algae-type LHCA2 | ICQU01000026 | AT1G45474.1 | LHCA5 | photosystem 1 light harvesting complex gene 5 | Cre12.g508750.t1.2 | LHCA2 | Light-harvesting protein of photosystem I |
| Gene10884 | LHCA9 | ICQU01000011 | AT1G45474.1 | LHCA5 | photosystem 1 light harvesting complex gene 5 | Cre07.g344950.t1.2 | LHCA9 | Light-harvesting protein of photosystem I |


| Gene ID | NSAF (fmol) | Category | Gene annotation in this study | Accesson No. | Best-Hit Gene in Arabidopsis |  | Best-Hit Gene in Chlamydomonas |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | Gene ID | Gene Name | Gene ID | Gene Name |
| Gene11306 | 4526 | LHC | LHCBM | ICQU01000014 | AT2G05100.1 | LHCB2.1 | Cre01.g066917.t1.1 | LHCBM1 |
| Gene10292 | 4034 | PSII | PSBA | ICQU01000008 | ATCG00020.1 | PSBA | NP_958377.1 | PSBA |
| Gene11501 | 3411 | PSI | PSAF | ICQU01000021 | AT1G31330.1 | PSAF | Cre09.g412100.t1.2 | PSAF |
| Gene11156 | 3322 | LHC | LHCP | ICQU01000012 | AT5G54270.1 | LHCB3 | Cre03.g156900.t1.2 | LHCBM5 |
| Gene11585 | 2942 | LHC | LHCA2 | ICQU01000023 | AT3G47470.1 | LHCA4 | Cre10.g452050.t1.2 | LHCA4 |
| Gene4825 | 2883 | PSII | PSBB | ICQU01000003 | ATCG00680.1 | PSBB | NP_958388.1 | PSBB |
| Gene11867 | 2773 | PSI | PSAE | ICQU01000029 | AT2G20260.1 | PSAE-2 | Cre10.g420350.t1.2 | PSAE |
| Gene11865 | 2654 | PSI | PSAH | ICQU01000028 | AT1G52230.1 | PSAH-2 | Cre07.g330250.t1.2 | PSAH |
| Gene4841 | 2605 | PSII | PSBD | ICQU01000004 | ATCG00270.1 | PSBD | NP_958420.1 | PSBD |
| Gene3547 | 2468 | Others |  |  | AT5G59970.1 |  | Cre12.g506350.t1.2 | HFO18 |
| Gene11916 | 2444 | PSI | PSAG | ICQU01000031 | AT1G55670.1 | PSAG | Cre12.g560950.t1.2 | PSAG |
| Gene9022 | 2440 | PSI | PSAD | ICQU01000006 | AT1G03130.1 | PSAD-2 | Cre05.g238332.t1.1 | PSAD |
| Gene11404 | 2412 | LHC | LHCB5 | ICQU01000019 | AT4G10340.1 | LHCB5 | Cre16.g673650.t1.1 | LHCB5 |
| Gene10541 | 2301 | LHC | LHCB4 | ICQU01000009 | AT2G40100.1 | LHCB4.3 | Cre17.g720250.t1.2 | LHCB4 |
| Gene11715 | 2235 | LHC | algae-type LHCA2 | ICQU01000026 | AT1G45474.1 | LHCA5 | Cre12.g508750.t1.2 | LHCA2 |
| Gene6781 | 1782 | PSII | PSBC | ICQU01000005 | ATCG00280.1 | PSBC | NP_958422.1 | PSBC |
| Gene11340 | 1707 | LHC | LHCSR | ICQU01000017 | AT3G61470.1 | LHCA2 | Cre08.g367500.t1.1 | LHCSR3.1 |
| Gene6617 | 1577 | Others |  |  | AT3G61320.1 |  | Cre06.g261750.t1.2 |  |
| Gene3504 | 1569 | PSII | PSAB | ICQU01000002 | ATCG00340.1 | PSAB | NP_958404.1 | PSAB |
| Gene7944 | 1382 | Others |  |  | AT3G61320.1 |  | Cre06.g261750.t1.2 |  |
| Gene11703 | 1355 | LHC | LHCA1 | ICQU01000025 | AT3G54890.1 | LHCA1 | Cre06.g283050.t1.2 | LHCA1 |
| Gene10723 | 1232 | PSII | PSBO | ICQU01000010 | AT3G50820.1 | PSBO-2 | Cre09.g396213.t1.1 | PSBO |
| Gene11536 | 1228 | LHC | LHCA3 | ICQU01000022 | AT1G61520.1 | LHCA3 | Cre11.g467573.t1.1 | LHCA3 |
| Gene10884 | 1219 | LHC | LHCA9 | ICQU01000011 | AT1G45474.1 | LHCA5 | Cre07.g344950.t1.2 | LHCA9 |
| Gene7515 | 1169 | Others |  |  | AT5G04180.1 | ATACA3 | Cre09.g415700.t1.2 | CAH3 |
| Gene11308 | 1066 | LHC | LHCP | ICQU01000015 | AT5G54270.1 | LHCB3 | Cre04.g232104.t1.1 | LHCBM3 |
| Gene7423 | 858 | Others |  |  |  |  |  |  |
| Gene8340 | 839 | Others |  |  | AT5G17170.1 | ENH1 | Cre12.g510400.t1.1 | CPLD30 |
| Gene9362 | 780 | Others |  |  | AT4G38970.1 | FBA2 | Cre05.g234550.t1.2 | FBA3 |
| Gene8224 | 529 | Others |  |  | AT5G23860.1 | TUB8 | Cre12.g549550.t1.2 | TUB2 |
| Gene8089 | 512 | Others |  |  | ATCG00490.1 | RBCL | NP_958405.1 | RBCL |


|  |  |  |  |  | Best-Hit Gene in Arabidopsis |  | Best-Hit Gene in Chlamydomonas |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Gene ID | NSAF(fmol) | Category | Gene annotation in this study | Accesson No. | Gene ID | Gene Name | Gene ID | Gene Name |
| Gene11306 | 7753 | LHC | LHCBM | ICQU01000014 | AT2G05100.1 | LHCB2.1 | Cre01.g066917.t1.1 | LHCBM1 |
| Gene10292 | 6458 | PSII | PSBA | ICQU01000008 | ATCG00020.1 | PSBA | NP_958377.1 | PSBA |
| Gene10541 | 3371 | LHC | LHCB4 | ICQU01000009 | AT2G40100.1 | LHCB4.3 | Cre17.g720250.t1.2 | LHCB4 |
| Gene4825 | 3021 | PSII | PSBB | ICQU01000003 | ATCG00680.1 | PSBB | NP_958388.1 | PSBB |
| Gene11404 | 1991 | LHC | LHCB5 | ICQU01000019 | AT4G10340.1 | LHCB5 | Cre16.g673650.t1.1 | LHCB5 |
| Gene11865 | 1757 | PSI | PSAH | ICQU01000028 | AT1G52230.1 | PSAH-2 | Cre07.g330250.t1.2 | PSAH |
| Gene6781 | 1495 | PSII | PSBC | ICQU01000005 | ATCG00280.1 | PSBC | NP_958422.1 | PSBC |
| Gene11938 | 1341 | PSII | PSBR | ICQU01000033 | AT1G79040.1 | PSBR | Cre06.g261000.t1.2 | PSBR |
| Gene3547 | 1317 | Others |  |  | AT5G59970.1 |  | Cre12.g506350.t1.2 | HFO18 |
| Gene11585 | 1159 | LHCI | LHCA2 | ICQU01000023 | AT3G47470.1 | LHCA4 | Cre10.g452050.t1.2 | LHCA4 |
| Gene11867 | 1142 | PSI | PSAE | ICQU01000029 | AT2G20260.1 | PSAE-2 | Cre10.g420350.t1.2 | PSAE |
| Gene11916 | 1105 | PSI | PSAG | ICQU01000031 | AT1G55670.1 | PSAG | Cre12.g560950.t1.2 | PSAG |
| Gene4841 | 1095 | PSII | PSBD | ICQU01000004 | ATCG00270.1 | PSBD | NP_958420.1 | PSBD |
| Gene7779 | 1091 | Others |  |  | ATCG00430.1 | PSBG | Cre12.g492300.t1.2 | NUO10 |
| Gene11308 | 1084 | LHC | LHCP | ICQU01000015 | AT5G54270.1 | LHCB3 | Cre04.g232104.t1.1 | LHCBM3 |
| Gene9022 | 1080 | PSI | PSAD | ICQU01000006 | AT1G03130.1 | PSAD-2 | Cre05.g238332.t1.1 | PSAD |
| Gene11715 | 976 | LHC | algae-type LHCA2 | ICQU01000026 | AT1G45474.1 | LHCA5 | Cre12.g508750.t1.2 | LHCA2 |
| Gene10884 | 937 | LHC | LHCA9 | ICQU01000011 | AT1G45474.1 | LHCA5 | Cre07.g344950.t1.2 | LHCA9 |
| Gene11501 | 926 | PSI | PSAF | ICQU01000021 | AT1G31330.1 | PSAF | Cre09.g412100.t1.2 | PSAF |
| Gene11349 | 902 | LHC | LHCA2 | ICQU01000018 | AT3G61470.1 | LHCA2 | Cre16.g687900.t1.2 | LHCA7 |
| Gene11703 | 858 | LHC | LHCA1 | ICQU01000025 | AT3G54890.1 | LHCA1 | Cre06.g283050.t1.2 | LHCA1 |
| Gene7515 | 822 | Others |  |  | AT5G04180.1 | ATACA3 | Cre09.g415700.t1.2 | CAH3 |
| Gene11156 | 789 | LHC | LHCP | ICQU01000012 | AT5G54270.1 | LHCB3 | Cre03.g156900.t1.2 | LHCBM5 |
| Gene2597 | 781 | Others |  |  | ATCG01100.1 | NDHA |  |  |
| Gene11934 | 741 | PSII | PSBW | ICQU01000032 | AT2G30570.1 | PSBW |  |  |
| Gene4851 | 714 | Others |  |  | ATCG01110.1 | NDHH | Cre09.g405850.t1.1 | NU07 |
| Gene11718 | 690 | Others |  |  | ATCG00420.1 | NDHJ | Cre07.g327400.t1.1 | NUO9 |
| Gene9021 | 683 | Others |  |  | ATCG01070.1 | NDHE | Cre09.g402552.t1.1 | NU011 |
| Gene11761 | 676 | Others |  |  |  |  |  |  |
| Gene6617 | 618 | Others |  |  | AT3G61320.1 |  | Cre06.g261750.t1.2 |  |
| Gene11650 | 605 | PSI | PSAL | ICQU01000024 | AT4G12800.1 | PSAL | Cre12.g486300.t1.2 | PSAL |
| Gene8813 | 572 | Others |  |  | AT1G20020.1 | ATLFNR2 | Cre11.g476750.t1.2 | FNR1 |
| Gene11139 | 565 | Others |  |  | AT2G28720.1 |  | Cre13.g590750.t1.2 | HTB21 |
| Gene10723 | 565 | PSII | PSBO | ICQU01000010 | AT3G50820.1 | PSBO-2 | Cre09.g396213.t1.1 | PSBO |
| Gene11808 | 539 | PSII | PSBQ | ICQU01000027 | AT4G05180.1 | PSBQ-2 | Cre08.g372450.t1.2 | PSBQ |
| Gene8340 | 531 | Others |  |  | AT5G17170.1 | ENH1 | Cre12.g510400.t1.1 | CPLD30 |
| Gene10856 | 527 | Others |  |  | AT5G58260.1 |  |  |  |
| Gene11340 | 526 | LHC | LHCSR | ICQU01000017 | AT3G61470.1 | LHCA2 | Cre08.g367500.t1.1 | LHCSR3.1 |
| Gene5104 | 522 | Others |  |  | AT1G15980.1 | NDF1 |  |  |


|  |  |  |  |  | Best-Hit Gene in Arabidopsis |  | Best-Hit Gene in Chlamydomonas |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Gene ID | NSAF(fmol) | Category | Gene annotation in this study | Accesson No. | Gene ID | Gene Name | Gene ID | Gene Name |
| Gene11703 | 9063 | LHC | LHCA1 | ICQU01000025 | AT3G54890.1 | LHCA1 | Cre06.g283050.t1.2 | LHCA1 |
| Gene11916 | 6111 | PSI | PSAG | ICQU01000031 | AT1G55670.1 | PSAG | Cre12.g560950.t1.2 | PSAG |
| Gene11867 | 5182 | PSI | PSAE | ICQU01000029 | AT2G20260.1 | PSAE-2 | Cre10.g420350.t1.2 | PSAE |
| Gene11501 | 5101 | PSI | PSAF | ICQU01000021 | AT1G31330.1 | PSAF | Cre09.g412100.t1.2 | PSAF |
| Gene11536 | 4709 | LHC | LHCA3 | ICQU01000022 | AT1G61520.1 | LHCA3 | Cre11.g467573.t1.1 | LHCA3 |
| Gene10884 | 4541 | LHC | LHCA9 | ICQU01000011 | AT1G45474.1 | LHCA5 | Cre07.g344950.t1.2 | LHCA9 |
| Gene11865 | 2669 | PSI | PSAH | ICQU01000028 | AT1G52230.1 | PSAH-2 | Cre07.g330250.t1.2 | PSAH |
| Gene11349 | 2593 | LHC | LHCA2 | ICQU01000018 | AT3G61470.1 | LHCA2 | Cre16.g687900.t1.2 | LHCA7 |
| Gene9022 | 2205 | PSI | PSAD | ICQU01000006 | AT1G03130.1 | PSAD-2 | Cre05.g238332.t1.1 | PSAD |
| Gene11585 | 1980 | LHC | LHCA2 | ICQU01000023 | AT3G47470.1 | LHCA4 | Cre10.g452050.t1.2 | LHCA4 |
| Gene11715 | 1861 | LHC | algae-type LHCA2 | ICQU01000026 | AT1G45474.1 | LHCA5 | Cre12.g508750.t1.2 | LHCA2 |
| Gene11306 | 1695 | LHC | LHCBM | ICQU01000014 | AT2G05100.1 | LHCB2.1 | Cre01.g066917.t1.1 | LHCBM1 |
| Gene3547 | 1333 | Others |  |  | AT5G59970.1 |  | Cre12.g506350.t1.2 | HFO18 |
| Gene3504 | 1286 | PSI | PSAB | ICQU01000002 | ATCG00340.1 | PSAB | NP_958404.1 | PSAB |
| Gene11650 | 1115 | PSI | PSAL | ICQU01000024 | AT4G12800.1 | PSAL | Cre12.g486300.t1.2 | PSAL |
| Gene11761 | 914 | Others |  |  |  |  |  |  |
| Gene11139 | 868 | Others |  |  | AT2G28720.1 |  | Cre13.g590750.t1.2 | HTB21 |
| Gene4841 | 822 | PSII | PSBD | ICQU01000004 | ATCG00270.1 | PSBD | NP_958420.1 | PSBD |
| Gene3244 | 820 | PSI | PSAA | ICQU01000001 | ATCG00350.1 | PSAA | NP_958375.1 | PSAA |
| Gene10292 | 783 | PSII | PSBA | ICQU01000008 | ATCG00020.1 | PSBA | NP_958377.1 | PSBA |
| Gene9567 | 782 | Others |  |  |  |  |  |  |
| Gene6617 | 765 | Others |  |  | AT3G61320.1 |  | Cre06.g261750.t1.2 |  |
| Gene7515 | 730 | Others |  |  | AT5G04180.1 | ATACA3 | Cre09.g415700.t1.2 | CAH3 |
| Gene4825 | 682 | PSII | PSBB | ICQU01000003 | ATCG00680.1 | PSBB | NP_958388.1 | PSBB |
| Gene10541 | 675 | LHC | LHCB4 | ICQU01000009 | AT2G40100.1 | LHCB4.3 | Cre17.g720250.t1.2 | LHCB4 |
| Gene5134 | 575 | Others |  |  |  |  |  |  |
| Gene9362 | 538 | Others |  |  | AT4G38970.1 | FBA2 | Cre05.g234550.t1.2 | FBA3 |
| Gene6781 | 535 | PSII | PSBC | ICQU01000005 | ATCG00280.1 | PSBC |  |  |


[^0]:    Suga, M., and Shen, J.-R. (2020) Structural variations of photosystem I-antenna supercomplex in response to adaptations to different light environments. Current Opinion in Structural Biology. 63: 10-17.

    Swingley, W.D., Iwai, M., Chen, Y., Ozawa, S., Takizawa, K., Takahashi, Y., et al. (2010) Characterization of photosystem I antenna proteins in the prasinophyte Ostreococcus tauri. Biochimica Et Biophysica Acta Bba - Bioenergetics. 1797: 14581464.

