

**Supplemental Table 1** Ka/Ks analysis of *OsTT3.1/LpTT3.1* and *OsTT3.2/LpTT3.2*

| Gene1          | Gene2          | Ka          | Ks          | Ka/Ks       | Evolutionary selection |
|----------------|----------------|-------------|-------------|-------------|------------------------|
| <i>LpTT3.1</i> | <i>OsTT3.1</i> | 0.046431568 | 0.50551727  | 0.091849618 | Purifying              |
| <i>LpTT3.2</i> | <i>OsTT3.2</i> | 0.119958113 | 0.785088532 | 0.152795651 | Purifying              |

**Supplemental Table 2** Primers used in PCR amplification of *OsTT3.1/LpTT3.1* and *OsTT3.2/LpTT3.2*

| Primer name            | Sequence (5'–3')         |
|------------------------|--------------------------|
| <i>LpTT3.1</i> forward | ATGCAGCGGCGGCGGG         |
| <i>LpTT3.1</i> reverse | AACATCATATACGGGCATGCGTTC |
| <i>LpTT3.2</i> forward | ATGGCTTCCTCCATCGC        |
| <i>LpTT3.2</i> reverse | CAATGTTTTATCCTTCCTCTCTA  |

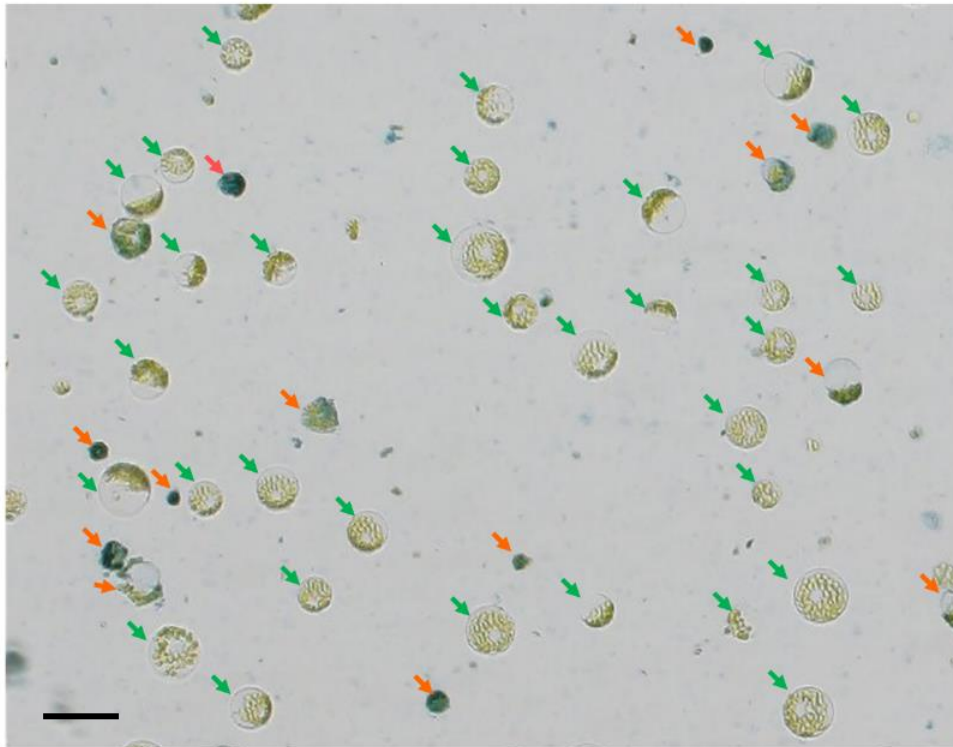
## A

|           |                                                                                  |     |
|-----------|----------------------------------------------------------------------------------|-----|
| LpTT3.1   | MQRRACTWAGVGKTAQAAAAHAALFCFTLLALVDGRDYSWWIIFIFLWLFHVSVAARGRESMPAFSLPHGRHWAPCH    | 80  |
| OsTT3.1   | MQRRACTWAGVGKTAQAAAAHAALFCFTLLALVDGRDYSWWIIFIFLWLFHGVARGRESMPAFSLPHGRHWAPCH      | 80  |
| Consensus | mqrraqtwagvgktaqaaaahaalfcftllal vdgrt yswwiifiplwlfh argfrsmpapslphgrhwapch     |     |
| LpTT3.1   | SVVAAPLLIAFELLLCIYLESVRVRNCPVDMKIVFLPLITFEVIILDNFRMCALMPGDEESMSDEAIWETLPHFWVA    | 160 |
| OsTT3.1   | SVVAAPLLIAFELLLCIYLESVRVRNCPVDMKIVFLPLITFEVIILDNFRMCALMPGDEESMSDEAIWETLPHFWVA    | 160 |
| Consensus | s vaaplliafellllciyles rv p vd kivflpll feviil dnfrmc almpgdeesmsdeaiwetlphfwva  |     |
| LpTT3.1   | ISMVFLIAATFTLLKLSGIVGALGWDLFINYGIAECFAFLVCTRWFNPMIHREPTGEASSSSAIRYRDWESGLVLP     | 240 |
| OsTT3.1   | ISMVFLIAATFTLLKLSGIVGALGWDLFINYGIAECFAFLVCTRWFNPMIHREPTGEASSSSAIRYRDWESGLVLP     | 240 |
| Consensus | ismvfliaattftllklsgdvgalgwldfinygiaecfaflvctrwfnpmih p geassss airyrdwesgl lp    |     |
| LpTT3.1   | SLEDHEERECGLPDIGGHMKIPIVFCVLLCMRLEGTTPSPA YIPIFALFSPFILOQAGVLFSLGRIVEFVLLLRN     | 320 |
| OsTT3.1   | SLEDHEERECGLPDIGGHMKIPIVFCVLLCMRLEGTTPSPA YIPIFALFSPFILOQAGVLFSLGRIVEFVLLLRN     | 320 |
| Consensus | sledhe er cglpdiggh mkipiv fcvllcmrlegtppsa yipifalfsplfiloqagvlfsl rl ekvlllrn  |     |
| LpTT3.1   | GEVSPNYLTSSKVRDCFAFLHGSRLLGWWSIDEGSKEEQARLFYTESGYNTFCGYPEVVRKMPKDLAEVWRLOA       | 400 |
| OsTT3.1   | GEVSPNYLTSSKVRDCFAFLHGSRLLGWWSIDEGSKEEQARLFYTESGYNTFCGYPEVVRKMPKDLAEVWRLOA       | 400 |
| Consensus | gvpvsnlyt sskvrdcfafh gsrllogwswidsgskeeqarlfytes gyntf gyppevv kmpk dlaeevwrloa |     |
| LpTT3.1   | ALGEQSEITKTCQOEYERLQNEKVLICRYEGEICMVLLPCRHRITLCKSCSDCKRCPCICRVEIDERMAVYD         | 472 |
| OsTT3.1   | ALGEQSEITKTCQOEYERLQNEKVLICRYEGEICMVLLPCRHRITLCKSCSDCKRCPCICRVEIDERMAVYD         | 472 |
| Consensus | algeqseitk t qe erlqnekvlricryegeicmvllpcrhrtlck csdkck cpicr pi erm vyd         |     |

## B

|           |                                                                                 |     |
|-----------|---------------------------------------------------------------------------------|-----|
| LpTT3.2   | MASSAFASCAITLHPFAIVSAAVAGEGCRVLLAVPEPRSATSMRCVG...ILALRCSALEDPG.....            | 66  |
| OsTT3.2   | MASSAFAS...VVVPEVQARVAAVAVGEICRVLLAYTELLSGSARFVVGRLAFRCSALEDPGPSDGGEGNGFVV      | 77  |
| Consensus | mass a as p v v aa gp crvlla p s r v lap rcsaledpg                              |     |
| LpTT3.2   | LAGSEEGKKKSGSVKQARGREVRRLIFASKKTRSIILNALTVIYASDIFVLKEVEALTFAVFNMRVFWVAIPFP      | 145 |
| OsTT3.2   | EEGSEEVVRKEQERRRQRRFVWRRLIFASKKTRSIILNALTVIYASDIFVLKEVEALTFAVFNMRVFWVAIPFP      | 157 |
| Consensus | g ee k r r rpvrllifaskktrsi ilnaltviyasdipvlkevealt pavfnmrffvv aipf p          |     |
| LpTT3.2   | FMIRAFGDRIRNGLLELGVWISLAYLAQAIGLITSDAGRASFMAFIVIVVPLIDGIFGATIPKLTWFGAIVSVIGVGL  | 225 |
| OsTT3.2   | FMIRAFGDRVRNGLLELGVWISLAYLAQAIGLITSDAGRASFMAFIVIVVPLIDGIFGATIPKLTWFGAIVSVIGVGL  | 237 |
| Consensus | f ira gdr rn glelg w slayl qaigli sdagrasf aftvivvplidgifga ipkltwfgaivs g gl   |     |
| LpTT3.2   | LECGGSPPCVGIVLNFFSAVFFGIHMLRTEQISRSTDKKKELLSFEVIVVAFSSILWFLFKDAFVEVDFSSFESWTFG  | 305 |
| OsTT3.2   | LECGGSPPCVGIVLNFLAAVFFGIHMLRTEQISRSTDKKKELLSFEVIVVAFSSILWFLFKDSYVIVVDFSSFESWTFG | 317 |
| Consensus | lecggsppcvgdvlnf avffgihmlrteqisrstdkkkf llsfevlvaf s lwflkd v v d sfeswtfg     |     |
| LpTT3.2   | MLWDSARSFEPWIPALYTGVLSTLCMWAEMVAMGVSATETAIVYGLEPVWGAAFAWELLGERWDNAAWGAALVLCGSL  | 385 |
| OsTT3.2   | MELDTATSFEPWIPALYTGVLSTLCMWAEMVAMGVSATETAIVYGLEPVWGAAFAWELLGERWDNAAWGAALVLCGSL  | 397 |
| Consensus | m d a sfpwipalytgvl st lcmwaemvam vsatetaivyglepvwgaafawellgerwdnaaw gaalvlcgs  |     |
| LpTT3.2   | TVQLFGSAPEKSKKVEERSFNLETTLKQDELSLSVIFVDSRKNIQSRLERKDKTL                         | 442 |
| OsTT3.2   | TVQLFGSAPEKSKKVTSCNLETTLKQDELSLSVIFVDSRKNIQSRLERKDKTL                           | 454 |
| Consensus | tvqlfgsapekskkv rs n let lk qd ls ls ipvdsrknigs lerkdktl                       |     |

**Supplemental Figure 1** Sequence alignments of *OsTT3.1/LpTT3.1* (A) and *OsTT3.2/LpTT3.2* (B)



**Supplemental Figure 2** A representative photo taken for protoplasts' viability observation after heat stress. The protoplasts were stained with Evans blue for 1 min and then observed under a light microscope. The 'green' arrows mark the viable cells with intact round shapes, while those in 'red' mark the dead or broken protoplasts with dark blue staining. The black bar in the photo represents 36  $\mu\text{m}$ .

**Supplemental data 1** Enzymatic hydrolysate (20 mL)

| <b>Solution name</b>      | <b>Dosage</b>                          | <b>Method</b>                             |
|---------------------------|----------------------------------------|-------------------------------------------|
| 0.6M D-Mannitol           | 2.186 g                                | metage                                    |
| 10mM MES·H <sub>2</sub> O | take 1 mL of 0.2M MES·H <sub>2</sub> O | 0.2M MES·H <sub>2</sub> O mother solution |
| Cellulase                 | 0.3 g                                  | metage                                    |
| Macerozyme                | 0.15 g                                 | metage                                    |

**Supplemental data 2** MES·H<sub>2</sub>O (100 mL)

| <b>Solution name</b>      | <b>Dosage</b> | <b>Method</b>                                                                                        |
|---------------------------|---------------|------------------------------------------------------------------------------------------------------|
| 0.2M MES·H <sub>2</sub> O | 4.265 g       | weigh 4.265 g MES·H <sub>2</sub> O, deionized water fixed volume 100mL, with KOH to adjust pH to 5.7 |

**Supplemental data 3** 0.1% BSA and 3.4 mM CaCl<sub>2</sub> (100 mL)

| <b>Solution name</b>    | <b>Dosage</b>                       | <b>Method</b>                                                                                               |
|-------------------------|-------------------------------------|-------------------------------------------------------------------------------------------------------------|
| 0.1% BSA                | 0.02 g                              | metage                                                                                                      |
| 3.4mM CaCl <sub>2</sub> | take 34 µL of 2 M CaCl <sub>2</sub> | 2M CaCl <sub>2</sub> : weigh 29.345 g CaCl <sub>2</sub> ·2H <sub>2</sub> O, deionized water volume to 100mL |

**Supplemental data 4 W5 (200 mL)**

| <b>Solution name</b>                                   | <b>Dosage</b>                         | <b>Method</b>                                                                                                                   |
|--------------------------------------------------------|---------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| 154mM NaCl Solution                                    | take 15.4mL of 2M NaCl                | 2M NaCl: weigh 11.7gNaCl, deionized water volume to 100mL                                                                       |
| 2mM MES·H <sub>2</sub> O Solution                      | take 1mL of 0.2M MES·H <sub>2</sub> O | 0.2M MES·H <sub>2</sub> O: weigh 4.265 g MES·H <sub>2</sub> O, deionized water fixed volume 100mL, with KOH to adjust pH to 5.7 |
| 125mM CaCl <sub>2</sub> Solution                       | take 12.5mL of 2M CaCl <sub>2</sub>   | 2M CaCl <sub>2</sub> : weigh 29.345 g CaCl <sub>2</sub> ·2H <sub>2</sub> O, deionized water volume to 100mL                     |
| 5mM KCl Solution                                       | take 1mL of 1M KCl                    | 1M KCl: weigh 0.7455g KCl, deionized water volume to 100mL                                                                      |
| Deionized water volume to 200mL and filter sterilized. |                                       |                                                                                                                                 |

**Supplemental data 5 MMG (20mL)**

| <b>Solution name</b>                                   | <b>Dosage</b>                           | <b>Method</b>                                                                           |
|--------------------------------------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------|
| 0.6M D-Mannitol                                        | 2.186 g                                 | metage                                                                                  |
| 4mM MES·H <sub>2</sub> O                               | take 400μL of 0.2M MES·H <sub>2</sub> O | 0.2M MES·H <sub>2</sub> O solution                                                      |
| 15 mM MgCl <sub>2</sub> Solution                       | take 300μL of 1M MgCl <sub>2</sub>      | 1M MgCl <sub>2</sub> : weigh 2.033g MgCl <sub>2</sub> , deionized water volume to 100mL |
| Deionized water volume to 20 mL and filter sterilized. |                                         |                                                                                         |

**Supplemental data 6 PEG-4000 Solution (20mL)**

| <b>Solution name</b>                                                                     | <b>Dosage</b> | <b>Method</b> |
|------------------------------------------------------------------------------------------|---------------|---------------|
| 0.6 M D-Mannitol                                                                         | 2.186 g       | metage        |
| PEG 4000                                                                                 | 8g            | metage        |
| Deionized water volume to 20mL.Cool to room temperature after bathing at 42°C for 30min. |               |               |