

Supplementary information

A PETase enzyme synthesised in the chloroplast of the microalga *Chlamydomonas reinhardtii* is active against PET and polystyrene

Giulia Di Rocco^{*1}, Henry N. Taunt², Marcello Berto¹, Harry O. Jackson², Daniele Piccinini¹, Alan Carletti¹ and Saul Purton²

¹Department of Life Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy.

²Algal Research Group, Department of Structural and Molecular Biology, University College London, Gower Street, London, United Kingdom

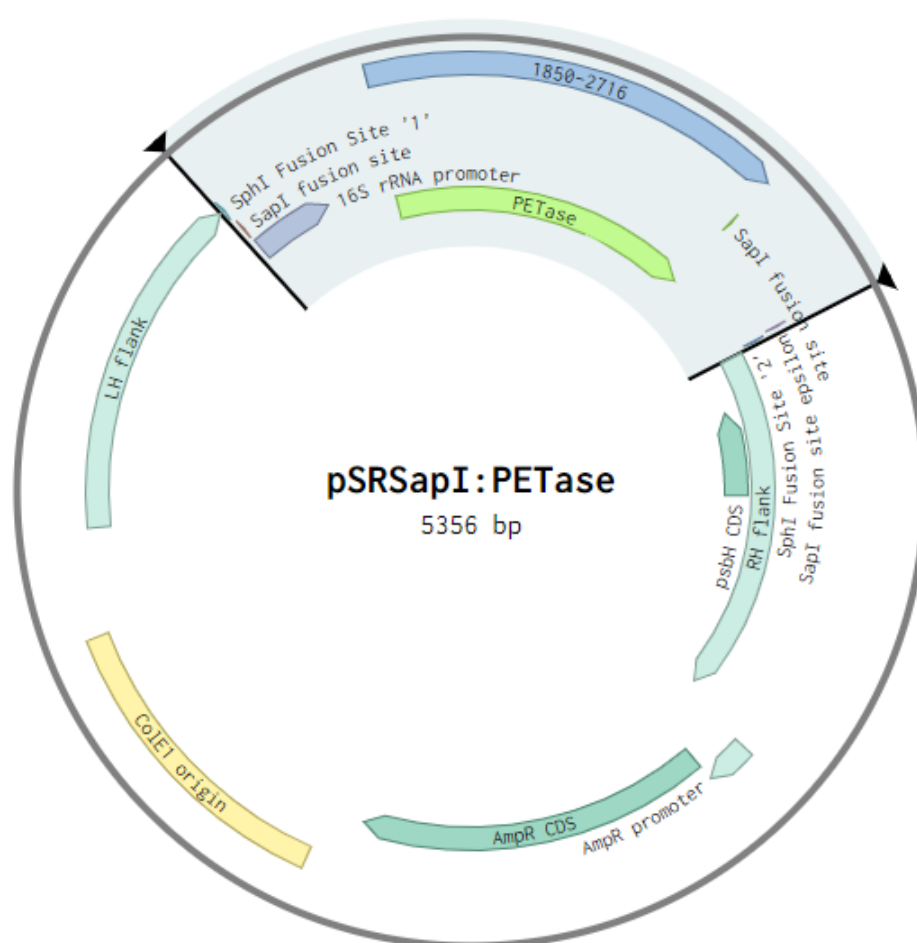


Figure S1: Transformation vector pSRSapI containing the PETase coding sequence. The entire construct is highlighted in blue and includes SapI and SphI sites added for cloning, the 16S rRNA promoter. The left and right flanks for homologous recombination and integration into the *C. reinhardtii* plastome are in green with the right flank containing *psbH* for phototrophic selection (see Figure 1A).

Matched peptides shown in **bold red**.

#PETase

```
1 MNFPRASRLM QAAVLGGLMA VSAAATAQTN PYARGPNPTA ASLEASAGPF
51 TVRSFTVSRP SGYGAGTVYY PTNAGGTVGA IAIVPGYTAR QSSIKWWGPR
101 LASHGFVVIT IDTNSTLDQP SSRSSQMAA LRQVASLNGT SSSPIYGKVD
151 TARMGVMGWS MGGGGSLISA ANNPSLKAAA PQAPWDSSTN FSSVTVPPTLI
201 FACENDSIAP VNSSALPIYD SMSRNAKQFL EINGGSHSCA NSGNSNQALI
251 GKKGVAVMKR FMDNDTRYST FACENPNSTR VSDFRTANCS
```

Figure S2: Sequence coverage (27%) of complete PETase sequence. The 27 residue N-terminal peptide cleaved during targeting to the thylakoid lumen is not found within the detected peptides, as predicted.

Matched peptides shown in **bold red**.

#MDH

```
1 MSLQSSIRAD SNCTLPNNPV CVLLPVDFIV AAMASSTSSA MAKWAAQAAR
51 GFAAAAPSSG KGRKVAVLGA AGGIGQPLSM LMKMNSQVSS LSLYDIAGTP
101 GVAADVSHIN TKAQVKGFDK DGLAEALRGC DLVIIPAGVP RKPQMTRDDL
151 FKINAGIVRD LVTAVGQHCP GAVLNIISNP VNSTVPIAAE QLKKMGVYDK
201 RKVMGVTTLD VVRAKTFYAE KNGLDVASVD VPVVGGHAGV TILPLFSQAT
251 PKATMSAEVL DALTKRTQDG GTEVVQAKAG KGSATLSMAY AAALFADSCL
301 RGLNGAPVVE CTYVESTVTD APYFASKVKL STEGVDKIHD LGPLSDYEKA
351 GLKAMPELL ASIEKGVQFV KGA
```

Figure S3: Sequence coverage (44%) for mitochondrial malate dehydrogenase precursor from *Chlamydomonas reinhardtii* (UniProtKB: locus MDHM_CHLRE, accession [Q42686](#)). Cleaved transit peptide = residues 1–56.

Matched peptides shown in **bold red**.

#Cyt C

```
1 MSTFAEAPAG DLARGEKIFK TKCAQCHVAE KGGGHKQGPN LGGLFGRVSG
51 TAAGFAYSKA NKEAAVTWGE STLYEYLLNP KKYMPGNKMV FAGLKKPEER
101 ADLIAYLKQA TA
```

Figure S4: Sequence coverage (33%) of apocytochrome c precursor from *Chlamydomonas reinhardtii* sequence. (GenBank: [M35173](#)).

Table S5. Primers used to confirm integration and homoplasmy (see Figure 1A&B).

Primers	
P1 (F1.long)	5'-GTCATTGCGAAAATACTG-3'
P2 (rbcL.Fn)	5'-CGGATGTAACTCAATCGGTAG-3'
P3(RY-psaR)	5'-AACTATTTGTCTAATTTAATAACC-3'