Supplementary Materials

Supplementary Fig. 1 Construction of plasmids for gene disruption. (A) Construction of plasmids for Δ*tthb178* and Δ*recJ* disruptions. The thermostable kanamycin-resistance gene, *HTK*, was amplified by PCR using pUC18/*HTK* plasmid as a template. The 500-bp DNA fragments from upstream and downstream of *tthb178* or *recJ* gene were amplified by PCR using *Thermus thermophilus* HB8 genomic DNA as a template. The DNA fragments were treated with KpnI and PstI, and then ligated into the pGEM-T Easy vector to obtain pGEM-T Easy/Δ*tthb178*::*HTK* and pGEM-T Easy/Δ*recJ*::*HTK* plasmids. (B) Construction of the plasmid for disruption of *recJ* by inserting the thermostable hygromycin-B-resistance gene, Hyg^R. The Hyg^R was amplified by PCR from pHG305 plasmid (accession number: AB470102). The amplified fragment was treated with KpnI and PstI, and then ligated into the corresponding site of pGEM-T Easy/Δ*recJ*::*HTK* to obtain pGEM-T Easy/ Δ*recJ*::Hyg^R plasmid.

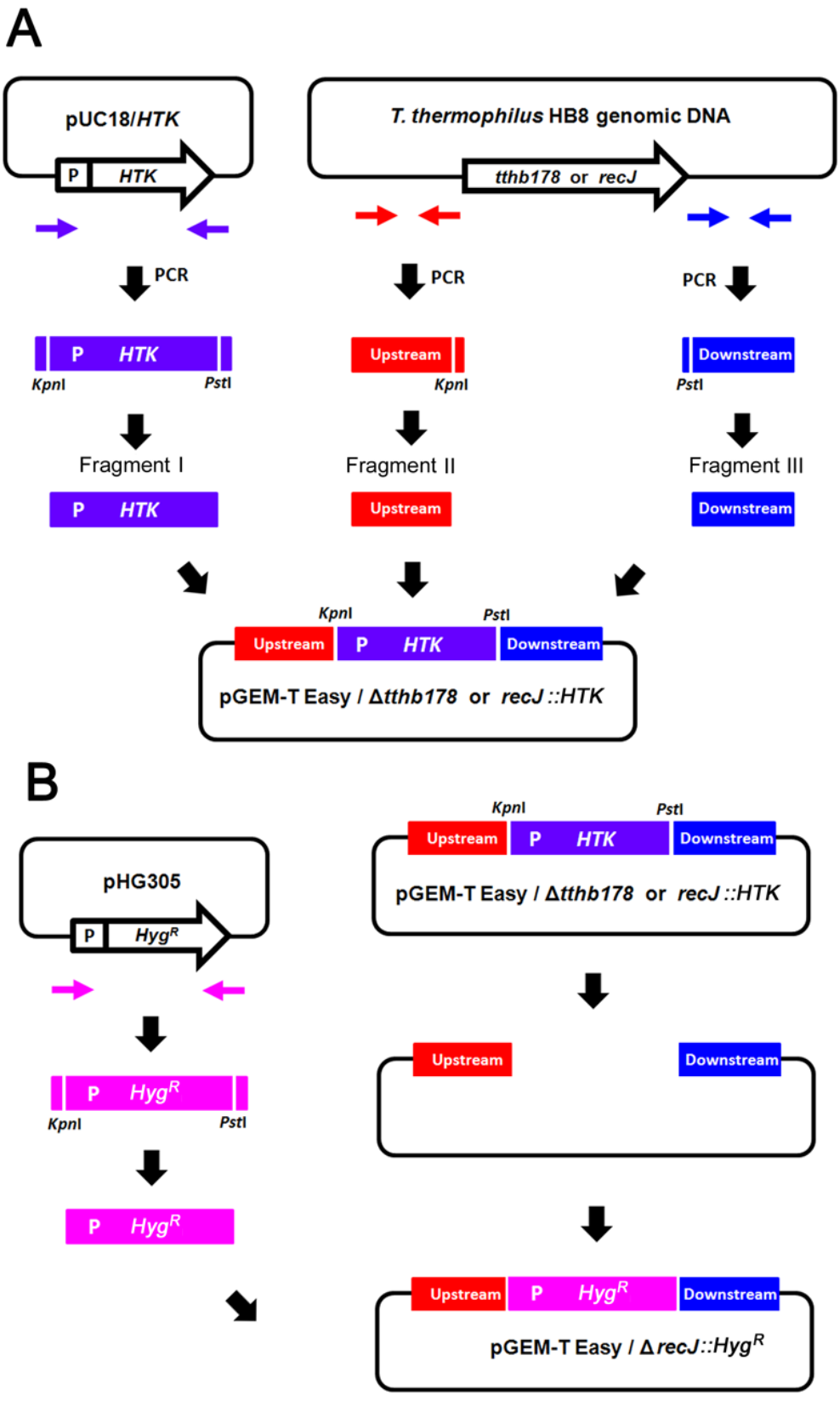
Supplementary Fig. 2. The *tthb178* (A)- and *recJ* (B)-disruptions were confirmed by PCR. The coding region of each gene in the disruptant genomic DNA was amplified by PCR, using the wild-type genomic DNA as a control. On the basis of the lengths of amplified DNA products, we concluded that disruptions were carried out as desired.

Supplementary Fig. 3. The absence of mRNAs of *tthb178* and *recJ* was confirmed by RT-PCR according to the procedure described in Materials and Methods. The cDNAs of *tthb178* (A) and *recJ* (B) were synthesised using the primer sets 5'-ACCTCTACGCCTTCCTCCTC-3' and 5'-CTCCTTGATTCTCTGGGCGG-3', and 5'-TTGGAAAACGGGGTGGAGGTG-3' and 5'-AAGAGGAGGAAGAGGGGCTCG-3', respectively. The abbreviations used are: *M*, DNA size marker; *C*, a positive control using wild-type genomic DNA as a template; *W*, cDNA synthesised from wild-type total RNA; *T*, cDNA from *Δtthb178* total RNA; *R*, cDNA from *Δrecj* total RNA; *D*, cDNA from double disruptant total RNA; *subscript c*, a negative control without reverse transcriptase. The lengths of synthesised cDNAs of *tthb178* and *recJ* were designated to be 332- and 634-bp, respectively.

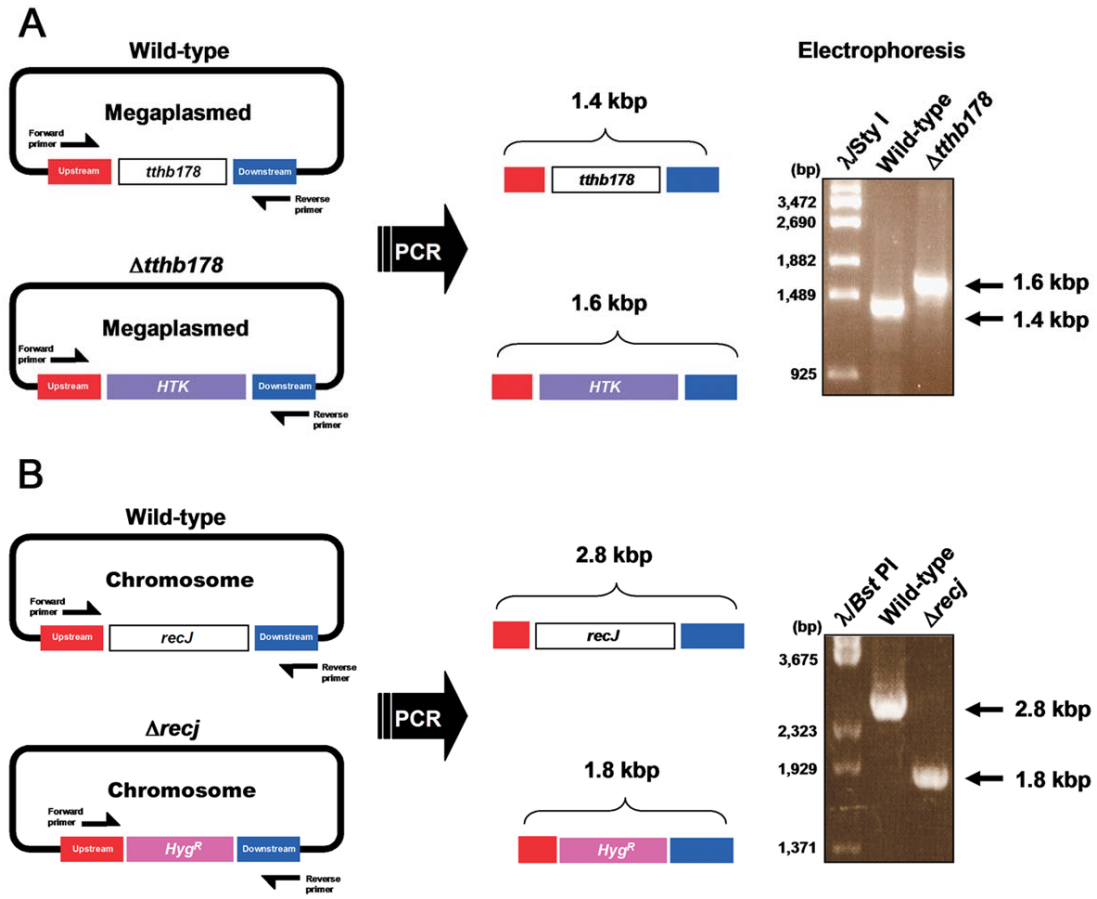
Supplementary Fig. 4. Transcription of *tthb178* in *T. thermophilus* HB8 cells. (A) The growth curve of WT *T. thermophilus* HB8. The absorbance at 660 nm was monitored and plotted against the cultivation time. The data represent the average of

three independent experiments, and each bar indicates the standard deviation. (B) The result of the RT-PCR analysis. Electrophoresis was carried out on 2% agarose gel. The numbers above the lanes correspond to those in (A). The plus (+) and minus (−) signs mean the addition and non-addition of reverse transcriptase during the RT-PCR, respectively. M, M′, and P mean the ϕ174/*Hinc*II DNA size marker, λ/*Hind*III DNA size marker, and the *tthb178* fragment amplified by PCR using genomic DNA as a template, respectively. The arrow indicates the amplified fragments of *tthb178*.

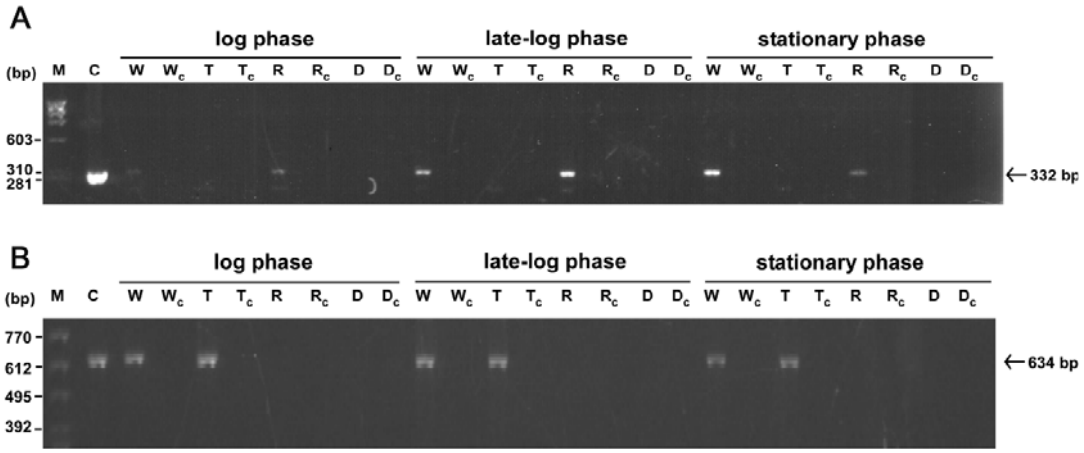
Supplementary Fig. 5. The elution profile of the exonuclease activity. (A) Elution profile of TTHB178 from Superdex 75 HR column was monitored by the absorbance at 280 nm. (B) The eluted fractions were subjected to SDS-PAGE. The peptide mass fingerprinting mass spectrometry revealed that the detected bands were TTHB178. (C) Exonuclease activity of the each fraction. The 2 μl of each fraction was reacted with 21-mer ssDNA at 37°C for 60 min.



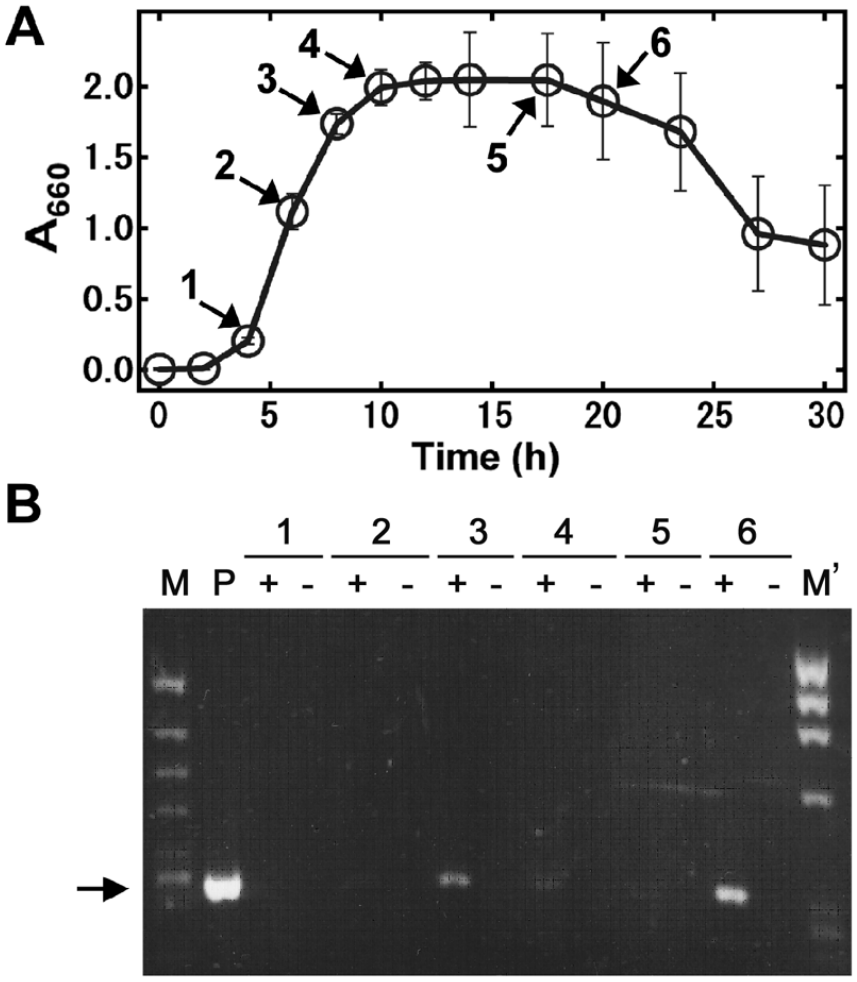
Supplementary Figure 1



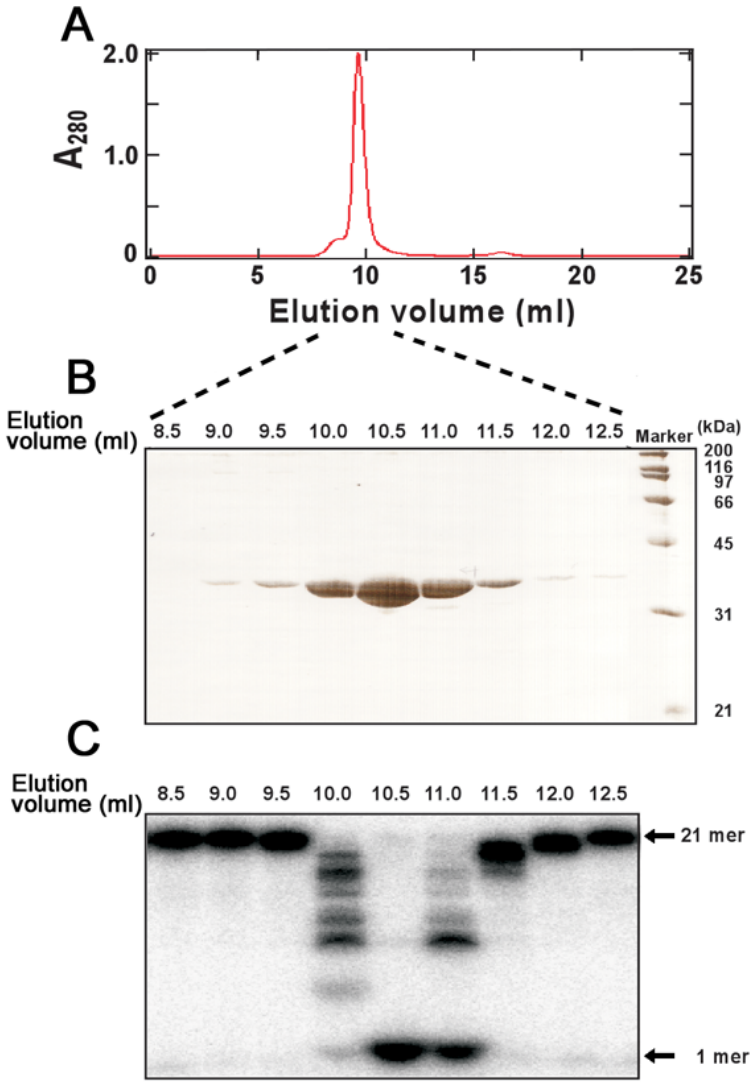
Supplementary Figure 2 Shimada *et al*.



Supplementary Figure 3 Shimada *et al*.



Supplementary Figure 4 Shimada *et al.*



Supplementary Figure 5 Shimada *et al.*

Supplementary Table 1

Distibution of single-stranded DNA-specific exonucleases (ssExos). ssExos were searched for using the amino acid sequences of following enzymes as query in BLAST search: *E. coli* exonuclease I, *E. coli* exonuclease VII, *E. coli* exonuclease X, *E. coli* RecJ, *E. coli* RecD, *H. sapiens* EXO1, *H. sapiens* MRE11, *H. sapiens* TREX1, *H sapiens* TREX2, *H. sapiens* Altemis, *H. sapiens* WRN exonuclease, *H. sapiens* exo/endonuclease-G, *H. sapiens* PIF1, *H. sapiens* DNase III, *H. sapiens* Apoptosis enhancing exonuclease, and *S. cerevisiae* DIN7. The codes in parenthesis indicate accession numbers of each protein.

Species	3'-5' ssExo	5'-3' ssExo	Bi-polar ssExo
Thermus thermophilus HB8	TTHB178 (BAD71974)	RecJ (BAD70990)	Not identified
Escherichia coli	exonuclease X (ACI82135) exonuclease I (AAA19938)	RecJ (AAA62789) RecD (AAG48658)	exonuclease VII (AAA24766)
Deinococcus radiodurans	DnaQ-like (NP_051628) DnaQ-like (NP_880692)	RecJ (ACX31683) RecJ-like (NP_294850)	exonuclease VII (Q9RXW9)
Bacillus subtilis	exonuclease I (CAA66997) DnaQ-like (O05231)	RecJ (O32044) RecJ-like (O31903) Putative 5'-3' exonuclease (P54161)	exonuclease VII (P54521)
Pyrococcus furiosus	Not identified	RecJ (AAL82179) RecJ-like (AAL80523)	Not identified
Thermococcus kodakaraensis	Not identified	RecJ (YP_183665) RecJ-like (YP_182568)	Not identified
Saccharomyces cerevisiae	MRE11 (NP_013951) EXO1 (NP_014676)	PIF1 (NP_013650) PIF1-like (NP_011896) DIN7 (CAA94102)	Not identified
Homo sapiens	MRE11 (NP_005582) EXO1 (NP_006018) TREX1 (NP_057465) TREX2 (NP_542432) Apoptosis enhancing exonuclease (AAH20988) DNase III (CAB50866)	Altemis (NP_001029030) Exo/Endonuclease-G (NP_005098) PIF1 (NP_079325)	WRN exonuclease (AAR05448)