

Supplementary materials

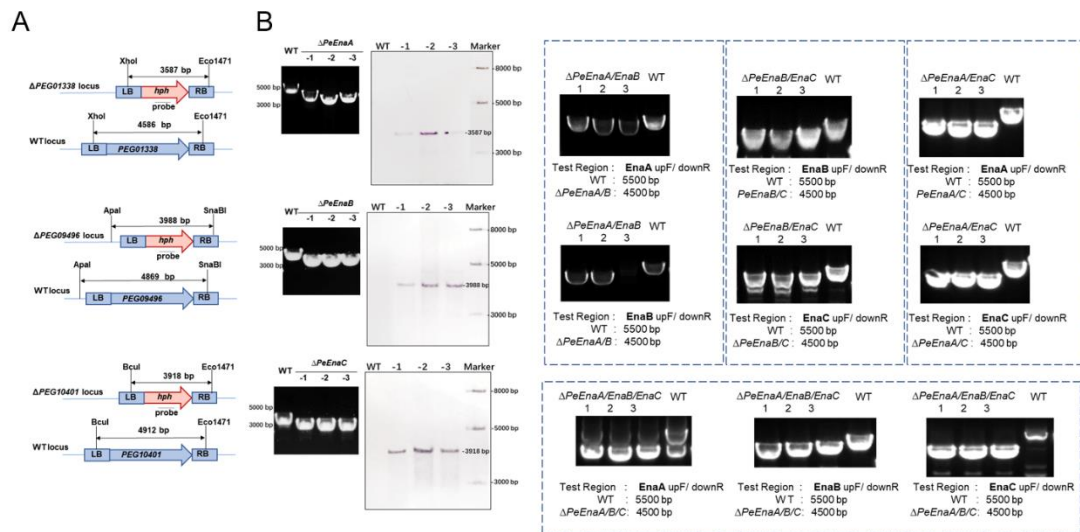


Figure S1 Knockout of *PeEnaA*, *PeEnaB*, and *PeEnaC* in *P. expansum*. **A.** *PeEnaA*, *PeEnaB*, and *PeEnaC* deletion strategy used by homologous recombination. **B.** PCR detection and Southern Blot of Δ *PeEnaA*, Δ *PeEnaB*, and Δ *PeEnaC* knockout mutants. WT means wild-type strain.

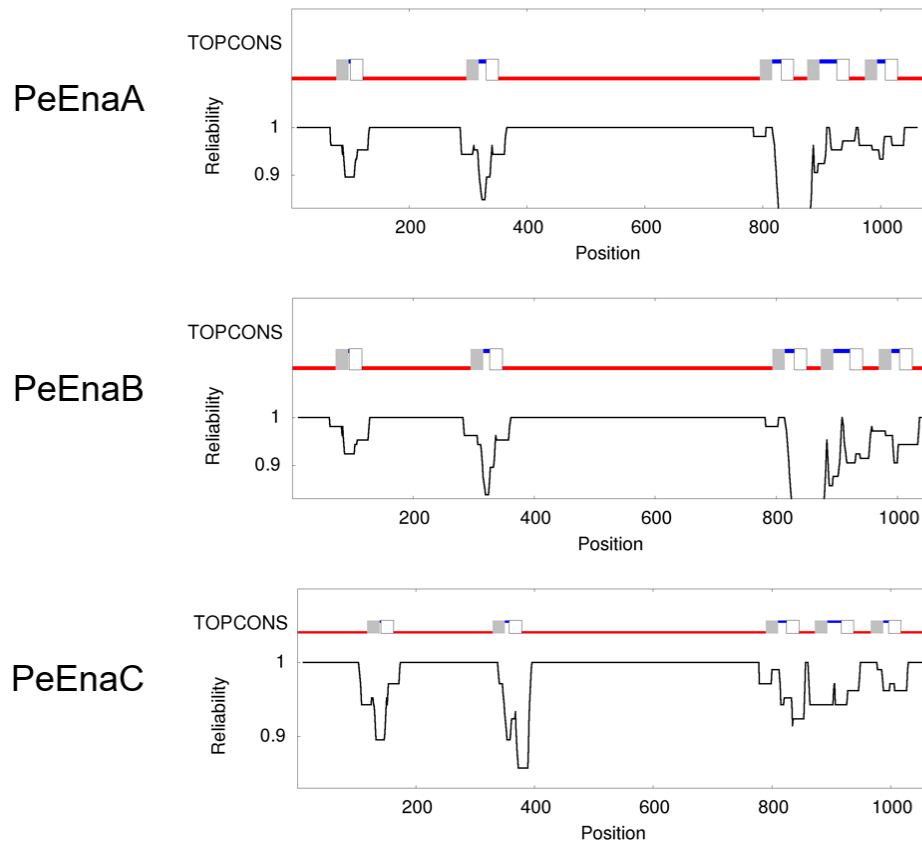


Figure S2 The transmembrane topology of the PeEna proteins. The transmembrane topology was determined by manual assertion deduced from a combination of prediction using the transmembrane topology web server TOPCONS.

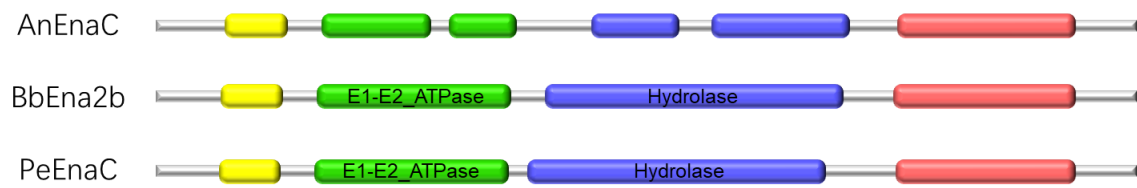
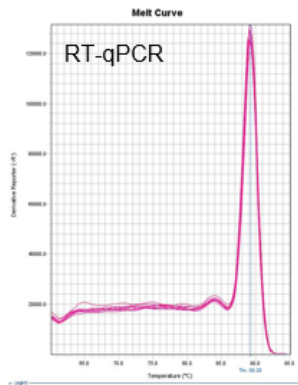
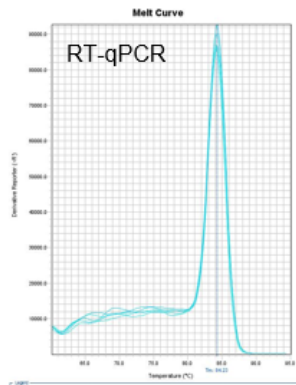


Figure S3 Functional conserved domain analysis of Ena family proteins via Pfam database. The cation-ATPase-N (pfam00690) domain is marked in red, the E1-E2_ATPase (pfam00122) domain is marked in green, the halo acid dehalogenase-like hydrolase (HAD) (COG4087. pfam00702) domain is marked in blue, and Cation_ATPase_C (pfam00689) domain is marked in yellow.

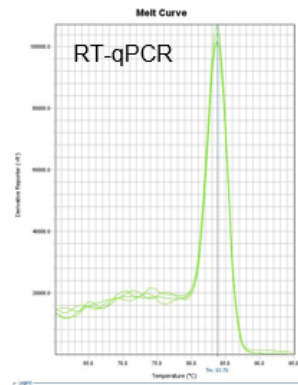
PeEnaA



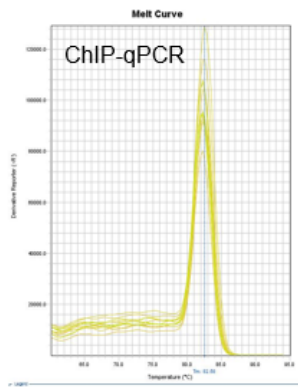
PeEnaB



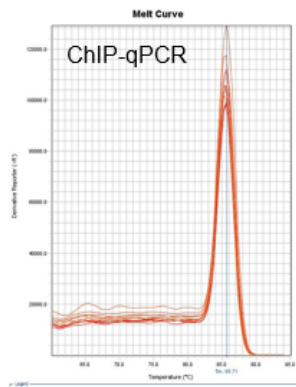
PeEnaC



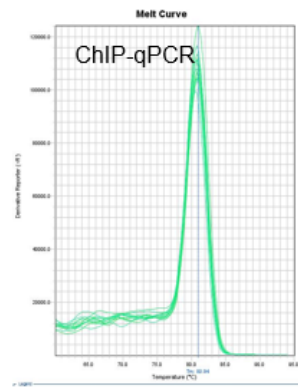
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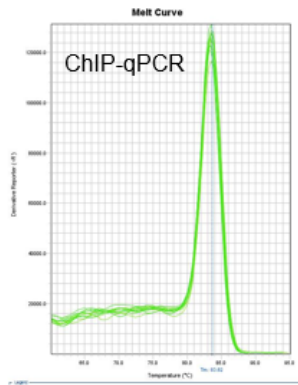
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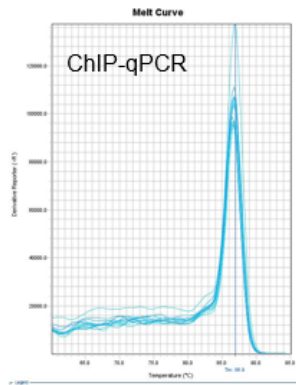
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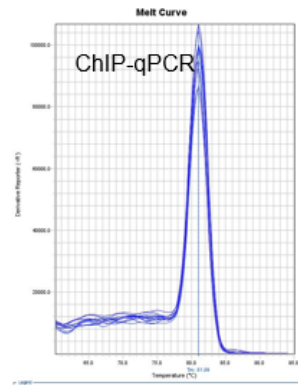
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PeEnaC-1



PeEnaC-2



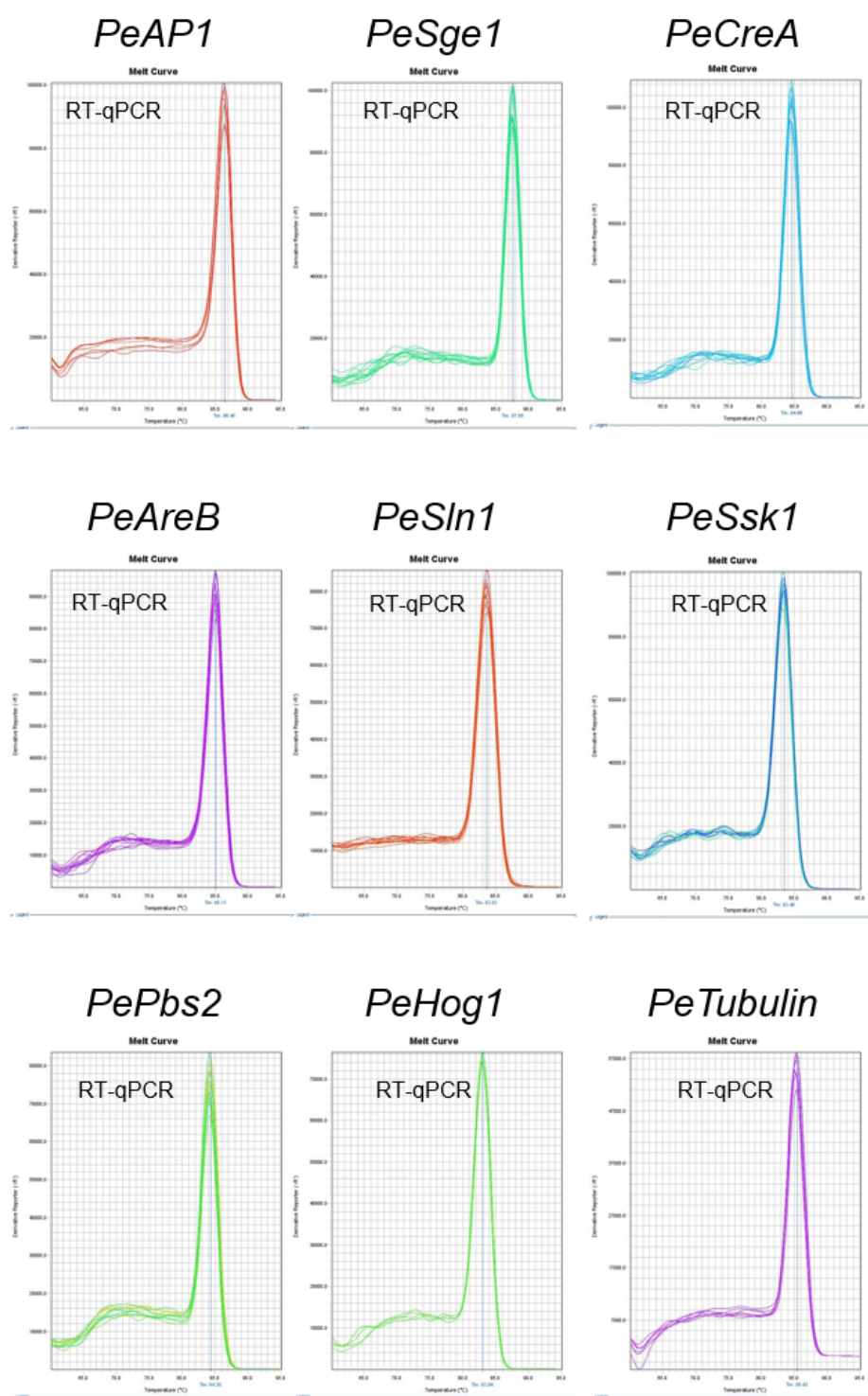


Figure S4 The melting curves of representative genes in this study.

Table S1 The primers used for construction and identification of gene deletion, complementation and eGFP tag strains in this study.

Primer name	Primer sequence (5'-3')
EnaA-up-F	GAATTCGAGCTCGGTACCCGGGTGCCCCGCATGGCTGTGC GTCGTCG
EnaA-up-R	CCACAGCTGCAGGTCTGACTCTAGATTTCGCGATTTCGCAA ATGGTAAGA
EnaA-down-F	AGTAACCATGCATGGTTGCCTAACTCAATAGATTTACTC AAATGAACAAC
EnaA-down-R	GCCAGTGCCAAGCTTCGGCGCGCCCAAATGCCGGAAGA GTTTGCAGAA
EnaC-up-F	GAATTCGAGCTCGGTACCCGGGGTTGTGTTTGAGCAAAA AAGTAGC
EnaC-up-R	CCACAGCTGCAGGTCTGACTCTAGATCAATAAGATTGTAG GAAAGAAAG
EnaC-down-F	AGTAACCATGCATGGTTGCCTAACTCTGGAAAACCTGTC TGA CTGAT
EnaC-down-R	GCCAGTGCCAAGCTTCGGCGCGCCTGATCATTCAACGTT GAATAAACG
EnaB-up-F	GAATTCGAGCTCGGTACCCGGGAGGAACAGCGTCTATGT TGATGCG
EnaB-up-R	CCACAGCTGCAGGTCTGACTCTAGATGGATATTGTCGTTC GATGGGTAG
EnaB-down-F	AGTAACCATGCATGGTTGCCTAACTCCGAATGCTTTCCCT GGCCCAACAT
EnaB-down-R	GCCAGTGCCAAGCTTCGGCGCGCCTATGGCGATGTAATT CAACTTCGC
EnaA-test-F	GTATGCATGAAGCGCGGGGGTTTG
EnaC-test-F	ACAGGAAGATGCAGTGGGCTTCGC
EnaB-test-F	TGGCCCAGCTGAGATGAGCCATTC
HPH-F	TCACCCCCATCTCAACTCCA
HPH-R	TGCTCCATACAAGCCAACCAC
NEO-F	ATGATTGAACAAGATGGATTGC
NEO-R	TCAGAAGAAGTTCGTCAAGAAG
Nat-F	TCCTTCACCAACGACACCGTCTTCC
Nat-R	CGTCCGATTCGTTCGTCCGATTCGTC
EnaA-Com-F	AACTCCATCACATCACAAGAGCTCTCGCAGGAAGCCCAA AAAGGCTCT
EnaA-Com-R	CTCCTCGCCCTTGCTCACTCTAGATTTGAAGATTACTTGT TCATTA
EnaB-Com-F	AACTCCATCACATCACAAGAGCTCATGTCAGACGAGAAG CCTGGCA

EnaB-Com-R	CTCCTCGCCCTTGCTCACTCTAGATTAGGGTTGCTCCGGG ATCTC
EnaC-Com-F	AACTCCATCACATCACAAGAGCTCATAGAGGTA ACTCCCG ATGTCGA
EnaC-Com-R	CTCCTCGCCCTTGCTCACTCTAGACTTCAGTTCCACGCTT GCATTCGC

Table S2 Formulation of the citrate-phosphate buffer

pH	0.2 mol/L Na ₂ HPO ₄ (mL)	0.1 mol/L Citric acid (mL)
3	4.11	15.89
5	10.30	9.70
8	19.45	0.55

Note:

Preparation of MM media at pH 5 and 8: Prepare 2× MM medium and the above citrate-phosphate buffer, and preheat them at 80°C, respectively. Mix the medium and the buffer in a ratio of 1:1 and spread the plates.

Preparation of CY media at pH 3, 5, and 8: Prepare 2× CY liquid medium and the above citrate-phosphate buffer, Mix them in a ratio of 1:1.

Table S3 The primers used for RT-qPCR in this study.

Name	Sequence (5'-3')	Annealing temperatures (°C)	Accession number
<i>EnaA</i> - RegionA	F: CTGGAGAAGTAGGTGGCTTTGAA R: CTCGCCCCGGTCCCCACGCGACAAT	60	PEX2_013210 (PEG01338)
<i>EnaA</i> - RegionB	F: ATGTGCCCAGAGTCTTTTGC GTCA R: TCGATGGCGAAAGATAAGCGGCT	60	PEX2_013210 (PEG01338)
<i>EnaB</i> - RegionA	F: TGCTCCTTCCCAGTGTTGTTGTG R: GCTGTGAAC TTGCCTCGTCTTG C	60	PEX2_039950 (PEG09496)
<i>EnaB</i> - RegionB	F: TGGAGCAAGACTGTTGAAGTGGC R: GACAGACATCGGAATCTGGTGGG	60	PEX2_039950 (PEG09496)
<i>EnaC</i> - RegionA	F: CCACGACTGCCTAGAAGCCAAGT R: TCCGACGGGAGTGTTCAAAGATA	60	PEX2_085740 (PEG10401)
<i>EnaC</i> - RegionB	F: CCCTCTTAGGGTTGGAAGCAAGT R: GTAGCAAGGCGGACGAAGGTGTA	60	PEX2_085740 (PEG10401)
<i>PeLaeA</i>	F: TCGAAGCTCTTCCGGACATG R: AATGCAATGCGGTCAATCTG	60	PEX2_035880 (PEG08484)
<i>PeVeA</i>	F: GAAGATACTTTCGGCCTTGATGA R: TCGCGATAAGCAGGATAAGGA	60	PEX2_043190 (PEG11106)
<i>PeVelB</i>	F: TTGTTGTCTGGGTCCG TCAA R: CCTGGGATGAGGGATTTCG T	60	PEX2_031850 (PEG02453)
<i>PeAP1</i>	F: GAGCCTTGTTGCC CAGATTGATA R: GTTCACGACTACACCACCTCA	60	PEX2_065210 (PEG01534)
<i>PeSge1</i>	F: ATGGAATGTACTTCGGGCTGCTT R: TAGTGGGTCGTTGGATGGGATAA	60	PEX2_061550 (PEG11642)
<i>PeCreA</i>	F: TCACGATACTTCGCCCCGACTCAA R: GAAGGCACGGTCGCAAAGAGGAC	60	PEX2_022240 (PEG01401)
<i>PeAreB</i>	F: CTATACCCTCATAATGGCGACCAC R: TTCAAGAAGAGTCCACATGCGTTG	60	PEX2_003790 (PEG07853)
<i>PeSln1</i>	F: CCCAGACCGACTCAAGTATCAAC R: GCTATCTAGCAGAATGTCACCTCCC	60	PEX2_054790 (PEG04458)
<i>PeSsk1</i>	F: CGATCAATACAATTCTTCCTTGCC R: GCTGGGATTGGAGTTTGAGCTTTC	60	PEX2_072810 (PEG02228)
<i>PePbs2</i>	F: GGAAGTGGGTTATCTGCCTCGTC R: TGCCCTGTTGAAGCAGCAGTATG	60	PEX2_005030 (PEG09483)
<i>PeHog1</i>	F: CACCGCTCTTCAAATCACCGACAG R: TGCCATCAAACGGCTTCCCTATC	60	PEX2_060120 (PEG11493)
<i>PePatA</i>	F: AAAGGCCGGTG CATTGATC R: TTGGAGGCTTTGGTGAGCAT	60	PEX2_082810 (PEG03683)
<i>PePatB</i>	F: GCCAGGCTATGCGATTGAGT R: GCTGGAACCCTGTCCATTGT	60	PEX2_082800 (PEG03684)

<i>PePatC</i>	F: TCCACCTGCGAATATCCCTTA R: CATCGCCAGTGCCATTTTC	60	PEX2_082790 (PEG03685)
<i>PePatD</i>	F: ATGAGATTCTGTCTGCGCAAAG R: CTACCCAAGCGGGATGAGATT	60	PEX2_082780 (PEG03686)
<i>PePatE</i>	F: CATTCTCATCGGGCCTGAGT R: TCGAAGCTCTTCCGGACATG	60	PEX2_082770 (PEG03687)
<i>PePatF</i>	F: GCGAGTGAATTTCGGCCAAT R: GTCCGACCCAAAGGATGAAG	60	PEX2_082760 (PEG03688)
<i>PePatG</i>	F: CGGCCGTCTTGAAGGAAAT R: CTTGCCGTAGCGGGTGAATA	60	PEX2_082750 (PEG03689)
<i>PePatH</i>	F: CATTTATCGGCGGTGTTCTGA R: GATCAACGCTTGACGATAGC	60	PEX2_082740 (PEG03690)
<i>PePatI</i>	F: GCAAACCTCATTCGCAAGGA R: TGGTTCTTGCCATCGATCAC	60	PEX2_082860 (PEG03676)
<i>PePatJ</i>	F: CGCCAGACATAACCGCCATA R: TTTGGTCGATCGGGACTGTT	60	PEX2_082870 (PEG03675)
<i>PePatK</i>	F: GACGCTGGGCTACTGGATTG R: TCGTGCGTGAGGCCAGTAT	60	PEX2_082880 (PEG03674)
<i>PePatL</i>	F: GCAGGAGATCCGTTTCAGACA R: CCACTGACCGACGGTTACAAC	60	PEX2_082850 (PEG03677)
<i>PePatM</i>	F: ACCCACAGCTGCACATGGA R: AGCGAGAAGAGGCGGAAGA	60	PEX2_082820 (PEG03681)
<i>PePatN</i>	F: CGTTCGATGTCGCTAGCAAA R: GGCGATAATCACGTCAATTCG	60	PEX2_082830 (PEG03680)
<i>PePatO</i>	F: TCGCCTCCTGGTGTGTATCTT R: AAGCGTGCCCAGTCATTCAG	60	PEX2_082840 (PEG03678)
<i>β-tubulin</i>	F: CTCCAGCTCGAGCGTATGAAC R: GGCTCCAAATCGACGAGAAC	60	PEX2_027410 (PEG11155)