

## *Supplementary Material*

### **Secreted expression of a hyperthermophilic $\alpha$ -amylase gene from**

### ***Thermococcus* sp. HJ21 in *Bacillus subtilis***

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## Plasmids used in this study

Plasmids	Description	Source
pET32a-Amy	<i>amy</i> gene donor	Laboratory stock
pHT01	<i>E. coli</i> - <i>B. subtilis</i> shuttle vector	Laboratory stock
pHT01-Amy	pHT01 with <i>amy</i> gene	This study
pHCMC04	P <sub><i>xylA</i></sub> promoter and XylR repressor donor	Laboratory stock
pHTP <sub><i>xylA</i></sub> -Amy	pHT01-Amy with the P <sub><i>xylA</i></sub> promoter and XylR repressor inserted in <i>SacI/BamHI</i> site	This study
pHTP43-Amy	pHT01-Amy with the P43 promoter inserted in <i>SacI/BamHI</i> site	This study
pHTP <sub><i>hag</i></sub> -Amy	pHT01-Amy with the P <sub><i>hag</i></sub> promoter inserted in <i>SacI/BamHI</i> site	This study
pHT43	signal peptide S <sub><i>amyQ</i></sub> donor	Laboratory stock
pHTP <sub><i>xylA</i></sub> -S <sub><i>amyQ</i></sub> -Amy	pHTP <sub><i>xylA</i></sub> -Amy with the signal peptide S <sub><i>amyQ</i></sub> inserted in <i>BamHI</i> site	This study
pHTP <sub><i>xylA</i></sub> -S <sub><i>Epr</i></sub> -Amy	pHTP <sub><i>xylA</i></sub> -Amy with the signal peptide S <sub><i>Epr</i></sub> inserted in <i>BamHI</i> site	This study
pHTP <sub><i>xylA</i></sub> -S <sub><i>YncM</i></sub> -Amy	pHTP <sub><i>xylA</i></sub> -Amy with the signal peptide S <sub><i>YncM</i></sub> inserted in <i>BamHI</i> site	This study

**Primers used in this study**

Primer	Sequence (5' →3')	Restriction site
P1	<u>TCTAGA</u> ATGAACAGGGGTATATTGGCGGCAC	<i>Xba</i> I
P2	<u>TCTAGAT</u> CAGCCCACCCACAGTAGCTCC	<i>Xba</i> I
P3	<u>GAGCTC</u> CTAACTTATAGGGGTAACAC	<i>Sac</i> I
P4	<u>GGATCC</u> CATTTCCTCCCTTTGATTTTATG	<i>Bam</i> HI
P5	<u>GAGCTC</u> AGCTTCGTGCATGCAGG	<i>Sac</i> I
P6	<u>GGATCC</u> AAGCTTCTGTTATTAATCTTGTC	<i>Bam</i> HI
P7	<u>GAGCTC</u> AAAATAACCGTGCAGCAGCG	<i>Sac</i> I
P8	<u>GGATCC</u> TGTTTTGTTCCCTCCCTGAATATG	<i>Bam</i> HI
P9	<u>TCTAGA</u> ATGATTCAAAAACGAAAGCGGACAG	<i>Xba</i> I
P10	CTTAAGACAA CCAGGGCGGCTGATGTTTTTG	
P11	CAA AAACATCAGCCGCCCTGGTTGTCTTAAG	
P12	<u>TCTAGAT</u> CAGCCCACCCACAGTAGCTCC	<i>Xba</i> I
P13	<u>TCTAGA</u> ATGAAAAACATGTCTTGCAA	<i>Xba</i> I
P14	GTTTTCAAGGGTCTCCGCATGAGCGAGAGGG	
P15	CCCTCTCGCTCATGCGGAGACCCTTGAAAAC	
P16	<u>TCTAGA</u> ATGGCGAAACCACTATCAAAA	<i>Xba</i> I
P17	GTTTTCAAGGGTCTCAGCGTCTGCCGCGGGT	
P18	ACCCGCGGCAGACGCTGAGACCCTTGAAAAC	
P19	GCTCCTGCATACATGAGTACACCGG	
P20	TCAGCCCACCCACAGTAGCTCC	

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P21 CCTACGGGAGGCAGCAG

P22 ATTACCGCGGCTGCTGG

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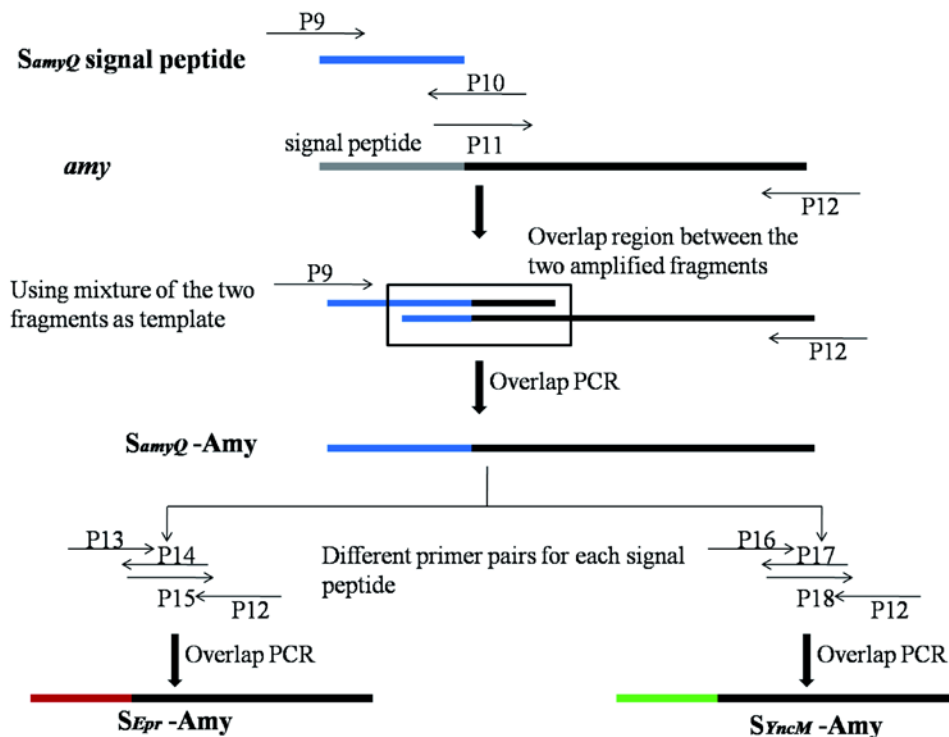


Figure S1

**Figure S1. Schematic diagram of the overlap PCR protocol.** Blue rectangle, red rectangle, green rectangle, grey rectangle, and amy represent the signal peptides  $S_{amyQ}$ ,  $S_{Epr}$ ,  $S_{YncM}$ , the native signal peptide of  $\alpha$ -amylase, and the coding sequence of  $\alpha$ -amylase, respectively. Approximate positions of the primers are indicated with arrows. Signal peptide  $S_{amyQ}$  was cloned from plasmid pHT43 as an upstream fragment using primer pair P9/P10, and the *amy* gene lacking its signal peptide was amplified as a downstream fragment using primer pair P11/P12. A mixture of the two fragments was used as a template and amplified by primers P9 and P12 to generate the fusion amyase  $S_{amyQ}$ -Amy. The same method was used to generate

the other two fusion amyloses,  $S_{Epr}$ -Amy and  $S_{YncM}$ -Amy.

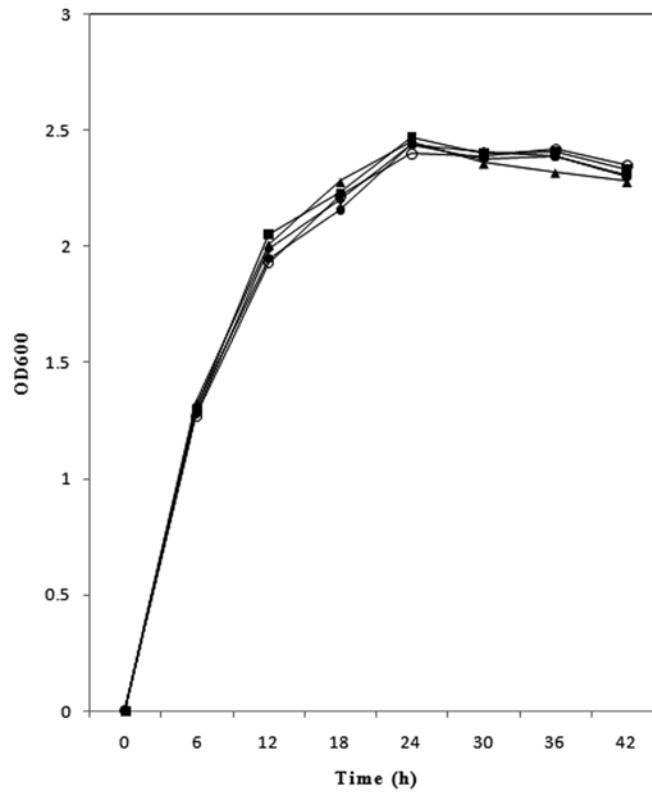


Figure S2

**Figure S2. Growth curves of *B. subtilis* DB104 harboring plasmids with different signal peptides when cultured in LB.** The black diamond, black square, black triangle, and black circle represent the OD<sub>600</sub> of *B. subtilis* DB104 harboring pHTP<sub>xylA</sub>-Amy, pHTP<sub>xylA</sub>-S<sub>Epr</sub>-Amy, pHTP<sub>xylA</sub>-S<sub>YncM</sub>-Amy, and pHTP<sub>xylA</sub>-S<sub>amyQ</sub>-Amy, respectively. The white circle represents the OD<sub>600</sub> of *B. subtilis* DB104 harboring pHTP<sub>xylA</sub>-Amy without xylose induction.