

**A new strategy to express the extracellular  $\alpha$ -amylase from *Pyrococcus furiosus*  
in *Bacillus amyloliquefaciens***

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Supplementary Table S1. Primers

Primer	Sequence (5'–3')
PamyA-F	<u>TCTAGAG</u> ATCTCAAGCCCGCTTTTTT
PamyA-R	<u>CTCGAGG</u> CCATTTACGGCTGATGTTT
pUBC19-CX-up	GGTACAAGAAAAACGAGGAAAGATGC
pUBC19-CX-down	TCGTATGTTGTGTGGAATTGTGAGC
SP-F	ATGAGAGGGAGAGGAAACATGA
SP-R	<u>CTCGAGG</u> GCTGATGTTTTTTGTAATCGGCAAAC
P43-F	<u>TCTAGAT</u> GATAGGTGGTATGTTTTTCG
P43-R	TCCTCTCCCTCTCATGTGTACATTCCTCTCTTACC
P16-F	<u>TCTAGAC</u> CGATAATTCCAATTTTCAT
P16-R	<u>CTCGAG</u> TCCTCCACCTCCCATATCTC
HN-F	<u>GTCGACA</u> AACTTGAGGCGATCACATC
HN-R	<u>GAATTC</u> TTTCAATTCTCCGGGGAATC
Cm-F	<u>GAATTC</u> CTAGGAGCTGGCGTAATAGCGAAG
Cm-R	<u>GAGCTC</u> CTGCAGGTGAGCGAGGAAGCGGAAGA
HC-F	<u>GAGCTC</u> GGTAATAAAAAAACACCTCCAAGCTG
HC-R	<u>GGTACC</u> ATAAGAGAGGAGCTTGTTGAAG
pNZT-F	AGCTCCTCTCTTATGGTACCCAATTCGCCCTATAGTGAGT
pNZT-R	ATCGCCTCAAGTTTGTGCGACATTTTTAAAGTATGTATACA
Genome-F	AAACTTGAGGCGATCACATCTG
Genome-R	ATAAGAGAGGAGCTTGTTGAAG
AmyA-F	ATGAGAGGGAGAGGAAACATGATTC
AmyA-R	TTATTTCTGAACATAAATGGAGACG
PFA-F	CTCGAGCATCACCACCACCATC
PFA-R	CTGCAGTTATCCAACACCACAGTAG

Underscored nucleotides indicate sites of restriction digestion.

## Design of two synthetic *pfa* genes

The optimization of GenScript (*opfa*) focused on the codon optimization, involving transcription, mRNA processing and stability, translation and protein folding ([http://www.genscript.com/codon\\_opt.html?src=gssb](http://www.genscript.com/codon_opt.html?src=gssb)). According to our BAA protein expression pattern-mimicking strategy, *mpfa* was designed to mimic BAA as many aspects as possible, which included the mRNA structure of BAA in addition to codon optimization. As shown in Figure S1, the mRNA structure of *opfa* was different with that of BAA while the mRNA structure of *mpfa* was similar with that of BAA.

**Figure S1 the mRNA structures of BAA, *opfa* and *mpfa*.** All the mRNA structure were calculated on the website (<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>).”

