Additional file 1

Secretion of the cytoplasmic and high molecular weight *β*-galactosidase of *Paenibacillus wynnii* with *Bacillus subtilis*

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Keywords: *Bacillus subtilis,* protein secretion, recombinant enzyme production, *β*-galactosidase, bioreactor

Table S1: Oligonucleotides used in this study.

Name	Sequence (5'→3')
O1	ctagtgaaaaagatgttgatgttagcttttacatttcttttggctttgactatccatgtaggggg
O2	cgcgccccctacatggatagtcaaagccaaaagaaatgtaaaagctaacatcaacatctttttca
P1	cgtctcacgcgcaggcgcgtaaaaaacttgtctattcacc
P2	ccttgtctacaaaccccttaaaaacg
P3	tcgagtctctacggaaatagcg
P4	catcgataagctcaggcagtcaggatccg
P5	cctggaattcgccaaagctgtaatggctg
P6	gcactagtcattcctctcttacctataatgg
P7	cgtctcacgcgccgtaaaaaacttgtc
P8	gcctcgagcaccattctaatcgtaaatc

Table S2: Signal peptides used in this study.

Signal peptide	Amino acid sequence	Origin
YoaW	VKKMLMLAFTFLLALTIHVGGAQA	*B. subtilis* 168
AprE	VRSKKLWISLLFALALIFTMAFGSTSSAQA	*B. subtilis* 168
PhoD	VAYDSRFDEWVQKLKEESFQNNTFDRRK FIQGAGKIAGLSLGLTIAQSASA	*B. subtilis* 168
GlmU	VDRRDNGGQYMDKRFAVVLA	*B. licheniformis*

Table S3: Plasmids used in this study.

Plasmid	Application
Bs_Pgeo_SPaprE_prom pMA	Provides modular cloning cassette
20AC44OP_bGalPW_opt_Bs_pMK-RQ	Provides optimized *β*-gal-Pw gene
pET20b - *β*_Gal_ *P.wynnii*_His6	Provides native *β*-gal-Pw gene
BNspeBif3	Vector backbone
pLF_P_{43}_YoaW	Negative control
pLF_P_{AprE}_AprE	Negative control
pLF_ P_{43}_YoaW_ *β*-gal-Pw	*β*-gal-Pw expression
pLF_ P_{43}_YoaW_ *β*-gal-Pw_opt	*β*-gal-Pw expression
pLF_ P_{43}_AprE_ *β*-gal-Pw_opt	*β*-gal-Pw expression
pLF_ P_{43}_PhoD_ *β*-gal-Pw_opt	*β*-gal-Pw expression
pLF_ P_{43}_GlmU_ *β*-gal-Pw_opt	*β*-gal-Pw expression
pLF_P_{AprE}_YoaW_ *β*-gal-Pw	*β*-gal-Pw expression
pLF_P_{AprE}_YoaW_ *β*-gal-Pw_opt	*β*-gal-Pw expression
pLF_P_{AprE}_AprE_*β*-gal-Pw_opt	*β*-gal-Pw expression
pLF_P_{AprE}_PhoD_*β*-gal-Pw_opt	*β*-gal-Pw expression
pLF_ P_{AprE} GlmU_*β*-gal-Pw_opt	*β*-gal-Pw expression

Table S4: Strains used in this study.

Strain	Genotype and properties	Source
Escherichia coli XL1 Blue	*endA1 gyrA96* (nal^R) thi-1 *recA1 relA1 lac* glnV44 F'[:Tn10 proAB+ *lacIq* Δ(l*acZ*)M15] hsdR17(rK - mK +)	Bullock et al., 1987
Bacillus subtilis 168	*trpC2*	Burkholder & Giles, 1947
B. subtilis SCK6	Erm^R *, his nprR2 nprE18* Δ*aprA3* Δ*eglS102* Δ*bglT bglSRV lacA*::P_{xylA} -*comK*	Zhang & Zhang, 2011
*Bs*Anc	*B. subtilis* SCK6 with plasmid pLF_P_{AprE}_AprE (negative control)	This work
*Bs*AY1	*B. subtilis* SCK6 with plasmid pLF_P_{AprE}_YoaW_*β*-gal-Pw	This work
*Bs*AY2	*B. subtilis* SCK6 with plasmid pLF_P_{AprE}_YoaW_*β*-gal-Pw_opt	This work
*Bs*AP2	*B. subtilis* SCK6 with plasmid pLF_P_{AprE}_PhoD_*β*-gal-Pw_opt	This work
*Bs*AG2	*B. subtilis* SCK6 with plasmid pLF_P_{AprE}_GlmU_*β*-gal-Pw_opt	This work
*Bs*AA2	*B. subtilis* SCK6 with plasmid pLF_P_{AprE}_AprE_*β*-gal-Pw_opt	This work
*Bs*43nc	*B. subtilis* SCK6 with plasmid pLF_P_{43}_YoaW (negative control)	This work
*Bs*43Y1	*B. subtilis* SCK6 with plasmid pLF_P_{43}_YoaW_*β*-gal-Pw	This work
*Bs*43Y2	*B. subtilis* SCK6 with plasmid pLF_P_{43}_YoaW_*β*-gal-Pw_opt	This work
*Bs*43P2	*B. subtilis* SCK6 with plasmid pLF_P_{43}_PhoD_*β*-gal-Pw_opt	This work
*Bs*43G2	*B. subtilis* SCK6 with plasmid pLF_P_{43}_GlmU_*β*-gal-Pw_opt	This work
*Bs*43A2	*B. subtilis* SCK6 with plasmid pLF_P_{43}_AprE_*β*-gal-Pw_opt	This work

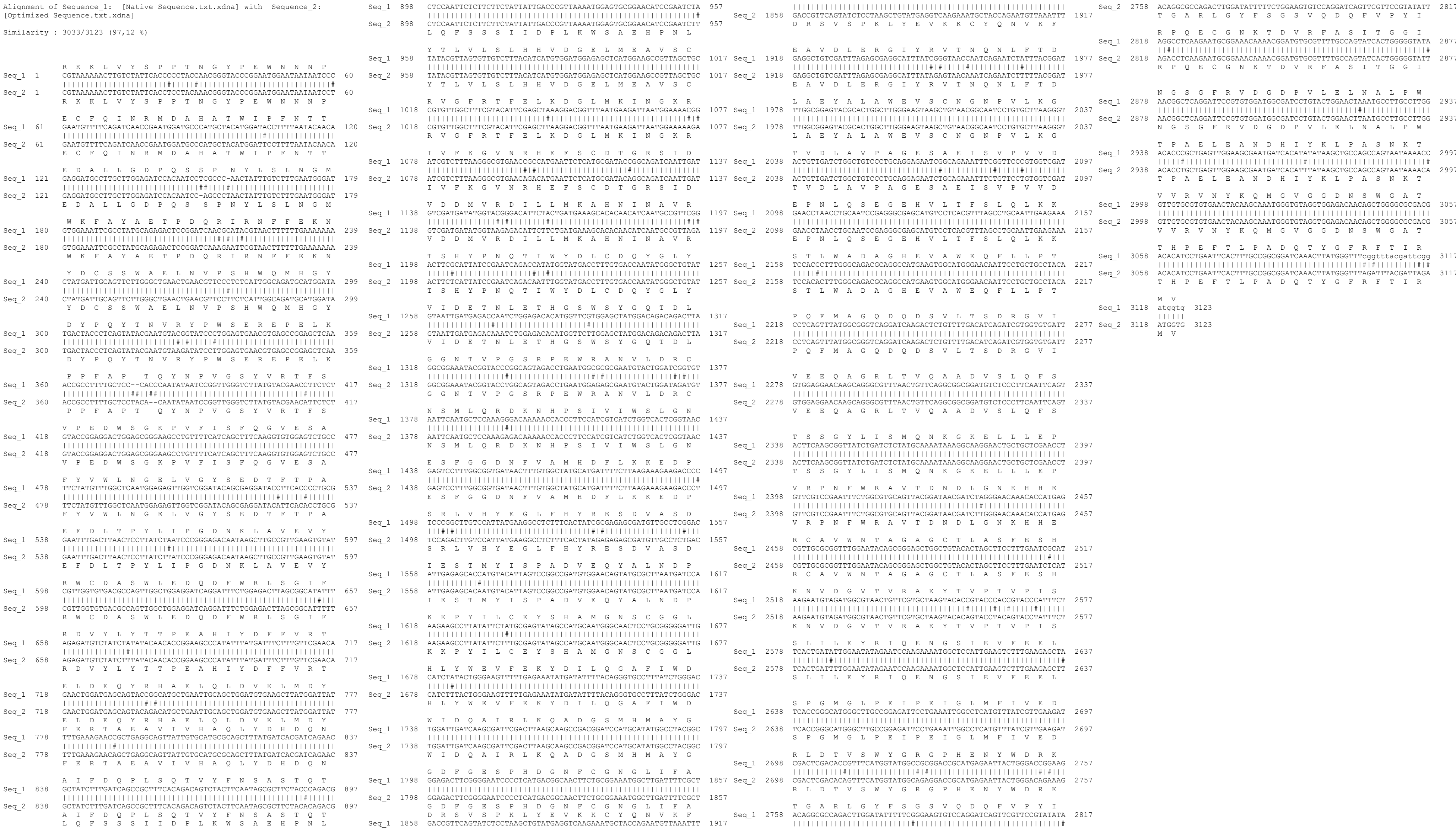


Figure S1: Alignment of native *β*-gal-Pw sequence (Seq1) and codon-optimized *β*-gal-Pw sequence (Seq2).

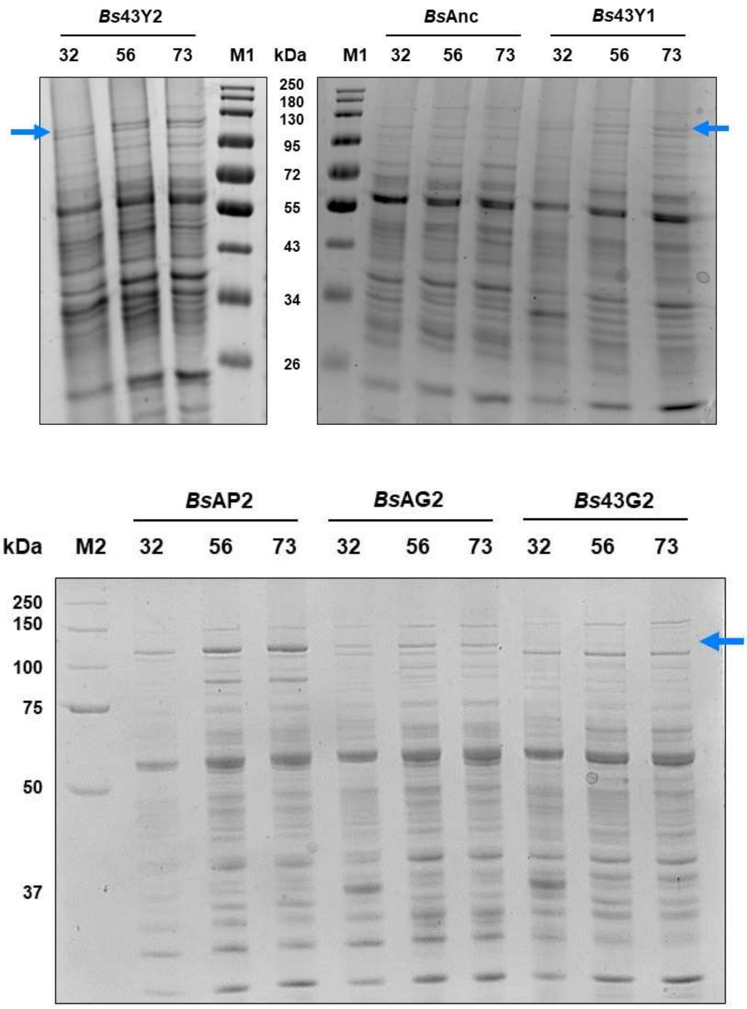


Figure S2: SDS PAGEs of the secretomes of the different *B. subtilis* strains during shake flask cultivation. The culture supernatant was analyzed after 32 h, 56 h and 73 h of cultivation. The blue arrow indicates the 120 kDa band corresponding to the size of the *β*-gal-Pw. An amount of 8 µg of protein was loaded in each lane. M1=Biorad Precision Plus Protein™ Unstained Protein Standards; M2=NEB Color Prestained Protein Standard Broad Range (10-250 kDa)

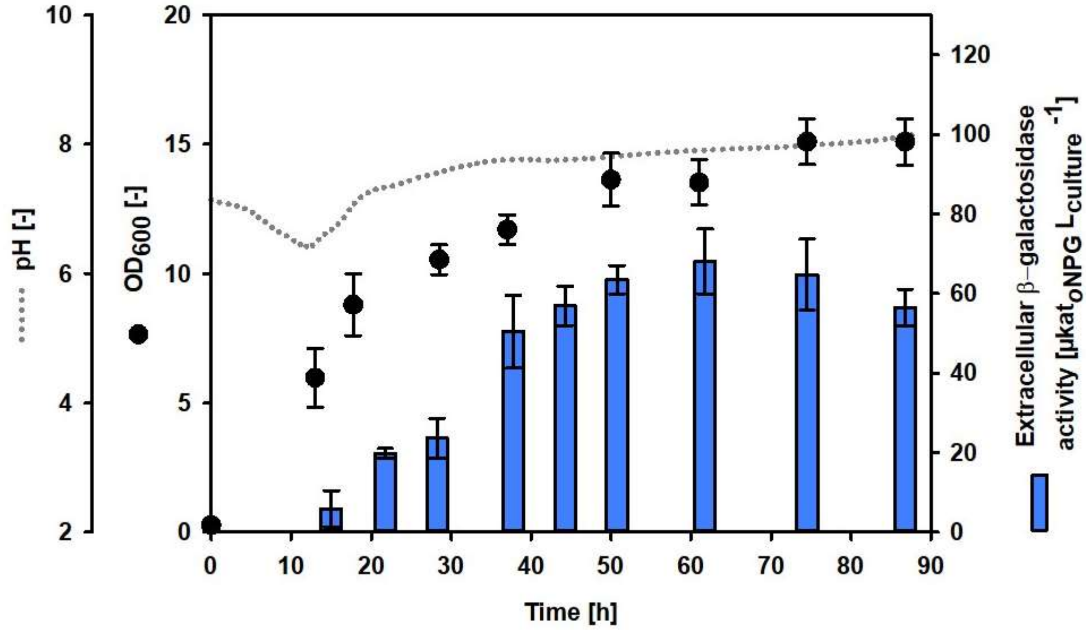


Figure S3: Repetition of the bioreactor cultivation of *Bs*AP2 for secretory *β*-gal-Pw production. Cultivation was done in duplicates at 30 °C, without pH control and with a working volume of 0.8 L.

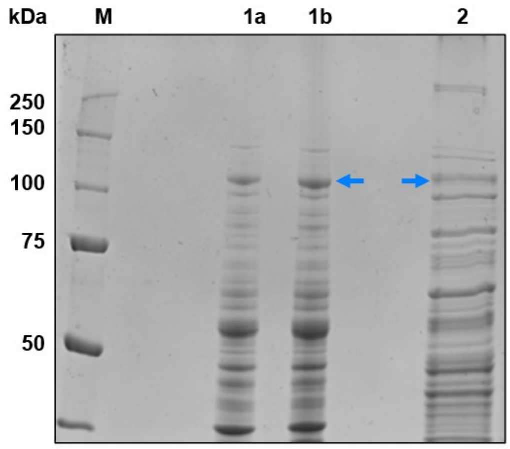
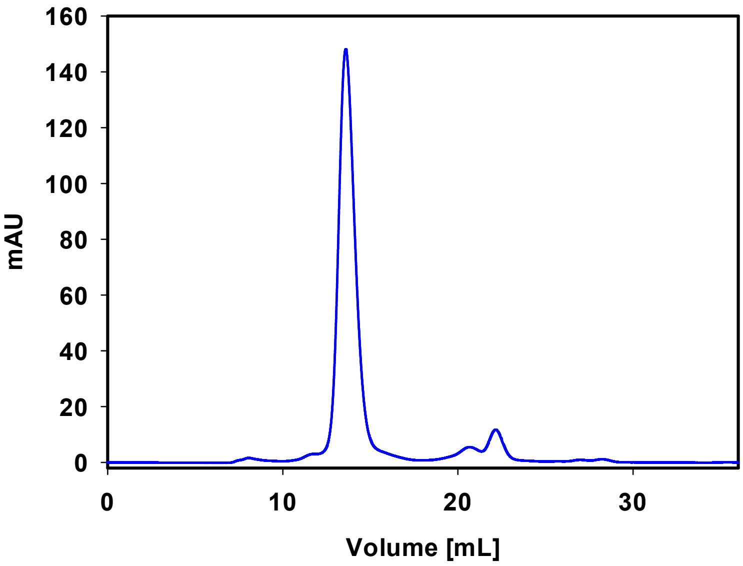


Figure S4: SDS PAGE of secretome (1) and intracellular proteome (2) of *Bs*AP2. The bands (blue arrow) corresponding to a size of 120 kDa were analyzed by MS. Supernatant and cell free extract after 73 h of shake flask cultivation were analyzed. The supernatant sample was loaded twice (1a and b). An amount of 8 % SDS gel was used and 8 µg of protein was loaded. M = Marker

Figure S5: MS analyses results of (A) intracellular and (B) extracellular *β*-gal-Pw (C) purified *β*-gal-Pw. The *β*-gal-Pw in the supernatant of the bioreactor cultivation of *Bs*AP was purified.

A



B

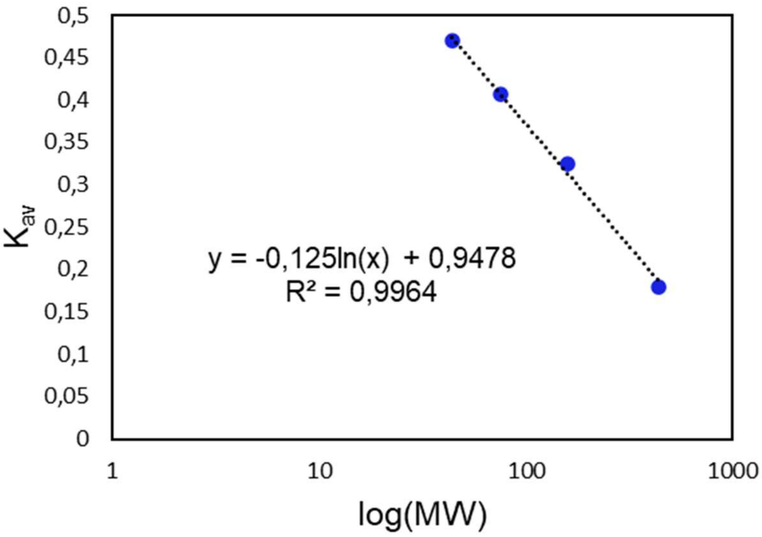


Figure S6: Chromatogram of size exclusion chromatography of purified *β*-gal-Pw (A) and the calibration curve generated (B). The *β*-gal-Pw was purified from the supernatant of the bioreactor cultivation of *Bs*AP2.

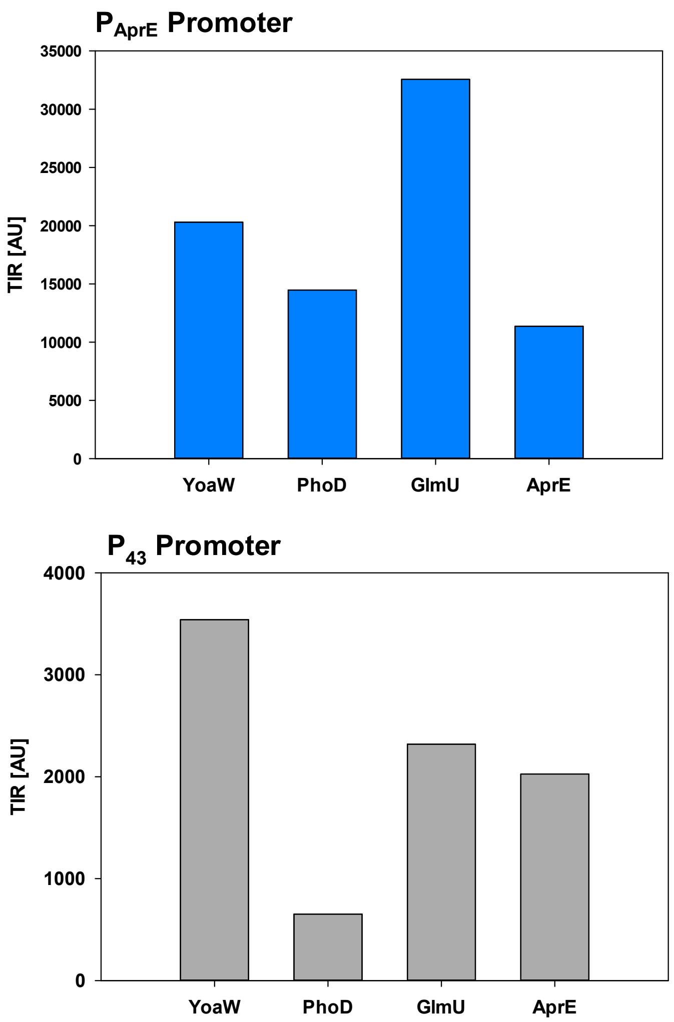
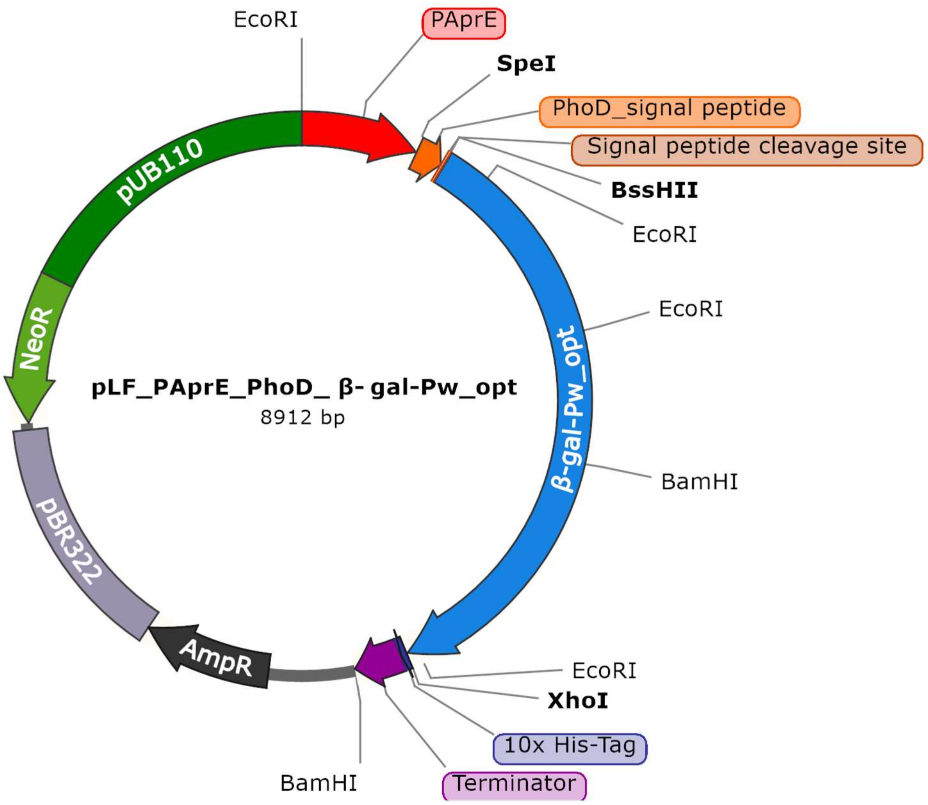


Figure S7: Translation initiation rates calculated with the RBS Calculator v2.1. for the different constructs. 100 nt upstream and 35 nt downstream of the start codon were considered. AU = Arbitrary units.

A



B

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Figure S8: Plasmid map (A) and DNA sequence (B) of plasmid pLF_P_{AprE}_PhoD_*β*-gal-Pw_opt.

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