Random and combinatorial mutagenesis for improved total production of secretory target protein in *Escherichia coli*

Supplementary material

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Table S1: Primers used in this study

Primer	Sequence (5' → 3')
NdeI-Tfu0937-F	GGAATTCCATATGACCAGCCAAAGCACCAC
EcoRI-Tfu0937-R	CCGGAATTCTCATTACTCTTGACCAAAAATACC
BamHI-Lipase6B-F	GATCGGATCCATGGCGGAGCACAACC
Lipase6B-EcoRI-R	GATCGAATTCTTAGTTCGTGTTCTGG
BamHI-RFP-F	ACGGGATCCGCGAGTAGCGAAGACG
EcoRI-RFP-R	ACCGGAATTCTTAAGCACCGGTGGAGTG
OsmY-DirEv-F	GTACCATATGACCATGACCCGTC
OsmY-DirEv-R	CCGCGGATCCGCTACCTTTGGTCTTC
OsmY-V191E-FP	GTTGACGGCGAGAAGAGCGTTAAAAACGATCTGAAGACCAAAGGTA
OsmY-V191E-RP	AACGCTCTTCTCGCCGTCAACCGCTTTCGCGATGCTCTCCGCACGA
OsmY-S154R-FP	ATCGTTCCGCGCCGTCACGTGAAGGTTGAAACCACCGACGGCGTGG
OsmY-S154R-RP	CACGTGACGGCGCGGAACGATATCGTCCGCCAGCAGTTTCGCCTTA
OsmY-L6P-FP	ATGACCCGTCCGAAGATTAGCAAAACCCTGCTGGCGGTGATGCTGA
OsmY-L6P-RP	GCTAATCTTCGGACGGGTCATGGTCATATGTATATCTCCTTCTTAA

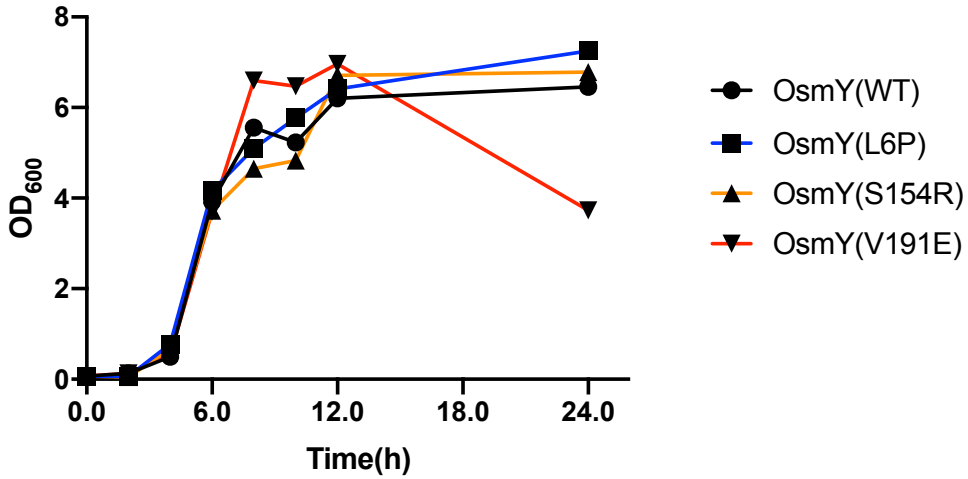


Figure S1: Growth curve of single site-directed mutants measured at 600 nm. Cells were cultivated in 20-mL flask cultures using 2×TY auto-induction media at 37 °C. Culture was inoculated using 1:100 dilution and 0.5 mL culture was sampled at various time points for cell density (OD_{600}) measurement. Wildtype and variants are represented using different colours; OsmY(WT) – black line, OsmY(L6P) – blue line, OsmY(S154R) – orange line and OsmY(V191E) – red line.

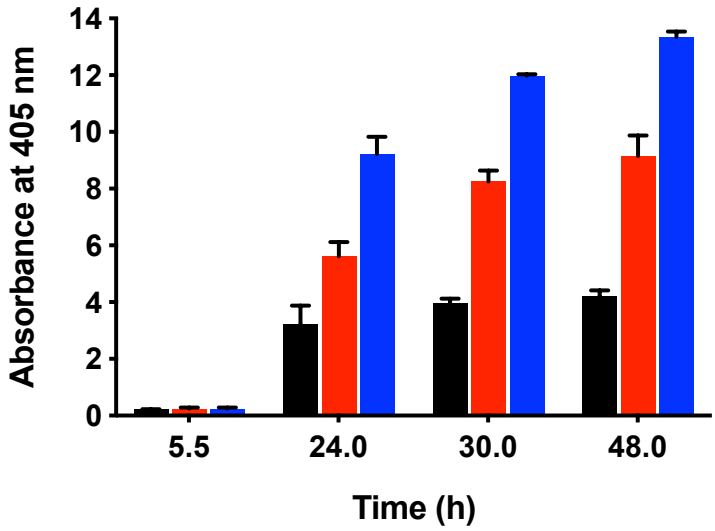


Figure S2: Characterization of improved variants in 50-mL flask cultures. The activity of extracellular OsmY-Tfu0937 was determined with the pNPG assay using an absorbance at 405 nm. Cultures were inoculated using 1:100 dilution. OsmY(WT) type and variants were expressed using C41(DE3) in 2TY auto-induction media and at 37 °C and 200 rpm. The activities of wildtype and variants are represented using different colours; OsmY(WT) (black), OsmY(TOA4) (red) and OsmY(M3) (blue). Experiments were performed in triplicates.

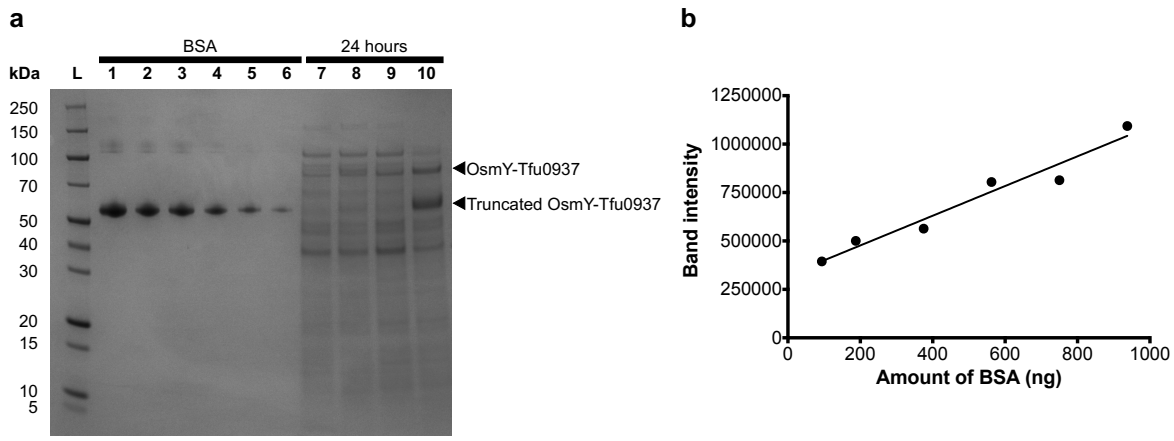
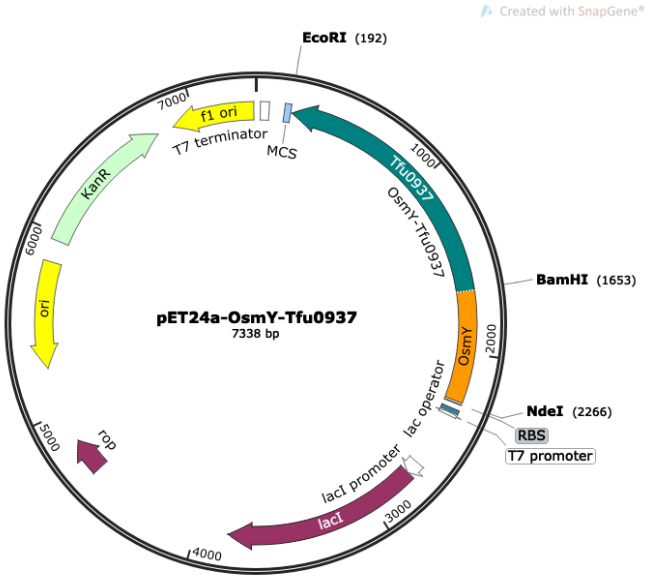


Figure S3: **(a)** SDS-PAGE of BSA standards and spent medium from 24 h cultivation. Lane L: protein marker, Lane 1: 250 µg/mL BSA, Lane 2: 200 µg/mL BSA, Lane 3: 150 µg/mL BSA, Lane 4: 100 µg/mL BSA, Lane 5: 50 µg/mL BSA, Lane 6: 25 µg/mL BSA, Lane7: OsmY(WT)-Tfu0937 in BL21(DE3), Lane 8: OsmY(M3)-Tfu0937 in BL21(DE3), Lane 9: OsmY(WT)-Tfu0937 in C41(DE3), Lane 10: OsmY(M3)-Tfu0937 in C41(DE3). **(b)** Calibration curve for protein quantification, obtained by densitometry of the BSA standards in (a) using ImageJ software.

a



b

MTMTRLKISKTLLAVMLTSAVATGSAYAENNAQTT
NESAGQKVDSSMNKVGNFMDDSAITAKVKAALVDH
DNIKSTDISVKTDQKVVTLSGFVESQAQAEEAVKV
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EIKAKLLADDIVPSRHVKVETTDGVVQLSGTVDSQ
AQSDRAESIAKAVDGVKSVKNDLKTKGSGSTSQST
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YRDDVALMRELGVGAYRFSIAWPRIQPEGKGTPVE
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AGGWPNRDTAKRFADYAEIVYRRLGDRITNWNTLN
EPWCSAFLGYASGVHAPGRQEPAAALAAAHHLMLG
HGLAAAVMRDLAGQAGRSVRIGVAHNQTTVRPYTD
SEADRDAARRIDALRNRIFTEPLVKGRYPEDLIED
VAAVTDYSFVQDGDLKTISANLDMMGVNFYNPSWV
SGNRENGGSDRLPDEGYSPSVGSEHVVEVDPGLPV
TAMGWPIDPTGLYDTLTRLANDYPGLPLYITENGA
AFEDKVVDGAVHDTERIAYLDSHLRAAHAAIEAGV
PLKGYFAWSFMDNFEWALGYGKRFGIVHVDYESQT
RTVKDSGWWYSRVMRNGGIFGQE**

Figure S4: **(a)** Plasmid map of pET24a-OsmY-Tfu0937. **(b)** Amino acid sequence of OsmY-Tfu0937. The OsmY wildtype sequence is shown in orange, Tfu0937 sequence in green, and GSGS linker sequence in black.