SUPPLEMENTARY INFORMATION

Acoustic Biosensors for Ultrasound Imaging of Enzyme Activity

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CONTENTS

Plasmid Name	Description and Purpose	Transcriptional regulators	Output gene product(s)	Insertions/Tags (including linkers)	Reference Infor-mation
WT C-His Ana GvpC in pET28a	Ana *gvpC* used as wild-type control for TEV and calpain sensor	pT7, LacO	WT C-His Ana GvpC	SLE-His6 at C-terminus	Addgene ID# 85732
WT N-His Ana GvpC in pET28a	Ana *gvpC* used as wild-type control for ClpXP sensor	pT7, LacO	WT N-His-Ana-GvpC	G-His6-SG at N-terminus	Addgene ID# 153294
C-His-GvpC-TEV	Ana *gvpC* with TEV cleavage site	pT7, LacO	C-His Ana GvpC with TEV cleavage site	SLE-His6 at C-terminus, GSGSGSG-ENLYFQG-SGSGSG in GvpC repeat 2	Addgene ID# 153296
C-His-GvpC-Calpain	Ana *gvpC* with calpain cleavage site	pT7, LacO	C-His Ana GvpC with calpain cleavage site	SLE-His6 at C-terminus, GSGSG-QQEVYGMMPRD-GSGSG in GvpC repeat 2	Addgene ID# 153295
N-His-GvpC-ssrA	Ana *gvpC* with ssrA degradation tag	pT7, LacO	N-His Ana GvpC with ssrA degradation tag	G-His6-SG at N-terminus, SG-AANDENYALAA at C-terminus	Addgene ID# 153297
pBEST_OR2_OR1-Pr-UTR1_ClpP-T500	*clpP* plasmid for use in the cell-free TX-TL system	OR2-OR1-Pr	ClpP		Addgene ID# 153302
pACYC-FLAG-dN6-His	*clpX* plasmid for use in the cell-free TX-TL system	pT7, LacO	Flag-ClpXdeltaNLinkedHexamer-His6	Flag tag at N-terminus His6 at C-terminus L20 linkers	Addgene ID# 22143
pET28a_T5-ARG1	Original acoustic reporter gene construct (*ARG*$_{wt}$)	pT5, LacO	Ana GvpA, WT Ana GvpC, Mega GvpR-U		Addgene ID # 106476

pET28a-T5-ASG_ClpXP	Acoustic sensor gene for ClpXP (*ASG*$_{ClpXP}$)	pT5, LacO	Ana GvpA, dGvpC, Mega GvpR-U	SG-AANDENYALAA at C-terminus	Addgene ID# 153299
pKD3	Frt-flanked cat cassette for recombineering		CAT		Addgene ID # 45604
pKD46	Plasmid that carries the Lambda Red recombineering system	pBAD, araBAD operon	Gam, Beta, Exo		Coli Genetic Stock Center
pE-FLP	*FLP* recombinase to remove the integration module in reombineering	pE	FLP		Addgene ID # 45978
araBAD-BCD20-ClpP-BCD17-ClpX	Expression of *clpX* and *clpP* under araBAD promoter	pBAD, araBAD operon	ClpX, ClpP		Addgene ID # 153301
pTetR-BCD2-Ana GvpC	Wild-type Ana *gvpC* under Tet promoter	pTet, TetO	WT Ana GvpC		Addgene ID # 153298

Supplementary Table 1: List and features of genetic constructs used in this study.

pKD3[1] was a gift from Barry L. Wanner (Addgene plasmid # 45604 ; http://n2t.net/addgene:45604 ; RRID:Addgene_45604). pKD46[1] was obtained from the Coli Genetic Stock Center (CGSC, https://cgsc.biology.yale.edu/Site.php?ID=64672).

pE-FLP[2] was a gift from Drew Endy & Keith Shearwin (Addgene plasmid # 45978;http://n2t.net/addgene:45978 ; RRID:Addgene_45978).

The pBEST_OR2-OR1-Pr-UTR1_ClpP-T500 was a gift from Zachary Sun and Richard Murray[3].

pACYC-FLAG-dN6-His[4] was a gift from Robert Sauer (Addgene plasmid # 22143 ; http://n2t.net/addgene:22143 ; RRID:Addgene_22143)

The pTARA backbone was modified to make the araBAD-BCD20-ClpP-BCD17-ClpX and pTEtR-BCD2-Ana GvpC constructs. pTARA[5] was a gift from Kathleen Matthews (Addgene plasmid # 31491; http://n2t.net/addgene:31491 ; RRID:Addgene_31491).

REFERENCES FOR SUPPLEMENTARY INFORMATION:

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3. Zachary, Z.S., Jongmin, K., Vipul, S. & Richard, M.M. Protein degradation in a TX-TL cell-free expression system using ClpXP protease. *bioRxiv* (2015).
4. Martin, A., Baker, T.A. & Sauer, R.T. Rebuilt AAA + motors reveal operating principles for ATP-fuelled machines. *Nature* **437**, 1115-1120 (2005).
5. Wycuff, D.R. & Matthews, K.S. Generation of an AraC-araBAD promoter-regulated T7 expression system. *Analytical Biochemistry* **277**, 67-73 (2000).