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Acoustic biosensors for ultrasound imaging of enzyme activity

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SUPPLEMENTARY INFORMATION

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Plasmid Name	Description and Purpose	Transcript ional regulators	Output gene product(s)	Insertions/Tags (including linkers)	Reference Infor- mation
WT C-His Ana GvpC in pET28a	Ana <i>gvpC</i> 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 <i>gvpC</i> 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 <i>gvpC</i> 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 <i>gvpC</i> with calpain cleavage site	pT7, LacO	C-His Ana GvpC with calpain cleavage site	SLE-His6 at C-terminus, GSGSG-QQEVYGMMPR D-GSGSG in GvpC repeat 2	Addgene ID# 153295
N-His-GvpC-ssrA	Ana <i>gvpC</i> 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	<i>clpP</i> plasmid for use in the cell-free TX-TL system	OR2-OR1-Pr	ClpP		Addgene ID# 153302
pACYC-FLAG-dN6-His	<i>clpX</i> 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 (<i>ARG_{WT}</i>)	pT5, LacO	Ana GvpA, WT Ana GvpC, Mega GvpR-U		Addgene ID # 106476

pET28a-T5-ASG_ClpXP	Acoustic sensor gene for ClpXP (<i>ASG_{ClpXP}</i>)	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	<i>FLP</i> recombinase to remove the integration module in recombineering	pE	FLP		Addgene ID # 45978
araBAD-BCD20-ClpP-BCD17-ClpX	Expression of <i>clpX</i> and <i>clpP</i> under araBAD promoter	pBAD, araBAD operon	ClpX, ClpP		Addgene ID # 153301
pTetR-BCD2-Ana GvpC	Wild-type Ana <i>gvpC</i> under Tet promoter	pTet, TetO	WT Ana GvpC		Addgene ID # 153298

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

pKD3¹ was a gift from Barry L. Wanner (Addgene plasmid # 45604 ; <http://n2t.net/addgene:45604> ; RRID:Addgene_45604). pKD46¹ was obtained from the Coli Genetic Stock Center (CGSC, <https://cgsc.biology.yale.edu/Site.php?ID=64672>).

pE-FLP² 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 Murray³.

pACYC-FLAG-dN6-His⁴ 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. pTARA⁵ was a gift from Kathleen Matthews (Addgene plasmid # 31491; <http://n2t.net/addgene:31491> ; RRID:Addgene_31491).

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