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Supporting Information

Development of a Whole-cell Biosensor for the Determination of Tyrosine in Urine for Point-of-care Diagnostics

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Table S1. Bacterial strains used in this study

Strain	Relevant genotype	Source
***E. coli* DH5α**	F⁻ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169 hsdR17(rK-mK+) λ⁻	Purchased from Protech Technology
YCY_737	*E. coli* DH5α *pLacUV5-tyrR-ParoF-rfp-PtyrP-GFP*	pYCY_737 transformed into *E. coli* DH5α
YCY_795	*E. coli* DH5α *P14-tyrR-ParoF-RFP-PtyrP-GFP*	pYCY_795 transformed into *E. coli* DH5α
YCY_815	*E. coli* DH5α *pTet-tyrR-ParoF-rfp-PtyrP-GFP*	pYCY_815 transformed into *E. coli* DH5α
YCY_851	*E. coli* DH5α *P14-tyrR-ParoF-RFP-PtyrP+3-GFP*	pYCY_851 transformed into *E. coli* DH5α
YCY_890	*E. coli* DH5α *P14-tyrR-ParoF-RFP-PtyrP+3-RBS30-GFP*	pYCY_890 transformed into *E. coli* DH5α
YCY_891	*E. coli* DH5α *P14-tyrR-ParoF-RFP-PtyrP+3-RBS34-GFP*	pYCY_891 transformed into *E. coli* DH5α
YCY_930	*E. coli* DH5α *P14-tyrR-ParoF-RBS30-RFP-PtyrP+3-RBS30-GFP*	pYCY_930 transformed into *E. coli* DH5α

Table S2. Plasmids used in this study

Plasmid	Relevant genotype	Source
pYCY_019	pBbE2k plasmid with *TetR(rev)-pTetR/A(rev)-RFP, Kan^R*	[1]
pYCY_020	pBbE5k plasmid with *LacI(rev)-pLacI(rev)-pLacUV5-RFP, Kan^R*	[1]
pYCY_658	pBbE5k plasmid with *ParoF-rfp, Kan^R*	PCR fragments of *ParoF* were amplified with primer 583 and 584, from *E. coli* DH5α as the template. The PCR products were digested with EcoRI and BamHI, and cloned into plasmid pYCY_020 with EcoRI and BglII.
pYCY_663	pBbE5k plasmid with *PtyrP-gfp, Kan^R*	PCR fragments of *PtyrP* were amplified with primer 585 and 586, from *E. coli* DH5α as the template. The PCR products were digested with EcoRI and BamHI, and cloned into plasmid pYCY_020 with EcoRI and BglII.
pYCY_681	pBbE5k plasmid with *pLacUV5-tyrR-ParoF-rfp, Kan^R*	PCR fragments of *tyrR* were amplified with primer 603 and 588, from *E. coli* DH5α as the template. The PCR products were digested with EcoRI and BamHI, and cloned into plasmid pYCY_658 with EcoRI and BglII.
pYCY_682	pBbE5k plasmid with *pLacUV5-tyrR-PtyrP-gfp, Kan^R*	PCR fragments of *tyrR* were amplified with primer 603 and 588, from *E. coli* DH5α as the template. The PCR products were digested with EcoRI and BamHI, and cloned into plasmid pYCY_663 with EcoRI and BglII.
pYCY_712	pBbE5k plasmid with *P14-RFP, Kan^R*	DNA fragments of P14 were annealed with primer 631 and 632,

		and cloned into plasmid pYCY_20 with AatII and EcoRI.
pYCY_734	pBbE5k plasmid with *P14-tyrR-ParoF-RFP, Kan^R*	DNA fragments of *tyrR-ParoF-RFP* from pYCY_681 were digested with EcoRI and XhoI, and subcloned into plasmid pYCY_712.
pYCY_737	pBbE5k plasmid with *pLacUV5-tyrR-ParoF-rfp-PtyrP-gfp, Kan^R*	PCR fragments of *PtyrP-GFP* were amplified with primer 637 and 639, from pYCY_663 as the template. The PCR products were digested with BglII and XhoI, and cloned into plasmid pYCY_681 with BamHI and XhoI.
pYCY_795	pBbE5k plasmid with *P14-tyrR-ParoF-RFP-PtyrP-GFP, KanR*	PCR fragments of *PtyrP-GFP* were amplified with primer 637 and 639, from pYCY_663 as the template. The PCR products were digested with BglII and XhoI, and cloned into plasmid pYCY_734 with BamHI and XhoI.
pYCY_815	pBbE2k plasmid with *pTet-tyrR-ParoF-rfp-PtyrP-gfp*	DNA fragments of *tyrR-ParoF-RFP-PtyrP-GFP* from pYCY_795 were digested with EcoRI and XhoI, and subclone into plasmid pYCY_19.
pYCY_851	pBbE5k plasmid with *P14-tyrR-ParoF-RFP-PtyrP+3-GFP, Kan^R*	Mutation of PtyrP+3 were mutated with primer 695 and 696, from pYCY_795 as the template.
pYCY_890	pBbE5k plasmid with *P14-tyrR-ParoF-RFP-PtyrP+3-RBS30-GFP*	DNA fragments of RBS30 were annealed with primer 715 and 716, and cloned into plasmid pYCY_851 with BglII and NdeI.
pYCY_891	pBbE5k plasmid with	DNA fragments of RBS34 were annealed with primer 717 and 718,

	P14-tyrR-ParoF-RFP-PtyrP+3-RBS34-GFP	and cloned into plasmid pYCY_851 with BglII and NdeI.
pYCY_930	pBbE5k plasmid with *P14-tyrR-ParoF-RBS30-RFP-PtyrP+3-RBS30-GFP*	Deletions RBS were used primer 763 and 764, and insertions DNA fragments of RBS30 with primer 772 and 773.

Table S3. Primers used in this study

Primer	Sequence (5'→3')
0583_EcoRI-BglII-ParoF_for	TTTTGAATTCttttAGATCTagggagtgtaaatttatctatacagaggtaag
0584_ParoF-BamHI_rev	TTTTGGATCCgatggcgatcctgtttatgctc
0585_EcoRI-BglII-PtyrP_for	TTTTGAATTCttttAGATCTgcctagcgtagcgattgcc
0586_PtyrP-BamHI_rev	TTTTGGATCCgctttcttctgtcctgacgatctt
0588_tyrR-ter-BamHI_rev	TTTTGGATCCaaaaaaaaccccgccctgtcaggggcggggttttttttttttactcttcgttcttcttctgactcag
0603_EcoRI-RBS-tyrR_for	TTTTGAATTCtttaagaaggagatatatttatgcgtctggaagtcttttgtga
0631_P14 promoter (AT)_for	CttgacaattaatcatccggctcgtataatgtgtggaG
0632_P14 promoter (AT)_rev	AATTCtccacacattatacgagccggatgattaattgtcaaGACGT
0637_BglII-term_for	TTTTAGATCTaaaaaaaaaccccgcccctga
0639_GFP-BamHI-XhoI_rev	TTTTCTCGAGtttGGATCCttacttgtacagctc
0695_PtyrP+3 (m)_F	TCGtatgtaacgtcggtttgac
0696_PtyrP+3 (m)_R	tggtgtaaatataaatgtacaataaaaag
0715_BglII-RBS30-NdeI 5'fw	GATCTtctagagATTAAAGAGGAGAAAtacCA

0716_BglII-RBS30-NdeI 3'rw	TATGgtaTTTCTCCTCTTTAATctctagaA
0717_BglII-RBS34-NdeI 5'fw	GATCTtctagagAAAGAGGAGAAAtacCA
0718_BglII-RBS34-NdeI 3'rw	TATGgtaTTTCTCCTCTTTctctagaA
0763_890 ParoF-dRBS-RFP F'w	CATATGGCGAGTAGCGAAG
0764_890 ParoF-dRBS-RFP R'w	AGATCCGATGGCGATCCT
772_ParoF-RBS30-RFP 5'fw	ttaatctctagaCATATGGCGAGTAGCGAAG
773_ParoF-RBS30-RFP 3'rw	ctctttaatcAGATCCGATGGCGATCCT

Table S4. TyrR protein levels and their induced fluorescence outputs.

in Tyr	P14	PLacUV5*	Ptet
GFP	1.440	1.056	0.980
RFP	0.522	0.056	0.174
in Phe	P14	PLacUV5*	Ptet
GFP	0.969	1.385	1.088
RFP	0.952	0.811	0.983

The fluorescence induction folds of GFP/RFP in the presence of Tyr and Phe at 100 μM when TyrR was driven by P14, PlacUV5, and Ptet promoters.

Table S5. Comparison of the current method with recent reported studies in Tyr detection.

Method	Linear range (μM)	LOD (μM)	Analysis time	Ref.
GC-MS	5.0-160	1.6	18 min (excluding pretreatment steps)	[2]
HPLC-UV	1.25-80	0.31	7 min (excluding pretreatment steps)	[3]
Electrochemical sensor	1-100	0.39	4 min	[4]
LC-MS	5.5-110	2.2	7 min (excluding pretreatment steps)	[5]
Cell-based	5-150	4.72	5 hr	This work

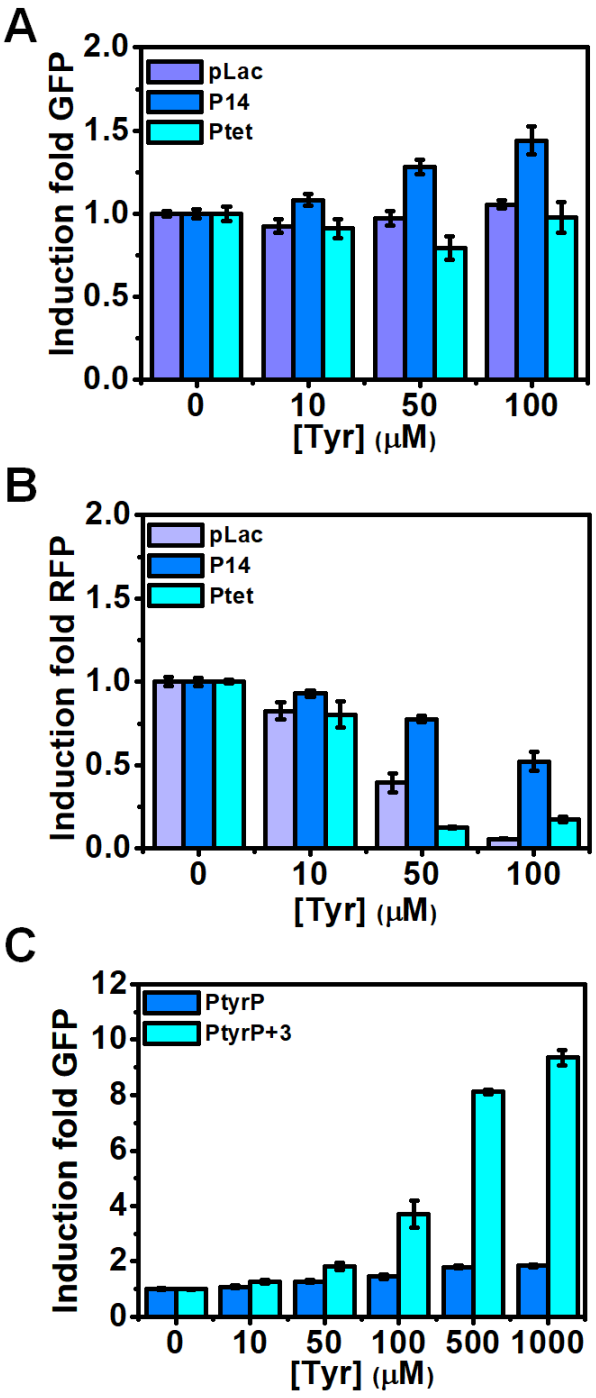

Figure S1 The fluorescence induction folds of (A) GFP and (B) RFP of plasmids carrying various promoters for TyrR expression. (C) The induction folds of GFP of cells carrying P14-TyrR-PtyrP/PtyrP+3-GFP plasmids.

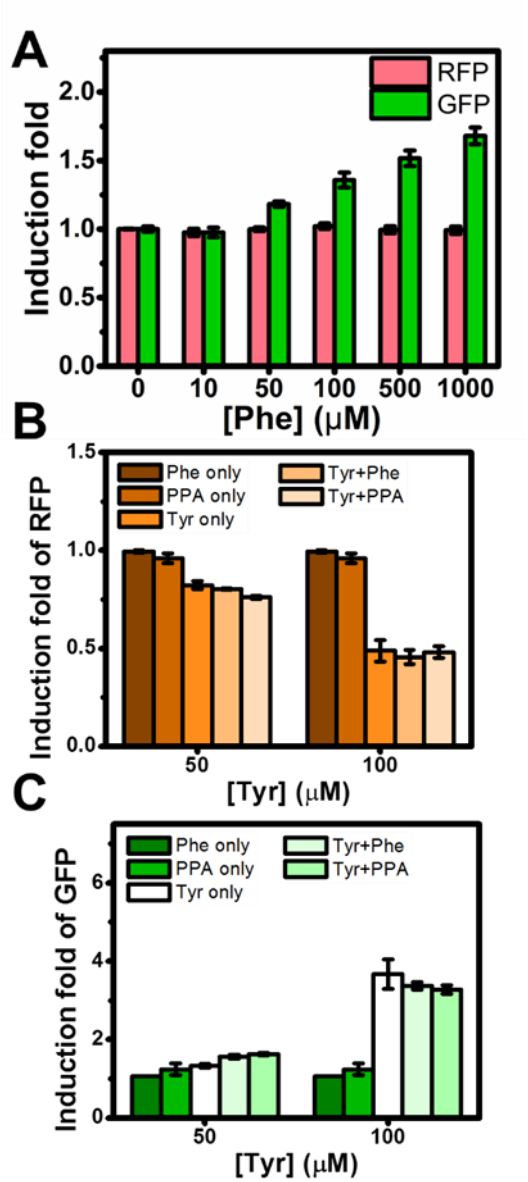

Figure S2. (A) Induction fold of sensor cells in the presence of Phe at various concentrations. (B) RFP and (C) GFP interference test of sensor cells in the co-existence of Phe and PPA.

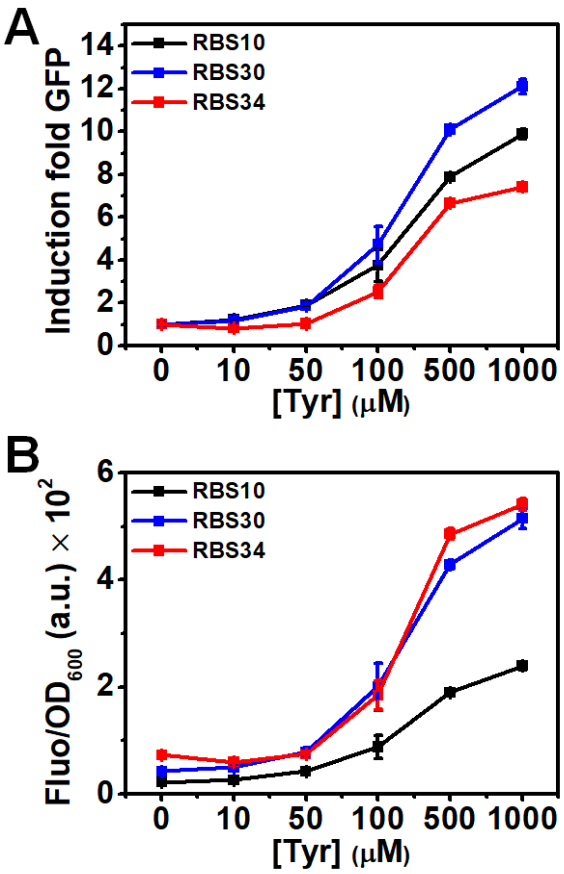

Figure S3. Comparison of (A) induction folds and (B) fluorescence intensities/OD_{600} of 24 h post-induction of various ribosome-binding sites.

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