

Supplementary file 1:

A) Linear DNAs used in this study.

Name	Description	Notes
pJ23119-tetO-BCD2-phlF-ssrA(LAA)	# 3n2, 5n1, 4n	
pLacI-BCD2-tetR-ssrA(LAA)	& 5n1, 4n	
pLambdaCI-BCD2-lacI-ssrA(LAA)	% 5n1	
pPhlF-BCD2-srpR-ssrA(LAA)	# 3n2, 5n1, 4n	
pSprR-BCD2-lambdaCI-ssrA(LAA)	# 5n1	
pSrpR-BCD2-tetR-ssrA(LAA)	# 3n2	
pPhlF-BCD2-srpR	# 3n2/no-ssrA	
pSrpR-BCD2-tetR	# 3n2/no-ssrA	
pTetR-BCD2-phlF	# 3n2/no-ssrA	
pSrpR-BCD2-lacI-ssrA(LAA)	# 4n	
pBetI-BCD7-QacR-ssrA(LAA)	# 5n2	
pPhlF-BCD7-srpR-ssrA(LAA)	# 5n2	
pQacR-BCD7-tetR-ssrA(LAA)	# 5n2	
PSrpR-BCD7-BetI-ssrA(LAA)	# 5n2	
pTetR-BCD7-phlF-ssrA(LAA)	# 5n2	
pJ23151-BCD7-betI	\$ transfer fxns	
pJ23151-BCD7-lacI	\$ transfer fxns	
pJ23151-BCD7-lambdaCI	\$ transfer fxns	
pJ23151-BCD7-phlF	\$ transfer fxns	
pJ23151-BCD7-qacR	\$ transfer fxns	
pJ23151-BCD7-srpR	\$ transfer fxns	
pJ23151-BCD7-tetR	\$ transfer fxns	
pLacI-BCD2-sfGFP-ssrA(LAA)	& test reporter	
pLambdaCI-BCD2-sfGFP-ssrA(LAA)	% test reporter	
pPhlF-BCD2-sfGFP-ssrA(LAA)	# test reporter	
pSrpR-BCD2-sfGFP-ssrA(LAA)	# test reporter	
pTetR-BCD2-sfGFP-ssrA(LAA)	# test reporter	

Promoter from Stanton et al. (2014)

\$ Promoter from Anderson promoter panel

% Promoter from Elowitz and Leibler (2000)

& Promoter from Lutz and Bujard (1997)

B) Plasmids used in this study.

Name	Description	Resistance	Copy number	Notes
pZS1	% Original repressilator plasmid	ampR	pSC101	
pZS1 w/ OR2* mutation	%	ampR	pSC101	minimize passages
pZE21-GFP(AAV)	% Original repressilator reporter (pTetO1)	kanR	colE1	
pZE21-eGFP(ASV)	% pZE21-GFPAAV with eGFP replacement	kanR	colE1	
pET21a(+)-Histag-Cerulean	Expression vector for Cerulean purification	ampR	colE1	c. Transcriptic Inc.
pET21a(+)-Histag-Citrine	Expression vector for Citrine purification	ampR	colE1	c. Transcriptic Inc.
pET21a(+)-Histag-mCherry	Expression vector for mCherry purification	ampR	colE1	c. Transcriptic Inc.
pTetR(r)-BCD7-Citrine	% transfer fxns	kanR	pSC101*	
pTetR-BCD7-Citrine	# transfer fxns	kanR	pSC101*	c. Transcriptic Inc.
pSrpR-BCD7-Citrine	# transfer fxns	kanR	pSC101*	c. Transcriptic Inc.
pQacR-BCD7-Citrine	# transfer fxns, in vitro reporter	kanR	pSC101*	c. Transcriptic Inc.
pPhlF-BCD7-Citrine	# transfer fxns, in vitro reporter	kanR	pSC101*	c. Transcriptic Inc.
pLacI-BCD7-Citrine	& transfer fxns	kanR	pSC101*	c. Transcriptic Inc.
pLacI(r)-BCD7-Citrine	% transfer fxns	kanR	pSC101*	
pCI(OR2*)-BCD7-Citrine	* transfer fxns	kanR	pSC101*	
pCI-BCD7-Citrine	% transfer fxns	kanR	pSC101*	
pBetI-BCD7-Citrine	# transfer fxns	kanR	pSC101*	c. Transcriptic Inc.
3n1	oscillator plasmid	kanR	pSC101*	minimize passages
3n2	oscillator plasmid	kanR	pSC101*	minimize passages
5n1	oscillator plasmid	kanR	pSC101*	minimize passages
5n2	oscillator plasmid	kanR	pSC101*	minimize passages
pBetI-BCD7-phlF-ssrA(LAA)	# for building 3n1	ampR	pSC101*	
pBetI-BCD7-qacR-ssrA(LAA)	# for building 5n2	ampR	pSC101*	
pLacO1-BCD7-tetR-ssrA(LAA)	& for building 5n1	ampR	colE1	amplify in lacI repressor strain
pLambdaCI-BCD7-lacI-ssrA(LAA)	% for building 5n1	ampR	colE1	amplify in lambdaCI repressor strain
pPhlF-BCD7-srpR-ssrA(LAA)	# for building 3n1, 3n2, 5n1, 5n2	ampR	pSC101*	
pQacR-BCD7-tetR-ssrA(LAA)	# for building 5n2	ampR	pSC101*	
pSrpR-BCD7-betI-ssrA(LAA)	# for building 3n1, 5n2	ampR	pSC101*	
pSrpR-BCD7-lambdaCI-ssrA(LAA)	# for building 5n1	ampR	pSC101*	
pSrpR-BCD7-tetR-ssrA(LAA)	# for building 3n2	ampR	pSC101*	
pTetR-BCD7-phlF-ssrA(LAA)	# for building 3n2, 5n1, 5n2	ampR	colE1	amplify in tetR repressor strain

pPhlF-BCD20-sfGFP-ssrA(LAA)	# 1 color strong reporter used in study	ampR	colE1	
pPhlF-BCD22-sfGFP-ssrA(LAA)	# 1 color weak reporter used in study	ampR	colE1	
pPhlF-BCD20-Citrine-ssrA(LAA)	# for building 3-color reporter plasmid	ampR	colE1	
pSrpR-BCD20-Cerulean-ssrA(LAA)	# for building 3-color reporter plasmid	ampR	colE1	
pTetR-BCD20-mCherry-ssrA(LAA)	# for building 3-color reporter plasmid	ampR	colE1	
3-color BCD20 reporter, pPhlF/pSrpR/pJ23119-tetO	3 color reporter plasmid	cmR	colE1	minimize passages
pTetR(r)-Citrine(ASV)	% in vitro reporter	kanR	colE1	
pTetR(r)-Cerulean(ASV)	% in vitro reporter	kanR	colE1	
pLacI(r)-mCherry(ASV)	% in vitro reporter	kanR	colE1	
pLacI(r)-Cerulean(ASV)	% in vitro reporter	kanR	colE1	
pCI-Citrine-(ASV)	% in vitro reporter	kanR	colE1	
pLacI(r)TetR(ASV)	% for initial conditions experiment	kanR	colE1	
pTetR(r)-CI(ASV)	% for initial conditions experiment	kanR	colE1	

- # Promoter from Stanton et al. (2014)
- \$ Promoter from Anderson promoter panel
- % Promoter from Elowitz and Leibler (2000)
- & Promoter from Lutz and Bujard (1997)
- * Promoter from Rosenfeld et al. (2005)

C) Strains used in this study.

Name	<i>E. coli</i> type	Resistance	Notes
Rosetta2	JS006	cmR	
pZS1 + pZE21-GFP(AAV)	JS006	kanR ampR	
pET21a(+)-Histag-Citrine	BL21-DE3	ampR	
pET21a(+)-Histag-Cerulean	BL21-DE3	ampR	
pET21a(+)-Histag-mCherry	BL21-DE3	ampR	
3n1 + pPhlF-BCD20-sfGFP-ssrA(LAA)	JS006	kanR ampR	minimize passages
3n1 + pPhlF-BCD22-sfGFP-ssrA(LAA)	JS006	kanR ampR	minimize passages
3n2 + pPhlF-BCD20-sfGFP-ssrA(LAA)	JS006	kanR ampR	minimize passages
3n2 + pPhlF-BCD22-sfGFP-ssrA(LAA)	JS006	kanR ampR	minimize passages
5n1 + pPhlF-BCD20-sfGFP-ssrA(LAA)	JS006	kanR ampR	cells unhealthy, minimize passages
5n1 + pPhlF-BCD22-sfGFP-ssrA(LAA)	JS006	kanR ampR	minimize passages
5n2 + pPhlF-BCD20-sfGFP-ssrA(LAA)	JS006	kanR ampR	minimize passages
5n2 + pPhlF-BCD22-sfGFP-ssrA(LAA)	JS006	kanR ampR	minimize passages
3n2 + 3-color BCD20 reporter, pPhlF/pSrpR/pJ23119-tetO	JS006	kanR cmR	minimize passages
pZS1 + pZE21-eGFP(ASV)	JS006	kanR ampR	
pZS1 w/ OR2* mutation + pZS21-eGFP(ASV)	JS006	kanR ampR	

D) DNA concentrations used in experiments.

Experiment	DNA and concentration	Type of DNA
Repressilator orig./O_R2*, 3color	Repressilator pZS1 or pZS1 w/ O _R 2* mutation, 0.5 nM (if not otherwise indicated)	Plasmid
	pTetR(r)-Cerulean(ASV), 5 nM	Plasmid
	pLacI(r)-mCherry(ASV), 5 nM	Plasmid
	pCI-Citrine(ASV), 5 nM	Plasmid
Repressilator, initial conditions	Reaction in nano-reactor:	
	Repressilator pZS1, 5 nM	Plasmid
	pLacI(r)-Cerulean(ASV), 5 nM	Plasmid
	pTetR(r)-Citrine-(ASV), 5 nM	Plasmid
	Pre-synthesis reaction (CI):	
	pTetR(r)-Citrine(ASV), 5 nM	Plasmid
	pTetR(r)-CI(ASV), 5 nM	Plasmid
	Pre-synthesis reaction (TetR):	
	pLacI(r)-Cerulean(ASV), 5 nM	Plasmid
	pLacI(r)TetR(ASV), 5 nM	Plasmid
Response curve measurements	Promoter plasmid: pXXX-BCD7-Citrine, 1 nM	Plasmid
	Repressor template: pJ23151-BCD7-XXX, 0-2.5 nM	Linear
	Repressor reporter: pJ23151-BCD7-Cerulean, 0-2.5 nM	Linear
3n1	3n1 oscillator plasmid, 5 nM	Plasmid
	pPhlF-BCD7-Citrine, 2.5 nM	Plasmid
3n2	pJ23119-tetO-BCD2-phlF-ssrA(LAA), 1.5 nM	Linear
	pPhlF-BCD2-srpR-ssrA(LAA), 12 nM	Linear
	pSrpR-BCD2-tetR-ssrA(LAA), 24 nM	Linear
	pTetR(r)-Cerulean(ASV), 5 nM	Plasmid
3n2/no-ssrA	pJ23119-tetO-BCD2-phlF, 1.5 nM	Linear
	pPhlF-BCD2-srpR, 12 nM	Linear
	pSrpR-BCD2-tetR, 24 nM	Linear
	pTetR(r)-Cerulean(ASV), 5 nM	Plasmid
4n	pJ23119-tetO-BCD2-phlF-ssrA(LAA), 0.75 nM	Linear
	pLacI-BCD2-tetR-ssrA(LAA), 6 nM	Linear

	pPhlF-BCD2-srpR-ssrA(LAA), 6 nM	Linear
	pSrpR-BCD2-lacI-ssrA(LAA), 12 nM	Linear
	pTetR(r)-Cerulean(ASV), 2.5 nM	Plasmid
	pLacI(r)-mCherry(ASV), 2.5 nM	Plasmid
	pPhlF-BCD7-Citrine, 2.5 nM	Plasmid
5n1	pJ23119-tetO-BCD2-phlF-ssrA(LAA), 1.1 nM	Linear
	pLacI-BCD2-tetR-ssrA(LAA), 16.8 nM	Linear
	pLambdaCI-BCD2-lacI-ssrA(LAA), 1.4 nM	Linear
	pPhlF-BCD2-srpR-ssrA(LAA), 5.6 nM	Linear
	pSrpR-BCD2-lambdaCI-ssrA(LAA), 11.2 nM	Linear
	pCI-Citrine(ASV), 3 nM	Plasmid
	pTetR(r)-Cerulean(ASV), 2.5 nM	Plasmid
5n1, plasmid DNA	5n1 oscillator plasmid, 5 nM	Plasmid
	pTetR(r)-Cerulean(ASV), 5 nM	Plasmid
	pLacI(r)-mCherry(ASV), 5 nM	Plasmid
	pCI-Citrine(ASV), 5 nM	Plasmid
5n2	pBetI-BCD7-QacR-ssrA(LAA), 1 nM	Linear
	pPhlF-BCD7-srpR-ssrA(LAA), 12 nM	Linear
	pQacR-BCD7-tetR-ssrA(LAA), 4 nM	Linear
	pSrpR-BCD7-BetI-ssrA(LAA), 24 nM	Linear
	pTetR-BCD7-phlF-ssrA(LAA), 4 nM	Linear
	pTetR(r)-Cerulean(ASV), 2.5 nM	Plasmid
	pQacR-BCD7-Citrine, 2.5 nM	Plasmid