A Sigma Factor Toolbox for Orthogonal Gene Expression in *Escherichia Coli*

Enabling Modular Control of Complex Heterologous Pathways

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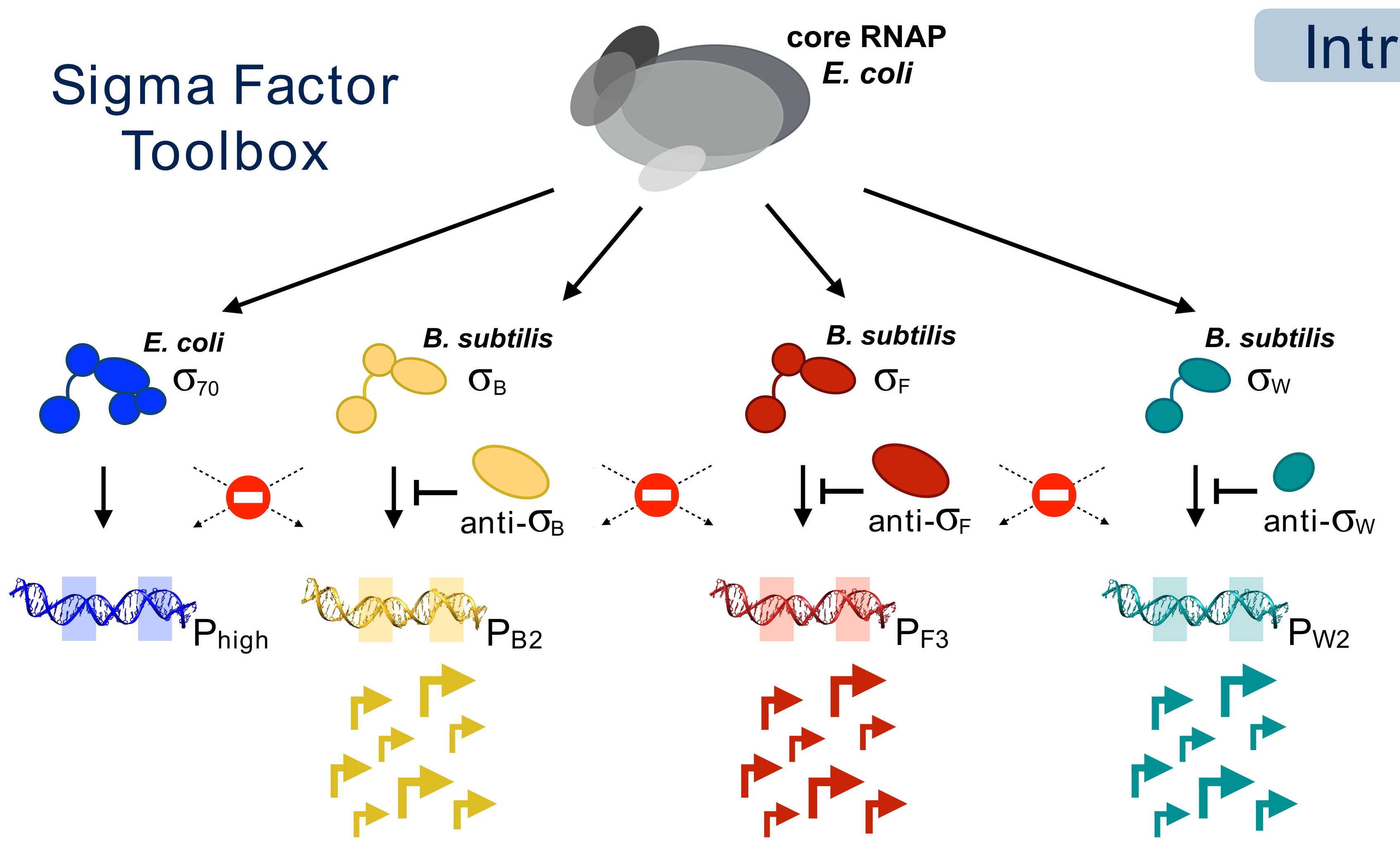
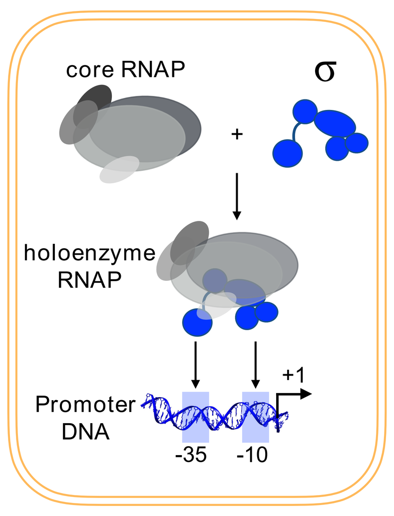
Sigma Factor Toolbox

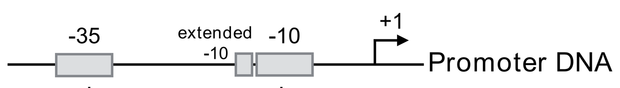


Introduction

Microorganisms as mini-factories can constitute an interesting **environmental friendly alternative** for the synthesis of various (bio)chemicals. **Synthetic biology**, that utilizes engineering principles to design **novel biological systems**, may contribute significantly to the transition to this bio-based approach. To construct controllable genetic networks, **well-defined parts** are required that enable **independant and tunable gene expression**. This work contributes to the expansion of the synthetic biology toolbox for the model organism *E. coli* by creating **orthogonal expression systems based on heterologous sigma (σ) factors**. Furthermore, specific inhibition of **σ** factor activity is obtained by introducing the corresponding anti-**σ** factor. Finally, **orthogonal promoter libraries** were generated for three **σ** factors to enable gene expression **fine-tuning for multiple expression modules**.

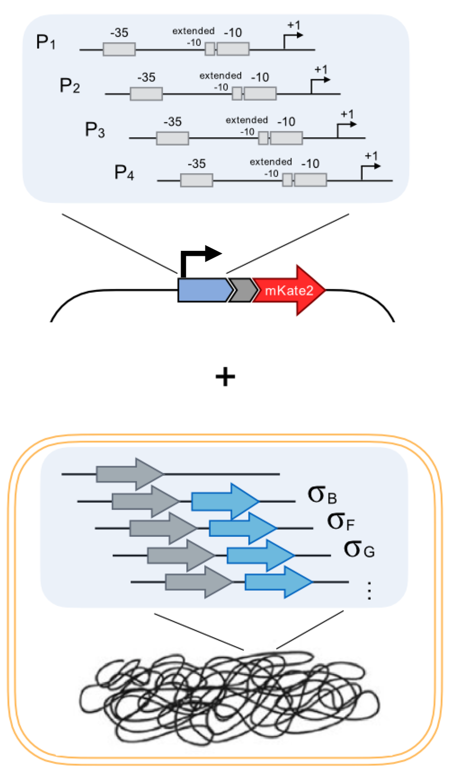
Set of orthogonal sigma factors





Species	σ-factor	Consensus	Function
E. coli	σ^{70}	TTGACA - 17nt - TATAAT	Housekeeping genes
	σ^{S}	TTGACA - 17nt - TATACT	Stationary phase
B. subtilis	σ^{A}	TTGACA - 17nt - TATAAT	Housekeeping genes
GROUP 3	$\sigma^{B}(/\sigma^{C})$	GTTTaa - 13-15nt - GGGWAW	General stress response
GROUP 3	σ^{F}	NGTATWWN - 14nt - GKNNANNNTW	Early forespore gene expression
GROUP 3	σ^{G}	GHATA - 17-18nt - MAWAMTA	Late forespore gene expression
GROUP 4	σ^{M}	tgaAAC - 16-17nt - CGTC	Extracytoplasmic Function
GROUP 4	σ^{W}	tgaAAC - 16-17nt - CGTM	Extracytoplasmic Function
GROUP 4	σ^{X}	tgtaACtttt - 12-13nt - CGWC	Extracytoplasmic Function

Bacteria possess a **single RNA polymerase** (RNAP) that consists of a core to which **different σ factors** may bind. The **σ** factor directly binds the promoter to **selectively initiate transcription**. A bacterium generally has several alternative sigma factors that function as global regulators of gene expression. Some **σ** factors do recognize each other's promoters but others are **very specific** and show **no cross-talk**. They are **orthogonal**.



Heterologous sigma factor

Promoters	/	B	B S201R R240H	F	G	M A130V	M E73K	W	W L94F	X T152P
P_{no}	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
P_{low}	0.18	0.18	0.18	0.15	0.15	0.18	0.14	0.17	0.21	0.17
P_{mid}	0.31	0.32	0.30	0.26	0.29	0.32	0.26	0.29	0.38	0.27
P_{high}	1.00	1.03	0.96	0.85	0.94	1.03	0.83	0.98	1.11	0.89
P_{B1}	0.01	0.88	0.35	0.54	0.02	0.00	0.00	0.00	0.01	0.01
P_{B2}	0.01	0.21	0.15	0.01	0.01	0.01	0.00	0.00	0.00	0.01
P_{B3}	0.01	0.04	0.01	0.07	0.04	0.01	0.01	0.01	0.01	0.01
P_{F1}	0.02	0.04	0.01	1.85	0.73	0.02	0.01	0.01	0.01	0.02
P_{F2}	0.07	0.04	0.03	1.91	0.76	0.04	0.03	0.02	0.03	0.04
P_{F3}	0.02	0.01	0.01	0.51	0.27	0.07	0.05	0.01	0.01	0.01
P_{G1}	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.00
P_{G2}	0.01	0.00	0.00	0.01	0.16	0.00	0.00	0.00	0.00	0.00
P_{G3}	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00
P_{M1}	0.00	0.00	-0.01	0.00	0.00	0.88	0.30	0.11	0.06	0.21
P_{M2}	0.20	0.20	0.20	0.17	0.17	0.13	0.18	0.21	0.19	0.22
P_{M3}	0.01	0.00	0.00	0.01	0.01	0.87	0.38	0.08	0.11	0.06
P_{M4}	0.07	0.06	0.02	0.02	0.03	1.66	0.68	0.02	0.18	0.17
P_{W1}	0.02	0.02	0.01	0.00	0.01	0.04	0.04	0.30	0.38	0.02
P_{W2}	0.04	0.01	0.01	0.01	0.01	1.18	0.51	0.39	0.43	0.01
P_{W3}	0.03	0.05	0.05	0.05	0.04	0.15	0.13	0.48	0.48	0.05
P_{W4}	0.00	0.00	0.00	0.00	0.01	0.14	0.02	0.24	0.21	0.00
P_{X1}	0.02	0.01	0.02	0.01	0.02	1.78	0.57	0.01	0.01	0.27
P_{X2}	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.07	0.00	0.09
P_{X3}	0.38	0.35	0.31	0.29	0.28	1.68	0.75	0.22	0.28	0.44

normalized FU/OD (0–2)

Six **σ factors from *Bacillus subtilis*** together with naturally occuring cognate promoters were **analyzed for orthogonality in *E. coli***. Most heterologous **σ** factors function orthogonally towards the host and each other. Up to four of the **σ** factors assayed can be combined to control different genes independently without any cross-talk.

Repression by anti-sigma factor

Heterologous sigma factors

	B	B S201R R240H	F	M A130V	M E73K	W	W L94F	X T152P
Anti-B	21.14	4.45	2.05				0.87	0.94
Anti-F	1.10	1.37	32.80				0.64	1.39
Anti-M	0.91	1.04	0.99	0.94	1.67		0.63	1.27
Anti-W	1.02	1.18	0.91			9.07	4.47	1.11
Anti-X	1.05	1.20	1.02				0.93	3.96

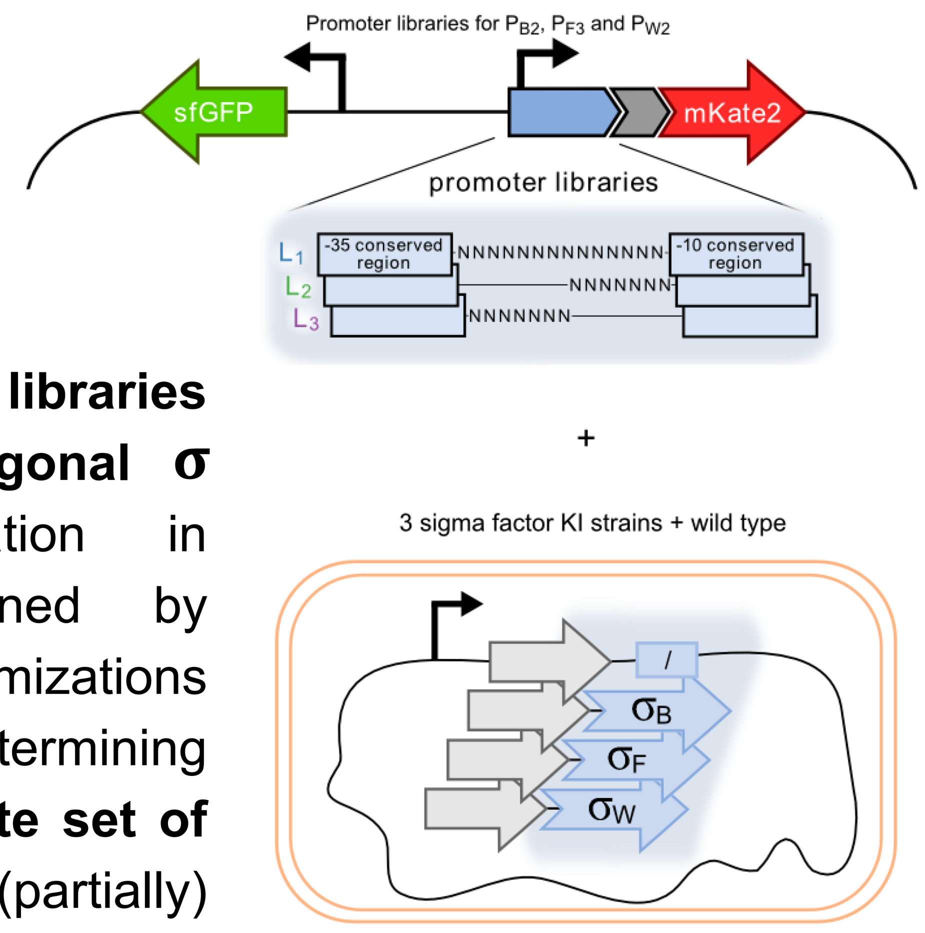
Fold repression (0–35)

Orthogonality in the functioning of the anti-**σ** factors is shown as **activity of the factors in absence/presence of the anti-σ**. **σ** factor B, F and W activity can be fully repressed while in the conditions tested, other **σ** factors only show partial repression.

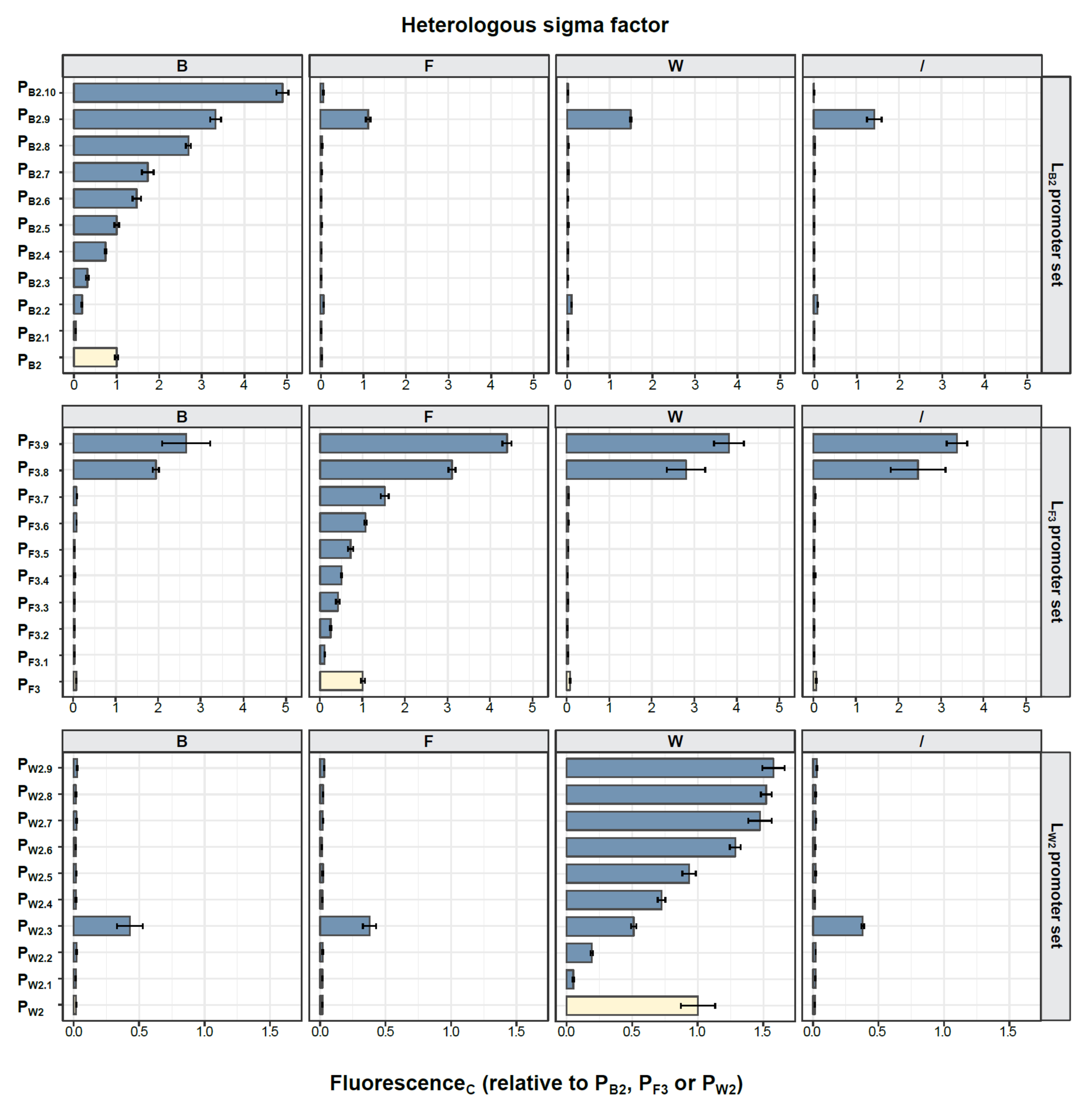
Orthogonal promoter libraries

Heterologous sigma factor

Promoter	/	B	F	W
P_{B2}	0.01	0.21	0.01	0.00
P_{F3}	0.02	0.01	0.51	0.01
P_{W2}	0.04	0.01	0.01	0.39



To enable **tunability**, **promoter libraries** were built for three fully **orthogonal σ factor-promoter pairs**. Variation in expression levels was obtained by introducing DNA sequence randomizations in between the **σ**-specificity determining boxes. After cell sorting, a **discrete set of promoters** was selected, (partially) **retaining the orthogonal features**.



Heterologous sigma factor

Promoters	B	F	W
$P_{B2.10}$	534.89	7.00	2.35
$P_{B2.9}$	2.33	0.79	1.05
$P_{B2.8}$	93.01	1.33	1.09
$P_{B2.7}$	72.92	1.12	1.40
$P_{B2.6}$	89.78	1.42	1.01
$P_{B2.5}$	51.37	1.30	1.44
$P_{B2.4}$	48.43	1.60	1.50
$P_{B2.3}$	22.58	1.36	1.21
$P_{B2.2}$	2.04	0.76	1.15
$P_{B2.1}$	2.18	1.03	1.22
P_{B2}	50.84	1.57	1.31
$P_{F3.9}$	0.79	1.30	1.13
$P_{F3.8}$	0.79	1.26	1.14
$P_{F3.7}$	1.66	35.09	1.05
$P_{F3.6}$	2.05	31.82	1.19
$P_{F3.5}$	1.12	28.83	1.44
$P_{F3.4}$	1.09	17.65	0.96
$P_{F3.3}$	1.29	20.61	1.44
$P_{F3.2}$	1.42	13.78	1.07
$P_{F3.1}$	1.03	4.57	1.40
P_{F3}	0.97	14.94	1.14
$P_{W2.9}$	0.89	1.07	55.65
$P_{W2.8}$	0.89	1.12	74.95
$P_{W2.7}$	1.07	1.01	70.63
$P_{W2.6}$	0.97	0.93	80.28
$P_{W2.5}$	0.90	1.06	45.98
$P_{W2.4}$	1.23	1.30	51.24
$P_{W2.3}$	1.13	0.99	1.34
$P_{W2.2}$	1.05	0.91	9.05
$P_{W2.1}$	0.86	0.89	2.68
P_{W2}	1.20	1.21	65.65

Activation ratio (0, 1, 25, 50, 75, 100, 600)

The Sigma Factor Toolbox :

- Orthogonal **σ** factors to independently control gene expression
- Orthogonal anti-**σ** factors for specific repression
- Promoter libraries for fine-tuning

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