

**Title:****Testing the functionality of SIGEX duo-directional reporter plasmid pSPPH21 using an inducible promoter**

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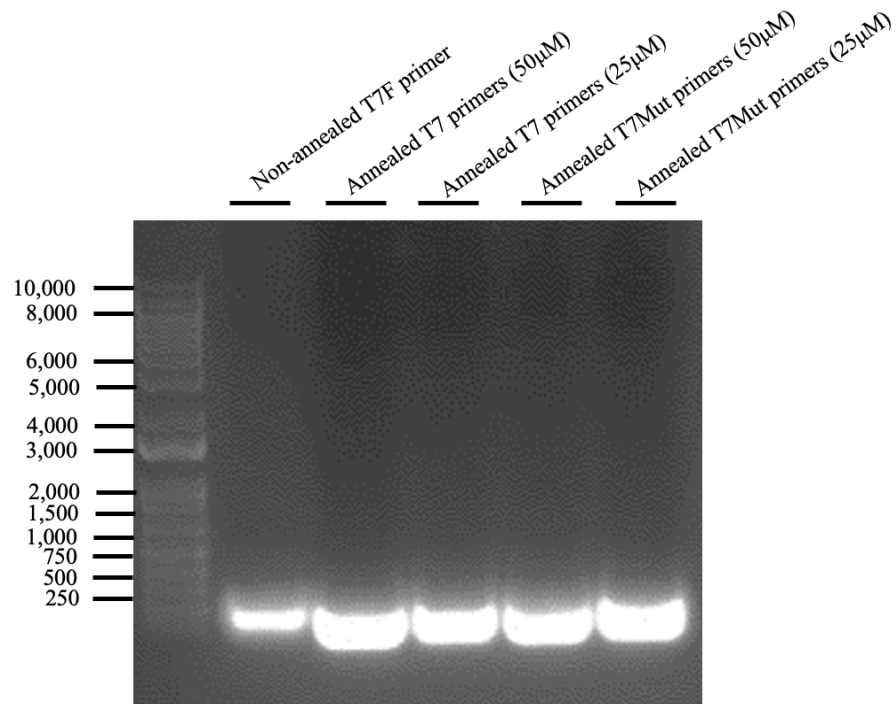
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**Supplemental:****Table S1.** Glossary of promoter, plasmid, and colony abbreviations.

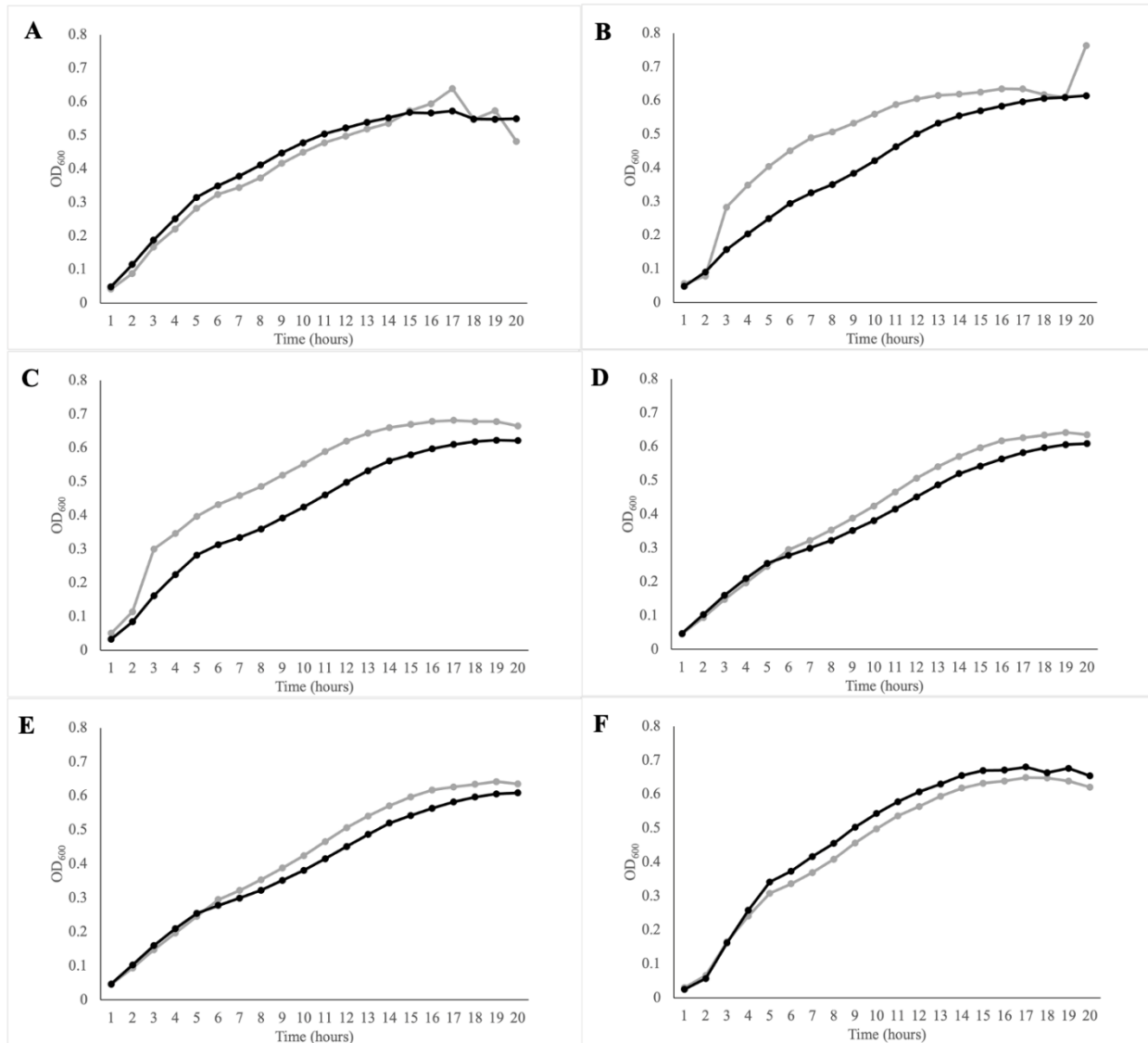
<b>Abbreviation</b>	<b>Sample Type</b>	<b>Description</b>
T7	Promoter	T7 inducible promoter insert
T7Mut	Promoter	T7Mut constitutively active promoter insert
pSPPH21	Plasmid	Promoterless pSPPH21 vector constructed by Abrishamkar et al. featuring fluorescence reporter genes, GFP and RFP, flanking the NruI site.
pT7	Plasmid	Recombinant pSPPH21 vector with the T7 inducible promoter blunt-end ligated into the NruI site.
pT7Mut	Plasmid	Recombinant pSPPH21 vector with the T7Mut inducible promoter blunt-end ligated into the NruI site.
pT7# DH5 $\alpha$ colony	Colony	Resulting DH5 $\alpha$ transformant colony obtained from ligating the T7 inducible promoter insert into the pSPPH21 vector.
pT7Mut# DH5 $\alpha$ colony	Colony	Resulting DH5 $\alpha$ transformant colony obtained from ligating the T7Mut inducible promoter insert into the pSPPH21 vector.
pT7# BL21(DE3) colony #	Colony	Resulting BL21(DE3) transformant colony obtained from the pT7 vector isolated from a certain pT7# DH5 $\alpha$ colony.
pT7Mut# BL21(DE3) colony #	Colony	Resulting BL21(DE3) transformant colony obtained from the pT7Mut vector isolated from a certain pT7Mut# DH5 $\alpha$ colony.

**Table S2.** Sequences of primers used throughout the project. All primers were ordered from IDT. Lac promoter sequences were obtained from pET28a(+) plasmid map on SnapGene.

<b>Purpose</b>	<b>Sequences</b>
Creating inducible promoter	Forward (T7F): (5'- TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCAA GCTT-3')
	Reverse (T7R): (5'- AAGCTTGGAATTGTTATCCGCTCACAATTCCCCTATAGTGAGTCGTA TTA-3')
Creating constitutive promoter	Forward Mutant (T7MutF): (5'- TAATACGACTCACTATAGGGGAATTTGAAGCGGATAACAATTCCAA GCTT-3')
	Reverse Mutant (T7MutR): (5'- AAGCTTGGAATTGTTATCCGCTTCAAATTCCCCTATAGTGAGTCGTAT TA-3')
Sequencing clones	Forward (pSPPH21F): (5' - GGCGTATCACGAGGCAGAATTTC - 3')
	Reverse (pSPPH21R): (5' - GGAAGCCTGCATAACGCGAAG - 3')
	Forward (T7FP): (5' -ATTTCGAACTCGTGACCGTT- 3')
	Reverse (T7RP): (5' -ACTGACAGAAAATTTGTGCC- 3')



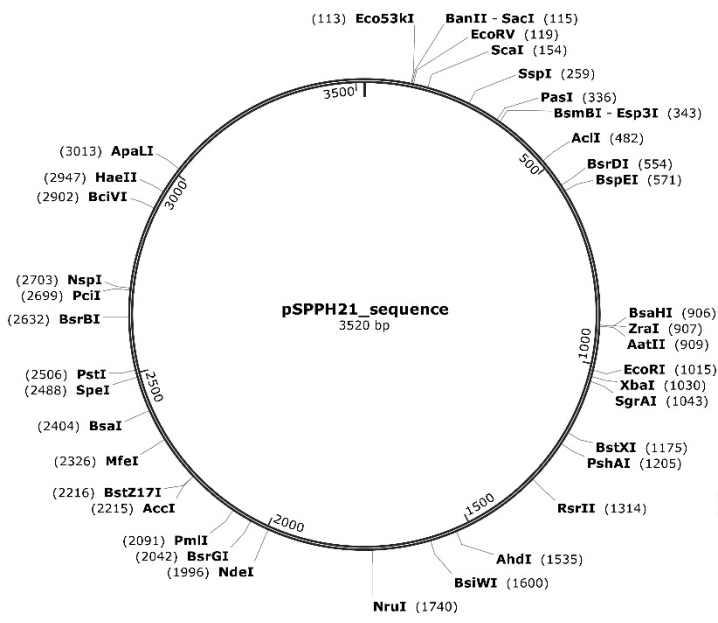
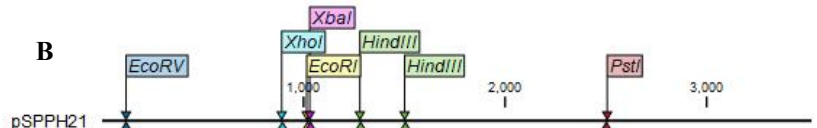
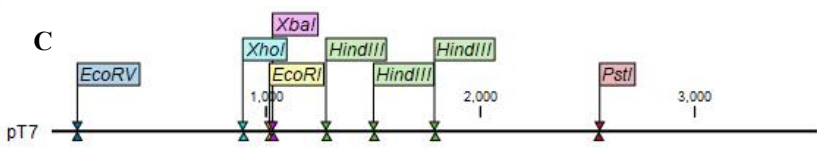
**Figure S1.** Gel electrophoresis of pre-annealed and annealed T7 and T7Mut primers. Custom primers were purchased from IDT and rehydrated in a duplex buffer to a concentration of 100µM. Forward and reverse primer pairs were annealed in a 1:1 ratio to final concentrations of 25µM and 50µM. The mini gel was 0.8% agarose with SYBR Red (ThermoFisher) and run at approximately 100V for 30 minutes before imaging using GelDoc UV imaging system (BioRad).



**Figure S2.** Growth curves of five pT7 (A-E) and one pT7Mut (F) BL21 transformants. Bacterial growth was monitored with a BioTek microplate reader measuring OD<sub>600</sub> at one hour intervals over a 20 hour period. OD<sub>600</sub> measurements were blanked using LB broth. Gray lines represent 0.5mM IPTG induced samples, while black lines represent uninduced controls (n=2).

A		
pT71-4_BL21_For	ATGANTCCTTTGATAACGCTCTTCGGAGGAAGCCATTACTAGAGTTTCTCCTCTTTAATTCG	84
pT7_For_reference	tgaactcctttgataaacgctcttcggaggaagccattactagagtttctcctcctttaatTCG	1740
pT71-2_BL21_For	ATGANTCCTTTGATAACGCTCTTCGGAGGAAGCCATTACTAGAGTTTCTCCTCTTTAATTC	82
pT71-3_BL21_For	TGAACTCCTTTGATAACGCTCTTCGGAGGAAGCCATTACTAGAGTTTCTCCTCTTTAATTCG	81
	* *****	
pT71-4_BL21_For	<b>AAGCTT</b> ----- <b>GGAATGTTATCGGATAACAATCCAAGCTT</b> CGATACTAGA	131
pT7_For_reference	<b>TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATCCAAGCTT</b> CGAattaa--	1798
pT71-2_BL21_For	<b>AAGCTT</b> CGATAC-----TAGAGT--	100
pT71-3_BL21_For	<b>AAGCTT</b> CGATAC-----TAGAGT--	99
	*	
pT71-4_BL21_For	GTCACACAGGAAAGTACTAGATGCGTAAAGGAGAAGAAGCTTTTCACTGGAGTTGTCCCAA	191
pT7_For_reference	--agaggagaaaTACTAGAGatgcgtaaaggagaagaacttttcaactggagttgtcccaa	1856
pT71-2_BL21_For	--CACACAGGAAAGTACTAGATGCGTAAAGGAGAAGAAGCTTTTCACTGGAGTTGTCCCAA	158
pT71-3_BL21_For	--CACACAGGAAAGTACTAGATGCGTAAAGGAGAAGAAGCTTTTCACTGGAGTTGTCCCAA	157
	** ** *****	
B		
pT71-4_BL21_Rev	TCTCCTTTACGCATCTAGTACTTTCCTGTGTGACTCTAGTATCG <b>AAGCTTGGAAATTGTTA</b>	104
pT7_Rev_reference	tctcctttacgcatCTTAGTAtttctc---ctctttaatTCG <b>AAGCTTGGAAATTGTTA</b>	1796
pT71-2_BL21_Rev	TCTCCTTTACGCATCTAGTACTTTC-----	68
pT71-3_BL21_Rev	TCTCCTTTACGCATCTAGTACTTTC-----	75
	***** **	
pT71-4_BL21_Rev	<b>TCCGATAACAATTC</b> ----- <b>AAGCTT</b> <b>TA</b> ATTAAAGAGGAGAACTCTAGTA	151
pT7_Rev_reference	<b>TCCGCTCACAATTC</b> CCCTATAGTGAGTCGTATTA <b>CGA</b> attaaagaggagaaactctagta	1856
pT71-2_BL21_Rev	-----CTGTGTGACTCTAGTATCG <b>AAGCTT</b> <b>GGA</b> ATTAAAGAGGAGAACTCTAGTA	119
pT71-3_BL21_Rev	-----CTGTGTGACTCTAGTATCG <b>AAGCTT</b> <b>CGA</b> ATTAAAGAGGAGAACTCTAGTA	126
	* *****	
pT71-4_BL21_Rev	ATGGCTTCTCCGAAGACGTTATCAAAGAGTTCATGCGTTTCAAAGTTCGTATGGAAGGT	211
pT7_Rev_reference	atggcttctccgaagacgttatcaaagagttcatgcgtttcaaagttcgtatggaaggt	1916
pT71-2_BL21_Rev	ATGGCTTCTCCGAAGACGTTATCAAAGAGTTCATGCGTTTCAAAGTTCGTATGGAAGGT	179
pT71-3_BL21_Rev	ATGGCTTCTCCGAAGACGTTATCAAAGAGTTCATGCGTTTCAAAGTTCGTATGGAAGGT	186
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**Figure S3.** Sequencing results of the isolated pT71 BL21 colonies 2-4, aligned with the pT7 *in silico* reference sequence using the Clustal Omega tool, in the forward (A) and reverse (B) orientation. The pT7 *in silico* reference sequence contains the pSPPH21 backbone vector with the T7 inducible promoter inserted in a 5' → 3' orientation flanked by the NruI sites. The underlined letters indicate the NruI site. The inducible promoter sequence in the forward orientation (green bolded) and reverse complementary orientation (blue bolded) are indicated. Potential errors in base calling are indicated in red bolded letters, due to the bases possibly representing the NruI site bases instead. The \* indicates a match between the bases, while a lack of this indicates a mismatch between the bases. The horizontal lines indicate gaps in the sequence alignment analysis. The letter 'N' represents regions where the bases could not be deciphered correctly during Sanger sequencing.

**A****B****C**

**Figure S4.** Restriction enzyme sites map of the pSPPH21 plasmid demonstrated by SnapGene

(A) and CLC Genomics (B). Restriction enzyme sites map of the pT7 *in silico* plasmid with CLC Genomics (C), indicating one additional HindIII site (green box) from the T7 promoter insert at 1785 bp.