**Title:**

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

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**Supplemental:**

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

| Abbreviation | Sample Type | Description |
|---|---|---|
| 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# DH5α colony | Colony | Resulting DH5α transformant colony obtained from ligating the T7 inducible promoter insert into the pSPPH21 vector. |
| pT7Mut# DH5α colony | Colony | Resulting DH5α 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# DH5α colony. |
| pT7Mut# BL21(DE3) colony # | Colony | Resulting BL21(DE3) transformant colony obtained from the pT7Mut vector isolated from a certain pT7Mut# DH5α 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.

| **Purpose** | **Sequences** |
|---|---|
| Creating inducible promoter | Forward (T7F): (5'-TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCAAGCTT-3')<br><br>Reverse (T7R):<br>(5'-AAGCTTGGAATTGTTATCCGCTCACAATTCCCCTATAGTGAGTCGTATTA-3') |
| Creating constitutive promoter | Forward Mutant (T7MutF):<br>(5'-TAATACGACTCACTATAGGGGAATTTGAAGCGGATAACAATTCCAAGCTT-3')<br><br>Reverse Mutant (T7MutR):<br>(5'-AAGCTTGGAATTGTTATCCGCTTCAAATTCCCCTATAGTGAGTCGTATTA-3') |
| Sequencing clones | Forward (pSPPH21F):<br>(5'- GGCGTATCACGAGGCAGAATTTC - 3')<br><br>Reverse (pSPPH21R):<br>(5'- GGAAGCCTGCATAACGCGAAG - 3')<br><br>Forward (T7FP):<br>(5' -ATTTCGAACTCGTGACCGTT- 3')<br><br>Reverse (T7RP):<br>(5' -ACTGACAGAAAATTTGTGCC- 3') |

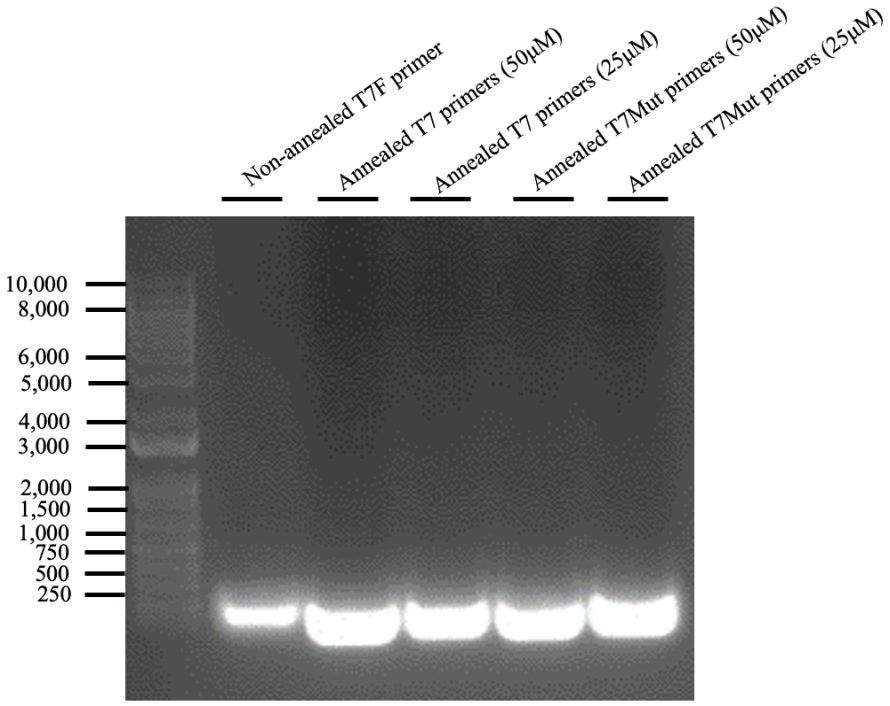


**Figure S1.** Gel electrophoresis of pre-annealed and annealed T7 and T7Mut primers. Custom primers were purchased from IDT and rehydrated in a duplex buffer to a concentration of 100µM. Forward and reverse primer pairs were annealed in a 1:1 ratio to final concentrations of 25µM and 50µM. The mini 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).

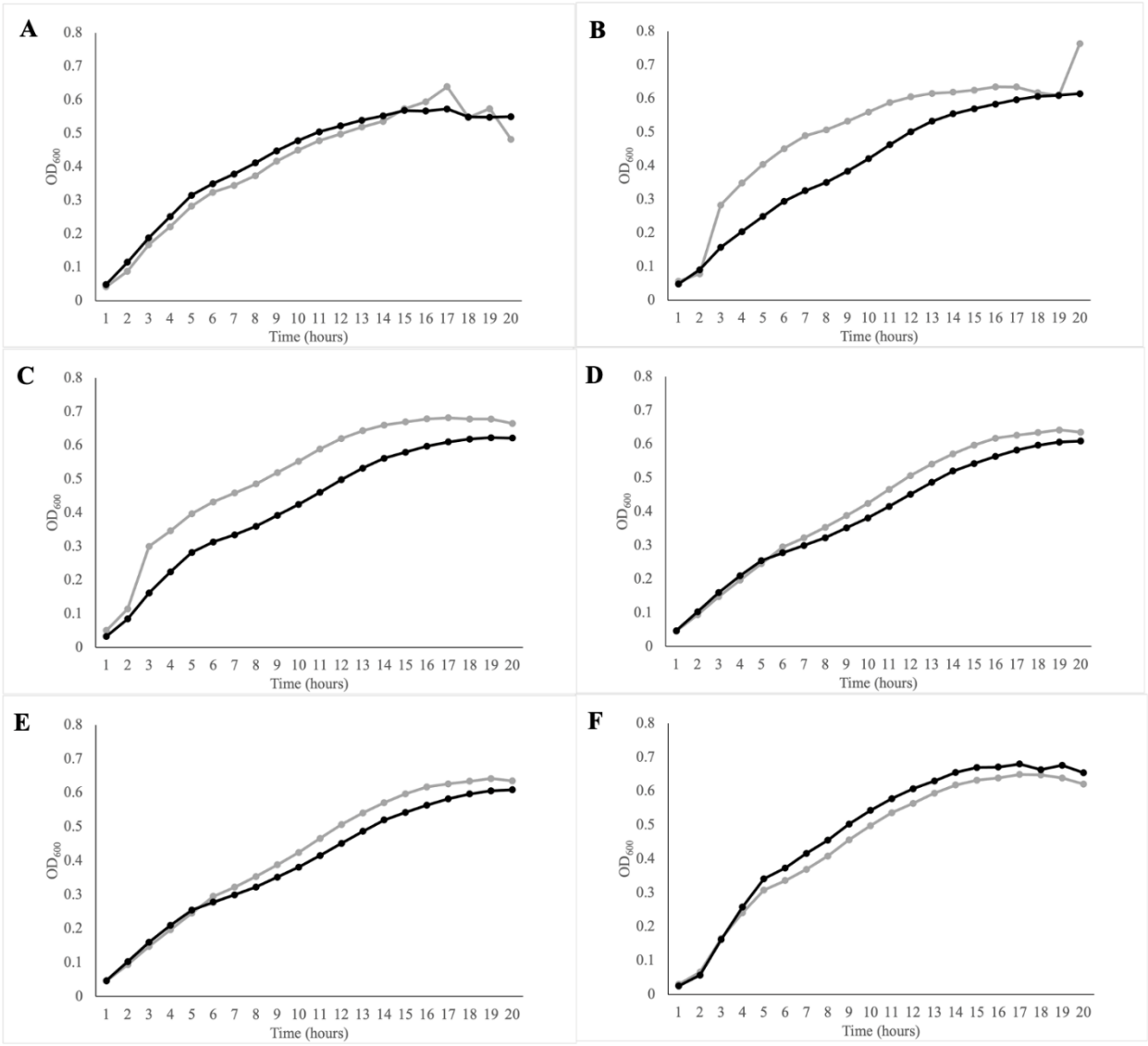


**Figure S2.** Growth curves of five pT7 (**A-E)** and one pT7Mut (**F**) BL21 transformants. Bacterial growth was monitored with a BioTek microplate reader measuring $OD_{600}$ at one hour intervals over a 20 hour period. $OD_{600}$ measurements were blanked using LB broth. Gray lines represent 0.5mM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


**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 contains the sequence the pSPPH21 backbone vector with the T7 inducible promoter sequence 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.

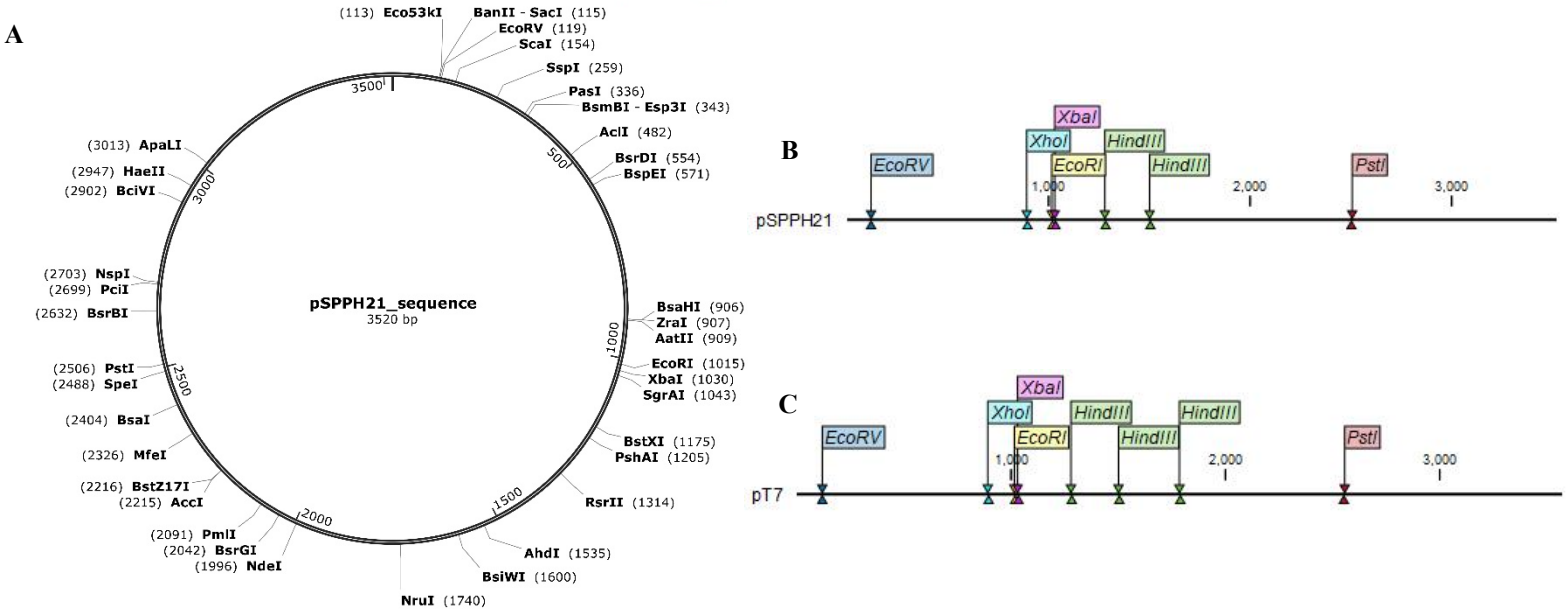


**Figure S4.** Restriction enzyme sites map of the pSPPH21 plasmid demonstrated by SnapGene (**A**) and CLC Genomics (**B**). Restriction enzyme sites map of the pT7 *in silico* plasmid with CLC Genomics (**C**), indicating one additional HindIII site (green box) from the T7 promoter insert at 1785 bp.