An improved tetracycline-inducible expression system for fission yeast

Xiao-Hui Lyu

Introduction

In *Schizosaccharomyces pombe*, the thiamine-repressible *nmt1* promoter and its weakened variants have been the most commonly used inducible expression system for the last 30 years[1]. However, this system has drawbacks including long induction time, incompatibility with rich media, and requirement for medium switch. Tetracycline-inducible promoters do not have such limitations but have not been widely used due to low expression levels at the induced state.

Recently, we have constructed a new tetracycline-inducible system based on a promoter named *enotetS*, which is constructed by replacing a 19-bp sequence in the promoter of the *eno101* gene with a single 19-bp *tet* operator sequence[2]. The *enotetS* promoter is fully induced within a few hours upon the addition of the inducer anhydrotetracycline, and reaches an expression level higher than that of the strong *adh1* promoter.

Furthermore, for applications that need lower expression levels, we construct four weakened variants of the *enotetS* promoter by inserting GC-rich sequences of different lengths, which inhibit expression to different degrees[3]. The resulting promoter series spans a broad range of expression levels, matching the range of the *nmt* promoter series but providing more intermediate levels. For ease of use, we construct plasmid vectors that contain both a tetracycline-inducible promoter and a cassette expressing the TetR repressor. This system enhances the ability to manipulate gene expression in fission yeast.

Plasmids information

(Expression level: PenotetS> PenotetSW1> PenotetSW2> PenotetSW3> PenotetSW4**)**

plasmid	GCK File Name
pDB5318	pAde6-pCMV-tetR-PenotetS-mECtrine-ADH1 terminator-hphMX
pDB5319	pAde6-pCMV-tetR-PenotetSW1-mECtrine-ADH1 terminator-hphMX
pDB5320	pAde6-pCMV-tetR-PenotetSW2-mECtrine-ADH1 terminator-hphMX
pDB5321	pAde6-pCMV-tetR-PenotetSW3-mECtrine-ADH1 terminator-hphMX
pDB5322	pAde6-pCMV-tetR-PenotetSW4-mECtrine-ADH1 terminator-hphMX

How these plasmids were constructed:

1. Replace a 19-bp sequence between the TATA box and TSS of *Peno276*[4] (refined from *Peno101*) with a 19-bp *tet* operator sequence to get the *enotetS* promoter.
2. Integrate the *enotetS* promoter into the SIV plasmid.
3. A cassette expressing the TetR repressor is inserted in reverse upstream of the *enotetS* promoter.
4. Insert different stem-loop sequences at the 5' UTR (before the *NheI* restriction site).

Notes for using these plasmids:

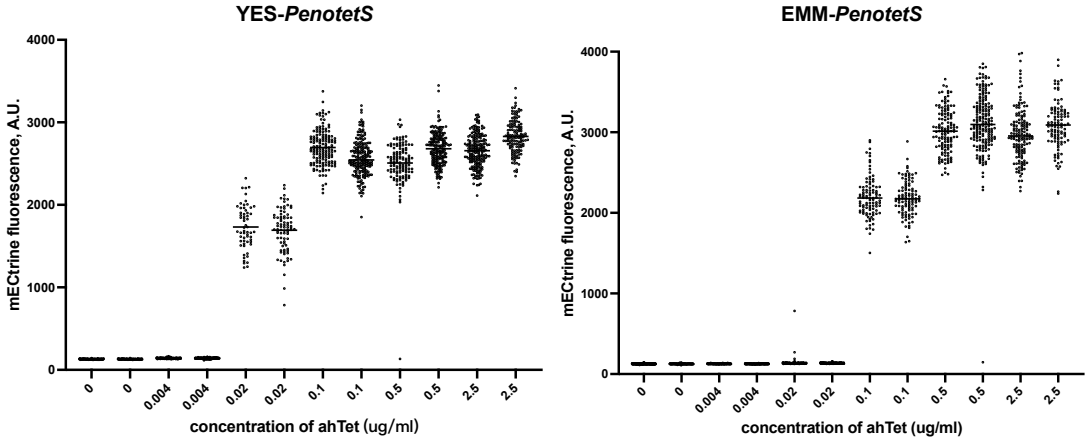
1. Restriction site for changing different tag: *NheI & BglII*
2. Linearization Site: *NotI*

Results

To characterize the expression level and other related features of the *enotetS* series promoters, we integrated the above plasmids (pDB5318-pDB5322) into LD1 (*h⁻ leu1-32 ura4-D18*) and quantified the mECtrine fluorescence detected by dragonfly confocal microscopy.

(The following data may be update.)

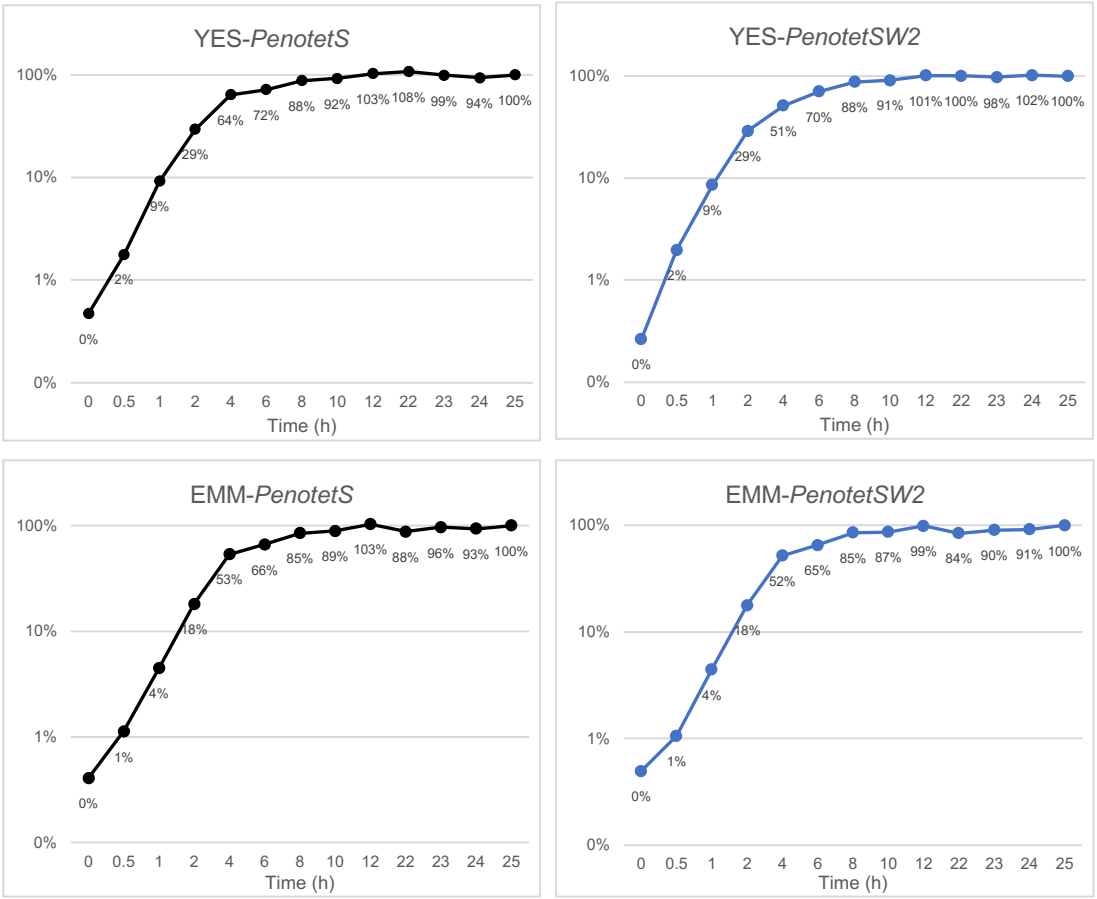
1. The effect of ahTet concentration on PenotetS expression



2.5 μg/ml of ahTet is a suitable concentration in both YES and EMM medium.

2. Fluorescence induction kinetics

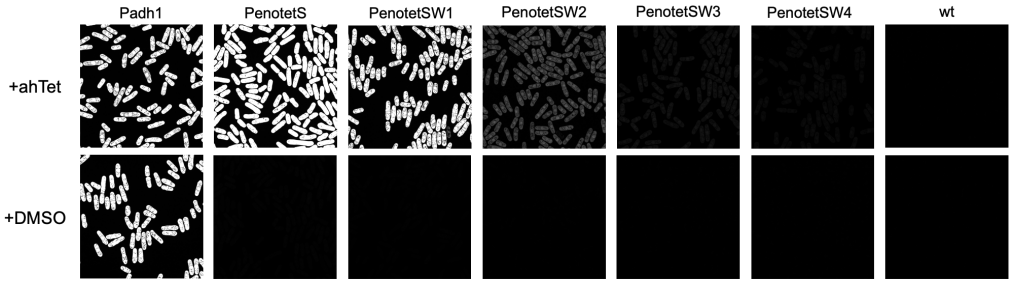
Cells were induced with 2.5 μg/ml ahTet and sampled at different time. The expression levels at different time points were normalized to the last time point.



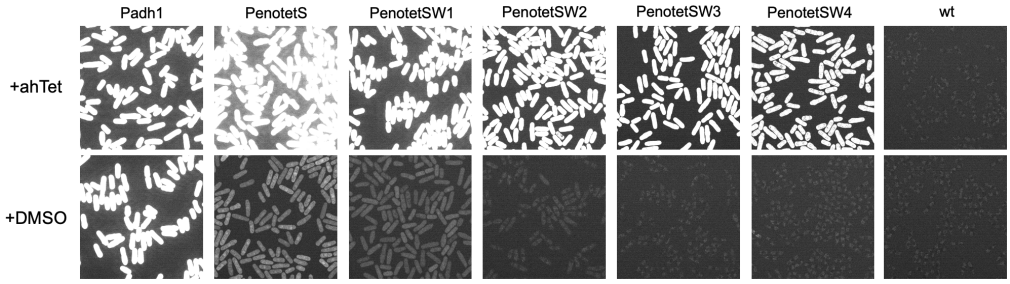
3. Comparison of expression levels

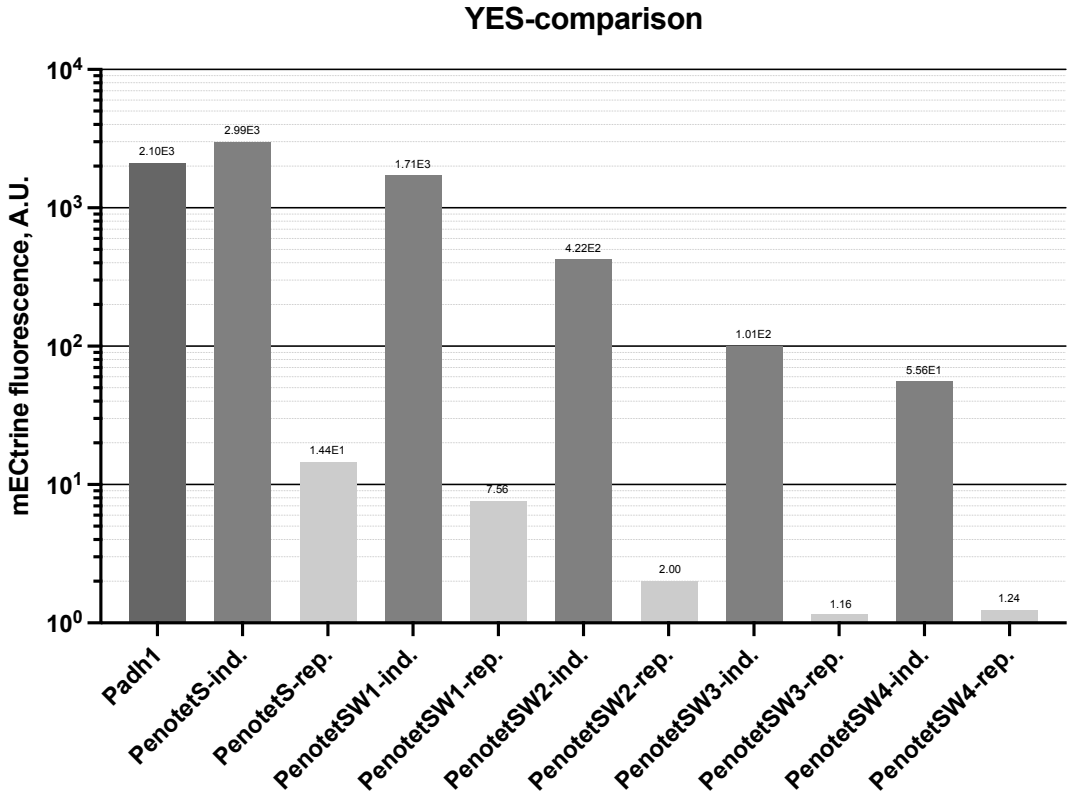
YES

Low scale:



High scale:

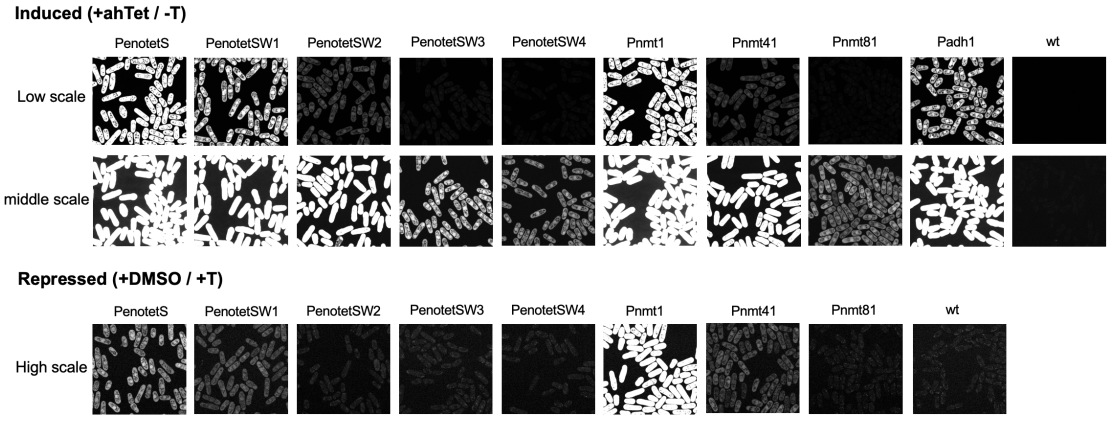


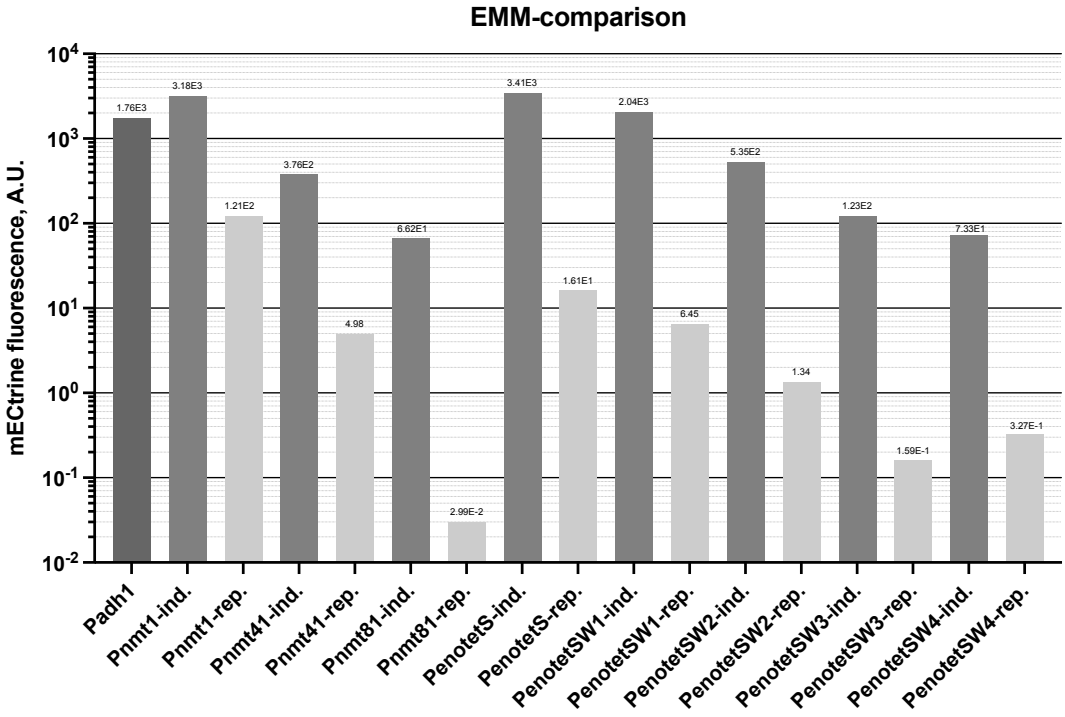


Expression level (induced):

PenotetS > Padh1 > PenotetSW1 > PenotetSW2 > PenotetSW3 > PenotetSW4

EMM





Expression level (induced):

PenotetS > *Pnmt1* > *PenotetSW1* > *Padh1* > *PenotetSW2* > *Pnmt41* > *PenotetSW3* > *PenotetSW4* > *Pnmt81*

Reference

1. Basi, G., Schmid, E. & Maundrell, K. TATA box mutations in the Schizosaccharomyces pombe nmt1 promoter affect transcription efficiency but not the transcription start point or thiamine repressibility. *Gene* **123**, 131–136 (1993).
2. Murphy, K. F., Balázsi, G. & Collins, J. J. Combinatorial promoter design for engineering noisy gene expression. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 12726–12731 (2007).
3. Lamping, E., Niimi, M. & Cannon, R. D. Small, synthetic, GC-rich mRNA stem-loop modules 5' proximal to the AMG start-codon predictably tune gene expression in yeast. *Microb. Cell Factories* **12**, 74 (2013).
4. Wang, H. *et al.* Identification and refinement of two strong constitutive promoters for gene expression system of Schizosaccharomyces pombe. *World J. Microbiol. Biotechnol.* **30**, 1809–1817 (2014).