A New Generation of Inducible All-In-One Tet-On-ORF/shRNA Vectors

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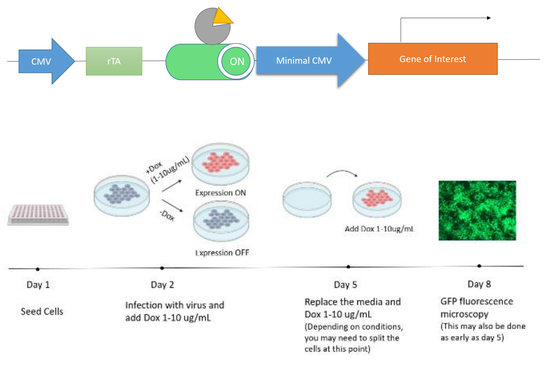
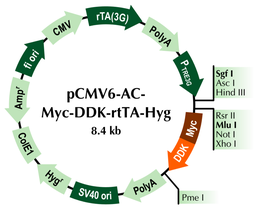
Introduction

Tet-On is a powerful inducible system and also a classical tool to regulate gene expression in mammalian cells. It has also been applied to regulate Pol III-driven transcription, such as shRNA or gRNA driven by U6 or H1 promoter. However, there are unresolved technical issues that limit its applications. 1). The Tet-On inducible system is composed of Tet regulatory protein and inducible gene expression cassettes, however co-transfection of two vectors will cause the imbalance of the two functional groups in transfected cells. 2). Due to the limited insert size and the unique structure of the lentivector, it poses a great challenge to incorporate all functional cassettes into a single lentiviral transfer plasmid. Our All-in-one new system is built upon the tetracycline activator protein, Tet-On 3G, combined with a new plasmid structure, which could tightly regulate the downstream transcription of gRNA or shRNA. The responsiveness of our system to Doxycycline regulation is also dramatically improved compared with the current versions. The new Tet-On system is further optimized into a compact structure to be compatible with the Lenti-virus package (All-In-One Lenti-Tet-On system), which still keeps the leaky expression at an undetectable level. Combined with all these features, the new generation of the system imposes a wide application in the genomic editing fields.

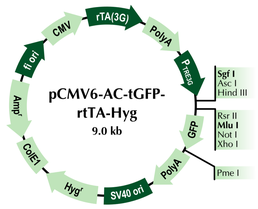
Procedure and Results



A. All-In-One Tet-On

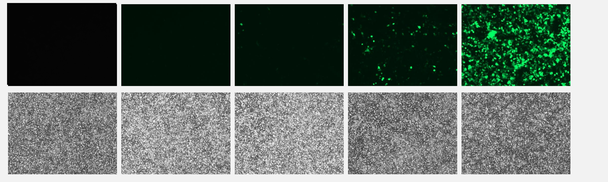


PS100134 (PS100124)



PS100133 (PS100125)

Dose-dependent GFP expression in HEK293T cells



The Stable Cell Lines With All-In-One Inducible Vectors

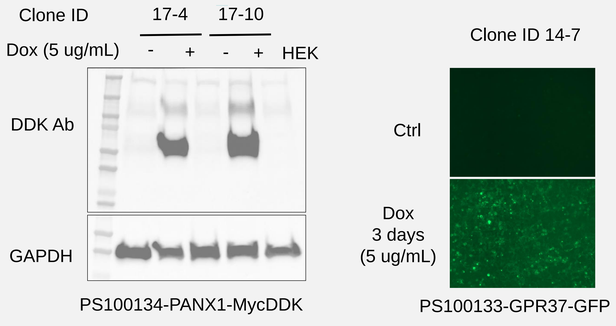
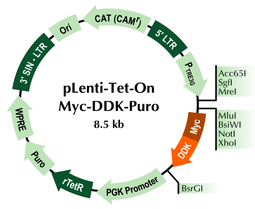
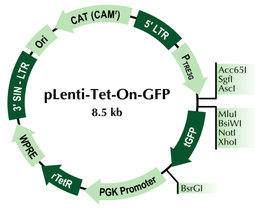


Fig.1. Human PANX1 and GPR37 cDNA genes were cloned into a mammalian Tet-On inducible vector PS100134 and PS100133 respectively. HEK293T cells were transfected with a PS100134-PANX1-MycDDK and PS100133-GPR37-GFP construct (0.5 µg DNA/well of a 12-well plate). Single stable HEK293T colonies were established after 4 weeks of hygromycin drug selection at 200 µg/mL and then treated with or without doxycycline (Dox) at 5 µg/mL for 3 days. The expression of the target genes was analyzed with Western Blot and GFP fluorescence correspondingly.

B. All-In-One Lenti-Tet-On

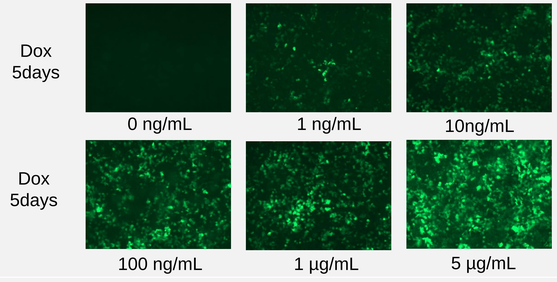


PS100131

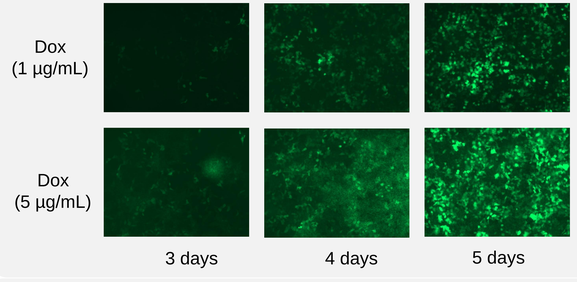


PS100128

Dose-dependent GFP expression in HEK293T cells



Time-Course GFP expression in HEK293T cells



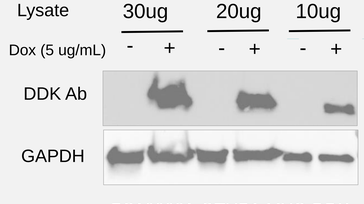
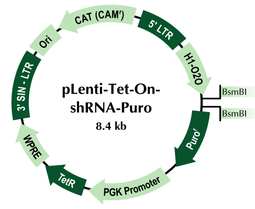


Fig.2. **Stable cell lines with Lenti inducible vector PS100131**. The OTUB1 cDNA genes were cloned into PS100131 and HEK293T was transduced with the lentiviral particles. The clonal protocol as in Fig.1 was followed afterward, and the target gene expression was analyzed with Western Blot.

C. All-In-One Lenti-Tet-On-shRNA



PS100130

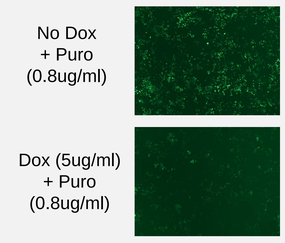


Fig.3. Dox-induced knocking down of GFP gene in HEK293T with GFP stable cell line. The cells were transduced with lentiviral particles of inducible GFP shRNA and treated with 10 µg/ml Dox at Day 0 and 0.8 µg/ml Puro at Day 3 post-transduction. The decrease of the GFP expression was observed on Day 6 post-transduction.

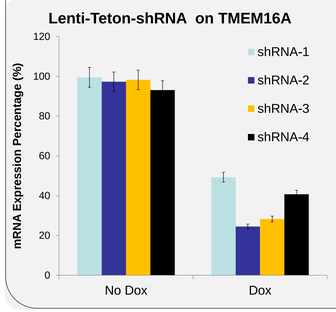


Fig.4. Dox-induced knocking down of TMEM16A gene in HEK293T cells. HEK293T cells were transduced with lentiviral particles of inducible TMEM16A shRNA and treated with 10 µg/ml Dox at Day 0 and 0.8 µg/ml Puro at Day 3 post-transduction. Total RNA was extracted at Day 6 post-transduction and mRNA expression was analyzed by qRT-PCR. Dox treatment led to about 70% reduction in gene expression of CDC25A gene.

Conclusions

1. The all-in-one inducible vectors are effective tools for temporal control of gene expression in mammalian cells, which exhibit the highest specificity with almost zero leaky problems.
2. The current Lenti-Tet-On-shRNA vector is based on H1-2O2 or U6-2O2 promoters and 1st-G tetracycline repressor TetR, which shows promising results for future tests on Tet-On 3G and the new Tet responsive promoter H1-4O4.

Gossen, M. & Bujard, H. (1995) Efficacy of tetracycline-controlled gene expression is influenced by cell type. BioTechniques 89:213-215