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Construction and validation of over-expression of RE1-silencing transcription factor (REST) using PiggyBac transposon inducible vector system in HEK293FT cells

Norhazlin Jusoh [1,2], Norshariza Nordin [1,3], Nurul Ain Nasim Mohd Yusof [4], Pike-See Cheah [3,5] and King-Hwa Ling [1,3]*

[1] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[2] Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch, Tapah Campus, 35400 Tapah Road, Perak, Malaysia.
[3] Malaysian Research Institute on Ageing (MyAgeing®), Universiti Putra Malaysia, Selangor, Malaysia.
[4] Pluripotent Stem Cell Laboratory, Haematology Unit, Cancer Research Centre, Institute for Medical Research (IMR), Block C, National Institute of Health (NIH) Malaysia, Setia Alam, Malaysia.
[5] Department of Human Anatomy, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
* Correspondence: lkh@upm.edu.my ; Tel.: +603-8947 2564

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Abstract: RE1-silencing transcription factor (REST), a key regulator of neural gene expression, modulates ion channel function, neurotransmitter receptor activity, and synaptic plasticity, and its dysregulation has been implicated in neurodegenerative diseases. However, stable overexpression of REST in mammalian cells remains technically challenging, highlighting the need for an efficient and controllable delivery platform. Here, we constructed and validated a PiggyBac-based inducible vector system for regulatable REST overexpression in HEK293FT cells as a proof of concept. The REST-FLAG-P2A-GFP construct was assembled using NEBuilder HiFi DNA Assembly and validated by restriction fragment length polymorphism (RFLP), Sanger sequencing, and whole-plasmid sequencing. HEK293FT cells were transfected with REST-FLAG-P2A-GFP and SB100X, together with Xlone_GFP plasmids, using polyethylenimine (PEI), followed by doxycycline induction at 24 and 48 hours post-transfection. REST expression was confirmed by eGFP fluorescence imaging, while blasticidin resistance supported stable transgene integration for up to 5 days. Western blot analysis further verified inducible REST overexpression, detecting REST protein at approximately 130 and 200 kDa, with 1.45-fold ($p < 0.01$) and 1.56-fold ($p < 0.05$) increases, respectively, compared with uninduced cells. Collectively, these findings demonstrate the utility of the PiggyBac transposon system for stable and inducible expression of transcription factors in mammalian cells and establish a platform for future studies of REST function and gene regulatory mechanisms.

Keywords: PiggyBac; RE1-Silencing Transcription Factor (REST); Molecular cloning; Transfection.

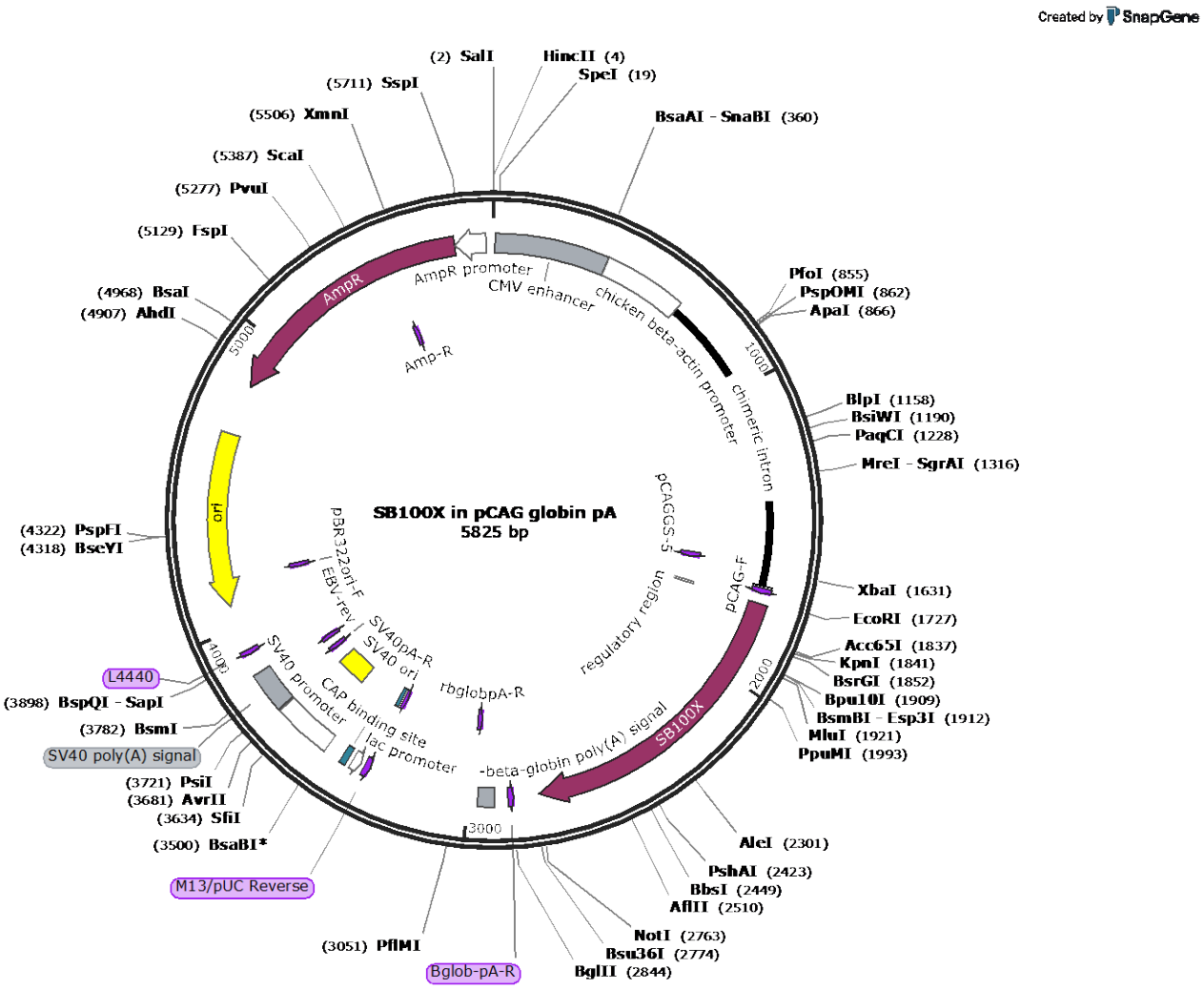

Figure S1. SBSleeping Beauty (SB100X) plasmid as the transposase.

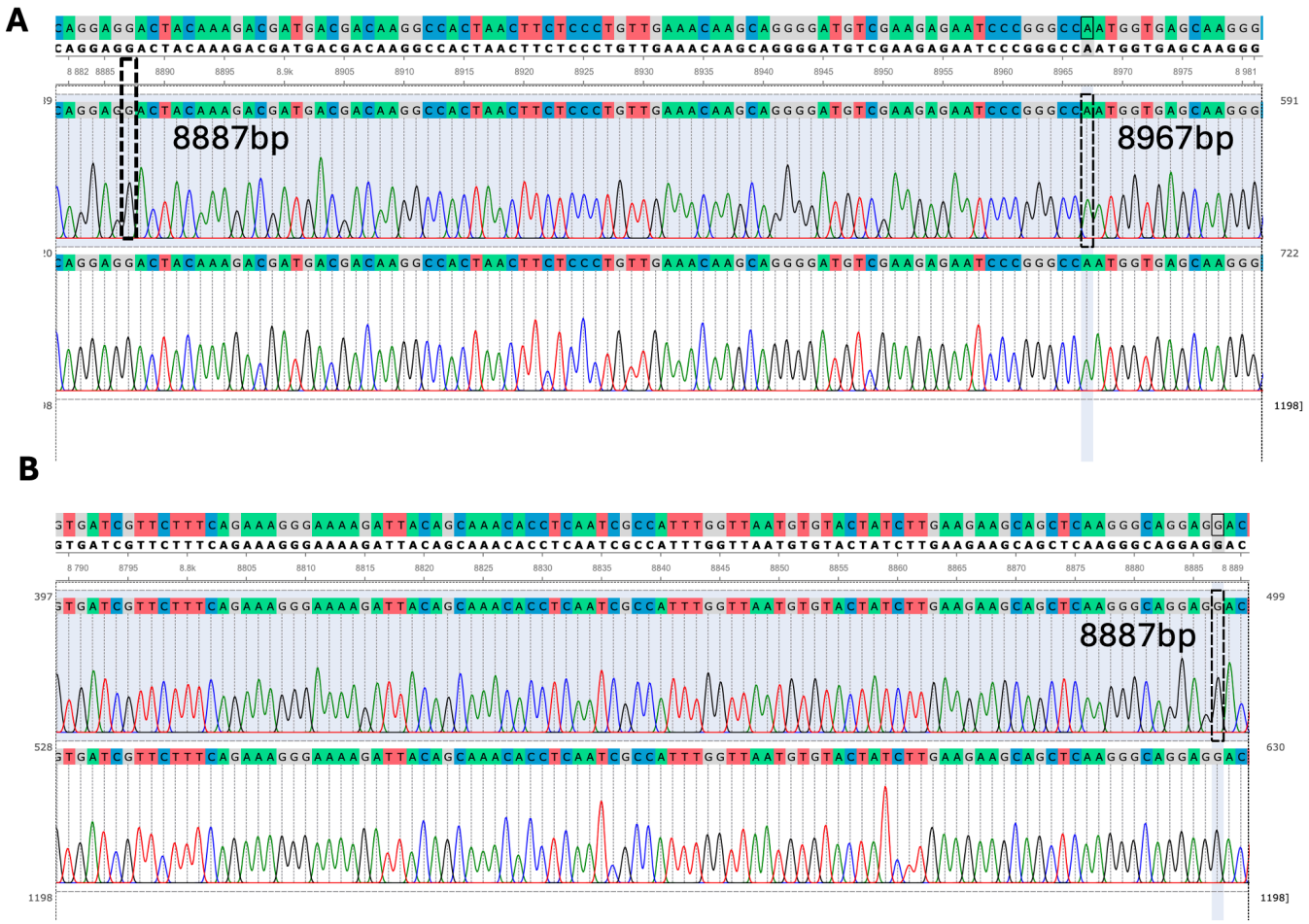

Figure S2. Sanger sequencing validation of key cloned regions in the REST-FLAG-P2A-GFP recombinant construct. (A) Representative Sanger sequencing chromatogram showing the FLAG-P2A region of the recombinant construct. The sequence spanning approximately 8887 bp to 8967 bp confirms the expected nucleotide composition across the inserted FLAG tag and P2A peptide region. Dashed lines indicate the validated boundaries of the sequenced segment. (B) Representative Sanger sequencing chromatogram showing the junction between the REST coding sequence and the beginning of the FLAG sequence. The sequence trace confirms the correct continuity, orientation, and in-frame fusion of REST with the downstream FLAG tag. Together, these chromatograms verify accurate assembly of the recombinant REST-FLAG-P2A-GFP construct at the key insertion and junction regions.