Development of a tetracycline-inducible lentiviral vector with an instant regulatory system

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Tian Yang

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Abstract

Lentiviral vectors, originally derived from human immunodeficiency virus, provide highly efficient viral gene delivery vehicles. Lentiviral vectors often use a constitutive promoter to drive the expression of a therapeutic gene. To regulate the expression of a therapeutic gene, a regulatory system such as Tet-On needs to be established in the target cell lines to produce a regulatory protein, reverse Tet-responsive transcriptional activator (rtTA). The expressed rtTA binds to the tetracycline responsive element (TRE) in the promoter in response to doxycycline and activates transcription of gene of interest. A hypothesis in this study is based on the speculation that a basal leaky expression of rtTA in the bi-directional TRE vectors allows instantly inducible expression of a gene of interest and thereby avoids the time-consuming procedures for generating Tet-On cell lines. Based on this hypothesis, a novel lentiviral vector has been developed to examine an instant induction of PP2Cβ as a target gene. Three instantly inducible bicistronic lentiviral vectors [pLenti-Bi-TRE-Tet-on (V), pLenti-Bi-TRE-Tet-on-PP2Cβ WT (WT), pLenti- Bi-TRE-Tet-on-PP2Cβ MUT (MUT)] were constructed and characterised to assess the usefulness of these vectors. Transient transfection of both WT and MUT vectors into HEK293T cells showed a great induction of PP2Cβ expression upon 24 h of 1 μM doxycycline treatment. The result promises the use of these vectors as a mammalian expression plasmid with a feature of inducible target gene expression. However, viral infection studies involving lentiviral packaging and infection procedures did not show a reproducible expression of rtTA or PP2Cβ in HEK293T cells. Therefore, the inducibility of viral transduction needs to be improved for the future studies of PP2Cβ in primary cells.

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Abbreviations

A	absorbance
AD	activation domain
APS	ammonium persulfate
ATP	adenosine triphosphate
attL	Left integration attachment site
attR	Right integration attachment site
b	path length
bp	base pair
BAD	Bcl-2-associated death promoter
BSA	bovine serum albumin
CA	capsid
CAK	CKD-activating kinase
cDNA	complementary deoxyribonucleic acid
CDKs	cyclin-depend protein kinase
CFU	colony-formation unit
CIP	calf intestinal alkaline phosphatase
CMV	cytomegalovirus
Cp/Ct	crossing point
CFU	colony-formation unit
DBD	DNA binding domain
DMEM	Dulbecco's Modified Eagle medium
DMSO	dimethyl sulphoxide
DNase	deocyribonuclease
dNTP	deoxyribonucleotide triphosphate
Dox	doxycycline
DTT	dithiothreitol
ε	extinction coefficient
EDTA	ethylene diamine tetra-acetic acid
FBS	fetal bovine serum
FCS	fetal calf serum
GFP	green fluorescent protein
GPCR	G protein-coupled receptor
GUSB	beta glucuronidase

HEK 293T	human embryonic kidney 293T cell line
hES	human embryonic stem cell
HIV	human immunodeficiency virus
HRP	horse radish peroxidase
IN	integrase
IκB	NFκB inhibitory binding partner
IKK	IκB kinase
IL-1β	Interleukin-1β
JUK	Jun N-terminal kinase
kb	kilo base
LB	Luria-Bertani
LTR	long terminal repeat
LV	Lentiviral vector
M	molar
MA	membrane associated matrix
MAPK	mitogen-activated protein kinase
MCS	multiple cloning site
MEKK	MAP kinase kinase kinase
MLK	mixed lineage kinase
mM	millimolar
MKK	MAPK kinase
MKKK	MKK kinase
MTK1	MAP three kinase 1
MUT	vector containing mutant PP2Cβ
NC	nuclear capsid
NCBI	national centre for biotechnology information
NFκB	nuclear factor kappa B
nm	nanometer
nM	nanomolar
nt	nucleotide
OD	optical density
PEG	polyethylene glycol
Pen/Strep	penicillin-streptomycin
PIC	pre-integration complex
PMSF	phenylmethanesulfonyl fluoride

RNAi	RNA interference
PP2Cβ	protein phosphatase type 2C isoform beta
PPMs	protein phophatase magnesium-dependent enzymes
PPM1B	PP2Cβ
PPPs	phospho-protein phosphatases
PR	protease
PTPs	protein tyrosine phosphatases
PVDF	polyvinylidene fluoride transfer membrane
qPCR	quantitative real-time PCR
RCR	replication competent recombinant
RCV	replication competent virus
RE	restriction endonuclease
RHD	Rel homology domain
RNase	ribonuclease
RIN	RNA integrity number
RRE	Rev-responsive element
RT	reverse transcription/reverse transcriptase
RT-	reverse transcriptase free reaction
RT-qPCR	reverse transcription quantitative real-time PCR
rTetR	Reverse TetR
rtTA	tetracycline-responsive transcriptional activator
SAPKs	stress signaling pathways
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SIN	self-inactivating vector
SU	Surface/envelop glycoprotein
TAK1	TGFβ-activated kinase 1
TBST	Tris-buffered saline with Tween 20
TEMED	N,N,N',N'-tetramethylethylenediamine
tetO	tet operator
TetR	Tet represor protein
TGFα	Trasnforming growth factor α
TGFβ	transforming growth factor β
TLR	toll-like receptor
TM	Trans membrane protein

Tm	melting temperature
TNFα	tumor necrosis factor
TRE	tetracycline-response element
TREmod	modified TRE-response element
tTA	tetracycline-controlled transactivator
U	units
μM	micromolar
UV	ultraviolet
V	vector only/no PP2Cβ control vector
VSV-G	vesicular stomatitis virus G glycoprotein
WT	vector containing wild type PP2Cβ
ψ	packaging signal

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