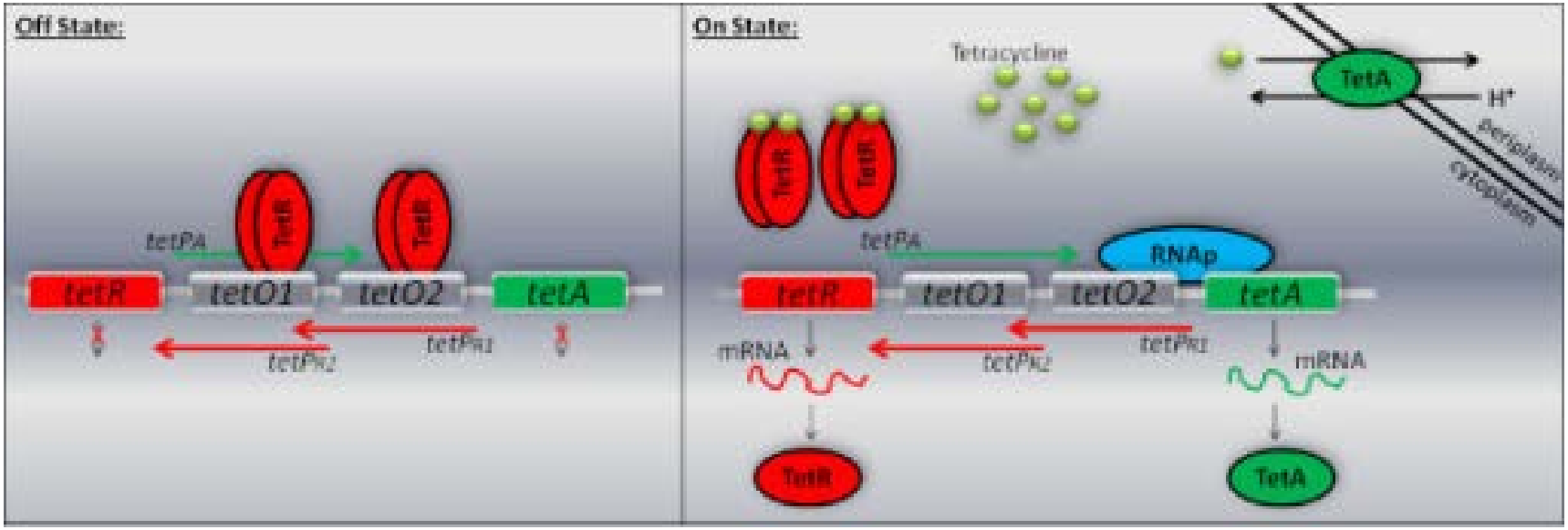
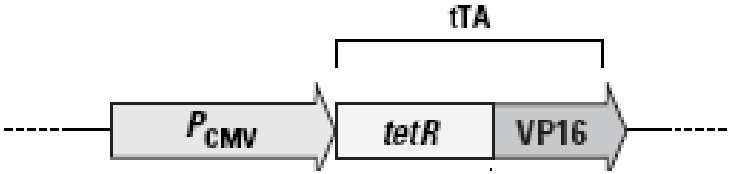
Tet-Off® and Tet-On® Gene Expression Systems


Off State:
On State:
Tetracycline
TetA
H+
periplasm
cytoplasm
TetR
TetR
tetPA
tetR
tetO1
tetO2
tetA
tetPR1
tetPR2
RNAp
mRNA
mRNA
TetR
TetA

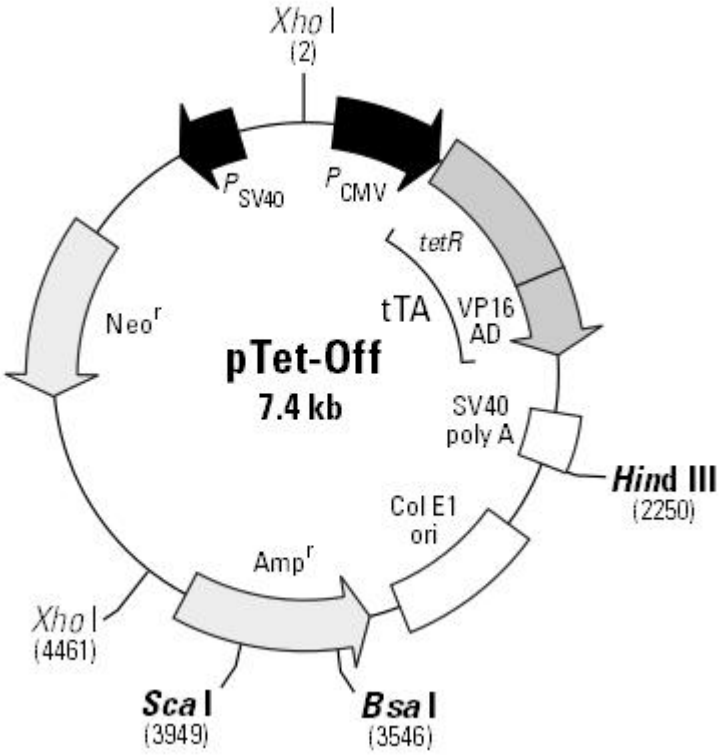
The difference between Tet-On and Tet-Off is not whether the transactivator turns a gene on or off, as the name might suggest; rather, **both proteins activate expression**. The difference relates to their respective **response to doxycycline** (Dox, a more stable tetracycline analogue); Tet-Off activates expression in the absence of Dox, whereas Tet-On activates in the presence of Dox.

Tet-Off



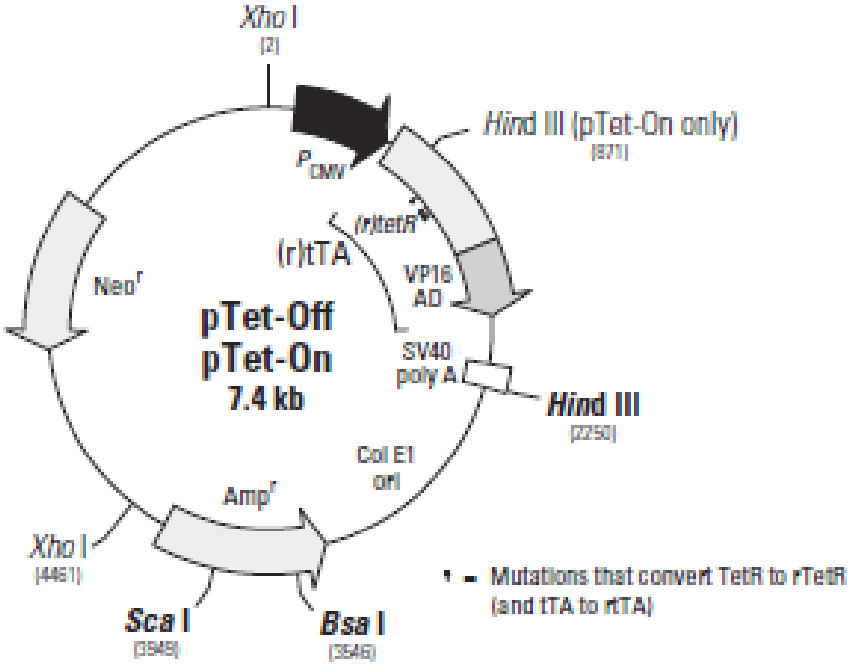
fusion of amino acids 1–207 of TetR and the C-terminal 127 a.a. of the Herpes simplex virus VP16 activation domain (AD). Addition of the VP16 domain converts the TetR from a transcriptional repressor to a transcriptional activator.

tetracycline-controlled transactivator (**tTA**)



Tet-On

reverse Tet repressor (rTetR) which was created by four amino acid changes in TetR→ reverse tTA

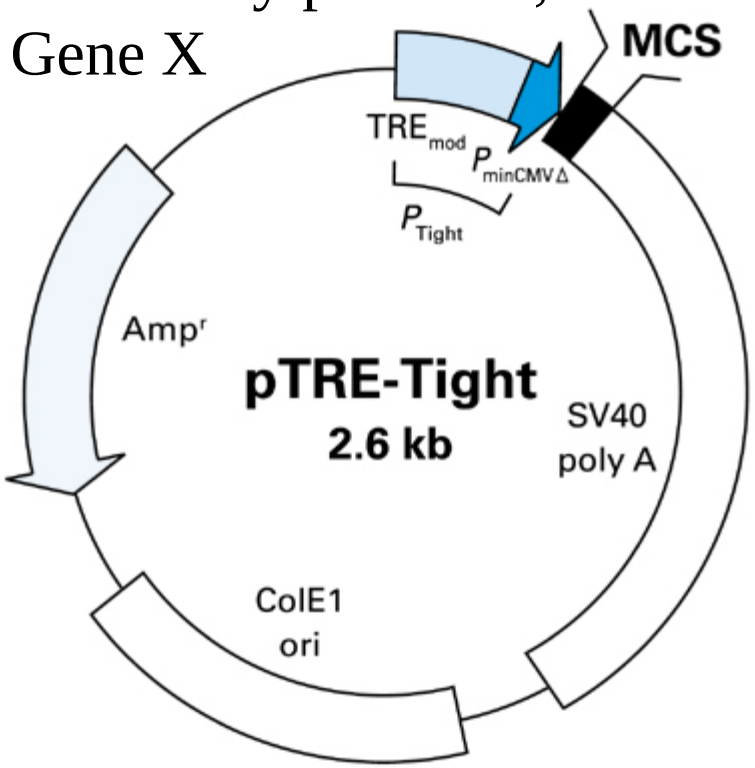


Tetracycline vs. Doxycycline

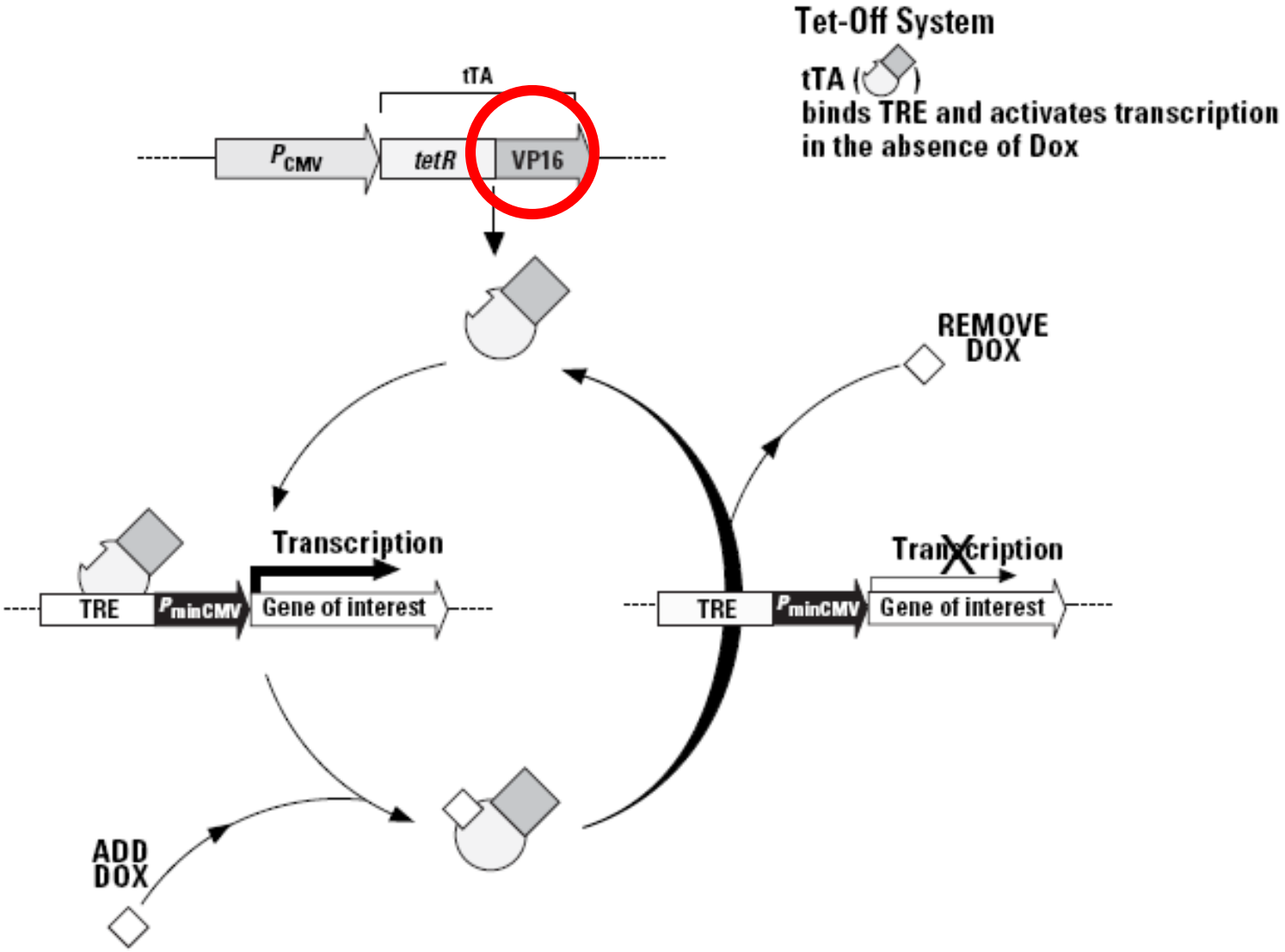
The Tet-On System is only responsive to Dox, not Tc (Gossen & Bujard, 1995). In contrast, Tet-Off systems respond equally well to either Tc or Dox. We recommend that you use Dox for all Tet System experiments, in part because a significantly lower concentration of Dox is required for complete activation or inactivation (0.01–1 µg/ml Dox vs. 1–2 µg/ml Tc). In both systems, the antibiotics are used at concentrations far below cytotoxic levels for either cell culture or transgenic studies. In addition, Dox has a longer half-life (24 hours) than Tc (12 hours). Thus, for the Tet-Off System, you may prefer to use Dox for long-term maintenance of antibiotic levels and switch to Tc in preparation for induction.

The second critical component is the response plasmid which expresses a gene of interest (**Gene X**) under control of the **tetracycline-response element, or TRE.**

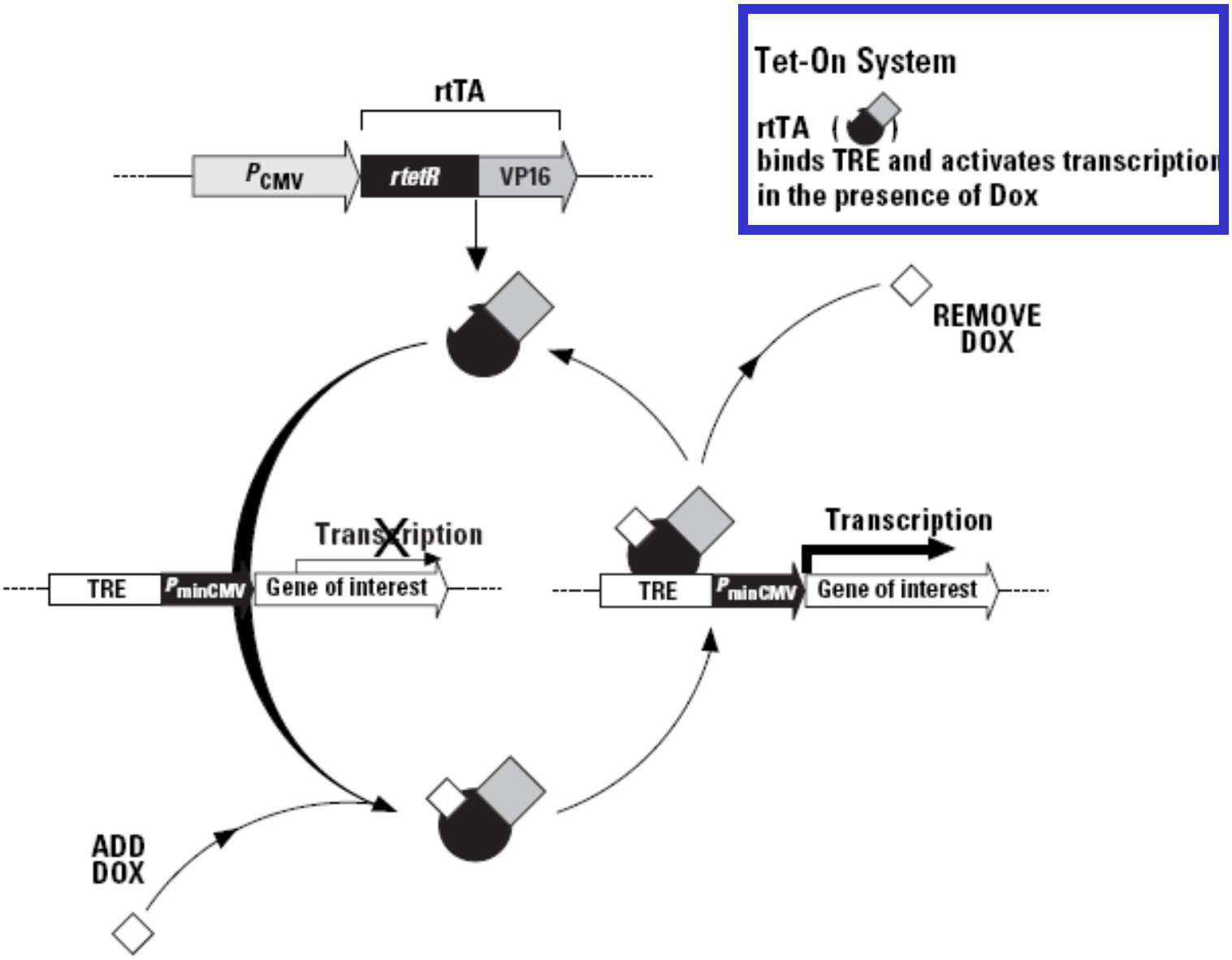
TRE=seven direct repeats of a 42-bp sequence containing the19-bp tet operator sequence (tetO), located just upstream of the minimal CMV promoter (PminCMV). Lacking the strong enhancer elements normally associated with the CMV immediate early promoter, there is extremely low background expression of Gene X



Tet-Off


Tet-Off System
tTA ( )
binds TRE and activates transcription
in the absence of Dox
tTA
P_{CMV}
tetR
VP16
REMOVE
DOX
Transcription
TRE
P_{minCMV}
Gene of interest
Transcription
TRE
P_{minCMV}
Gene of interest
ADD
DOX

Tet-On


Tet-On System
rtTA
binds TRE and activates transcription
in the presence of Dox
rtTA
P_{CMV}
rtetR
VP16
REMOVE
DOX
Transcription
TRE
P_{minCMV}
Gene of interest
Transcription
TRE
P_{minCMV}
Gene of interest
ADD
DOX

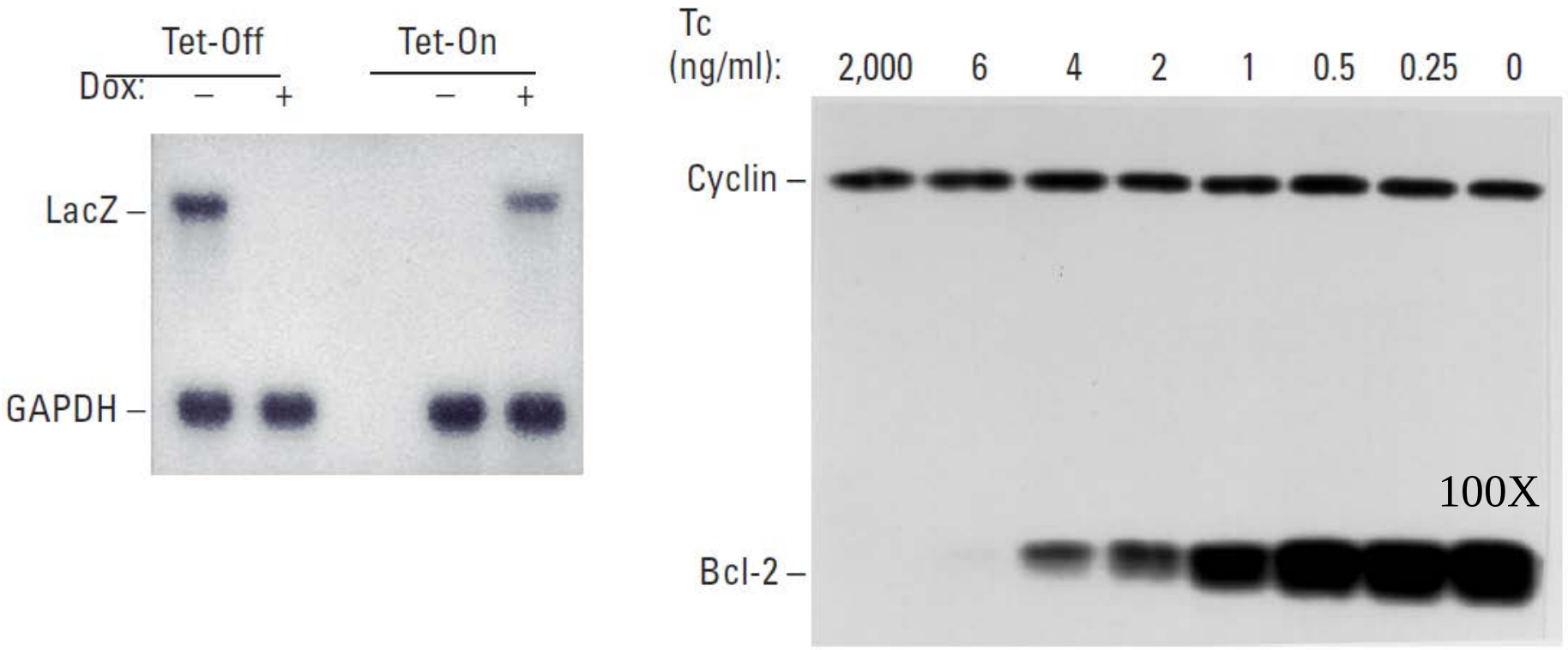


Figure 1. Inducible on/off control of gene expression in the Tet Systems. Panel A. Double-stable cell lines were developed by stably transfecting HeLa Tet-Off or HeLa Tet-On cells with a plasmid containing *E. coli* lacZ under control of the Tet response element (TRE). Cells were cultured +/– 1 µg/ml Dox. For Northern analysis, 10 µg of total RNA was loaded per lane, and the blot was hybridized simultaneously with probes to lacZ and the GAPDH housekeeping gene (Gossen *et al.*, 1995; reprinted with permission of the author). **Panel B.** HeLa S3 Tet-Off cells were stably transfected with a plasmid expressing Bcl-2 under control of the TRE and grown in the presence of the indicated amounts of Tc. A Western blot containing 100 µg of total protein from each condition was probed with human Bcl-2-specific and human cyclin-B1-specific mouse monoclonal antibodies. Based on scanning densitometry, removal of Tc gave ~100-fold induction of Bcl-2. For details, see Yin & Schimke (1995).

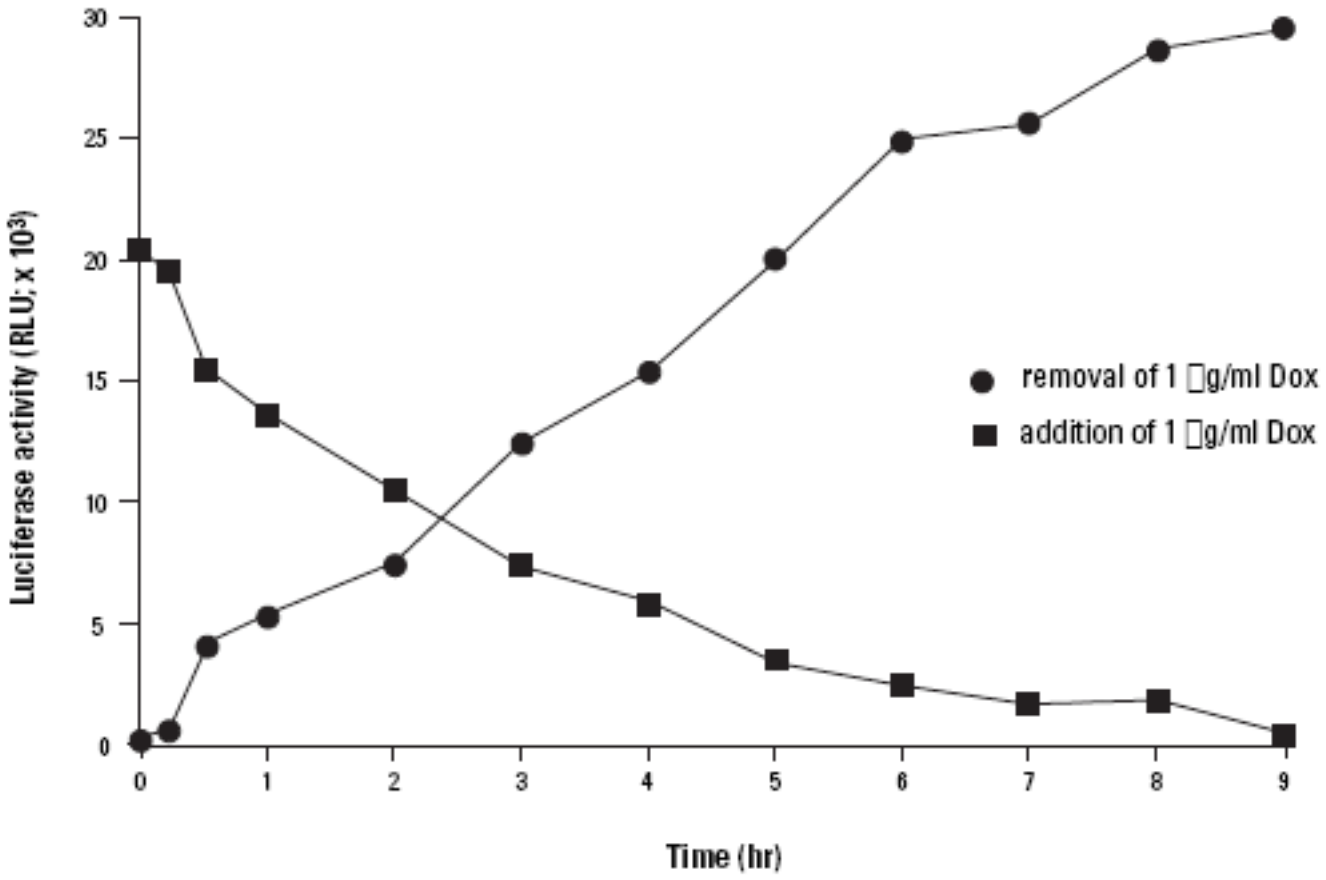


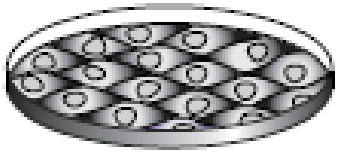
Figure 3. Luciferase expression is rapidly induced in a Tet-Off cell line in response to removal of Dox. The CHO-K1-EGFP-Luc Tet-Off control cell line expresses the tTA and contains a stably integrated copy of the firefly luciferase gene under control of the TRE. Luciferase activity was continuously monitored with a fluorescent imaging plate reader (FLIPR, Molecular Devices Corp.) after addition or removal of 1 µg/ml Dox from the culture medium (Cunningham et al., 1997).

The ultimate **goal** in setting up a functional Tet System is creating a **double-stable Tet cell line** which contains both the regulatory and response plasmids.

Perform pilot experiments

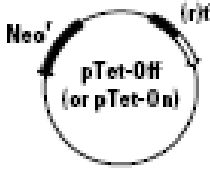
(Section VII; ~ 3 weeks)

Host cell line

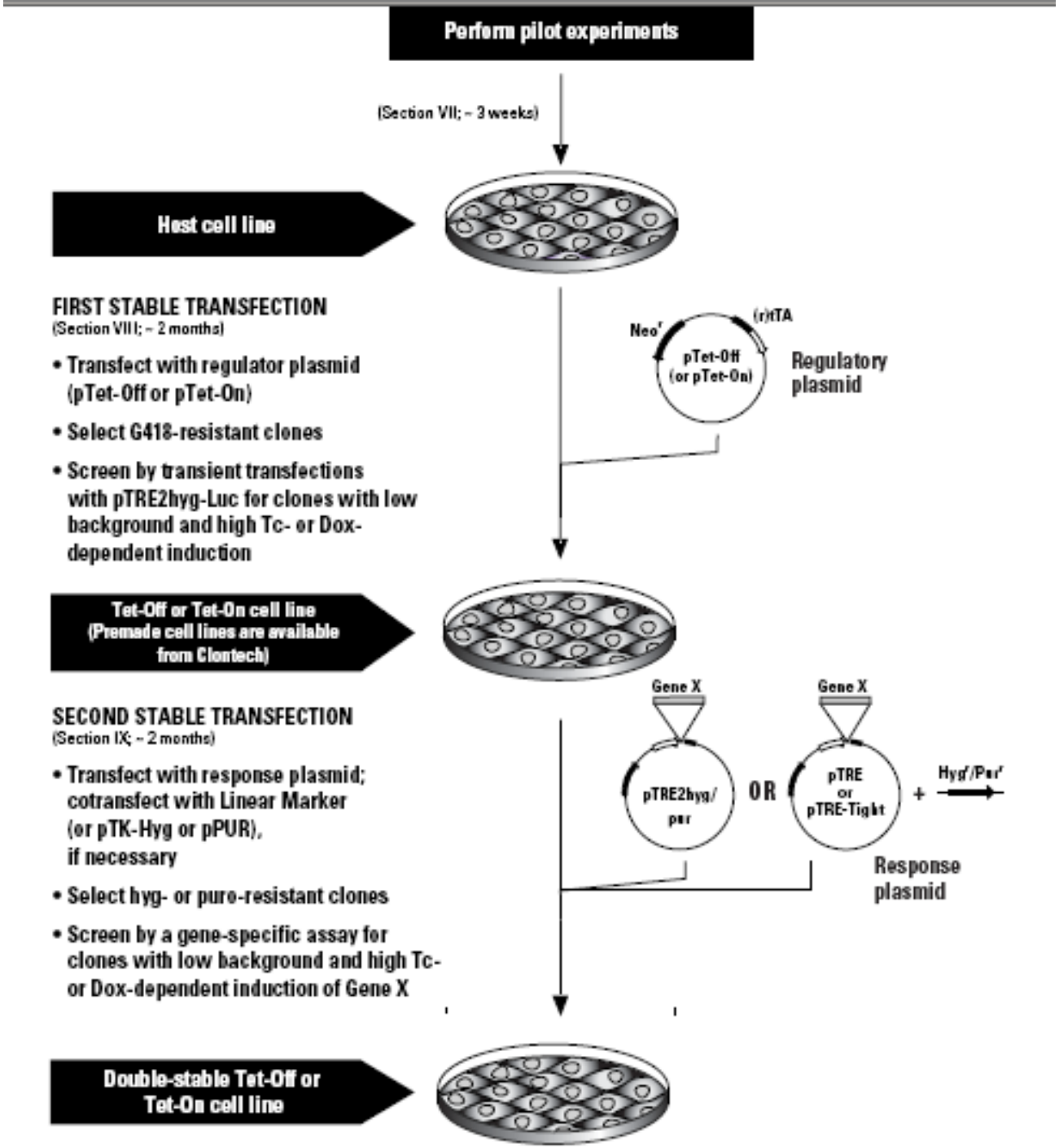


FIRST STABLE TRANSFECTION
(Section VIII; ~ 2 months)

- Transfect with regulator plasmid (pTet-Off or pTet-On)
- Select G418-resistant clones
- Screen by transient transfections with pTRE2hyg-Luc for clones with low background and high Tc- or Dox-dependent induction



Regulatory plasmid


Perform pilot experiments
(Section VII; ~ 3 weeks)
Host cell line
FIRST STABLE TRANSFECTION
(Section VIII; ~ 2 months)
• Transfect with regulator plasmid (pTet-Off or pTet-On)
• Select G418-resistant clones
• Screen by transient transfections with pTRE2hyg-Luc for clones with low background and high Tc- or Dox-dependent induction
Neo'
(r)tTA
pTet-Off (or pTet-On)
Regulatory plasmid
Tet-Off or Tet-On cell line (Premade cell lines are available from Clontech)
SECOND STABLE TRANSFECTION
(Section IX; ~ 2 months)
• Transfect with response plasmid; cotransfect with Linear Marker (or pTK-Hyg or pPUR), if necessary
• Select hyg- or puro-resistant clones
• Screen by a gene-specific assay for clones with low background and high Tc- or Dox-dependent induction of Gene X
Gene X
pTRE2hyg/ pur
OR
Gene X
pTRE or pTRE-Tight
+
Hyg'/Pur'
Response plasmid
Double-stable Tet-Off or Tet-On cell line

Advantages of the Tet Systems

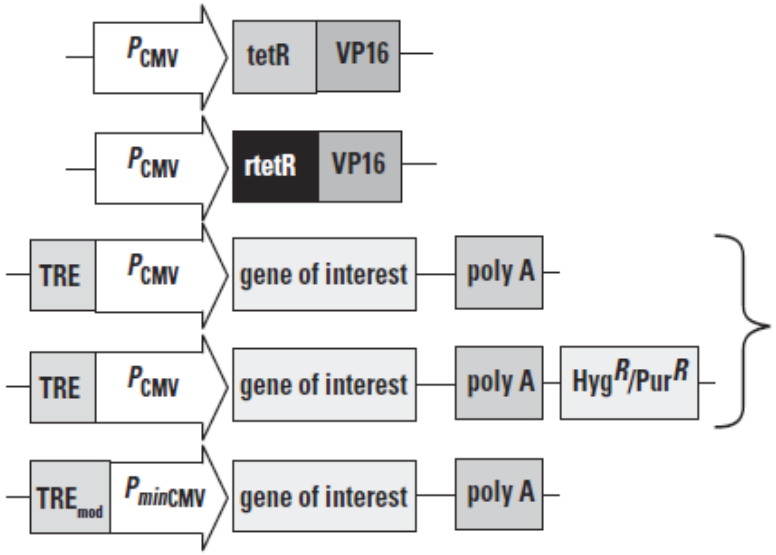
• **Extremely tight on/off regulation.** Background, or leaky, expression of Gene X in the absence of induction is extremely low with pTRE or its variants (Figure 1). For the lowest background expression, use pTRE-Tight Vectors.

• **No pleiotropic effects.** When introduced into mammalian cells, the prokaryotic regulatory proteins (TetR or rTetR, the prokaryotic precursors to tTA and rtTA) act very specifically on their target sequences, presumably because these regulatory DNA sequences are nonexistent in eukaryotic genomes.

• **High inducibility and fast response times**. With the Tet Systems, induction can be detected within 30 minutes using nontoxic levels of inducer. Induction levels up to **10,000-fold** have been observed.

• **High absolute expression levels.** Maximal expression levels in the Tet systems can be higher than expression levels obtained from the CMV promoter or other constitutive promoters.

Basic Vectors



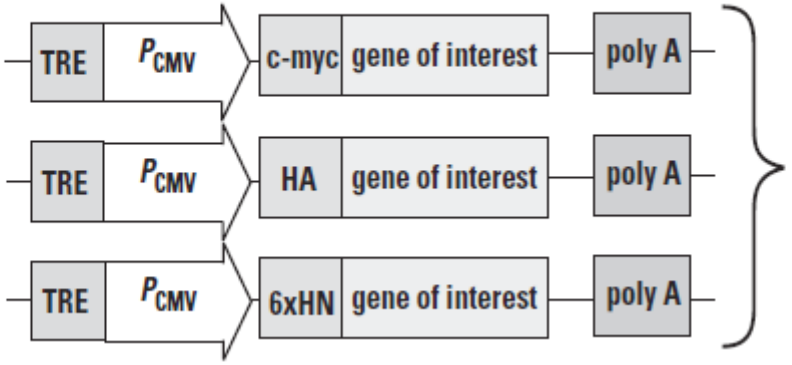
Regulator vector for use in Tet-Off system

Regulator vector for use in Tet-On system

Response plasmids encoding the Tet Responsive Element (TRE) for use in either Tet-Off or Tet-On

Response plasmid encoding a modified Tet Responsive Element (TRE_{mod}) for use in either Tet-Off or Tet-On

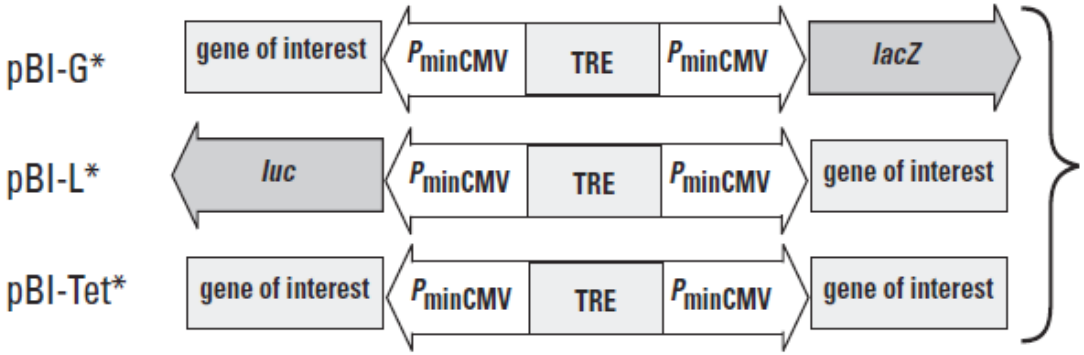
Tagged Vectors



Response plasmids for use in either Tet-Off or Tet-On System

Used for screening with antibodies or for purification

Bidirectional Tet Vectors



Response vectors for monitoring expression of a target gene via expression of a coregulated reporter