Published in final edited form as:

Anal Biochem. 2010 January 15; 396(2): 167–172. doi:10.1016/j.ab.2009.09.043.

Factors modulating expression of *Renilla* luciferase from control plasmids used in luciferase reporter gene assays¹

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Abstract

The *Renilla* luciferase gene is commonly used as an internal control in luciferase-based reporter gene assays to normalize the values of the experimental reporter gene for variations that could be caused by transfection efficiency and sample handling. Various plasmids encoding *Renilla* luciferase under different promoter constructs are commercially available. The validity of the use of *Renilla* luciferase as an internal control is based on the assumption that it is constitutively expressed in transfected cells and that its constitutive expression is not modulated by experimental factors that could result in either the upregulation or the downregulation of the amounts of the enzyme produced. During the past ten years, a number of reports have appeared that identified a variety of conditions that could alter the basal constitutive expression of *Renilla* luciferase. The use of *Renilla* luciferase in those circumstances would not be valid and an alternative way of normalization would be necessary. This review covers the factors that have been reported thus far as modulating the expression of *Renilla* luciferase from plasmid constructs.

Keywords

luciferase; *Renilla*; reporter gene; internal control; normalization

Renilla luciferase

Renilla luciferase is an enzyme that catalyzes the chemical reaction which is responsible for the green bioluminescence of *Renilla reniformis*, a soft coral also known as sea pansy and a member of the class Anthozoa [1]. *In vitro*, the enzyme catalyzes the oxidative decarboxylation of coelenterazine (*Renilla* luciferin) in the presence of O₂, resulting in the emission of blue light (with an emission maxima of 480 nm), and the formation of oxyluciferin and CO₂ as the reaction products [2]. The *in vivo* bioluminescence results from the transfer of energy from oxyluciferin to *Renilla* green fluorescent protein which then emits green light with an emission maxima of 509 nm [3]. The enzyme exists as a single polypeptide chain of 35 KDa in the active form [4]. The enzyme is immediately active following translation without requiring post-translational modifications [4]. Leoning et al recently carried out crystallographic studies on

¹Research in the laboratory of J.A.H. is supported by a grant from the Alliance for Lupus Research. A.S. was a Research Fellow supported by an NIAMS Rheumatology Training Grant, T32 AR050947.

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Renilla luciferase at a resolution of 1.4 Å and reported that the enzyme demonstrated a classic/ β -hydrolase fold [5].

The cDNA for the *Renilla* luciferase enzyme was first cloned by Lorenz et al, who determined that the protein contains 311 amino acid residues and who also expressed the cloned gene in *E. coli* [6]. Subsequently, the same group demonstrated the expression of the cloned enzyme in mammalian cells transfected either transiently or stably and suggested its potential use as a marker for mammalian gene expression [7]. The recombinant *Renilla* luciferase expressed in mammalian cells was found to be identical to the native protein both in terms of molecular weight and immunological reactivity [7]. The enzyme has subsequently been developed for use as a reporter gene in mammalian cell culture systems [8]. *Renilla* luciferase has also been shown to be useful as a bioluminescent reporter for gene expression in *Candida albicans* [9]. Furthermore, the enzyme has been used as a reporter gene in live mice using optical imaging devices [10-12].

***Renilla* luciferase expressing plasmids**

The substrate of *Renilla* luciferase, coelenterazine, is distinct from the substrate of a luciferase found in the firefly *Photinus pyralis*, whose substrate is known as D-luciferin. *Renilla* luciferase does not have any effect on the substrate of firefly luciferase [13]. Because of this substrate distinction, *Renilla* luciferase has been adopted for use as an internal control for luciferase-based assay systems of mammalian gene expression. In these assays the *Renilla* luciferase is expressed under the control of a constitutive promoter and the firefly luciferase is expressed under the experimental promoter and the assays are carried out in a single-tube format [8]. This system, which was developed by Promega Corporation, allowed for the normalization of the results of the firefly luciferase assay measurements, hence reducing the variability that could be caused by differences in transfection efficiency and in sample handling. The *Renilla* luciferase plasmids are available in a variety of promoter configurations, including the herpes simplex virus type 1 thymidine kinase (HSV-1 TK) promoter (pRL-TK and pHRG-TK), the SV40 early enhancer/promoter (pRL-SV40 and pHRL-SV40) and CMV immediate-early enhancer/promoter (pRL-CMV and pHRL-CMV) [8]. Some promoterless constructs are also available, such as pRL-null and pHRG-B.

The utility of an internal control plasmid is dependent on the fact that the encoded protein is expressed in a constitutive manner without being influenced by experimental factors. One of the most widely used internal control plasmids in luciferase-based reporter gene assays is the pRL-TK plasmid, a plasmid that expresses *Renilla* luciferase under the control of the HSV-1 TK promoter [8]. A typical pattern of *Renilla* luciferase activity from a single experiment for Jurkat cells and from another experiment for HeLa cells is presented in Fig. 1 (our unpublished observations). Jurkat cells were transfected with a fixed amount of the pRL-TK plasmid by electroporation and they were then immediately treated with various doses of 1,25-dihydroxyvitamin D3 for 32 hours. HeLa cells were transfected with identical amounts of the pRL-TK plasmid and beginning at five hours after transfection, they were treated with various doses of 1,25-dihydroxyvitamin D3 for 24 hours. At the end of treatment the cells were harvested and *Renilla* luciferase assays were carried out. The results presented in Fig.1 for both cell types show that there was no relationship between *Renilla* luciferase activity and the dose of vitamin D. The sample-to-sample variation is minimal and is believed to be due to differences in transfection efficiency and sample handling. In such a system where there is no effect of the experimental conditions on *Renilla* luciferase activity, the use of the reporter gene as an internal control for normalization will be valid.

Over the past ten years, several reports have appeared in the biomedical literature that have pointed out various experimental factors that could alter the constitutive expression of

Renilla luciferase under the various promoters used and therefore making the use of *Renilla* luciferase as an internal control invalid in those cases. This review covers all the literature identified by Medline search and that reported experimental factors that suppressed (Table 1) or induced (Table 2) the expression of *Renilla* luciferase. A number of factors were shown not to alter the expression of *Renilla* luciferase; a list of some of these factors identified during the literature survey is presented in Table 3. The use of the plasmids listed in Table 3 as internal controls under the experimental conditions mentioned would be legitimate. For example, Kogai and colleagues reported that treatment with all-*trans* retinoic acid failed to induce *Renilla* luciferase from the pRL-TK and pRL-SV40 plasmids in MCF-7 human breast carcinoma cells despite causing an increased expression of firefly luciferase, making these two plasmids good internal controls in this system [14].

The promoter structure of *Renilla* luciferase in the pRL-TK plasmid

The pRL-TK plasmid, which carries the *Renilla* luciferase gene under the HSV-1 TK promoter [15], is the most widely used internal control plasmid in luciferase reporter gene assays. The HSV-1 TK inset in the pRL-TK plasmid is 753 base pairs long and consists of the nucleotides from -724 to +29 region of the TK gene [15]. There are three *cis*-acting elements in the promoter region of the HSV-1 TK gene that are involved in both constitutive and induced expression of the TK gene (Fig. 2): a proximal element containing a TATA box (at -12 to -29), the first distal element (at -47 to -61) and the second distal element (at -80 to -105) [16-18]. The general transcription factor TFIID binds to the TATA box. The first distal element has a GC-box, which is a binding site for the transcription factor Specificity Protein-1 (sp1), and the second distal element contains binding sites for sp1 and CCAAT-transcription factor (CTF) [17;19].

Transfection of a complete copy of the HSV-1 TK gene into mammalian cells leads to a constitutive expression of the gene [20-22]. During a productive viral infection by HSV-1, induced expression of the TK gene occurs as a result of activation in *trans* by some of the immediate early genes such as Infected Cell Polypeptide-4 (ICP-4) [17]. ICP-4 induces TK by interacting with the general transcription factors [23] or by decreasing the affinity of binding of cellular negative transcription factors to the TK promoter [24]. Experimental factors that alter the constitutive expression of *Renilla* luciferase from the pRL-TK plasmid do so probably by interacting with these three *cis*-acting elements or other unidentified regulatory elements in the TK promoter.

Factors that suppress the expression of transfected *Renilla* luciferase (Table 1)

The following factors have been shown to suppress the constitutive expression of *Renilla* luciferase. Using *Renilla* luciferase in those circumstances for normalization could result in a falsely increased normalized value of the experimental reporter gene.

Nuclear receptors

In African green monkey kidney fibroblast cells (CV-1), Everett and colleagues showed that the transfection of a plasmid expressing one of the following nuclear receptors markedly suppressed the expression of *Renilla* luciferase under the HSV-1 TK promoter: the peroxisome proliferator activator receptor alpha (PPAR α), chicken ovalbumin upstream promoter transcription factor-1 (COUP-TFI), apolipoprotein A1 regulatory protein-1 (ARP-1 or COUP-TFII), and hepatocyte nuclear factor-4 (HNF-4) [25]. The repression of *Renilla* luciferase was, respectively, 92%, 95%, 93% and 81% compared to the *Renilla* luciferase activity in control samples. Potential binding sites for ARP-1 and COUP-TFI were identified within the HSV-1 TK promoter, the chimeric intron located between the HSV-1 TK promoter and the *Renilla*

luciferase gene, and also within the luciferase gene of the pRL-TK plasmid. Moreover, the COUP-TF proteins have been described as being able to bind to DNA in a promiscuous manner [26]. It is interesting to note that the steroid/thyroid/retinoid superfamily of nuclear receptors mentioned above were found to also affect the expression of firefly luciferase from various plasmids. In CV-1 cells, transfected PPAR α or ARP-1 suppressed the expression of firefly luciferase from the pRSV-Luc plasmid, whereas transfection of COUP-TFI caused an induction of firefly luciferase and HNF-4 had no effect. On the other hand, in the same cell type, PPAR α and HNF-4 caused suppression of firefly luciferase expression from the pSV40-Luc plasmid while COUP-TFI and ARP-1 caused activation. The simultaneous use of the mentioned firefly luciferase and *Renilla* luciferase plasmids in CV-1 cells together with cotransfection of these nuclear receptors will lead to erroneous interpretations.

Dexamethasone

Ibrahim et al showed that dexamethasone treatment of CV-1 cells transfected with a plasmid encoding the glucocorticoid receptor (GR) suppressed the expression of *Renilla* luciferase from the pRL-TK plasmid [27]. A similar effect was noticed when a plasmid that encodes the *Renilla* luciferase under the thromboxane synthase (TS) promoter (pRL-TS) was transfected into CV-1 cells. The use of pRL-TK and pRL-TS in those circumstances resulted in an apparent induction of the activity of the firefly luciferase that had glucocorticoid response elements by 12-fold and 47-fold, respectively. Dexamethasone also inhibited the expression of *Renilla* luciferase from pRL-TK in L6 skeletal muscle cells; however, intriguingly this inhibitory effect was lost when the cells were stably transfected with GR. In addition, there was no effect on *Renilla* luciferase activity when the cells were transfected with the pRL-TS plasmid. The mechanism of inhibition of expression from the pRL-TK plasmid by dexamethasone could probably be due to the inhibition of the transactivation functions of sp1 since dexamethasone has been reported to inhibit the binding of sp1 to DNA [28].

Type I Interferons

In the human melanoma cell line SK-MEL-28, both interferon alpha (IFN α) and interferon beta (IFN β) caused significant inhibition of expression of *Renilla* luciferase from the codon-modified plasmid phRL-TK, the inhibition being in the range of 18-19% [29]. This inhibition did not appear to be caused by increased cytotoxicity, as there was no increase in the percentage of apoptotic cells. Since treatment with these interferons did not lower the levels of *Renilla* luciferase mRNA, it was suggested that the inhibition occurred in a post-transcriptional manner. Treatment with the type I interferons also resulted in decreased levels of firefly luciferase by 30% for IFN α and 60% for IFN β . As a result of the effects on the levels both of *Renilla* luciferase and firefly luciferase, IFN α treatment caused an apparent decrease in normalized firefly luciferase activity by 30%, whereas IFN β caused an apparent decrease of 40%. Type I interferon treatment did not alter the expression of green fluorescent protein (GFP) based on the measurement of mean fluorescent intensity, indicating that GFP could be an alternative internal control in these circumstances.

15-deoxy- $\Delta^{12,14}$ -prostaglandin J2 (15d-PGJ2)

In Human Embryonal Kidney-293 (HEK293 cells), Zhang et al showed that the cyclopentenone prostaglandin 15d-PGJ2, which is a natural ligand of PPAR-, inhibited the expression of *Renilla* luciferase from phRG-TK and phRG-B plasmids by 5- to 6-fold. This inhibition was achieved at concentrations that did not induce cell toxicity and it occurred in a dose-dependent manner [30]. Also, 15d-PGJ2 treatment inhibited the levels of *Renilla* luciferase expressed from the pRL-TK plasmid by 2-fold. The authors did not find typical PPAR- binding sites within the *Renilla* plasmids; so, the mechanism of inhibition of *Renilla* luciferase is not clear.

Factors that induce the expression of transfected *Renilla* luciferase (Table 2)

The following experimental variables have been reported to upregulate the expression of *Renilla* luciferase from various promoters. Using *Renilla* luciferase in those circumstances for normalization could result in a falsely decreased normalized value of the experimental reporter gene.

Specificity Protein-1 (sp1)

Osborne and Tonissen showed that transfected sp1 caused a twelve-fold induction of the expression of *Renilla* luciferase from pRL-TK in the MM96L malignant melanoma cell line [31]. There are two sp1 binding consensus sites in the proximal region of the HSV-1 TK promoter (Fig. 2). Increased binding of sp1 to those sites could contribute to the induction of *Renilla* luciferase by transfected sp1. This is supported by the fact that overexpression of sp1 in other cell types has been reported to induce the expression of genes whose promoters contain GC-boxes[32]. Co-transfection of sp1 did not cause an increase in expression in the absence of the HSV-1 TK promoter region. However, removal of the HSV-1 TK promoter resulted in reduction of *Renilla* activity to background levels; therefore, the pRL-null plasmid will not be useful as an internal control in the MM96L cell lines. The authors reported potential additional sp1 binding sites within the HSV-1 TK promoter. Removal of the distal 275 bp region from the HSV-1 TK promoter in the pRL-TK plasmid was associated with only 3-fold induction of *Renilla* activity following sp1 transfection, suggesting that there could be functional sp1 binding sites in this region.

Androgens

In CV-1 cells, Ibrahim and colleagues showed that dihydrotestosterone (DHT) in the presence of transfected androgen receptor (AR) induced the expression of *Renilla* luciferase from pRL-TK by two-fold [27]. DHT did not induce the expression of *Renilla* luciferase when the pRL-TS plasmid was used. In the LNCaP prostatic carcinoma cell line isolated from a lymph node metastasis and which is responsive to androgens, Mulholland et al showed that the androgen analog R1881 caused an increase, up to eleven-fold in some cases, in the expression of *Renilla* luciferase from pRL-TK, pRL-TS, phRG-TK, phRL-TK, phRG-B, phRL-SV40 and phRL-CMV plasmids, with expression from the pRL-TK plasmid being induced 8-fold [33]. Even the promoterless vector phRG-B was induced by R1881, although the induction was much less (2-fold). The induction of *Renilla* luciferase by R1881 from pRL-TK and phRL-CMV was augmented by co-transfection of androgen promoter (AR) or an empty vector (pcDNA3.1). The authors identified two potential androgen response elements within the sequences of the various *Renilla* vectors.

In cell lines that do not normally express AR, such as MCR7 breast carcinoma cells, PC3 prostate carcinoma cells and SW480 colon adenocarcinoma cells, R1881 treatment did not induce expression from the phRL-TK plasmid. There was no induction of expression from the pRL-TK and pRL-TS plasmids in PC3 cells by R1881, even after transfection with the AR. In another study, Hanson et al showed that R1881 induced expression from the pRL-SV40 plasmid in LNCaP cells by 5-fold and this effect was augmented by transfection of a plasmid coding for the Wilms Tumor-1 (WT1) protein [34]. The synergistic effect of WT1 and R1881 was probably due to the binding of both AR and WT1 to the SV40 promoter region [34]. In the same study, it was shown that R1881 alone did not induce expression from the pRL-null vector but there was a strong induction when R1881 treatment was carried out in the presence of transfected WT1 protein.

Human T-cell leukemia virus type 1 (HTLV-1) Tax protein

Kubota and colleagues showed that in NIH 3T3 murine embryonic fibroblasts and in HeLa human cervical carcinoma cells, transfection of the HTLV-1 Tax protein induced the expression of *Renilla* luciferase from the pRL-TK plasmid [35]. In COS-7 monkey kidney fibroblastic cells the induction by Tax was only modest, whereas there was no induction in human chondrocytic HCS-2/8 cells. Removal of the HSV-1 TK promoter abolished the responsiveness to Tax. By deletion analysis, the authors showed that the sp1 sites, but not the CTF site, within the HSV-1 TK promoter were needed for the induction of *Renilla* luciferase by Tax. This observation is in agreement with previous reports which showed that Tax protein enhances the DNA binding activity of sp1 thus resulting in transactivation [36;37]. The differences in the effects of Tax on the pRL-TK plasmid among various cell types could be due to differences in the cellular amounts of sp1 [35].

WT1 protein

In LNCaP cells Hanson and co-workers showed that transfection of WT1 upregulated the expression of *Renilla* luciferase from pRL-SV40 (by 2.5-fold) [34]. The effect of WT1 on the pRL-SV40 is in agreement with a previous study that reported that WT1 activates the SV40 promoter [38]. Interestingly, transfected WT1 also induced expression from the promoterless pRL-null plasmid by 1.6- to 2.2-fold in LNCaP cells. Also, as mentioned above, the androgen analog R1881 and WT1 synergistically induced expression from the pRL-SV40 and pRL-null plasmids. The effect of WT1 on the *Renilla* luciferase plasmids was shown to be dependent on the zinc finger domain of WT1. The WT1 induction of *Renilla* luciferase did not occur in androgen-insensitive cells such as HEK293 cells and PC3 prostatic carcinoma cells. Based on those observations the authors indicated that the effects of WT1 and androgens required an intact androgen signaling pathway.

GATA-binding protein-4 (GATA4) and GATA-binding protein-6 (GATA6)

In COS-7 cells, Ho and Strauss reported that transfection of a plasmid expressing either one of the two GATA6 isoforms caused a five- to eight-fold induction of the expression of *Renilla* luciferase from the pRL-TK plasmid and a three-fold induction of expression from the pRL-SV40 plasmid [39]. Transfection of GATA4 also caused an induction of *Renilla* luciferase from these plasmids. Consensus GATA binding sites were identified in the chimeric intron located downstream of the promoter in both pRL-TK and pRL-SV40 plasmids and also within the HSV-1 TK promoter of the pRL-TK plasmid. Deletion of the chimeric intron abolished the induction from the pRL-SV40 by GATA6 or GATA4 and reduced the induction from the pRL-TK plasmid by GATA6 to 2.6-fold. Since a similar chimeric intron is also found in several other *Renilla* luciferase plasmids, including pRL-CMV and pRL-null, the use of those plasmids as an internal control in the presence of transfected GATA4 or GATA6 could lead to erroneous interpretations.

Nurr77 family nuclear receptors

Matuszyk and colleagues showed that in PC12 rat pheochromocytoma cells, transfection of the orphan nuclear receptors Nur77, Nurr1 or Nor-1 induced expression from the pRL-TK plasmid [40]. Use of pRL-TK as an internal control resulted in an apparent suppression of expression from the FasL promoter. The authors identified a potential Nur77 site within the HSV-1 TK promoter. The promoterless plasmid phRG-B which carries a modified *Renilla* luciferase gene was found to be a valid internal control for Nurr1.

Adenoviral E1A protein

Thavathiru and Das showed that transfection of the 12S form of E1A protein increased the expression of *Renilla* luciferase from the pRL-TK plasmid in Saos-2 human osteosarcoma cells

by 5-fold [41]. Using pRL-TK as an internal control plasmid in that case resulted in an apparent repression of the expression from the human proliferating cell nuclear antigen promoter. The authors did not find potential E1A responsive regions in the pRL-TK sequence.

Skeletal Muscle Nascent Polypeptide-Associated Complex (skNAC)

Sims and colleagues reported that, the muscle-specific transcription factor skNAC, when transfected into C3H10T1/2 mouse embryonic mesenchymal cells, caused a nearly nine-fold induction of expression from the pRL-TK plasmid [42]. Transfected skNAC was also reported to induce expression from various promoters even in the absence of consensus binding sites [42]. The use of pRL-TK as an internal control in the presence of transfected skNAC resulted in the apparent lack of skNAC effect on the myoglobin promoter. However, by using normalization involving flow cytometry for transfected GFP and total protein measurement, the authors were able to demonstrate the induction of the myoglobin promoter by skNAC.

Phorbol 12-myristate 13-acetate (PMA)

Shifera and Hardin showed that PMA treatment induced expression of *Renilla* luciferase from the pRL-TK plasmid in HEK293 cells nearly five-fold [43]. There was not such an effect when PMA treatment was carried out on HeLa cells transfected with the pRL-TK plasmid. The induction occurred in a dose- and time-dependent manner. The authors showed that the activation of protein kinase C (PKC) and of the JNK and ERK pathways, both of which are downstream effectors of PKC [44-47], was necessary but not sufficient for the induction of *Renilla* luciferase from the HSV-1 TK promoter found in the pRL-TK plasmid. The ability of PMA to induce expression of *Renilla* luciferase was attributed to a cooperative interaction of JNK and ERK pathways with the adenoviral protein E1A whose encoding gene is integrated into the genome of HEK293 cells [48;49]. Therefore, the pRL-TK plasmid would not be a good internal control in E1A-expressing systems when phorbol treatment is employed.

Activated Ras

Behre et al showed that transfected oncogenic Ras induced expression from the pRL-CMV plasmid by 4-fold in CV-1 cells [50]. Use of pRL-CMV resulted in the apparent abolishing of the transactivation effect of activated Ras on the human macrophage-colony stimulating factor (M-CSF) promoter. However, in these cells Ras did not induce expression from the pRL-null plasmid, which was shown to be a valid internal control in the presence of activated Ras. Oncogenic Ras has also been reported to activate other reporter constructs that contain the HSV-1 TK promoter such as pBLCAT2 and pTK81-luc [50;51], indicating that the pRL-TK will not be useful as an internal control in the presence of activated Ras.

Concluding remarks

In experimental systems where the *Renilla* luciferase activity is either suppressed or induced by one or more of the experimental factors, an alternative means of normalization is needed. In such cases, one could use another *Renilla* luciferase plasmid that is not affected by the factor in question. For example, one could use the promoterless pRL-null plasmid as an internal control in systems in which the plasmid gives expression levels which are well above the assay background [50]. But caution will be needed even in this case, as some factors have been shown to modulate expression from the pRL-null plasmid. Other approaches for normalization that could be considered include the flow cytometric or fluorimetric quantitation of GFP expression [52;53], the measurement of total protein, and the use of the β -galactosidase assay [54]. In summary, it is always advisable to first test the effects of the various experimental factors on the internal control within the context of the planned experimental system before carrying out the actual studies.

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Abbreviations used

HEK293	Human Embryonal Kidney-293
TK	thymidine kinase
HSV	herpes simplex virus
TS	thromboxane synthase
SV40	simian virus-40
GR	glucocorticoid receptor
AR	androgen receptor
IFN	interferon
CMV	cytomegalovirus
sp1	specificity protein-1

DHT	dihydrotestosterone
PMA	phorbol 12-myristate 13-acetate
JNK	Jun N-terminal kinase
ERK	extracellular signal-regulated kinase
PKC	protein kinase C

Fig.1.

For the Jurkat cell experiment, 12 million cells were electroporated per sample with 1 g of pRL-TK and, immediately after transfection, the indicated amount of vitamin D3 diluted in ethanol was added. The cells were harvested 32 hours after transfection and lysed in 250 l of Passive Lysis Buffer (Promega, Madison, WI) and luciferase assay was carried out on 100 l of cell lysate using the Stop & Glo Reagent (Promega, Madison, WI). For the HeLa cell experiment, 300,000 cells were seeded per well on a six-well plate on the day prior transfection and the cells were transfected using Lipofectamine (Invitrogen, Carlsbad, CA) with 20 ng of the pRL-TK plasmid per well. Vitamin D treatment was added 5 hours after transfection. The cells were harvested after treatment for 24 hours and lysed in 200 l of Passive Lysis Buffer; luciferase assay was carried out on 100 l of lysate. Ethanol was added to the 0 nM samples. The Y-axis value RLU refers to relative luminescence units.

Fig. 2.

The promoter elements in the HSV-1 TK insert in the pRL-TK plasmid. The numbers below the horizontal line representing a portion of the HSV-1 TK gene indicate nucleotide positions in relation to the transcription start site which is at +1. The four known transcription factor binding sites on the HSV-1 TK promoter are shown by shaded boxes. The transcription factors that bind to those sites are depicted as ovals. CTF stands for CCAAT-transcription factor and sp1 stands for Specificity Protein-1.

Table 1Experimental factors that suppress expression from *Renilla* luciferase plasmids

Cell line ^a	<i>Renilla</i> Luc. Vector	Promoter	Experimental factor
CV-1[27]	pRL-TK	HSV-1 TK	dexamethasone + GR ^b
	pRL-TS	TS	dexamethasone + GR ^b
L6 [27]	pRL-TK	HSV-1 TK	dexamethasone
HEK293[30]	phRG-TK	HSV-1 TK	15d-PGJ ₂
	pRL-TK	HSV-1 TK	15d-PGJ ₂
	phRG-B	None	15d-PGJ ₂
CV-1 [25]	pRL-TK	HSV-1 TK	PPAR α ^b
			ARP-1 ^b
			HNF-4 ^b
			COUP-TF1 ^b
SK-MEL-28 [29]	phRL-SV40	SV40	IFN α
			IFN β

^a Reference indicated between brackets^b transfected

Table 2Experimental factors that enhance expression from *Renilla* luciferase plasmids

Cell line ^a	<i>Renilla</i> Luc. Vector	Promoter	Experimental factor
MM96L [31]	pRL-TK	HSV-1 TK	sp1 ^b
CV-1 [27]	pRL-TK	HSV-1 TK	DHT +AR ^b
CV-1 [50]	pRL-CMV	CMV	Ras ^b
C3H10T1/2 [42]	pRL-TK	HSV-1 TK	skNAC ^b
COS-7 [39]	pRL-TK	HSV-1 TK	GATA4 ^b
	pRL-SV40	SV40	GATA4 ^b
	pRL-TK	HSV-1 TK	GATA6 ^b
	pRL-SV40	SV40	GATA6 ^b
PC12 [40]	pRL-TK	HSV-1 TK	Nur77 ^b
	pRL-TK	HSV-1 TK	Nurr1 ^b
	pRL-TK	HSV-1 TK	Nor-1 ^b
LNCaP [33]	pRL-TK	HSV-1 TK	R1881
	pRL-TS	TS	R1881
	phRG-TK	HSV-1 TK	R1881
	phRL-TK	HSV-1 TK	R1881
	phRG-B	None	R1881
	phRL-SV40	SV40	R1881
	phRL-CMV	CMV	R1881
Saos-2 [41]	pRL-TK	HSV-1 TK	E1A ^b
LNCaP [34]	pRL-SV40	SV40	WT1 ^b
	pRL-SV40	SV40	R1881
	pRL-null	none	WT1 ^b
	pRL-null	none	R1881+WT1 ^b
NIH 3T3 [35]	pRL-TK	HSV-1 TK	HTLV-1 Tax ^b
HeLa [35]	pRL-TK	HSV-1 TK	HTLV-1 Tax ^b
COS-7 [35]	pRL-TK	HSV-1 TK	HTLV-1 Tax ^b
HEK293 [43]	pRL-TK	HSV-1 TK	PMA

^aReference indicated between brackets^btransfected

Table 3Experimental factors that do not affect expression from *Renilla* luciferase plasmids

Cell line ^a	<i>Renilla</i> Luc. Vector	Promoter	Experimental factor
CV-1 [27]	pRL-TS	TS	DHT + AR ^b
L6 [27]	pRL-TS	TS	dexamethasone
LLCPK1 [27]	pRL-TS	TS	dexamethasone + GR ^b
HepG2 [27]	pRL-TS	TS	dexamethasone
Jurkat [27]	pRL-TS	TS	dexamethasone
CV-1 [50]	pRL-null	none	Ras ^b
MCF-7 [14]	pRL-TK	HSV-1 TK	All- <i>trans</i> retinoic acid
	pRL-SV40	HSV-1 TK	All- <i>trans</i> retinoic acid
PC12 [40]	phRG-B	none	Nurr ^b
MCF-7 [33]	phRL-TK	HSV-1 TK	R1881+AR ^b
SW480 [33]	phRL-TK	HSV-1 TK	R1881+AR ^b
PC3 [33]	phRL-TK	HSV-1 TK	R1881+AR ^b
	pRL-TK	HSV-1 TK	R1881+AR ^b
	pRL-TS	TS	R1881+AR ^b
LNCaP [34]	pRL-null	none	R1881
PC3 [34]	pRL-null	none	WT1 ^b
HEK293 [34]	pRL-null	none	WT1 ^b
SK-MEL-28 [29]	phRL-SV40	SV40	IFN γ
HCS-2/8 [35]	pRL-TK	HSV-1 TK	HTLV-1 Tax ^b
HeLa [43]	pRL-TK	HSV-1 TK	PMA

^aReference indicated between brackets^btransfected