A procedure to standardize CAT reporter gene assay

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To assay eukaryotic DNA sequences for their transcriptional regulatory activity, these sequences are routinely linked to a reporter gene (e.g., CAT gene coding for chloramphenicol acetyltransferase) transfected into cells and transient expression of the reporter gene is monitored (1). For comparison cotransfection with a second reporter gene, e.g. the gene coding for β-galactosidase is frequently done (2). Expression of the cotransfected control plasmid, however, is likely to be modulated by transacting enhancer or silencer DNA elements. This potential artefact was reported recently (3).

Here, we show that the level of CAT activity can be normalized to the amount of CAT plasmid DNA introduced into recipient cells by DNA transfection. Cells of a human B-cell line were transfected with various recombinant CAT DNA constructs using DOTAP reagent (Boehringer Mannheim). Two days after transfection, cells were extensively washed and equal numbers of cells were lysed. To determine the amount of CAT enzyme synthesized, 135 µl of the crude cell lysate (150 µl) were subjected to CAT ELISA (Boehringer Mannheim). For monitoring the level of plasmid DNA actually transferred into recipient cells, 15 µl of the lysate were incubated with RNase A (100 µg/ml), and subsequently with Proteinase K (100 µg/ml), each for 30 min at 37°C. Two volumes of 20×SSC were added, the DNA was dotted in a series of dilutions onto Hybond N membrane (Amersham Buchler), and hybridized to ^{32}P-labeled pCAT DNA as probe (Figure 1). Intensities of the hybridization signals indicate the amount of transferred plasmid DNA present in crude cell extract compared to a standard. The level of CAT enzyme (determined by CAT ELISA) or of CAT activity (monitored by thin layer chromatography of reaction products) is now normalized to the relative amount of transferred CAT DNA (Table 1). Small amounts of CAT plasmid DNA in the transfection mixture result in low amounts of DNA introduced into the cells (Figure 1, lanes 4–6). However, the quotient of the CAT enzyme level present in the cell extract relative to the amount of CAT plasmid DNA transferred into the cells is constant under these conditions. Obviously, this procedure is done under the condition that cells with similar growth parameters and cultures with the same cell densities are transfected. On the other hand, this protocol does not need cotransfection of a second plasmid and, therefore, does not exhibit the potential artefact in determination of transacting enhancer or silencer activities.

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Table 1. Standardization of CAT assays after transfection of various CAT plasmids into human lymphoid cells

plasmid DNA transfected	amount of DNA transfected (µg)	relative amount of DNA transferred	CAT enzyme per cell extract (pg)	normalized CAT expression
pMMTV-CAT	5.0	<0.03	12.5	>416
pfos-CAT	5.0	0.1	10.0	100
pCAT	5.0	0.33	1.5	4.5
pHCMV-CAT	5.0	1	38.0	38
pHCMV-CAT	1.0	0.45	19.0	42
pHCMV-CAT	0.5	0.33	13.0	43

Human lymphoid cells (10^7 cells) were transfected with the amount of DNA indicated. Cell extracts were prepared and the DNA was dotted in series of 3-fold dilutions onto nylon membrane. As shown in Figure 1, hybridization with CAT DNA as a probe reveals plasmid DNA actually transferred into lymphoid cells and present in the cell extracts. Hybridization intensities indicate the amount of transferred plasmid DNA compared to a standard. The cell extract with the highest amount of transferred plasmid DNA was used as relative standard within this series of transfection assays and was set equivalent to 1 (Figure 1, lane 4). CAT expression (determined by CAT ELISA) is then normalized to the relative amount of transferred DNA.

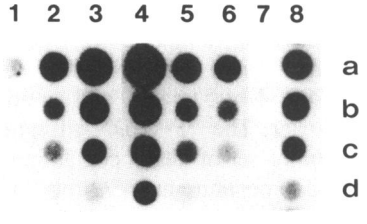

Figure 1. DNA dot blot analysis of transfected CAT plasmid DNA. Human lymphoid cells (10^7 cells) were transfected with DNA (5 µg each) of different CAT plasmids: pMMTV-CAT (lane 1), pfos-CAT (lane 2), pCAT (lane 3). Cells transfected with pMMTV-CAT were incubated in presence of dexamethasone (10^{-6} M). Furthermore, 10^7 cells were transfected with various amounts of pHCMV-CAT DNA (5 µg; 1 µg; 0.5 µg) (lanes 4–6). DNA of cell extracts was transferred in serial dilutions (30 µl, 10 µl, 3 µl, 1 µl of diluted cell extract; lanes a–d) onto Hybond N membrane. As controls, lambda DNA (lane 7) and pCAT DNA (lane 8) were dotted as well (500 pg, 250 pg, 125 pg, 60 pg; lanes a–d). The membrane was hybridized to ^{32}P-labeled pCAT DNA as probe.