SELECTION OF A CONTROL REPORTER VECTOR FOR THE DUAL-LUCIFERASE REPORTER ASSAY FOR TRANSCRIPTION ACTIVATION

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Reporter vectors are used in transient transfection experiments to study the effect of transcription factors on gene expression. The experimental reporter vector containing tested target elements for transcription factors is usually co-transfected together with a second reporter vector, which is used as an internal control of transfection efficiency. Normalization of the levels of transcription of the experimental reporter gene to the internal control reporter gene minimizes the variability of the obtained results caused by differences in the transfection efficiency between different samples of transfected cells. Co-transfection with other vectors or applied treatments should not affect the activity of the control reporter. For this reason, an appropriate control reporter vector should be chosen carefully. The Dual-Luciferase Reporter (DLR) assay system offers the sequential measurement of activities of experimental and control reporters in the same cell lysates, obtained from cells co-transfected with two reporter vectors encoding distinct reporter luciferases: firefly luciferase (encoded by the experimental vector); and \textit{Renilla} luciferase (encoded by the control vector). There are several choices for commercially available vectors containing the \textit{Renilla} luciferase gene, and it should be determined which vector works well in a given study. We showed that for the orphan nuclear receptors of the Nur77 family, phRG-B (a promoterless reporter vector containing the synthetic \textit{Renilla} luciferase gene) is a better alternative than pRL-TK (a vector containing the native \textit{Renilla} luciferase gene under the transcriptional control of the herpes simplex virus thymidine kinase promoter region), because the proteins of the Nur77 family affect the expression of luciferase encoded by the reporter gene of pRL-TK but not the phRG-B internal control vector.