

Transcription Factors GFC-Transfection Array

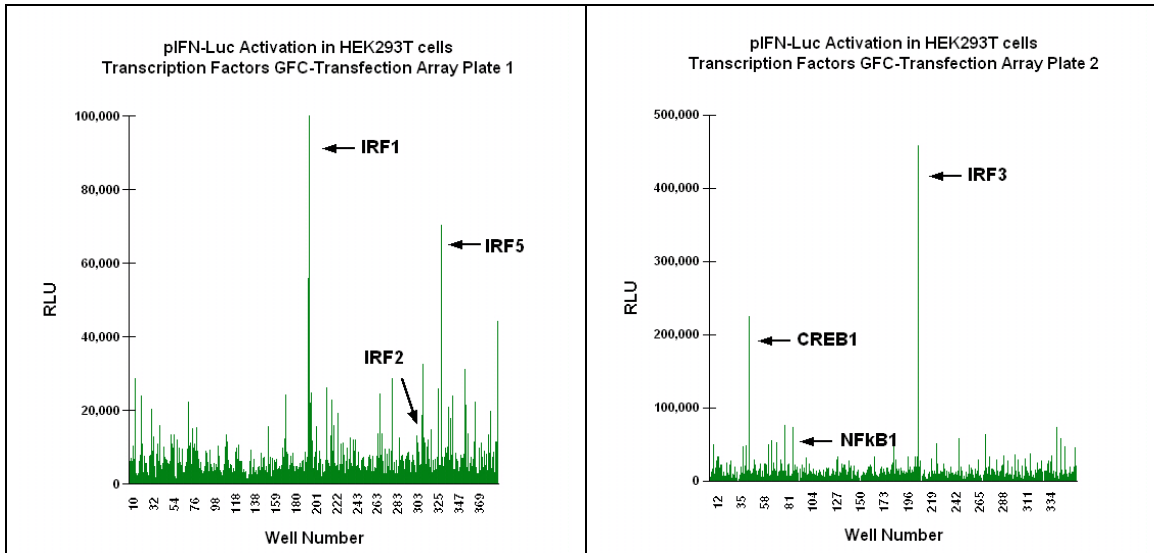


Figure 1. Effects of genes from the Transcription Factors GFC-Transfection Array on the activity of the Interferon- β 1-promoter (pIFN-luc) measured by a reporter gene assay.

The Transcription Factors GFC-Transfection Array was tested for activation of a reporter plasmid containing the Interferon- β 1 promoter driving expression of luciferase (pIFN-Luc). The array was reverse transfected to HEK293T cells using TurboFectin 8.0, and luciferase activity was scored 48 hours later using BriteLite luciferase substrate (Perkin Elmer). Luciferase activity for each of the cDNAs in the array is plotted against the sample number. Several of the cDNAs known to activate the pIFN-Luc reporter are indicated. Additional genes (known and novel interactors) that activate the IFN- β 1 promoter or inhibit its intrinsic activity were also detected and are indicated on the graph.

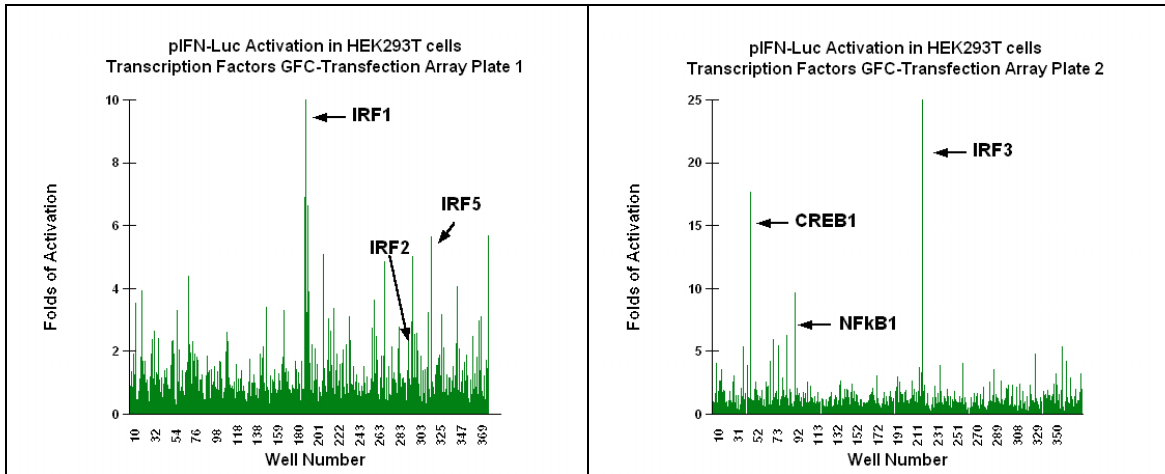


Figure 2. Summary of six (6) independent pIFN-luc reporter gene assays with the Transcription Factors GFC-Transfection Array

High throughput screening assays generate a large sum of data. To reduce the noise from assay-to-assay variation, results are best presented in terms of fold activation (reporter gene activity divided by the activity of the control). To increase the accuracy of an assay, and to reduce the number of false positive or false negative results, the assay should be repeated in several (3 or more) independent assays. Genes that repeatedly show activation higher (or lower) than the determined threshold are likely to be true modifiers.

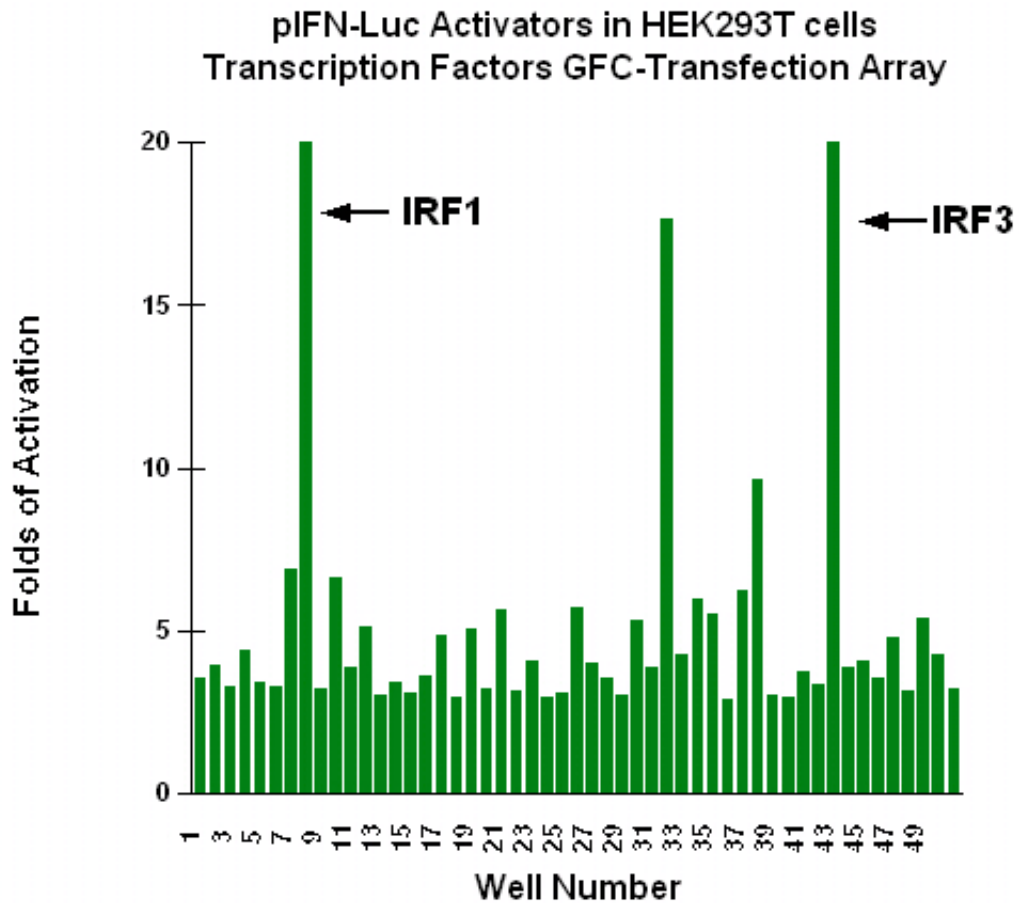


Figure 3. Positive modifiers (activators) lead of the pIFN promoter in HEK293T cells from the Transcription Factors GFC-Transfection Array

The Transcription Factors GFC-Transfection array was used in 6 independent reporter gene assays with pIFN-luciferase in HEK293T cells. Of the 704 genes tested, fifty-one (51) activated the IFN promoter by more than 3 folds (Fig 8-3a). Two clones activated the reporter by 10-20 folds and two others by more than 60 folds. The activator genes include both known and novel activators of the IFN pathway.

pIFN-Luc Negative Modulators in HEK293T cells
Transcription Factors GFC-Transfection Array

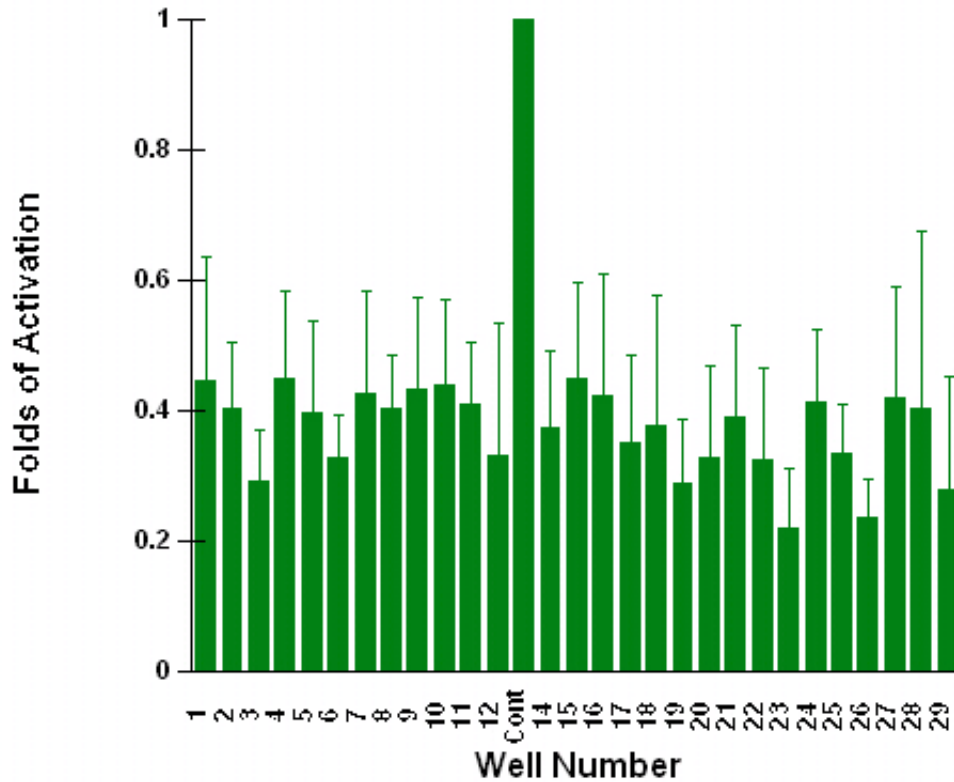


Figure 4. Negative modifiers (inhibitors) leads of the pIFN promoter in HEK293T cells from the Transcription Factors GFC-Transfection Array

Clones in the Transcription Factors GFC-Transfection Array can also down regulate the activity of the reporter gene. A reporter assay with pIFN-luciferase revealed 28 clones that reproducibly inhibit the intrinsic activity of the Interferon- β 1 promoter in HEK293T cells by more than 50%. The results are a summary of 6 independent reporter gene assays with corresponding standard deviation.

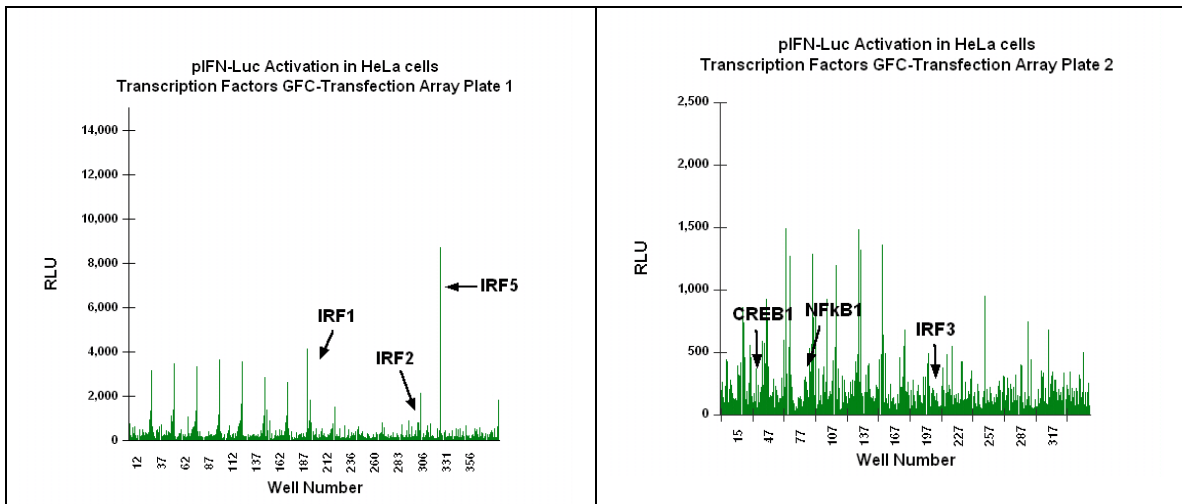


Figure 5. Effects of genes from the Transcription Factors GFC-Transfection Array on the activity of the Interferon- β 1-promoter (pIFN-luc) measured in HeLa cells by a reporter gene assay.

The Transcription Factors GFC-Transfection Array was tested for activation of the pIFN reporter gene plasmid in HeLa cells. In these cells, the activation was much lower than in HEK293T cells, with the highest activation given by IRF5, followed by IRF1 and IRF2. Thus, the results of the same assay can vary significantly depending on the cell type used and its genetic background.

Activations of a non-mammalian promoter in reporter gene assay using the Transcription Factors GFC-Transfection Array

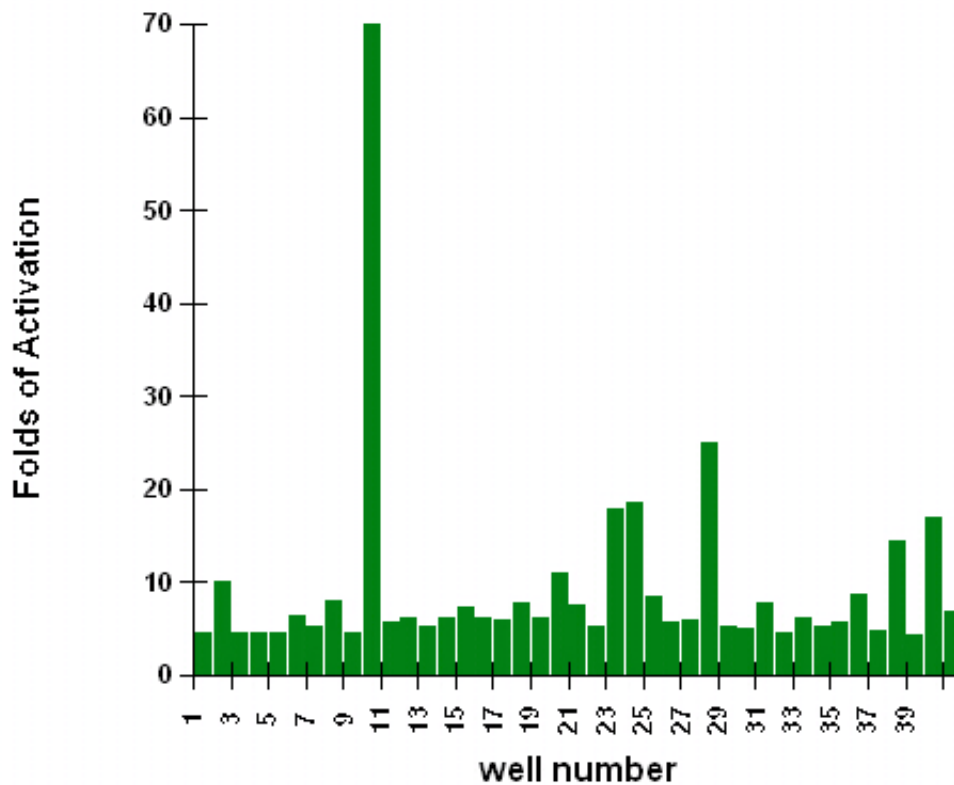


Figure 6. Effects of genes from the Transcription Factors GFC-Transfection Array on the activity of a non-mammalian promoter measured in HEK293T cells by a reporter gene assay.

The Transcription Factors Array can be used for screening of promoters or genes of non-human origin for which no activators are known. In this assay, we used the Transcription Factors Array in three independent experiments looking for activation of a non-mammalian promoter. Very few transcription factors are known in this organism, and none are known to activate this promoter. Using the GFC-Transfection Array, we identified several potential activators of the promoter including one cDNA that increased reporter activity by over 70 folds. These findings will lead to further inquiry and discovery of important elements in promoters of this organism, and provide clues to identify homologous transcription factors from this organism. Without the use of GFC-Transfection Arrays, it will be extremely difficult to obtain such discoveries.