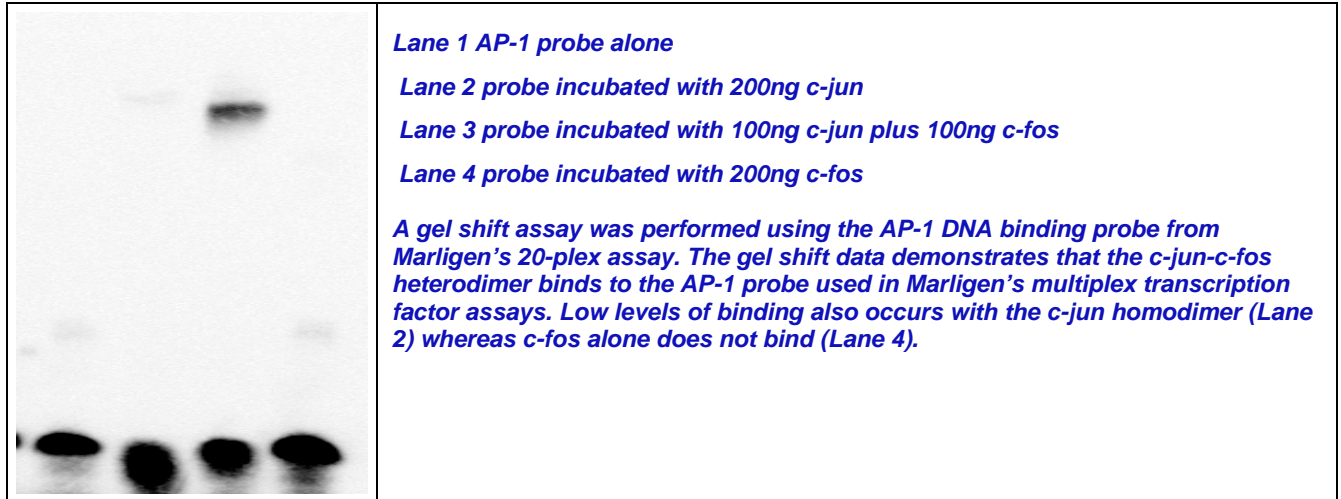


DNA BINDING SITE INFORMATION FOR MARLIGEN'S MULTIPLEX TRANSCRIPTION FACTOR ASSAYS

AP-1 (also called TRE/AP-1, TPA Response Element or Activating Protein 1)

The 12-O-Tetradecanoyl Phorbol 13-Acetate Response Element/Activating Protein 1 (AP-1) site 5'-TGAGTCA-3' binds homo- and hetero-complexes of transcription factor proteins from the AP-1 family. The AP1 family is composed of the subfamilies Jun, Fos, Maf, NF-E2, and CRE-BP/ATF; the most archetypical AP-1 complex is a c-Jun/c-Fos heterodimer. AP-1 modulates the expression of many genes regulation in response to different physiological and pathological stimuli, including cytokines, growth factors, stress signals, bacterial and viral infections, as well as oncogenic stimuli. Studies in genetically modified mice and cells have highlighted a crucial role for AP-1 in a variety of cellular events involved in normal development or neoplastic transformation causing cancer. AP-1 binding proteins are targets considered within the pharmaceutical industry.



AP2 (Activator Protein 2, Activating Enhancer Binding Protein 2)

The Activating Protein 2 (AP2) binding site 5'-TAGCCCCCAGGCGAT-3' binds AP2 proteins. Activating Enhancer Binding Protein 2, Activator Protein 2, Transcription Factor AP2 (TFAP2) can act as an activator or repressor depending on the promoter it binds. TFAP2, also referred to as AP2, has 3 forms: α , β , γ . The AP2 proteins bind DNA as homo- or heterodimers with each other and are involved in cell differentiation and development.

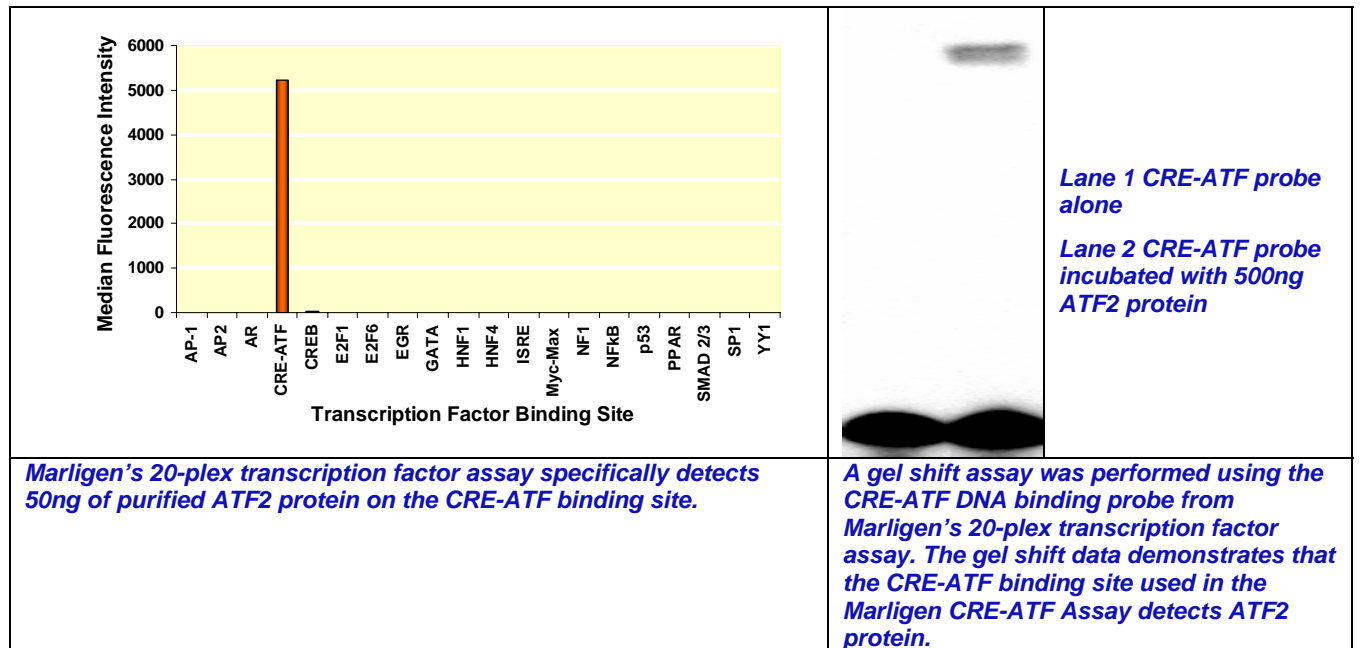
AR (Androgen Receptor)

The Androgen Receptor (AR) site 5'-GGTACAGGGTGTCT-3' site termed ARE (Androgen Response Element) binds AR. AR (NR3C4) is a steroid hormone nuclear receptor activated by androgens (i.e. testosterone, dihydrotestosterone). In the absence of ligand, the androgen receptor may weakly associate with nuclear components but hormone binding greatly increases receptor affinity. The hormone-receptor complex binds to DNA sequences upstream of transcriptional start sites to control the development, differentiation, and function of male reproductive and accessory tissues. A well-established target of the androgen receptor is the gene for IGF-1. The androgen receptor also appears affect rapid changes in the transcription of genes independent of its ability to interact with DNA. Direct interactions of the androgen receptor with cytoplasmic proteins have been reported. Specifically, the androgen receptor may affect ion transport as well as the phosphorylation state of other transcription factors. Androgen receptor defects are associated with a variety of diseases including androgen insensitivity syndrome, X-linked spinal and bulbar muscular atrophy, infertility male syndrome, Reifenstein syndrome, and possibly metastatic prostate cancer.

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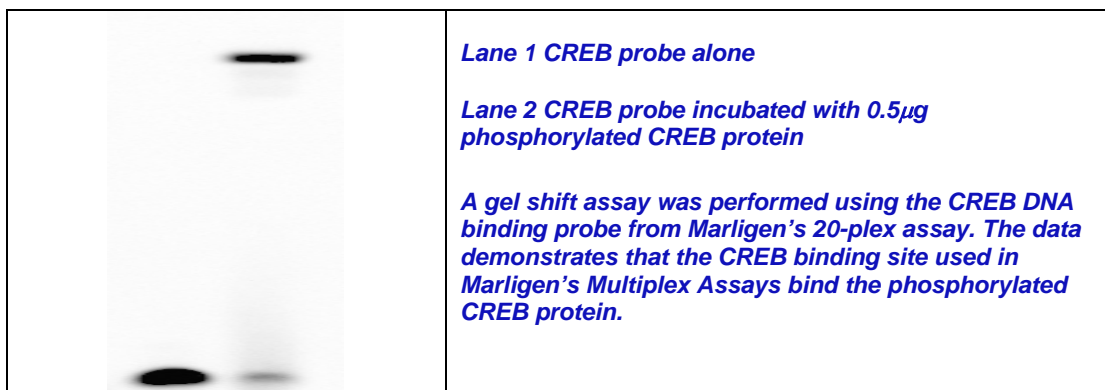
CRE-ATF1 (also called Cyclic AMP Response Element or Activating Transcription Factor 1)

The cyclic AMP Response Element/Activating Transcription Factor 1 (CRE-ATF1) site 5'-TGACGTCA-3' will bind many proteins from the AP-1 and CREB family. These proteins can form homo- and heterodimers on this site. Activating Transcription Factor 1 (ATF1) is a transcriptional activator, and one of many transcription factors that bind to this regulatory element.



CREB (cyclic AMP Response Element Binding Protein)

The CREB assay detects transcription factors that have been shown to be responsive to intracellular levels of cyclic AMP. This cyclic AMP Response Element Binding Protein (CREB) site 5'-TTACGTAA-3' complexes with homo- and heterodimers and multimers that can form using combinations of a variety of proteins including CREB, CRE-BP, and ATF proteins from both the AP1 and CREB families. There are many factors that determine the binding specificity and function of CREBs, including the presence of co-activator proteins and phosphorylation state of CREB proteins. The CREB binding proteins mediate cAMP, growth-factor-dependent and calcium-dependent gene expression via binding to this site. The CREB binding element is an important indicator of signals propagated by hormones, growth factors, and neurotransmitters. CREB binding proteins also function in growth-factor-dependent cell survival, glucose homeostasis, and in learning and memory.



E2F1-5 (Adenovirus E2 Gene Binding Factors 1-5)

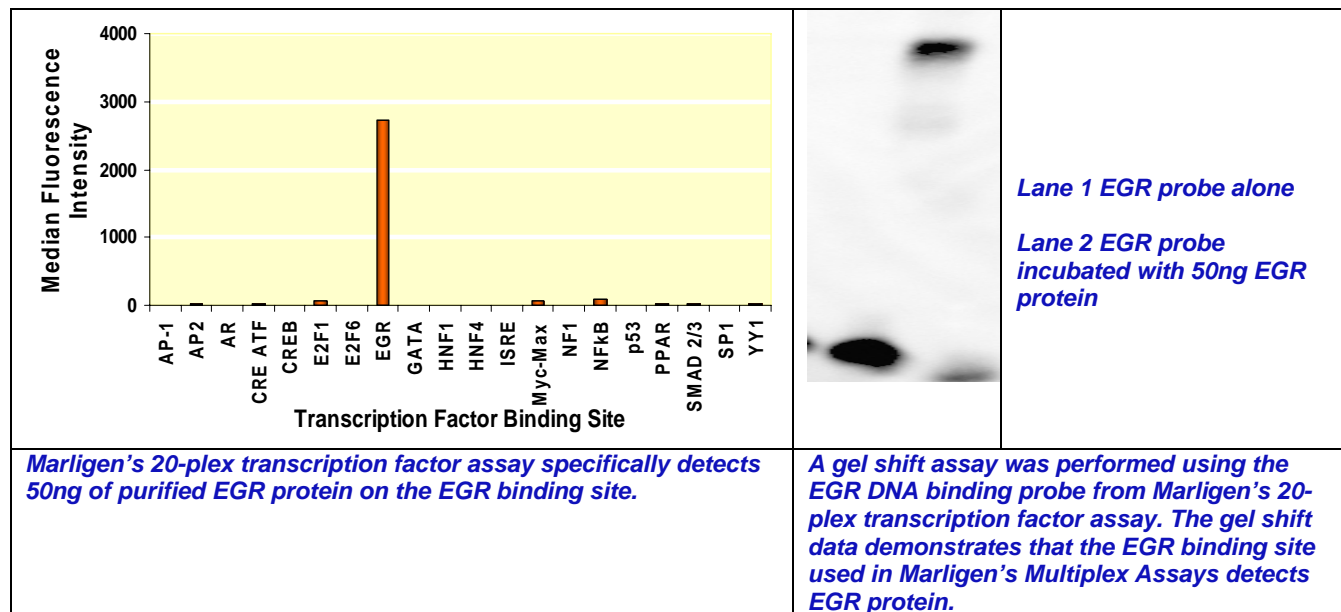
The Adenovirus E2 Gene Binding Factors 1-5 (E2F1-5) site 5'-TTTCCGCGC-3' recognizes E2F1-5 of the E2F family. E2Fs are a family of transcription factors involved in cell cycle regulation that include E2Fs1-8. E2Fs 1-5 bind DNA as homo- or heterodimers with DP1/2. Interactions with Retinoblastoma protein (RB1) and other related proteins (p130, p107) determine the function of E2F proteins.

E2F6 (Adenovirus E2 Gene Binding Factor 6)

The Adenovirus E2 Gene Binding Factors 6 (E2F6) site 5'-TTTCCC GC-3' is another form of the E2 site that preferentially binds the E2F6 protein. E2F6 is a transcription factor from the E2F family involved in cell cycle regulation. It is considered that in many cases E2F6 works antithetically to E2F1-5 having opposing effects on cell cycle regulation or repressing the expression of genes transactivated as a consequence of E2F1-5 expression.. E2F6 binds DNA as homo- or heterodimers with DP1/2. Interactions with the Retinoblastoma protein (RB1) and other related proteins (p130, p107) determine the activation state of E2F6.

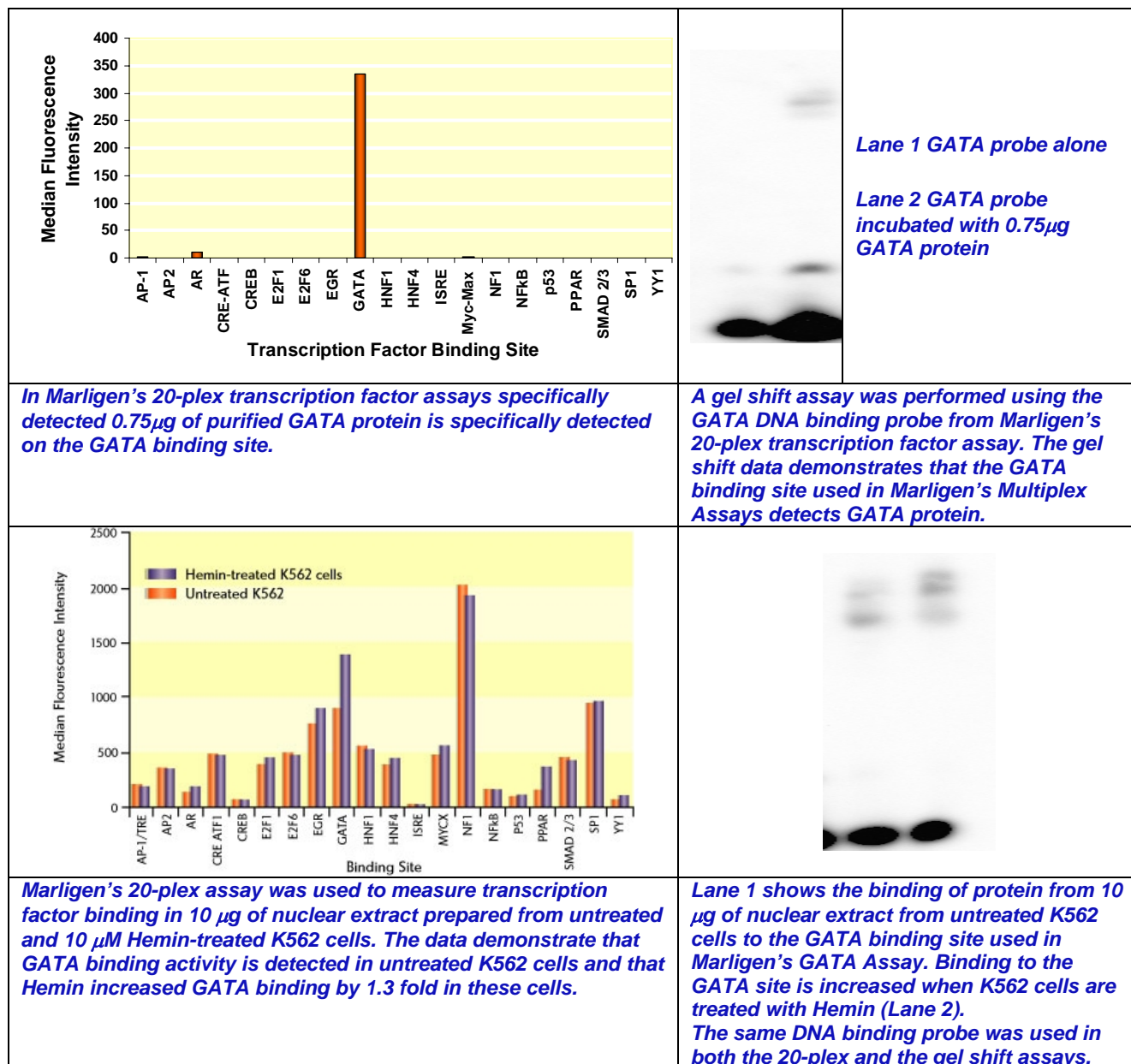
EGR (Early Growth Response)

The Early Growth Response (EGR) site 5'-CGCCCCGC-3' recognizes proteins from the EGR family of transcriptional activators. Four EGR proteins are well established: Egr1 (ZNF225, ZIF268, NGFI-A, Krox-24); Egr2 (Krox-20); Egr3; and Egr4 (NGFI-C). EGR-1 and EGR-2 are nuclear proteins with zinc finger DNA binding domains closely resembling that of the well-characterized transcription factor, SP-1. In several cases binding sites for EGR overlap or coincide with those for SP-1. One function of Egr proteins may be to downregulate promoters by competing with Sp1 for binding to overlapping regulatory elements. EGR-1 is induced by PMA and contributes to the up-regulation of the megakaryocyte-specific gene CD41a. EGR1 is an immediate early gene in T-cell activation and can regulate transcription synergistically with NF-ATc. A connection of EGR proteins with Wilms' tumors has been described.



GATA (Globulin Transcription Factor)

The Globulin Transcription Factor (GATA) site 5'-TCAGATAAGA-3' binds GATA transcription factors. GATA transcription factors are transcriptional activators that function as a family of genes with specific cell and tissue distribution. There are at least 6 forms of GATA proteins. Different tissue specificity has been shown for each GATA form. GATA1 is found in megakaryocytes and erythroid cells like K562; GATA2-3 are found in endothelial cells and T-lymphocytes including Jurkat and HL-60 cells; GATA4-6 are found in the heart, smooth muscle tissue, and gastrointestinal tissues.



HNF1 (Hepatocyte Nuclear Factor 1)

The Hepatocyte Nuclear Factor 1 α (HNF1 α) site 5'-GTTAAT-3' binds HNF1 proteins. HNF1 α is also referred to as TCF1 (Transcription Factor 1), LF-B1 (Liver-specific Transcription Factor 1), MODY3 (Maturity Onset Diabetes of the Young 3), & APF (Albumin Proximal Factor). HNF1 α forms homo- or heterodimers on DNA with HNF1 β (TCF2, LF-B3, MODY5, HNF2, or vHNF1) and functions as an activator in intestine and liver specific genes.

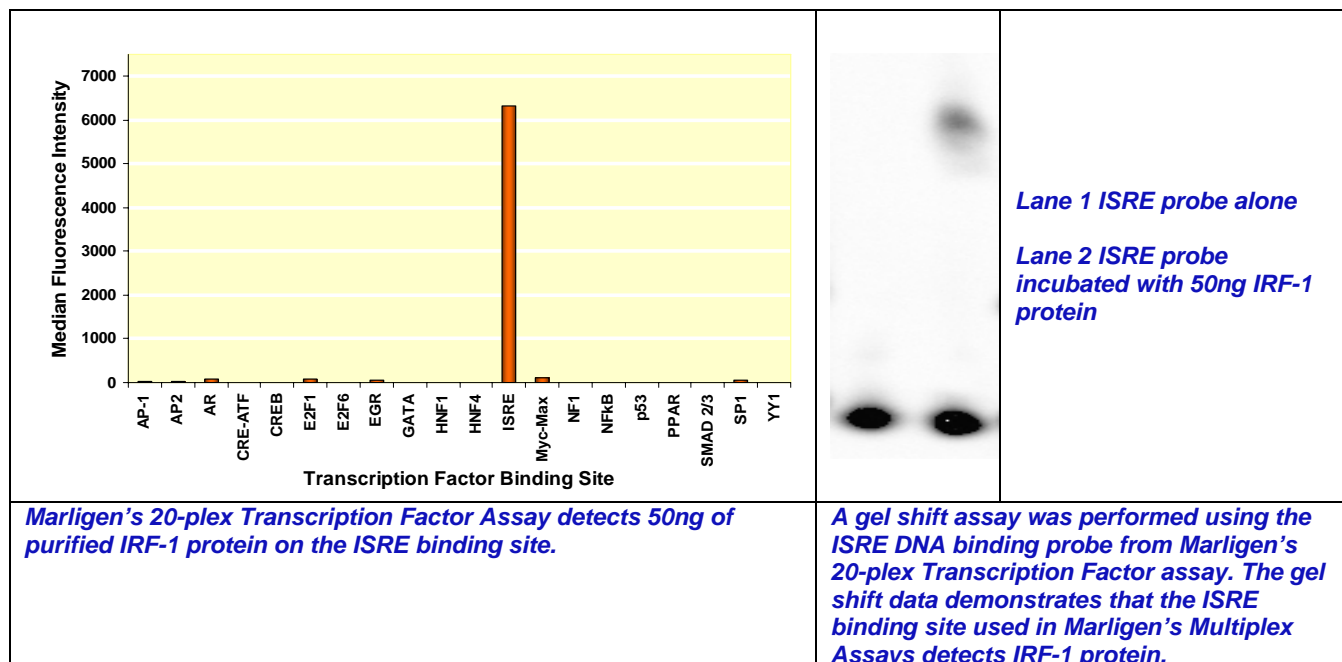
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HNF4 (Hepatocyte Nuclear Factor 4)

The Hepatocyte Nuclear Factor 4 (HNF4) site 5'-AGGTCA-3' binds HNF4 proteins. HNF4 is a nuclear orphan receptor that binds to DNA as homo- or heterodimers with PPAR gamma, SMAD2/3 or RXR. There are 9 variants of HNF4 that include α , β , and γ . HNF4 is also known as NR2A1, MODY1 (Maturity Onset Diabetes of the Young 1), and TCF14 (Transcription Factor 14). HNF4 is an activator that functions with HNF1 in liver and intestine to regulate specific gene expression.

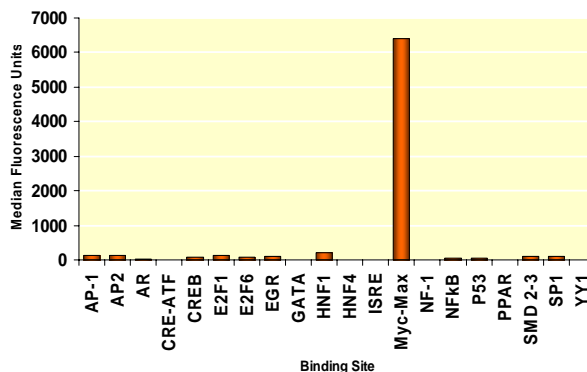
ISRE (Interferon Stimulated Response Element)

The Interferon Stimulated Response Element (ISRE), 5'-GAAAATGAAACT-3', is activated in response to binding of Interferon Regulatory Factors (IRFs) induced by interferon. There are 9 different IRFs: IRFs 1,2,3,4 (Liver-specific IRF-LSIRF), 5, 6, 7, 8 (ICSBP) & 9 (ISGF3 γ p48). ISGF3 (Interferon Stimulated Gene Factor 3) binds ISRE as a heterodimer of ISGF3 α (heterodimer of STAT1-p91&2-p84) and ISGF3 γ (IRF9-p48). IRFs can function as activators and repressors and bind ISRE as homo- and heterodimers. The ISRE site is similar to GAS (Gamma Interferon Activated Sequence) and ICS (IRF Consensus Sequence).

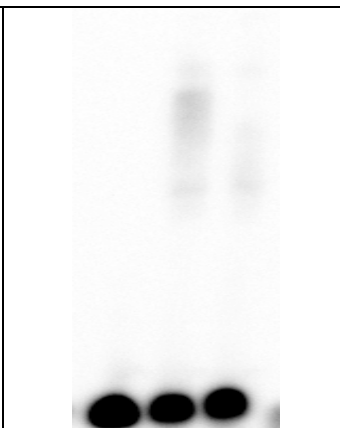
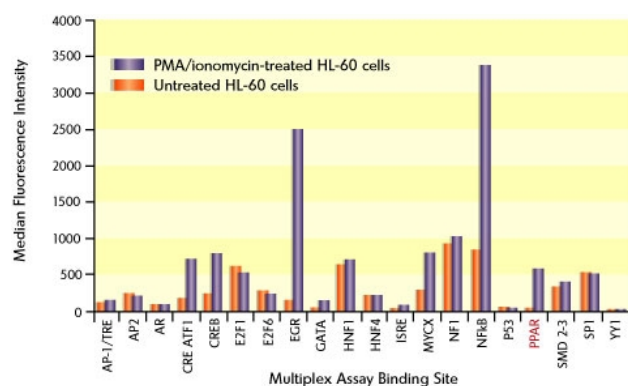


Myc-Max

The Myc-Max site 5'-ACCACGTGGT-3' binds transcription factor complexes containing c-Myc/Max proteins. Different transcription factors from the bHLH-ZIP class of proteins can interact with Myc or Max to form homo- or hetero-complexes that bind with varying affinity to the Myc-Max binding site. Examples of proteins that may participate in complexes localized on the myc-max site include: Myc (c-Myc, N-Myc, L-Myc, B-Myc, and v-Myc) and Mad/Max (Max1&2, Mad1-4, and Mxi1.) It is widely accepted that the Myc-Max binding proteins regulate genes associated with the cell cycle. Activation of myc genes also has been associated with cancer and there is strong evidence that myc has a major role in the pathogenesis of Burkitt's lymphoma.



Pure max protein specifically binds to the Myc-Max DNA binding site when assayed on Marligen's 20-plex Assay.



Lane 1; Myc-Max probe alone

Lane 2 Myc-Max probe incubated with 10 µg of nuclear extracts from PMA/ionomycin-treated HL-60 cells

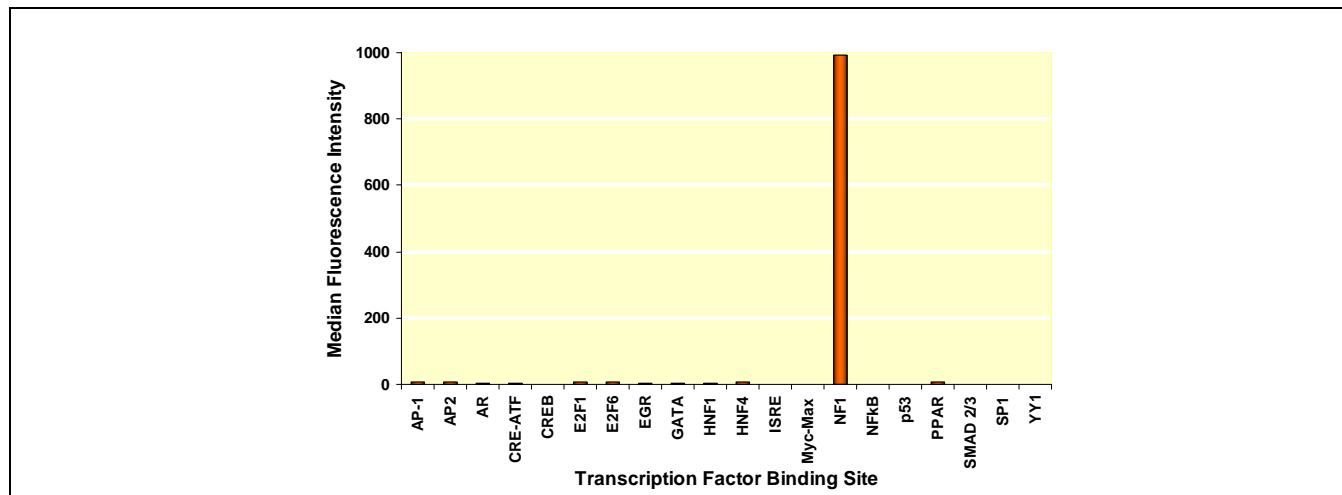
Lane 3 Myc-Max probe incubated with untreated HL-60 cells

Marligen's 20-plex assay was used to measure transcription factor binding in 10 µg of nuclear extract prepared from untreated and HL-60 cells treated with 100nM PMA plus 500 nM ionomycin for 24 hours. The data demonstrate that Myc-Max binding activity in HL-60s is increases with this treatment. The Myc-Max site is labeled MYCX in the graph above.

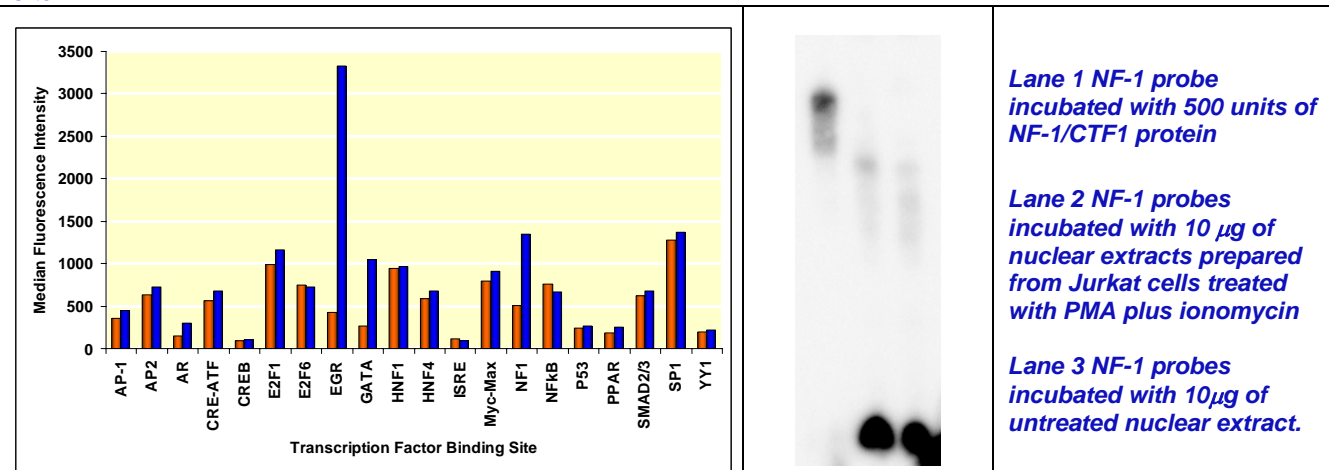
Data shows that there is an increase in DNA binding to the Myc-Max site with PMA/ionomycin treatment. The same DNA binding probe was used in Marligen's multiplex assay and the gel shift assay.

NF-1

The core nuclear factor-1 (NF-1/CTF-1) binding site 5'-TGGNNNNNGCCAA-3' binds to proteins of the NF-1 family. Currently there are 4 genes (NF-1A, NF-1B, NF-1C and NF-1X) known to contribute diversity within the NF-1 gene family. At least 6 splice variants of NF-1C also have been identified. NF-1 proteins are transcriptional activators facilitate assembly of basal transcription complexes through direct interaction with TFIIB. NF-1 is expressed in a wide variety of cell types with the exception of B-cells and T-cells. Chick embryo fibroblasts overexpressing NF-1 proteins are resistant to transformation by the nuclear oncogenes jun, fos, junD, myc and qin but are readily transformed by cytoplasmic oncogenes such as src, raf, ras and fps.



Marligen's 20-plex Transcription Factor Assay detects 6 units of purified NF-1/CTF-1 protein on the NF-1 binding site.



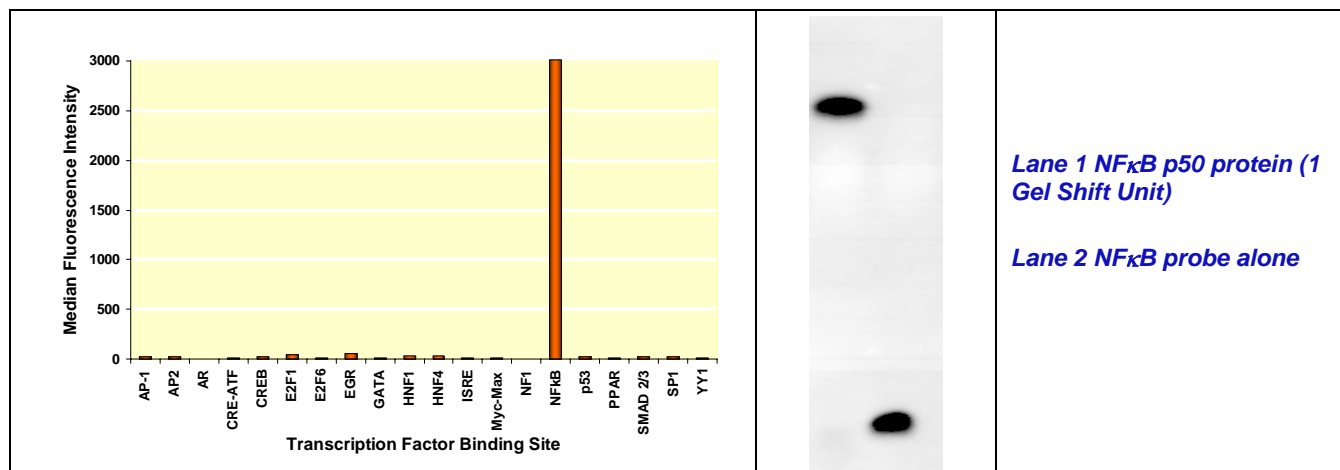
Marligen's 20-plex assay was used to measure transcription factor binding in 10 µg of nuclear extract prepared from untreated and PMA/ionomycin-treated Jurkat cells. The data demonstrate that NF-1 binding activity was increased 2 fold in these cells with PMA and ionomycin treatment.

Gel shift assays were performed using the NF-1 DNA binding probe from Marligen's 20-plex assay. The gel shift data demonstrated that the NF-1 probe used in Marligen's assay binds the NF-1/CTF1 protein. The data also shows that an increase in binding to this site is observed when Jurkat cells are stimulated with PMA/ionomycin.

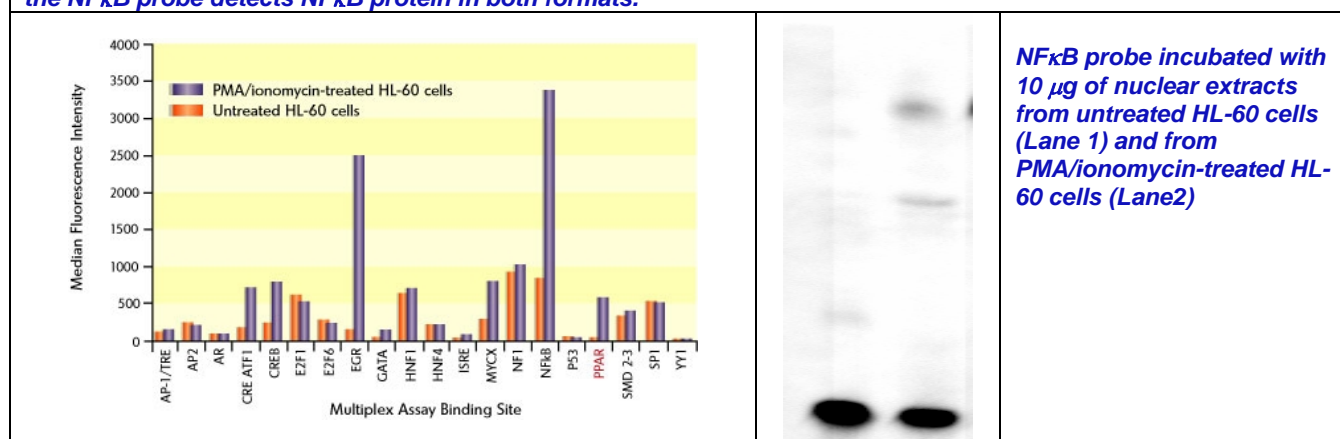
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NFκB (NUCLEAR FACTOR KAPPA BETA)

Complexes comprising homo or heterodimers of proteins from the NFκB or rel family will bind to the NFκB site (5'-AGGGGACTTTCCCA-3'). Transcription factors of the Rel NFκB family are ubiquitous in cells and are activated in response to signals that affect cell growth, differentiation, inflammation and apoptosis. These proteins are critical in the regulation of immune responses. NFκB transcription factors are induced by many stimuli such as TNF or PMA that affect PKA or PKC mediated signals. The NFκB transcription factor was originally identified as a protein complex consisting of a 65 kDa DNA binding subunit and an associated 50 kDa protein. Findings that NFκB proteins are connected to various signaling pathways that affect important biological responses have made them a high priority as pharmaceutical targets.



The left panel shows a gel shift assay of recombinant p50 protein binding to Marligen's NFκB probe. The right panel shows 0.4 GSU of the same p50 protein measured on Marligen's NFκB Assay. The data demonstrates that the NFκB probe detects NFκB protein in both formats.



Marligen's 20-plex assay was used to measure transcription factor binding in 10 μg of nuclear extract prepared from untreated and PMA/ionomycin-treated HL-60 cells. The data demonstrate that NFκB binding activity was increased 3 fold in these cells with PMA and ionomycin treatment.

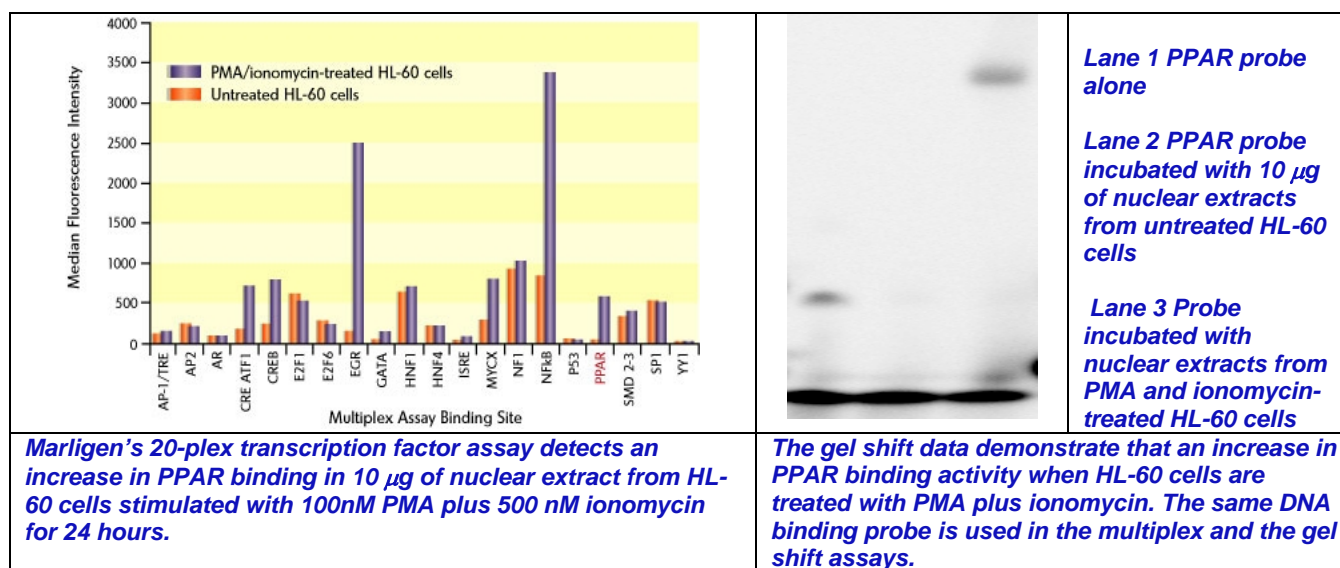
The gel shift data shows that an increase in binding to the NFκB binding site is observed when HL-60 cells are treated with PMA/ionomycin.

p53 (Tumor Protein p53, TP53)

The Tumor Protein p53 (p53) site 5'-GGGCATGT-3' binds homotetrameric p53 protein which activates gene transcription. High levels of wild type p53 is known to cause cell cycle arrest or apoptosis functions. P53 is considered to be a tumor suppressor gene and a high frequency of P53 mutation is associated with cancer. Consistent with a role for P53 as a tumor suppressor gene is that cells or tissues lacking functional P53 are genetically unstable and more prone to tumours.

PPAR (Peroxisome Proliferator-Activated Receptor)

The Peroxisome Proliferator-Activated Receptor (PPAR) site also known as the Peroxisome Proliferation Response Element (PPRE) 5'-TGACCTTGACCT-3' binds transcription factors from the PPAR family as homo- and heterodimers. Three PPAR isotypes called PPAR-alpha, PPAR-beta/delta and PPAR-gamma have been identified. The PPARs have a DNA binding domain and a ligand-binding domain that has specificity for prostanoids, fatty acids, fibrates, and thiazolidinediones. Once activated by ligand binding, PPARs bind to DNA at peroxisome proliferator response elements (PPREs) within genes and modulate transcription. The PPARs display differential tissue distribution with PPAR-alpha and PPAR-gamma having involvement in the pathogenesis of chronic diseases such as diabetes, obesity and atherosclerosis. There is substantial evidence that different ligands may determine the specificity of PPARs interaction with particular coactivators, and drugs such as the hypolipidaemic fibrates and the insulin sensitizing thiazolidinediones (pioglitazone and rosiglitazone). Less is known regarding PPAR-beta/delta, but it is implicated in embryo implantation, tumorigenesis in the colon, reverse cholesterol transport, and recently in skin wound healing. PPAR is a target for many biotechnology and pharmaceutical companies because of its involvement in many chronic diseases.

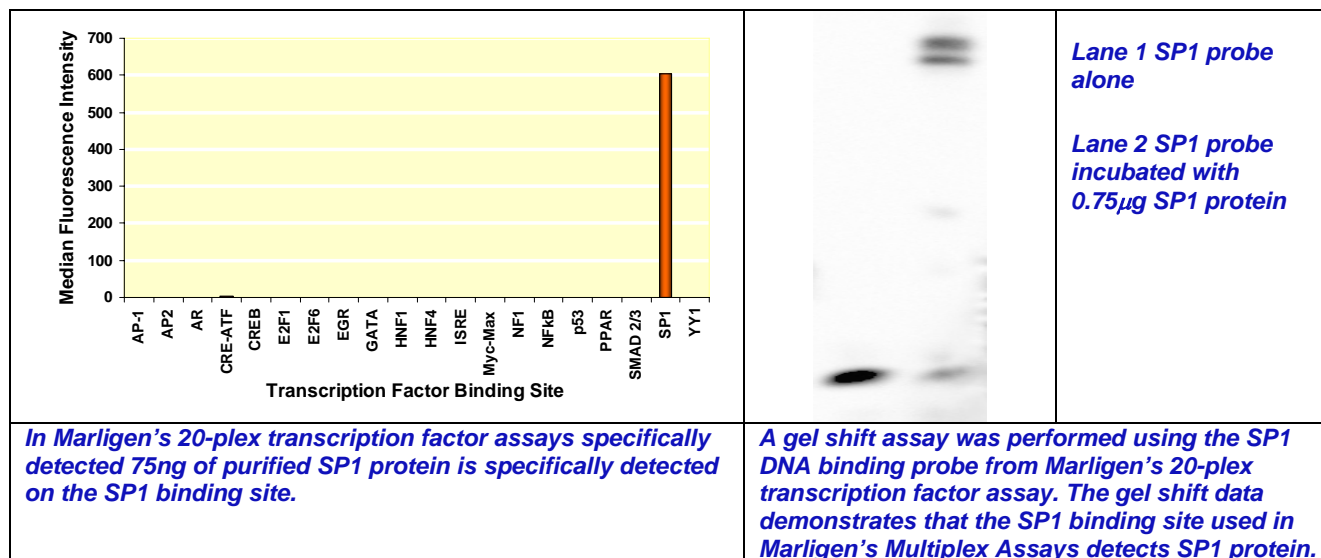


SMAD2/3 (Mothers Against DPP Homolog 2/3, MADH 2/3)

The SMAD 2/3 site 5'-AGCCAGACA-3' also known as the TCE (TGF β control element) binds a complex of SMAD proteins that affect gene transcription. The SMAD complex comprises two receptor regulated SMADS (R-SMAD2/3) and a the SMAD-5 co-SMAD. R-SMADs 2 & 3 are regulated by TGF β and activin type 1 receptor kinases. Once activated in the cytoplasm by their ligand, R-SMAD2/3 complexes with SMAD4 and translocates to the nucleus to activate transcription. The SMAD2/3/4 complex participates in the regulation of TGF- β responsive genes and has been implicated as a tumor suppressor with roles in the regulation of cell cycle control, Mutations in SMAD2 and SMAD3 proteins have been associated with specific cancers.

SP1 (Stimulating Protein 1, Specificity Protein 1, Simian Virus-40 Protein 1)

The Stimulating Protein 1 (SP1) site 5'-CGGGGCGGGGC-3' is a GC-box promoter element of specific genes. SP1 can either repress or activate transcription depending on the transcription factors it associates with. Other members of the SP family of transcription factors include SP2-8. SP1 and SP3 bind to similar sites and control the function of a wide variety of genes.



YY1 (Yin Yang 1)

The Yin Yang 1 (YY1) site 5'-CGGCCATCT-3' overlaps the transcription start site in gene promoters. YY1 is also referred to as NF-E1 and NMP1 (Nuclear Matrix Protein 1). It can act as either an activator or repressor depending on the transcription factors it associates with. YY1 plays an important role in development and differentiation and because of its multifunctional characteristics; it exhibits control over a large number of cellular and viral genes.