

1 Supplement 1

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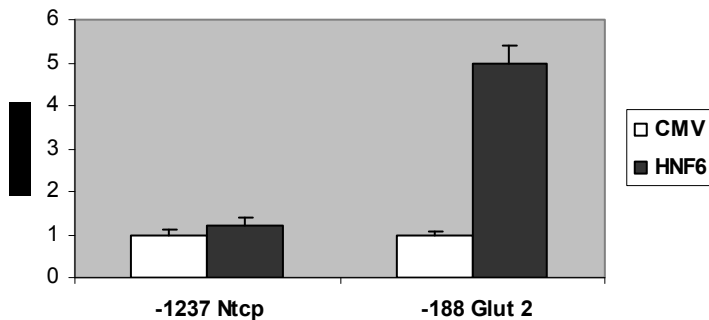
3 HNF6 does not activate *ntcp* promoter:

4 To examine if *ntcp* is an authentic HNF6 transcriptional target gene, HepG2 cells were
5 cotransfected with the -1237 *ntcp* promoter construct (GenBank accession number GI:14280236)
6 bearing the 758/-770 potential HNF6 binding site (TTTATTGACTgTT for *ntcp* compared to HNF6
7 consensus binding motif of DHWATTGAYTWWD where D=not C; H= not G; W= A or T; Y= C or
8 T) along with expression vectors consisting of the CMV promoter driving the expression of mouse
9 HNF-6 or CMV control expression vectors (400 ng) (1). The CMV-Renilla luciferase plasmid was
10 used to normalize for transfection efficiency (25 ng). As positive control the -188 *glut-2* promoter
11 construct with known HNF6-responsive site for HNF6 transcriptional activation (2) was also
12 assayed.

13 Luciferase activity was measured from protein extracts following 24 hours of transfection in
14 triplicates from 2 separate experiments using the Dual-Luciferase Assay System (Promega). The
15 mean fold induction of *ntcp* or *Glut-2* promoter driven luciferase activity by pCMV-H6 was
16 calculated relative to luciferase values from *ntcp* or *Glut-2* promoter transfected with pCMV control
17 plasmid.

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21 1. **Rausa F, Samadani U, Ye H, Lim L, Fletcher CF, Jenkins NA, Copeland NG, and**
22 **Costa RH.** The cut-homeodomain transcriptional activator HNF-6 is coexpressed with its target
23 gene HNF-3 beta in the developing murine liver and pancreas. *Dev Biol* 192: 228-246, 1997.

24 2. **Rausa FM, Tan Y, and Costa RH.** Association between hepatocyte nuclear factor 6 (HNF-
25 6) and FoxA2 DNA binding domains stimulates FoxA2 transcriptional activity but inhibits HNF-6
26 DNA binding. *Mol Cell Biol* 23: 437-449, 2003.

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