Integrated Approach for the Identification of Human Hepatocyte Nuclear Factor 4alpha Target Genes Using Protein Binding Microarrays

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Hepatology, 2010

In this file:

Supplemental Figures S1-S7

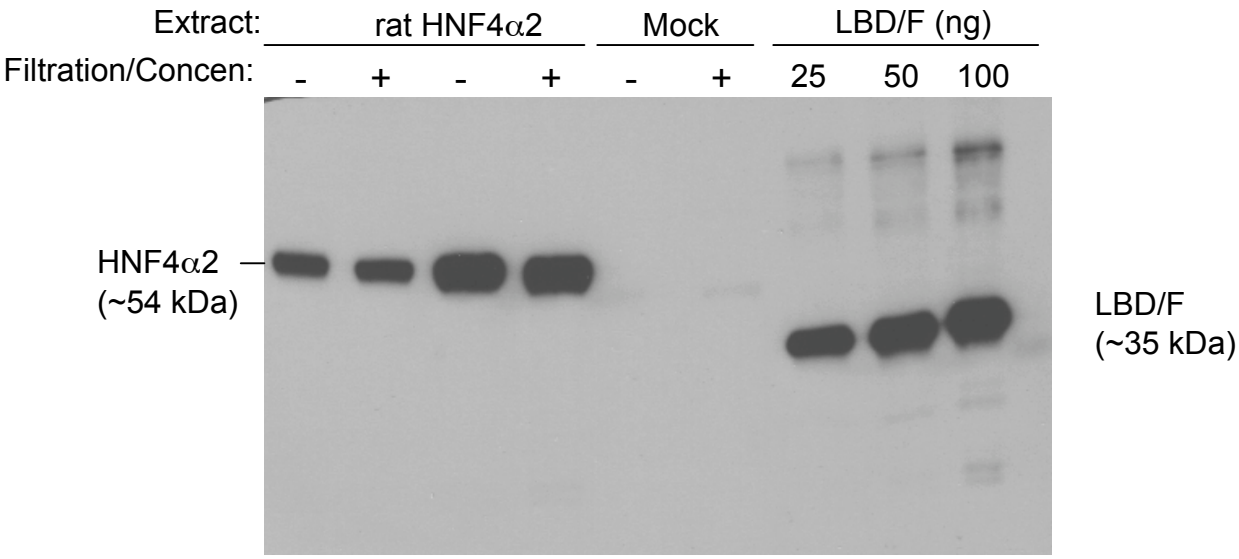

Figure S1. Specificity of HNF4α antibody used in PBMs.
Immunoblot (IB) of crude nuclear extracts (~5 μg per lane) from COS-7 cells transfected with pMT7.rHNF4α2 expressing full length rat HNF4α2 or mock-transfected probed with the affinity purified C-terminal antibody (α445). Mock transfected COS-7 cells do not show a detectable level of HNF4α, or any other cross reacting bands. Known quantities (25, 50, and 100 ng) of purified, recombinant LBD/F (ligand binding/F domain) allow for approximate quantification of HNF4α. Extracts applied to the PBMs were filtered and concentrated (see main text for details). IB analysis of the other antibodies (commercial mouse monoclonals) used to develop the PBMs (αCTD and αNTD) gave equally excellent, specific signals (blots not shown).

Supplemental Figure S1
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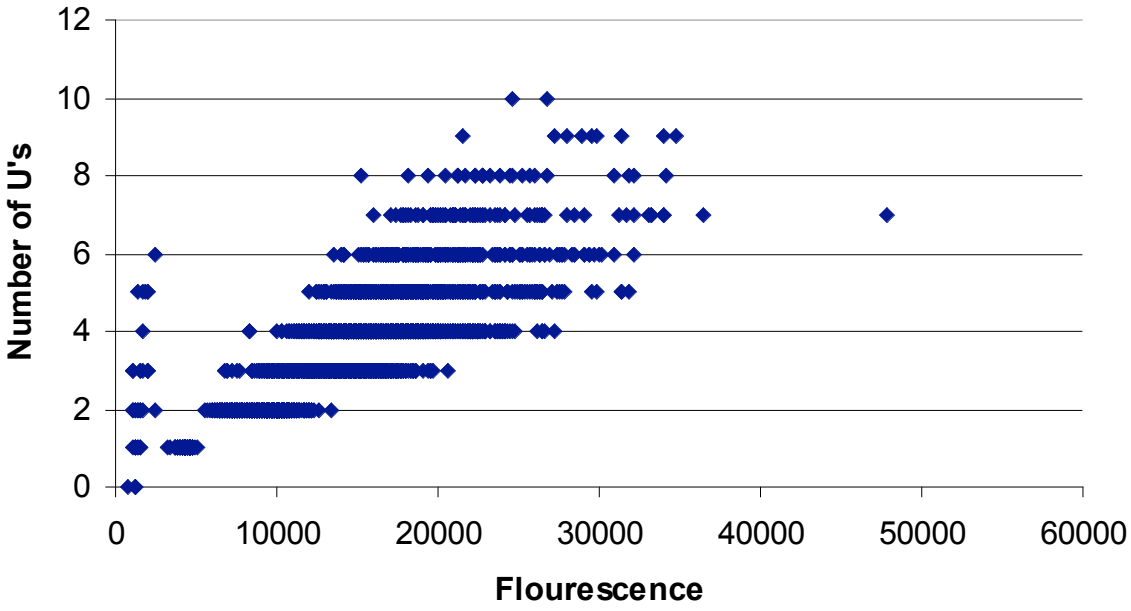

Figure S2. Linear relationship between Cy3 incorporation and number of A's in the variable region.
Scatter plot of Cy3-mediated fluorescence (arbitrary units) in DNA extended on PBM1 in the presence of dUTP-Cy3 and the number of uracils incorporated, based on the number of adenines in the variable region (see Fig. 1 for oligo design and Supplemental Table S2A for a complete list of sequences on PBM1).

Supplemental Figure S2
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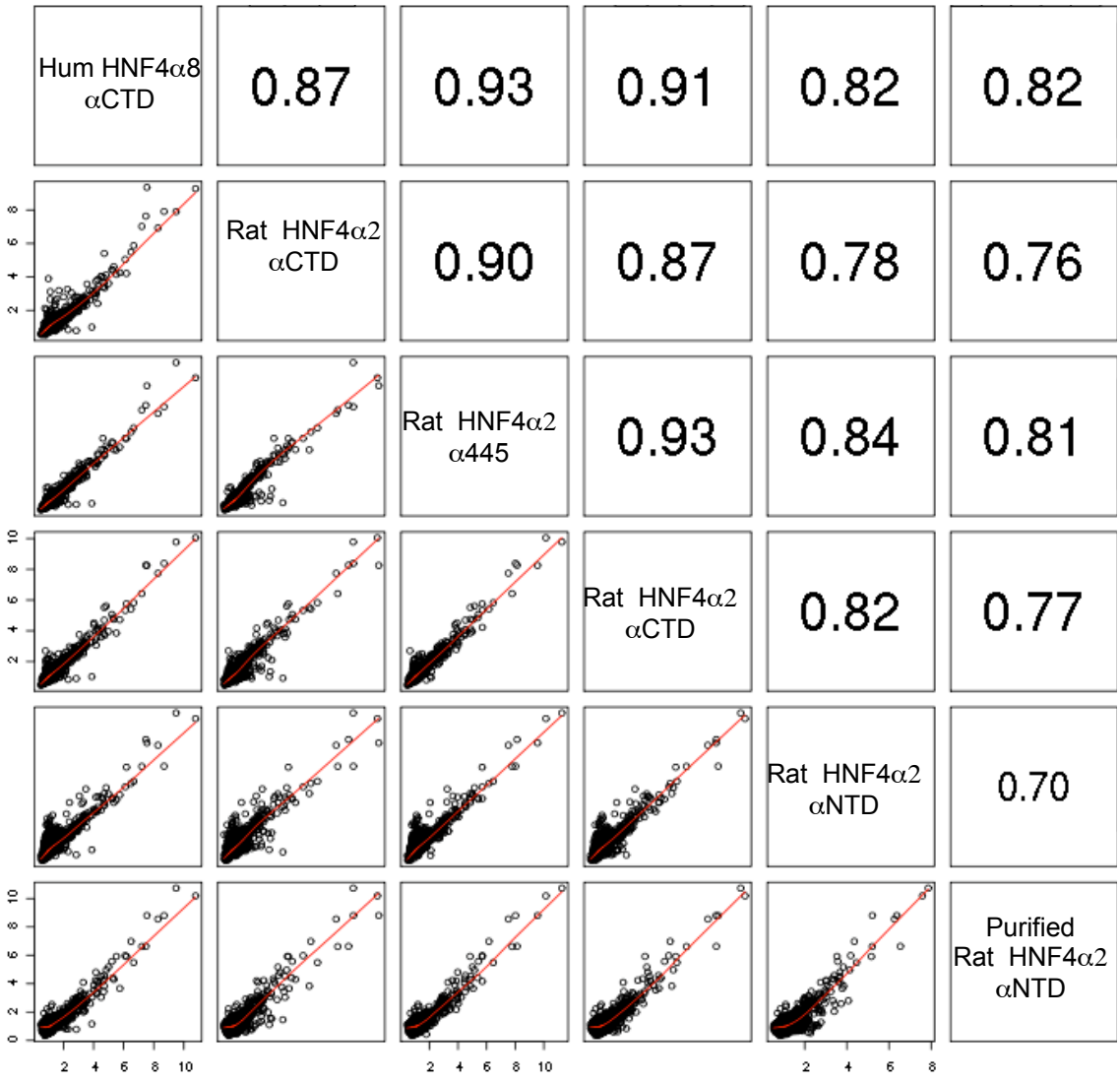

Figure S3. Reproducibility of PBM1.
Scatter plot matrix of normalized fluorescence illustrating reproducibility of PBM1 across different HNF4α isoforms, species and antibodies. The plot shows that the intensities are highly reproducible and there is no significant difference between isoforms (HNF4α2 vs. HNF4α8), species (rat vs human) or antibodies (α445 vs. αCTD vs. αNTD) used in the arrays. All protein samples are from crude nuclear extracts from transfected COS-7 cells except for the "purified" material (see main text for details). Numbers, correlation coefficients, R squared.

Supplemental Figure S3
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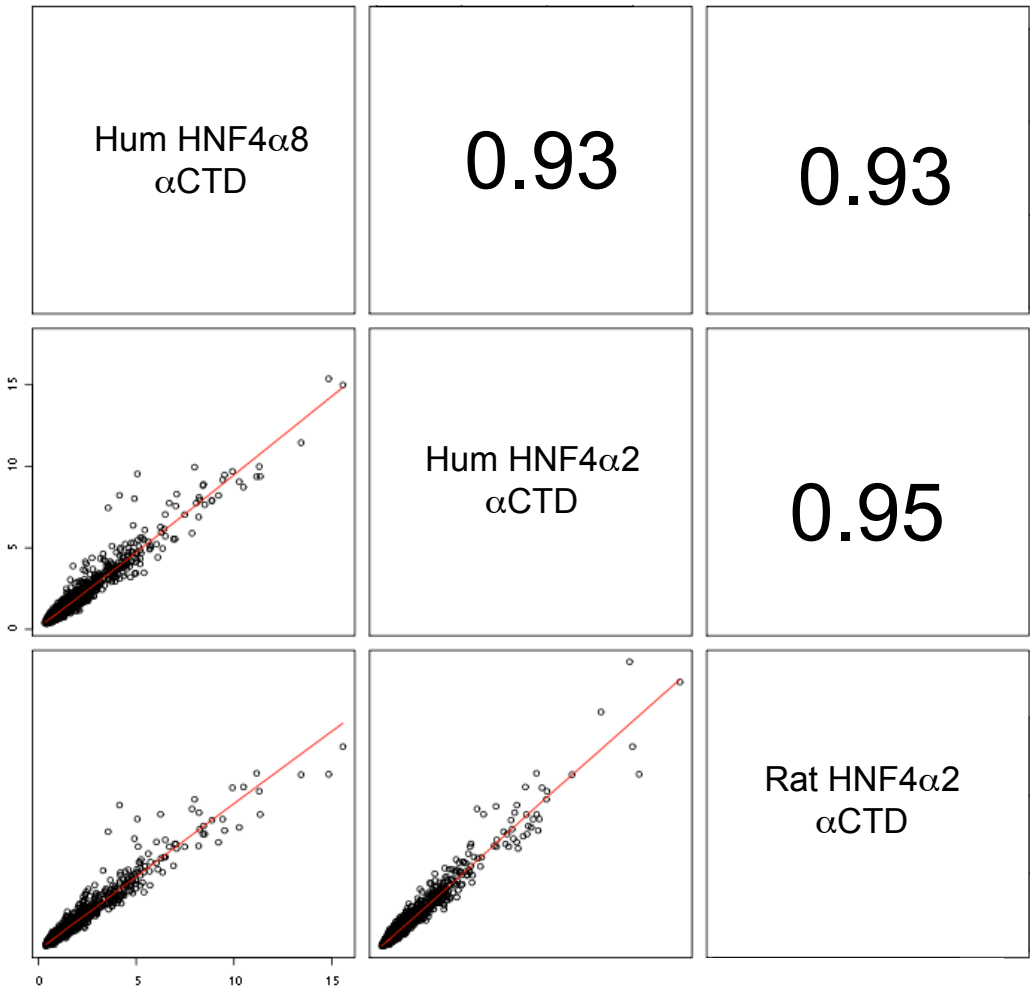

Figure S4. Reproducibility of PBM2.
Scatter plot matrix of normalized fluorescence illustrating reproducibility of PBM1 across different HNF4α isoform and species. The plot shows that the intensities are highly reproducible and there is no significant difference between isoforms (HNF4α2 vs. HNF4α8) or species (rat vs. human) or between the arrays. Numbers, correlation coefficients, R squared. Increased correlation in PBM2 vs. PBM1 (see Fig. S3) is attributed to an improvement in array treatment conditions.

Supplemental Figure S4
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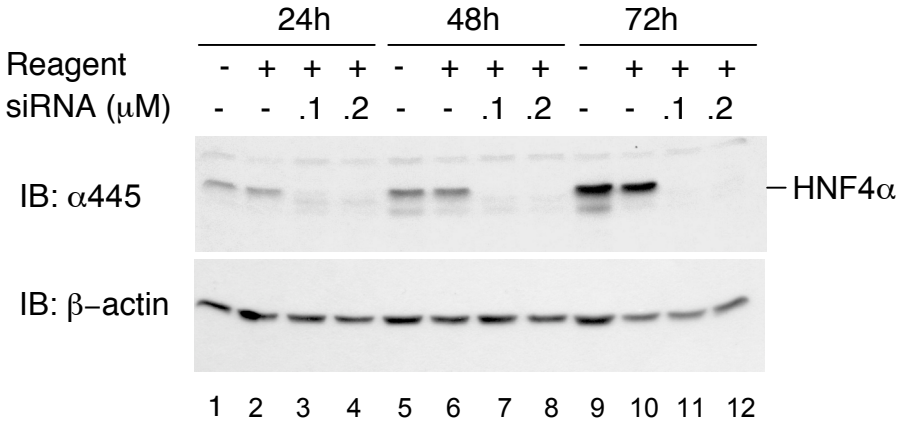

Figure S5. RNAi Knockdown of HNF4α in HepG2 cells.
Immunoblot (IB) analysis with affinity purified antibody α445 to HNF4α showing a decrease in human HNF4α protein upon treatment of HepG2 cells with siRNAs directed against HNF4α1 as described in Materials and Methods in the main text. Reagent, transfection reagent. Cells were harvested at the indicated times after siRNAs were introduced. 20 μg total protein of whole cell extracts were loaded per lane.

Supplemental Figure S5
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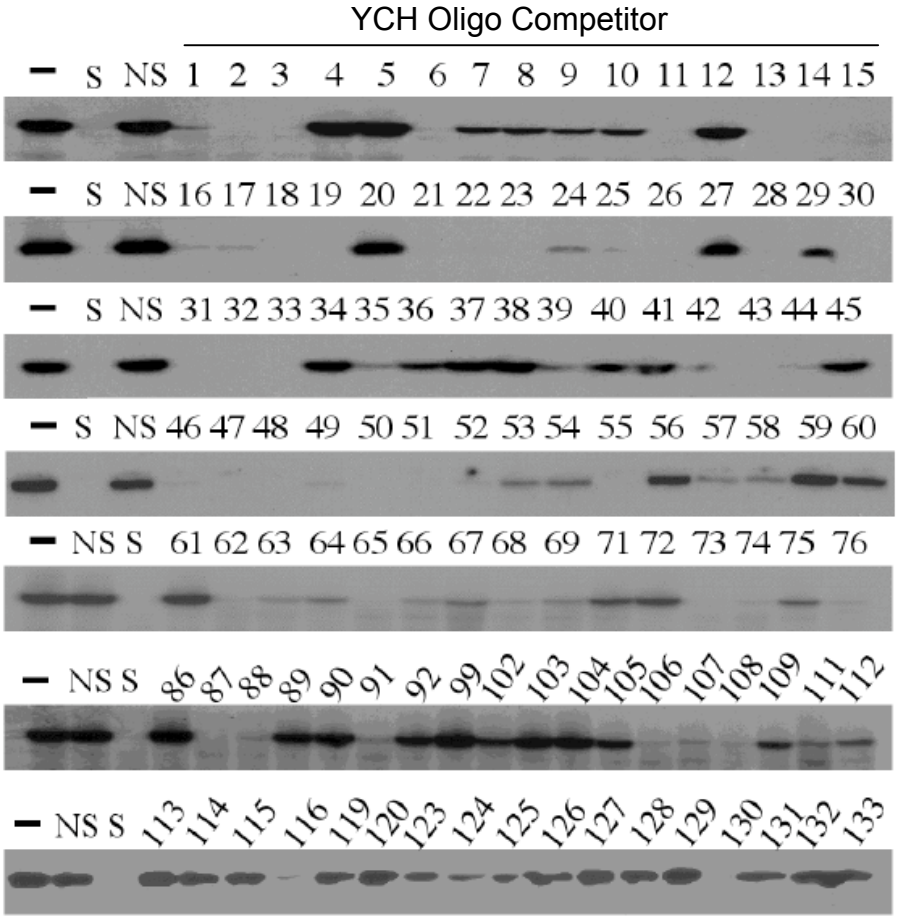

Figure S6. Gel shift results used in Fig. 3C to compare to PBM results
Gel shifts were performed as described in Supplemental Materials and Methods using crude nuclear extracts from COS-7 cells transfected with pMT7.HNF4α1 (rat) and the ApoB.-85.-47 double-stranded oligonucleotide as a ^{32}P-labelled probe. Unlabeled YCH oligonucleotide competitors (YCH1-133) as well as a specific competitor (S, ApoB.-85.-47) and a nonspecific competitor (NS, 175 TTR) were added to the shift reactions in 100-fold molar excess. Shown are the HNF4α:DNA complexes: binders are represented by a lack of a shift band; nonbinders contain a shift band analogous to the control lacking a competitor oligonucleotide (-). Not shown is the free probe which is in excess in all reactions. See Supplemental Table S8 for sequence of ApoB.-85.-47, 175TTR and the YCH oligo's. Results from these competitions were compared to PBM2 results in Fig. 3C in the main text.

Supplemental Figure S6
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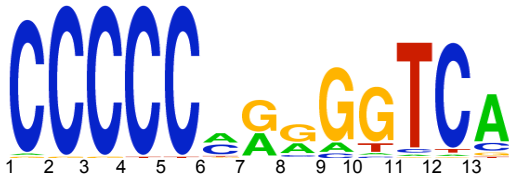
A

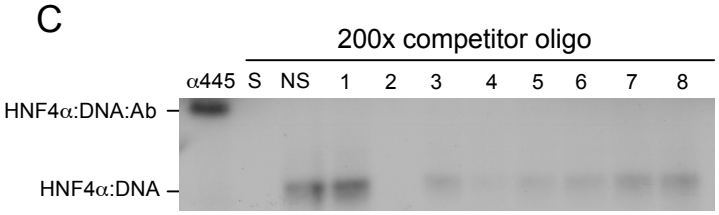
Noncanonical PWM -- this study



B

Noncanonical PWM -- Badis et al, 2009 (Figure S9)

C



Competitor oligo's:

Oligo	Description	Test Sequence
1	Nonbinding seq.	AGCTAGCTAGCTA
2	Canonical seq.	GGGTCAAAGTCCA
3	Noncanonical Consensus	CCCCCAGGGGTCA
4	Noncanonical seq. #1	CCCCCAAGGGTCA
5	Noncanonical seq. #2	CCCCCCAAGGTCA
6	Noncanonical seq. #3	CCCCCCAGGTTCA
7	Badis Noncanonical seq. #1	CCCCAGGGGTCAA
8	Badis Noncanonical seq. #2	ATATAGGGGTCAA

Figure S7. Binding of HNF4α to noncanonical sequences.
(**A**,**B**) Position weight matrices (PWMs) of noncanonical binding sequences from PBM results from this paper (**A**) and Badis et al. 2009 (**B**). The PBMs used by Badis et al. resolve only up to 8-mers while the PBMs used in this study can resolve 13-mers. (**C**) Gel shift assay performed as described in Supplemental Materials and Methods using crude nuclear extracts from COS-7 cells transfected with pMT7.HNF4α2 (human) and the ApoB.-85.-47 double-stranded oligonucleotide as a ^{32}P-labelled probe. Unlabeled oligonucleotide competitors were added in 200-fold molar excess. Left, gel with HNF4α:DNA and supershift (HNF4α:DNA:Ab) complexes indicated. S, specific competitor (ApoB.-85.-47); NS, nonspecific competitor 175 TTR. α445, affinity purified antibody to HNF4α. Not shown is the free probe which is in excess in all reactions. Right, legend with descriptors and test sequence for Oligo's 1-8 used in the competitions. See Supplemental Table S8 for complete sequences of all oligo's.

Results: Gel shift analysis reveals that HNF4α2 binds noncanonical sequences identified by PBM although the binding is not as strong as to canonical sequences (e.g., oligo 2).

Badis G, Berger MF,Philippakis AA, Talukder S, Gehrke AR, Jaeger SA, Chan ET, et al. Diversity and complexity in DNA recognition by transcription factors. Science 2009;324:1720-1723

Supplemental Figure S7
Bolotin et. al. 2010