ENCODE Antibody Validation Documentation
Transcription factor: Hepatocyte nuclear factor 3, alpha; forkhead box A1 (GeneID 3169)

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Transcription factor: FOXA1 (GeneID 3169; ~49 kDa)

Antibody: FOXA1 (C-20), Santa Cruz Biotechnology (sc-6553)
Goat polyclonal, epitope mapping at C-terminus of FOXA1 of human origin
Web: http://www.scbt.com/datasheet-6553-hnf-3alpha-beta-c-20-antibody.html

Validation 1: Immunoblot Analysis

For an antibody to meet ENCODE validation standards, a single band of the predicted size, or a band of no less than half the total signal, must be detected in a lane on a Western blot.

a. Vendor immunoblot analysis

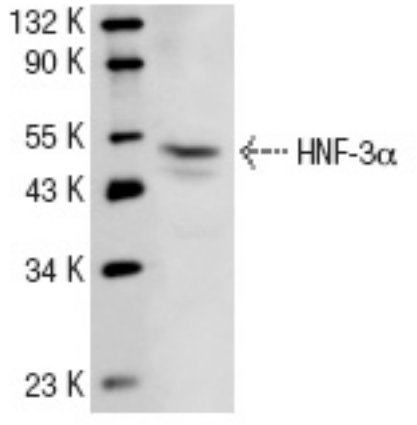

Figure Legend: Western blot analysis of FOXA1 expression in HepG2 whole cell lysate.

b. Myers Lab immunoblot analysis

Western blot protocol

Whole cell lysates were immunoprecipitated using primary antibody, and the IP fraction was loaded on a 12% acrylamide gel and separated with a Bio-Rad PROTEAN II xi system. After separation, the samples were transferred to a nitrocellulose membrane using a Bio-Rad Trans-Blot Electrophoretic Transfer system. Standard western blot protocol was used to probe the membrane with the primary antibody (same antibody as used for IP), and an HRP-conjugated secondary antibody and SuperSignal West Femto solution (Thermo Scientific) were used to detect the immunoprecipitated proteins.

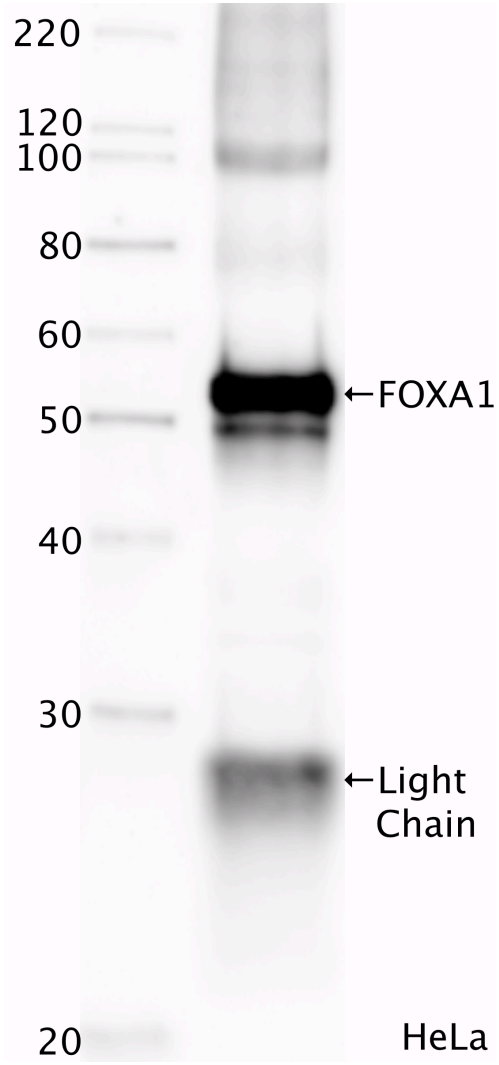

Figure Legend: FOXA1 immunoblot: IP-western with sc-6553 FOXA1 antibody in whole cell lysate of HeLa. Light chain of IgG is indicated, and FOXA1 band is indicated at ~50 kDa.

Validation 2: Immunoprecipitation with multiple antibodies against different parts of the target protein

ENCODE data standards allow for secondary validation of antibodies by performing ChIP with multiple antibodies against different parts of the target protein. A statistically significant overlap of targets constitutes validation.

FOXA1 (C-20) sc-6553 is a goat polyclonal antibody, with epitope mapping at the C-terminus of FOXA1 of human origin. A second antibody used in ChIP-seq experiments on FOXA1 in our lab is FOXA1 (Q-6) sc-101058, a mouse monoclonal antibody raised against recombinant FOXA1 of human origin. Irreproducible Discovery Rate (IDR) analysis results for two ChIP-seq experiments using these two antibodies, each in HepG2, are as follows:

At IDR 0.01: 14930 peaks are significant
At IDR 0.05: 18842 peaks are significant
At IDR 0.1: 21519 peaks are significant

These results indicate significant overlap between the two ChIP-seq libraries.

References

Li Q, Brown JB, Huang H, Bickel PJ. Measuring Reproducibility of High-throughput experiments (Submitted to the Annals of Applied Statistics)