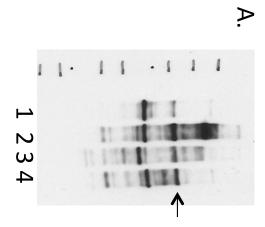
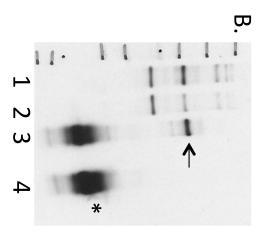
ENCODE DCC Antibody Validation Document

Date of Submission 06/15/2012				
Name: Trupti Kawli	Email: trupti@stanford.edu			
Lab				
Antibody Name: ZC3H11A (NB100-74650) Target: ZC3H11A (Entrez Gene 9877)				
Antibody Name. 2CSTTTA (NBT00-74030)	raiget. 2CSTITA (Little2 Gene 9077)			
Company/				
Source: Novus Biolo	ogicals			
Catalog Number, database ID, laboratory NB100-74650	Lot Number A1			
This is a rabbit polyclonal antibody generated	using an epitope that maps to a region between residue 250			
Antibody Description:				
ZC3H11A is a C3H1-type zinc finger protein. Z	C3H11A has been identified as a protein that is			
	r ATR. The function of ZC3H11A remains uncharacterized.			
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Species Target Human	Species Host Rabbit			
Validation Method #1 Immunoprecipitation	Validation Method #2 IP-Mass Spec			
Purification Affinity	Polyclonal Polyclonal			
Method	Monoclonal			
Van day UDI				
Vendor URL: http://www	v.novusbio.com/ZC3H11A-Antiba∓			
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A. Western blots on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2), HeLaS3 (Lane3), and HepG2 (Lane4). B. Immunoprecipitation was performed on nuclear lysates from K562 cells using antibody NB100-74650 against ZC3H11A. Lane1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with NB100-74650. Lane 3: Bound material from immunoprecipitation with NB100-74650. Lane 4: Bound material from control immunoprecipitation with rabbit IgG. Arrow indicates band of expected size (89kD) that is highly enriched in the specifically immunoprecipitated fraction. Bands indicated by * in K562 immunoprecipitate is IgG light chains. Validation #1 Analysis Comment: A band of ~89kD is detected by Western blotting with NB100-74650 in multiple human cell lines. Immunoprecipitation from K562 nuclear lysate efficiently enriches a single protein of ~89KD. Based on these observations, this antibody meets this ENCODE criterion. Insert Validation Image (click here)





Immunoprecipitation of ZC3H11A from K562 cells using NB100-74650. Lane 1: input nuclear lysate, Lane 2: material immunoprecipitated with NB100-74650, Lane 3: material immunoprecipitated using control IgG. Bands A was excised from the gel and subject to analysis by mass spectrometry. IP followed by mass spectrometry: Briefly, protein was immunoprecipitated from K562 whole cell lysates using NB100-74650, and the IP fraction was loaded on a 10% polyacrylamide gel (NuPAGE Bis-Tris Gel) and separated with an Invitrogen NuPAGE electrophoresis system. The gel was silver-stained, gel fragments corresponding to the bands indicated were excised and destained using the SilverSNAP Stain for Mass Spectrometry (Pierce). Then proteins were trypsinized using the in-gel digestion method. Digested proteins were analyzed on an LTQ-Orbitrap (Thermo Scientific) by the nanoLC-ESI-MS/MS technique. Peptides were identified by the SEQUEST algorithm and filtered with a high confidence threshold (Protein false discovery rate < 1%, 2 peptides per protein minimum). We report 71 different proteins identified in band A. Of the specifically immunoprecipitated proteins, ZC3H11A is the most abundant (242 peptides). Based on these observations, this band is likely due to the presence of immunoprecipitated ZC3H11A and NB100-74650 meets the ENCODE standard for validation by this criterion.

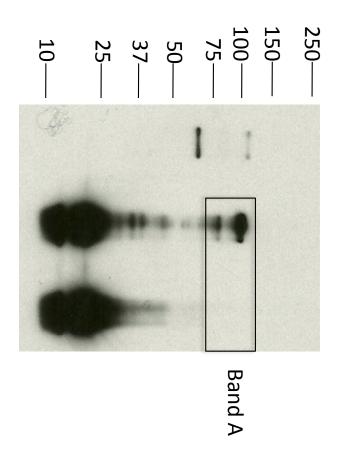
Validation #2 Analysis

Insert Validation Image (Click here)	
	1

Submit by Email

Immunoprecipitation assay (IP) + mass spectrometry assay





Lane 1 Input lysate

Lane 2 Bound material from IP

Lane 3 Bound material from IP using non-specific IgG

Lane

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X201114. Ram A. Journal of Common Contamining protein 11A 349 NOT IN CONTROL (II X20111A. Ram A. Soform 5 of Interfection enhancer-binding factor 3 381 NOT IN CONTROL (II X2011A. Ram A. Soform 5 of Interfection enhancer-binding factor 3 381 NOT IN CONTROL (II X2011A. Ram A. Alf-Pelippendent RNA helicase A. Soform 5 of Interfection RNA helicase Plant RNA helicase DDXS Soform 5 of Interfection RNA helicase DDXS	Spectrum	Name of Protein	Count of peptides	Ratio(ZC3H11A/IgG Control)
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