## **ENCODE DCC Antibody Validation Document**

Date of Submission 09/12/2012	
Name: Dr. Flo Pauli	Email: fpauli@hudsonalpha.org
Lab Myers	
Antibody Name: Pbx3	Target: epitope mapping at the N-terminus of Pbx 3 of hum
Company/ Source:	ruz Biotechnology
Catalog Number, database ID, laboratory sc-891	Lot Number H0307
Antibody Description: Rabbit polyclonal IgG, epitope mapping at t	the N-terminus of Pbx 3 of human origin
Target Description: GeneID: 5090. 47 kDa.	uence 5'-ATCAATCAA-3' (RefSeq).
Species Target Human	Species Host Rabbit
Validation Method #1 Western Blot	Validation Method #2
Purification Method	Polyclonal/ Monoclonal
Vendor URL: http://da	atasheets.scbt.com/sc-891.pdf
ference (PI/ blication formation)	
ease complete the following for antibodies to histone mo our specifications are not listed in the drop-down box, ase write-in the appropriate information	difications:
stone Name AA modified	AA Position Modification

## Western blot protocol:

Whole cell lysates were immunoprecipitated using primary antibody (sc-891), 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 with an Invitrogen iBlot system. Blotting with primary (same as that used for IP) and secondary HRP-conjugated antibodies was performed on an Invitrogen BenchPro 4100 system. Visualization was achieved using SuperSignal West Femto solution (Thermo Scientific).

Validation #1 Analysis

Results: Band of expected size visualized, representing strongest signal in the lane.

Figure legend: IP-western with sc-891 in WCL (whole cell lysates) of GM12878 and HeLa; PM=protein marker. PBX3 band is indicated.



