Broad Institute - Encode3 Secondary Antibody Validation

Millipore 05-689 Lot DAM1810805

Target: CBX5_HP1.alpha

Approved name: chromobox 5

<u>Function</u>: CBX5 is one of several Cbx proteins involved in the regulation of heterochromatin. Like other Cbx proteins, it has a structural motif, the chromodomain, involved in binding to methyl-lysine. It functions in heterochromatic gene silencing and depends on SUV39H1 (KMT1A), the writer of the H3K9 methyl mark.

Member of complex: Not relevant to this validation.

References:

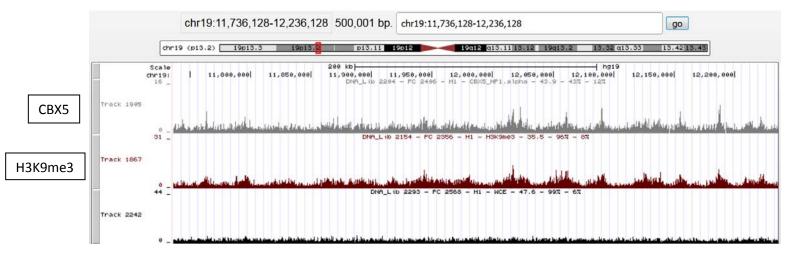
- 1. Nature. 2001. 410(6824):120-4. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, Kouzarides T. PMID: 11242054
- 2. Nature. 2001. 410(6824):116-20. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. PMID: 11242053
- 3. J Biol Chem. 2011. 286(1):521-9. Recognition and specificity determinants of the human cbx chromodomains. Kaustov L, Ouyang H, Amaya M, Lemak A, Nady N, Duan S, Wasney GA, Li Z, Vedadi M, Schapira M, Min J, Arrowsmoth CH. PMID: 21047797

Antibody being validated:

- 1. Millipore 05-689 Lot DAM1810805 [Mouse monoclonal, Protein G chromatography purified]
- 2. Broad Alias: PchAb 530
- 3. Immunogen: Between residues 2 and 191
- 4. https://www.encodeproject.org/antibodies/ENCAB329LKX/

This validation relies on the use of antibodies to a chromatin regulator (CBX5) and a histone modification (H3K9me3) in H1 cells, and the demonstration that highly similar patterns of enrichment are obtained with each antibody. The first track shown used an antibody to CBX5 (PchAb 530, ENCAB329LKX), and the second track shown used an antibody to H3K9me3 previously characterized to Encode standards (PchAb 125-V, ENCAB000APY).

Overall correlation score: 0.9155



Antibody being compared (H3K9me3):

- 1. Abcam ab8898 Lot# 877391 (rabbit polyclonal, affinity purified)
- 2. Broad Alias: PchAb 125-V
- 3. Immunogen: Synthetic peptide within Human Histone H3 aa 1-100 (N terminal) (tri methyl K9) conjugated to Keyhole Limpet Haemocyanin (KLH)
- 4. https://www.encodeproject.org/antibodies/ENCAB000APY/