Broad Institute - Encode3 Secondary Antibody Validation

Abcam ab8898 Lot: GR36126-3

Target: H3K9me3

Approved name: Anti-trimethyl-Histone H3 (Lys 9)

Function: Histone H3 is one of the five main histone proteins involved in the structure of chromatin in eukaryotic cells. Featuring a main globular domain and a long N-terminal tail, H3 is involved with the structure of the nucleosome. The N-terminal tail of histone H3 protrudes from the globular nucleosome core and can undergo several different types of epigenetic modifications that influence cellular processes. These modifications include the covalent attachment of methyl or acetyl groups to lysine and arginine amino acids and the phosphorylation of serine or threonine. Lysine 9 trimethylation is associated with heterochromatin and low-complexity genomic regions.

References:

1. Science. 2001. 293(5532):1074-80. Translating the histone code. Jenuwein T, Allis CD. PMID: 11498575.

Antibody being validated:

1. Abcam ab8898 Lot: GR36126-3 [Rabbit polyclonal, affinity purified]

2. Broad Alias: PchAb 402

3. Immunogen: Synthetic peptide within Human Histone H3 aa 1-100 (N terminal) (tri methyl K9) conjugated to Keyhole Limpet Haemocyanin (KLH)

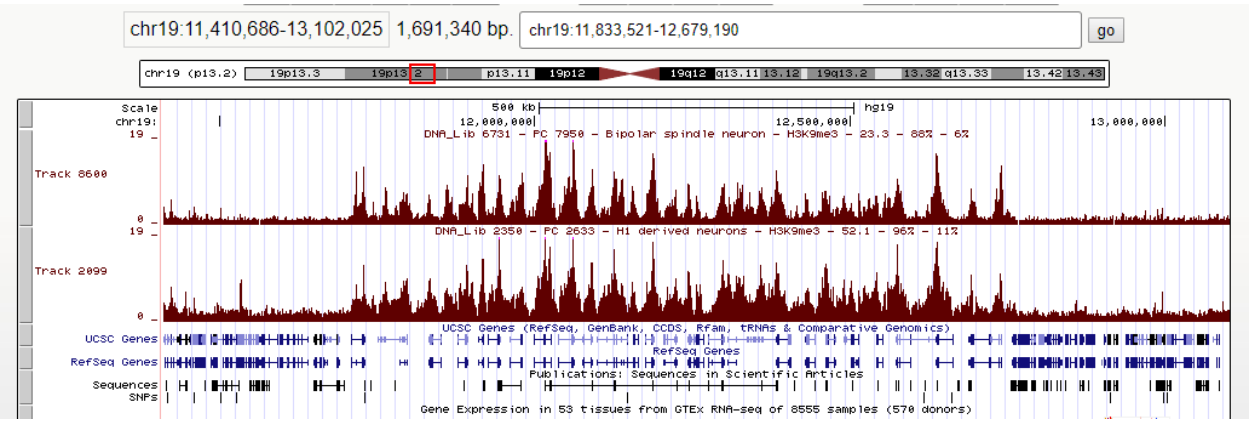
4. https://www.encodeproject.org/antibodies/ENCAB000AUR/

This validation relies on the use of antibodies to a histone modification (H3K9me3) in in two related cell types (H1 derived neurons and bipolar spindle neurons), demonstrating highly similar patterns of enrichment are obtained with each antibody. Please note that the two antibodies originate from two lots of the same vendor antibody part number. The first track shown used an antibody to H3K9me3 previously characterized to Encode standards (PchAb 1259, ENCAB369JSU, bipolar spindle neurons), and the second track shown used an antibody to H3K27me3 (PchAb 402, ENCAB000AUR, H1 derived neurons). Please note, the genomewide correlation of the following two tracks is slightly lower than the regular standard but the tracks were obtained from two related cell types, rather than from a single cell type. There are not data available to us to attempt the ChIP-ChIP comparison in a single cell type.

Genomewide correlation = 0.703

ENCAB369JSU

ENCAB000AU



<u>Antibody being compared (H3K27me3)</u>:

1. Abcam ab8898 Lot: GR131093-3 [Rabbit polyclonal, affinity purified]
2. Broad Alias: PchAb 1259
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/ENCAB369JSU/