Broad Institute - ENCODE4 Secondary Antibody Validation Diagenode C15410210 Lot: A2359-00234P Target: CTCF

Approved name: CCCTC-binding factor

Function: CTCF functions in concert with other subunits of the cohesion complex to insulate domains of active and inactive chromatin from one another, and to create boundaries between topologically associating chromosome domains.

Other complex members: CTCF complexes with cohesion complex members, particularly including STAG2 (SA2).

References:

1. Proc Natl Acad Sci U S A. 2008. 105(24):8309-14. CTCF physically links cohesion to chromatin. Rubio ED, Reiss DJ, Welcsh PL, Disteche CM, Filippova GN, Baliga NS, Aebersold R, Ranish JA, Krumm A. PMID: 18550811

2. Mol Cell Biol. 2011. 31(11):2174-83. Specific sites in the C terminus of CTCF interact with the SA2 subunit of the cohesin complex and are required for cohesin-dependent insulation activity. Xiao T, Wallace J, Felsenfeld G. PMID: 21444719

3. Nat Rev Genet. 2014. 15(4):234-46. CTCF: an architectural protein bridging genome topology and function. Ong CT, Corces VG. PMID: 24614316

Antibody being validated for use in the mouse:

1. Diagenode C15410210 Lot: A2359-00234P [Rabbit polyclonal]
2. Broad Alias: PchAb 1378
3. Immunogen: Four KLH coupled peptides modeled on the sequence of CTCF
4. https://www.encodeproject.org/antibodies/ENCAB719MQZ/

This document is intended to validate an antibody (ENCAB719MQZ) for use in the mouse that is already validated for use in the human. The validation relies on the comparison of ChIP-seq data obtained using ENCAB719MQZ with ChIP-seq data obtained using a different antibody (ENCAB247TYO) that is already validated for use in the mouse.

The antibody being validated is a rabbit polyclonal antibody that was raised against four KLH coupled peptides modeled on the sequence of human CTCF. The previously validated antibody being used for comparison is a rabbit polyclonal antibody that was raised against a peptide derived from the N terminal region of human CTCF. Notably, the amino-acid sequence of mouse and human CTCF is 100% identical in the zinc finger region of the protein [residues 266 - 577] and only differs by two conservative substitutions (at positions 4 and 189) from positions 1

through 265. In effect, it is likely that the immunogens for both antibodies are based on regions of the protein that are 100% identical between human and mouse.

The top four tracks in the figure below were collected at the Broad Institute mapping center using the antibody proposed here for validation, i.e. ENCAB719MQZ. The tissue type represented is mouse hippocampus at 2 months (four replicates).

The bottom three tracks in the figure below represent released Encode data, and were collected using the previously validated antibody (ENCAB247TYO); these correspond to the following files submitted to Encode by Hudson Alpha:

https://www.encodeproject.org/files/ENCFF538QDO/

Mus musculus strain B6NCrl forebrain tissue postnatal (0 days)

https://www.encodeproject.org/experiments/ENCSR677HXC/

https://www.encodeproject.org/files/ENCFF323QPQ/

Mus musculus strain B6NCrl midbrain tissue postnatal (0 days)

https://www.encodeproject.org/experiments/ENCSR985ZTV/

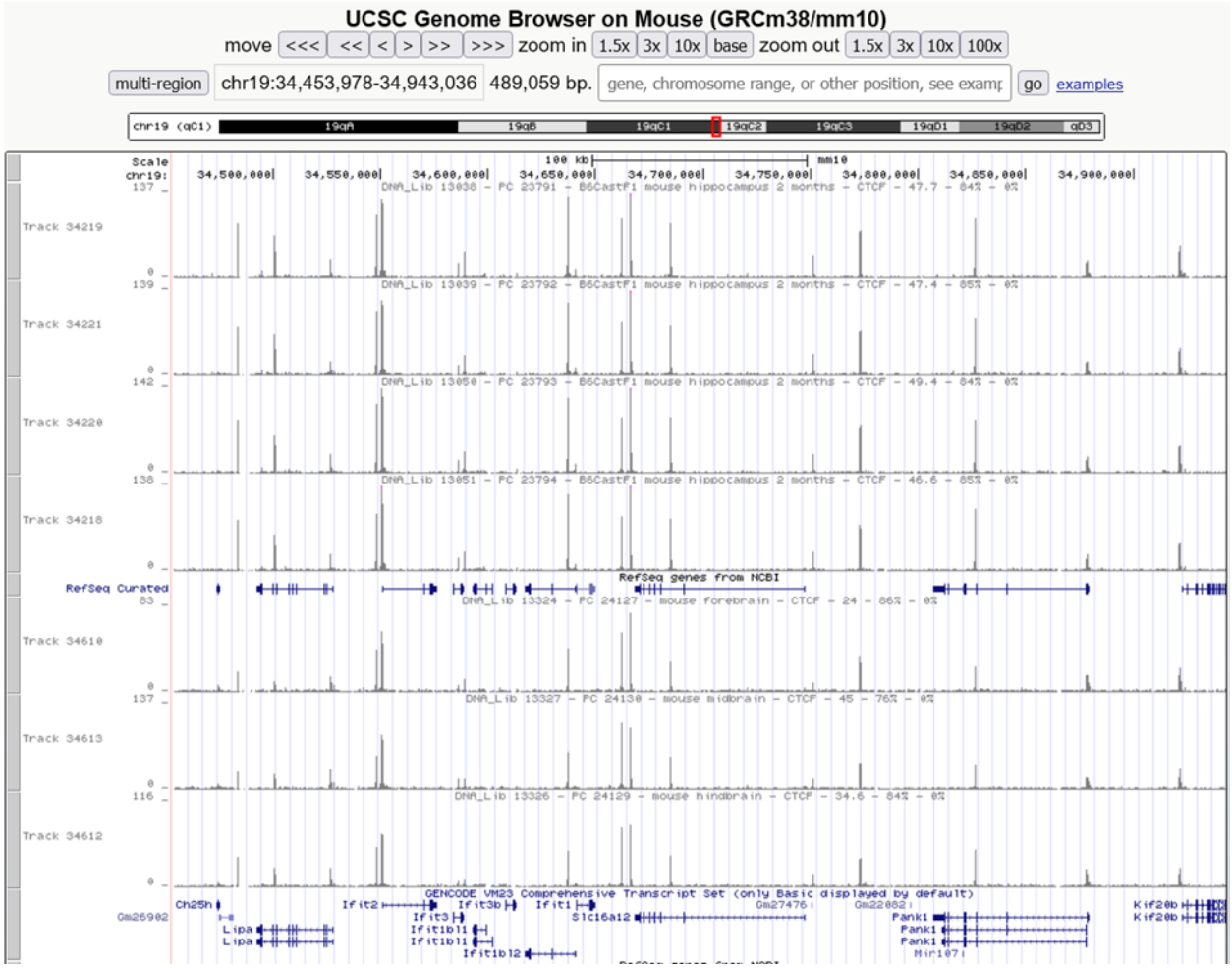
https://www.encodeproject.org/files/ENCFF024MXS/

Mus musculus strain B6NCrl hindbrain tissue postnatal (0 days)

https://www.encodeproject.org/experiments/ENCSR150RGT/

Genome wide, the CTCF maps generated using the two different antibodies in two distinct tissue types (both from mouse brain) have peak - peak overlap concordance of 85%.

Method: CTCF ChIP-seq BAM files were segmented using Homer (factor mode), creating BED files indicating peak locations. The tracks compared were the Broad Institute track from B6 x Cast F1 mouse hippocampus at 2 months of age, and the Hudson Alpha track from newborn mouse forebrain, corresponding to the 1st and 5th tracks in the accompanying figure. The peaks in each BED file were ranked based on the read count from the corresponding BAM file, and the top 25K peaks were selected from each. GRanges was used to determine peak - peak overlap between the two trimmed peak sets. Peaks were counted as overlapping if they overlapped by at least 100 base pairs. 21,279 peaks out of 25,000 were found to overlap by at least 100 base pairs (85.1%).



Antibody being compared (CTCF):

1. Active Motif 61311 Lot: 23913002 [Rabbit polyclonal, affinity purified]
2. Broad Alias: PchAb 1444
3. Immunogen: Peptide within the N-terminal region of human CTCF.
4. https://www.encodeproject.org/antibodies/ENCAB247TYO/