Broad Institute - Encode3 Secondary Antibody Validation

Cell Signaling Technology 3418BF Lot 2 Target: CTCF

Approved name: CCCTC-binding factor

<u>Function</u>: 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:

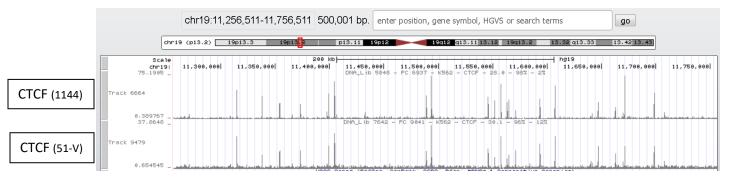
- Proc Natl Acad Sci U S A. 2008. 105(24):8309-14. CTCF physically links cohesion to chromatin. Rubio ED, Reiss DJ, Welcsh PL, Disteche CM, Filippova GN, Baliga NS, Aebersold R, Ranish JA, Krumm A. PMID: 18550811
- Mol Cell Biol. 2011. 31(11):2174-83. Specific sites in the C terminus of CTCF interact 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. <u>Nat Rev Genet</u>. 2014._15(4):234-46._CTCF: an architectural protein bridging genome topology and function._Ong CT, Corces VG._PMID: 24614316

Antibody being validated:

- 1. Cell Signaling Technology 3418BF Lot 2 [Rabbit monoclonal]
- 2. Broad Alias: PchAb 1144
- 3. Immunogen: extreme carboxy terminus of the human CTCF protein

This validation relies on the use of two different antibodies raised against different, non-overlapping peptides, both derived from the sequence of human CTCF, and the discovery of highly correlated patterns of enrichment by ChIP-Seq in K562 cells. The polyclonal antibody being validated (ENCAB000AXX) was raised against a 17 residue long synthetic peptide located 52 residues away from the C-terminus of CTCF. In contrast, the monoclonal antibody was raised against a peptide derived from the extreme C-terminus of CTCF.

Overall correlation score = 0.976



Antibody being compared (CTCF):

- 1. Millipore 07-729 Lot: DAM1472197 [Rabbit polyclonal, unpurified]
- 2. Broad Alias: PchAb 51-V
- 3. Immunogen: KLH-conjugated, synthetic peptide corresponding to amino acids 659-675 (C-TNQPKQNQPTAIIQVED) of human CCCTC-binding factor (CTCF) with a N-terminal cysteine added for conjugation purposes. The full length human protein is 727 residues long.
- 4. https://www.encodeproject.org/antibodies/ENCAB000AXX/