

# Broad Institute - Encode3 Secondary Antibody Validation

Target: REST

## **Antibodies being validated:**

### **PchAb 448**

1. Millipore 17-641 Lot: NG1875688 [Rabbit polyclonal, affinity purified]
2. Broad Alias: PchAb 448
3. Immunogen: made against a GST fusion protein corresponding to amino acids 801-1097 of human REST
4. <https://www.encodeproject.org/antibodies/ENCAB584CZQ/>

### **PchAb 1223**

1. Millipore 17-641 Lot: 2384024 [Rabbit polyclonal, affinity purified]
2. Broad Alias: PchAb 1223
3. Immunogen: made against a GST fusion protein corresponding to amino acids 801-1097 of human REST
4. <https://www.encodeproject.org/antibodies/ENCAB937ZMF/>

REST is a sequence specific binding factor. As such, the validation standard for REST antibodies is distinct from the chromatin regulator validation standard under which the remainder of our antibody validation dossiers are being constructed. The present document does not intend to substitute for the full computational analysis for the enrichment of the recognition sequence for REST in peaks discovered using ChIP-seq. The present document will merely illustrate the tracks we have obtained using two distinct lots of a particular antibody, and will present a single illustration of the occurrence of a perfect occurrence of the REST recognition sequence in tight proximity to a ChIP-seq peak, using a particular locus [CHRN2 (cholinergic receptor nicotinic beta 2 subunit)] identified in the classic literature on the genome-wide occurrence of the recognition motif of the neuron-restrictive silencing factor (REST). Many thousands of other such illustrations could also be presented.

**Approved name:** RE1 silencing transcription factor

**Function:** REST is a protein that acts as a transcriptional repressor, repressing neuronal genes in non-neuronal tissues. Specifically, REST binds a DNA sequence element NRSE (neuron-restrictive silencer element). REST is part of the Kruppel-type zinc finger transcription factor family, which regulate gene expression through a set of zinc finger DNA-binding protein.

**Member of complex:** N/A

**Other complex members:** N/A

**References:**

1. Proc Natl Acad Sci USA. 1996. 93(18):9881-6. Identification of potential target genes for the neuron-restrictive silencer factor. Schoenherr CJ, Paquette AJ, Anderson DJ. [PMID: 8790425](#)
2. Cell. 1995. 80(6):949-57. REST: a mammalian silencer protein that restricts sodium channel gene expression to neurons. Chong JA, Tapia-Ramírez J, Kim S, Toledo-Aral JJ, Zheng Y, Boutros MC, Altshuler YM, Frohman MA, Kraner SD, Mandel G. [PMID: 7697725](#)

**Supporting figures from literature:**

Table 1. Genes with NRSE-like sequences

Gene	Sequence comparison	Binding activity*	Silencing activity*	Location†
Consensus‡	TTCAGCACACGGACAGCGCC			
Neuronal genes				
Rat SCG10	-----G--T---	+	+	5'-Reg
Rat type II sodium channel§	----A-----A--	+		5'-Reg
Human synapsin§	-----G-----T---	+	+	5'-Reg
Rat BDNF§	-----TT-----A--	+		Intron
Rat NMDA rec. 1	-----T-----AT-	+	+	5'-UTR
Human nicotinic ACh rec. β2¶	-----	+	+	5'-UTR
Chicken β4-tubulin	-----G-----	+	+	Intron
Chicken middle neurofilament	-----T--	+		5'-Reg
Human glycine rec.	-----G---T-	+	+	5'-UTR
Rat glycine rec.	-----T-A-----T--	+		5'-UTR
Rat synaptophysin	-C-----GT-----A--	+	+	Intron
Human L1¶	-----G-----AA	+		Intron
Rat atrial natriuretic peptide	-----ACG-	+		3'-UTR
Mouse calbindin¶	AG-----G-----	+		5'-UTR
Rat GABA-A rec. δ subunit	-----GA---G---GA	+	+	Intron
Rat nicotinic ACh rec. α7	AG---G--G--C---A---	-	-	5'-UTR
Mouse P-Lim	-----G-----			5'-UTR
Mouse Hes-3	GG-----			Coding
Human CRF	-----G-----			Intron
Human olfactory rec.	G-----CA-----			Coding
Mouse synaptotagmin IV	-----T-----A--A			5'-UTR
Mouse AMPA rec.	--T-----G-----T			5'-Reg
Rat VGF	-----GCT-----			5'-UTR
Rat proenkephalin	-----AC---G--G---			Intron

**Figure 1.** Table 1 from Schoenherr publication referenced above, listing genes with neuron-restrictive silencer element (NRSE) like sequences. Note arrow indicating to nicotinic receptor (CHRN2 – cholinergic receptor nicotinic beta 2 subunit). This locus has a perfect match to the canonical binding motif of REST, and was identified in the classic literature on the genome-wide occurrence of the recognition motif of the neuron-restrictive silencing factor (REST). Many thousands of other such illustrations could also be presented.

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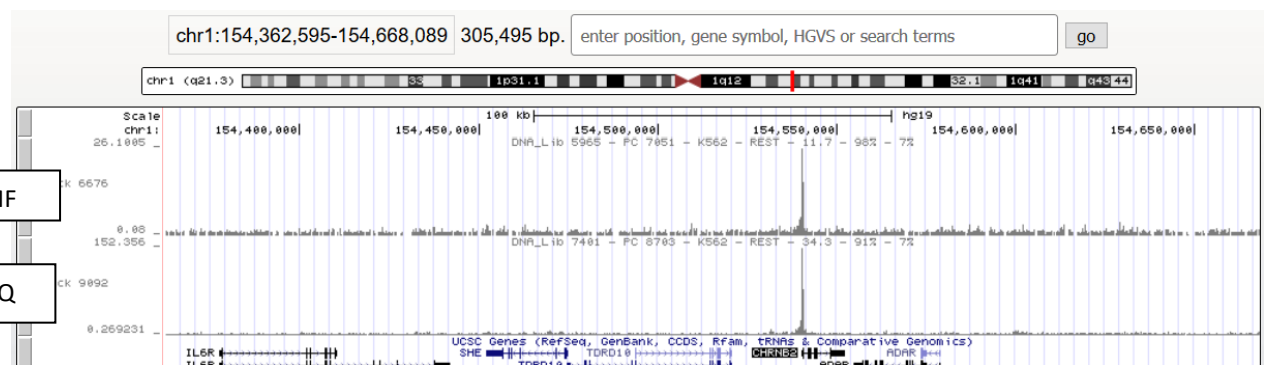
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CCCGACGCTCGTCTGGCCGTCTGGCCCTCAGTACTAAGCAGGCCCTGAC
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GTGTGTTCTGCAGAGTTGCAGAGGGGA

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**Figure 2.** The sequence corresponds to the 1479 bases centered on the ChIP-seq peak that is coincident with the 5' end of the CHRN2 locus. The blue highlight illustrates a 100% identity with the canonical recognition motif of REST in the approximate center of the ChIP-seq peak.

**Supporting Gene Tracks:**

These track images rely on the use of antibodies to a sequence specific binding factor REST (from two lots of the same antibody) in K562 cells, and demonstrate highly similar patterns of enrichment obtained with each antibody. The tracks provide an example of a locus with a ChIP-seq peak and a REST binding site from Schoenherr *et al* (1996).



Additionally, these images demonstrate the same tracks with the same REST antibodies at the CHRN2 locus (cholinergic receptor nicotinic beta 2 subunit), and demonstrate similar enrichment patterns, illustrating the exact 1,479 base pair locus shown in the DNA sequence figure above.

