

Jensen Lab: Honours Projects 2012

Discipline of Biochemistry

The laboratory's long-term research interest is the study of post-transcriptional gene regulation in the developing vertebrate nervous system, and in particular, how the metabolism of pre-mRNA and mRNA changes as cells transition from immature precursors to committed post-mitotic neurons. The precise control of protein expression is absolutely critical in biology, and the key decisions about which genes are turned on or off at any one moment control the proper growth and maturation of an organism during development. Many gene expression programs are highly complex and controlled by regulating transcription of individual genes.

However, transcription is just the start of the process to produce an active protein. In vertebrates pre-mRNAs almost always contain introns, which must be accurately excised. Capping and polyadenylation are also regulated processes, as is the export of the mature mRNA from the nucleus. Finally, protein factors bound to specific mRNAs in the cytoplasm can dictate whether that message is translated or not, the half-life of the mRNA, and also the localization of the mRNA to a specific sub-cellular destination for highly localized protein expression.

The laboratory's approach to understanding these processes has been to start not with the individual mRNAs themselves, but with the factors that control message fate: RNA binding proteins. A principal interest of the lab is elucidating the role of the Hu/ELAV family of RNA binding proteins in the processes of post-transcriptional gene regulation. There are four Hu genes: HuA, which is expressed ubiquitously, and three so-called neuronal Hu (nHu) genes, HuB, HuC and HuD, which make protein only in neurons. The Hu proteins have been shown to stabilize the half-life and regulate the translation of a variety of mRNAs, and the nHu proteins can induce a neuronal phenotype in cells when expressed both *in vitro* and *in vivo*. It is likely that the nHu proteins play an important role in the post-transcriptional regulation of mRNAs that are critical for the commitment of a cell to the neuronal phenotype.

We have developed a novel technique called CLIP that allows the identification of genuine, *in vivo* RNA targets for any chosen RNA binding protein (see Figure 1). In brief, the fundamental principle of the method is the use of UV photocrosslinking to "freeze" RNA-protein interactions in live cells or tissue. These stable complexes can be purified and the RNA sequence surrounding the site of crosslinking identified by

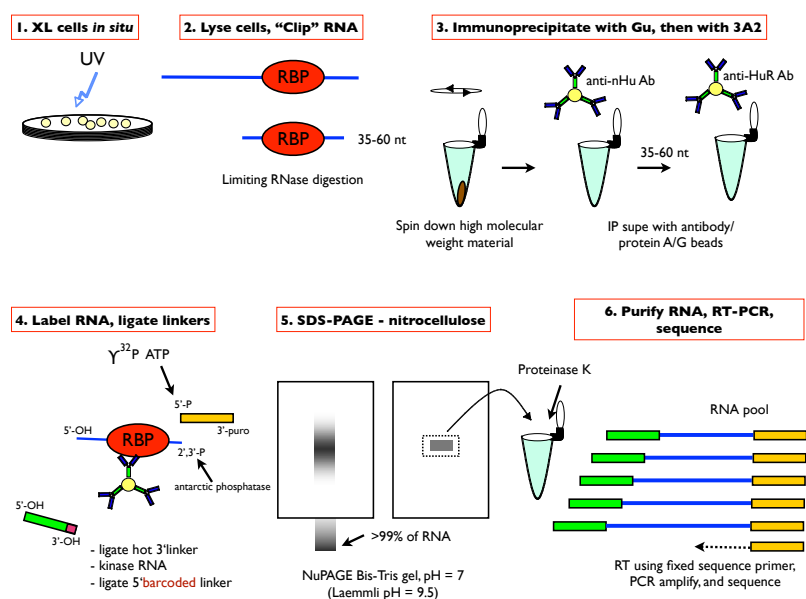


Figure 1. The CLIP Method.

sequencing; thus both the identity of the mRNA and the site of interaction with the RNA binding protein are known.

The laboratory has recently performed a number of CLIP experiments using antibodies specific to the nHu proteins or to HuA alone, and we have carried out these experiments in both neuronal precursor-like cells and in post-mitotic neurons. The raw output of each of these distinct CLIP experiments is a pool of small RNAs (40-120 nt long) that represent the site of Hu interaction within each mRNA. These “CLIP tag” pools were sequenced using an Illumina Genome Analyzer Iix, and generated over 25 million individual reads. We have been able to identify and map over 85% of these tags to “hotspots” in thousands of different messages (see Figure 2 for an typical mRNA targeted by Hu). In general, we are looking for an Honours student in 2012 to help us analyze and pursue this Hu-mRNA interaction data. In particular, we have a number of hypotheses concerning the functional consequences of Hu interaction with a message, and the student will pursue these hypotheses using mRNA reporter systems developed in the lab.

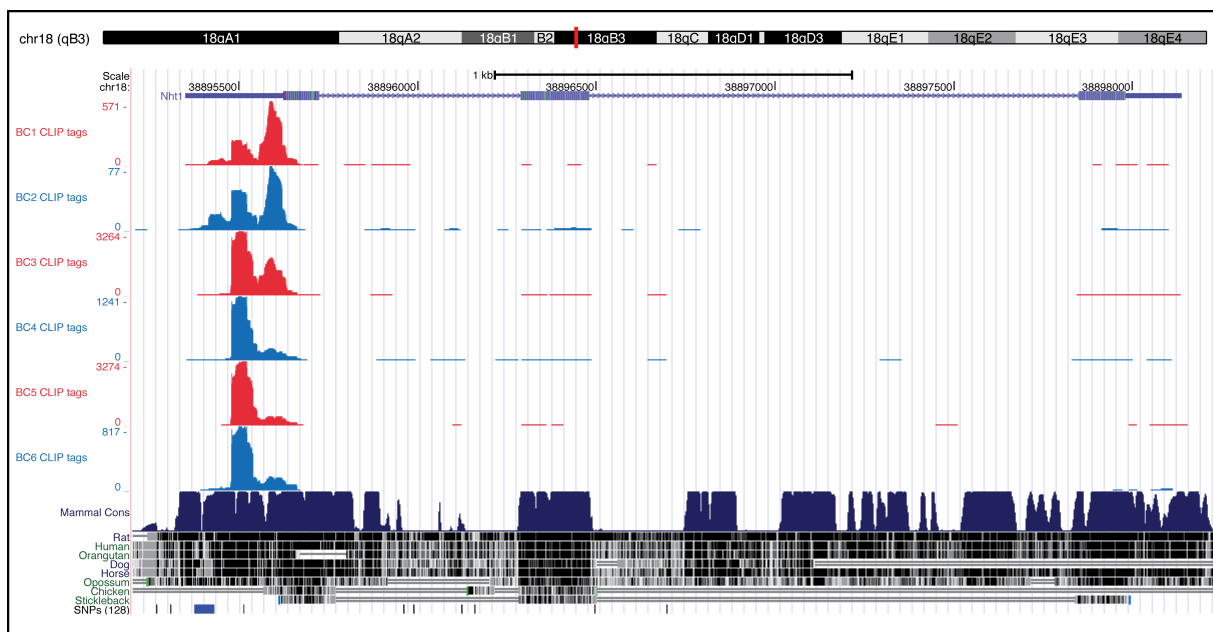


Figure 2. Histograms showing CLIP tag distributions in Nht 1.