What is the difference between IP and input?

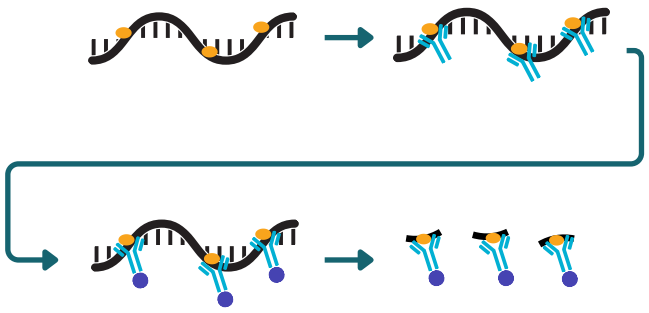
In the field of RNA therapeutic research, drug developers often need to understand how a specific RNA binding protein (RBP) interacts with a specific RNA target. Protein interactions on mRNA can be highly dynamic, with proteins binding and dissociating rapidly. This makes it difficult to study a specific interaction. Fortunately, crosslinking and immunoprecipitation (CLIP) assays can help developers overcome this challenge. Crosslinking creates a strong bond between mRNA and proteins, creating a snapshot of mRNA and protein interactions in both time and space. Isolation of these mRNA-protein complexes can help drug developers study these interactions. For high-quality, reproducible data, two RNA libraries are required: IP and input.

This FAQ explains more about what these two libraries are and their roles in immunoprecipitation.

IP measures protein binding

The IP library is created from RNA that binds to the target protein through a process called immunoprecipitation or antibody pulldown. First, a specific antibody is used to enrich for crosslinked mRNA-protein complexes containing the protein of interest. This enriched pool of mRNA-protein complex is then size-selected based on the weight of the targeted RNA-binding protein.

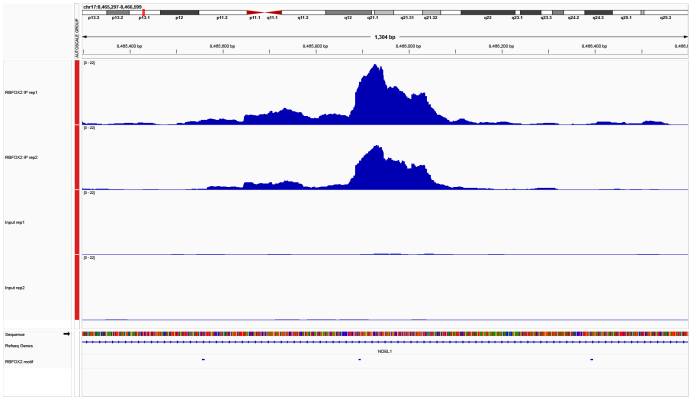
Afterward, the samples are washed under stringent conditions, removing nonspecific protein-RNA complexes. RNA isolated from the remaining protein-RNA complexes are used to create the IP library.



Input is a negative control

The input is a negative control that is created from the total cell lysate before immunoprecipitation. The input contains crosslinked mRNA protein complexes but is not specific to the protein of interest. Like the IP, the input is size-selected to ensure the background RNA is specific to your targeted protein. After the IP library is prepared, both the IP and input libraries are sequenced and compared to each other to identify sites of protein binding.

To pinpoint where proteins bind with confidence, drug developers must identify regions that are more enriched in the IP library compared to the input library. Typically, drug developers evaluate the level of enrichment in the IP library over the input library for each detected read cluster. Any cluster that meets a predefined significance threshold, usually involving a minimum fold change and p-value, is considered a peak and represents a high-confidence binding site. Clusters without significant enrichment in the IP over the input may originate from nonspecific immunoprecipitation and may not represent a biologically-relevant binding site.



In this example, the IP has a signifcantly larger peak than the input, so the protein binding site is in the IP library.

Immunoprecipitation at Eclipsebio

At Eclipsebio, we use crosslinking and immunoprecipitation approaches in our next-generation sequencing eCLIP assays. miR-eCLIP+ locates miRNA and siRNA binding and discovers any off-targets. RBP-eCLIP identifies where and how proteins bind to RNA. m6A-eCLIP maps methylated bases at a single-nucleotide resolution across the transcriptome, and it can be paired with eSENSE m6A to reduce the input required to only 2 µg total RNA.

Interested in how crosslinking and immunoprecipitation can improve your RNA insights? Contact Eclipsebio today to learn more.