What is crosslinking?

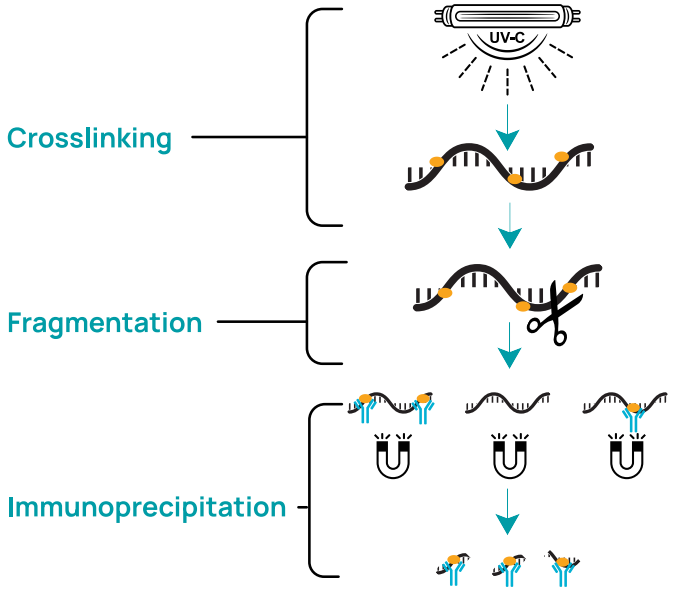
The binding of many regulatory factors to mRNA, such as miRNAs or proteins, is highly dynamic. A regulatory protein will bind, perform its action, and then rapidly dissociate. In order to capture these interactions, it is often necessary to perform crosslinking to capture the interactions.

Crosslinking

One of the most common ways to perform crosslinking on RNAs is to use ultraviolet (UV) light.

When mRNA is exposed to 254-nm ultraviolet light, the nucleotide bases in the strand form covalent bonds with proteins that are bound to the RNA. The bound protein complexes remain in place on the RNA, allowing for a snapshot in time of protein dynamics.

For most workflows, the crosslinked RNA is then fragmented for further analysis. This fragmentation is critical for next-generation sequencing-based approaches for RNA characterization, such as Eclipsebio's RBP-eCLIP assay.



Immunoprecipitation

After crosslinking and fragmentation, immunoprecipitation is performed to isolate only the RNA fragments that are bound to the target protein. During the immunoprecipitation process, a selective antibody is used to bind to the target of interest. Depending on the protocol, a magnet is used to enrich the antibodies that are now attached to the proteins and RNA.

Once the targeted antibody-protein groups are pulled down by the magnet, the samples are washed, leaving behind only the protein-RNA complexes for the desired protein. This bound mRNA can be then used for sequencing or other analysis.

At Eclipsebio, we used our enhanced crosslinking immunoprecipitation (eCLIP) in multiple services.

Researchers can also perform crosslinking by baking mRNA in an oven at 80°C, but this method is less common.

Crosslinking Immunoprecipitation at Eclipsebio

With **miR-eCLIP+**, we identify miRNAs and siRNAs, showing where they bind and discovering an off-targets.

With **RBP-eCLIP**, we discover where and how proteins bind to RNA.

With **m6A-eCLIP**, we locate specific methylated bases at a single-nucleotide resolution across the transcriptome.

Interested in using one of our eCLIP capabilities? Contact us to learn more.