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Dialysis-Based Method for Affinity Mapping of Chromatin Interactions

Researchers at Stanford have developed methods for preparing chromatin from cells for downstream genomic chromatin mapping.

Chromatin profiling strategies are widely used to map the genomic location of chromatin elements, such as histone post-translational modification and chromatin associated proteins. Though there are various different techniques to perform this profiling, they each tend to suffer from the same drawbacks, which limit their utility under certain conditions. For instance, some assays include incubating permeabilized cells with enzymes, where the nuclear envelope retains the chromatin structure and interacting biomolecules. However, this precludes anucleated cells, such as bacteria, or in cells undergoing cell division. In addition, substantial wash and centrifugation steps can introduce unwanted variability and produce negative impacts on chromatin quality that can hamper downstream analysis. Therefore, there is a need in the art for improved methods for sample preparation for chromatin mapping with lower starting material, minimal handling steps, and a non-reliance on the nuclear membrane.

Stage of Research

The inventors have developed a single vessel (i.e. a "one-pot") method for preparing samples for genome wide mapping of protein-DNA interactions. Compatible workflows can include DiMeLo-seq, or other methods such as CUT&RUN or CUT&TAG. In this method, wash steps have been replaced with a series of dilution and/or precipitation steps that enable addition of components that would otherwise interfere with downstream workflow steps above a certain concentration.

Stage of Development

Research - in vitro

Applications

- Determining the genomic location of at least one biomolecule-genomic DNA interaction.
- Sample preparation with DiMeLo-seq.
- Chromatin profiling approaching using tethered enzymes such as CUT&RUN.

Advantages

- The one-pot or single vessel method can drastically shorten the overall time and workload.
- Lower sample requirements.
- Minimized handling steps.
- Allow chromatin preparation in the absence of a nuclear envelope.
- Provide reliable performance in downstream profiling techniques.

Innovators

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