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Functional mapping of m⁶A RNA modifications in cancer

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We use this protocol and it's working

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Disclaimer

This overview protocol is provided for research use only. Individual experimental procedures may require optimization depending on experimental conditions.

Abstract

This protocol provides an overview of the experimental workflows used in this study, including chromatin immunoprecipitation (ChIP), m6A RNA immunoprecipitation (MeRIP-seq), SELECT-based site-specific m6A validation, and immunofluorescence assays. Detailed step-by-step protocols for each procedure are provided separately and linked below.

Before start

- All experiments were performed using standard molecular biology and cell culture practices.
- Detailed reagent compositions, incubation conditions, and quality control steps are described in the individual protocols linked below.
- Users should consult the corresponding protocol for step-by-step instructions and optimization guidance.

Chromatin immunoprecipitation (ChIP)

- 1 This workflow describes ChIP experiments performed in 22Rv1 cells with or without *CHD9* knockdown, followed by DNA purification and ChIP-seq library preparation.

Detailed protocol:

Chromatin immunoprecipitation (ChIP) in 22Rv1 cells

<https://www.protocols.io/edit/chromatin-immunoprecipitation-chip-in-22rv1-cells-hibjb4akp>

ATAC-seq for chromatin accessibility profiling

- 2 This workflow describes an assay for transposase-accessible chromatin using sequencing (ATAC-seq) to profile chromatin accessibility in cultured cells.

Detailed protocol:

ATAC-seq library preparation in cultured cells

<https://www.protocols.io/edit/atac-seq-library-preparation-in-cultured-cells-hib7b4arp>

m⁶A RNA immunoprecipitation (MeRIP)

- 3 This workflow describes m⁶A RNA immunoprecipitation using fragmented total RNA from cell lines, followed by purification of m⁶A-enriched RNA for downstream assays.

Detailed protocol:

m⁶A methylated RNA immunoprecipitation (MeRIP) in cell lines

<https://www.protocols.io/edit/m6a-merip-hibsb4anf>

Senescence-associated β -galactosidase staining

- 4 This workflow describes senescence-associated β -galactosidase staining assays used to assess cellular senescence by light microscopy.

Detailed protocol:

Senescence β -galactosidase staining assay in cultured cells

<https://www.protocols.io/edit/senescence-galactosidase-staining-assay-in-culture-hib5b4aq7>

SELECT-based site-specific m⁶A validation



- 5 This workflow describes SELECT (Single-base Elongation- and Ligation-based qPCR Amplification) assays used for site-specific validation of m⁶A modifications at defined RNA loci.

Detailed protocol:

SELECT assay for site-specific m6A detection

<https://www.protocols.io/edit/select-based-quantification-of-site-specific-m6a-m-hibxb4app>

Immunofluorescence staining and imaging

- 6 This workflow describes immunofluorescence staining and imaging procedures used to assess protein localization and subcellular distribution in cultured cells.

Detailed protocol:

Immunofluorescence staining and imaging in cultured cells

<https://www.protocols.io/edit/immunofluorescence-staining-and-confocal-microscop-hib4b4aqx>