CUT&RUN for advanced **MeRIP** applications

EpigenTek Group Inc.
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Product Summary

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	CUT&RUN MeRIP Kit	CUT&RUN MeRIP-Seq Kit
Catalog No.	P-9018	P-9016
Purpose	Enrich short RNA fragments containing m^6A methylation sites for downstream analysis (PCR, NGS)	m^6A RNA enrichment + library preparation for NGS
Kit Size	24 rxns	12 and 24 rxns
Starting Material	Isolated RNA	
Input Amount	1 - 20 µg per rxn 10 µg optimal 500 ng minimum	
Protocol Time	< 3 h	< 6 h
Antibody Notes	Highly-specific and MeRIP-grade anti-m6A antibody is included with kit and not cross-reactive to unmethylated A	

m^{6}A RNA Modification

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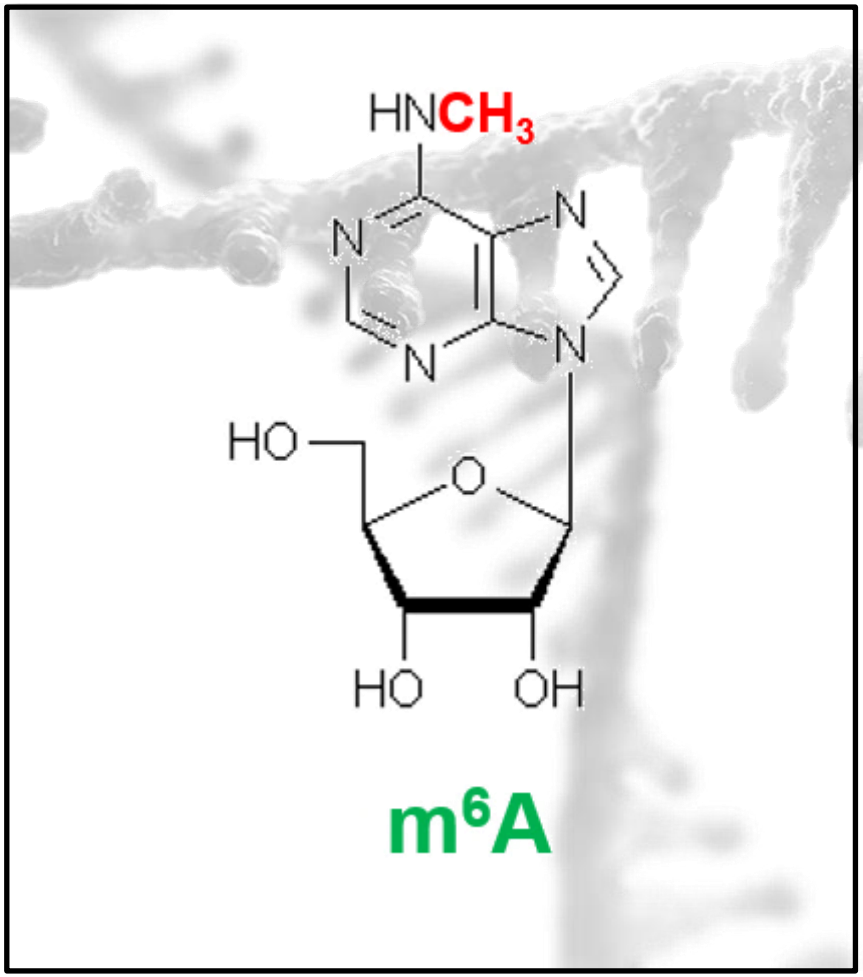

N^6-Methyladenosine

- "The fifth RNA base"
- Most common and abundant eukaryotic RNA modification
- Accounts for >80% of all RNA methylation
- Found mainly in mRNA; also observed in non-coding species (tRNA, rRNA, miRNA, etc.)
- Functions
  - RNA metabolism
  - Cell differentiation
  - Immunity
  - Inflammation
  - Circadian clock
  - Viral life cycle

MeRIP-seq

Methylated **R**NA **I**mmuno**P**recipitation **Seq**uencing

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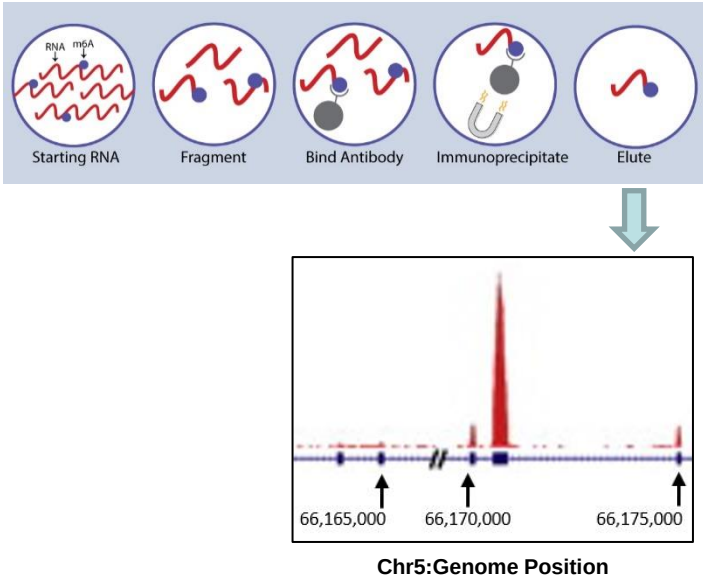
- First method to detect m^{6}A on transcriptome-wide level
- Couples m^{6}A RNA immunoprecipitation with NGS
- Allows for high-throughput localization of modified sites from enriched m^{6}A-containing RNA fragments

Procedure

- RNA extraction
- RNA fragmentation
- Binding of magnetic bead-coupled antibody to sheared RNA
- Immunoprecipitation of m^{6}A RNA fragments
- RNA separation from bound antibody and elution
- Reverse transcription to generate cDNA
- Library construction
- Sequencing



CUT&RUN

Cleavage **U**nder **T**argets and **R**elease **U**sing **N**uclease

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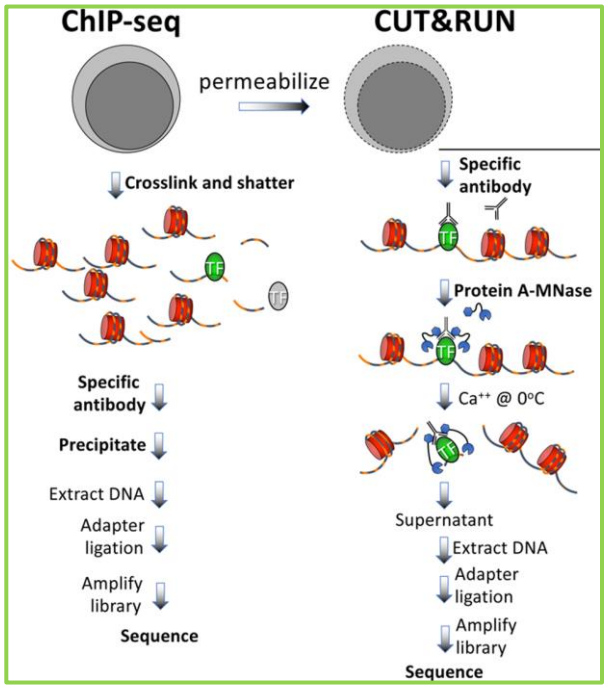
- Recent innovation in protein/DNA interaction studies
- Originally developed to resolve the shortcomings of ChIP
  - Large amount of starting material
  - Long protocol time (several days)
  - Limited resolution



Kaya-Okur et al., 2020

- **EpigenTek** has developed a **new MeRIP method** using CUT&RUN technology for improved m6A enrichment

CUT&RUN m^{6}A MeRIP

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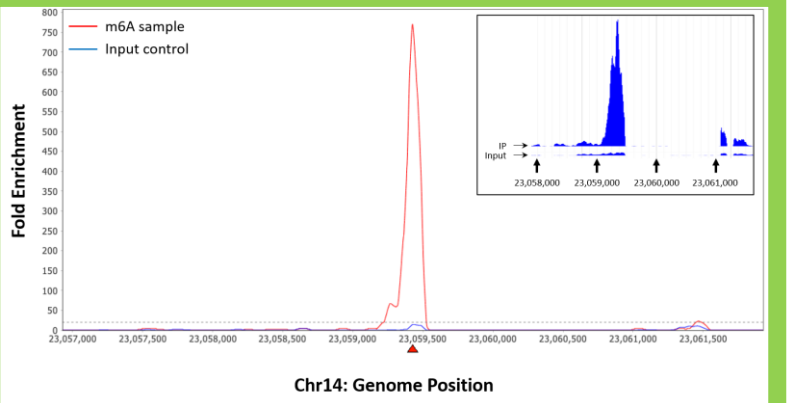
- **Advantages**
  - Higher enrichment
  - Lower input
  - Reduced background
  - Faster, more streamlined procedure
- **Cleavage under targets**
  - Simultaneous fragmentation of immunocaptured RNA and cleavage/removal of any sequences in both ends of target m^{6}A-containing sequences
  - RNA regions occupied by antibody are unaffected
- **Generation of short RNA fragments only bound with anti-m^{6}A antibody**
  - High resolution mapping



The m^{6}A peak distribution within the human *ACIN1* transcript from samples processed with **CUT&RUN m^{6}A MeRIP** (EpigenTek cat. #P-9018) correlates well with public database records (inset).

P-9018 CUT&RUN MeRIP Procedure

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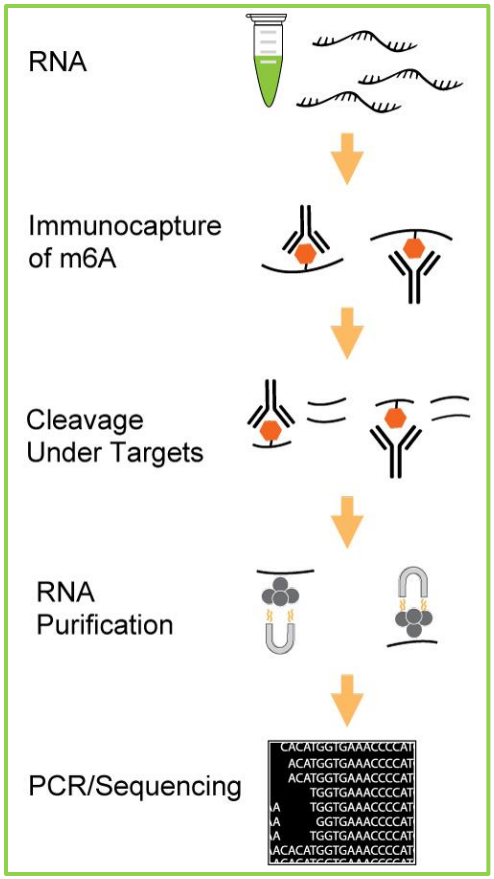
- Isolated and purified total RNA from any species
- m^{6}A RNA immunocapture with highly-specific anti-m^{6}A antibody (included in kit)
- Cleavage under targets (in situ)
- Wash away unbound RNA (via antibody affinity magnetic beads)
- Enriched m^{6}A RNA release, purification (via RNA affinity magnetic beads), and elution

The enriched m^{6}A RNA is now ready for downstream analysis (PCR, NGS)



P-9016 CUT&RUN MeRIP-Seq Kit

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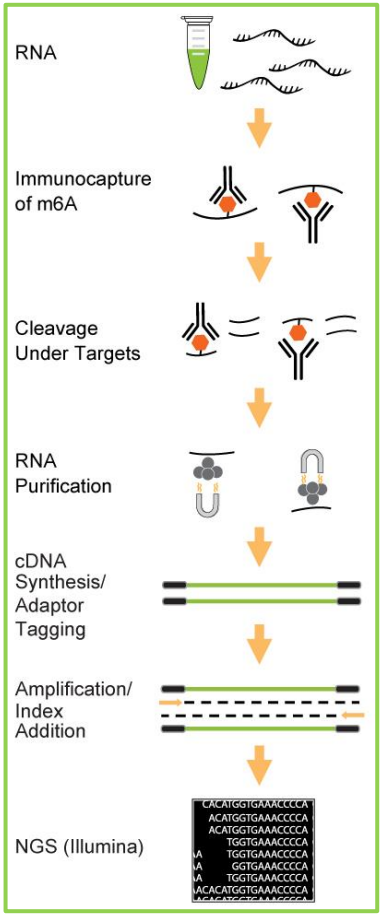
- Same features as P-9018 **+ library prep**
- Reagents included for:
  - **Reverse transcription** of released enriched RNA
  - **Adaptor ligation**
  - **Amplification** of purified, adaptor-ligated cDNA for NGS library construction



Key Features

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- Fast, streamlined procedures
- Sonication-free fragmentation
- Cleavage and immunocapture processed **in same single-tube**
  - Minimized sample loss
  - Low input amount
- Unbound RNA cleavage/removal in situ using unique nucleic acid cleavage enzyme mix with low sequence bias
- RNA cleaved at both ends, directly adjacent to antibody binding site
  - Enrichment of shorter RNA fragments for more reliable identification of m^6A RNA methylation sites
  - Minimized immunocapture/sequencing background
  - High-resolution mapping

For more information, please visit:

www.epigentek.com

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