Application of CUT&RUN and CUT&Tag chromatin mapping assays in agricultural research

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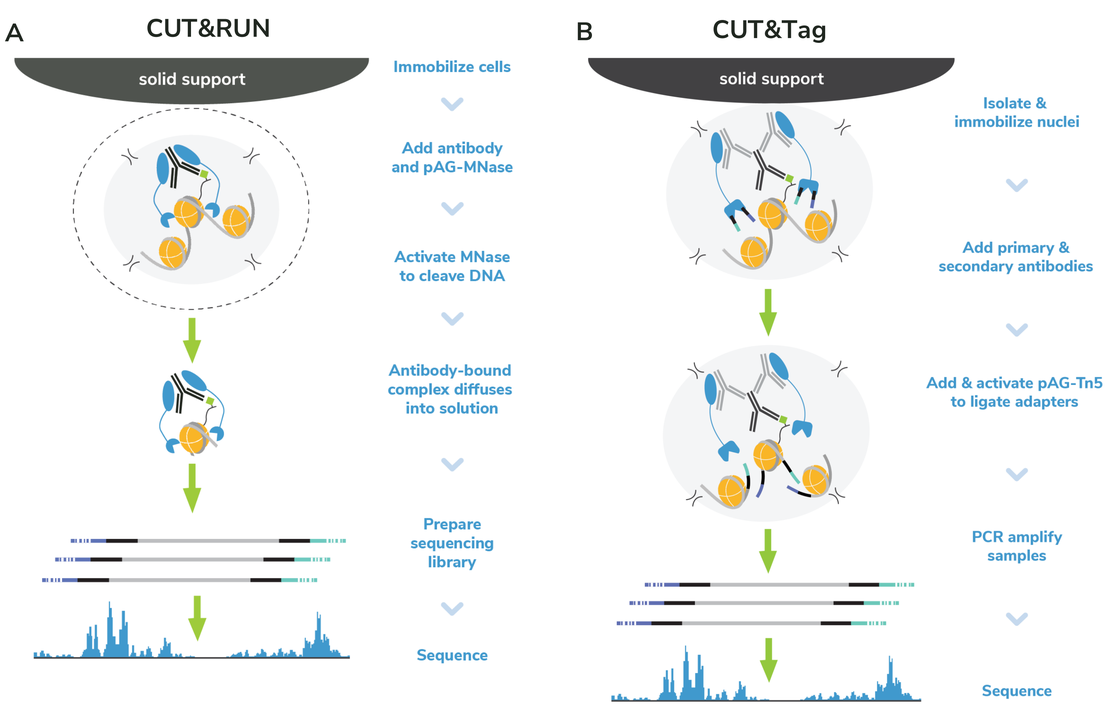
Epigenomic mapping provides key insights into gene regulation, but commonly used ChIP approaches are insufficient

- Gene regulation studies focus on RNA-seq, which only reveal the outcomes – not the mechanisms
- **Epigenomics is the solution**: mapping the location of chromatin features provides mechanistic insights that are central to cell fate and function
- Existing epigenomic technologies, such as ChIP-seq, are inadequate for plant and agriculture research due to high cell number requirements, low-throughput protocols, and high costs

CUTANA™ CUT&RUN and CUT&Tag chromatin mapping assays are enabling advanced epigenetics research

- CUT&RUN and CUT&Tag are immunotethering-based chromatin mapping approaches
- Fast, user-friendly protocol performed using intact cells / nuclei coupled to magnetic beads



Compared to ChIP-seq, CUT&RUN and CUT&Tag generate higher quality data with >100X fewer cells and >10X reduced sequencing depth, with 70% cost savings

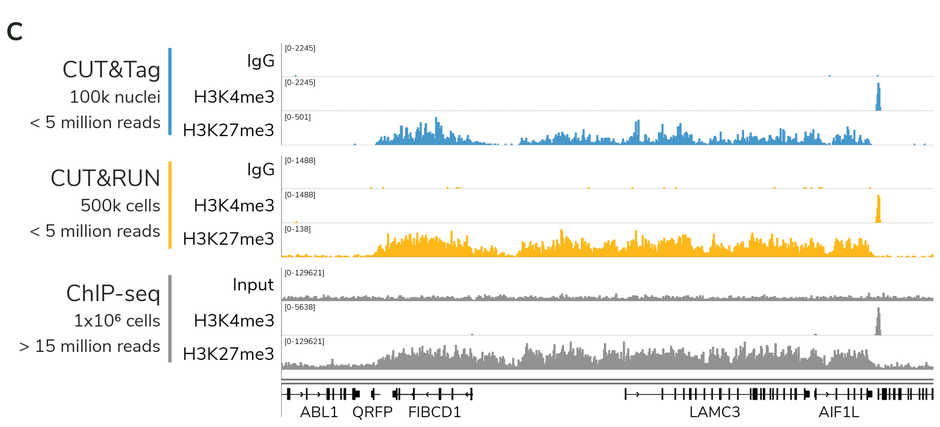
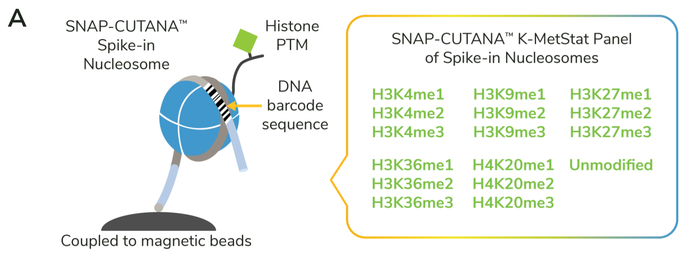


Figure 1: CUTANA™ CUT&RUN **(A)** and CUT&Tag **(B)** workflows for chromatin mapping. **(C)** CUT&RUN and CUT&Tag data generated from K562 cells compared to ChIP-seq data (ENCODE).

Identification of highly specific and efficient antibodies for CUT&RUN and CUT&Tag using innovative spike-in controls

- More than 70% of antibodies against histone post-translational modifications (PTMs) cross-react with related PTMs and/or show poor enrichment efficiency (chromatinantibodies.com)
- We developed SNAP-CUTANA™ nucleosome spike-in controls to help identify best-in-class histone PTM antibodies for CUT&RUN and CUT&Tag chromatin mapping assays

SNAP-CUTANA™ Spike-ins: the only control to directly validate antibodies in CUT&RUN + CUT&Tag



- ✓ Add to bead-bound cells / nuclei before adding antibody
- ✓ Perform CUT&RUN or CUT&Tag
- ✓ Examine recovery of spike-in controls in sequencing data

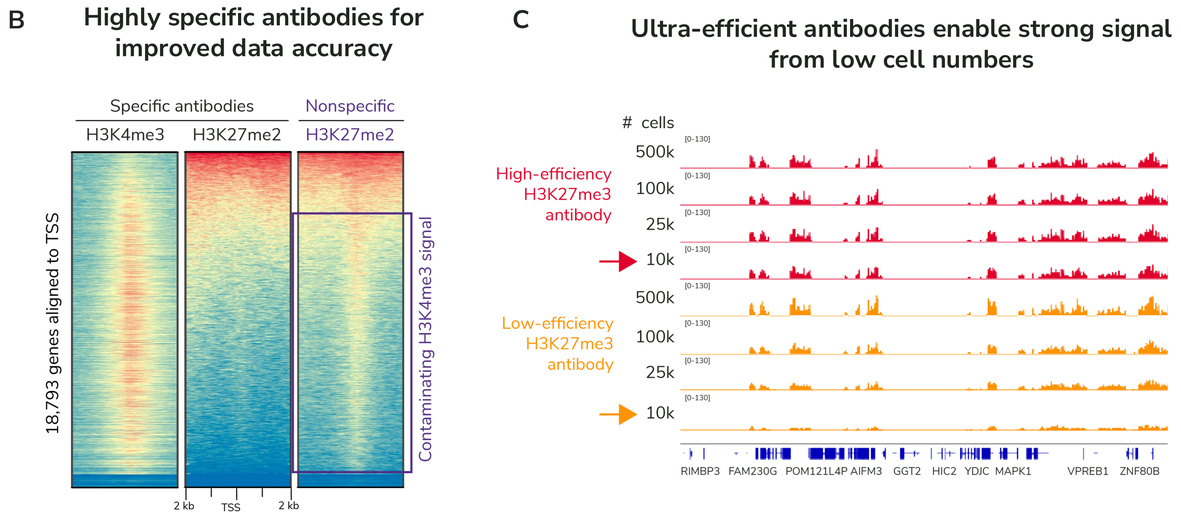


Figure 2: (A) The SNAP-CUTANA™ K-MetStat Panel is a pool of 16 DNA-barcoded nucleosomes carrying widely-studied histone lysine methylation PTMs. **(B)** Antibody specificity defined by the K-MetStat Panel is reflected in CUT&RUN profiles (K562 cells). An H3K27me2 antibody flagged as cross-reactive with H3K4me3 shows extensive off-target signal in CUT&RUN sequencing data. **(C)** Highly efficient antibodies are required to generate reliable profiles from low cell numbers (K562 cells).

Workflow optimization and assay automation are supported by unique nucleosome spike-in controls

Platform comparison	ChIP-seq	CUTANA™ CUT&RUN and CUT&Tag	Implications for automation
Sample input	Fragmented chromatin	Intact cells or nuclei	Streamlined workflow
Required cells	>1 million	500k - 5k	More data from precious samples
Defined controls	Uncommon	SNAP-CUTANA™ Spike-ins	Standardized protocols
Seq depth (reads)	>30 million (mammalian)	3-5 million (mammalian)	Greater multiplexing
Assay cost (per reaction)	~$225	~$72	70% cost savings
Background	High	Low	Better data quality
Experimental throughput	Low	High	Compatible with 96-well plates

Automation halves hands-on time and increases throughput >8-fold.

SNAP-CUTANA™ Spike-ins are key to CUT&RUN automation, enabling:

- **Lower cell numbers** via identification of high-quality antibodies
- **96-well plate optimization** by validating workflow success
- **High reproducibility** by monitoring technical variation and flagging failed reactions

CUTANA™ CUT&RUN and CUT&Tag assays are empowering next-generation agricultural research

Optimized sample prep for plant CUT&RUN and CUT&Tag

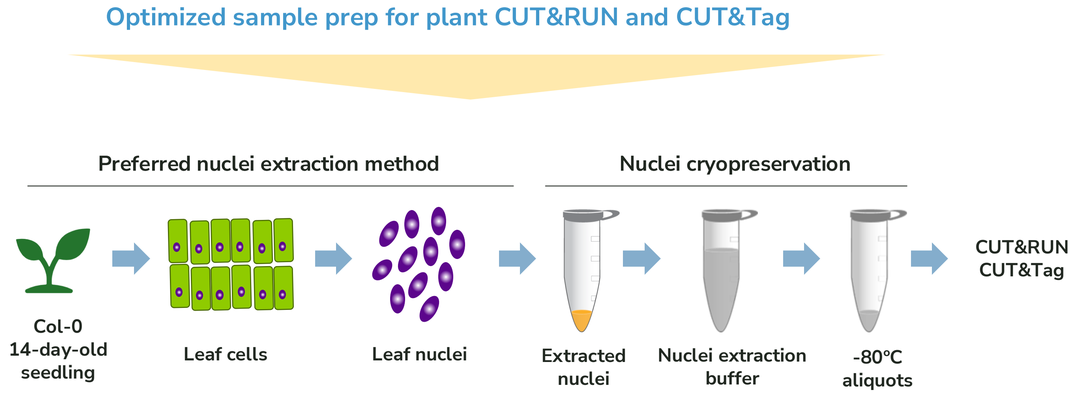


Figure 3: Extraction of *Arabidopsis thaliana* nuclei upstream of CUTANA™ CUT&RUN and CUT&Tag assays. Cryopreservation in Nuclei Extraction Buffer (EpiCypher 21-1026) preserves plant nuclei integrity and provides flexibility to perform the assay across multiple days and/or collaborative sites.

Both CUT&RUN and CUT&Tag are compatible with plants cells

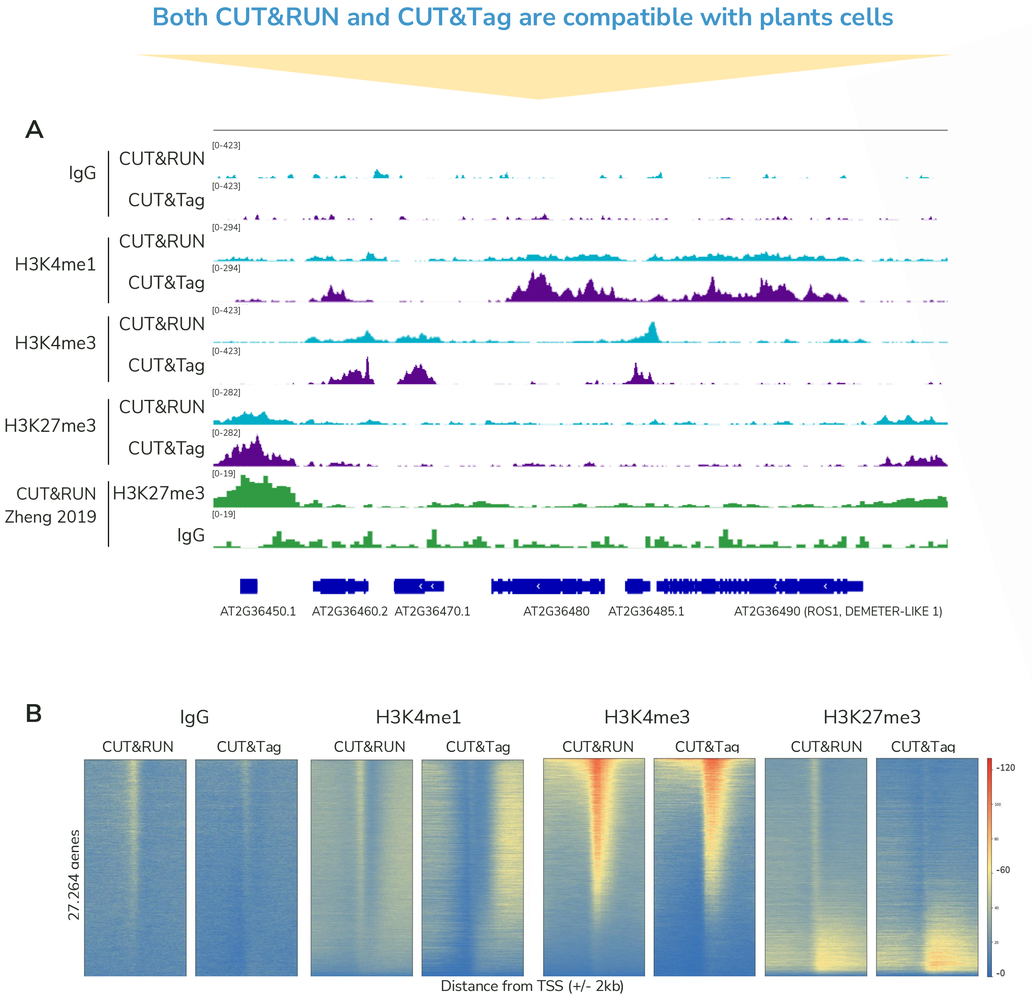


Figure 4: CUTANA™ CUT&RUN and CUT&Tag assays map the epigenomic landscape of *Arabidopsis thaliana* seedlings. 500,000 nuclei were used for CUT&RUN assays, while 100,000 nuclei were used in CUT&Tag. (**A**) IGV tracks of histone PTMs. H3K27me3 data align with previously published CUT&RUN tracks (Zheng, 2019). (**B**) Heatmaps show PTM signal relative to transcription start sites (TSS). Genes ordered by H3K4me3 signal intensity.

Conclusions

- CUTANA™ CUT&RUN and CUT&Tag are compatible with plant samples, generating robust profiles for histone PTMs in repressive and open chromatin regions
- Extraction of nuclei is recommended, using EpiCypher's Nuclei Extraction Buffer and protocol
- Preliminary data suggest that CUT&Tag may have greater signal-to-noise vs. CUT&RUN

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