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ESTABLISHMENT OF ASSAY LIMITS FOR DIFFERENTIAL GENE EXPRESSION AND DNA METHYLATION TO CHARACTERIZE SPECIFICITY OF EPIGENETIC EDITING

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INTRODUCTION

- Epigenetic silencing is a novel therapeutic modality which leverages DNA methylation with the intention to durably silence target genes without cutting or nicking the DNA.
- A comprehensive analytical approach was developed to establish robust metrics for genome-wide specificity assessment of epigenetic silencers.
- CRMA-1001 is an epigenetic silencer under development for the treatment of chronic hepatitis B.

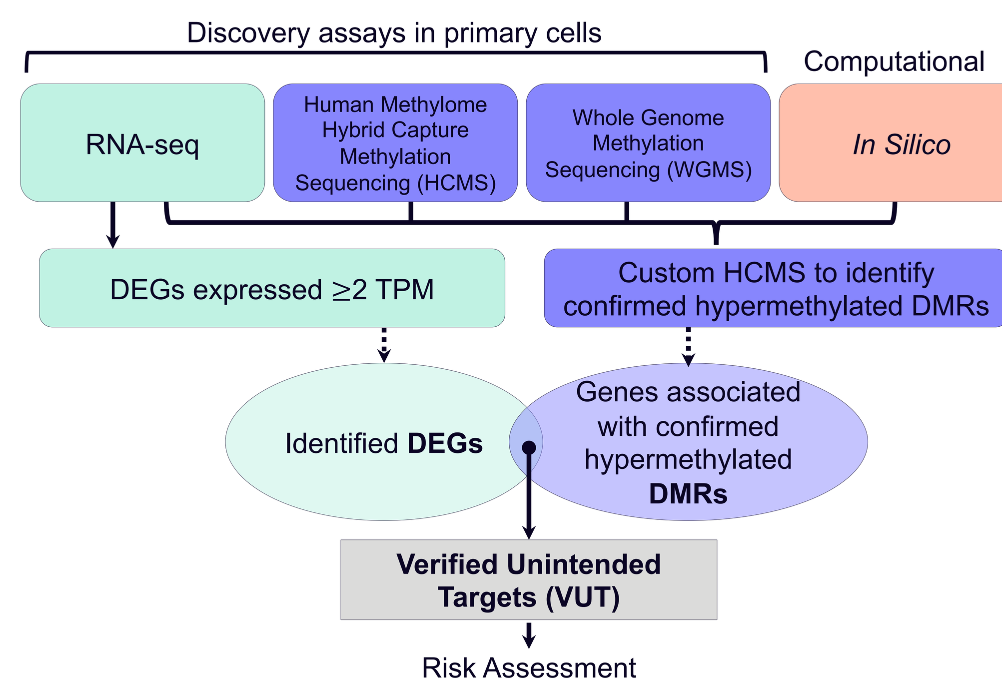
METHODS

Control RNA and DNA experiments: Utilized to determine the assay limits and data quality requirements for RNA-seq and DNA methylation assays.

***In vitro* experiments with tool epigenetic silencer:** To establish appropriate thresholds and parameters for nominating differentially expressed genes (DEG) and differentially methylated regions (DMR) in vitro in the Hep3B cell line.

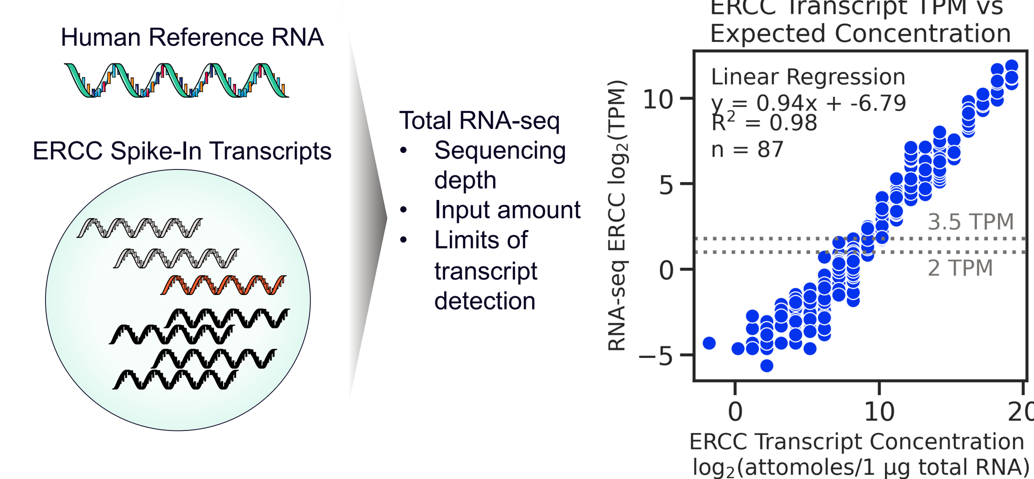
Primary cell experiments with CRMA-1001: Data quality requirements and analysis parameters were used to assess the specificity of CRMA-1001 in primary human cells.

Framework for evaluating specificity of epigenetic silencers



RESULTS

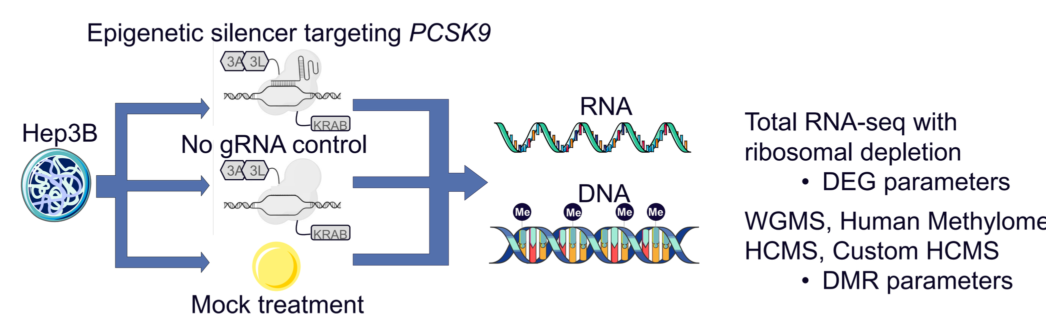
Quantification limits for detecting transcripts using RNA-seq



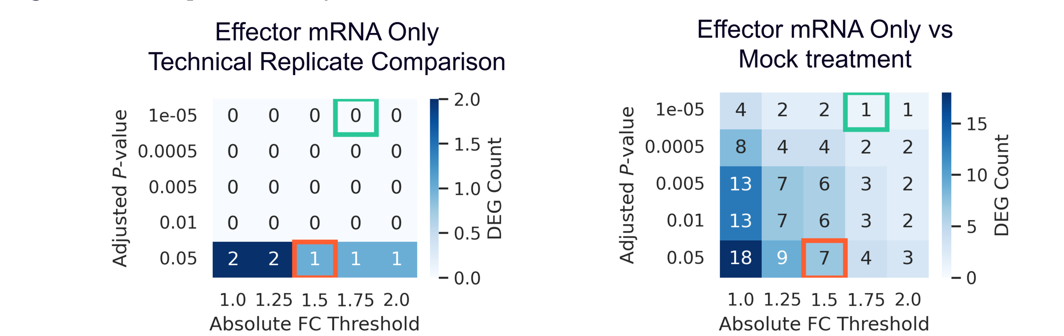
Control RNA experiment utilized human reference control RNA and the ERCC Spike-In Mix1 containing 92 synthetic transcripts not found in the human transcriptome, each at a known concentration, spanning a 20-log2 range of concentrations.

Linear regression of ERCC transcript TPM vs expected concentration. 3.5 TPM corresponded to the transcript with the minimum concentration detected in all replicates. A threshold of 2 TPM was selected for DEG filtering as a TPM above which genes are considered expressed in total RNA-seq datasets. [1]

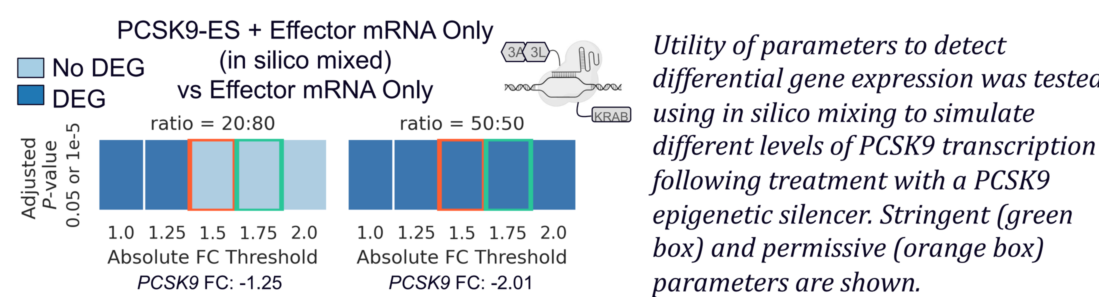
Parameters for detecting differential gene expression using a tool epigenetic silencer



Hep3B cells were treated with PCSK9-ES, or controls (mock or effector mRNA only [no gRNA control]-treatment).

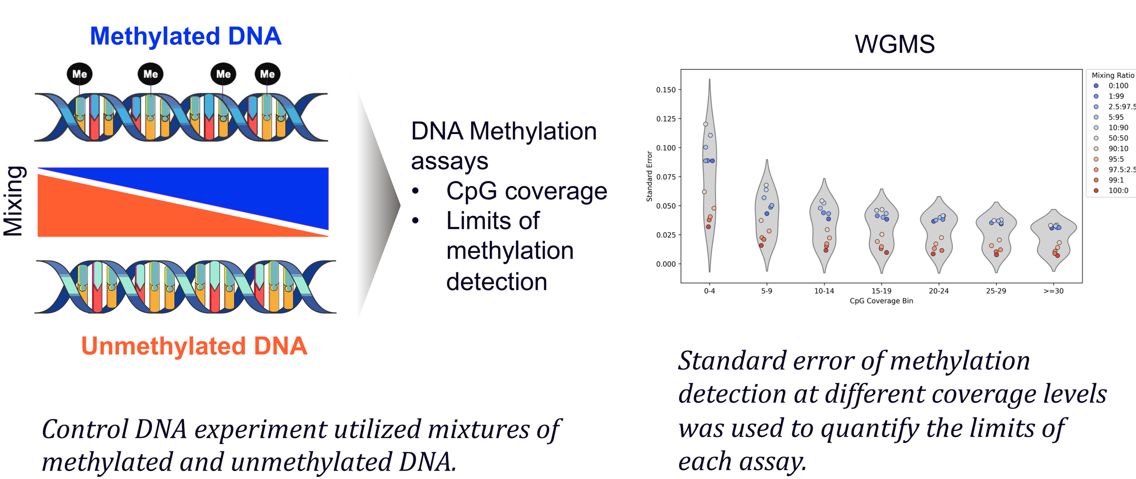


Comparison between technical replicates of the effector mRNA only treatment or between mock- and effector mRNA only treatment identifies stringent (green box) and permissive (orange box) parameters for identification of DEGs.



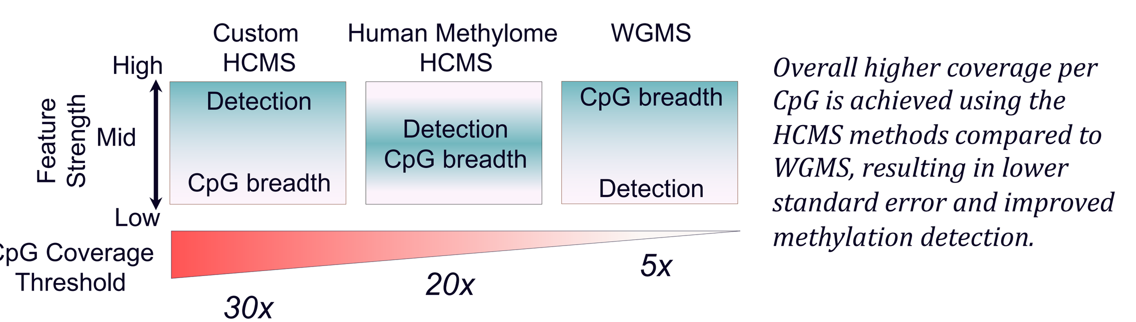
Utility of parameters to detect differential gene expression was tested using in silico mixing to simulate different levels of PCSK9 transcription following treatment with a PCSK9 epigenetic silencer. Stringent (green box) and permissive (orange box) parameters are shown.

Quantification limits for detecting DNA methylation levels using EM-seq assays



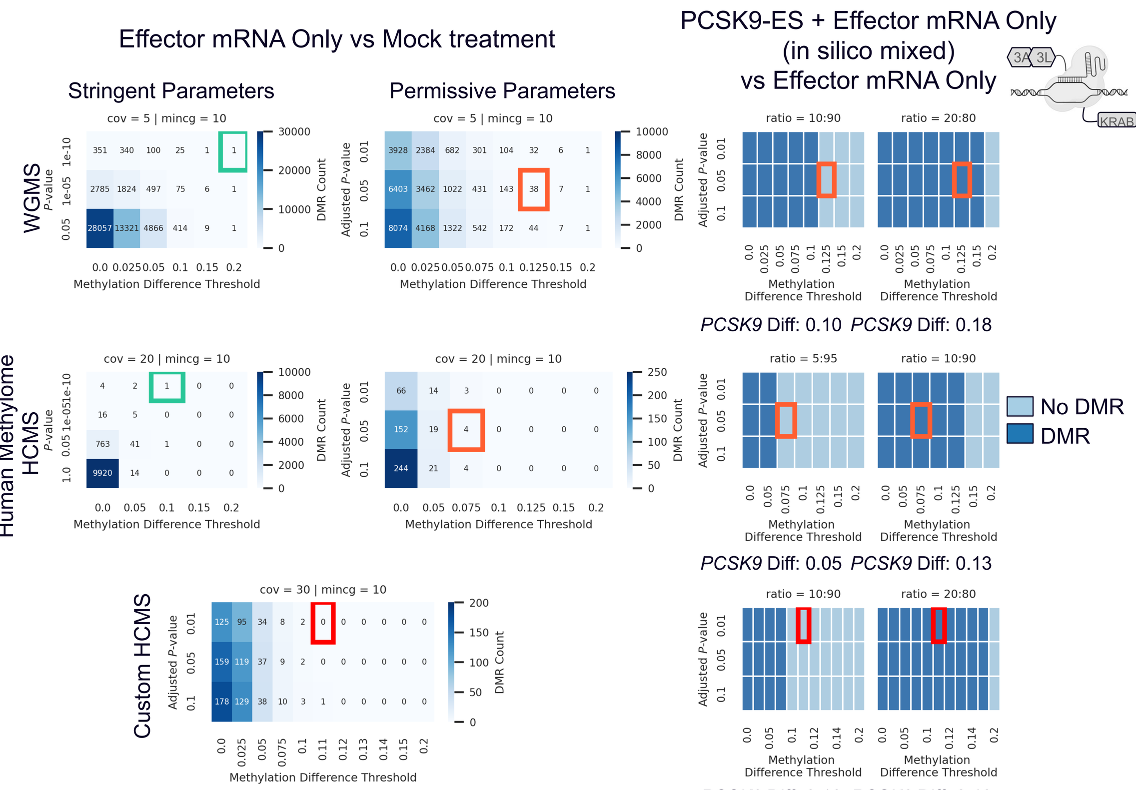
Control DNA experiment utilized mixtures of methylated and unmethylated DNA.

Standard error of methylation detection at different coverage levels was used to quantify the limits of each assay.



Overall higher coverage per CpG is achieved using the HCMS methods compared to WGMS, resulting in lower standard error and improved methylation detection.

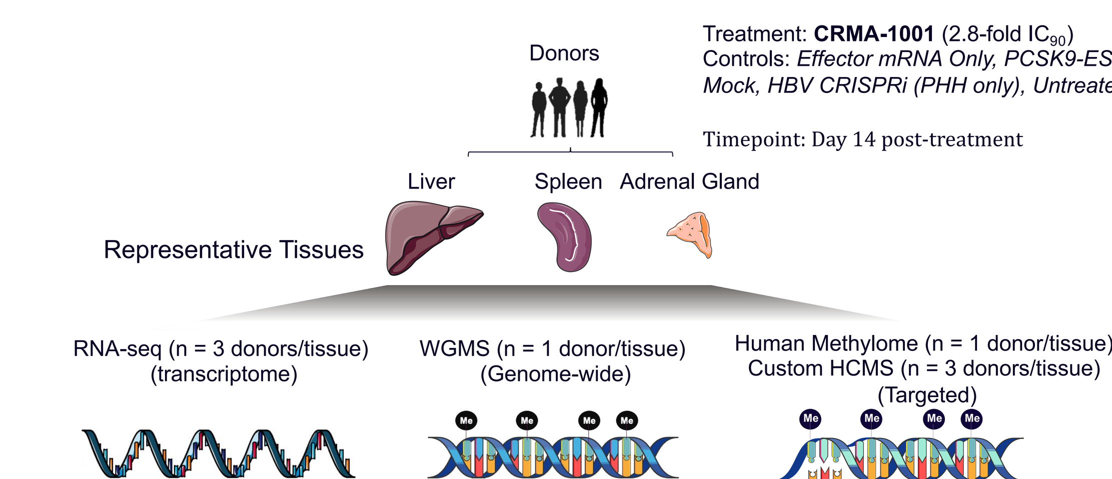
Parameters for detecting differential DNA methylation using a tool epigenetic silencer

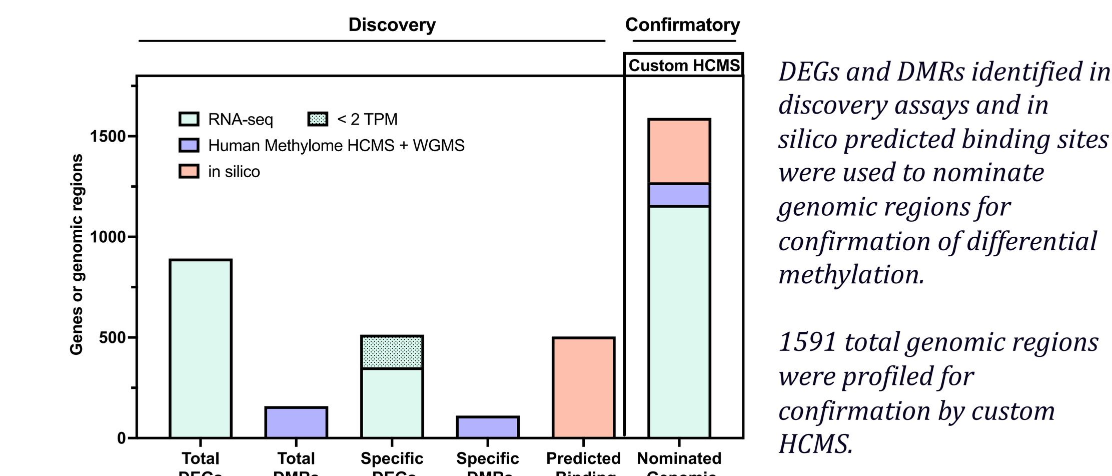


Comparisons between controls identified parameters (boxes) for identification of DMRs using each DNA methylation assay. Two sets of parameters (termed "stringent" [green] and "permissive" [orange]) were selected for site nomination from the discovery assays.

Utility of selected parameters (boxes) to detect differential methylation were tested using in silico mixing to simulate different levels of PCSK9 promoter methylation following epigenetic silencing.

Nomination of potential unintended target sites of CRMA-1001 in primary human cells

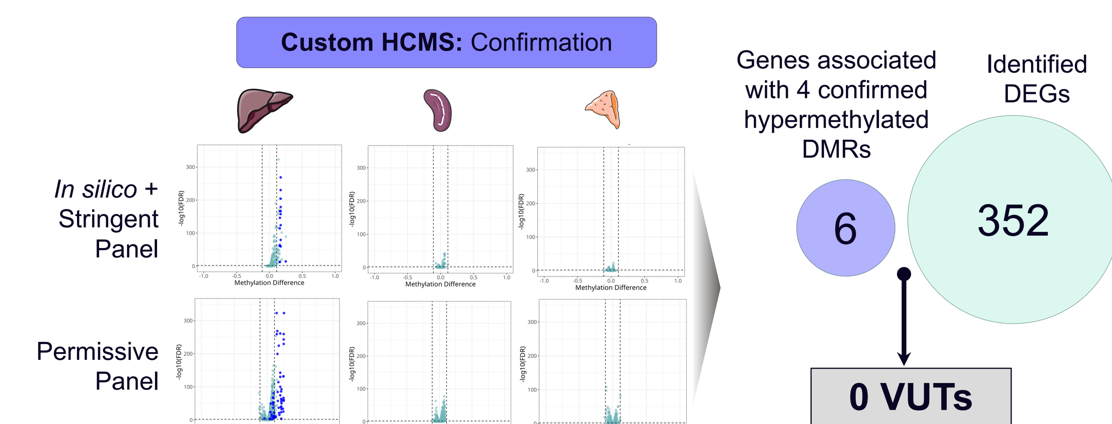




DEGs and DMRs identified in discovery assays and in silico predicted binding sites were used to nominate genomic regions for confirmation of differential methylation.

1591 total genomic regions were profiled for confirmation by custom HCMS.

Zero verified unintended targets identified for CRMA-1001 in primary cell studies



Zero VUTs identified by comparing genes in proximity to confirmed DMRs and DEGs with > 2 TPM expression in primary cell studies with CRMA-1001.

REFERENCES

1. Wagner GP, Kin K, Lynch VJ. A model based criterion for gene expression calls using RNA-seq data. *Theory Biosci.* 2013;132(3):159-164. doi:10.1007/s12064-013-0178-3

CONCLUSIONS

- These results demonstrate a robust standard for quantification of intended and unintended methylation and gene expression changes induced by epigenetic silencers.
- Application of these standards to CRMA-1001 in liver, spleen, and adrenal gland primary cells identified zero verified unintended targets consisting of both differential gene expression and differential DNA hypermethylation.

ACKNOWLEDGEMENTS

- Thank you to the nChroma Bio team, collaborators and partners

CONTACT INFORMATION

- For additional information on this abstract, please contact: info@nchromabio.com

- For additional details on **CRMA-1001 preclinical development**, please see: *Preclinical pharmacology and safety of CRMA-1001, a novel epigenetic editor for chronic hepatitis B that demonstrates HBV surface antigen loss in animal models via precise HBV DNA methylation* **(Poster #1118)**
- For additional details on **In Vivo Pharmacology**, please see: *Epigenetic editors targeting chronic Hepatitis B achieve HBV surface antigen loss with a single course of treatment in multiple HBV mouse models* (**Poster #1262**)