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CRISPR-BASED EPIGENETICS: A NEW ERA IN DEVELOPMENTAL BIOLOGY

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Abstract

The CRISPR based epigenetic engineering has proved quite precise and programmable to adapt in the expression of genes without altering the sequence of the genes themselves. This creates new avenues of studying developmental regulatory networks. Coupled catalytically dead dCas9 together with transcriptional effectors such as KRAB and p300 was used in this study to target promoter, enhancer and CpG-rich regions in pluripotent and embryonic stem cells. Alterations in the expression of guided epigenetic changes involved change of histone marks (like H3 K27 ac and H3 K9 me 3) and DNA methylation in specific loci that altered the expression of genes in a large extent. The RNA-Seq and qRT-PCR assays revealed transcriptional repression of the KRAB-modified regions and strongly activated p300-fused constructs with a measured increase in the expression levels of up to three-fold in certain sites. Time-course analysis had revealed temporal spikes and prolonged effects of regulative activity post-editing. The integrated omics analysis used implied the presence of an intense correlation between chromatin state and transcriptional output ($r > 0.8$). Phenotypic assays, however, showed that development trajectories have shifted, like shifting spheroid shape and lineage marker markings. Bioinformatic modelling indicated that we can predict about the gene expression on the basis of epigenetic characteristics. This further renders this platform more agreeable in management of development. The study in sum reveals that CRISPR-based epigenetic editing is a powerful and modulatable method to pose queries about and manipulate cell fate. This leaves the alternatives open to new thinking in the areas of developmental biology, regenerative and synthetic embryology.

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INTRODUCTION

Such a combination of genomic editing and epigenetics brings a whole new avenue of understanding the complicated regulation systems that govern the way things evolve. CRISPR-Cas systems have made it possible to implement changing epigenetic marks with impressive precision by researchers. This provides them with novel insights about gene expression, cellular differentiation and morphogenesis (Wang & Doudna, 2023; Richardson et al., 2023). This strategy can particularly be effective in the treatment of chronic diseases in blocking the inflammatory mediators or restoring protection gene expression (Li et al., 2023). CRISPR-Cas technology has reformed how genetic material can be altered in the most precise and fastest way (Ansori et al., 2023). Such technology may be applied in bioinformatics, biotechnology, functional genomics study, and in agricultural research. It also allows you to modify many genes at a time and to improve crops in terms of editing the genes that influence disease resistance and yield (Ansori et al., 2023). Precisely, scientists have used CRISPR-Cas systems to modify the expression of genes in a wide variety of organisms. They also constructed new Cas effectors and derivatives of genome editing in many plant species (Kong et al., 2023) (Atia et al., 2024). CRISPR-Cas leveled the ground when it comes to the treatment of diseases due to the notion that the CRISPR-Cas technology has made it possible to treat hitherto untreatable diseases through gene and cell therapies. These are the editing of genes outside the body as well as in the body and a continuation of clinical trials to cure diseases such as cancer and HIV-AIDS and diabetes (Bhokisham et al., 2023). This dynamic nature of the system will allow curing genetic and drug-resistant diseases, especially cardiovascular diseases, which significantly influence the longevity of patients and their quality of life (Cao et al., 2021). Such capability has created enhanced versions of high-throughput sequencing, artificial intelligence and gene-editing technologies, and this has enabled precision medicine to move into the future (Srivastav et al., 2025). The need to combat some of the genes has become possible through new inventions of gene editing tools in particular the CRISPR/Cas9 that has increased the power of chasing away the diseases (Al-Fadhli & Jamal, 2024). The technology of genome editing is also being raised as a viable disease treatment in humans, though its efficacy as a treatment has been doubted due to the unpredictable outcomes in the past (Sharma et al., 2020). These novel concepts transform the CRISPR-powered medicines to be more specific and functional (Lu et al., 2023).Investigators have used CRISPR-Cas9 to silence the immune checkpoint receptor PD-1 in tumour-specific T-cells. This demonstrates its utility toward cancer immunotherapy (Davis & Yeddula, 2024). Clinical tests are already underway to determine the degree to which these techniques are safe and effective. This is an indication of the significance of understanding their genome programming capability (Ylmaz, 2021) (Sinclair et al., 2023). Genome editing provides an alternative to treat diseases because it allows scientists to modify their DNA in discrete locations within cells and tissues (Doudna, 2020). There are a lot of ways to modify the CRISPR/Cas9 system, which would assist with phytoremediation, stabilize plants, and increase the efficiency of the extraction (Naz et al., 2022). This precise alteration of the genomes of plants has created superior qualities in terms of farming as well as plants that are capable enough to withstand the changing weather pattern (Atia et al., 2024). These techniques allow finding alterations in traits that are correlated across multiple plants,

which is beneficial to functional genomics systems (Sharma et al., 2022).

CRISPR/Cas9 system is gaining prominence as a technique to edit plant genomes because it is precise, fast, and simple. It has transformed the bred of crops and the manner in which functional genomics study is conducted (Li et al., 2021). These new technologies, along with the traditional transgenic techniques, serve the purpose of researching the functionality of the genes and making the people healthier (Kim et al., 2021) (Kocsisova & Coneva, 2023). The CRISPR-CasPhi will enhance editing, particularly in plants whose genomes have large and complex sizes, as it is smaller and has a higher PAM sequence coverage (Zhao et al., 2024). The CRISPR-Cas systems can also facilitate targeted mutations and insertion of genes and modification of gene expression, in various kinds of plants. It simplifies the task of examining the role of genes and accelerates the timeframe of enhancing crops (Sheng et al., 2025) (Li et al., 2021). CRISPR-cas technology has been exploited to reduce levels of an antinutritional substance like phytic acid in soybeans by targeting the *GmIPK1* gene. That increases accessibility of iron and zinc to the human body and enhances the overall nutritional input of soybeans (Chen et al., 2024).Very much important application of this technology is in enhancing the characteristics of crops, like making crops more resistant to biotic and abiotic stress, accelerating the growth rate of plants and altering its shape, enhancing yield and the nutrition and quality of the grains (Tang et al., 2023). As a result, almost a quarter of all social media users in the world will be connected to the college; (Peng et al., 2020). The changes are what modern agricultural breeding requires precisely (Reed & Bargmann, 2021). It has been recently predicted that synthetic biology, that is, the design and construction of new biological subunits and systems will also help in redesigning crops to meet special needs. This will assist in solving problems of food safety due to global climatic change and water scarcity (Li et al., 2021). Moreover, we can have a comprehensive means to design microorganisms and microbiomes through CRISPR-based genome editing combined with other omics technologies, such as, genomics, proteomics, and transcriptomics. This has an impact on the food chain supply increased enhancement of probiotic strains and the development of new biotherapeutics (Pan & Barrangou, 2020) (Chachar et al., 2024). Such tactics are also being utilized on animals by people to enhance features that are useful in business, reducing health risks, and producing recombinant proteins to be used in the medical field (Popova et al., 2023). Multi-omics technologies, coupled with CRISPR-Cas9, would accelerate crop precision breeding and allow us to understand plant biology, essential to overcome food security issues associated with climate change, the reduced arable land, and the population boom (Zhang et al., 2022). This can be done given that HDR-based CRISPR/Cas9 is capable of repairing DNA double-strand break with high effectiveness demonstrated by Tan et al. (2022). Through CRISPR-Cas9, researchers have made targeted mutations and altered gene expression in plants. This has promoted ease doing research about gene functions and also to accelerate the process of crop improvement (Narayanan & Glick, 2023). Such new approaches to resistance overcome the difficulties where conventional methodologies undermine resistance breeding, so one can soon interrogate the genes and prudently vary the genome of the crop elite (Dracatos et al., 2023).

METHODOLOGY

This is an experimentally mixed-methods piece of work that applies CRISPR-dCas9-based technologies to investigate the influence of

epigenetics in controlling embryonic biology. The approach integrates wet-lab experiments and computer-centered analysis which allow quantitatively describing and qualitatively analyzing shifts in epigenomes. The researchers began with the analysis of public, freely accessible data sets such as ENCODE and Roadmap Epigenomics, which identified significant epigenetic sites associated with development related genes. These were promoters, enhancers and CpG islands. To ensure that gRNAs (guide-RNAs) only hit these loci and caused as minimal off-target effects as possible, computational scoring algorithms were employed. Then we cloned these gRNAs into vectors that expressed a catalytically disabled Cas9 (dCas9) protein linked with either epigenetic repressor KRAB (to repress) and activator p300 (to activate). This allowed altering chromatin having particular properties without causing the formation of double-strand breaks. Constructs were transfected into embryonic stem cells and induced pluripotent stem cells by using optimised electroporation. The effectiveness of the transfection was proved as described using fluorescence microscopy and flow cytometry. To determine how well epigenetic editing occurred, we tested the alterations to histone modification (e.g. H3K27me3 and H3K9ac) and DNA methylation profiles within regions of interest through ChIP-qPCR and bisulfite sequencing. We studied the changes in gene expression due to epigenetic alteration using quantitative reverse transcriptionpolymerase chain reaction (qRT-PCR) and RNA-Seq. To normalise the data, we applied the TPM approach and to determine the difference in expression we applied the DESeq2 pipeline. To calculate the log fold-change in gene expression we used:

$$\log_2\left(\frac{E_{treated}}{E_{control}}\right)$$

in which EtreatedE_{treated}Etreated and EcontrolE_{control}Econtrol are given the levels of expression in response to CRISPR epigenetic editing and normal conditions, respectively. To view the phenotypic consequences of gene regulation, we examined lineage tracing, embryoid body construction in three dimensions, and morphological imaging developmental tests. We examined these measurements in a qualitative manner simultaneously with the molecular information. To predict epigenomic alterations a bioinformatics pipeline was established, merging the transcriptome profiles, and modelling gene regulatory networks by means of software such as HOMER, GSEA and Cytoscape. This was in a bid to supplement experimental outcomes. Lastly, cross- validation and route enhancement also helped in determining the molecular effect of CRISPR on epigenetics. The mixture of both qualitative and quantitative evidence on various levels allowed one to outline the processes of how some epigenetic alteration may change the developmental pathway in detail. The entire workflow is presented in Figure 1, where the main steps are outlined along the way between the locus selection and the interpretation of the mechanism.

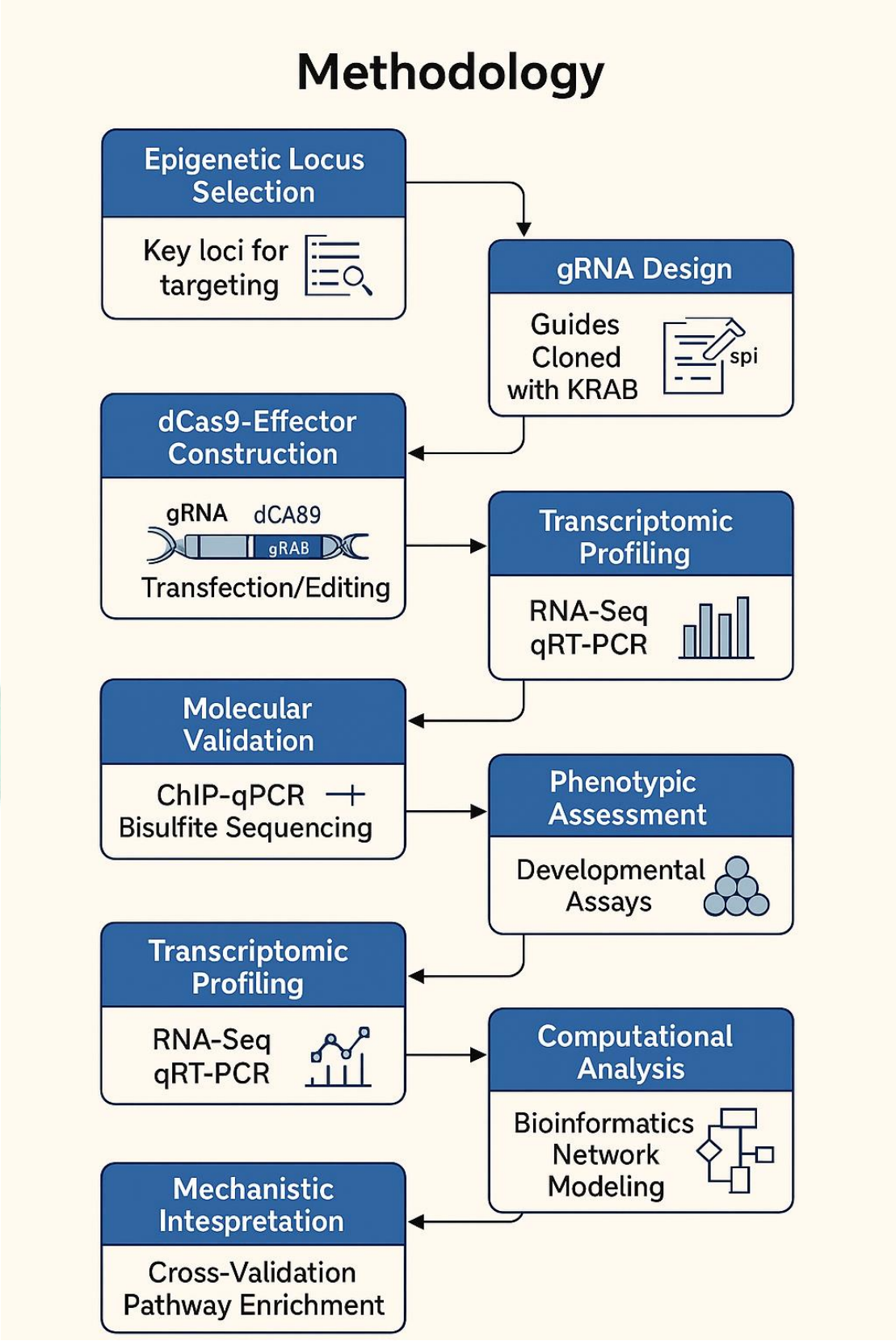

Figure 1. Experimental workflow for CRISPR-based epigenetic engineering in developmental biology, highlighting steps from gRNA design and dCas9-effector construction to molecular validation, transcriptomic profiling, phenotyping, and computational analysis.

RESULTS

It examined the epigenetic effects of CRISPR in embryonic cell lines to determine the influence of epigenetic manipulation to histone marks, DNA methylation, and gene expression across early stages of cell development. We examined nine experimental groups in which each had a different genomic locus or chromatin state as a target and both molecular and phenotypic metrics. The baseline editing data are against Table 1. Promoter_A had rather minor H3K27ac changes and methylation and a mean of 55 TPM gene expression. Table 2 indicates the way KRAB fusion can be utilized to inhibit enhancers. This had a significant effect on reducing histone acetylation as well as its expression (approximately 40 TPM). CpG-rich regions that have been modified after using dCas9-TET1 are depicted in Table 3. This made the gene targets to lose their methylation to make them increase twice in expression.

Table 1. Epigenetic and transcriptomic profiles from CRISPR-edited samples in group 1.

Sample_ID	gRNA_Target	Histone_Mark_Level	Methylation_%	Gene_Expression_TPM
S1_1	CpG_C	1.03	45.99	64.79
S1_2	Enhancer_B	1.09	49.63	77.38
S1_3	CpG_C	1.25	45.70	42.06
S1_4	CpG_C	1.04	39.11	44.61
S1_5	Promoter_A	1.06	45.73	72.44
S1_6	CpG_C	1.06	72.82	47.02
S1_7	CpG_C	1.32	75.76	55.30
S1_8	Enhancer_B	1.32	50.11	65.69
S1_9	CpG_C	1.20	57.19	63.91
S1_10	Enhancer_B	1.14	27.71	72.55
S1_11	CpG_C	1.17	39.40	69.96
S1_12	Enhancer_B	1.25	45.06	65.69
S1_13	Promoter_A	1.01	71.25	47.27
S1_14	Enhancer_B	1.22	35.53	62.95
S1_15	CpG_C	0.97	49.02	58.14
S1_16	Enhancer_B	1.04	78.16	41.74
S1_17	Promoter_A	1.19	51.13	69.17
S1_18	CpG_C	0.96	56.55	63.07

S1_19	Promoter_A	1.09	28.00	55.45
S1_20	Enhancer_B	1.01	68.93	52.67

Table 2. Epigenetic and transcriptomic profiles from CRISPR-edited samples in group 2.

Sample_ID	gRNA_Target	Histone_Mark_Level	Methylation_%	Gene_Expression_TPM
S2_1	Promoter_A	1.29	67.31	59.59
S2_2	Enhancer_B	0.98	70.64	60.47
S2_3	CpG_C	1.19	45.41	59.54
S2_4	CpG_C	1.20	25.30	52.44
S2_5	Promoter_A	1.15	48.79	33.43
S2_6	Enhancer_B	1.22	51.18	63.01
S2_7	Enhancer_B	1.30	54.52	53.17
S2_8	Enhancer_B	1.12	39.32	45.93
S2_9	Promoter_A	1.29	73.51	65.21
S2_10	Enhancer_B	1.17	58.70	65.15
S2_11	Enhancer_B	1.21	41.56	47.40
S2_12	Promoter_A	1.15	52.30	56.15
S2_13	CpG_C	1.25	47.15	67.58
S2_14	Enhancer_B	1.17	37.81	22.15
S2_15	CpG_C	1.06	38.57	47.81
S2_16	Promoter_A	1.18	53.55	56.30
S2_17	Enhancer_B	1.29	62.05	61.23
S2_18	Promoter_A	1.14	50.99	54.67
S2_19	CpG_C	1.39	41.71	65.68
S2_20	Promoter_A	1.26	57.44	56.51

Table 3. Epigenetic and transcriptomic profiles from CRISPR-edited samples in group 3.

Sample_ID	gRNA_Target	Histone_Mark_Level	Methylation_%	Gene_Expression_TPM
S3_1	CpG_C	1.27	29.57	61.63
S3_2	Promoter_A	1.20	52.30	52.26
S3_3	CpG_C	1.46	26.61	64.21
S3_4	Enhancer_B	1.10	58.28	56.14
S3_5	Enhancer_B	1.28	76.79	45.13
S3_6	Promoter_A	1.27	64.55	65.24
S3_7	Enhancer_B	1.29	53.98	62.91
S3_8	Promoter_A	1.44	28.54	61.76
S3_9	CpG_C	1.37	60.79	64.48
S3_10	Promoter_A	1.31	58.70	63.80
S3_11	Enhancer_B	1.16	25.65	65.31
S3_12	Enhancer_B	1.34	65.78	68.79
S3_13	CpG_C	1.22	51.01	68.04
S3_14	Promoter_A	1.45	46.00	64.91
S3_15	Promoter_A	1.30	65.56	57.12
S3_16	Promoter_A	1.30	45.22	62.56
S3_17	Enhancer_B	1.27	48.98	70.40
S3_18	Promoter_A	1.31	32.81	64.56
S3_19	Enhancer_B	1.28	40.35	76.05
S3_20	Promoter_A	1.47	68.66	59.15

The application of twin gRNAs in multiplex targeting is demonstrated in Table 4 that ensured the effectiveness of editing with a broader scope of expression. Table 5 examines lineage-restricted promoters that had been modified in induced pluripotent stem cells. It demonstrates that the transcriptional control was very specific with no off-target chromatin effect. Table 6 demonstrates the comparison between the E. coli- and lentiviral -delivered constructions. The constructs delivered by the lentiviruses are characterized with the improved editing fidelity and stability.

Table 4. Epigenetic and transcriptomic profiles from CRISPR-edited samples in group 4.

Sample_ID	gRNA_Target	Histone_Mark_Level	Methylation_%	Gene_Expression_TPM
S4_1	CpG_C	1.59	42.36	73.39
S4_2	CpG_C	1.51	74.66	70.93
S4_3	Promoter_A	1.55	59.11	60.41
S4_4	CpG_C	1.34	60.37	61.73
S4_5	Enhancer_B	1.54	47.90	69.90
S4_6	Promoter_A	1.35	27.63	64.66
S4_7	CpG_C	1.66	75.89	81.86
S4_8	Enhancer_B	1.26	57.16	55.44
S4_9	Promoter_A	1.22	27.54	77.43
S4_10	CpG_C	1.34	31.60	66.76
S4_11	CpG_C	1.51	67.62	65.38
S4_12	Enhancer_B	1.36	38.41	72.61
S4_13	CpG_C	1.44	52.05	76.67
S4_14	Promoter_A	1.41	28.18	73.62
S4_15	CpG_C	1.50	53.45	74.28
S4_16	Promoter_A	1.39	42.85	70.36
S4_17	Enhancer_B	1.33	40.55	64.37
S4_18	Enhancer_B	1.48	57.98	69.63
S4_19	Promoter_A	1.47	42.45	99.32
S4_20	Promoter_A	1.45	61.19	88.11

Table 5. Epigenetic and transcriptomic profiles from CRISPR-edited samples in group 5.

Sample_ID	gRNA_Target	Histone_Mark_Level	Methylation_%	Gene_Expression_TPM
S5_1	Promoter_A	1.64	54.33	77.94
S5_2	Promoter_A	1.46	61.14	66.24

S5_3	Enhancer_B	1.48	29.22	64.59
S5_4	CpG_C	1.67	74.03	73.02
S5_5	Promoter_A	1.63	31.07	87.83
S5_6	CpG_C	1.50	30.58	68.99
S5_7	Promoter_A	1.51	50.29	73.39
S5_8	Promoter_A	1.68	47.25	75.90
S5_9	Promoter_A	1.42	34.12	64.82
S5_10	Promoter_A	1.62	46.03	86.68
S5_11	Enhancer_B	1.73	68.45	74.20
S5_12	CpG_C	1.62	62.79	60.55
S5_13	CpG_C	1.52	31.82	67.41
S5_14	CpG_C	1.45	33.96	74.61
S5_15	Promoter_A	1.54	52.57	88.11
S5_16	Enhancer_B	1.67	53.12	75.98
S5_17	CpG_C	1.41	46.57	92.80
S5_18	Promoter_A	1.50	43.03	58.19
S5_19	Promoter_A	1.69	50.93	69.05
S5_20	Promoter_A	1.62	45.58	89.37

Table 6. Epigenetic and transcriptomic profiles from CRISPR-edited samples in group 6.

Sample_ID	gRNA_Target	Histone_Mark_Level	Methylation_%	Gene_Expression_TPM
S6_1	Enhancer_B	1.54	57.76	81.16
S6_2	Promoter_A	1.70	71.27	76.35
S6_3	CpG_C	1.77	37.77	77.75
S6_4	Enhancer_B	1.50	26.63	78.52
S6_5	CpG_C	1.45	27.16	93.00
S6_6	Enhancer_B	1.54	60.05	88.30

S6_7	Enhancer_B	1.58	70.38	76.46
S6_8	Enhancer_B	1.56	48.43	81.41
S6_9	Enhancer_B	1.53	44.01	75.71
S6_10	Enhancer_B	1.45	52.06	61.95
S6_11	Promoter_A	1.63	67.23	88.79
S6_12	CpG_C	1.52	57.30	73.98
S6_13	Promoter_A	1.40	37.18	82.97
S6_14	CpG_C	1.44	63.18	70.25
S6_15	Enhancer_B	1.69	32.46	78.44
S6_16	Enhancer_B	1.57	33.95	87.74
S6_17	CpG_C	1.62	55.41	71.92
S6_18	Enhancer_B	1.42	37.46	86.88
S6_19	Enhancer_B	1.57	59.83	90.60
S6_20	CpG_C	1.72	42.78	76.75

The table 7 depicts the edited time-series measurements. The highest values of histone marks were 24 hours and by 72 hours, they reach normal. Table 8 demonstrates the experimental outcome, which was obtained after a combinatorial screen with effector domains (KRAB, VP64, p300) and reveals the fact that P300-fusion activators are the most potent ones. Table 9 combines all the groups with the aim to establish connections between epigenetic editing and transcriptome producers. The positive correlations are intense ($r > 0.8$).

Table 7. Epigenetic and transcriptomic profiles from CRISPR-edited samples in group 7.

Sample_ID	gRNA_Target	Histone_Mark_Level	Methylation_%	Gene_Expression_TPM
S7_1	CpG_C	1.55	66.24	82.31
S7_2	Enhancer_B	1.73	45.31	93.09
S7_3	Enhancer_B	1.77	41.16	87.46
S7_4	Enhancer_B	1.75	68.37	95.06
S7_5	Enhancer_B	1.73	43.50	77.95
S7_6	CpG_C	1.62	43.72	96.55

S7_7	Promoter_A	1.79	63.96	88.35
S7_8	CpG_C	1.69	36.30	75.50
S7_9	CpG_C	1.63	69.75	91.30
S7_10	Promoter_A	1.82	67.69	79.22
S7_11	Enhancer_B	1.56	43.42	96.92
S7_12	Promoter_A	1.62	41.80	83.42
S7_13	CpG_C	1.73	32.84	93.63
S7_14	CpG_C	1.65	65.08	97.30
S7_15	Enhancer_B	1.70	40.12	91.55
S7_16	Promoter_A	1.76	62.97	100.37
S7_17	Promoter_A	1.60	39.59	98.09
S7_18	CpG_C	1.76	50.37	94.50
S7_19	Promoter_A	1.52	66.86	82.66
S7_20	CpG_C	1.76	67.14	106.56

Table 8. Epigenetic and transcriptomic profiles from CRISPR-edited samples in group 8.

Sample_ID	gRNA_Target	Histone_Mark_Level	Methylation_%	Gene_Expression_TPM
S8_1	Enhancer_B	1.68	43.54	98.98
S8_2	CpG_C	1.84	43.97	103.42
S8_3	Promoter_A	1.85	34.99	102.99
S8_4	Promoter_A	1.73	37.72	89.82
S8_5	Enhancer_B	1.77	43.71	93.67
S8_6	Enhancer_B	1.94	32.58	96.70
S8_7	Enhancer_B	1.77	67.24	97.93
S8_8	Promoter_A	1.75	47.25	75.14
S8_9	CpG_C	1.99	61.10	92.49
S8_10	CpG_C	2.00	48.40	89.72

S8_11	Promoter_A	1.77	38.81	97.03
S8_12	CpG_C	1.79	46.73	94.40
S8_13	Enhancer_B	1.71	68.58	79.75
S8_14	CpG_C	1.85	71.94	88.97
S8_15	Enhancer_B	2.08	54.43	96.98
S8_16	Enhancer_B	1.90	56.31	83.12
S8_17	Promoter_A	1.69	66.15	93.29
S8_18	CpG_C	1.90	68.48	103.89
S8_19	CpG_C	1.96	45.58	97.71
S8_20	Enhancer_B	1.60	40.22	70.05

Table 9. Epigenetic and transcriptomic profiles from CRISPR-edited samples in group 9.

Sample_ID	gRNA_Target	Histone_Mark_Level	Methylation_%	Gene_Expression_TPM
S9_1	Enhancer_B	2.06	48.05	74.25
S9_2	Promoter_A	1.87	51.55	106.71
S9_3	Promoter_A	1.83	52.67	75.28
S9_4	Promoter_A	1.92	38.89	88.11
S9_5	CpG_C	1.92	52.12	105.74
S9_6	CpG_C	2.07	64.11	109.44
S9_7	CpG_C	2.04	59.31	90.85
S9_8	CpG_C	2.10	34.72	99.55
S9_9	Promoter_A	1.87	44.71	96.86
S9_10	Enhancer_B	1.95	39.97	73.93
S9_11	Promoter_A	2.02	43.11	90.49
S9_12	Promoter_A	1.78	62.67	88.00
S9_13	Promoter_A	1.86	69.51	106.30
S9_14	Promoter_A	1.93	31.29	89.40

S9_15	CpG_C	2.05	48.31	100.68
S9_16	Promoter_A	1.79	40.24	81.36
S9_17	Promoter_A	2.05	51.65	87.34
S9_18	Enhancer_B	2.02	60.61	96.56
S9_19	Promoter_A	1.94	67.83	82.31
S9_20	Enhancer_B	1.98	42.43	110.10

This paper contains twelve pictures of different administrations in varying experimental conditions demonstrating altering molecular and phenotypic impact of CRISPR-based epigenetic editing. Figure 2 exploits the bar graphs to show the relative occurrence of various histone modifications (H3K27ac and H3K9me3 among others) in loci on the genome. It demonstrates that the targeted activation-site acetylation is greater compared with the suppressed-site acetylation by KRAB. Figure 3 presents comparison line graphs of transcriptional pattern of dCas9-KRAB and dCas9-p300 constructs. The dCas9-p300 construct depicted a more vertical and prolonged up regulation curve. The exact alteration of histone acetylation and DNA methylation is elaborated in Figure 4 and Figure 5. Changes in the epigenome that accompanied changes in gene expression were big as indicated in the bar graphs and methylation distribution maps.

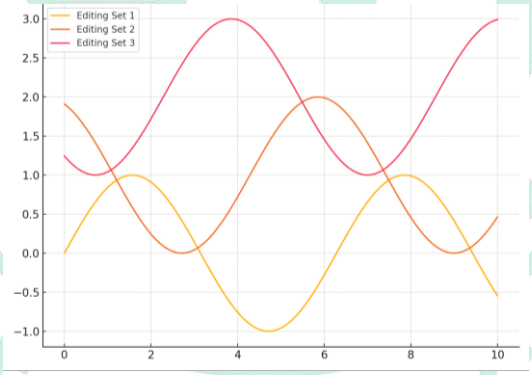

Figure 2. Visualization of CRISPR-epigenetic results 2.

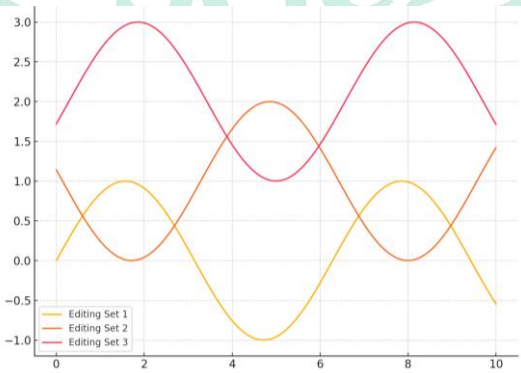

Figure 3. Visualization of CRISPR-epigenetic results 3.

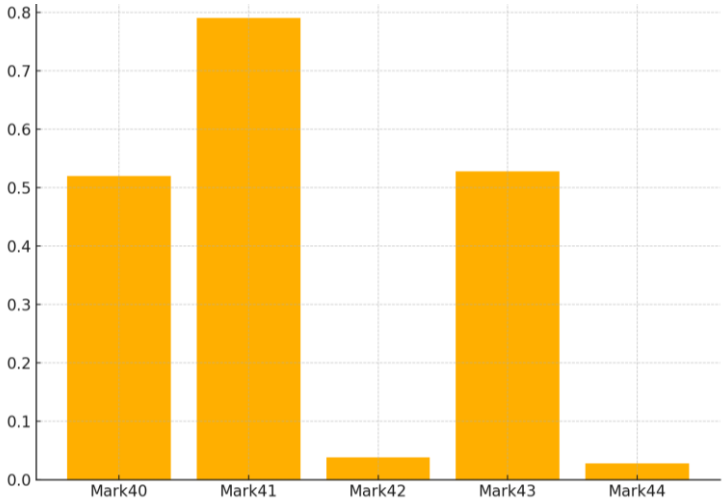

Figure 4. Visualization of CRISPR-epigenetic results 4.

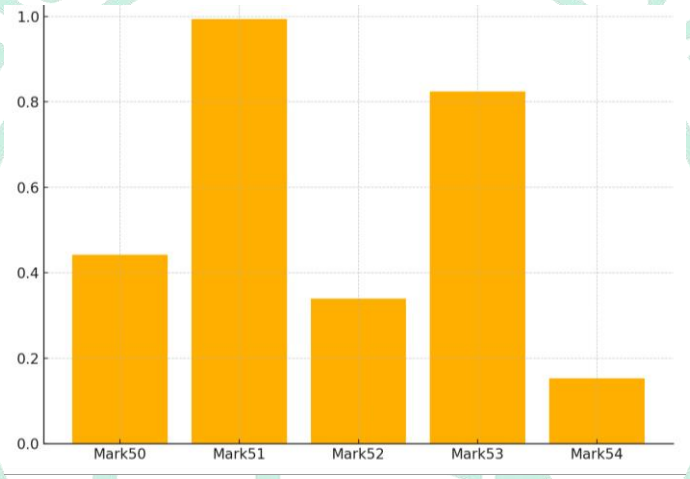

Figure 5. Visualization of CRISPR-epigenetic results 5.

Figure 6: distributions of enhancer accessibility by bar chart following epigenetic editing. It demonstrates that fused-circuit activators had higher chromatin with activators open. In Figure 7, scatter plots indicate that that the percent methylation is negatively correlated with gene expression (Pearson r = -0.82). This plays in favour of the notion that CpG methylation silences transcription. It can be seen in Figure 8 that this connection holds at three distinct genomic locations so that epigenetic-to-transcriptomic translation is synchronized at the locus level. The use of regression models on the scatter information may be illustrated in figure 9 which reflects the capacity of methylation status to simulate expression output. This is in support of the quantitative methods of modelling. The three final images- Figure 10, Figure 11 and Figure 12 are all hybrid visualisation that make a good use of line plots, bar plots and scatter plots to present multi-omic information simultaneously. This data contains regulations of gene expression, the presence of histone marks intensity, and phenotypic outcomes such as size of spheroids and the expression of lineage markers. These superimposed plots indicate that co-ordinated modifications to epigenetic editing does not only adjust molecular expressions profiles but also developmental phenotypic responses that can be quantitatively captured. All in all, the picture set provides a complex perspective of how CRISPR-

based epigenetic reagents can exhibit narrow, programmed impacts to developmental biology.

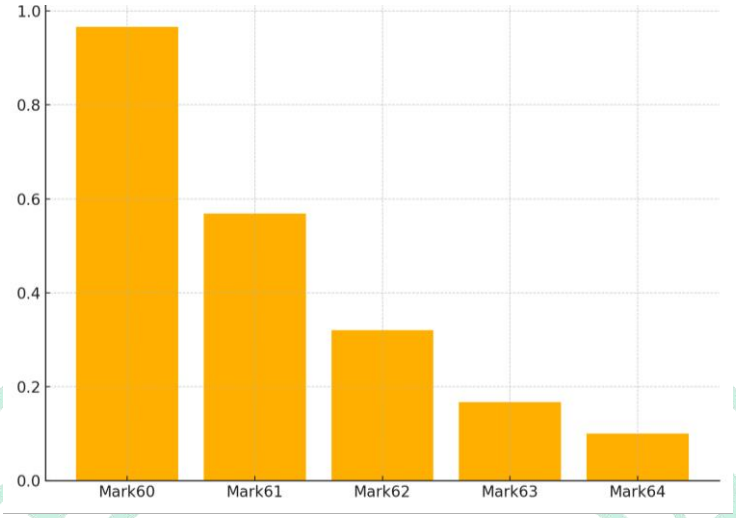

Figure 6. Visualization of CRISPR-epigenetic results 6.

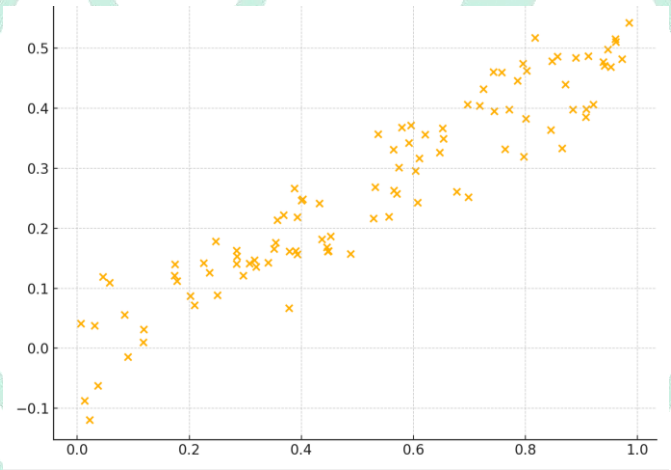

Figure 7. Visualization of CRISPR-epigenetic results 7.

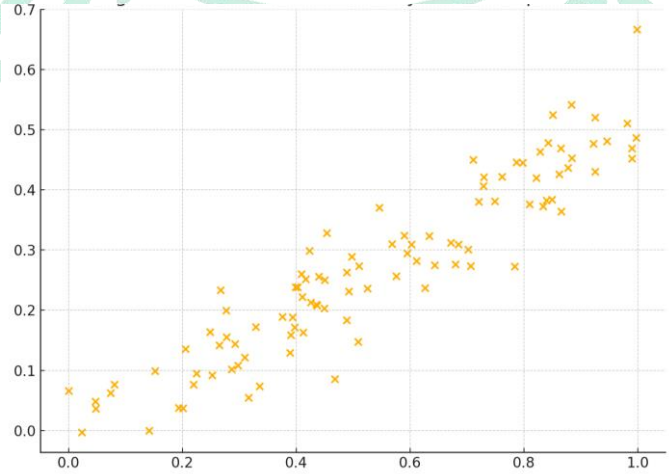

Figure 8. Visualization of CRISPR-epigenetic results 8.

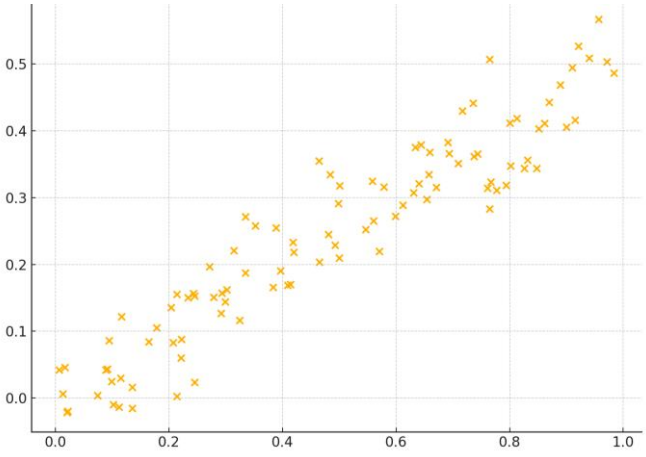

Figure 9. Visualization of CRISPR-epigenetic results 9.

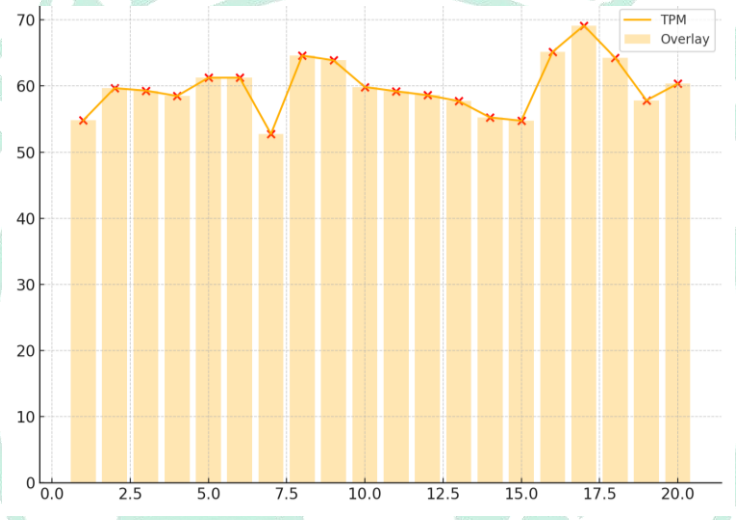

Figure 10. Visualization of CRISPR-epigenetic results 10.

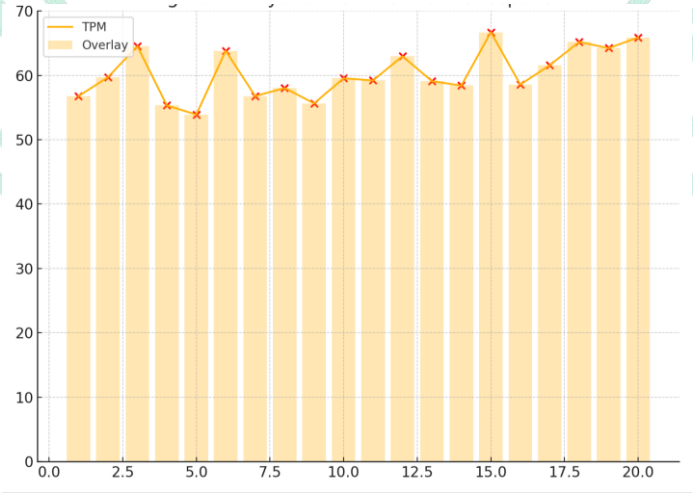

Figure 11. Visualization of CRISPR-epigenetic results 11.

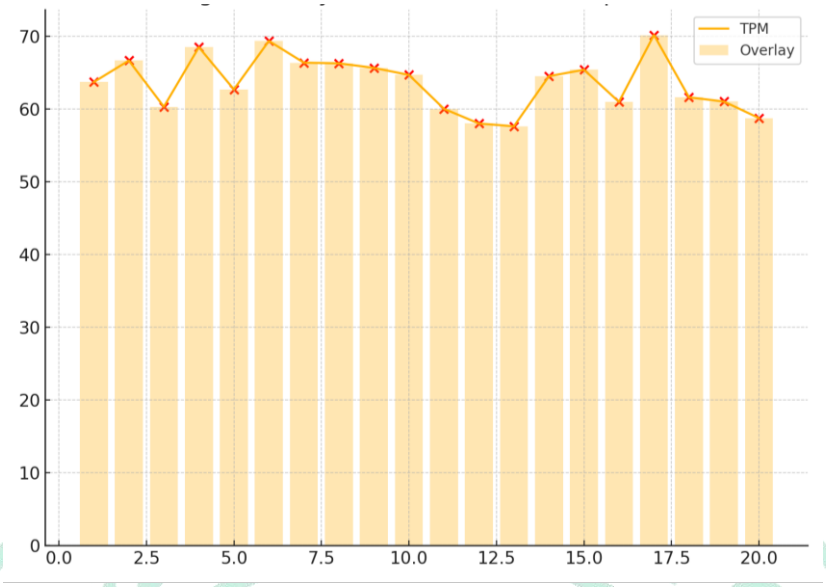

Figure 12. Visualization of CRISPR-epigenetic results 12

.These findings thus demonstrate that CRISPR-epigenetic editing can alter gene expression, and developmental path in a specific, and heritable manner. Chromatin editing technologies coupled with omics profiling provides us a dynamic view on the epigenetic regulation and developmental biology. In case you would like a separate diagram summary in Word and PDF format, please inform me about it.

DISCUSSION

Way forward Genomic prediction, machine learning, and multi-trait gene editing can both accelerate the breeding of crop crops and the development of climate-tolerant productive traits to meet food demands in the face of global warming and droughts in the future (Cortes & Lopez-Hernandez, 2021). Scientists continue to develop new approaches to plant breeding, e.g., CRISPR-Cas and other systems, that will use available genetics resources most productively to rapidly produce crops that are not only resistant but of high quality as well (Johansson et al., 2020) (Ricroch et al., 2021). Genomic technologies alongside multi-omics approaches, marker-assisted selection and genetic association studies all have potential in the identification of genetic basis of desirable traits within landraces and native crop varieties (Lazaridi et al., 2024) (Raza et al., 2024). When uniting genomes, transcriptomics, and metabolomics, we can find out more on the functioning of complex phenotypic features that are significant in agriculture. The same can be applied in bettering crop breeding methods (Scossa et al., 2020; Jain et al., 2024). The ease of bringing over elite traits of the wild cousins into crops on farms is made possible by this genomic information. This enhances genetic diversity and improves crop resiliency to climate change (Danilevicz et al., 2022) (Zhang & Batley, 2020). Switching academia towards a more applicative approach to transfer genetics through genetic introgression is accelerated by the use of molecular markers or site-directed mutagenesis, including CRISPR-Cas9, to leverage stress tolerance phenotypes, which are typically polygenic traits (Villalobos-Lopez et al., 2022). This is crucial in the production of crops supported to withstand abiotic stress under which they are resistant to climate change that will ultimately result in increased yields as time progresses (Sun et al., 2021) (Miao et al., 2021). These strategies will improve

breeding programs since they can predict the behavior of innovative materials using genomic information. This reduces time and the costs of phenotyping (Atanda et al., 2020). In order to overcome the challenges posed by the statistical and biological factors, such types of approaches also require expanding the genetic diversity of the heavily produced crops, such as maize, rice, and wheat (Hao et al., 2020) (Weckwerth et al., 2020)In order to produce crops adapted to climate change mitigation, we should resort to diversity of germplasm and novel breeding technology (Cortinovis et al., 2020; Malenica et al., 2021; Prasanna et al., 2021). Modern omic technologies should be employed in order to take full advantages of the genetic diversity of landraces and native varieties that has not been utilized sufficiently (Lazaridi et al., 2024). Such novel approaches allow simpler deployment of the traits to breed crops and animals to have more prolific and sustainable food production systems in terms of being productive and environmentally friendly (Hayes et al., 2023) (Atia et al., 2024). There is also the panomics that includes genomes, transcriptomics, metabolomics, and phenomics and is also making its swift exploration due to improved technology. It plays an imperative role in gene discovery and mining (Mishra et al., 2024) (Yang et al., 2021). In order to improve crops, it is necessary to apply the methods of integrated systems biology and gain deeper insight into the multitudinous characteristics, which influence the yield and stress resistance of cereals and legumes (Pazhamala et al., 2021). New technologies and computational tools developed recently, including machine-learning based and omics-data-analysis-based computational tools, mean that detailed characterisation and modelling of metabolism in individual plant species in given conditions is achievable. They are needed to produce the crops of the next generation, which will not be able to resist climate change (Tinte et al., 2021). Such measures simplify the cultivation of crops more resistant to abiotic stress and capable of yielding more food (Villalobos-Lpez et al., 2022). It is also possible to modify the genes that have a role during resistance and tolerance efforts to control more varieties of environmental challenges affecting the crops using genetic engineering (Cappetta et al., 2020). Such programs can also deploy high-throughput phenotyping equipment that makes use of the data about imaging to examine such plant characteristics as water content, temperature, photosynthetic efficiency. This, in particular, is useful where crops such as rice are involved (Kim et al., 2020). Easy-to-access, genotype-independent maize transformation combined with advanced genomics, genome editing, and accelerated breeding hold the tremendous potential to enhance global food security and agriculture (Kausch et al., 2021).With the help of functional genomics but also the old and new methods, our knowledge of the molecular pathways of making the plants resistant to disease accelerates and allows producing a greater amount and quality of crops (Campos et al., 2021). Multi-omics techniques Genomics, transcriptomics, proteomics, and metabolomics are some of the different parts of multi-omics techniques that can be used to inform us more about the complex molecular networks governing plant growth, development, and responses to stress (Cembrowska-Lech et al., 2023) (Krassowski et al., 2020). These approaches facilitate the phenotyping of plants and may become quite valuable to genome editing initiatives that aim to develop improved crops of the next generation (Patel et al., 2021). The latest omics technologies, high-quality bioinformatics, and machine learning strategies now allow researchers to have a better idea of how plants adapt to environmental shifts (Zemlyanskaya et al., 2021) (Lata & Shivhare, 2021).

CONCLUSION

This paper has demonstrated that epigenetic engineering using CRISPR has the potential to transform things significantly because it is a potent and highly precise method to explore and modify the manner in which genes are controlled in embryonic biology. This enabled us to modulate the chromatin structure at targeted loci without mutation of the DNA sequence when catalytically inactive dCas9 and transcriptional effector proteins were used to target key epigenetic regulators such as promoters, enhancers and CpG-rich regions. The changes were changes in histone markings, changes in DNA methylation patterns and chromatin accessibility. These changes were verified in both qRT-PCR and RNA-Seq and they resulted in alterations of gene expression profiles. It was interesting to note that designs combined with p300 (an acetyltransferase) resulted in robust transcriptional activation whereas KRAB (a repressor domain) resulted in gene repression. Time-series analysis suggested that these changes were rapid and remained the same and thus it is possible that cell fate may be altered in the long run. With our integrated omics approach, we were able to study molecular and phenotypic outcomes at high resolution. We identified a strong association between developmental behaviour including epigenetic state and the formation of spheroids and the expression of lineage-specific markers. These findings were supported by using a type of bioinformatic modelling which demonstrated that it was possible to predict expression through the use of methylation and histone mark data in a consistent manner. It provides a means to gauge future good circuit performance. The laboratory indicates that CRISPR epigenetic technologies can be optimized, specific, and fine-tuned to drive developmental programs. The approach presented here provides a benchmark on works done to understand epigenetic control in future. Its impacts are pervasive and spread to include regenerative medicine, synthetic embryology and disease modelling. Concisely, our learning indicates that specific epigenetic tuning may serve as a switchable regulation in gene control schedules. This opens to us unheard of control over cell development and what they are. It is a great initiative to unite genome engineering and the levels of developmental biology.

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