Functional Validation of SNPs in Specific Traits by Utilizing Molecular Tools

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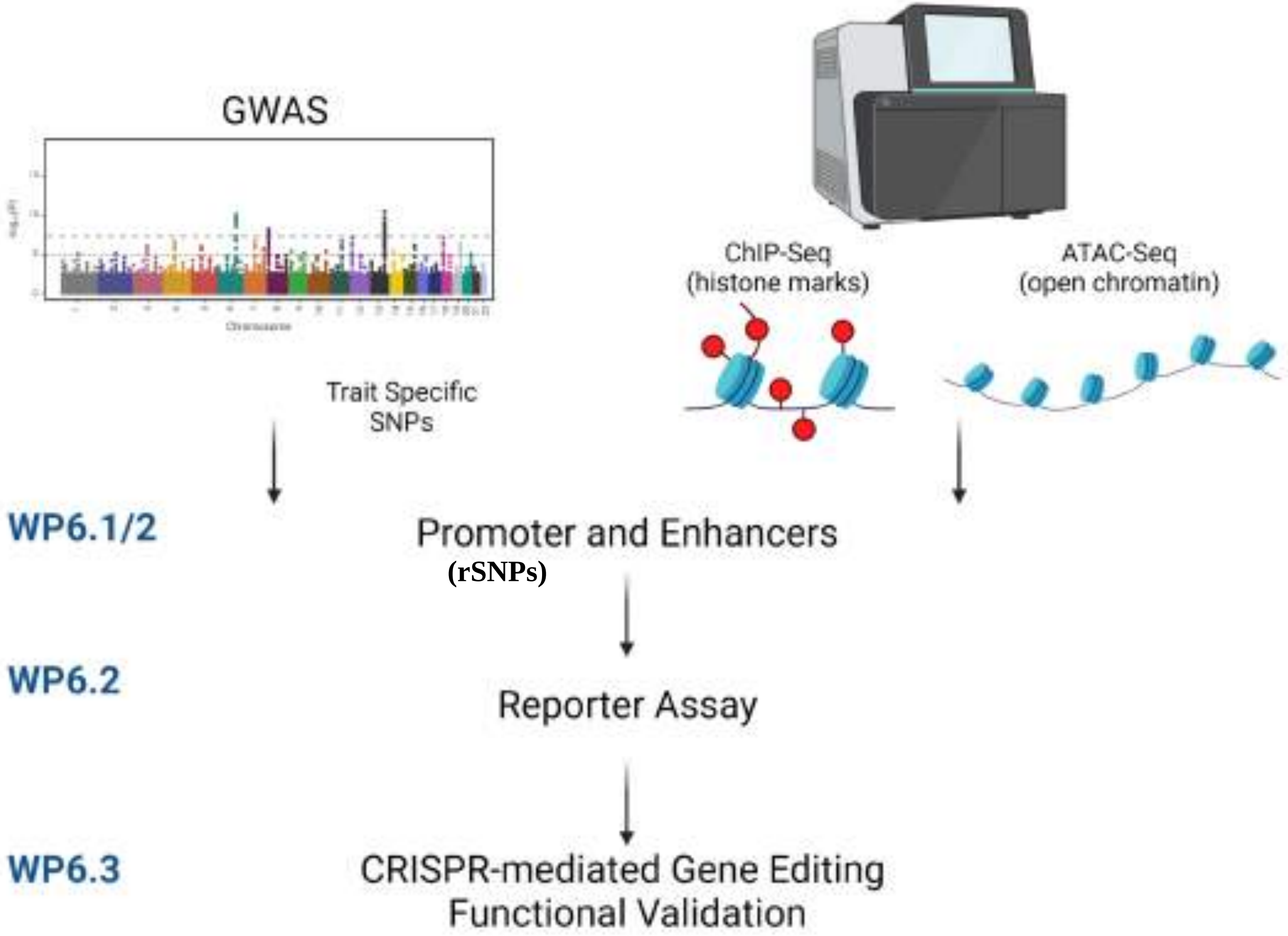
Background

-Genomic data allows us to identify regulatory elements and SNPs in key tissues of different livestock species, including cow

-How regulatory elements and SNPs influence gene expression and subsequently modulate phenotype

Our Strategy



GWAS

ChIP-Seq
(histone marks)

ATAC-Seq
(open chromatin)

Trait Specific
SNPs

WP6.1/2 Promoter and Enhancers
(rSNPs)

WP6.2 Reporter Assay

WP6.3 CRISPR-mediated Gene Editing
Functional Validation

Selection of SNPs

Promoter (rSNPs)

Existing NGS data

WP4
Beef and Daily trait GWAS

WP2
RNAseq, ATAC-seq

Transcription factor binding site analysis

31 different rSNPs

Functional Analysis
promoter test
mutant test

3 different rSNPs

CRISPR

Functional Validation

Enhancer

Existing NGS data

WP4
Beef trait GWAS

WP2
RNAseq, ATAC-seq

Transcription factor binding site analysis

4 putative enhancers

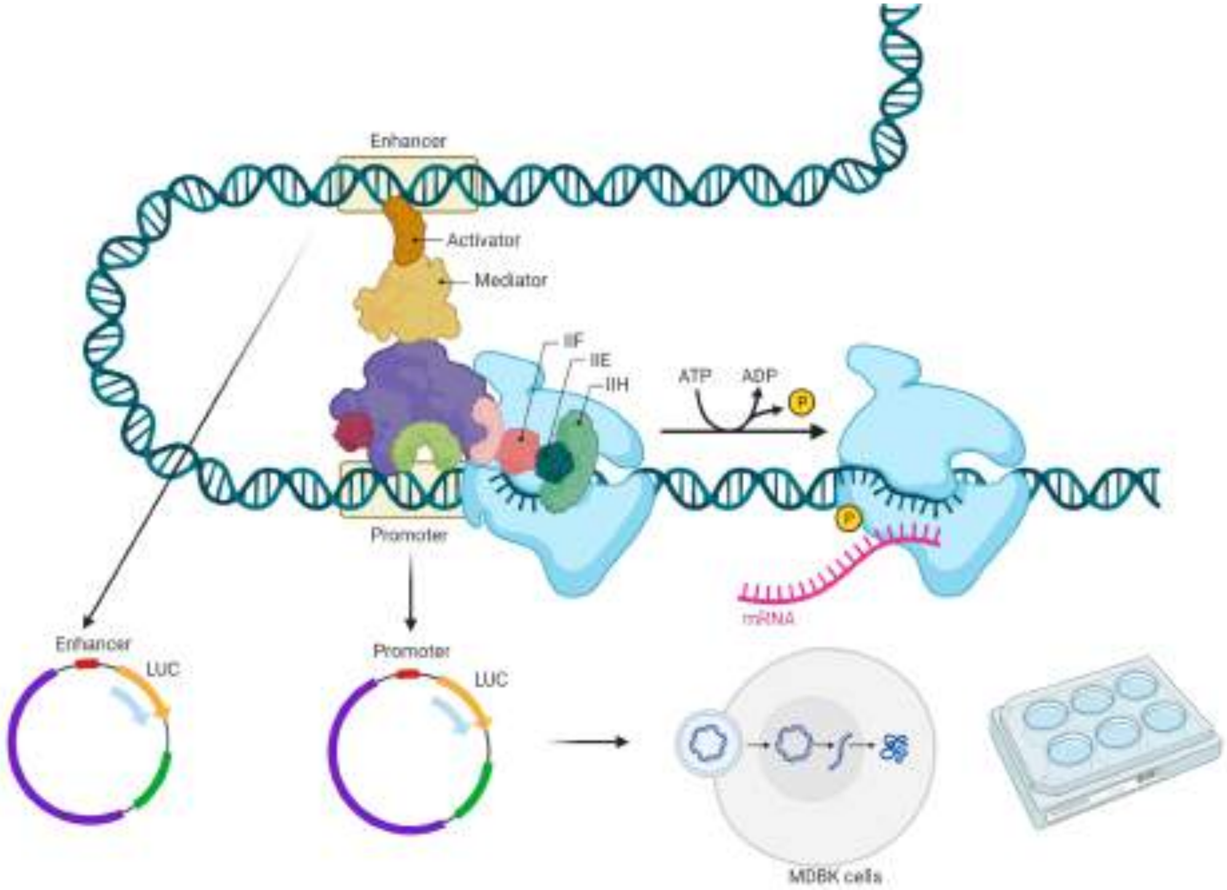
Functional Analysis
enhancer test
mutant test

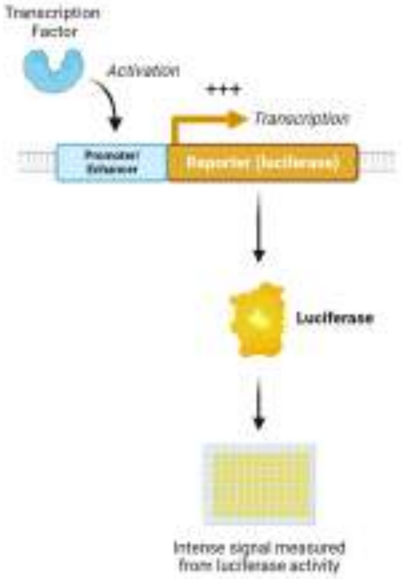
3 different regions

CRISPR

RNA seq

Testing Enhancers and Promoters



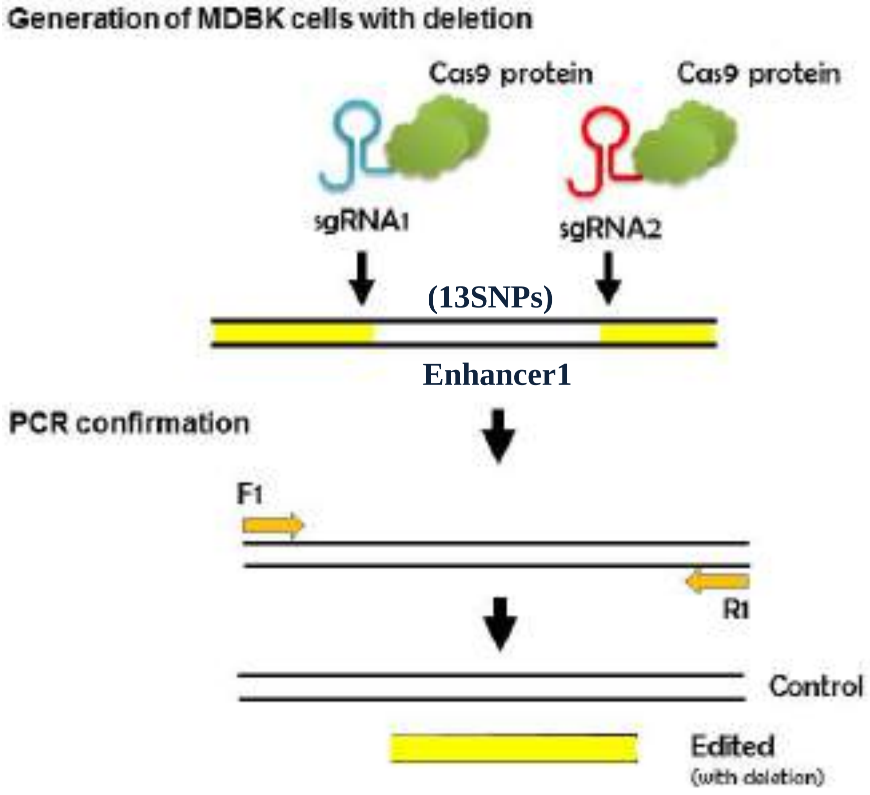


Enhancer1 has enhancer activity

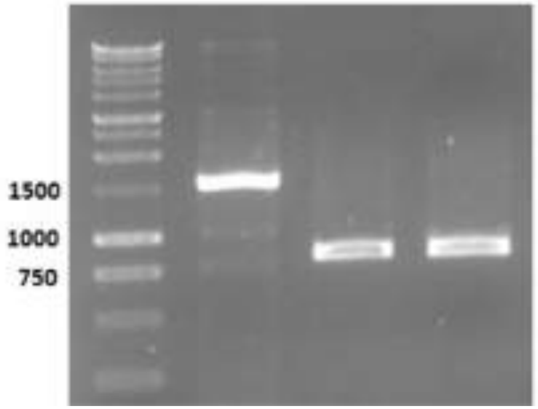
	SNPs	Identified method	Promoter /Enhancer	Enhancer activity (N4)
Enhancer1	13 SNPs	ATAC-enhancer ChIP	Enhancer	2.13
Enhancer2	1SNP_t/c	ATAC-enhancer ChIP	Enhancer	4.06
Enhancer3	1SNP_a/c	ATAC-enhancer ChIP	Enhancer	2.64
Enhancer4	1SNP_t/c	ATAC-enhancer ChIP	Enhancer	0.98
MT/WT				Activity MT/WT (N6)
Enhancer2	1SNP_t/c	ATAC-enhancer ChIP	Enhancer	0.81
Enhancer3	1SNP_a/c	ATAC-enhancer ChIP	Enhancer	0.23

CRISPR-mediate deletion of SNPs cluster element in Enhancer 1

Strategy of CRISPR-mediated deletion of Enhancer1 in MDBK cells



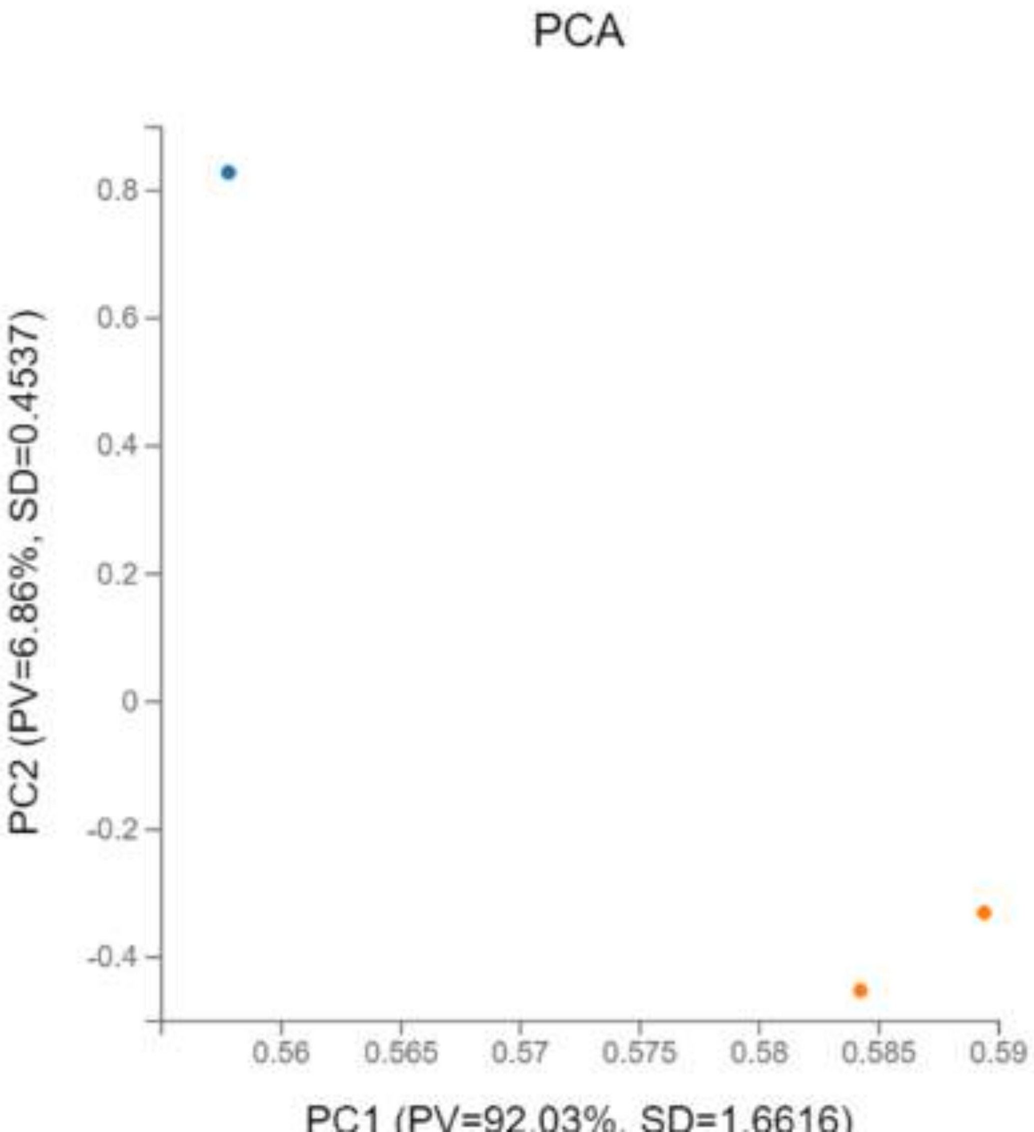
Confirmation of CRISPR-mediated editing



WT Clone 1 Clone2

Enhancer1 SNPs
Deletion Mutant

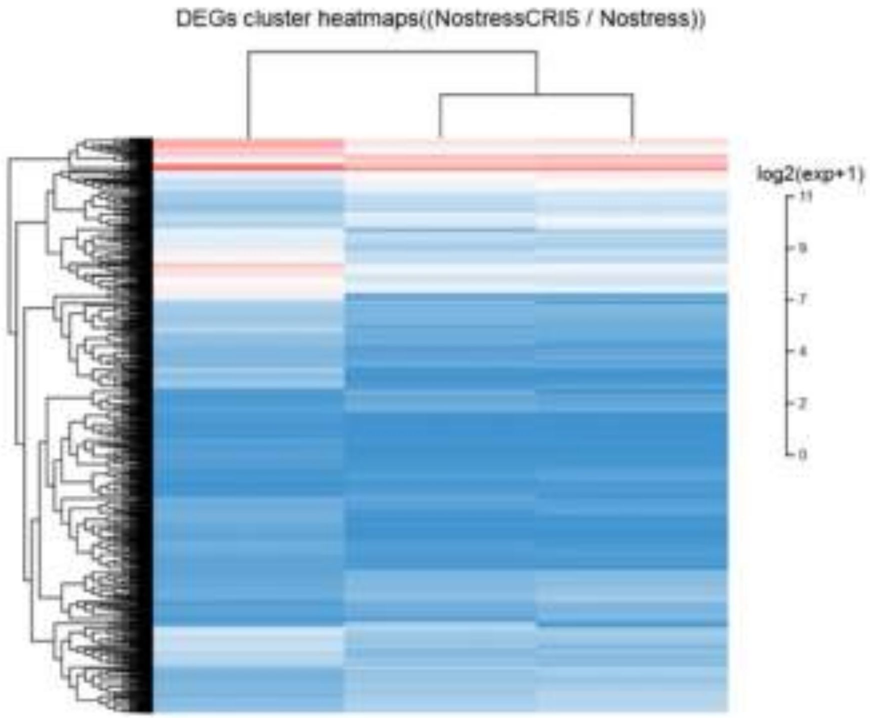
630 Genes are altered in Enhancer1 SNPs deletion mutants



● WT

● CRISPR
(del SNPs)

630 Genes are altered in Enhancer1 deletion mutants



WT

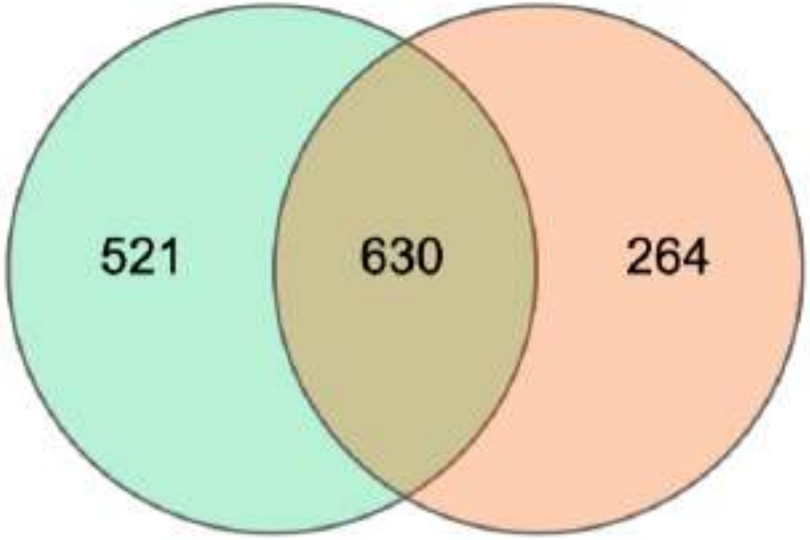
Clone 1

Clone2

Enhancer1
Deletion Mutant

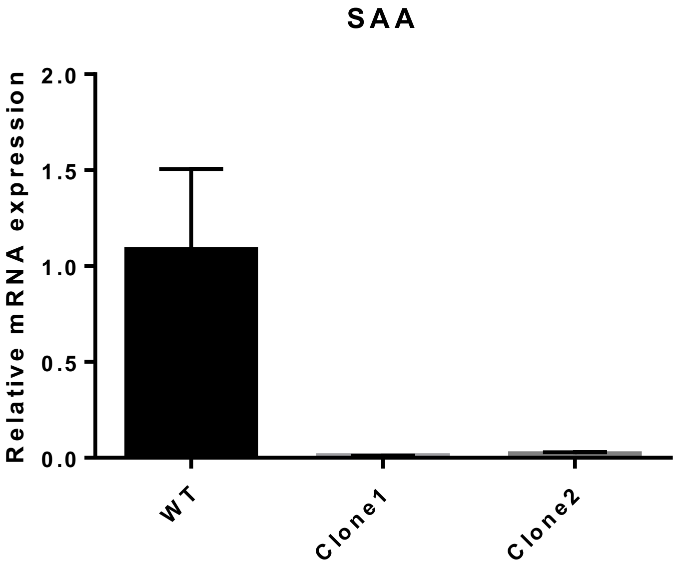
Clone 1

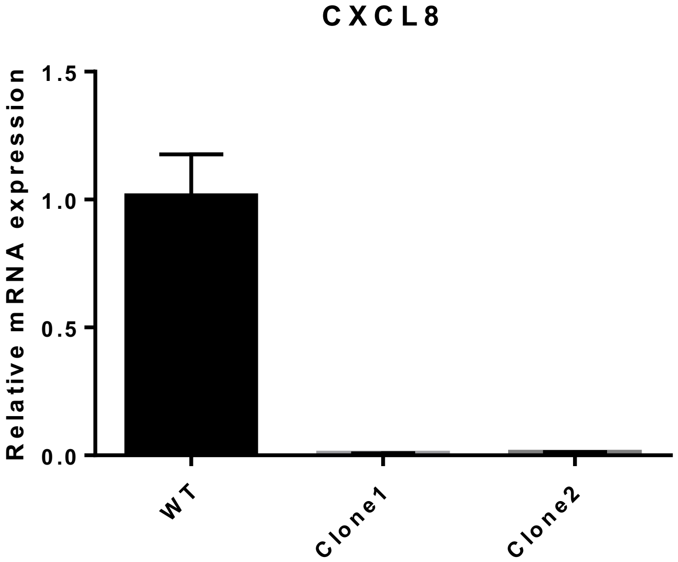
Clone2



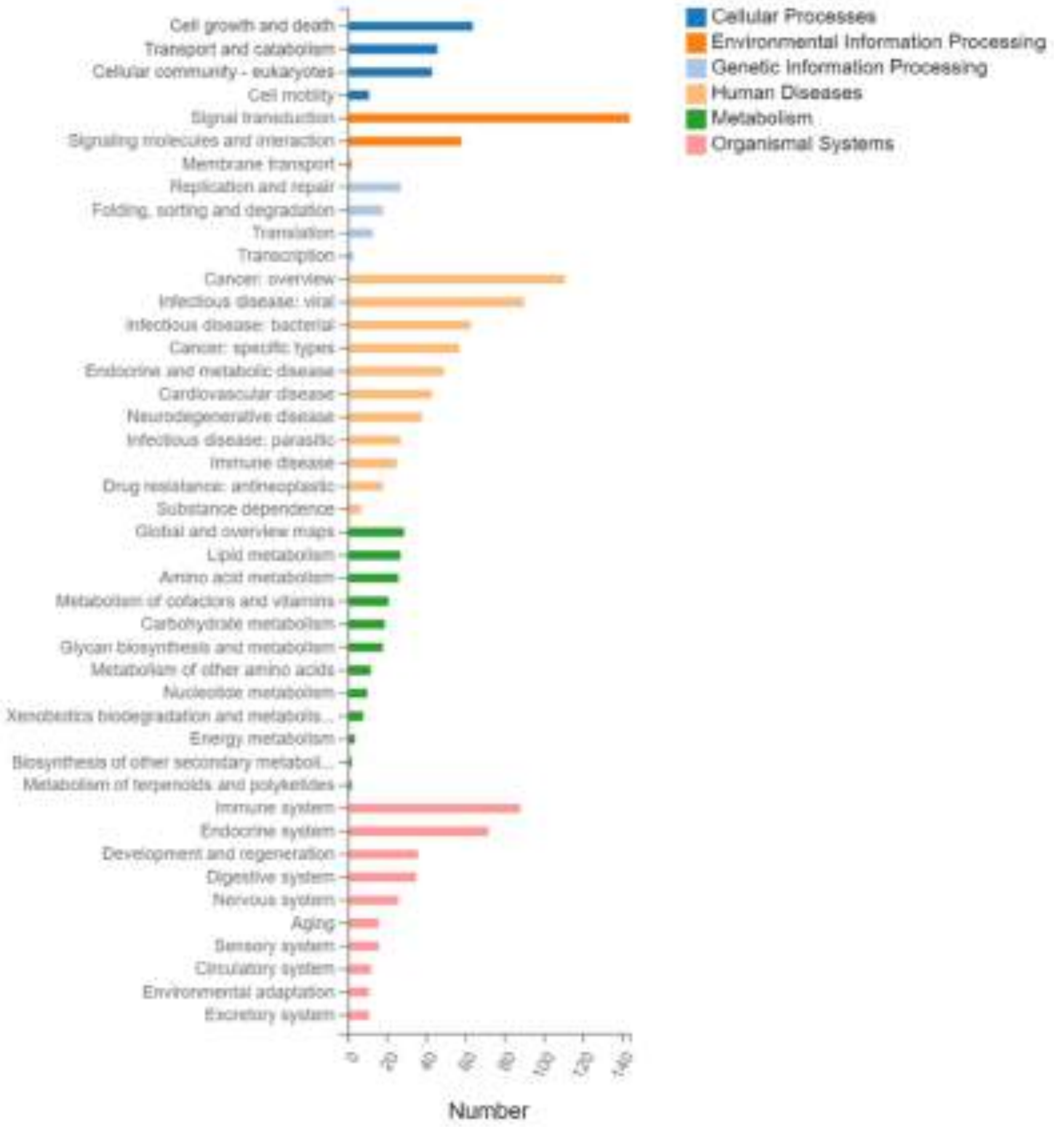
Significantly changed gene expression in two CRISPR (Enhancer1 deletion) clones compared to MDBK WT (FDR<0.001)

Validation with qPCR confirms the accuracy of RNAseq





Cell growth and cycle pathway are affected by the lack of 13SNPs in Enhancer1 region



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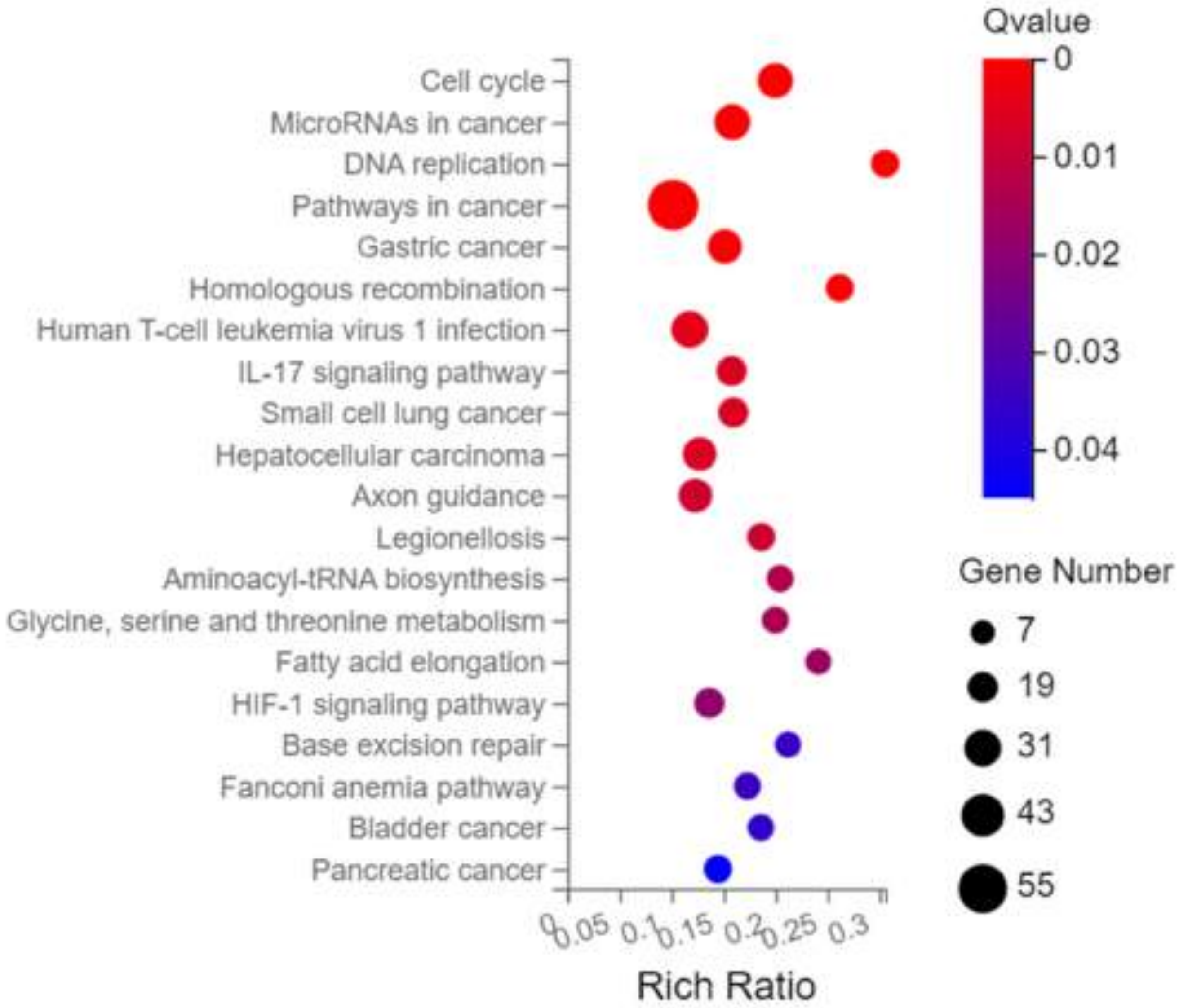
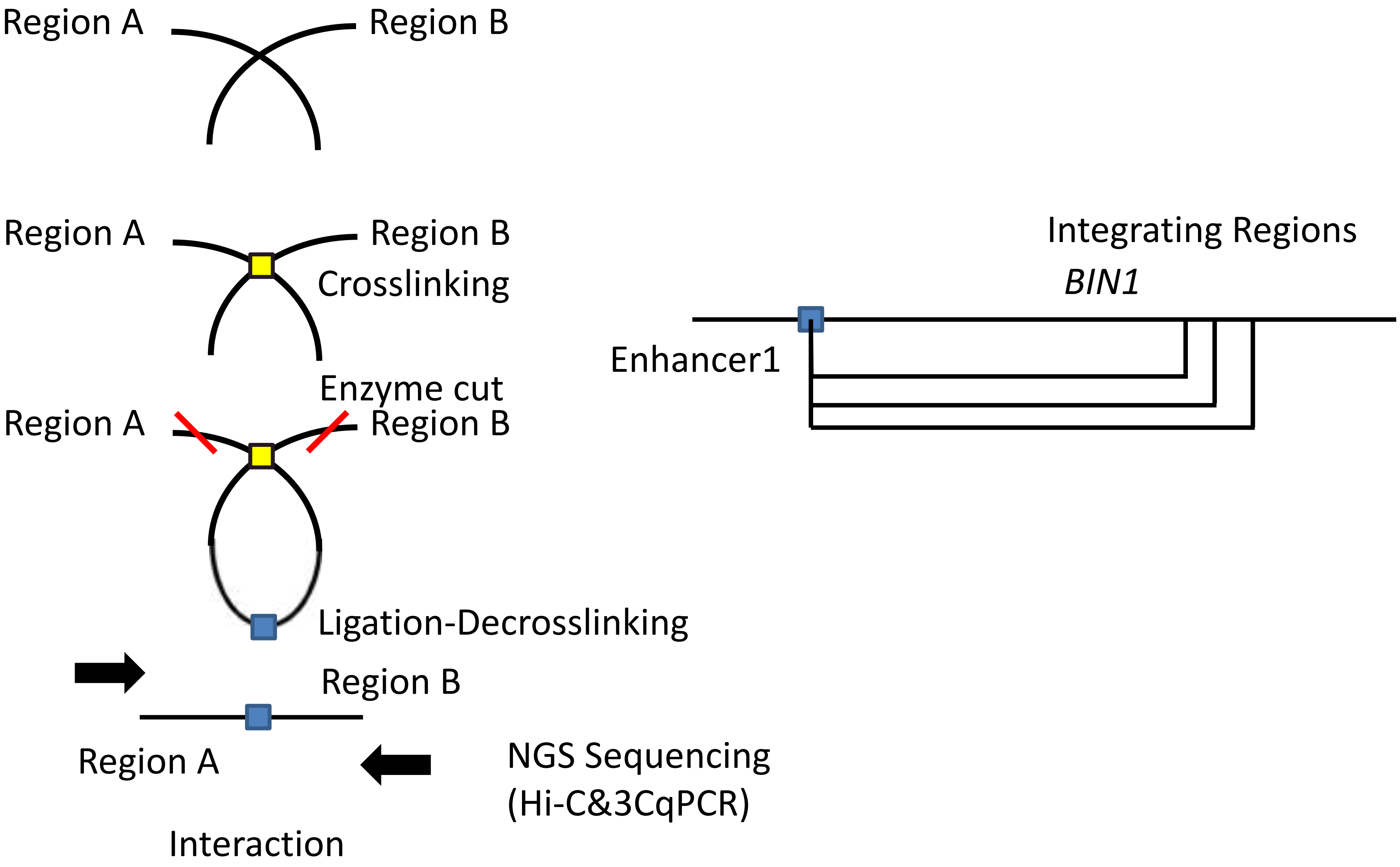
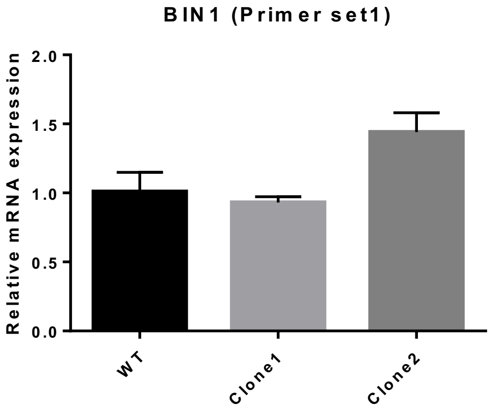


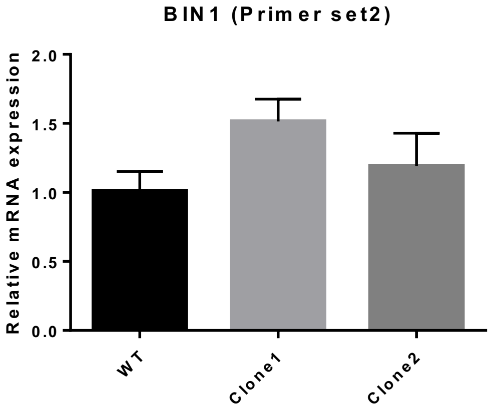
HiC and 3C assay reveals the interaction of the Enhancer1 with *BIN1*

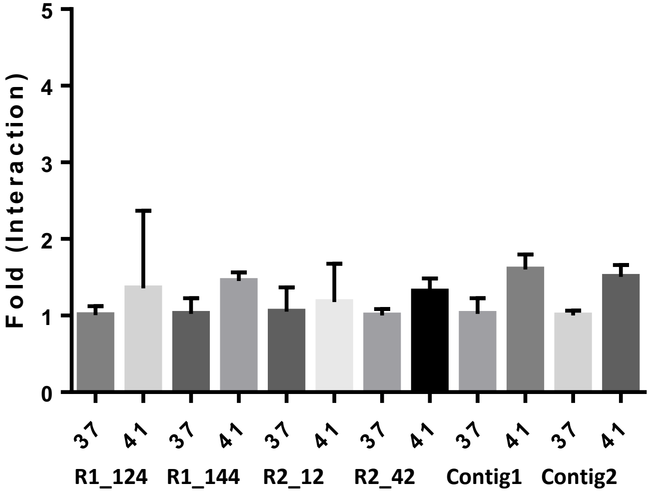


HiC and 3C assay reveals the interaction of the Enhancer1 with *BIN1*

Gene Name	Reads	Interaction Enhnacer	Interaction
BIN	R1_74	Enhancer1	Yes
BIN	R1_108	Enhancer1	Yes
BIN	R1_124	Enhancer1	Yes
BIN	R1_144	Enhancer1	Yes
BIN	R1_268	Enhancer1	Yes
BIN	R2_12	Enhancer1	Yes
BIN	R2_42	Enhancer1	Yes
BIN	R2_186	Enhancer1	Yes
BIN	R2_324	Enhancer1	Yes
BIN	Contig1	Enhancer1	Yes
BIN	Contig2	Enhancer1	Yes
BIN	Contig3	Enhancer1	Yes







Conclusion

We have generated more than 10 SNPs deletion mutants in regulatory elements using CRISPR-mediated gene editing

Beef trait-specific SNPs in Enhancer 1 element deletion showed 630 gene expression alterations including cell cycle and growth

We have identified chromosomal interaction between Enhancer1 and *BIN* in normal and stressed conditions

Members in WP6

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Hiroaki Taniguchi	Institute of Genetics and Animal Breeding of the Polish Academy of Sciences (IGHZ)	WP6.4 Leader
Joanna Stojak	Institute of Genetics and Animal Breeding of the Polish Academy of Sciences (IGHZ)	WP6.3/4
Dominique Rocha	Institut National de la Recherche Agronomique (INRAE)	WP6.2 Leader
Mathieu Charles	Institut National de la Recherche Agronomique (INRAE)	WP6.1 Leader
Mélissa Poncet	Institut National de la Recherche Agronomique (INRAE)	WP6.2
Caroline Mörke	Farm Animal Biology (FBN)	WP6.3
Doreen Becker	Farm Animal Biology (FBN)	WP6.3
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