CLASS_ACT_1

Framework for interpretation of individual disease-associated variants

- **Single nucleotide polymorphisms (SNPs) is the nucleotide variations associated with disease**

- **Genome-wide association studies (GWAS) have successfully identified thousands of common genetic variants associated with complex diseases (http://www.ebi.ac.uk/gwas/)**

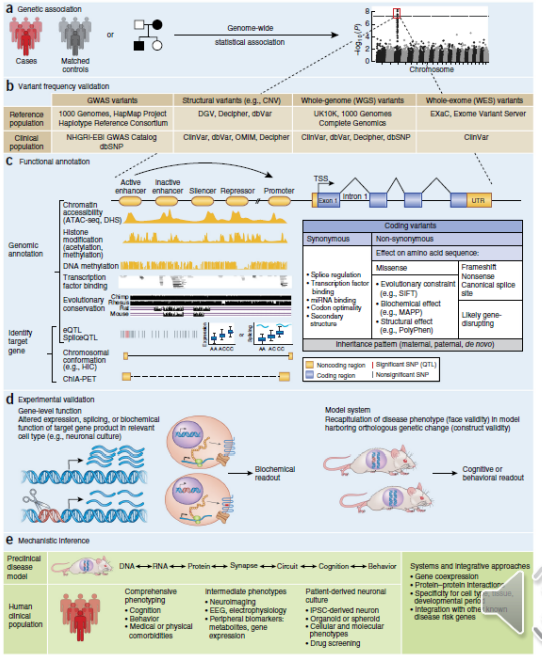
- **Functional annotation: to define genomic regulatory regions by genome-wide integration data**

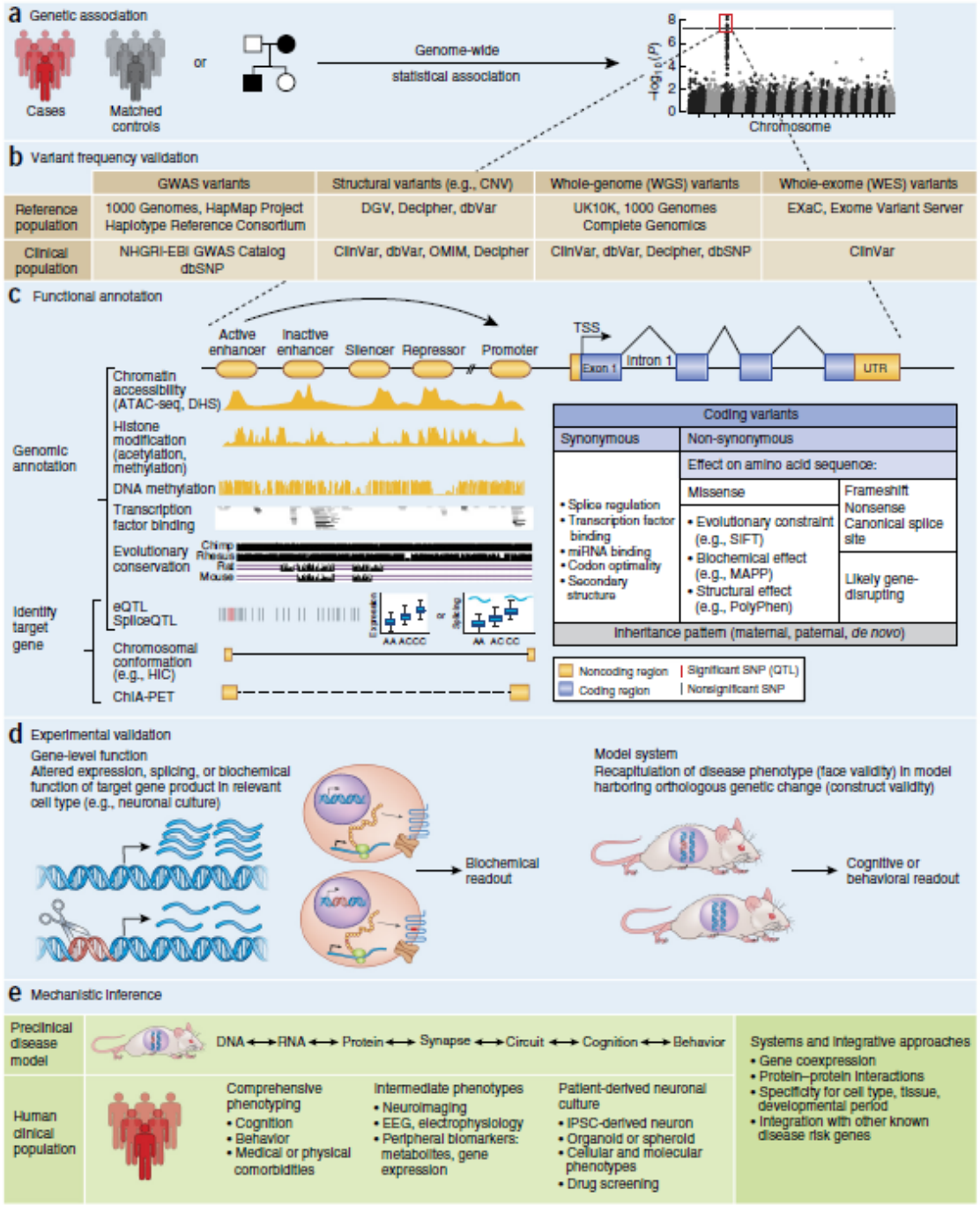
- **Experimental validation**

- **Disease Animal models**

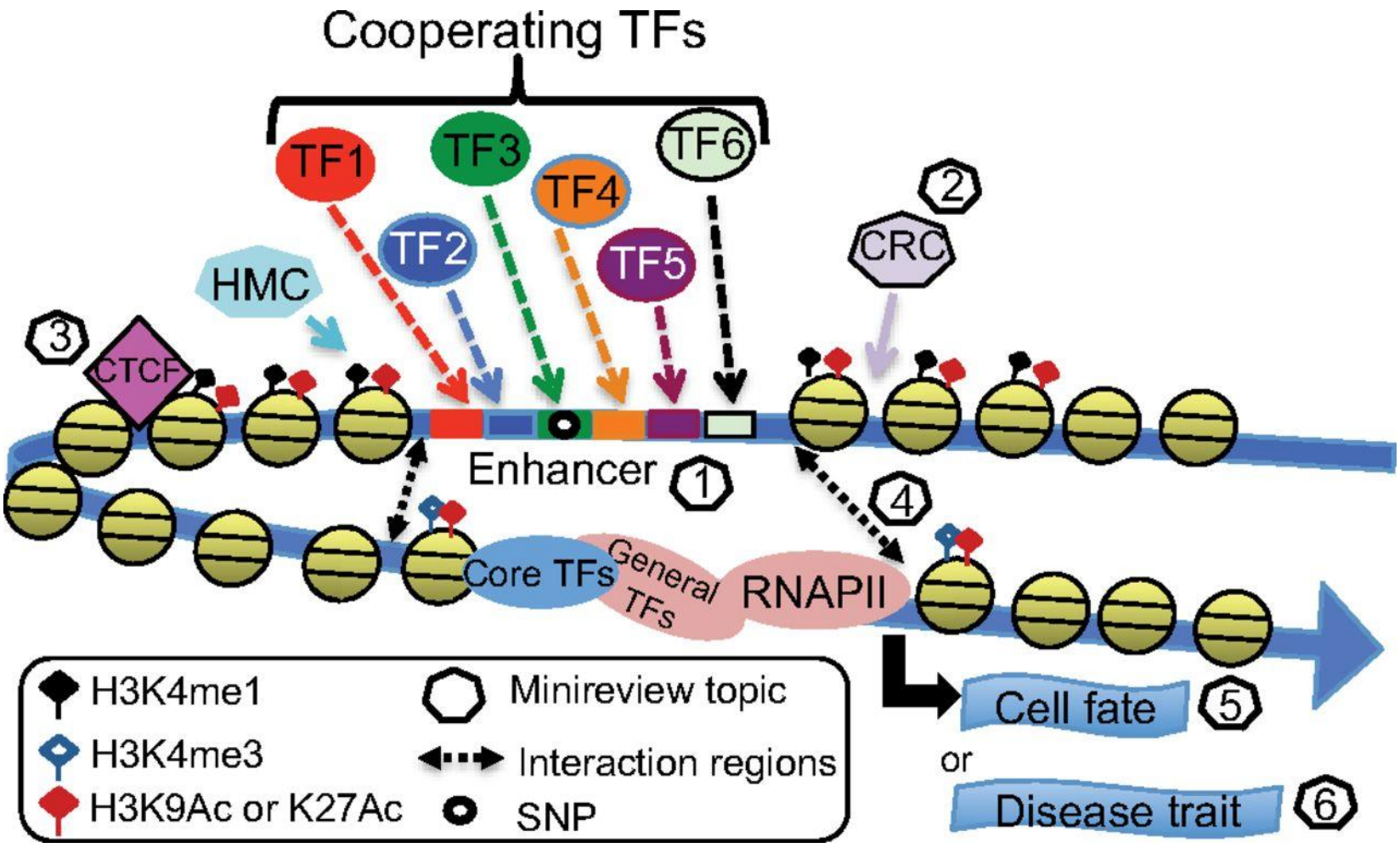
- **Correlation between molecular mechanisms and disease symptoms**

- **Drug Discovery**




a Genetic association
Cases
Matched controls
or
Genome-wide statistical association
Chromosome
b Variant frequency validation
GWAS variants
Structural variants (e.g., CNV)
Whole-genome (WGS) variants
Whole-exome (WES) variants
Reference population
1000 Genomes, HapMap Project Haplotype Reference Consortium
DGV, Decipher, dbVar
UK10K, 1000 Genomes Complete Genomics
ExaC, Exome Variant Server
Clinical population
NHGRI-EBI GWAS Catalog dbSNP
ClinVar, dbVar, OMIM, Decipher
ClinVar, dbVar, Decipher, dbSNP
ClinVar
c Functional annotation
Active enhancer
Inactive enhancer
Silencer
Repressor
Promoter
TSS
Exon 1
Intron 1
UTR
Genomic annotation
Chromatin accessibility (ATAC-seq, DHS)
Histone modification (acetylation, methylation)
DNA methylation
Transcription factor binding
Evolutionary conservation
Chimp
Rhesus
Rat
Mouse
Identify target gene
eQTL
SpliceQTL
Chromosomal conformation (e.g., HiC)
ChIA-PET
Coding variants
Synonymous
Non-synonymous
Effect on amino acid sequence:
Missense
Frameshift
Nonsense
Canonical splice site
Likely gene-disrupting
• Splice regulation
• Transcription factor binding
• miRNA binding
• Codon optimality
• Secondary structure
• Evolutionary constraint (e.g., SIFT)
• Biochemical effect (e.g., MAPP)
• Structural effect (e.g., PolyPhen)
Inheritance pattern (maternal, paternal, de novo)
Noncoding region
Coding region
Significant SNP (QTL)
Nonsignificant SNP
d Experimental validation
Gene-level function
Altered expression, splicing, or biochemical function of target gene product in relevant cell type (e.g., neuronal culture)
Biochemical readout
Model system
Recapitulation of disease phenotype (face validity) in model harboring orthologous genetic change (construct validity)
Cognitive or behavioral readout
e Mechanistic inference
Preclinical disease model
DNA ↔ RNA ↔ Protein ↔ Synapse ↔ Circuit ↔ Cognition ↔ Behavior
Human clinical population
Comprehensive phenotyping
• Cognition
• Behavior
• Medical or physical comorbidities
Intermediate phenotypes
• Neuroimaging
• EEG, electrophysiology
• Peripheral biomarkers: metabolites, gene expression
Patient-derived neuronal culture
• iPSC-derived neuron
• Organoid or spheroid
• Cellular and molecular phenotypes
• Drug screening
Systems and integrative approaches
• Gene coexpression
• Protein–protein interactions
• Specificity for cell type, tissue, developmental period
• Integration with other known disease risk genes

Genome-wide characterizations of regulatory regions.



Peggy J. Farnham J. Biol. Chem. 2012;287:30885-30887

To create the mutation in the plasmid we can use **site directed mutagenesis** method and inserting the SNP in the region of interest using primers that are specific for that region. we can insert this mutation in the promoter region or in the enhancer region.

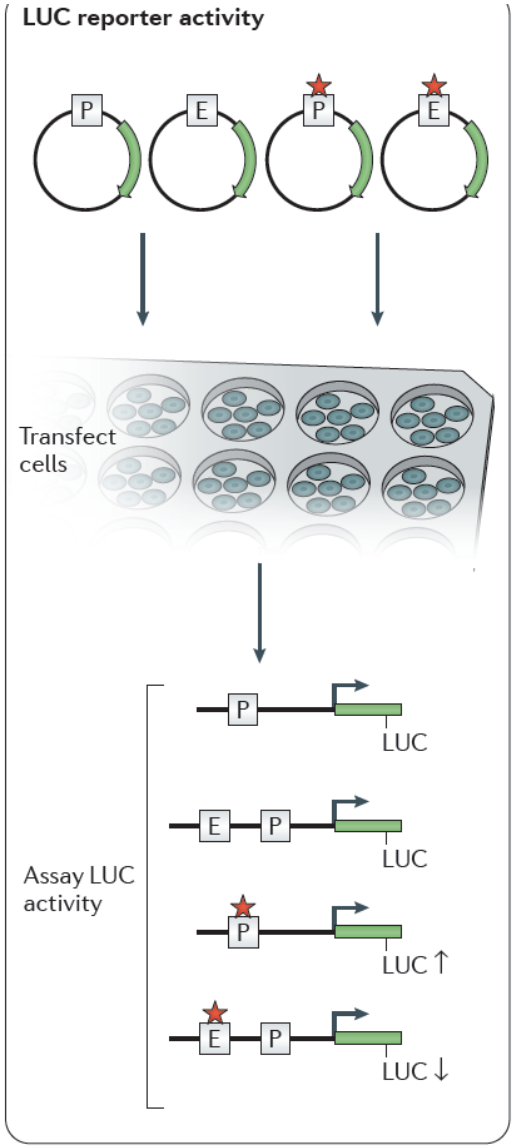
The samples in this experiment are: one **negative control** in which the cells are transfected with **empty vector**, one **positive control** in which the cells are transfected with the plasmid containing luciferase that is under the control of **constitutive active promoter**. Positive and Negative control are important to test if luciferase assay work in a correct manner.

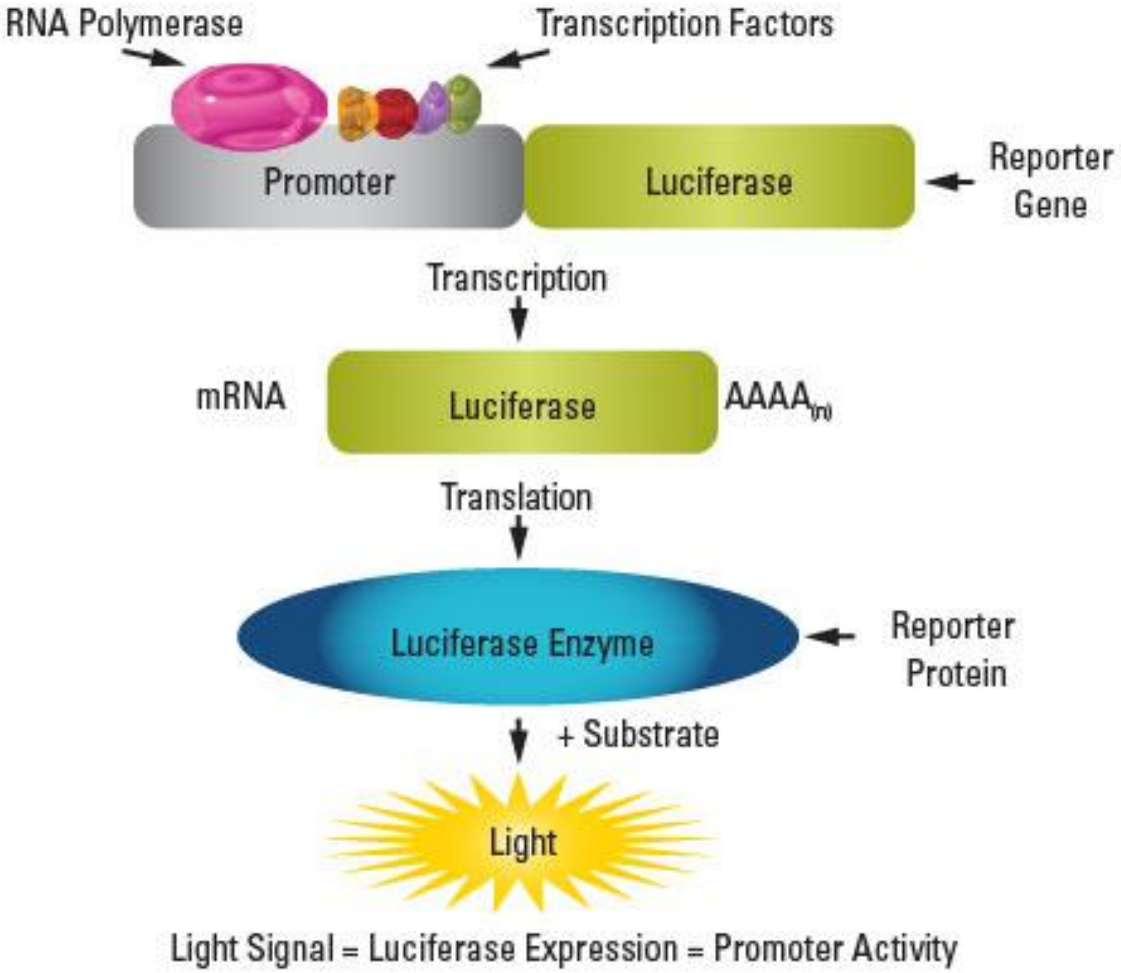
EXPERIMENT: COMPARISON BETWEEN WILD TYPE AND MUTANT.

Promoter and Enhancer wild type do not induce transcription
SNP in the Promoter increases trascription
SNP in the Enhancer decrease the trascription

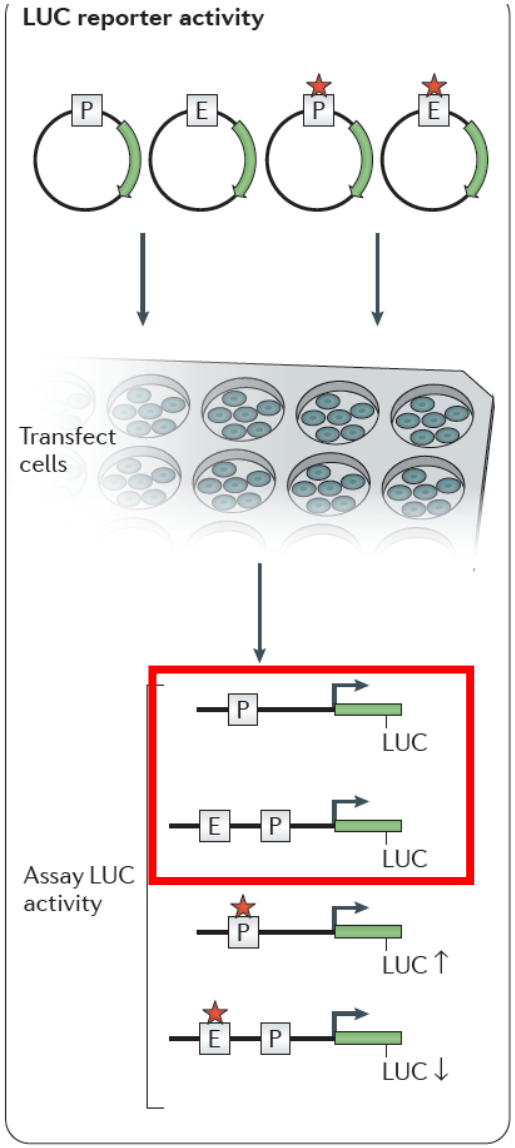
Specifically, in this picture they had two samples mutated, one has a SNP on the promoter and the other has a SNP on the enhancer. From the results showed here I can see that the mutation on the promoter doesn't affect the luciferase expression while the mutation on the enhnacer affected it and there was no light emitted in this second case.

In order to test if SNP has a role in the transcription rate by alteration of TFBS, luciferase assay can be used





In order to test if SNP has a role in the transcription rate by alteration of TFBS, luciferase assay can be used



To test enhancer and promoter with SNPs:

Question?

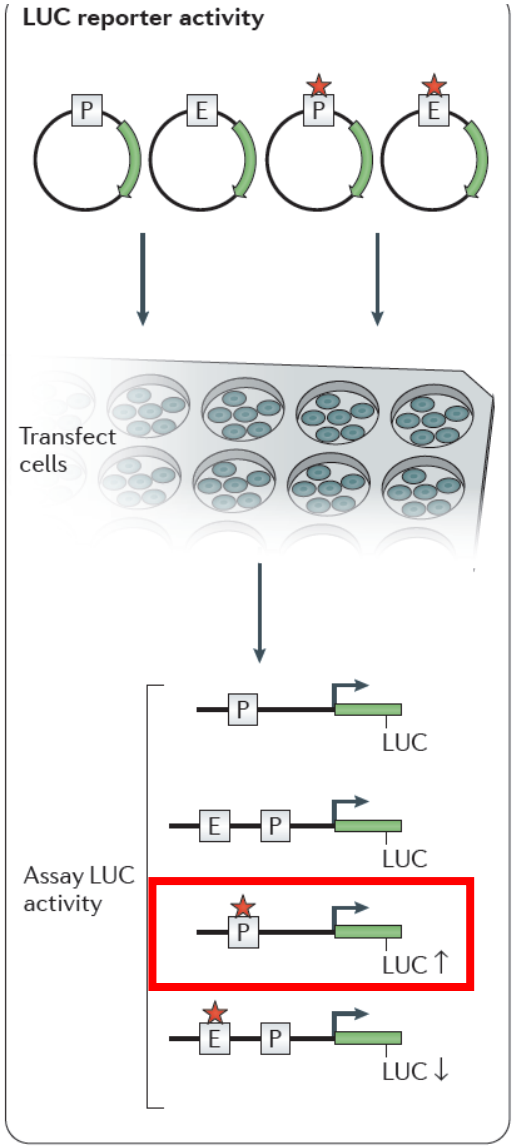
Is the SNP in the promoter or in enhancer able to change trascription activation?

How?

Does Transcription Increase or Decrease?

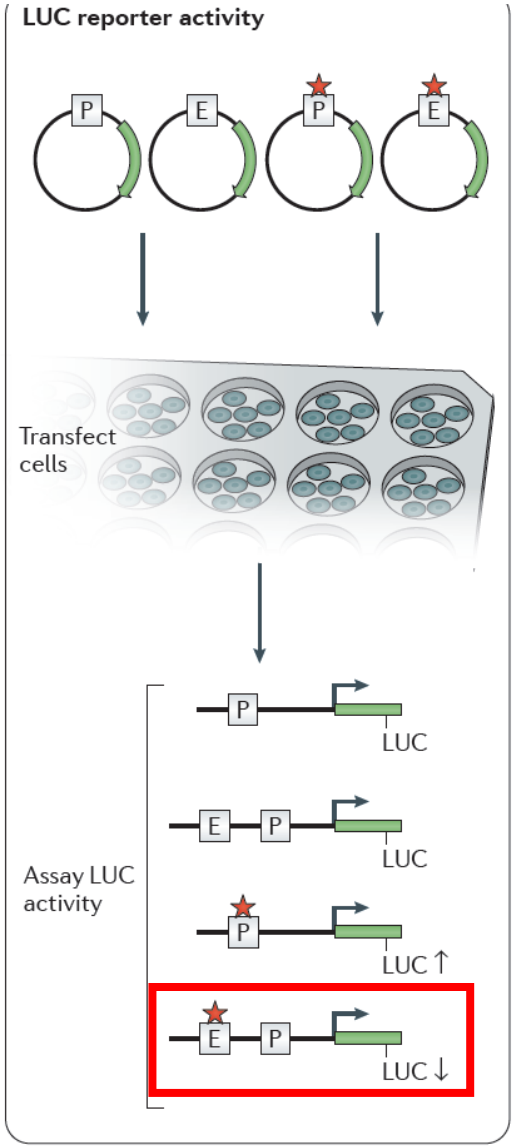
Promoter and Enhancer wild type do not induce transcription

In order to test if SNP has a role in the transcription rate by alteration of TFBS, luciferase assay can be used



SNP in the Promoter increases trascription

In order to test if SNP has a role in the transcription rate by alteration of TFBS, luciferase assay can be used



SNP in the Enhancer decrease the trascription

Question 2

Incorrect Mark 0.00 out of 1.00

Progesterone (Pg) bound Progesterone Receptor (PgR) induces cell growth acting on Cyclin A gene. Single nucleotide variant is present at upstream to TSS, therefore when Progesterone level is higher we can see cell growth arrest.

What is the impact of SNP on Cyclin A expression after treatment with high Pg level?

Select one:

- a. SNP increases Pg sensitivity to induce Cyclin A expression ✗ Pg hormone at high level inhibit cell growth and cyclin A, therefore SNPs decreases Pg sensitivity
- b. SNP does not have effect on Pg sensitivity to induce Cyclin A expression
- c. SNP decreases Pg sensitivity to induce Cyclin A expression
- d. SNP regulates PgR expression to induce Cyclin A expression
- e. SNP inhibits PgR transcription to induce Cyclin A expression

SNP is not present in the consensus sequence for Pg receptor, therefore it is not direct inhibition of Pg receptor binding

Question 2

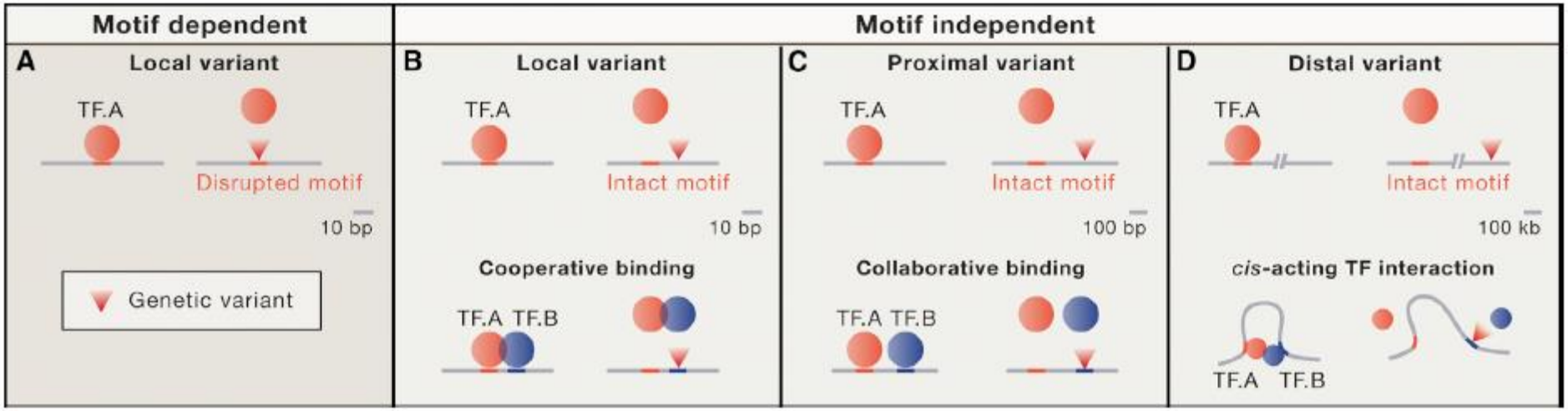
Incorrect Mark 0.00 out of 1.00

Progesterone (Pg) bound Progesterone Receptor (PgR) induces cell growth acting on Cyclin A gene. Single nucleotide variant is present at upstream to TSS, therefore when Progesterone level is higher we can see cell growth arrest.

What is the impact of SNP on Cyclin A expression after treatment with high Pg level?

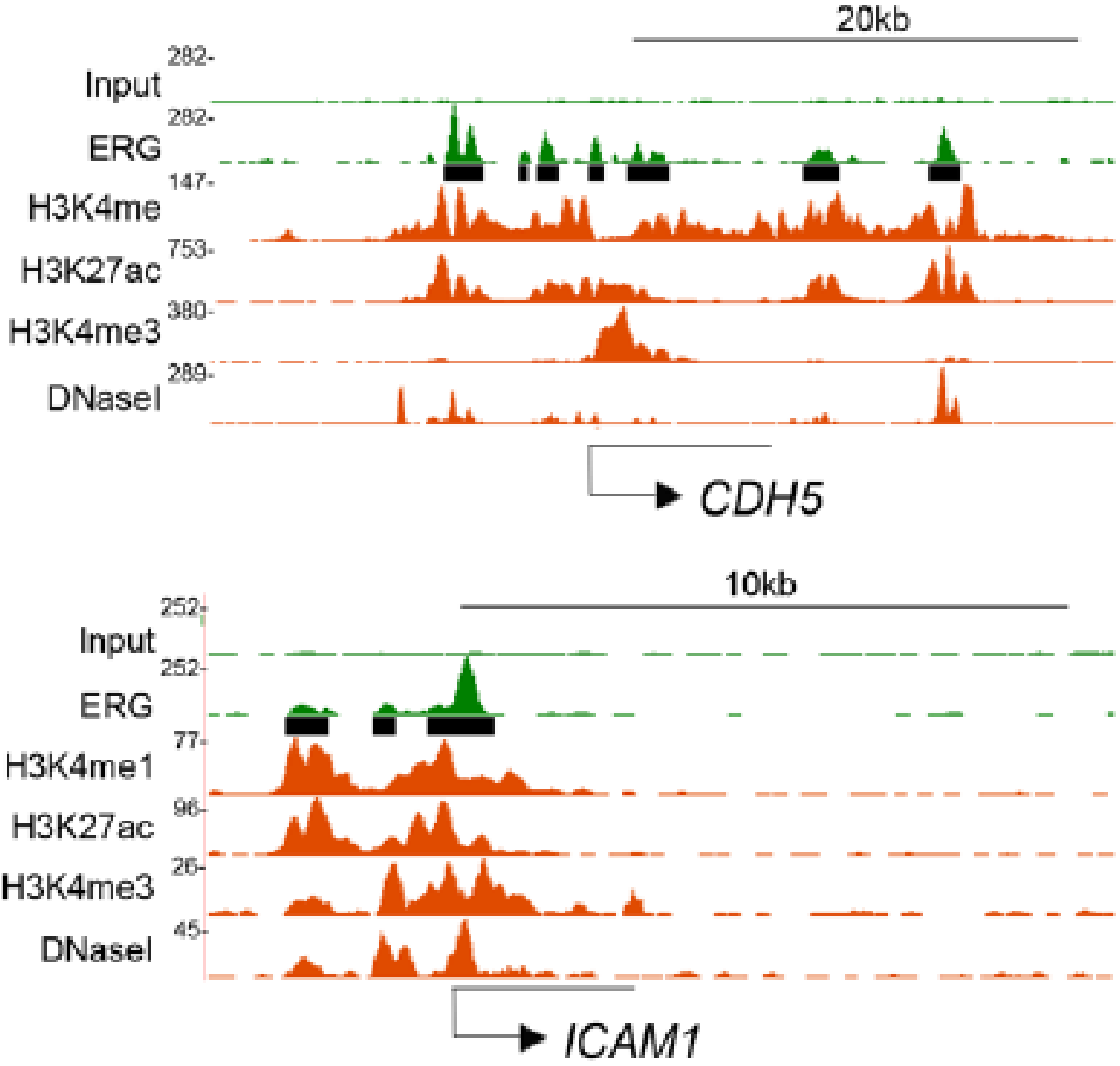
Select one:

- a. SNP increases Pg sensitivity to induce Cyclin A expression
- b. SNP decreases Pg sensitivity to induce Cyclin A expression
- c. SNP inhibits PgR transcription to induce Cyclin A expression ✗ SNP acts on Cyclin expression by the inhibition of PgR binding
- d. SNP does not have effect on Pg sensitivity to induce Cyclin A expression
- e. SNP regulates PgR expression to induce Cyclin A expression


Motif dependent
Motif independent
A
Local variant
TF.A
Disrupted motif
10 bp
Genetic variant
B
Local variant
TF.A
Intact motif
10 bp
Cooperative binding
TF.A TF.B
C
Proximal variant
TF.A
Intact motif
100 bp
Collaborative binding
TF.A TF.B
D
Distal variant
TF.A
Intact motif
100 kb
cis-acting TF interaction
TF.A TF.B

CLASS_ACT_2

Looking at this ChIP-seq expreiment we can say that ERG regulates CDH5 and ICAM1 because in this genes, ERG peaks mapped to regions of DNase I hypersensitivity, a marker of accessible open chromatin. ERG genomic loci overlapps with enriched histone marks of active promoters (H3K4me3 and H3K27ac) and enhancers (H3K4me1 and H3K27ac). In ICAM1 this markers are on the TSS, while in CDH5 the histone markers are also on gene body. So we Can conclude that the binding of ERG at this sites regulates the chromatin state and gene expression.


20kb
Input
282-
ERG
282-
H3K4me
147-
H3K27ac
753-
H3K4me3
380-
DNaseI
289-
CDH5
10kb
Input
252-
ERG
252-
H3K4me1
77-
H3K27ac
96-
H3K4me3
26-
DNaseI
45-
ICAM1

A fragment of the *TAL1* enhancer containing either the wild-type sequence or each of the mutant alleles was cloned upstream of luciferase as a promoter. Constructs were nucleofected into Jurkat cells, together with either control siRNA, or two independent siRNAs targeting MYB. Firefly luciferase activity was measured to test the enhancer activity of this fragment in reporter assays and normalized to renilla luciferase to control for cell number and transfection efficiency. Corresponding immunoblots for MYB and tubulin (as housekeeping gene) are shown below.

In Jurkat cells, fragments containing each of the seven different indel mutations robustly increased reporter activity a lot more than the wild-type fragment. Moreover, the activity of each of the mutant reporters was markedly reduced after MYB knockdown, indicating that the enhancer activity imparted by the mutations was indeed mediated by MYB.

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ONCOGENIC SUPER-ENHANCERS linked to SNPs IN TUMOR PROGRESSION

ONCOGENE REGULATION

An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element

Marc R. Mansour,[1,2] Brian J. Abraham,[3*] Lars Anders,[3*] Alla Berezovskaya,[1] Alejandro Gutierrez,[1,4] Adam D. Durbin,[1] Julia Etchin,[1] Lee Lawton,[3] Stephen E. Sallan,[1,4] Lewis B. Silverman,[1,4] Mignon L. Loh,[5] Stephen P. Hunger,[6] Takaomi Sanda,[7] Richard A. Young,[3,8†] A. Thomas Look[1,4†]

In certain human cancers, the expression of critical oncogenes is driven from large regulatory elements, called super-enhancers, that recruit much of the cell's transcriptional apparatus and are defined by extensive acetylation of histone H3 lysine 27 (H3K27ac). In a subset of T-cell acute lymphoblastic leukemia (T-ALL) cases, we found that heterozygous somatic mutations are acquired that introduce binding motifs for the MYB transcription factor in a precise noncoding site, which creates a super-enhancer upstream of the *TAL1* oncogene. MYB binds to this new site and recruits its H3K27 acetylase–binding partner CBP, as well as core components of a major leukemogenic transcriptional complex that contains RUNX1, GATA-3, and TAL1 itself. Additionally, most endogenous super-enhancers found in T-ALL cells are occupied by MYB and CBP, which suggests a general role for MYB in super-enhancer initiation. Thus, this study identifies a genetic mechanism responsible for the generation of oncogenic super-enhancers in malignant cells.

BACKGROUND

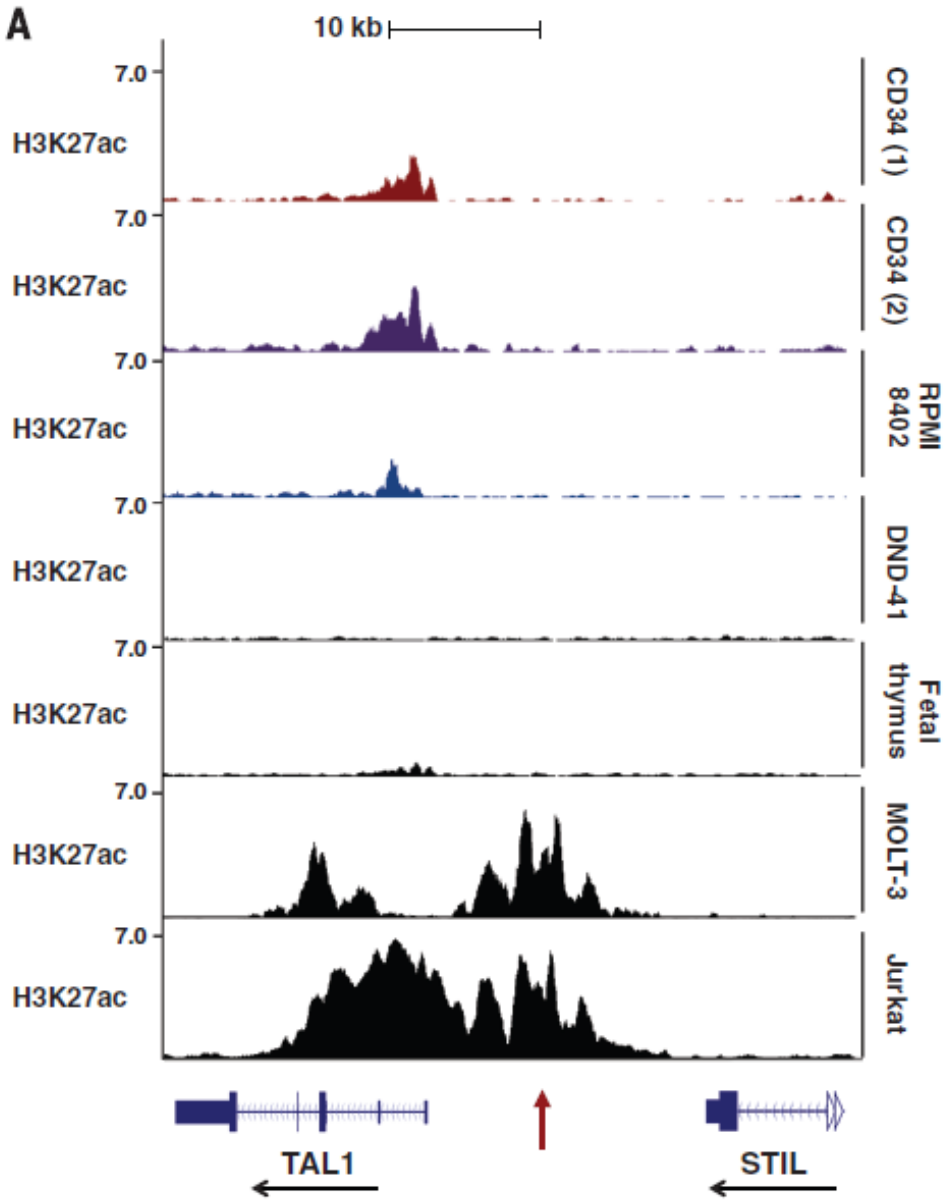
Super-enhancers (SE) upstream TAL1

MYB form Leukemogenic Transcriptional Complex

MYB binds T-ALL cells SEs

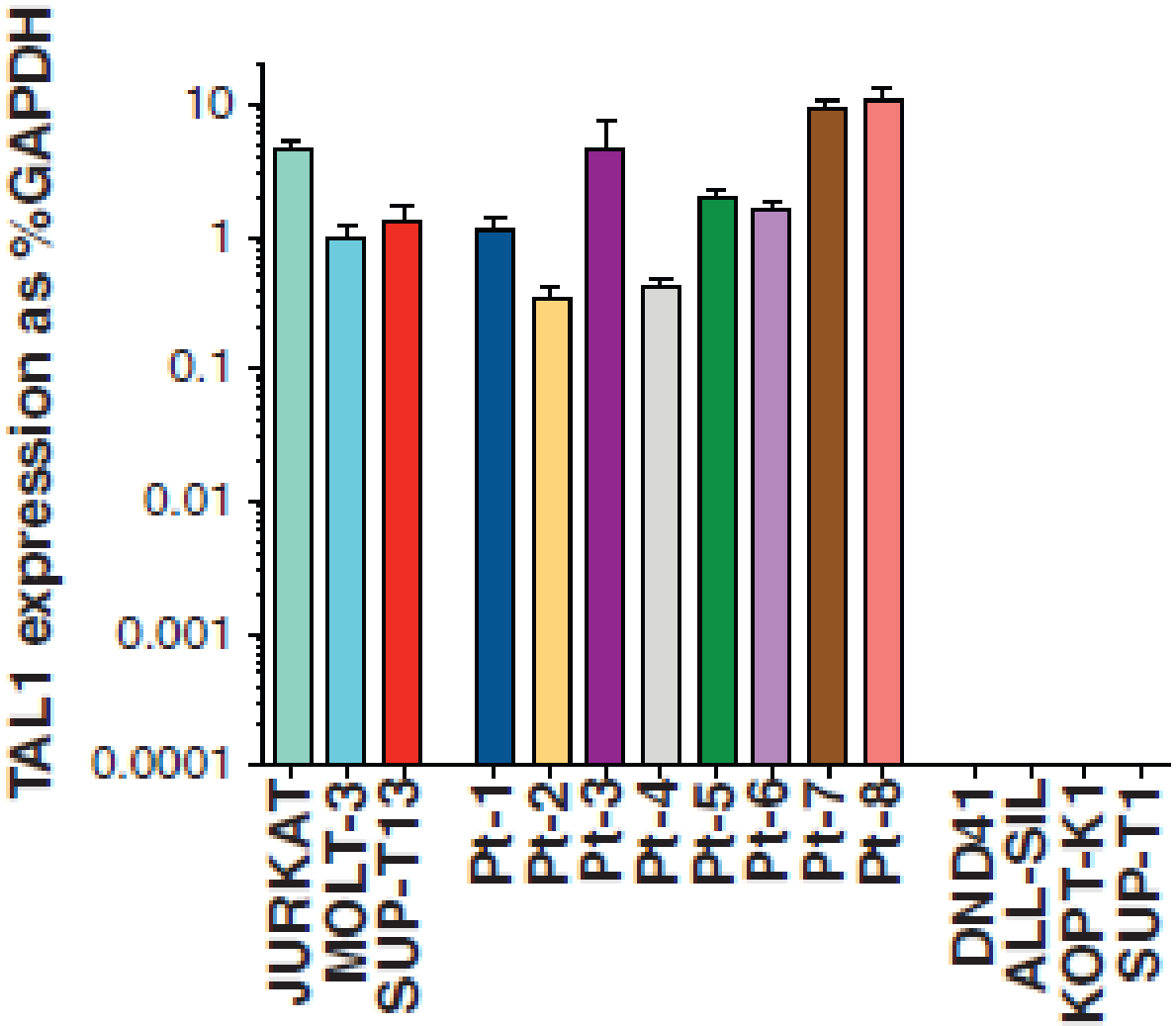
CONCLUSION

ChIP-Seq profile for H3K27ac (active enhancer mark) in different cell lines



Sequence alignments of the –7.5 kb site showing wild-type (WT) sequences in **black** and inserted sequences in **red** for Jurkat and MOLT-3 T-ALL cell lines and eight pediatric T-ALL patients. hg19, human genome build 19.

TAL1 mRNA expression as determined by quantitative polymerase chain reaction (PCR) and expressed as percentage of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).



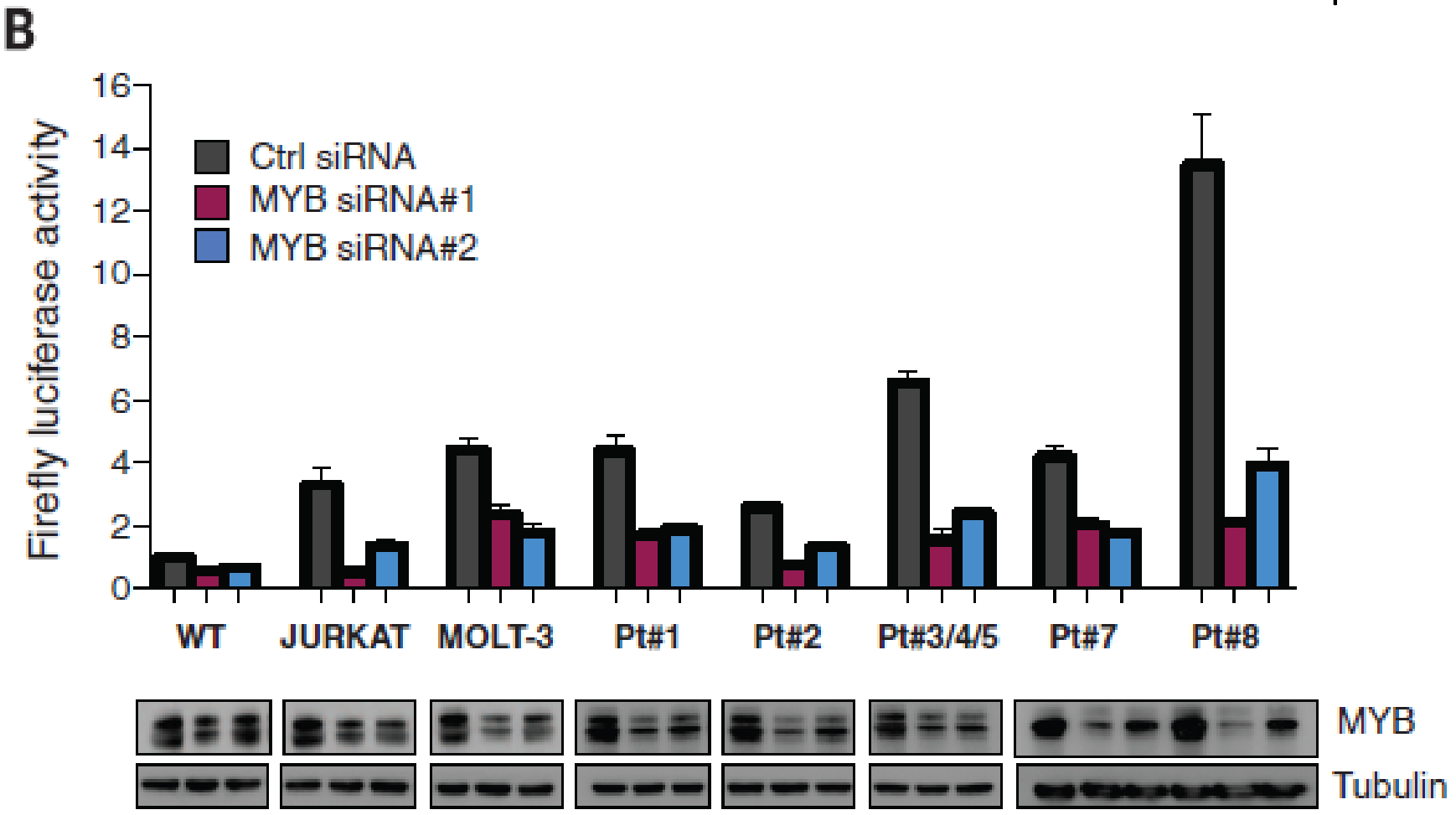
Mutations in TAL1 SE show consensus sequence for MYB, transcription factor.

A Myb primary motif

WT	GACGTA
Jurkat	[GACGGTTA]GGA[AACGGTA]
MOLT-3	GACGGTTA
Patient #1	GACCGTTTA
Patient #2	GCCGTTA
Patient #3	GACCGTTA
Patient #4	GACCGTTA
Patient #5	GACCGTTA
Patient #6	GACGGTTA
Patient #7	GACGGTTA
Patient #8	GACGGTTTA

TAL1 enhancer TRANSCRIPTION ACTIVITY USING LUCIFERASE ASSAY

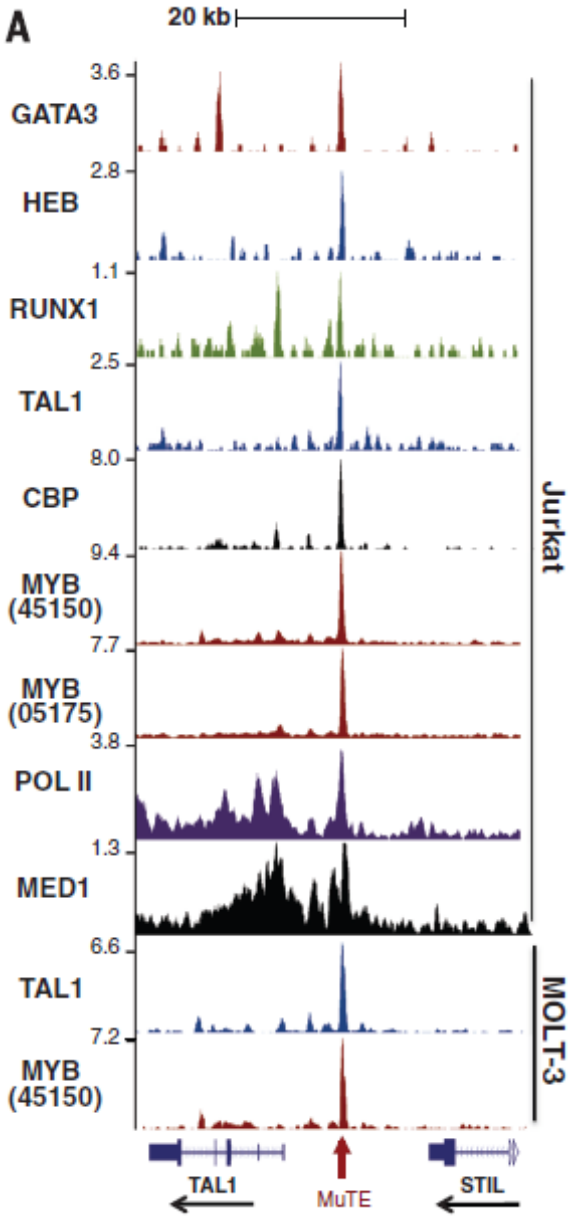
MYB binds the mutant TAL1 enhancer site and is a member of the TAL1 complex



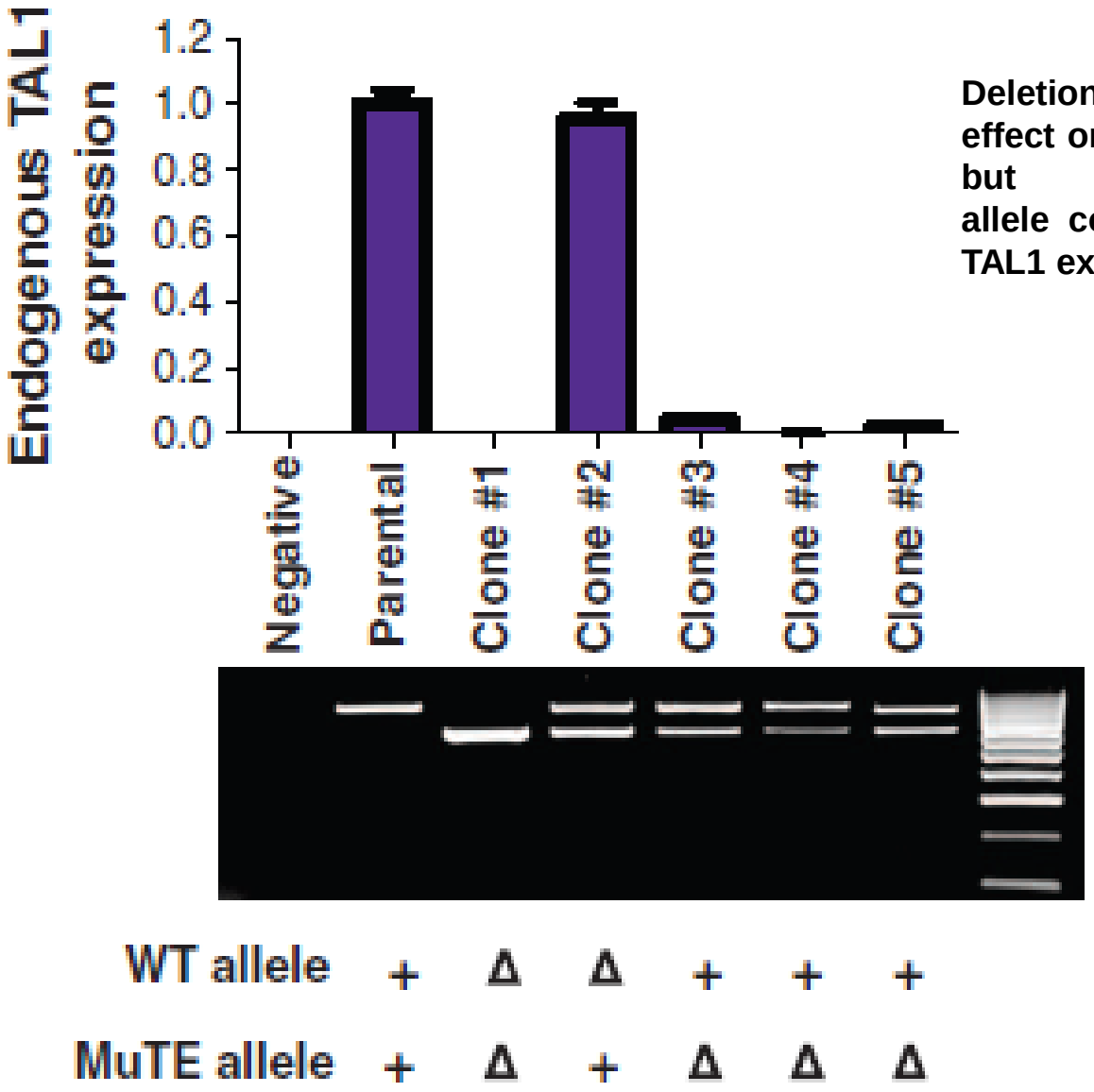
They take into account eight samples, for each sample they perform three condition:

1.the ctrl siRNA, that is the positive controll, it allows to verify that the luciferase works, it rapresent the normal level of activation of the superenhancer in the presence if MYB. And it is also a control for the role of siRNA.
2.MYB siRNA#1
3.MYB siRNA#2
This is a luciferase assay used to verify if and in which samples MYB is important in the activation of the superenhacer.
In the bottom there is a western blot that analyse the level of MYB expretion in each samples and condition. Tubulin is used as control.
These are two different siRNA against the same TF, this is important to be sure that the effects are caused by the inhibition of MYB and not by other interference.From the results we can see that the level of luciferese activity in the WT do not change: MYB do not bind to the superenhacer.
In the other seven samples MYB can bind to the superenhacer and, in fact, in the first condition the expression level of the luciferase is high, expetially in patient 8, but, when MYB is inhibited by a siRNA, it cannot bind the superenhancer anymore and so the activity of the luciferase drop-down to the level we can see in WT.
The presence of the WB below is another evidence of the fact that siRNA inhibit specifically MYC.

MYB binds the mutant TAL1 enhancer (MuTE) site and is a member of the TAL1 complex



Targeted deletion of 177 to 193 bp of the mutant (CRISPRCas9), but not wild-type, allele in Jurkat cells abrogates expression of endogenous TAL1



Deletion of the wild type allele had no effect on endogenous TAL1 mRNA levels, but deletion of the mutant allele completely abrogated endogenous TAL1 expression

AGAROSE GEL ELECTROPHORESIS

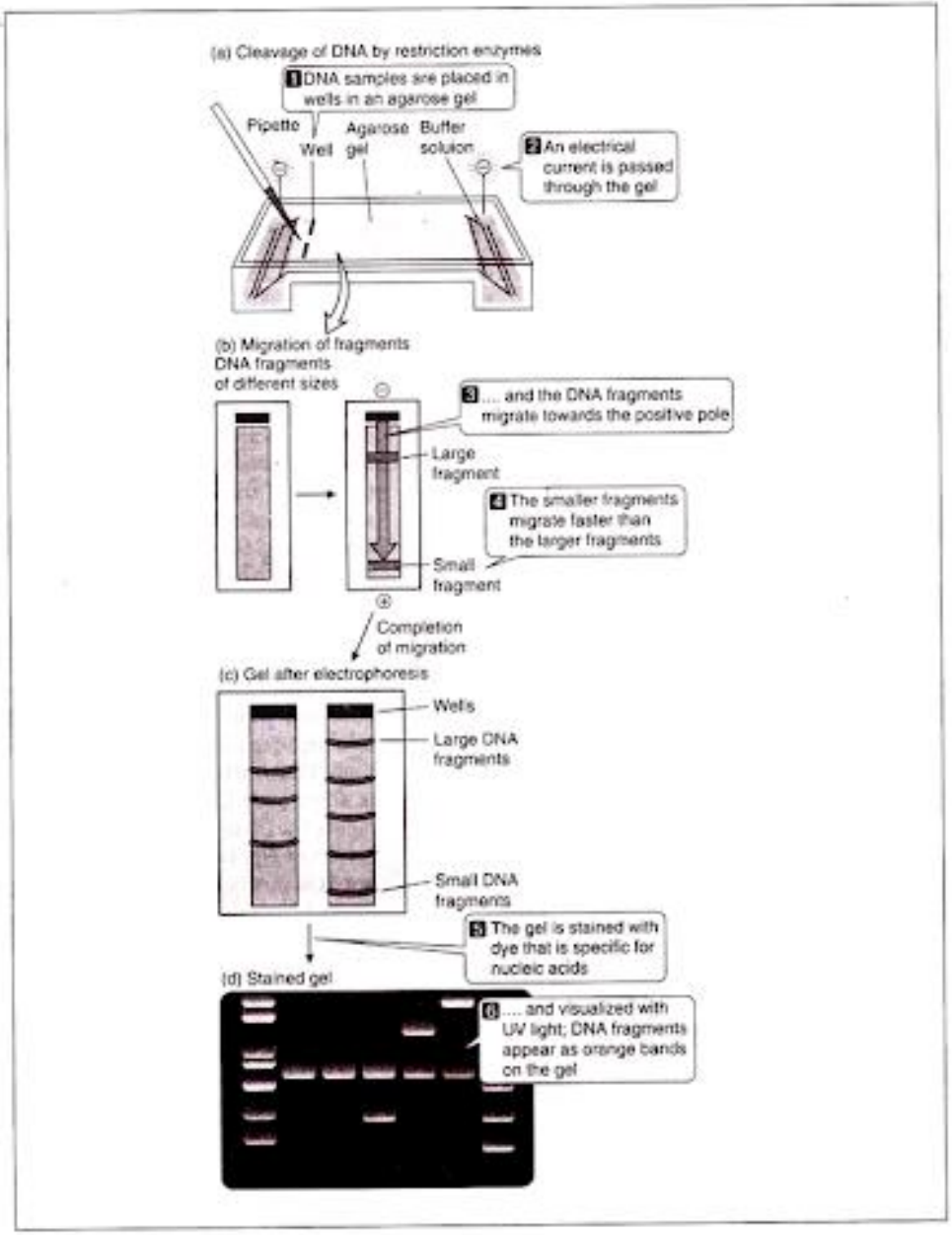
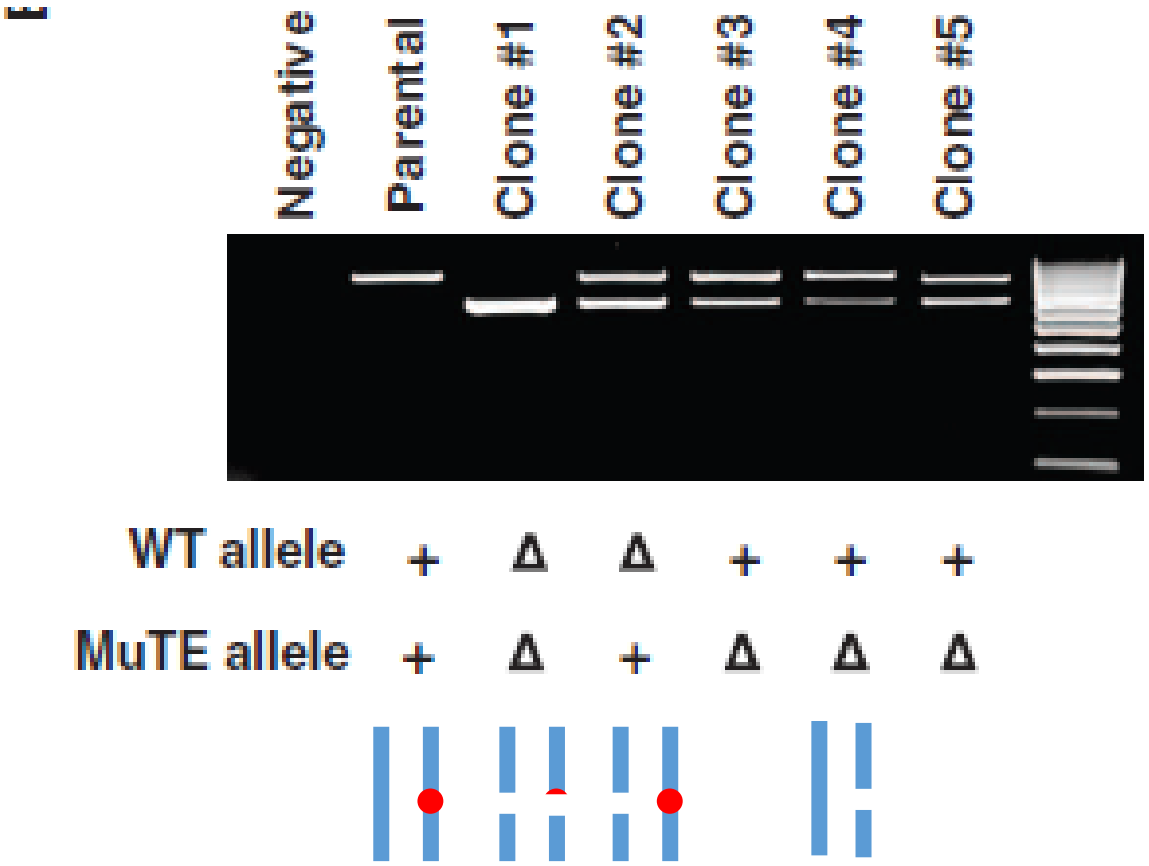


Fig. 3.13: Agarose gel electrophoresis

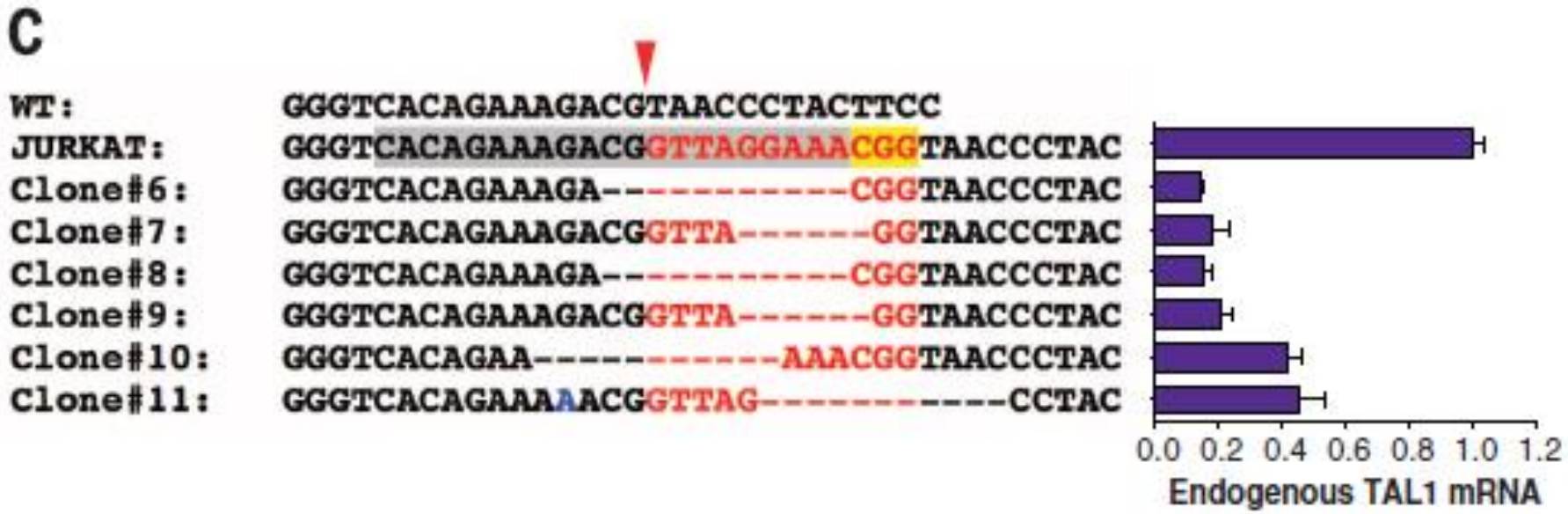
Agarose gel of products from PCR amplification across the MuTE site for CRISPR-Cas9 Jurkat clones



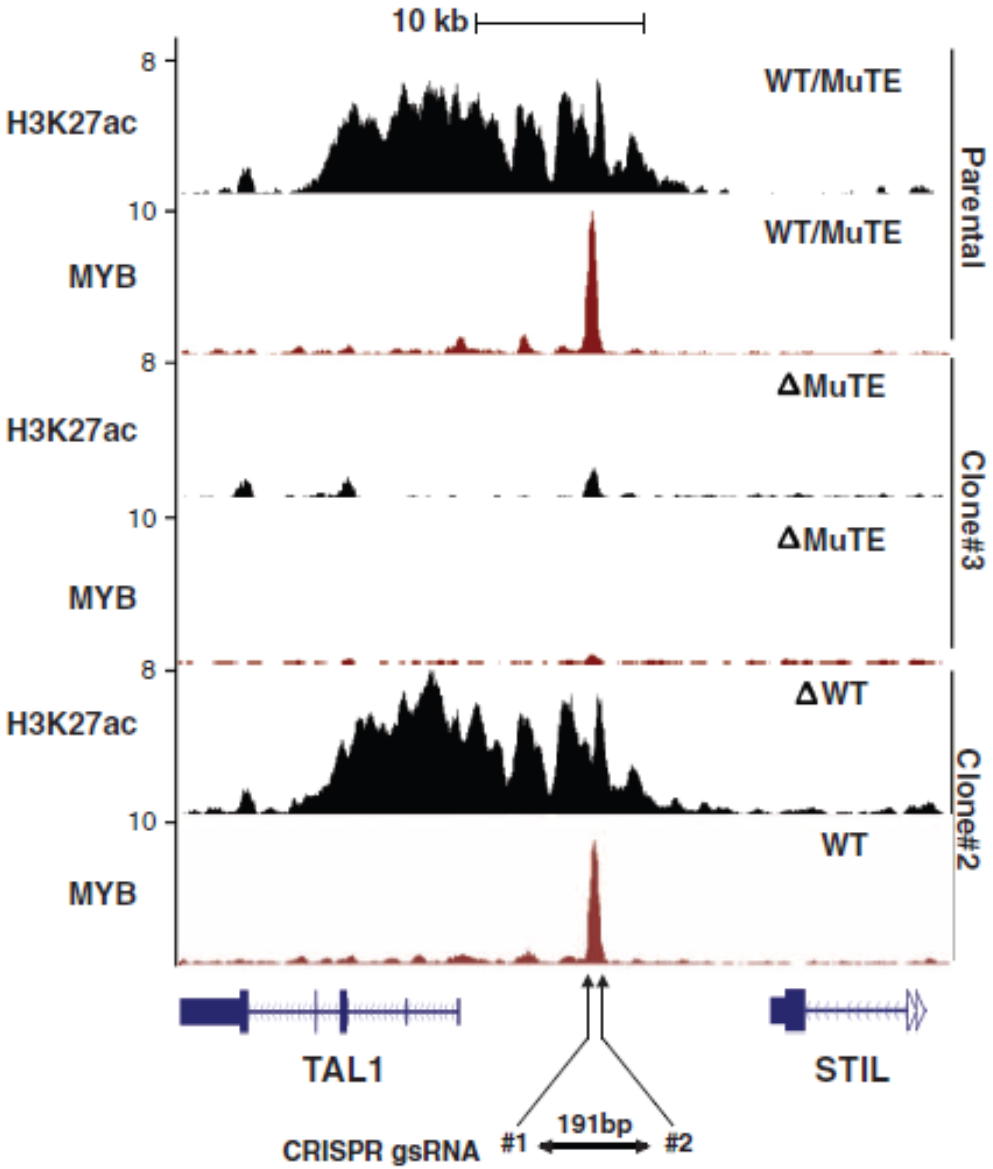
Two alleles:
WT and Mutant

Fragments
Deletion by CRISPR-Cas9

Targeted deletion of 177 to 193 bp of the mutant (CRISPRCas9), but not wild-type, allele in Jurkat cells abrogates expression of endogenous TAL1



ChIP-seq tracks for H3K27ac and MYB at the STIL-TAL1 locus from selected CRISPR-Cas9 clones



Deletion of the wild type allele had no effect on H3K27ac signal and MYB binding, but deletion of the mutant allele completely abrogated H3K27ac signal and MYB binding

LUCIFERASE ACTIVITY AND DELETION OF SPECIFIC ENHANCER