

# **Comparing the effect of mutations in two different versions of the *eve* stripe 2 enhancer**

Francheska López Rivera

Group meeting

February 25, 2015

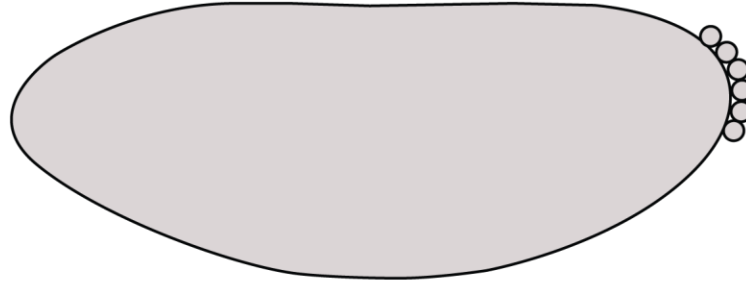
# Goals for this meeting

- Introduction and previous results
- Results after merging data from different stains
- Discussion about how to communicate the story

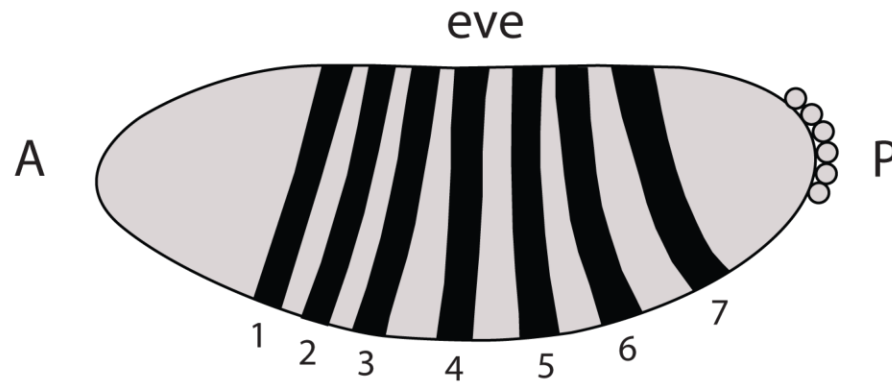
# Enhancers control gene expression in animals.



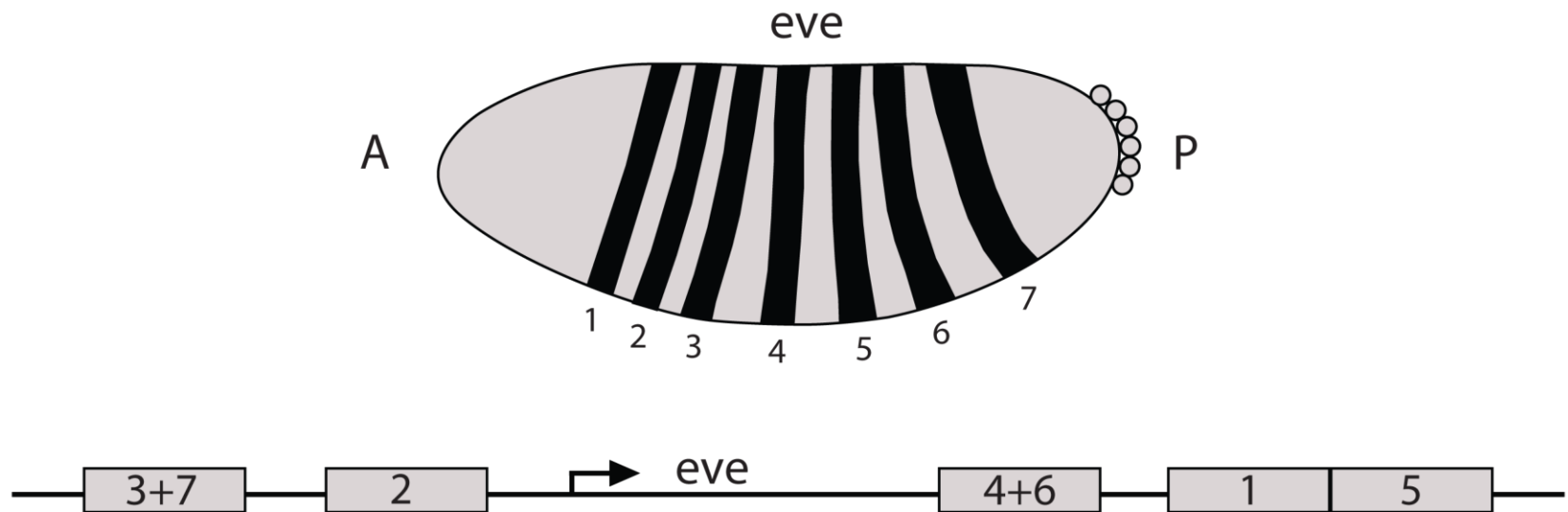
We use the *Drosophila melanogaster* embryo as  
our system.



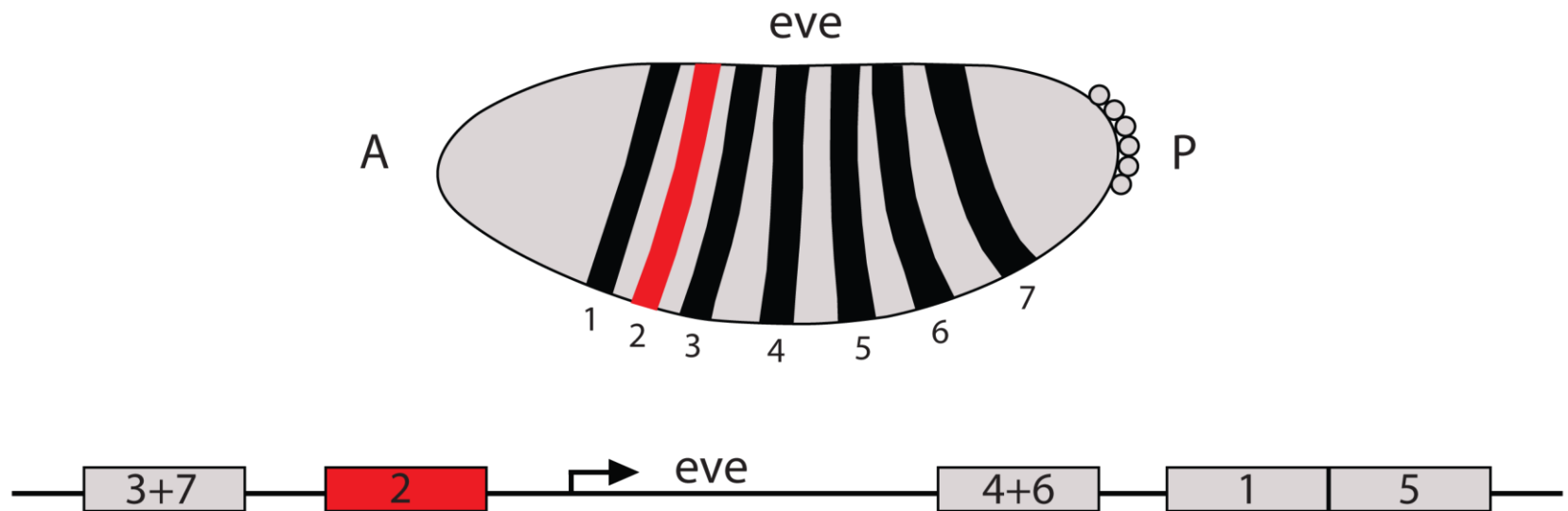
We focused on the *even-skipped* (*eve*) stripe 2 enhancer in *Drosophila melanogaster* embryos.



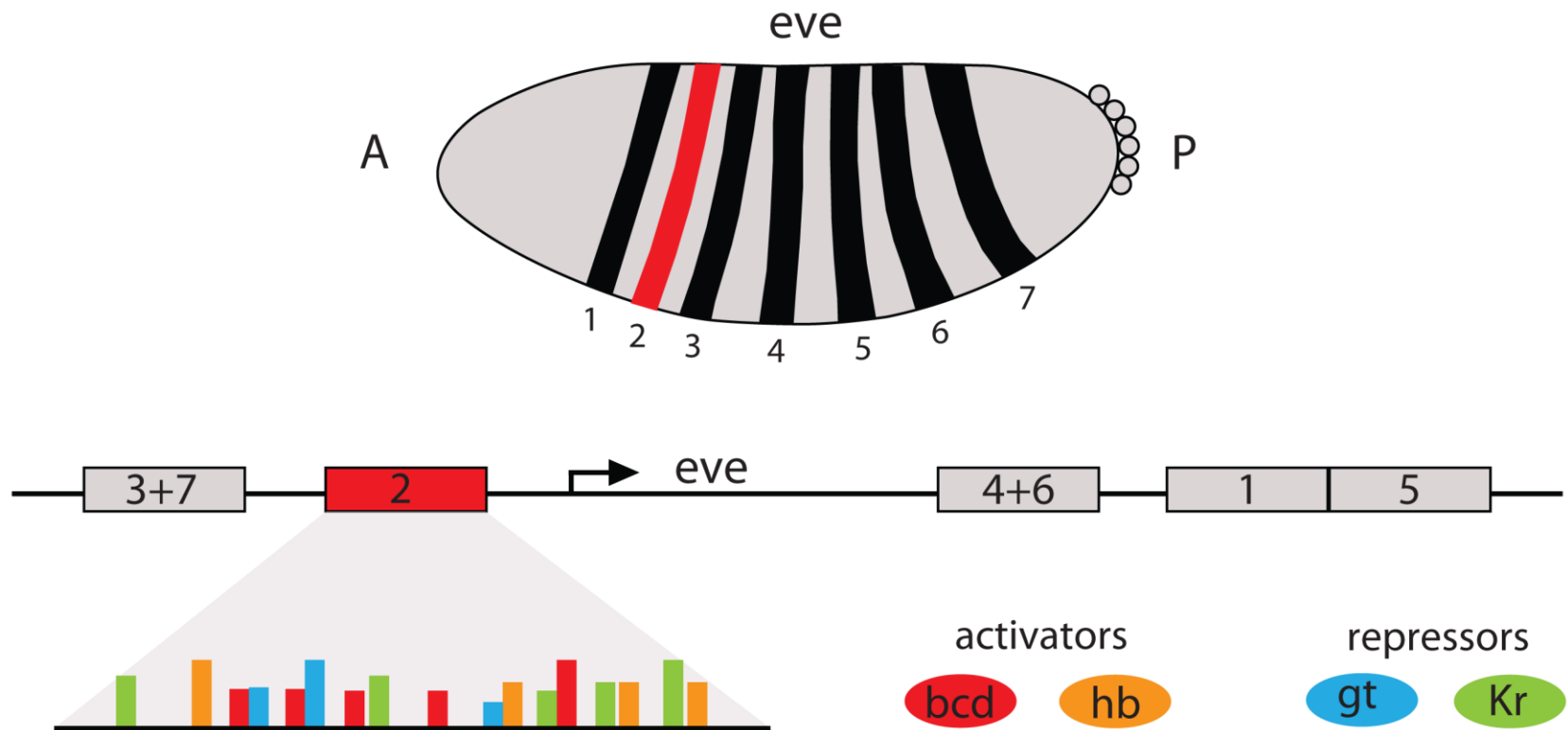
We focused on the *even-skipped* (*eve*) stripe 2 enhancer in *Drosophila melanogaster* embryos.



We focused on the *even-skipped* (*eve*) stripe 2 enhancer in *Drosophila melanogaster* embryos.

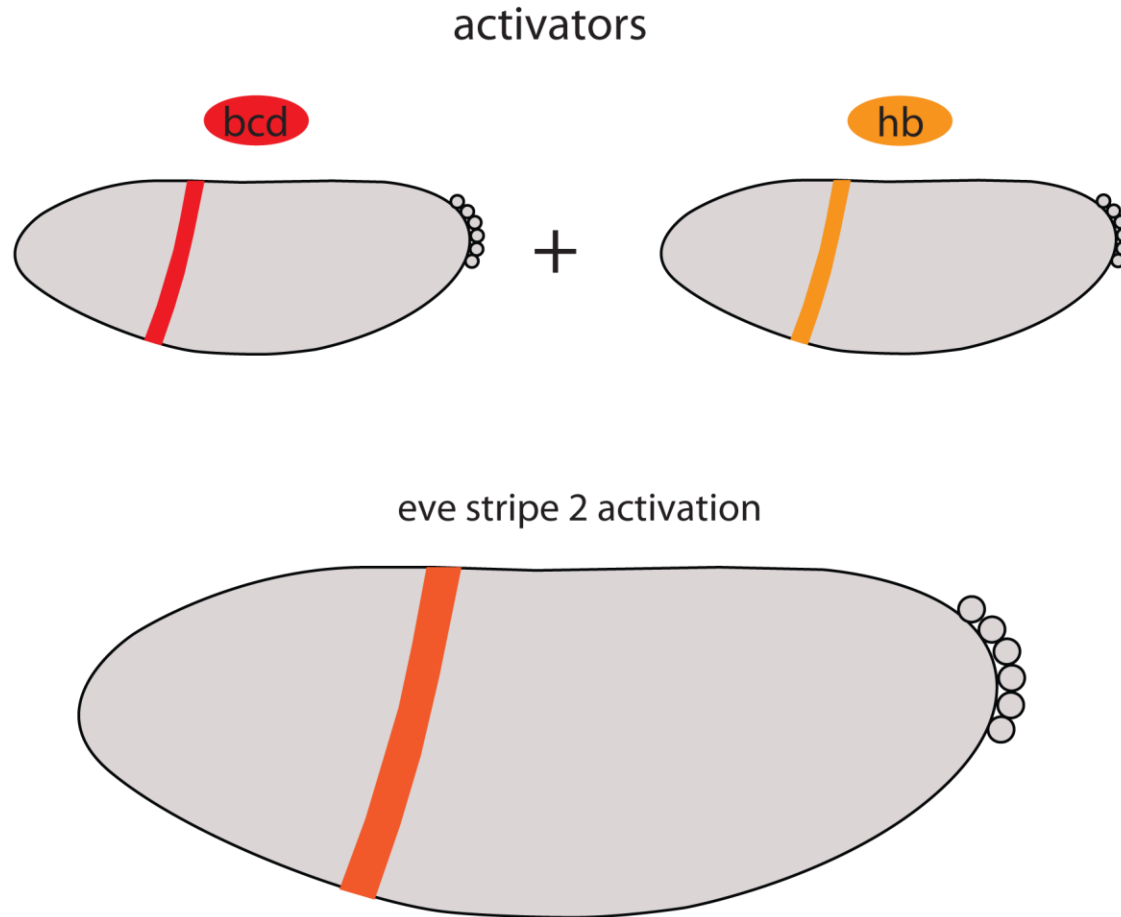


We focused on the *even-skipped* (*eve*) stripe 2 enhancer in *Drosophila melanogaster* embryos.

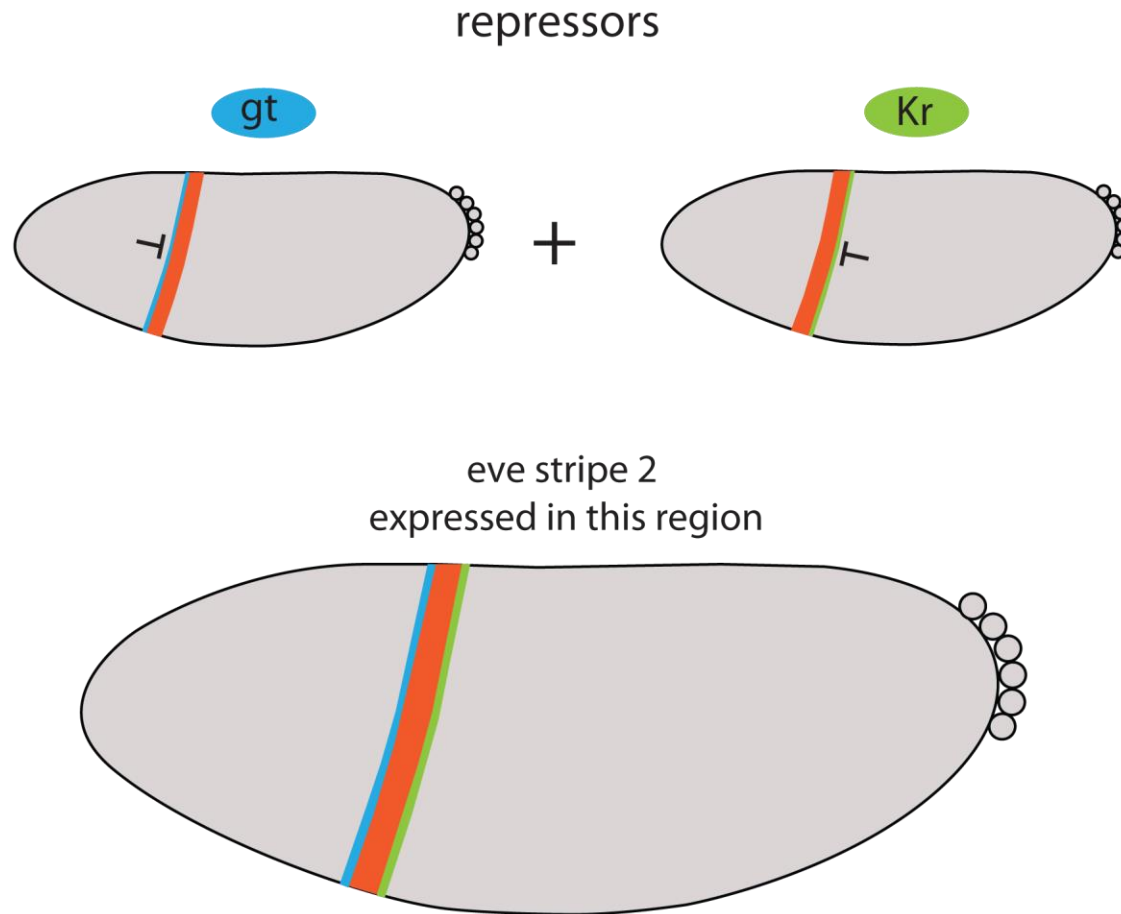




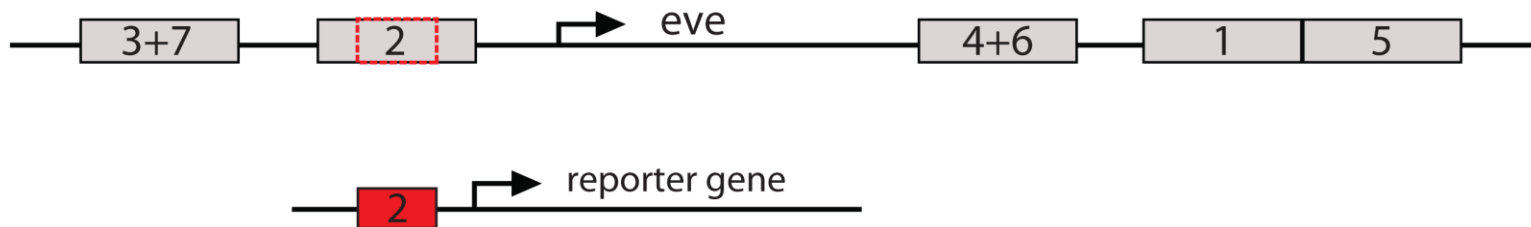
The position of *eve* stripe 2 is determined by four transcription factors.



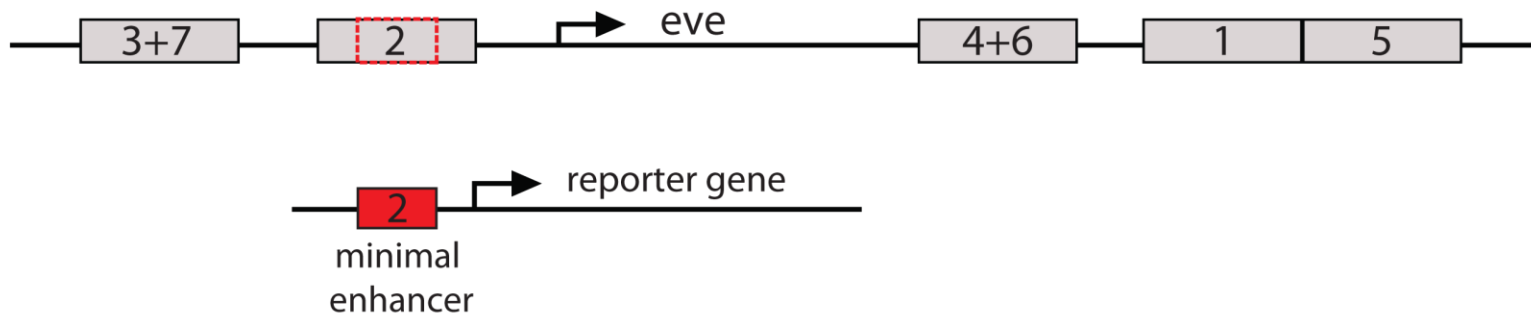
The position of *eve* stripe 2 is determined by four transcription factors.



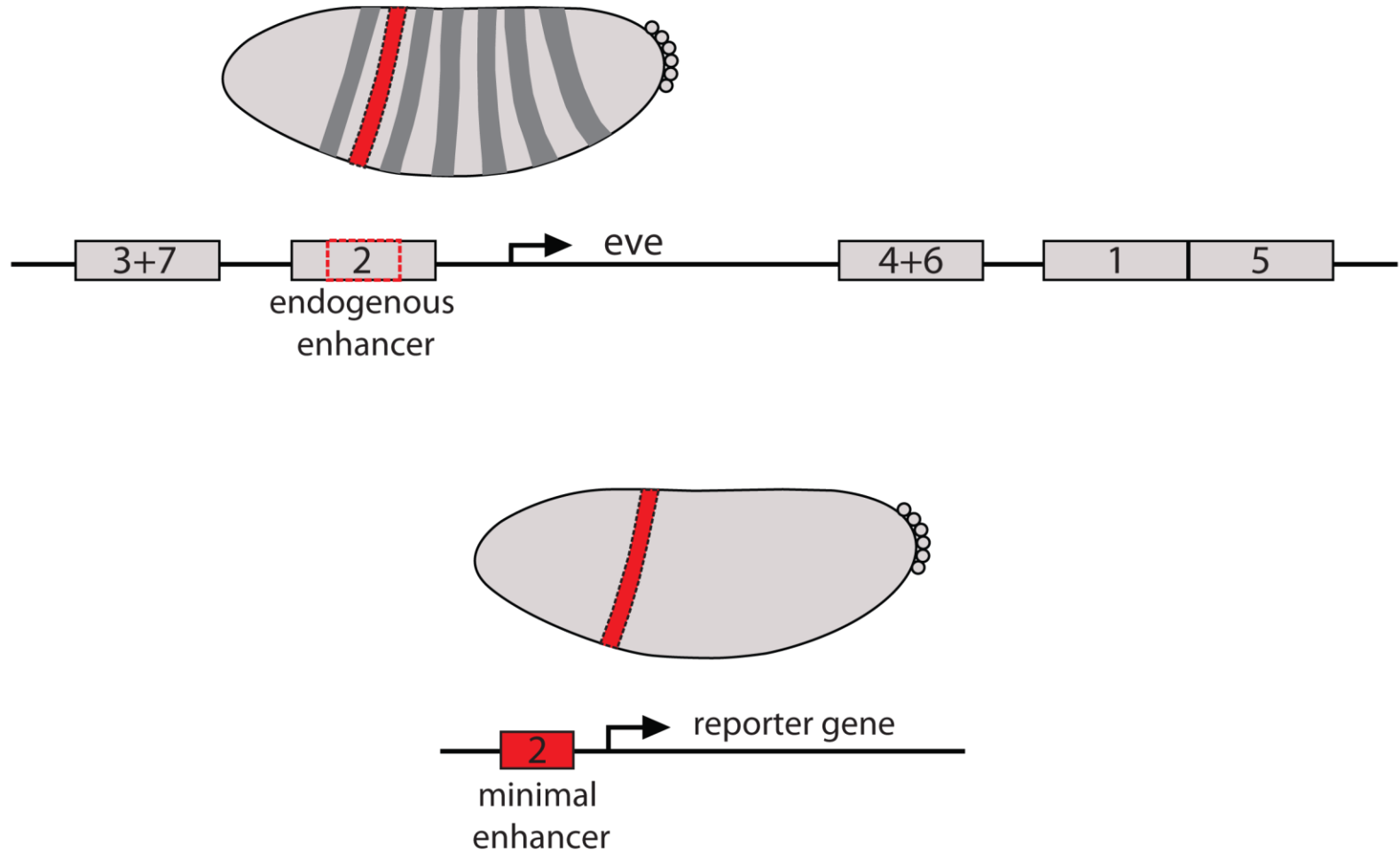
Reporter constructs have been used to study the *eve* stripe 2 enhancer.



Minimal reporter constructs have been used to study the *eve* stripe 2 enhancer.



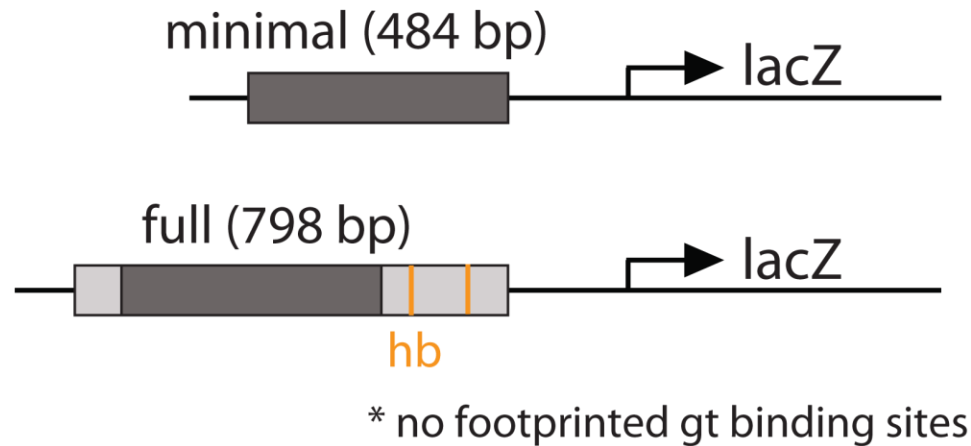
Minimal reporter constructs have been used to study the *eve* stripe 2 enhancer.



# What is the role of sequences flanking the *eve* stripe 2 enhancer?

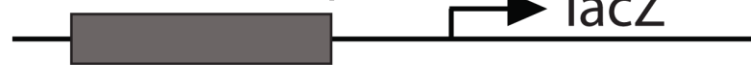


# What is the role of sequences flanking the *eve* stripe 2 enhancer?



# We mutated *eve* stripe 2 minimal and full enhancers.

minimal (484 bp)



full (798 bp)



hb

\* no footprinted gt binding sites

mutated minimal



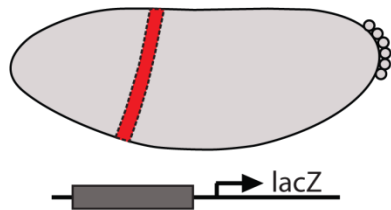
mutated full



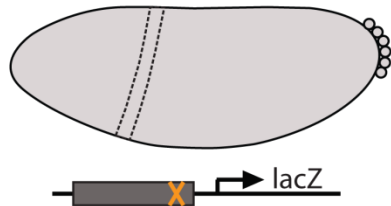


# Mutations on important *eve* stripe 2 enhancer binding sites affected *eve* stripe 2 expression.

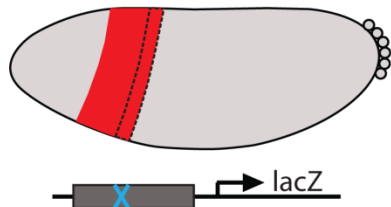
Previous work (Arnosti et. al)  
(minimal)



wt



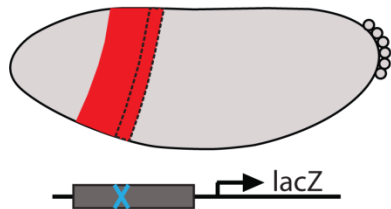
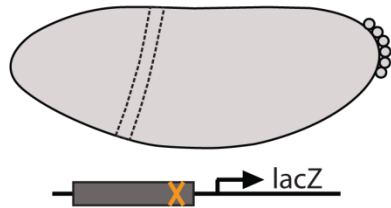
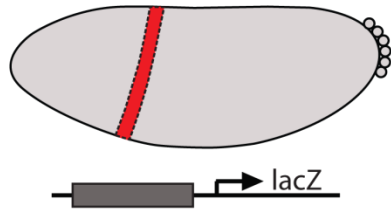
$\Delta hb$   
(activator)



$\Delta gt$   
(repressor-  
anterior boundary)

# We expect flanking sequences to compensate for mutations if they contain binding sites.

Previous work (Arnosti et. al)  
(minimal)

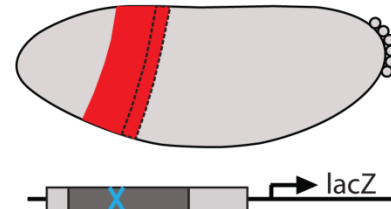
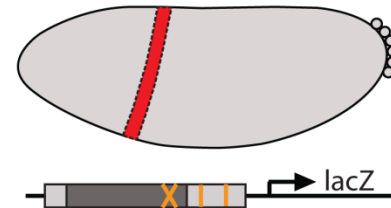
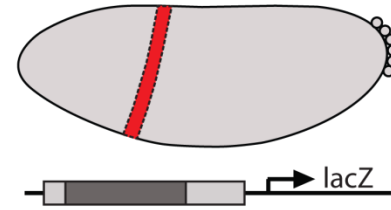


wt

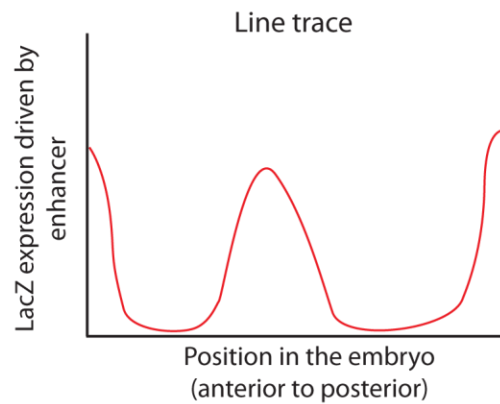
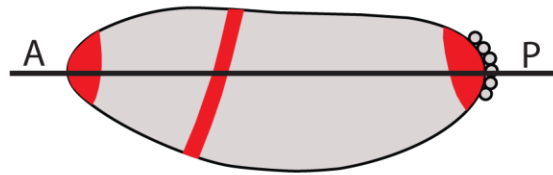
$\Delta hb$   
(activator)

$\Delta gt$   
(repressor-  
anterior boundary)

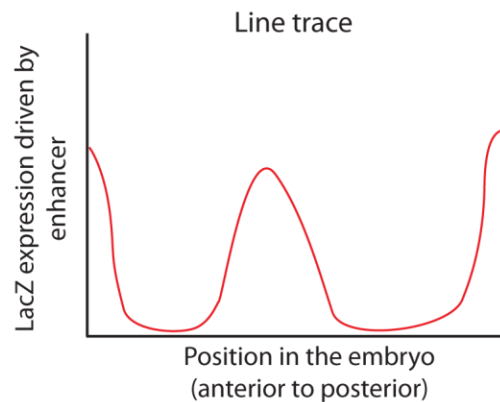
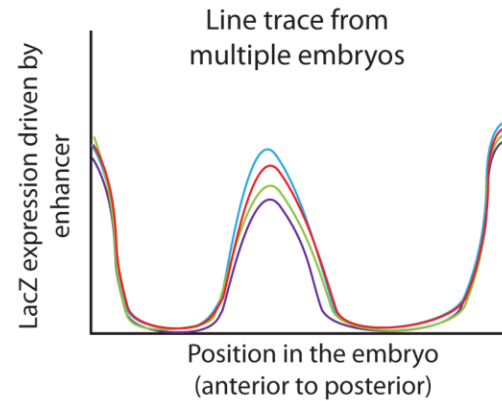
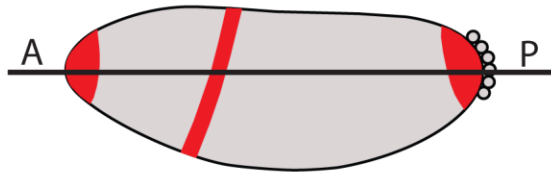
Hypothesis  
(full)



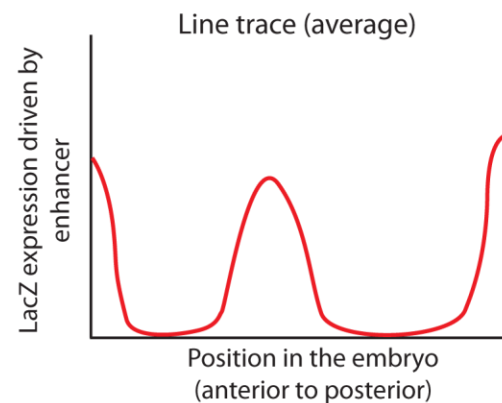
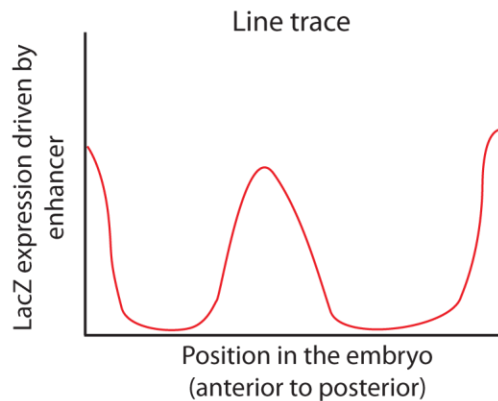
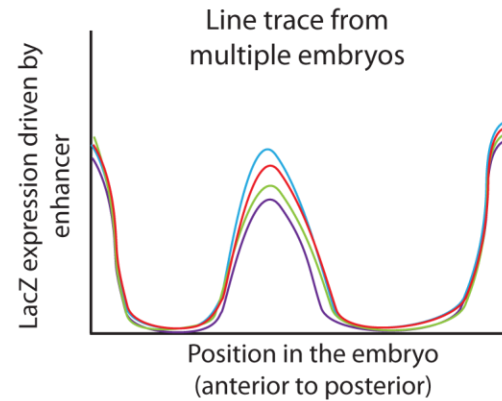
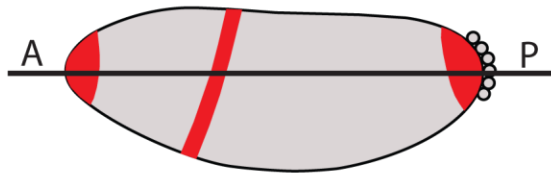
# Data will be presented as line traces.



# Data will be presented as line traces.

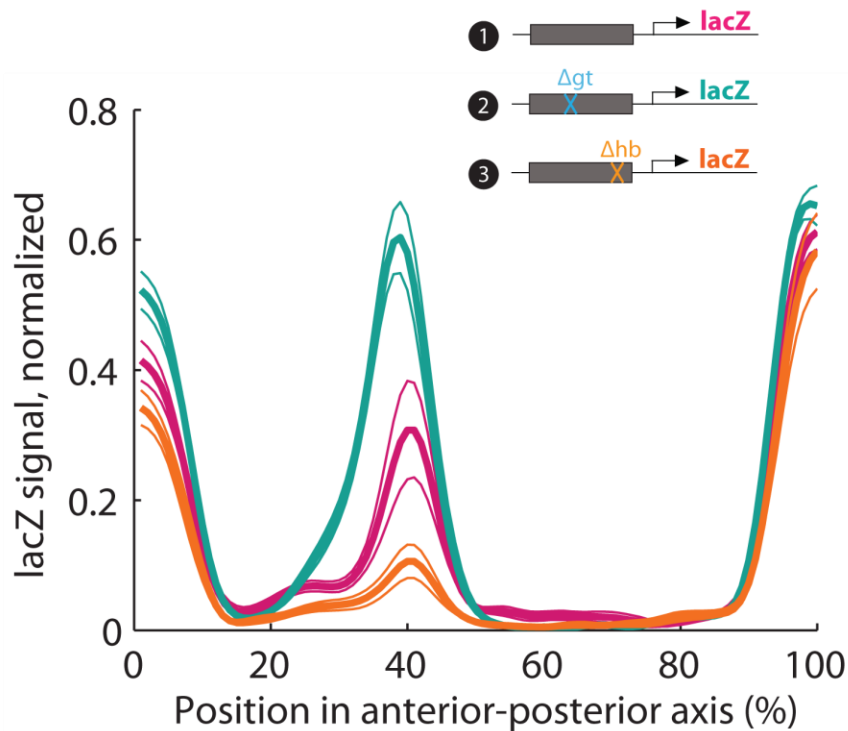


# Data will be presented as line traces.



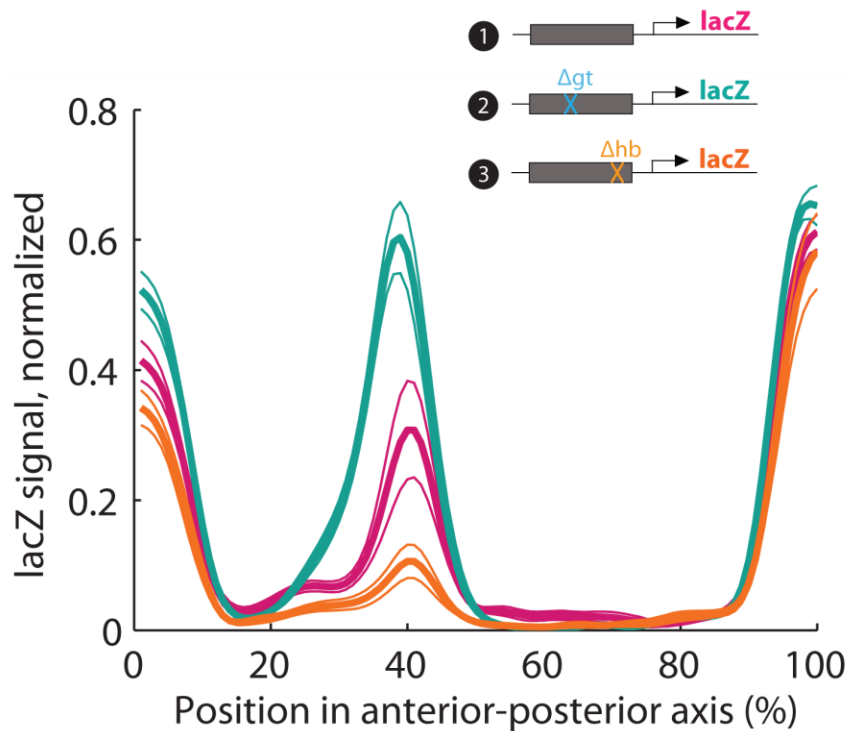
# Minimal enhancer levels are consistent with previous observations.

LacZ expression driven by minimal  
eve stripe 2 enhancer

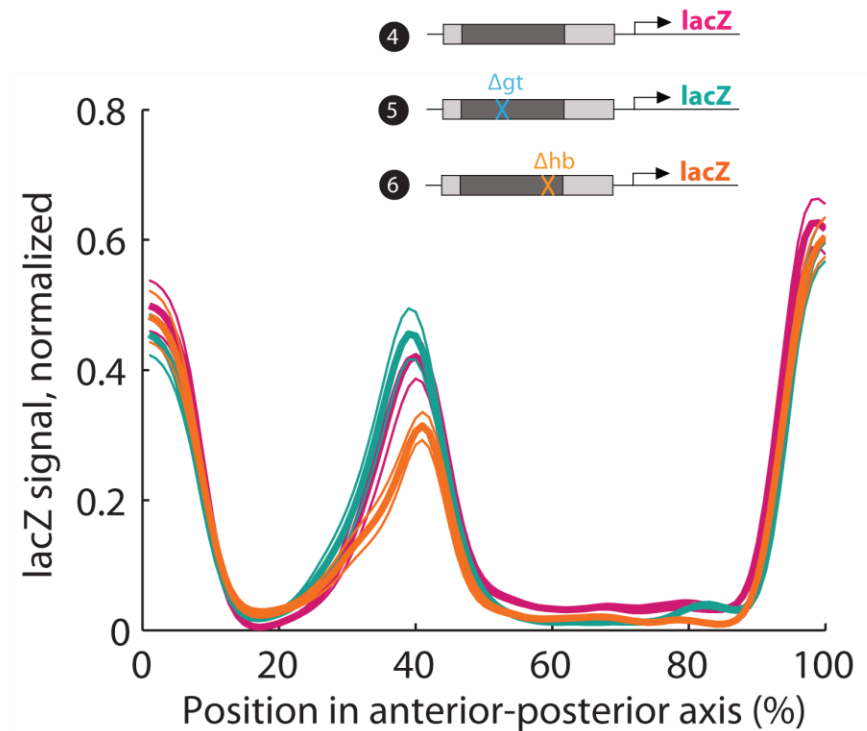


# Flanking sequences compensate for mutations even in the absence of footprinted binding sites.

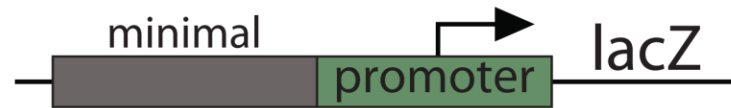
LacZ expression driven by minimal eve stripe 2 enhancer



LacZ expression driven by full eve stripe 2 enhancer



We want to identify the sequence features responsible for compensation.



a) Distance from promoter



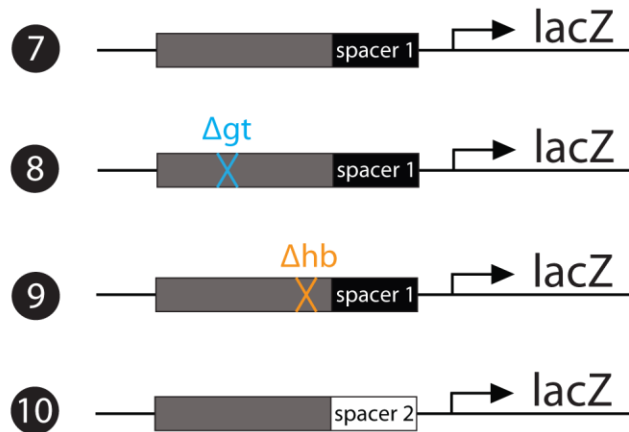
b) Additional binding sites





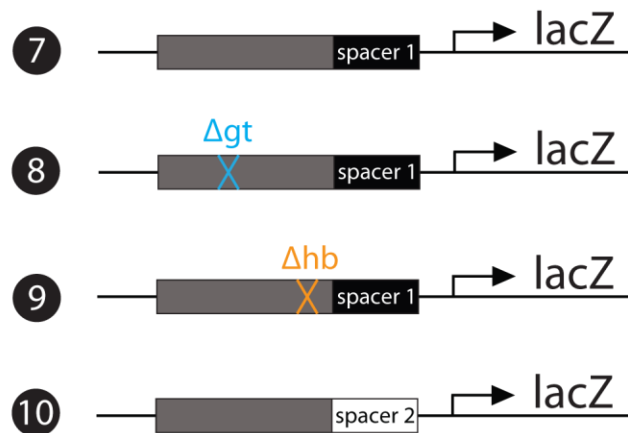
# We designed constructs to test for distance from the promoter.

a) Distance from promoter

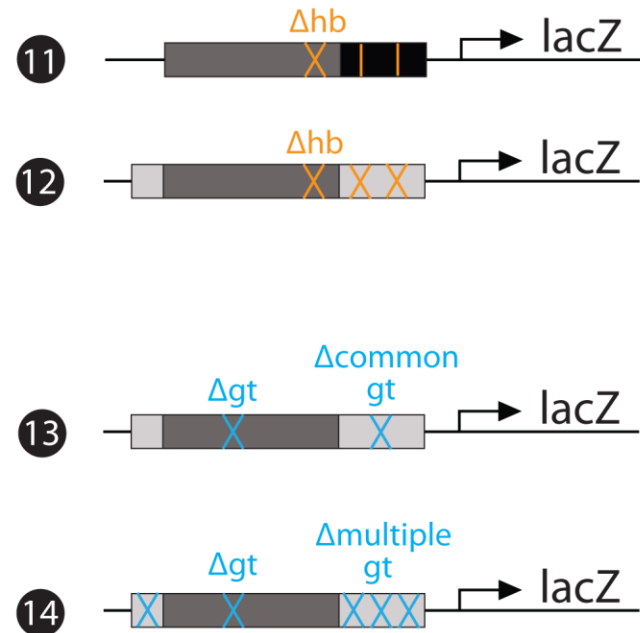


# We designed constructs to test for additional hb or gt binding sites.

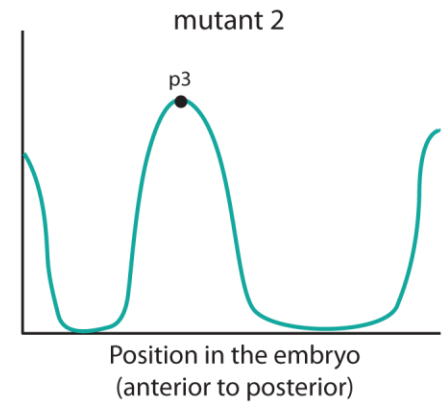
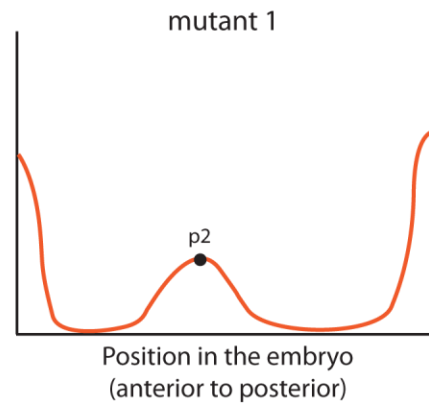
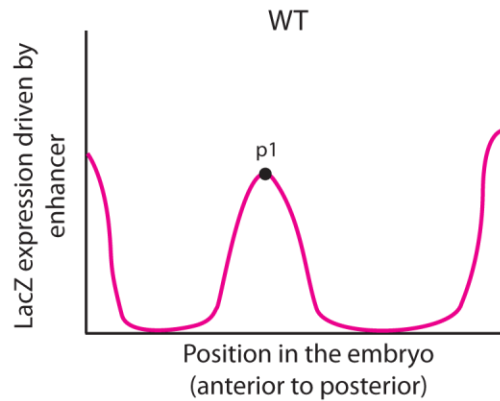
a) Distance from promoter



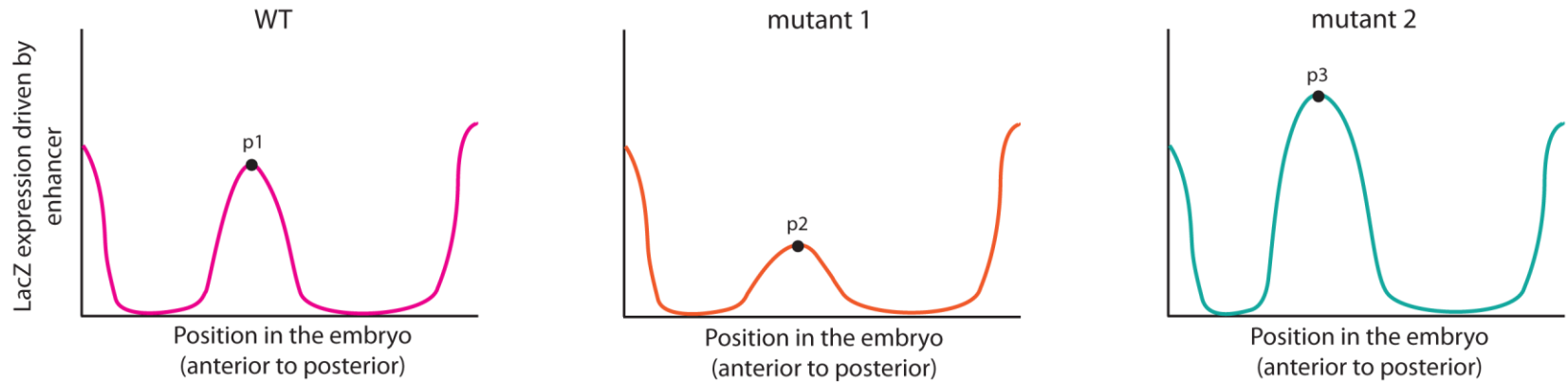
b) Additional binding sites



Data will be presented as ratios of the peak mean value of two constructs.

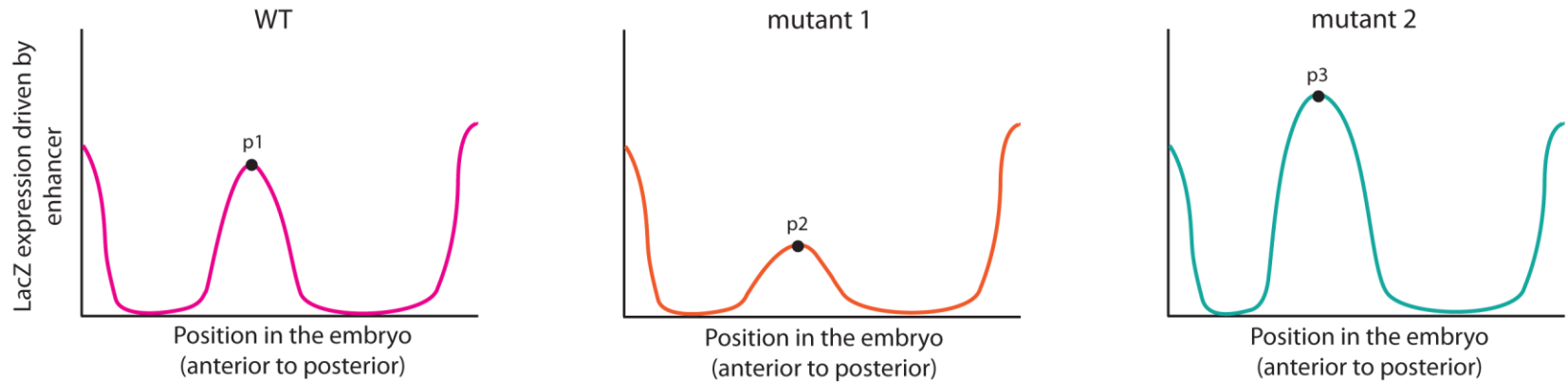


# Data will be presented as ratios of the peak mean value of two constructs.



	A	B	C	D	
1		p1	p2	p3	
2	Stain 1	0.21	0.11	0.41	
3	Stain 2	0.25	0.1	0.45	
4	Stain 3	0.15	0.05	0.5	
5					

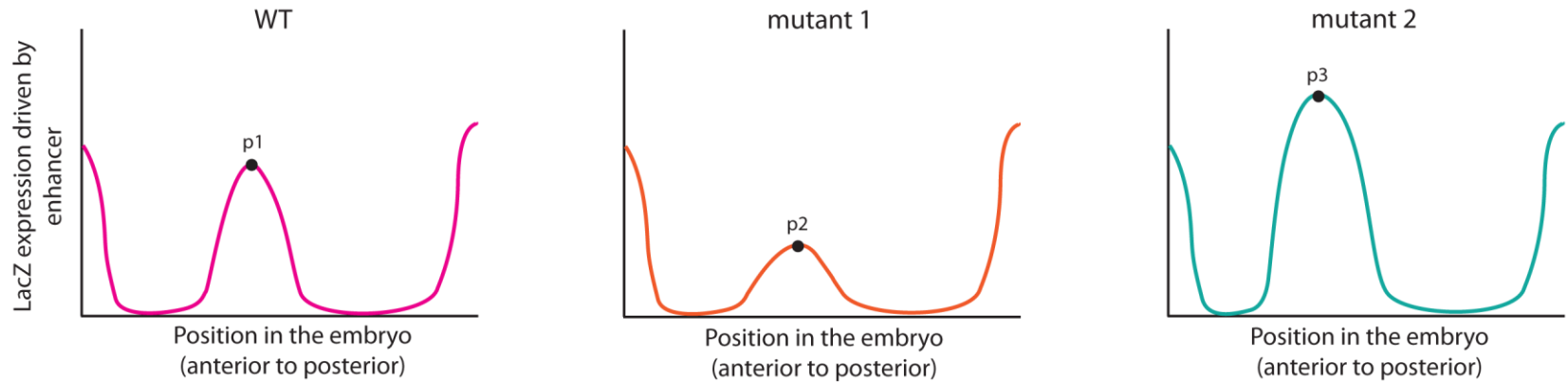
# Data will be presented as ratios of the peak mean value of two constructs.



	A	B	C	D	
1		p1	p2	p3	
2	Stain 1	0.21	0.11	0.41	
3	Stain 2	0.25	0.1	0.45	
4	Stain 3	0.15	0.05	0.5	
5					

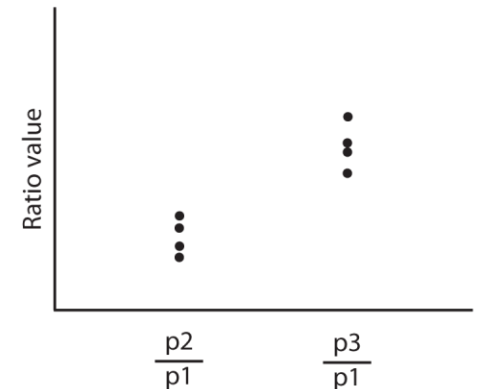
$$\text{Ratio: } \frac{p2}{p1}$$

# Data will be presented as ratios of the peak mean value of two constructs.



	A	B	C	D	
1		p1	p2	p3	
2	Stain 1	0.21	0.11	0.41	
3	Stain 2	0.25	0.1	0.45	
4	Stain 3	0.15	0.05	0.5	
5					

$$\text{Ratio: } \frac{p2}{p1}$$



# Data discussion



Thank you!

