ChIP analysed by q-PCR

Bernadett Papp

(PhD student

EMBL

Heidelberg, Germany)

ChIP I.

studying interactions between endogenous proteins and their native chromatin target site in vivo

1. Formaldehyde crosslink

- Live cells treated with formaldehyde to generate reversible chemical crosslinks between proteins / proteins and DNA located close proximity (2A).

! crosslinking time (over- and undercrosslinking)

! epitope masking, sonication problems

2. Sonication

- Crosslinked cells sonicated to shear the chromatin into uniformly sized pieces of DNA with well-defined lengths.

! optimal fragment length

! Reproducibility

3. Immunoprecipitation

- Fragmented chromatin is subjected to immunoprecipitation with an antibody against the protein of interest under highly stringent conditions.

! antibody quality (affinity purified if possible)

! optimalization of the antibody/chromatin ratio to achieve max IP efficiency

! preimmunserum

ChIP II.

4. Reversion of the crosslink

- Several stringent washes reduce the background and the product of the IP is de-crosslinked by proteinaseK and heat treatment.

5. Extraction of the immunoprecipitated DNA

! purity (affecting the qRT-PCR)
! contamination (DNA)
! Loss of DNA (DNAse)

6. q-PCR !!!!!!!

- *If a protein under investigation is associated with a specific genomic region in vivo, DNA fragments from this region should be enriched in the immunoprecipitate compared to other portions of the genome.*
= The presence and the quantity of relevant genomic regions in the IP are determined

- It is not possible to compare the amounts of proteins within a primer pair from one IP to another using different antibodies since different antisera have different precipitation efficiencies. Comparisons should be made only on the same protein in 2 different genomic regions.

ChIP III.

- The sample values are compared to those obtained from known amounts of input DNA.
(Input is the total of chromatin-DNA used for one IP.)
(Serial dilutions of genomic DNA is used for the standard curve.)

! primer design, quality control of the PCR
! ctrl regions (pos and neg)
! Input material

7. Data analysis and interpretation

- The results can be plotted in diagrams as percentage of the input with the different genomic regions on the X axis.

! Independent experiments from independent chromatins
! Diagram types (% of Input versus fold enrichments over preimmune/ctrl regions)
! Substraction of background, normalization

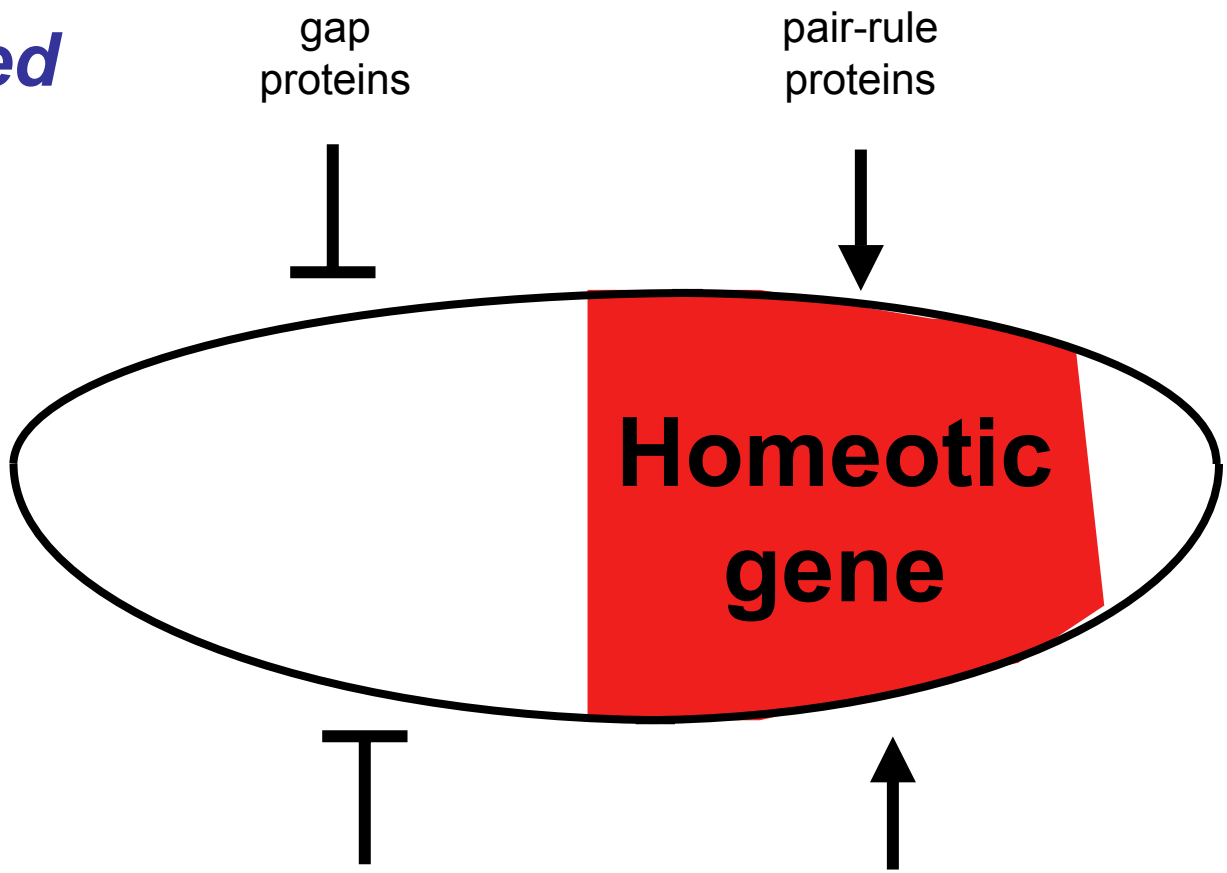
! Interpretation of the data !!!!

"Negative is negative. Positive? Antibody IPs what is IPs."

ChIP studies on a HOX gene regulated by Polycomb group and trithorax group proteins

INITIATION

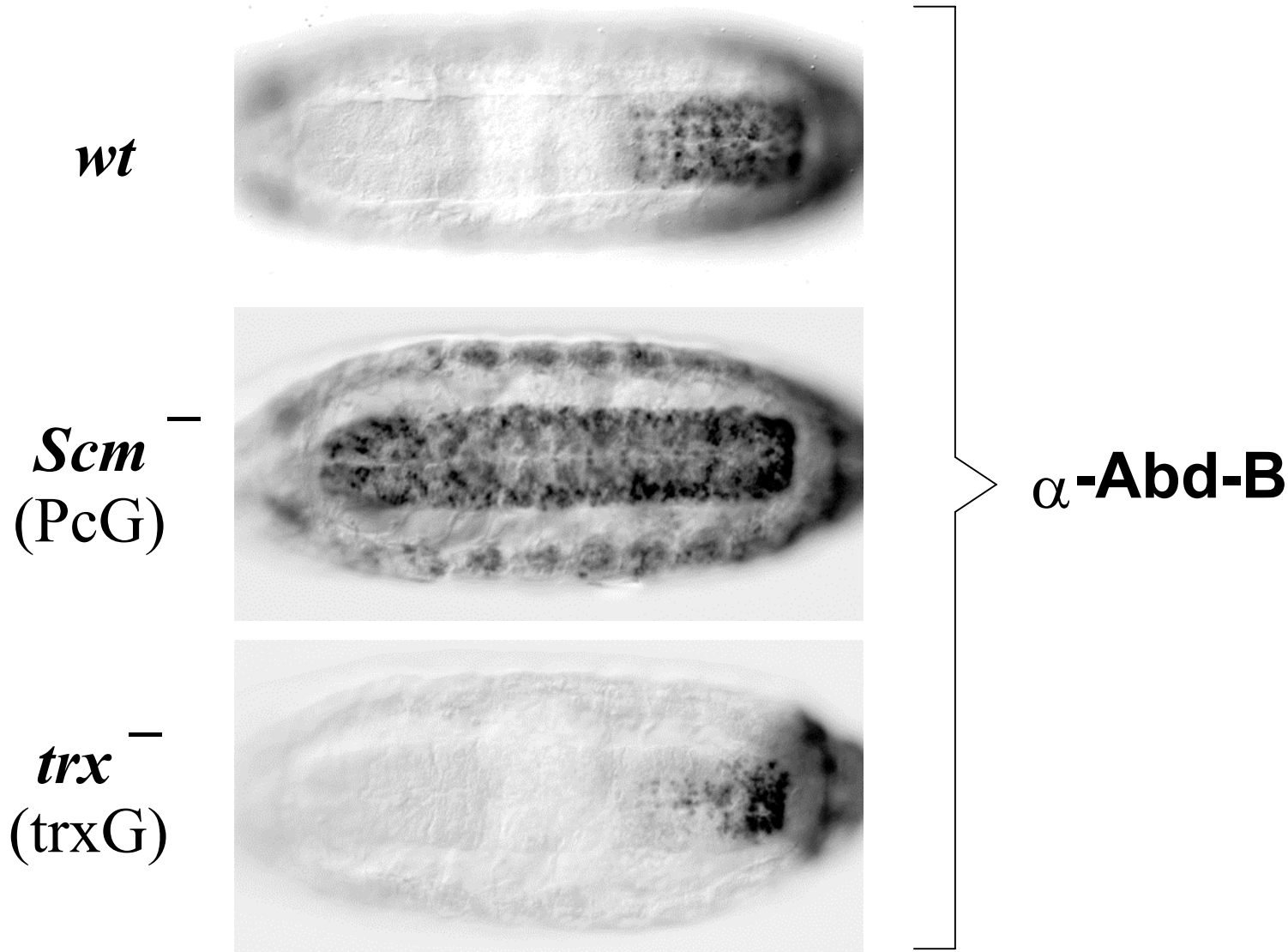
localized



ubiquitous

MAINTENANCE

Control of HOX gene expression by Polycomb group (PcG) and trithorax group (trxG) regulators

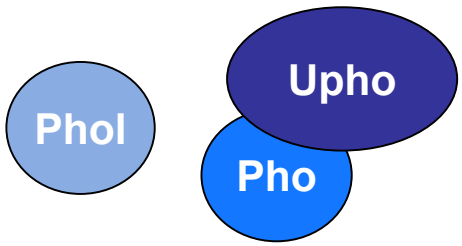


Polycomb response elements (**PRE**s)

- silencers

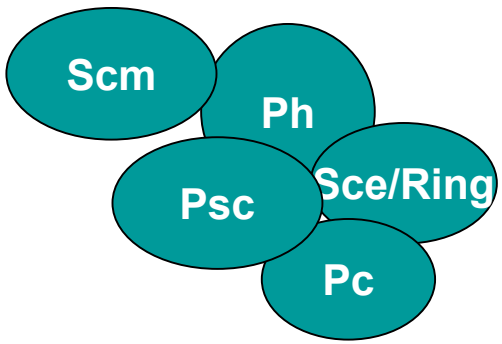
- anchor sites for Polycomb group proteins

Polycomb group (PcG)



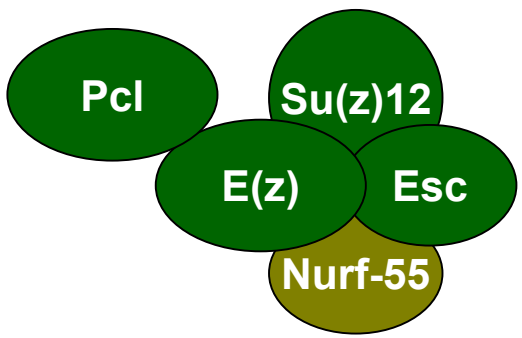
PhoRC

(DNA-binding)



PRC1

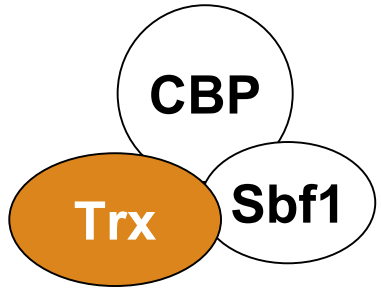
(*Inhibition of Chromatin Remodeling*)



PRC2

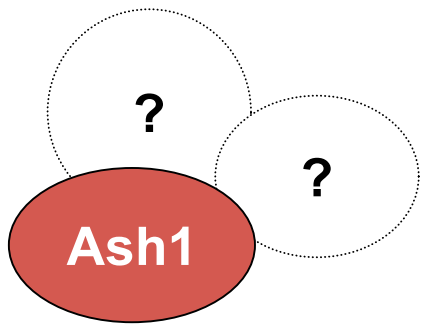
(*HMTase K27 H3*)

trithorax group (trxG)



TAC1

(*HMTase K4 H3*)

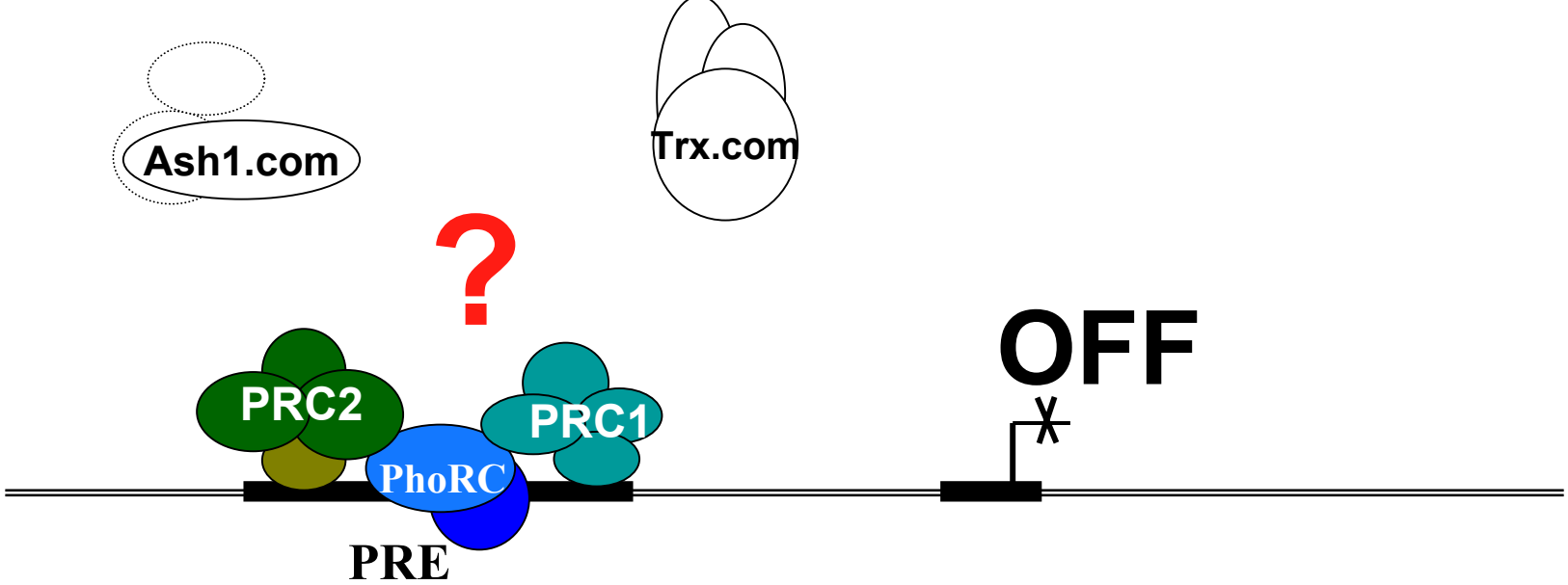


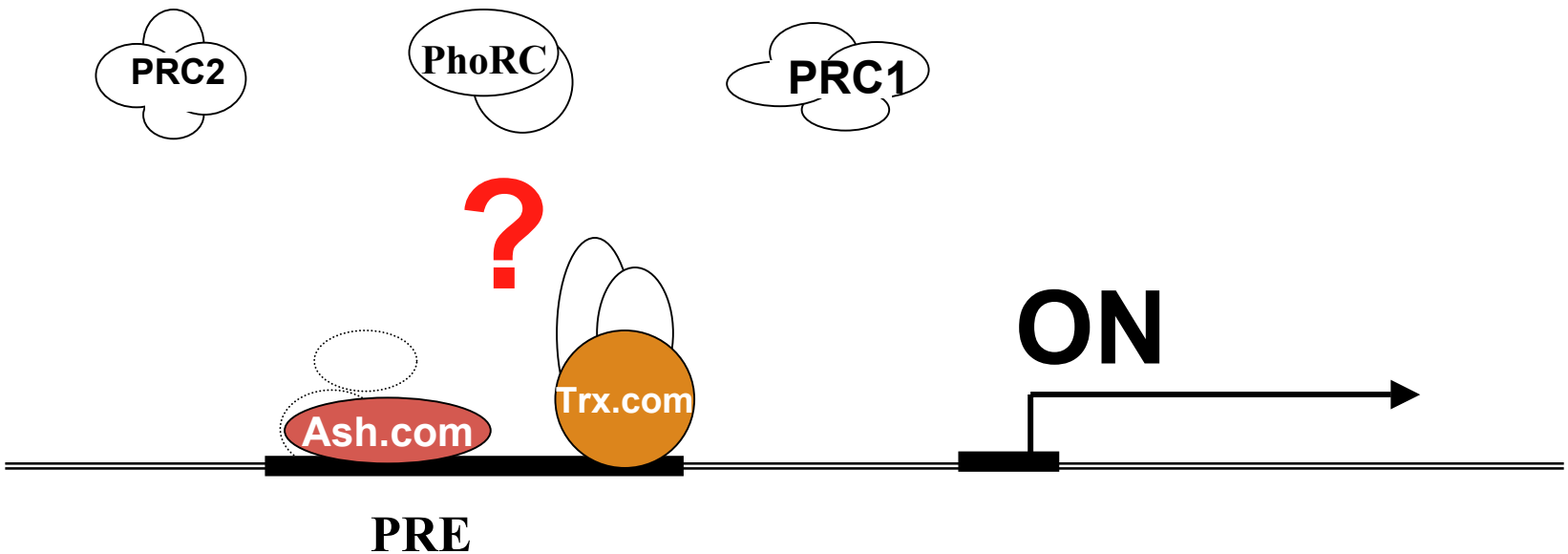
ASH.com

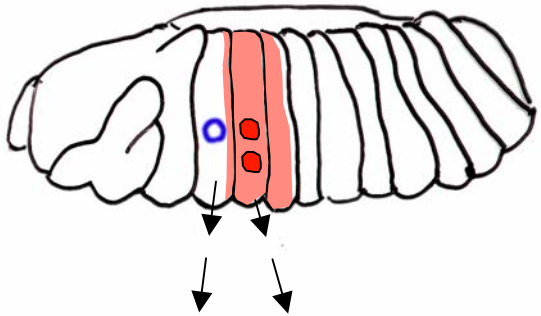
(*HMTase K4/K9 H3 K20 H4*)

OFF state

ON state


Ash1.com
Trx.com
?
PRC2
PRC1
PhoRC
OFF
PRE

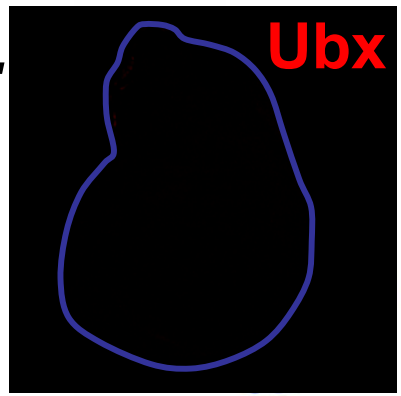

PRC2
PhoRC
PRC1
?
Trx.com
Ash.com
ON
PRE



cell divisions

Ubx ***OFF***

wing disc

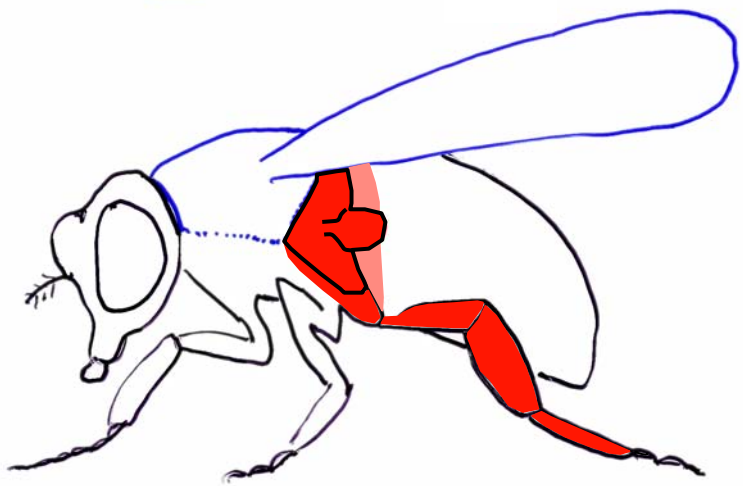




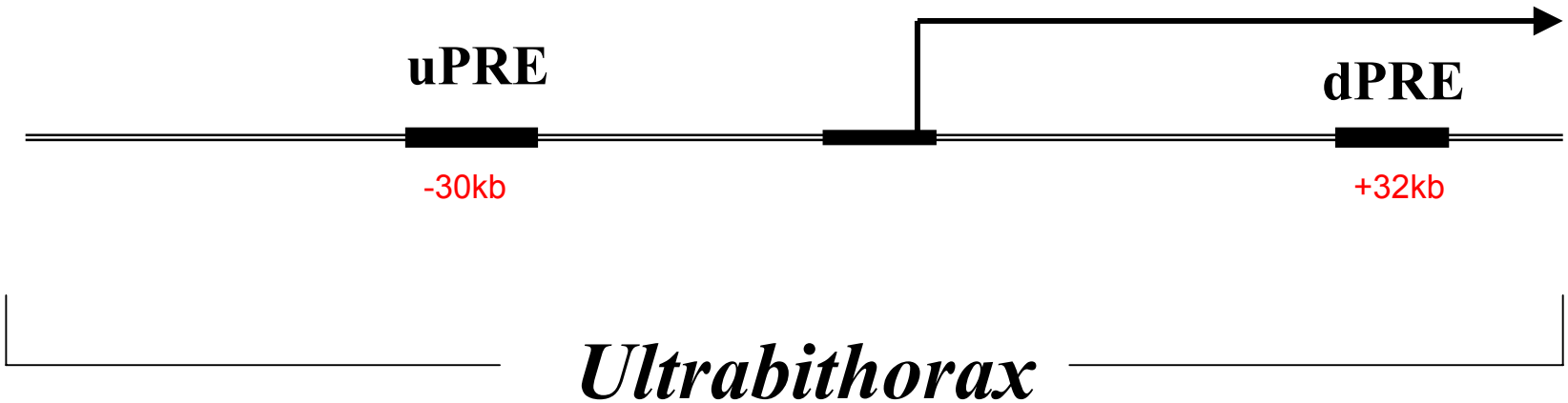
Ubx ***ON***

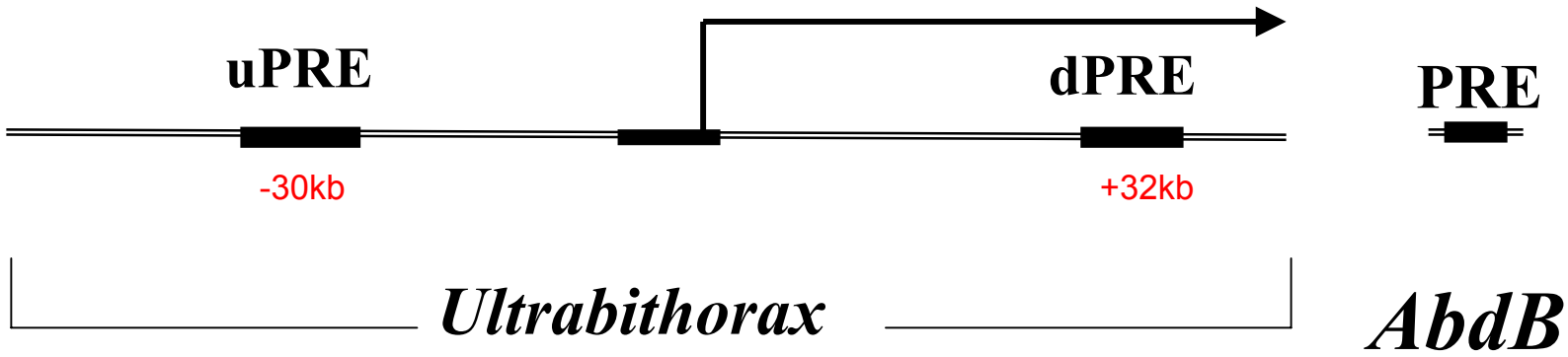
haltere disc
+
3rd leg disc

X-ChIP

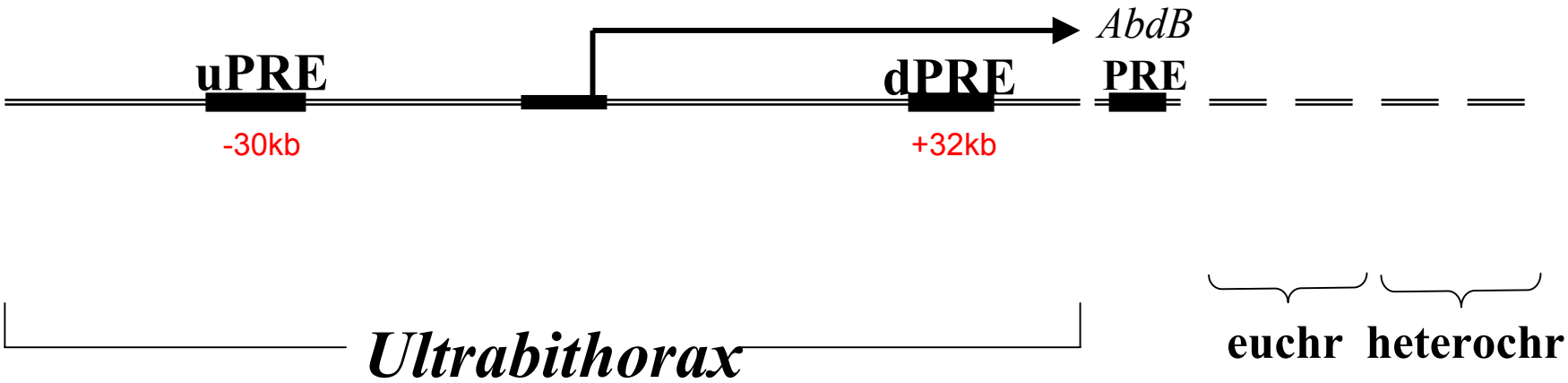


X-ChIP

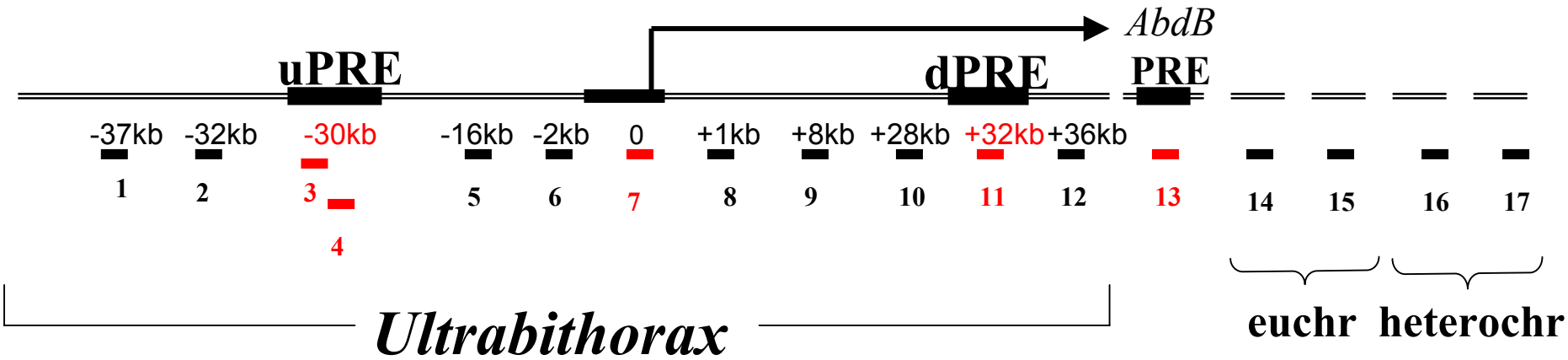

uPRE
dPRE
-30kb
+32kb
Ultrabithorax



Wing	OFF	OFF
Haltere/3rd leg	ON	OFF

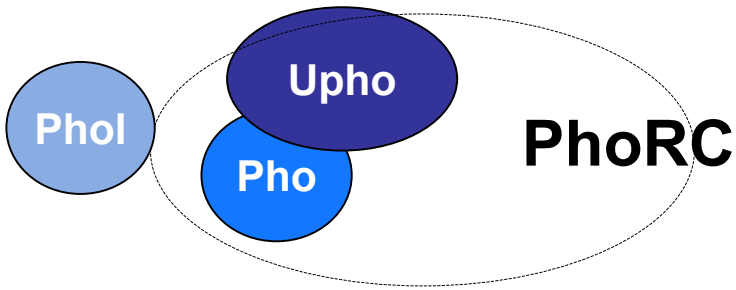


Wing	OFF	OFF	-
Haltere/3rd leg	ON	OFF	-

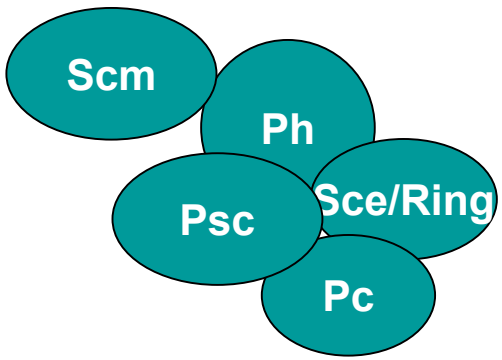


Wing	OFF	OFF	-
Haltere/3rd leg	ON	OFF	-

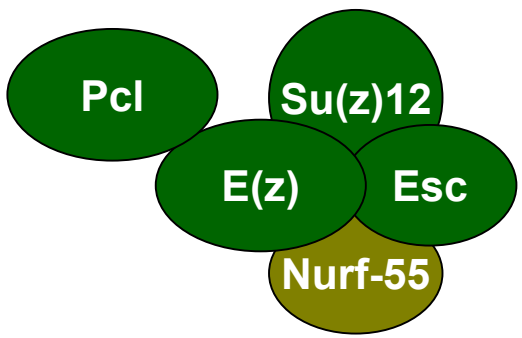
Polycomb group (PcG)



PhoRC



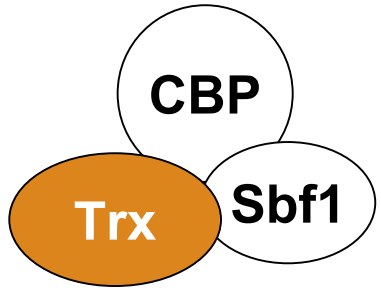
PRC1



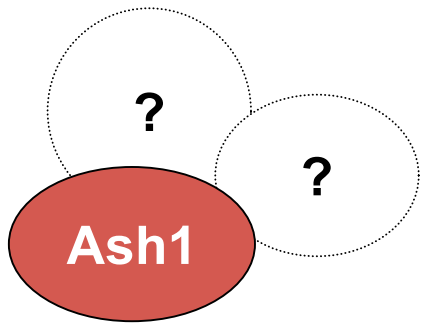
PRC2

OFF state

trithorax group (trxG)

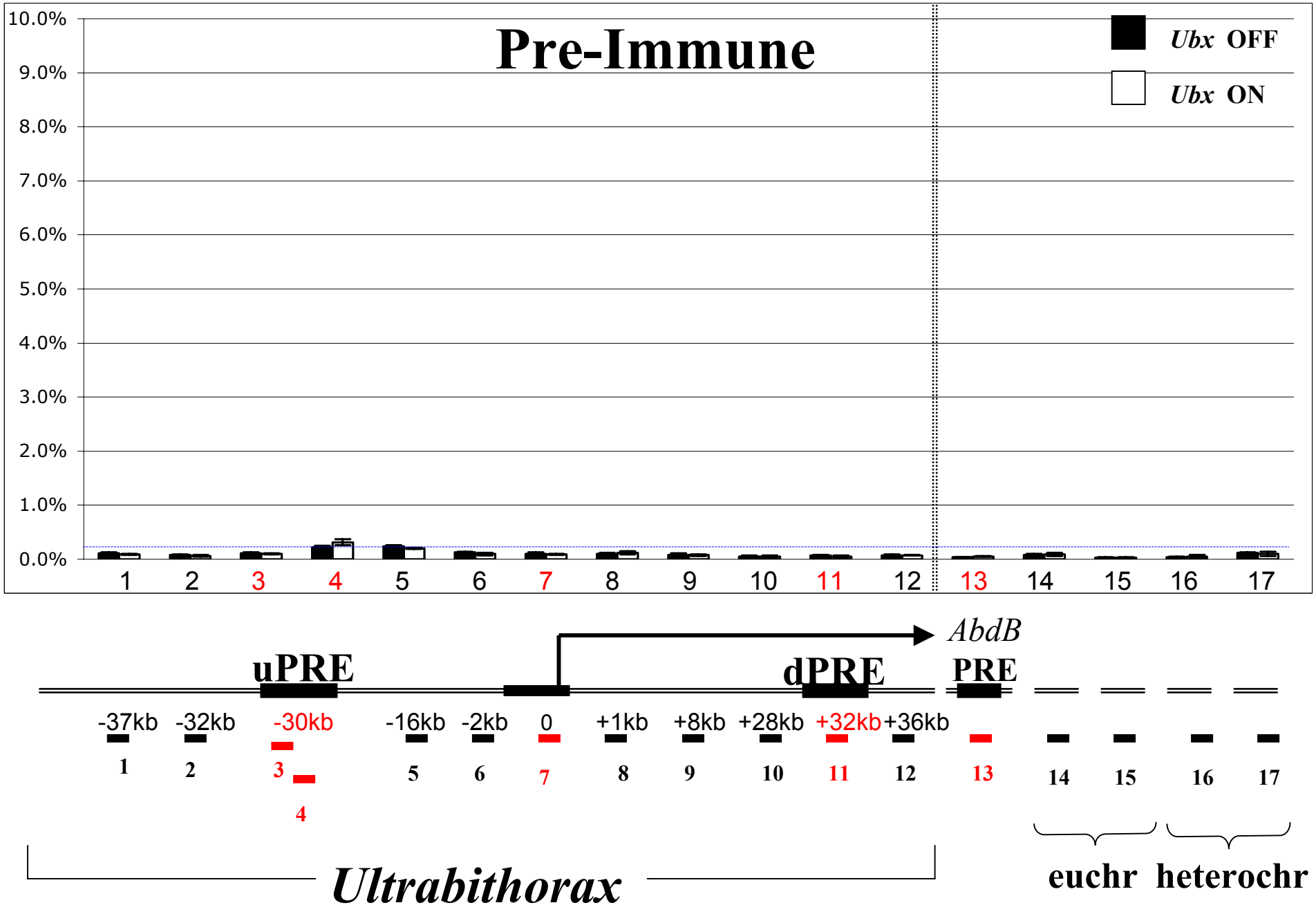


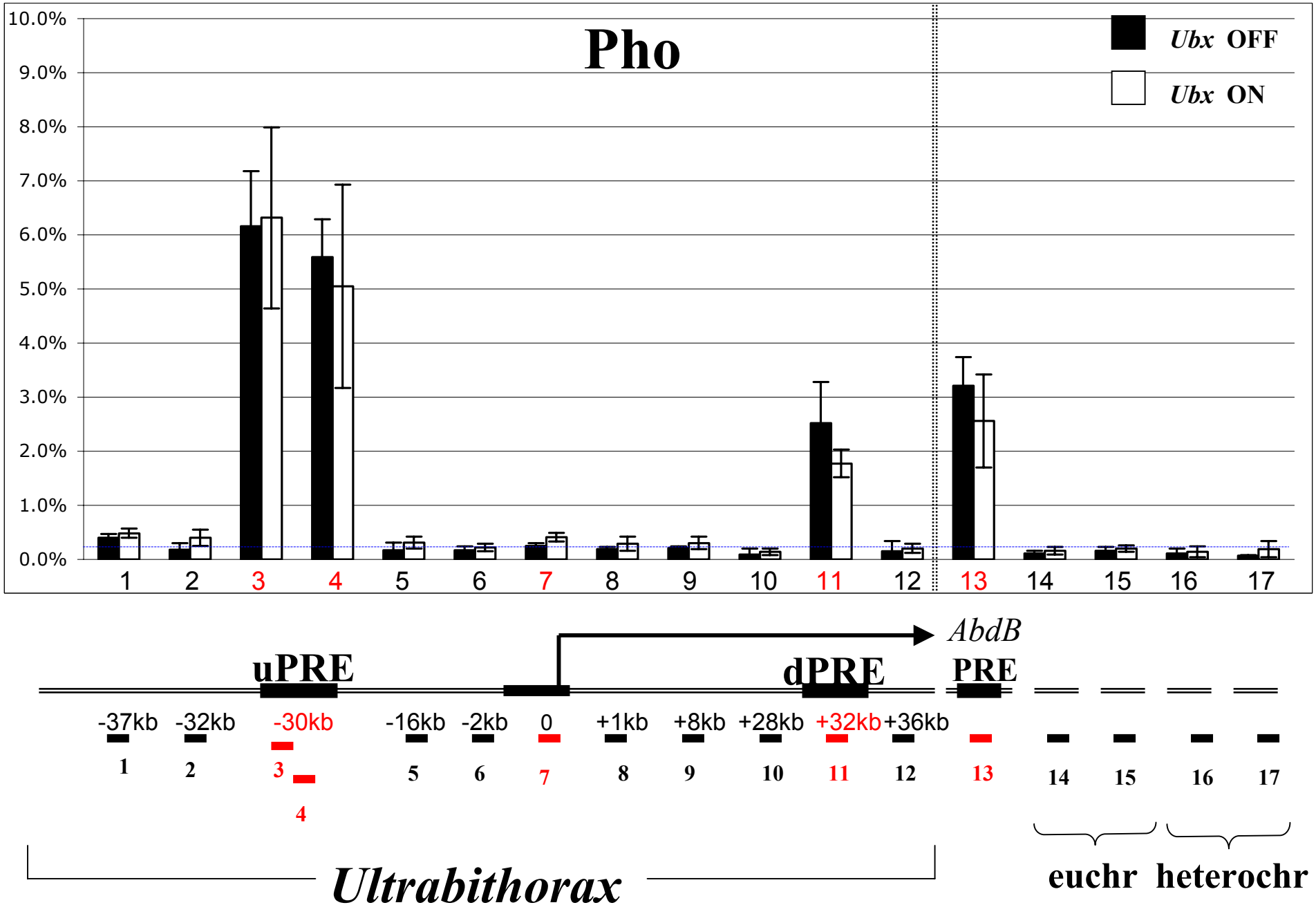
TAC1



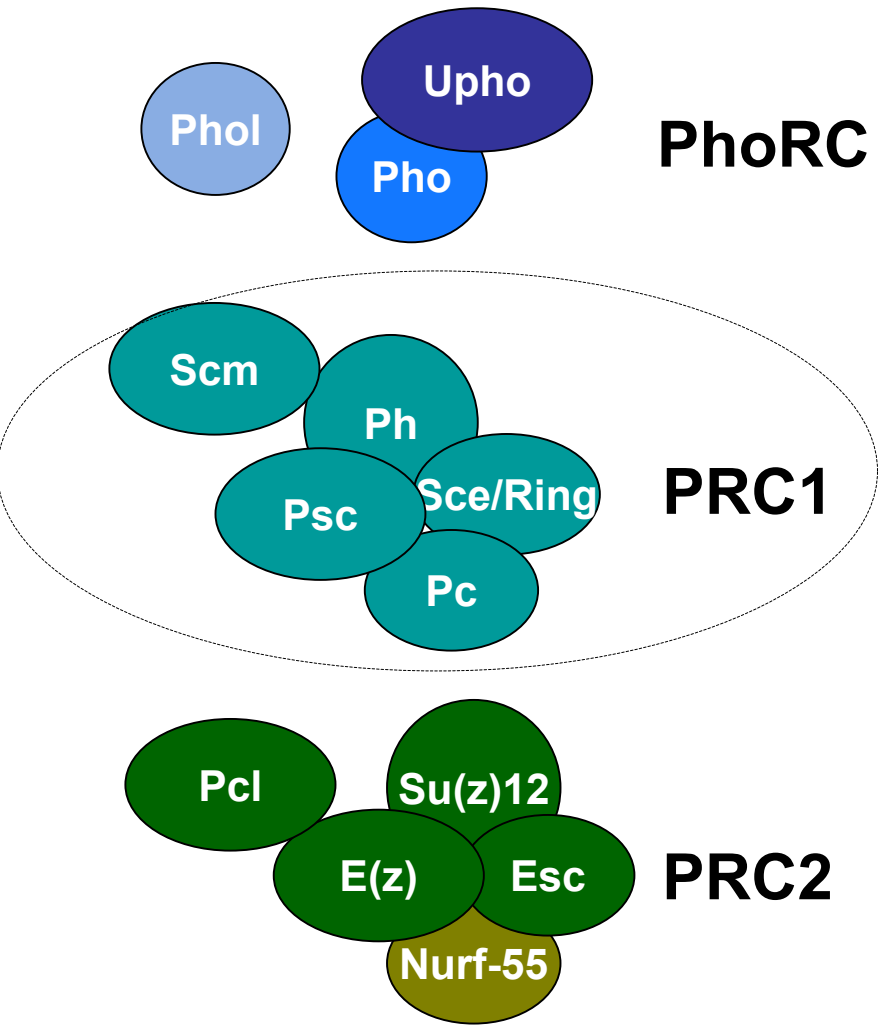
ASH.com

ON state

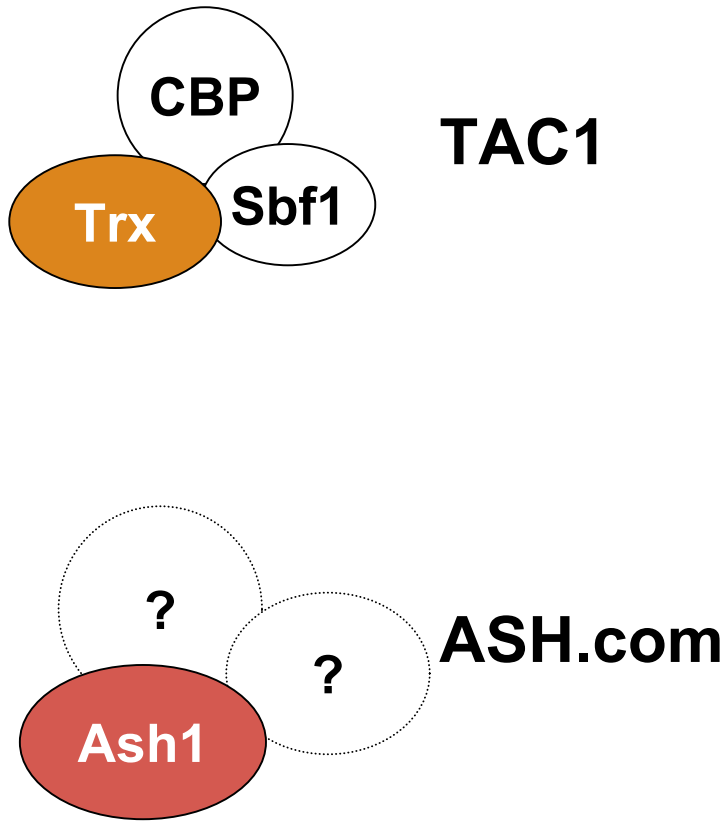

Pre-Immune
Ubx OFF
Ubx ON
10.0%
9.0%
8.0%
7.0%
6.0%
5.0%
4.0%
3.0%
2.0%
1.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr


Pho
Ubx OFF
Ubx ON
10.0%
9.0%
8.0%
7.0%
6.0%
5.0%
4.0%
3.0%
2.0%
1.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr

Polycomb group (PcG)

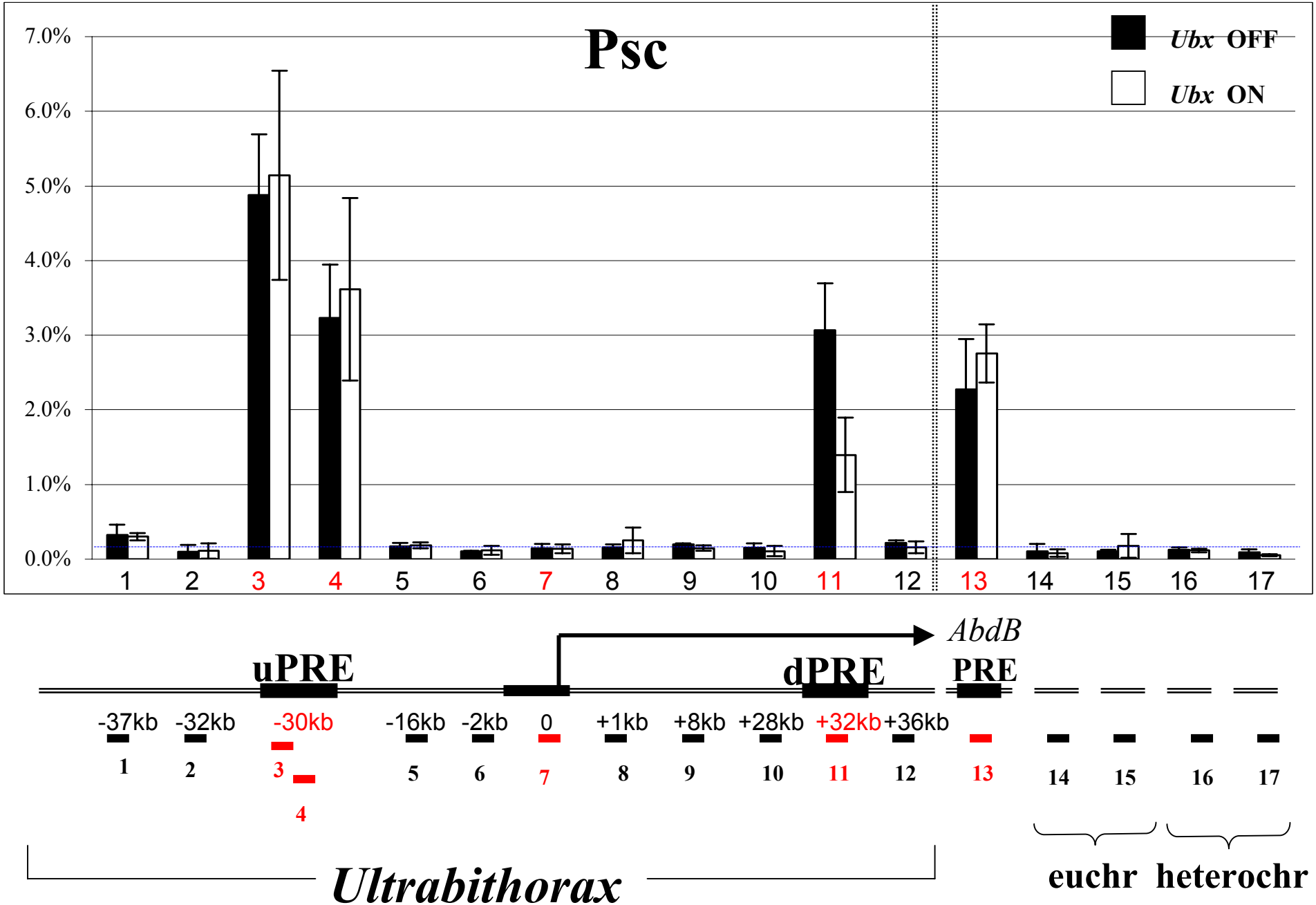


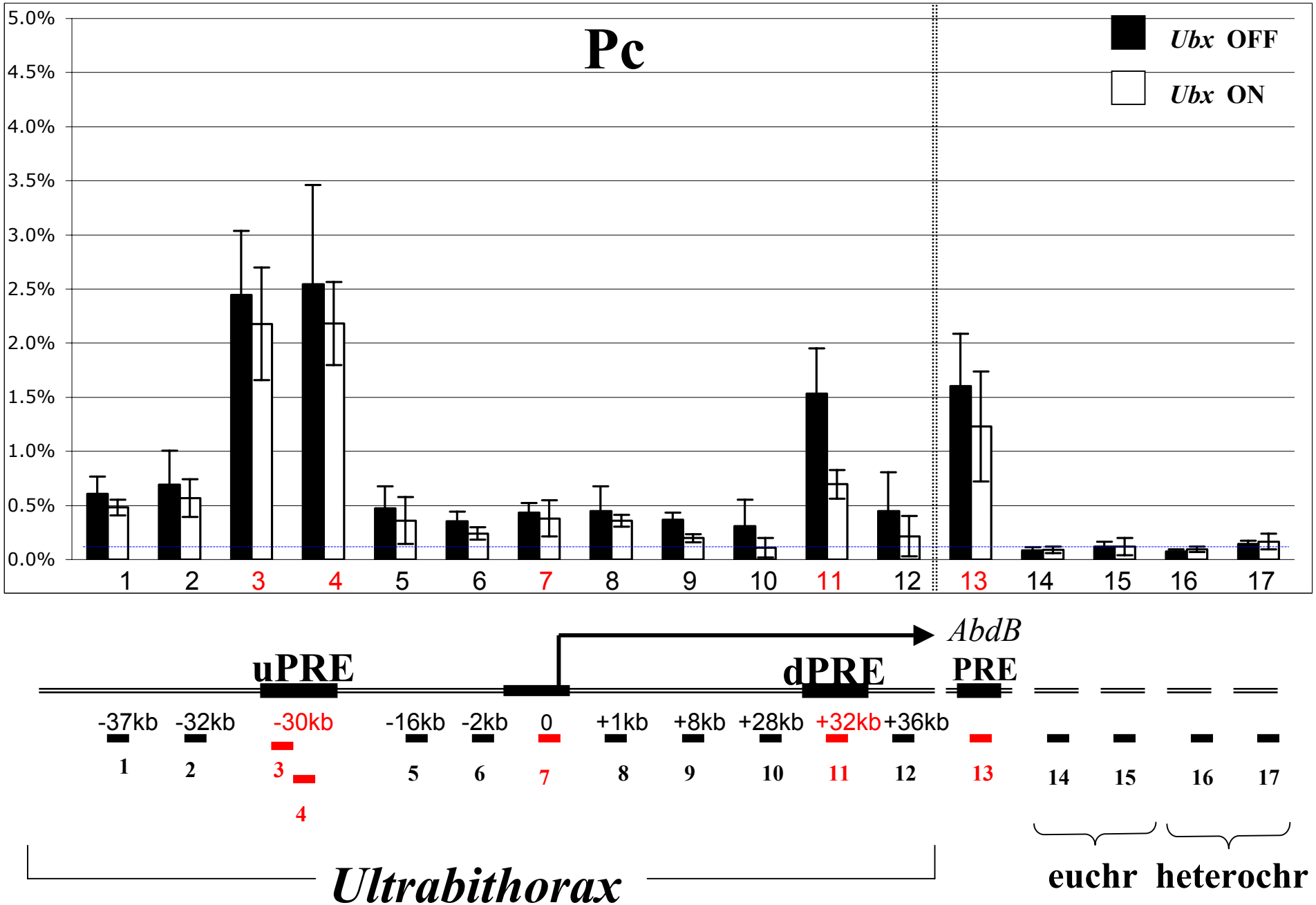
trithorax group (trxG)



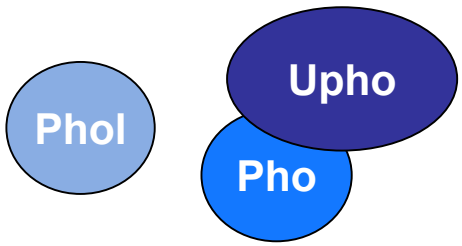
OFF state

ON state

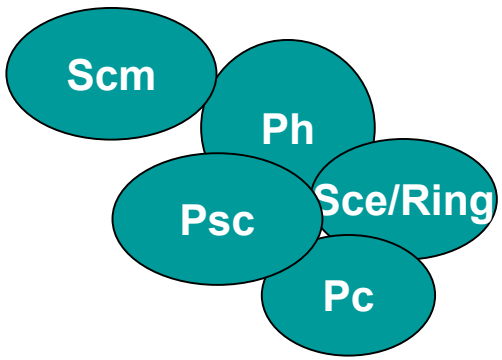

Psc
Ubx OFF
Ubx ON
7.0%
6.0%
5.0%
4.0%
3.0%
2.0%
1.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr


Pc
Ubx OFF
Ubx ON
5.0%
4.5%
4.0%
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr

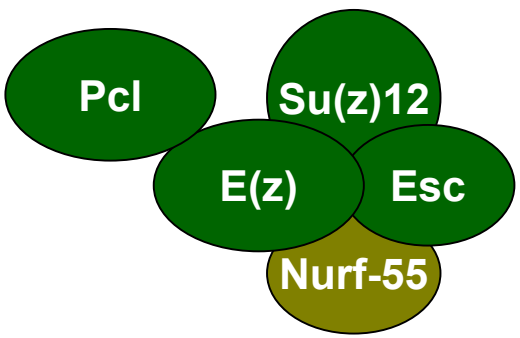
Polycomb group (PcG)



PhoRC



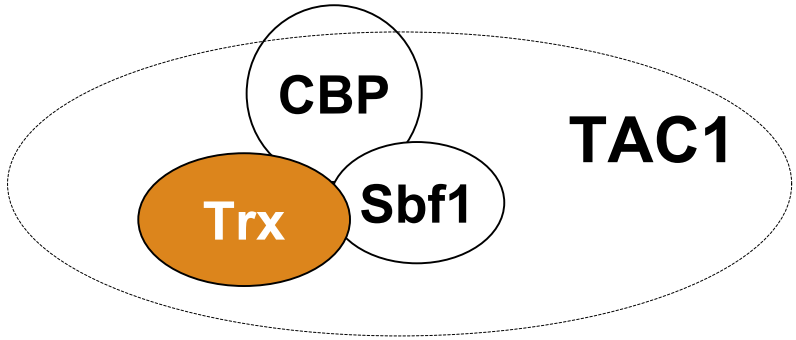
PRC1

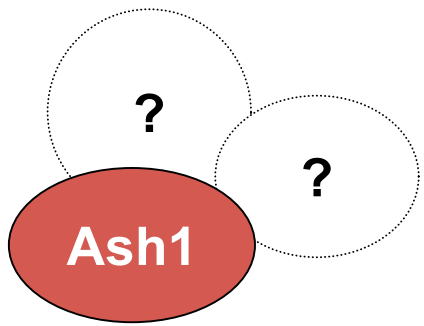


PRC2

OFF state

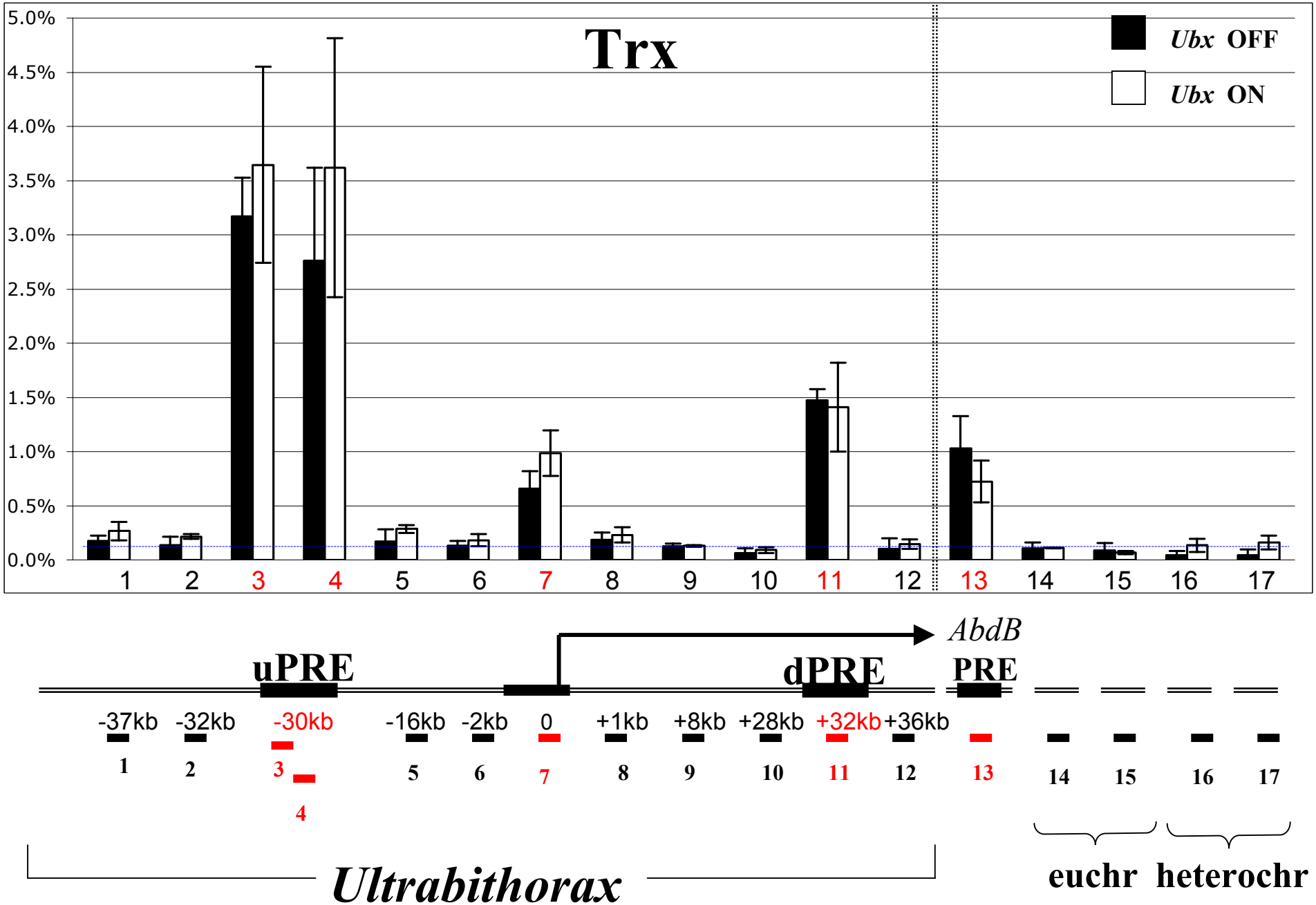
trithorax group (trxG)

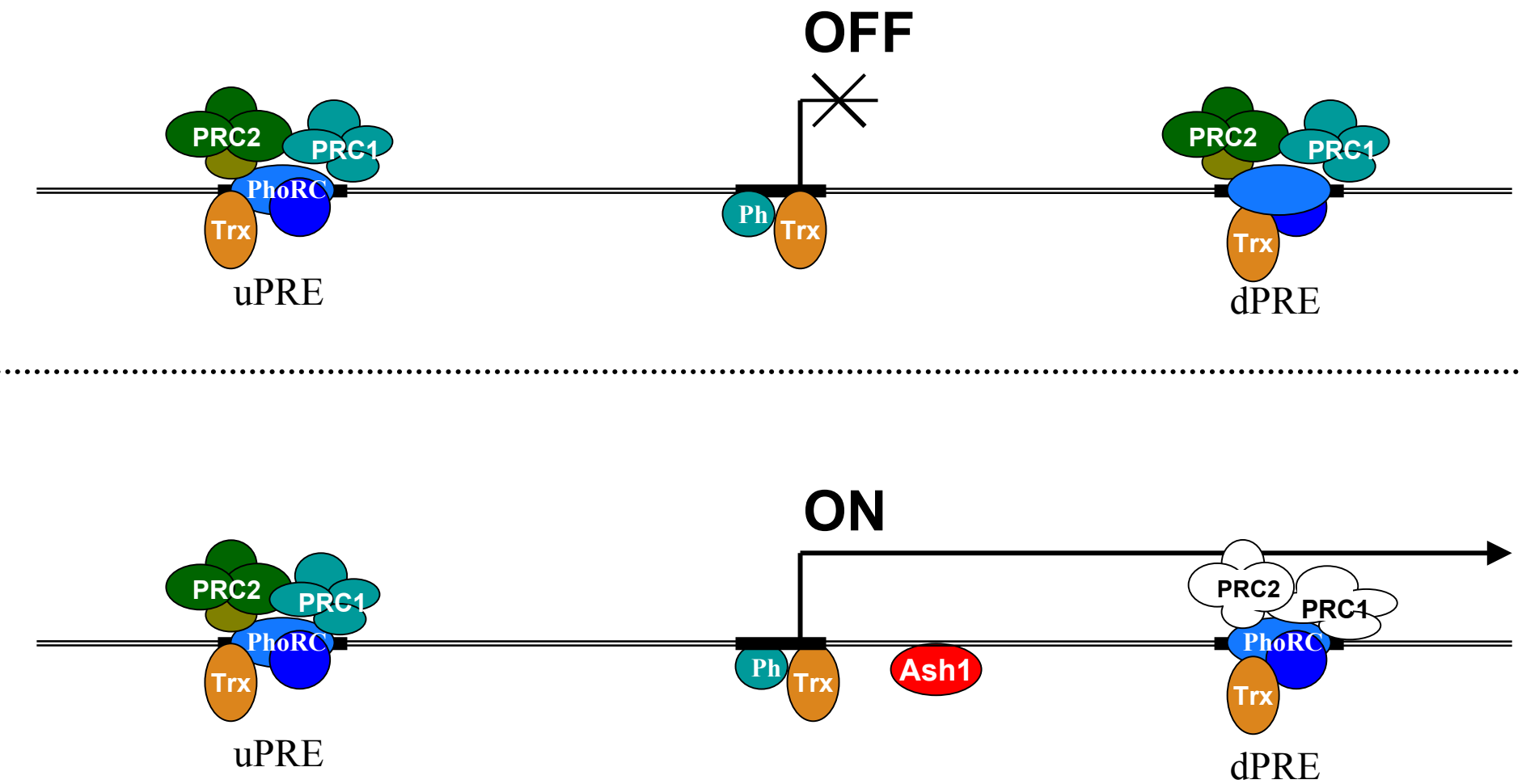


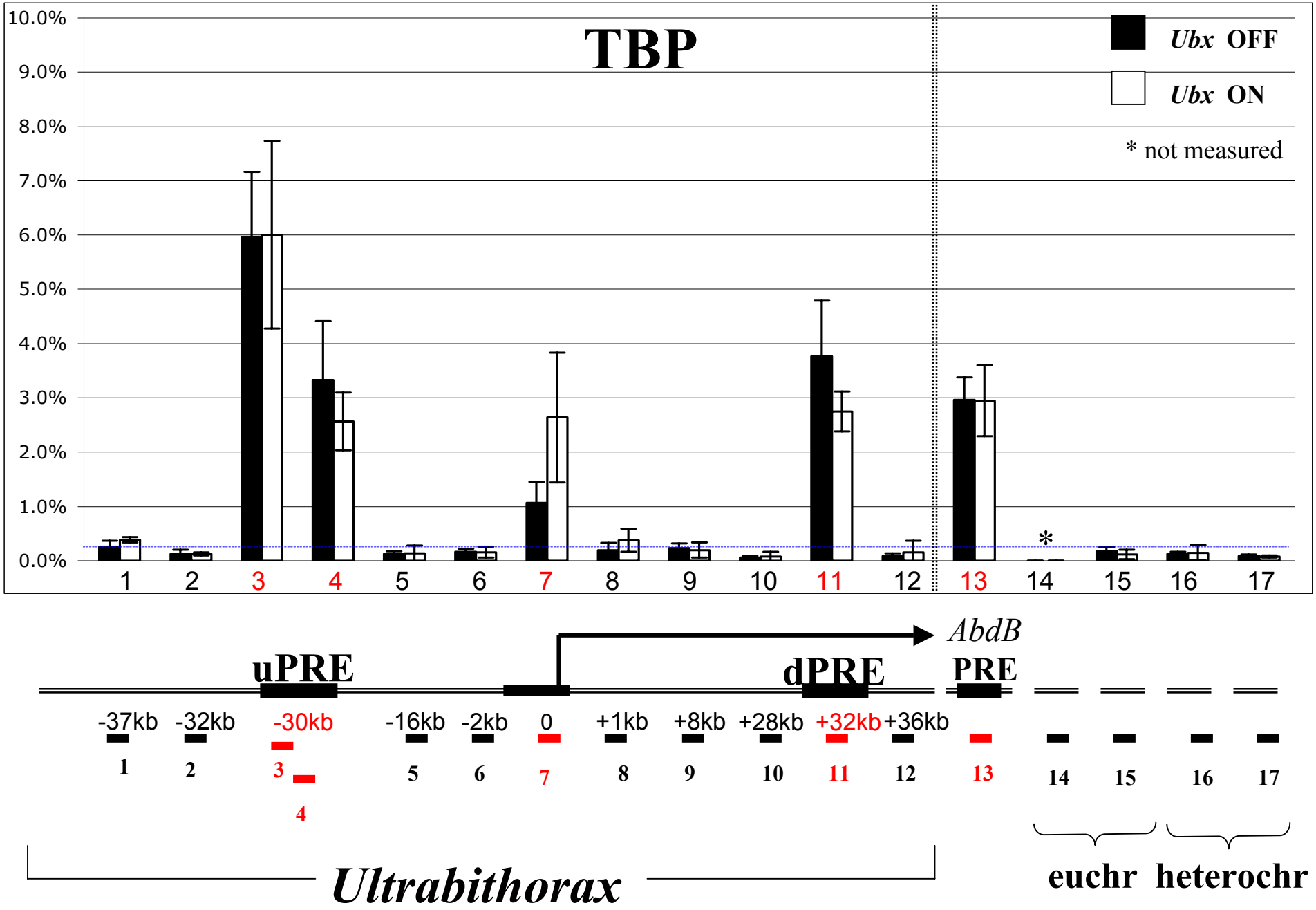


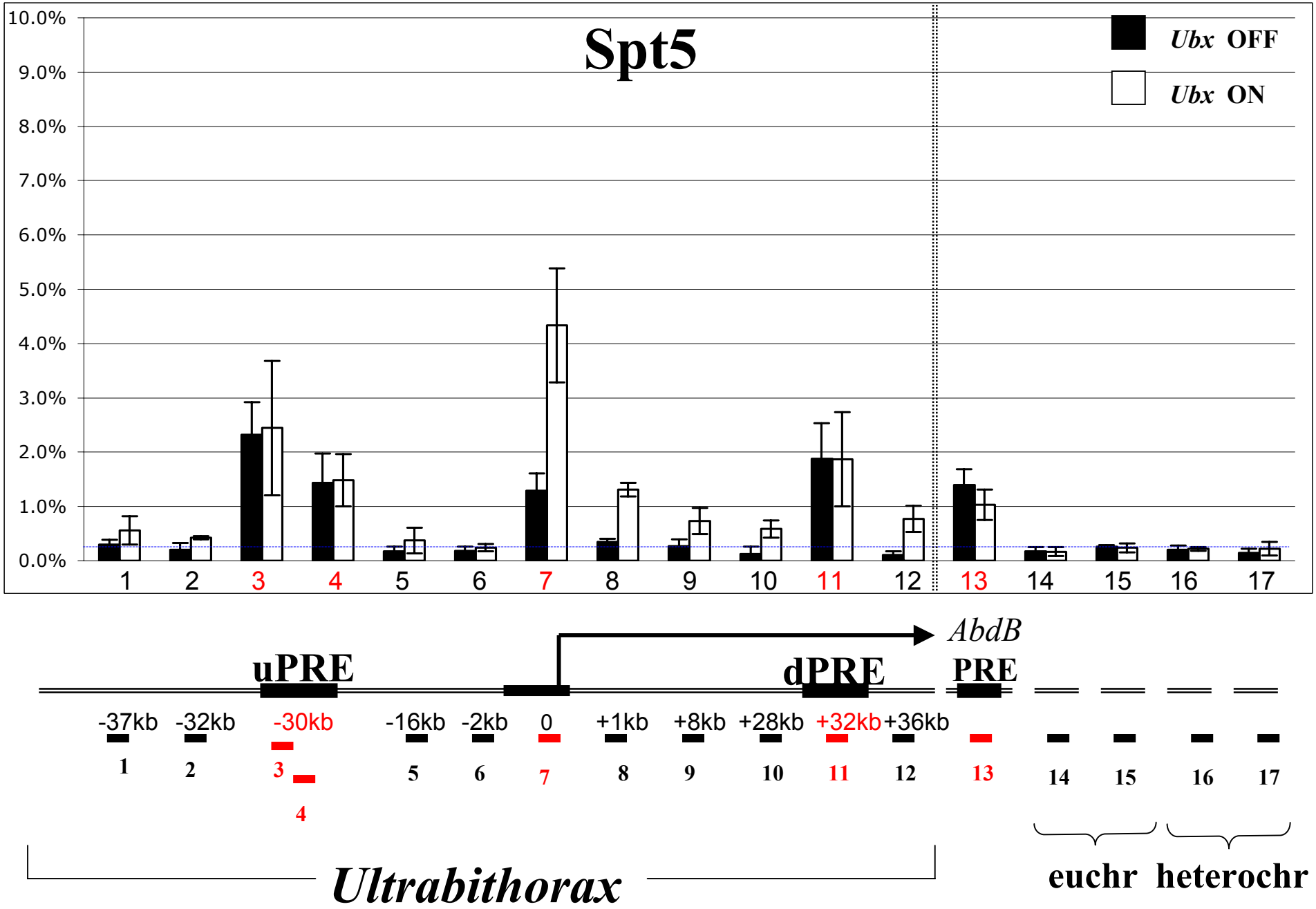
ASH.com

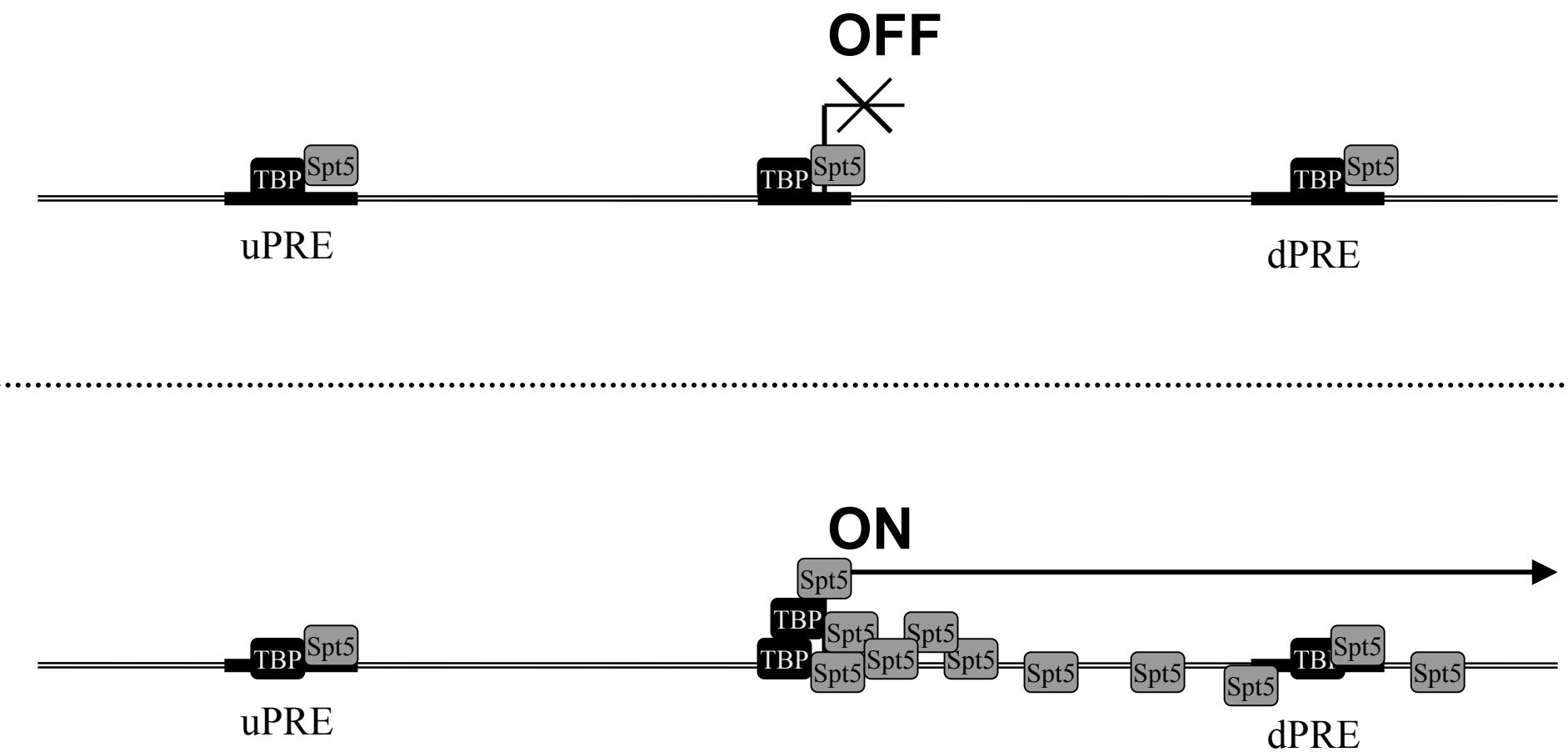
ON state


Trx
Ubx OFF
Ubx ON
5.0%
4.5%
4.0%
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr

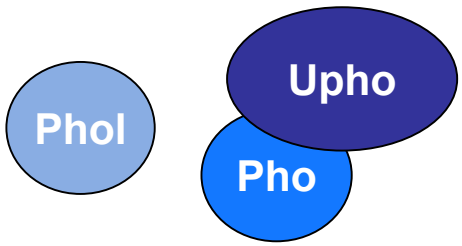

OFF
PRC2
PRC1
PhoRC
Trx
uPRE
Ph
Trx
PRC2
PRC1
Trx
dPRE
ON
PRC2
PRC1
PhoRC
Trx
uPRE
Ph
Trx
Ash1
PRC2
PRC1
PhoRC
Trx
dPRE


TBP
Ubx OFF
Ubx ON
* not measured
10.0%
9.0%
8.0%
7.0%
6.0%
5.0%
4.0%
3.0%
2.0%
1.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr


Spt5
Ubx OFF
Ubx ON
10.0%
9.0%
8.0%
7.0%
6.0%
5.0%
4.0%
3.0%
2.0%
1.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr

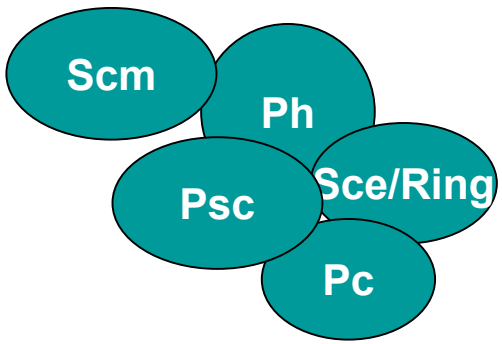

OFF
TBP
Spt5
TBP
Spt5
TBP
Spt5
uPRE
dPRE
ON
Spt5
TBP
Spt5
Spt5
TBP
Spt5
Spt5
Spt5
Spt5
Spt5
Spt5
TBP
Spt5
Spt5
Spt5
uPRE
dPRE

Polycomb group (PcG)



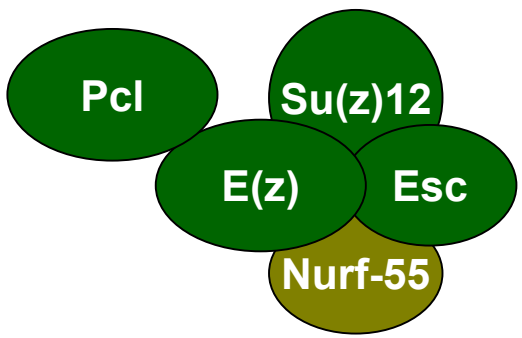
PhoRC

(DNA-binding)



PRC1

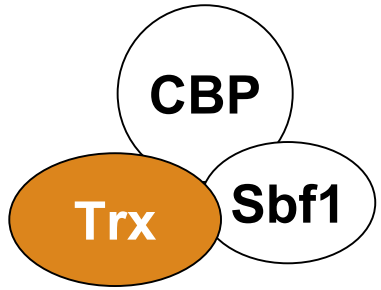
(*Inhibition of Chromatin Remodeling*)



PRC2

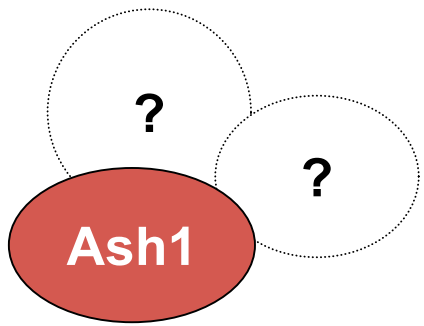
(*HMTase K27 H3*)

trithorax group (trxG)



TAC1

(*HMTase K4 H3*)

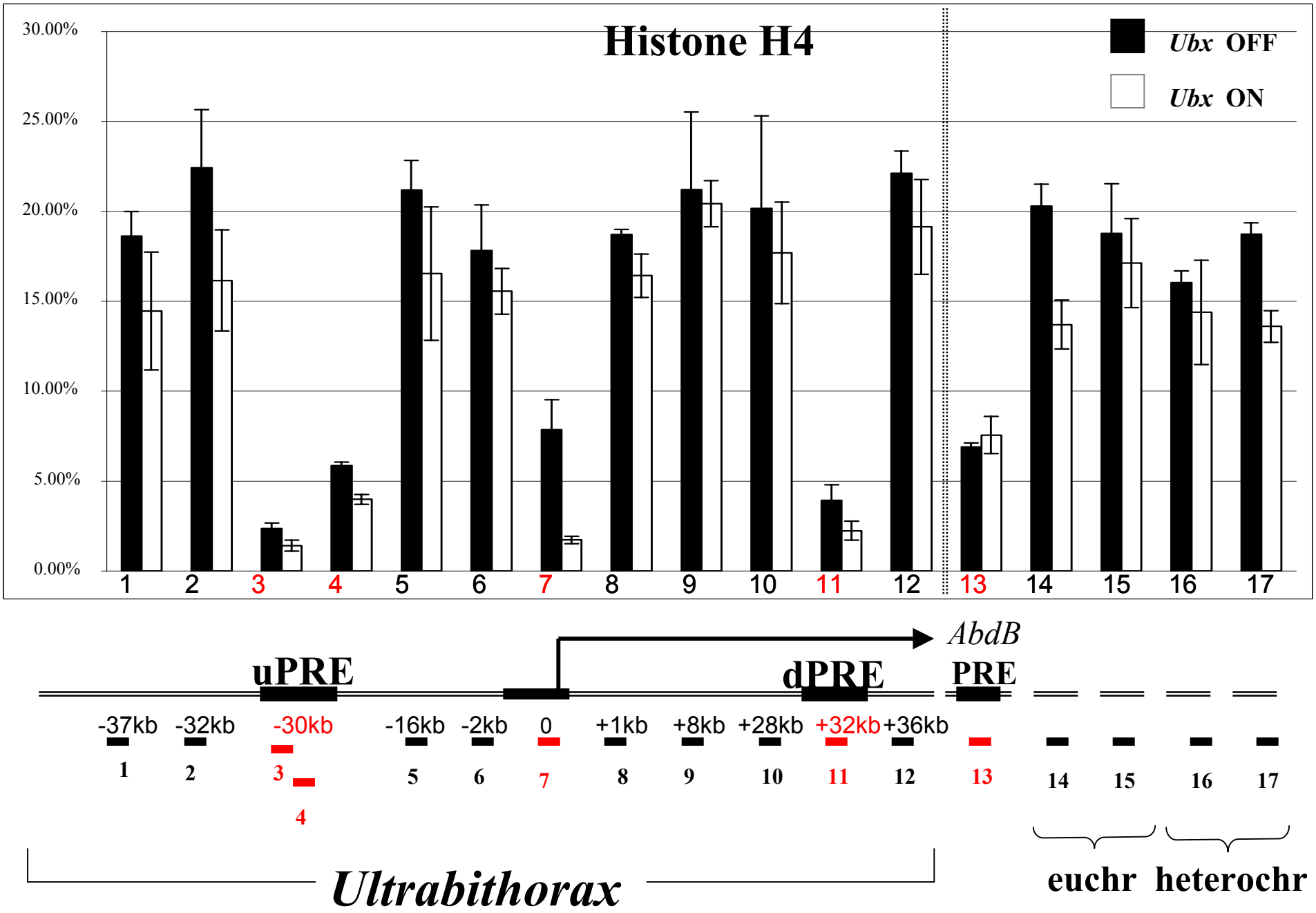


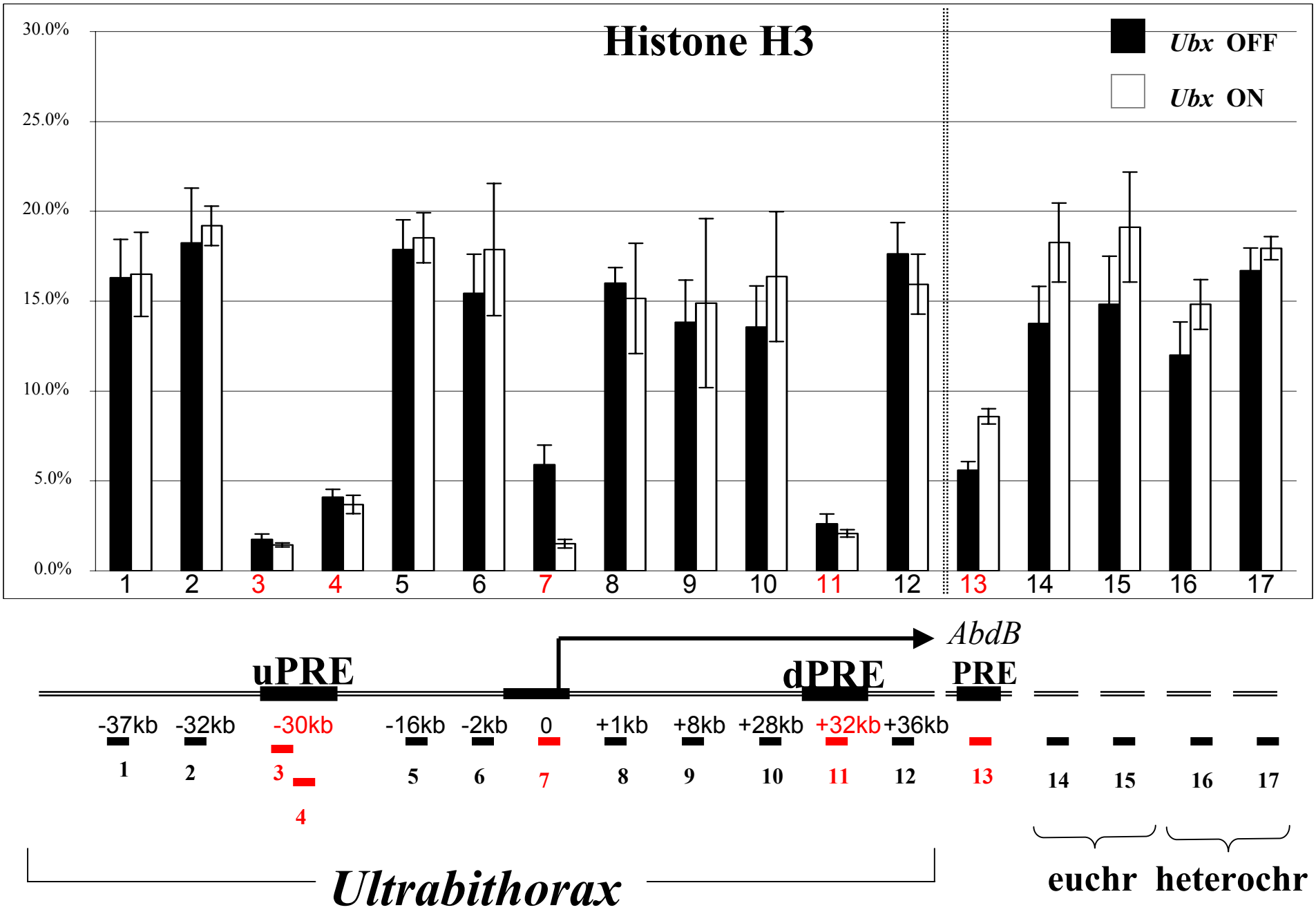
ASH.com

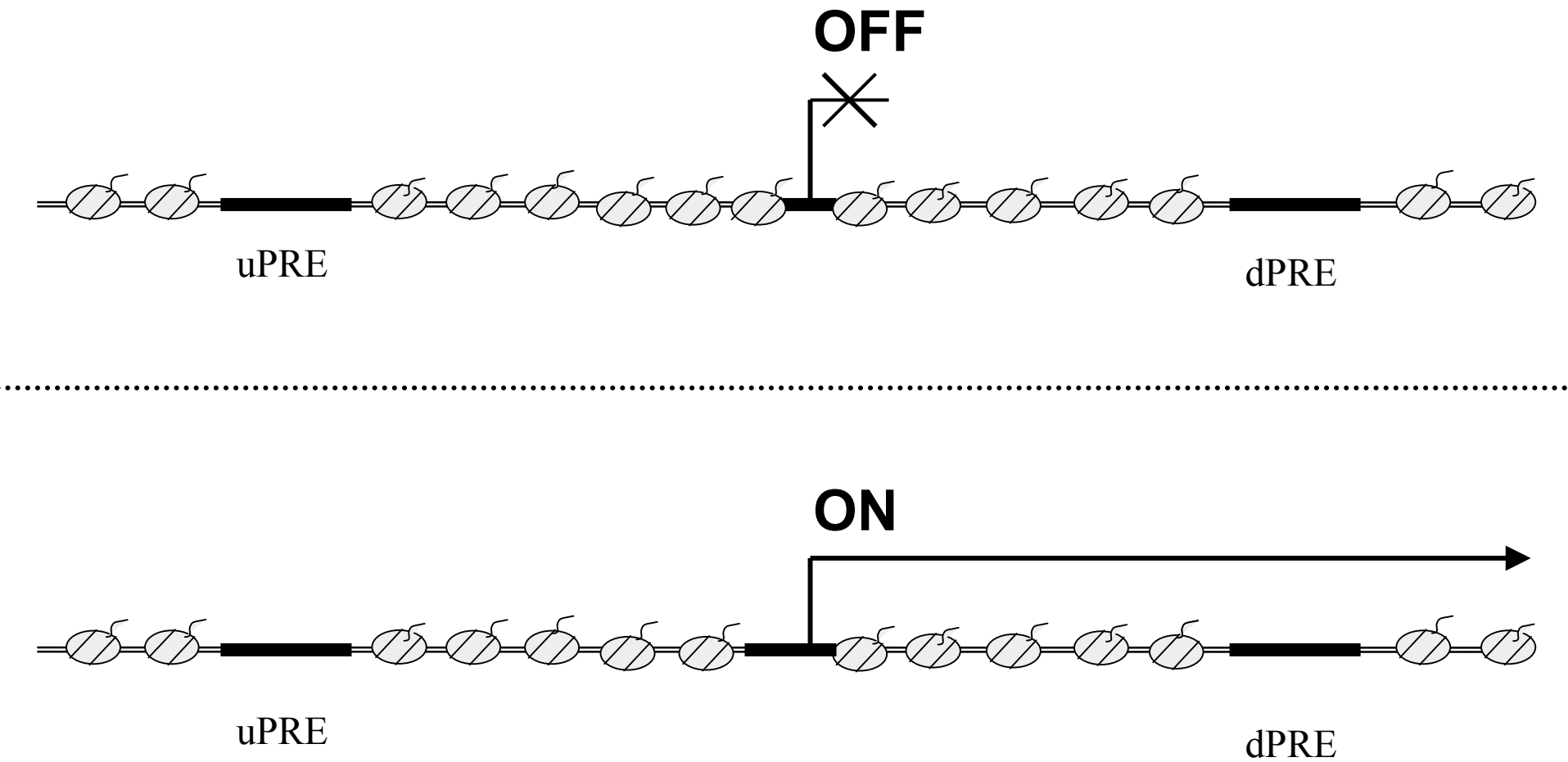
(*HMTase K4/K9 H3 K20 H4*)

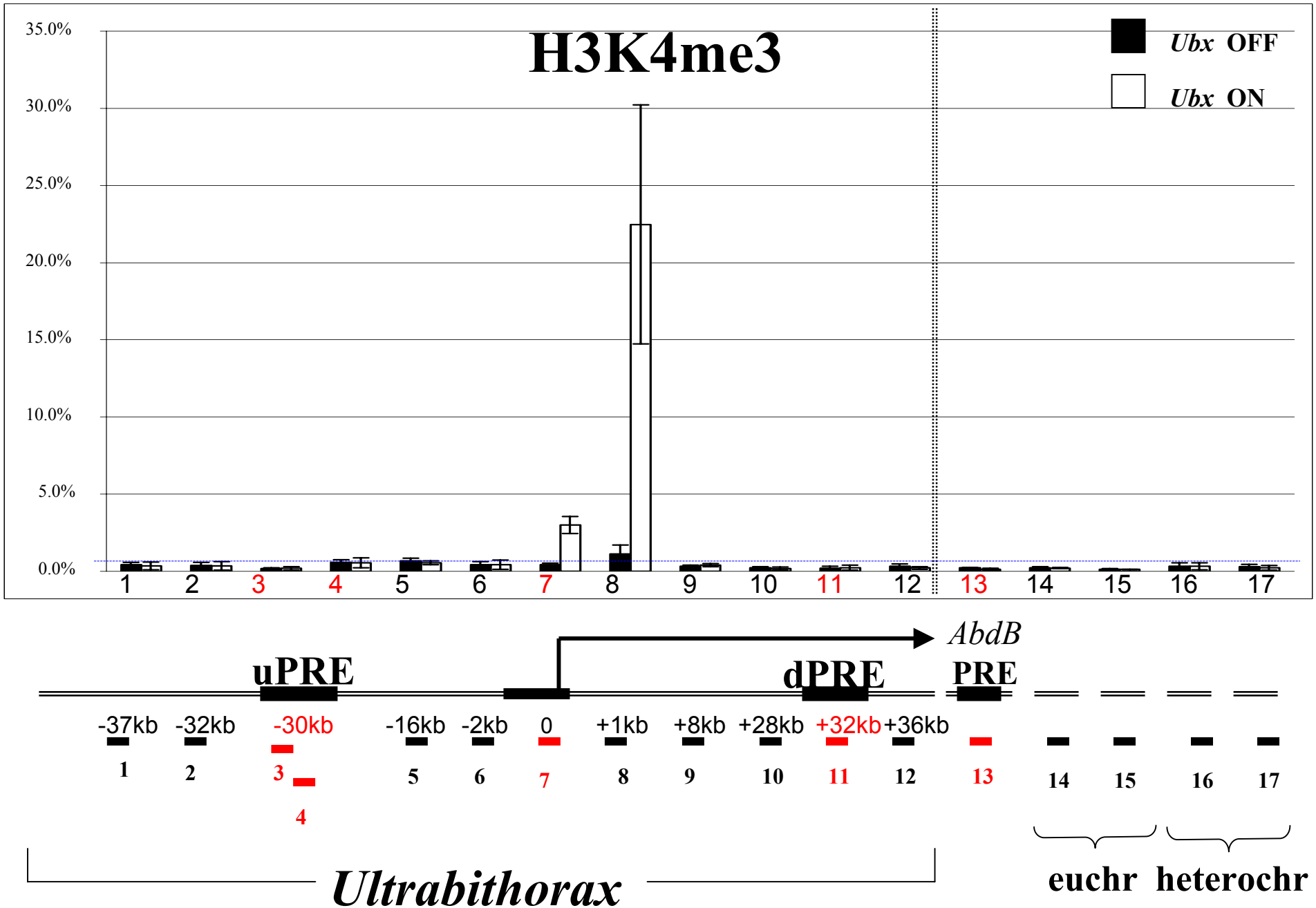
OFF state

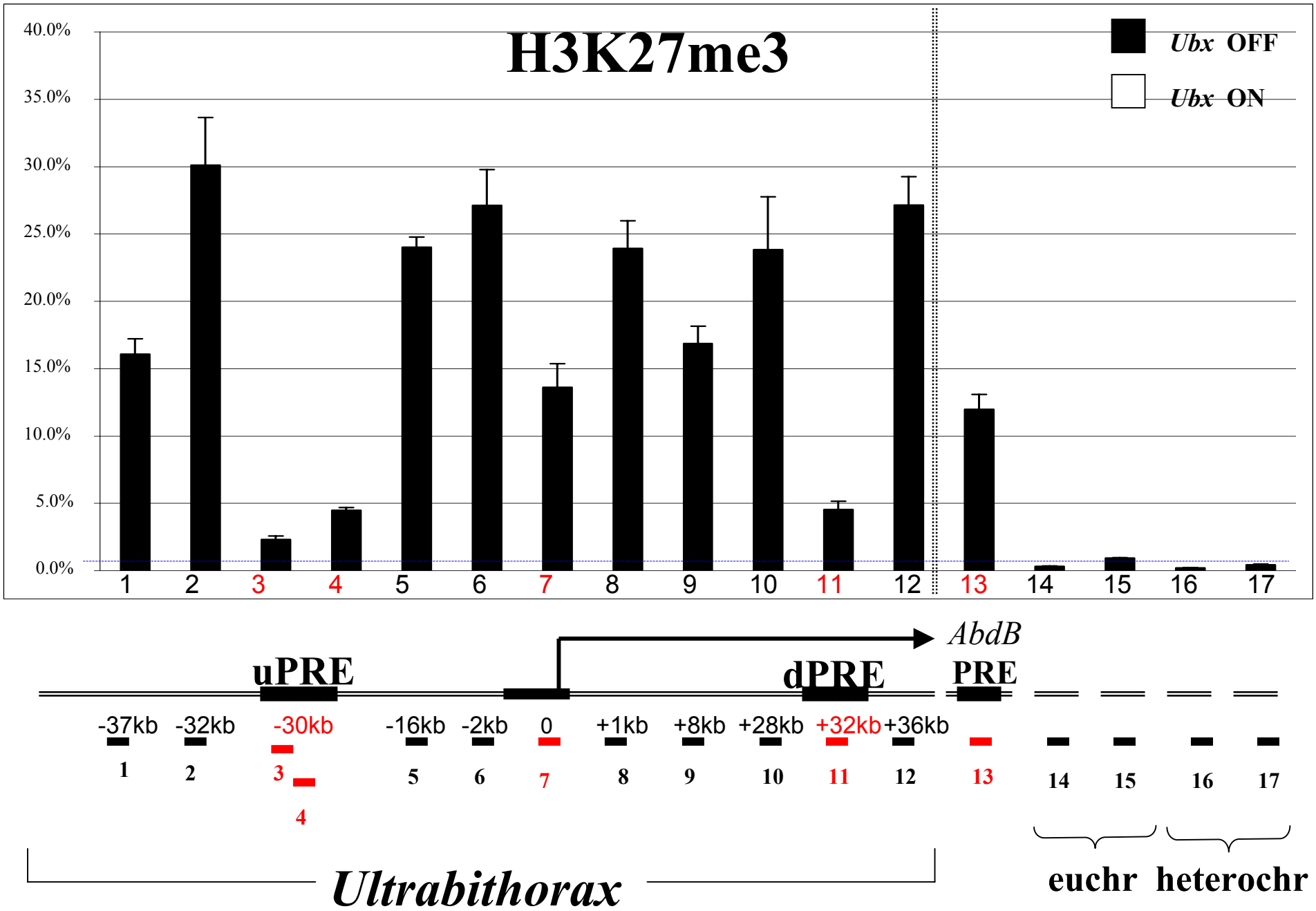
ON state

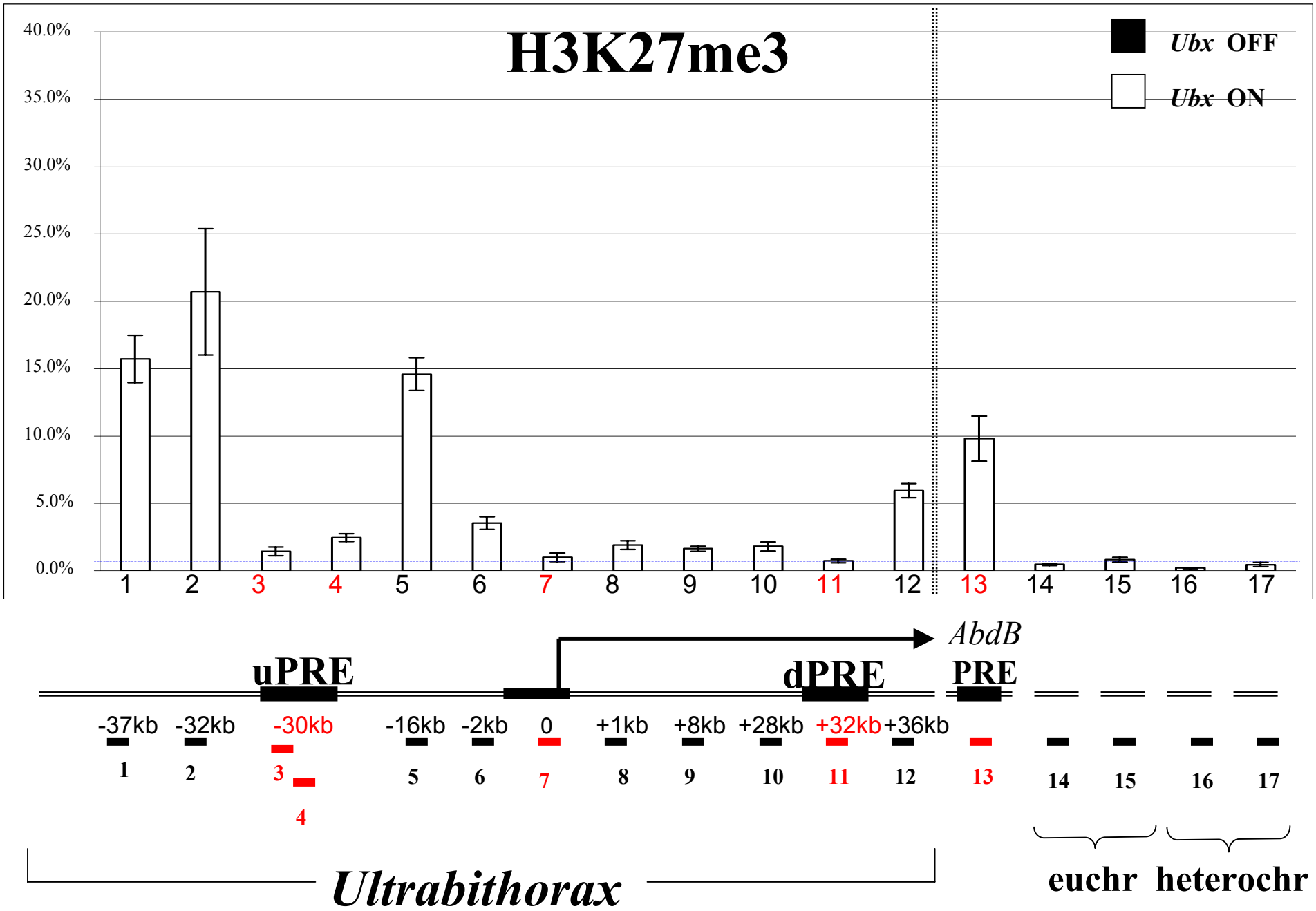

Histone H4
Ubx OFF
Ubx ON
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr

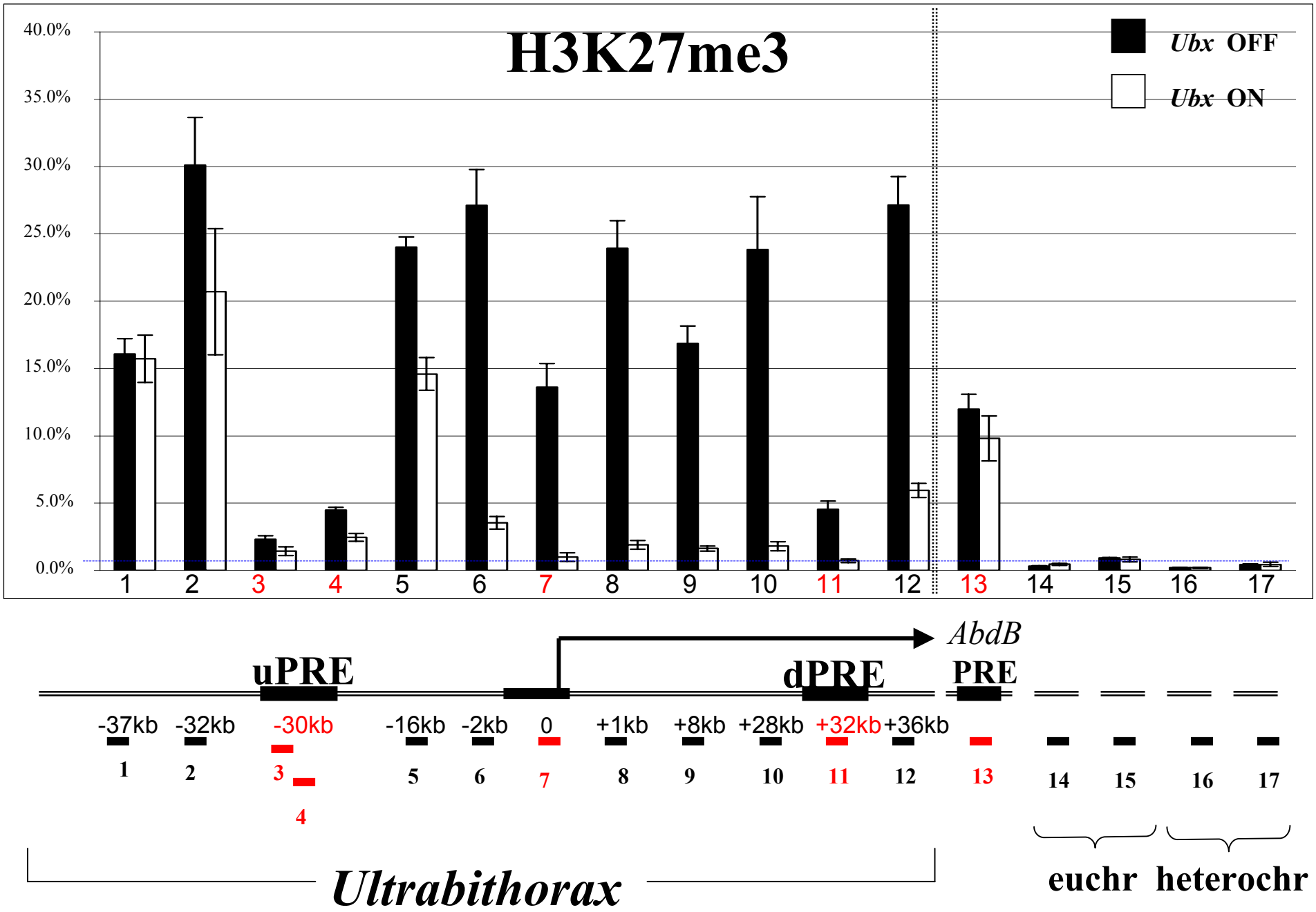

Histone H3
Ubx OFF
Ubx ON
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr

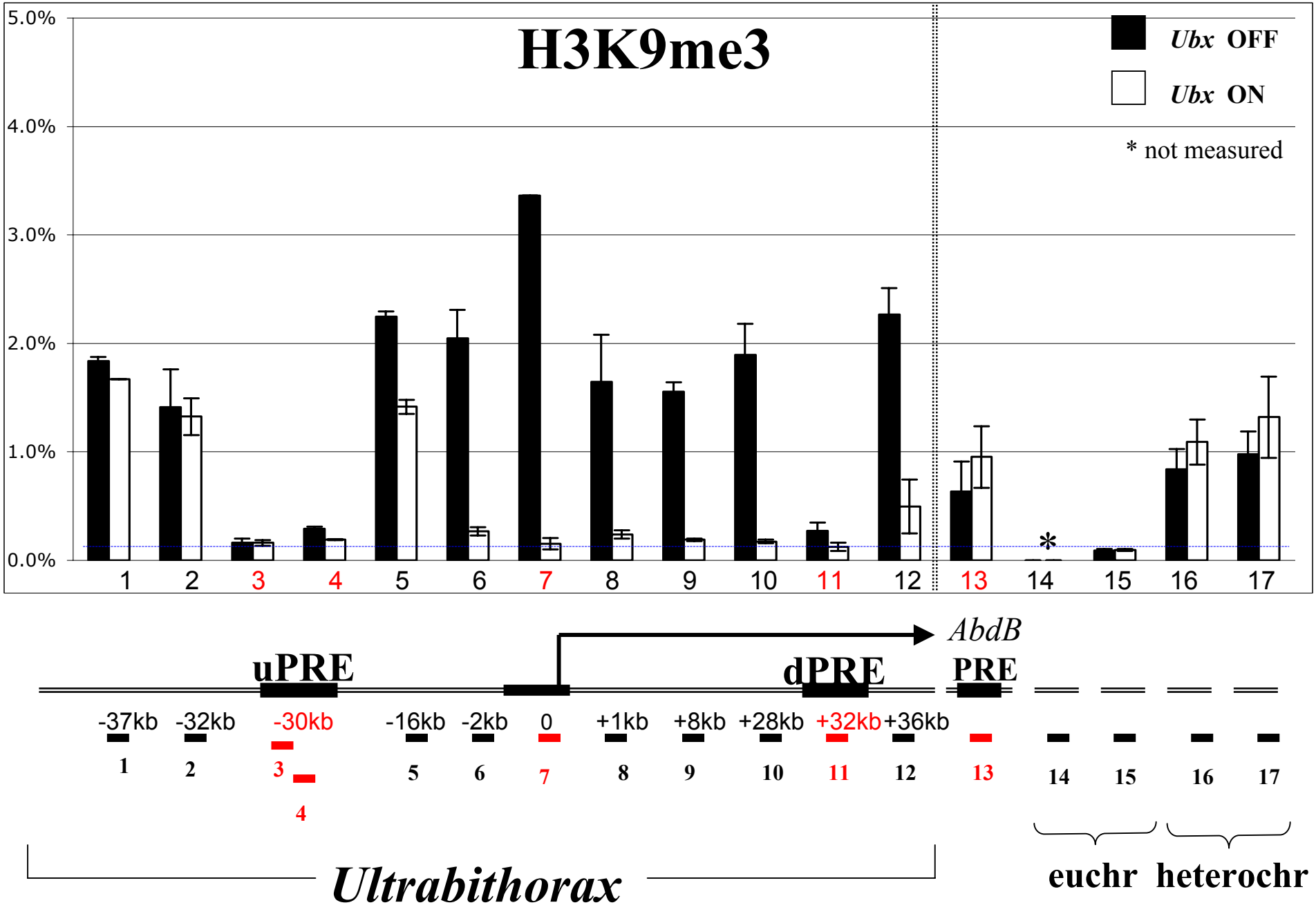

OFF
uPRE
dPRE
ON
uPRE
dPRE

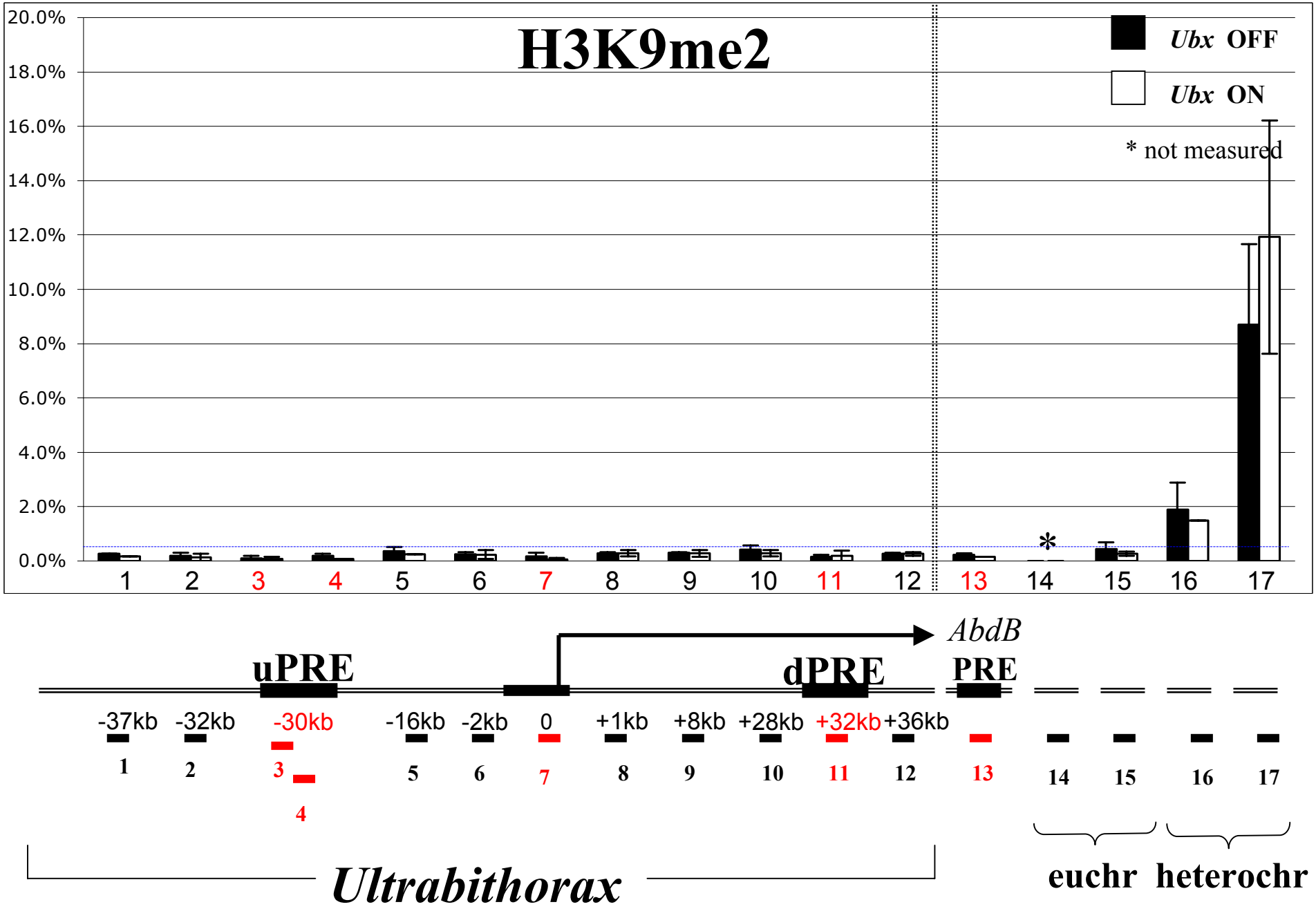

H3K4me3
Ubx OFF
Ubx ON
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr

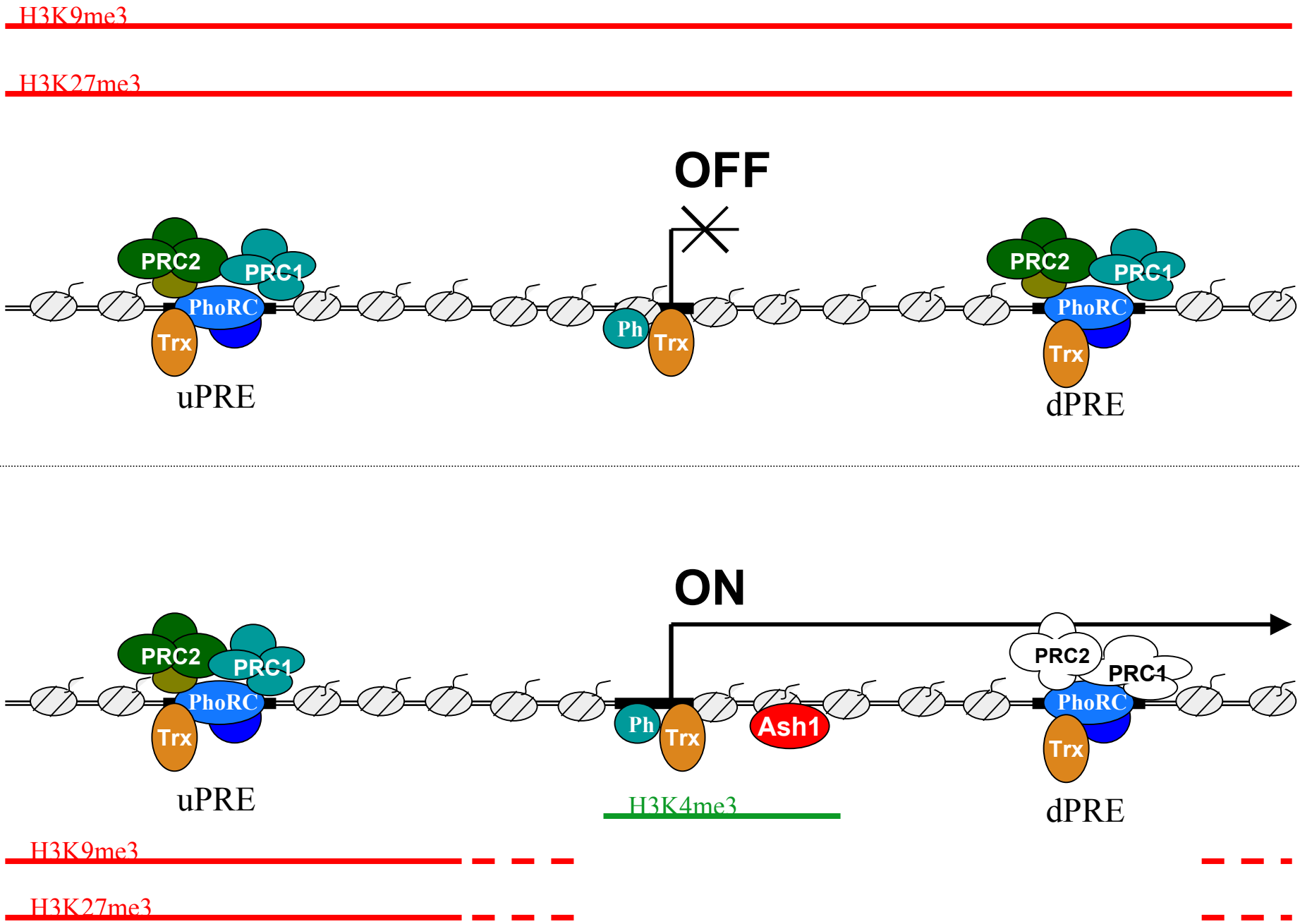

H3K27me3
Ubx OFF
Ubx ON
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr


H3K27me3
Ubx OFF
Ubx ON
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr


H3K27me3
Ubx OFF
Ubx ON
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr


H3K9me3
Ubx OFF
Ubx ON
* not measured
5.0%
4.0%
3.0%
2.0%
1.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr


H3K9me2
Ubx OFF
Ubx ON
* not measured
20.0%
18.0%
16.0%
14.0%
12.0%
10.0%
8.0%
6.0%
4.0%
2.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
AbdB
uPRE
dPRE
PRE
-37kb -32kb -30kb -16kb -2kb 0 +1kb +8kb +28kb +32kb +36kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Ultrabithorax
euchr heterochr


H3K9me3
H3K27me3
OFF
PRC2
PRC1
PhoRC
Trx
Ph
Trx
PRC2
PRC1
PhoRC
Trx
uPRE
dPRE
ON
PRC2
PRC1
PhoRC
Trx
Ph
Trx
Ash1
PRC2
PRC1
PhoRC
Trx
uPRE
H3K4me3
dPRE
H3K9me3
H3K27me3

Acknowledgements

