Insertional mutagenesis in the wheat fungal pathogen *Zymoseptoria tritici* using the fungal *TC1-mariner* transposon *impala*

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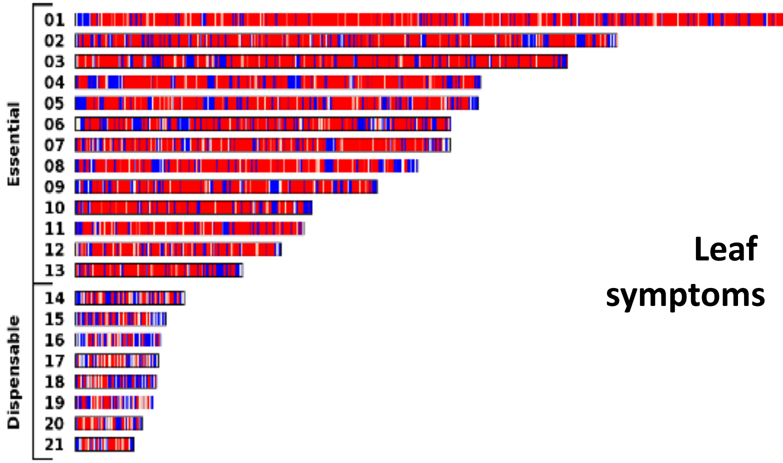
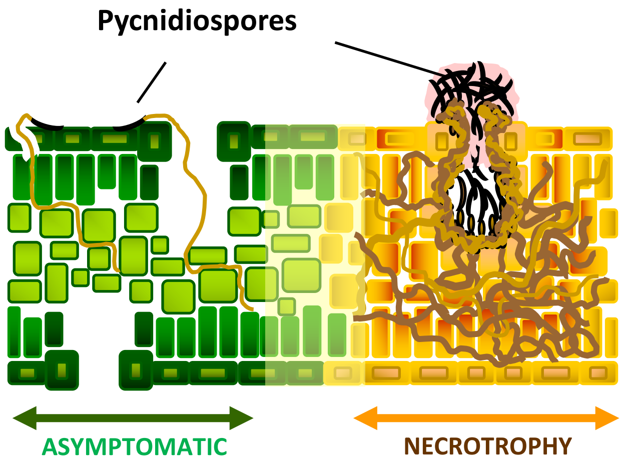
Zymoseptoria tritici, a fungal pathogen of wheat

**Fully sequenced genome 21 chromosomes,
39.7 Mb, 10.952 genes, 18 % transposons**

**Haploid hemi-biotrophic ascomycete
Life cycle: sexual and asexual reproduction**



**Leaf
symptoms**



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**Genetics and genomics tools
Transformation, reverse genetics using Ku70 deficient strains, cellular biology
Large infection RNAseq datasets,
GWAS, populations genomics, experimental evolution**

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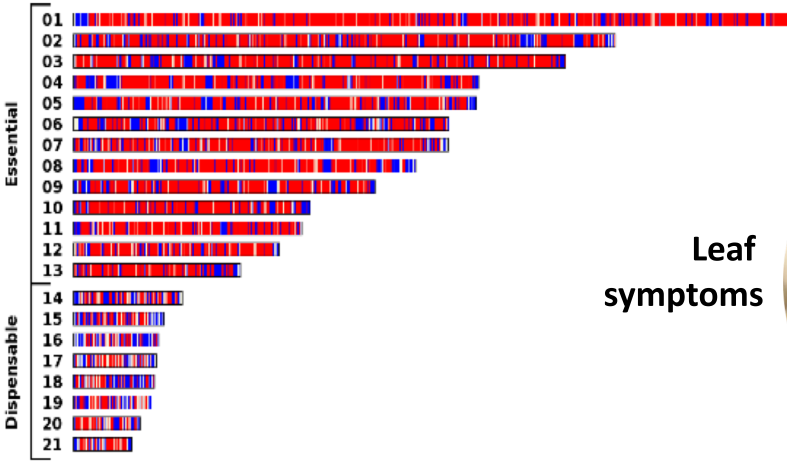

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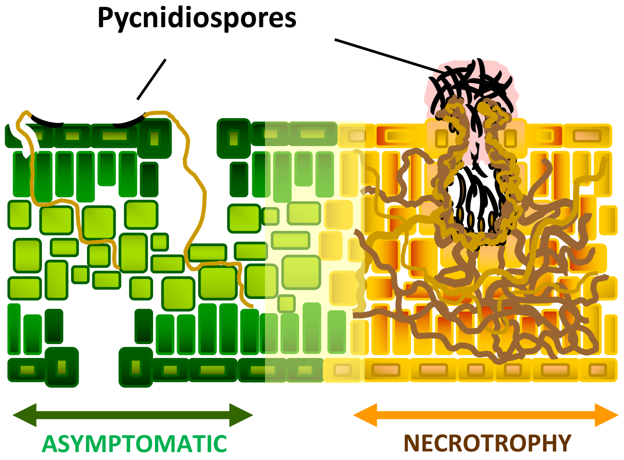


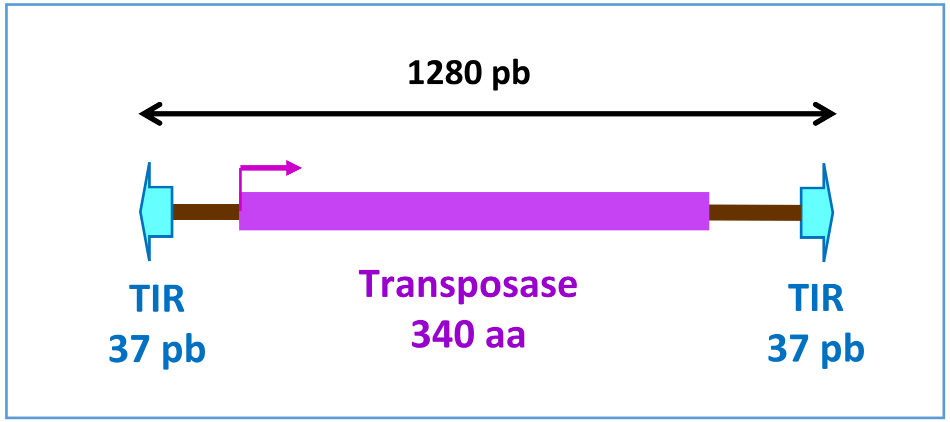
leaf

Genetics and genomics tools
Transformation, reverse genetics using Ku70 deficient strains, cellular biology
Large infection RNAseq datasets,
GWAS, populations genomics, experimental evolution

But no transposon-based insertional mutagenesis

Impala, a tool for insertional mutagenesis in fungi

- *impala,* a fungal *TC1-Mariner* DNA transposon from *Fusarium oxysporum*
- *impala* excise and re-insert by a cut and paste mechanism
- re-insertion in host genome as a single copy at a dinucleotide TA site



TIR: Terminal Inverted Repeats

- Successfully used for insertional mutagenesis in several fungi :
Aspergillus nidulans, Aspergillus fumigatus, Fusarium moniliforme, Fusarium graminearum, Magnaporthe oryzae, Penicillium griseoroseum (all mutants tagged)

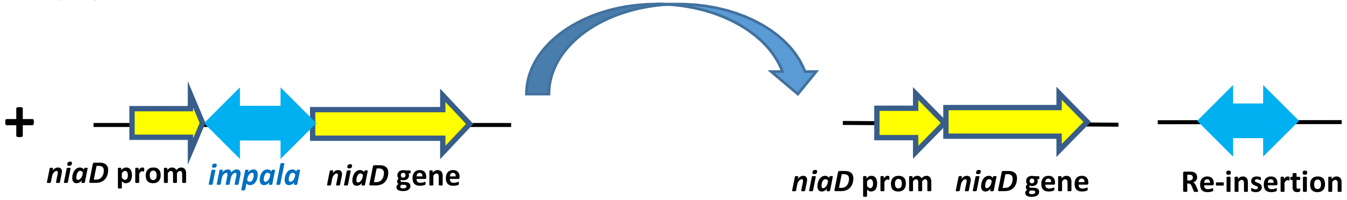
Dufresne and Daboussi (2010) Methods Mol. Biol. 638:41-54.

impala transposition : monitoring excision

niaD::impala vector
Introduced in a
Z. tritici Nia- mutant

Growth
on nitrate
glucose
medium

Nia -



impala excision

impala revertants

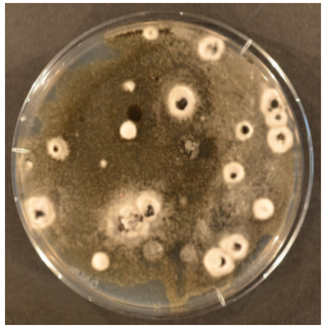
niaD prom *niaD* gene

niaD restored

Re-insertion
of *impala*
in host
genome

Growth
on nitrate
glucose
medium

Nia +



Nia+ revertants on nitrate glucose medium
(2 months)

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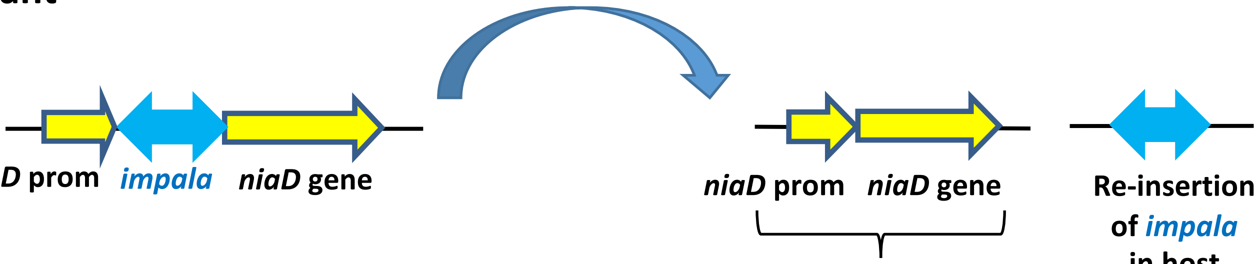


impala **excision**

impala **revertants**

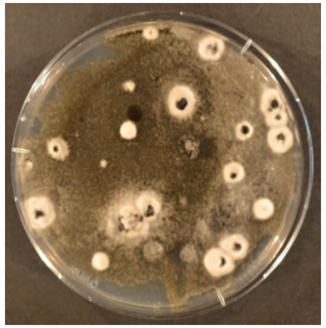
niaD **restored**

Re-insertion
of *impala*
in host
genome

90%

Growth
on nitrate
glucose
medium

Nia +



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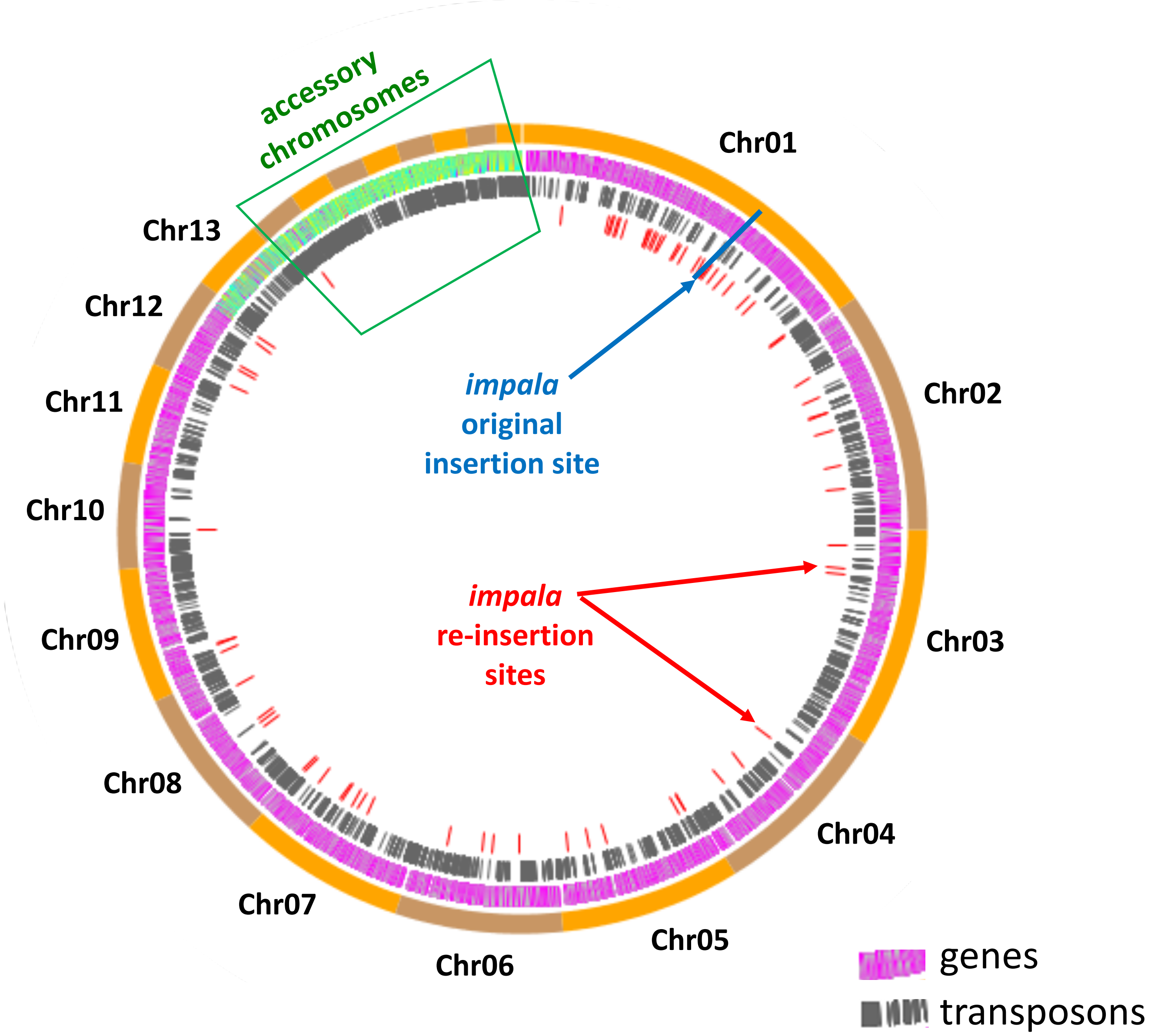
impala transposition : insertion pattern ?

- Insertion of *impala*
  in all core chromosomes
  (Chr01-12)

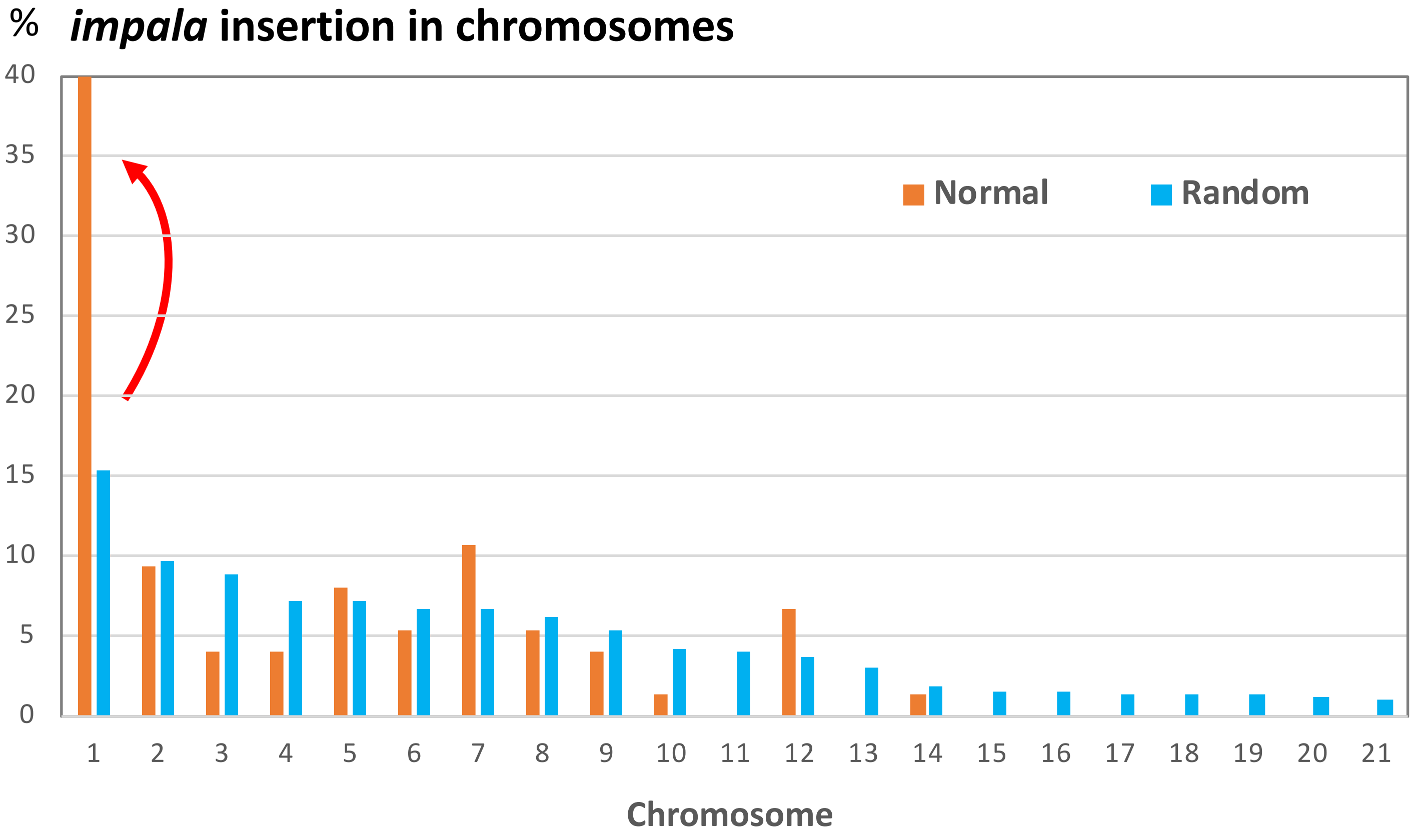
- Only one insertion
  in accessory chromosomes
  1 / 9 expected according to size

accessory chromosomes:
enriched in hetero-chromatin marks
(H3K27me3; H3K9me3)
and transposons,
few expressed genes



74 insertions rescued by LM-PCR

impala transposition : insertion pattern ?



Positive bias toward chromosome 1 2.5 x random

impala:niaD **vector in chromsome 1**

local jump?

impala insertion : coding vs non-coding

	number	%
Promoter	43	58
5'UTR	10	14
Exons	5	7
Introns	2	3
3'UTR	2	3
TEs	1	1
Other	11	15
Total	74	

Preference for genes : 84 % of insertions
(genes = 40 % of the genome)

Low insertion frequency in native transposons
(TEs = 18 % of the genome)

impala insertion in genes : where ?

impala insertion in genes

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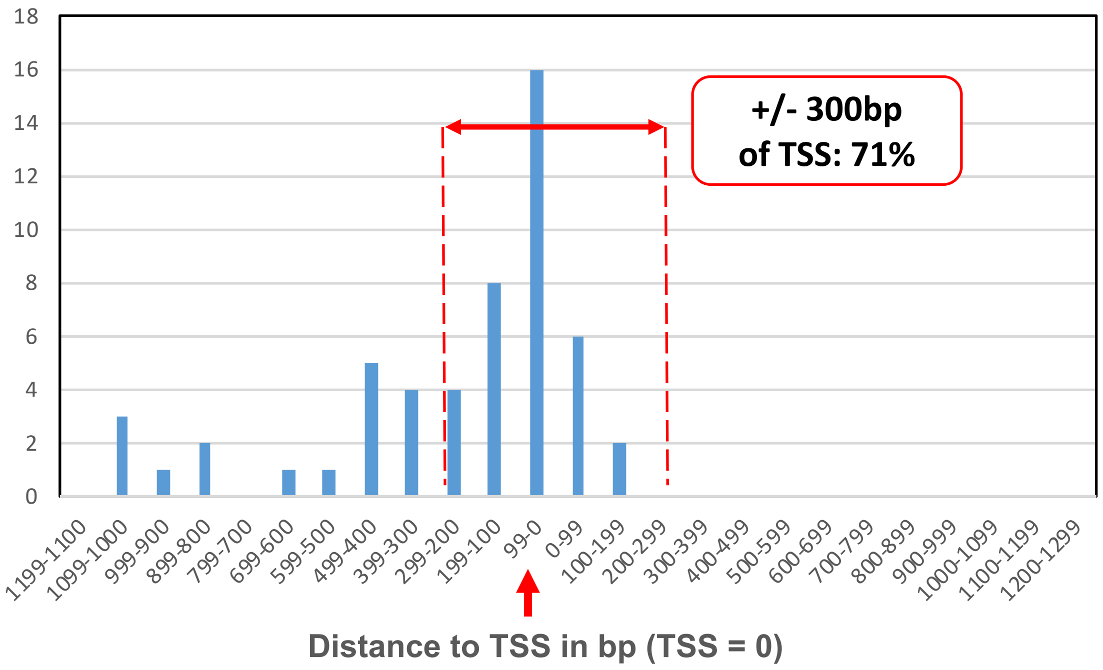
genes
84 %

5' gene : 72 %

impala insertions in promoters and 5'UTRs



+/- 300bp
of TSS: 71%

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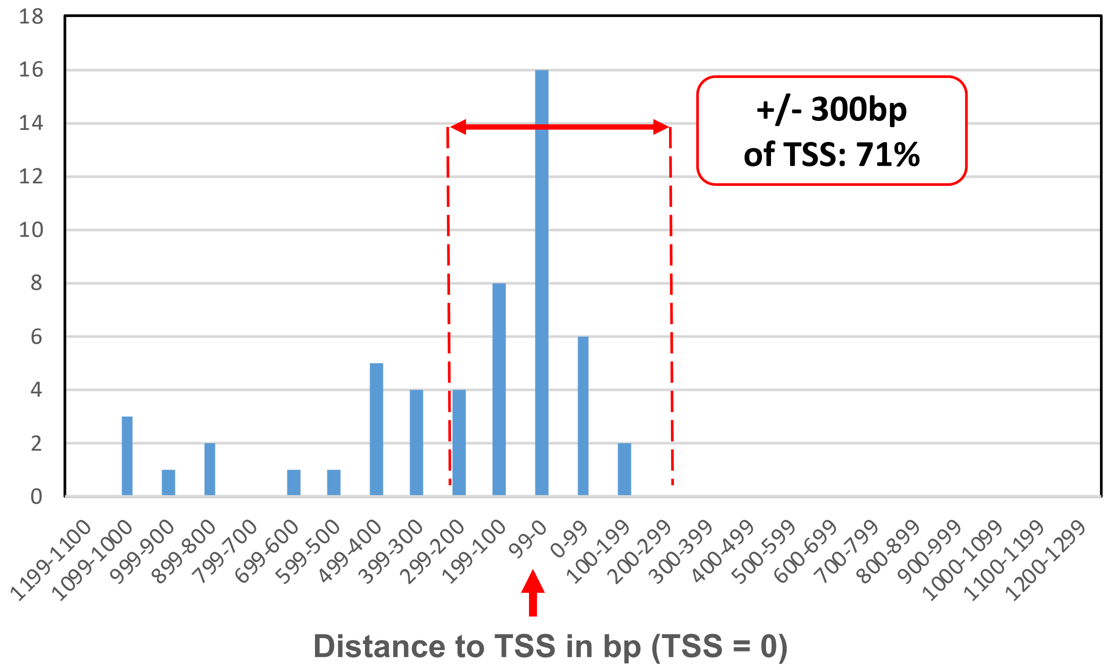


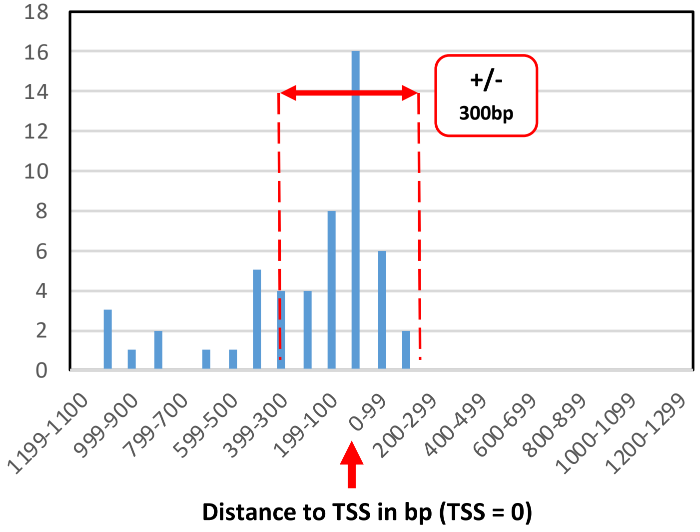
+/- 300bp
of TSS: 71%

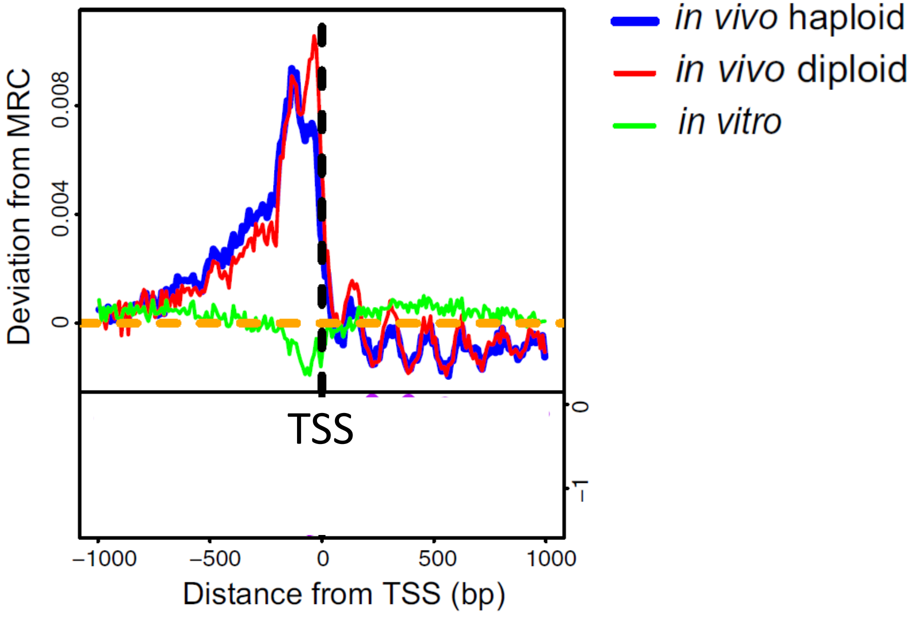
50% of all insertions are near TSS (transcription start site)

impala transposition : preference for TSS ?

Is such pattern observed for heterologous transposition in other DNA transposons ?
(here *Hermes* from insect, hAT family related to TC1-mariner)

***impala* insertions in promoters and 5'UTRs**





Hermes* heterologous transposition in the yeast *S. cerevisae

Gangadharan et al. 2010 PNAS 107(51), 21966–21972

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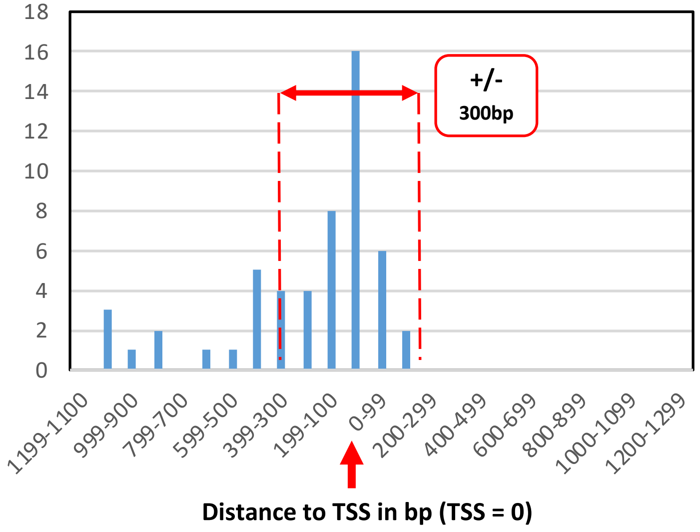

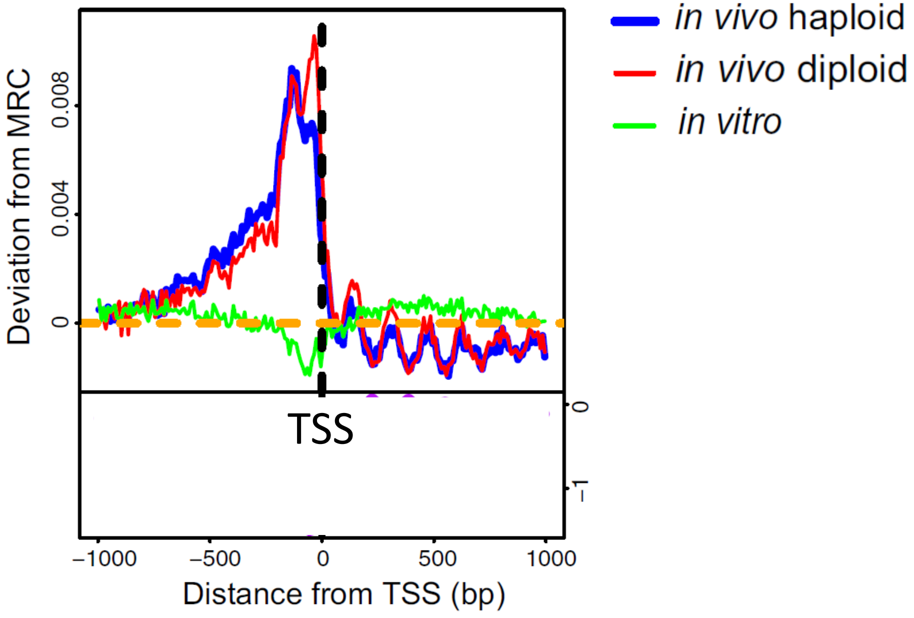

Hermes **heterologous transposition in the yeast** ***S. cerevisae***

Yes,
Hermes **has also an insertion preference for TSS**

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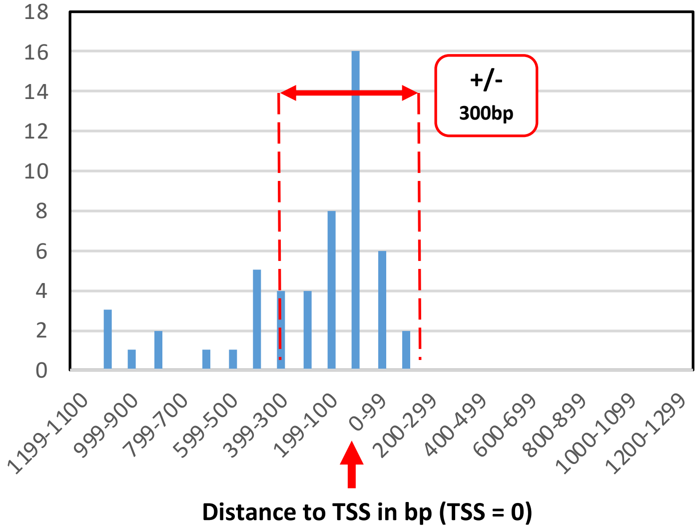

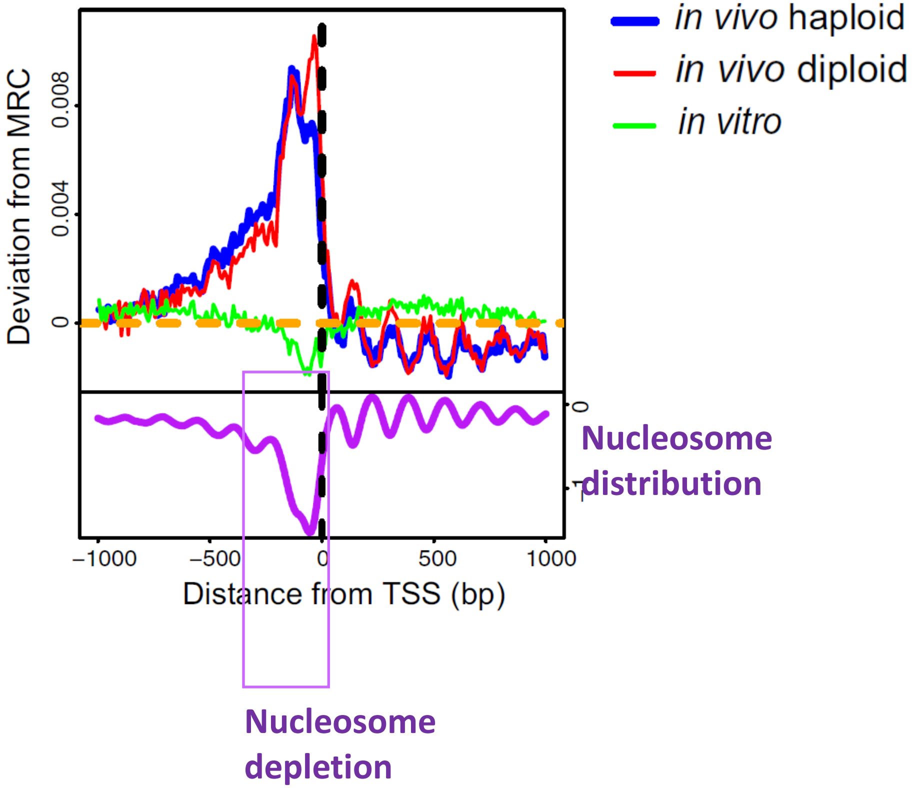

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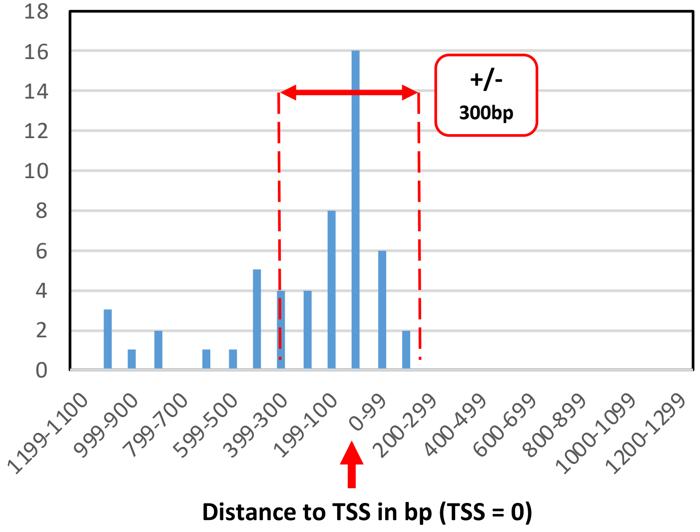
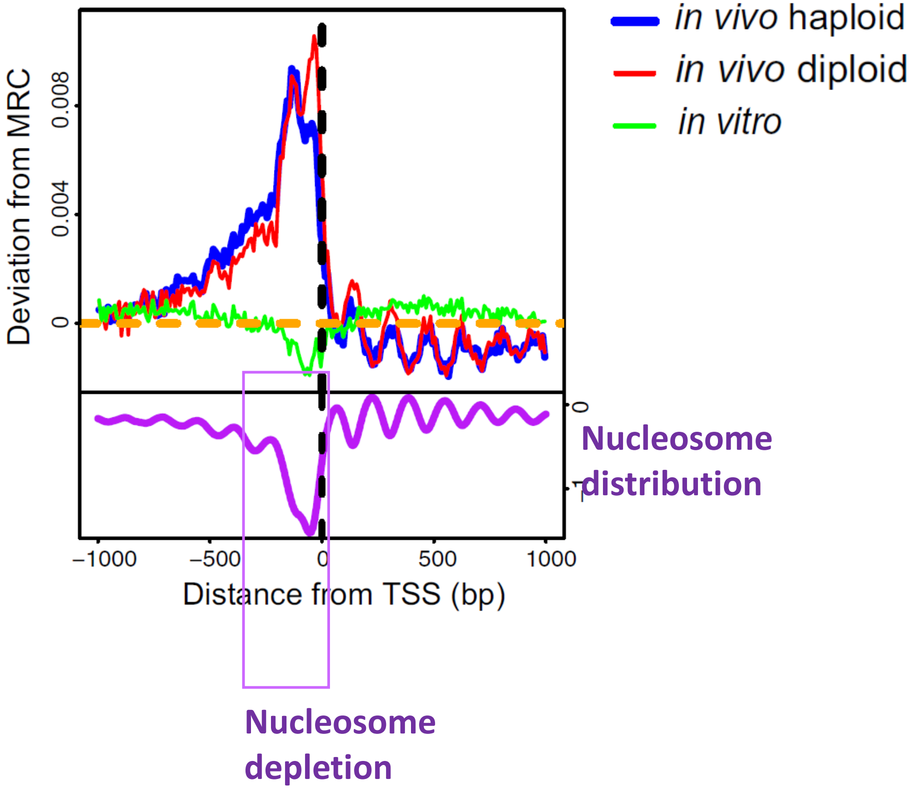




Hermes
inserts mostly
in the region before TSS
depleted in nucleosomes
Open chromatin ?

Similar mechanisms
for *impala* ?

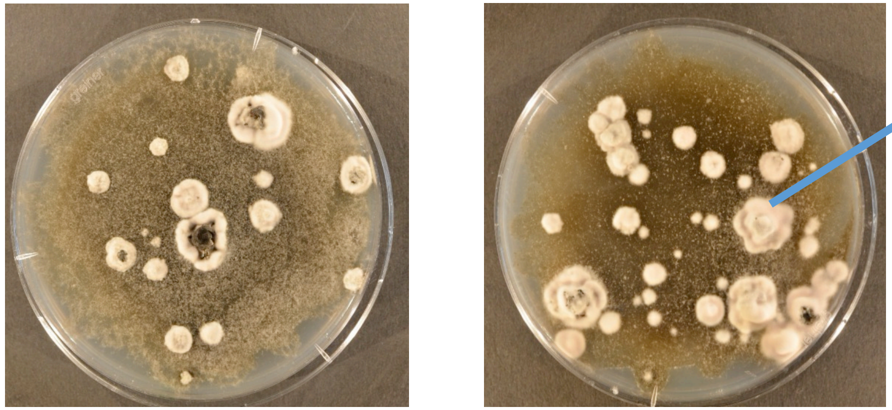
Gangadharan et al. 2010 PNAS 107(51), 21966–21972

Chromatin landscape and *impala* transposition ?

Modification of chromatin landscape using trichostatin-A (TSA, histone deacetylase inhibitor)

Hypothesis: histone deacetylase inhibition = increase in histone H3 acetylation = chromatin opening

Nia+ revertants at 60 dpi



Nitrate medium

Nitrate medium
+ **trichostatin A**
0,3 µM
(sub-lethal dose)

TSA Nia+ revertants
Same *impala* excision and insertion rates as control

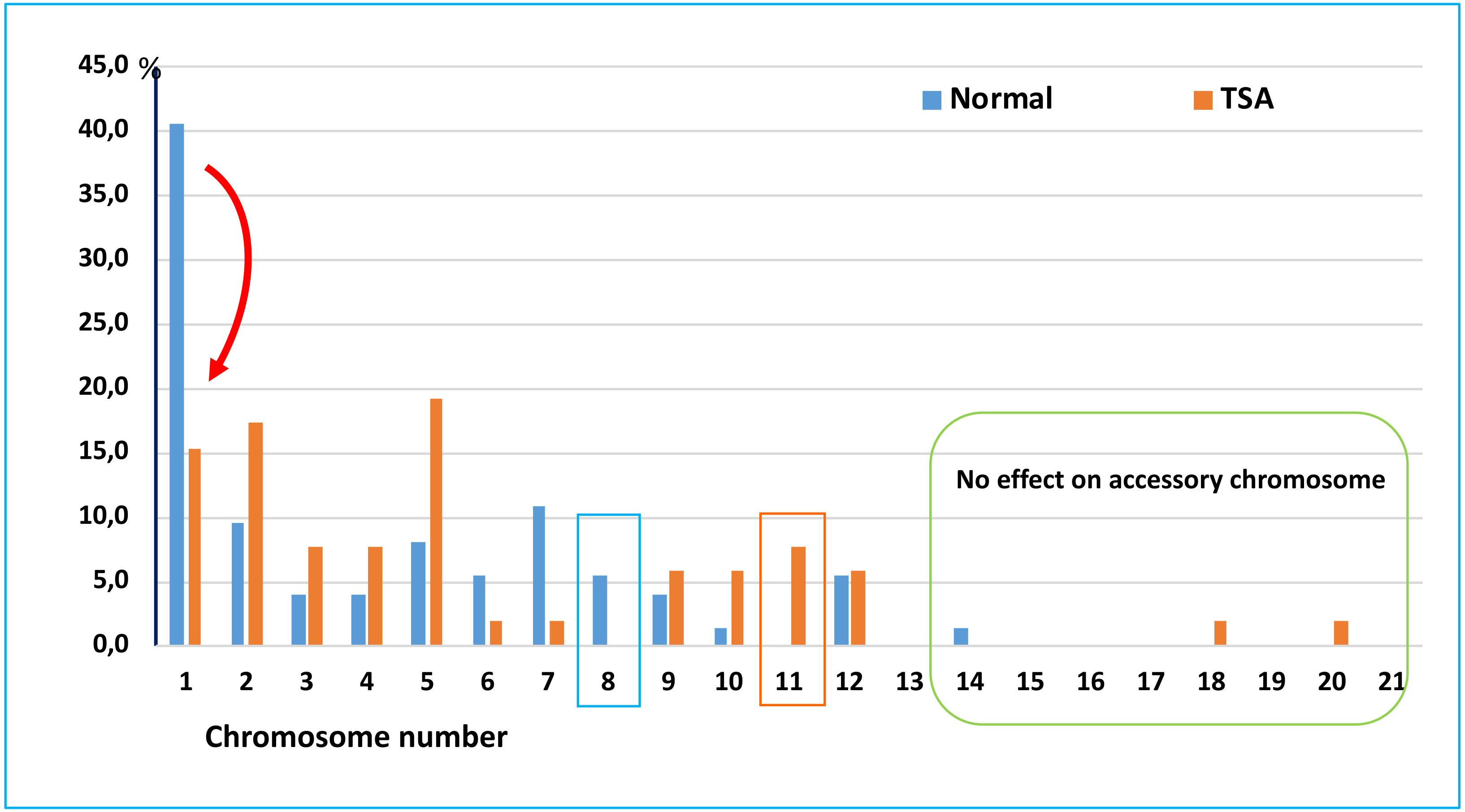
impala transposition : chromatin effect ?

Modification of chromatin during *impala* transposition by inhibiting histone deacetylase (TSA)

Effect of TSA on *impala* re-insertion

Chromosomal pattern

reduced bias toward chromosome 1



No effect on accessory chromosome

impala transposition : chromatin effect ?

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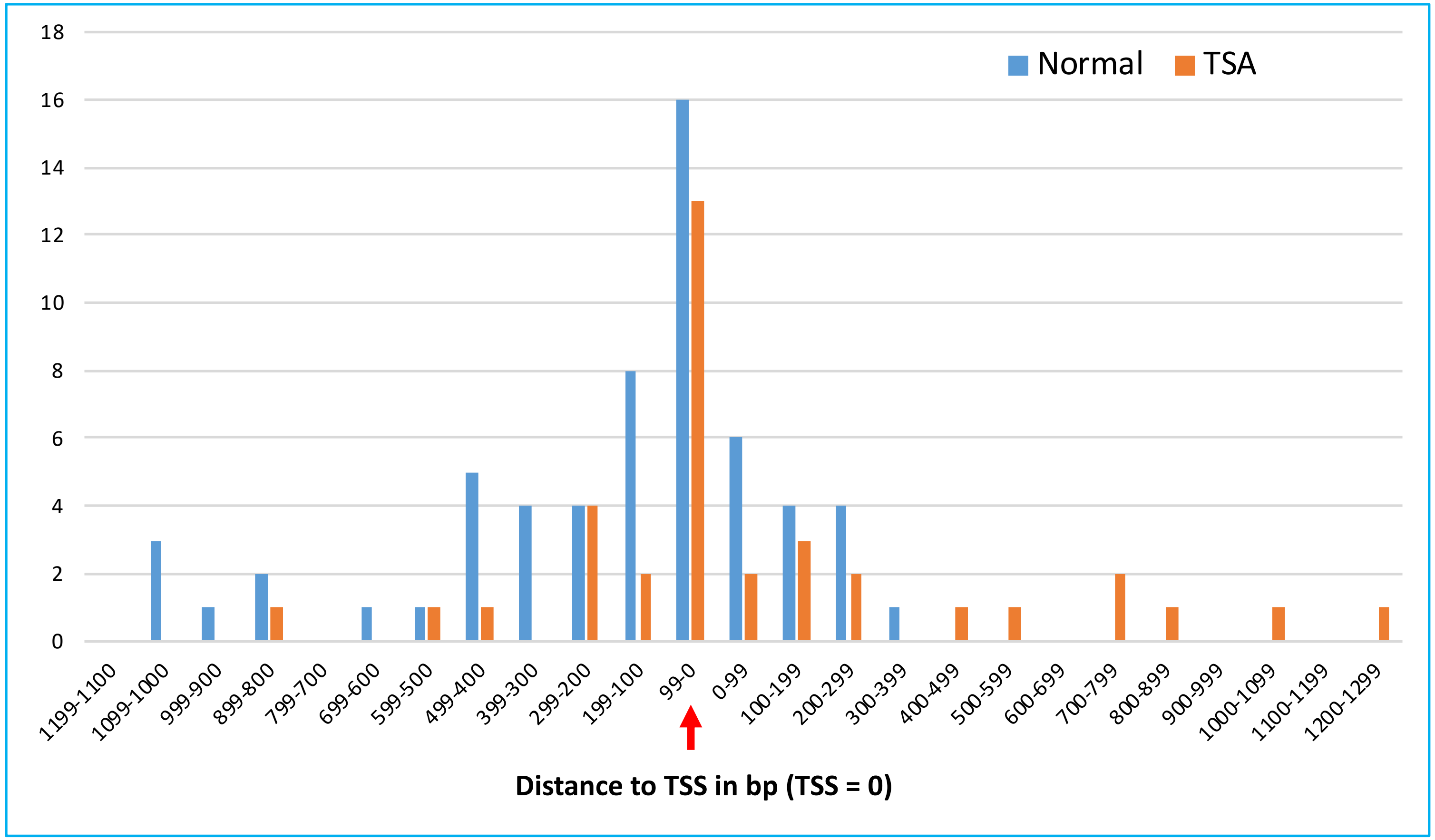
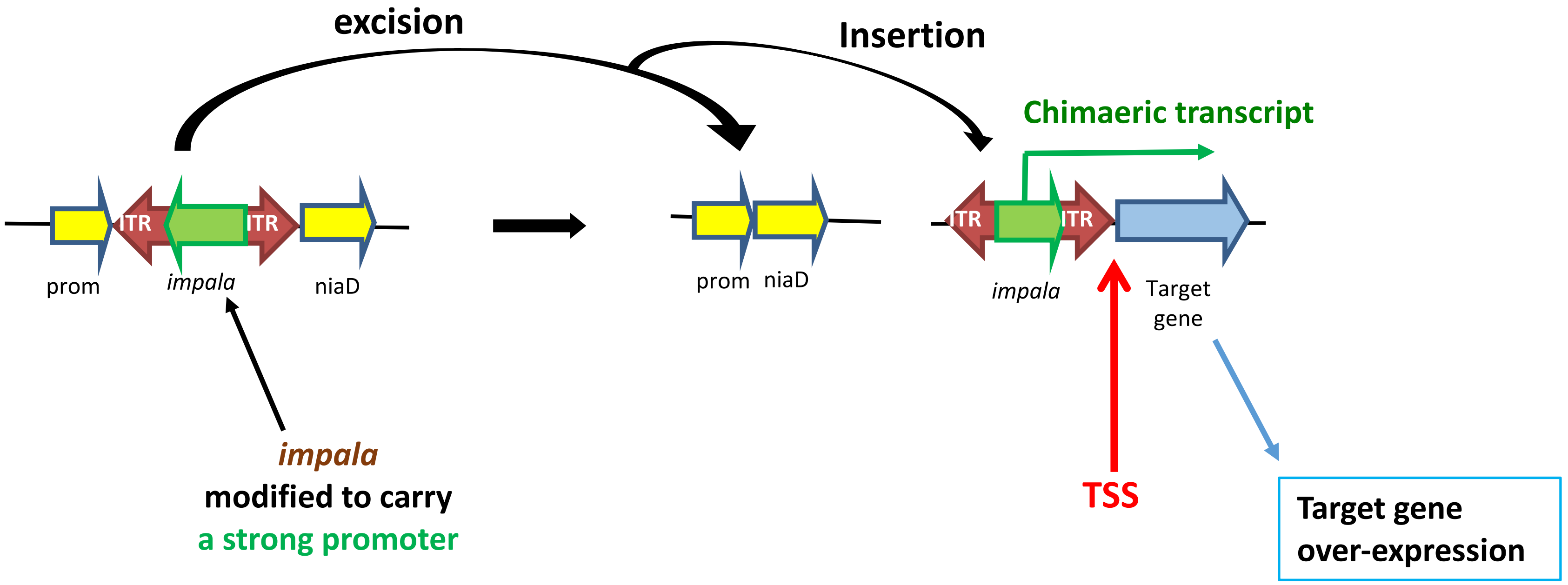
Gene level pattern

Reduced bias toward TSS



impala insertion near TSS: a tool for activation tagging



impala
modified to carry
a strong promoter

Target gene over-expression

impala insertion near TSS: a tool for activation tagging

Transposition of a chimaeric *impala:pGpd*

rev	TRF	Site	TA	Target gene	insertion in	*pGpd* vs gene transcript	insertion effect	dist TSS	gene fonction
1157	316	1:1978814	+	MSTRG.733.1	5'UTR	opposite	inactivation	- 54 bp	unknown
1193	326	3:3207994	+	Mycgr3T103943	5'UTR	opposite	inactivation	- 133 bp	unknown
1200	326	6:2369989	+	Mycgr3T44402	first exon	same	activation	- 456 b	TFIID su12
1043	300	5:1192048	+	Mycgr3G93193	5'UTR	opposite	inactivation	- 79 bp	unknown
1049	301	12:897652	+	Mycgr3T77474	promoter	same	activation	+ 40 bp	AA transport

Preference for TSS maintained (80 %)

impala transposition in *Zymoseptoria tritici*

impala is active in _Z. tritici_

efficient nitrate reductase-based excision assay
high rate of _impala_ excision and insertion

impala insertion pattern

mostly in core chromosomes, no hot spots (random ?)

insertion in genes (84 %) near TSS (50%)
= preference for genes expressed during reversion assay ?

impala insertion near TSS
= preference for nucleosome depleted region ?

Chromatin landscape and _impala_ transposition

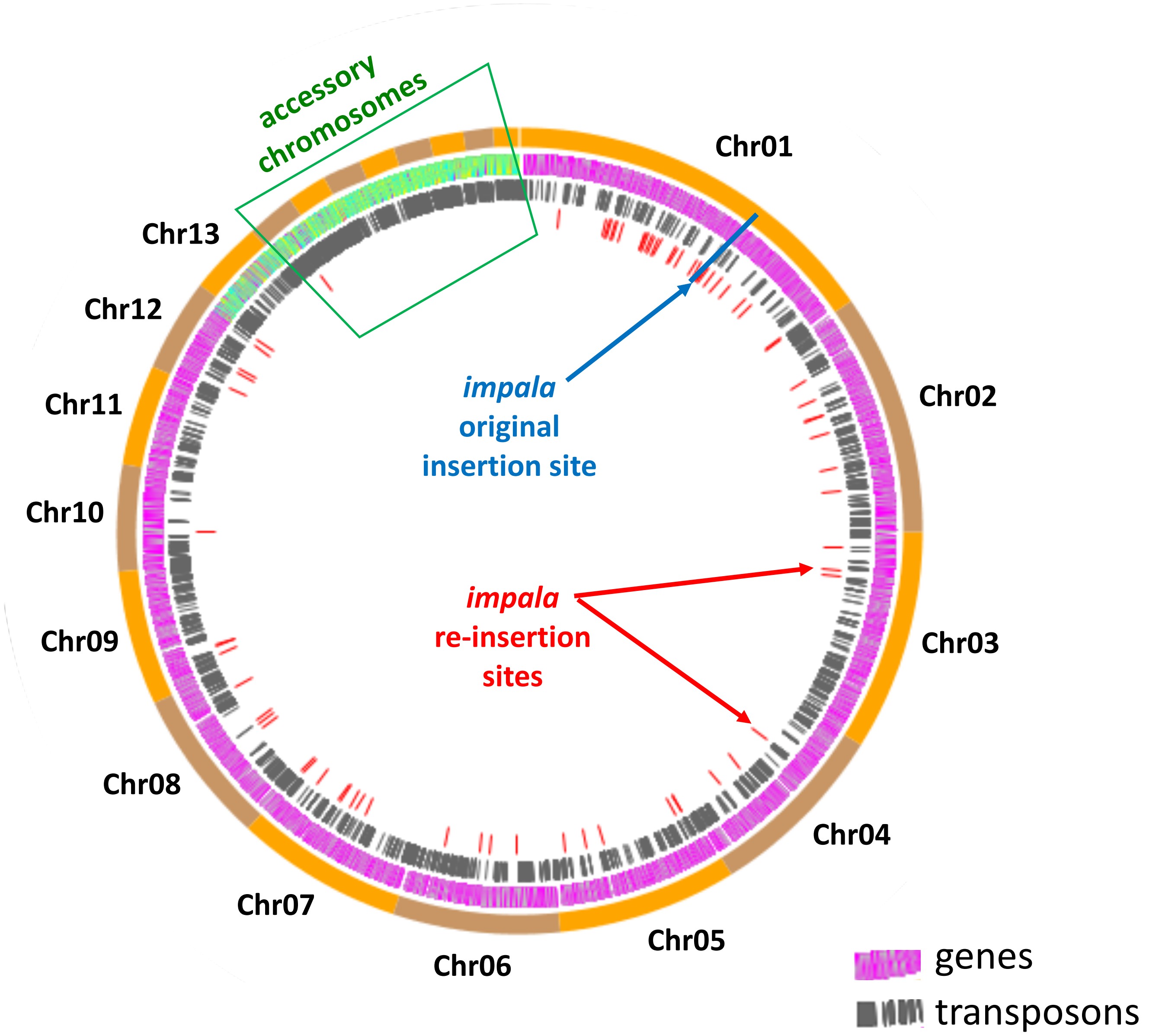
HDAC inhibition modifies _impala_ insertion pattern
= impact of chromatin opening on _impala_ access to DNA ?

impala transposition : insertion pattern ?

- Insertion of *impala* in all core chromosomes (Chr01-12)

- Only one insertion in accessory chromosomes
  1 / 9 expected according to size

accessory chromosomes:
enriched in hetero-chromatin marks (H3K27me3; H3K9me3) and transposons,
few expressed genes
only 1/10 of core chromosome genes
expected insertions according to the number of expected genes / Mb
= 1 insertion



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impala
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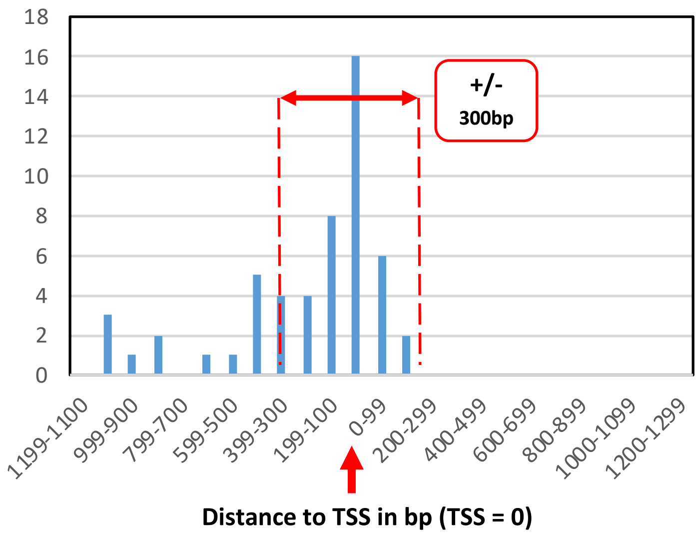

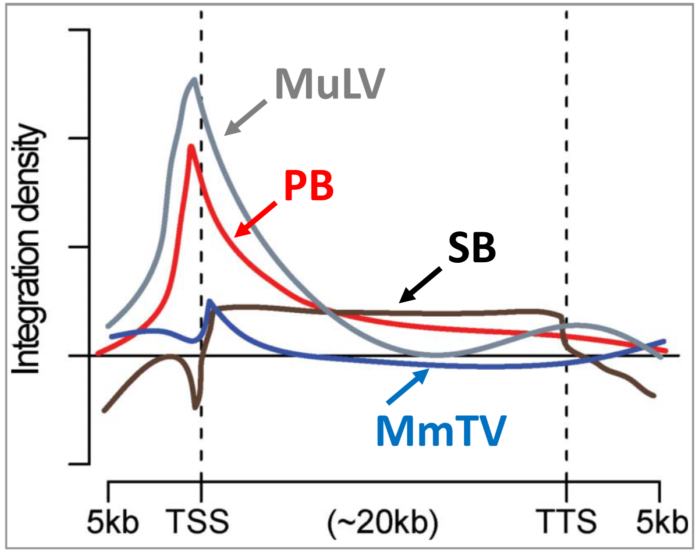

De Jong et al. 2014 Mobile Genetic Elements 4:6, 1-6
Heterologous transposition in mammalien cells

Animal *TC1 mariner*
SB: *Sleeping Beauty*
PB: *PiggyBac*

Animal retrovirus
MMTV: retrovirus
MuLV: retrovirus

only PiggyBac