

Lezione XIX-XX

giovedì 17-XI-2011

corso di genomica

Laurea Magistrale Biotecnologia Industriale

aula 8

orario : Martedì ore 14.00 - 16.00

Giovedì ore 13.00 - 15.00

libro consigliato Zanichelli “Introduz. Alla genomica” di
Arthur M.Lesk

D. Frezza

Analisi 3C intercluster HoxA

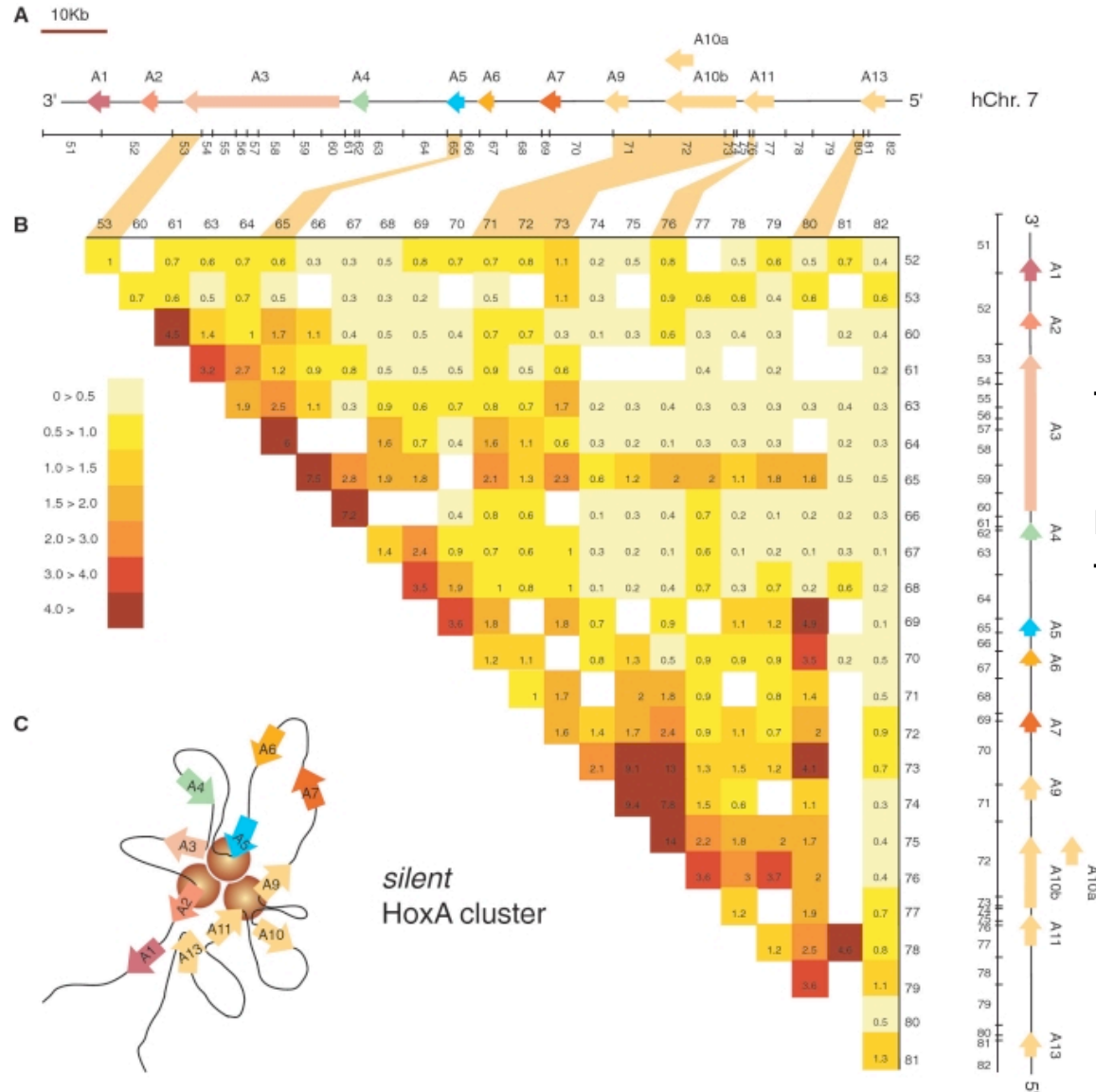
Analisi sui cluster inattivi

The three dimensional architecture of Hox cluster silencing
Maria A.Ferraiuolo et al.

Nucleic Acid Research vol 38 n.21 2010, pp 7472-7484

L'articolo è sulla didattica web come gli altri

interactions cluster Hox A



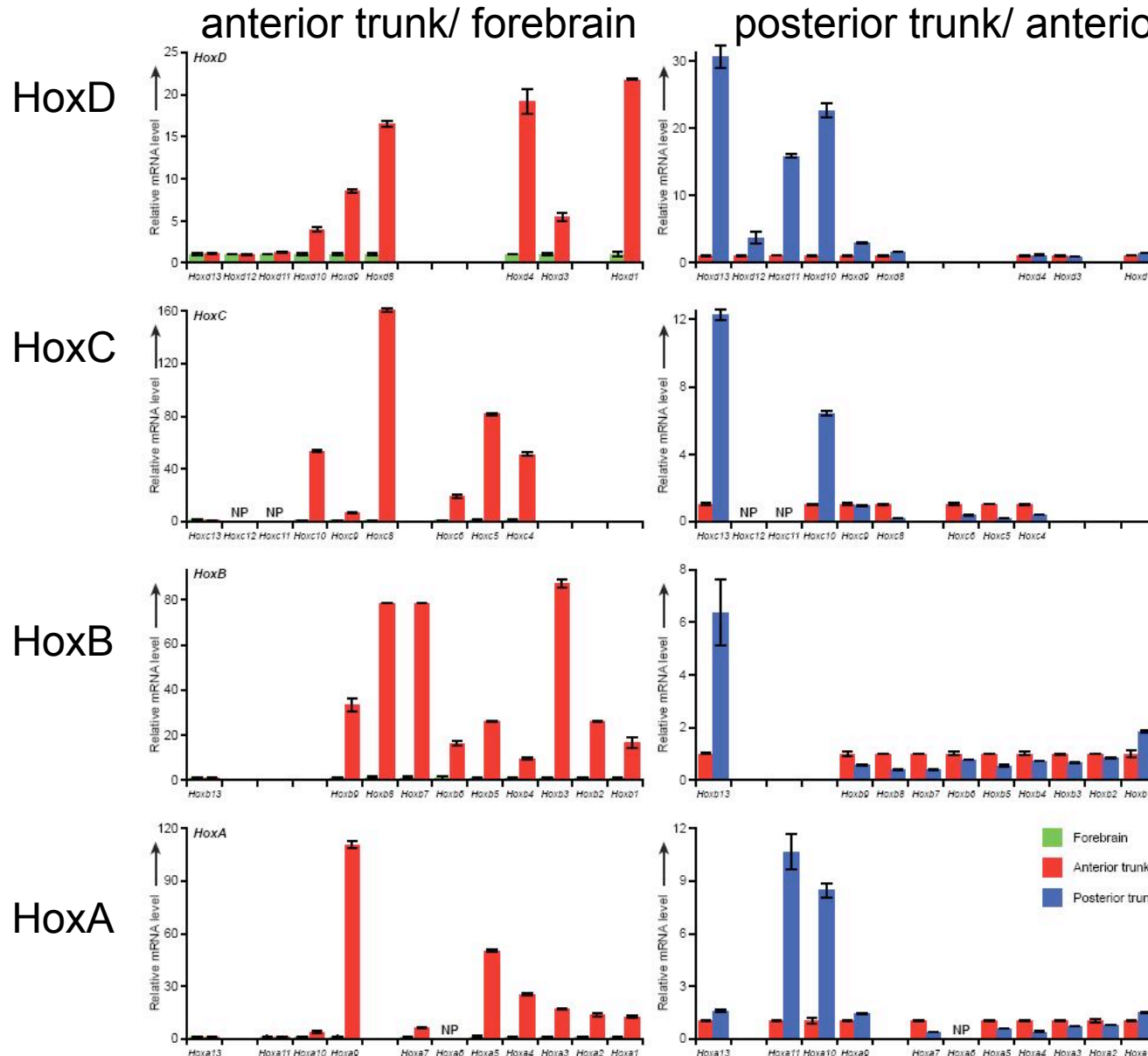
Esempio di interazioni in un cluster inattivo

The transcriptionally silent HoxA cluster is organized into multiple chromatin loops in the human genome

Come si ricavano le strutture dei looping

Figura 2 dell'articolo di Ferraiuolo su NAR

espressione normalizzata



Articolo Science di
Noordermeer et al
14 oct. 2011

normalizzazione rispetto
alla regione inattiva

Valori inattivi <50

Valori attivi:

-tronco anteriore >50

- tronco posteriore 2x
tronco anteriore

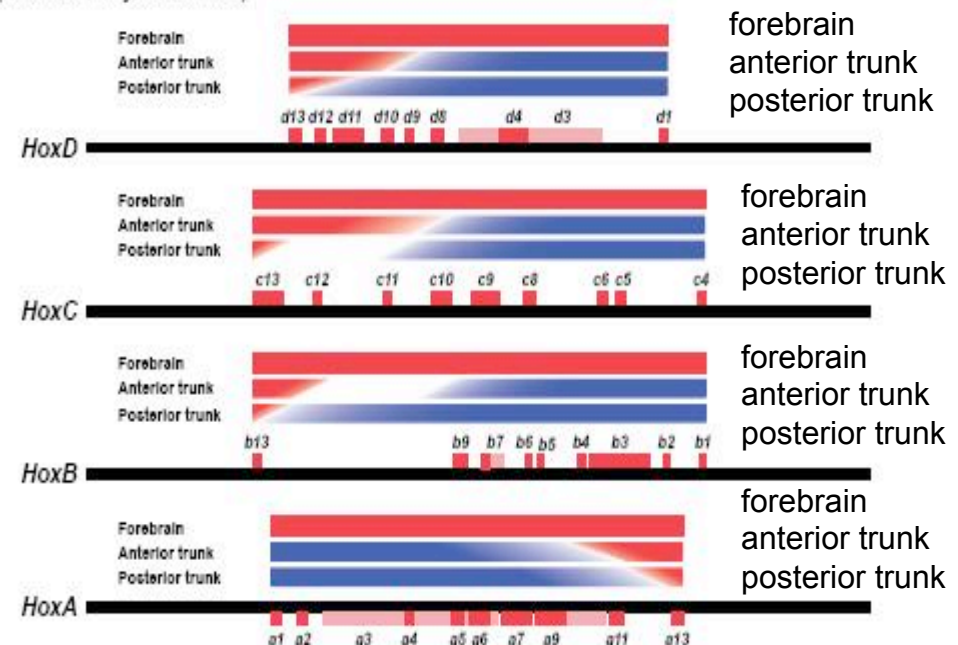
come si normalizzano i valori di trascrizione

Decision scheme for activity state
(based on Affymetrix data)

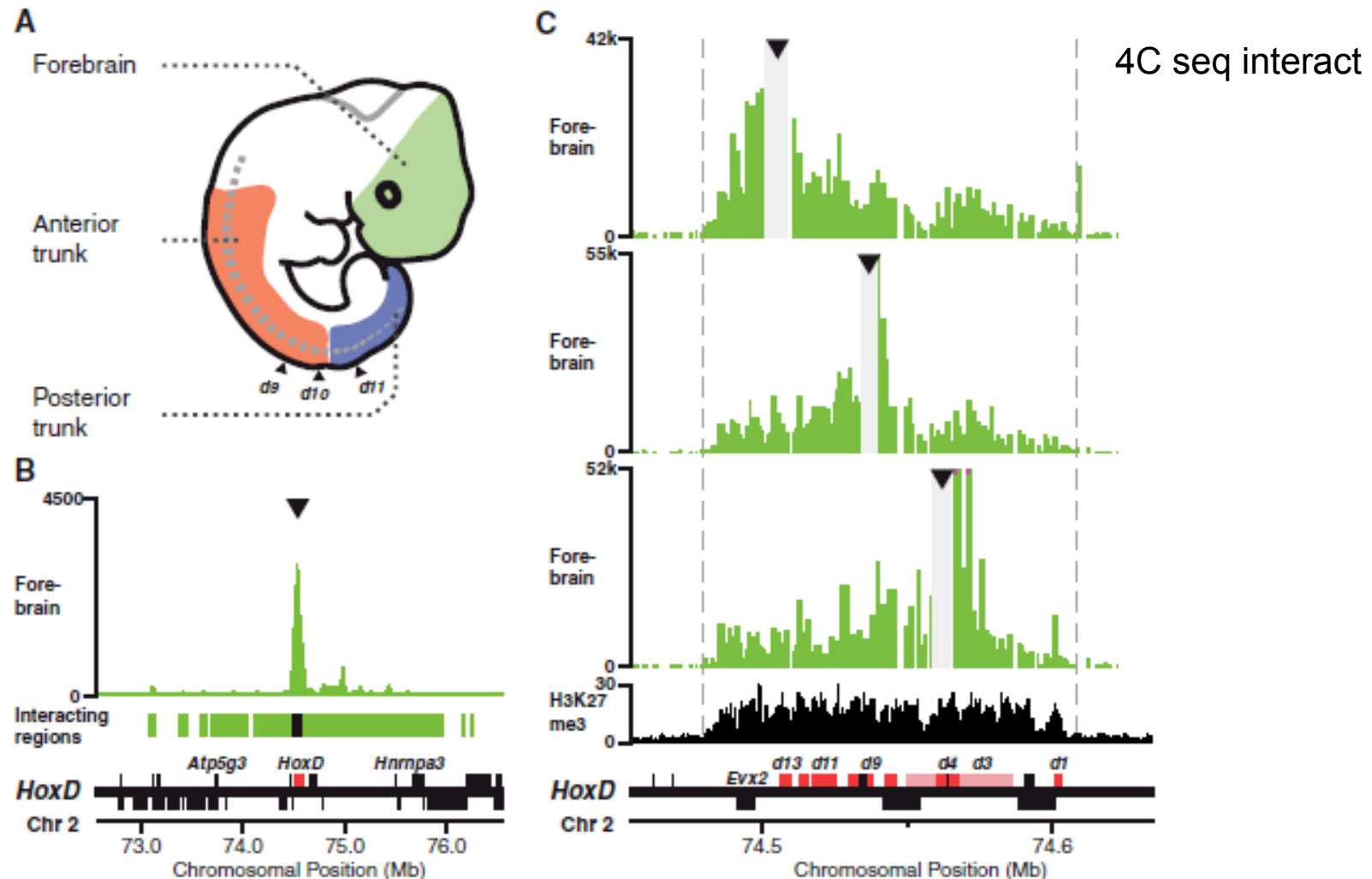
	Anterior trunk	Posterior trunk
Inactive	Value Anterior trunk < 50	Value Posterior trunk < 50
Being activated	Value Anterior trunk > 50 Value Posterior trunk > 2x Anterior trunk	Value Posterior trunk > 50 Value Anterior trunk < 50
Active	Value Anterior trunk > 50 Value Posterior trunk < 2x Anterior trunk	Value Posterior trunk > 50 Value Anterior trunk > 50



Activity state of *Hox*-genes
(based on Affymetrix data)



Espressione del cluster Hoxd



Multiplex 4C-seq (Multiplex Circular Chromosome Conformation Capture - sequencing)

Preparation of 4C library (circular 3C library)

Tecnica 4C

*Nla*III digestion of
cross-linked DNA

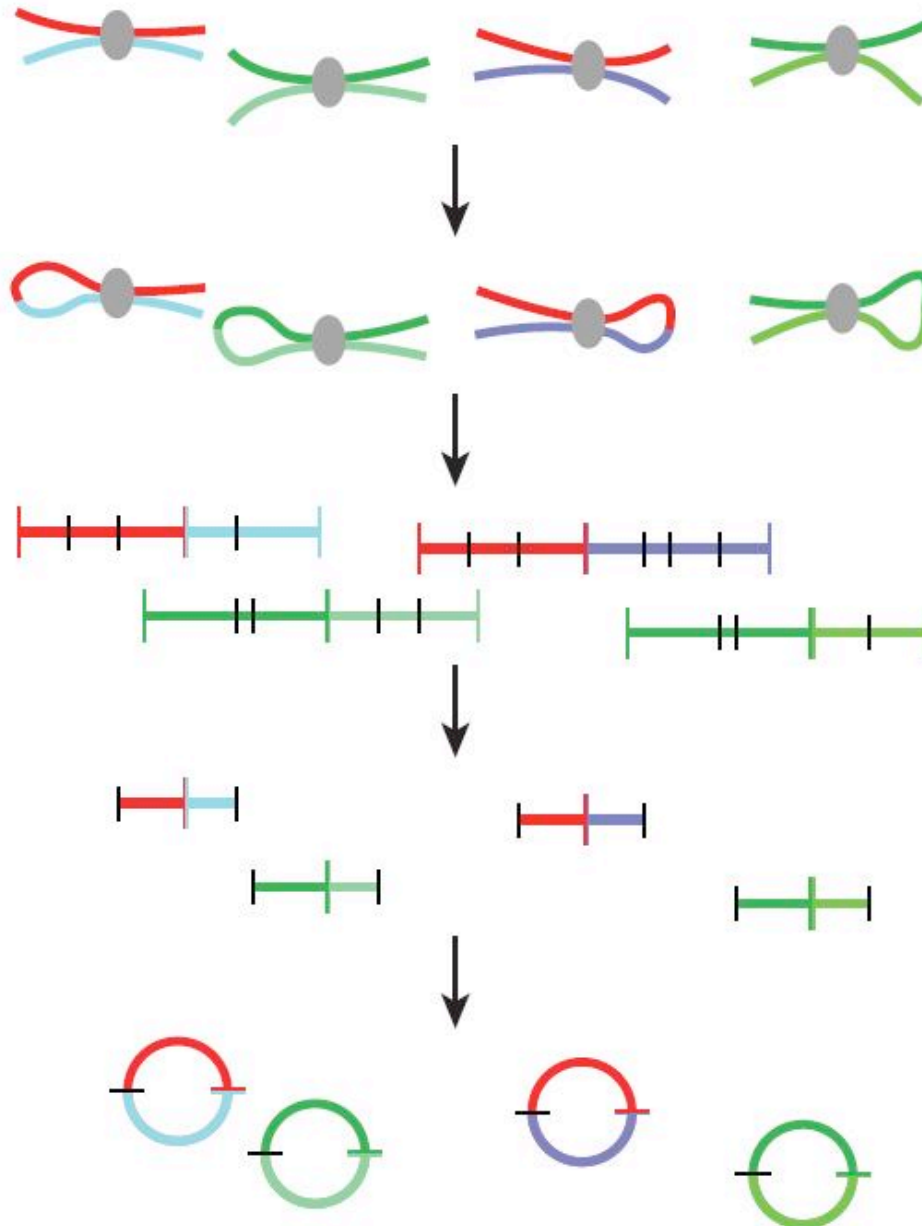
Dilution

Ligation

Reversal of
cross-links

*Dpn*II digestion
religated DNA

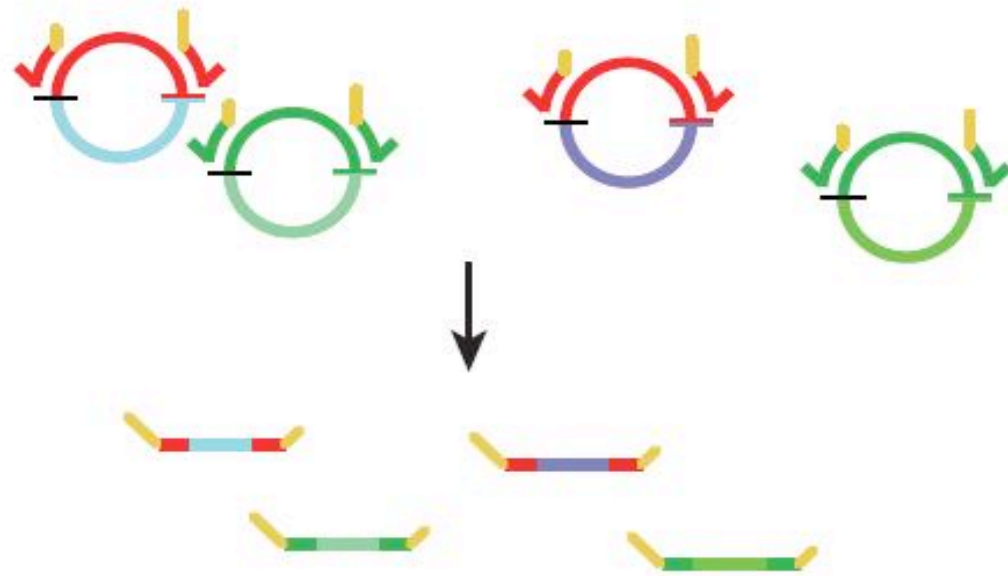
Circularization
(second ligation)



metodo 4C: dalla sequenza nota a quella interagente

Preparation of PCR-amplified 4C-sequencing material

PCR amplification
(with primers containing
Illumina adapters)



sequenze dagli adapters

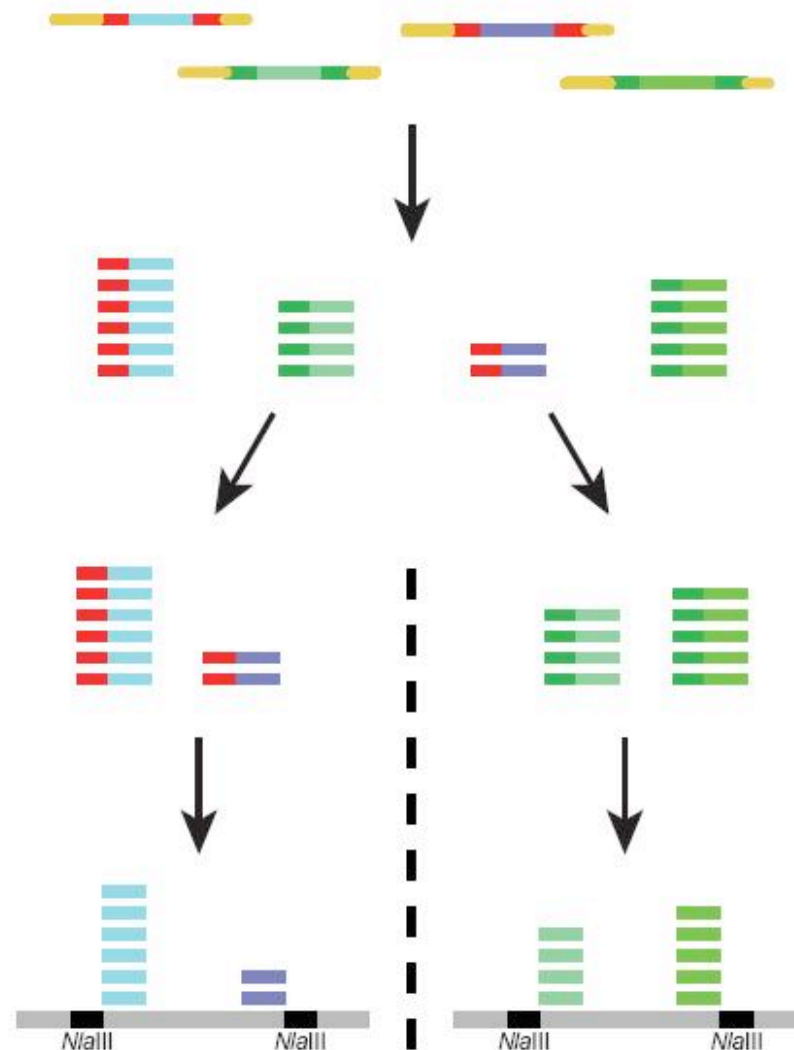
Mapping of Illumina (Solexa) reads

Multiplex Illumina
sequencing (76 bp reads)

De-multiplexing:
separation based on
PCR primer sequence

Removal of PCR
primer sequence

Mapping of reads



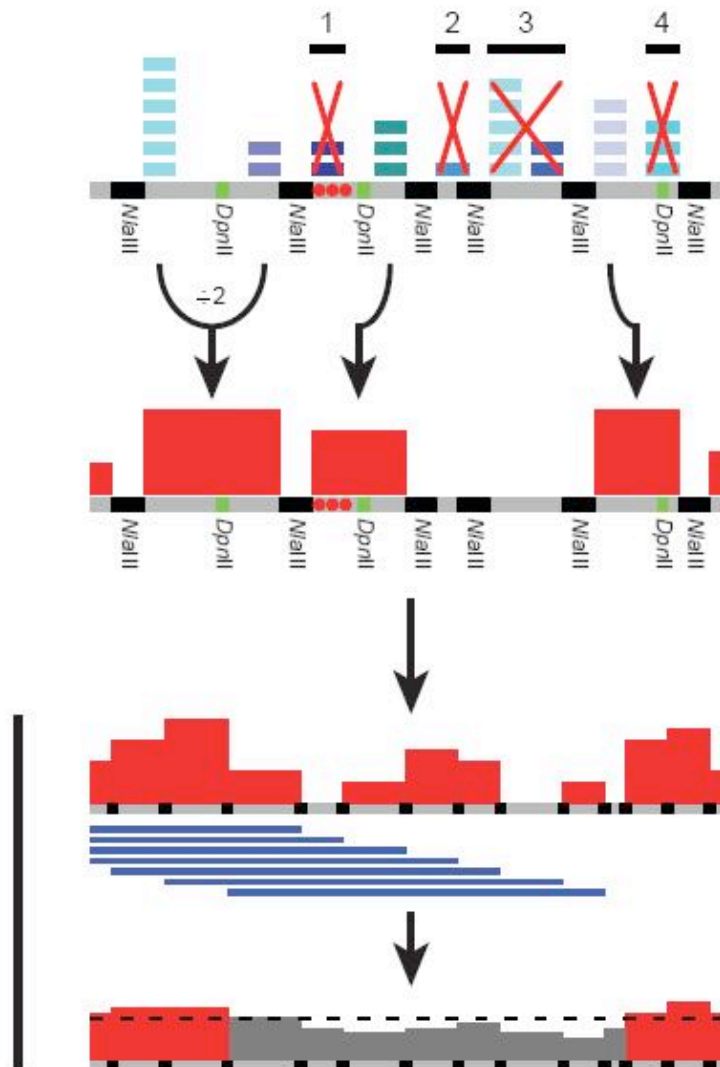
Visualization of interacting fragments and regions

mapping

Removal of non-informative reads

Translation to interacting restriction fragments

Smoothing of data and identification of interacting regions



Non-informative reads:

1. repeat sequences
2. too short *Nla*III fragment
3. absence of *Dpn*II site (secondary cutter) in *Nla*III fragment
4. *Dpn*II site too close to *Nla*III site

Running mean algorithm
Window size: 29 informative fragments
FDR: 0.05

Outline of the Multiplex 4C-seq procedure and data analysis.

profile of the inactive cluster in forebrain

3D intracluster high resolution in inactive forebrain HoxD

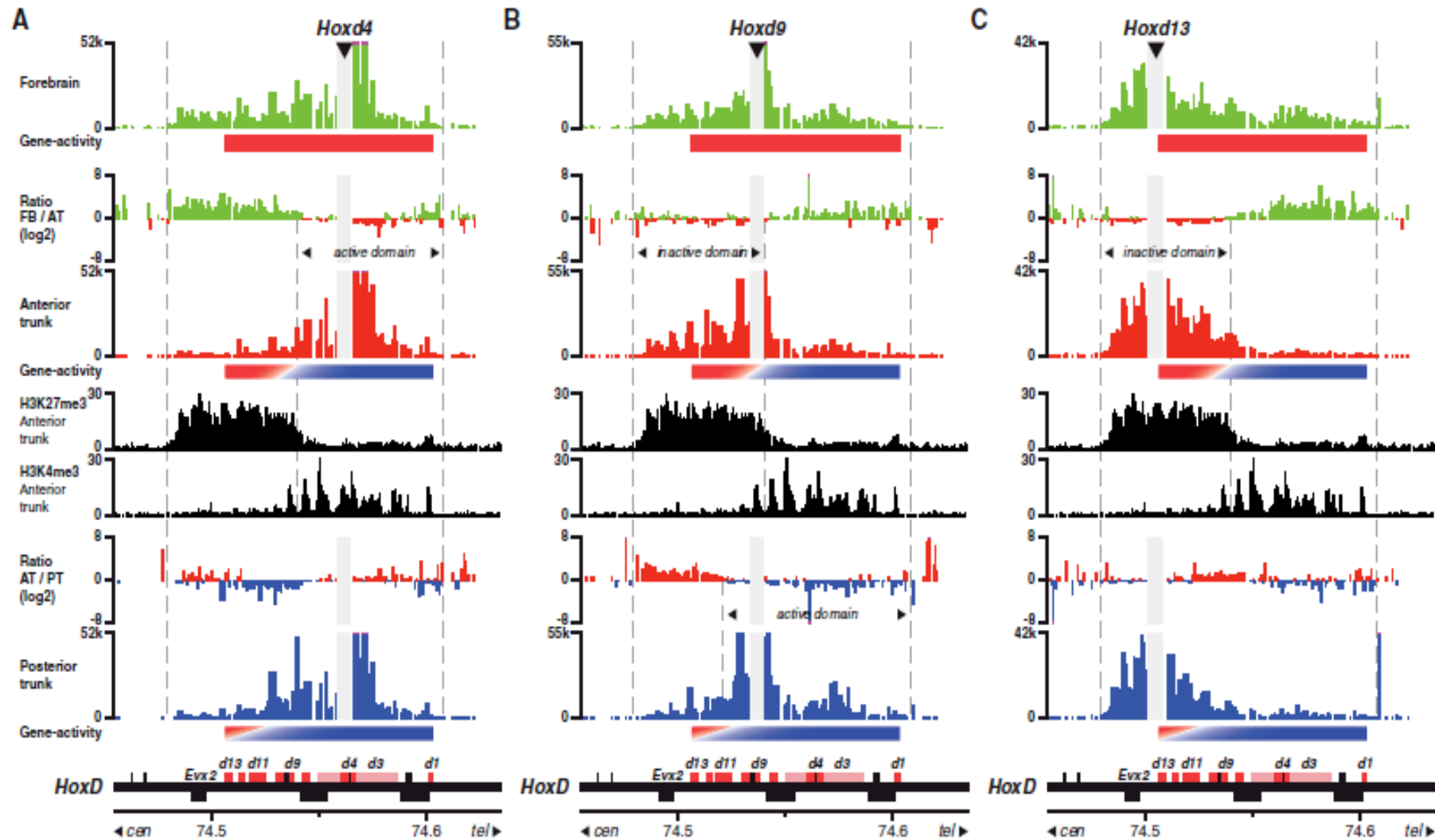
Si osservano associazioni 3D di domini (comparabili) a partire da *Evx2* fino a poche kb al 3' di *HoxD1*. Analoga situazione con 7 o 3 marcatori per gli altri cluster Hox (fig S7-8). I cluster silenti formano compartimenti 3D separati dalle flanking reg. Poca organizzazione specifica nei domini che suggeriscono contatti random. I domini associati corrispondono al pattern di metilazione H3k27me3 (fig S7-8-9) con stato represso.

Vedi fig 1 in basso la metilazione diffusa su tutto il cluster

Organizzazione ed espressione

centromer

telomer



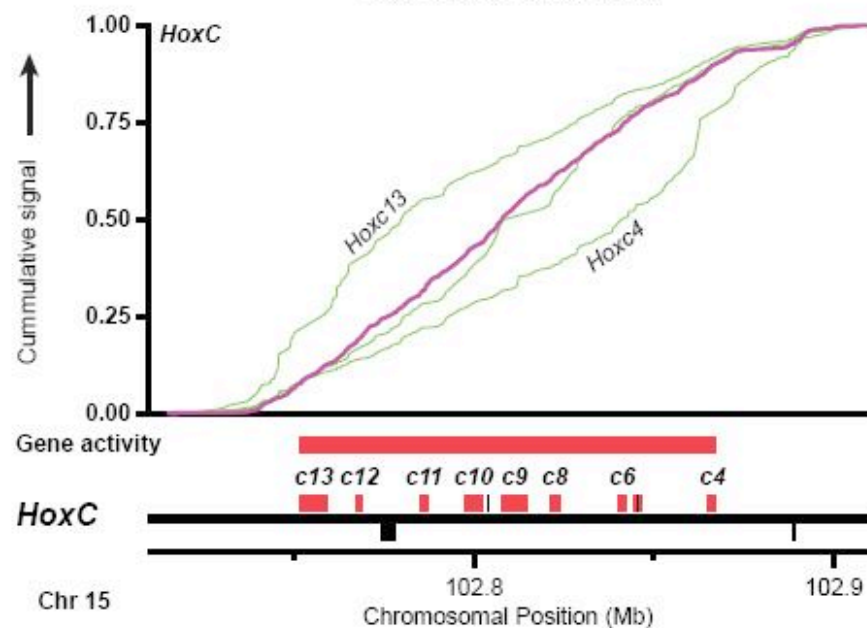
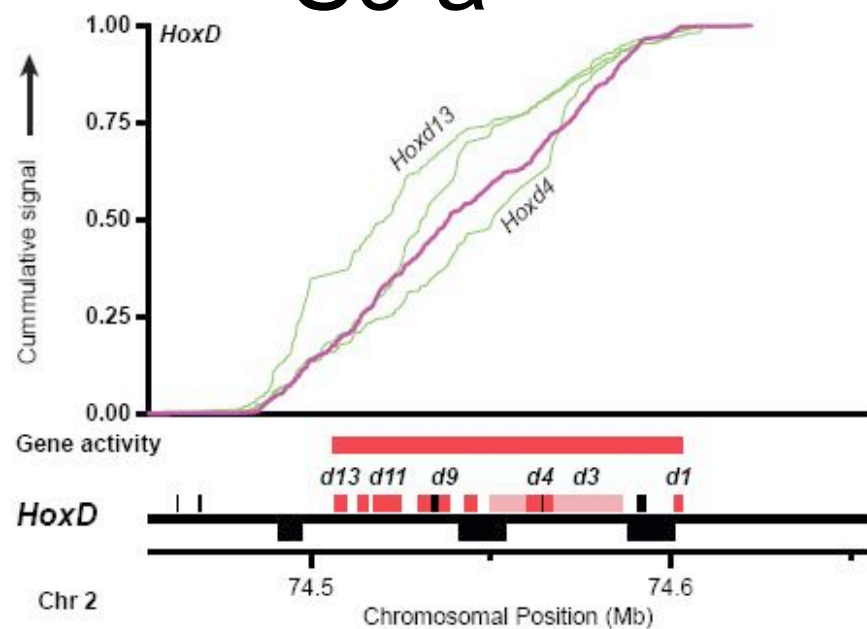
Ratio of interactions below each profile viewpoint (repr H3K27me3/ permiss H3K4me3)

analisi dell'architettura di HoxD in tessuto anteriore e posteriore

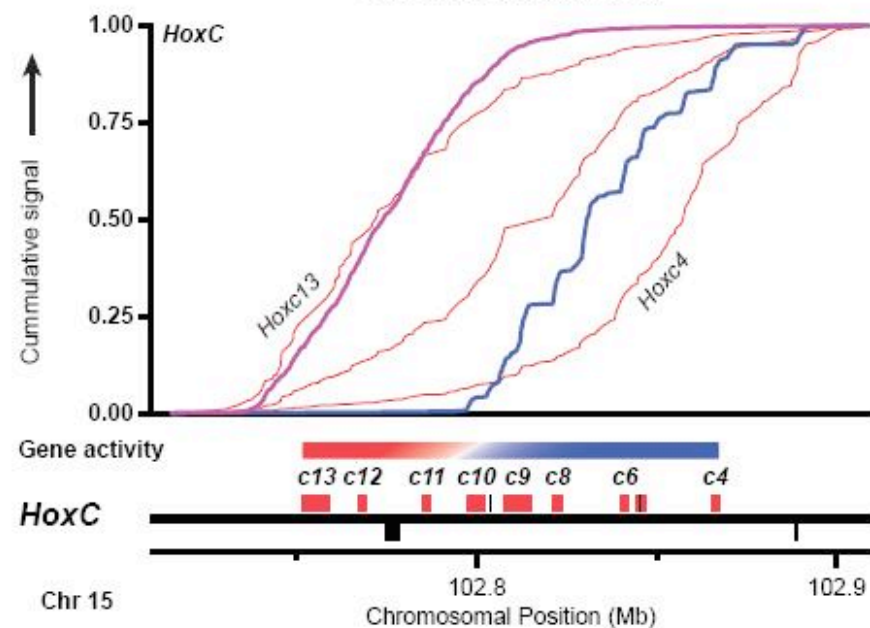
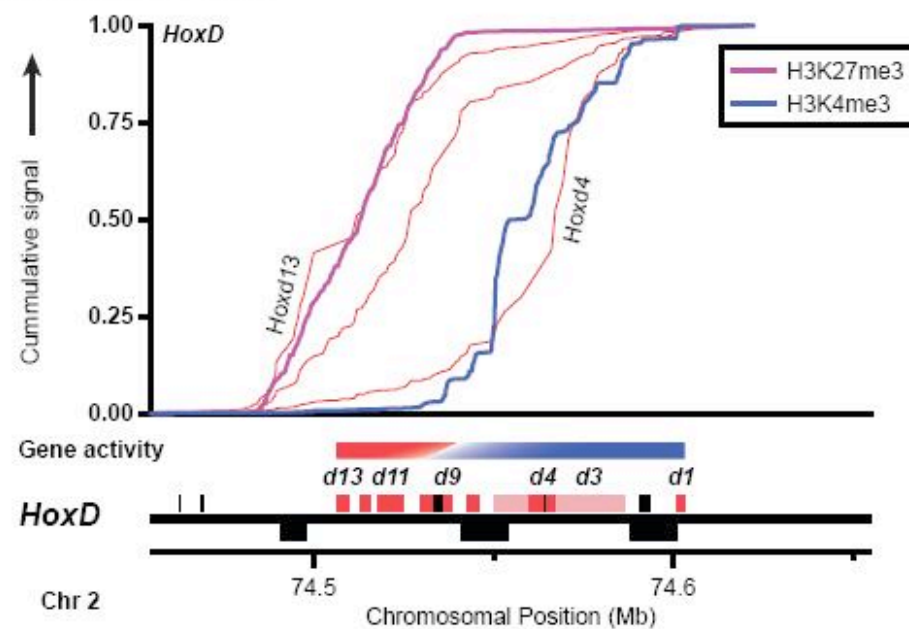
Allo stadio osservato E10.5 i cluster sono attivi su tessuto anteriore (rosso) e posteriore (blue) e non su forebrain (verde). Profilo bimodale di associazione che divide il cluster in due compartimenti 3D. Il confine tra i due compartimenti è collocato in punti diversi con overlap tra HoxD9 nel tronco anteriore e posteriore vedi fig 2 (leggere pag 223 fine seconda e terza colonna)
fig2 ; S9 ed S10

S9 a

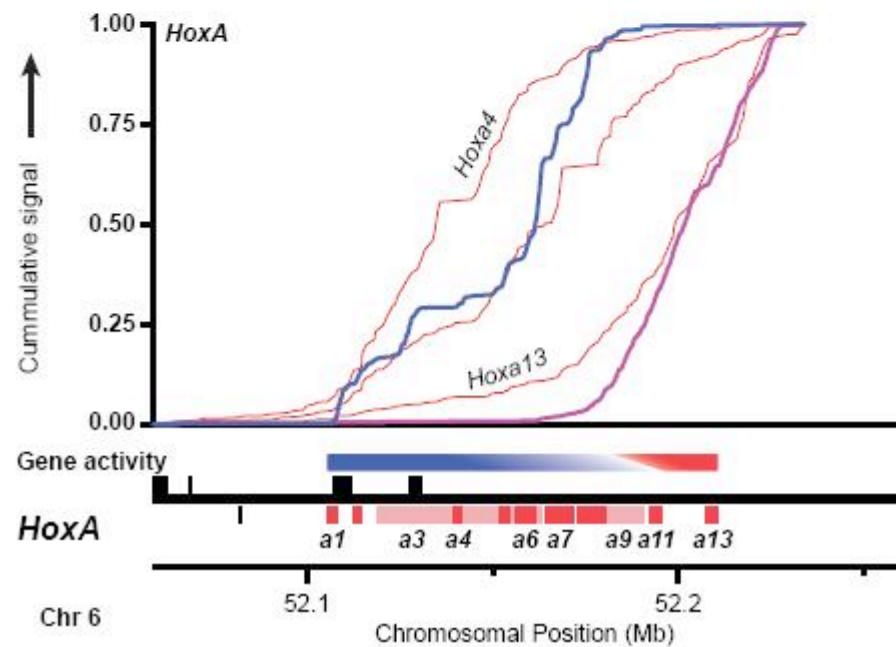
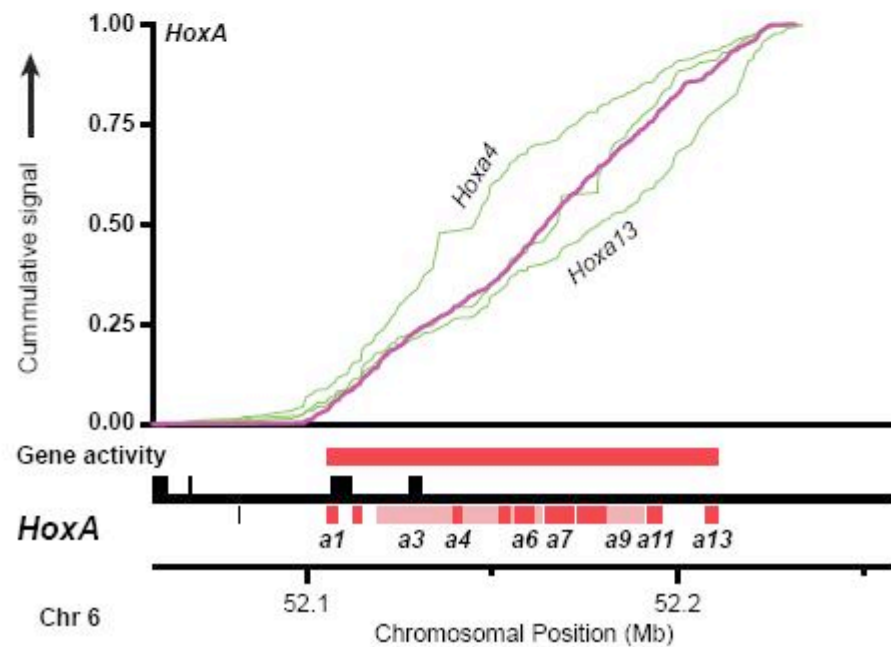
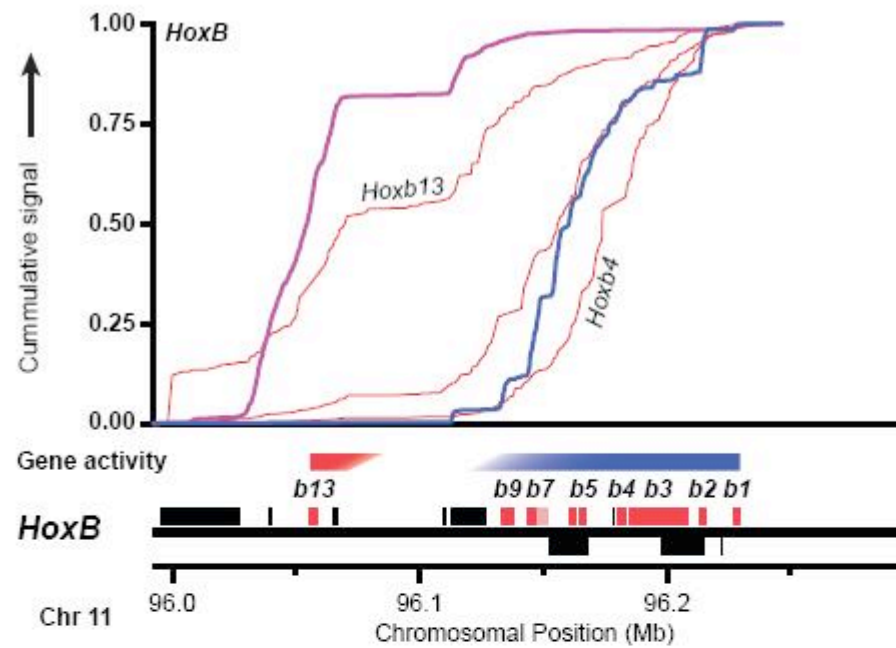
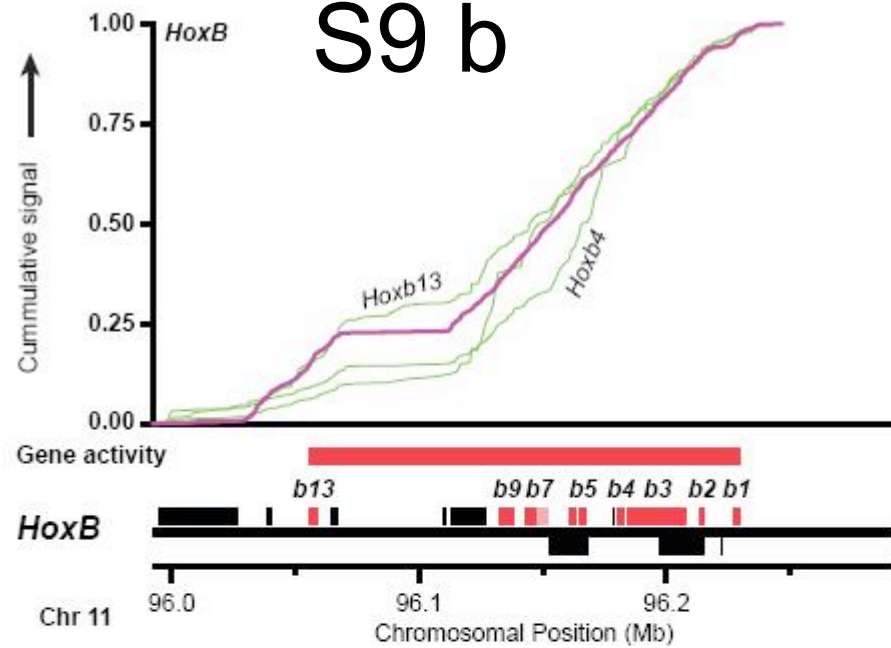
Forebrain



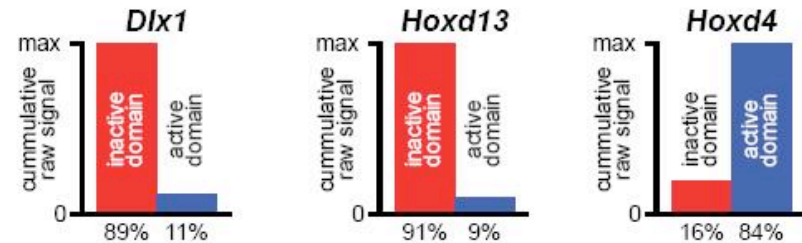
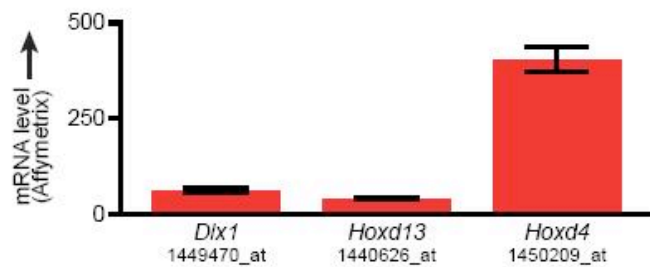
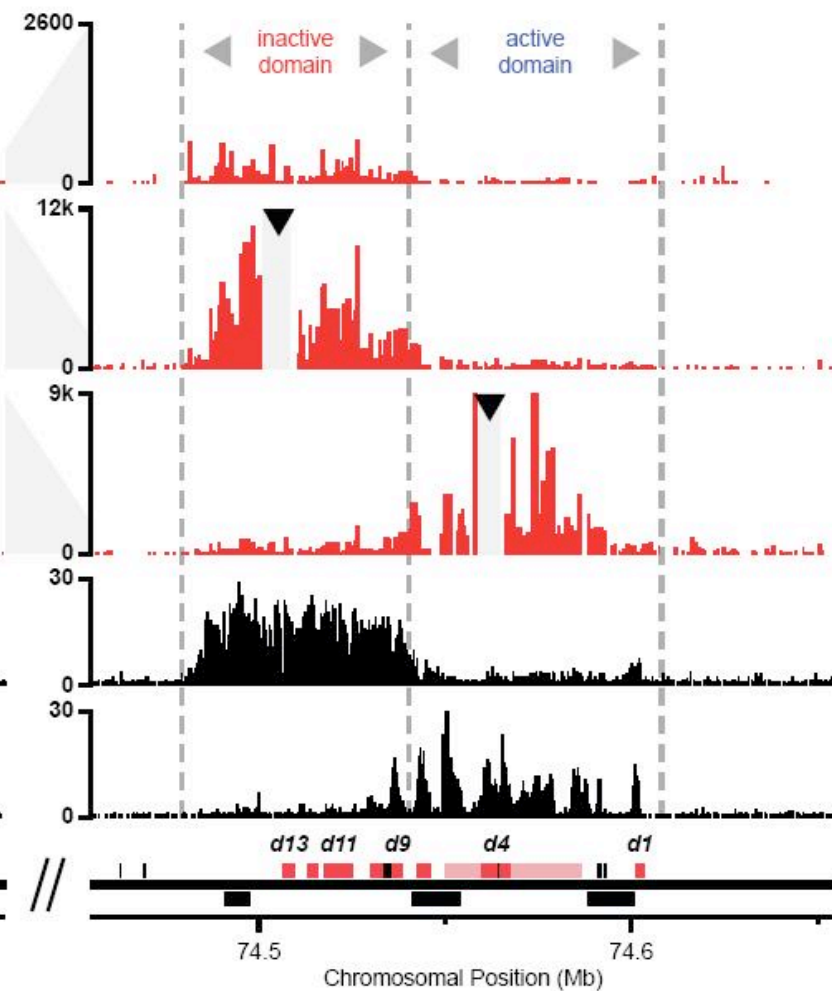
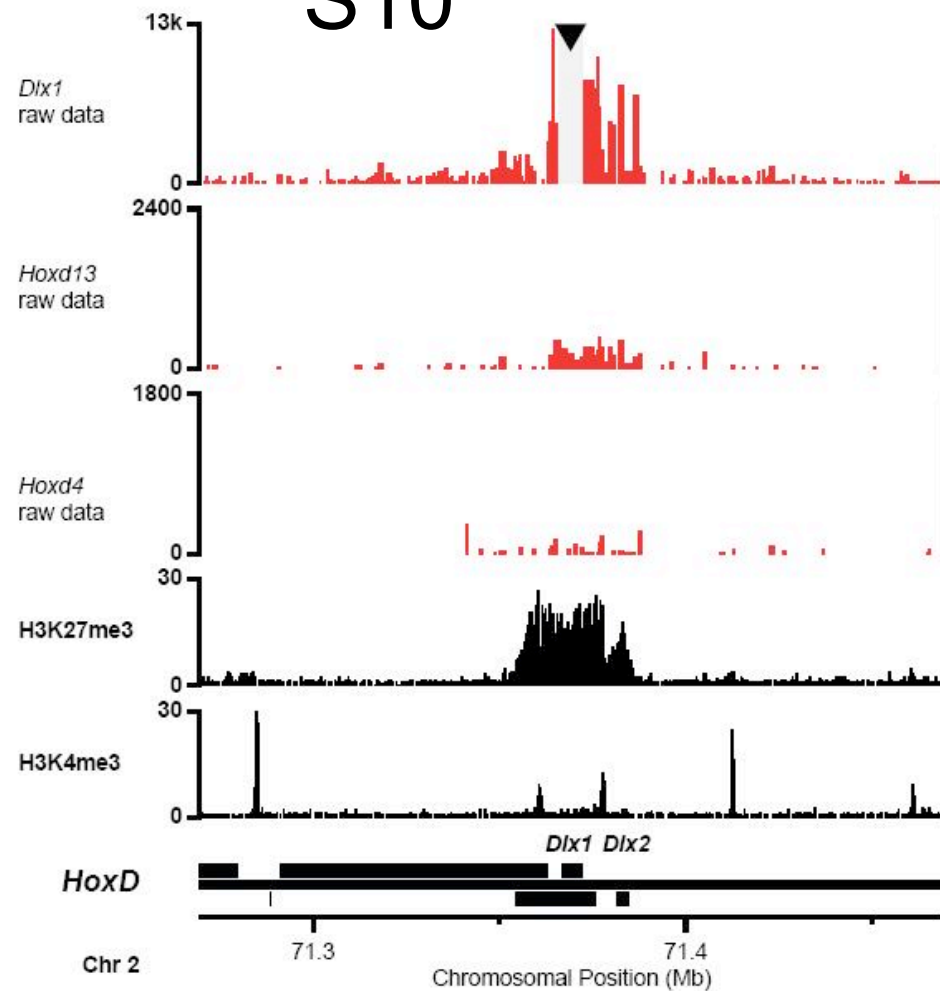
Anterior trunk



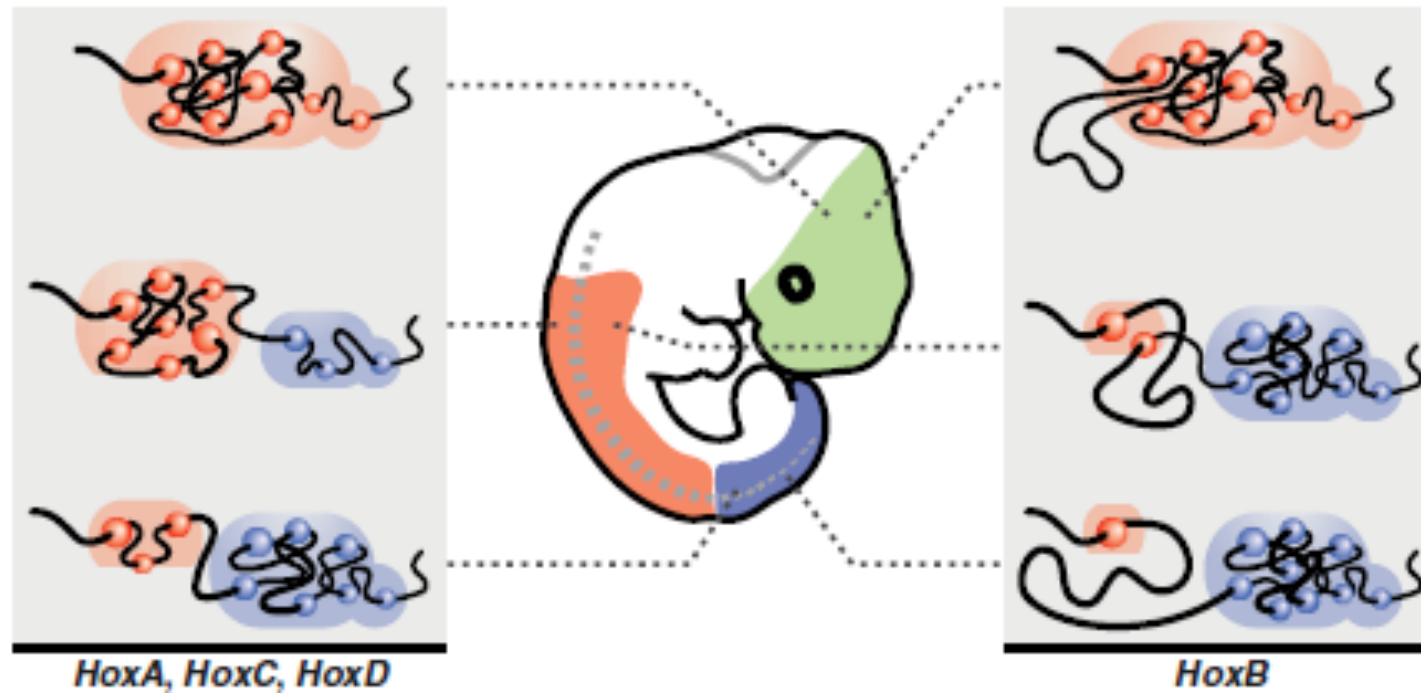
S9 b



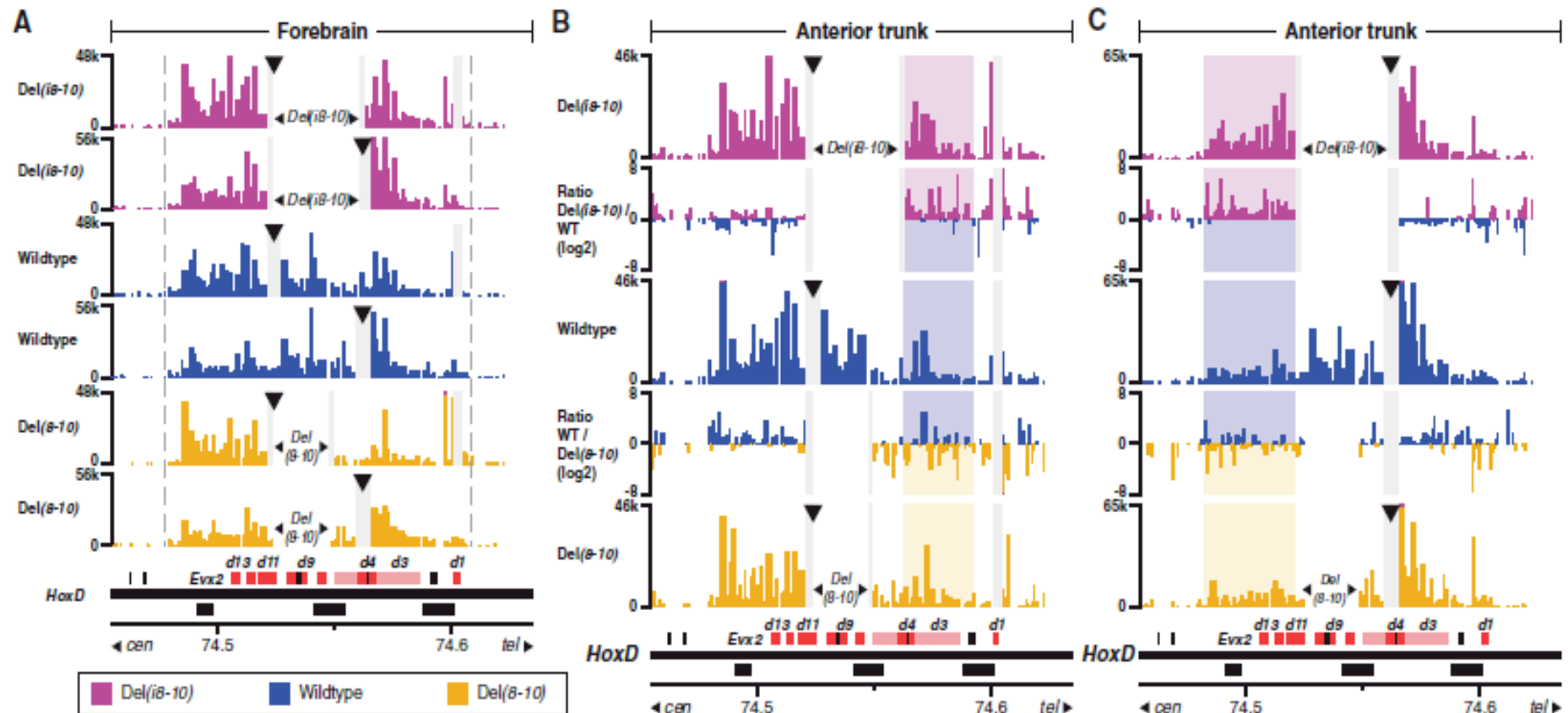
S10



schema 3D ed espressione colineare



espressione ectopica (fuori luogo) di HoxD delet. mut.



Prossimo articolo : Erez Lieberman-Aiden et al.

Comprehensive mapping of long range interactions reveals folding principles of the human genome

Science 9 Ottobre 2009, 326 (5950) pp 289-293

