FaceBase2 Spoke

Epigenetic landscapes and regulatory divergence of human craniofacial traits

Joanna Wysocka & Licia Selleri

Our team members:

Licia Selleri
(UCSF)

Sara Prescott
(Stanford, now at Harvard)

Tomek Swigut
(Stanford)

Ian Welsh
(UCSF)

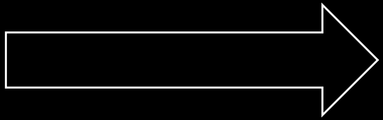
Hannah Long
(Stanford)

Our aims:

AIM1: To characterize epigenetic landscapes and transcriptomes of human and chimpanzee Cranial Neural Crest Cells (CNCCs) and to identify conserved and species-specific cis-regulatory elements

AIM2. To analyze activity of candidate human-specific craniofacial enhancers *in vivo.*

Challenge: how do regulatory elements encode human traits?

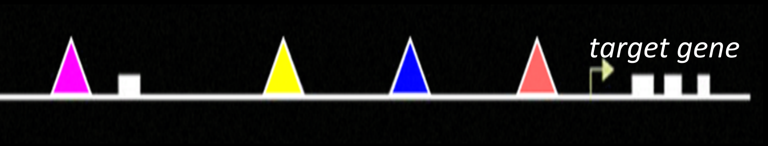


genotype

development

phenotype

enhancers

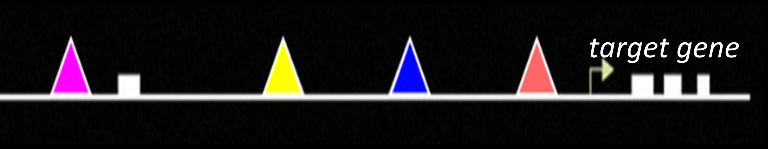


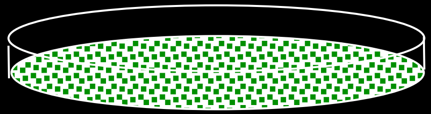
Challenge: how do regulatory elements encode human traits?

development

genotype → phenotype

enhancers





ability to systematically identify cell type-specific regulatory elements

ability to model aspects of human development in the dish

craniofacial development

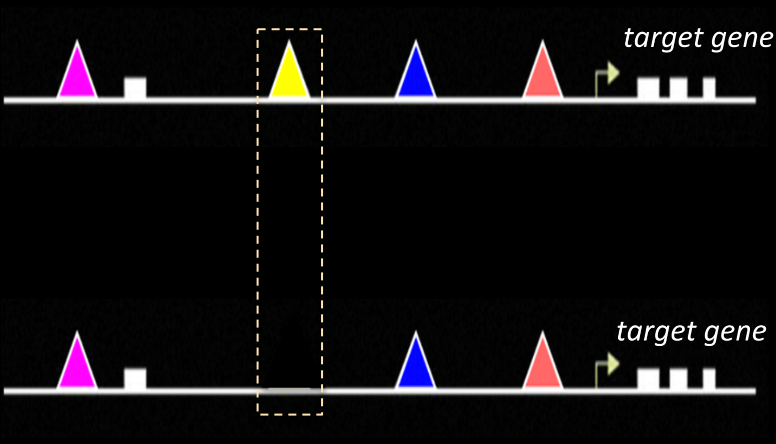
Studying evolution of closely related species is a powerful tool for uncovering genotype-phenotype connections

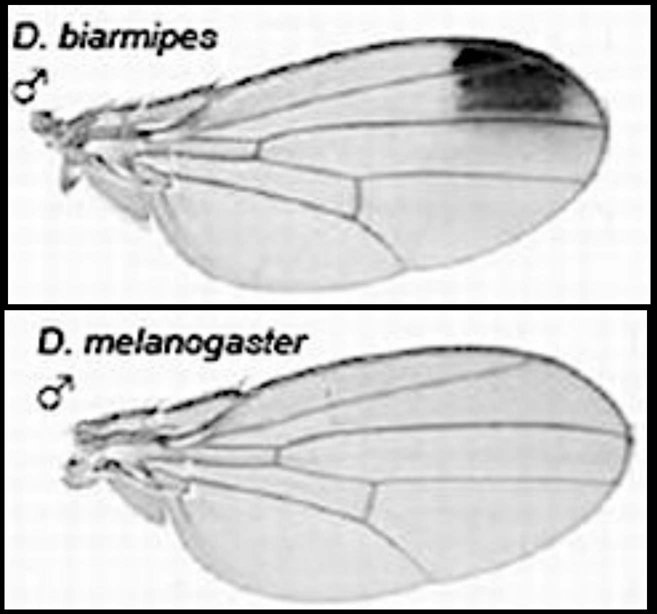
development

genotype → phenotype

enhancers

wing patterning in Drosophila





Sean Carroll, David Kingsley, Mike Levine & others

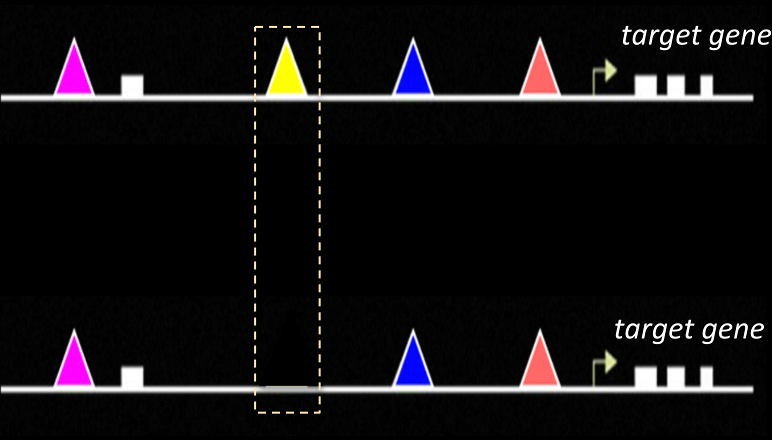
'Cellular anthropology': Using higher primate cellular models to study enhancer landscape evolution

development

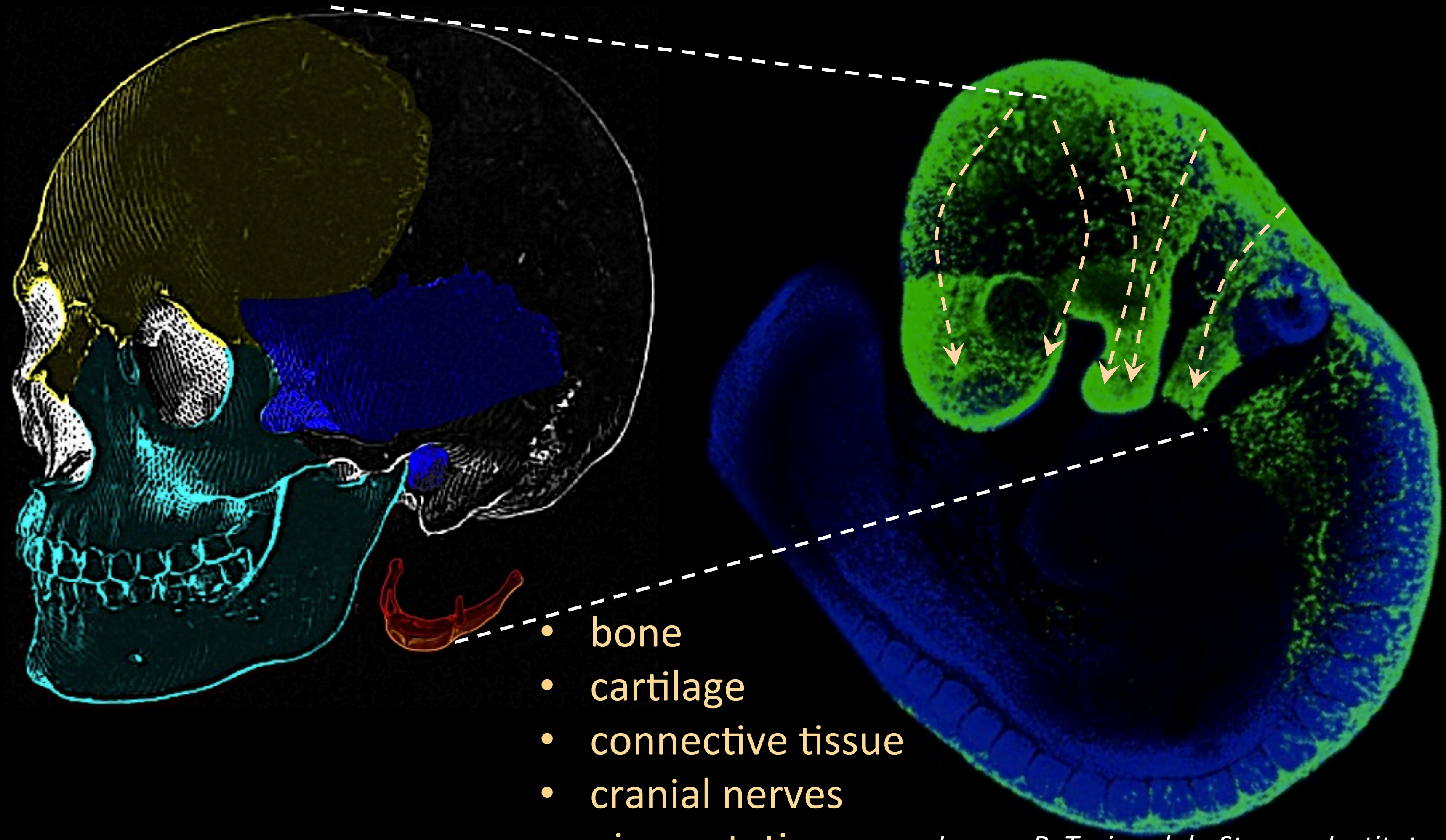
genotype → phenotype

enhancers

?



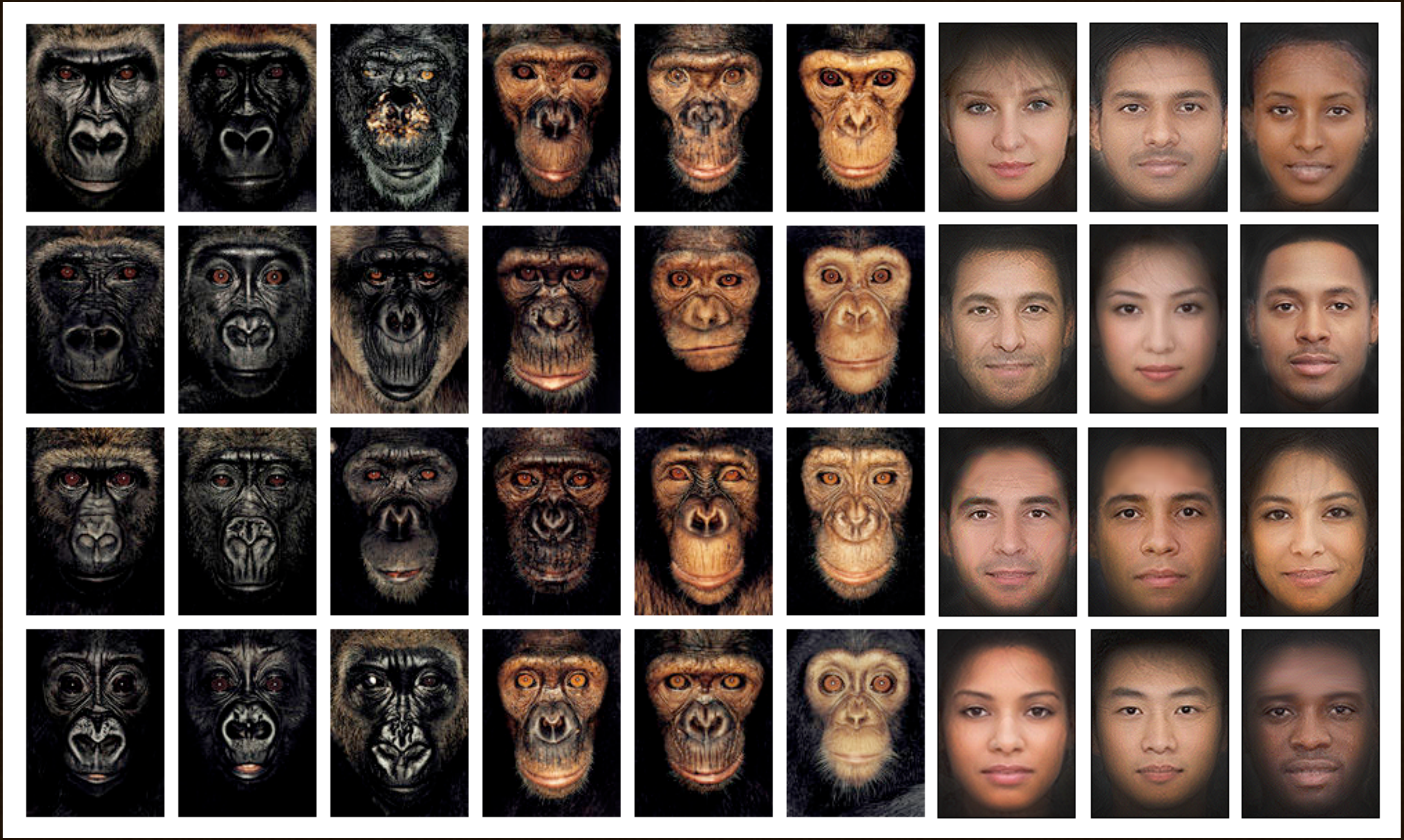
Most of our face and head is derived from the cranial neural crest



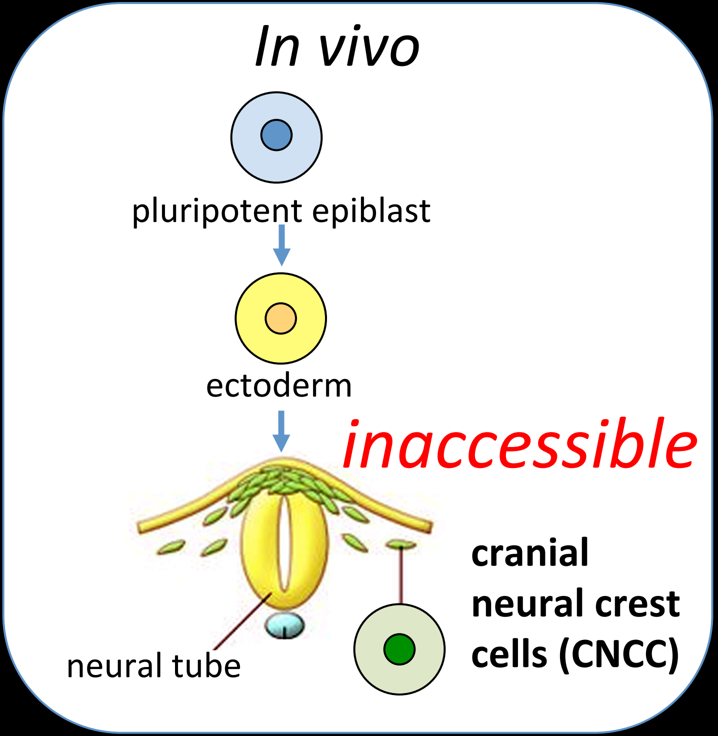
- bone
- cartilage
- connective tissue
- cranial nerves
- pigmentation

Image: P. Trainor lab, Stowers Institute

Cranial neural crest derived structures played a key role in human evolution

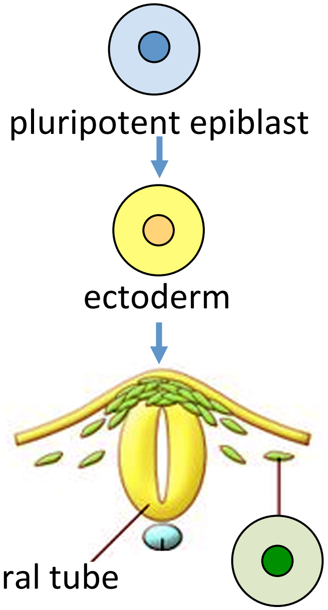


How can we access cranial neural crest cells from higher primates?



An *in vitro* model of human cranial neural crest cell formation

In vivo



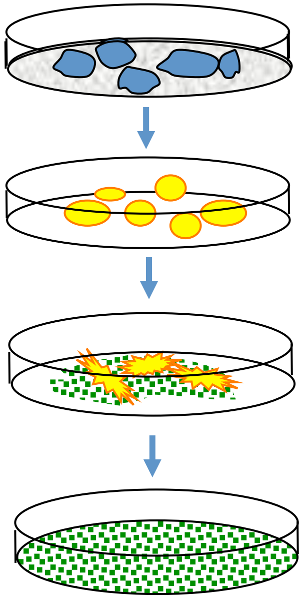
pluripotent epiblast

ectoderm

neural tube

cranial neural crest cells (CNCC)

In vitro

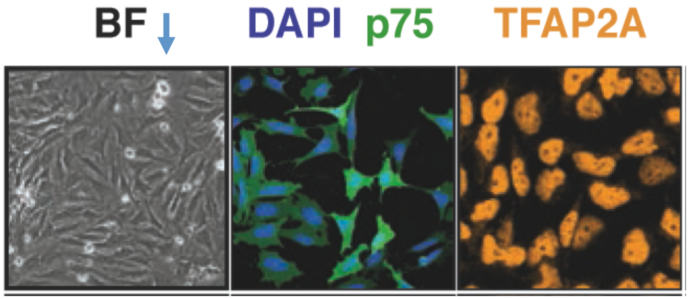


hESC or iPSC

neuroepithelium

induction & emigration of CNCC

maintenance of human **CNCC**

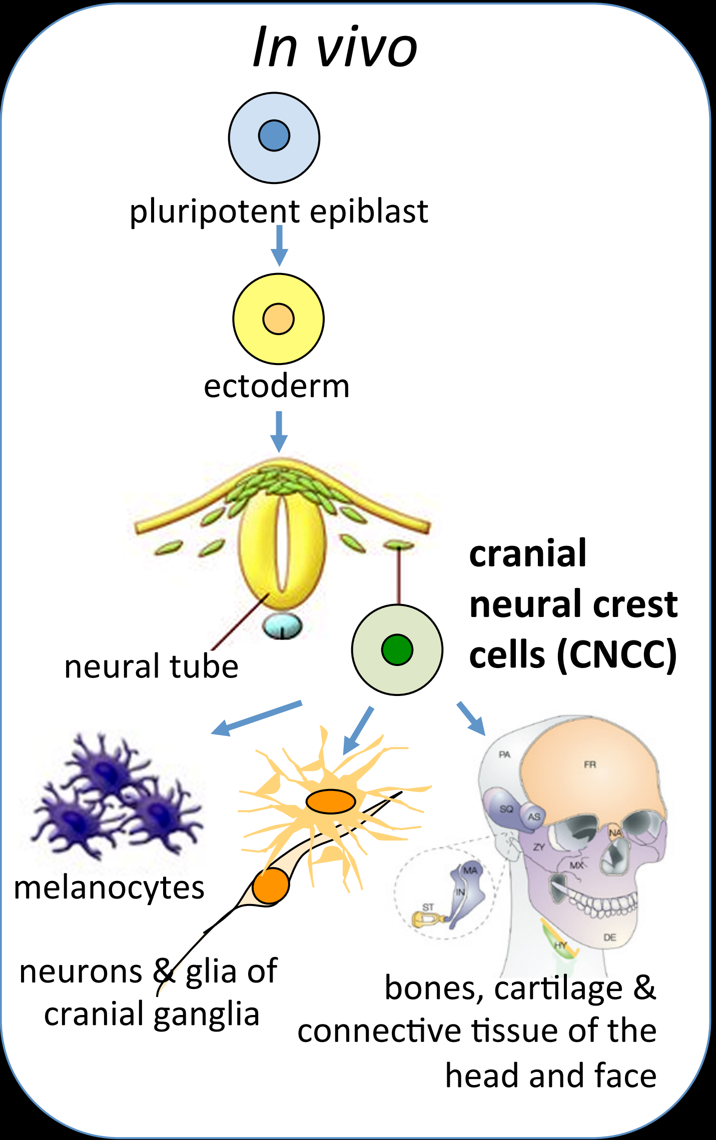


p75+, TFAP2A+, TWIST1+, SOX9+, SOX10+, DLX1/2+, HOX-

Bajpai et al., Nature 2010

Rada-Iglesias, Bajpai et al., Cell Stem Cell, 2012

In vitro derived human CNCC exhibit transcriptional and cellular characteristics of the NC *in vivo*



In vivo

pluripotent epiblast

ectoderm

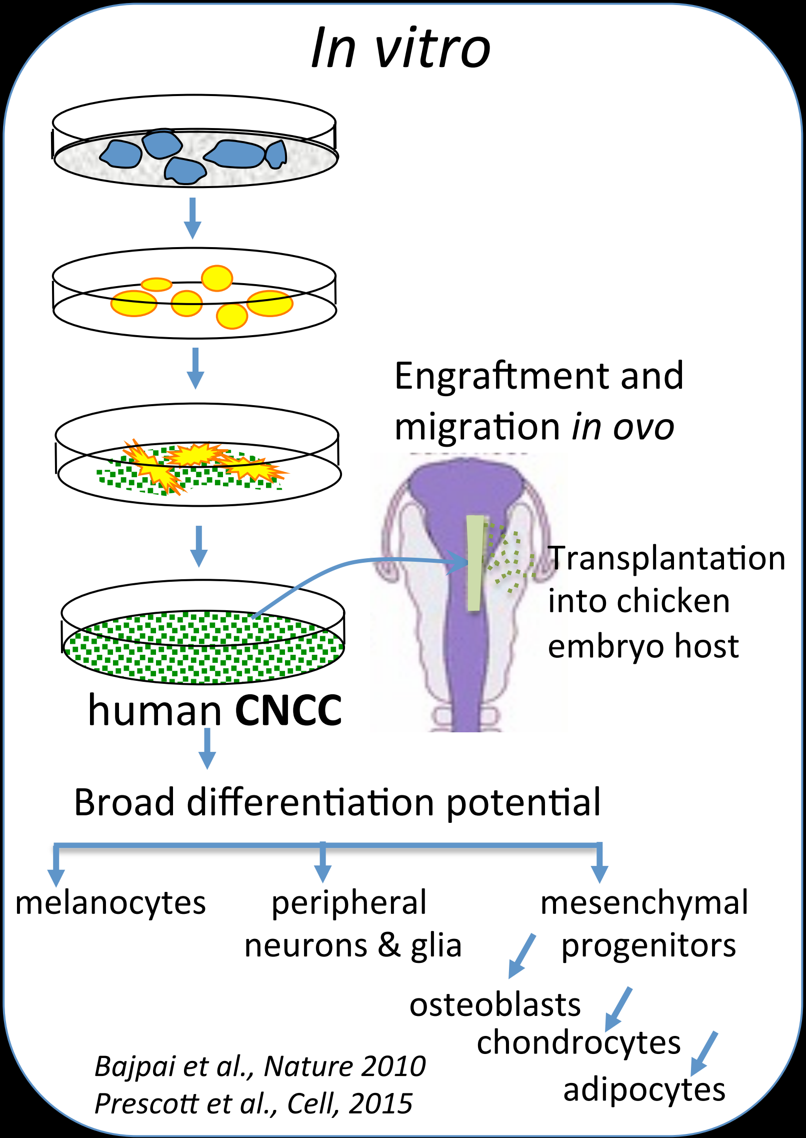
neural tube

cranial neural crest cells (CNCC)

melanocytes

neurons & glia of cranial ganglia

bones, cartilage & connective tissue of the head and face



In vitro

Engraftment and migration *in ovo*

Transplantation into chicken embryo host

human **CNCC**

Broad differentiation potential

melanocytes

peripheral neurons & glia

mesenchymal progenitors

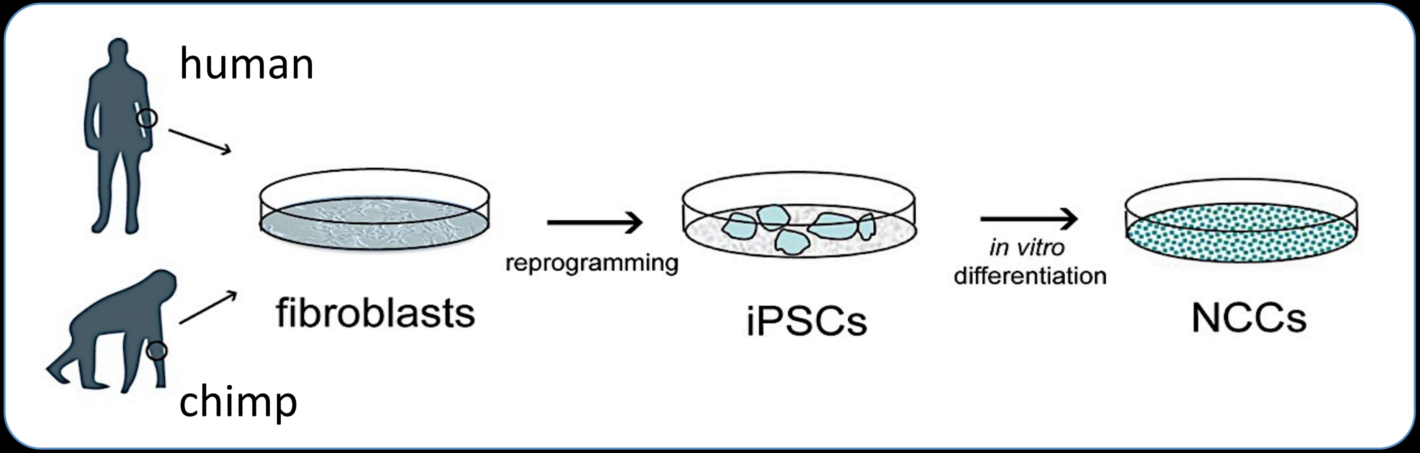
osteoblasts

chondrocytes

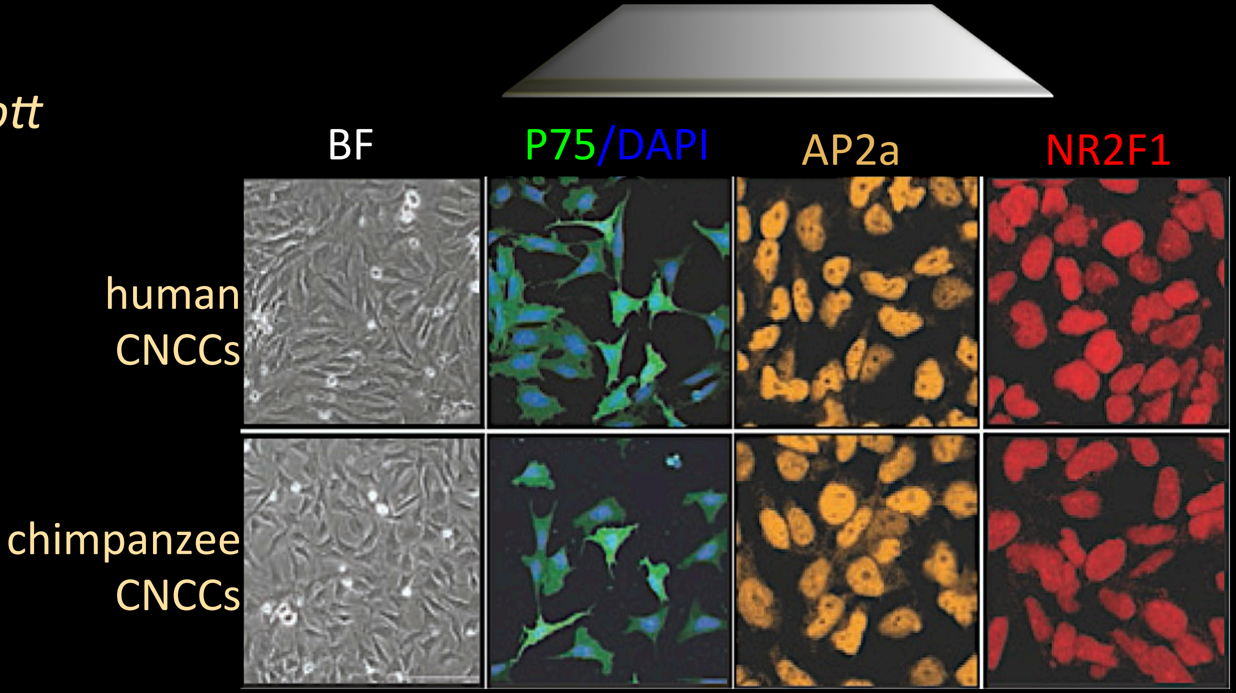
adipocytes

Bajpai et al., Nature 2010
Prescott et al., Cell, 2015

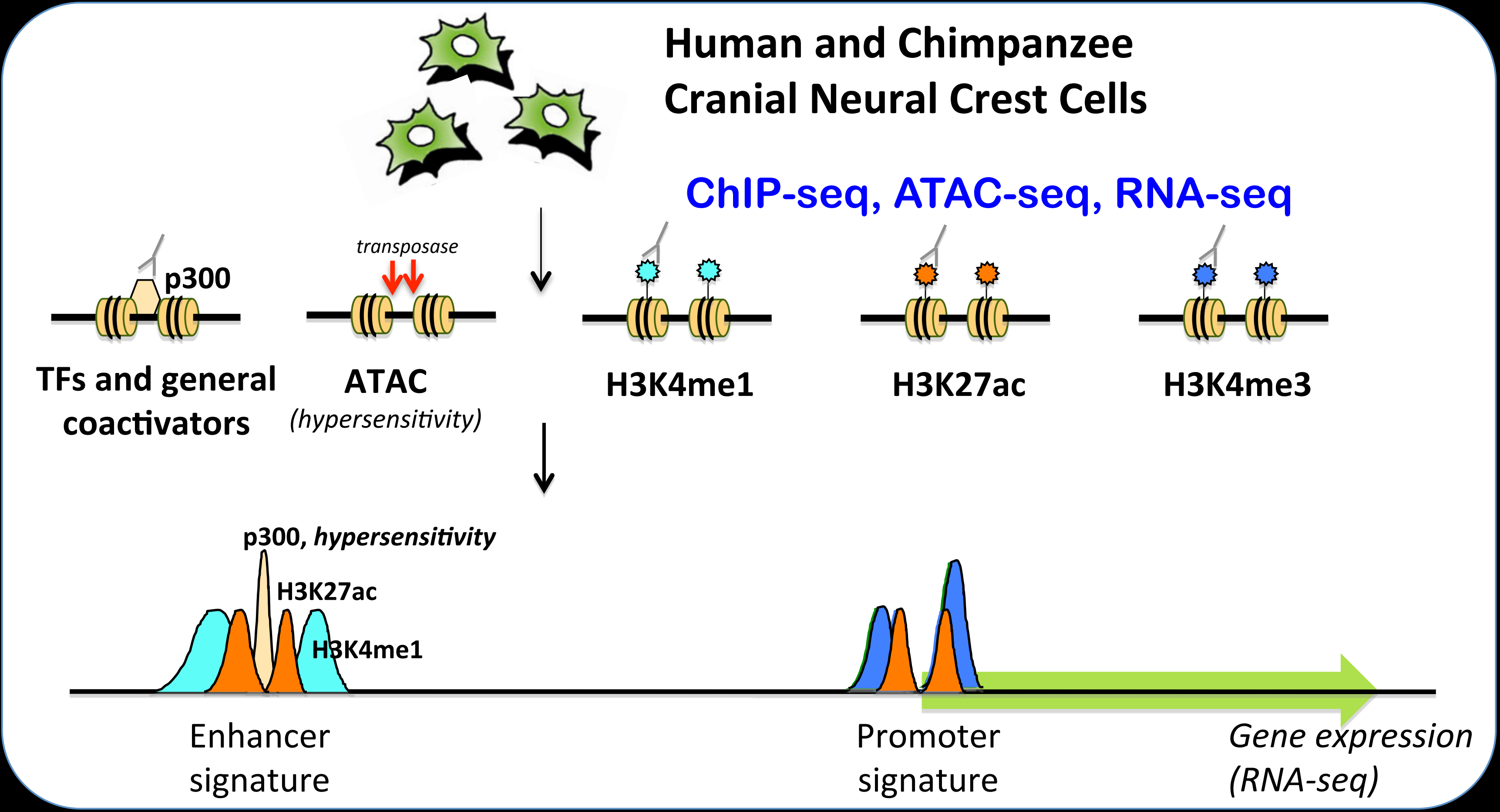
Establishment of the chimpanzee cranial neural crest model



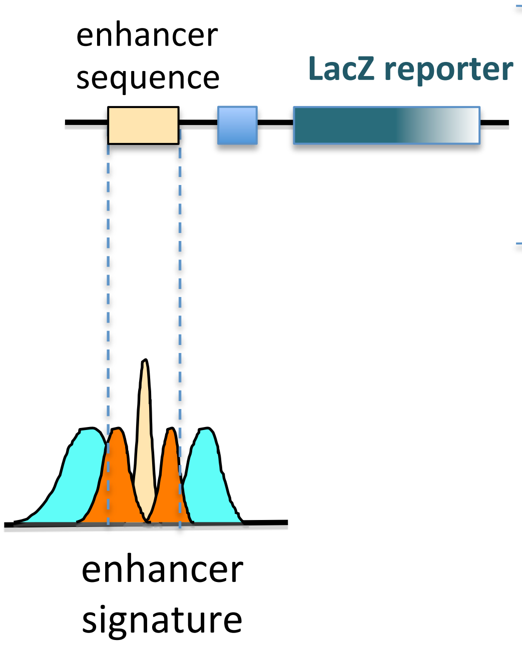
Sara Prescott



Epigenomic strategy for systematic annotation of primate cranial neural crest enhancers



Epigenomically mapped human CNCC enhancers drive craniofacial gene expression *in vivo*

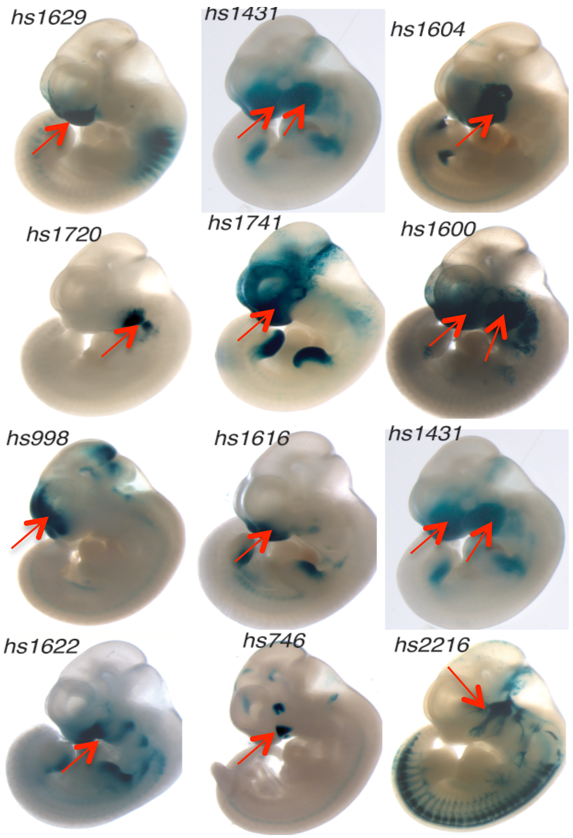


enhancer sequence

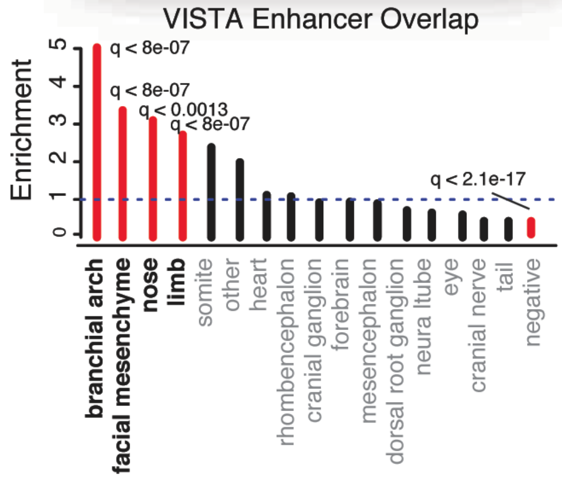
LacZ reporter

enhancer signature

Mouse embryo LacZ staining (e11.5)

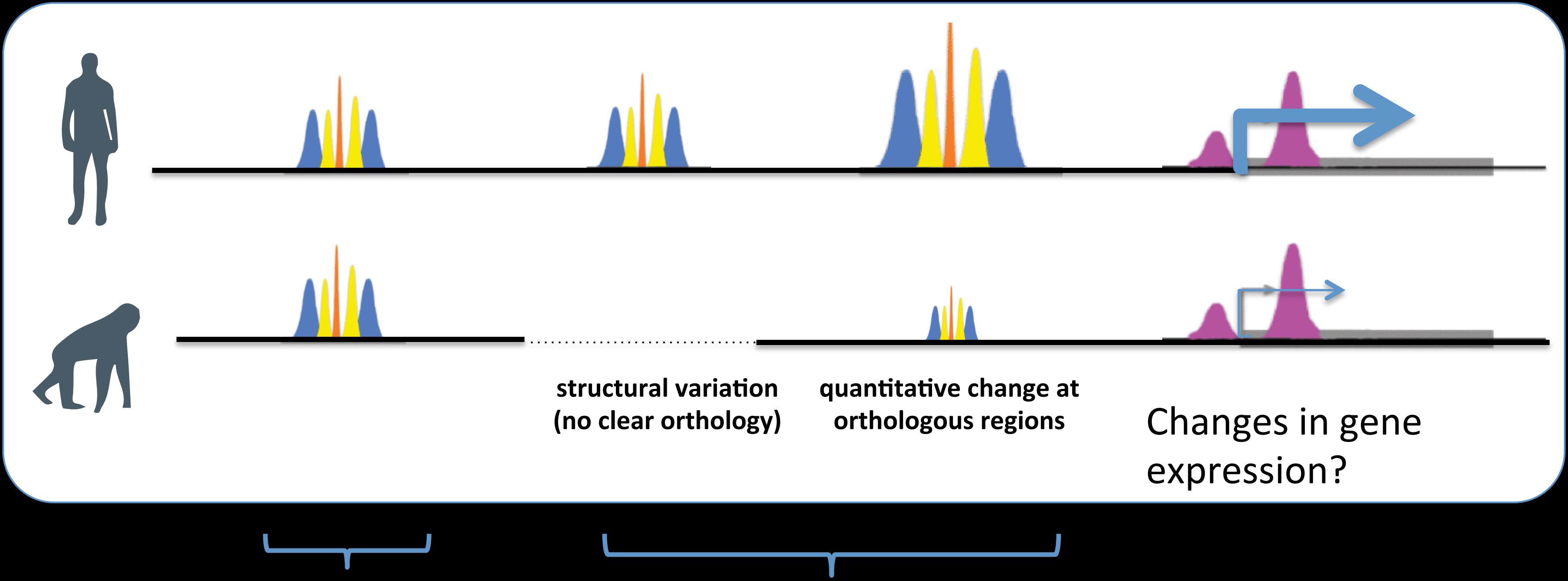


VISTA Enhancer Browser



247 regions overlapping active CNCC enhancers tested by the VISTA Enhancer Browser (L. Pennacchio, A. Visel)

Can we use epigenomics to experimentally map regulatory divergence in human and chimp neural crest cells?



structural variation (no clear orthology)

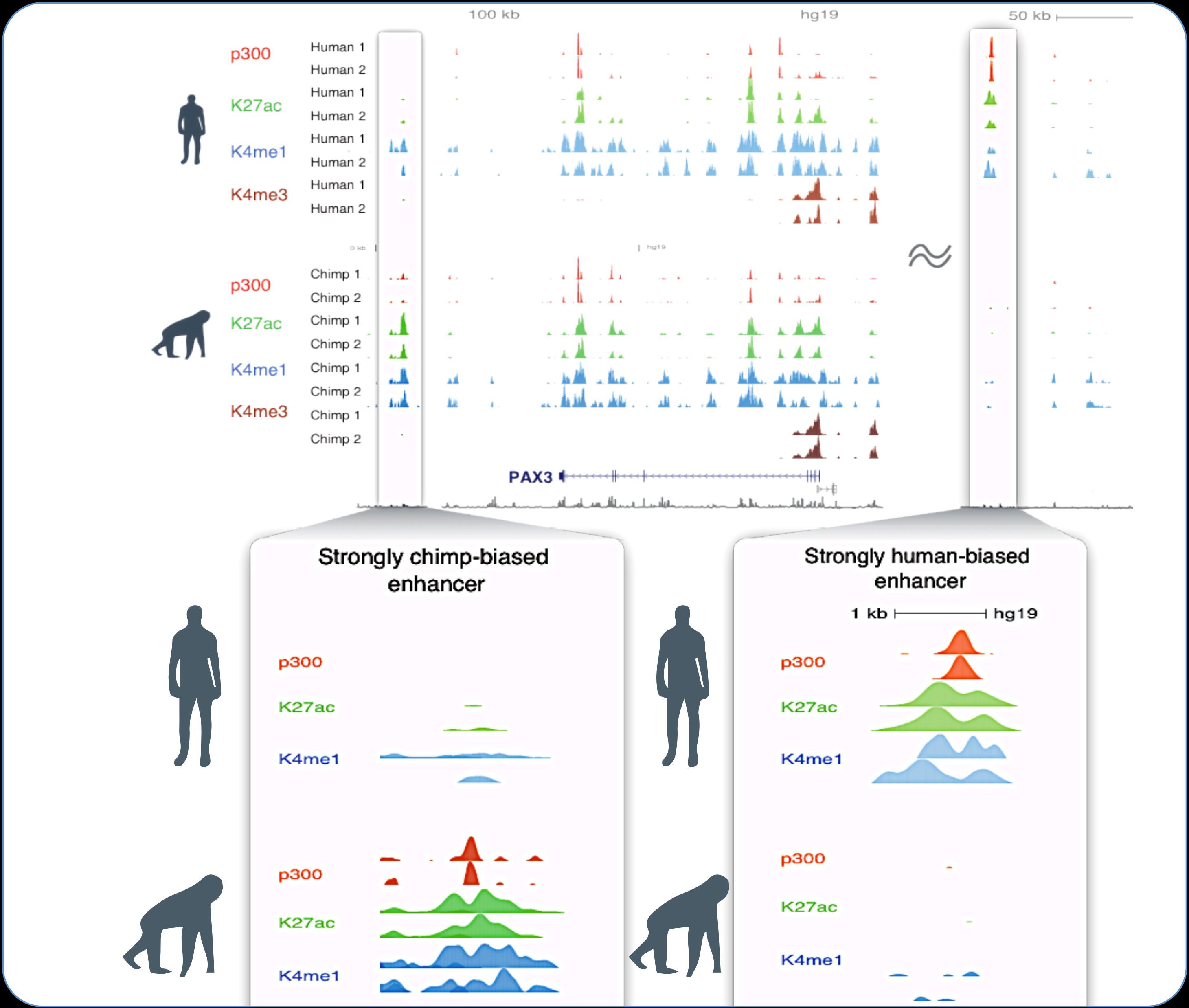
quantitative change at orthologous regions

Changes in gene expression?

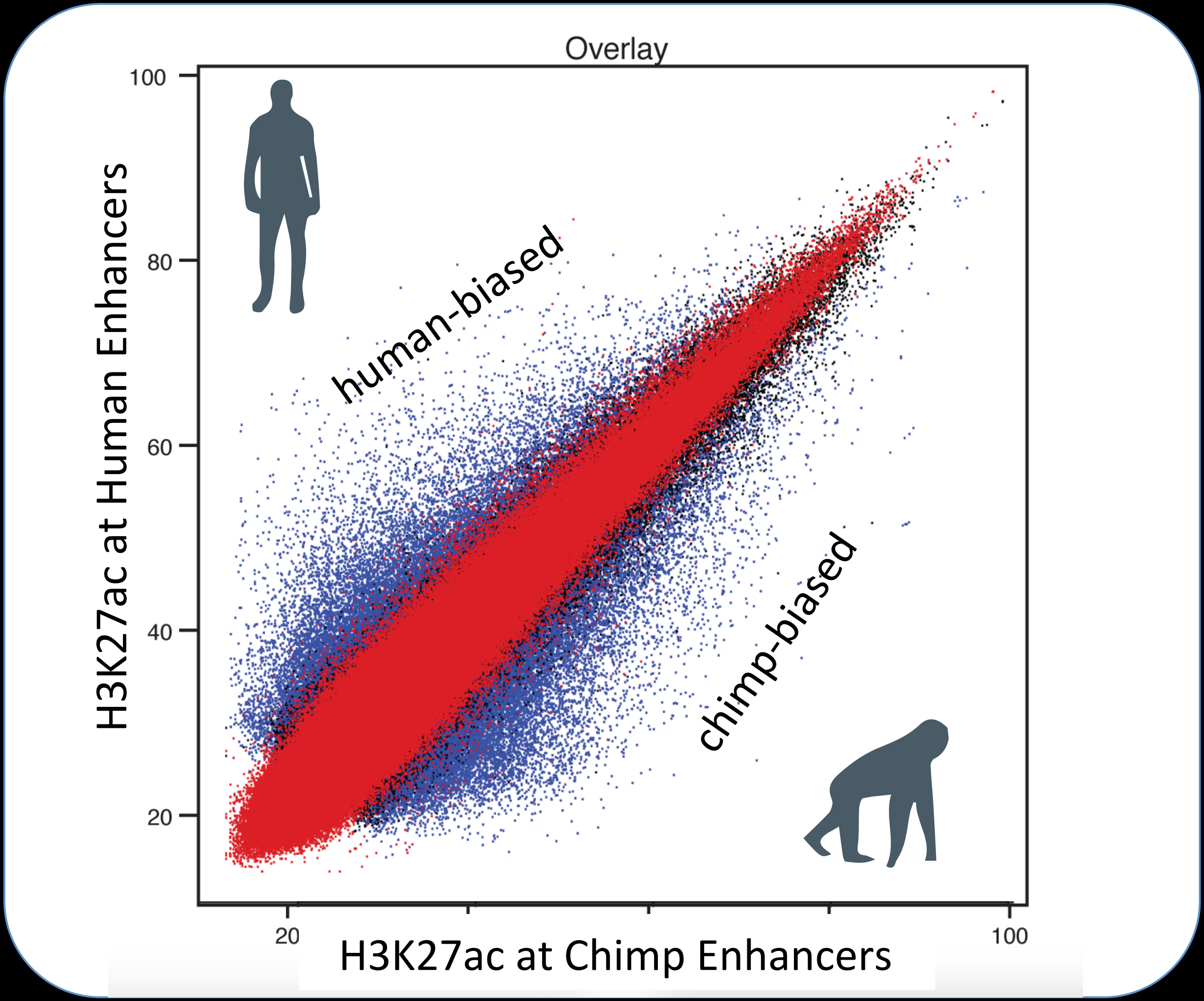
“Invariant” enhancers

“Species-biased” enhancers

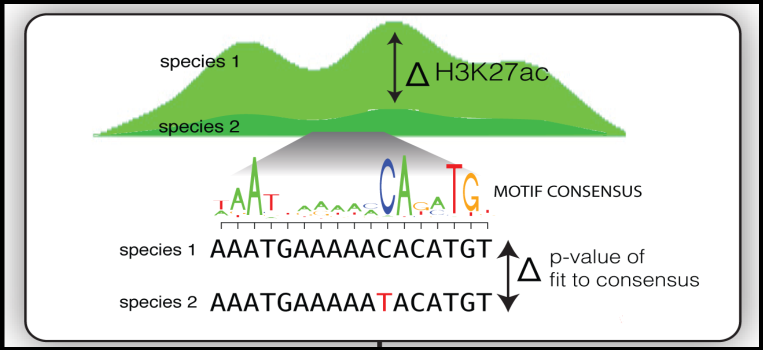
Discovery of species-biased enhancers



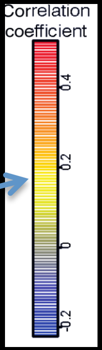
Quantitative epigenomic comparisons at orthologous regions identify species-biased enhancers genome-wide



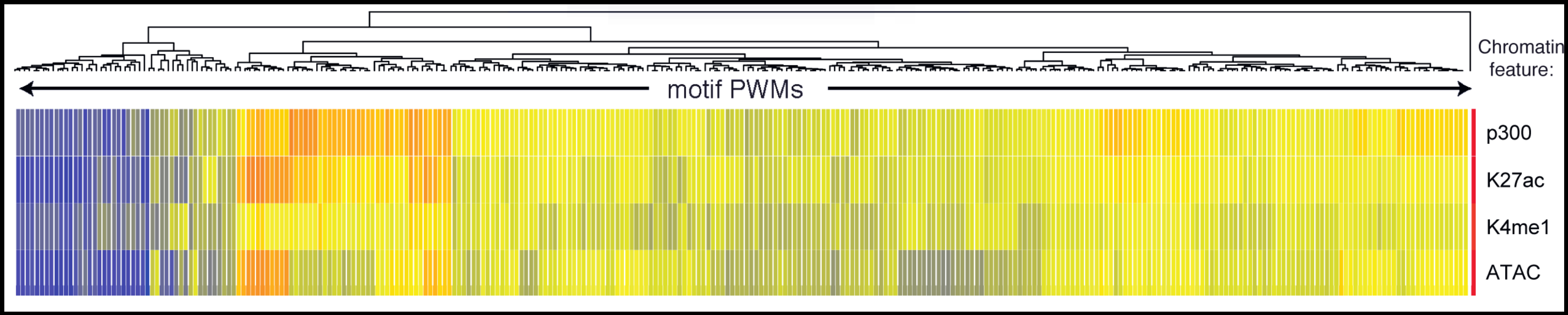
Can changes in specific transcription factor motifs explain epigenomic divergence?



Correlation coefficient

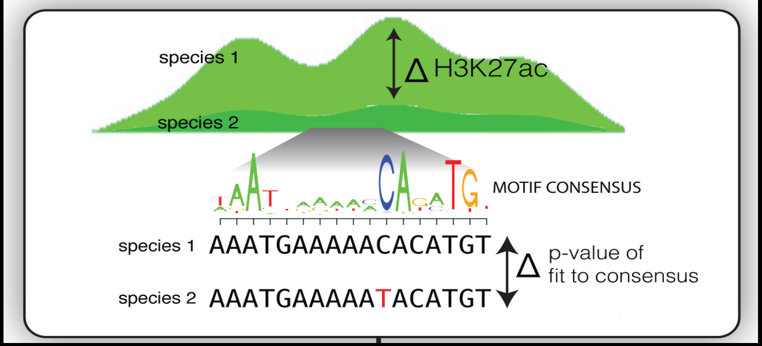


Correlation between motif changes and divergence of ChIP enrichments between species



Using interspecies genetic diversity like a large-scale enhancer mutagenesis screen

Can changes in specific transcription factor motifs explain epigenomic divergence?



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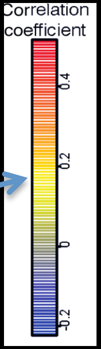

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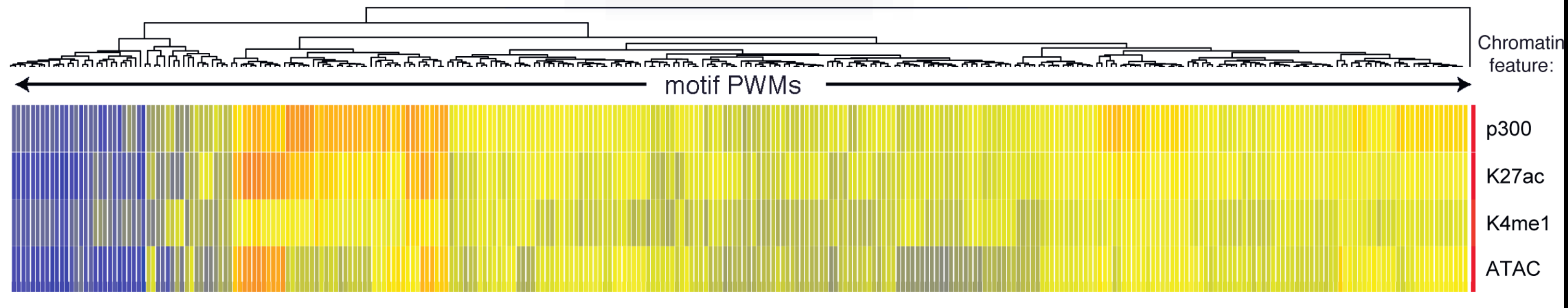
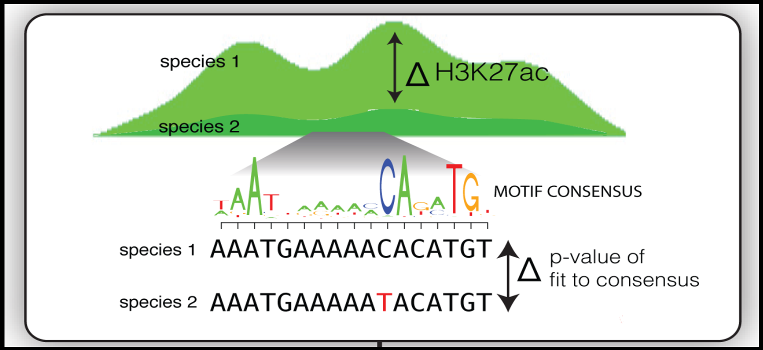


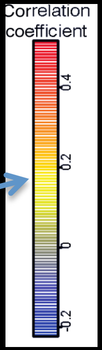
motifs **negatively** correlated with gain of active signatures (repressors)

motifs **positively** correlated with gain of active signatures (known and novel NC TFs)

Surprise: A singular outlier motif strongly predictive of permissive chromatin states



Correlation coefficient



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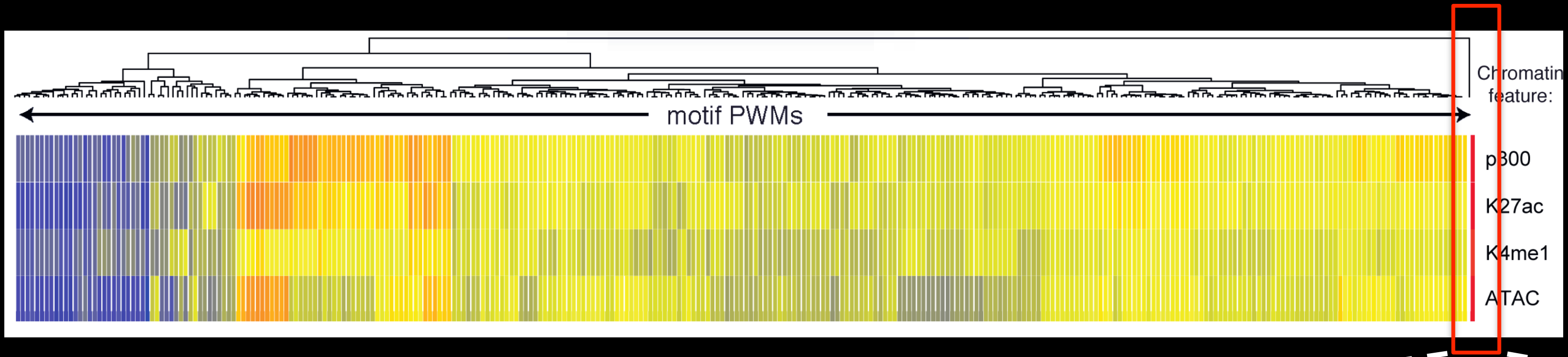
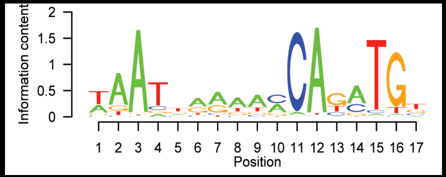


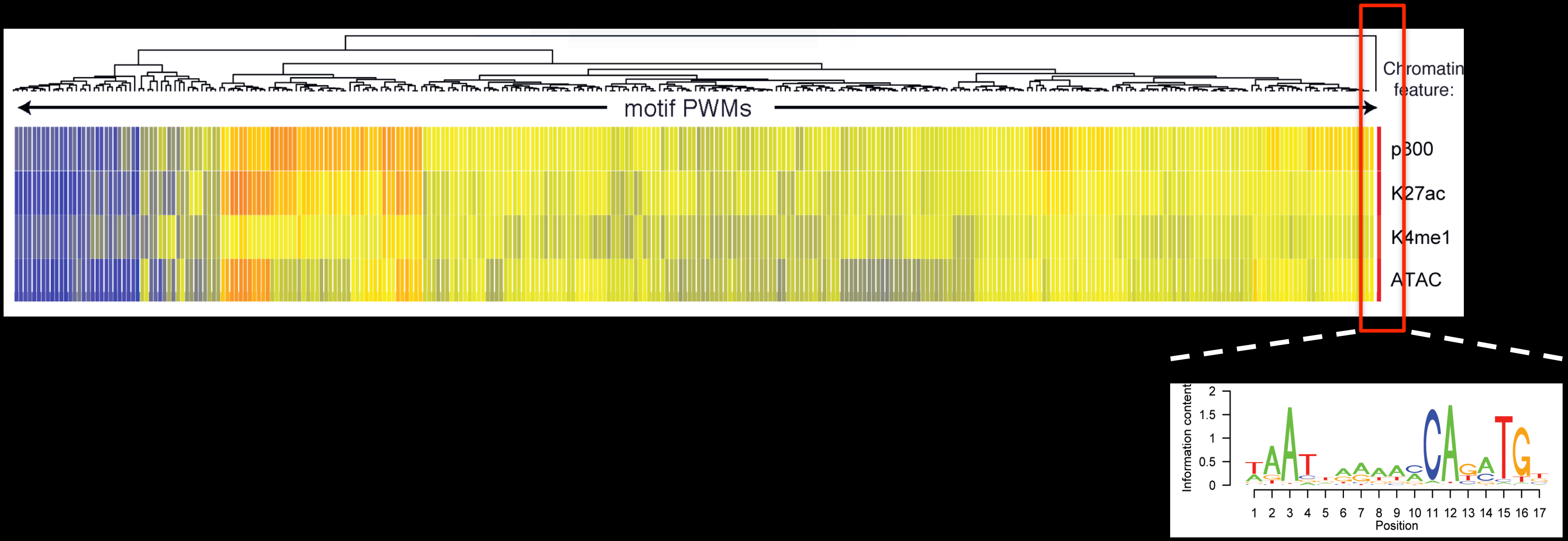


‘Coordinator’ – long novel motif

Surprise: A singular outlier motif strongly predictive of permissive chromatin states

‘Coordinator’ :

- Highly predictive of binding of other TFs
- Enriched at CNCC-selective enhancers as compared to pleiotropic enhancers, and at species-biased enhancers compared to invariant ones
- Sufficient to drive expression in reporter assays in CNCCs



‘Coordinator’ – long novel motif

Does the Coordinator motif license enhancer activity in the primate neural crest?

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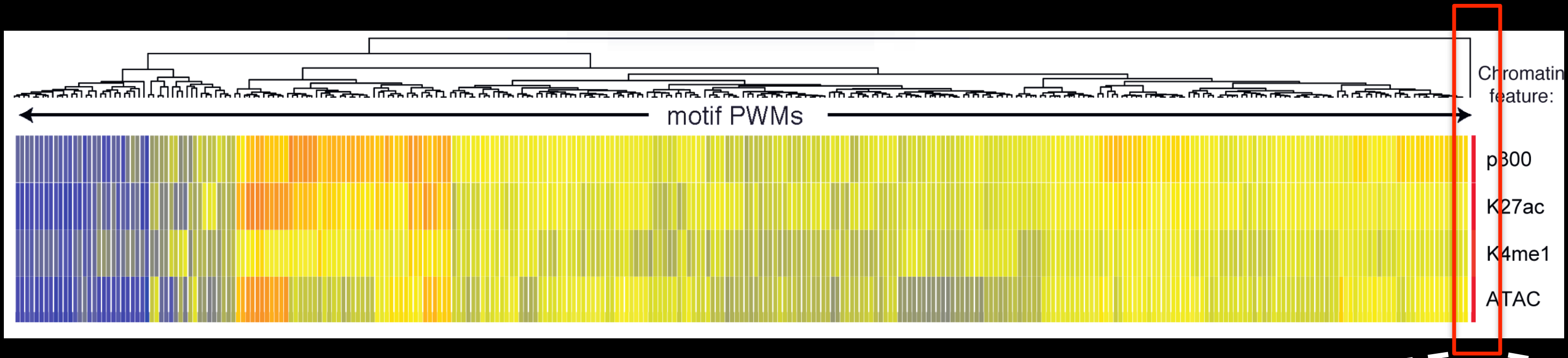
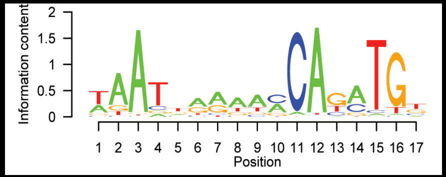


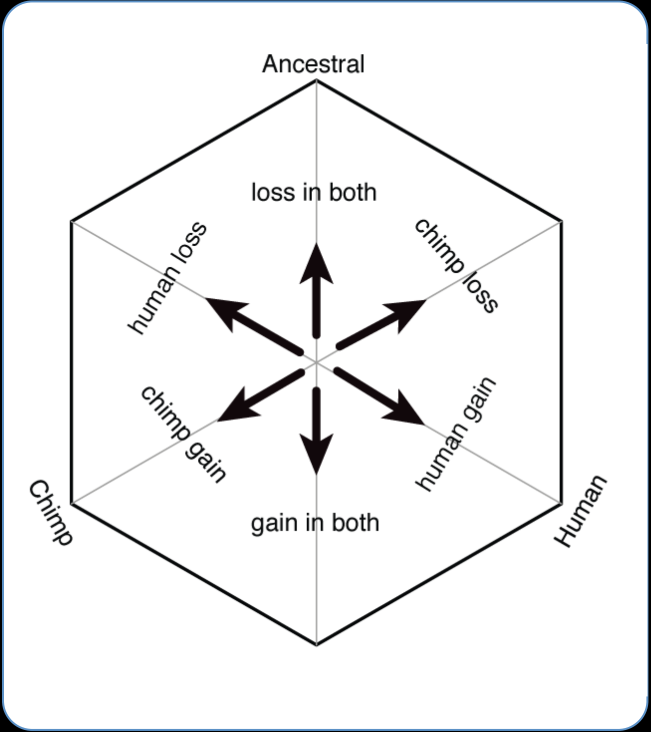
Does the Coordinator motif function in the primate neural crest like the TAGteam motif in *Drosophila* embryo, which binds a pioneer factor Zelda?
(*Ch. Rushlow, M. Eisen and others*)



‘Coordinator’ – long novel motif

Analysis of changes in Coordinator motif relative to ancestral states

Changes in Coordinator relative to ancestral state

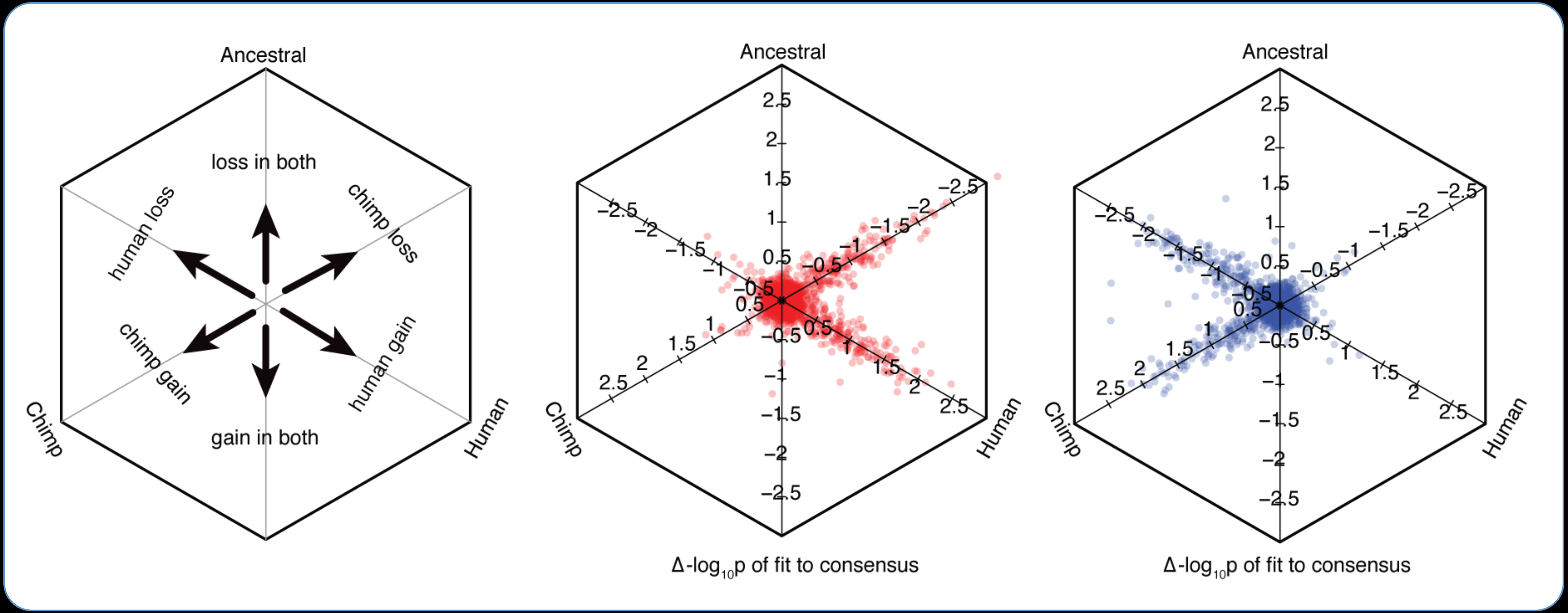


# Analysis of changes in Coordinator motif relative to ancestral states reveals equal contribution of gains and losses

Changes in Coordinator relative to ancestral state

Coordinator motif changes at human-biased enhancers

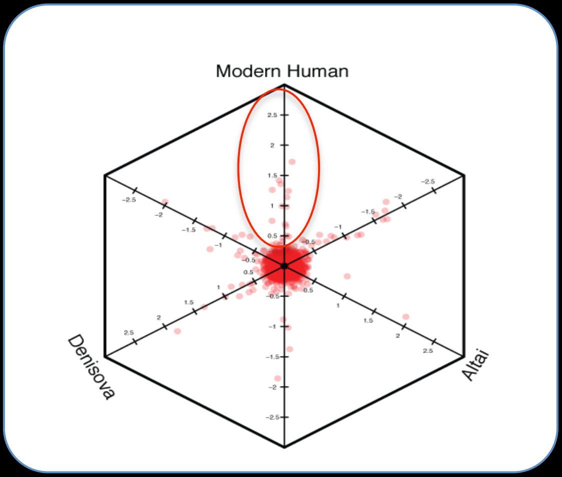
Coordinator motif changes at chimp-biased enhancers



Most changes in Coordinator motif occurred prior to split of humans from other hominins, but some appear human-specific

human skull

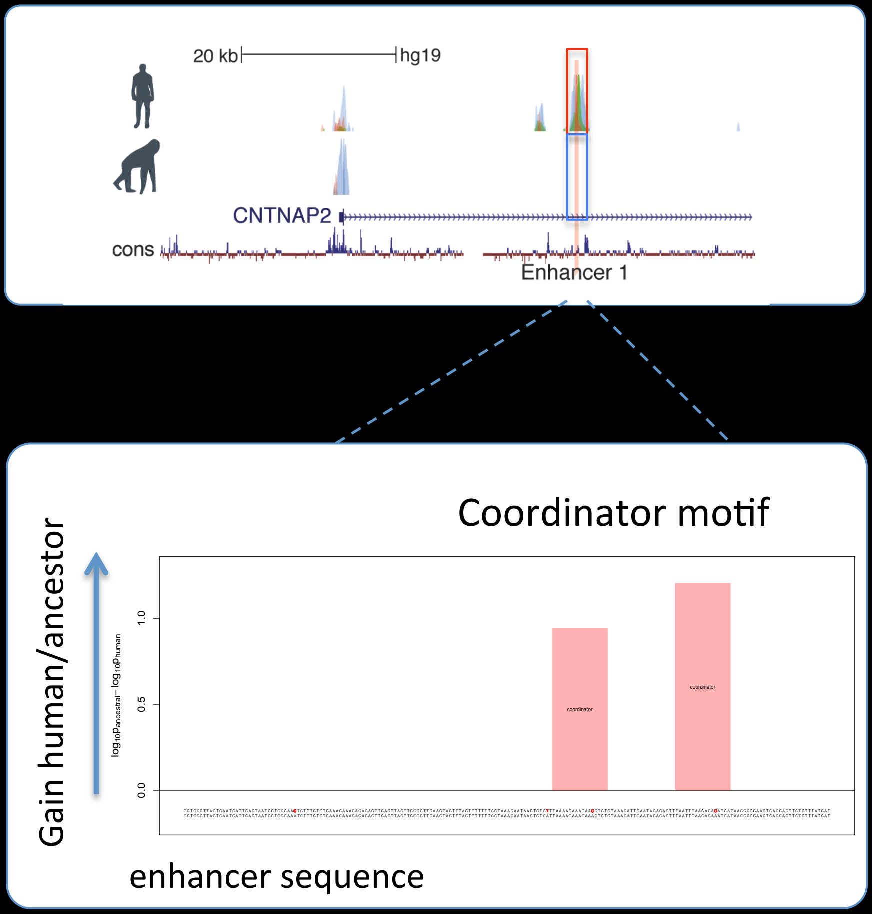
?



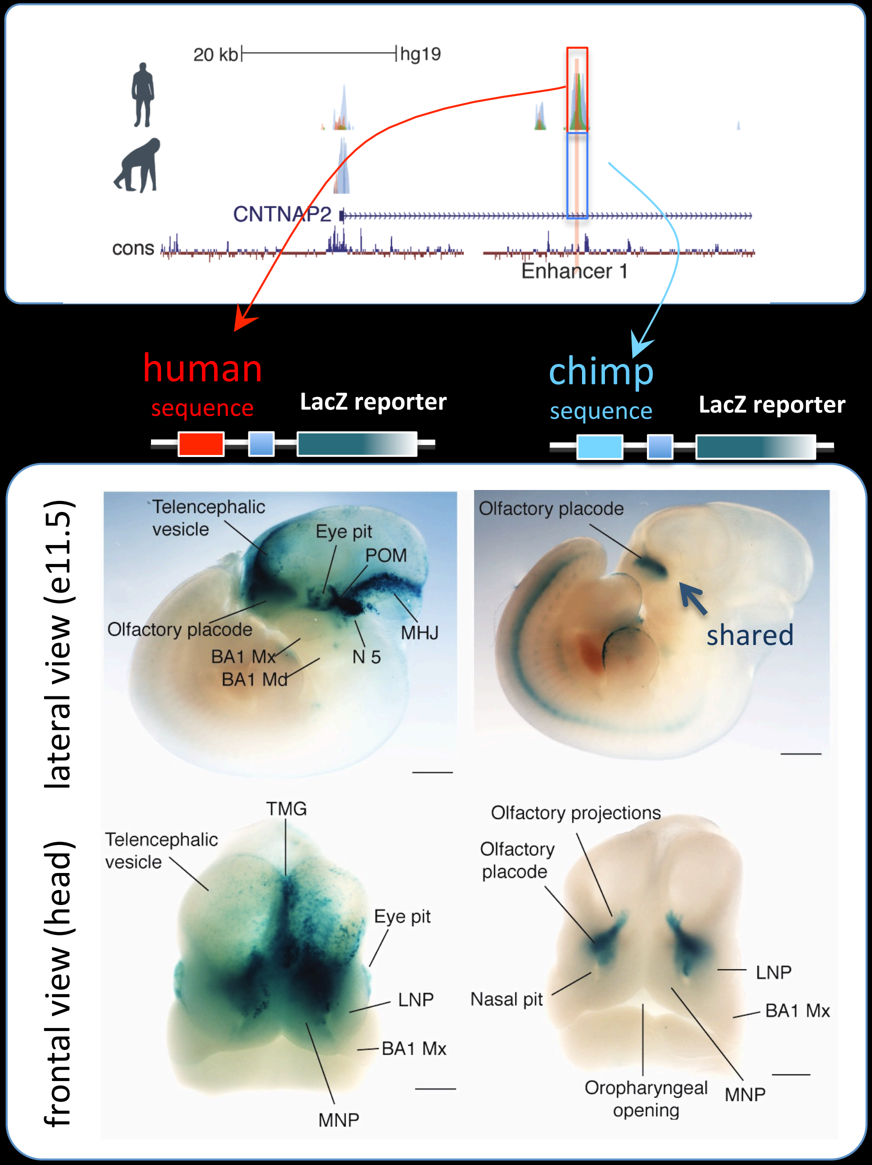
Coordinator motif changes
at <u>human-biased</u> enhancers

Neanderthal skull
(to scale)

An example of human-biased enhancer with human-specific gains of the Coordinator motif



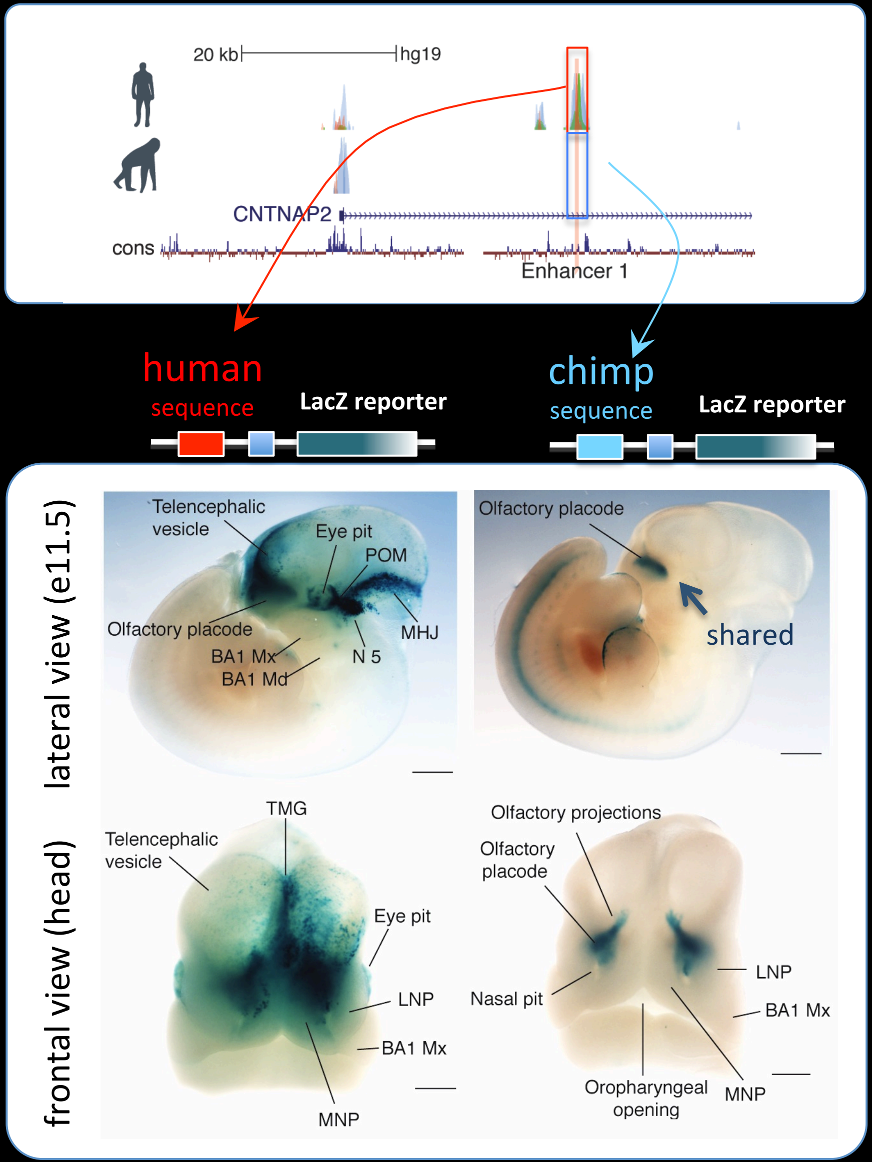
Species biased enhancers show divergent activity in head structures *in vivo*

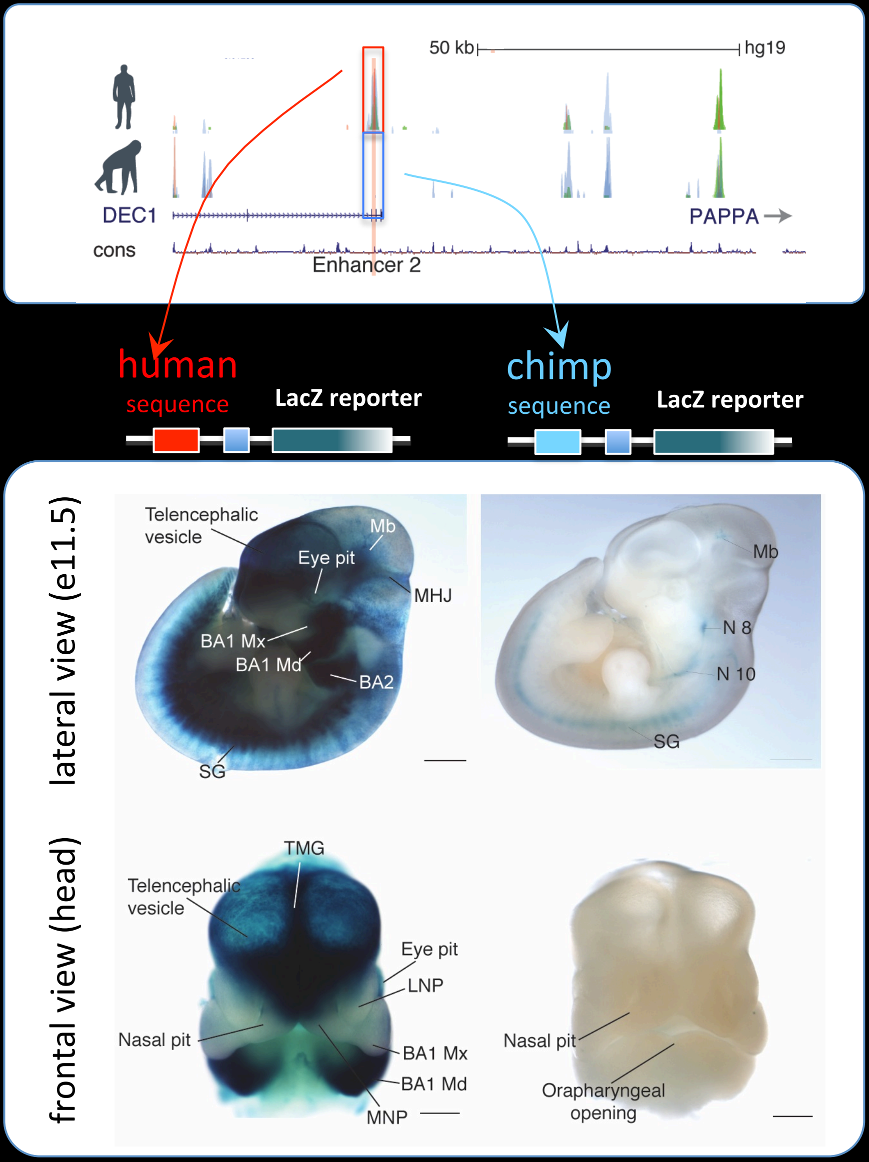


Licia Selleri

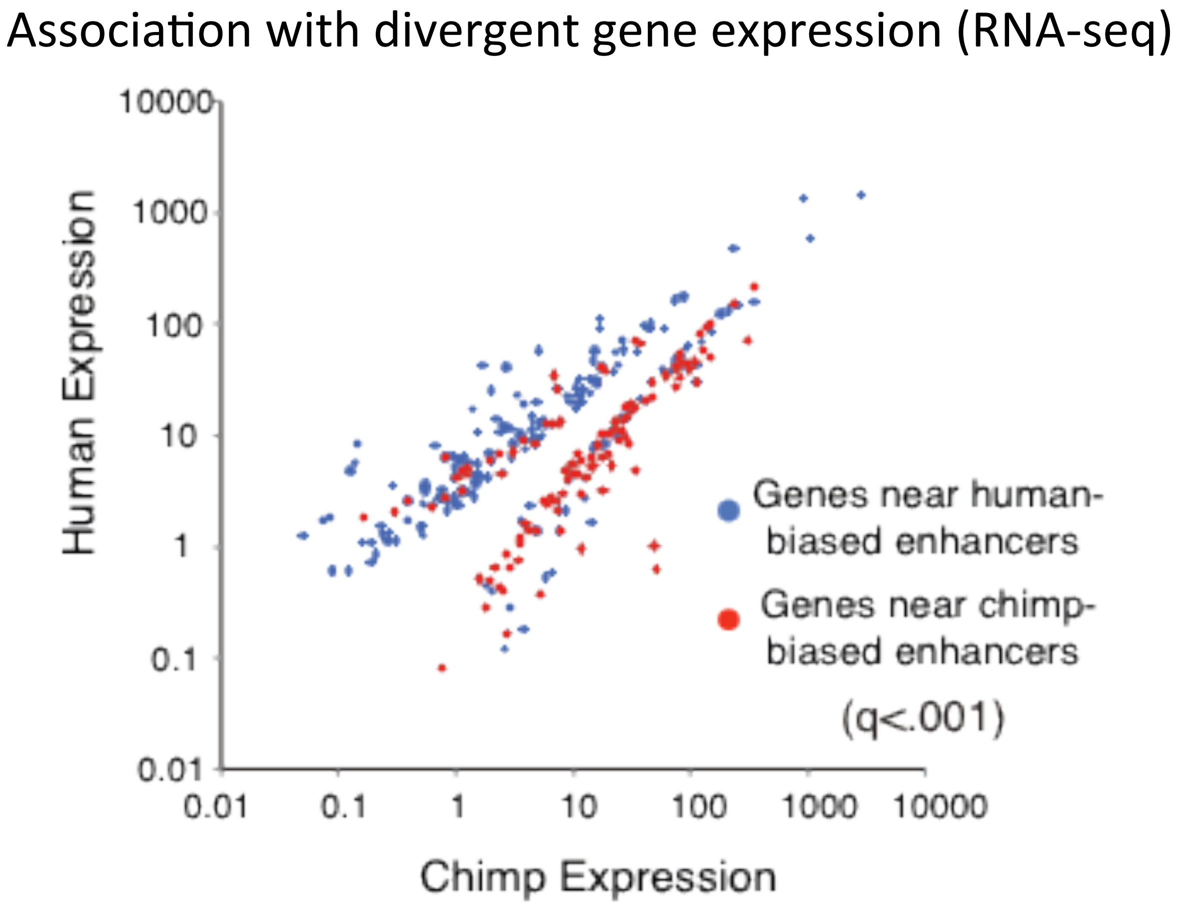
(UCSF)

Species biased enhancers show divergent activity in head structures *in vivo*



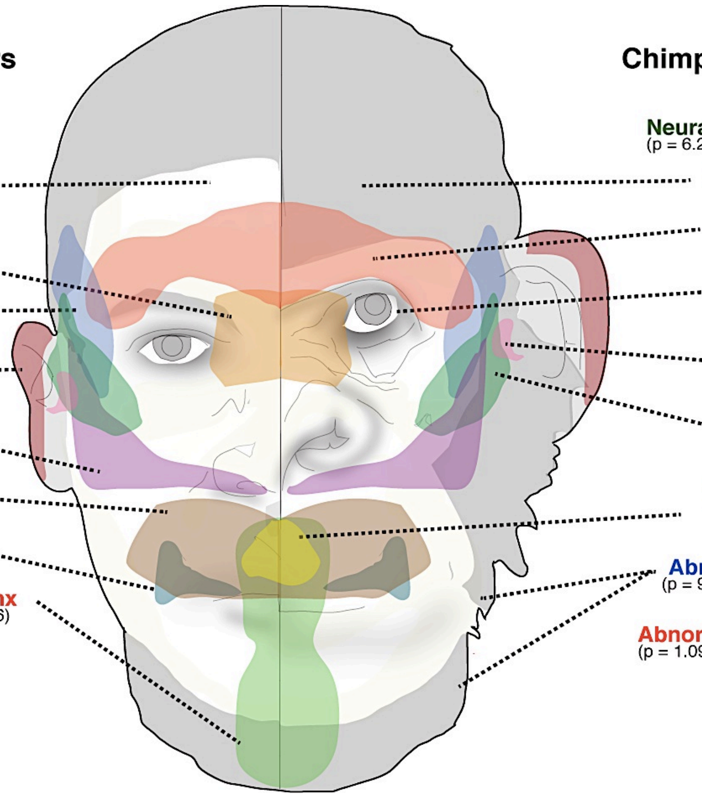


Species-biased enhancers are associated with genes showing species-biased expression in the neural crest



Association with divergent gene expression (RNA-seq)

Ontology annotation of species-biased enhancers reveals associations with genes involved in development and malformation of various craniofacial structures



Human-Biased Enhancers

Head development
(p = 1.8150e-3)

Abnormal frontal bone morphology
(p = 2.4005e-6)

Hypotelorism
(p = 1.9659e-5)

Pterygoid bone hypoplasia
(p = 5.9320e-6)

Small earlobe
(p = 2.8384e-2)

Small basisphenoid bone (skull base)
(p = 5.4149e-7)

Decreased maxillary shelf size
(p = 2.5332e-6)

Odontogenesis
(p = 3.8073e-4)

Abnormality of the pharynx
(p = 2.7420e-6)

Chimp-Biased Enhancers

Neural crest formation
(p = 6.2532e-5)

Abnormal neurocranium morphology
(p = 1.0341e-6)

Frontal bossing
(p = 2.5071e-4)

Blue sclerae
(p = 3.5665e-4)

Absent tympanic membrane
(p = 1.1974e-6)

Abnormal squamosal bone morphology
(p = 5.4816e-6)

Hypoplasia of the epiglottis
(p = 1.0983e-4)

Abnormal branchial arch morphology
(p = 9.6781e-5)

Abnormality of the adrenal glands
(p = 1.0982e-2)

Human Phenotype
Mouse Phenotype
Biological Process

Examples of species-biased genes with dosage-dependent effects on facial morphology

Chimp biased in expression and enhancer landscapes:

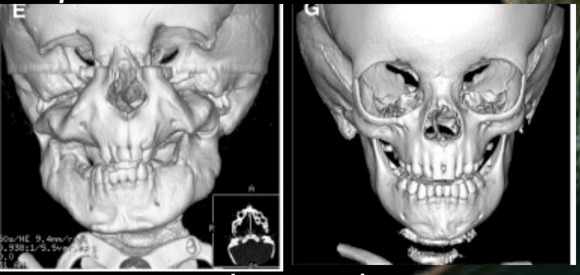
PAX3

- haploinsufficiency in humans associated with reduced jaw size and pigmentation, depressed nasal bridge *(Waardenburg syndrome)*

EDNRA

- heterozygous mutations in humans are associated with a striking transformation of skull shape

patient *normal*



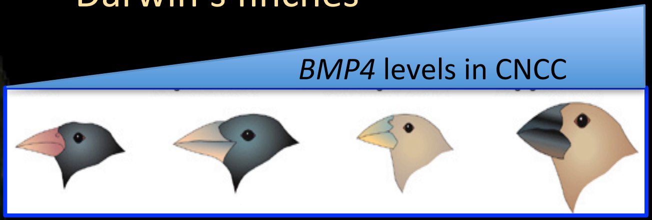
Gordon et al., 2015

Examples of species-biased genes with dosage-dependent effects on facial morphology

Human biased in expression and enhancer landscapes:

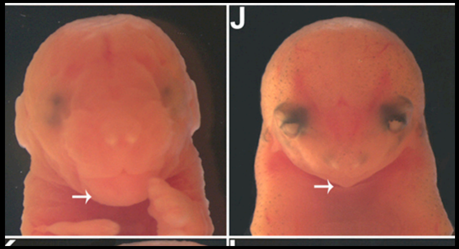
BMP4

- variation of beak morphology in Darwin's finches



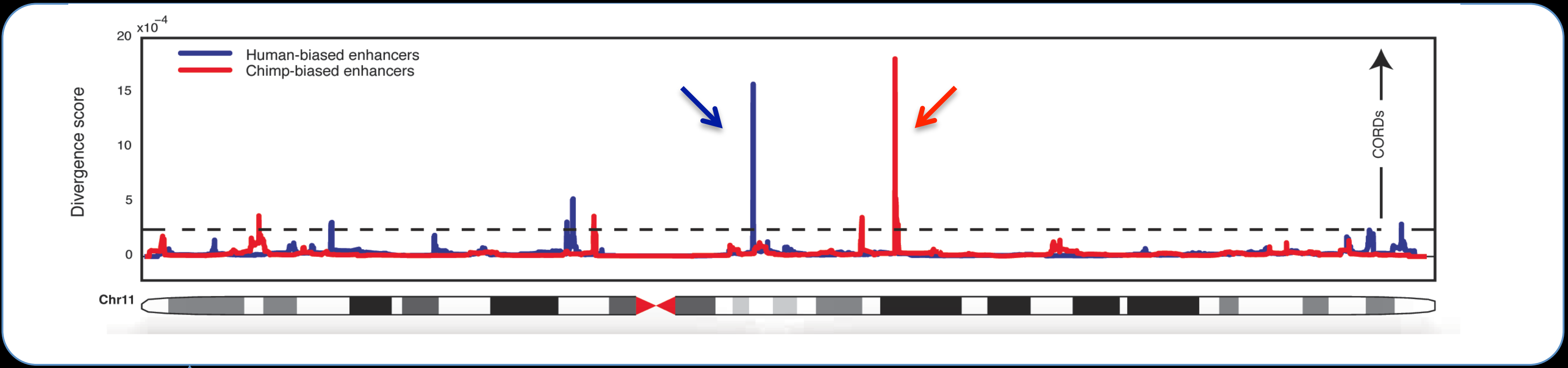
- elevated expression of *BMP4* in the mouse CNCC results in jaw size reduction and rounding of the skull shape

control *BMP4 OE*



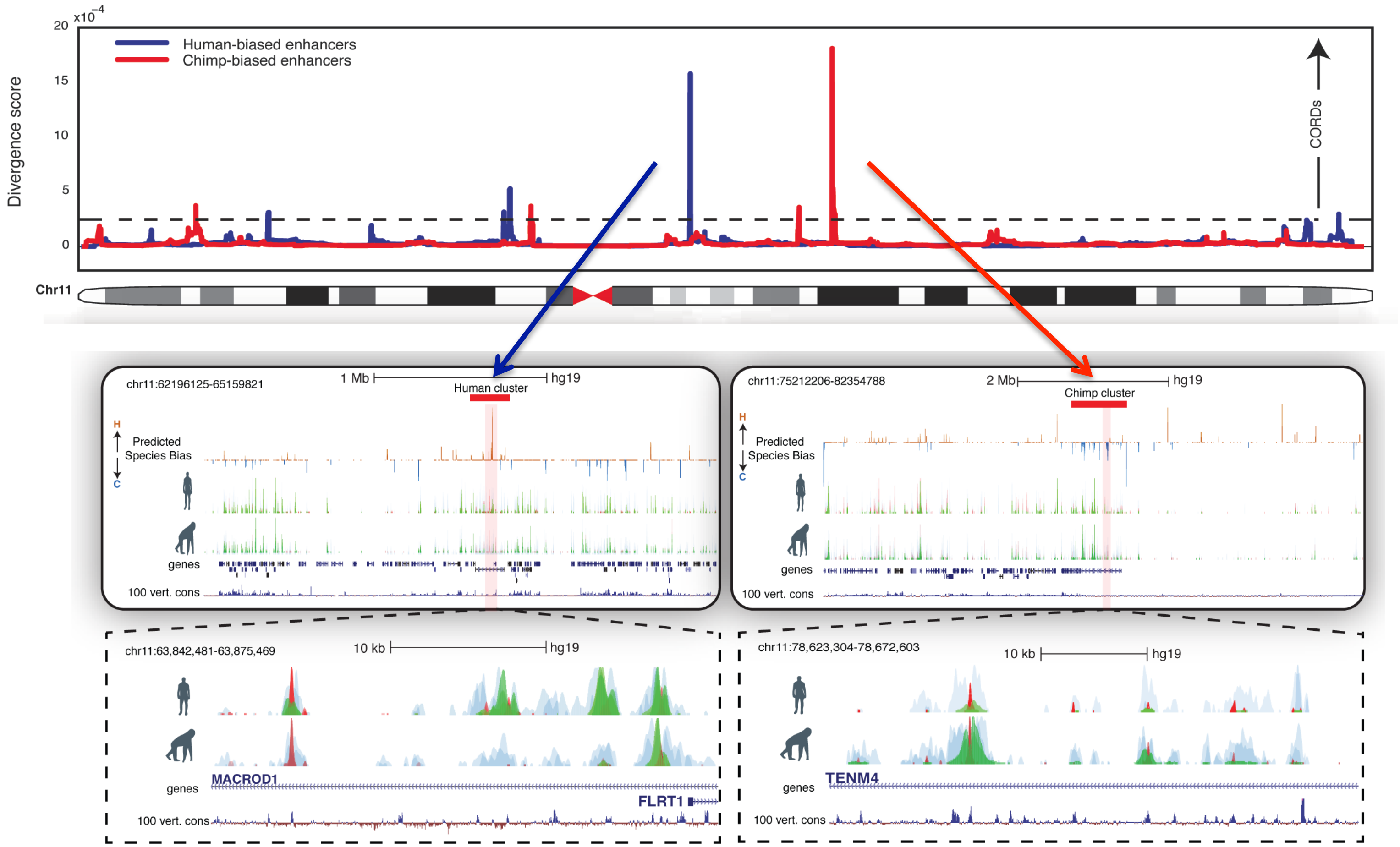
Bonilla-Claudio et al., 2012

Species-biased enhancers are not distributed evenly across chromosomes

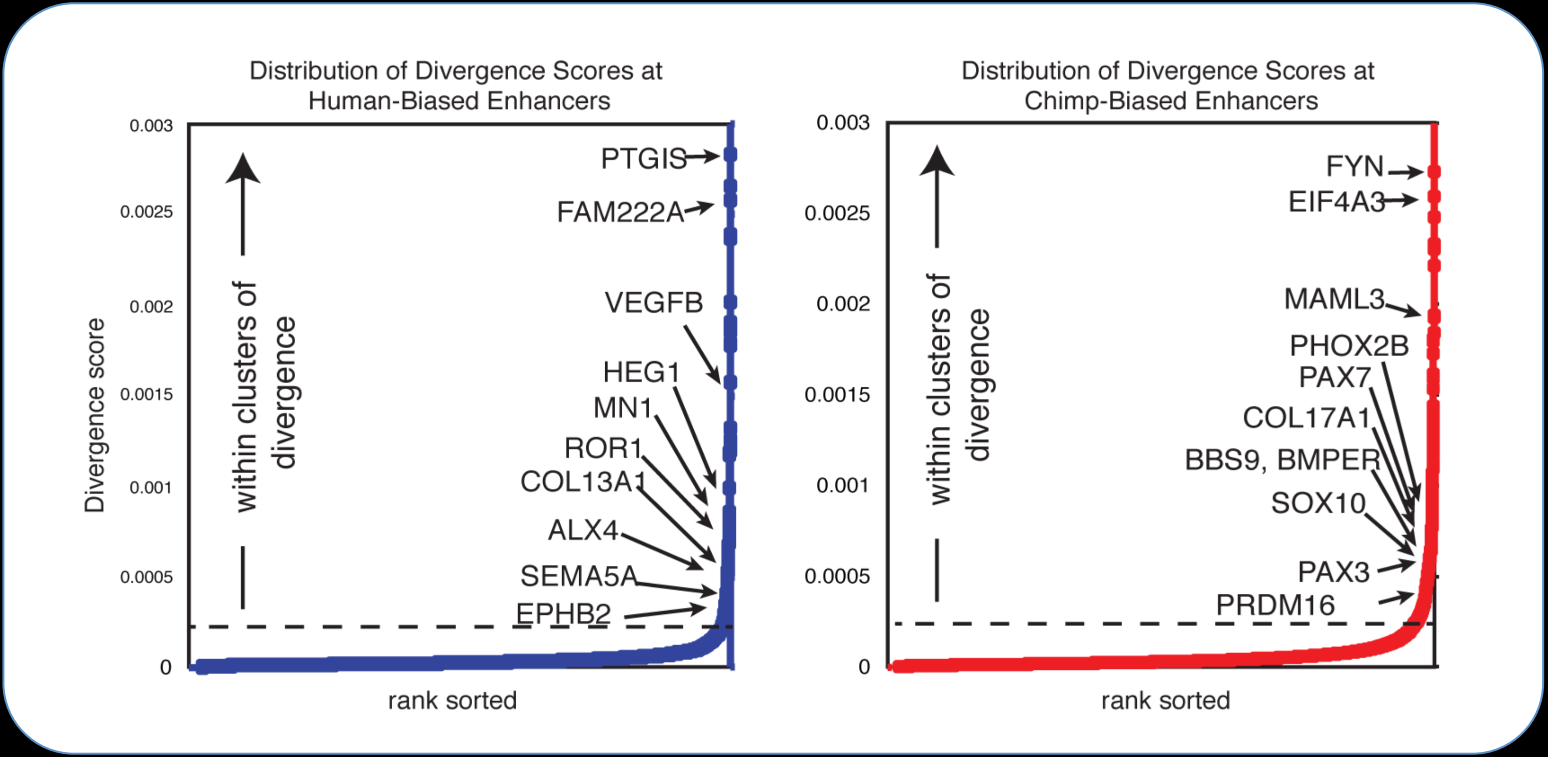


Quantitative measure of enhancer bias across chromosome 11

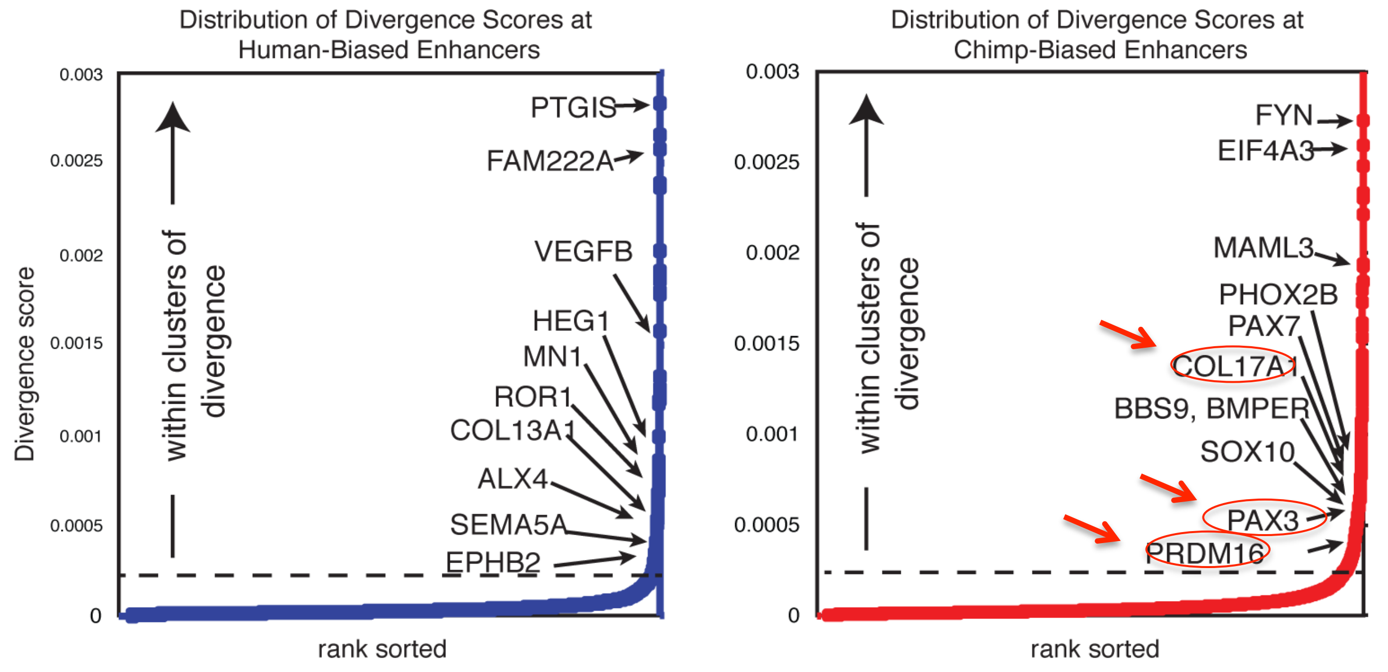
About 10% of species-biased enhancers fall within clusters of high regulatory divergence



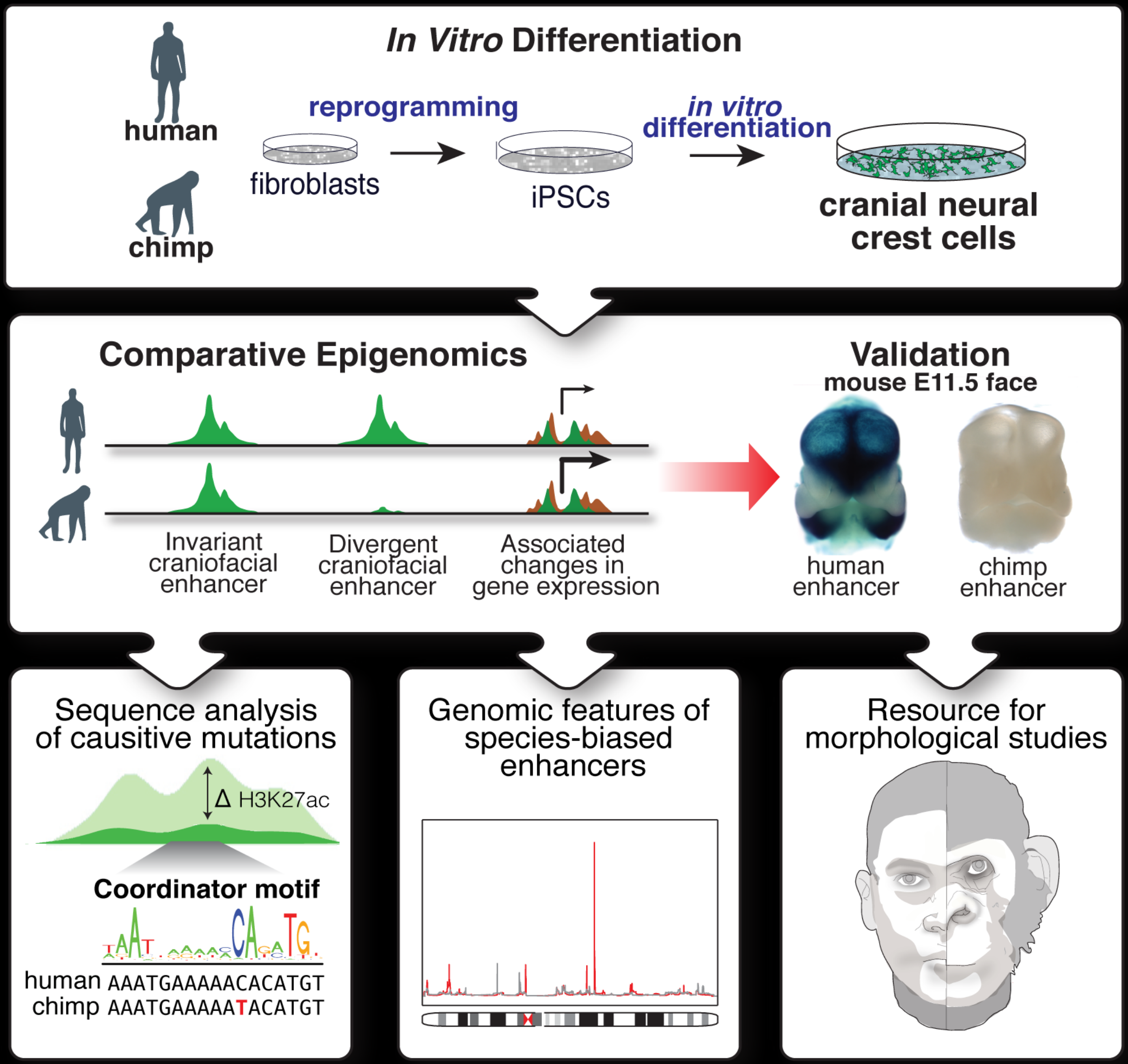
Clusters of high regulatory divergence can be identified across the whole genome



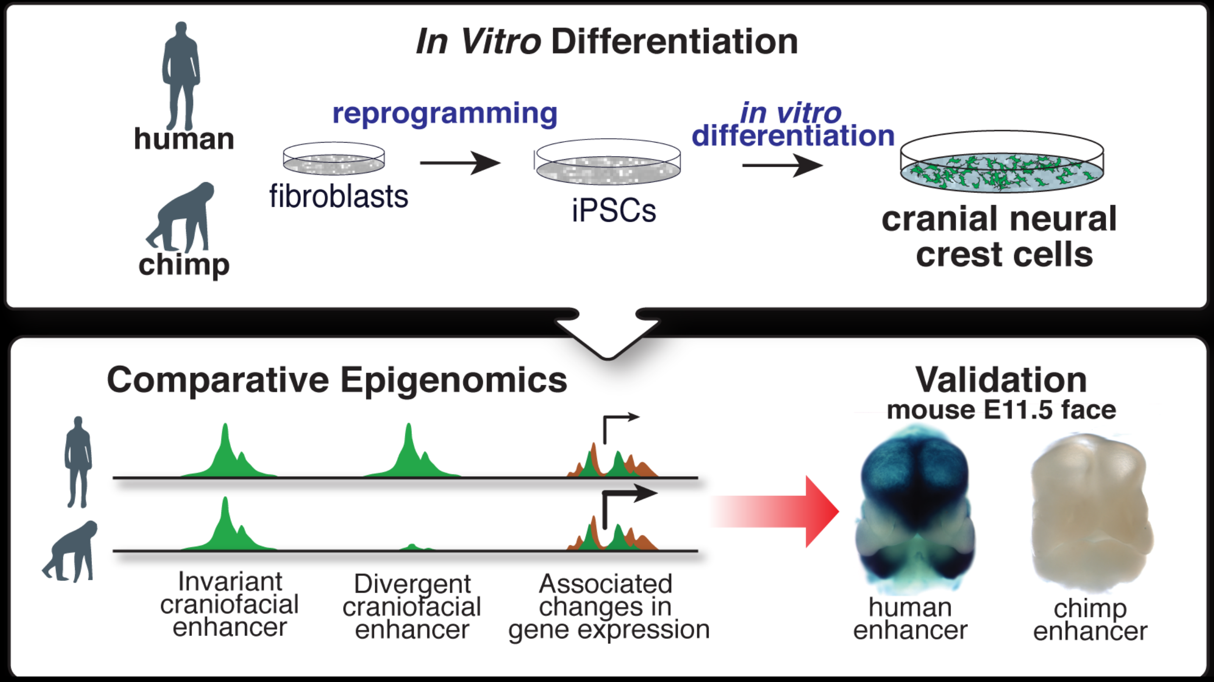
Clusters of high regulatory divergence overlap loci implicated in normal-range human facial variation in GWAS studies



Summary :


In Vitro Differentiation
human
chimp
reprogramming
fibroblasts
iPSCs
in vitro differentiation
cranial neural crest cells
Comparative Epigenomics
Invariant craniofacial enhancer
Divergent craniofacial enhancer
Associated changes in gene expression
Validation
mouse E11.5 face
human enhancer
chimp enhancer
Sequence analysis of causitive mutations
Δ H3K27ac
Coordinator motif
human AAATGAAAAACACATGT
chimp AAATGAAAAATACATGT
Genomic features of species-biased enhancers
Resource for morphological studies

FaceBase deliverables :



- ChIP-seq: Histone modifications (H3K27ac, H3K4me1, H3K4me3, H3K27me3), coactivator (p300), transcription factors (NR2F1, TFAP2A)
- chromatin accessibility (ATACseq)
- gene expression (RNAseq)

three different human lines and two chimp lines; many datasets in replicates to increase robustness

5 constructs completed; more in progress for 2016/2017

Collaboration with the A. Visel spoke

Functional manipulation?