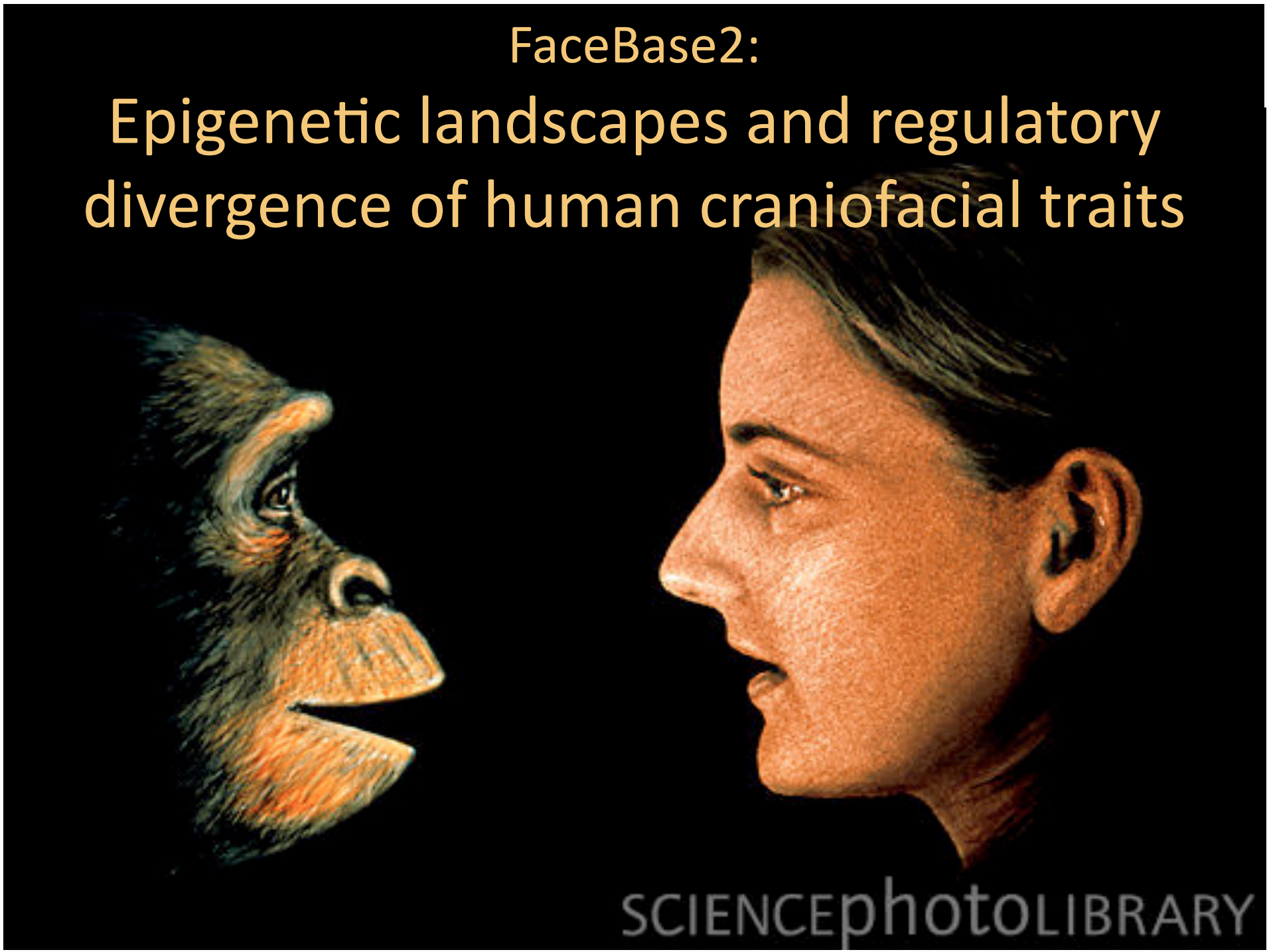
FaceBase2:

Epigenetic landscapes and regulatory divergence of human craniofacial traits



Team members at the meeting:

Licia Selleri
(Weill Cornell School of Medicine)

Sara Prescott
(Stanford)

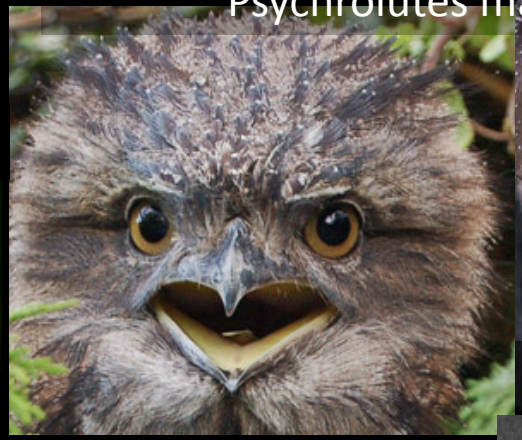
Tomek Swigut
(Stanford)

Cranial neural crest and fantastic variation of vertebrate craniofacial form

Gekko gecko

Psychrolutes marcidus

Anoplogaster cornuta

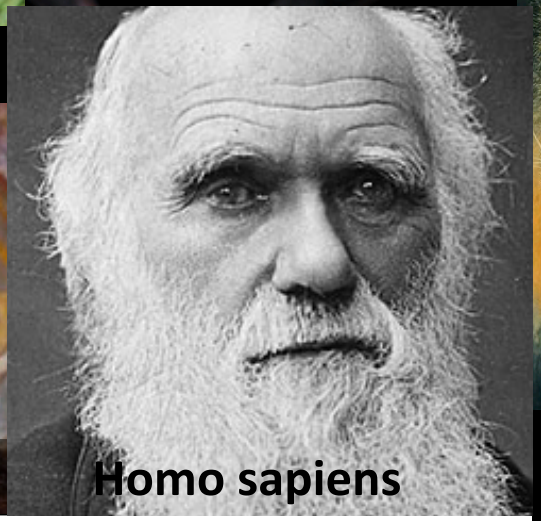


Podargus strigoides

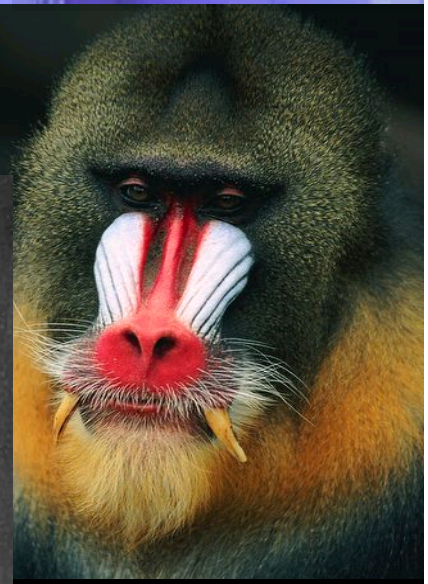
Coelops robinsoni

Balaeniceps rex

Lepidobatrachus laevis



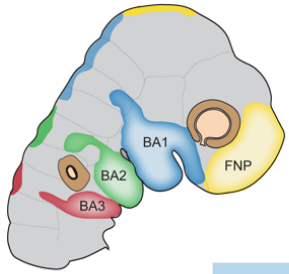
Homo sapiens



Mandrillus sphinx

What (molecularly) makes our faces human?

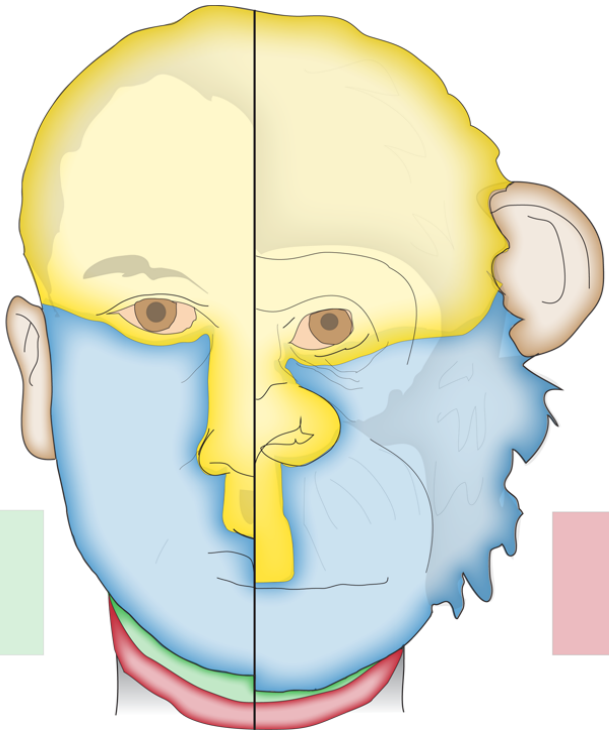
Cranial neural crest derived structures are strong targets of selective pressure and are divergent between humans and chimps



All arches:
Hair and Pigmentation
Bone density

Branchial Arch 1 (BA1)
Jaw and palate shape
Middle ear and auditory apparatus
Motorsensory control of face and mouth (CN V)

Branchial Arch 2 (BA2)
Muscles and nerves of facial expression
Middle ear muscles and bones

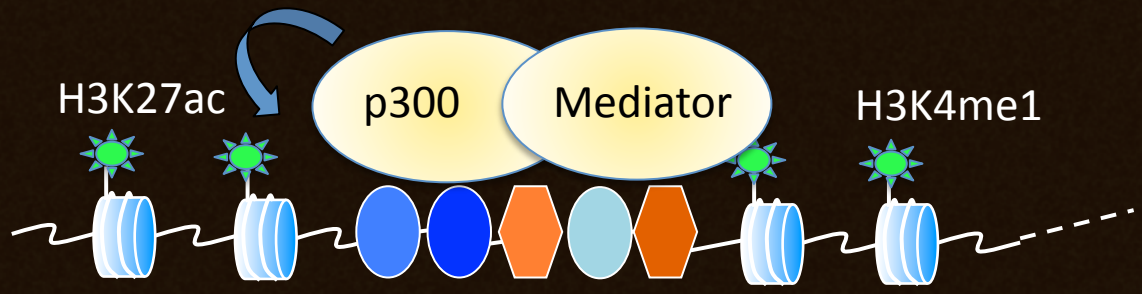


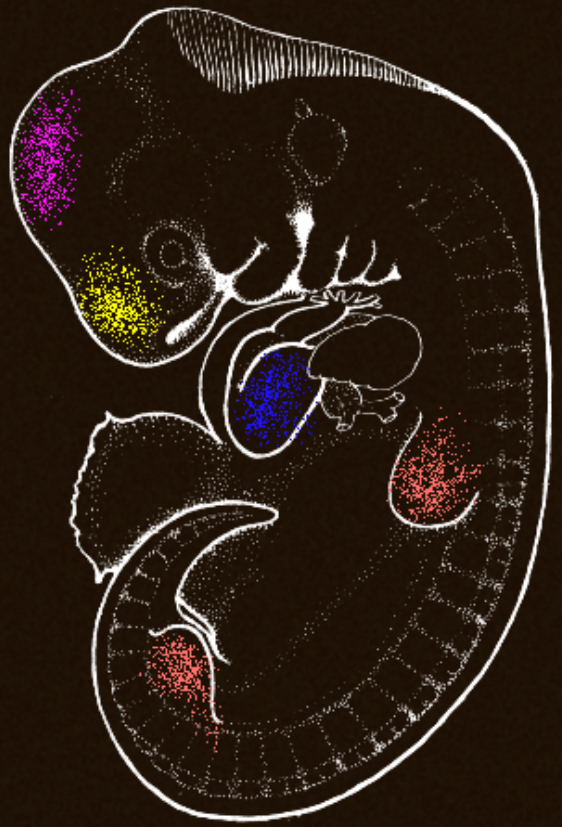
Frontonasal prominence (FNP)
Brow and forehead shape
Nasal bridge morphology
Eye position
Philtrum size
Tuning of olfaction

Placodal derivatives
Ear size, shape and orientation
Visual acuity

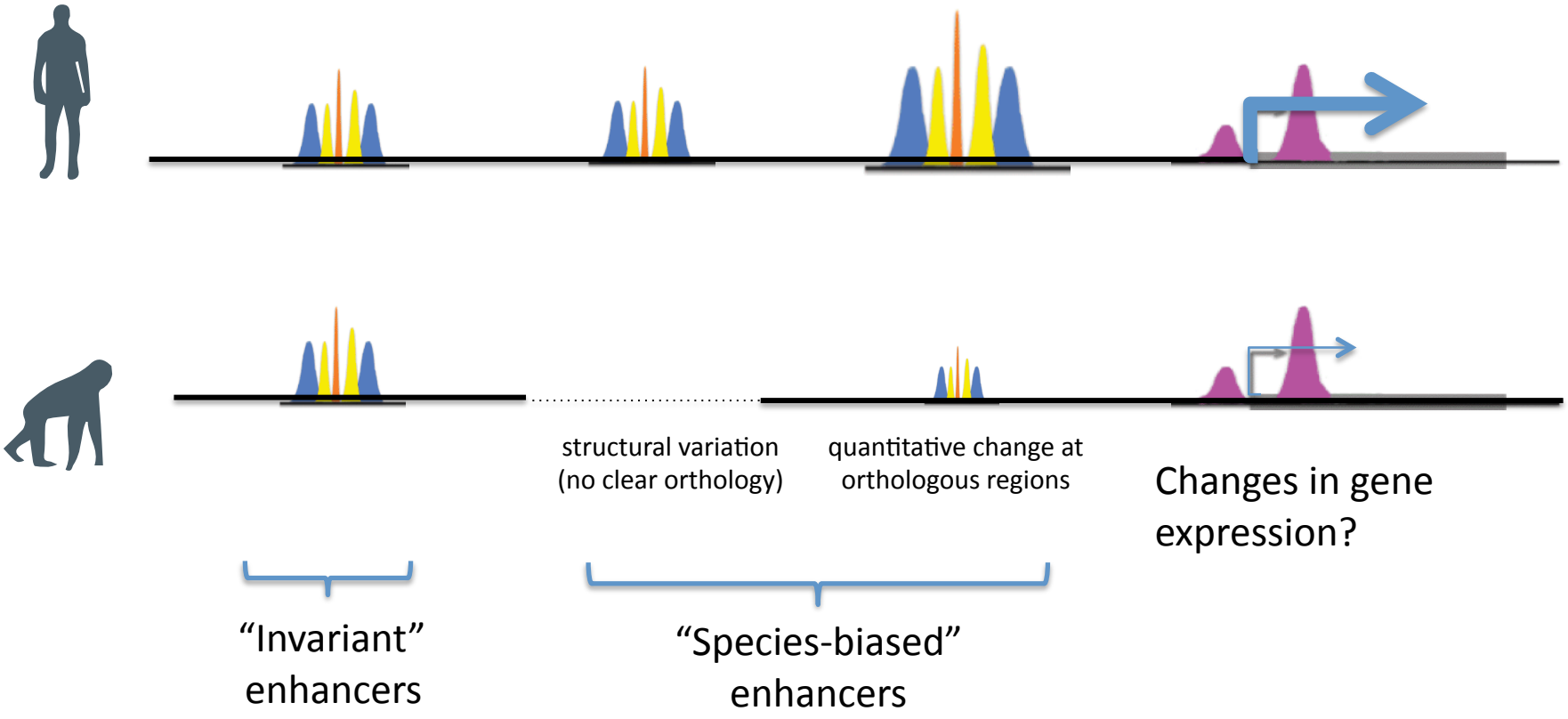
Branchial Arch 3 (BA3)
Parathyroid and thymus development
Throat morphology and innervation

Cis-regulatory change is thought to underlie most of the morphological divergence between closely related species





Can we systematically map cis-regulatory divergence in human and chimp neural crest cells?



Our aims:

AIM1: To characterize epigenetic landscapes and transcriptomes of human and chimpanzee cNCCs and to identify conserved and species-specific cis-regulatory elements

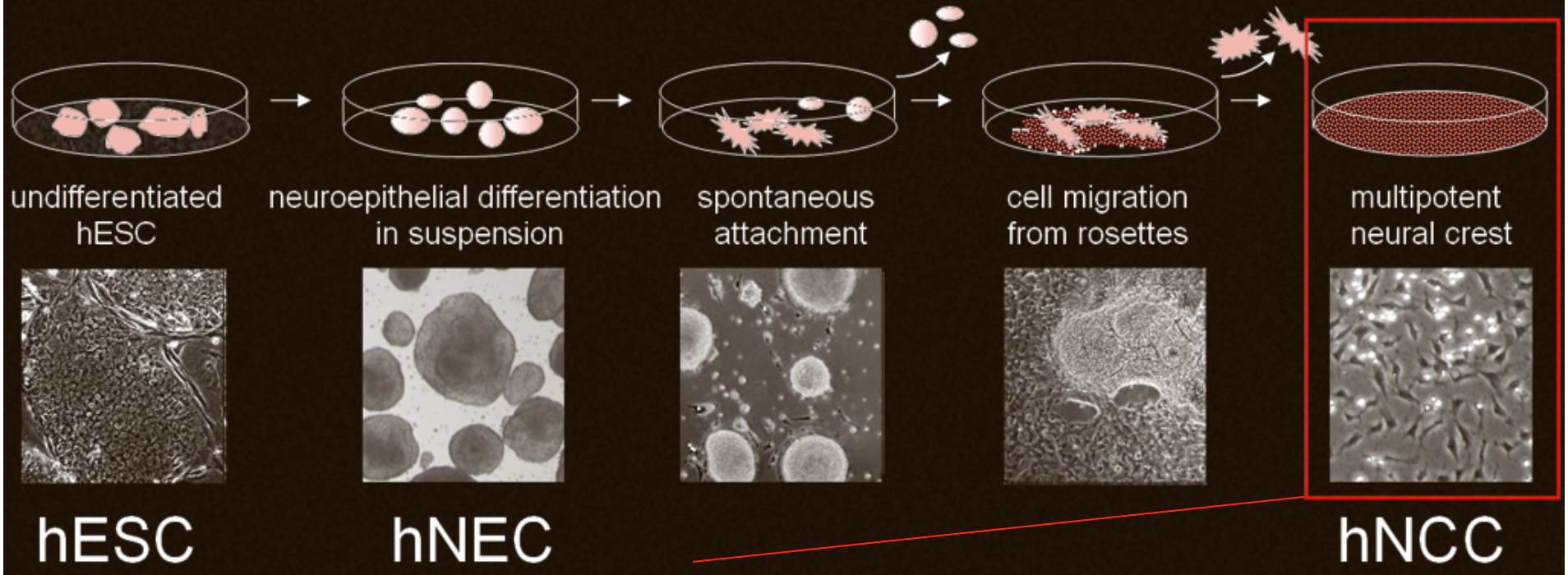
AIM2. To analyze activity of candidate human-specific craniofacial enhancers *in vivo.*

Human embryonic stem cell-based model of cranial neural crest formation

embryonic stem cells

neurectoderm

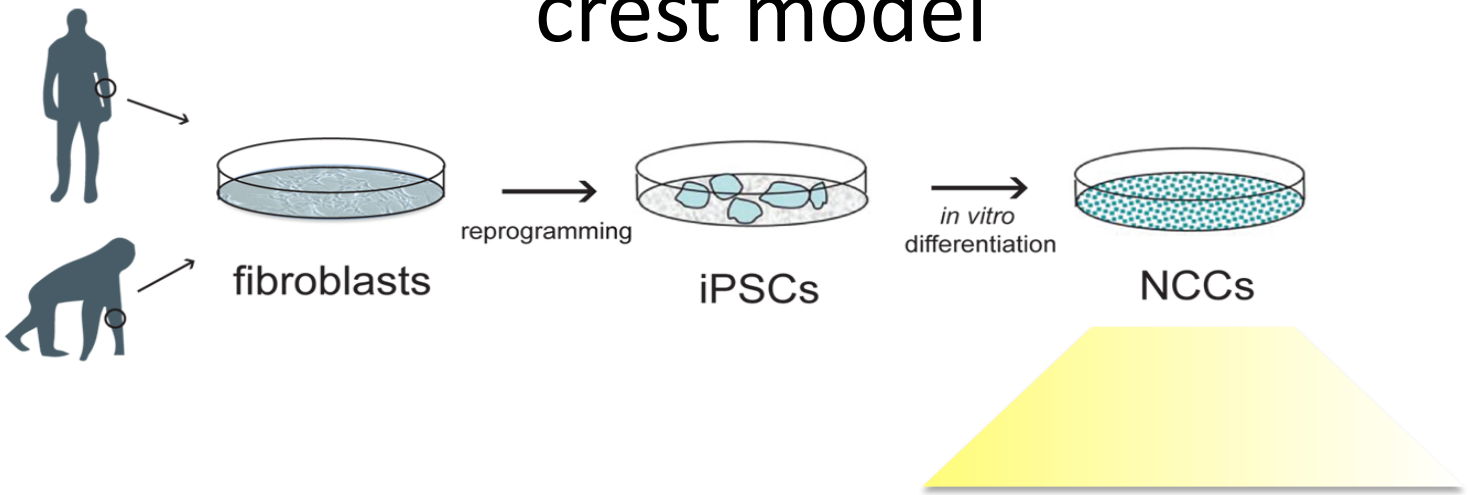
neural crest cells



Bajpai et al., Nature, 2010
Rada-Iglesias et al. Cell Stem Cell, 2012

- NC gene expression (p75+, TFAP2A+, SOX9+, ..)
- migration in ovo
- neurogenic, mesenchymal and melanocytic potential

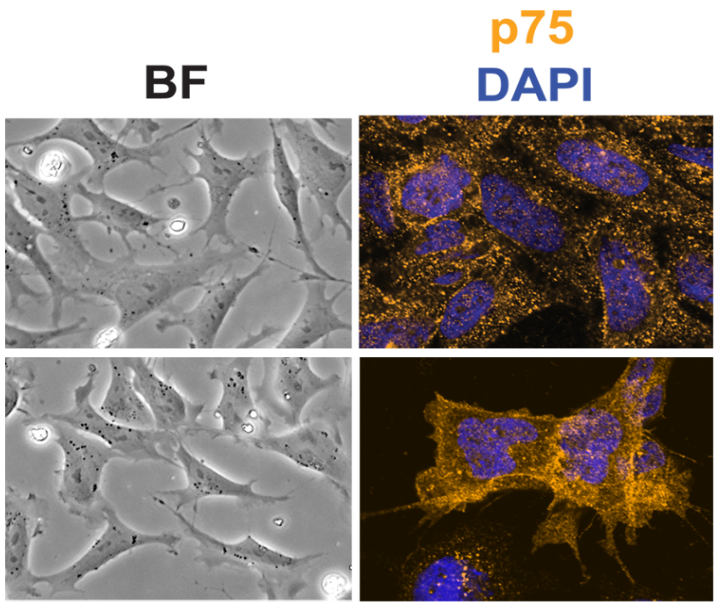
Establishment of the chimpanzee cranial neural crest model



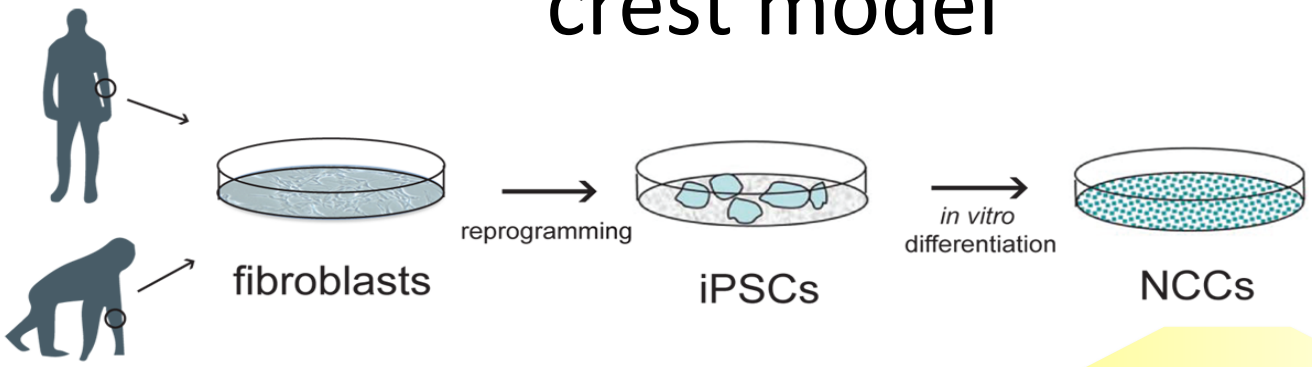
Sara Prescott

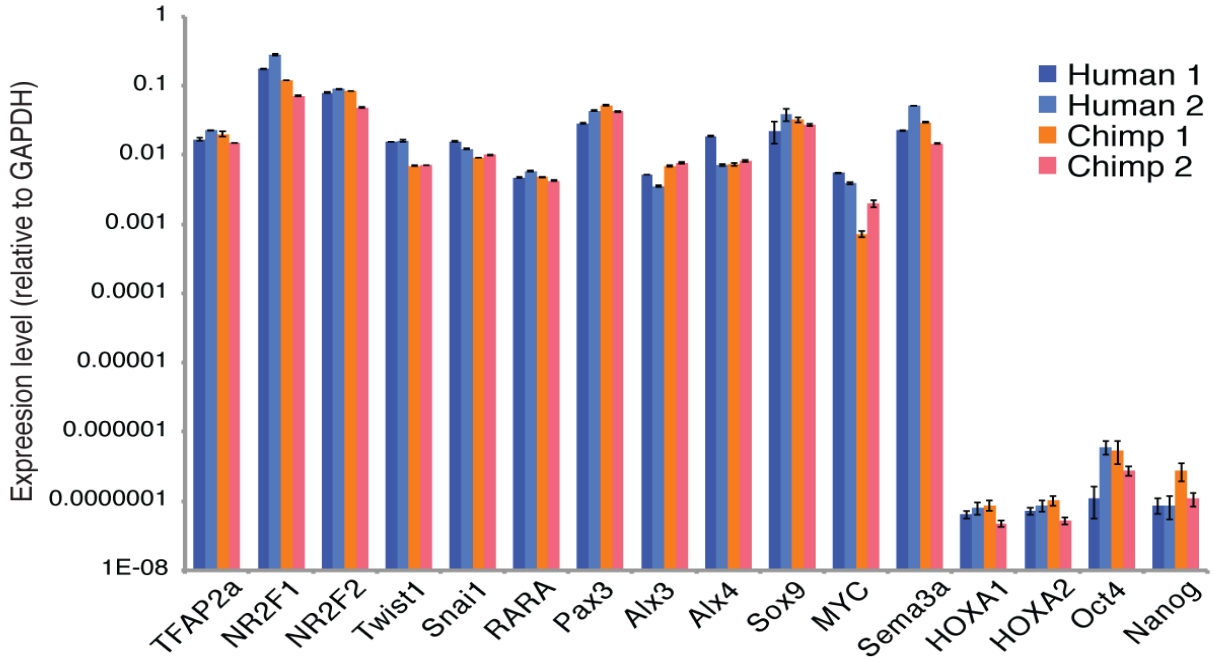
human NCCs

chimp NCCs

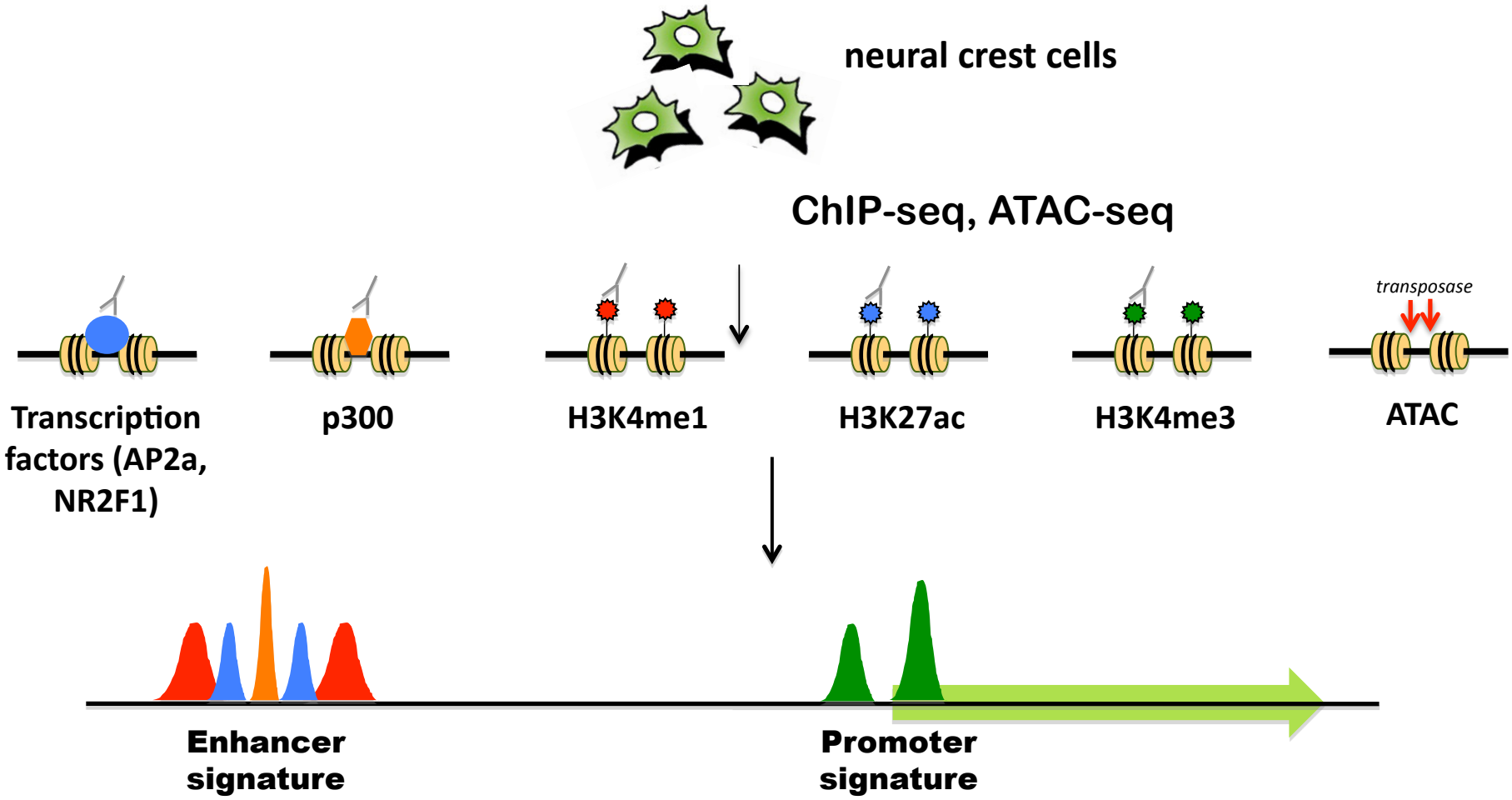


Establishment of the chimpanzee cranial neural crest model

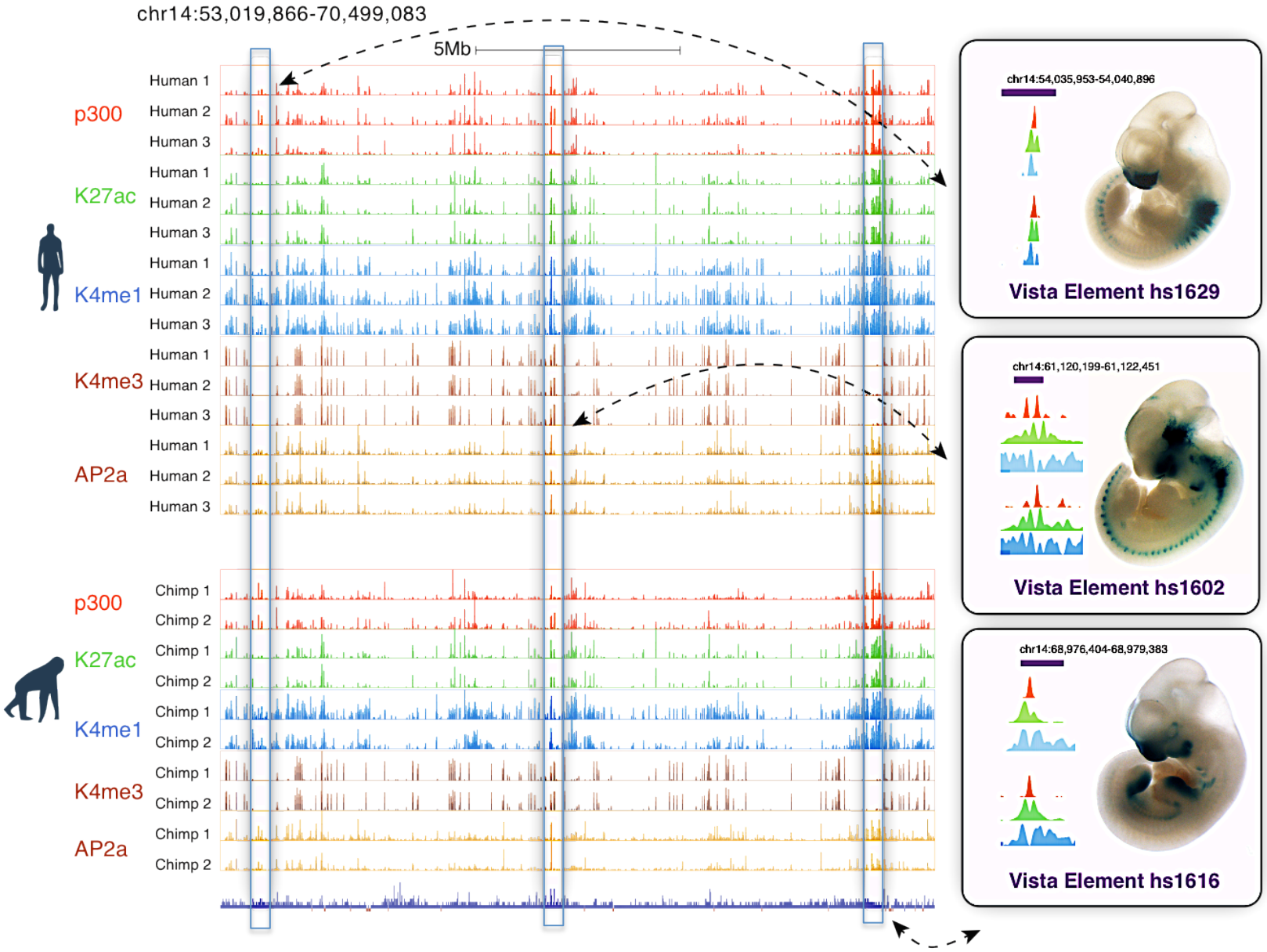




Generate comprehensive epigenomic maps of human and chimp neural crest cells

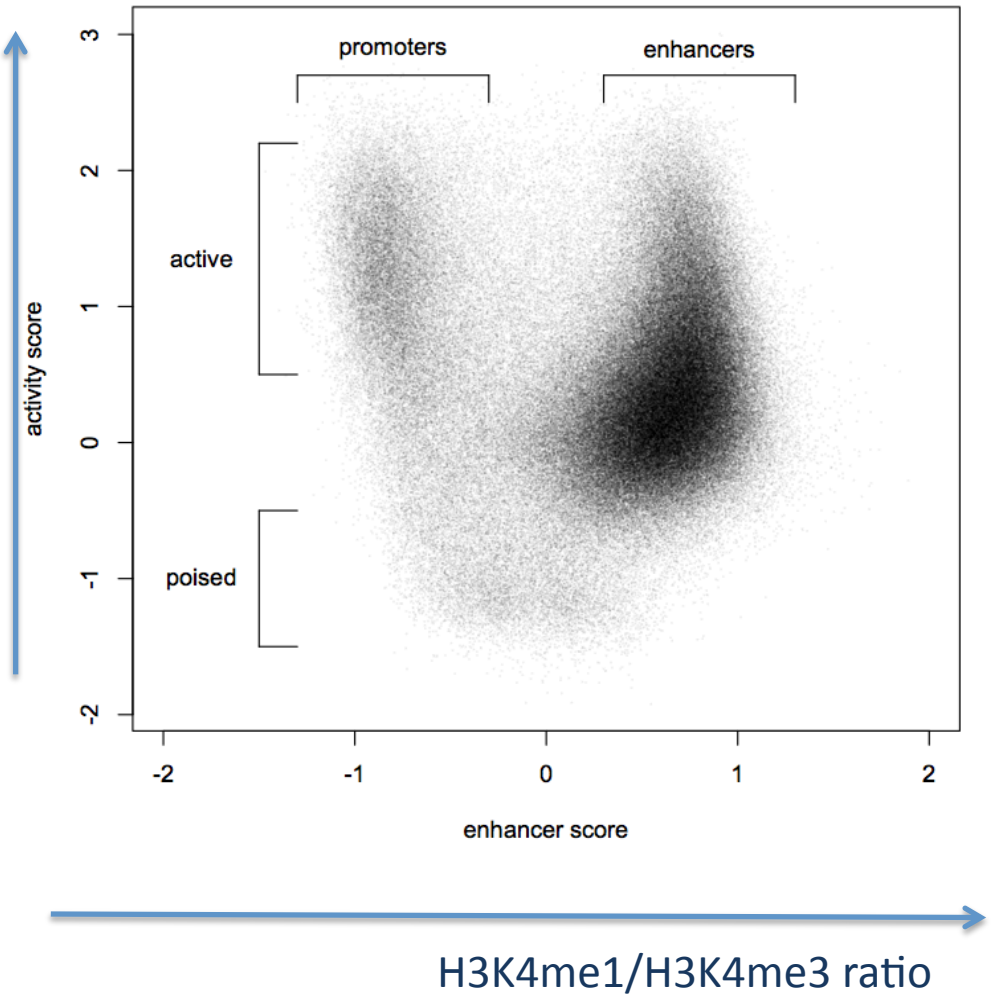


Observed epigenomic patterns are generally well conserved between human and chimp



Most mapped elements correspond to putative active enhancers

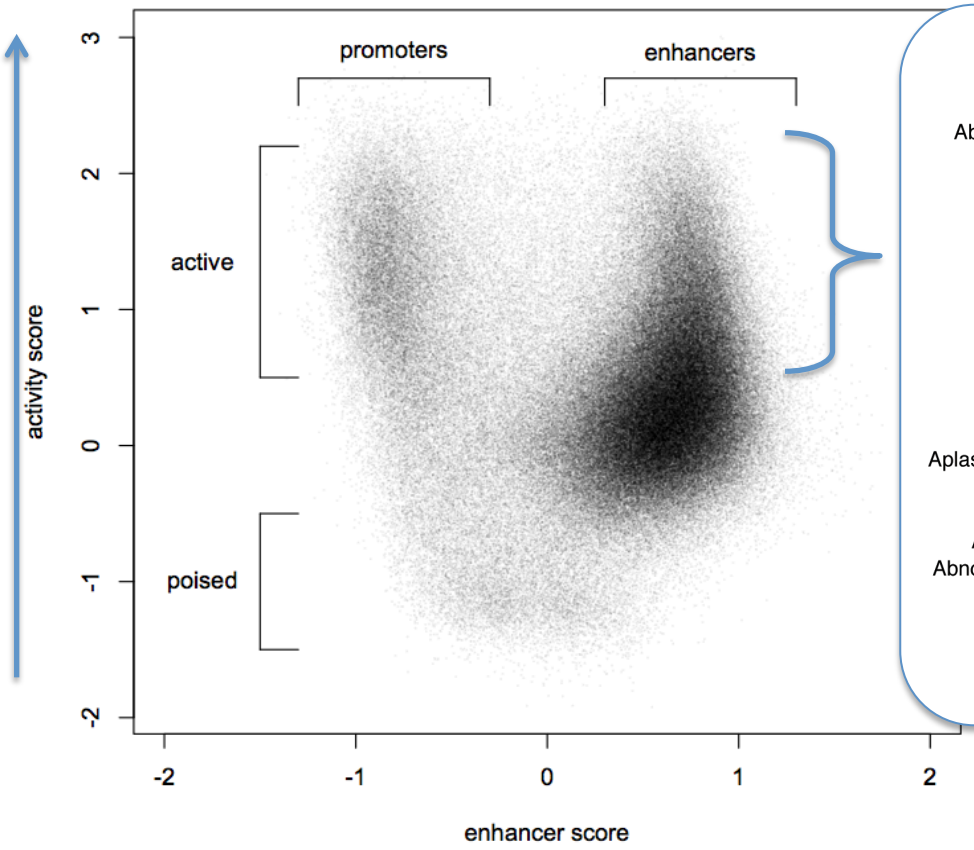
H3K27ac

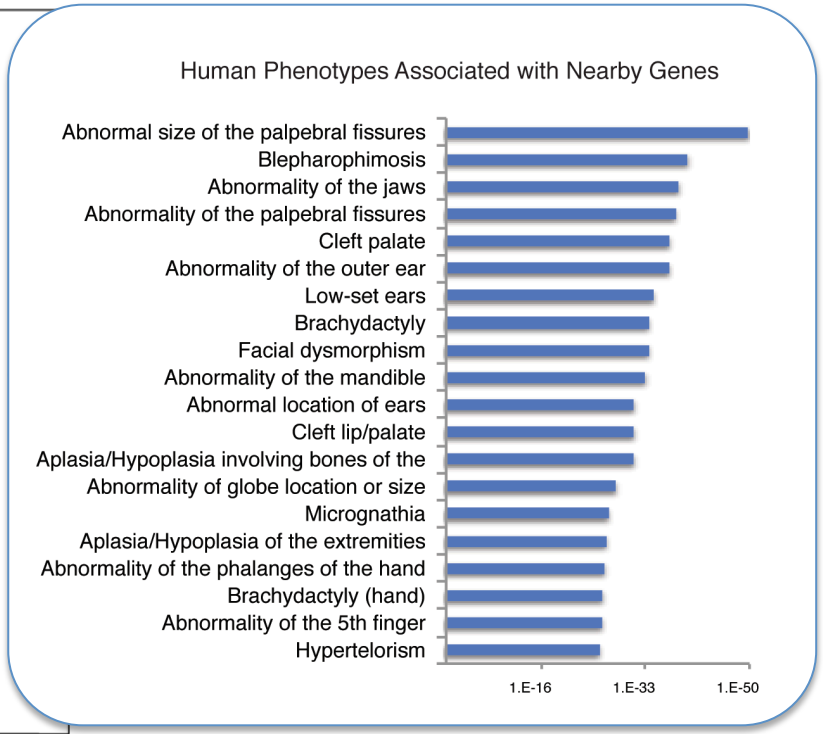


H3K4me1/H3K4me3 ratio

Mapped active enhancer elements are enriched for craniofacial ontologies

H3K27ac

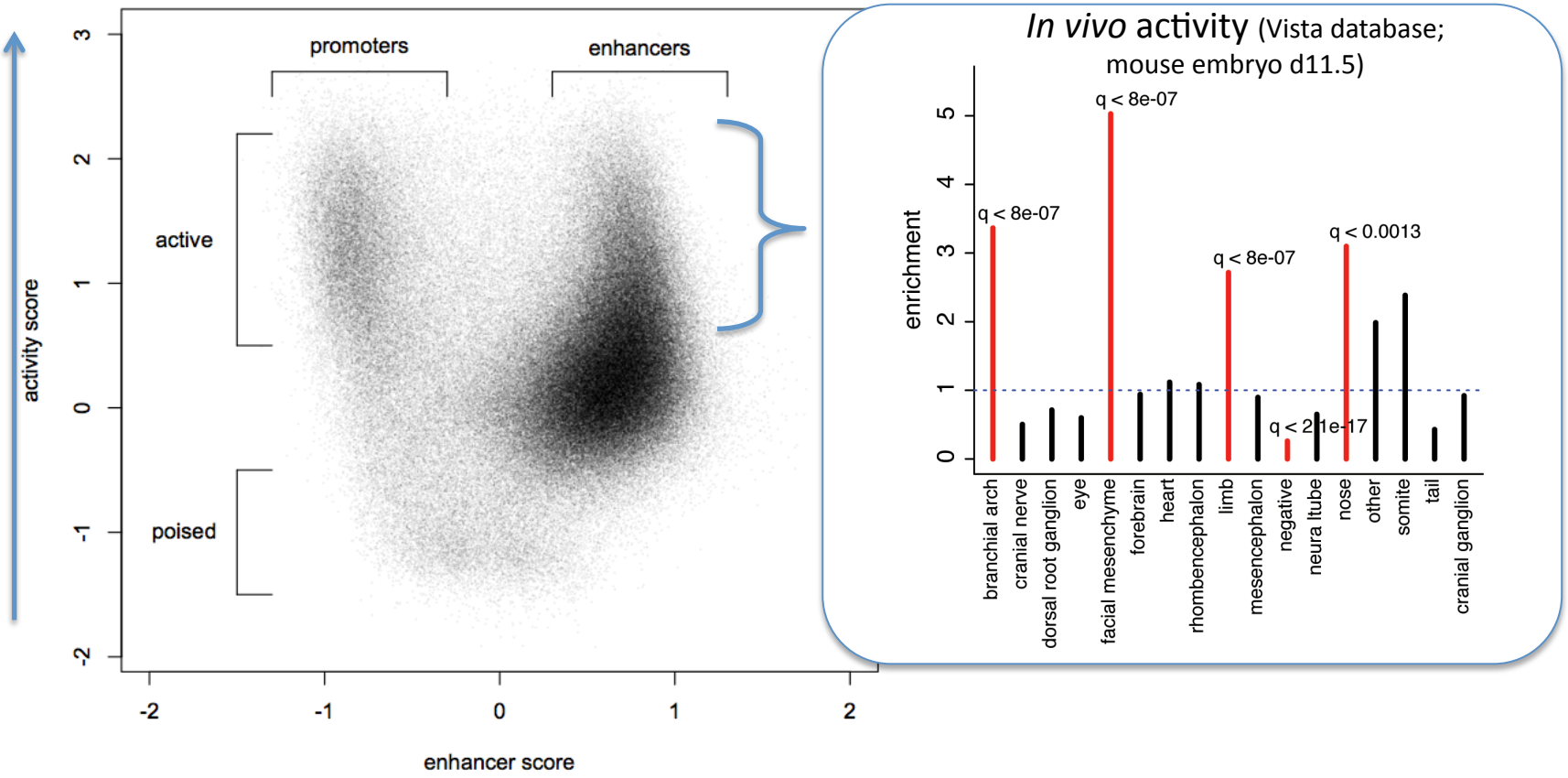




H3K4me1/H3K4me3 ratio

Mapped active enhancer elements drive facial expression *in vivo*

H3K27ac

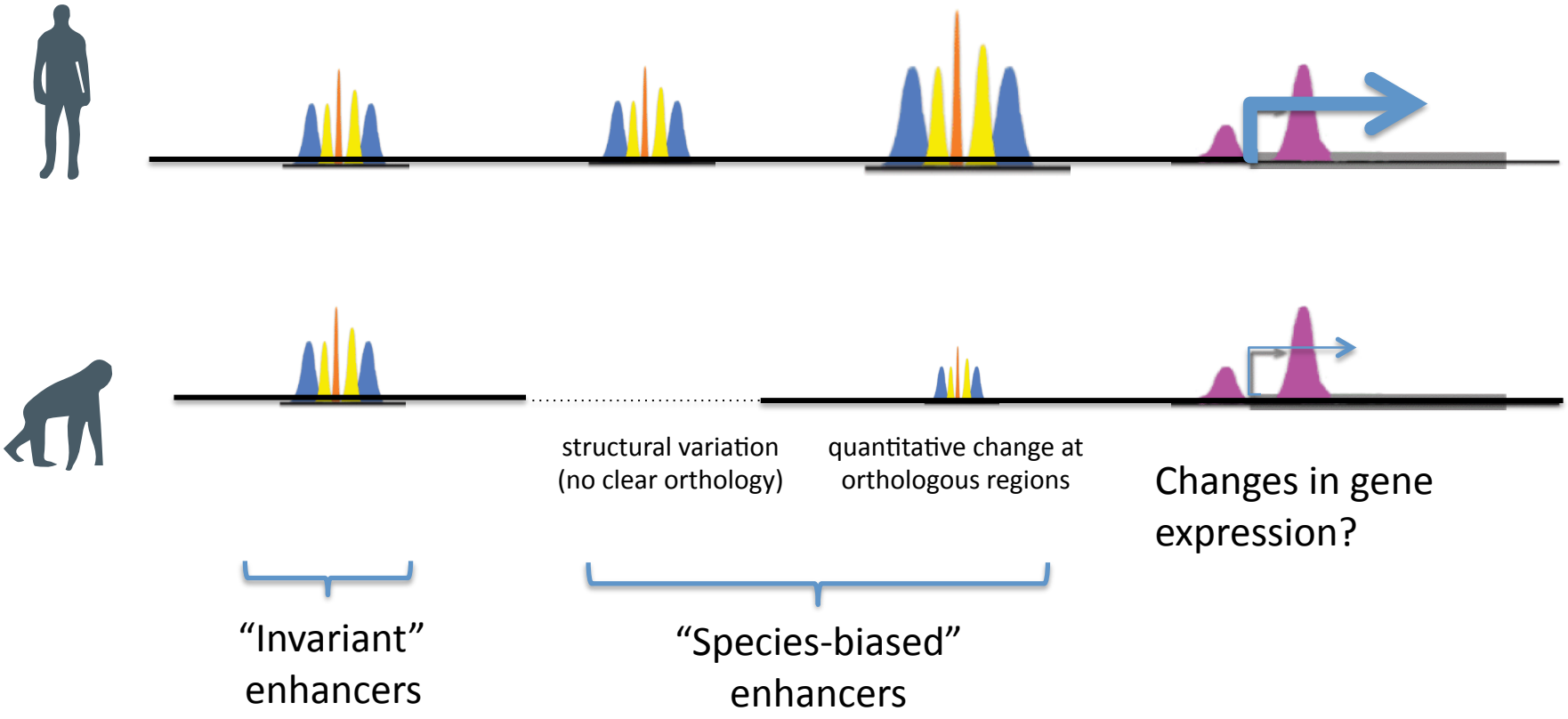


Upcoming plans:

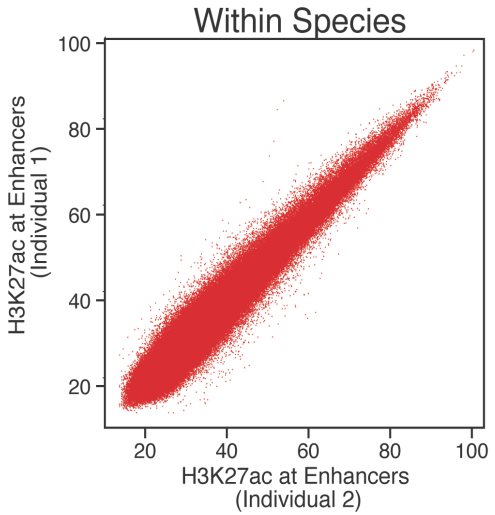
Mid-2015:

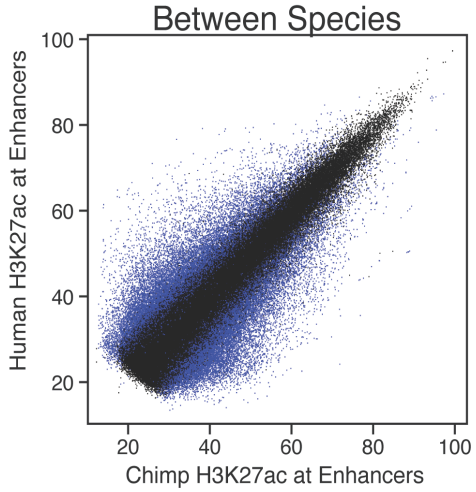
- Deposit all human CNCC ChIP-seq (TFAP2a, NR2F1, p300, H3K4me1, H3K4me3, H3K27ac, H3K27me3) and ATAC-seq raw sequencing reads to the NCBI SRA repository (30-40 libraries) (three different genetic backgrounds)
- Deposit minimally processed ChIP-seq and ATAC-seq results (wig files) to the Hub for visualization in the FaceBase genome browser
- Deposit metadata sheets with detailed descriptions of data and QC

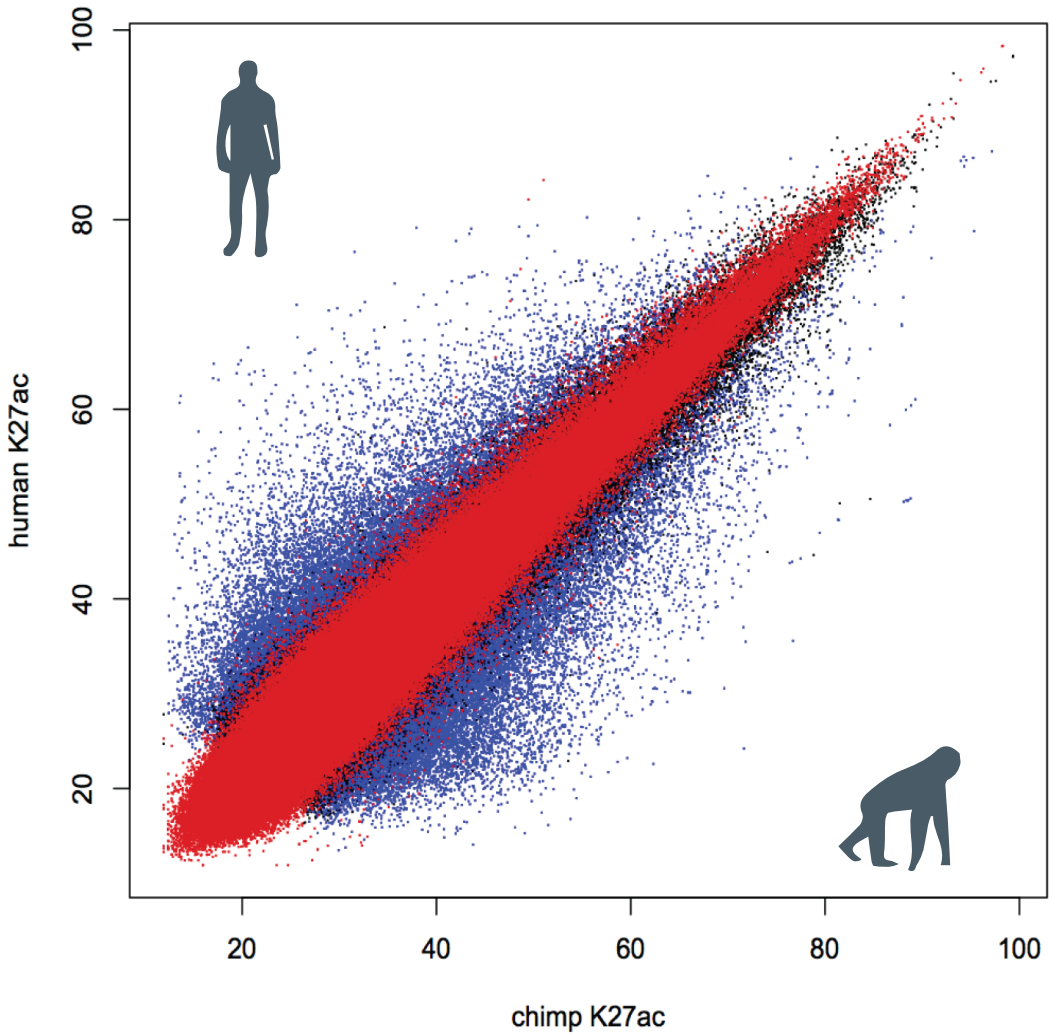
Using epigenomics to experimentally map regulatory divergence in human and chimp neural crest cells



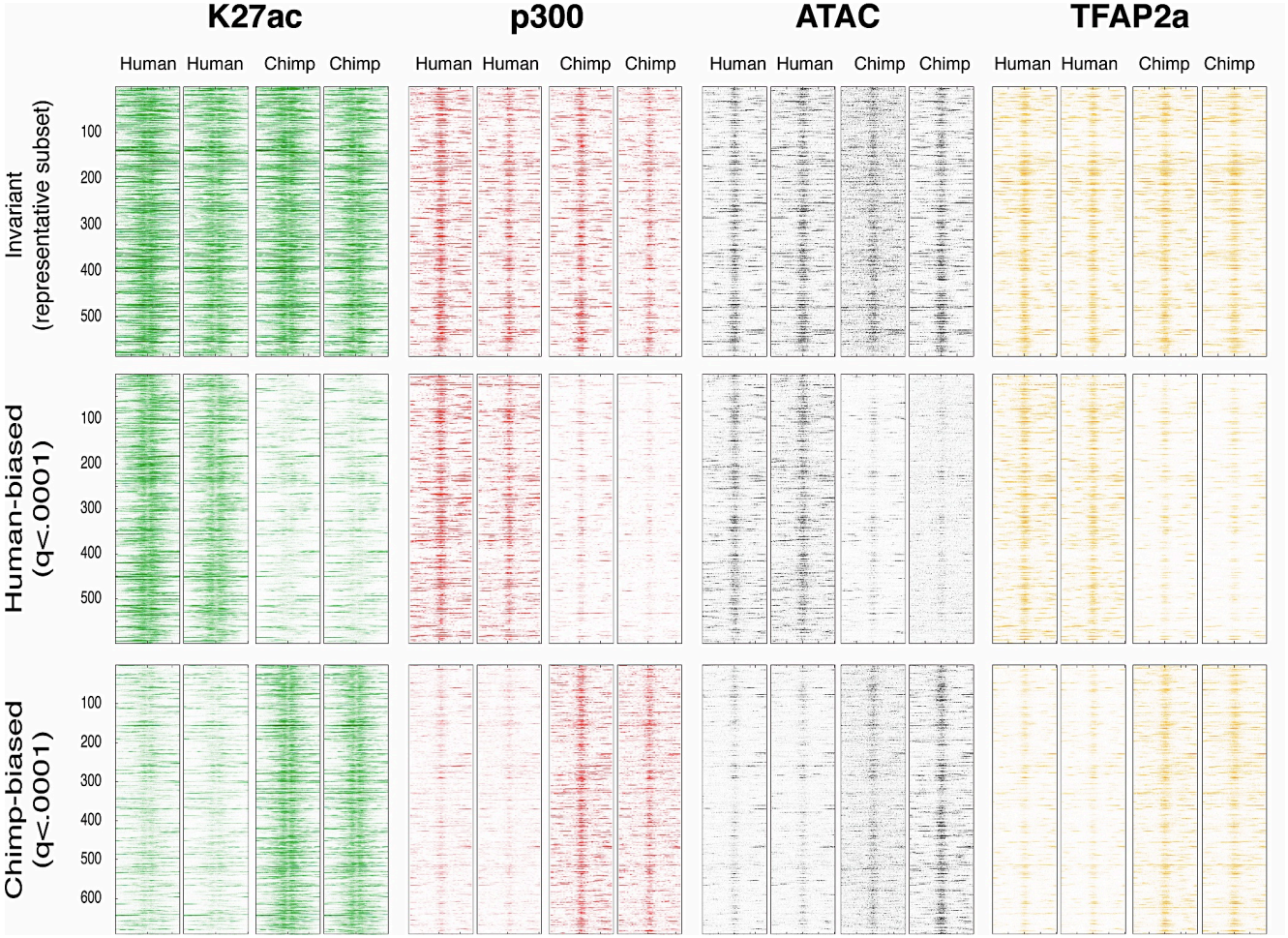
Quantitative comparisons of H3K27ac levels at orthologous regions identify species-biased enhancers



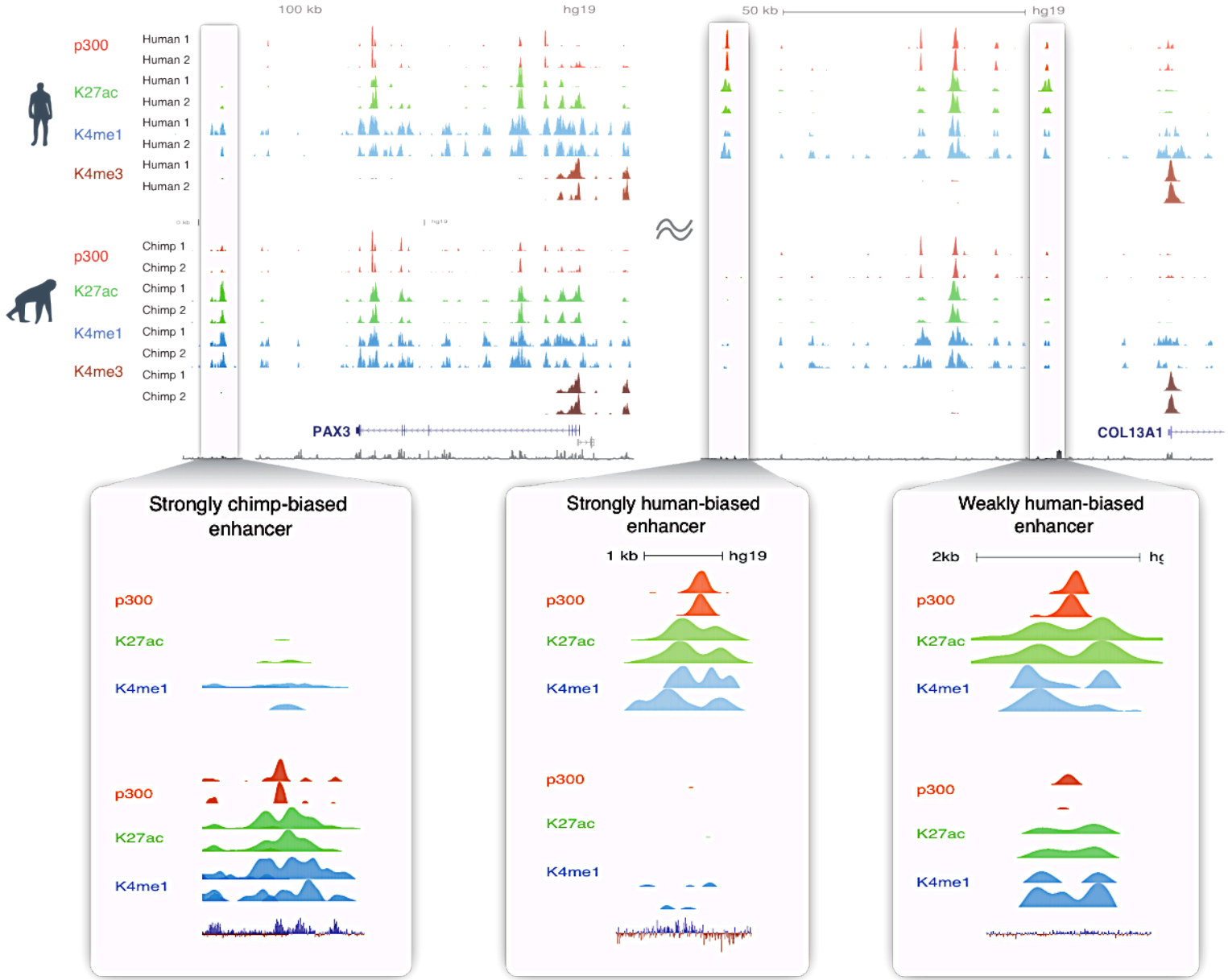




H2K27ac status alone is predictive of biases in transcription factor and p300 binding and chromatin accessibility

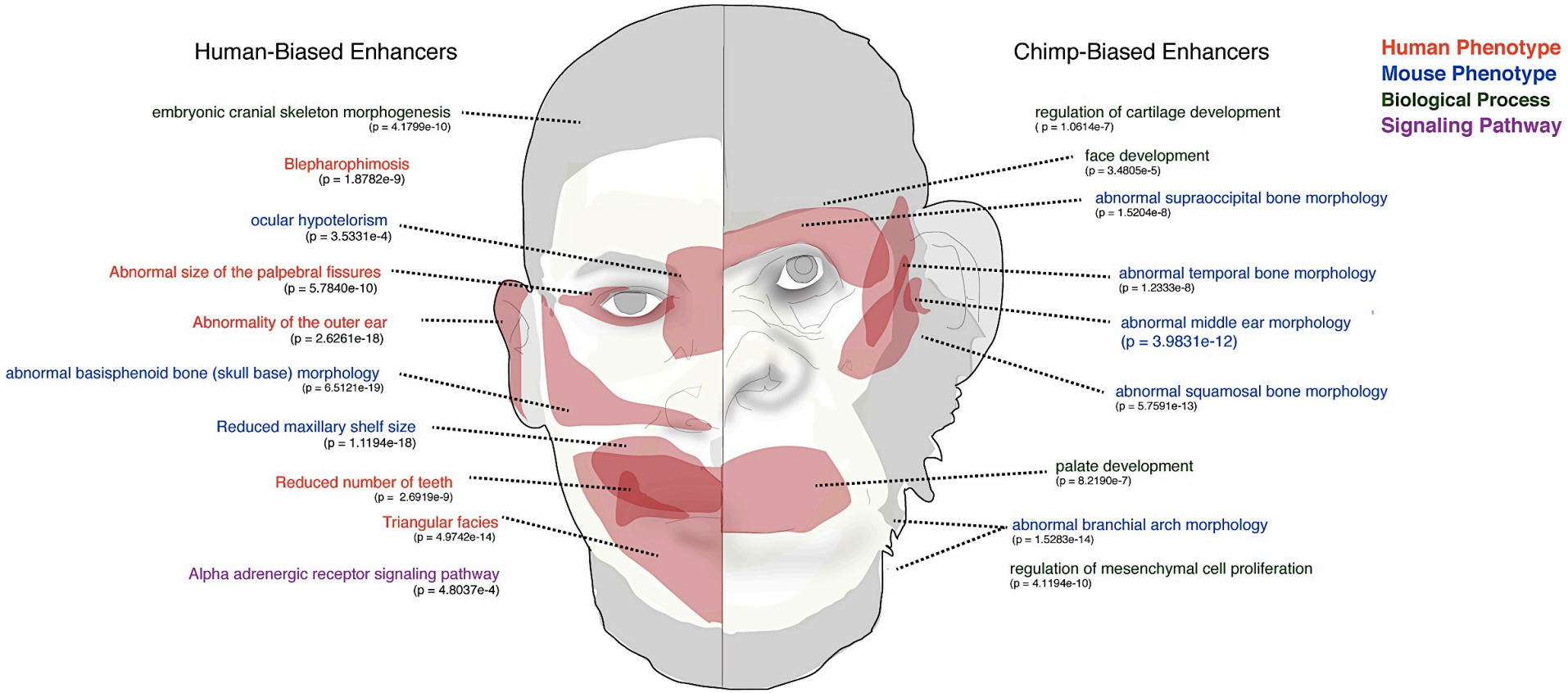


Examples of species-biased enhancers

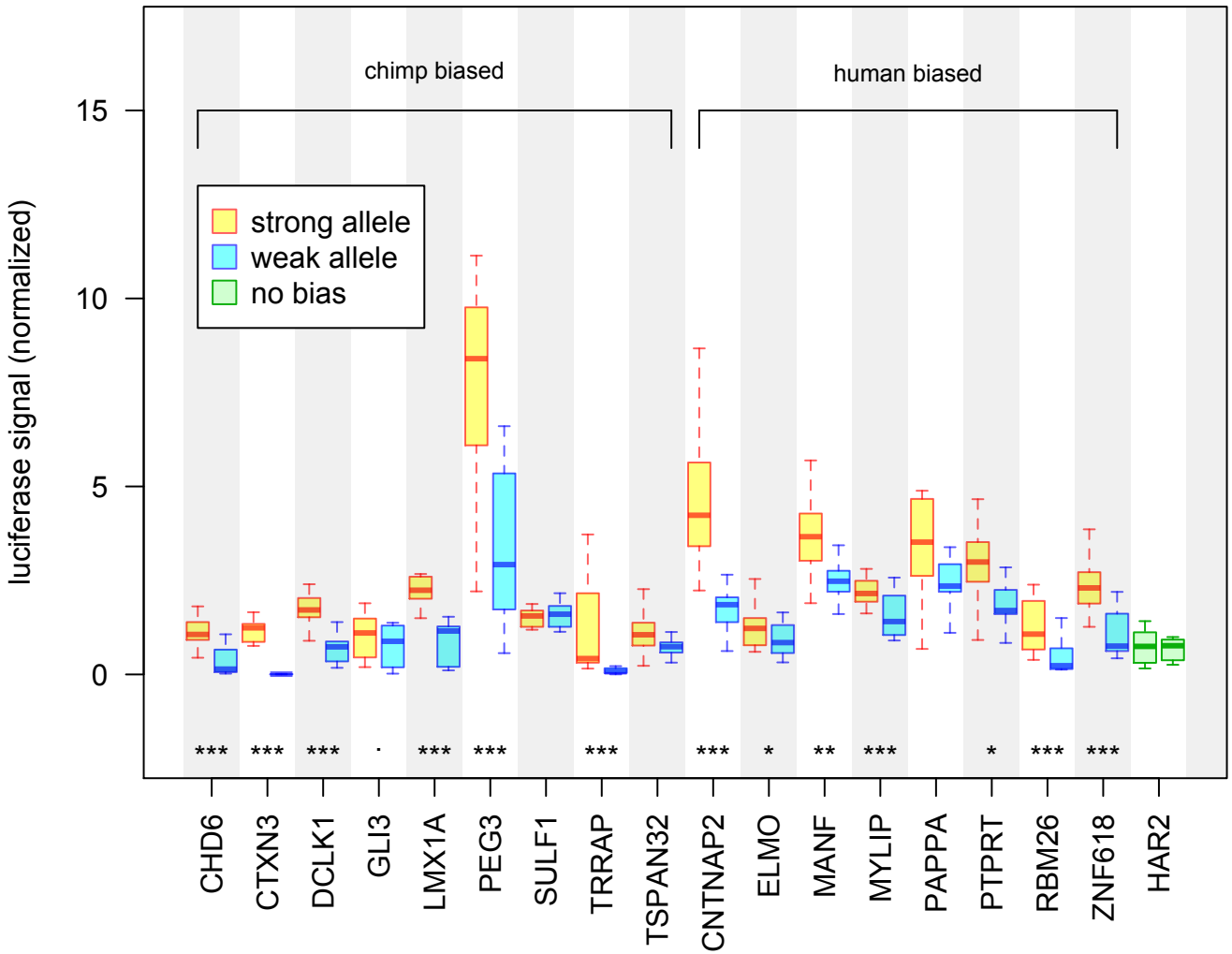


Species-biased enhancers are associated with genes that affect craniofacial structures divergent in humans and chimps

(often in a dosage dependent manner)



We can validate epigenomic enhancer predictions and species-specific biases in activity using luciferase reporter assays in human and chimp CNCCs



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

Use transient transgenesis reporter assays in mice to test human-biased candidate enhancer regions (and, where applicable, their chimp orthologs)

Licia Selleri

Ongoing Analyses of Transgenic Mouse Embryos for Divergent Chimp/Human Enhancers

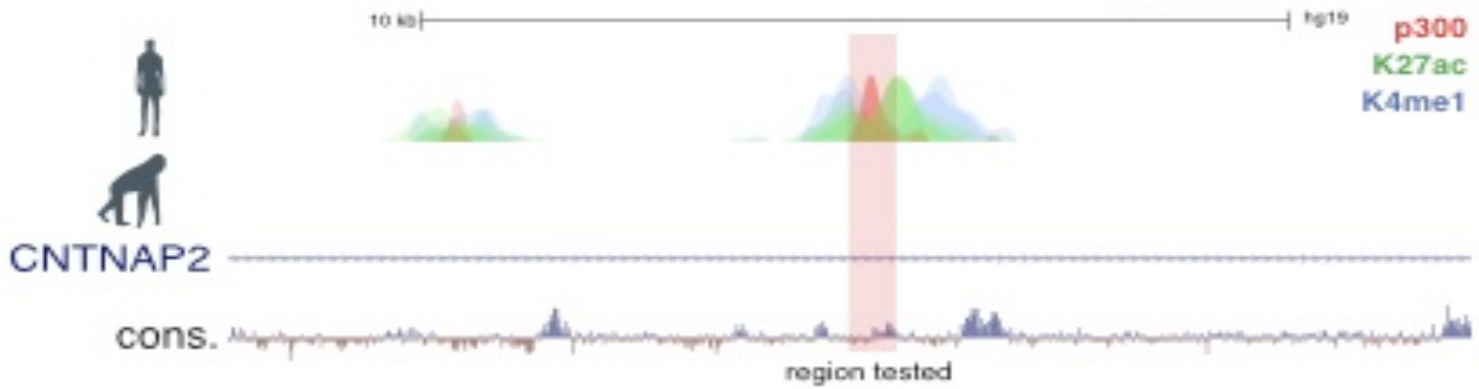
[Analyses Complete: 4 PCR(+), 2 LacZ (+) Tg Embryos] CNTNAP2x3-Chimp
(1 more injection round needed)
[Analyses Complete: 6 PCR(+), 5 LacZ (+) Tg Embryos] CNTNAP2x3-Human

[Analyses Ongoing: 3 PCR(+), 1 LacZ (+) Tg Embryos] PAPPAx3-Chimp
(1 more injection round needed)
[Embryo Harvesting In Progress] PAPPAx3-Human

[Analyses Ongoing; 4 PCR(+), 4 LacZ (+) Tg Embryos] LRP6x3-Human

Ongoing Analyses of Transgenic Mouse Embryos for Divergent Chimp/Human Enhancers

[Analyses Complete: 4 PCR(+), 2 LacZ (+) Tg Embryos] CNTNAP2x3-Chimp
(1 more injection round needed)
[Analyses Complete: 6 PCR(+), 5 LacZ (+) Tg Embryos] CNTNAP2x3-Human



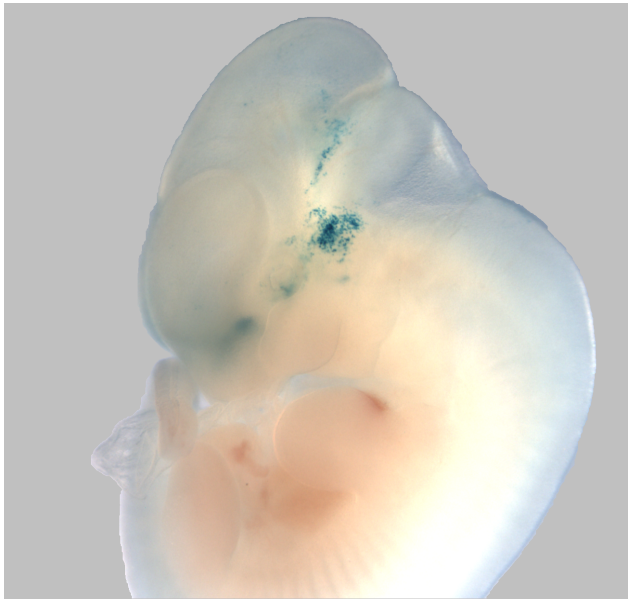
Observed expression domains are highly reproducible despite variation in intensity

Human *CNTNAP2* enhancer spatial expression patterns in E11.5 tg mouse embryos

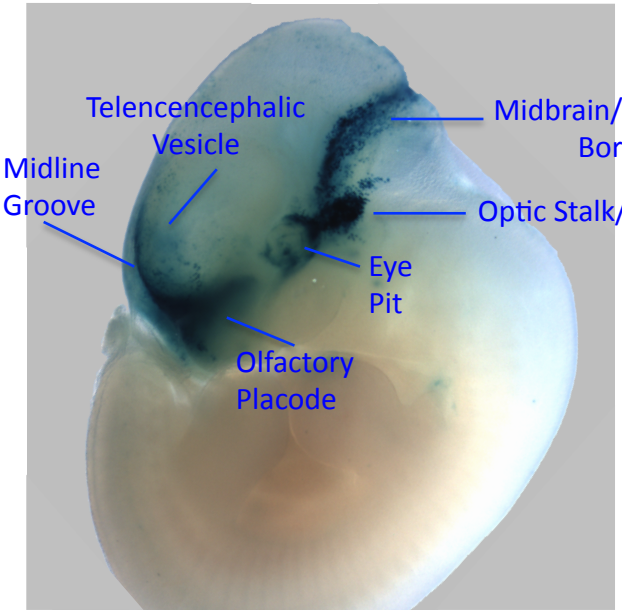
5/5 tg embryos show these expression domains despite variation in intensity; 2 are shown

Sagittal views

Hum8



Hum30

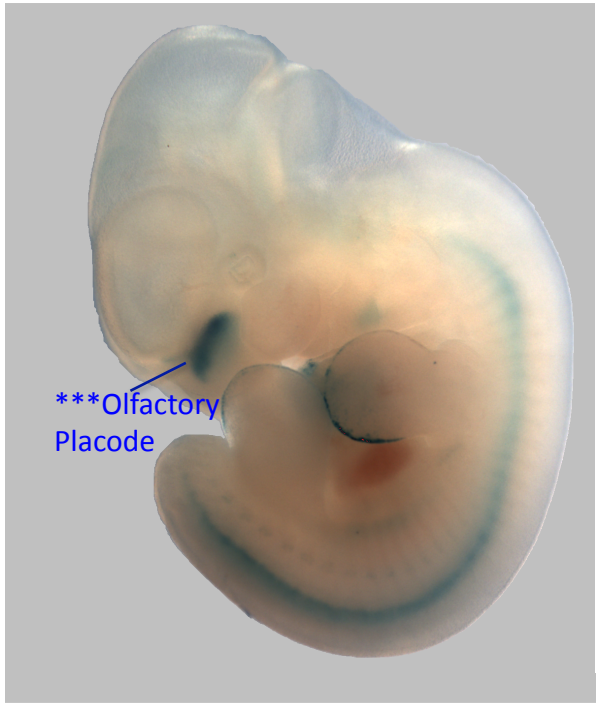


Intensity of Staining

Potential Gain of Head Expression Domains of Human *CNTNAP2* Enhancer Compared to Chimp Enhancer

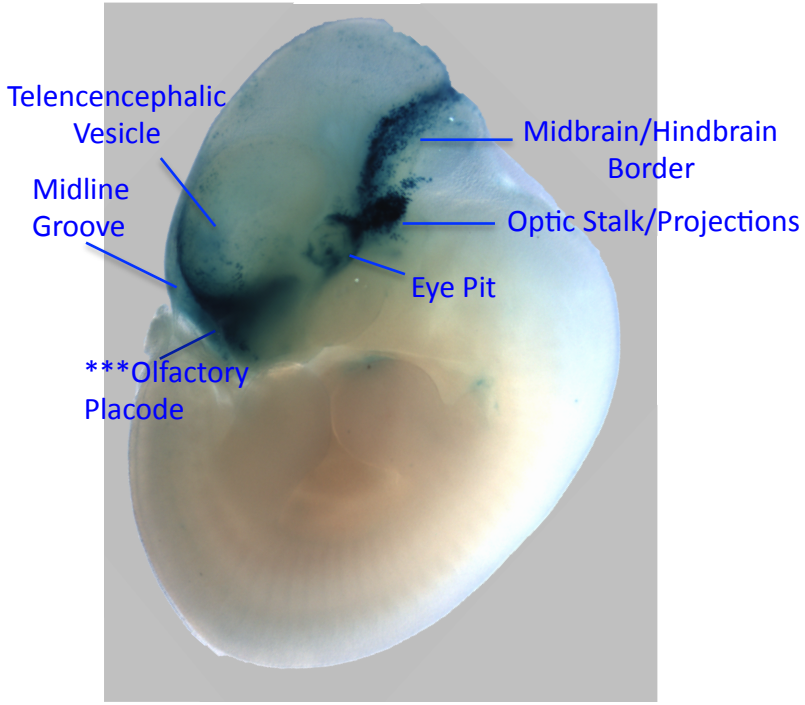
Orthologous chimp sequence

Chimp 32



Human *CNTNAP2* enhancer

Hum30



*****Olfactory Placode: Domain Common to Chimp and Human**

Upcoming plans:

Mid-2015:

- Analyze expression patterns and image first five reporter constructs
- Clone and inject next round of constructs

Acknowledgements:

Licia Selleri
(Weill Cornell School of Medicine)

Robert Aho (Weill Cornell School of Medicine)

Rusty Gage and Carol Marchetto
(Salk Institute)

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(Stanford)

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NIDCR

Tomek Swigut
(Stanford)