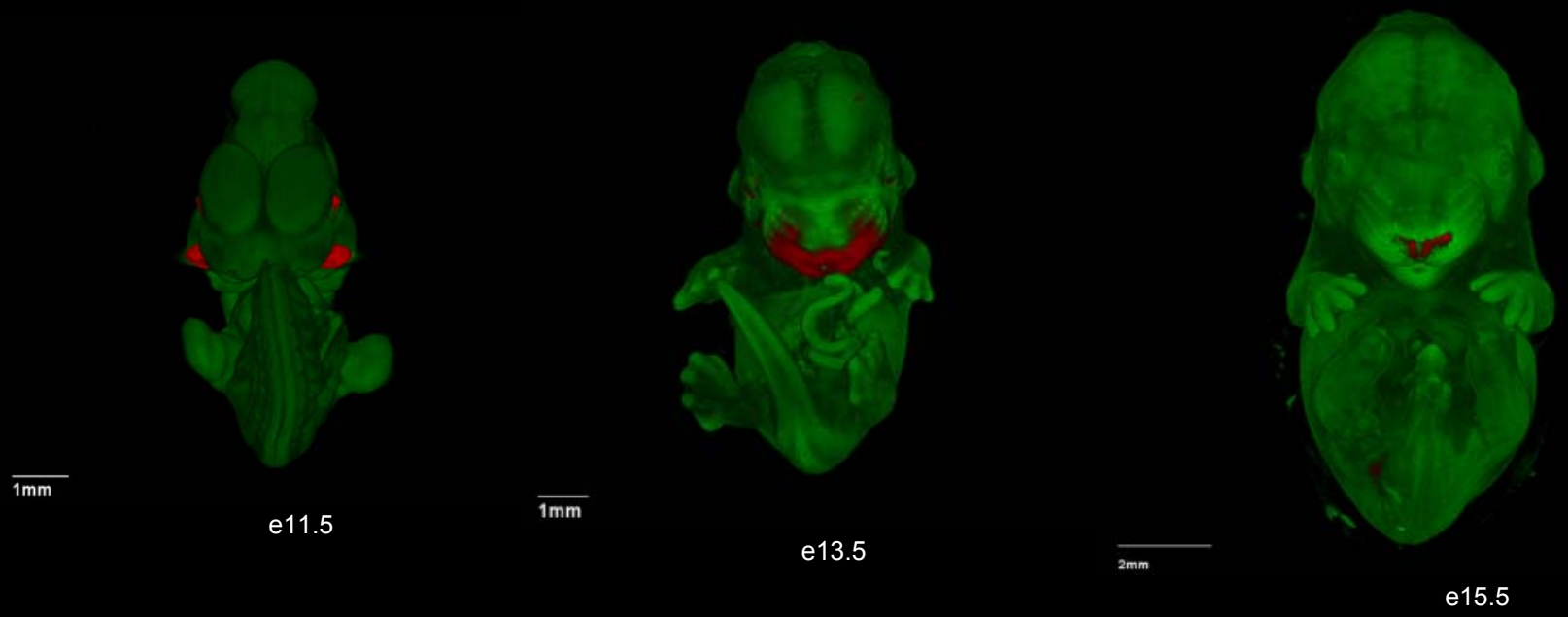
Genomic and Transgenic Resources for Craniofacial Enhancer Studies

January 2015 Update



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FaceBase

NIH National Institute of Dental and Craniofacial Research

Project Team

Lawrence Berkeley National Lab

Experimental postdocs: [Catia Attanasio, Alex Nord], Han Wu, Evgeny Kvon
Computational postdocs: Yoko Yuzawa (data liaison!), Iros Barrozzi
Other Senior Staff: Diane Dickel, Len Pennacchio, Eddy Rubin
Molecular Biology and Mouse Transgenics: Jennifer Akiyama, Veena Afzal, Brandon Mannion, Cathy Pickle, Ingrid Plaijzer-Frick

MRC Human Genetics, Edinburgh, UK - *Optical projection tomography*
David FitzPatrick, Harris Morrison

HDBR, Newcastle, UK – *Human fetal tissues*
Steven Lisgo

University of Calgary, Canada - *Morphometry*
Benedikt Hallgrimsson, Denise Liberton

University of Southern California – *KO analysis*
Yang Chai

FaceBase

Outline

BACKGROUND

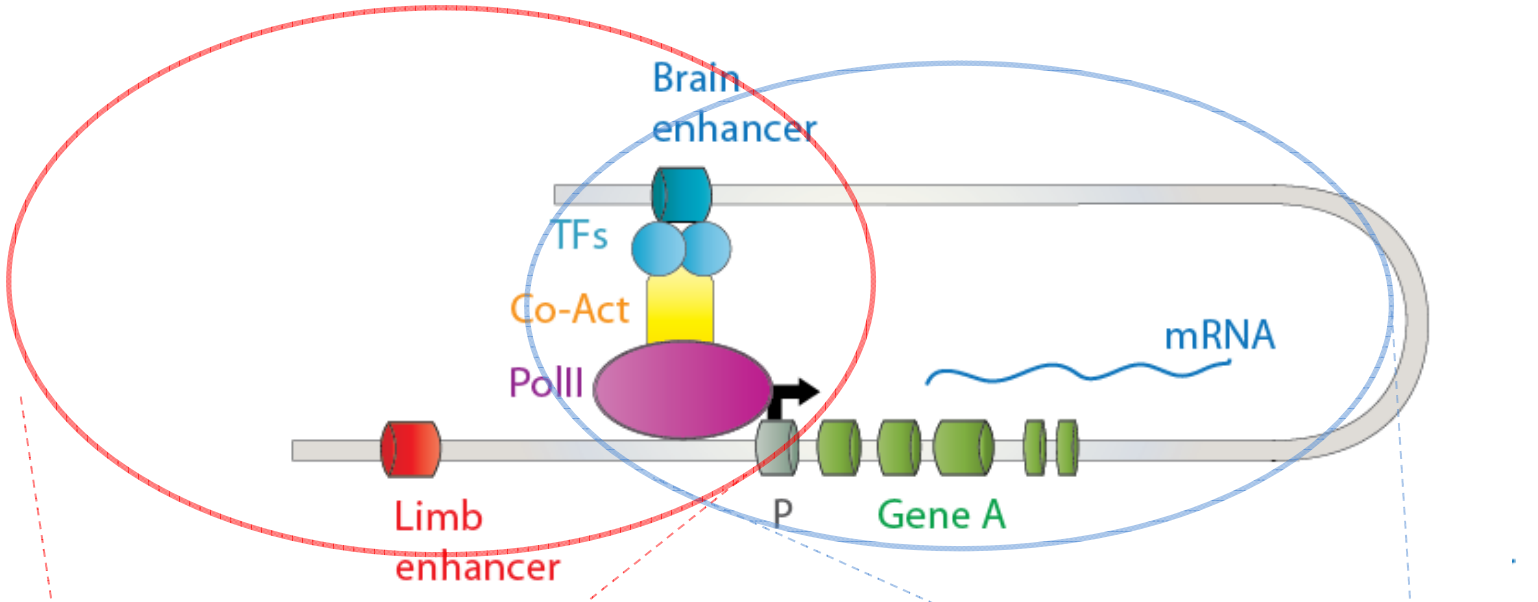
FACEBASE 2 – Progress

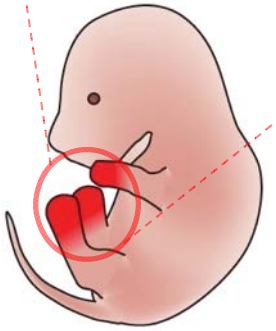
RNA-seq and ChIP-seq from mouse face regions

RNA-seq and ChIP-seq from human craniofacial tissue

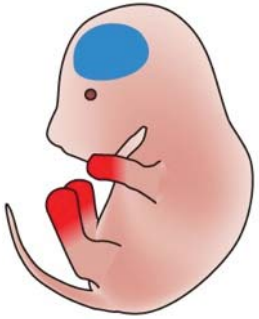
Transgenic enhancer validation/characterization

Distant-acting enhancers dictate tissue-specific gene expression

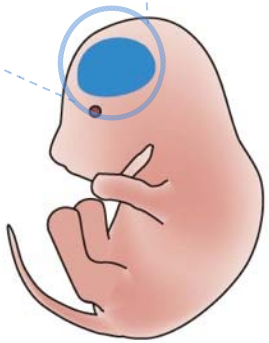




Limb
Enhancer

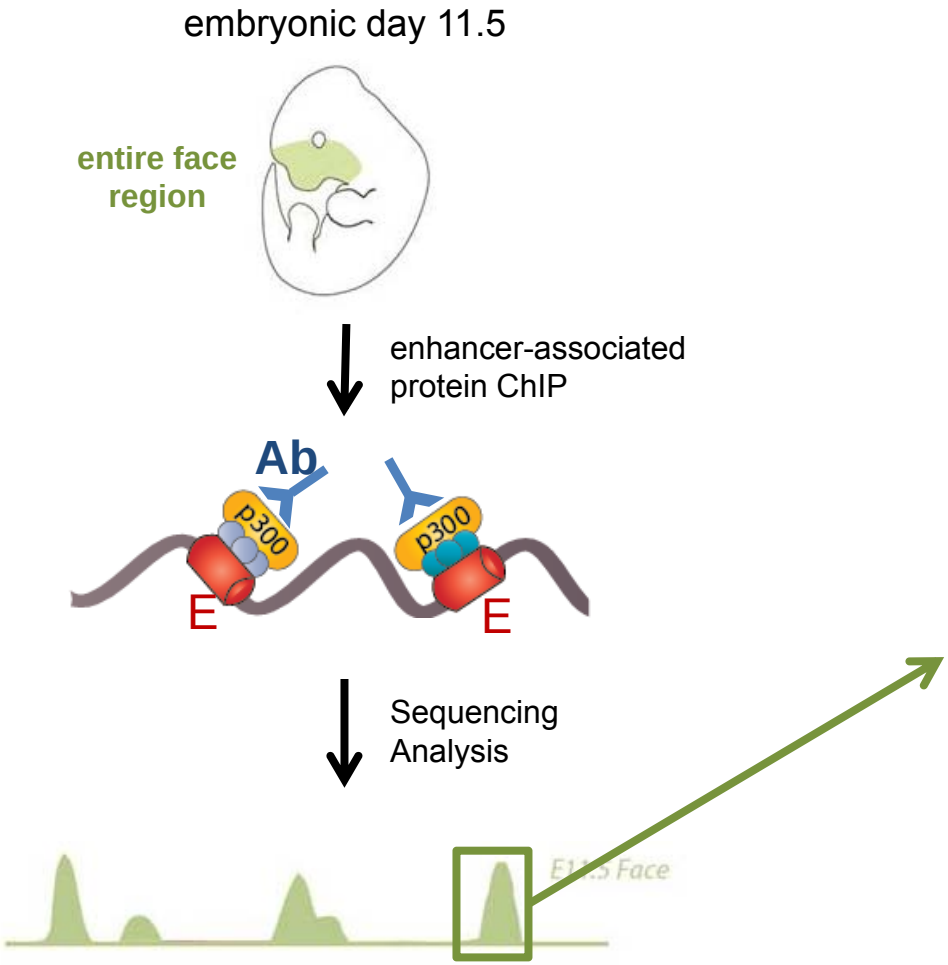


Gene Expression
mRNA: Limb AND Brain



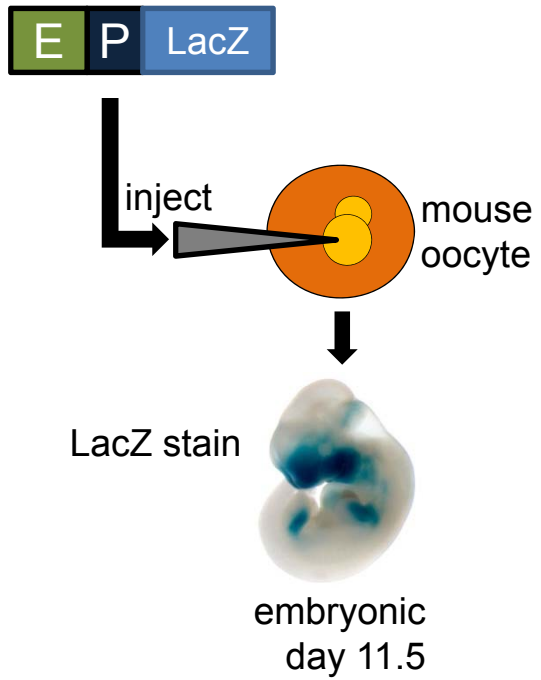
Brain
Enhancer

Enhancer mapping by tissue-ChIP-seq



Thousands of craniofacial candidate enhancers

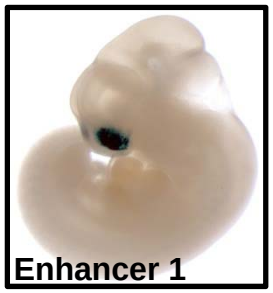
Characterize in Transgenic Mouse Embryos

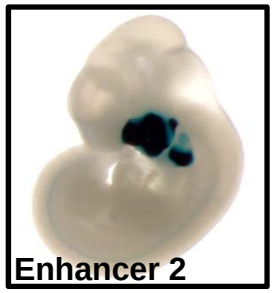


Transgenic Characterization

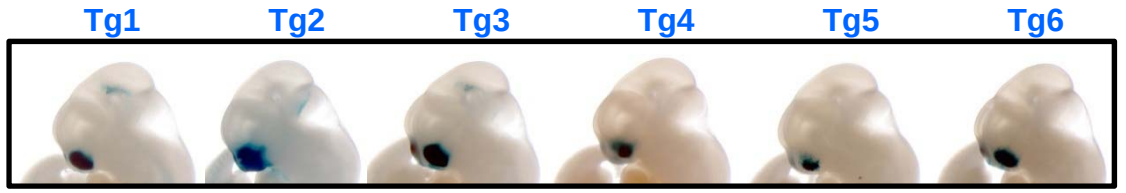
3 of ~200 craniofacial enhancers

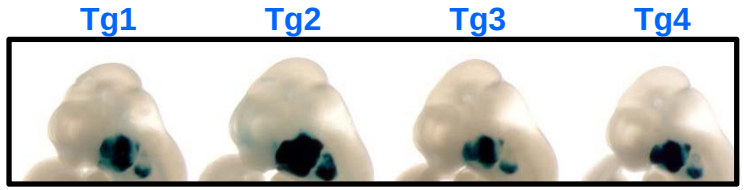
different spatial patterns

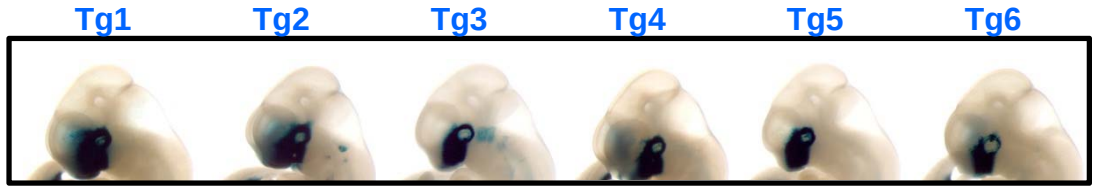




Independent transgenic F0 embryos with same construct:







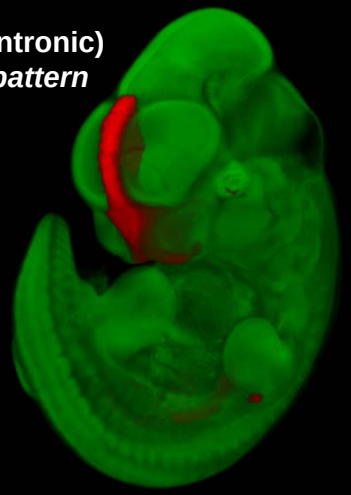
reproducibility of patterns

(minimum: 3 embryos)

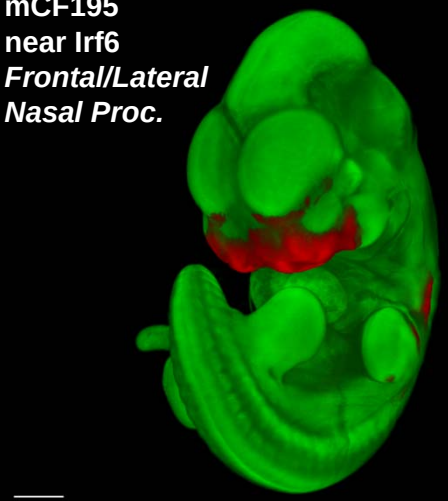
OPT imaging (enhancer:background)

OPT imaging: David FitzPatrick/Harris Morrison, Edinburgh

mCF121
Abca4 (intronic)
Midline pattern

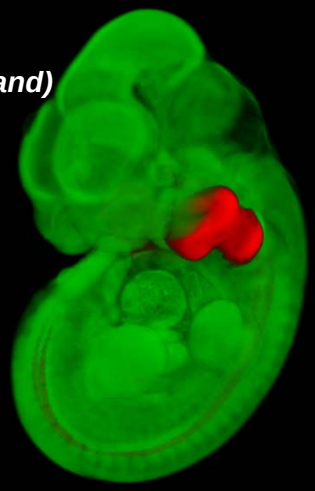


mCF195
near Irf6
Frontal/Lateral Nasal Proc.

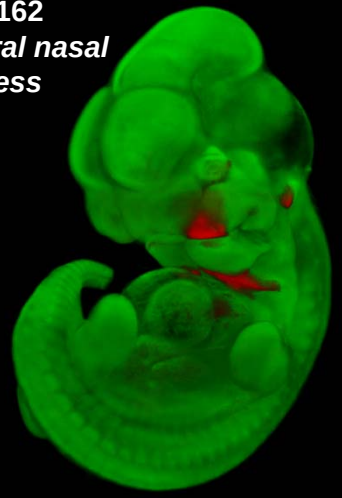


mCF171
Mn1
Branchial arches

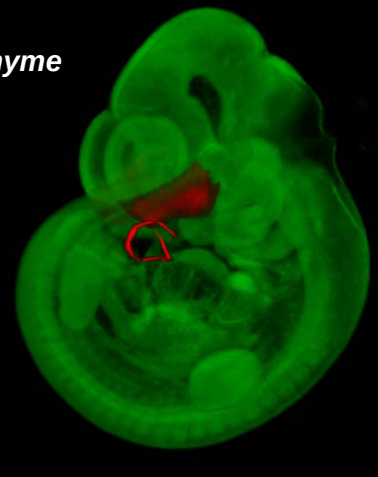
mCF90
First BA
(Max and Mand)



mCF162
Lateral nasal process

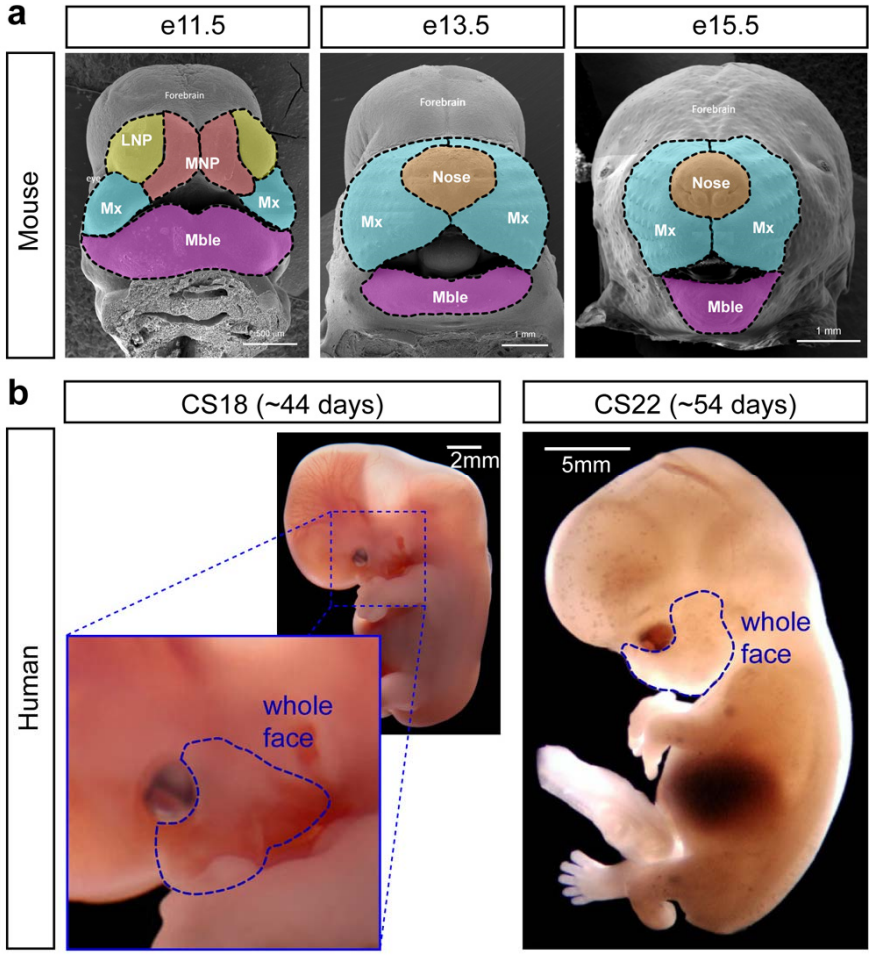


mCF208*
Facial Mesenchyme



FACEBASE 2: Specific Aims

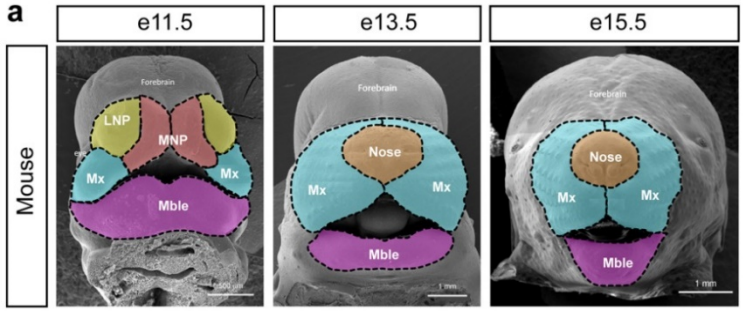
Aim 1: Genome-wide enhancer Activity Mapping via ChIP-seq of Mouse and Human Craniofacial Tissues



- Critical developmental windows
  - MOUSE maxillary, mandibular, medial/lateral nasal processes at e11.5, e13.5, and e15.5
  - HUMAN face at cs18 and cs22
- ChIP-seq: histone modifications for promoters, enhancers, and repressed chromatin
- rRNA-depleted total RNA: mRNA and most non-coding RNA species

FACEBASE 2: Specific Aims

Aim 1: Progress Mouse Tissues



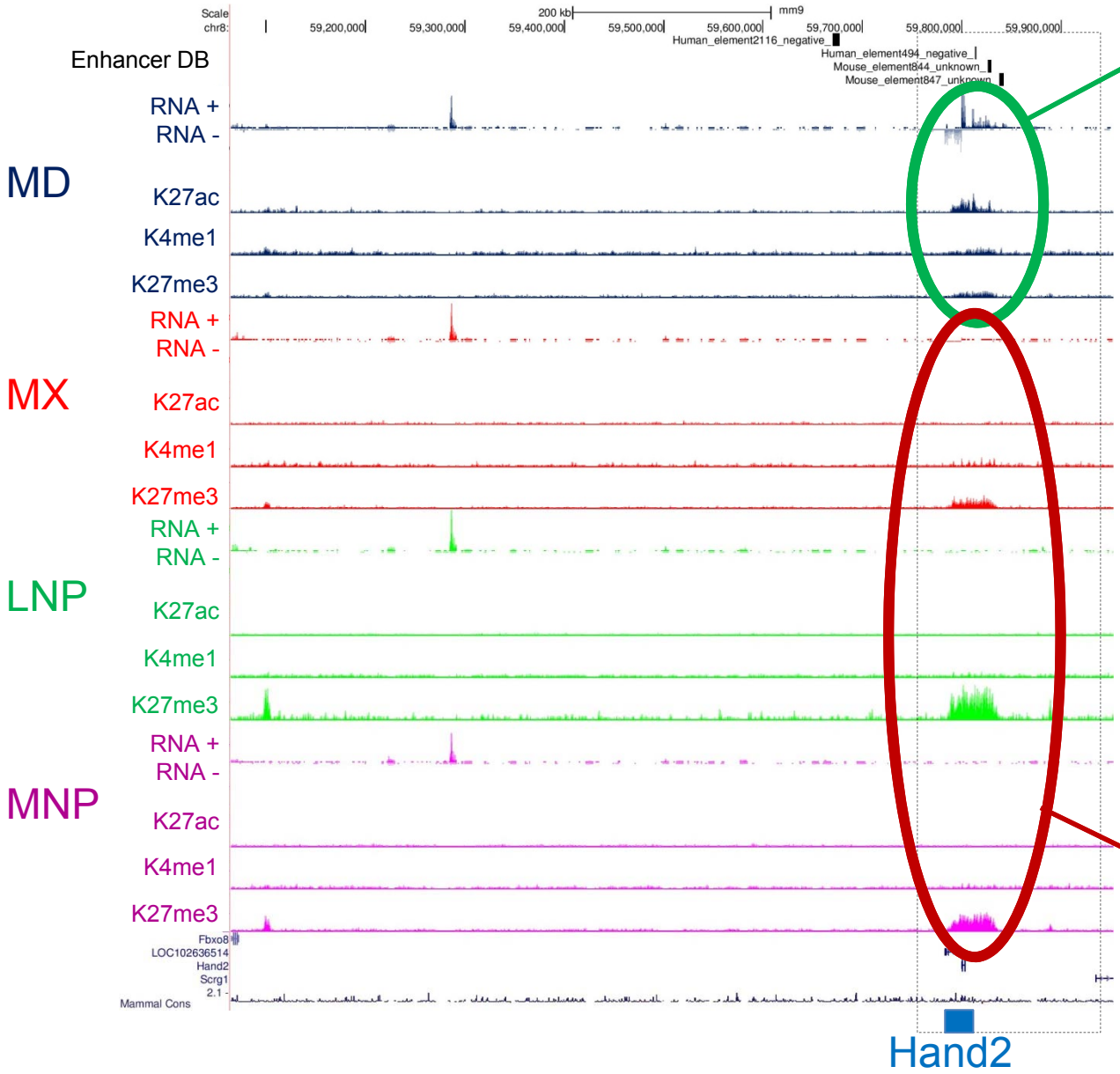
Mouse Tissues

Stage	*Tissue*	*RNA-seq*	*H3K4me1*	*H3K27ac*	*H3K27me3*
E11.5	Mandibular process	√	√	√	√
	Maxillary process	√	√	√	√
	Lateral nasal prominence	√	√	√	√
	Medial nasal prominence	√	√	√	√

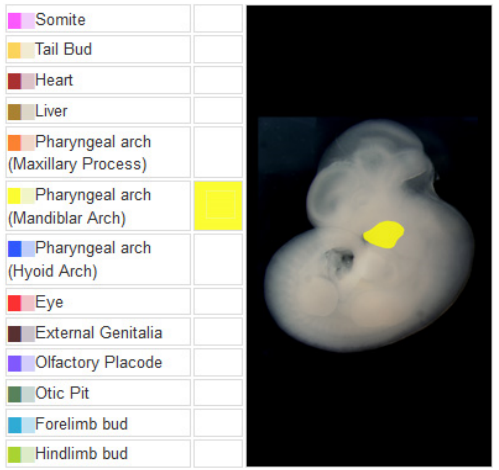
Stage	*Tissue*	*RNA-seq*	*H3K4me1*	*H3K27ac*	*H3K27me3*
E13.5	Mandibular process	√			
	Maxillary process	S			
	Nose	S			

√: RNA-Seq or ChIP-Seq finished including QC and primary data analysis
S: Currently in sequencing

Histone signatures at genes correlate with subregional gene expression



"Active" histone signature
+ H3K27ac
-- H3K37me3



E11.5 ISH data:
http://embrys.jp

"Repressed" histone signature
-- H3K27ac
+ H3K37me3

Enhancer predictions?

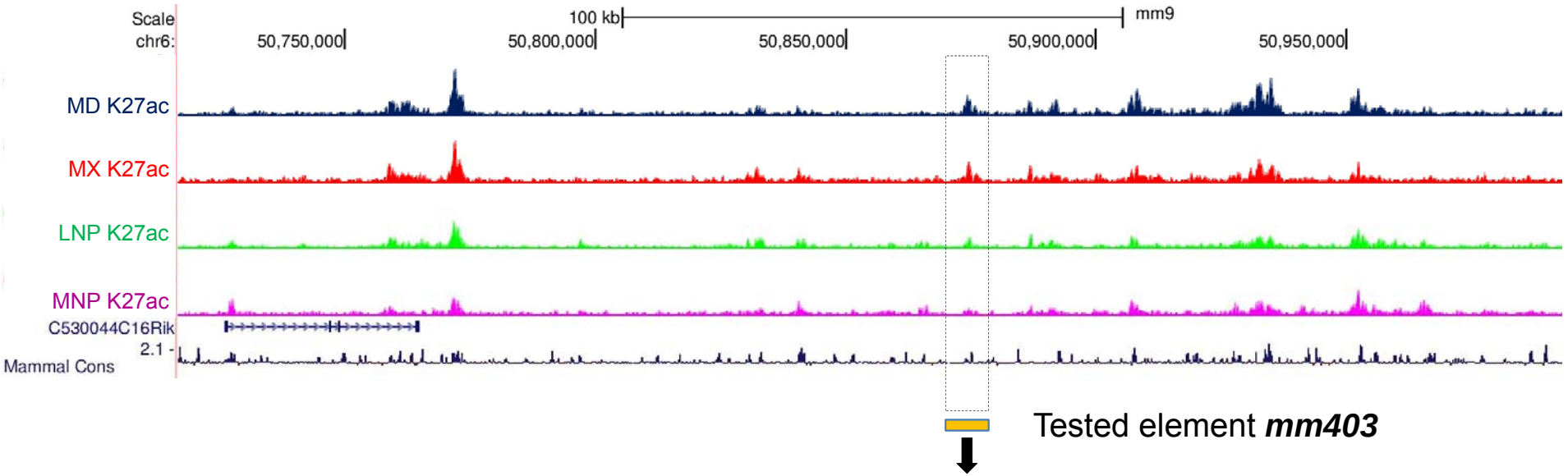
First-pass analysis: GREAT ontology analysis

(shown: top 1000 distal MNP H3K27ac peaks)

Top 10 Mouse Phenotypes associated with candidate enhancers

Term Name	Binom P-Value	Binom Fold Enrichment	Binom Observed Hits
abnormal craniofacial development	1.9E-36	3.1	162
abnormal maxilla morphology	6.4E-36	4.7	98
abnormal viscerocranium morphology	7.6E-34	3.2	145
abnormal cranium morphology	8.9E-34	2.7	178
abnormal craniofacial morphology	1.1E-33	2.2	256
abnormal craniofacial bone morphology	1.8E-33	2.7	181
abnormal axial skeleton morphology	2.4E-33	2.3	232
abnormal neurocranium morphology	8.6E-33	3.4	128
abnormal skeleton morphology	1.2E-32	2.1	280
abnormal limbs/digits/tail morphology	1.5E-32	2.3	224

Differential H3K27ac signal correlates with subregional *in vivo* enhancer activity

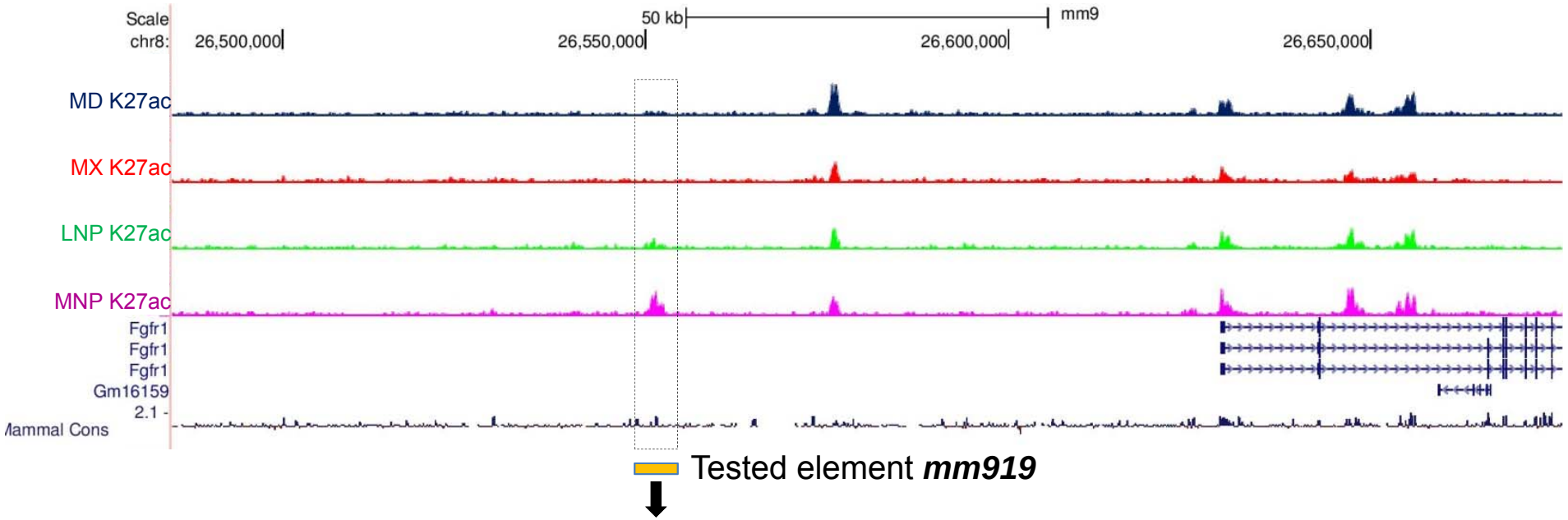


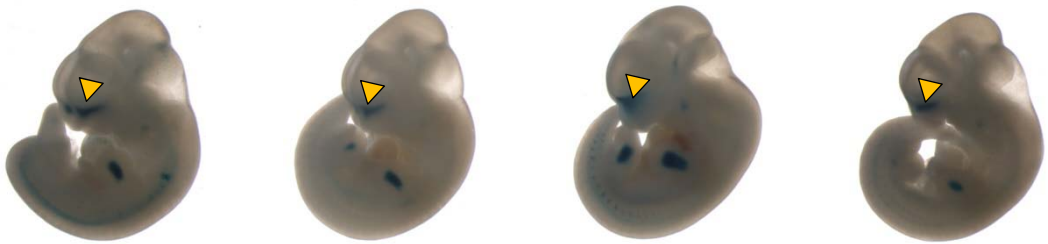
Tested element ***mm403***

In vivo LacZ pattern
Reproducibility: 10/11

Pattern includes MD/MX
but not LNP/MNP

Differential H3K27ac signal correlates with subregional *in vivo* enhancer activity

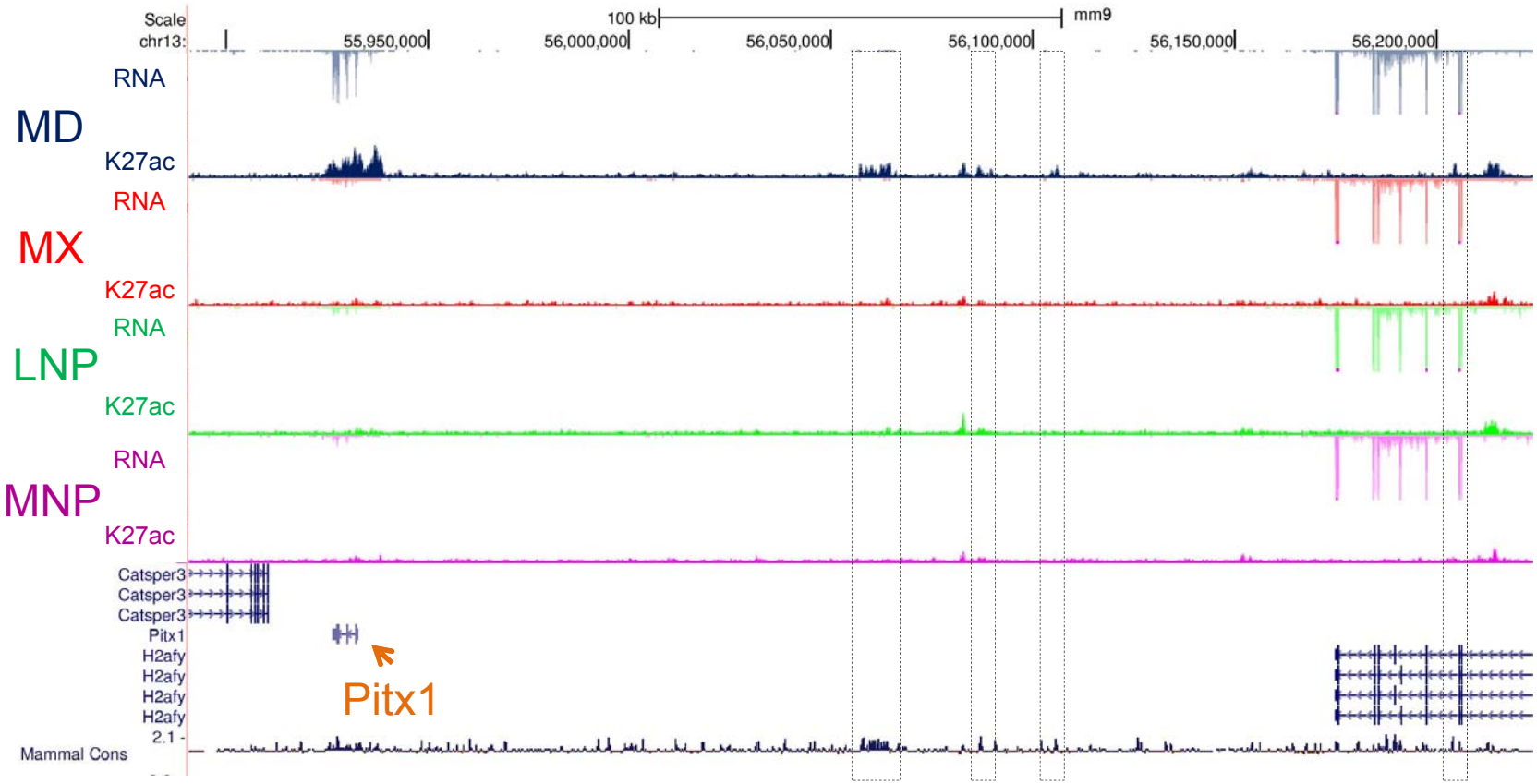


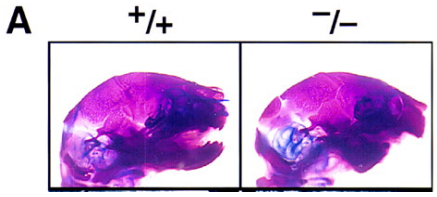


In vivo LacZ pattern
Reproducibility: 10/11

Pattern includes LNP/MNP but not MX/MD

H3K27ac signature identify candidate enhancers near genes with facial phenotypes



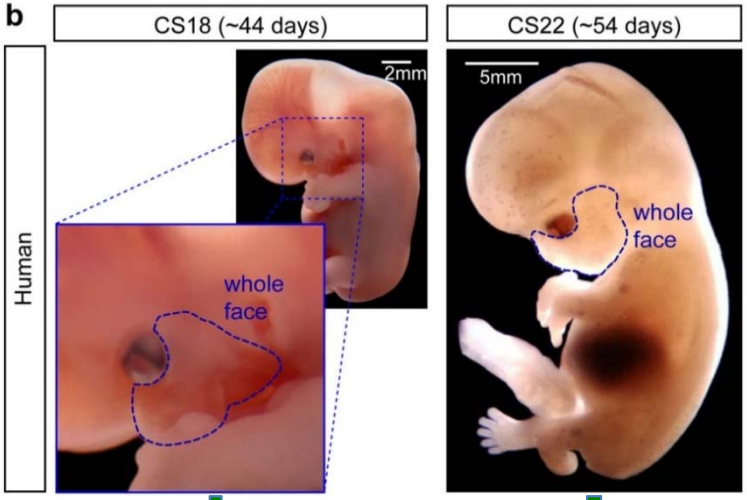


Pitx1 gene KO: severely reduced mandible

Szeto DP et al. Genes Dev. 1999

FACEBASE 2: Specific Aims

Aim 1b: Progress human tissues

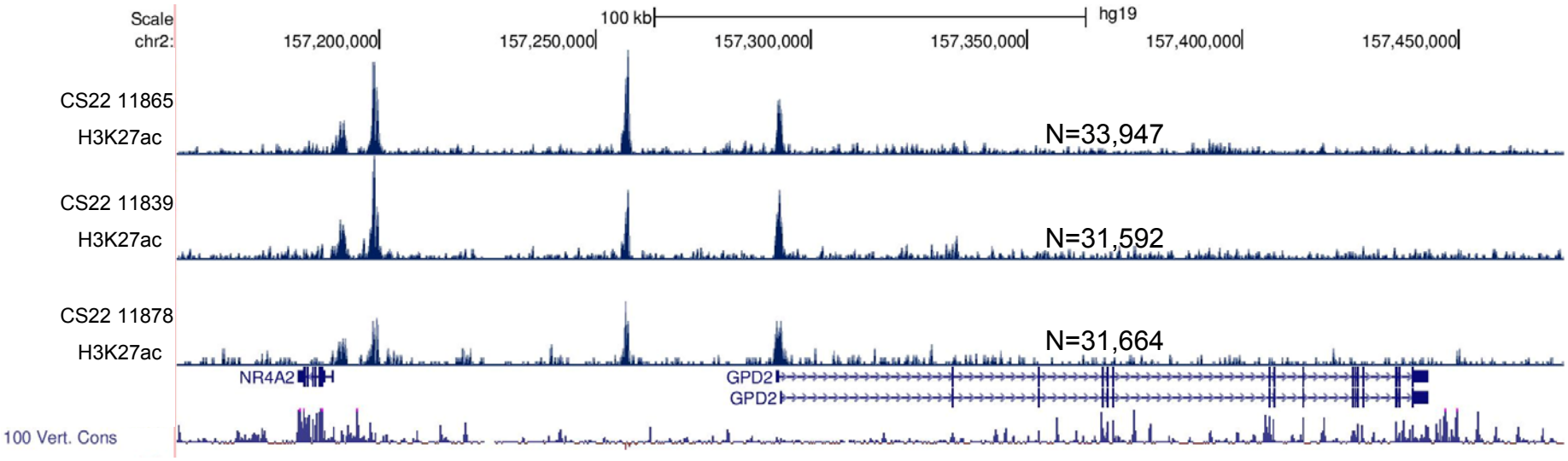


Human Tissue (whole face)

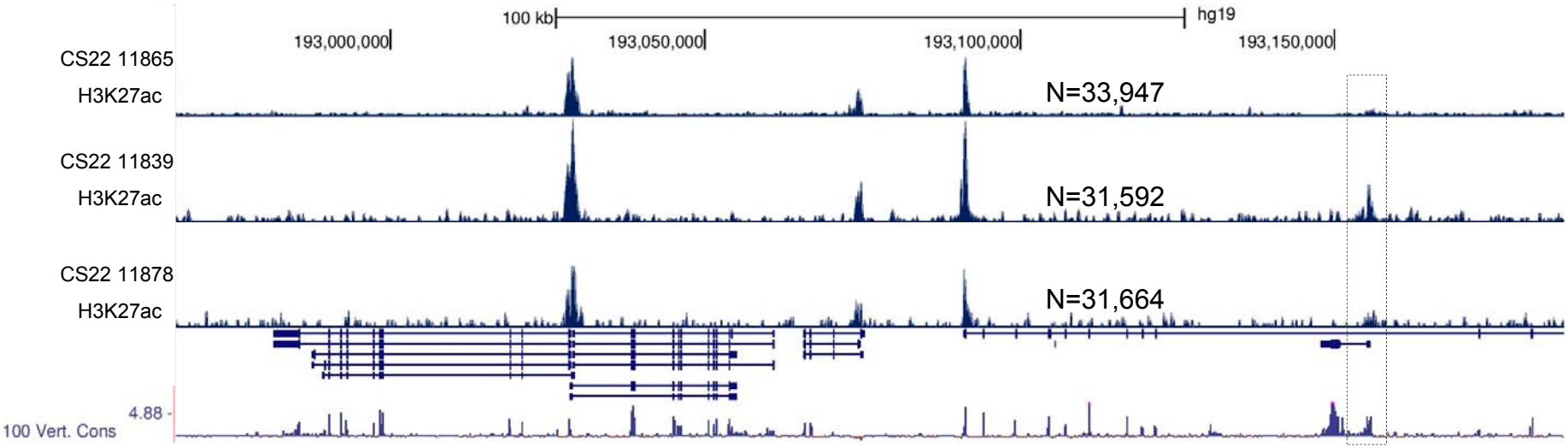
Stage	*ID*	*RNA-Seq*	*H3K4me1*	*H3K27ac*	*H3K27me3*
CS18	11904	S	S	S	
CS22	11839	√		√	
CS22	11865	√		√	√
CS22	11878	√		√	√
CS22	11940	√	S	S	
CS22	11954				
CS23	11884	√	S	S	S
CS23	11846				

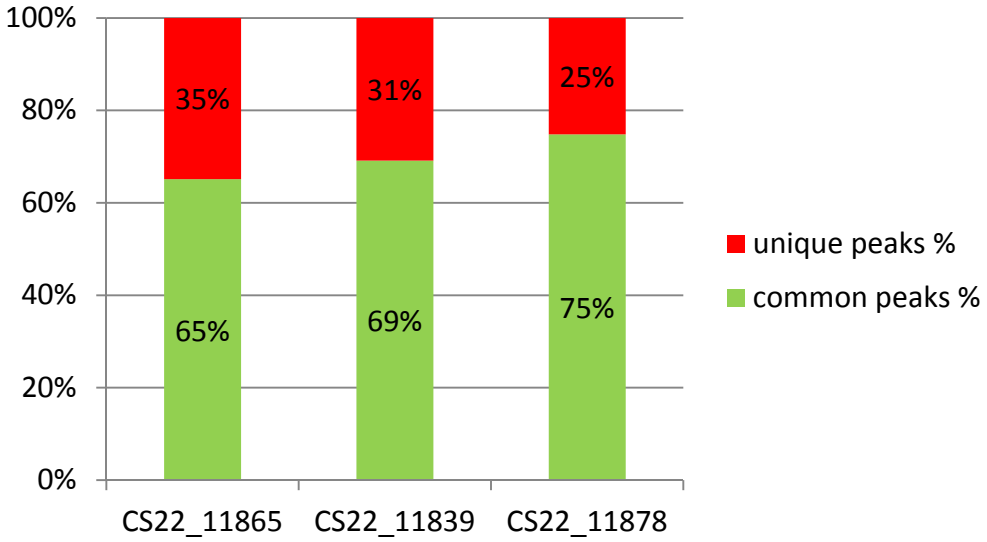
√: RNA-Seq or ChIP-Seq finished including QC and primary data analysis
S: Currently in sequencing

ChIP-Seq data sets from CS22 samples

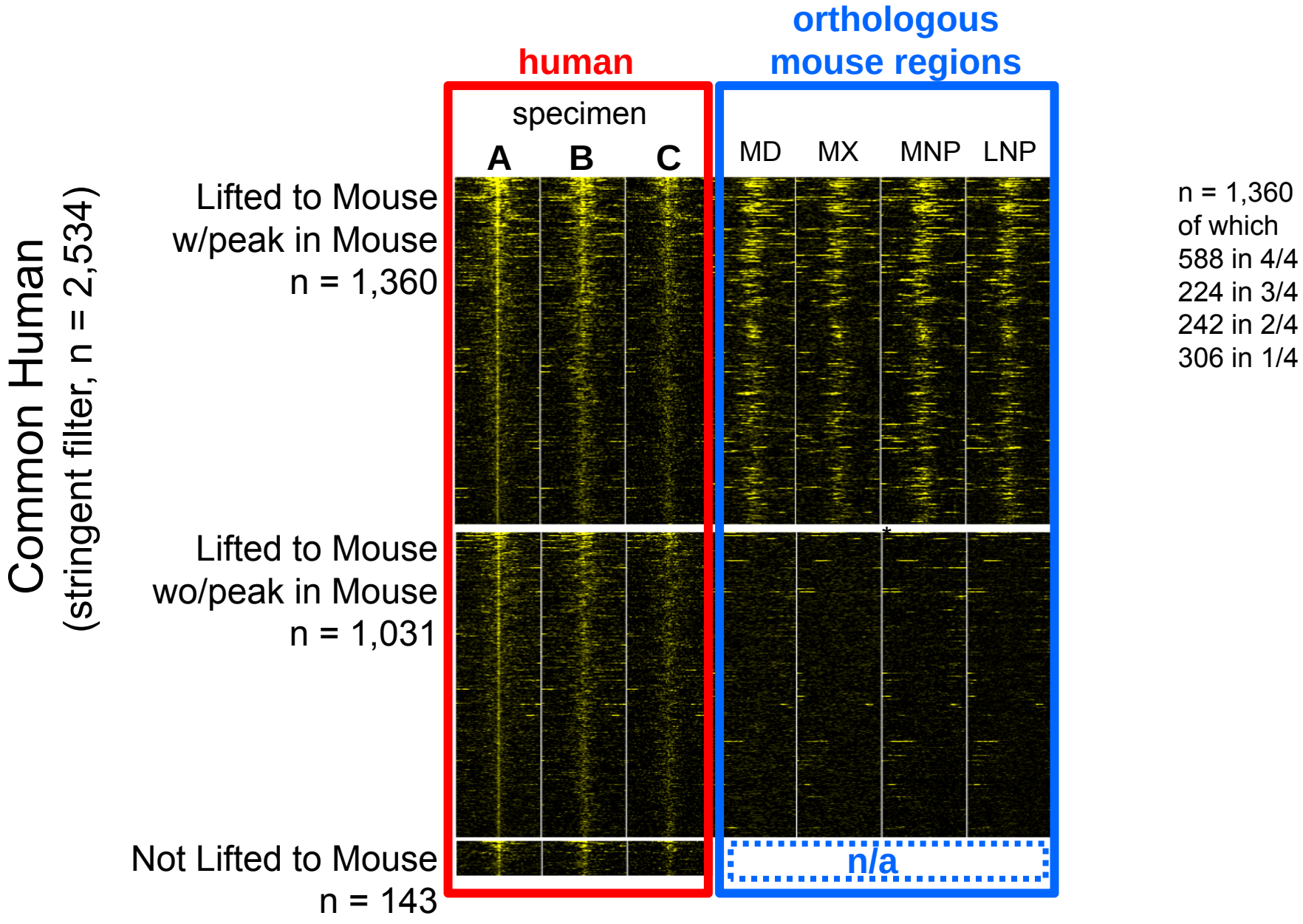


ChIP-Seq data sets from CS22 samples





Conservation of peaks across species



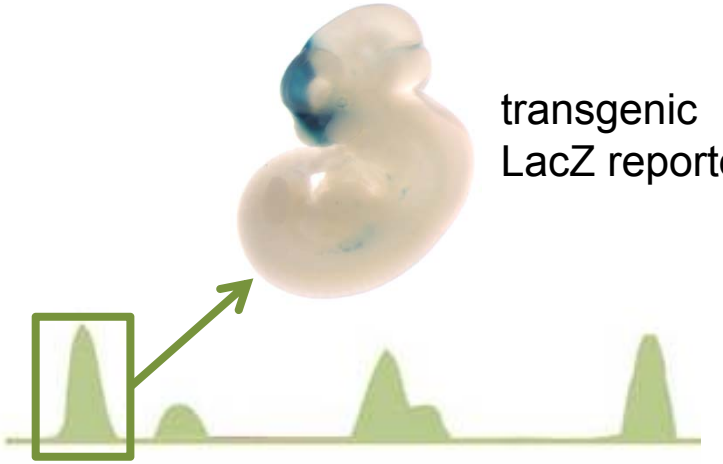
Aim 1

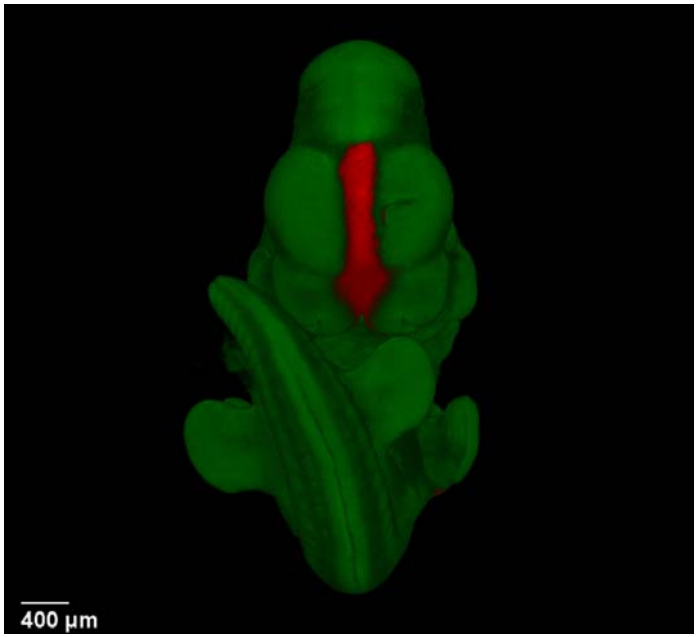
In progress:

- Correlate histone vs. expression data
- Systematic intersection of histone data with previously obtained transgenic data
- Subregional correlations of histone vs. expression data
- Examination of variation across individuals in human samples
- Identification of species-specific peaks (human vs. mouse)

FACEBASE 2: Specific Aims

Aim 2: Transgenic Assays of Candidate Enhancer Sequences



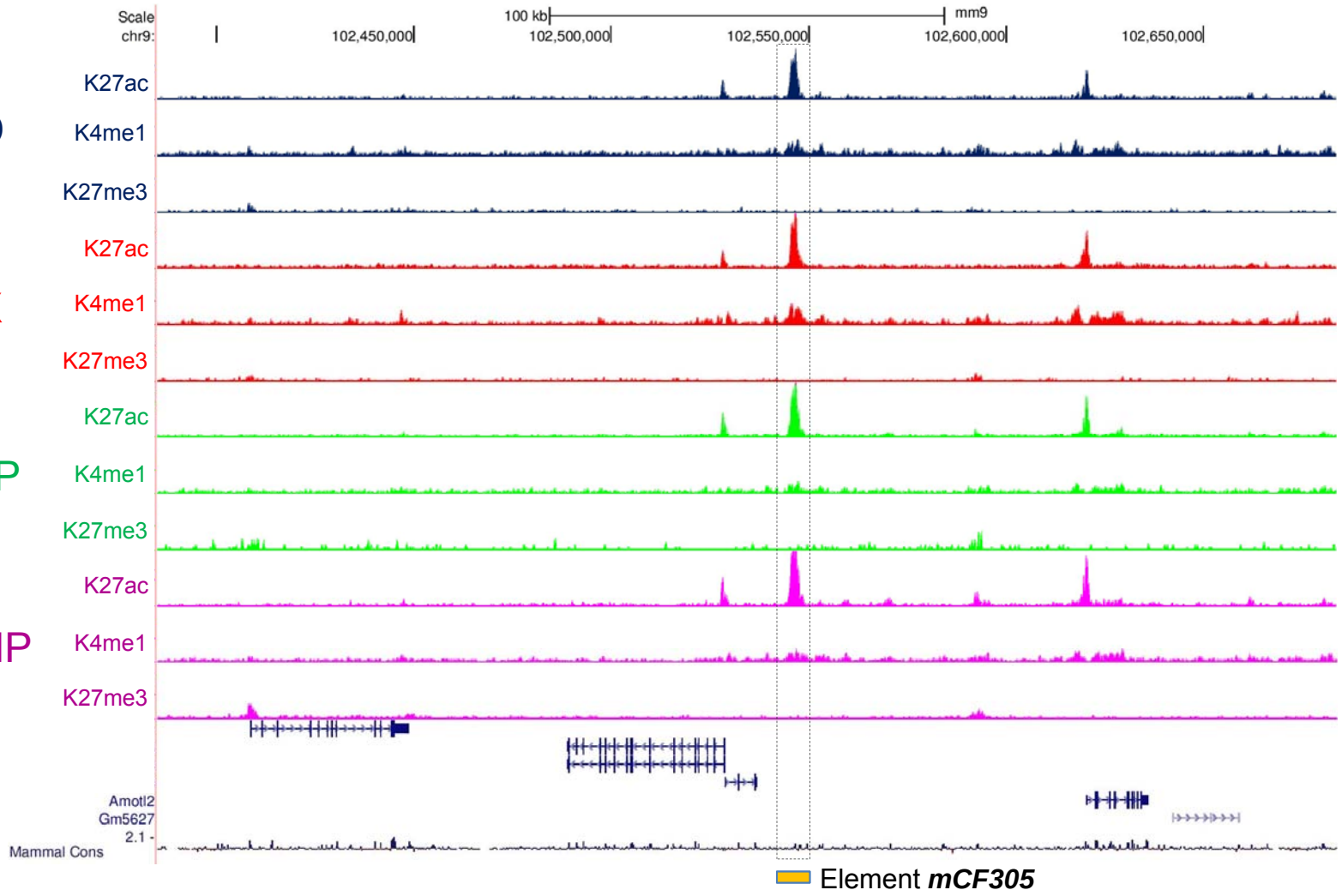


- Candidate sequences could be:
  - from developmental mouse studies
  - from human studies of CF birth defects
  - from human studies of normal variation
  - risk alleles of known CF enhancers
- ~25 transgenic experiments per year
- all positives will be OPT imaged

As in FaceBase 1:
We make this capability available to other FaceBase investigators and are looking forward to collaborate!

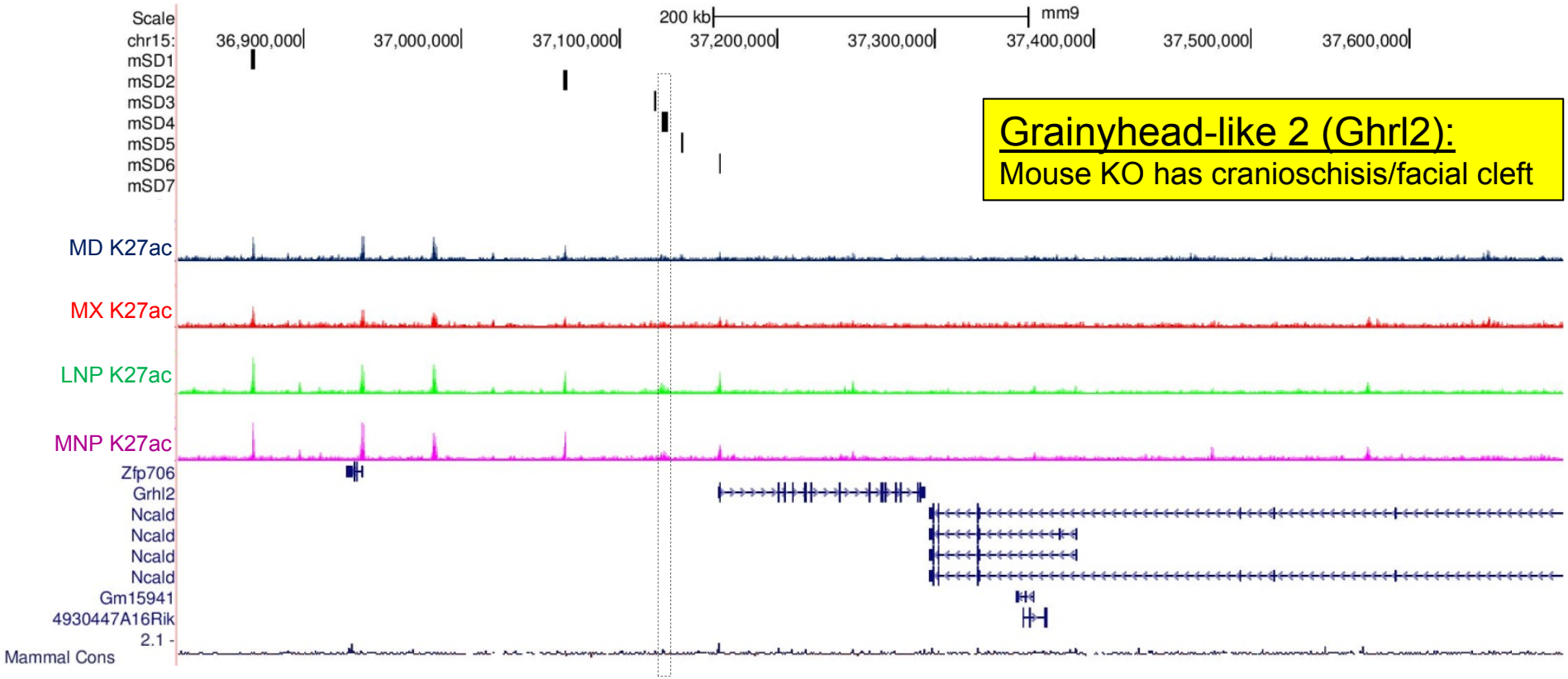
Testing enhancer predictions from new histone data sets

- The first batch of 16 such elements are in the transgenic pipeline for *in vivo* testing;
- The second batch for transgenics will be focused on candidates associated with craniofacial malfunctions



Using histone data to scan loci of interest for enhancers

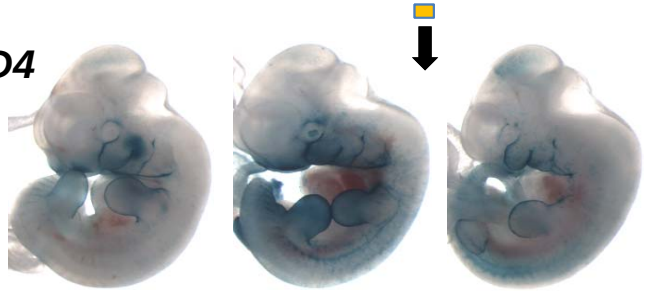
Example: Screening for enhancers near *Ghrl2*
With Sebastian Dworkin, Monash U.



Grainyhead-like 2 (Ghrl2):
Mouse KO has cranioschisis/facial cleft

Tested element ***mSD4***

In vivo LacZ pattern
Reproducibility: 6/7



- Highly reproducible ectodermal enhancer (consistent with *Ghrl2* expression)
- Remarkably similar to an enhancer of IRF6 we found earlier with Brian Schutte

Aim 2

In progress:

- Evaluation of palate-specific enhancers
  (still based on FaceBase 1 data sets)
- Evaluation of new histone-only enhancer predictions
- Considering: species-specific enhancers
  (comparison mouse-human)
- Collaborative testing at individual loci

FaceBase Investigators and beyond:

Please approach us with requests and suggestions for transgenic testing!