#### Genomic and Transgenic Resources for Craniofacial Enhancer Studies May 2016 Update



e15.5

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# Outline

### BACKGROUND

### **Data Generation Progress**

- Chromatin and RNA-seq data from mouse face regions
- Chromatin and RNA-seq data from human craniofacial tissue
- Transgenic enhancer validation/characterization
- OPT

### **Analysis, Interactions and Collaborations**

Integrative analysis of human and mouse chromatin data Human-specific craniofacial enhancers (Wysocka) Grhl2 enhancers (Dworkin)



# Distant-acting enhancers dictate tissue-specific gene expression



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### **Enhancer mapping by tissue-ChIP-seq**





### **Transgenic Characterization**

#### 3 of ~200 craniofacial enhancers



### **OPT imaging (enhancer:background)**

OPT imaging: David FitzPatrick/Harris Morrison, Edinburgh



400 µm

mCF90 First BA (Max and Mand)







400 µm

Mn1

mCF208\* Facial Mesenchyme

# FACEBASE 2: Specific Aims

#### Aim 1: Genome-wide enhancer Activity Mapping via ChIP-seq of Craniofacial Tissues



- Critical developmental windows
  - MOUSE maxillary, mandibular, medial/lateral nasal processes at e11.5, e13.5, and e15.5
  - HUMAN 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
- ATAC-seq: assay for open chromatin new



# FACEBASE 2: Specific Aims

Aim 1: Progress Mouse Tissues



#### **Mouse Tissues**

Stage	Tissue	RNA-seq	H3K4me1	H3K27ac	H3K27me3	
E11.5	Mandibular process	V	V	V	V	
	Maxillary process	V	V	V	V	
	Lateral nasal prominence	V	V	V	V	1
	Medial nasal prominence	V	V	V	v	

Stage	Tissue	RNA-seq	H3K4me1	H3K27ac	H3K27me3
E13.5	Mandibular process	V	V	٧	V
	Maxillary process	v	V	v	V
	Nose	v	V	v	V

 $\sqrt{2}$  RNA-Seq or ChIP-Seq finished including QC and primary data analysis



### Improved ChIP-seq protocols



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# FACEBASE 2: Specific Aims

Aim 2: Transgenic Assays of Candidate Enhancer Sequences



- Candidate sequences:
  - Scanning loci of interest for enhancers (developmental biology, human genetics)
  - Testing predictions of region-specific enhancer activity
- 26 transgenic experiments in 2015
- 62% positive for craniofacial structures

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



# FACEBASE 2: Specific Aims

Aim 2: Optical projection tomography data (OPT)



- 43 available in FaceBase Hub
- 49 more generated (David FitzPatrick Lab)

### **Analysis, Interactions and Collaborations**



Systematic identification of enhancers with sub-regional activity patterns





### Integrative analysis of chromatin and RNA-seq data



# Candidate regions currently being tested in transgenics



### **Human-Specific Craniofacial Enhancers**

#### (collaboration with Wysocka group)



### Conservation of peaks across species



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n = 1,360 of which 588 in 4/4 224 in 3/4 242 in 2/4 306 in 1/4 Using tissue-ChIP-seq data to support identification of human-specific craniofacial enhancers



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### Preliminary results

×.

JW6 element



Reproducibility: 6/7

Chimp

In progress



### **Collaborative Testing of Enhancers**

(example: Seb Dworkin, Monash University)



#### Using histone data to scan loci of interest for enhancers

Example: Screening for enhancers near *Grhl*2 With Sebastian Dworkin, Monash U.



Tested element **mSD4** In vivo LacZ pattern Reproducibility: 6/7

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- Highly reproducible ectodermal enhancer (consistent with *Grhl2* expression)
- Remarkably similar to an enhancer of IRF6 we found earlier with Brian Schutte

# Ectodermal enhancer of Grhl2



FaceBase Investigators and beyond: Please approach us with requests and suggestions for transgenic testing!

## **Project Team**

#### Lawrence Berkeley National Lab

Experimental postdocs: Cailyn Spurrell, Marco Osterwalder, Evgeny Kvon Computational postdocs: Yoko Yuzawa (data liaison), Iros Barozzi Other Senior Staff: Diane Dickel, Len Pennacchio Molecular Biology and Mouse Transgenics: Jennifer Akiyama, Veena Afzal, Brandon Mannion, Cathy Pickle, Ingrid Plaijzer-Frick, Momoe Kato, Tyler Garvin, Elizabeth Lee

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

HDBR, Newcastle, UK Steven Lisgo

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

#### University of Southern California – KO analysis Yang Chai

Major Spoke Collaborator: Wysocka Lab

