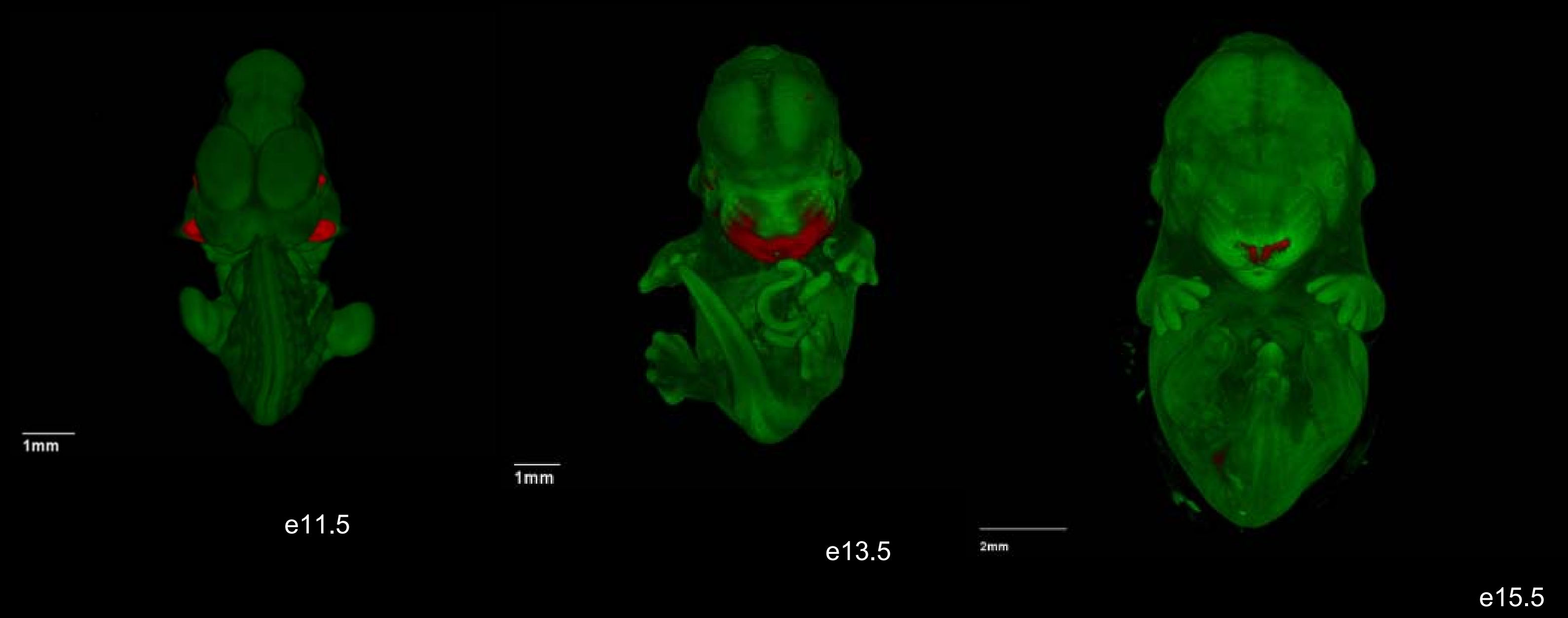
Genomic and Transgenic Resources for Craniofacial Enhancer Studies

May 2018 Update



FaceBase

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School of Natural Sciences
University of California, Merced

Poster

sc RNA-Seq

Sudha Rajderkar

ChIP-/RNA-seq

Cailyn Spurrell

Informatics

Matthew Blow

Valentina Snetkova

Outline

Data Generation Progress

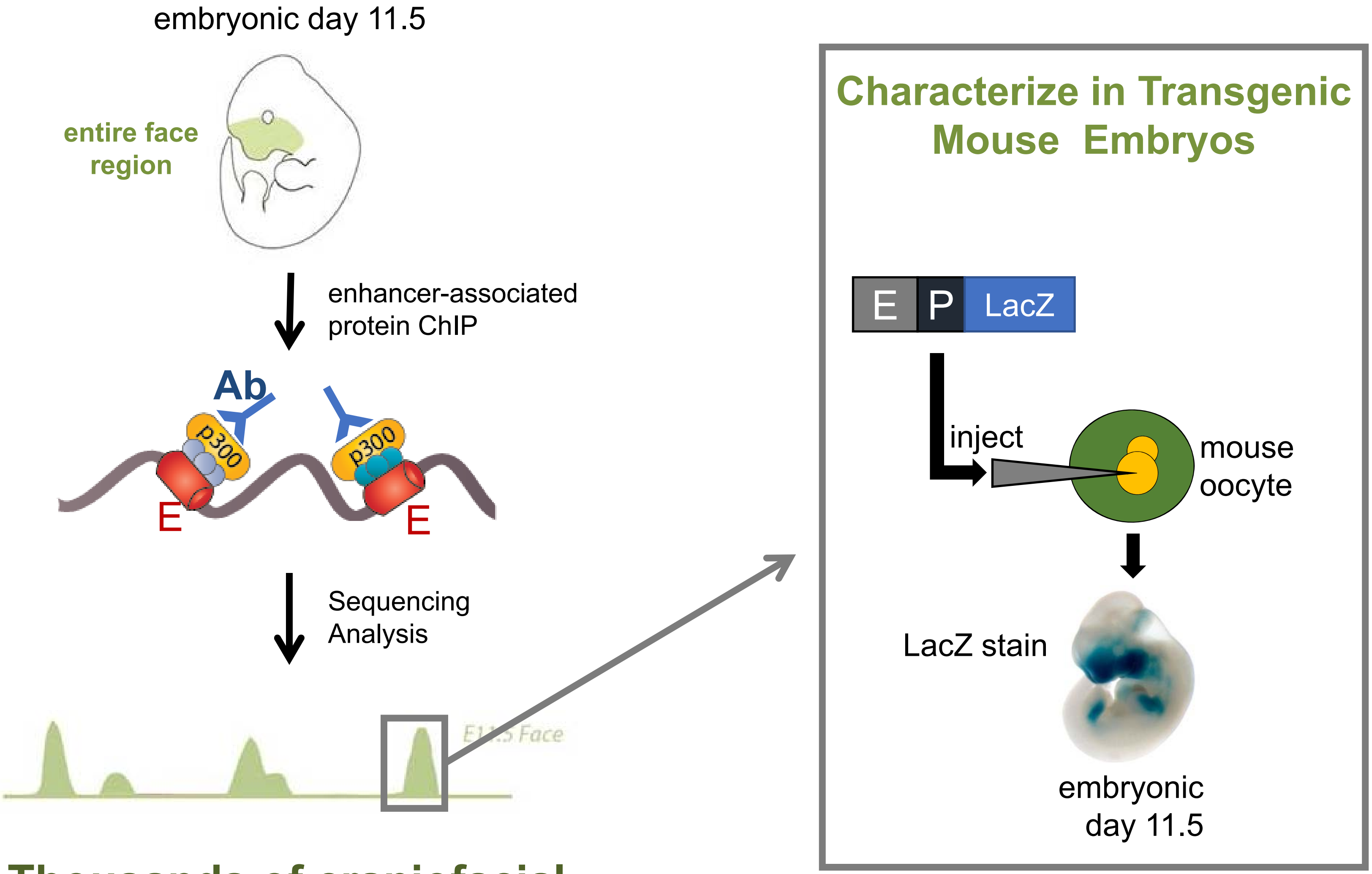
Chromatin and RNA-seq data from **human** and **mouse** craniofacial tissue, transgenic validation and OPT

Analysis, Interactions and Collaborations

Mapping Enhancers at Single Cell Resolution

Centralized FaceBase Bioinformatics Pipeline

Enhancer Mapping by Tissue-ChIP-seq



embryonic day 11.5

entire face region

enhancer-associated protein ChIP

Sequencing Analysis

Characterize in Transgenic Mouse Embryos

inject

mouse oocyte

LacZ stain

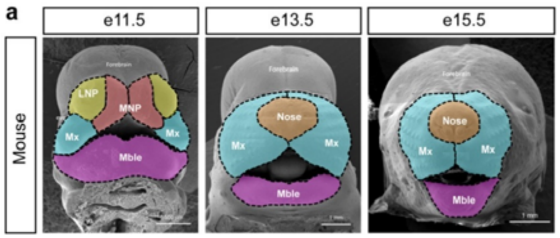
embryonic day 11.5

Thousands of craniofacial candidate enhancers

Data Generation Progress

FACEBASE 2: Specific Aims

Aim 1a: Progress Mouse Tissues



Mouse Tissues

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

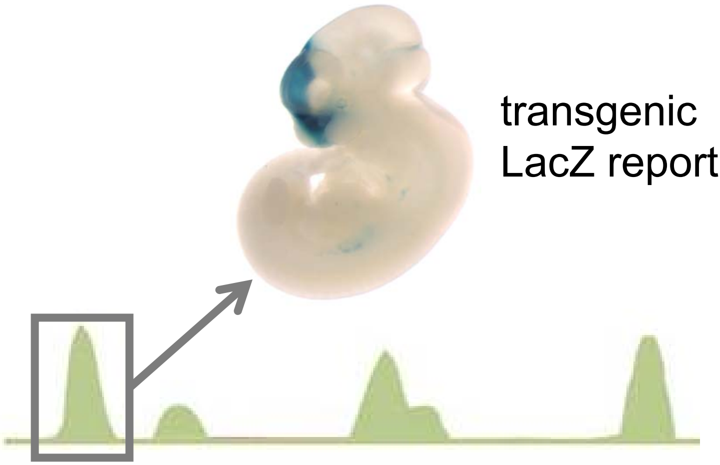
Stage	*Tissue*	*RNA-seq*	*H3K4me1*	*H3K27ac*	*H3K27me3*
E13.5	Mandibular process	√	√	√	√
	Maxillary process	√	√	√	√
	Nose	√	√	√	√

Stage	*Tissue*	*RNA-seq*	*H3K4me1*	*H3K27ac*	*H3K27me3*
E15.5	Mandibular process	√	√	√	√
	Maxillary process	√	√	√	√
	Nose	√	√	√	√

- major milestones complete
- in progress: replicates for E15.5

FACEBASE 2: Specific Aims

Aim 2: Transgenic Assays of Candidate Enhancer Sequences



- Candidate sequences:
  - Candidate regions from developmental mouse studies
  - Risk variants/alleles from human studies of CF birth defects (GWAS and Mendelian)
  - Variants linked to normal variation in GWAS
- 20 transgenic experiments in 2017
- 45% positive for craniofacial structures

We make this capability available to other FaceBase investigators and are looking forward to collaborating!

Ernest Orlando Lawrence
Berkeley National Laboratory

FACEBASE 2: Specific Aims

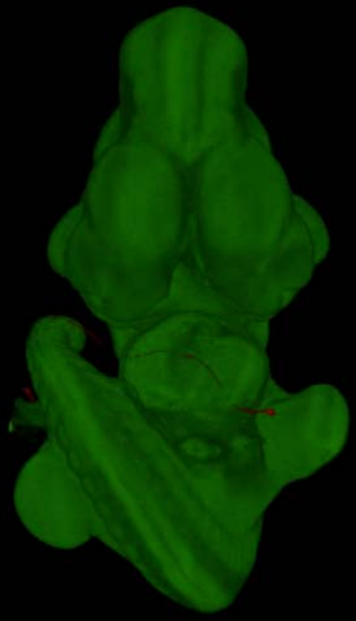
Aim 2: Optical projection tomography data (OPT)

enhancer: **background**

43 available in FaceBase Hub

74 more generated to date

hs59
Nasal process
Branchial arch
Intragenic to *RPGRIP1L*
Near *IRX3/4*



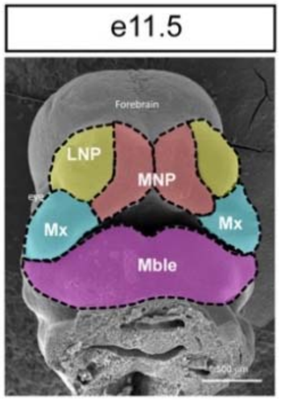
hs921
Mandibular process

Intragenic to *AGAP1*
Near *GBX2*

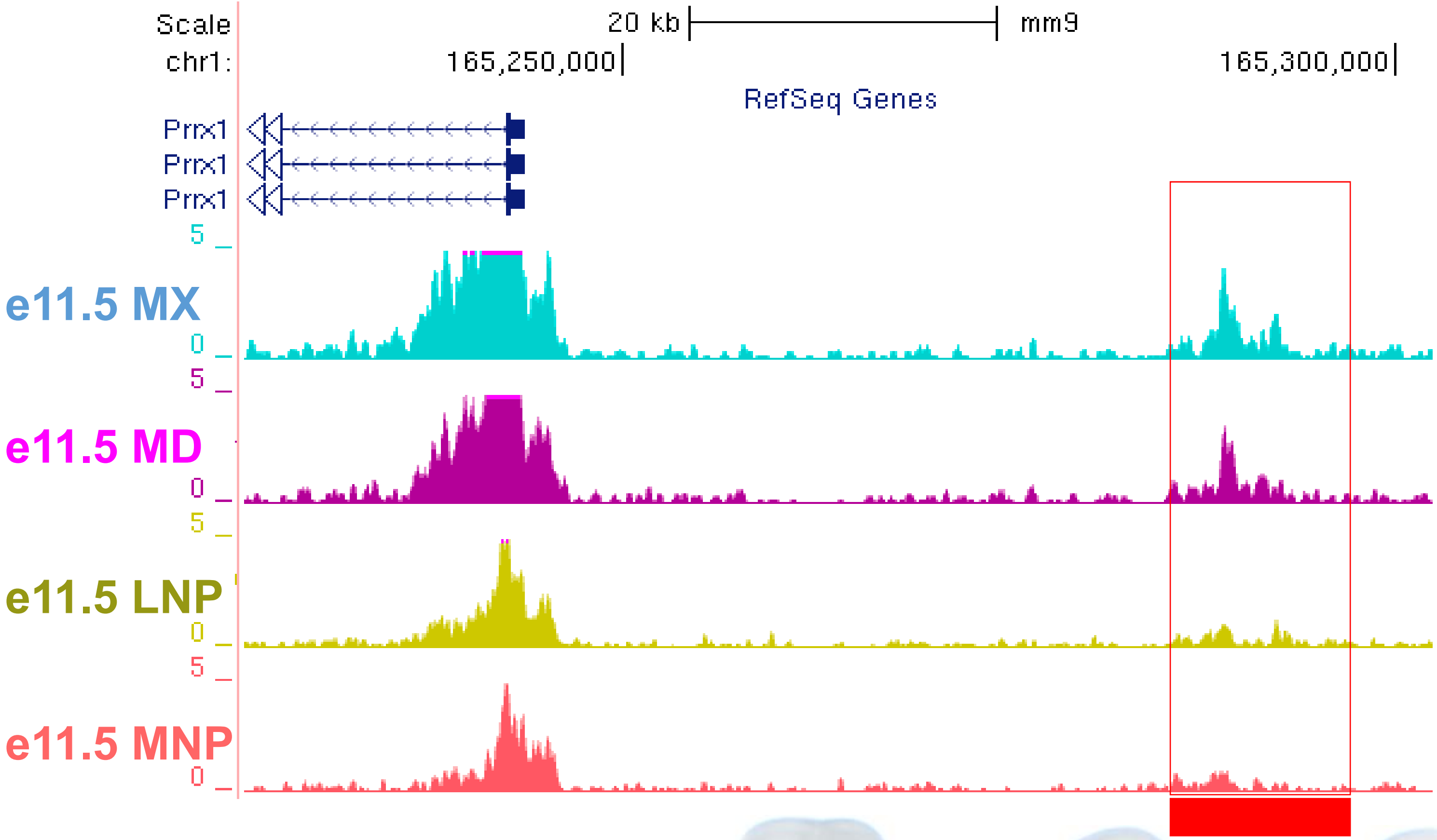
MRC Human Genetics, Edinburgh, UK - David FitzPatrick, Harris Morrison

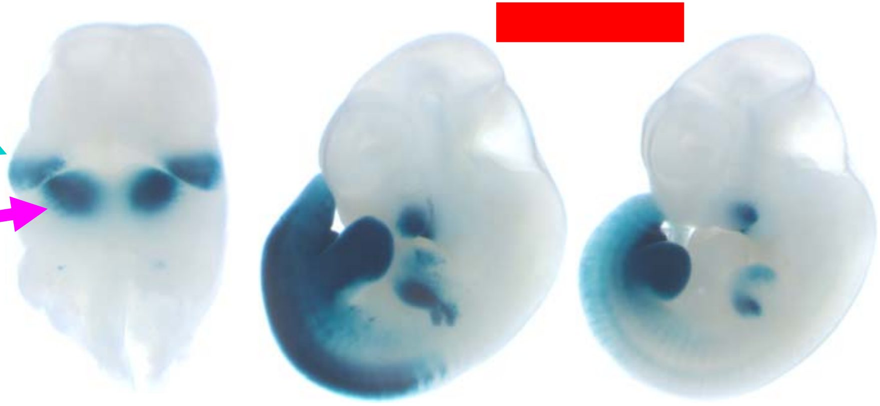
Analysis, Interactions and Collaborations

Integrative analysis identifies MX/MD-specific enhancer near *Prrx1*



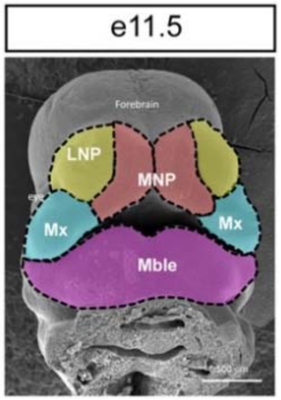
Shown:
H3K27ac
only



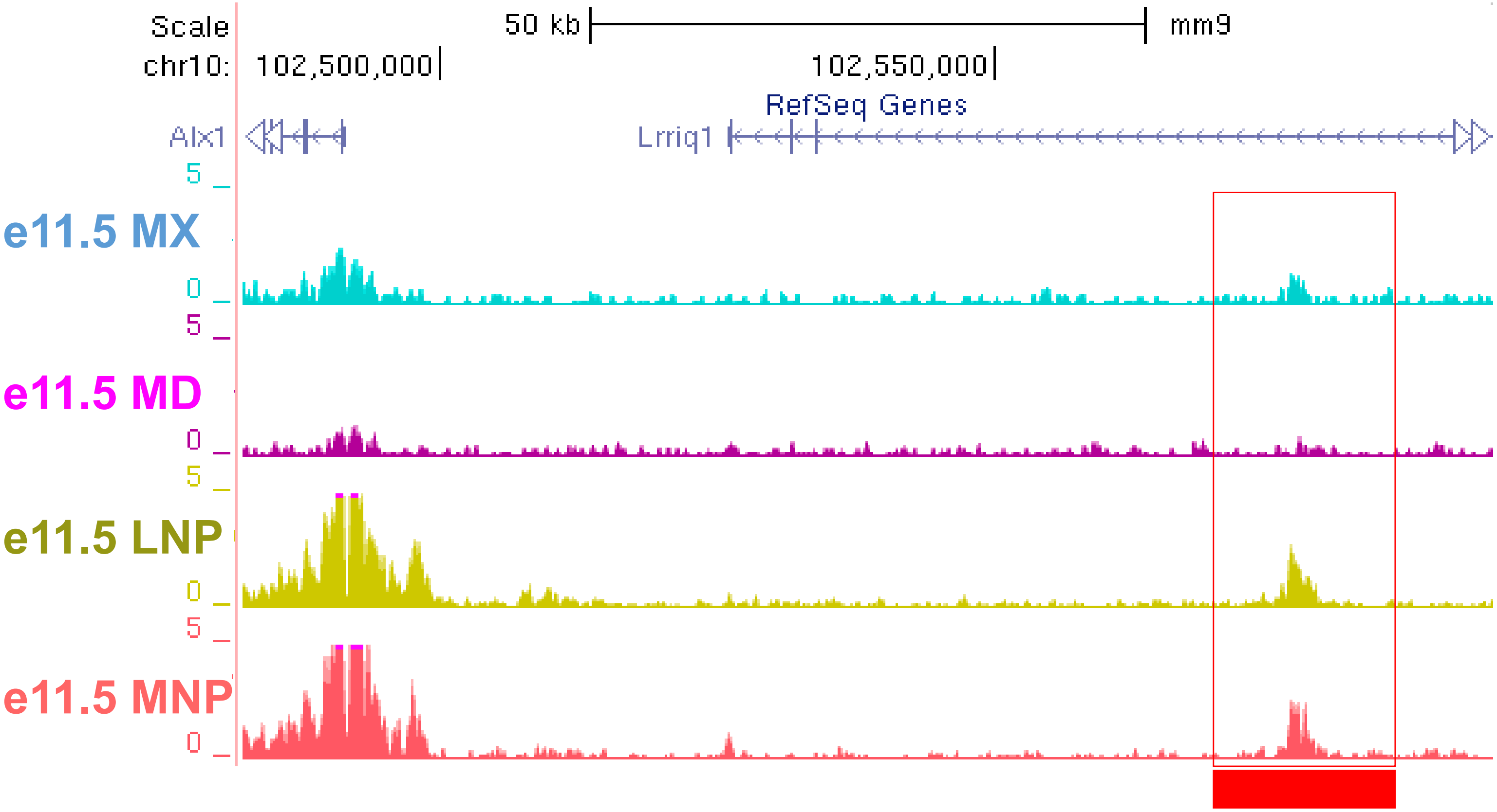


In vivo LacZ pattern
Reproducibility: 4/6

Integrative analysis identifies NP-specific enhancer near *Alx1*



Shown:
H3K27ac
only



Larger transgenic series in progress!

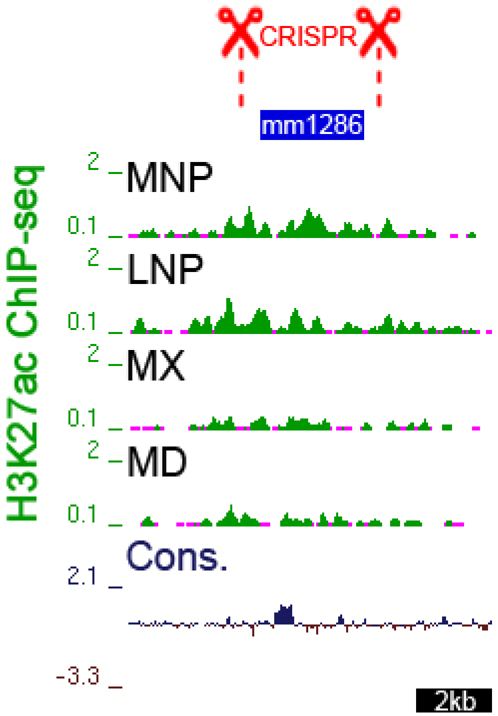
In vivo LacZ pattern
Reproducibility: 7/8

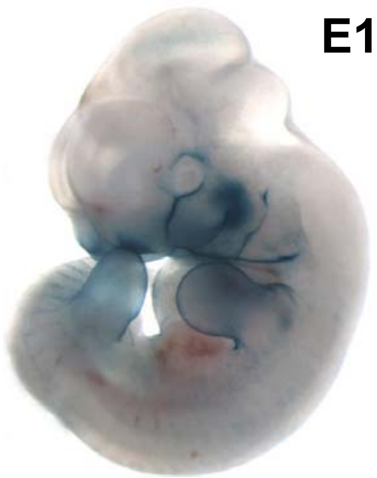
Ernest Orlando Lawrence Berkeley National Laboratory

Collaborations and Community Resources

(Example: Enhancer Landscape of GHRL2 - with Seb Dworkin)

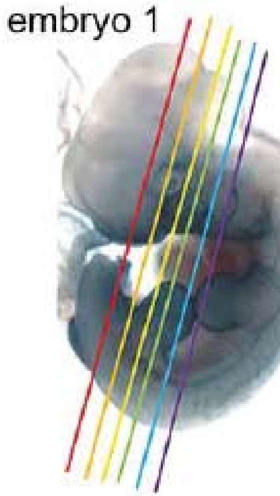
***Grhl2* -34kb**

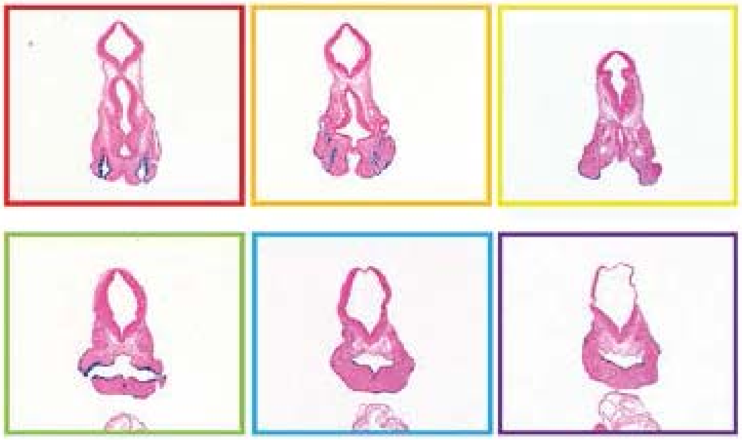




Grhl2 has essential roles in epithelial morphogenesis and epidermal development.

The mm1286 enhancer is active in craniofacial epithelia at E11.5.





Enhancer KO line generated and transferred to **Dworkin Lab** for detailed molecular and phenotypic analysis

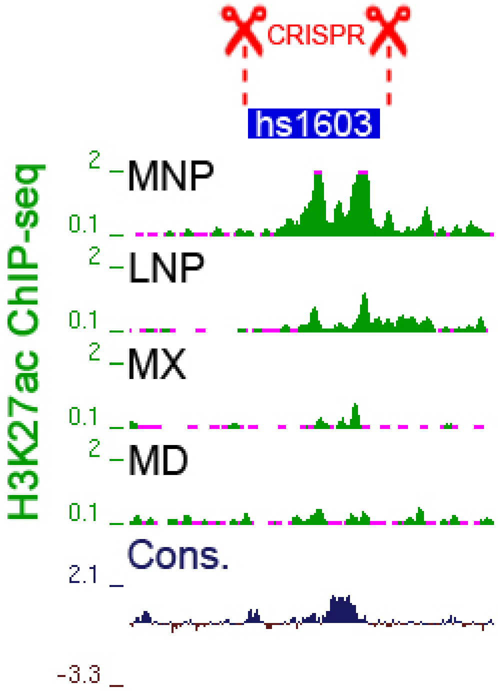
Sebastian Dworkin

La Trobe University, Melbourne

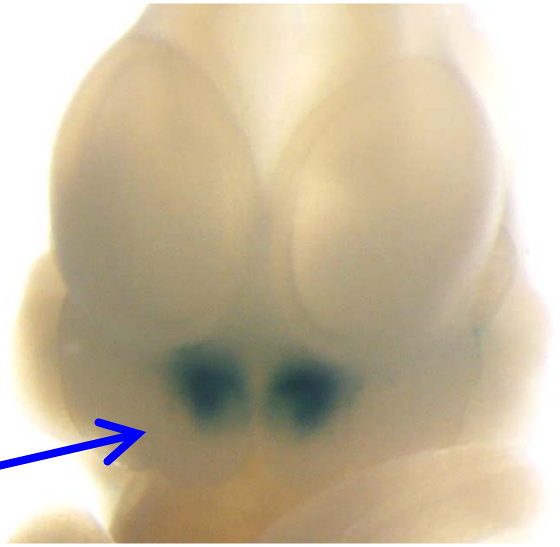
Enhancer Function in Known CF Loci

(Example: SOX5 - with Benedikt Hallgrimsson)

***Sox5* +710kb**

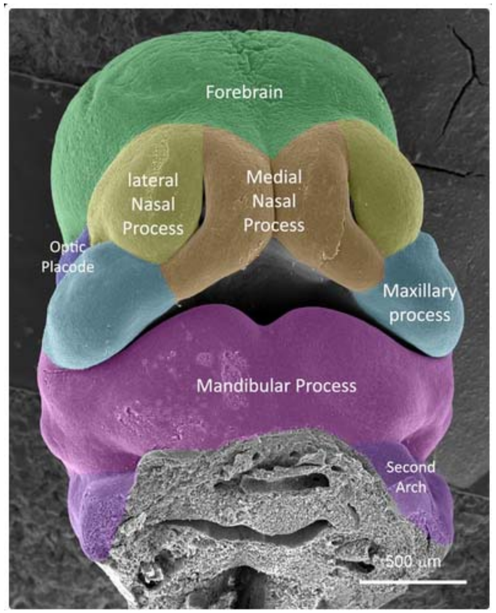


E11.5



>40 skulls from Enhancer KOs and WT controls at p56 shipped to **Hallgrimsson lab**, micro-CT analysis of results in progress

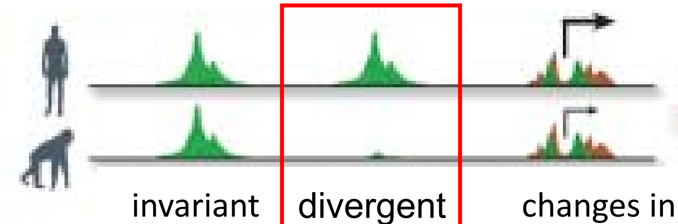
Also: LNP/MNP enhancer near *Bmp4*



LAMB-SHAFFER SYNDROME (OMIM: 616803): associated with ***Sox5***;
→ facial dysmorphologies (e.g. **nasal bridge defects**)

In vivo Functional Characterization and Validation of Enhancers Driving Morphological Difference Between Human and Chimp Face

ATAC-seq, ChIP-seq, RNA seq from in vitro differentiated NCCs*



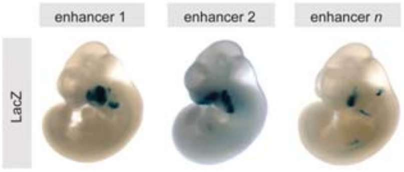
invariant enhancer

divergent enhancer

changes in gene expression

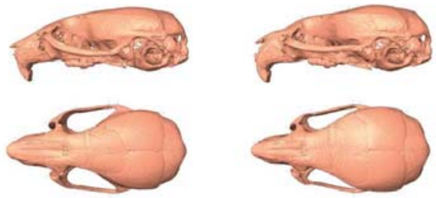
Part 1

Characterization using transgenic reporters in mice



Part 2

Functional validation using CRISPR/Cas9 enhancer swap and phenotyping in mice

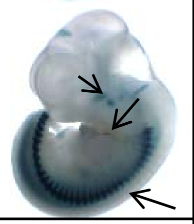


Part 3

With Wysocka/Selleri spoke project

*Prescott *et al.*, Cell 2015

Validation of Enhancers with Differential Activity

Name	Human	Chimp	Mouse	Human	Putative Target Gene
JW1				Limb (9/10), Periocular Mesenchyme (4/10)	*Prrx1/Prx1/MHox*
JW3				Trigeminal (6/8), Telencephalon (8/8)	*Edn3*
JW5				Face, Heart, Somites/DRG, and Otic vesicle (6/7)	*Otos*
JW6			No blues; 6tg by PCR	Heart, Facial mesenchyme, Hindbrain, Mesenchyme	*Mylip*

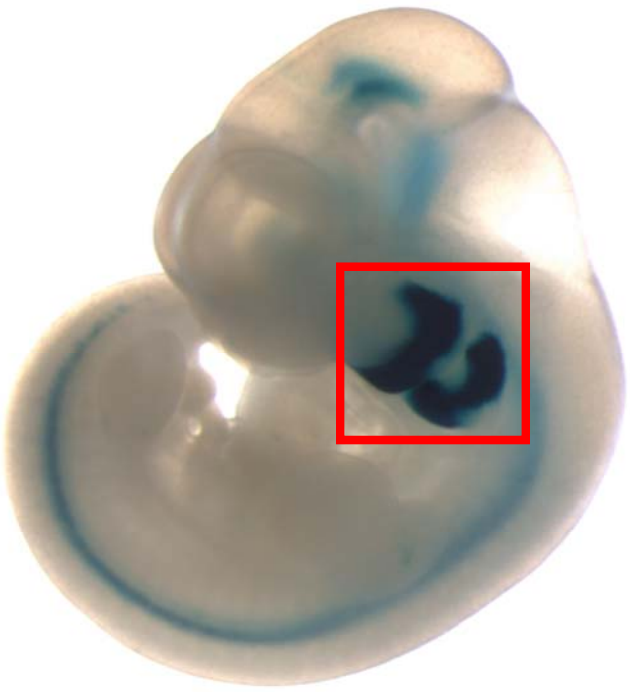
Knock-in in progress

Also: Sox9 – see Licia's presentation

With Wysocka/Selleri spoke project

Mapping Enhancers at Single Cell Resolution

What Cell Type is This Enhancer Active In?



Enhancers likely define specific cell populations

…But typically don't have cell type information

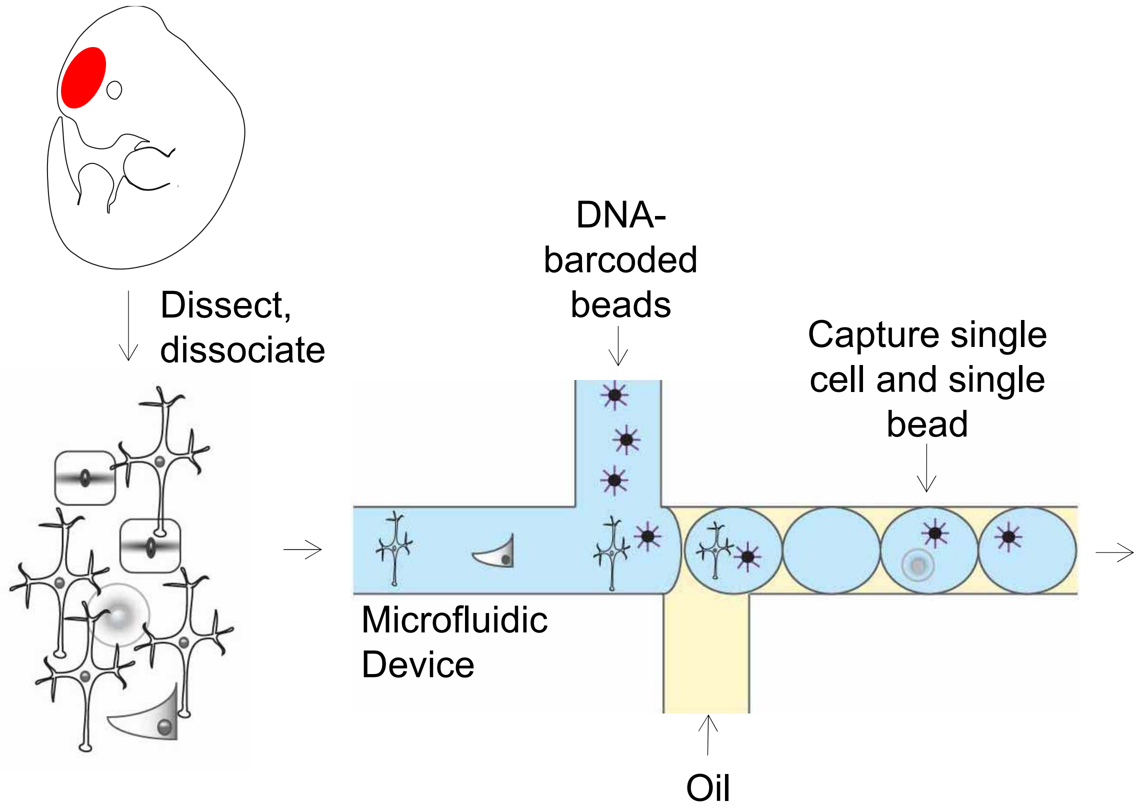
ChIP-Seq, RNA-Seq:

Resolution historically limited to bulk tissue

Transgenic assay:
Determining cell type requires laborious immunofluorescence labeling

Can emerging single cell technologies solve this problem?

Single Cell RNA-seq is Becoming Routine



Dissect, dissociate

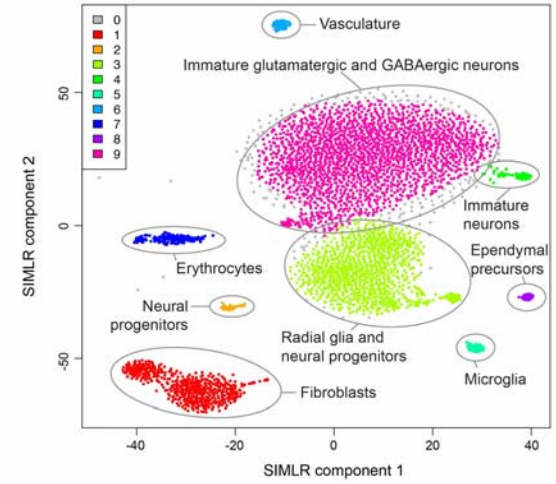
DNA-barcoded beads

Capture single cell and single bead

Microfluidic Device

Oil

RNA-seq on >4,700 single brain cells



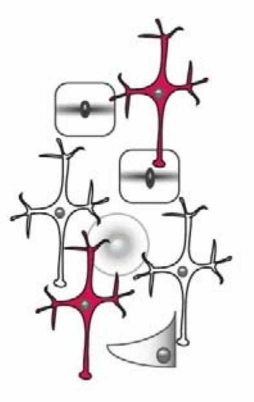
e.g., Drop-seq, 10X Chromium

Dickel *et al*. (2018) Cell

Single Cell RNA-seq is Becoming Routine

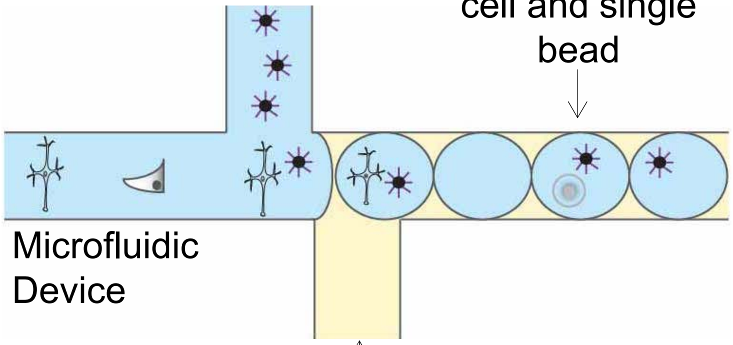
Enhancer active in **forebrain** → Enhancer active in **forebrain immature glutamateric/GABAergic neurons**

Dissect, dissociate



DNA-barcoded beads

Capture single cell and single bead

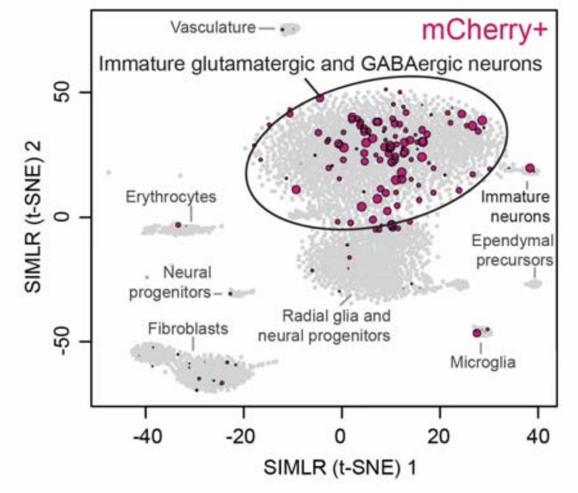


Microfluidic Device

Oil

e.g., Drop-seq, 10X Chromium

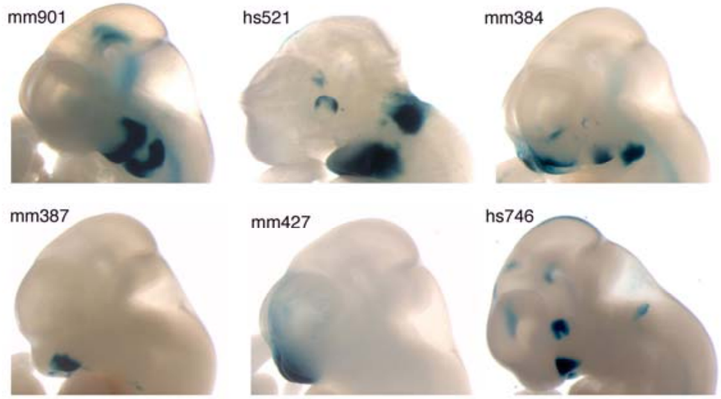
Mapping mCherry onto RNA-seq on >4,700 single brain cells



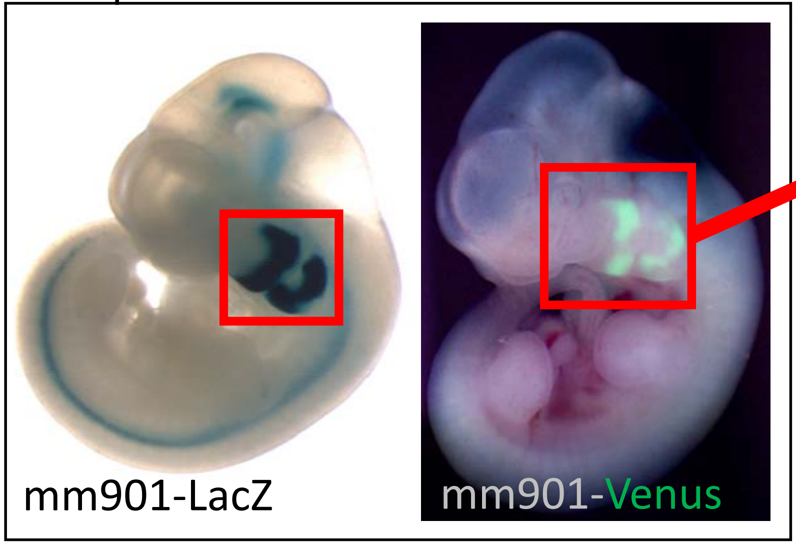
Dickel *et al*. (2018) Cell

Single Cell Profiling of E11.5 Face: 28,000+ Cells

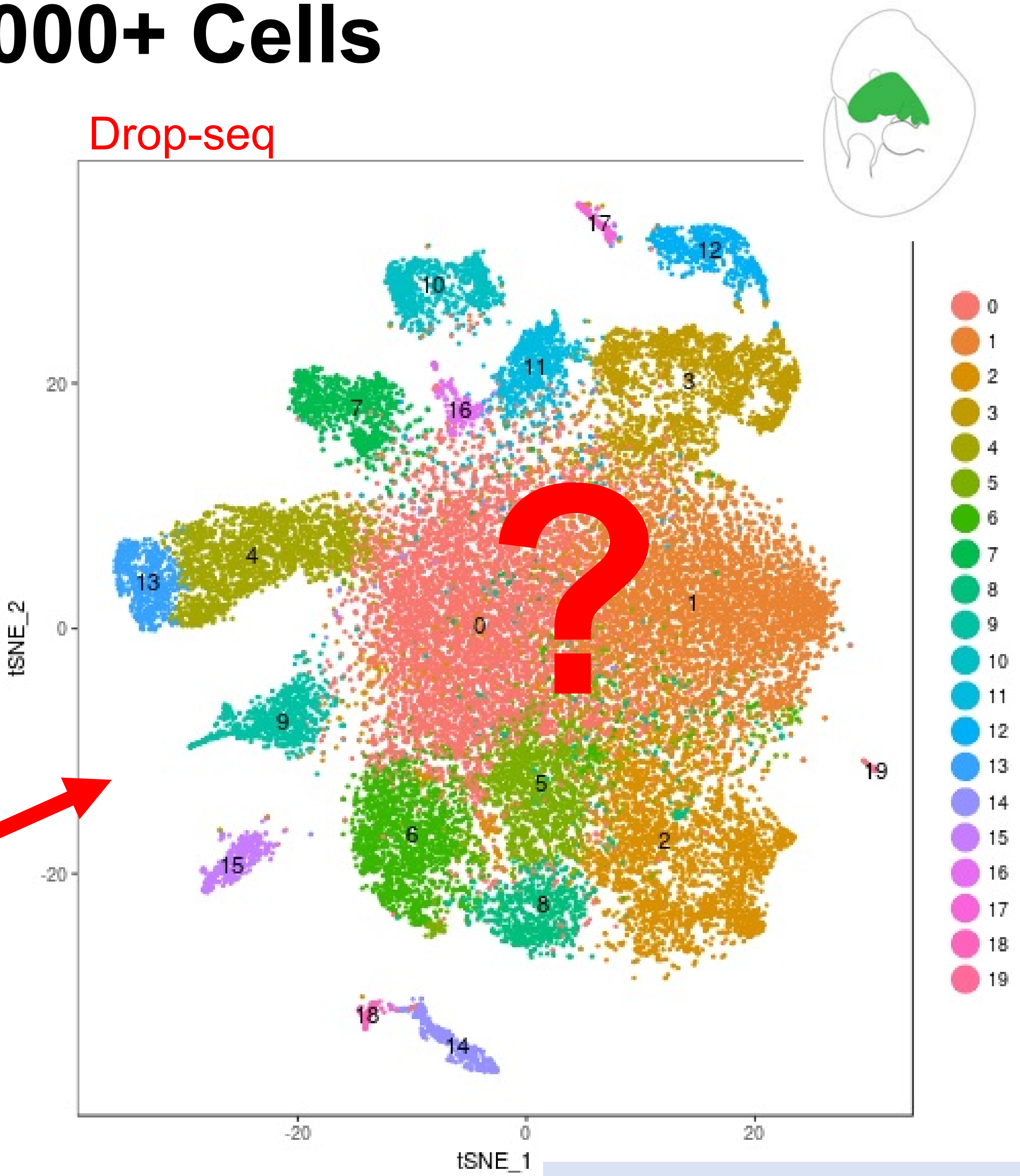
Pilot Study: Six Craniofacial Enhancers



Example: mm901



Drop-seq



Visel Lab, unpublished

FaceBase Standardized Analysis Pipelines for ChIP-seq and RNA-seq data

FaceBase Standardized Analysis

Challenge:

- Several FaceBase spokes produce ChIP-seq and/or RNA-seq data
- Each lab has been using their favorite analysis pipeline (in most cases in-house developed/tweaked)
- Limits comparison across data from different spokes and hurdle to integrative analysis

Goal:

Need to analyze data in a uniform and reproducible way

Solution:

Analyze data using ENCODE pipelines on DNAnexus

Using ENCODE Pipelines at DNAnexus

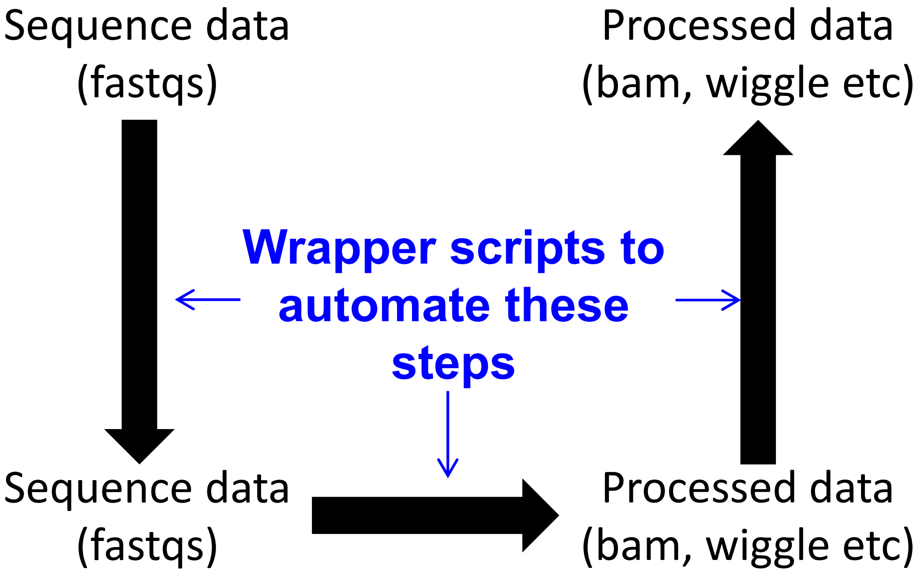
- Platform for genomics data analysis
- Built on Amazon cloud
- Implements analysis tools and workflows including ENCODE pipelines
- Command line interface for automation

DNAnexus

https://www.dnanexus.com

General FaceBase / DNAnexus analysis workflow

FaceBase



Sequence data
(fastqs)

Processed data
(bam, wiggle etc)

Wrapper scripts to automate these steps

Sequence data
(fastqs)

Processed data
(bam, wiggle etc)

DNAnexus

BERKELEY LAB
ERNEST ORLANDO LAWRENCE
BERKELEY NATIONAL LABORATORY

Status of Pipeline Development

Complete:

- Metadata requirements established and implemented
- Data transfer protocols established
- (Most) wrapper scripts established
- Test data sets successfully processed

In progress:

- Additional validation and comparison with in-house analyses and replicated data sets
- Streamlining of data transfer (currently temporarily stored at LBNL)
- Ensure human data is properly controlled
- Review display of data in FaceBase

Project Team

Lawrence Berkeley National Lab

Experimental postdocs: Sudha Rajderkar, Cailyn Spurrell, Marco Osterwalder, Evgeny Kvon,
Computational postdocs: Valentina Snetkova, Guy Kelman, 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, Stella Tran

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

FaceBase