GENETIC TOOLS AND RESOURCES FOR OROFACIAL CLEFTING RESEARCH

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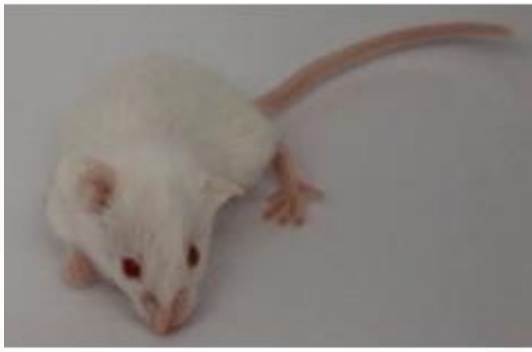
The Jackson Laboratory

FaceBase Annual Meeting
Iowa City, IA
May 1, 2013



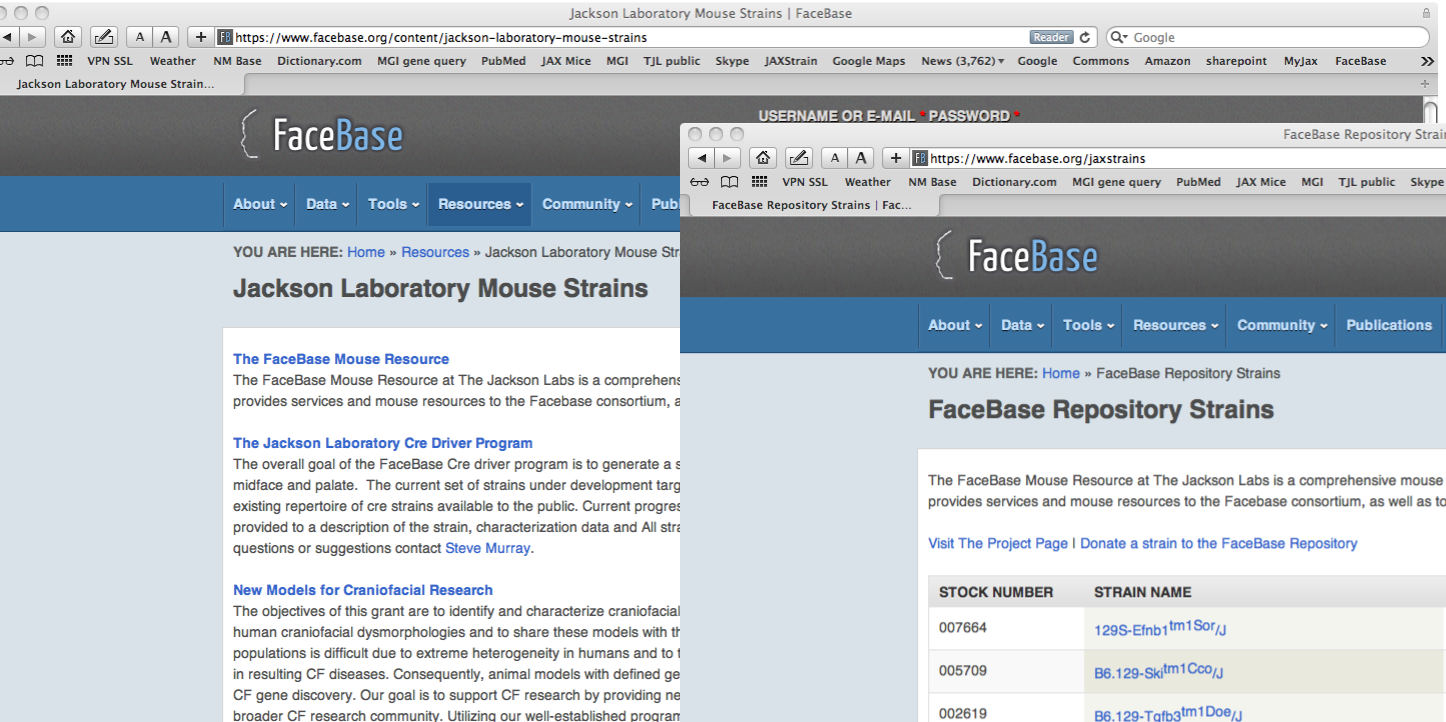
Aims

- Aim 1: Generate inducible and constitutive Cre recombinase driver strains as genetic tools for orofacial clefting research.
- Aim 2: Provide a repository for importation, cryopreservation, genetic quality control, and distribution of new and existing mouse models and tool strains important for orofacial clefting research.

- Supplement Aims:
  - 1) Discover new craniofacial mouse mutants leveraging our spontaneous mutant program, ongoing ENU mutagenesis screens and the KOMP2 program at JAX.
  - 2) Map and identify the causative gene for spontaneous and ENU mutants.
  - 3) Perform broad phenotypic analysis of these mutants to maximize value to the scientific community.

FaceBase Repository

Reporting time	# strains held	# mice shipped	# strains	# investigators	# institutions
Year 2	54	1572	27	383	185
Year 3	65	1240	35	378	182
Year 4	71	1158	42	343	155

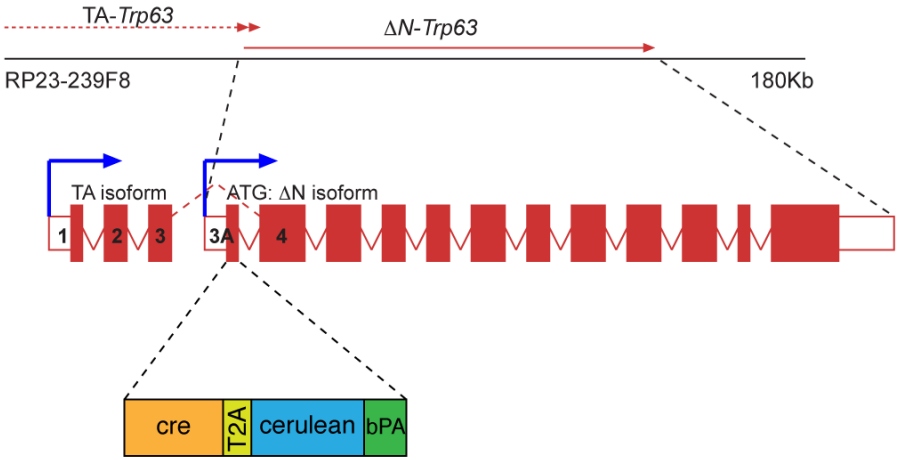


Cre project

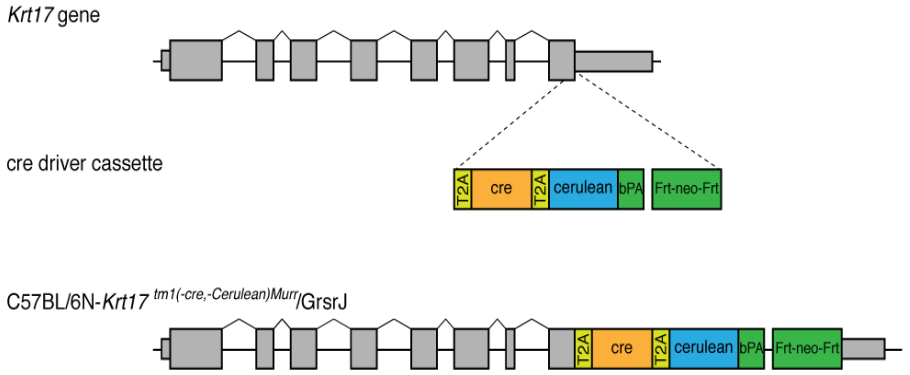
- Produced 41 transmitting lines representing 11 individual drivers/targets.
- Initiated production of 16 lines (microinjections of completed constructs or targeted clones)
- Completed characterization of 6 lines
- 5 lines public or in process
- ALL lines (multiple founders) with clear, specific activity will be made public
- Continued charaterization uncovering “better” lines, i.e. those with more robust activity in the intended target tissue.
- We will continue to inject this spring/summer with a target of 15 total additional injections by August

Cre project: basic strategies

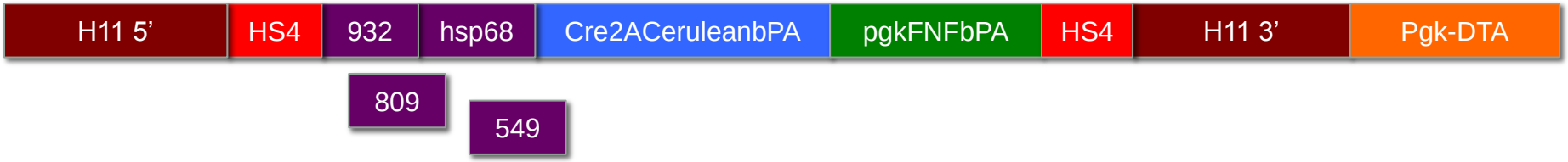
BAC transgenic:



Knockin allele:



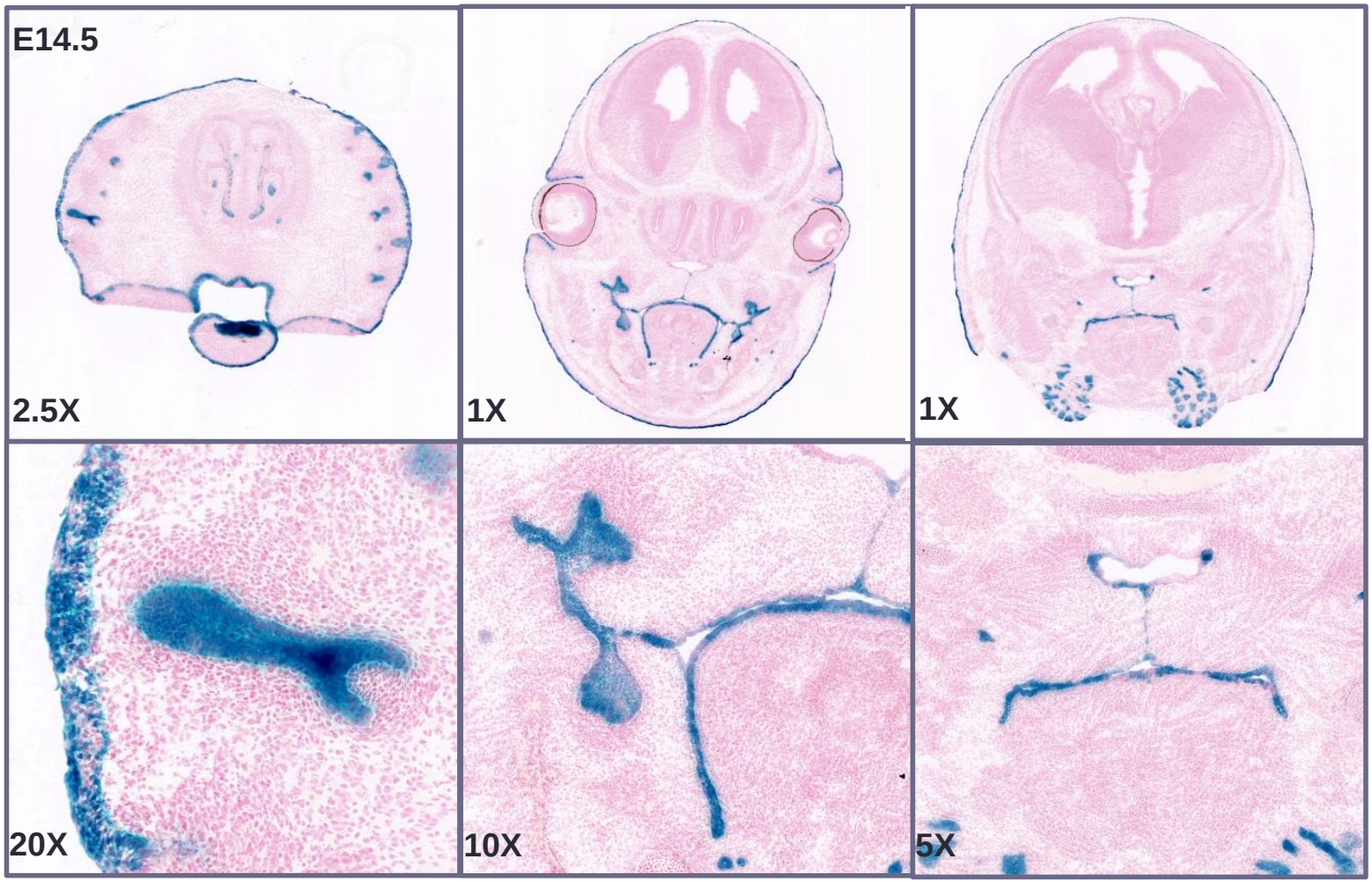
Highly-conserved enhancer element knockin:



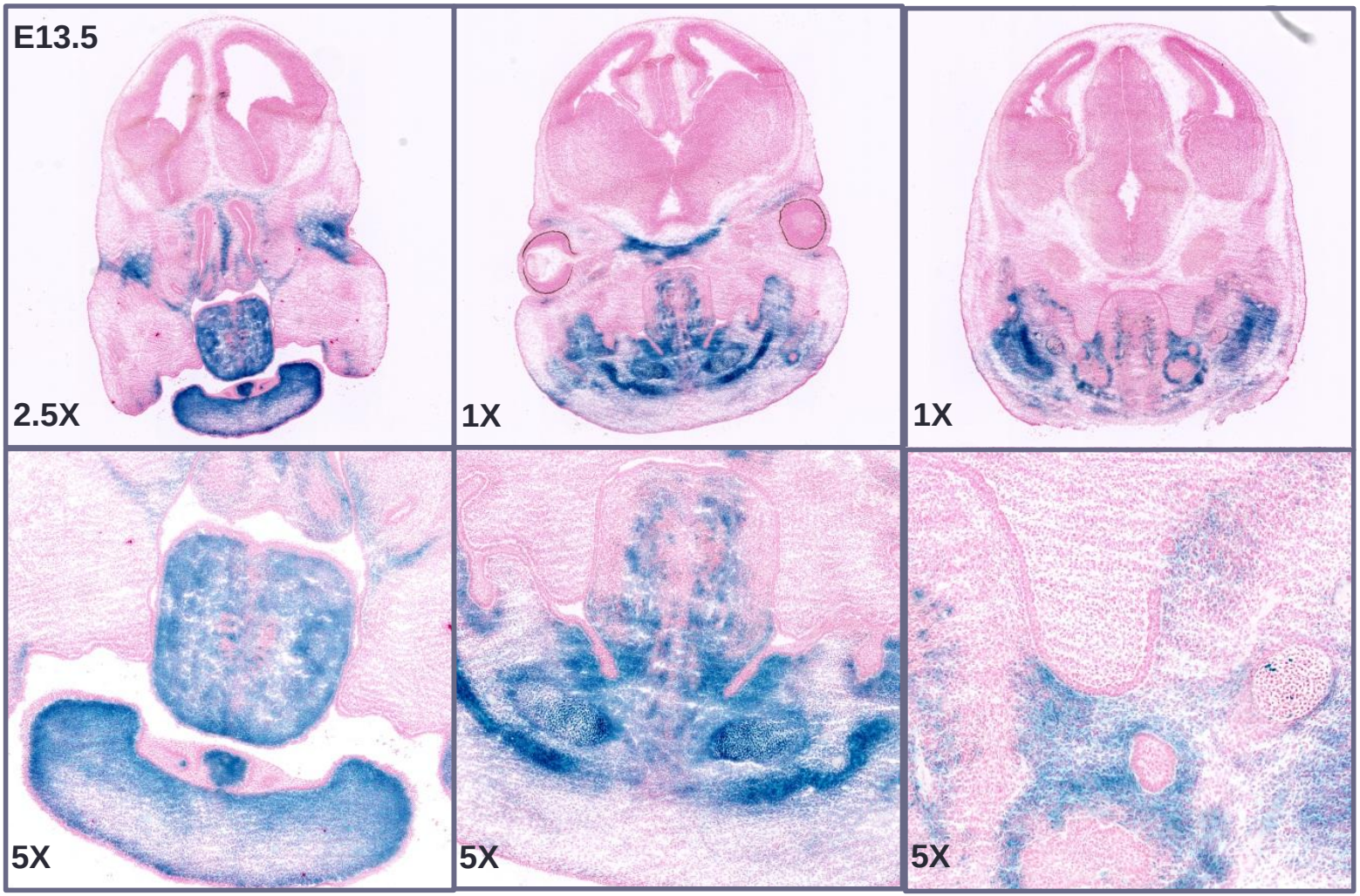
BAC transgenic Cre progress

Strain	Cloning	Injection	Founders	Characterization	Available?
Tbx22-Cre	Complete	Complete	Yes: 5 transmitting lines	Complete (3 lines)/ Underway (2 lines)	**Yes**
Krt6A-Cre	Complete	Complete	Yes: 4 transmitting lines	Complete (4 lines)	**In Process**
dNp63-CreERT2	Complete	Complete	Yes: 9 transmitting lines	**Comp**lete (4 lines)/ Underway (5 lines)	Pending
dNp63-Cre	Complete	Complete	Yes: 8 transmitting lines	Complete (4 lines)/Underway (4 lines)	**Yes** (2 lines)
Lhx8-Cre	Complete	Complete	**Pups born**		
Tbx22-CreER	Complete	**Complete**			
Fgf17-Cre	Complete	**Complete**			
Shox2-Cre	Complete	**Complete**			
Shox2-CreERT2	Complete	Scheduled			
Dlx2-Cre	Complete	Complete	**Pups born**		
Six6-Cre	Complete	**Complete**			
Dlx1-Cre	Complete	Scheduled			
Satb2-Cre	Complete	Scheduled			
Fgf15-Cre	Underway	Planned			
Pax7-Cre	Underway	Planned			
Dlx5-Cre	Underway	Planned			

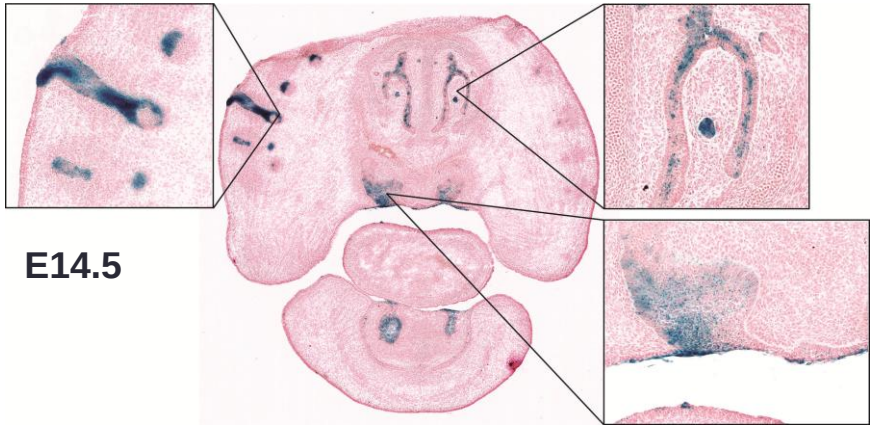
C57BL/6J-Tg(Trp63,-cre,-Cerulean)4Grsr/GrsrJ

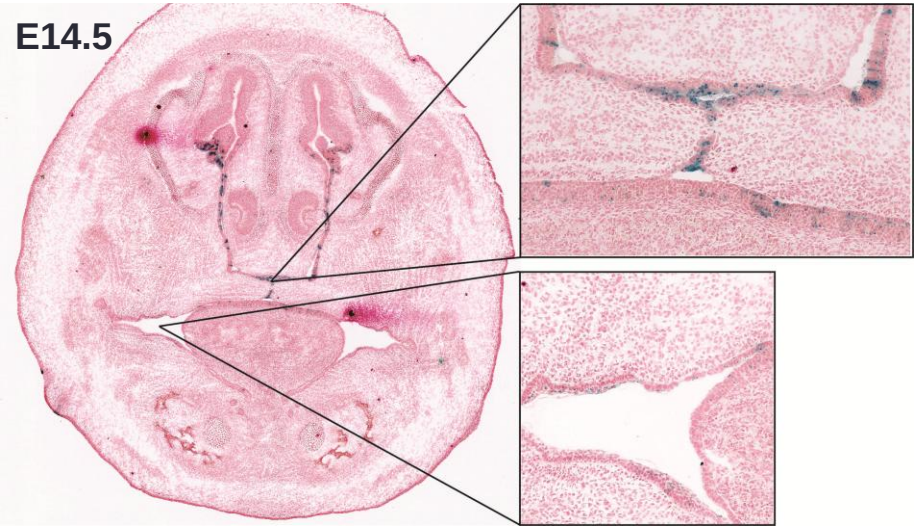


C57BL/6J-Tg(Tbx22,-cre,-mCherry)1Grsr/GrsrJ

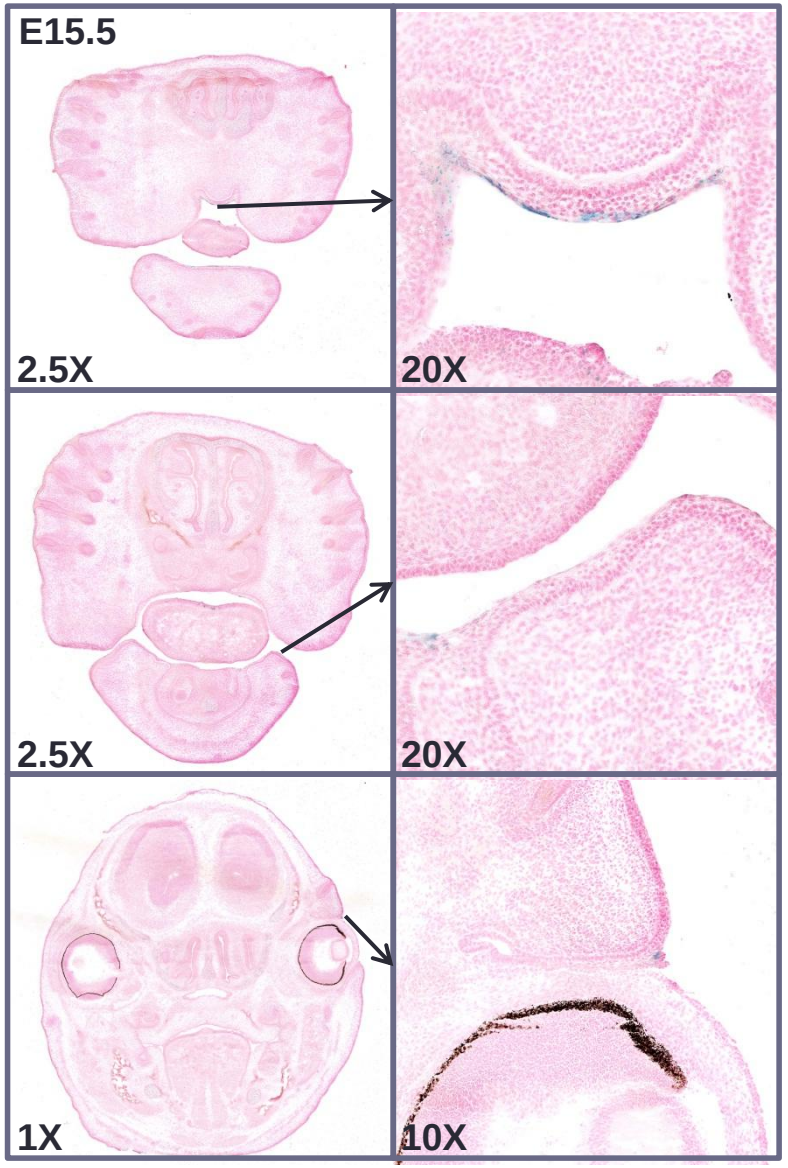


Krt17-cre knockin:





Krt6-cre BAC Tg, Founder #3841



Transgenic and knock-in Cre progress

Strain	Cloning	Injection	Founders	Characterization	Available?
Dlx2-CreIRESmCherry	Complete	Complete	**Yes: 7 transmitting lines**	Complete (1 line)/ Underway (6 lines)	**Pending**
Tg(HS4-HCES932-Cre2ACerulean)	Complete	Complete*	**Yes: 1 transmitting line**	Underway (1 line)	

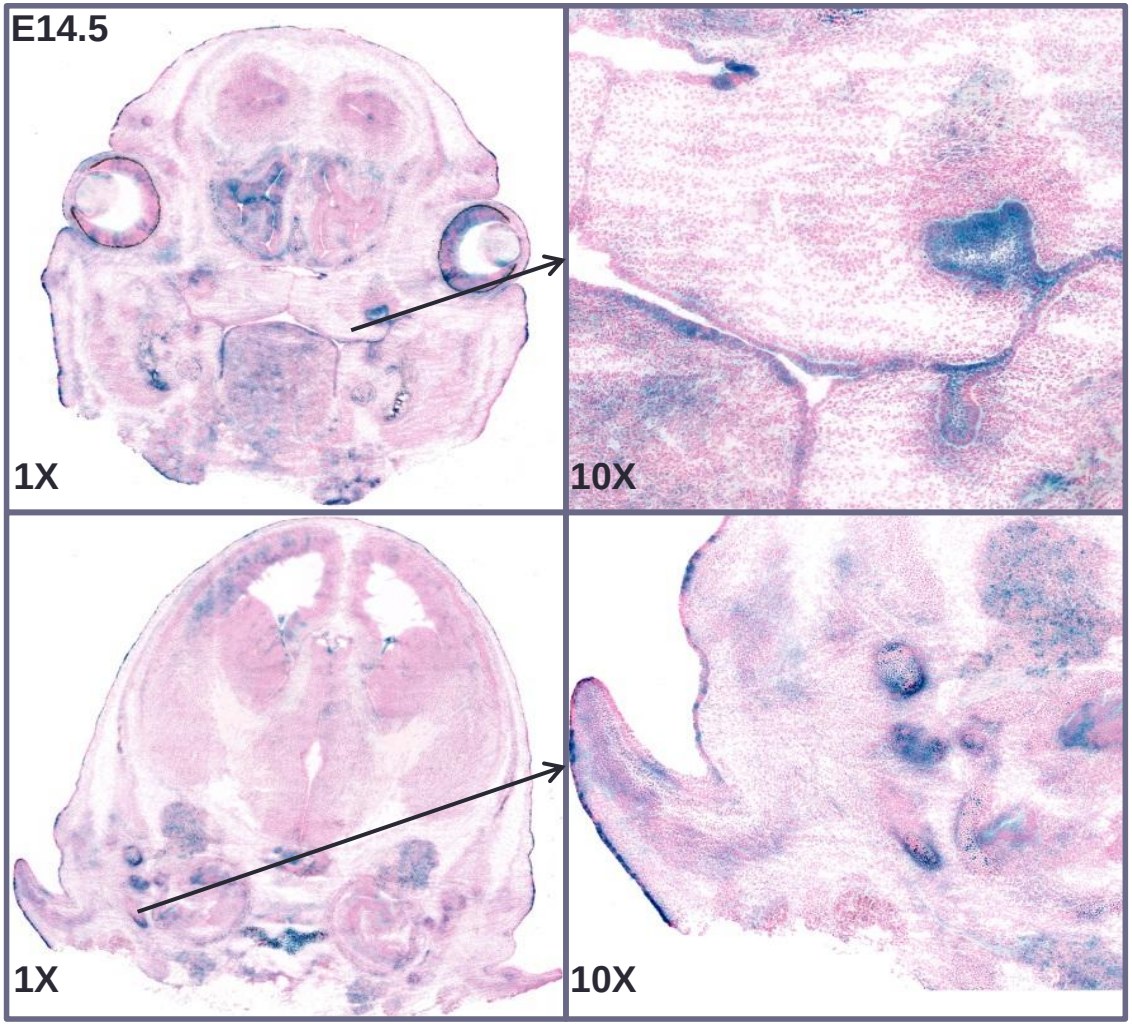
*Repeat injection complete

Strain	Cloning	Injection	Chimeras	GLT	Characterization	Available?
Krt17-2ACre2ACerulean	Complete	Complete	Yes	**Yes**	**Complete**	**Yes**
Krt6a-2ACre2ACerulean	Complete	Complete (3)*	**Yes (Perfect Host)**	**Pending****		
Lhx8-2ACre2ACerulean	Complete	Complete (3)*	**Yes (Perfect Host)**			
H11-HCES932-Cre2ACerulean	Complete	Complete (2)*	**Yes**	**Yes**	Underway	
H11-HCES809-Cre2ACerulean	Complete	On hold				
H11-HCES644-Cre2ACerulean	Complete	On hold				

*Multiple injections performed, second pending

**ES-derived pups born: genotype pending

Dlx2-cre Tg, Founder #904



JAX Cre Driver Project

The overall goal of the FaceBase Cre driver program is to generate a set of novel mouse tool strains to facilitate genetic analysis of the developing midface and palate. The current set of strains under development target structures and cell populations that are not effectively covered by the existing repertoire of cre strains available to the public. Current progress towards the release of these strains is indicated in the table, with links provided to a description of the strain, characterization data and All strains will be distributed by the JAX Repository. If you have any comments, questions or suggestions contact Steve Murray.

Additional JAX resources

- Other FaceBase tool strains at JAX
- All Cre strains at JAX
- JAX Cre strain characterization
- Other FaceBase Repository mouse models

BAC Transgenics

Project	Driver (gene/enhancer)	Allele Type	Construct Complete?	Injection?	Founders?	Characterization?	Available?
dNp63CreERT2_2ACerulean	Trp63	BAC Tg	Yes	Yes	Yes	In Progress	In Progress
dNp63Cre_2ACerulean	Trp63	BAC Tg	Yes	Yes	Yes	Yes	Yes
Founder 3428			Yes	Yes	Yes	Yes	Yes
Founder 3430			Yes	Yes	Yes	Yes	Yes
Tbx22CreIRESmCherry	Tbx22	BAC Tg	Yes	Yes	Yes	Yes	Yes
Founder 391			Yes	Yes	Yes	Yes	Yes
Krt6aCre_2ACerulean	Krt6a	BAC Tg	Yes	Yes	Yes	Yes	In Progress
Tbx22CreERT2_2ACerulean	Tbx22	BAC Tg	Yes	In Progress	No	No	No
Lhx8Cre_2ACerulean	Lhx8	BAC Tg	Yes	In Progress	No	No	No

C57BL/6J-Tg(Trp63,-cre,-Cerulean)4Grsr/GrsrJ

Stock Number: 018792

C57BL/6J-Tg(Trp63,-cre,-Cerulean)3Grsr/GrsrJ males were mated to female Rosa 26 reporters (stock no

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dNp63CreERT2_2ACerulean	Trp63	BAC Tg	Yes	Yes	Yes	In Progress	In Progress
dNp63Cre_2ACerulean	Trp63	BAC Tg	Yes	Yes	Yes	Yes	Yes
Founder 3428			Yes	Yes	Yes	Yes	Yes
Founder 3430			Yes	Yes	Yes	Yes	Yes
Tbx22CreIRESmCherry	Tbx22	BAC Tg	Yes	Yes	Yes	Yes	Yes
Founder 391			Yes	Yes	Yes	Yes	Yes
Krt6aCre_2ACerulean	Krt6a	BAC Tg	Yes	Yes	Yes	Yes	In Progress
Tbx22CreERT2_2ACerulean	Tbx22	BAC Tg	Yes	In Progress	No	No	No
Lhx8Cre_2ACerulean	Lhx8	BAC Tg	Yes	In Progress	No	No	No

C57BL/6J-Tg(Trp63,-cre,-Cerulean)4Grsr/GrsrJ

Strain Name: **C57BL/6J-Tg(Trp63,-cre,-Cerulean)4Grsr/GrsrJ**

Stock Number: **018792**

Availability: **In Progress**

Register Interest

These *Trp63-cre/cerulean* transgenic mice express cre recombinase and the cerulean variant of green fluorescent protein (GFP) driven by transformation related protein 63 (*Trp63*) promoter/enhancer elements. They may be useful for generating conditional mutations for studying craniofacial development.

Strain Information

Donating Investigator Steve Murray, The Jackson Laboratory

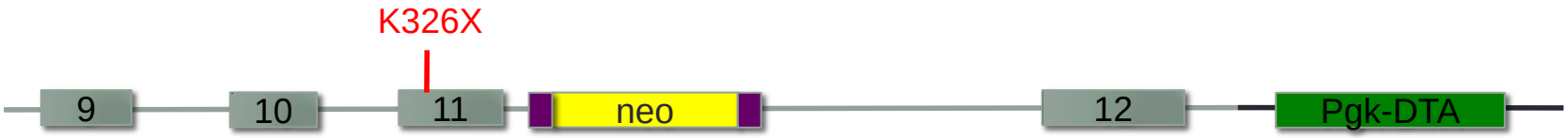
Description

Mice hemizygous for the *Trp63-cre/cerulean* transgene express cre recombinase and the cerulean variant of green fluorescent protein (GFP) under direction of transformation related protein 63 (*Trp63*) promoter/enhancer elements. Uniform *cre* activity has been detected at E14.5 throughout both oral and cranial epithelium. Oral epithelial expression includes anterior and posterior secondary palatal shelf epithelium, oral and nasal epithelial triangles, medial edge epithelium, primary palate epithelium, and tooth buds. Patchy *cre* activity is observed in nasal epithelium. Uniform *cre* activity is also seen in submandibular gland epithelium. When these mice are bred with mice containing a *loxP*-flanked sequence, *cre*-mediated recombination will result in deletion of the floxed sequences in the *cre*-expressing cells of the offspring.

Mouse models of human clefting alleles

- ARHGAP29
  - Candidate gene for 1p22 CLP GWAS hit (alternative to ABCA4)
  - Engineered point mutation identical to nonsense mutation (K326X) found in human CLP case

Arhgap29 point mutation allele

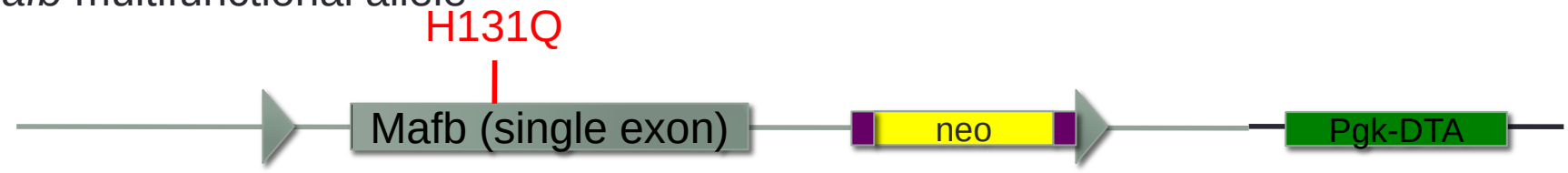


- Germline transmission successful, colony expanded.
- First surviving litter from intercross: only 6 pups, no homs
  - 8 units set up: however significant litter attrition.
  - Timed matings planned as more females are generated.
- FLP cross underway to generate neo-excised allele

Mouse models of human clefting alleles

- MAFB
  - Candidate gene for 20q12 CLP signal.
  - Generating bi-functional allele (H131Q variant and floxed)

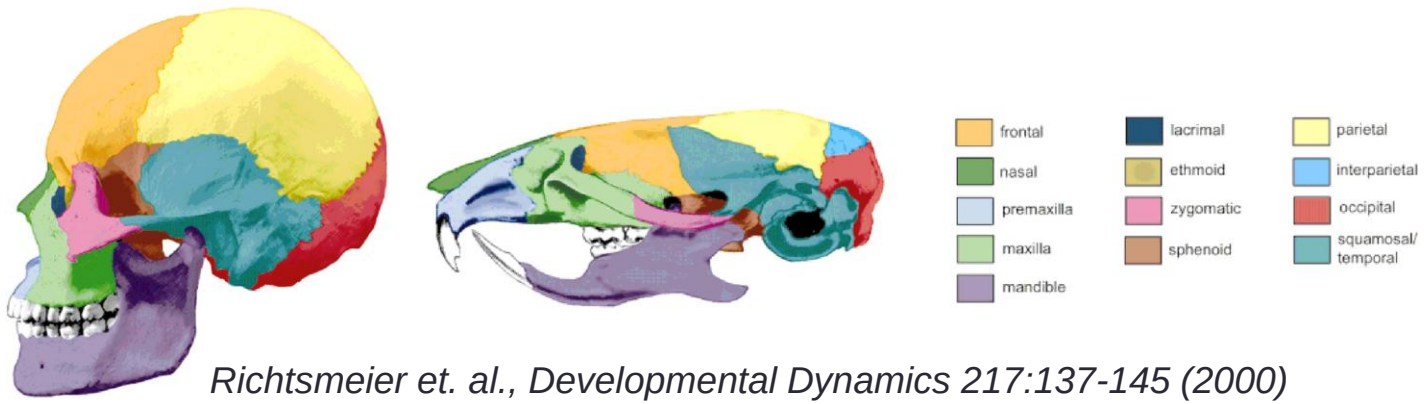
Mafb multifunctional allele



- Germline transmission successful, colony expanded
- Preliminary breeding results of $Mafb^{H131Q}$ intercross: 9/9/4 (no obvious phenotypes in homozygotes)
- FLP and Cre crosses underway to generate Mafbdel and neo-excised alleles
- Will generate, evaluate and compare phenotypes of $Mafb^{del/del}$, $Mafb^{del/H131Q}$ *and* $Mafb^{H131Q/131Q}$ allelic series

New Models for Craniofacial Research

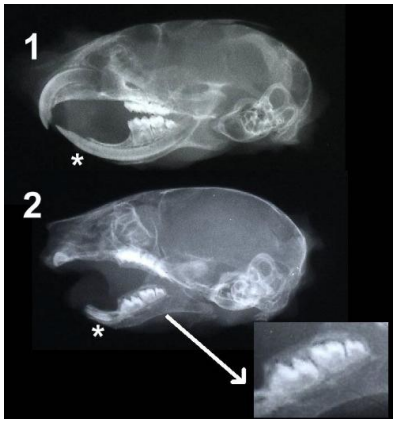
- Multiple sources
  - Spontaneous:
  - ENU induced: recessive cleft palate mutants from saturation ENU screen. 11 identified, 4 currently being mapped and sequenced
  - KOMP2
- Full range of craniofacial phenotypes
- Identify causative gene, characterize, distribute
- “Resources” on FaceBase site – existing models

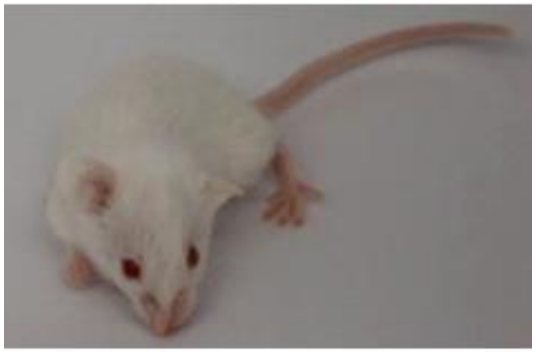


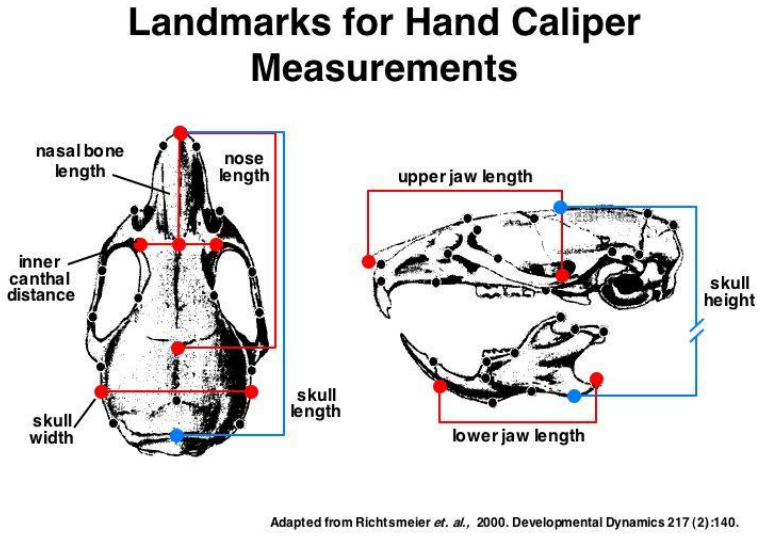
Richtsmeier et. al., Developmental Dynamics 217:137-145 (2000)

New spontaneous models in progress

- 46 currently
  - both dominant & recessive
  - 10 have known causative gene
  - Others: heritability testing, mapping, sequencing
  - Characterization pipeline
    - Skull & skeletal morphology
      - x-rays, microCT
    - Sight, hearing, dentition
    - Embryonic phenotyping







New Mutant Workflow: leveraging Mouse Mutant Resource infrastructure

New mutant arrives from production colony

Test for heritability, determine mode of inheritance

If not heritable, then discard

- Basic phenotyping
- Genetically map to a 30-60 cM chromosomal sub-region
- Cryopreserve sperm or embryos
- **Exome and/or whole genome sequencing

Publish to Web and / or primary literature, distribute information directly if collaborator exists

Strains sequenced to date	Mutations discovered	Datasets in progress
229*	50	69

**Newly introduced as of 2009

KOMP2 Embryonic Lethality Pipeline

GLT/tm1b allele

Het x het mating

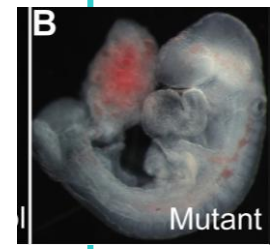
<50% expected homozygous mutants at genotype: "SUBVIABLE"

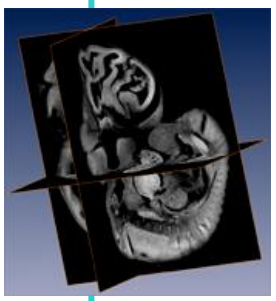
No homozygous mutants at genotype: "LETHAL"

>50% expected homozygous mutants at weaning: "VIABLE"

Homozygotes

Breed/ship cohorts





Dissection & genotyping

Gross morphology (embryo and placenta): selected imaging Minimum standard MP terms

Optical Projection Tomography (embryos & placenta)

E9.5

< 50% expected hom

Dissection & genotyping

Gross morphology (embryo and placenta): selected imaging Minimum standard MP terms

E12.5

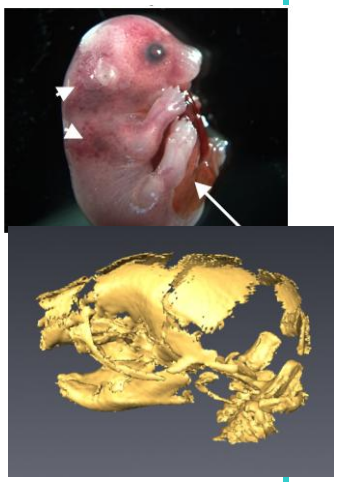
> 50% expected hom

Dissection & genotyping

Gross morphology (embryo and placenta) Minimum standard MP terms

High-resolution microCT (skull and other skeletal elements)

E18.5



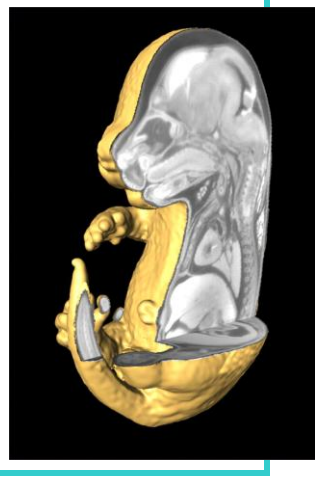
> 50% expected hom

Dissection & genotyping

Gross morphology (embryo and placenta): selected imaging Minimum standard MP terms

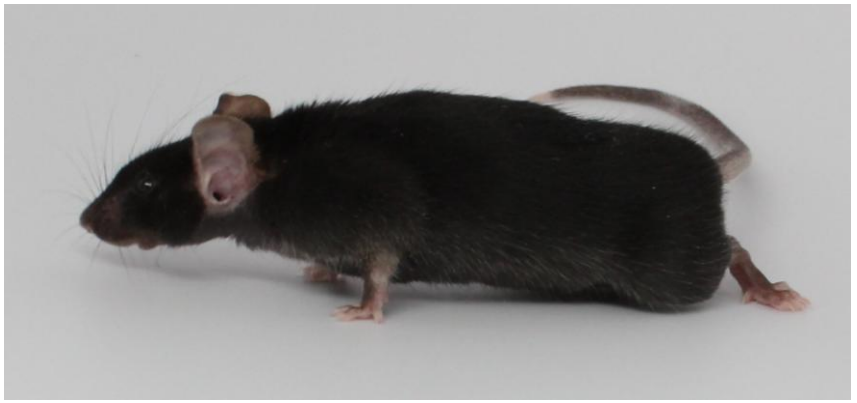
High-resolution microCT (iodine contrast)

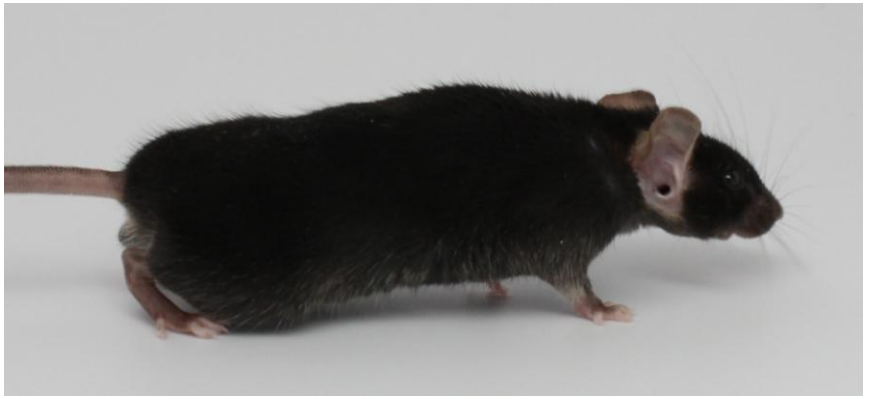
E15.5



Red outline indicates proposed analyses

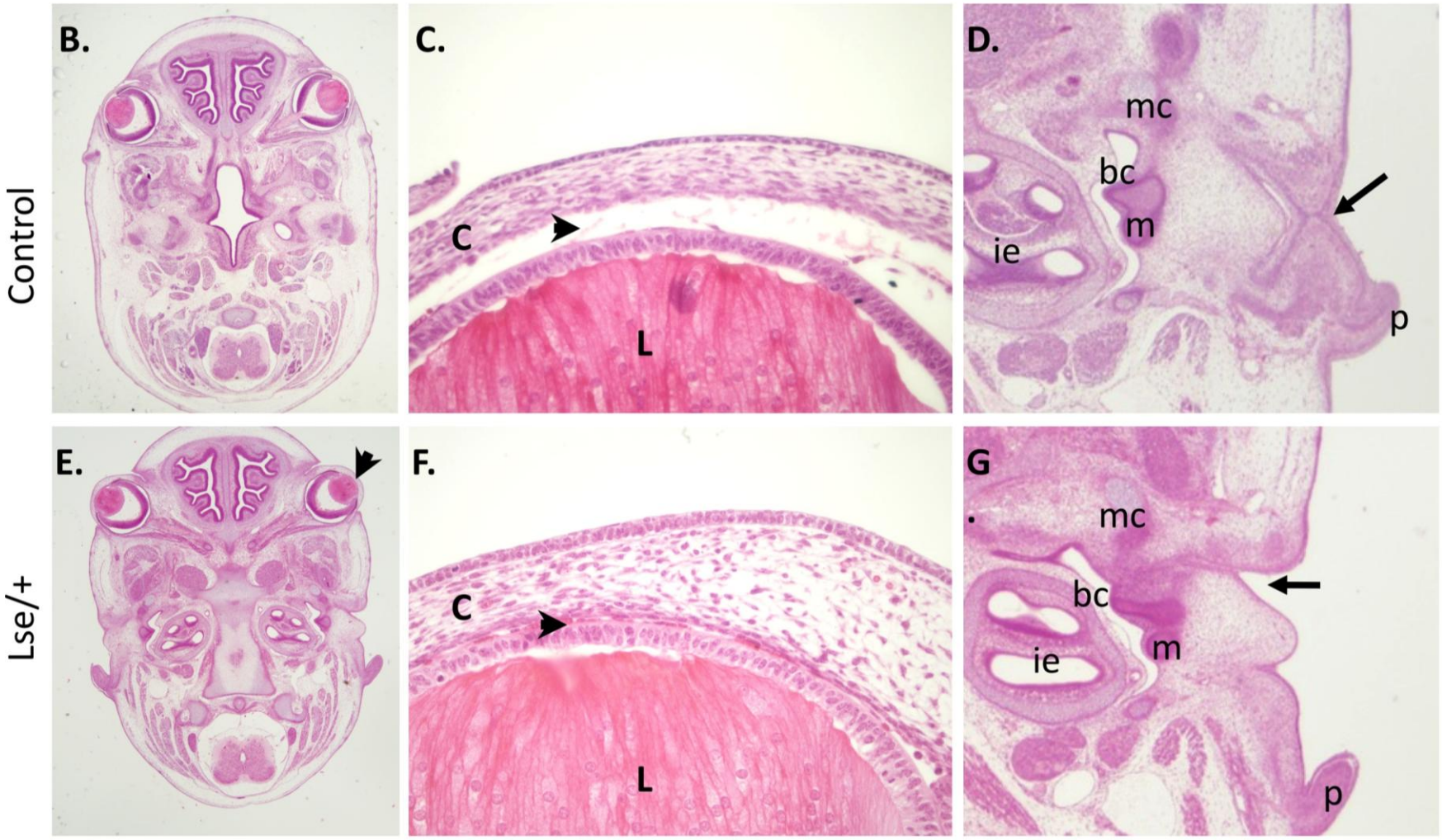
Low-set ears *(Lse)*





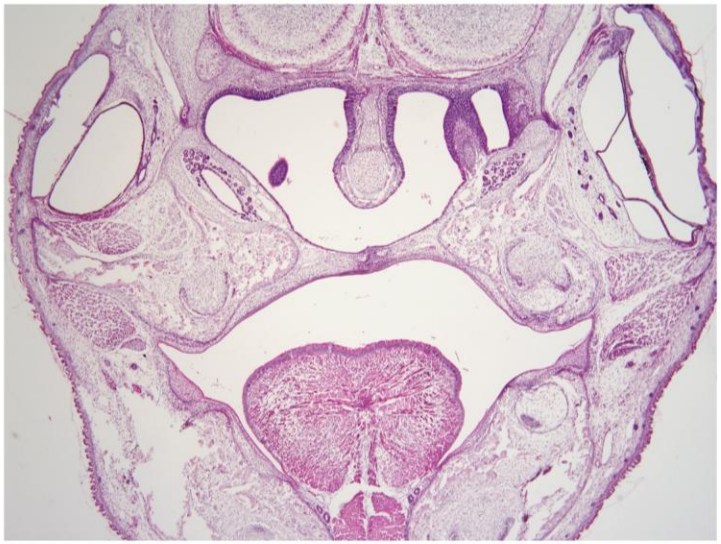
- First identified in 1983!
- Dominant mutation characterized by ventrally shifted external ear structures, bulging eyes, adult-onset corneal opacity and a shortened lifespan.
- Lse homozygotes are not viable: recently determined to be due to secondary cleft palate.
- Maps to proximal chromosome 7: FGF3, FGF4 and FGF15 in region, but no mutations in coding sequence.
- Regional array capture and sequencing of 5Mb of proximal chromosome 7: no non-synonymous coding SNPs

Lse: eye and ear defects



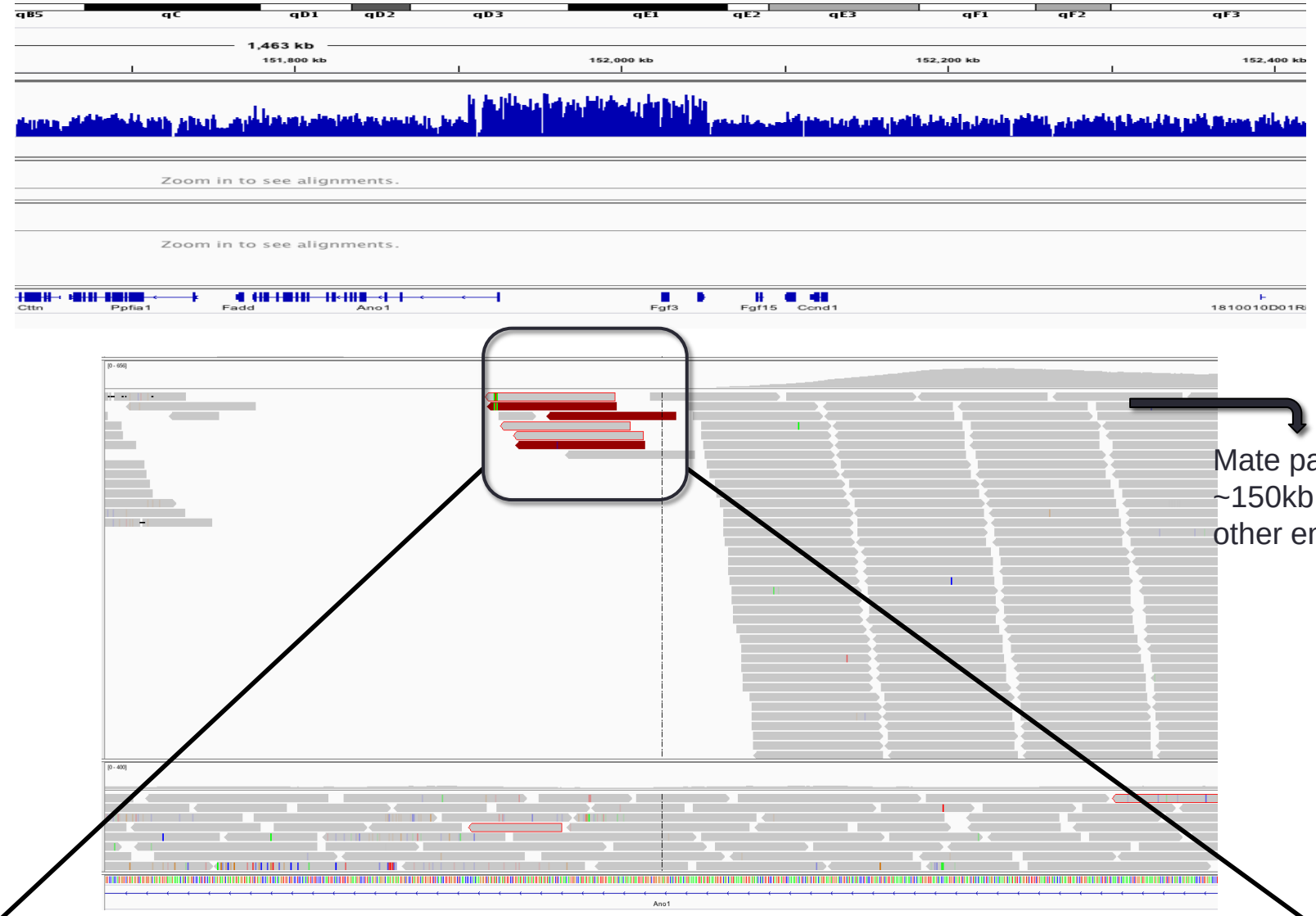
Lse: semidominant cleft palate

Control



Lse/+

Proximal end of Chromosome 7 CNV



Mate pairs map ~150kb distal: other end of CNV

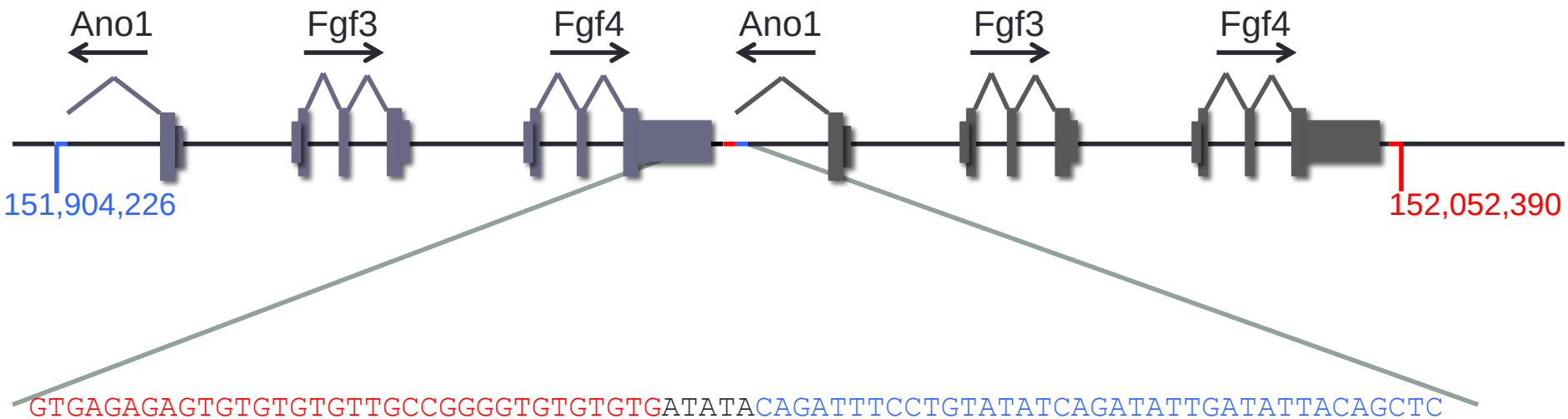
Unmapped mate pair 3' reads (- strand):

GAGCTGTAATATCAATATCTGATATACAGGAAATCTGTATATCACACACACCCCGGCAACACACACACTCTCTCAC

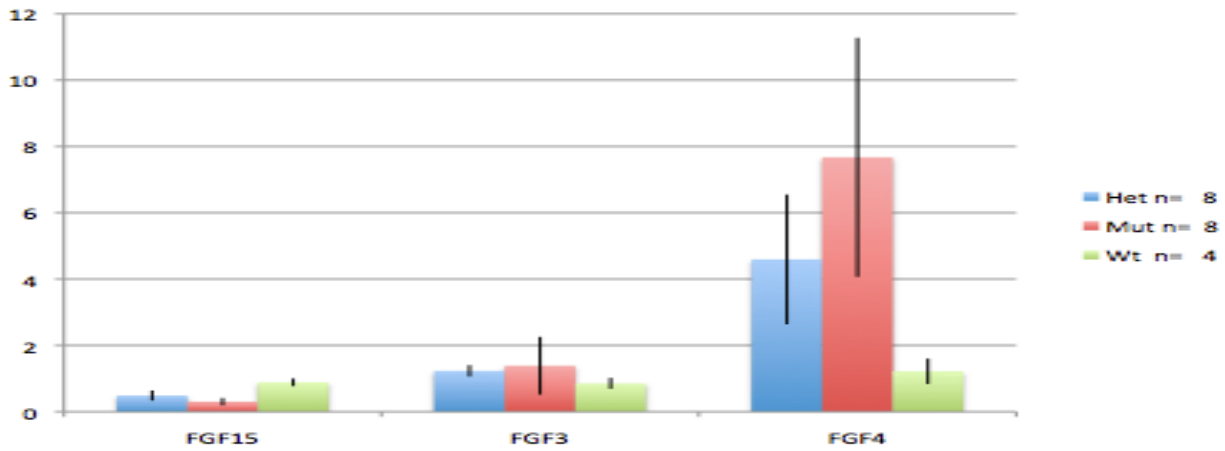
TGTATATCACACACACCCCGGCAACACACACACTCTCTCACAATCTTTCTGTGCTGGAGATGACCAATTCAAAAGC

CTGTAATATCAATATCTGATATACAGGAAATCTGTATATCACACACACCCCGGCAACACACACACTCTCTCACAAT

Lse: Duplication on distal Chromosome 7



148,164 bp duplication



Summary

- Repository continues to grow, distributing strains to over 300 investigators
  - Limited number of “high demand” strains
  - Growth and value relies on community participation
- Cre progress has ramped up and pipeline is flowing: strains will steadily move to public availability supported by FaceBase Repository
  - On track to overproduce
  - KOMP2 program has helped enhance pipeline throughput
- New model program well underway and producing new craniofacial mutant models for the community
  - Spontaneous program already identifying new mutants/genes
  - ENU yielding ~5 new CP mutants/quarter: mapping sequencing of first set underway
  - KOMP2 program identifying new lethal strains this spring/summer: phenotyping pipeline ready to take advantage

The team

- FaceBase Team
  - Jocelyn Sharp
  - Caleb Heffner; Chris Durkin, Polyxeni Gudis
  - Leslie Goodwin, Judy Morgan, Herb Pratt, Leslie Haynes, Harold Coombs
  - Cathy Lutz and the JAX Repository team

FaceBase
A Resource For Craniofacial Researchers

- JAX Scientific Services
- Funding: DE020052, OD011185, RR026117