Transcriptome Atlases of the Craniofacial Sutures

1U01DE024448-01, Spoke Project

RFA-DE-14-004, FaceBase 2: Craniofacial Development and Dysmorphology – Dataset, Tool, and Resource Development

Key Personnel

Multiple Principal Investigators

Greg Holmes, Ph.D.
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Harm van Bakel, Ph.D.
(Icahn School of Medicine at Mount Sinai, New York)

Co-Investigator

Michael Donovan, Ph.D., M.D.
(Icahn School of Medicine at Mount Sinai)

Subcontract-Principal Investigator

Steven Potter, Ph.D.
(Cincinnati Children's Hospital Medical Center)

Overall Goal

To generate comprehensive gene expression atlases of 11 major craniofacial sutures at embryonic stages of the wild-type mouse and craniosynostosis mouse models.

Significance

Suture function is critical for normal craniofacial development

Defective/pathologic activity of suture genes can cause craniosynostosis and midface hypoplasia, but the genetic cause in most cases of these conditions is unknown

A complete knowledge of suture gene expression would further our understanding of suture function and craniofacial development and aid in predicting genes involved in craniosynostosis and other craniofacial dysplasias

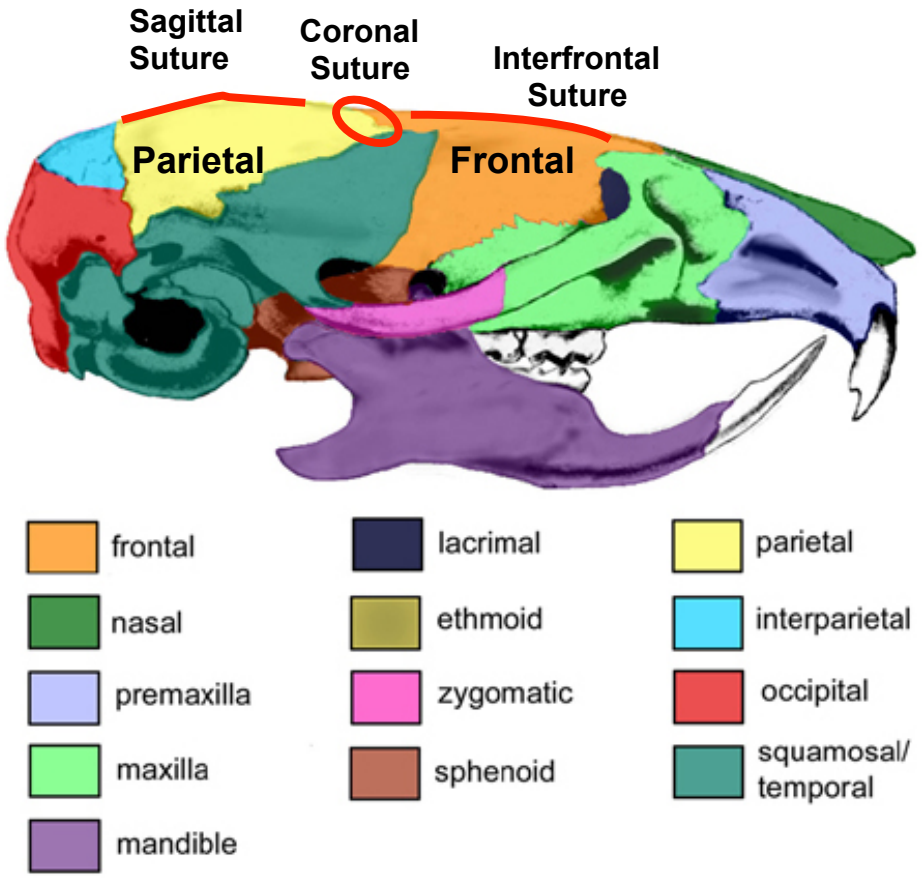
Specific Aims

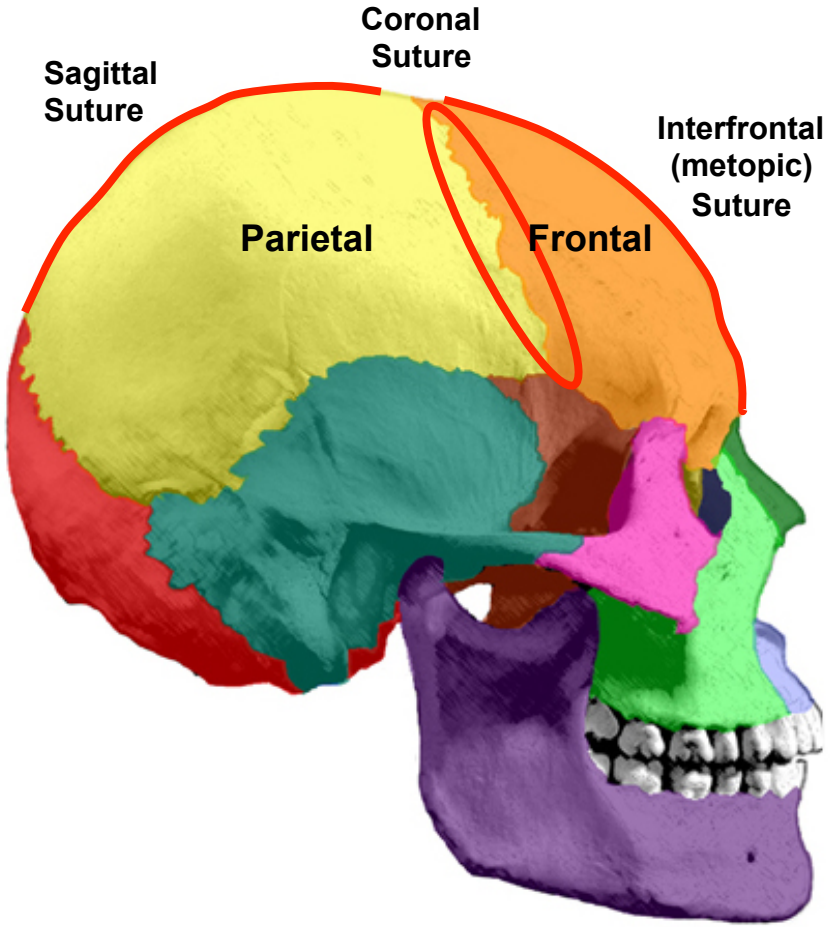
Aim 1: To isolate the subregions of eleven murine craniofacial sutures using laser capture microdissection (LCM) to create a gene expression atlases at two (or three) embryonic stages.

Aim 2: To use next-generation sequencing (NGS) to create gene expression atlases for murine WT (C57BL/6J) craniofacial suture subregions.

Aim 3: To use NGS to create gene expression atlases for craniofacial suture subregions in Apert and Saethre-Chotzen mouse models.

Homology of murine and human craniofacial bones and sutures

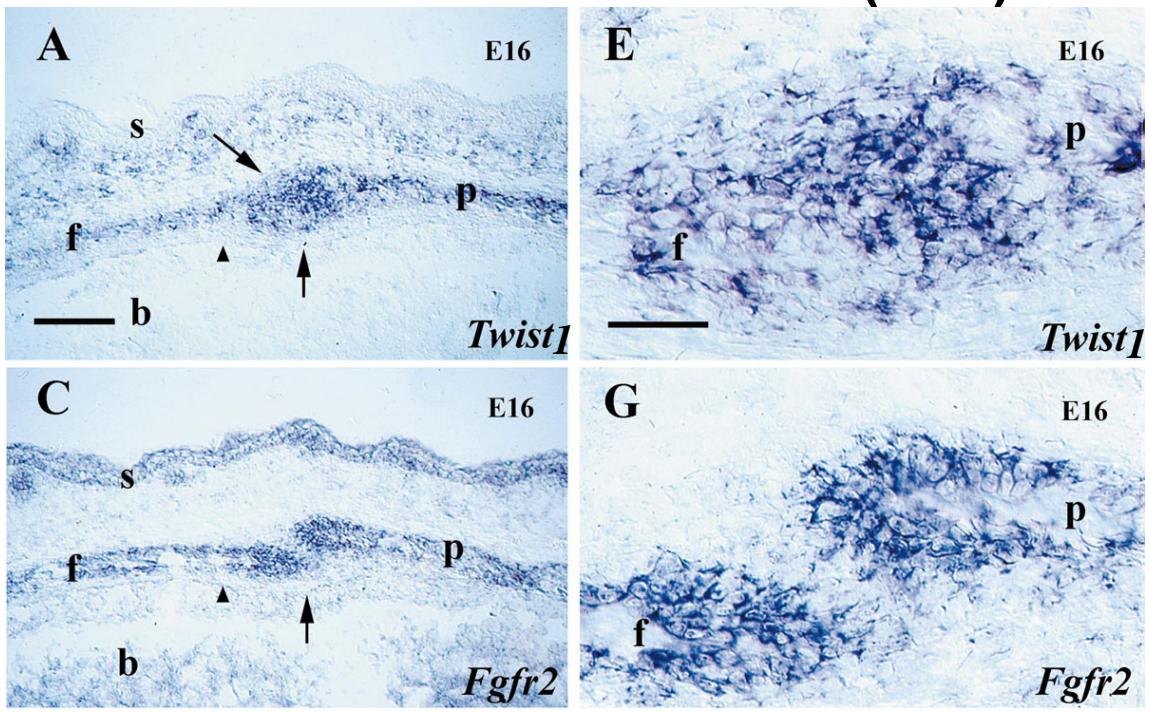




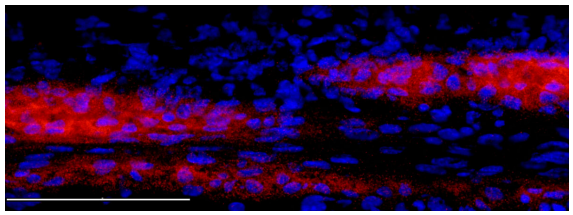
Richtsmeier *et al*, 2000

Gene expression distinguishes suture subregions: mesenchyme & osteogenic fronts

Murine Coronal Suture (E16)



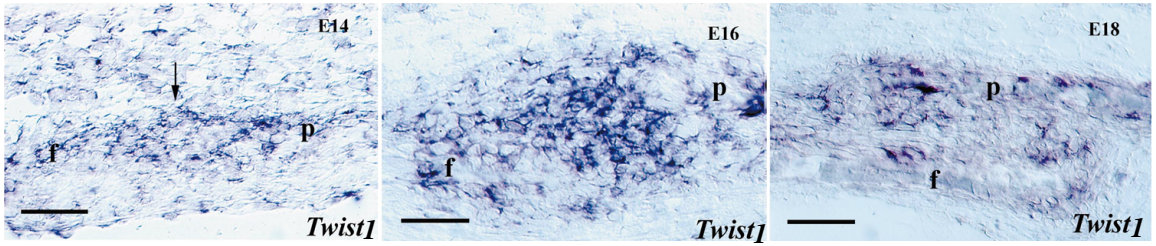
Johnson *et al*, 2000



Alkaline phosphatase (osteogenic fronts, osteoblasts)

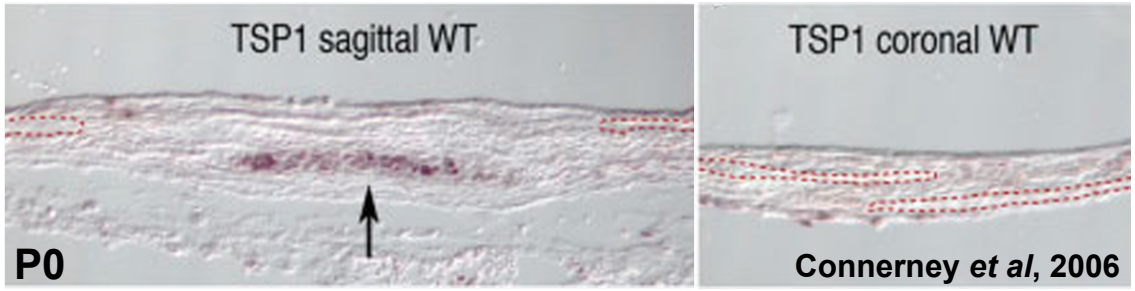
Expression varies temporally and spatially across sutures

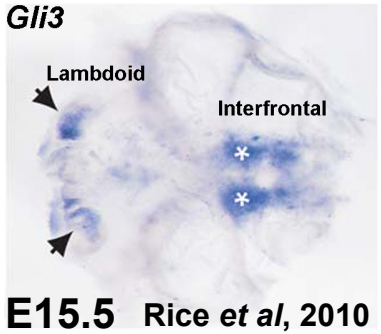
***Twist1* expression varies over time in coronal suture mesenchyme:**



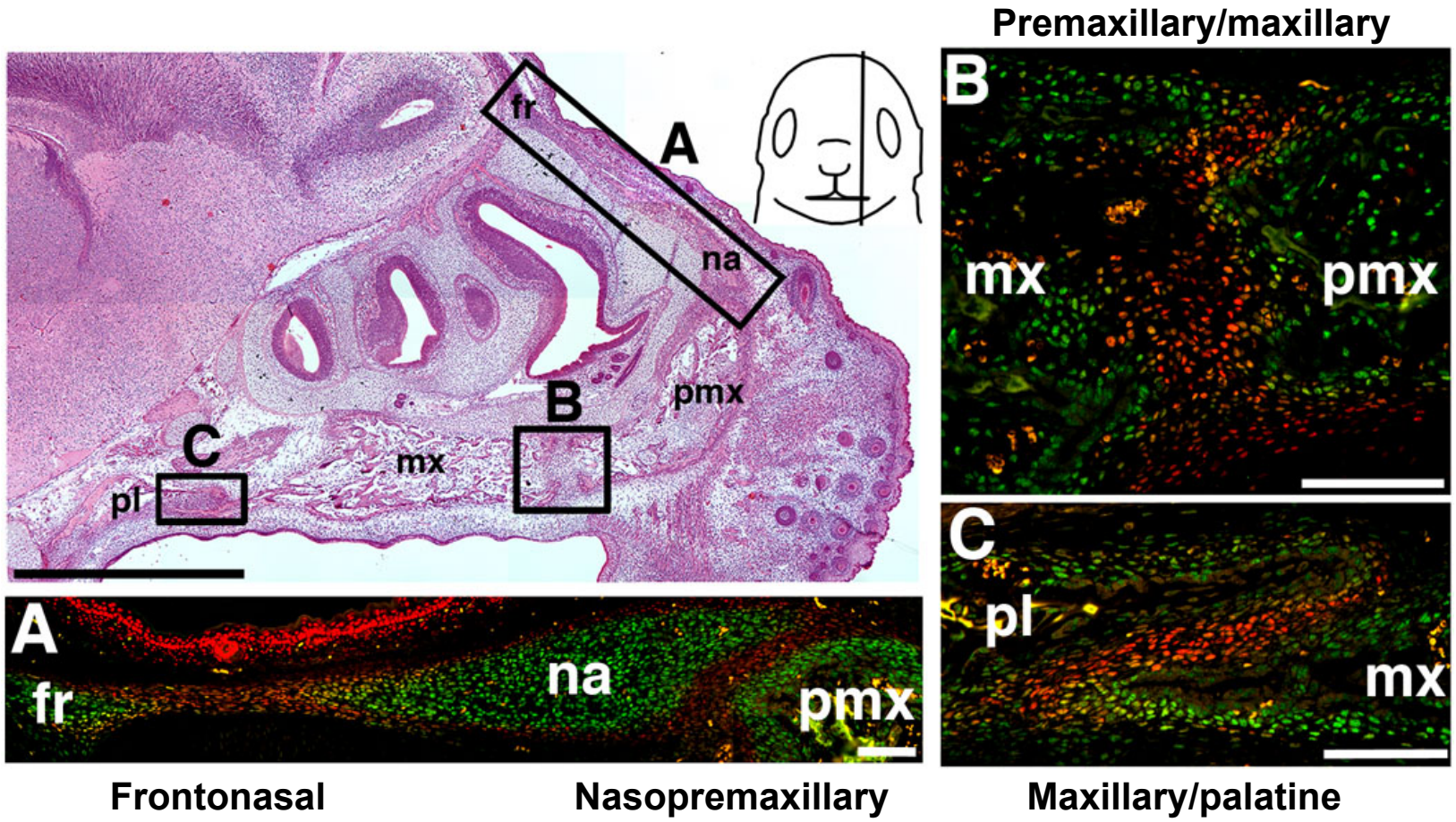
Johnson *et al*, 2000

***Thrombospondin1* and *Gli3* expression is suture specific:**





New sutural genes: *Bcl11b*

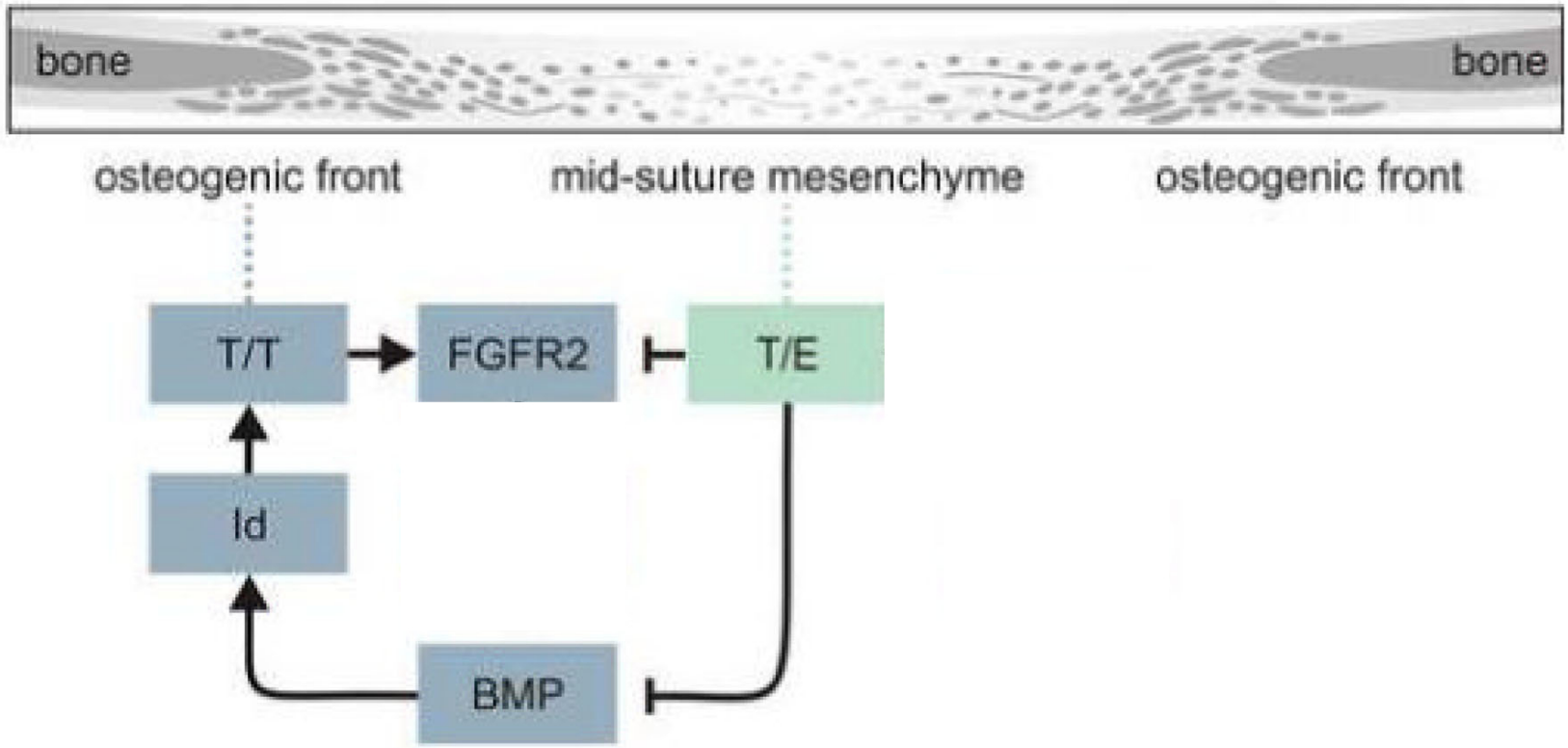


E16.5 Mouse

IHC: BCL11B, RUNX2

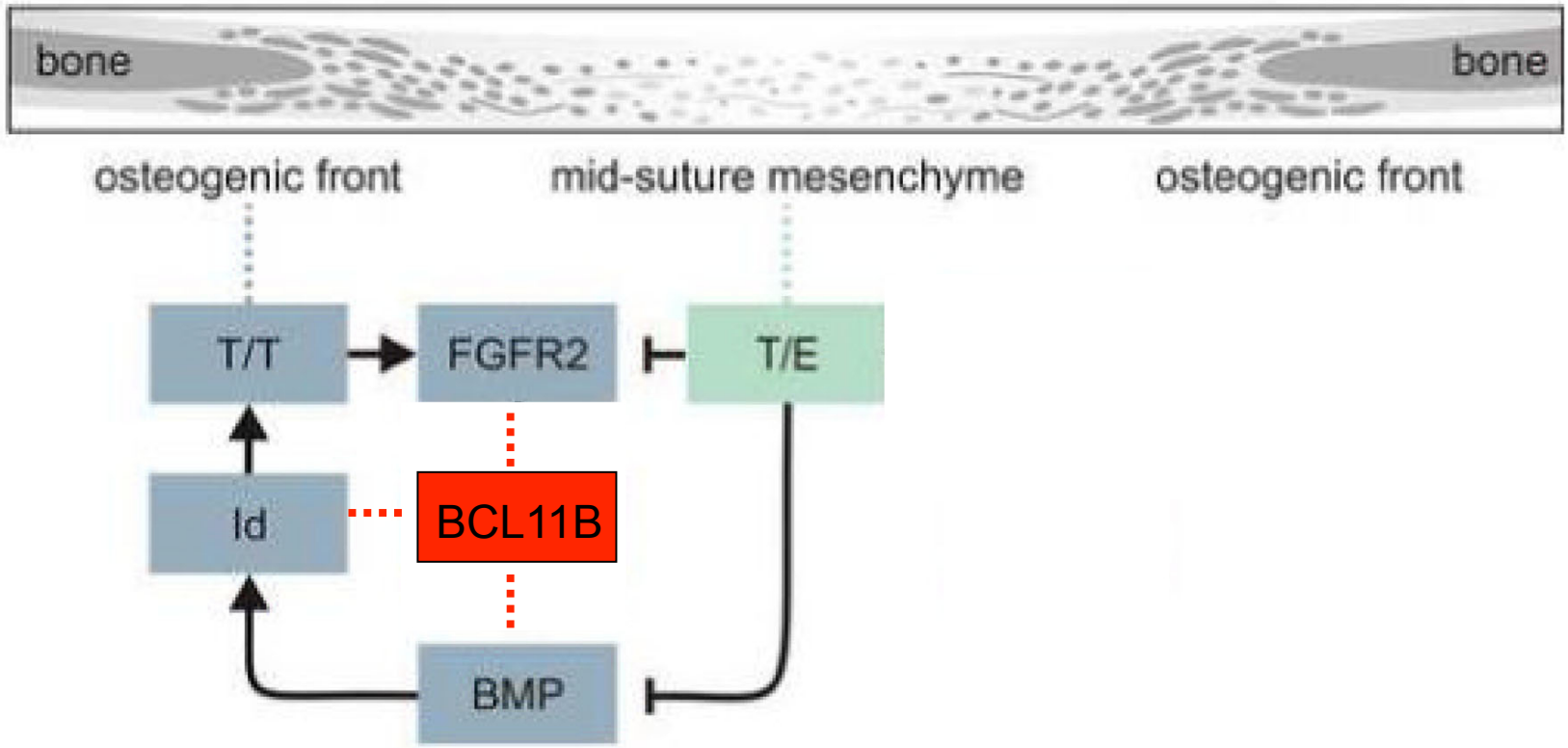
Holmes *et al*, 2015

TWIST1 regulation of *Fgfr2* and suture formation



Connerney *et al*, 2008

TWIST1 regulation of *Fgfr2* and suture formation



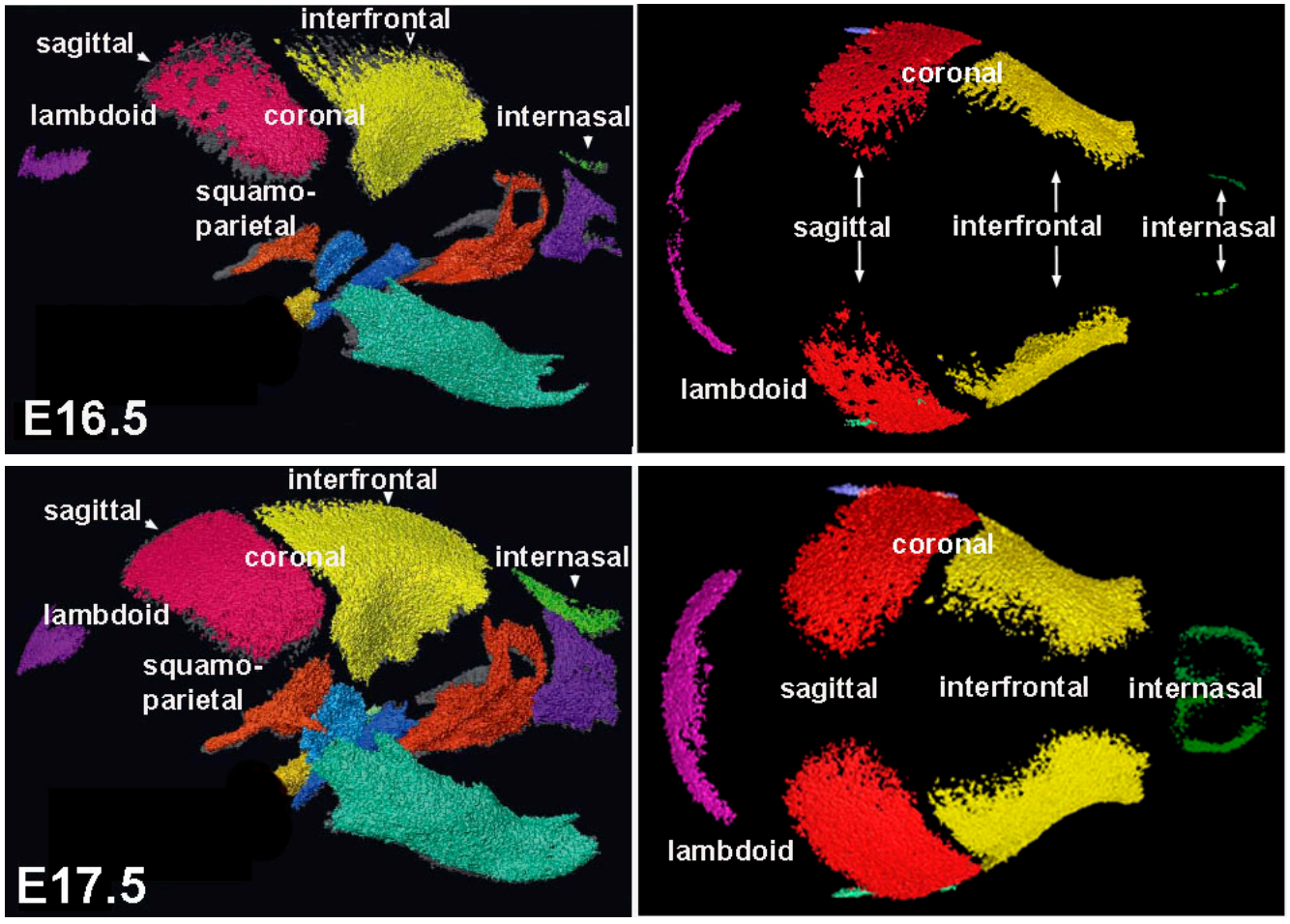
Connerney *et al*, 2008

Specific Aims

Aim 1: To isolate the subregions of eleven murine craniofacial sutures using laser capture microdissection (LCM) to create a gene expression atlases at two (or three) embryonic stages.

Cranial Sutures for Atlases at E16.5 and E18.5

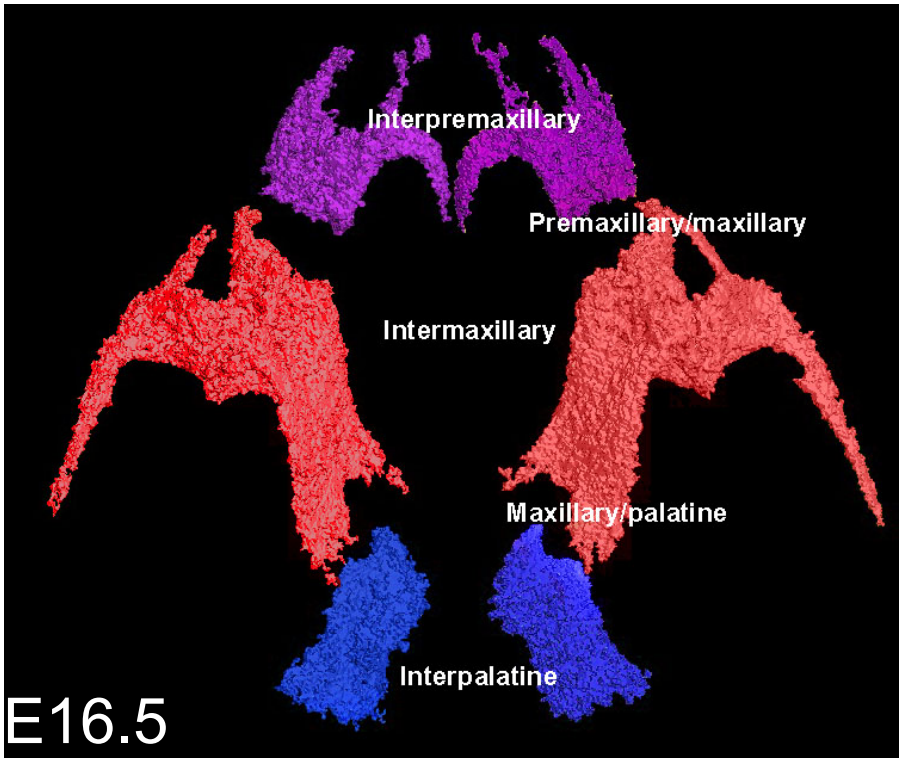
Suture
Interfrontal (IF)
Sagittal (S)
Coronal (C)
Lambdoid (L)
Squamoparietal (SQ)

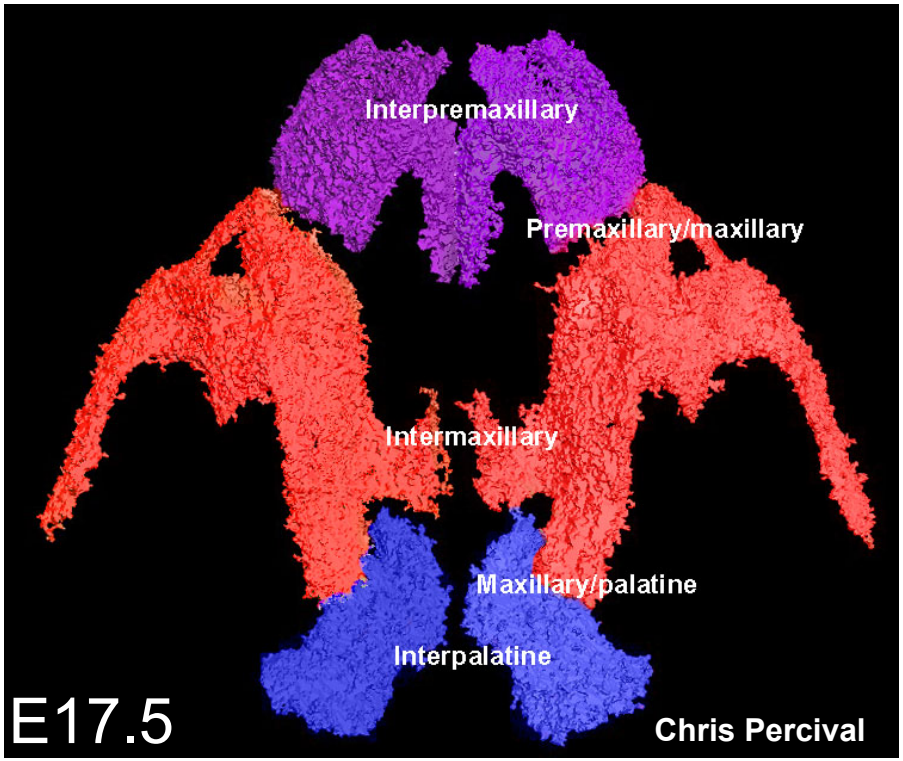


Chris Percival

Facial Sutures for Atlases at E16.5 and E18.5

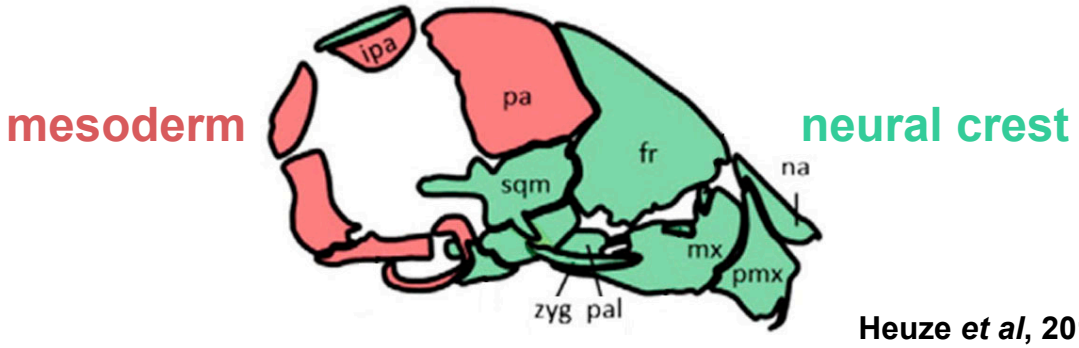
Internasal (IN)
Interpremaxillary (IPM)
Intermaxillary (IM)
Interpalatine (IP)
Premaxillary/maxillary (PM)
Maxillary/palatine (MP)





Sutures for Atlases: Lineage

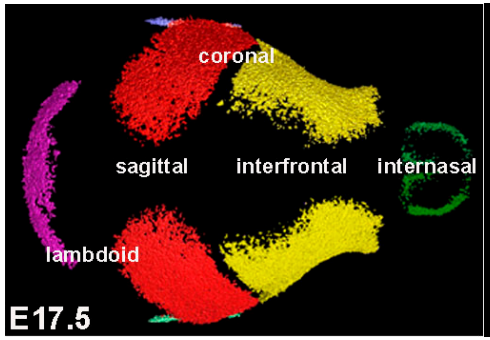
Suture	Location	Lineage
Interfrontal (IF)	Cranial	Neural crest
Sagittal (S)	Cranial	Mixed
Coronal (C)	Cranial	Mixed
Lambdoid (L)	Cranial	Mesoderm
Squamoparietal (SQ)	Cranial	Mixed
Internasal (IN)	Facial	Neural crest
Interpremaxillary (IPM)	Facial	Neural crest
Intermaxillary (IM)	Facial	Neural crest
Interpalatine (IP)	Facial	Neural crest
Premaxillary/maxillary (PM)	Facial	Neural crest
Maxillary/palatine (MP)	Facial	Neural crest

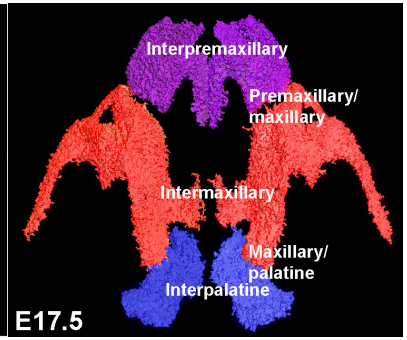


Heuze *et al*, 2014

Sutures for Atlases: Bone identity

Suture	Bone Pair	Bone Pair Homology
Interfrontal (IF)	Frontals	Homologous
Sagittal (S)	Parietals	Homologous
Coronal (C)	Frontal/Parietal	Non-homologous
Lambdoid (L)	Parietal/Interparietal	Non-homologous
Squamoparietal (SQ)	Parietal/Squamous	Non-homologous
Internasal (IN)	Nasals	Homologous
Interpremaxillary (IPM)	Premaxillaries	Homologous
Intermaxillary (IM)	Maxillaries	Homologous
Interpalatine (IP)	Palatines	Homologous
Premaxillary/maxillary (PM)	Premaxillary/Maxillary	Non-homologous
Maxillary/palatine (MP)	Maxillary/Palatine	Non-homologous



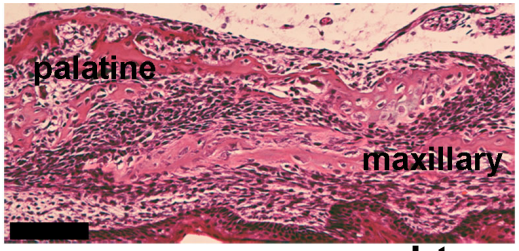


Chris Percival

Sutures for Atlases: Structure

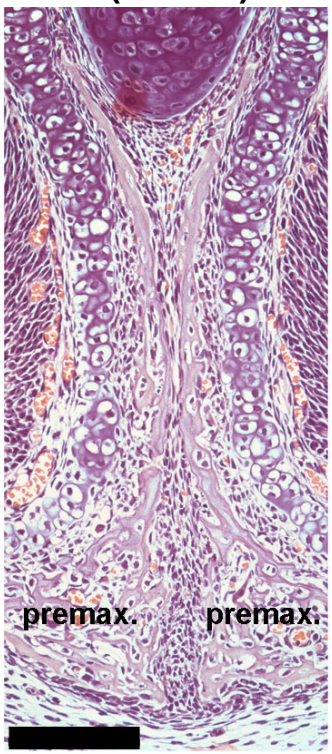
Suture	Structure
Interfrontal (IF)	End-to-end
Sagittal (S)	End-to-end
Coronal (C)	Overlapping
Lambdoid (L)	Overlapping
Squamoparietal (SQ)	Overlapping
Internasal (IN)	End-to-end (broad)
Interpremaxillary (IPM)	End-to-end (broad)
Intermaxillary (IM)	End-to-end
Interpalatine (IP)	End-to-end
Premaxillary/maxillary (PM)	End-to-end
Maxillary/palatine (MP)	Overlapping

overlapping



end-to-end

end-to-end (broad)



Greg Holmes

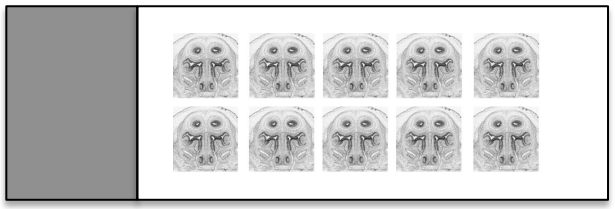
Versatility of atlases for expression discovery

This range of sutures will allow for mesenchymal versus osteogenic gene expression discovery with specificity for:

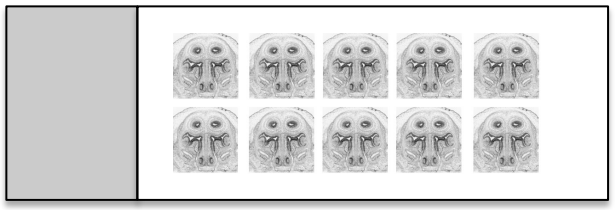
- **Individual sutures**
- **Lineage (mesoderm versus neural crest)**
- **Individual bones**
- **Structure**
- **Timing**

Laser Capture Microdissection

Sectioning of fresh-frozen embryonic heads

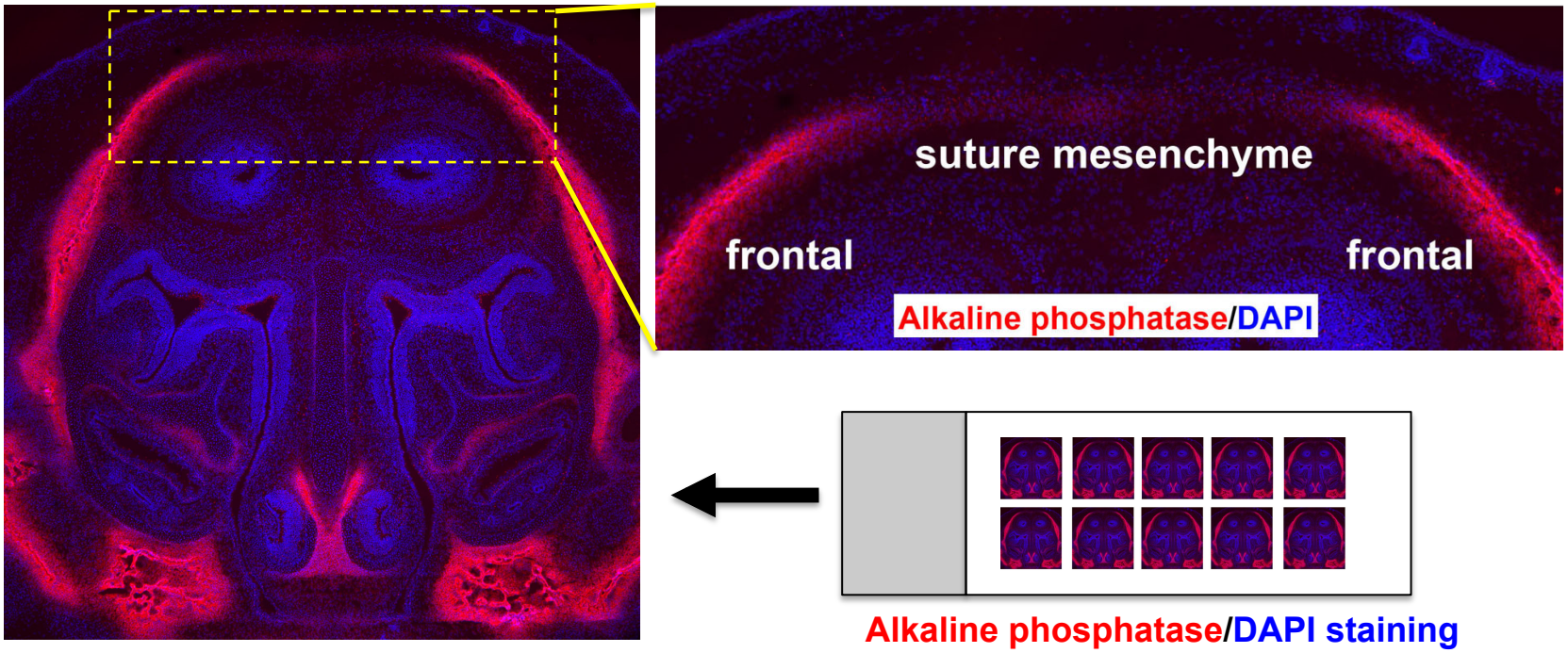


5-10 PEN membrane slides



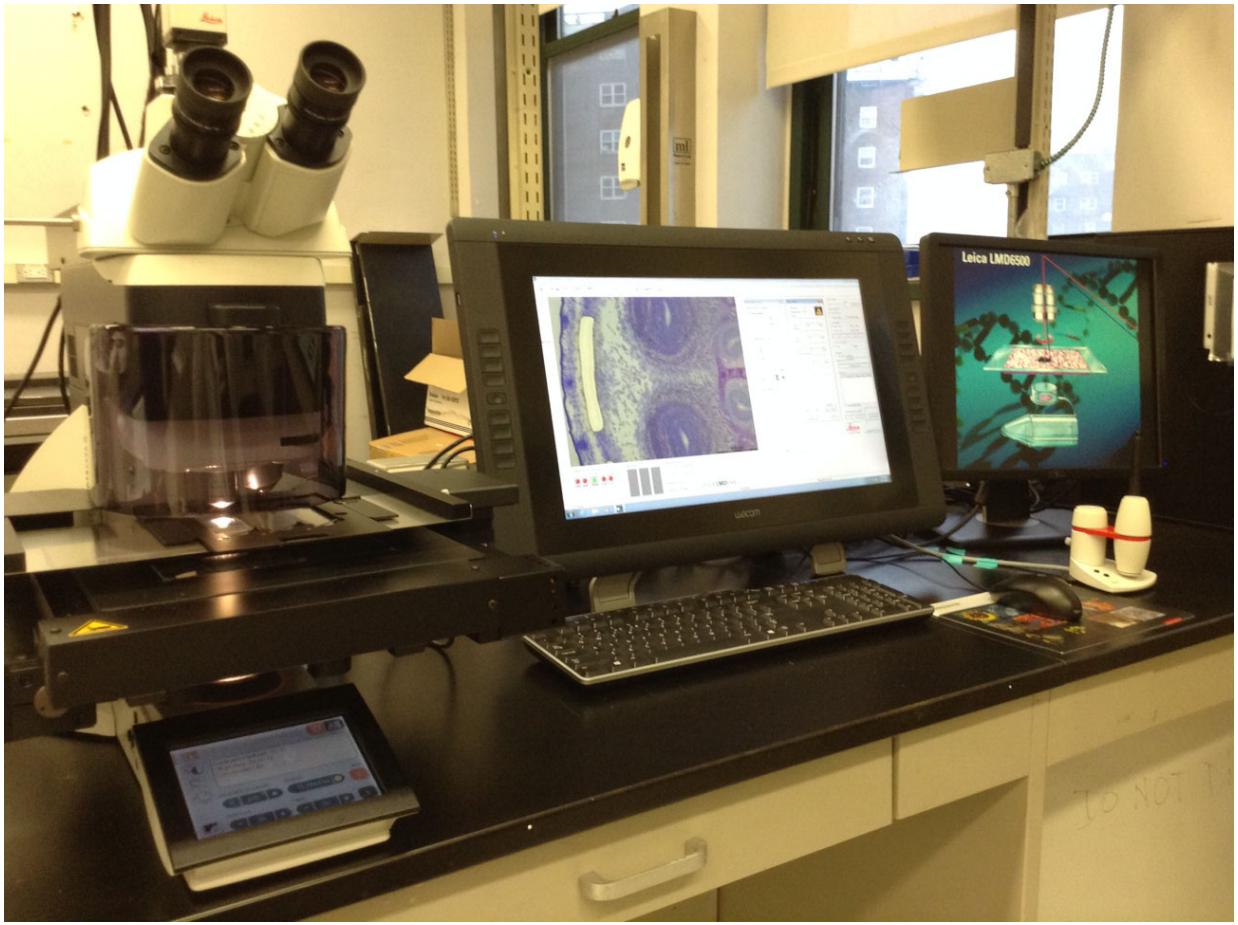
1 Superfrost slide (Guide slide; every 5th or 10th section)

Guide Slide: E16.5 Interfrontal suture



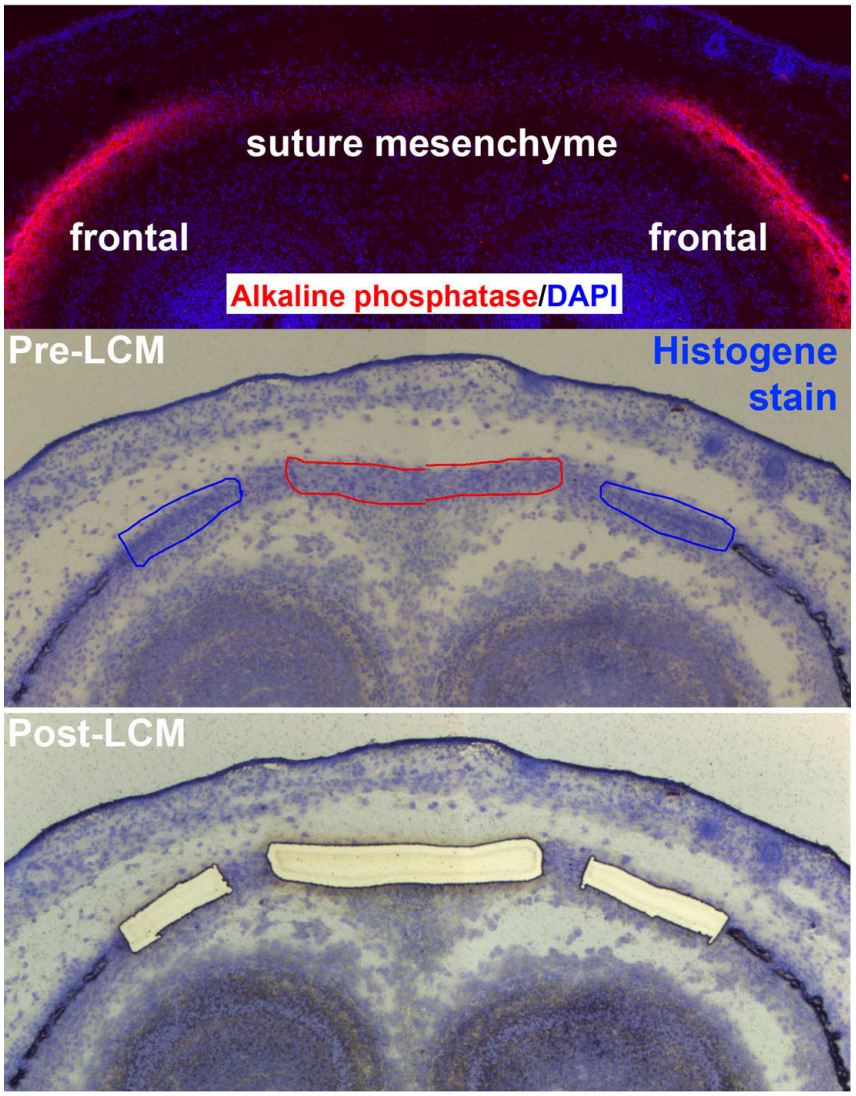
-Alkaline phosphatase distinguishes preosteoblasts/osteoblasts from suture mesenchyme

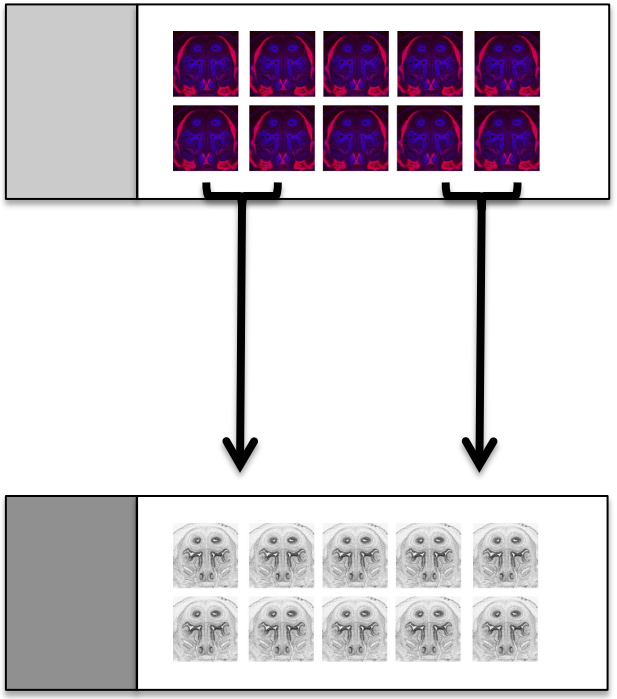
Leica LMD 6500



Pathology Shared Resource Facility, Mount Sinai Hospital

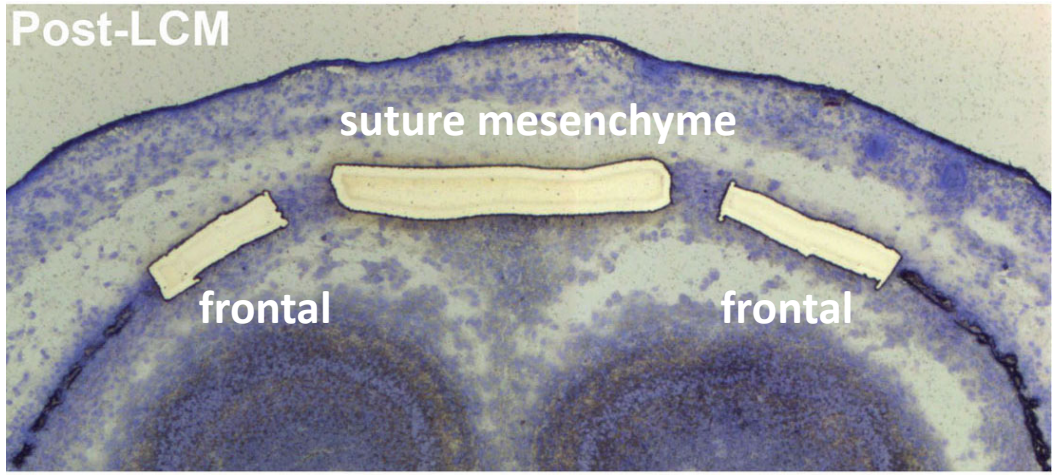
E16.5 Interfrontal suture LCM





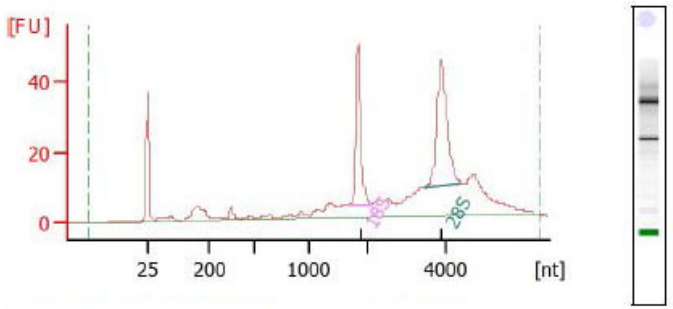
2-3 slides

E16.5 Interfrontal suture LCM



Laser Capture Microdissection → RNA extraction → Bioanalyzer

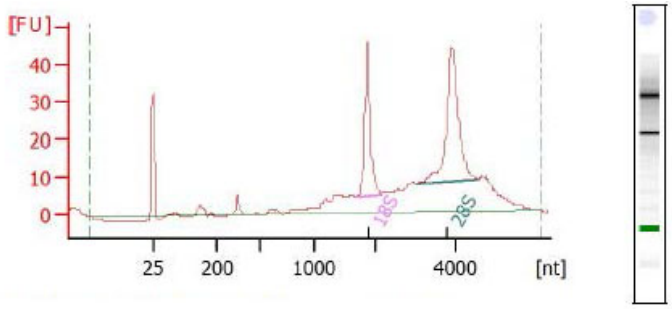
suture mesenchyme



RNA Concentration: 1,130 pg/µl

RNA Integrity Number (RIN): 8.6 (B.02.08)

frontals (combined)



RNA Concentration: 1,133 pg/µl

RNA Integrity Number (RIN): 8.1 (B.02.08)

Specific Aims

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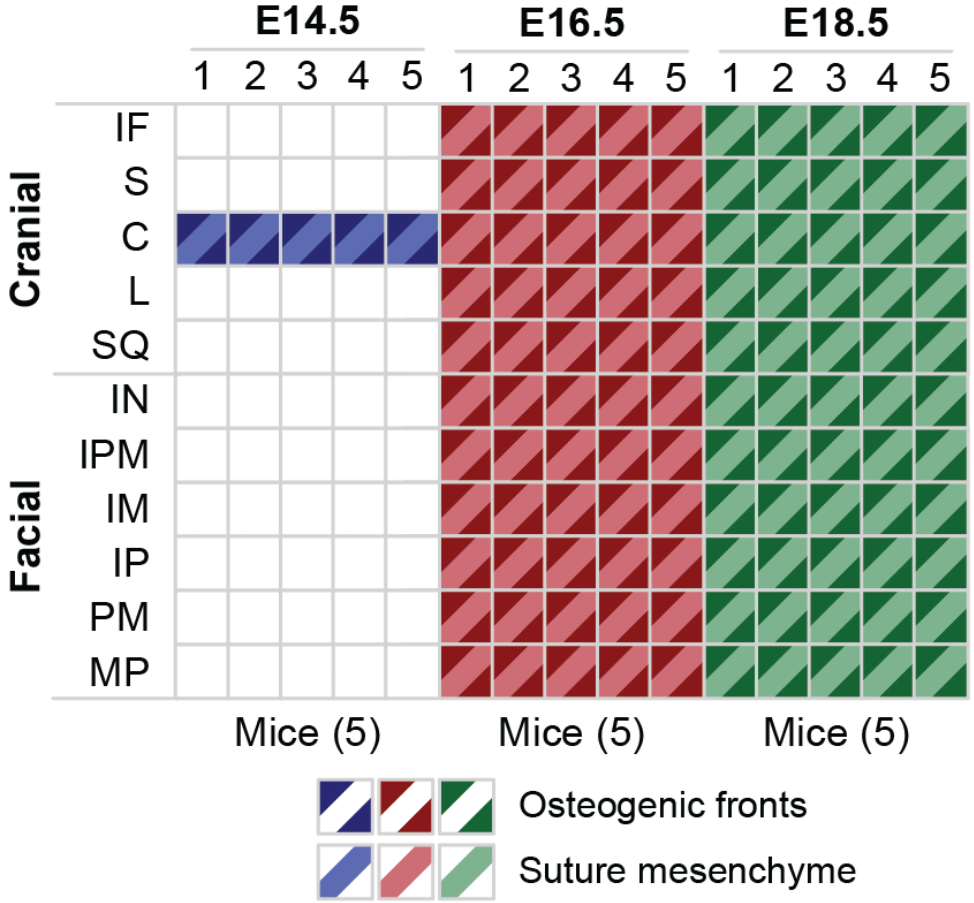
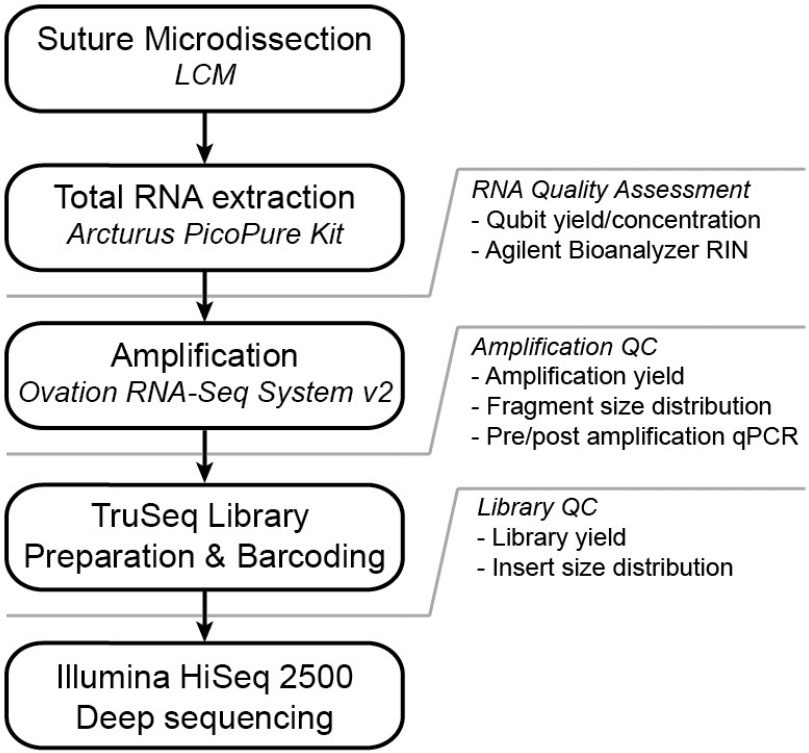
Aim2: Next Generation Sequencing

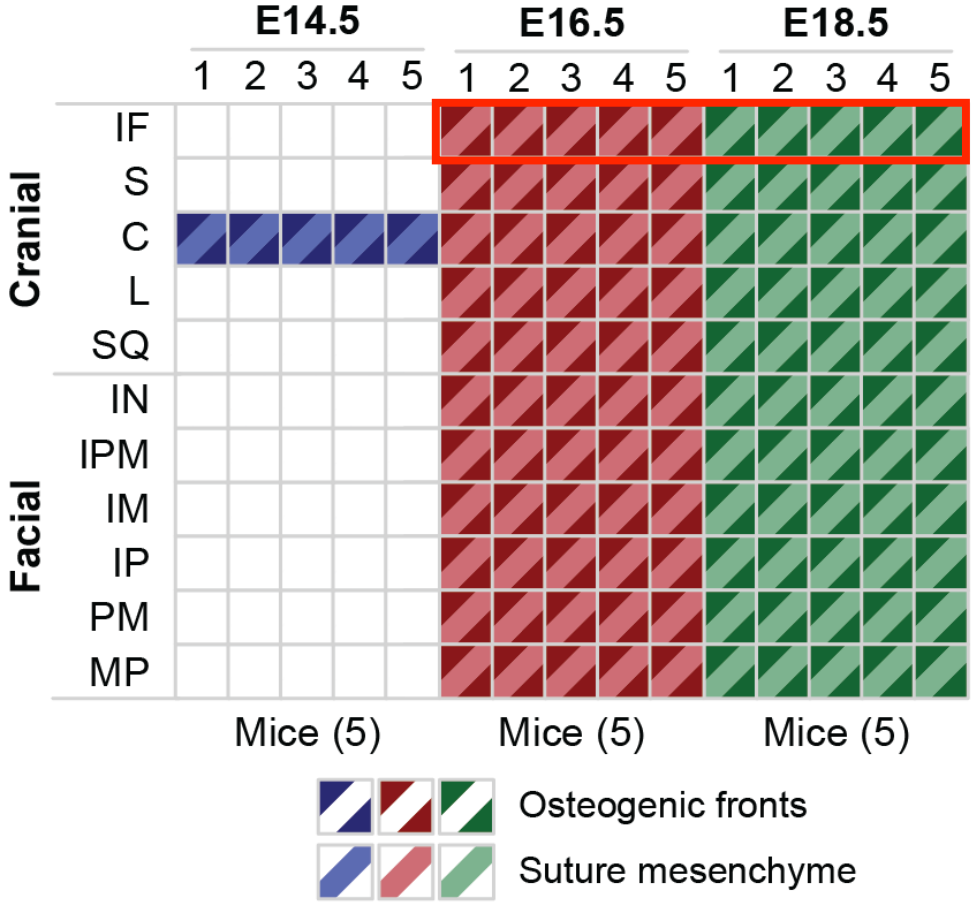
Experiment overview: WT

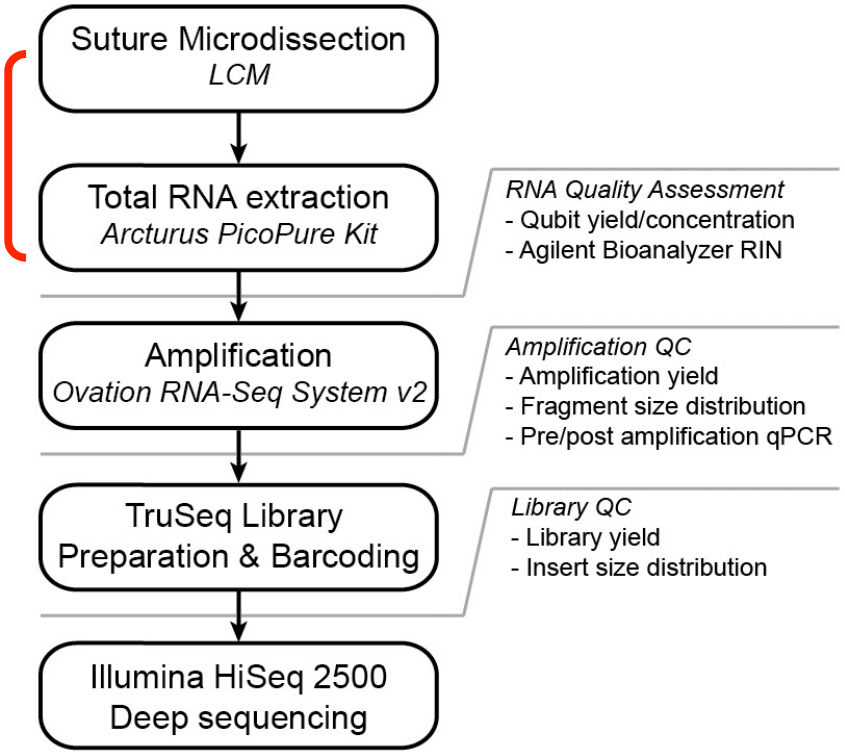




Aim2: Next Generation Sequencing

Experiment overview: WT





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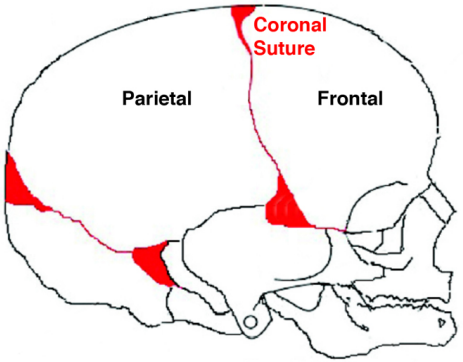
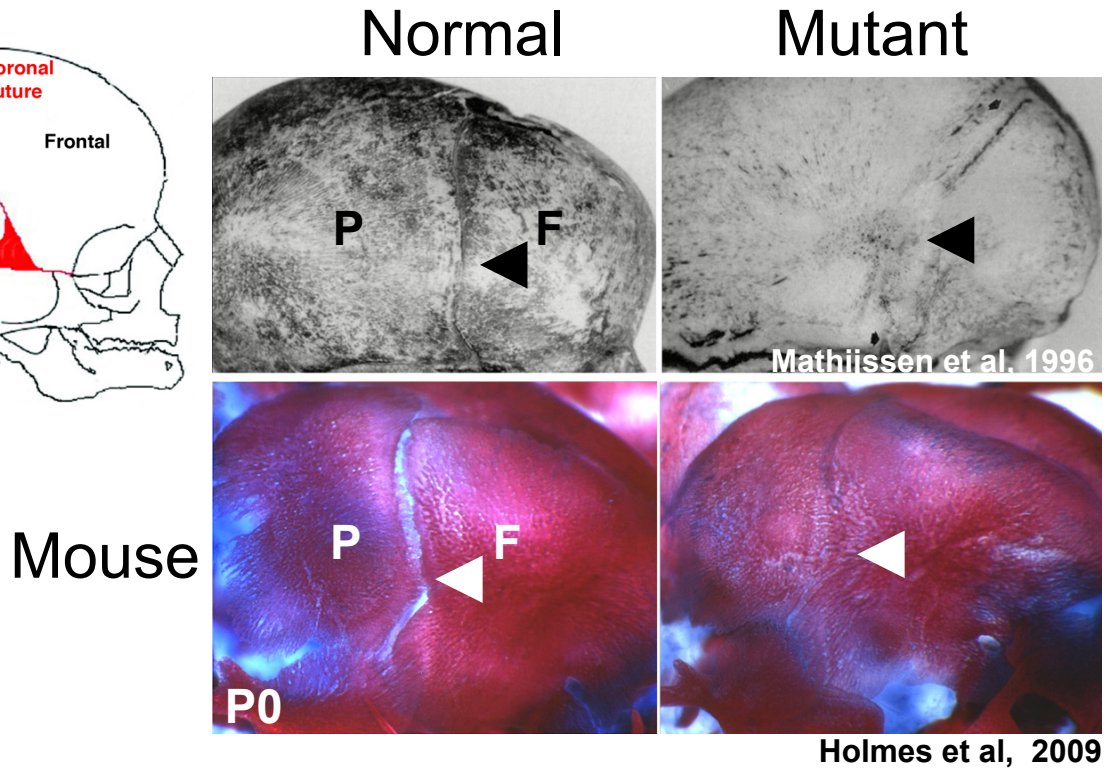
Apert *Fgfr2*$^{+/S252W}$ Mouse Model

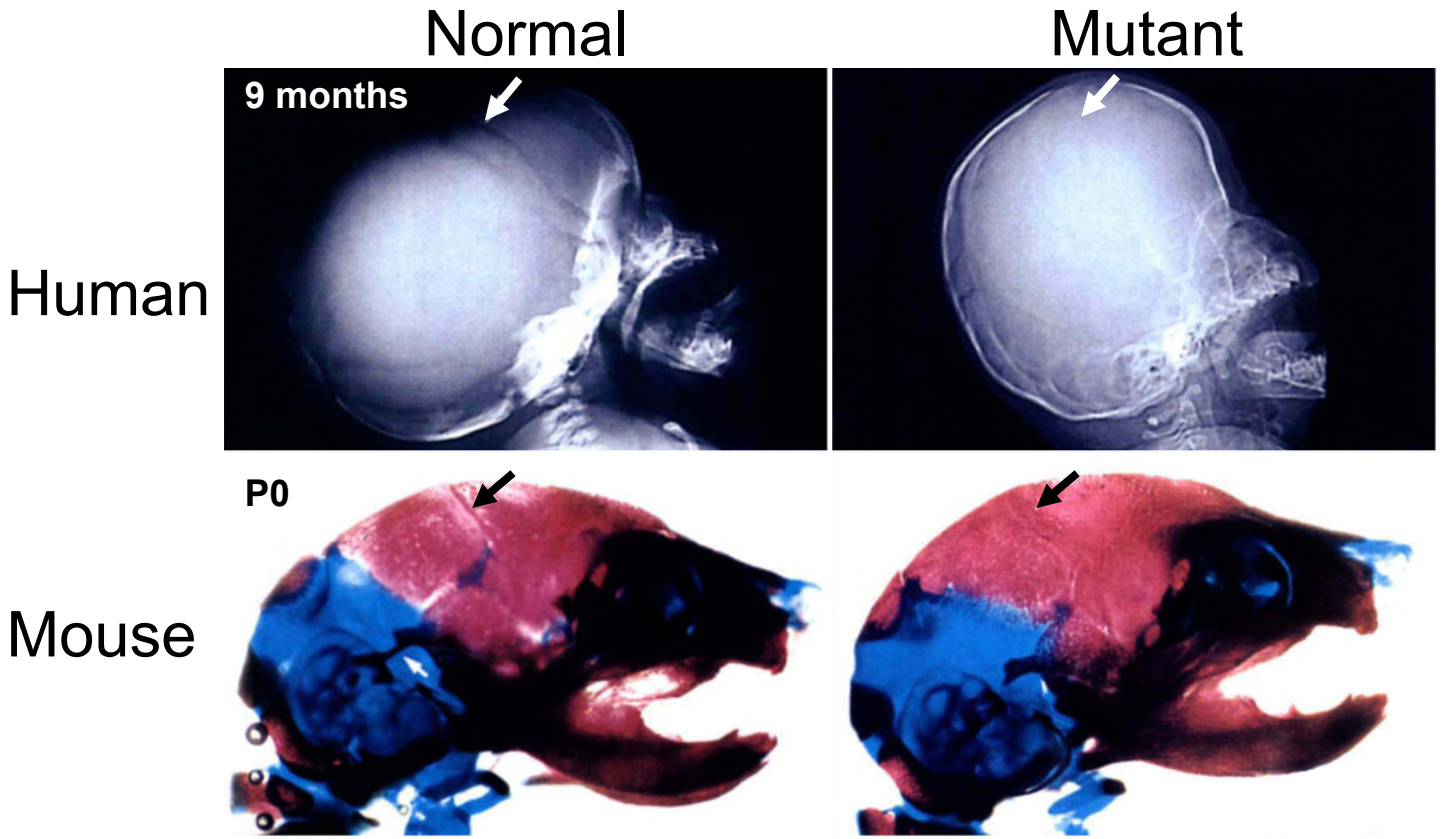




Holmes et al, 2009

- Coronal suture fusion
- Interfrontal suture wider at P0
- Premaxillary/maxillary and maxillary/palatine fusion initiated by P0, and more extensive facial suture fusion seen postnatally
- Fgfr2 is extensively expressed in osteogenic fronts/preosteoblasts of craniofacial bones
- All sutures to be assessed at E16.5 and E18.5, and at E14.5 for the coronal suture

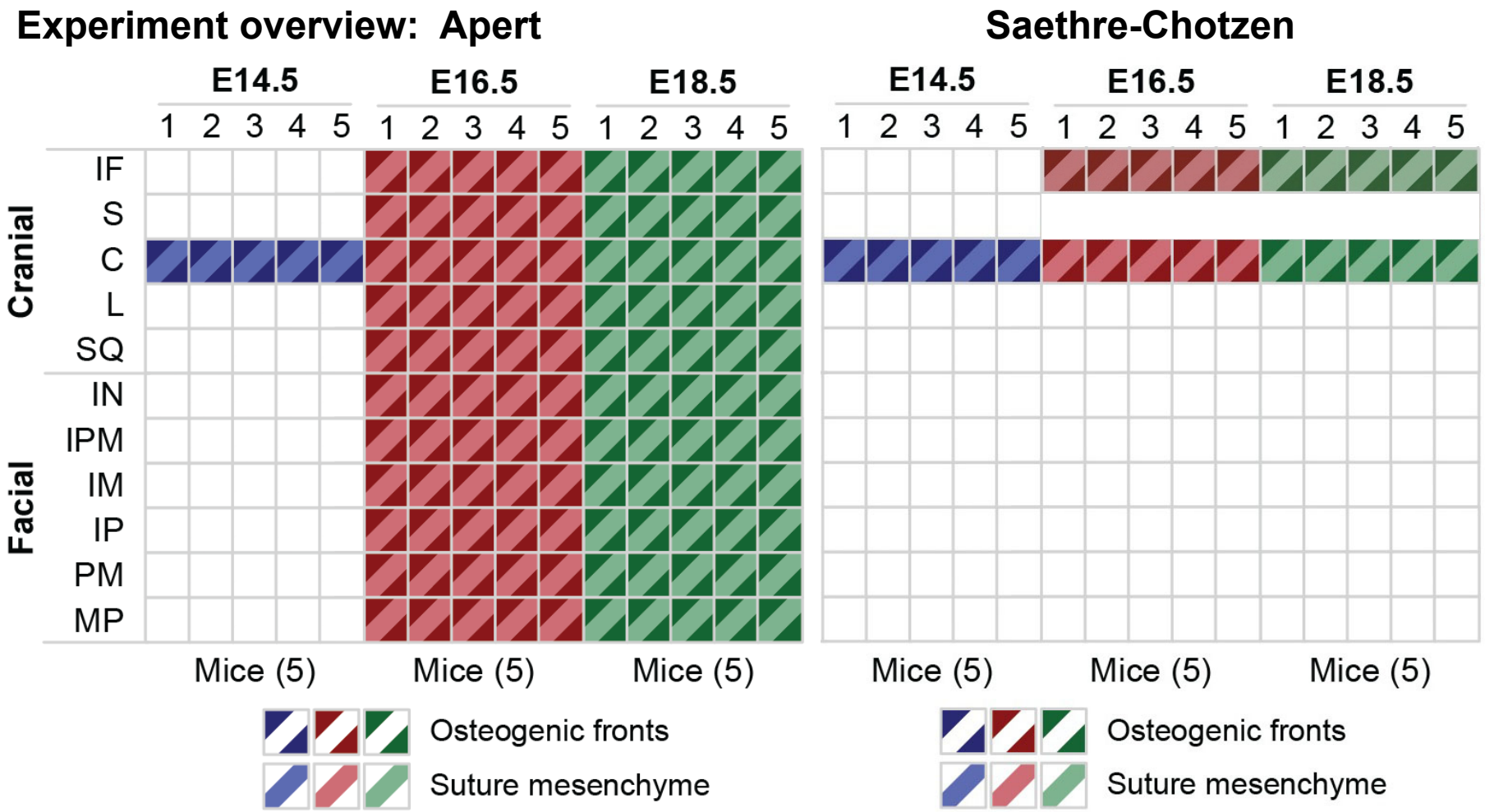
Saethre-Chotzen *Twist1*$^{+/-}$ Mouse Model



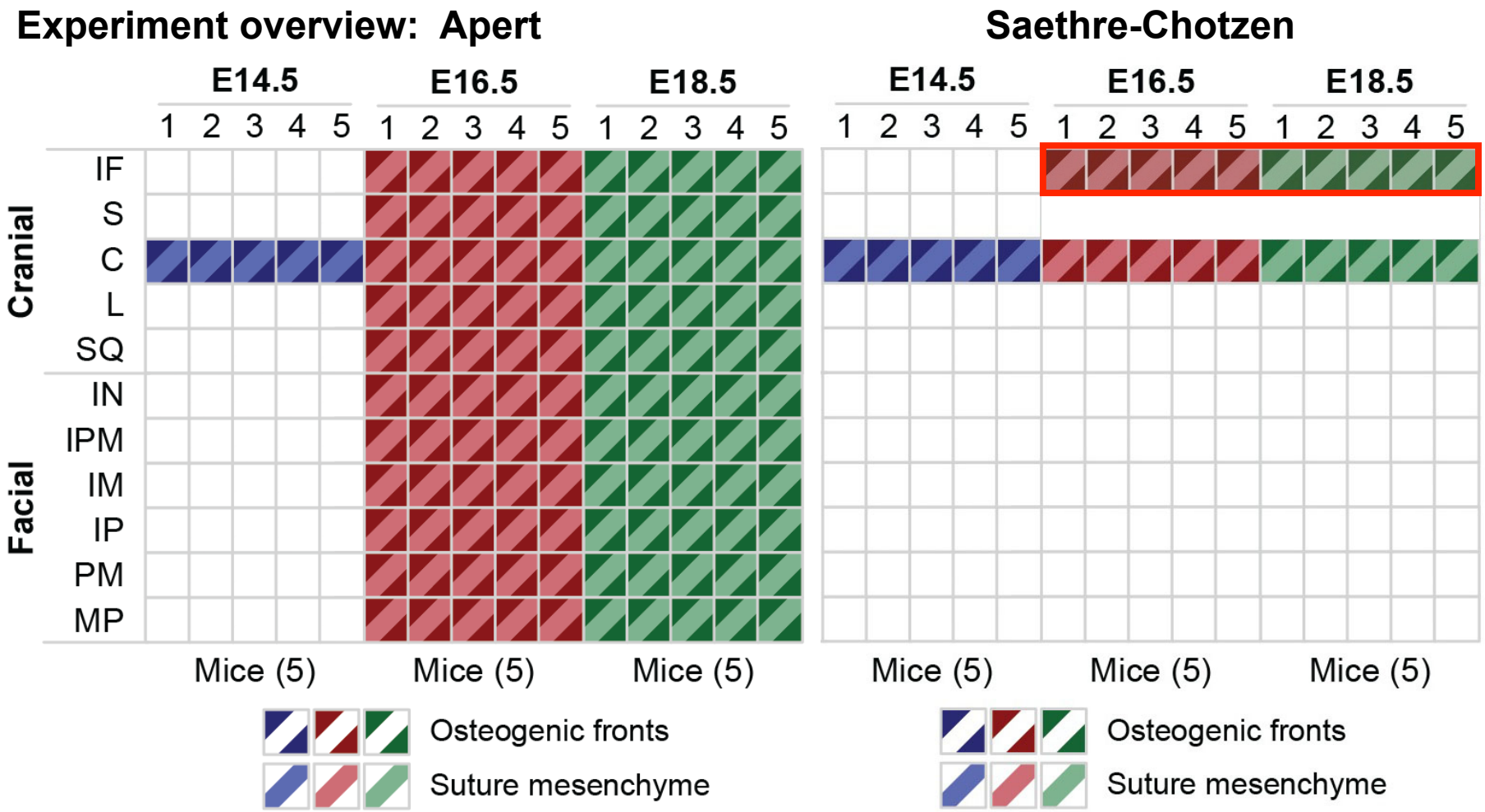
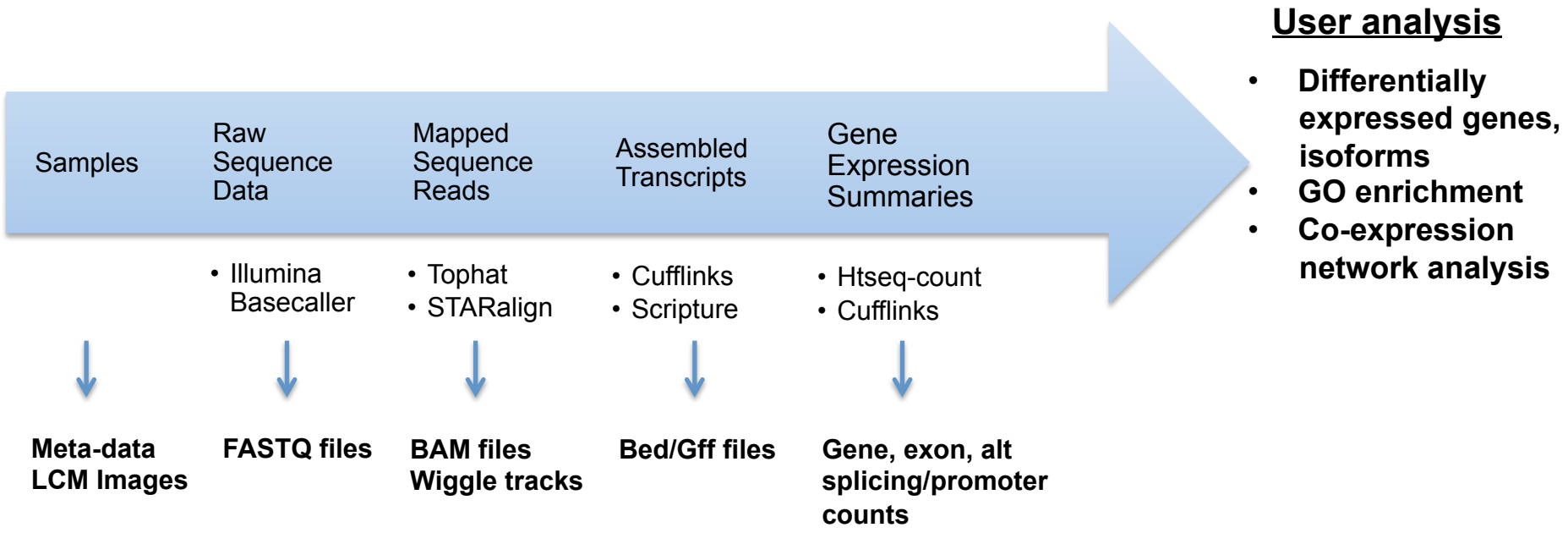
- **Coronal suture fusion**
- **Interfrontal suture wider at P0**
- **TWIST1 interacts with the Fgfr2, Ephrin, and Notch pathways in the suture**
- **The coronal and interfrontal sutures to be assessed at E16.5 and E18.5, and at E14.5 for the coronal suture**

El Ghouzzi et al, 1997

Aim 3: Next Generation Sequencing



Aim 3: Next Generation Sequencing



Deliverable Transcriptome Data for FaceBase 2