

# *2016 FaceBase Annual Meeting*



## *Integrated research of functional genomics and craniofacial morphogenesis*

PI: Yang Chai

University of Southern California

### **Team Members:**

Bridget Samuels

Thach-Vu Ho

Jill Harunaga

Shery Park

Zoe Johnson

Jingyuan Li

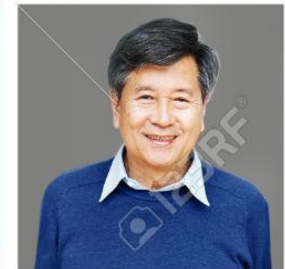
Pedro Sanchez

Paul Thomas (bioinformatics)

Jeremy Green (KCL)

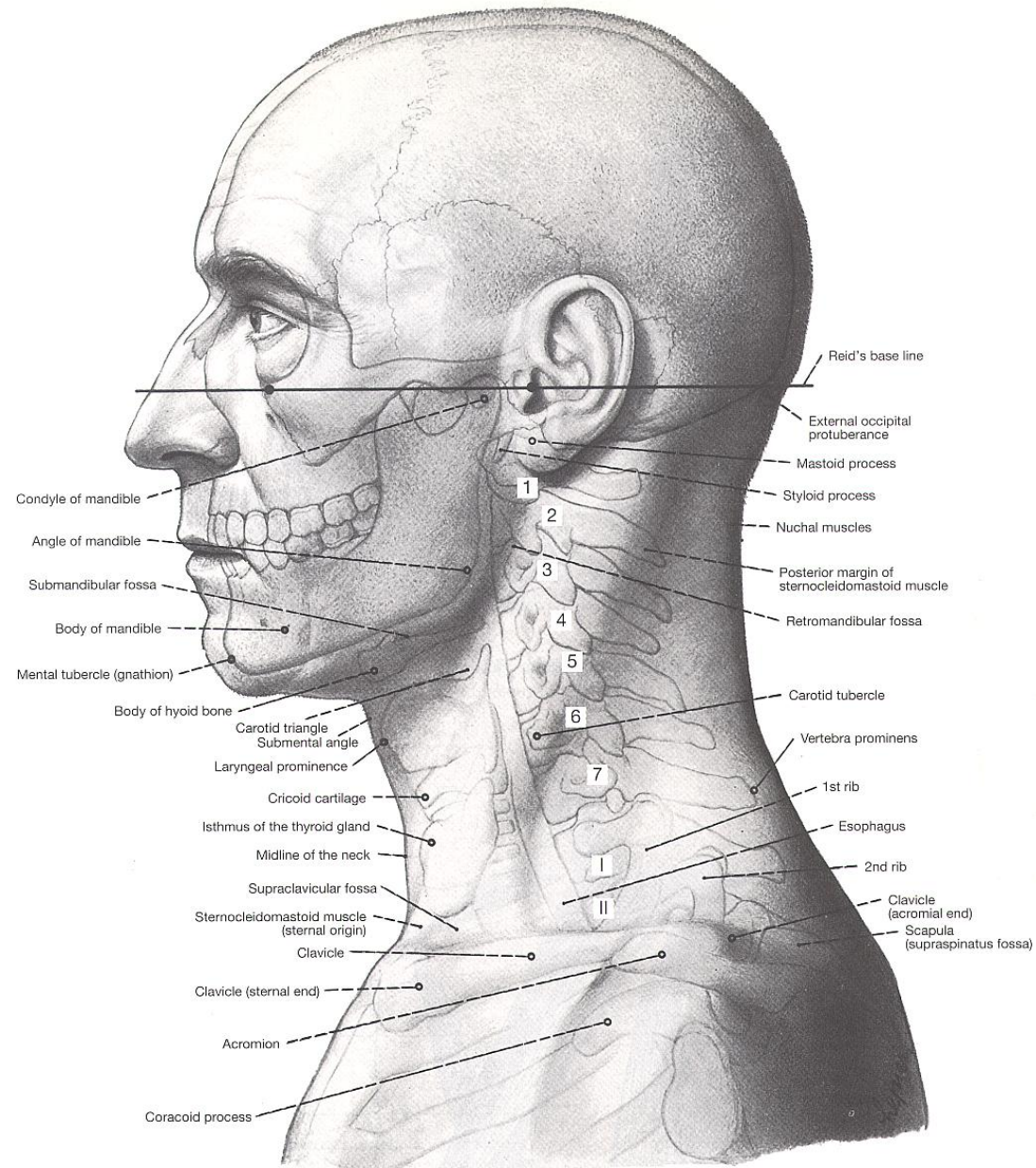
***U01 DE024421 NIDCR, NIH***

*Our face  
is our  
identity*



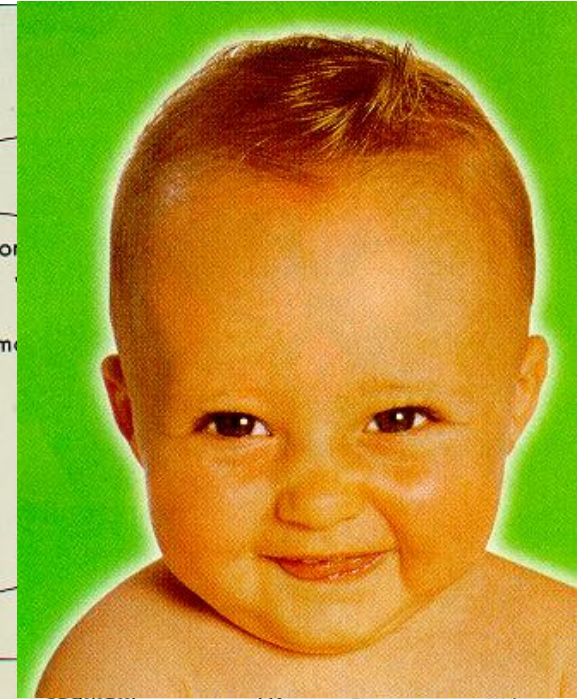
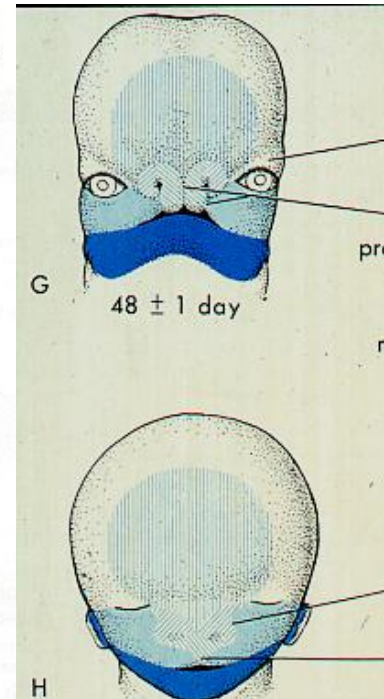
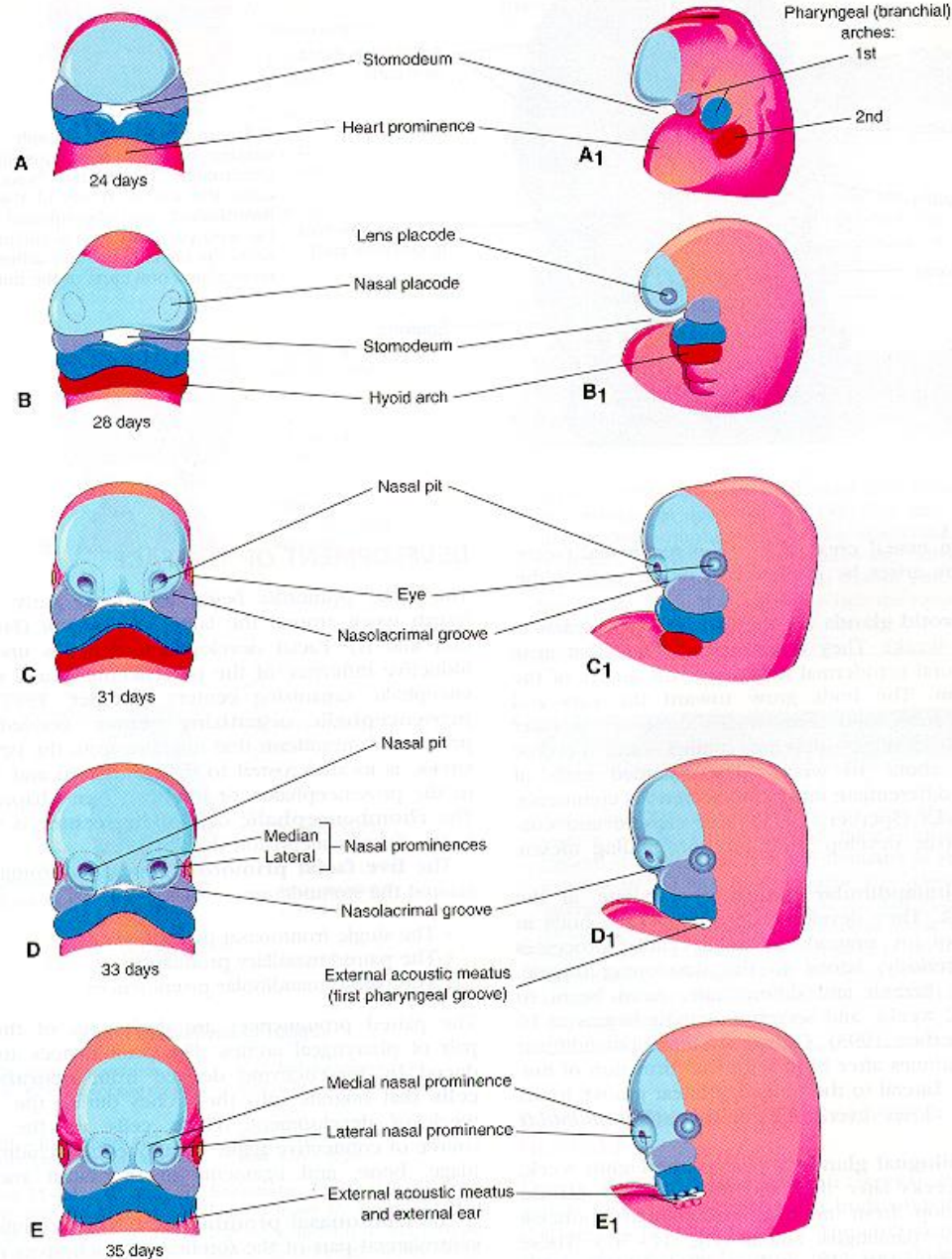


# *Craniofacial bones determine our facial appearance*



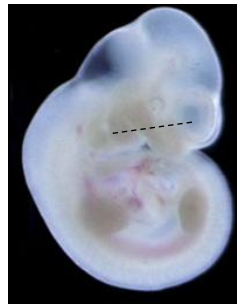


# Different cellular contributions to facial development





# Palate, tongue, and mandible development



E10.5



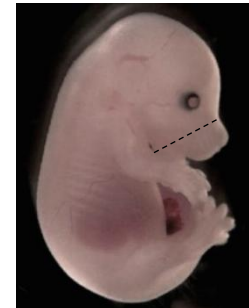
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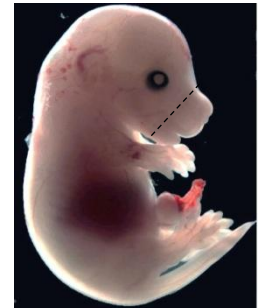
E12.5



E13.5

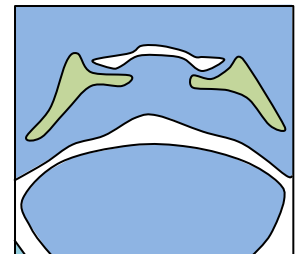
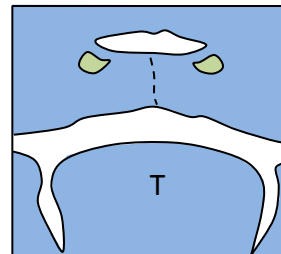
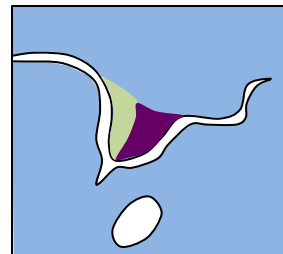
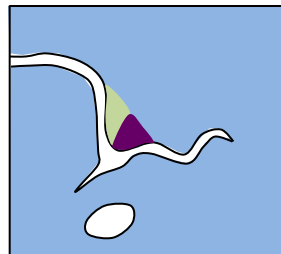
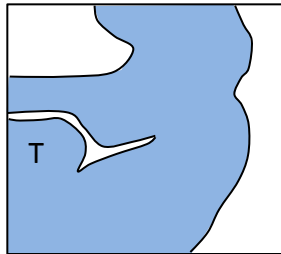
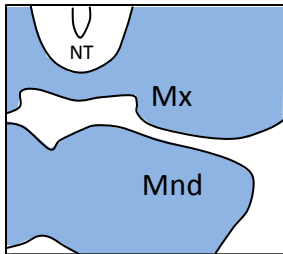


E14.5

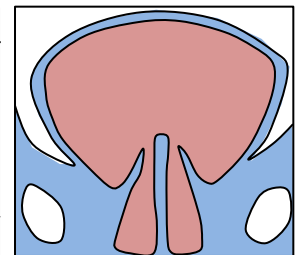
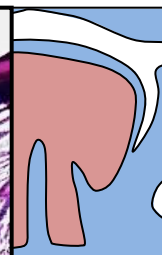
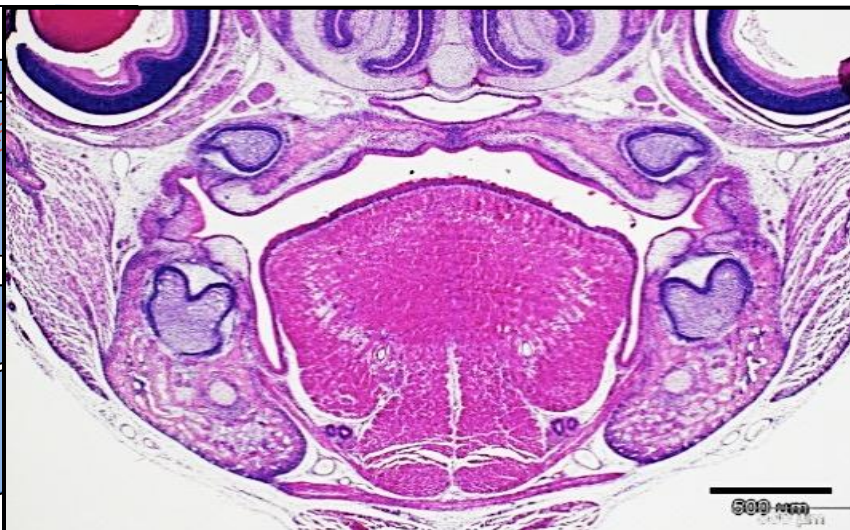
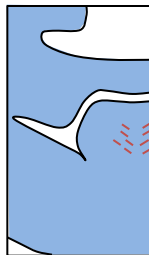
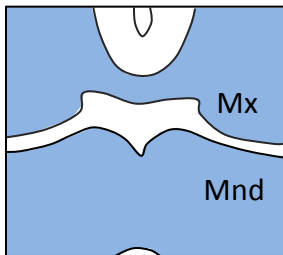


E15.5

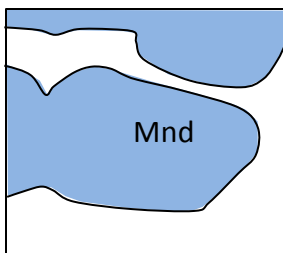
Palate



Tongue



Mandible



## *Specific Aims*

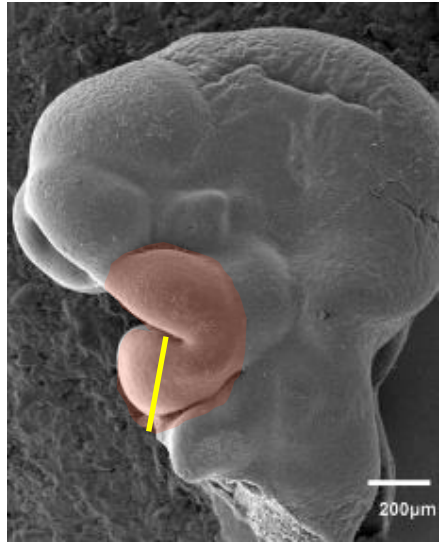
1. Global and specific gene expression analyses and 3D imaging study
  - (1) General gene expression profiles during mandible development using microarray/RNA-Seq
  - (2) Specific gene expression: define sub-domains within the developing mandible and correlate this information with cell fate analysis
  - (3) 3D imaging and ontology development
2. Gene expression, cell fate and 3D imaging during maxilla development

## *Our goals*

*1.To provide a foundation for the investigation of the molecular regulatory mechanisms of mandible/maxilla morphogenesis*

*2.To build collaborations for dental, oral, and craniofacial tissue/organ regeneration*

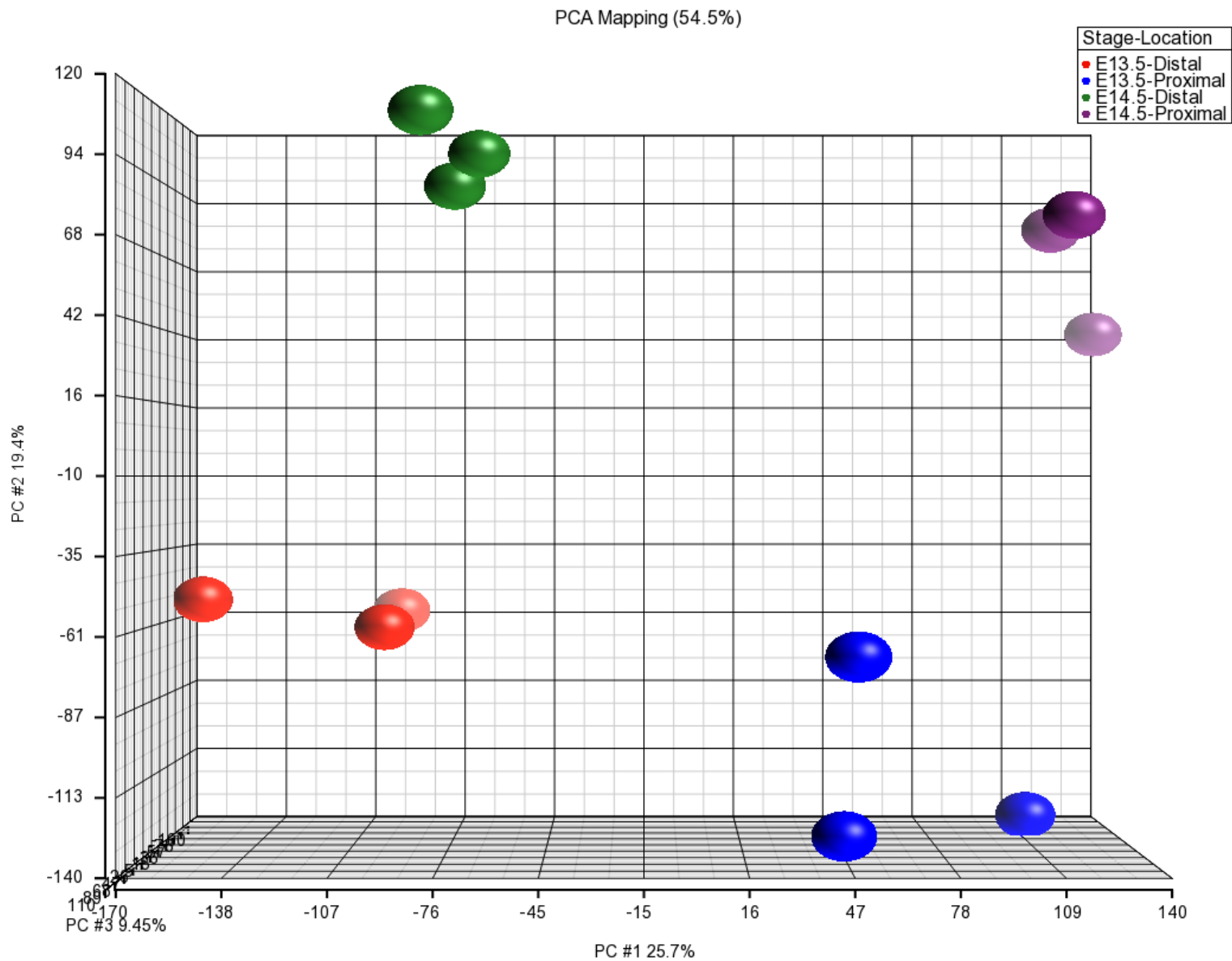
# Gene expression profile analyses



E10.5

| MicroArray for FaceBase2.0 |                             |          |  |              |          |          |
|----------------------------|-----------------------------|----------|--|--------------|----------|----------|
|                            | Mandible                    |          |  | Maxilla      |          |          |
|                            | Proximal                    | Distal   |  | Proximal     | Distal   |          |
| <b>E10.5</b>               | 3                           | 3        |  | <b>E10.5</b> | 3        | 3        |
| <b>E11.5</b>               | 6                           | 6        |  | <b>E11.5</b> | 6        | 6        |
| <b>E12.5</b>               | 3                           | 3        |  | <b>E12.5</b> | 3        | 3        |
|                            | GSE67985                    | GSE67985 |  |              | GSE67985 | GSE67985 |
| <b>E13.5</b>               | 3                           | 3        |  | <b>E13.5</b> | 3        | 3        |
|                            | GSE67985                    | GSE67985 |  |              | GSE67985 | GSE67985 |
| <b>E14.5</b>               | 3                           | 3        |  | <b>E14.5</b> | 3        | 3        |
|                            | GSE67985                    | GSE67985 |  |              | GSE67985 | GSE67985 |
|                            | Queued for submission       |          |  |              |          |          |
|                            | Submitted to GEO # GSE67985 |          |  |              |          |          |





Principal Component Analysis shows good separation between mandibular sample groups

# FaceBase Microarray Data

**Select**

- E10.5\_Mnd\_D  E11.5\_Mnd\_D  E12.5\_Mnd\_D  E13.5\_Mnd\_D  E14.5\_Mnd\_D
- E10.5\_Mnd\_P  E11.5\_Mnd\_P  E12.5\_Mnd\_P  E13.5\_Mnd\_P  E14.5\_Mnd\_P
- E10.5\_Max\_D  E11.5\_Max\_D  E12.5\_Max\_D  E13.5\_Max\_D  E14.5\_Max\_D
- E10.5\_Max\_P  E11.5\_Max\_P  E12.5\_Max\_P  E13.5\_Max\_P  E14.5\_Max\_P

**Compare**

- Distal vs. Proximal
- Mandible vs. Maxilla
- E10.5 vs. E11.5
- E11.5 vs. E12.5
- E12.5 vs. E13.5
- E13.5 vs. E14.5

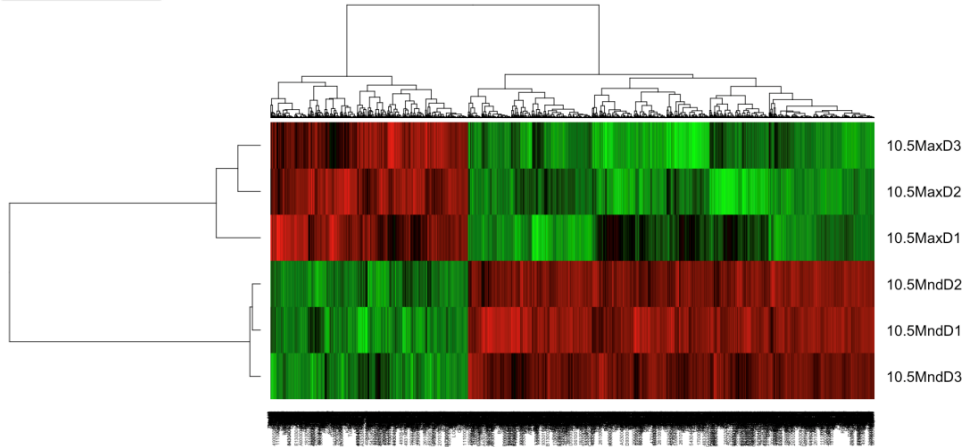
**Absolute Fold Change Cutoff**

**Adjusted P-Value Cutoff**

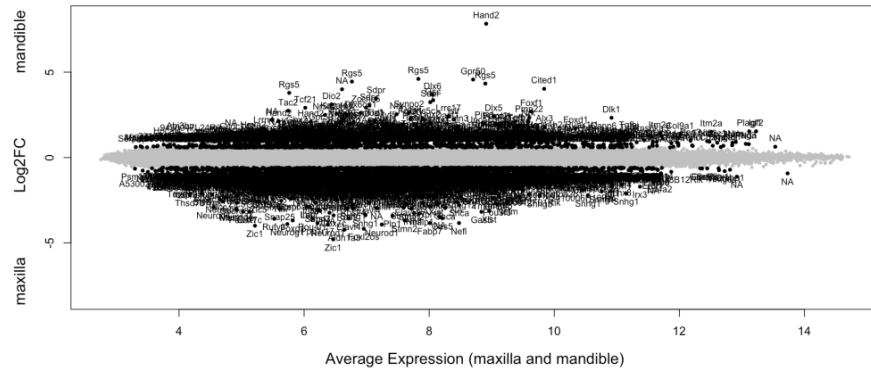
**Max. Number of DE Probesets**

Submit (will take a few minutes the first time!)

Download heatmap



Download MA plot



Download table

|   | Probeset     | Gene  | Ave.Expr | Log2FC | FC     | Adj.P   | Description   |
|---|--------------|-------|----------|--------|--------|---------|---|
| 1 | 1436041_at   | Hand2 | 8.91     | 7.83   | 228.05 | 3.5e-06 | heart and neural crest derivatives expressed transcript 2 |
| 2 | 1420940_x_at | Rgs5  | 7.83     | 4.61   | 24.34  | 5.0e-06 | regulator of G-protein signaling 5                        |
| 3 | 1455498_at   | Gpr50 | 8.70     | 4.57   | 23.72  | 2.3e-03 | G-protein-coupled receptor 50                             |

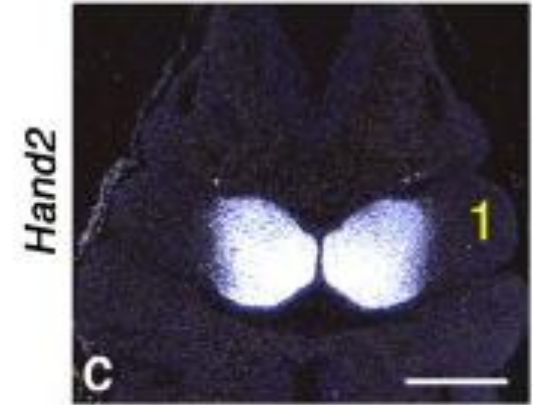
# *Top 20 differentially expressed genes between distal region of mandible and maxilla at E10.5*

| Rank | Probeset     | Gene    | Avg. Expr. | Log2 FC | Fold change | Adj. P-Value | Description   |
|------|--------------|---------|------------|---------|-------------|--------------|---|
| 1    | 1436041_at   | Hand2   | 8.91       | 7.83    | 228.05      | 3.5e-06      | heart and neural crest derivatives expressed transcript 2                       |
| 2    | 1420940_x_at | Rgs5    | 7.83       | 4.61    | 24.34       | 5.0e-06      | regulator of G-protein signaling 5  |
| 3    | 1455498_at   | Gpr50   | 8.70       | 4.57    | 23.72       | 2.3e-03      | G-protein-coupled receptor 50   |
| 4    | 1420941_at   | Rgs5    | 6.76       | 4.45    | 21.82       | 6.5e-05      | regulator of G-protein signaling 5  |
| 5    | 1417466_at   | Rgs5    | 8.90       | 4.33    | 20.09       | 5.5e-06      | regulator of G-protein signaling 5  |
| 6    | 1449031_at   | Cited1  | 9.84       | 4.02    | 16.27       | 3.5e-06      | Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1 |
| 7    | 1446812_at   |         | 6.61       | 4.00    | 15.96       | 2.5e-05      |   |
| 8    | 1420942_s_at | Rgs5    | 5.76       | 3.79    | 13.80       | 2.5e-05      | regulator of G-protein signaling 5  |
| 9    | 1452507_at   | Dlx6    | 8.05       | 3.68    | 12.86       | 1.7e-03      | distal-less homeobox 6  |
| 10   | 1416779_at   | Sdpr    | 7.15       | 3.45    | 10.94       | 9.7e-04      | serum deprivation response  |
| 11   | 1421412_at   | Gsc     | 8.06       | 3.36    | 10.24       | 3.0e-03      | goosecoid homeobox  |
| 12   | 1416778_at   | Sdpr    | 8.02       | 3.24    | 9.46        | 6.3e-04      | serum deprivation response  |
| 13   | 1418937_at   | Dio2    | 6.44       | 3.11    | 8.65        | 7.5e-05      | deiodinase, iodothyronine, type II  |
| 14   | 1443832_s_at | Sdpr    | 7.04       | 3.06    | 8.34        | 8.3e-05      | serum deprivation response  |
| 15   | 1437355_at   | Zcchc5  | 6.99       | 2.92    | 7.57        | 6.5e-05      | zinc finger, CCHC domain containing 5   |
| 16   | 1417447_at   | Tcf21   | 6.02       | 2.91    | 7.54        | 2.5e-04      | transcription factor 21   |
| 17   | 1419411_at   | Tac2    | 5.75       | 2.74    | 6.67        | 7.5e-05      | tachykinin 2  |
| 18   | 1434939_at   | Foxf1   | 9.63       | 2.70    | 6.51        | 8.3e-05      | forkhead box F1   |
| 19   | 1435399_at   | Synpo2  | 7.68       | 2.63    | 6.21        | 5.1e-03      | synaptopodin 2  |
| 20   | 1438799_at   | Dlx6os1 | 6.91       | 2.61    | 6.13        | 1.4e-04      | distal-less homeobox 6, opposite strand 1                                       |

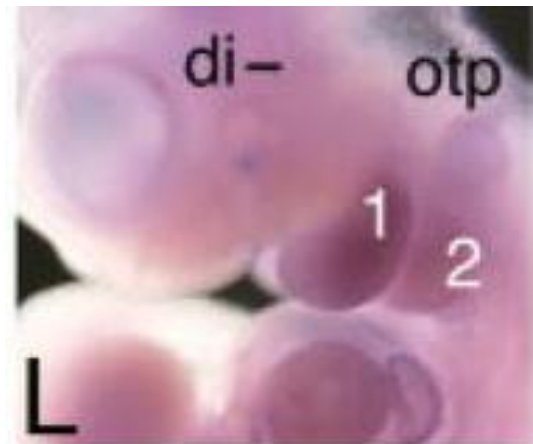


# Validating our microarray data and generating new hypotheses to investigate the patterning of the branchial arch

| Rank | Probeset     | Gene    | Avg. Expr. | Log2 FC | Fold change | Adj. P-Value | Description   |
|------|--------------|---------|------------|---------|-------------|--------------|---|
| 1    | 1436041_at   | Hand2   | 8.91       | 7.83    | 228.05      | 3.5e-06      | heart and neural crest derivatives expressed transcript 2                       |
| 2    | 1420940_x_at | Rgs5    | 7.83       | 4.61    | 24.34       | 5.0e-06      | regulator of G-protein signaling 5  |
| 3    | 1455498_at   | Gpr50   | 8.70       | 4.57    | 23.72       | 2.3e-03      | G-protein-coupled receptor 50   |
| 4    | 1420941_at   | Rgs5    | 6.76       | 4.45    | 21.82       | 6.5e-05      | regulator of G-protein signaling 5  |
| 5    | 1417466_at   | Rgs5    | 8.90       | 4.33    | 20.09       | 5.5e-06      | regulator of G-protein signaling 5  |
| 6    | 1449031_at   | Cited1  | 9.84       | 4.02    | 16.27       | 3.5e-06      | Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1 |
| 7    | 1446812_at   |         | 6.61       | 4.00    | 15.96       | 2.5e-05      |   |
| 8    | 1420942_s_at | Rgs5    | 5.76       | 3.79    | 13.80       | 2.5e-05      | regulator of G-protein signaling 5  |
| 9    | 1452507_at   | Dlx6    | 8.05       | 3.68    | 12.86       | 1.7e-03      | distal-less homeobox 6  |
| 10   | 1416779_at   | Sdpr    | 7.15       | 3.45    | 10.94       | 9.7e-04      | serum deprivation response  |
| 11   | 1421412_at   | Gsc     | 8.06       | 3.36    | 10.24       | 3.0e-03      | gooseoid homeobox   |
| 12   | 1416778_at   | Sdpr    | 8.02       | 3.24    | 9.46        | 6.3e-04      | serum deprivation response  |
| 13   | 1418937_at   | Dio2    | 6.44       | 3.11    | 8.65        | 7.5e-05      | deiodinase, iodothyronine, type II  |
| 14   | 1443832_s_at | Sdpr    | 7.04       | 3.06    | 8.34        | 8.3e-05      | serum deprivation response  |
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| 16   | 1417447_at   | Tcf21   | 6.02       | 2.91    | 7.54        | 2.5e-04      | transcription factor 21   |
| 17   | 1419411_at   | Tac2    | 5.75       | 2.74    | 6.67        | 7.5e-05      | tachykinin 2  |
| 18   | 1434939_at   | Foxf1   | 9.63       | 2.70    | 6.51        | 8.3e-05      | forkhead box F1   |
| 19   | 1435399_at   | Synpo2  | 7.68       | 2.63    | 6.21        | 5.1e-03      | synaptopodin 2  |
| 20   | 1438799_at   | Dlx6os1 | 6.91       | 2.61    | 6.13        | 1.4e-04      | distal-less homeobox 6, opposite strand 1                                       |



**Hand2 expression at E10.5**  
Barbosa et al., 2007 Dev. Biol.



**Dlx6 expression at E10.5**  
Ruest et al., 2003, Development

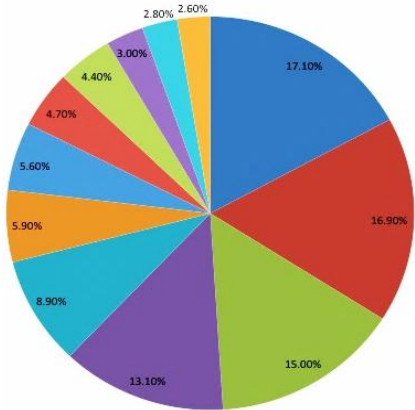
# Gene expression profile along the distal-proximal axis in the developing mandible

C

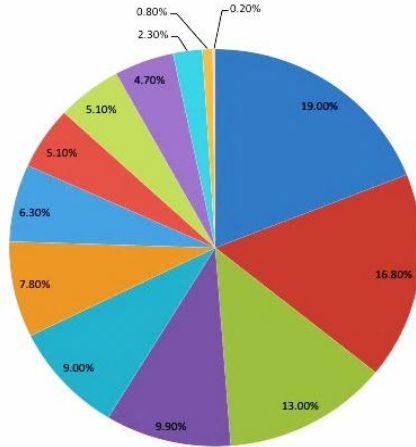
## Downregulated genes

## Upregulated genes

Biological processes

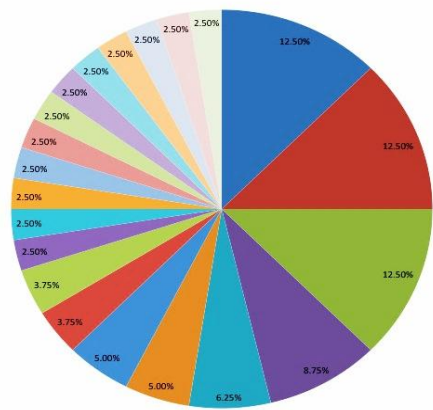


- metabolic process (GO:0008152)
- cellular process (GO:0009987)
- developmental process (GO:0032502)
- biological regulation (GO:0065007)
- multicellular organismal process (GO:0032501)
- immune system process (GO:0002376)
- response to stimulus (GO:0050896)
- cellular component organization or biogenesis (GO:0071840)
- localization (GO:0051179)
- reproduction (GO:0000003)
- biological adhesion (GO:0022610)
- apoptotic process (GO:0006915)

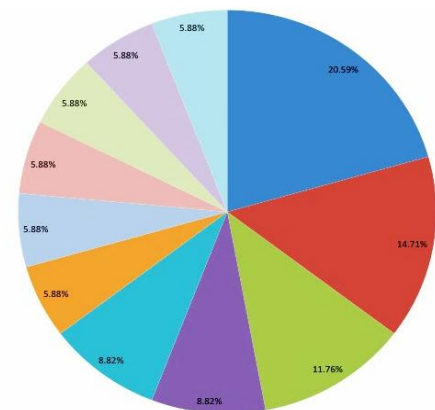


- cellular process (GO:0009987)
- metabolic process (GO:0008152)
- developmental process (GO:0032502)
- multicellular organismal process (GO:0032501)
- biological regulation (GO:0065007)
- localization (GO:0051179)
- response to stimulus (GO:0050896)
- biological adhesion (GO:0022610)
- immune system process (GO:0002376)
- cellular component organization or biogenesis (GO:0071840)
- reproduction (GO:0000003)
- apoptotic process (GO:0006915)
- growth (GO:0040007)

Pathways



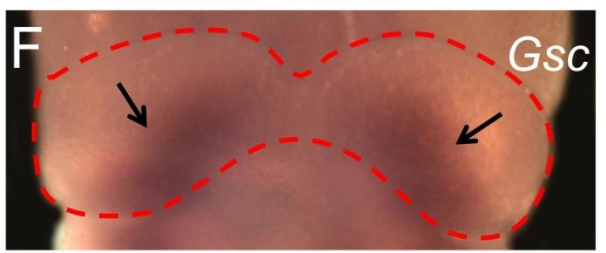
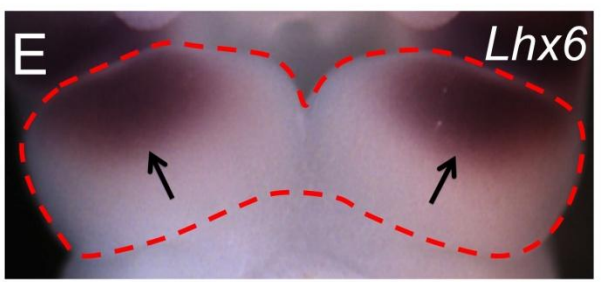
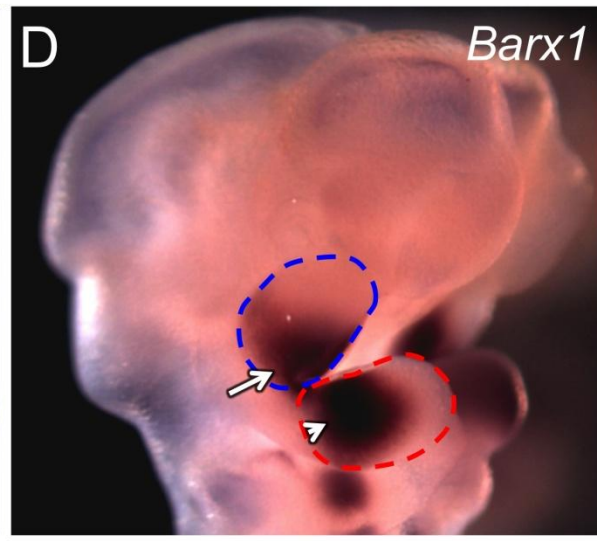
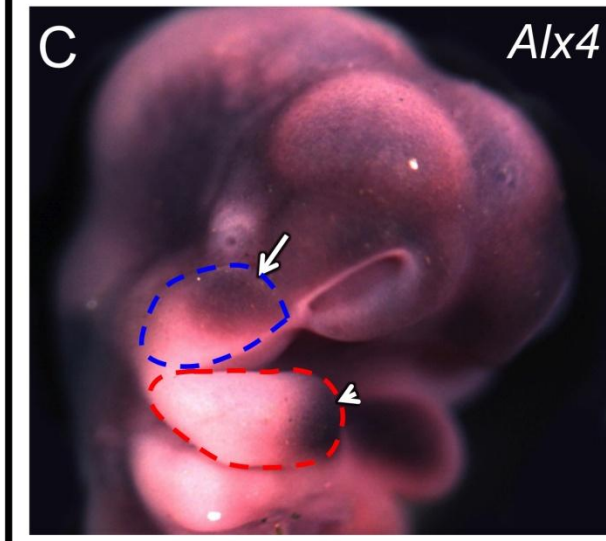
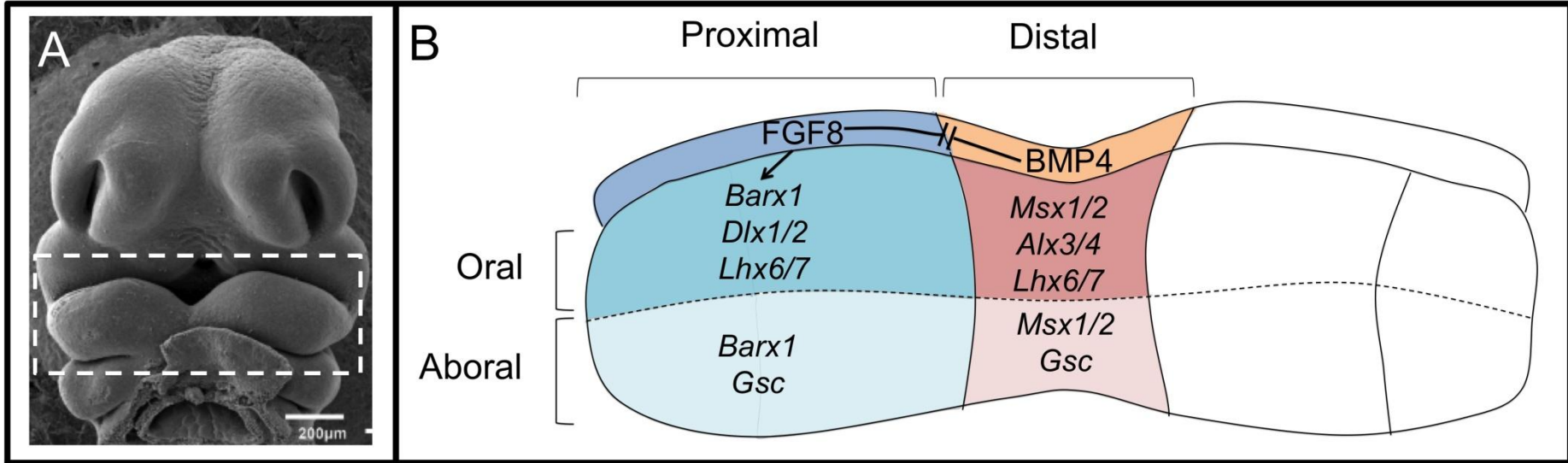
- Integrin signaling pathway (P00034)
- Inflammation mediated by chemokine and cytokine signaling pathway (P00031)
- Wnt signaling pathway (P00057)
- Cytoskeletal regulation by Rho GTPase (P00016)
- Nicotinic acetylcholine receptor signaling pathway (P00044)
- Ionotropic glutamate receptor pathway (P00037)
- Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway (P00026)
- Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway (P00027)
- Gonadotropin releasing hormone receptor pathway (P06664)
- Beta2 adrenergic receptor signaling pathway (P04378)
- Beta1 adrenergic receptor signaling pathway (P04377)
- De novo purine biosynthesis (P02738)
- Angiogenesis (P00005)
- Huntington disease (P00029)
- FGF signaling pathway (P00021)
- Parkinson disease (P00049)
- Cadherin signaling pathway (P00012)
- Blood coagulation (P00011)
- Dopamine receptor mediated signaling pathway (P05912)
- Dopamine receptor mediated signaling pathway (P05912)
- Angiotensin II-stimulated signaling through G proteins and beta arrestin (P05911)
- Corticotropin releasing factor receptor signaling pathway (P04380)
- Angiotensin II-stimulated signaling through G proteins and beta arrestin (P05911)
- Corticotropin releasing factor receptor signaling pathway (P04380)



- Gonadotropin releasing hormone receptor pathway (P06664)
- Wnt signaling pathway (P00057)
- Cadherin signaling pathway (P00012)
- TGF-beta signaling pathway (P00052)
- Alzheimer disease-presenilin pathway (P00004)
- Oxytocin receptor mediated signaling pathway (P04391)
- Nicotinic acetylcholine receptor signaling pathway (P00044)
- Ionotropic glutamate receptor pathway (P00037)
- Integrin signaling pathway (P00034)
- Inflammation mediated by chemokine and cytokine signaling pathway (P00031)
- Huntington disease (P00029)
- Angiogenesis (P00005)
- Huntington disease (P00029)
- FGF signaling pathway (P00021)
- Parkinson disease (P00049)
- Cadherin signaling pathway (P00012)
- Blood coagulation (P00011)
- Dopamine receptor mediated signaling pathway (P05912)
- Dopamine receptor mediated signaling pathway (P05912)
- Angiotensin II-stimulated signaling through G proteins and beta arrestin (P05911)
- Corticotropin releasing factor receptor signaling pathway (P04380)
- Angiotensin II-stimulated signaling through G proteins and beta arrestin (P05911)
- Corticotropin releasing factor receptor signaling pathway (P04380)

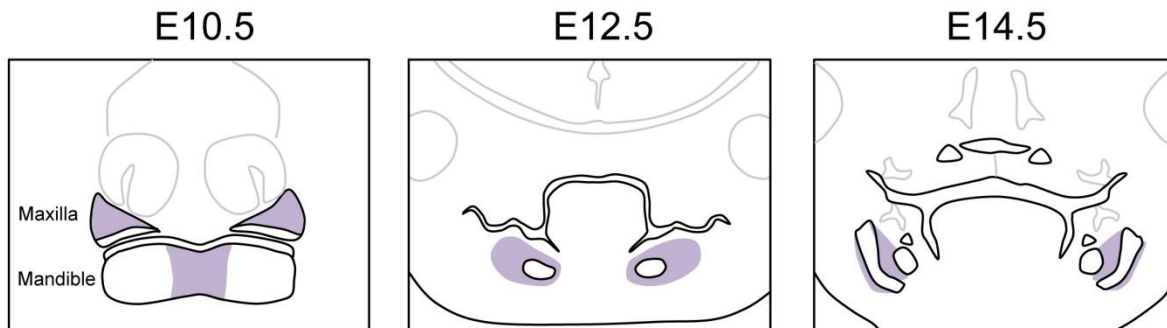
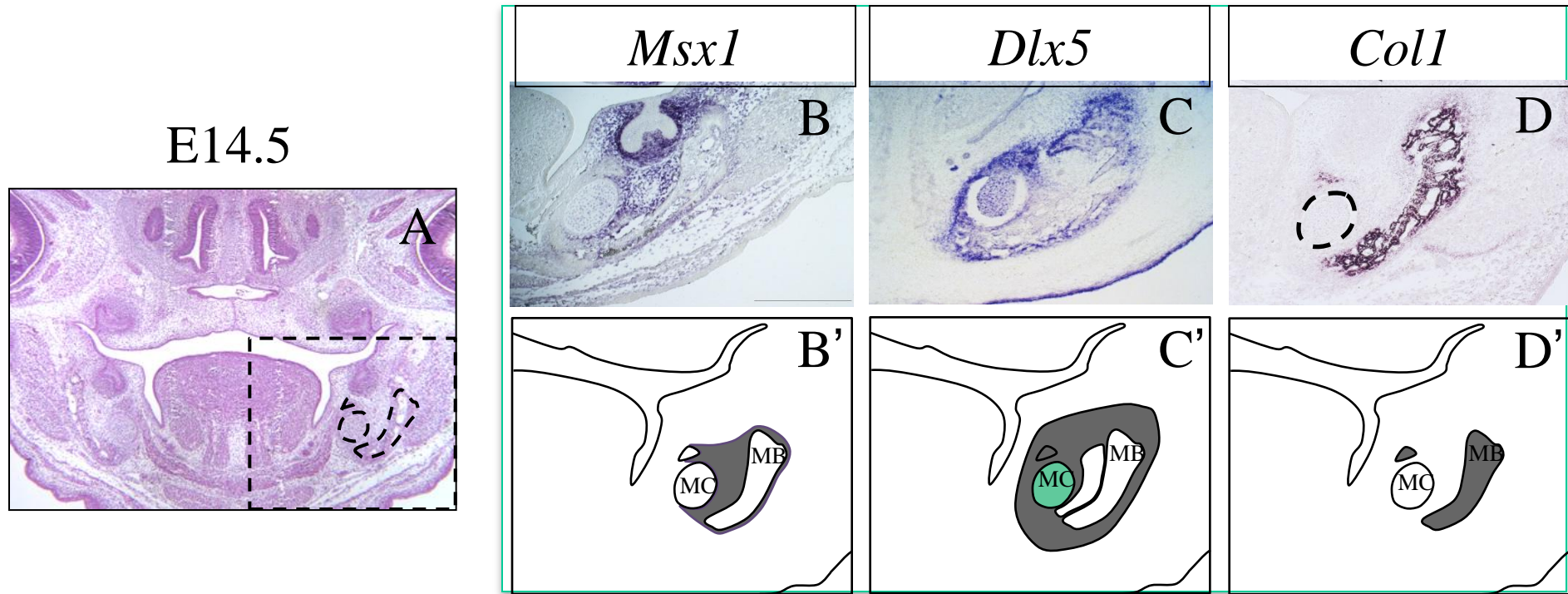
# Gene expression and patterning of the branchial arch

## Early patterning of the maxillary and mandibular arches

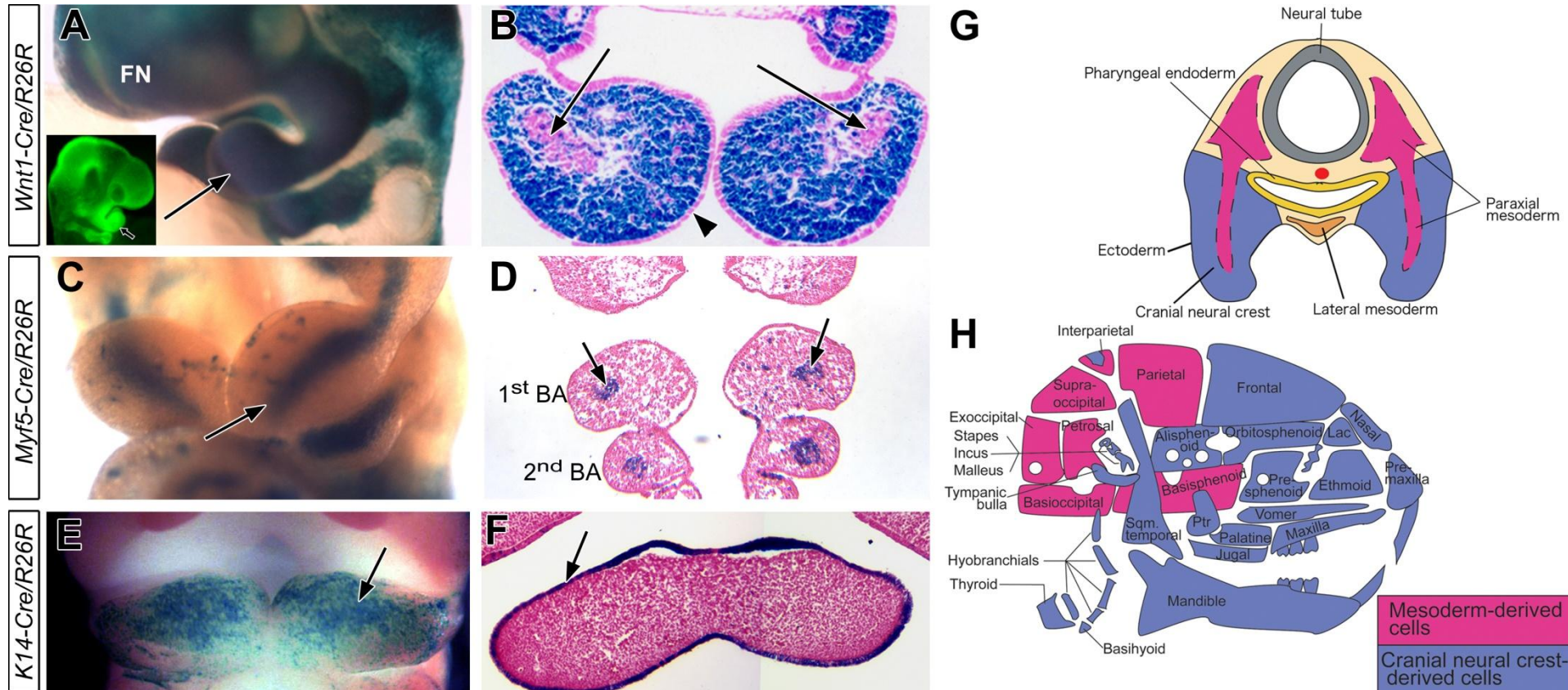




# *Molecular identity of the proximal region of the developing mandible*



# Genetic cell lineage analyses

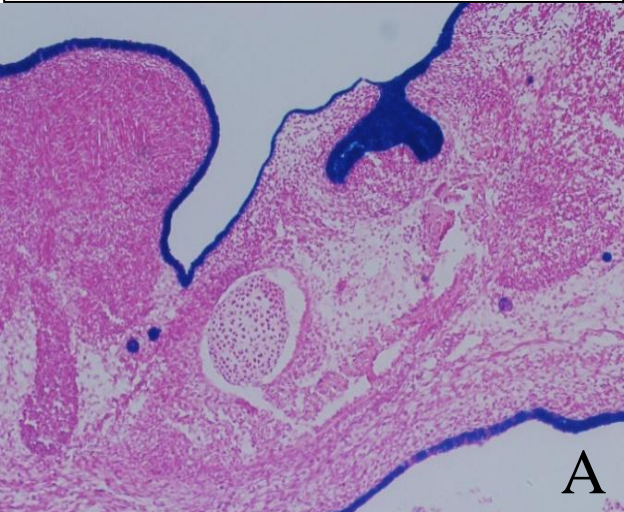


E10.5



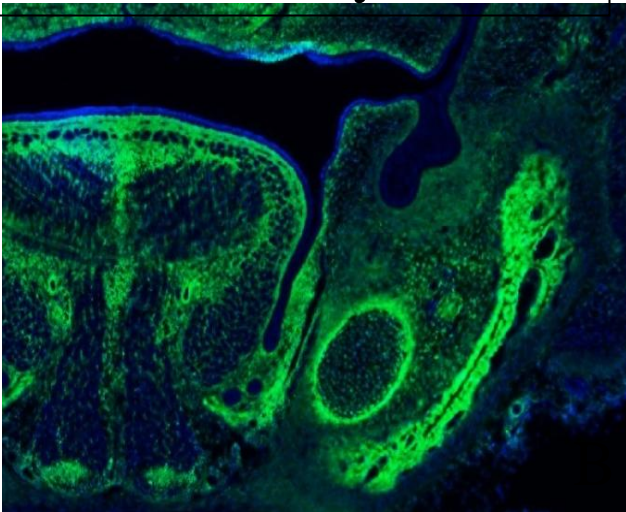
# Cell lineage analysis during mandible development

Epithelium



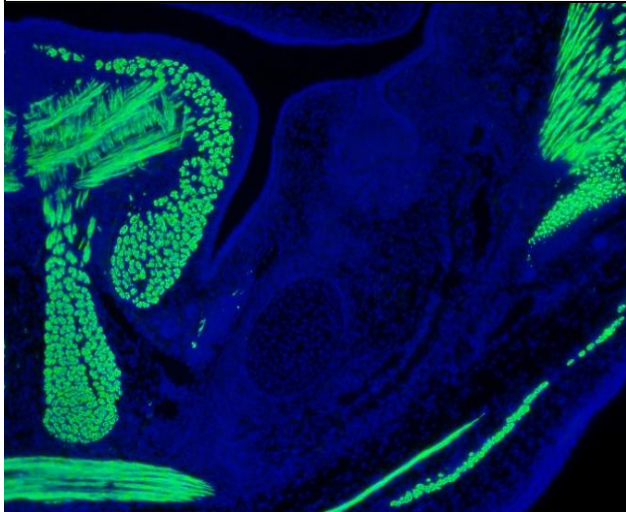
*K14-Cre;R26R*

CNCC-derived mesenchyme



*Wnt1-Cre;ZsGreen*

Myogenic cells



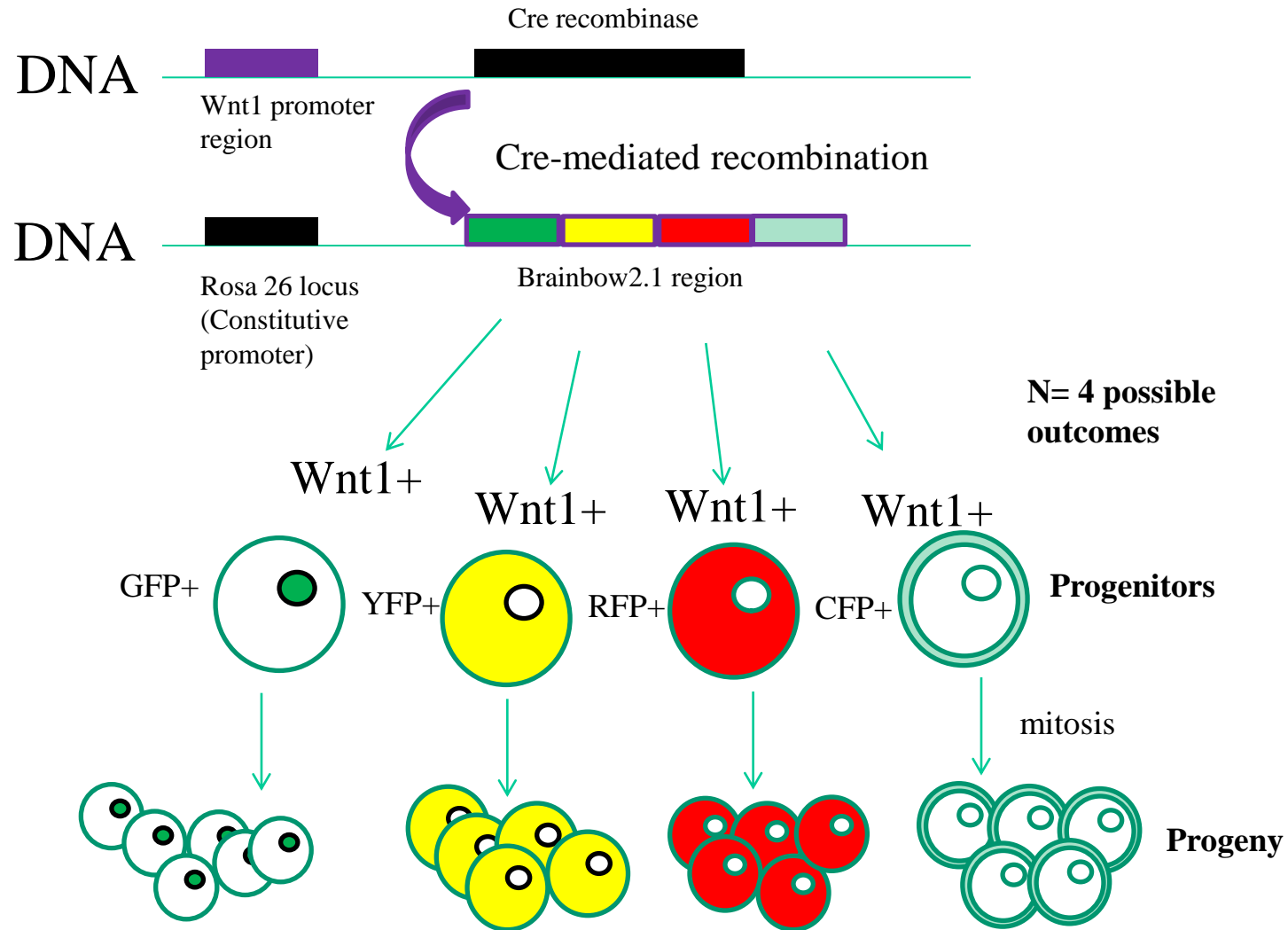
*Myf5-Cre;ZsGreen*

E14.5



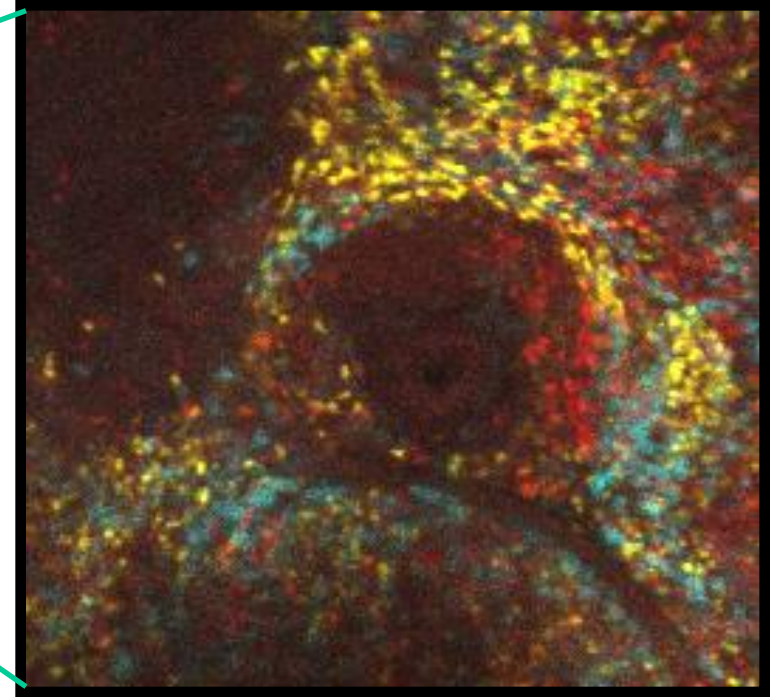
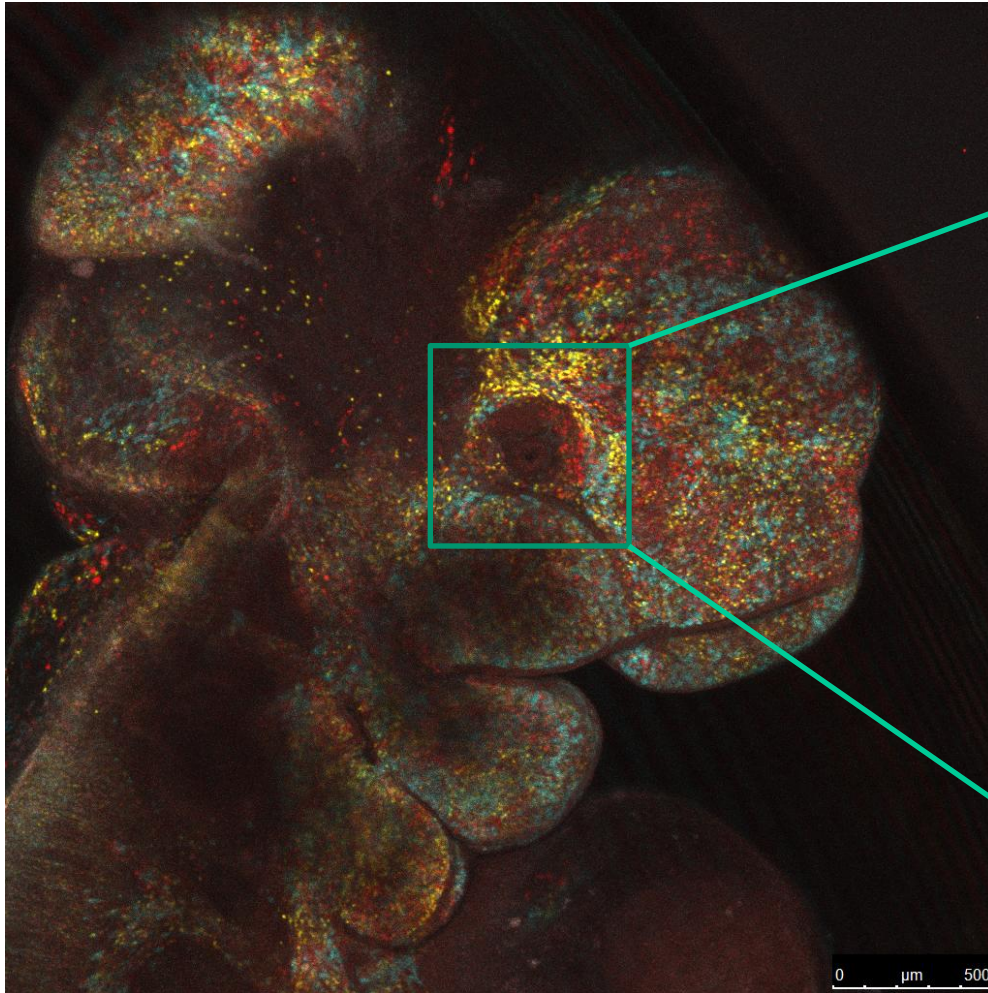
*Cell lineage tracing using Brainbow mice: an opportunity to trace a single progenitor cell and its progeny through tissue-specific multicolor stochastic recombination*

*Wnt1-cre; Brainbow2.1<sup>fl/+</sup>*



E10.5

*Wnt1-cre; Brainbow2.1<sup>fl/+</sup>*



N= 4 in *Brainbow2.1<sup>fl/+</sup>*

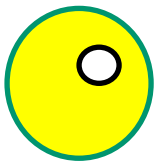
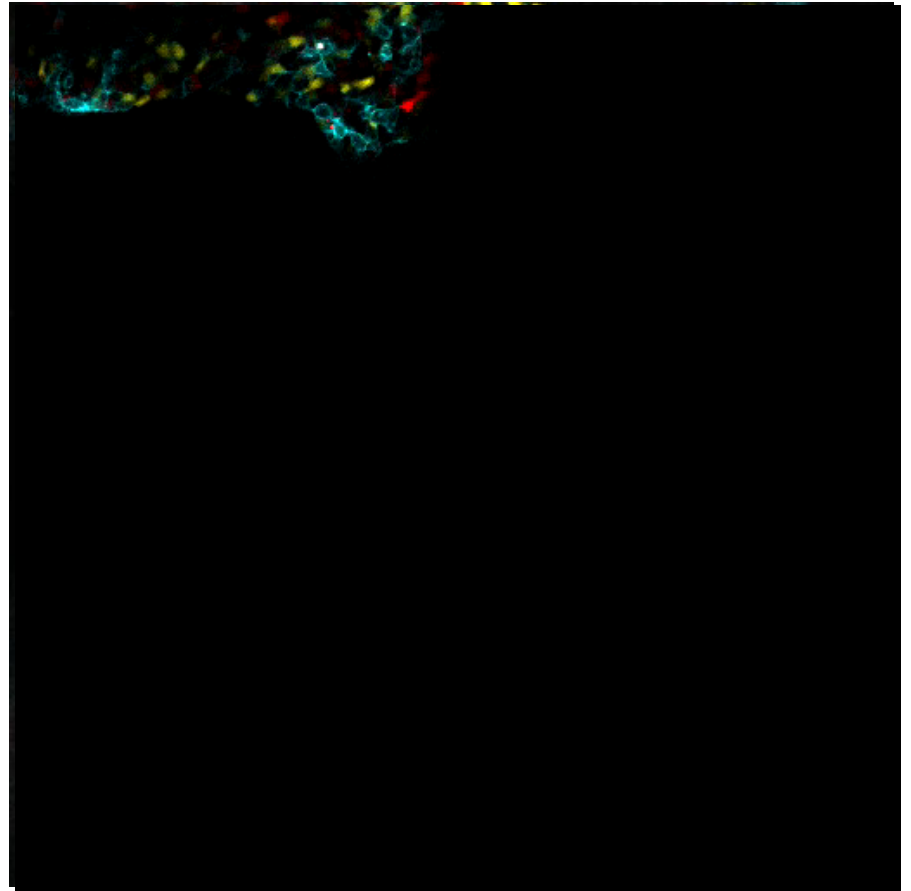
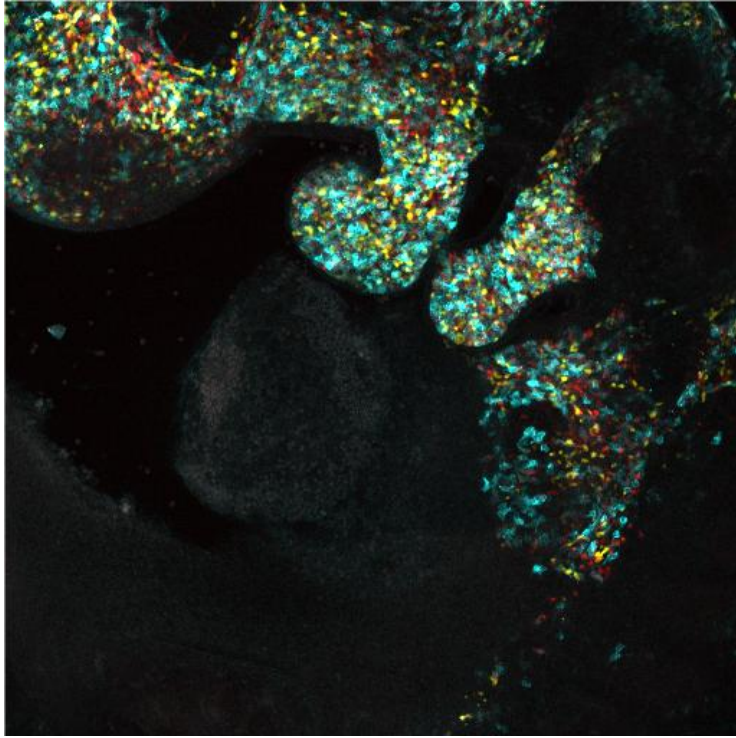
p= probability that 2 adjacent cells of the same color derive from the same progenitor

n=number of possible recombination

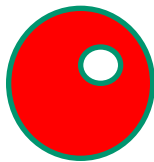
**If n=High then p=High**  
**If n=Low then p= Low**

*Wnt1-cre; Brainbow2.1<sup>fl/fl</sup>*

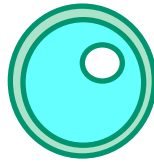
E9.5



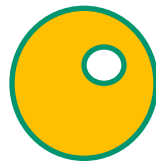
Yellow



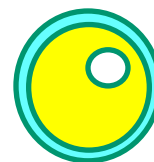
Red



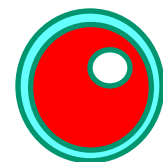
Cyan



Orange



Yellow/Cyan

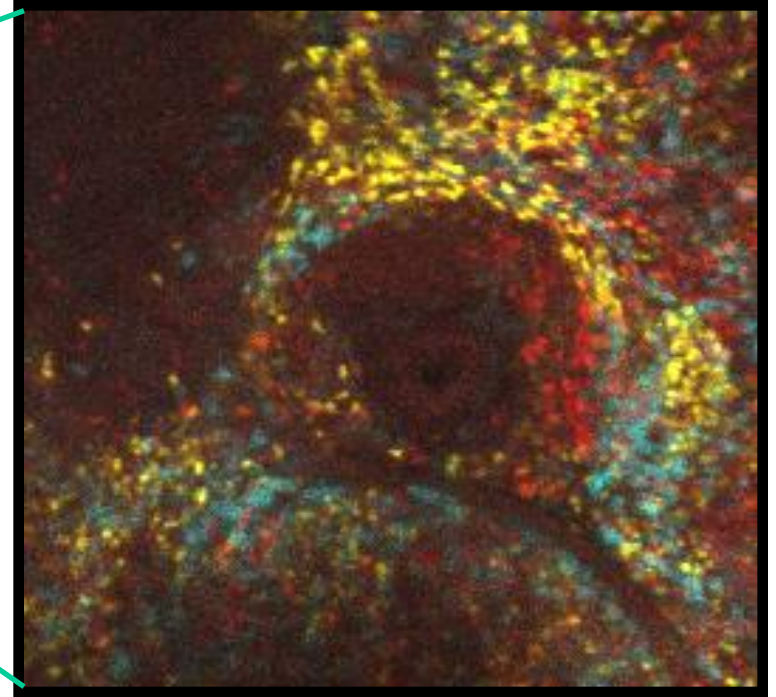
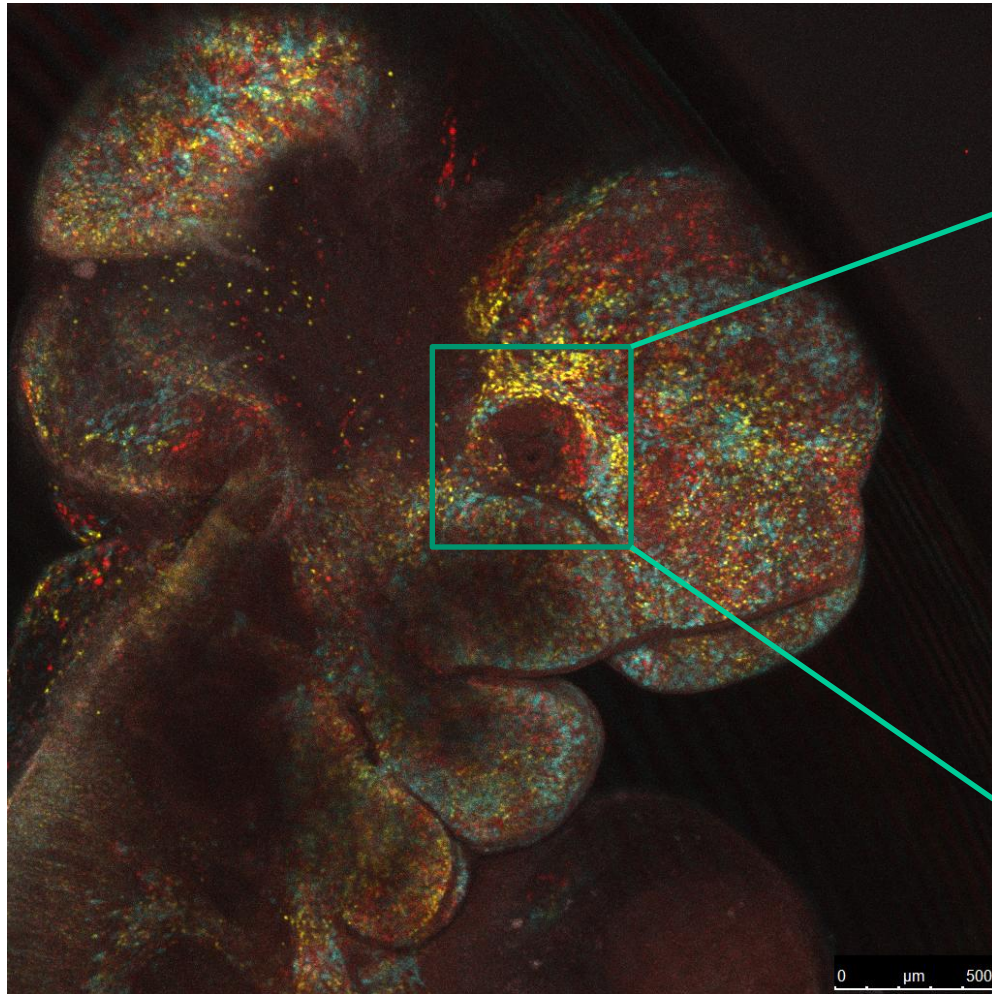


Red/Cyan



E10.5

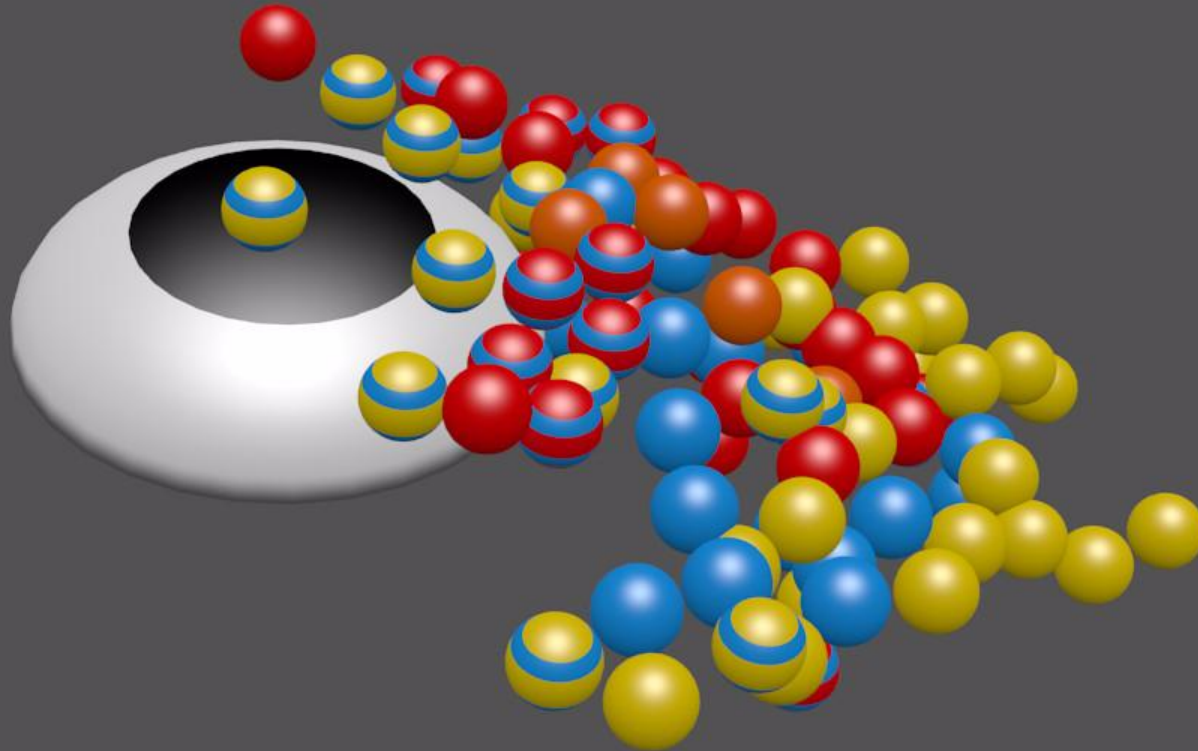
*Superficial structure: the eye*



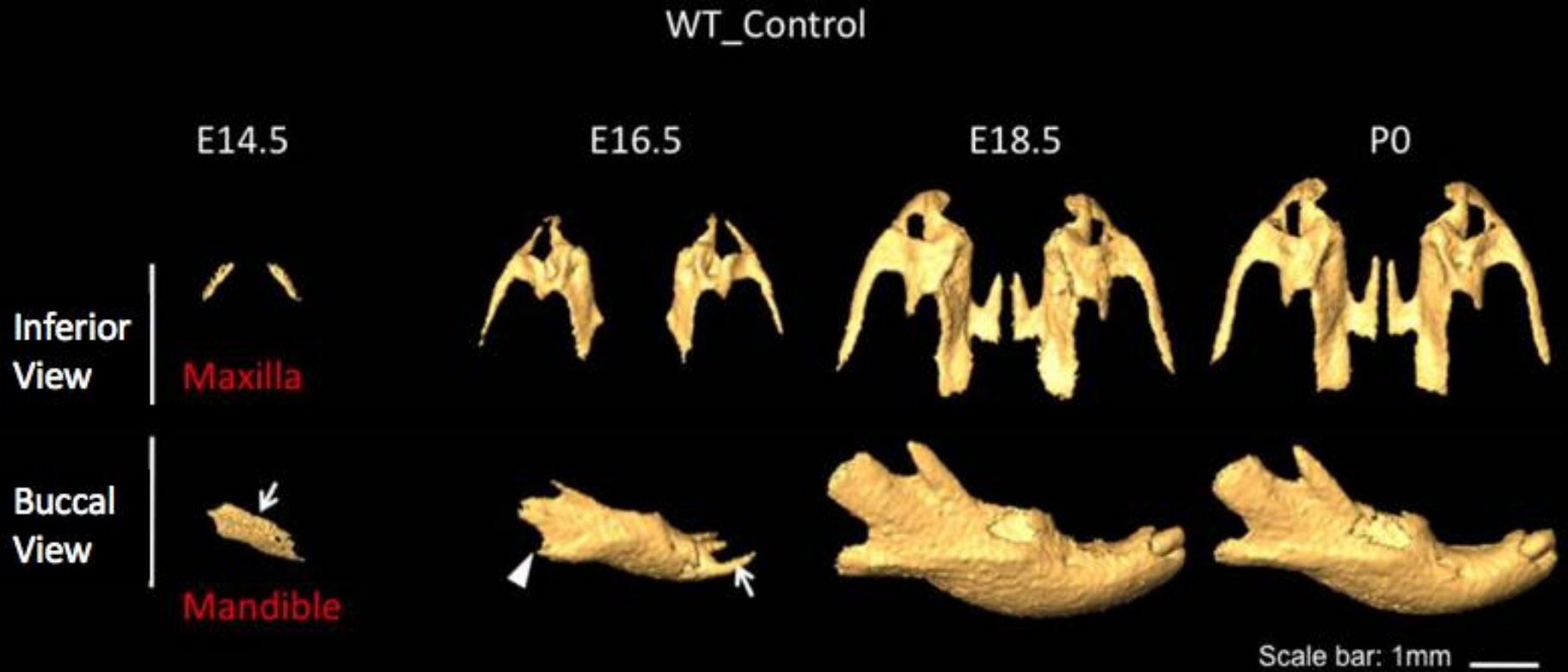
Same progenitor?

E9.5

*Superficial structure: cells above the eye*

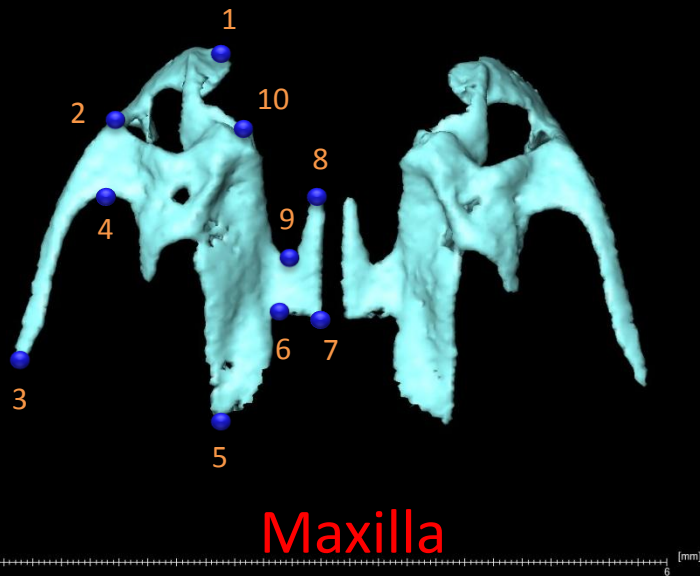


# *Morphogenesis of maxilla and mandible*





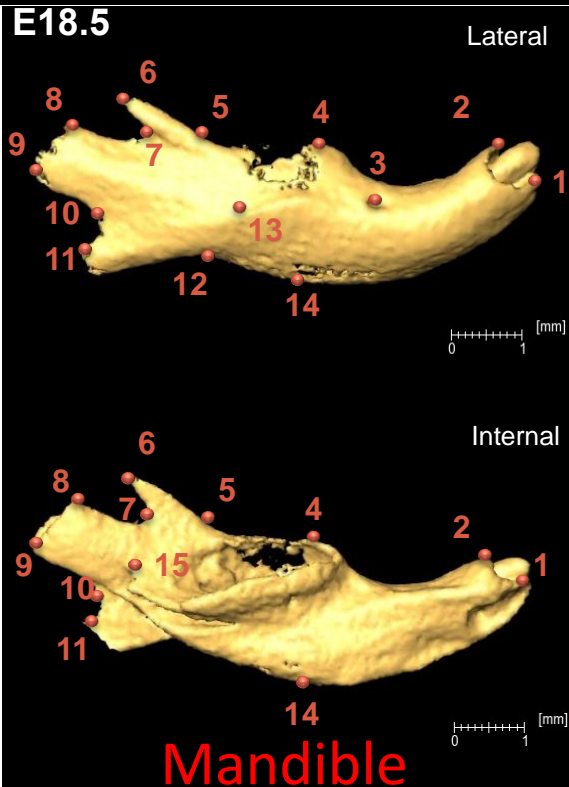
E18.5



Maxilla

1. Anterior point of maxilla
2. Lateral inferior intersection of frontal and zygomatic process
3. Junction point of zygoma with zygomatic process
4. Anterio-medial point to zygomatic process
5. Posterior point of maxilla
6. Posterior-lateral point of the palatine process
7. Posterior-medial point of the palatine process
8. Most Anterior-medial point of palatine process
9. Anterior-lateral point of palatine process
10. Medial point of premaxillary-maxillary suture

E18.5



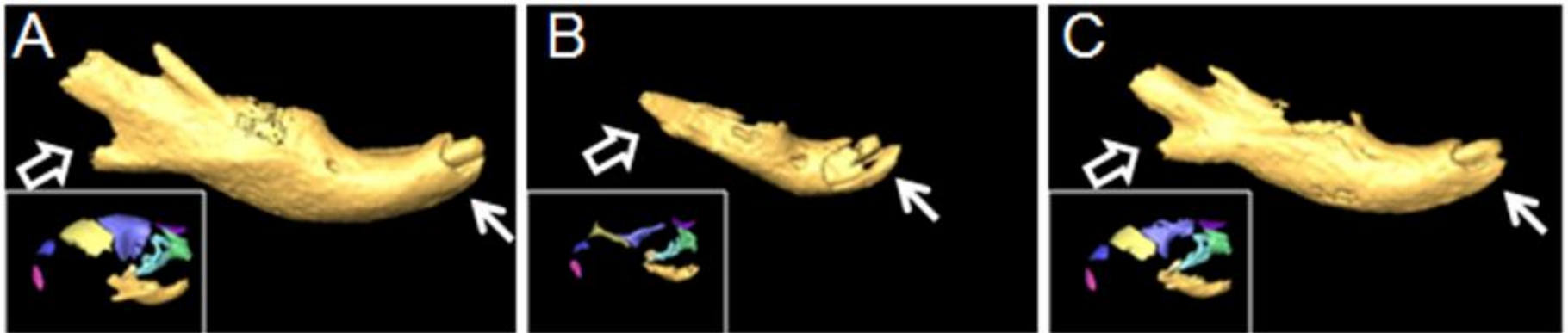
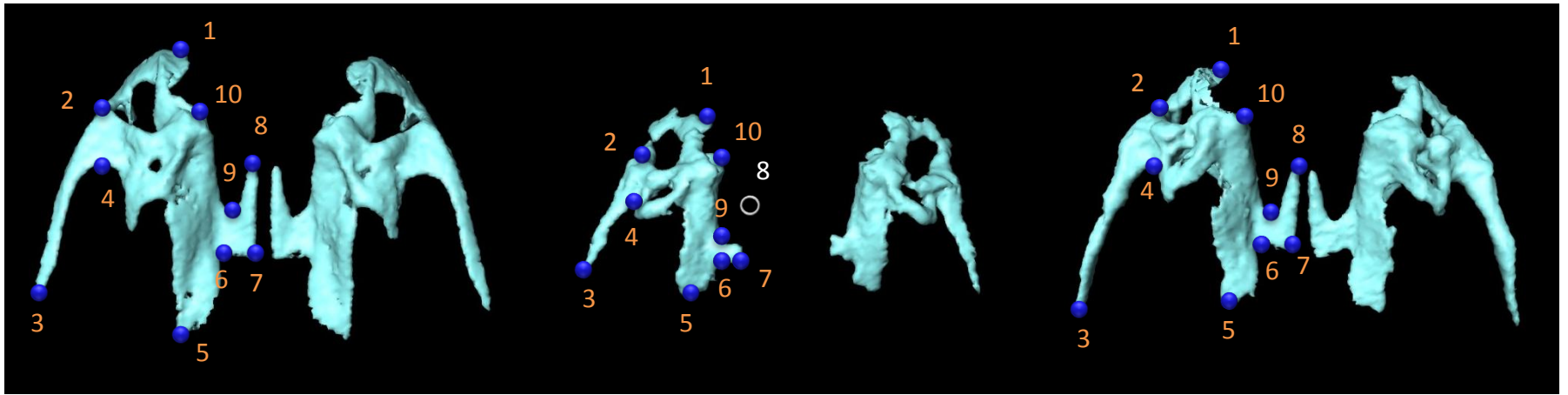
Mandible

1. Most anterior point of mandible
2. Anterior-superior point of mandible
3. Mental foramen
4. Molar alveolus of dentary
5. Anterior junction of mandibular ramus and body
6. Superior tip of coronary process
7. Most inferior point of mandibular notch
8. Anterior point of condylar process
9. Posterior point of condylar process
10. Superior point of angular process
11. Secondary cartilage of angular process
12. Inferior junction of mandibular ramus and body
13. Midpoint of external oblique ridge
14. Inferior point of mandibular body
15. Mandibular foramen

*Control*

*Wnt1Cre;Tgfbr2<sup>fl/fl</sup>*

*Wn1Cre;Tgfbr2<sup>fl/fl</sup>;Tgfbr1<sup>fl/+</sup>*



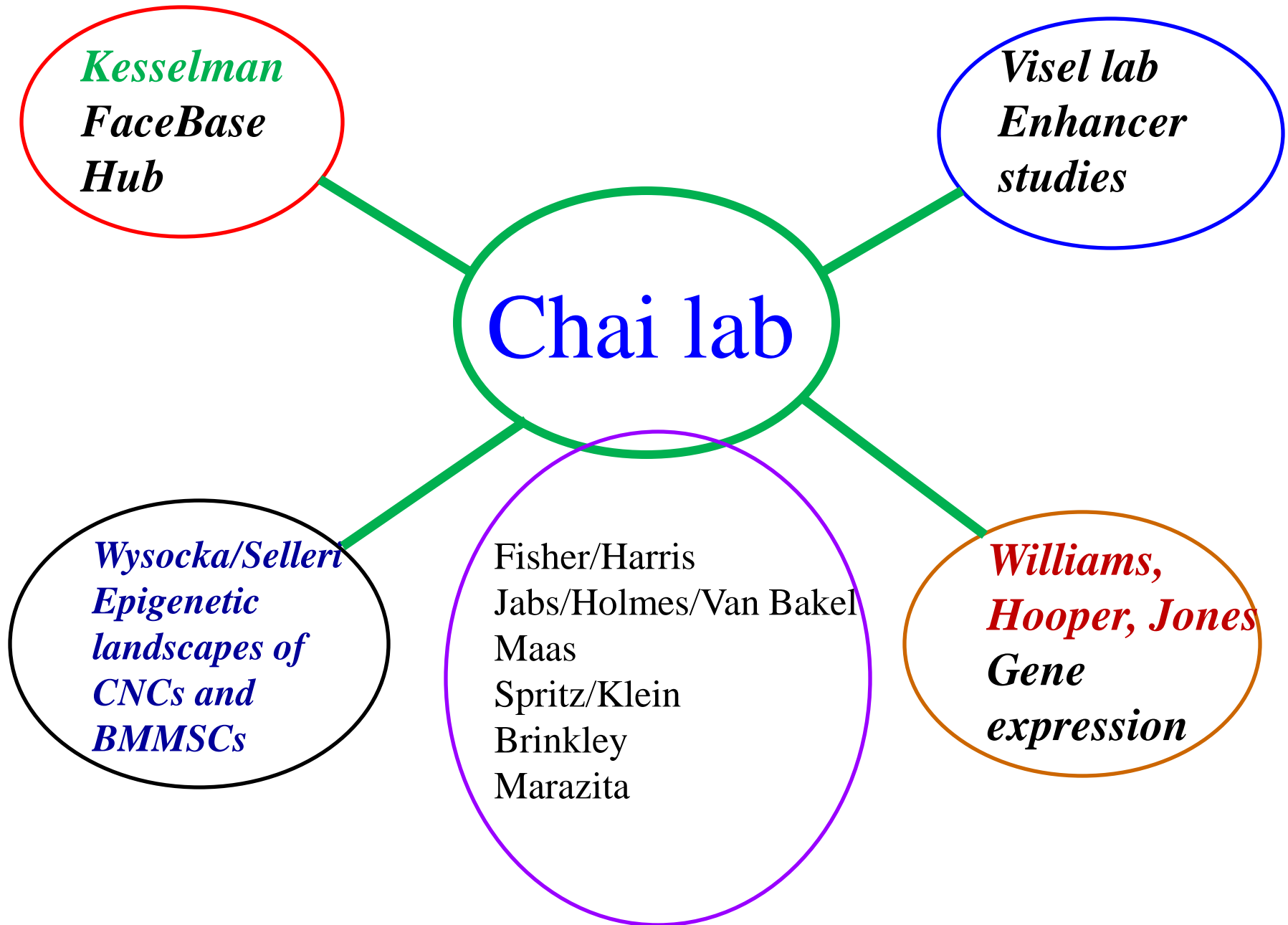
# ***OUR MILESTONES***

## **MILESTONES**

|   | <u><b>Year 1</b></u> | <u><b>Year 2</b></u> | <u><b>Year 3</b></u> | <u><b>Year 4</b></u> | <u><b>Year 5</b></u> |
|---|----------------------|----------------------|----------------------|----------------------|----------------------|
| SA 1-1: Microarrays (mandibles)         | 50 control arrays    |                      | 75 mutant arrays     |                      |                      |
| SA 1-1: In situ analysis (mandible)     | 8 genes              | 7 genes              | 7 genes              | 7 genes              | 2 genes              |
| SA 1-2: Cell lineage tracing (mandible) | 24 controls          |                      |                      |                      |                      |
| SA 1-2: 3D imaging (mandible)           | 12 controls          |                      | 36 mutants           |                      |                      |
| SA 2-1: Microarrays (maxillas)          | 10 control arrays    |                      | 20 mutant arrays     |                      |                      |
| SA 2-1: In situ analysis (maxilla)      | 7 genes              | 7 genes              | 7 genes              |                      |                      |
| SA 2-2: Cell lineage tracing (maxilla)  | same as in SA1-2     |                      |                      |                      |                      |
| SA 2-2: 3D imaging (maxilla)            | 12 controls          |                      | 36 mutants           |                      |                      |
| SA 2-2: Heat maps (mandible + maxilla)  | 4 controls           | 12 mutants           |                      |                      |                      |



# Collaborations and interactions



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