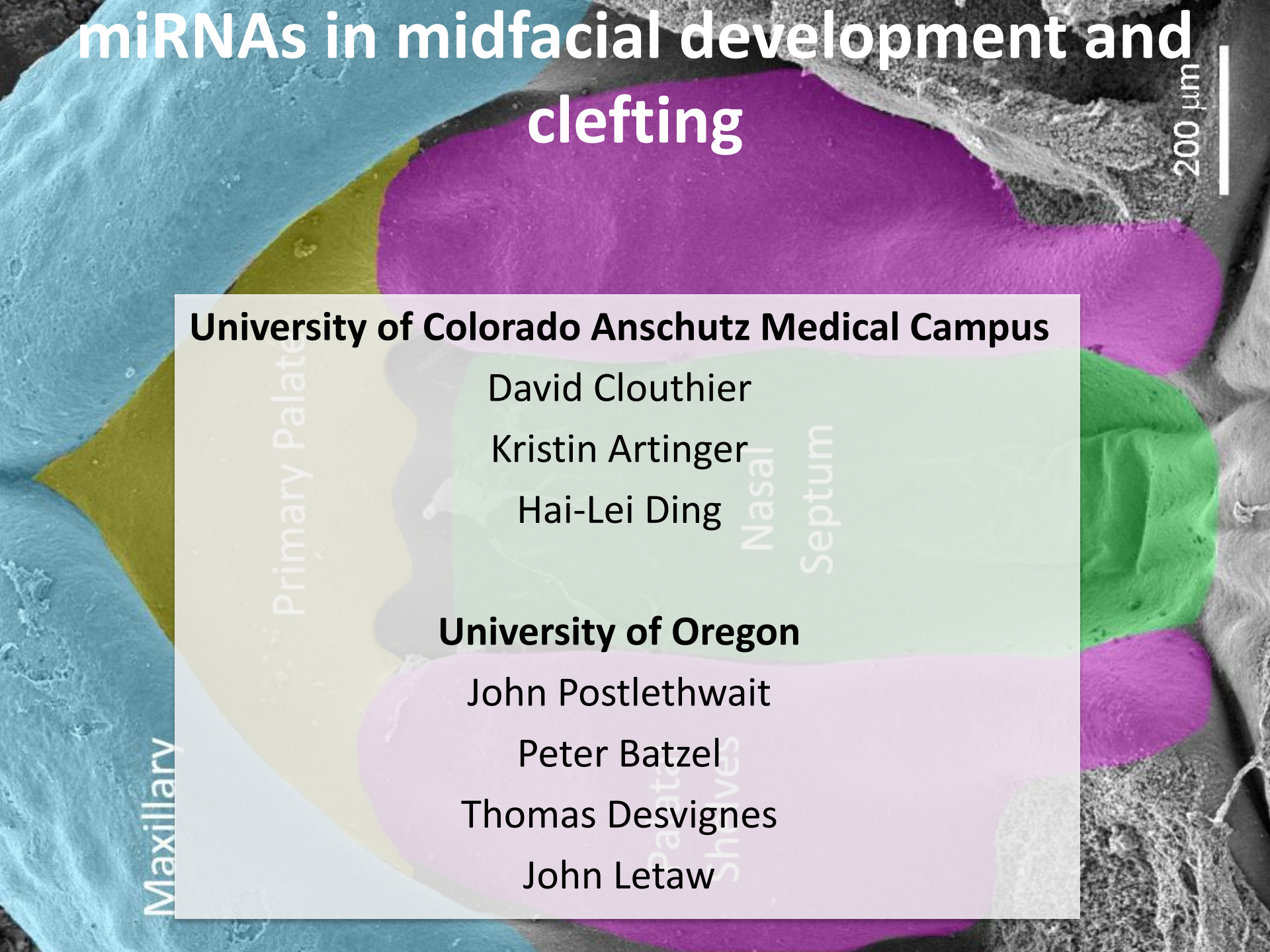
miRNAs in midfacial development and clefting



University of Colorado Anschutz Medical Campus

David Clouthier

Kristin Artinger

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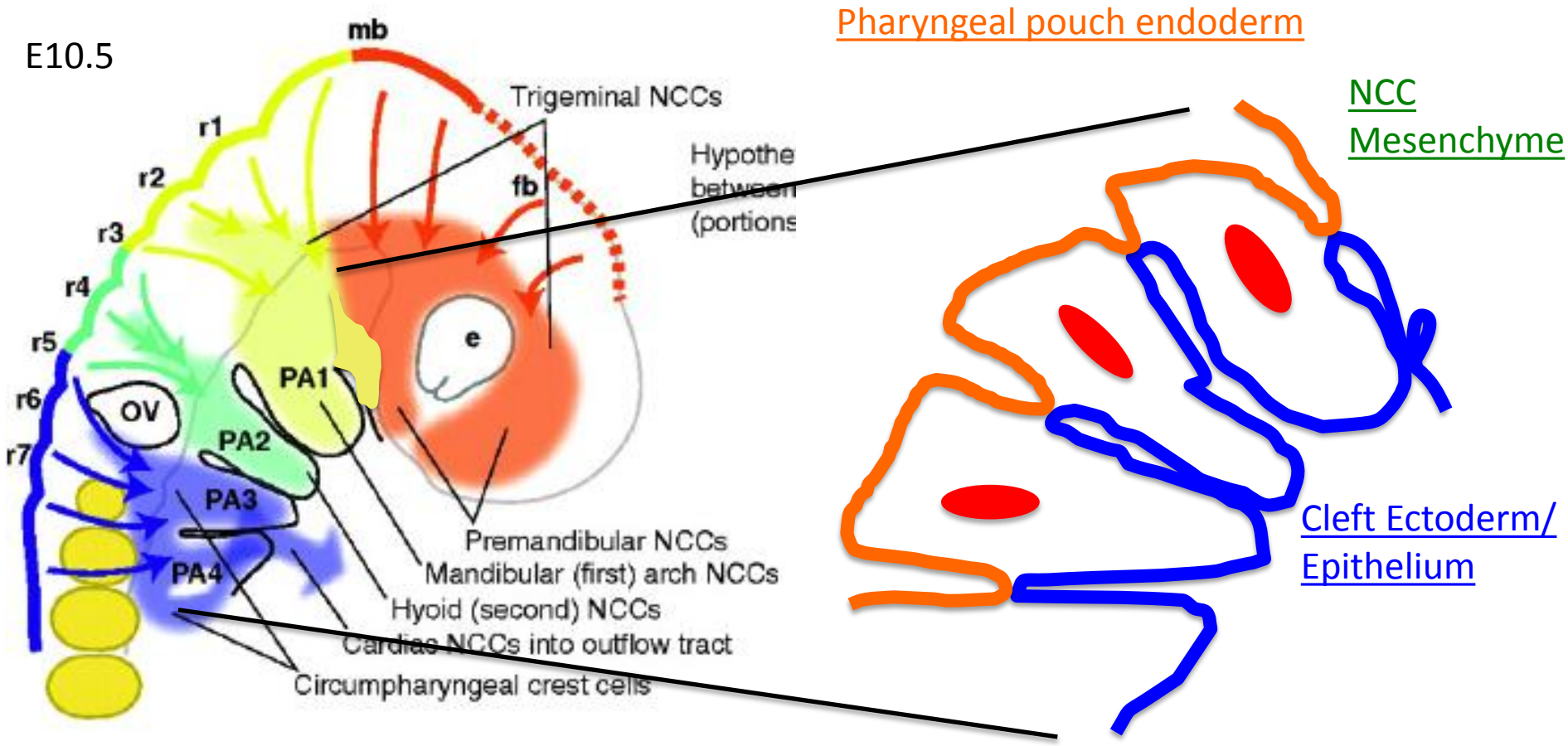
John Letaw

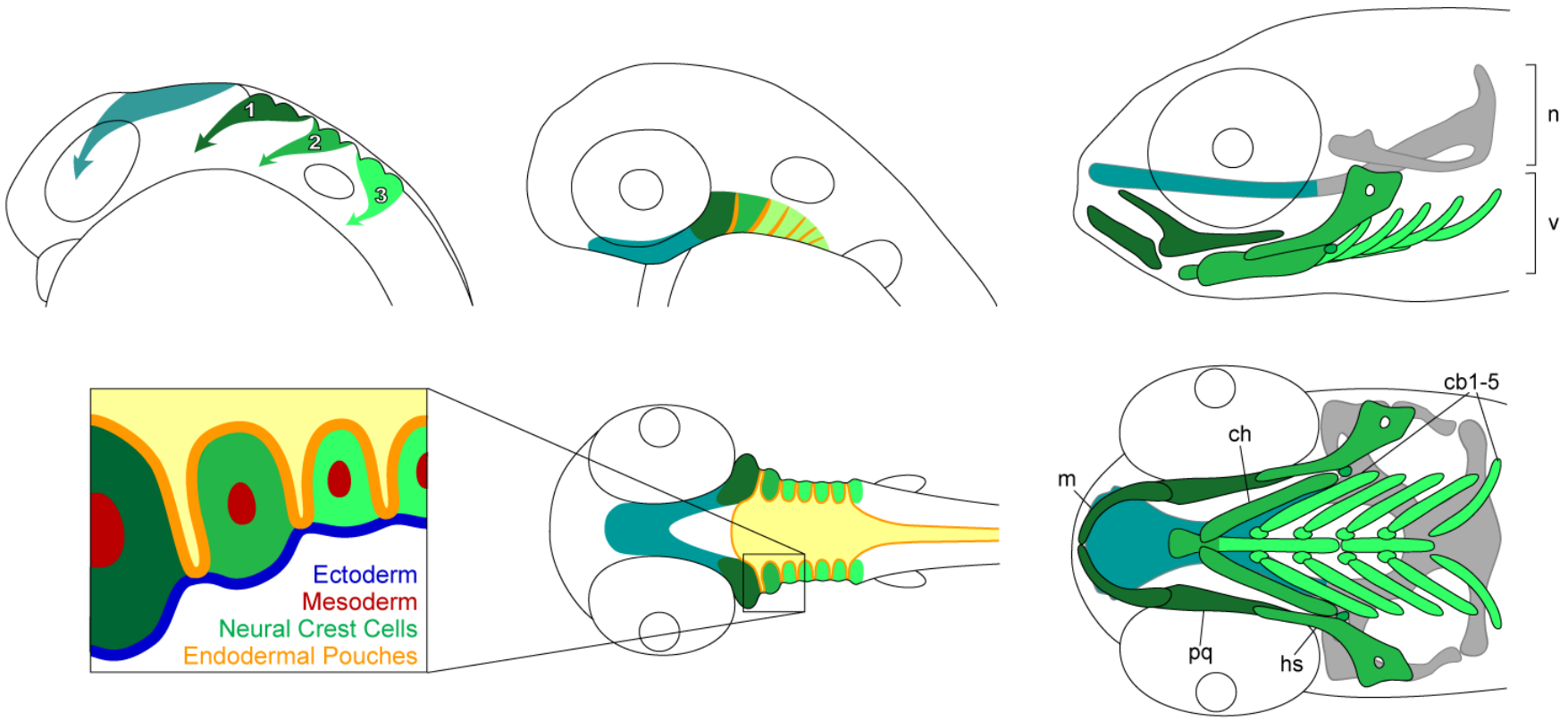
Mouse NCCs migrate into pharyngeal arches and interact with endoderm and ectoderm



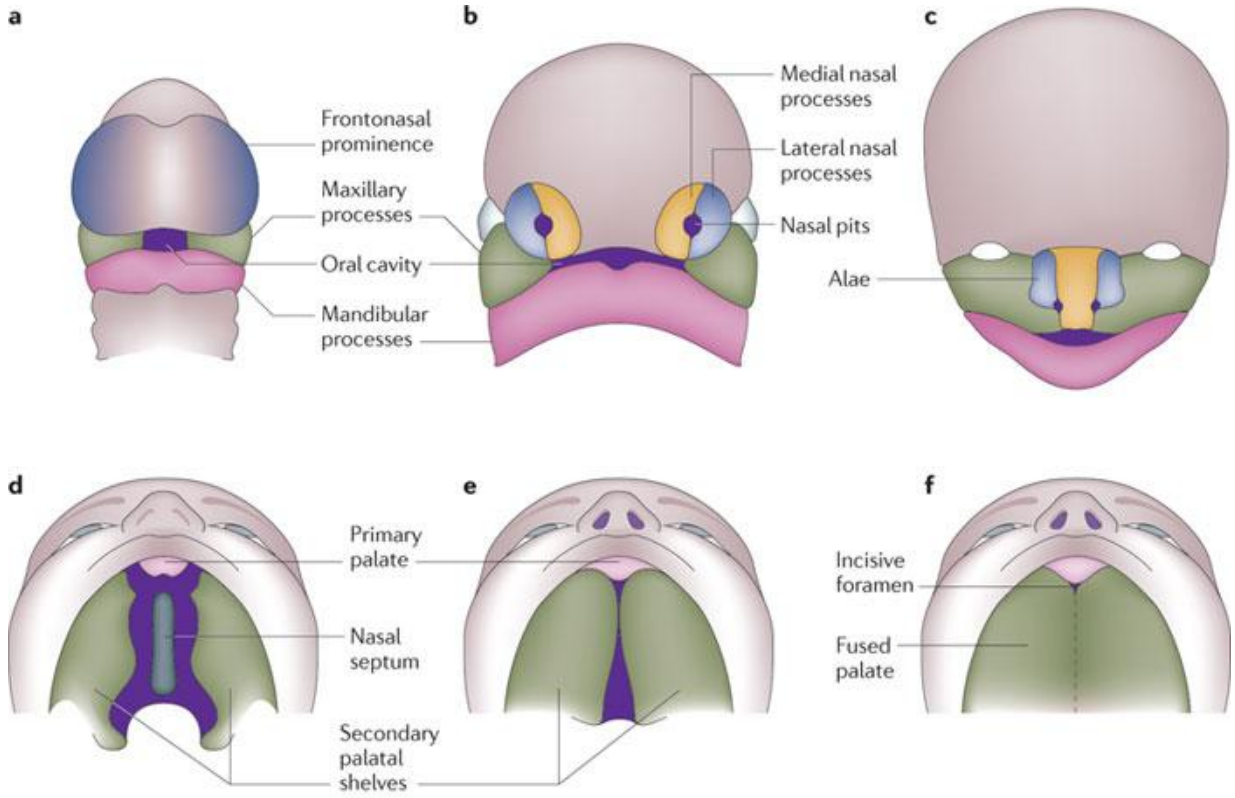
Sambasian et al, 2011 Development 138, 2401

: mesodermal core

Neural Crest Cells (NCCs) Differentiate into Cartilage and Bone of the Face

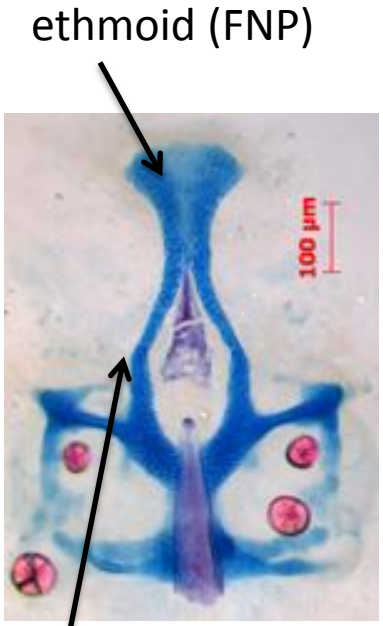


Discrete embryonic prominences form coordinately during vertebrate craniofacial development



Nature Reviews | Genetics

Dixon et al (2011)
Nature Review Genetics



Do miRNA sub-populations control region-specific craniofacial morphogenesis?

Aim 1: Deep sequencing to characterize mature miRNA expression in different tissues during craniofacial development

- Physical dissection in mouse followed by miRNA-seq:
  - FNPs, Maxillae, Palatal shelves
- "Genetic dissection" in zebrafish

Aim 2: Expression analysis in mouse and zebrafish

Aim 3: Test functional significance of tissue-specific differences

- Inject zebrafish embryos to test results of gain- and loss-of-function

Uploaded data to Hub so far

1. All raw data and bioinformatic processing from mouse miRNA-seq:
   E10.5 FNP, MAX
   E11.5 FNP, MAX
   E12.5 FNP, MAX, PAL
   E13.5 FNP, MAX, PAL
   E14.5 FNP, MAX

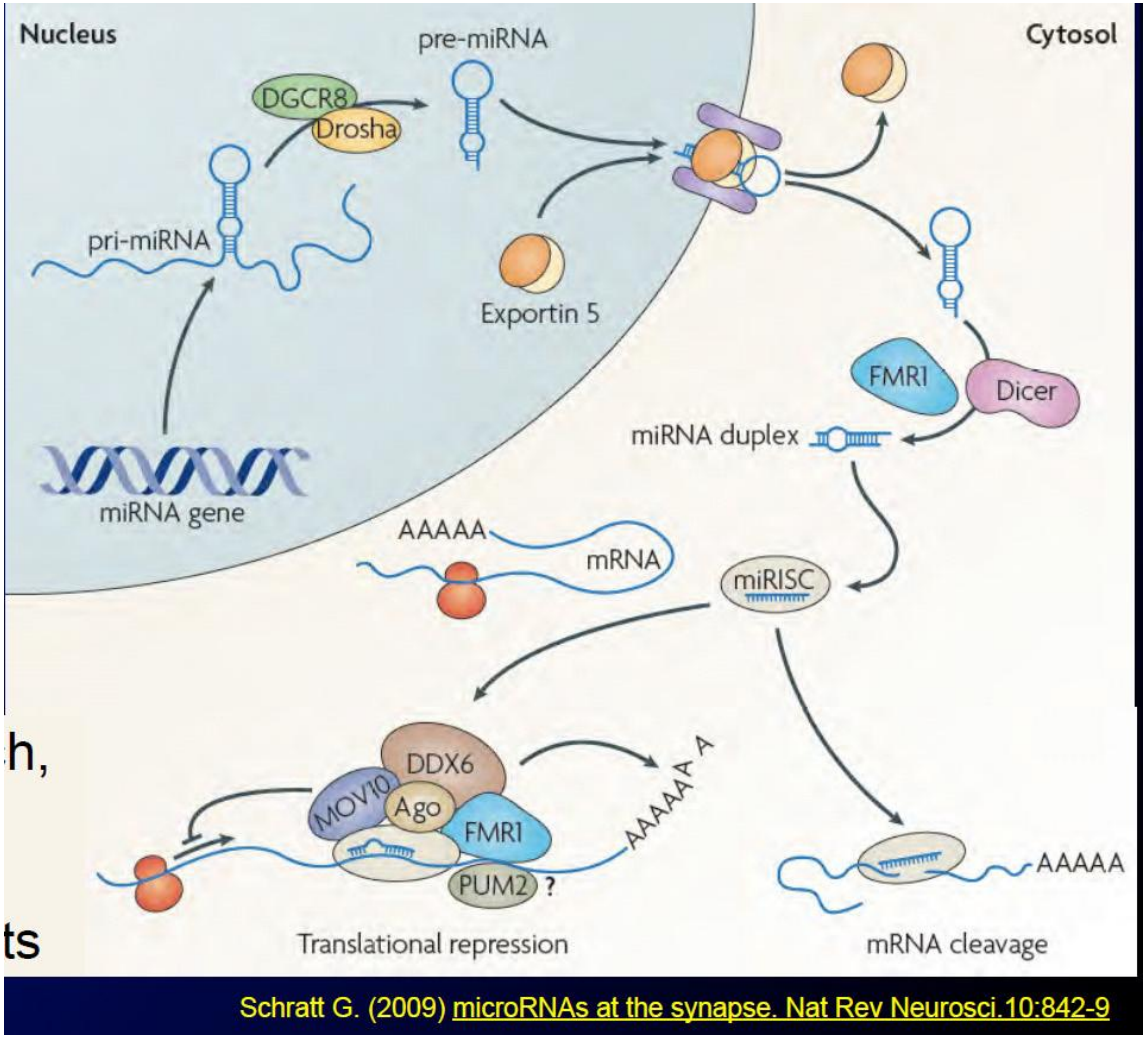
2. Biological replicates and new bioinformatic processing-Uploaded in one month

3. Expression of 20 miRNAs examined in mouse frontal sections- 10 sections per probe throughout the head

4. Expression of ~20 miRNAs examine in zebrafish whole mount in situ hybridization

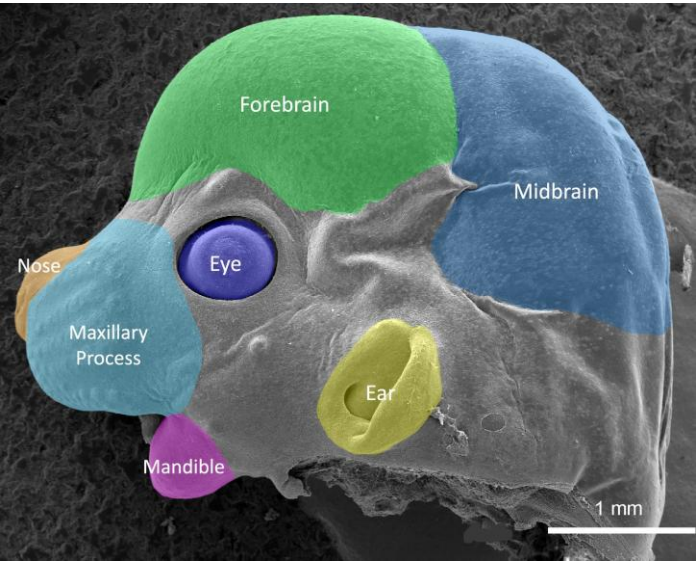
5. ~16 functional analysis in zebrafish by overexpression and knockdown

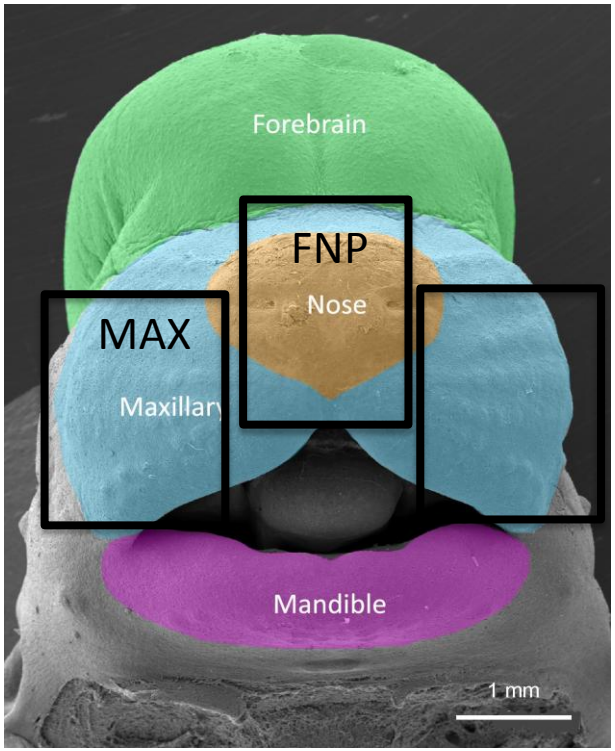
Overview of miRNA biogenesis

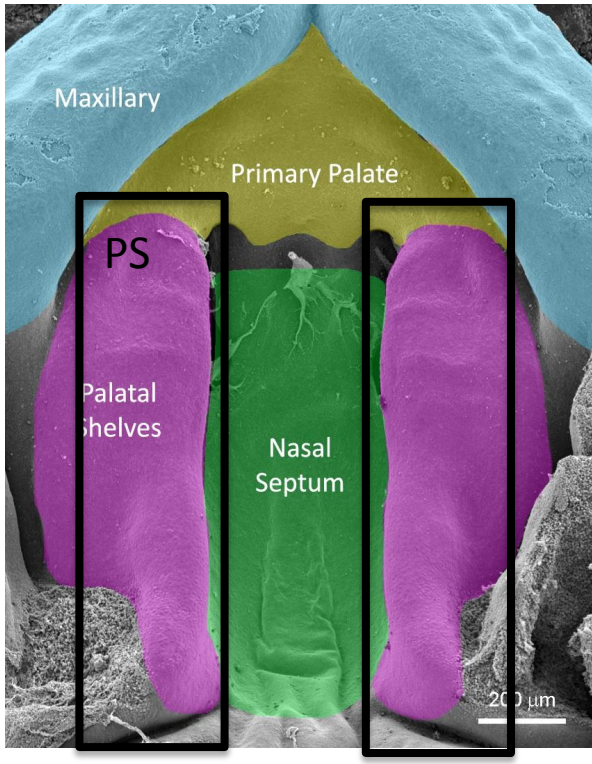


Schratt G. (2009) microRNAs at the synapse. Nat Rev Neurosci.10:842-9

Isolation of mouse midfacial and palatal shelves by microdissection at E10.5-14.5



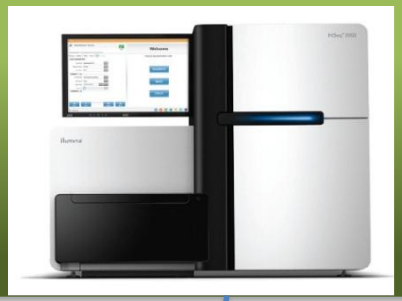




E10.5-14.5

Iwata, Bringas and Chai, FaceBase

Pipeline Flowchart



Illumina FASTQ

Quality Filter

FASTX-Toolkit

Remove Adapter/Barcode

Output FASTA

python

Remove reads < 14bp or > 30bp

Count reads

Remove reads with fewer than 10 counts

NCBI BLAST

BLASTn to reference genome

BLAST results

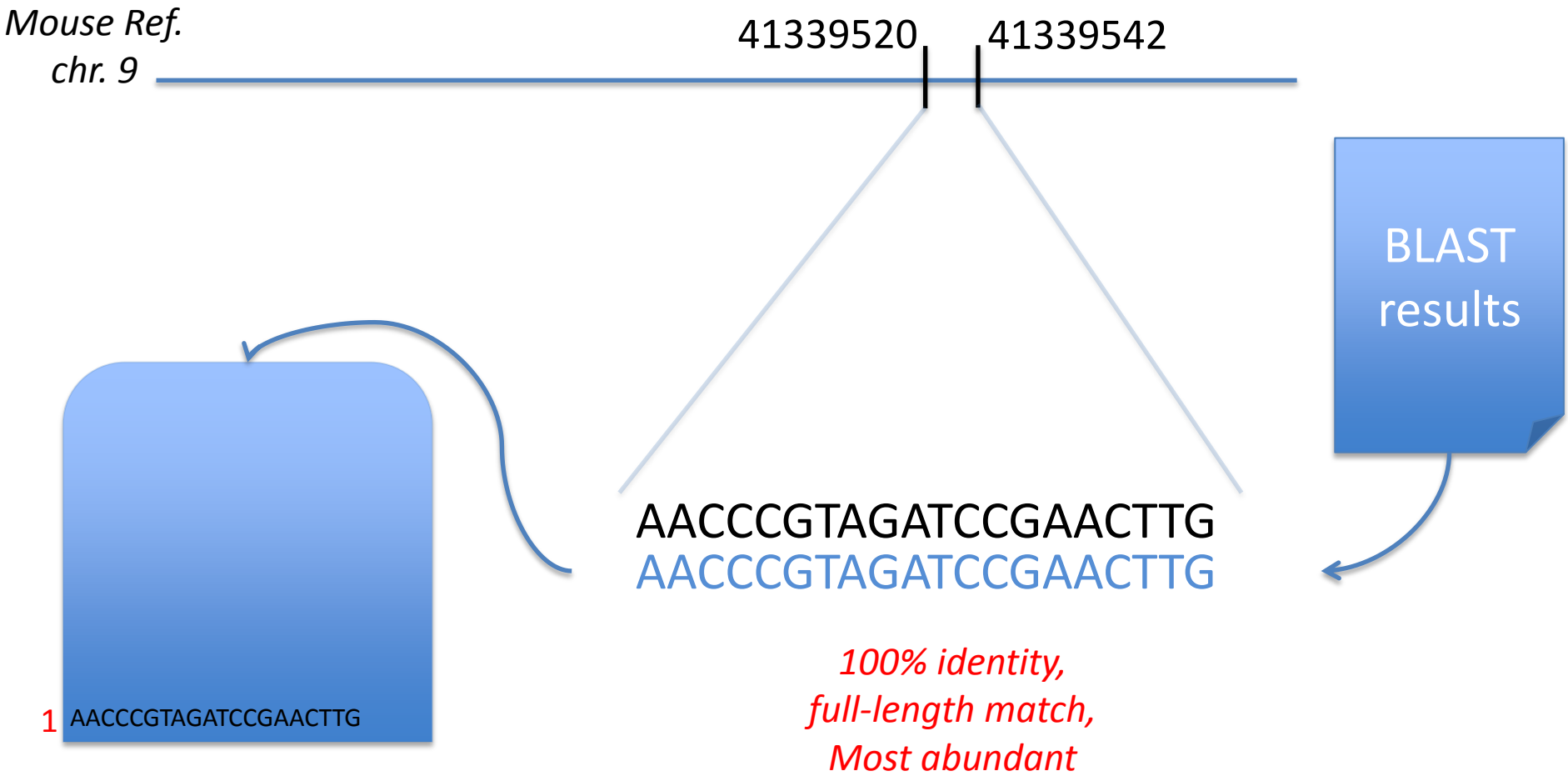
Sequence Binning

Excel/R Output

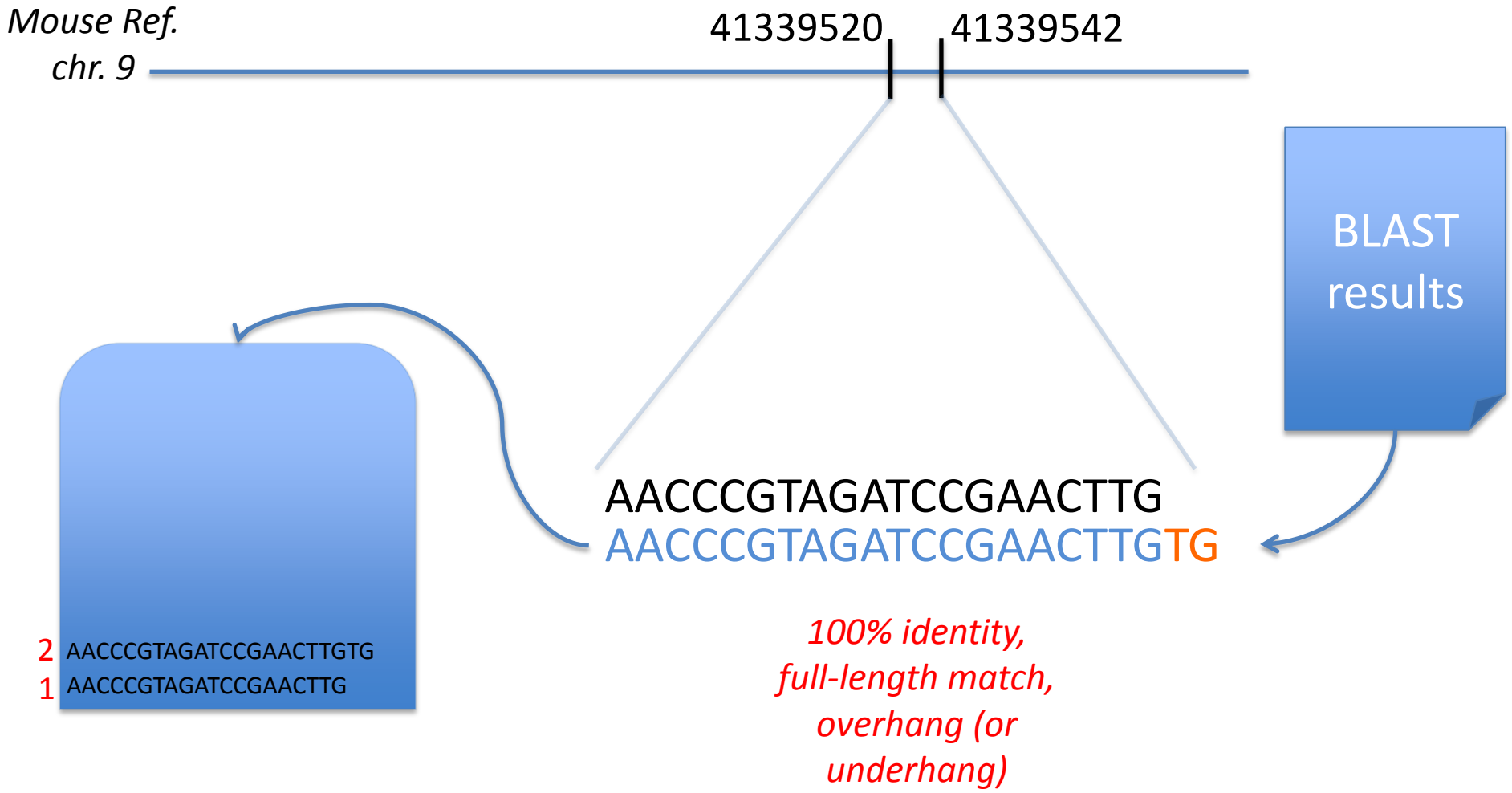
Mirbase Annotations

Species	Count
Human	2042
Mouse	1281
Zebrafish	247

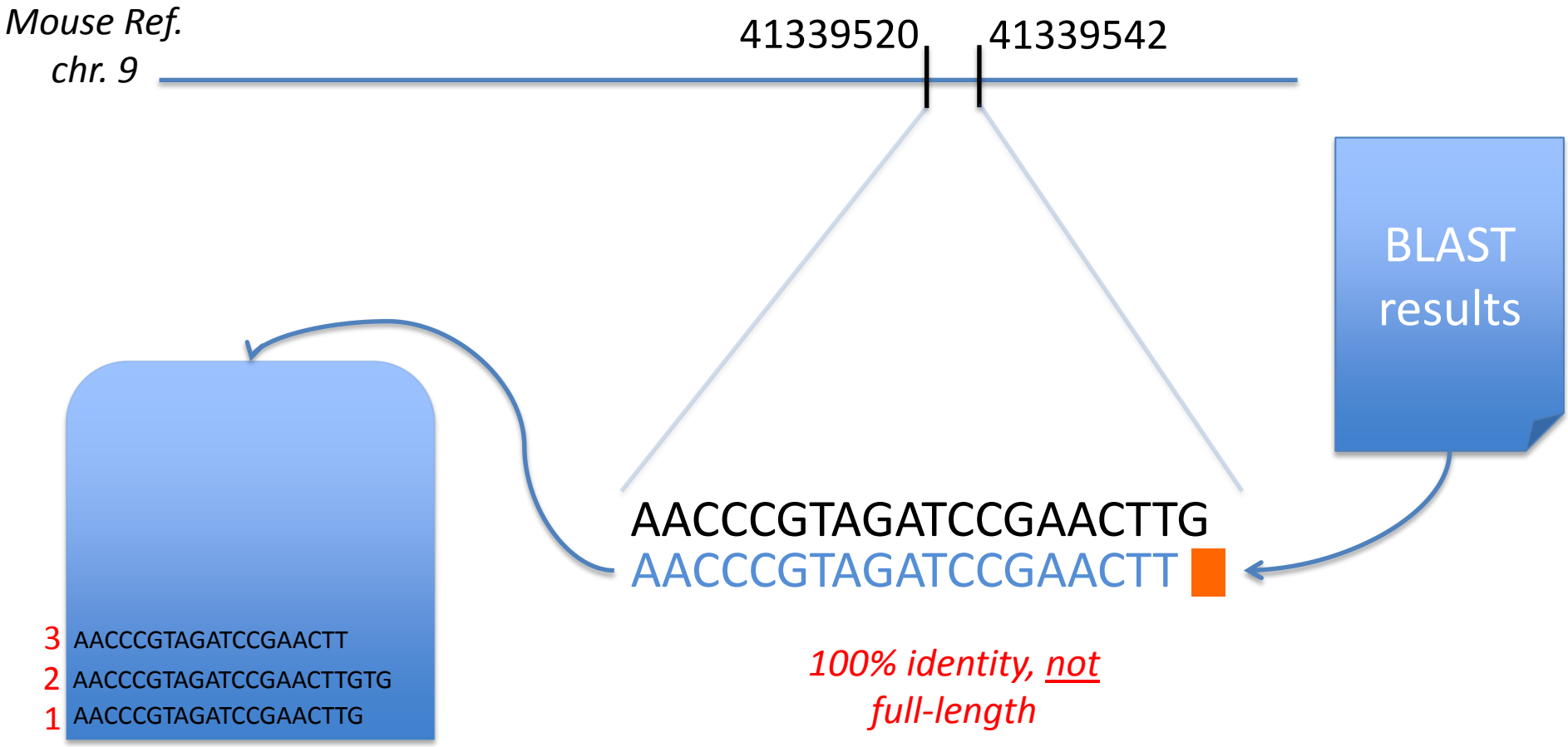
Binning (Full Length Perfect Matches)



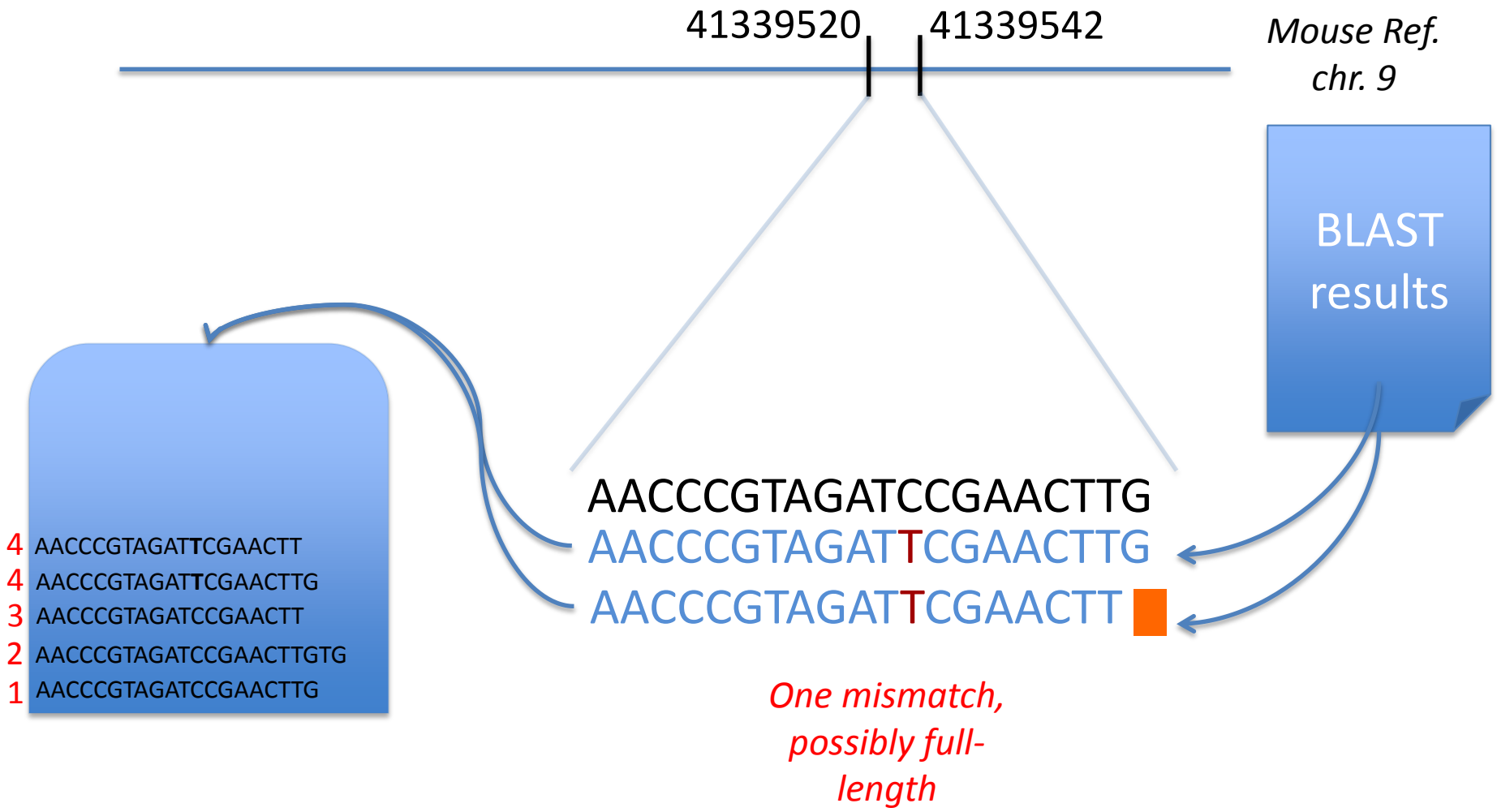
Binning (Full Length Perfect Matches)



Binning (Not Full-Length)



Binning (One mismatch)



Pileup and Associated Read Depth

Annotation	Genomic Location	MAX1 10.5	MAX2 10.5	MAX3 12.5	MAX4 12.5	Starting Bin Seq.
mir-100	[9:41339520-41339541]	12852	11241	267688	254306	AACCCGTAGATCCGAACTTG

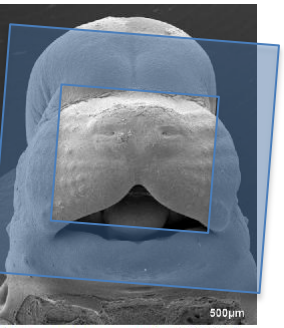
P-value	P-adjusted
2.44E-14	1.23E-11

	10.5FNP10F	10.5FNP2012	10.5MAX10M	10.5MAX2012	12.5FNP2011	12.5MAX2011	12.5MAX2012
Raw Record Count	3,032,971	8,271,576	4,653,960	6,039,877	16,409,966	15,042,410	12,256,346
Unique Sequences	4,263	9,715	5,870	7,541	9,001	8,713	7,788
Compressed Bins	5,750						

Expression profiles of miRNAs in mice

miRNA	Embryonic Age	Expression Profile
miR-10b	E13.5	spinal cord, DRG, intestine
miR-20a	E13.5	eye, lung, brain
miR-23b	E12.5, E13.5	naris, vibrissae, TG, DRG, palatal shelf, maxilla, tongue, nasal and tongue epithelium, tongue muscle, incisor, otic capsule, malleus, diencephalon, limb muscle, mesothelium, intervertebral space, foregut, gastrointestinal neurons
miR-24-1	E12.5, E13.5	vibrissae, TG, DRG, nasal epithelium, maxilla, tongue, incisor, limb muscle, gastrointestinal neurons, mesothelium, intervertebral space, pinna
miR-34b	E13.5	brain
miR-122a	E13.5	liver
miR-124-3	E13.5	brain, FNP, TG, pancreas, liver, craniofacial mesenchyme, intestinal neurons
miR-128-1	E13.5	no expression
miR-128-2	E12.5, E13.5	maxilla, TG, DRG, tongue, diencephalon, telencephalon, ocular muscles, limb, mesothelium, ribs
miR-133b	E12.5, E13.5	vibrissae, maxilla, TG, tongue, intervertebral space, hyoid, ocular muscles, diencephalon, muscle, mesothelium, ribs
miR-153	E13.5	brain
miR-195	E13.5	no expression
miR-666	E12.5, E13.5	FNP, DRG, ocular muscle, tongue, mandible, vertebrae, mesothelium, ribs, liver, spinal cord, limb
miR-15a	E12.5	tongue, oral and nasal epithelium, nasal mesenchyme, limb, eye, brain, liver, intestine
miR-27b	E12.5	tongue, limb, rib, pena, TG, nasal epithelium, eye, vibrissae, brain, facial cartilage, intestinal neuron
miR-130a	E12.5	tongue, rib, limb, intestine, facial cartilage, nasal epithelium, maxilla
miR-130b	E12.5	tongue, intestine, facial cartilage, nasal epithelium, maxilla
miR-206	E12.5	tongue, rib, limb, eye, maxilla, nasal epithelium
miR-335	E12.5	tongue, brain

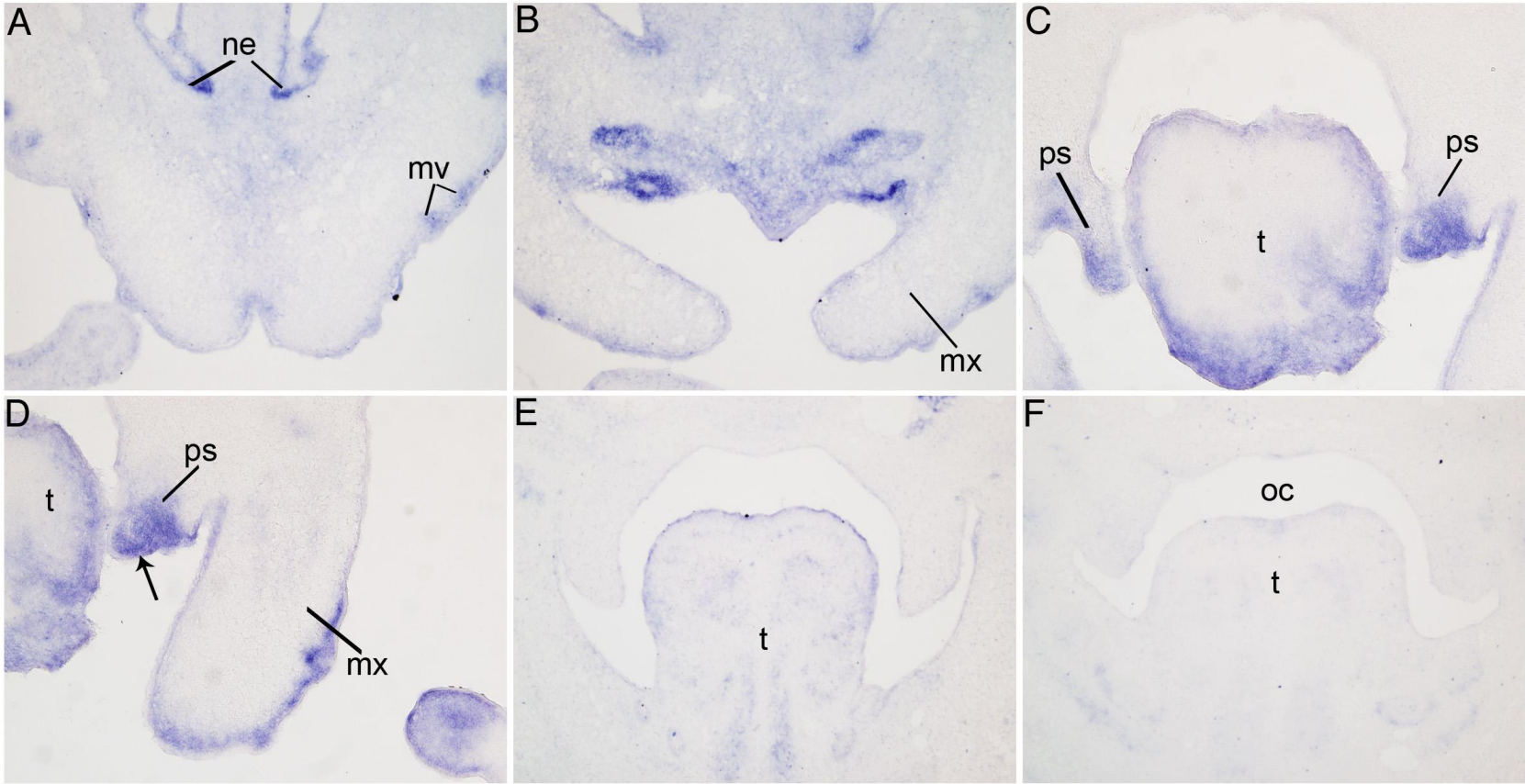
miR-23b is expressed in mouse facial structures at E12.5



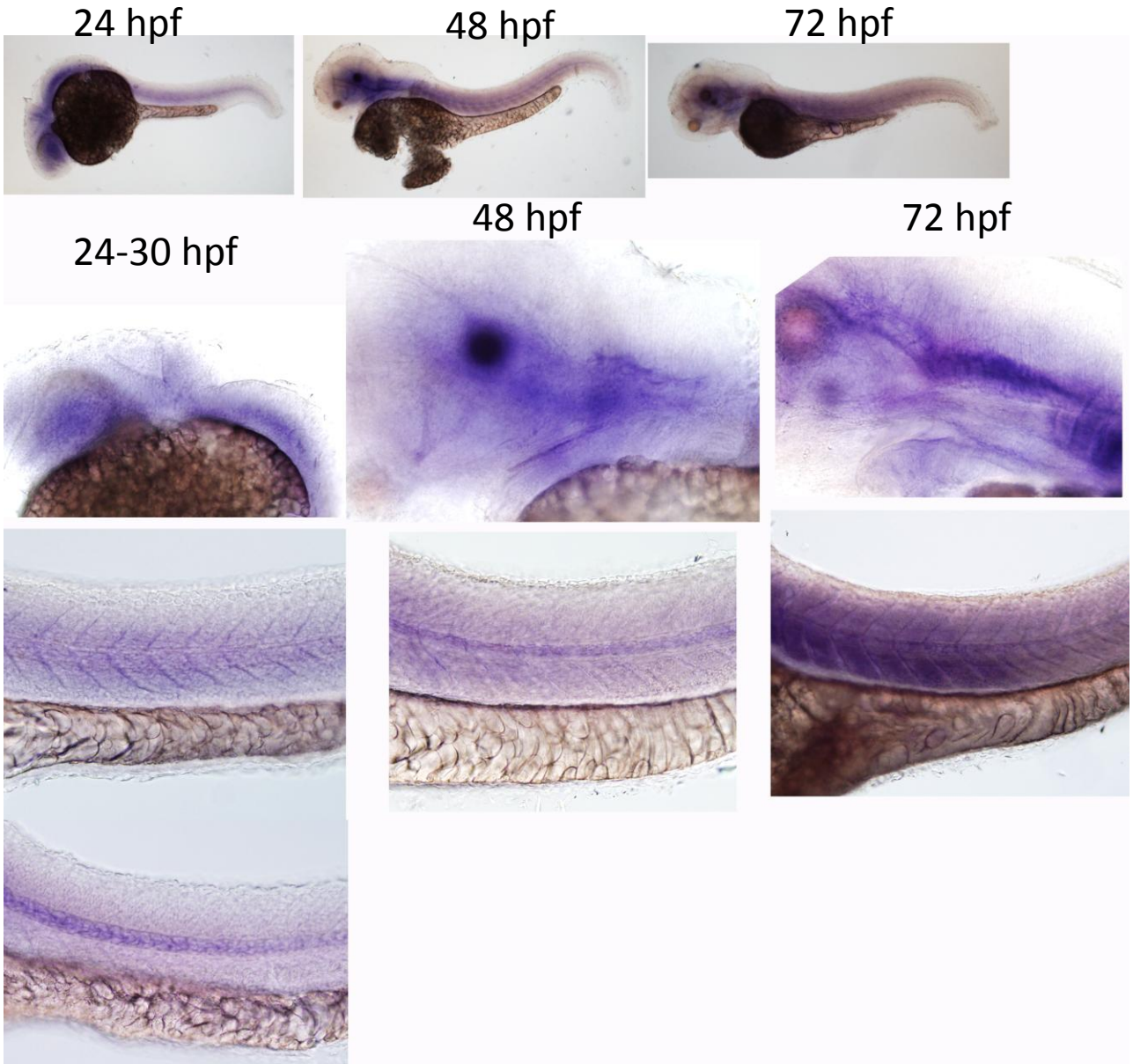
Fetal mouse (mus musculus) at E13.5, SEM image. Iwata J, Bringas P, Chai Y. USC

Maxilla enriched:

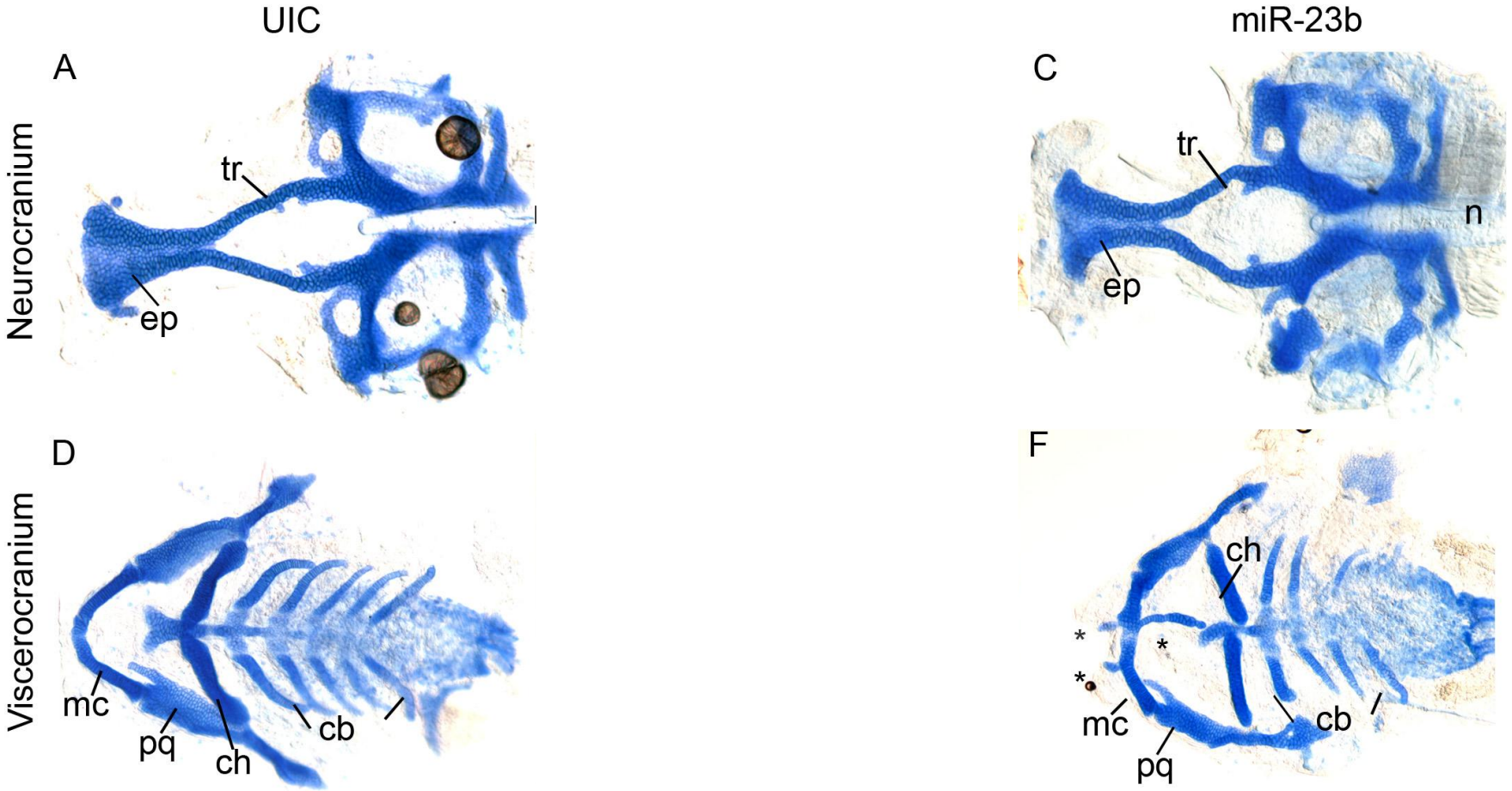
miR-23b



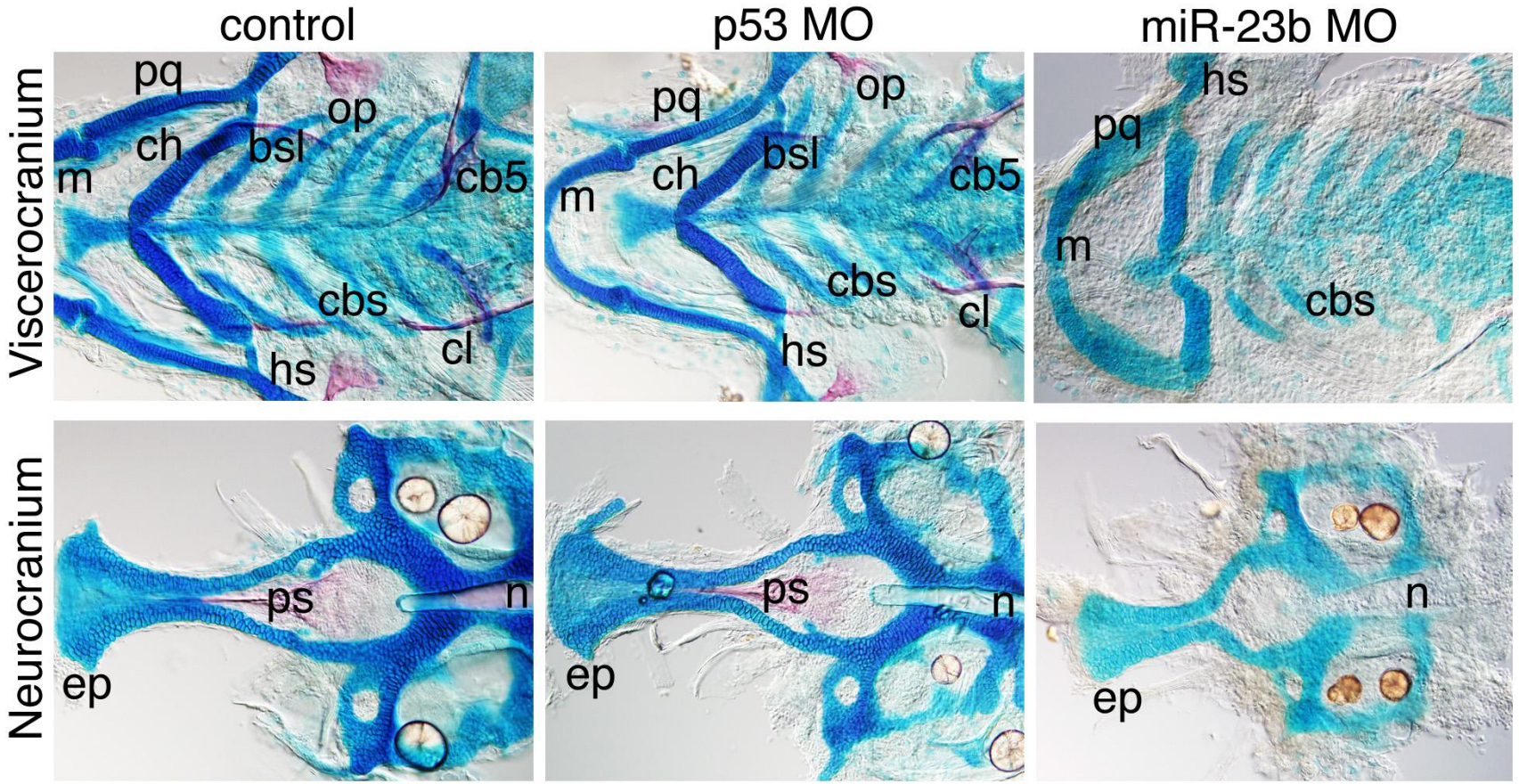
miR-23b is expressed in zebrafish facial structures as well as notochord and trunk muscle



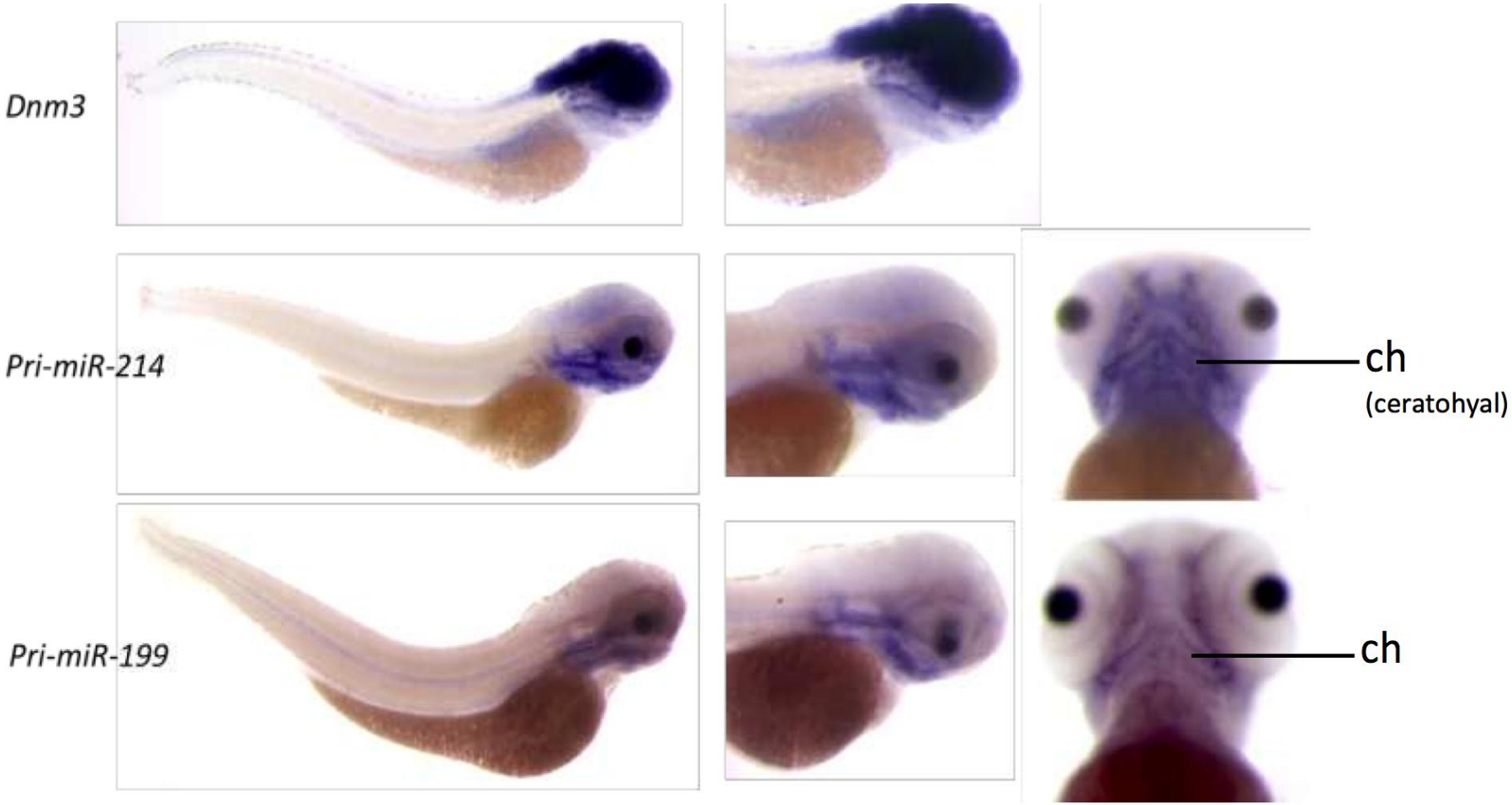
miR-23b overexpression results in a cleft or size reduction in the ethmoid plate in zebrafish



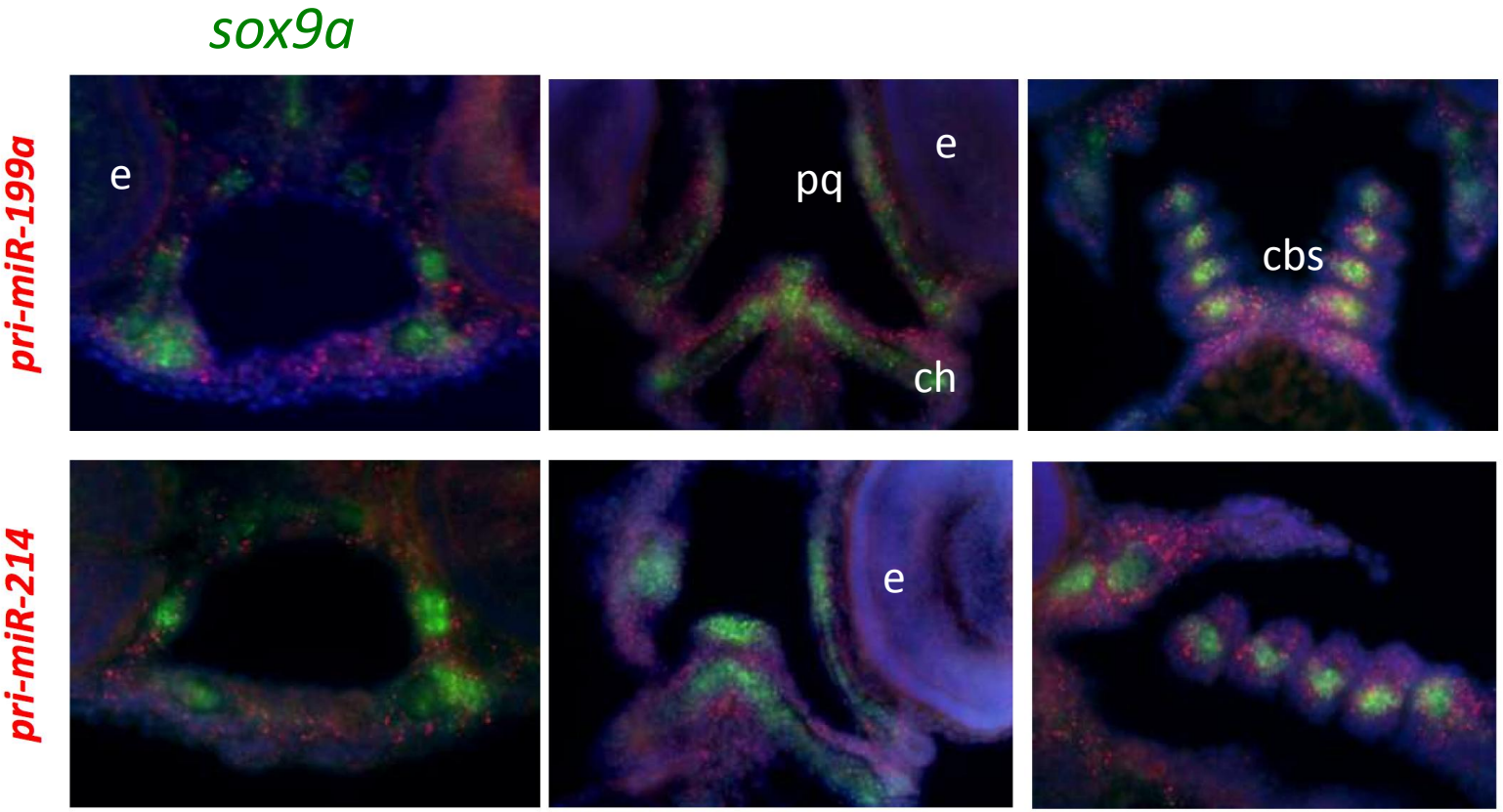
miR-23b knockdown results in a size reduction in Meckel's cartilage and the ethmoid plate in zebrafish



miR214/199 are expressed in developing cartilage

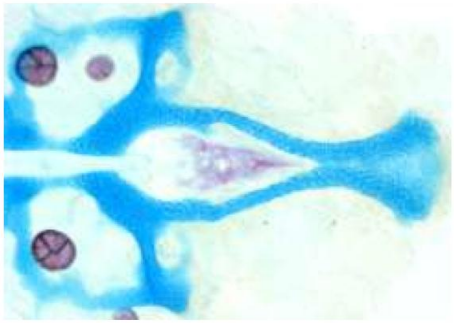


miR214/199 are expressed in an opposite pattern to *sox9a*, but is co-expressed with *sox9b*

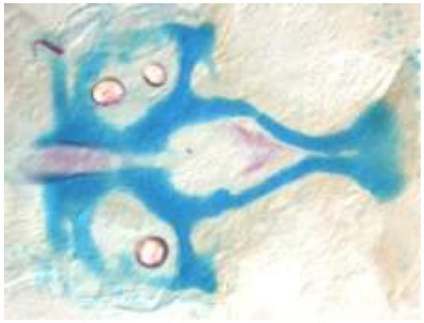


miR214/199 overexpression results in Trabeculae and Ceratobranchial defects

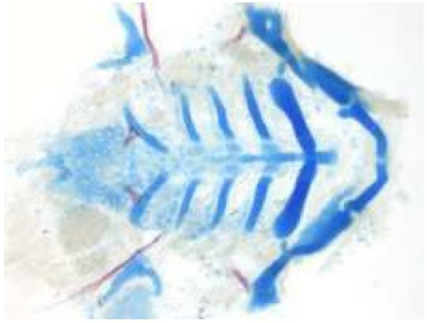
UIC



miR-214 OE
30-50uM



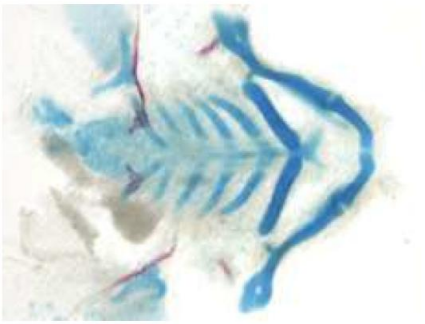
Trabeculae junction and midline defects



Mostly loss of 1cb

miR-199 OE
10-30uM

Trabeculae junction and ep shape



Mostly loss of 1cb

miR214/199 co-OE
3/1uM

Increased phenotype

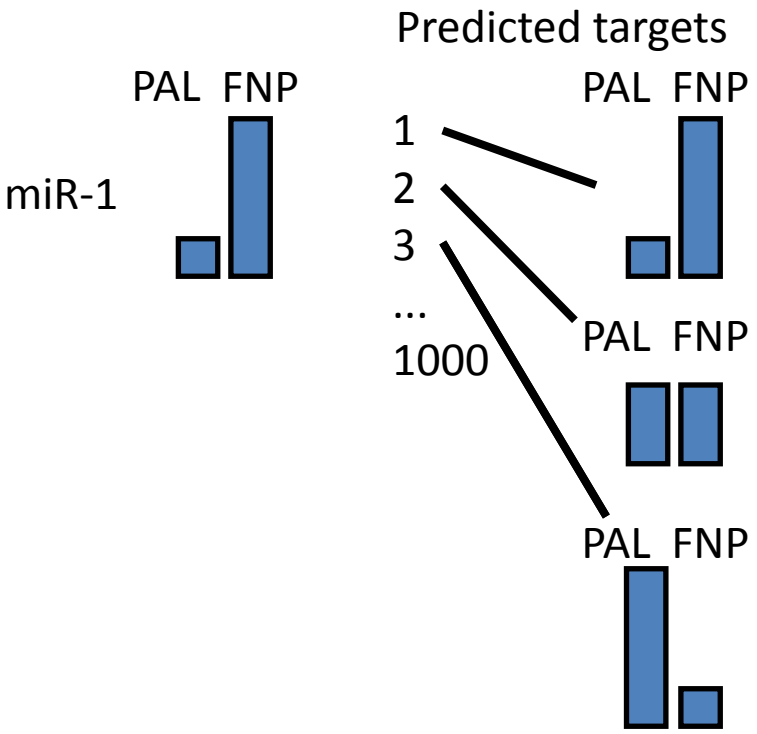
Profound delay in formation

Summary

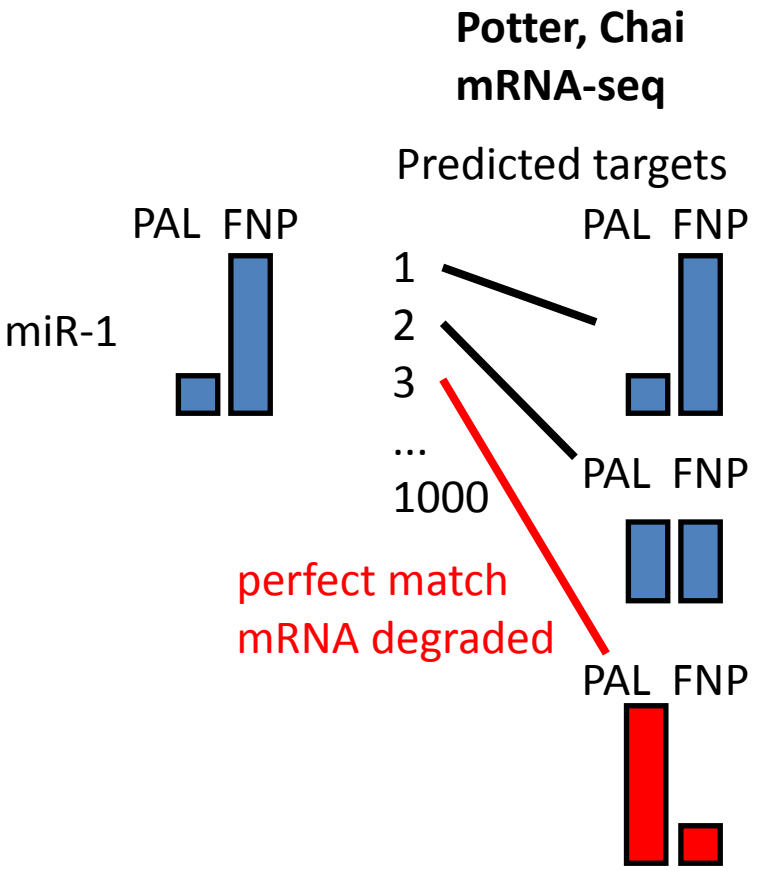
- RNA-seq data illustrates the complexity in miRNA expression in midfacial region.
- miRNA expression in mouse and zebrafish shows specific expression.
- Functional analysis of selected miRNAs in zebrafish suggests a role for miR133b in palatal development, miR23b in cartilage formation and miR214/199 in Trabeculae and Ceratobranchial development.
- Co-expression studies with genes that are potential targets suggests gene regulatory networks in midface development.

Spoke project interactions

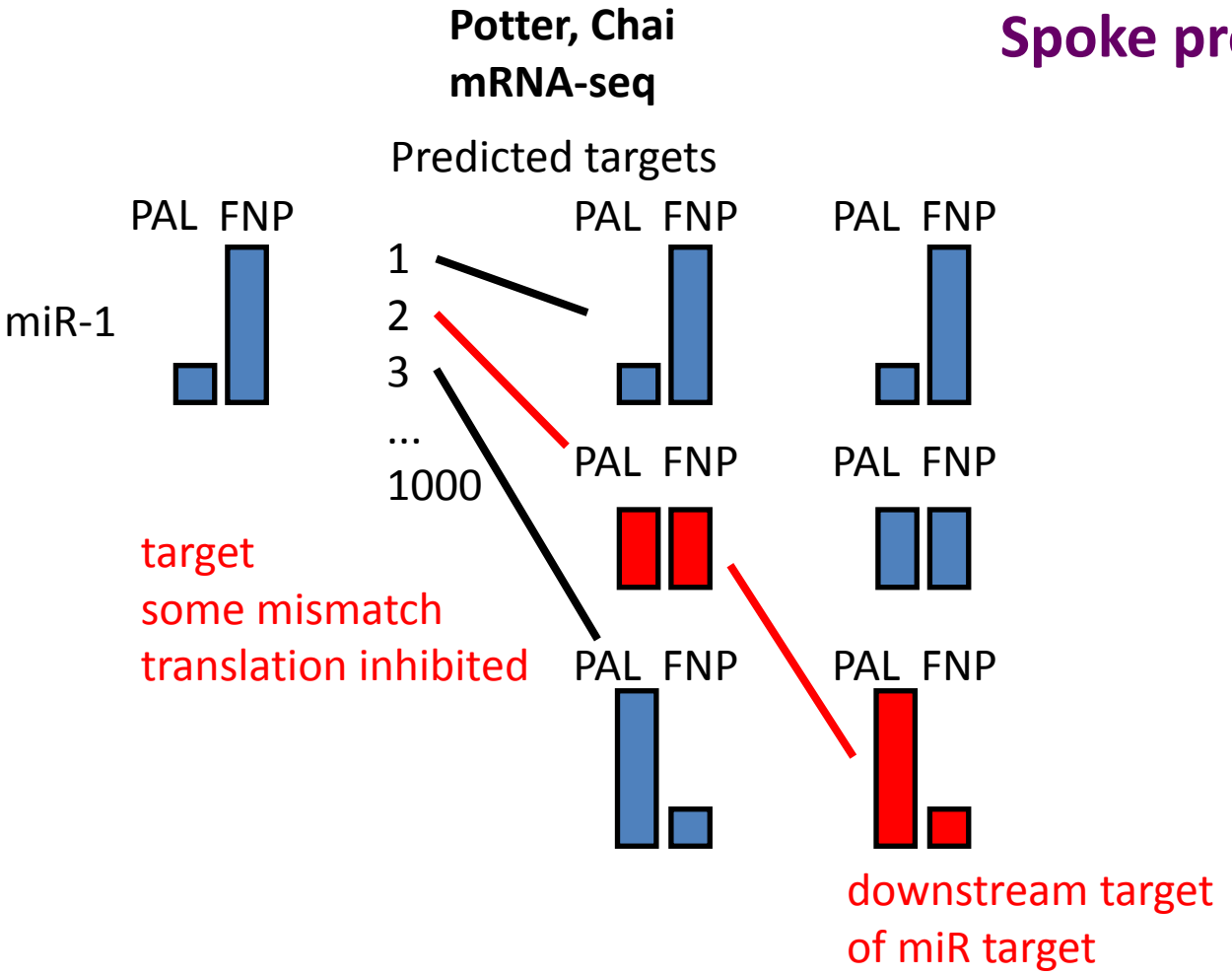
Potter, Chai
mRNA-seq



Spoke project interactions



Spoke project interactions



Acknowledgements

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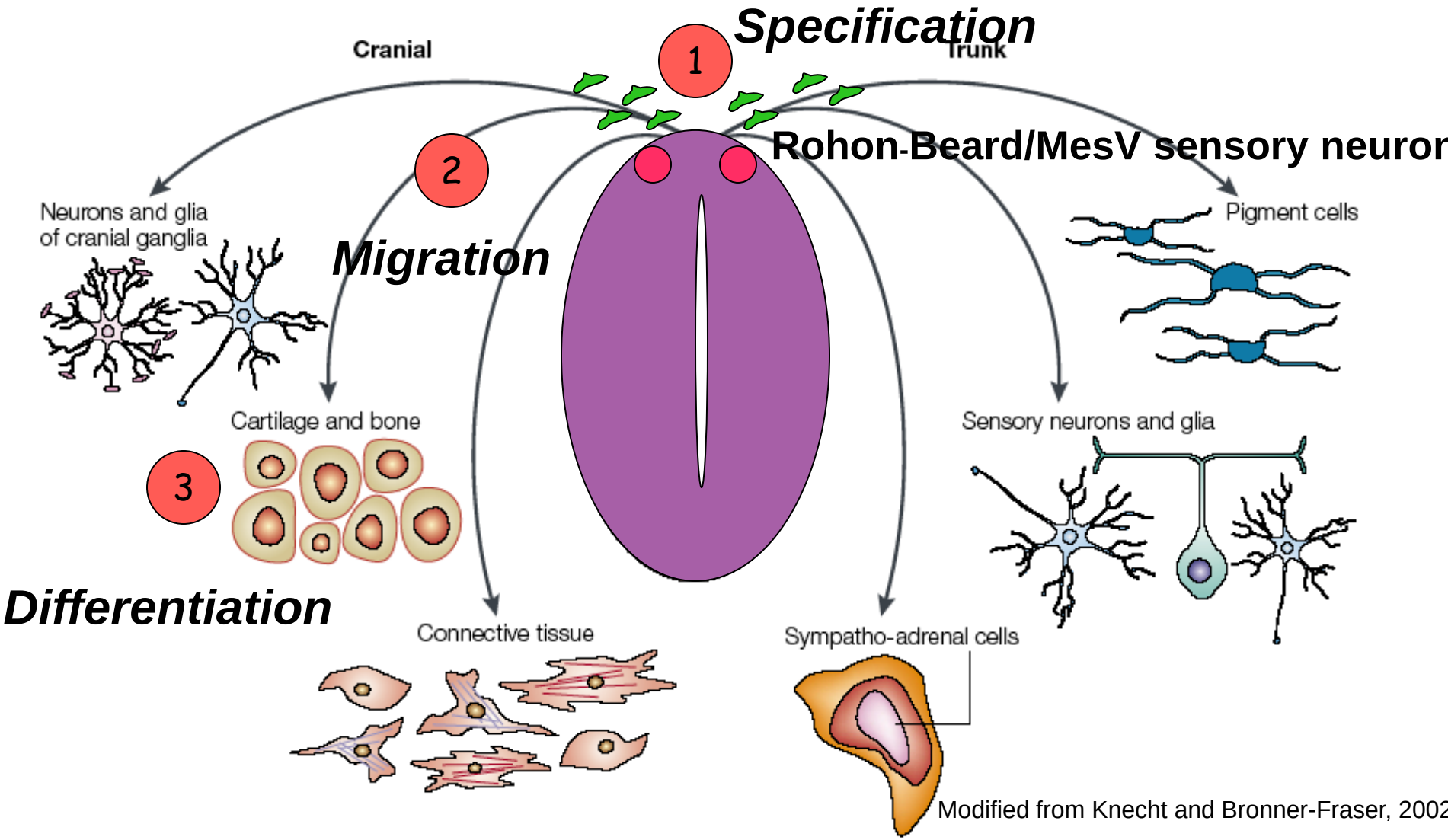
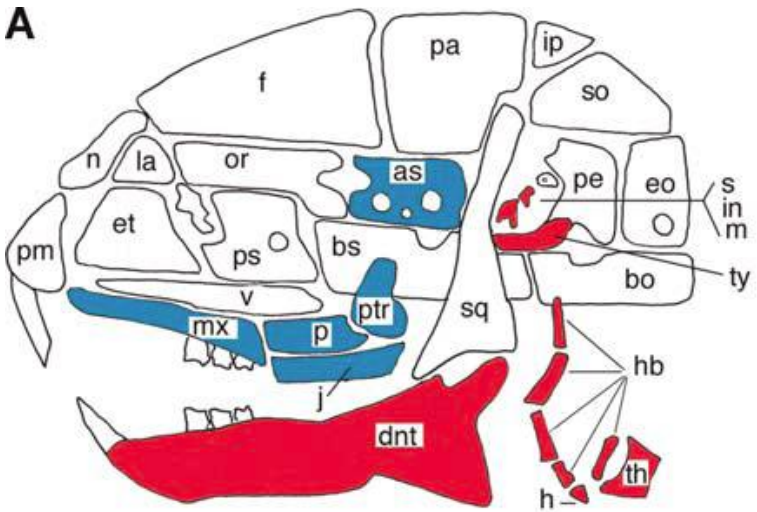
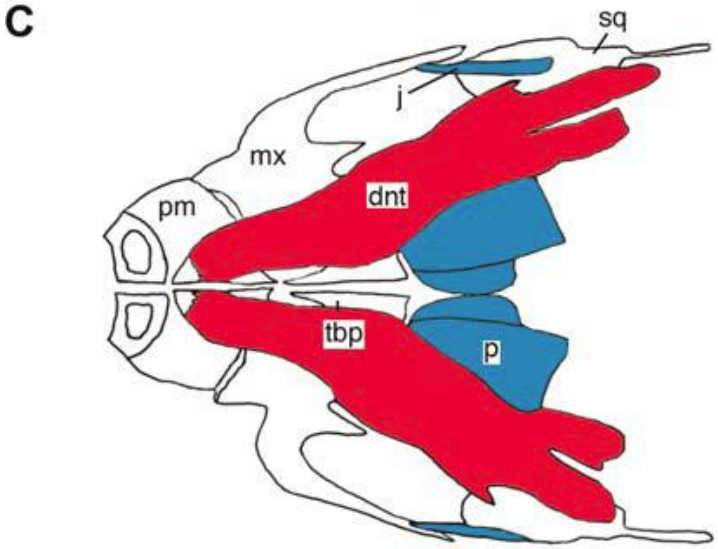
Neural Crest Cells form Multiple Derivatives during Development

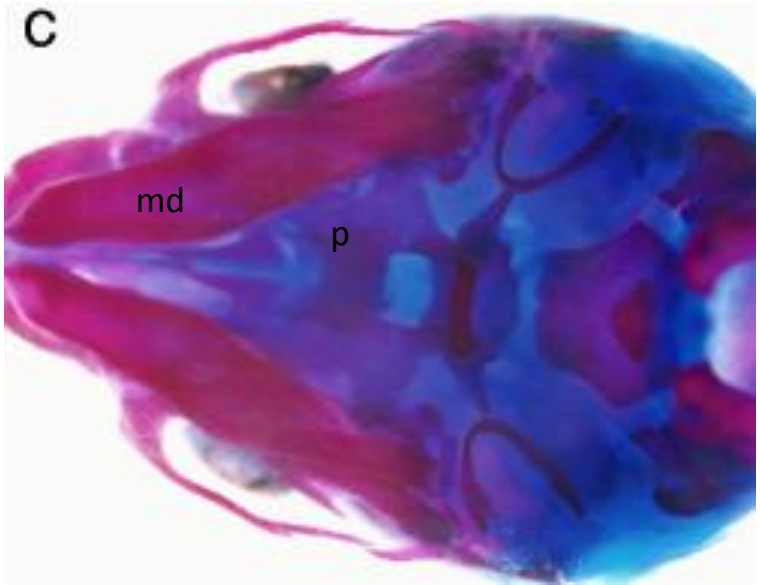


Modified from Knecht and Bronner-Fraser, 2002

Mouse NCCs migrate into pharyngeal arches and form the craniofacial skeleton

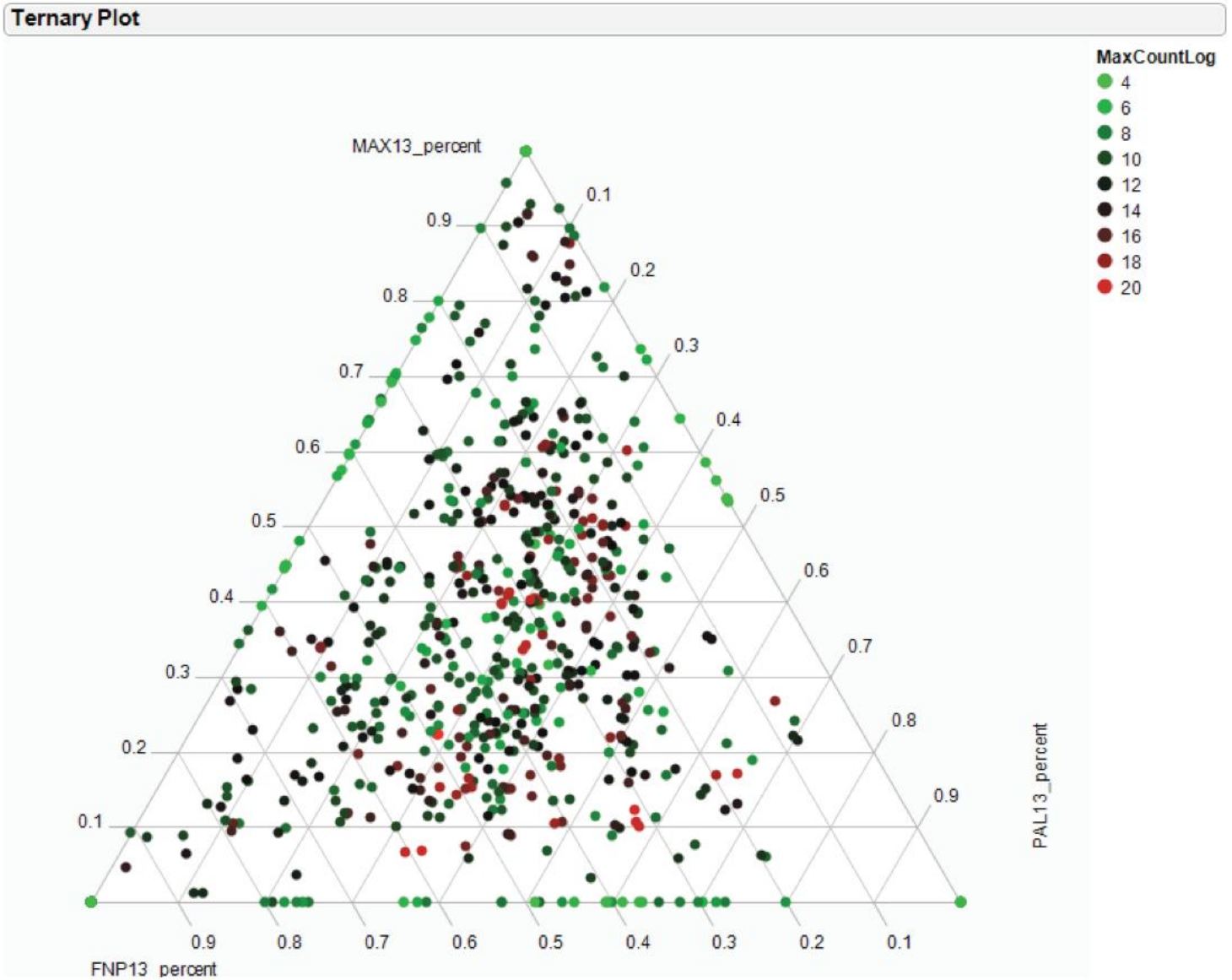




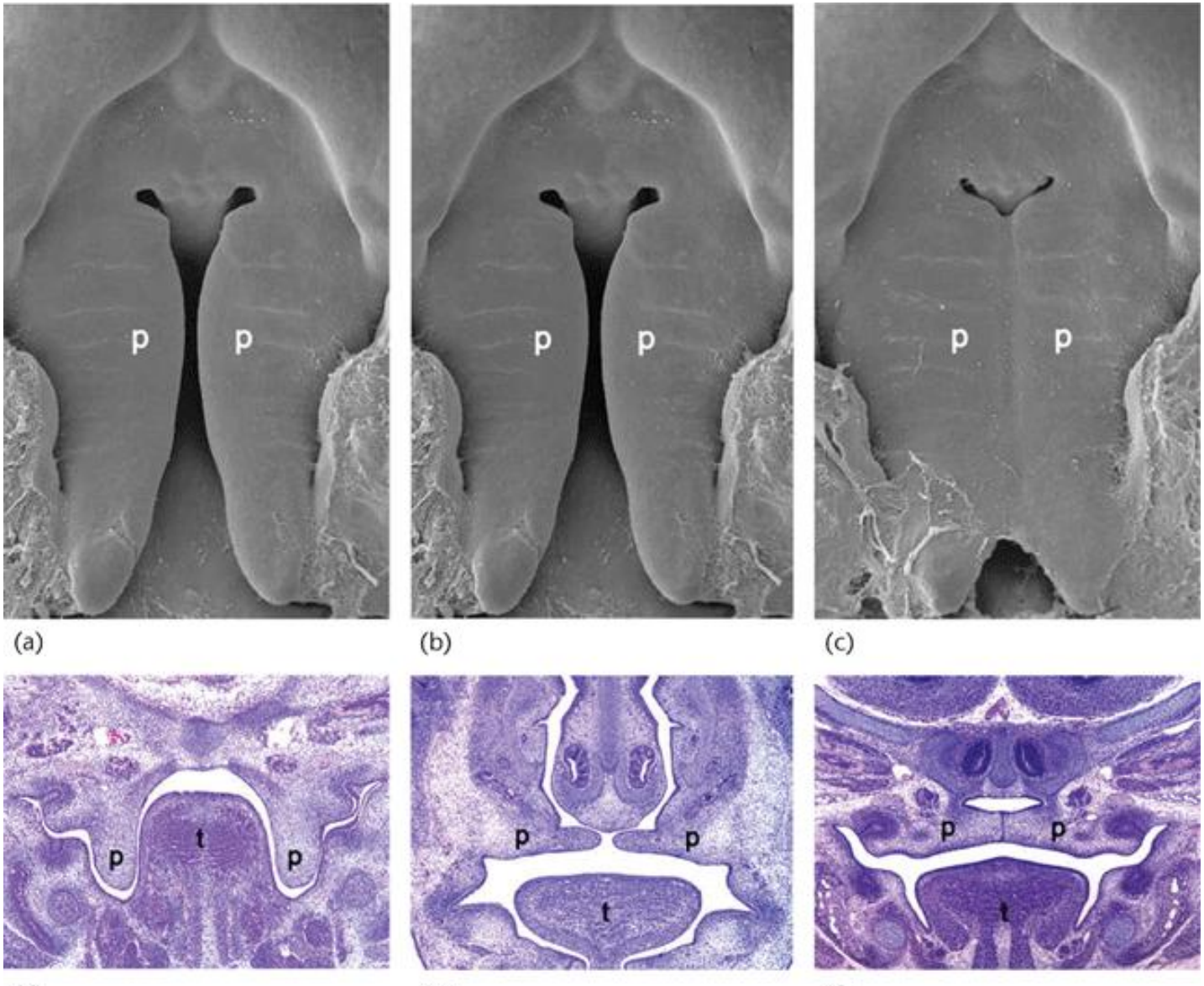


Clouthier et al, 2010 AJMG 152A, 2967

Ternary plot of miRNA abundance in different midfacial tissues at E13.5

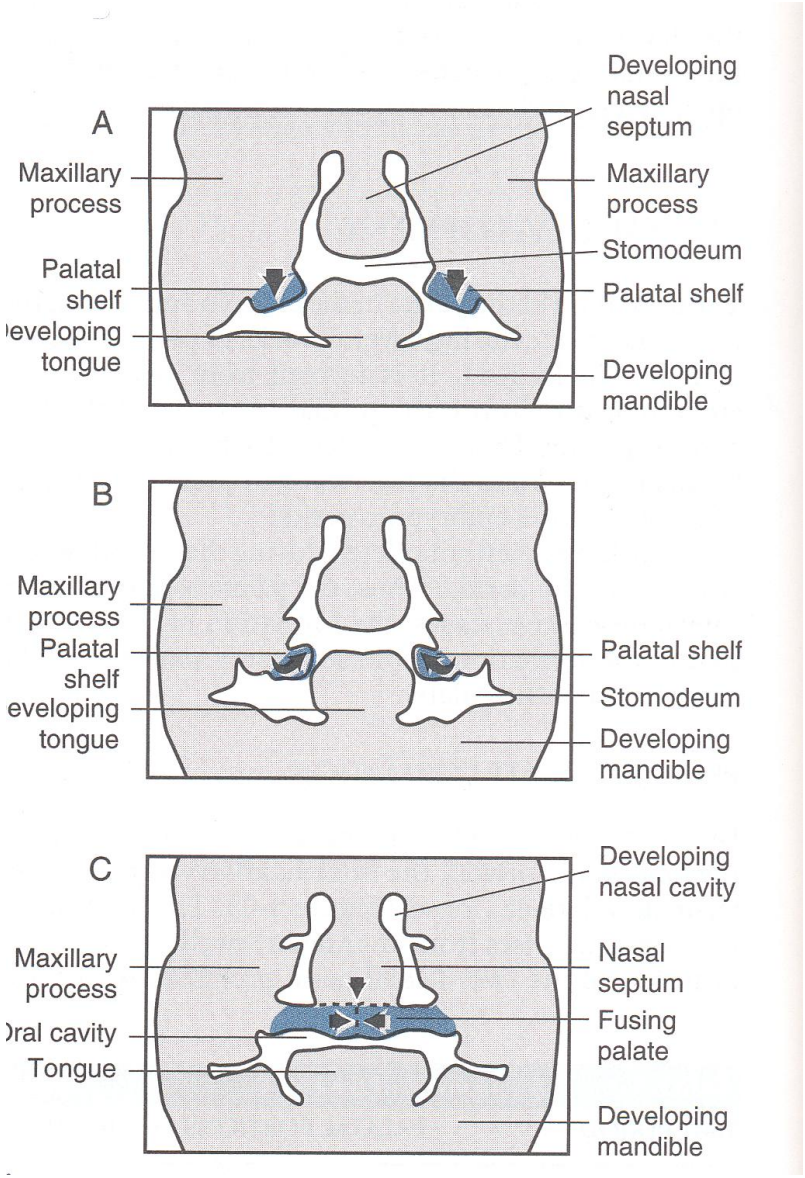


Palatal Development in Mammals

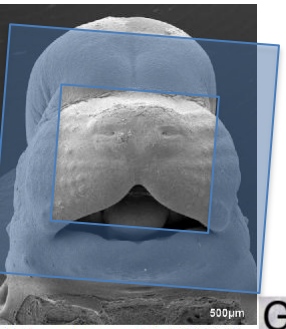


Craniofacial Defects and Cleft Lip and Palate Thomason and Dixon, 2009

Palatal Development in Mammals



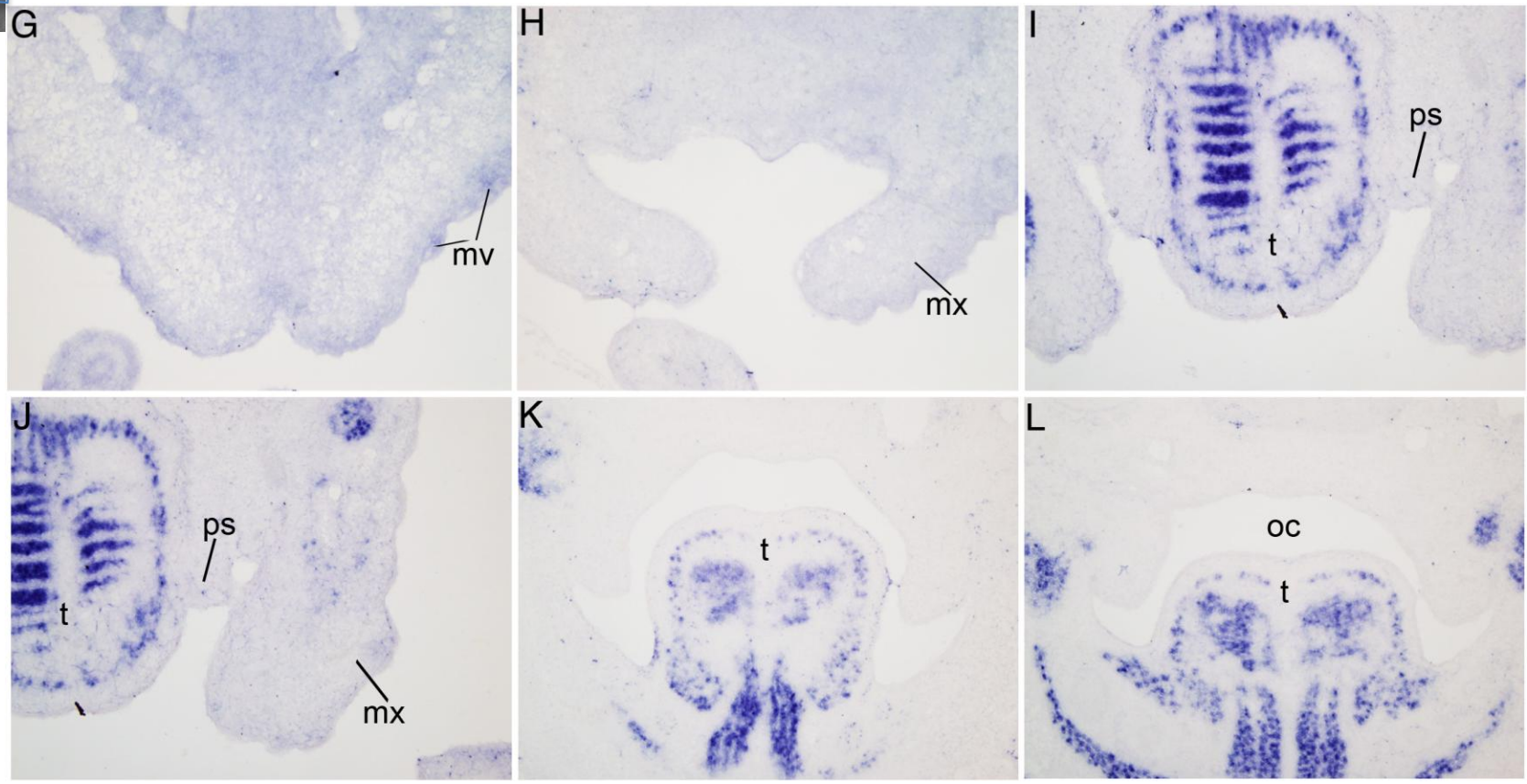
miR-133b is expressed in mouse facial structures at E12.5



Fetal mouse (mus musculus) at E13.5, SEM image. Iwata J, Bringas P, Chai Y. USC

Palate enriched:

miR-133b



miR-133b is expressed in zebrafish trunk and facial muscle