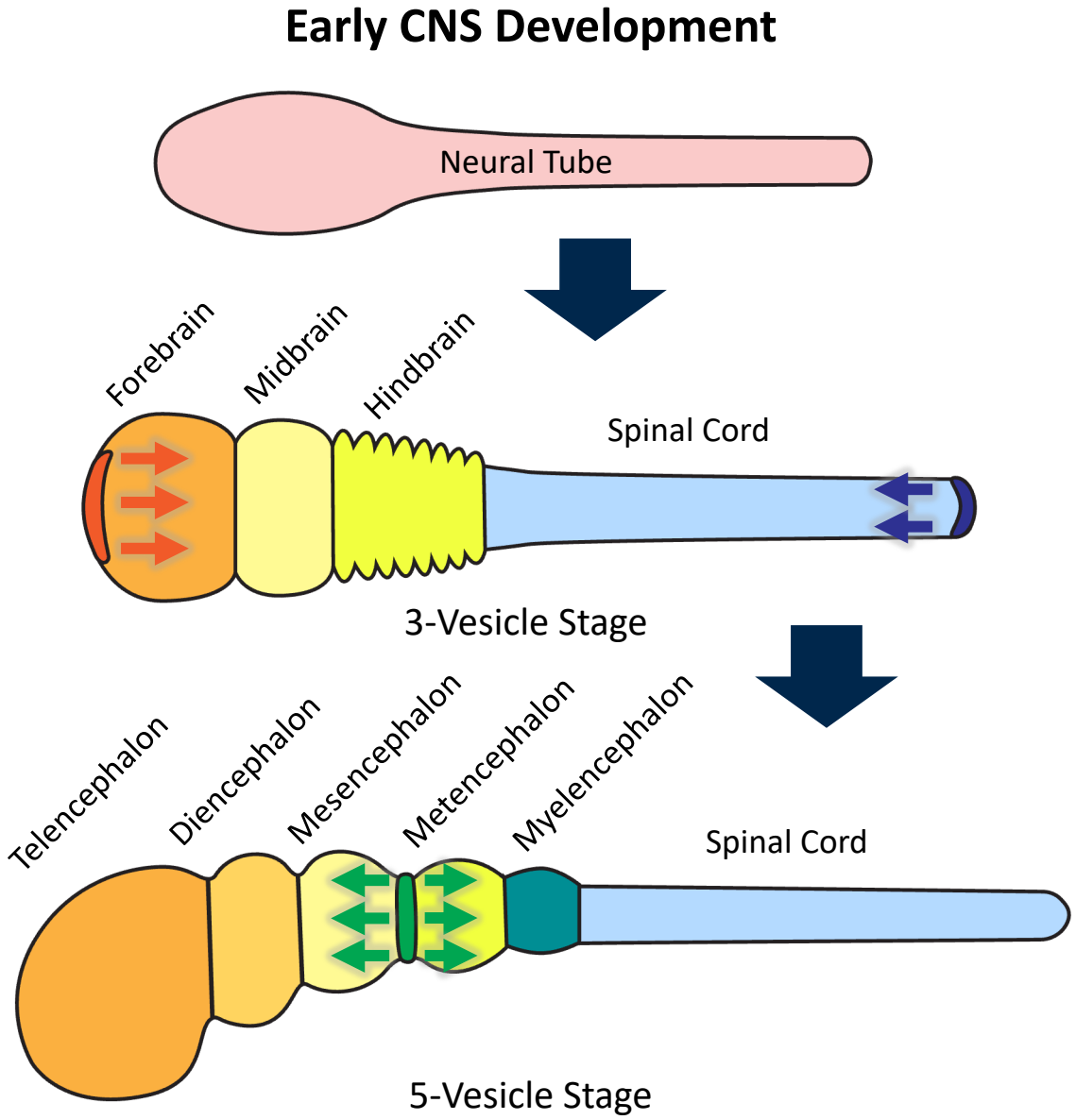
Background

Patterning in Biology

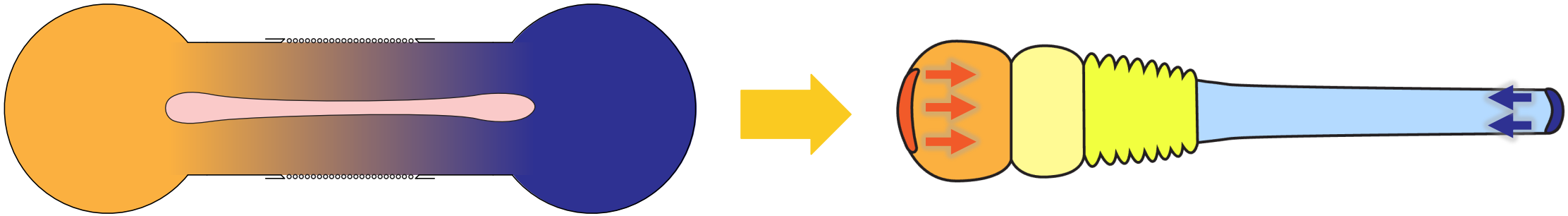
Generation of complex organizations through cell fate decisions

Rostrocaudal (RC) Patterning
of neural tube



Background

Recapitulate CNS patterning using engineering tools



Achieve patterning

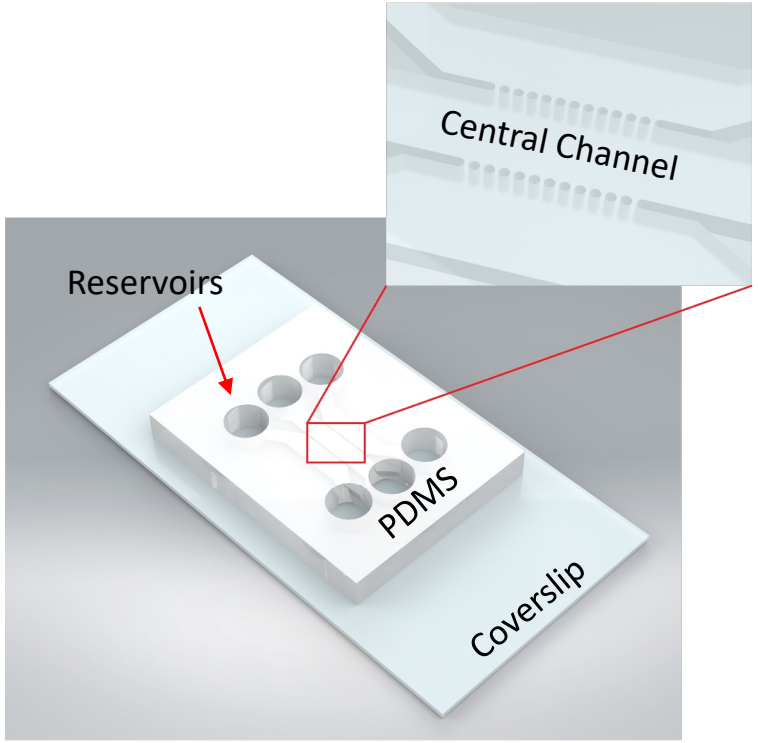
Patterning achieved

?

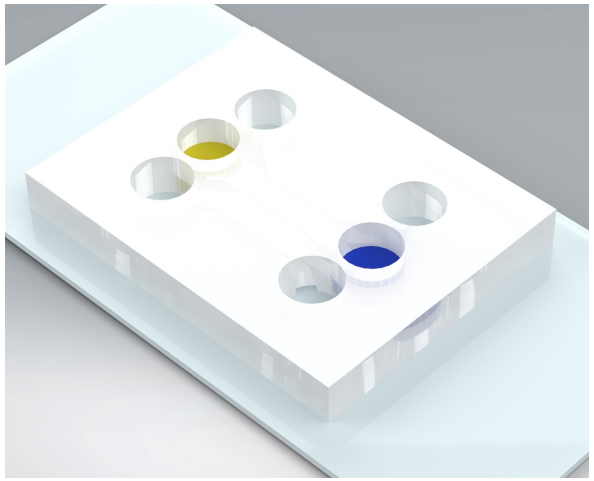
Microfluidics generate chemical gradient

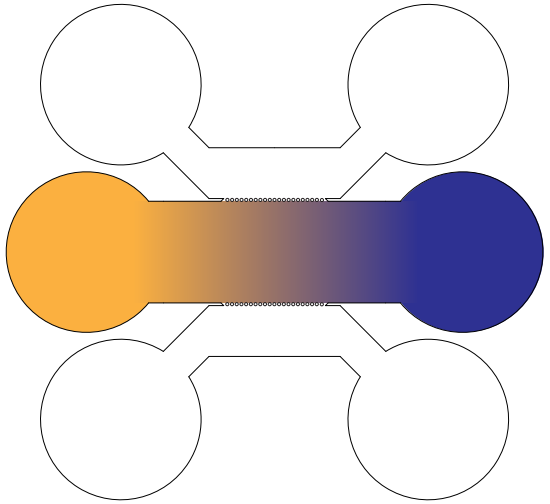
Differential secretion of soluble factors

Neural Tube

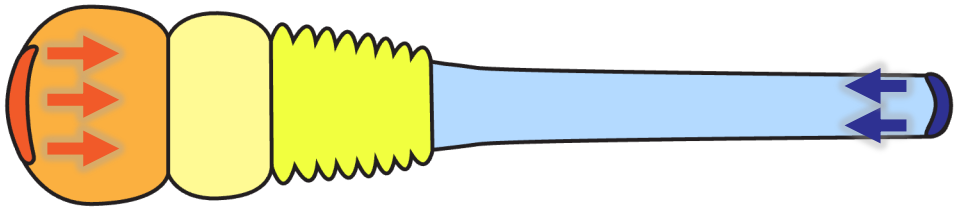


Microfluidic Device



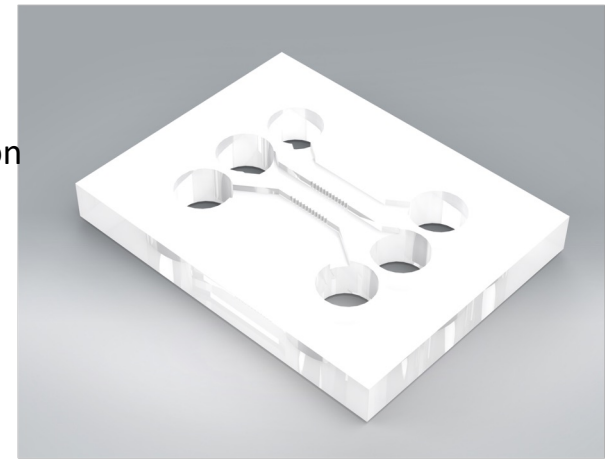


Recapitulate
Rostrocaudal
Patterning

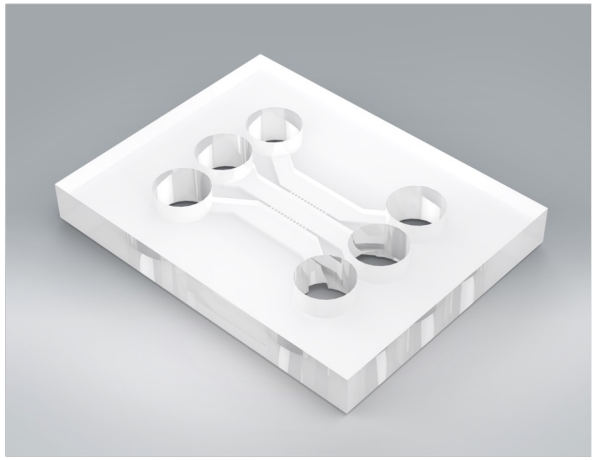


Neural Tube

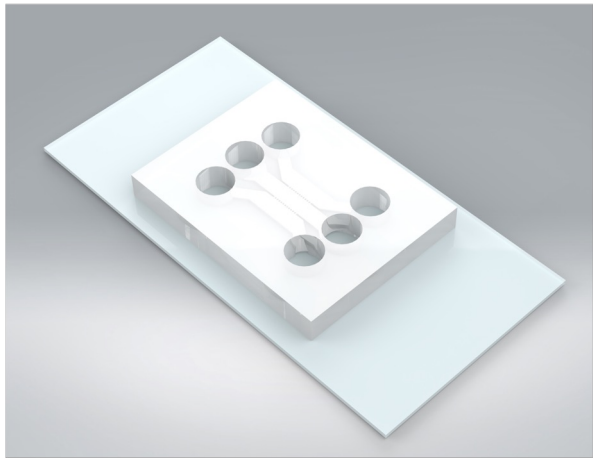
Molded from Patterned Silicon Wafer



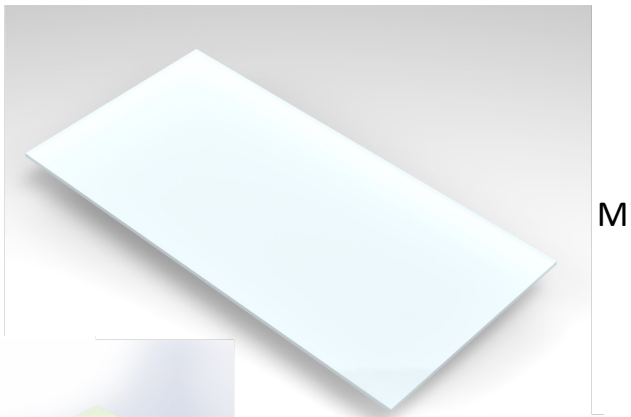
UV Clean



Bonding

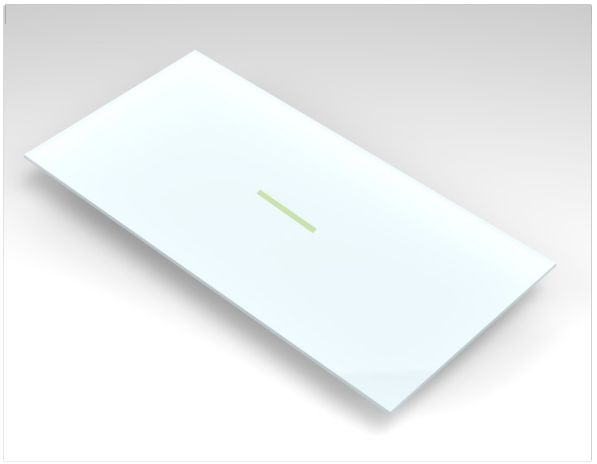


UV Ozone Activate



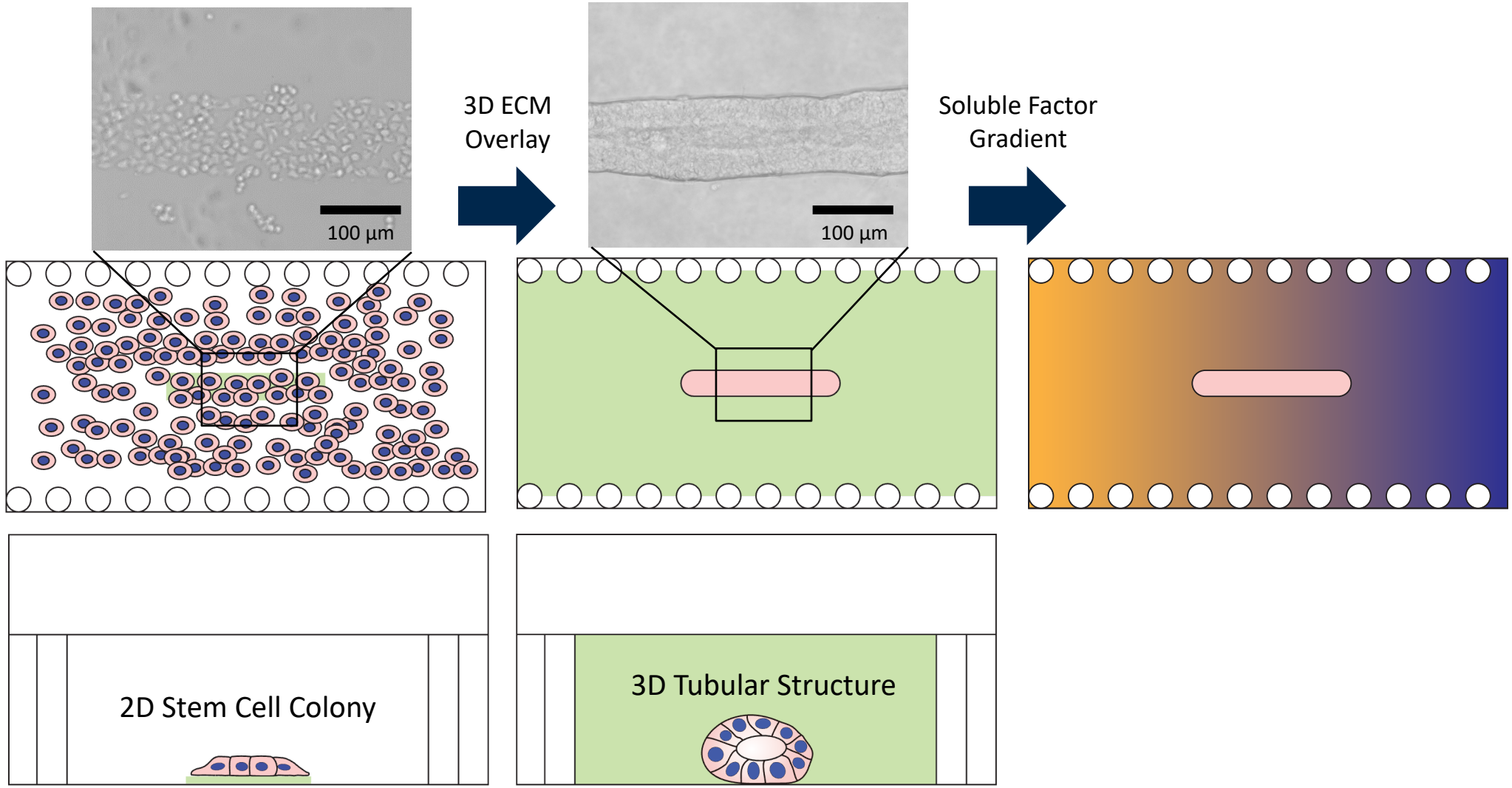
Coat with Diluted Extracellular Matrix (ECM)

Micro-contact Printing

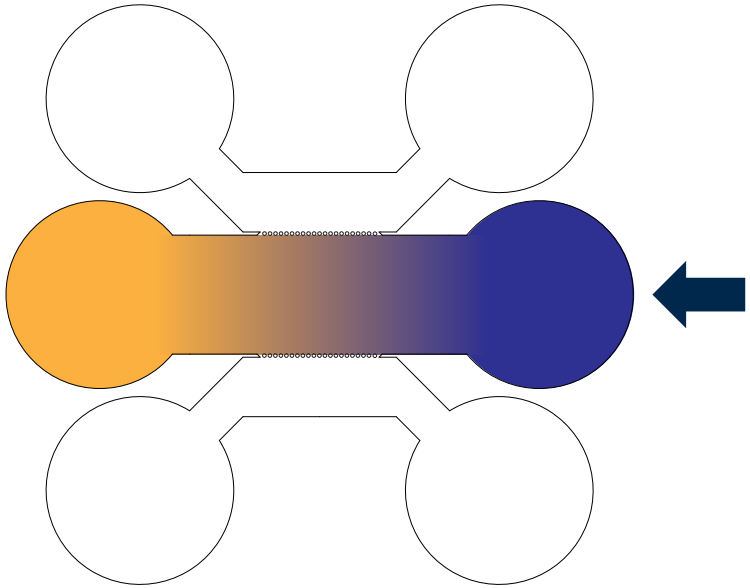


Neural Tube

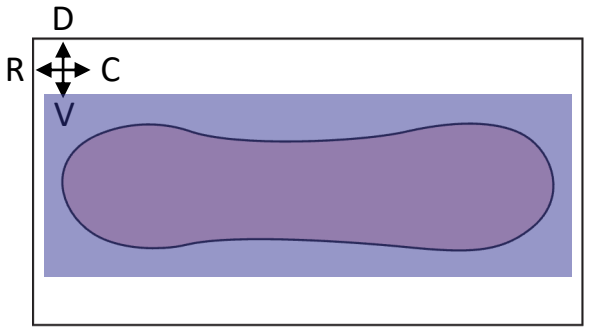
Human embryonic stem cells (**hESC**): cells that capable of forming any tissues within the adult body

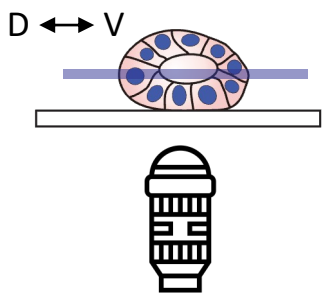


Neural Tube

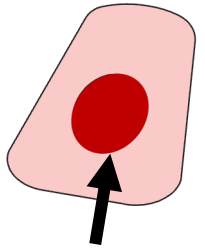


CHIR + RA
+ FGF8

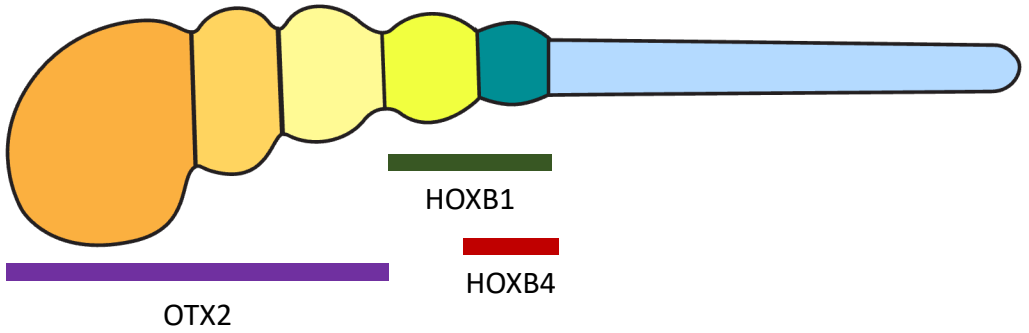


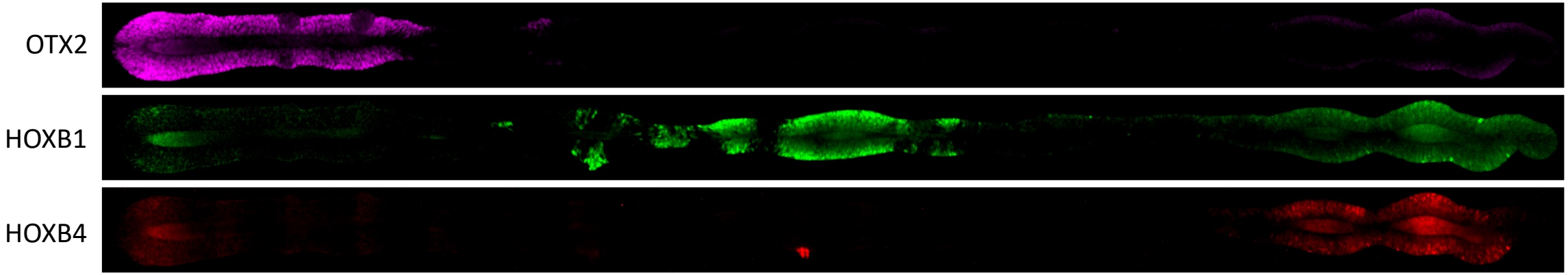


Confocal microscopy enables imaging at a **cross-section**.

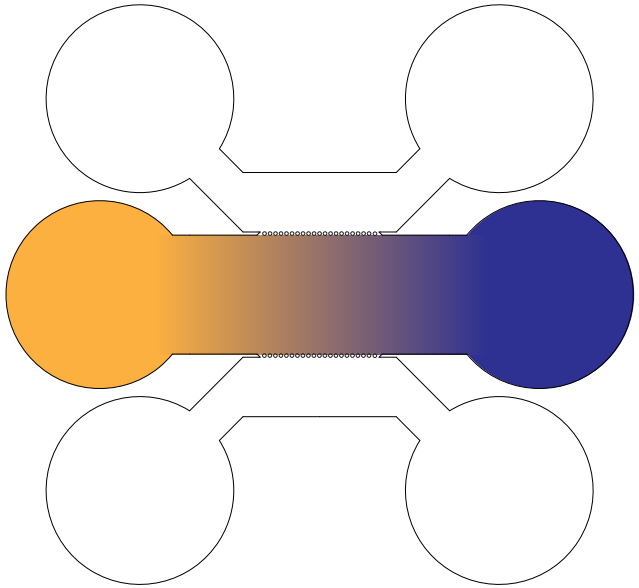


Region-specific Transcription Factors (Markers) in **Nucleus** Indicate **Cell State**

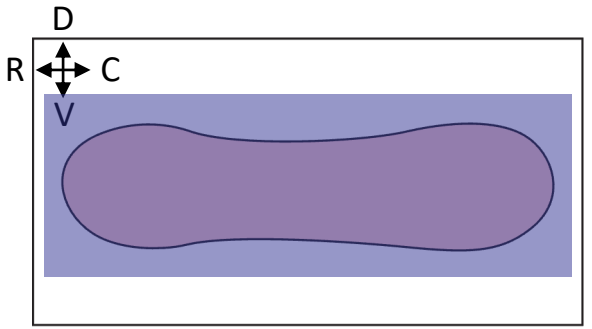


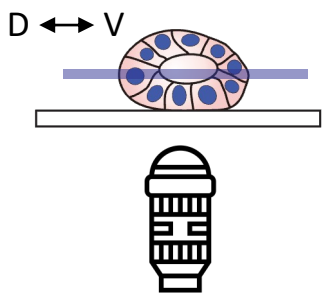


Neural Tube



CHIR + RA
+ FGF8





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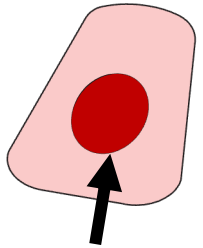
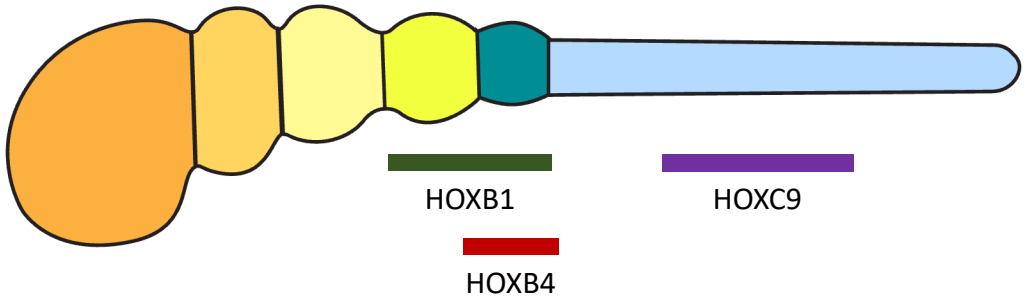
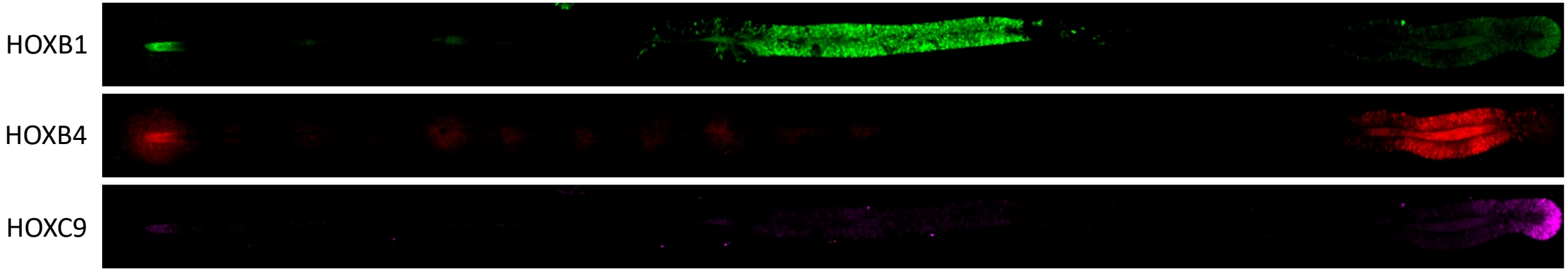
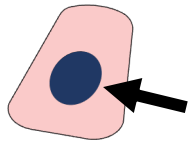


Region-specific Transcription Factors (Markers) in **Nucleus** Indicate **Cell State**

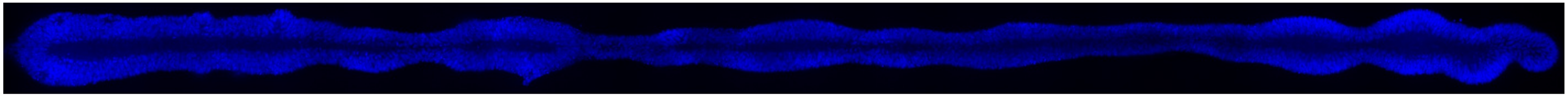




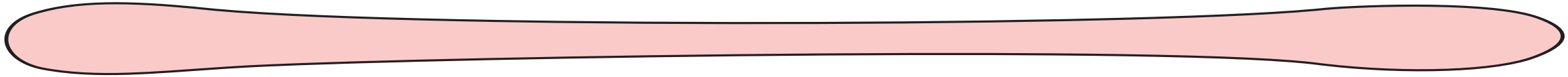
Neural Tube

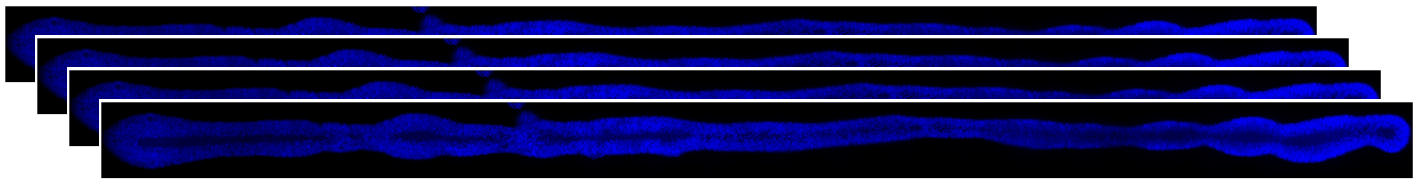


DAPI is universal nuclear marker



Object Identification





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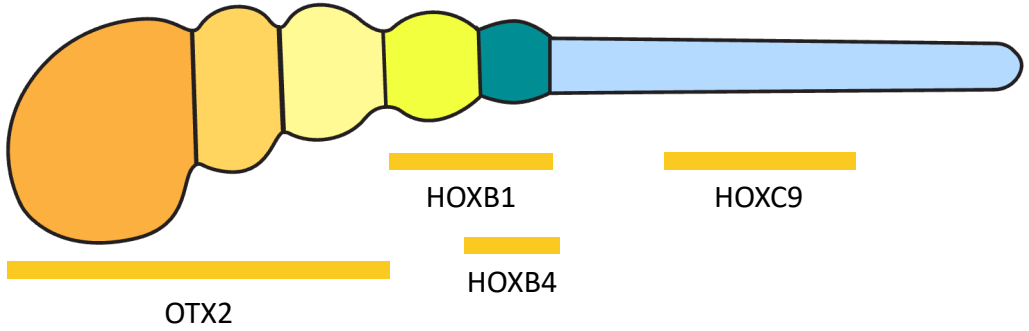
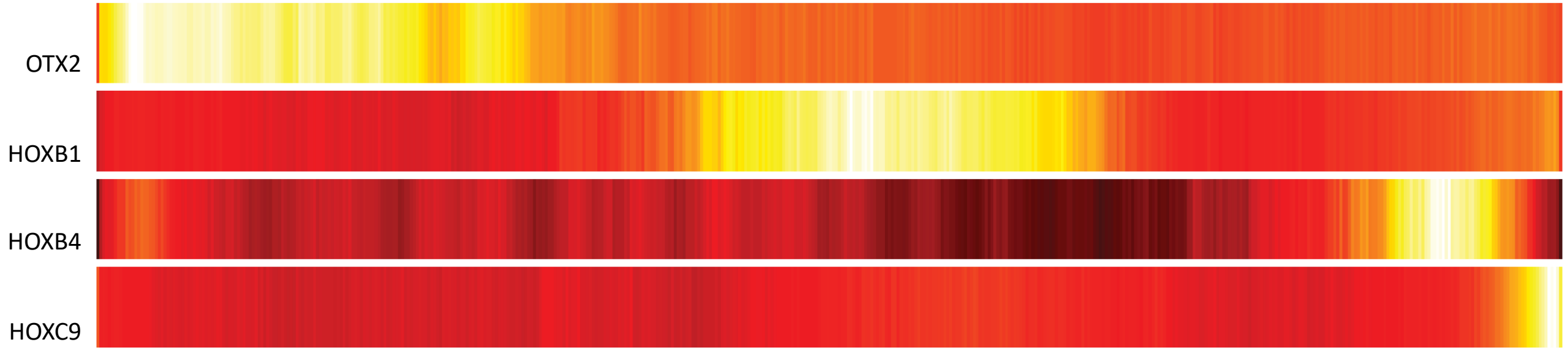
Scalable approach to quantify regional marker localization

Neural Tube

Neural tubes demonstrate **consistent** and **appropriate** localization of regional markers



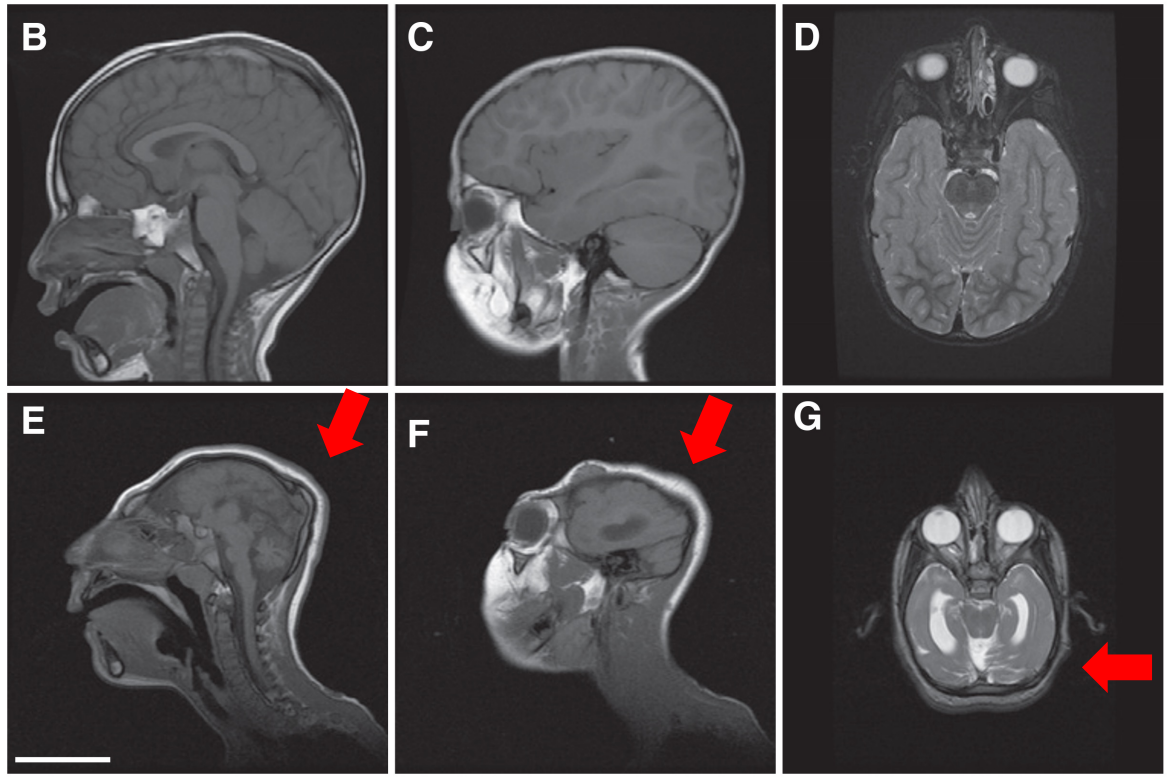
Averaged Heatmap of Marker Expression (N > 50)



NDE1-KO

Mutation in NDE1 Gene ← Microcephaly

Microcephaly is a medical condition where the patients have **smaller than normal head** size.



Normal (2 years)

Microcephaly (4.5 years)

Genetic evidence suggests mutation in **NDE1** gene could result in microcephaly[1].

[1] Alkuraya et al. "Human mutations in NDE1 cause extreme microcephaly with lissencephaly." The American Journal of Human Genetics (2011).

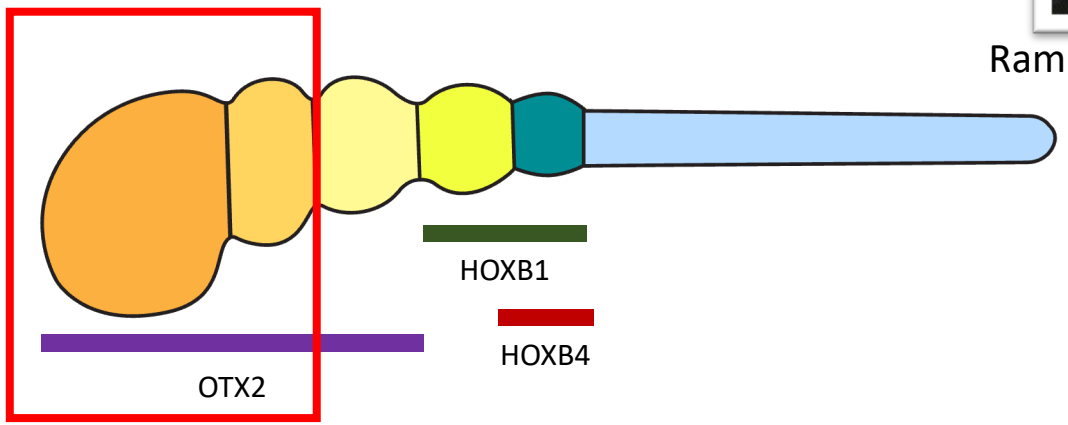
NDE1-KO

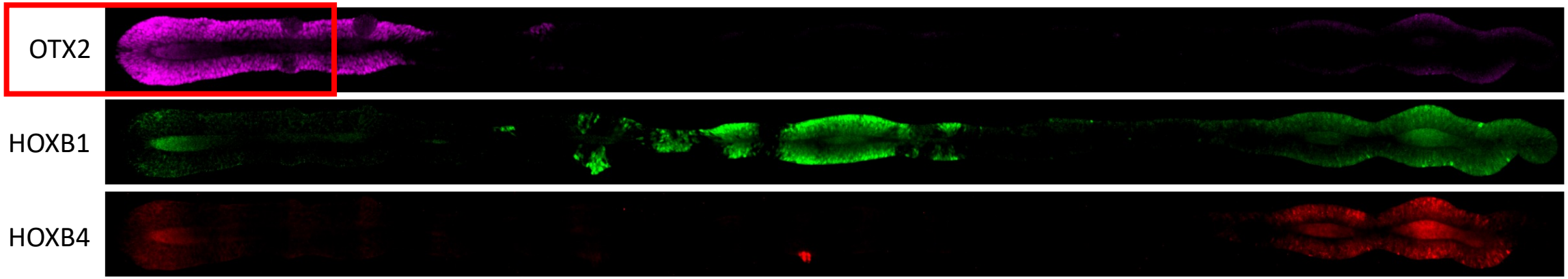
Inhibit NDE1 Gene → ? → Microcephaly

Study the effects of knocking-out NDE1 on RC patterning and forebrain formation

Prof. Orly Reiner

Rami Yair Tshuva





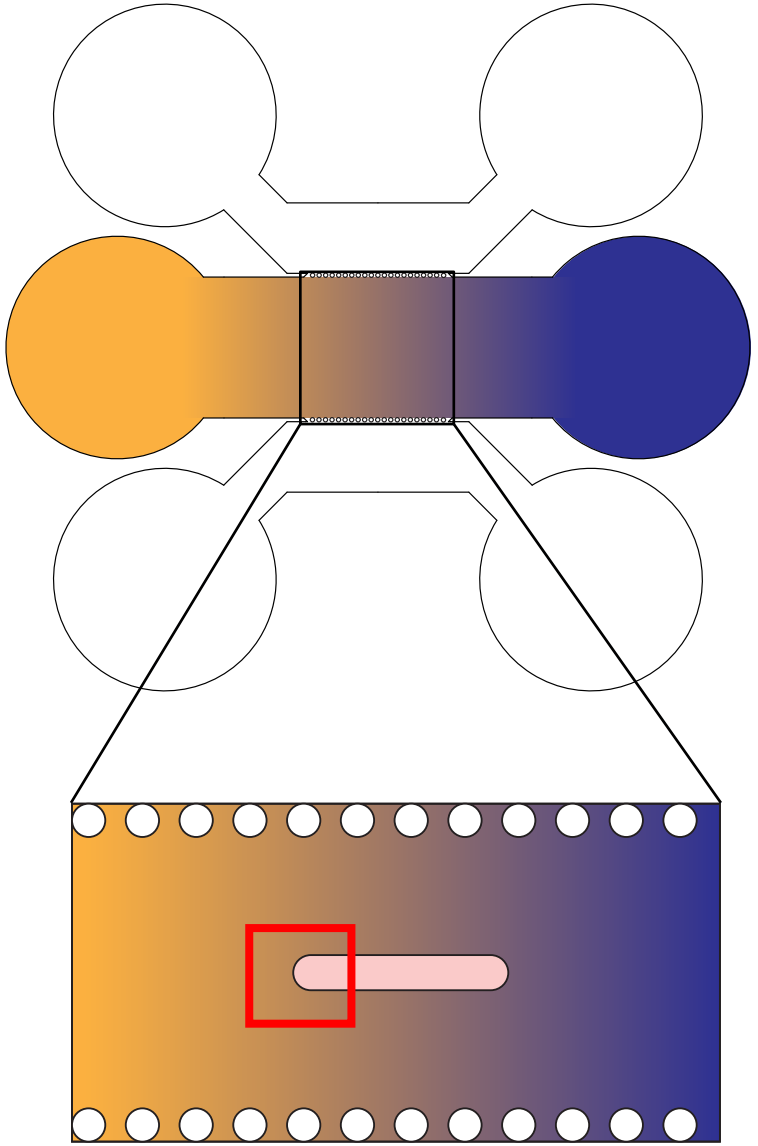
NDE1-KO

Knock out NDE1 gene in human embryonic stem cells

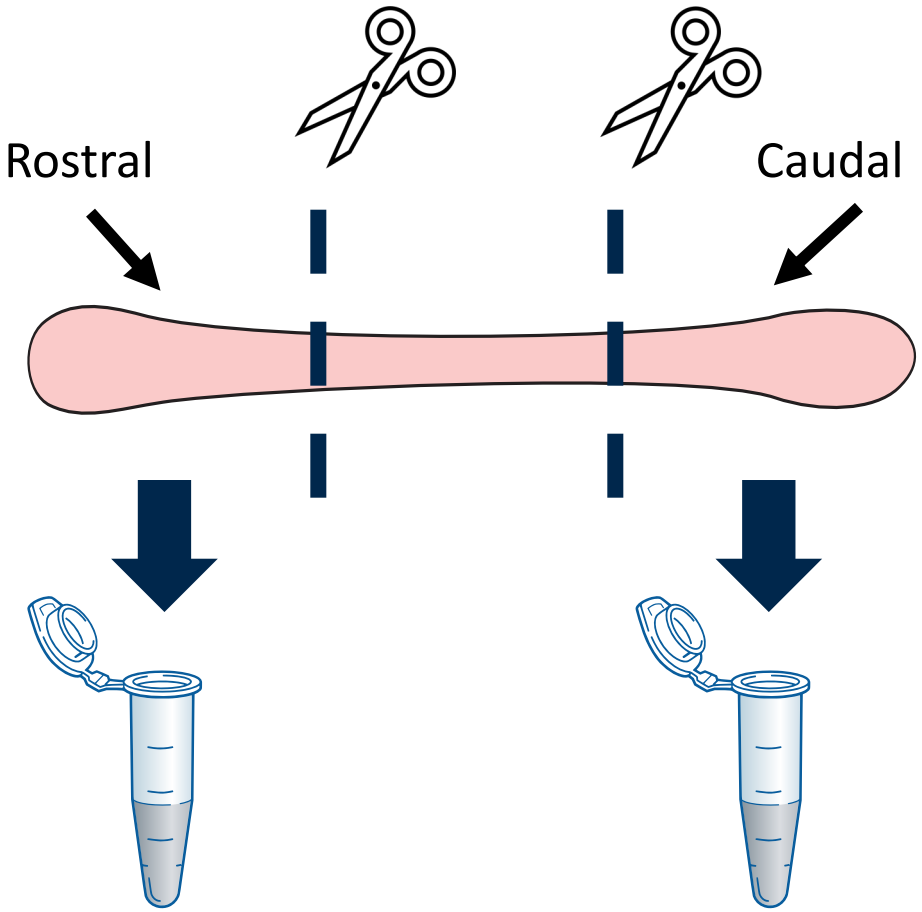
- NDE1-KO vs. control

Perform microfluidic-based patterning

- Evaluate forebrain development

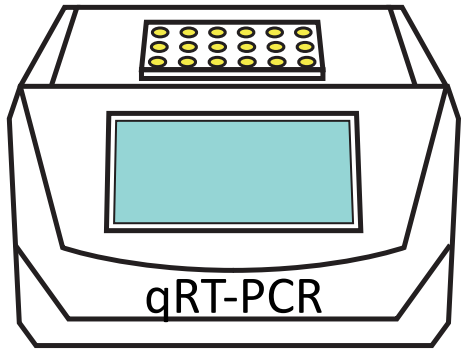


NDE1-KO



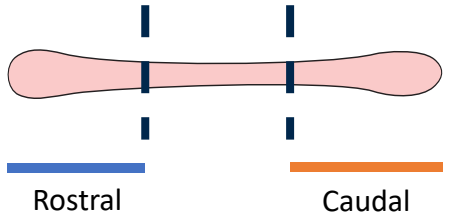
qRT-PCR (Real-time reverse transcription polymerase chain reaction): **quantitative** measurement of DNA/RNA fragments

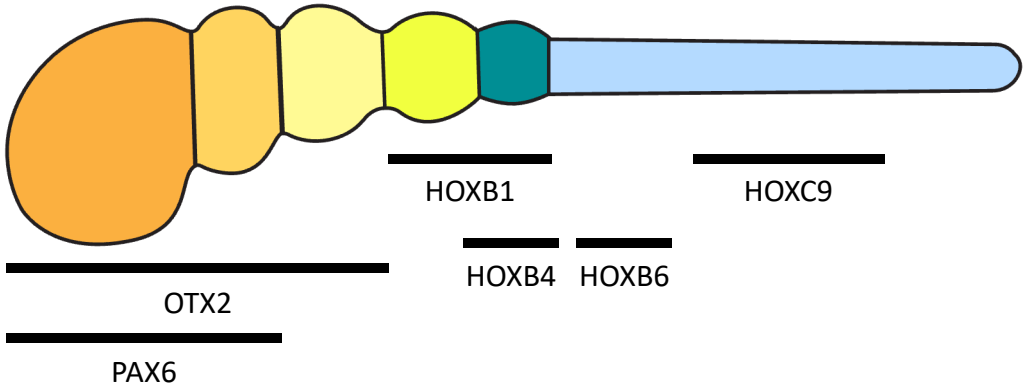
PCR enables quantitative comparison between control and NDE1-KO cell lines.

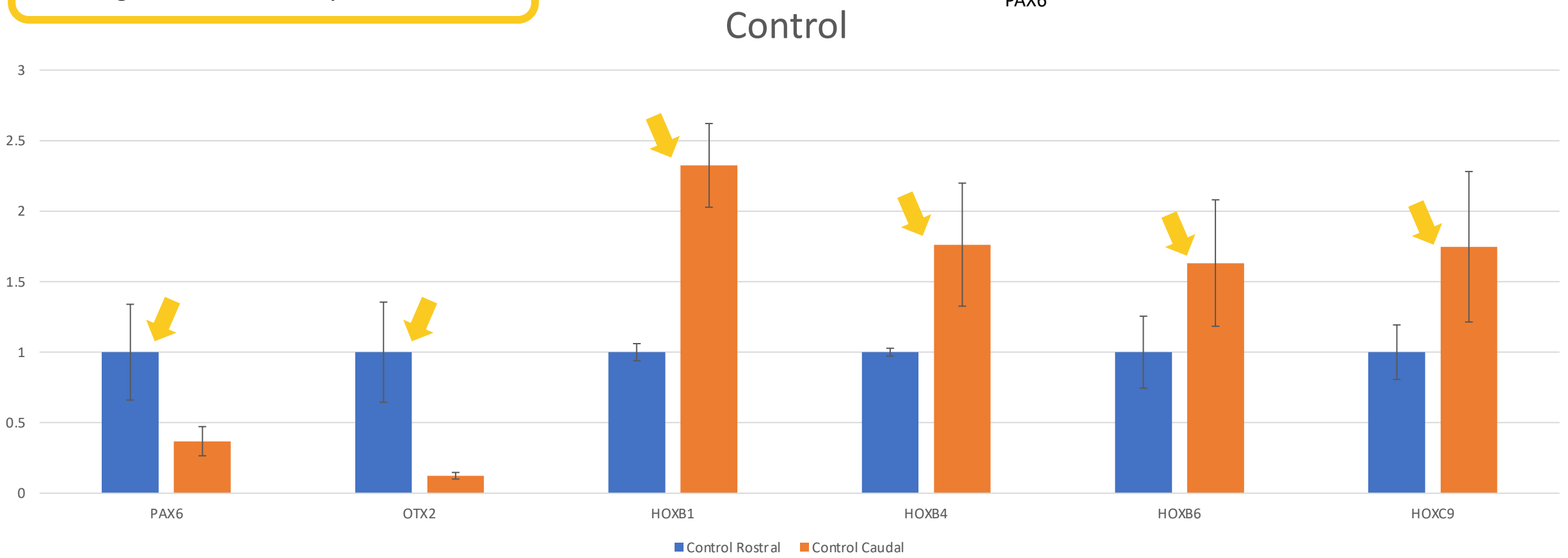


NDE1-KO

Consistent upregulation of rostral and caudal genes in their respective section

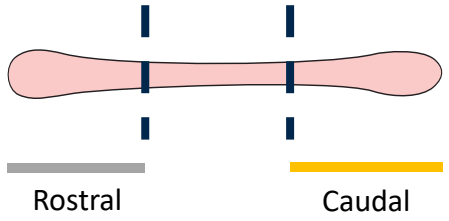


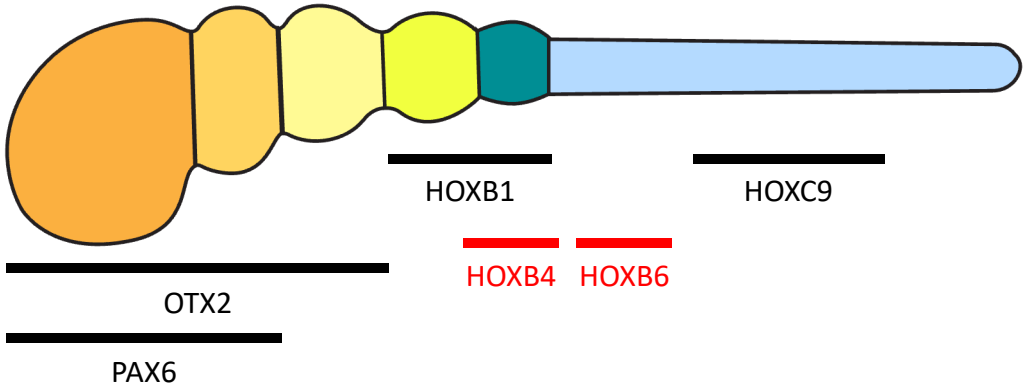


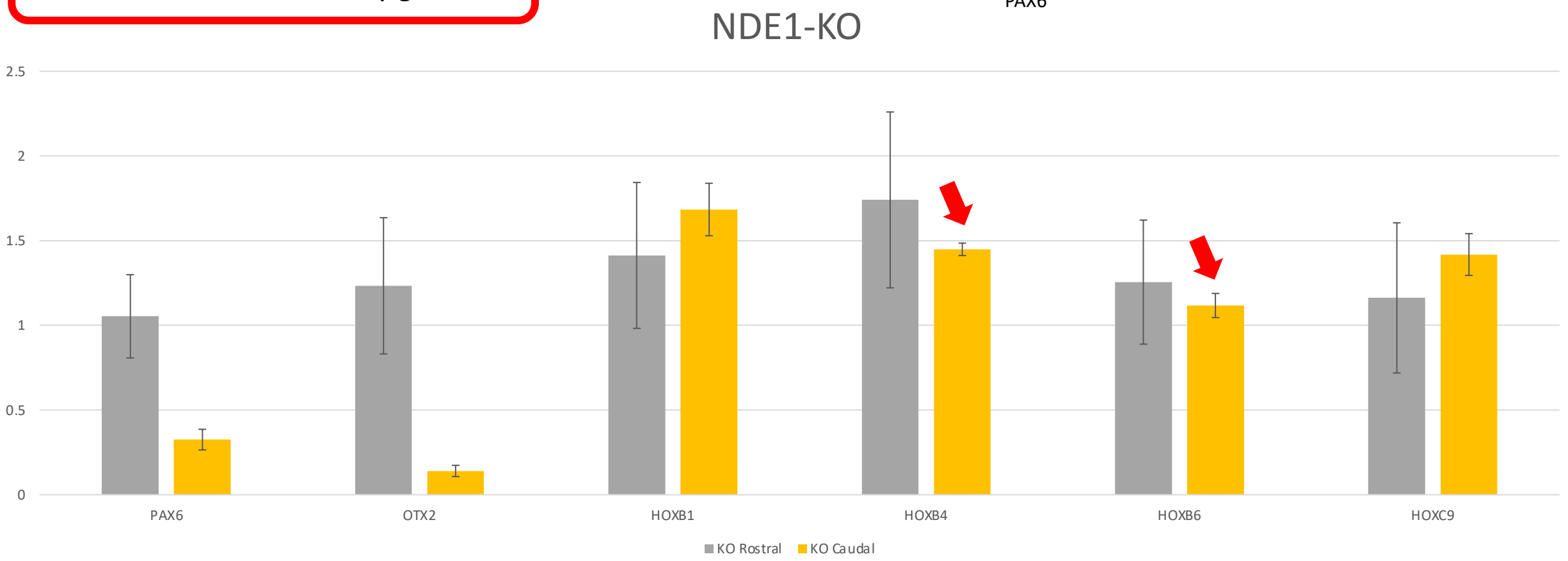


NDE1-KO

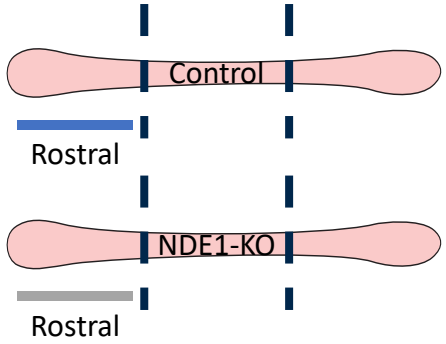
NDE1 knock out disrupts the localization of HOX-family genes

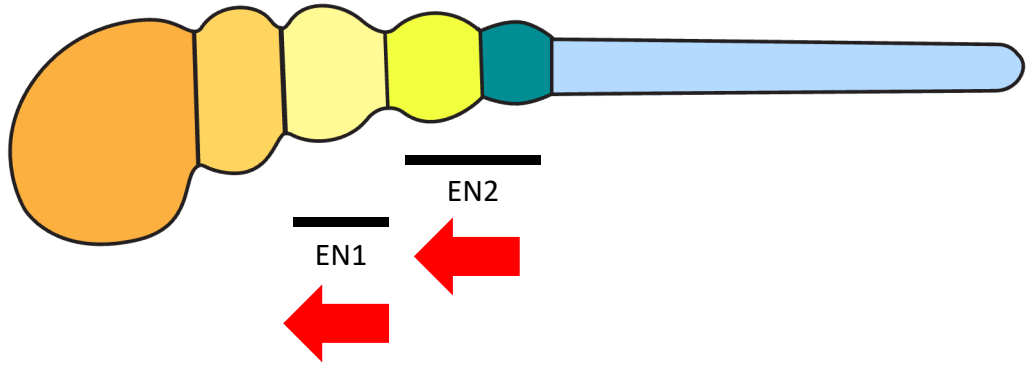


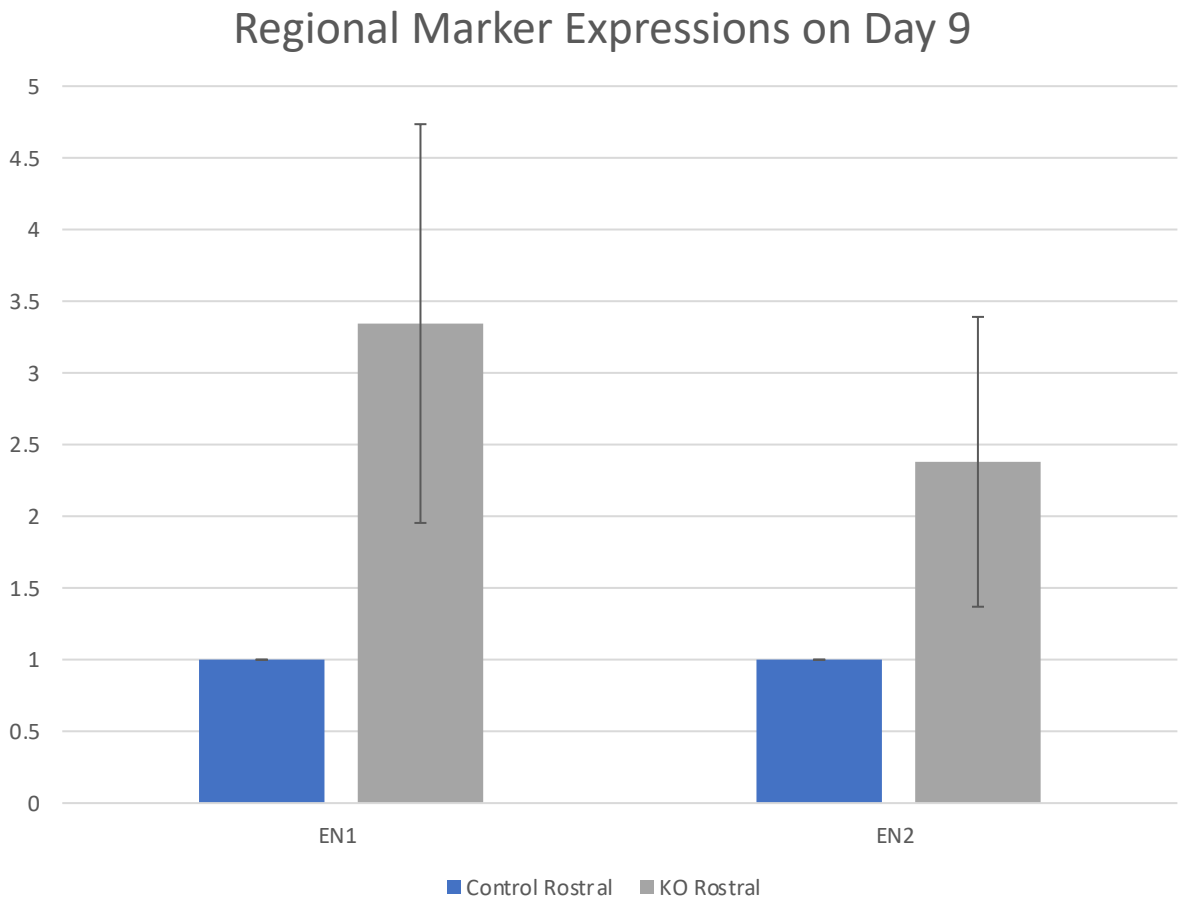




NDE1-KO







NDE1 knock out causes the rostral shift of midbrain to hindbrain regions

Inhibit NDE1 Gene through Knock-out → Disruption to neural tube RC patterning → Abnormal forebrain development