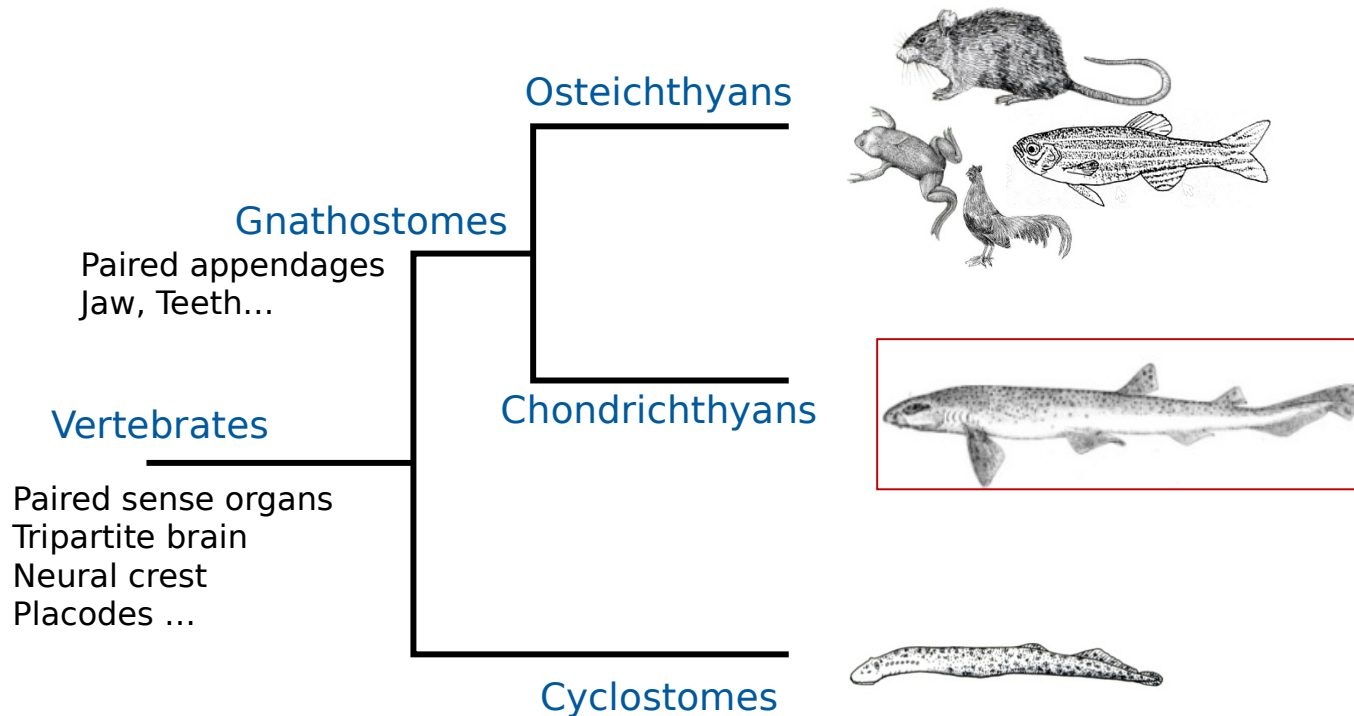


From confocal imaging to 3D RNA profiling:
The catshark model



S. Mazan
Développement et évolution des vertébrés BIOM
07/12/2021, Banyuls

The catshark: chondrichthyan!



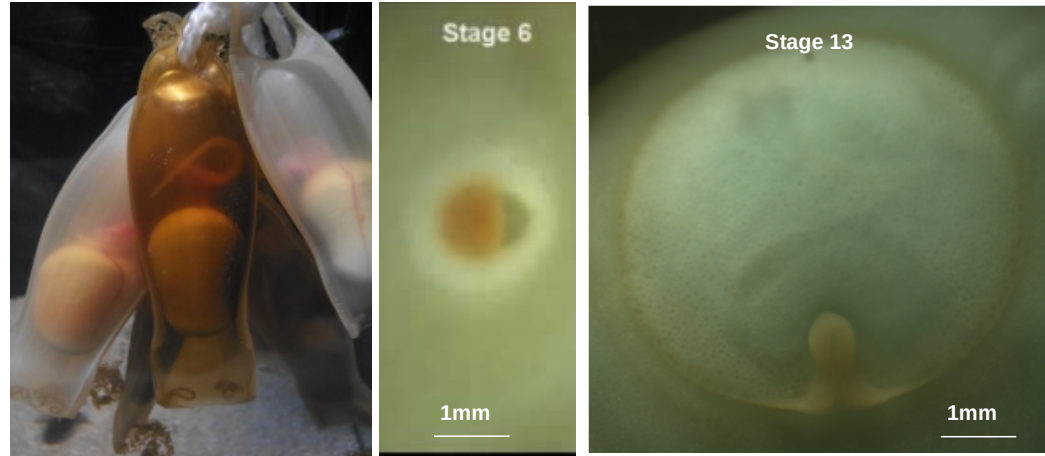
- A key phylogenetic position
- Generality of mechanisms inferred from mouse or zebrafish
- Blueprint of ancestral gnathostomes
- Origin of gnathostome innovations

Broad characteristics



- Abundant species on European coasts
 - Easy to maintain in laboratory conditions
 - Overlong generation time
 - Rate of egg production/female: loose seasonality, 2 eggs every two weeks
 - Local production: 1000-2000 eggs per year
- One of the very few experimentally tractable chondrichthyan species

Developmental characteristics



Clichés: R. Lagadec, B. Godard

- Slow : hatching after 5-6 months at 16°C
- Telolecithal egg
- Large blastoderm, large embryo size
- Relatively inaccessible: collagen egg case
- Embryo development outside egg case:
only from advanced orgagenesis stages

Methodological tools

Evolution of :
gene/organ profiles
neuronal connectivity patterns
developmental mechanisms
regulatory landscapes

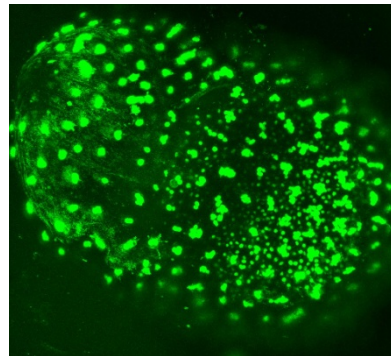
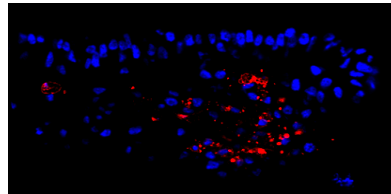
Descriptive approaches



Cliché: M. Coolen

- ISH
- IHC
- Neuronal tract tracing
- Section/whole-mount confocal imaging

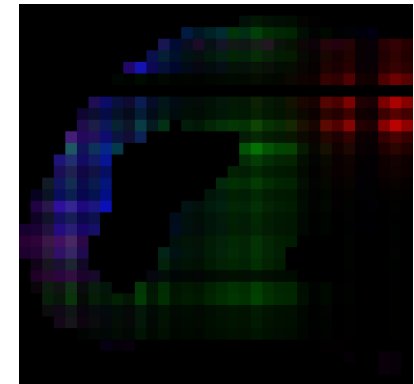
Experimental approaches



Clichés: B. Godard & R. Lagadec

- Cell tracing using lipophilic dyes
- BrdU incorporation
- Functional analyses of signaling pathways

Genomics/transcriptomics

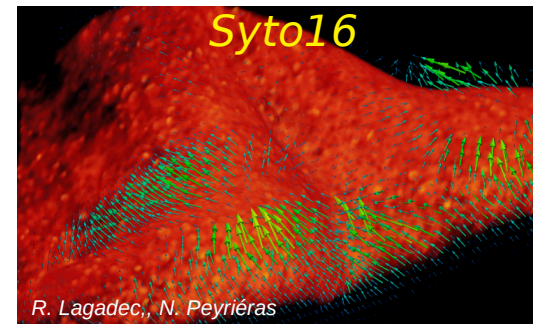
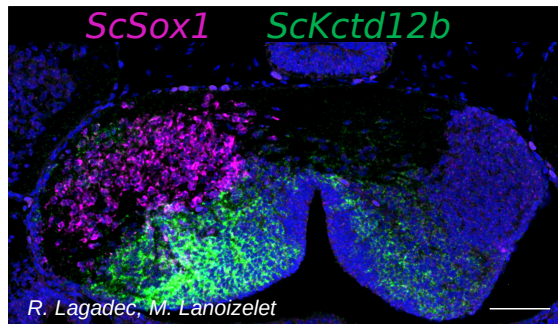


Data: H. Mayeur & R. Lagadec

- scRNA-seq
- ATAC-seq
- Spatial transcriptomics

➤ Experimentally accessible evo-devo model

Imaging catshark embryos

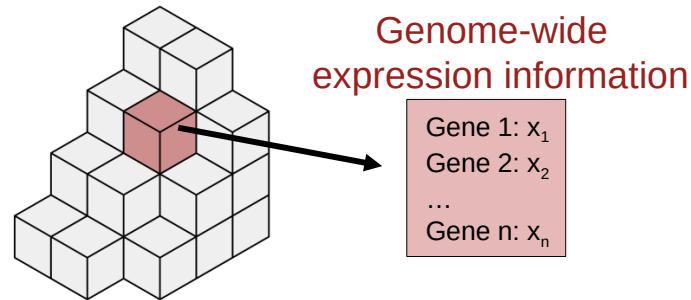


- Fluorescent IHC, ISH on sections : +++
no background or autofluorescence
- Time-lapse imaging : +/-
major limitations due to embryo inaccessibility, non-transparency
- 3D imaging : ++
Clearing protocols to optimize depending on stage/tissue/tracers
Size possibly challenging
Light sheet microscopy set-ups to optimize

3D RNA profiling: RNA tomography

Objective: genome wide 3D RNA profile of embryo, organ, tissue

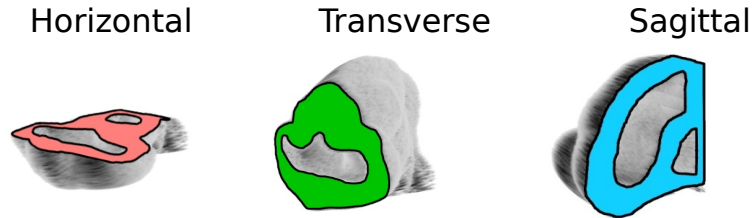
Generation of 3D digital model: n voxels



Multiple questions in developmental biology
Comprehensive cross-species comparisons
No a priori knowledge needed

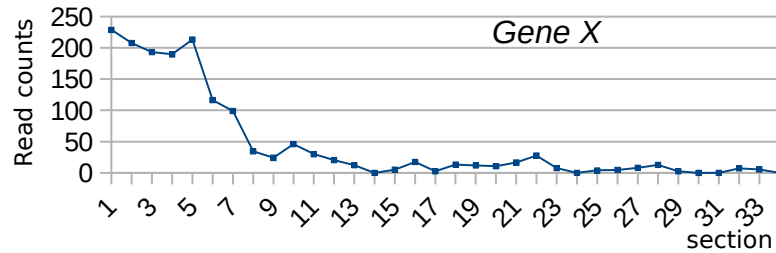
RNA tomography: 3 main steps

1. Sectioning across 3 planes

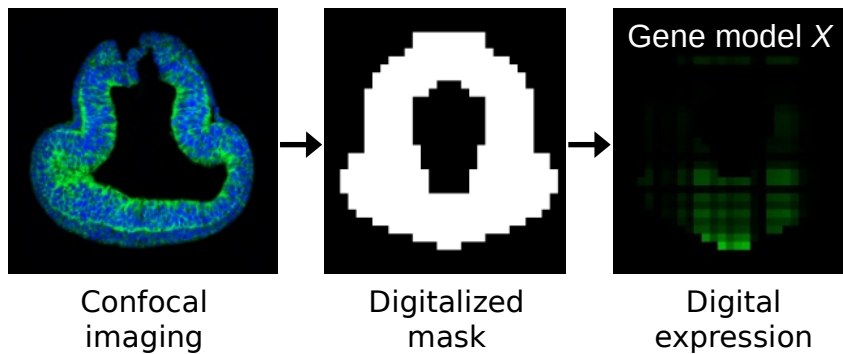


2. Illumina library construction from serial sections – sequencing

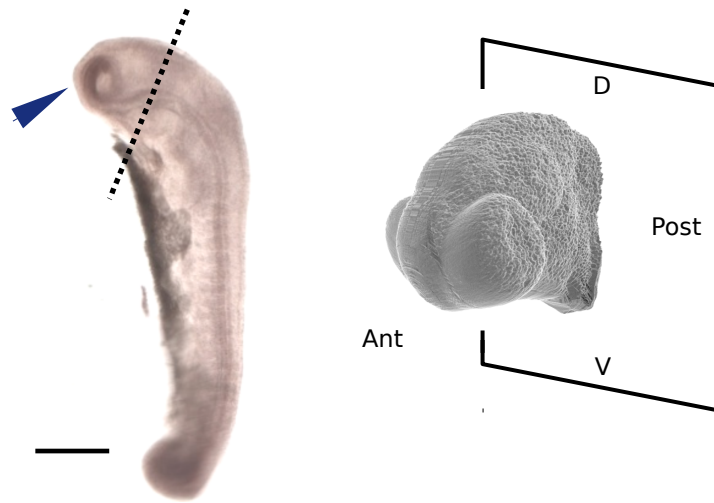
1D profile along each plane



3. Projection on 3D digital model of tissue (Iterative Proportional Fitting)

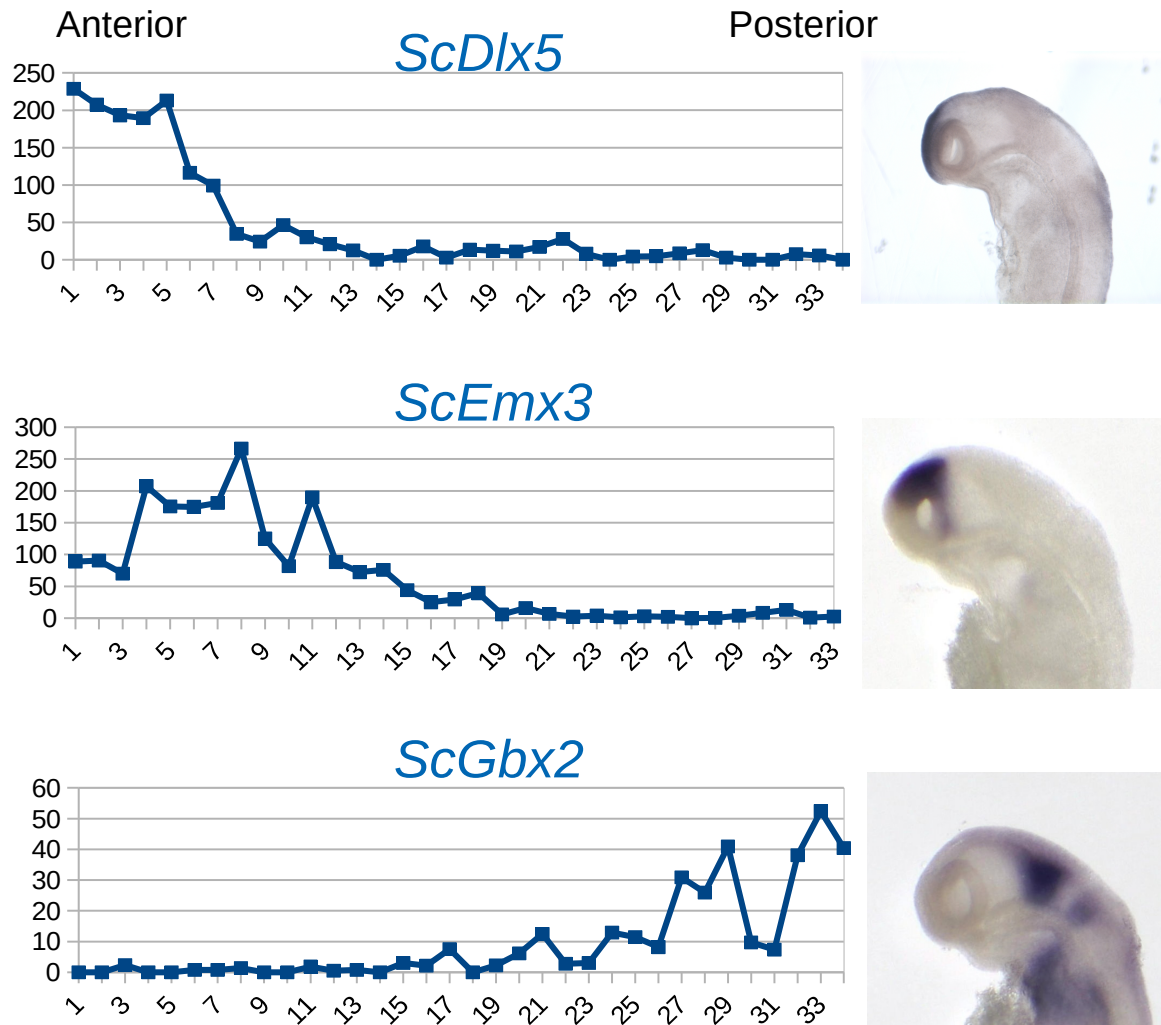


Generation of a 3D RNA profile: embryonic catshark head, stage 17



- Sequencing: total of 97 sections (18 μm)
- PE100: 90 M reads
- Expression information:
 - 30 000 genes (21 000 coding)
 - 12725 voxels

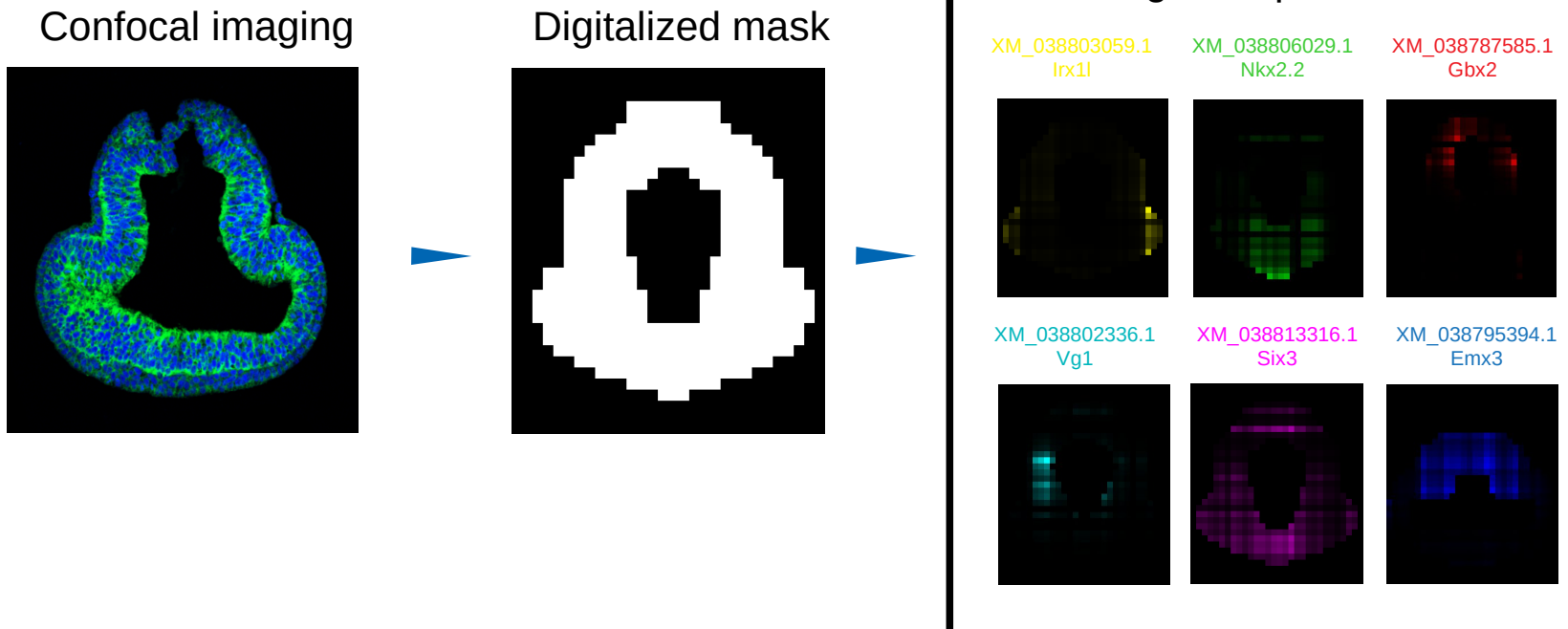
Regionalized profiles along each axis



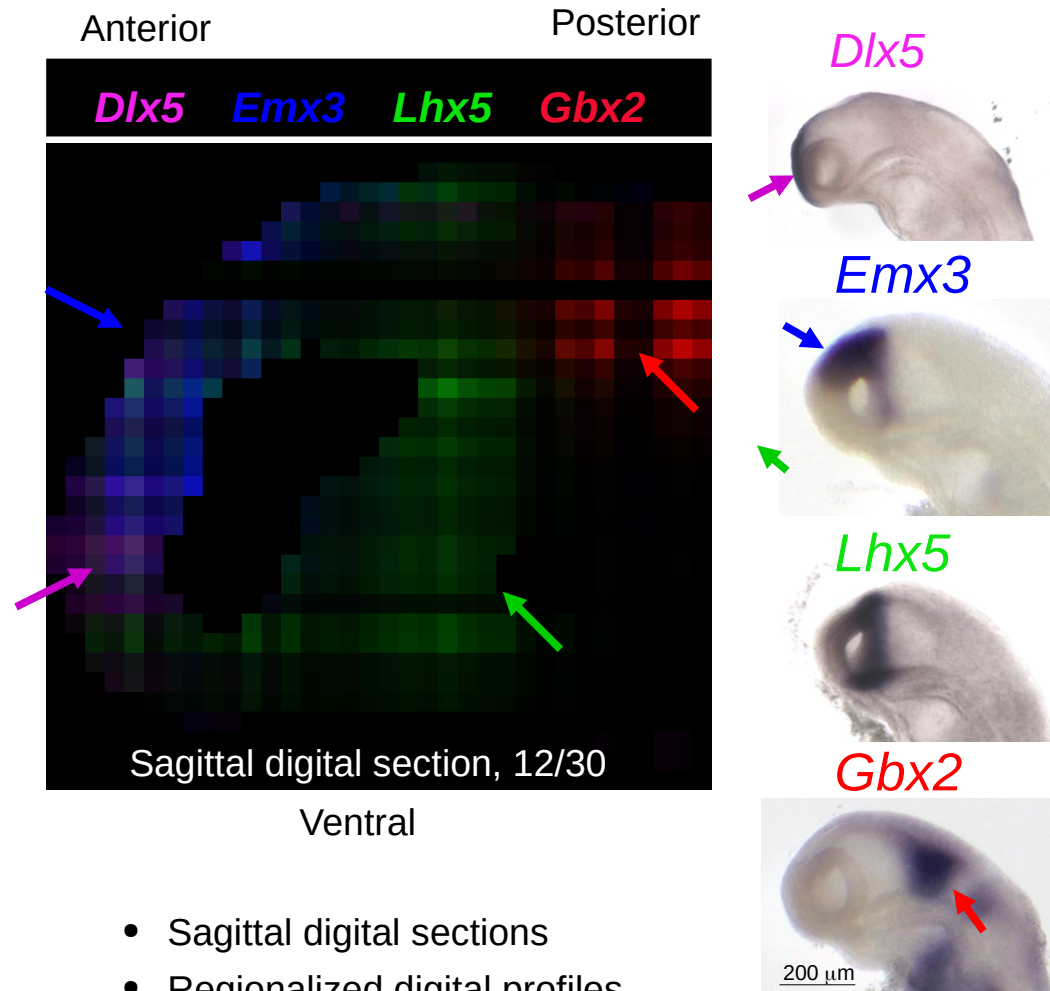
- Consistent with ISH results

Generation of a genome-wide 3D RNA profile

- Confocal imaging and generation of 3D mask
- Projection of section reads onto mask
- 12725 voxels with quantified expression data for 20040 gene models

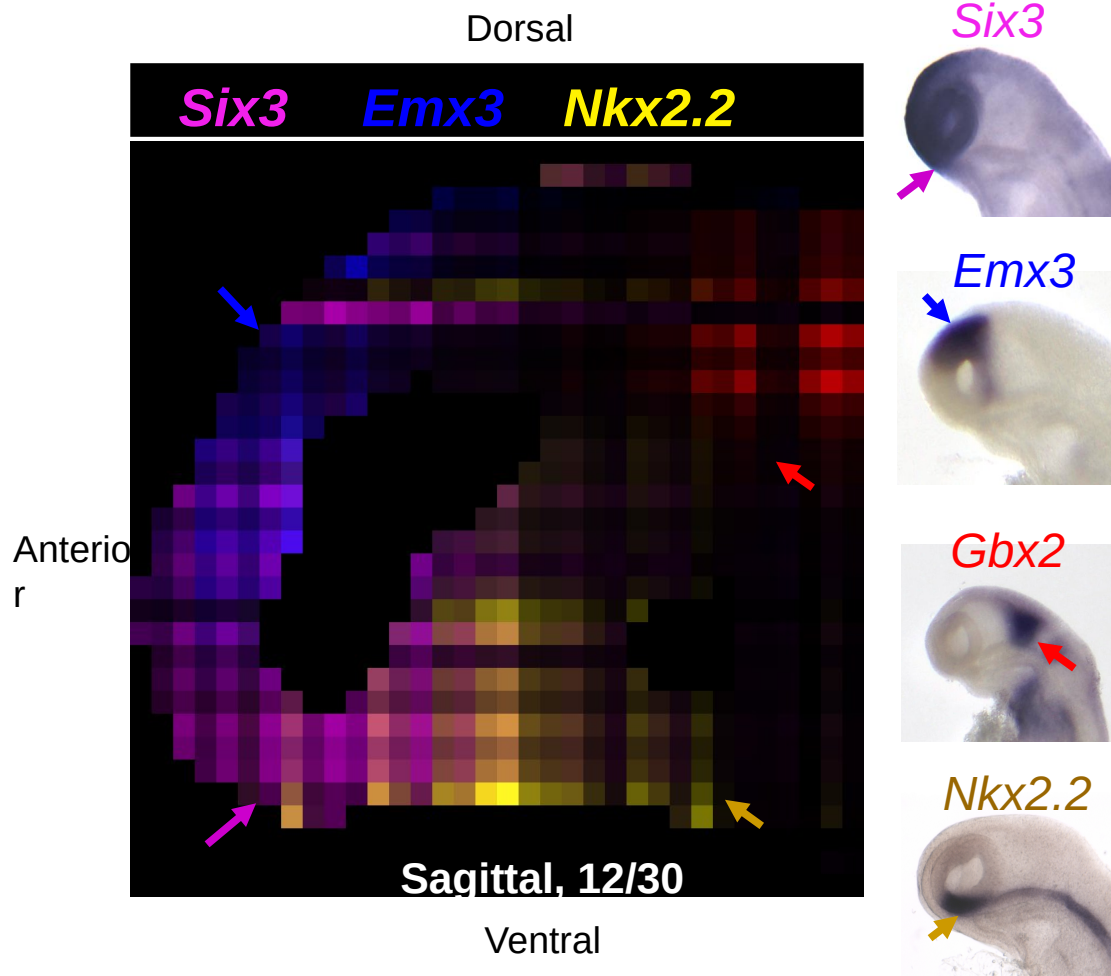


Validation: regionalized profiles along AP axis



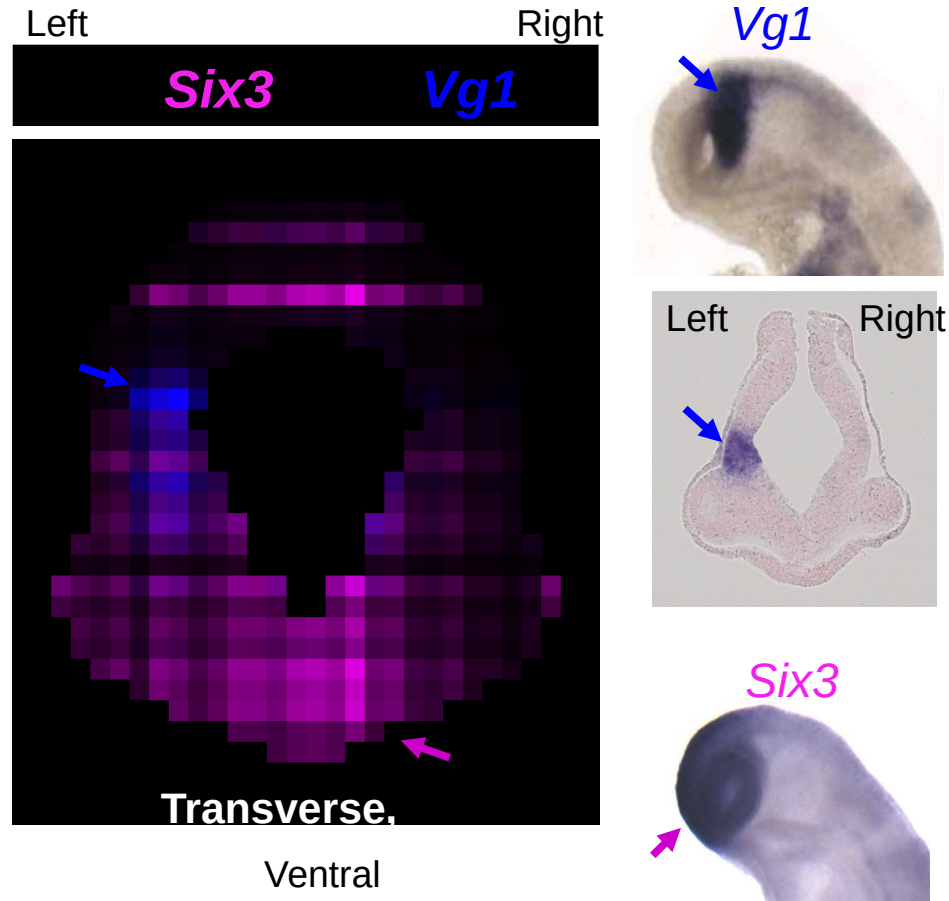
- Sagittal digital sections
- Regionalized digital profiles
- Consistent with ISH results

Validation: regionalized profiles along DV axis



- Consistent with ISH results

Validation: regionalized profiles along LR axis



- Transverse digital sections
- Left-restricted digital profiles
- Consistent with ISH results

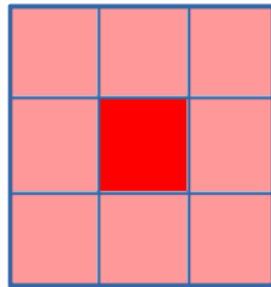
Extracting novel information

Pearson correlation

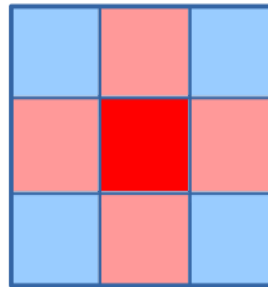
$$r = \frac{\sum (x - m_x)(y - m_y)}{\sqrt{\sum (x - m_x)^2 \sum (y - m_y)^2}}$$

1. Autocorrelation

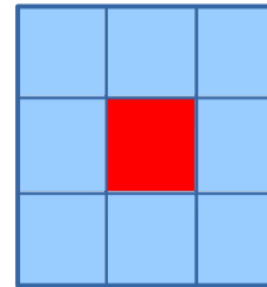
Objective: search for regionalized profiles



Autocorrelation > 0



Autocorrelation = 0

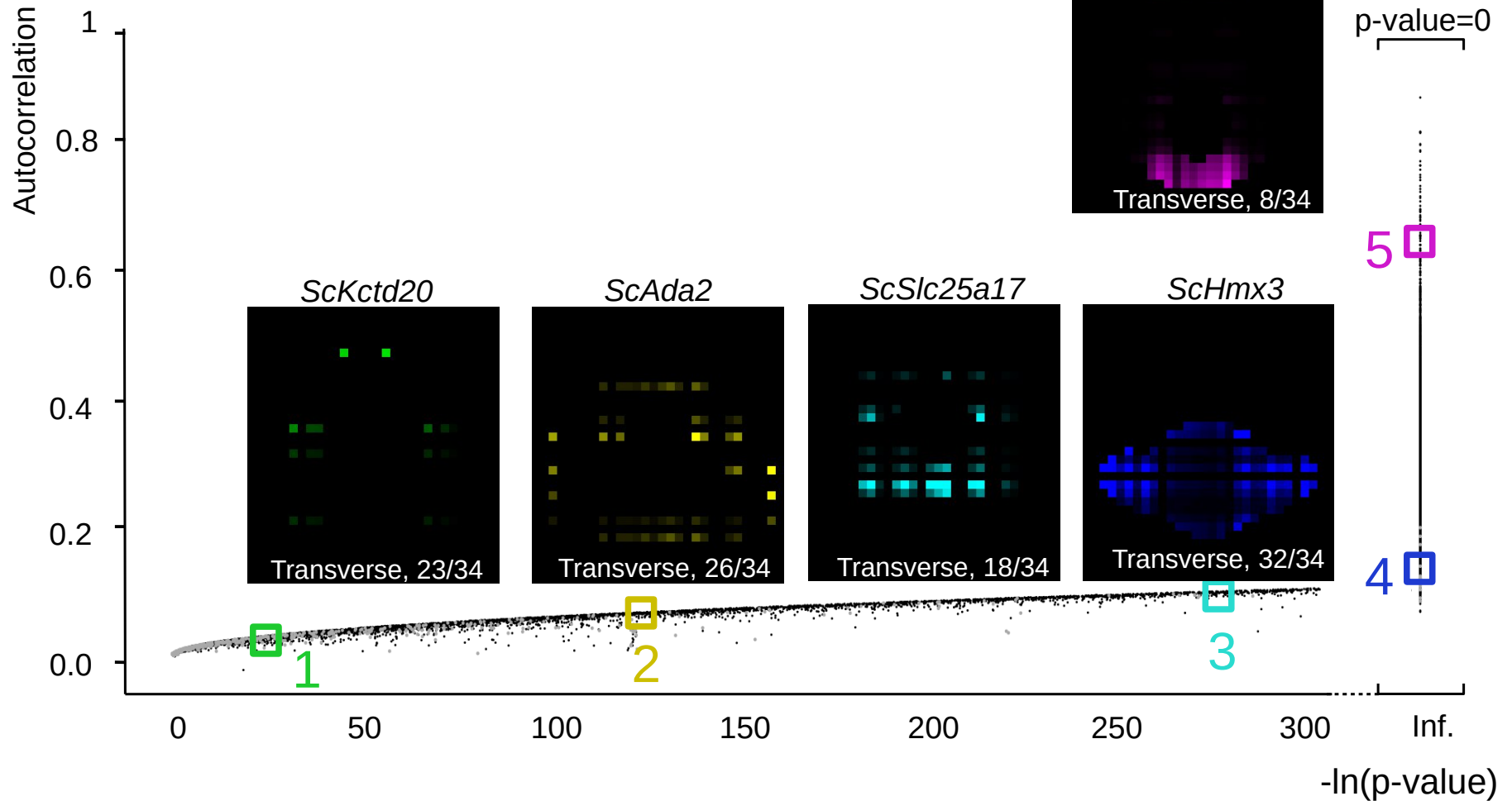


Autocorrelation < 0

- Pearson correlation between expression of each pair of “neighboring” voxels
- Autocorrelation value and P-value for each gene

A quantified regionalization indicator

- High autocorrelation/p-value=0: Highly regionalized profiles
- Low autocorrelation/high p-value: dispersed positive voxels
- More than 50% genes with regionalized digital profiles



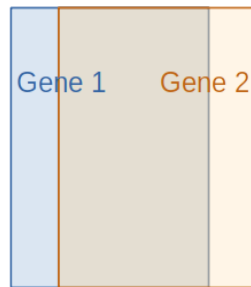
Extracting novel information

2. Correlation

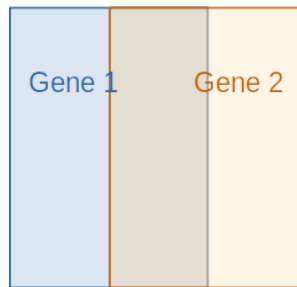
Pearson correlation

$$r = \frac{\sum (x - m_x)(y - m_y)}{\sqrt{\sum (x - m_x)^2 \sum (y - m_y)^2}}$$

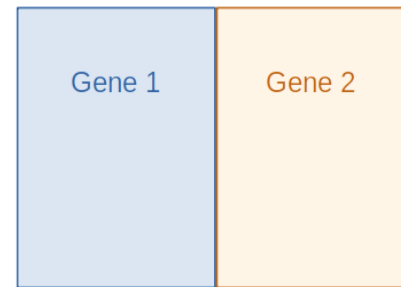
Objective: characterize the gene repertoire expressed in a given territory



Correlation > 0



Correlation = 0

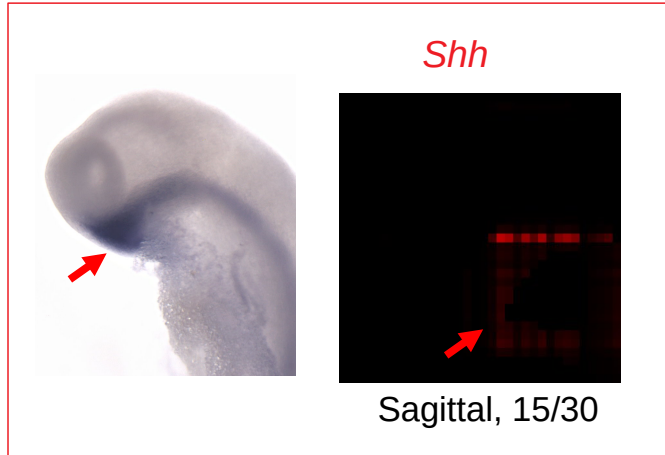


Correlation < 0

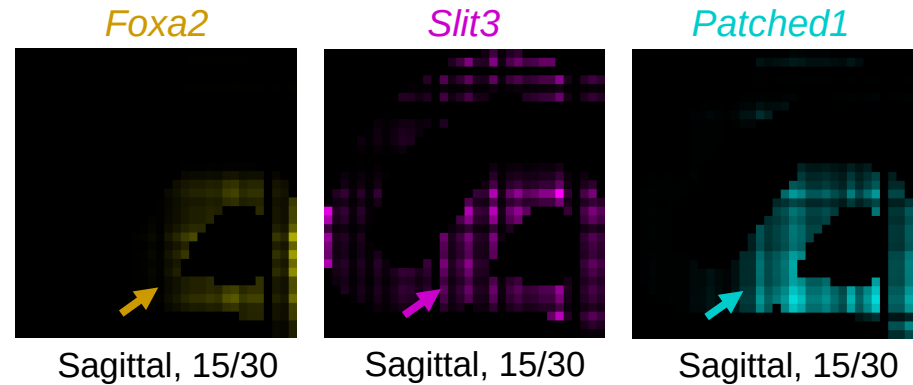
- Pearson correlation between expressions of a reference marker and other genes in all voxels
- List of correlation values for all genes relative to the reference marker

Application: search of genes correlated to a reference marker

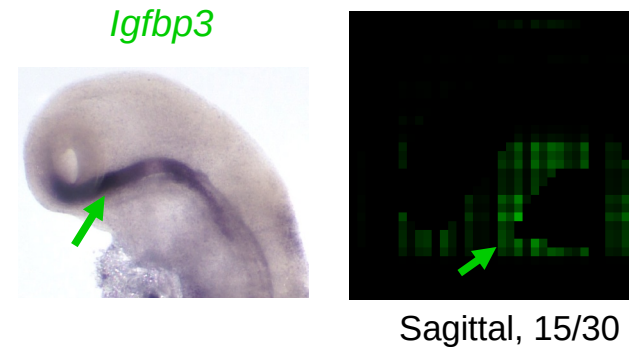
Shh (ventral midline)



Correlation with *Shh* > 0.4 (ventral midline)



- 15 genes with Pearson correlation > 0.4
- Include known midline markers or *Shh* targets such as *Foxa2*, *Slit3* or *Patched1*
- Novel markers, such as *Igfbp3*



- A relevant tool to identify novel regional markers

RNA tomography in catshark: conclusions



- Generation of a reference dataset in the catshark
- Bioinformatic tools to extract novel information
- Limitations:
resolution, best suited to polarised profiles, minimal organ size needed
- Advantages:
genome-wide information, robust and fast, few experimental adaptations needed, cost effective, applicable to a broad range of species/organs
- Ideally suited to large organs /embryos such as in catshark
- Novel perspectives in a broad range of organisms

Thanks to:

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Development and evolution of vertebrates

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