

Single cell transcriptome analysis in *Ciona intestinalis*

2016 07 11

**Ritsuko SUYAMA
Filipe TAVARES-CADETE
Garth ILSLEY
(Luscombe Unit)**



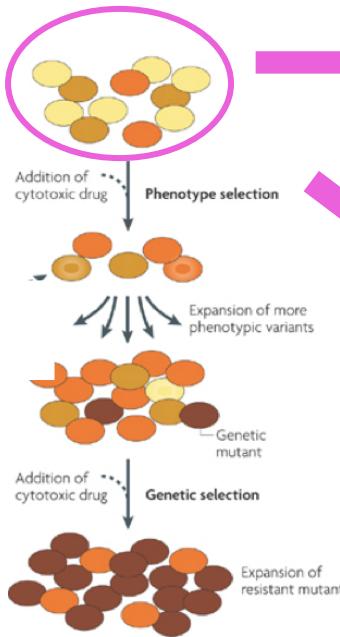
OIST

OKINAWA INSTITUTE OF SCIENCE AND TECHNOLOGY GRADUATE UNIVERSITY

Single cell transcriptome analysis

Advantages of scRNA-Seq

1) Heterogeneity



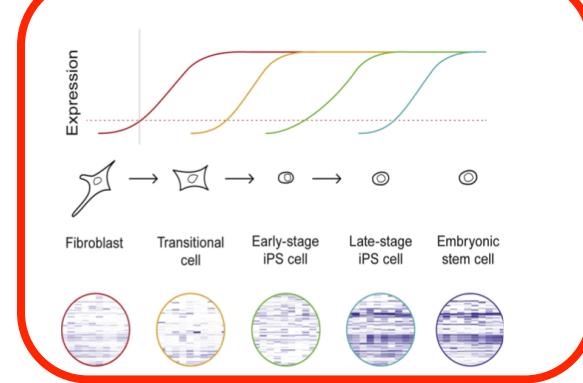
Average expression of
Cell population

Individual
analysis
by scRNA-Seq

Brock A et al., 2009

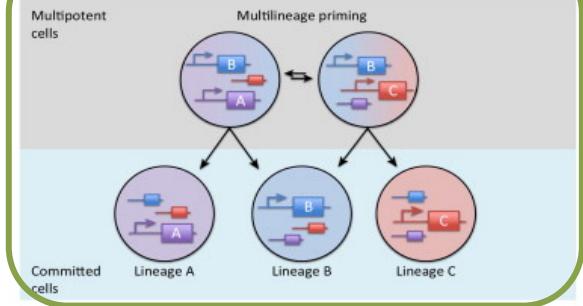
- 1) Dissect cellular heterogeneity by individual gene expression
- 2) Dynamic changes in expression during reprogramming
- 3) Delineate the process of lineage commitment

2) Reprogramming



Daniel H Kim et al., 2015

3) Lineage commitment



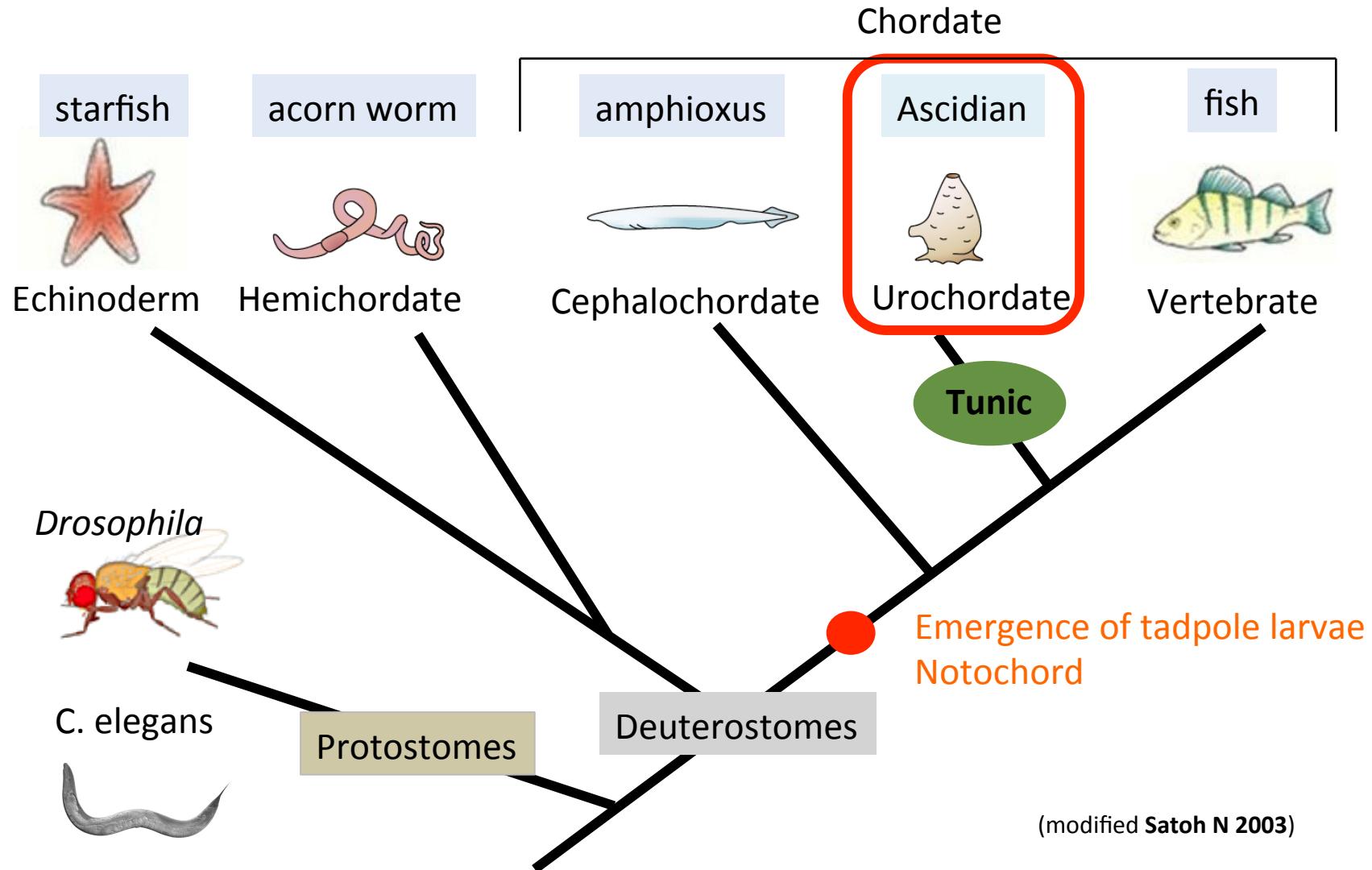
Nimmo RA et al., 2015

Our model: *Ciona intestinalis*

Ciona intestinalis
(Ascidian, sea squirt)



The evolution of Chordates



Tadpole-type larva of the ascidian *Ciona intestinalis*

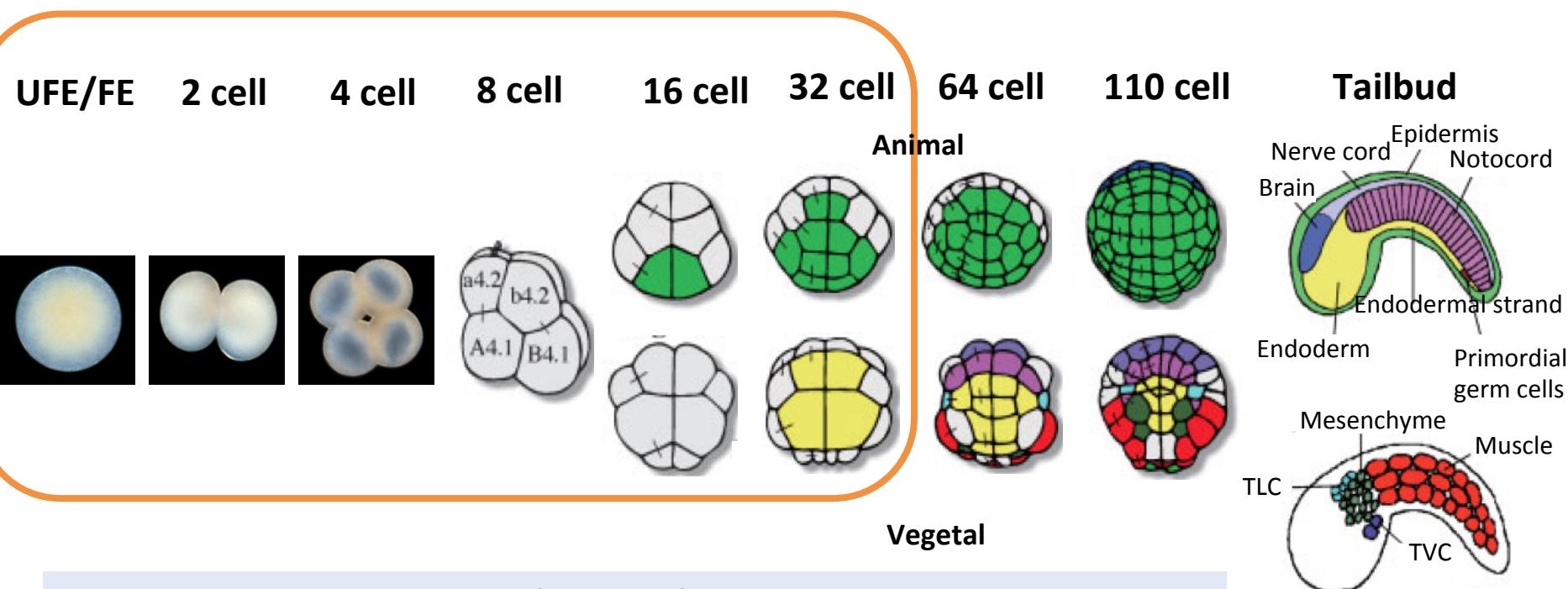


- Low cell number in tadpole larvae.
(~2600 cells)
- The blastomeres of early ascidian embryos are large and easy to manipulate.
- The developmental cell fate is defined.

(Satoh N 2003)

Developmental fate of *Ciona* embryo

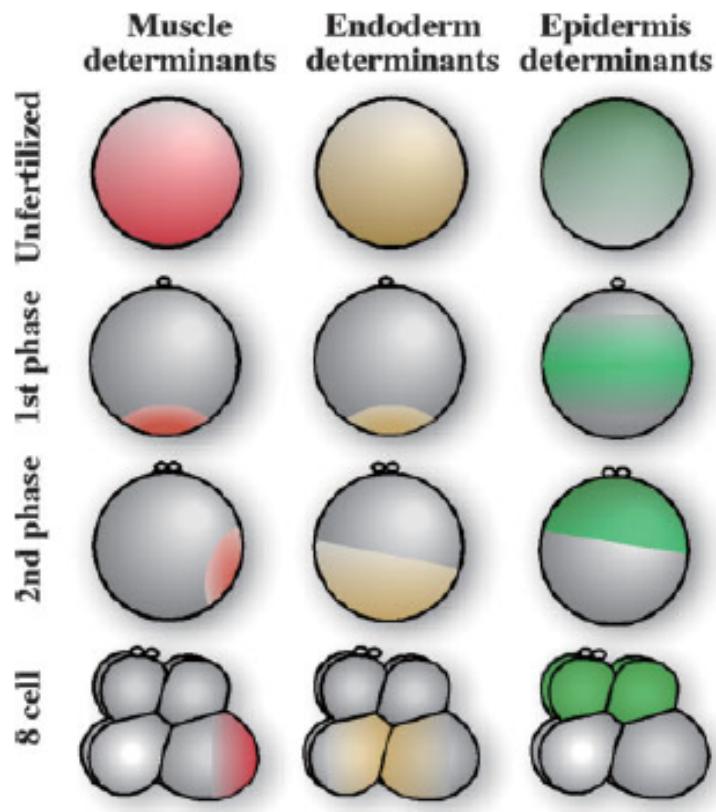
- Lineage and molecular events in *Ciona* embryo



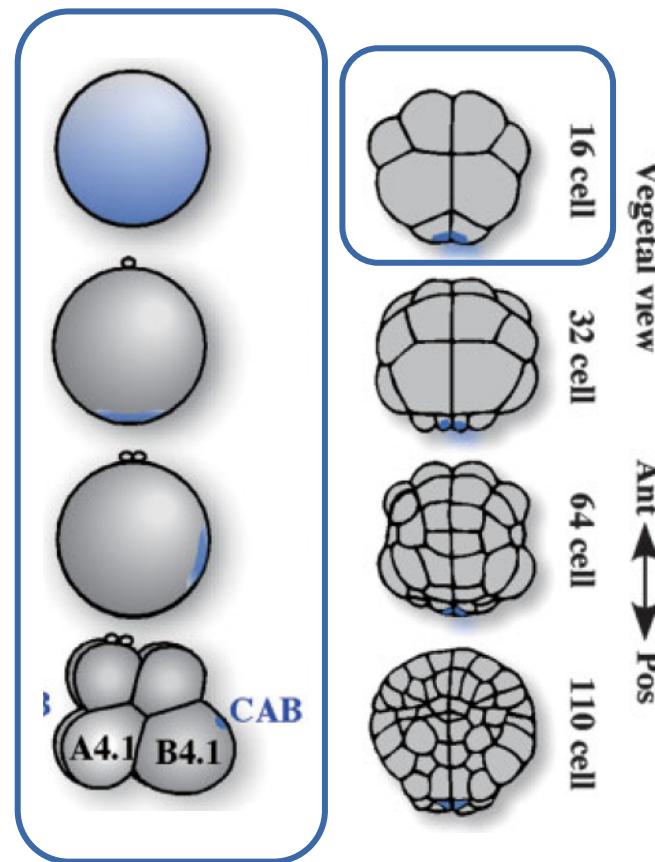
- Low cell number in tadpole larvae (~2600 cells).
- The blastomeres of early ascidian embryos are large and easy to manipulate (150um).
- The developmental cell fate is defined.

(Nishida H 2005)

Localization of maternal factors at the egg stage determine later cell lineage

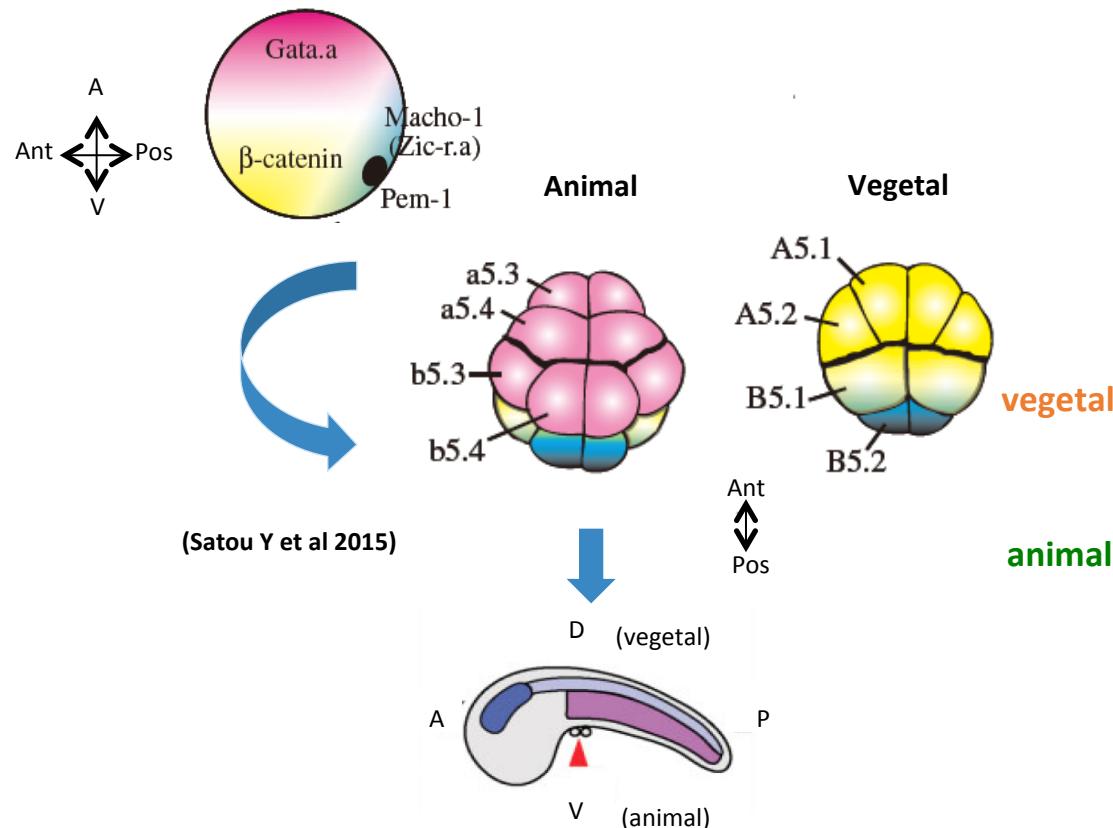


Postplasmic mRNA/PEM is localized to the posterior pole: posterior development

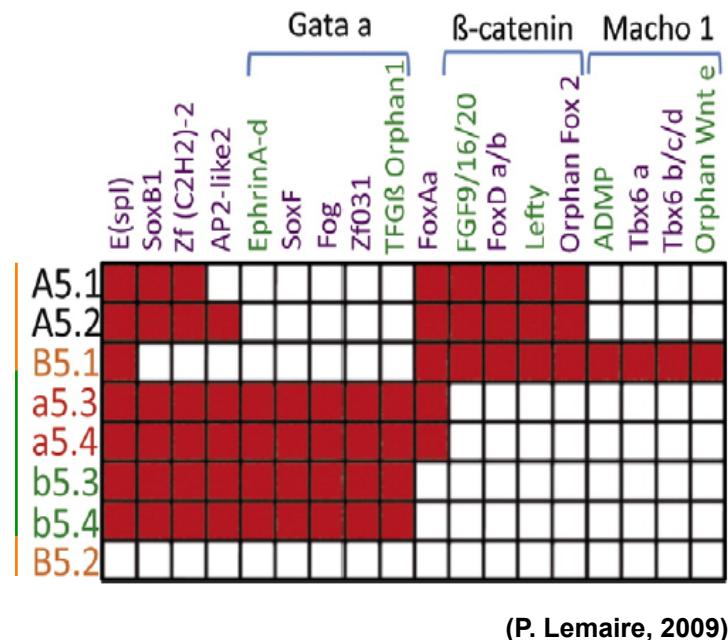


Maternal genes determine body axis and specify zygotic expression patterns

(1) Maternal genes : Postplasmic mRNA/PEM [B5.2 localized]



(2) Zygotic genes



- Define A-P axis
- Unequal cleavage at the posterior pole
- Formation of primordial germ cell

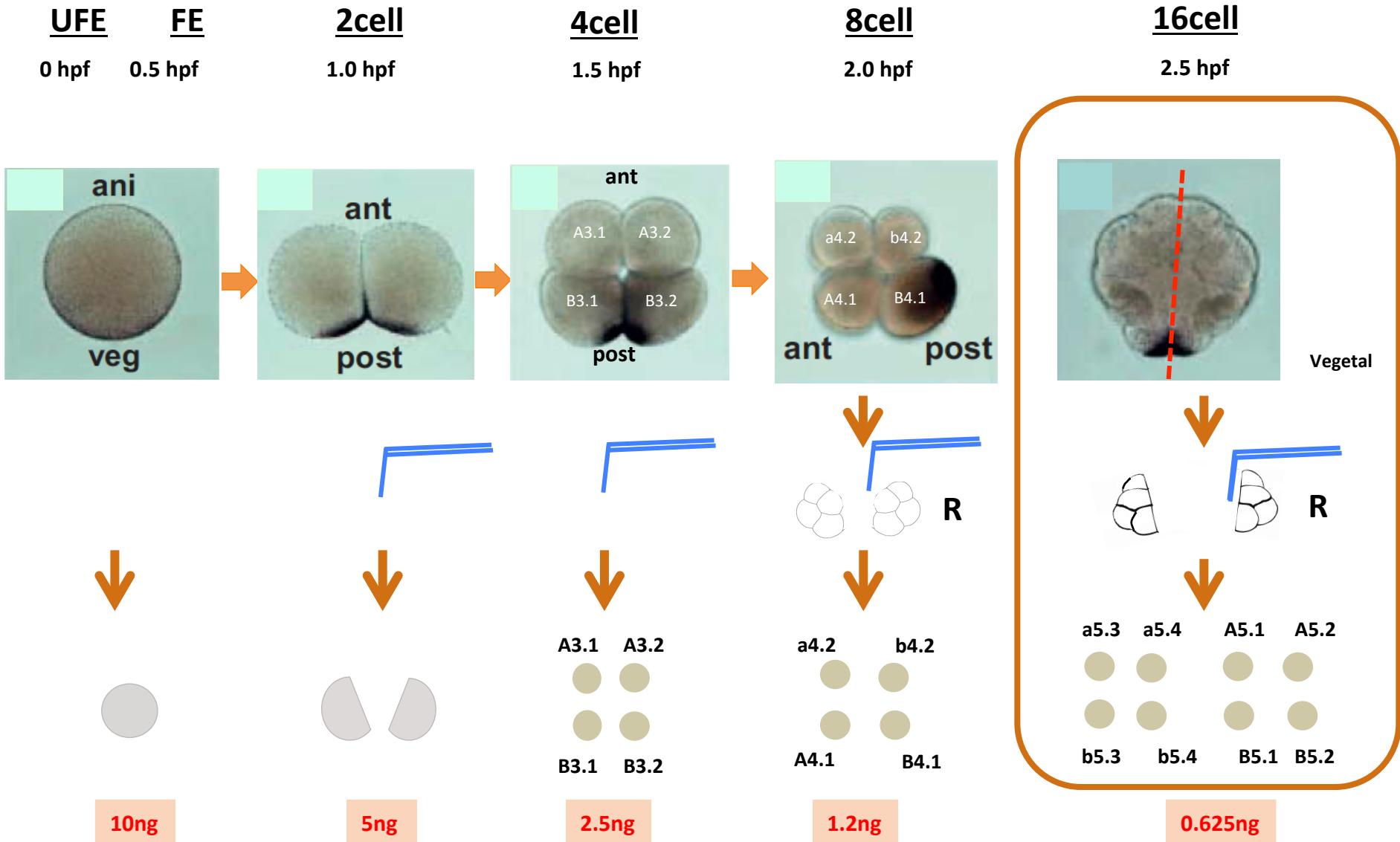
- Gata- α : ectoderm specification
- β -catenin : endoderm specification
- Macho-1: muscle specification

Project objectives

Study *Ciona* development at the single-cell level

- Establish single cell RNA-Seq in *Ciona* embryo.
- Measure changes in gene expression during embryonic development.
- Identify new cell lineage determinants.
- Explore new patterns and genes with cell-specific expression.

Experimental design



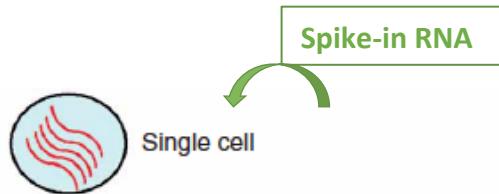
How to dissect single cells



Tang's Method in NGS

STEP1

- Cell lysis
(5~6hrs)



- cDNA synthesis
(~1hr)



- Primer removal
(1.5hr)



- Poly(A) tailing
(1hr)



- Second-strand cDNA synthesis
(1hr)



- PCR amplification [1st] (5hrs)



+UP1

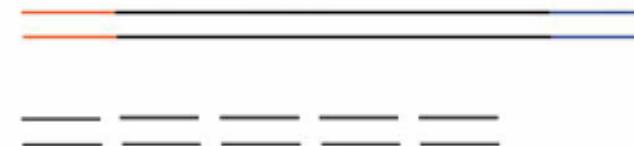
- PCR amplification [2nd] (5~6hrs)



+AUP1, 2

STEP2

- DNA shearing
(3~4hrs)



- Adaptor ligation
(7~8hrs)



- PCR amplification
(3hrs)

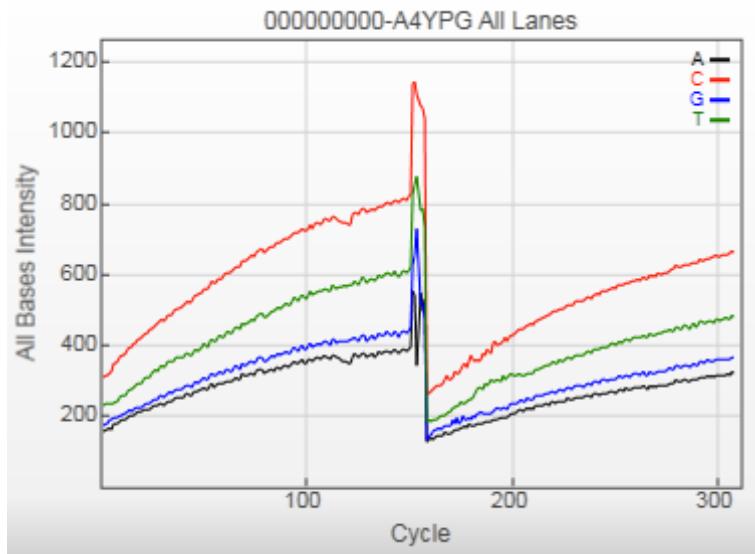


NGS analysis

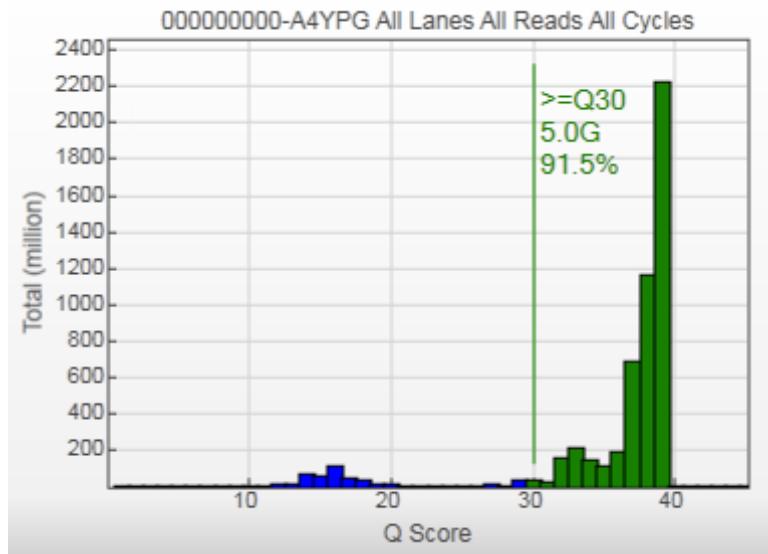
(Tang, F. et al., 2010)

MiSeq sequencing analysis by Tang's

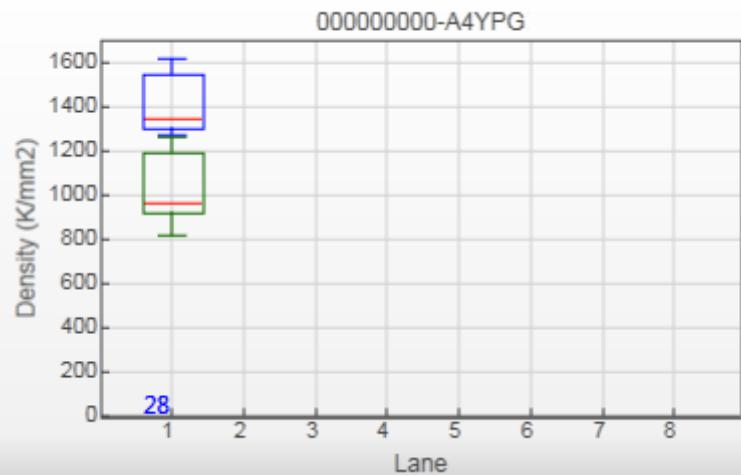
Signal intensity



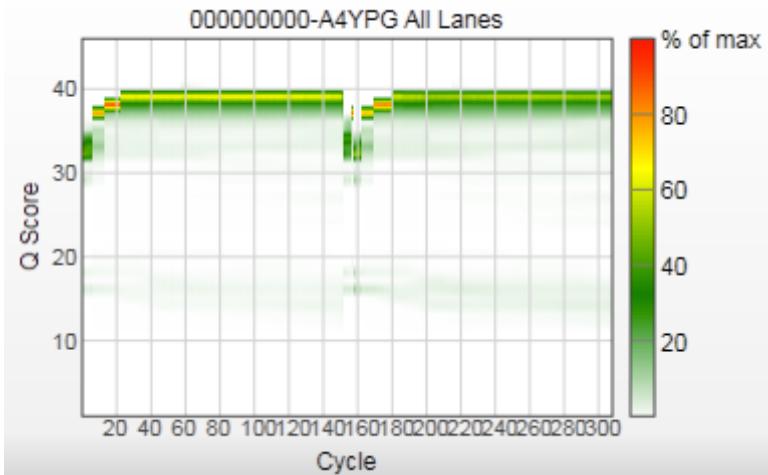
Q30 score distribution = Error (0.1%)



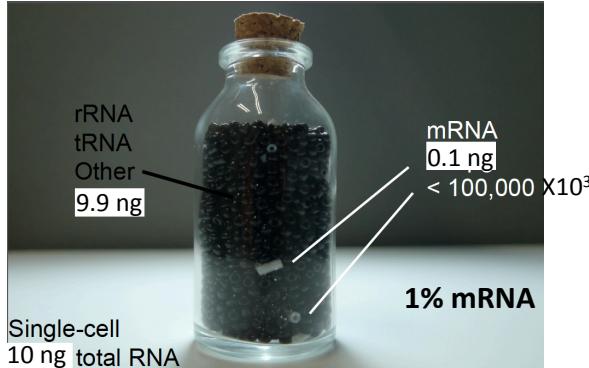
Cluster density



Q score heat map



MiSeq provides sufficient coverage for single-cell samples



<2013_illumina_rna-seq_quartz-seq>

Tang's

Synthesized cDNA

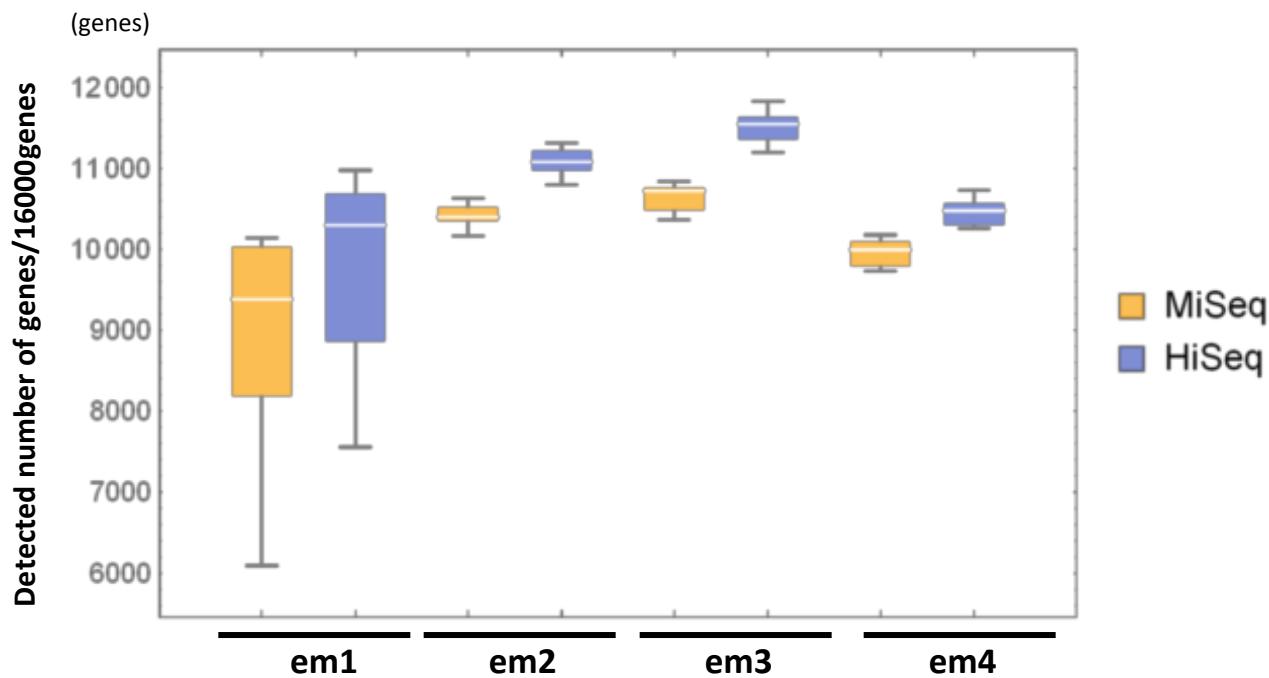
Full length cDNA amplification by PCR

cDNA fragmentation

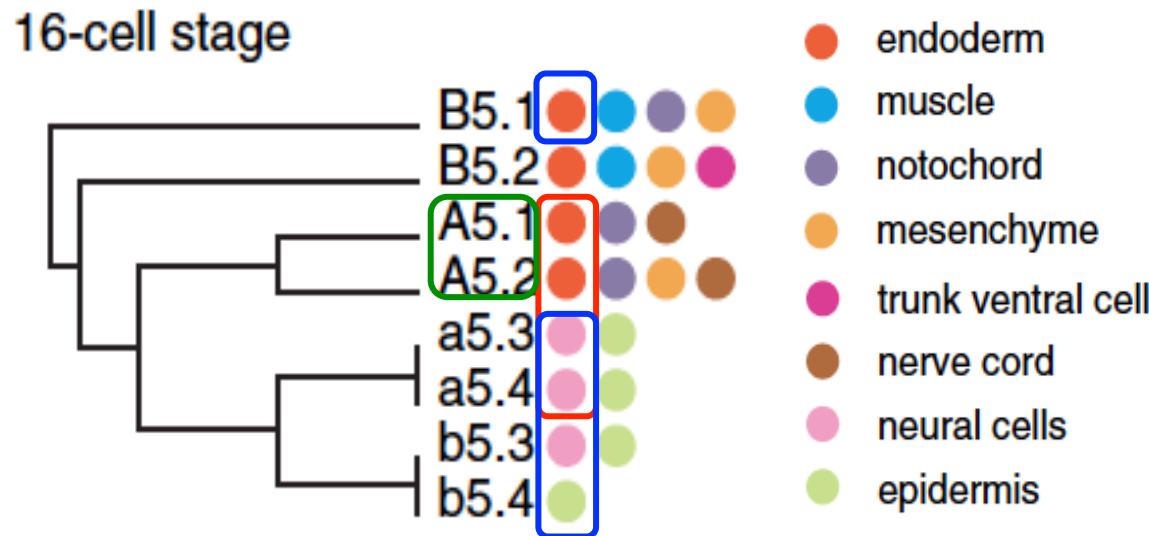
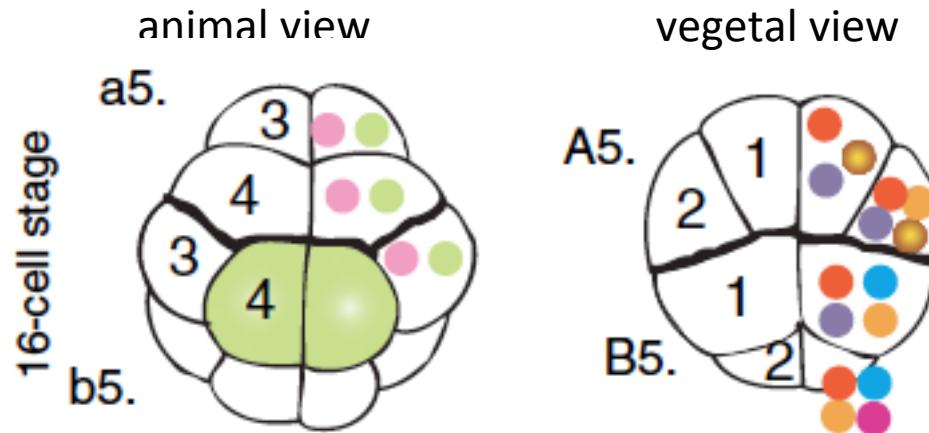
Attached adaptor for NGS and Amplification by PCR

MiSeq: 2 million reads/sample

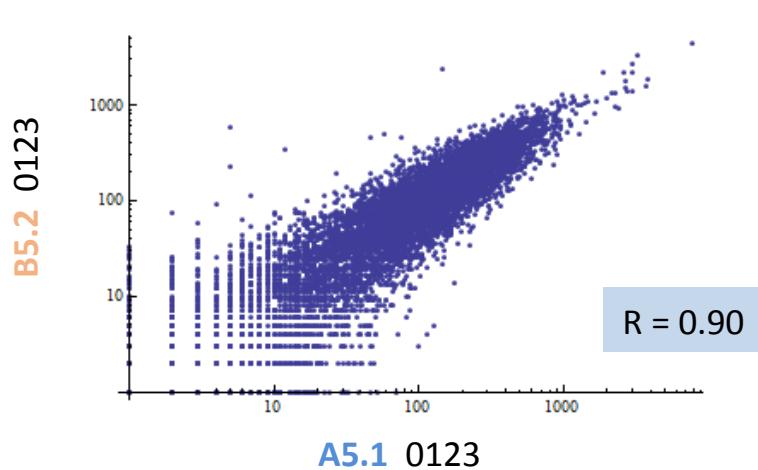
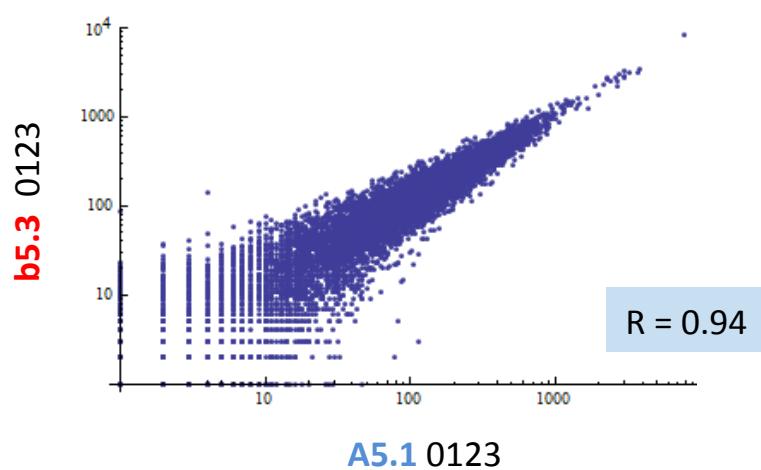
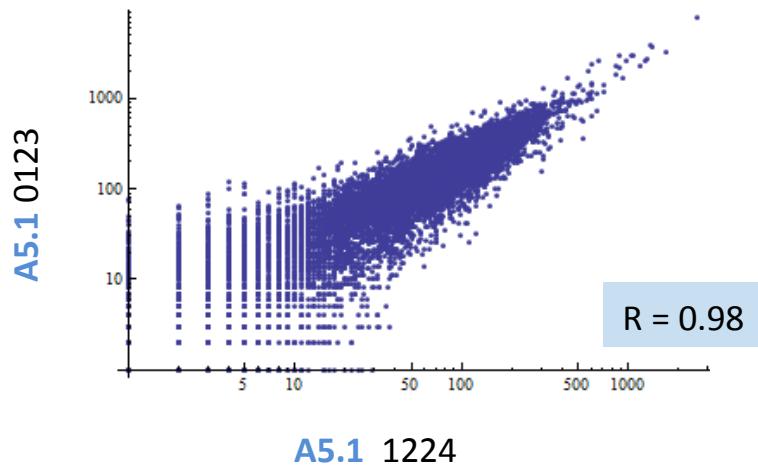
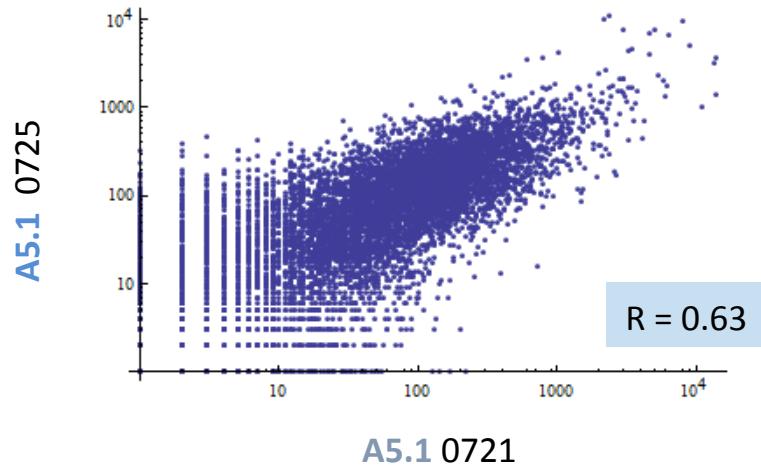
HiSeq: 20 million reads/sample



Expression pattern at 16 cell stage

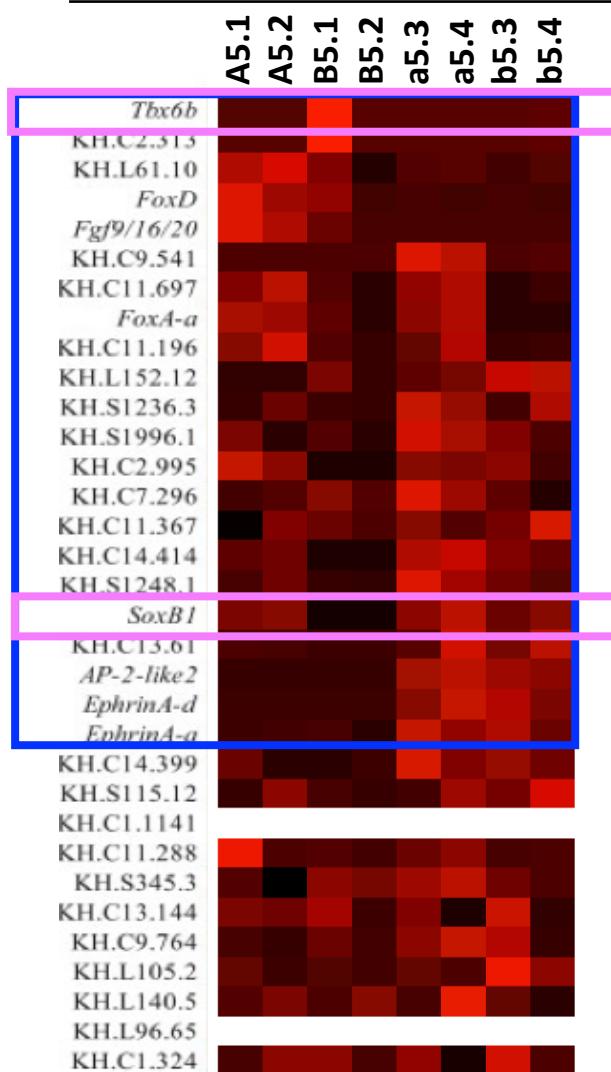


Correlation between experiments

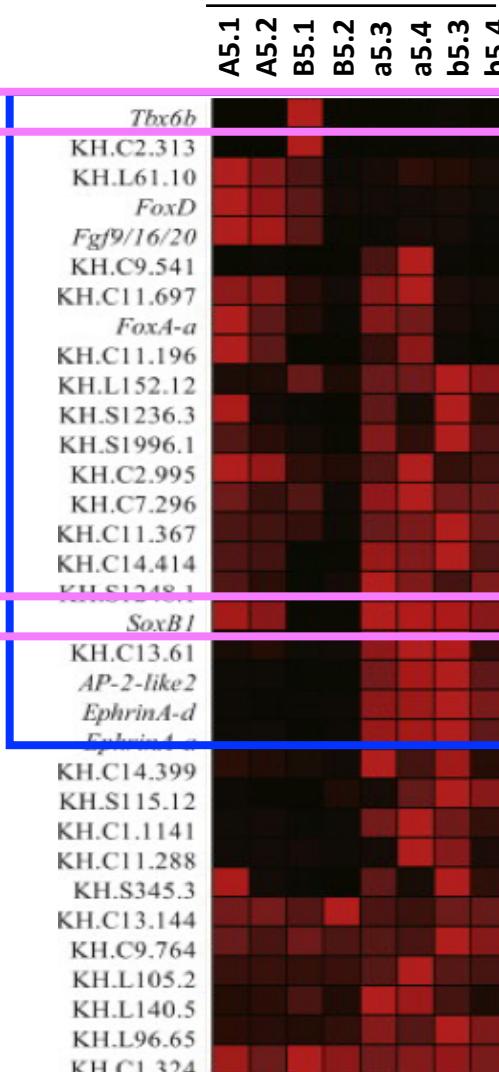


Comparison of the gene expression profile to Microarray and *in situ* data

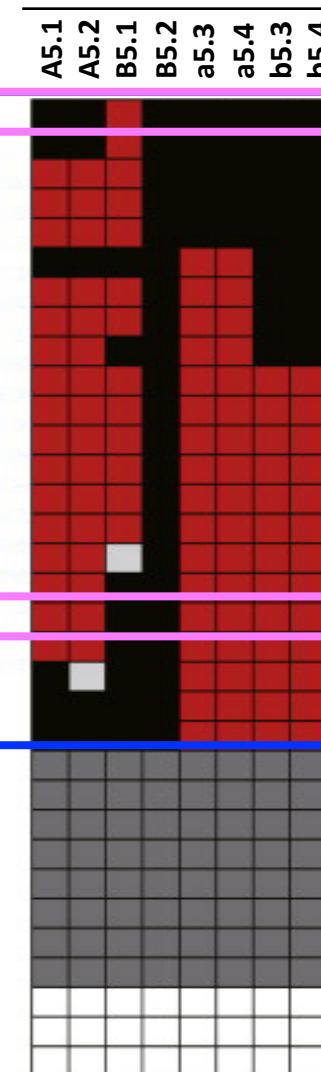
Single cell : RNA-Seq (Averaged)



Microarray

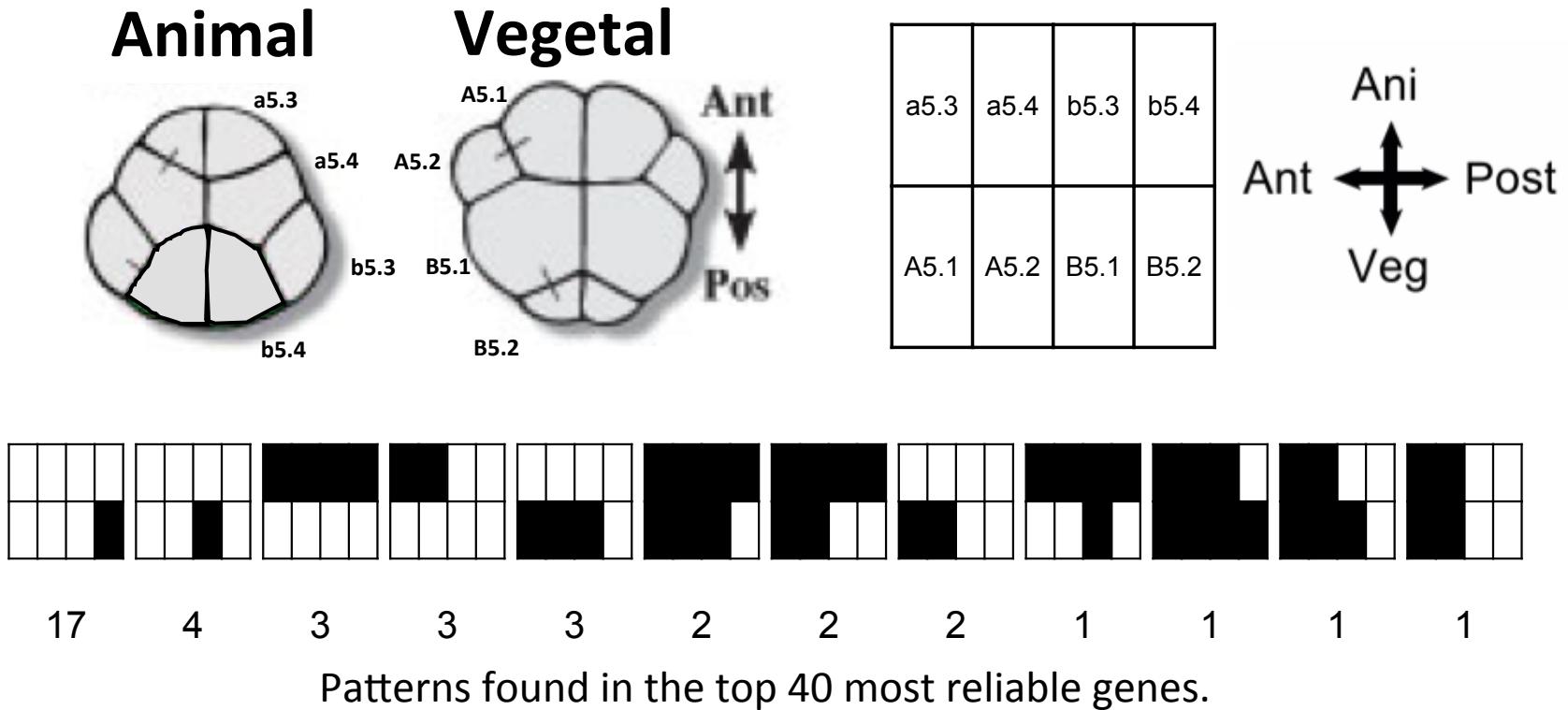


In situ hybridization

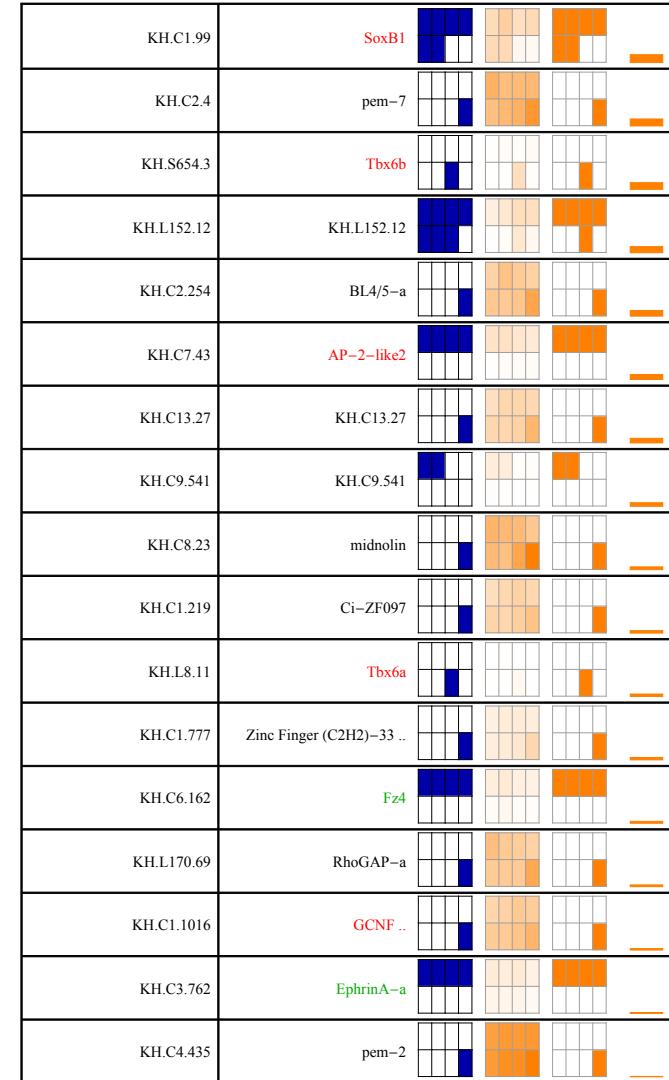
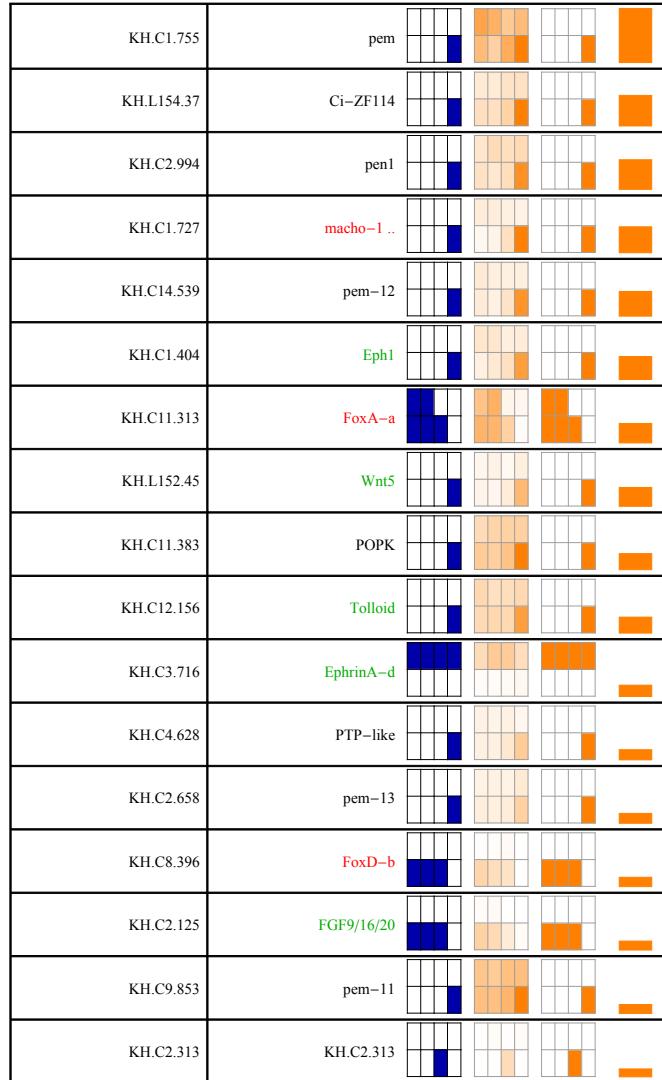


(Matsuoka et al., 2013)

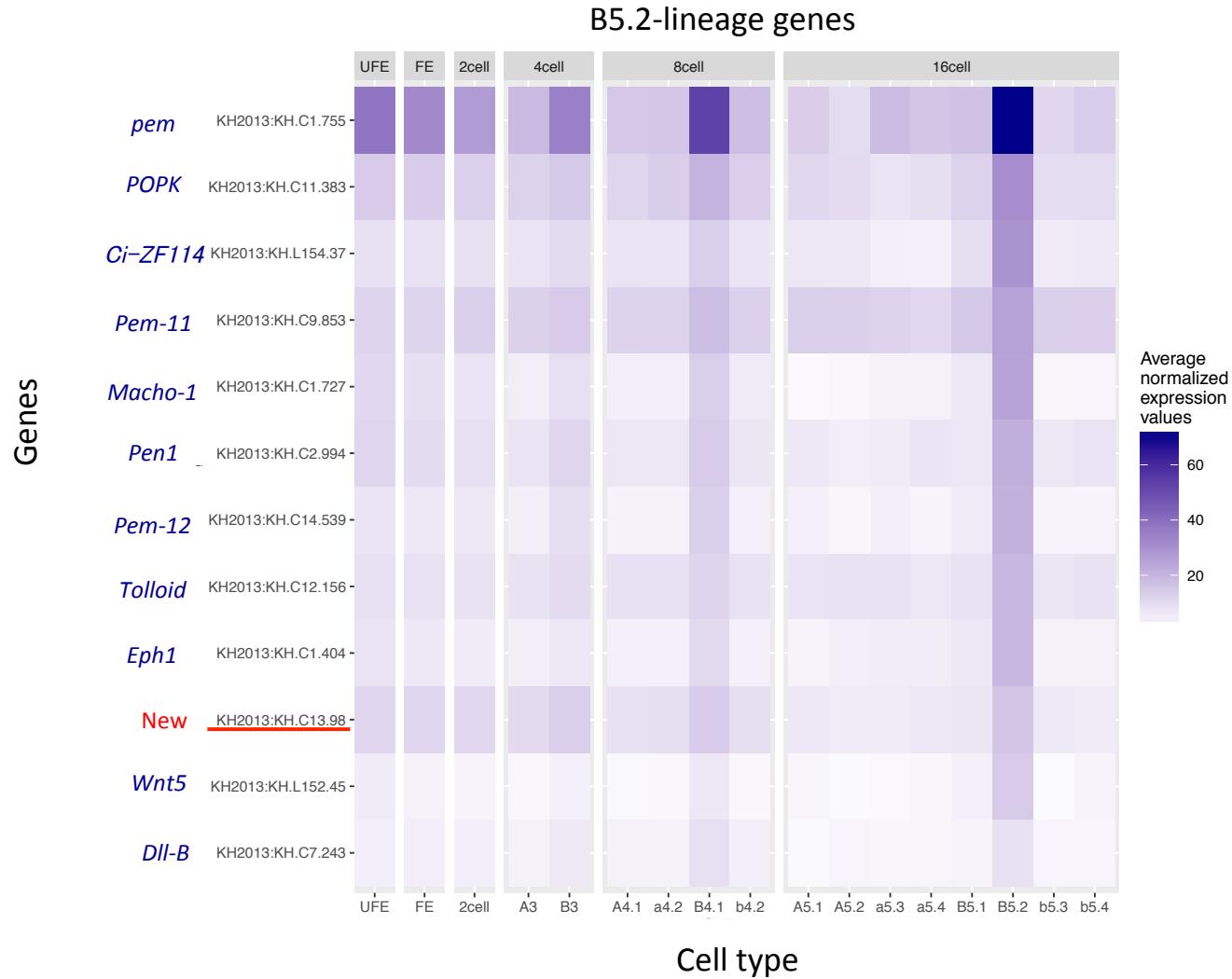
Patterns after transcription is active



High-reliability gene validation



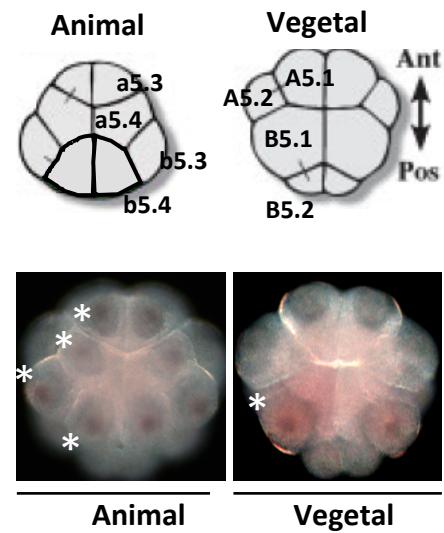
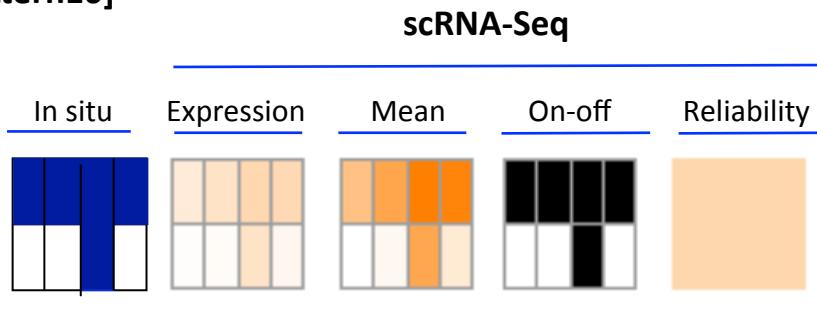
Strong signal from B5.2 lineage



New pattern validation

Zygotic genes : New pattern

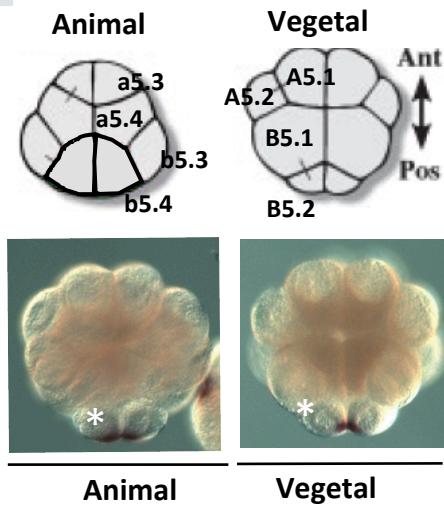
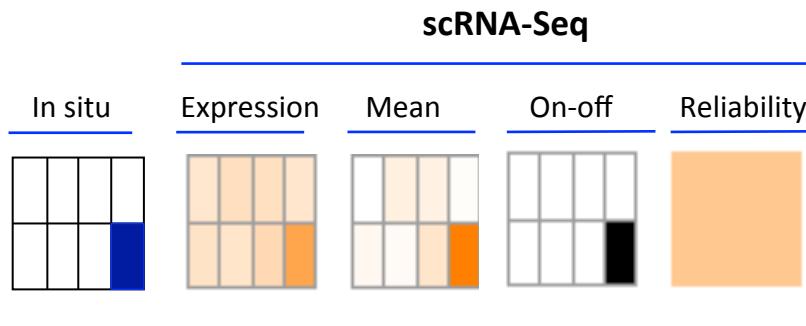
[KH.L152.12: Pattern10]



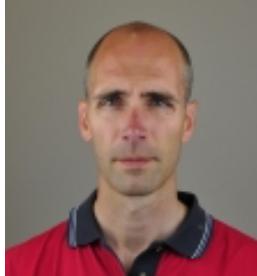
New pattern validation

Maternal genes : Postplasmic mRNA/PEM [B5.2 localized]

[KH.C13.98: Pattern1]



Acknowledgements



OIST Sequence Facility

JAPAN National BioResources Project

