ROUND TABLE DISCUSSIONS ON THE NEW AND DEVELOPING TOOLS
FOR EMERGING MODEL SYSTEMS IN EVO-DEVO

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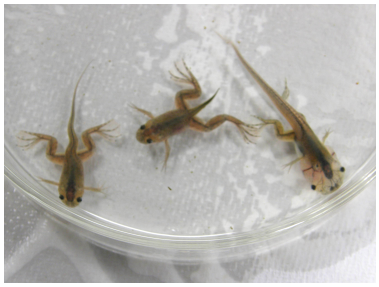
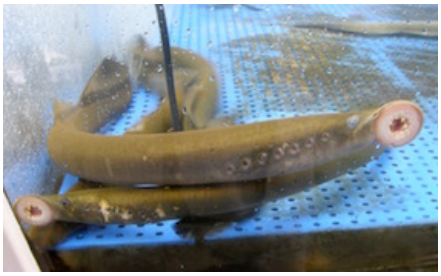
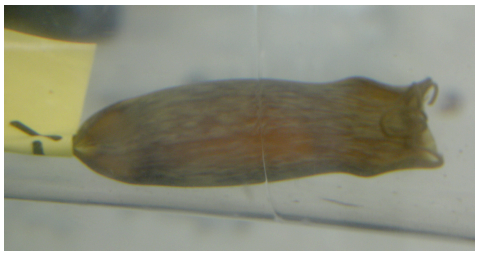
"Techniques for transient functional analyses of genes and cells" (mainly vertebrates)


Methods applicable to the species in which the establishment of stable transgenic lines is not practical

(lamprey, shark, chick, turtle,)

① Lineage tracing by dye injection

② Reporter assay (enhancer/promoter + reporter gene)

Japanese river lamprey, *Lethenteron japonicum*

- Cyclostomes (extant jawless vertebrates)
- 7 gill pores, median fins
- No jaw
- No paired limb

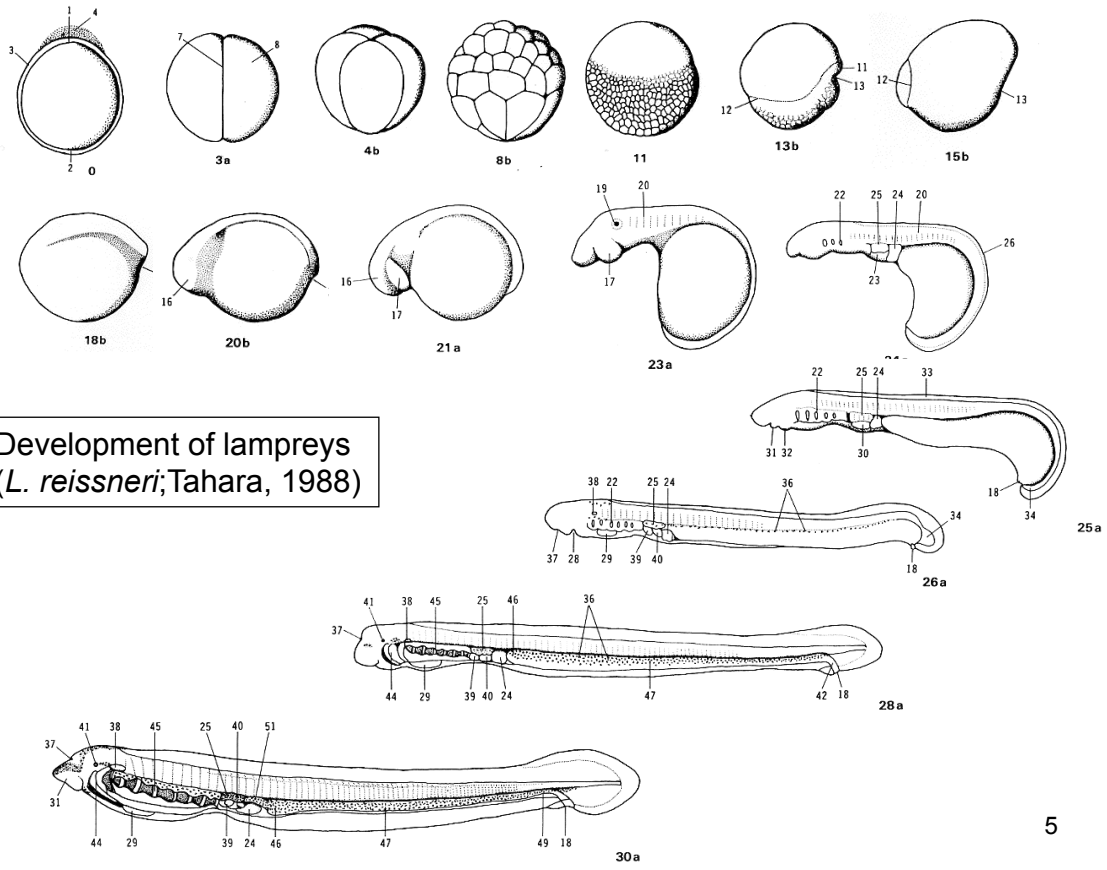
The lamprey adults are about 40 cm in body length. Their basic body plan does not differ from that of gnathostomes (e.g., dorsal spinal cord, brain, notochord, vertebrae, cranium, and pharyngeal arches). The pattern of early embryogenesis is much similar to that of amphibians; the egg contains a large amount of yolk and undergo holoblastic (complete) cleavage.
L. japonicum is a parasitic lamprey species which can be caught in rivers in Northern regions of Japan. Eggs and embryos are available only from May through July.

Fertilization of the lamprey

eggs

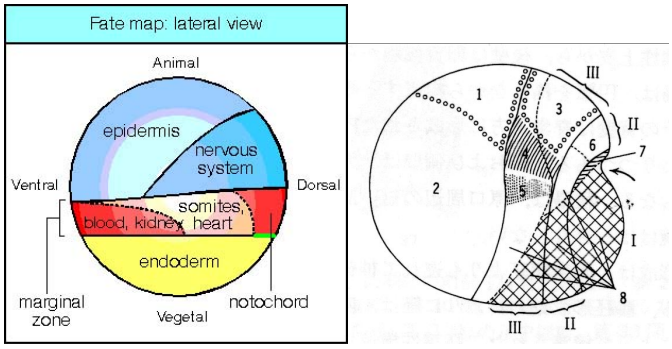
sperm

- Season = May-July
- ~80,000 eggs/spawn
- Egg size = ~1 mm



Development of lampreys
(*L. reissneri*;Tahara, 1988)

The fate map of lamprey gastrula was published in 1934. The regionalization of cells with different fates largely coincides with that in *Xenopus* fate map.



Fate map of lamprey gastrula (Weissenberg, ‘34)
1.Neural tube
2.Epidermis
3.Notochord
4.Somites
5.Lateral plate
6.Prechordal plate
7.Liver
8.Endoderm

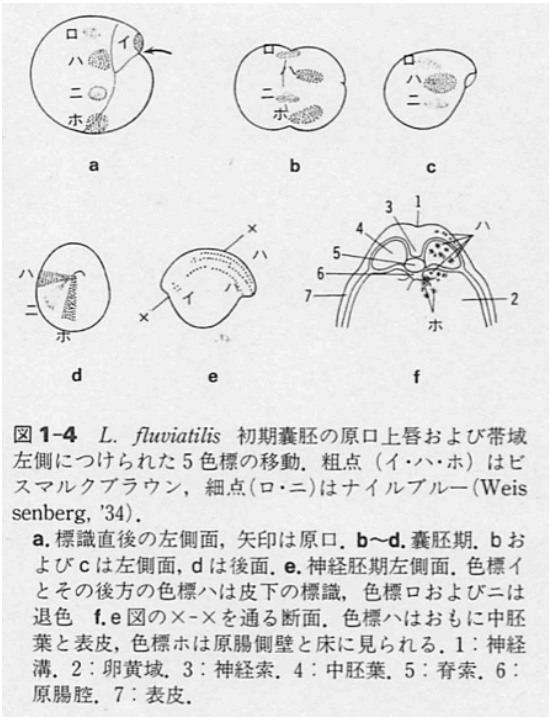
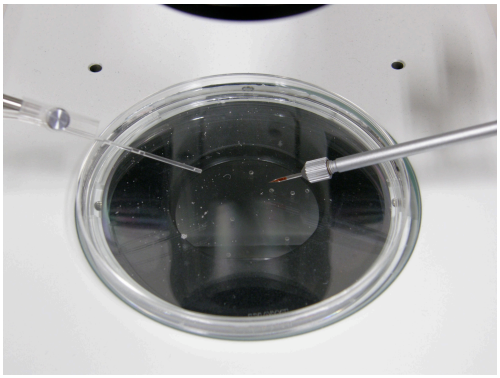
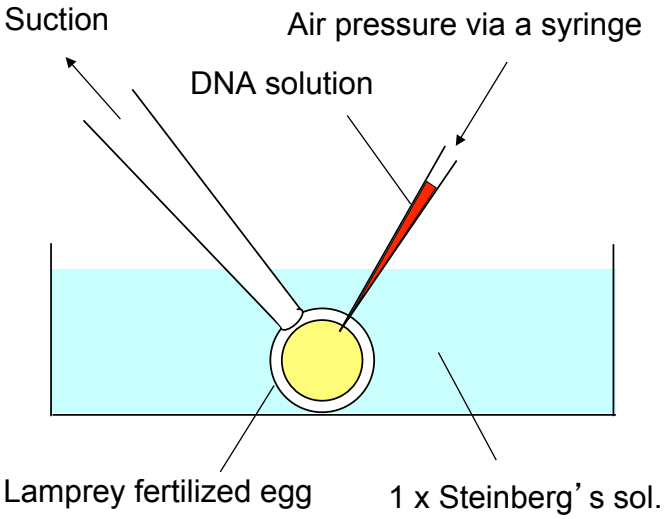


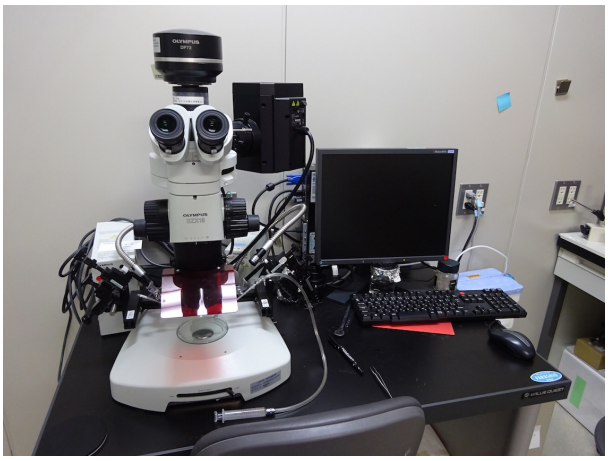
図 1-4 *L. fluviatilis* 初期嚢胚の原口上唇および帯域左側につけられた5色標の移動．粗点（イ・ハ・ホ）はビスマルクブラウン，細点（ロ・ニ）はナイルブルー（Weissenberg, '34）．
a. 標識直後の左側面，矢印は原口．**b～d.** 嚢胚期．b およびcは左側面，dは後面．**e.** 神経胚期左側面．色標イとその後方の色標ハは皮下の標識，色標ロおよびニは退色　**f.** e図の×-×を通る断面．色標ハはおもに中胚葉と表皮，色標ホは原腸側壁と床に見られる．1：神経溝．2：卵黄域．3：神経索．4：中胚葉．5：脊索．6：原腸腔．7：表皮．

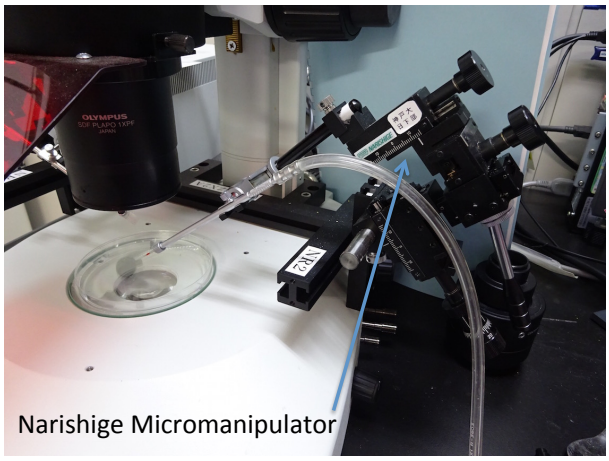
「脊椎動物の発生」培風館

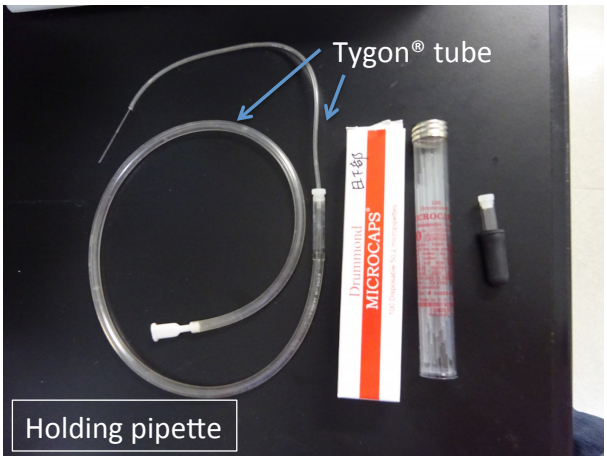
Microinjection into lamprey eggs

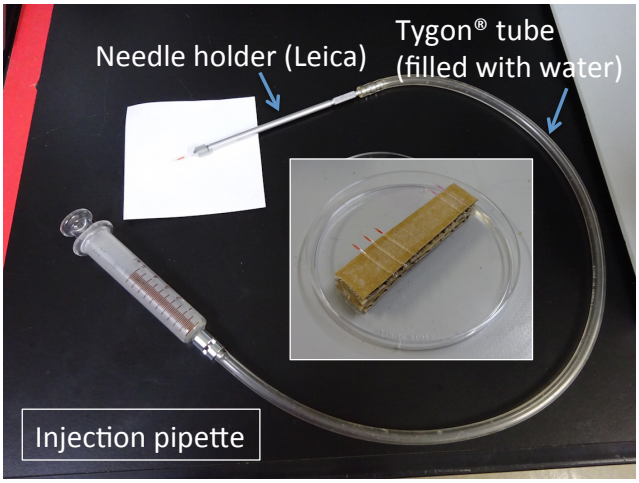












Reporter assay (enhancer/promoter)

Purpose

Identify tissue-specific enhancer
Search genomic sequence driving tissue-specific expression - conserved non-coding elements (CNEs)

mark living cells in living embryos
can use promoter sequence of other species - functional conservation

Method

- prepare reporter gene construct (enhancer/promoter + GFP or lacZ) plasmids

- injection at 1- to 2- cell stages (fish, frog), electroporation (chick)

Technical tips

- efficiency of reporter expression - depends on the promoter used (structural protein or regulatory protein, such as TFs)

- sometimes minimal promoter combined

- Confirmation of genomic integration – may not be necessary

Analysis (F0)

signal strength
number of positive embryos
Statistics

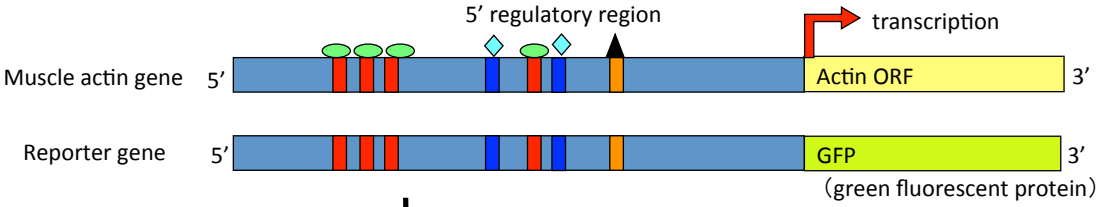
Advantage

Results can be assayed in F0

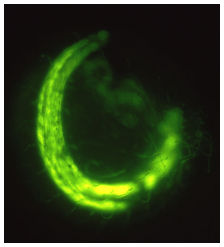
Disadvantage

- signal intensity and pattern (mosaic) vary
- Genomic integration confirmation?
  (Clipping fins – extract DNA?)

5’ regulatory region of muscle actin gene



Microinjection into eggs



OlMA1-GFP

OlMA1-DsRed

Funtional analysis of 5’ upstream regions of medaka actin genes (Kusakabe et al., 1999)

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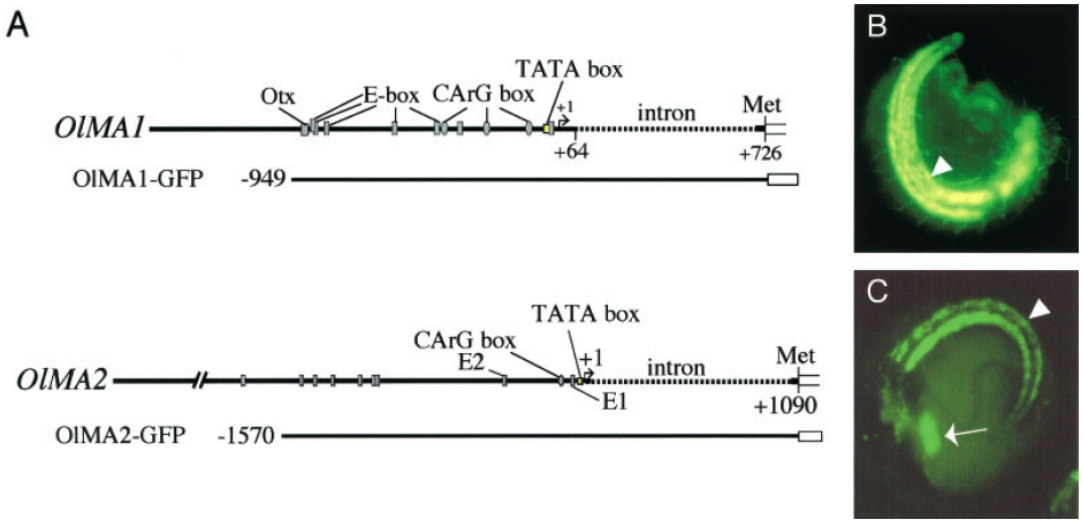
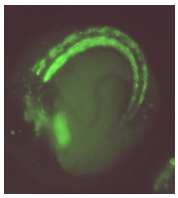


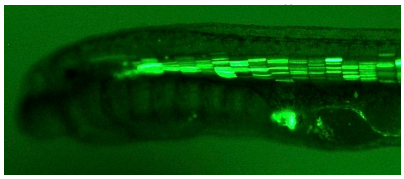
Fig. 1. Structures and expression of medaka muscle actin-GFP constructs used in this study. **A.** The genomic structures of endogenous medaka actin genes (top) and corresponding fusion gene constructs (Kusakabe et al., ’99). Broken lines correspond to the first intron of the gene. “Met” indicates the start Met codon of actin open reading frames (ORF). In all the fusion genes, the actin ORF was replaced with the *GFP* ORF, as indicated by a white box. **B.** A five-day medaka embryo injected with an OlMA1-GFP fusion construct. Strong signal is seen in somitic muscle (white arrowhead). C. A four-day medaka embryo injected with OlMA2-GFP fusion construct. GFP expression is strong in both somitic (white arrowhead) and cardiac (white arrow) muscles.

Introduction of OlMA1-GFP DNA in the lamprey

Expression of OlMA2-GFP
in the lamprey embryo
(Kusakabe et al., 2003)



OlMA2-GFP



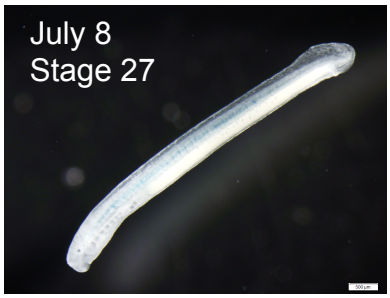
Lamprey (cyclostome)

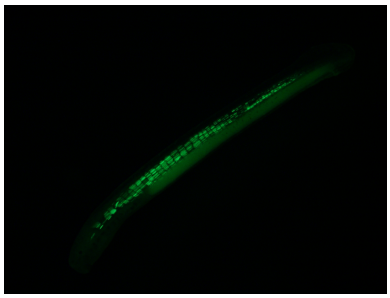
Medaka (teleost)

Human (mammal)

Common ancestor
of gnathostomes

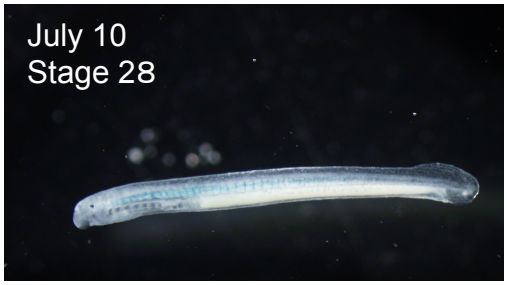
Common ancestor of vertebrates

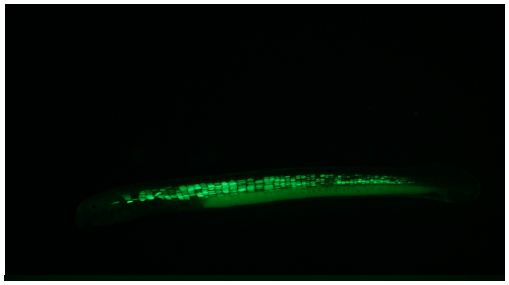


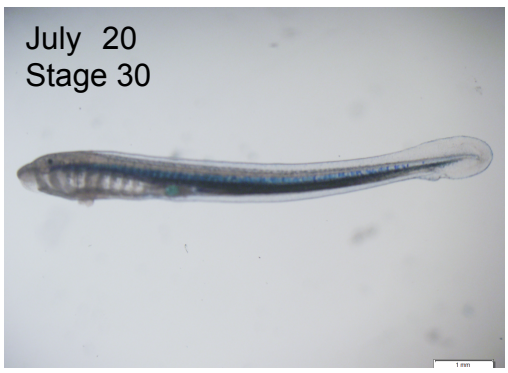


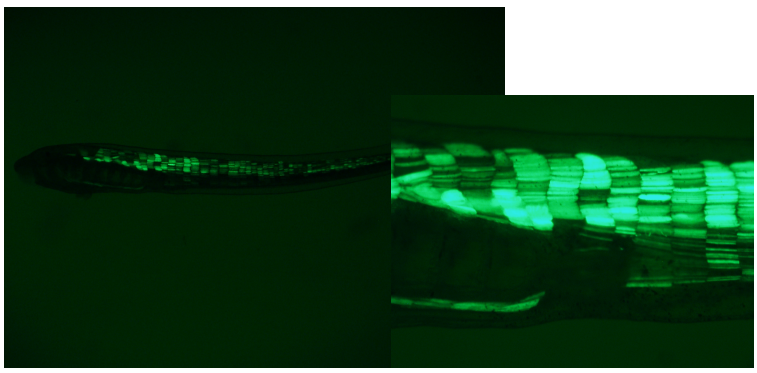
Observation of OlMA1-Kaede

June 8 Fertilization & injection
Incubation at 9℃
July 3 Stage 26
Move to 16℃









Lineage tracing (dye injection - membrane associated)

- traditionally, ink
- DiI (most commonly used, lasts long) (used in various organisms)
- PKH26 (comes with diluent)

purpose

- trace behavior of living cells (cell lineage, axon extension)

technical tips

- solvent (soybean oil, DMSO, EtOH, dH2O)
- how to fill the needle ?
- how to stabilize the embryo (anaesthetization, holding pipet, holding dish, wet paper) ?
- how long the label lasts ?

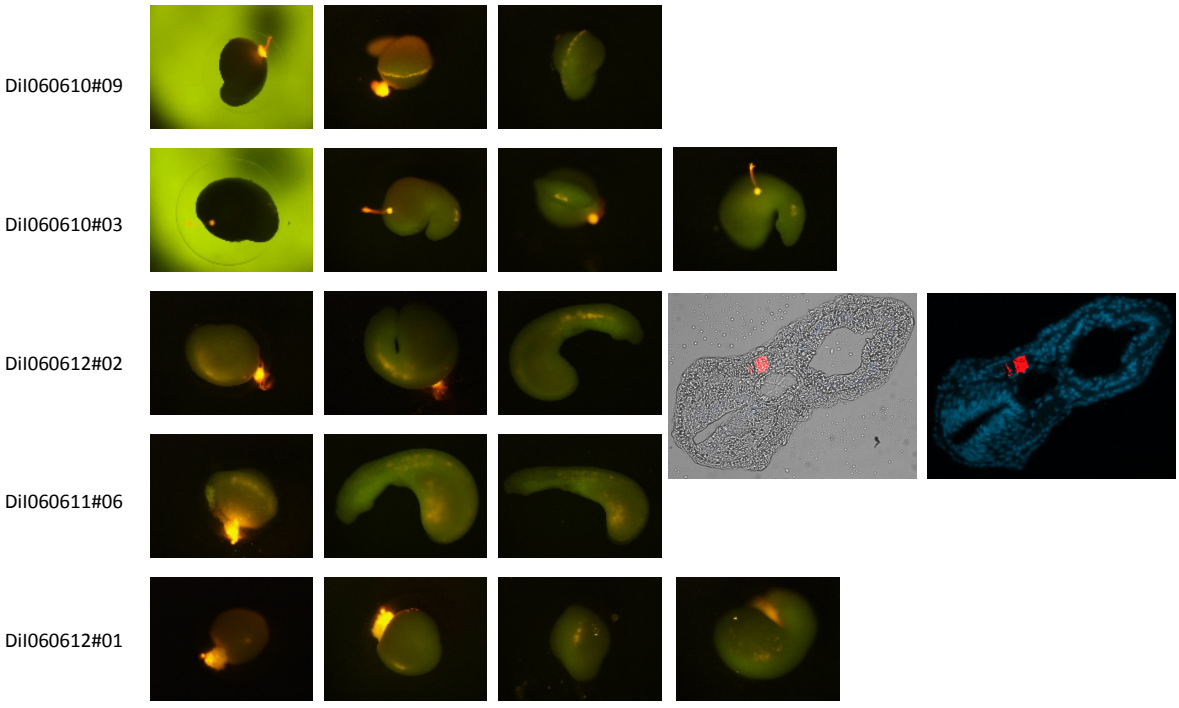
advantage

- can label any kind of cells in any organisms
- no molecular technique necessary

disadvantage

- heavily label the cells in the surface (epidermis etc.)
- not cell-type specific

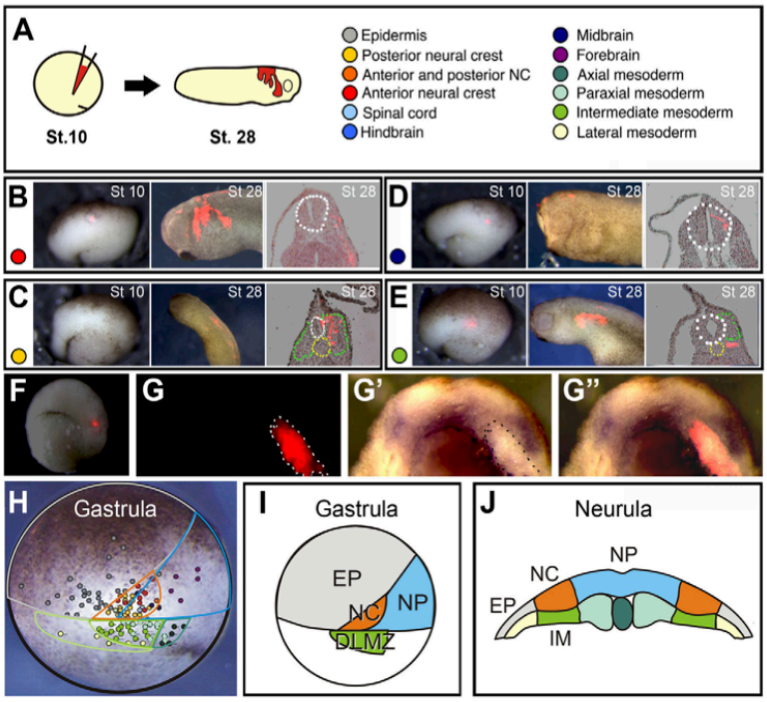
Example embryos injected at gastrula stage



Development 136, 771-779 (2009) doi:10.1242/dev.029017

Differential requirements of BMP and Wnt signalling during gastrulation and neurulation define two steps in neural crest induction

Ben Steventon, Claudio Araya, Claudia Linker, Sei Kuriyama and Roberto Mayor*



PNAS

Body wall development in lamprey and a new perspective on the origin of vertebrate paired fins

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