Modern Biotechnology

Module 3: Lineage tracing technologies

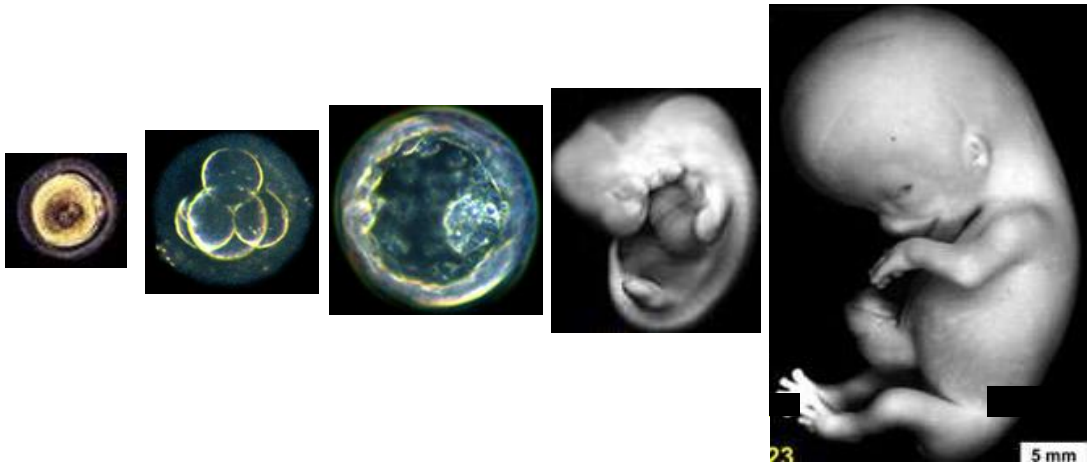
2018.3.28

Prof. Huang Wei

How life starts from a single cell?

Emerging of different cell types: differentiation
Growth and movement of “each seeding cell”: pattern formation

To understand human development



https://star-cosmos.com/starcomsos_blog/wp-content/uploads/2009/08/human-body.jpg

How to experimentally observe the progeny of single cell?
Lineage tracing (fate mapping)!

What lineage tracing is?

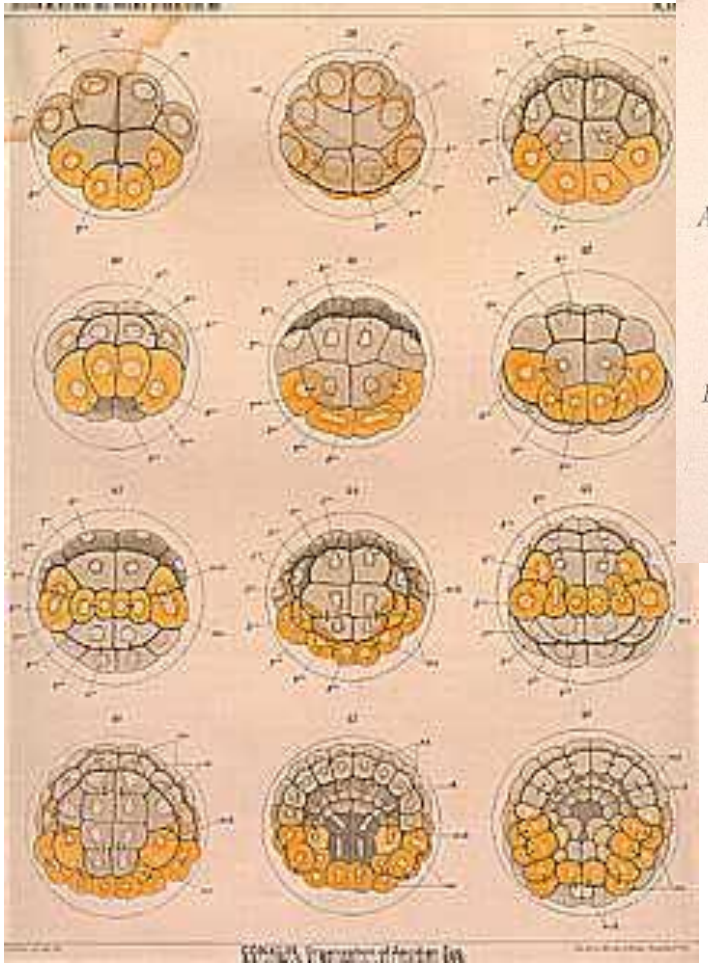
1. The identification of all progeny of a single cell
2. Originated from development biology
3. Now also an essential tool for studying
    1. Stem cells in adult mammalian tissue
    2. Tumor cells in adult mammalian tissue
4. Provides a powerful means of understanding tissue development, homeostasis and disease

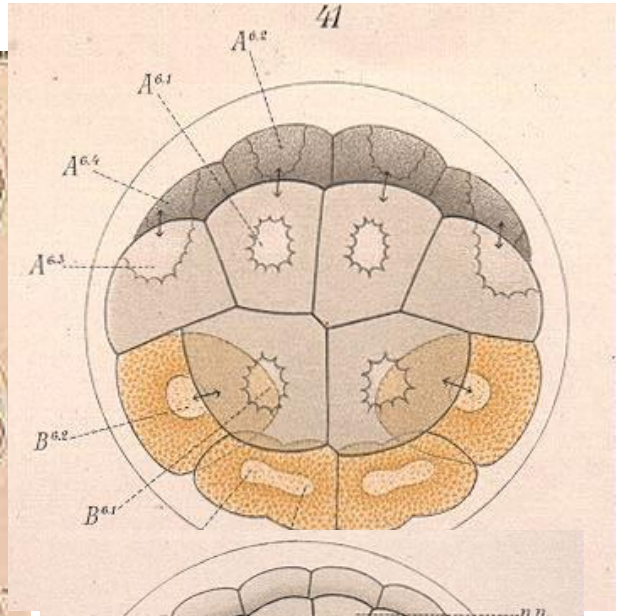
The development of lineage tracing techniques

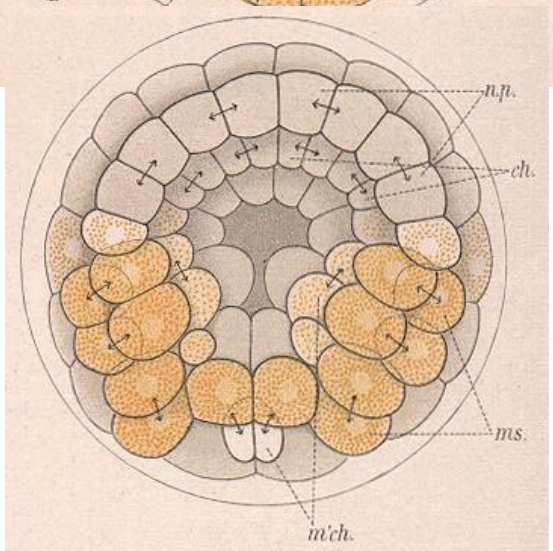
1. Direct observations with microscopy
    1. *All the cells all the time*
2. Labeling cells with chemical or other markers,
    1. *signal diluted out*
3. Permanent genetic makers, signals are stable
    1. By viral transduction or DNA transfection
    2. Genetic Mosaics
    3. Cell marking by genetic recombination
4. Stochastic generation of multiple markers

Direct observations with microscopy

In 1905, Edwin Conklin published a remarkable fate map of the ascidian(海鞘) embryo. He studied early cleavages by light microscopy.

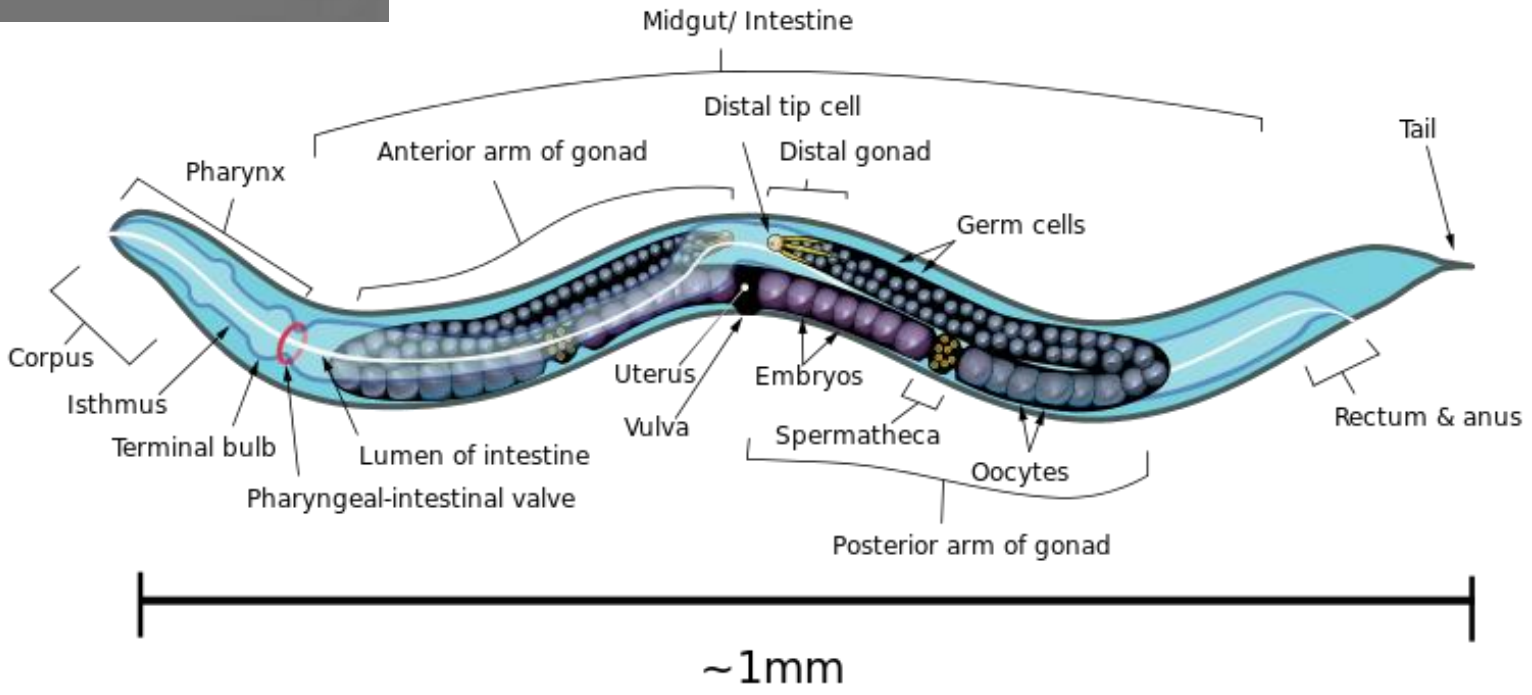




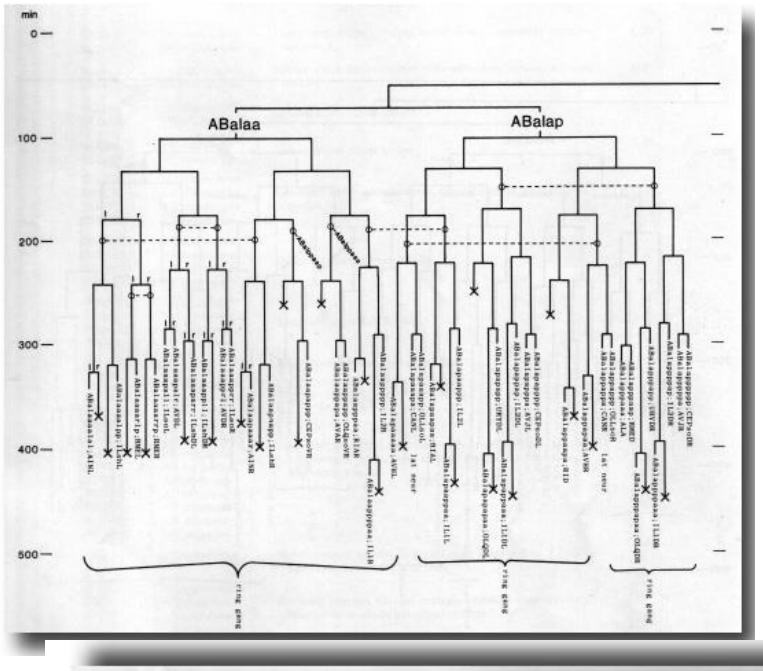


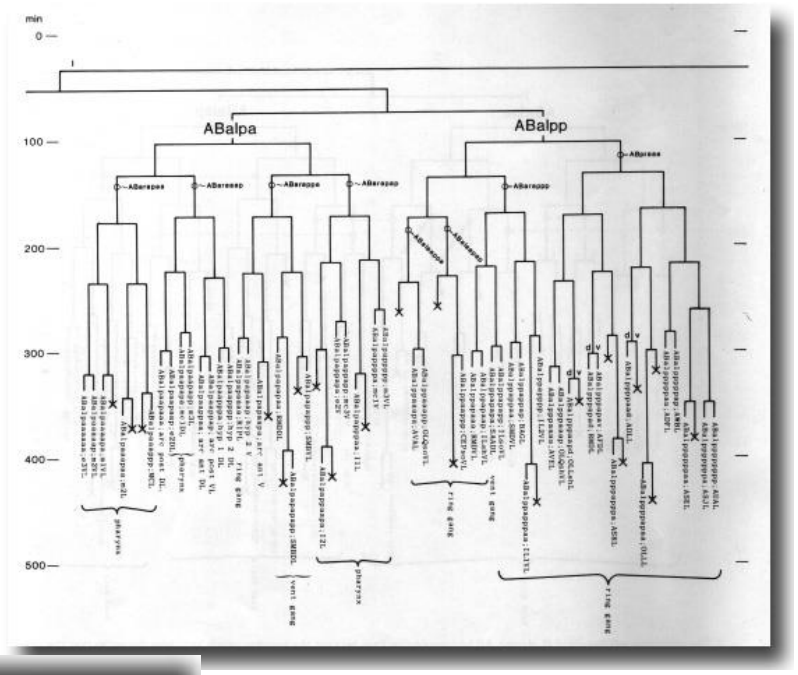
Direct observations with microscopy

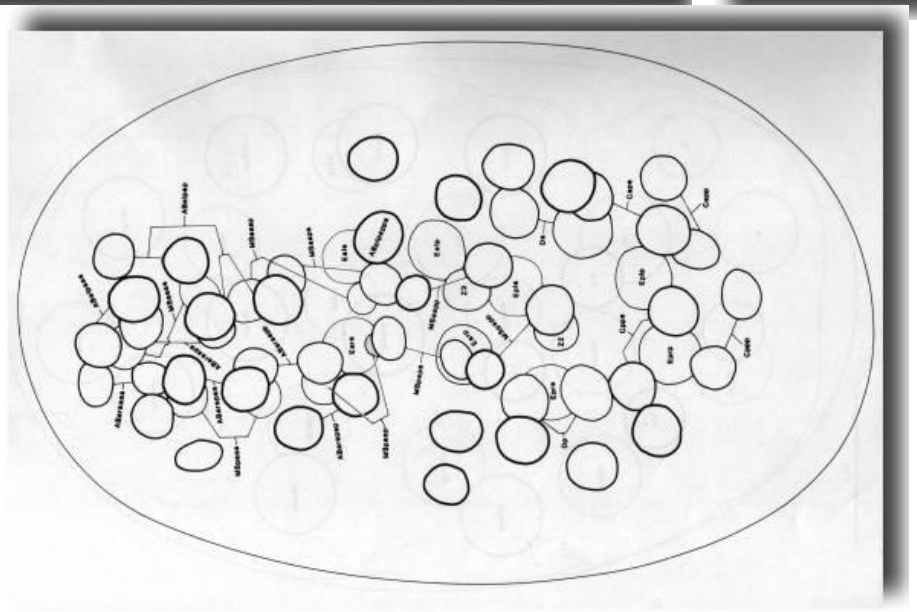
In 1985, John Sulston used light microscopy (DIC) to determine the fate of every single cell in the C. elegans embryo. He won Nobel prize in Physiology and Medicine in 2002.



Only A fraction of the whole fate map is shown here







These results took generations of biologists for ~100 years.

Summary of the traditional direct observation methods

Advantages:

Easy, noninvasive

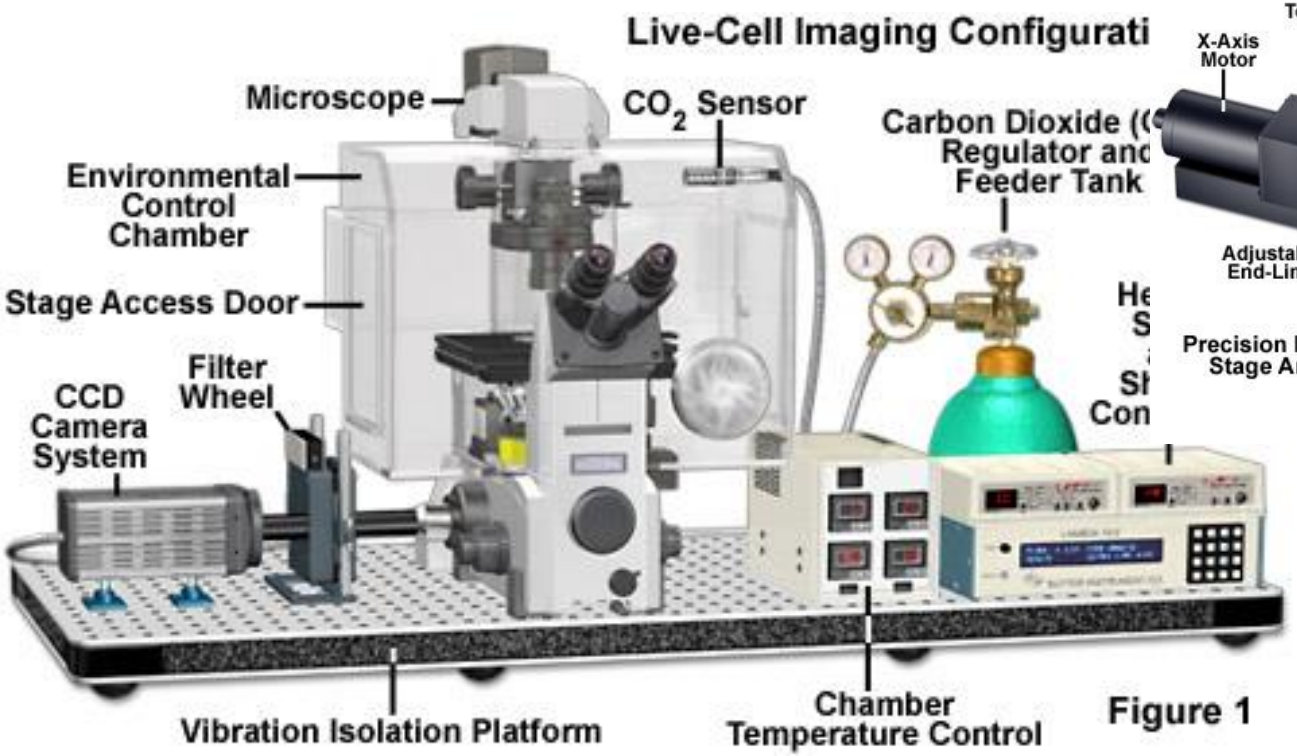
Limitations:

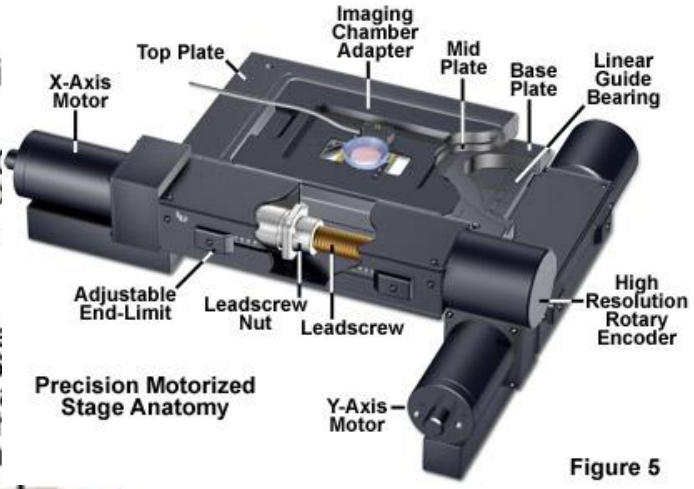
Transparent embryo, small number of cells

Manual recording and analysis

Overcome the limits

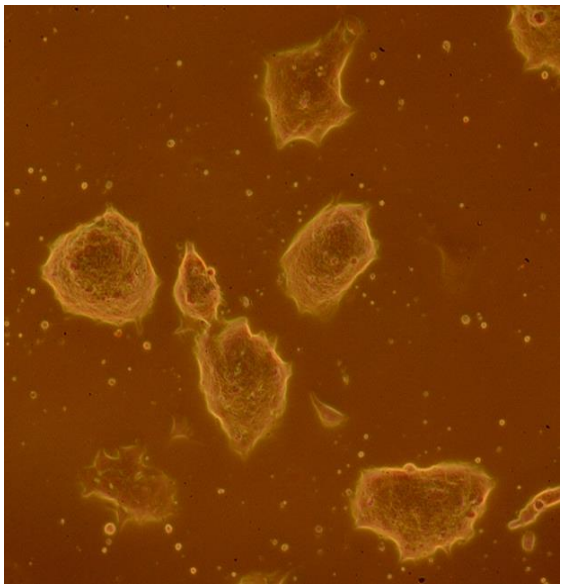
- Automatic time-lapse fluorescent microscopy:
  - automatic taking images of the same positions repetitively, many cells
  - Suitable for cell culture, 2D, transparent
  - 一科107





Example: tracking the dedifferentiation of cell back to ES cells in 2D culture

Oct4^{+}, **Nanog^{+}**, Sox2^{+}
Gata6^{-}, Gata4^{-}, Lam1β^{-}

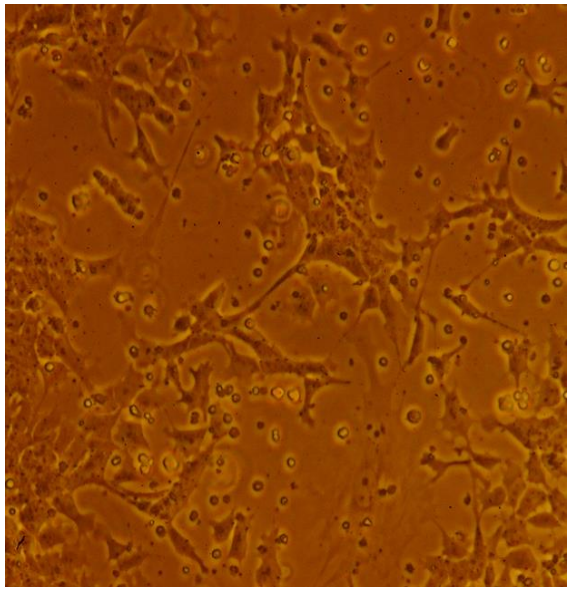


on 2D surface
+
retinoic acid
(RA)

?

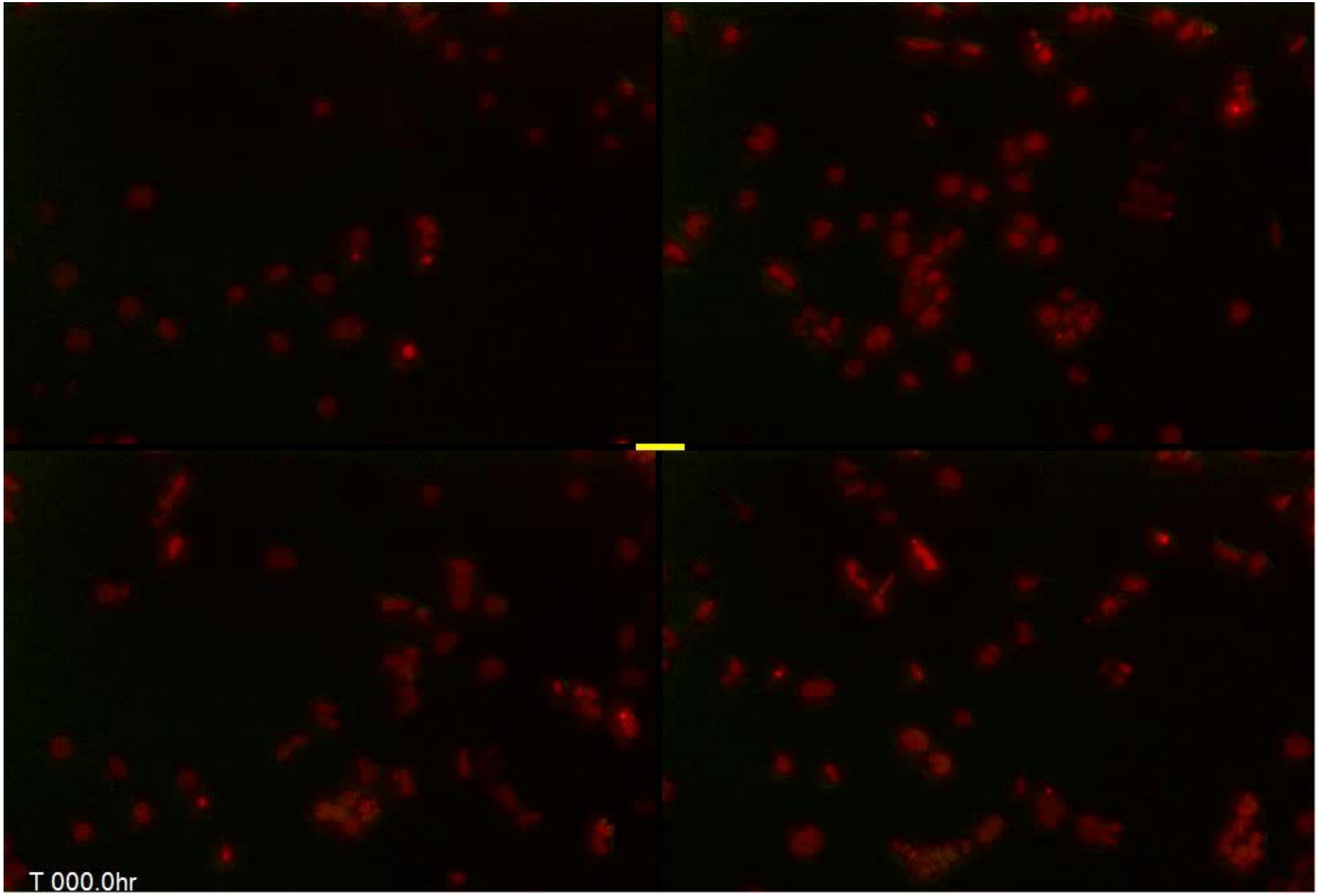
reversibility

Gata6^{+}, Gata4^{+}, Lam1β^{+}
Oct4^{-}, **Nanog^{-}**, Sox2^{-}



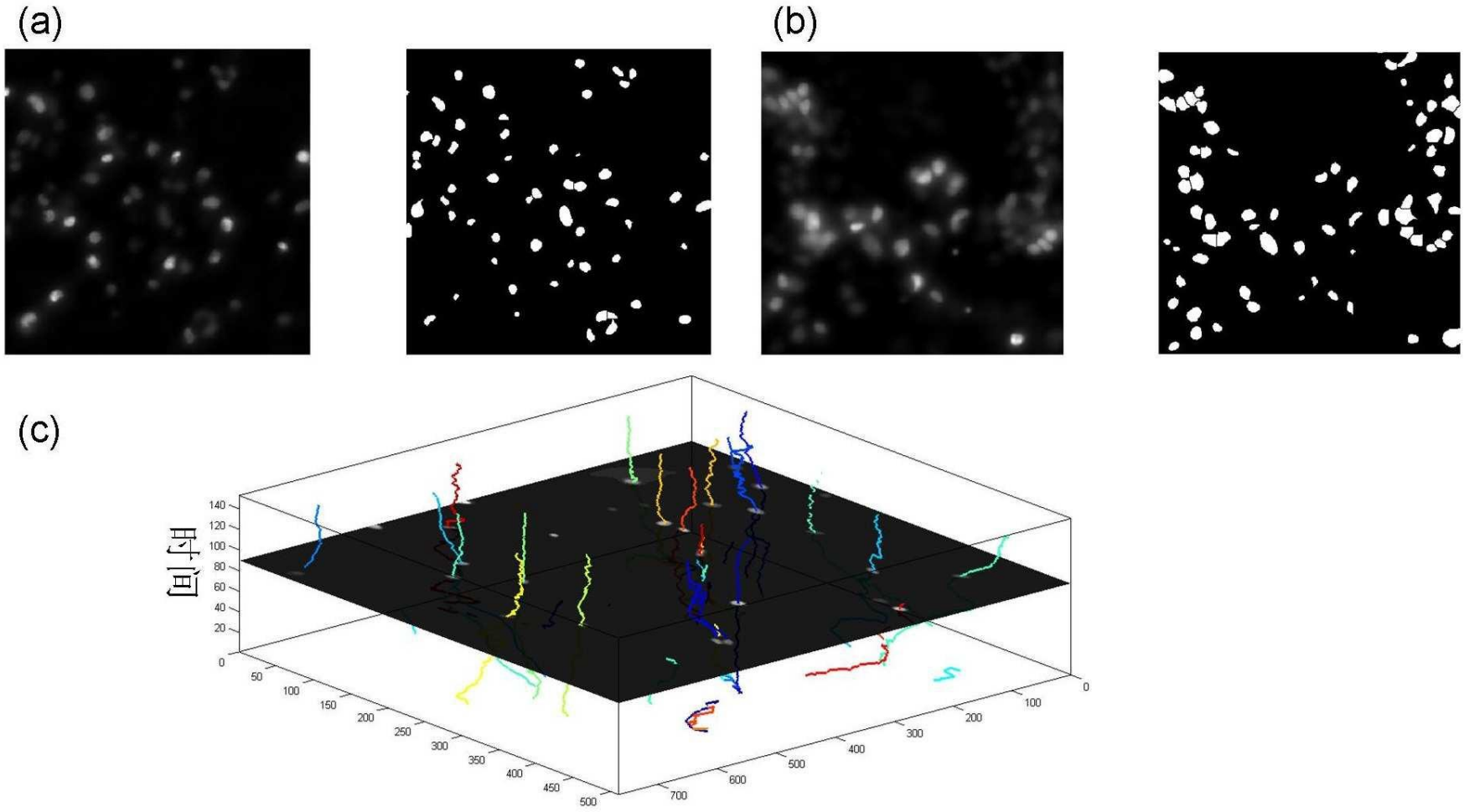
Genetic label for cell type identification: Nanog-GFP
Cell tracking: nuclear-targeted mCherry

Direct observation of dedifferentiation and nanog-dependent proliferation



Red: cell nuclei labeled with mCherry; Green: Nanog-GFP; Yellow: overlay

Additional technology development:
image-based automatic cell identification and tracking
Utilizing the nuclear targeted mCherry signal



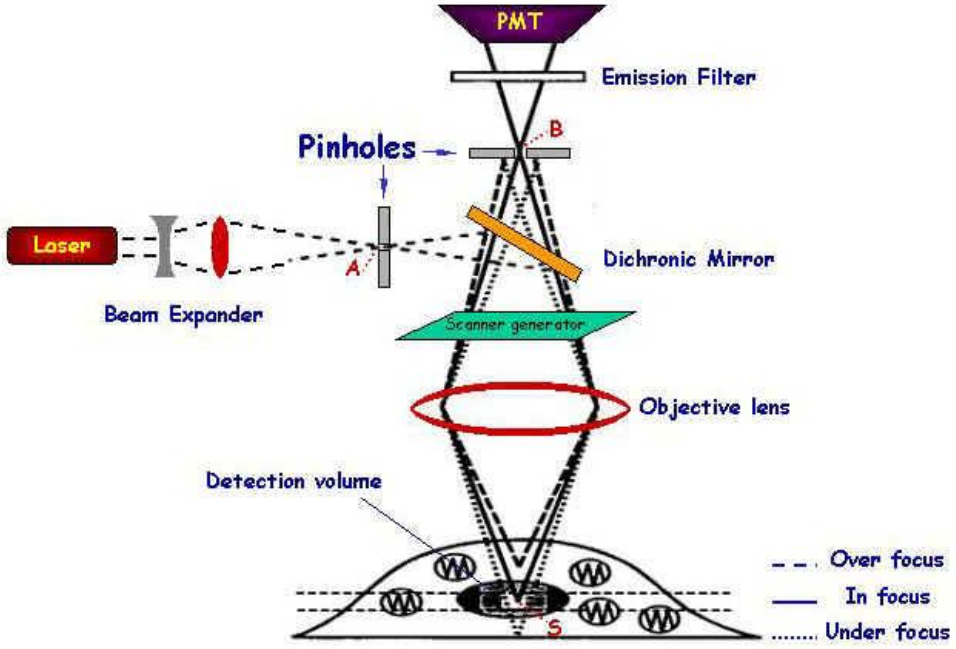
What about 3D embryo?

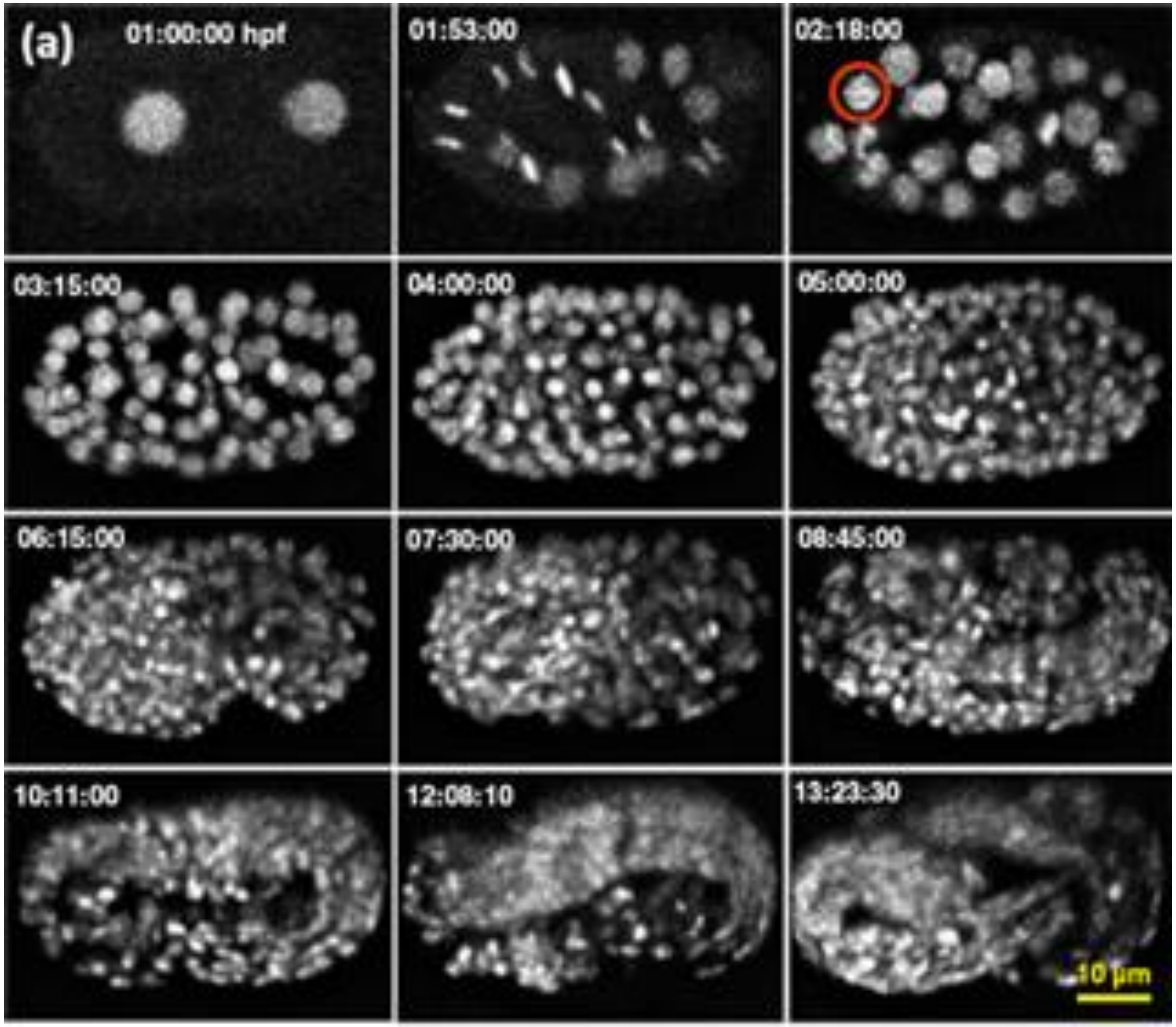
Let’s go back to C elegans:

40x25x25μm, 1-~1000 cells

Confocal microscopy: remove out-of-focus signal

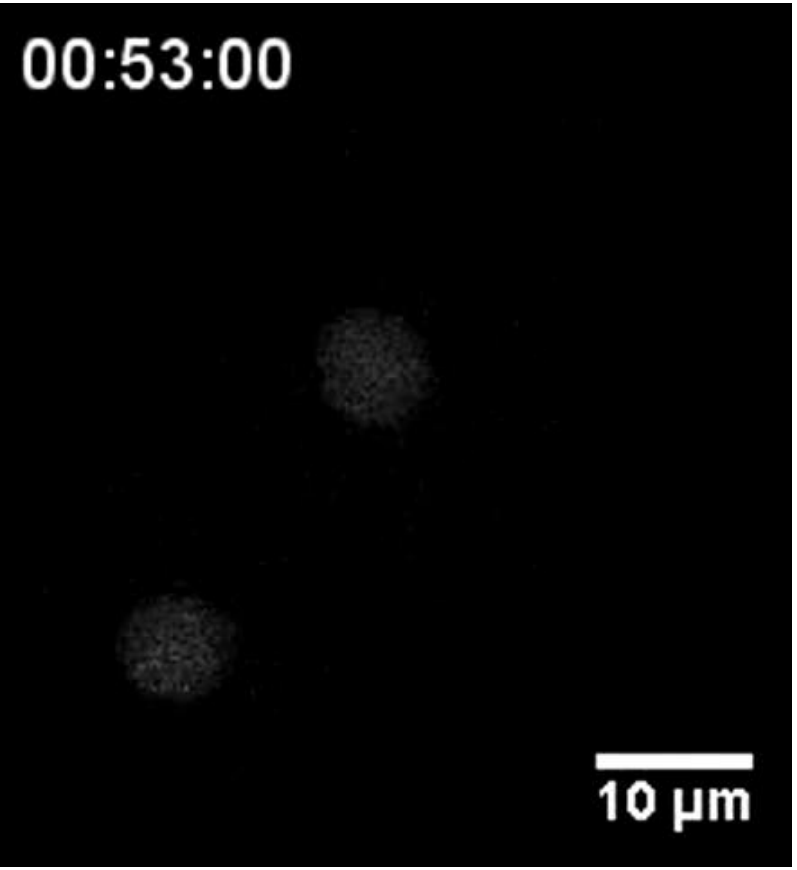
——一科系公共平台 一共四台

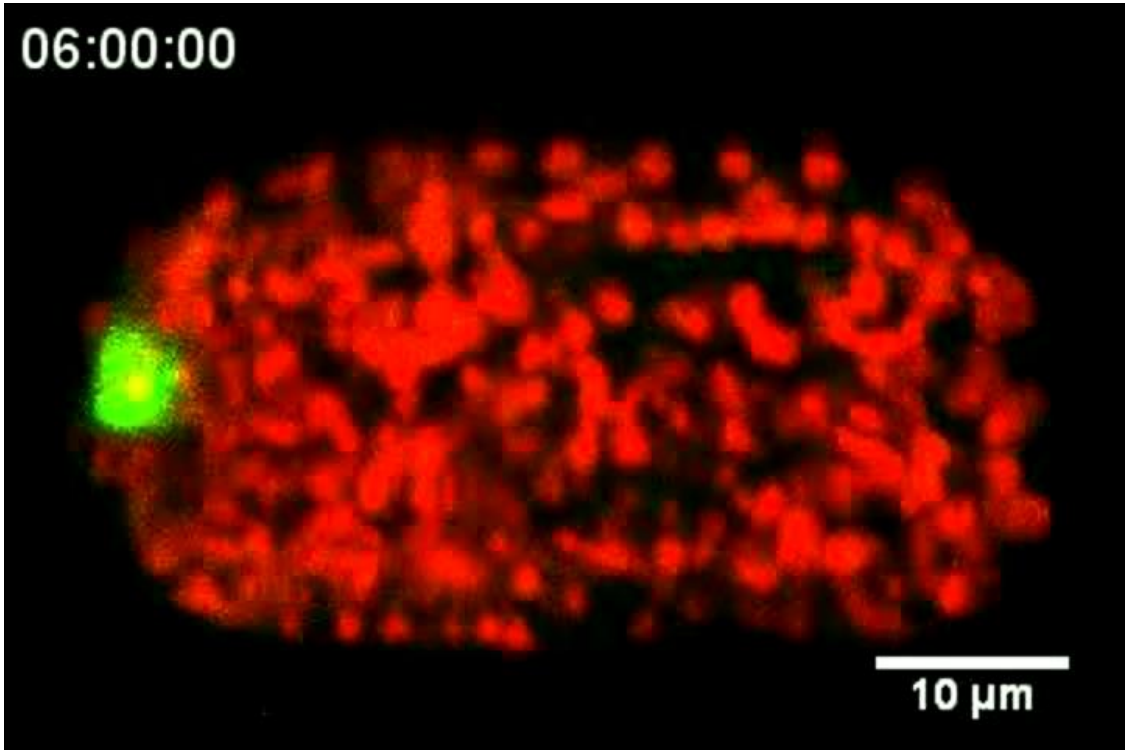




High-speed nuclear imaging in the nematode embryo. Embryos with GFP-histones were volumetrically imaged from the two-cell stage until hatching.

Confocal microscopy and automatic image process reduces the work of lineage tracing to hours





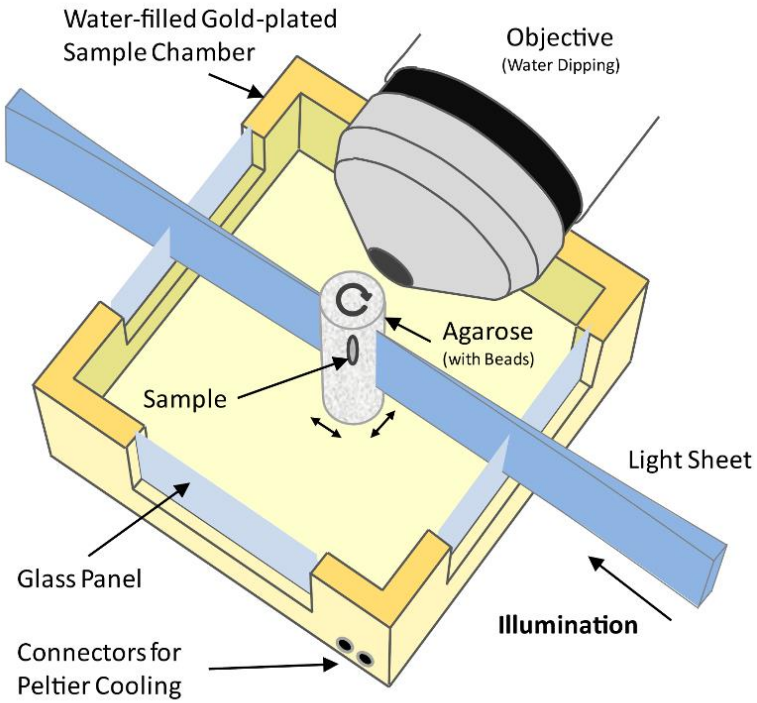
Development of new microscopy for lineage tracing of larger embryo with more cells

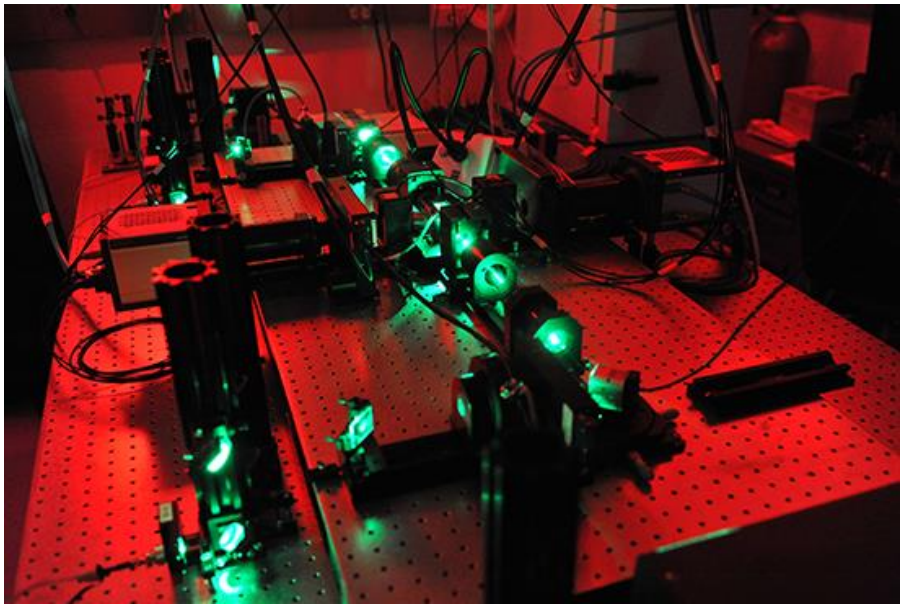
Limitations of traditional confocal microscopy:

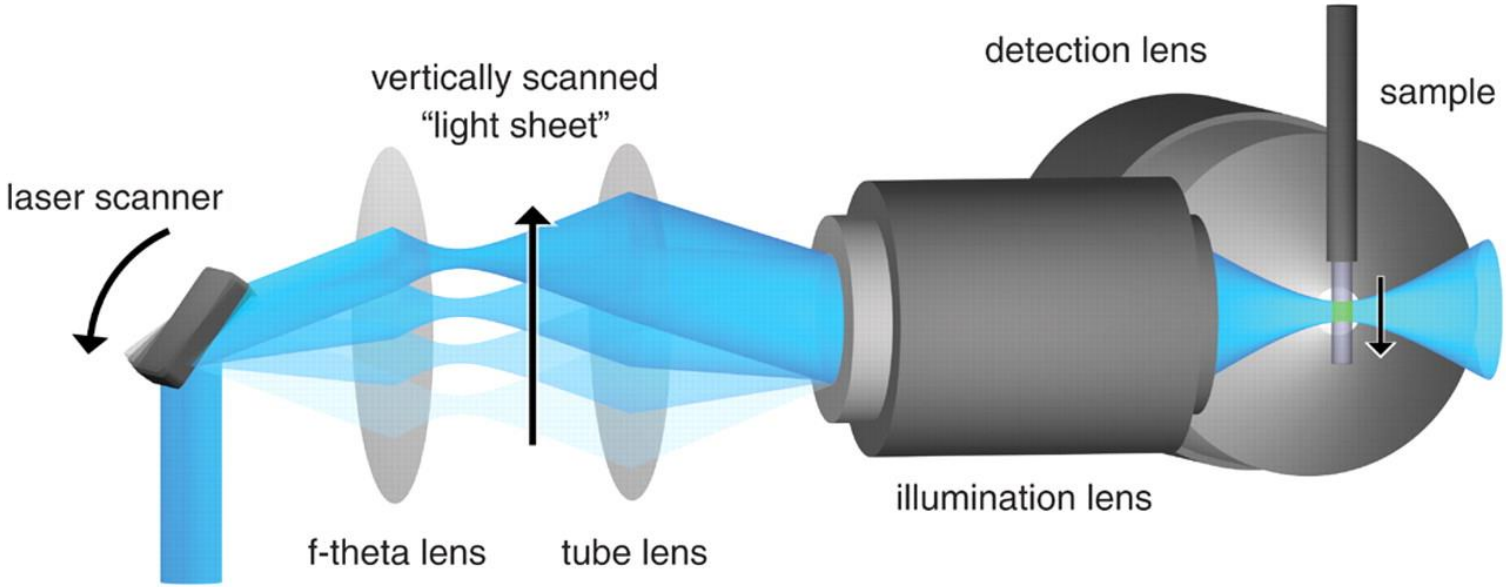
1. Limited light penetration (z~50-100μm) and thick embryo:
   Solution: two-photon, infrared, better penetration
   ——一科107，两台

2. Slow speed and fast moving cells-lost tracking of cells:
   Solution: light sheet microscopy
   not scan mode, fast
   illuminate only the imaged z-section, less photo damage
   ——正在购置的前期

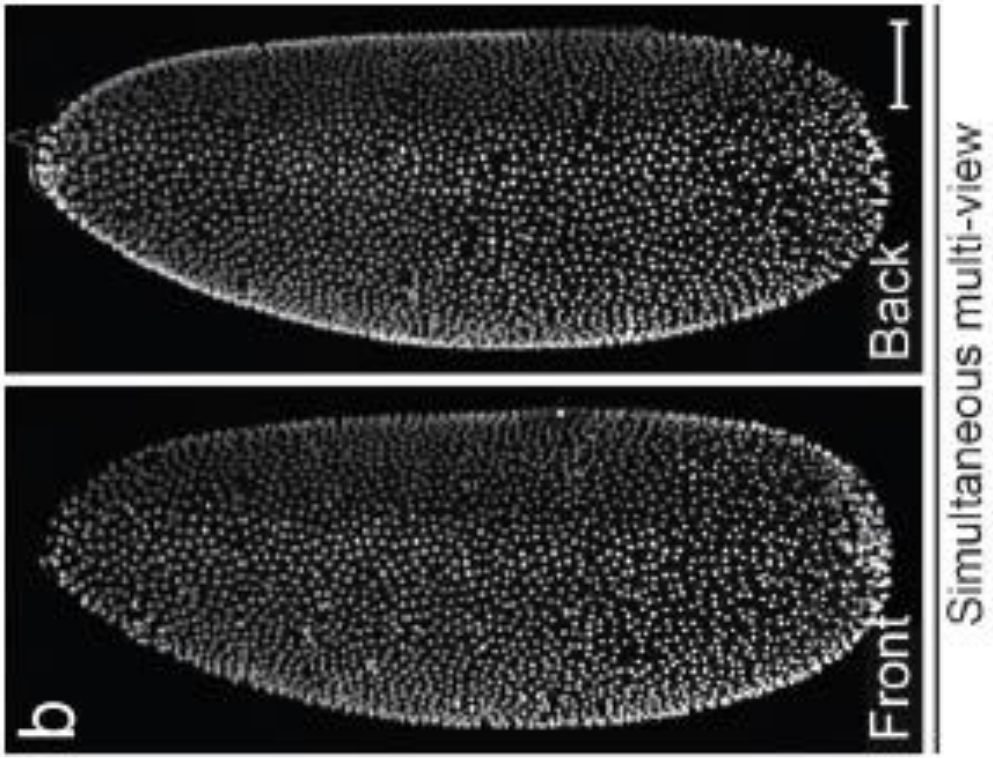
A light sheet microscopy



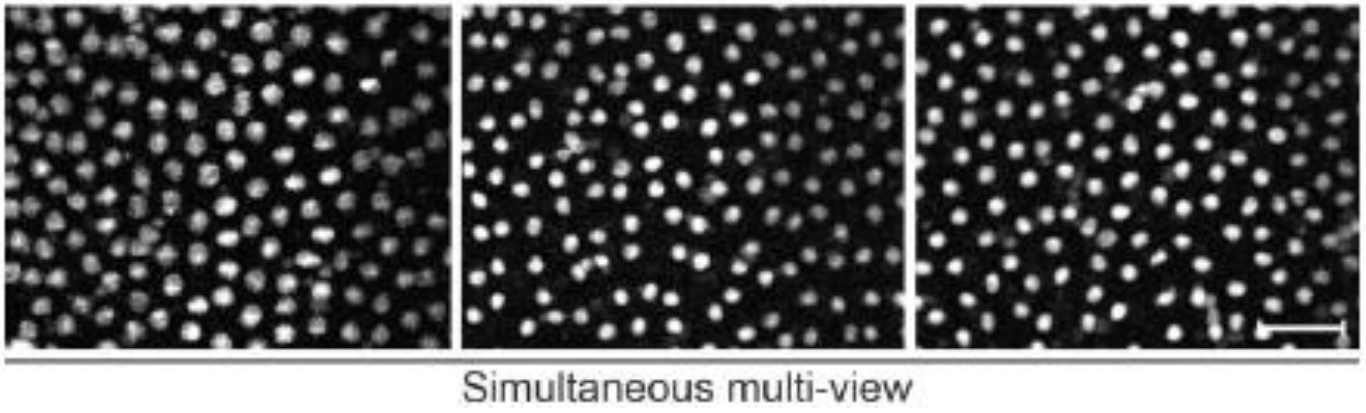




Imaging the whole drosophila embryo every 30 seconds!



Up to 10TB images everyday!



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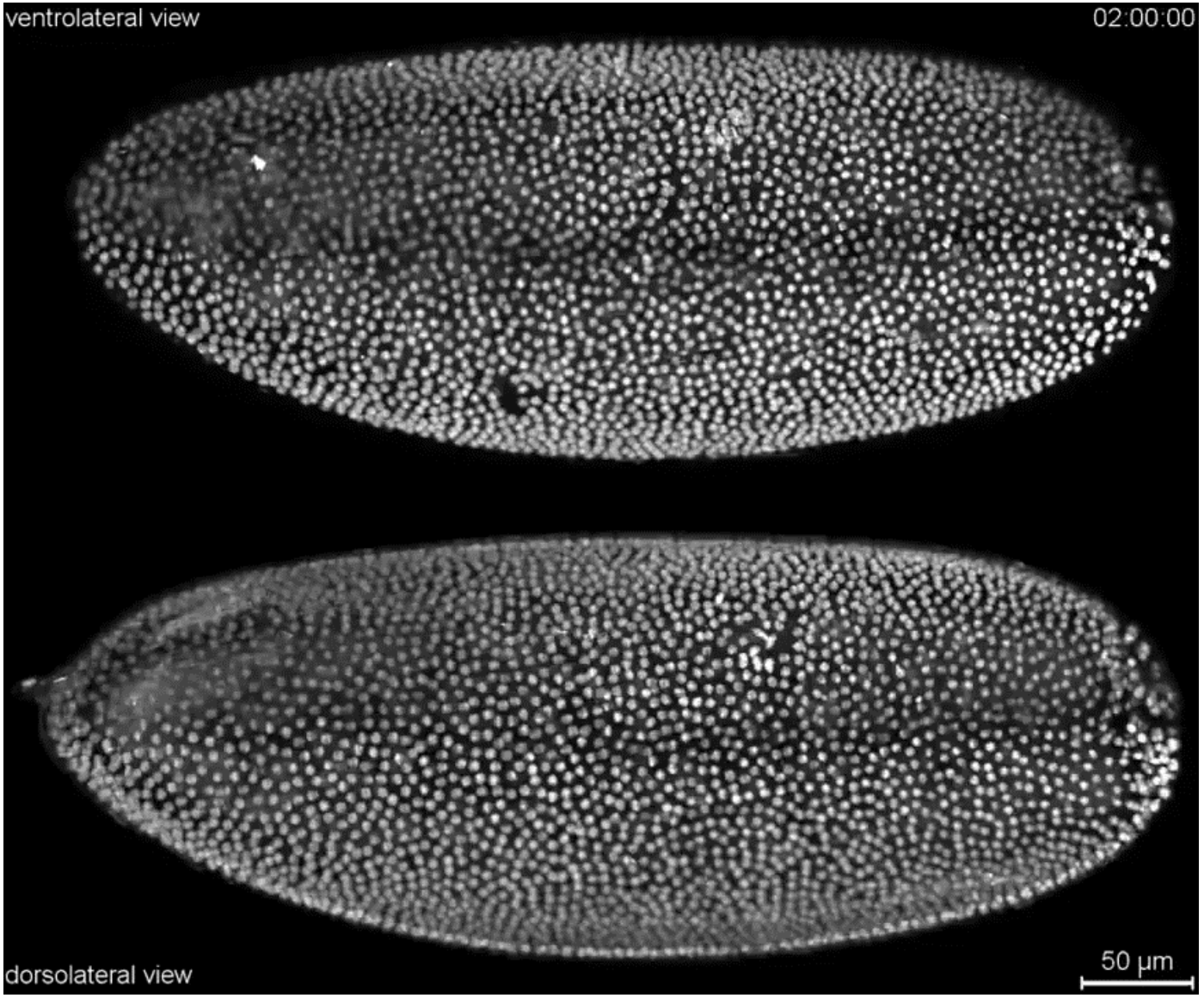
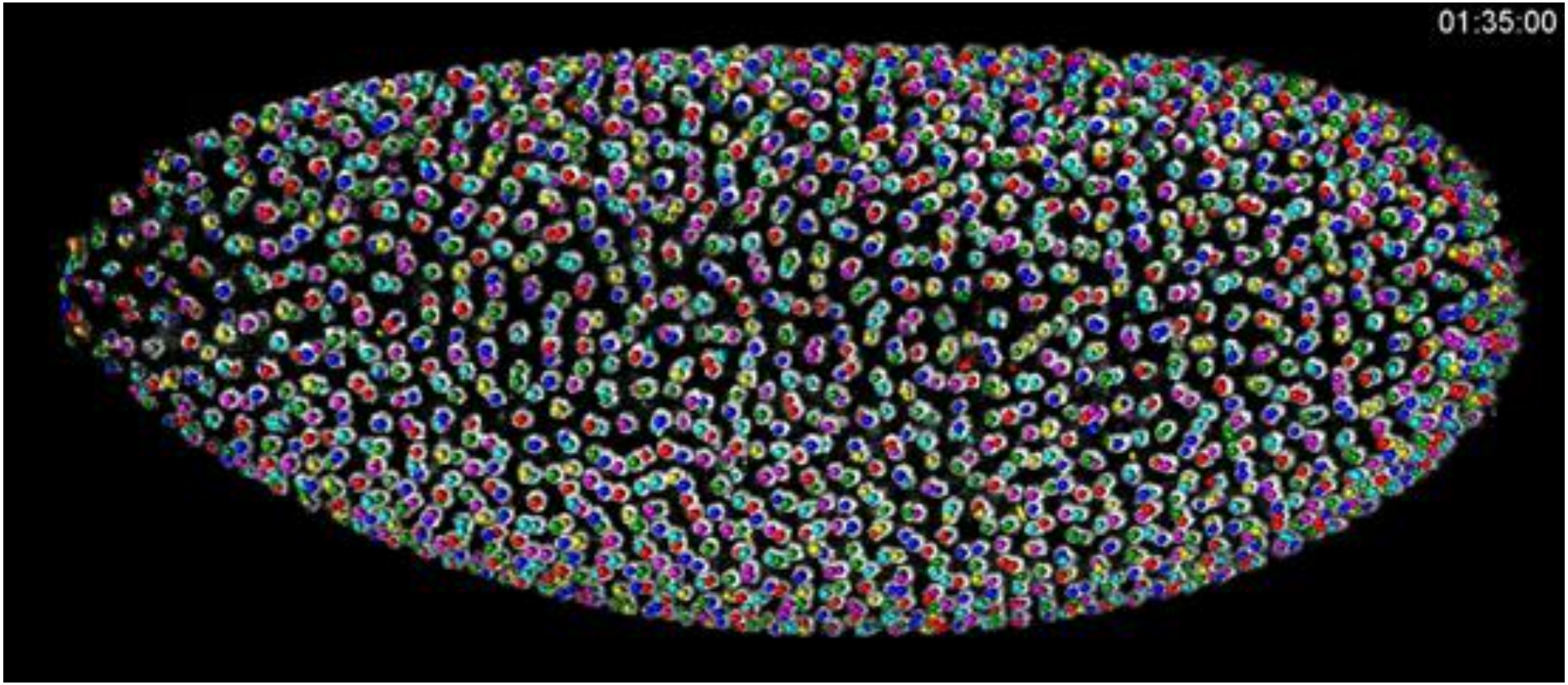


Automatic lineage tracing in thousands of cells



The limitation even with the most advanced microscopy

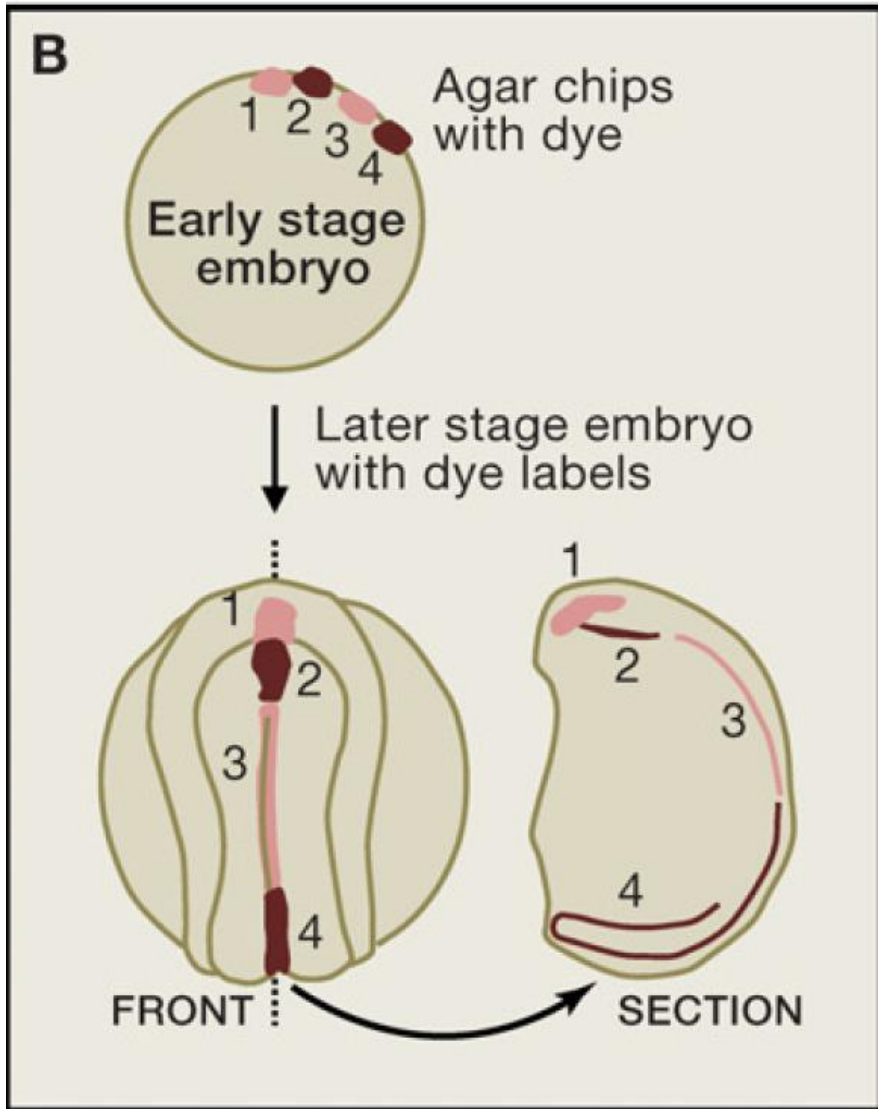
1. Cells are not accessible to direct observation
2. Cell density is extremely high
3. Time duration is too long

Scientists have adapted indirect methods to mark single cell, then observe the static distribution late. Reconstitution of the dynamic process is performed afterwards

The difference in the following methods are mainly in the labeling

Labeling cells with chemical or other markers

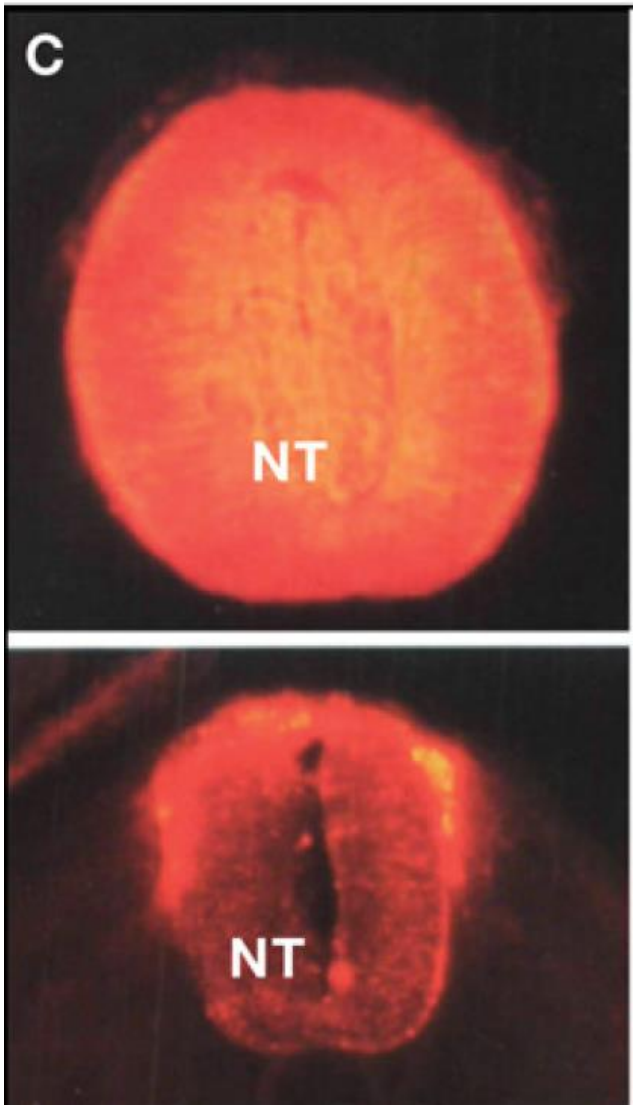
In early 1900s, ‘vital dye” which label cells without killing them, were used to directly mark cells in amphibian embryos and follow the fate of their progeny during gastrulation (Voigt 1929).
In a way, as the dye would be diluted over time, this method is similar to pulse-chase assay.



Labeling cells with chemical or other markers

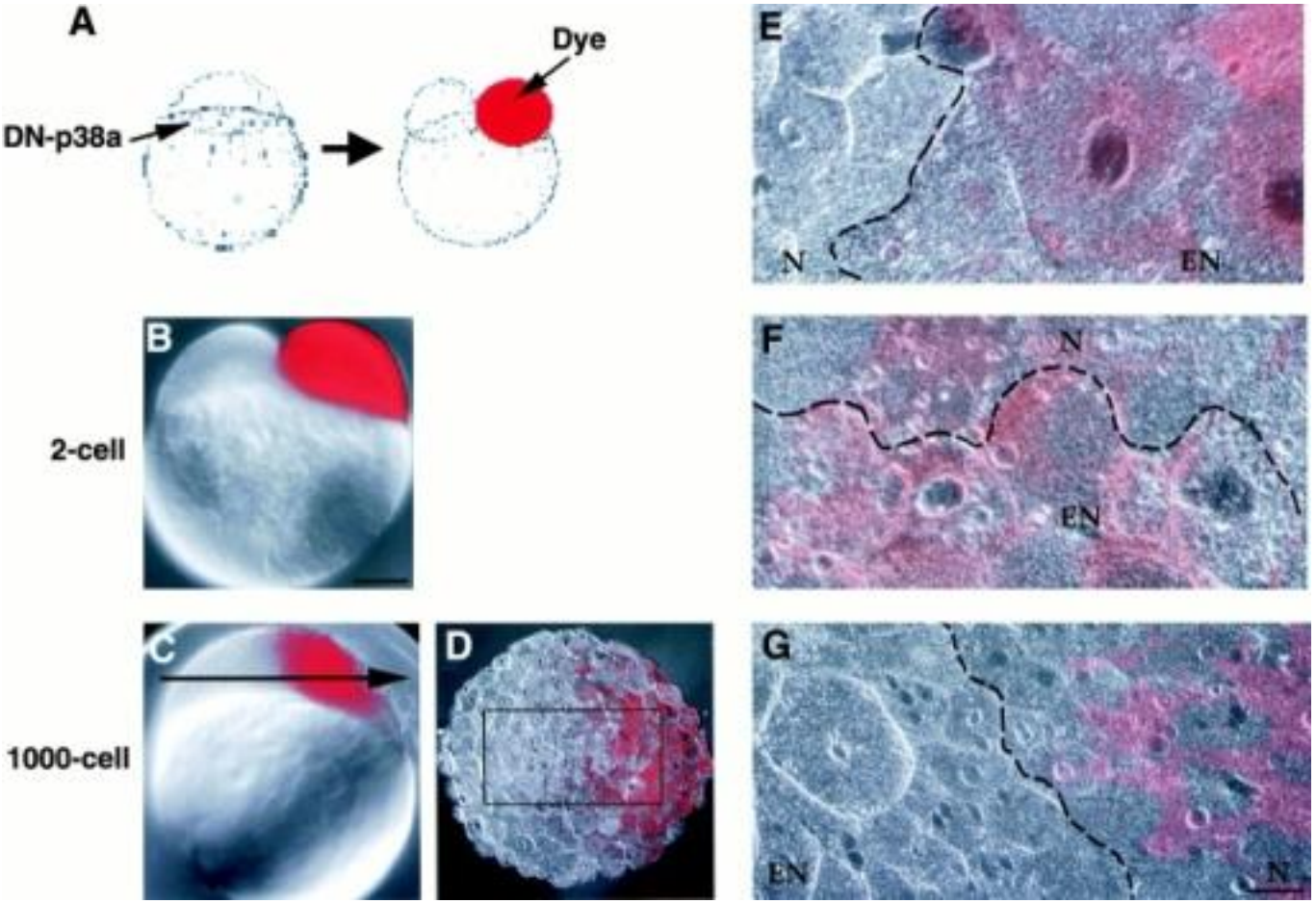
Lipid-soluble dye, such as Dil and DiO are still widely used to label plasma membrane and used for lineage tracing.

Tracing of neural crest cell in chick embryo



Labeling cells with chemical or other markers

Direct microinjection of dye into single cell



The limitation of chemical dyes

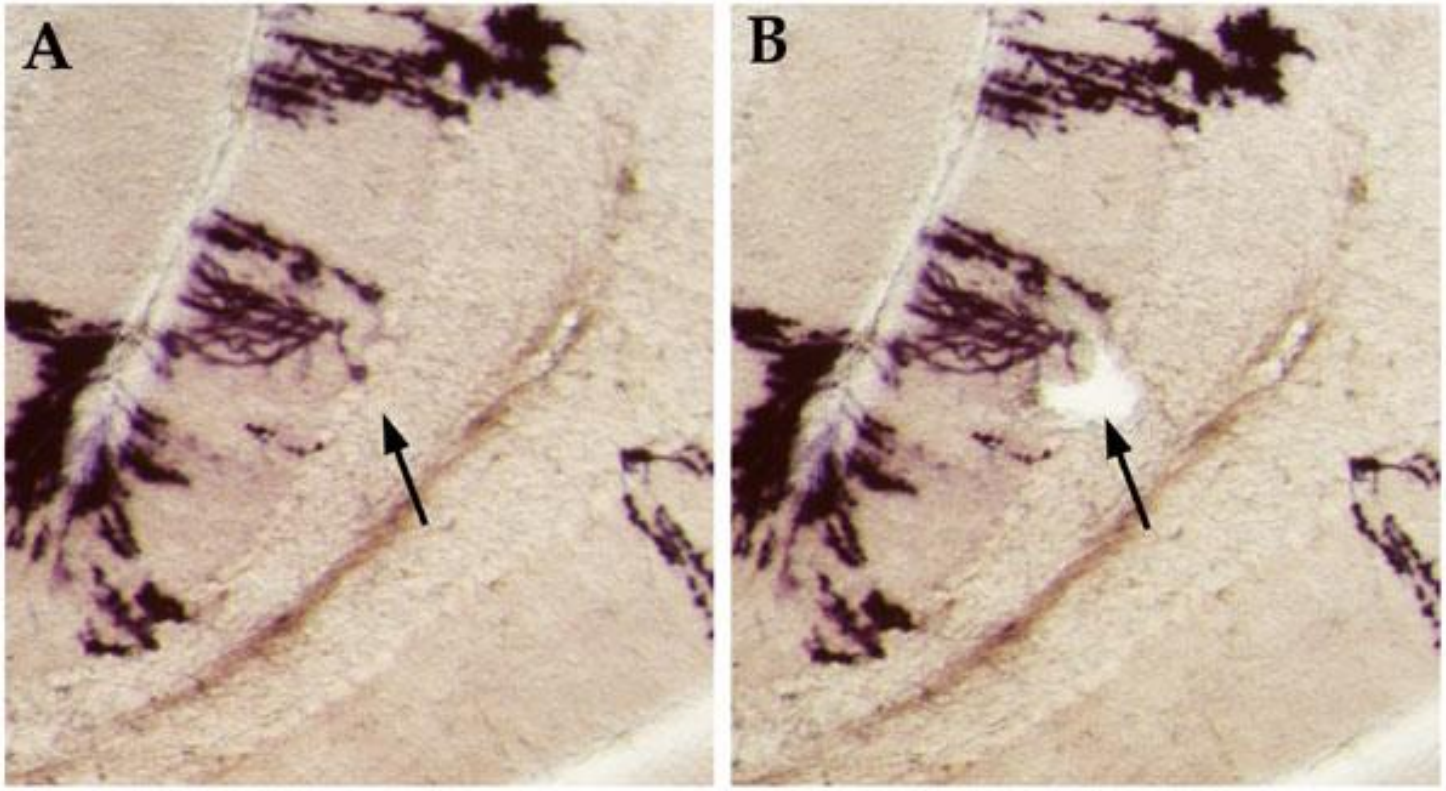
Dye dilution due to cell proliferation:
the longer it is followed, the weaker the signal is

Dye could spread to neighboring cells

How to permanently label a single cell so that all the progeny retent the same signal

Genetic marker is permanent and localized!

Permanent genetic marker introduced by retrovirus



The alkaline phosphatase-positive (AP-positive) cells are present in a chick cerebellar section.

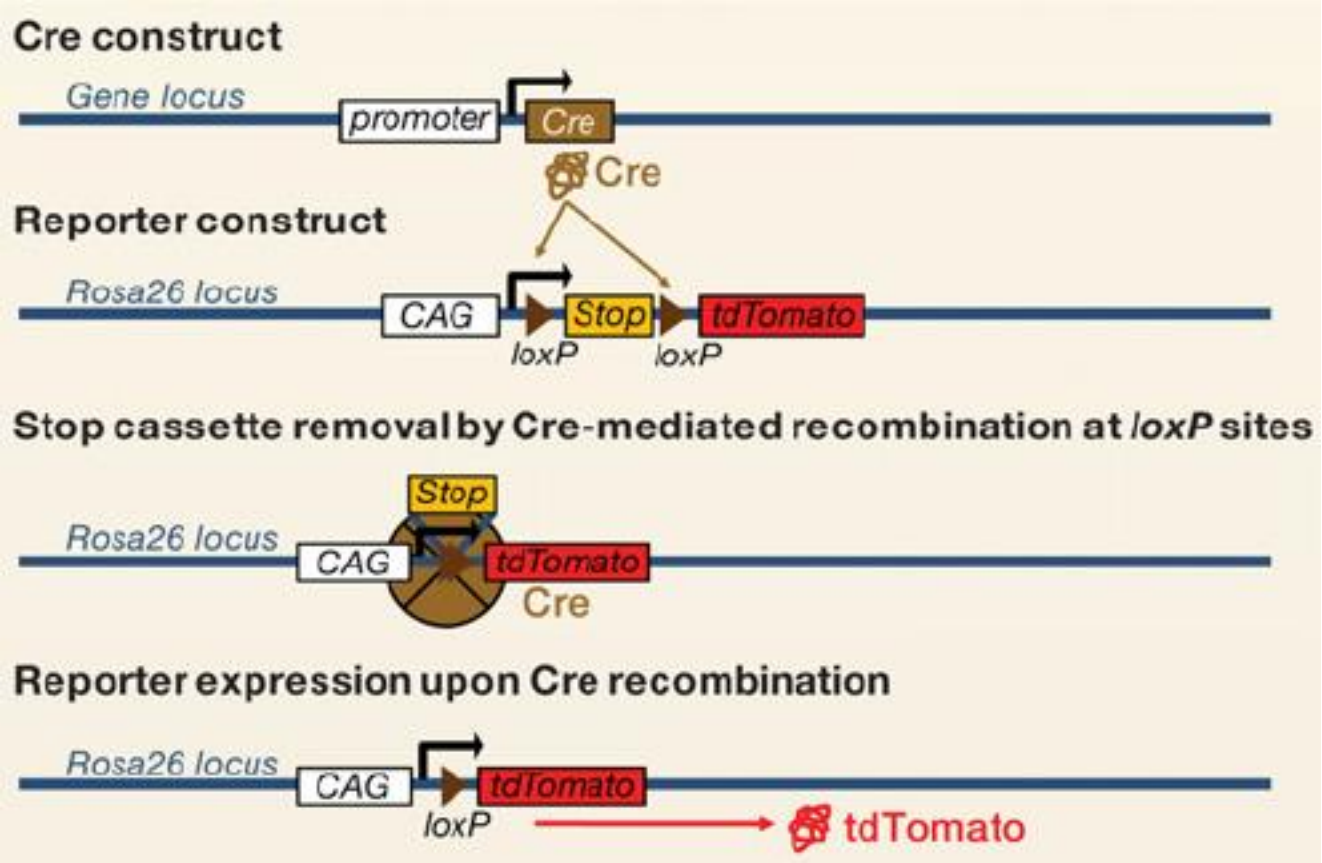
Limitation: only dividing cells are labeled with retrovirus
retroviral vector might be spontaneously silenced.

Cell marking by genetic recombination

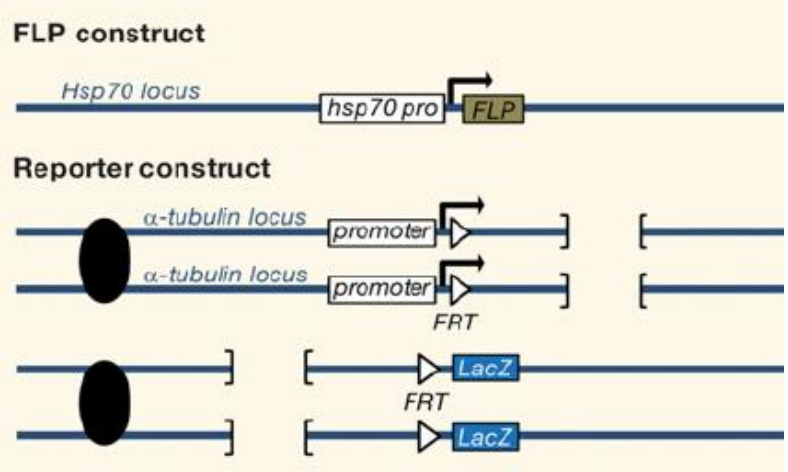
1. It has been used since early 1990s and now the prefer approach.
2. A recombinase enzyme is expressed in a cell- or tissue-specific manner to activate the expression of a conditional reporter gene.
3. Flp-FRT and Cre-LoxP recombination are two widely used site-directed recombination technology.
4. Flp (flippase) is derived from baker's yeast S. cerevisiae, FRT is the flippase recognition target sequence.
5. Cre recombinase and its recognition site LoxP are derived from bacteriophage P1.

The genetic elements in the Cre-LoxP system

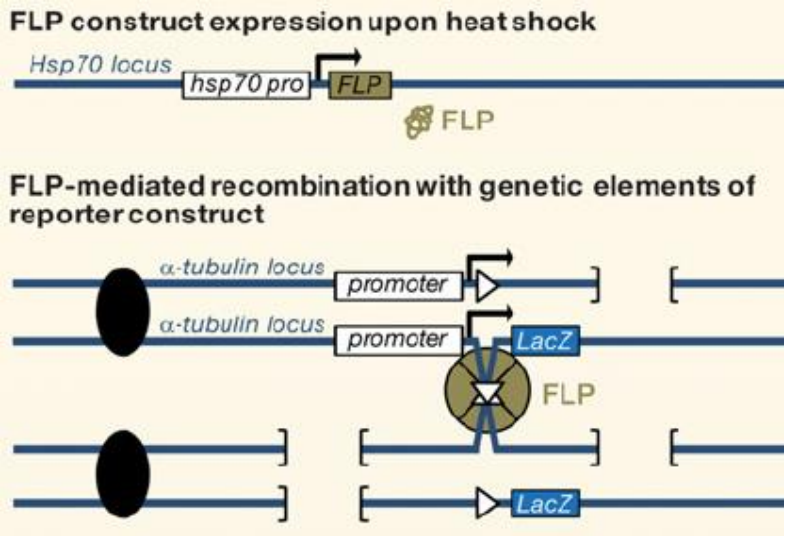
Cre is expressed under control of a tissue- or cell-specific promoter.

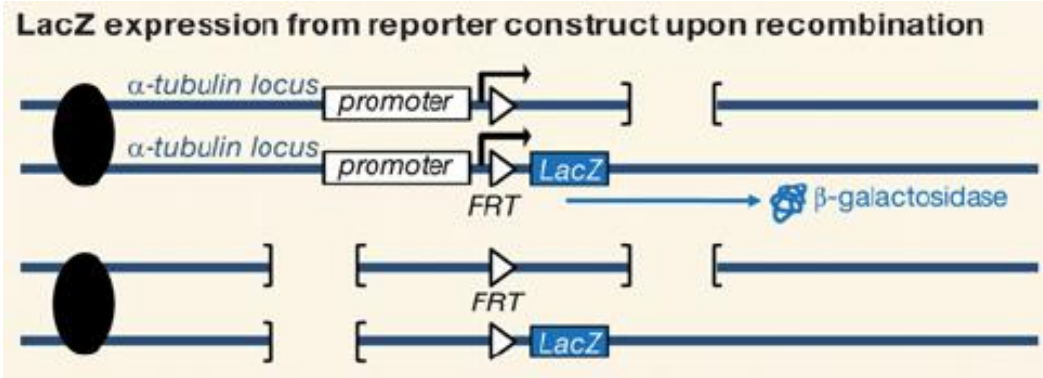


The genetic elements in the FLP-FRT system

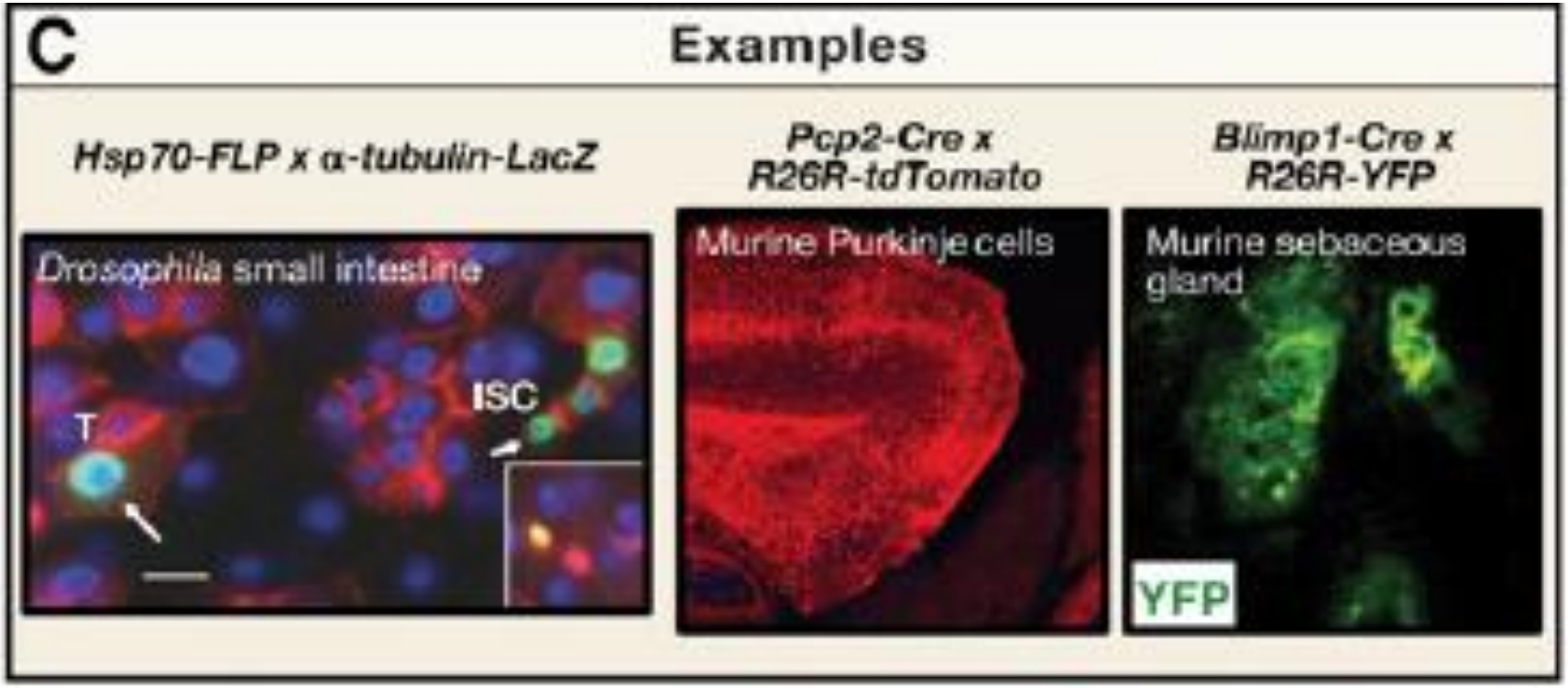


Mostly used in drosophila, FLP is usually under control or a heat shock-inducible or cell-specific promoter.





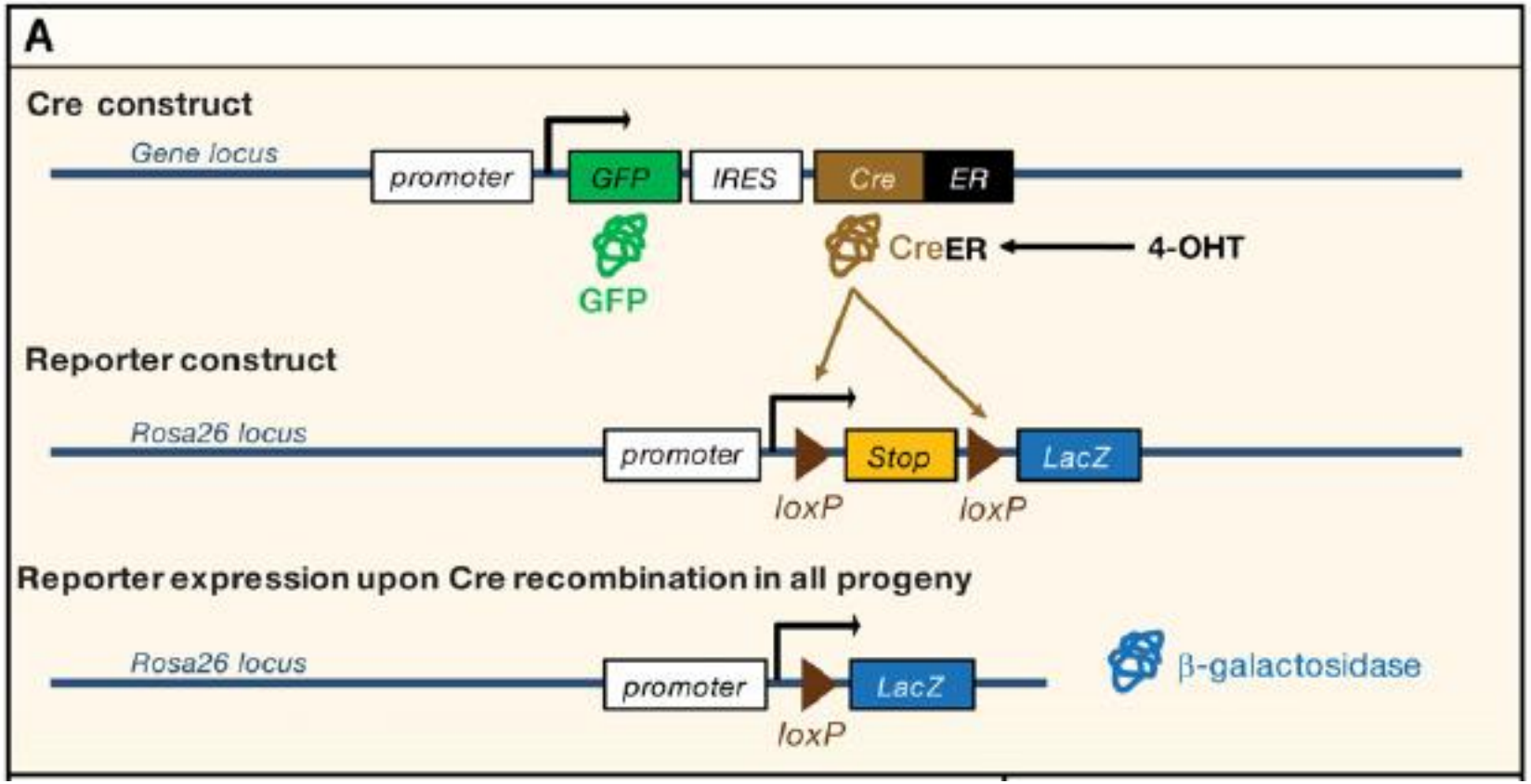
Examples


C
Examples
Hsp70-FLP x α-tubulin-LacZ
Pcp2-Cre x R26R-tdTomato
Blimp1-Cre x R26R-YFP
Drosophila small intestine
T
ISC
Murine Purkinje cells
Murine sebaceous gland
YFP

Key points in using these systems

- The recombination frequency needs to be tuned low enough to trace single cells or high enough to label entire cell population.
- Recombinase can be controlled “spatially” via cell-specific promoter.
- Recombinase can also be controlled “temporally” via inducible promoter.

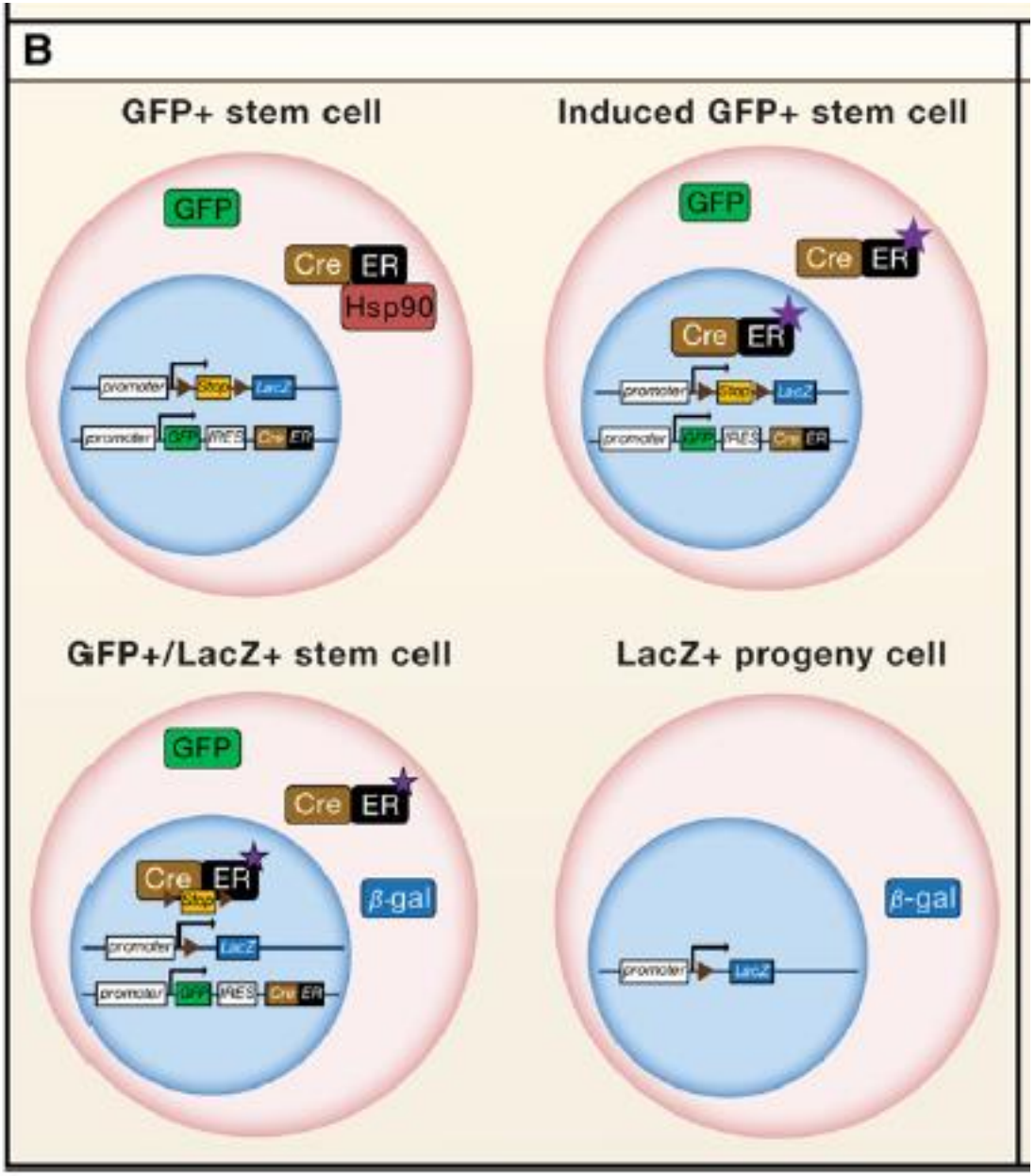
An estrogen receptor based inducible recombination



4-OHT, tamoxifen is an synthetic estrogen analog.

An estrogen receptor based inducible recombination

1. CreER is kept inactive in the cytoplasma by heat shock proteins.
2. Upon binding of tamoxifen, CreER is release from Chaperone and enter nucleus.
3. Cre recombines loxP sites to remove the stop cassette enabling expression of LacZ expression.
4. All the GFP+/LacZ+ progeny express LacZ as a genetic mark.
5. CreER itself could be controlled by another inducible promoter to reduce leakage.



Limitation of recombinase-based lineage tracing

1. Only a small fraction of cells should be marked at a time, and a large number of repeat experiments need to be carried out to obtain the large fate map.

2. If the cells are heterogeneous, repeat single cell labeling might not provide entire picture.

3. Multiple different marks would be very helpful.

Stochastic generation of multiple markers

“Over the brainbow”

nature Vol 450|1 November 2007|doi:10.1038/nature06293

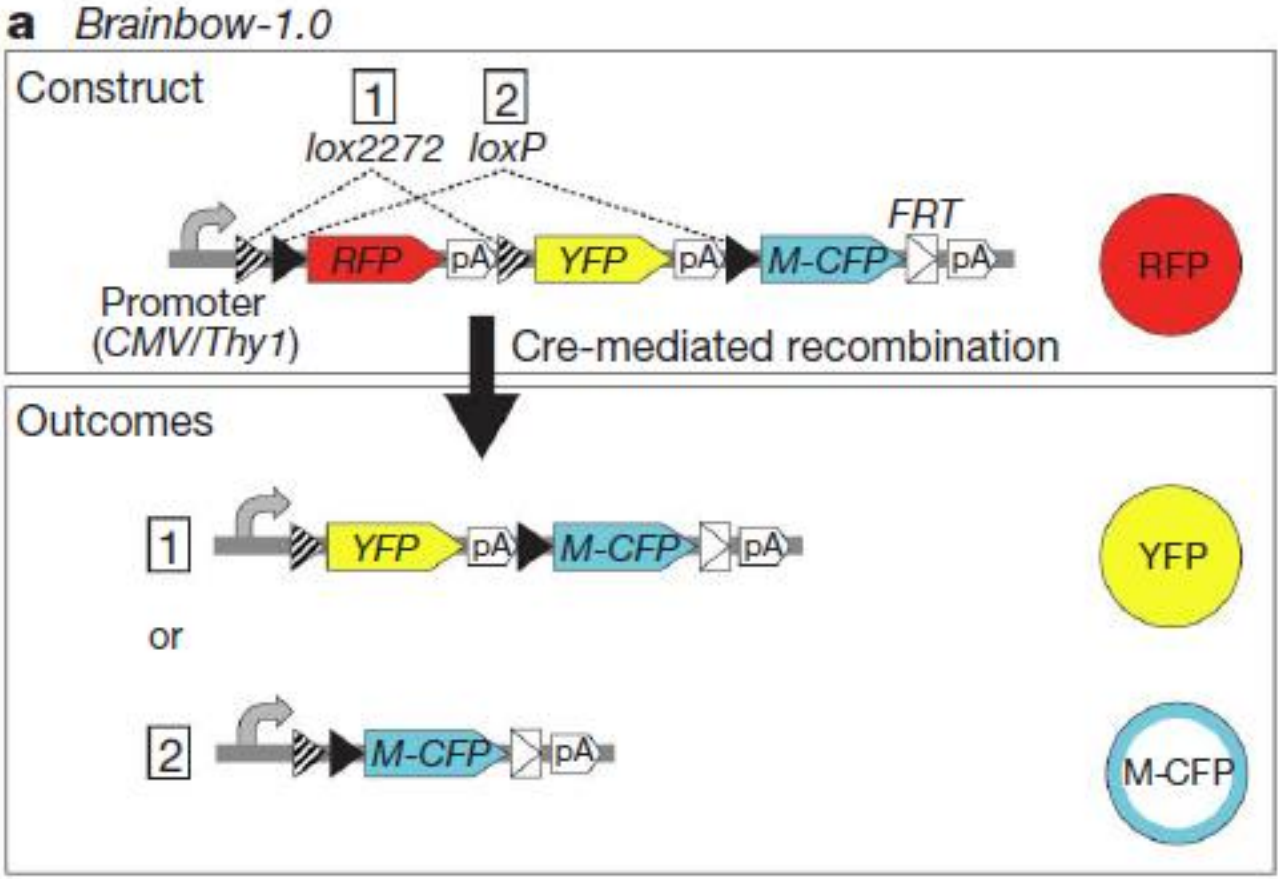
ARTICLES

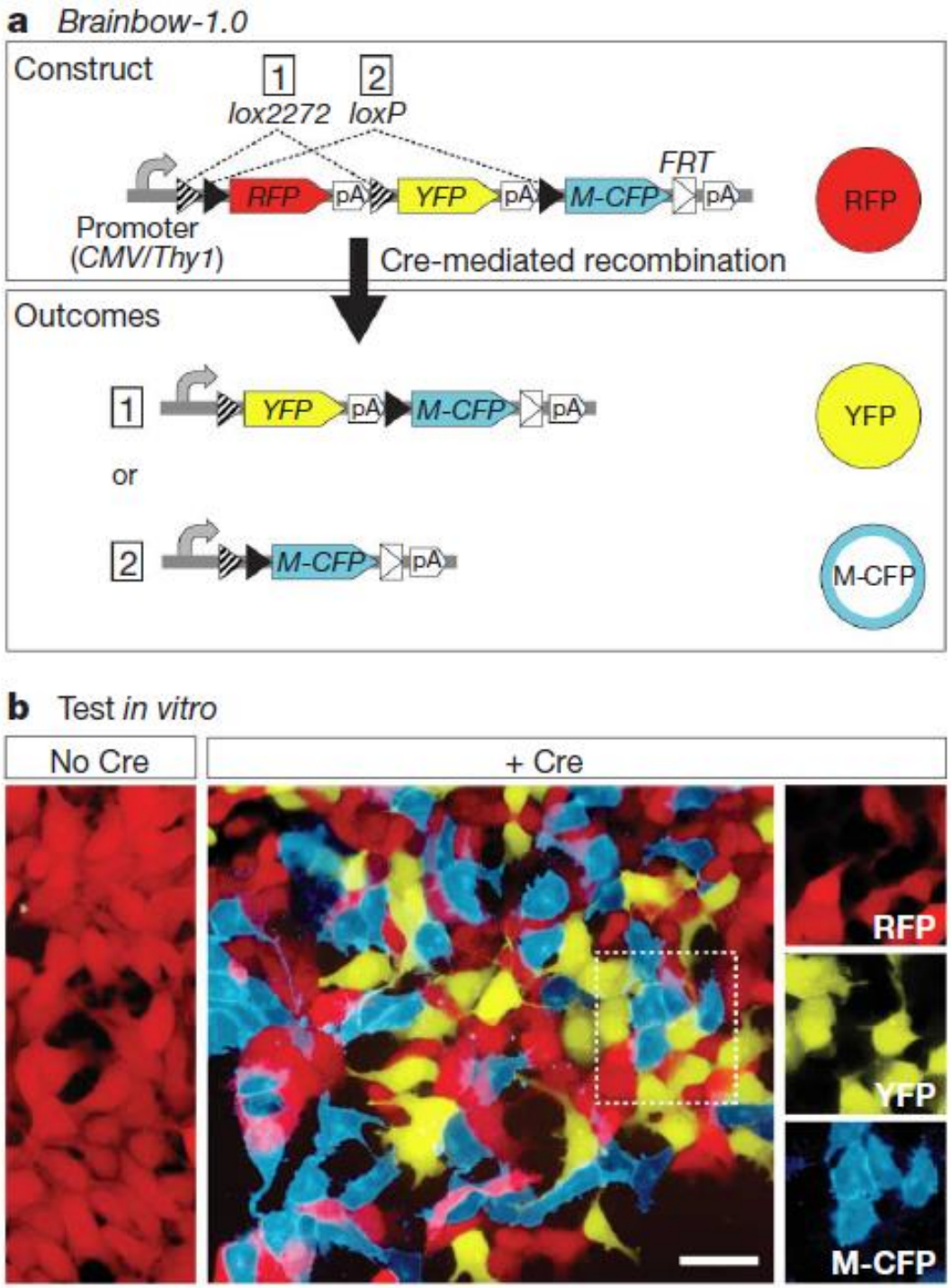
Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system

Jean Livet[1], Tamily A. Weissman[1], Hyuno Kang[1], Ryan W. Draft[1], Ju Lu[1], Robyn A. Bennis[1], Joshua R. Sanes[1] & Jeff W. Lichtman[1]

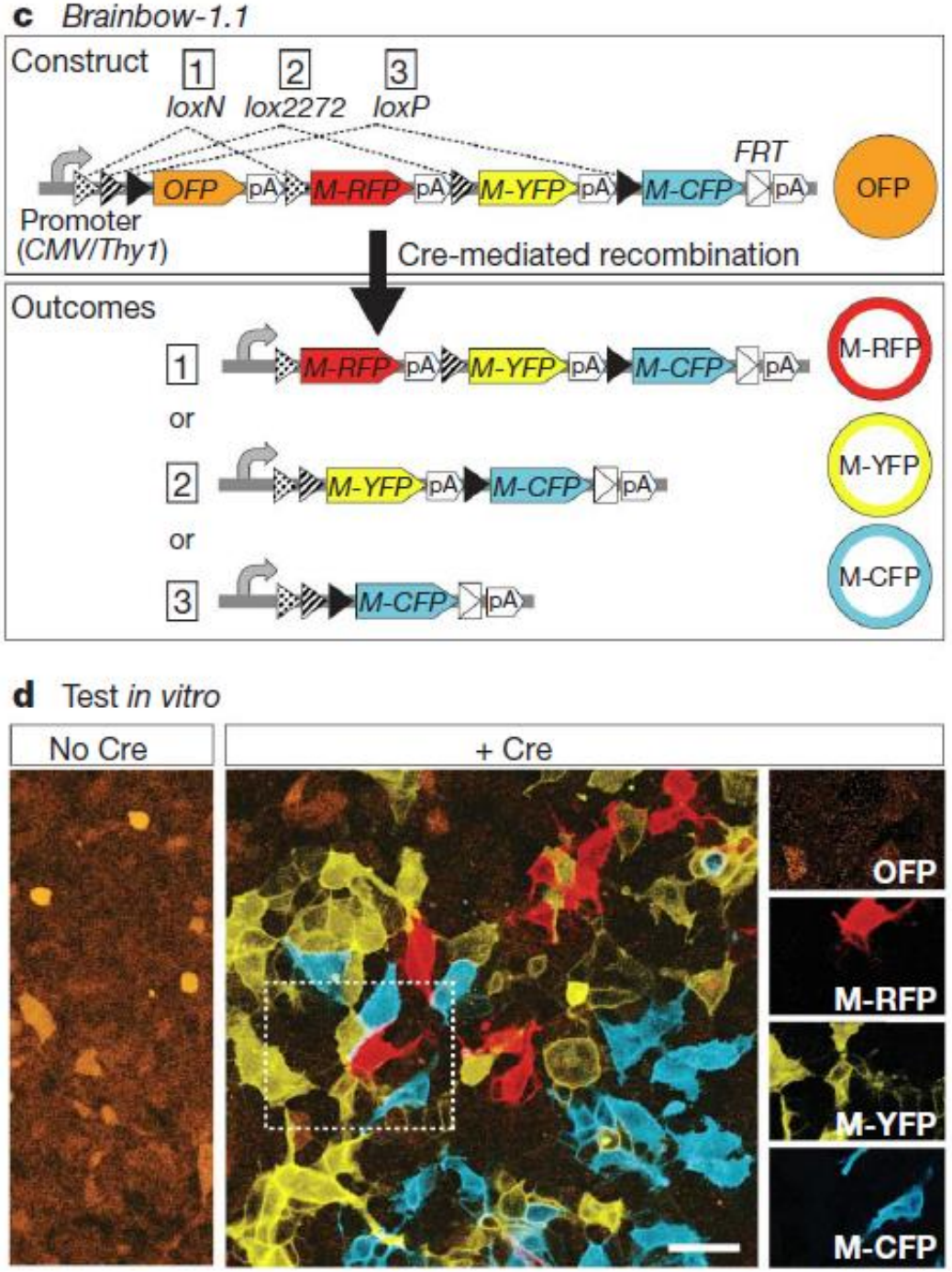
The strategies

Generating multiple reporters (different FPs) stochastically
There are a number of lox variants that can be recognized individually by Cre, but they are not compatible with each other: such as loxP and lox2272



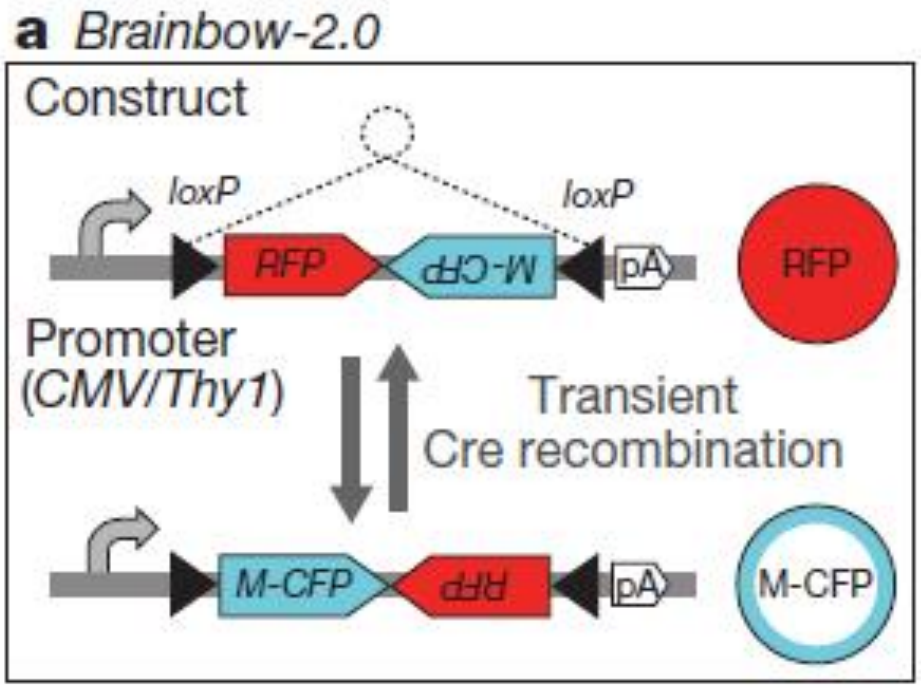

a Brainbow-1.0
Construct
1
2
lox2272
loxP
FRT
RFP
pA
YFP
pA
M-CFP
pA
Promoter
(CMV/Thy1)
Cre-mediated recombination
RFP
Outcomes
1
YFP
pA
M-CFP
pA
YFP
or
2
M-CFP
pA
M-CFP
b Test in vitro
No Cre
+ Cre
RFP
YFP
M-CFP

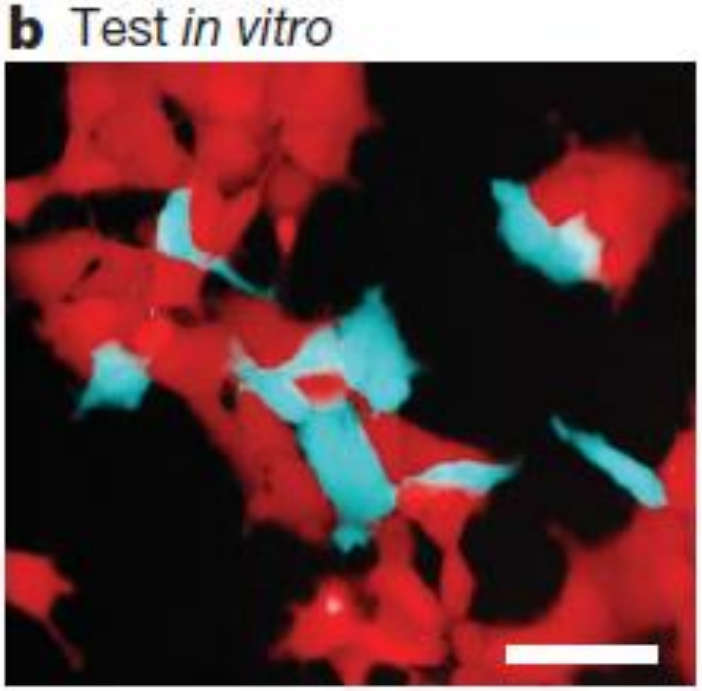
Brainbow 1.1
4 colors

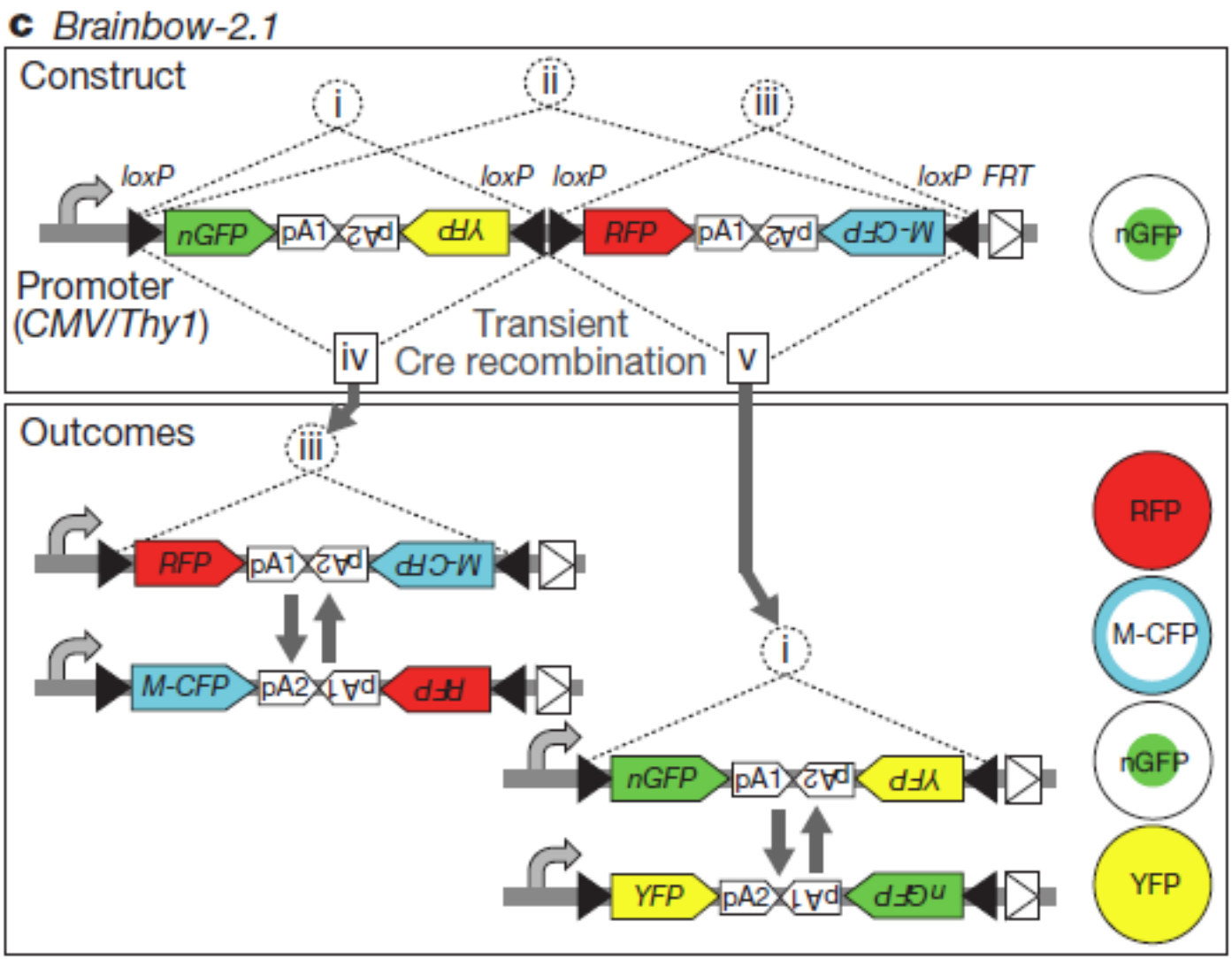


The strategies

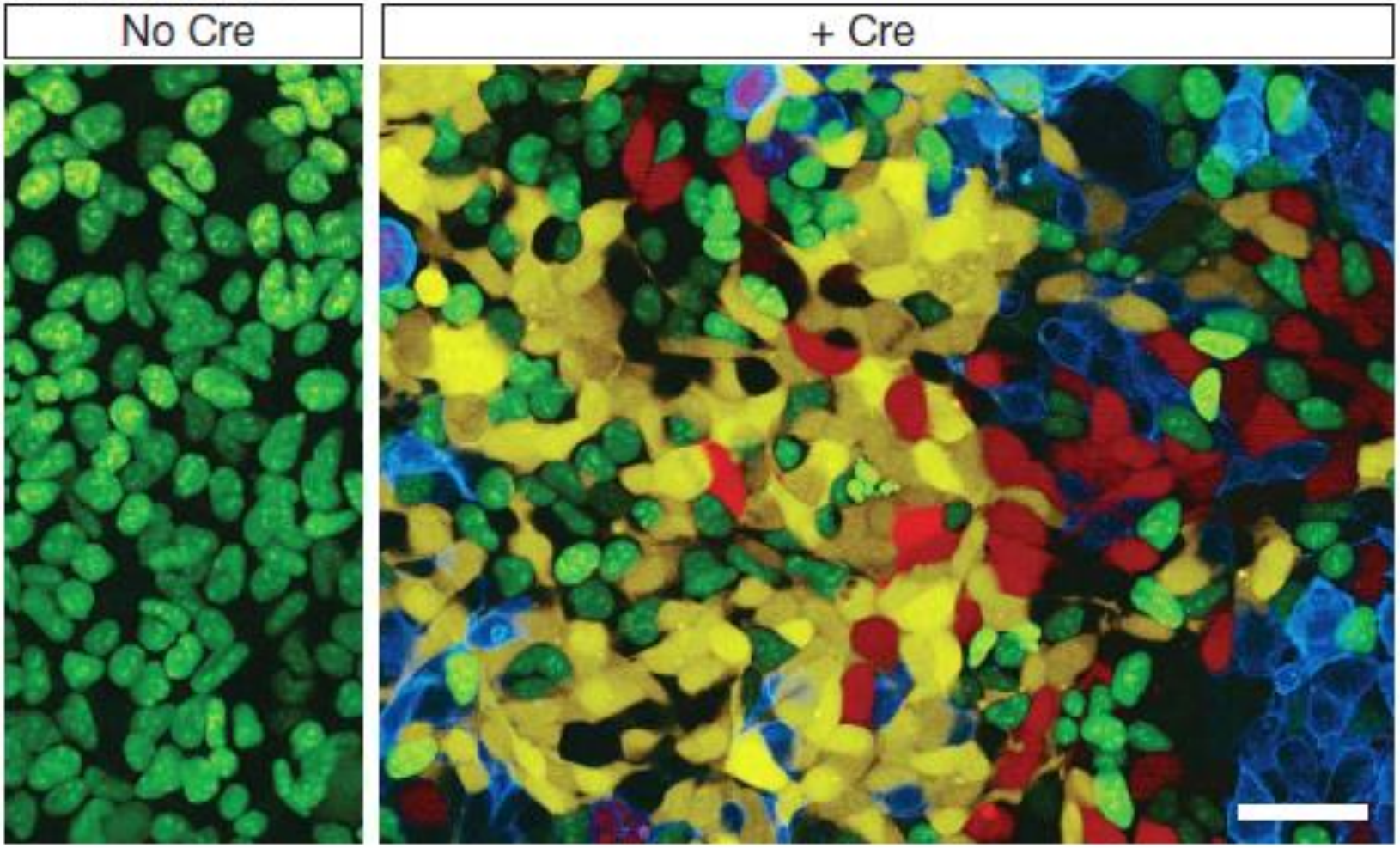
For the lox pair with same direction, Cre removes it out. For the lox pair of opposite directions, Cre flips it around.



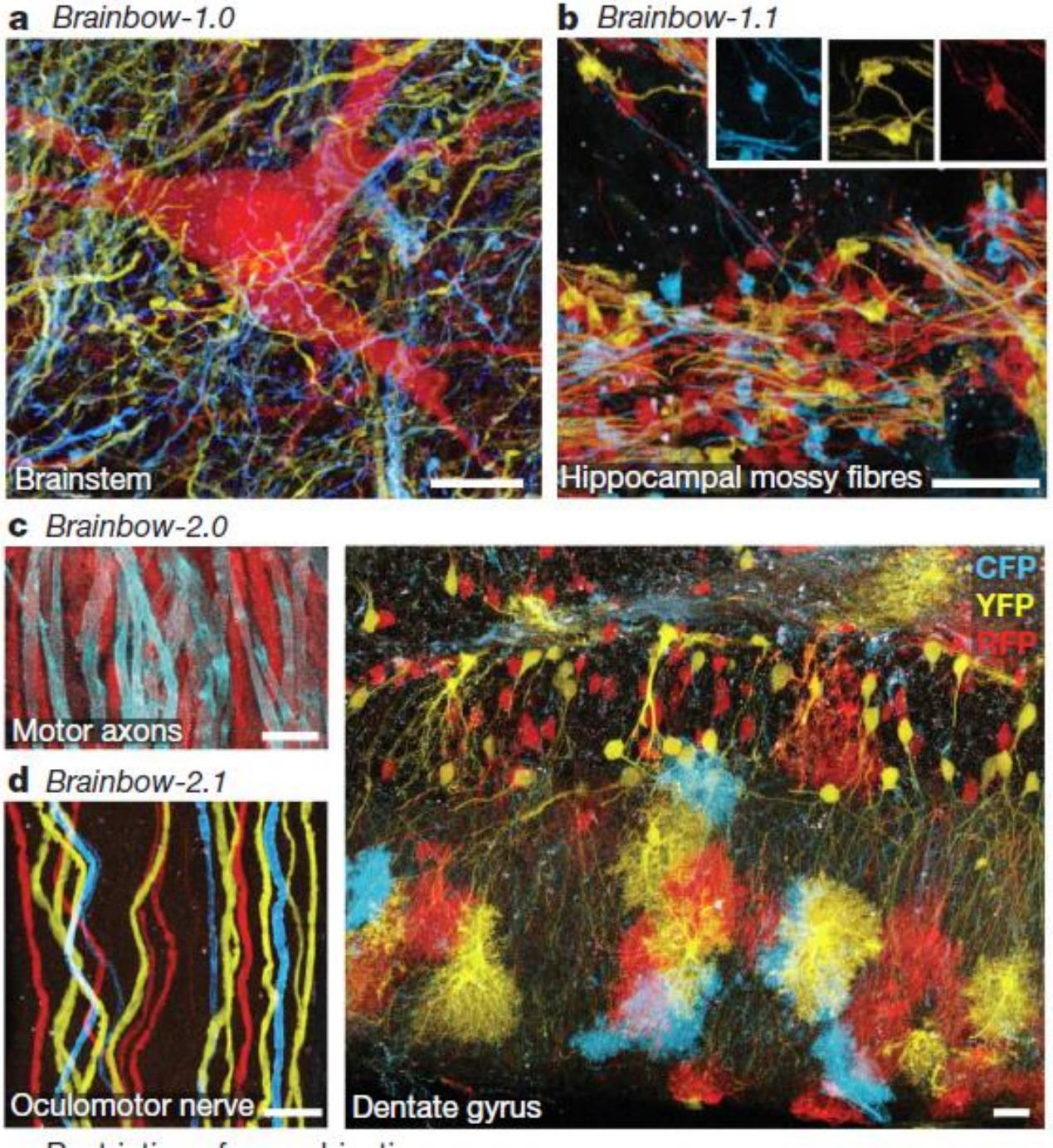



c Brainbow-2.1
Construct
i
ii
iii
loxP
loxP
loxP
loxP
FRT
nGFP
pA1
pA2
YFP
RFP
pA1
pA2
M-CFP
nGFP
Promoter
(CMV/Thy1)
Transient
Cre recombination
iv
v
Outcomes
iii
RFP
pA1
pA2
M-CFP
M-CFP
pA2
pA1
RFP
i
nGFP
pA1
pA2
YFP
YFP
pA2
pA1
nGFP
RFP
M-CFP
nGFP
YFP

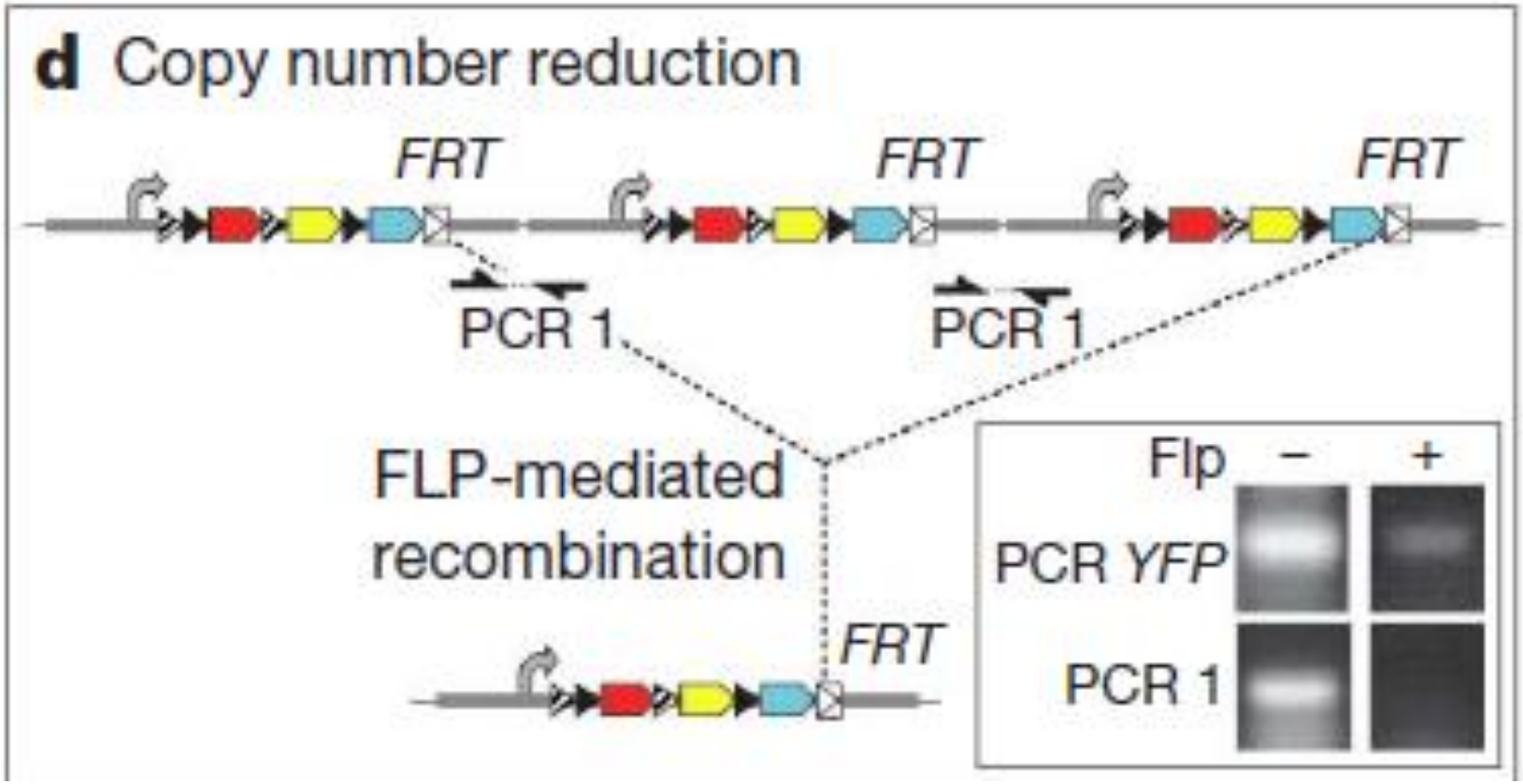
d Test *in vitro*

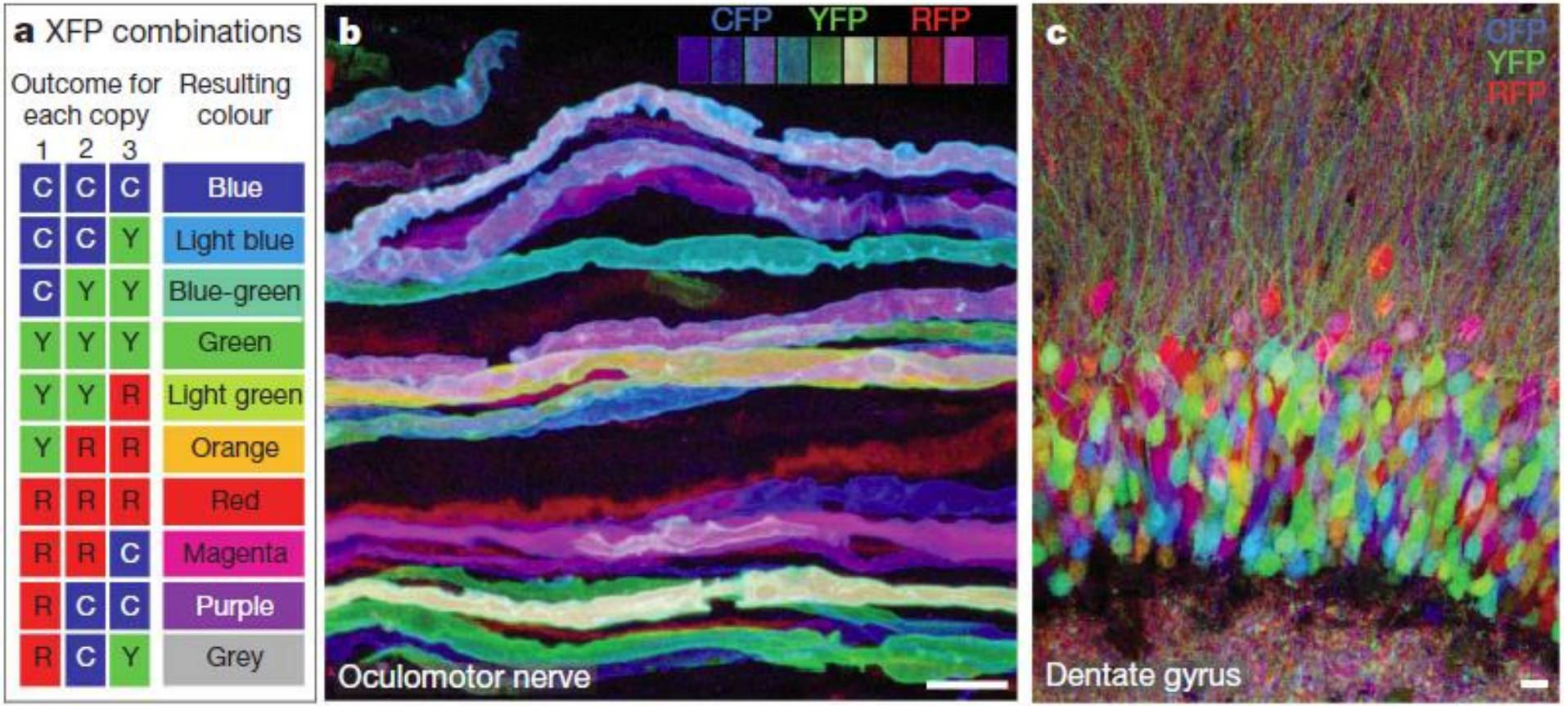


Some results



Using FLP-FRT to change brainbow copy number




a XFP combinations
Outcome for each copy
Resulting colour
1 2 3
C C C Blue
C C Y Light blue
C Y Y Blue-green
Y Y Y Green
Y Y R Light green
Y R R Orange
R R R Red
R R C Magenta
R C C Purple
R C Y Grey
b
CFP YFP RFP
Oculomotor nerve
c
CFP
YFP
RFP
Dentate gyrus

Decision tree for choosing an appropriate lineage tracing strategy