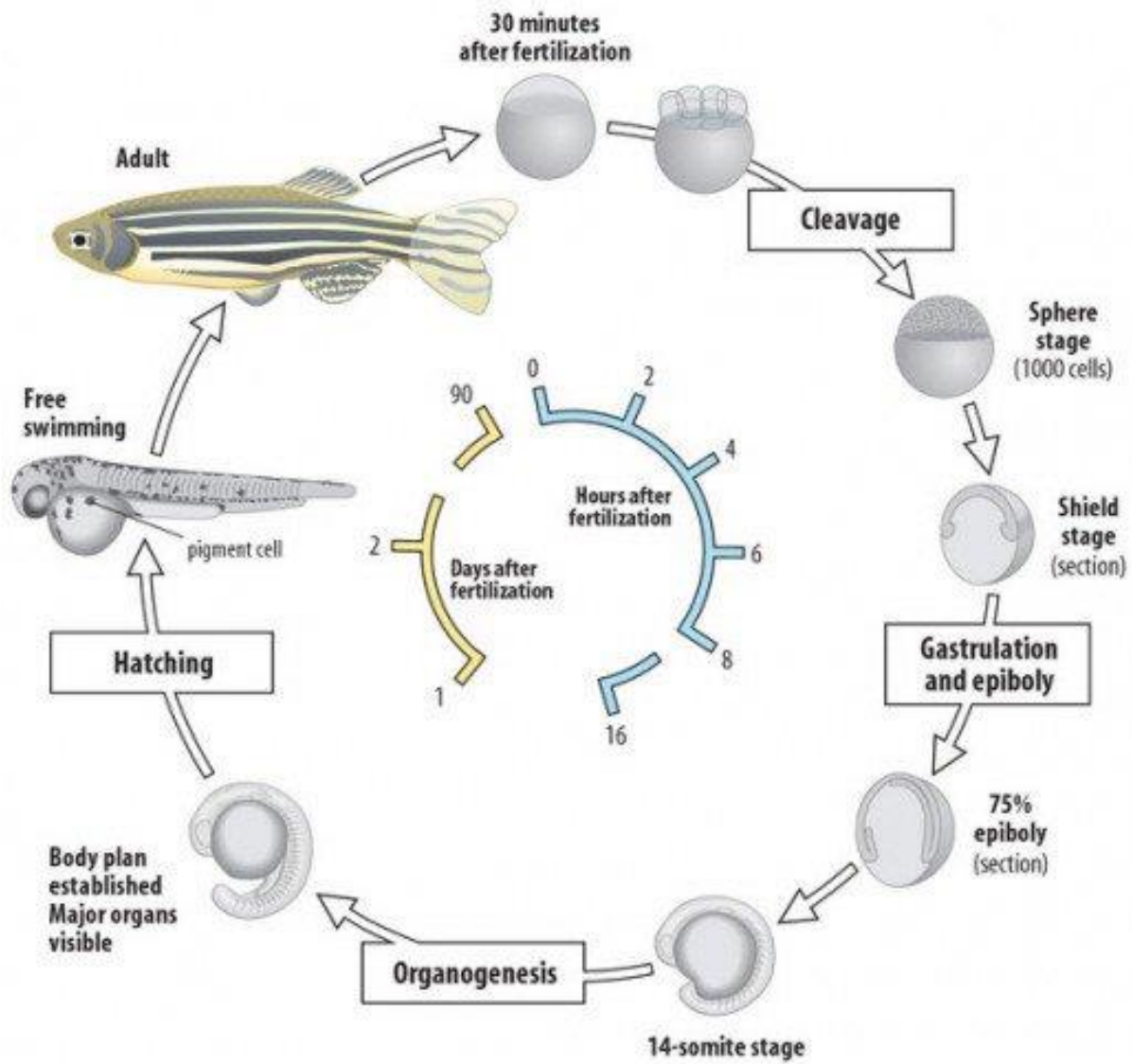


# Zebrafish, a model of choice for biomedical research

Yoav Gothilf  
Dept. Neurobiology, Tel Aviv University  
yoavgothilf@gmail.com



30 minutes after fertilization

Adult

Cleavage

Sphere stage (1000 cells)

Shield stage (section)

Gastrulation and epiboly

75% epiboly (section)

14-somite stage

Organogenesis

Body plan established Major organs visible

Hatching

Free swimming

pigment cell

Hours after fertilization

Days after fertilization

0

2

4

6

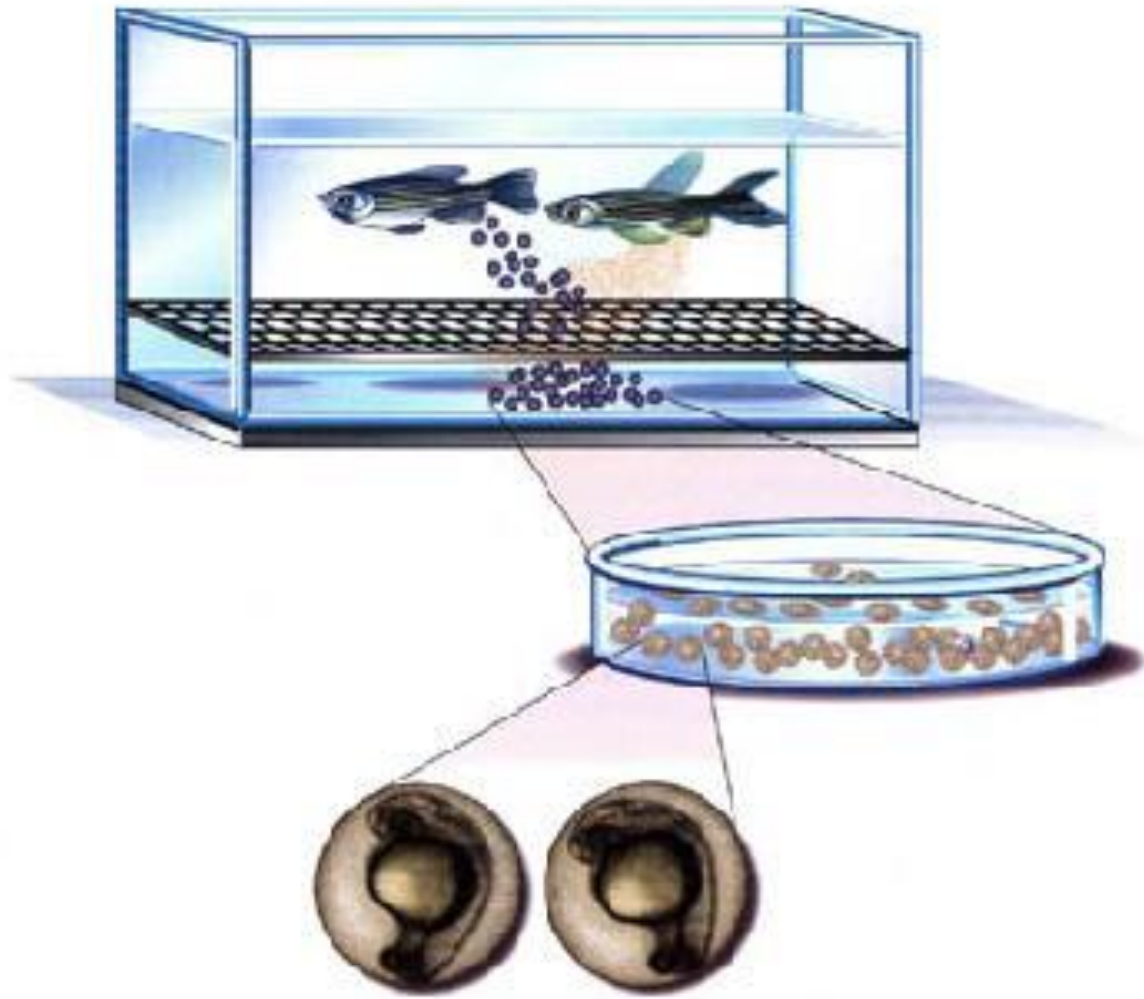
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16

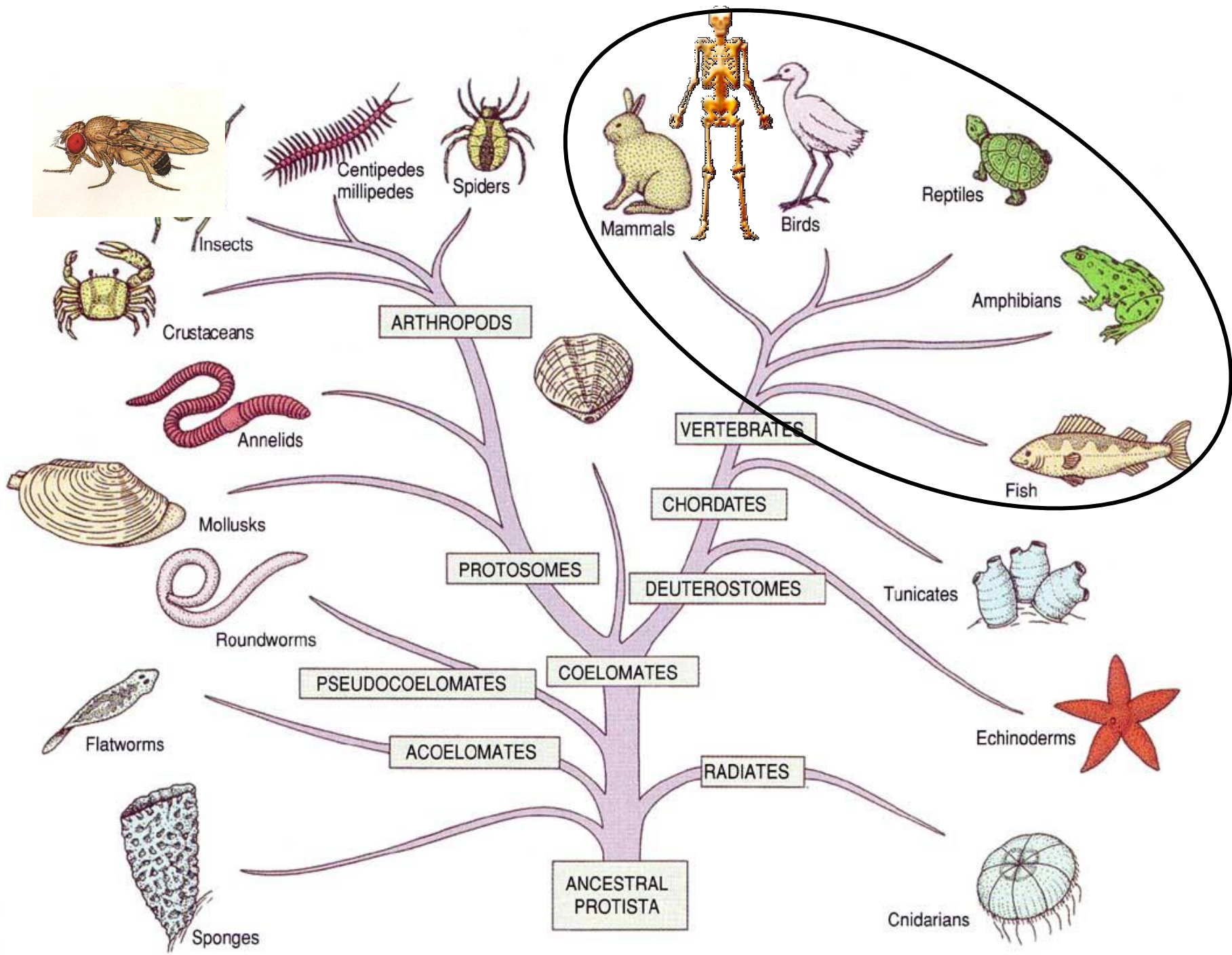
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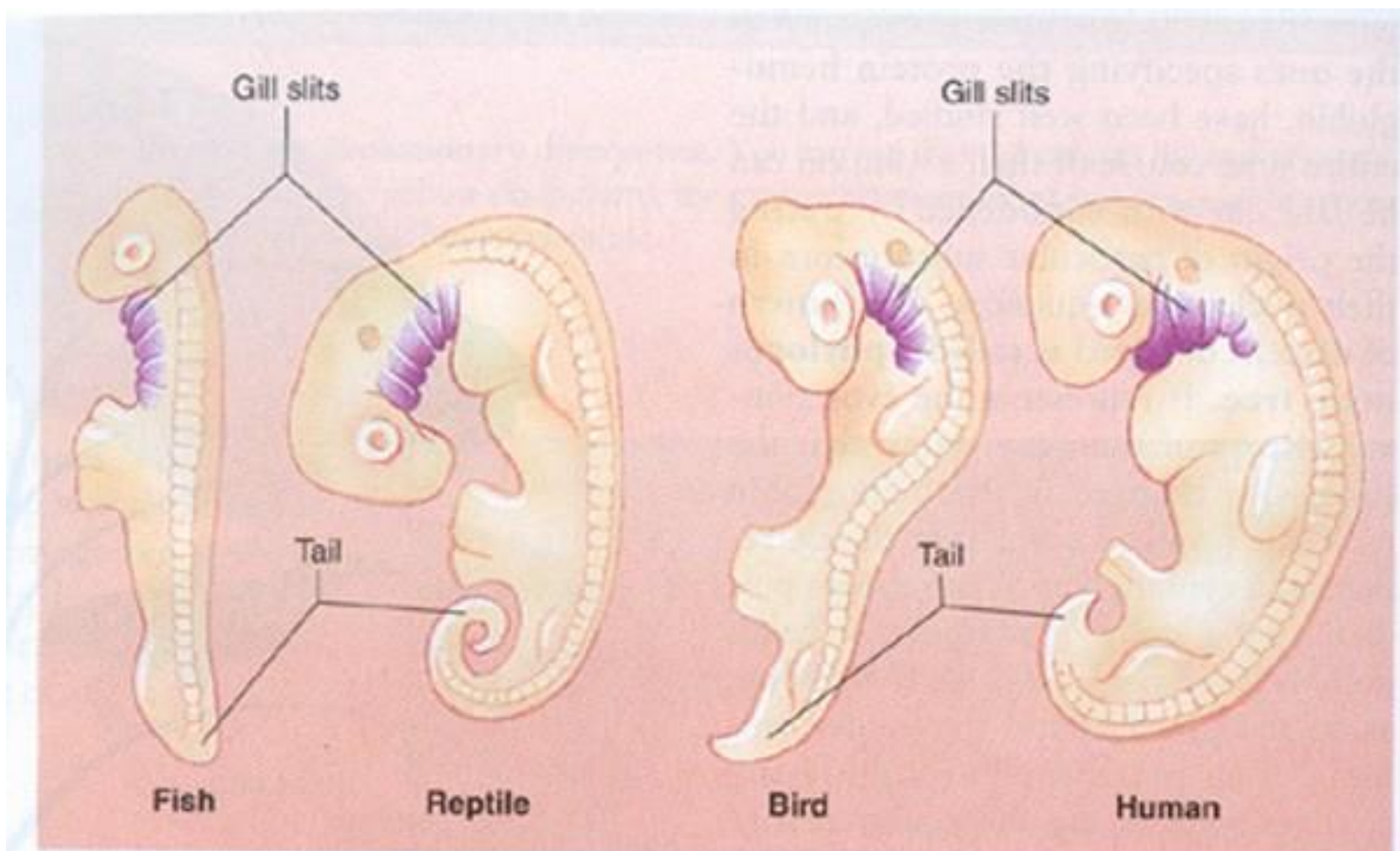
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1



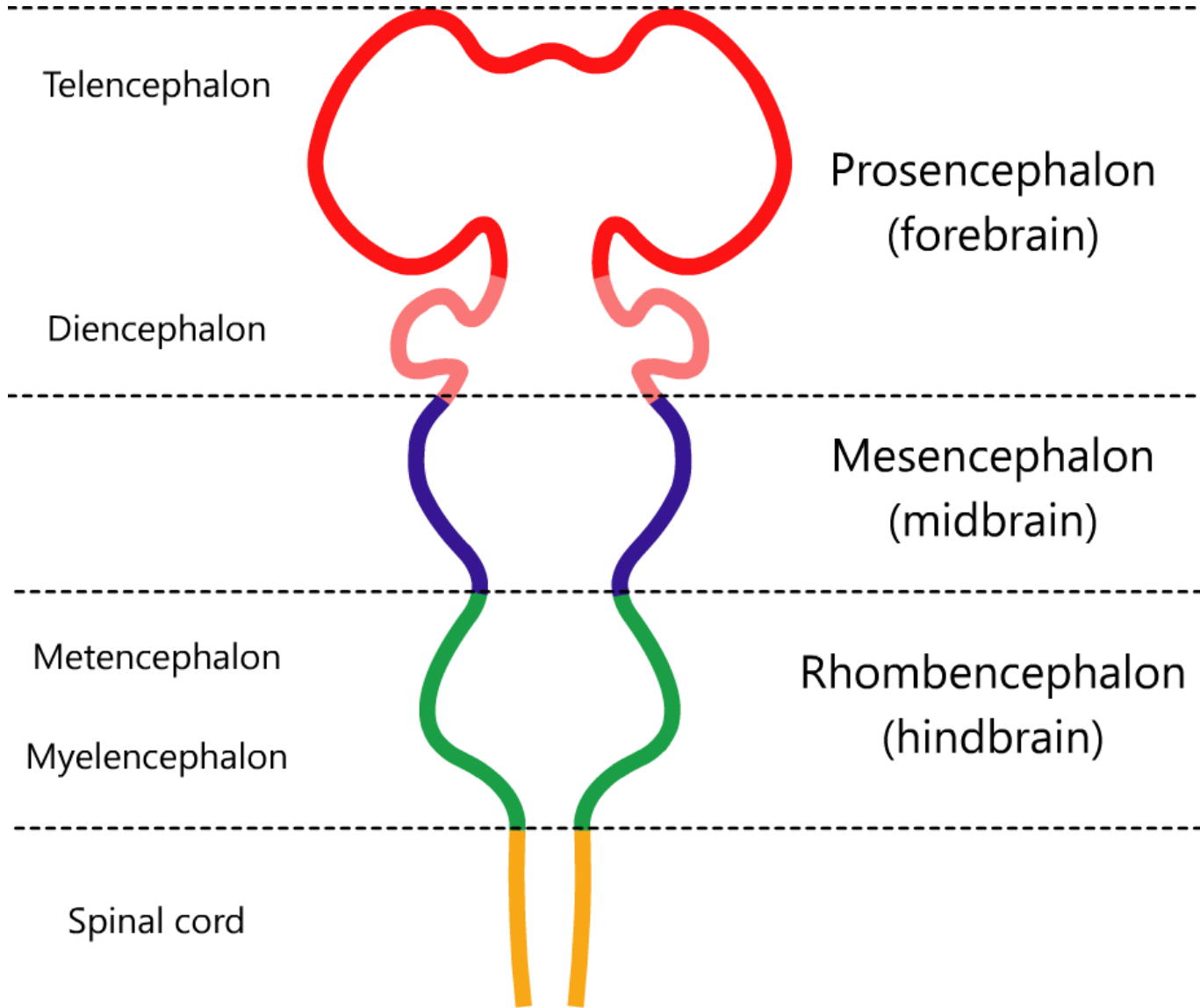
<https://youtu.be/4c-Kw4timVA>





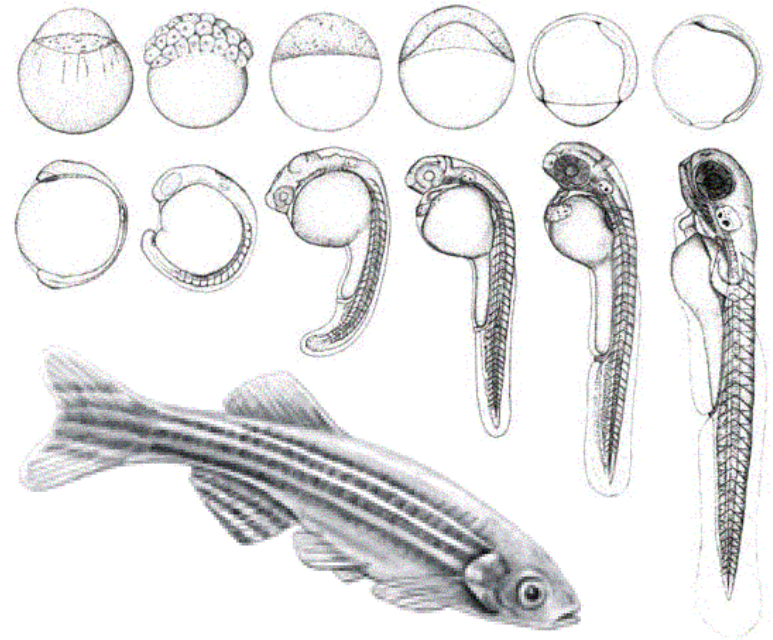
**FIGURE 20.18**

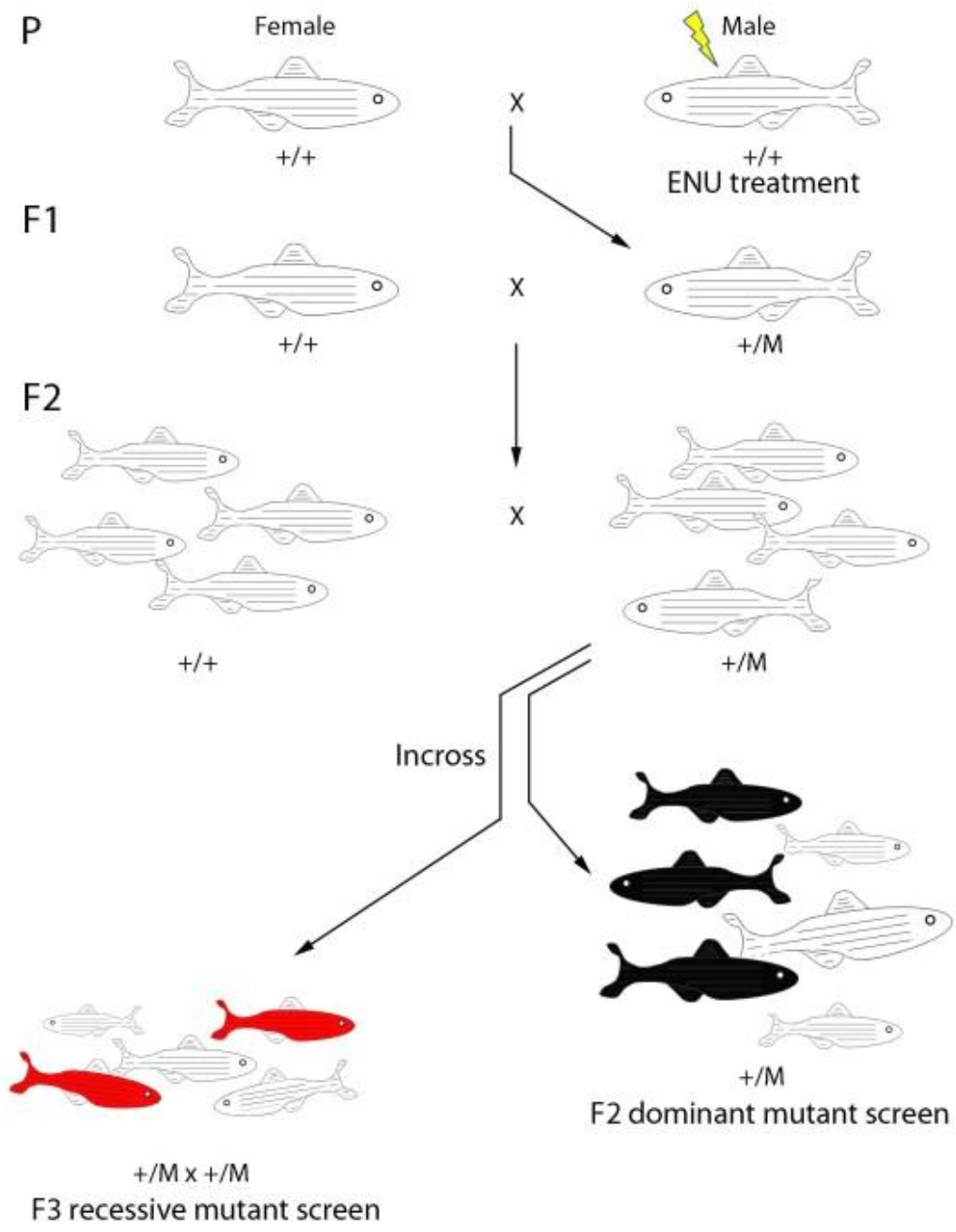
**Our embryos show our evolutionary history.** The embryos of various groups of vertebrate animals show the features they all share early in development, such as gill slits (*in purple*) and a tail.



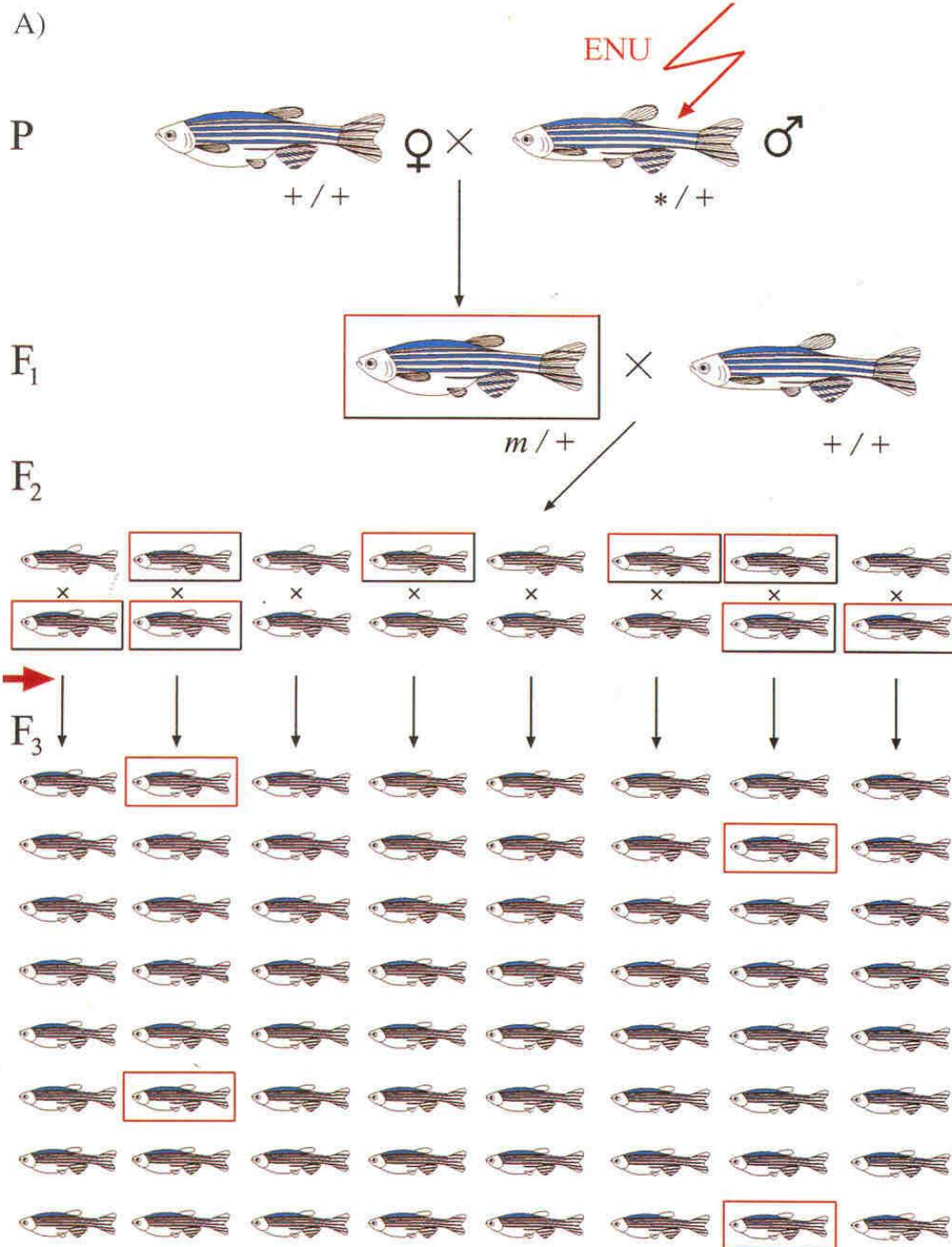
# Zebrafish

- Vertebrate
- External fertilization
- Many embryos
- Transparent embryos
- Fast development
- Short generation time
- Easy to maintain

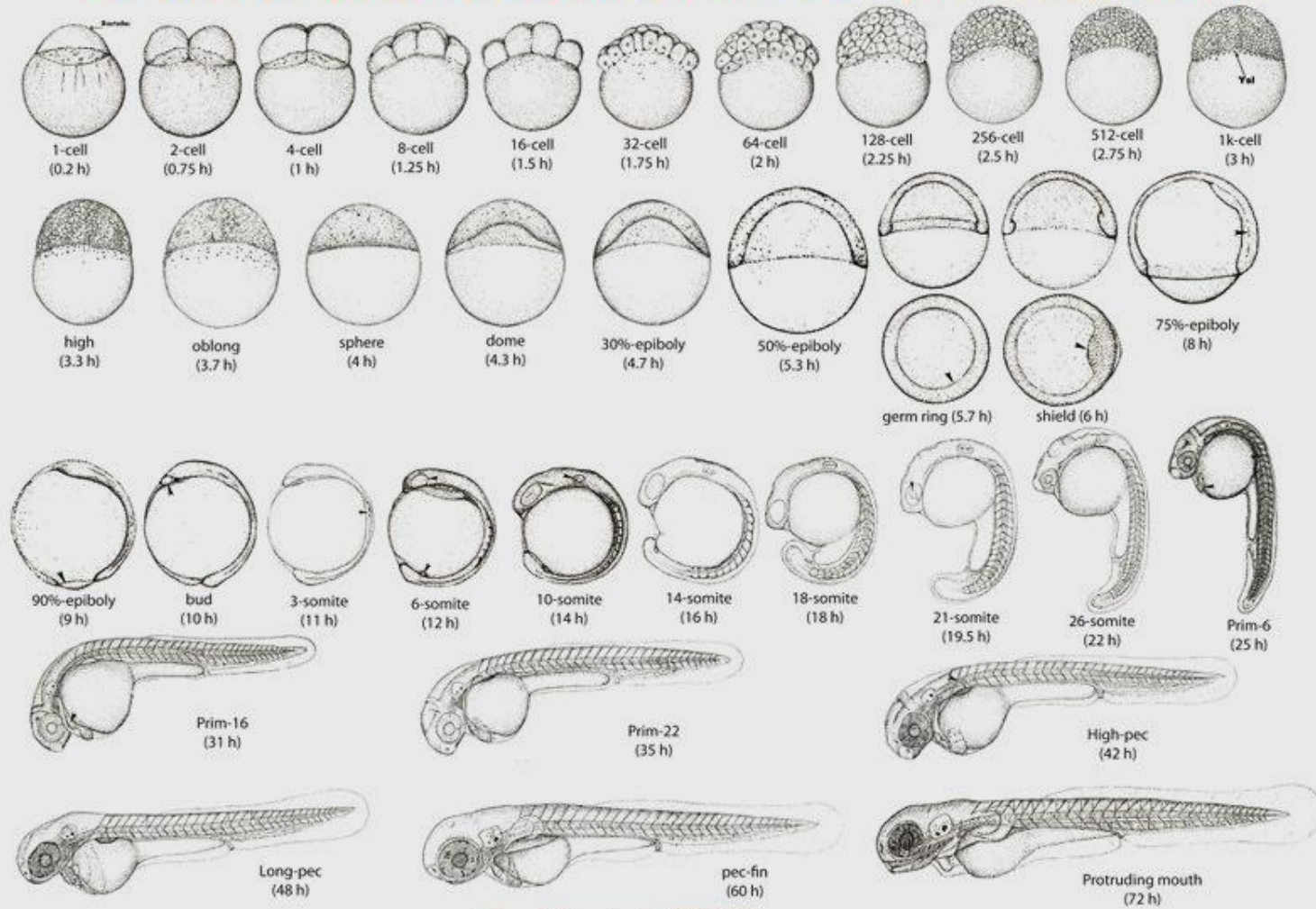








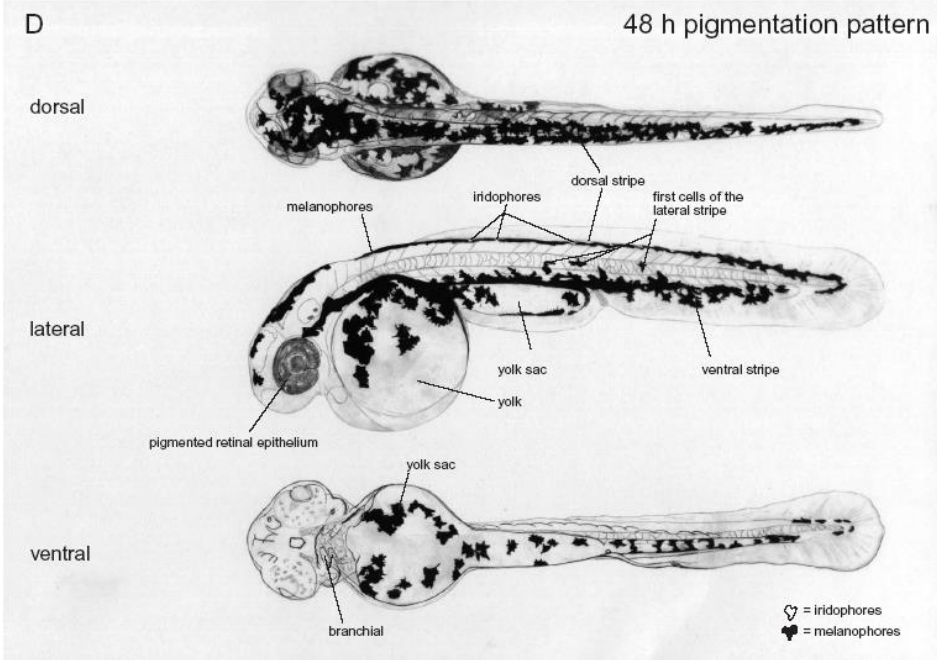
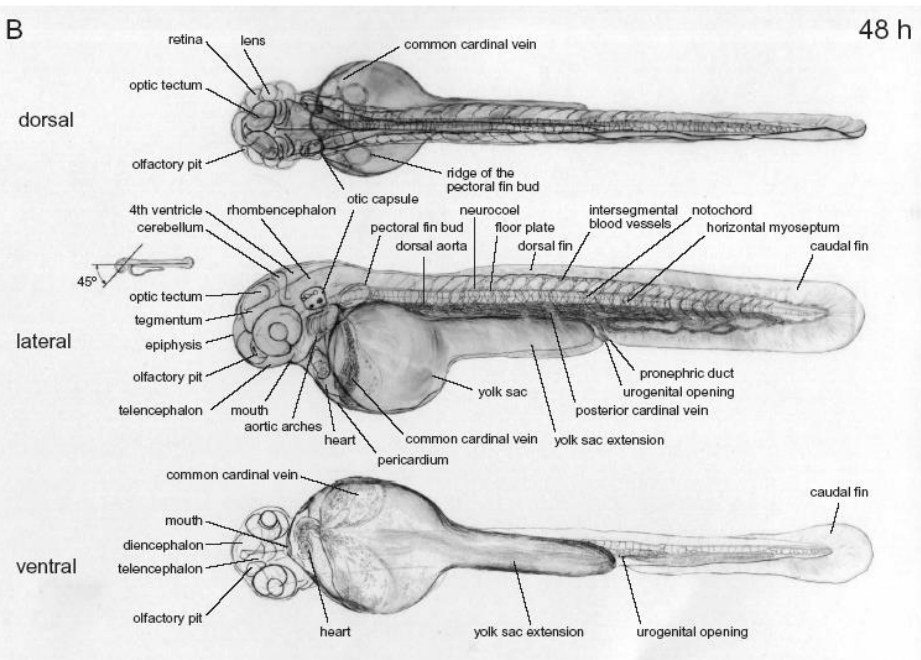
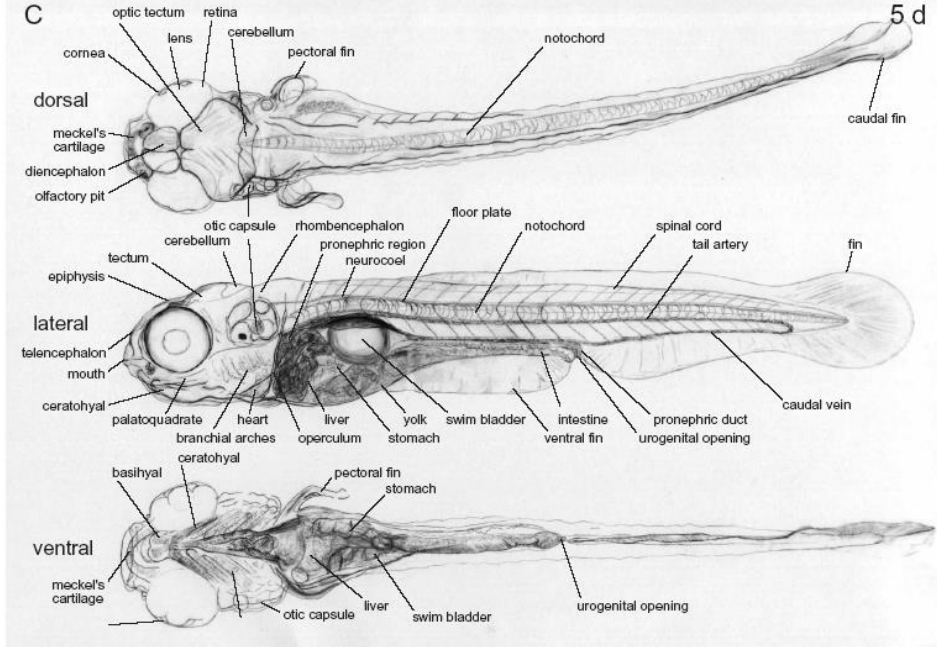
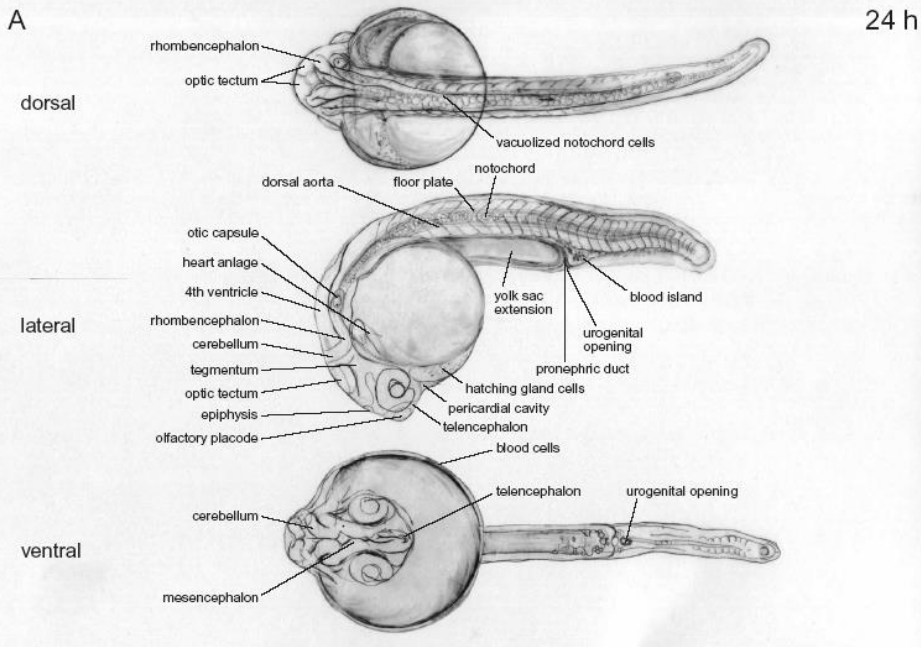
# STAGES OF EMBRYONIC DEVELOPMENT OF THE ZEBRAFISH

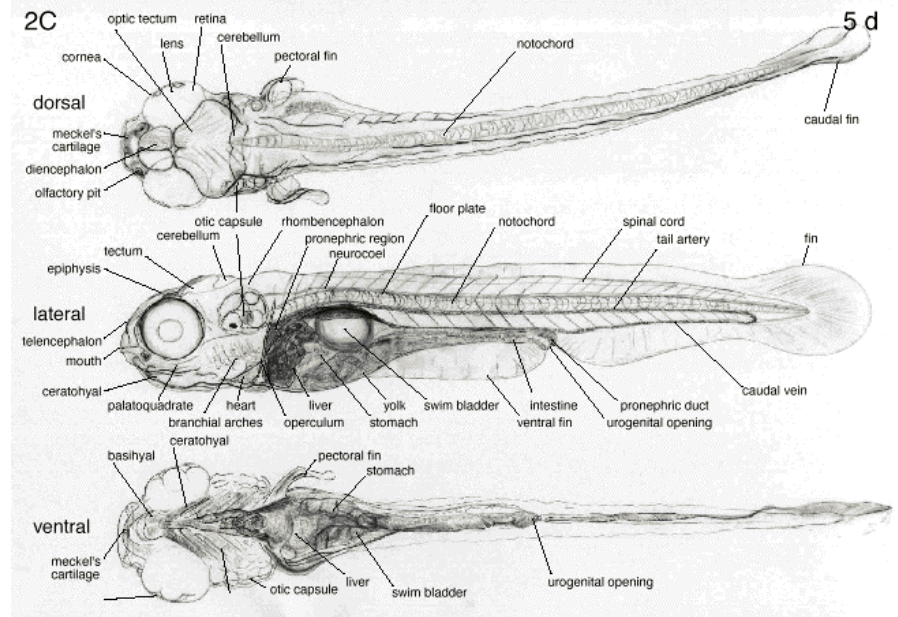
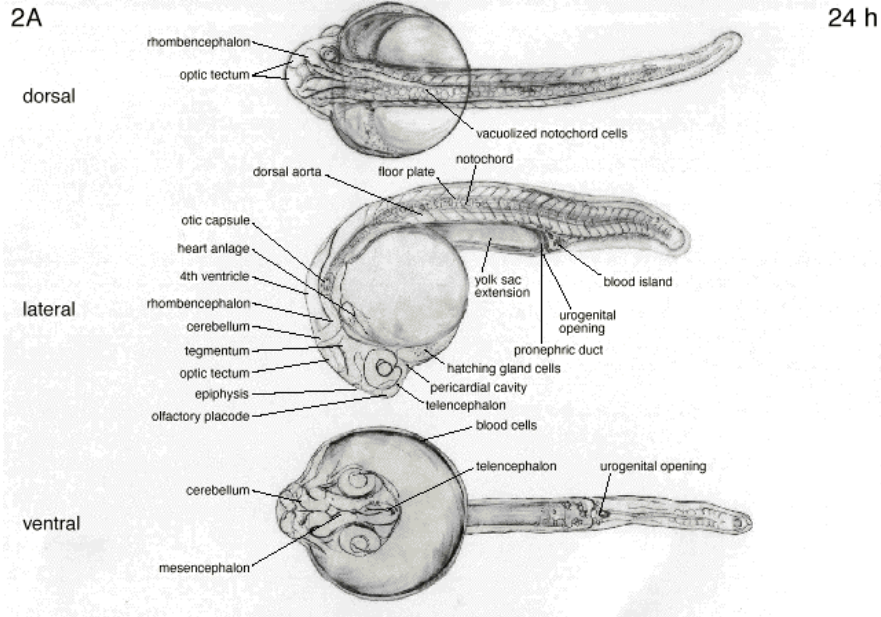


sandeepkhat@ece.ucsb.edu

Systems Biomaging Lab, ECE, UCSB  
sybil.ece.ucsb.edu

Source <http://www.uoneuro.uoregon.edu/k12/zfk12.html>







# Development

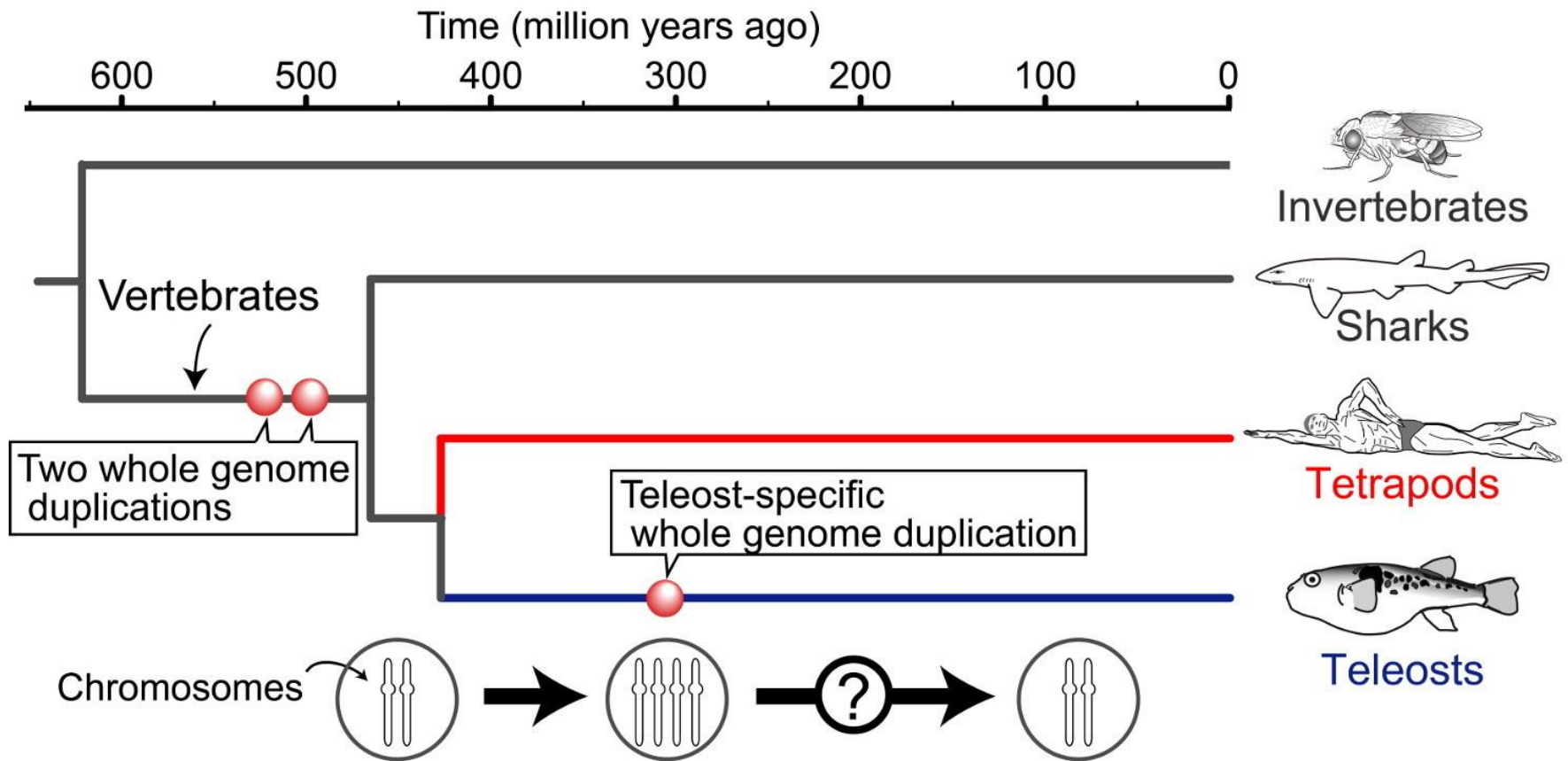
Volume 123

December 1996

Zebrafish Issue

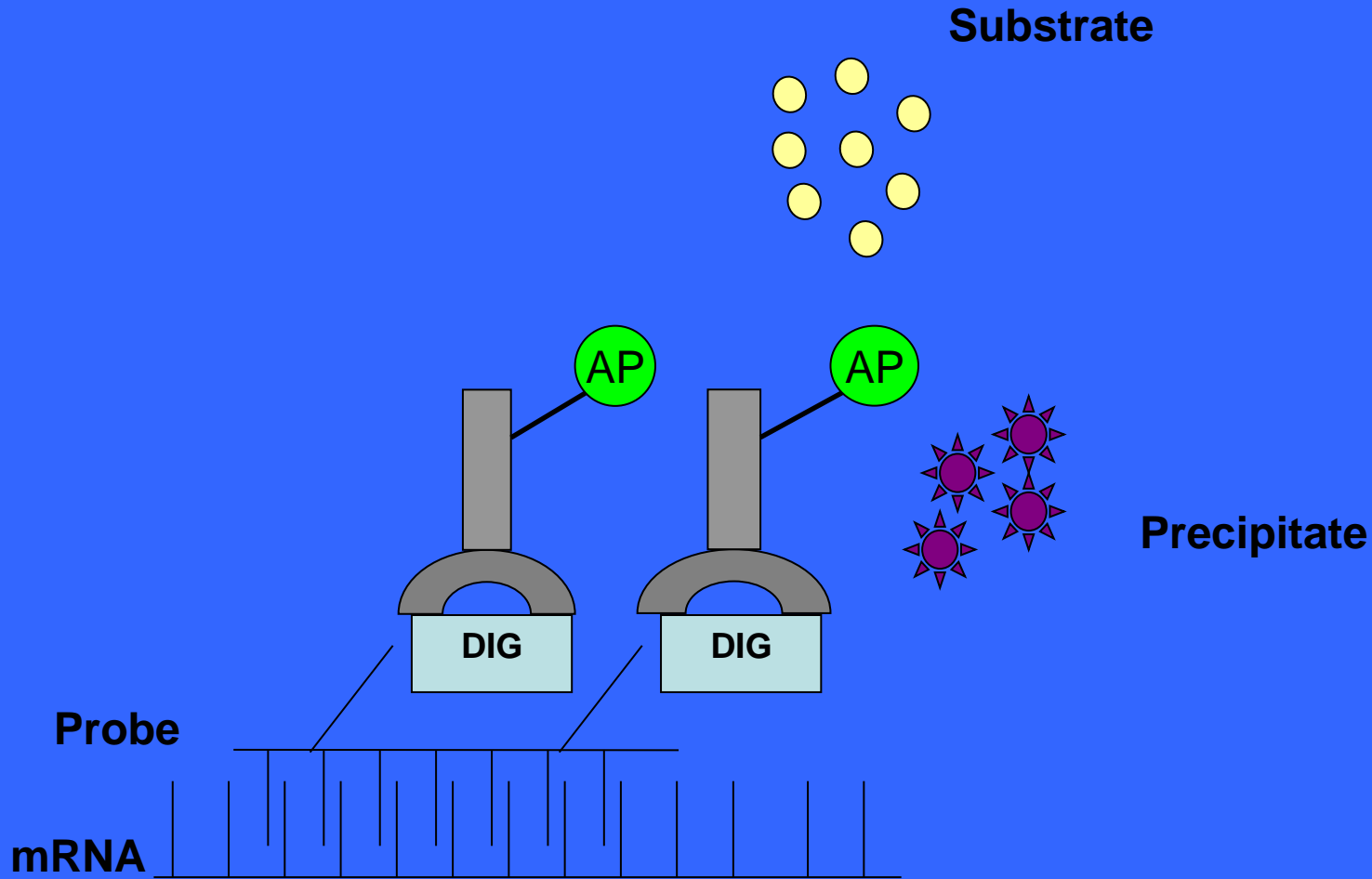
# Zebrafish, a model of Choice

- Vertebrate
- Large number of accessible transparent embryos
- Fast development and short generation time
- Developmental mutants
- Gene expression analysis
- Transgenesis
- Gene knockdown, knockout and knockin
- Behavioral tests
- Imaging capabilities

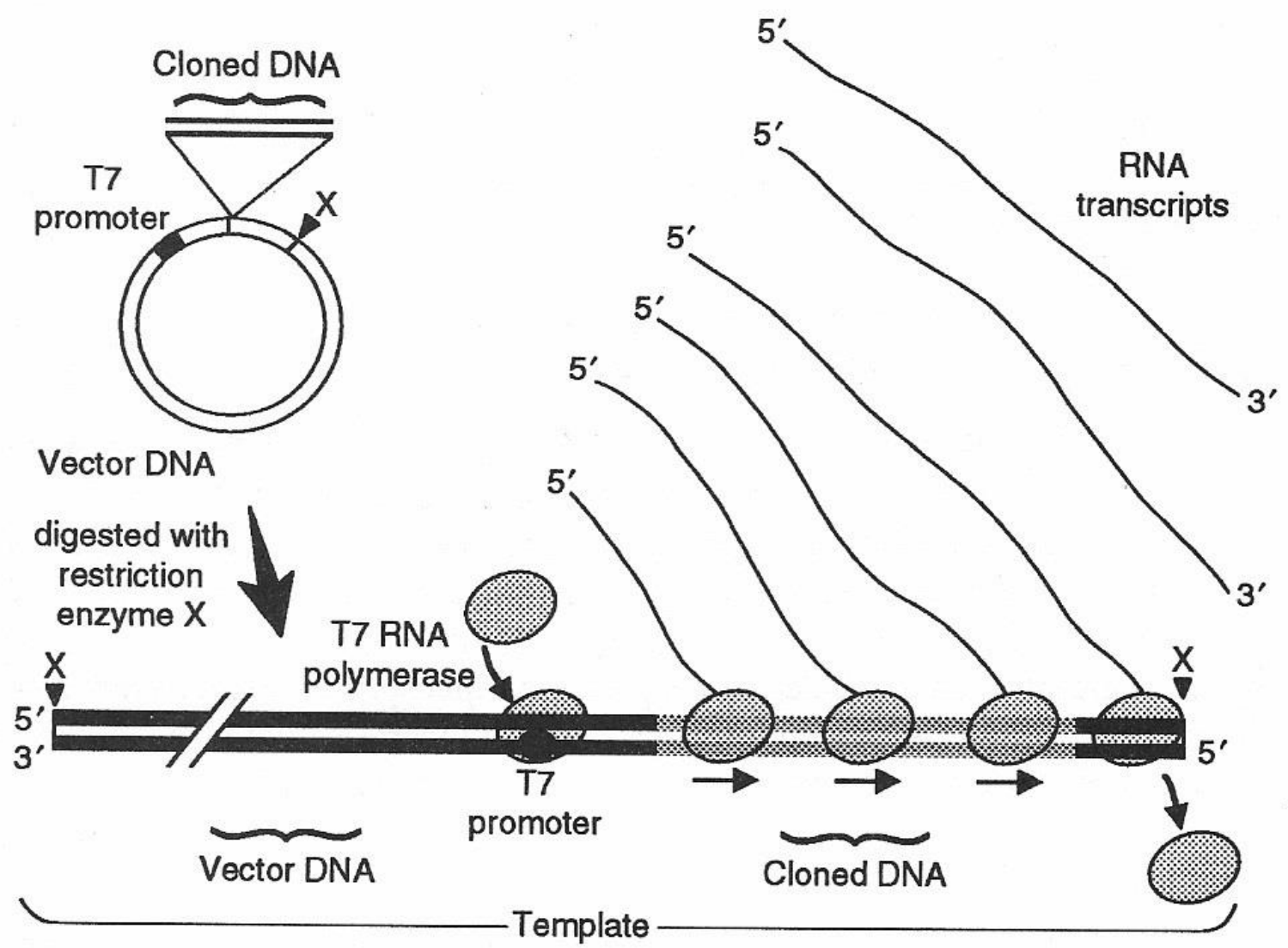


Whole genome duplication is a rare evolutionary event that has played a dramatic role in diversification

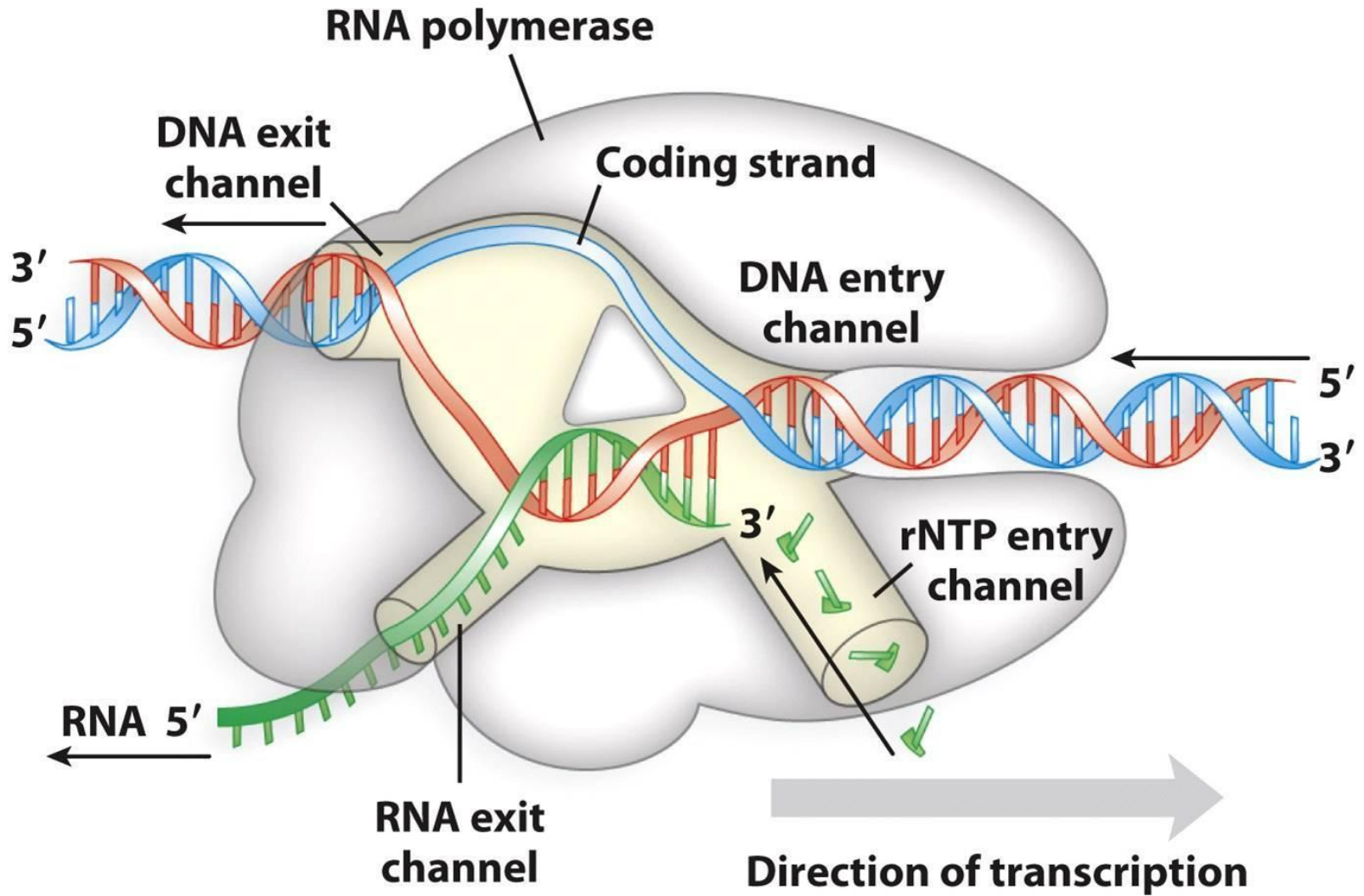
# Whole mount *in situ* hybridization





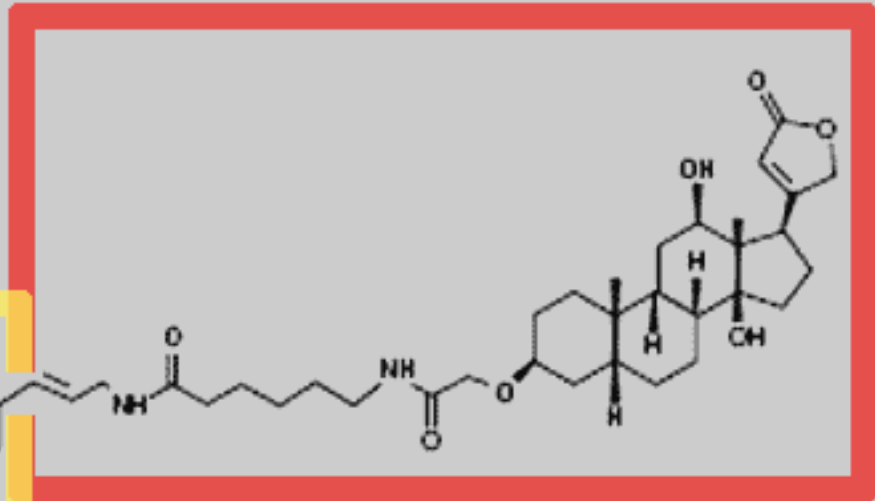
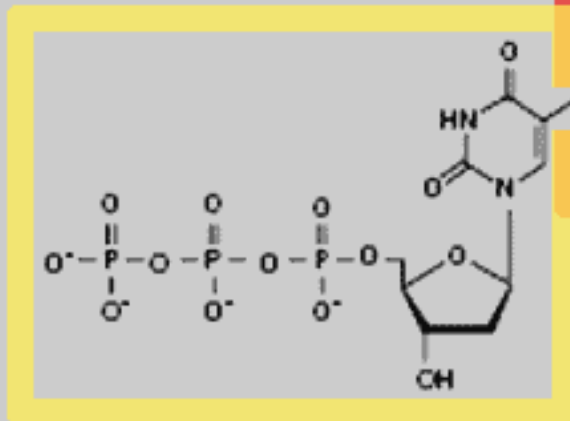


**Figure 3.8.1** Runoff transcripts.



**Figure 15-14**  
*Molecular Biology: Principles and Practice*  
 © 2012 W. H. Freeman and Company

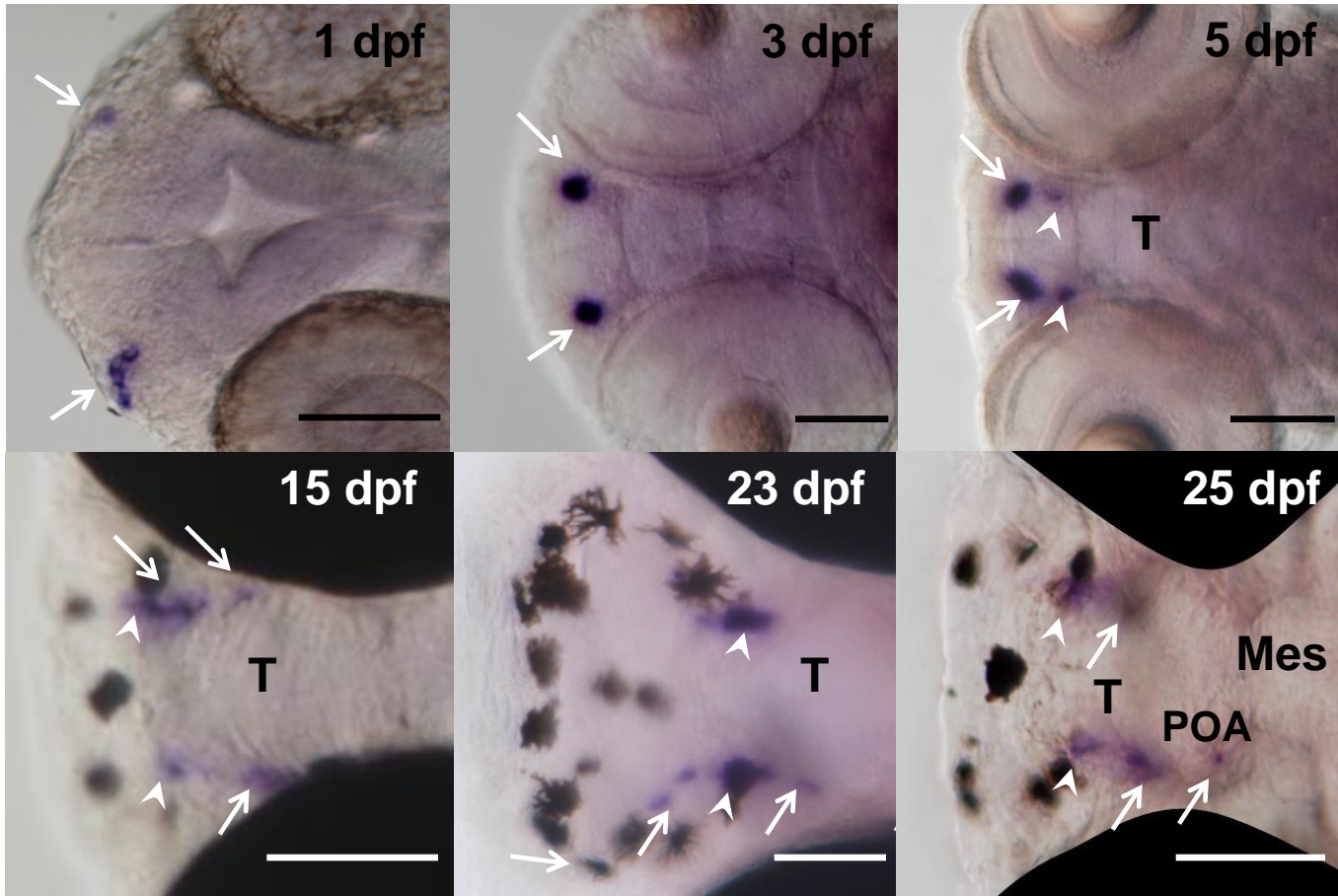
dUTP



Digoxigenin

Digoxigenin (DIG) is a steroid found exclusively in the flowers and leaves of the plants *Digitalis purpurea*, *Digitalis orientalis* and *Digitalis lanata* (foxgloves), where it is attached to sugars

# GnRH3 mRNA localization during zebrafish development

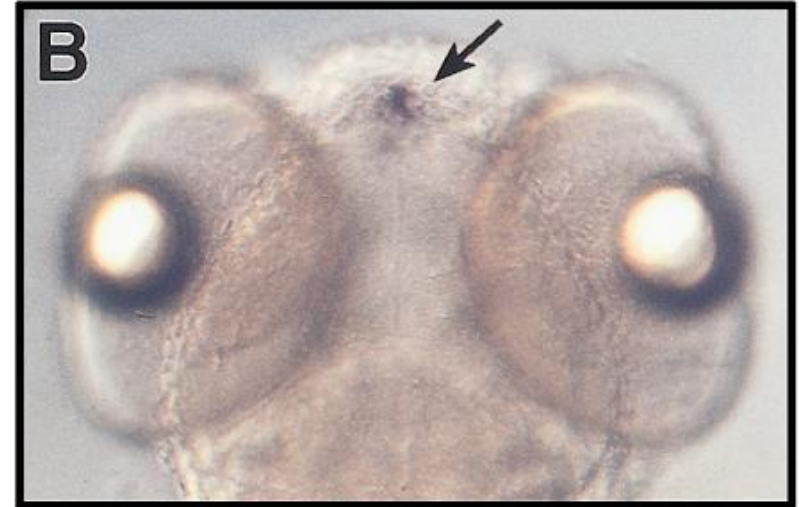
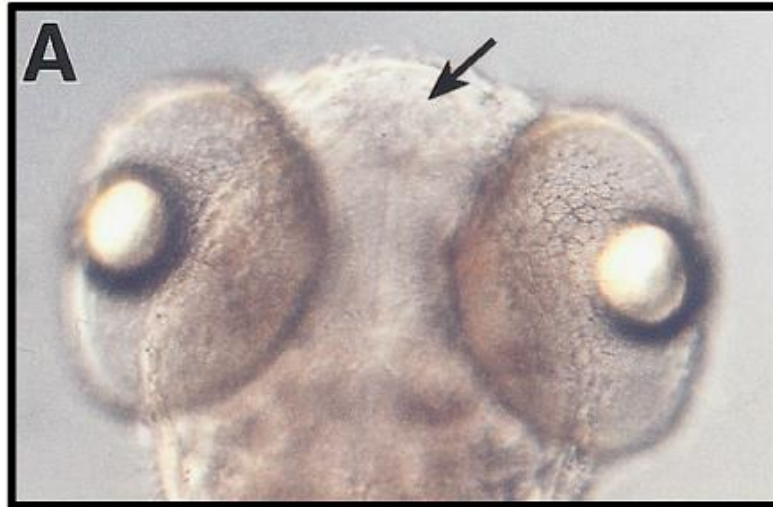


# AANAT2 mRNA expression in zebrafish larvae

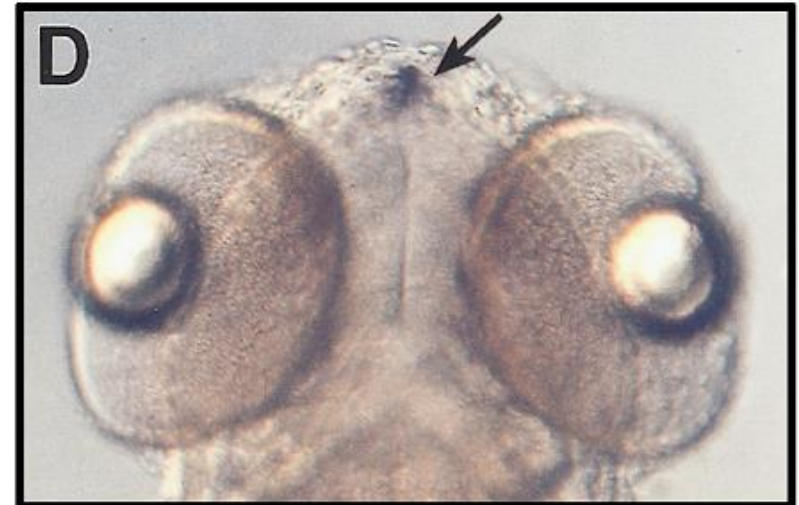
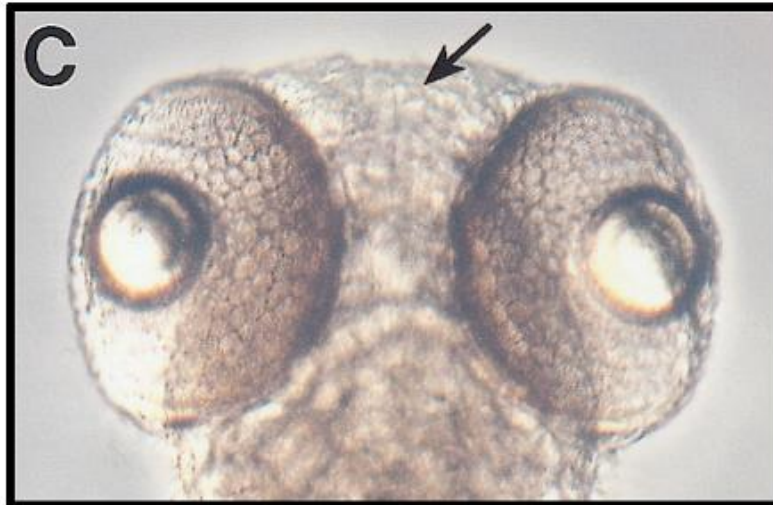
ZT6

ZT18

LD

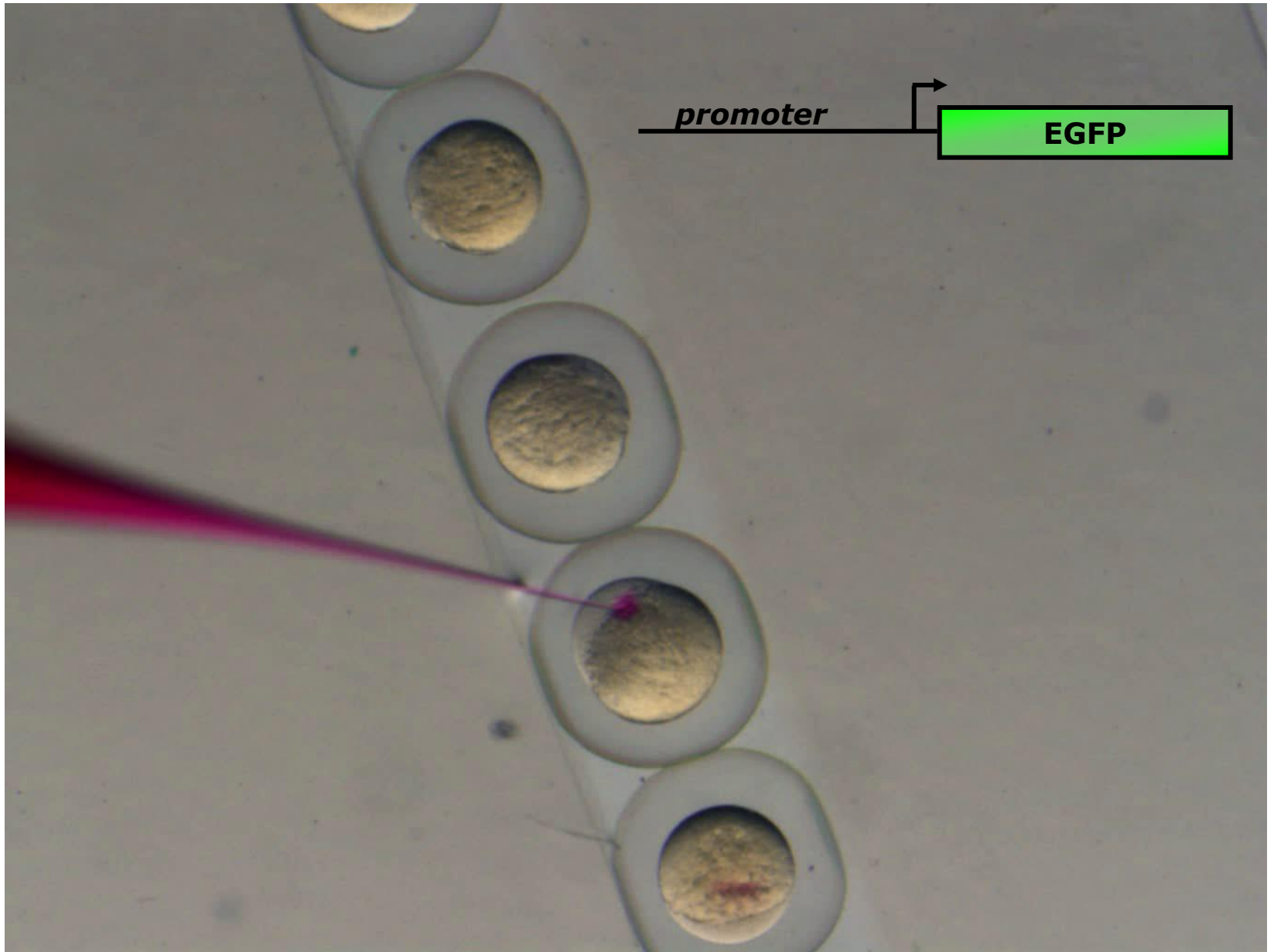


DD

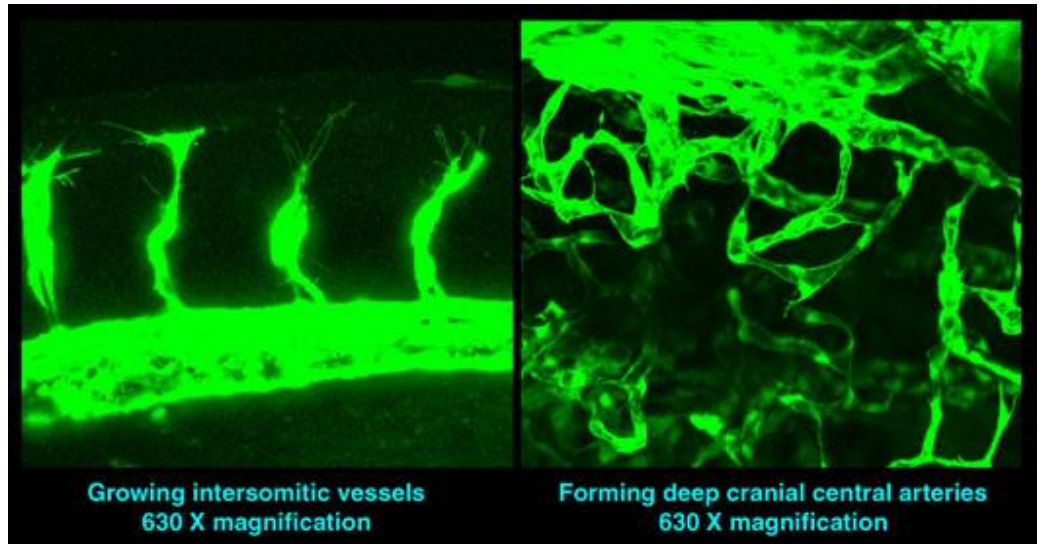
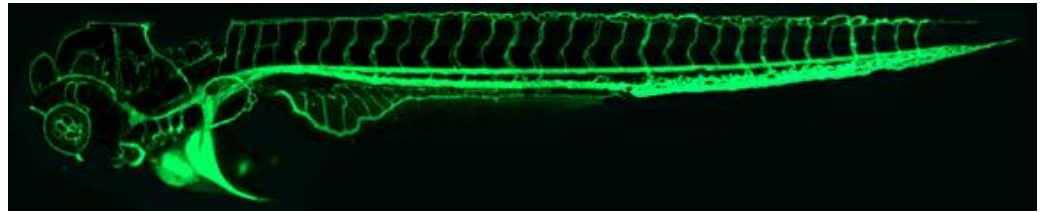
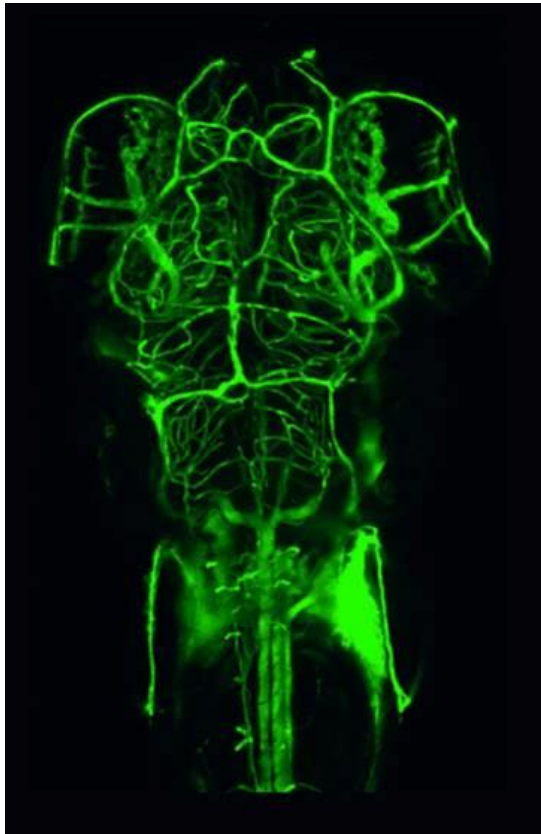


Transgenesis:

Method development and utilization for  
visualizing and manipulating neuros



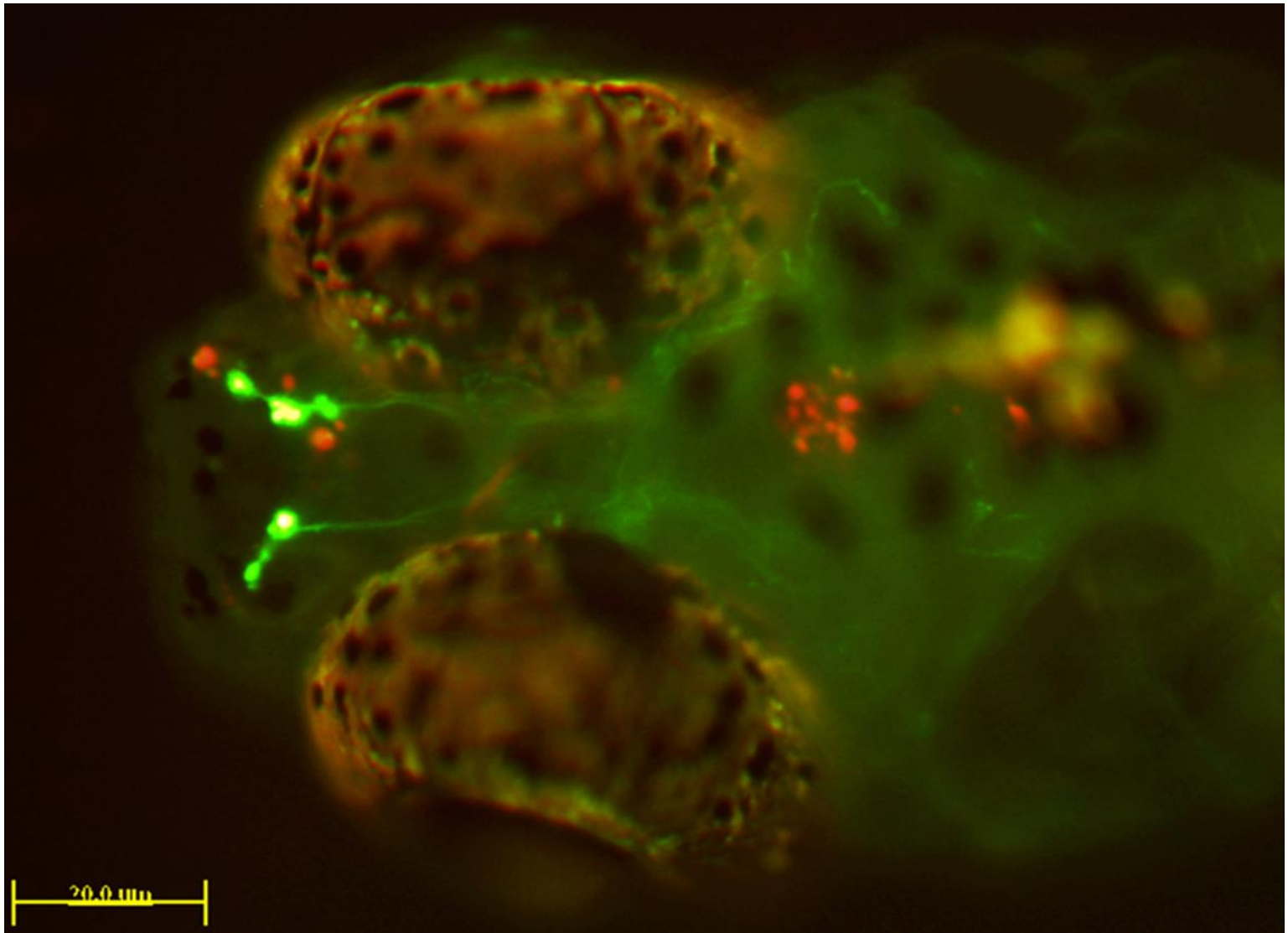
# Transgenic fish in which GFP is driven by vascular-specific promoters

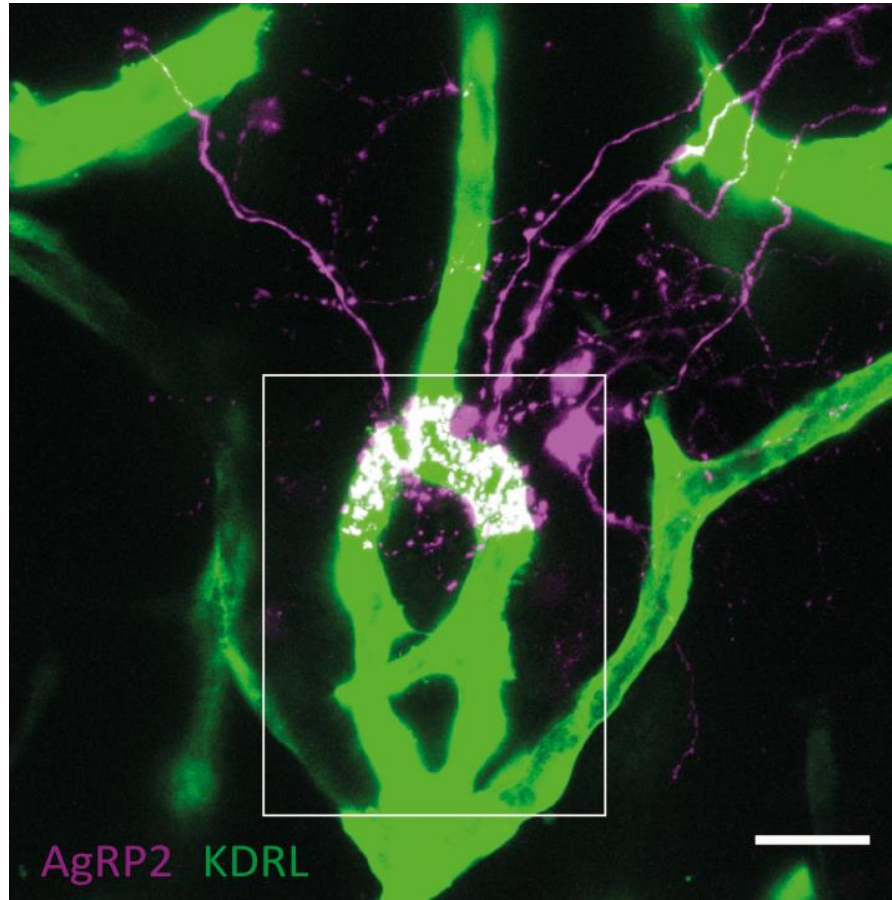


<https://youtu.be/yk7TWOtrphM>

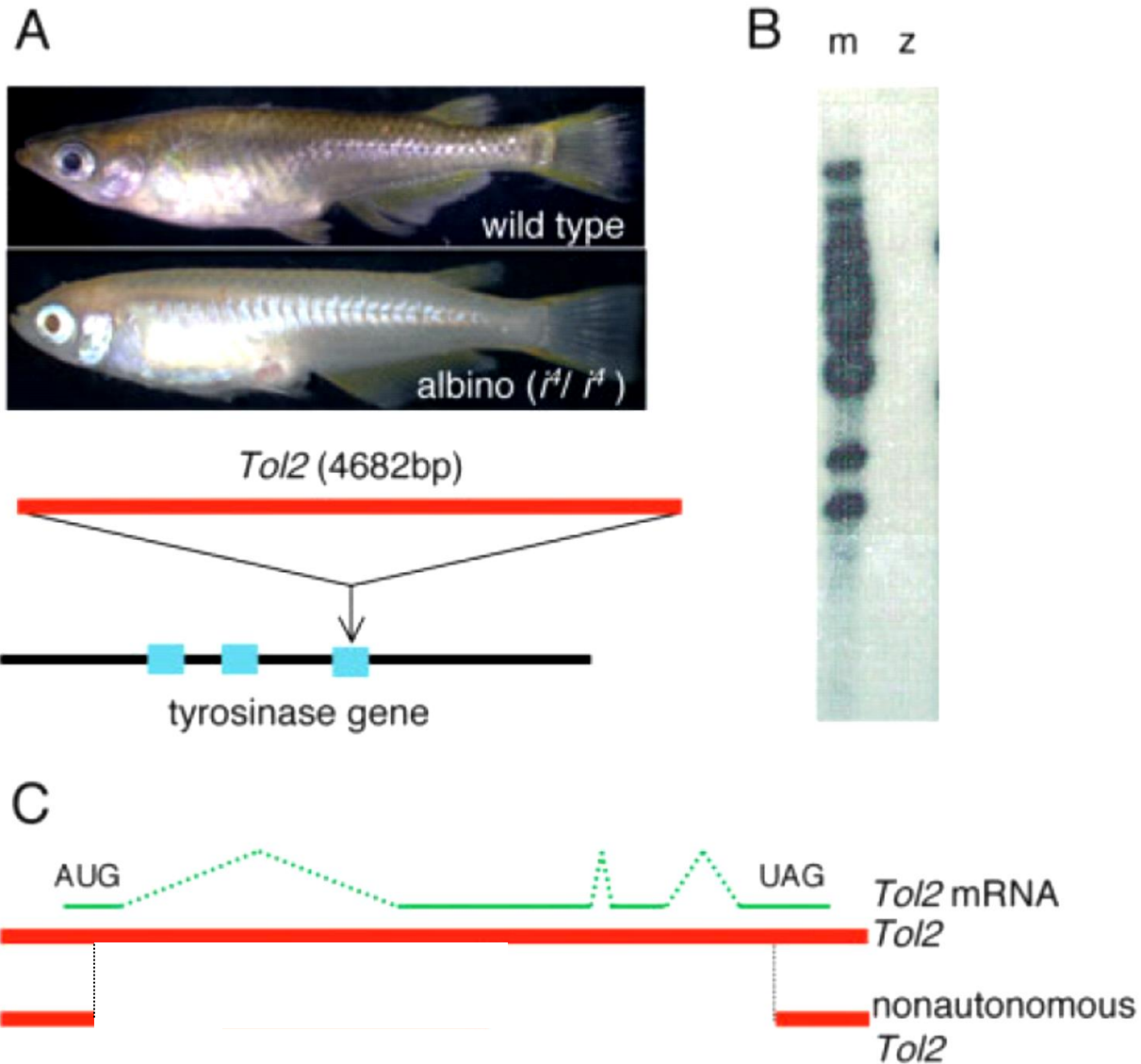


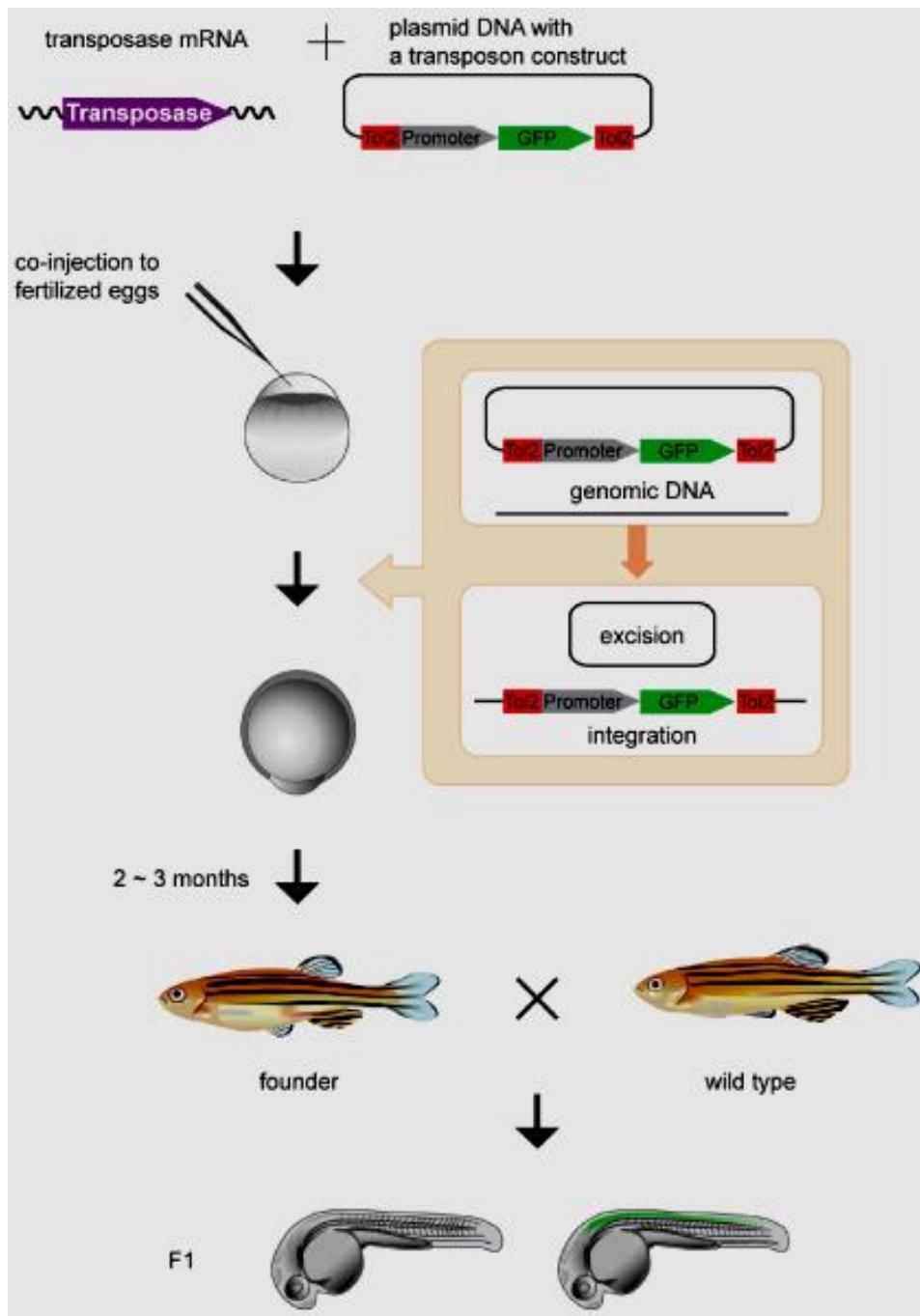




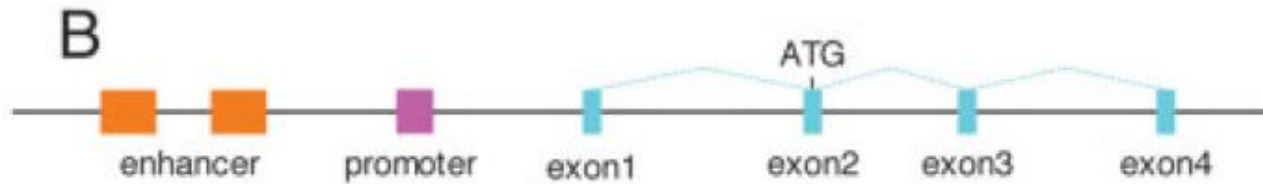


# Discovering the Tol2 transposase system

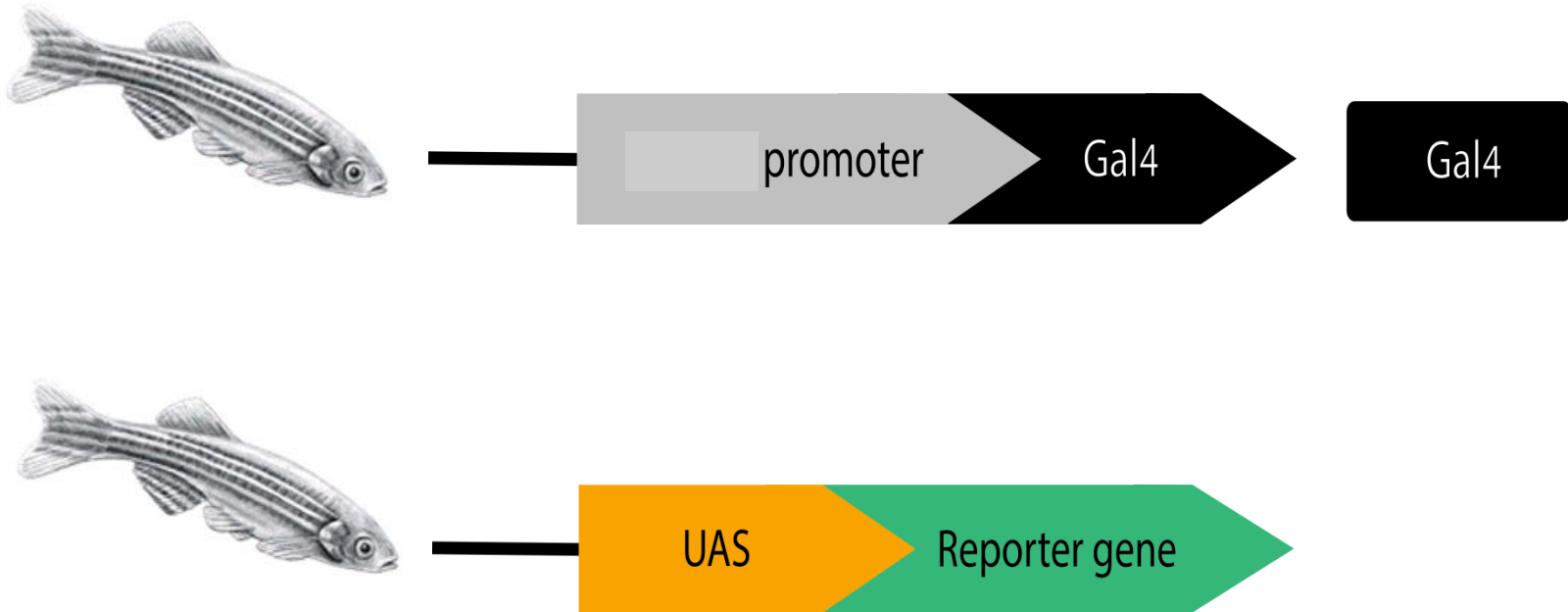




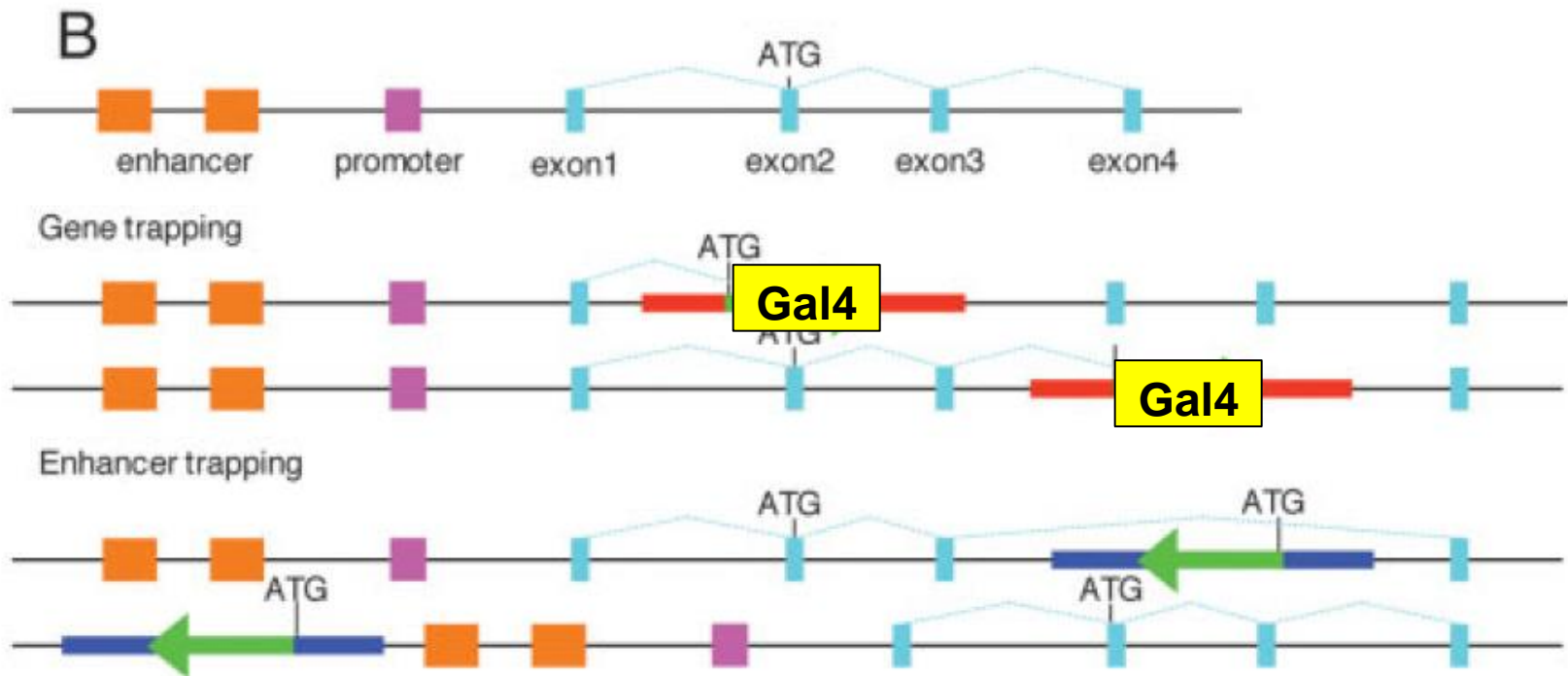
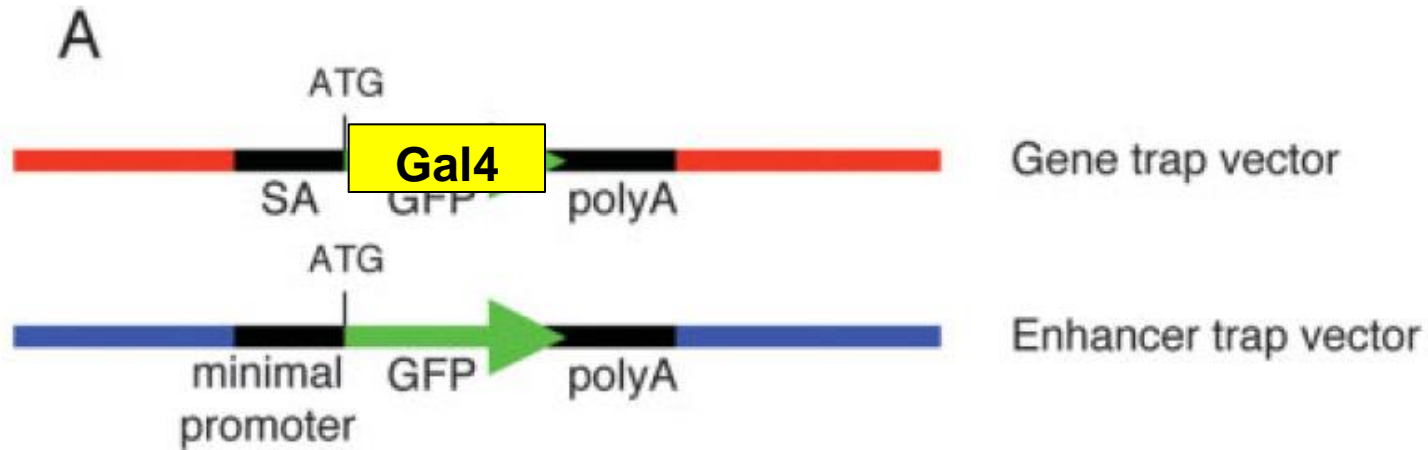
# Gene and enhancer trap using the Tol2 system



# The GAL4-UAS system

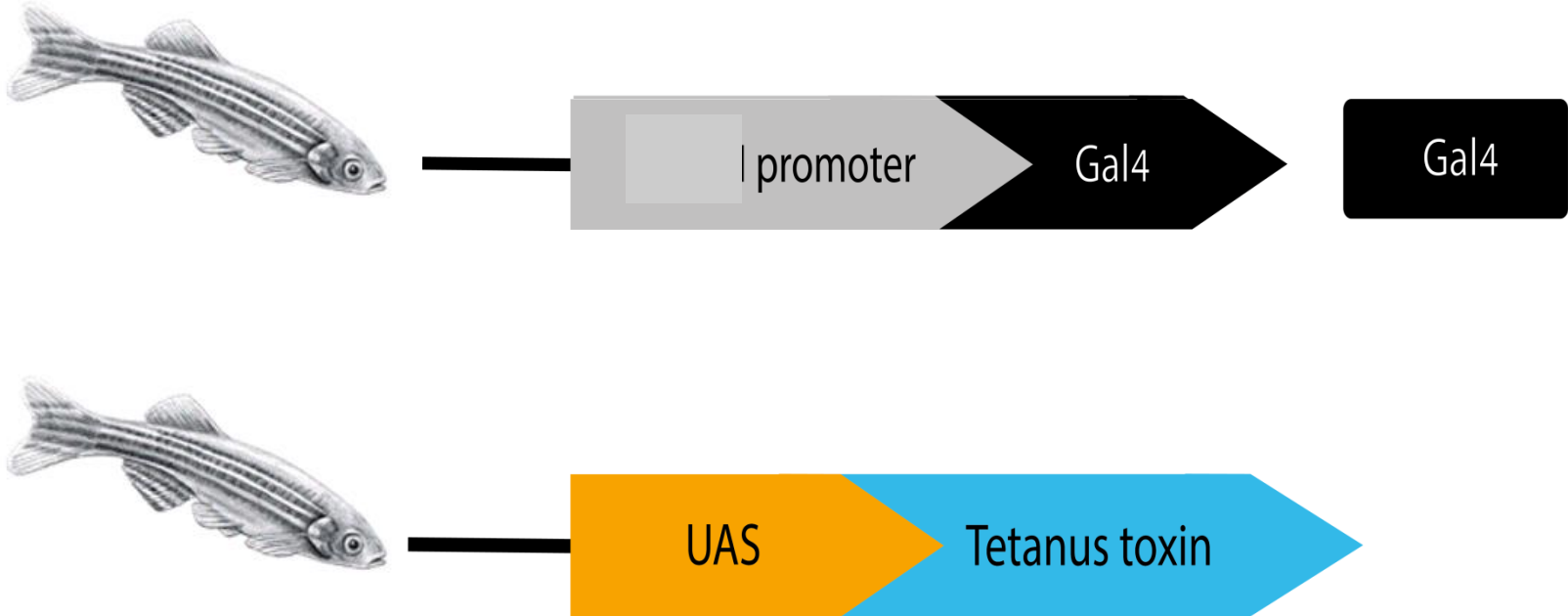


# Gene and enhancer trap using the Tol2 system

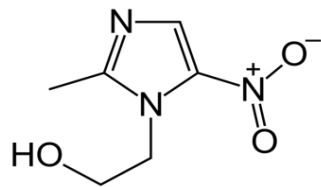
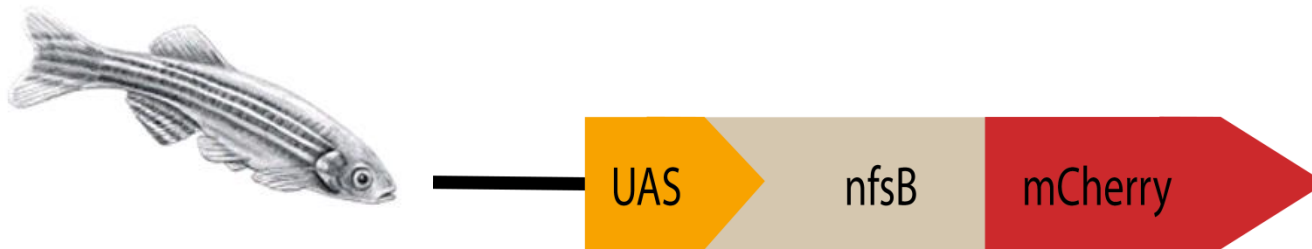




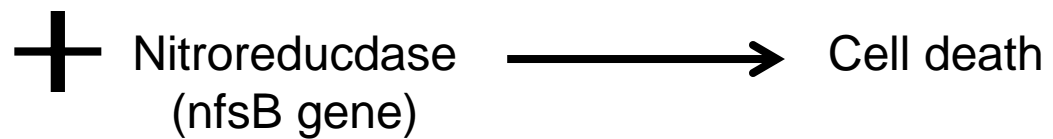
# Specific neuronal inhibition



# Neuronal ablation

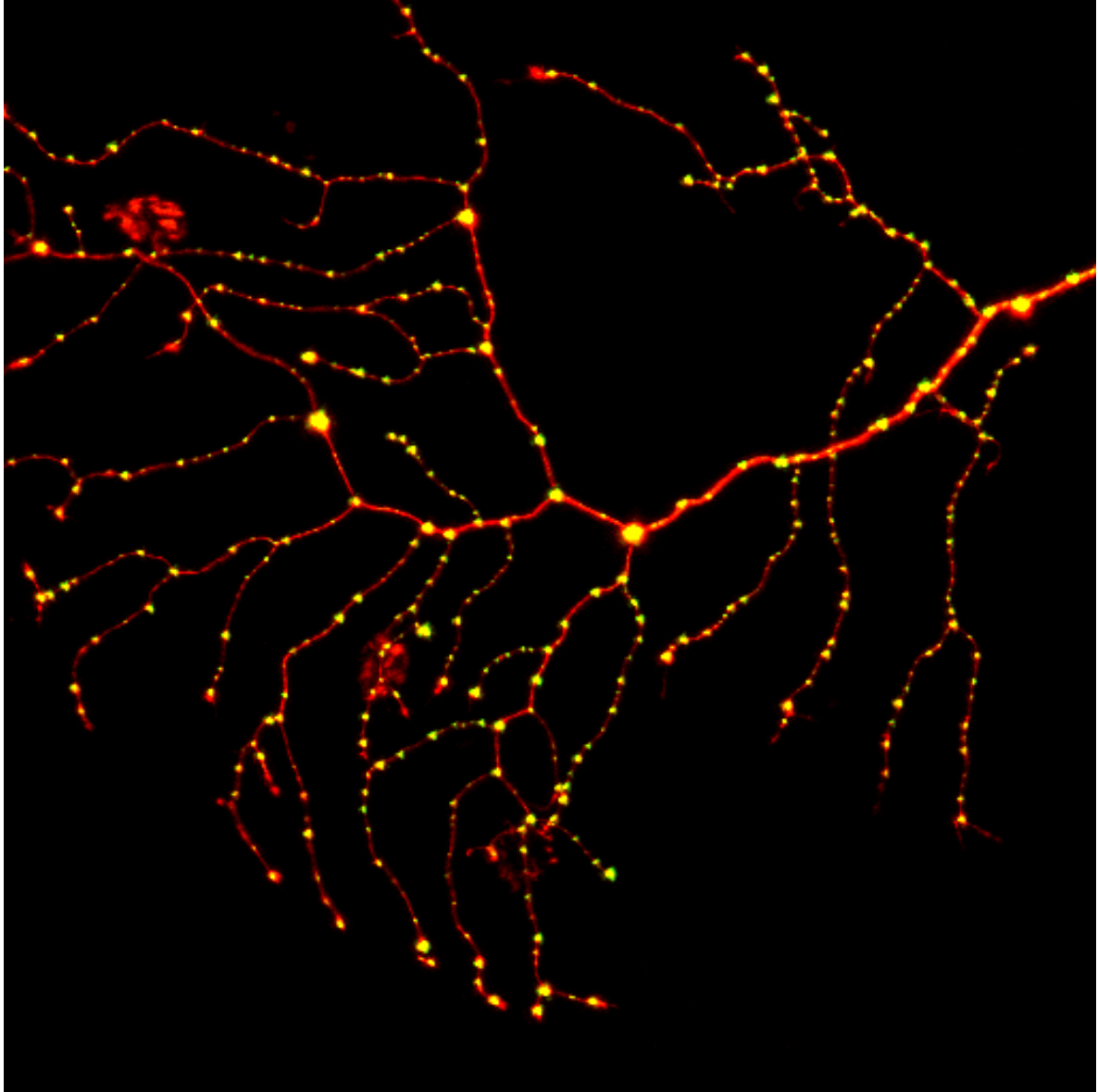


Metronidazole

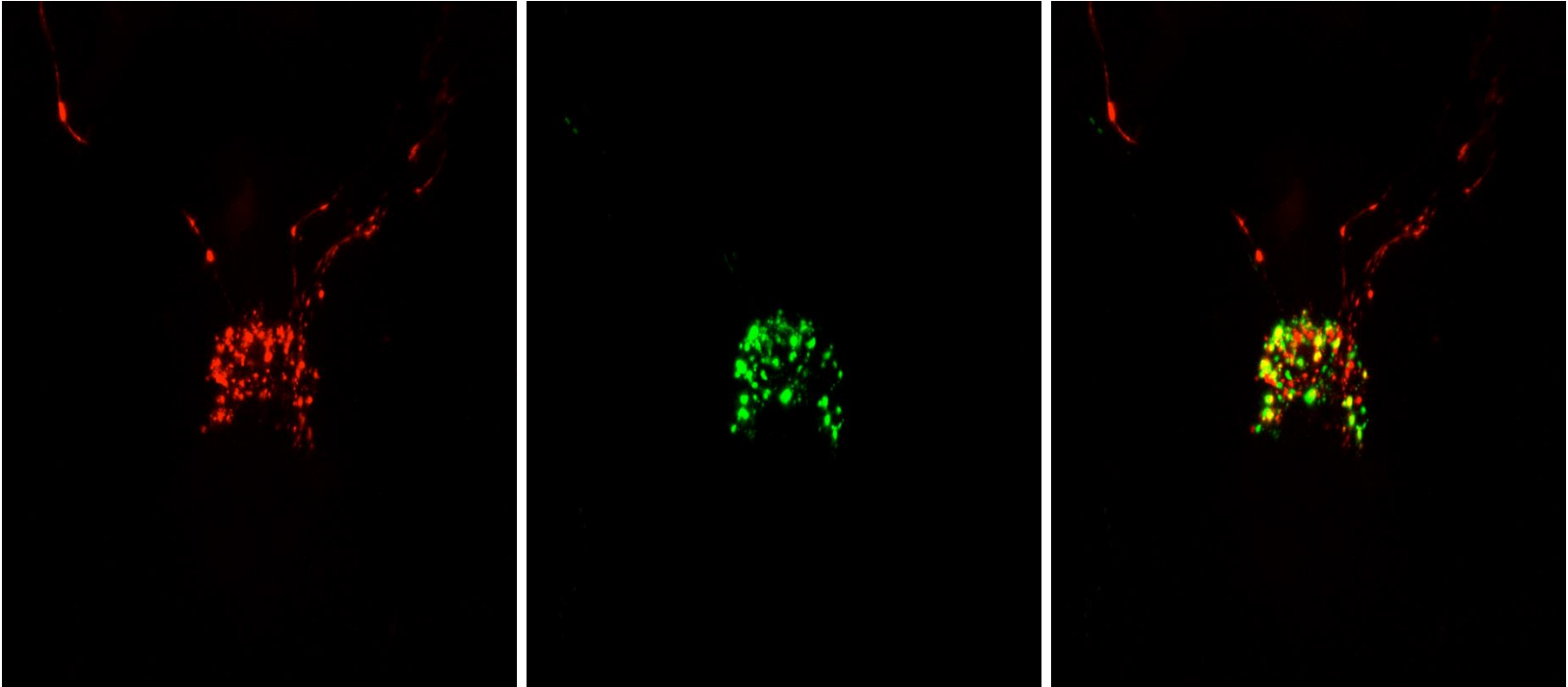


# Synaptic plasticity

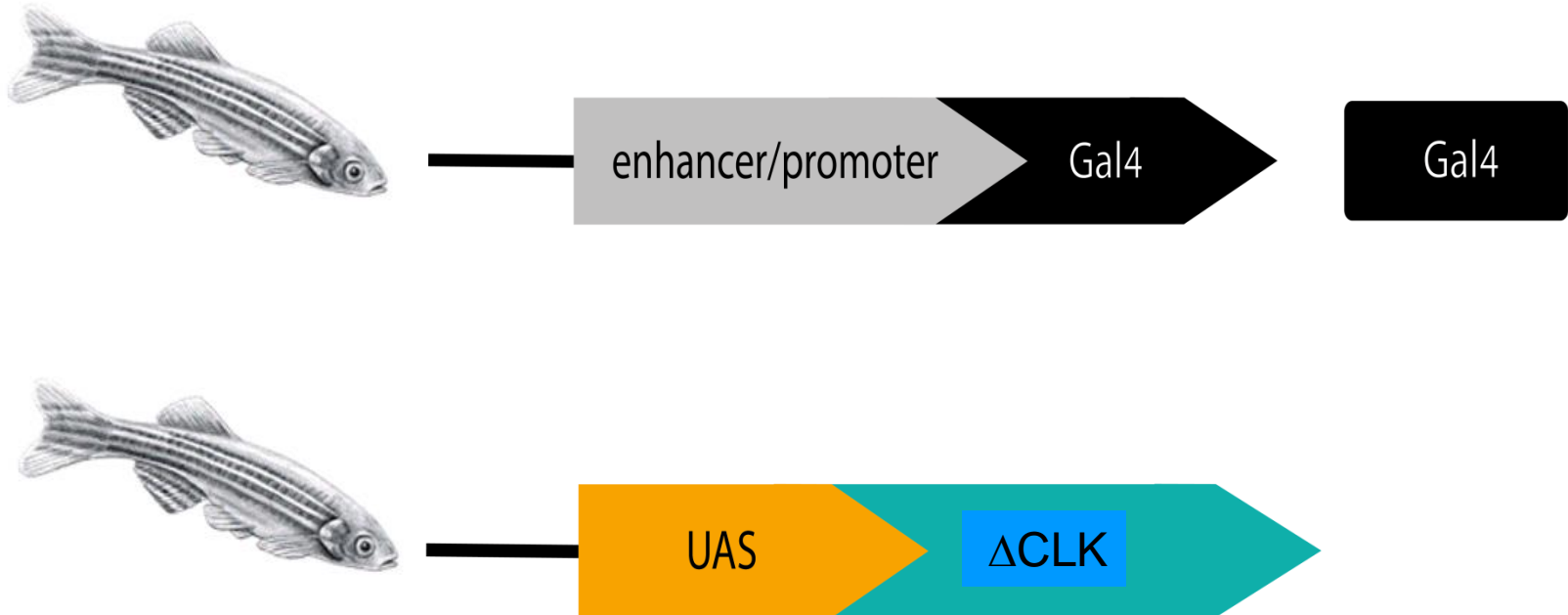




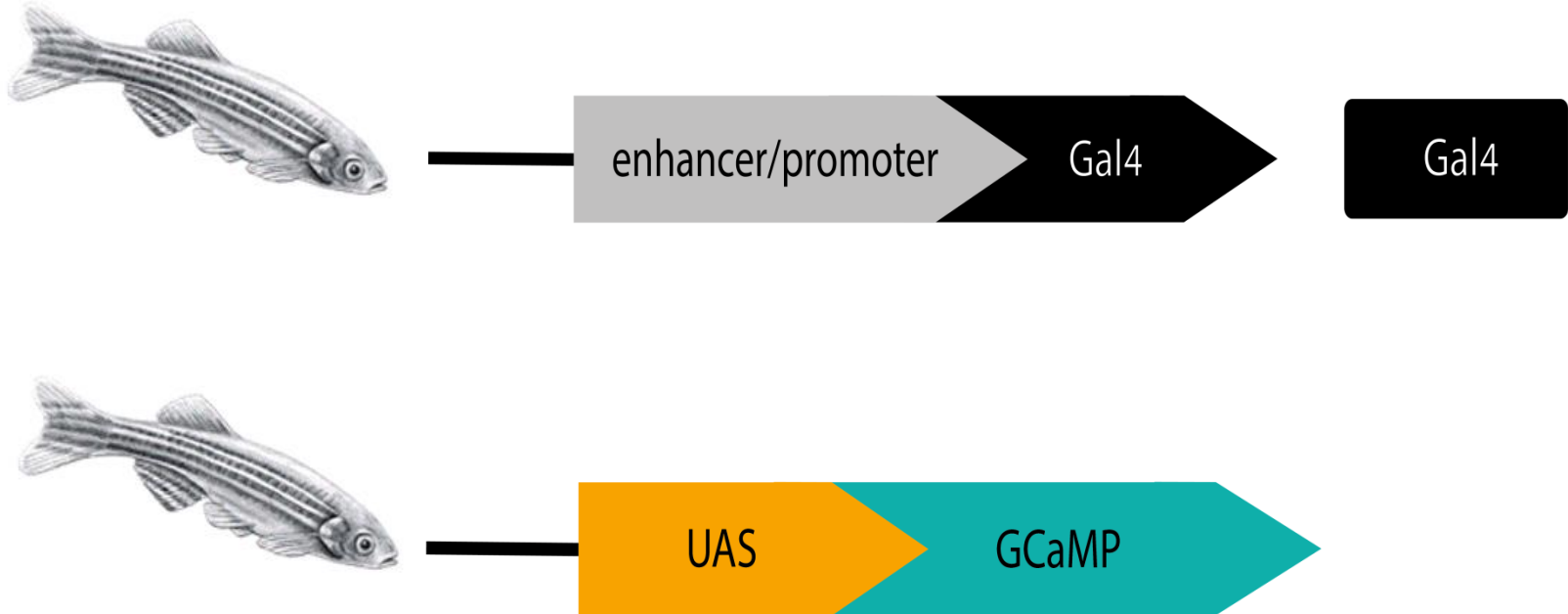
*Lior Appelbaum, BIU*



# Blocking the clock

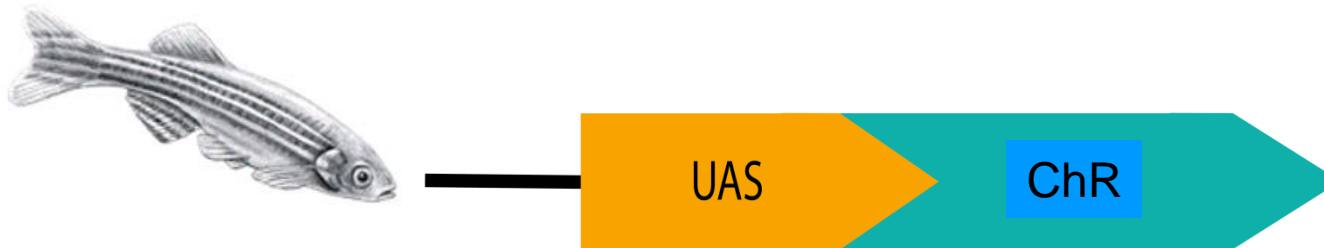


# Seeing neurons in action, using Ca<sup>++</sup> sensor



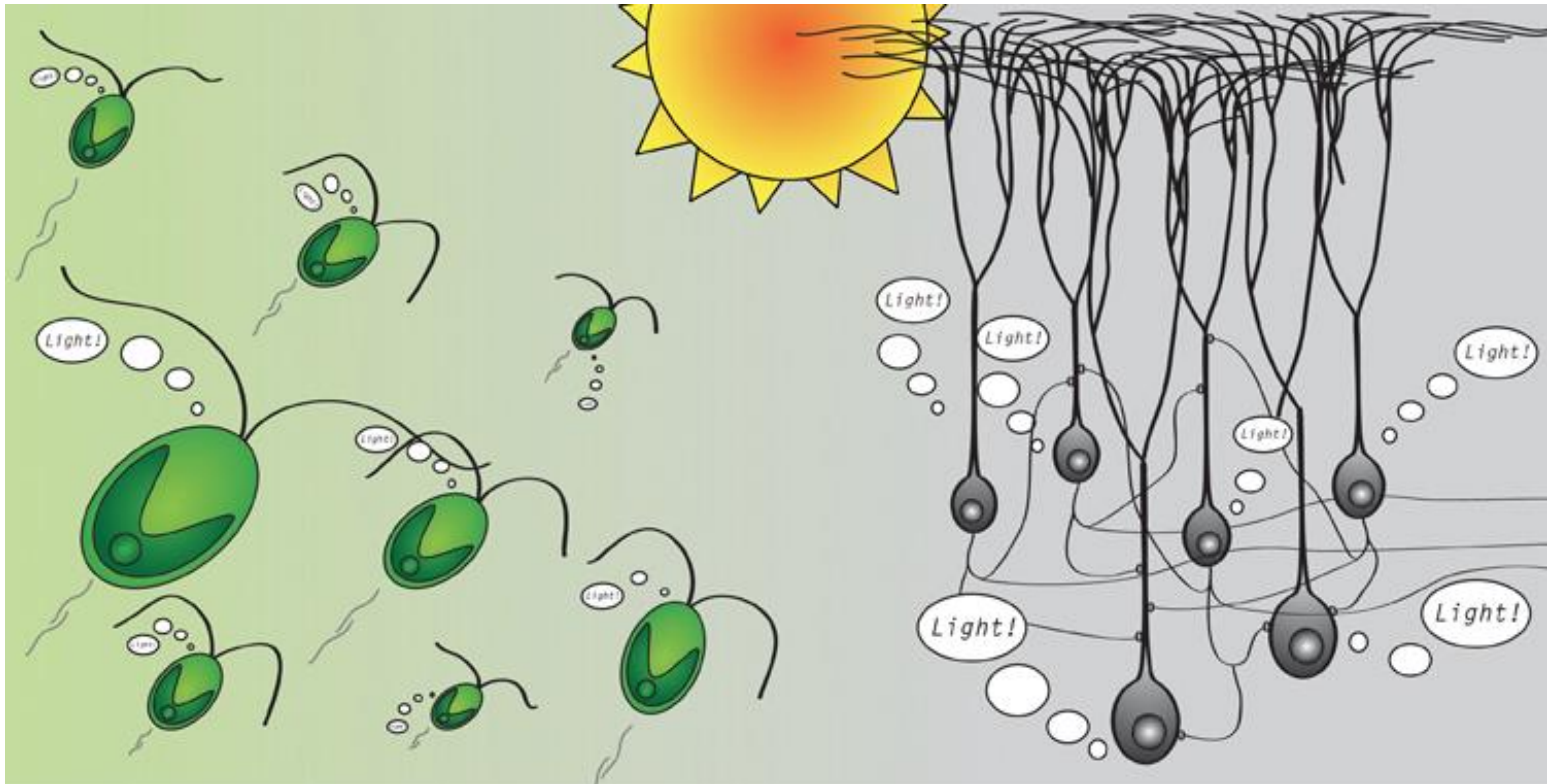
<https://www.youtube.com/watch?v=1Q-g1uCvYOA>

# Activating neurons

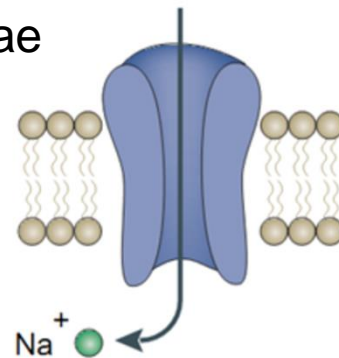




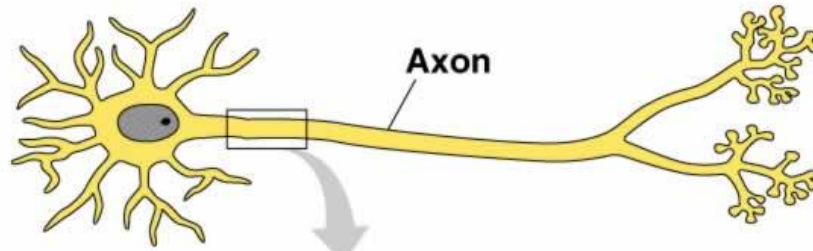
# Optogenetics



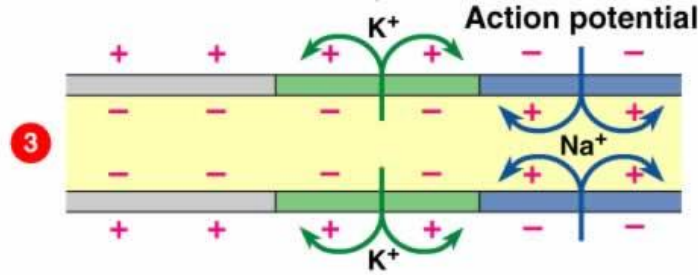
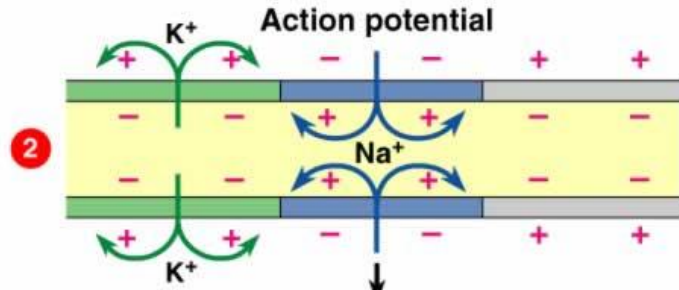
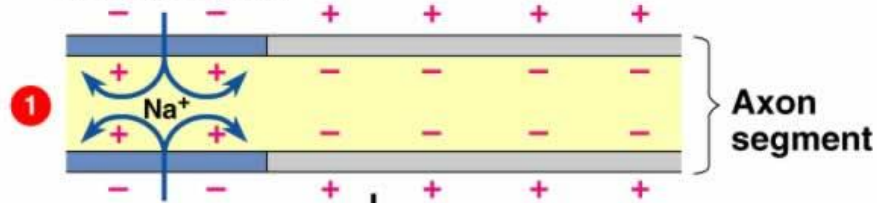
Light sensitive single-cell algae



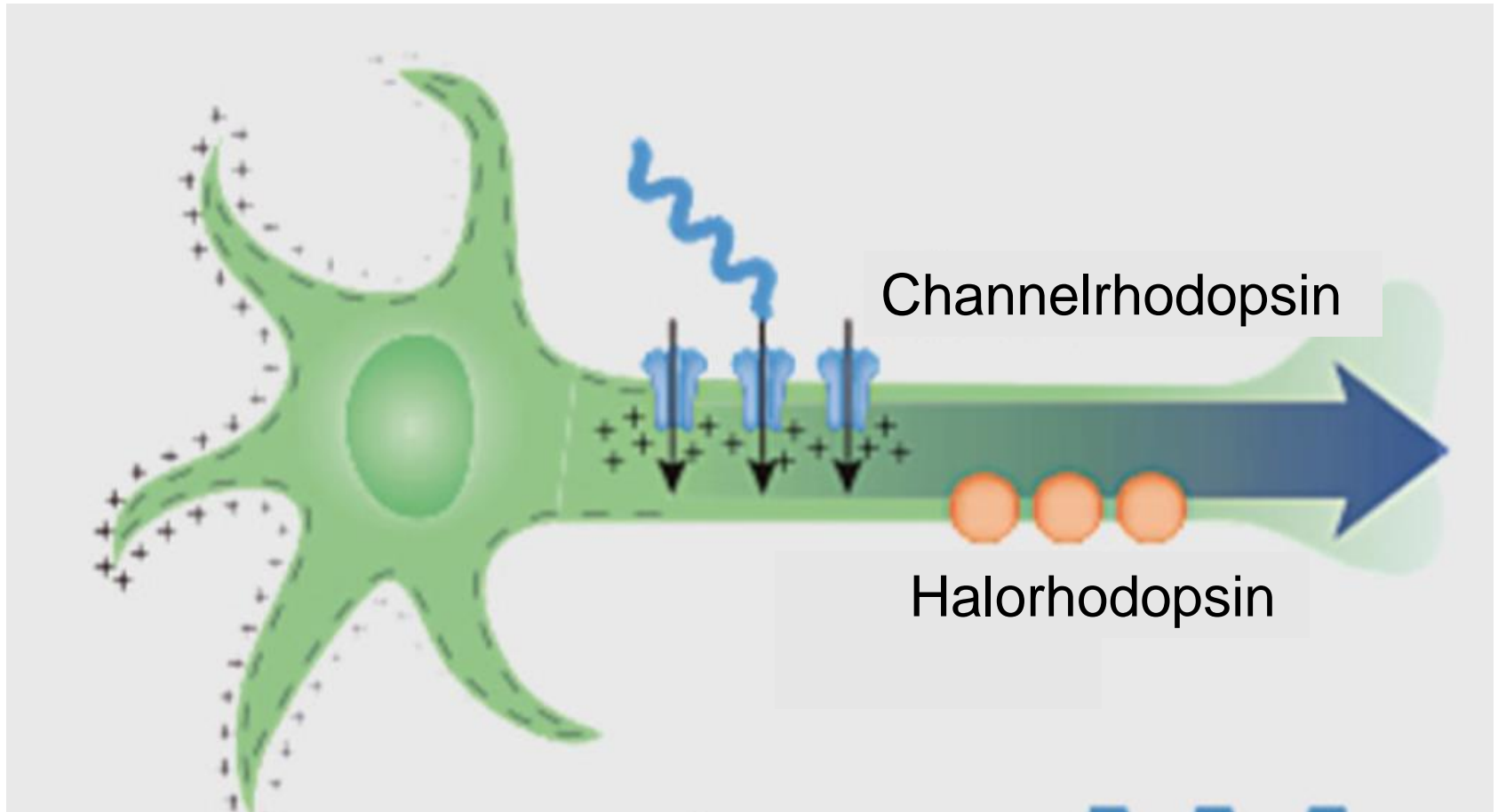
Light sensitive sodium channel called channelrhodopsin



**Action potential**

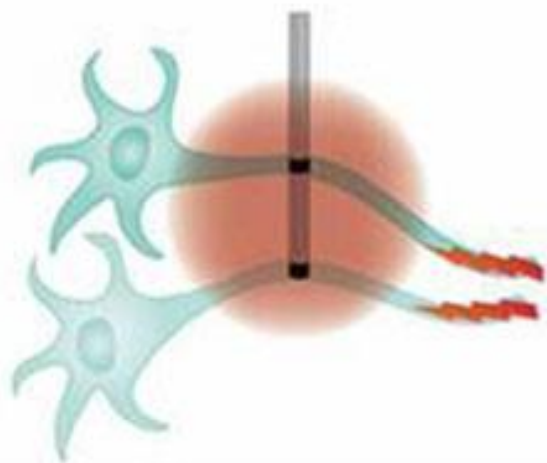


# Generation of action potential by light pulse



The blue-light sensitive Channelrhodopsin and the yellow light-activated chloride pump halorhodopsin together enable activation and silencing of neural activity

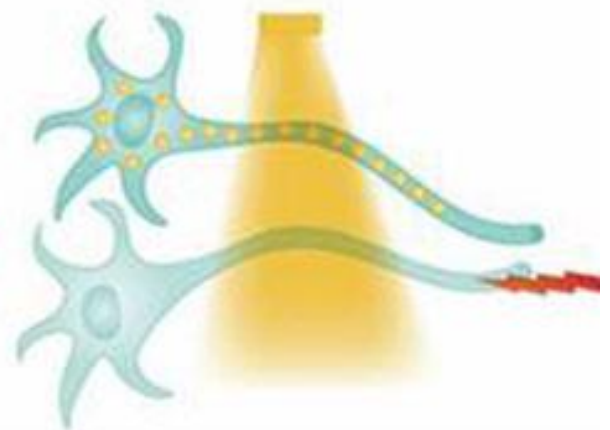
Electrical stimulation

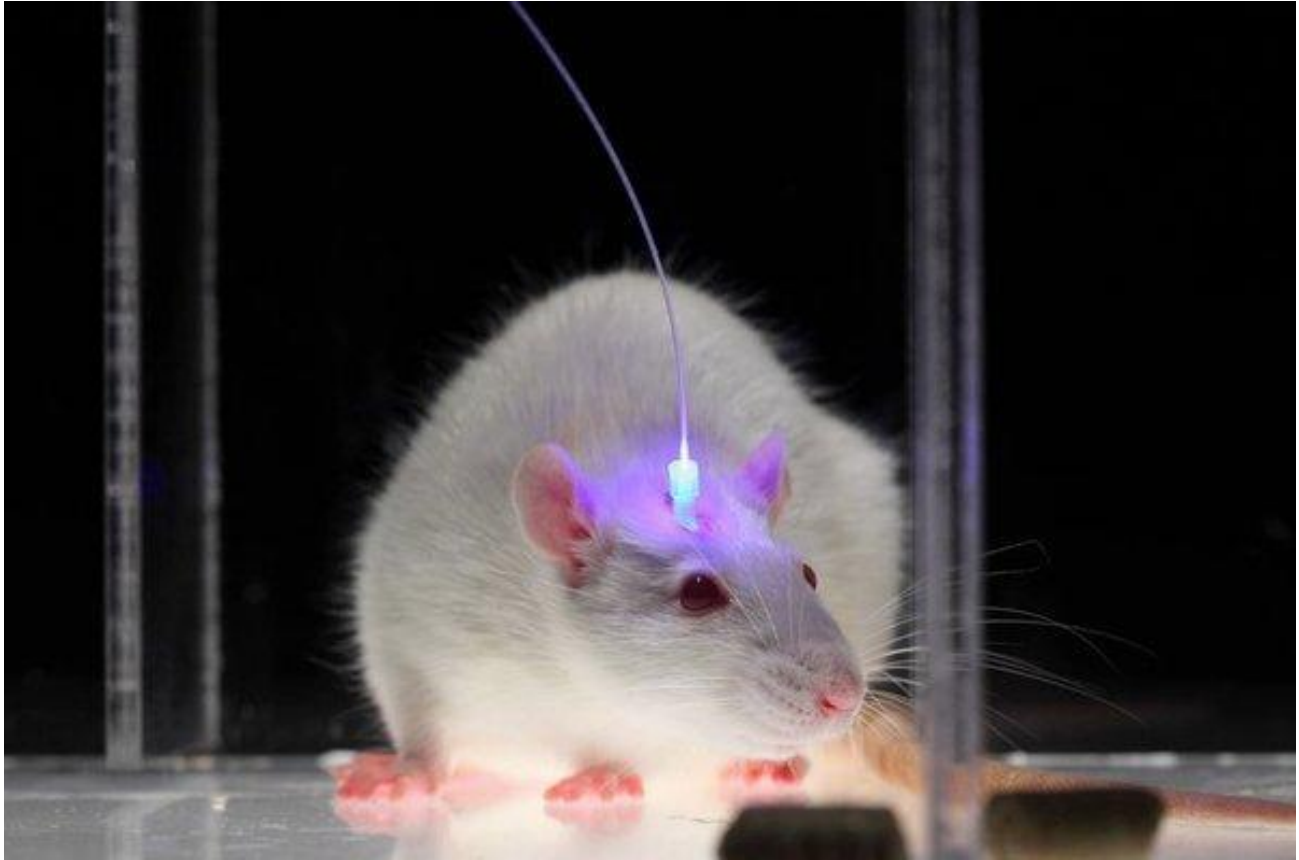


Optogenetic excitation

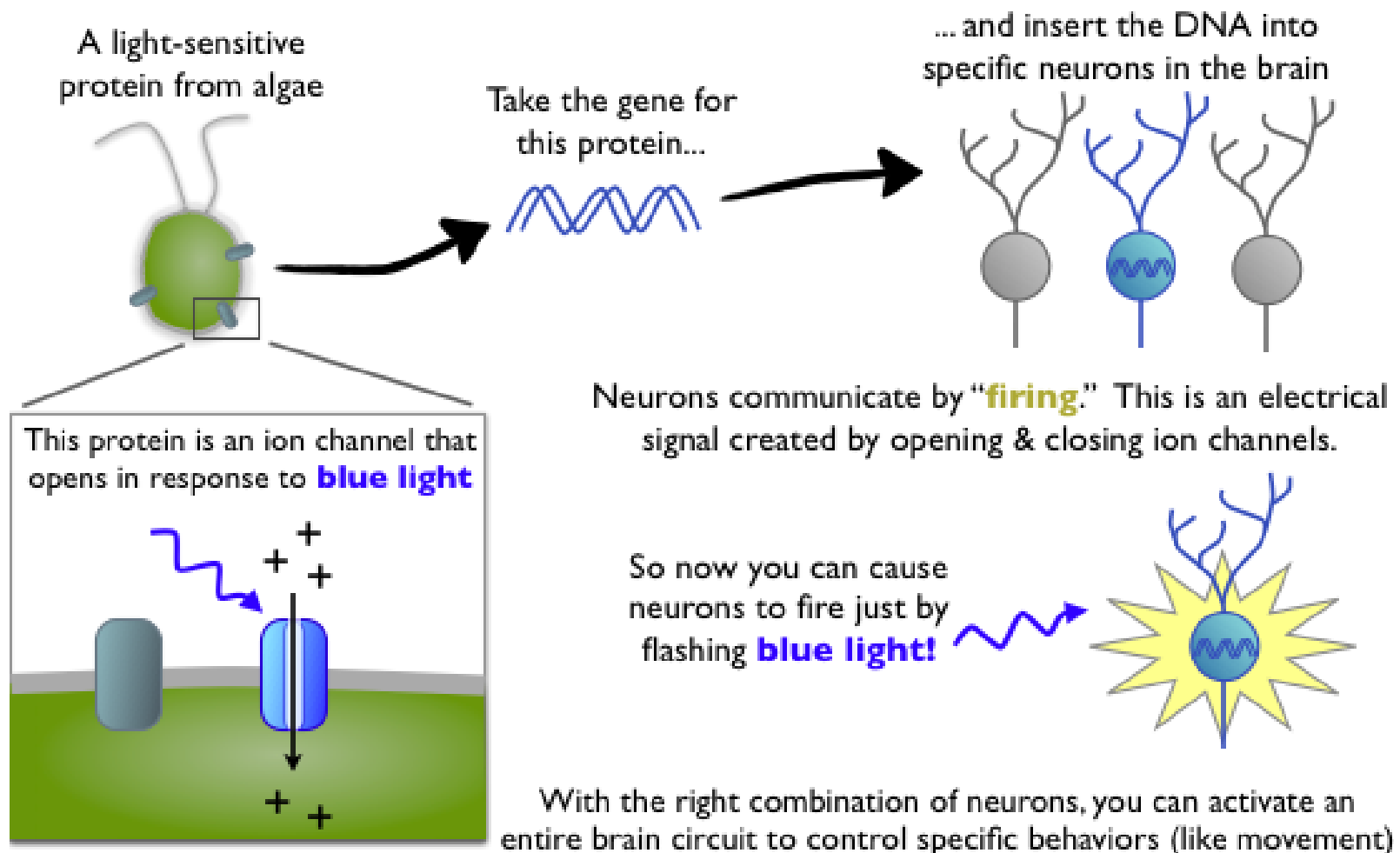


Optogenetic inhibition





# How optogenetics works



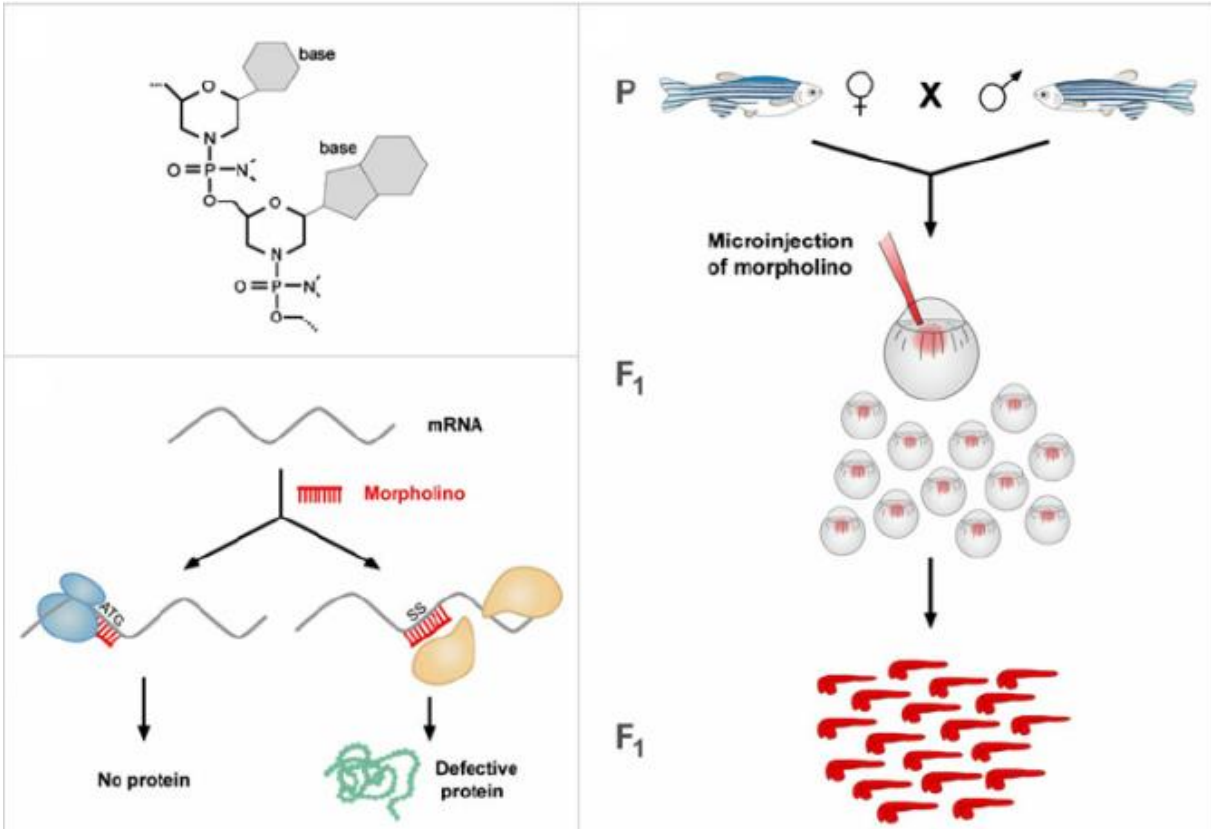
<https://www.youtube.com/watch?v=l64X7vHSHOE>

[https://www.youtube.com/watch?v=rfEKc\\_0iaJo](https://www.youtube.com/watch?v=rfEKc_0iaJo)

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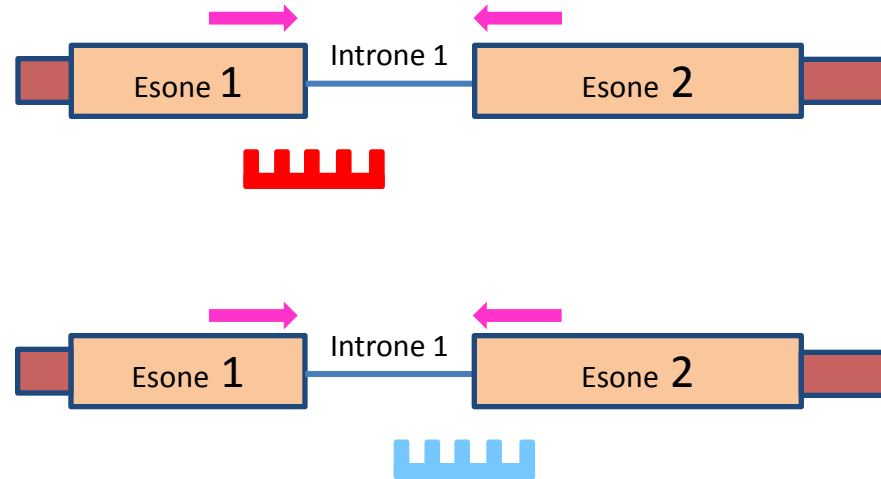
# Gene knockdown for studying gene function

Using morpholino-modified oligonucleotides (MO)



# Gene knockdown for studying gene function

Using morpholino-modified oligonucleotides (MO)





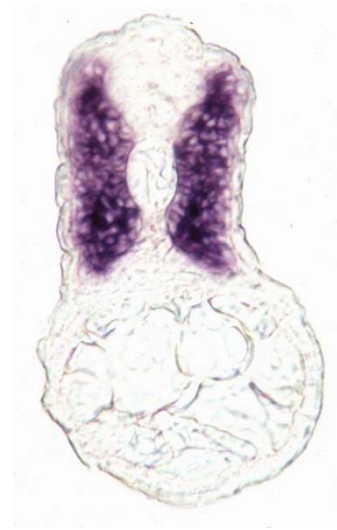
# Muscle-specific expression of *Smyd1*



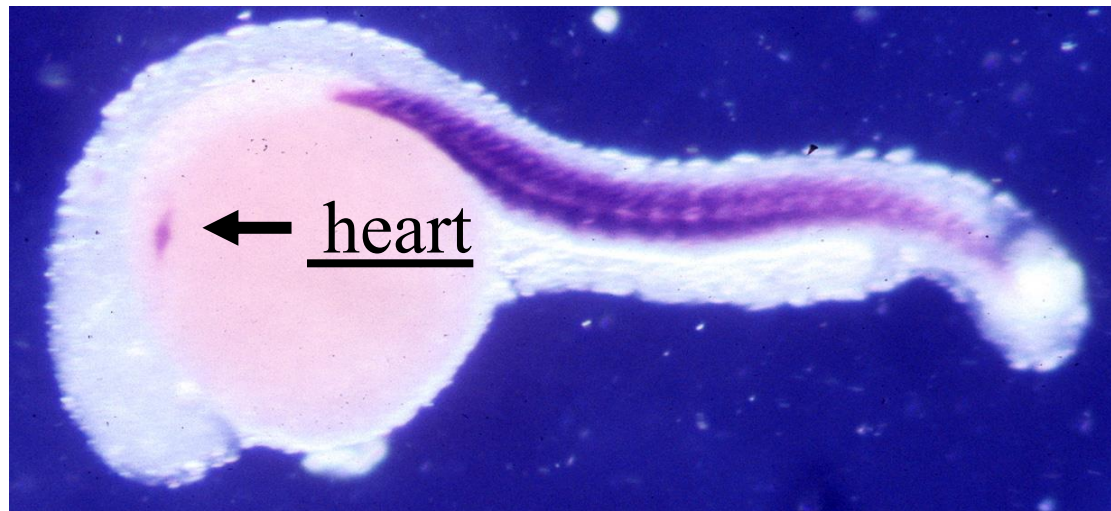
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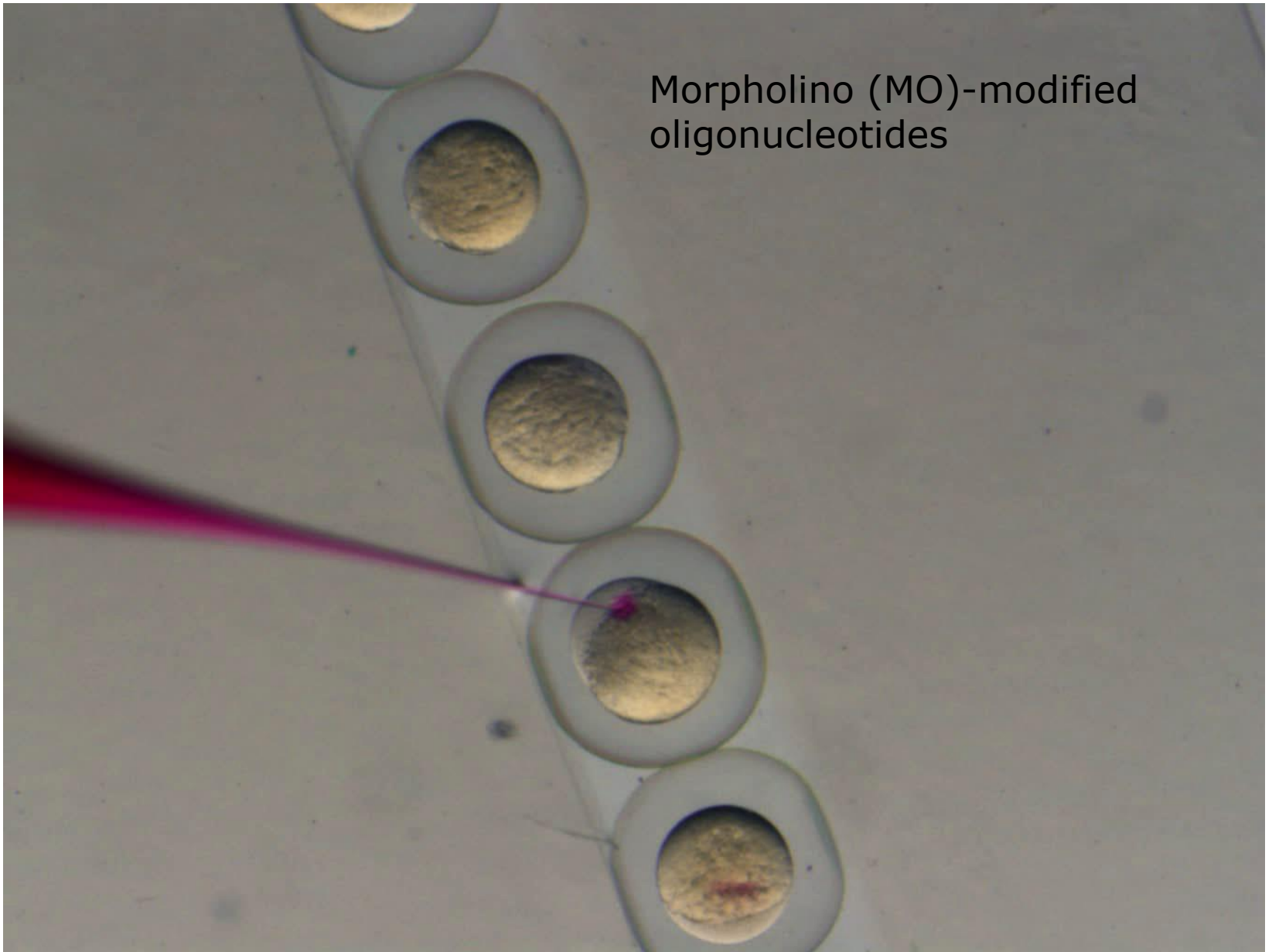
24h



24h

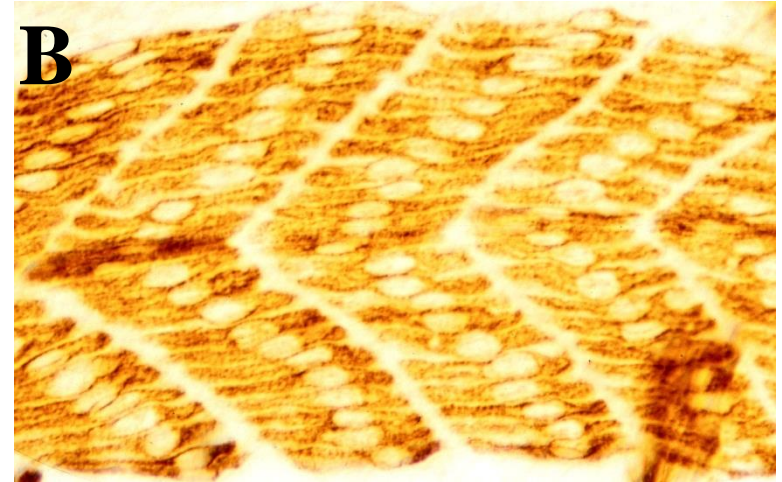
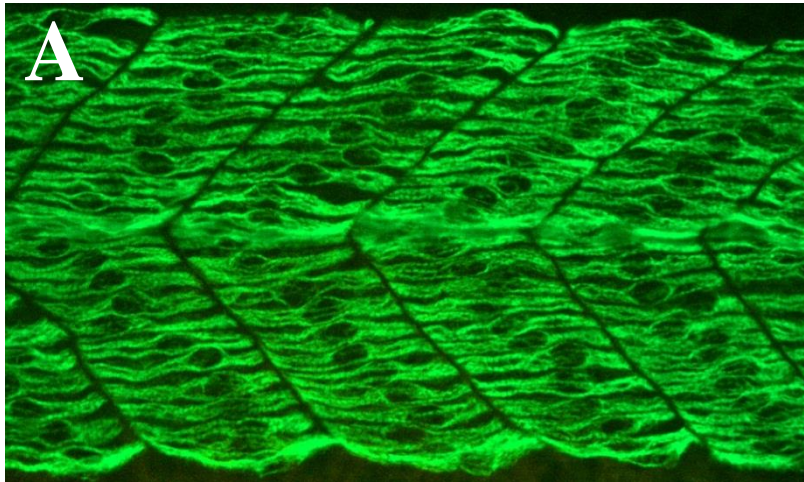


Morpholino (MO)-modified oligonucleotides

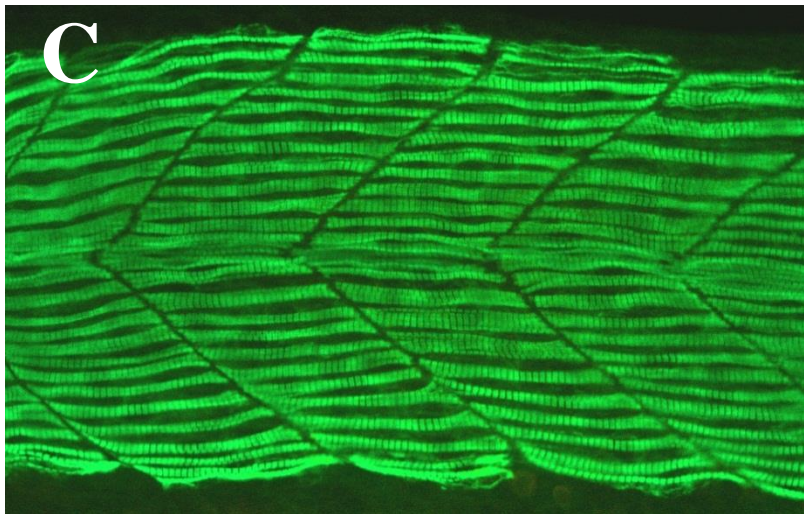


# Knockdown of *Smyd1* expression disrupts muscle cell differentiation

**MO**



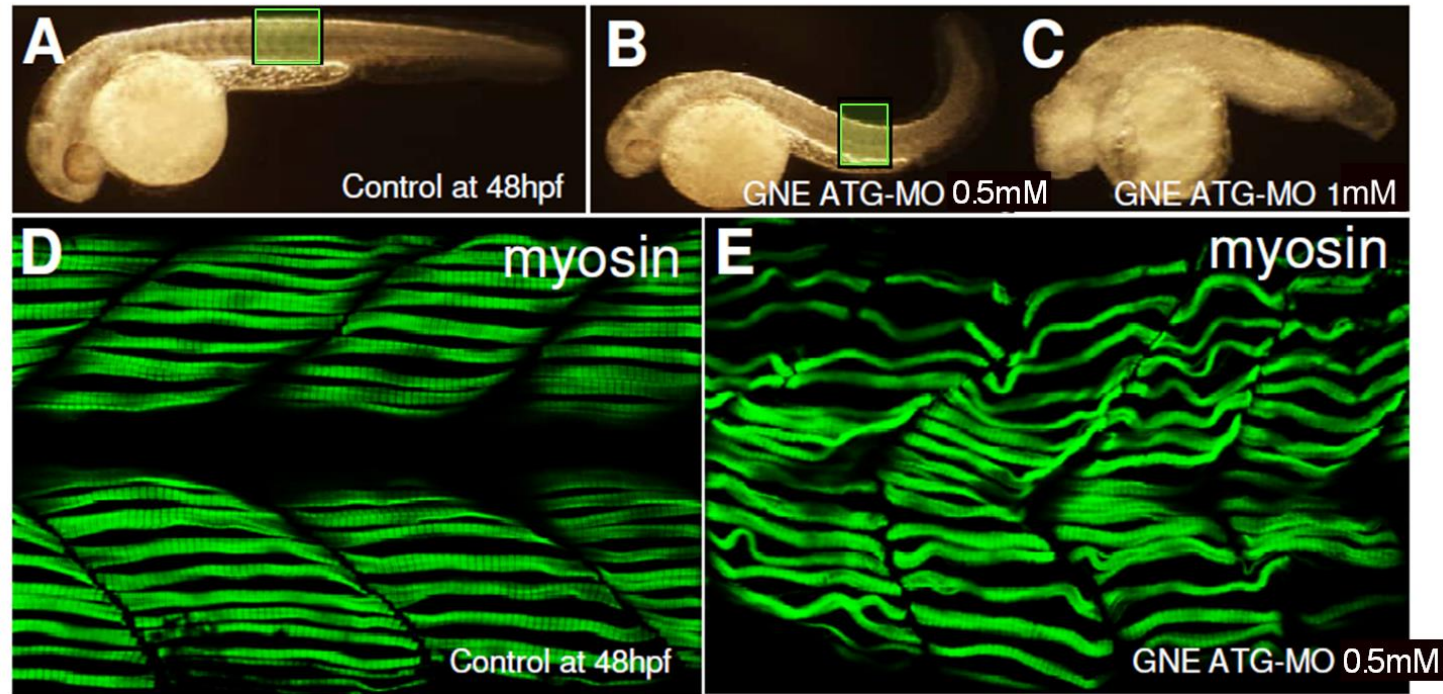
**Control**



Knock down of Smyd1:  
Larvae are paralyzed and  
heart doesn't beat



# GNE-mediated Hereditary Inclusion Body Myopathy



GNE (UDP N-Acetylglucosamine 2-epimerase/N-Acetylmannosamine kinase)

# *GNE* MO knockdown phenotypes

1mM Splice Morpholino - 3dpf



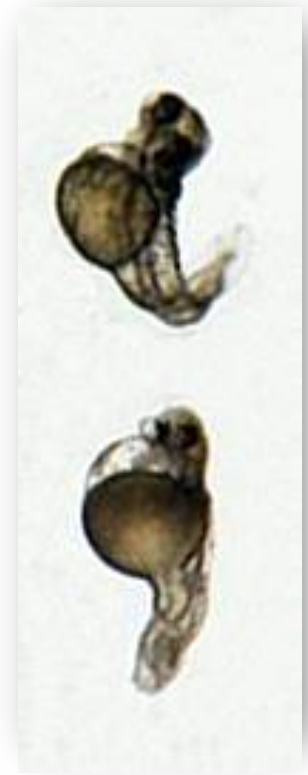
Control



Mild

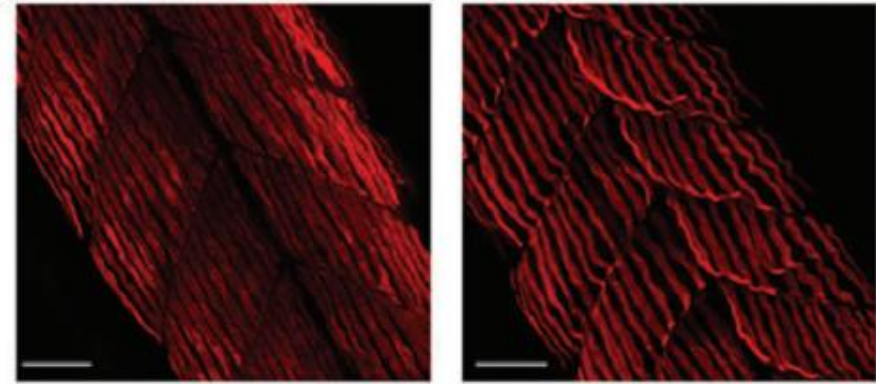
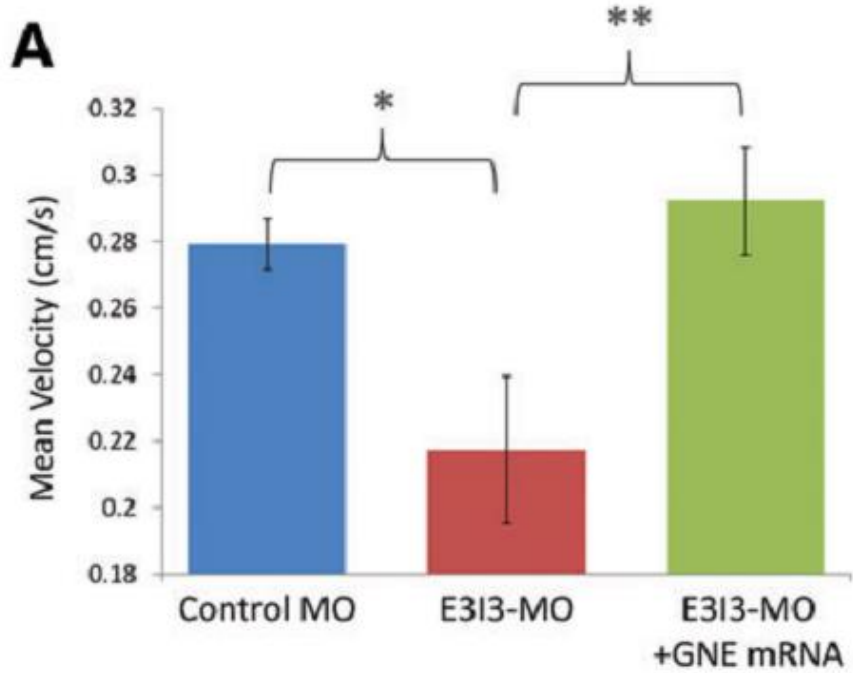


Intermediate



Severe

# Larvae locomotor activity

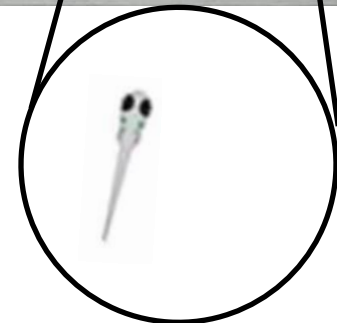
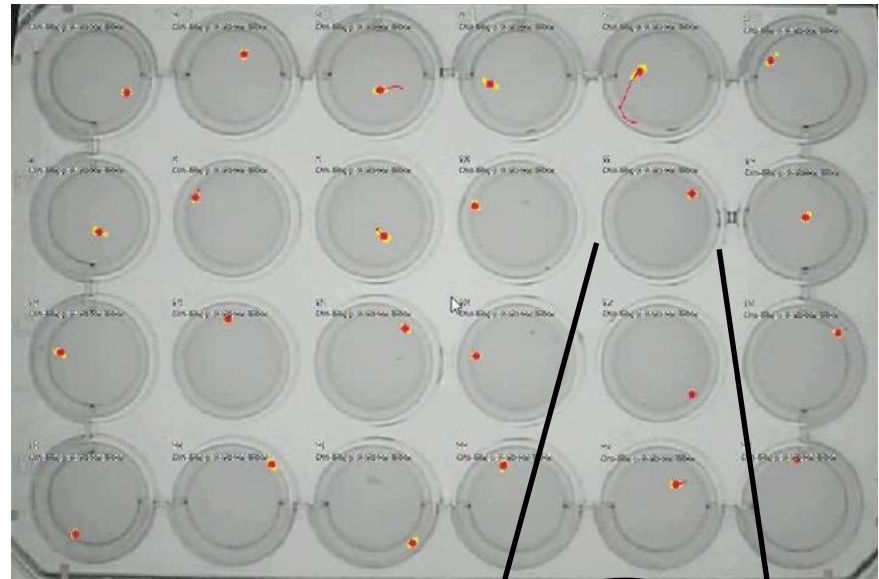


Immunostaining of 7 dpf larvae with slow muscle myosin antibody

# Monitoring locomotor activity of zebrafish larvae

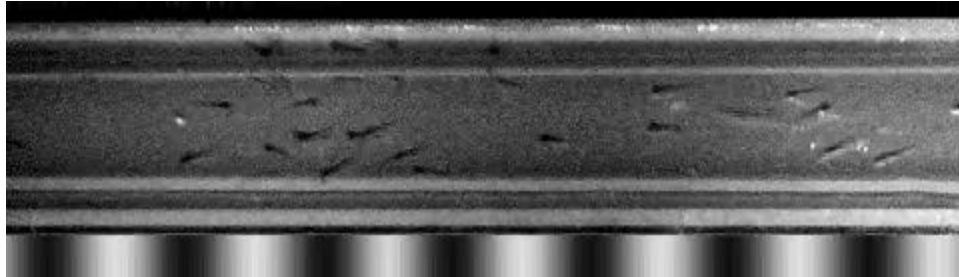


Danio Vision, Noldus



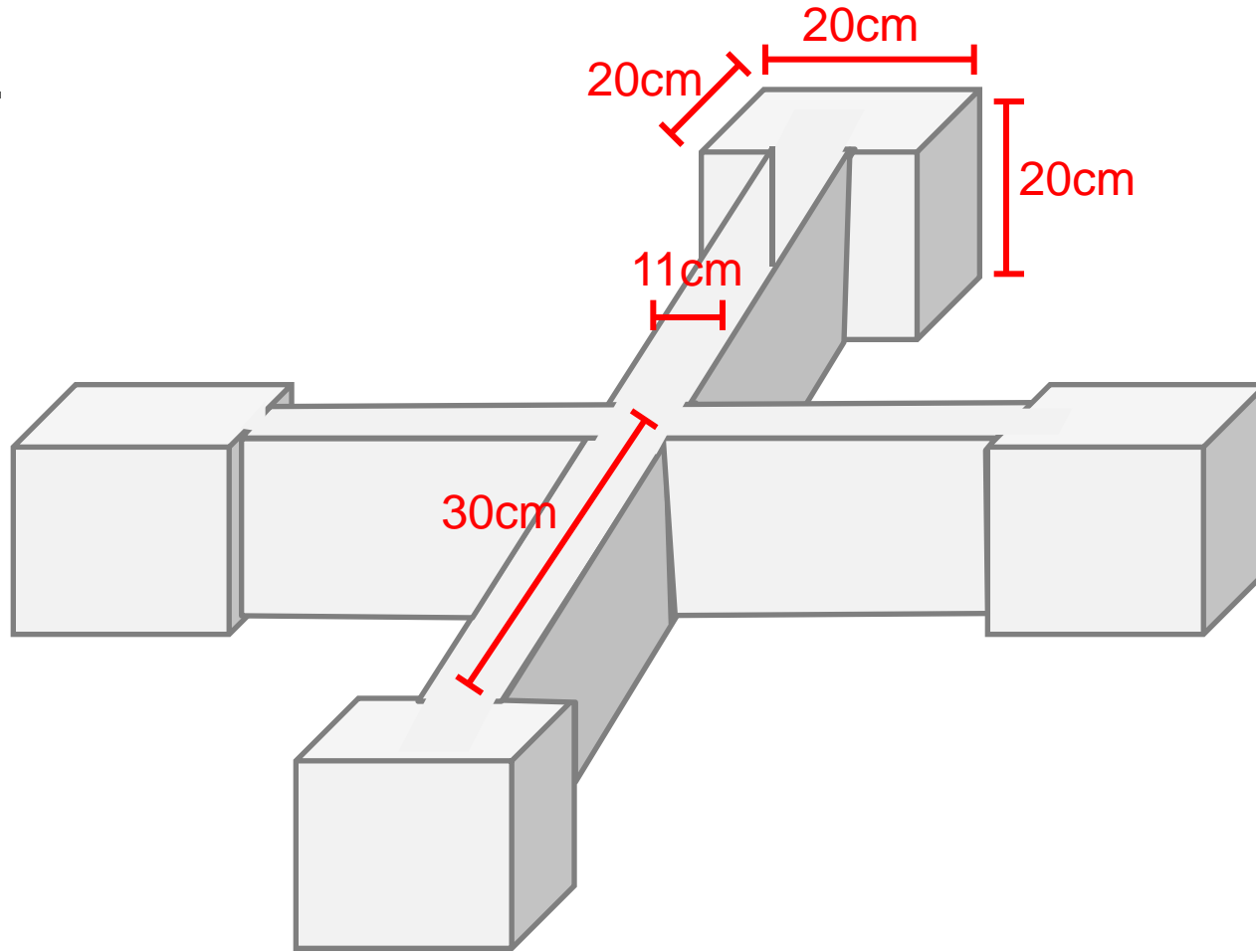


# Vision test

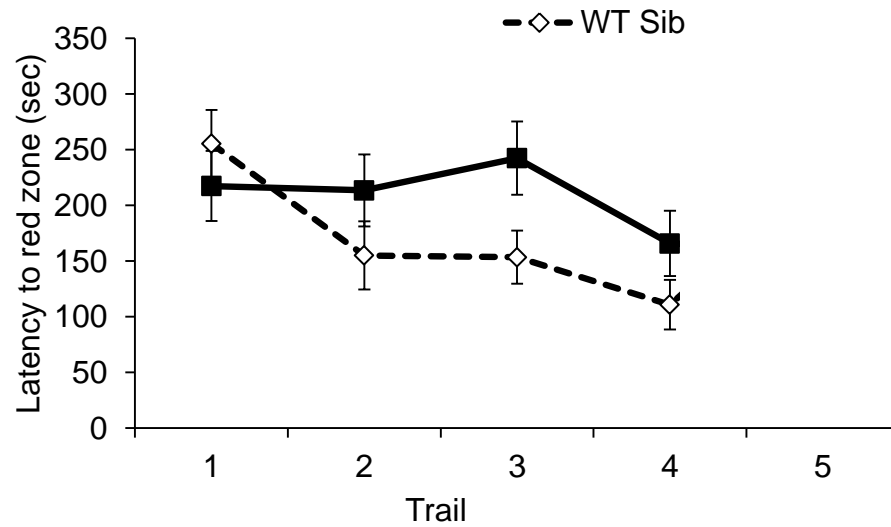
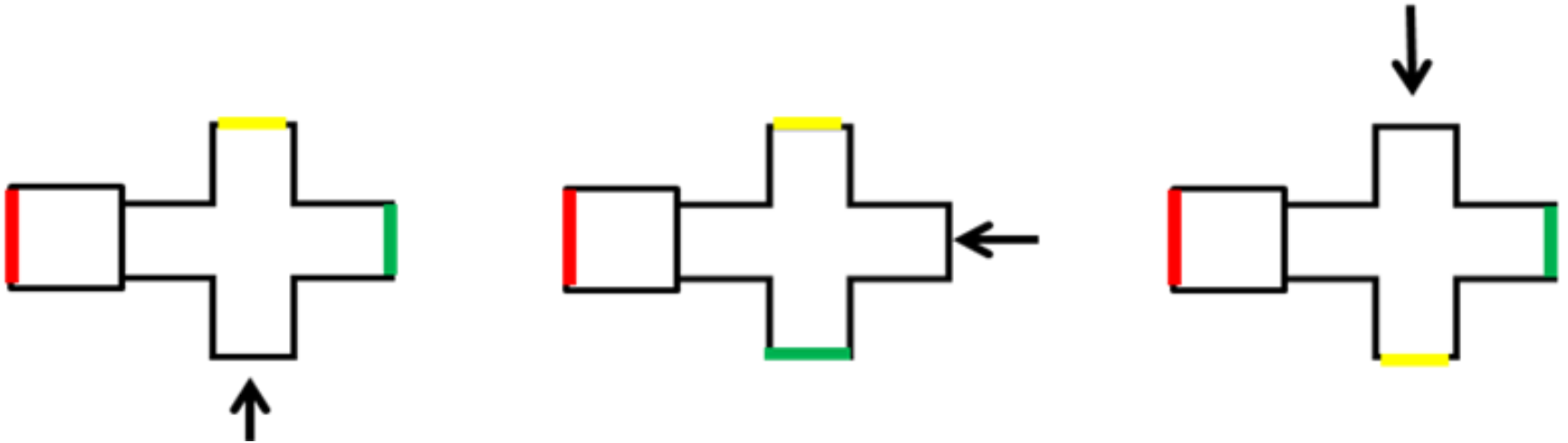


# IQ test for fish

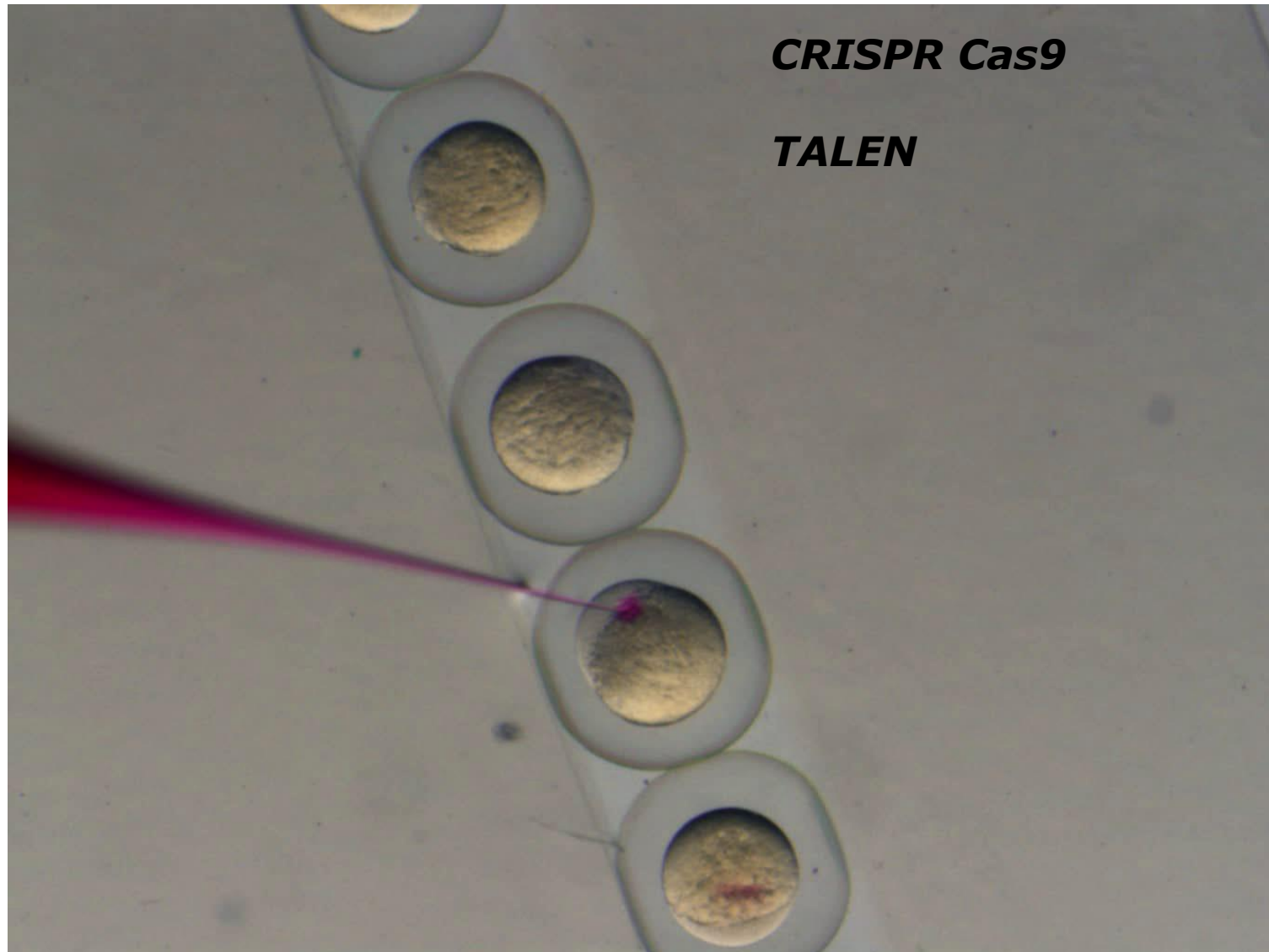
A.



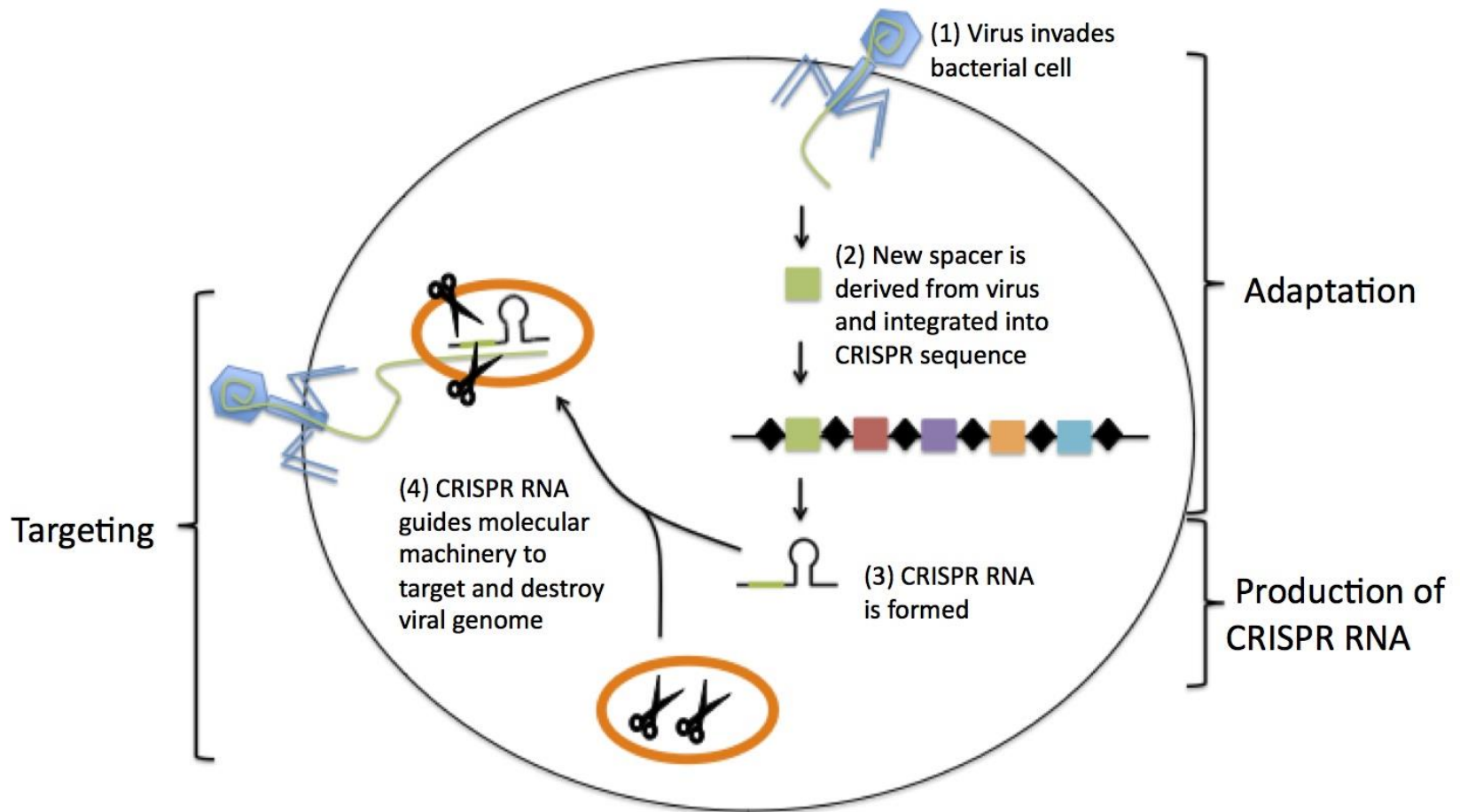
# IQ test for fish

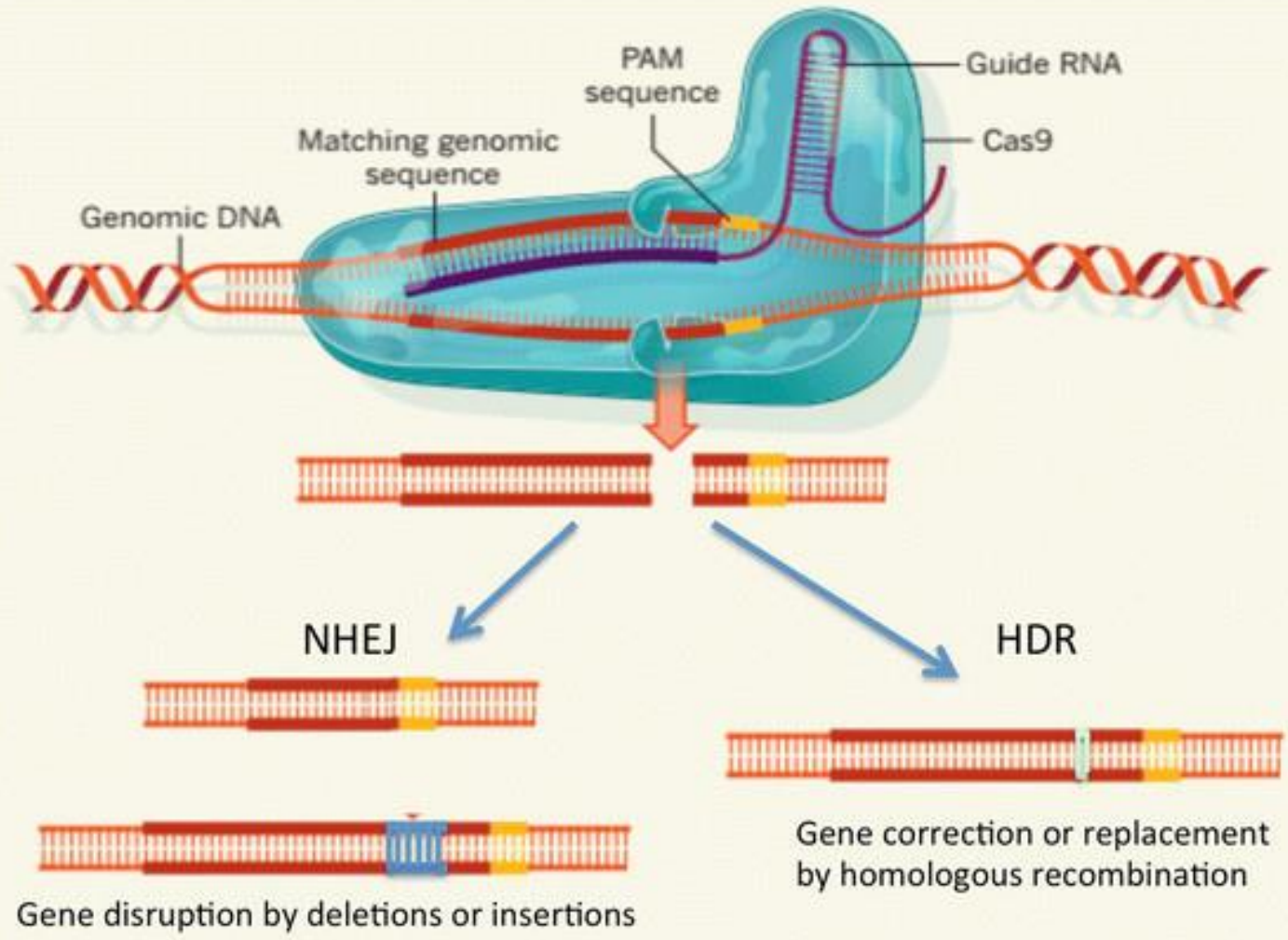


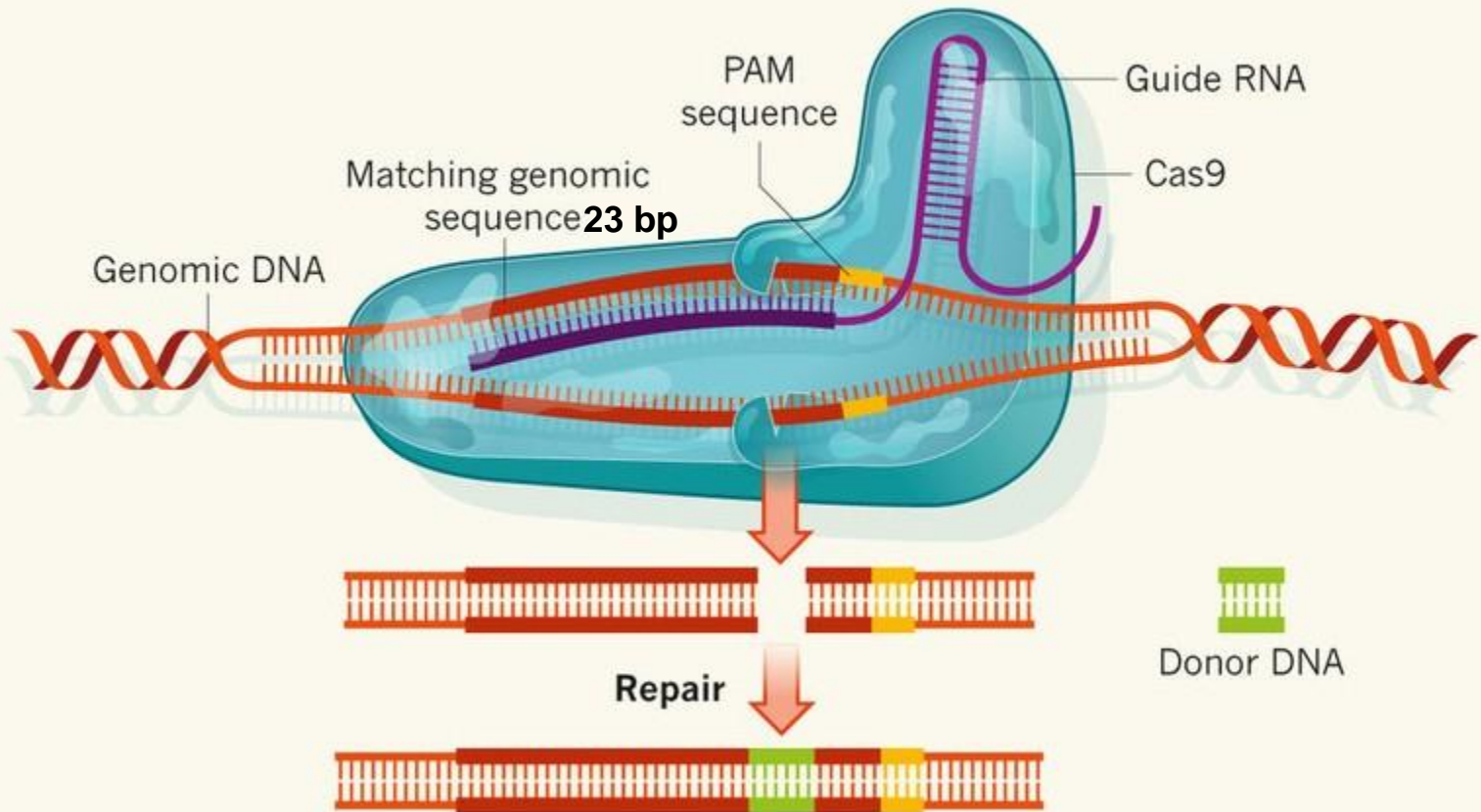
# Gene knockout for studying gene function



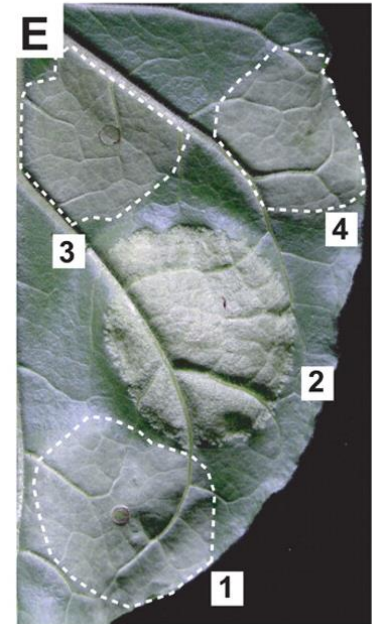
# clustered regularly interspaced short palindromic repeats (CRISPR)



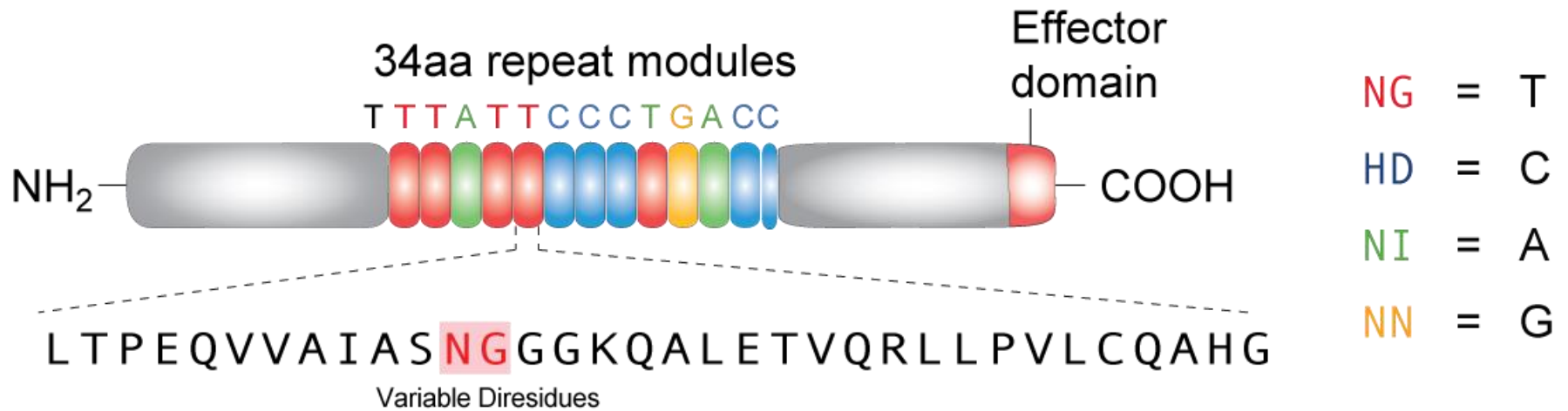




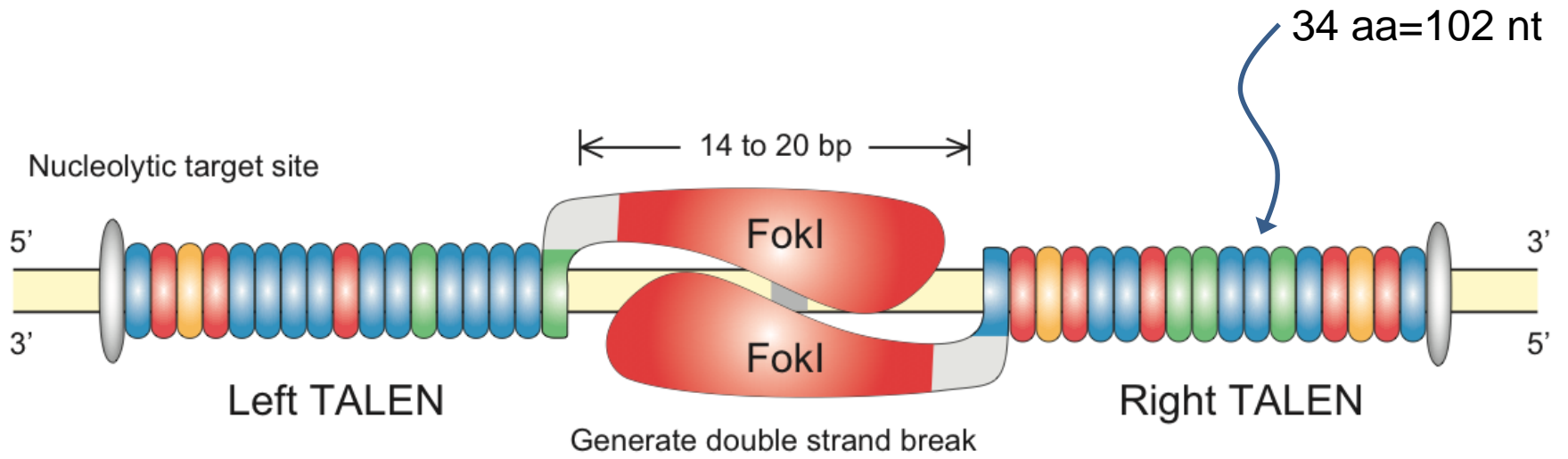
- Cell size modifications
- Transcription Activator-Like Effector - TALE



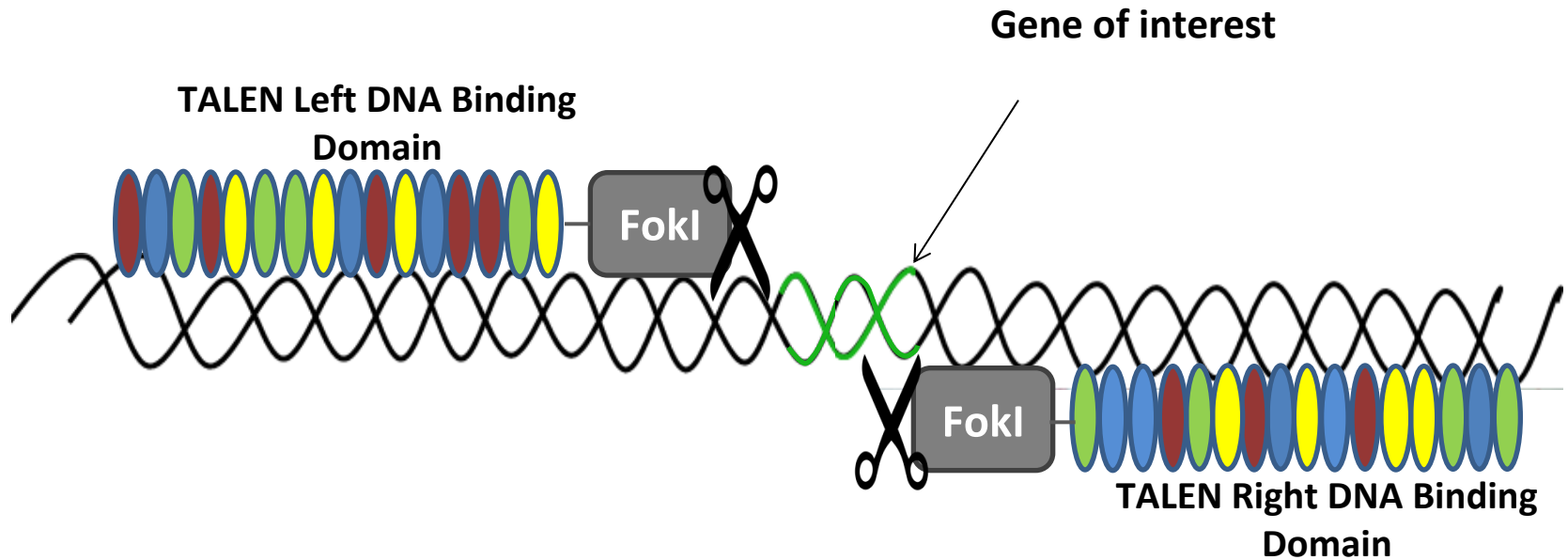




- TALE DNA-targeting domains contain 13-28 repeats of 34 aa each.
- Each repeat displays a different pair of residues at positions 12 & 13 (RVD) that associates preferentially with different nucleotides.
- H-histidine; D-aspartate; N-asparagine; G-glycine; I-Isoleucine

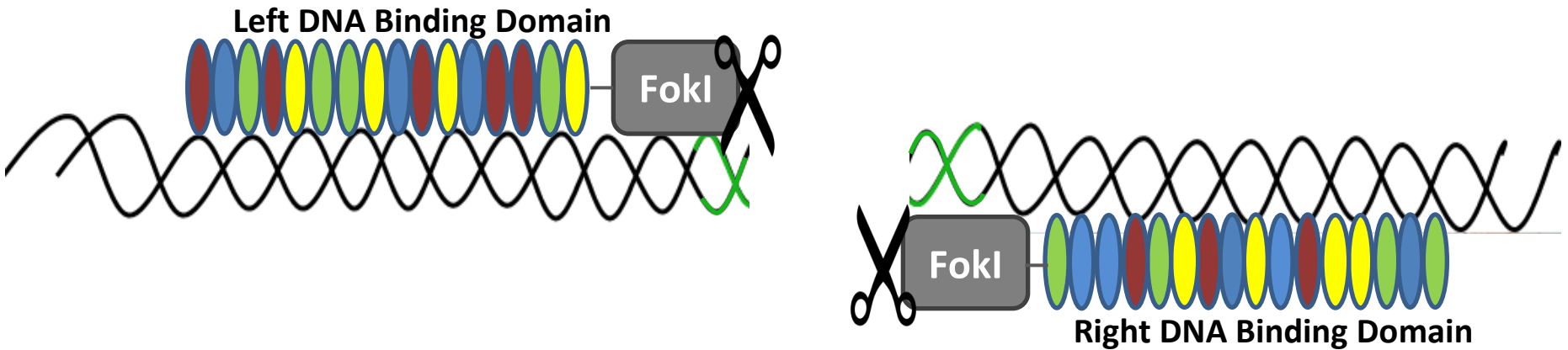


# TALEN-mediated Gene Knockout



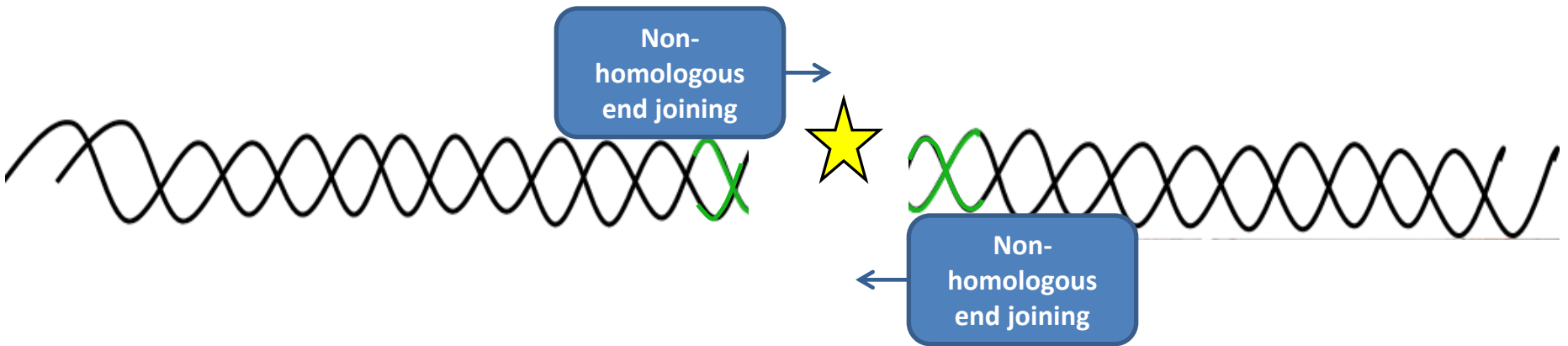
**TALEN domains bind to a gene of interest and FOKI nucleases induce a double-stranded break.**

# TALEN-mediated Gene Knockout



**TALEN domains bind to a gene of interest and FOKI nucleases induce a double-stranded break.**

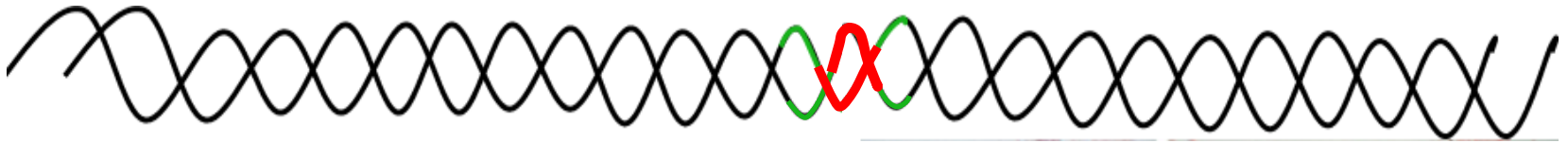
# TALEN-mediated Gene Knockout



**Non-homologous end joining (NHEJ) repair system introduces indels (insertions and/or deletion) into the gene sequence.**

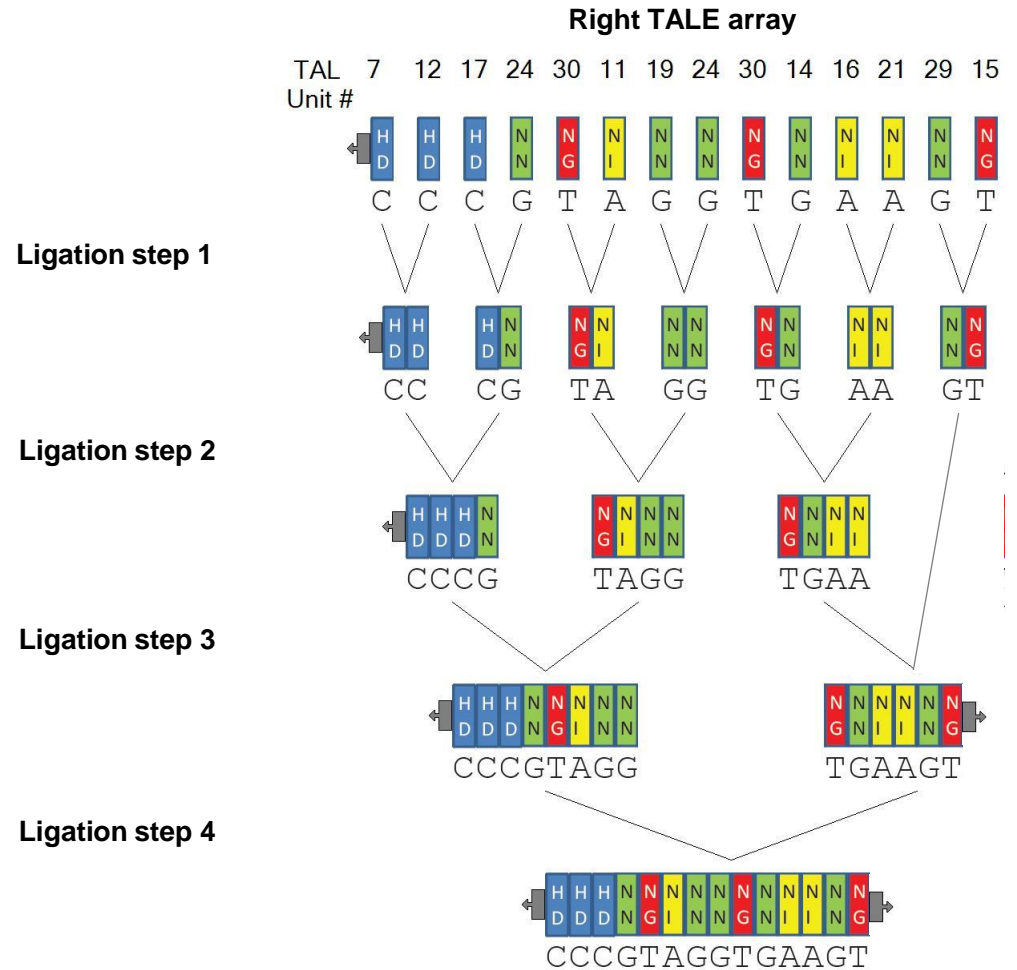
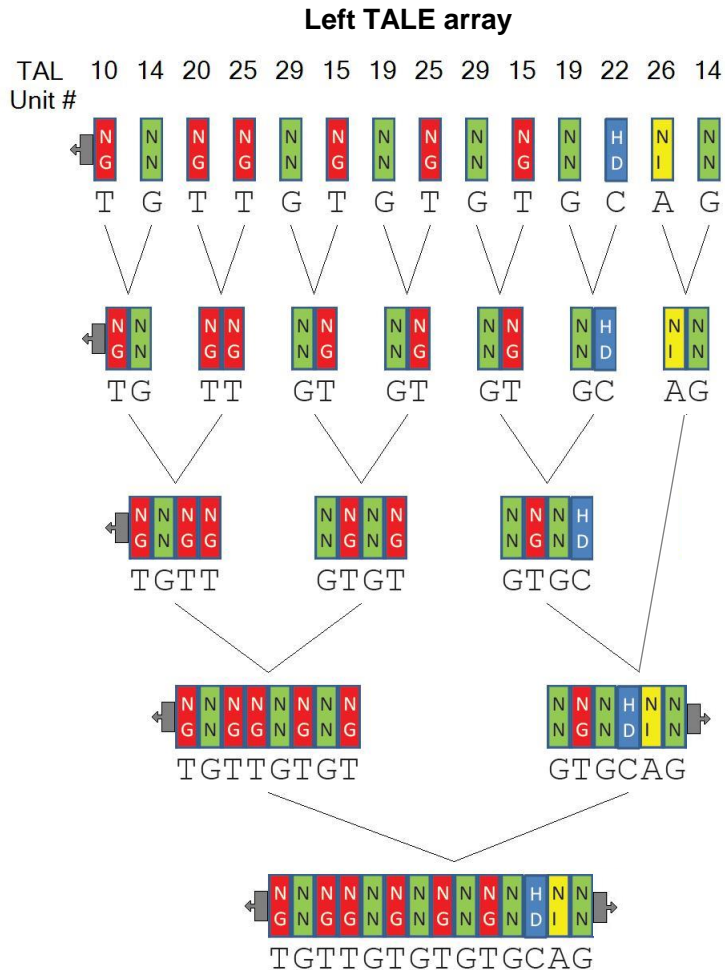
# TALEN-mediated Gene Knockout

Mutated (inherited) gene sequence



**Non-homologous end joining (NHEJ) repair system  
introduces indels (insertions and/or deletion) into the gene  
sequence.**

# Construction of TALE arrays



- What are the advantages and disadvantages of zebrafish as a model for human genetic diseases?
- Whole genome duplication: What could be the consequence and why is this a consideration?
- What are the advantage of CRISPR over TALEN?
- What are the advantage of TALEN over CRISPR?
- Which of the two systems is mostly used in zebrafish research?