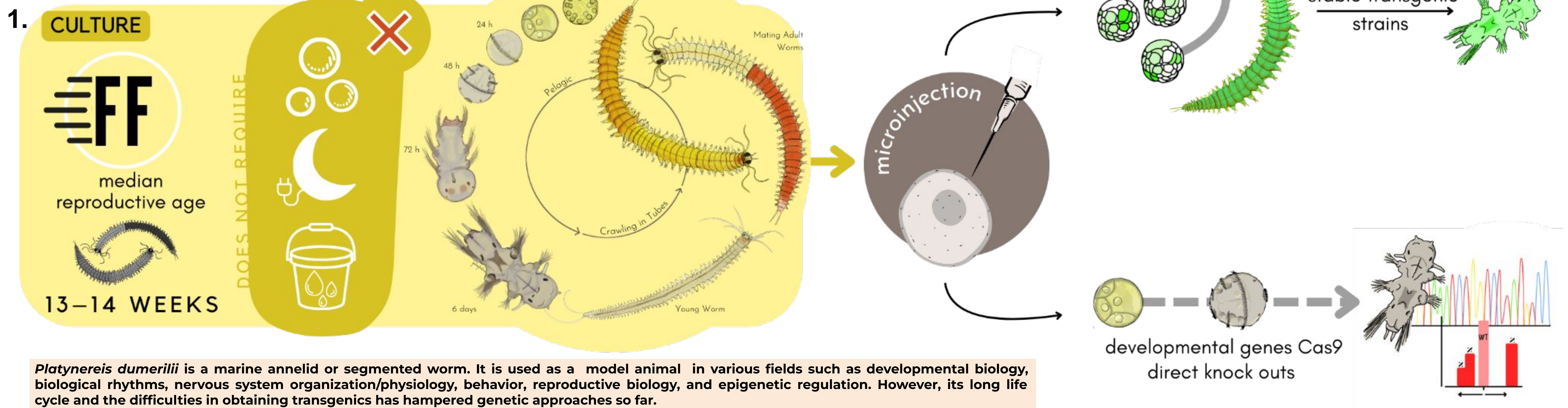
TRANSGENESIS AND GENOME EDITING IN THE MARINE MODEL PLATYNEREIS DUMERILII

Mathieu Legras[1,2], Giulia Ghisleni[3], Antoine Prevel[1], Duygu Özpolat[4], Guillaume Balavoine[1]

1. CNRS/Institut Jacques Monod
2. CNRS/Institut des Neurosciences Paris Saclay
3. University of Milano-Bicocca
4. Washington University in St. Louis

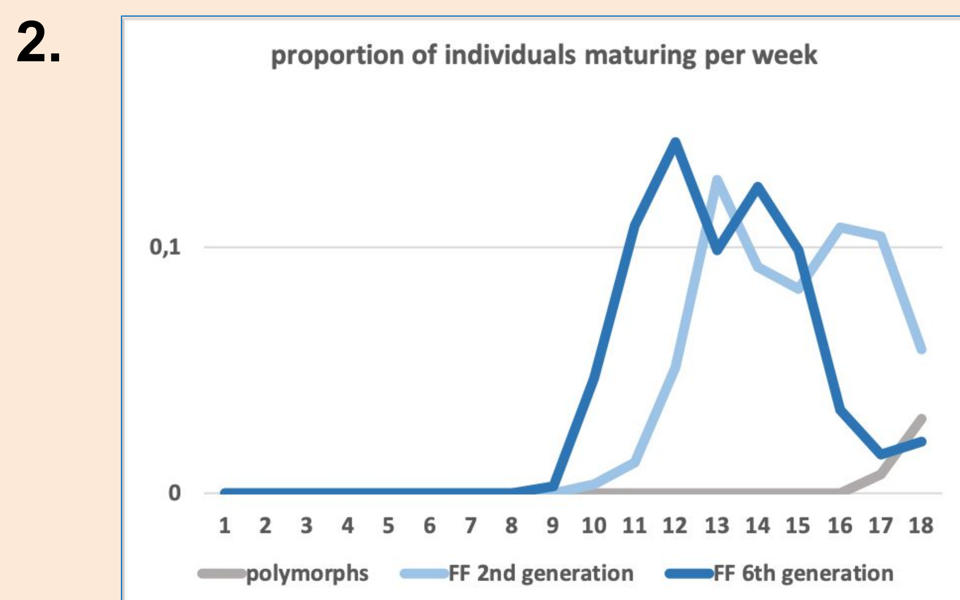
GRAPHICAL ABSTRACT



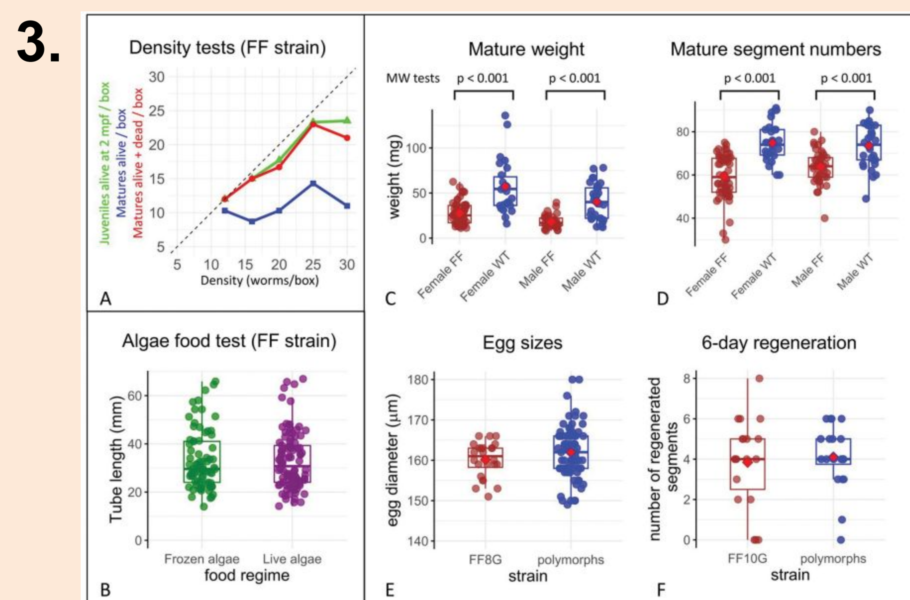
Platynereis dumerilii **is a marine annelid or segmented worm. It is used as a model animal in various fields such as developmental biology, biological rhythms, nervous system organization/physiology, behavior, reproductive biology, and epigenetic regulation. However, its long life cycle and the difficulties in obtaining transgenics has hampered genetic approaches so far.**

FAST REPRODUCING STRAIN AND CULTURE PROTOCOL

2.



3.



4.

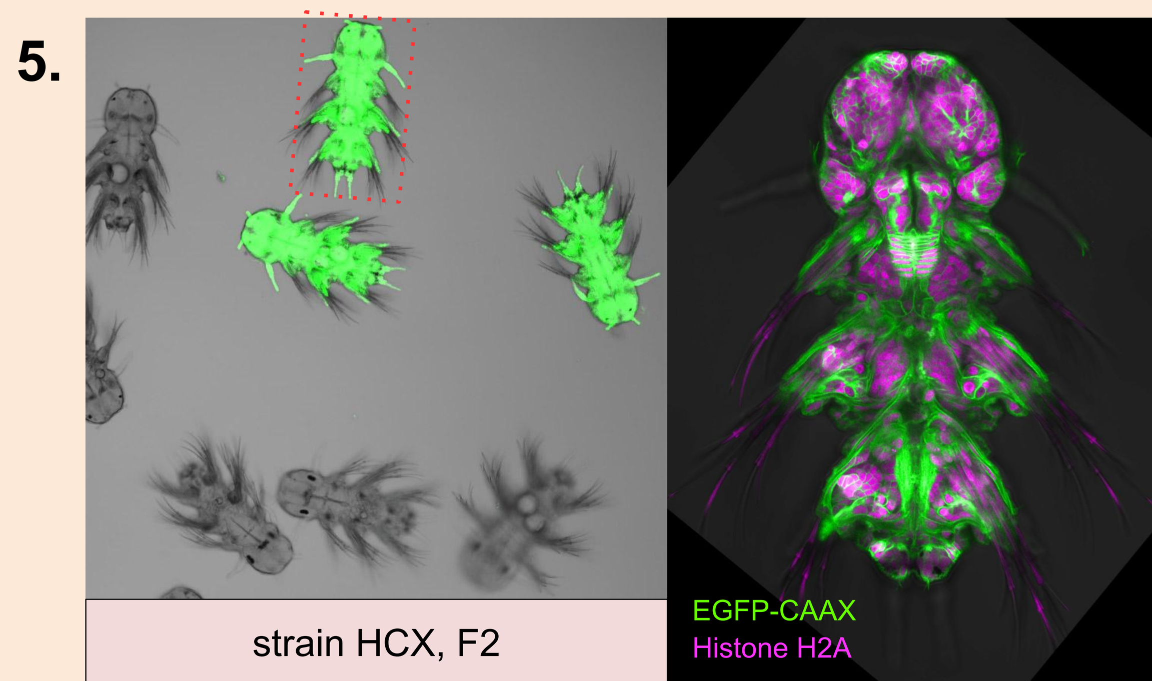
	"Fast Forward" protocol	"Traditional" protocol
Temperature	20°C	18°C
Day / night (hours)	16 / 8	16 / 8
Moonlight	not necessary	required for sexual metamorphosis synchronization
Density (worms / m²)	375	alternance of high density (>2000) and low density (500)
Food	Microalgae, Sera micron, spinach, Tetramin in finely ajusted quantities	Microalgae, Sera micron, spinach, Tetramin
Selection of healthy juveniles	required	not required
Change of water	every 2 months	every two weeks
Bubbling	not required	required for high density
Average mature weight (mg)	27.7 (female) 18.1 (male)	57.2 (female) 40.1 (male)
Number of segments	59.3 (female) 63.8 (male)	74.8 (female) 73.5 (male)
Average mature age (weeks)	13.2	>18
Egg size (μm)	161	162

Platynereis dumerilii **is known for its relatively long life cycle (25-35 weeks before reaching maturation). We selected early reproducing individuals and simplified the culture protocol (food regimen, worm density per box, removal of artificial moonlight or reduced water changes), reducing the average age of mature worms to 13-14 weeks (fig. 2).**

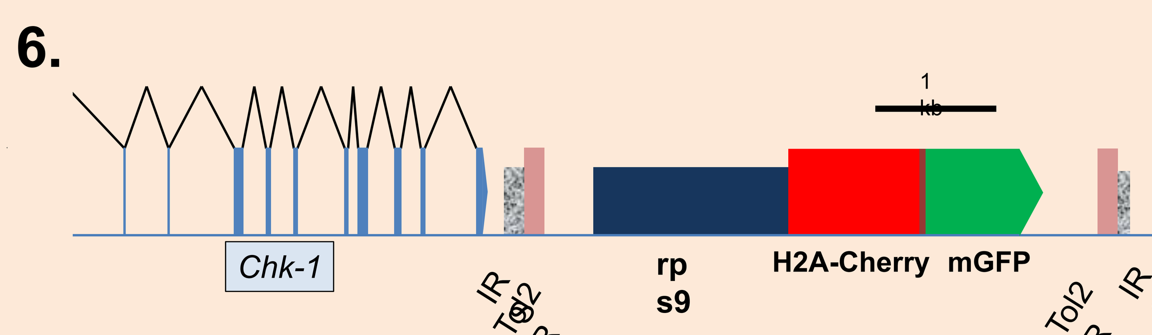
The density of worms per box is crucial for the rapid development of ***Platynereis dumerilii*** **(fig. 3). The Fast Forward (FF) strain produces smaller adults, but no differences are observed concerning egg sizes or regeneration ability (fig. 4).**

STABLE TRANSGENESIS

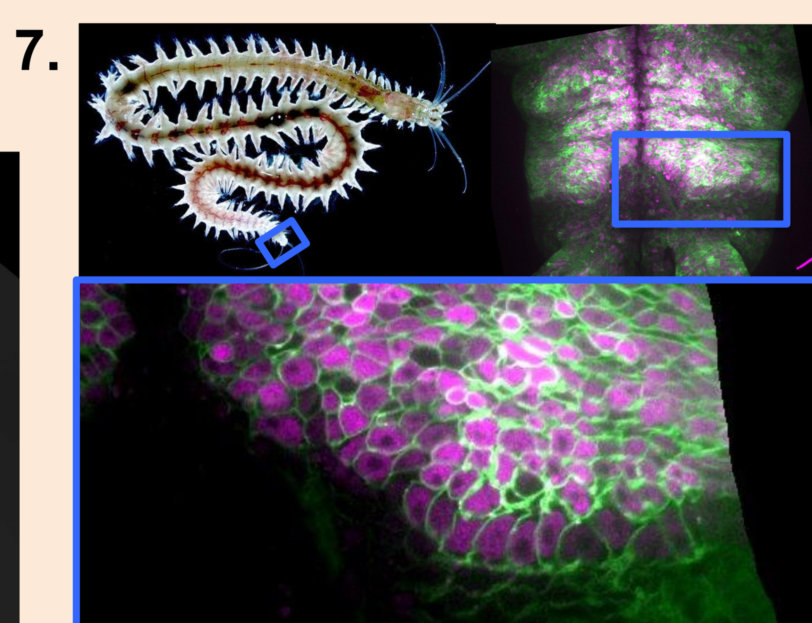
5.



6.



7.

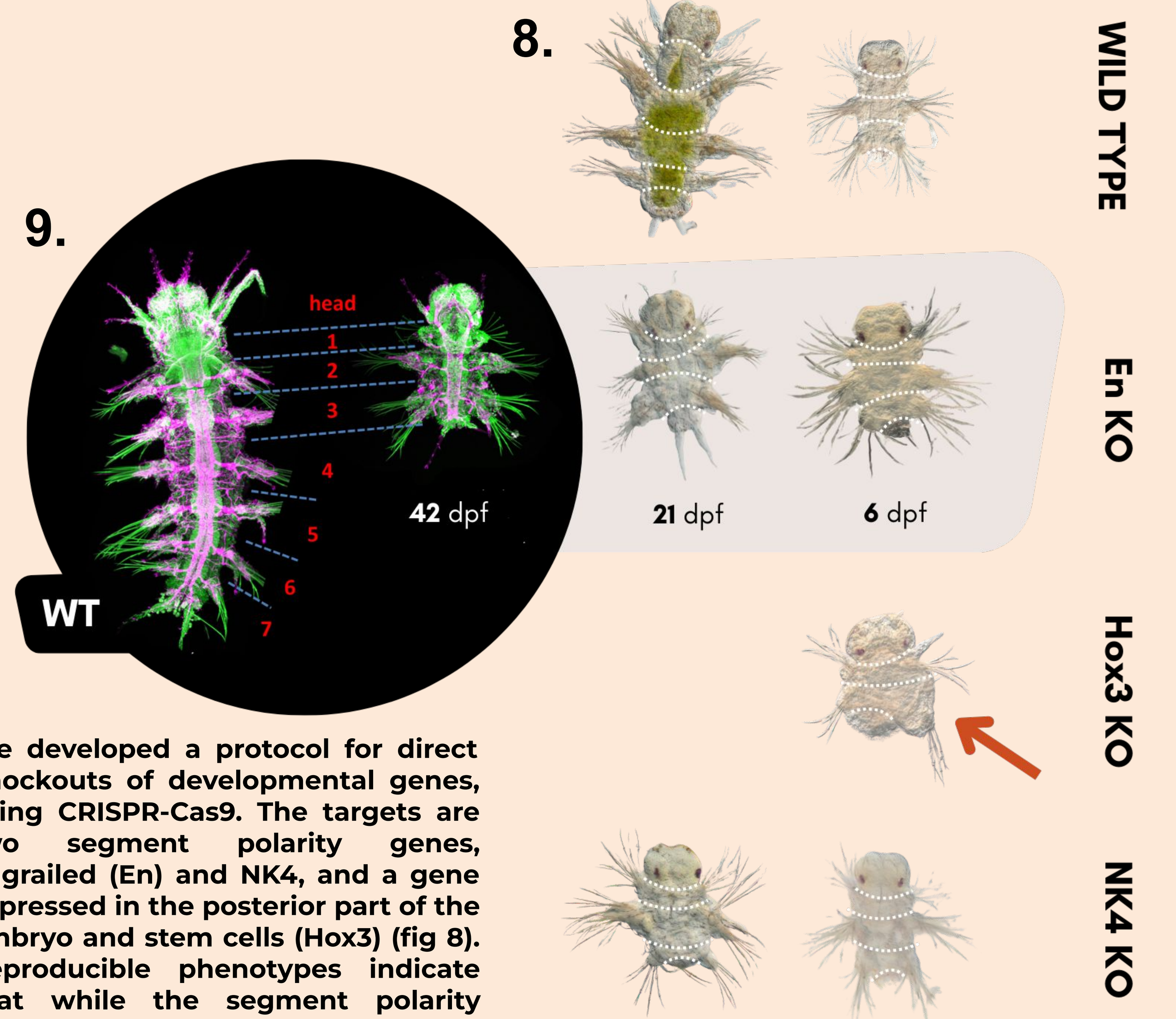


Platynereis dumerilii **has resisted efforts for wide scale transgenesis so far, due to the efficiency of its genomic immunity. Most transgenes are silenced in the germ line of the worm. By using procedures designed to limit this silencing response with a transposase protocol, we obtained a first stable strain that expresses fluorescent membrane and nuclear proteins (fig 5), under the control of a strong ubiquitous promoter (rps9). The transgene insertion in the genome has been mapped near the** ***Chk-1*** **ortholog gene (fig 6). This strain is ideal to follow the activity of the posterior stem cells (yellow arrowheads) responsible for the lifelong growth of the worm (fig 7).**

KNOCK OUTS OF ESSENTIAL GENES

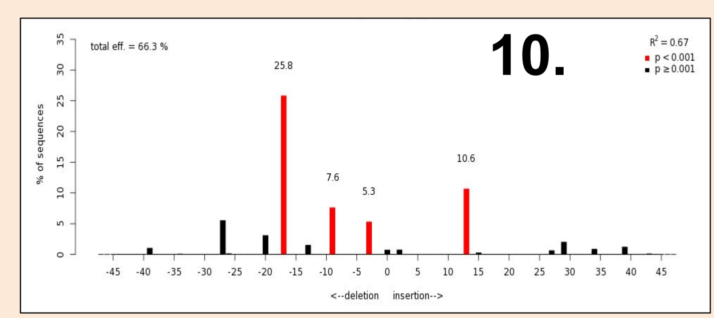
8.

9.



We developed a protocol for direct knockouts of developmental genes, using CRISPR-Cas9. The targets are two segment polarity genes, engrailed (En) and NK4, and a gene expressed in the posterior part of the embryo and stem cells (Hox3) (fig 8). Reproducible phenotypes indicate that while the segment polarity genes are dispensable for the larval segment formation, they are necessary for the subsequent posterior addition of segments (fig 9). Hox3 disruption leads to posterior body mis patterning (red arrow). The genotyping of mutations is assessed on individual crisprant by using the software TIDE (Brinkman et al, 10.1093/nar/gku936) (fig 10).

10.



CONCLUSIONS and PERSPECTIVES

The acceleration of the life cycle of ***Platynereis*****, which can be as quick as 11 weeks with the new protocol, allows for better feasibility of reverse genetics experiments. Genomic and transcriptomic studies will be performed to understand the specificities of the FF strain. The simplification of the culture protocol also enables wider adoption of this model organism by new labs.**

The creation of the first ubiquitously expressed transgenic strain in ***Platynereis*** **is a major breakthrough. The chromosomal location of the transgene provides a "safe harbor" for the insertion of other transgenes, which will be targeted using the CRISPR-Cas9 protocol. The efficient insertion of new transgenes by knock-in depends on two factors: the high cutting efficiency of Cas9 at the targeted site and a high probability of homologous recombination when the cut is performed. We have developed a very efficient protocol for using Cas9 in knock-out experiments, achieving a mutation efficiency of nearly 100%. This is the first step in optimizing a future knock-in protocol.**

The development of ***Platynereis*** **as a convenient model for reverse genetics and transgenesis will give new impetus to biological studies in marine animals. Additionally, it is strategically placed among the lesser-studied lophotrochozoans, the third branch of the bilaterians.**

2024 EURO EVO DEVO Helsinki
EURO EVO DEVO
cnrs
Institut Jacques Monod
NeuroPSI Paris-Saclay Institute of Neuroscience
Fondation ARC pour la recherche sur le cancer
UFRJ
Università degli Studi di Milano Bicocca
Washington University in St. Louis