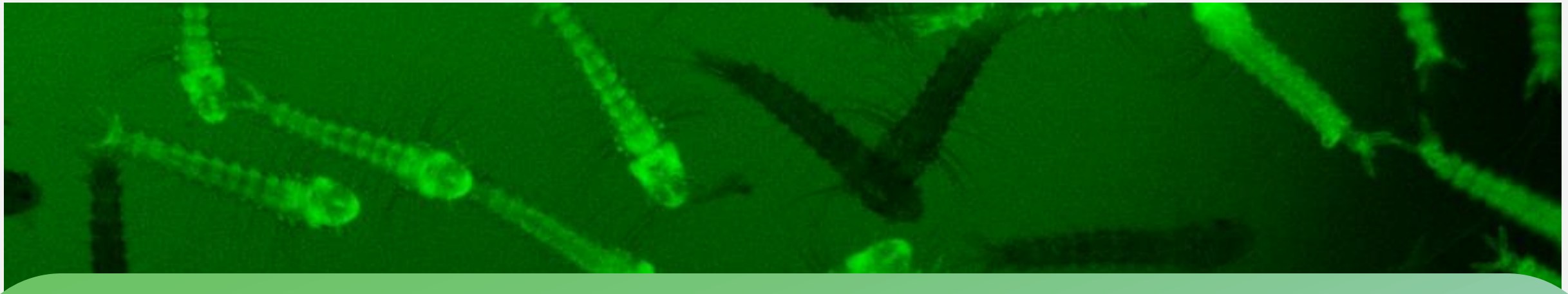
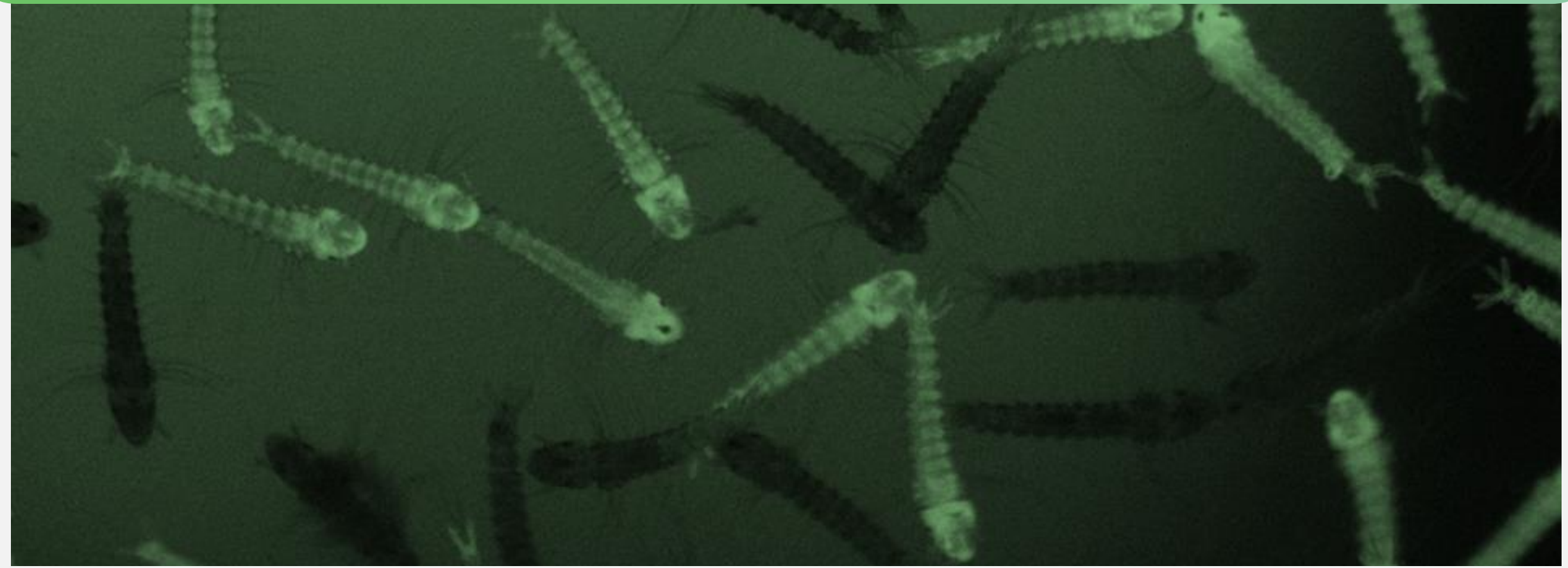


Development of Genetic Sexing Strains for the application of the Sterile Insect Technique in Aedes albopictus *and* Aedes aegypti

Célia Lutrat

celia.lutrat@outlook.com

INTRODUCTION



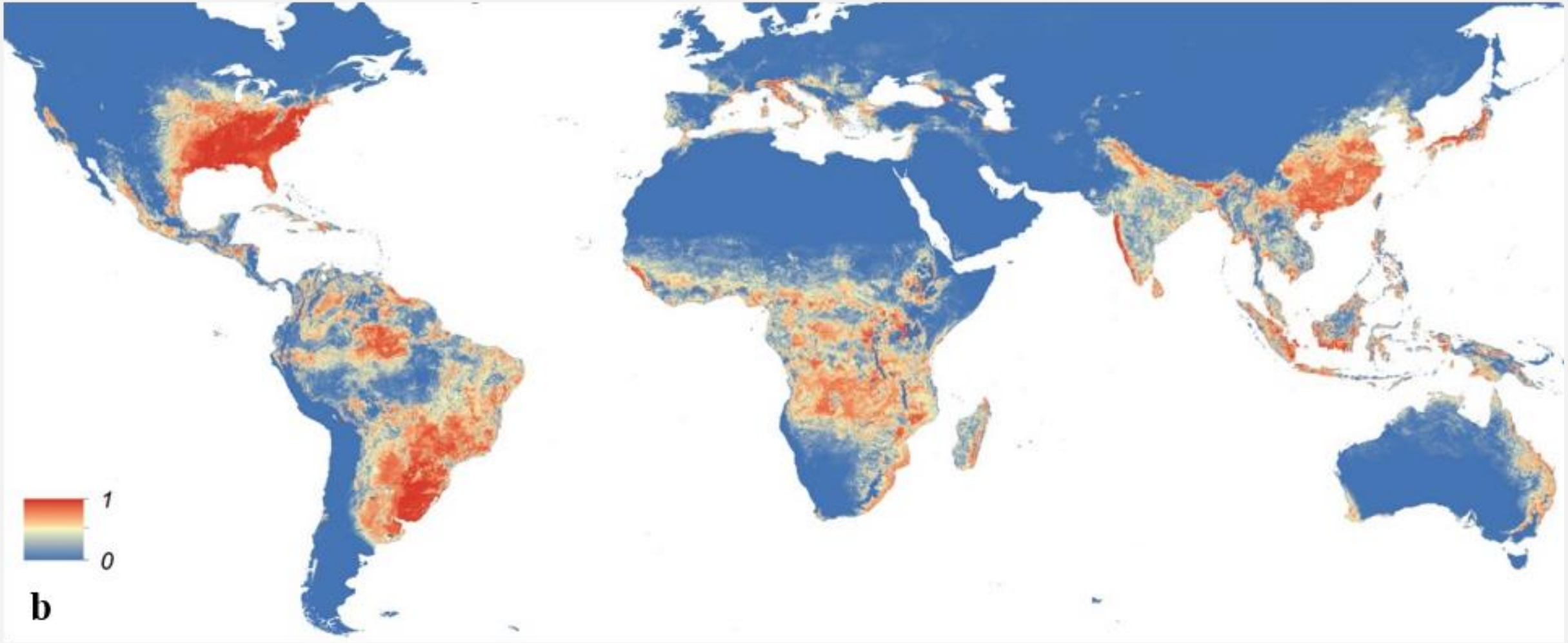
INTRODUCTION

Aedes albopictus

Jim Occi, BugPics, Bugwood.org

INTRODUCTION

World distribution of *Aedes albopictus*



Kraemer et al. 2015
Reiter 1998

INTRODUCTION

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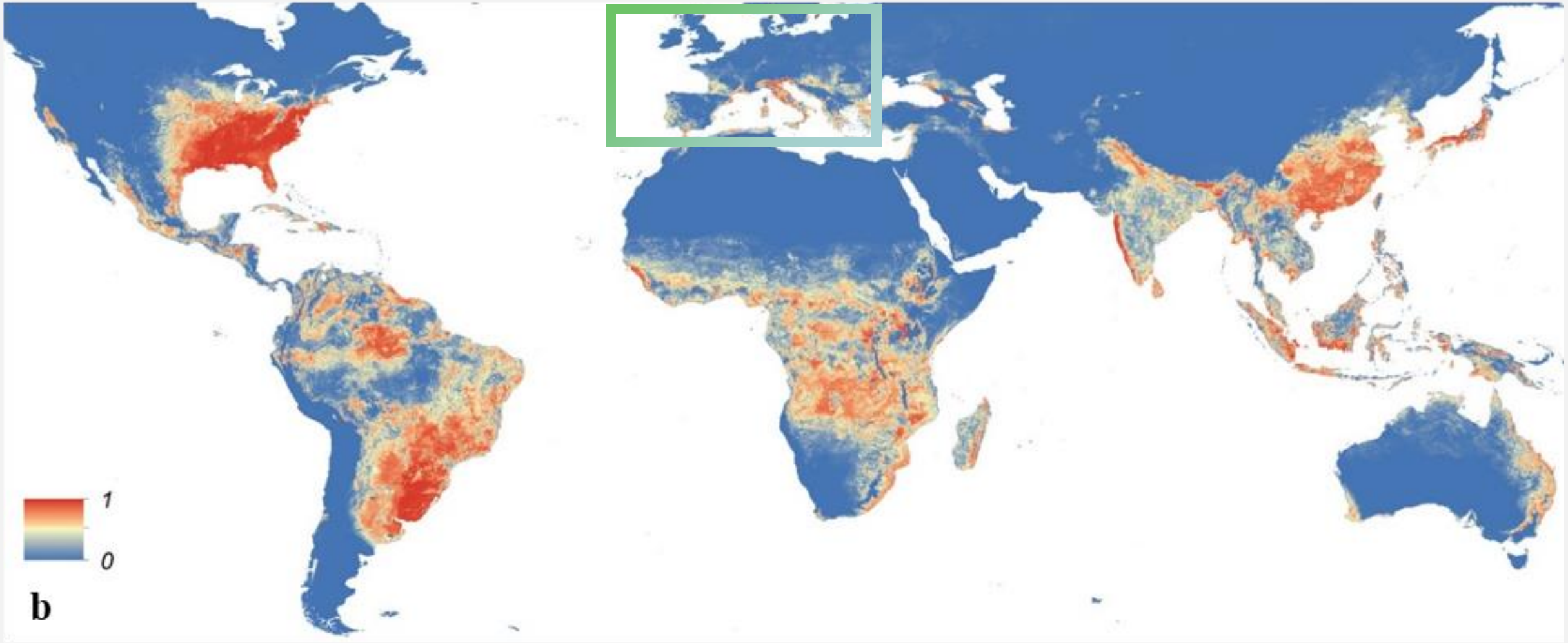

Kraemer et al. 2015

INTRODUCTION

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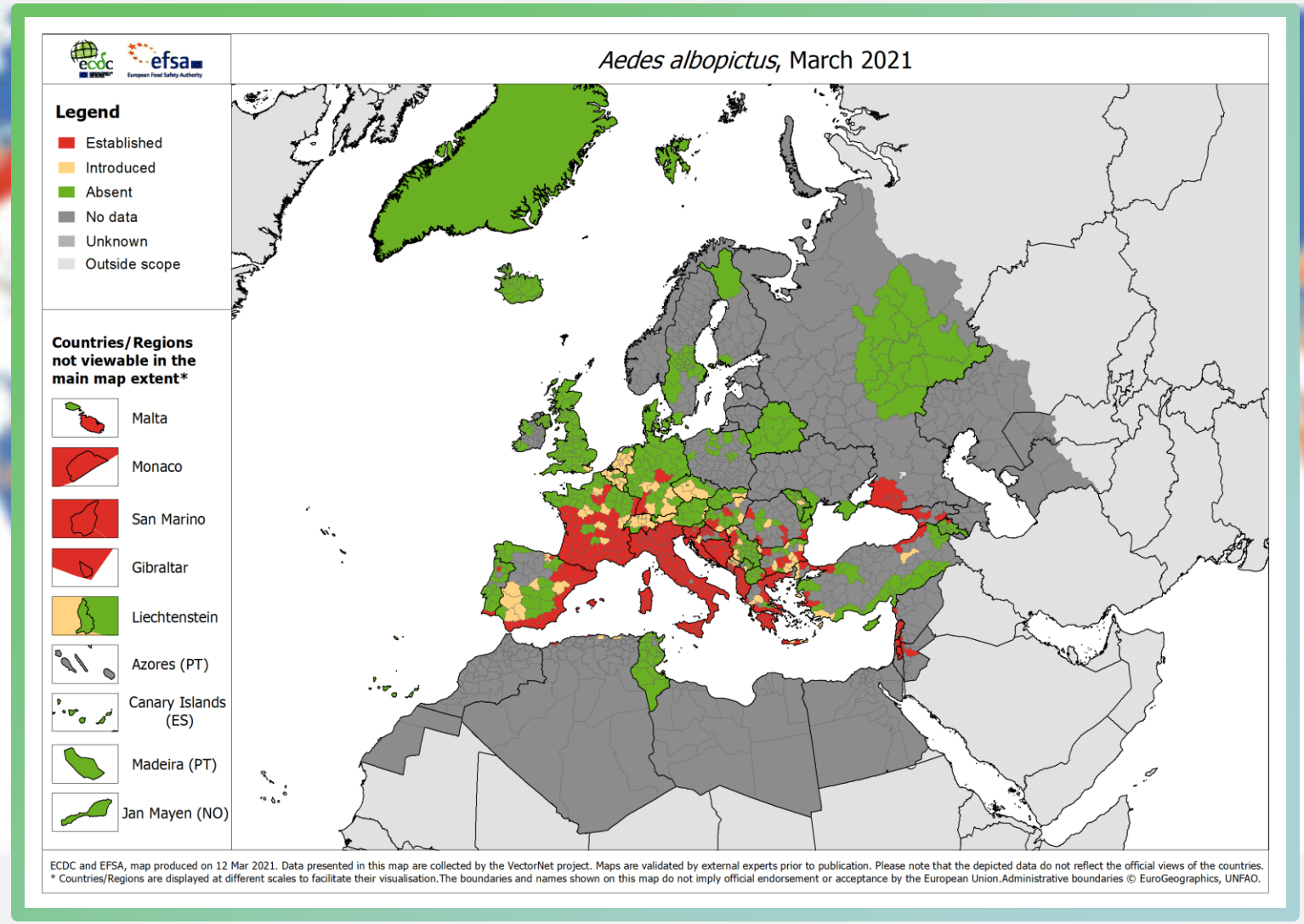


ECDC 2021

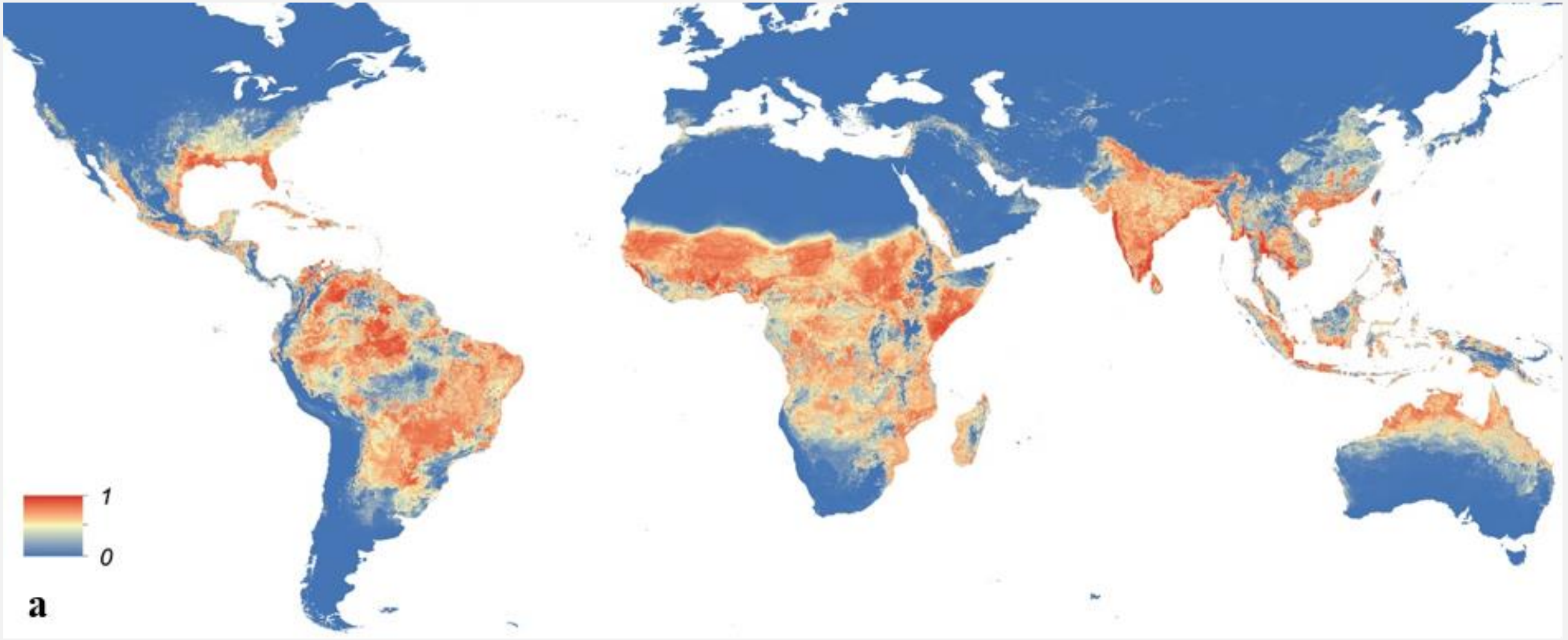
INTRODUCTION

Aedes aegypti

James Gathany - PHIL, CDC

INTRODUCTION

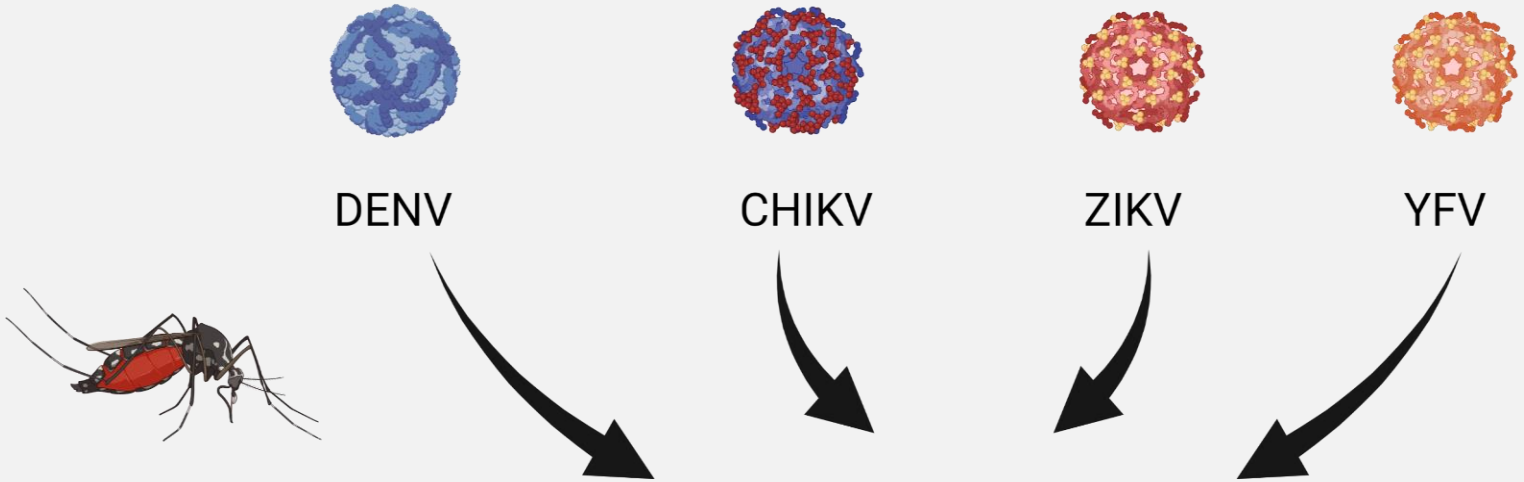
World distribution of *Aedes aegypti*



Kraemer et al. 2015
Soghigian et al. 2020

INTRODUCTION

Aedes-borne viruses cause severe illnesses costing over 2M DALYs annually



WHO Global Vector Response 2017-2030, Puntasecca et al. 2021

INTRODUCTION

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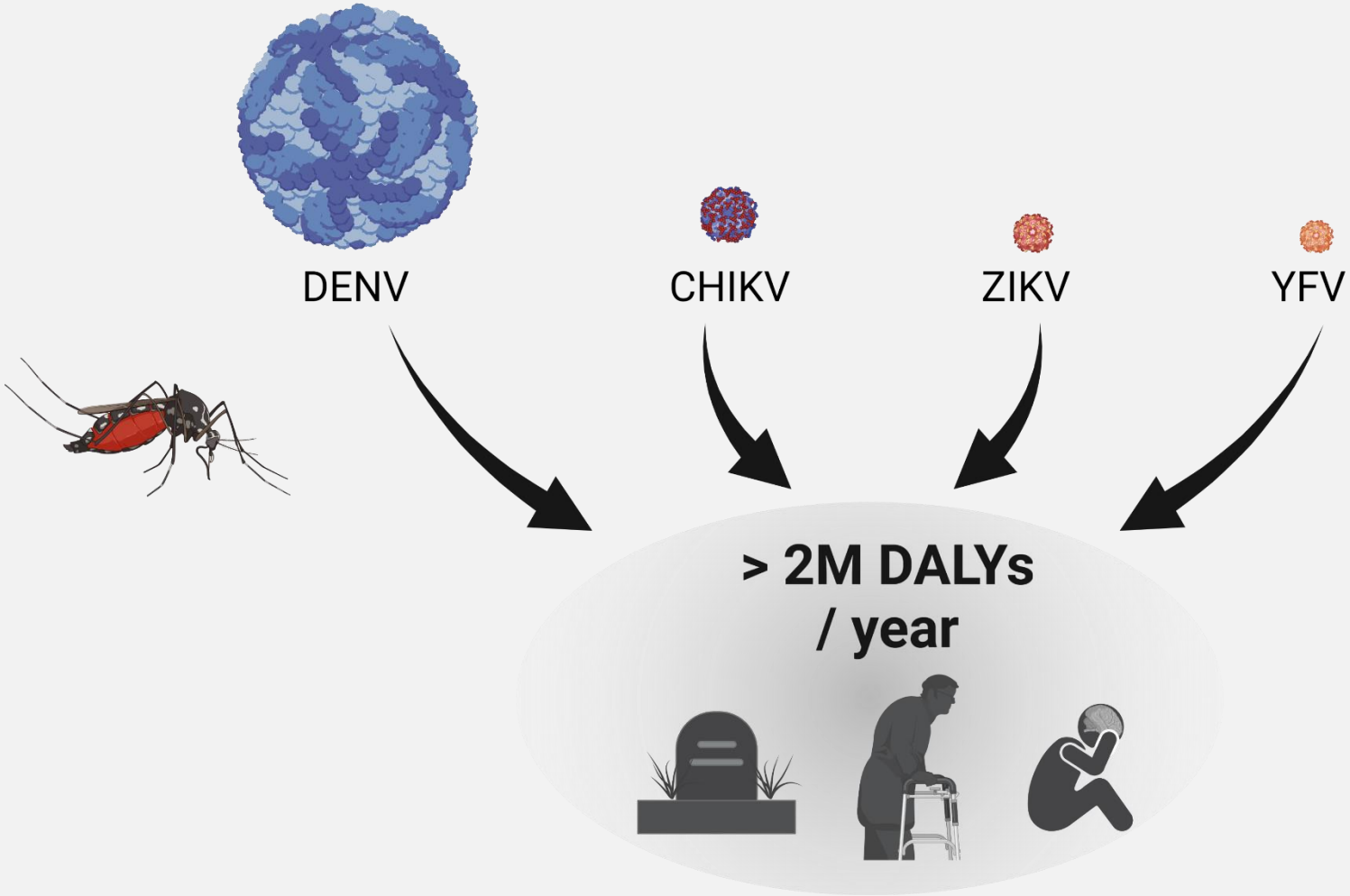
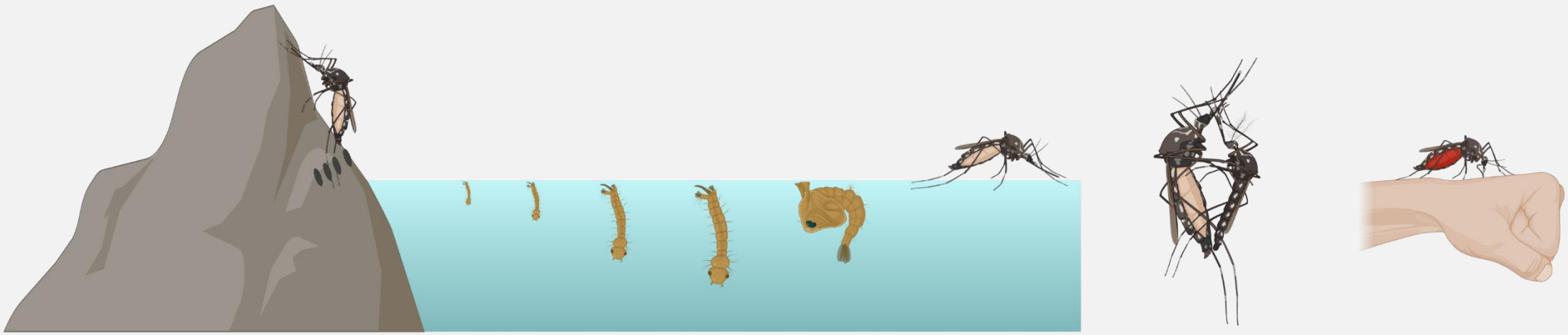


WHO Global Vector Response 2017-2030, Puntasecca et al. 2021

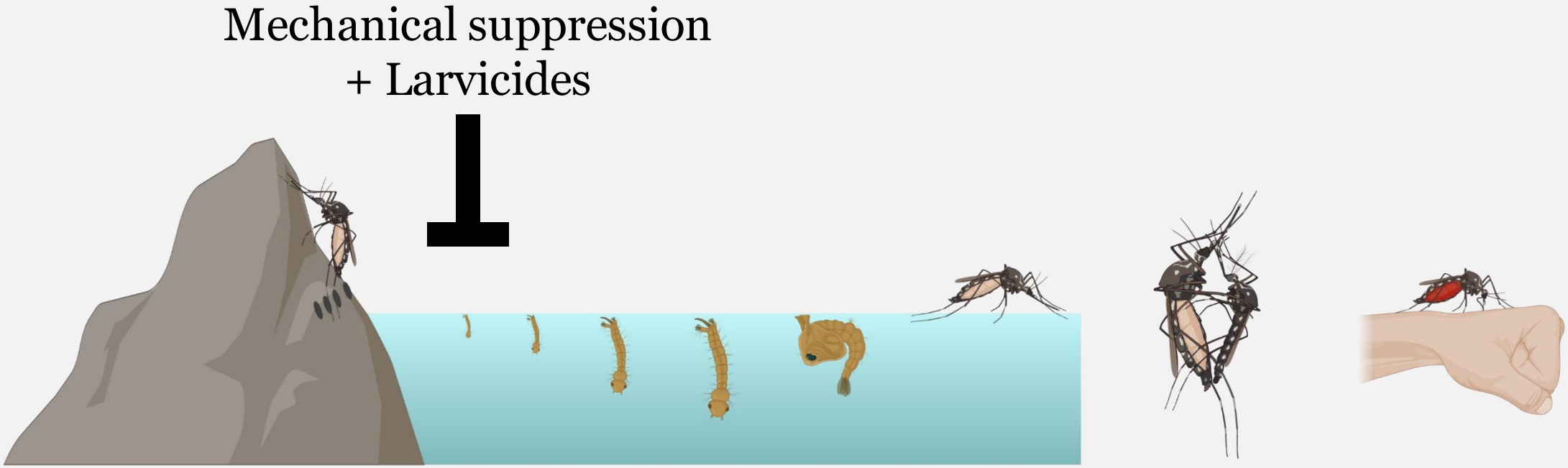
INTRODUCTION

Life cycle of *Aedes* mosquitoes



INTRODUCTION

Current vector control against *Aedes* mosquitoes



Labbé et al. 2011, Reiter 2007

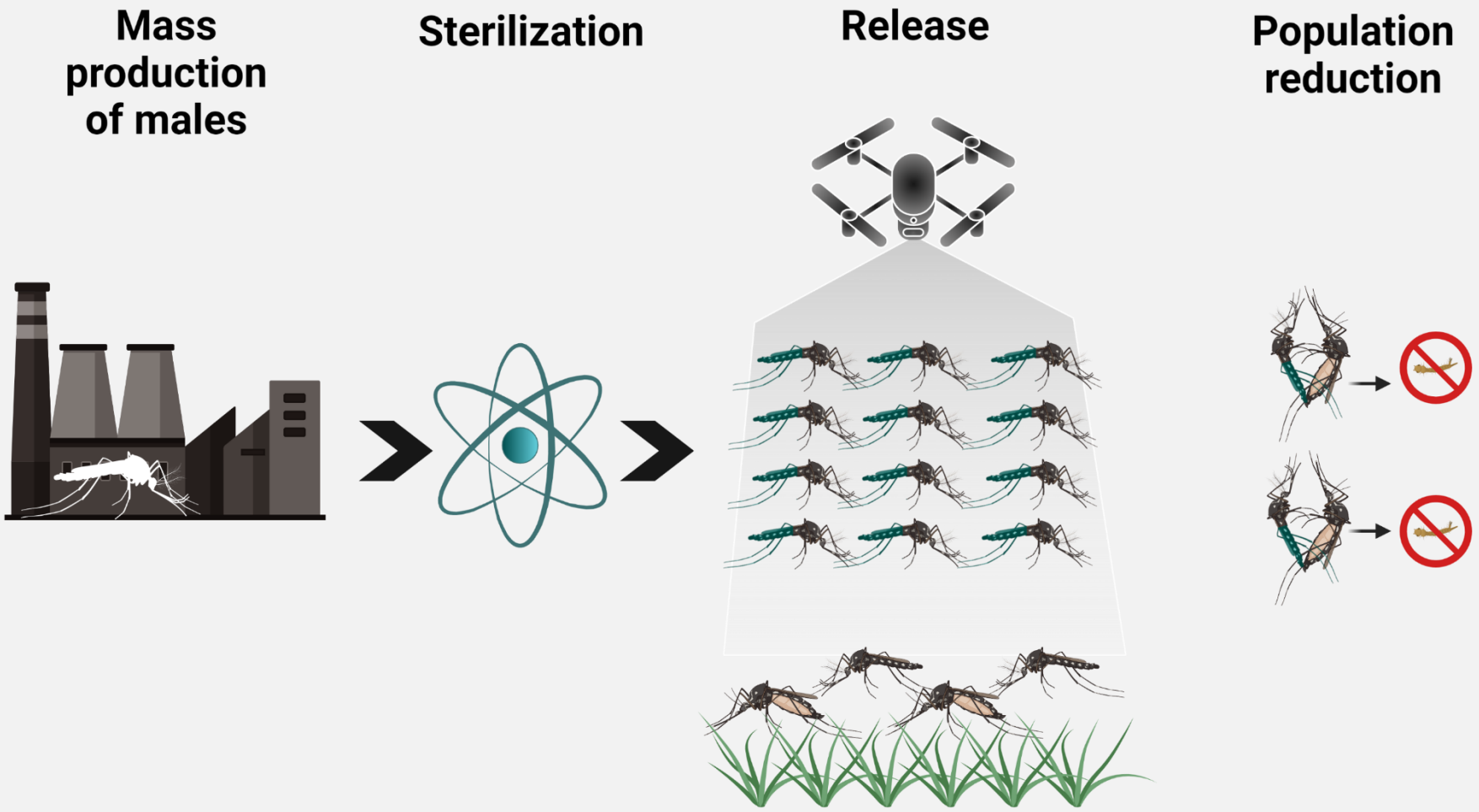
INTRODUCTION

Current vector control against *Aedes* mosquitoes

Labbé et al. 2011, Beketov et al. 2013, Antwi et Reddy 2015, ANSES 2020

INTRODUCTION

The Sterile Insect Technique (SIT) is a promising alternative



Knippling 1955, Bellini et al. 2013

INTRODUCTION

Sex separation is the main bottleneck to *Aedes* SIT

Rendon et al. 2004, Papathanos et al. 2018

INTRODUCTION

Aedes sex dimorphism

♀

♂

Moorefield 1951

INTRODUCTION

Current sexing methods exploit the size dimorphism at the pupal stage

Fay et Morlan 1959

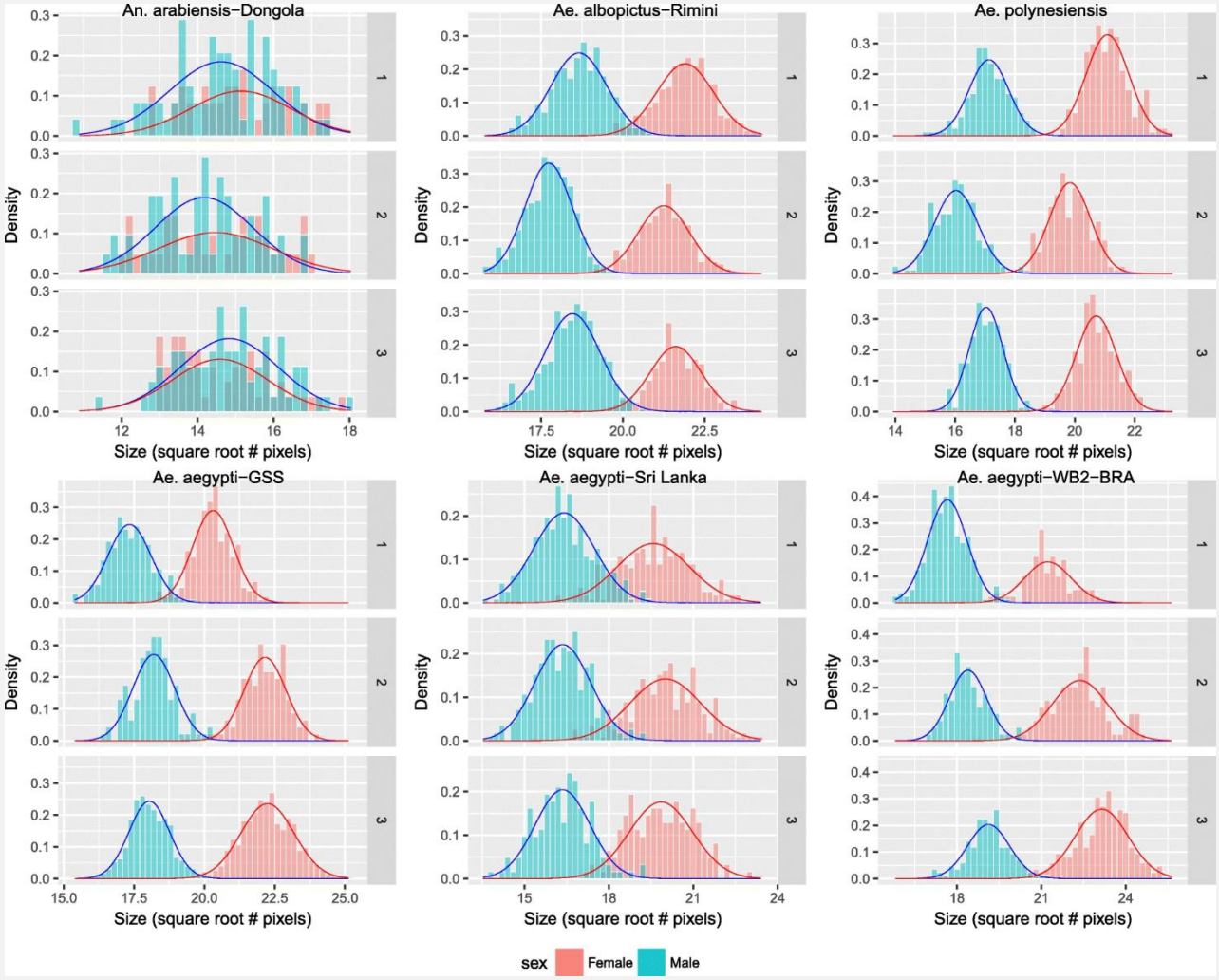
INTRODUCTION

Current sexing methods exploit the size dimorphism at the pupal stage

Fay et Morlan 1959, Mamai et al. 2020

INTRODUCTION

Current sexing methods exploit the size dimorphism at the pupal stage



Zacarés et al. 2018, Mamai et al. 2020

INTRODUCTION

30% male recovery

≤1% female contamination

1800 pupae /min

Bellini et al. 2013

INTRODUCTION

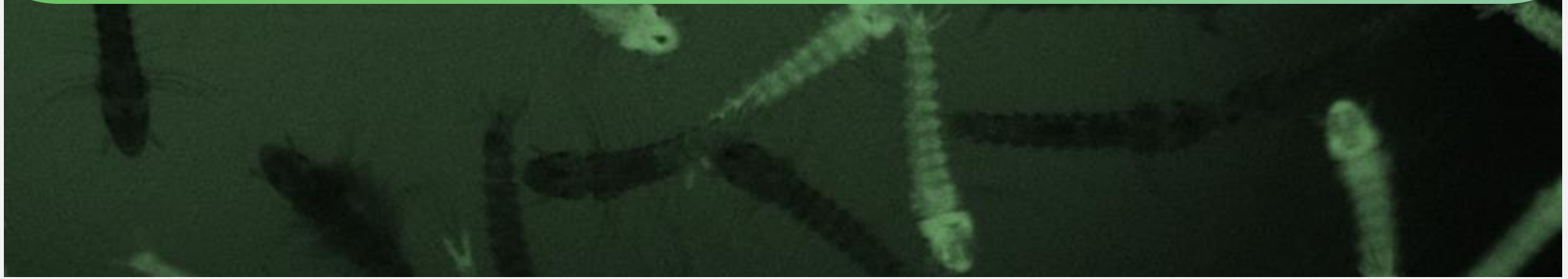
30% male recovery
≤1% female contamination
1800 pupae /min

Up to 70% male recovery
≤1% female contamination
? pupae /min

Bellini et al. 2013, Zheng et al. 2019

OBJECTIVE:

Improve the cost-efficiency ratio of *Aedes* sex separation



OBJECTIVE

Comparison of the sexing methods developed during the last 15 years

Trends in Parasitology

Volume 35, Issue 8, August 2019, Pages 649-662

Review

Sex Sorting for Pest Control: It's Raining Men!

Célia Lutrat [1, 2, 3], David Giesbrecht [4], Eric Marois [5], Steve Whyard [4], Thierry Baldet [1, 2], Jérémy Bouyer [1, 6]

OBJECTIVE

7 criteria for a good sexing method

Sorting stage

OBJECTIVE

7 criteria for a good sexing method

Sorting stage

Male recovery

OBJECTIVE

7 criteria for a good sexing method

Sorting stage

Male recovery

Female contamination

OBJECTIVE

7 criteria for a good sexing method

Sorting stage

Male recovery

Female contamination

Sorting speed

OBJECTIVE

7 criteria for a good sexing method

Sorting stage

Male recovery

Female contamination

Sorting speed

Initial investment

OBJECTIVE

7 criteria for a good sexing method

Sorting stage

Male recovery

Female contamination

Sorting speed

Initial investment

Consumable cost

OBJECTIVE

7 criteria for a good sexing method

Sorting stage

Male recovery

Female contamination

Sorting speed

Initial investment

Consumable cost

Acceptability

OBJECTIVE

3 strategies identified

Conditional female death / conditional masculinization

OBJECTIVE

3 strategies identified

Conditional female death / conditional masculinization

Converting females into males by RNAi

OBJECTIVE

3 strategies identified

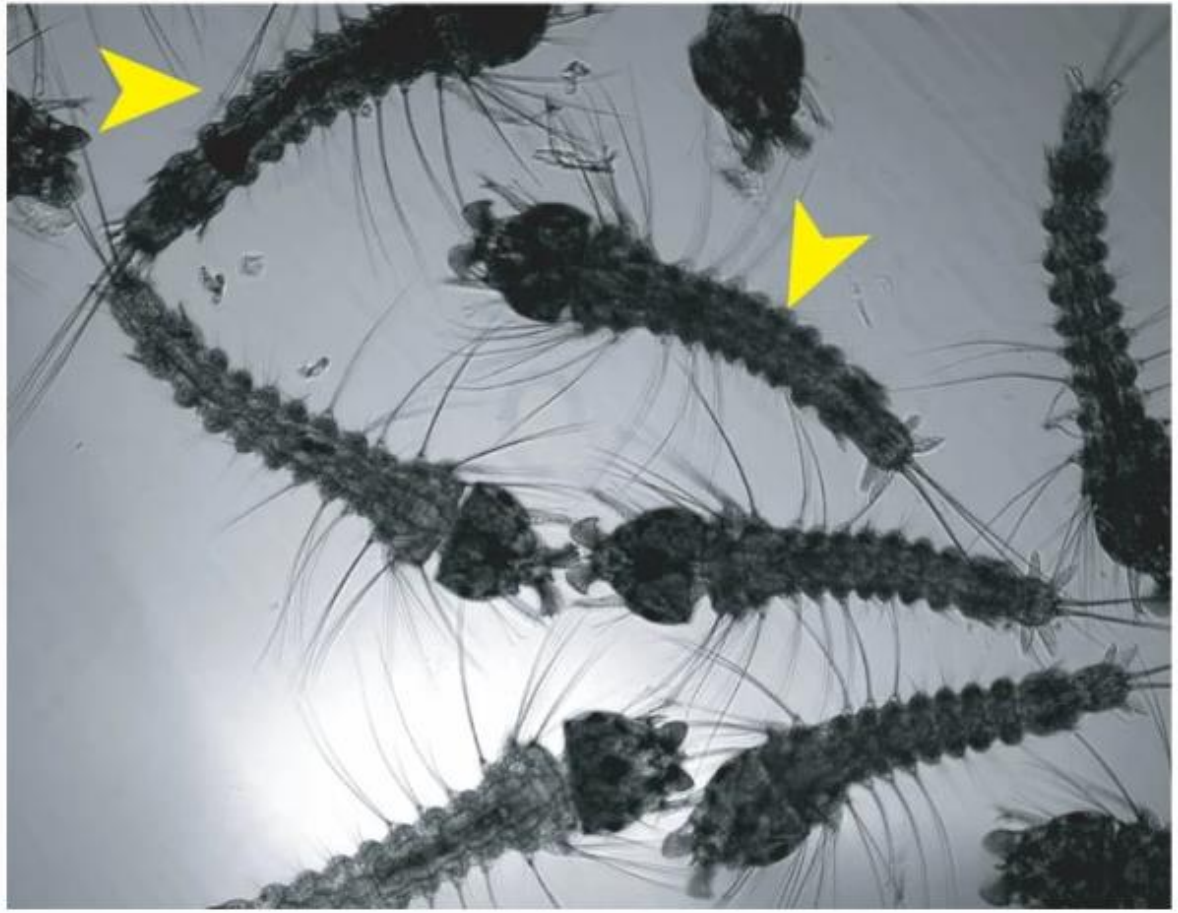
Conditional female death / conditional masculinization

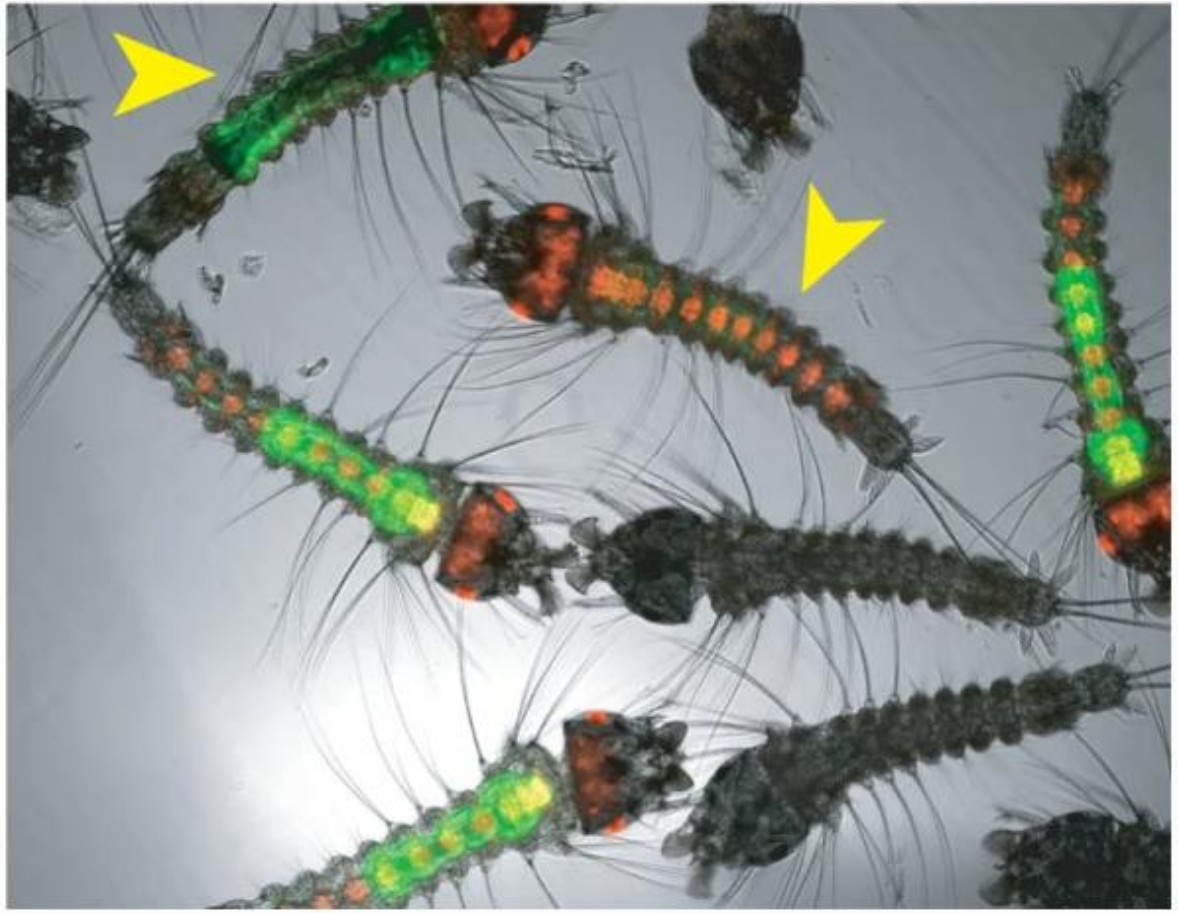
Converting females into males by RNAi

Sex-specific expression of a fluorescence marker

OBJECTIVE

This approach has already been developed in *Anopheles* mosquitoes

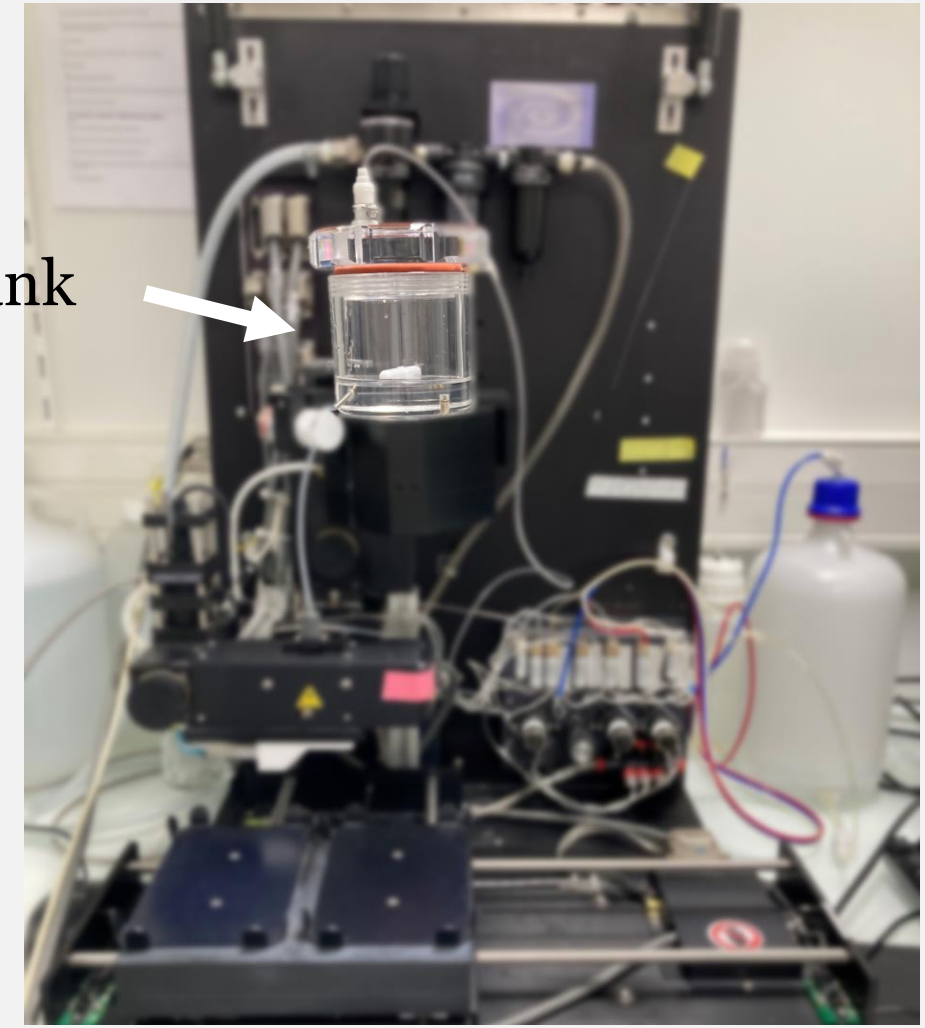




Marois et al. 2012

OBJECTIVE

Neonate larvae can be sorted based on fluorescence using a COPAS device



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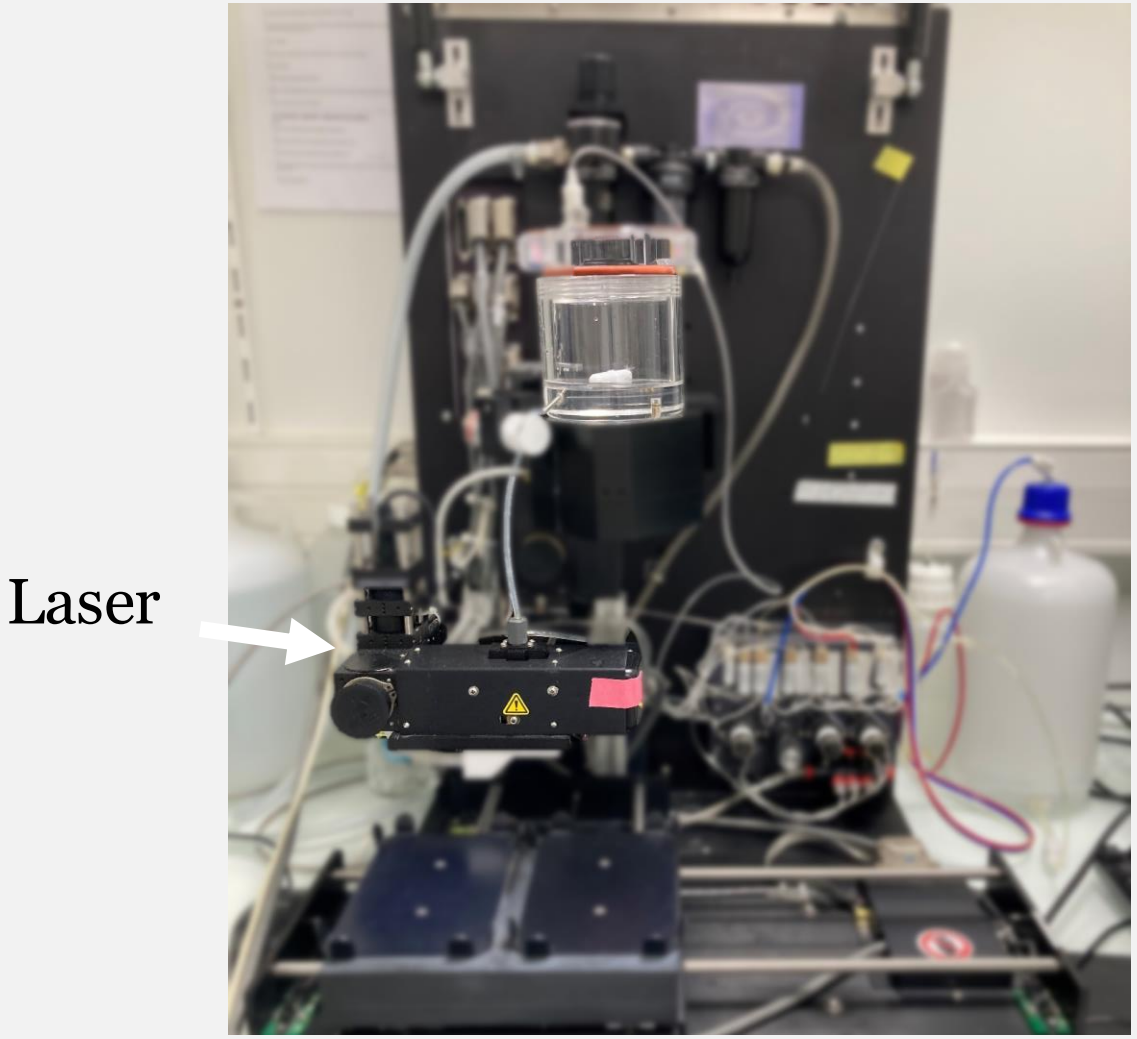

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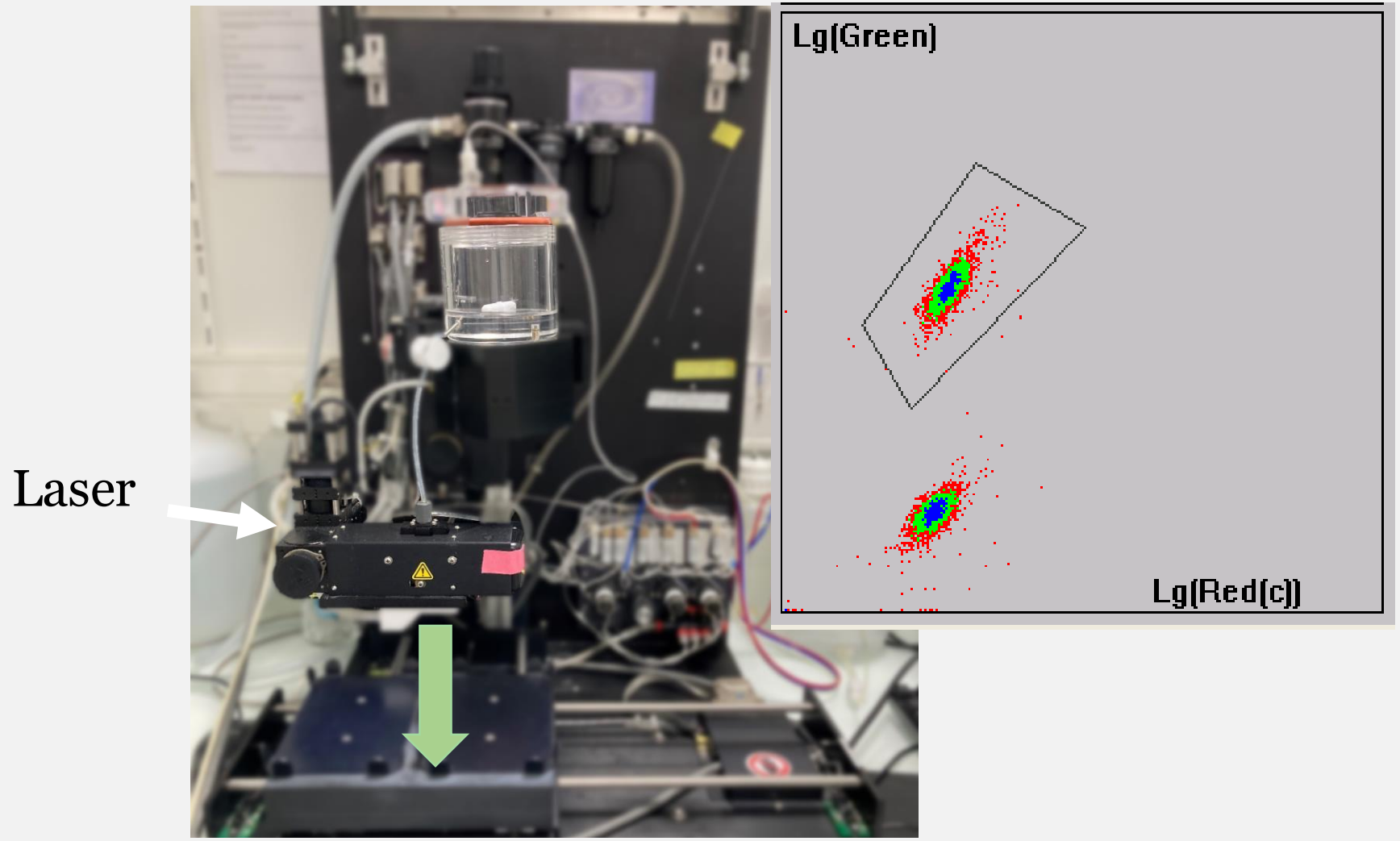
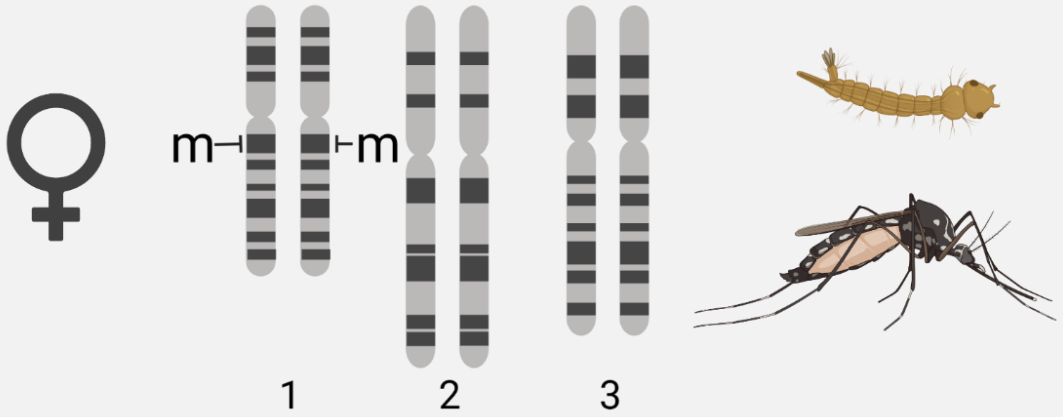
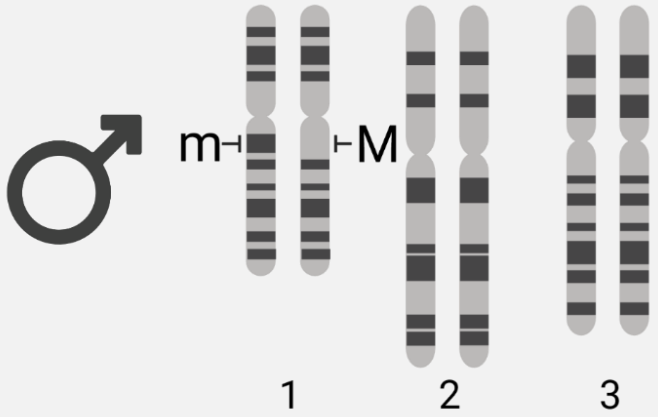
Neonate larvae can be sorted based on fluorescence using a COPAS device



OBJECTIVE

Developing Genetic Sexing Strains carrying a sex-specific fluorescent markers





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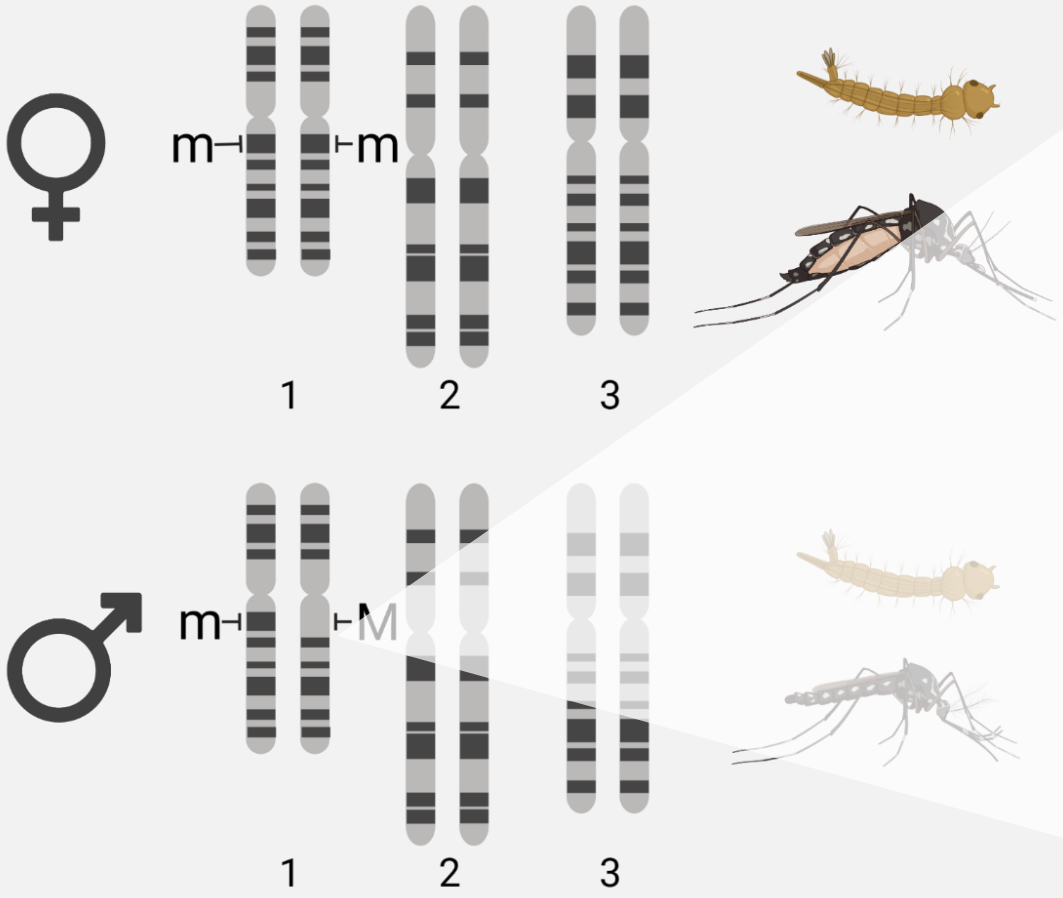

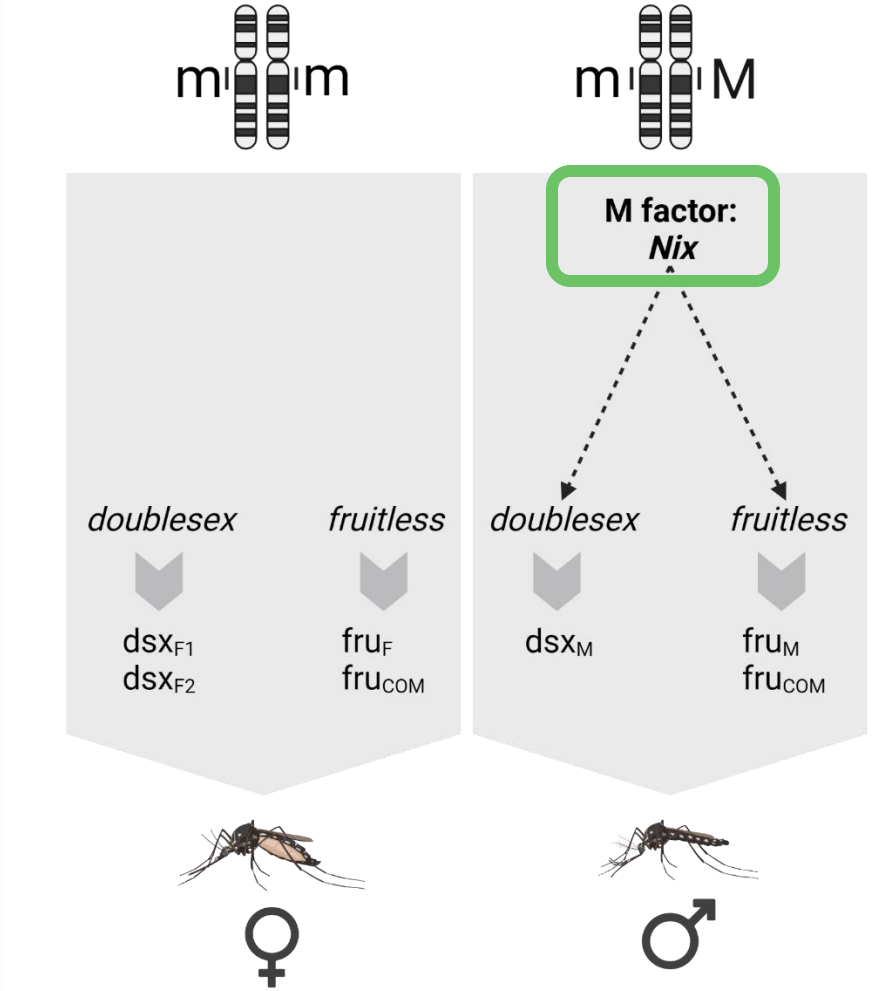

OBJECTIVE

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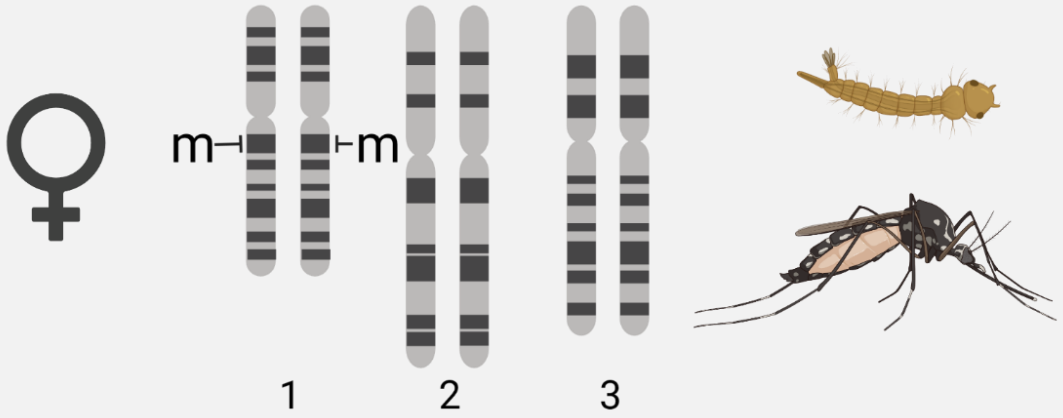
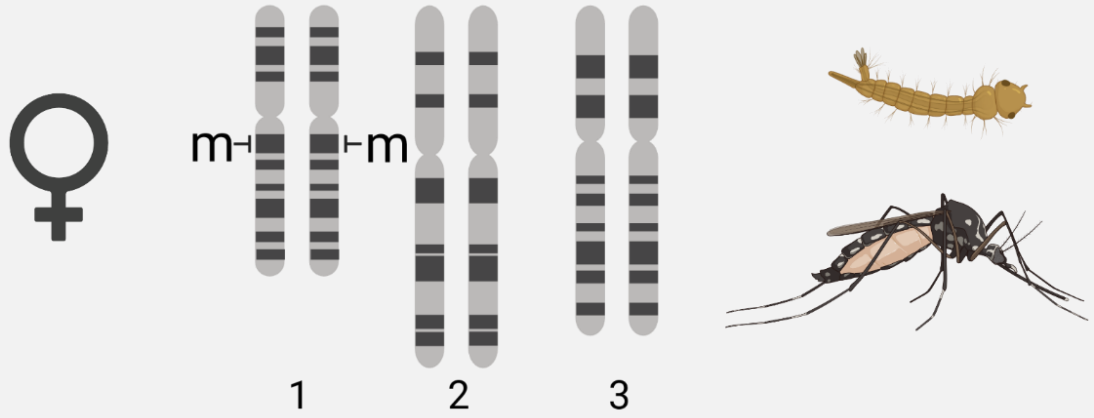
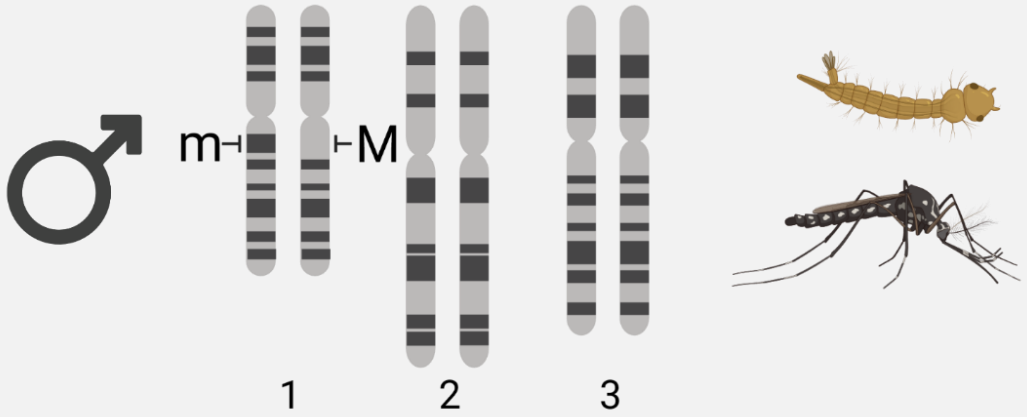
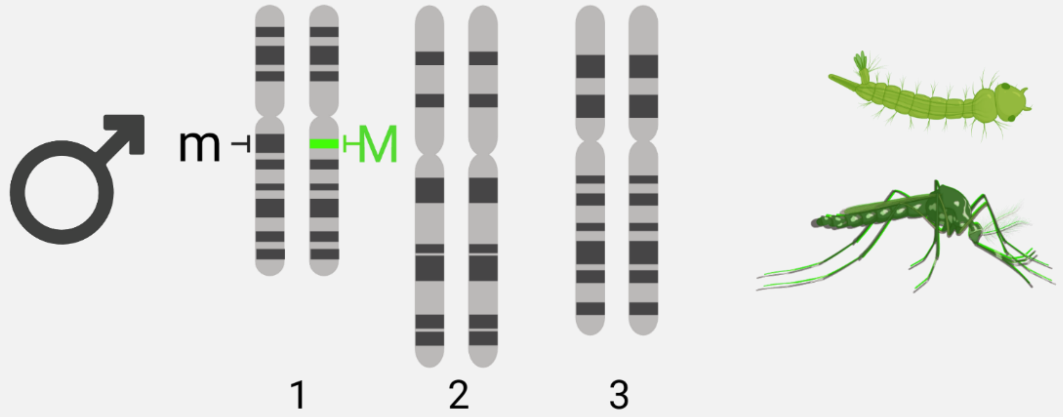


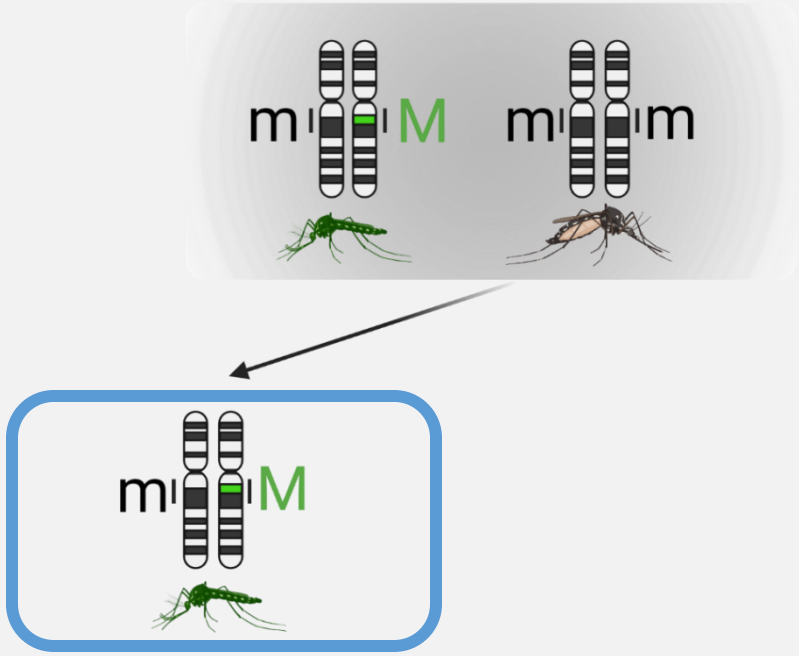






OBJECTIVE

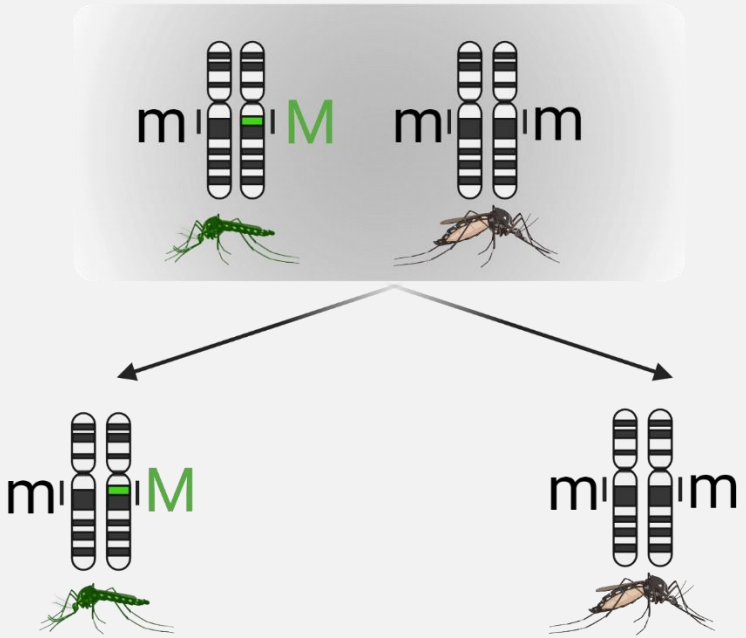
2 GSS allow 2 uses: transgenic or non-transgenic



Transgenic males

OBJECTIVE

2 GSS allow 2 uses: transgenic or non-transgenic



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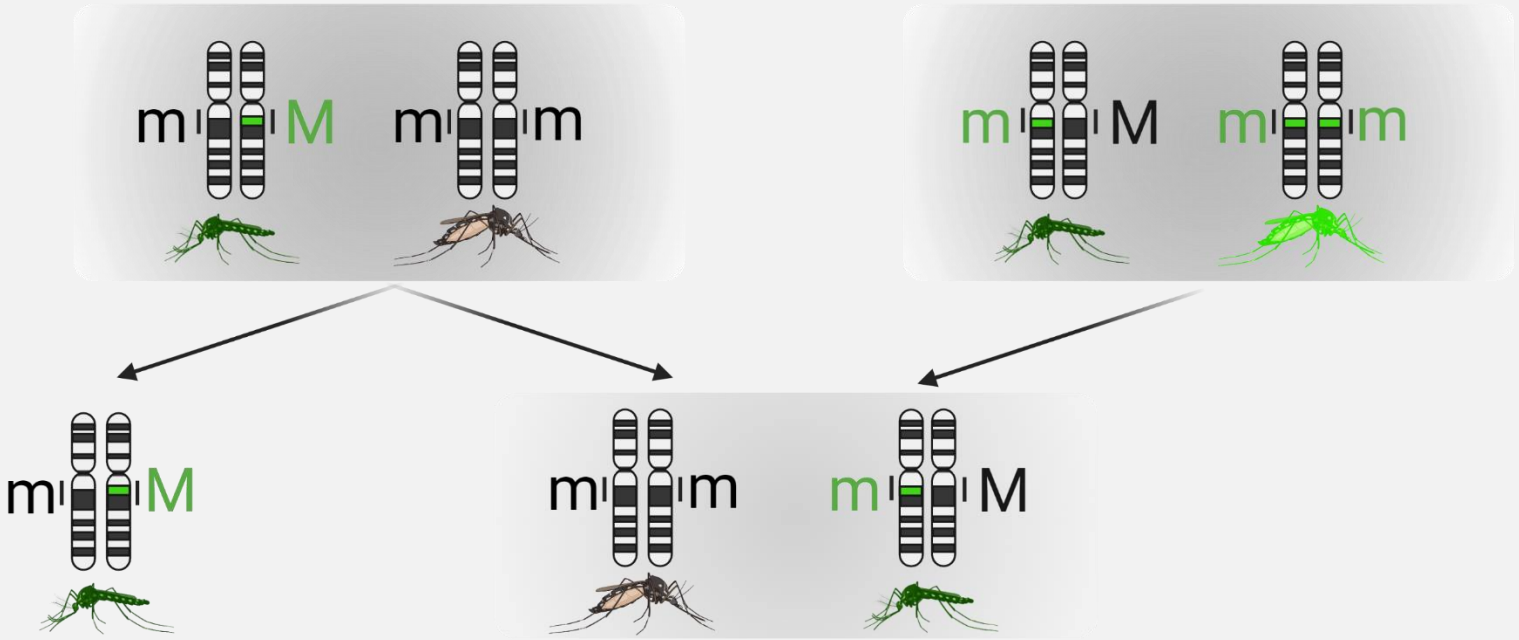

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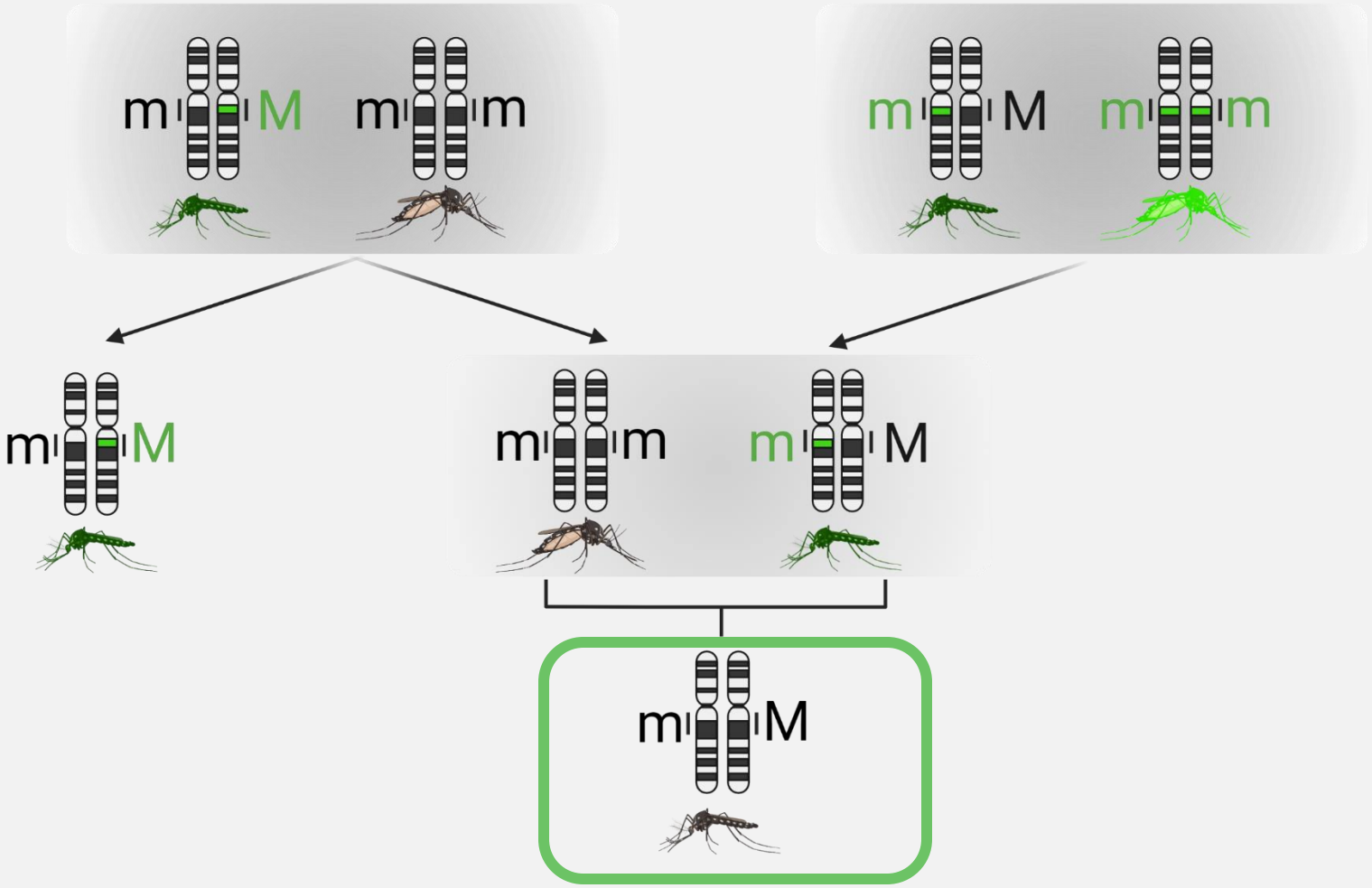

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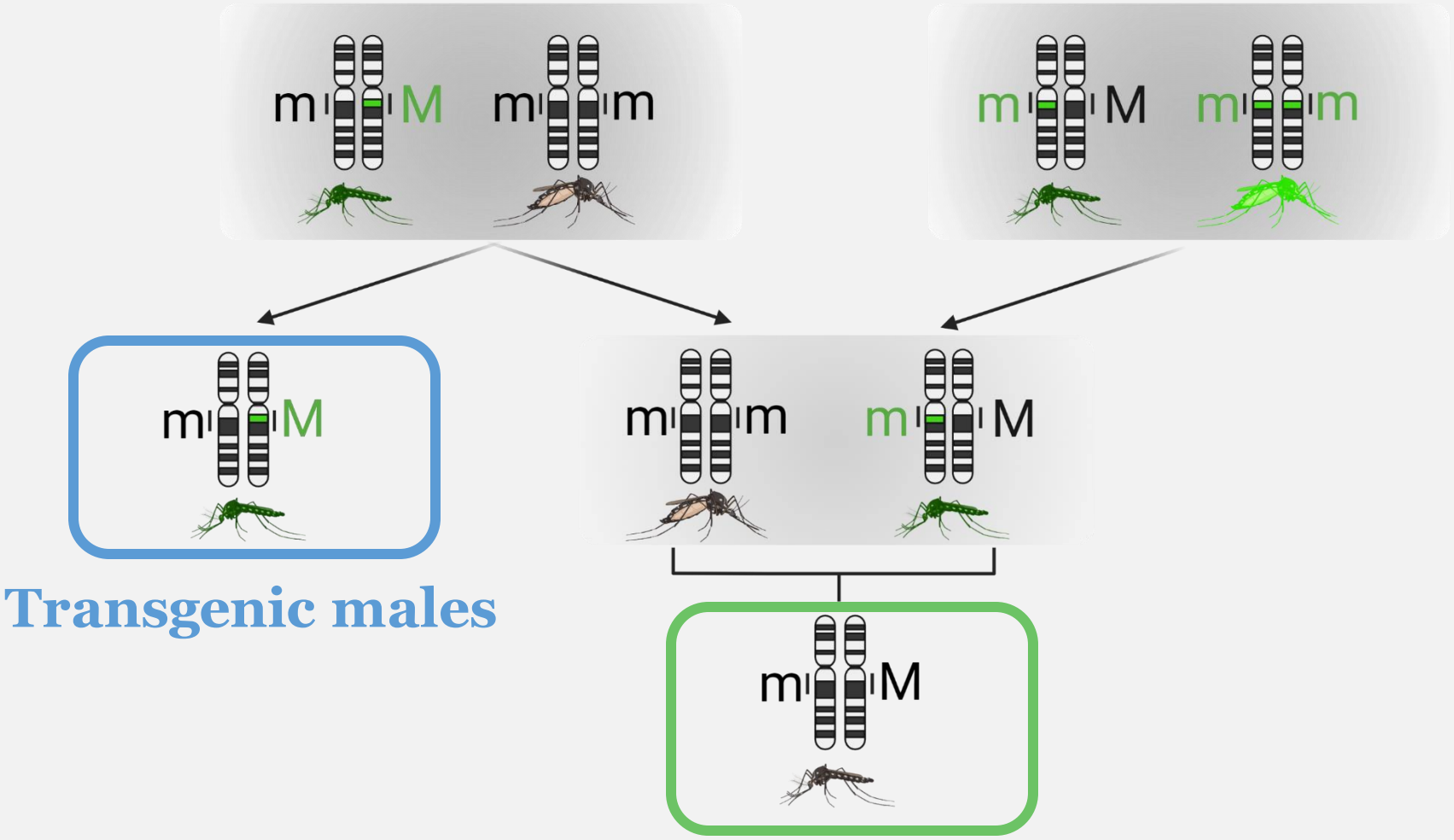
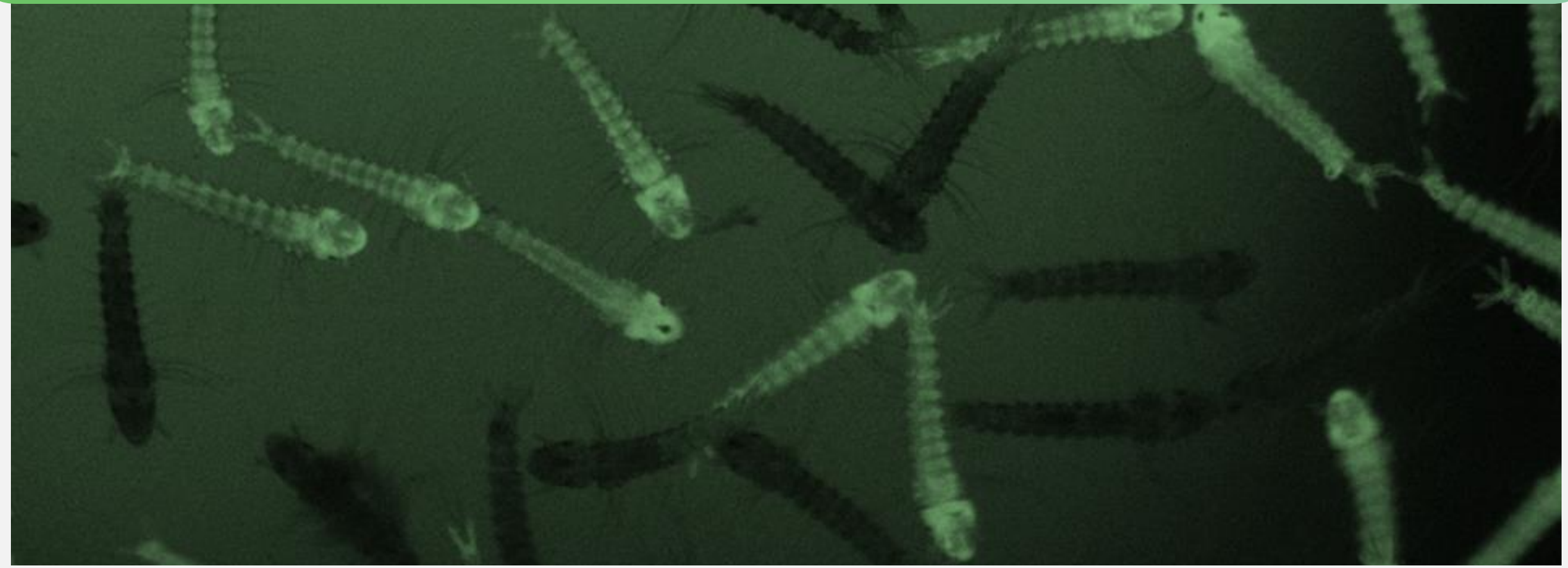
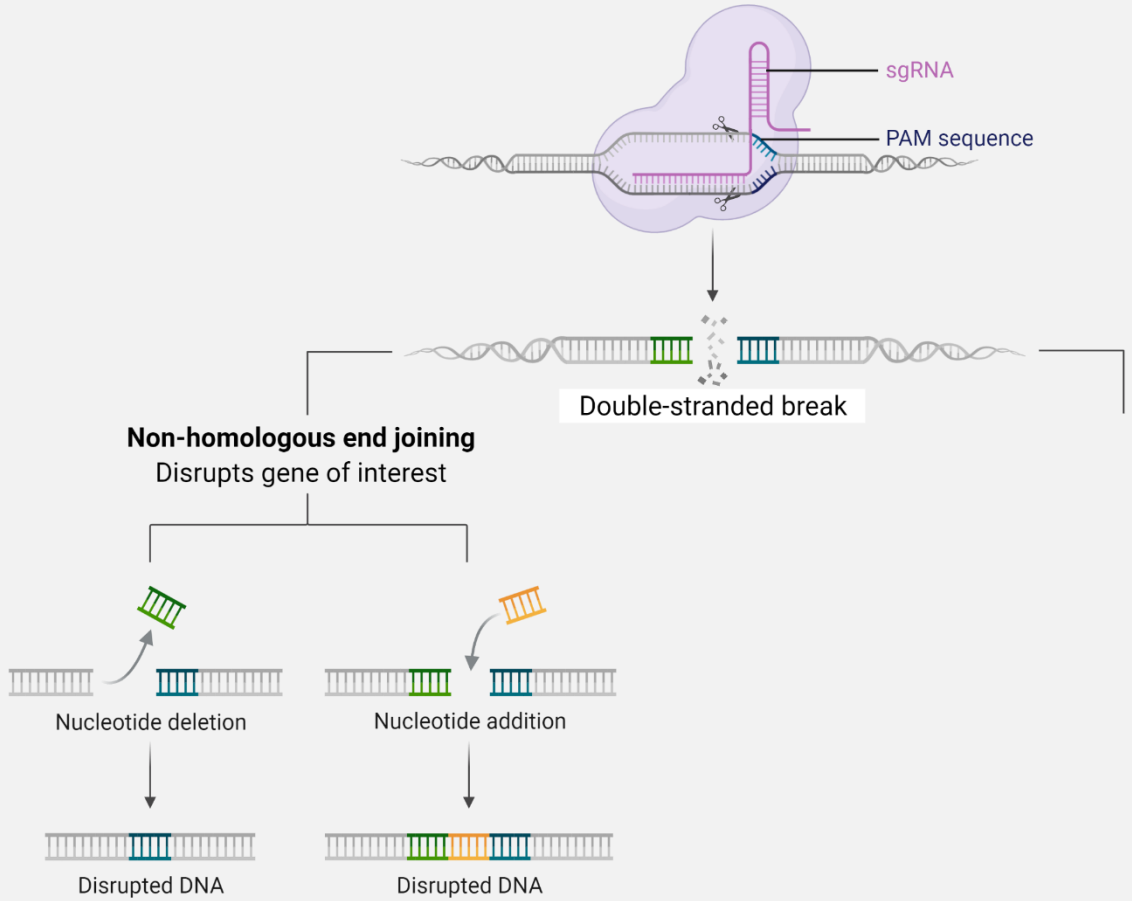


METHODS



METHODS

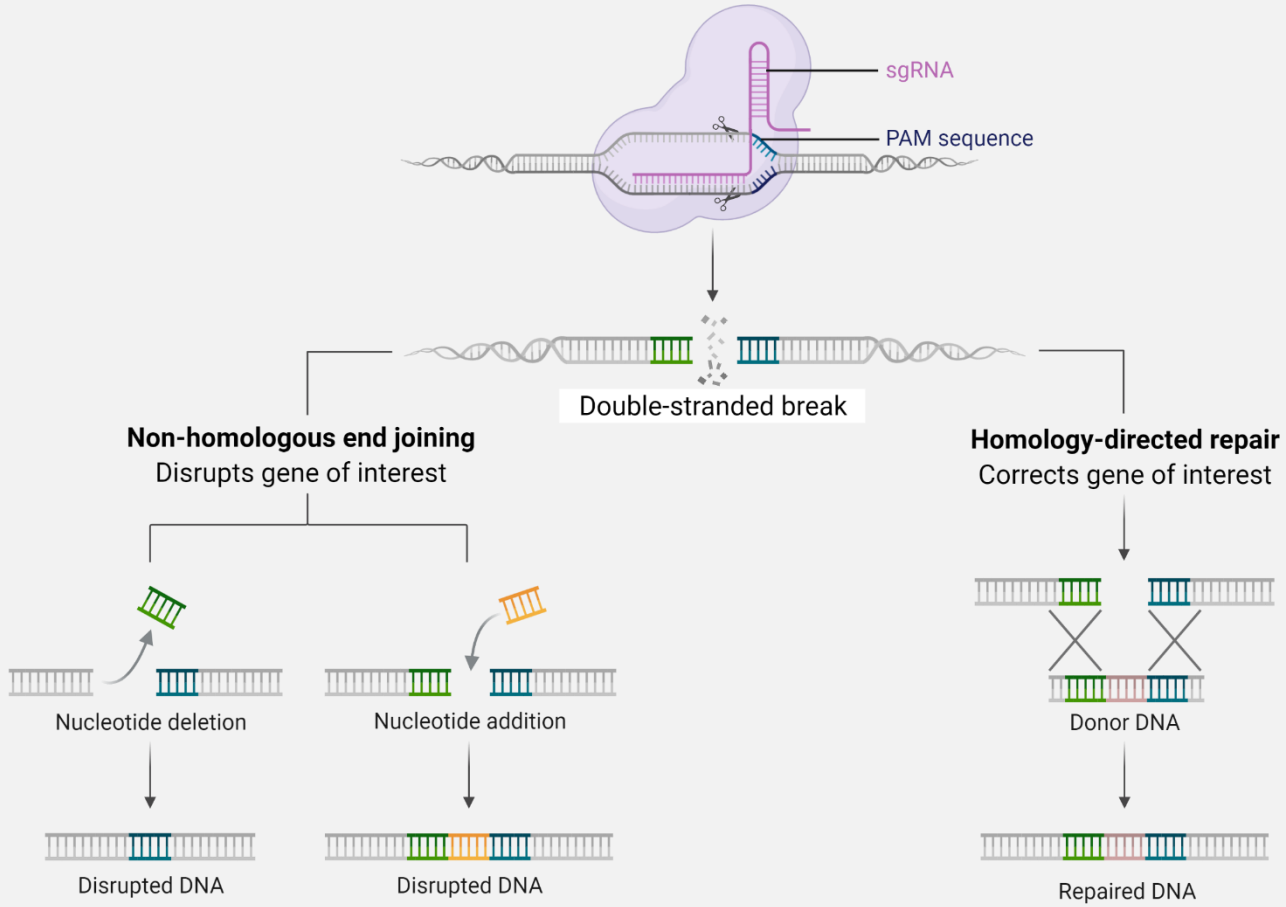
CRISPR-Cas9 allows targeted genome editing



Reviewed in Adli 2018

METHODS

CRISPR-Cas9 allows targeted genome editing



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METHODS

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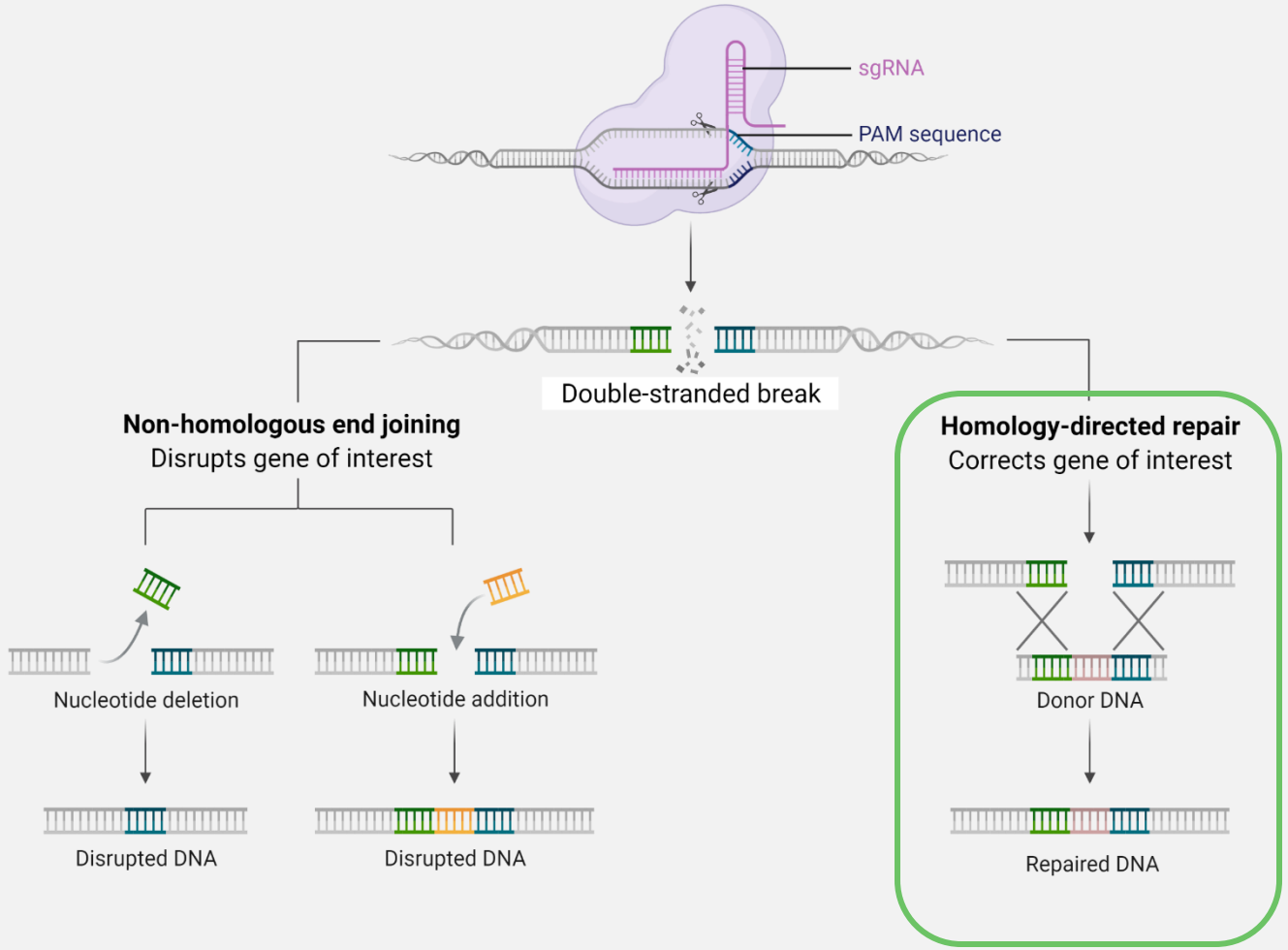
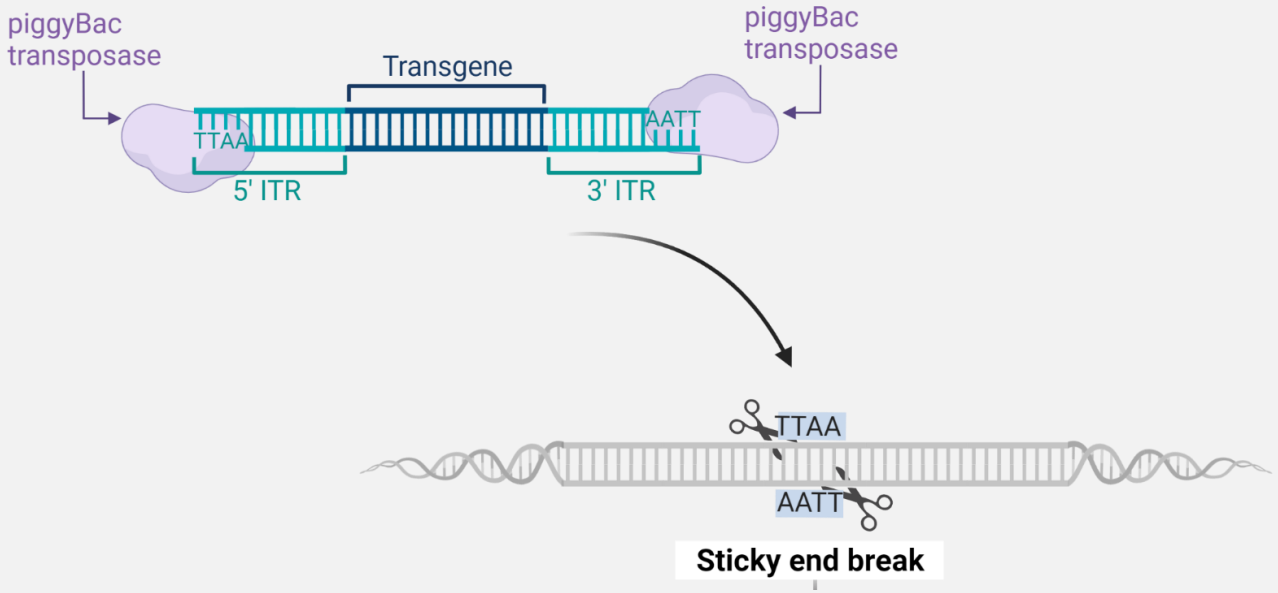


Reviewed in Adli 2018

METHODS

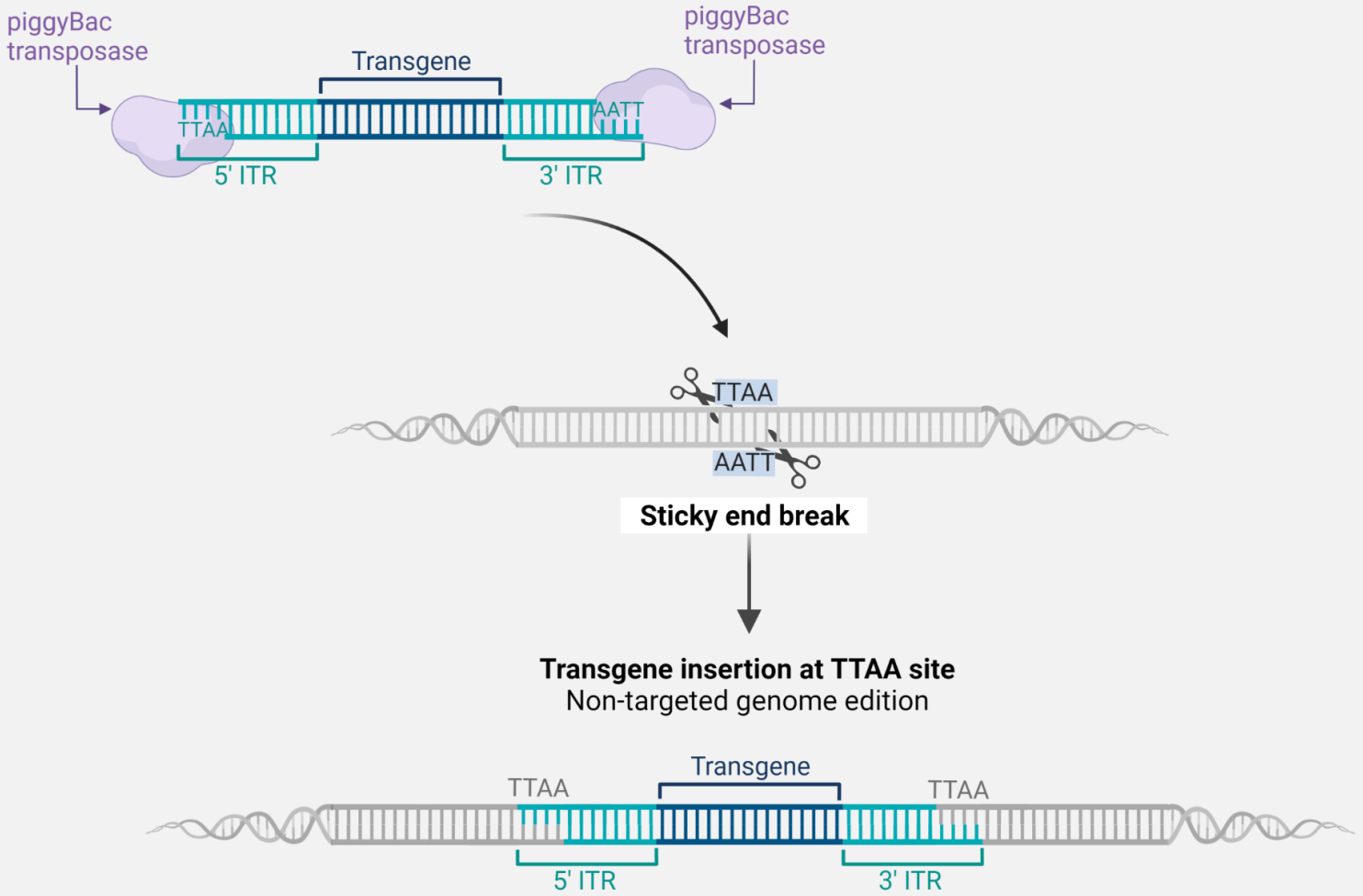
piggyBac allows non-targeted genome editing



Fraser et al. 1995; Fraser et al. 1996

METHODS

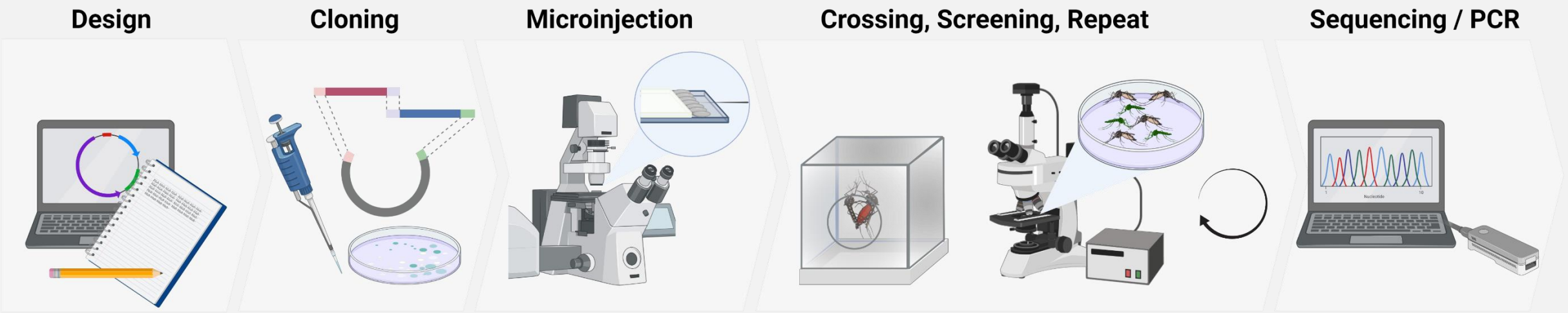
piggyBac allows non-targeted genome editing



Fraser et al. 1995; Fraser et al. 1996

METHODS

Development of transgenic lines



METHODS

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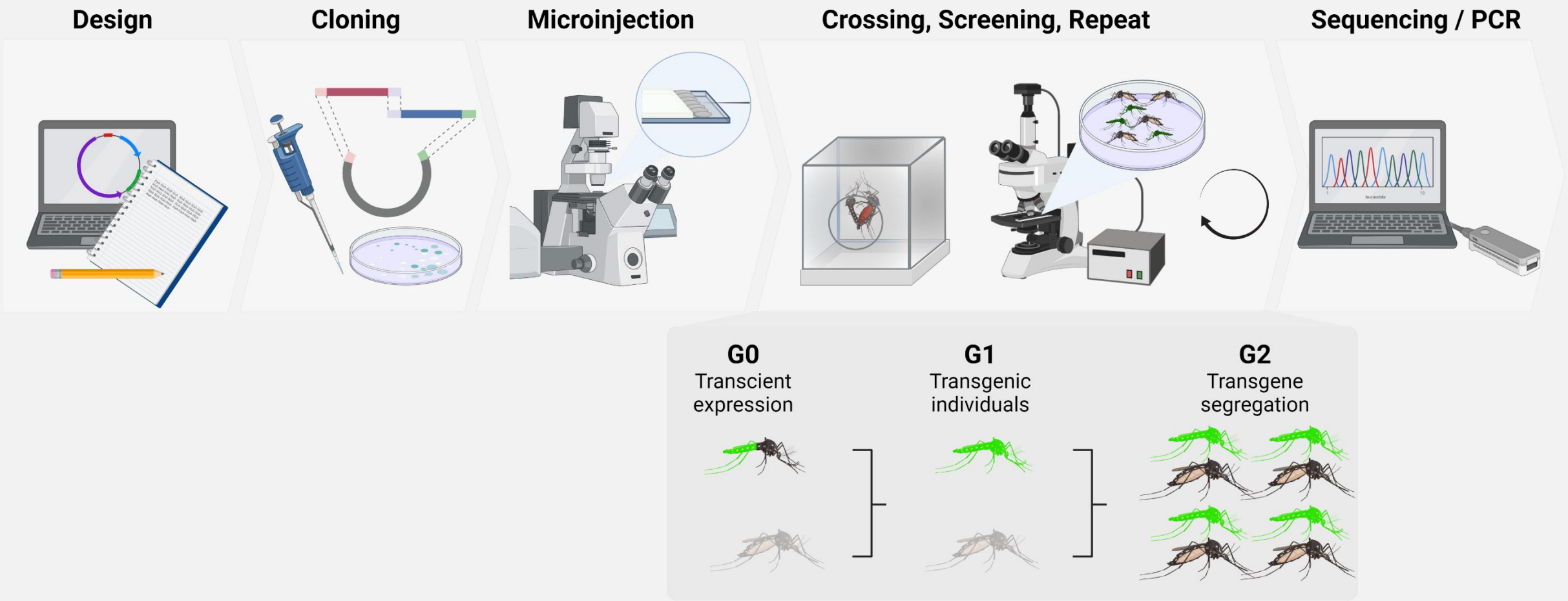

METHODS

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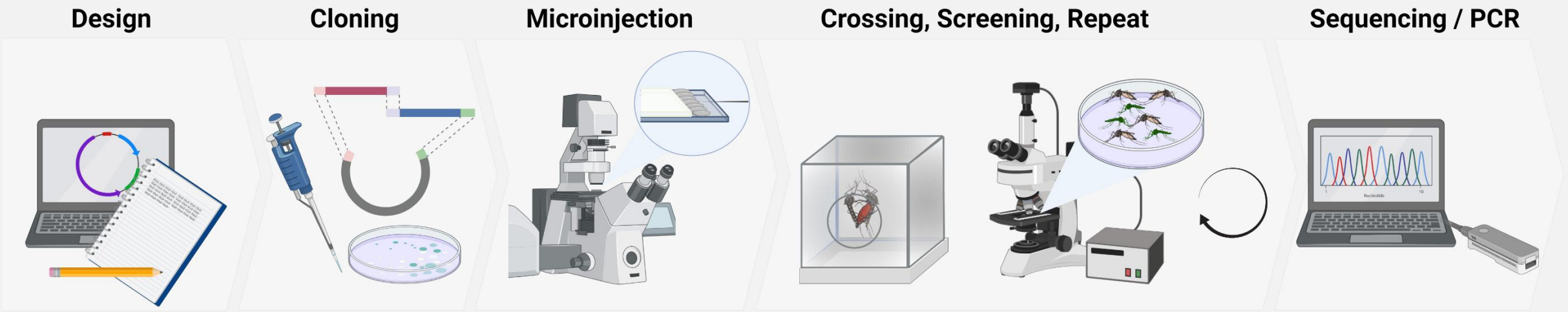
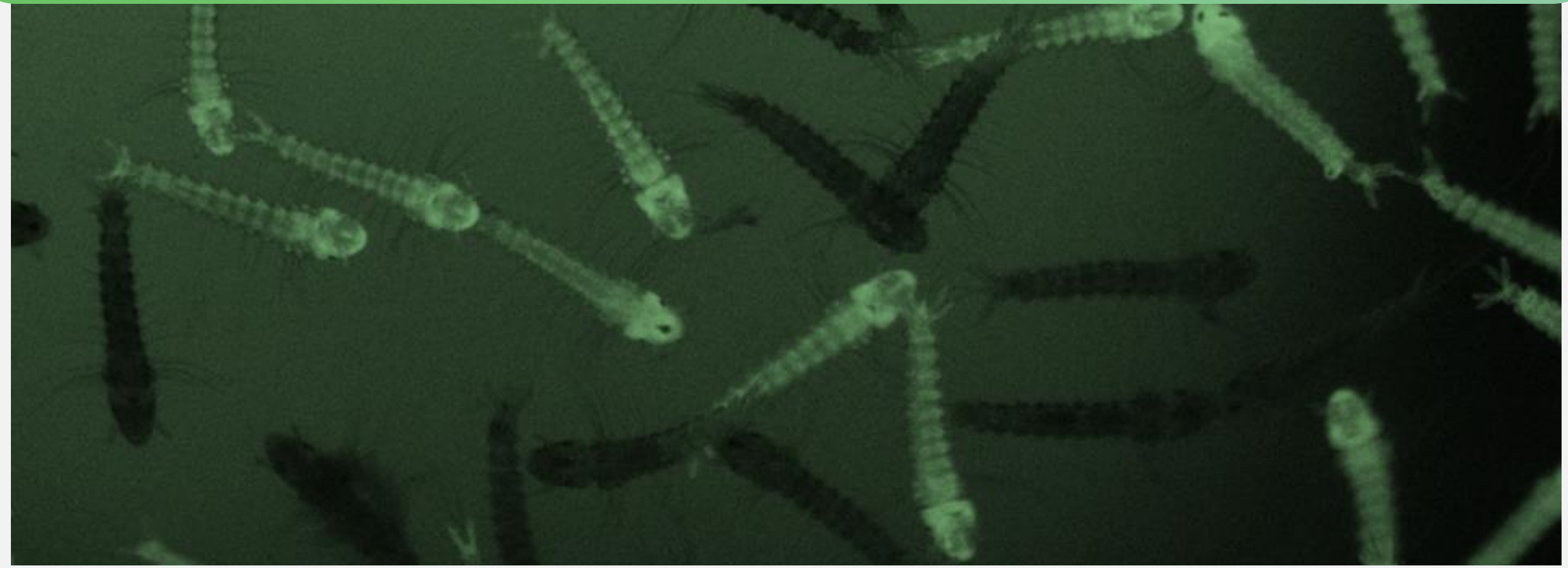


RESULTS

Developing GSS in *Aedes aegypti*

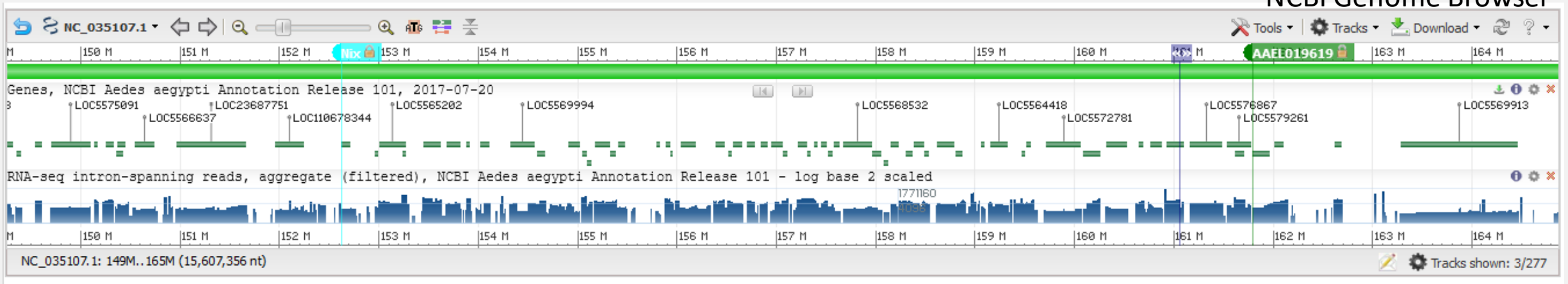


RESULTS – *AEDES AEGYPTI*

Target identification

Chromosome 1

NCBI Genome Browser



Matthews et al. 2018

RESULTS – *AEDES AEGYPTI*

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Chromosome 1

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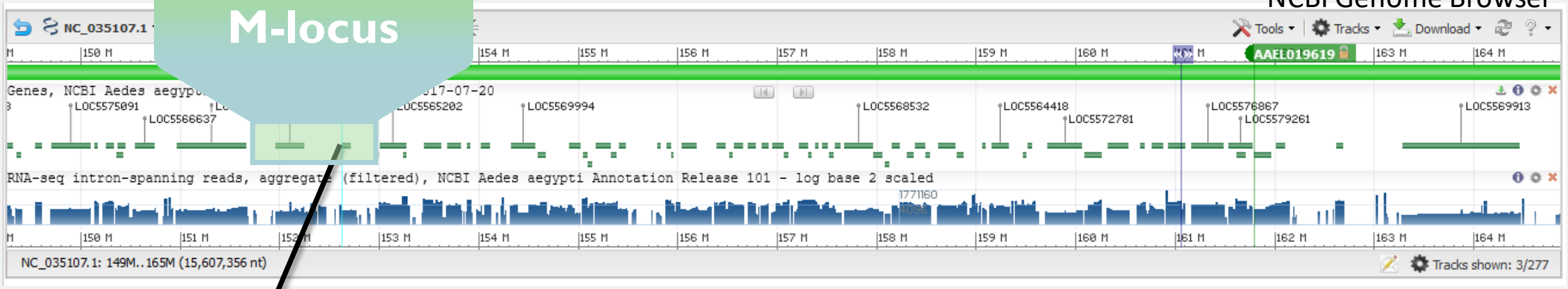

M-locus:
Nix

Hall et al. 2014, Hall et al. 2015, Matthews et al. 2018

RESULTS – *AEDES AEGYPTI*

Target identification

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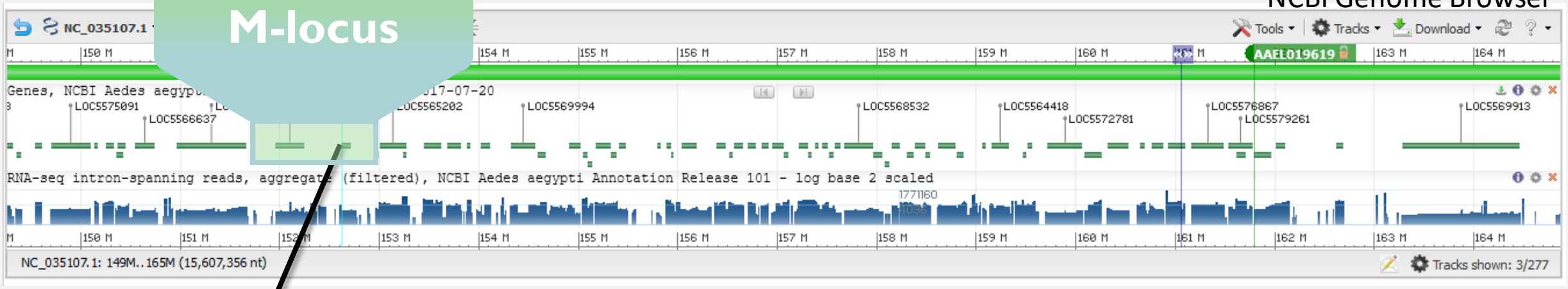
NCBI Genome Browser



M-locus:
Nix

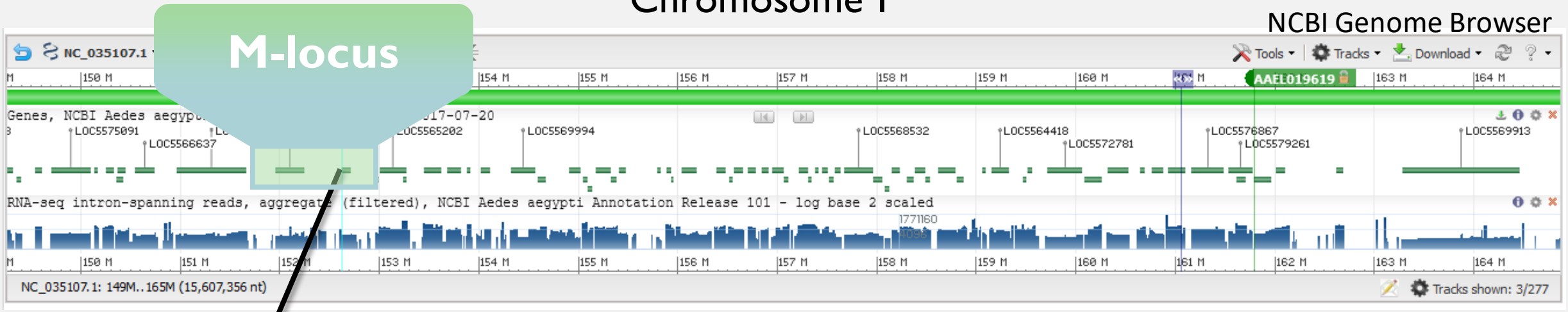
- in the intron

RESULTS – *AEDES AEGYPTI*

Target identification

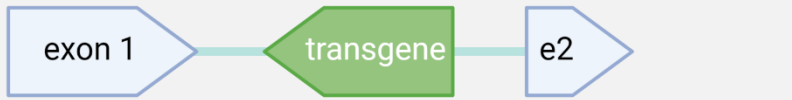
Chromosome 1

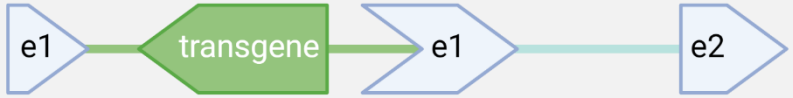
NCBI Genome Browser



M-locus:
Nix

- in the intron
- as an artificial intron





RESULTS – *AEDES AEGYPTI*

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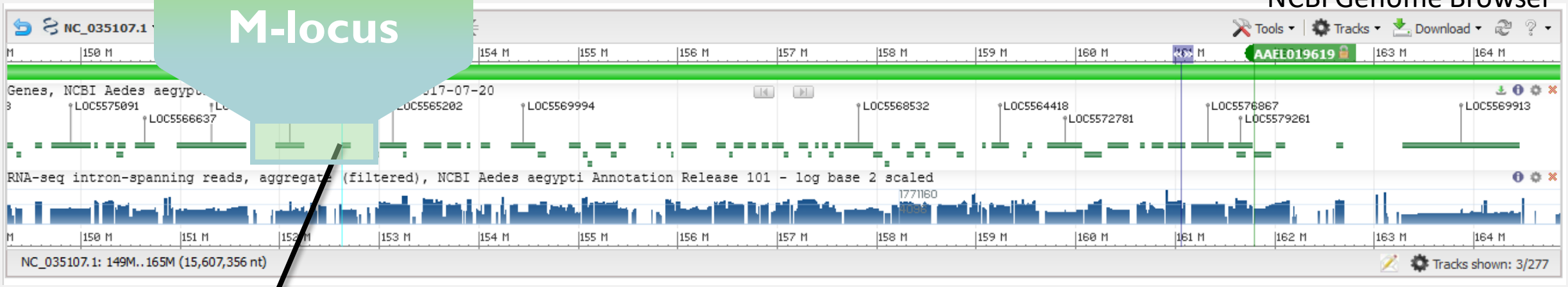
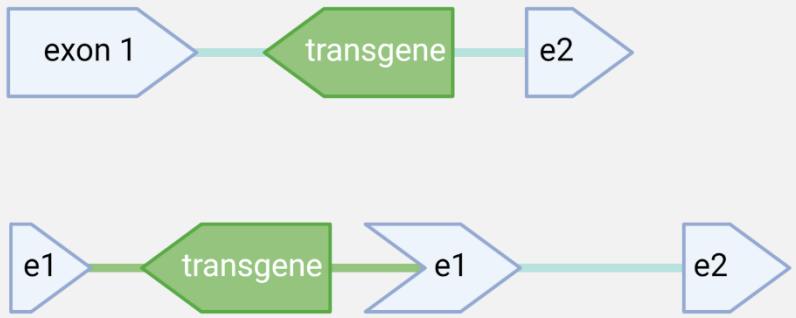


M-locus:
Nix

- in the intron
- as an artificial intron



Masculinization gene

Central to the M-locus

Highest linkage between maleness and fluorescence

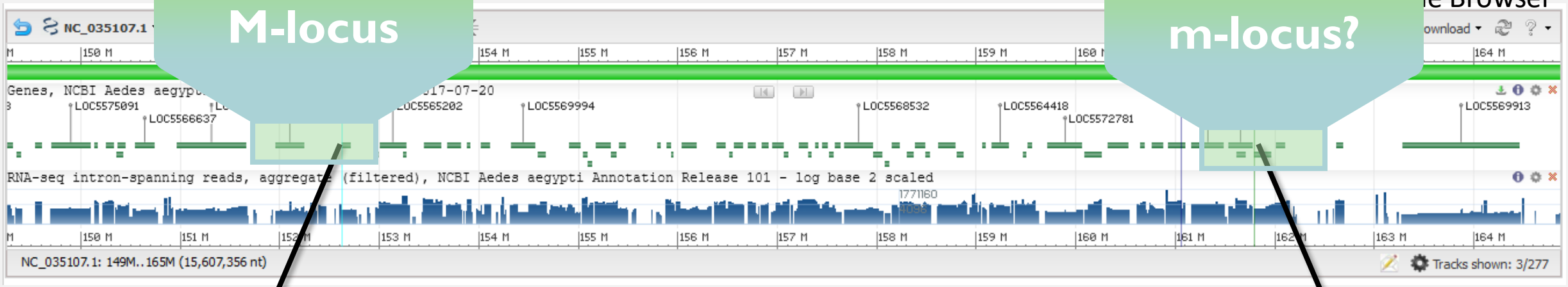
RESULTS – *AEDES AEGYPTI*

Target identification

Chromosome 1

M-locus

m-locus?



M-locus:
Nix
- in the intron
- as an artificial intron

Gene linked to the m-locus (chromosome quotient analysis, A. Fontaine)

Probably misablembled on the M-bearing chromosome 1

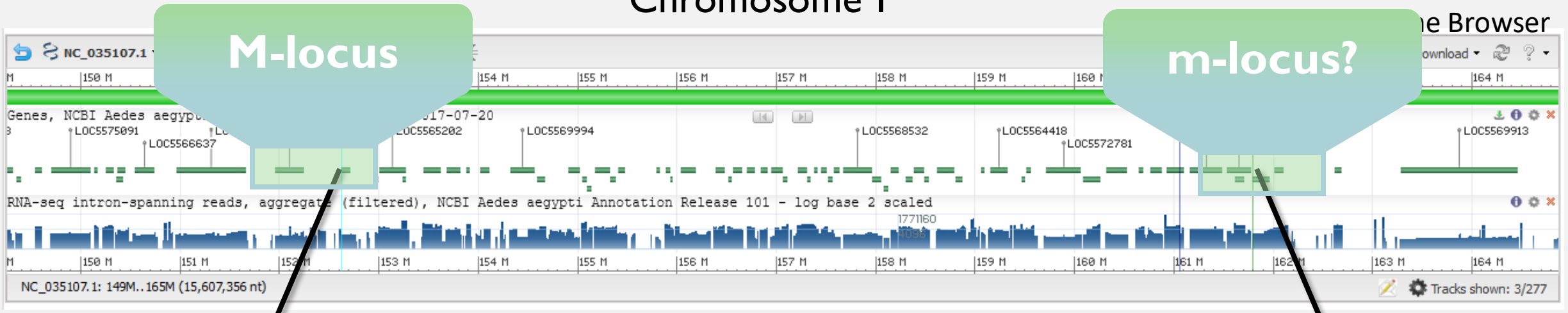
m-locus:
AAEL019619
- in an intron
- as an artificial intron

Fontaine et al. 2017

RESULTS – *AEDES AEGYPTI*

Target identification

Chromosome 1



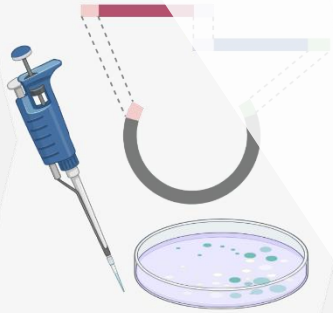
M-locus:
Nix
- in the intron
- as an artificial intron

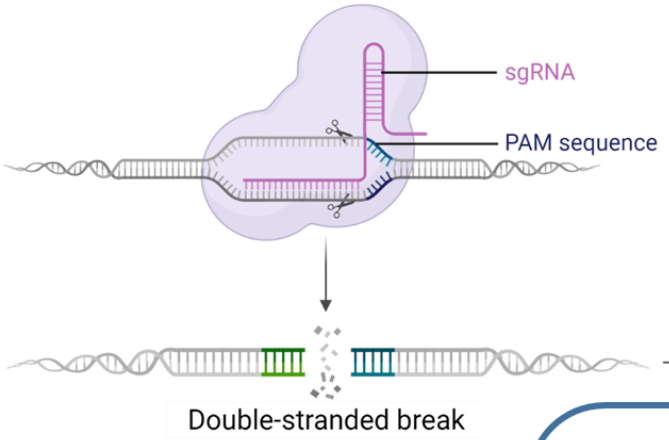
m-locus:
AAEL019619
- in an intron
- as an artificial intron

RESULTS – *AEDES AEGYPTI*

CRISPR-Cas9 approach

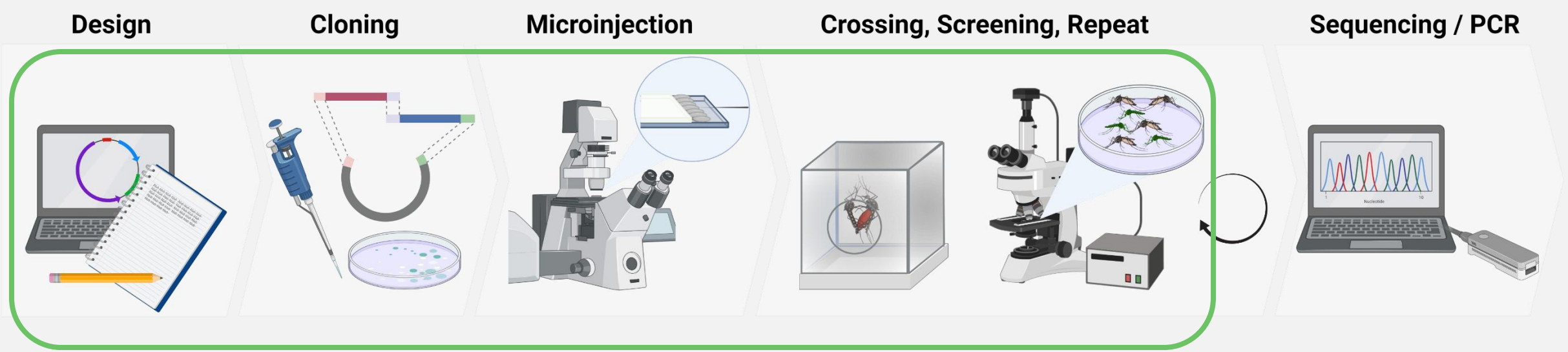
Design **Cloning** **Microinje**





RESULTS – *AEDES AEGYPTI*

CRISPR-Cas9 approach

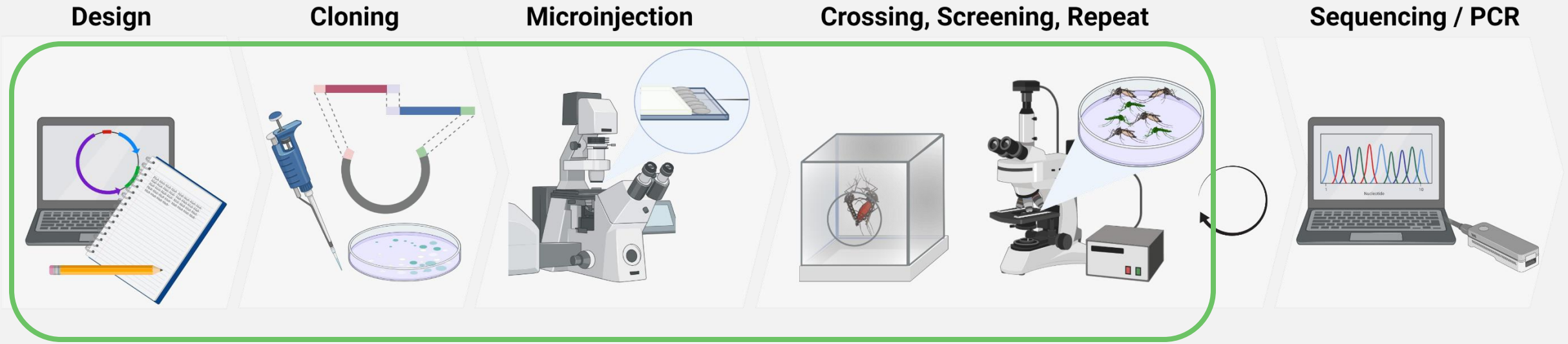


sgRNAs
+ repair plasmids

RESULTS – *AEDES AEGYPTI*

CRISPR-Cas9 approach

200 Cas9-expressing eggs

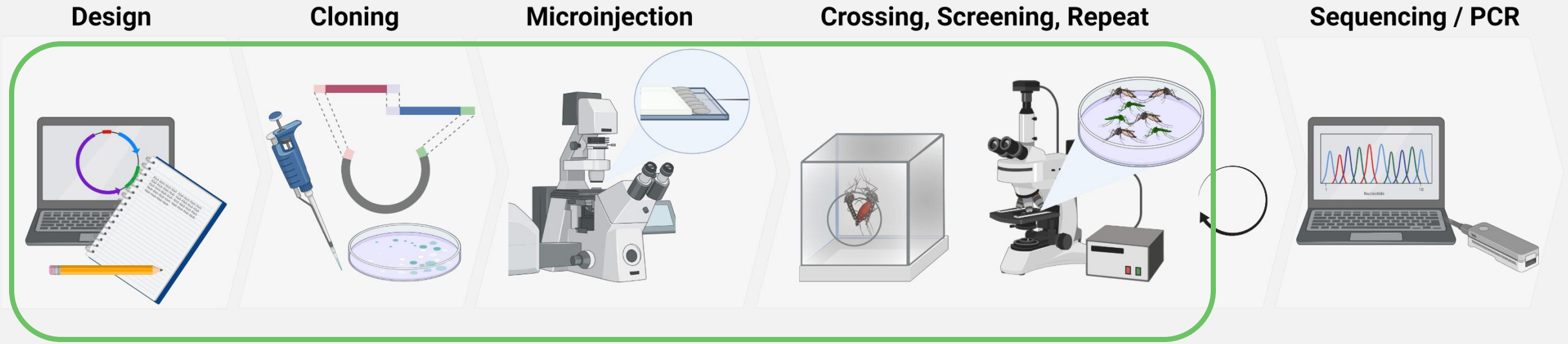


sgRNAs
+ repair plasmids

RESULTS – *AEDES AEGYPTI*

CRISPR-Cas9 approach

200 Cas9-expressing eggs



sgRNAs + repair plasmids

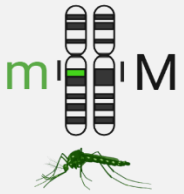
1 transgenic larva in pooled G1!

RESULTS – *AEDES AEGYPTI*

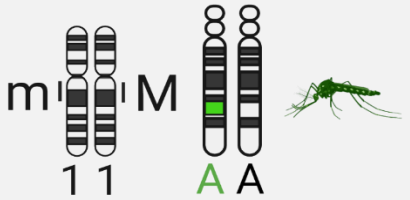
Interpretation

M-linked insertion

m-linked insertion



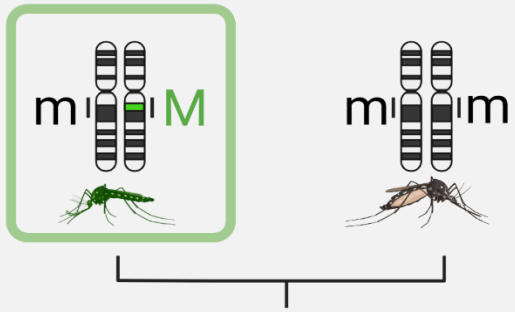
Autosomal insertion



RESULTS – *AEDES AEGYPTI*

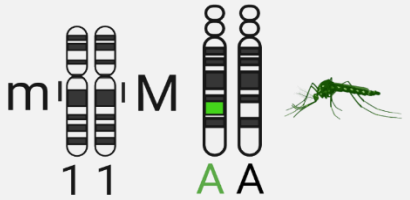
Interpretation

M-linked insertion



m-linked insertion

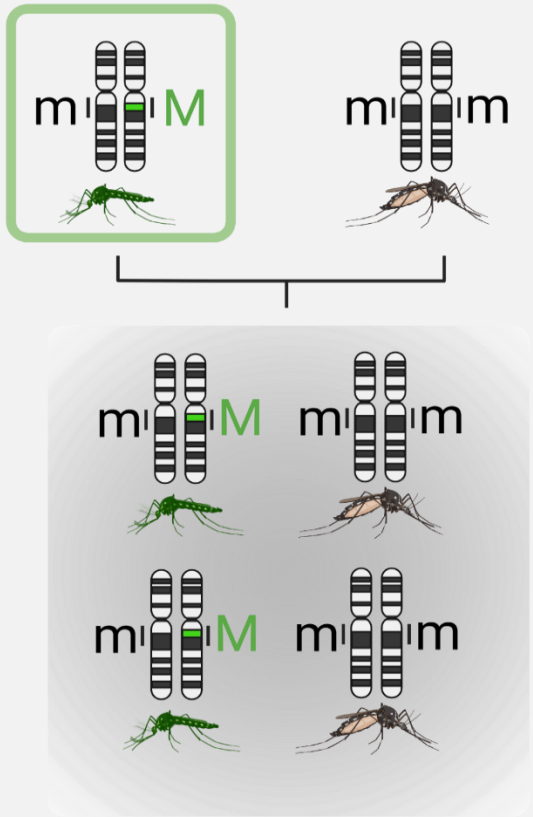
Autosomal insertion



RESULTS – *AEDES AEGYPTI*

Interpretation

M-linked insertion



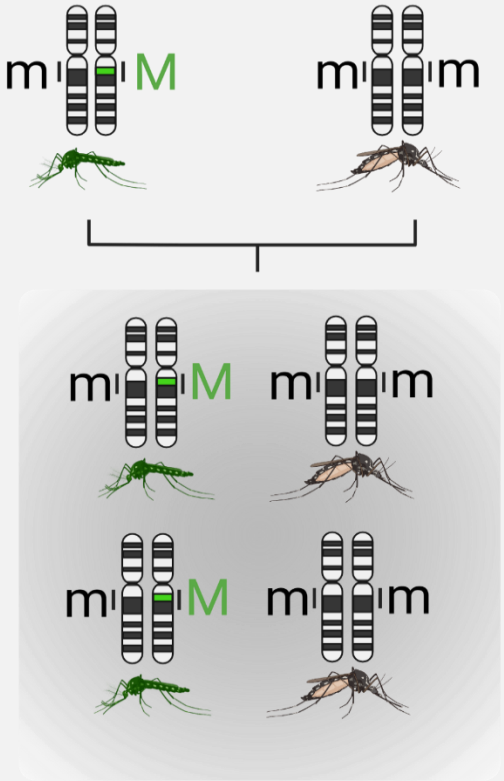
m-linked insertion

Autosomal insertion

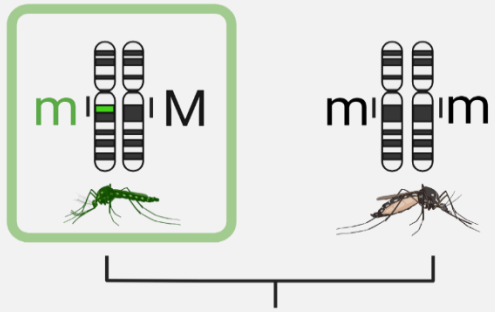
RESULTS – *AEDES AEGYPTI*

Interpretation

M-linked insertion



m-linked insertion

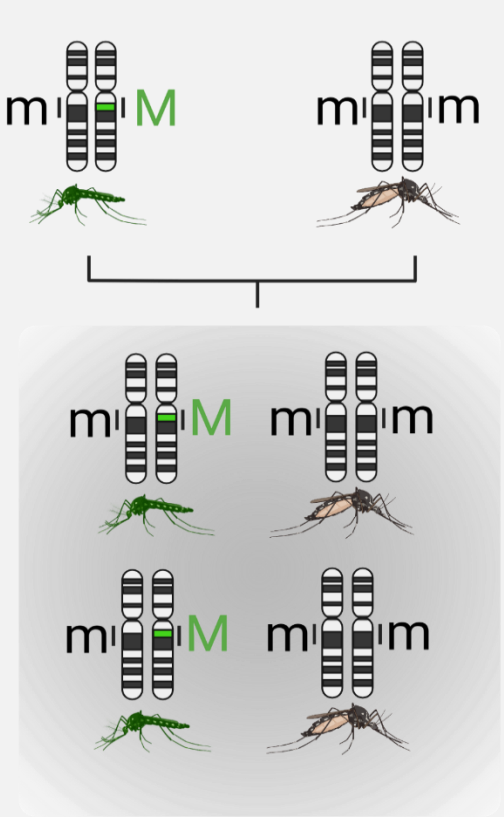


Autosomal insertion

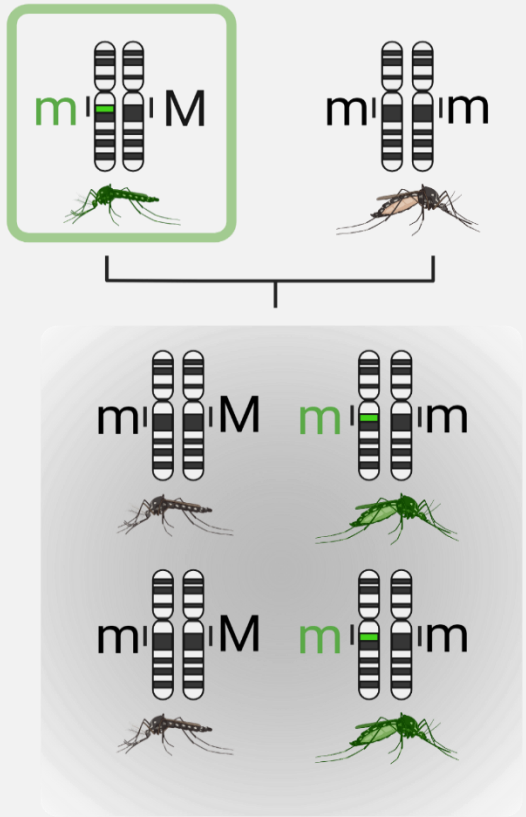
RESULTS – *AEDES AEGYPTI*

Interpretation

M-linked insertion



m-linked insertion

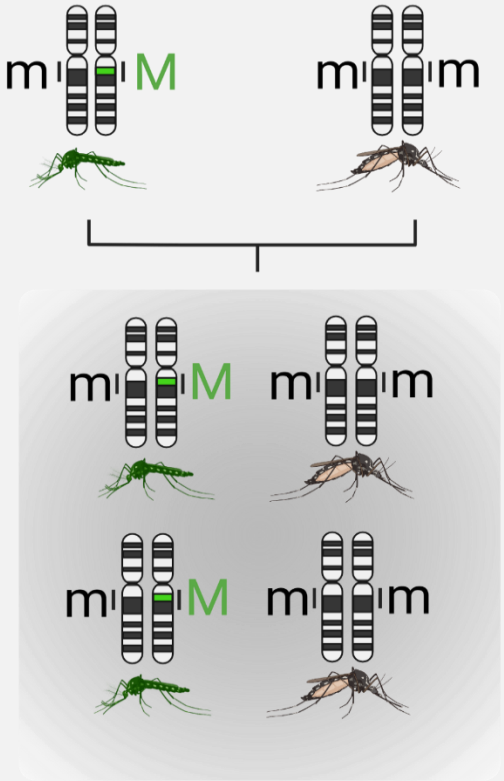


Autosomal insertion

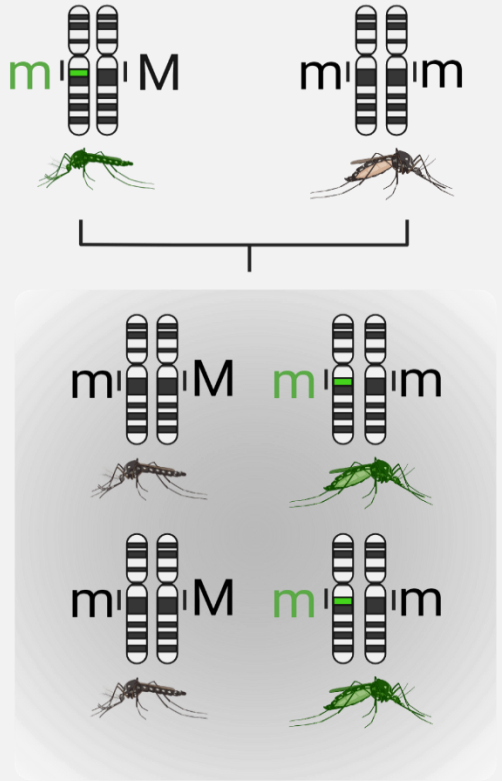
RESULTS – *AEDES AEGYPTI*

Interpretation

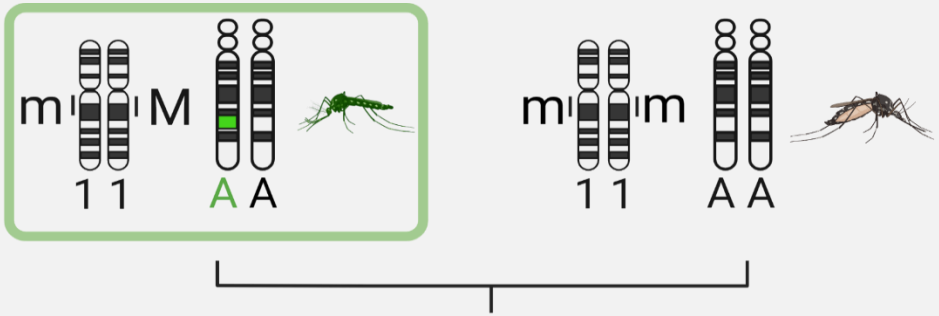
M-linked insertion



m-linked insertion



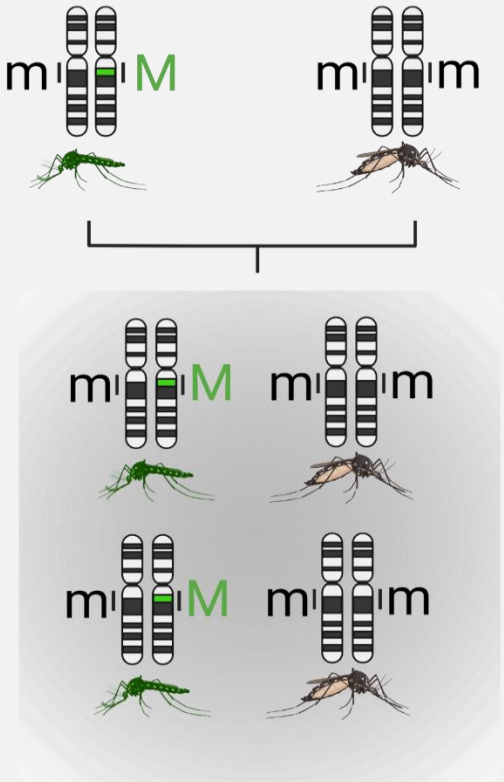
Autosomal insertion



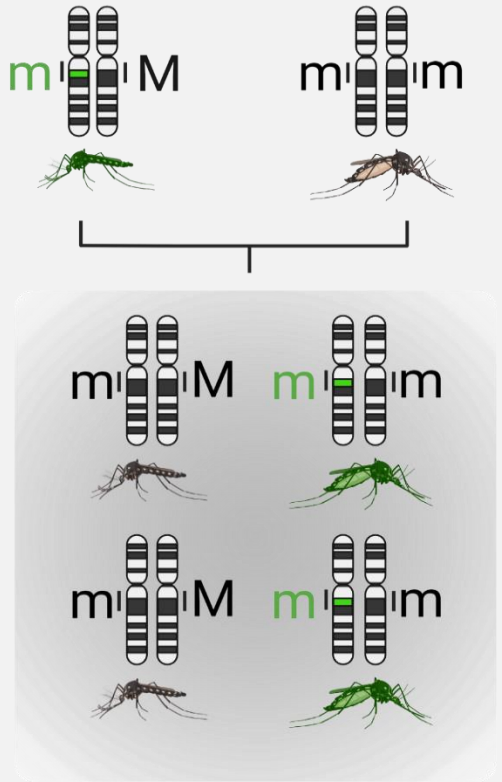
RESULTS – *AEDES AEGYPTI*

Interpretation

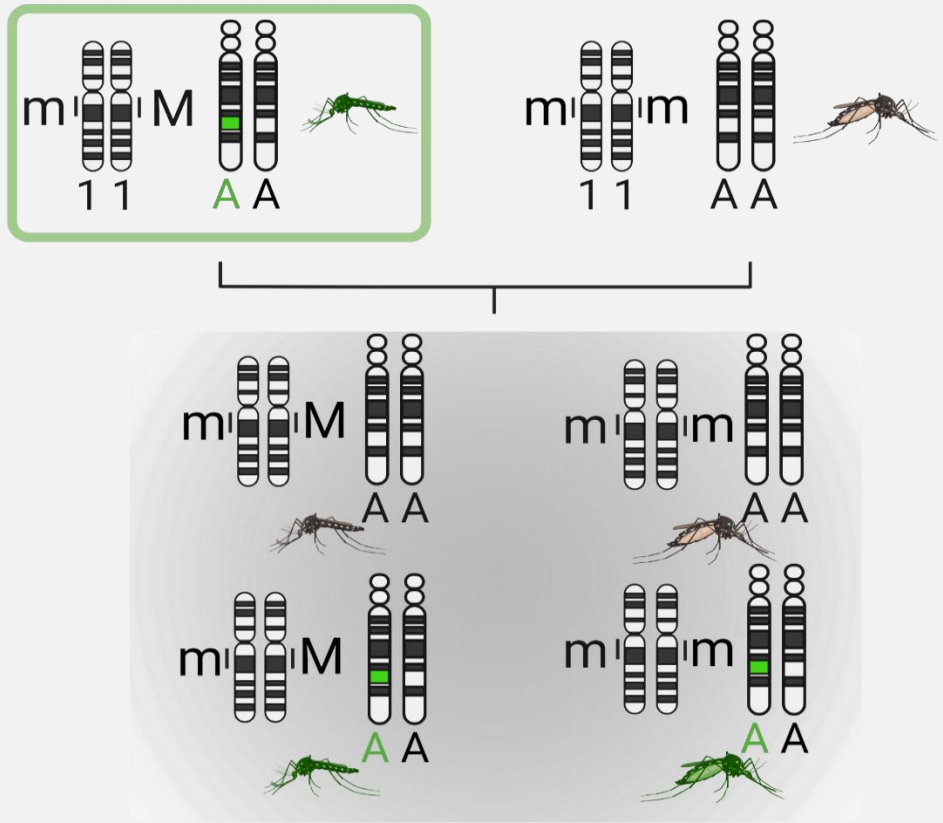
M-linked insertion



m-linked insertion



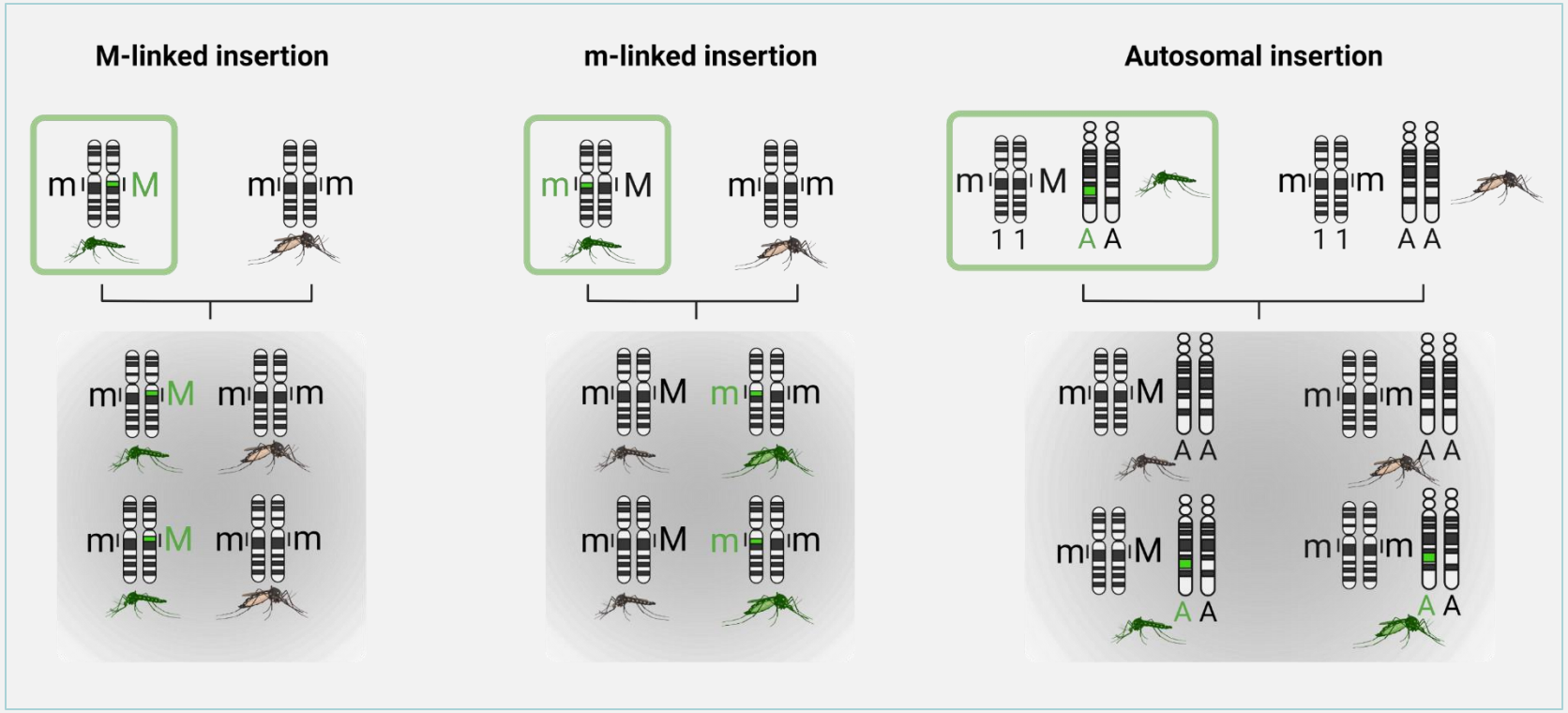
Autosomal insertion



RESULTS – *AEDES AEGYPTI*

Interpretation

Offspring phenotype

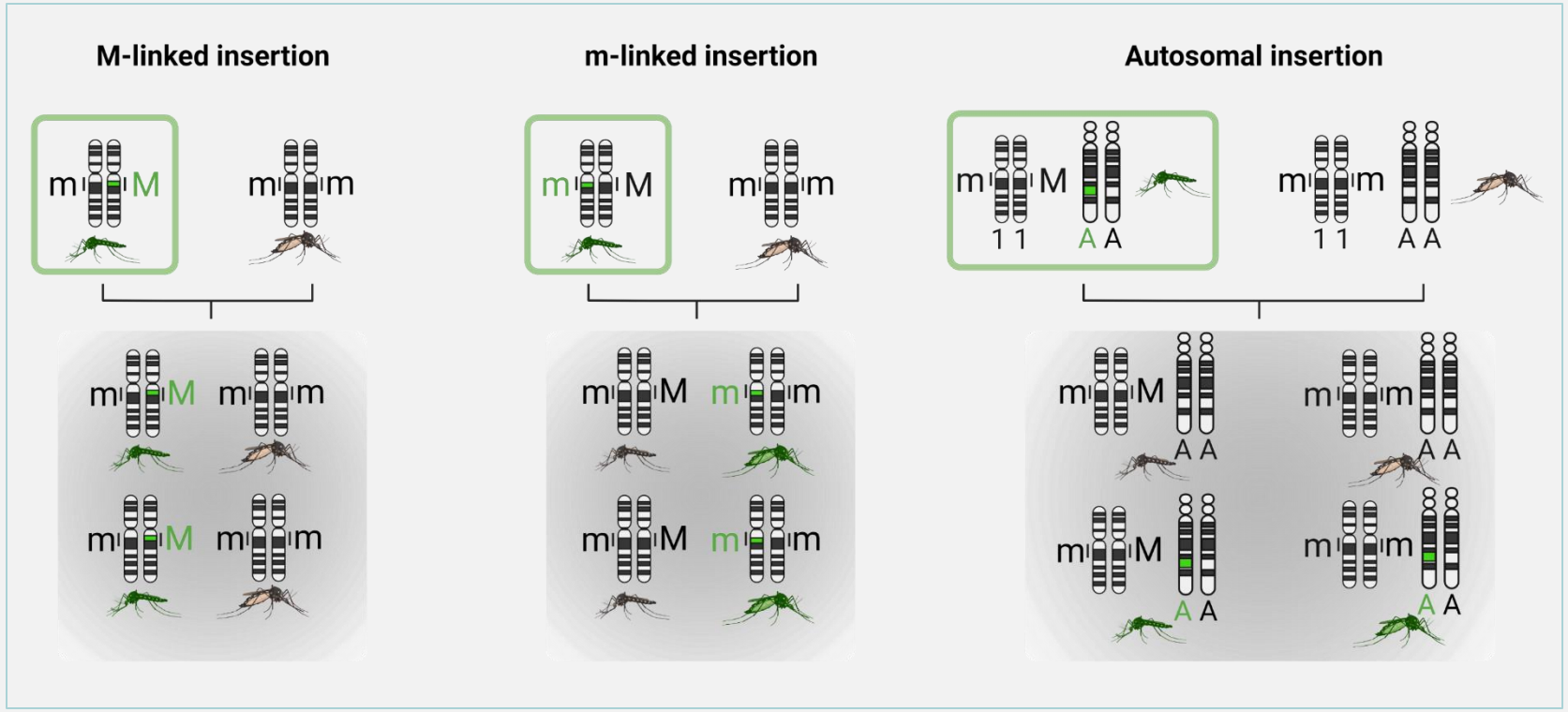


RESULTS – *AEDES AEGYPTI*

Interpretation

Father genotype

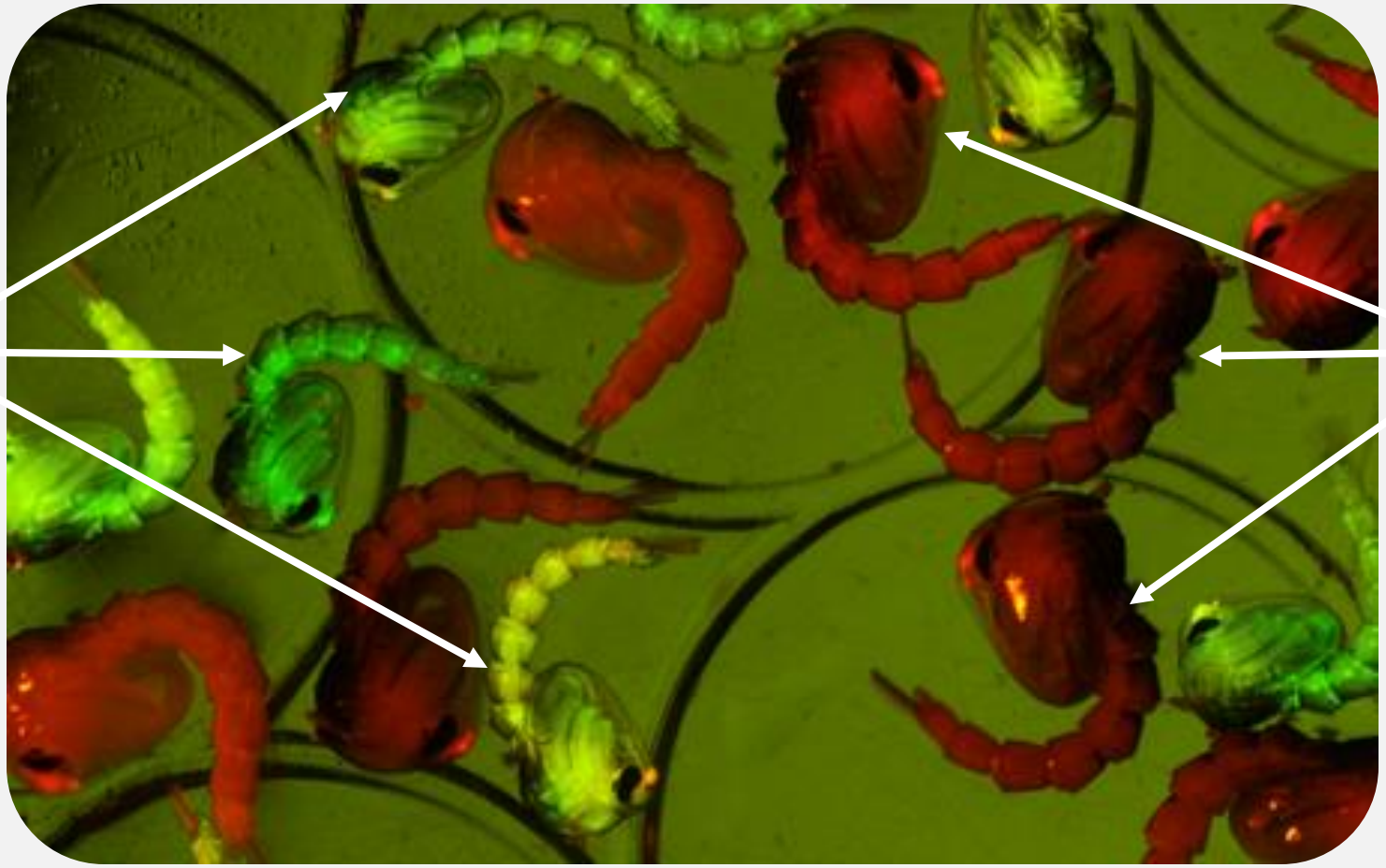
Offspring phenotype



RESULTS – *AEDES AEGYPTI*

Obtaining of an M-linked line

Males
GFP



Females
non-GFP *

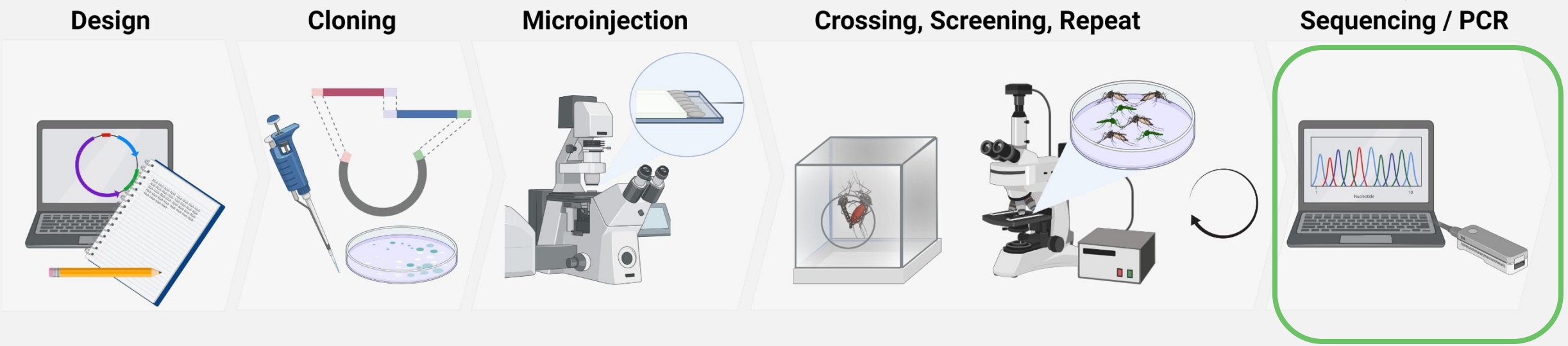
*DsRed = Cas9 marker

All males express GFP ➔ M-linkage

RESULTS – *AEDES AEGYPTI*

Obtaining of an M-linked line

PCR failed
➔ sequencing



Gilpatrick et al. 2020

RESULTS – *AEDES AEGYPTI*

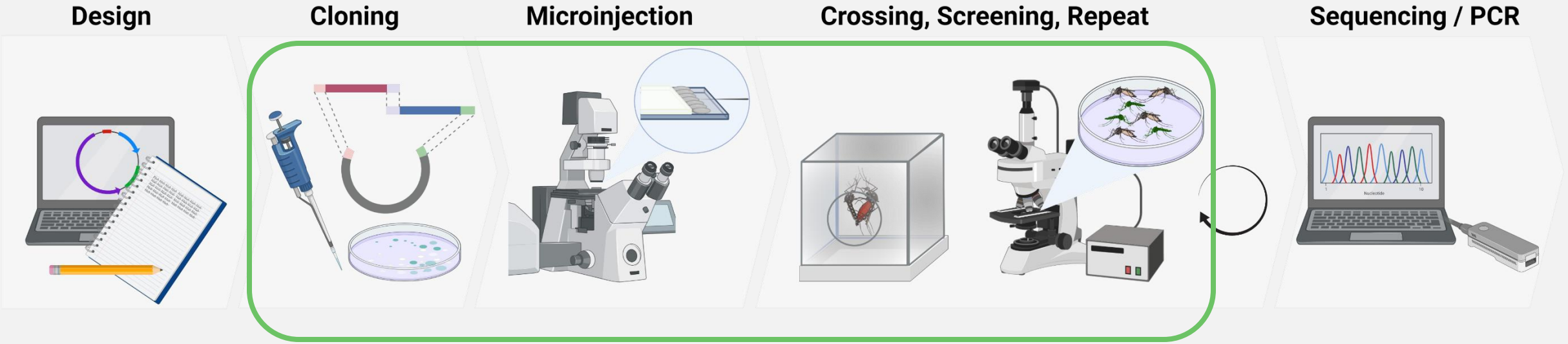
Obtaining of an M-linked line

All males express GFP ➔ M-linkage

RESULTS – *AEDES AEGYPTI*

2nd round AAEL019619

600 Cas9-expressing eggs



Expressed gRNAs targeting AAEL019619

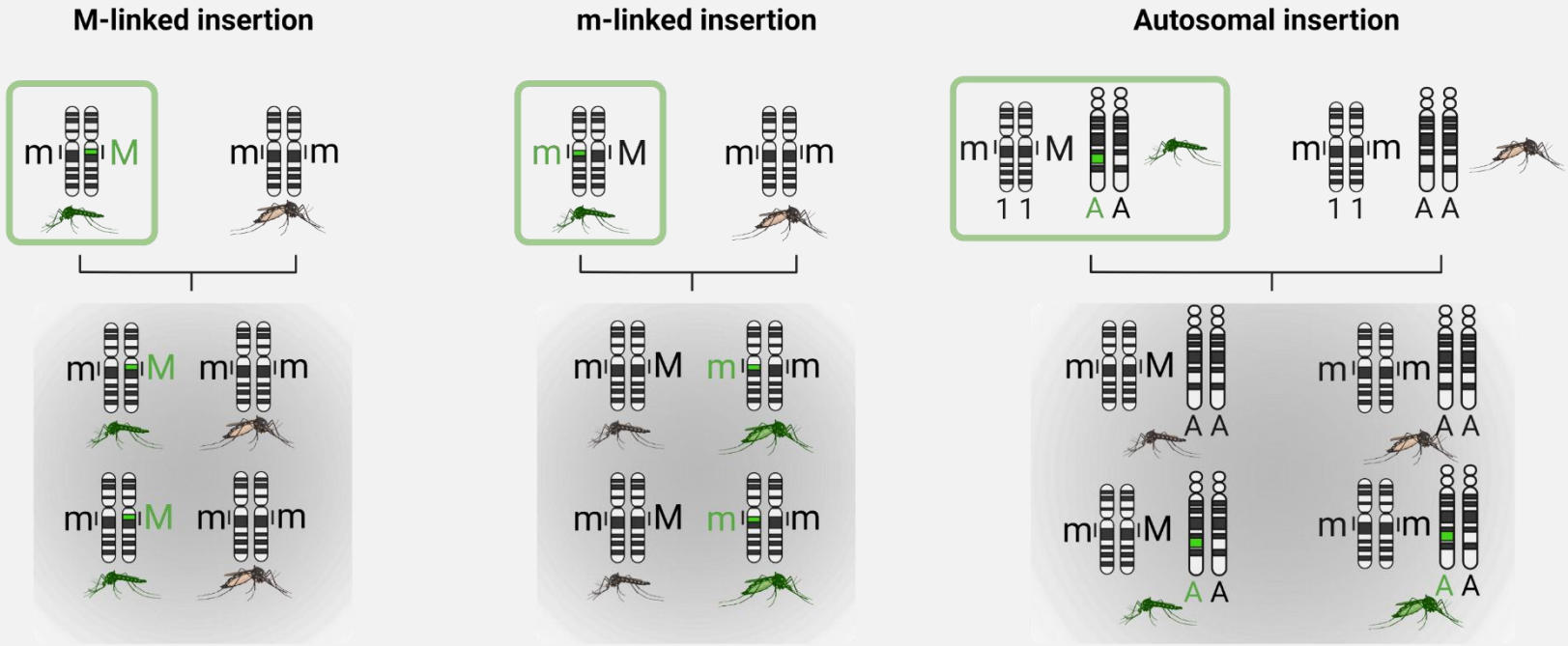
25 transgenic larvae in G1

RESULTS – *AEDES AEGYPTI*

2nd round AAEL019619

14 transgenic fathers

11 transgenic mothers

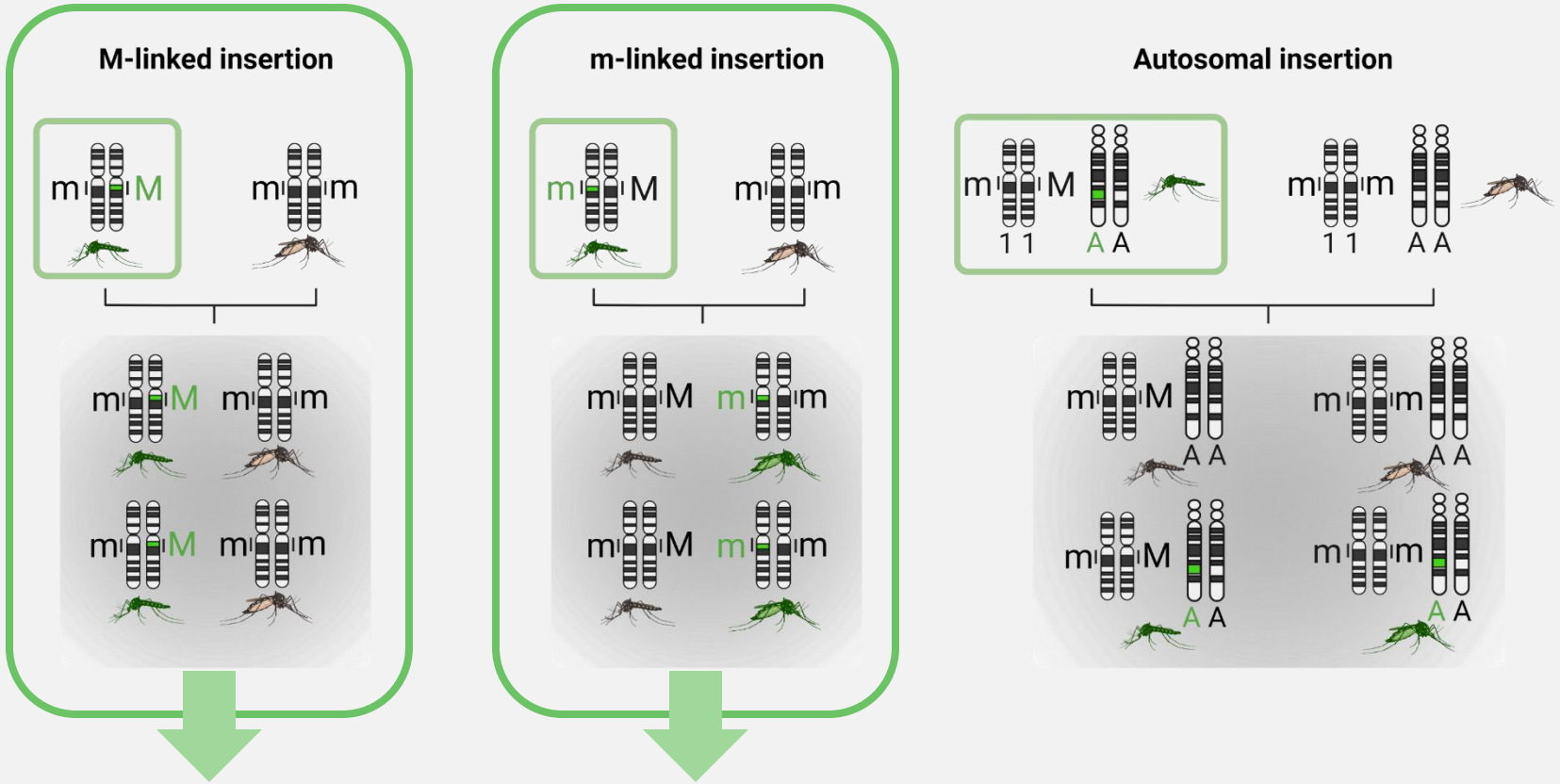


RESULTS – *AEDES AEGYPTI*

2nd round AAEL019619

14 transgenic fathers

11 transgenic mothers



10 M-linkages

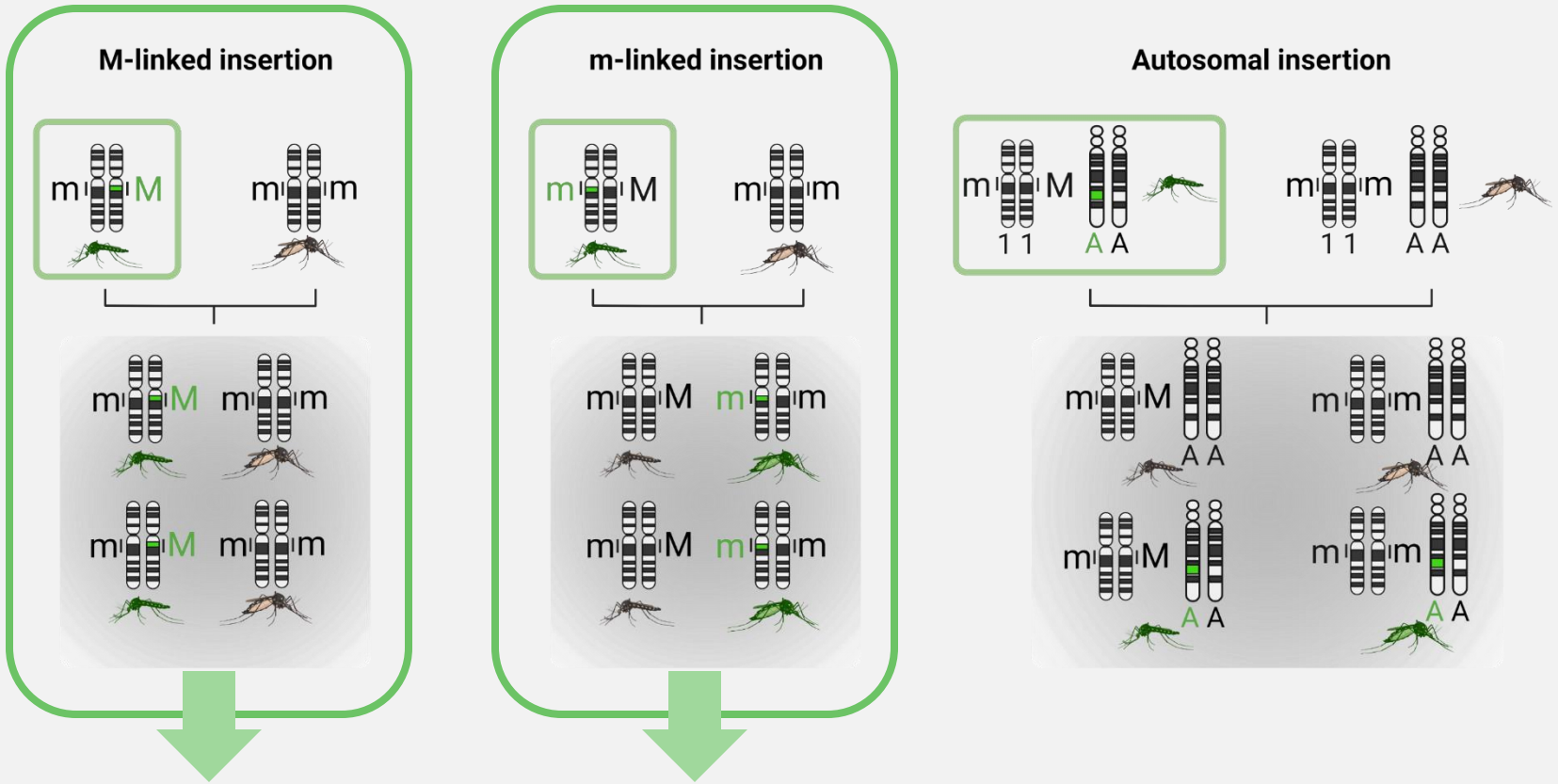
4 m-linkages

RESULTS – *AEDES AEGYPTI*

2nd round AAEL019619

14 transgenic fathers

11 transgenic mothers



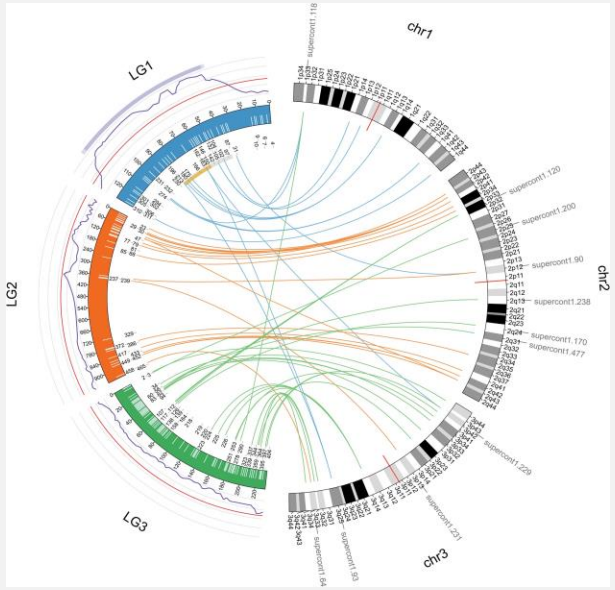
10 M-linkages

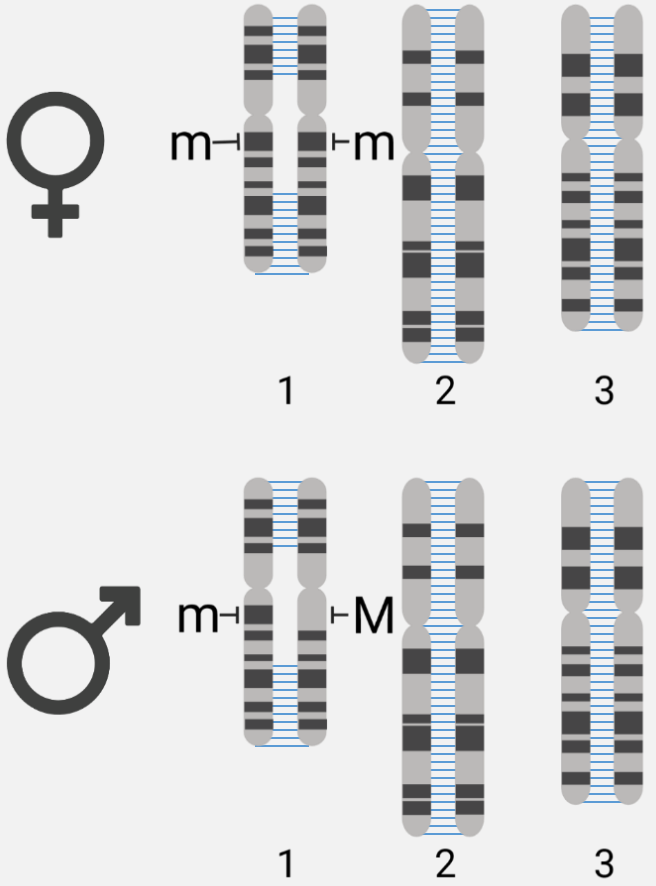
4 m-linkages

11 m-linkages

RESULTS – *AEDES AEGYPTI*

AAEL019619 is located at the neighboorhood of both sex loci

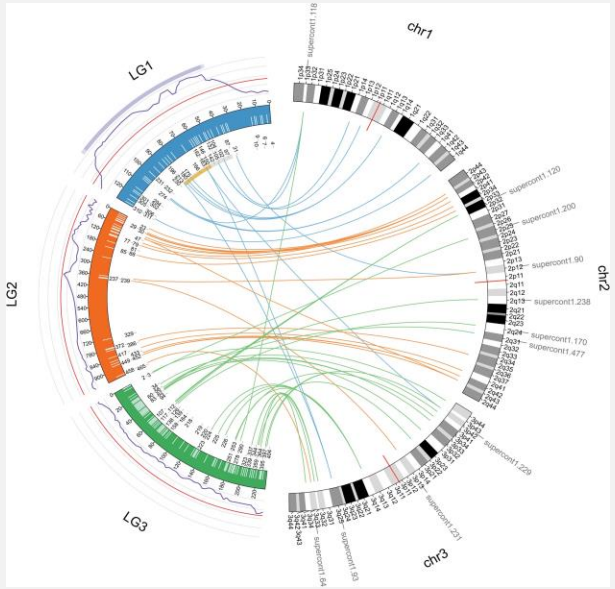


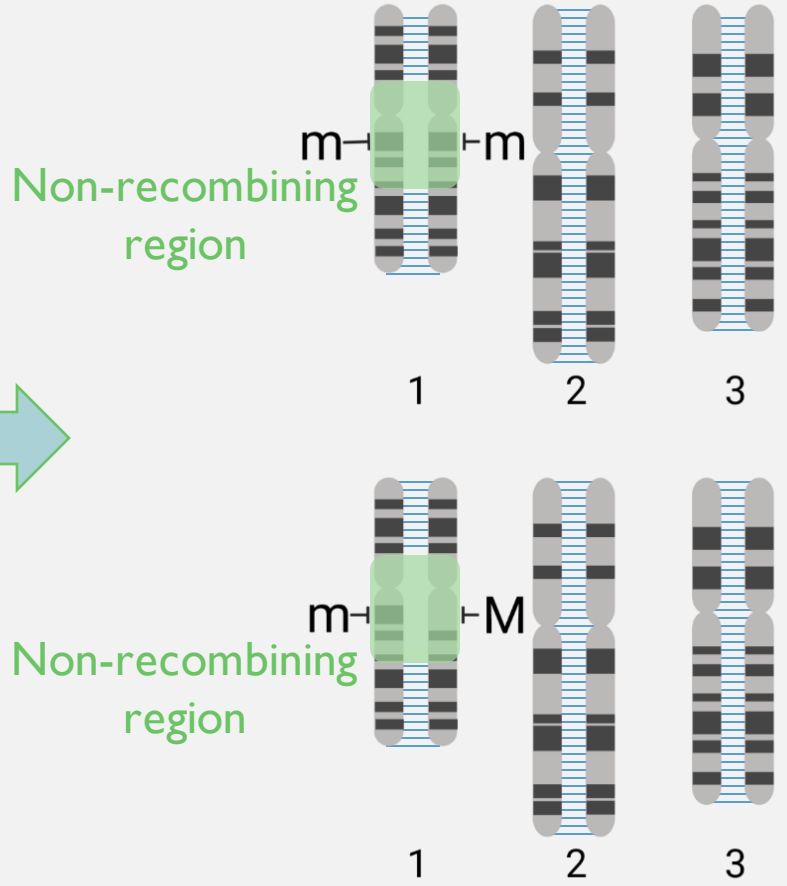


Fontaine *et al.* 2017

RESULTS – *AEDES AEGYPTI*

AAEL019619 is located at the neighboorhood of both sex loci

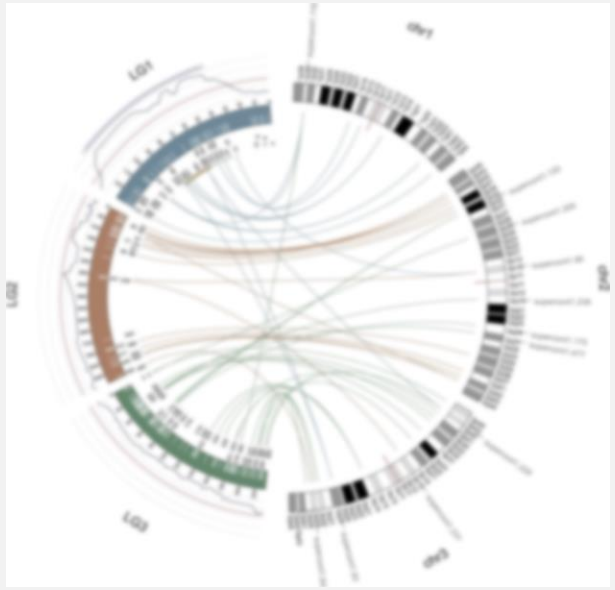


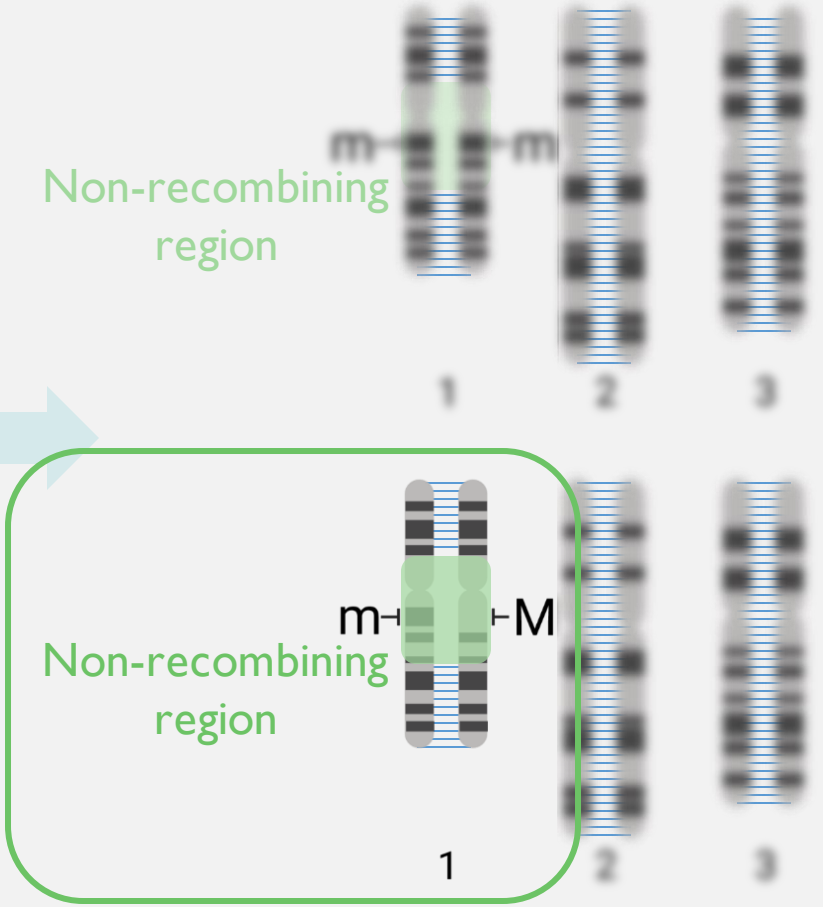


Fontaine *et al.* 2017

RESULTS – *AEDES AEGYPTI*

AAEL019619 is located at the neighboorhood of both sex loci

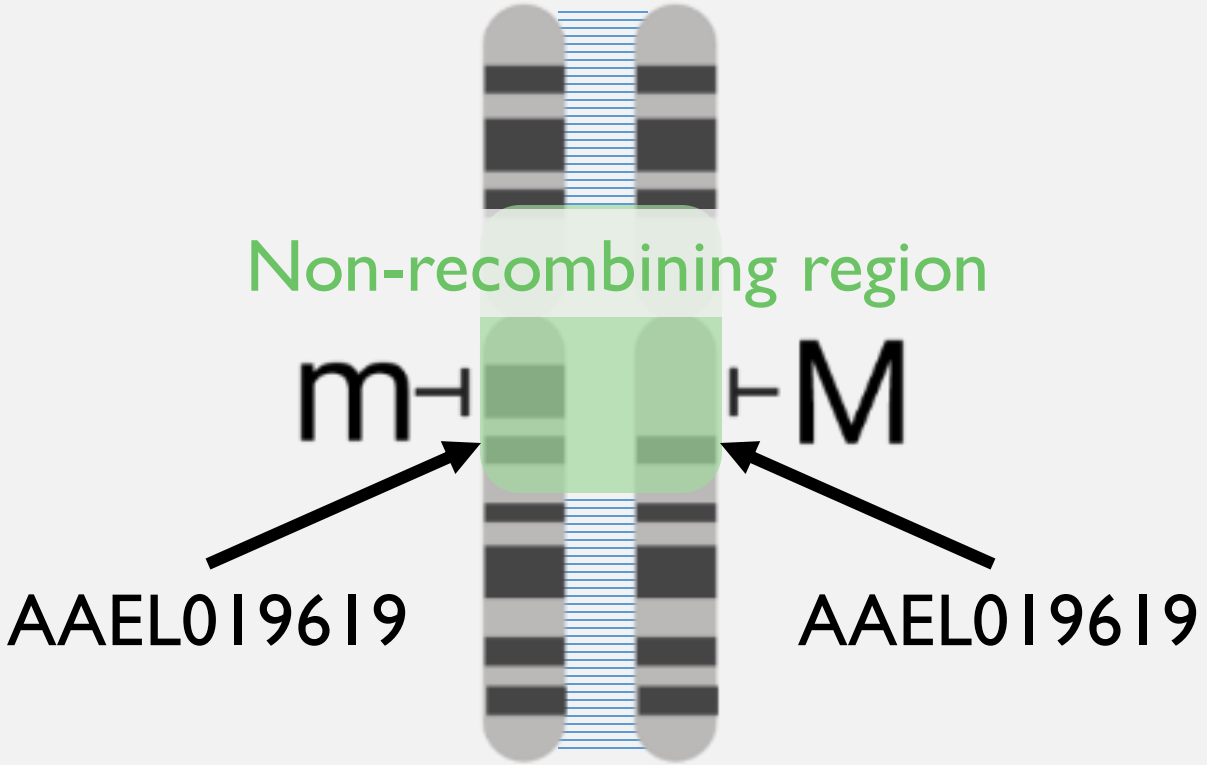




Fontaine *et al.* 2017

RESULTS – *AEDES AEGYPTI*

AAEL019619 is located in the non-recombining region near <u>both</u> sex loci



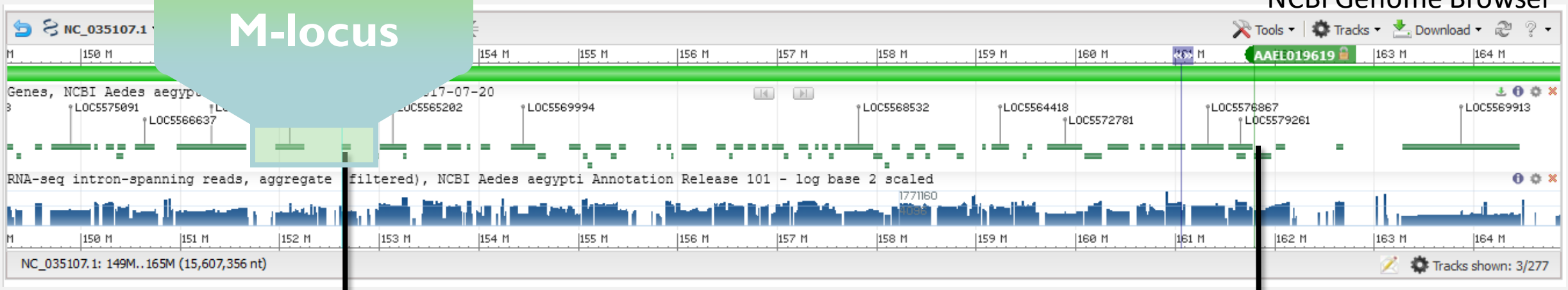
Fontaine *et al.* 2017

RESULTS – *AEDES AEGYPTI*

AAEL019619 is located at the neighboorhood of both sex loci

Chromosome 1

NCBI Genome Browser



M-locus

Nix

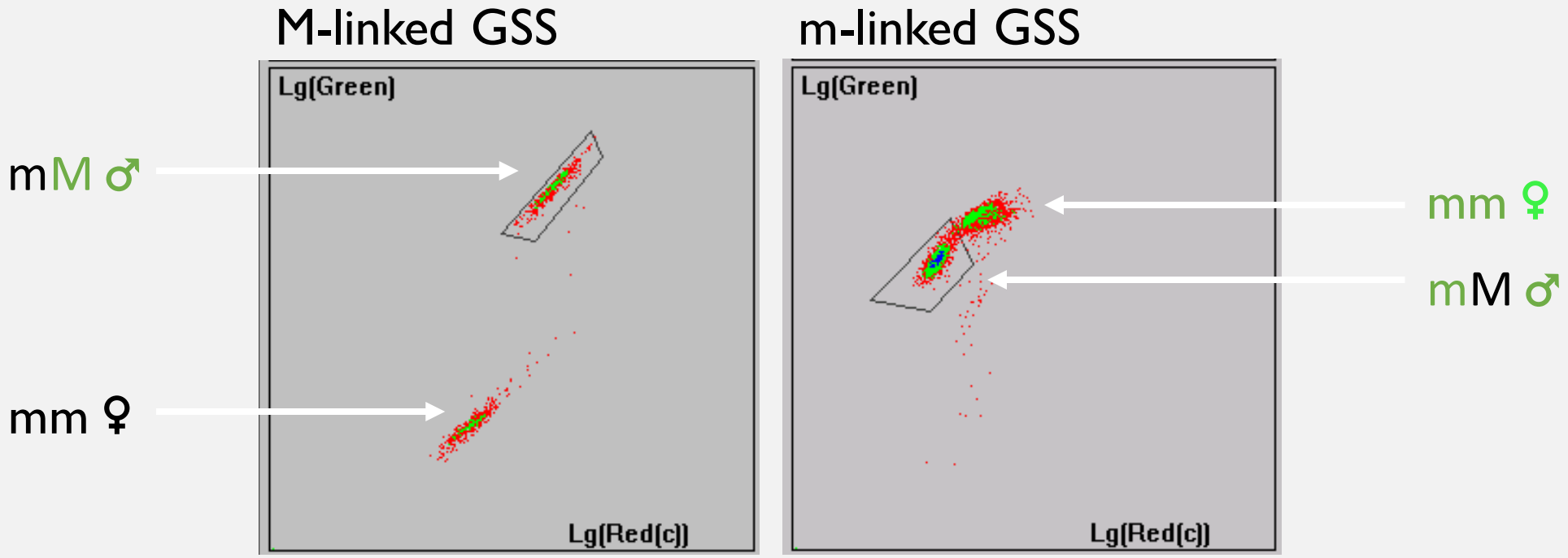
9 Mbp

AAEL019619

Not a misassembly!

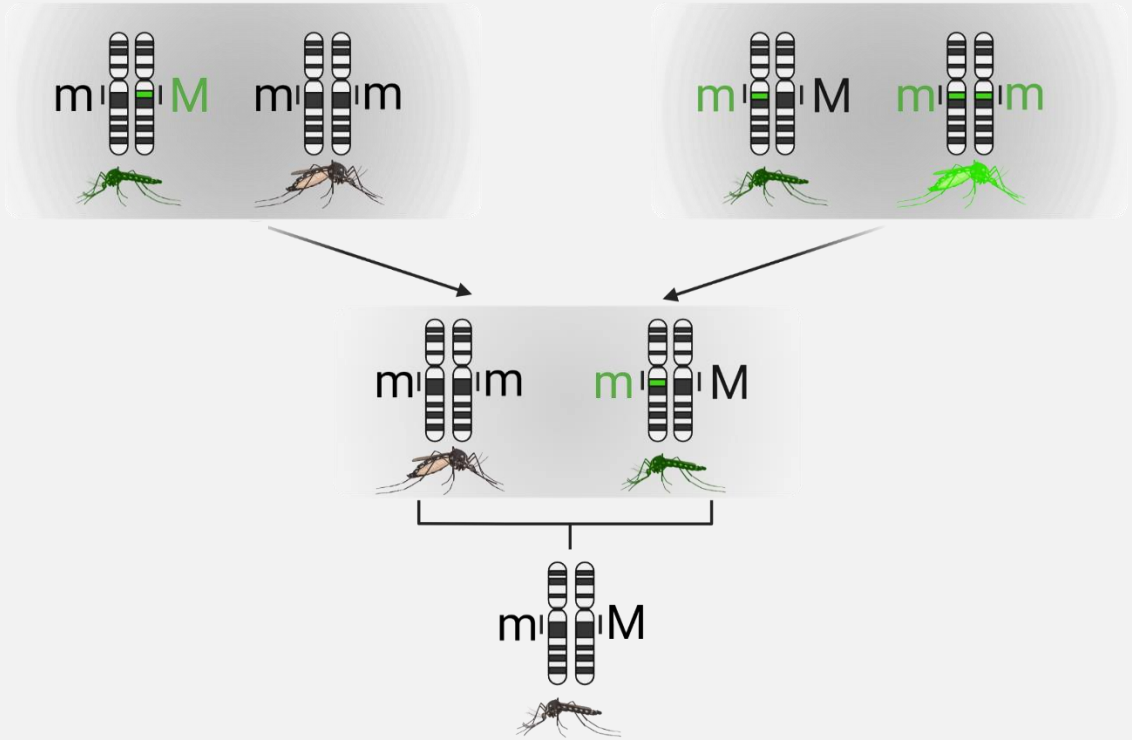
RESULTS – *AEDES AEGYPTI*

Both the M and m-linked lines can be sorted by COPAS



RESULTS – *AEDES AEGYPTI*

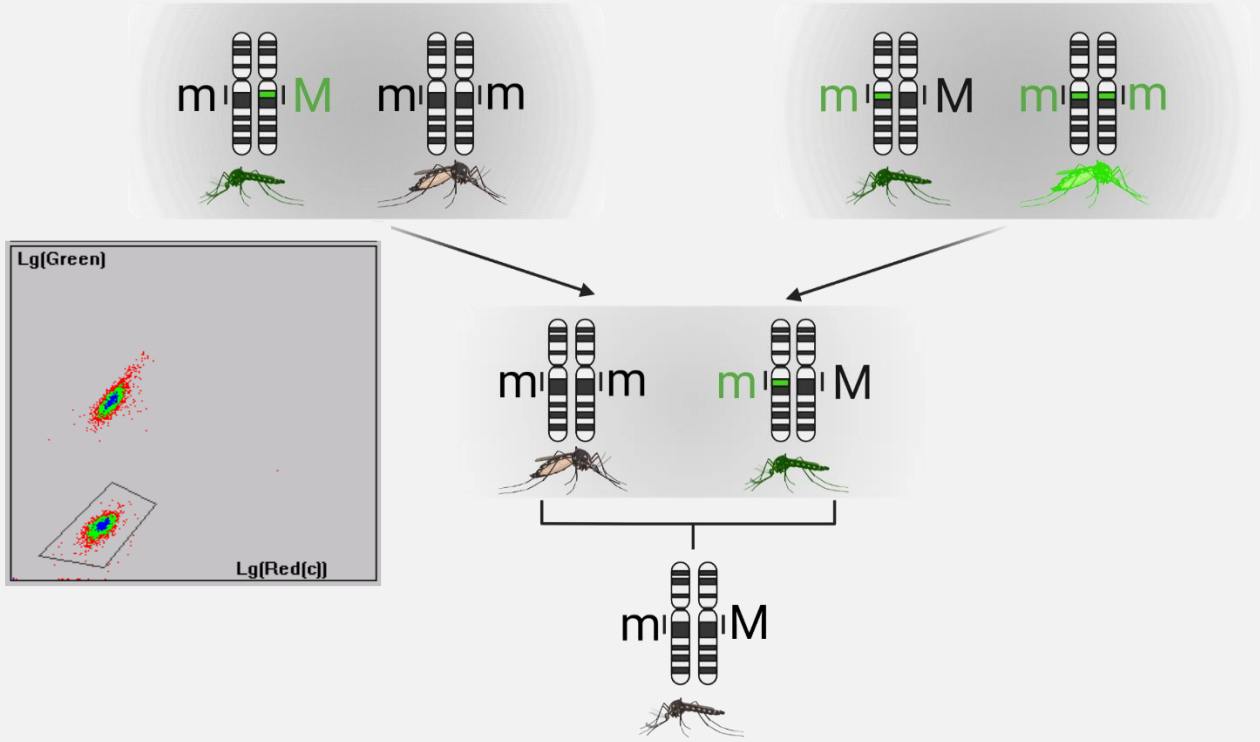
Obtaining non-transgenic males



RESULTS – *AEDES AEGYPTI*

Obtaining non-transgenic males

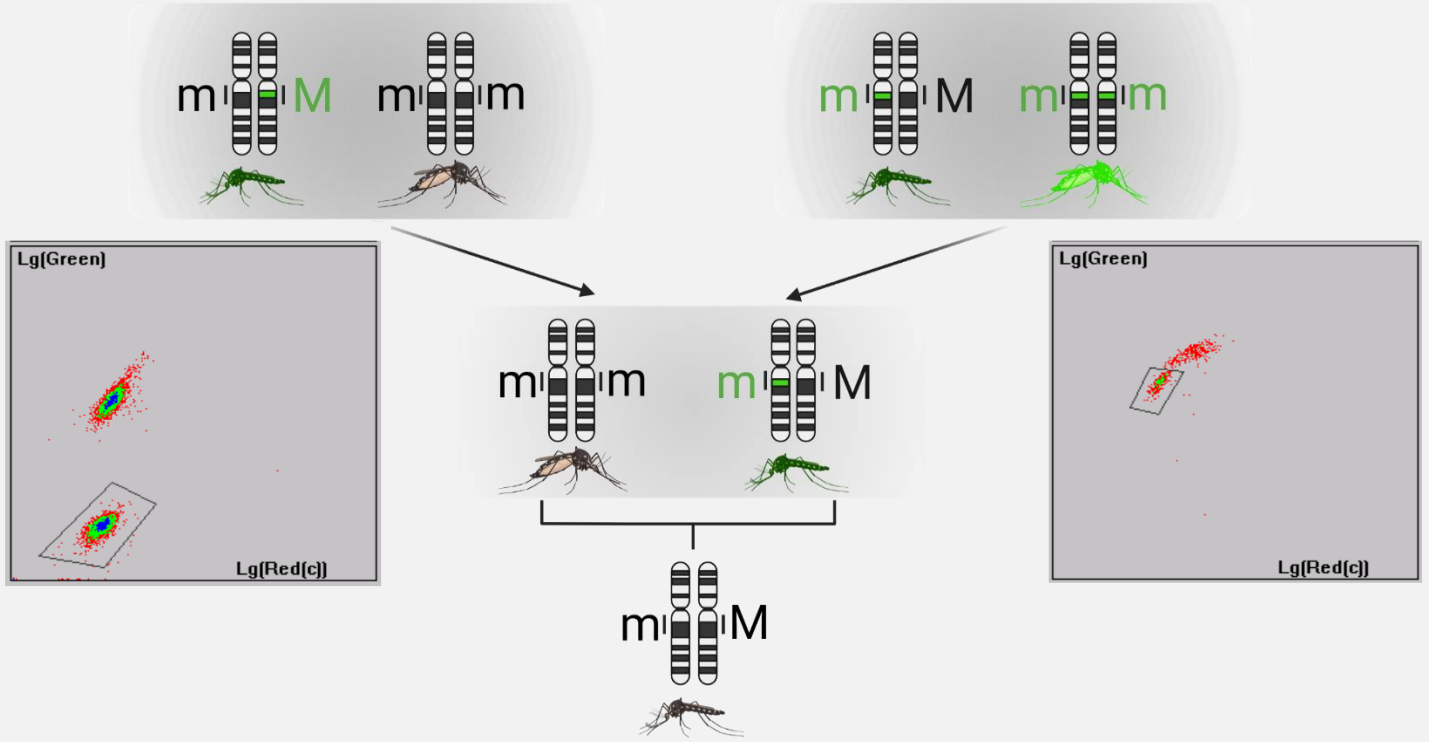
1,000 non-transgenic females from the M-linked GSS



RESULTS – *AEDES AEGYPTI*

Obtaining non-transgenic males

1,000 non-transgenic females from the M-linked GSS

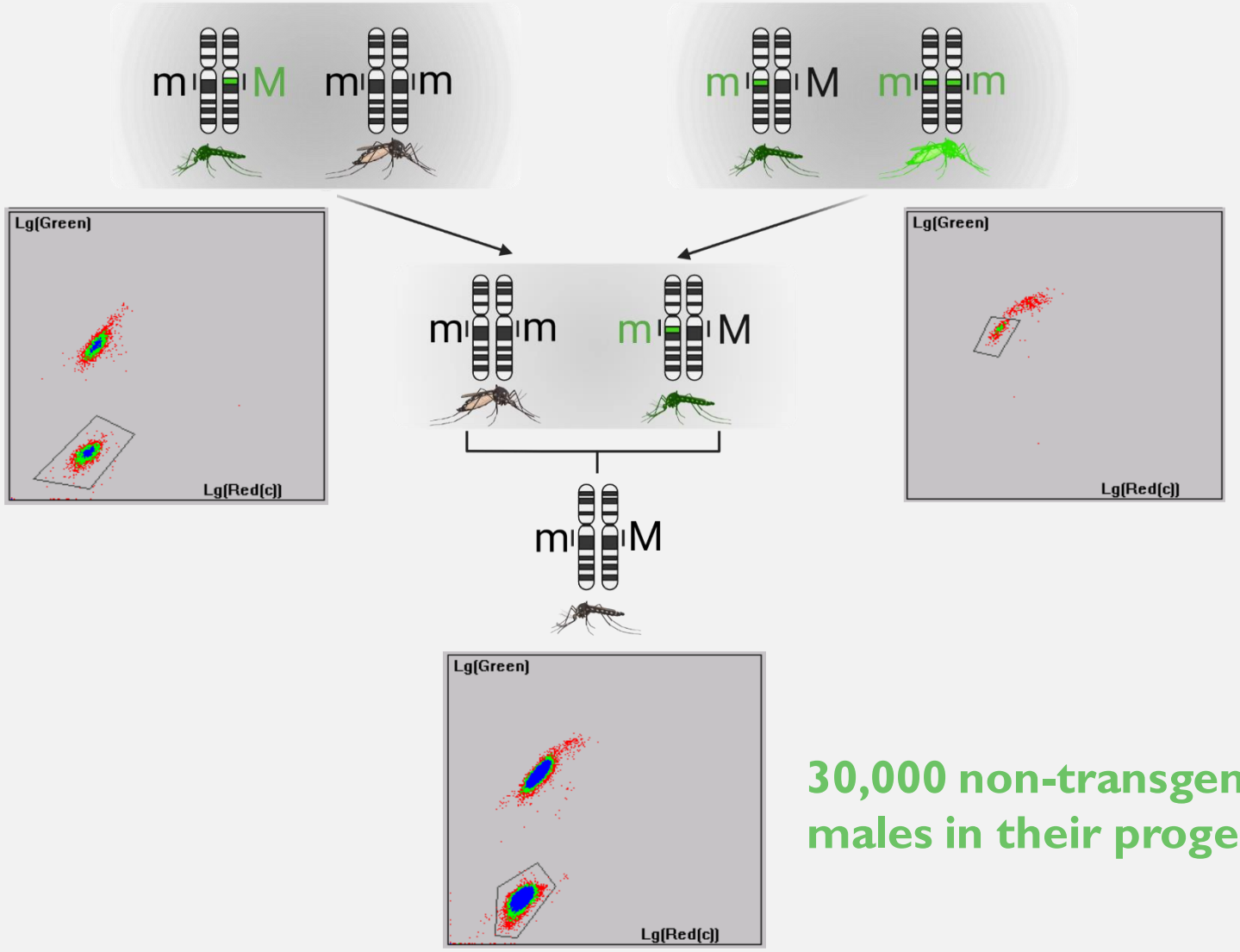


300 hemizygous transgenic males from the m-linked GSS

RESULTS – *AEDES AEGYPTI*

Obtaining non-transgenic males

1,000 non-transgenic females from the M-linked GSS

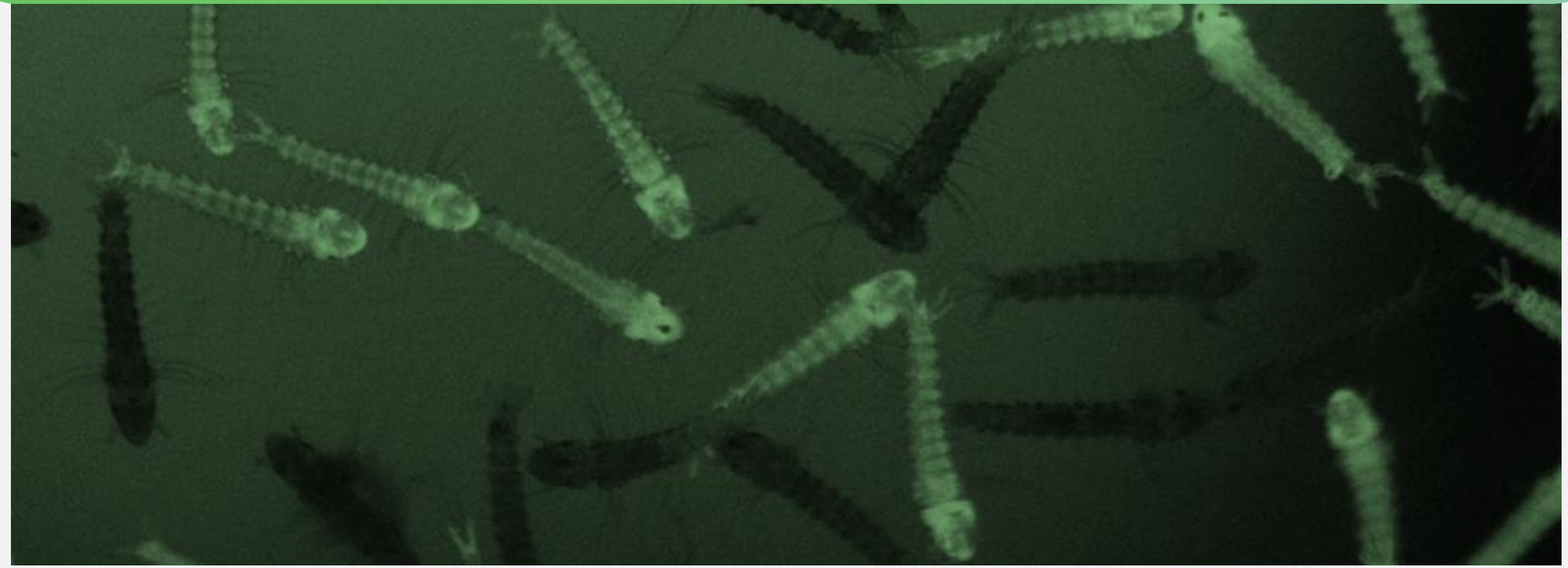


300 hemizygous transgenic males from the m-linked GSS

30,000 non-transgenic males in their progeny

RESULTS

Developing GSS in *Aedes albopictus*



RESULTS – *AEDES ALBOPICTUS*

In *Aedes albopictus*, there are no chromosome-long scaffolds.

Reference genome Aalbo_primary.1

None (2019). Isolate: FPA.

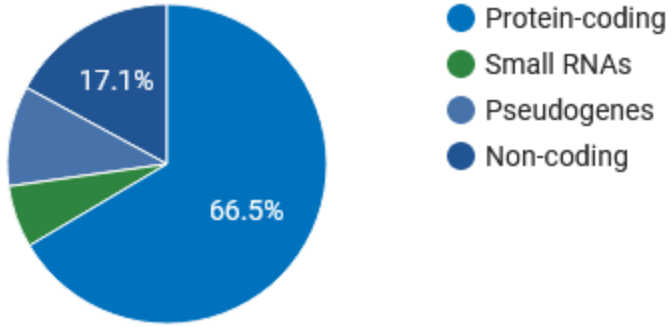
RefSeq GCF_006496715.1

Download

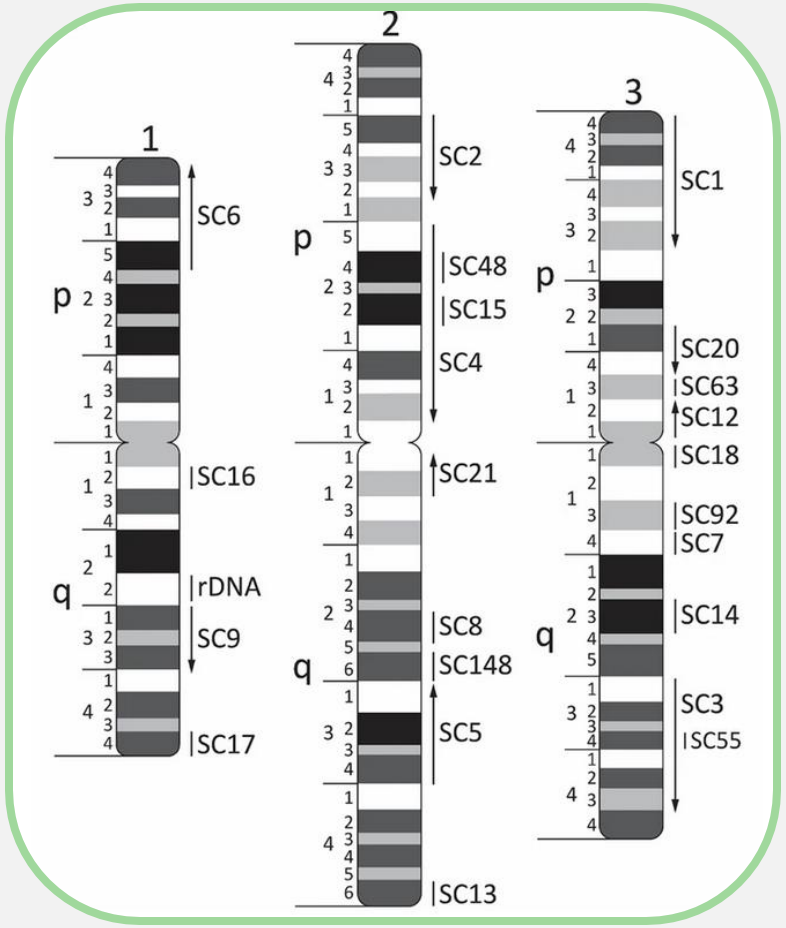
Genome size	2.5 Gb
Contig N50	1.2 Mb
Genes	40,494

NCBI Annotation Release 102 Jul 3, 2019

Current gene set



View all genes

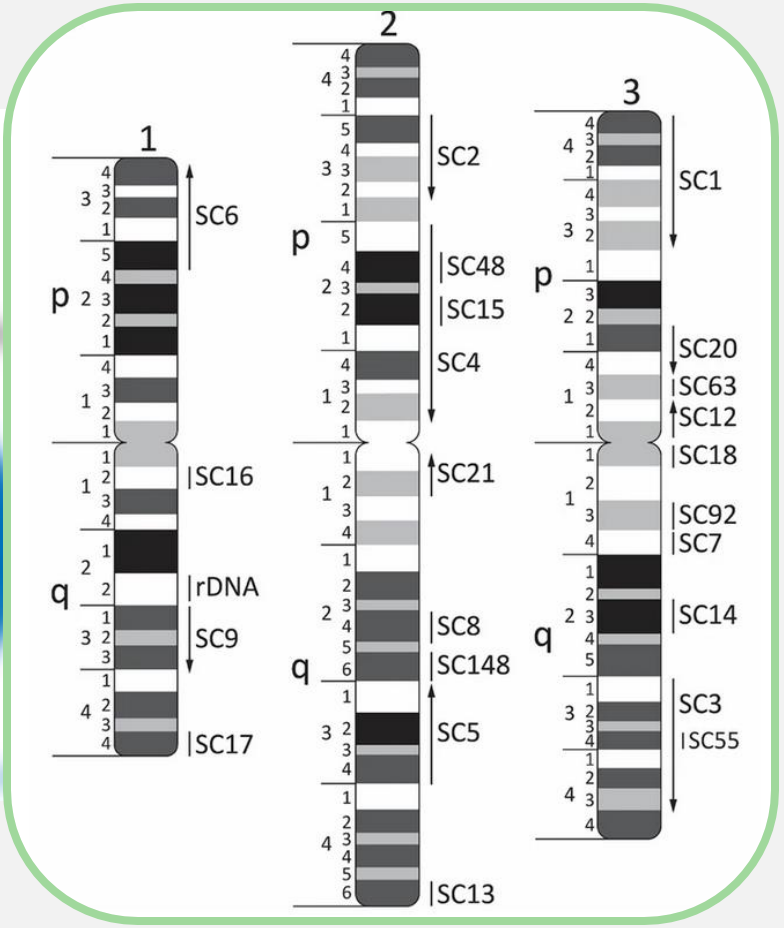


NCBI, Palatini et al. 2020

RESULTS – *AEDES ALBOPICTUS*

In this genome, *Nix* belongs to scaffold 261, which is <1Mbp

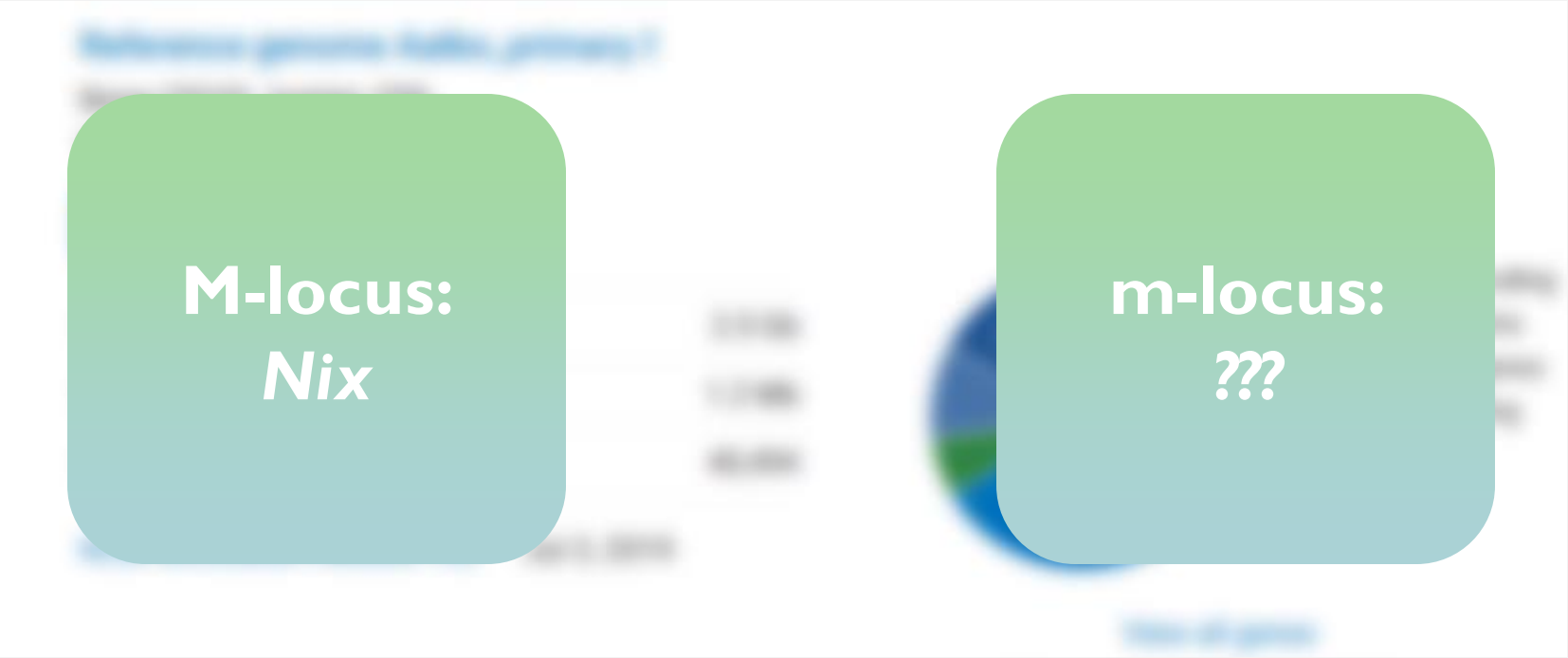
M-locus:
Nix



NCBI, Palatini et al. 2020

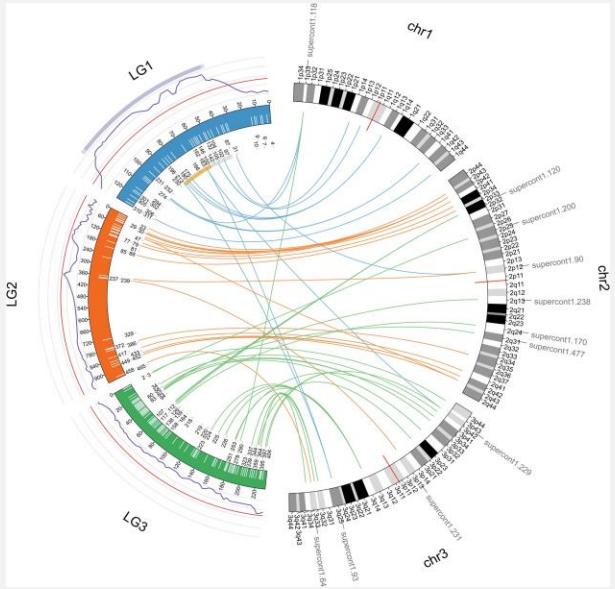
RESULTS – *AEDES ALBOPICTUS*

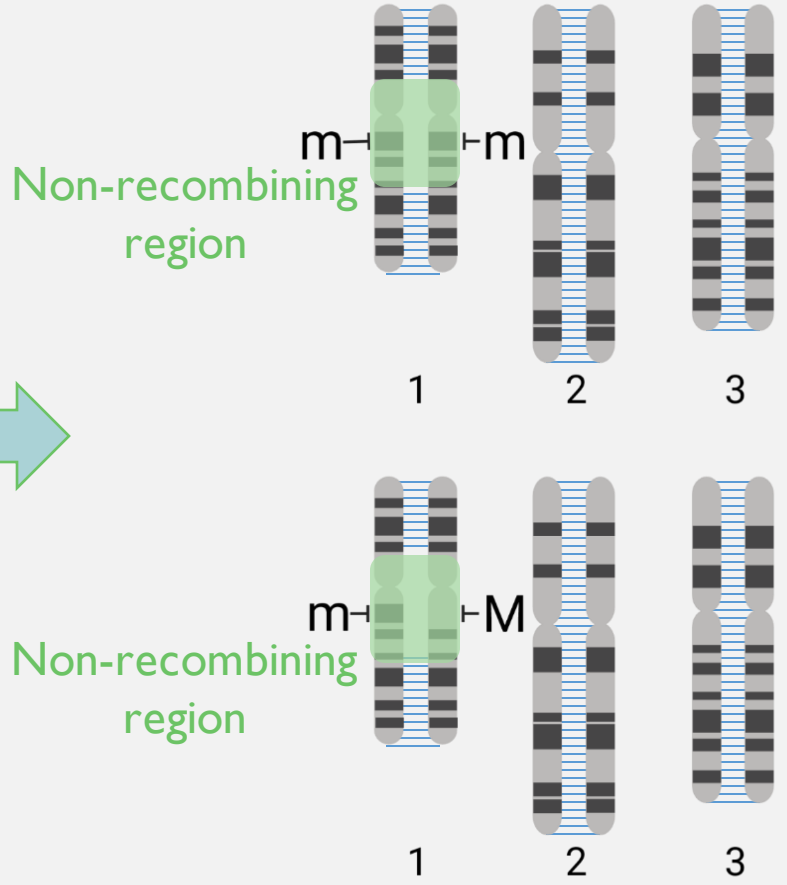
There is no information about the m-locus



RESULTS – *AEDES ALBOPICTUS*

If the non-recombining region is the same in *Ae. albopictus*, there is a ~5% chance to randomly land in a sex locus by non-targeted approaches

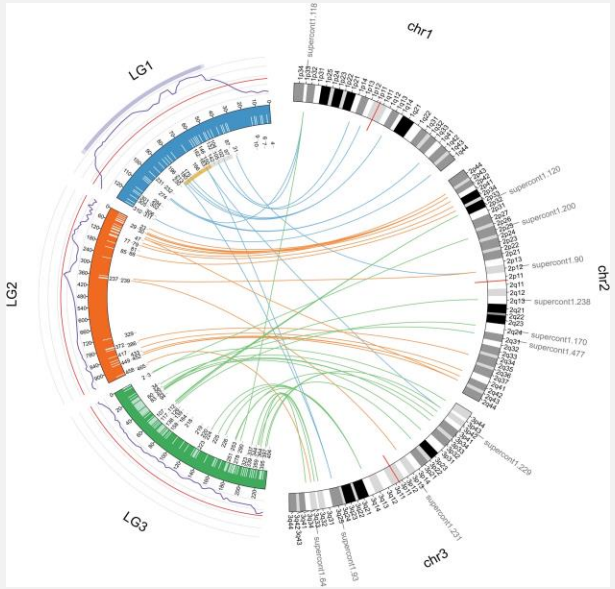


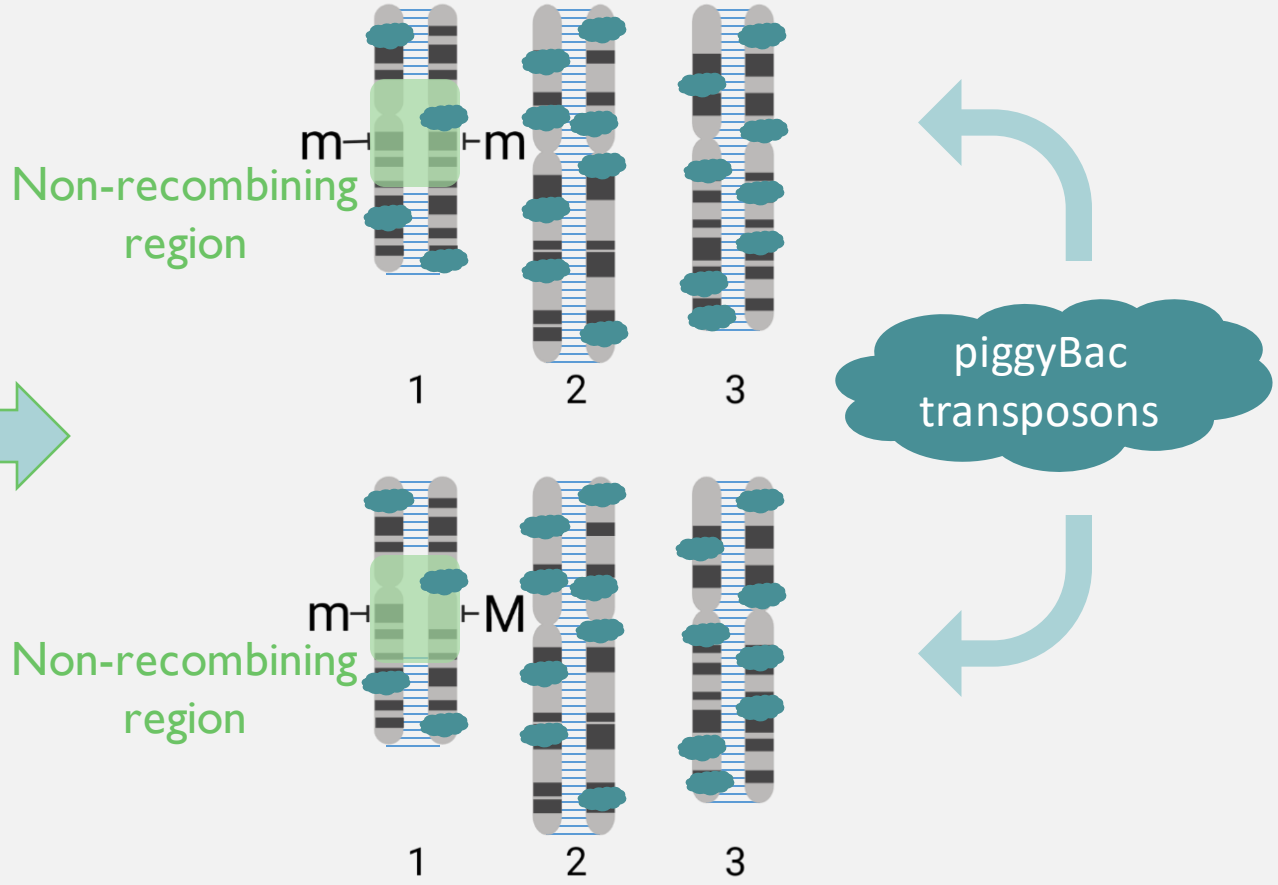


Fontaine *et al.* 2017

RESULTS – *AEDES ALBOPICTUS*

If the non-recombining region is the same in *Ae. albopictus*, there is a ~5% chance to randomly land in a sex locus by non-targeted approaches

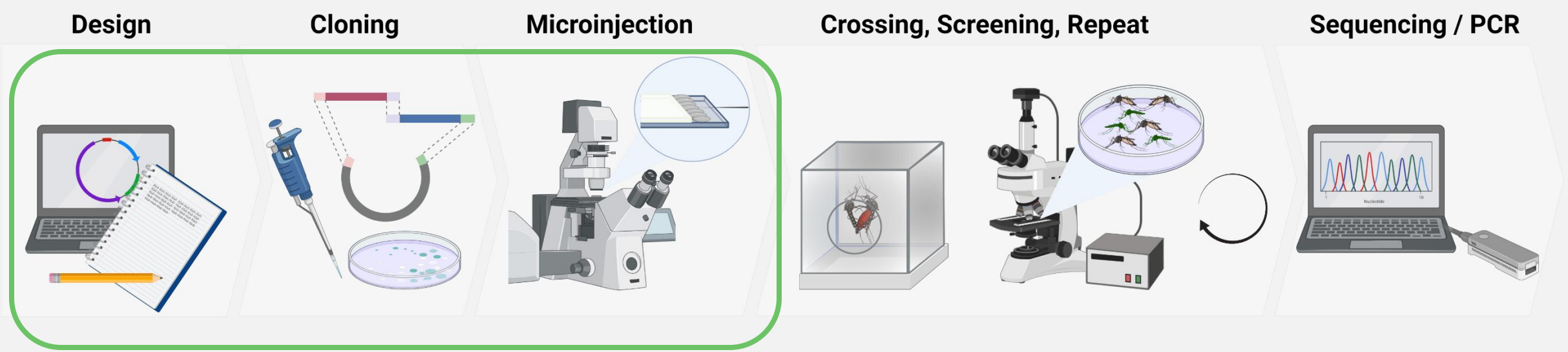




Fontaine *et al.* 2017

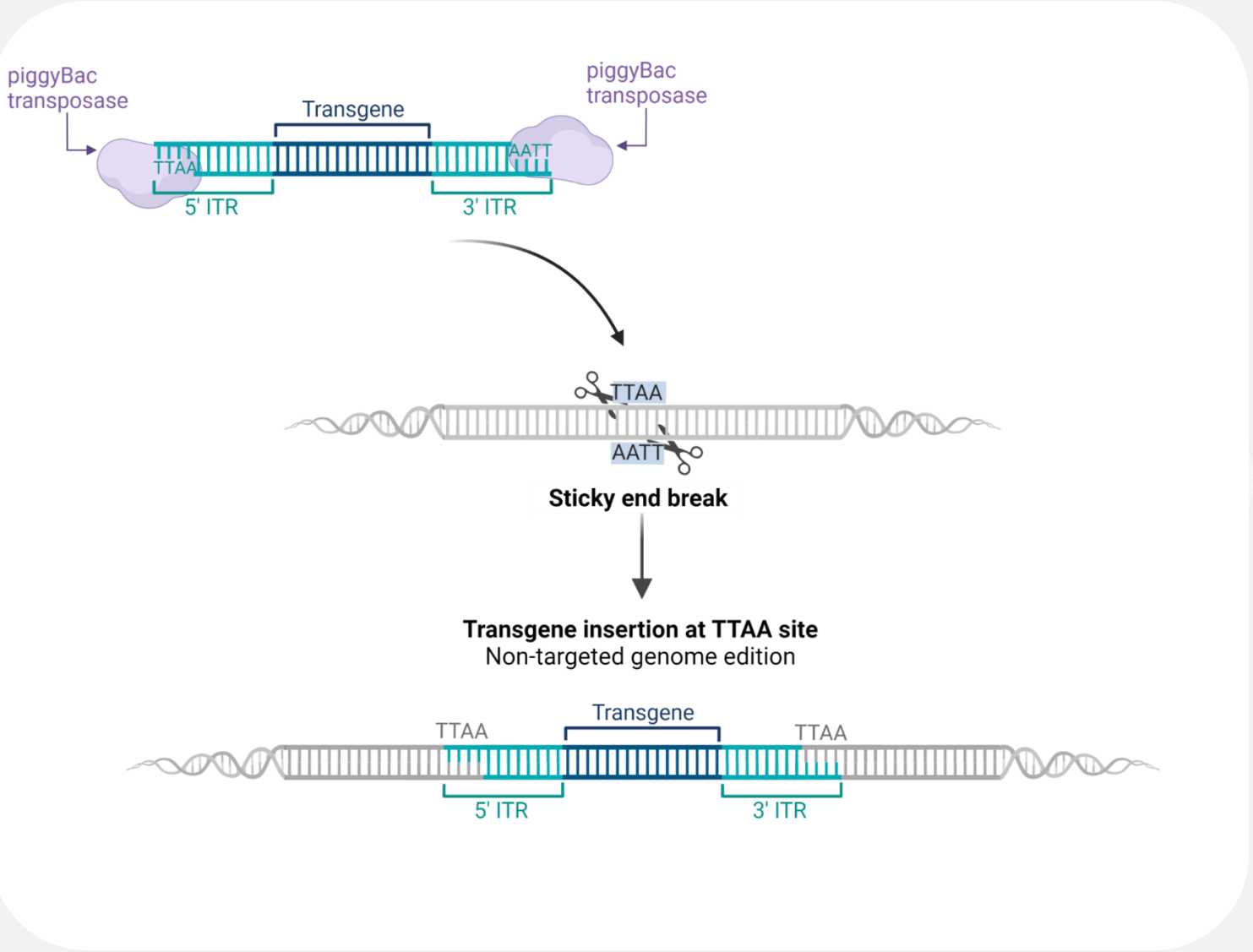
RESULTS – *AEDES ALBOPICTUS*

piggyBac approach



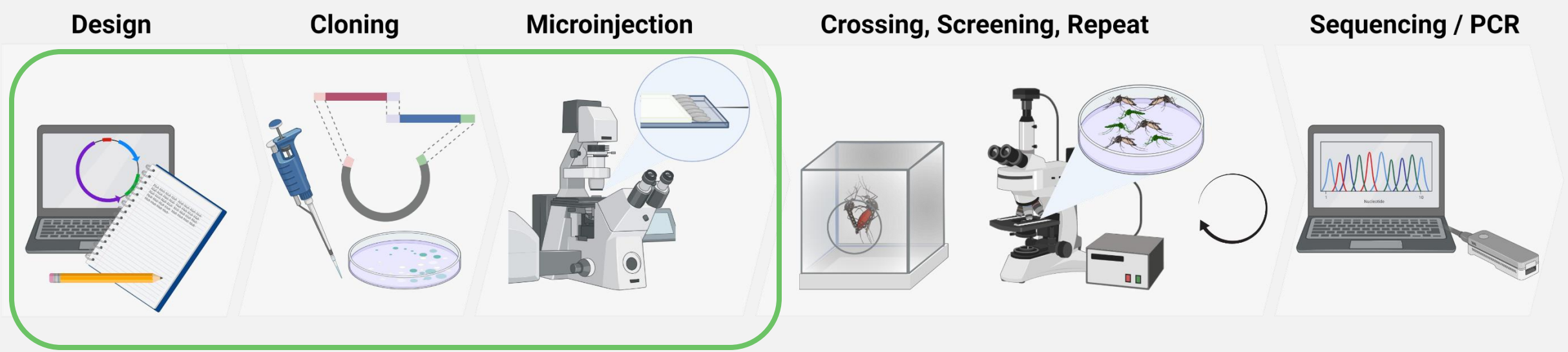
RESULTS – *AEDES ALBOPICTUS*

piggyBac approach



RESULTS – *AEDES ALBOPICTUS*

piggyBac approach

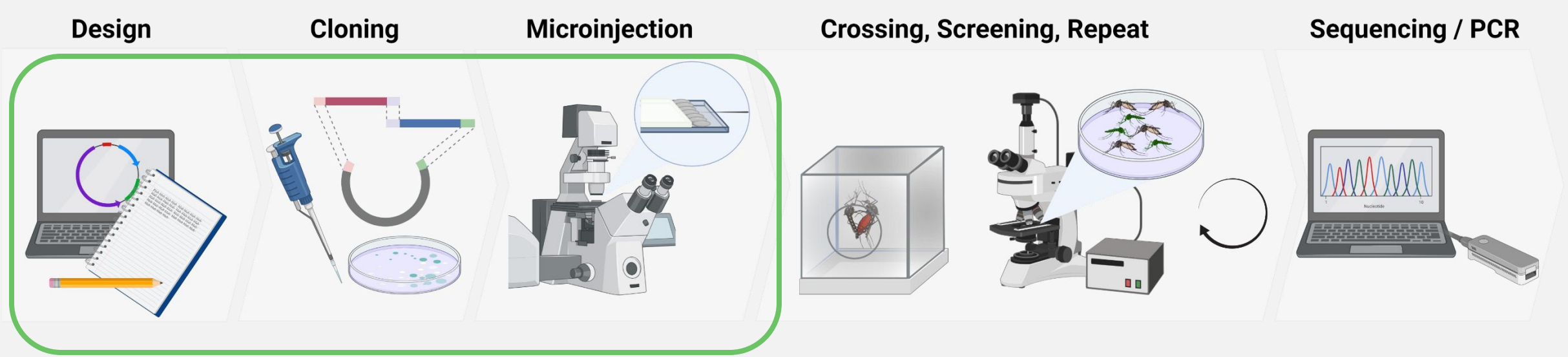


6 piggyBac plasmids encoding different fluorochromes

RESULTS – *AEDES ALBOPICTUS*

piggyBac approach

Injection of 100s of WT eggs



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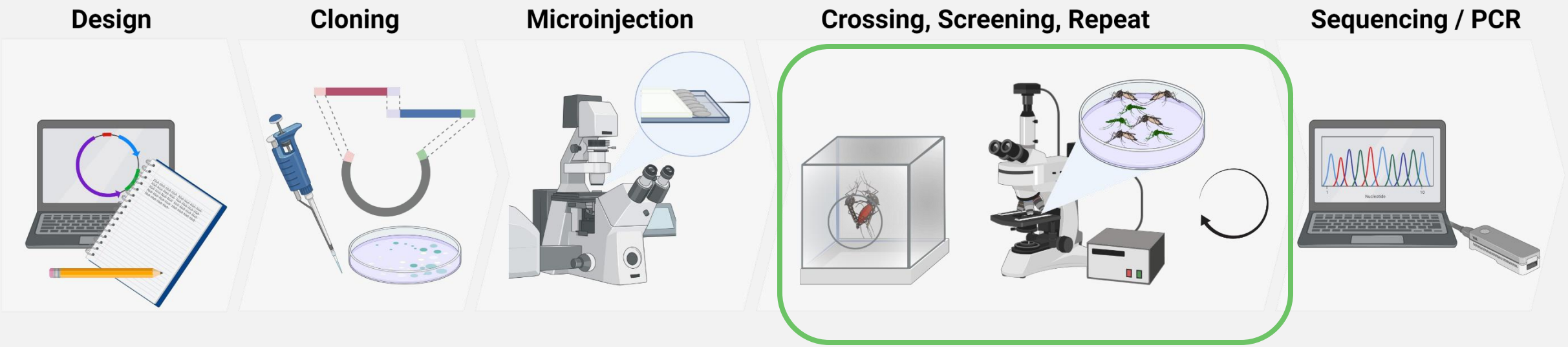
RESULTS – *AEDES ALBOPICTUS*



RESULTS – *AEDES ALBOPICTUS*

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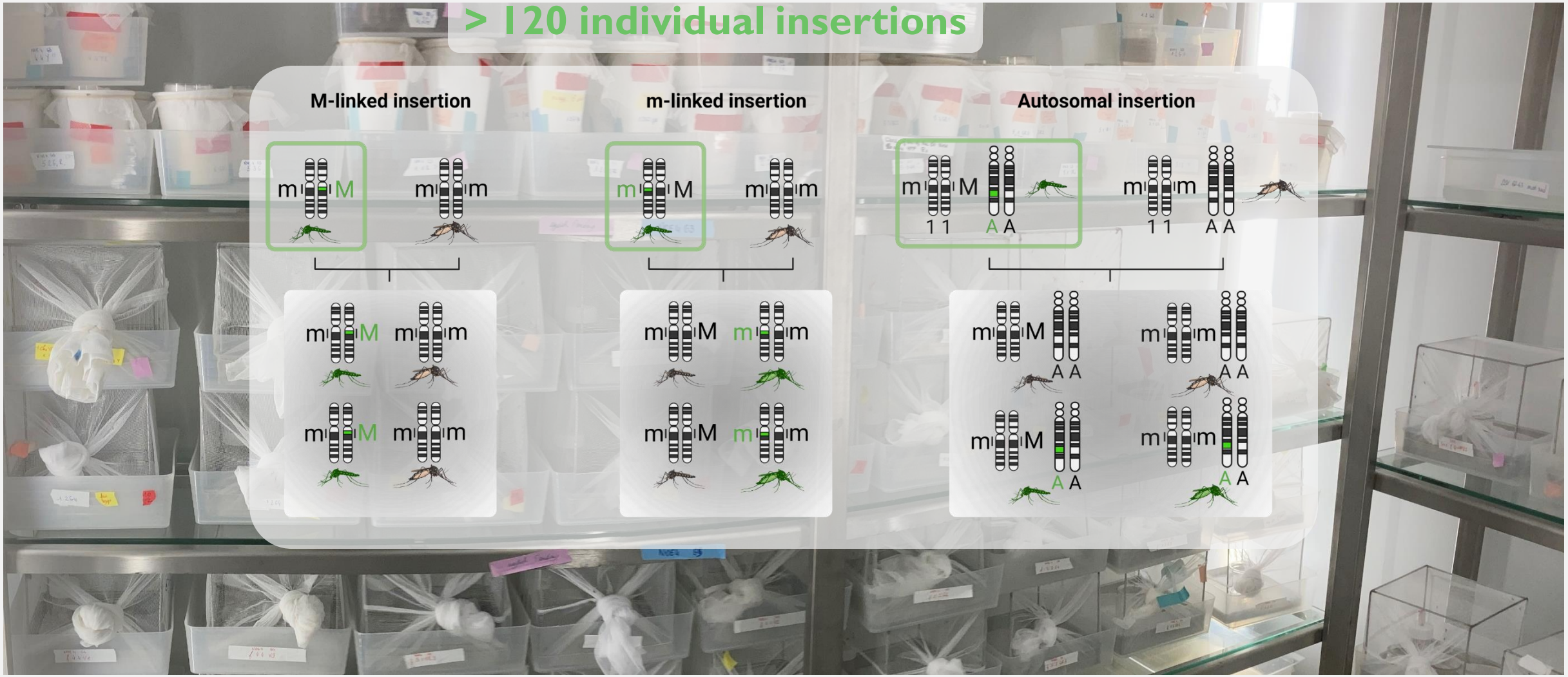
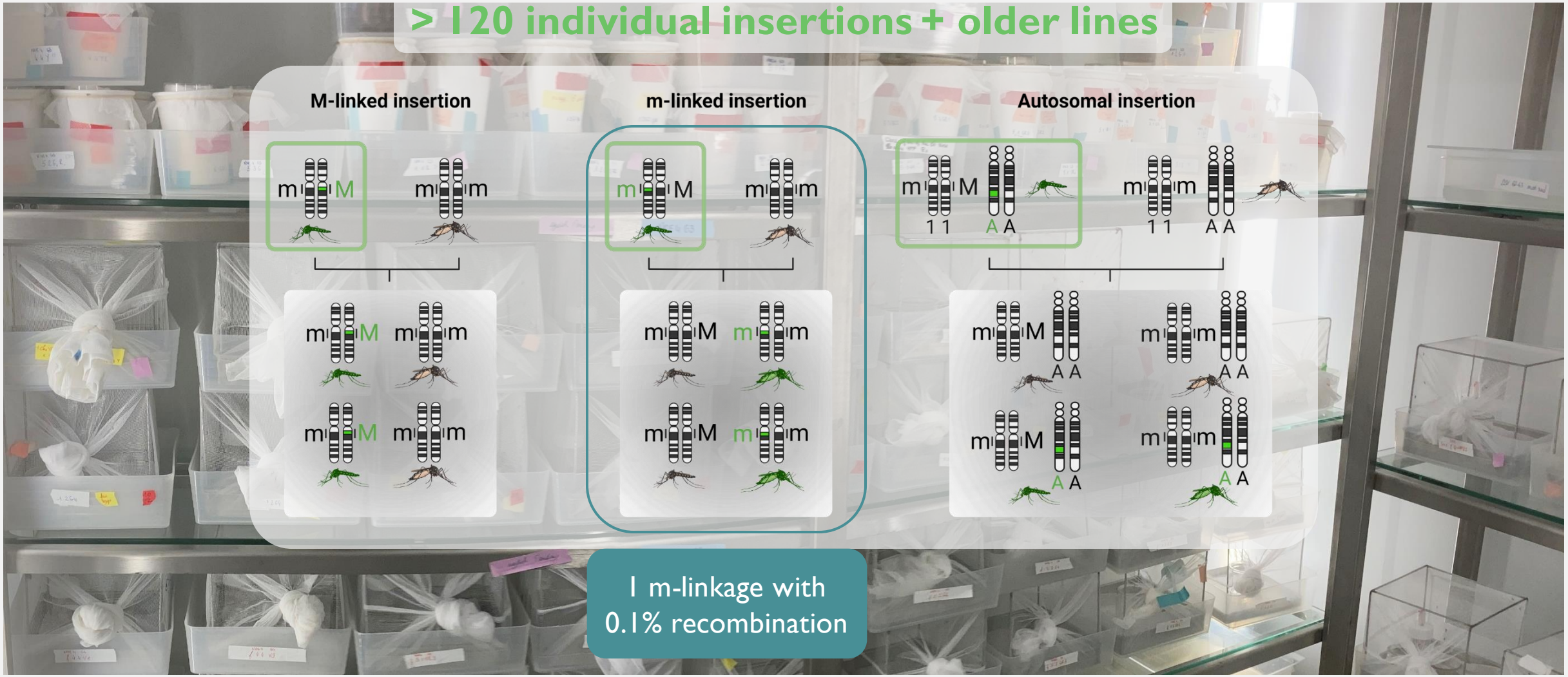
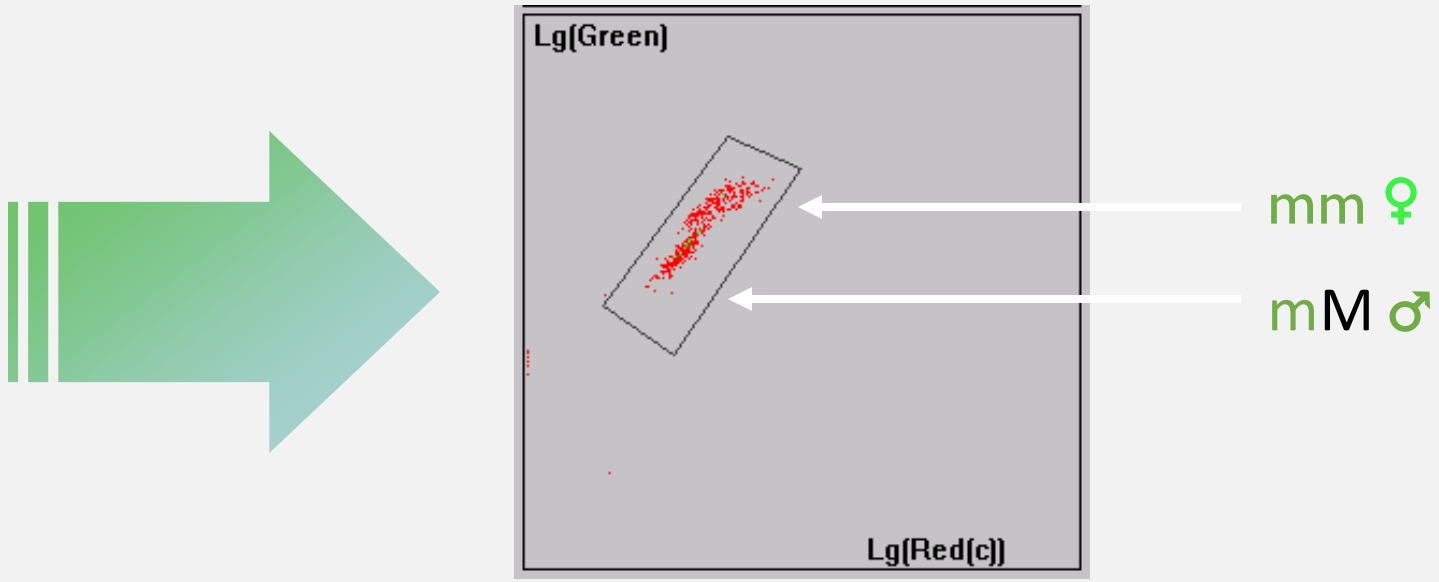


RESULTS – *AEDES ALBOPICTUS*



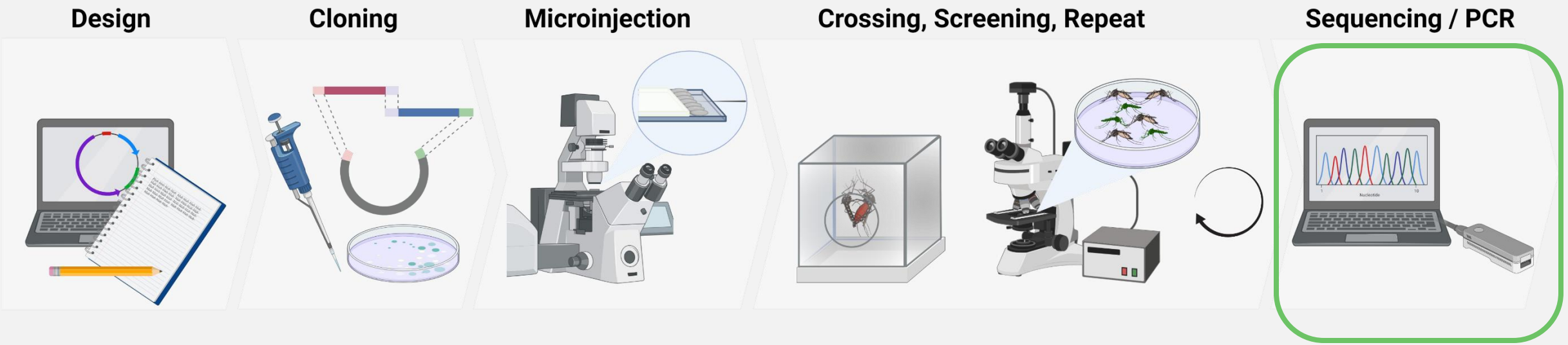
RESULTS – *AEDES ALBOPICTUS*

This m-linked line allows sex separation of neonate larvae



RESULTS – *AEDES ALBOPICTUS*

Targeted sequencing recovered the sequence of the insertion locus



No clear mapping
Scaffold 9 and/or 39?

RESULTS – *AEDES ALBOPICTUS*

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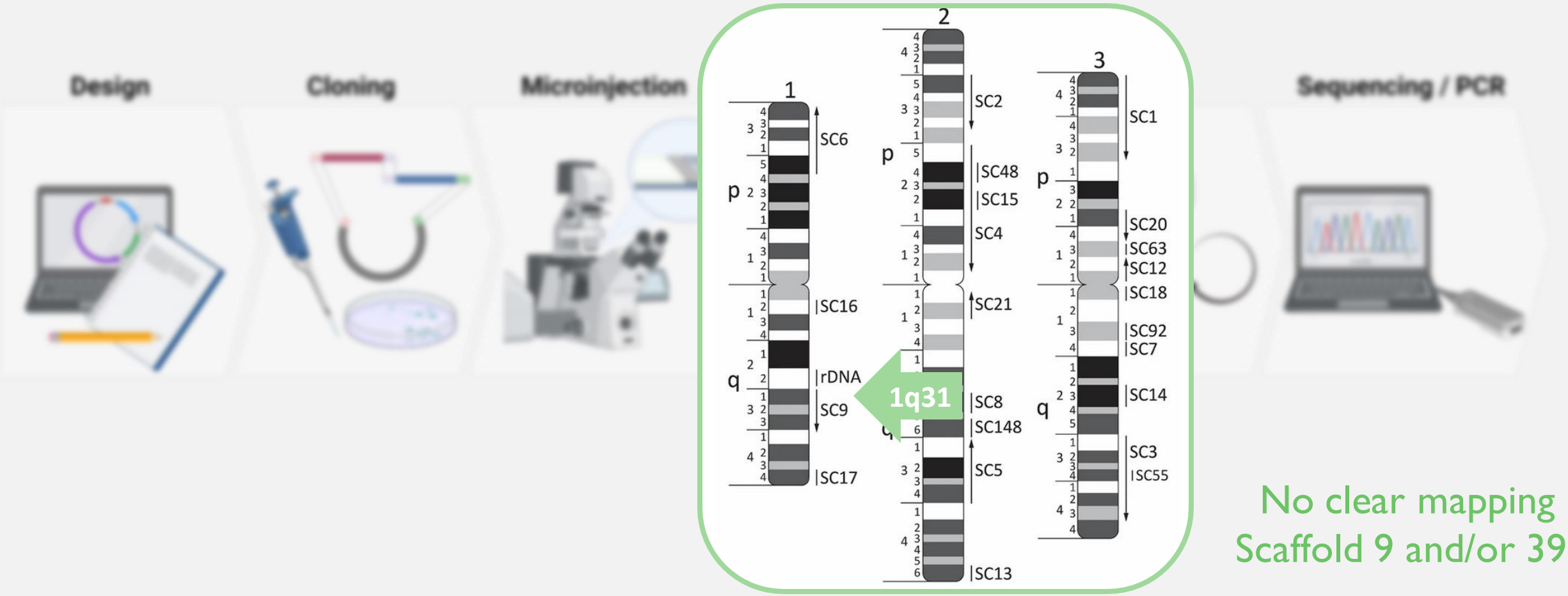
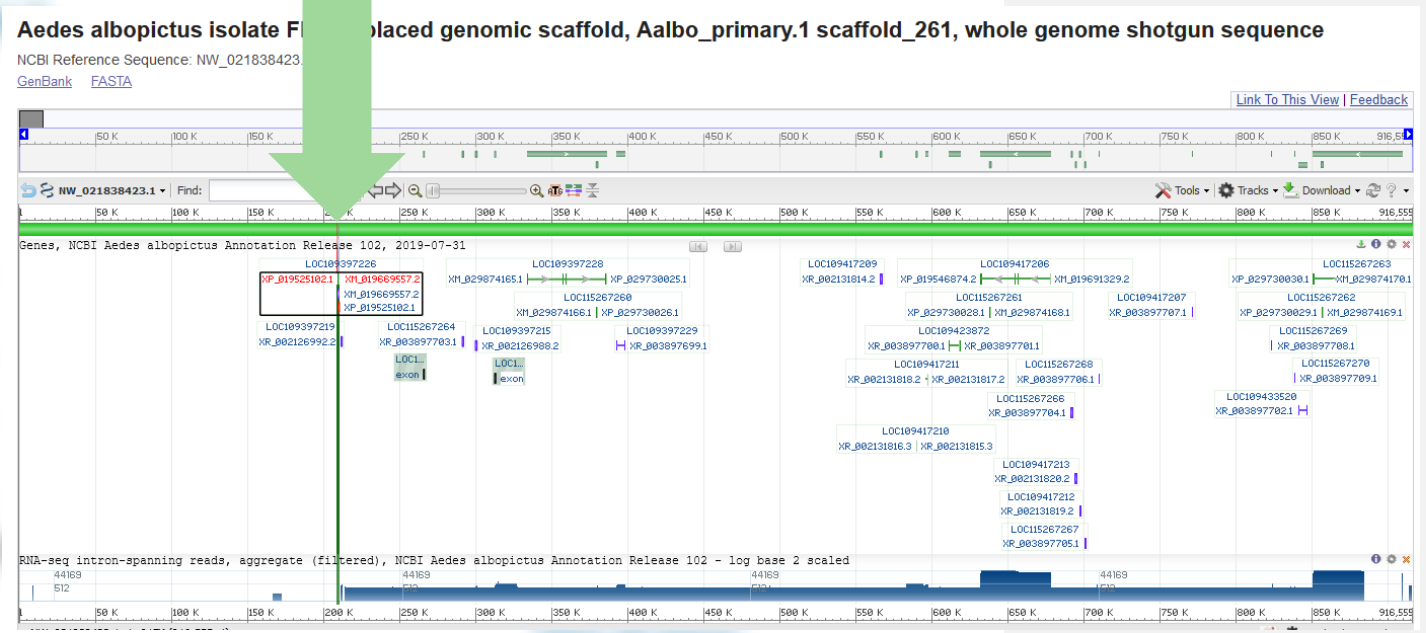


No clear mapping
Scaffold 9 and/or 39?

RESULTS – *AEDES ALBOPICTUS*

Finding targets for the M-locus

M-locus:
Nix is the only gene we know



RESULTS – *AEDES ALBOPICTUS*

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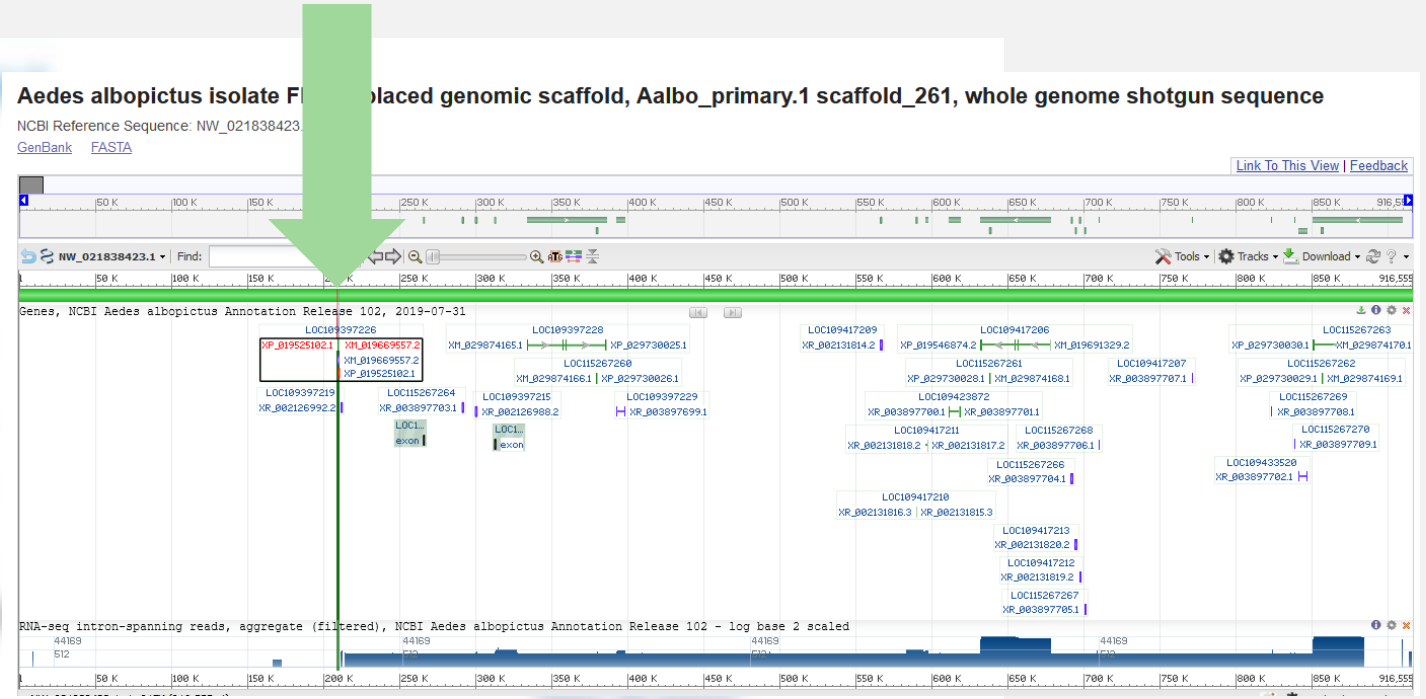
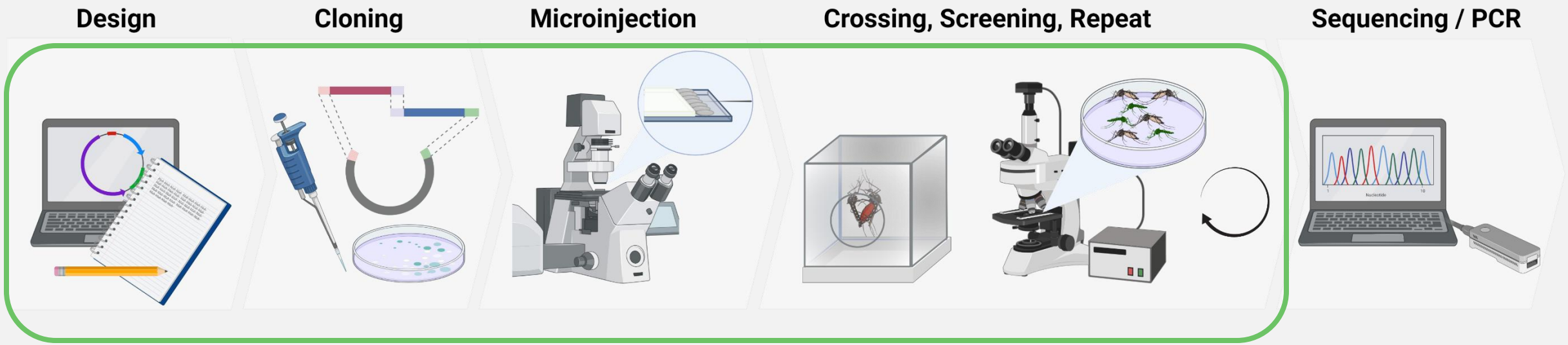


?

No male-specific gene

RESULTS – *AEDES ALBOPICTUS*

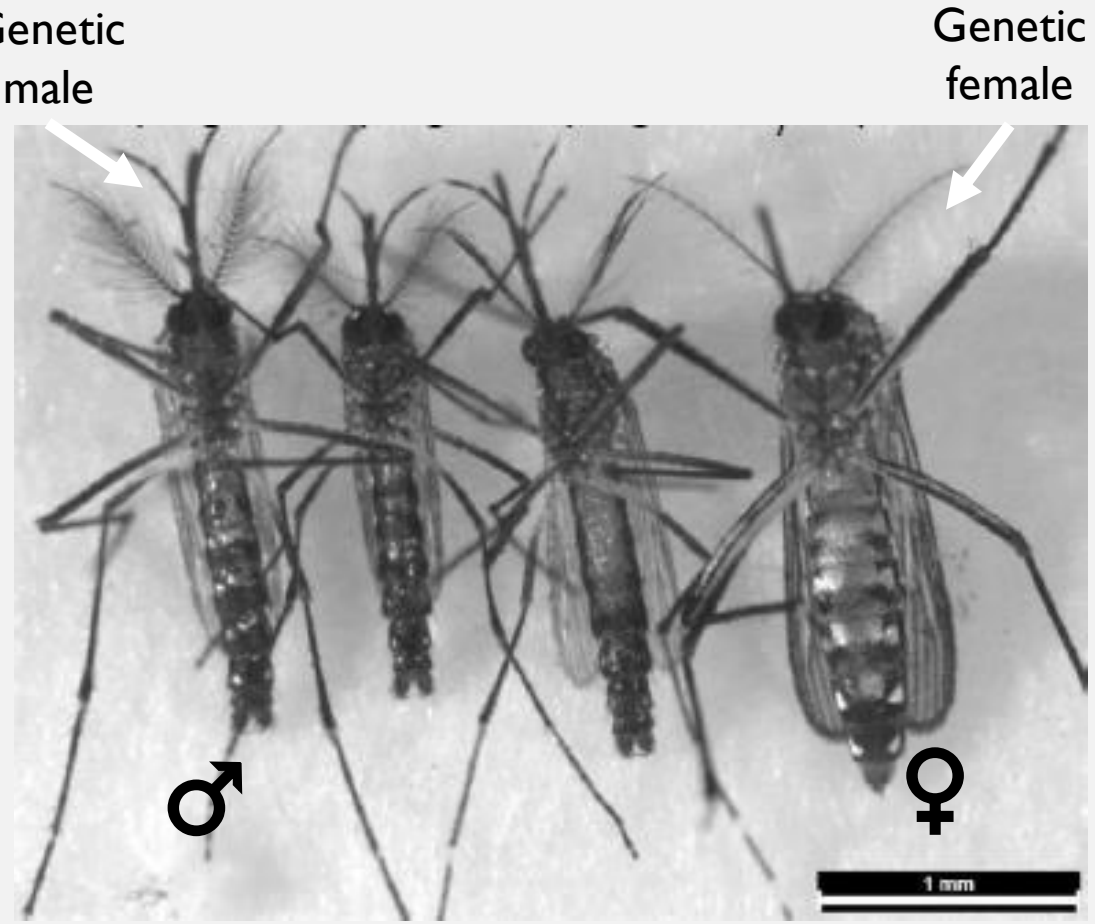
Targeting *Nix* by CRISPR-Cas9 failed



No transgenic

RESULTS – *AEDES ALBOPICTUS*

If you can't reach the M-locus: create your M-locus!

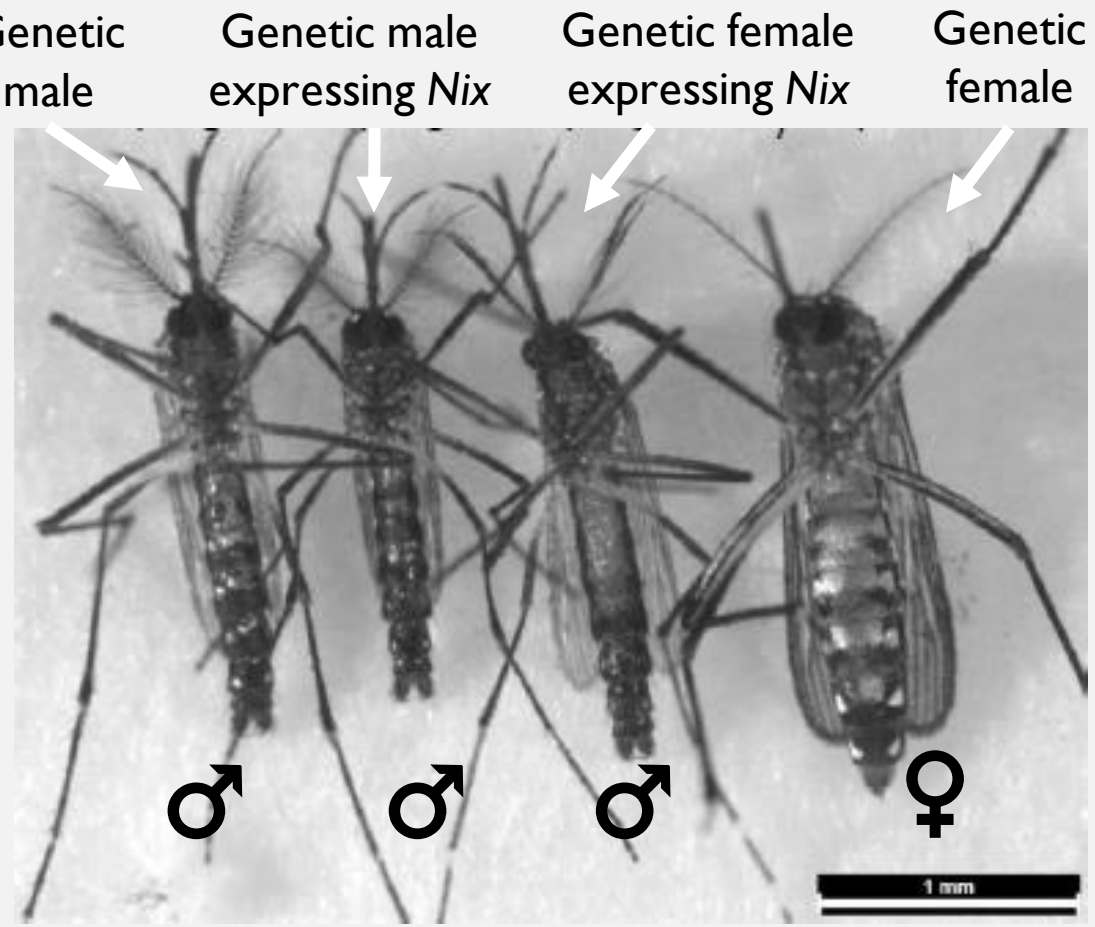


Aryan et al. 2020

RESULTS – *AEDES ALBOPICTUS*

If you can't reach the M-locus: create your M-locus!

Aryan *et al.* (2020) showed in *Ae. aegypti* that *Nix* alone could turn genetic females into fertile phenotypic males



Aryan et al. 2020

RESULTS – *AEDES ALBOPICTUS*

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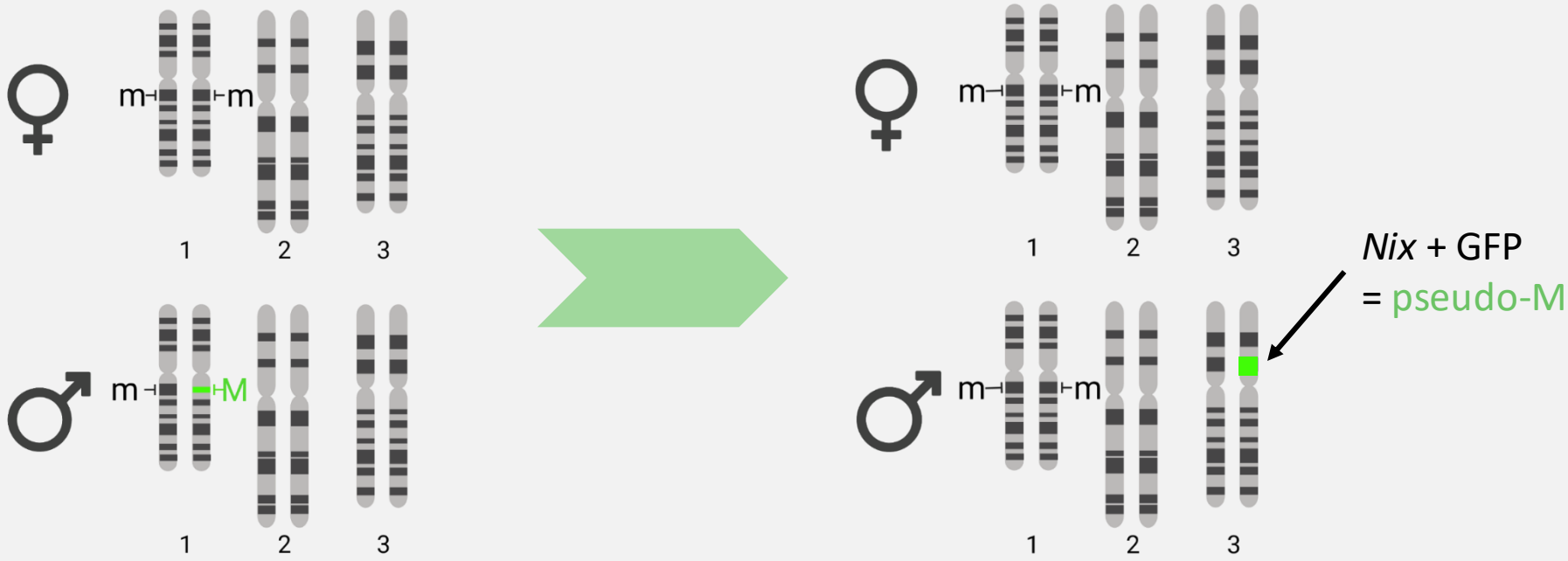
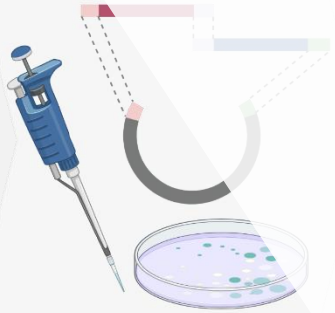


RESULTS – *AEDES ALBOPICTUS*

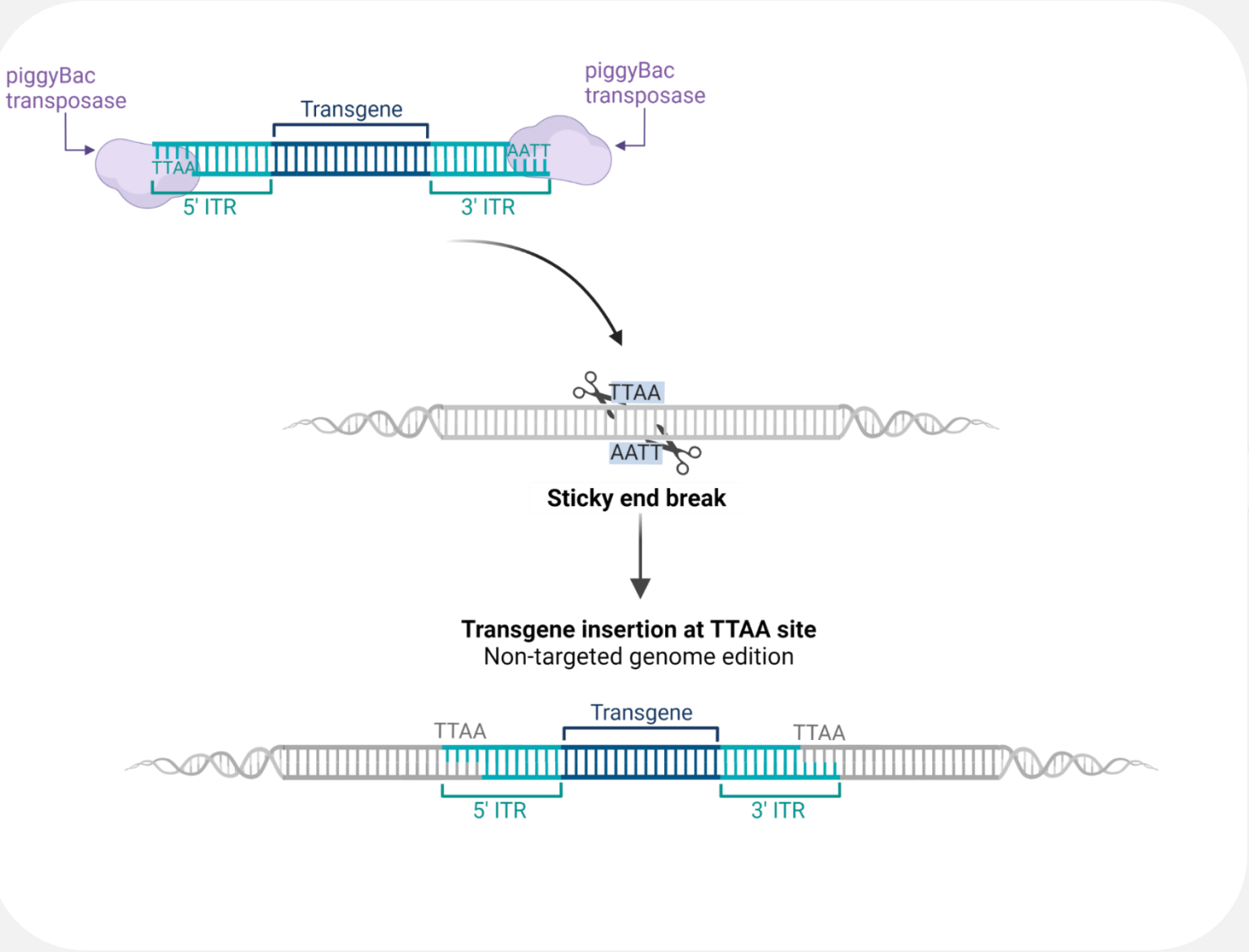
piggyBac approach

Design

Cloning



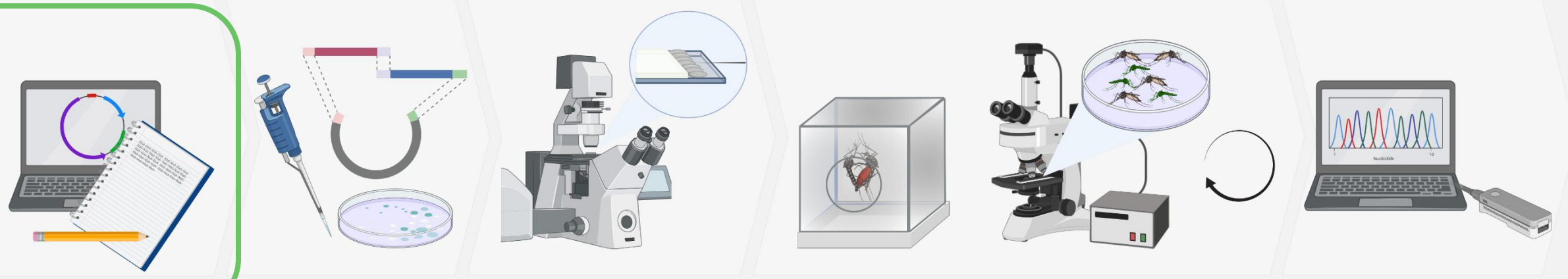
Micro

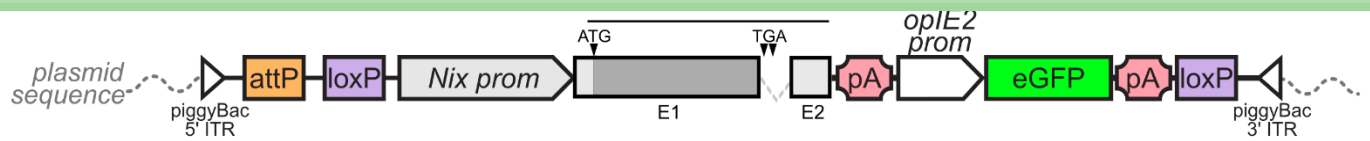


RESULTS – *AEDES ALBOPICTUS*

piggyBac approach

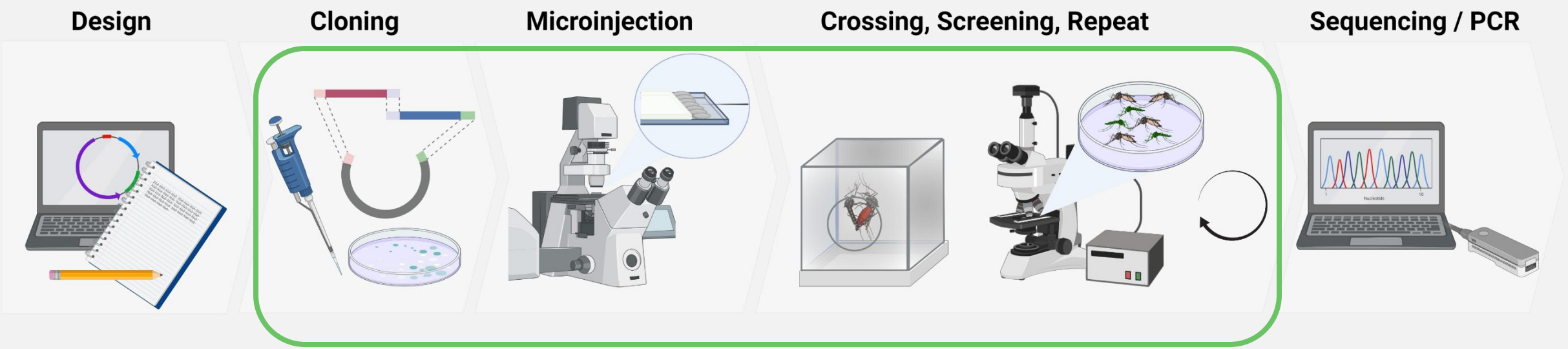
Design | **Cloning** | **Microinjection** | **Crossing, Screening, Repeat** | **Sequencing / PCR**





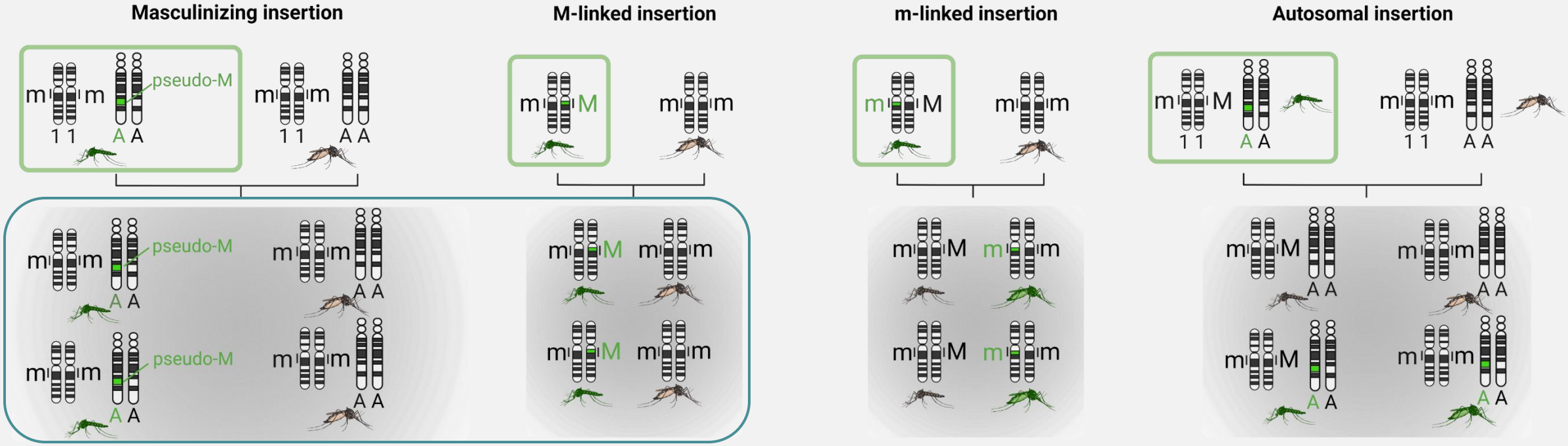
RESULTS – *AEDES ALBOPICTUS*

22 transgenic males
9 transgenic females



RESULTS – *AEDES ALBOPICTUS*

12 individual males

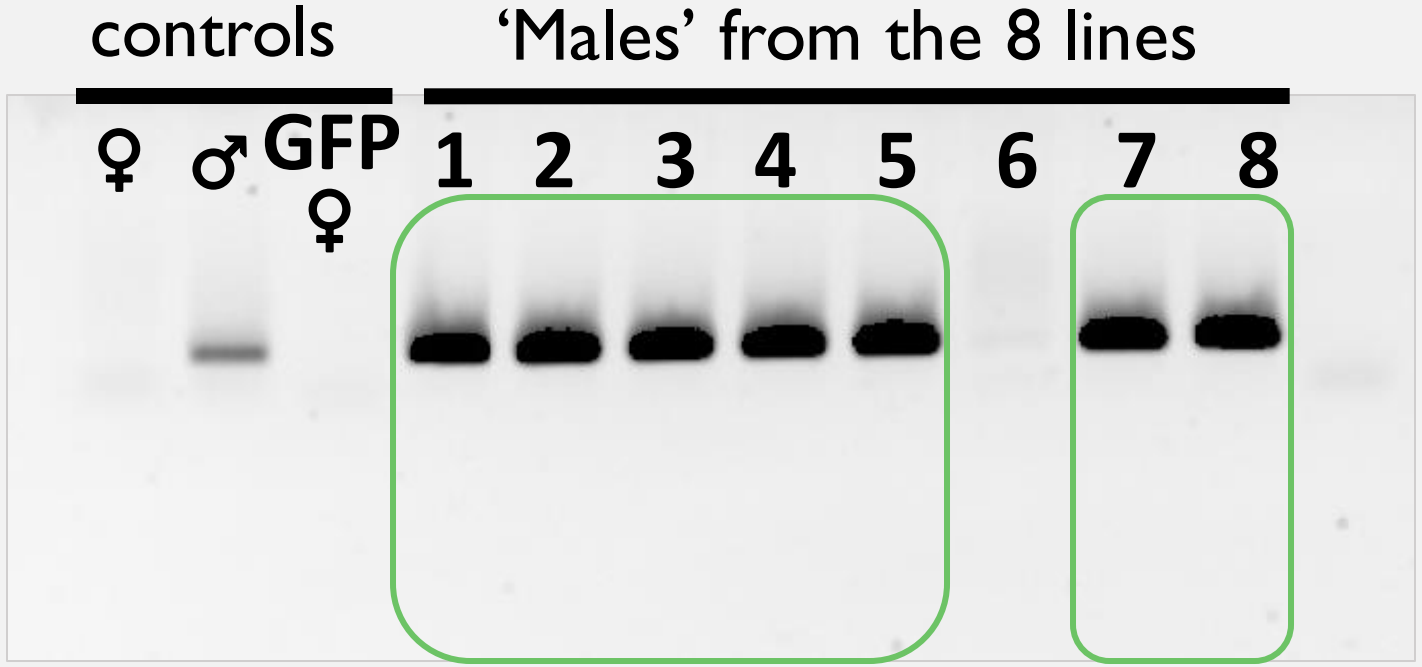


8 lines with transgenic sons and non-transgenic daughters

RESULTS – *AEDES ALBOPICTUS*

Amplification of the endogenous *Nix* gene

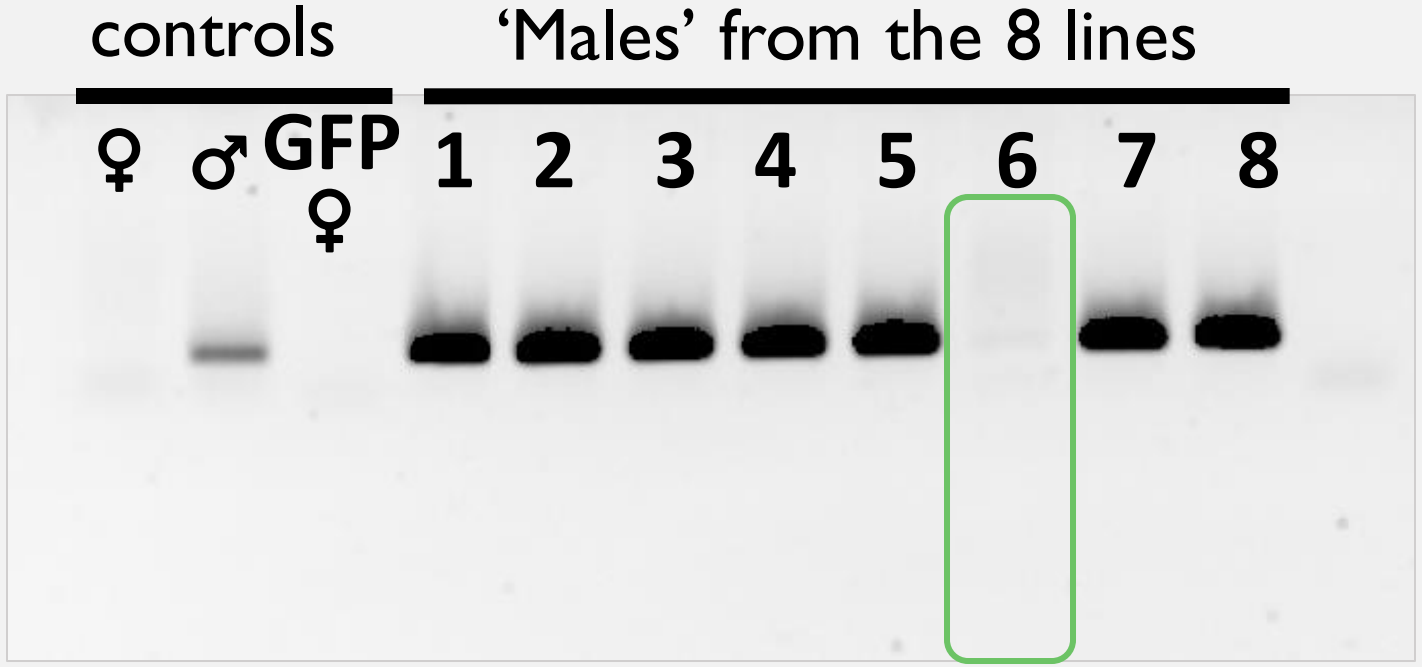
RESULTS – *AEDES ALBOPICTUS*



Amplification of the endogenous *Nix* gene

7 out of 8 carry it: M linkage

RESULTS – *AEDES ALBOPICTUS*



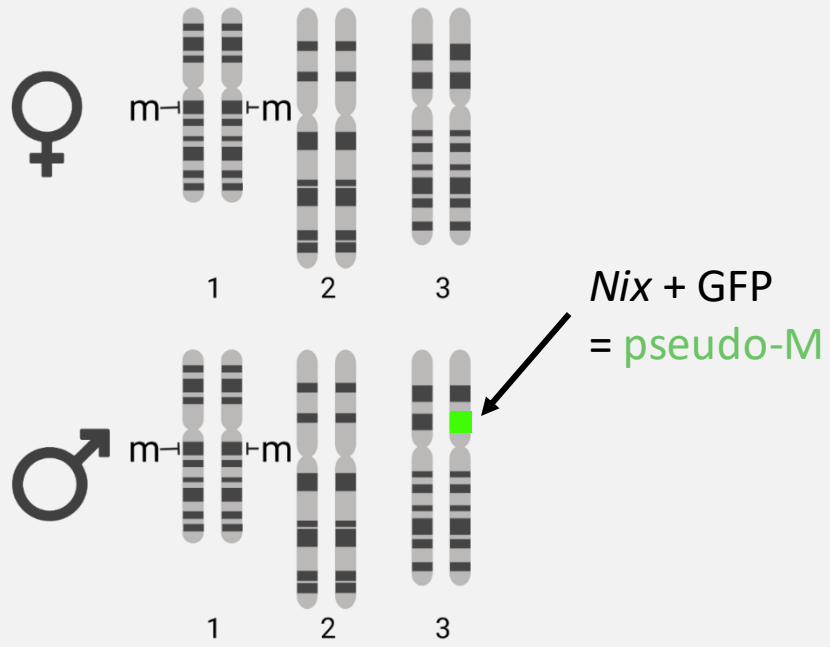
Amplification of the endogenous *Nix* gene

7 out of 8 carry it: M linkage

1 does not: pseudo-M linkage

RESULTS – *AEDES ALBOPICTUS*

Hypotheses



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RESULTS – *AEDES ALBOPICTUS*

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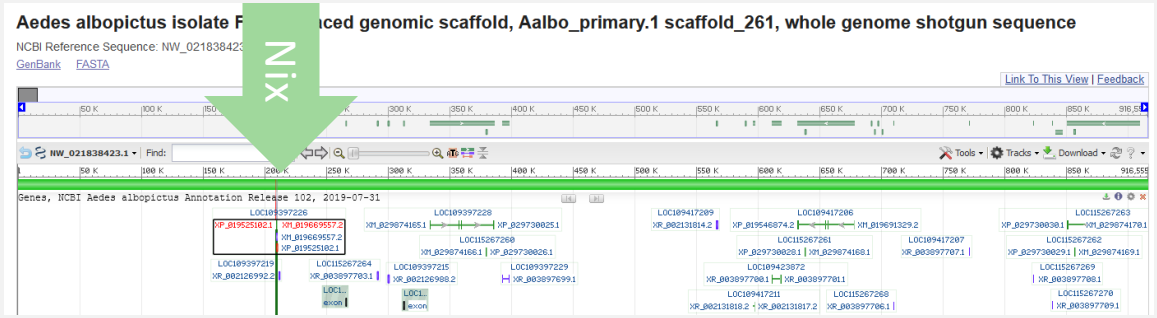
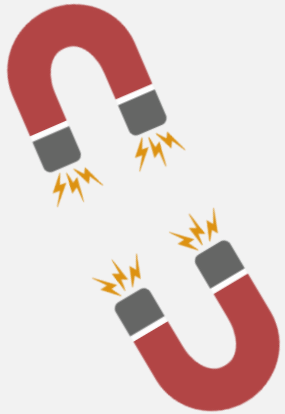
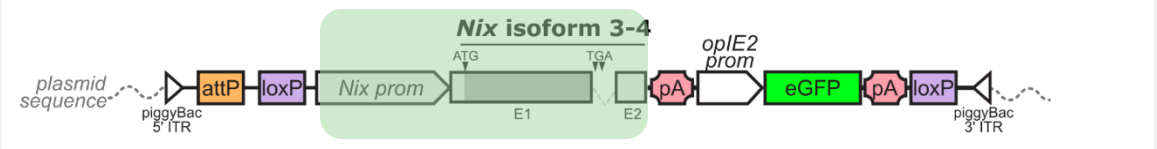




Sequence homology
➔ homing



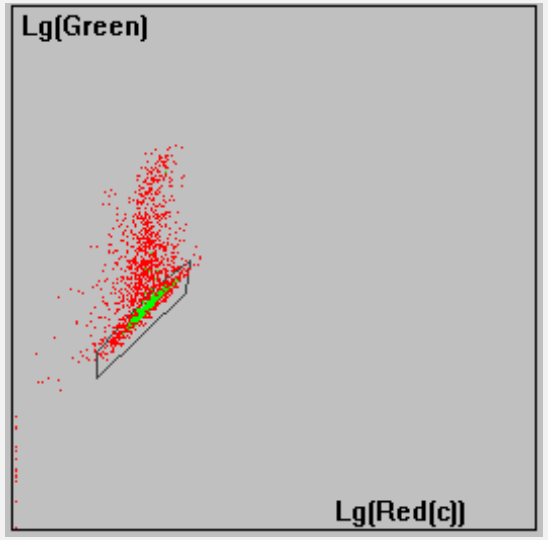
Amplification of the endogenous *Nix* gene

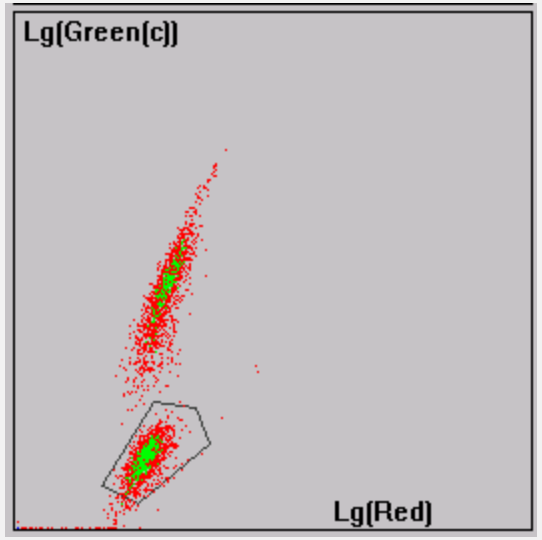
7 out of 8 carry it:
M linkage

I does not:
pseudo-M linkage

RESULTS – *AEDES ALBOPICTUS*

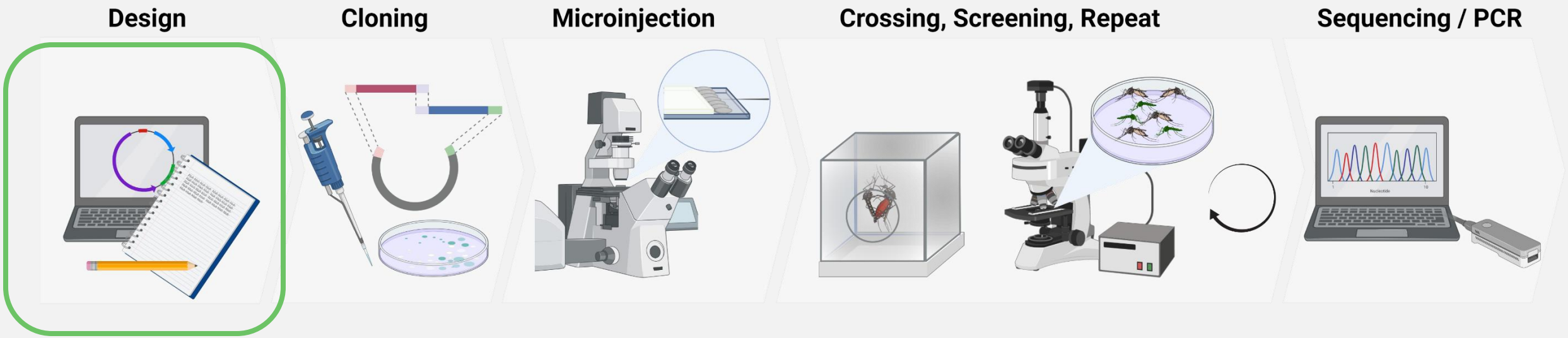
Issue: GFP is weak and unevenly expressed under the OpIE2 promoter

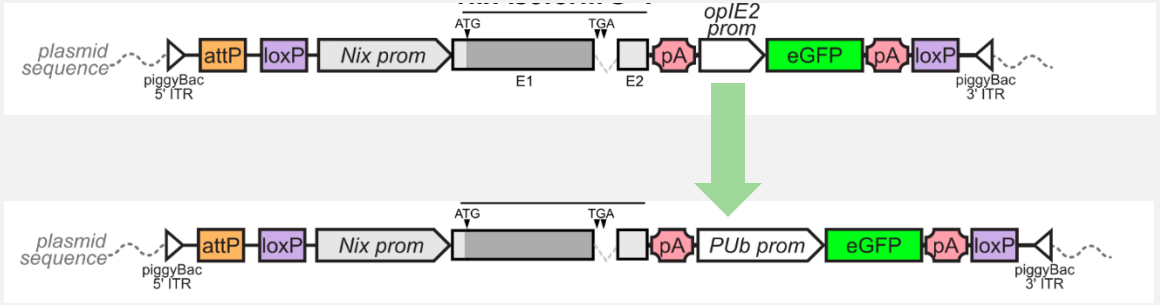




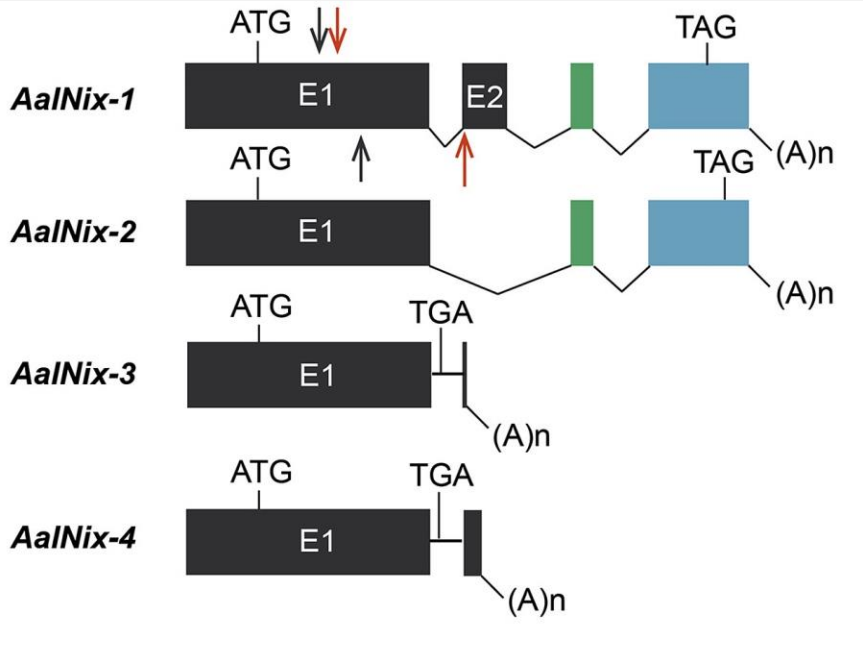
RESULTS – *AEDES ALBOPICTUS*

Solution: replacing OpIE2 with PUb promoter



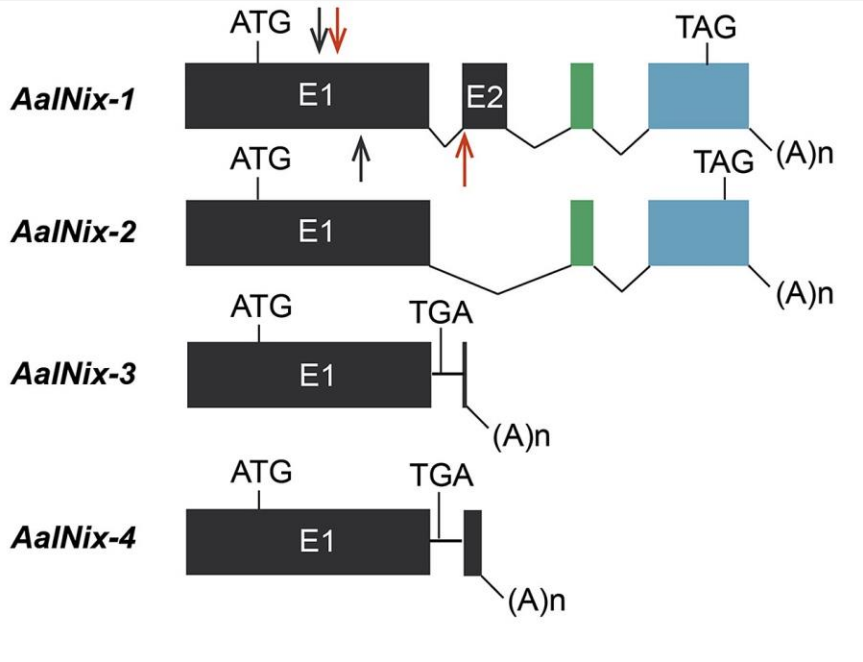


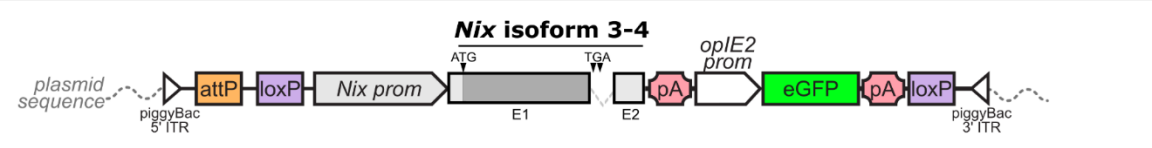
RESULTS – *AEDES ALBOPICTUS*

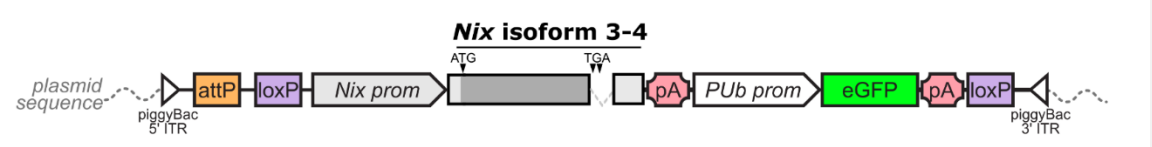


Liu et al. 2020

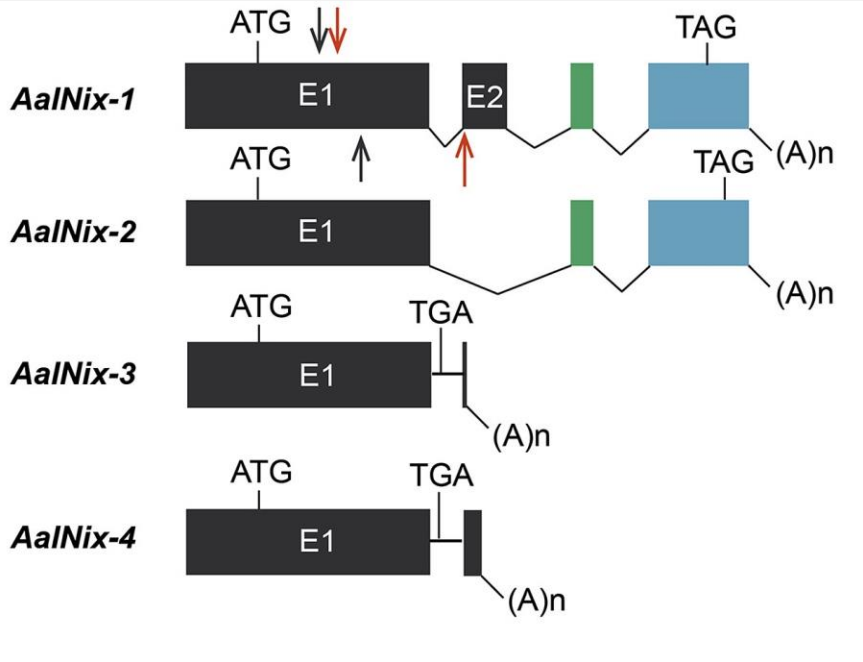
RESULTS – *AEDES ALBOPICTUS*

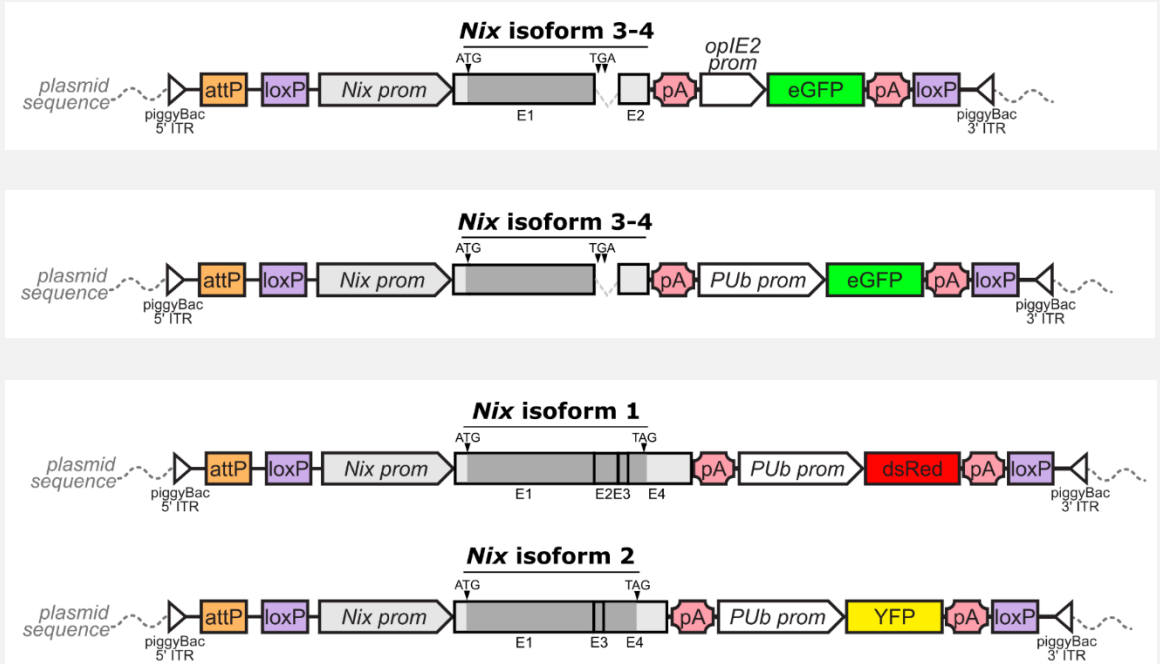






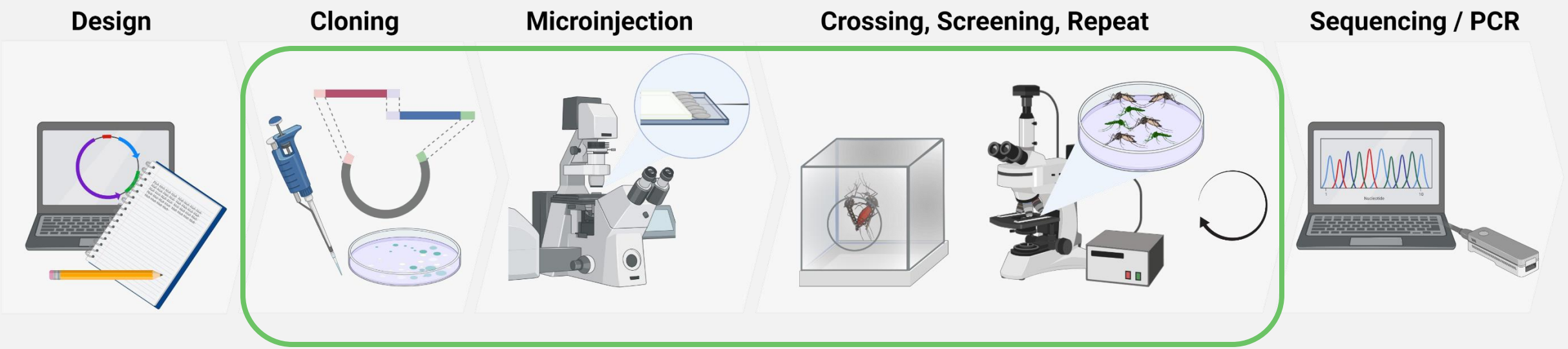
RESULTS – *AEDES ALBOPICTUS*





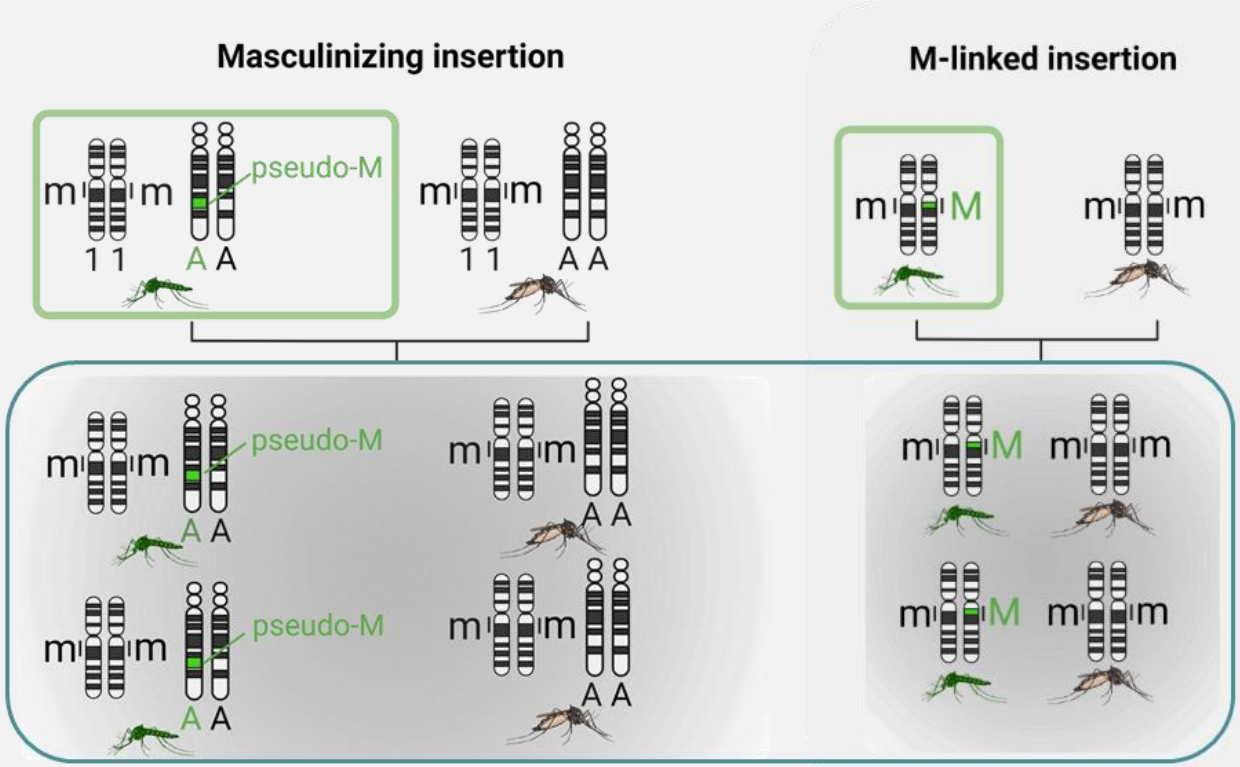
RESULTS – *AEDES ALBOPICTUS*

> 100 transgenic individuals
~85% of males



RESULTS – *AEDES ALBOPICTUS*

13 single-male lines
with 100% male fluorescence



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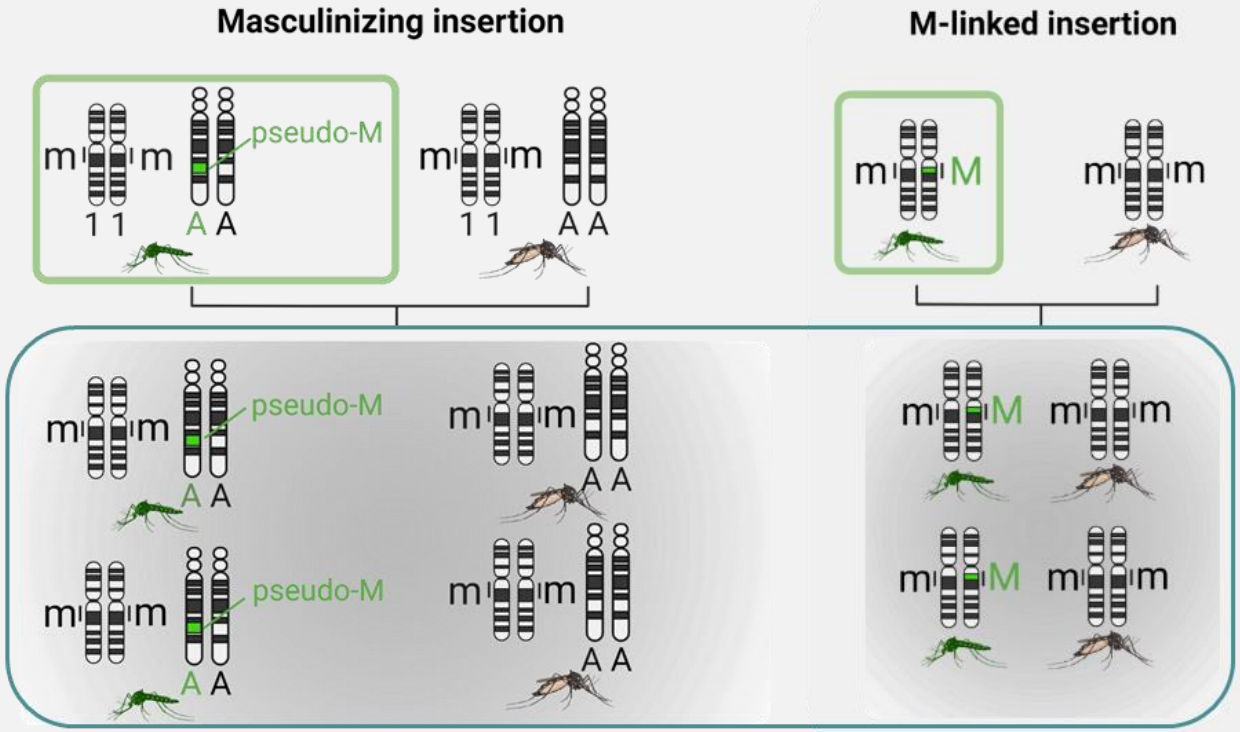

Amplification of the endogenous *Nix* gene

RESULTS – *AEDES ALBOPICTUS*

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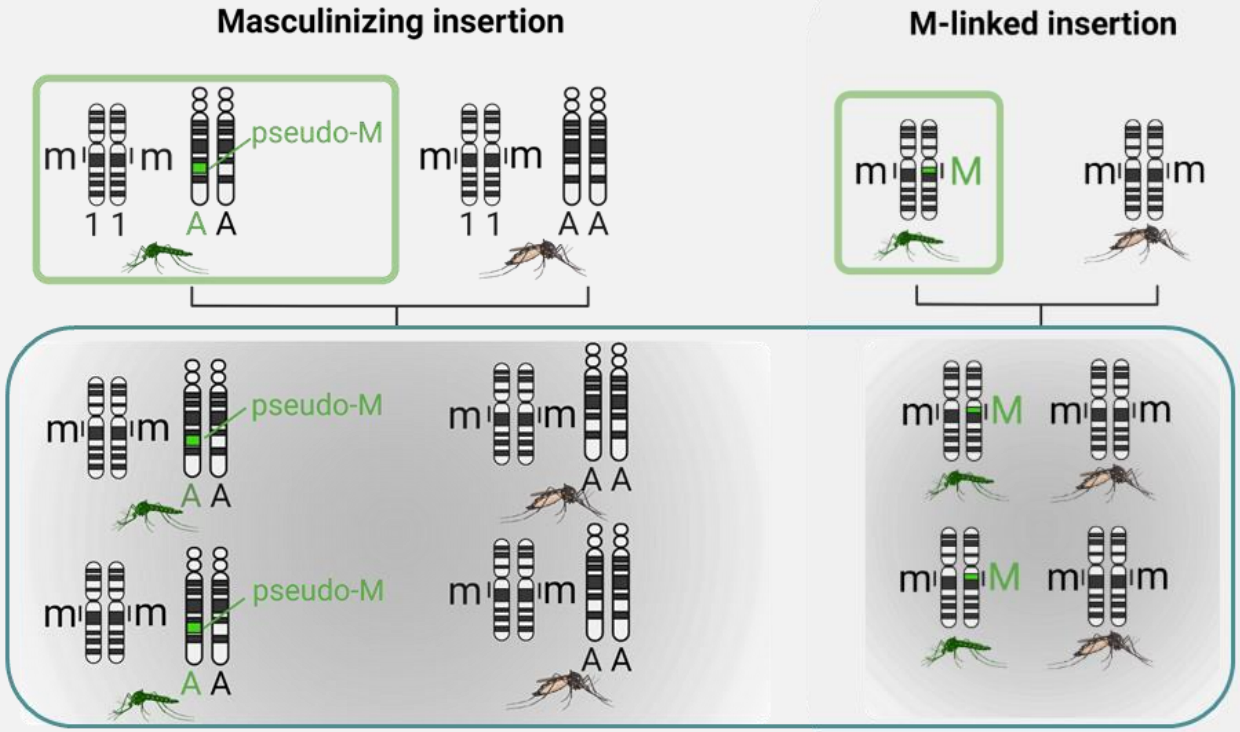

Amplification of the endogenous *Nix* gene

4 out of 13 were pseudo-M linked (GFP)

RESULTS – *AEDES ALBOPICTUS*

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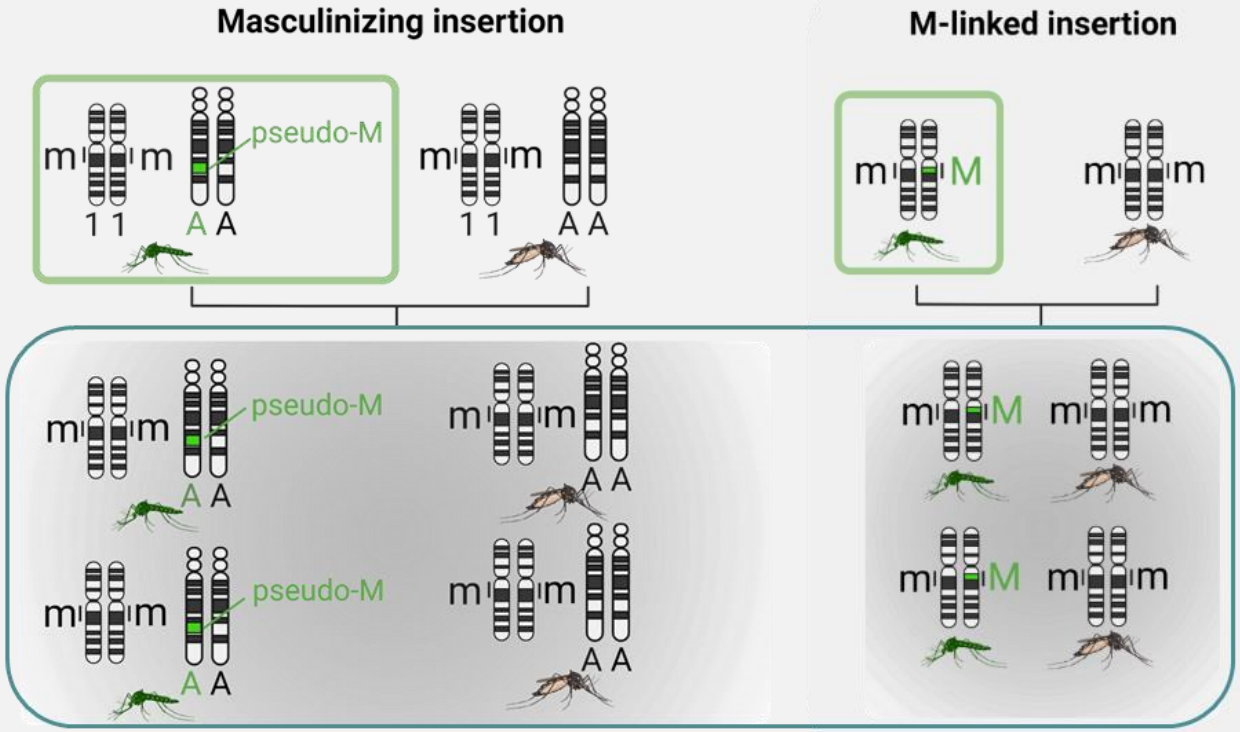


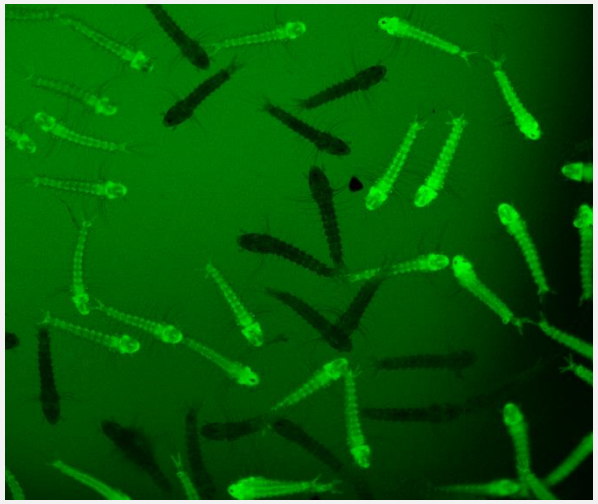
Amplification of the endogenous *Nix* gene

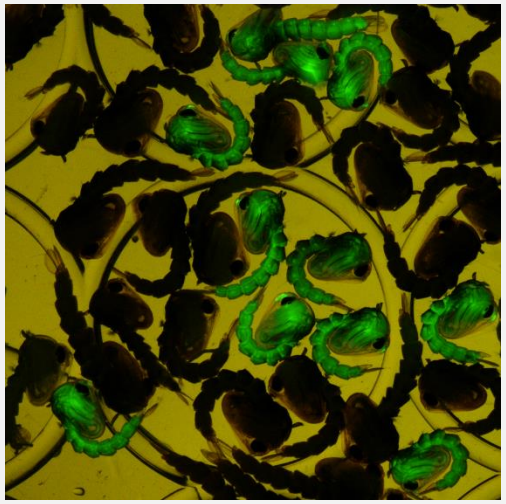
4 out of 13 were pseudo-M linked (GFP)

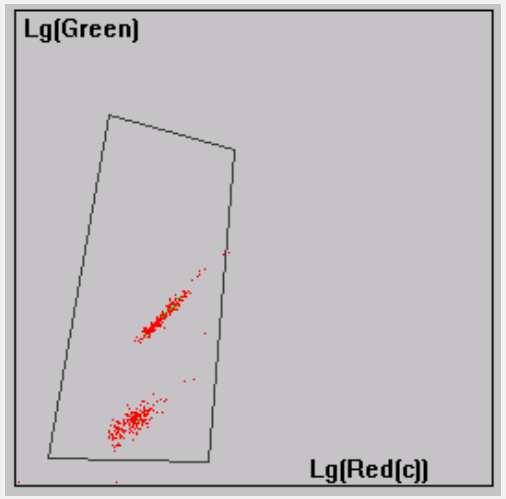
7 out of 13 were M linked (YFP and/or DsRed)

RESULTS – *AEDES ALBOPICTUS*

Selection of an M-linked GSS







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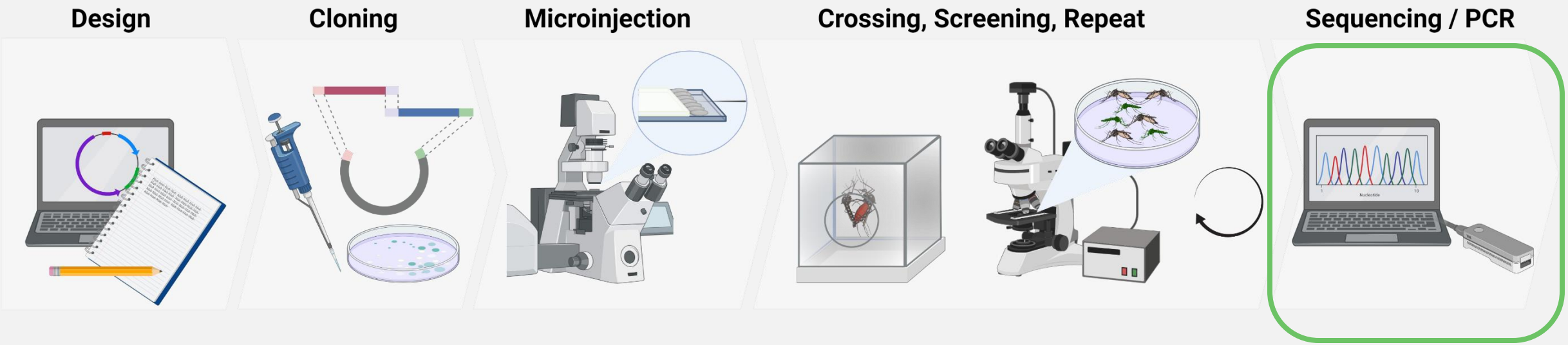
RESULTS – *AEDES ALBOPICTUS*

Selection of an M-linked GSS

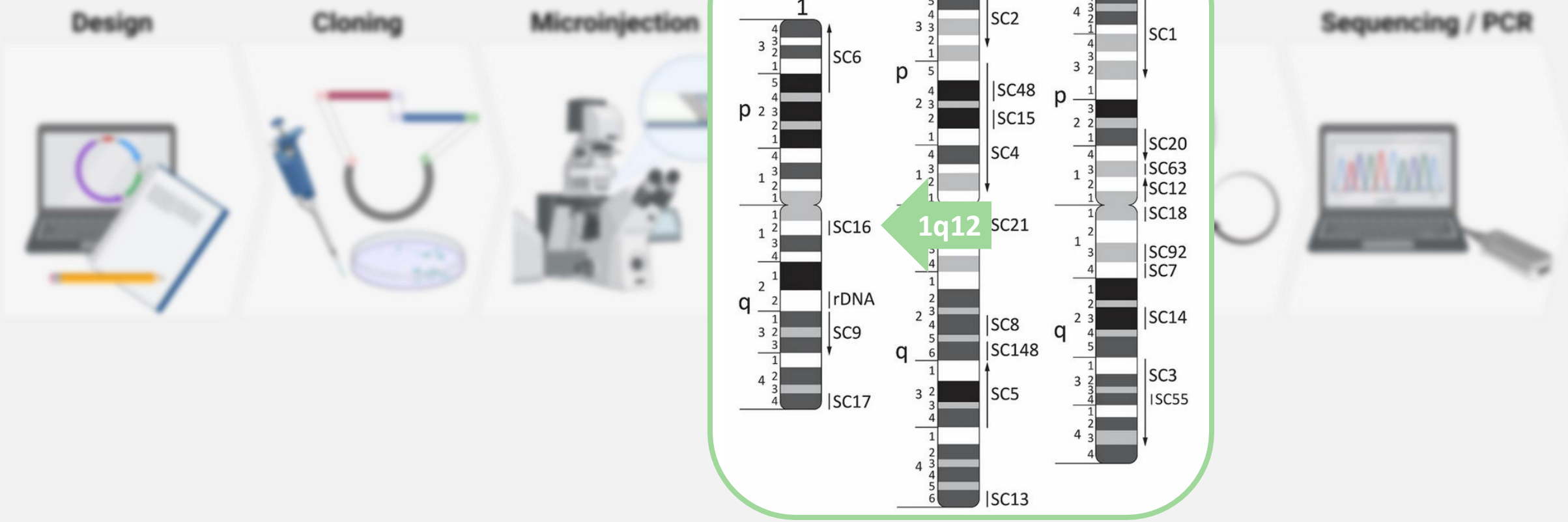
Mapped to scaffold 16



RESULTS – *AEDES ALBOPICTUS*

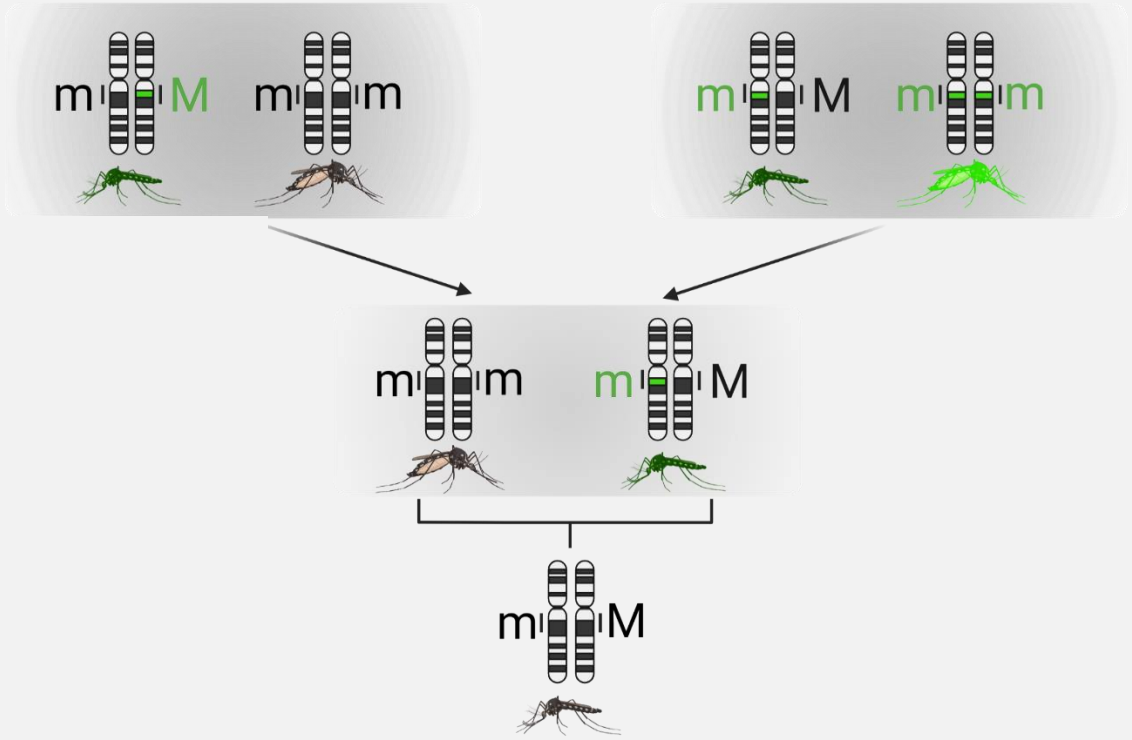
Selection of an M-linked GSS

Mapped to scaffold 16



RESULTS – *AEDES ALBOPICTUS*

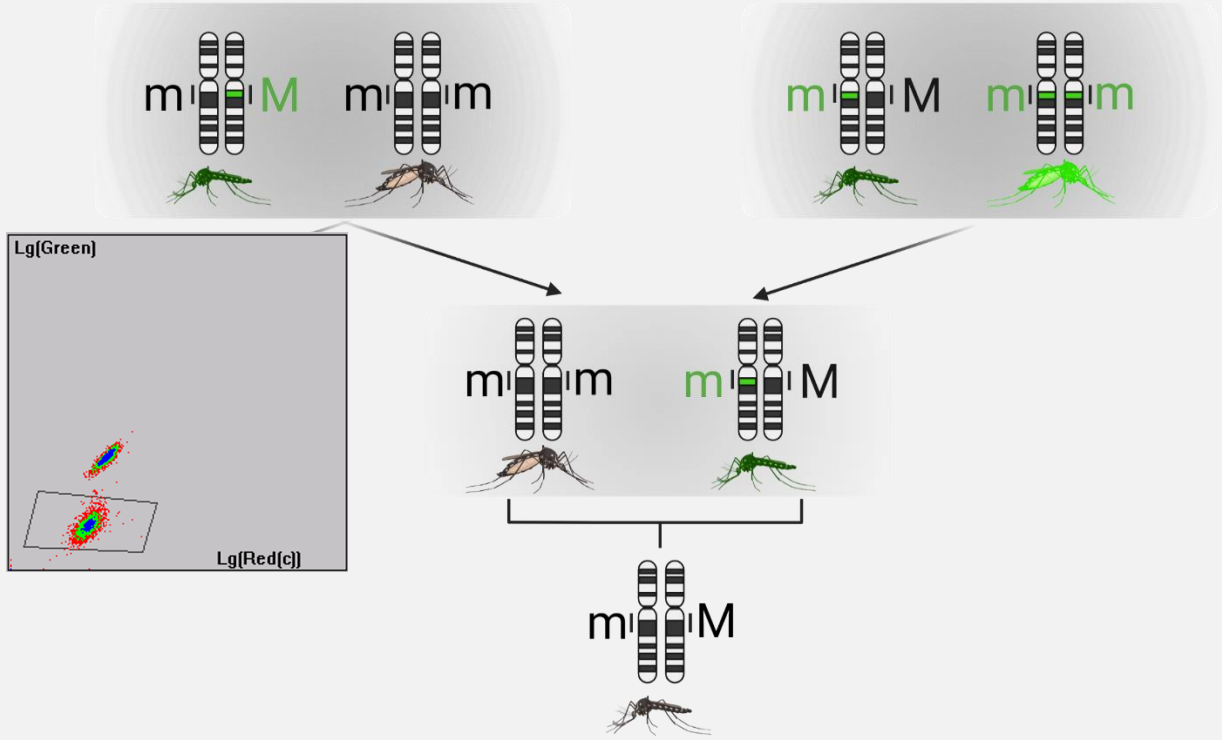
Obtaining non-transgenic males



RESULTS – *AEDES ALBOPICTUS*

Obtaining non-transgenic males

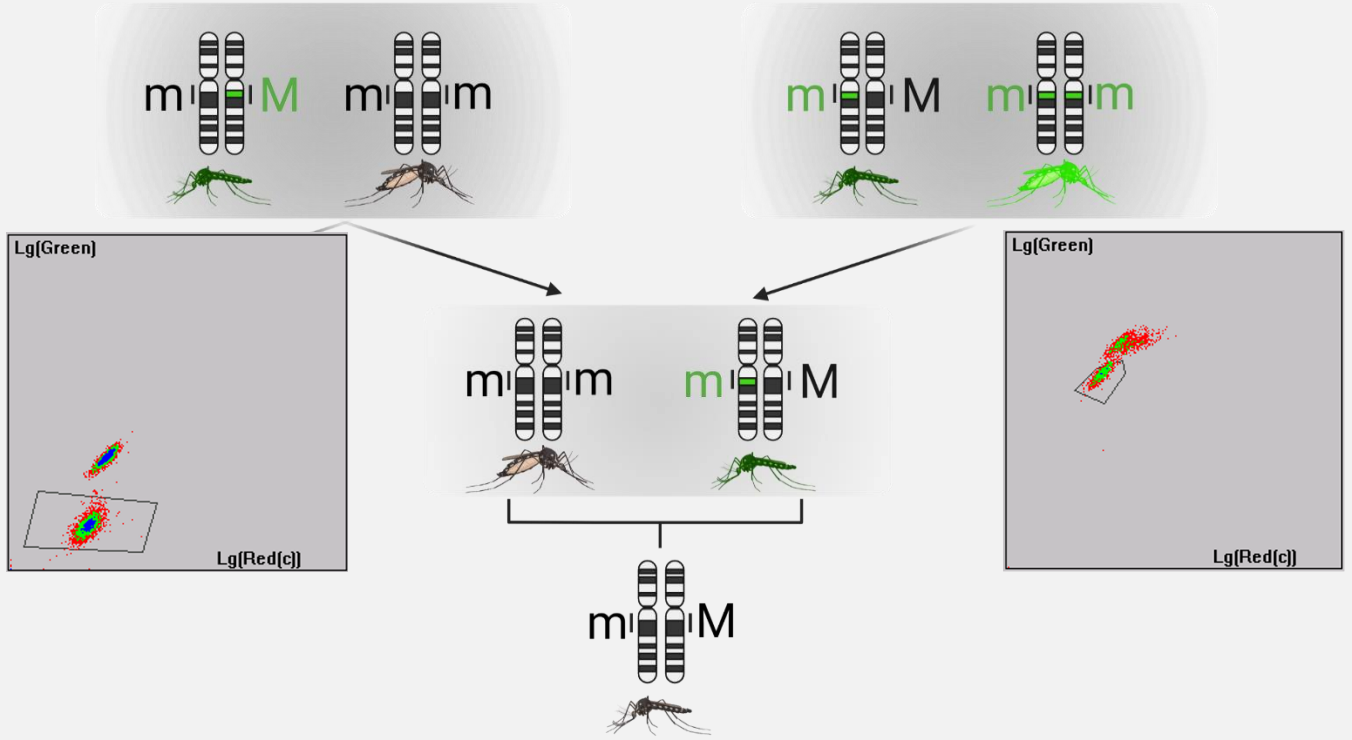
850 non-transgenic females from the M-linked GSS



RESULTS – *AEDES ALBOPICTUS*

Obtaining non-transgenic males

850 non-transgenic females from the M-linked GSS

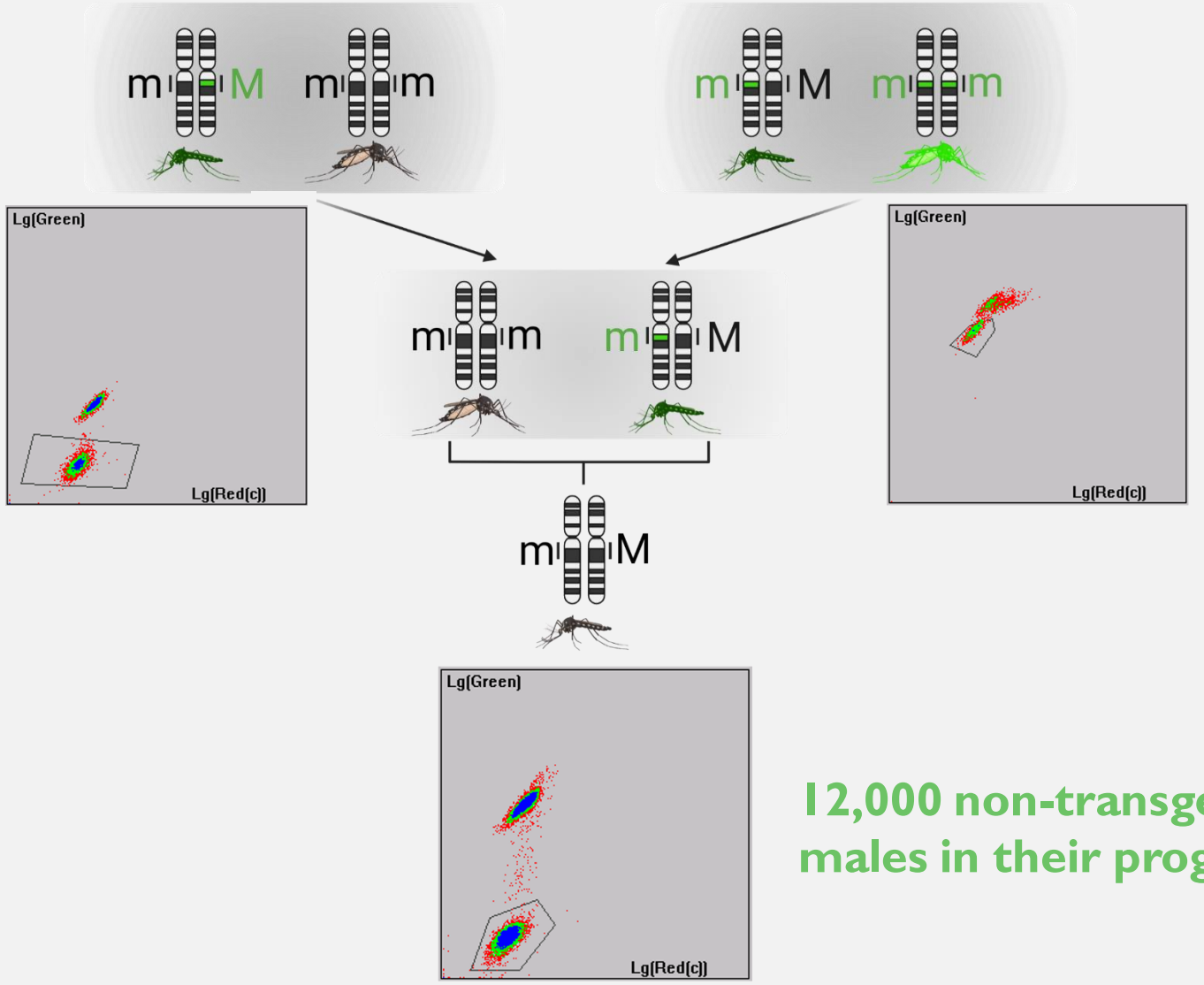


250 hemizygous transgenic males from the m-linked GSS

RESULTS – *AEDES ALBOPICTUS*

Obtaining non-transgenic males

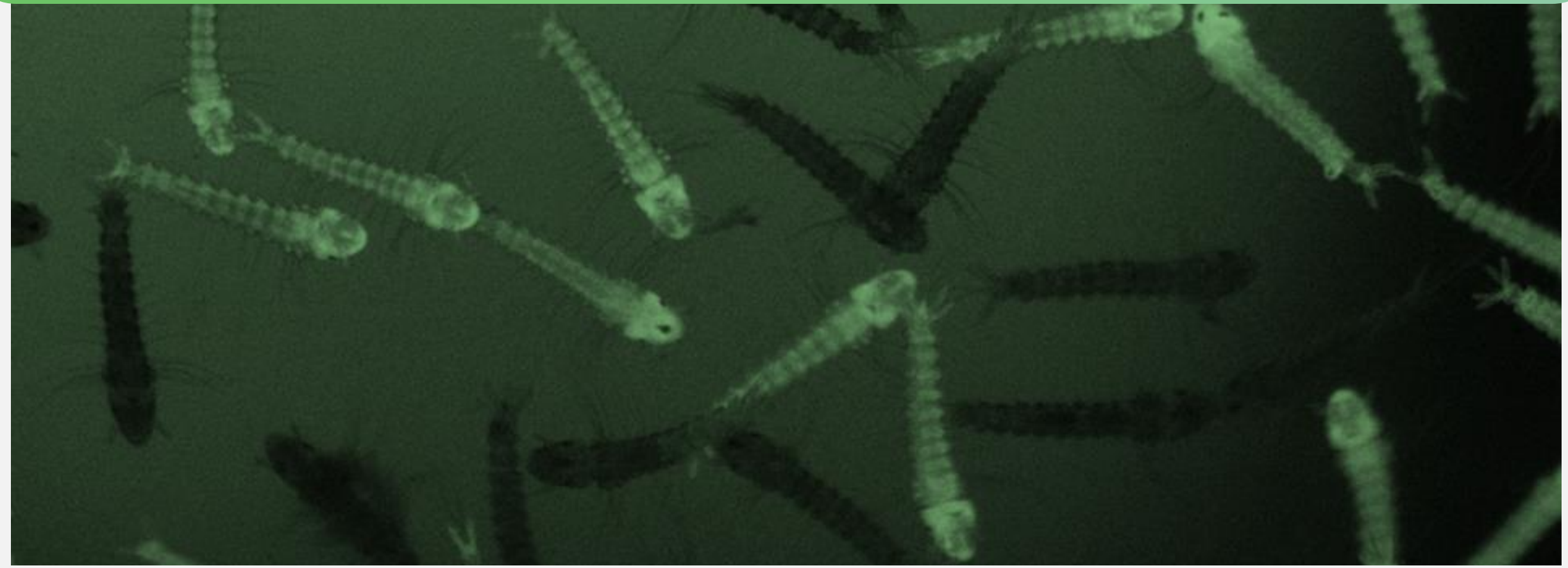
850 non-transgenic females from the M-linked GSS



250 hemizygous transgenic males from the m-linked GSS

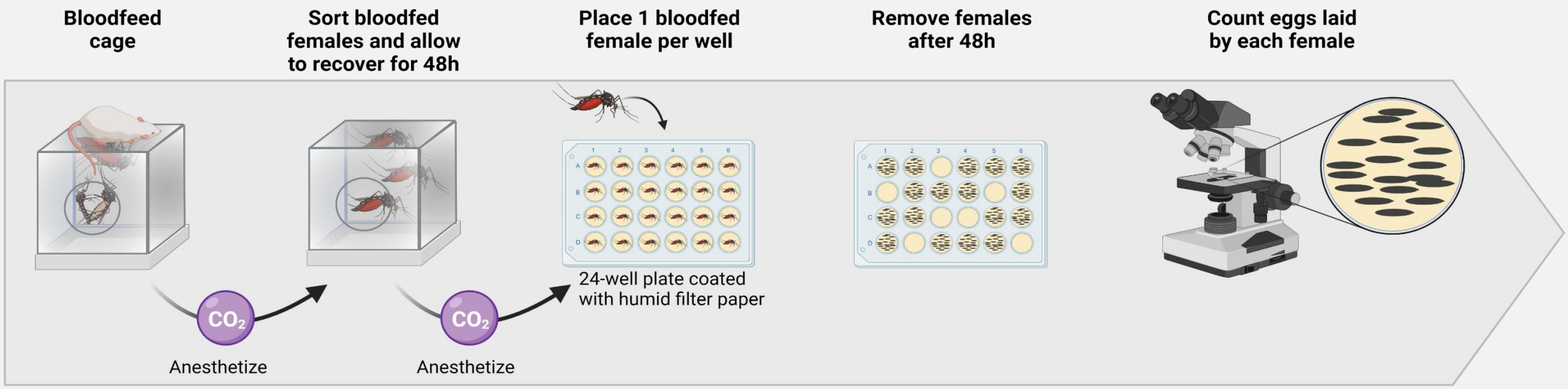
12,000 non-transgenic males in their progeny

RESULTS – PERFORMANCE OF THE GSS



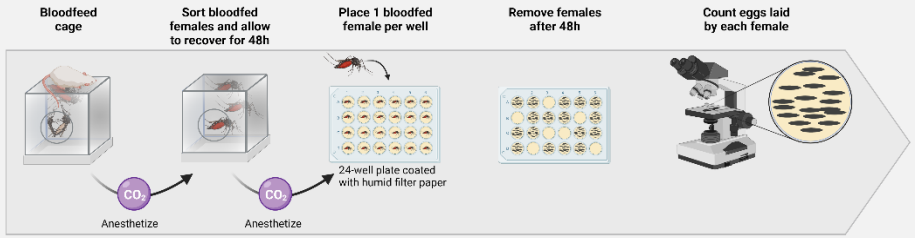
RESULTS – PERFORMANCE OF THE GSS

Fecundity

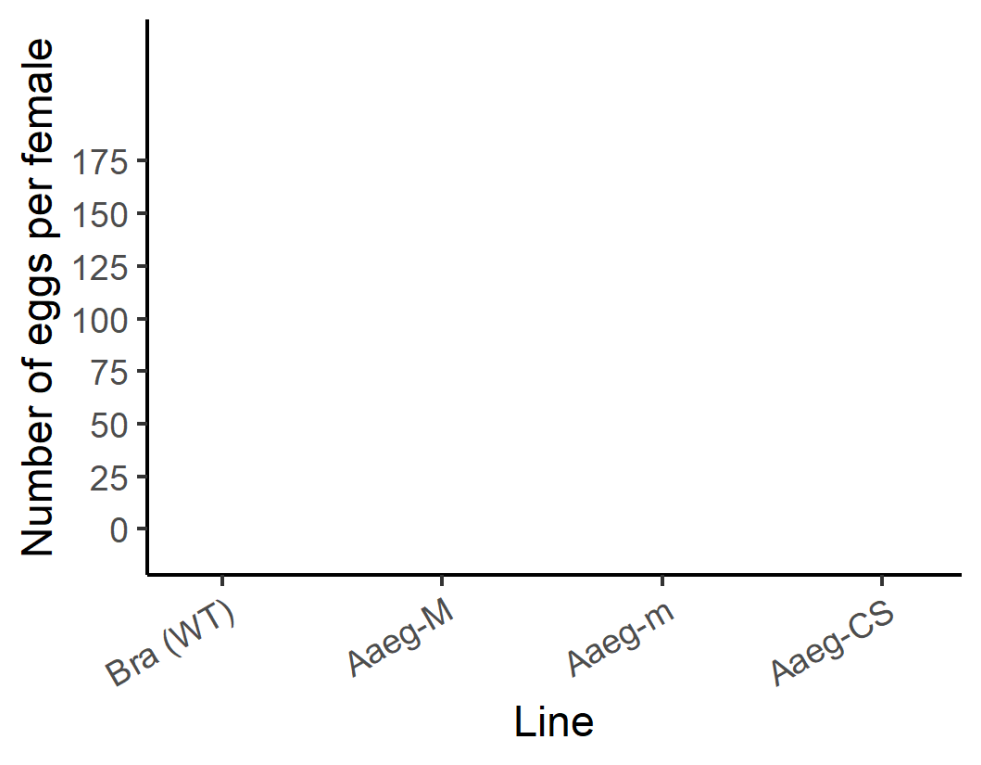


RESULTS – PERFORMANCE OF THE GSS

Fecundity

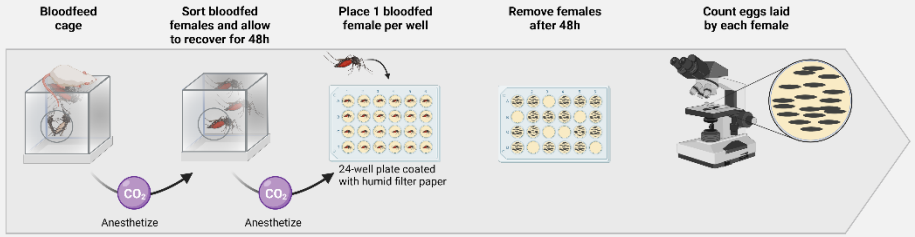


Aedes aegypti

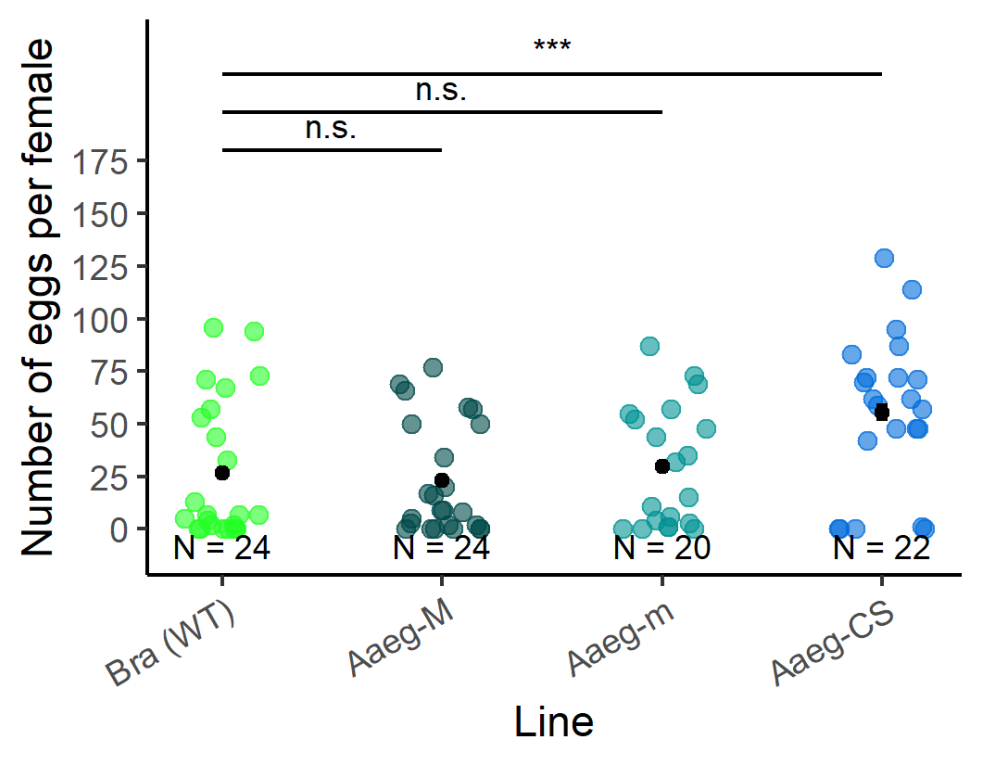


RESULTS – PERFORMANCE OF THE GSS

Fecundity

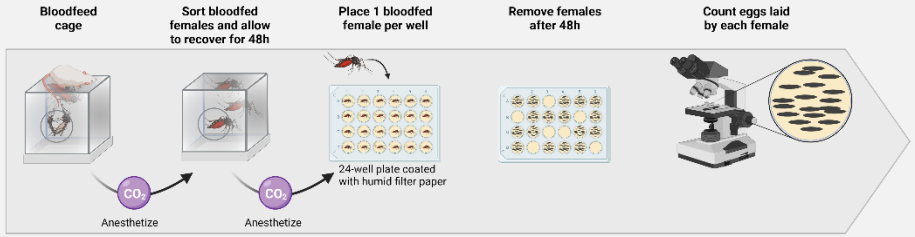


Aedes aegypti

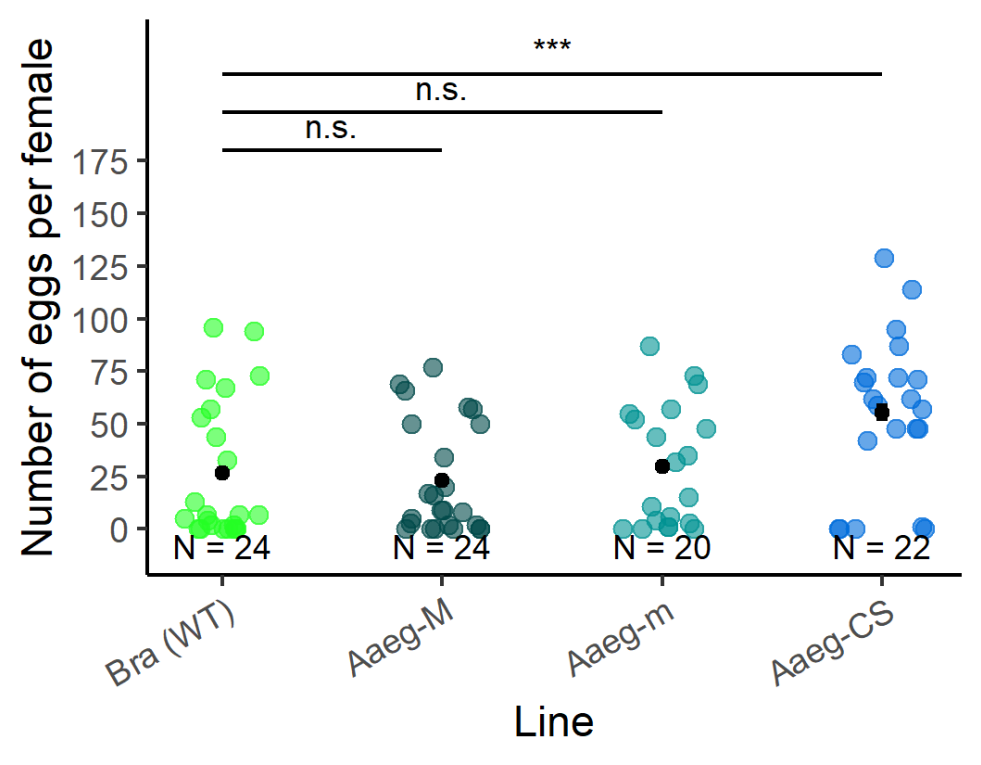


RESULTS – PERFORMANCE OF THE GSS

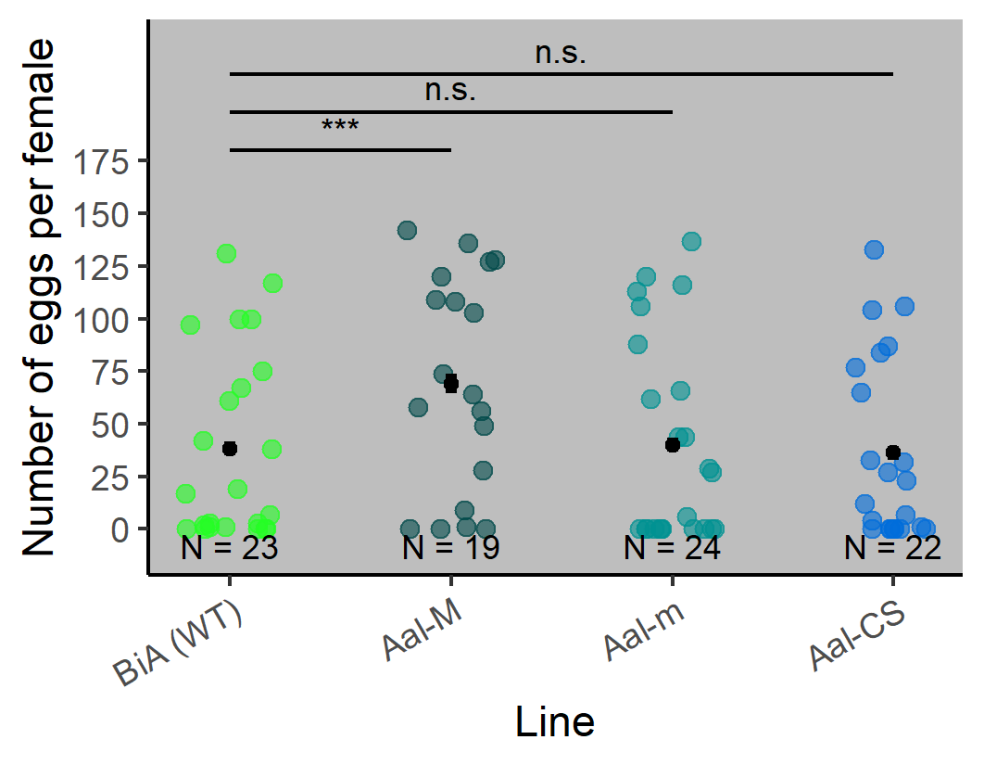
Fecundity



Aedes aegypti



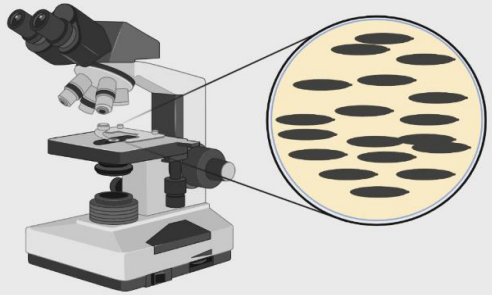
Aedes albopictus



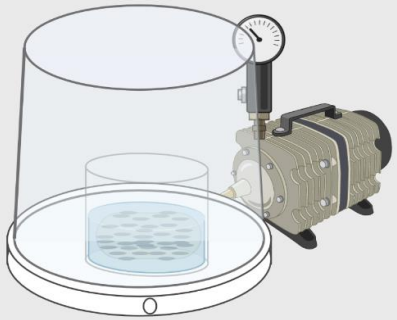
RESULTS – PERFORMANCE OF THE GSS

Egg hatching rate

Count eggs

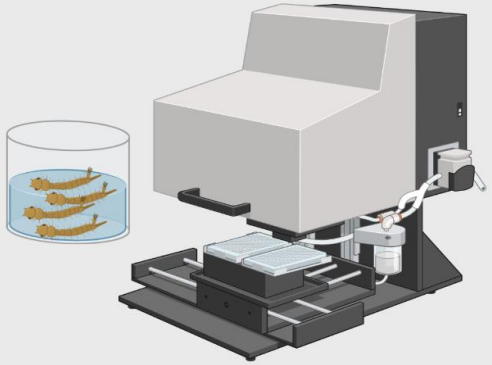


Submerge eggs and apply a vaccuum

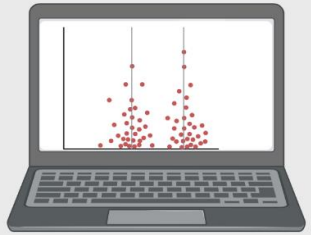


30' vaccuum

Count larvae using COPAS

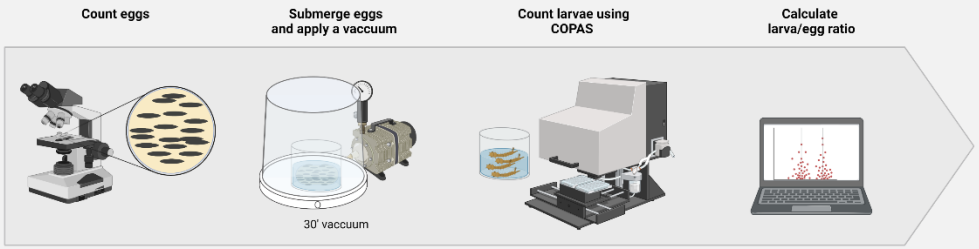


Calculate larva/egg ratio

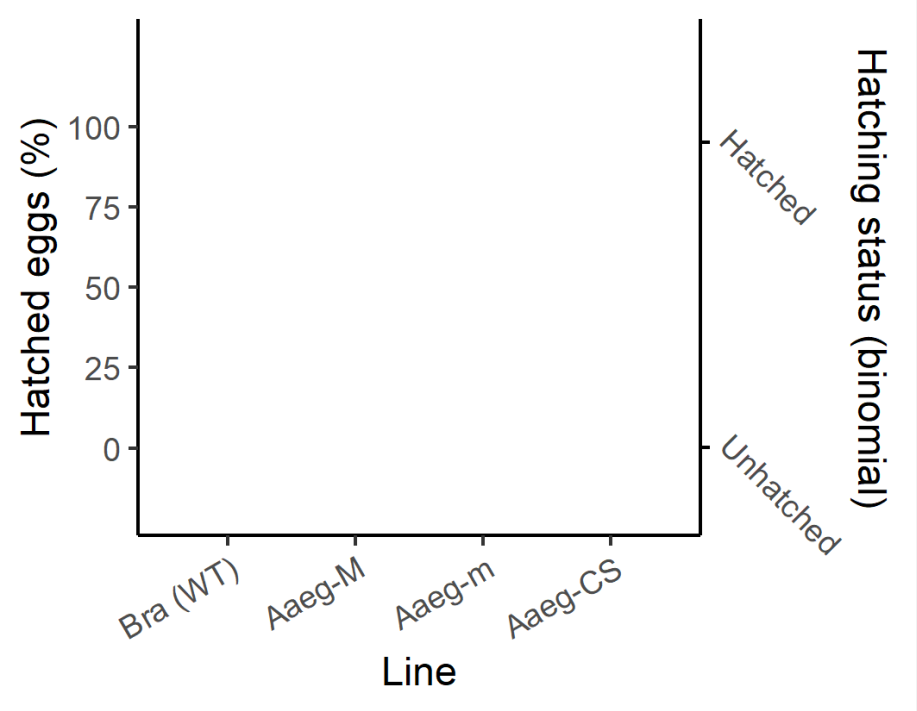


RESULTS – PERFORMANCE OF THE GSS

Egg hatching rate

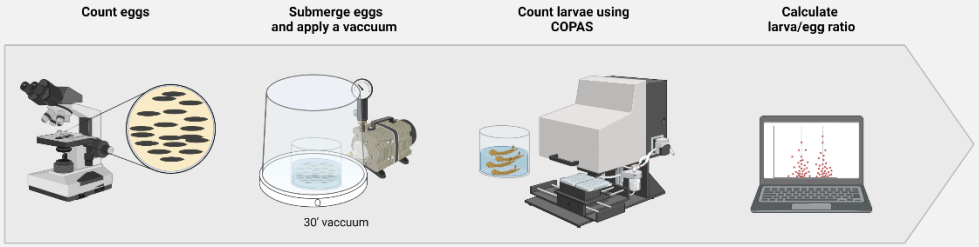


Aedes aegypti

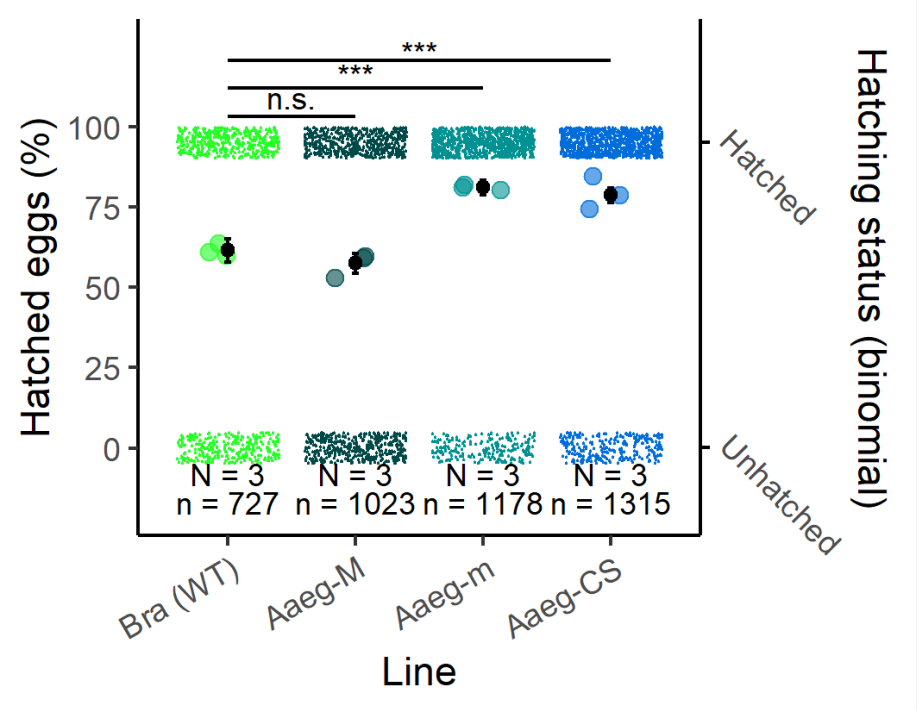


RESULTS – PERFORMANCE OF THE GSS

Egg hatching rate

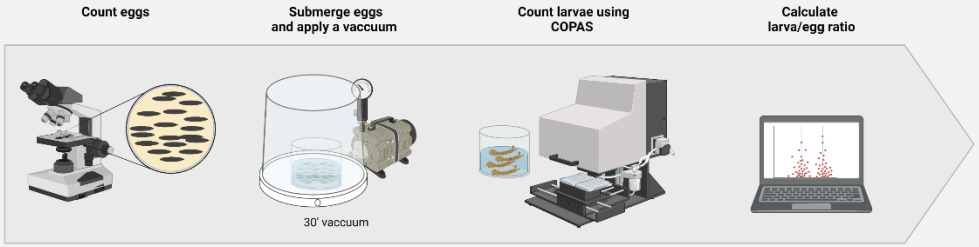


Aedes aegypti

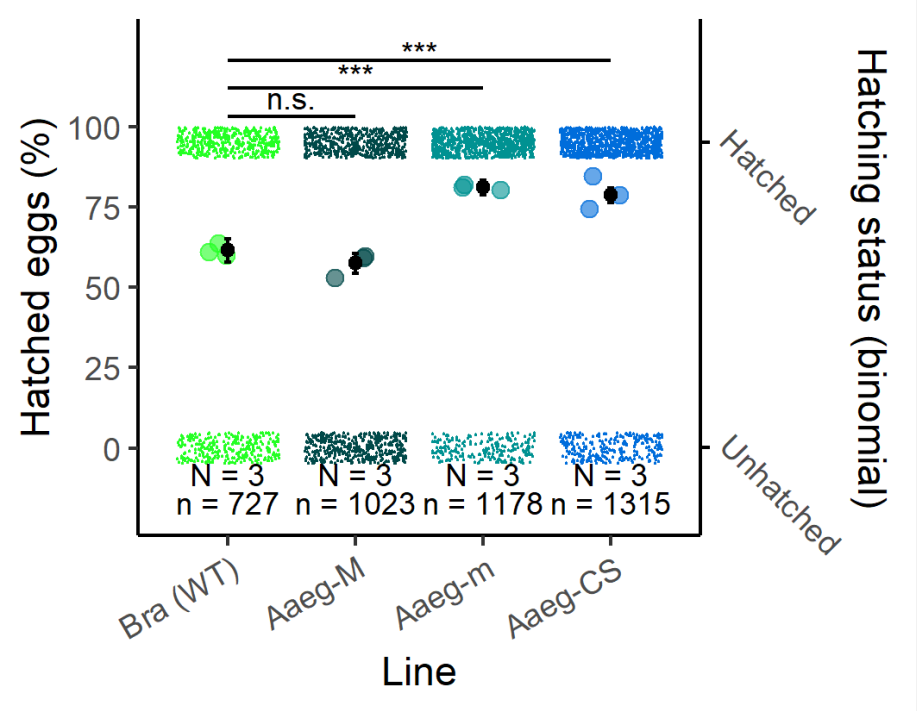


RESULTS – PERFORMANCE OF THE GSS

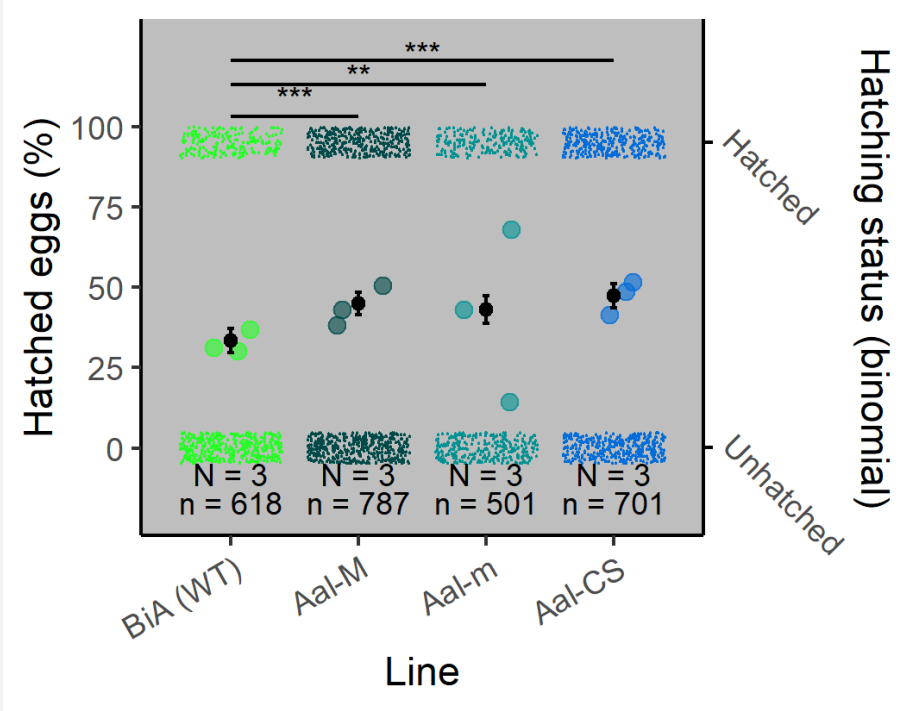
Egg hatching rate



Aedes aegypti



Aedes albopictus



RESULTS – PERFORMANCE OF THE GSS

Male competitiveness

Mix equal numbers of WT females,
WT males and transgenic males

Equal competitiveness
50% of the females mate with a WT male
50% of the females mate with a transgenic male

Result:
75% non-transgenic
25% transgenic

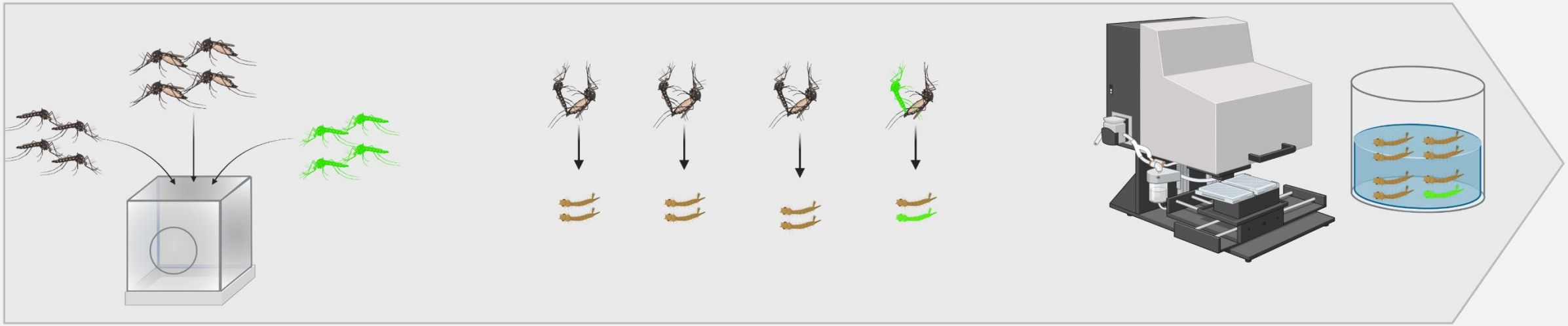
RESULTS – PERFORMANCE OF THE GSS

Male competitiveness

Mix equal numbers of WT females, WT males and transgenic males

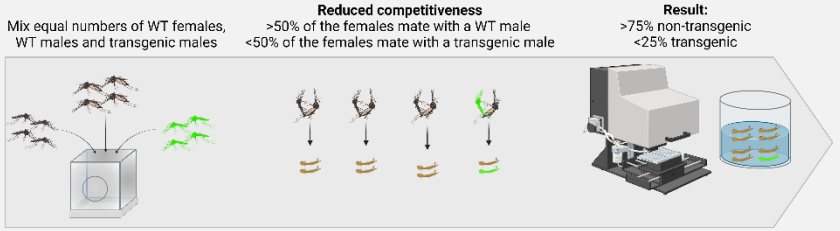
Reduced competitiveness
>50% of the females mate with a WT male
<50% of the females mate with a transgenic male

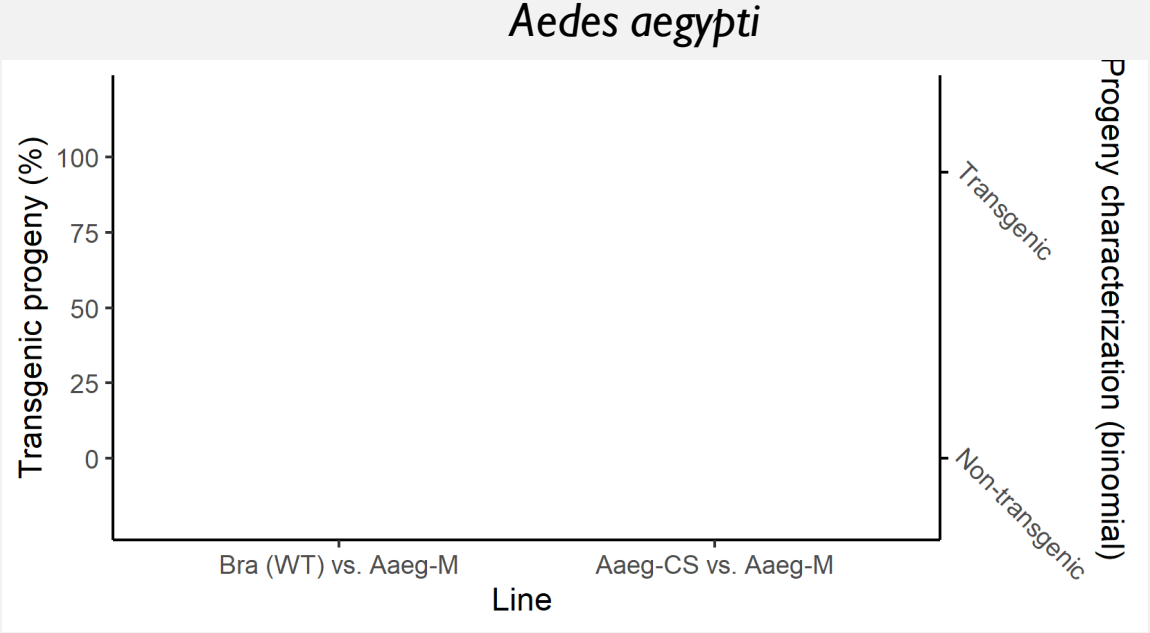
Result:
>75% non-transgenic
<25% transgenic



RESULTS – PERFORMANCE OF THE GSS

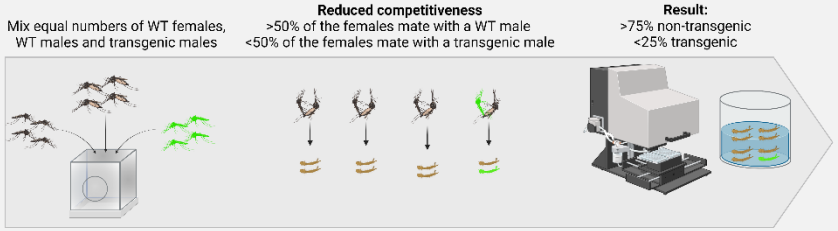
Male competitiveness

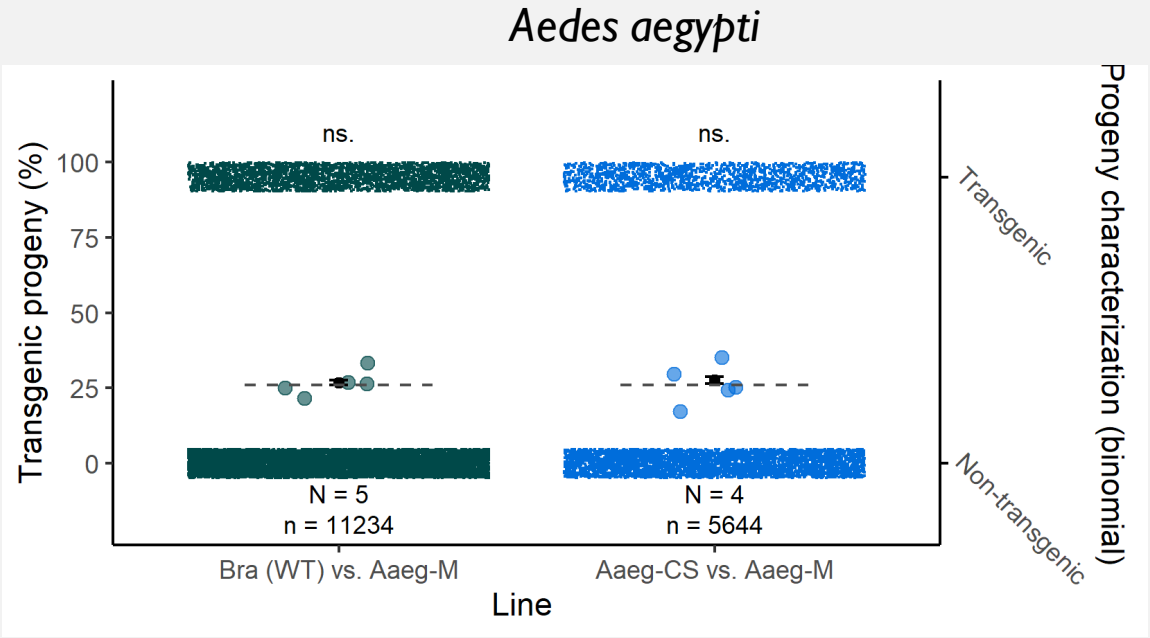




RESULTS – PERFORMANCE OF THE GSS

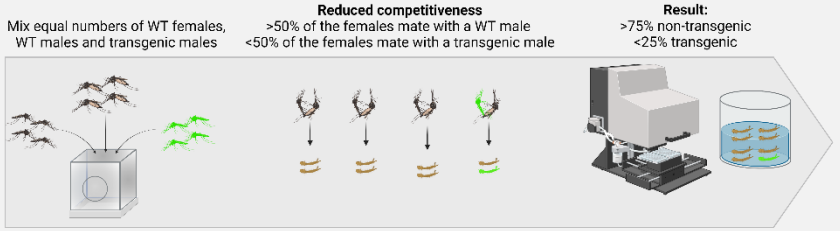
Male competitiveness



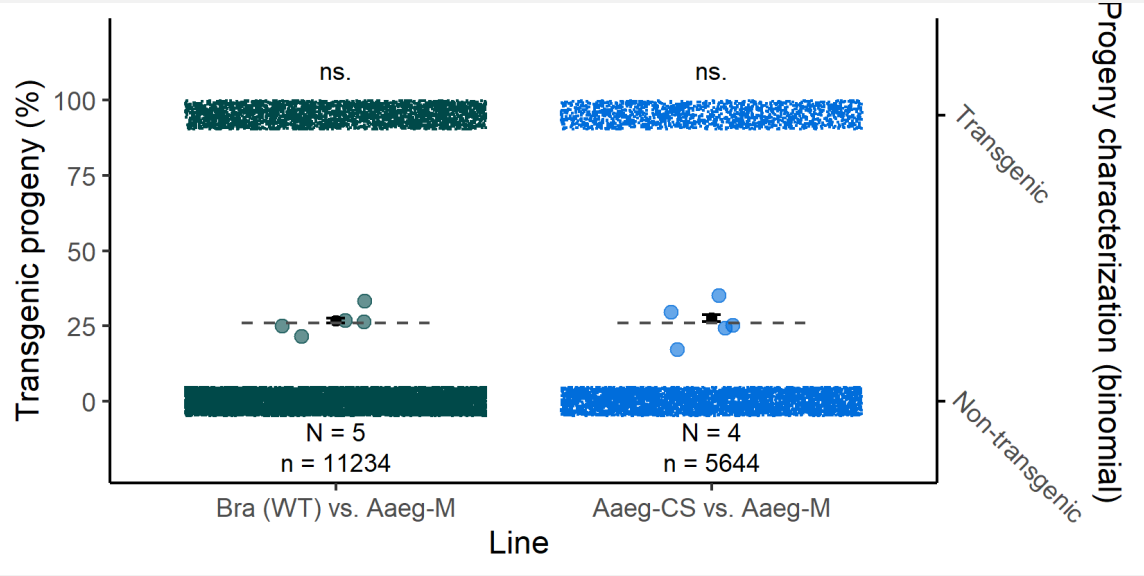


RESULTS – PERFORMANCE OF THE GSS

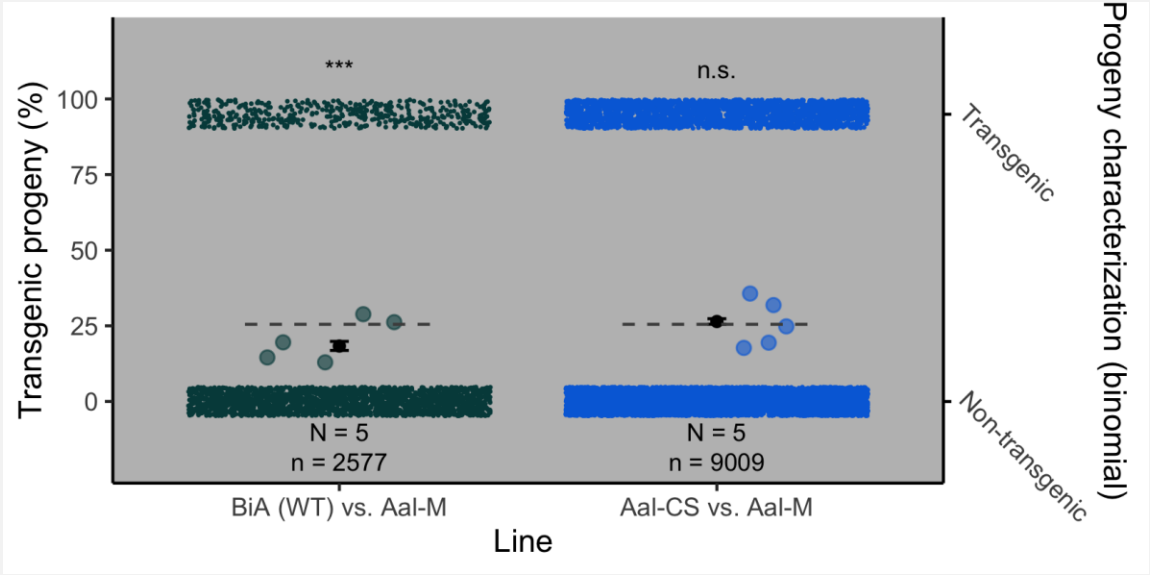
Male competitiveness



Aedes aegypti



Aedes albopictus



RESULTS

Valorisation

communications biology

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Transgenic expression of *Nix* converts genetic females into males and allows automated sex sorting in *Aedes albopictus*

Célia Lutrat ✉, Roenick P. Olmo, Thierry Baldet, Jérémy Bouyer & Eric Marois ✉

Just out in Communications Biology!

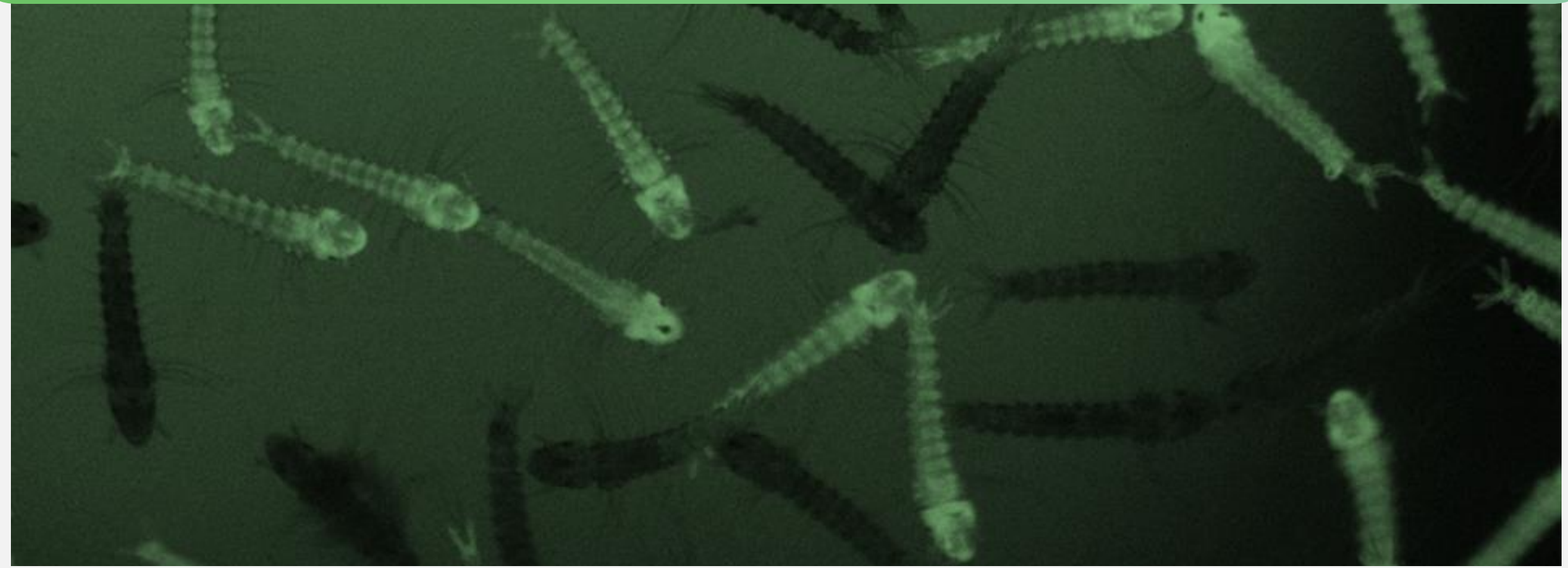
Combining two Genetic Sexing Strains allows sorting of non-transgenic males for *Aedes* genetic control

Authors

Célia Lutrat [1,2,3,4,*], Myriam Burckbuchler [4], Roenick Proveti Olmo [4], Rémy Beugnon[5,6], Albin Fontaine[7], Thierry Baldet [1,8], Jérémy Bouyer [1,9,10,†], Eric Marois [4,†,*]

Soon to be submitted

CONCLUSION



CONCLUSION

7 criteria for a good sexing method

Sorting stage

Male recovery

Female contamination

Sorting speed

Initial investment

Consumable cost

Acceptability

CONCLUSION

Evaluating our sexing method

Sorting stage
Neonate larvae

CONCLUSION

Evaluating our sexing method

Sorting stage
Neonate larvae

Male recovery
70%

CONCLUSION

Evaluating our sexing method

Sorting stage	Male recovery	Female contamination
Neonate larvae	70%	0-0.1%

CONCLUSION

Evaluating our sexing method

Sorting stage
Neonate larvae

Male recovery
70%

Female contamination
0-0.1%

Sorting speed
60 larvae / sec

CONCLUSION

Evaluating our sexing method

Sorting stage
Neonate larvae

Male recovery
70%

Female contamination
0-0.1%

Sorting speed
60 larvae / sec

Initial investment
+++

CONCLUSION

Evaluating our sexing method

Sorting stage
Neonate larvae

Male recovery
70%

Female contamination
0-0.1%

Sorting speed
60 larvae / sec

Initial investment
+++

Consumable cost
Decreased

CONCLUSION

Evaluating our sexing method

Sorting stage
Neonate larvae

Male recovery
70%

Female contamination
0-0.1%

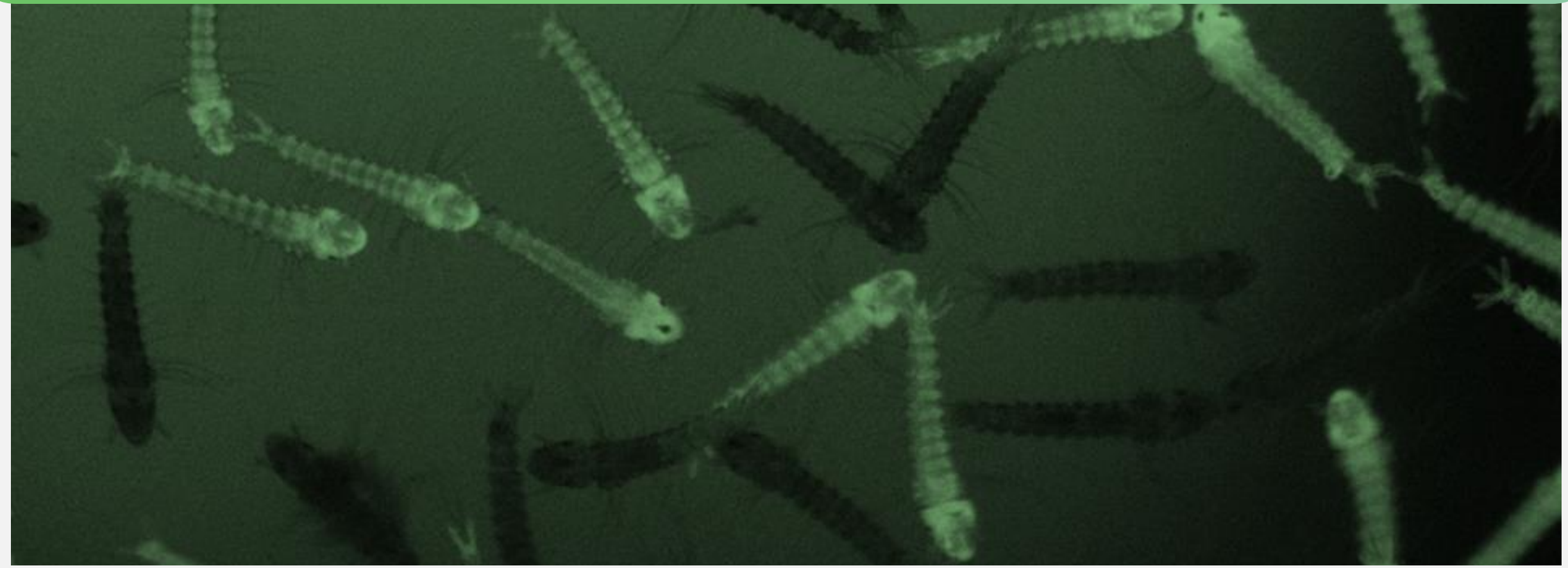
Sorting speed
60 larvae / sec

Initial investment
+++

Consumable cost
Decreased

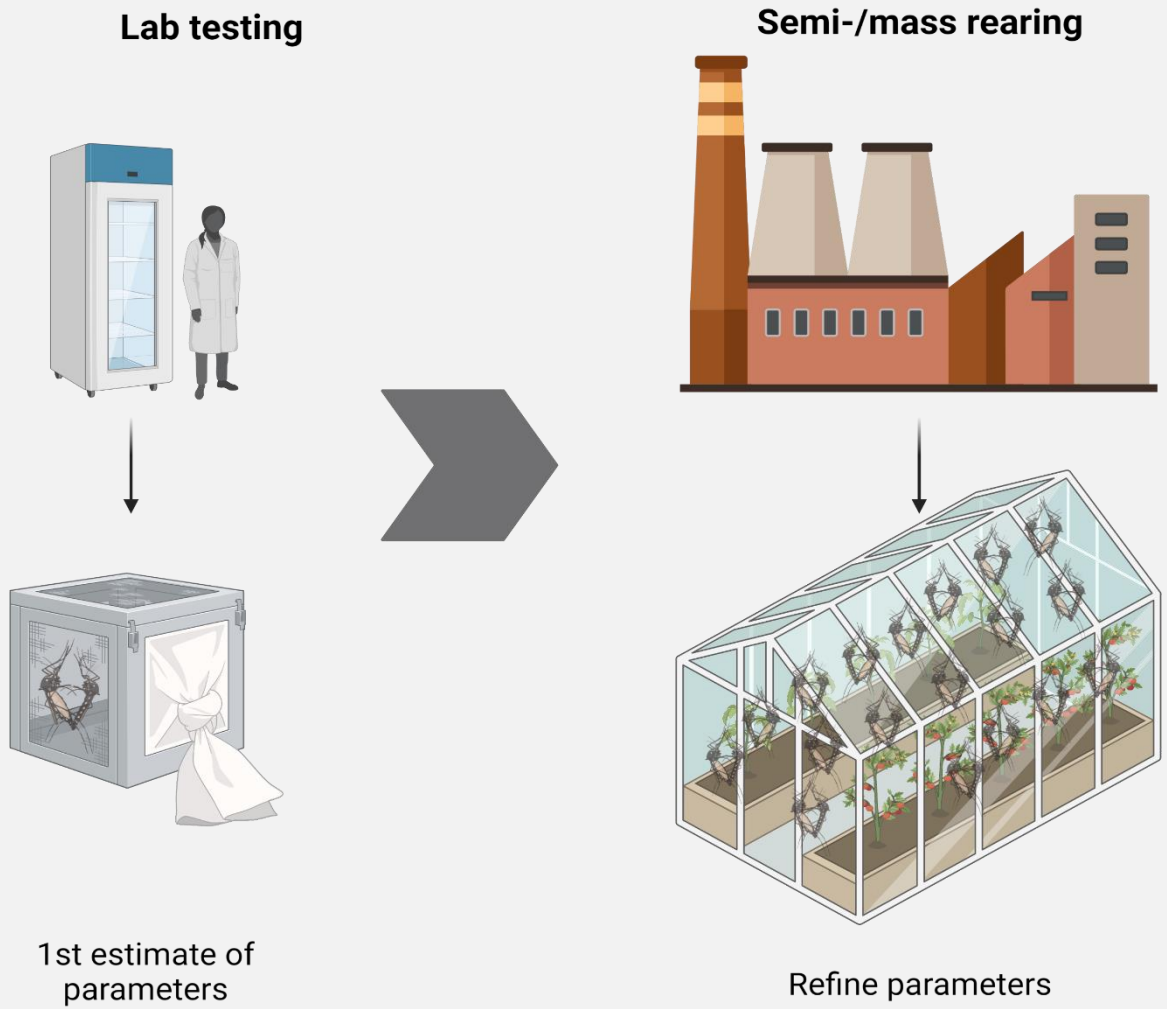
Acceptability
To be defined

PERSPECTIVE

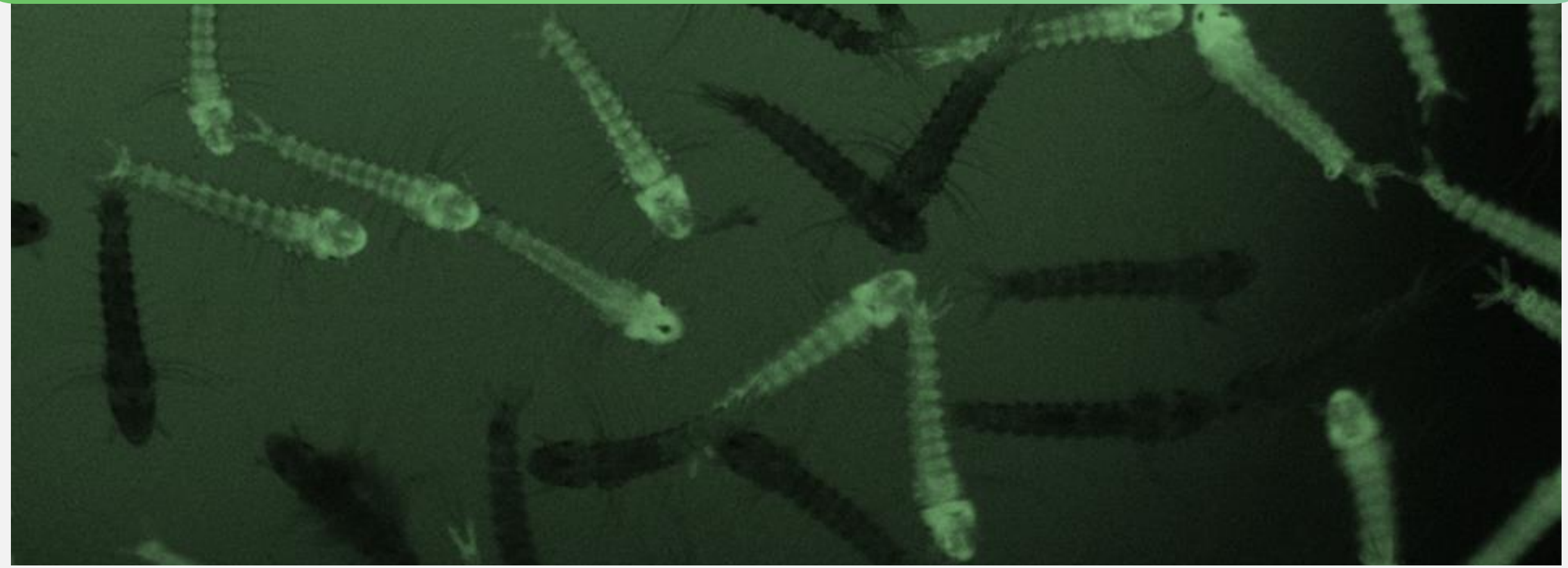


PERSPECTIVE

Going further



ACKNOWLEDGEMENTS



ACKNOWLEDGEMENTS

CIRAD UMR ASTRE
Collectif vecteurs

IBMC UPR9022
Equipe moustiques

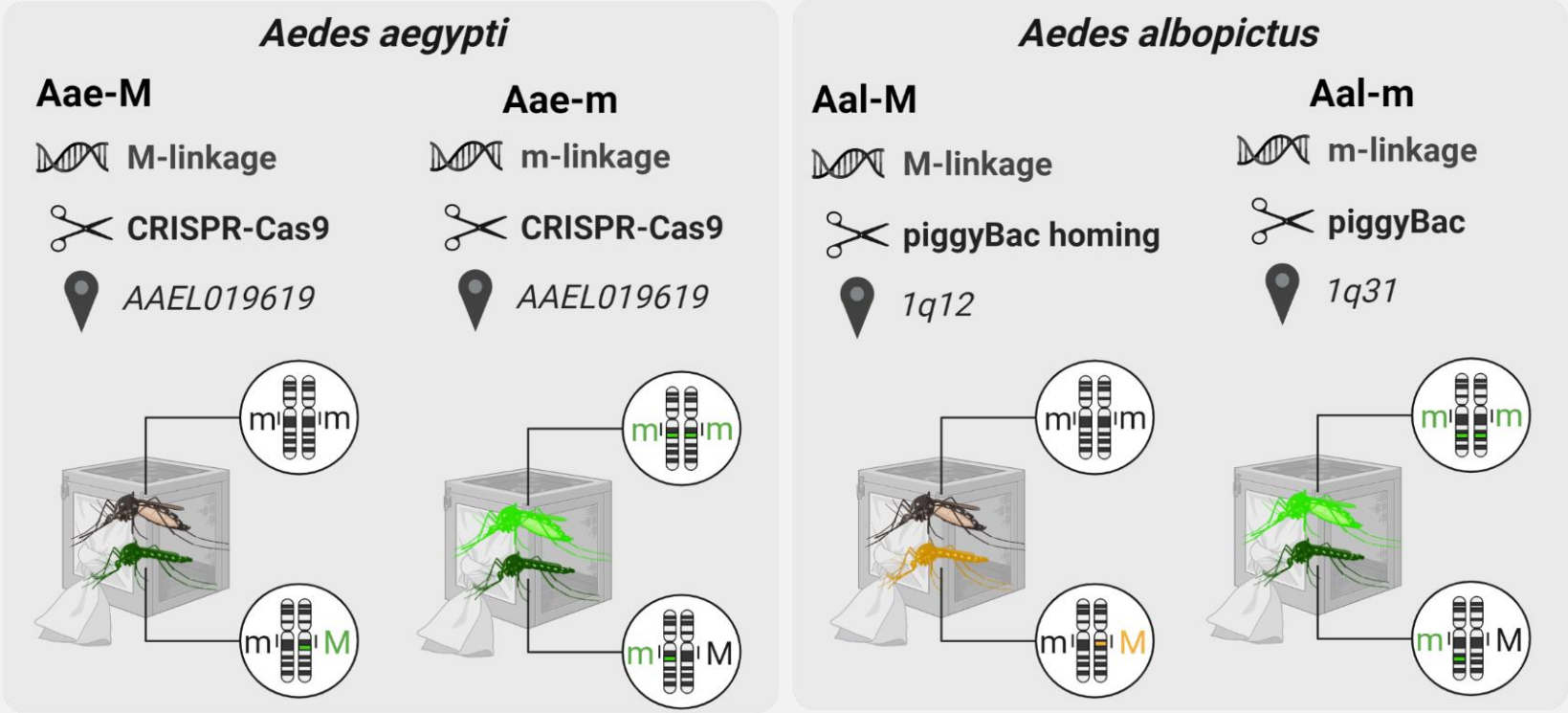
Graphical figures were designed on Biorender.com. Data figures were plotted on R.

Funding sources: EU ERC CoG—682387 REVOLINC to J.B., ANR grants #ANR-11-EQPX-0022, #ANR-19-CE35-0007 GDaMO and # 18-CE35-0003-02 BAKOUMBA to E.M. No funding was received from Union Biometrica.

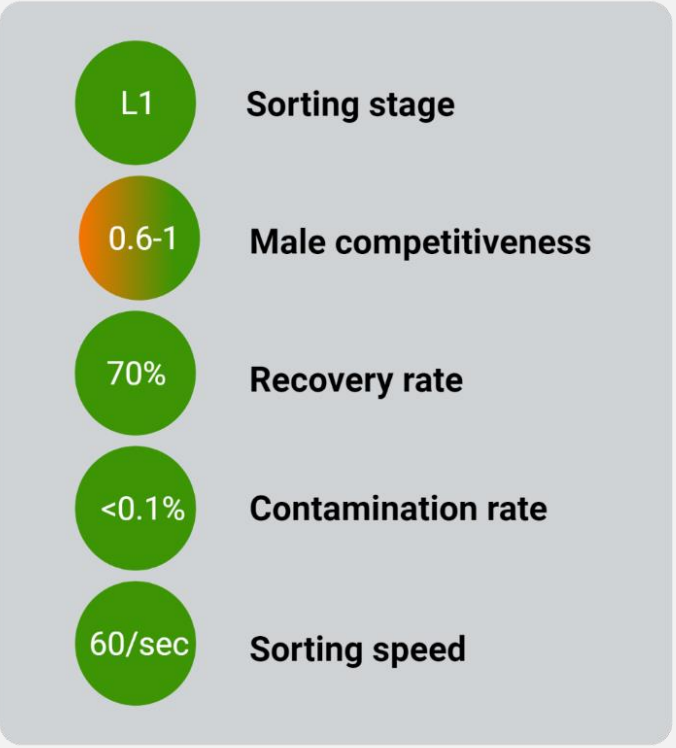
SUMMARY

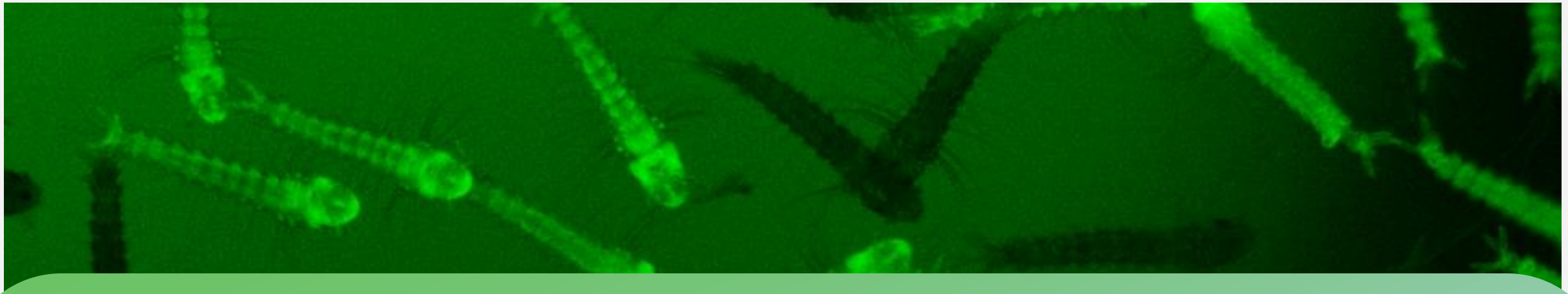
Questions, collaborations:
celia.lutrat@outlook.com

DEVELOPMENT



PERFORMANCE





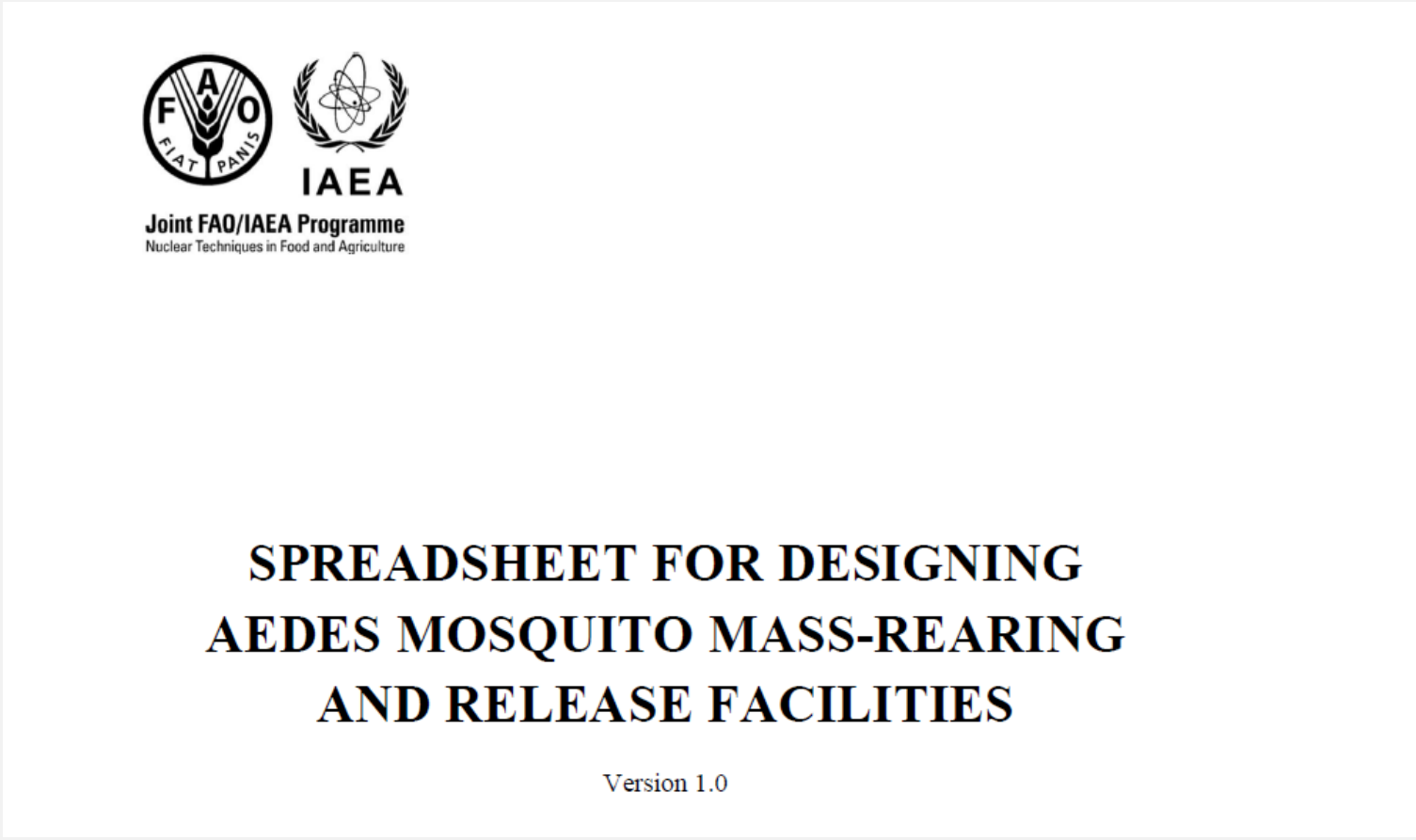
Développement de méthodes innovantes de sexage pour l'application de la Technique de l'Insecte Stérile chez les moustiques du genre Aedes

Célia Lutrat

celia.lutrat@outlook.com

PERSPECTIVE

Economical interest



FAO and IAEA 2019

PERSPECTIVE

Economical interest

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

SPREADSHEET FOR DESIGNING AEDES MOSQUITO MASS-REARING AND RELEASE FACILITIES

Version 1.0

FAO and IAEA 2019
WHO 2018

PERSPECTIVE

Economical interest

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

SPREADSHEET FOR DESIGNING AEDES MOSQUITO MASS-REARING AND RELEASE FACILITIES

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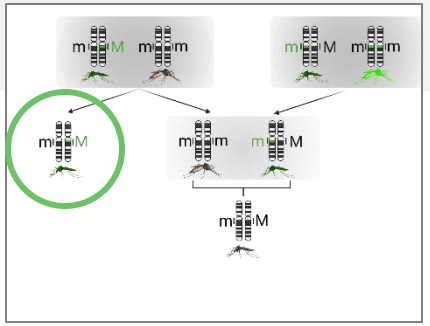

Joint FAO/IAEA Programme
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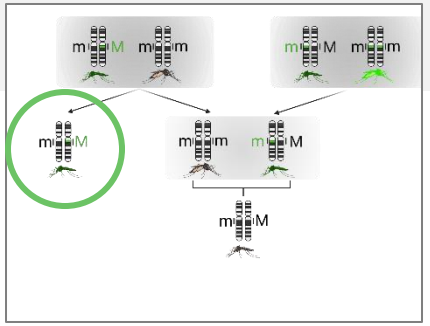

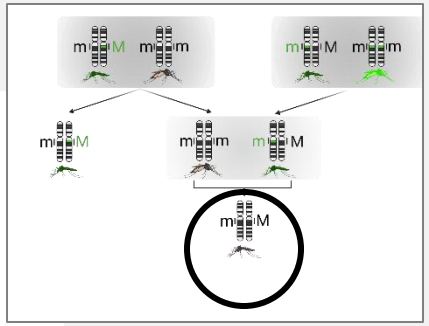

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WHO 2018

PERSPECTIVE

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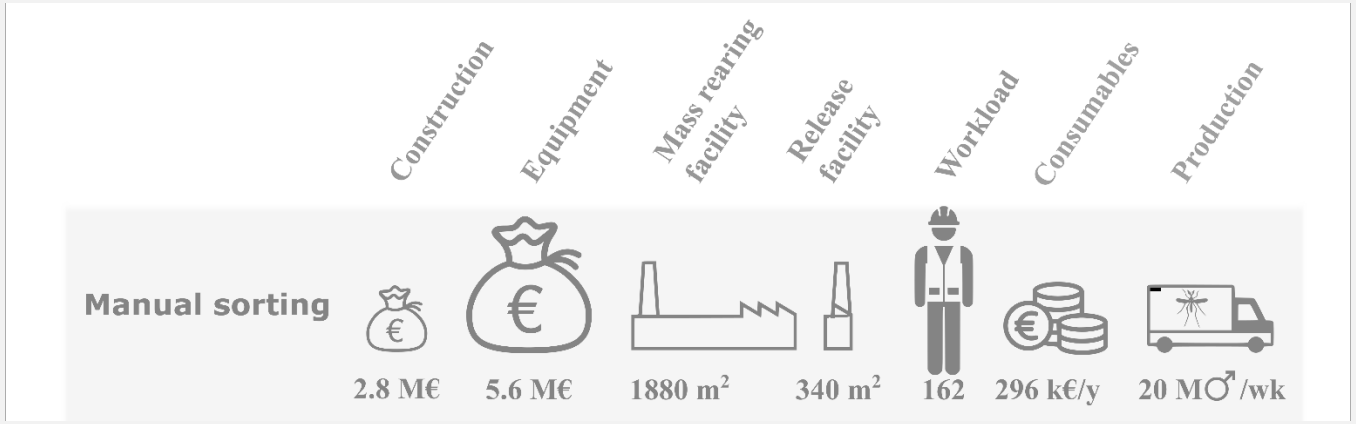

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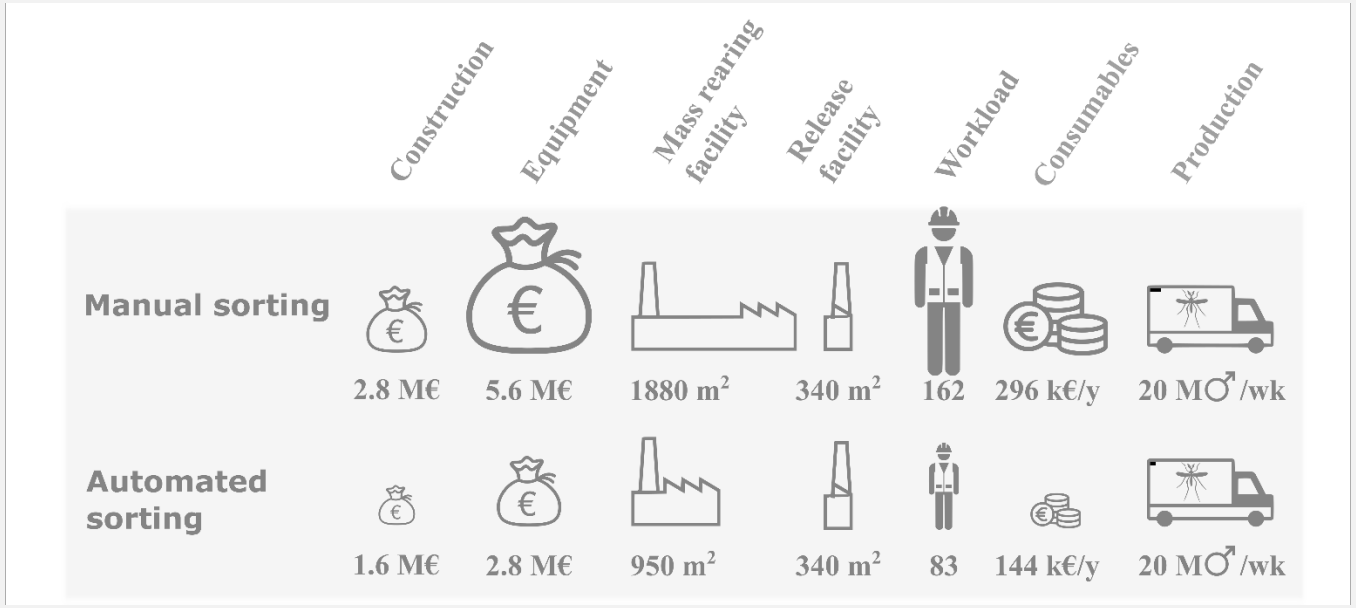

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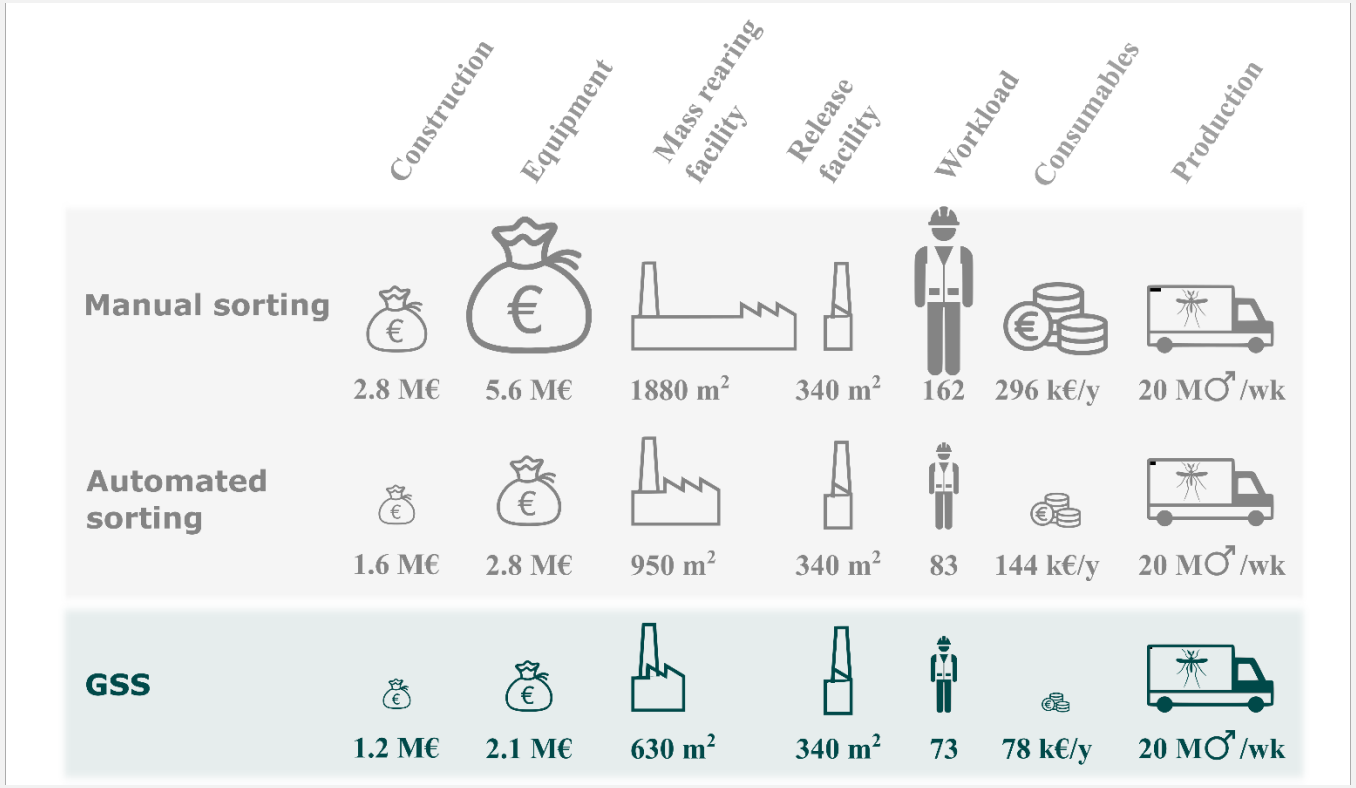
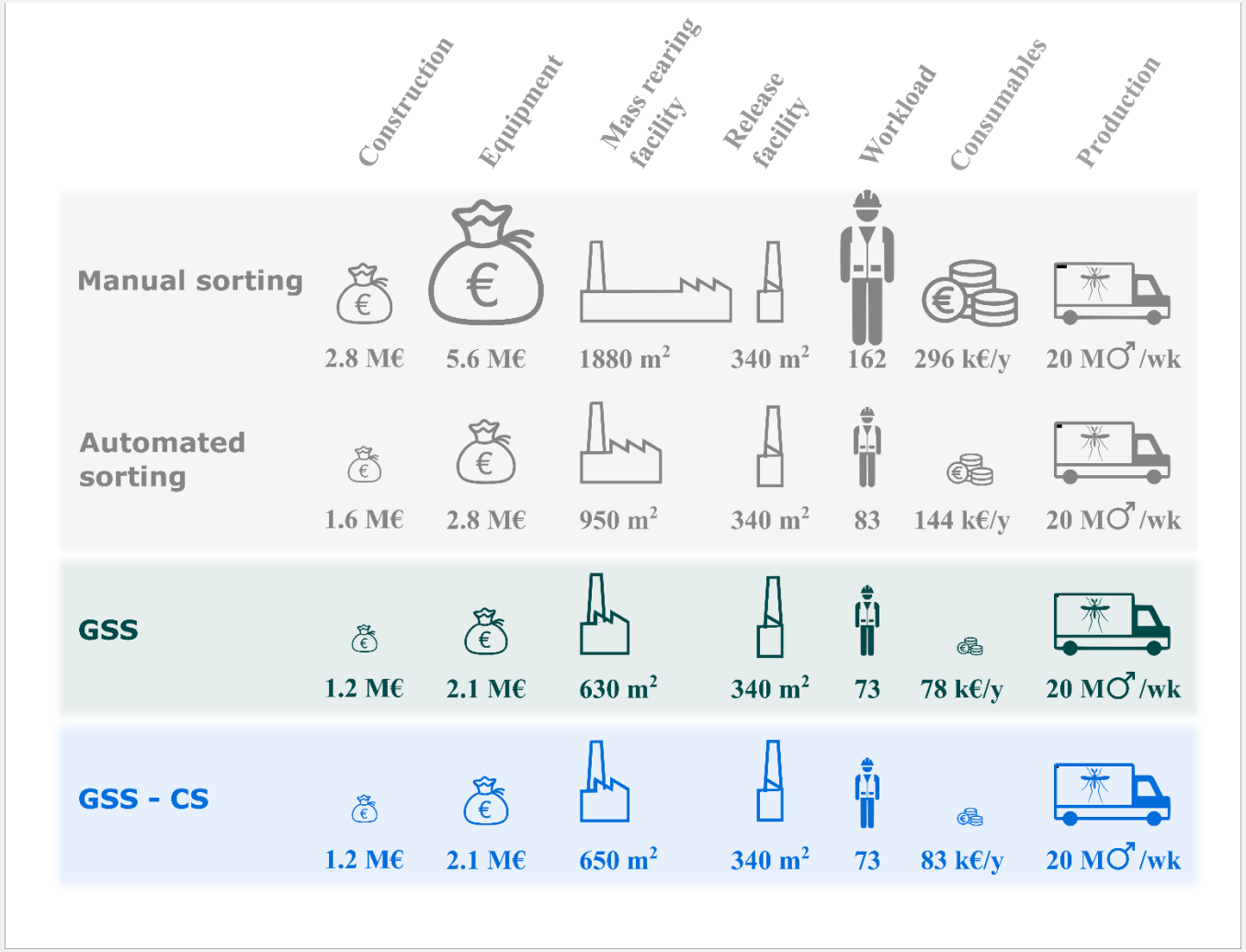
Economical interest



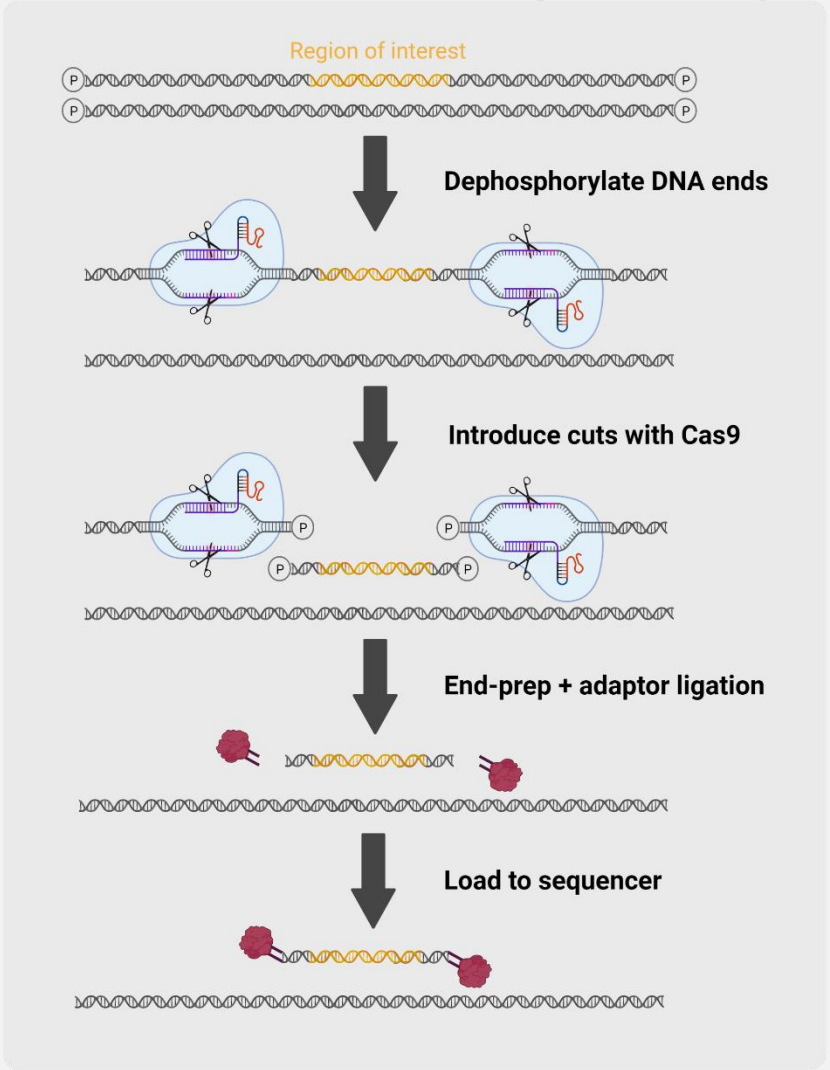
PERSPECTIVE

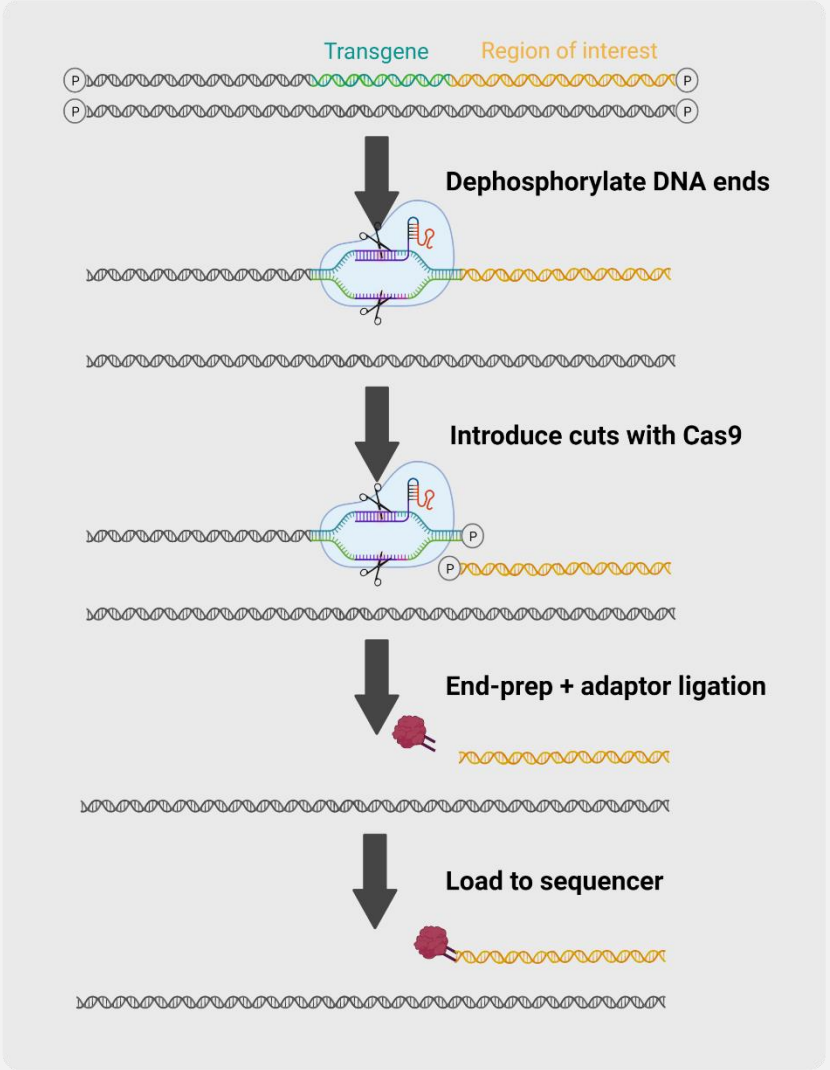
Economical interest



SUPPLEMENTARY

Adaptation of the nCATS sequencing method

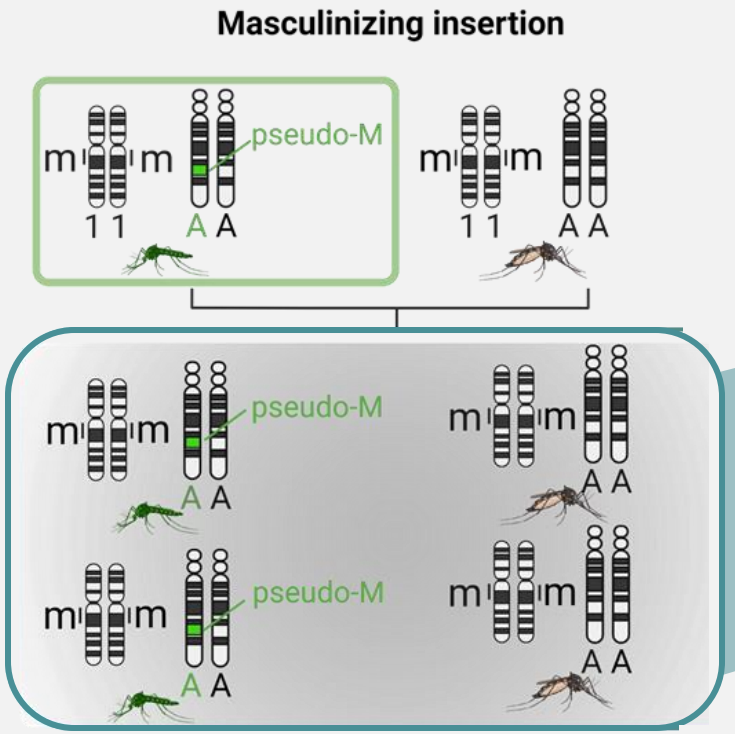




Adapted from Gilpatrick et al. 2018

SUPPLEMENTARY

Nix ectopic expression results



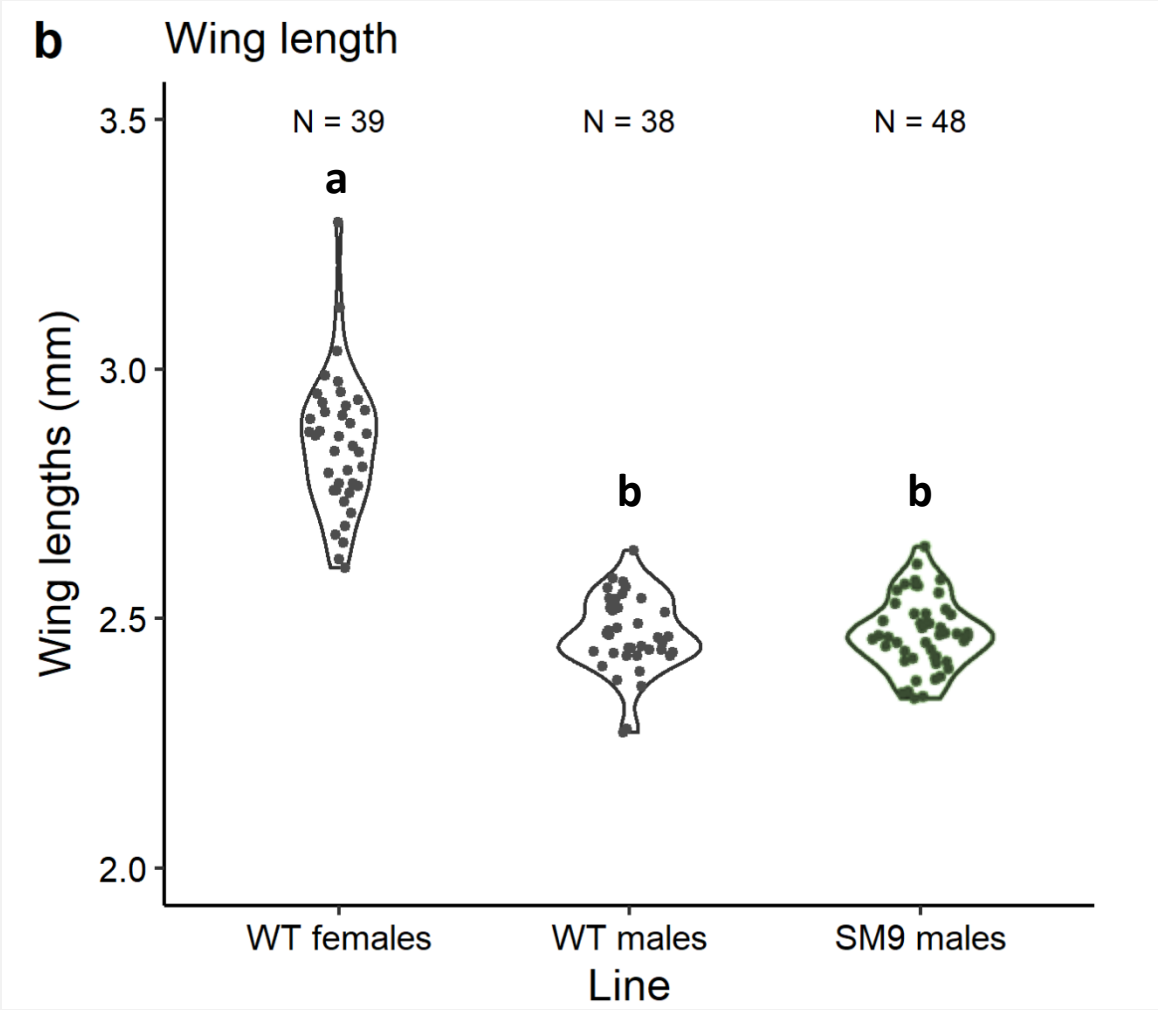
Amplification of the endogenous *Nix* gene

4 out of 13 were pseudo-M linked (GFP)

7 out of 13 were M linked (YFP and/or DsRed)

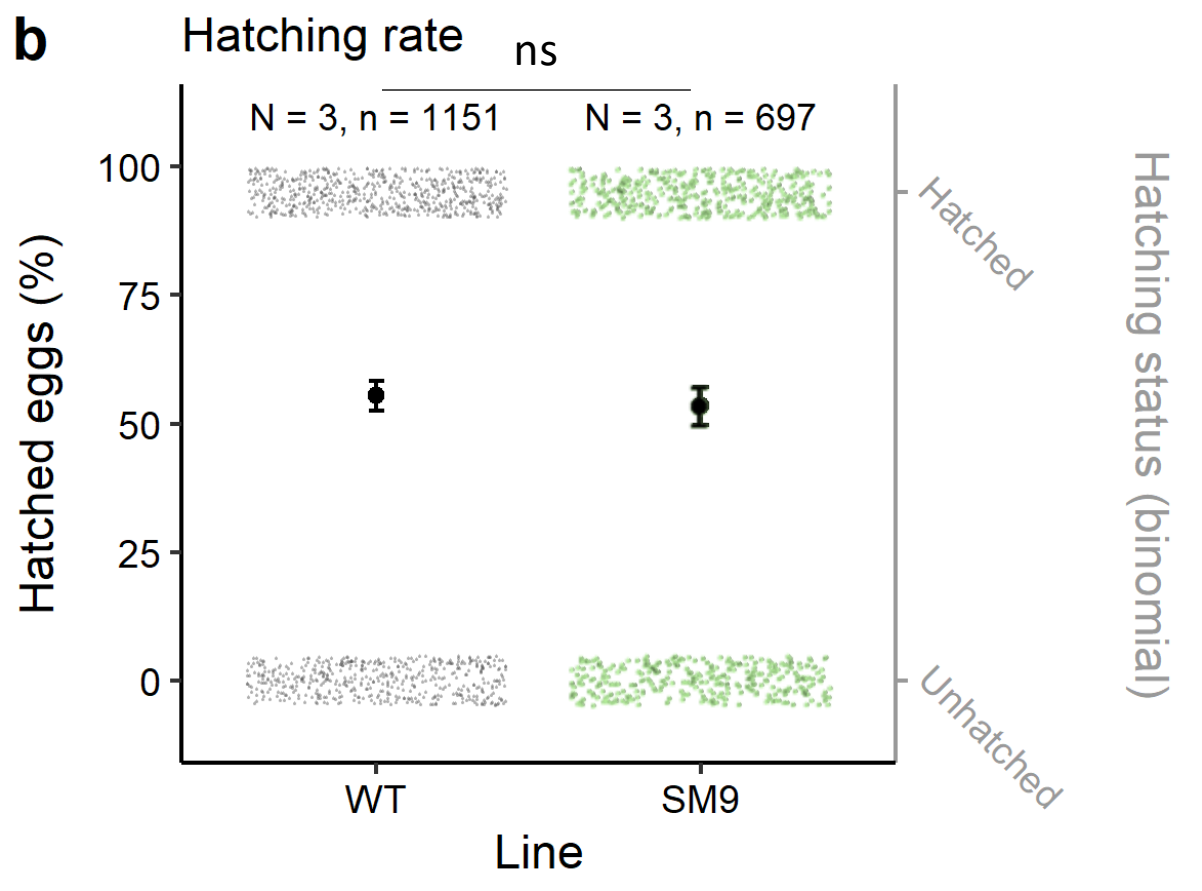
SUPPLEMENTARY

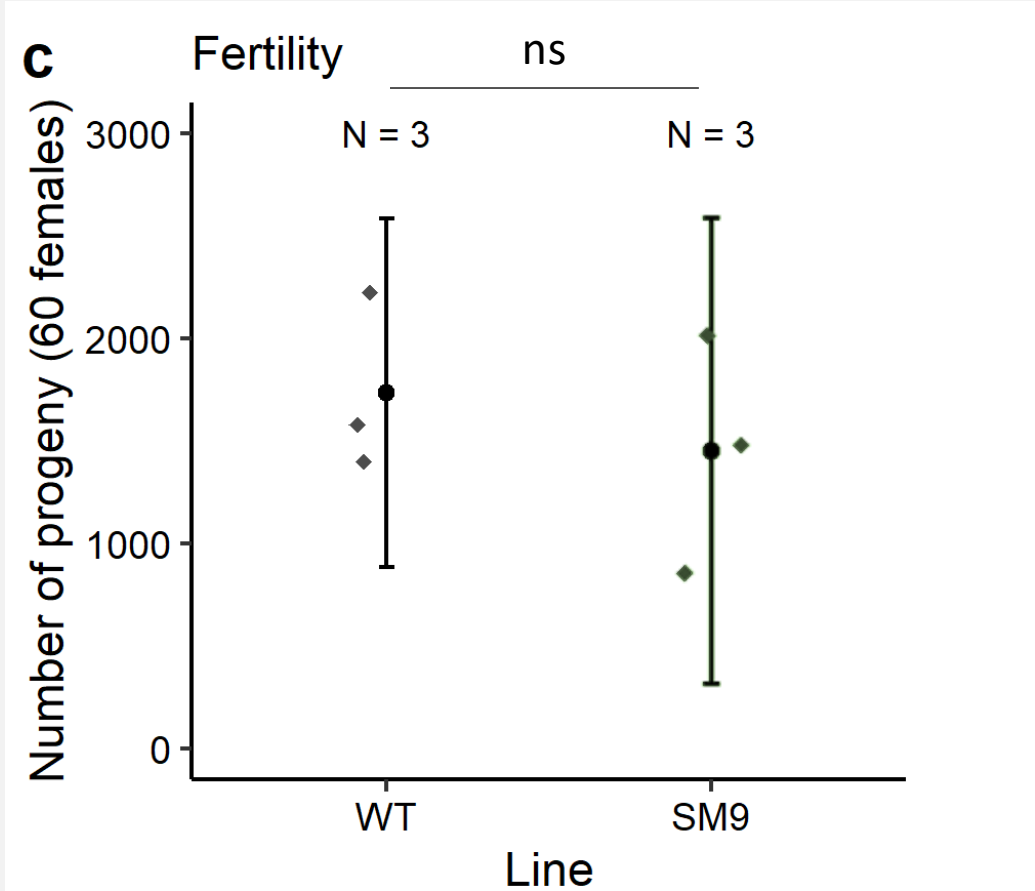
Nix ectopic expression results: size dimorphism



SUPPLEMENTARY

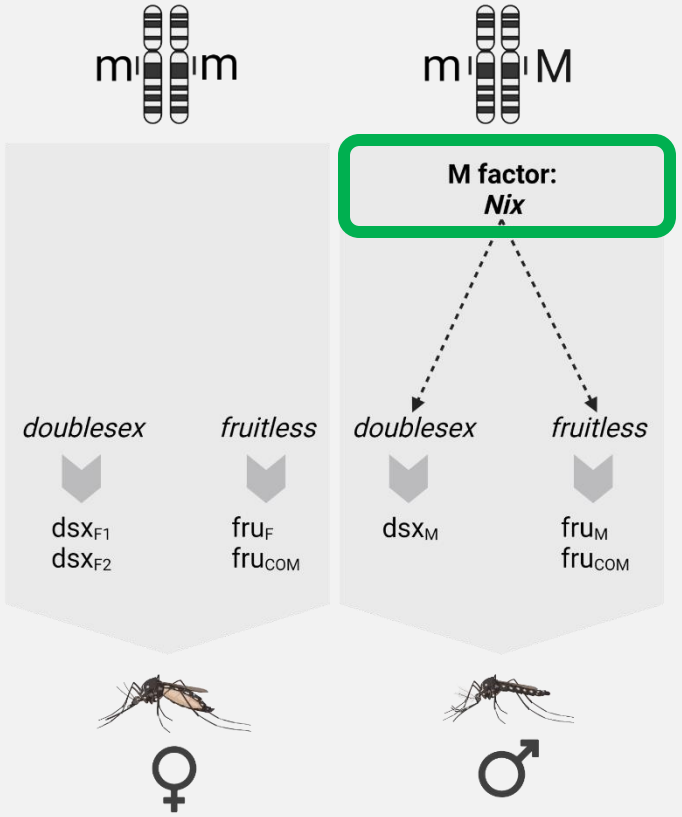
Nix ectopic expression results: fertility



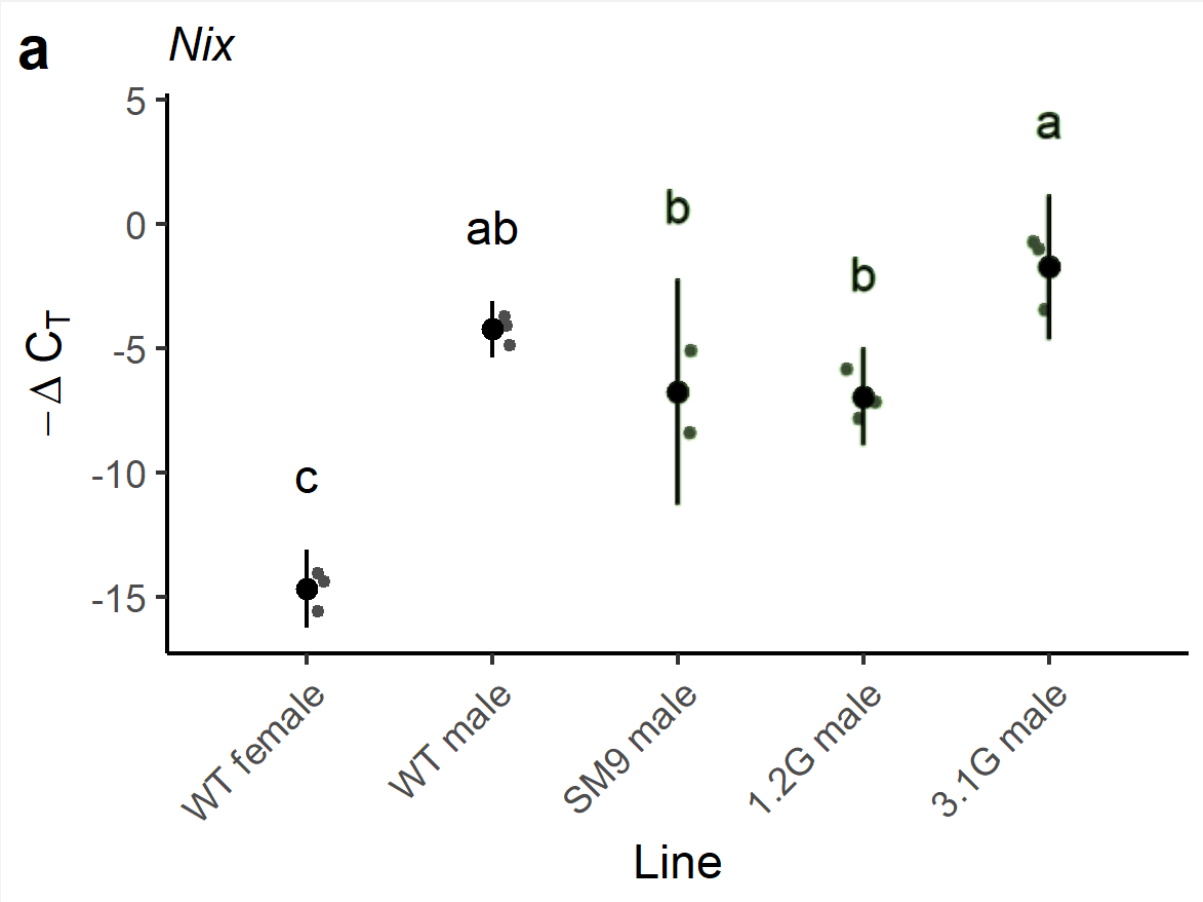


SUPPLEMENTARY

Nix ectopic expression results: expression levels of genes of interest

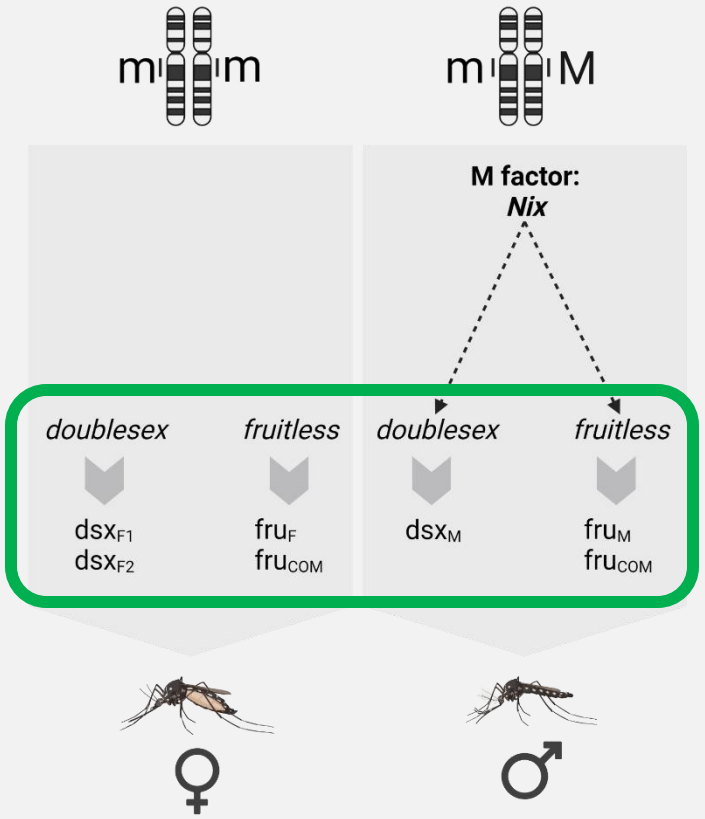


RT-qPCR

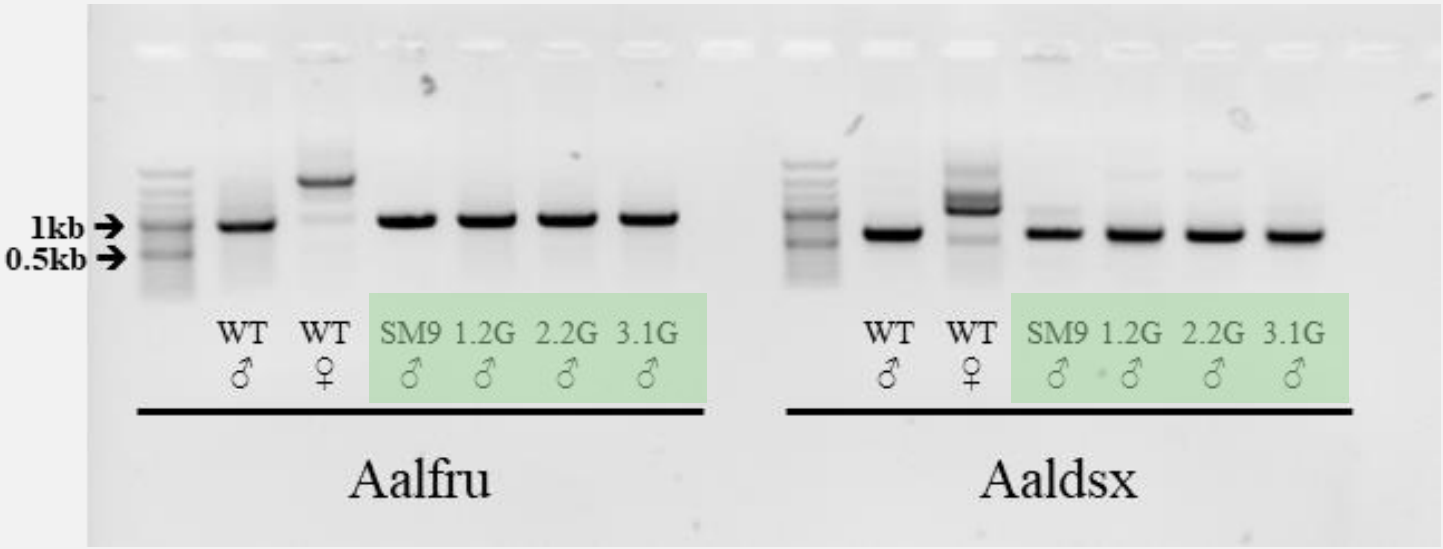


SUPPLEMENTARY

Nix ectopic expression results: expression levels of genes of interest

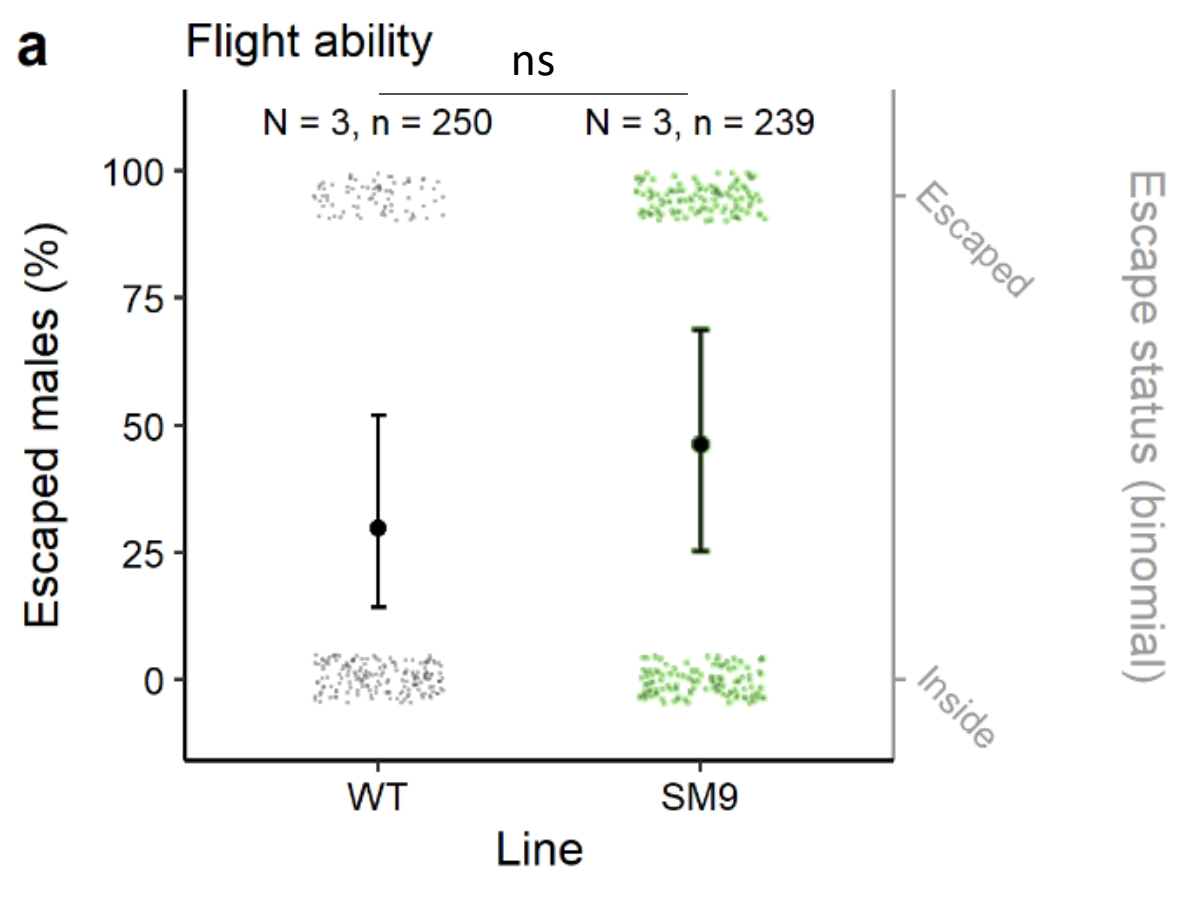


RT-PCR



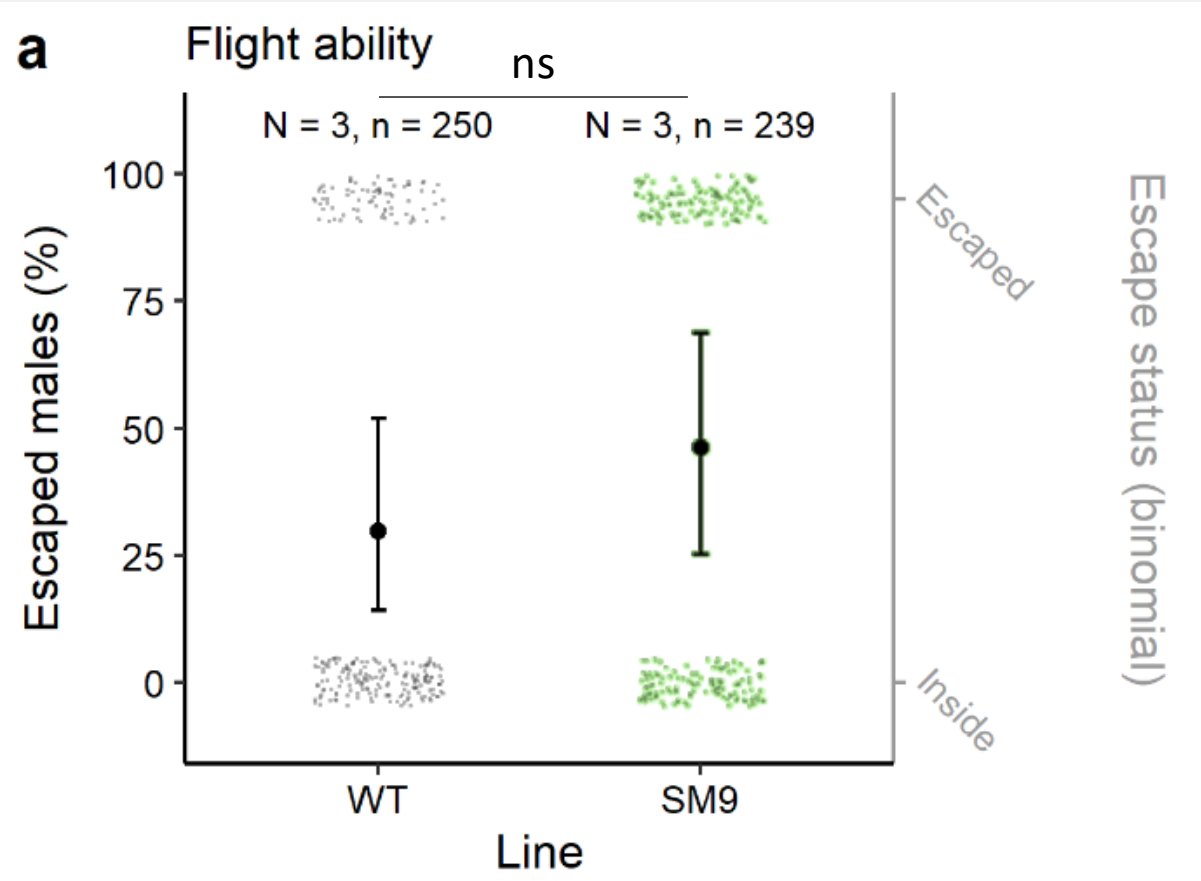
SUPPLEMENTARY

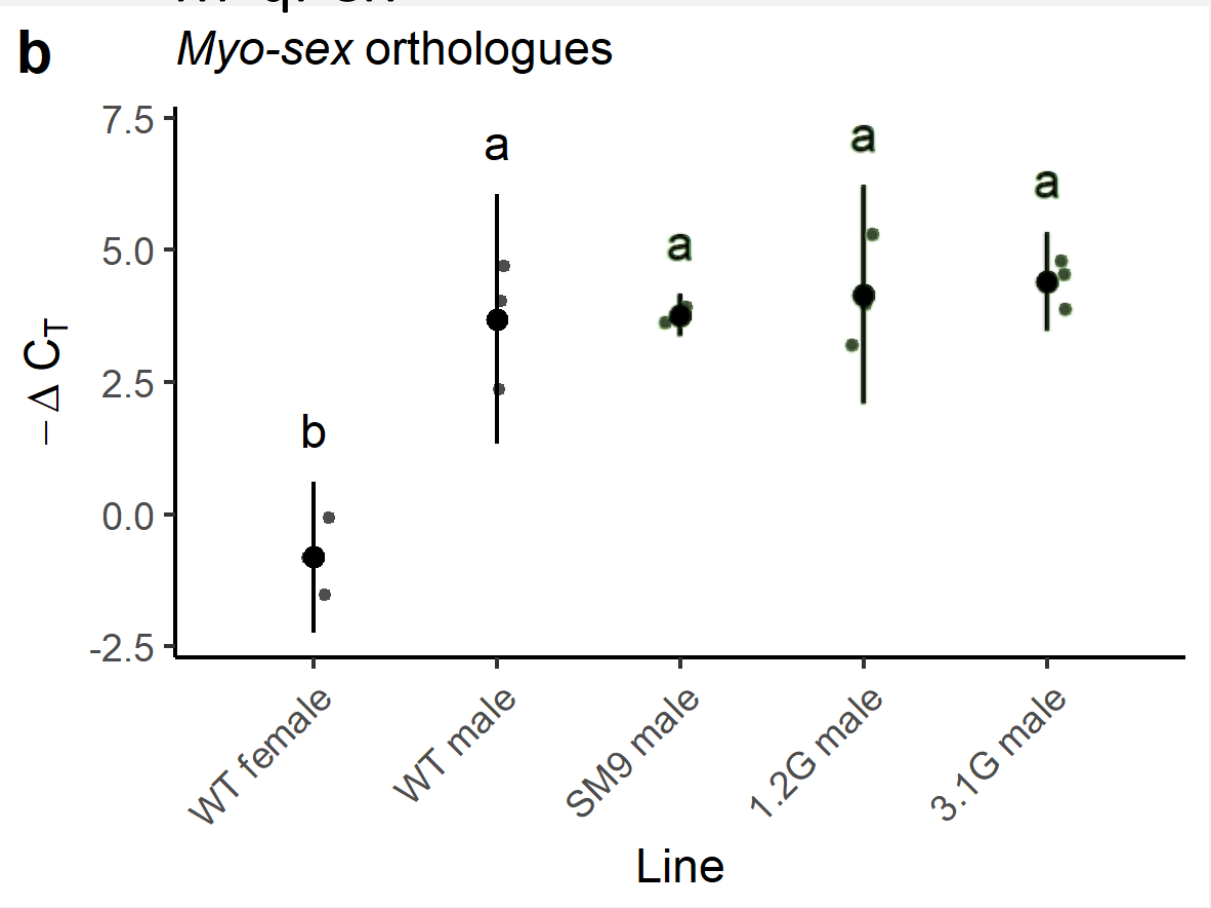
Nix ectopic expression results: expression levels of genes of interest



SUPPLEMENTARY

Nix ectopic expression results: expression levels of genes of interest

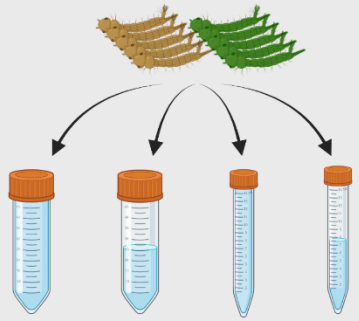




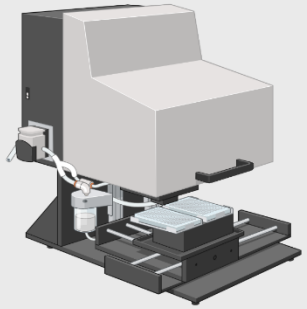
SUPPLEMENTARY

COPAS sorting speed

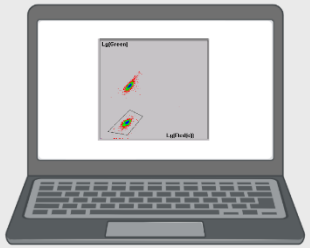
Prepare samples of a defined number of larvae at different concentrations



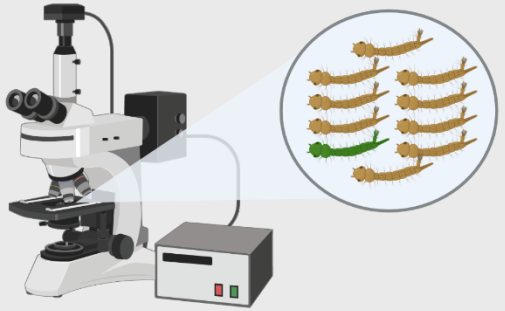
Sort by COPAS



Determine recovery rate

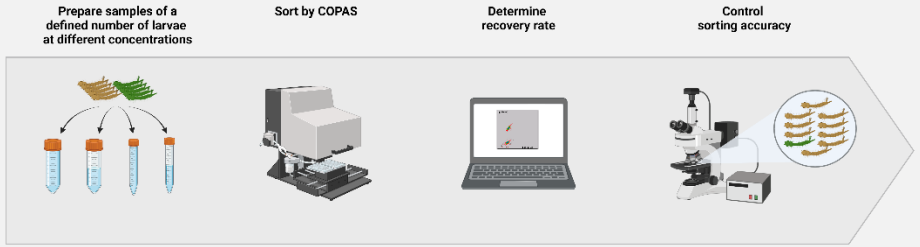


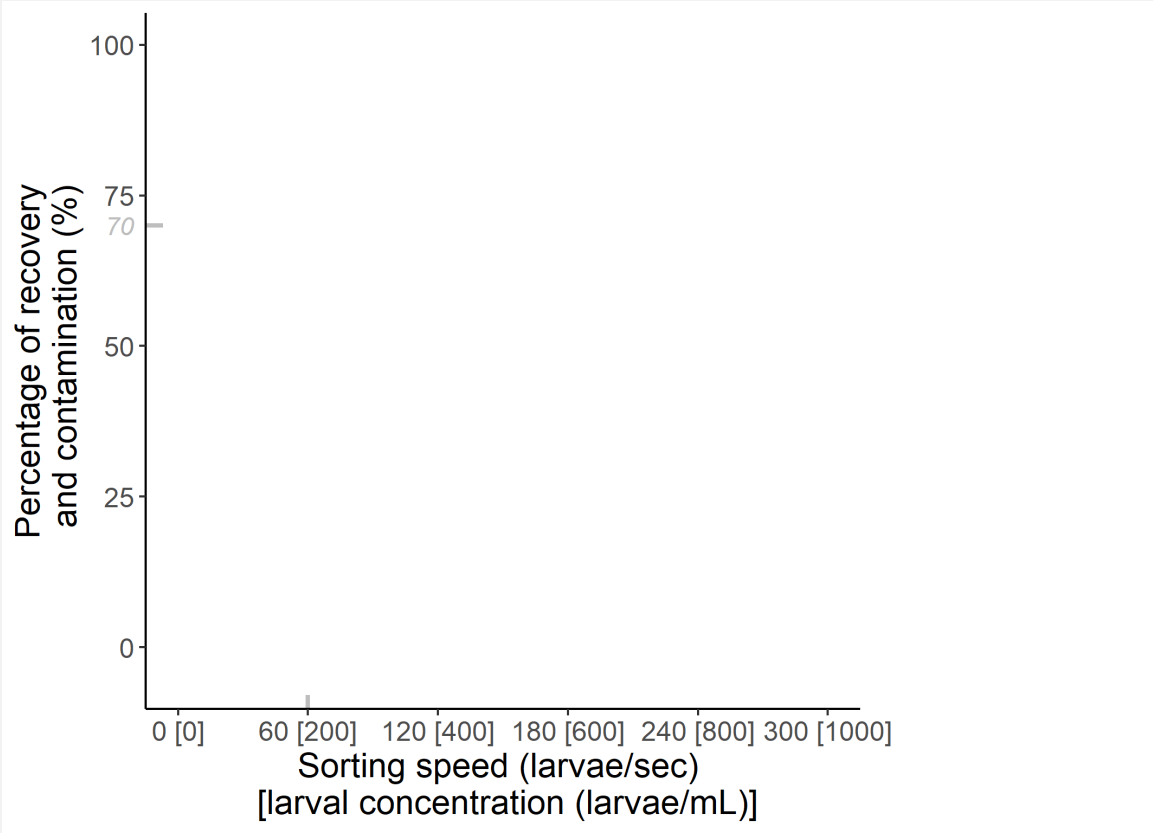
Control sorting accuracy



SUPPLEMENTARY

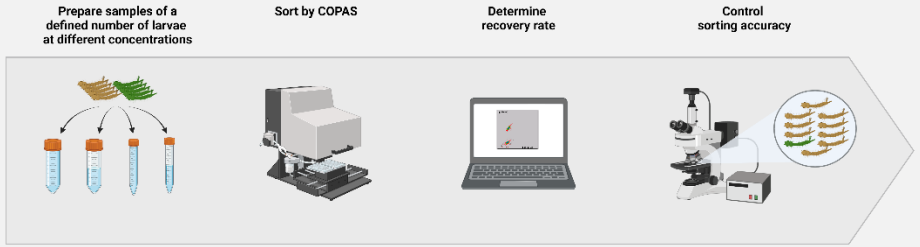
COPAS sorting speed

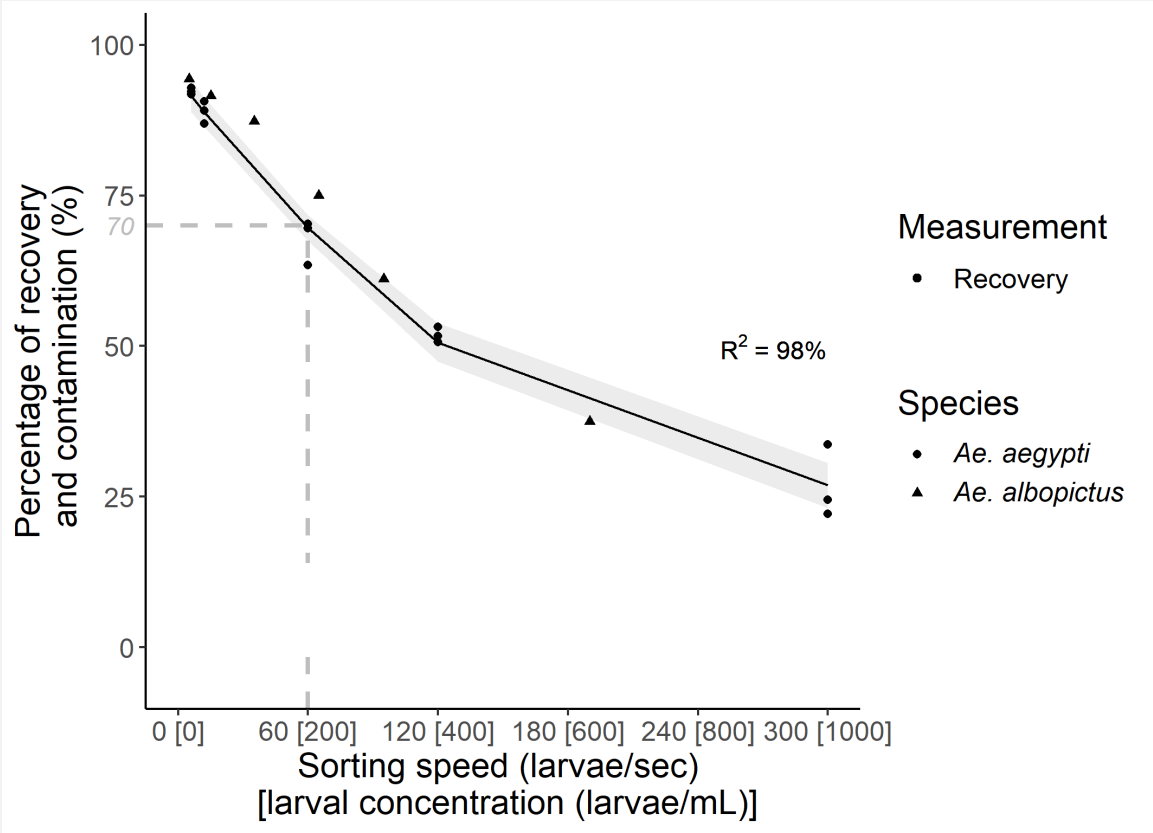




SUPPLEMENTARY

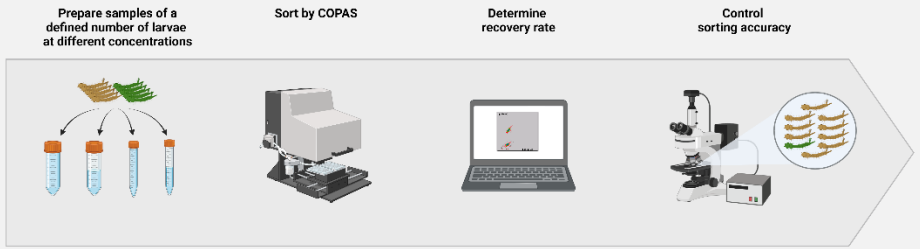
COPAS sorting speed

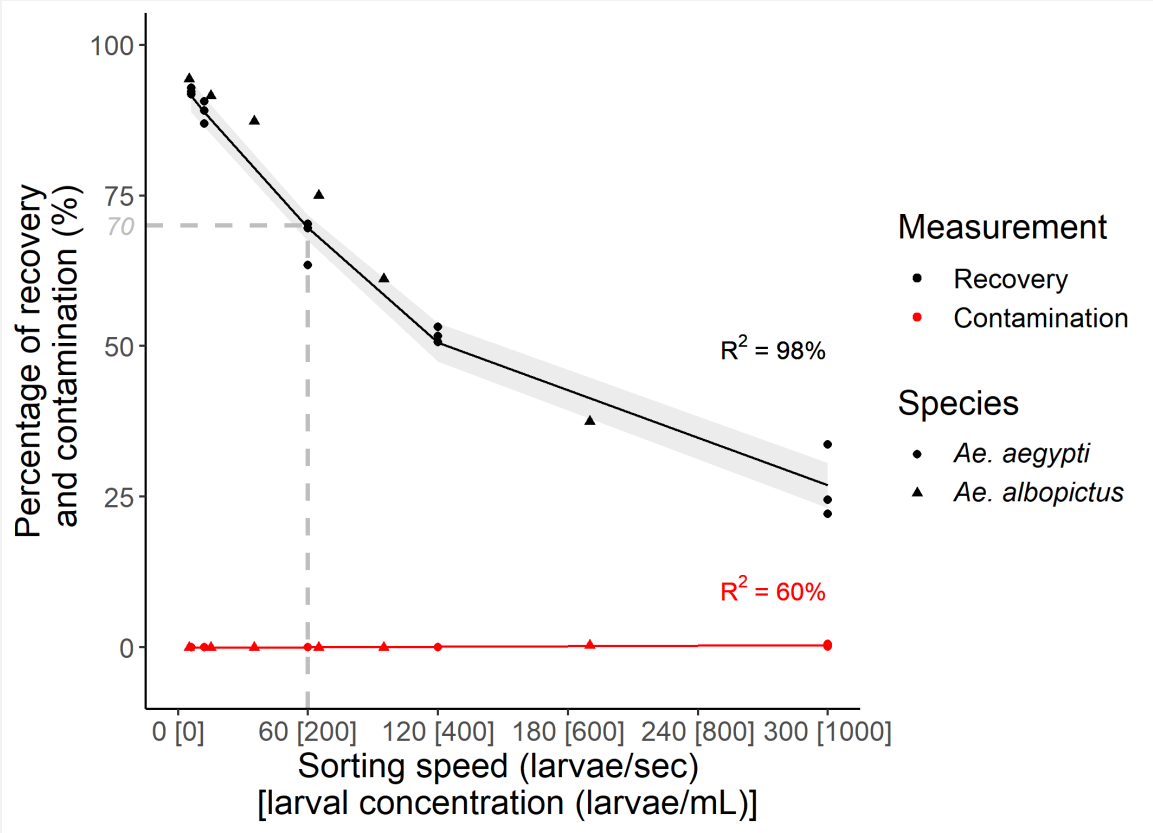




SUPPLEMENTARY

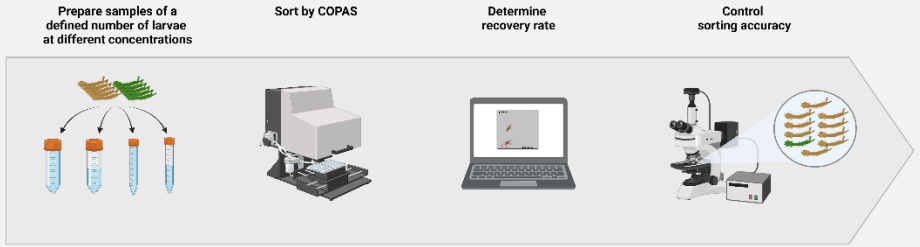
COPAS sorting speed

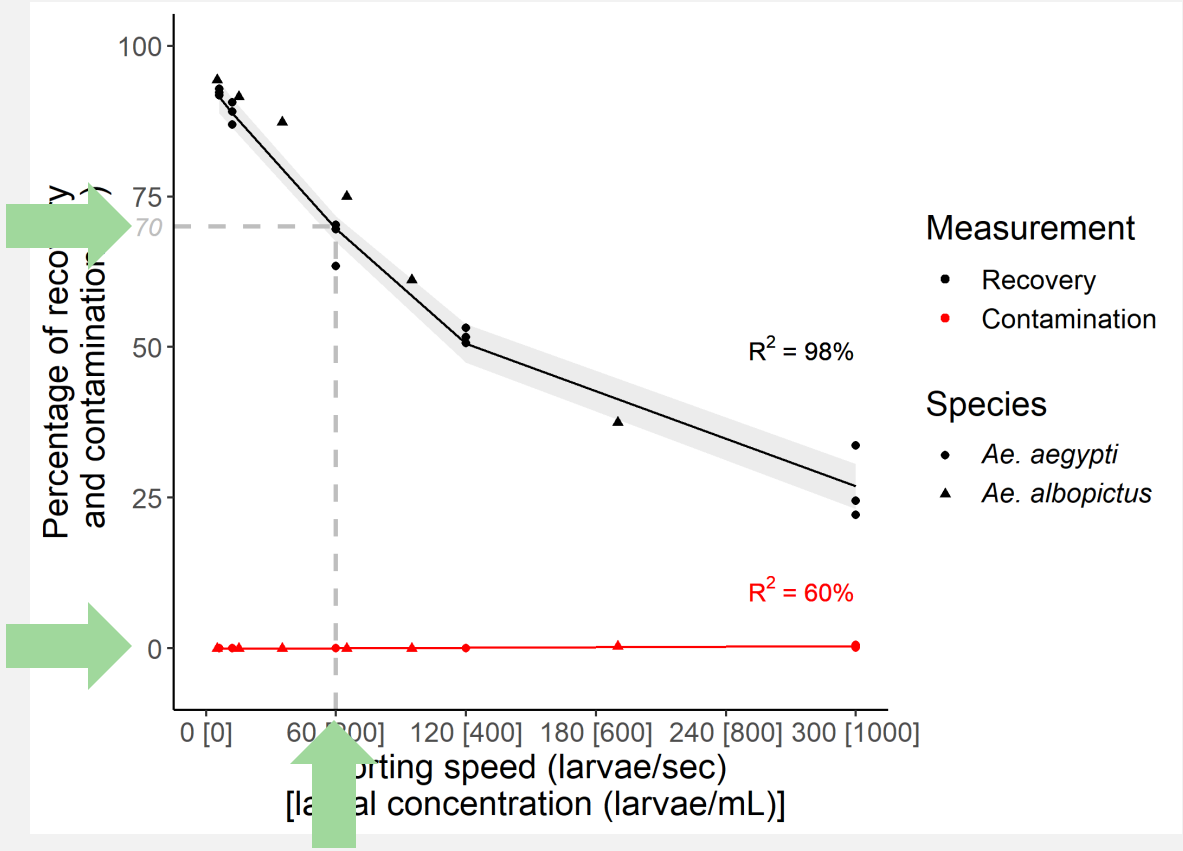




SUPPLEMENTARY

COPAS sorting speed



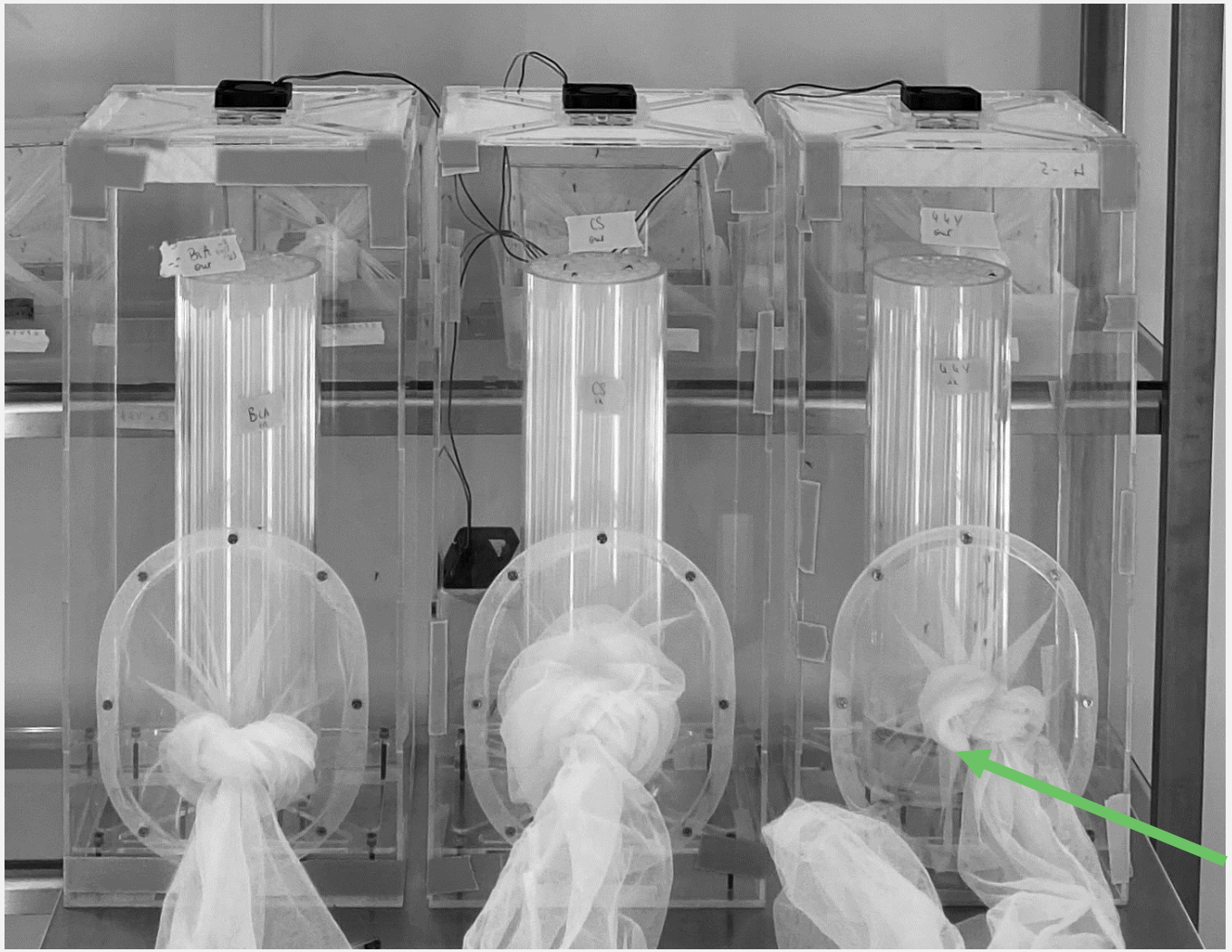


At 60 larvae / sec:
70% recovery
0% contamination

1 million males:
<15 hours

SUPPLEMENTARY

Male flight ability

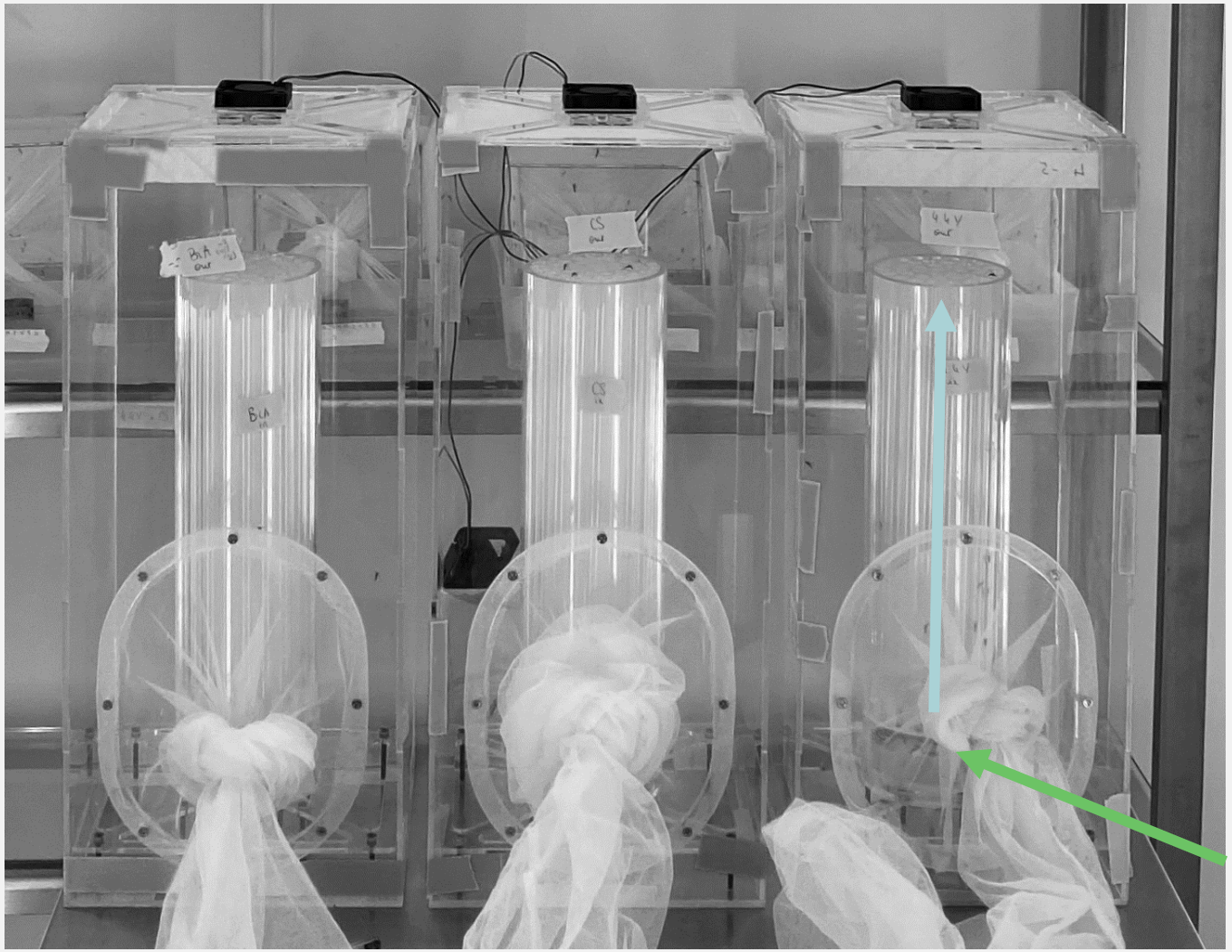


Insert mosquitoes
in the bottom chamber

Culbert et al. 2018

SUPPLEMENTARY

Male flight ability



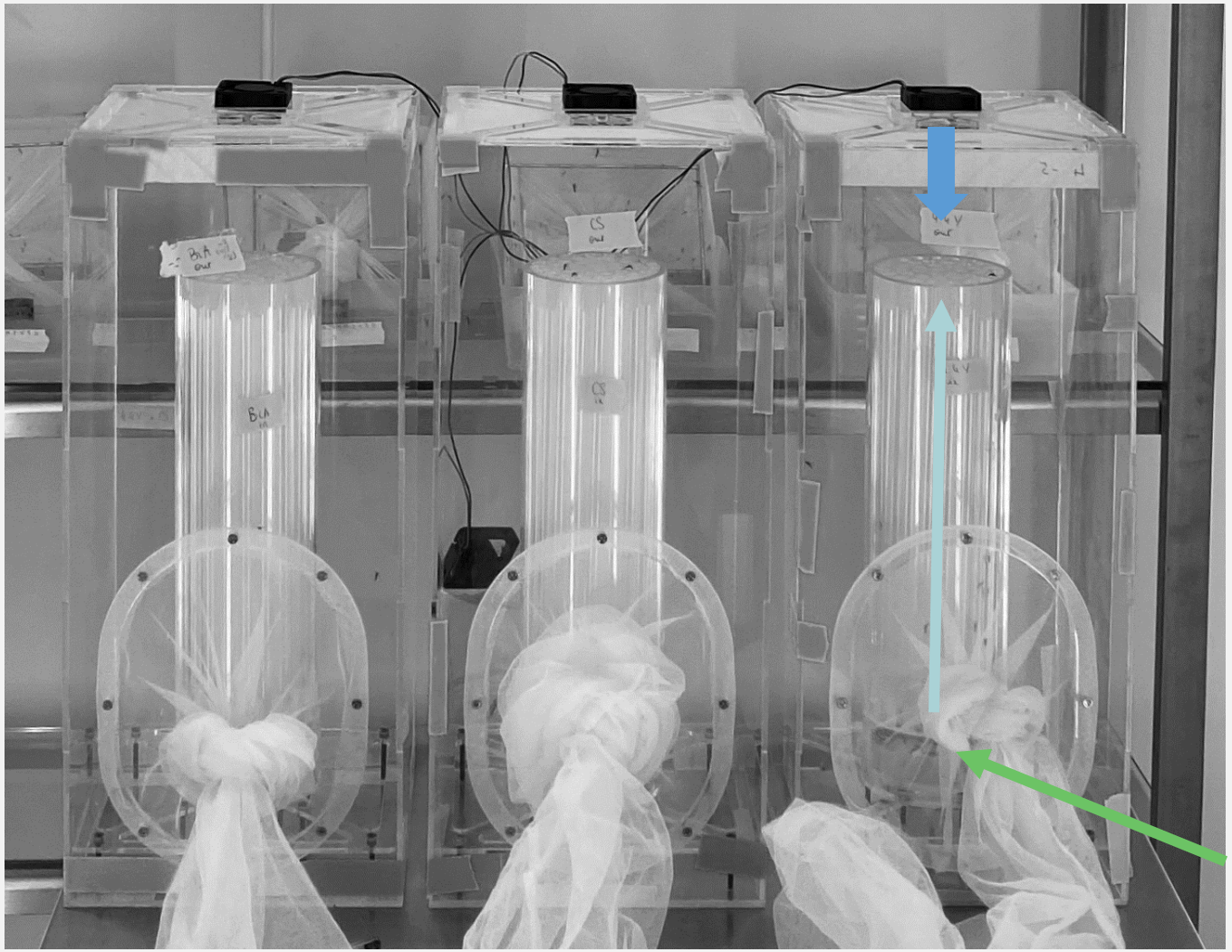
Let them fly up the tubes

Insert mosquitoes
in the bottom chamber

Culbert et al. 2018

SUPPLEMENTARY

Male flight ability



The fan blows air in the opposite direction, making it harder

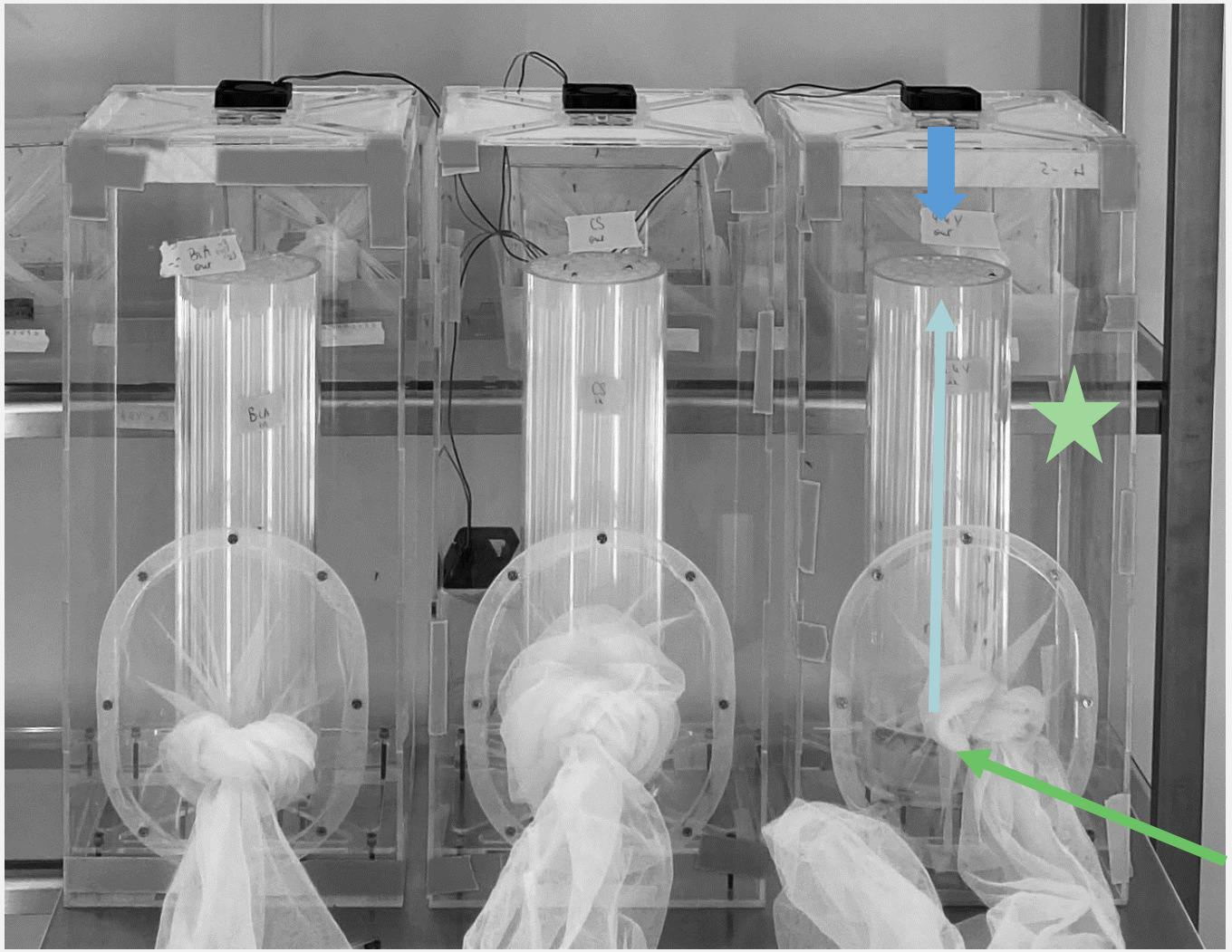
Let them fly up the tubes

Insert mosquitoes
in the bottom chamber

Culbert et al. 2018

SUPPLEMENTARY

Male flight ability



The fan blows air in the opposite direction, making it harder

The ones that managed to make it to the bigger chamber: successed

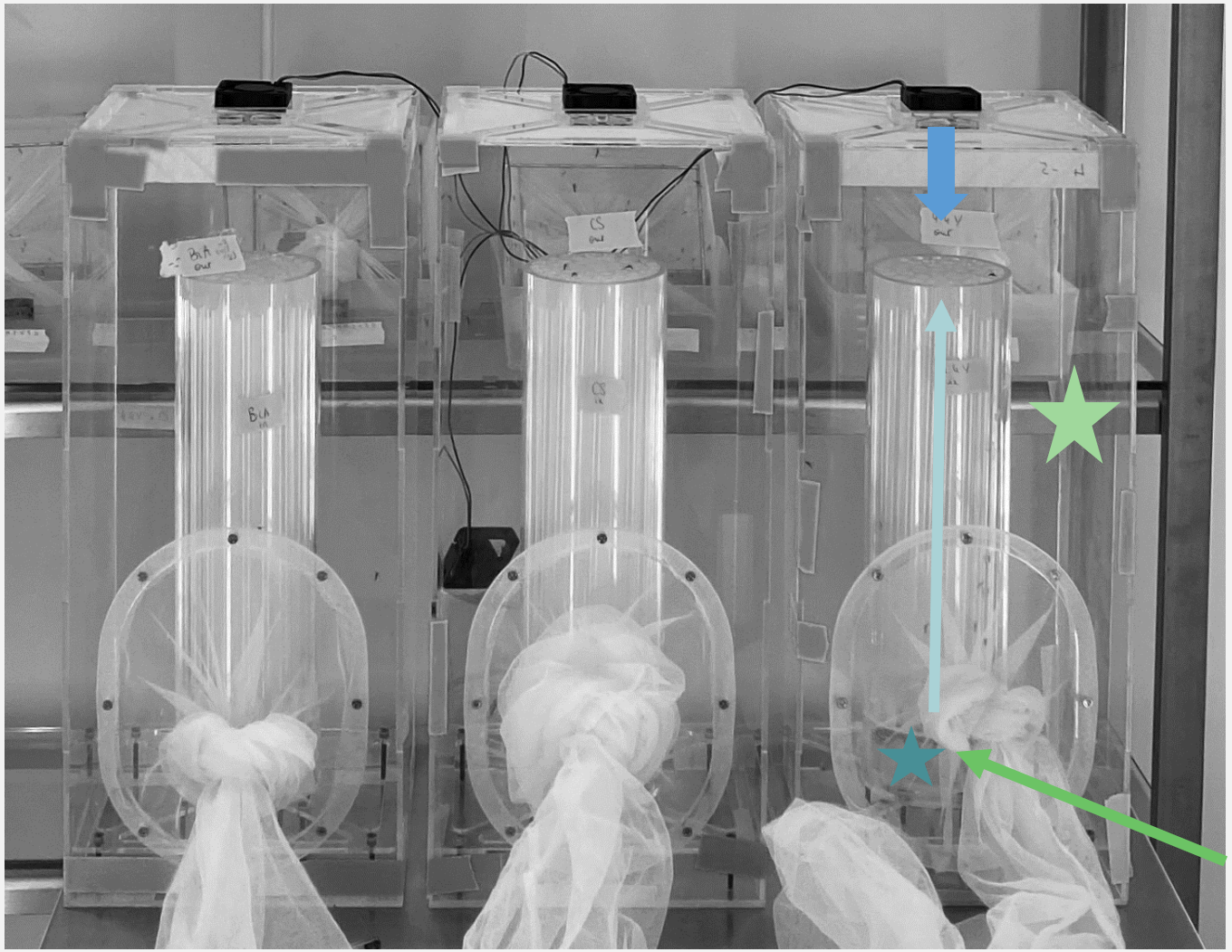
Let them fly up the tubes

Insert mosquitoes in the bottom chamber

Culbert et al. 2018

SUPPLEMENTARY

Male flight ability



The fan blows air in the opposite direction, making it harder

The ones that managed to make it to the bigger chamber: successed

Let them fly up the tubes

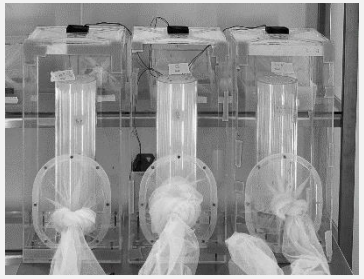
The ones that stayed in the tube or in the bottom chamber: failed

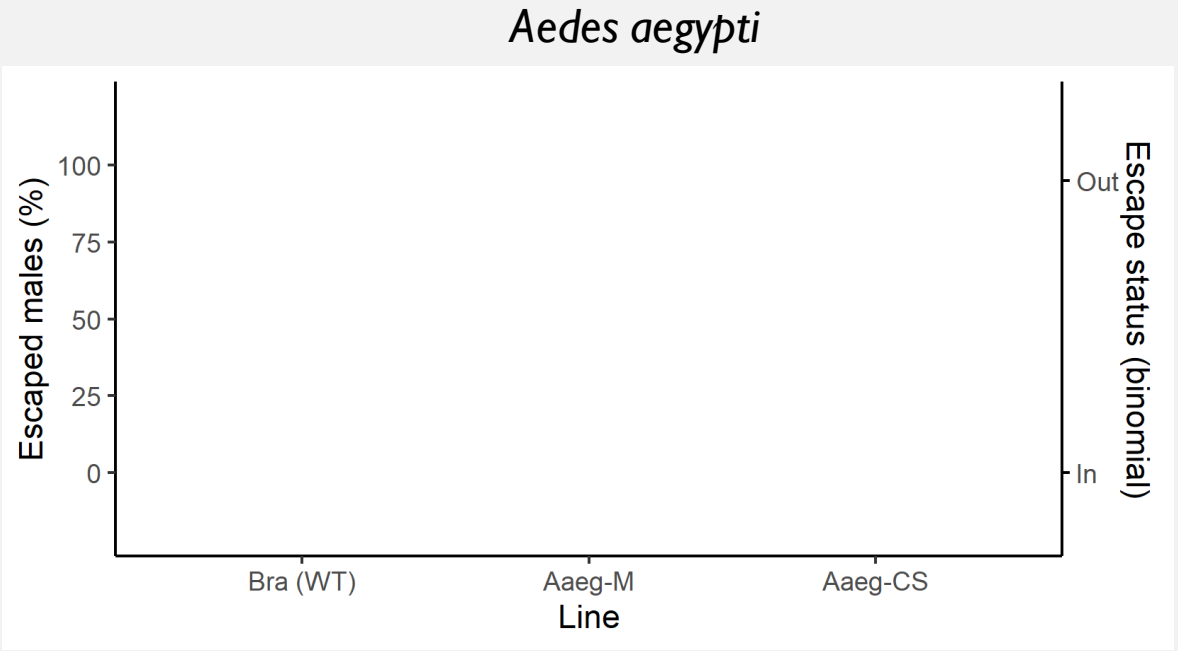
Insert mosquitoes in the bottom chamber

Culbert et al. 2018

SUPPLEMENTARY

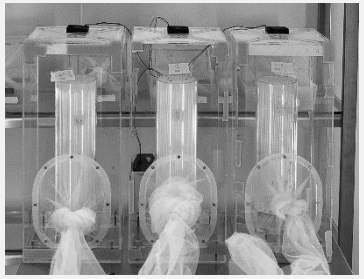
Male flight ability - GSS

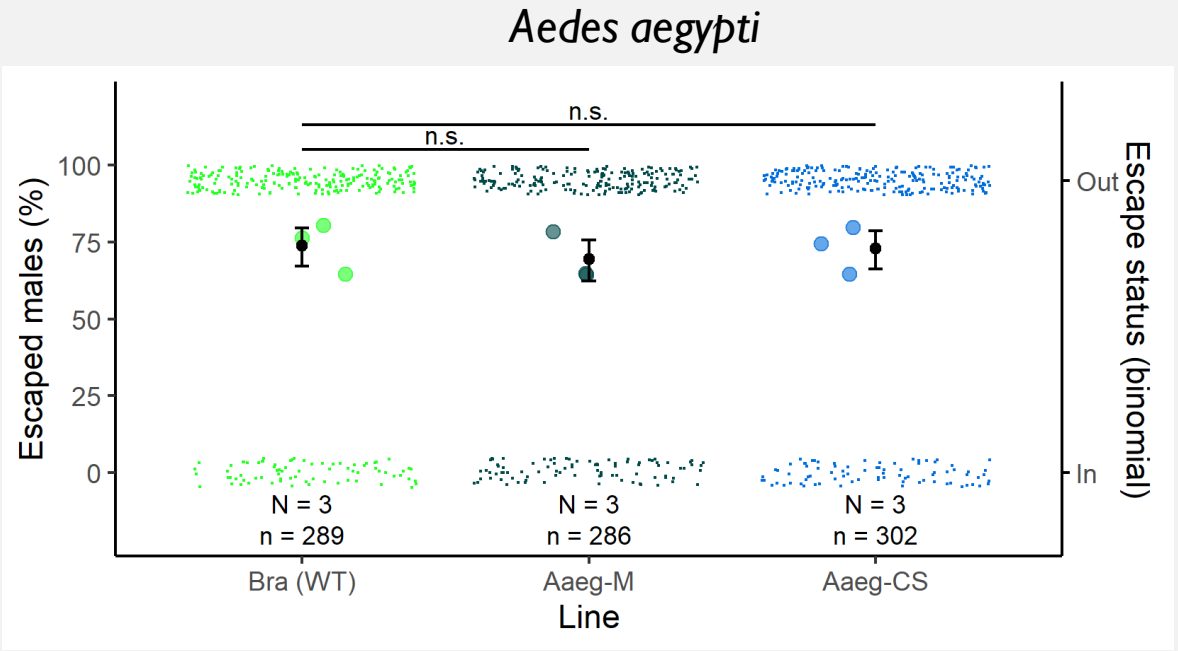




SUPPLEMENTARY

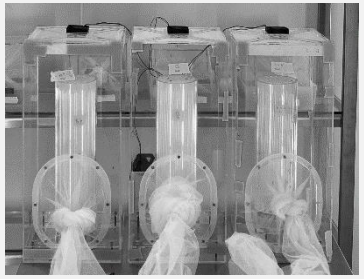
Male flight ability - GSS



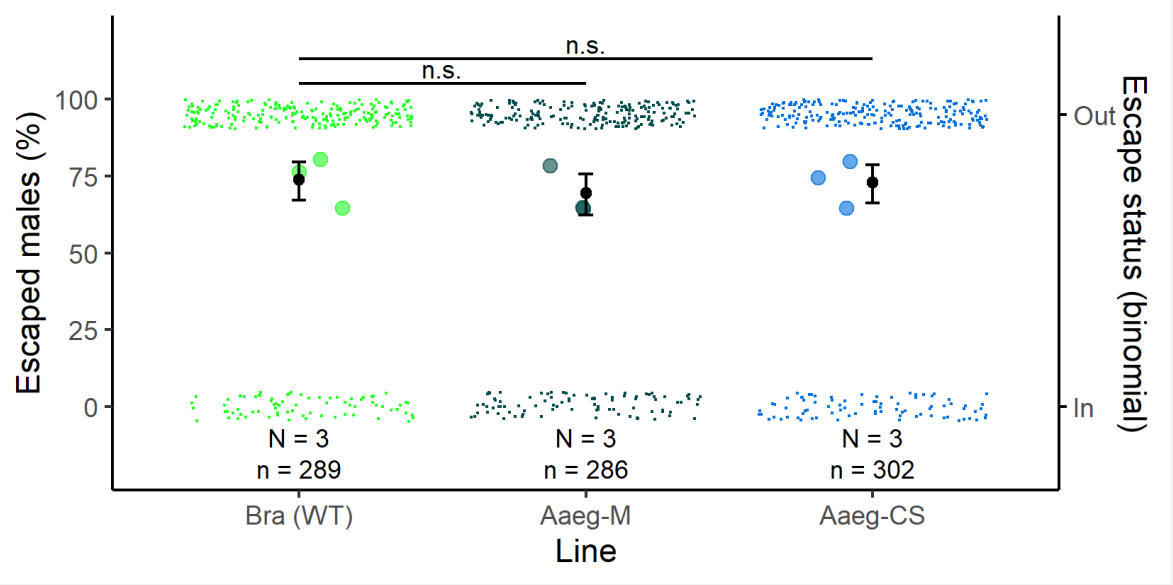


SUPPLEMENTARY

Male flight ability - GSS



Aedes aegypti



Aedes albopictus