Institute of Animal Sciences
Agricultural Research Organization

POULTRY by HUMINN

מינהל המחקר החקלאי - מכון וולקני
Agricultural Research Organization - Volcani Institute

Poultry Science Association
2023 Annual Meeting Program

Towards a Humane and Sustainable Solution:
A Genetic Model to Eliminate Male Chicks Culling in the Egg Industry

Yuval Cinnamon

The GOLDA project

The hens that lay eggs from which only WT females hatch

Why do we need GOLDA?

Layer & Broiler Breeders

The poultry industry is the major contributor of dietary protein, worldwide

THE PROBLEM

Unwanted by the egg and meat industries, ~7 billion male chicks are culled around the world annually by inhumane and costly methods

INHUMANE

7 Billion Day-Old Male Chicks

COSTLY

~7 Billion US$

A global 50% increase in demand for eggs from 2020 to 2035 will intensify the issue of male chick culling at a faster pace

- Major sustainability problem
- The EU are committed to ban the culling of day-old male chicks
- The US is also committed, but still seeking for a practical and viable solution

Number-wise, this is most devastating animal welfare issue worldwide

What are the requirements for a viable solution?

Requirements for an ideal solution

The solution should be highly accurate, very fast, safe to the layers and cost-effective

1 Methods must be applicable to eggs prior to setting for incubation.

2 Methods must not affect the integrity of egg-shells and the interior of eggs.

3 Eggs containing male blastoderm cells need to be used for reasonable purposes.

4 Applied methods should have no negative effects on embryonic development.

5 Rate of hatchability of chicks must not be affected.

6 Necessary technical equipment must be available and its use economically feasible.

7 Methods must be applicable to large numbers of eggs.

8 Sorting by gender must be possible within short periods of time.

9 Methods must be f ree of any negative effect on female reproduction.

10 Post-embryonic physical and behavioral development of chicks remains unchanged.

11 Methods must be acceptable f rom a humanitarian and ethical point of view.

12 The level of errors must be minimal and acceptable to the industry.

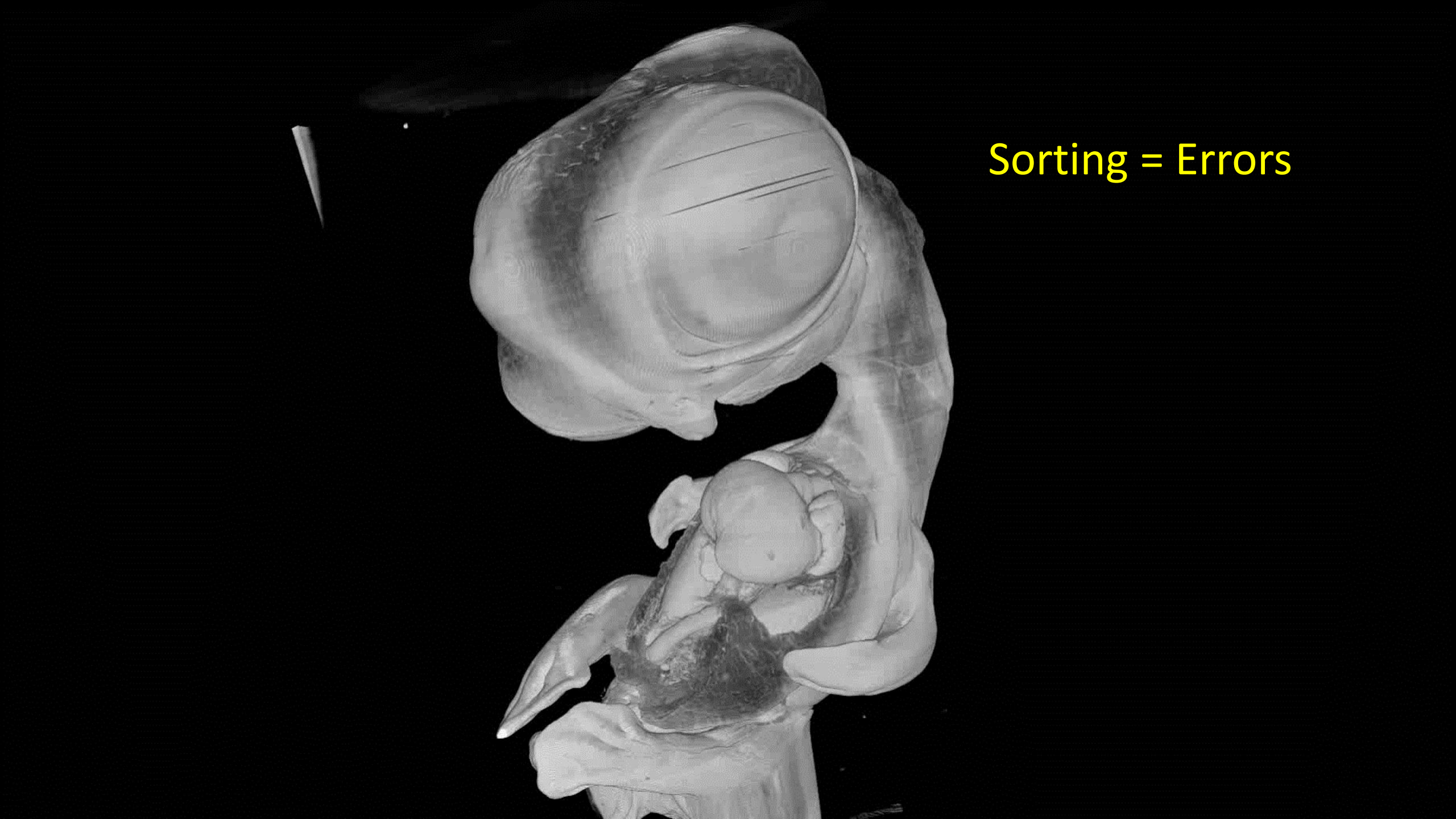
13 There must be an accepted solution to unwanted hatched males

Kaleta and Redmann (2008)

Addressing the Culling of Day-Old Male Chicks

Previous and ongoing attempts:

- Attempts to bias the sex ratio
- Development of dual purpose breeds
- **Sorting strategies**


Sorting = Errors

Addressing the Culling of Day-Old Male Chicks

Previous and ongoing attempts:

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Despite all attempts, to date there are no viable solution.

POULTRY WORLD

Henner Schönecke, chairman of Bundesverband Ei, which is the branch organisation for the German layer sector. Photo: Koos Groenewold

Germany: ‘Only a few hatcheries will survive’

27-04 | Market trends/analysis | Article

FOUNDATION FOR FOOD & AGRICULTURE RESEARCH®

Egg-Tech Prize Request for Applications

Open Opportunity

Hatcheries have no use for male chicks, so billions of them are immediately culled every year, a practice increasingly seen as unethical and unsustainable. Image credit: iStock/N-sky

Egg-Tech Prize for tech solutions to end male chick culling remains unclaimed as 'no applicants met the criteria'

April 20, 2023 Elaine Watson

Organizers of the $6 million Egg-Tech Prize—a high-profile global competition to find scalable technologies to sex chicks before they hatch—have not yet issued a final award because "none of the applicants met the criteria," *AFN* has learned.

Addressing the Culling of Day-Old Male Chicks

Previous and ongoing attempts:

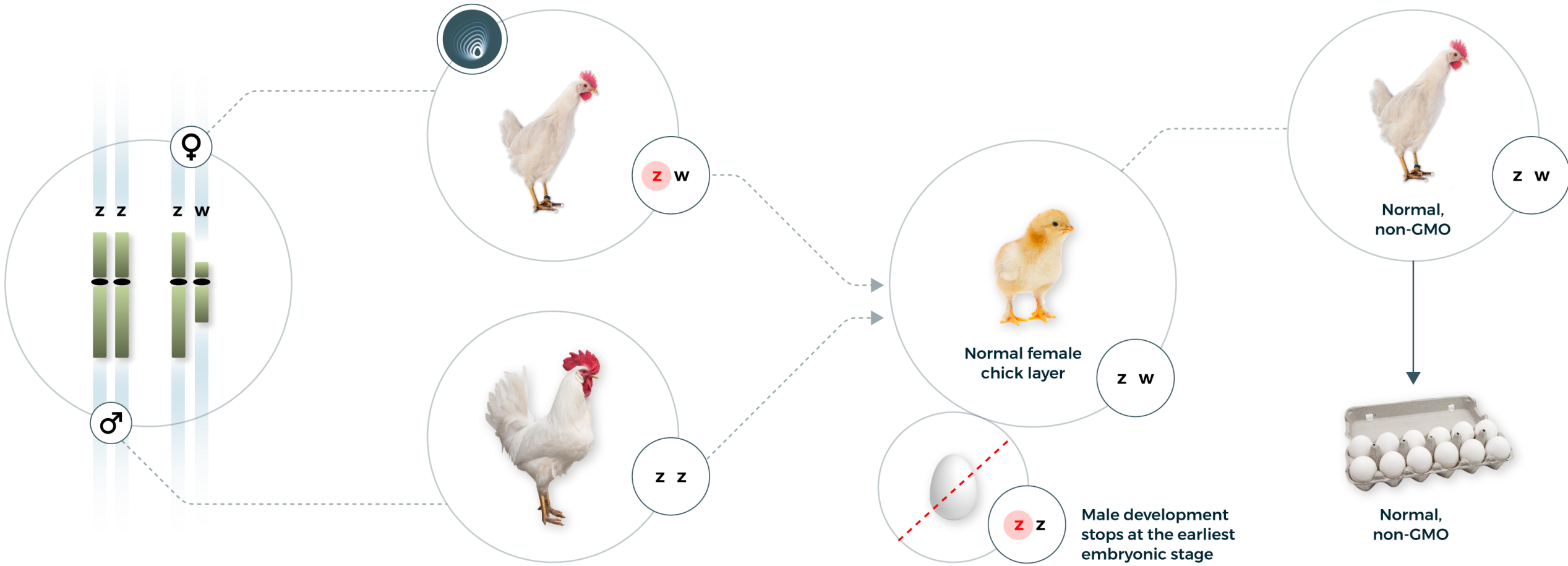
- Attempts to bias the sex ratio
- Development of dual purpose breeds
- **Sorting strategies**

Despite all attempts, to date there are no viable solution.

The rationale behind our solution is based on two key insights:

1. Practically, there are no differences between male and female embryos
2. Chickens' sex is determined by sex chromosomes, so they are key to the solution.

Genetic Solution to End the Culling of Day-Old Male Chicks



REGULATORY CLEARANCE

efsa EUROPEAN FOOD SAFETY AUTHORITY

- The European Commission has confirmed that the layer hens and the table eggs produced by NRS Poultry technology are wild-type (i.e., non-GMO).

- As a result, the table eggs produced by NRS Poultry Technology can be marketed immediately in all EU member states without any restrictions or special labeling.

FDA U.S. FOOD & DRUG ADMINISTRATION

Center for Veterinary Medicine

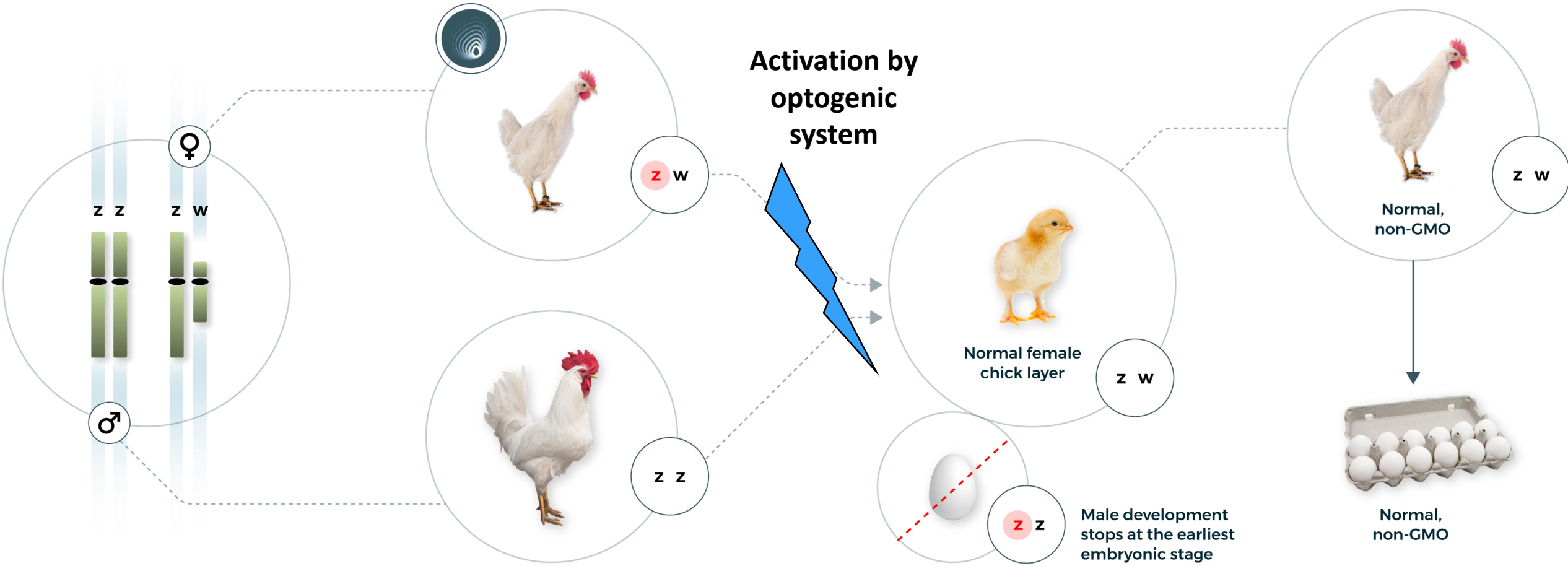
The CVM has indicated in a written response that they "…may consider exercising enforcement discretion for some of the activities/phases described…".

Implying that the wild-type layer hens and table eggs could be exempt from regulatory approval once sufficient evidence is provided.

COMPASSION in world farming

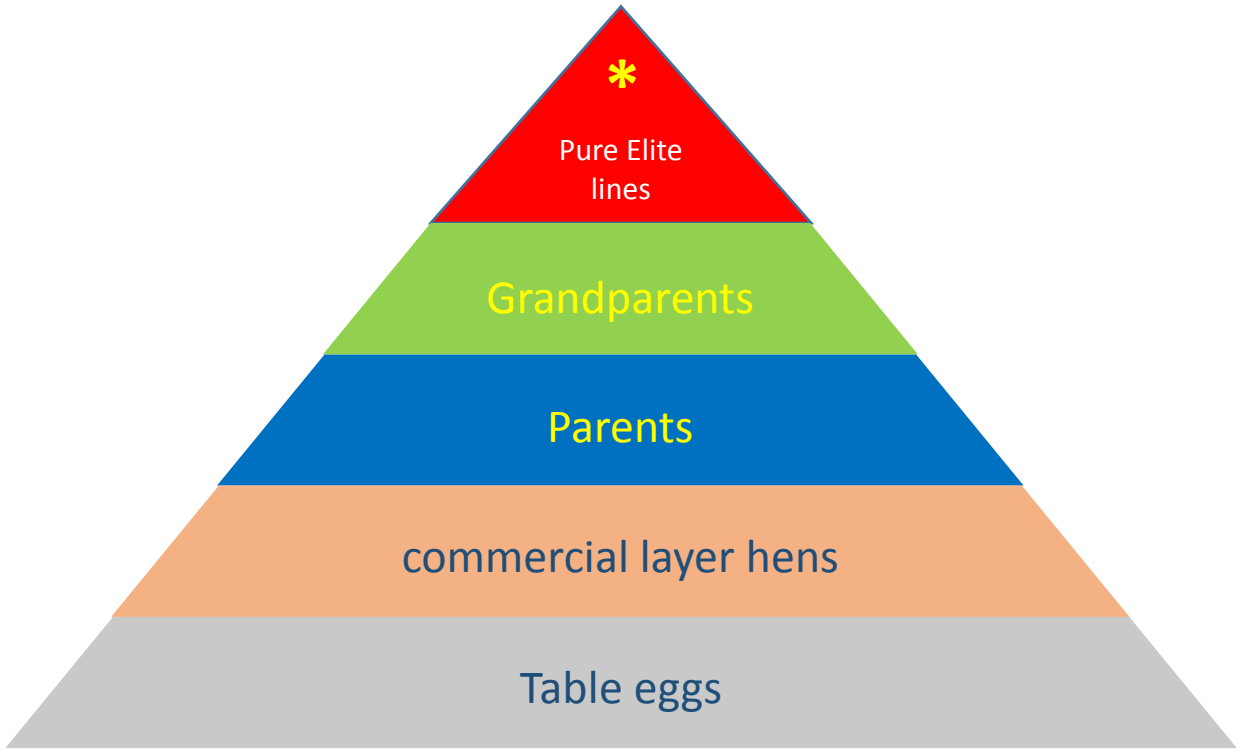
ciwf.org

Genetic Solution to End the Culling of Day-Old Male Chicks

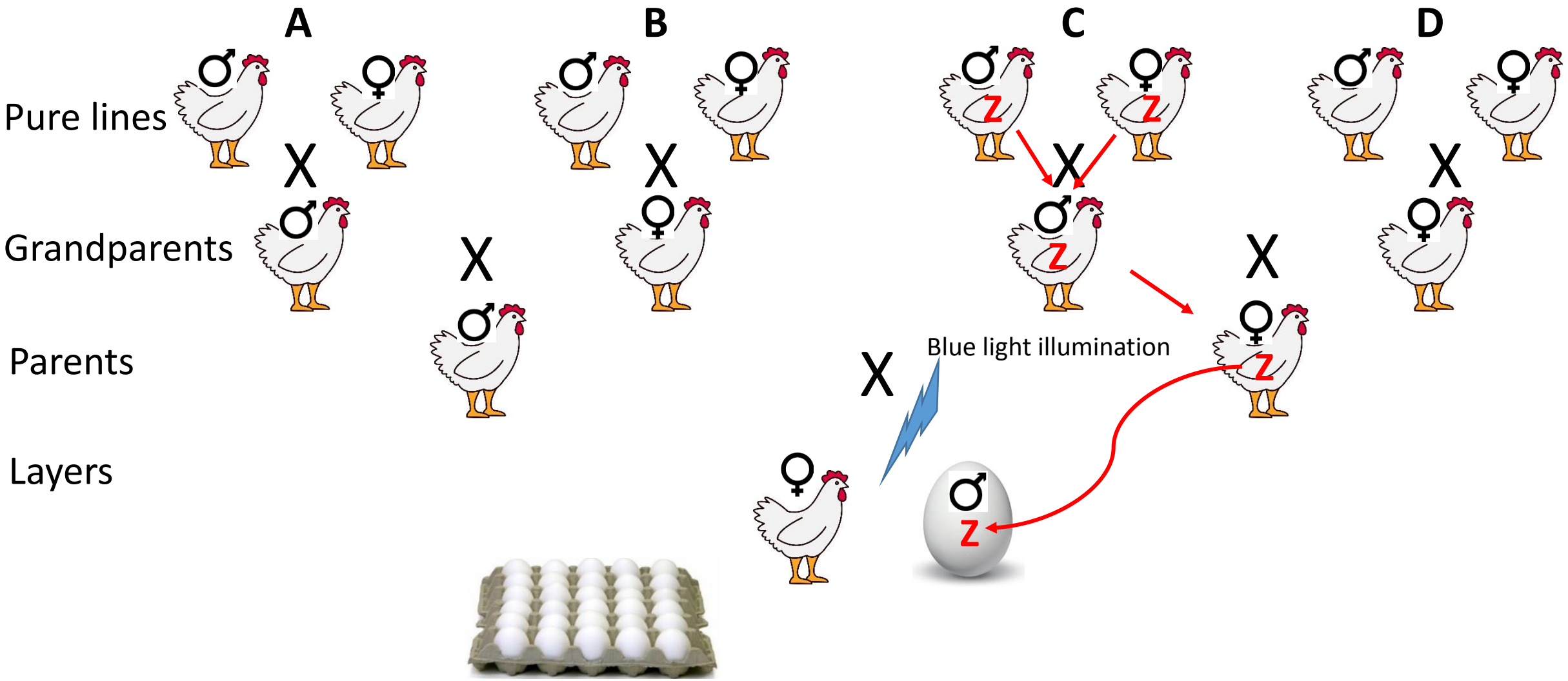


How does the solution integrates with the breeding program of the genetic lines?

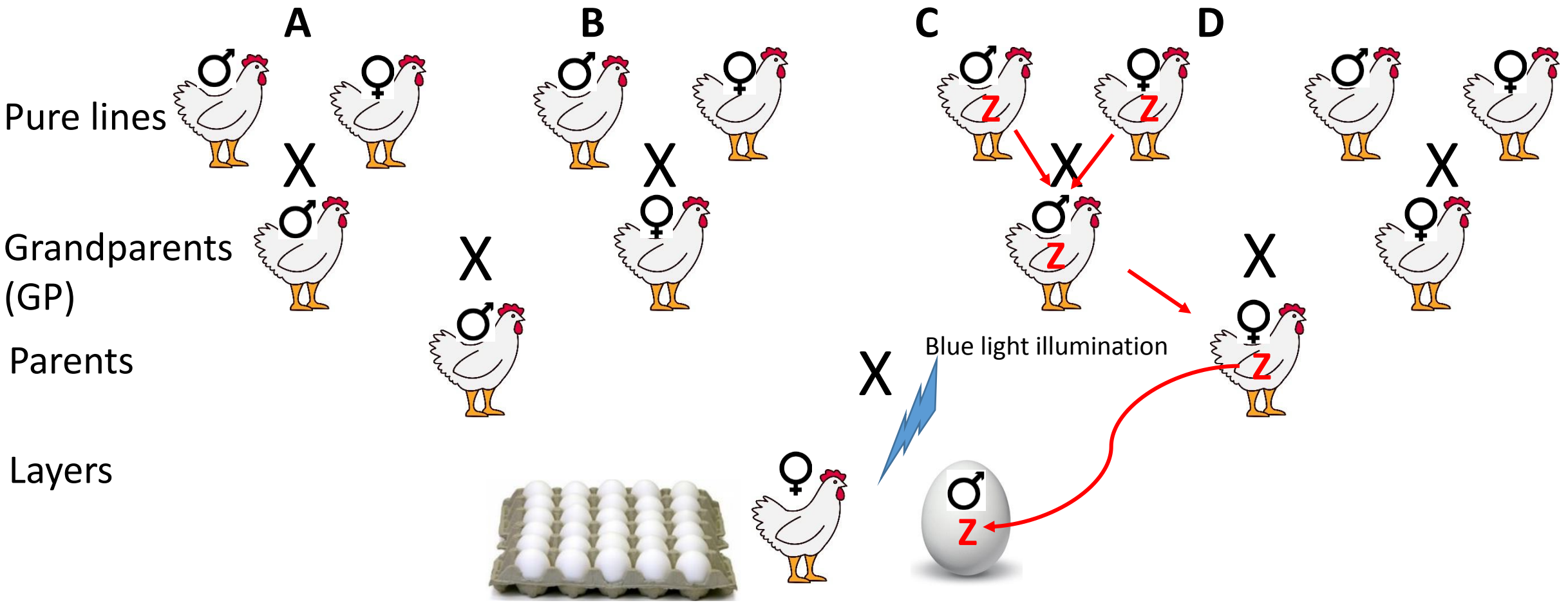
The production pyramid



Integration into Breeding Program



Integration into Breeding Program



What is the workflow of generating genetically transformed chickens?

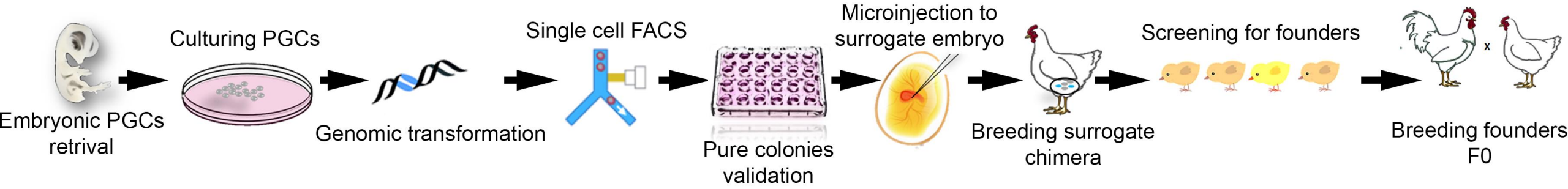
Generating Genetically Transformed Chickens

Primordial Germ Cells (PGCs) are unique embryonic cells that transfer genetic information across generations.

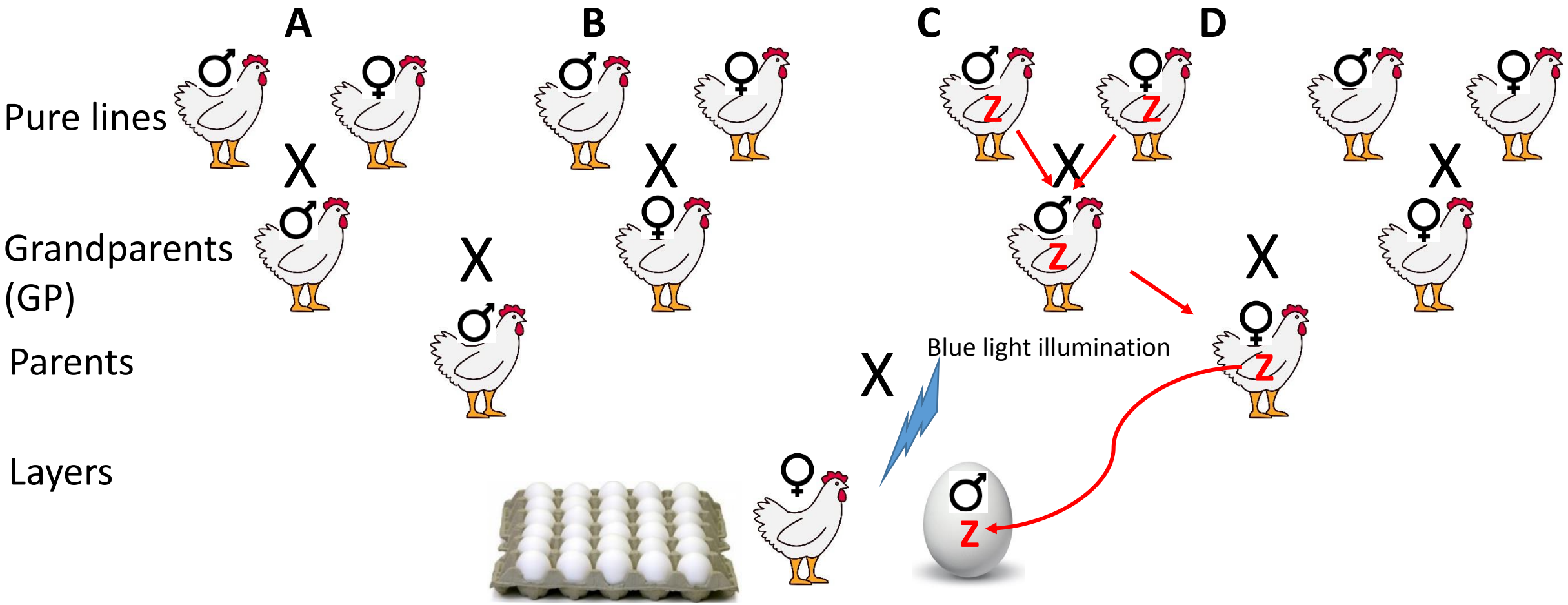
PGCs in the embryo

- In males, PGCs will colonize the testes → Upon sexual maturity PGCs will differentiate into **sperm**
- In females, PGCs will colonize the ovary → Upon sexual maturity PGCs will differentiate into **eggs**

PGCs → **Sperm**
PGCs → **Eggs**



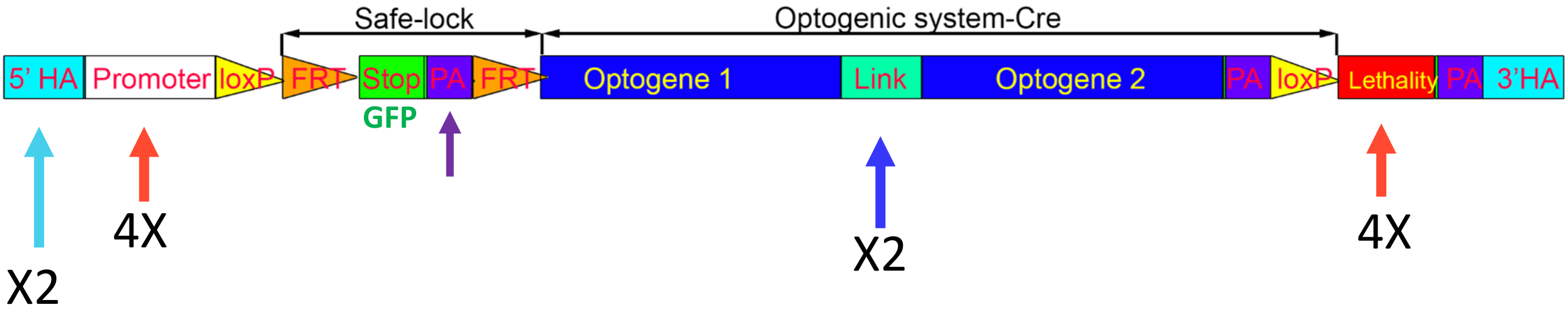
Integration into Breeding Program



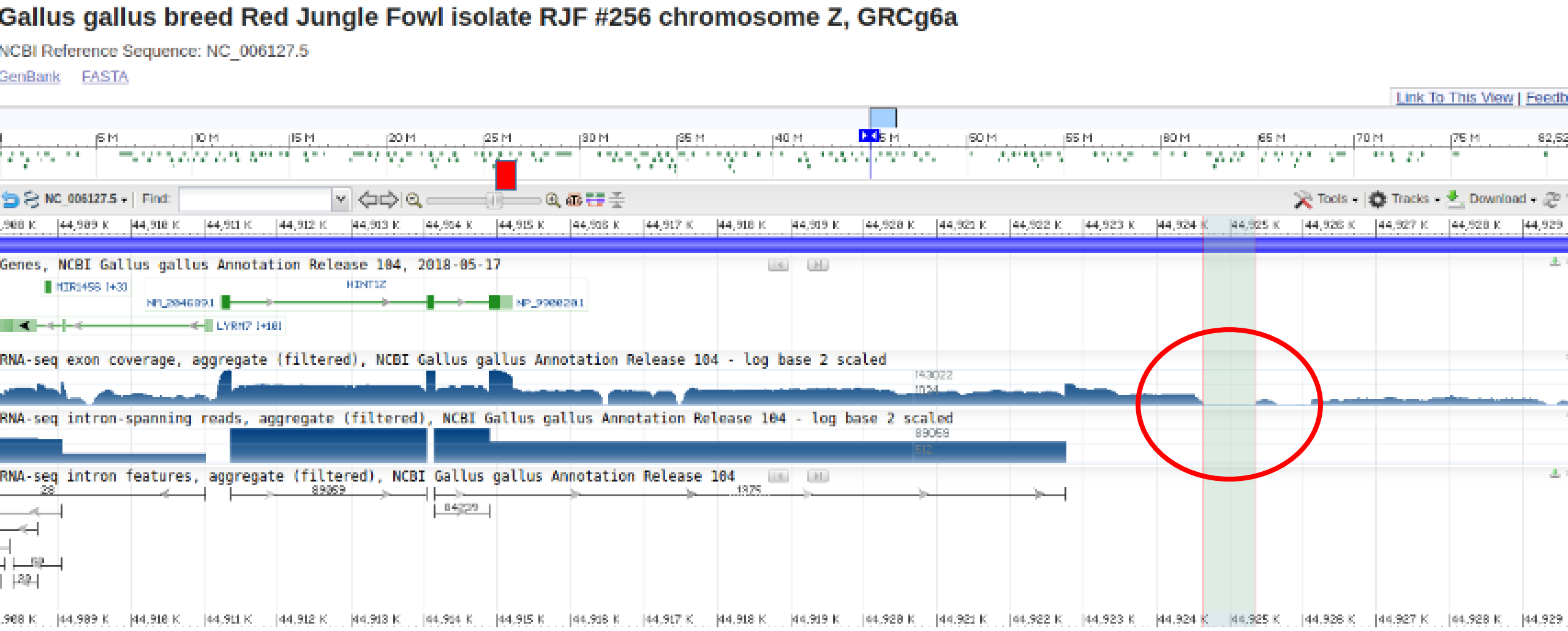
What are the requirements for the targeting vector to be introduced on the Z chromosome?

Requirements from the HolyGrail

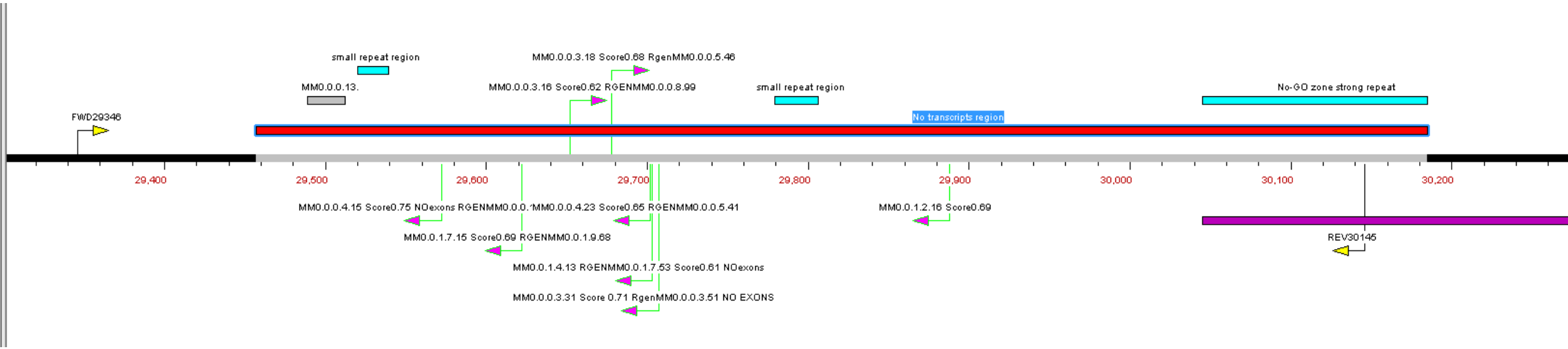
1. Integration to the Z chromosome
2. Single ubiquitous promoter
3. Light inducible molecular “switch” – Optogenic system
4. Gene to stop embryogenesis
5. Security “Safe-lock” element to prevent unintended activation



Integration site on the Z chromosome

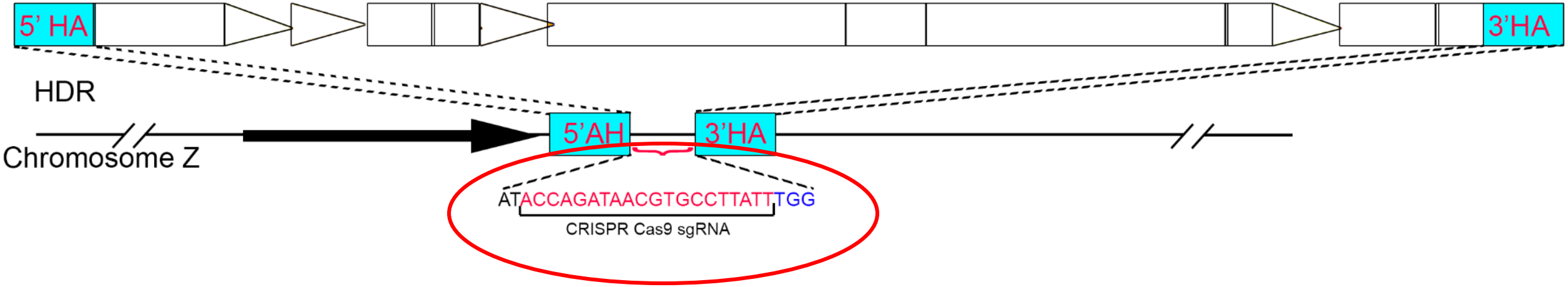


Integration site on the Z chromosome

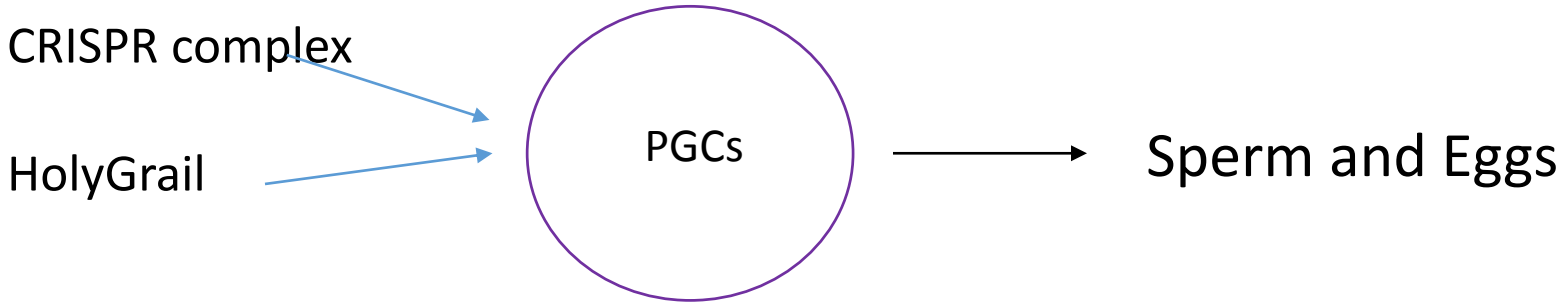


Integration to the Z chromosome

The integration site on the Z chromosome

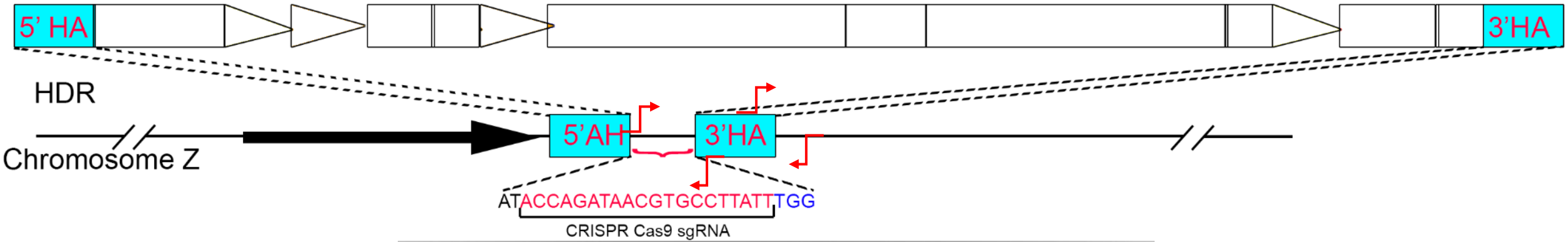


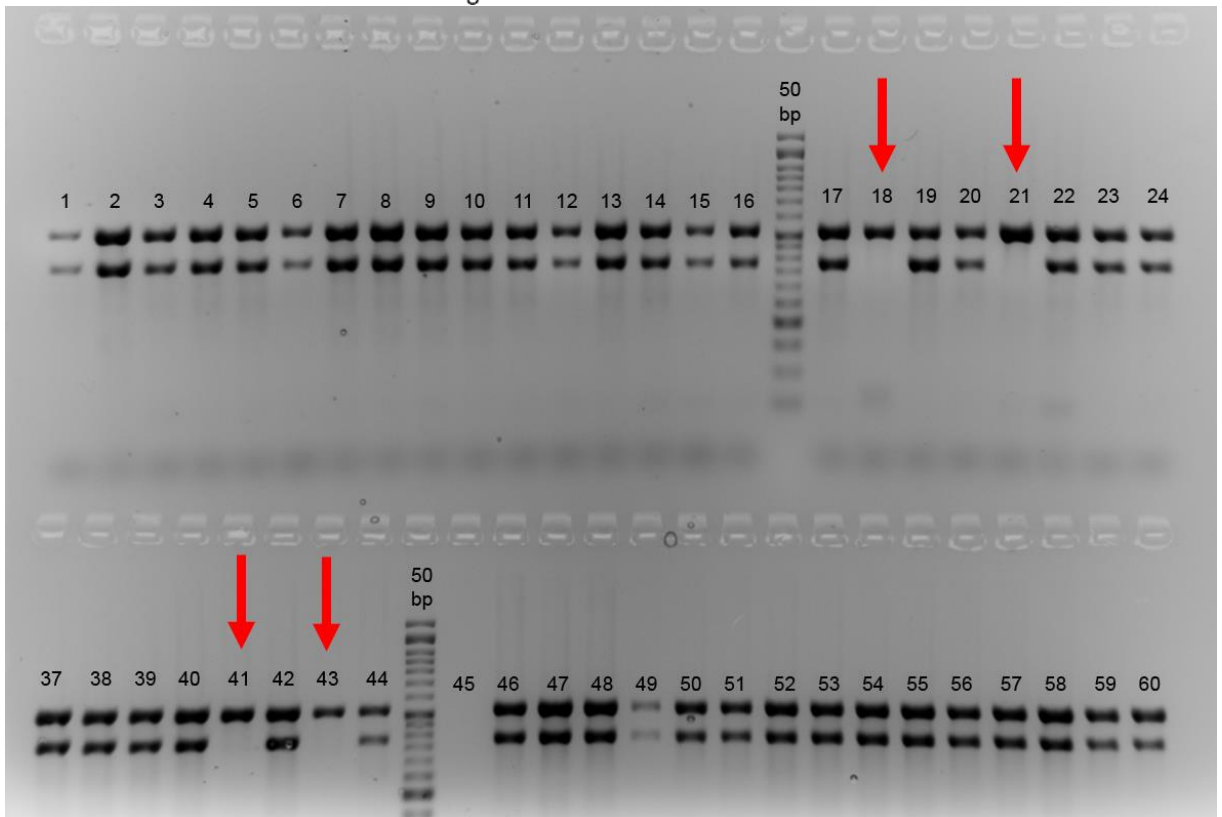
Co-transfection



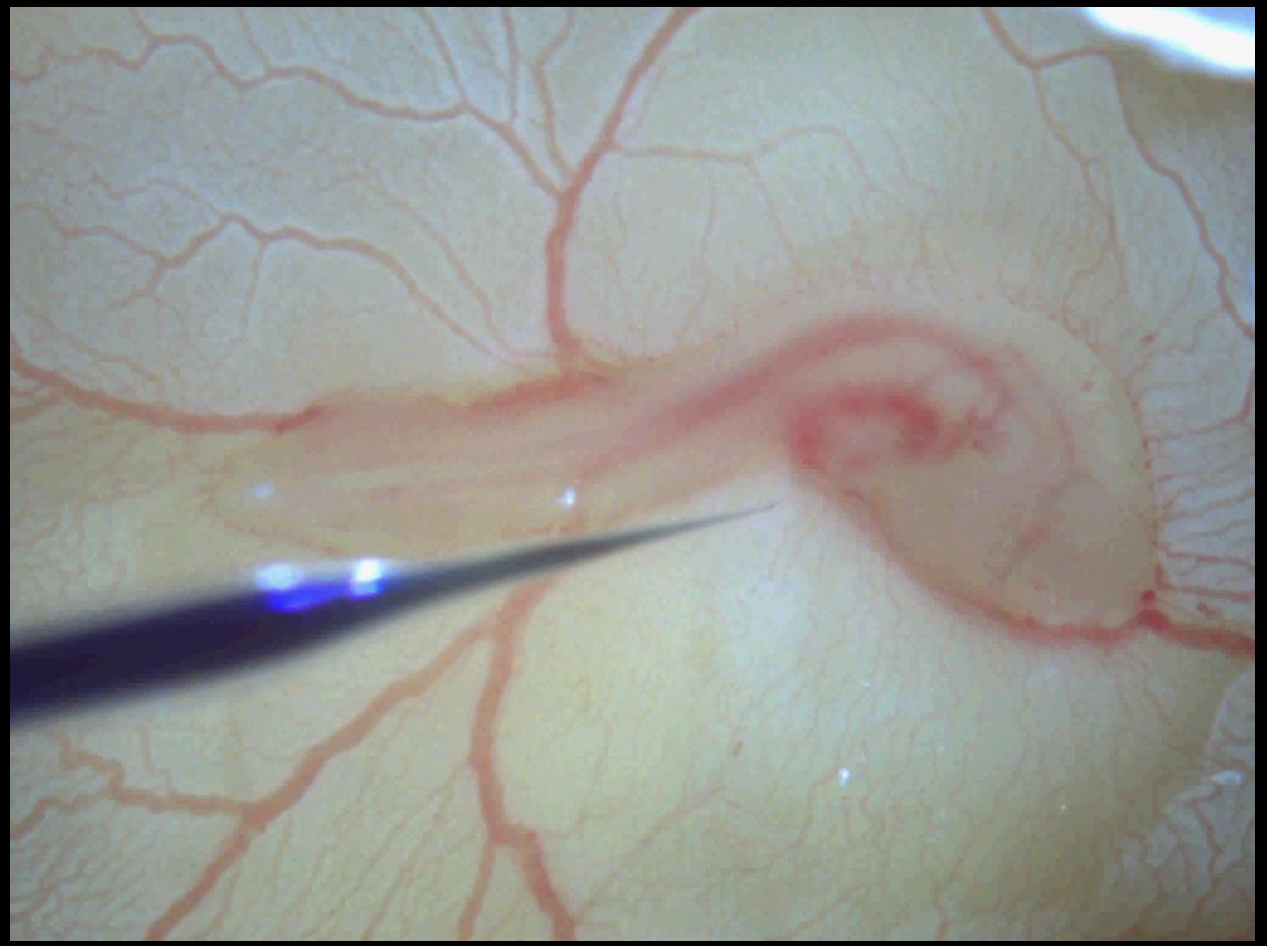
Integration to the Z chromosome

The integration site on the Z chromosome

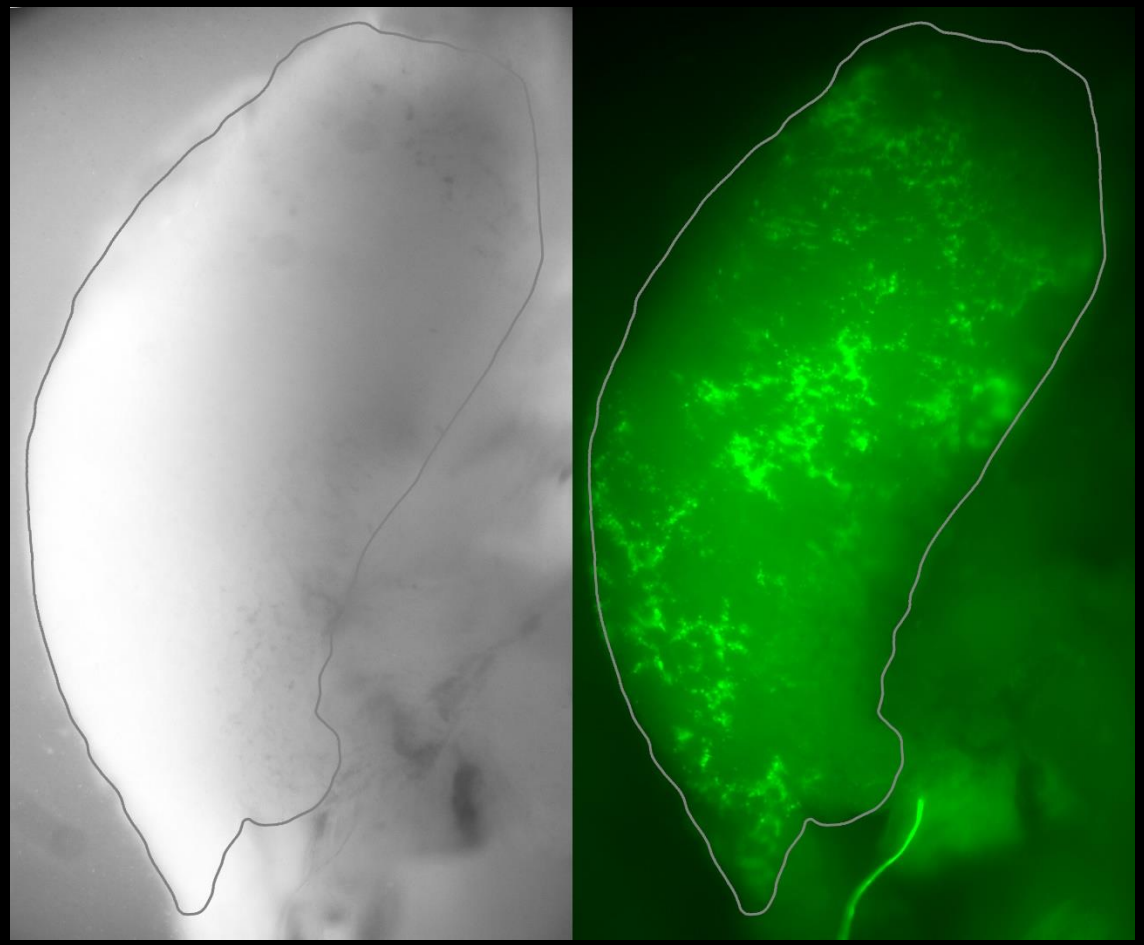


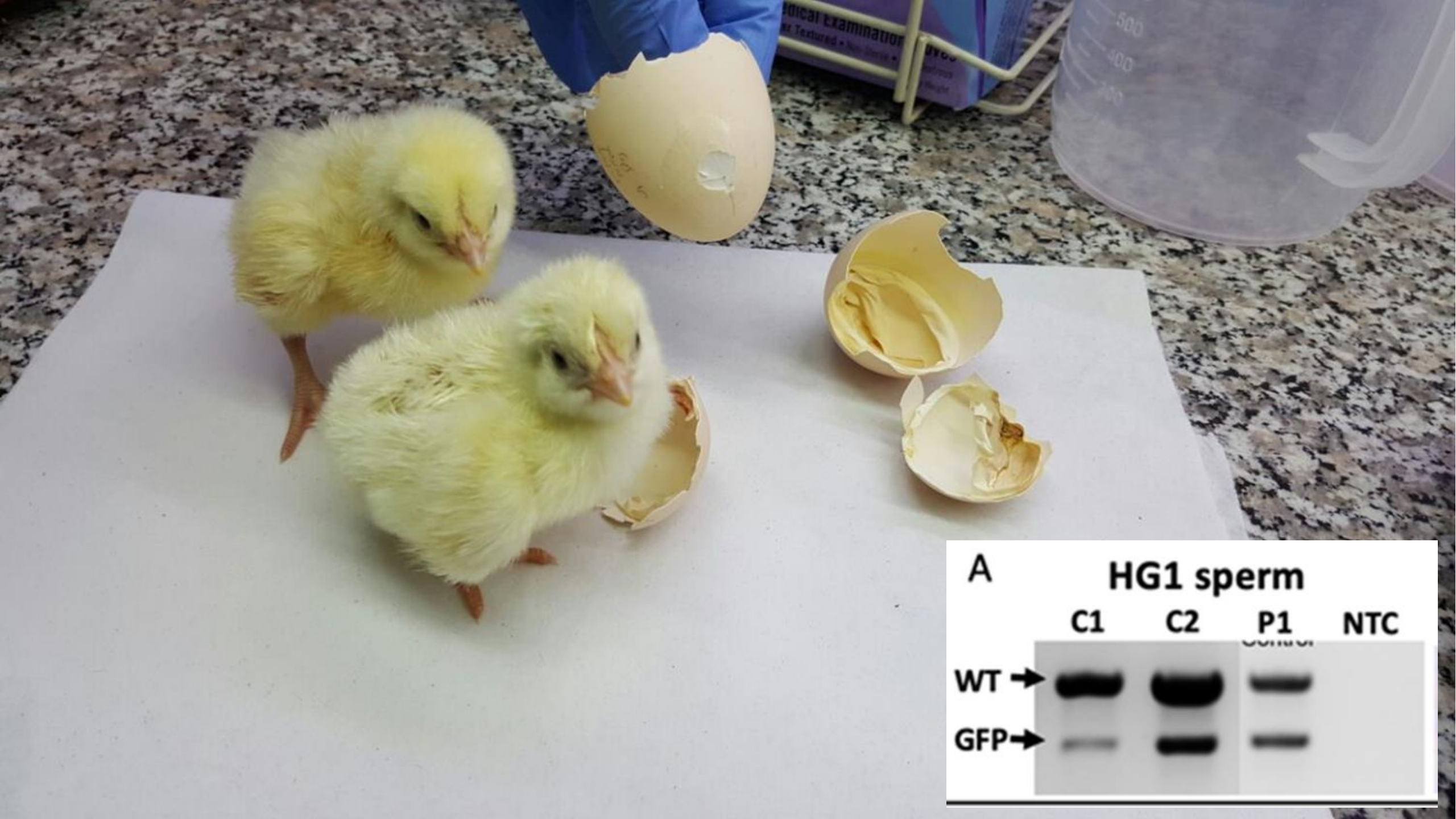


Injection of genetically transformed PGCs



Colonization of PGCs in the gonads

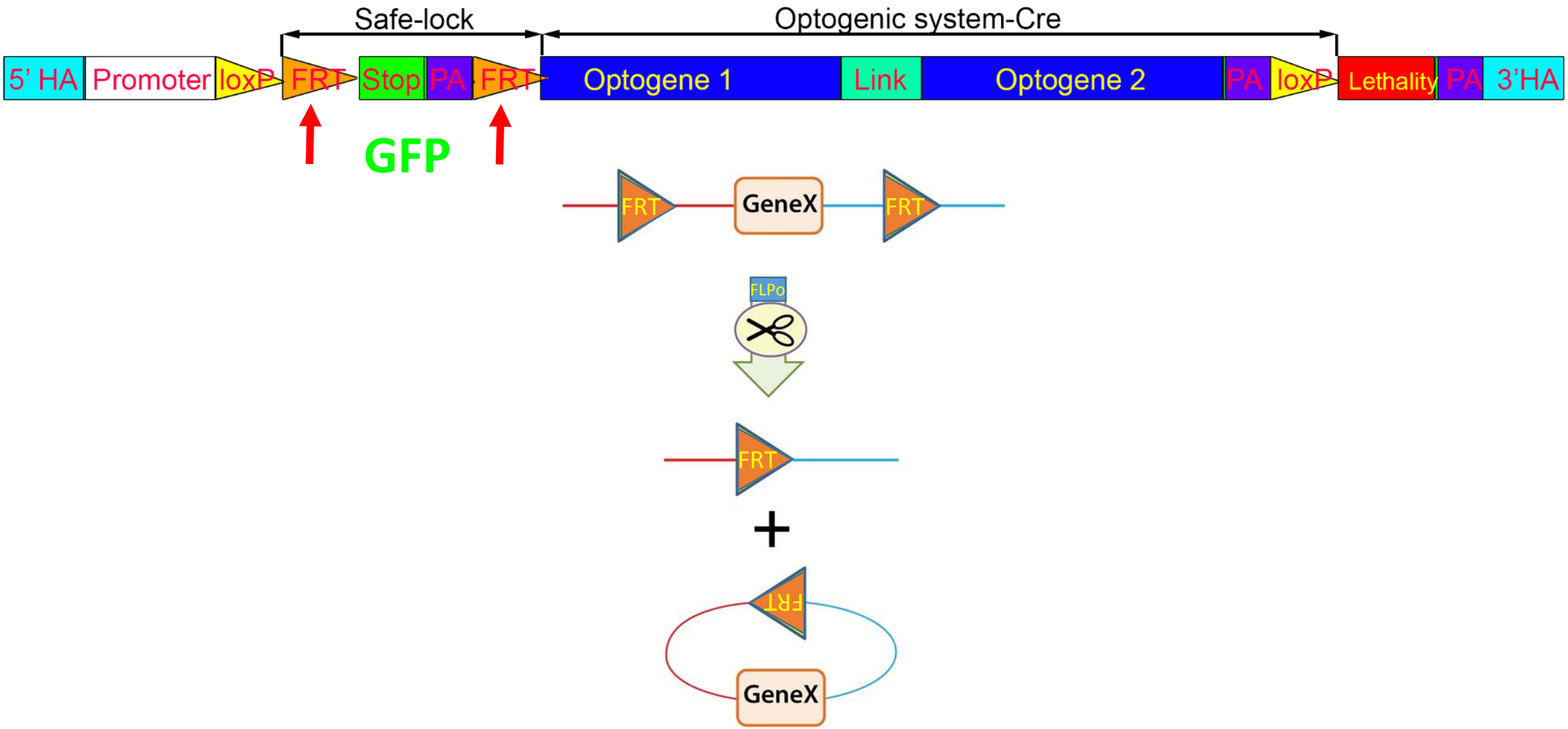


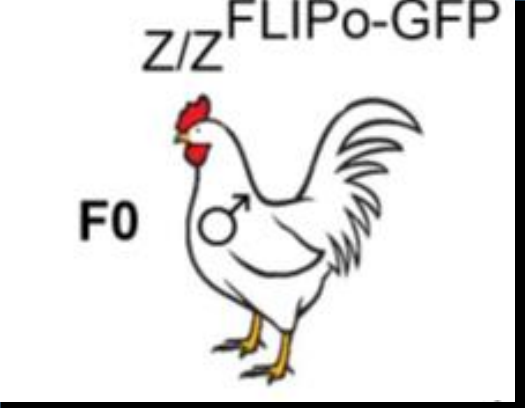

A
HG1 sperm
C1
C2
P1
NTC
WT
GFP

CoronChick – The HolyGrail Founder

Design of the HolyGrail construct

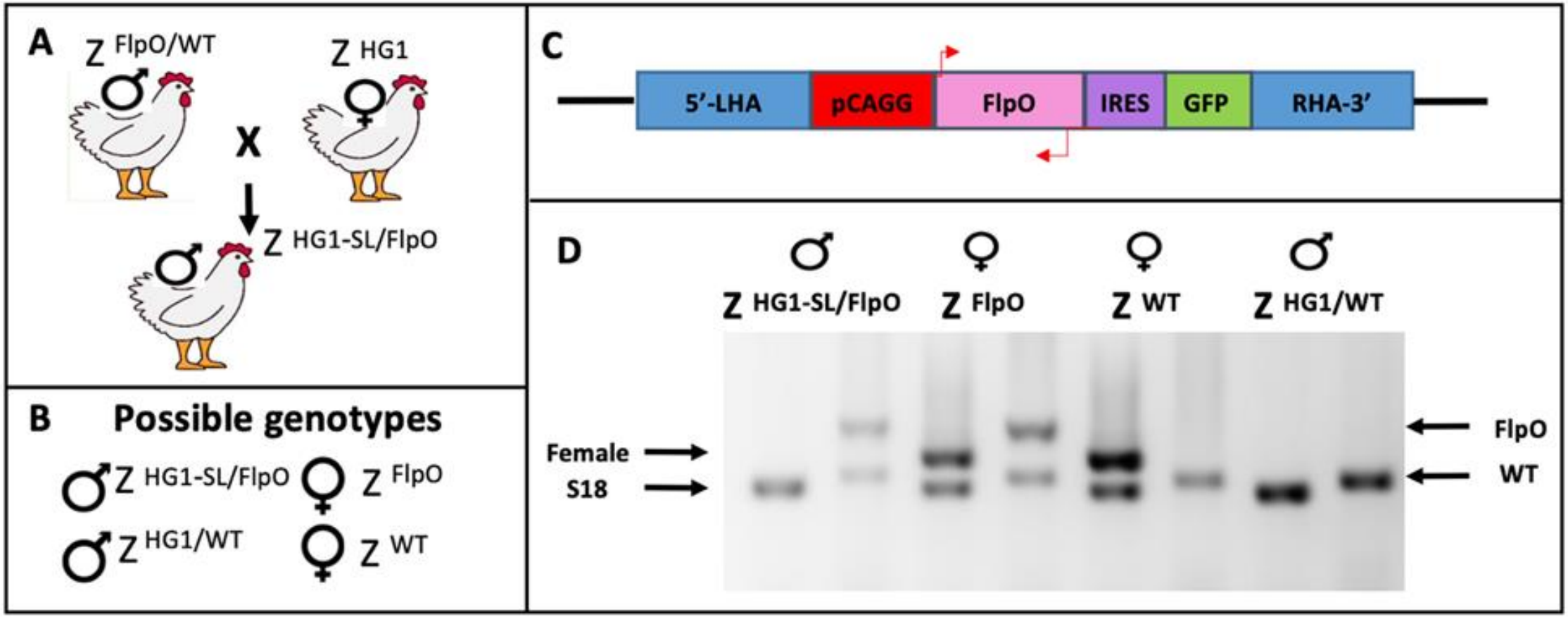
The “Safe-lock” mechanism

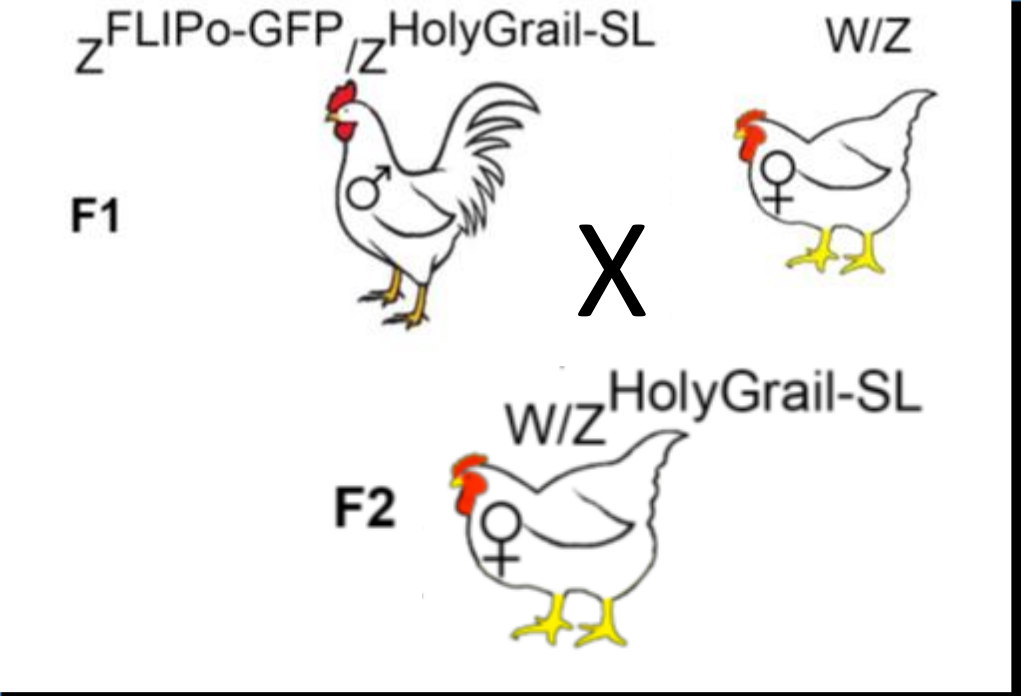




FlipChick – GFP positive FLPo

WT GFP negative


A
Z FlpO/WT
Z HG1
X
Z HG1-SL/FlpO
B
Possible genotypes
♂ Z HG1-SL/FlpO
♀ Z FlpO
♂ Z HG1/WT
♀ Z WT
C
5'-LHA
pCAGG
FlpO
IRES
GFP
RHA-3'
D
♂ Z HG1-SL/FlpO
♀ Z FlpO
♀ Z WT
♂ Z HG1/WT
Female
S18
FlpO
WT

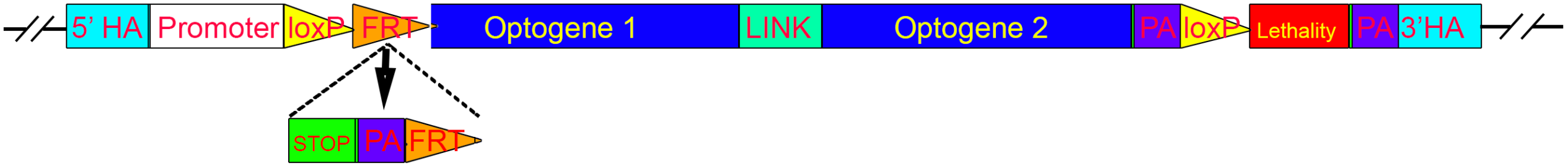

ZFLIPo-GFP/ZHolyGrail-SL
W/Z
F1
X
W/ZHolyGrail-SL
F2

W/Z HolyGrail-SL

F2

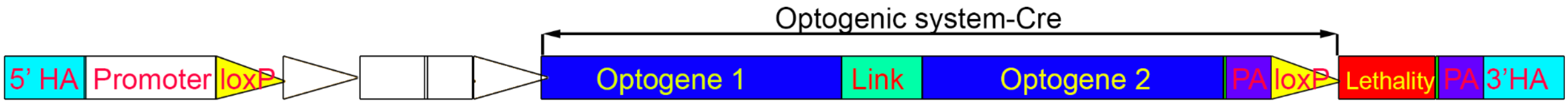
GOLDA

The GOLDA genome – The HolyGrail without the GFP “Safe-Lock” mechanism



So what is the Opotogenic molecular switch?

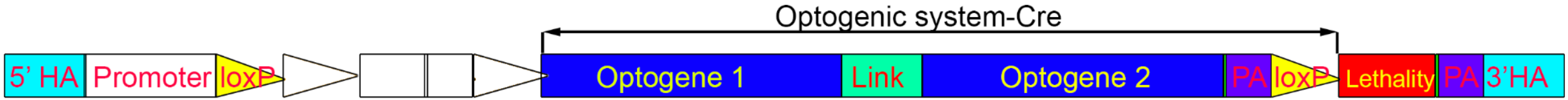
The light-inducible molecular - The optogenic system



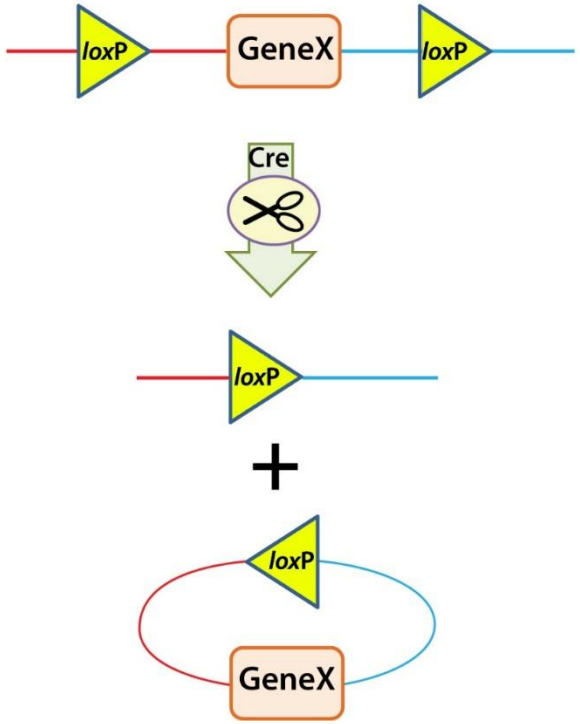
What are optogenes?

Design of the HolyGrail construct

The molecular light-inducible switch - Optogenic system

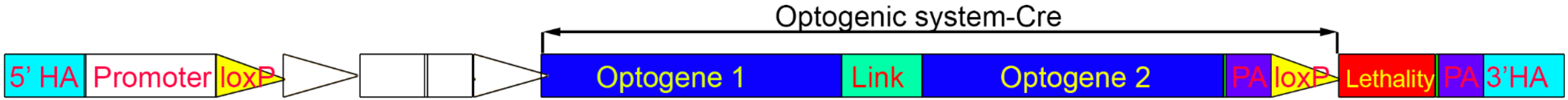


The Cre loxP system

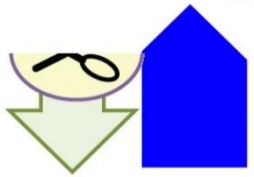


Design of the HolyGrail construct

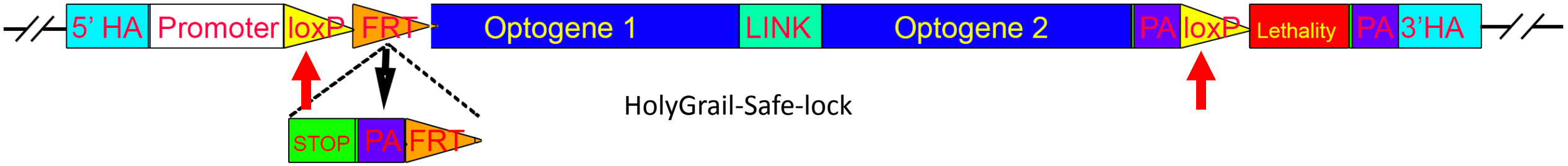
The molecular light-inducible switch - Optogenic system



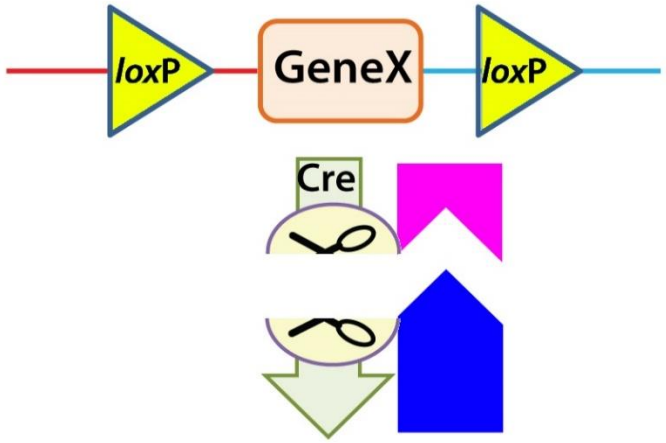
Combining Optogenes with Cre



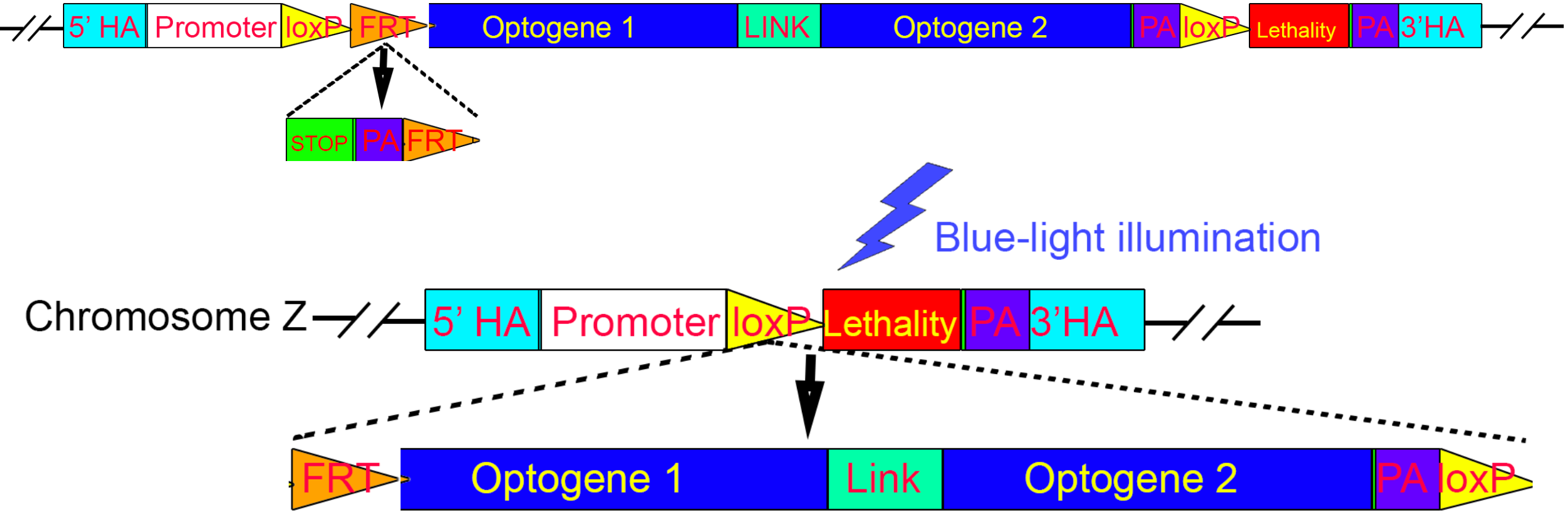
The GOLDA genome – The HolyGrail without the GFP “Safe-Lock” mechanism



HolyGrail-Safe-lock



Light-activation of the lethality inducing gene



BMP-inhibitor, Noggin – the lethality inducing gene

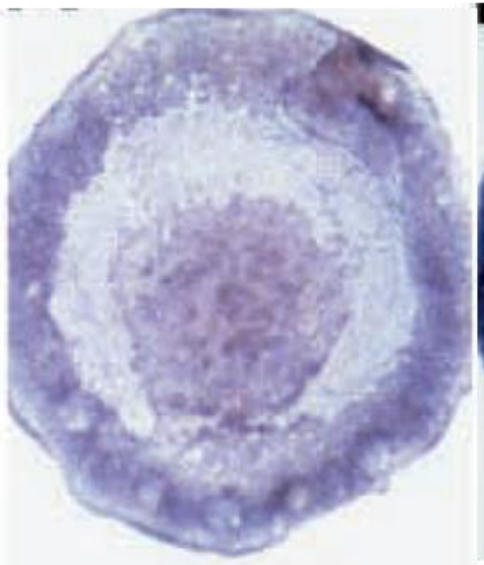
Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse

Glenn Winnier,[1] Manfred Blessing,[1,2] Patricia A. Labosky, and Brigid L.M. Hogan

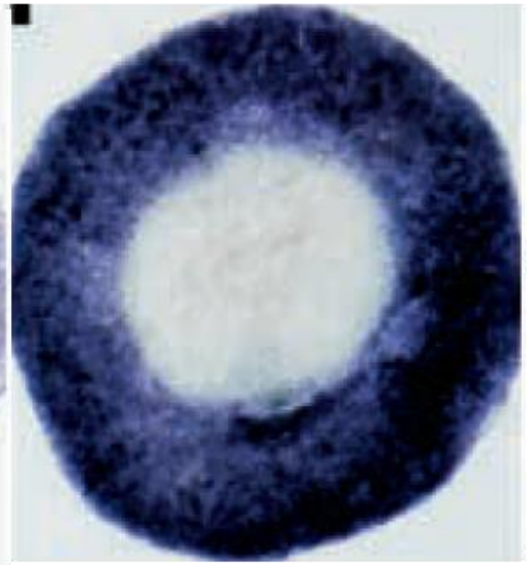
Howard Hughes Medical Institute and Department of Cell Biology, Vanderbilt University Medical School, Nashville, Tennessee 37232-2175 USA

GENES & DEVELOPMENT 9:2105-2116, 1995

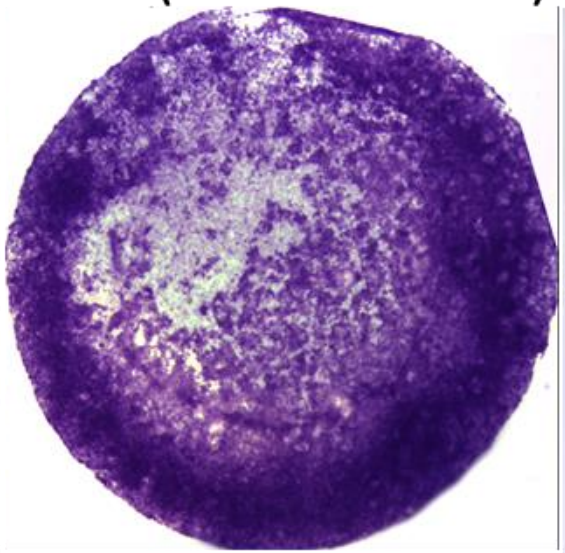
BMP4



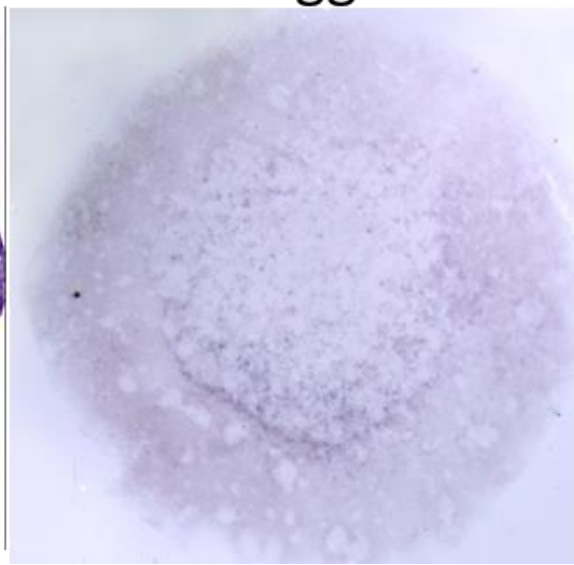
BMP7



ID2 (BMP effector)

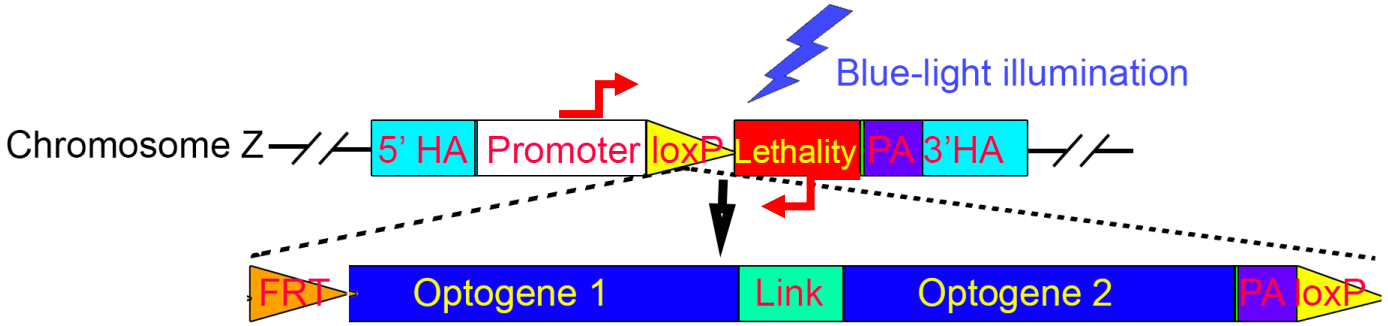


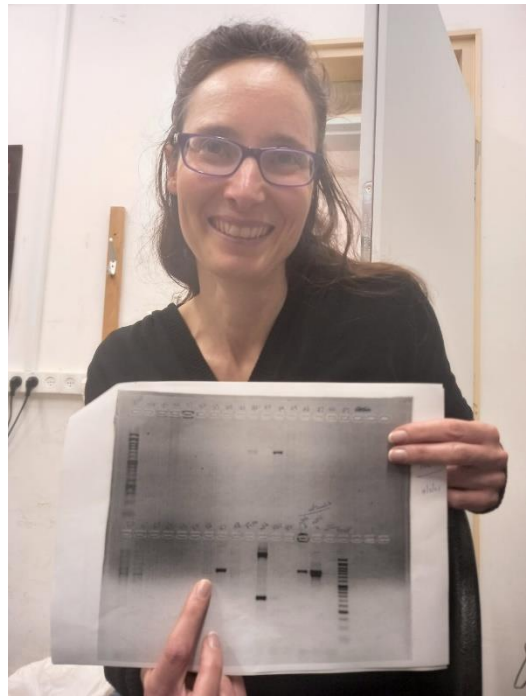
Noggin



Noggin @24h

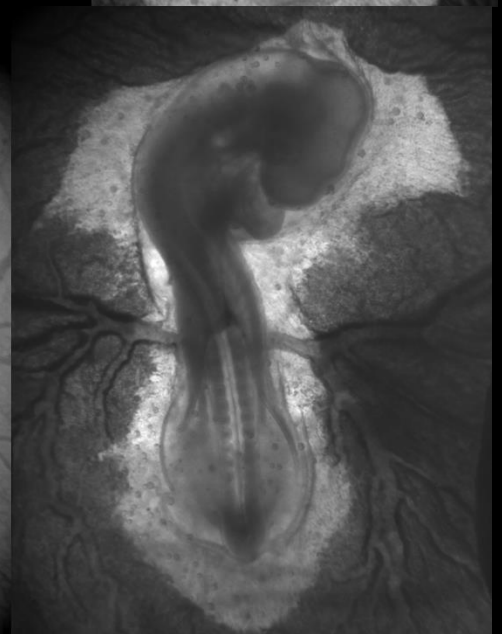
PCR validation to confirm the activation of the Lethality-inducing mechanism



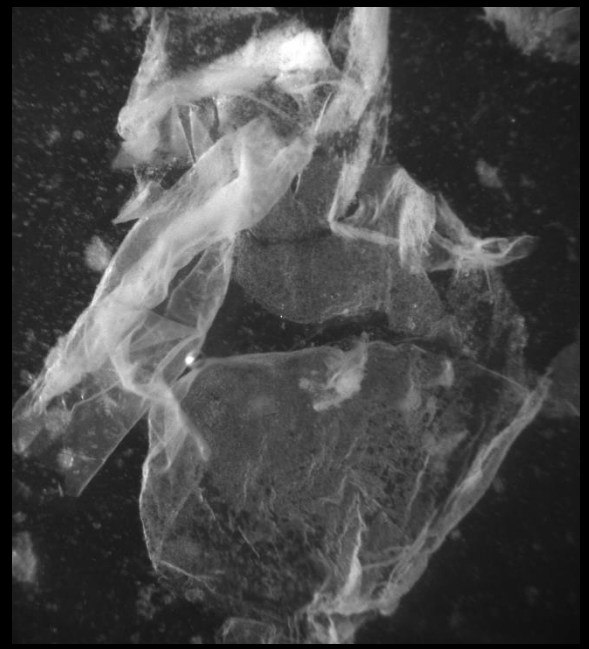


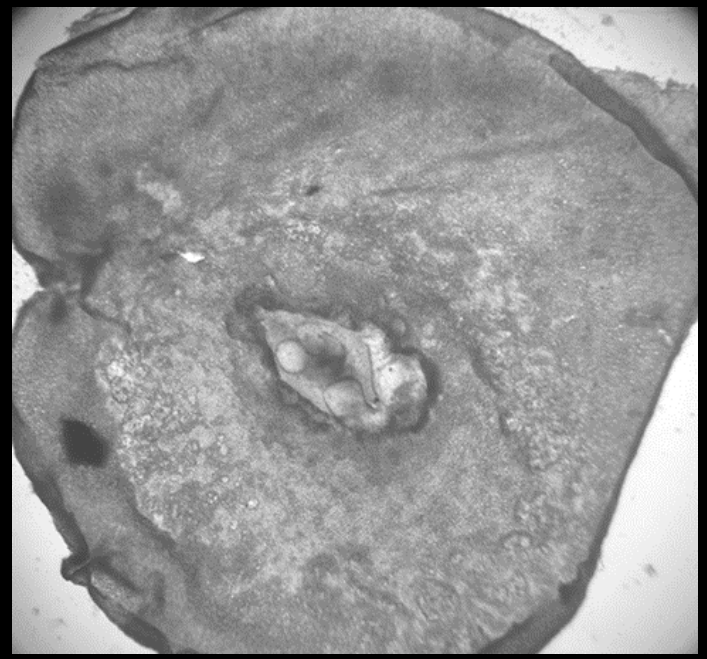
GOLDA's embryos, following illumination and 72h of incubation

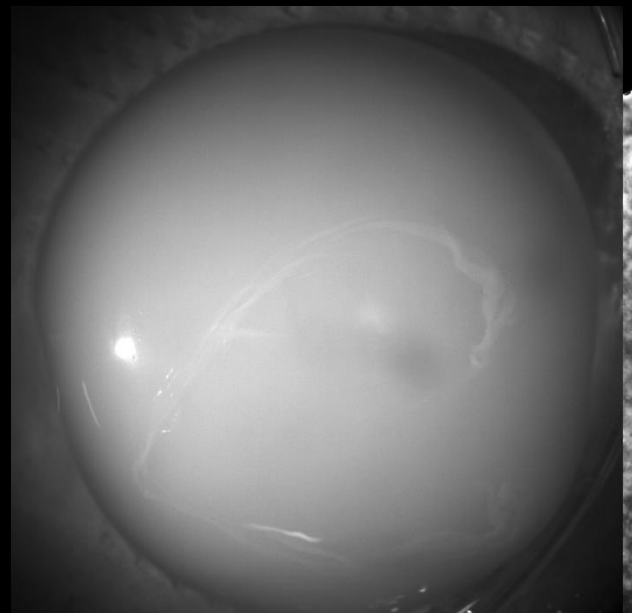
Normal Females

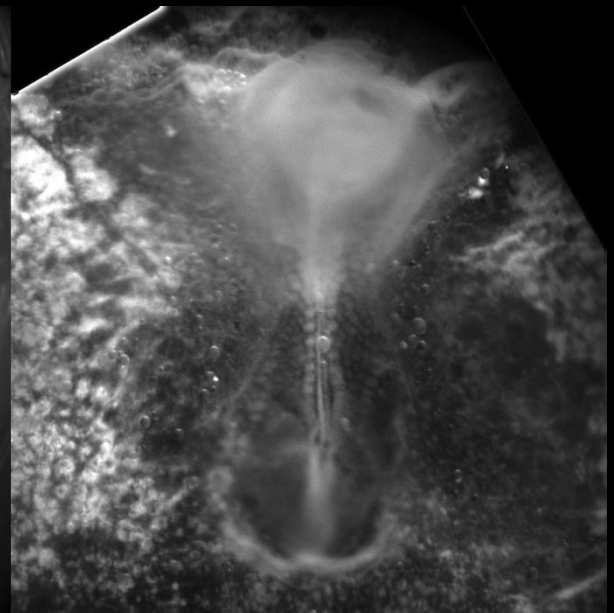


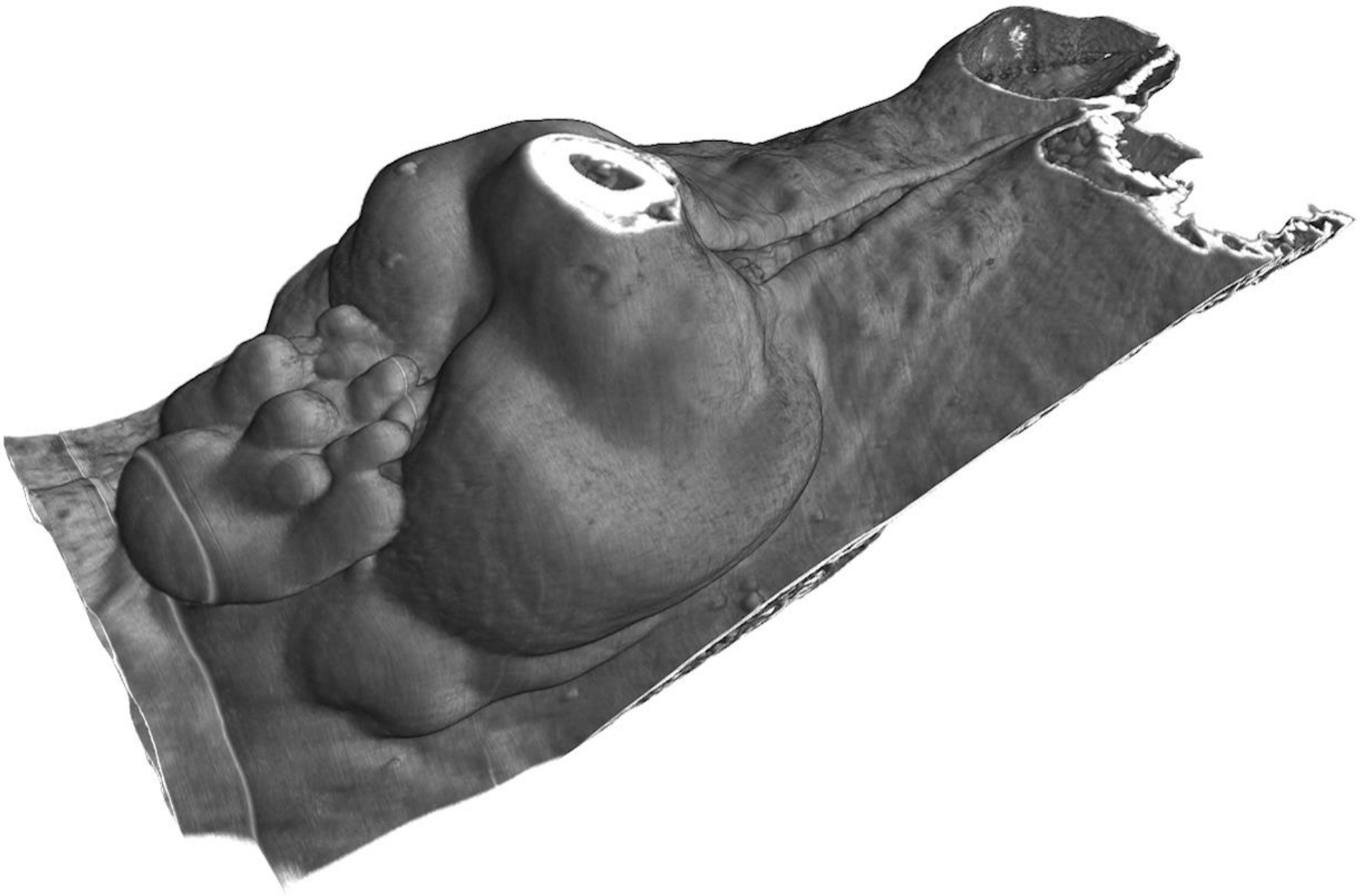
Malformed and dead males







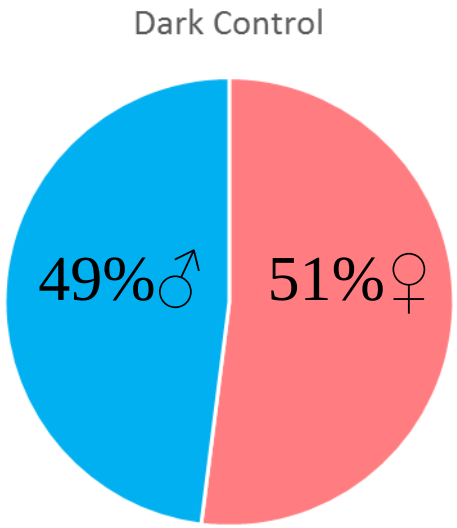


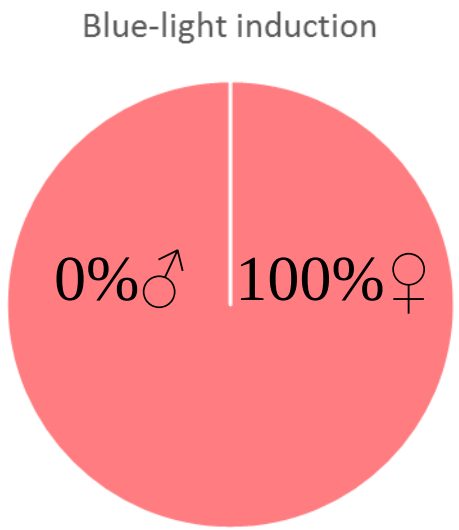


Hatchability Analysis of Blue-Light Illuminated GOLDA eggs

	♀			♂		
	Total	Hatched	Hatched %	Total	Hatched	Hatched %
Dark Control	102	90	88%	82	71	87%
Blue-light	267	173	65%	208	0	0%

Distribution of the hatched chicks





Current status and future prospective of the research

- Fine tuning the illumination process to ensure that no males hatch

Light intensity X Duration = Illumination dosage

- Performances

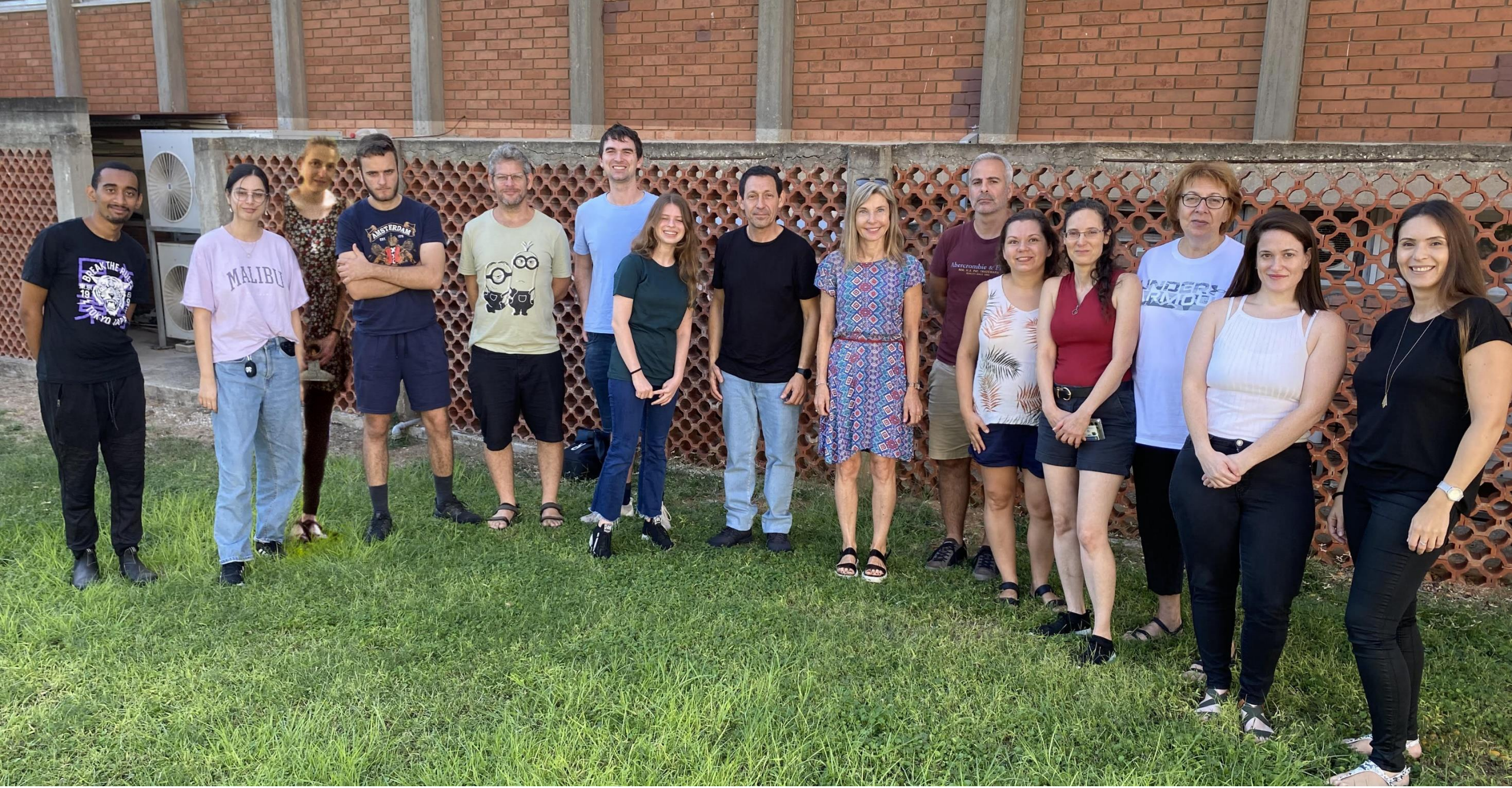
- Excluding male eggs, infertile and early embryonic mortality eggs

Genetic Solution to End the Culling of Day-Old Male Chicks

Advantages:

- Reliability and Accuracy: Genetic Solution with 100% Accuracy
- Compatibility with Current Breeding Program
- Maintenance of Genetic Diversity of the Pure Lines
- Easy Integration with Hatchery Workflow
- The Illumination can take place (maybe) during storage, or pre-incubation.
- Male Embryos Cease Development at an Early Stage
- High Nutritional Value of Male Eggs
- No Need for Egg Sampling
- No Expensive Sorting Technologies Required (Hyperspectral, MRI, Mass Spectrometry, etc.)
- Applicable to a Variety of Chicken Strains and Egg Types/Colors
- Elimination of Timely and Costly Sorting of Male Chicks
- Environmental and Sustainability Benefits
- Saves 50% of Energy and Space Required for Incubation
- No Need to Dispose Dead Males
- The end product – The layers and the table eggs are Non-GMO and identical to the currently used

The Practice of Sorting and Culling Billions of Male Chicks Will Come to An End



Always seeking for highly motivated excellent scientists to join our team: yuvalcinnamon@gmail.com

Collaborations:

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Head of the Poultry and Aquaculture Dept.

The Agricultural Research Organization – The Volcani Institute.

Dr. Dimitri Dima Shinder

Poultry Farm

Mark Ruzal

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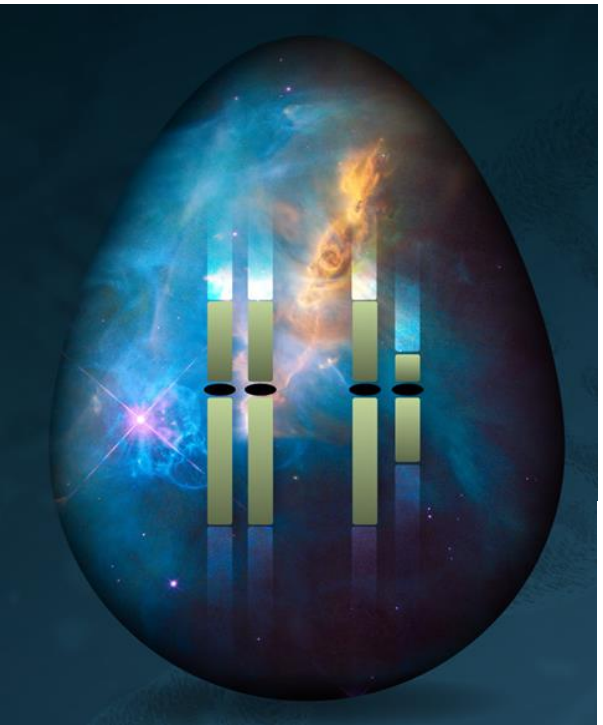
Sergey Oblezin

Thank you!



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