

Reproductive Sterility as a Tool for Prevention and Control of Invasive Aquatics

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**USDA currently allows only *P. brigesii* to be sold
and shipped in the US**



Pomacea brigesii

Will leave aquatic plants intact

Produced in Florida

**Some established populations
recorded in USGS database**



Asolene spixi

Will eat aquatic plants

No longer in trade

**No established populations
recorded in USGS database**

Can reproductively sterile *P. brigesii* and *A. spixi* be produced as new ornamental snail products?



Pomacea brigesii

Sterile *P. brigesii* could be sold without any requirement for USDA approval.

Is there a potential market for sterile *P. brigesii*?

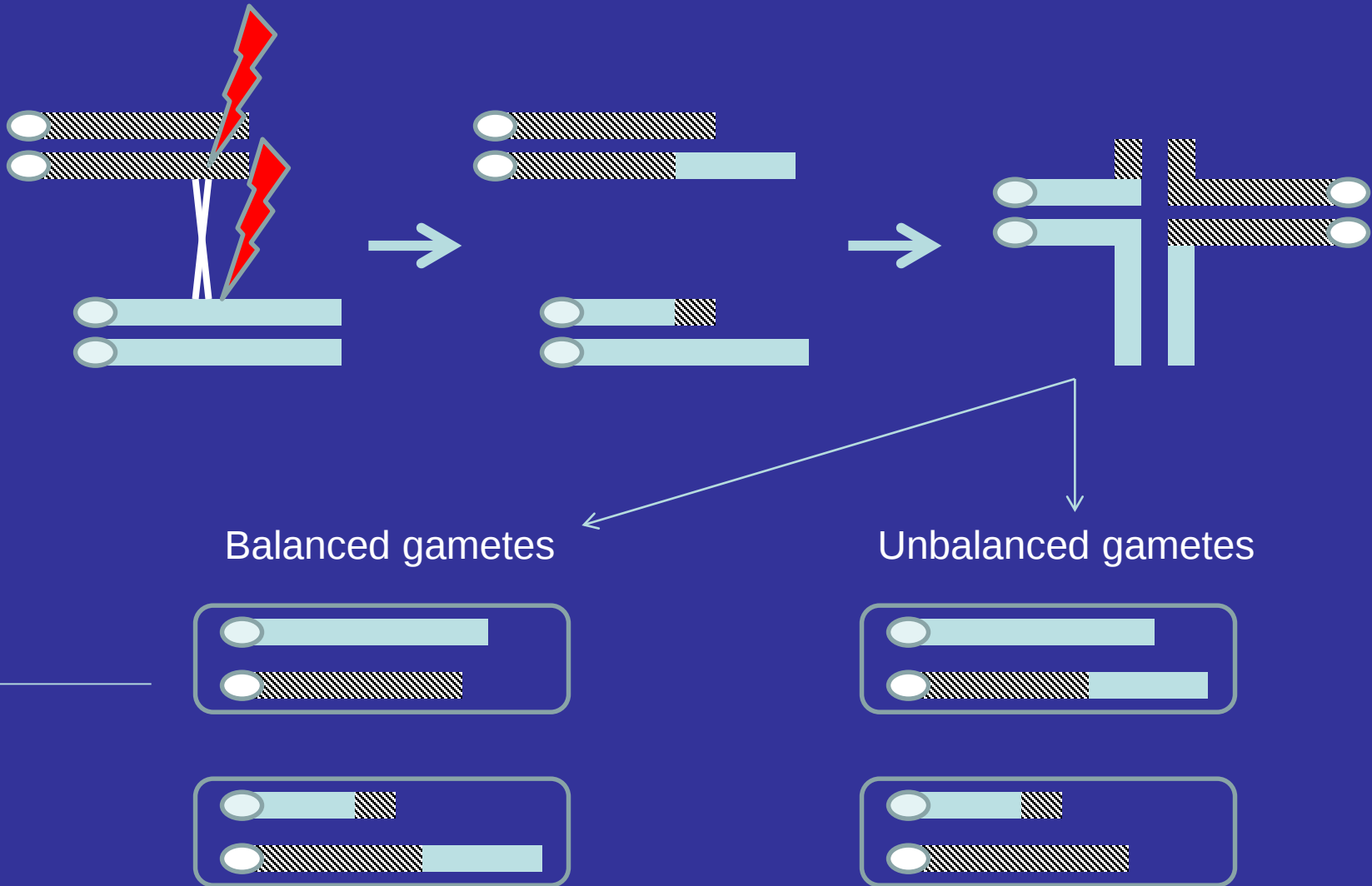


Asolene spixi

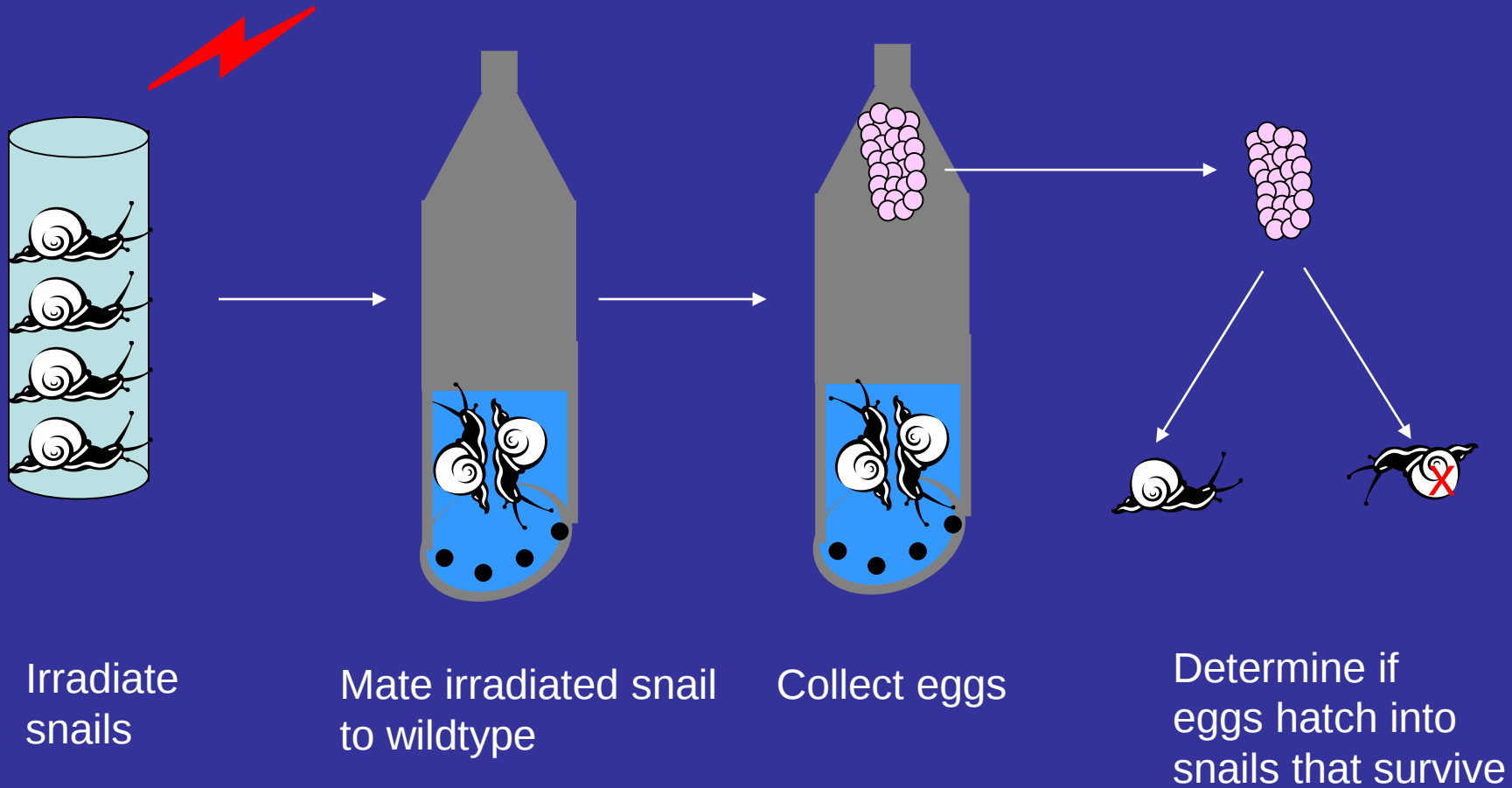
Sterile *A. spixi* cannot be sold without USDA approval.

Is there a potential market for sterile *A. spixi*?

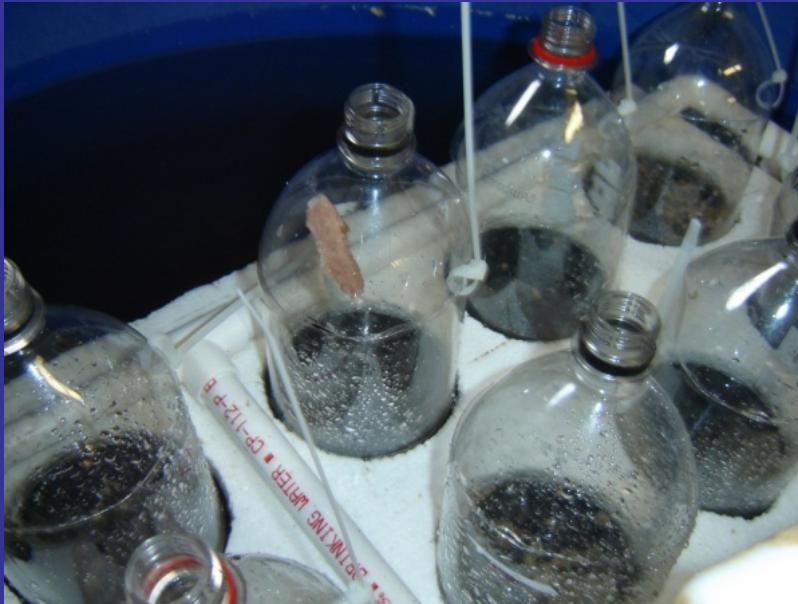
Irradiation of Chromosomes Produces Translocations That Pair Abnormally During Meiosis



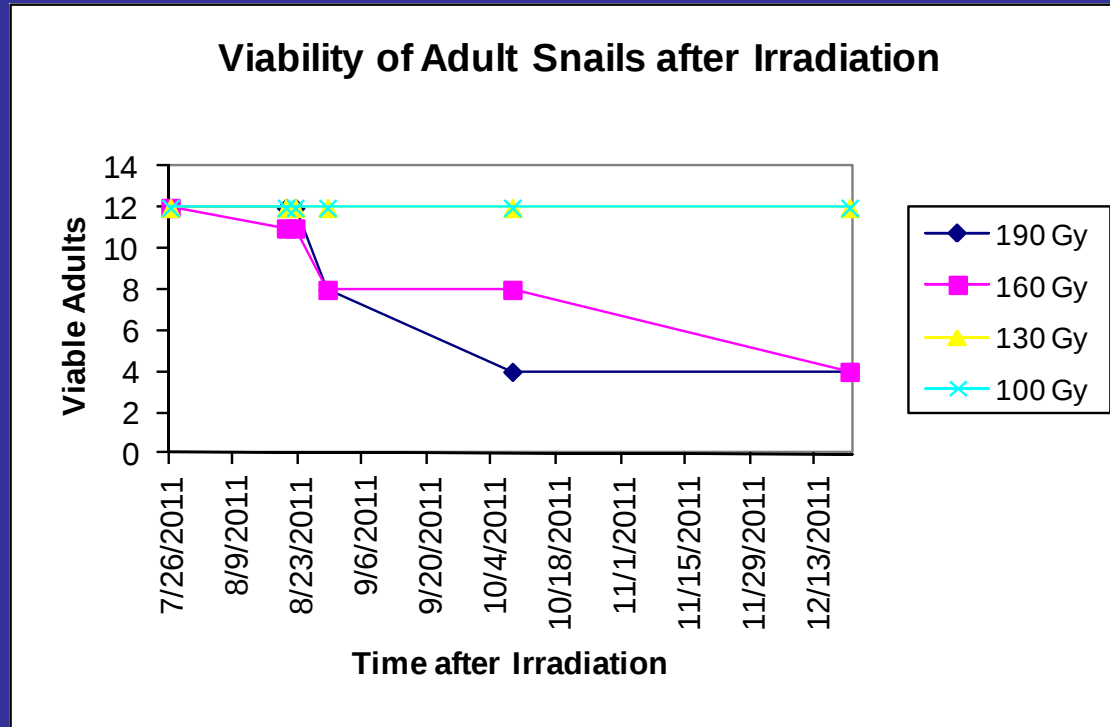
What dose of radiation (x-rays) will render snails reproductively sterile?



Snails Matings Produce Eggs at the Top of Mating Chambers



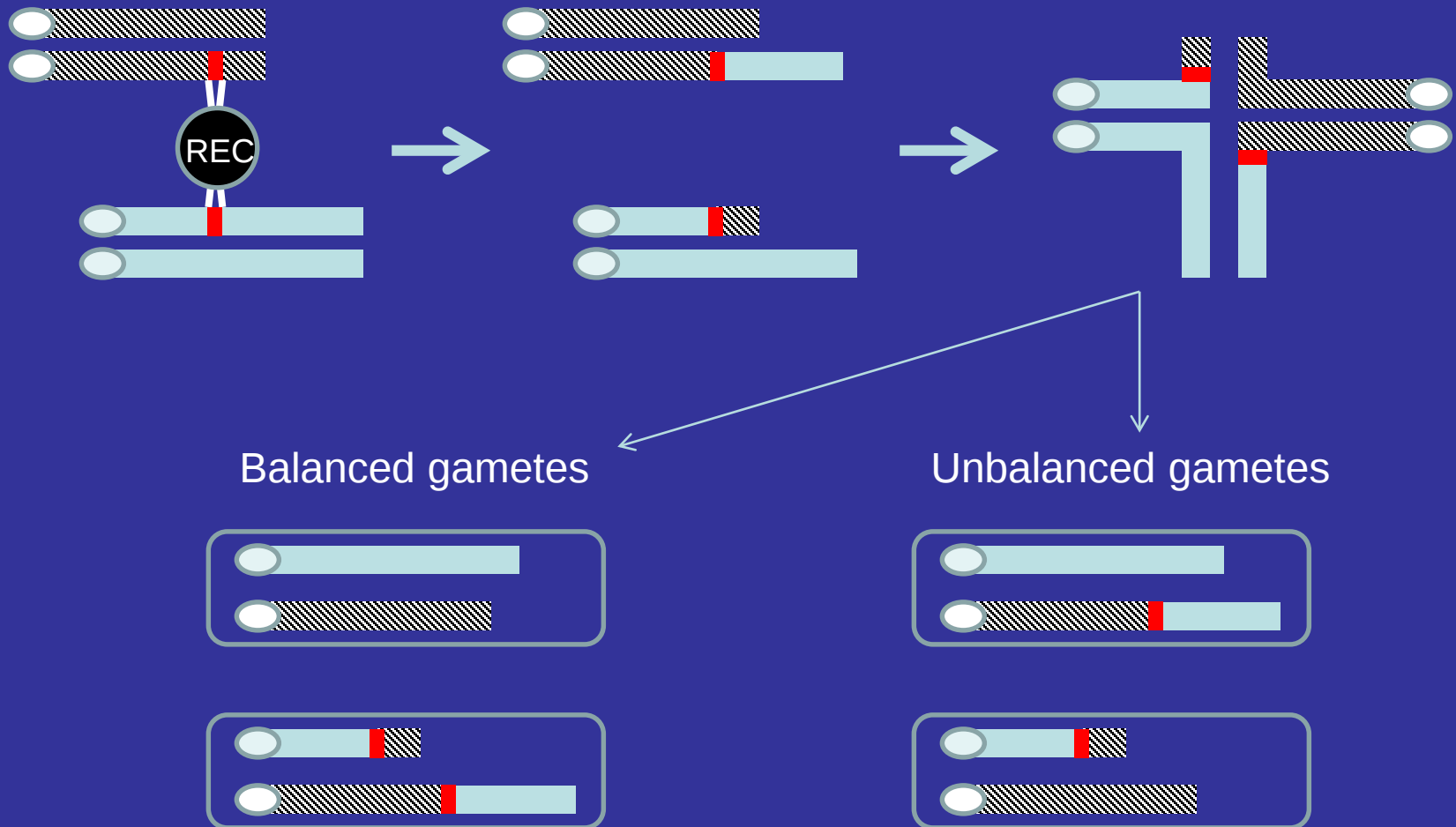
Viability of Irradiated *P. brigesii* Adults Decreases at Doses of Radiation Above 130 Gy.



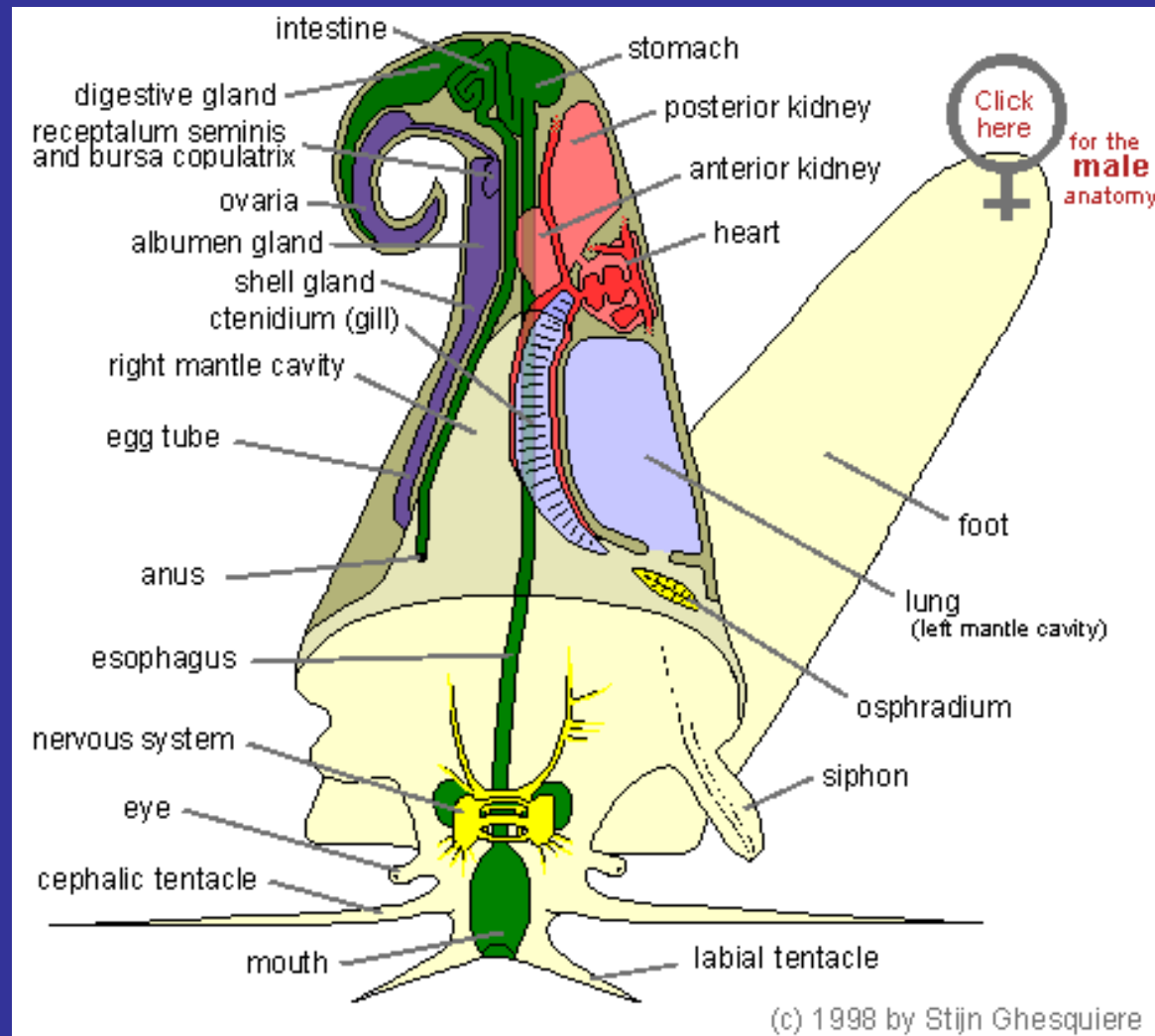
Fertility is reduced in irradiated snails in two ways:

1. Egg production is decreased
2. Fertility of eggs is reduced

Recombination Between Chromosomes can Produce Translocations That Pair Abnormally During Meiosis

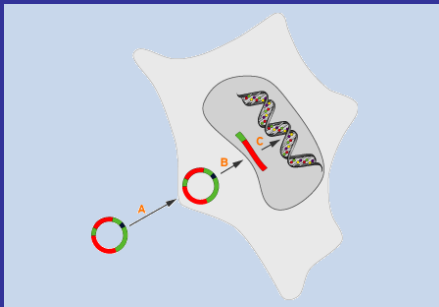


Reproductive Tissue of Snail is Located in the Spiral Shell Tip.



Getting DNA Components Into Snails

Transfect DNA
Into Snail Tissue



Mate Transfected
Snail

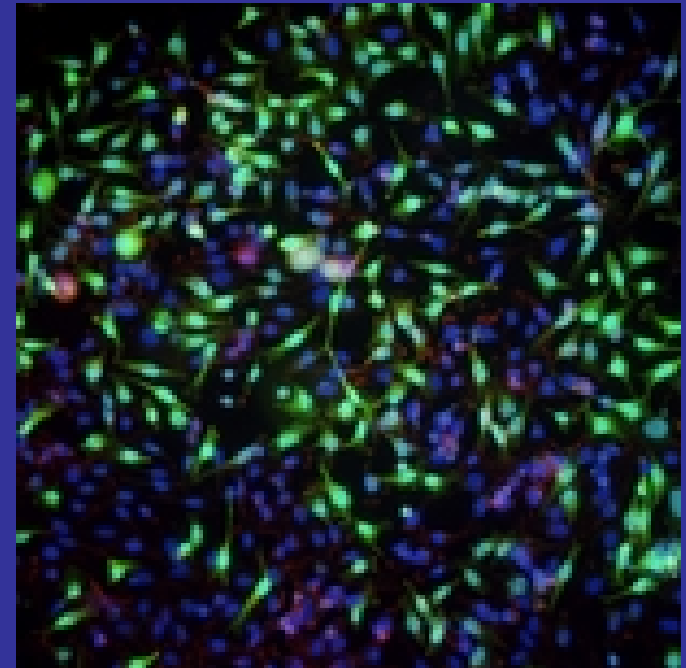
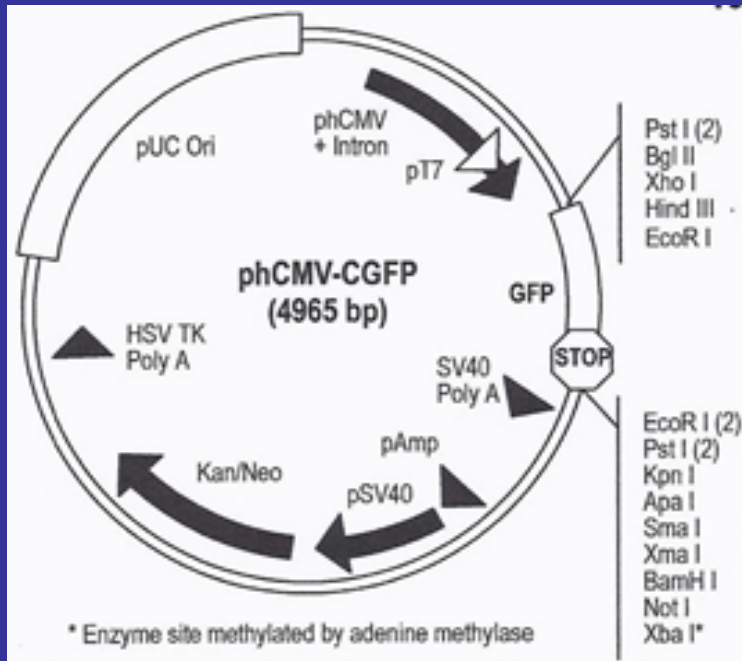


Collect eggs
for DNA



Detect Gene Expression
Detect DNA by PCR

Plasmid DNA with Green Fluorescent Protein (GFP) Gene

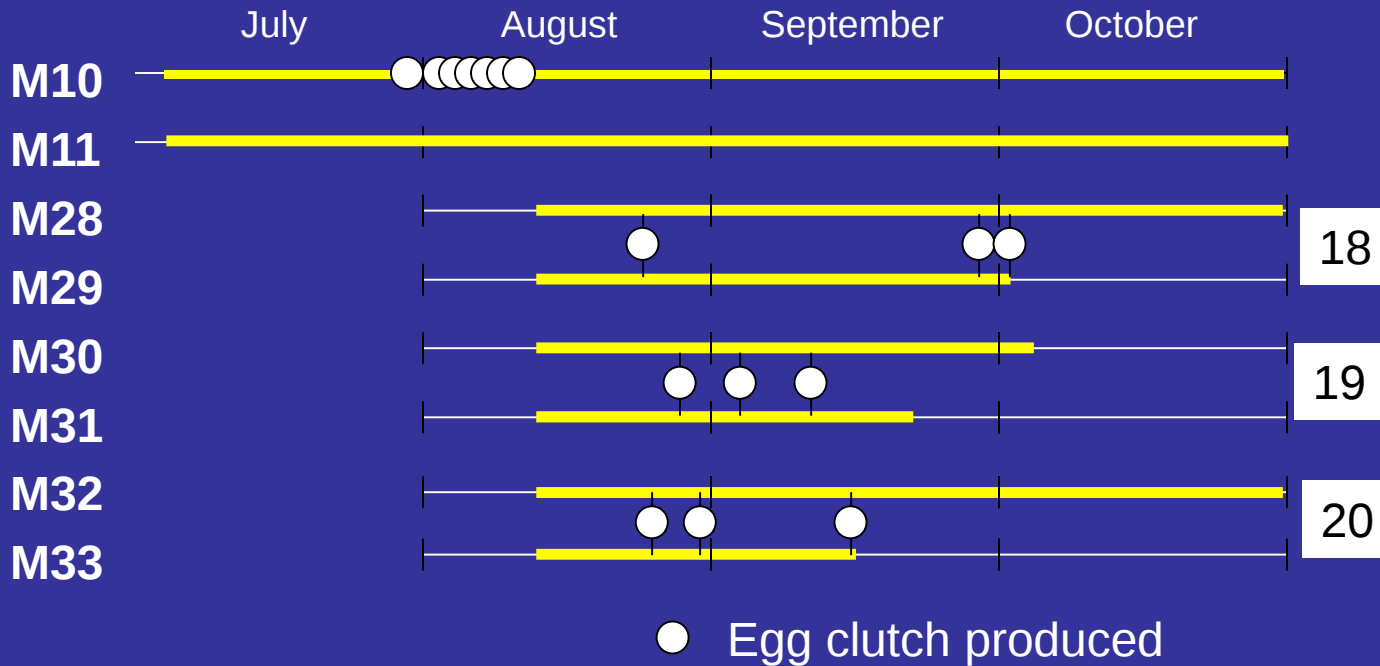


Mammalian cells expressing GFP

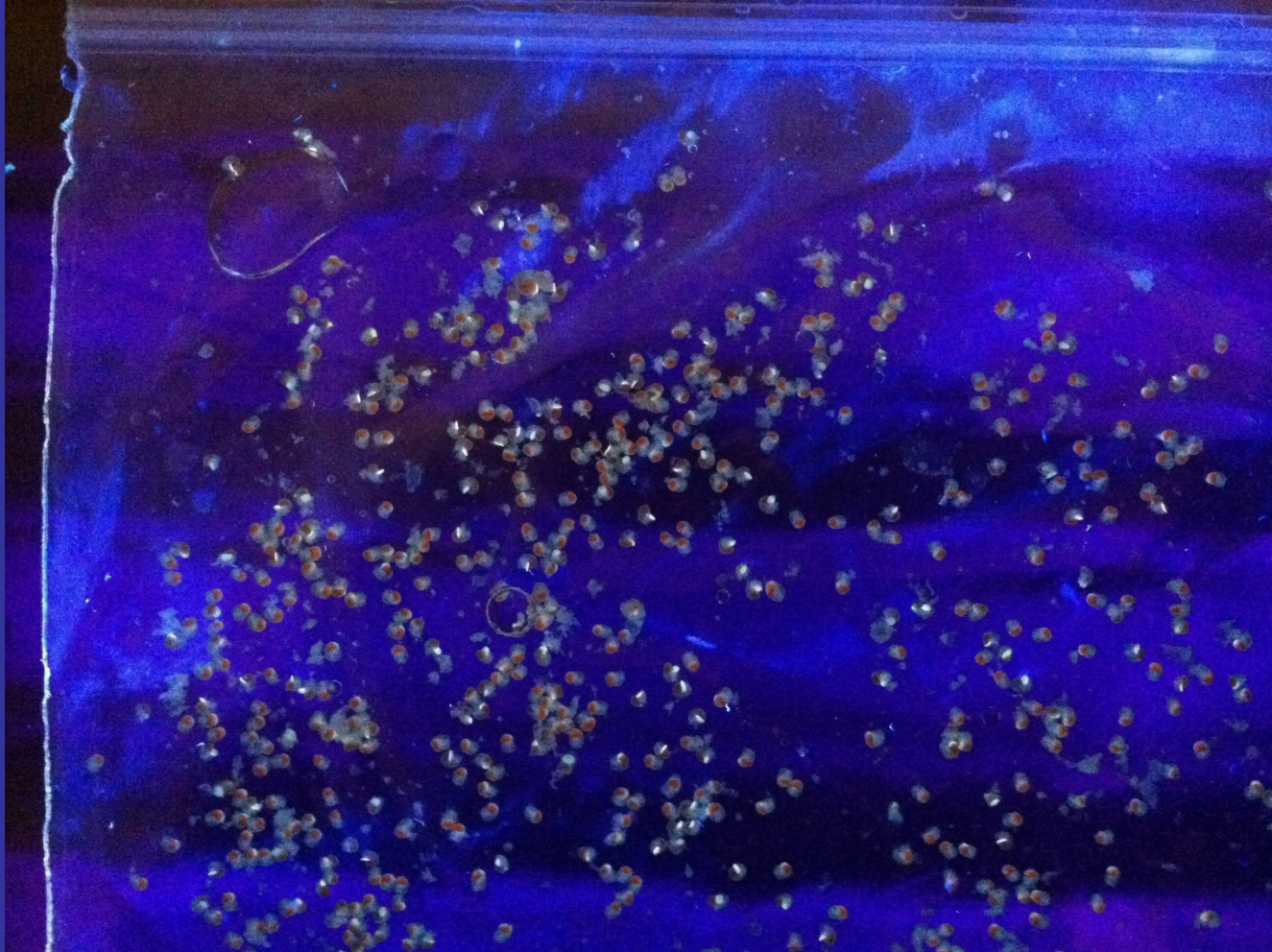
Can GFP plasmid DNA be introduced into snails so that they can be easily identified by green fluorescence?

Males Transfected with DNA are Viable and Fertilize Females To Produce Viable Eggs

Male Snails Transfected with GFP Plasmid DNA



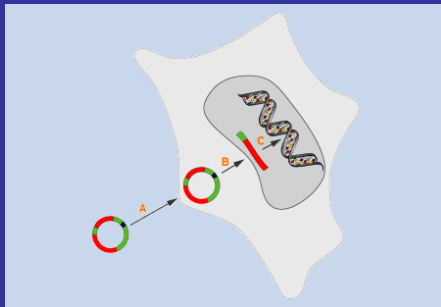
GFP-Transfected Snail Hatchlings Can be Viewed Under UV Light to Look for Green Fluorescence



No green fluorescent hatchlings have been detected as yet .

PCR Analysis of GFP-Transfected Snails

Transfect DNA
Into Snail Tissue



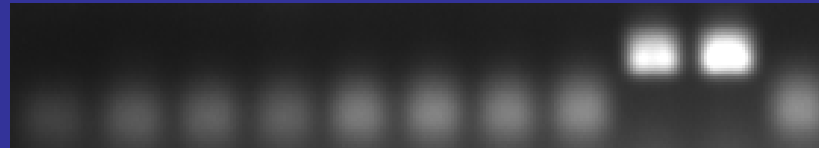
Mate Transfected
Snail



Collect eggs
for DNA



1 2 3 4 6 7 8 9 10 11 12



← GFP

- | | |
|-------|--------------------------------------|
| 1-9 | Hatchlings of GFP-Transfected Snails |
| 10-11 | Positive Control (GFP Plasmid) |
| 12 | Negative Control (No DNA) |

No GFP positive hatchlings at yet

Conclusions

Mortality is high when snails are irradiated to produce translocation chromosomes.

Directed recombination is being investigated as an alternative to irradiation treatment to produce chromosomal translocations.

Techniques for getting DNA integrated into snail genomic DNA and expressed are being investigated.