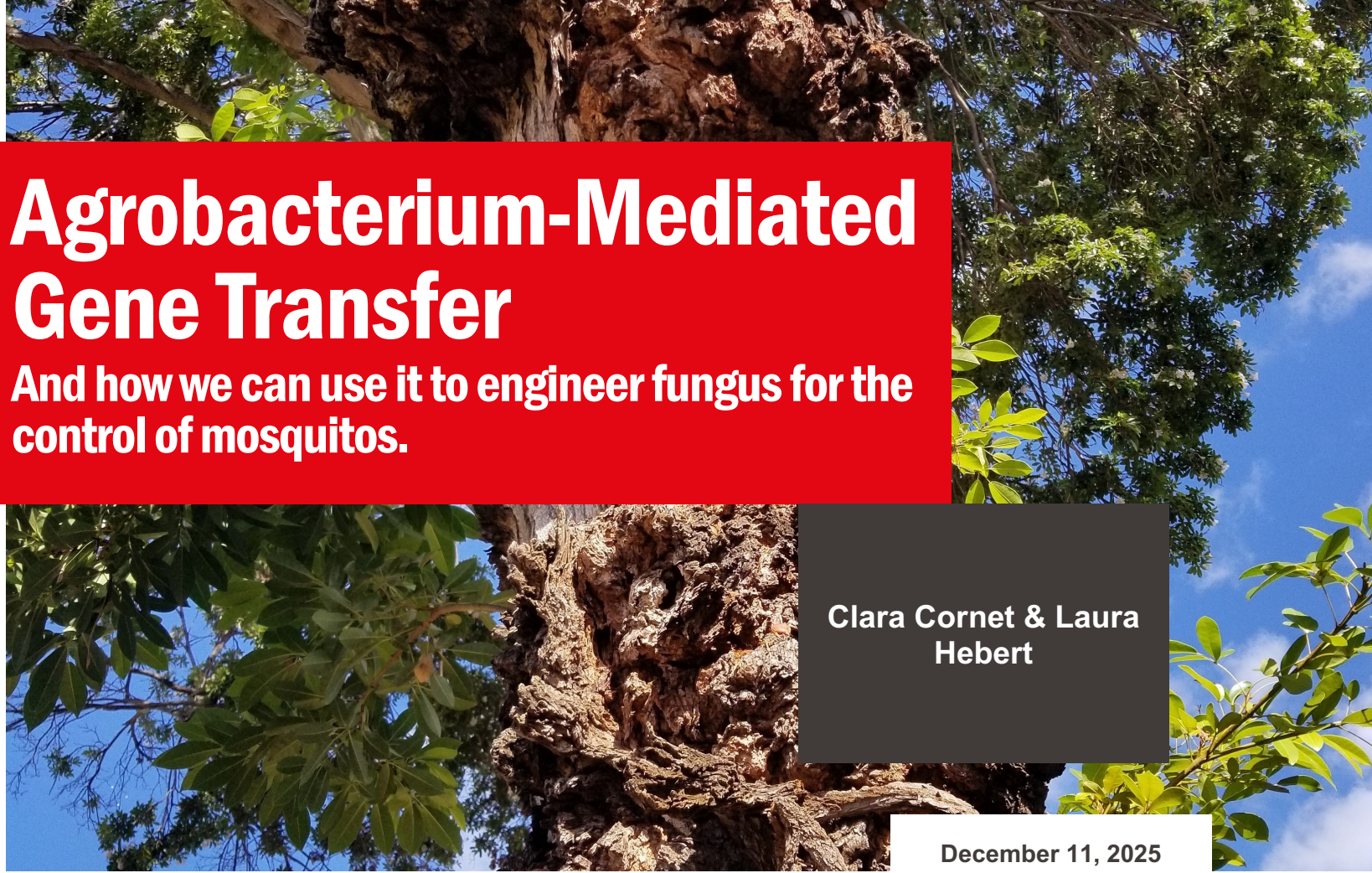
Agrobacterium-Mediated Gene Transfer

And how we can use it to engineer fungus for the control of mosquitos.



Clara Cornet & Laura Hebert

December 11, 2025

École polytechnique fédérale de Lausanne

École
polytechnique
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de Lausanne

OUTLINE

What is it?

How does it work?

How do we use it?

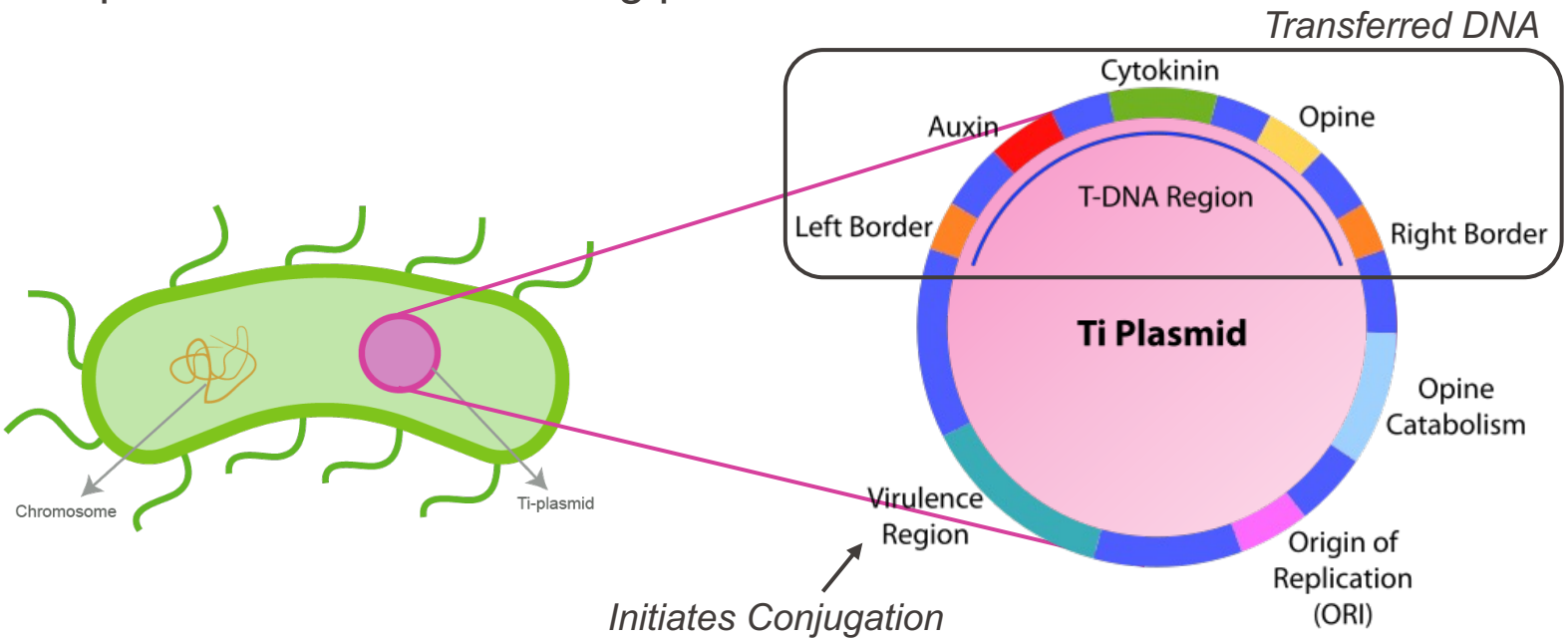
Conclusion

What are agrobacterium?

- **Soil pathogenic bacterium**
- **Rod shaped**, **gram negative**
- Known for naturally **inserting** their **DNA into plants**
- "Natural genetic engineer" = vector in the production of transgenic plants
- Properties started being used for **cloning** in 1981

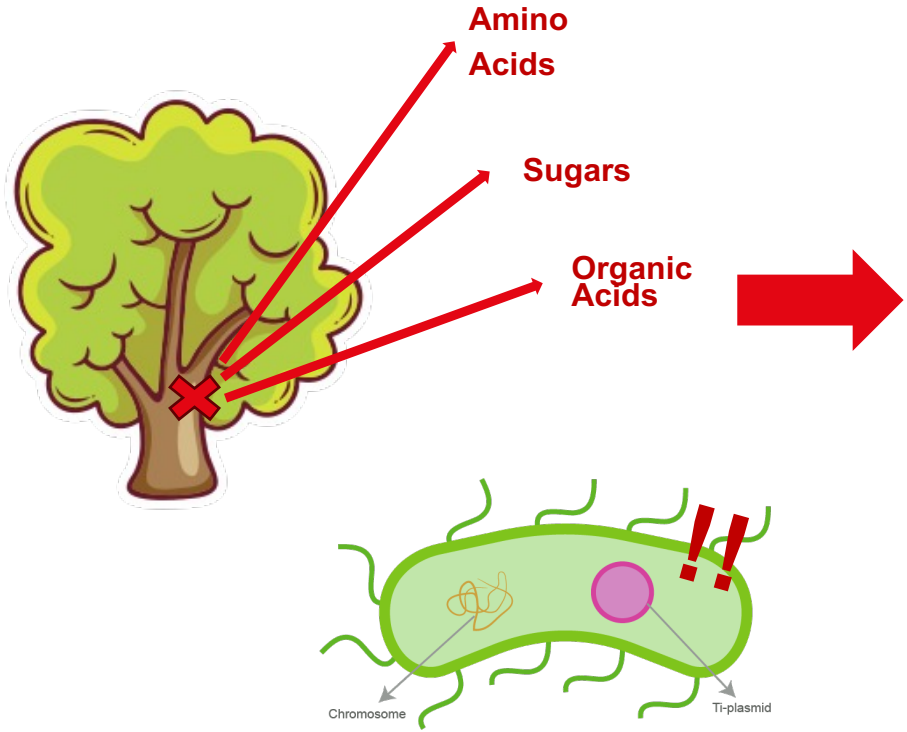
What are agrobacterium?

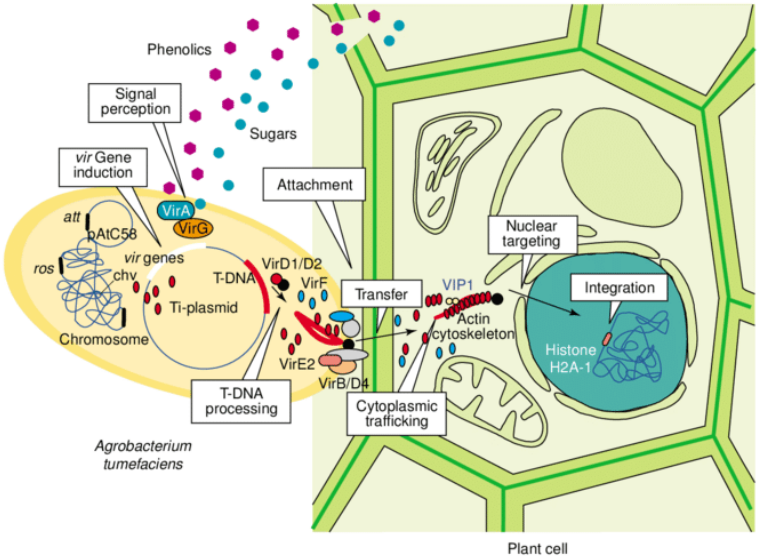
- Ti plasmid = Tumor inducing plasmid



(Kroemer, 2021)(Wikipedia contributors, 2025)

What is agrobacterium-mediated gene transfer?





(Kroemer, 2021)(Gelvin, S. B., 2003)

What is agrobacterium-mediated gene transfer?

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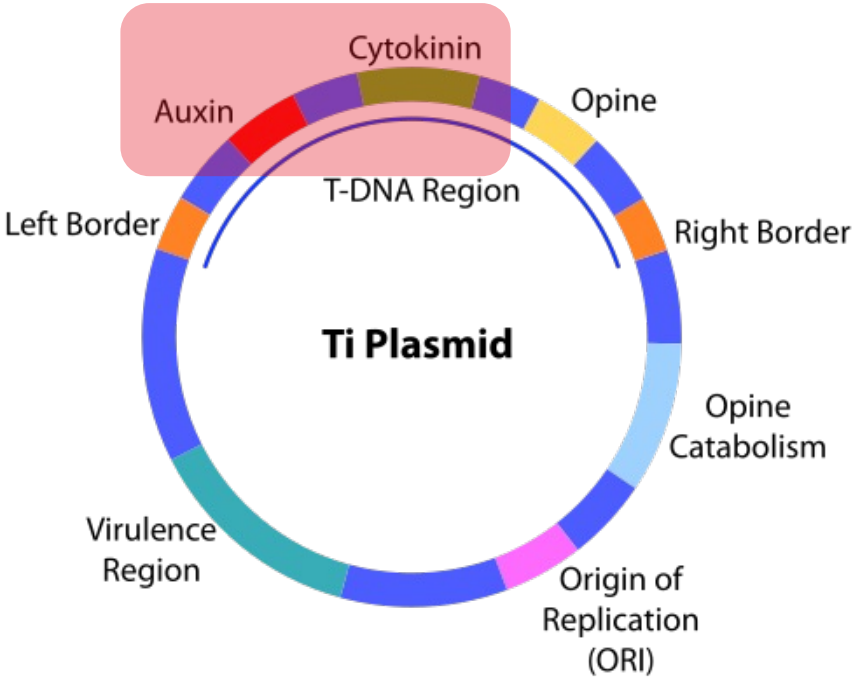
Normal Ti Vector

(theCrux, 2023)(Jeschke & Starikov, 2022)

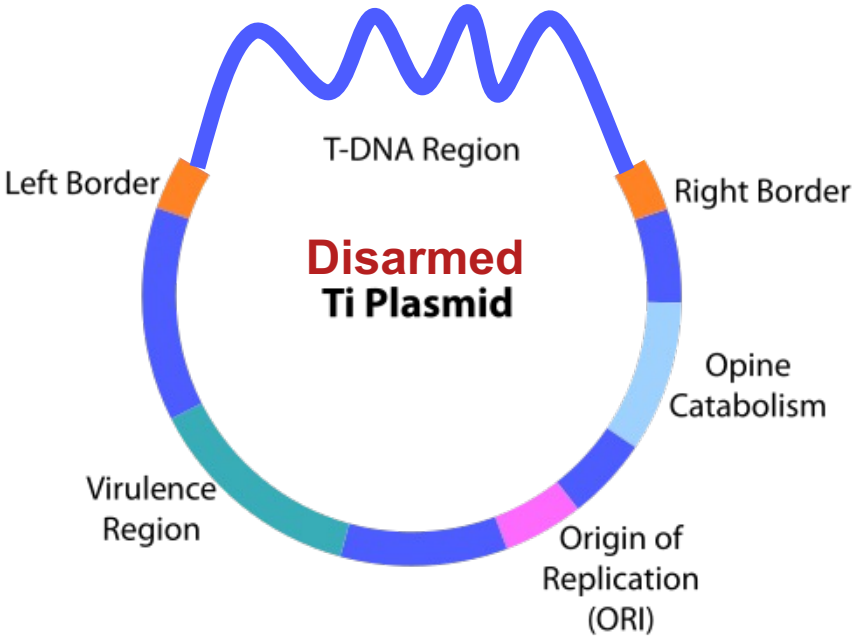
- Oncogenes = armed



(Wikipedia contributors, 2025)

Disarmed Ti Vector

- Oncogenes = armed
- If oncogenes are removed = disarmed → Tumors can not form
- **T-DNA depends on the recognition of the border repeats**
  - Removing Auxin and Cytokinin still keeps the T-DNA functional
- Can add any DNA in their place!



(theCrux, 2023)(Jeschke & Starikov, 2022)

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Plant Cloning via Binary Vectors

(theCrux, 2023)(Fang et al., 2006)

- Virulence genes can be on a different plasmid than t-DNA → vir genes can act in trans
- Only the LB and RB are required to define the T-DNA segment that gets exported

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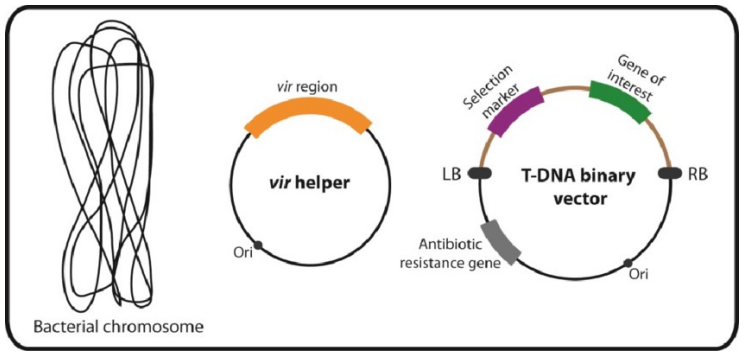
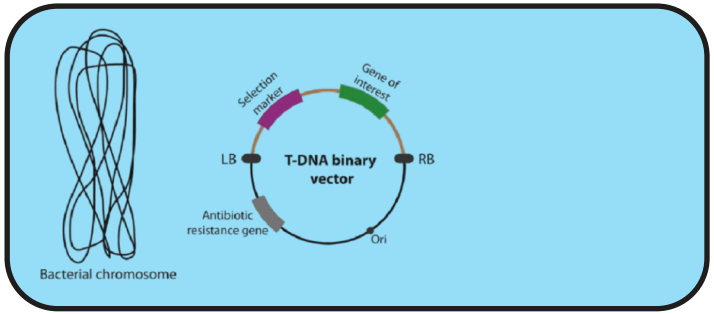


Modified agrobacterium cell

Plant Cloning via Binary Vectors

(qASh education, 2020)

- *E.coli* is an ideal cloning host
- ➢ Grow a shuttle vector in *E.coli*
  - Can replicate in E.coli and Agrobacterium as it contains 2 replication origins



E.coli cell

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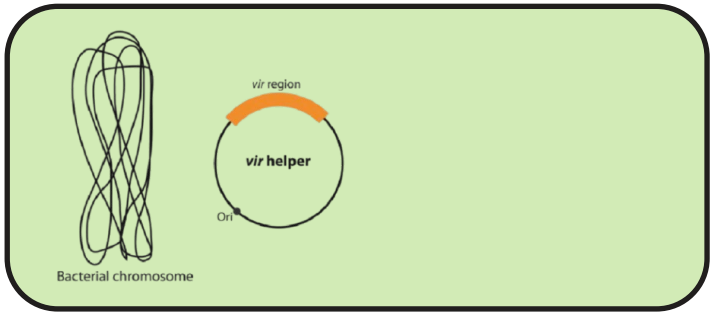
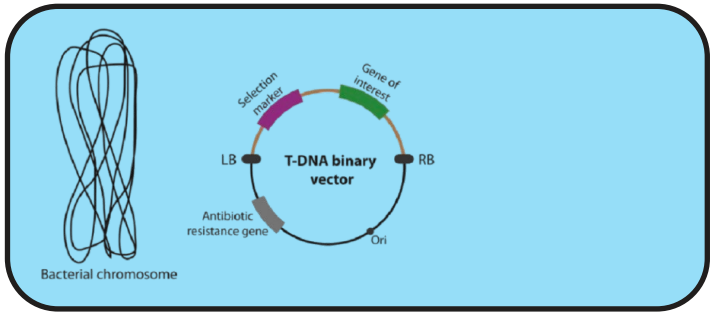


Agrobacterium cell



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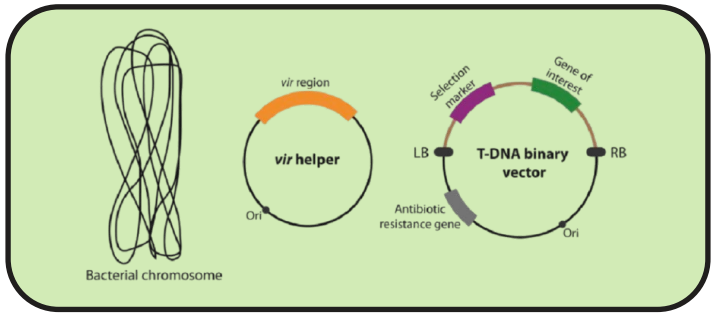

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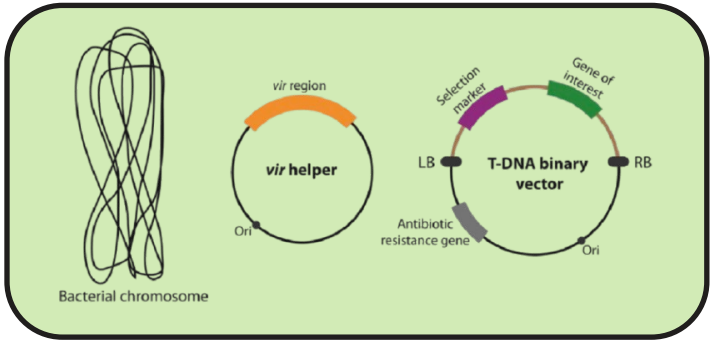

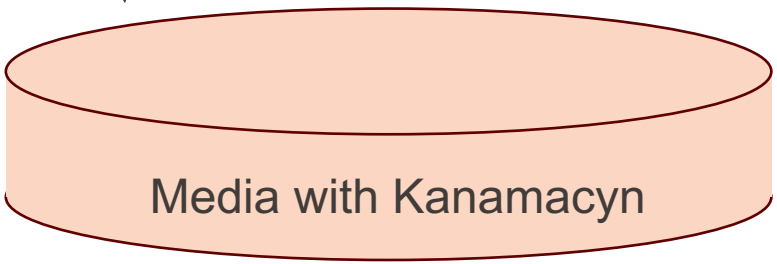

*taking the example where the T-DNA binary vector is pBIN19 (selection marker = neo R and Antibiotic resistance gene = kan R)

Plant Cloning via Binary Vectors

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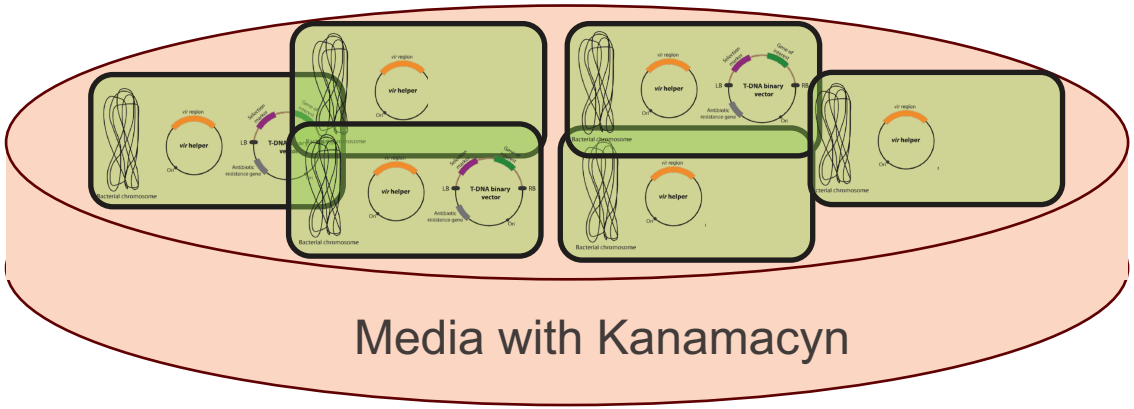

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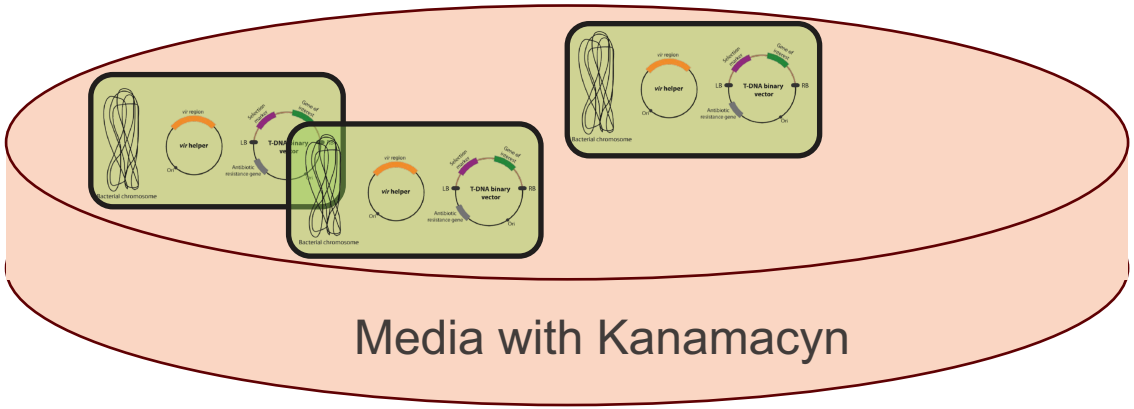
Plant Cloning via Binary Vectors

- Only cells with both the plasmid, *especially the T-DNA plasmid that contains the Kanamacyn resistance gene*, will be able to grow



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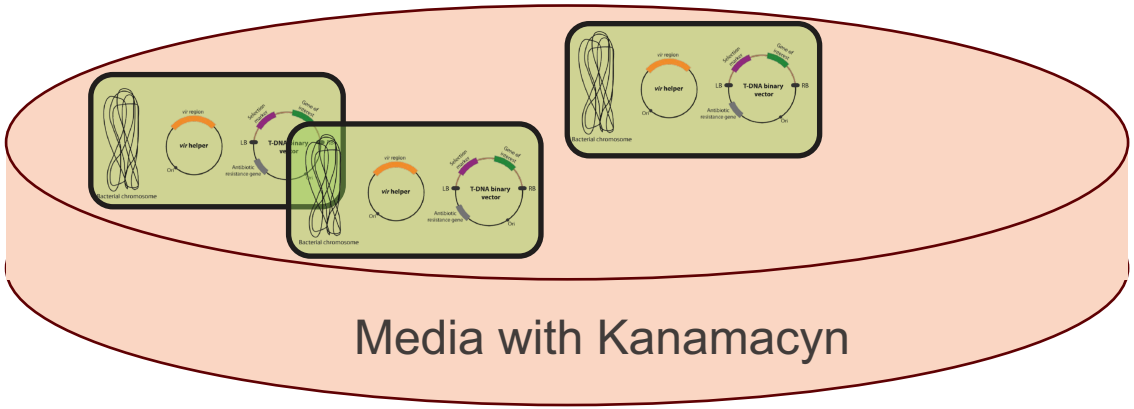

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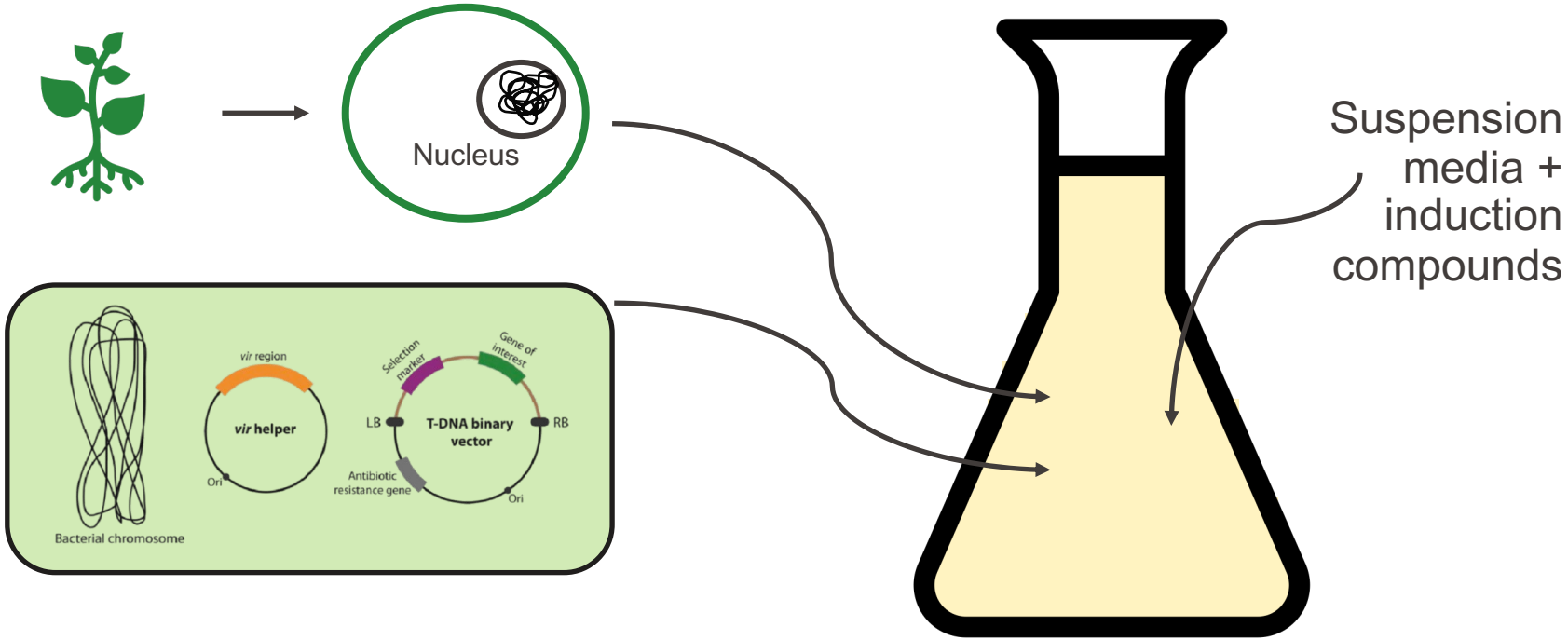

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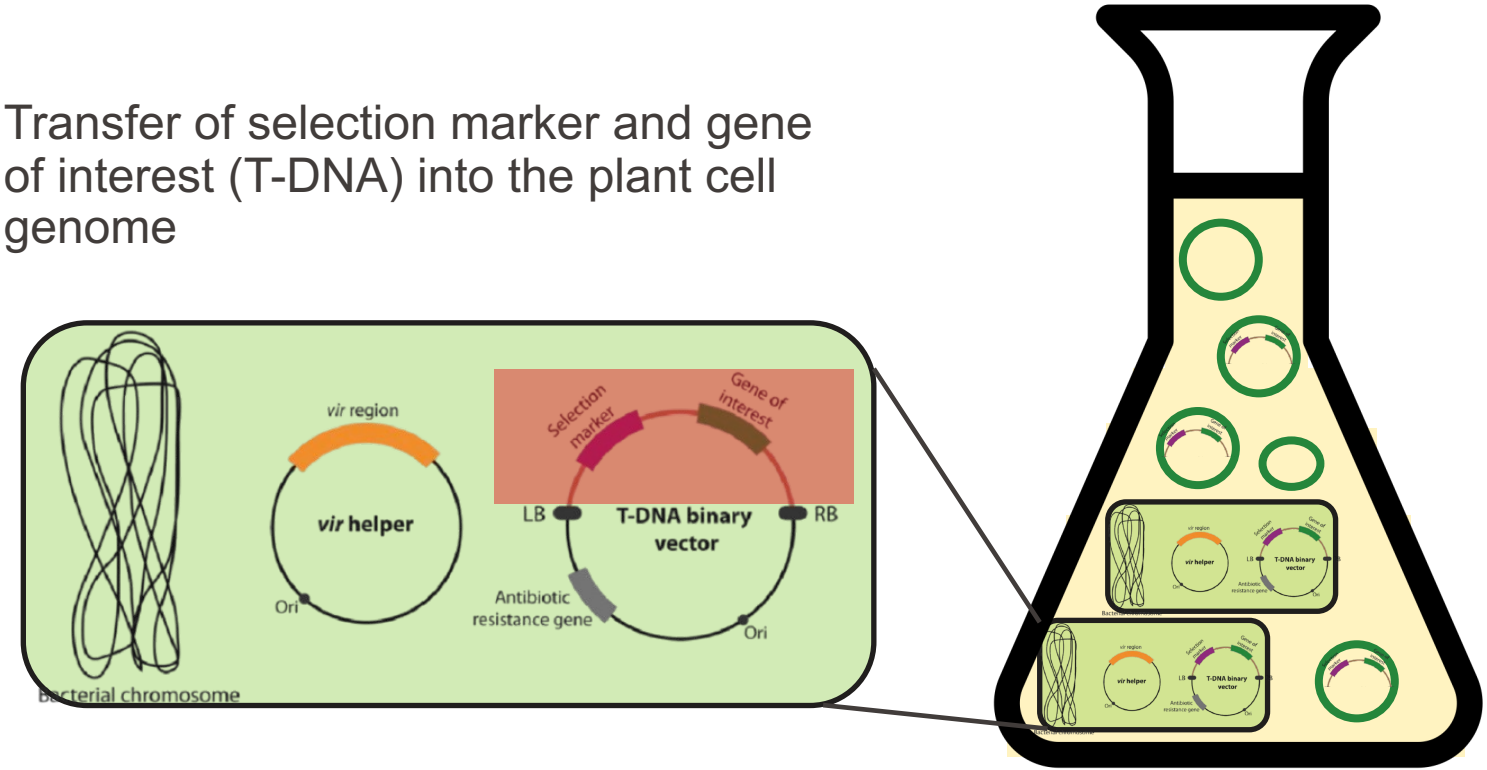
Plant Cloning via Binary Vectors

- Transfer of selection marker and gene of interest (T-DNA) into the plant cell genome



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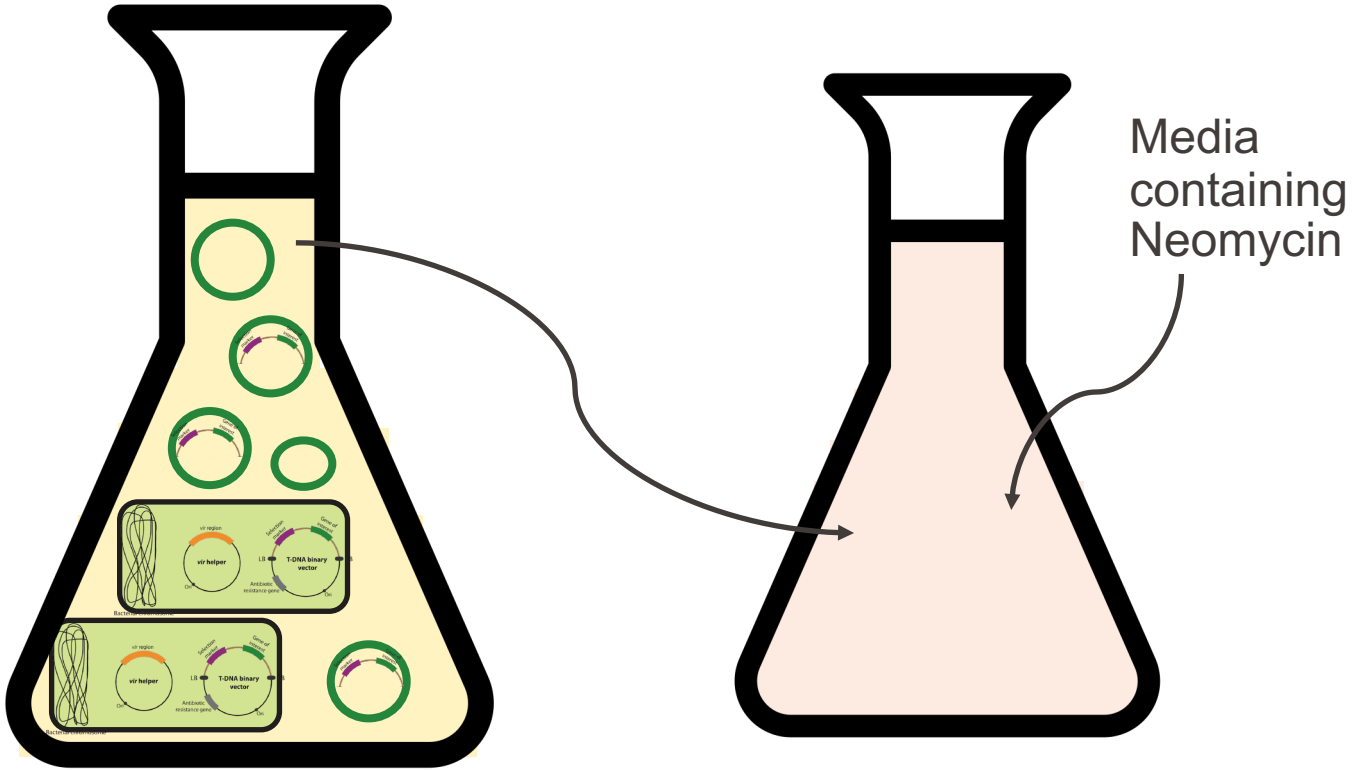

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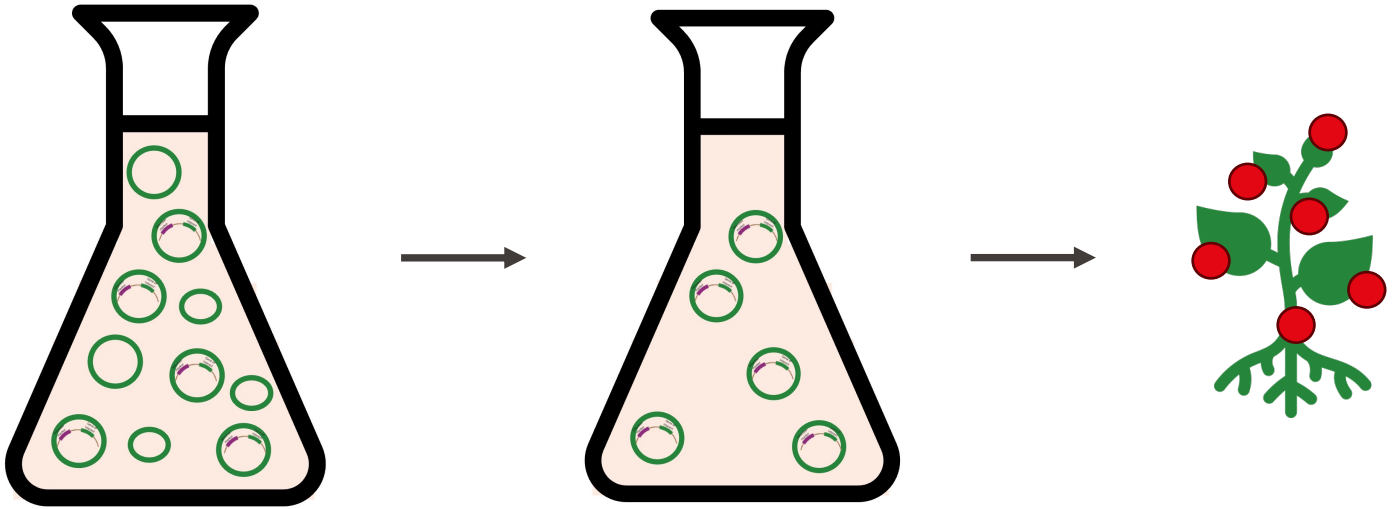
Plant Cloning via Binary Vectors

- Upon incubation, transformed plant cells with DNA insert and selectable marker will be selected.



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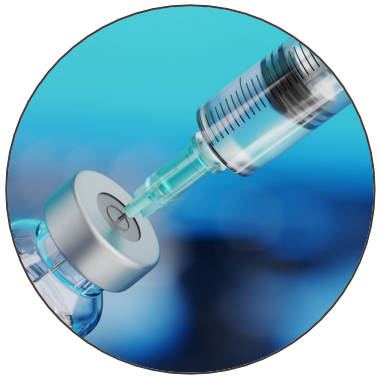
Where can we apply it?

(Sapkota, 2022)



Pharmaceutical Products

Biomonitoring

Agriculture

OUTLINE

What is it?

How does it work?

How do we use it?

Conclusion

How can we use agrobacterium-mediated gene transfer?

Article | Published: 24 October 2025

Engineered *Metarhizium* fungi produce longifolene to attract and kill mosquitoes

Dan Tang, Jiani Chen, Yubo Zhang, Xingyuan Tang, Xinmiao Wang, Chaonan Yu, Xianxian Cheng, Junwei Zhang, Wenqi Shi, Qing Zhen, Shuxing Liu, Yizhou Huang, Jiali Ning, Guoding Zhu, Meichun Zhang, Juping Hu, Etienne Bilgo, Abdoulaye Diabate, Sheng-Hua Ying, Jun Cao ✉, Raymond J. St. Leger ✉, Jianhua Huang ✉ & Weiguo Fang ✉

Nature Microbiology (2025) | Cite this article

Mosquitos are the world's deadliest animal.

Mosquitos are becoming resistant to chemical insecticides.

Let's set the scene...

(Tang et al., 2025)

Article | Published: 24 October 2025

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What type of fungus could be used?

(Tang et al., 2025)

- Some fungal species can infect and kill insects.
  - Spores can attach, penetrate and multiply inside the insect, ultimately killing the host.

- *Metarhizium robertsii* – “***robert***”
  - particularly good at attracting insects.
  - not very good at killing mosquitos.

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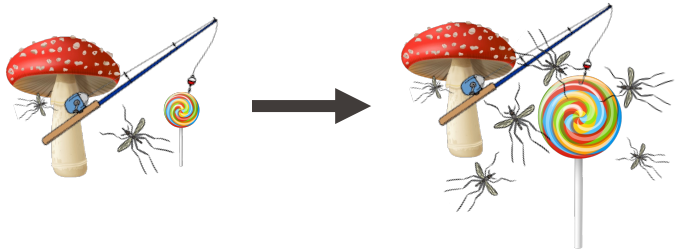
How can we make "*ping*" More attractive?



Let's zoom in on the experimental procedure

(Tang et al., 2025)

- Finding what allows "*robert*" to be so attractive.
  - Insects use olfaction to find food, lay eggs and find nectar.

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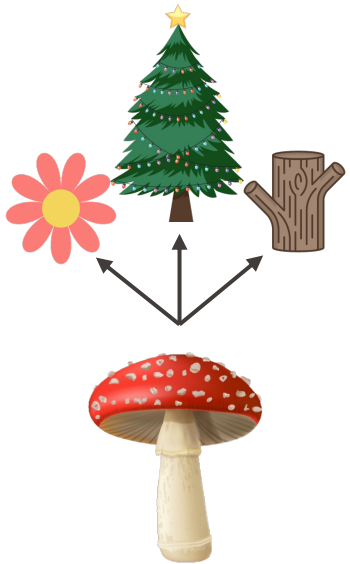

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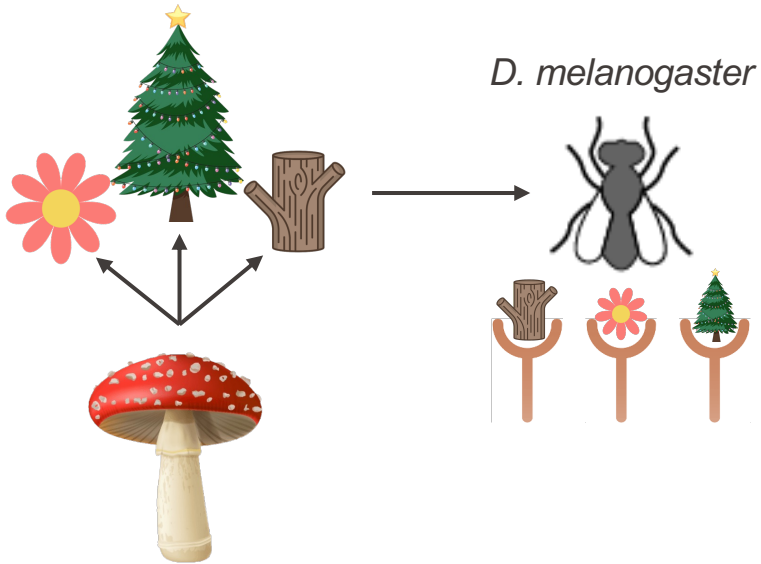

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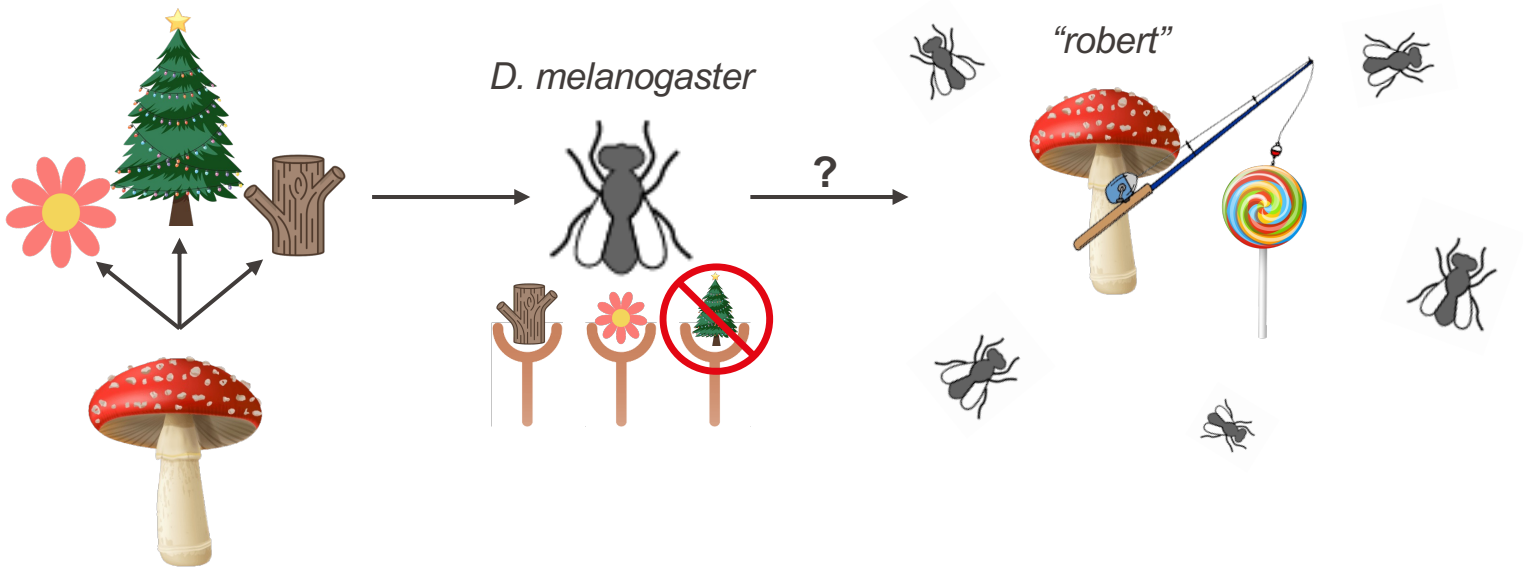

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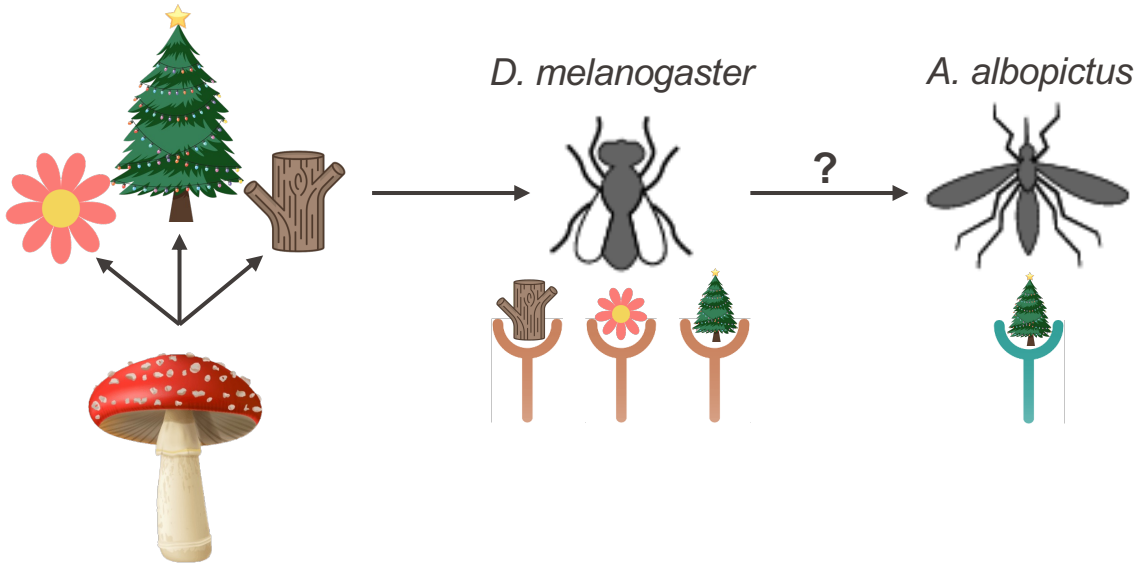

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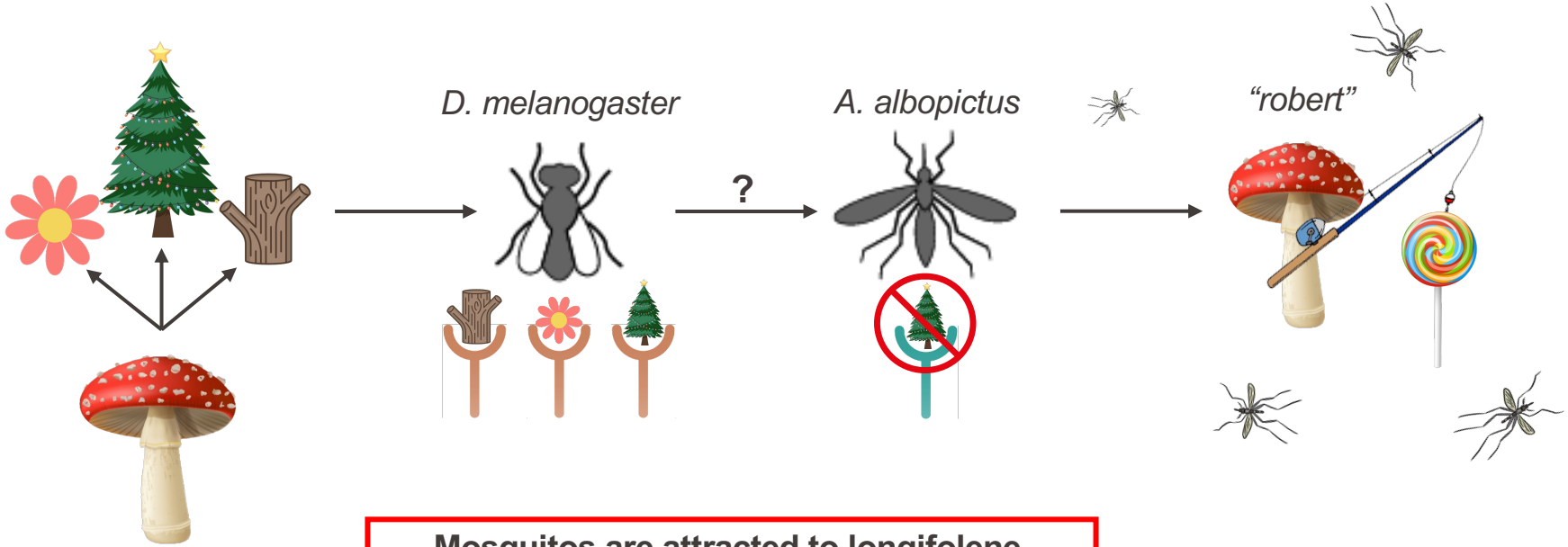
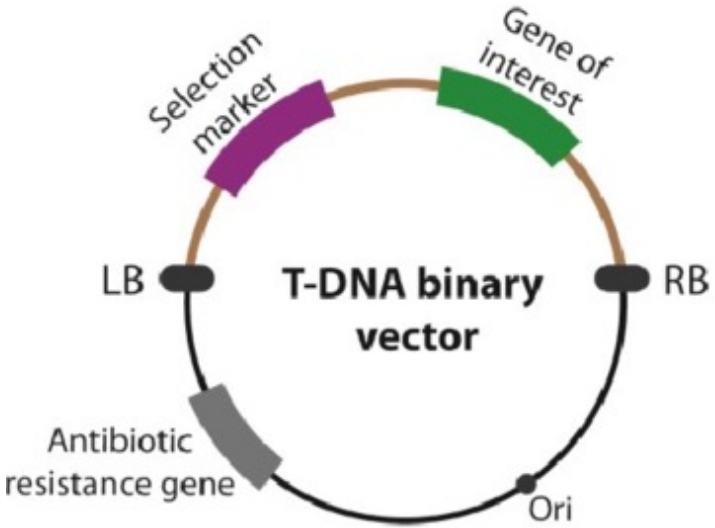


Mosquitos are attracted to longifolene.

Let's zoom in on the experimental procedure

(Tang et al., 2025)

- Cloning longifolene synthase into "*ping*".
  - Upregulation of longifolene production.



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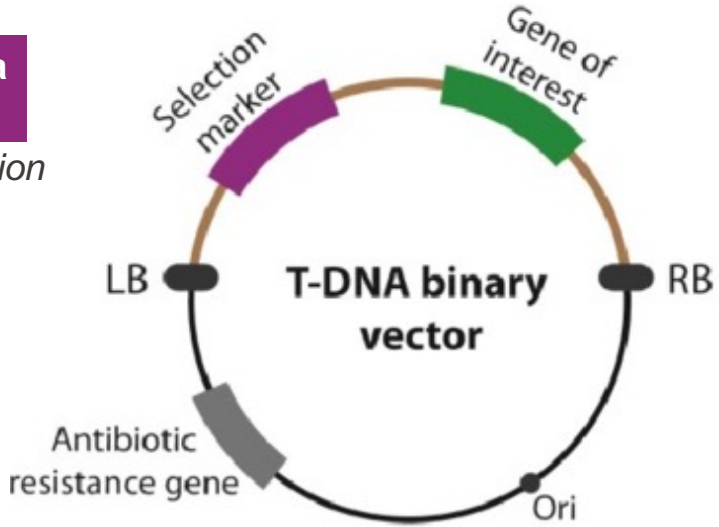
Sulfonylurea Resistance

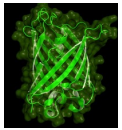
For fungi selection



Constitutive Promoter — ON

Longifolene Synthase

Green Fluorescent Protein



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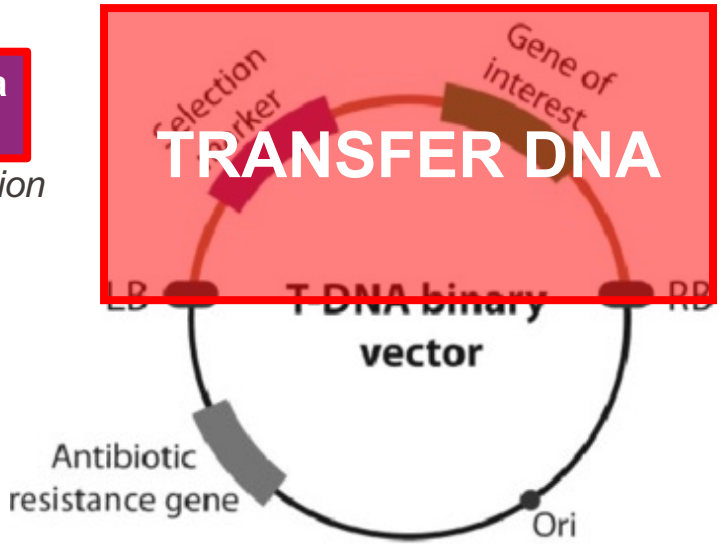

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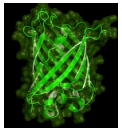

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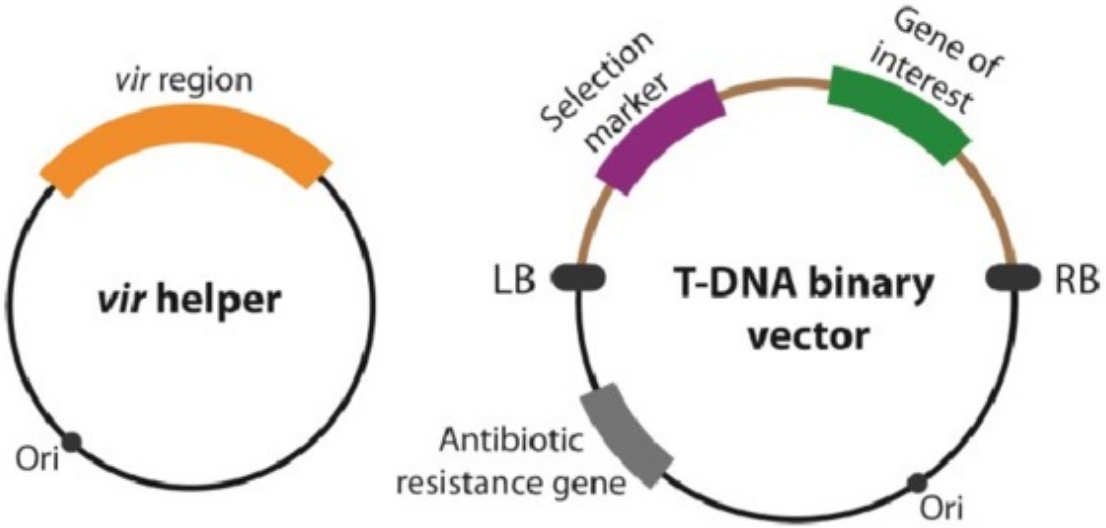
Virulence Factors

Cut out TDNA
Send T DNA to new cell



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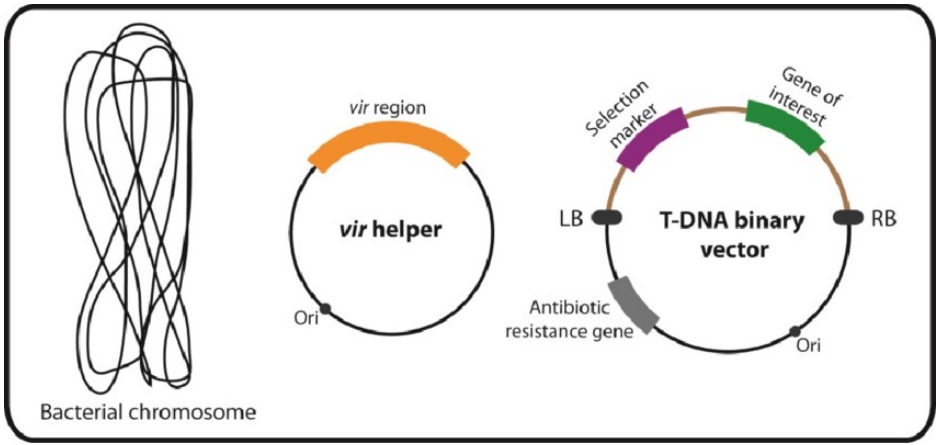
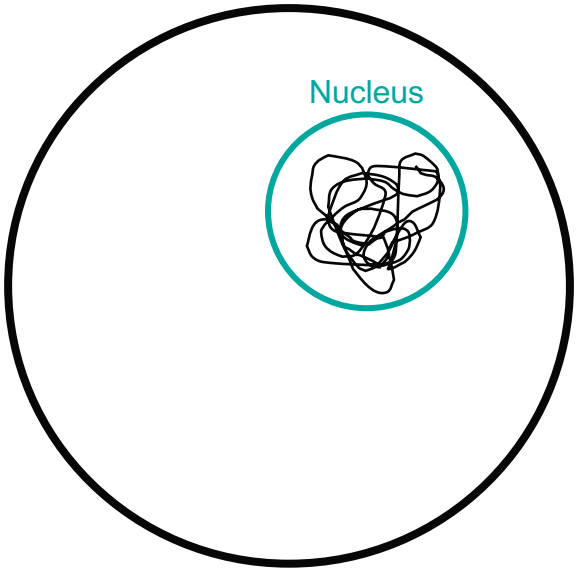


A. Tumefaciens agrobacterium cell



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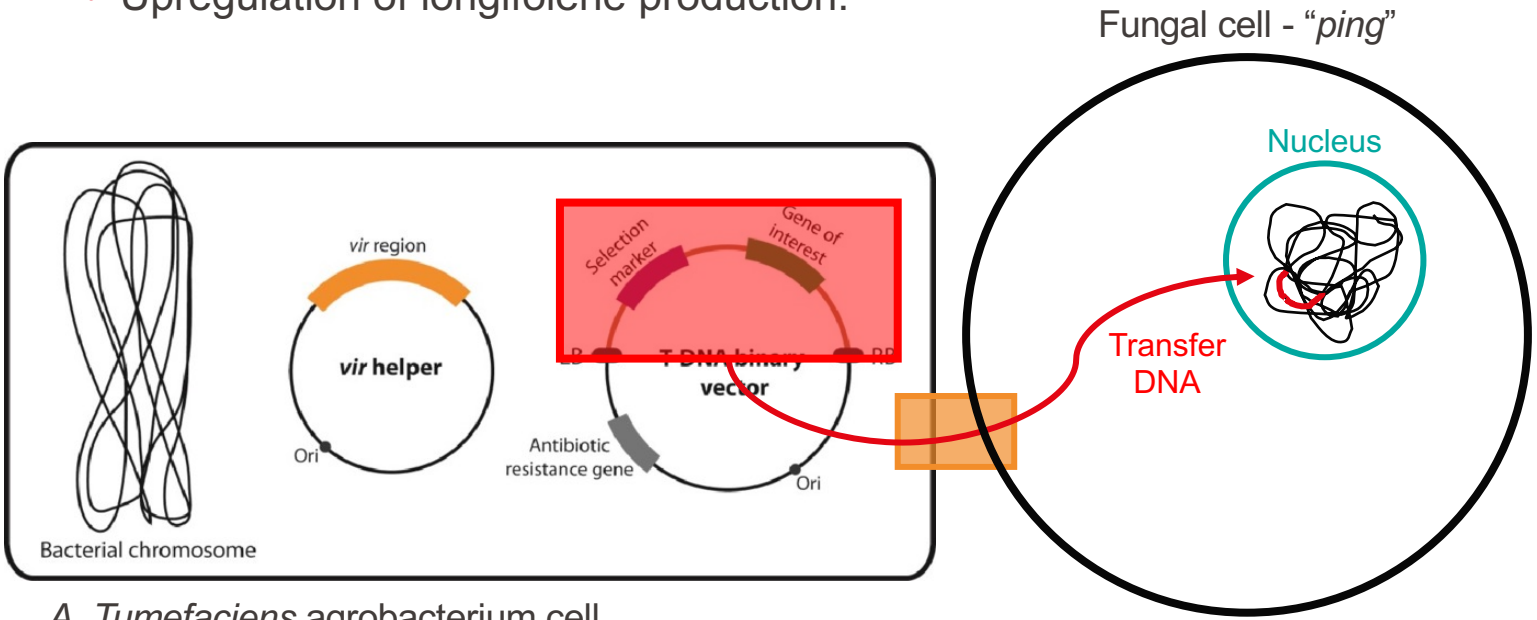

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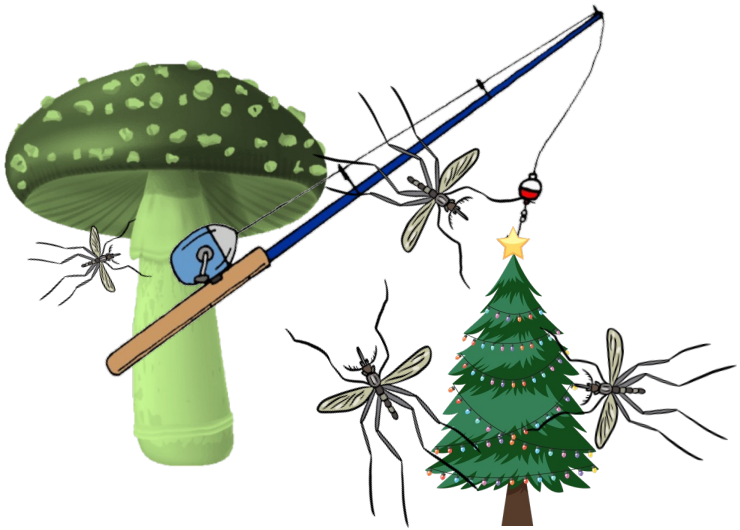

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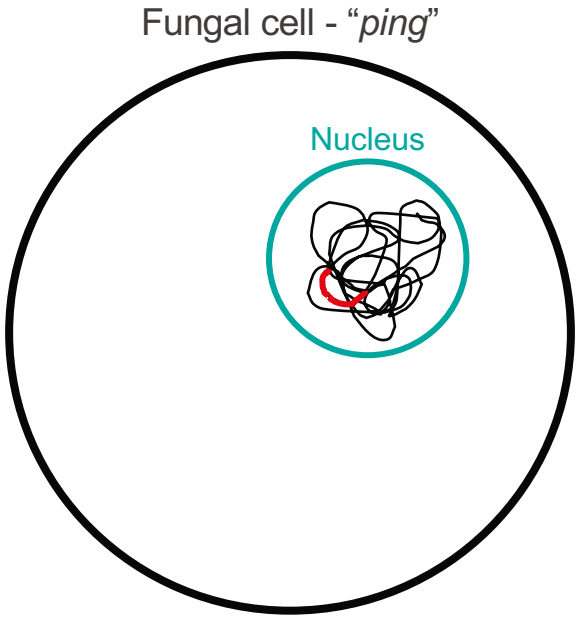

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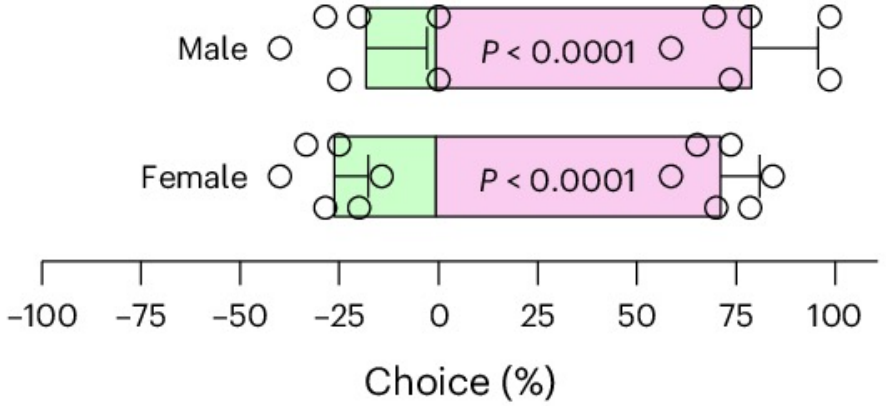
Let's zoom in on the experimental procedure

(Tang et al., 2025)

- Assessing the engineered fungus' capabilities compared to wild type.
  - How well the mechanism works in more complex ("smelly") environments.

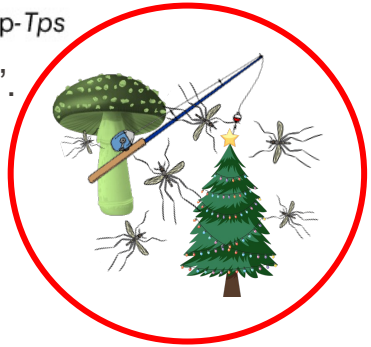


WT

Wild type "*ping*".

Mp-*Tps*

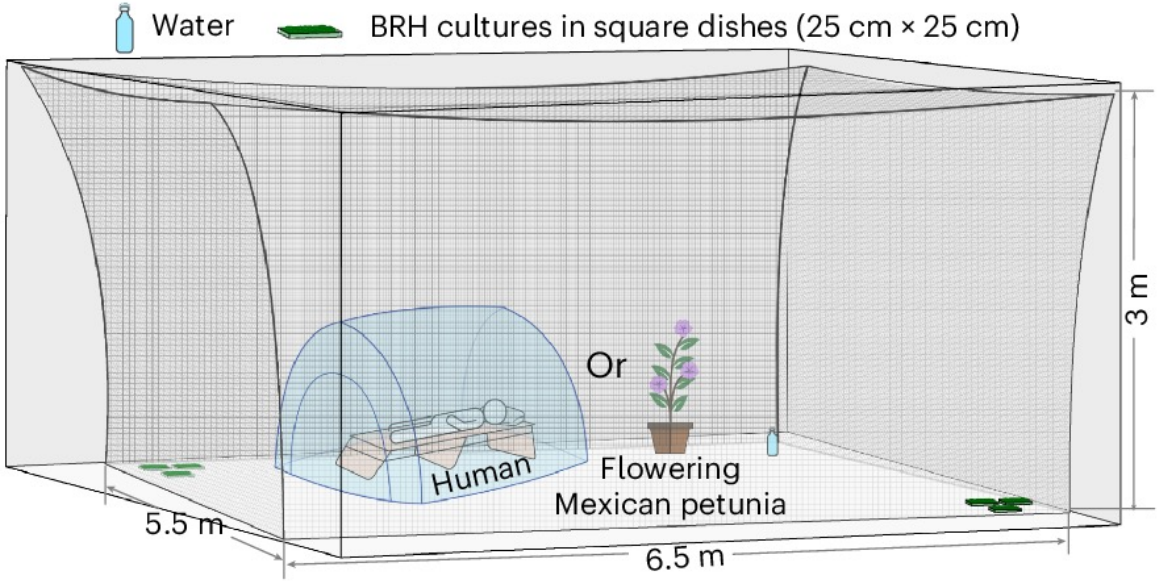
Modified "*ping*".



Let's zoom in on the experimental procedure

(Tang et al., 2025)

- Assessing the engineered fungus' capabilities compared to wild type.
  - How well the mechanism works in more complex ("smelly") environments.



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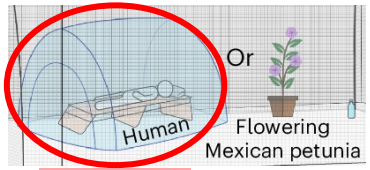
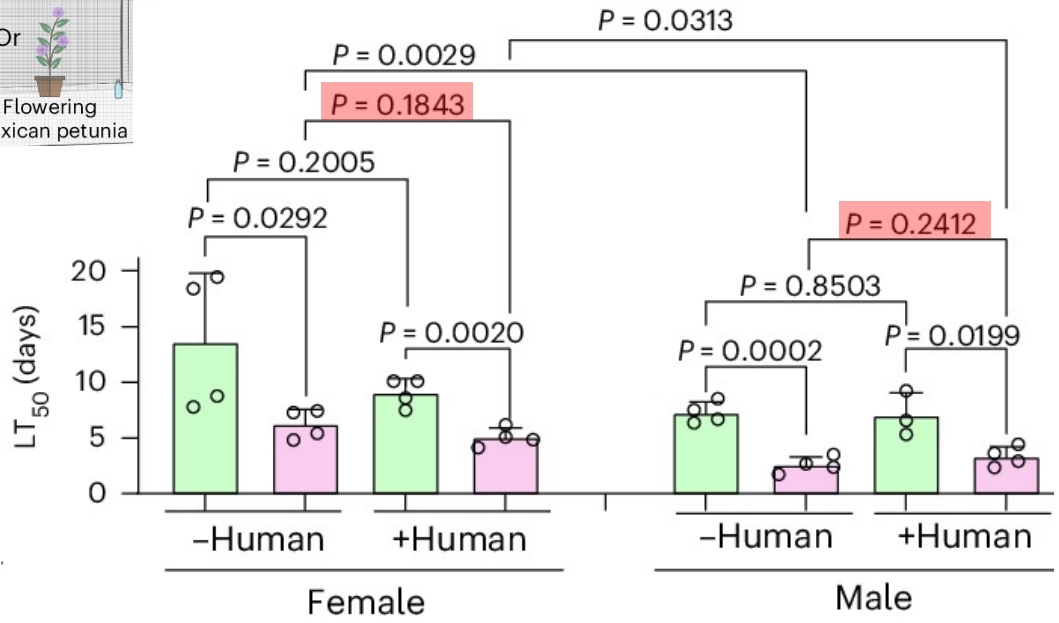
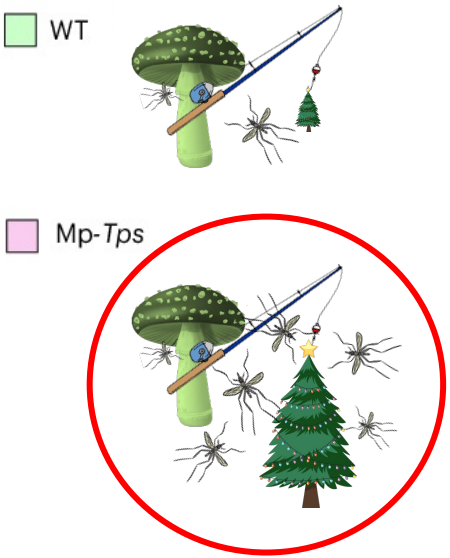


No Impact





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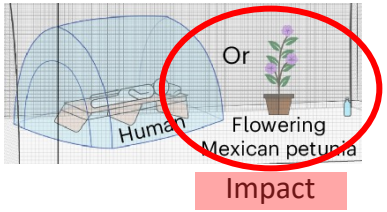

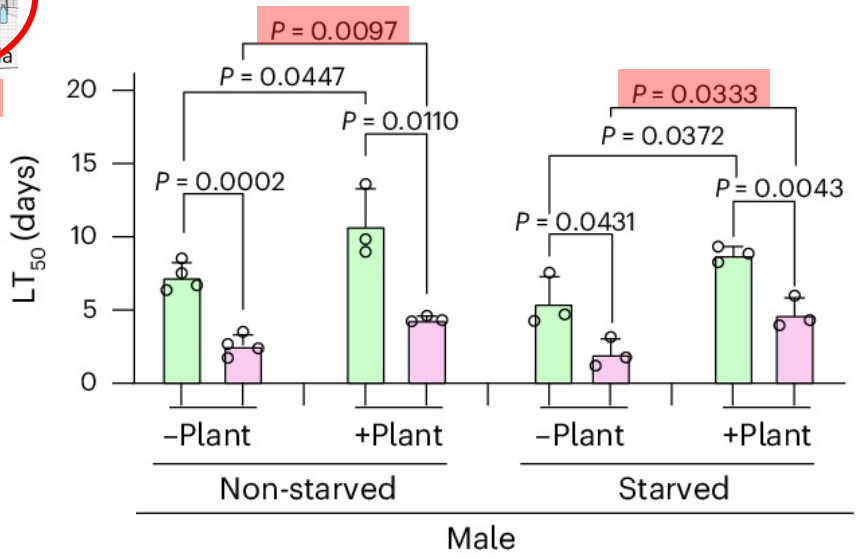

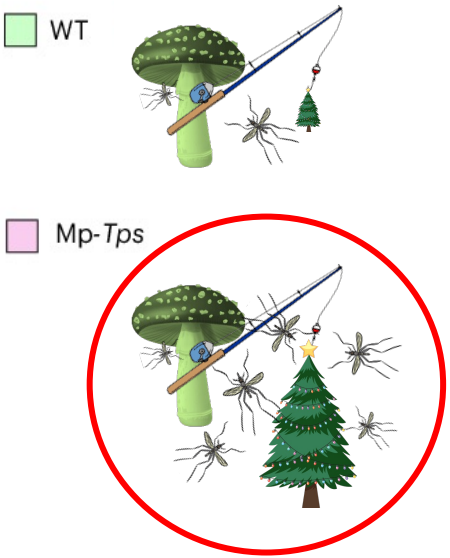

Limitations of the study

- The tested environment was **not as complex as real-world situations**
  - Outdoor environments have many more different smells
  - Need to conduct field trials

- Could this be used outside of a lab?
  - Would require further engineering
    - **Reduce environmental persistence**
      - Reduce resistance to UV exposure
      - Reduce ability to colonize plants
  - Would require further testing on **non-target impacts**
    - Harming insects beyond mosquitos

OUTLINE

What is it?

How does it work?

How do we use it?

Conclusion

Conclusion

- Agrobacterium-mediated gene transfer is a **useful molecular biology tool**.
  - Takes advantage of natural microbial adaptations.
  - Allows the engineering of plant and fungal cells.
- **Many different applications**.
  - Ex. Engineering fungal strains for improved biocontrol of mosquitos.
- As with many cloning techniques **concerns arise**.
  - Possible release to the environment has unknown consequences
    - Need to establish methods to reduce prevalence in environment (ex. “kill switches”).
    - For now, restriction to the laboratory.

Thank you

Are there any questions?

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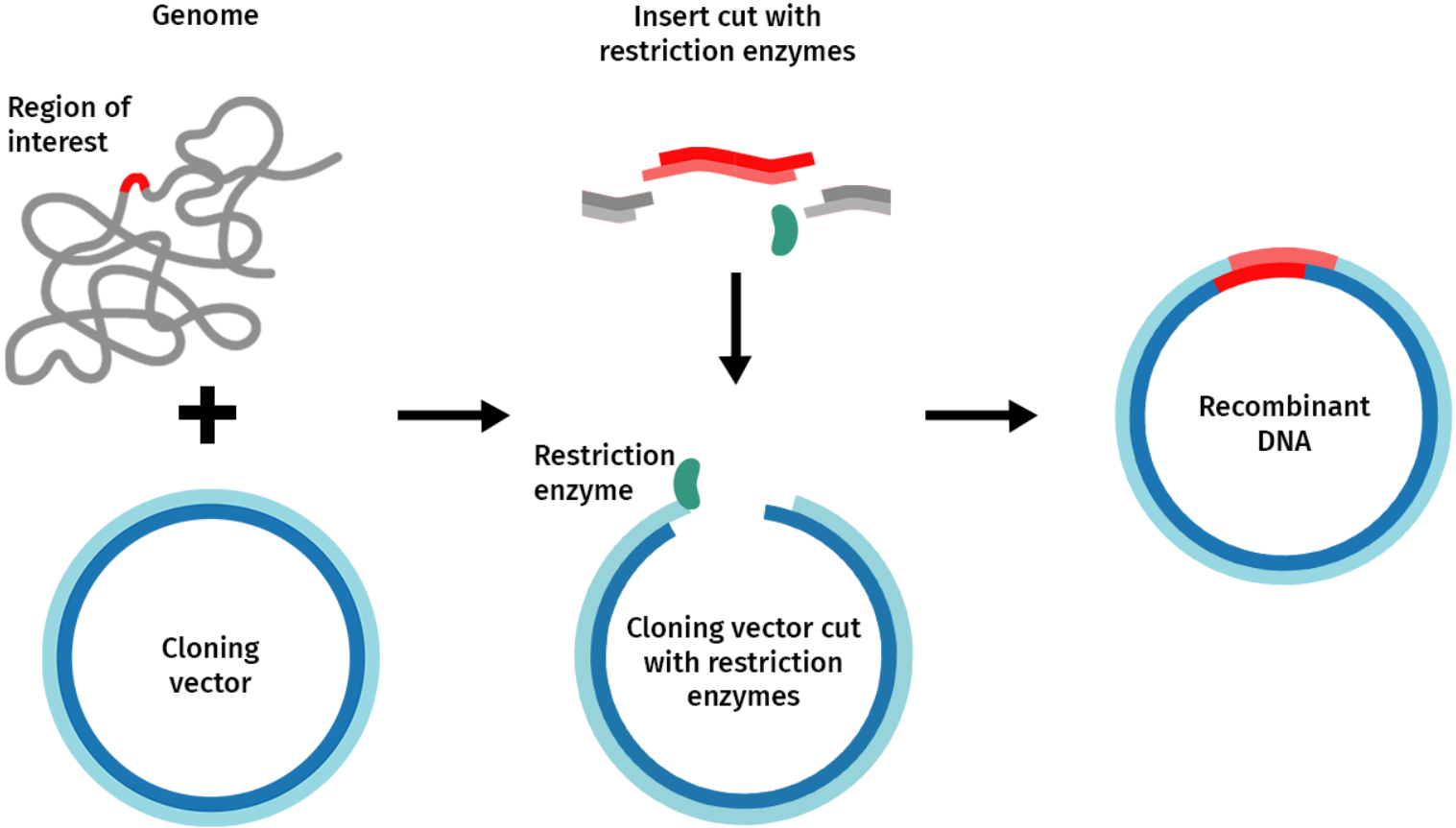
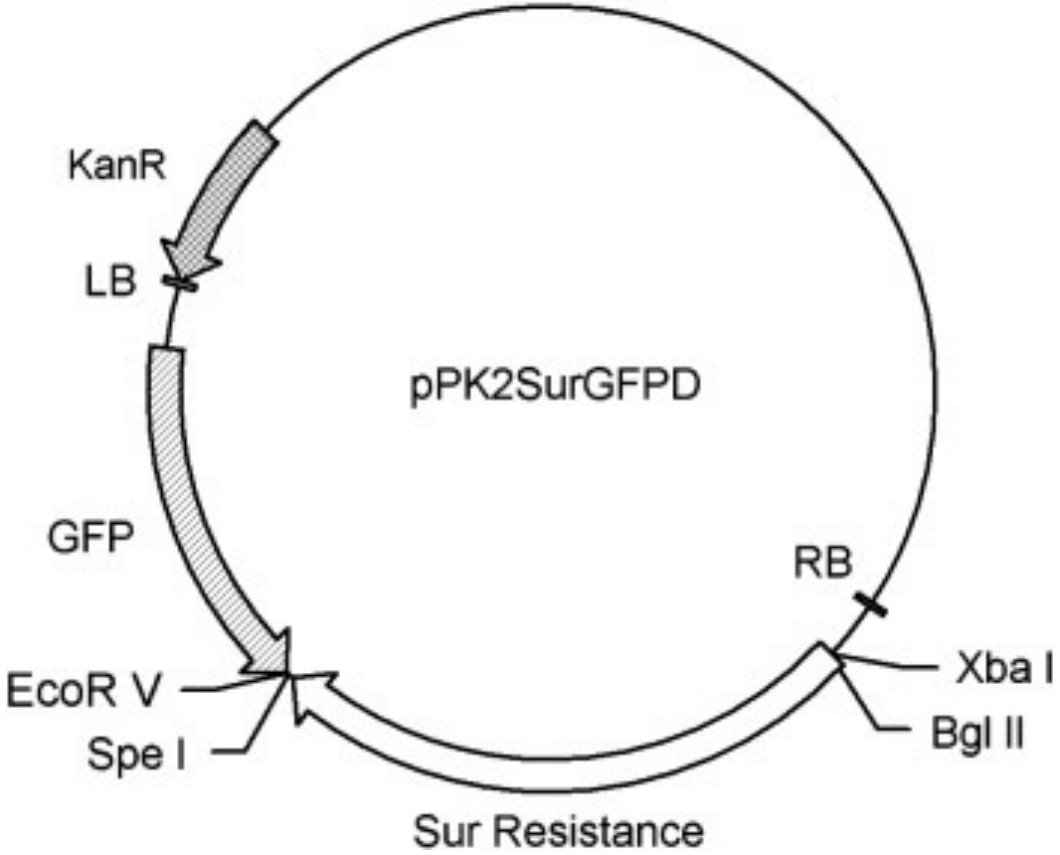
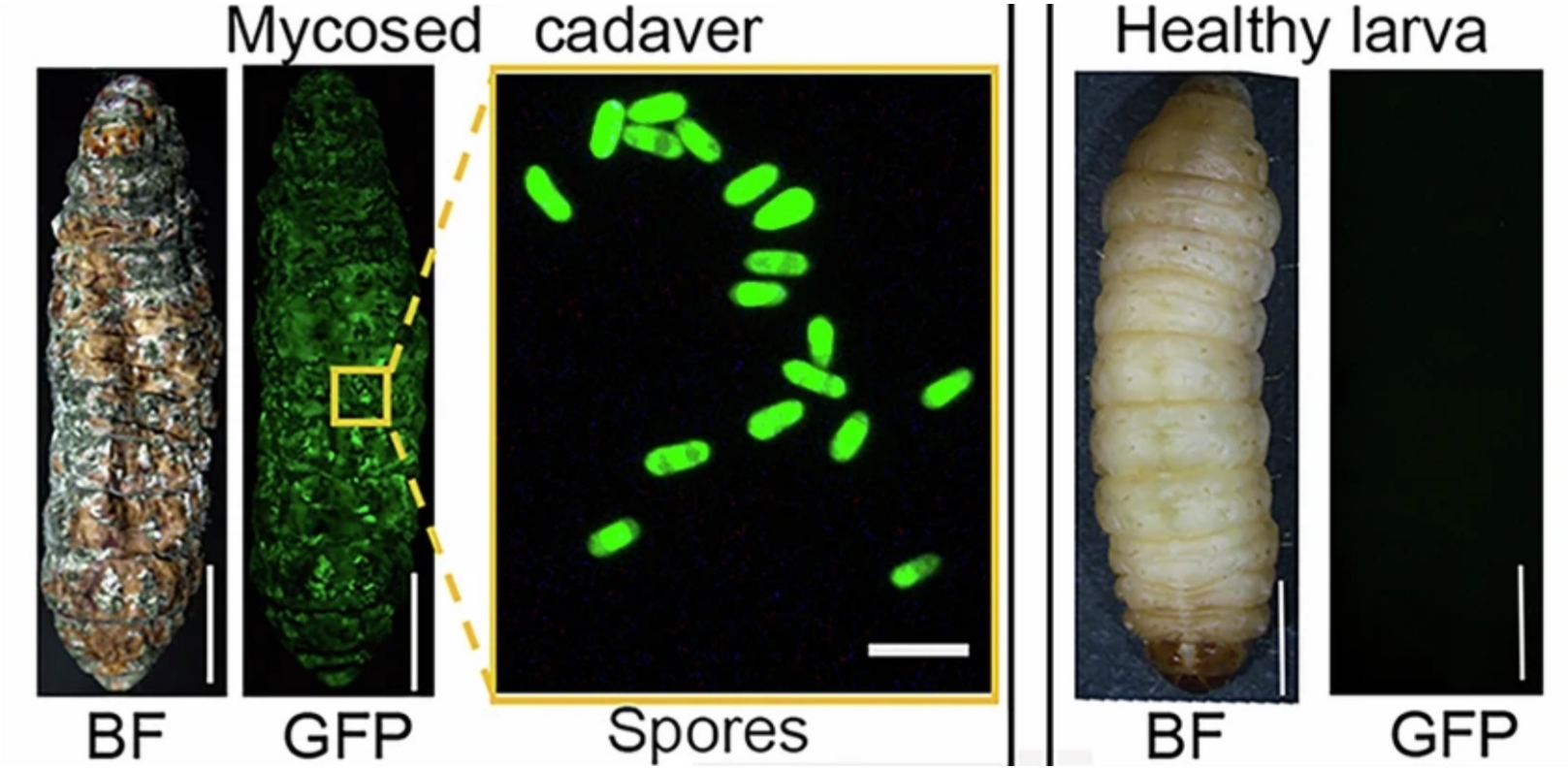

Genome
Region of interest
Cloning vector
Insert cut with restriction enzymes
Restriction enzyme
Cloning vector cut with restriction enzymes
Recombinant DNA


KanR
LB
pPK2SurGFPD
GFP
RB
EcoR V
Xba I
Spe I
Bgl II
Sur Resistance


Mycosed cadaver
Healthy larva
BF
GFP
Spores
BF
GFP