# Conditional-Lethal Transgenic Screwworm

## Development of a Male-only Strain

Felix D. Guerrero, John E. George, John B. Welch

Knipling-Bushland USDA, ARS US Livestock Insects Research Laboratory, Kerrville, TX, USA

# Overall Project Objectives

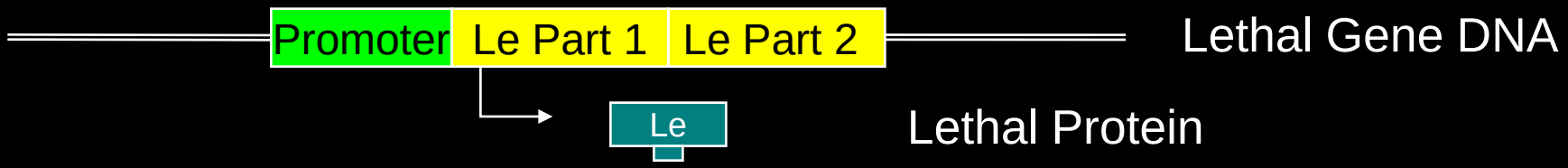
A. Transgenic strain of flies in which female lethality can be selectively turned on early in the life cycle.

B. Horn fly originally to be used as the model insect to develop technology at Kerrville.

C. Screwworm transgenesis in Panama.

# Genetic Engineering Terms

Gene Promoter: A DNA sequence which regulates the activity of an adjacent protein coding region.




Tetracycline: An inexpensive well-known antibiotic that can bind to specific proteins and prevent their function.

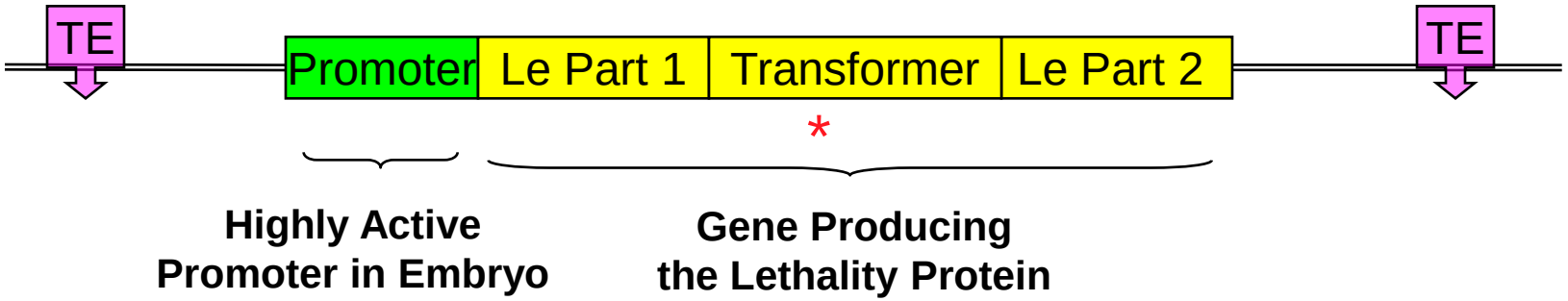
Transposable Element: A segment of DNA capable of moving from one location on a chromosome to another, within the same or different organisms, thus, transferring genetic characteristics.

# Transgenic Fly System Components

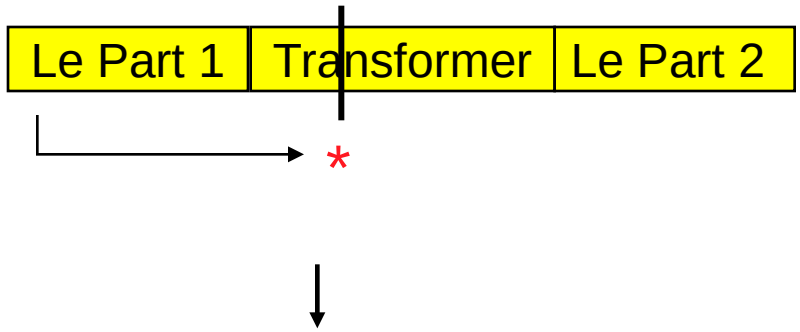
- Gene promoter that is highly active in embryonic stage
- Tetracycline (Tet)
- Lethality-causing protein (Le) which tetracycline can bind to and inactivate
- Gene region (Transformer) controlling sex-specific synthesis of lethal-causing protein
- Transposable element (TE) to transfer gene system into screwworm genome

# Female Conditional Lethal System

## DNA Construct

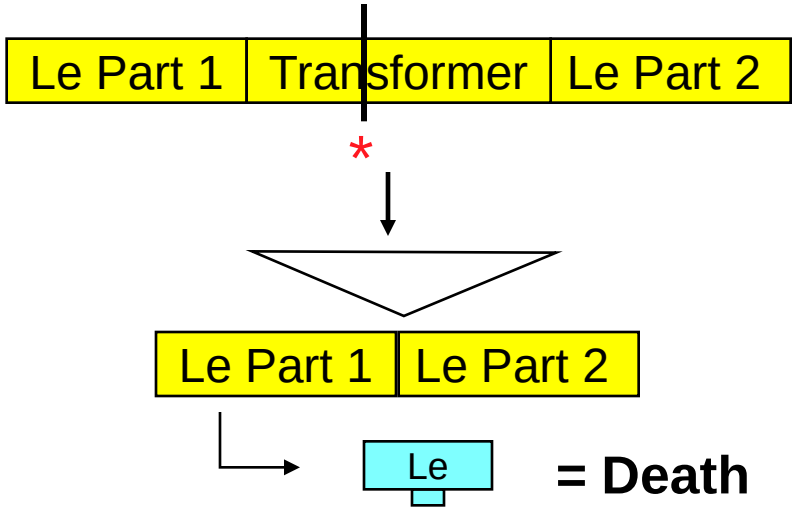



## Males




**No Le protein = no lethality**

## Females




**= Death**

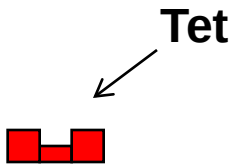
# Female Conditional Lethal System

Microinject embryos with DNA to produce transgenics

Select and maintain colony with tetracycline in growth media

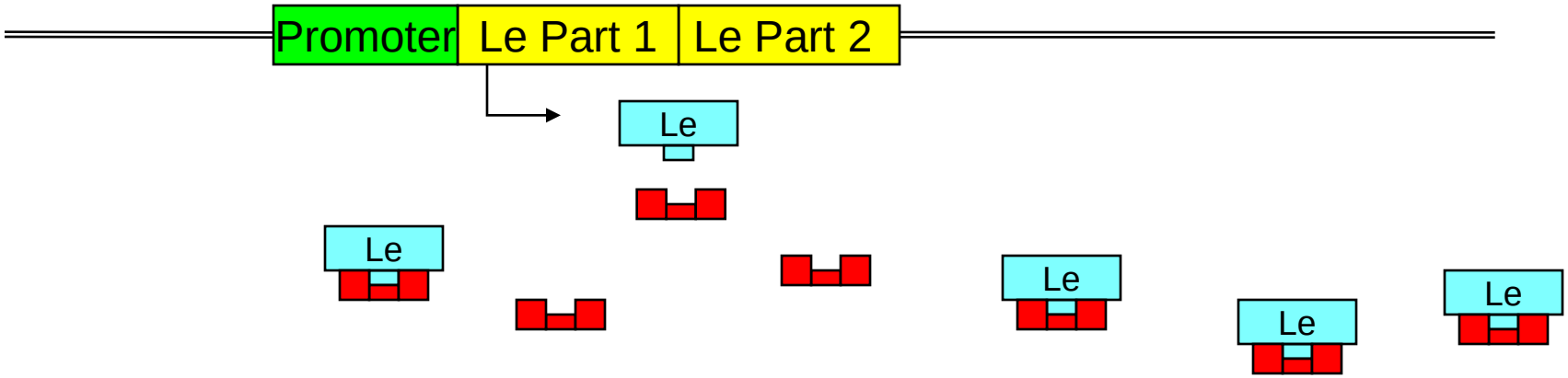
# Female Conditional Lethal System

## Tetracycline Present




**Males** = no Le product

**Females** = Le is made but Tet binds and inactivates

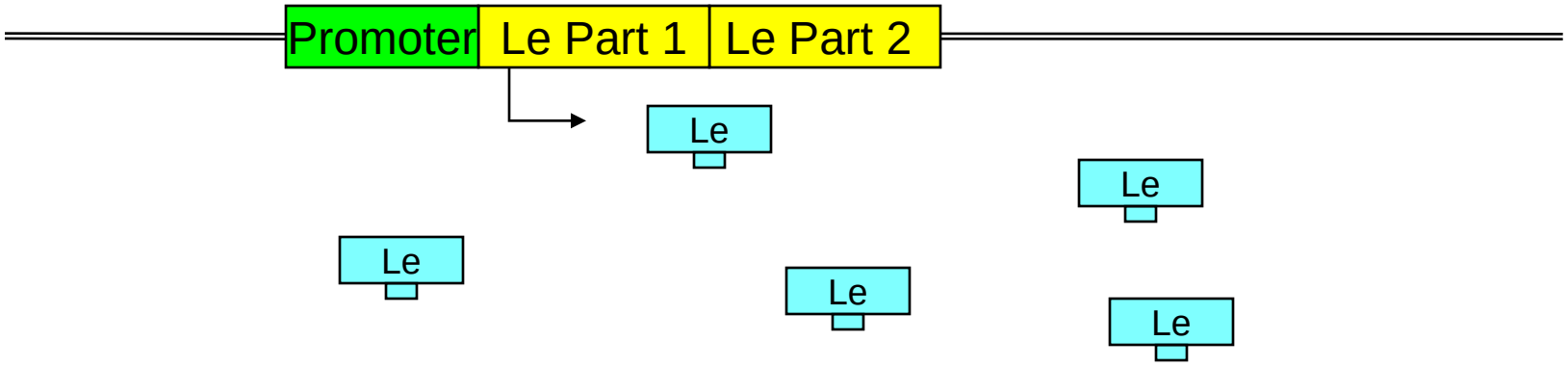


# Female Conditional Lethal System

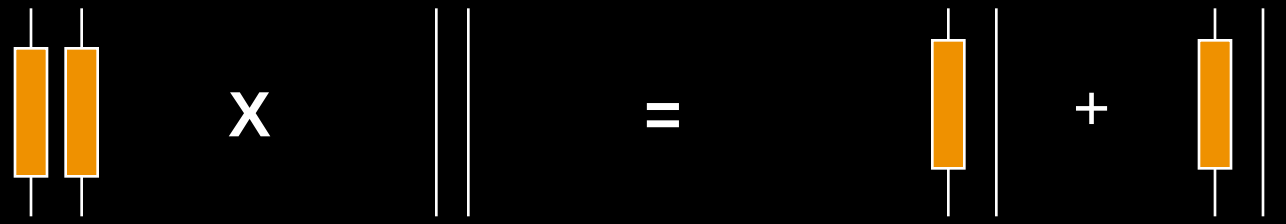
## Tetracycline Absent

**Males** = no Le product

**Females** = Le is made and causes lethality in embryo



# Male-only Strain Advantages




- Irradiation:
  - Dose can be reduced = male fitness
  - The absolute need is eliminated
  - Safety factor if radiation problems occur
- By not rearing females:
  - Save growth media costs
  - Plant capacity effectively doubled

# Project Timeline (5-yr duration)

Funds became available March 20, 2007

## Year 1 (3/20/07-3/19/08)

- ✓ Horn fly/screwworm gene sequence database
  - DNA Libraries/sequencing (egg, 1st instar and adult stages)
  - DNA database assembly
- Fill two postdoc positions
  - Hiring delay for molecular biologist postdoc
- ✓ Equip lab/obtain permits for horn fly transgenesis

# Original Year 2 Targets

A. Demonstration of horn fly transformation with piggybac transposon system and GFP

B. Cloning and sequencing of horn fly genes for female conditional lethal genetic system

C. Synthesis of screwworm cDNA and genomic libraries

# Contingency Plan Redirection

Fragility of Horn Fly Embryos (< 5% survival)

↓

Obtain C. macellaria colony as substitute model (> 50% survival)

↙ ↘

New IBC Approvals

↓

Transform in Kerrville
w/GFP
w/lethal sys

Direct jump to Year 4 Objectives

↓

Train Panama Tech in Kerrville (8/09)
+
Microinjections of *C. hominivorax*
in Panama (2009?)

# Project Timeline (cont.)

## Year 2 (3/20/08-3/19/09)

- ✓ Synthesis of horn fly genomic libraries (FG)
- ✓ Demonstrate horn fly transgenesis success w/GFP
  - problems led to redirection to secondary screwworm (AR)
- Assemble components of lethal gene system
  - (in progress) (JW)

# Project Timeline (cont.)

## Year 3 (3/20/09-3/19/10)

- *C. macellaria* transformation with Female-lethal system (AR)
  - Awaiting lethal system component final construction (JW, AR)
- Evaluate fitness of transformed *C. macellaria*
  - √ GFP transformed flies (AR, JW)
  - Female-lethal system components not yet available (JW, AR)
- Train Panama scientist(s) on transgenesis (AR)
  - √ Mario Vasquez June 8-19 In Kerrville
  - Second trip to Kerrville is budgeted.....when??
  - Four week trip to Panama in proposal.....when??
- Initiate cloning *C. hominivorax* DNA system components (JW)
  - Contingency if medfly/olive fly system fails

# Project Timeline (cont.)

## Year 4 (3/20/10-3/19/11)

- Complete assembly of *C. hominivorax* homologues (JW)
- Screwworm transgenesis with GFP (in Panama)
  - 4 week trip to Panama budgeted (AR)
  - 2 week trip to Panama budgeted (AR or JW)
- Evaluation of female-lethal transformed *C. macellaria* (AR, JW)

# Project Timeline (cont.)

## Year 5 (3/20/11-3/19/12)

- Screwworm transformation with conditional lethal system
- Evaluate transgenic screwworm lines (in Panama)