Construction of Piggy^{Bac} Vector for Screwworm transformation

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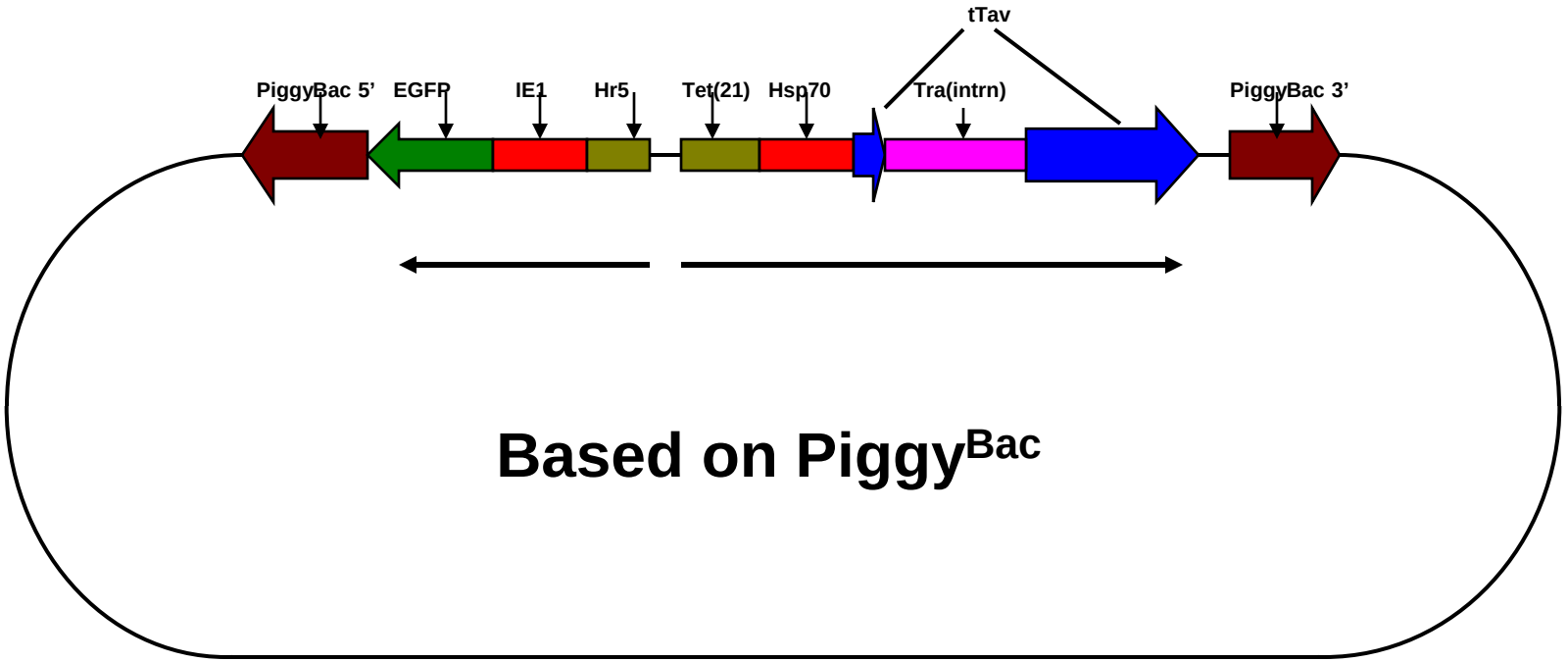
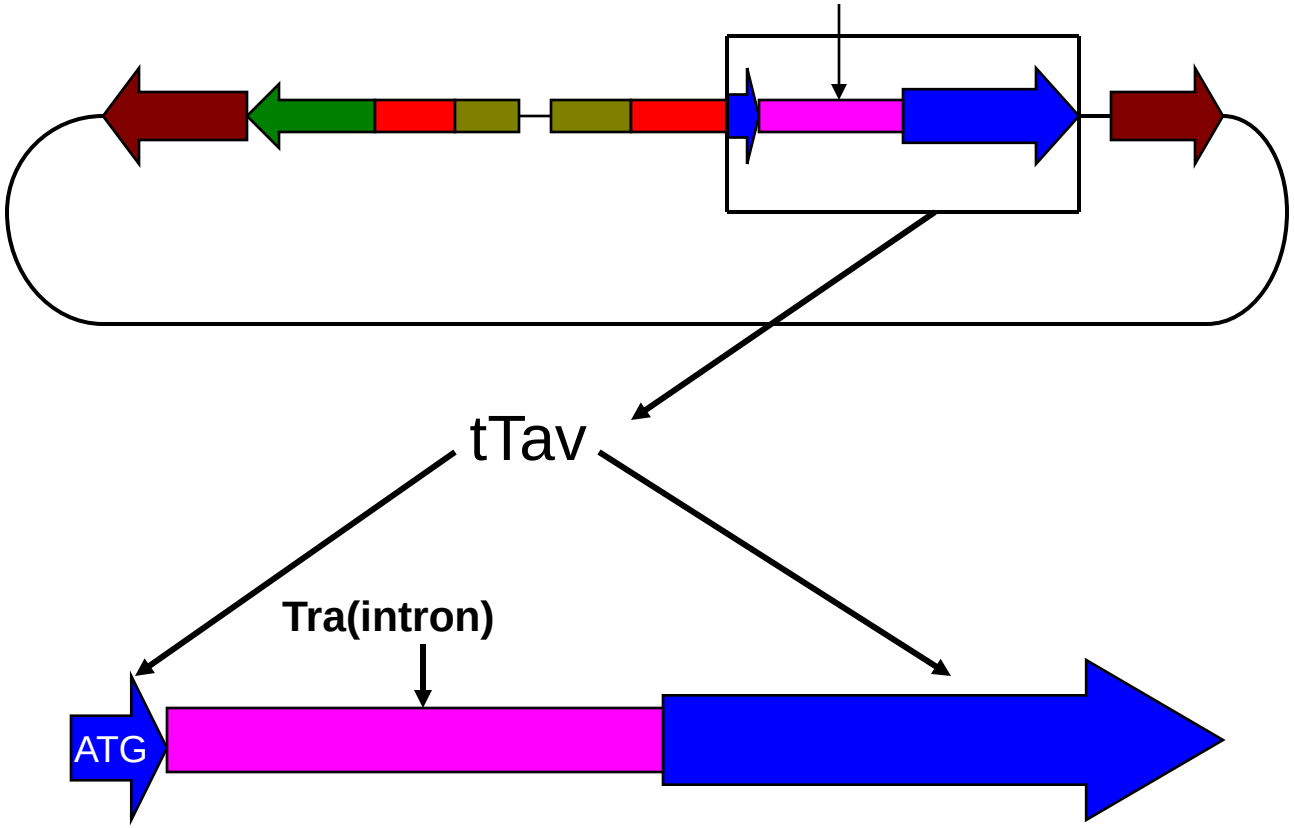
What is the Construction

- The plasmid carrying the foreign genes
- Using as vector to transform foreign genes into embryos

Construction

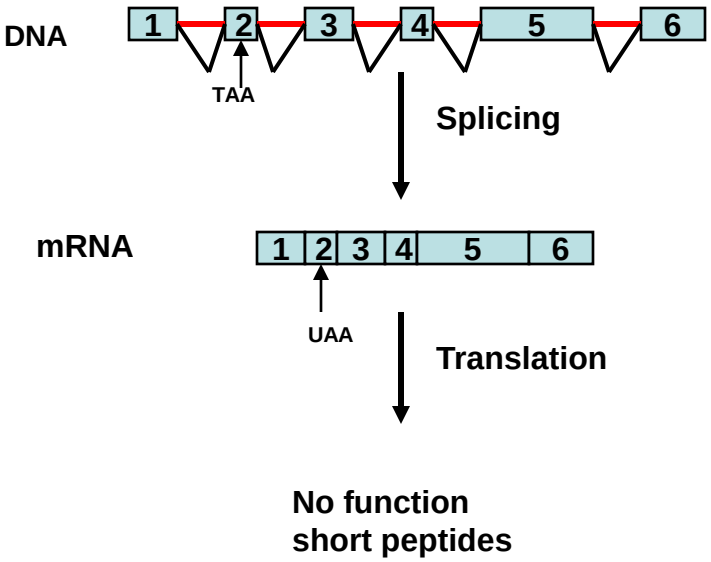


Tra-intron (Transformer)

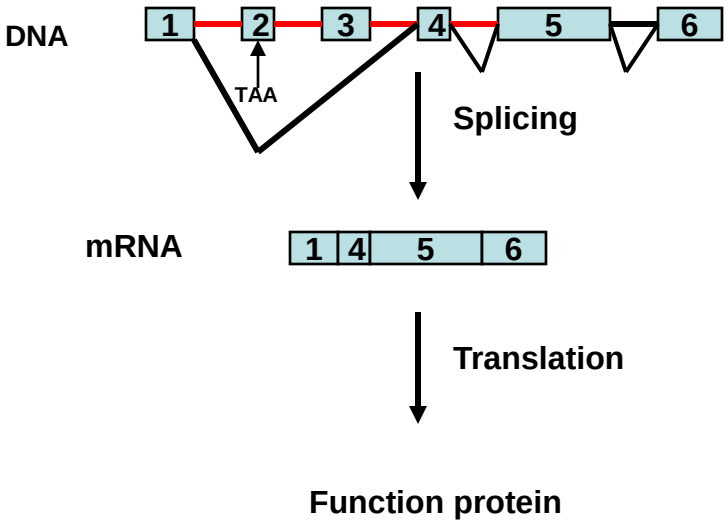


- Tra (transformer) is sexual determination gene
- Tra gene splicing differs in male and female flies
- Tra gene splicing is controlled by its first intron

Tra gene splicing in flies



Transformer gene splicing in male flies



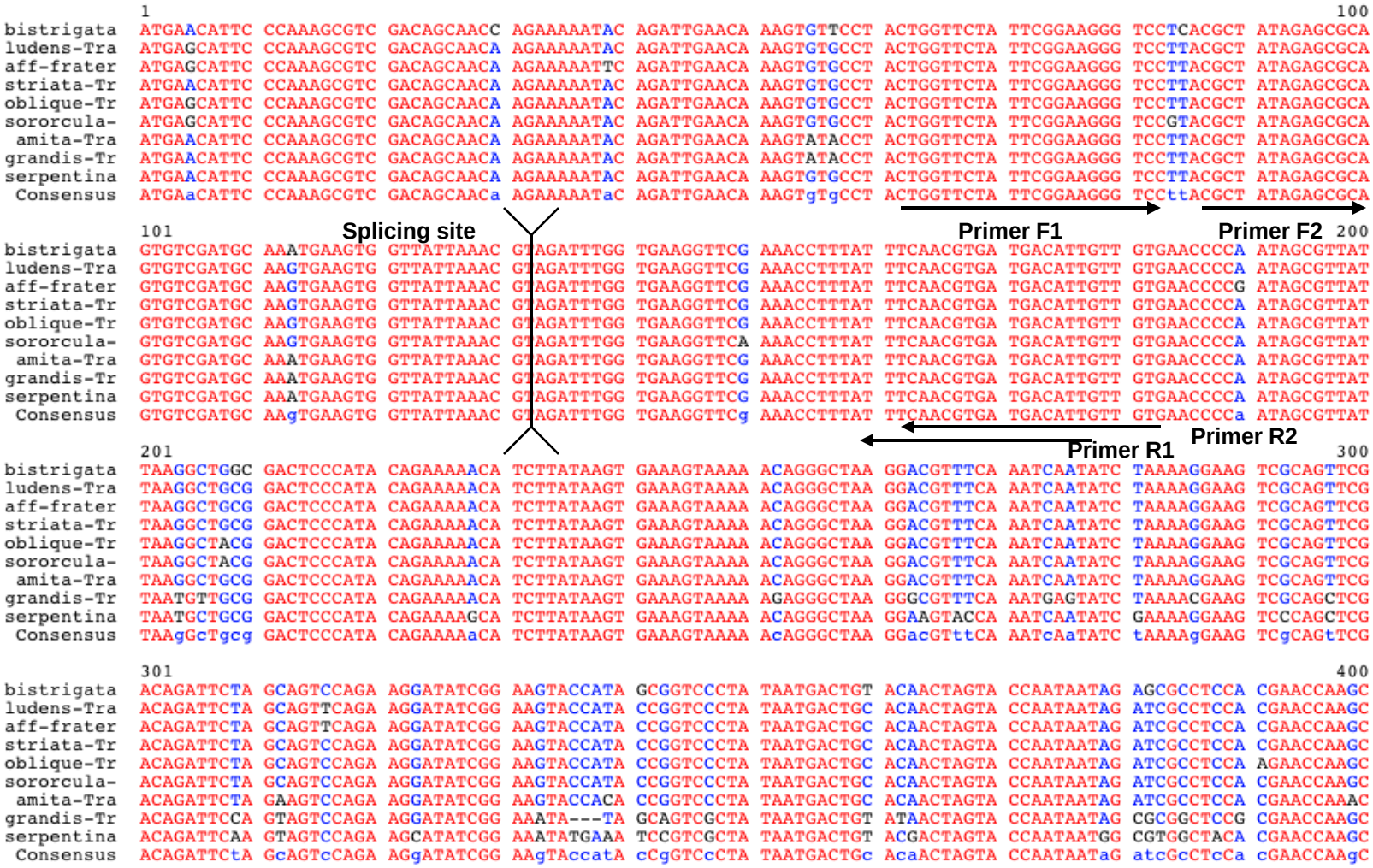
Transformer gene splicing in female flies

Cloning the tra-intron

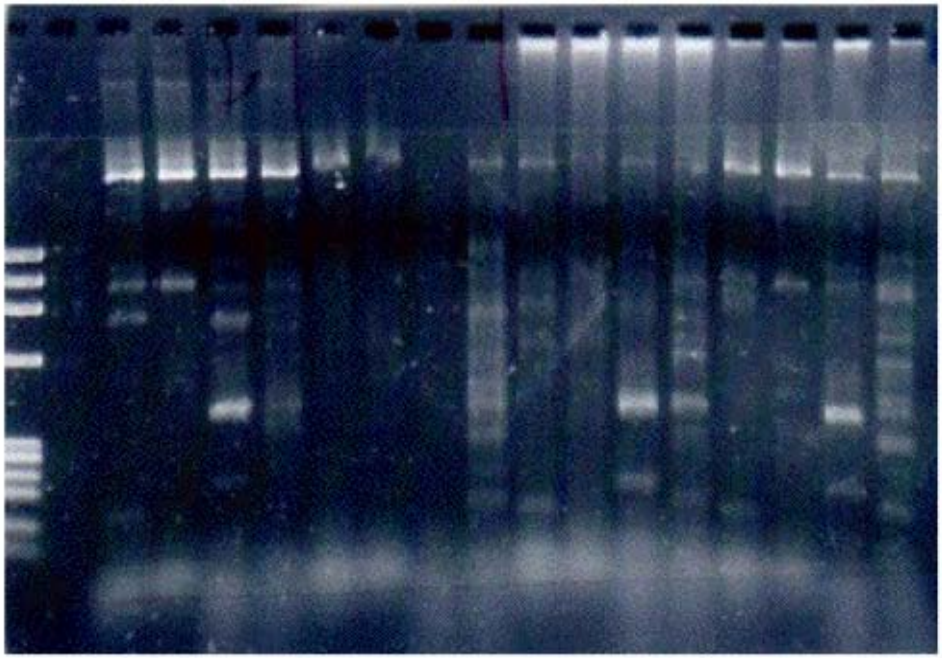
- **Using Caribbean fruit fly** *(Anastrepha suspensa (Lowe))* **as target to clone the *tra* intron**
  - **Found entire transformer gene sequence from nine Caribbean fruit fly species**
  - **Aligned the transformer genes, designed PCR primers**
  - **Cloned the first intron**

- **We also found the tra-intron sequence information for Olive fruit fly** *(Bactrocera oleae)* **from GenBank**

Transformer gene of Caribbean Fruit Fly



Cloning the first intron of the *transformer* gene from Caribbean Fruit Fly



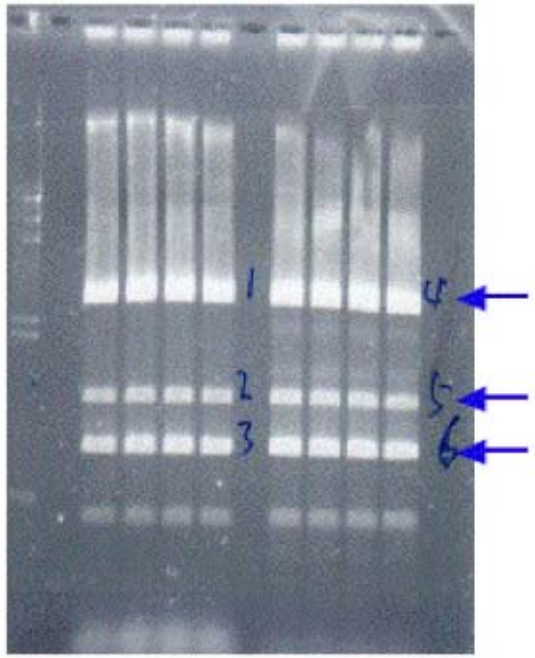
Lane 1: Ladder

Lane 3-6: Sample #1 with four pairs primers

Lane 7-10: Sample #2 with four pairs primers

Lane 11-14: Sample #3 with four pairs primers

Lane 15-19: Sample #4 with four pairs primers

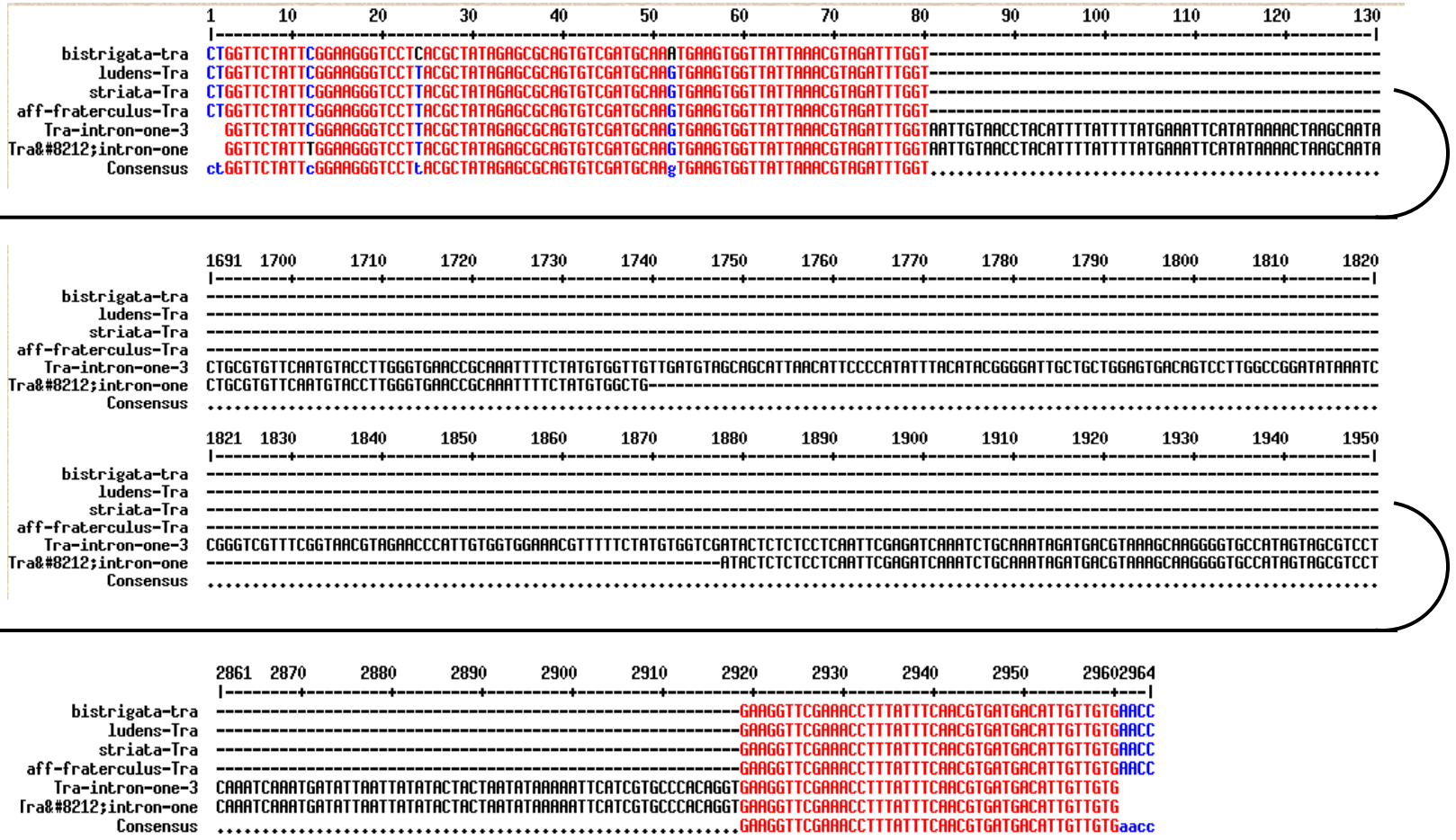


Lane 1: Ladder

Lane 3-6: Sample #1 with Primer F1.R1

Lane 8-11:Sample #1 with primer F2/R1

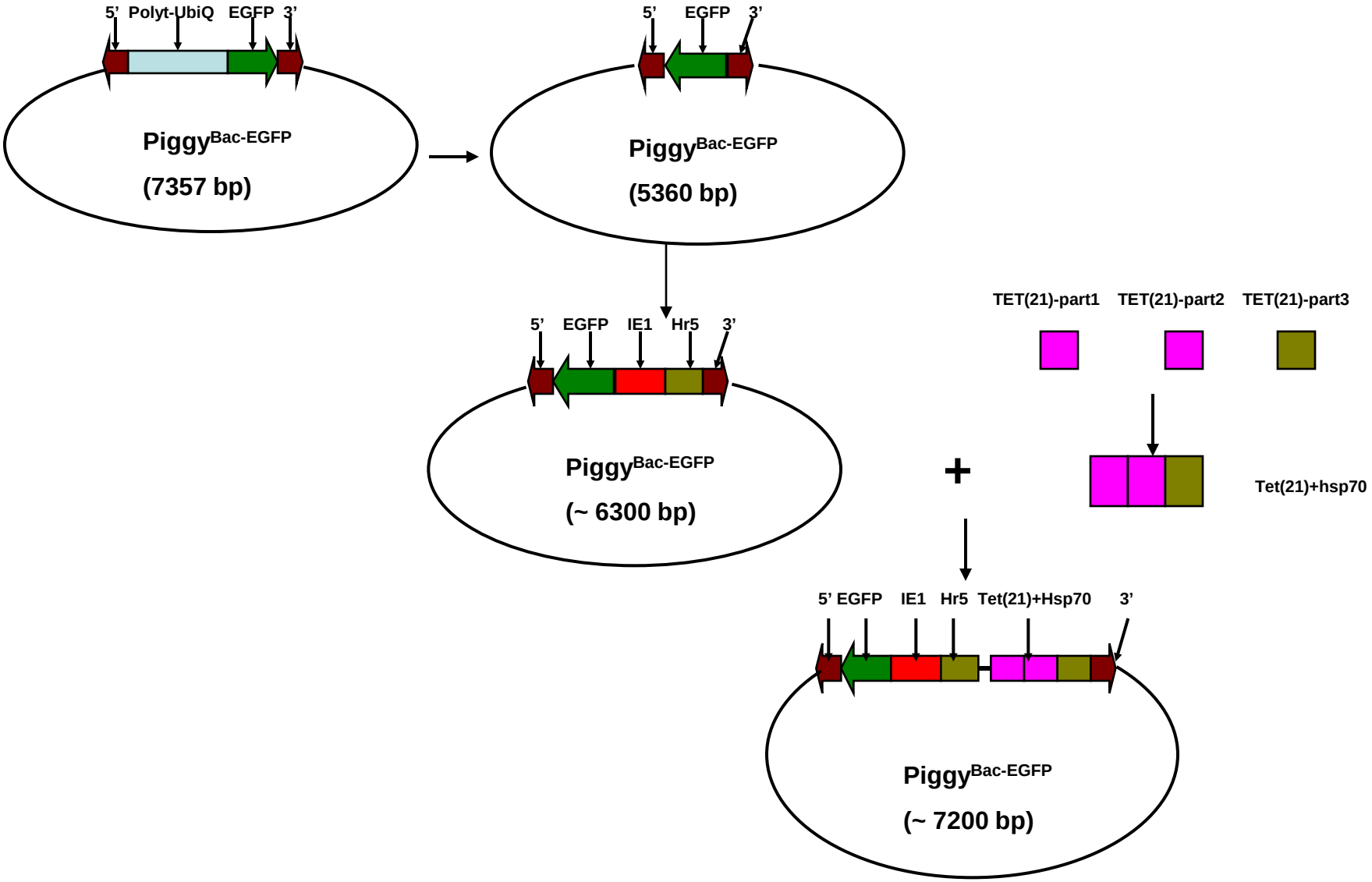
Cloning the intron of *tra* gene



Cloning of intron for *tra* gene

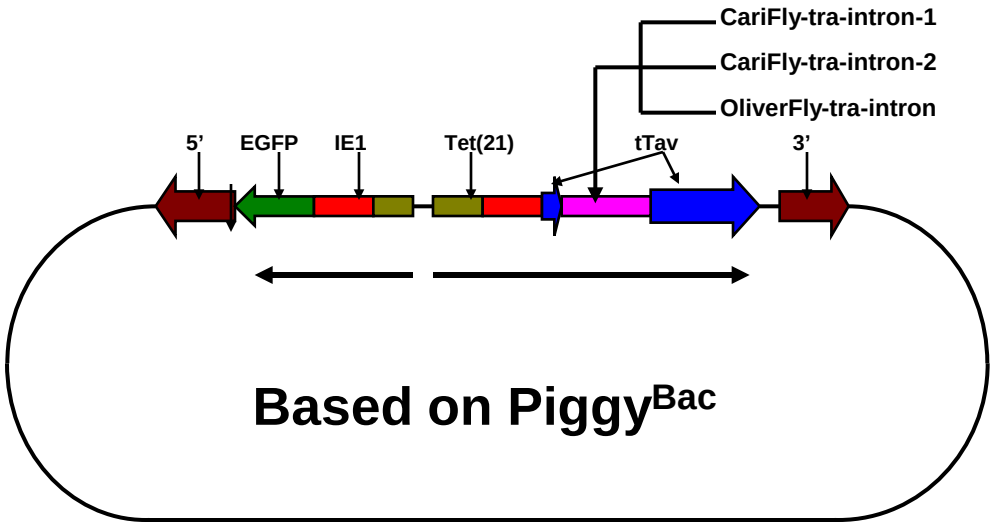
- **Three first introns of *tra* gene in hand**
  - **The first intron of *tra* gene from Olive fruit fly**
  - **The first intron from Caribbean fruit fly (2819 bp)**
  - **The first intron from Caribbean fruit fly (2953 bp)**

Construction of Vector



Next Steps

- **Finishing the vector constructions**



- **Evaluation for potential roles of the three *tra* introns in the female conditional lethal genetic system**

Thank you very much