

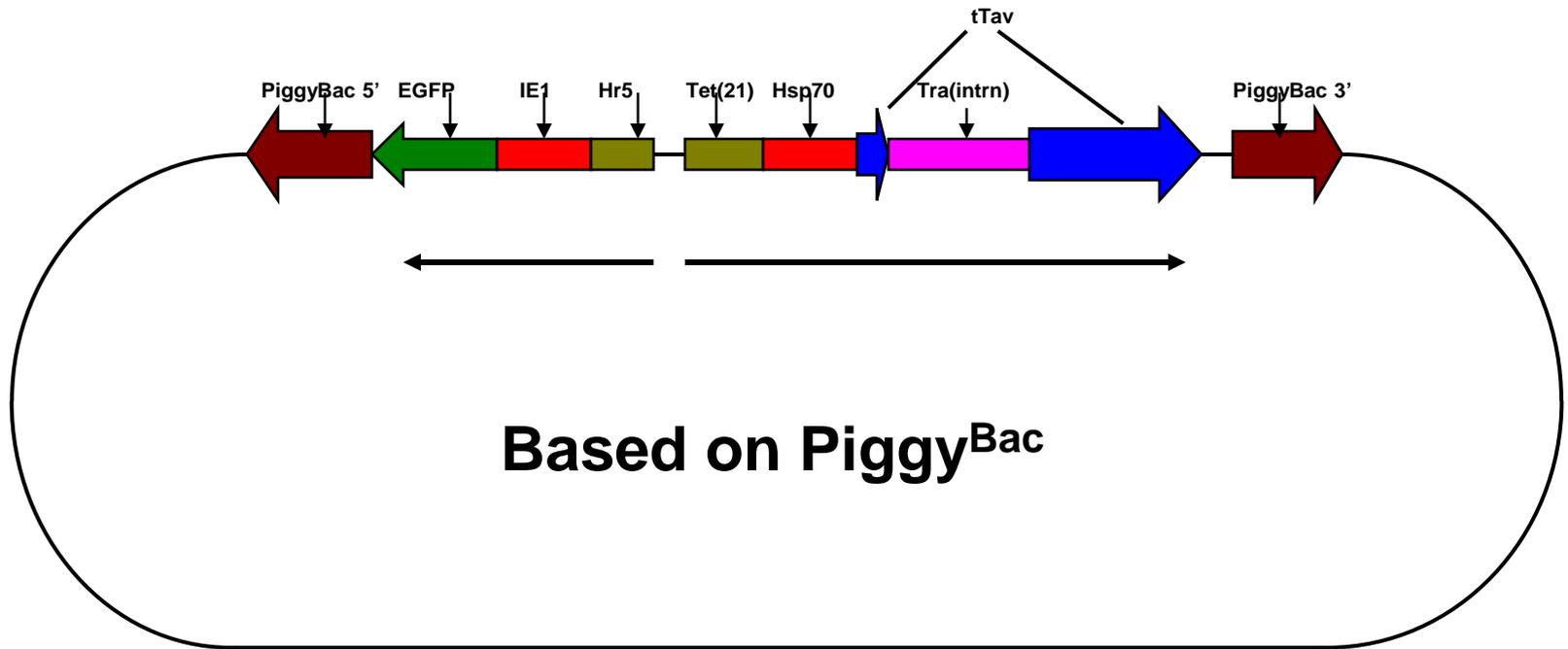
# **Construction of Piggy<sup>Bac</sup> Vector for Screwworm transformation**

Ju Wang

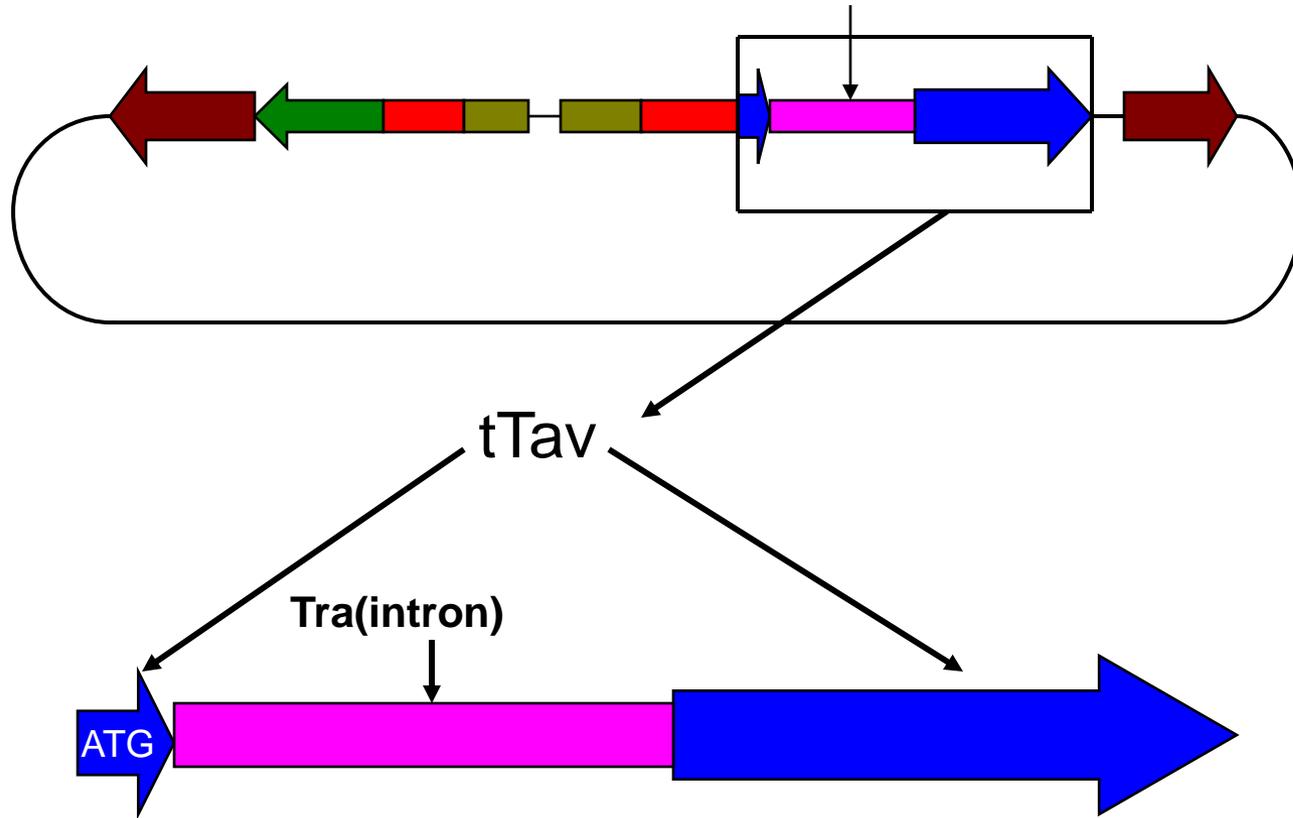
# What is the Construction

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

# Construction

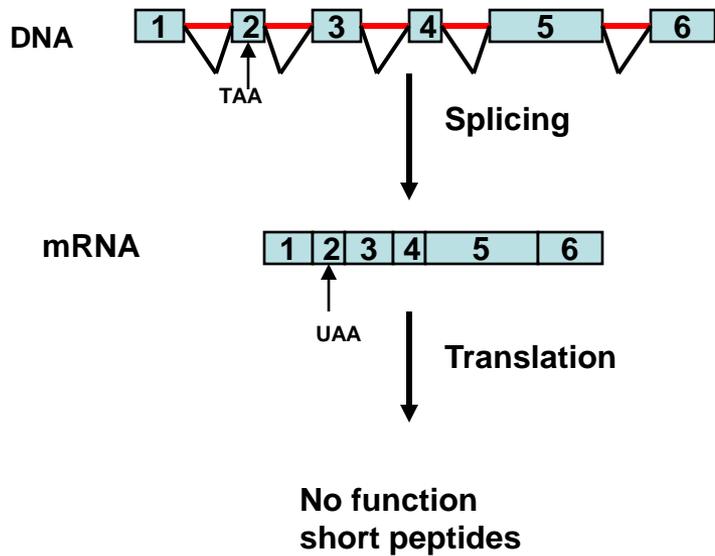


# Tra-intron (Transformer)

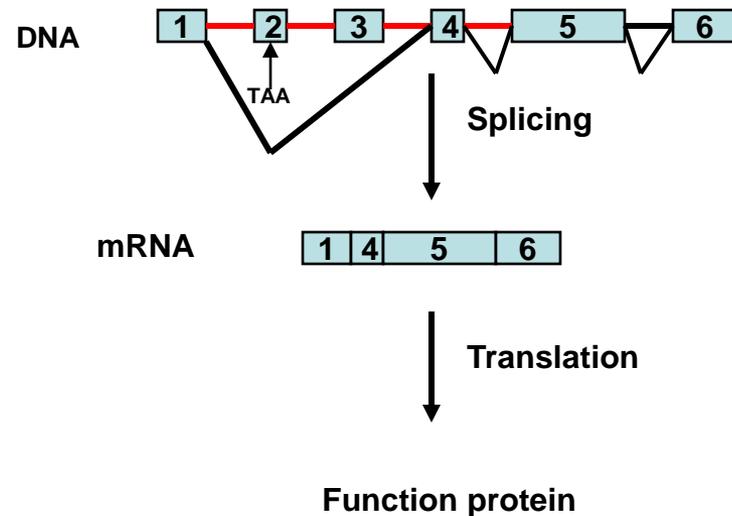


- Tra (transformer) is sexual determination gene
- Tra gene splicing differs in male and female flies
- Tra gene splicing is controlled by its first intron

# *Tra* gene splicing in flies



**Transformer gene splicing in male flies**



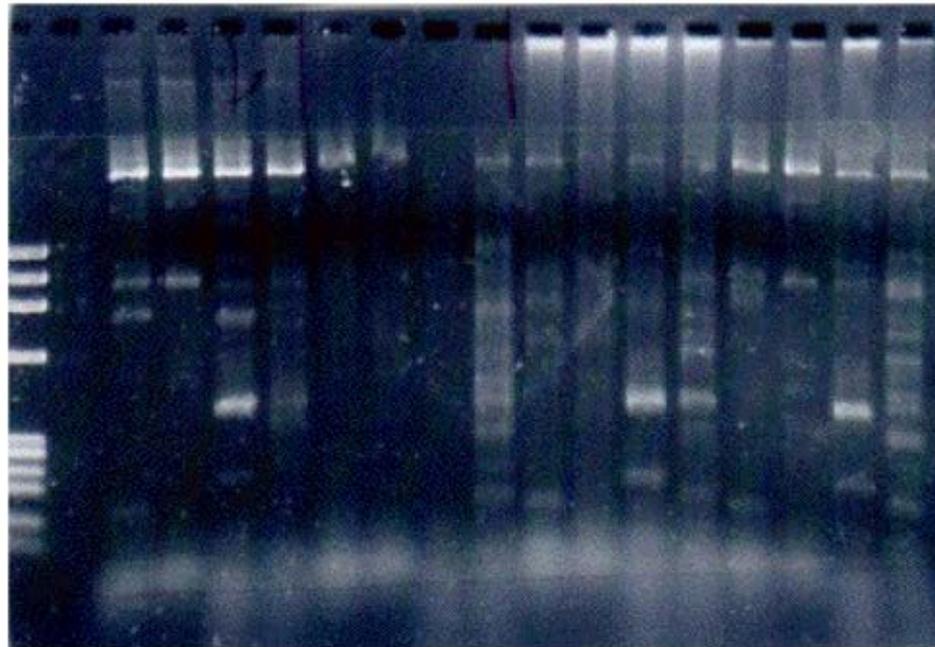
**Transformer gene splicing in female flies**

# 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**



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



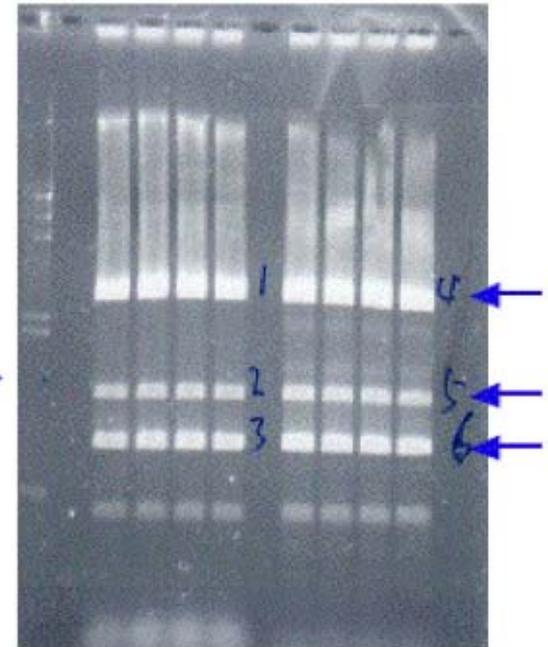
Lane 1: Ladder

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 5-11: Sample #1 with primer F2/R1

# Cloning the intron of *tra* gene

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
bistrigata-tra	CTGGTCTATTCGGAGGGTCTCACGCTATAGAGCGCAGTGTGATGCARAATGAAGTGGTTATTAACGTAGATTGGT													
ludens-Tra	CTGGTCTATTCGGAGGGTCTCACGCTATAGAGCGCAGTGTGATGCARAATGAAGTGGTTATTAACGTAGATTGGT													
striata-Tra	CTGGTCTATTCGGAGGGTCTCACGCTATAGAGCGCAGTGTGATGCARAATGAAGTGGTTATTAACGTAGATTGGT													
aff-fraterculus-Tra	CTGGTCTATTCGGAGGGTCTCACGCTATAGAGCGCAGTGTGATGCARAATGAAGTGGTTATTAACGTAGATTGGT													
Tra-intron-one-3	GGTCTATTCGGAGGGTCTCACGCTATAGAGCGCAGTGTGATGCARAATGAAGTGGTTATTAACGTAGATTGGTAAATTGTAACCTACATTTATTTATGAARATTCATATAAACTAAGCAATA													
Tra#8212;intron-one	GGTCTATTCGGAGGGTCTCACGCTATAGAGCGCAGTGTGATGCARAATGAAGTGGTTATTAACGTAGATTGGTAAATTGTAACCTACATTTATTTATGAARATTCATATAAACTAAGCAATA													
Consensus	ctGGTCTATTCGGAGGGTCTCACGCTATAGAGCGCAGTGTGATGCARAATGAAGTGGTTATTAACGTAGATTGGT.....													

	1691	1700	1710	1720	1730	1740	1750	1760	1770	1780	1790	1800	1810	1820
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
bistrigata-tra	-----													
ludens-Tra	-----													
striata-Tra	-----													
aff-fraterculus-Tra	-----													
Tra-intron-one-3	CTGCGTGTTCAATGTACCTTGGGTGAACCGCAAAATTTCTATGTGGTGTGTGATGTAGCAGCATTAAACATTCACATACGCGGATGCTGCTGGAGTGACAGTCTTGCCCGGATATAAATC													
Tra#8212;intron-one	CTGCGTGTTCAATGTACCTTGGGTGAACCGCAAAATTTCTATGTGGCTG-----													
Consensus	.....													

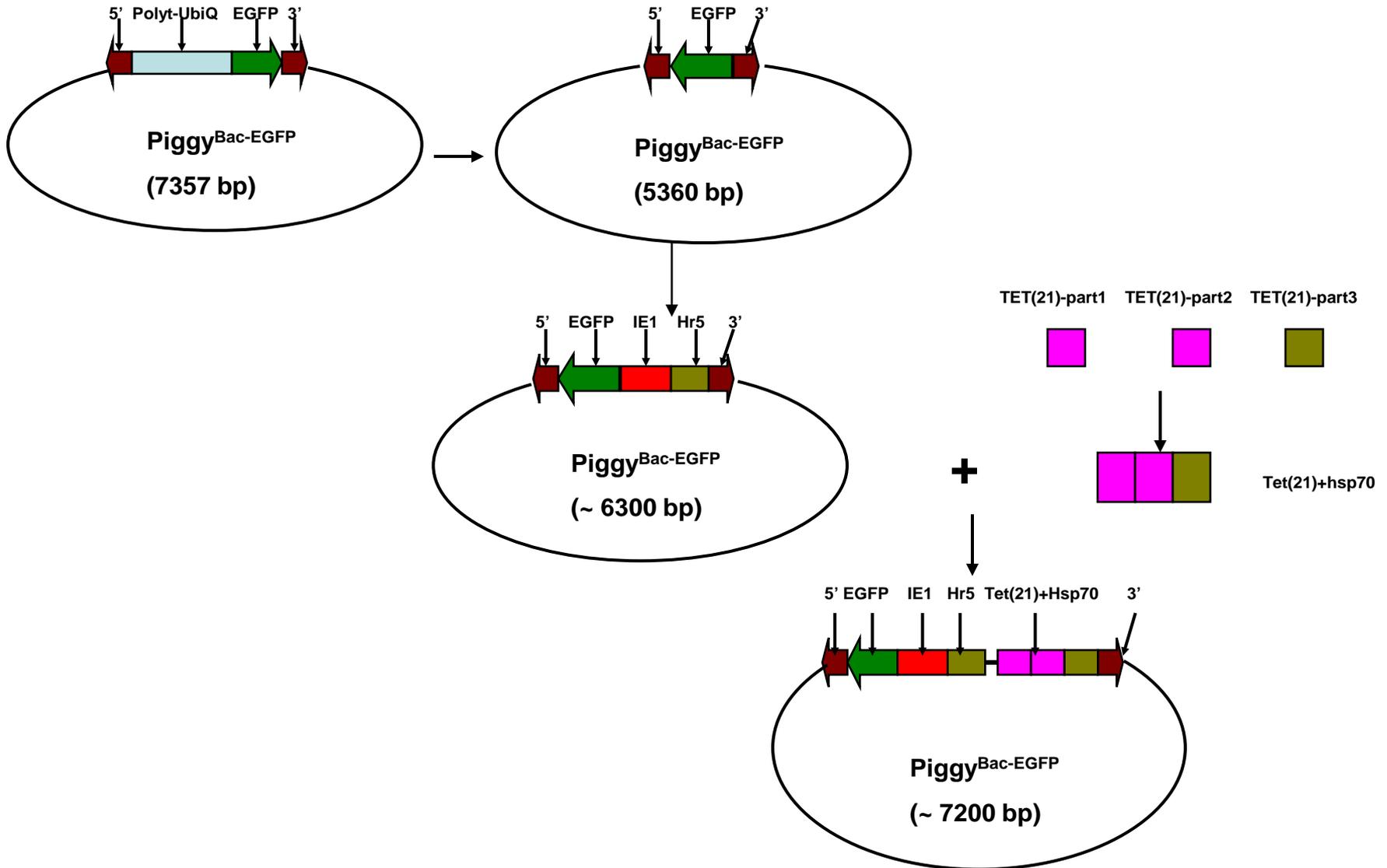
	1821	1830	1840	1850	1860	1870	1880	1890	1900	1910	1920	1930	1940	1950
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
bistrigata-tra	-----													
ludens-Tra	-----													
striata-Tra	-----													
aff-fraterculus-Tra	-----													
Tra-intron-one-3	CGGGTCGTTTTCGGTACGTAGAACCCATTGTGGTGGAAACGTTTTTCTATGTGGTGCATCTCTCCCTCAATTCGAGATCAAAATTCGCAAAATAGATGACGTAAGCAGGGGTGCCATAGTAGCGTCT													
Tra#8212;intron-one	-----ATACTCTCTCCCTCAATTCGAGATCAAAATTCGCAAAATAGATGACGTAAGCAGGGGTGCCATAGTAGCGTCT													
Consensus	.....													

	2861	2870	2880	2890	2900	2910	2920	2930	2940	2950	2960	2964
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----											
bistrigata-tra	-----GAAGGTTCGAAACCTTTATTTCAACGTGATGACATTGTTGTGARCC											
ludens-Tra	-----GAAGGTTCGAAACCTTTATTTCAACGTGATGACATTGTTGTGARCC											
striata-Tra	-----GAAGGTTCGAAACCTTTATTTCAACGTGATGACATTGTTGTGARCC											
aff-fraterculus-Tra	-----GAAGGTTCGAAACCTTTATTTCAACGTGATGACATTGTTGTGARCC											
Tra-intron-one-3	CAATCAAAATGATATTAATTATATACTACTAATATAAAATTCATCGTGCCACAGGTGAAGGTTTCGAAACCTTTATTTCAACGTGATGACATTGTTGTG											
Tra#8212;intron-one	CAATCAAAATGATATTAATTATATACTACTAATATAAAATTCATCGTGCCACAGGTGAAGGTTTCGAAACCTTTATTTCAACGTGATGACATTGTTGTG											
Consensus	.....GAAGGTTCGAAACCTTTATTTCAACGTGATGACATTGTTGTGaacc											

# Cloning of intron for *tra* gene

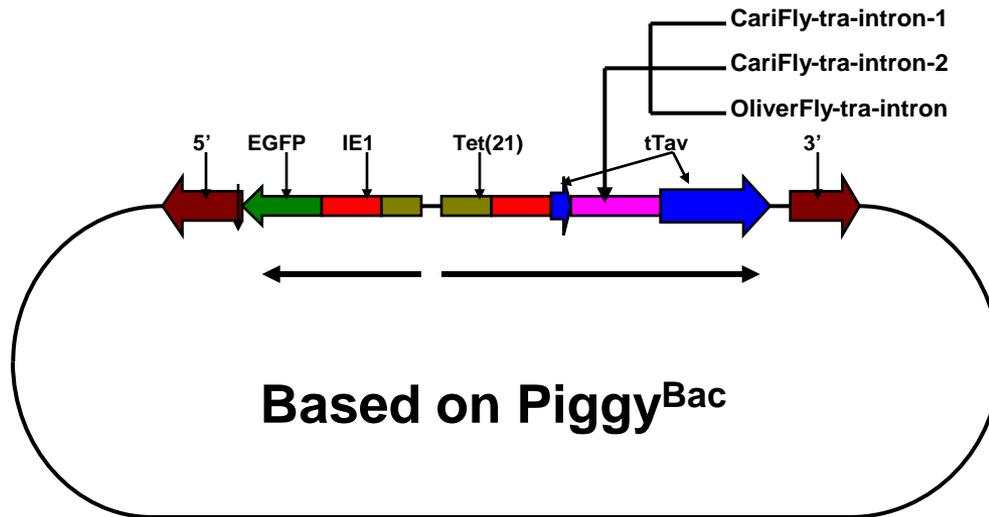
- **Three first introns of *tra* gene in hand**
  - **The first intron of *tra* gene from Olive fruit fly**
  - **The first intron from Caribbean fruit fly (2819 bp)**
  - **The first intron from Caribbean fruit fly (2953 bp)**

# 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