**BAPC-CRISPR/Cas9 System for Heritable Gene-Knock OUT:**

Asian Citrus Psyllid

In 2017, the Genome of *Diaphorina citri*, the Asian citrus psyllid, and the Official Gene Set, OGS_v2 were used to design psyllid control strategies. [www.citrusgreening.org](http://www.citrusgreening.org)

We used the CRISPR/Cas9, Clustered regularly interspaced palindromic repeats (CRISPR) & CRISPR associated proteins (Cas9).

**System to disrupt gene expression in psyllids.** The targeted gene was the Thioredoxin gene, TRX, in *D. citri*, deletions of 220bp and 505 bp in the 3’ region of the TRX gene. The knockout psyllids had longer development time (~6 d) longer to eclose to adult, lower fecundity (3-5 eggs/ wk/female) versus (2-3 eggs/day/female control); and shorter Adult life spans post eclosion (8-9 d) versus (11-14 d) of mock injected controls.

CRISPR/Cas9

How the genome editor works

1. A cell is transfected with a DNA plasmid that expresses both the Cas9 protein and a sequence of guide RNA (gRNA) which matches that of the gene of interest.
2. Cas9 identifies the corresponding DNA sequence on the host cell’s genome, and cuts both strands of DNA.
3. The cell’s attempt to repair the break effectively silences the targeted gene by joining the cleaved DNA back together, using a process called non-homologous end joining (NHEJ).
4. OR
5. A faulty gene can be ‘corrected’ with a replacement segment of DNA, or a new gene altogether can be introduced. If a modified piece of DNA whose flanking regions match the target sequence is also supplied, then there is a good chance that it will recombine with the host DNA when the cut is made, thus introducing a new or replacement gene. This pathway is known as homology directed repair (HDR).

What next?

- **CRISPR/Cas9**
- **Delivery of lethal dsRNAs in insect diets by branched amphiphilic peptides**
- **Successful plasmid injection with Active ACP Promoter expressing GFP (Green)**
- **Branched amphiphilic peptide capsules**
- **Branched amphiphilic peptides self-assemble into capsules or vesicles.**

**CRISPR/Cas9, designed to Thioredoxin**

- Two guide RNA, gRNAs (Dharmacon).
- Cas9 Protein
- BAPC, ~ 4.2 fmoL/nL (Tomich, KSU).
- Nanojet III, (Dummond)

**CRISPR/Cas9 System for Heritable Gene-Knock OUT:**


In the future, each pest could be modified internally or externally, to no longer a pest. Activity against pest vectors could be designed and delivered through several strategies, including directly infecting the pest by introducing genetically engineered bacteria with antibiotic resistance genes, or via an active ACP promoter expressing GFP (green).

**References:**

1) Hunter, W.B. 2018. MPM, Modified – Pest Management to reduce insect vectors (in prep).

**Branched amphiphilic peptide capsules (BAPC)**

Branched amphiphilic peptides self-assemble into capsules or vesicles. The peptide nano-spheres are comprised of equimolar proportions of two branched peptide sequences bis(FLIVI)-K-KKKK that self-assemble to form a bi-layer delimited Capsules, which are readily absorbed by Cells. BAPC described in detail Tomich et al, 2014;

Methods:

Cas9 protein was designed and then purchased as two gRNAs, (Dharmacon). Microinjections used to coinject Cas9 protein and two sgRNAs, with 0.1 ng BAPC mol/nL (Tomich, KSU).

The TRX treated psyllid adults lifespan averaged 8.5 d post eclosion compared to controls injected with buffer, which averaged 16 d. Average mortality averaged 58%-69% across treatments at 3 d post injections. Interestingly of the few F1 eggs oviposited, post TRX injection near ovaries of an F0 adult female which also were KO positive, when collected as nymphs, one 4th instar out of six analyzed, was TRX KD positive. Surprisingly the development of a second psyllid from eggs, NOT injected, but oviposited from a TRX-injected psyllid ovaries may produce stable KO offspring.

Thus, it appears that 1) purposefully co-inject Cas9 protein and two sgRNAs, with 0.1 ng BAPC, into the region of the adult female psyllid ovaries may produce stable KD offspring. 2) A CRISPR – BAPC combination method would provide a much easier gene-editing strategy for insects where it has not been feasible to conduct egg injections. Previous egg injections trials in psyllids over a one year period, and thousands of eggs, did not produce any positive KO results, and few psyllids. Very expensive.

**CONCLUSIONS**

- **CRISPR/Cas9 works in psyllid nymphs, and adults, microinjections.**
- **BAPC-CRISPR/Cas9 system works for Adult Ovary Injection modifies next generation, resulting in Heritable, gene editing.**
- **TRX knockout, produces psyllids with slower development, reduced adult lifespan, and reduced fecundity.**
- **CRISPR/Cas9 provides a system for population modification management, MPM (2018).** **Future prospects: to produce Non-Vector Psyllids, HLB.**