BAPC-CRISPR/Cas9 System for Heritable Gene-Knock OUT: Asian Citrus Psyllid

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Wayne B. Hunter[1], Maria T. Gonzalez [1], John Tomich [2]

1. USDA--ARS, 2001 S. Rock Rd, Ft. Pierce, FL, USA. **2**. Kansas State University, KSU, Manhattan, KS, USA.

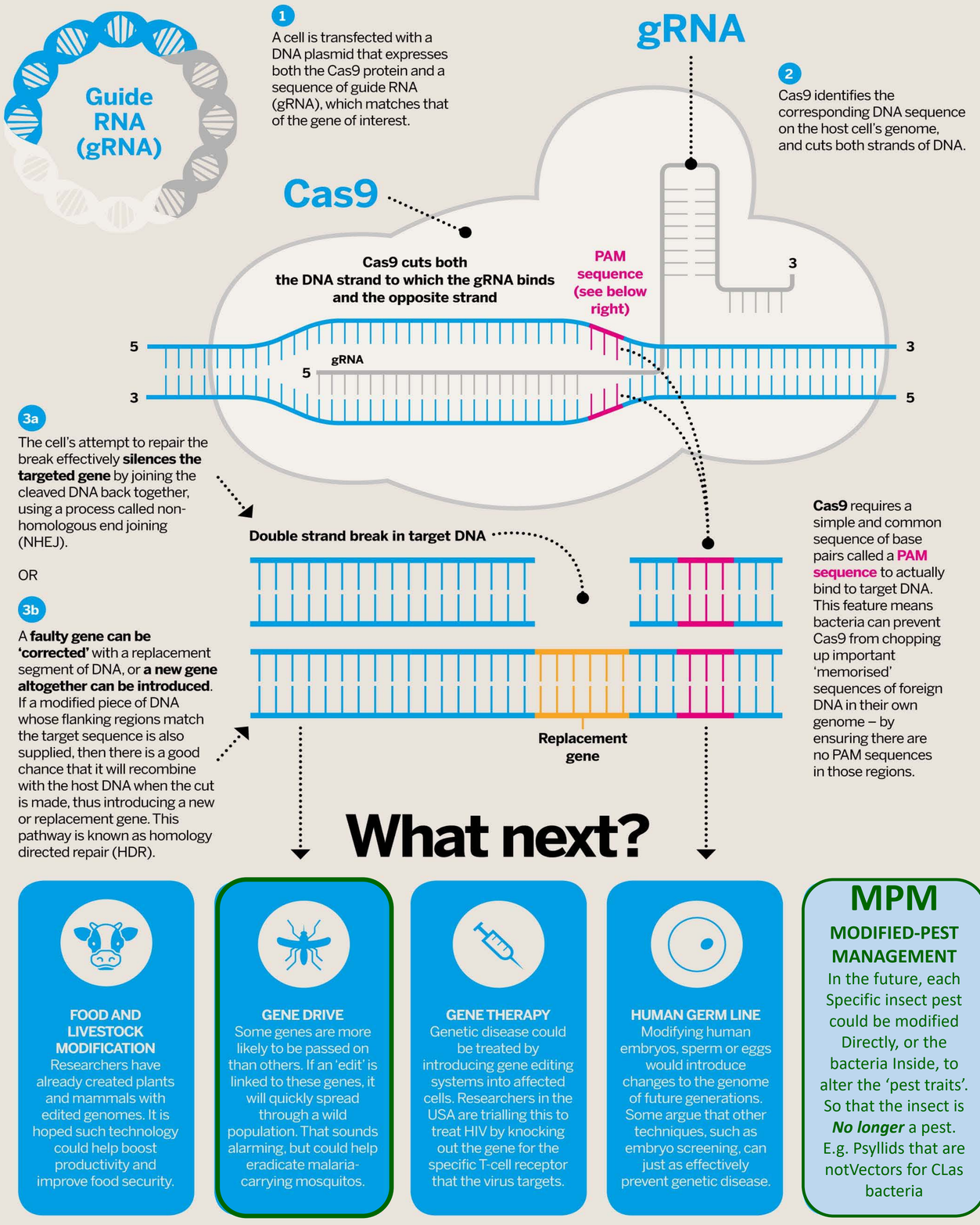
In 2017. the Genome of *Diaphorina citri*, the Asian citrus psyllid, and the Official Gene Set, OGS_v2 were used to design psyllid control strategies. [www.citrusgreening.org]
We used the CRISPR/Cas9, Clustered regularly interspaced palindromic repeats (CRISPR) & CRISPR associated proteins(Cas).
System to disrupt gene expression in psyllids. The targeted gene was the Thioredoxin gene, TRX, in *D. citri,* deletions of 220bp and 505 bp in 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 lifespans post eclosion (8-9 d) versus (11-14 d) of mock injected controls.

Asian citrus psyllid, *Diaphorina citri*, Kuwayama.

Conduct gene knockout, which Slowed development, reduce Fecundity, Shortened Adult Lifespan post eclosion.

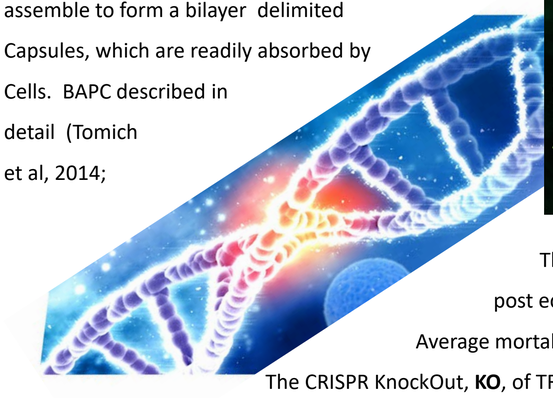
CRISPR/Cas9, designed to Thioredoxin

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



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 bilayer delimited Capsules, which are readily absorbed by Cells. BAPC described in detail (Tomich et al, 2014;



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. The CRISPR KnockOut, **KO**, of TRX resulted in psyllids with: Longer development times, Shorter adult lifespans , Reduced fecundity. There is room for a lot of improvement with this technique. Interestingly of the few F_1 eggs oviposited, post TRX injection near ovaries of an F_0 adult female which also were KO positive, when collected as nymphs, one 4th instar out of six analyzed, was TRX KO positive. Surprisingly the development of a second generation psyllid from eggs, NOT injected, but oviposited from a TRX-injected **F_0 adult female,** had the missing TRX-KO sequence. **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 KO 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.**

Methods:
Cas9 protein was designed and then purchased as two gRNAs, (Dharmacon). Microinjections used Nanojet III (Dummond), purchased tips with filament. Solutions used as recommended on kits. Conc. BAPC, 1.9 nL x 10^{16}, per vol. injected/psyllid. Psyllid gene TRX, data mined from DIACI_1.01 genome: www.citrusgreening.org.

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