Developing CRISPR-Cas9 based genome-editing tools in entomopathogenic nematode *Steinernema carpocapsae*

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Abstract

Steinernema carpocapsae is parasitic to insects and mutualistic to bacterial symbionts *Xenorhabdus nematophila*, therefore is valuable to study naturally-occurring microbial symbiosis. As genetic tools in *Xenorhabdus* bacteria proved to be powerful to reveal the mechanisms underlying bacteria-host interactions, the lack of genetic tools on *Steinernema* nematodes hampered the study in the molecular pathways regulating the host side of the symbiotic conversation. Here we present our attempts to establish a CRISPR-Cas9 based approach to introduce mutations in *S. carpocapsae* genes predicted to encode collagens in the nematode cuticle. We are presenting our progress in genome-editing of *S. carpocapsae* and current efforts aiming to develop CRISPR-Cas9 co-conversion markers in *S. carpocapsae* by introducing mutations that cause distinctive and heritable phenotypes. Our CRISPR-Cas9 based tools will open a new revenue to study molecular pathways in host-microbes signaling particularly in naturally occurring parasitic and mutualistic symbiosis.

Figure 1. The insect-nematode-bacteria tripartite symbiosis includes both parasitic and mutualistic symbiosis.

Steinernema **nematodes are valuable models for studying both mutualistic and pathogenic symbiosis.**

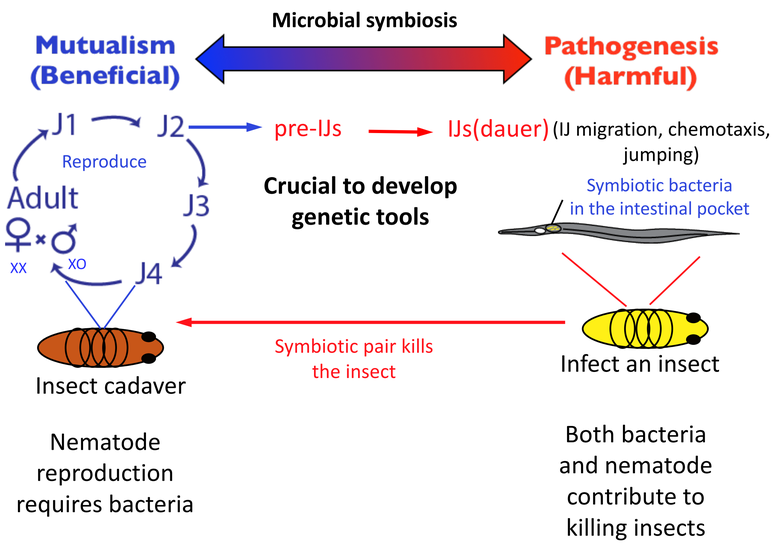

Figure 2. We choose cuticle collagen gene homologues to be targets of CRISPR-Cas9 based genome-editing in *S. carpocapsae*.

(A). We target homologous genes that might encode collagens in *S. carpocapsae* cuticle thus are likely to cause visible phenotypes .

SC-dpy-10 (X-linked) R70C target

```
S. carpocapsae  LNELDFGKLVLPLNRTERQAYN
C. elegans      ALELQYGKMRMTGNRTARGAYG
C. briggsae     ALELQFGKMKLSGNRTARGAYG
                **::**: :  *** * **.
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SC-rol-6.1 (non X-linked) R71C target

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SC  GAGTASNRVRRQQYGGYGATGVQPP
CE  GAGTPSNRQRRQAYGGYGATGSQPS
    **** *** *** ******** **
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(B). We aim to develop a CRISPR-Cas9 method with template-based homologous repair to create missense mutations in SC-*dpy-10* and SC-*rol6.1*.

SC-dyp-10 repair template as an example.

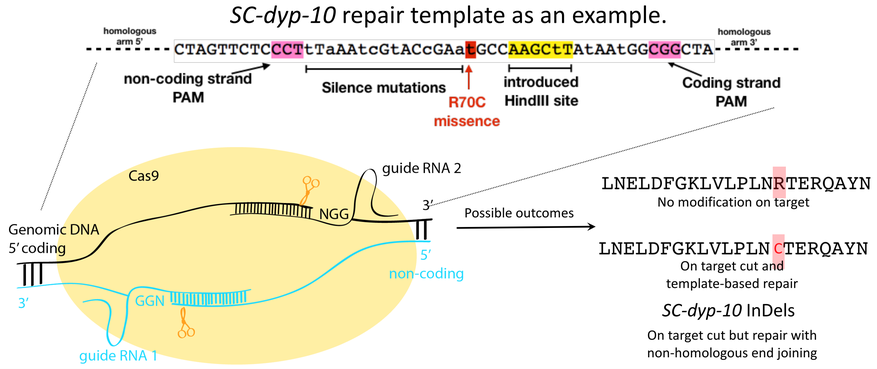
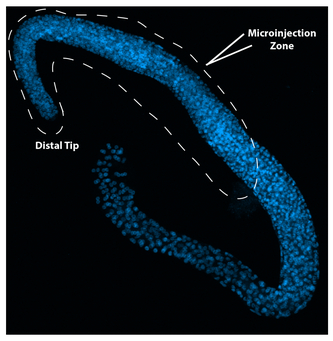


Figure 3. We perform gonadal microinjection to deliver CRISPR-Cas9 reagents.

(A). Gonadal arm of the adult female *S. carpocapsae* female might have a syncytia (z-stack video available upon request).



Left: One of the two gonadal arms dissected from an adult female stained with DAPI.

S. carpocapsae features long gonadal arms with highly packed germ cells.

Zone of microinjection in our preliminary trials is circled with dashed line.

Gonadal flow was observed during injection.

(B). A flow chart for gonadal microinjection in *S. carpocapsae* adult females.

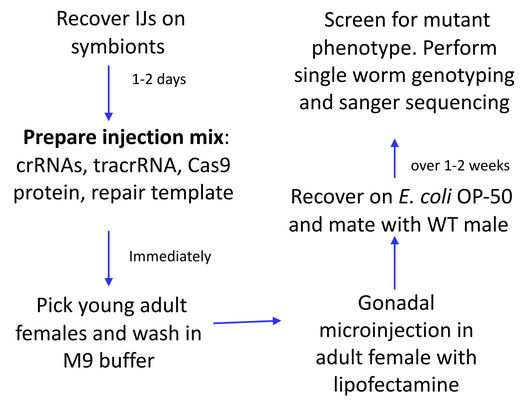
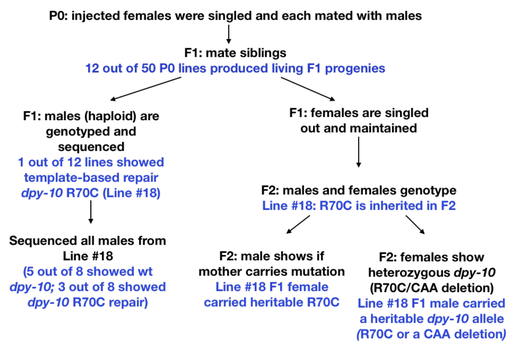


Figure 4. SC-*dpy-10* R70C mutation could be confirmed by sanger sequencing in F1 and F2 progenies.

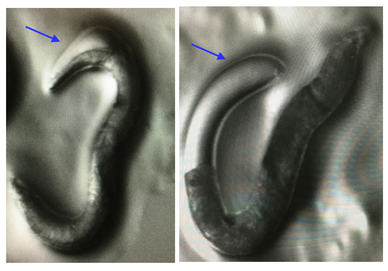


Out or 1 line that showed Template-based repair:

1. *SC-dpy-10* R70C (X-linked) is detected in Line #18: 3/8 F1 males, at least 1/8 F1 female.
2. CAA deletion (non template-based repair) occurred in 1/16 F1 progenies.
3. Both R70C missense and CAA deletion in SC-*dpy-10* are inherited in F2.
4. No obviously visible phenotype is associated with *SC-dpy-10* (R70C) mutants

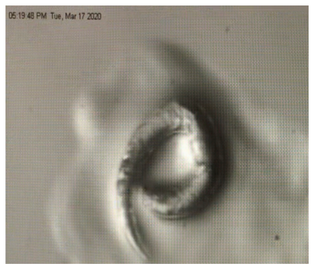
Figure 5. We observed cuticle-associated phenotypes in F1 progenies after our attempts to modify SC-*rol 6.1* by gonadal microinjection (Blisters are indicated by blue arrows).

Blister phenotypes



F1 adult male F1 adult female

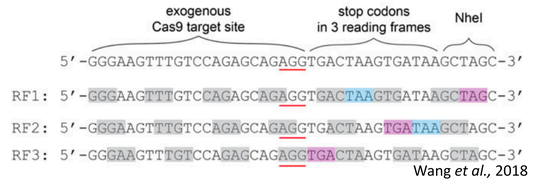
Roller phenotype



F1 adult male
(video available upon request)

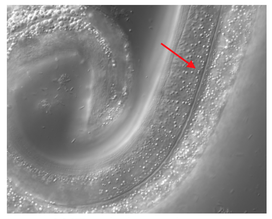
Figure 6. Current effort to better develop cuticle-associated mutants as *S. carpocapsae* Co-CRISPR markers (Alae: red arrow; Annuli: yellow arrow).

(A). Adapting the *C. elegans* "STOP-IN" cassette to create *S. carpocapsae* null mutants.

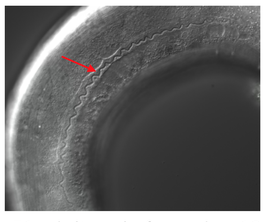


Wang *et al.*, 2018

Preliminary trial of STOP-IN in *S. carpocapsae* showed modified alae structure



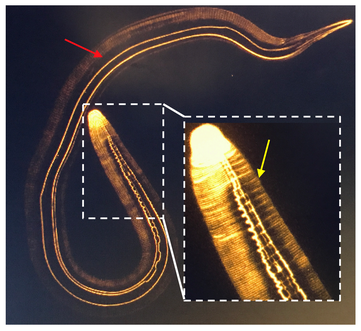
WT adult male: Continuous and straight alae



F1 adult male from *dpy-10* Stop-In microinjection : Alae is zig-zagged

(B). Developing methods to characterize cuticle structures in *S. carpocapsae* wildtype and mutants.

Preliminary trial of DiI staining *S. carpocapsae* cuticle.



A representative IJ stained with DiI showing alae and annuli.

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