Enhancing enhancer studies in non-traditional insect models: A new suite of reporter vectors for diverse insect species

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1. Enhancer-reporter assays limited in insects

Quick look

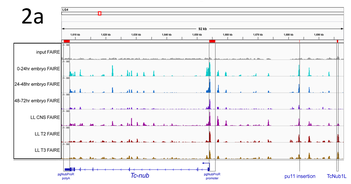
- Enhancers control where and when genes are expressed
- Reporter assays are crucial to studying this control
- Right now this is difficult outside of *Drosophila*
- We need different promoters and new constructs

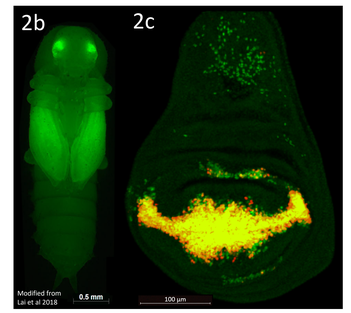
Fig1. Enhancer-reporter assay. (a) A hypothetical gene is expressed under the control of three enhancers in the legs, abdomen and wing. (b) Enhancer-driven transgene expression via the Dm-Hsp70 core promoter reveals the distinct functional roles of each enhancer. Unfortunately, the lack of functionality of the *Dm-Hsp70* in other insects has largely limited the use of enhancer-reporter assays to *Drosophila.*

2. A Drosophila Synthetic Core Promoter (DSCP) reports beetle enhancer activity in flies

Quick look

- We wanted to study beetle (*Tribolium castaneum*) enhancers
- A promoter (DSCP) works with beetle enhancers in flies
- The DSCP could be used to make new reporter constructs which function outside of *Drosophila*





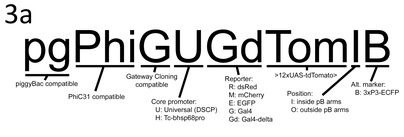
- In the red flour beetle *Tribolium castaneum*, our labs had established both chromatin (FAIRE-seq) and sequence (SCRMshaw) –based enhancer prediction, with no means of testing the function of potential enhancers.
- To address this, we chose *nubbin* (*nub*), a conserved wing gene between flies and beetles, as a case study for the establishment of a Cross-Species Reporter Assay (CSRA).
- Utilizing open chromatin profiling (FAIRE-seq) of the *Tc-nub* locus (2a) near the insertion site of a *nub* enhancer trap line *pu11*(2b), we chose several potential *Tc-nub* enhancers that were open in the thorax at the last larval stage for activity evaluation in the *Drosophila* wing.
- A Drosophila Synthetic Core Promoter (DSCP) was used as an alternative to the *Dm-Hsp70*.
- We found an enhancer (TcNub1L;2a) which drove expression with DSCP in the *Drosophila* wing.
- We needed enhancer-reporter constructs for testing TcNub1L activity in flies and beetles.

3. We created new constructs with the DSCP and additional molecular tools

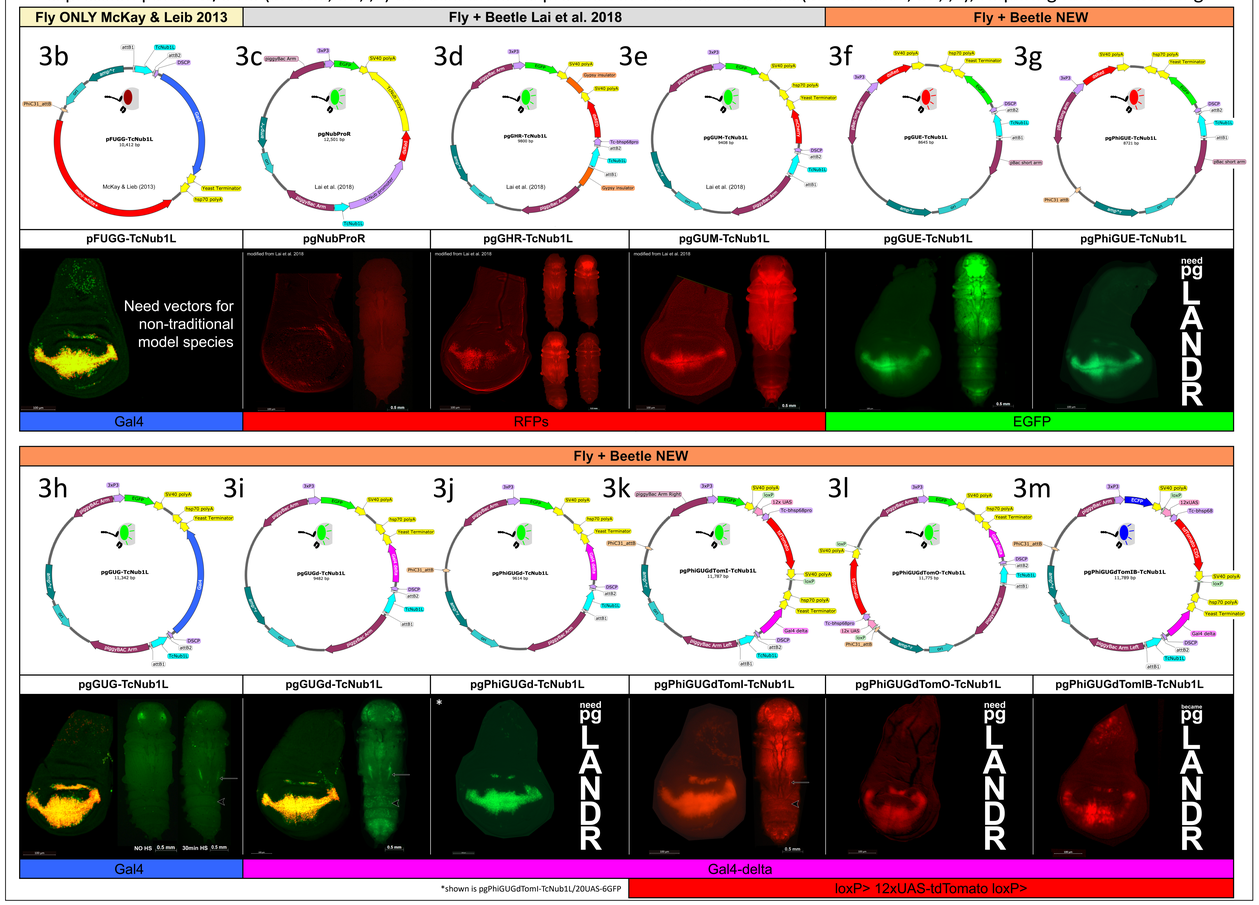
Quick look

- Using the DSCP we made a suite of new vectors with…
- Different color reporters and markers, Gal4-UAS, and both transposon and site-directed integration.

3a



- The construct used in our previous study in *Drosophila,* pFUGG(3b), uses fly-specific tools; we needed new constructs for use in beetles.
- We developed 3 constructs utilizing piggyBac (pB) integration, the universal 3xP3 eye marker, and three different promoters:
  - The endogenous *Tc-nub* promoter in pgNubProR(2a, 3c), the *Tc-bhsp68* in pgGHR(3d), and the DSCP in pgGUM(3e).
- The *Tc-nub* promoter failed to drive expression in flies or beetles(3c), the *Tc-bhsp68* drove decent expression in flies (3d), but suffered from severe position effects in beetles (3d), but **the DSCP drove robust TcNub1L expression in the developing wings of flies and beetles (3e).**
- **The ability of the DSCP to function across >300Mya of evolutionary distance suggests great potential for use in other species.**
- Using the DSCP, we created a new suite of reporter vectors for use in flies, beetles, and other insects, with additional molecular tools (3a,e-m).
- These reporters utilize **new fluorescent reporters and markers (3f-g,k-m), Gal4-UAS (3h-m), and PhiC31 integration (3g,j-m).**
- A deletion variant of the Gal4 protein, **Gal4-delta (Gal4d/Gd) is required for use in *Tribolium* (3h,i,k).** Gal4d can drive part of the TcNub1L expression patter w/o HS (arrows, 3h,i,k) but also drives expression in a subset of fat cells(arrowheads, 3h,i,k), requiring further tweaking.



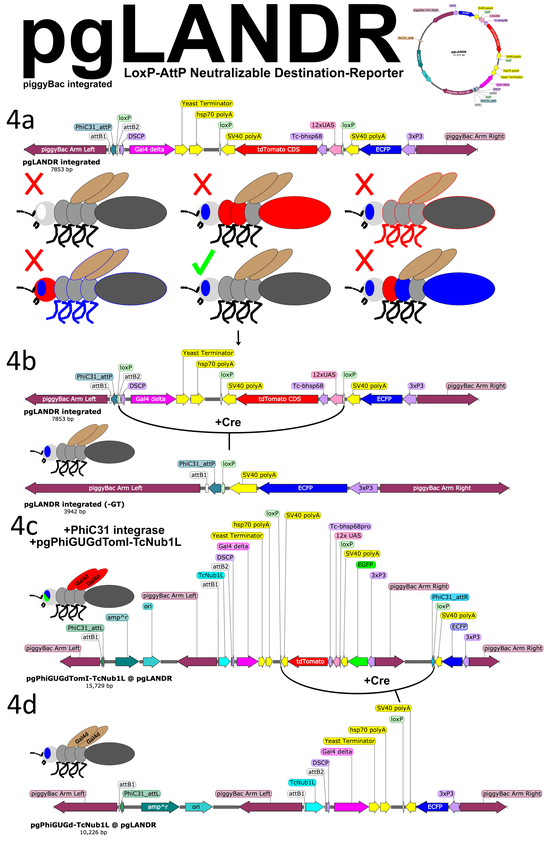
*shown is pgPhiGUGdTomI-TcNub1L/20UAS-6GFP

4. pgLANDR – a versatile landing site for PhiC31 integration

Quick look

- Random insertions can break genes, or cause undesirable effects on a reporter
- These pitfalls can be avoided by using site-directed PhiC31 integration
- pgLANDR provides a versatile PhiC31 landing site for use in a variety of insect species

pgLANDR
piggyBac integrated LoxP-AttP Neutralizable Destination-Reporter



- In order to utilize the function of our new PhiC31-compatible reporter constructs (3g,j-m), we need to integrate landing sites into suitable locations within various insect genomes.
- For this purpose we developed pgLANDR (pgBac LoxP-AttP Neutralizeable Destination-Reporter).
- pgLANDR utilize random pBac transposon integration into various locations of the genome, and reports position effects with removable, enhancer-less DSCP-Gal4d and UAS-tdTomato (4a).
- Once integration events free of position effects are detected, those transgenic lines can be crossed to a Cre source to remove the reporter(4b).
- **This leaves you with a 3xP3-ECFP marked landing site (4b).**
- PhiC31 integration of one of our new vectors, pgPhiGUGdTomI-TcNub1L (3k), yields a transgenic marked with EGFP + ECFP fluorescence in the eye, expressing Gal4d and tdTomato in the wings (4c).
- Recombination between loxP sites inside and outside the insertion yield an **ECFP-marked Gal4 driver** suitable for a variety of downstream analyses (4d).

5. Summary

- Enhancer reporter assays in insects have been limited mainly to *Drosophila.*
- We have created a variety of new reporter constructs utilizing **new fluorescent proteins, Gal4-UAS, and PhiC31 integration for use in non-*Drosophila* insects.**
- **In the future** we plan to establish **PhiC31 integration in beetles using pgLANDR**, and further improve our Gal4-UAS system.
- **These enhancer-reporter constructs provide tools with applications ranging from pest management to molecular biology** research, and will greatly **enhance the study of enhancers in non-traditional insect models.**

Acknowledgements

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