A panel of fluorophore-tagged daf-16 alleles
Ulkar Aghayeva¹, Abhishek Bhattacharya¹ and Oliver Hobert¹§
¹Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University, New York

**Description**

*daf-16* encodes a broadly expressed transcription factor that functions in a wide variety of developmental and physiological processes (Lin *et al*., 1997; Ogg *et al*., 1997; Tissenbaum, 2018), including in the nervous system (Kim and Webb, 2017). DAF-16 protein shows highly dynamic cytoplasmic to nuclear translocation, which, in the past, has been visualized using multicopy constructs, which can produce potential overexpression artifacts (such as those described in (Henderson and Johnson, 2001)). To avoid such overexpression effects, the generation of fluorescently tagged *daf-16* allele would be useful. Similarly, the generation of conditional alleles of *daf-16* would be useful to resolve a number of open questions in regard to the focus of *daf-16* action. To address both issues, we recently generated an mNeonGreen tagged *daf-16* allele that also contained an auxin-inducible degron (Bhattacharya *et al*., 2019; Zhang *et al*., 2015). This allele, *daf-16*(ot853[maf-16::mNG::AID]), enabled us to provide a proof of concept for neuron-type specific *daf-16* depletion (Bhattacharya *et al*., 2019). One issue with this allele has been that due to the emission spectrum of its fluorescent tag (mNeonGreen), it cannot be used in conjunction with *gfp*-based phenotypic read-outs.

We have now genome-engineered three additional fluorophore tagged *daf-16* alleles, some with or without additional tags (FLAG tag or AID degron) (Fig.1). Like the previously published ot853 allele, we CRISPR/Cas9-engineered the ot821 allele (mKate2 tag) with the SEC method (Dickinson *et al*., 2015) and the ot971 (gfp tag) and ot975 (mNeptune2.5::AID) alleles with the so-called Mello method (Dokshin *et al*., 2018), using pDD282 (Dickinson *et al*., 2015) and pEY56 (kindly provided by Eviatar Yemini, based on Addgene plasmid #51310 pcDNA3-mNeptune2.5) as templates. All four alleles show similar expression and subcellular localization patterns. In Figure 1 we show the nuclear localized DAF-16 protein in dauers, generated by a standard crowding/starvation protocol (as described in Bhattacharya *et al*., 2019).

We note that this tagging approach also provides a rare opportunity to assess the intensities of fluorophores side-by-side: We find that GFP expression is brighter than that of mNeonGreen, while mKate2 is clearly weaker and mNeptune2.5 even
weaker.

All strains are available at the CGC.

**Reagents**

OH16024: daf-16(ot971[daf-16::gfp])

OH13908: daf-16(ot821[daf-16::mKate2::3xFlag])

OH14125: daf-16(ot853[daf-16::mNG::AID])

OH16029: daf-16(ot975[daf-16::mNeptune2.5::AID])

**Acknowledgments:** We thank Chi Chen for expert assistance in nematode injections and Ev Yemini for providing the mNeptune2.5 template plasmid.

**References**


**Funding:** This work was funded by a grant from the NINDS (1 R21 NS115442-01)

**Author Contributions:** Ulkar Aghayeva: Investigation, Writing - review and editing. Abhishek Bhattacharya: Investigation. Oliver Hobert: Conceptualization, Funding acquisition, Project administration, Supervision, Writing - original draft, Writing - review and editing.

**Reviewed By:** Patrick Hu

**History:** Received January 1, 2020 Accepted January 6, 2020 Published January 7, 2020

**Copyright:** © 2020 by the authors. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Citation:** Aghayeva, U; Bhattacharya, A; Hobert, O (2020). A panel of fluorophore-tagged daf-16 alleles. microPublication Biology. https://doi.org/10.17912/micropub.biology.000210