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LAIDLAW SCHOLARS

NEUROGENETICS OF VOCAL COMMUNICATION

Knocking out the Language-Associated Gene

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- FOXP2 is crucial for neurodevelopment in humans. Altered FOXP2 expression has been found to affect language and speech development.
- Bats such as *Phyllostomus discolor* are emerging as models for the study of the neurogenetics of vocal communication.
- We seek to develop a *FoxP2* knockout bat to shed light on biological and evolutionary mechanisms of FoxP2 in vocal production learning.
- This study aimed to verify the efficacy of chosen guide RNA to lead Cas9 to cleave in the correct position in the *FoxP2*/*FOXP2* sequence in bat and human DNA .
- Chosen guides successfully drove Cas9 cleavage in the correct positions in both species' DNA. The success of this protocol provides support for further study in vitro, hopefully leading to a CRISPR-Cas9-directed knockout of *FoxP2* in vivo.

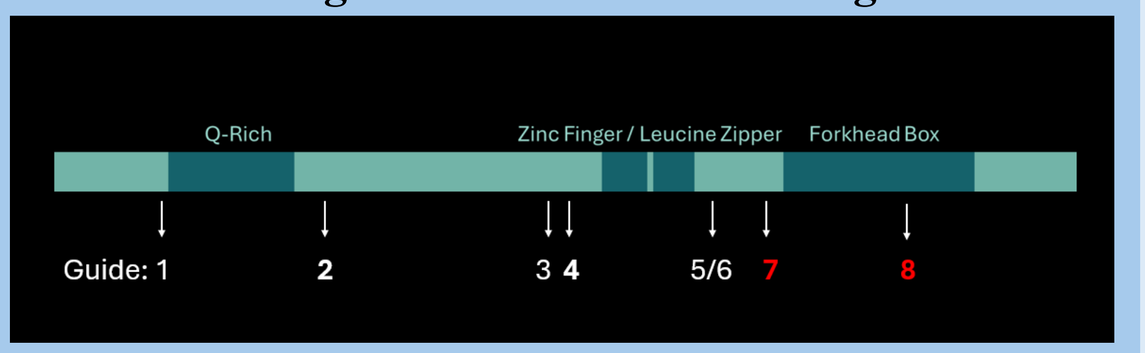
Bats have mammalian brain structures, allowing for evolutionary comparison of FoxP2 mechanisms with humans.

Bats are vocal production learners, allowing for insight into FoxP2's role in language and speech development.

FoxP2 is a transcription factor. A mutation in the forkhead box, which codes for the DNA binding domain, would lead to knockout.

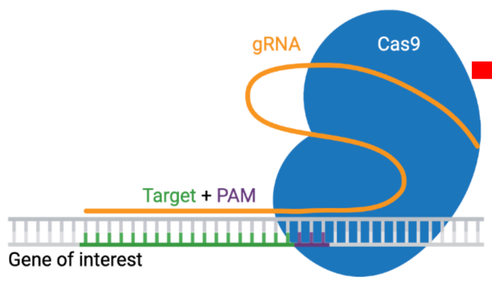
FoxP2 knockout of a mammalian vocal production learner would shed light on mechanisms of FoxP2 in vocal production learning, with potential applications in human education and healthcare.

Choosing Guides for Cas9 Cleavage



The forkhead box codes for the DNA binding domain of FoxP2. Guides 7 and 8 should guide Cas9 to cut in the forkhead box region. FoxP2, as a translation factor, depends on binding to DNA to perform its function. Successful cleavage and sequence alteration in this region should result in loss of function.

Methods & Conditions



Cleavage (DNA digest) plan: DNA/guide combinations

Bat DNA

Guide 7 | Guide 8

Human DNA

Guide 7 | Guide 8

Guide 4 – positive control

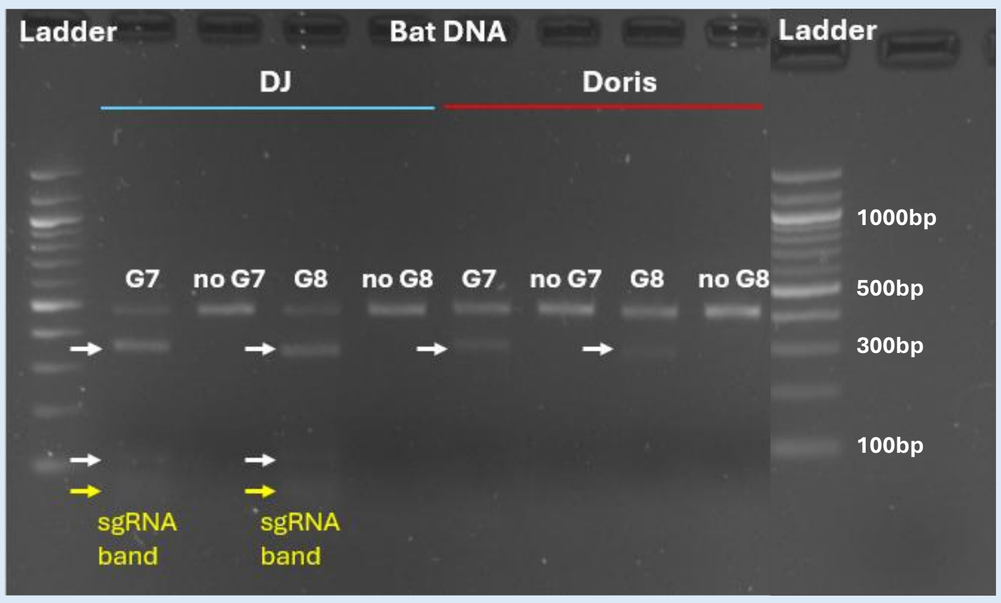
Fragmentation

DNA digest: cleavage by Cas9 of DNA sample occurs, resulting in two fragments. Predicted sizes of fragments are calculated ahead of digest, to check that guide RNA is guiding Cas9 to cut at the correct position.

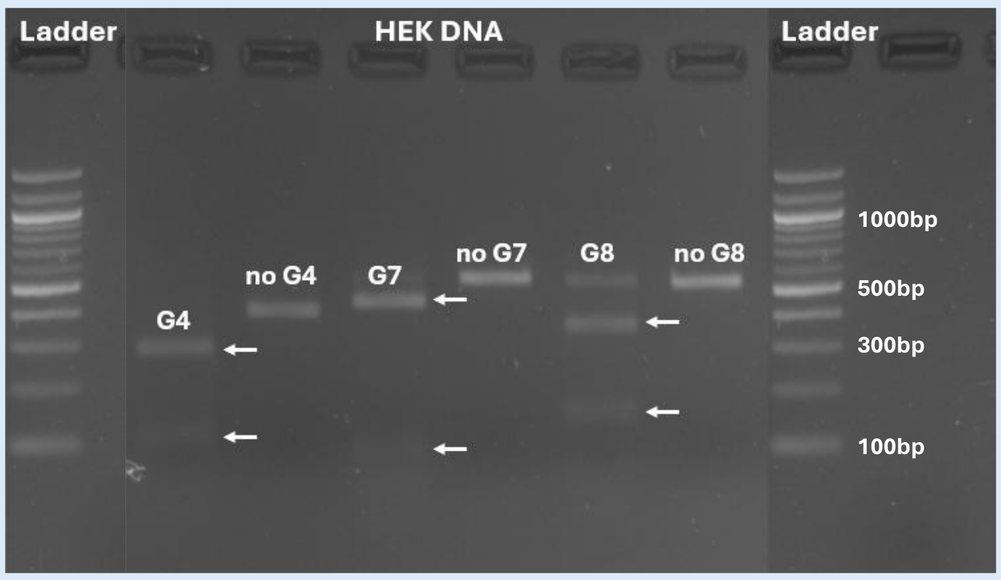
Digested DNA samples are run on an agarose gel. Differently sized fragments will run through the gel at different speeds, separating into distinct bands.

Results

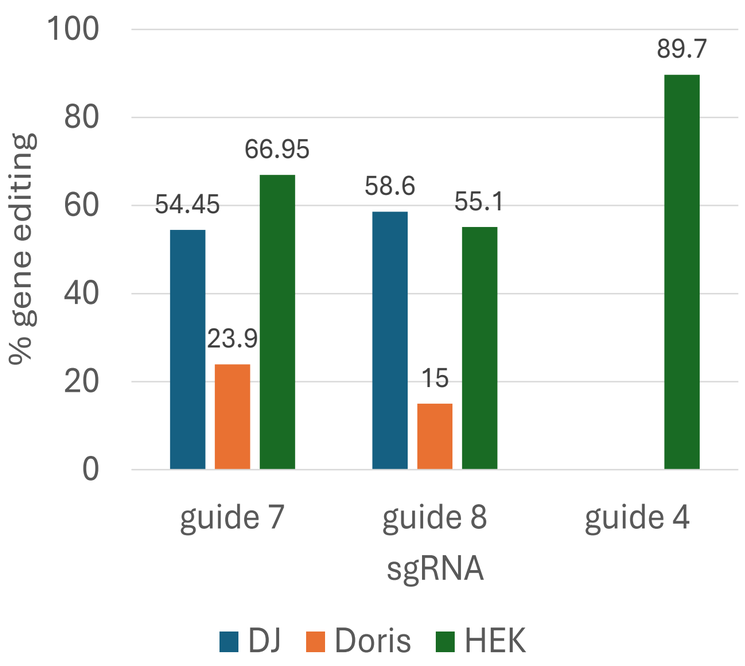
Gel analysis of DNA digest



DNA digest demonstrated digestion of bat wing punch DNA in both guide 7 and guide 8 conditions, and not the guideless (Cas9-only) control.



Gel analysis of DNA digest found efficient guide 7 and guide 8 cleavage of HEK DNA. Positive control guide 4 cleavage in HEK DNA was successfully replicated.



Discussion and Implications

Having verified chosen guide's ability to guide Cas9 to cleave in the correct spot, a T7E1 analysis will be performed to verify that Cas9 cleavage is driving alteration of the sequence. Once this is verified, testing in bat cells and individuals may proceed.

FOXP2 is a significant transcription factor involved in reaction cascades throughout the body. Its in vivo study is crucial in building understanding about its mechanisms. Beyond vocal learning, FOXP2 has roles in prenatal development, cell proliferation, morphogenesis and immunity. Continued study of the genetic and molecular mechanisms of FOXP2 may enable the development of better treatment methods for conditions where its activity is implicated.

References

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