**BK Channel Clustering in Slo-1 mutants**

By Dylan Lee

Alcohol abuse is a big problem in society. Alcoholism is thought to have a strong genetic basis at the same times we do not understand the mechanisms by which alcohol causes intoxication. It has been useful to use C Elegans as model organism to study ethanol’s effect on a molecular level. There have been several proposed targets by which ethanol acts on the brain. One of the targets is a voltage gated potassium pump called the BK(~~Burger King~~ Big Postassium) channel.

It is known through( Davies et al) that the bk channel is an ethanol target. It has been observed that mutants of slo-1 have an ethanol resistant phenotype. Through *(*Davis 2012) we know that there exists a single cytostolic residue that is responsible for increased ethanol tolerance in all ethanol tolerant slo-1 mutants. It is unknown the mechanism by which ethanol effects the bk channel and it is not understood how the slo-1 mutants increased tolerance works.

The BK Channel is commonly found on neuron and muscle cells. It has been observed through the use of gfp that the bk channel is usually found in a clustered phenotype. Recently it has been shown that a dystrophin mutation acted on the bk channel. The dystrophin mutation caused the worms to express the bk channels on neurons and muscle cells in a diffuse phenotype. The worms with this mutation had an ethanol tolerance comparable to that of a slo-1 mutant. (Oh KH, Kim H)

**It would be useful to see whether or not slo-1(T381) bk channels form a clustered phenotype on neurons and muscle cells**. In order to see whether or not this is the case I propose mutating worms with gfp attached to their slo-1 channels at the 381st threonine and then using microscopy to observe a clustered or not phenotype.

**Experimental Methods.**

To confirm whether or not the t381 mutation forms clusters. Worms(slo-1(cim105[slo-1::GFP])(Oh KH, Kim H) will be mutagenized using crispr and homology directed repair then microscopy will be used to confirm a clustered phenotype or not.

**Crispr Methods**

Crispr works by taking a generated marker(sgRNA) and using the marker as a guide to make a double stranded break using the cas9 enzyme. The composed of sgRNA attached to the cas 9 protein. Sg RNA is made up of two components crRNA and tracRNA. crRNA is an rna strand that acts as a target for where to make our double stranded cut. tracRNA binds the crna to the cas 9 proteins that makes the double stranded cut. To select the location of the crRNA sequence we need to find a pam or a short sequence NGG or NCC that will be directly preceded in the 5’ direction by the template that will be used to make our crRNA. One codon upstream of our pam region will be where the double stranded break will occur. Once we have assembled our sgrna sequence we need to insert said sequence into a plasmid with the sequence for cas 9 already in it. Such a plasmid exists P eft-3::Cas9::tbb-2–3′ UTR and PU6::sgRNA(our own custom printed crRNA + tracRNA) and can be ordered off of add gene.



figure 1

The design of the plasmid used to insert cas 9 into c elegans genome.

(Dickinson et al 2014)

Yellow = sequence to change

Electric blue = t381 mutation T→ I

bright green = mutation sites for pcr screen

red= crrna =CCACAGUGUCGGGGACACUUGGG

red = crna template = cccaagtgtccccgacactgtgg

purple = homology arms for repair template

pink = pam sequence

babyblue = end of homology arms.

Black = cut site

ActtagcgaatgtaactgaaaattacttttctgctttgaaaaattttgctaatttaacacccattttgcccgaaatcacaatttttcaaaattcaacactatttaggcccgaaatcggcctgaaaacggcctgaactgacttttcagaacacagtcaatatgggtgtcattgaaacggaaatttcacggagatttcaattttgctaggtaaaactgtcctgtgtacccgatgtacccaagtgtccccgacactgtggggacaaactcaaaatccttcaatttacatcgaattttttgattttttcactaattctccaattttcagGCCATGTTTGCAAGTTACGTACCAGAAATTGCCGATTTGATTGGAAACCGGCAAAAATACGGTGGGGAGTACAAAGGAGAGCACGGGAAGAAGCACATAGTGGTCTGTGGCCATATCACCTACGATTCGGTGTCCCATTTTCTTCAAGATTTCCTACACGAGGACCGTGATGACGTGGATGTCGAAGTGGTGTTTTTGCATCGTGTCGTGCCGGATTTGGAGCTGGAAGGCTTGTTTAAGCGGCATTTCACAAAAGTCGAATTTTTCACGGGGACTGTCATGGATTCTCTGGATCTTAGCAGAGTCAAGgtacactttatttttagcttaaatttttctgaaagaacgcaaaaattttaaaaagttgcatcaaaaatcaagtagaaaccttcaaaactgactgagttatgcgacttttaaaaaagttacccctgtggggacaattttgggacatctggcctcctaggcaatttttaagcaaaatatacaaattttttacaaaatttccgtaaatttgacacccat

figure 2

**HDR(Homology Directed Repair)**

To create the edits to the bk channel. We are creating a cut 5’ upstream of the exon where the t381 mutation is. Then we will be making an edit using HDR( homology directed repair). We will be using a repair template that has five edits. Only one edit will change a residue the rest will exist to screen for the mutation using pcr. For HDR to occur a cut needs to be made near the site that is going to be repaired and a repair template needs to be created for homologous recombination to occur.

The repair template that will be used to create the T381 mutation can be described as follows.

A stretch of dna will be printed the ends highlighted in green fig 3 will be exposed to restriction enzymes and ligated into a plasmid p:unc 119.

NNNNGGGGAGTACAAAGGAGAGCACGGGAAGAAGCACATAGTGGTCTGCGGGCACATAATCTACGATTCGGTGTCCCATTTTCTTCAAGATTTCCTACACGANNNN

Green=restriction enzyme palindromic regions.

Purple =Homology arm

Yellow and blue=Region to be change

Blue = end of the homology arms.

Fig 3

**MICRO INJECTION**

The crispr plasmid along with the repair template will be injected using microinjection into the gonad of the worm slo-1(cim105[slo-1::GFP]).

**PCR Screen**

Once the worm has been injected its progeny will be grown up and seperated. Once the progeny f1 of the original worm has matured and laid eggs of its own. The f1 generation’s worms will be lysed and the dna will be extracted.

To see if transgenesis was successful a screen of the animals using pcr will be carried out. To do this primers were created. A start primer was made based on the region that was being modified an end primer was made for a random region about 1000 bp down stream of start.

start primer : TGTGGCCATATCACC

end primer :taagaagaaaatcttaaaa

These primers create one product 1109 base pairs long verified with biobike in a wild type:gfp worm

If this product cannot be created from the progeny of the worm then we can say that transgenesis has taken place because our modification will have broken the region the start primer would have bound to. If this product can be created than we can be confident that transgenesis has not taken place.

**Microscopy**

Once the animals are screened using pcr the animals that carry the T381 mutation will be grown into adults and microscopy will take place.

For Microscopy an animal will be moved under the microscope illuminated with blue light and then the the dorsal cord(nerve) the sacrolemma(muscle cell) and the egg laying nerve will be observed under magnification. The puncta that appear or do not appear should then be counted on each of the different areas. Compare the number of puncta to the numbers in (Oh KH, Kim H).

**Strains used**

A strain of worm with gfp attached to the end of its slo-1 gene was used.

slo-1(cim105[slo-1::GFP])(Oh KH, Kim H)

Davies AG, Pierce-Shimomura JT, Kim H, et al. A Central Role of the BK Potassium Channel in Behavioral Responses to Ethanol in C. elegans. *Cell*. 2003;115(6):655-666. doi:10.1016/s0092-8674(03)00979-6.

Davis SJ, Scott LL, Hu K, Pierce-Shimomura JT. Conserved single residue in the BK potassium channel required for activation by alcohol and intoxication in C. elegans. *J Neurosci*. 2014;34(29):9562–9573. doi:10.1523/JNEUROSCI.0838-14.2014

Dickinson DJ, Ward JD, Reiner DJ, Goldstein B. Engineering the Caenorhabditis elegans genome using Cas9-triggered homologous recombination. *Nat Methods*. 2013;10(10):1028–1034. doi:10.1038/nmeth.2641

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