Drosophila Gene Expression with the WDS Gene

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Genetics 324

3 March 2018

**Introduction**

CRISPR-Cas9 is a genome editing system that occurs naturally in bacteria (What are genome 2018). Crispr stands for clustered regularly interspaced short palindromic repeats and Cas9 is an enzyme (Gao 2018). Scientists use it to target certain sequences and edit DNA at precise locations (Zhang 2017). This can become a huge advantage to the medical field in curing and cutting out mutations and diseases. It can also assist scientists in creating desired genes and outcomes in other cells. For now, only somatic cells are legal to genetically alter because of the large health and ethical concerns with germline cells (Zhang 2017).

In this research, CRISPR-Cas9 will be paired with the gene WDS in Drosophila; which stands for will die slowly (Hollmann 2002). CRISPR will target areas of the Drosophila’s DNA and edit the strands to silence the WDS gene. This results in 100% lethality before the end of the pupil stage (Zhu J-yi 2017). Since we will not have time to test this we decided to test gene expression. We hypothesized that after editing the gene with WDS the expression will be lowered dramatically which poses the question: how does WDS paired with CRISPR cause changes in gene expression in cells?

**Materials and Methods**

*Cloning guide RNA sequences into pAc-sgRNA-Cas9*

In a .5mL microcentrifuge tube the following components were added: sterile water, pAc-sgSNA-Cas9, 10x NEB buffer 3.1, and BspQI enzyme. The reactions were mixed by gently pipetting up and down, and later incubated at 50C for 2 hours and -20C after 4 hours. In a microcentrifuge tube the following components were added: Oligo 1 (100uM), Oligo 2 (100uM), 10X T4 Ligation Buffer (NEB), T4 Polynucleotide kinase, and Nuclease-free water. The tube was annealed in a thermocycler for 30mins at 37C, 5min at 95C, and then ramped down to 25C at 5C/min. Quick calf intestinal alkaline phosphate (NEB) is added to the .5mL tube of BspQI digest. It is then incubated at 37C for 10 min. After incubation, Binding buffer is added and mixed by pipetting. The mixture is then added to a spin filter column and spun for 1 minute at 13,000 rpm. The flow through is discarded and DNA Wash Buffer is added to the spin filter and spun again for 1 min. This step is repeated, and the spin filter is transferred to a new 1.5 mL tube. Sterile water is added to the center of the spin filter, and the product is placed on a nanodrop to measure the DNA concentration and 260/280 values. The previously annealed oligos is diluted in 1:10 sterile water. In a new microcentrifuge the following components were added: the digested vector, the annealed oligos, 10X T4 Ligation Buffer (NEB), T4 DNA Ligase (NEB), and nuclease-free water. The tube is then incubated at room temperature for 10 minutes. The ligation reaction from before is added to a tube that contains the NEB 5-alpha Competent *E. coli* cells. The tube is mixed by gently flicking the tube 4-5 times, and then placed on ice for 30 minutes. The tube is then placed in a water bath at 42C for exactly 30 seconds to heat shock the mixture. Immediate place on ice for 5 minutes after. Pipette the SOC into the mixture. Incubate the tube at 37C for 45 minutes and then shake vigorously at 250 rmp. Warm selection plates made of LB containing ampicillin to 37C. Mix the cells by flicking the tube and inverting it, spread 50-100microliters of each dilution onto a selection plate, and incubate overnight at 37°C.

*Colony PCR*

Six colonies were chosen from the agar plates and placed into six separate PCR tubes with the tip of a pipette. Next in a 1.5mL tube, a master mix was made by mixing nuclease-free water, pAc Primer mix, sgRNA for oligo 1, and Q5 Hot Start High-Fidelity 2x Master Mix. The mix was vortexed for a few seconds to collect the liquid. Master mix was added to each PCR tube. The original pAc-sgRNA-Cas9 was added to the N tube and all tubes were collected and placed in a PCR machine for thermocycling. Initial denaturation was for 30 seconds at 98C; 30 cycles were done for 5 seconds at 98C, 15 seconds at 52C, and 20 seconds at 72C. Final extension was for 2 minutes at 72C and then the tubes were held at 4C.

*Gel electrophoresis*

The electrophoresis chamber was filled with the gel and the 1X TAE buffer was poured over. Quick-Load Purple 100kb DNA ladder was loaded into the first well. 6X loading dye was added to each PCR tube and 10 microliters of each sample was loaded into separate wells in the gel chamber. The lid was placed, and the electricity was turned on. Once the gel was done it was looked at under a UV light.

*Plasmid Purification*

A frozen pellet of DH5alpha *E. coli* was obtained from the liquid cultures prepared during colony PCR. Plasmids were purified using the GeneJET Plasmid Miniprep Kit (ThermoFisher Scientific). A nanodrop is then used to determine the concentration and purity of the DNA. Once the calculations are done, purified DNA, water, and U6For sequencing primer was added to the sequencing tube.

**Results**

The purpose of this experiment was to test the effects of Cas9 genome editing paired with the gene Wds. The gene codes for many molecular functions, biological processes, and cellular components. This helped us with determining what functions could be mutated later on (table 1).

Further analysis of the DNA was taken with a nanodrop machine. The PCR purification for both colonies 260/280 values were normal levels, but the concentration for colony one was extremely low compared to colony two. For the first colony the 260/280 value was 1.89 and the concentration was 43.7. For the second colony the 260/280 value was 1.89 and the concentration was 475.2. These results were the only results that came back positive, and/or what we expected.

Figure 1 shows the chromatograms of both colonies after sequencing. The original sequence we used for the colonies was CT CGCTGCAGCC GAACT, and after sequencing it was shown that we had no clear results. The data came back inconclusive, and we could not determine whether or not the sequence was there.

Figure 2 also supports that the sequence was not there. It is shown on the gel electrophoresis capture that the two colonies did not have strong base pair results. In lane 1 was the ladder showing where the colonies should have travelled to, and lane 4 is a negative control group showing what a normal electrophoresis with no DNA is supposed to look like. Nether lane 2 or 3 showed very much DNA and resulted in looking more like the negative control.

**Discussion**

CRISPR-Cas9 is a genome editing system that occurs naturally in bacteria (What are genome 2018). Crispr stands for clustered regularly interspaced short palindromic repeats and Cas9 is an enzyme (Gao 2018). In this research, we used CRISPR-Cas9 with the gene WDS in Drosophila cells. WDS stands for will die slowly, and CRISPR will target areas of the Drosophila’s DNA and edit the strands to silence the WDS gene (Hollmann 2002). Our research question was how does WDS paired with CRISPR cause changes in gene expression in cells? It was hypothesized that after editing the gene with WDS the expression would be lowered dramatically. After inserting the selected gene sequence, PCR purification, and other steps listed above, it was concluded that the sequence was not present in the vector.

Many factors could have caused this result. The most likely cause of this result was that the plasmid didn’t even get cut open. It’s not possible to see whether it cuts the plasmid it is just assumed it worked, and there is a strong possibility that it never even happened.

Thus, the future direction with CRISPR is endless. Other lab studies could conduct this experiment and be careful of no cross-contamination and try to make sure they cut open the plasmid. Once they are sure that both steps happened they could grow the cells and chart and analyze WDS’s affect on the cells. Also, they could pair CRISPR with other genes and see the direct affects it has on other insects or even animals. The possibilities of CRISPR are boundless and I think it is important that everyone utilizes this system.

**Figures**

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| --- | --- | --- |
| **Molecular Functions** | **Biological Processes** | **Cellular components** |
| Histone acetyltransferase activity | Chromatin remodeling | Ada2/Gcn5/Ada3 transcription activator complex |
| Protein binding | Eye development | MLL3/4 complex |
|  | Haltere development | NSL complex |
|  | Histone acetylation | Nucleus  |
|  | Histone H3-K4 methylation | Set1C/COMPASS complex |
|  | Positive regulation of proteasomal ubiquitin-dependent protein catabolic process  |  |

**Table 1: Functions, Processes, and components of the gene WDS.**





**Figure 1. Chromatogram.** The top chromatogram was for the first colony that was sequenced, and the bottom was for the second colony. Both show that the results were inconclusive with the sporadic and overlapped sequence.

 

**Figure 2. Gel electrophoresis.** In lane one was the ladder, lane two and three were colonies one and two, and the last lane was a negative control group. The ladder showed the base pairs that result with strong and good DNA, and the negative control shows what no DNA looks like. Ours resulted in little to no DNA concluding that the sequence wasn’t there.

**Citations:**

Gao R, Feyissa BA, Croft M, Hannoufa A. 2018 Feb. Gene editing by CRISPR/Cas9 in the obligatory outcrossing Medicago sativa. Planta. [accessed 2018 Mar 2]. <https://www.ncbi.nlm.nih.gov/pubmed/29492697>

Hollmann M, Simmerl E, Schäfer U, Schäfer MA. 2002 Dec. The essential Drosophila melanogaster gene wds (will die slowly) codes for a WD-repeat protein with seven repeats. Molecular genetics and genomics : MGG. [accessed 2018 Mar 2]. <https://www.ncbi.nlm.nih.gov/pubmed/12471440>

What are genome editing and CRISPR-Cas9? - Genetics Home Reference. 2018 Feb. U.S. National Library of Medicine. [accessed 2018 Mar 2]. <https://ghr.nlm.nih.gov/primer/genomicresearch/genomeediting>

Zhang F. 2017 Aug 21. Questions and Answers about CRISPR. Broad Institute. [accessed 2018 Mar 2]. [https://www.broadinstitute.org/what-broad/areas-focus/project spotlight/questions-and-answers-about-crispr](https://www.broadinstitute.org/what-broad/areas-focus/project%09spotlight/questions-and-answers-about-crispr)

Zhu J-yi, Fu Y, Nettleton M, Richman A, Han Z. 2017. eLife. [accessed 2018 Mar 2]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5300701/