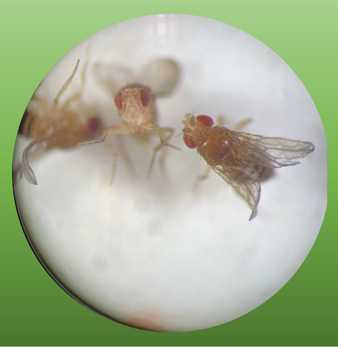
Fingerprinting in recombinant flies



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Introduction:

Drosophila melanogaster share almost 75% of the disease genome with humans and are great model systems used to understand cancer. The oncogene Stit in *Drosophila melanogaster* is equivalent to the Ret (Rearranged during transfection) gene in humans. Our principal goal is to understand how the oncogene Stit effects the growth and migration of cancer in Drosophila. We investigate this by having two different insertion sites of the oncogene Stit; 2A4 (M3) and 6C12 (pCary), these are separate fly stocks at the fly lab. We crossed the two stocks to each other to create recombinant flies which will have a stronger expression of the oncogene Stit, containing both insertion sites (2A4 + 6C12). We do not know if this recombination has occurred. We wish to perform a PCR to confirm the potential recombinants, and then look for a stronger expression of Stit.

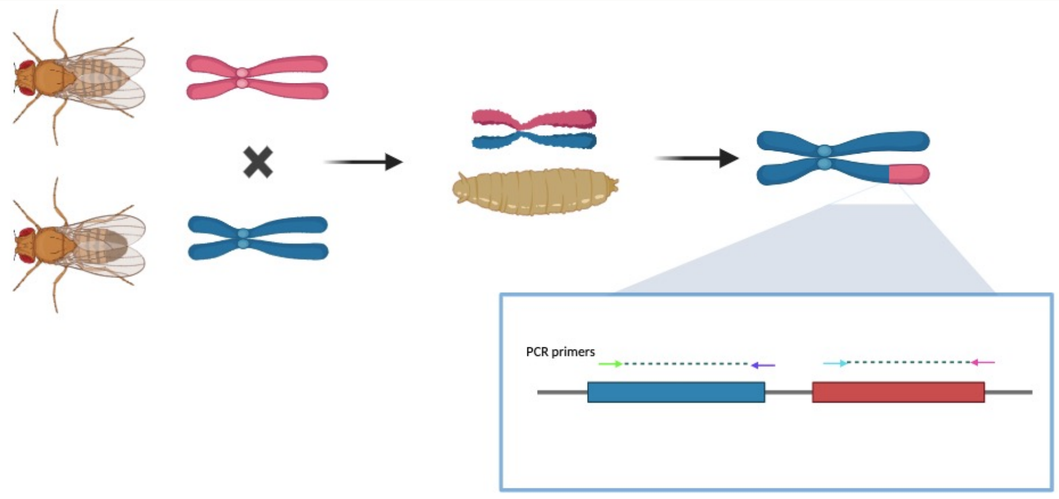
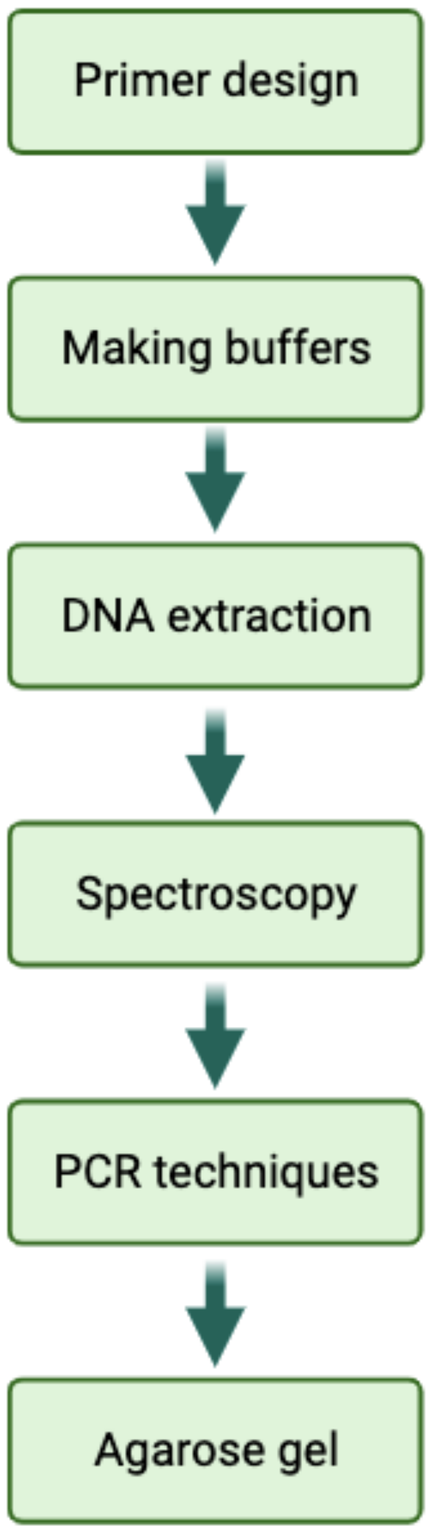


Figure 1: *an illustration of the crossing of two flies to create a recombinant fly with both the target genes on the same chromosome. The outlined square is the PCR explained.*

Methods:



Primers were designed in Benchling, where we decided on ordering five primers; two for the 6C12 and three for the 2A4, due to the uncertain orientation of one insertion site.

To methods/buffers were tested; one from the protocol given and Berkeley project method. Berkeley's method seemed more promising and consist of 100mM Tris-HCl, 100mM NaCl, 100mMEDTA and 0.5% SDS.

We tried out two methods. This included the DNA extraction part. Where the key difference was the LiCl/Kac solution that was added in Berkeley's method.

We tried adding RNase to tests 6-9 to see if that changed the DNA concentration of the PCR samples. Measured the amount of DNA with a spectroscopy machine, before and after adding the RNase.

The target genes were amplified using PCR . The gradient on the PCR machine was adjusted for each test trying to find optimal temperature.

Amplified PCR samples were analyzed on a 2% agarose gel to confirm the bands size was correct according to the expected band size.

Results:

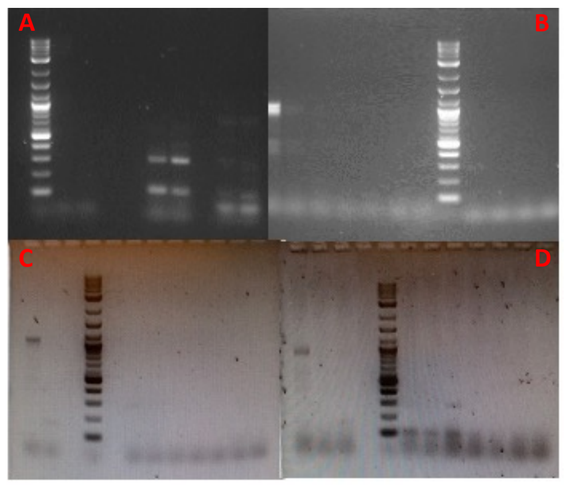


Figure 2: *Gel images of tests 1-5 (test 2 and 3 are on the same image(B)).*

Figure 2 illustrates five tests conducted on the controls, where the temperature gradient on the PCR machine was varied to determine the optimal temperature. The results indicate that the pCary displayed bands in all images, with appearances at temperatures of 56˚C (D), 58.1˚C (Twice, B and C) and 59˚C (A).

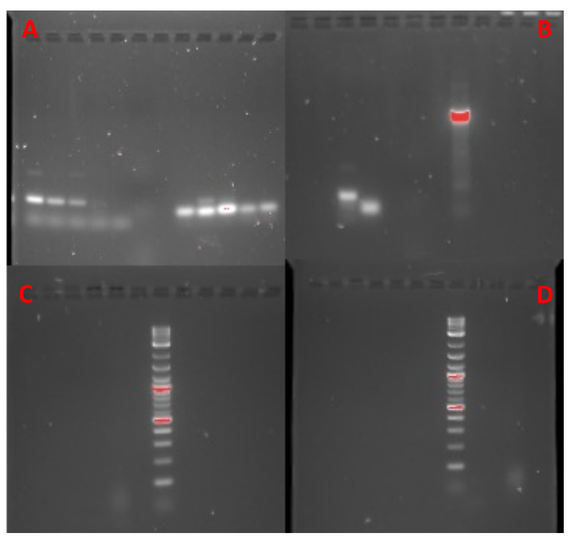


Figure 3: *Gel images of tests 6-9, where RNase was added.*

Figure 3 shows limited success with PCR samples treated with RNase for genomic DNA isolation. Test A and B were same in procedure; however, in Test B, we doubled the amount of DNA added to the PCR samples, resulting in a strong band on the pCary (B). In Figure 3 (A and B) the DNA ladder was not visible due to using the incorrect sample. In Tests C and D, we utilized different primers to determine the presence of Stit in the DNA samples. These tests also had the same procedure, except for a longer elongation time in Test D.

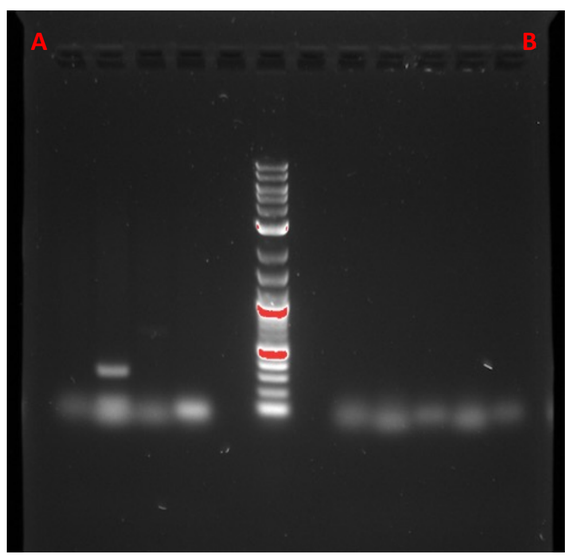


Figure 4: *Gel images of test 10 and 11 combined on one agarose gel.*

In figure 4A (left of the DNA ladder) we tried testing the primers to see if they were compatible/working. The expected outcome is no bands (negative control experiment), however, as you can see we got a band, which suggests that the primers are not functioning as expected. In figure 4B (right of the DNA ladder) we performed a PCR of fly DNA containing both Stit insertions (transheterozygous animals). This was another test to se if the primers were working for us to confirm both Stit insertions are present. There were no bands, indicating that the primers are not working.

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REFERENCES

1. E. Greenwald et. al (2019): Elements of transgenesis: a primer
2. E. Jay Rehm et. al (2020) Berkeley Drosophila Genome Project

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Conclusion:

The agarose gel results have shown a diverse range of observations. Figure 2 identified specific temperature-related band patterns, while Figure 3 had limited success, with procedural inconsistencies like the invisible DNA ladder. Particularly, Figures 4A and 4B indicated primer inefficiency, as unexpected bands appeared and Stit insertions weren't conclusively confirmed. These outcomes suggest a need for designing new, more effective primers. Another potential "next step" could be to compare expression level of protein on a western blot, with an antibody.

The team from left to right*: Sandra Ninzima, Nora Solheim, Fergal M. O'Farrell, Villö Balázs, Aurelia E. Lewis, Katherine L.P. Downham, Maria John and Elizabeth M. Blackley*