IBERS Biology: Parasites and Drug Screening Workshop 2 (Molecular Biology – Down to the DNA)

Aims:

In this workshop we will assess the drug resistance status of the nematodes we looked at in the first workshop at the molecular level, to confirm the results you obtained from the drug screening assay. For this we can extract DNA from the nematode worm samples and then quantify the DNA. We can then set up a virtual Polymerase Chain Reaction (PCR).

Click on the Panopto lecture (link below) to hear Dr Liz Hart give a brief introduction to the principles of molecular biology you will be exploring
https://aberystwyth.cloud.panopto.eu/Panopto/Pages/Viewer.aspx?id=0f9b29d5-3801-4fd1-91e1-1f41d45b5da2

Step 1. Extract the DNA

In our labs we can extract the DNA from the worm samples using a Qiagen DNeasy Blood and Tissue kit. These kits are designed for the rapid purification of total DNA from a variety of samples.

The sample of nematode is first placed into a 1.5ml eppendorf tube for the DNA extraction.

Read through the protocol below to see how we extract our DNA using the kit:

1. Add 200 µl buffer AL and mix by vortexing. Place the samples in the heating block (set at 56^{o}C) for 10 minutes.
2. Add 200ul ethanol and mix by vortexing.
3. Pipet the mix into a DNeasy spin column and centrifuge at 8000 rpm for 1 minute.
4. Discard the flow through.
5. Place the spin column in a new collection tube. Add 500 µl buffer AW1. Centrifuge for 1 min at 8000 rpm.
6. Discard the flow through.
7. Place the spin column in a new collection tube. Add 500 µl buffer AW2. Centrifuge for 3 minutes at 14,000 rpm.
8. Discard the collection tube and the flow through.
9. Place the spin column in a new 1.5 ml microcentrifuge tube.
10. Elute the DNA by adding 200 µl of buffer AE onto the spin column membrane. Incubate for 1 minute at room temperature.

11. Centrifuge for 1 minute at 8000 rpm. **Keep the flow through! This is where your DNA is!**

Step 2. Quantify the DNA

To quantify the DNA in our samples we use a Nanodrop spectrophotometer. We record our results and use the quantification to determine how much DNA from our samples we need to include in our PCR.

You can learn more about using the nanodrop here:

http://www.youtube.com/watch?v=xtMbDtWL0P4

Step 3. PCR.

For the PCR step we need to make up a PCR mix to load on to the PCR machine. This needs to include 50 -100ng of DNA, a PCR master mix (this includes the DNA polymerase in a buffer containing dNTPs and $MgCl_2$) water and a forward and reverse primer to amplify the region of DNA that we are interested in. The samples are then run in the thermal cycler (PCR machine) on a Standard PCR program.

Click on the link below to access the virtual PCR lab and learn more about PCR and find out what all of the components of the PCR master mix do.

https://learn.genetics.utah.edu/content/labs/pcr/

Step 4. Agarose Gel Electrophoresis.

Following the PCR step we can then run out a small amount of our PCR product on an agarose gel to check we have the correct size for our product. We measure the size of the product in base pairs (bp).

Have a look at the following videos to see how to set up an agarose gel and then run out the PCR product:

1.How to set up an agarose gel

https://www.youtube.com/watch?v=vq759wKCCUQ

2. Running an agarose gel

https://www.youtube.com/watch?v=KKmiKKMDDhY

Step 5. Sequencing

The DNA that was extracted can then be sent for sequencing. Have a look at the video below to learn about Sanger sequencing.

https://www.youtube.com/watch?v=e2G5zx-OJIw

In our next workshop (Workshop 3) we will explore how to use bioinformatics to interpret the results of our PCR and to explore whether our nematodes are susceptible or resistant to the anthelmintic drugs we tested in workshop 1.

Before you go, just for fun, A PCR music parody:
http://www.youtube.com/watch?v=2tjU59ChVmU