Practical 2 (WEEK 4) supplementary material

Determining the sex of the domestic chicken (PART I)

This document provides supplementary information and activities that will support your learning of the practical, and poses some questions for you to consider. This will help you in your understanding of key processes, and your ability to address concepts as part of the poster assessment task.

In Prac 2 (pp 19-38), there are two specific tasks:

Task A – *Pipette use*
Task B – *DNA extractions from muscle, blood and feather*

The following information and activities are provided below to support your understanding of these tasks.

NOTE: You are not required to submit this worksheet and your answers, rather we will discuss as part of the prac session.

TASK A: Pipette use (prac manual pp. 20-24)

Pipettes are an important tool used in molecular biology and you need to make sure that you are familiar with their use. This is important as pipetting errors account for most errors experienced in molecular biology and adding an incorrect amount of reagent can result in your experiment failing. Pipette volumes generally range from 10 mL down to 0.1 µL (microlitres). Most often, the volumes you will need to dispense with pipettes will be less than 1 mL or 1000 µL. There are four common pipettes that are used in molecular biology (i.e. P1000, P200, P20 and P10) which dispense different volume ranges. It is helpful to start thinking of a µL as an everyday unit. To get your mind nice and 'warm', try to answer the following questions:

Activity 1: Using pipettes to measure volumes

1. If there are 1000 µL in 1 mL, how many µL are there in 1L?
2. How many µL are there in 0.225 mL?
3. How many µL are there in 0.001 mL?
4. If 1 ml of water weights 1 gram, then how much do the following weigh in grams:
   - 500 µl
   - 1000 µl
   - 1 µl
   - 1000 nl (nanolitres)

TASK B: DNA extractions from muscle, blood and feather (prac manual pp. 25-34)

In this prac, a commercial DNA purification kit (Qiagen DNeasy Blood & Tissue Kit) is used to purify DNA from chicken blood, muscle and feathers. The amount and quality of DNA extracted will often vary between different tissue types, as well as by how the samples are collected, stored and prepared. It is therefore important to ensure that samples are prepared appropriately (according to the tissue type) and that optimal amounts are used for DNA extraction.

Following preparation, the cells of a tissue sample are *lysed* (broken apart) to release the DNA using the enzyme proteinase K, buffer AL and/or ATL. The lysate is then loaded onto a DNeasy Mini Spin Column (which comprises a silica gel) whereby, during centrifugation, DNA is selectively bound to the membrane as contaminants pass through. Remaining contaminants (e.g. protein, RNA, salt) and enzyme inhibitors are subsequently removed by two wash steps (using buffers AW1 and AW2), and the DNA eluted from the column using water or buffer, ready for use.

Note: Buffer ATL contains an anionic surfactant (sodium dodecyl sulfate, SDS) which disrupts protein bonds, while buffer AL contains a chaotropic agent (guanidine hydrochloride) which promotes cell lysis, inactivates/denatures proteins and promotes DNA binding. AW1 is a stringent wash buffer (which also contains guanidine hydrochloride as well as ethanol) and removes residual proteins, while AW2 is a tris-based ethanol solution that removes excess salt.

Activity 2: DNA extraction

1. What are the 5 common steps in any DNA extraction procedure? HINT: The first step is sample *preparation* followed by…

2. Why is only 20 mg of muscle tissue needed? What might be the consequence of adding more muscle tissue to tube for downstream extraction?

3. After removing the tip of the feather that surrounds the blood spot (visible or not), why then cut the tip length-wise?

4. Proteinase K and Buffer AL are used in the extraction of all 3 tissue types, yet ALT is only used for extraction of muscle and feather.
    a. What role does proteinase K play?
    b. What role does ATL play?
    c. What role does AL play?

5. What are the consequences if cell *lysis* is not complete after incubation with these associated buffers?

6. After the DNA is on the spin column and is *washed* with buffers AW1 and AW2 buffers, why is it important for the spin column to then be completely dried (removing residual ethanol)) before *elution*?

7. Why is it important to elute the DNA into a fresh tube in the final step?

8. Why elute in 100 µL? What happens if you only elute in 25 µl?

9. Give one pro and one con for the different types of sample collection - destructive sampling, invasive sampling and non-invasive sampling.