WELCOME

SLE254 Practical 2

(Sexing chickens – Part I)

Pracs 2-4

Week 4 - Prac 2
- Pipette practice
- DNA extraction

(Blood/ Muscle tissue/ Feather)

↓

Week 6 - Prac 3:
- Visualise extracted DNA product on agarose gel and estimate concentration of extracted DNA using the Nanodrop instrument.
- Amplify the CHD 1 gene using PCR.

↓

Week 8 - Prac 4:
- Visualise PCR product on agarose gel.
- Analyse gel to determine the sex of your sample/s.

Assessment task

- 1x scientific poster (32%) of the total mark.
- Record all your results and any changes in the methods.
- Due date: **8pm Friday 20th Septembe**r.
- We will discuss poster requirements in more detail in classes.

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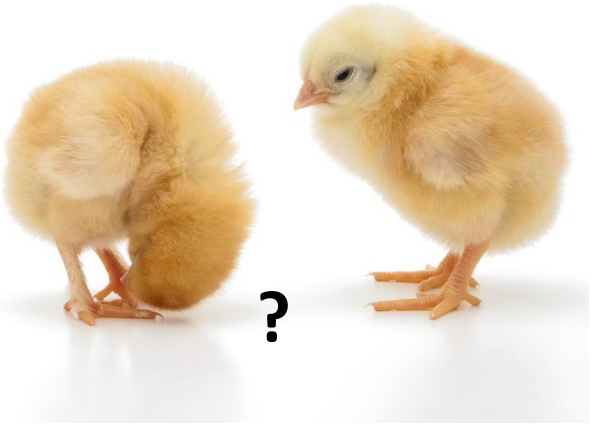

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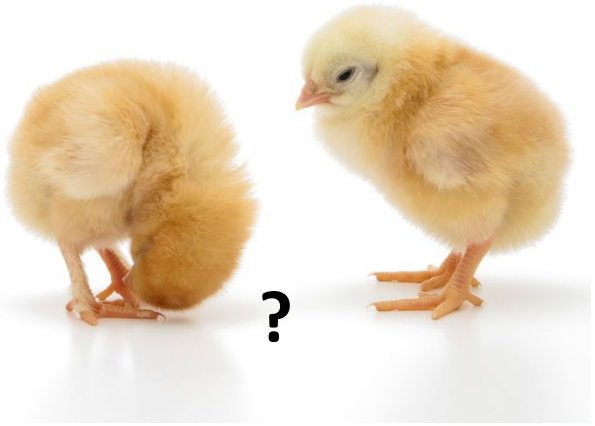
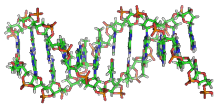
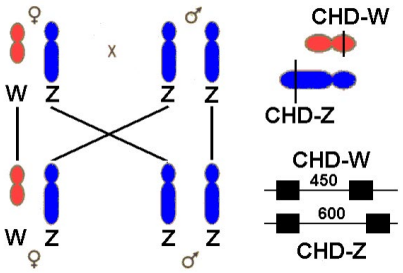


- Universal genetic markers can be used to reliably sex almost all bird species.
  - Size/variation of the chromo-helicase-DNA binding gene (CHD1) carried on the sex chromosome
  - males are homogametic (ZZ chromosomes)
  - females are heterogametic (WZ chromosomes)





A molecular test for bird gender

Source: SM Carr (2008); mun.ca/biology/scar/Bird_sexing.html

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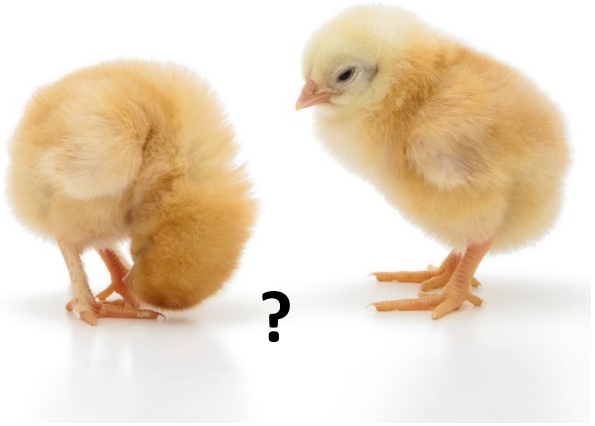

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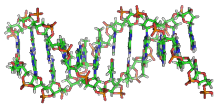
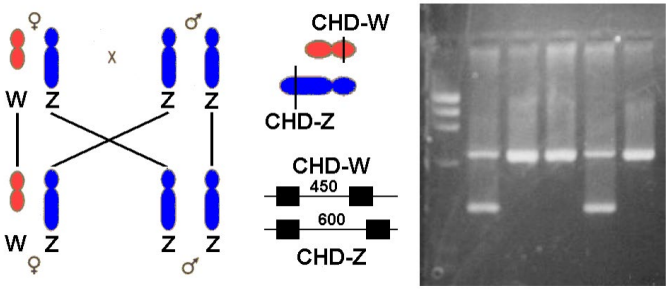


- Process involves:
  - DNA extraction
  - PCR amplification of CHD1 gene
  - Visualisation of PCR products to determine sex.



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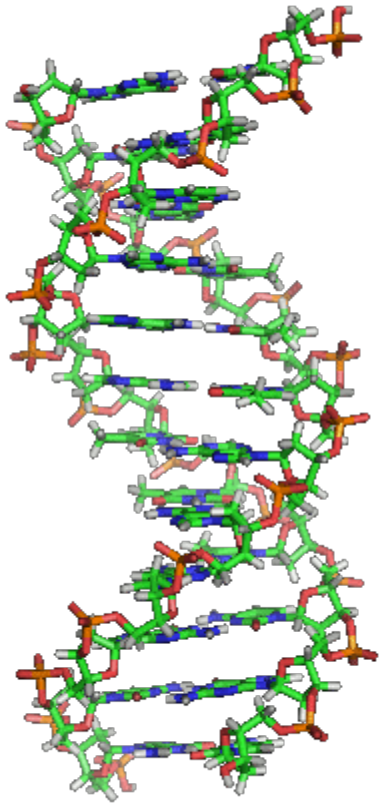
DNA sampling: quality and quantity



DNA samples

We need to be able to **SOURCE** DNA from individuals in order to look at their genetics:

Three types of sampling:

1. *Destructive*
2. *Non-destructive* (invasive)
3. *Non-invasive*

1. Destructive sampling

- The animal is killed in order to obtain the tissues necessary for genetic analysis
- Necessary in order to obtain enough DNA to do the analysis
- e.g. allozyme variation
  - Small mammals
  - Reptiles
  - Birds

2. Non-destructive sampling or Invasive sampling

- The animal is usually captured, and a biopsy or blood sample is taken *invasively* (small sample)
- Some invasive sampling strategies do not require catching the animal
- e.g. whales using a biopsy dart gun

3. Non-invasive sampling

- Good approach for many species which may be of significance:
  - conservation concern (e.g. endangered)
  - cryptic / elusive (e.g. hard to catch)
- Source of DNA is left behind and collected without having to catch or disturb the animal

3. Non-invasive sampling

- feathers
- skins
- bones
- eggshells
- scats
- swabs

DNA degrades overtime & may not be reliable for analysis

Which sampling method to use?

- Depends what you are doing?
  - How much DNA do you need?
  - What quality does it need to be?

- Ethical considerations
  - Replacement, reduction, refinement

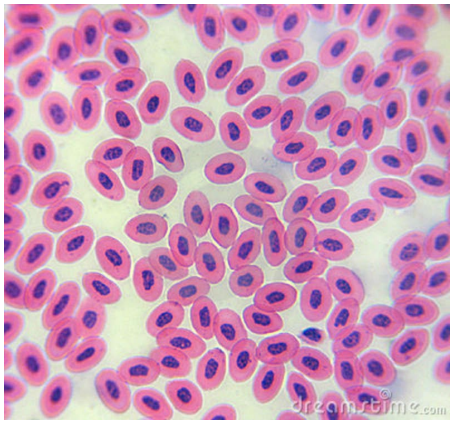
Different sample types and approaches

Muscle
destructive sampling

Blood
Invasive but non-destructive sampling

Feather
Non-invasive/non-destructive

Which do you predict will provide the most DNA? Or the best quality DNA?



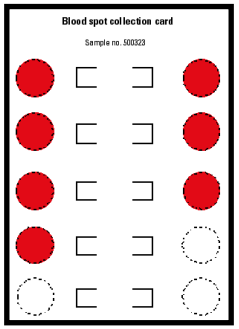
Chicken blood smear

What do you notice about this blood smear?

Has this changed your opinion about the above questions?

DNA quality

The quality of DNA affected by:



- Method of preservation
  - Stabilisation buffers, ethanol, formalin, blood spot cards

- Length of preservation
  - DNA degrades over time

Photo: P. Cianfaglione; http://avianmusing.blogspot.com

- Method of extraction
  - Chemical or physical lysis;

  in-house or commercial extraction kits

- Type & length of storage
  - Liquid nitrogen, -80°C, -20°C, fridge (4°C), room temperature

High stability
(expensive)

ideal

Low stability

How the session will run

- You will work together in groups to extract DNA from 3 different sample types.
  - The DNA samples will be used in subsequent pracs.
  - Please make sure your tubes are clearly labelled and placed in the appropriate rack/box at the end of the session (your demos will advise you here).

- It is important you actively take part in and have an understanding of the procedures as they will support your ability to put together your Assessment task (poster).
  - Take notes! And think about each sample and the amount of DNA it is likely to yield. Will it influence the downstream PCR assay?

- Demonstrators will be available to assist you in answering questions & further guiding you through the activities.
  - We will use the last 20-30 min of the prac to run through any specific questions

Prac 2 – Part A (Pipetting)

We will go through a quick demo on pipette handling!

(See lab manual pp 20-24)

- Complete questions on p 20.

Prac 2 – Part B (DNA extraction)

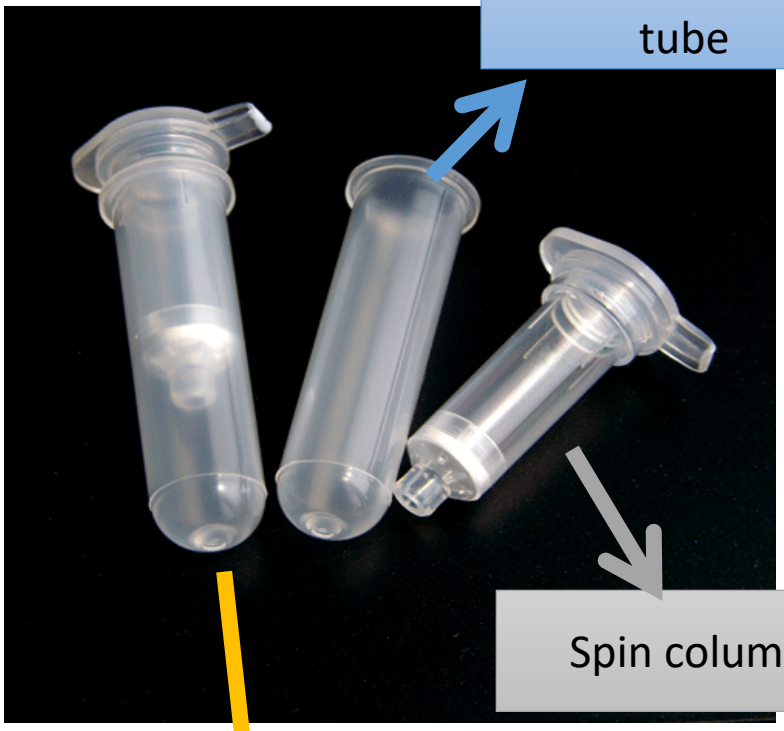
Reagents	Labelled	Blood (pp 26-28)	Muscle (pp 29-31)	Feather (pp 32-33)
Proteinase K	**PK**	X	X	X
PBS	**$PBS_{(B)}$**	X		
RNaseA	**$RNA_{(M,B)}$**	x	X	
Buffer AL	**$AL_{(B,M,F)}$**	X	X	X
Buffer ATL	**$ALT_{(M,F)}$**		X	X
95% Ethanol	**$E_{(B,M,F)}$**	X	X	X
Buffer AW1	**AW1**	X	X	X
Buffer AW2	**AW2**	X	X	X
Buffer AE	**$AE_{(B,M,F)}$**	X	X	X

Prac 2 – Part B (DNA extraction)

	Blood	Muscle	Feather	Reminder
Sample preparation	Blood card - cut 1 hole punch sized	20mg tissues - About the size of match head	Small section -Refer to pg 34	Discard razorblade in yellow sharps container
Incubate tube 56˚C for 30 min	Step 7	Step 3	Step 3	
Change new 2ml collection tube	Step 14	Step 9 and 10	Step 7 and 8	Mark a **"C"** beside the number
Change 1.5ml microfuge tube	step 16	Step 12	Step 10	Mark a **"C"** beside the number
While waiting for incubation, 1st record your group name and your sample details to the excel sheet prepared, 2nd try practical 2 questions (p 35). After finishing your extraction bring your samples to us.				

Prac 2 – Part B (DNA extraction)

1.5 ml Microcentrifuge tube



Spin column + collection tube

Prac 2 – Part B (DNA extraction)

Vortex mixer

Mini centrifuge

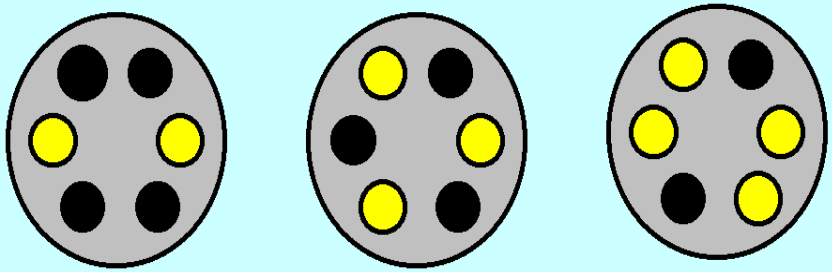
Incubator/ Hot plate

CENTRIFUGE
Proper Balancing

The diagrams below indicate balanced loading of 2, 3 and 4 sample tubes in a centrifuge.

Represents *sample tubes* in the holders

Represents *empty* holders



Centrifuge

- **Always make sure to balance the centrifuge and to cover the lid before centrifugation**

Sample labelling

MAKE SURE TO LABEL YOUR TUBES!

Group initials:

- John Hotwings & Colonel Sanders = JHCS

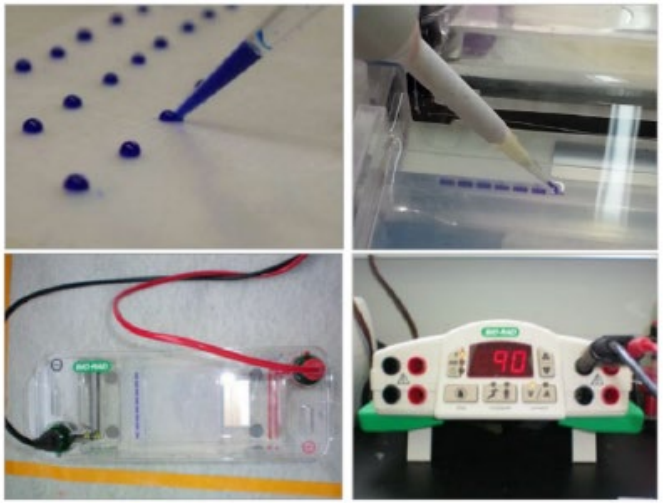
Sample type:

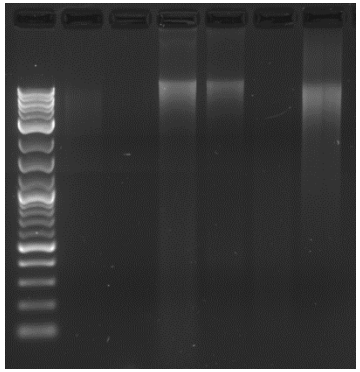
- B = Blood
- F = Feather
- M = muscle

For example for a blood sample label = JHCS-B

Assessing DNA quality…Week 6 (Prac 3)

Gel electrophoresis





Spectrophotometry (NanoDrop)