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SLE254 The Practical Report (Sexing chickens - pracs 2-4)



Determining the sex of the domestic chicken (Gallus gallus)

WHY?

Many species like chickens are sexually monomorphic and cannot easily be distinguished by phenotype when young.

HOW?

Analysis of the sex-chromosome-specific gene locus CHD (chromo-helicase-DNA binding gene, CHD1), carried on the W & Z chromosomes (CHD-W & CHD-Z).

- males are homogametic (ZZ chromosomes)
- females are heterogametic (ZW chromosomes)

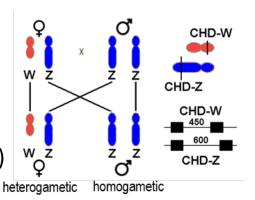
BY WHAT PROCESS?

PCR amplification of CHD1 from sample DNA extracts and visualisation by gel electrophoresis.

- males yield 2× CHD1Z (~600 bp)
- females yield 1× CHD1Z (~600 bp) & 1× CHD1W (~450 bp)



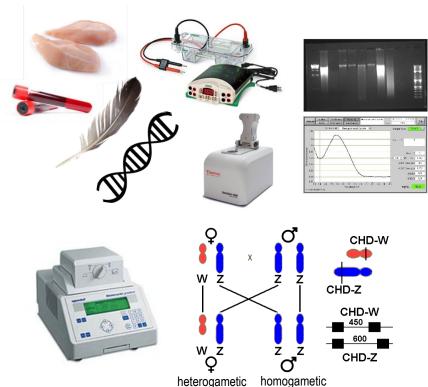




Comprise conserved exons, with introns of different lengths

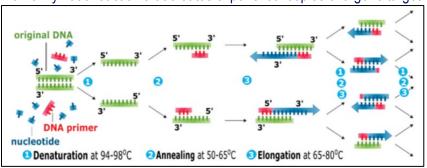
DNA extraction. Pracs 2&3:

assessment & CHD1 gene PCR amplification



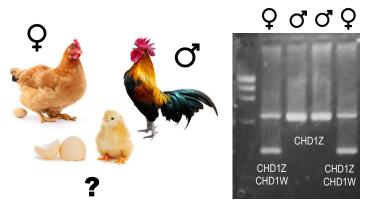
PCR (Polymerase Chain Reaction)

- an enzymatic reaction that creates exponential copies of a gene target.



Prac 4:

PCR product visualisation & interpretation



Source: modified from SM Carr (2008); mun.ca/biology/scar/Bird sexing.html

Assessment task – Chicken prac poster

(Worth 32%, Due 8pm Friday 22nd September)

Poster format comprising:

- Introduction & Aims
- Methods
- Results
- **Discussion & Conclusions**
- References



Which samples are most likely to be successful in PCR?

Overview

Assessment task – Chicken prac poster

Worth 32%, Due 8pm Friday 20th September

Poster format comprising:

- Introduction & Aims
- Methods
- Results
- Discussion & Conclusions
- References



Based on the Chicken practical series

Requires you to report on the utility and approach taken in the use of particular molecular techniques for the sexing of animal species - using the domestic chicken (Gallus gallus) as an example.

You must prepare and submit your poster as an <u>individual</u> assessment task.

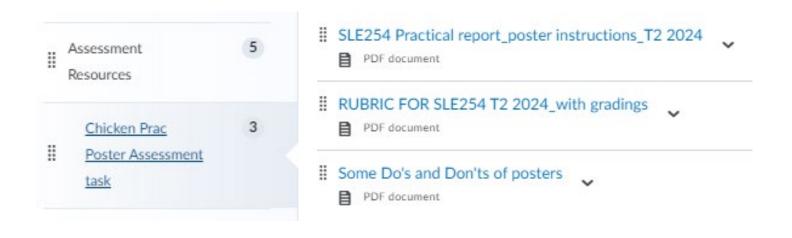
Submit to the assignment drop box

(no emailed hard copy assignments will be accepted)

Note: marks will be deducted at a rate of 5% per day. No marks will be given after 7 days. If you need an extension, please complete the online extension request form.

Instructions for the assessment task (poster)

A detailed set of instructions and a rubric is provided on the unit site (details allocation of marks) – it is essential you consult these!

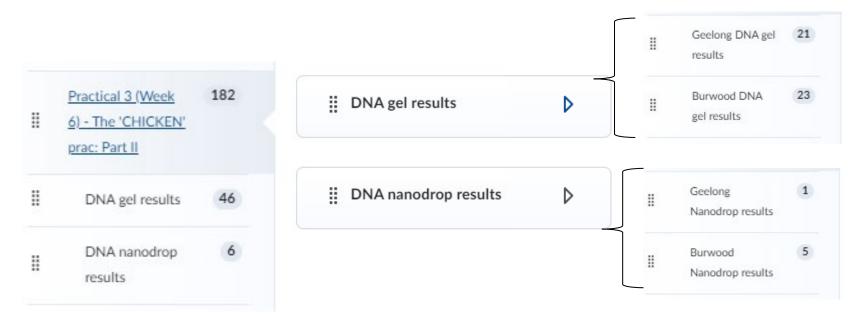


Plagiarism is taken very seriously. Check your work in Turnitin!

Please read the Rules of Plagiarism in Appendix 2 of the poster instructions

Results for the assessment task (poster)

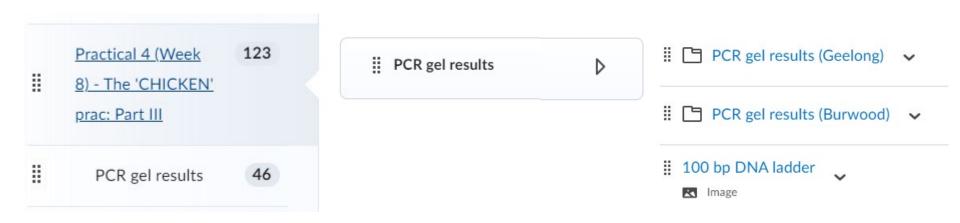
All results generated throughout the pracs are available on the unit site.



Prac 3 – DNA gel images and nanodrop results.

Results for the assessment task (poster)

All results generated throughout the pracs are available on the unit site.



Prac 4 – PCR gel images.

NB: These we be uploaded at the end of Week 8.

Title, Introduction & Aims

- Your title should be informative and convey an accurate description of what your report is about in context with the outcomes. Be descriptive, yet concise. Good titles are typically less than 15 words.
- The introduction is an account of the current scientific research area, setting the scene for the scientific problem to be addressed. <u>DO NOT just copy the introduction from the prac manual.</u>
 - Give context e.g. Some species like chickens are monomorphic and hard to distinguish sex phenotypically.....we can use molecular techniques like PCR to do this.....explain how this is done...
 - Chickens follow a Z-W type sex determination system which means......difference in males and females are.....
 - State what you aim to determine (i.e. the sex of the chicken) and what you expect......

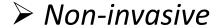
Rationale & utility of the approach:

Sampling

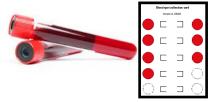
> Destructive



> Non-destructive (invasive)









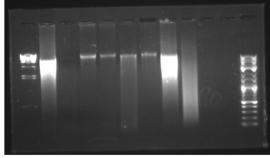


DNA Quality & quantity

- > Sample type
- Preservation method & duration
- Extraction method

Can influence PCR success or failure

Agarose gel electrophoresis



Nanodrop



High or low quality (HMW/LMW), sheared/ fragmented, high or low yield, contaminated?

Methods

- The methods is a very succinct statement of the procedures used to generate the data. DO NOT just copy the methods from the prac manual.
- You should detail key steps, e.g. sample collection, processing, DNA extraction, and PCR analysis and visualisation.
 - Refer to relevant references to avoid re-writing whole slabs of text, e.g. "The method employed to extract DNA from samples followed that of Hogan et al. (2020), which briefly included"
- The methods should comprise concise paragraphs (**not dot points**) like that of a scientific paper:

e.g. "For PCR, 10µl reaction volumes were prepared and comprised 30 ng DNA, 1 x PCR buffer, 1.6 mM MgCl2, 0.2 mM of each dNTP, 0.4 mM of each forward and reverse primer and 0.05 U of Taq DNA polymerase (Type/company)". "Reactions were subsequently subjected to 94°C for 3 min + (94°C for 30 sec + 60°C for 30 sec + 72°C for 30 sec) x 25 + 72°C for 10 min + 15°C for ∞ ".

Methods

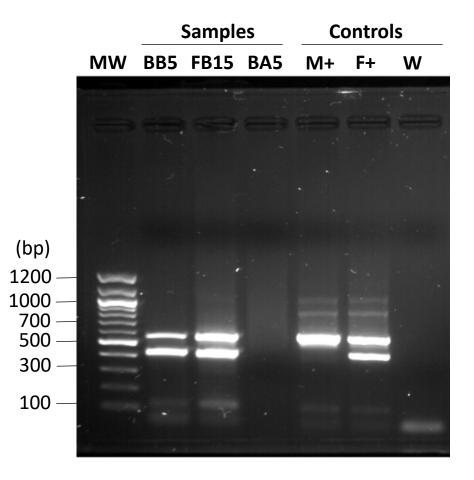
- Do not provide lists of materials.
- Avoid use of irrelevant steps, e.g. "I put my gloves on before..." or "I then disposed of the tube".
- Write in past-tense.

Results

- The results section should visually present the data/results, and state the key findings (do not discuss them)
 - e.g. summary data like mean <u>+</u> standard deviations of DNA concentrations should be provided in a table with corresponding text stating that "From Table 1, it was observed that the highest concentration of DNA was obtained from blood samples".
- You may choose to separate it into 2 sections dealing with DNA extraction and PCR separately.
 - Include a copy of the accompanying gel image which should be clearly annotated (i.e. to include sample ids, markers etc), labelled (e.g. "Figure 1"), and have a caption that details what is depicted, e.g. "Figure 1 Agarose gel electrophoresis of PCR products obtained from amplification of the W and Z genes from chicken blood samples".
- State any other findings, e.g. comparisons between groups, in relation to controls ...etc.

Results

Figure 1. Agarose gel electrophoresis of PCR products obtained from total DNA extracted from blood (BB5, BA5) and feather (FB15) samples taken from two domestic chickens. Experimental controls include gDNA from a male (M+) and a female (F+) chicken, and water (no template control). MW – 100 bp ladder.



You have access to a reasonable number of gel images as part of the class dataset. As such, alongside your own gel image, you may wish to convert the rest of the data into a table of e.g. % Males/Females per sample type.

Discussion & Conclusions

- As it suggests, you need to discuss your results. Don't just restate what they were i.e. state what the results mean and why.
 - Findings should be discussed in relation to the scientific literature (citing a min. of 5 refs in the text).
 - Should comprise concise paragraphs (not dot point format)
- Start with key findings, e.g. were their great differences between sample types? maybe suggest why this might be.....
- Conclusion needs to bring everything back together. Restate aims and if they were reached or not and why, and provide some suggestions for future directions/work.
 - Remember: no new findings or results should be added at this point.

References

- References should be in Harvard style and should have correct and consistent formatting.
 - Reference section can be smaller font size.
 - Comprise a list of at least 5 or more relevant peer-reviewed papers
 - Reference the practical manual if cited in the methods
 - Reference any other sources of information (websites) if cited in the text
- Further instructions for referencing is given at the end of the Poster instruction document in Appendix 1.

Format

- Use PowerPoint to make your poster
- Make a new presentation with a single slide (portrait or landscape)
 - From slide "layout" (under the home tab) choose a completely blank slide and adjust the slide size to poster dimensions (70 cm \times 100 cm)
- Poster should be predominantly visual
- Keep your writing to a minimum by using concise sentences and avoiding irrelevant information.
- Don't use any fonts smaller than 16 point and make sure your images are large enough and clear
- Word count should be about 800 1000 words (excluding figure/table captions and references)
- Use headlines to make it easy to read
- Use graphics colour and fonts effectively to achieve a consistent and clean layout

Poster with a simple layout

- Layout easy to follow
- Text :
 - Maybe a little too small?
 - Maybe a little too much?
- Slightly boring, but does the job

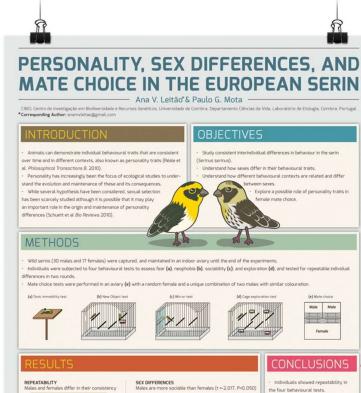
AOPA 2016 Poster title goes here, containing strictly only the essential number of words...

Author's Name(s) goes here¹, Author's Name(s) goes here², Author's Name(s) goes here⁴

Name of institution/workplace goes here Name of institution/workplace goes here Name of institution/workplace goes here

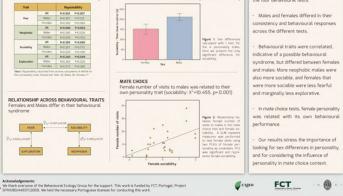
HINGOSCHOIL	nesolts.		Discussion
First feep your poster within the following limits: Sile: Al) Crientation: Portrait (yerbial) The page size of the gooter template is AD (((bk-11) feet), portrait (yerbial) formust. Do not change this page size. Most printers can solie-to-fit a smaller or larger size, when printing. Introduction – The introduction should passent the reasoning behind the project which you are describing/investigation. This mayor that the reasoning behind the project which you are describing/investigation. This mayor that the reasoning the project which you are describing/investigation, this mayor that the temperature size your introduction ybould finel able to predict what your investigation will be. At the terms time your introduction should allow someone who is not an expect so understand why you did this experiment. Simply highlight this text and replace.	Ties for making a successful poster— *Re-entits your paper into poster former is. Simplify eventhing, avoid data overfoil *Headings of mine fisher is words should be in upper and lower traes, not all capitals. *Never do whole sentences in capitals or underline to stress, your poster leave breathing space around your poster leave breathing apace around your poster. *When laying out your poster leave breathing apace around your post Dob overcoved your poster. *Thy using photographs or coloured graphs. Avoid long numerical tables. Importing / inserting files. *The best type of image files to insert are IPEG or Tiffs, IPEG is the preferred format. *Be aware of the quality/evolution of the image to avoid placetion when the image is printed. *For simple graphs use MS Socie, or do the graph directly in Present John in a scientific graphing programs (se, Sigma Pfor, Prices, LPSS, Statistical should be seved as IPIG or Tiff if possible. *Results - Titls section provides the reader with a clear, concess summary of the data you collected and the results of any stabilities for the results as you go along. Simply highlight this sext end replace.		Priving and Laminating. Once you have completed your pooler, it's a good lides to produce an All size draft point to check it yourself and proof read. If possible, show your privined poster to a colleague/friend/family-member for a look with Triefs eyes. Once you are heapy with the poster, send it vie email to eventuffscopulorgia as by 5:00pm Monday 12° September 2016. The ADRA Office will prior the poster for you and take it to the Congress. Discospon – This is the section in which you can interpret the results of the investigation and discuss their meeting. It is important that your discussion relates the results of the investigation and discuss their meeting. It is important that your discussion relates the results to the loss relates the results are yout have led to clear, on a takes the results to the loss relates the results are yout have led to clear, on a takes to the questions related initially, so your discussion, from hight also discuss any limitations of the investigation, from high also discuss any limitations of the investigation. Don't just conducte that further investigation, and in the product possibly be – be widely as to what the further research could possibly be – be widely to to what the time. Simply highlight this text and replace.
			THE SECTION OF THE SE
Method			Conclusion
How to use this poster template Simply highlight this text and replace it by typing in your seen text, or copy and pasts your text from a MS Word document or a PowerPoint, slide			Conclusion - Review the main findings and results, and express them in general terms. This part is also for bury negders who don't have time to read all of your findings, and for readers
presentation. The body text / fort size should be between 3 & and 32 points. Arial, relivetice, Califert or equivalent. Keep body vest left aligned, do not justify text. The colour of the text, fittle and poster bedgeround can be changed to the colour of your choice. Section headings can be moved as and down to accommodate the lost boxes.	Figure, sable or picture	Capazino de la certa Otras en Trans Confesso de Transi de Asana de aprovisión de como de aprovisión de como de	who want to road on ownsiew of the findings before deciding whether to read the findings in detail. Simply highlight this test and replace.
Marhod - The method section describes in detail the operations performed by the investigator. The method must consider energin indemnation for the reader to be able to repeat the experiment, but it should not include any inclevant details. Simply highlight this text and replace.	Eigente, faible or picture Common to be on to Tomas or Tomas fore Roman or application trade: () 6-23 points, on the longity of the volume in case of given today more fore 2 of a foreign with		References Antonice - standard referencing: If you have a large number of references, change forcing souther
	Cognitive to the pel to Divine or Dans New Annies or manimum, make, forecast, M. and N. aponto- disple aligned for reflect in a Again to a regal in Capital and right or the may religion the garages graph or galantic	Figure, hable or picture	to between 18 and 24. This section should take no more than is of this solumn. Simply highlight this text and replace.

Nice poster with a simple, yet visual layout

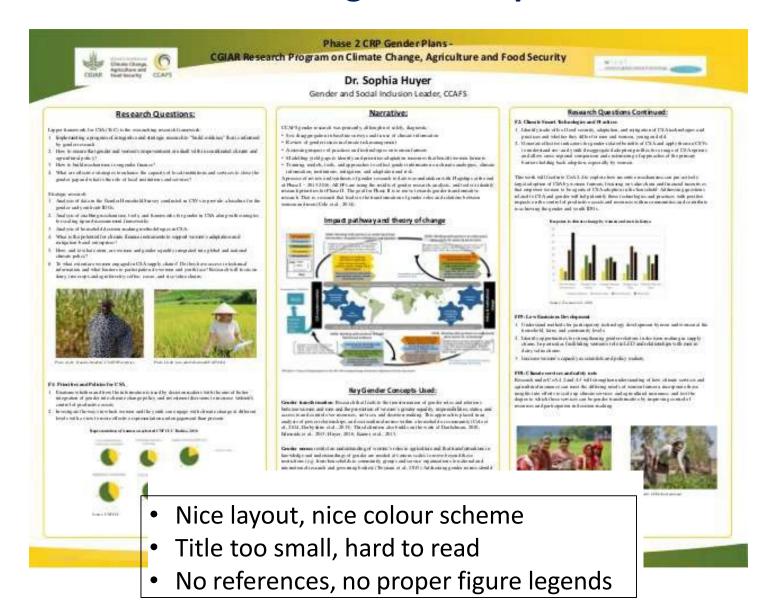


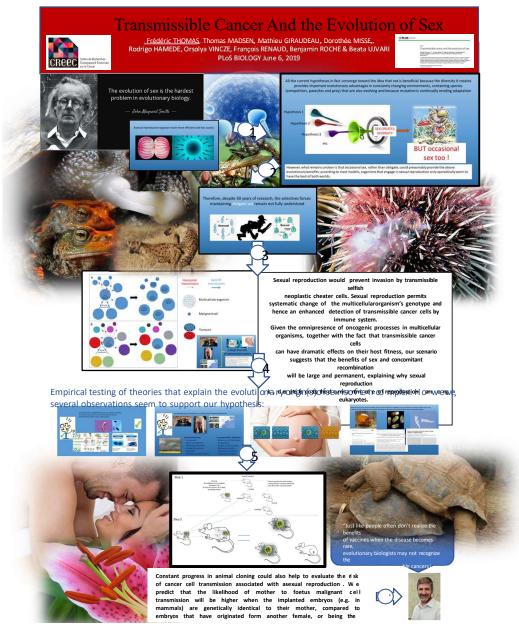
Replace this with Discussion and conclusions

Replace this with References



What's wrong with this poster?





What about this one?

- Confusing layout, hard to follow
- Text hard to see:
 - blue text on blue background
 - font too small
 - white text overlapping images
- Images too small
- No references

Nice poster with a well structured and visual layout

Seasonal, sex, and age specific variations in immune gene expression profiles underlie cancer progression in Tasmanian devils (Sarcophilus harrisii)

Nynke Raven¹, Rodrigo Hamade², Marcel Klaassen¹, Frédérick Thomas³, Thomas Madsen¹, Beata Ujvari¹

Background

Tasmanian devil facial tumour disease (DFTD) has reduced devil populations by >85% in the last 20 years. Research has recently discovered devils are showing signs of adaptation to DFTD1, including changes in allele frequency in cancer related genes?, surviving longer once infected with DFTD3 and even regression and clearing of DFTD4, all indicating the importance of the devil immune system in fighting the disease.

However, factors other than disease also affect immune function, and thus can concomitantly impact DFTD progression and outcomes. Such factors are:

- Seasonal variation: altered immune function and switching between adaptive and innate immune responses across
- . Sex differences in immune function and strategies; females tend to mount and maintain stronger immune
- · Impact of co-infection with multiple parasites8
- Variation in response to overall health and body condition⁶

Tasmanian devils show sex specific differences in response to DFTD10, suffer from immunosenescence11, are regularly infected with parasites 12 and show seasonal hormonal variation connected with reproduction 13



Aim: Investigate the factors influencing the Tasmanian devil immune system and consequently, DFTD susceptibility and progression

samples collected seasonally (IgG, IgM, IgE, IgA, CD4, CD8, MHCC2).

Methods

Genes: 3 innate immune genes (CD11, CD16, NKG2D), 7 adaptive immune genes

from West Pencil Pine forest. Factors: Sex, Season, DFTD infection, Age, Ticks, Head width and Weight. Interaction between head width and weight as proxy for body condition. Syrs, 40 animals with DFTD, Correlations between variables measured and taken into account when interpreting results.

Gene expression: via RT-qPCR, Normalization using Pfaffl

Statistical analysis in R

Principal component analysis (gehinlot) Linear mixed models (Ime4) Model analysis (glmulti) Factors normalized and scaled for comparisons



Results

- Strongly correlated genes:
- IgA & CD8 · IgM & IgG & NKG2D
- Together, PC1 and PC2 account for 66% of variation in dataset

Key findings of Multi-model analysis model estimates for PC1 and PC2 models in top 2 AIC

- · DFTD infection and season in all top 20,000 models Strongest effects with DFTD, DFTD*Head and

DFTD*Sex, all statistically significant

- Age and DFTD present in all top 20,000 models. · Strongest effects for DFTD infection, highly significant

Female devil MHC class 2 immune gene expression is stable throughout the year, while male expression fluctuates, highest in summer, lowest

Devils display immunosenescence. with IgG expression lower when infected with DFTD



Discussion

This study has shown strong correlation between the expression of several immune genes in Tasmanian devils, a result not surprising as many immune genes share common pathways.

The expression of the immune genes was affected by DFTD infection, season and age.

In general, female immune function is stable throughout the year, while male immune function fluctuates in relation to the breeding season. The collapse of the male immune system following the breeding season (when involved in fighting and mate guarding) might make males more susceptible to DFTD infection. The stable immune function of females throughout the year, potentially provides better protection against DFTD. This could explain higher male susceptibility to DFTD, the lack of males surviving to older age and why most of the DFTD regressions have been discovered in females.

Immunosenescence was confirmed in devils, also showing that DFTD accelerates the attenuation of the immune function. The study highlights the complexity of factors driving DFTD progression and susceptibility, and that further studies are needed to better derstand the factors driving the immune system of Tasmanian and hence DFTD epidemiology.



Nice poster with a well structured and visual layout

Transmissible cancers: Are they rare, or do we just fail to recognise them?

Georgina Bramwell¹, Aaron Schultz¹, Craig Sherman¹, Marcel Klaassen¹, Rodrigo Hamede^{1,2}, Thomas Madsen¹, Frédéric Thomas³, Beata Ujvari^{1,2}

¹Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Australia ²School of Natural Sciences, University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia. ³CREEC, MIVEGEC, UMR IRD/CNRS/UM 5290, 34394 Montpellier, France



Introduction

Disseminated neoplasia (DN) has been reported in the literature since the 1960's in 15 different species of bivalves all over the world1 (Fig. 1). The group of bivalves that have been affected by DN are mussels, cockles, clams and oysters. In all species affected, DN presents as abnormally proliferating cells, particularly haemocytes². A recent study in the USA found that DN has the capability of acting as a transmissible cancer3. Up until this discovery it was believed that transmissible cancers were rare and only affected two mammalian species (Tasmanian devils and dogs)4 however the recent discovery raises the question, are

Analysing previous research on DN revealed that there are many differences between techniques and study design, indicating the possibility that evidence supporting transmissible cancer could have been missed (Figs 2-3). Based on reviewing the inconsistencies in the literature, here we propose a step-by-step guide to determine the presence/absence of transmissible cancers in bivalves



The Problem

Data collection

- Tissue type
- · Most use both haemolymph and solid tissue Solid tissue ranges from the whole
- individual to the mantle, gill and stomach
- Some only use haemolymph or a transverse section of all organs
- Sample location
- Always mentions but not always exact Longitudinal samples
- Varies between monthly, annually, randomly or only once

Diagnostic techniques







Possible outcomes

- Phenotypic
- One paper mention a size difference⁶
- One paper mentions pale, watery tissue²⁶
- Bivalve Health

The Solution - Systematic/consistent data collection

Target key species

Optimal = 20-24°C

Salinity levels²¹

environments

cell death)

Cancer thrives in hypoxic

40°C = 100% sarcoma cel

• Low = 0.5% (100% sarcoma

• High = 35% (50% sarcoma cel

• 1-2% O2 or below in tumours2

anaerobic oxygen production²

· Bivalves can switch to

Collect samples

· Total abundance of DN often

Analysis

Future directions Revisit museum specimens

Histological analysis

- Cloning
- · Min. 100 sample size per

Appearance/size

helow 10%2

- Measure initial weight and size
- · Note any mantle recession /watery tissue/discolouration2

Tissue required for histology

· Both haemolymph and transverse section of all organs

neoplasia specific genes

· To determine neoplasia

- · To separate
- host/neoplasia sequences (Fig. 4)
- BLAST sequences · If transmissible cancer, neoplasia Figure 4. Example

sequence will match between individuals

Need multiple genes - (without paralogues and NUMts) Possible genes include³

mtCOI, EF1a, rDNA ITS Phylogenetic analysis

To identify the lineage

PCR/gel screening

Queensland museum · Contains approx. 200 species

. University of Cambridge

Contains approx. 100,000 lots







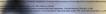
· Study showed success to detec mussels using PCR and qPCR25 sea stars using droplet PCR26 (Fig

Transferability

· Potential artificial transfer

- · "Just like people often don't realize the benefits of vaccines when the disease becomes rare, evolutionary biologists may not recognize the critical role of transmissible cancers in shaping animal evolution as these cancers are very rare' [Prof James DeGregori].
- . Transmissible cancers may not be as rare as originally thought as DN may actually be a transmissible cancer due to inconclusive/inconsistent research. A step-by-step guide can be a solution to detect transmissible cancers.
- If common, similar to other parasites and pathogens, transmissible cancers can/will have a





Some useful resources

The following links can provide advice on effective poster design:

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http://guides.nyu.edu/posters
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http://www.personal.psu.edu/drs18/postershow/

https://www.behance.net/gallery/2284120/SCIENTIFIC-POSTER

https://www.youtube.com/watch?v=AwMFhyH7_5g