HOW TO WRITE A PROPER SCIENTIFIC PAPER

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REQUIREMENTS OF A SCIENTIFIC PAPER

Scientific papers are for sharing your own original research work with other scientists or for reviewing the research conducted by others.

A standard format is used for writing scientific papers, because –

- It allows the author to efficiently communicate scientific findings to the broad community of scientists in a uniform manner.
- It allows the paper to be read at several different levels, thereby enabling you to target a wider audience pool.

Traits of a good scientific paper –

- They are highly readable — that is, clear, accurate, and concise.
- They must be able to convince their audience that the research presented is important, valid, and relevant to other scientists in the same field.

STRUCTURING YOUR SCIENTIFIC PAPER

Most journal-style scientific papers are subdivided into the following sections:
| Title | Authors (and their affiliations) | Introduction | Materials and Methods | Results (with Tables and Figures) | Discussion | Acknowledgments | References | Appendices |

Experimental Process	Section of Paper
What did I do in a nutshell?	Abstract
What is the problem?	Introduction
How did I solve the problem?	Materials and Methods
What did I find out?	Results
What does it mean?	Discussion
Who helped me out? (optional)	Acknowledgement
Whose work did I refer to?	Literature Cited (References)
Extra information (optional)	Appendices

TITLE, AUTHORS' NAMES, AND THEIR INSTITUTIONAL AFFILIATIONS

Title

- The title needs to be specific enough to describe the contents of the paper, but not so technical that only specialists will understand.
- The title usually describes the subject matter of the article. However, it is sometimes more effective to have a title that summarizes the results.

Authors

- The person who did the work and wrote the paper is generally listed as the first author of a research paper.
- For published articles, other people who made substantial contributions to the work are also listed as authors.

CASE STUDY: MY MASTER'S RESEARCH PROJECT

- **Title: A detailed characterization of the *Nkx3.1* null mutant mouse model**

- **Context:**

  - *Nkx3.1* encodes a transcriptional repressor that acts as a negative regulator of prostate growth.
  - The *Nkx3.1* null mutant mouse recapitulates the early stages of human prostate carcinogenesis, so it could serve as a good model to understand prostate cancer initiation.

- **Goal:**

  - Gains insights into the role of *Nkx3.1* in prostate differentiation and organ integrity.
  - Determine consequences of *Nkx3.1* loss on the prostate immune microenvironment.

ABSTRACT (AKA SUMMARY)

- An abstract summarizes, in one paragraph, the major aspects of the entire paper in the following prescribed sequence:
  - The ***question(s) you investigated*** (or purpose) – Introduction
  - The ***experimental design*** and ***methods*** used – Methods (no excessive details)
  - The ***major findings*** including ***key quantitative results*** or ***trends*** – Results.
  - A brief summary of your ***interpretations*** and ***conclusions*** – Discussion.

- Length – 100 to 250 words.
- Limit your statements concerning each segment of the paper to two or three sentences.

- ***DON'T INCLUDE references, illustrations, or abbreviations.***
- ***ALWAYS write your abstract after you are done writing every other part of your paper.***

INTRODUCTION: WHAT TO INCLUDE?

Introduction serves the following purposes:

- Establish the context of the work being reported by discussing the relevant primary research literature and summarizing our current understanding of the problem being investigating.
- State the purpose of the work in the form of the hypothesis, question, or problem you investigated.
- Briefly explain your rationale and approach and, whenever possible, the possible outcomes your study can reveal.

Exemplifying through the case study:

- **Introduce background information** on prostate cancer (*e.g.* statistics, risk factors and progression, *etc.*) and the *Nkx3.1* gene (*e.g.* function, relevance to prostate cancer, *etc.*).
- **Purpose**: Determine role of Nkx3.1 in prostate differentiation, organ integrity, and immune microenvironment.
- **Rationale**: Nkx3.1 is deleted in 80-85% of all human prostate tumor samples.
- **Approach**: Analysis of changes in histology and protein expression patterns.
- **Possible outcome:** Discover the potential role of *Nkx3.1* in prostate cancer initiation.

MATERIALS AND METHODS

- Mention the organism studied, and if relevant, their pre-experiment handling and care, and when and where the study was carried out.
- For field studies, provide a description of the study site.
- What experiments did you do in order to answer your scientific question?
  Provide protocols and enough information here to allow another scientist to repeat your experiment.
- Mention how the data was analyzed (*e.g.* quantitative analysis, statistical procedures, *etc.*).
- Mention relevant ethical considerations (if any).

Hematoxylin and eosin staining protocol

1. Deparaffinization of slides:
a. Xylene - 5 minutes x 3 changes (change bath each time)
b. 100% ethanol - 3 minutes x 2 changes
c. 95% ethanol - 2 minutes
d. 70% ethanol - 2 minutes
e. 50% ethanol - 2 minutes
2. Wash slides in running water in sink – 3 minutes.
3. Dip slides in Surgipath Harris Hx (Hematoxylin) – 8 minutes.
4. Wash slides in running water briefly.
5. Dip slides very briefly in acid alcohol.
6. Wash slides in running water.
7. Blue the slides in lithium carbonate – 20 dips.
8. Wash slides in running water.
9. Dip slides in eosin – 20 seconds.
10. Wash in running water until clear.
11. 95% ethanol - 3 minutes
12. 100% ethanol – 3 minutes
13. 100% ethanol – 3 minutes
14. Xylene – 5 minutes x 3 changes (change bath each time).
15. Coverslip using Permount mounting medium: Clean coverslip using compressed air, place slide flat on a paper towel, and put a few drops of Permount on the coverslip.
17. Lower the coverslip slowly towards the slide gently, trying to avoid air bubbles on the slide,
18. Gently wipe the excess xylene from the underside of the slide using a paper towel.
19. Dry slides in hood.

Immunofluorescence staining protocol

1. Deparaffinization of slides (same as H&E staining).
2. Wash sections with distilled water – 2 minutes.
3. Antigen retrieval- Immerse slides into boiling citrate buffer, pH 6.0 - 10 minutes.
4. Wait until slides are cooled down. Wash the slides in PBS for 5 minutes.
5. Endogenous peroxidase blocking (This step could be excluded if HRP-enzymatic reaction is not involved in subsequent staining procedures). Incubate sections in 3% H_2O_2/methanol for 20 minutes.
6. Wash sections with distilled water – 5 minutes.
7. Wash sections with PBS – 5 minutes.
8. Permeabilization- incubate slides in 0.2% PBS-Tween 20 – 15 minutes x 2 changes.
9. Wash sections with PBS – 5 minutes x 2 changes.
10. Circle the sections with DAKO pen (hydrophobic pen).
11. Serum blocking: incubate sections in blocking solution (10% normal serum in PBS – serum origin is species in which secondary antibody is raised in).
12. Primary antibodies: incubate sections in the mixture of primary antibodies at 4 degrees Celsius for overnight in a dark humid chamber (Antibody diluent: 5% normal serum in PBS – serum origin is species in which secondary antibody is raised).
13. Wash sections with PBS – 5 minutes x 3 changes.
14. Secondary antibodies- **CASE-1-** Incubate sections in the mixture of fluorescent conjugated secondary antibodies at room temperature for 1 hour (Antibody diluent: 5% normal serum in PBS – serum origin is species in which secondary antibody is raised). **CASE-2-** In the case involving tyramide amplification, incubate sections in the mixture of HRP- and fluorescent conjugated secondary antibodies for 45 minutes.
15. Wash sections with PBS – 5 minutes x 3 changes. For **CASE-1** proceed to step 16 while for **CASE-2**, proceed to step 17.
16. **(CASE-1)** Mount with fluorescent mounting medium and cover sections with coverslip. Press on coverslip to remove excess mounting medium. Seal coverslip with nail polish.
17. Prepare tyramine amplification solution as suggested by the manufacturers protocol. Apply section with tyramide amplification solution for 6 minutes.
18. Wash sections with PBS – 5 minutes x 3 changes.
19. Do as stated in step 16.
20. Store slides in dark at 4 degrees Celsius.

Immunohistochemical staining protocol

Steps 1-3 are the same as the immunofluorescence staining protocol.
4. Wait until slides are cooled down. Wash the slides in 0.1% PBS-Tween 20 (PBST) - 5 minutes x 2 changes.
6. Rinse slides on shaker in distilled water for 10 minutes.
7. Rinse slides on shaker in PBS pH 7.4 for 10 minutes.
8. Serum blocking (same as Step 11 on immunofluorescence staining protocol).
9. Primary antibodies: incubate sections in the mixture of primary antibodies at 4 degrees Celsius for overnight (Antibody diluent: 1% normal serum in PBS – serum origin is species in which secondary antibody is raised).
10. Wash slides in PBST - 5 minutes x 3 changes.
11. Make ABC media 30 minutes prior to usage: Add 2 drops of solution A and 2 drops of solution B for every 5 ml of PBS. Add ABC solution to tissue, and incubate in dark, humid chamber at room temperature for 45 minutes.
12. Wash slides in PBST – 5 minutes x 3 changes.
13. Development in NovaRed solution: For every 5 ml of distilled water, add 3 drops of solution 1, then 2 drops each of solution 2, 3 and 4. Add 2-3 drops of NovaRed solution per section to develop. Check at microscope when signal is optimal.
14. Stop reaction by dipping slides immediately into distilled water after development.
15. Incubate slides in distilled water for 5 minutes on shaker.
16. Hematoxylin staining: dip slides in hematoxylin for 4-6 seconds, then stop reaction by dipping slides immediately into distilled water.
17. Incubate slides in distilled water for 5 minutes on shaker.
18. Lithium carbonate: dip slides in lithium carbonate 5 times.
19. Incubate slides in distilled water for 5 minutes on shaker.
20. Remove from water and allow slides to dry in hood overnight.
21. Dip slides in xylene.
22. Coverslip using Permount mounting medium: Clean coverslip using compressed air, place slide flat on a paper towel, and put a few drops of Permount on the coverslip.
23. Lower the coverslip slowly towards the slide gently, trying to avoid air bubbles on the slide,
24. Gently wipe the excess xylene from the underside of the slide using a paper towel.
25. Dry slides in hood.

RESULTS: PRESENTING THE DATA

- Objectively present the results, without interpretation, in an orderly and logical sequence using both text and illustrative materials (Tables and Figures).
- Do NOT discuss or interpret the results - that goes in the Discussion.
- Only include the key data, tables and graphs in Results.
- The results section always begins with text, reporting the key results and referring to your figures and tables as you proceed.

Sections of the anterior prostate tissue from *Nkx3.1*$^{+/+}$ (Wild type) and *Nkx3.1*$^{-/-}$ (null mutant) aged 4 and 12 months were IF stained using anti-CK5 and anti-CK8 antibodies, which selectively label basal cells and luminal cells in the prostate, respectively. The final count data obtained from the analysis of these stains were converted to percentage, and have been tabulated in Table-1 and Table-2.

Table-1: Percentage of CK5^{+} and CK8^{+} cells in the anterior prostate tissue of 4-month-old *Nkx3.1*$^{+/+}$ mice (Mouse 1 and 2) and *Nkx3.1*$^{-/-}$ mice (Mouse 3 and 4).

	Genotype	% CK5	% CK8
Mouse-1	*Nkx3.1*$^{+/+}$ (Wild type)	13.10	86.90
Mouse-2	*Nkx3.1*$^{+/+}$ (Wild type)	14.50	85.50
Mouse-3	*Nkx3.1*$^{-/-}$ (Null mutant)	14.80	85.20
Mouse-4	*Nkx3.1*$^{-/-}$ (Null mutant)	11.10	88.90

Table-2: Percentage of CK5^{+} and CK8^{+} cells in the anterior prostate tissue of 12-month-old *Nkx3.1*$^{+/+}$ mice (Mouse 4) and *Nkx3.1*$^{-/-}$ mice (Mouse 5 and 6).

	Genotype	% CK5	% CK8
Mouse-4	*Nkx3.1*$^{+/+}$ (Wild type)	16.80	83.20
Mouse-5	*Nkx3.1*$^{-/-}$ (Null mutant)	20.02	79.98
Mouse-6	*Nkx3.1*$^{-/-}$ (Null mutant)	16.29	83.71

Each Table or Figure must include a brief description of the results being presented and other necessary information in a legend.

Tables and Figures are assigned numbers separately and in the sequence that you will refer to them from the text

Table legends go above the Table

Table-3: CK5 and CK8 expression in SVP2 positive cells in the anterior prostate of 4-month-old (Mouse 1 and 2) and 12-month-old (Mouse 3 and 4) *Nkx3.1*$^{-/-}$ mice.

	Age	% SVP2 CK5	% SVP2 CK8
Mouse-1	4 months	0.52	99.48
Mouse-2	4 months	0.00	100.00
Mouse-3	12 months	0.00	100.00
Mouse-4	12 months	0.00	100.00

Figure legends go below the Figure

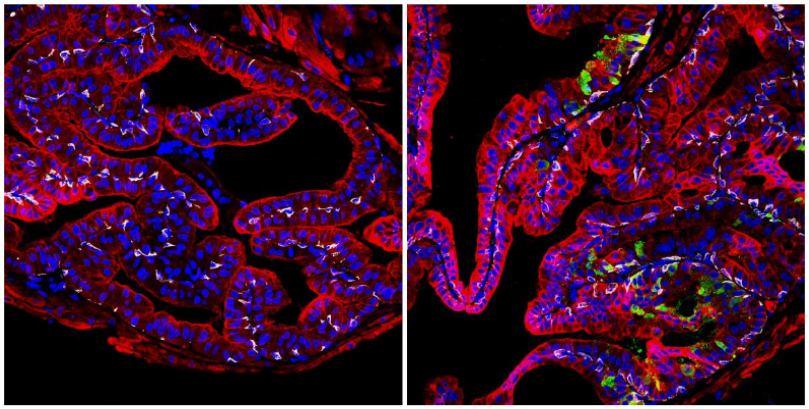

Figure- 2A Figure- 2B

Figure-2: Results of CK5-CK8-SVP2 IF staining done on 4 month old mice: A) Wild type mice B) *Nkx3.1*$^{-/-}$ mice. Blue = DAPI, White = CK5, Red = CK8, Green = SVP2.

DISCUSSION: INTERPRETING THE DATA

- **Include your interpretation of the experimental results in light of what was already known about the subject of the investigation.**
- **Convey to the audience how our understanding of the topic/field might have changed after taking your results into consideration.**
- **How do these results relate to the original question?**
  - How can my results establish the link between *Nkx3.1* and prostate cancer initiation?
- **Do the data support your hypothesis?**
  - Does my data show that *Nkx3.1* causes the initiation of prostate cancer? If so, how?
- **Are your results consistent with what other investigators have reported? If your results were unexpected, try to explain why.**
- **Is there another way to interpret your results?**
  - *e.g.* my results could be implying that *Nkx3.1* is actually just hampering prostate development
- **What further research would be necessary to answer the questions raised by your results?**
  - *e.g.* use conditional mutants instead of germline mutants
- **Conclude with: How do your results fit into the big picture?**

HOW TO PROPERLY CITE LITERATURE

- Proper referencing is required for any information, material, illustrations, *etc.* that you include in your scientific paper that did not originate from your own work.
- If you do not reference you are committing plagiarism, which is a punishable offense.
- Within the body of the text, there are several different ways to reference:
  1. According to Plassman *et al.* (2008), there are several different……
  2. Since then ducks (Indigo and Mauve, 1994) and platypuses (Magenta, *et al.*, 1995)……
  3. Harry Potter was a wizard[1] and so was Gandalf[2]……

  If you use (1) or (2) and the paper being referenced has one or two author(s), include the last name of the author(s) and the year of publication. If the paper has more than two authors, include the last name of the first author + *et al.*, followed by the year of the publication.
- If you use (1) or (2), you should list the citations in alphabetical order in the References section. If you use (3), should arrange them according to their order of appearance in the text (1, 2, 3, *etc.*)
- Always rephrase the words when referencing someone else's work. If you cannot rephrase, use quotes (*e.g.* According to Muse (2001), "80% of inmates were insane")

PREPARING THE REFERENCES SECTION

- You can prepare the reference section in the following ways (ordered by ease):
  1. Use a referencing software e.g. Endnote (best option), RefWorks, etc.
  2. Derive citation from the journal website.
  3. Manually prepare the properly structured citation.
- Styles of citation:
  - **APA:** Alibali, M. W. (1999). How children change their minds: Strategy change can be gradual or abrupt. *Developmental Psychology, 35,* 127-145. http://pitt.libguides.com/c.php?g=12108&p=64730
  - **MLA:** Doggart, Julia. "Minding the Gap: Realizing Our Ideal Community Writing Assistance Program." *The Community Literacy Journal* 2.1 (2007): 71-80. Print. http://pitt.libguides.com/c.php?g=12108&p=64731

APPENDIX: EXTRA INFORMATION

- Show calculations for how the results were derived.

Protein Yield calculations.

Table-2 calculations
Buffer-2: 10.9/35 x 100% = 31.14%
Buffer-3: 21.4/35 x 100% = 61.14%

Table-3 calculations
Filtered: 29.71/35 x 100% = 84.85%
Unfiltered: 9.90/35 x 100% = 28.29%

Table-4 calculations
4°C: 24.50/35 x 100% = 70%

Table-5 calculations
Dialyzed protein: 25.54/35 x 100% = 73%

Table-6 calculations
35 µg/ml: 23.46/35 x 100% = 67%

- Mention the recipe of the solutions that were used in the experiments.

M9 minimal media recipe

For every litre of M9-

Na_2HPO_4- 6g
KH_2PO_4- 3g
NaCl- 0.5g
$N^{14}H_4Cl$- 1.0 g
pH- 7.5
$CaCl_2$- 1 ml of 0.1 M solution.
$MgSO_4.7H_2O$- 1 ml of 1 M solution.
40% Glucose (dextrose)- 10 mL
Ampicillin- 100 mg
Vitamins- 2 ml
Biotin & Thiamine- 1 ml each of 10 mg/ml stock solution.

USEFUL WEBSITES AND MATERIALS

- http://abacus.bates.edu/~ganderso/biology/resources/writing/HTWsections.html
- http://www.columbia.edu/cu/biology/ug/research/paper.html
- http://www.nature.com/scitable/ebooks/english-communication-for-scientists-14053993/118519636#headerAndCitation
- Victoria E. McMillan, Writing Papers in the Biological Sciences, Bedford Books, Boston, 1997
- Robert S. Day, How to Write and Publish a Scientific Paper, 4th edition, Oryx Press, Phoenix, 1994.