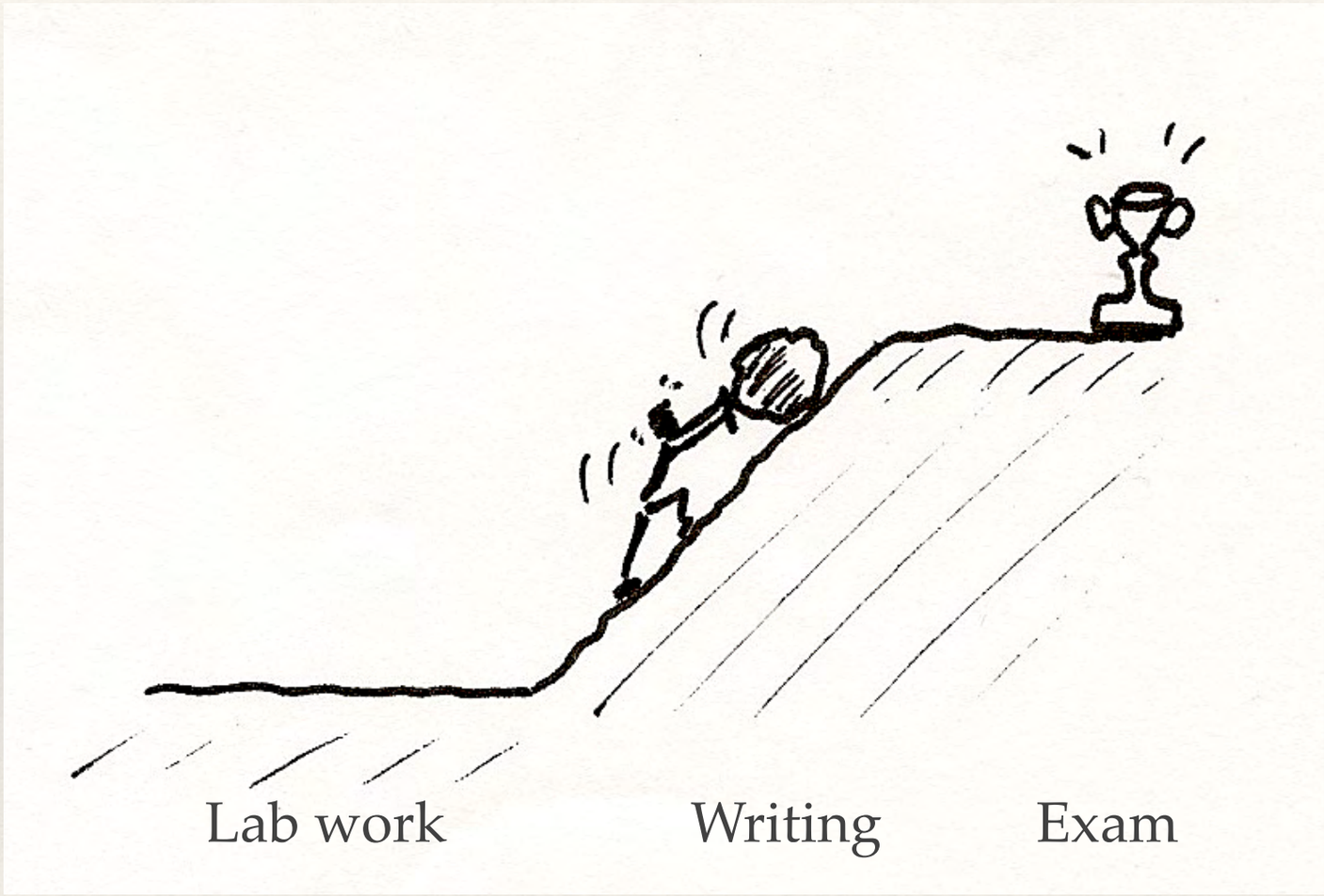
A supervisors perspective:

How to write a Master thesis

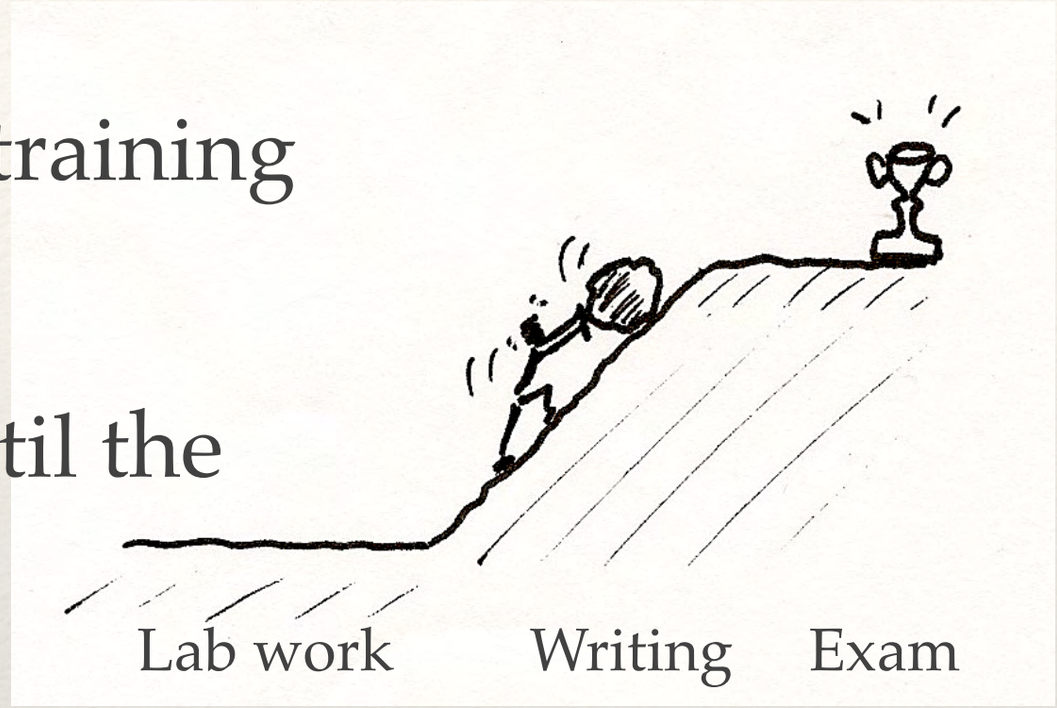
Odd Stokke Gabrielsen

A common experience



General

- Most students have quite limited training in academic writing
- Start early to write - don't wait until the practical work is over
- Writing is clearing your thoughts, may give you a better grasp of your own research work and new and better ideas how to progress
- Ask your supervisor how to start and how to get feedback



One way of doing it ….

Training in writing a master thesis

- One week focused on writing (Intro, methods, results so far) - with feedback

Language

- academic written english

- writing skills

Language - remember KISS

- **KISS = keep it short and simple**
- Convey the message clearly and simply using the minimum number og words
- Keep the sentences short:
  - easy to write
  - easy to read
  - easy to move
  - easy to punctuate
  - An average of 15 words or less per sentence

People (sensors) are slow at understanding things

- It takes people time to understand things
- They may not be experts in your specific research topic
- If they have to read it again and again, then it becomes boring and …
- Try to re-word your explanations

An example

Abstract

Altered post-translational protein modifications (PTMs) and misregulation of transcription factors (TFs) have long been associated with cancer development. Many of the TFs are modified at multiple sites by a diverse set of PTMs. Key modifications are phosphorylation, often linked to cellular signalling, and SUMOylation, usually causing repression of transcription. Different PTMs on the same protein may be connected through crosstalk. SUMOylation of TFs has been extensively studied, but our current understanding of SUMO-linked PTMs is limited.

Here, we have studied PTM crosstalk on the proto-oncogenic transcription factor c-Myb by investigating the link between SUMO conjugation and phosphorylation. We show that SUMO conjugated to c-Myb directs the recruitment of the nuclear homeodomain-interacting protein kinase 1, HIPK1, making the SUMOylated protein a preferred kinase substrate. The SUMO-binding property of HIPK1 is dependent on an active kinase domain. The SUMO-docking property extends to HIPK2 and CtBP1. The functional consequence of HIPK1 phosphorylating c-Myb, tested on a chromatin embedded reporter, was found to be highly dependent on the coactivator p300. Together, HIPK1 and p300 partly derepress the SUMO-repressed c-Myb, suggesting that the HIPK1-induced phosphorylations counteract the effect of SUMOylation.

The nuclear kinases HIPK1 and HIPK2 appear to preferentially phosphorylate targets that are SUMOylated, as observed for c-Myb and CtBP1. This represents a novel mechanism of PTM crosstalk between SUMOylation and phosphorylation.

Furthermore, HIPK1 in cooperation with p300 partly derepresses the SUMO-repressed c-Myb, suggesting that this crosstalk modulates the negative effect of SUMOylation.

Abstract

Many transcription factors are modified at multiple sites by a diverse set of post-translational modifications (PTMs), which may be connected through crosstalk. Here, we have studied PTM crosstalk on the proto-oncogenic transcription factor c-Myb by investigating the link between SUMO conjugation and phosphorylation. We show that SUMO conjugated to c-Myb directs the recruitment of the nuclear homeodomain-interacting protein kinase 1, HIPK1, making the SUMOylated protein a preferred kinase substrate. Similar SUMO-docking is observed with HIPK2 and CtBP1. When tested on a chromatin-embedded reporter, HIPK1 activated c-Myb in the presence of the coactivator p300. Our studies identify a novel mechanism of PTM crosstalk between SUMOylation and phosphorylation.

Avoid long sentences

- The average length of a sentence in English has become shorter and shorter over the centuries
  - Shakespeare's time: 45 words per sentence
  - 150 years ago: 29 words per sentence
  - Today 15-18 words per sentence are recommended
  - Goal in academic language: < 25 words per sentence
- The longer your sentence, the greater the chance it will be misunderstood
- Breaking up long sentences
  - look for link words such as "and", "as well as", "moreover", "in fact", "although", … and break up the long sentence with a full stop and beginning of a new sentence

Delete unnecessary words

- Example:
  - In order to... better: To...
  - Due to the fact that... better: Because...
  - Lower amount of... better: Less...
  - Similar in nature to... better: Like
- To start sentences:
  - not start with also, and, but,..
  - keep it grammatically simple

Word order

- Basic word order in English: (1) Subject (2) verb (3) direct object and (4) indirect object
- Put the subject before the verb
  - *wrong*: In the survey participated 350 subjects
  - *correct*: Three hundred and fifty subjects participated in the survey
  - *wrong*: Of particular interest was the sugar transporter, because …
  - *correct*: The sugar transporter was of particular interest, because ….
- Keep the subject and verb as close as possible to each other

a, an or the

- In English, singular nouns are almost always preceded by a, an or the
  - Literature versus the literature
  - In case versus in the case

Active - passive

- Traditionally, practical reports in science are written in an impersonal style that supports the notion of being objective. The procedure, for example, is written impersonally:
  - 'The calorimeter was calibrated ... ' and 'After crystallisation, the purple residue was dissolved in ... '
- By avoiding any reference to a specific investigator, the report seeks to convey that the investigation has been carried out and reported on objectively, in an unbiased manner.

Use of tenses

- A MSc-thesis is written **largely in the past tense**. As a general rule, use the past tense when describing what was done and then reporting the results.
- **Exceptions** to the 'past tense' rule include:
  - Using the **present** tense if you are making a **general statement** about something that applies through time. For example, 'Standard practice is to allow the calorimeter to cool overnight to equilibrate to ambient conditions.'
  - Employing the **present** tense in the Method or Results section if you refer to a table or graph. For example 'The table shows ... '.
  - Adopting the **present** tense in the Discussion and/or Conclusion if commenting on some aspect of your results, or making suggestions for improvement. For example, 'Taken overall, the results show ... ' or 'It is suggested that ... '.
  - Using the **future** tense in the Discussion if referring to something that will take place in the future. For example, 'In the next growing season the procedure will be repeated but with modifications, taking into account . .. '.

Collect and use standard phrases

- Read papers in your field, select those you find easy to read and note for yourself general and useful phrases
  - General expressions are not plagiarism
- Other sources:
  - www.phrasebank.manchester.ac.uk

Before you write - Think!

- What are the main questions you are answering?
- Which results give answers to these questions?
- Formulate your story around these answers.

Clear logic - problem and answers

A MSc-thesis is a scientific contribution

- Defining a problem
- Defining one or a few hypotheses
- Planning an approach - an analysis

- This should be briefly stated in “aim of the work”
- Results - telling about your analysis and results
- Discussion of how the problem has been solved
- Conclusions

Your written story - not like this

much better like this

The overall logic - easily recognized structure

- A problem - some hypotheses - results - discussion and conclusion.
- Recognized in the title
- Structuring the summary

- Logic found clearly stated in the “aim of the work”
- Defining subchapters in the Results section
- Defining subchapters in the Discussion section

An example

- Title
- Aim of the work
- Results
- Discussion

Design of an inducible system to control SUMOylation of the transcription factor c-Myb *in vivo*

The thesis is divided into three parts, based on three key questions.

1. Can SUMOylation of c-Myb be induced *in vivo* by heterodimerization of c-Myb and Ubc9?
2. Can deSUMOylation of c-Myb be induced *in vivo* by heterodimerization of c-Myb and SENP1?

f c-Myb be used to address
and SUMO-dependent possess
pendent protein interaction?

Another example

- Title

- Aim of the work
- Results
- Discussion

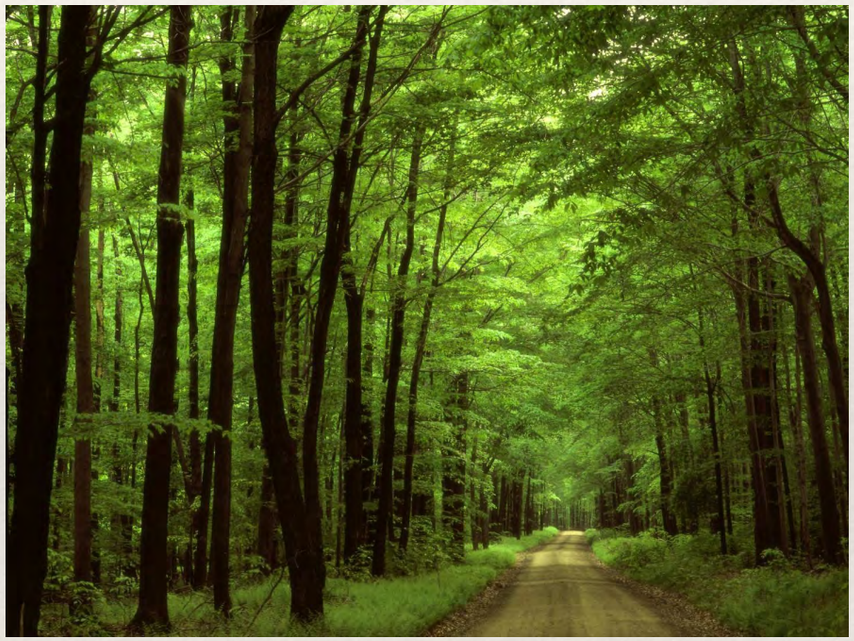
Studying the interaction between c-Myb and the menin/MLL system

This study is divided in two main parts with several questions to be addressed in each part. Most of the questions are approached by interaction studies and primarily GST pulldown experiments.

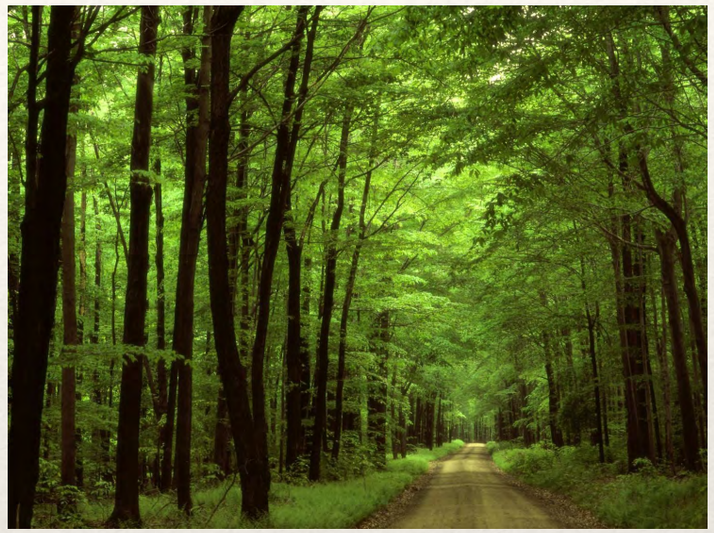
Part 1 is a detailed study of the interaction between c-Myb and menin and consists of five main questions:

1. To which regions of c-Myb does menin bind?
2. Is the interaction between c-Myb and menin direct?
3. Is the interaction between c-Myb and menin dependent on a post-translational

Structure helps the reader

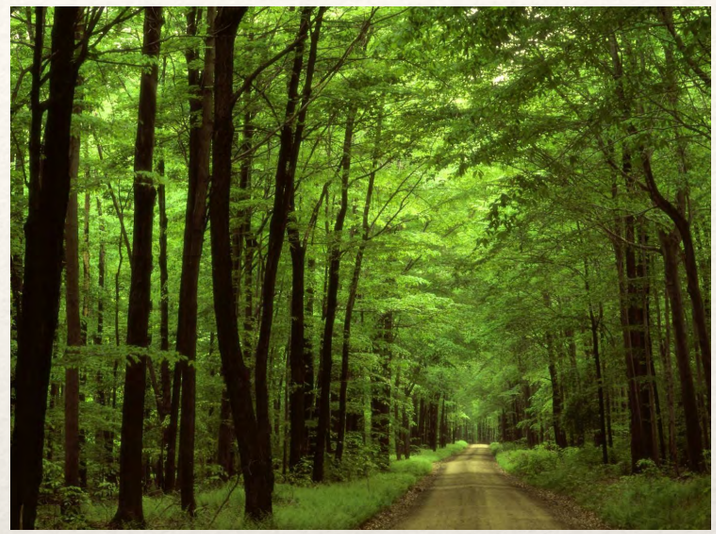


Help the reader by using clear leaders



- Some examples of leaders:
  - The problem is... (describe the problem)
  - To solve this problem... (describe what you did)
  - The most important finding is...
  - Let us consider the example of...
  - Comparing table 1, 2 and 3, we see that...

Helping your reader with small phrases …



- Having found that …, we next asked whether …
- To determine if …, we next examined …
- Our previous studies had shown that … x suggested that … To test this we set up..
- Since it has been shown previously that …but that … we wondered whether …
- In addition to performing … experiments … we also determined …
- To confirm that … were indeed …, we carried out …
- The experiments reported above demonstrate that …, but it is possible that …
- To verify and extend this result, we used a second assay …

Remind your reader of where we have been and now are going

Helping your reader along - examples

Where we come from …

Where we are going …

- **After construction** of all plasmids and expression of the proteins, **the interaction studies could begin**. **One of the goals** of this study was to map the menin-interacting domain of c-Myb. **Furthermore, we asked** whether the interaction was direct or if it was dependent on ….

Part II

The standard sections

A classical subdivision

- Title
- Summary
- Introduction - leading up to “aim of the work”
- Materials and Methods
- Results
- Discussion
- References
- Appendix

Title

- A given title when you start
- *… your research may have changed the problem ….*
- A good title finalized in the last moment

Writing the abstract

- The abstract is the thesis in one paragraph. There are different abstract styles. One example is:
  - The concept of ... is introduced and the problems ... are outlined.
  - However, ... is not fully understood
  - Here, such-and-such ... is addressed ... is observed. ... This is supported by ... In addition, we show ...
  - Conclusion, impact ... These findings define ... Our research demonstrates that ... Overall, our data uncover mechanisms of a critical ...

Abstract - summary

- An abstract **summarises** the investigation's context, aim, method, results and conclusion for the investigation. As in a published research paper, an abstract captures the **important features**. It gives the reader sufficient information to decide whether the report is of interest and should be read.
- An abstract is normally between 150 and 300 words
- Typically, the abstract gives:
  - the **context** for the investigation
  - the **aim**(s) of the investigation
  - what was carried out (**method**)
  - what was discovered (**results**)
  - what was concluded (**conclusion**).
- A well-written abstract has a balance of the above features. Being brief, the abstract **does not include a discussion**. Traditionally, it does not cite references.

An example

The context

- The c-Myb transcription factor is a key regulator of haematopoiesis. Its activity is regulated by a large network of protein interactions and post-translational modifications. ATF7IP is a transcriptional cofactor previously implicated both as a repressive and activating modulator of other transcription factors, depending on site and context. A recent yeast two-hybrid screening in our lab identified ATF7IP as a putative interaction partner of c-Myb.

Aims

.. hypotheses

- In this thesis, the interaction between ATF7IP and c-Myb was investigated and validated using GST-pulldown analyses. We found ATF7IP to associate with c-Myb in a SUMO-enhanced manner. Initially we proposed two hypothetical models for the functional consequences of how ATF7IP modulates c-Myb function: The Epigenetic Repressor Model and The Mediator-like Activation Model. Using various reporter gene assays, we found no evidence for the first model, but ATF7IP-induced activation in two different reporter assays. We conclude that ATF7IP acts as a novel c-Myb coactivator, presumably by bridging enhancer-bound c-Myb to the general transcription apparatus, thereby promoting the initiation of c-Myb target gene expression.

Results

Conclusions

166 words

Abstract - summary

- To be written towards the end when the rest is finished
- The entrance door for the sensors
- Check: would I have understood this, if it was the first time I read anything on this topic?
- The background, the problem addressed and your results in a compact format written in a form that is easily understood for most persons with a bio-scientific background

Introduction

- The introduction provides the context for the rest of the thesis.
- Typically, it contains some or all of the following elements:
  - Why the investigation is important or useful.
  - The theoretical and research context for the investigation, citing relevant literature.
  - Key definitions and abbreviations.
- The aim(s) of the investigation, questions it seeks to answer or hypotheses it seeks to test.

Introduction

- How much background?
  - More than you think.
  - Give the reader something to latch on to.
  - Always explain once what the acronyms mean.

Introduction

- Two parts:
  - a longer Introduction section
  - and a shorter “Aims of the work”
- Introduction
  - Clear overview of the background, the relevant literature
  - Precise and insightful, still easy to read
  - Interesting discoveries and problems, what we know and what we don’t understand
  - not only a lexical list of information - boring
- Your research problem defined in **aims of work**
  - One of the most important parts of the thesis
  - Should be well written

Aim of the work

- The end of the Introduction
- One of the most important parts of the thesis
- Here is where you find your problem stated, your hypotheses defined, and your plan how to approach your work
- Should be well written
- Most sensors will read this with particular attention

Aim of the work - example

- Typically, the "Aims" gives:
  - briefly summarized the context for the investigation
  - clearly state the hypothesis and the aim(s) of the investigation
- briefly how you will approach the investigation
- should be written as if it was formulated before the work started

The thesis is divided into three parts, based on three key questions.

1. Can SUMOylation of c-Myb be induced *in vivo* by heterodimerization of c-Myb and Ubc9?

2. Can deSUMOylation of c-Myb be induced *in vivo* by heterodimerization of c-Myb and SENP1?

3. Can induced SUMOylation and deSUMOylation of c-Myb be used to address questions concerning c-Myb modification dynamics and SUMO-dependent possess like; SUMO-dependent phosphorylation and SUMO-dependent protein interaction?

In order to answer the key questions, is it also necessary to address whether the fusion

(Materials and) Methods

- Conventionally, a Method section (sometimes called Materials and Methods, or Procedure) gives enough detail so that a reader can repeat the investigation using the information provided.
- SOP - standard operating procedures - a new source
- General procedures in M&M - specifics in the Results section (in particular in Figure legends)

A full Method section typically contains:

- Experimental subjects.
  - Microbes, plants, animals or people that are the subjects of the investigation. Where appropriate, give precise information about their characteristics and how samples were obtained.
- Materials.
  - Chemicals (including detail of amounts, concentrations, physical form, and so on) and other media (such as a particular growth medium for microbes or plants).
- Conditions.
  - Physical factors, such as temperature and pressure, and any other factors that are likely to influence the outcome of the investigation.
- Apparatus.
  - Equipment of all kinds, including measuring and recording devices, used in carrying out the investigation.
- Procedure. What was done, how and, where appropriate, why.

A classical subdivision

- ✓ Title
- ✓ Summary
- ✓ Introduction - leading up to “aim of the work”
- ✓ Materials and Methods
- Results
- Discussion
- References
- Appendix

Results

- A results section does not normally contain raw data. Rather, it contains data that are presented and analysed in ways that respond to the investigation's aim(s).
- It is usual to guide the reader through the presented data, highlighting the points you wish to bring to the reader's attention, which will be referred to in the discussion and conclusion.
- The data are typically presented in numbered tables and/or graphs, which are referred to in the text.
  - A graph or chart - such as line graphs, scatter plots, bar charts and histograms - reveals relationships between variables in a visual form.
  - Data presented in tables or graphs may be accompanied by statistical analyses, together with their interpretation.

Results

- Overall structure shown in the headings
  - build your result section based more on logic than on historic progression
- For each experiment described, include the following:
  - Introduce why you are doing this experiment - the problem addressed
  - Approach - the set up of your experiment
  - What you observed - refer to a figure, table, graph ..
  - What did you conclude from this experiment
  - Transition to next experiment
- Tenses: an experiment is written largely in the past tense, while conclusions are written in the present tense

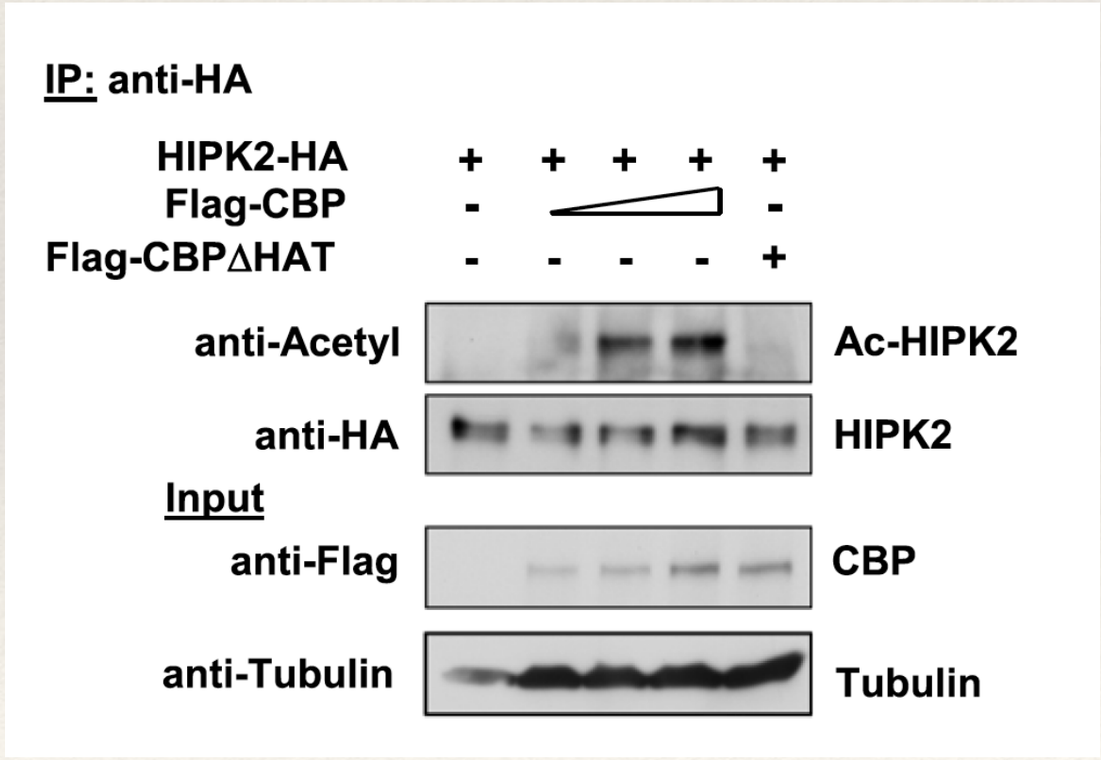
Results

- Show a critical, scientific attitude in your conclusions - don't try to push your data to support stronger conclusions than you have reasons for.
- Remember that you are evaluated not primarily from your experimental data and results, but from your approach, problem solving and your critical analysis of your data.
- Show that you have a acquired a mature research culture

Figures and tables

- Figures - what should be included? gels and blots

  - Framed picture(s)
  - Information around
  - Readable size
  - One common style throughout the thesis



Example

- The figure legend should be readable in itself independent of the running text
- Most details may be put into the figure legend

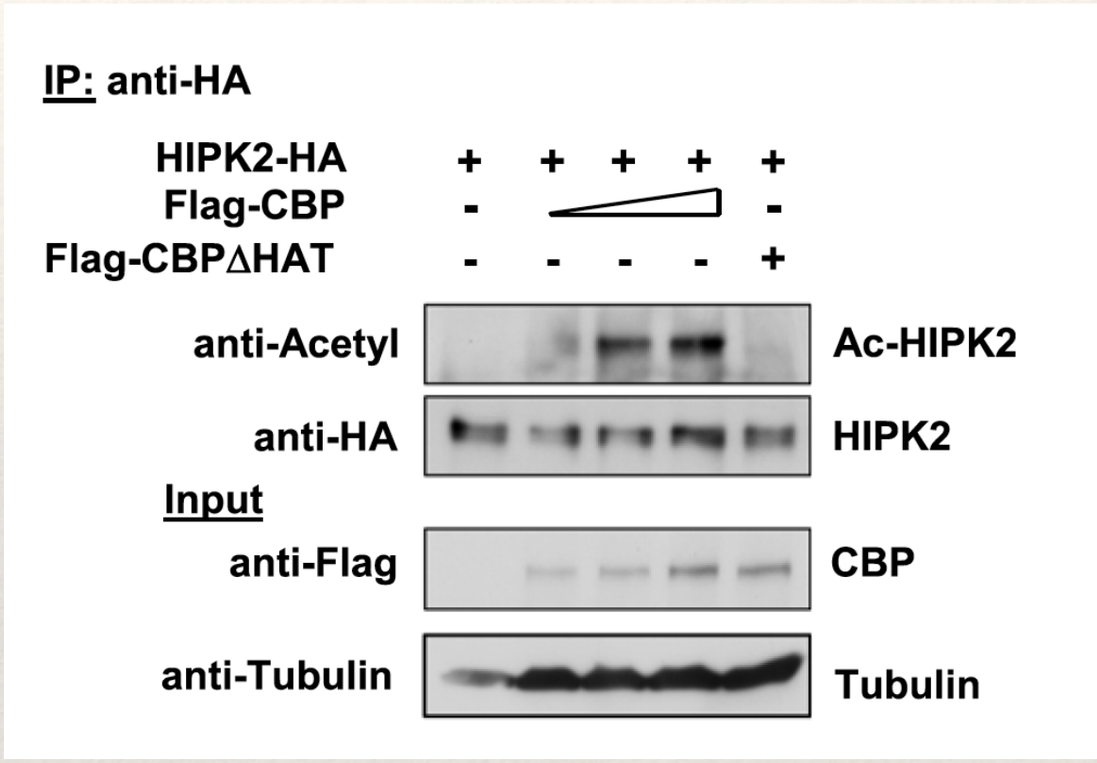


Figure 1. HIPK2 Is Modified by Acetylation

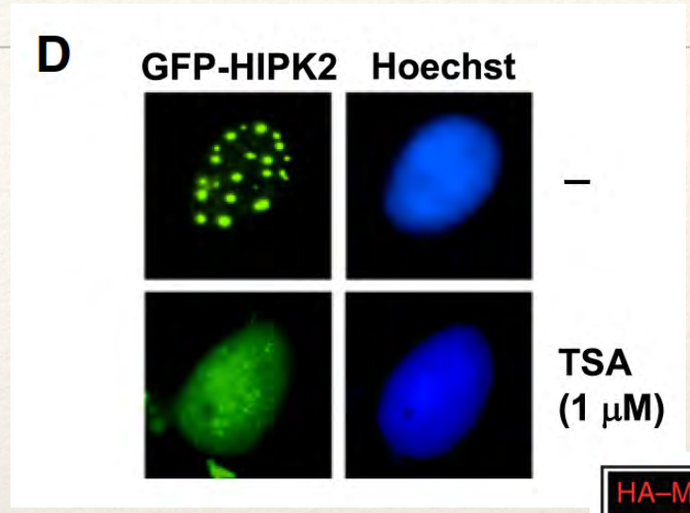
(A) 293T cells were transiently transfected to express the indicated Flag-tagged kinases along with YFP-tagged CBP as shown. A fraction of the cell lysates was tested for the correct expression of the transfected CBP by western blotting, as shown in the lower part. Equal amounts of protein in the remaining extracts were used for IP of the kinases with anti-Flag antibody. After elution of bound proteins in 1 × SDS sample buffer, protein acetylation was revealed by western blotting (WB) using an antibody recognizing acetylated lysines. The star indicates a nonspecific band.

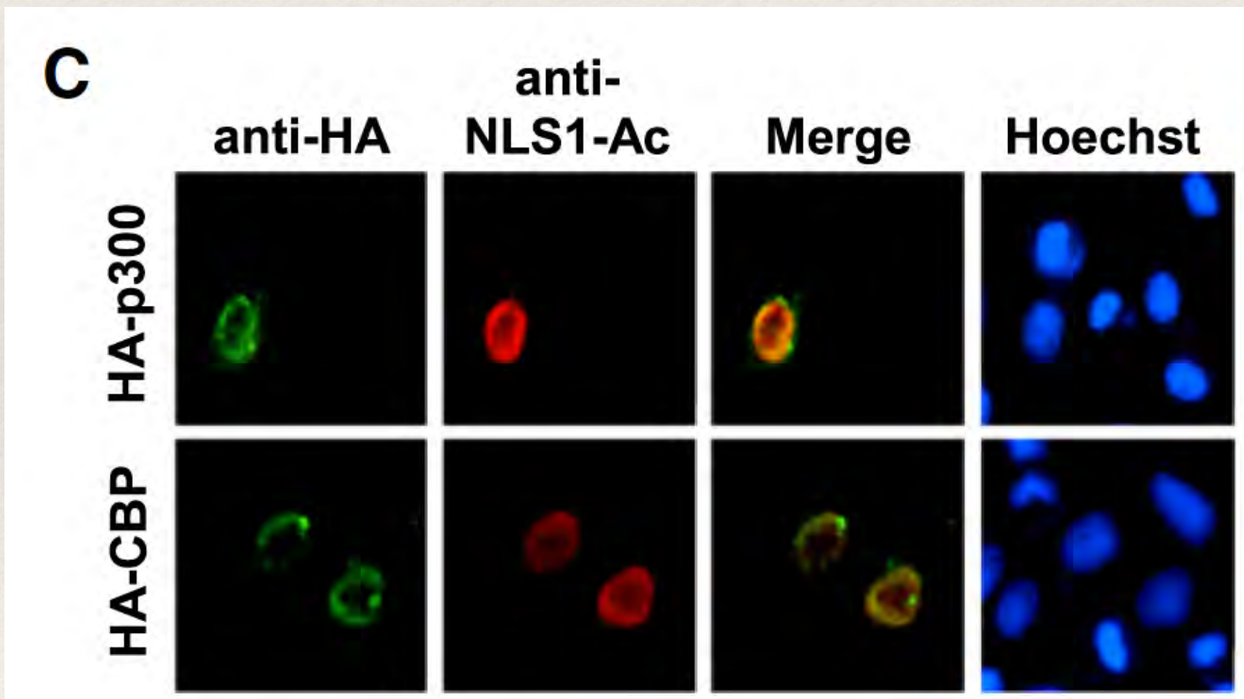
(B) 293T cells were transfected with Flag-tagged HIPK2 and the indicated acetyltransferases. One part of the lysates was used as an input and loading control (lower), whereas the remaining extract was used for IP of HIPK2 and western blot analysis to detect its acetylation.

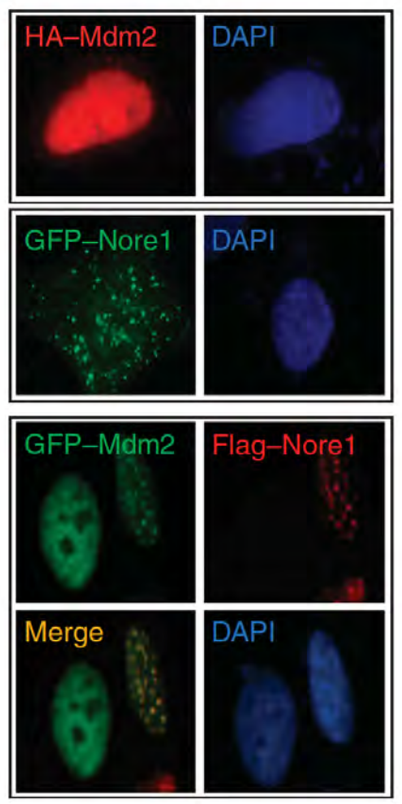
(C) 293T cells were transfected with expression vectors for HIPK2, increasing amounts of CBP, and a mutant thereof with a defect HAT activity (CBPΔHAT). Expression of the transfected proteins and HIPK2 acetylation was analyzed as in (B).

Figures - what should be included? microscopy

- (Framed) picture(s)
- Information around (transfections, Ab) or treatment
- Alternatively, text in the picture
- Readable size
- One common style throughout the paper
- Take a careful look into some publications from your own field of interest and notice how figures are set up



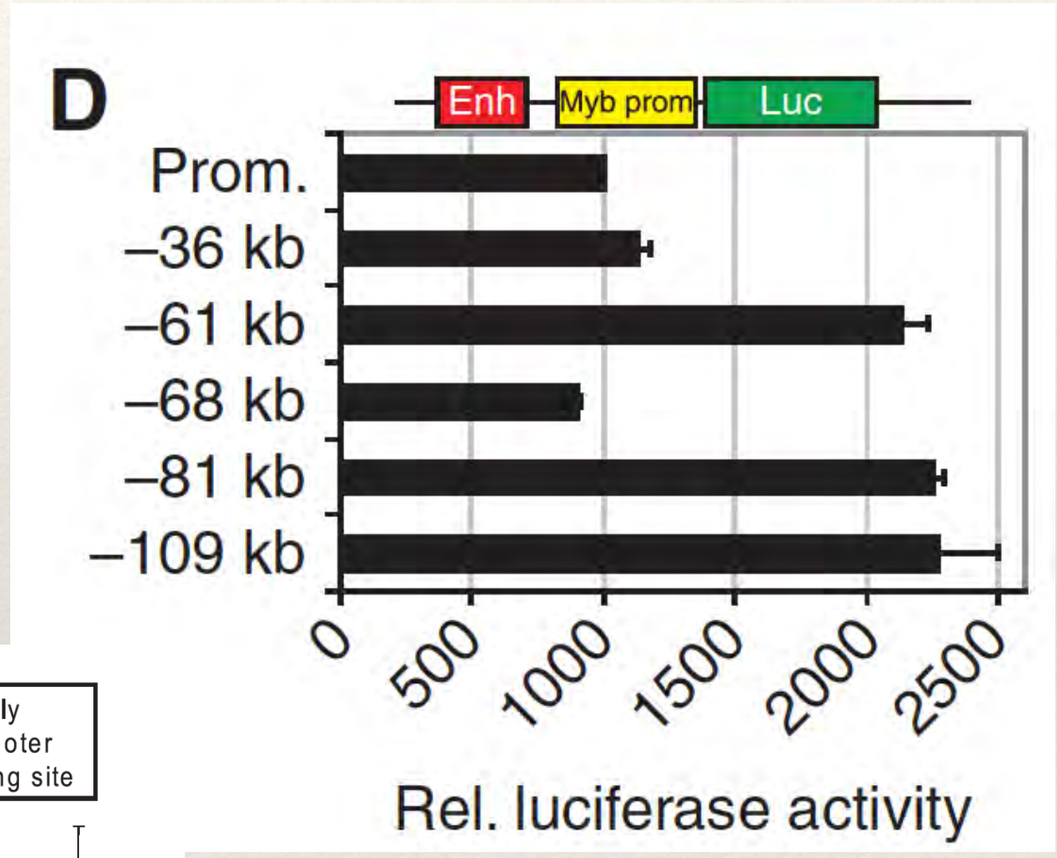


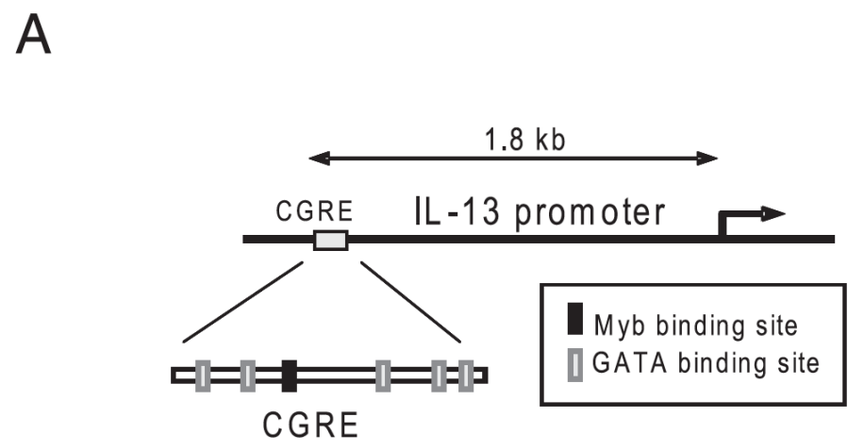


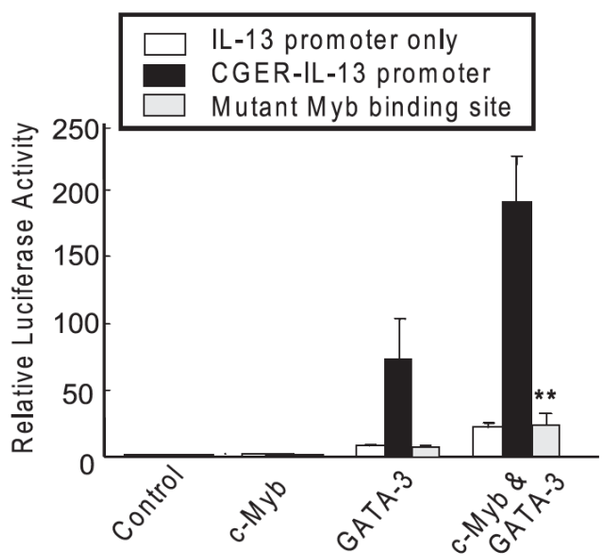
(C) U2OS cells transfected to express HA-tagged CBP or p300 were analyzed by indirect immunofluorescence for localization of the expressed HATs and endogenous acetylated HIPK2. Chromosomal DNA was stained with Hoechst, and the merged images indicate colocalization in yellow color.

Figures - what should be included? Numbers & measurements

- (Framed) graph(s)
- Horizontal or vertical
- Information around
- Illustration

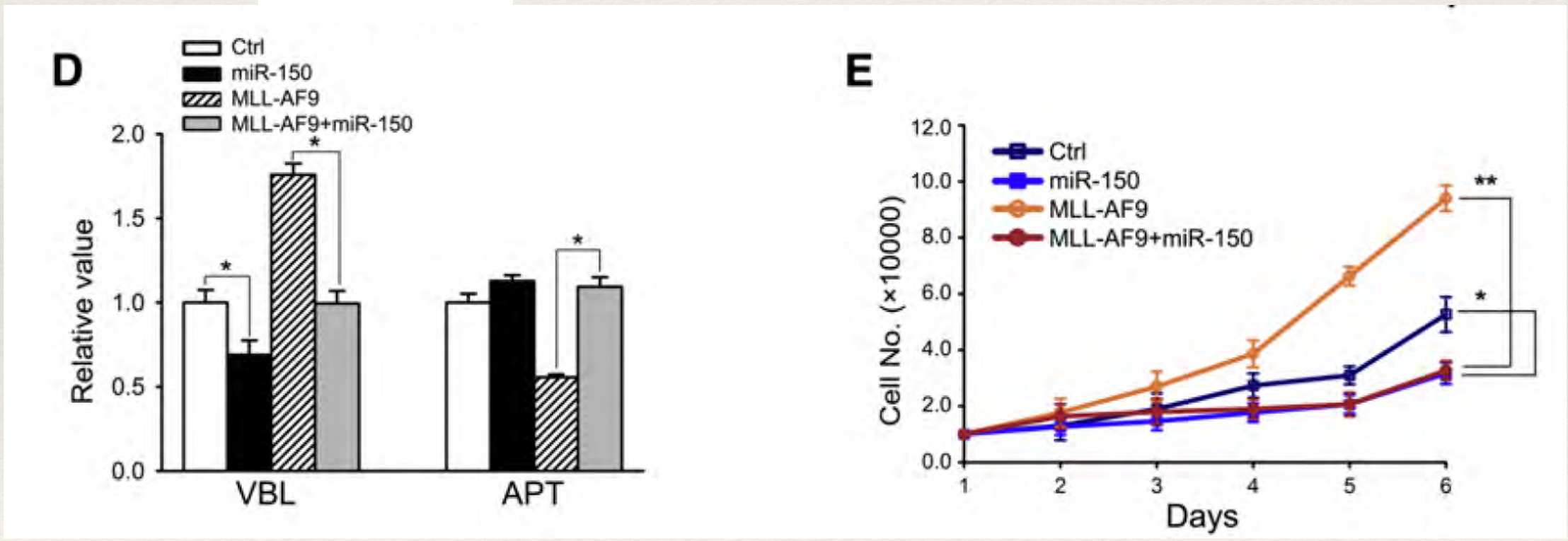






Statistics in figures

(D and E) Effects of miR-150 on viability/apoptosis (D) and proliferation (E) of mouse BM progenitor cells in the presence or absence of MLL-AF9. Mean ± SD values are shown. *p < 0.05; **p < 0.01.



Quantitative graphics

- Rules of thumbs
  - Always say something about the error in your measurements. Include error bars in your graphs.
  - Always show the initial point on your axes
  - Always scale % graphs to 100%.

Graphics

- Qualitative graphics
- Cartoons have enormous power. Everyone understands them, and they draw attention.
- Photos create understanding and sympathy.
- Pay attention to detail, color, contrast, fonts

A classical subdivision

- ✓ Title
- ✓ Summary
- ✓ Introduction - leading up to “aim of the work”
- ✓ Materials and Methods
- ✓ Results
- Discussion
- References
- Appendix

Results vs. Discussion

- Results: what you observed in your experiments
- Discussion: what you think

Discussion

- The discussion of a MSc thesis is typically a distinct section after the data have been presented. It includes some or all of the following items (with slight variations according to the discipline):
- A discussion of the results in response to the aim and in relation to other people's findings from the research literature.
- It critiques the investigation, revealing any limitations or errors, where possible with suggestions as to how they might be overcome.
- It discusses your main conclusions and contributions.
- It may give recommendations for further investigations.

References

- Learn EndNote !!
- … or some other automatic systems (Papers)

Conclusion

- A conclusion in a MSc thesis is typically a short paragraph. It may come at the end of the discussion, or in a separate section with its own heading, just after the discussion. The conclusion makes closing statements that draw together findings from the results and discussion.

Appendices

Appendix 1: Abbreviations

2KR	SUMO-negative c-Myb
3-FLAG	3 × FLAG-tag (Hydrophilic octapeptide tag)
aa	Amino acid
AF	Activator function
Amp	Ampicillin
AMV	Avian myeloblastosis virus
Aos1	Component of the SUMO E1
AP21967	Rapamycin derivate
APS	Ammonium persulfat
BCL_2	B-cell lymphoma 2
bp	Base pair
$BRCA_1$	Breast cancer
BSA	Bovine serum albumin
CBP	CREB binding protein
CCNA1	Cyclin A
CHD	Chromo-helicase DNA bindi
ChiP	Chromatin Immunoprecipitat

Appendix 2: Recipe

Agarose gel (0.8%)
100 ml 1×TAE buffer
0.8 g agarose

Agarose gel (1%)
100 ml 1×TAE buffer
1g agarose

50×TAE (500ml)
121 g Tris base (2M)
50 ml 0.5M EDTA (0.05M)
28.5 ml glacial acetic acid
Add dH_2O to 500 ml

1×TAE (500ml)
100 ml 50×TAE
Add dH_2O to 500 ml

1M Tris-HCl (1000 ml)
121.15 g Tris base
800 ml dH_2O
Adjust pH to 8.0 with HCl (≈ 45 ml)
Adjust volume to 1000 ml
Autoclave

50% Glycerol (100 ml)
50 ml 99.5% glycerol
50 ml dH_2O
Autoclave

Appendix 3: Kits and commercial agents

Reagent	Producer	Product number
Cell work		
FuGENE®6 Transfection reagent	Roche	11988387001
DMEM (1×)	GIBCO® Invitrogen	41956-039
DPBS (1×)	GIBCO® Invitrogen	14190-094
Fetal Bovine Serum	GIBCO® Invitrogen	10106-169
PenStrep (PS)	GIBCO® Invitrogen	15140122
Trypsine (1×)	GIBCO® Invitrogen	25300
Tryphan Blue Stain	Invitrogen	T10282
Countess™ cell counting chamber slides	Invitrogen	C10228
Western blotting		
ELC Plus Western Blotting detection system	Amersham	RPN2132
Redymatic, developer solution	Kodak	5023866
Redymatic, fix solution	Kodak	5023874
Super signal® WestDura Extended Duration	Thermo Scientific	#34076

Appendix 4: Primer sequences

For amplification of FRB

Forward FRB (A112)
gagaccttacgcgtgccaccATGgggtcagggATCCTCTGGCATGAGATGTGGCATG

Reverse FRB-HA (A113)
agccttcagagtcgactattaaagatctGCGTAGTCTGGTACGTCGTACGGATAACT

For amplification of FKBP

Forward FKBP1A (A116)
gtacggttCAGCTGtgatggggtcaGGAGTGCAGGTGGAAACCATCTCC

Reverse FKBP1A (A117)
cgtgcgtaagcggccgcttaTCATTCCAGTTTTAGAAGCTCCACATC

For amplification of hUbc9

A classical subdivision

- ✓ Title
- ✓ Summary
- ✓ Introduction - leading up to “aim of the work”
- ✓ Materials and Methods
- ✓ Results
- ✓ Discussion
- ✓ References
- ✓ Appendix

Final summary

- Take the writing seriously, most students will profit from an extra effort here
- Start early, ask your supervisor how, but the responsibility is yours
- Be sure that you are able to formulate precisely the problem and the hypotheses of your thesis. Think of the structure of your thesis.
- Look carefully into good examples
- Revisit your notes