

Problem 10-6 Your Own Introduction

Write a short Introduction based on your work. Be sure to follow the funnel shape. Include known, unknown, question, and experimental approach. Also consider including results, conclusions, and significance of the paper. Be sure to signal the parts of your Introduction.

CHAPTER 11**Materials and Methods****11.1 OVERALL**

The purpose of the Materials and Methods section is to describe the experimental approach used to arrive at your conclusions. Although most readers are not interested in the experimental details of your work and will therefore not read this section, some readers will want to repeat part or all of your procedures and will read the Materials and Methods section in great detail. Above all, reviewers will read this section very carefully to ensure that it contains sufficient detail to evaluate or repeat your work. You should write this section with great care because if your experimental approach appears faulty, incomplete, or unprofessional, your paper may get rejected.

11.2 CONTENT**MATERIALS AND METHODS GUIDELINE 1:**

Provide enough details and references to enable a trained scientist to evaluate or repeat your work.

MATERIALS AND METHODS GUIDELINE 2:

Include materials and methods, but not results.

The Materials and Methods section should cover

- Materials (drugs, culture media, buffers, gases, or apparatus used)
- Subjects (patients, experimental materials, animals, microorganisms, plants)

For medical studies include

- (a) Total number of subjects
 - (b) Number of subjects receiving treatment
 - (c) How subjects were selected
 - (d) Details such as sex and age if relevant
- Design (includes independent and dependent variables, experimental and control groups)
 - Procedure (what, how, and why you did something)

Define the materials and methods as precisely as you can. Do not forget to include your control experiments. Check and follow the detailed specifications found in the *Instructions to Authors* of the journal to which you plan to send your manuscript.

Note that the Materials and Methods section is unavoidably linked to the Results section. In the Materials and Methods section, you need to describe how you obtained the results you report. Vice versa, in the Results section, you need to provide results for everything you describe in Materials and Methods. Do not make the error of mixing in some of the results in this section except for necessary intermediate results that provide the information needed for the next logical experimental step of your study.

References

If your methods have not been reported previously, you must provide all of the necessary detail. If, however, methods have been previously described in a standard journal, provide only that literature reference.

Example 11-1

Referring to previously described methods

- a) Plasmids were isolated according to Braun (19).
- b) For the numerical calculations involved in this study, we used a state-of-the-art chemistry-climate model (CCM) developed at the National Center for Atmospheric Research (22).

If you modified a previously published method, provide the literature reference and give a detailed description of your modifications.

Example 11-2

Referring to described methods that were modified

Plasmids were isolated according to Braun (19) with minor modifications. Instead of dissolving DNA pellets in sterile water, pellets were dissolved in buffer A.

Be sure to quote original references, that is, references that actually provide the method you want to describe. Do not just list a reference that refers the reader to another paper.

Details and Technical Specifications

In the Materials and Methods section, you need to provide sufficient details and exact technical specifications such as temperature, pH, total volume, time, and quantities to ensure that scientists can repeat your work. Include trade names, manufacturer, model numbers, and lot numbers, if essential. Identify organisms with full taxonomic names.

Example 11-3 a Providing sufficient detail

To lyse the cells, we used 250 μ l of SDS-solubilization buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA pH 8.0, 1% SDS w/v, 1:100 100 mM Phenylmethylsulfonyl fluoride (PMSF), and 100x lysophosphatidic acid (LPA)). After lysis, cells were resuspended once the supernatant had been removed.

In Example 11-3a, the author provides good detail of the solubilization buffer but fails to do so for the resuspension buffer.

Revised Example 11-3

To lyse the cells, we used 250 μ l of SDS-solubilization buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA pH 8.0, 1% SDS w/v, 1:100 100 mM Phenylmethylsulfonyl fluoride (PMSF), and 100x lysophosphatidic acid (LPA)). After lysis, cells were resuspended in 100 μ l sterile saline after removal of the supernatant.

Here are a few more examples that do not provide sufficient detail:

Example 11-3

Providing sufficient detail

- b To classify native species, orchids were collected.
- c To identify genes with a high probability of having differential expression in adenomas and follicular carcinomas, we used statistical methods.
- d All samples were centrifuged.

Revised Example 11-3

- b To classify native species, orchids were collected in the Everglades during the month of January.
- c To identify genes with a high probability of having differential expression in adenomas and follicular carcinomas, we used parametric (t test) and nonparametric (Mann-Whitney U test) methods.
- d All samples were centrifuged at 5000xg for 30 min at 25 $^{\circ}$ C.

Whereas some Materials and Methods sections contain too little detail, others include unnecessary extra detail, such as the one shown in the next example:

Example 11-4**Unnecessary information in Materials and Methods**

CD4⁺ CD44^{high} T cell purification

We were interested in examining the gene expression of the anergic portion of T cells in Jak3 KO mice that are CD4⁺ CD44^{high}. To obtain the CD4⁺ CD44^{high} T cells used for RNA isolation, spleens were removed from Jak3 KO and Jak3 Het mice at 8 to 10 weeks of age. Splenocytes were isolated by homogenizing the tissue with frosted glass slides (Fisher Scientific, Pittsburgh, PA).

In this example, the first sentence of the paragraph is unnecessary. This sentence should have been given in the introduction of the paper. The purpose for the actual portion of the experiment can be found in the second sentence of the paragraph "To obtain ...". Thus, the first sentence can be omitted.

Revised**Example 11-4**

CD4⁺ CD44^{high} T cell purification

To obtain the CD4⁺ CD44^{high} T cells used for RNA isolation, spleens were removed from Jak3 KO and Jak3 Het mice at 8 to 10 weeks of age. Splenocytes were isolated by homogenizing the tissue with frosted glass slides (Fisher Scientific, Pittsburgh, PA).

Consider another example:

Example 11-5**Unnecessary information in Materials and Methods**

Cells were scraped out of the wells and resuspended in a 1.5 ml Eppendorf tube.

In the preceding example, we find a description for the tube size used, "in a 1.5 ml Eppendorf tube." Although for some experiments it may be important to mention the manufacturer for certain equipment if it is essential for the success of the experiment, a description of the tube size is usually considered unnecessary detail and should be avoided.

Revised**Example 11-5**

Cells were scraped out of the wells and resuspended in 100 μ l sterile saline.

Use of Parentheses

To provide enough details while maintaining good flow in your writing, parentheses are commonly used in the Material and Methods section. Often these technical specifications include manufacturer's names, lot numbers, names of machinery, and additional explanations and specifications.

Example 11-6**Use of parentheses**

- a 20 mg/ml trypsin (TPCK, bovine pancreas) dissolved in Z-buffer (10 mM Tris HCl pH 8.0, 120 mM NaCl, 50% (v/v) glycerol) was thermally denatured at 65 °C for 3 min.
- b To assess trends over time, we calculated relative abundances of species, averaging monthly capture data over 6-month periods (January–June and July–December).

Appendices**MATERIALS AND METHODS GUIDELINE 3:**

Place detailed description of procedures or other lengthy details in an appendix.

Rather than putting detailed descriptions of procedures or other lengthy details in the body of the paper, you should place them in an appendix. If so, this must be part of your plan for the paper, not an afterthought submitted with the proofs, because an appendix has to be reviewed with the rest of the paper. Alternatively, you may be able to send lengthy material as supplementary material to an archive recommended by the journal.

Aside from detailed descriptions of procedures, material included in appendices rather than in the main body of a research paper (or proposal) may include detailed calculations, algorithms, proofs, tables, plots, and images or large data sets for meta-analyses and comparisons. Many journals now maintain electronic archives of supplementary material, including original data. This arrangement allows authors to be both thorough overall and concise in the main body of the article.

11.3 ORGANIZATION**MATERIALS AND METHODS GUIDELINE 4:**

Arrange experimental details as protocols grouped in chronological order or by subsections.

MATERIALS AND METHODS GUIDELINE 5:

Signal and link the different topics.

MATERIALS AND METHODS GUIDELINE 6:

Explain the purpose for any procedure whose function is not clear.

The Materials and Methods section is usually a long section and typically covers various topics. These topics need to be organized and methods need to be described in logical order, including the sequence of the procedures for each method.

You can organize your Materials and Methods section by separating each group of actions into one or more paragraphs. Paragraphs on the same type of information can then be grouped into subsections. The sequence of events within these subsections is usually written in chronological order or from most to least important. Each subsection has its own subheading, which functions as a signal, naming the particular material, variable, or specific procedure. Although use of subsections is optional, it

usually simplifies and clarifies the presentation for the reader. Subsections can include one or more paragraphs.

Example 11-7

Materials and Methods subsection

Cultures. Samples taken from the tips and subcutaneous sections of aseptically removed IVs were cultured as previously described (12). To identify the sources of organisms that colonize IVs, swab cultures of surrounding skin were obtained at the time of IV insertion as well as at the time of IV removal. In addition, one or more peripheral blood samples were obtained. Isolated organisms were identified by standard microbiologic methods.

Check your target journal to find out what subheadings are commonly used in your field and construct your subsections accordingly. You may even consider using the same subheadings in Materials and Methods and in the Results section. Examples of common subheadings are

Analysis of X	Antibodies
Cell Cultures	Chemicals and Reagents
Cloning	Data Analysis
Materials	Outcome Measures
Plasmids	Protein Expression and Purification
Sequence Analysis	Statistical Analysis
Study Design	Study Population
Synthesis of Y	Treatment Protocol

Other ways to signal different topics within the Materials and Method section can be by topic sentences or transitions. Topic sentences can be used to signal the topic of a paragraph, especially within a subsection. Transitions are often placed at the beginning of the first sentence of a paragraph to link the paragraph to the previous one before introducing the topic of the remaining sentences in the paragraph. No signals are used if the topic becomes apparent from the subject and verb.

In all cases, it is important to ensure that the reader will understand why each procedure was performed and how each procedure is linked to the central question of the paper. Therefore, you should state the purpose or give a reason for any procedure whose function or relation to the question of the paper is not clear. Also, provide any background information that might be necessary to understand the experiments you performed. Statements of purpose or background are usually placed at the beginning of a paragraph and typically serve as topic sentences and transitions.

Example 11-8

- Topic sentences/Statement of purpose**
- a To purify prolyl 4-hydroxylase from human placenta, full-term human placentae were collected 30 min after delivery.
- b Next, a trait-by-trait correlation matrix was developed to assist in identifying clusters of related traits.

11.4 IMPORTANT WRITING PRINCIPLES FOR MATERIALS AND METHODS

Voice

MATERIALS AND METHODS GUIDELINE 7:

In the Materials and Methods section, passive voice is often preferred.

MATERIALS AND METHODS GUIDELINE 8:

Do not switch from one point of view to another for no apparent reason.

The Materials and Methods section is the one section in a research paper where often passive voice is preferred over active voice. The reason is twofold: It lets you emphasize materials or methods as the topic of your sentences, and readers do not need to know who performed the action.



Example 11-9

Use of voice

The principal investigator collected the different fungal species from various tepuis in Venezuela.



Revised Example 11-9

Different fungal species were collected from various tepuis in Venezuela.

It is easiest to write your entire Materials and Methods section from one point of view. The disadvantage is that if most sentences are written in passive voice, writing becomes dull. If you are more experienced in writing and are taking into consideration word location and cohesion, you may choose to write in both active and passive voice in the Materials and Methods section to make your writing more smooth, interesting, and clear.

What you should avoid at all costs, however, is changing back and forth from one point of view to another within one paragraph for no apparent reason. Such switches will unnecessarily confuse and distract readers.



Example 11-10

Use of voice

The assays were performed for 10 min at room temperature. We then added 10 ml of 95% ethanol.

ESL advice

In this example, the author switches from passive to active voice for no apparent reason. These types of switches are seen particularly often for ESL writers. It is easier for the reader to follow a passage if the passive voice is used consistently as in the revised example.



Revised Example 11-10

The assays were performed for 10 min at room temperature. Then 10 ml of 95% ethanol were added.