FINAL Subcloning report. Here is a more detailed version of your report

Cloning Project Report

Your report should follow the format of a typical scientific publication with the following sections:

Title and Authors

Give your report a title and list the members of your group who contributed to the results.

Introduction: (300 words max)

WHY you performed the work (and not because it was an assignment!): E.g. what is the purpose of exon swapping, why you are producing fusion proteins with FRET molecules (and what are those?), or epitope tags, etc. Think what and why.

Methods: (800 words max)

A summary of what you actually did to produce the results you obtained. Include images and flow charts of cloning project, sequences and maps of the starting components, the cloning areas and a complete final plasmid map. Describe (sequence, alignment and mp) of each primer. PROVE primers will not dimerize or other issues. EACH must have a complete and clear figure legend AND you MUST show that your project, if completed would be "correct" in terms of reading frame. Indicate all pertinent information on a final plasmid map. the expanded region of the MCS and your final product including the base pairs and amino acid sequence, gene accession name, full sequence of the new plasmid, a DNA sequence of just the coding region (any additional features should be highlighted) the amino acid sequence including any features. All snap gene files must be placed into a separate folder on your lab archives WITH AN APPROPRIATE title. "final plasmid" is NOT an appropriate title.

Methods Hints: The Materials and Methods (M&M) section should be written in paragraph format. Methods should NOT be written as lists of steps, as they might appear in your notebook or in a recipe. Avoid excessive detail. For example, DON'T state: "The solution was prepared by adding 5 µL of 200 mM NaCl to 95 µL of deionized water. " Instead, state: "The solution contained 10 mM NaCl." A reader who chooses to repeat your experiment may have his/her own way of preparing the solutions. The final concentrations of components are the important consideration. If you are using a published technique, you can cite the procedure without reproducing the detailed steps. In this course, you will probably find it convenient to frequently refer to procedures in the lab manual. If you are using a commercial kit, e.g. the Zyppyplasmid purification kit, you can state that you followed the manufacturer's instructions. In all cases, be sure to include any modifications to the published procedure. ***Rule of thumb: A good M&M section provides enough information for a trained professional to reproduce your experiments. Look at a publication for style!***

Results: (800 words max)

A summary of the actual results you obtained (including any photographs of gels follow a professional format - see images below for correct and incorrect examples). This is in the form of a narrative – not bullet points or some

summary table. ALL gels and figures must have a final figure legend.

Results Hints: The section contains a brief narrative that guides your reader through the figures and legends that present your experimental data. ***Figures*** *need to be clearly labeled and to be accompanied by a figure legend. The figure legend should have a title and include explanations of the different panels or graphs in the figure. The figure legend should be placed below the figure. A well-written legend contains enough information that an expert reader can understand the experiment shown in the figure from its legend (assuming that the reader also looks at the M&M section). Report the results of all your experiments, even if you think they are incorrect. Discuss any experimental problems that you encountered in the experiment and speculate how these could have affected the results. Compare your results to those posted by other groups on the data sharing site. If your results agree, you may feel more confident about your results. Propose further experiments to resolve any remaining questions.*

Discussion: (800 words max)

A complete discussion of the results (including your speculation as to why things did not work as you expected if that is relevant).

<u>If you did NOT finish the project a detailed description of what you would do INCLUDING sequencing primers, digest gels with controls and any other verification (refer to the notes for an idea of what should be done). If the project did not work – provide a description of how you would troubleshoot and if that would fail suggest a viable second approach.</u>

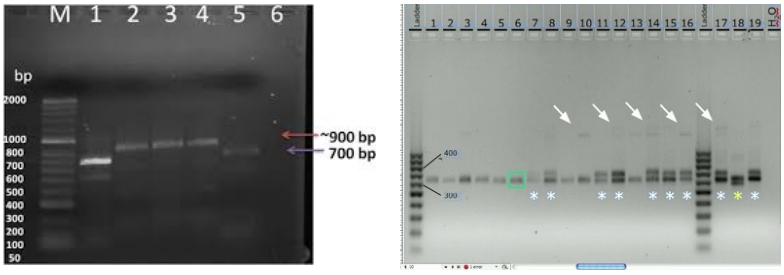
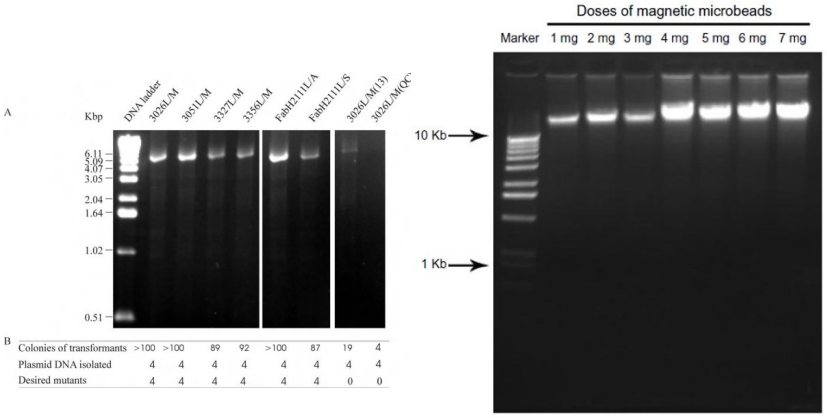


Figure 4. Agarose gel electrophoresis (FspI digests of *in vitro*φCR2.1 TOPO transformants):
Lane 1: Molecular Weight Marker.
Lane 2: Colony #1, PCR transposome inserted into 1.7 kb segment of TOPO.
Lane 3: Colony #2, PCR transposome inserted into 1.1 kb segment of TOPO.
Lane 4: Digested TOPO, expected bands at 1.0 kb, 1.1 kb, 1.7 kb.
Lane 5: Colony #1, PCR transposome inserted into 1.7 kb segment of TOPO.

The gels and figure legend above are bad. No, don't copy or even think about doing some of these things. What is wrong? Good question… text ON or IN the gel image. Arrows pointing to bands IN or ON the gel image. Lanes detected poorly and what is with those stars?

The gels below are fine! Notice the ladder on the left gel. That is great. I like how the lanes are labeled. Names or something indicating what is what… you can use numbers and then in the figure legend describe EACH number/lane. But what you see her looks better. If an arrow must be used, see how it is done in the gel on the right. ALSO make SURE your bands are right. IN the legend describe the source and volume

(or mass if possible) of each molecular weight ladder. What is the mass or volume loaded of samples – that goes in the legend. Because this may vary from gel to gel you can't really put that detail in the method… Oh, don't forget the percent gel, buffer system in the methods and legend. Lastly this is not a list or a bullet pointed thing.



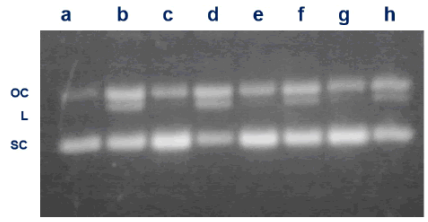


Figure 8: Agarose gel electrophoretic pattern of ethidium bromide stained pBR322 plasmid DNA after treatment with serotonin, melatonin and tryptophan in the absence and presence of copper. The reaction mixture (30 µl) contained 0.50 µg pBR322 DNA, 10 mM Tris-HCl (pH 7.5), indicated concentrations of the three metabolites and Cu(II). Incubation was carried out at 37°C for 1 hour. Lane a: DNA alone; Lane b: DNA + Cu(II) 100 µM; Lane c: DNA + serotonin (100 µM); Lane d: DNA + serotonin (100 µM) + Cu(II) 100 µM ; Lane e: DNA + melatonin (100 µM) Lane f: DNA + melatonin (100 µM) + Cu(II) 100 µM; Lane g: DNA + tryptophan (100 µM); Lane h: DNA + tryptophan (100 µM) + Cu(II) 100 µM. SC=Supercoiled DNA; OC= Open circular DNA; L=Linear DNA.