

Lab Report 2

Experiments 3-9

Lab Report 2 represents the bulk of your work in Genetic Engineering, and it will be big! There are many experiments to cover, lots of data to analyze, and lots details to consider. The table below is a list of each experiment, the required data, and the required analysis. In addition, you will need to describe each of the protocols that you used. If you pay careful attention to the table, you will come through this with a paper that you will be proud of and your will also have a sense of great satisfaction over the amount of work that you did this quarter.

Experiment	Required Data	Required Analysis
#3 Insertion of a Gene for Chloramphenicol Resistance from <i>Bacillus subtilis</i> into an <i>Escherichia coli</i> Plasmid	ligation gel	<ul style="list-style-type: none">• identification of bands in each lane• analysis of how BAP affects the ligation pattern in the gel
#4 Transformation of <i>Escherichia coli</i> With a Chimeric Plasmid	table of transformation colony counts	<ul style="list-style-type: none">• calculation of transformation and insertion frequencies• calculation of transformants/μg DNA• analysis of how BAP affects the transformation and insertion frequencies
#5 Screening of Transformants for Chimeric Plasmids	piggy-back gel of plasmid screens	<ul style="list-style-type: none">• identification of proximal and distal orientations• analysis of additional bands resulting from partial digestions
#6 Large-Scale Purification of Plasmids pRIT4501 and pRIT4502 by Cesium Chloride Density Gradient Centrifugation		

#7	Large-Scale Purification of Plasmids pRIT4501 and pRIT4502 by Qiagen Column Chromatograph		
#8	Verification of Purified Plasmids	verification gel OD readings from Cesium and Qiagen preps	<ul style="list-style-type: none"> • analysis of gel • calculation of DNA concentrations • calculation of DNA purity • comparison of Qiagen and CsC preps
#9	Construction of a Restriction Map of Plasmids pRIT4501 and pRIT4502	gels of restriction digests tables of distances/molecular wts standard curves	<ul style="list-style-type: none"> • map
#10	Construction of a Restriction Map of Plasmids pRIT4501 and pRIT4502	map of opposite orientation confirmation gel standard curve table of distances/molecular wts	<ul style="list-style-type: none"> • prediction of expected fragment sizes • demonstration that observed fragment sizes match predicted values

Required protocols:	restriction digestion gel electrophoresis BAP treatment ligation transformation	plasmid extraction by alkaline lysis plasmid extraction by tritonX-100 cesium chloride density gradient sedimentation Qiagen column chromatography
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Some Points to Remember

Abstract

A short summary of what you did and what you found out. The Abstract is not an introduction to the paper. It should be written as a stand-alone unit.

Introduction

The Introduction should be a short overview of what question you are asking and how you plan to approach the problem. The overall goal of the project could be expressed as an intent to compare heterologous gene expression in *Escherichia coli* and *Bacillus subtilis*. In order to do that you need to construct and characterize specialized plasmids. This paper deals with their construction. You are also interested in studying the effect of BAP on ligation and the efficacy of two different methods of plasmid preparation. Relevant background includes a description of pUC9 and pPL608, the function of BAP, and a brief discussion of the difference between Qiagen and CsCl protocols. You do not have to spill your guts about everything you know about recombinant DNA.

Methods and Materials

Methods and Materials is a written narrative in the past tense, passive voice. Exactly the way your high school English teacher told you not to write. Do not write it as a set of instructions or is a “first do this, then do that...” style. Do not present a diary of your afternoon in the lab: “While the DNA was being digested I prepared a gel...” You should write as though your readers have a basic background in molecular biology. Avoid trivial detail such as how you melt the agarose, tape the ends of the gel, etc. Gels can be made in a variety of ways, depending on the type of gel box that the reader is using. Be sure to include the formulas of all reagents. These can be in a separate section for reagents, or in parentheses following the first time they are mentioned.

Do not give experimental design. All protocols should be experiment-neutral. When in doubt, ask yourself “How would I run a Qiagen column? How would I do a BAP treatment? Etc.”

Results

Describe the experiments without going into details of procedure. The Results is a written narrative, not a list of tables and figures. Tables and figures should be numbered consecutively in the order in which they are discussed in the text. You may not have a figure or table if it is not referred to in the text. Gels should be oriented with the wells on top. Figure legends should be clear. It should not require any effort on the reader’s part to identify the contents of each lane. For example, the wells should be numbered so that the reader does not have to keep counting the wells to figure out what is in each.