

Table of Contents:

CONTENTS

Foreword
Acknowledgements
Note to Instructors
Instrumentation
Nomenclature

INTRODUCTION: Conceptual outline for experiments

PART I MANIPULATION OF DNA

Lab session 1: Getting oriented; Practicing with pipetmen
Lab session 2: Large scale purification of plasmid DNA
Lab session 3: Preparation of expression vector DNA (pET-41a(+), a GST fusion protein vector)
Lab session 4: Preparation of insert DNA (egfp)
Lab session 5: Preparation of transformation-competent cells and control transformation
Lab session 6: DNA ligation and transformation of Escherichia coli

PART II SCREENING TRANSFORMANTS

Lab session 7: Colony hybridizations
Lab session 7a: Interim laboratory session:
Lab session 7b: Colony hybridization: DNA probe
Lab session 7c: Colony hybridization: Monoclonal antibody probe
Lab session 8: Completion of colony hybridization with DNA probe
Lab session 9: Characterization of recombinant clones
Lab session 9a: Completion of colony hybridization with mAB probe
Lab session 9b: PCR screen
Lab session 9c: Visualization of green fluorescent protein: Part 1
Lab session 10: Further characterization of recombinant clones
Lab session 10a: Interim laboratory session:
Lab session 10b: Analysis of PCR screen results
Lab session 10c: Isolation and characterization of miniprep DNA from potential transformants (Restriction analysis of putative transformants)

Lab session 10d: Visualization of green fluorescent protein: Part 2

PART III EXPRESSION, DETECTION, AND PURIFICATION OF RECOMBINANT PROTEINS FROM BACTERIA

Lab session 11: Expression of fusion protein from positive clones and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Immunological analysis (Western blot): Part 1

Lab session 12: Expression of fusion protein from positive clones and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Immunological analysis (Western blot): Part 2

Lab session 13: Extraction of recombinant protein from Escherichia coli using a glutathione affinity column

Interim laboratory session: I. Laboratory exercise: Inoculate cultures for protein purification

Lab session 14: Analysis of purification fractions

Appendices: Equipment, Prep list, Making sense of orientation