Modern
Biology
Series

A Laboratory Course in Molecular Biology

11. Cloning a DNA Segment from Sheep

INSTRUCTOR MANUAL

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APPENDIX 1 - CONTENTS OF THE CHEMICAL PACKAGE

	Quantity
Electrophoresis	
Agarose	5 g
Gel Stain (methylene blue)	10 ml of a 1000 x solution
Electrophoresis Buffer (Tris-Acetate-EDTA)	50 ml of a 100 x solution
DNA Sample Buffer	600 µl - 2 tubes
DNA Standards	200 µl
DNA Manipulation	
Sheep DNA	350 µl (2mg/ml)
Plasmid pUC18 DNA - EcoR1 cut	130 µl (100 µg/ml)
EcoR1 Endonuclease	450 units (45µl) - 2 tubes
Endonuclease Buffer	1.2 ml - 2 tubes
DNA Ligase	15 units in 15 µl or in 7.5µl
Ligase Buffer	300 µl of a 2 x solution
1M NaCl	1 ml
Components for DNA elutors	
1.5ml tubes	8
0.5ml tubes	8
Transfer pipets	10
Glass wool	1 pack
Push pins	1 pack
Bacterial Transformation and Growth	
E. coli (Strain DH5α)	1.0 ml
E. coli - pUC18	250 µl
E. coli - pUC18 - Satellite	250 µl
$CaCl_2$	5 ml
Nutrient Broth	15 ml (2 tubes)
Nutrient Broth + Ampicillin	200 ml
Nutrient Agar + Ampicillin	400 ml
Xgal	20 mg
Xgal Solvent	1 ml
Sterile Petri Dishes	20
Sterile Transfer Pipets	50
Sterile Microtubes	25
Sterile Culture Tubes	25
Inoculating Loops	3 packs (12/pack)
Ampicillin	0.6 ml
Plasmid Isolation	
Quick Lysis Buffer	15 ml
SDS-NaOH	30 ml
Isopropanol	70 ml
Ammonium Acetate	18 ml

APPENDIX 2 - PREPARATION OF SOLUTIONS

I. ELECTROPHORESIS (LAB SESSION 1 AND 4)

Electrophoresis Buffer

The electrophoresis buffer is supplied as a 100-fold concentrate. To prepare the working buffer, add 35ml of the buffer concentrate to 3.5 liters of distilled or deionized water. Store the unused buffer in the refrigerator between electrophoretic runs. The buffer should be reused in the electrophoresis chamber for the two electrophoretic runs. However, fresh buffer should be used for the preparation of the agarose gels.

Staining Solution

The staining solution (methylene blue) is supplied as a 2000-fold concentrate. To prepare the working stain, add 1ml of the staining concentrate to 2 liters of distilled water. Please note, neither the concentrate nor the working dilution of the staining solution should be pipetted by mouth. Store the unused stain in a tightly capped bottle in the refrigerator.

II. ENZYMES

EcoR1 (Lab Session 1)

The restriction enzyme EcoR1 is provided in a glycerol solution. Immediately before use, add 220µl of restriction nuclease buffer to one of the tubes containing the EcoR1. Mix well the contents of the tube by rotating the tube on its side to ensure that the enzyme comes in contact with the buffer. Place the tube in an upright position in a beaker containing ice chips.

EcoR1 (Lab Session 4)

The restriction enzyme EcoR1 is provided in a glycerol solution. Immediately before use, add 300µl of restriction nuclease buffer to the remaining tube containing EcoR1. Mix well the contents of the tube by rotating the tube on its side to ensure that the enzyme comes in contact with the buffer. Place the tube in an upright position in a beaker with ice chips.

DNA Ligase (Lab Session 2)

The ligase (15 units) is provided in 15µl or in 7.5µl of a glycerol solution. Immediately before use, add 200µl of the 2 x ligase buffer to the tube containing the ligase. Mix the contents of the tube and place it in a beaker with ice chips.

III. BACTERIAL CULTURE MEDIA

Xgal-Ampicillin-Agar Plates (Lab Session 3)

Twenty mg of Xgal, 1ml of Xgal solvent (Dimethyl Formamide), one bottle containing 400ml of nutrient agar plus ampicillin and 20 petri dishes are supplied. Due to the unstable nature of ampicillin, additional ampicillin is also provided and should be added to the nutrient agar plus ampicillin mixture to ensure that sufficient antibiotic is present in the agar plates. The plates must be prepared at least one day before the laboratory session. To prepare the plates:

A. Loosen the cap on the bottle.

B. Place the bottle in a beaker of boiling water over a burner until the agar has liquefied. This should take about 20-25 minutes.

C. Remove the bottle from the bath and let cool at room temperature for about 10 minutes.

D. Pour the entire 1ml of the Xgal solvent into the tube containing Xgal, cap the tube and carefully shake until the Xgal is dissolved.

Note: The Xgal solvent, dimethyl formamide, is toxic at this concentration. The solvent should be handled with caution in a well vented area (a fume hood, if available) and the instructor should wear gloves.

E. Pour the entire 0.6ml of the ampicillin solution and the 1ml of Xgal solution into the bottle containing the nutrient agar, replace the cap, and swirl the bottle to mix the contents. The ampicillin, Xgal, and Xgal solvent will be found in 1.5ml tubes along with the other frozen components of this Chemical Package

F. Uncover the petri dishes, one at a time, and pour a thin layer (10-20ml) of agar into the lower half of each dish. Immediately replace the covers. Let the agar harden for about one day at room temperature. If desired, the plates can be stored in the refrigerator in an inverted position for at least two weeks.

Culture Tubes with Nutrient Broth-Ampicillin (Lab Session 3)

200ml of the broth is provided along with twenty-five 20ml sterile culture tubes. A sterile pipet can be used to add about 6ml of media to each tube. Alternatively, place a mark on each tube about 6cm from the bottom and fill the tube to the line with media. Immediately recap the tubes and store them in the refrigerator until needed.