

SAMPLE LAB EXAM

Recombinant DNA Technology

MBIO 4570 FINAL LAB EXAM

DATE: sample

PAGE: 1 of 2

TIME: 1.5 h

INSTRUCTOR Dr. L. Cameron

Student Name _____ Student Number _____

Answer exam questions in PEN ONLY.

Answer QUESTIONS ON EXAM PAPER.

Spacing has been removed for example exam.

- 10 1. Explain the function of each the following experimental steps or solutions.
- a) Heat shock at 43°C for 90 sec. Put on ice for 3 min. (*E. coli* transformation)
 - b) Plate on LB-AMP ((*E. coli* transformation)
 - c) Plasmid DNA preparation solutions:
 - (i) 0.2 M NaOH, 1% SDS
 - (ii) 200 mM NaCl, 20 mM Tris-HCl, pH 7.5, dilute 1:1 with 95% EtOH
 - (iii) cartridge
 - d) 0.4 M NaOH + 0.6 M NaCl (Southern Blot)
 - e) DIG-High Prime (contains hexanucleotide primers, dNTPs, dUTP-DIG-AP, Klenow and appropriate salts
 - f) standard hybridization solution (5x SSC, 1.0% (w/v) Blocking, 0.1% sarcosyl, 0.01 % sodium dodecyl sulfate (SDS).
 - g) guanidine isocyanate in the QC binding buffer of the QIAquick Gel Extraction kit
 - h) VMM medium
- 1 2. During *Rhizobium leguminosarum* genomic DNA preparation using the DNeasy Genomic DNA kit what two components are added to remove protein. Explain the specific function of each with respect to the step carried out.
- 1 3. Explain the function of the helper phage pRK600.
- 1.5 4. For a conjugation experiment (*E. coli* DH5 α (Str^s Pro⁻) containing pKNOCK rhamnose kinase fragment plasmid construct x *Rhizobium leguminosarum* Rlt100 Str^r) state the medium (be precise) required to select for homologous recombinants. Explain why.
- 2 5. a) What is the purpose of the Southern blot hybridization experiment (*Rhizobium leguminosarum* construct)? Answer question by presenting a completely labelled schematic diagram of experiment results. Include a figure title with all relevant information.
- 1 b) Why is it important that *Rhizobium leguminosarum* construct genomic DNA be isolated with care (ie fragment size >50 kb)? That is, how would the hybridization results change?
- 1.5 c) Outline how to determine the bp size of highlighted band on chemiluminescent film of Southern blot.

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- 1.5 6. After hybridization the final wash is ~40 ml ml 0.1x SSC containing 0.1% SDS (pre-warmed to 68°C). The blot is washed on the roller for 15 min at 68°C. Explain the function of the wash components and temperature with respect to DNA hybridization.
- 1 7. a) 1 kb Plus DNA ladder can only determine the bp size of _____ DNA.
b) How is it possible to visualize restriction digested DNA bands in an agarose gel using the Innotech MultiImage light cabinet?
- 0.5 8. a) What is the purpose of megaprimer amplification as used in your lab?
2 b) How does the megaprimer amplification differs from basic PCR amplification?
Answer this question using a diagram of megaprimer PCR at the molecular level.
- 1.5 c) Show all primers. State criteria for design of each primer.
- 1.5 9. What medium is used to select pBluescript ligated PCR product? Explain the function of each component with respect to experiment carried out in the MBIO 4570 lab.
- 1 10. After determining that you have a potential mutant rhamnose kinase by restriction digestion outline procedure to determine if the required bp change has occurred. State each procedure and/or process, details not required.