UNIVERSITETET I OSLO

Det matematisk-naturvitenskapelige fakultet

Exam in:
MBV4010 Arbeidsmetoder i molekylærbiologi og biokjemi I
MBV4010 Methods in molecular biology and biochemistry I
Day of exam: Friday 28 September 2012
Exam hours: 10.00 – 13.00
This examination paper consists of 4 pages.
Appendices: 3 (3 pages)
Permitted materials: Calculator
Make sure that your copy of this examination paper is complete before answering.

Read thoroughly through the entire problem before starting to answer the sub-questions.

Problem 1

A wide range of cellular proteins are methylated by lysine (K) specific methyltransferases (MTs), and a one such human enzyme, VCP-KMT, methylates VCP, an essential and highly abundant protein. For expression of recombinant protein in *E. coli*, you want to clone the gene encoding VCP-KMT into the plasmid pET-33b(+), which can be used for expressing proteins with N-terminal and/or C-terminal His-tag. For this purpose you will amplify the VCP-KMT gene from human cDNA by polymerase chain reaction (PCR). You will use PCR-primers that have non-annealing extensions that contain restriction sites which enable cloning of the VCP-KMT encoding gene into the plasmid pET-33b(+).
In Appendix 1 you find the DNA and protein sequence of VCP-KMT (229 amino acids; 690 nucleotides including stop codon), and in Appendix 2 you find a map of pET-33b(+).

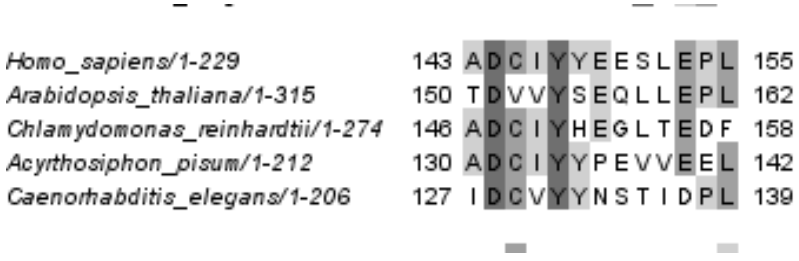
a)You want to generate a construct encoding VCP-KMT with a His-tag added to the **C-terminus**, and you want to minimize the inclusion of non-relevant sequence. You may assume that the VCP-KMT gene is not cleaved by any of the enzymes that cleave the pET-33b(+) cloning/expression region. Which restriction enzymes is it then preferable to use for the cloning? (Explain)

b) Design a pair of primers for performing the cloning outlined in a).To ensure efficient cleavage of the PCR-product by the restriction enzymes, the hexanuclotide ATATAT should be included at the 5' end of the primers. The part of the primer that anneals to the VCP-KMT sequence should be 18 nucleotides long.

c) You also want to make a pET-33b(+) based plasmid for the expression of VCP-KMT with an **N-terminal** His-tag. You should use the same criteria as in a and b, but in this case only the following restriction enzymes are available to you: NcoI, NdeI , NheI and XhoI. Design a primer pair for performing this cloning.

d) To investigate whether you have been successful in generating the two constructs described in a),b), and c), you will cleave plasmid isolated from candidate clones with the restriction enzymes NcoI and XhoI. Calculate the size of the fragments obtained in the case of the two constructs.

e) Below is shown a part of a sequence alignment of VCP-KMT orthologues from various organisms. Here you will find a conserved conserved tyrosine residue, which you want to mutate into tryptophane. Design a pair of primers for performing this mutagenesis using the QuikChange method. In the primers, the mutated region should be flanked by 12 nucleotides of non-mutated sequence on each side. Indicate the mutations that have been introduced. In appendix 3 you find the genetic code and a list of amino acid abbreviations.



Problem 2

You want to investigate whether there are two alternatively spliced mRNAs from a given gene in a given cell type in your model organism. You make a cDNA library from that tissue and get the following result after using primers designed to be specific for your gene and for an ACTIN gene in a PCR with genomic DNA and cDNA as templates. The primers used for you gene anneal to exon sequences flanking very short introns and the alternative splicing sites. The primers for ACTIN also anneal to exons flanking a short intron
Explain what has gone wrong or whether you have been successful in you experiment if:

a) you get a PCR product with both primer sets on genomic DNA, but no bands for the cDNA

b) you get PCR products of the expected sizes with the ACTIN primers on cDNA and on genomic DNA, but no PCR products on either template with you gene-specific primers

c) you get PCR products of the expected sizes with the ACTIN primers on both cDNA and genomic DNA, but only on the genomic DNA with your gene-specific primer set

d) you get PCR products of equal size with the ACTIN primers on both cDNA and genomic DNA, and with the gene-specific primers you get one band for genomic DNA, and two bands on the cDNA whereof one is of the same size as the genomic band and the other shorter than the genomic band

e) you get PCR products of the expected sizes with the ACTIN primers on both cDNA and genomic DNA, and with the gene-specific primers you get one band for genomic DNA, and two bands on the cDNA shorter than the genomic band

f) Still with the goal of investigating alternative splicing, explain shortly which of the following set ups you would chose for your next experiment in situations a) to e):
1) I will repeat the whole experiment, and make sure that I improve/change (*fill in here*)
2) I will repeat the PCR, but change (*fill in here*)
3) I will investigate the PCR product(s) with the (*fill in here*) method to be sure that (*fill in here*)

Problem 3

Answer briefly (2-3 sentences maximum) to the following questions.

a) Briefly describe the basic principles of real-time PCR.

b) Why is "codon usage" a concern when expressing a human protein in E. coli?

c) Explain the terms “Forward Genetics” and “Reverse Genetics”.

d) Describe/outline how TALE nucleases can be engineered to recognize specific DNA sequences.

e) What are isoschizomers?

Appendix 1

Protein and DNA sequence of VCP-KMT(229 amino acids; 690 nucleotides incl. stop codon).

```
  1   M  A  D  T  L  E  S  S  L  E  D  P  L  R  S  F  V  R  V  L
  1  ATGGCGGATACGCTGGAGTCCTCGCTGGAGGACCCACTGCGGAGCTTTGTGCGAGTTTTG

 21   E  K  R  D  G  T  V  L  R  L  Q  Q  Y  S  S  G  G  V  G  C
 61  GAGAAGCGGGATGGTACAGTGCTACGACTACAGCAGTATAGCTCCGGTGGCGTGGGTTGC

 41   V  V  W  D  A  A  I  V  L  S  K  Y  L  E  T  P  E  F  S  G
121  GTTGTGTGGGACGCTGCCATTGTCCTTTCTAAATACCTGGAAACGCCCGAGTTTTCTGGC

 61   D  G  A  H  A  L  S  R  R  S  V  L  E  L  G  S  G  T  G  A
181  GACGGGGCCCACGCGCTGAGCCGGCGGTCGGTGCTGGAGCTGGGTTCGGGCACCGGGGCC

 81   V  G  L  M  A  A  T  L  G  A  D  V  V  V  T  D  L  E  E  L
241  GTGGGGCTCATGGCTGCTACCCTCGGGGCTGATGTTGTAGTCACCGATCTTGAGGAATTG

101   Q  D  L  L  K  M  N  I  N  M  N  K  H  L  V  T  G  S  V  Q
301  CAAGACTTGCTGAAGATGAATATTAATATGAACAAGCATCTTGTCACTGGTTCTGTTCAA

121   A  K  V  L  K  W  G  E  E  I  E  G  F  P  S  P  P  D  F  I
361  GCCAAGGTACTGAAATGGGGGGAAGAAATAGAAGGCTTTCCTTCTCCACCCGACTTCATA

141   L  M  A  D  C  I  Y  Y  E  E  S  L  E  P  L  L  K  T  L  K
421  CTGATGGCCGACTGCATATACTATGAAGAGTCTTTGGAGCCATTGCTGAAAACTCTAAAA

161   D  I  S  G  F  E  T  C  I  I  C  C  Y  E  Q  R  T  M  G  K
481  GATATCAGCGGATTTGAAACTTGTATTATATGTTGTTATGAACAACGAACAATGGGGAAA

181   N  P  E  I  E  K  K  Y  F  E  L  L  Q  L  D  F  D  F  E  K
541  AATCCAGAAATTGAGAAAAAATATTTTGAGCTCCTTCAGCTAGATTTTGACTTTGAAAAA

201   I  P  L  E  K  H  D  E  E  Y  R  S  E  D  I  H  I  I  Y  I
601  ATTCCTTTGGAAAAACATGATGAAGAGTATCGAAGTGAAGATATTCATATTATATACATC

221   R  K  K  K  S  K  F  P  S  -
661  AGAAAGAAAAAATCGAAATTTCCATCGTGA
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Appendix 2

Map of the vector pET-33b(+)

pET-33b(+) Vector

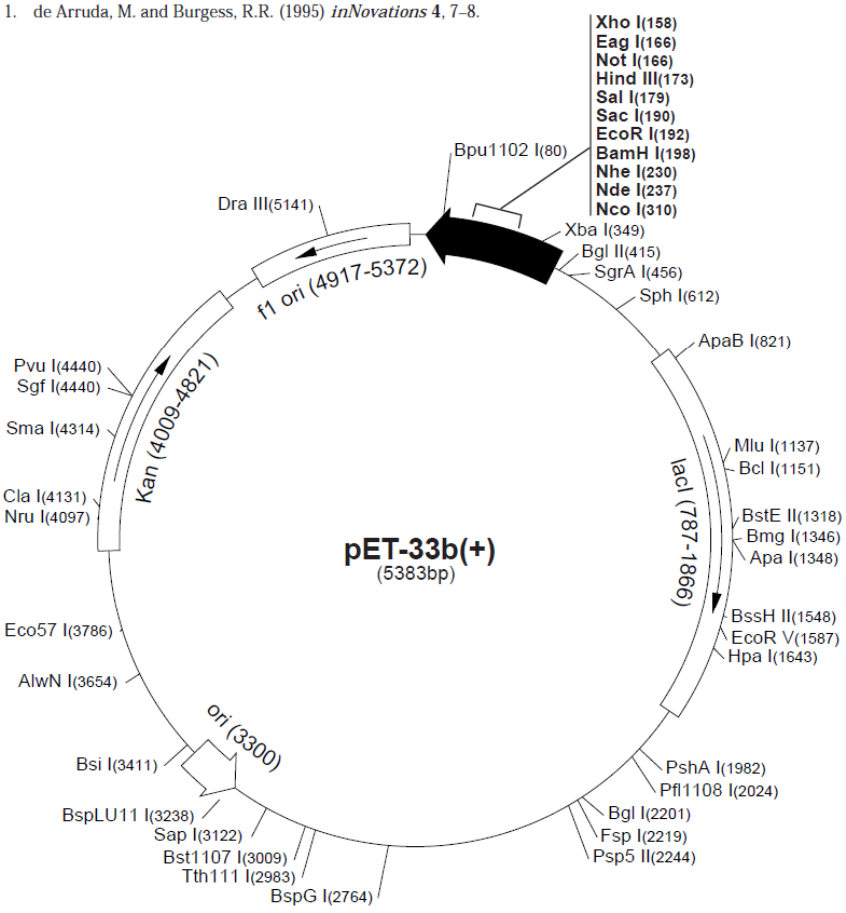
TB147 12/98

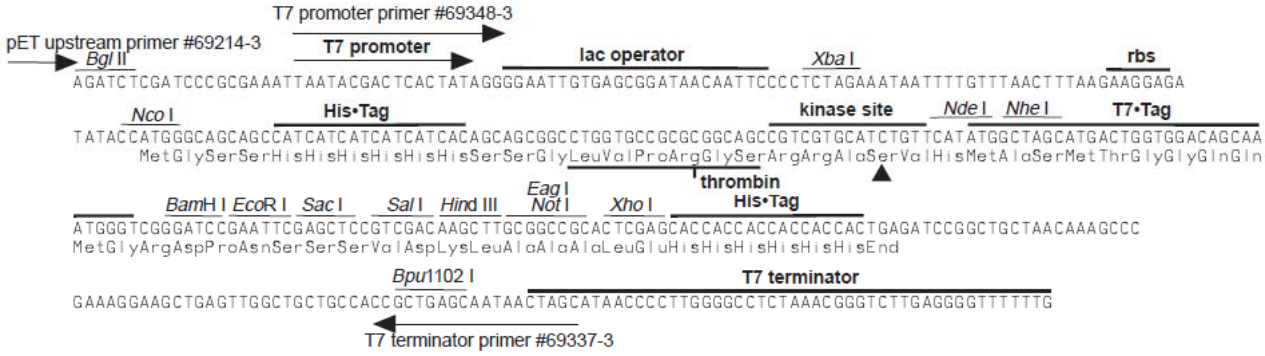
The pET-33b(+) vector (Cat. No. 69054-3) is derived from pET-28b(+) and carries a 15bp sequence encoding the protein kinase A (PKA) site RRASV, located between the thrombin cleavage and *Nde* I sites (1). Proteins expressed in pET-33b(+) can be easily purified by metal chelation chromatography (*via* either N- or C-terminal His•Tag® sequences) and efficiently labeled with ^{32}P- or ^{33}P-γATP and the catalytic subunit of cAMP-dependent protein kinase from heart muscle. Labeled proteins can be used as direct probes in protein-protein interaction studies. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circle map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the T7 terminator primer (Cat. No. 69337-3).

pET-33b(+) sequence landmarks

T7 promoter	384-400
T7 transcription start	383
His•Tag coding sequence	284-301
PKA site coding sequence	242-256
T7•Tag® coding sequence	206-238
Multiple cloning sites (*Bam*H I - *Xho* I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	787-1866
pBR322 origin	3300
Kan coding sequence	4009-4821
f1 origin	4917-5372

1. de Arruda, M. and Burgess, R.R. (1995) *inNovations* **4**, 7–8.

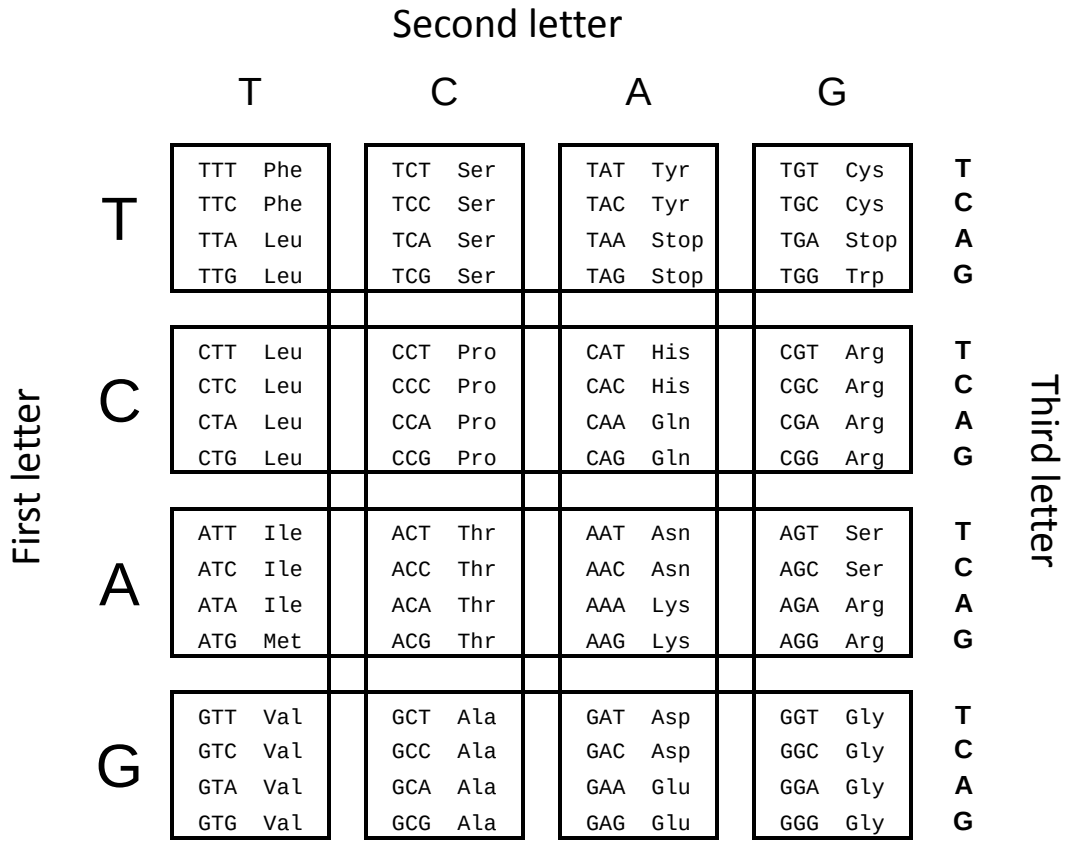




pET-33b(+) cloning/expression region

Appendix 3

The genetic code



Amino acid	Abbreviation (three letters)	Abbreviation (one letter)
Alanine	Ala	A
Cysteine	Cys	C
Aspartate	Asp	D
Glutamate	Glu	E
Phenylalanine	Phe	F
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Lysine	Lys	K
Leucine	Leu	L
Methionine	Met	M
Asparagine	Asn	N
Proline	Pro	P
Glutamine	Gln	Q
Arginine	Arg	R
Serine	Ser	S
Threonine	Thr	T
Valine	Val	V
Tryptophane	Trp	W
Tyrosine	Tyr	Y