UNIVERSITETET I OSLO

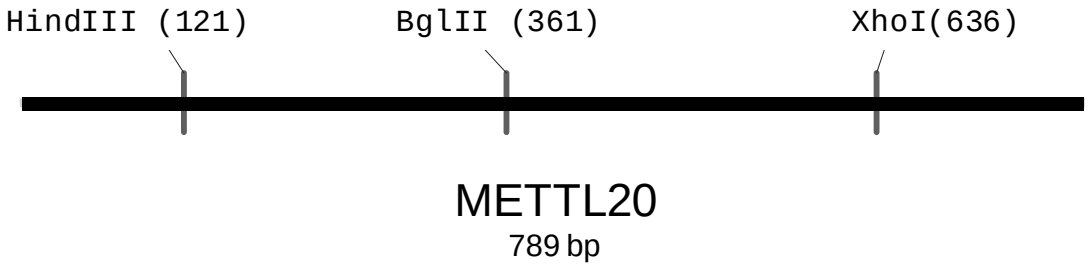
Det matematisk-naturvitenskapelige fakultet

Exam in:
MBV4010 Arbeidsmetoder i molekylærbiologi og biokjemi I
MBV4010 Methods in molecular biology and biochemistry I
Day of exam:. Friday 30 September 2011
Exam hours: 09.00 – 12.00
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

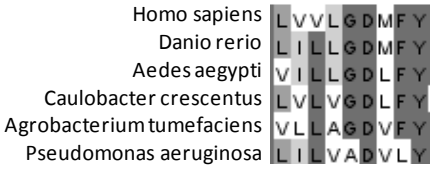
You are studying the uncharacterized human methyltransferase METTL20, and you want to clone the corresponding gene into a vector for stable expression in mammalian cells. For this purpose you will amplify the METTL20 gene from human cDNA by polymerase chain reaction (PCR), and you will use PCR-primers that have non-annealing extensions that contain restriction sites which enable cloning of the METTL20 gene into the plasmid pcDNA5/FRT.
In Appendix 1 you find the DNA and protein sequence of METTL20 (262 amino acids; 789 nucleotides including stop codon), and in Appendix 2 you find a map of pcDNA5/FRT. Below you also find a restriction map of the METTL20 gene.



a) You choose to use the restriction enzymes NheI (recognition sequence: GCTAGC) and ApaI (recognition sequence: GGGCCC) for the cloning, and you design PCR primers that contain recognition sites for these enzymes. 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 METTL20 sequence should be 18 nucleotides long. Indicate the sequence of the two primers you will use (written in the direction 5' to 3').

b) You cut the PCR-product as well as the vector pcDNA5/FRT with NheI and ApaI, and you use T4 DNA ligase to ligate the METTL20-containing fragment and the cut vector together. The resulting ligation mixture is then used to transform competent *E. coli*, and plasmid DNA is isolated from the resulting clones. If the cloning has been successful, what is the size of the smallest fragment you obtain when cleaving the plasmid with ApaI and HindIII?

c) Below is shown a sequence alignment of METTL20 orthologues from different eukaryotic and prokaryotic organisms. Only the region corresponding to Leu183-Tyr191 in human METTL20 is shown.



You will mutate a highly conserved amino acid residue in this region by using the following forward mutagenesis primer: GTTGTTCTTGGCGCCATGTTTTATGAT
In appendix 3 you find the genetic code and a list of amino acid abbreviations.
Indicate the mutations that have been introduced, both at the DNA and protein levels. The introduction of the mutation has been accompanied by the introduction of a 6-mer palindromic restriction site. Indicate this site.

Problem 2

a) Describe how total RNA can be isolated from plant cells and be used to synthesize first strand cDNA. Why is it particularly important to wear gloves and use pipette tips with filter when isolating RNA?
Explain in what steps and for what purpose the following are used:
i) liquid nitrogen
ii) RNaseH
iii) oligo(dT)
iv) RNasin
v) Reverse transcriptase.

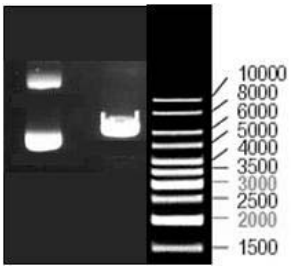
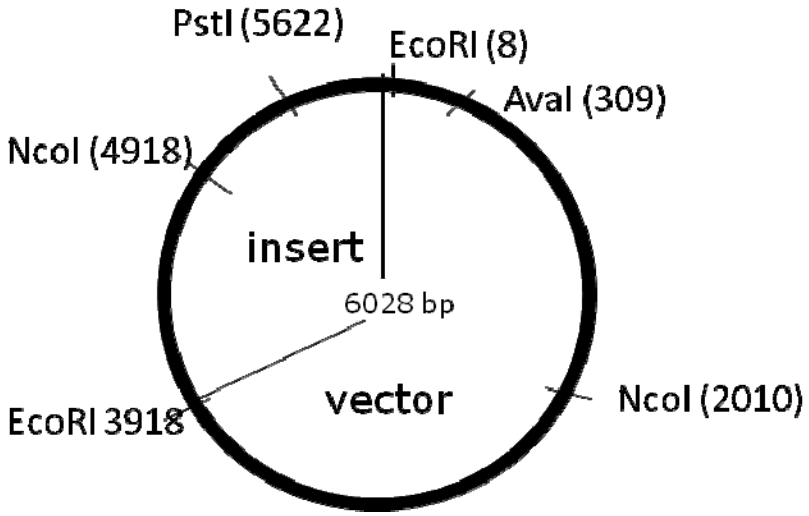


Fig. 1

b) You do a PCR on your cDNA and clone your cDNA product in the TOPO vector, and isolate the resulting plasmid from transformed *E. coli* cells. You run a sample of your plasmid prep on an agarose gel and two bands are seen, while when digesting with a restriction endonuclease with a single target sequence in the plasmid only one band is seen (Fig. 1). What do these three bands represent? What is the size of the plasmid as estimated from size marker (ladder)?

c) Choose one or more restriction enzymes that most unambiguously can determine the orientation of your insert, cf the maps below. What are the expected sizes of the restriction fragments resulting from your digestion?

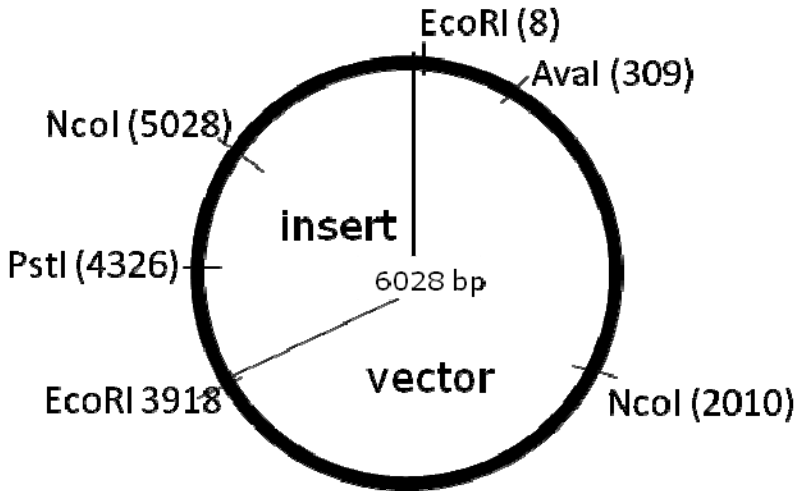
Orientation 1:



(Note: Problem 2 continues on next page)

(Problem 2, continued)

Orientation 2:



Problem 3

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

a) What is the role of a selectable marker, such as an antibiotic resistance gene, in DNA cloning?

b) What does it mean that two restriction enzymes have “compatible cohesive ends”?

c) When translating a DNA sequence into protein (*e.g.* on a computer), how many different reading frames can be applied? (Explain briefly)

d) What are Dicer proteins and what is their function?

e) In recent years, several high-throughput ("next generation") DNA sequencing technologies have been developed. Outline the basic principles of one of these technologies.

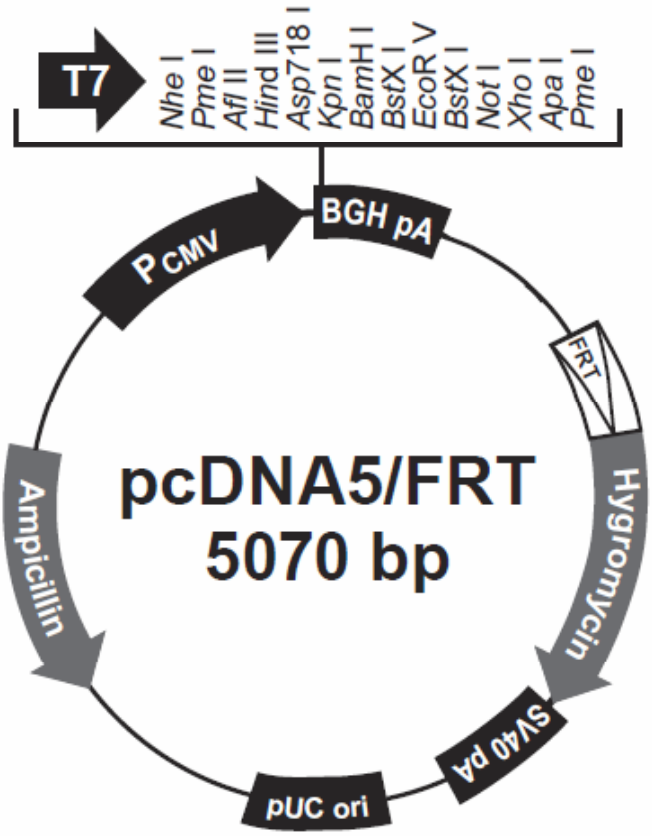
Appendix 1

Protein and DNA sequence of METTL20 (262 amino acids; 789 nucleotides incl. stop codon).

```
  1   M  A  L  S  L  G  W  K  A  H  R  N  H  C  G  L  L  L  Q  A
  1  ATGGCTTTGAGTCTAGGTTGGAAAGCACACAGGAACCACTGTGGTCTCCTCTTGCAGGCT

 21   L  R  S  S  G  L  L  L  F  P  C  G  Q  C  P  W  R  G  A  G
 61  CTGCGAAGCAGTGGTCTTCTCTTGTTTCCCTGTGGCCAGTGTCCCTGGAGAGGAGCTGGA

 41   S  F  L  D  P  E  I  K  A  F  L  E  E  N  T  E  V  T  S  S
121  AGCTTTTTGGACCCTGAGATAAAGGCTTTCCTGGAGGAGAACACTGAAGTCACCAGCAGT

 61   G  S  L  T  P  E  I  Q  L  R  L  L  T  P  R  C  K  F  W  W
181  GGTAGCCTCACCCCTGAAATCCAGTTGCGGCTTTTGACCCCCAGATGCAAATTCTGGTGG

 81   E  R  A  D  L  W  P  H  S  D  P  Y  W  A  I  Y  W  P  G  G
241  GAGAGAGCTGACCTGTGGCCCCACAGTGATCCTTACTGGGCAATCTACTGGCCAGGAGGC

101   Q  A  L  S  R  Y  L  L  D  N  P  D  V  V  R  G  K  S  V  L
301  CAAGCCCTGTCTAGGTATCTTTTGGATAATCCTGATGTTGTCAGAGGAAAATCTGTATTA

121   D  L  G  S  G  C  G  A  T  A  I  A  A  K  M  S  G  A  S  R
361  GATCTTGGGAGTGGATGTGGAGCTACAGCTATTGCTGCTAAGATGAGTGGGGCATCAAGG

141   I  L  A  N  D  I  D  P  I  A  G  M  A  I  T  L  N  C  E  L
421  ATCTTGGCCAATGACATAGACCCTATTGCAGGAATGGCTATTACACTAAATTGTGAATTG

161   N  R  L  N  P  F  P  I  L  I  Q  N  I  L  N  L  E  Q  D  K
481  AACAGACTGAATCCTTTTCCTATTTTAATCCAAAACATTTTGAATTTGGAACAAGATAAG

181   W  D  L  V  V  L  G  D  M  F  Y  D  E  D  L  A  D  S  L  H
541  TGGGACCTTGTTGTTCTTGGCGATATGTTTTATGATGAAGACCTTGCAGATAGTCTTCAT

201   Q  W  L  K  K  C  F  W  T  Y  R  T  R  V  L  I  G  D  P  G
601  CAGTGGCTGAAGAAGTGCTTCTGGACCTATAGAACTCGAGTACTGATTGGTGACCCTGGG

221   R  P  Q  F  S  G  H  S  I  Q  H  H  L  H  K  V  V  E  Y  S
661  CGGCCCCAGTTCAGTGGACACAGCATTCAGCATCACCTGCACAAAGTGGTAGAATATTCA

241   L  L  E  S  T  R  Q  E  N  S  G  L  T  T  S  T  V  W  G  F
721  CTTTTGGAGTCTACTAGGCAGGAAAACAGTGGACTGACAACAAGCACAGTGTGGGGTTTT

261   Q  P  -
781  CAGCCTTGA
```

Appendix 2

Map of the vector pcDNA5/FRT

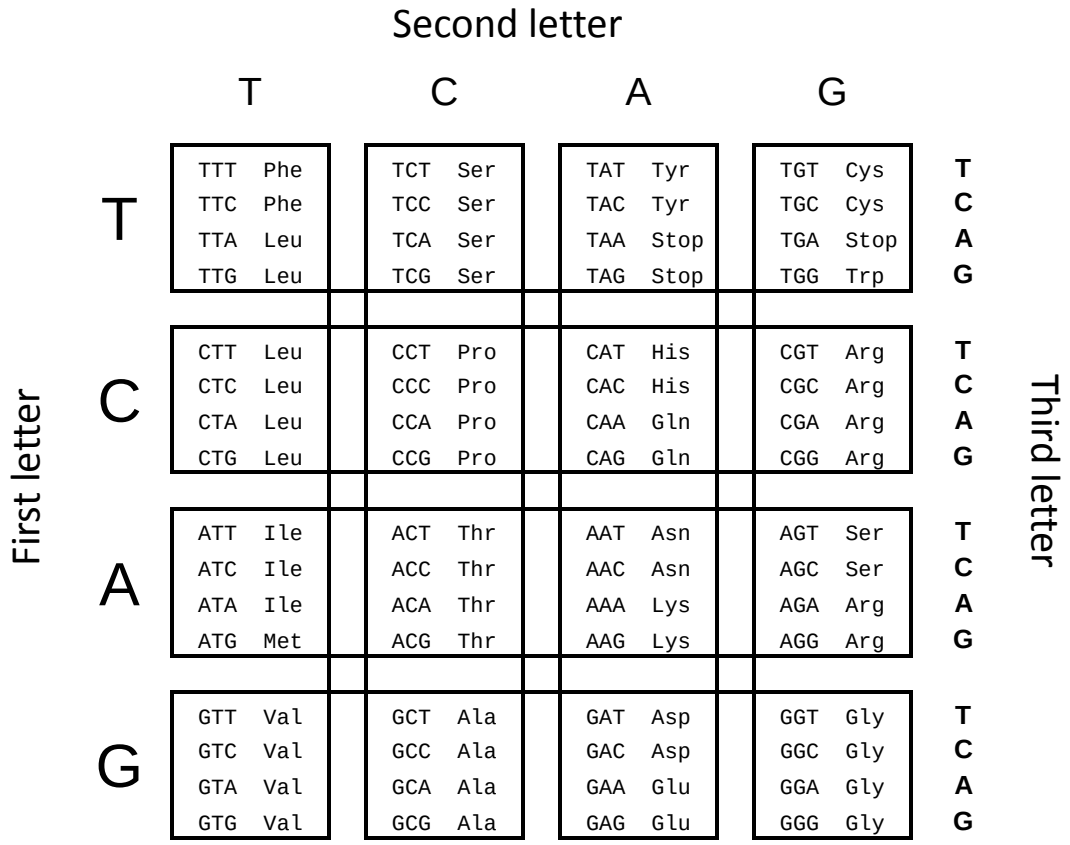


Comments for pcDNA5/FRT
5070 nucleotides

CMV promoter: bases 232-819
CMV forward priming site: bases 769-789
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1010
BGH reverse priming site: bases 1022-1039
BGH polyadenylation signal: bases 1028-1252
FRT site: bases 1536-1583
Hygromycin resistance gene (no ATG): bases 1591-2611
SV40 early polyadenylation signal: bases 2743-2873
pUC origin: bases 3256-3929 (complementary strand)
bla promoter: bases 4935-5033 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 4074-4934 (complementary strand)

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