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 27 September 2013
Exam hours: 9.00 – 12.00
This examination paper consists of 5 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)
You will be cloning the ALKBH8 protein from the plant Arabidopsis thaliana into the plasmid pET-28a(+) for expression and purification of recombinant protein. For this purpose you will amplify the ALKBH8 gene from Arabidopsis 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 PCR-product into pET-28a(+). Design primers for performing this cloning, in accordance with the following criteria:

- The part of each primer that anneals to the ALKBH8 sequence should be 18 nucleotides long.
- The restriction enzymes NdeI and BamHI should be used for the cloning
- Insert the ALKBH8 gene in such a way that the expressed protein will contain an N-terminal His-Tag (but no C-terminal tag), but the tag should be kept as short as possible.
- 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.

In Appendix 1, you will find a map of the pET-28a(+) plasmid, and in Appendix 2, the DNA and protein sequence of Arabidopsis ALKBH8, as well as a table indicating restriction enzymes that cleave in the ALKBH8 gene sequence.

b)
To investigate whether you have been successful in performing the cloning described in a), you will cleave plasmid isolated from candidate clones with following combination of restriction enzymes:

i) NcoI and XhoI. ii) HindIII and NdeI

Calculate the size of the fragments obtained in these two cases if the cloning has been successful.

In Appendix 1 you will find a table giving positions of restriction enzyme cleavage within the ALKBH8 cDNA sequence.

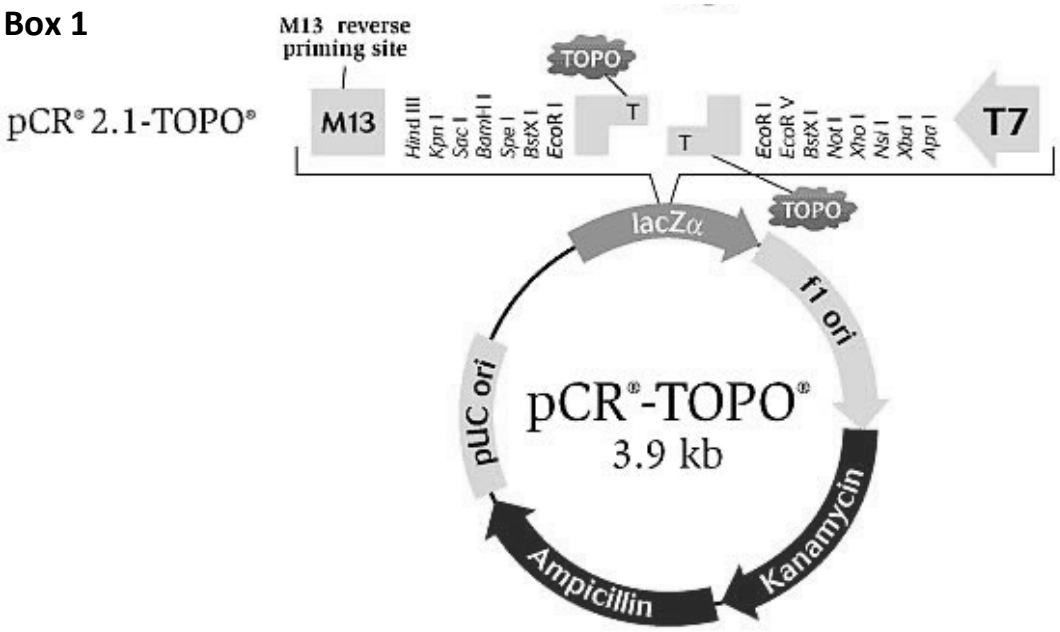
c)
By using the QuikChange method for site-directed mutagenesis, you will to mutate Asp226 in the ALKBH8 protein. For this purpose the following forward primer (as well as a complementary reverse primer) has been designed:
ATCGCCTCATATCGCGACACATTCAGCA
By comparing the mutant primer sequence to the wild-type sequence, indicate the mutations that will be introduced, both on the DNA and protein level. The introduction of the mutation(s) is accompanied by the introduction of a 6-nucleotide palindromic recognition site for a restriction enzyme. Indicate this site.

In Appendix 3, you will find the genetic code.

Problem 2

a)
You have made cDNA from plant tissue and want to amplify the *ASHR3* cDNA and clone it into the vector pCR 2.1-TOPO (see Box 1 for map). Explain how TOPO-cloning functions, and what the M13 priming site is used for. In the lab there are two different DNA polymerases you can use for the PCR reaction (see Box 1 for product information). Explain which one you would use.

Box 1



Product information:

DNA polymerase A

Taq DNA Polymerase catalyzes the incorporation of dNTPs into DNA. It requires a DNA template, a primer terminus, and the divalent cation Mg++. Taq DNA Polymerase contains a polymerization dependent 5'-3' exonuclease activity. It does not have a 3'-5' exonuclease and thus no proof reading function. Despite this, the enzyme synthesizes DNA in vitro with reasonable fidelity. This Taq polymerase leaves a 3' dA overhang on ~80% of the ends of PCR fragments).

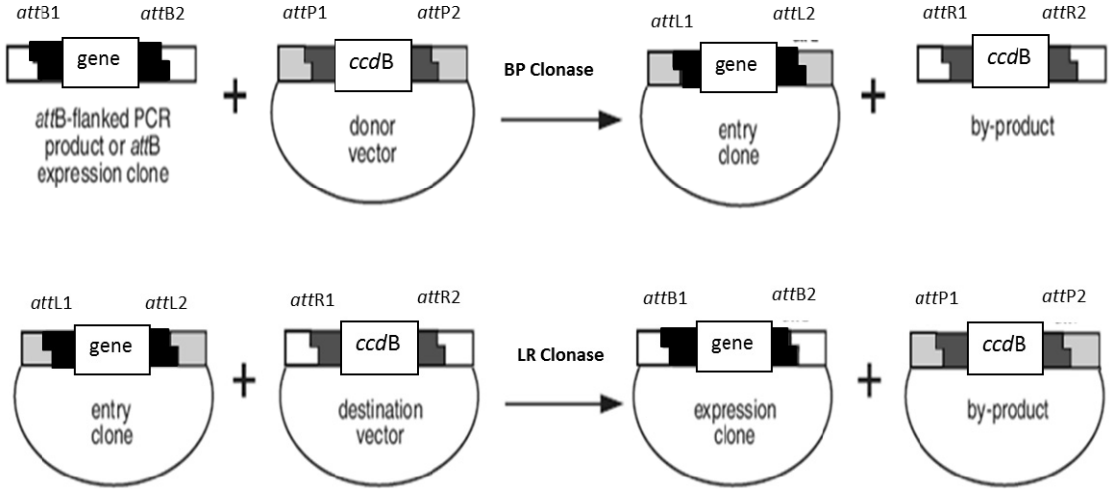
DNA polymerse B:

Thermo Scientific *Pfu* DNA Polymerase is a highly thermostable DNA polymerase from the hyperthermophilic archaeum *Pyrococcus furiosus*. The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'→3' direction. *Pfu* DNA Polymerase also exhibits 3'→5' exonuclease (proofreading) activity that enables the polymerase to correct nucleotide incorporation errors. It has no 5'→3' exonuclease activity.

Problem 2, continued

b)

You want to move your cDNA from the TOPO vector to make a Gateway Entry clone for ASHR3, with the aim of making an Expression clone afterwards. Explain the figure below. What is the function of the *att* sites? What is the difference between the BP and the LR clonases? What happens to the by-products and why?



c)

Translate the sequence below which encompasses the codons for the last six amino acids in ASHR3 and the stop codon. Make use of the genetic code table in Appendix 3.

```
.. ccgtaaaccgcagcaagattagct
```

You have a destination vector for making a fusions protein with a C-terminal GFP tag. Use the Gateway manual (Box 2) to make an *ASHR3* reverse *att* primer (*att*B2) suitable for making an ASHR3-GFP fusion protein.

Box 2

- To enable efficient Gateway® cloning, the reverse primer **MUST** contain the following structure:
  1. Four guanine (G) residues at the 5′ end followed by
  2. The 25 bp *att*B2 site followed by
  3. 18-25 bp of template- or gene-specific sequences
- If you wish to fuse your PCR product in frame with a C-terminal tag:
  1. The primer must include one additional nucleotide to maintain the proper reading frame with the *att*B2 region (see diagram below and **Example 2**, next page)
  2. Any in-frame stop codons between the *att*B2 site and your gene of interest must be removed

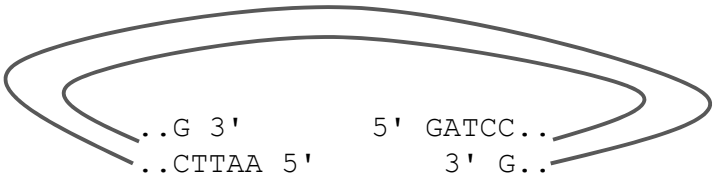
*att*B2 Reverse Primer:

5′-GGGG-AC-CAC-**TTT-GTA**-CAA-GAA-AGC-TGG-GTN--(template-specific sequence)-3′

*att*B2

Problem 3

a)
Below is schematically illustrated a plasmid vector that has been cut with the restriction enzymes EcoRI (GAATTC) and BamHI (GGATCC), resulting in sticky ends. Indicated are also five different linear double-stranded DNA fragments with sticky ends. Which one of these fragments can be efficiently ligated with the vector to form a circular plasmid? Explain.



i)
```
5' TTAA......
       ......CTAG 5'
```

ii)
```
5' AATT......
       ......GATC 5'
```

iii)
```
3' AATT......
       ......CTAG 3'
```

iv)
```
5' AATT......
       ......CTAG 5'
```

v)
```
3' TTAA......
       ......CTAG 5'
```

b)
Shown below are pairs of oligonucleotides, that are partially complementary to each other (the complementary sequence is underlined). For each pair, show how the two sequences can anneal to each other. In each of the two cases, what would be the result of the extension of the annealed DNA by a DNA polymerase (template-dependent, with proofreading activity) in the presence of dNTPs?

i)
```
5' CGTCAGAGTACC 3'
        and
5' TAGCATGGTACT 3'
```

ii)
```
5' TAGCATTGCACG 3'
        and
5' ATGCTATAGACG 3'
```

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

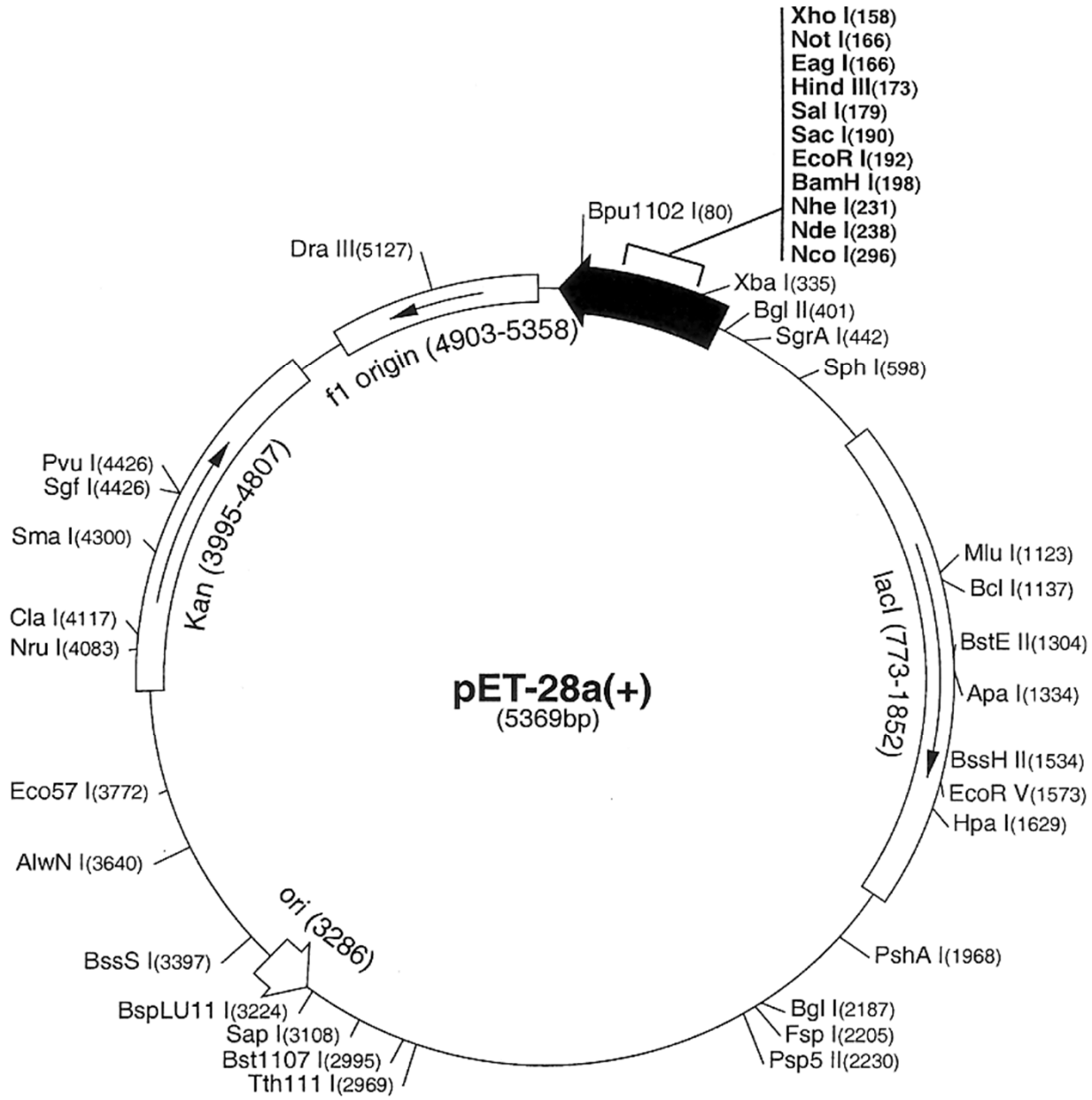
i)
How can a sequence search help you in identifying the (unknown) function of a protein?

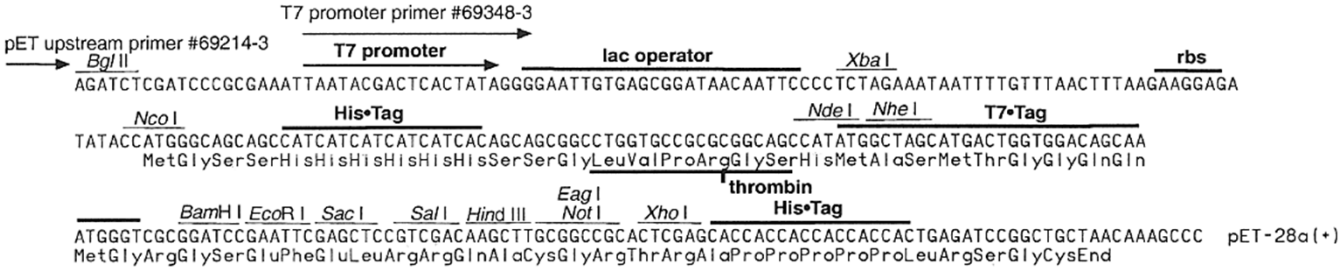
ii)
What is a DNA library?

iii)
Which three elements should be present in a plasmid used for DNA cloning?

iv)
What are the basic principles for monitoring gene expression using DNA microarrays?

Appendix 1. Map of plasmid pET28a(+)





Appendix 2. DNA and protein sequence of Arabidopsis ALKBH8

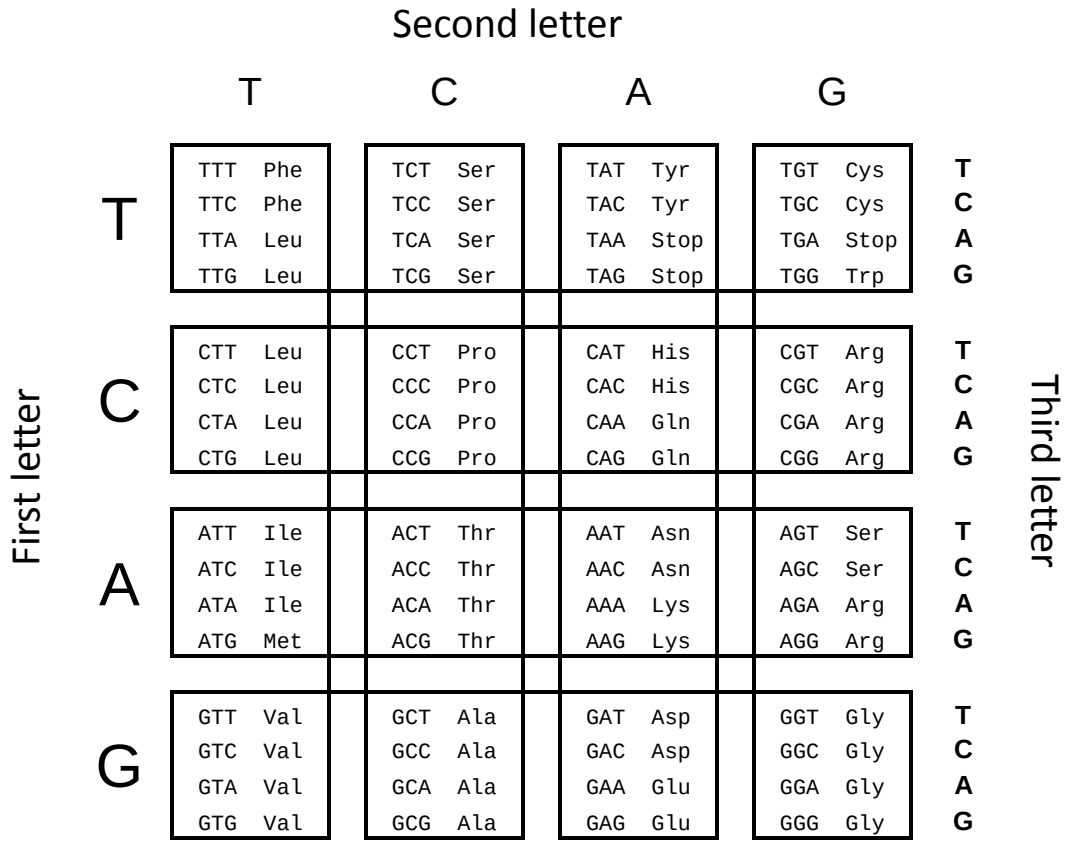
(344 amino acids, 1035 nucleotides incl. stop codon)

```
    1   M  V  Q  P  R  F  V  R  P  T  Q  S  S  P  S  S  I  S  G  E
    1  ATGGTTCAGCCGCGATTCGTCCGTCCGACGCAATCATCGCCATCTTCCATTTCTGGAGAA

   21   P  N  S  S  N  L  Y  V  A  N  C  G  P  A  V  G  L  T  H  N
   61  CCAAATTCATCAAATCTATACGTCGCGAATTGTGGACCGGCGGTTGGACTAACACACAAC

   41   A  I  A  A  V  F  A  E  F  G  E  V  N  G  V  Y  A  A  D  D
  121  GCAATCGCGGCGGTGTTCGCTGAGTTCGGTGAAGTAAACGGAGTCTACGCGGCGGATGAT

   61   S  G  V  R  V  I  V  S  F  A  D  P  F  S  A  K  A  A  L  E
  181  AGCGGCGTTCGTGTTATCGTCTCTTTCGCTGATCCCTTCTCCGCGAAAGCAGCTCTAGAA

   81   A  L  S  G  R  P  C  P  D  L  K  G  R  S  L  H  I  R  Y  S
  241  GCTTTGAGTGGTCGACCATGTCCTGACCTTAAAGGACGTTCTTTGCATATTCGATATTCA

  101   V  L  Q  L  P  S  E  V  N  D  C  V  P  V  S  L  I  D  S  E
  301  GTTCTTCAATTACCTTCCGAGGTGAATGATTGTGTTCCAGTGTCTTTGATTGATTCAGAG

  121   L  N  I  P  G  L  F  L  L  P  D  F  V  T  V  A  E  E  Q  Q
  361  TTGAATATTCCAGGGCTTTTCTTGTTACCTGATTTTGTCACTGTGGCAGAAGAACAGCAA

  141   L  L  A  A  V  D  A  R  H  W  I  G  L  A  K  R  R  V  Q  H
  421  TTGCTTGCAGCTGTTGATGCTCGACATTGGATTGGTCTTGCTAAACGGCGTGTTCAACAC

  161   Y  G  Y  E  F  C  Y  G  T  R  N  V  D  T  K  K  R  L  G  E
  481  TATGGATATGAGTTTTGTTACGGGACAAGAAATGTTGATACCAAAAAGCGTCTCGGTGAG

  181   L  P  S  F  V  S  P  I  L  E  R  I  Y  L  F  P  N  F  D  N
  541  CTTCCGTCGTTTGTTTCTCCTATACTTGAAAGAATCTATTTGTTCCCGAACTTTGACAAC

  201   G  S  A  S  L  N  L  D  Q  L  T  V  N  E  Y  P  S  G  V  G
  601  GGCTCAGCAAGCTTGAATTTGGACCAGTTAACGGTAAATGAGTACCCATCTGGTGTGGGA

  221   L  S  P  H  I  D  T  H  S  A  F  E  D  C  I  F  S  L  S  L
  661  TTATCGCCTCATATTGATACACATTCAGCATTTGAAGATTGCATATTCAGCCTTTCTTTA

  241   A  G  P  C  I  M  E  F  R  R  Y  S  V  S  T  W  K  A  S  T
  721  GCTGGTCCTTGTATTATGGAATTCAGAAGATACTCTGTTTCCACATGGAAAGCCTCAACT

  261   T  D  A  E  K  S  G  D  S  S  C  I  K  K  A  L  Y  L  P  P
  781  ACCGATGCTGAGAAGTCGGGTGACTCCTCTTGCATCAAAAAGGCCTTATATCTTCCTCCT

  281   R  S  M  L  L  L  S  G  E  A  R  Y  A  W  N  H  Y  I  P  H
  841  CGGTCTATGCTCTTATTATCCGGTGAAGCACGATATGCTTGGAATCACTACATTCCACAT

  301   H  K  I  D  K  V  K  D  K  V  I  R  R  S  S  R  R  V  S  F
  901  CACAAGATTGACAAGGTGAAGGATAAAGTGATCAGAAGAAGTTCTAGAAGGGTATCTTTC

  321   T  L  R  K  V  R  N  H  P  C  S  C  K  Y  P  Q  Y  C  D  S
  961  ACATTACGGAAGGTGAGAAATCATCCTTGCAGTTGTAAGTATCCACAATACTGTGACTCT

  341   Q  Q  Q  M  -
 1021  CAGCAACAAATGTAA
```

Enzyme	Cleavage site(s)
BclI	929
EcoRI	739
HindIII	239, 609
HpaI	629
MunI	418
NruI	85
PvuII	430
SalI	251
SspI	366
StuI	823
XbaI	234, 943

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