

# BCH3356 *Molecular Biology Laboratory*

Final exam: This exam is worth 30 % of the final mark for the course BCH3356

Name: \_\_\_\_\_

Student number: \_\_\_\_\_

## Instructions

*All questions should be answered within the space provided on THIS COPY EXAM.*

*You may use the endorsement of pages for your draft calculations.*

*Students are allowed to use a calculator.*

*Proctors will not answer any question during the exam.*

## Part I ( /30 marks): Underlying principles of molecular techniques

### 1. BCH3356 Crossword (10%)

- ACROSS
- Before proceeding with ligation, pTrcHisB was treated with a PHOSPHATASE.
- Type of membrane used for the electrophoretic transfer: PVDF
- Restriction enzymes are also called restriction ENDONUCLEASES
- The chemical added to a liquid culture to promote the overexpression of your recombinant protein was IPTG
- A negative control is commonly used to assess the BACKGROUND signal.
- The discrimination capacity of a test in detecting a given signal: SPECIFICITY
- The transfer of a cloned fragment of DNA from one vector to another: SUBCLONING
- The ion used to recharge the affinity chromatography column used to purify your recombinant T7 RNA polymerase : NICKEL
- The ligation strategy used in BCH3356 is considered as DIRECTIONAL.
- DOWN
- Number of steps per PCR amplification cycle: THREE
- The plasmid conformation that moves the faster on a gel is the SUPERCOILED conformation.
- Process of DNA melting: DENATURATION
- The name of the destination vector used for the ligation you performed is pTRCHISB.
- pH conditions used for the miniprep: ALKALINE
- Component of the lac operon that is constitutively expressed in host cells transformed with an empty or recombinant pTrcHisB: REPRESSOR
- PCR product: AMPLICON
- Is the primer that anneals onto the antisense DNA strand: FORWARD
- Ability of a cell to take up extracellular DNA: COMPETENCY
- Reformation of complementary strands of DNA: ANNEALING

1. 0.5 was subtracted for every missing or wrong answer.

2. Identify and explain the main procedural steps for the minipreparation of plasmid DNA by alkaline lysis. Molecular details are to be emphasized. Use a separate paragraph to describe each major step. (10%)

3 MARKS: Cell lysis with NaOH for denaturation of plasmid and chromosomal DNA

3 MARKS: Neutralization with K acetate: plasmid DNA reanneals faster than chromosomal DNA because the two strands of the plasmid DNA remain entangled due to supercoiling.

4 MARKS: Centrifugation to pellet chromosomal DNA and other aggregates including denatured proteins. Plasmid DNA is found in the supernatant.

3. Explain the underlying molecular principle for immobilized metal ion affinity chromatography (IMAC) you used to purify your recombinant his-tagged T7 RNA polymerase. Molecular details should be emphasized. Make sure to explain (1) why the his-tagged T7 RNA polymerase initially binds onto the beads inside the column and then (2) why it is eluted outside the column. (10%)

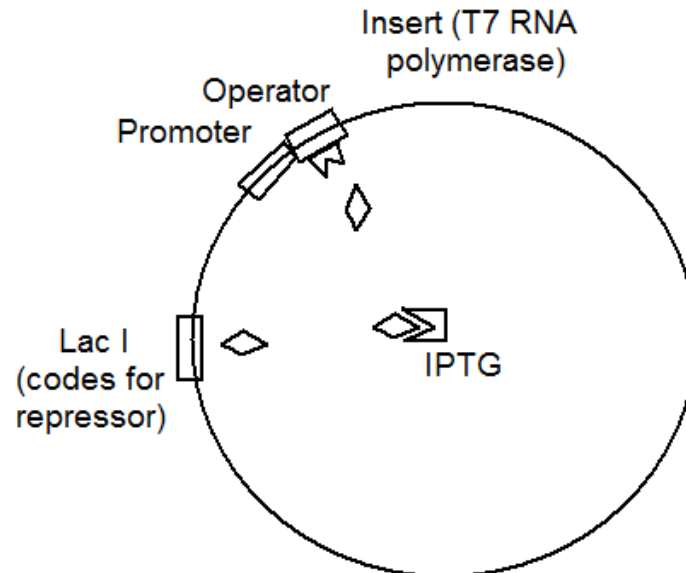
2 MARKS: Resin beads are covalently coated with the NTA chelator

2 MARKS: Column is charged with nickel ions that form chelation bounds with NTA.

3 MARKS: Proteins with his-tag can be retained within the column by forming chelation bounds with nickel ions.

4 MARKS: Elution of the his-tagged protein is done by increasing the imidazole concentration. Imidazole is identical to the histidine ring side chain and it competes with his-tag for accessing nickel ions.

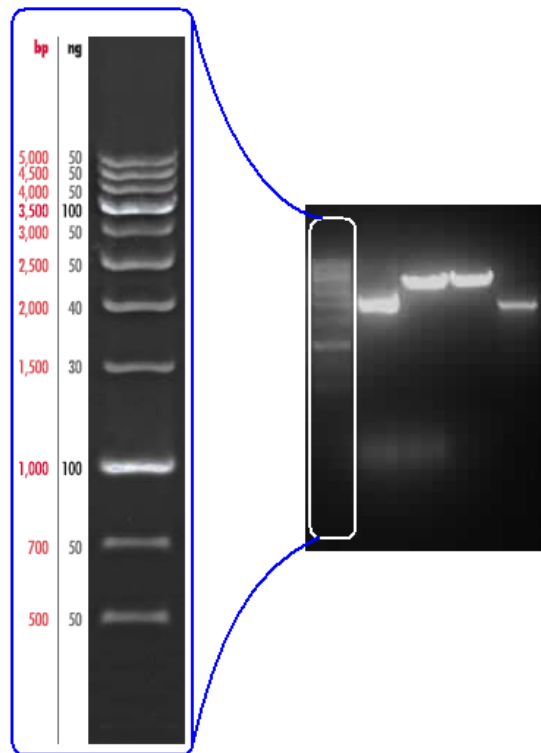
- 4 Explain the genetic components of the lac operon regulating the IPTG-inducible expression of T7 RNA polymerase in the TrcHisB/T7 and DH5a system you used during the semester. You should clearly explained how IPTG is able to induce protein expression. Use a diagram. (10%)



- The lac I repressor is constitutively expressed and binds onto the operator to prevent expression of the T7 RNA pol. Insert.
- IPTG binds with lac I repressor and frees the operator so transcription of T7 RNA polymerase insert can occur.
  - 2 MARKS: for lacI gene
  - 1 MARK for promoter
  - 2 MARKS for operator
  - 2 MARKS for T7 RNA pol. insert downstream the operator
  - -1 if a reference to the T7 RNA polymerase insert is missing
  - -1 for a reference to the three genes involved in galactose metabolism: lacZ, lacY and lacA (these 3 genes were missing from pTrcHisB/T7)
  - -1 if lac I was not incorporated in diagram
  - -2 if diagram was missing

## Part II ( /30 marks): Analytical skills

1. A group of students who completed the course last year had prepared an amplicon and a destination plasmid for a ligation procedure. Refer to their gel picture below to figure out the volumes to be used for ligation. You should assume that the amount of plasmid necessary for the ligation is 10ng with an insert:vector molar ratio of 5:1. That means that there should be 5 molecules of insert for every molecule of plasmid. Notice that the lengths for the plasmid vector and PCR amplicon used last year were 2961bp and 1851bp, respectively. Assume that the total volume of both the purified plasmid and purified amplicon was about 50ul. Explain your reasoning and calculations on next sheet then fill the summary table provided just under the gel. (15%)

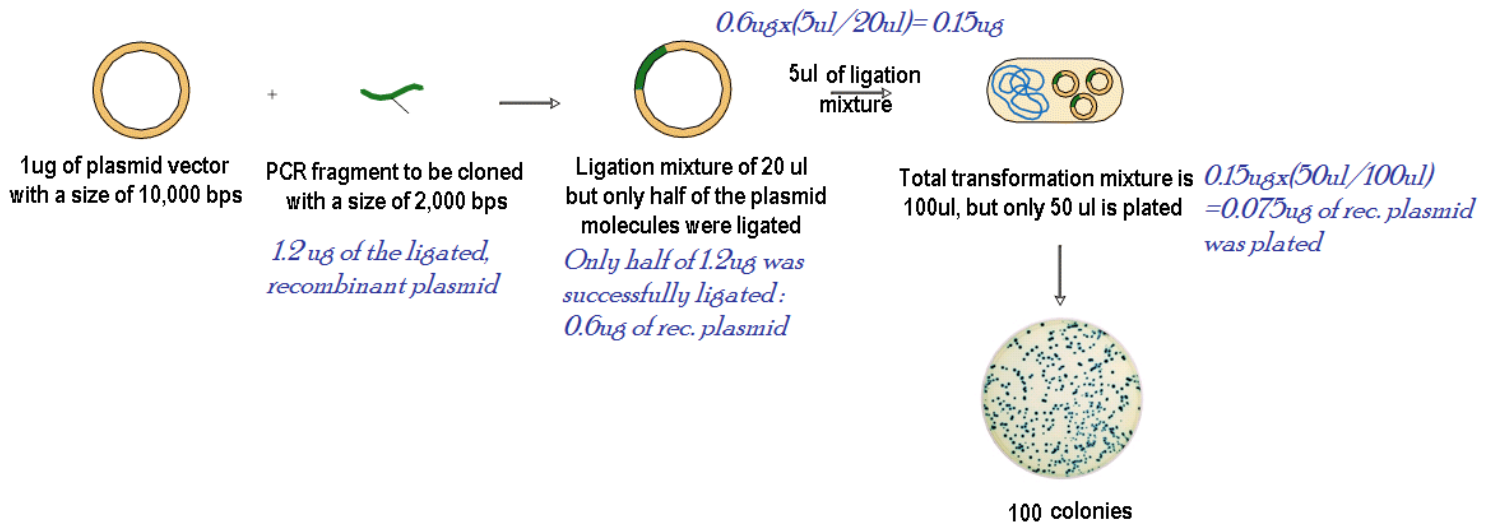


- 1) DNA ladder : 5ul
- 2) Undigested plasmid : 10 ul total (5ul + 4ul H2O +1ul loading buffer)
- 3) Digested, unpurified plasmid : 10ul total (5ul + 4ul H2O + 1ul loading buffer)
- 4) Digested, purified plasmid : 10ul total (5ul + 4ul H2O + 1ul loading buffer)
- 5) Digested, purified amplicon: 10ul total (5ul + 4ul H2O + 1ul loading buffer)

TREATMENT	LIGATION REACTION	H <sub>2</sub> O (μl)	XhoI/EcoRI open plasmid 25ng (μl)	Digested and purified PCR amplicon (μl)	Ligation buffer 5X (μl)	Ligase (1U/μl)	Total volume (μl)
I PCR product	Purified, dephosphorylated plasmid + digested and purified PCR amplicon					2	20

4 MARKS for estimate of insert amount ( $10\text{ng} \times 5 \times 1851\text{bp}/2961\text{bp} = \text{about } 31.2\text{ng}$  of insert; or 78ng if 25ng of plasmid were used)  
3MARKS for conversion of band intensities into DNA concentrations in purified insert and plasmid (lanes 4 and 5)  
3MARKS for correct volume answers

**Transformation yield (10%)** The figure below displays an overview for a ligation and transformation procedure.



- a. What's the transformation yield for that experiment? Make sure to use the appropriate units so that you can compare your experimental value with the expected yield. Show your calculations. 7 MARKS

100 colonies/0.075  $\mu\text{g}$  = 1333 cfu/  $\mu\text{g}$  of rec. plasmid DNA  
 -2 MARKS per calculation error  
 -2 MARKS if experimental units not provided

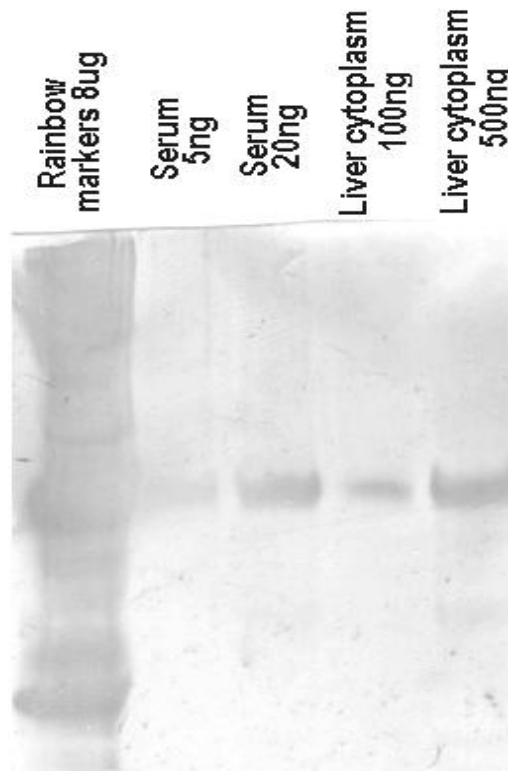
- b. Is the experimental transformation yield reasonable? In other words, is it too low or too high relative to the expected transformation yield assuming that competent cells had been treated with calcium ions and heat-shocked? 3 MARKS

Expected transformation yield is  $10^{+6}$  cfu/  $\mu\text{g}$  of plasmid DNA, but only 1333 cfu/  $\mu\text{g}$  was obtained: this is low!

2 MARKS for mention of the expected yield

1 MARK for comparison between experimental and expected values

**Western analysis (10 marks)** The Western analysis result obtained by one group of students from last year is displayed below. Assume that the band that was detected corresponds to rat serum albumin.



- c. Refer to the results displayed on the figure above to discuss the relative abundance of rat albumin between the two fractions that were investigated, i.e., serum and liver cytoplasm. Show your calculations and justify your reasoning. 3 MARKS

Intensities in lanes 3 (20 ng of serum) and 5 (500ng of liver cytoplasm) are similar although 25 times more protein was loading in lane 5! That means that the target, rat serum albumin, is 25 times less abundant in the liver cytoplasm fraction compared to the serum fraction. Similar comparison between lanes 2 (5ng of serum) and 4 (100ng of liver cytoplasm) gives a similar relative abundance.

-1.5 MARKS if a quantitative relative estimate was missing

- d. Estimate the sensitivity of the Western analysis. 3 MARKS

A band was detected with only 5ng of serum and 100 ng of liver cytoplasm. The sensitivity for the Western analysis was therefore  $\leq 5\text{ng}$  of serum protein and  $\leq 100\text{ng}$  of liver cytoplasm.

-1 MARK if the protein source (serum or liver cytoplasm) was not indicated

- e. Describe two experimental steps you could modify to improve the specificity of the Western analysis. 4 MARKS

2 MARKS per correct answer (increase washing temperature or length; longer blocking; more specific antibodies, ...)

Only the first two experimental steps were taken into consideration!

### Part III ( /30 marks): *Design of a subcloning procedure*

**Parental plasmid:** Presume that (1) the exact full length for the CDS of the thermotolerant DNA polymerase from *Thermus thermophilus* (Appendix 3) had already been cloned into M13mp18 (Appendix I) between the XmaI and PstI recognition sites.

**Destination plasmid:** It is desirable to transfer the insert coding for the complete CDS of the thermotolerant DNA polymerase from *Thermus thermophilus* from the parental plasmid into pTrcHisA (Appendix 2).

**Purpose:** You need to overexpress and purify the thermotolerant DNA polymerase for your own research.

- a. Design two primers that you would use to amplify the complete CDS of the thermotolerant DNA polymerase assuming that the goal is to complete the subcloning procedure. Provide a diagram showing the exact alignment positions of each of your two primers along the DNA template. Don't forget that your primers should be designed and engineered for protein expression. Justify your reasoning. 15 marks

#### For forward primer

```
Sense 5'ATG GAG GCG ATG CTT CCG CTC TTT G...3'
      5'ATG GAG GCG ATG CTT CCG C3'
Antisense 3'TAC CTC CGC TAC GAA GGC GAG AAA C...5'
```

Primer engineering with about 3 extra nucleotides and a restriction site (XhoI, SacI BglII, PstI, KpnI or EcoRI) at the 5' end. Zero, 1 or 2 extra nucleotides were necessary to maintain the insert in frame with the His-tag (XhoI: +0 nt; SacI: + 0 nt; BglII: + 2nts; PstI: +1 nt; KpnI: +2 nts; EcoRI: +2 nts).

#### For reverse primer

```
Position 3071 5'GAC TGG CTT TCC GCC AAG GGT TAG GGG GGC ...3' Position 3100 Sense
              3'CTG ACC GAA AGG CGG TTC CCA ATC5'
              3'CTG ACC GAA AGG CGG TTC CCA ATC CCC CCG ...5' Antisense
```

Primer engineering with about 3 extra nucleotides and a restriction site (SacI BglII, PstI, KpnI, EcoRI OR BstBI) at the 5' end. It is not necessary to add extra nucleotides to maintain the frame beyond the STOP codon!

Let's say we fit the insert between XhoI and EcoRI sites:

Forward primer: 5' NNN(CTCGAG)ATG GAG GCG ATG CTT CCG C3'

Reverse primer: 5' NNN(GAATTC)CTA ACC CTT GGC GGA AAG CCA GTC3'

5 MARKS for designing the part of each primer annealing onto the CDS

5 MARKS for the engineering of each primer (2x5=10 MARKS)

-2.5 MARKS if a primer was not positioned properly to subclone the CDS located between positions 590 and 3094 of the taq polymerase.

-2.5 MARKS if insert was out of frame

-2.5 MARKS per error

Let's assume now that the subcloning procedure was successfully completed with the two primers indicated in your answer to question a. You then want to verify the ligation detail at both ends of your insert by DNA sequencing. Design two sequencing primers you would use to confirm that your ligation had been done as expected. Use a diagram showing the exact annealing position of each of your two primers along the recombinant vector containing the complete CDS for the thermotolerant DNA polymerase. Also indicate the first 15 nucleotides you first expect for each of your two sequencing reactions. Explain your reasoning. 10 marks

The purpose was not to sequence the insert, but to sequence the 'ligation detail' or ligation boundaries. There are several possibilities.

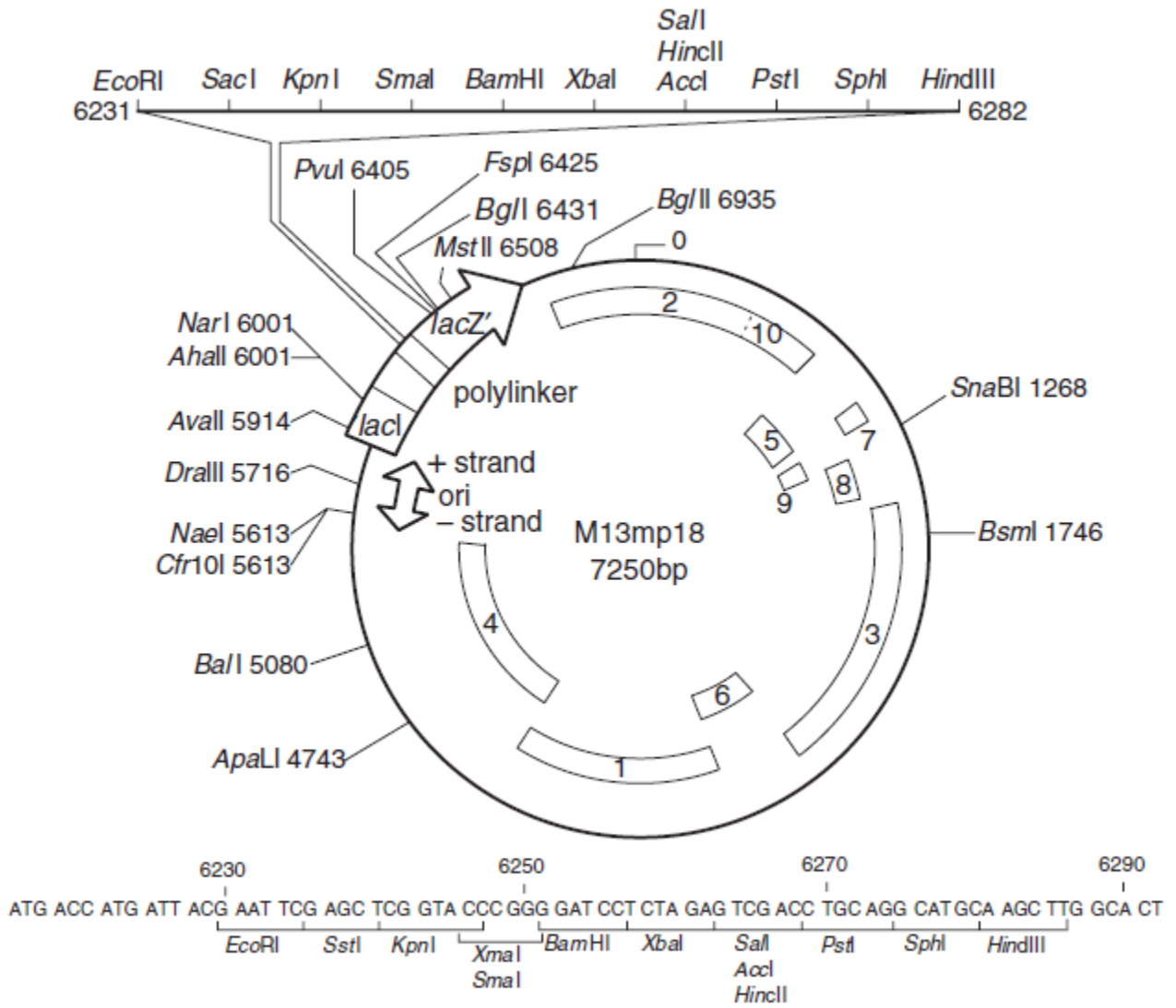
2.5 MARKS for each sequencing primer (total of 5 MARKS)

2.5 MARKS for the 15 first nts to be sequenced by each sequencing primer (total of 5 MARKS)

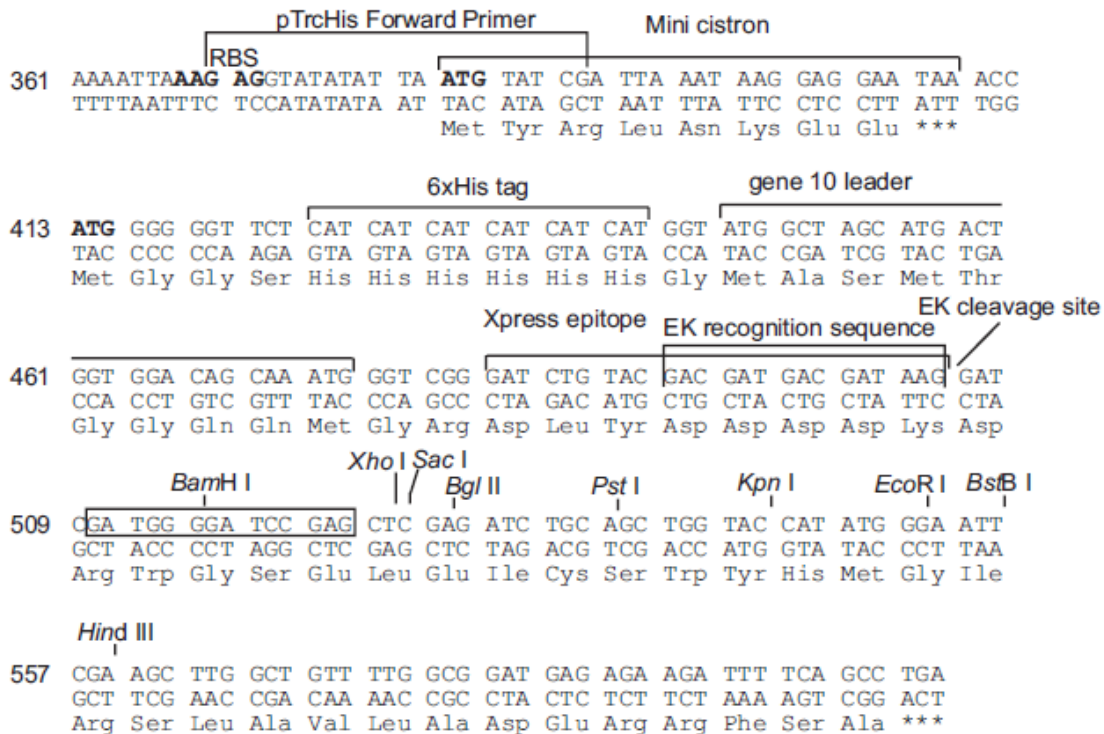
- b. What would be first 20 amino acids of your recombinant protein starting from the N-terminal end? 5 marks

The recombinant protein starts with the His-tag at its N-terminal end: Met-Gly-Gly-Ser-(His)<sub>6</sub>-Gly-Met-Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln (See pTrcHisA plasmid at positions 413-472).

Appendix I: Information relative to the vector M13mp18, a vector derived from a filamentous phage



Appendix 2: Polylinker region of the cloning vector pTrcHisA, a vector specialized for his-tagged protein expression. The full length of the vector is 4404.



### Recognition sequences

- BamHI: 5'-G↑G A T C C-3'  
3'-C C T A G↓G-5'
- XhoI: 5'-C↑T C G A G-3'  
3'-G A G C T↓C-5'
- SacI: 5'-G↑A G C T C-3'  
3'-C T C G A↓G-5'
- BglII: 5'-A↑G A T C T-3'  
3'-T C T A G↓A-5'
- PstI: 5'-C T G C A↑G-3'  
3'-G↓A C G T C-5'
- KpnI: 5'-G G T A C↑C-3'  
3'-C↓C A T G G-5'
- EcoRI: 5'-G↑A A T T C-3'  
3'-C T T A A↓G-5'
- BstBI: 5'-T T↑C G A A-3'  
3'-A A G C↓T T-5'
- HindIII: 5'-A↑A G C T T-3'  
3'-T T C G A↓A-5'

- Appendix 3: Sequence of the Thermotolerant DNA polymerase from *Thermus thermophilus*

```

FT      5'UTR      1..589
FT      CDS      590..3094
FT      3'UTR      3095..3221

1  tctagaggaa gcatgagcct caccctggca gacaaggtgg tctacgagga ggagatccag
61  aaaagccgct tcatcgccaa ggcggccccc gtggcctcgg aggaggaggg cttggcgttt
121 ttggccgaga accgggagcc tgaggccacc cacaacggcc acgcctacaa gatcggcctc
181 ctctaccgct tctctgacga cggggagccc tcgggcaccg caggcagggc cctcctccac
241 gccatagagg cccagggcct ggaccgggtg gcggctcctg tgggtgcgta cttcggcggg
301 gtgaagctcg gggccggggg gcttgtgctg gcttacgggg ggggtggcggc ggaggcctta
361 aggcggggcg ccaaggtccc cttgggtggag cgggtggggc tcgccttctc cgtgcccttc
421 gccgaggtgg gccgggtcta cgccctcctg gaggcccggc cccctgaagg ccgaggagac
481 ctacaccccg gaggcgtgcg cttcgccttc ctctcccca agcccgagcg ggaaggtttc
541 ctcagggcgc tcctggacgc caccggggga caggtggccc tggagtagca tggaggcgat
601 gcttccgctc tttgaaccca aaggccgggt cctcctgggt gacggccacc acctggccta
661 ccgcaccttc ttcgcctga agggcctcac cacgagccgg ggcgaaccgg tgcaggcggg
721 ctacggcttc gccaaagacc tcctcaagge cctgaaggag gacgggtaca aggcgctctt
781 cgtggtcttt gacgccaagg cccctcctct ccgccacgag gcctacgagg cctacaaggg
841 ggggagggcc ccgacccccg aggaattccc ccggcagctc gccctcatca aggagctggt
901 ggacctcctg gggtttacct gctcagaggt ccccggtac gaggcggacg acgttctcgc
961 caccctggcc aagaaggcgg aaaaggaggg gtacgaggtg cgcctcctca ccgccgaccg
1021 cgacctctac caactcgtct ccgaccgctc cgccgtcttc caccocgagg gccacctcat
1081 caccocgag tggctttggg agaagtacgg cctcaggccg gagcagtggg tggacttccg
1141 cgccctcgtg ggggacccct ccgacaacct ccccggggtc aagggcacgc gggagaagac
1201 cgccctcaag ctctcaagg agtggggaag cctggaaaac ctctcaaga acctggaccg
1261 ggtaaagcca gaaaacgtcc gggagaagat caaggccac ctggaagacc tcaggctctc
1321 cttggagctc tcccgggtgc gcaccgacct cccctgggag gtggacctcg cccaggggcg
1381 ggagcccagc cgggaggggc ttagggcctt cctggagagg ctggagtctg gcagcctcct
1441 ccacgagttc ggctcctcct agggccccgc cccctgggag gaggccccct ggcccccgcc
1501 ggaaggggcc ttcgtgggct tcgtcctctc ccgccccgag cccatgtggg cggagcttaa
1561 agccctggcc gcctgcaggg acggccgggt gcaccgggca gcagaccctc tggcggggct
1621 aaaggacctc aaggaggtcc ggggcctcct cgccaaggac ctgcctctct tggcctcgag
1681 ggaggggcta gacctcgtgc ccggggacga ccccatgctc ctgcctacc tcctggacce
1741 ctccaacacc acccccgagg ggggtggcgc gcgctacggg ggggagtgga cggaggacgc
1801 cgcccaccgg gccctcctct cggagaggct ccatcggaac ctcttaagc gcctcgaggg
1861 ggaggagaag ctcttttggc tctaccacga ggtggaaaag cccctctccc gggctcctggc
1921 ccacatggag gccaccgggg tacggcggga cgtggcctac cttcaggccc tttccttggg
1981 gcttgcggag gagatccgcc gctcagagga ggaggtcttc cgcttggcgg gccaccctt
2041 caacctcaac tcccgggacc agctggaag ggtgctcttt gacgagctta ggcttcccgc
2101 cttggggaag acgcaaaaga caggcaagcg ctccaccagc gccgcgggtg tggaggcctc
2161 acgggaggcc caccctatcg tggagaagat cctccagcac cgggagctca ccaagctcaa
2221 gaacacctac gtggaccccc tcccaagcct cgtccaccgc aggacgggcc gcctccacac
2281 ccgcttcaac cagacggcca cggccacggg gaggcttagt agctccgacc ccaacctgca
2341 gaacatcccc gtccgcaccc ccttgggcca gaggatccgc cgggccttcg tggccgaggc
2401 gggttggggc ttgggtggccc tggactatag ccagatagag ctccgcgtcc tcgcccacct
2461 ctccggggac gaaaacctga tcagggtctt ccaggagggg aaggacatcc acaccagac
2521 cgcaagctgg atgttcggcg tccccccgga ggccgtggac cccctgatgc gccggggcggc
2581 caagacggtg aacttcggcg tcctctacgg catgtccgcc cataggctct cccaggagct
2641 tgccatcccc tacgaggagg cgggtggcct tatagagcgc tacttccaaa gcttcccaa
2701 ggtgcggggc tggatagaaa agaccctgga ggaggggagg aagcggggct acgtggaaac
2761 cctcttcgga agaaggcgct acgtgcccga cctcaacgcc cgggtgaaga gcgtcagggg
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2881 gctcggcatg gtgaagctct tccccgcct ccgggagatg ggggcccgca tgctcctcca
2941 ggtccacgac gagctcctcc tggaggcccc ccaagcgcgg gccgaggagg tggcggcttt
3001 ggccaaggag gccatggaga aggcctatcc cctcgcctg cccctggagg tggaggtggg
3061 gatgggggag gactggcttt ccgccaaggg ttaggggggc cctgccttt agaggaagtt
3121 caaggggttg tcctcagaa acgcctccag ggaacgccc tctgggccta ccaggaggcc
3181 tttagcccca aaggtgcggg tgaaggcttc caggccctgg //

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## Appendix 4: Restriction Sites within the DNA sequence provided in appendix 3

#	Enzyme	Specificity	Sites & flanks	Cut positions (blunt - 5' ext. - 3' ext.)
1	AgeI	A <sup>▼</sup> CCGG <sub>A</sub> T	<a href="#">list</a>	*706/710
2	AleI	CACNN <sup>▼</sup> NNGTG	<a href="#">list</a>	2228
3	ApaLI	G <sup>▼</sup> TGCA <sub>C</sub>	<a href="#">list</a>	1589/1593
4	BamHI	G <sup>▼</sup> GATC <sub>C</sub>	<a href="#">list</a>	2373/2377
5	BcgI	<sub>▲</sub> NN <sup>▼</sup> (N) <sub>10</sub> CGA(N) <sub>6</sub> TGC(N) <sub>10</sub> <sub>▲</sub> NN <sup>▼</sup>	<a href="#">list</a>	*652/650+686/684
6	BclI	T <sup>▼</sup> GATC <sub>A</sub>	<a href="#">list</a>	#2478/2482
7	BfuAI	ACCTGCNNNN <sup>▼</sup> NNNN <sub>▲</sub>	<a href="#">list</a>	2343/2347
8	BmgBI	CAC <sup>▼</sup> GTC	<a href="#">list</a>	*1951
9	BsaI	GGTCTCN <sup>▼</sup> NNNN <sub>▲</sub>	<a href="#">list</a>	470/474
10	BsaWI	W <sup>▼</sup> CCGG <sub>W</sub>	<a href="#">list</a>	706/710
11	BsgI	GTGCAG(N) <sub>14</sub> <sub>▲</sub> NN <sup>▼</sup>	<a href="#">list</a>	731/729
12	BsmBI	CGTCTCN <sup>▼</sup> NNNN <sub>▲</sub>	<a href="#">list</a>	*1042/1046
13	BspHI	T <sup>▼</sup> CATG <sub>A</sub>	<a href="#">list</a>	2874/2878
14	BspMI	ACCTGCNNNN <sup>▼</sup> NNNN <sub>▲</sub>	<a href="#">list</a>	2343/2347
15	BsrFI	R <sup>▼</sup> CCGG <sub>Y</sub>	<a href="#">list</a>	*706/710
16	BsrI	ACTG <sub>A</sub> GN <sup>▼</sup>	<a href="#">list</a>	3077/3075
17	BtsI	GCAGTG <sub>A</sub> NN <sup>▼</sup>	<a href="#">list</a>	1130/1128
18	DraIII	CAC <sub>▲</sub> NNN <sup>▼</sup> GTG	<a href="#">list</a>	*1891/1888
19	EagI	C <sup>▼</sup> GGCC <sub>G</sub>	<a href="#">list</a>	*1582/1586
20	EcoP15I	CAGCAG(N) <sub>25</sub> <sup>▼</sup> NN <sub>▲</sub>	<a href="#">list</a>	1629/1631
21	HindIII	A <sup>▼</sup> AGCT <sub>T</sub>	<a href="#">list</a>	2689/2693
22	HpyCH4III	AC <sub>▲</sub> N <sup>▼</sup> GT	<a href="#">list</a>	2587/2586
23	KasI	G <sup>▼</sup> GCGC <sub>C</sub>	<a href="#">list</a>	*366/370
24	MslI	CAYNN <sup>▼</sup> NMRTG	<a href="#">list</a>	2228
25	NarI	GG <sup>▼</sup> CG <sub>CC</sub>	<a href="#">list</a>	*367/369
26	PfiFI	GACN <sup>▼</sup> N <sub>▲</sub> NGTC	<a href="#">list</a>	1046/1047
27	PvuII	CAG <sup>▼</sup> CTG	<a href="#">list</a>	2062
28	SbfI	CC <sub>▲</sub> TGCA <sup>▼</sup> GG	<a href="#">list</a>	1577/1573
29	SfoI	GGC <sup>▼</sup> GCC	<a href="#">list</a>	*368
30	SphI	G <sub>▲</sub> CATG <sup>▼</sup> C	<a href="#">list</a>	2932/2928
31	TspRI	<sub>▲</sub> MNCASTGNN <sup>▼</sup>	<a href="#">list</a>	1130/1121
32	Tth111I	GACN <sup>▼</sup> N <sub>▲</sub> NGTC	<a href="#">list</a>	1046/1047
33	XbaI	T <sup>▼</sup> CTAG <sub>A</sub>	<a href="#">list</a>	1/5
34	XmnI	GAANN <sup>▼</sup> NNTTC	<a href="#">list</a>	2897