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## APPENDIX 1

### Reference elements standard preparation

#### Manganese Standard Preparation

The reference element standard of manganese was prepared using Merck stock solution standard (Merck, Darmstadt, Germany) containing 1000 mg/L of the element. The concentration of calibration standard used for manganese determination in the meat samples ranged from 0.4 - 2.0 ppm. Deionized water was used as blank.

#### Calculations

Preparation of 0.4, 1.2 and 2.0 ppm of manganese standard respectively

$$\text{mL of stock solution required} = \frac{(\text{conc. of dilute standard}) \times (\text{vol. of dilute standard})}{(\text{conc. of stock solution})}$$

Where,

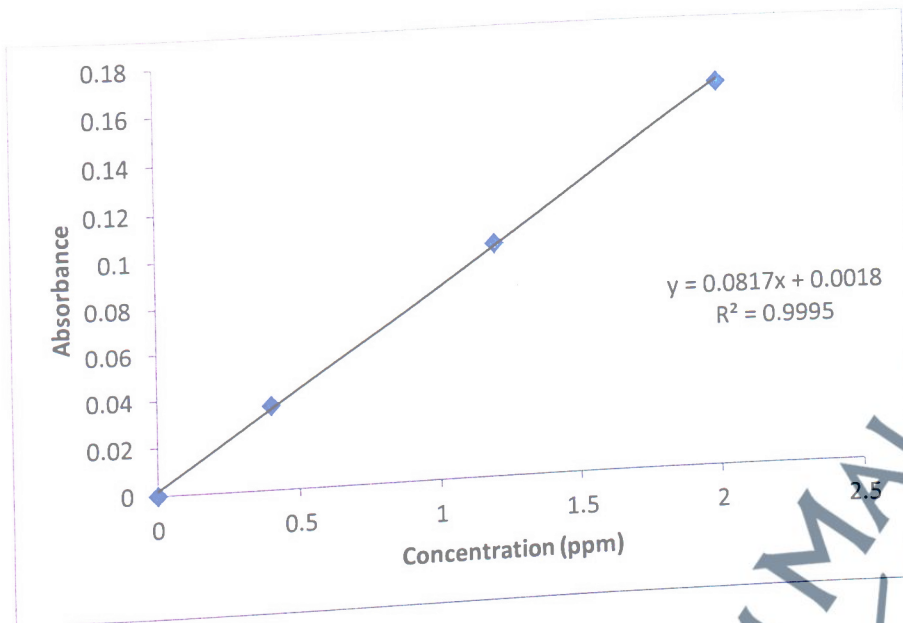
Concentration of dilute standard = 0.4, 1.2 and 2.0 ppm

Volume of dilute standard = 100 ml

Concentration of stock solution = 1000 ppm

mL of stock solution required to dilute 0.4, 1.2 and 2 ppm are 40, 120 and 200  $\mu$ l respectively.

Calibration Standard	Concentration of Standard (ppm)	Absorbance
1	0.000	0.000
2	0.400	0.036
3	1.200	0.101
4	2.000	0.164



Standard curve of Manganese (0.4-2.0 ppm)

### Calcium Standard Preparation

The reference element standard of calcium was prepared using Merck stock solution standard (Merck, Darmstadt, Germany) containing 1000 mg/L of the element. The concentration of calibration standard used for calcium determination in the meat samples ranged from 1 - 5 ppm. Deionized water was used as blank.

### Calculations

Preparation of 1, 3 and 5 ppm of calcium standard respectively

$$\text{mL of stock solution required} = \frac{(\text{conc. of dilute standard}) \times (\text{vol. of dilute standard})}{(\text{conc. of stock solution})}$$

Where,

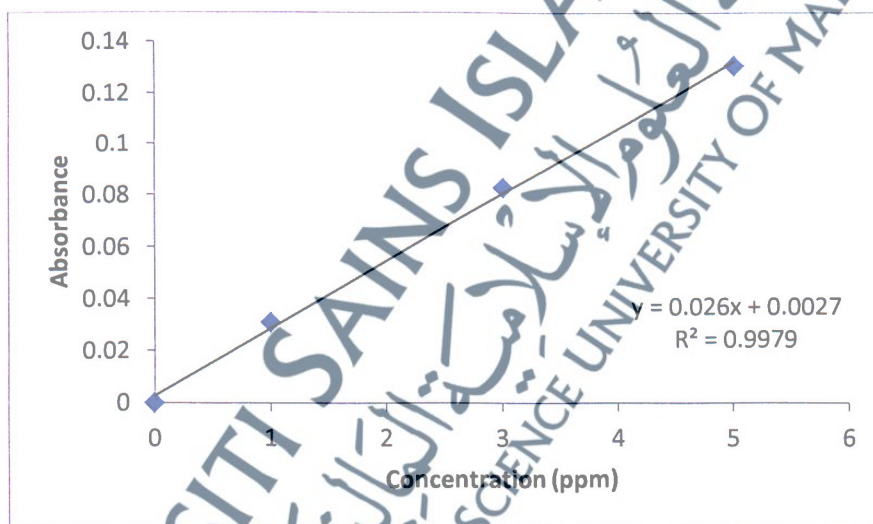
Concentration of dilute standard = 1, 3 and 5 ppm

Volume of dilute standard = 100 ml

Concentration of stock solution = 1000 ppm

mL of stock solution required to dilute 1, 3 and 5 ppm are 100, 300 and 500  $\mu$ l respectively.

Calibration Standard	Concentration of Standard (ppm)	Absorbance
1	0.000	0.000
2	1.000	0.081
3	3.000	0.083
4	5.000	0.131



Standard curve of Calcium (1-5 ppm)

### Copper Standard Preparation

The reference element standard of copper was prepared using Merck stock solution standard (Merck, Darmstadt, Germany) containing 1000 mg/L of the element. The

concentration of calibration standard used for copper determination in the meat samples ranged from 1 - 5 ppm. Deionized water was used as blank.

Calculations

Preparation of 1, 3 and 5 ppm of copper standard respectively

$$\text{mL of stock solution required} = \frac{(\text{conc. of dilute standard}) \times (\text{vol. of dilute standard})}{(\text{conc. of stock solution})}$$

Where,

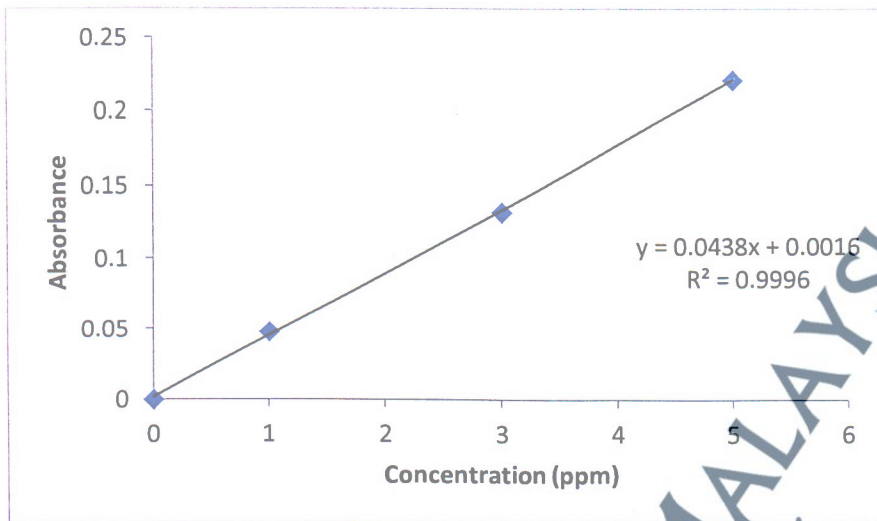
Concentration of dilute standard = 1, 3 and 5 ppm

Volume of dilute standard = 100 ml

Concentration of stock solution = 1000 ppm

mL of stock solution required to dilute 1, 3 and 5 ppm are 100, 300 and 500  $\mu$ l respectively.

Calibration Standard	Concentration of Standard (ppm)	Absorbance
1	0.000	0.000
2	1.000	0.048
3	3.000	0.013
4	5.000	0.221



Standard curve of copper (1-5 ppm)

### Iron Standard Preparation

The reference element standard of iron was prepared using Merck stock solution standard (Merck, Darmstadt, Germany) containing 1000 mg/L of the element. The concentration of calibration standard used for iron determination in the meat samples ranged from 2 - 6 ppm. Deionized water was used as blank.

### Calculations

Preparation of 2, 4 and 6 ppm of iron standard respectively

$$\text{mL of stock solution required} = \frac{(\text{conc. of dilute standard}) \times (\text{vol. of dilute standard})}{(\text{conc. of stock solution})}$$

Where,

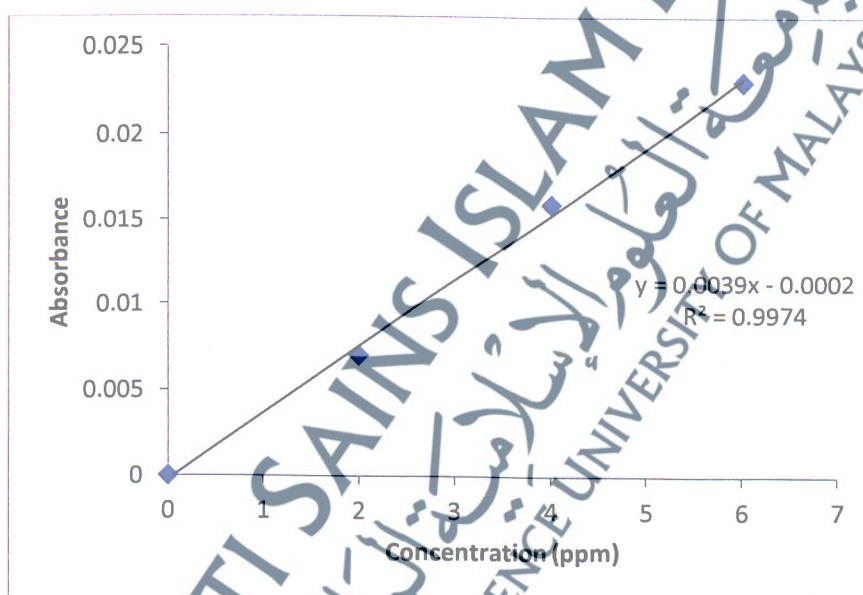
Concentration of dilute standard = 2, 4 and 6 ppm

Volume of dilute standard = 100 ml

Concentration of stock solution = 1000 ppm

mL of stock solution required to dilute 2, 4 and 6 ppm are 200, 400 and 600  $\mu$ l respectively.

Calibration Standard	Concentration of Standard (ppm)	Absorbance
1	0.000	0.000
2	2.000	0.007
3	4.000	0.016
4	6.000	0.023



Standard curve of iron (2-6 ppm)

### Magnesium Standard Preparation

The reference element standard of magnesium was prepared using Merck stock solution standard (Merck, Darmstadt, Germany) containing 1000 mg/L of the element.

The concentration of calibration standard used for magnesium determination in the meat samples ranged from 0.1 - 0.5 ppm. Deionized water was used as blank.

## Calculations

Preparation of 0.1, 0.3 and 0.5 ppm of magnesium standard respectively

$$\text{mL of stock solution required} = \frac{(\text{conc. of dilute standard}) \times (\text{vol. of dilute standard})}{(\text{conc. of stock solution})}$$

Where,

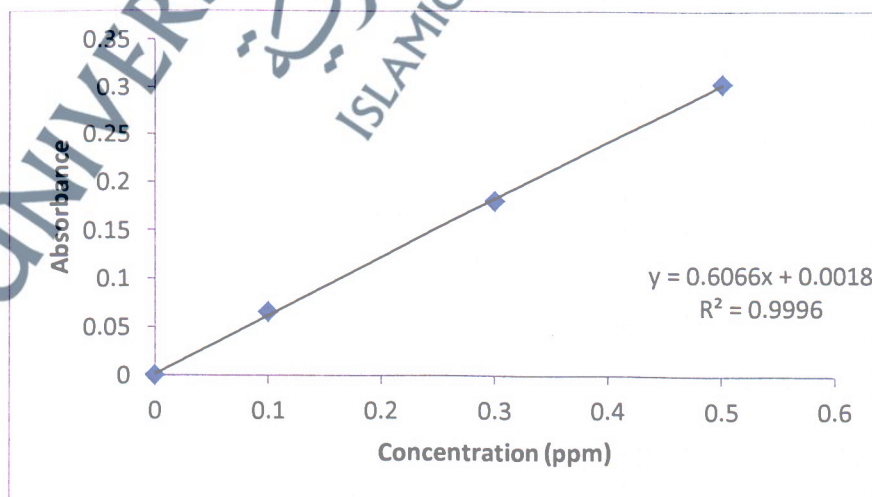
Concentration of dilute standard = 0.1, 0.3 and 0.5 ppm

Volume of dilute standard = 100 ml

Concentration of stock solution = 1000 ppm

mL of stock solution required to dilute 0.1, 0.3 and 0.5 ppm are 10, 30 and 50  $\mu\text{l}$  respectively.

Calibration Standard	Concentration of Standard (ppm)	Absorbance
1	0.000	0.000
2	0.100	0.066
3	0.300	0.181
4	0.500	0.306



Standard curve of Magnesium (0.1-0.5 ppm)

### Zinc Standard Preparation

The reference element standard of zinc was prepared using Merck stock solution standard (Merck, Darmstadt, Germany) containing 1000 mg/L of the element. The concentration of calibration standard used for zinc determination in the meat samples ranged from 0.3 - 1.0 ppm. Deionized water was used as blank.

### Calculations

Preparation of 0.3, 0.6 and 1.0 ppm of zinc standard respectively

$$\text{mL of stock solution required} = \frac{(\text{conc. of dilute standard}) \times (\text{vol. of dilute standard})}{(\text{conc. of stock solution})}$$

Where,

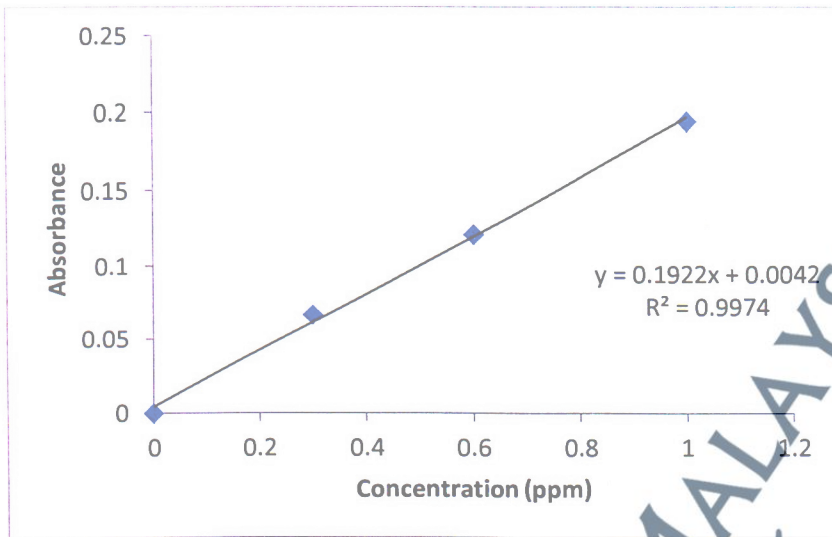
Concentration of dilute standard = 0.3, 0.6 and 1.0 ppm

Volume of dilute standard = 100 ml

Concentration of stock solution = 1000 ppm

mL of stock solution required to dilute 0.3, 0.6 and 1.0 ppm are 30, 60 and 100  $\mu$ l respectively.

Calibration Standard	Concentration of Standard (ppm)	Absorbance
1	0.000	0.000
2	0.300	0.067
3	0.600	0.121
4	1.000	0.194



Standard curve of Zinc (0.3-1.0 ppm)

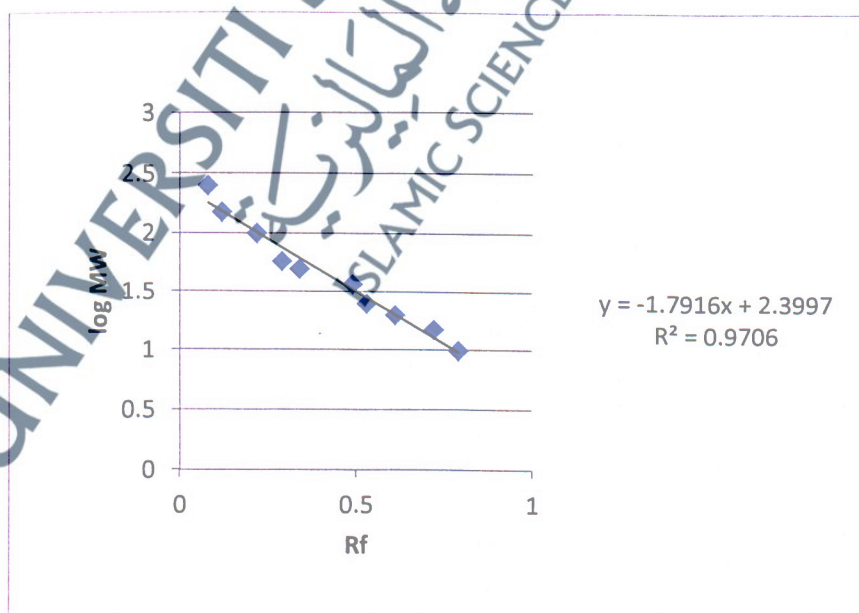
## APPENDIX 2

## Molecular Weight Determination of Bands

Migration distance of dye front: 9 cm

$R_f = \frac{\text{migration distance of protein}}{\text{migration distance of the dye front}}$

Band Numbers	Mw of Bands	Log Mw	Migration distance of Unknown Protein(cm)	$R_f$
1	10	1.00	7.1	0.79
2	15	1.18	6.5	0.72
3	20	1.30	5.5	0.61
4	25	1.4	4.8	0.53
5	37	1.57	4.4	0.49
6	50	1.70	3.1	0.34
7	75	1.76	2.6	0.29
8	100	2.0	2.0	0.22
9	150	2.18	1.1	0.12
10	250	2.40	0.72	0.08



Molecular Weight Determination of Protein bands of HM and NHM during refrigerated storage

Band Number	Migration distance of unknown protein	R <sub>f</sub>
1	1.2	0.13
2	2.4	0.27
3	2.7	0.30
4	2.9	0.32
5	3.1	0.34
6	3.3	0.42
7	4.7	0.52
8	7.6	0.84

From Figure 13:

$$y = -1.7916x + 2.3997$$

$x = R_f$  of unknown protein band

When  $R_f = 0.13$ ,

$$y = \log MW$$

$$MW = 10^y = 10^{-1.7916(0.13) + 2.3997}$$

$$MW = 146.8 \text{ KDa}$$

When  $R_f = 0.27$ ,

$$y = \log MW$$

$$MW = 10^y = 10^{-1.7916(0.27) + 2.3997}$$

$$MW = 83.4 \text{ KDa}$$

When  $R_f = 0.30$ ,

$$y = \log MW$$

$$MW = 10^y = 10^{-1.7916(0.30) + 2.3997}$$

$$MW = 72.8 \text{ KDa}$$

When  $R_f = 0.32$ ,

$$y = \log MW$$

$$MW = 10^y = 10^{-1.7916(0.32) + 2.3997}$$

MW = 67.1 KDa

When  $R_f = 0.34$ ,

$y = \log MW$

$$MW = 10^y = 10^{-1.7916(0.34) + 2.3997}$$

MW = 50.5 KDa

When  $R_f = 0.42$ ,

$y = \log MW$

$$MW = 10^y = 10^{-1.7916(0.42) + 2.3997}$$

MW = 44.0 KDa

When  $R_f = 0.52$ ,

$y = \log MW$

$$MW = 10^y = 10^{-1.7916(0.52) + 2.3997}$$

MW = 29.1 KDa

When  $R_f = 0.84$ ,

$y = \log MW$

$$MW = 10^y = 10^{-1.7916(0.84) + 2.3997}$$

MW = 7.7 KDa

Molecular weight of NHM and HM protein bands after SDS-PAGE

Samples	Number of Bands	Range of Molecular Weight (kDa)
NH1	6	44-146.8
NH9	8	7.7-146.8
H1	6	44-146.8
H9	8	29.1-146.8

<sup>a</sup>NH1= Non-Halal sample after 24 h refrigeration at 4°C, NH9= Non-Halal sample after 9 days of refrigerated storage at 4°C, H1=Halal slaughter sample after 24 h refrigeration at 4°C, H9= Halal slaughter sample after 9 days of refrigerated storage

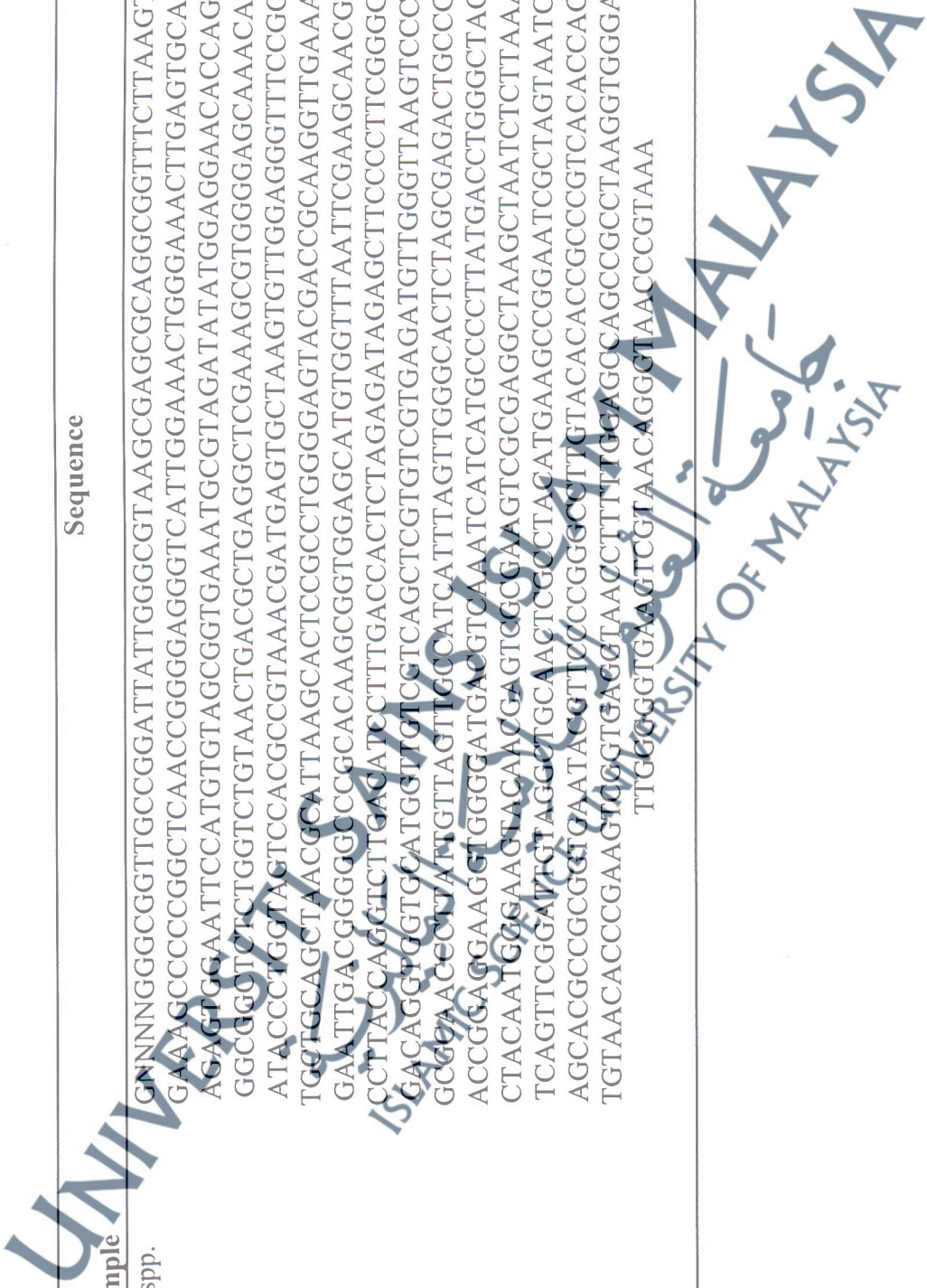


## Sequence

Sample

*E. spp.*

GNNNNGGGGGTTGCCGGATTATTGGGGCGTAAGCGAGCGCAGGGCGGTTTCTTAAGTCTGATGT  
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 AGAGTGGAAATTCCATGTGTAGCGGTGAAAATGCGTAGATAATGGAGGAAACACCAGTGGCGAA  
 GGCGGOTCTCTGGTCTGTA ACTGACGCTGAGGCTCGAAAAGCGTGGGGAGCAACACAGGATTAG  
 ATACCCCTGGTACTCCACGCCGTA AACGATGAGTGCTAAGTGTGGAGGGTTCCGGCCCTTCAG  
 TGGTGCAGCTAACCGCA TTAAGCACTCCGCCCTGGGGAGTACGACCGCCAAGGTTGAAACTCAAAG  
 GAAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGTTTAAATTCGAAGCAACGCGAAAGAA  
 CCTTACCAGGCTCTTGACATCC TTTGACCACCTCTAGAGATAGAGCTTCCCCCTCGGGGGCAAAGT  
 GACAGGTGGTGGATGTTGTCTCAGCTCGTCTCGTGAGATGTTGGGTTAAGTCCCGCAAACGA  
 GCGCAACECTATATGTTAGTTGCCATCATTTAGTTGGGCACCTCTAGCGAGACTGCCCGGTGACAA  
 ACCGCAAGAA GGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACCGTG  
 CTACAATGGGAACTACAACGAGTGCGGGAAGTCGCGGAGGCTAAGCTAATCTCTTAAAGCTTCTC  
 TCAGTTCGGATTGTAGGCTGCAACTCGGCTACATGAAGCCGGAAATCGCTAGTAATCGCCGGATC  
 AGCACGCCCGGTTGAATA CTTCCCGGGGCTTGTACACACCCGCCCTCACACCACGAGAGTT  
 TGTAACA CCCCAGGTTGGGTGAGGTAACCTTTTGGAGCCAGCCGCCCTAAGGTGGGATAGATGA  
 TTGGGGGTGAA GTCTGTAA CAGGTAACCCCGTAAA



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Sequence

Sample

*E. devriesei*

GNNGGGGCGGTTCCGGATTTTGGGCGTAAGCGAGCGCAGCGCGGTTTCTTAAGTCTGATGTG  
 AAAACCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAAGAG  
 GAGAGTGGAAATTCATGTGTAGCGGTGAAATGCCGTAGATATATGGAGGAACACCAGTGGCG  
 AAGCCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGAGCAAACAGGAT  
 TAGATAACCTGTGTAGTCCACGCCGTAACGATGAGTGCTAAGTGTGGAGGGTTTCCGCCCT  
 TTAGTGGCTGAGCTAACGCATTAAGCACTCCGCCCTGGGGAGTACGACCCGCAAGGTTGAAAC  
 TCAAAGGAAATTGACGGGGGCCCCGCAACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG  
 CGAAGAACCCTTACCAGGTOTTTGACATCCTTTTGACCACCTCTAGAGATAGAGCTTCCCCCTTCGG  
 GGGCAAGTGCACAGGTGGTGCATGGTTGTCGTCA GCTCGTGTGAGATGTTGGGTTAAG  
 TCCCGCAACGAGGGCAA CCGTTTATTGTTAGTTGCCATCATTAAGTTGGGCACCTAGCGAGA  
 CTGCCGCTGACAAACC GGAGGAAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGACC  
 TGGGCTACAGACCTGGTACAA TGGGA ACTACAACGAGTCGCGAAGTCGCGAGGCTAAGCTA  
 ATCTTTAAAGTTTCTCAGTTGGGATGTAGGCTGCAACTCGCCCTACATGAAGCCGGAAT  
 CGCTAGTAATCCGGGATCAGGACGCGCCGGTGAATAACGTTCCCGGGCCTTGTACACACCCGC  
 CCGTCACACCACGADAGTTTGTAAACACCCGAA GTCCGGTGAAGGTAACCTTTTGGAGCCAGCC  
 GCCTAAGGTGGGATAGATGATGGGGCTGAAGTCCGTAACAGGGGAAACCCGTAAAA