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## 23rd INTERNATIONAL BIOLOGY OLYMPIAD

8<sup>th</sup> – 15<sup>th</sup> July, 2012

SINGAPORE



PRACTICAL TEST 1

CELL & MOLECULAR BIOLOGY

***ANSWER KEY***

Total points: **100**

Duration: **90 minutes**

**Task (100 points)**

**Gene mapping by restriction endonuclease digestion of DNA fragments**

Part A. Confirmation of insertion of human DNA in a cloning plasmid. (80 points)

**Q1.1 (2 points × 8 + 1 point × 4 = 20 points)**

	Tube 1	Tube 2	Tube 3	Tube 4
DNA 'T' (with buffer)	10	10	10	10
RE1 ( <i>Nde</i> I)	0	1	0	1
RE2 ( <i>Eco</i> RI)	0	0	1	1
water	10	9	9	8
Total	20	20	20	20

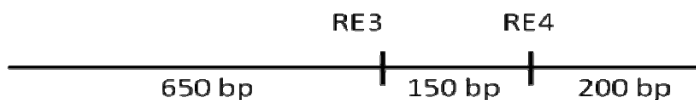
**Q1.2 (2 points × 5 = 10 points)**

a	b	c	d	e
✓	✗	✗	✗	✓

**Q1.3 (2 points × 5 = 10 points)**

a	b	c	d	e
✗	✓	✗	✓	✗

**Q1.4 (20 points)**



Either orientation

**Q1.5 (10 points)**

3 kbp —  
0.75 kbp —

1 2 3 4 5 6

500 bp  
300 bp

Lane 1: 1 kbp ladder (1 point)  
 Lane 2: uncut (supercoiled and relaxed form) (2 points)  
 Lane 3: Nde I – actual size: 2.57 kbp, 0.76 kbp (2 points for 2 bands).  
 Lane 4: EcoR I – 3.33 kbp (1 point for 1 band)  
 Lane 5: Nde I/EcoR I – actual sizes: 2.57 kbp, 0.49 kbp, 0.27 kbp (3 points for 3 bands)  
 Lane 6: 100-bp ladder (1 point)

**Q1.6 (1 point × 5 = 5 points)**

	RE1	RE2
Number of fragments	2 (1 point)	1 (1 point)
Estimated size	Any value between 2.5 to 3.0 kbp for the larger fragment, and 0.75 kbp for the smaller fragment – but their total must be close to the total given in the question, i.e., 3.33 kbp) (2 points)	3.33 kbp (any value between 3.0 to 3.5 kbp) (1 point)

**Q1.7 (1 point)**

Answer: 3.33 kbp (between 3 and 3.5 kbp) .

**Q1.8 (1 point)** [for having one correct tick; no points if there are more than 1 ticks]

Larger	Smaller	Same size
✓		

**Q1.9 (1 point)**

Answer: ✓ .

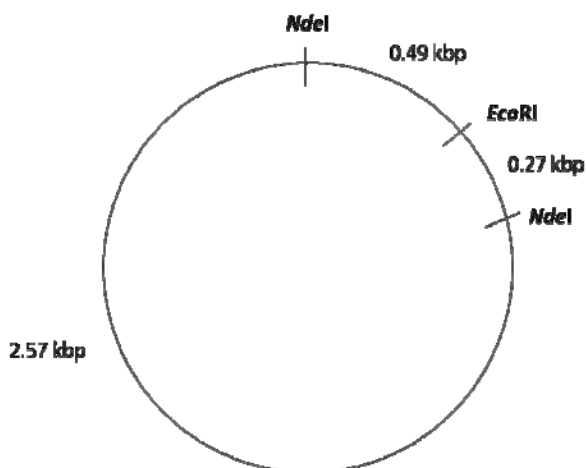
**Q1.10 (2 points)** [for having one correct tick; no points if there are more than 1 tick]

a	b	c
	✓	

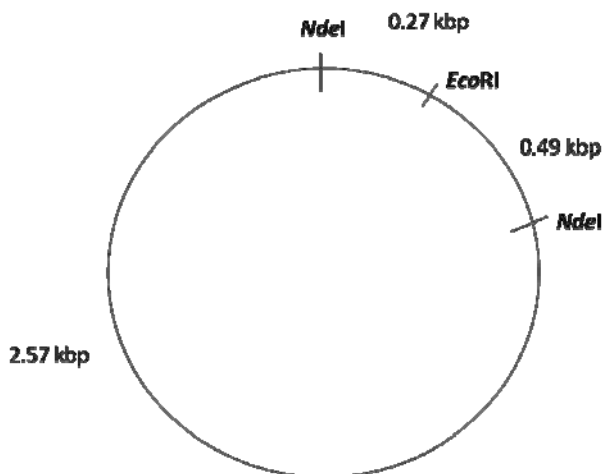
Part B. Determination of orientation by which fragment was inserted. (20 points)

**Q1.11 (20 points)**

1. **15 points.** 9 points for translating the observation in gels to writing down the estimate sizes of the 3 fragments generated by RE1 and RE2, i.e 2.5 to 3.0 kbp for the largest (3 points), 0.5 to 0.6 for the medium size (3 points) and 0.3 to 0.4 for the smallest fragment (3 points). However, their total size must be close to the total given in the question, i.e., 3.0 to 3.5 kbp (3 points) and the values must be consistent with the ones quoted in their answers to Q6 (3 points). Use enzyme names or RE1 and RE2).
2. **5 points** for proposing an alternative map (i.e., the relative position of EcoRI site to the flanking NdeI sites can be in two arrangements, see answers below).



With alternative model;



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PRACTICAL TEST 2

MICROBIOLOGY & BIOCHEMISTRY

*ANSWER KEY*

Total points: **100**

Duration: **90 minutes**

### Task I (50 points)

#### Bacteriophage: an effective agent in the killing of pathogenic bacteria

Part A. Effects of Phage and antibiotics on the killing of antibiotic-resistant *E. coli* (31 points)

**Q1.1 (1 point)**

Answer: 50-fold or 1/50

**Q1.2 (1 point)**

Answer: 10  $\mu\text{l}$

**Q1.3 (1 point)**

Answer: 10  $\mu\text{l}$

**Q1.4 (5 points  $\times$  3 = 15 points)**

	Tube 1	Tube 2	Tube 3	Tube 4
Diluted <i>E. coli</i> ( $2 \times 10^5$ cell/ml) in LB broth	0	20	20	20
bacteriophage stock ( $10^8$ pfu/ml) in deionized water	0	0	0	10
ampicillin stock (1 mg/ml) in deionized water	0	0	10	0
deionised water	10	10	0	0
LB broth	1000	970	970	970
Total ( $\mu\text{l}$ )	1000	1000	1000	1000

(5 points)

(5 points)

(5 points)



**Q1.5 (0.75 points × 2 + 1.5 points × 6 = 10.5 points)**

Tube	Absorbance reading at 595 nm	Blanked absorbance at 595 nm	Cell density (cells/ml)
1	0.143	0	0
2	0.312	0.166	$1.69 \times 10^6$
3	0.313	0.165	$1.71 \times 10^6$
4	0.157	0.015	$1.42 \times 10^4$

**Q1.6 (0.5 points × 5 = 2.5 points)**

a	b	c	d	e
x	✓	✓	x	x

Part B. Phage titre and multiplicity of infection (19 points)

**Q1.7 (2 points × 4 = 8 points)**

Plate	Dilution factor	Number of plaques observed	Calculated number of plaques in the original phage culture
A	$10^{-6}$	0	0
B	$10^{-5}$	2	200,000
C	$10^{-4}$	15	150,000
D	$10^{-3}$	153	153,000
E	$10^{-2}$	1560	156,000

**Q1.8 (3 points)**

$10^{-6}$	$10^{-5}$	$10^{-4}$	$10^{-3}$	$10^{-2}$
			✓	

**Q1.9 (4 points × 2 = 8 points)**

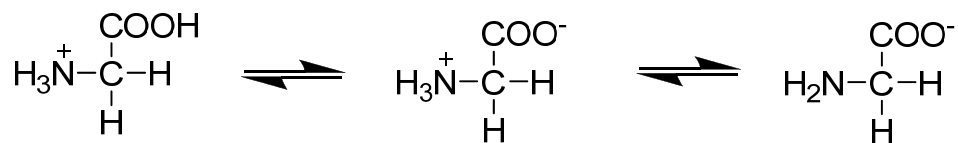
a	b
$3.06 \times 10^5$ pfu/ml	61

## Task II (50 points)

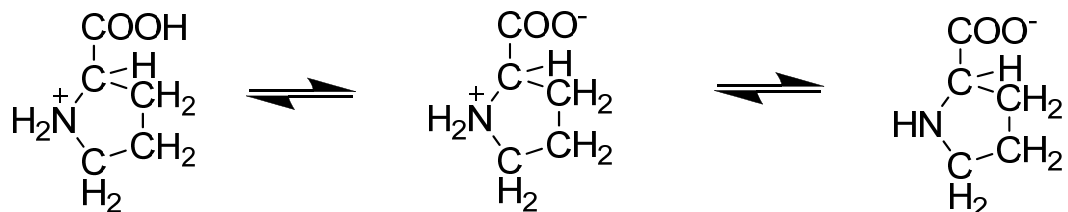
### Titration of an Amino Acid

**Q2.1 (3 points × 3 = 9 points)**

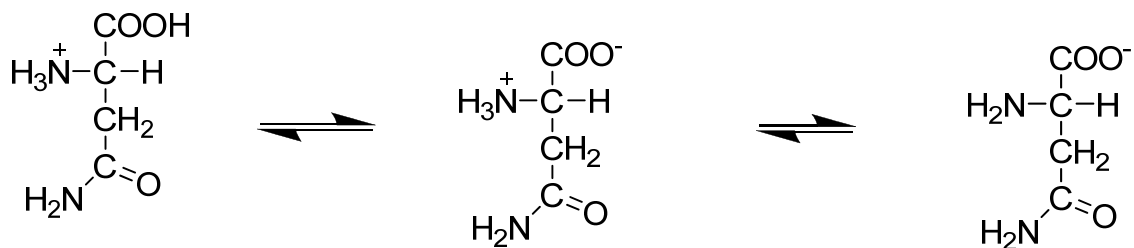
Glycine dissociation:



Proline dissociation:



Asparagine dissociation:



**Q2.2 (3 points × 2 = 6 points)**

Titration 1

Concentration of standardized NaOH: 0.3024 M

Starting volume of NaOH: 0.00 ml

Vol. NaOH added (ml)	pH
0.00	1.3
1.00	1.4
2.00	1.4
3.00	1.5
4.00	1.6
5.00	1.7
6.00	1.8
7.00	2.0
8.00	2.1
9.00	2.3
10.00	2.6
11.00	3.0
12.00	9.1
13.00	9.8
14.00	10.1
15.00	10.3
16.00	10.5
17.00	10.8
18.00	11.0
19.00	11.2
20.00	11.5
21.00	11.7
22.00	11.9
23.00	12.0
24.00	12.1
25.00	12.2

Titration 2

Concentration of standardized NaOH: 0.3024 M

Starting volume of NaOH: 0.00 ml

Vol. NaOH added (ml)	pH
0.00	1.4
1.00	1.4
2.00	1.5
3.00	1.6
4.00	1.7
5.00	1.8
6.00	1.9
7.00	2.1
8.00	2.2
9.00	2.4
10.00	2.7
11.00	3.2
12.00	9.4
13.00	9.9
14.00	10.2
15.00	10.4
16.00	10.6
17.00	10.8
18.00	11.0
19.00	11.3
20.00	11.6
21.00	11.8
22.00	12.0
23.00	12.1
24.00	12.2
25.00	12.3

**Q2.3 (6 points × 2 = 12 points)** Graphs 1 and 2.

Shape of graph: 2 points (buffering region containing pKa1); 2 points (inflexion point region containing pl); 2 points (buffering region containing pKa2)

**Q2.4 (2 points × 2 = 4 points)** Graphs 1 and 2.

Arrows to indicate: 2 points (finding and labeling pl – in middle)

**Q2.4.1 (2 points)**

Mean pl: 6.0 (2 pts: 5.8 – 6.2; 1 pt: 5.6 – 5.7 or 6.3 – 6.4)

**Q2.5 (4 points × 2 = 8 points)** Graphs 1 and 2.

Arrows to indicate: 2 points (finding and labeling pKa1 – in middle); 2 points (finding and labeling pKa2 – in middle).

**Q2.5.1 (2 points × 2 = 4 points)**

Mean pKa<sub>1</sub>: 1.9 (2 pts: 1.5 – 2.3; 1 pt: 1.1 – 1.4 or 2.4 – 2.7)

Mean pKa<sub>2</sub>: 10.9 (2 pts: 10.5 – 11.3; 1 pt: 10.1 – 10.4 or 11.4 – 11.7)

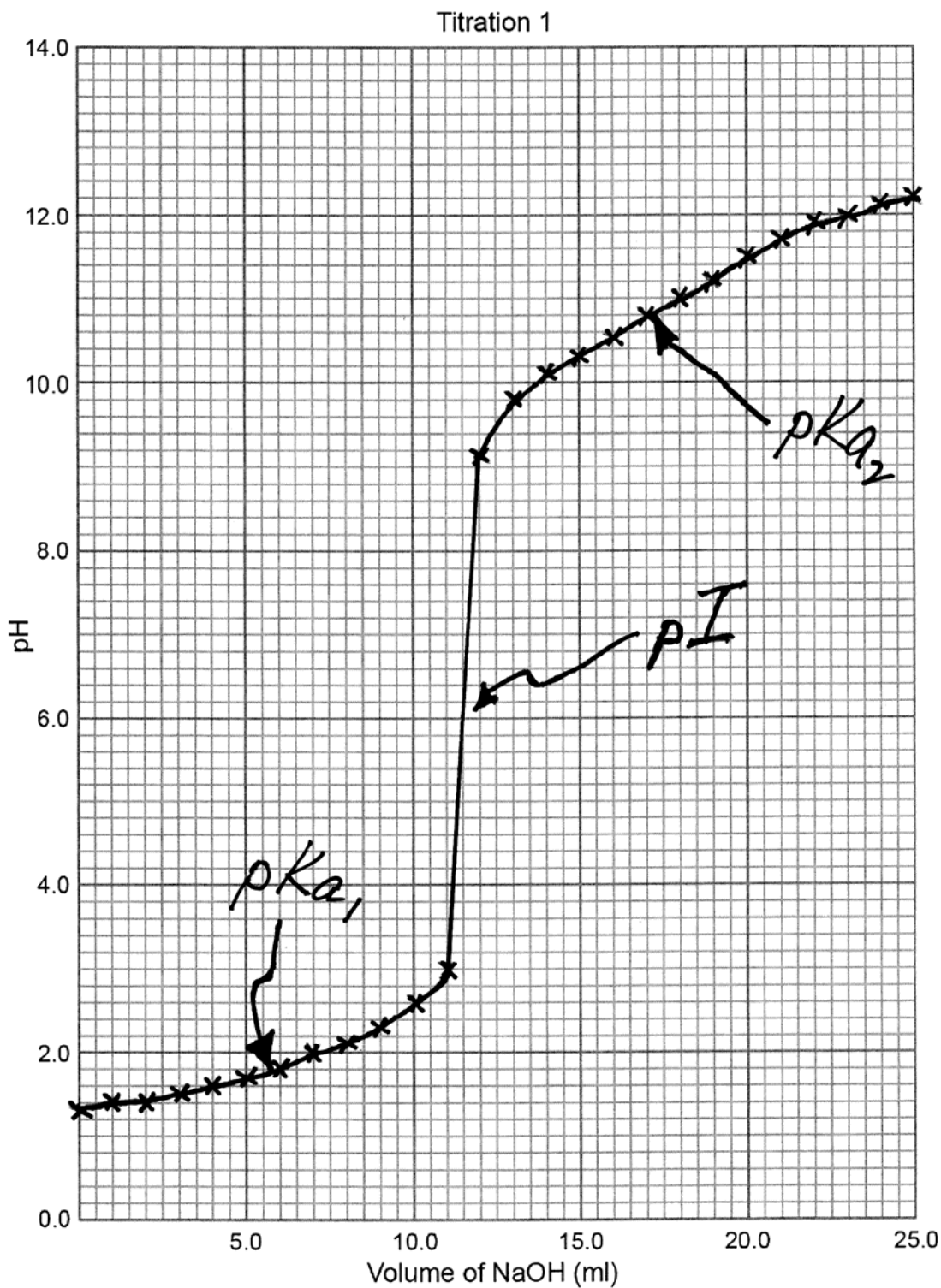
**Q2.6 (5 points)**

Answer: 115. (5 pts: based on volume of NaOH used to obtain value of pl in Q2.4, create formula in Excel to check if MW has been calculated correctly)

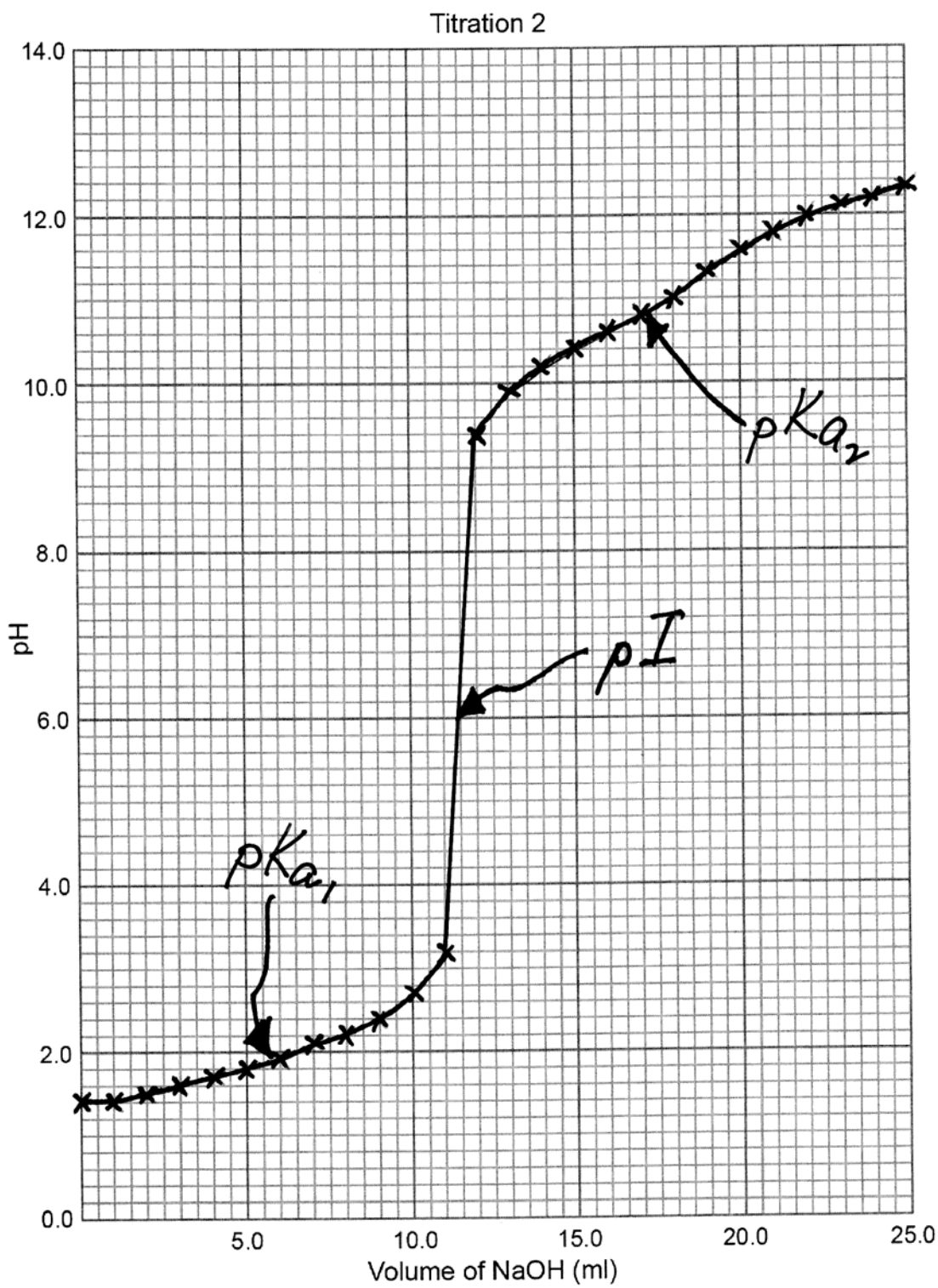
**Q2.7 (2 points)**

Answer: b.

Graph 1



Graph 2



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## 23rd INTERNATIONAL BIOLOGY OLYMPIAD

8<sup>th</sup> – 15<sup>th</sup> July, 2012

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**PRACTICAL TEST 3**

**PLANT DIVERSITY, ANATOMY & PHYSIOLOGY**

***ANSWER KEY***

Total points: **100**

Duration: **90 minutes**

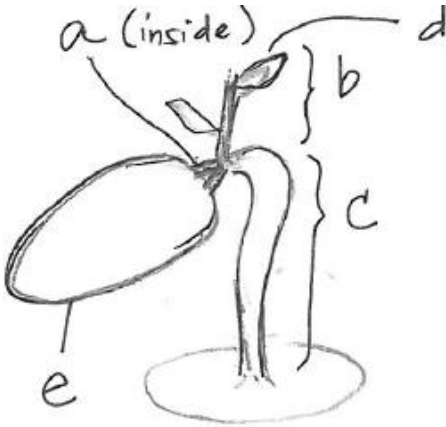
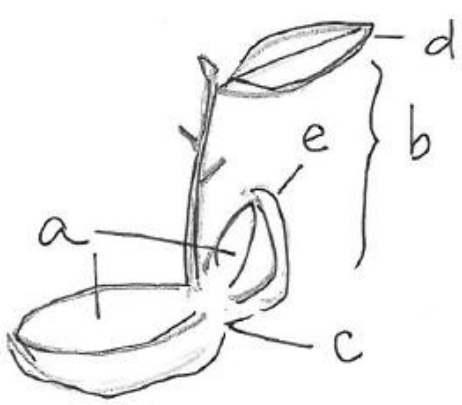
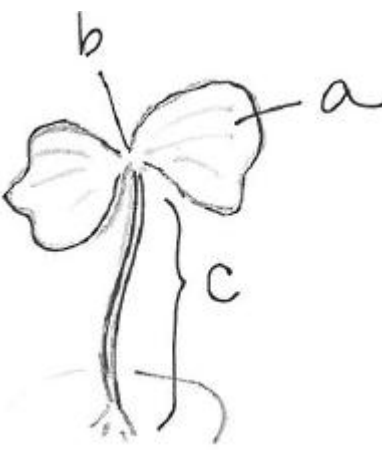
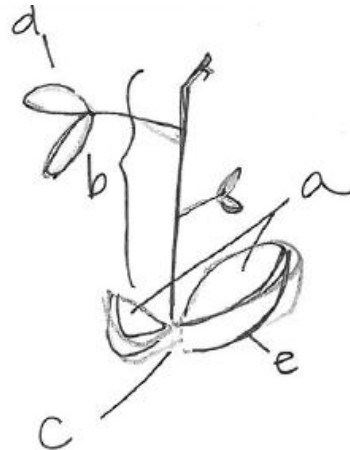


**Task I (60 points)**

**Plant diversity and anatomy**

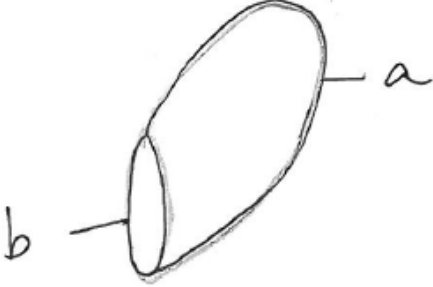




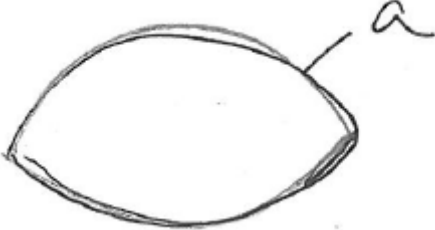
Part A. Morphology of seedlings (14.25 points)

**Q1.1 (0.5 points × 20 = 10 points; 2 points for quality of drawings; 2.25 points for not damaging specimens)**

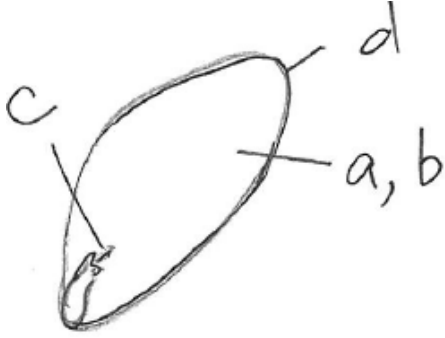
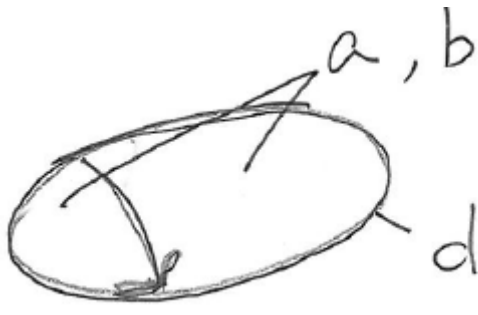
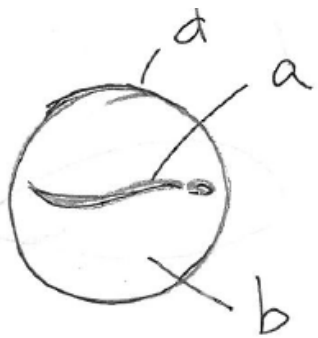
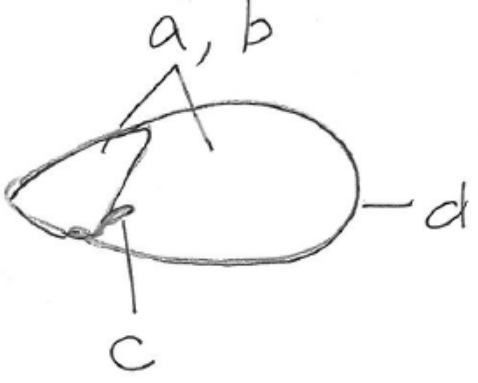
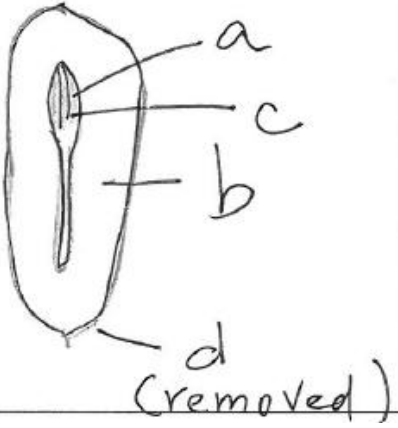
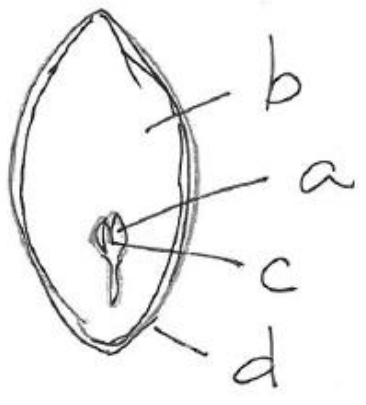
A	B
 <p>Absent: <u>b, d for some samples</u></p>	 <p>Absent: <u>c</u></p>
C	D
 <p>Absent: <u>d, e</u></p>	 <p>Absent: <u>c</u></p>

Part B. Seed morphology and anatomy (27.25 points)

**Q1.2 (0.25 points × 11 = 2.75 points)**

1	2
 <p>Absent: _____</p>	 <p>Absent: _____</p>
3	4
 <p>Absent: _____</p>	 <p>Absent: _____</p>
5	6
 <p>Absent: <u>a</u></p>	 <p>Absent: <u>b</u></p>

**Q1.3 (0.5 points × 24 = 12 points; 1 point for quality of drawings)**

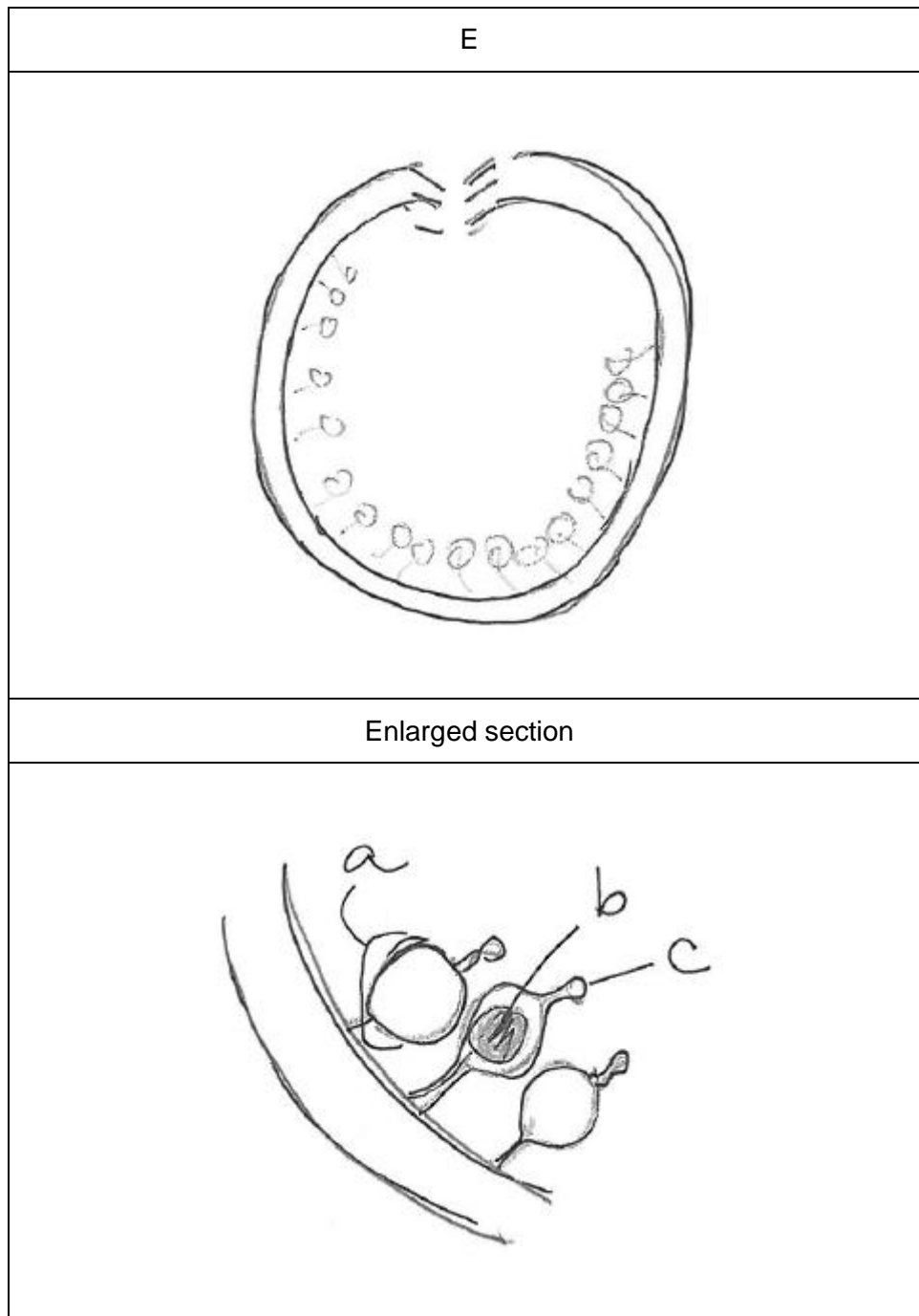
1	2
	
3	4
	
5	6
	

Q1.4 (0.5 points × 23 = 11.5 points)

Seed	a	b	c	d
1	2N	2N	2N	2N
2	2N	2N	2N	2N
3	2N	3N	2N	2N
4	2N	2N	2N	2N
5	2N	1N	2N	---
6	2N	1N	2N	2N

Part C. *Ficus* propagule (5 points)

Q1.5 (1+1+3 points)



Part D. Functional, ecological and phylogenetic aspects of seeds and seedlings (13.5 points)

**Q1.6 (0.5 points × 9 = 4.5 points)**

**Table 1**

Seeds	Family	Primary function of cotyledon*	Seed dry weight as % fresh weight	Probable germination pattern <sup>+</sup>	Climate of original habitat
1	Malvaceae	S	60%	R	Tropical / wet
2	Moraceae	S	45%	R	Tropical / wet
3	Malvaceae	P	80%	O	Tropical-Subtropical / dry
4	Sapindaceae	S	65%	R	Tropical / wet
5	Pinaceae	P	80%	O	Temperate / subtropical
6	Ginkgoaceae	***	55%	R	Tropical / wet
E	Moraceae	P	85%	O	Tropical / wet

\*\*\**Ginkgo* cotyledons remain embedded in the seed during germination

**Q1.7 (1 point × 5 = 5 points)**

a	b	c	d	e
x	x	x	✓	✓

**Q1.8 (1 point × 4 = 4 points)**

a	b	c	d
✓	--	--	x

**Task II (40 points)**

**Plant anatomy and physiology**

Part A. Anatomy of a plant stem (13 points)

**Q2.1 – Q2.3 (1 point × 3 = 3 points)**

Q2.1 (M or D)	Q2.2 (✓ or ×)	Q2.3 (C or P)
D	✓	P

**Q2.4 (0.5 points × 3 = 1.5 points)**

Shrub	Tree	Herb
×	×	✓

**Q2.5 (0.5 points)**

a	b	c	d	e
				✓

**Q2.6 (8 points)**

Quality of stem section <i>(for examiner's use only)</i>	
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CRITERIA	SCORE
Completeness of stem section Complete: 10 points; Incomplete: 5 points	/10
Staining of stem section Yes: 10 points No: 0 point	/10
Thickness of stem section Single layer (throughout): 40 points Single layer in (some areas): 30 points 2-3 layers of cells: 20 points >3 layers of cells: 10 points 5 bonus points if more than half the section meets criteria	/40
% intact cells in stem section 100% of cells: 20 points 80% of cells: 15 points 50% of cells: 10 points <5% of cells: 5 points	/20
% air bubbles in stem section 0 bubbles: 20 points <10 small bubbles: 15 points >10 small bubbles and some large bubbles: 10 points Numerous large bubbles, section obscured: 0 point	/20
<b>TOTAL</b>	<b>100</b>



Part B. Study of leaf epidermis and physiology (15 points)

(i) Lower epidermis

**Q2.7 (2 points)**

Answer: \_\_\_\_\_ ✓ \_\_\_\_\_ .

**Q2.8 (1.5 points × 2 = 3 points)**

	1	2	3	4	5	Mean
Length (µm)	120	100	100	150	150	124
Width (µm)	100	80	110	70	80	88

Acceptable Answers:

Length: 120–225 µm; Width: 80–130 µm

(Length: 125–225 µm; Width: 80–125 µm, Chimpan & Sipos; 2009)

(ii) Upper epidermis

**Q2.9 (2 points)**

Answer: \_\_\_\_\_ × \_\_\_\_\_ .

**Q2.10 (1.5 points × 2 = 3 points)**

	1	2	3	4	5	Mean
Length (µm)	180	220	240	210	200	210
Width (µm)	180	240	210	210	160	200

Acceptable Answers:

Length: 220 – 270 µm; Width: 160 – 230 µm

(Length: 225 – 250 µm; Width: 175 – 225 µm, Chimpan & Sipos; 2009)

Q2.11 (0.5 point × 3 = 1.5 points)

a	b	c
✓	x	✓

Q2.12 (1 point)

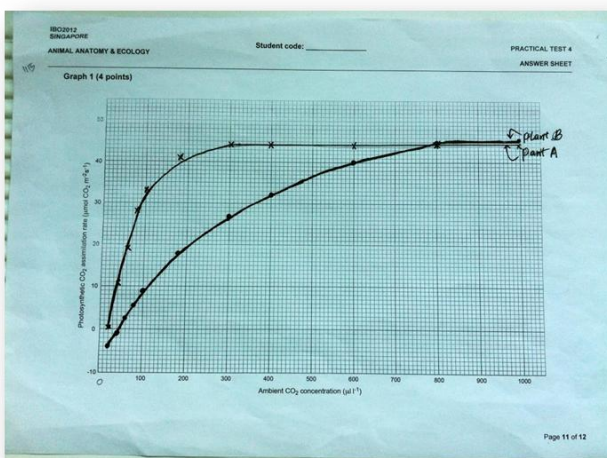
a	b	c
	✓	

Q2.13 (0.5 points × 5 = 2.5 points)

a	b	c	d	e
✓	✓	✓	x	✓

Part C. Interpretation of photosynthetic data from plants measured at different CO<sub>2</sub> concentrations (12 points)

Q2.14 (4 points) Graph 1



CRITERIA:

1. Plot
  - a. Accuracy (1 mark) – one point off, - 0.5 mark , two points off, -1 point
  - b. Differentiation of curves by different symbols or labels ( 1 point)
2. Smoothness of curves: 2 points, 1 point for each curve

**Q2.15 (0.5 points × 2 = 1 point)**

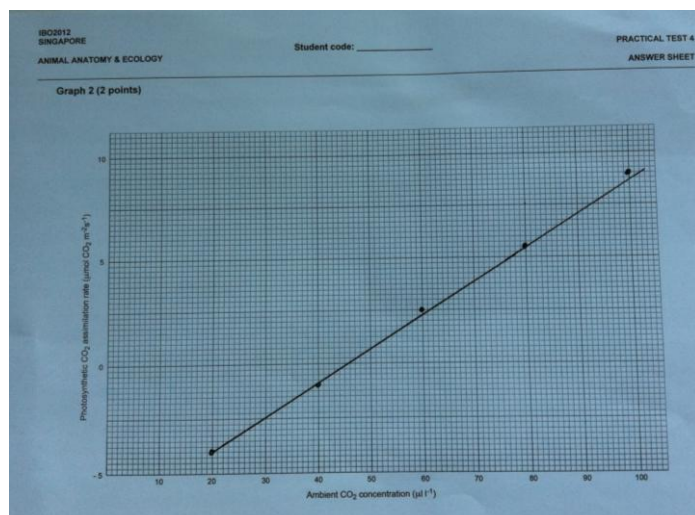
	C3	C4
A		✓
B	✓	

**Q2.16 (2 points)**

	A	B
Net photosynthetic CO <sub>2</sub> assimilation rate	42 ± 0.5 μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup>	20.5 ± 0.5 μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup>

(Note: without a unit, 0.5 points will be deducted)

**Q2.17 (2 points) Graph 2**



**CRITERIA:**

1. Plot Accuracy (1 point)
  - one point off, – 0.5 point,
  - two points off, –1 point
2. Straight line (1 point)

**Q2.18 (1 point)**

**Answer:**  $46 \pm 1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$

(Note: without a unit, 0.5 points will be deducted.)

**Q2.19 (1 point)**

increase	decrease	remain unchanged
✓		

**Q2.20 (1 point)**

increase	decrease	remain unchanged
	✓	

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**PRACTICAL TEST 4**

**ANIMAL ANATOMY & ECOLOGY**

***ANSWER KEY***

Total points: **100**

Duration: **90 minutes**

**Task I (20 points)**

**Anatomy of molluscs**

Answer the following questions:

**Q1.1 (3 points × 2 = 6 points)**

1	2
a	c

**Q1.2 (2 points × 2 = 4 points)**

1	2
1	1

**Q1.3 (2 points × 2 = 4 points)**

1	2
2	2

**Q1.4 (2 points × 2 = 4 points)**

1	2
c	c

**Q1.5 (0.4 points × 5 = 2 points)**

a	b	c	d	e
✓	✓	✓	x	x

**Task II (80 points)**

**Rank-abundance plots, ABC curves and community structure**

**Q2.1 (8 points × 4 = 32 points)** See Tables 1 – 2.

For each table:

- 4 pts for all correct answers in each column (Species and Abundance); minus ½ pt for every wrong answer until 0 point is reached.
- 2 pts for all correct answers in each column (Log<sub>10</sub> Abundance, Cumulative % Abundance and Cumulative % Biomass); minus ½ pt for every wrong answer until 0 point is reached.
- Pivot points: Total Abundance and Total biomass of species – 1 pt for correct answer; 0 pt for wrong answer.
- Note: Error Carried Forward (ECF) will be taken into consideration (correlated answers generated by Excel).

**Q2.2 (3 points × 4 = 12 points)** See Graphs 1 – 4.

**Q2.2.1 (1 point × 5 = 5 points)**

a	b	c	d	e
✓	x	✓	x	✓

**Q2.2.2 (2.5 points × 4 = 10 points)**

1	2	3	4
F	C	E	B

**Q2.2.3 (1.5 points × 4 = 6 points)**

Highly disturbed → undisturbed			
2	4	1	3

**Q2.2.4 (1 point × 10 = 10 points)**

1	2	3	4
B1, B6	A1, A2, B3, B4	A3, B7	B2, B5
or			
A3, B1, B6	A1, A2, B3, B4	B7	B2, B5

**Q2.2.5 and Q2.2.6 (2.5 points × 2 = 5 points)**

<b>Q2.2.5</b>	<b>Q2.2.6</b>
K	J

**Table 1. Community 1**

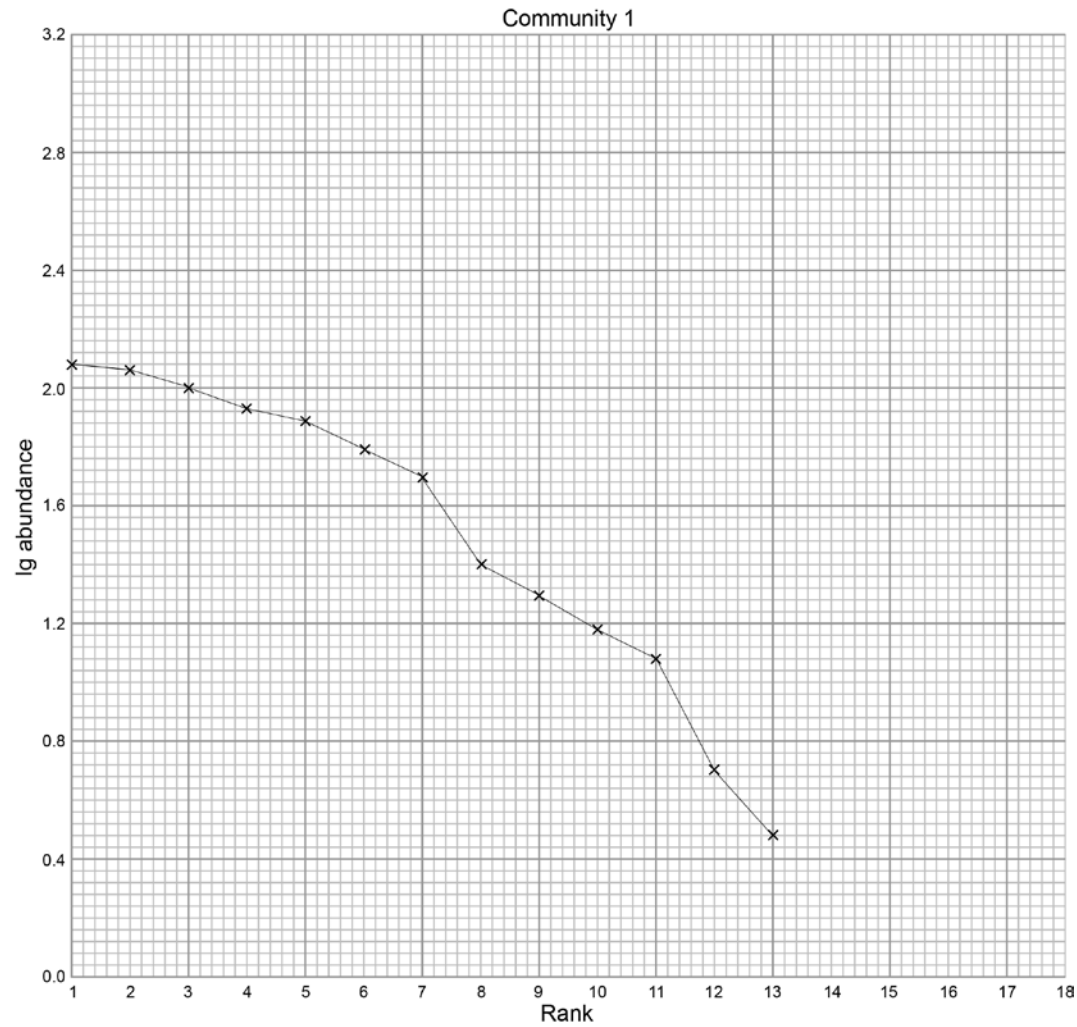
Species	Abundance	Rank	Log <sub>10</sub> (lg) Abundance	% Abundance	Cumulative % Abundance (+/- 0.10)	Biomass of individual	Total biomass of species	% Biomass	Cumulative % Biomass (+/-0.10)
N	120	1	2.08	17.39	17.39	1.00	120.00	10.05	10.05
D	115	2	2.06	16.67	34.06	2.80	322.00	26.98	37.03
I	100	3	2.00	14.49	48.55	1.25	125.00	10.47	47.50
P	85	4	1.93	12.32	60.87	1.70	144.50	12.11	59.61
F	78	5	1.89	11.30	72.17	1.15	89.70	7.51	67.12
K	62	6	1.79	8.99	81.16	4.00	248.00	20.78	87.90
E	50	7	1.70	7.25	88.41	1.30	65.00	5.45	93.34
B	25	8	1.40	3.62	92.03	0.90	22.50	1.88	95.23
M	20	9	1.30	2.90	94.93	1.50	30.00	2.51	97.74
H	15	10	1.18	2.17	97.10	1.35	20.25	1.70	99.44
J	12	11	1.08	1.74	98.84	0.05	0.60	0.05	99.49
G	5	12	0.70	0.72	99.57	0.80	4.00	0.34	99.82
A	3	13	0.48	0.43	100.00	0.70	2.10	0.18	100.00
<b>Total</b>	<b>690</b>						<b>1193.65</b>		



**Table 2. Community 2**

Species	Abundance	Rank	Log <sub>10</sub> (lg) Abundance	% Abundance	Cumulative % Abundance (+/- 0.10)	Biomass of individual	Total biomass of species	% Biomass	Cumulative % Biomass (+/-0.10)
J	1200	1	3.08	96.93	96.93	0.05	60.00	64.38	64.38
A	15	2	1.18	1.21	98.14	0.70	10.50	11.27	75.65
B	8	3	0.90	0.65	98.79	0.90	7.20	7.73	83.38
G	5	4	0.70	0.40	99.19	0.80	4.00	4.29	87.67
N	4	5	0.60	0.32	99.52	1.00	4.00	4.29	91.96
I	3	6	0.48	0.24	99.76	1.25	3.75	4.02	95.98
E	2	7	0.30	0.16	99.92	1.30	2.60	2.79	98.77
F	1	8	0.00	0.08	100.00	1.15	1.15	1.23	100.00
<b>Total</b>	<b>1238</b>						<b>93.20</b>		

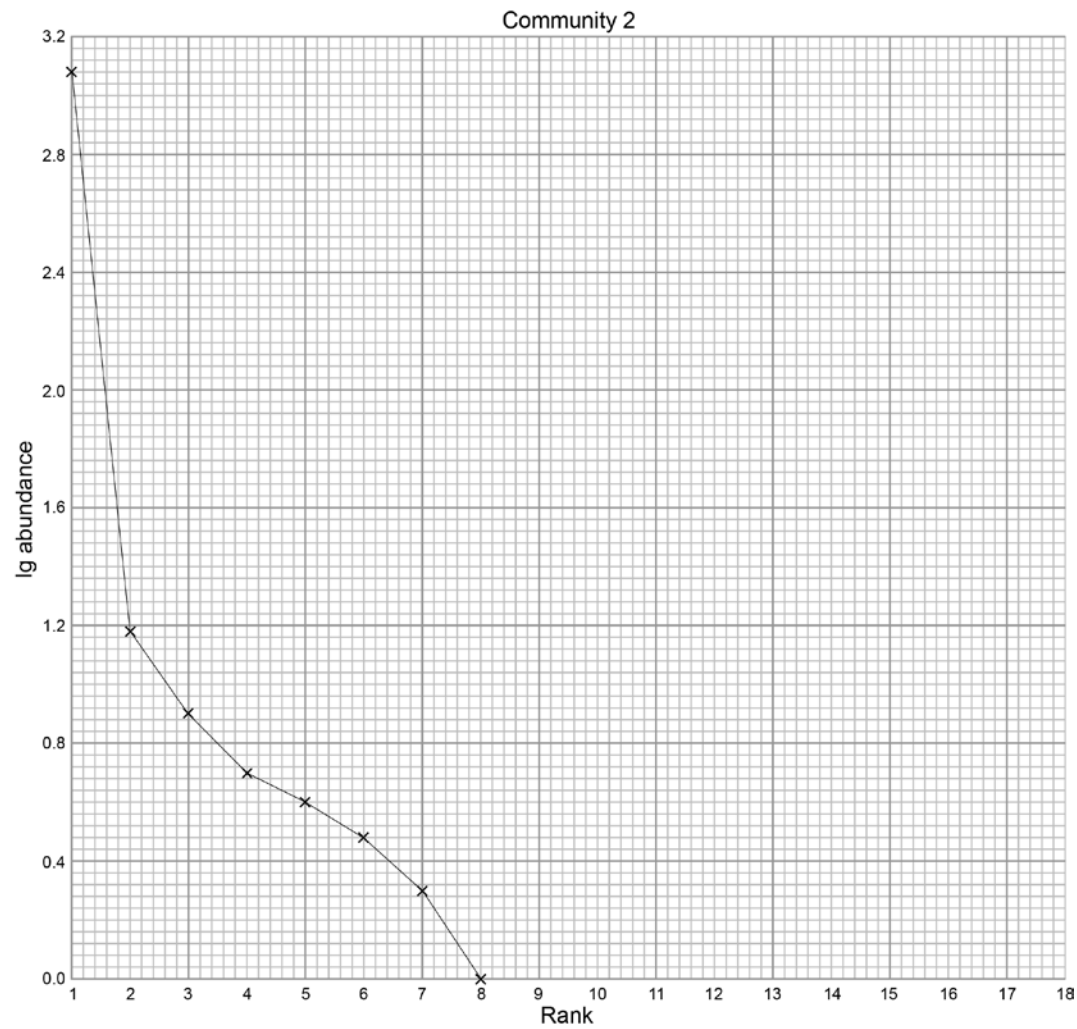
Graph 1



Provision made for Error Carried Forward (ECF).

Minus ½ point for every incorrect plotted point (within ONE square proximity) until 0 point is reached.

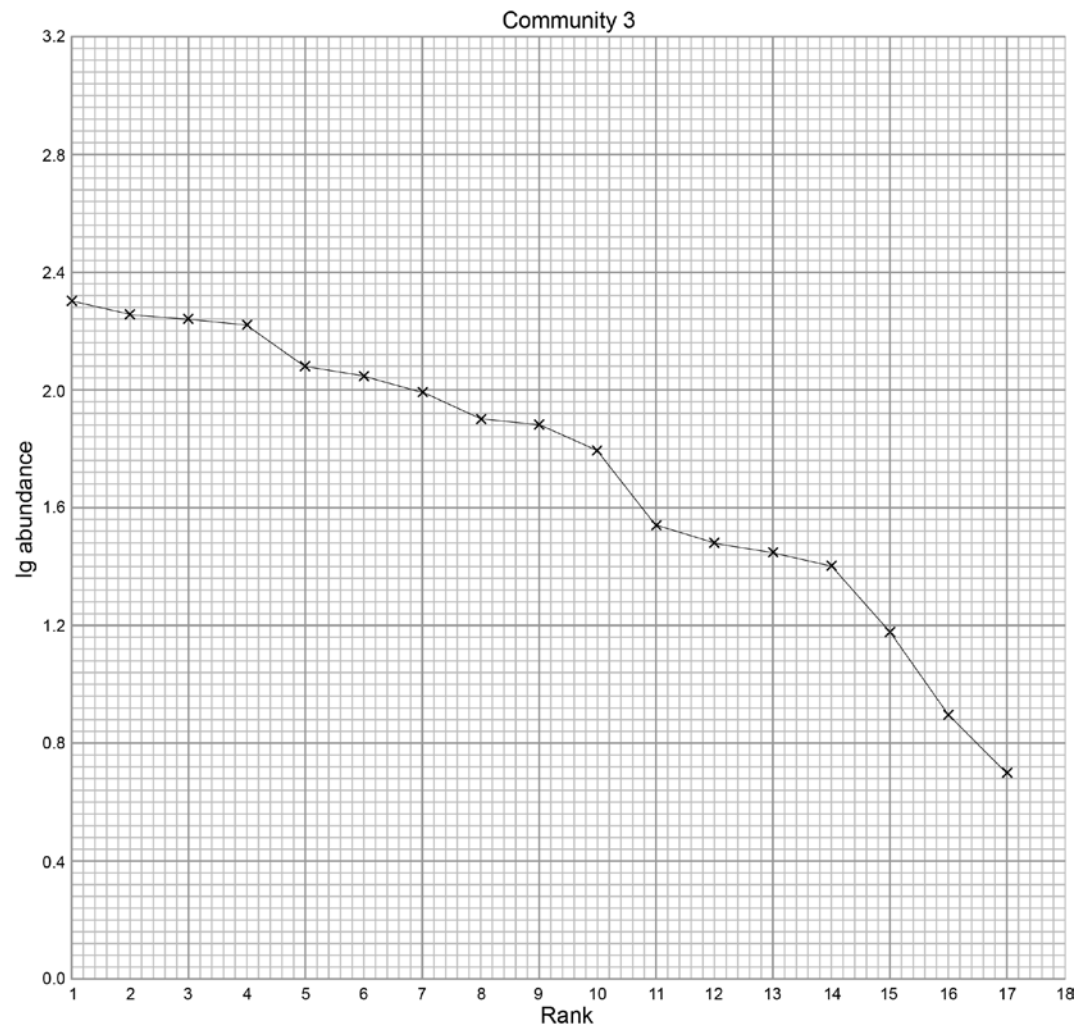
Graph 2



Provision made for Error Carried Forward (ECF).

Minus ½ point for every incorrect plotted point (within ONE square proximity) until 0 point is reached.

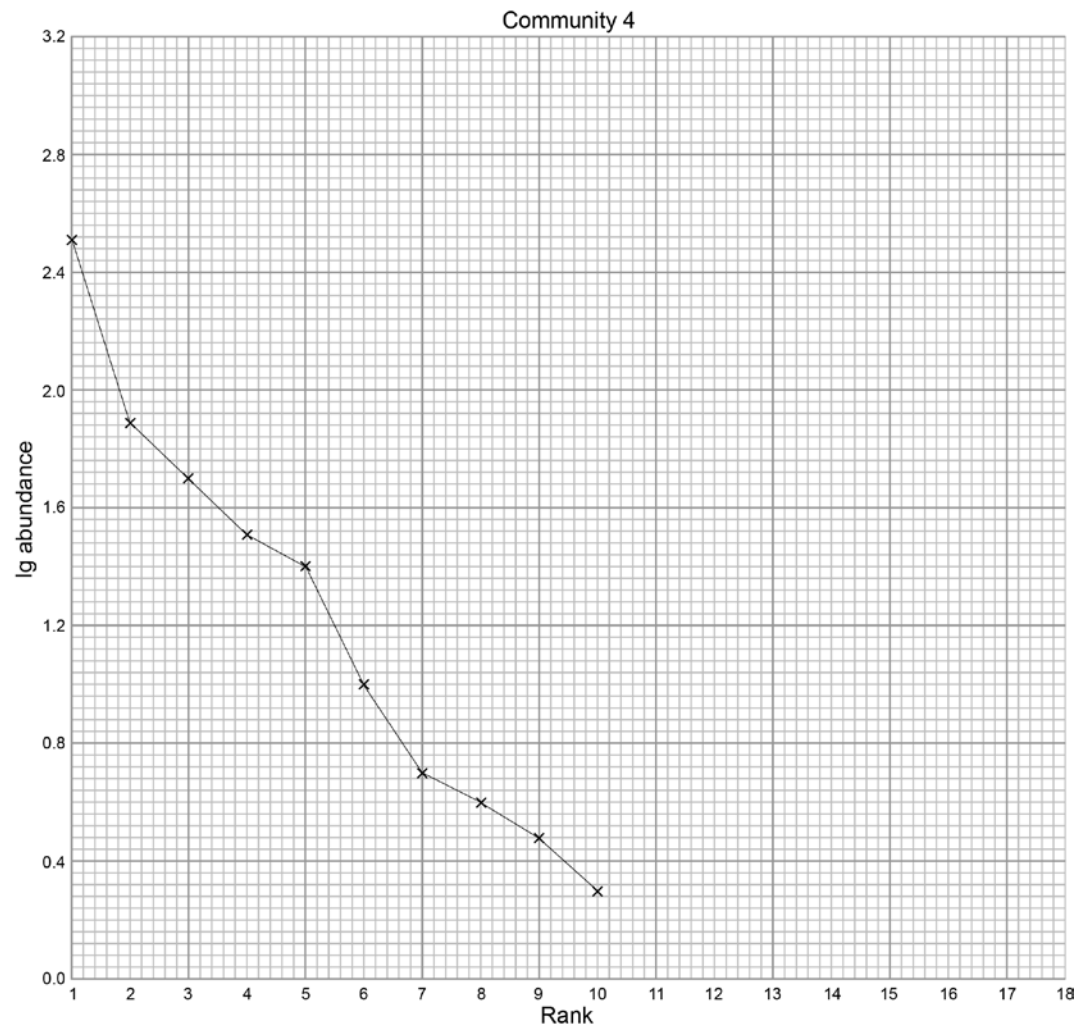
Graph 3



Provision made for Error Carried Forward (ECF).

Minus ½ point for every incorrect plotted point (within ONE square proximity) until 0 point is reached.

Graph 4



Provision made for Error Carried Forward (ECF).

Minus  $\frac{1}{2}$  point for every incorrect plotted point (within ONE square proximity) until 0 point is reached.