APPENDICES

Appendix 1: Bold's Basal Medium (Nichols and Bold, 1965)

	Stocks	per 400 mL
1.	$NaNO_3$	10.0 g
2.	$MgSO_4.7H_2O$	3.0 g
3.	K_2HPO_4	4.0 g
4.	KH_2PO_4	6.0 g
5.	$CaCl_2$	1.0 g
6.	NaCl	1.0 g
7.	Trace Element Solution	per 1 L
	$ZnSO_4$	8.82 g
	MoO_3	0.71 g
	$Co(NO_3)_2.H_2O$	0.49 g
	$MnCl_2$	1.44 g
	$CuSO_4.5H_2O$	1.57 g
	(Autoclave to dissolve)	
		per 10 mL
8.	H_3BO_4	1.14 g
9.	EDTA-KOH Solution	
	$EDTA.Na_2$	5.0 g
	KOH	3.1 g
		per 1 L
10.	$FeSO_4.7H_20$	4.98 g
	Concentrated HCL	1.0 mL

Preparation:

Stock solution 1 to 6	10 mL each
Stock solution 7 to 10	1 mL each

Make up to 1 L of deionised water
Adjust pH with 1 N of KOH to 6.8

Appendix 2: Data, results of one way ANOVA (Analysis of Variance), Newman-Keuls and correlation tests for growth characterisation of *Chlorella vulgaris* based on OD_{620} readings and cell count data of *Chlorella vulgaris*.

(a) Data of *C. vulgaris* based on OD_{620} readings.

Days / Replicates	R1	R2	R3	Sum	Average	Standard Deviation
0	0.021	0.028	0.025	0.074	0.0247	0.0035
2	0.098	0.093	0.093	0.284	0.0947	0.0029
4	0.166	0.166	0.128	0.460	0.1533	0.0219
6	0.271	0.295	0.282	0.848	0.2827	0.0120
8	0.369	0.318	0.348	1.035	0.3450	0.0256
10	0.459	0.505	0.480	1.444	0.4813	0.0230
12	0.501	0.549	0.525	1.575	0.5250	0.0240
14	0.559	0.583	0.573	1.715	0.5717	0.0121

(b) Data of *C. vulgaris* based on cell count (number of cells/mL).

Days/ Replicates	R1	R2	R3	Average	Standard Deviation
0	515000	455000	380000	450000	67638.75
2	1545000	1545000	1330000	1473333.333	124130.3
4	2767500	2212500	2842500	2607500	344129.3
6	5010000	5160000	5775000	5315000	405370.2
8	8572500	7845000	8077500	8165000	371559.2
10	9955000	10147500	10450000	10184166.67	249528.7
12	11472500	10450000	10595000	10839166.67	553253.6
14	13195000	12530000	11480000	12401666.67	864672.4

(c) One way ANOVA test of *C. vulgaris* based on OD_{620} readings.

Univariate Tests of Significance for OD_{620}
Sigma-restricted parameterization
Effective hypothesis decomposition

	SS	Degree of Freedom	MS	F	p
Intercept	2.303301	1	2.303301	7211.902	0.000000
Day	0.895048	7	0.127864	400.357	0.000000
Error	0.005110	16	0.000319		

(d) One way ANOVA test of *C. vulgaris* based on cell count data.

Univariate Tests of Significance for Cell Count
Sigma-restricted parameterization
Effective hypothesis decomposition

	SS	Degree of freedom	MS	F	p
Intercept	9.921169E+14	1	9.921169E+14	5098.230	0.000000
Day	4.451677E+14	7	6.359538E+13	326.800	0.000000
Error	3.113604E+12	16	1.946003E+11		

(e) Newman-Keuls test of *Chlorella vulgaris* based on OD_{620} readings

Newman-Keuls test; variable OD_{620}
Approximate Probabilities for Post Hoc Tests
Error: Between MS = .00032, df = 16.000

	Day	1	2	3	4	5	6	7	8
1	0		0.000338	0.000168	0.000185	0.000142	0.000164	0.000163	0.000175
2	2	0.000338		0.001121	0.000168	0.000185	0.000142	0.000164	0.000163
3	4	0.000168	0.001121		0.000159	0.000168	0.000185	0.000142	0.000164
4	6	0.000185	0.000168	0.000159		0.000715	0.000168	0.000185	0.000142
5	8	0.000142	0.000185	0.000168	0.000715		0.000159	0.000168	0.000185
6	10	0.000164	0.000142	0.000185	0.000168	0.000159		0.008772	0.000193
7	12	0.000163	0.000164	0.000142	0.000185	0.000168	0.008772		0.005750
8	14	0.000175	0.000163	0.000164	0.000142	0.000185	0.000193	0.005750	

(f) Newman-Keuls test of *Chlorella vulgaris* based on cell count data.

Newman-Keuls test; variable Cell Count
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 1947E8, df = 16.000

	Day	1	2	3	4	5	6	7	8
1	0		0.011946	0.000207	0.000185	0.000142	0.000164	0.000163	0.000175
2	2	0.011946		0.006364	0.000168	0.000185	0.000142	0.000164	0.000163
3	4	0.000207	0.006364		0.000159	0.000168	0.000185	0.000142	0.000164
4	6	0.000185	0.000168	0.000159		0.000159	0.000168	0.000185	0.000142
5	8	0.000142	0.000185	0.000168	0.000159		0.000190	0.000169	0.000185
6	10	0.000164	0.000142	0.000185	0.000168	0.000190		0.087889	0.000195
7	12	0.000163	0.000164	0.000142	0.000185	0.000169	0.087889		0.000641
8	14	0.000175	0.000163	0.000164	0.000142	0.000185	0.000195	0.000641	

(g) Correlation tests for growth characterisation of *Chlorella vulgaris* based on OD_{620} and cell count data of *C. vulgaris*.

Correlations (Growth curve)(correlation)
Marked correlations are significant at p <0 .05000

	Mean	Std.Dv.	r(X,Y)	r^2	t	p	N
OD_{620}	0	0					
Cell Count	6429479	4414803	0.987290	0.974742	29.13763	0.000000	24

Appendix 3: Data and test of means (t-test) results for the specific growth rate of *Chlorella vulgaris* based on optical density readings and cell count data.

(a) Specific growth rate of *Chlorella vulgaris* based on based on optical density readings and cell count data.

Specific Growth Rate/ Sample	R1	R2	R2	Average	SD
OD_{620}	0.1997	0.1625	0.2500	0.2041	0.0439
Cell Count	0.2827	0.3164	0.2611	0.2867	0.0279

(b) T-Test of *Chlorella vulgaris* based on based on optical density readings and cell count data.

(i)

Test of means against reference constant (value)								
	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
Specific Growth Rate (OD_{620})	0.204087	0.043928	3	0.025362	0.00	8.047009	2	0.015094

(ii)

Test of means against reference constant (value)								
	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
Specific Growth Rate (Cell Count)	0.286731	0.027894	3	0.016104	0.00	17.80445	2	0.003140

Appendix 4: Data, results of one way ANOVA (Analysis of Variance) and Newman-Keuls tests of *Chlorella vulgaris* when exposed to chloroamphenicol based on optical density readings and cell count data of *C. vulgaris*.

(a) OD_{620} data of *C. vulgaris* when exposed to chloroamphenicol.

Concentration / Day	0	2	4	6	8	10	Average	SD
Control	0.0270	0.0700	0.1420	0.3120	0.4830	0.5400	0.2623	0.2168
25 ug mL^{-1}	0.0290	0.0690	0.1140	0.2400	0.4270	0.5290	0.2347	0.2039
50 ug mL^{-1}	0.0270	0.0740	0.1420	0.2590	0.4220	0.5080	0.2387	0.1938
100 ug mL^{-1}	0.0230	0.0510	0.0930	0.1680	0.2910	0.3860	0.1687	0.1435
200 ug mL^{-1}	0.0310	0.0420	0.0300	0.0200	0.0220	0.0160	0.0268	0.0094
400 ug mL^{-1}	0.0260	0.0340	0.0270	0.0170	0.0180	0.0130	0.0225	0.0078

(b) Cell count data of *C. vulgaris* when exposed to chloroamphenicol.

Concentration/Day	0	2	4	6	8	10	Average	SD
Control	492001	1305305	2588043	5683211	8800585	9840053	4784866	3945884
25 ug mL^{-1}	520339	1266090	2077922	4362469	7789242	9597648	4268952	3705835
50 ug mL^{-1}	492892	1338175	2607040	4722622	7678058	9244961	4347291	3525952
100 ug mL^{-1}	420025	930150	1654322	3058730	5302618	7002288	3061356	2609698
200 ug mL^{-1}	563978	755183	546457	400808	364371	291399	487033	168588
400 ug mL^{-1}	496634	620432	492001	328915	290034	235732	410625	148219

(c) One way ANOVA test of *C. vulgaris* when exposed to chloroamphenicol based on OD_{620} readings.

Univariate Tests of Significance for $\log(OD_{620}+1)$
Sigma-restricted parameterization
Effective hypothesis decomposition

	SS	Degree of Freedom	MS	F	p
Intercept	0.127731	1	0.127731	43.29727	0.000000
Concentration	0.046344	5	0.009269	3.14187	0.021308
Error	0.088503	30	0.002950		

(d) One way ANOVA test of *C. vulgaris* when exposed to chloroamphenicol based on cell count data.

Univariate Tests of Significance for log(cell count+1) Chloroamphenicol concentration (cell count)
Sigma-restricted parameterization
Effective hypothesis decomposition

	SS	Degree of Freedom	MS	F	p
Intercept	1361.313	1	1361.313	8282.797	0.000000
Concentration	5.004	5	1.001	6.089	0.000527
Error	4.931	30	0.164		

(e) Newman-Keuls test of *C. vulgaris* when exposed to chloroamphenicol based on OD_{620} readings.

Newman-Keuls test; variable $\log(OD_{620}+1)$
Approximate Probabilities for Post Hoc Tests
Error: Between MS = .00295, df = 30.000

	Concentration	1	2	3	4	5	6
1	Control		0.964221	0.831002	0.769256	0.082123	0.098096
2	25ug/mL	0.964221		0.966800	0.483897	0.055646	0.084702
3	50ug/mL	0.831002	0.966800		0.735321	0.087616	0.113569
4	100ug/mL	0.769256	0.483897	0.735321		0.098166	0.198220
5	200ug/mL	0.082123	0.055646	0.087616	0.098166		0.953899
6	400ug/mL	0.098096	0.084702	0.113569	0.198220	0.953899	

(f) Newman-Keuls test of *C. vulgaris* when exposed to chloroamphenicol based on cell count data.

Newman-Keuls test; variable log(cell count+1) Chloroamphenicol concentration (cell count)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = .16435, df = 30.000

	Concentration	1	2	3	4	5	6
1	25ug/mL		0.937632	0.843105	0.012303	0.008399	0.955823
2	50ug/mL	0.937632		0.919162	0.015671	0.009702	0.990089
3	100ug/mL	0.843105	0.919162		0.010005	0.011836	0.619258
4	200ug/mL	0.012303	0.015671	0.010005		0.743951	0.007736
5	400ug/mL	0.008399	0.009702	0.011836	0.743951		0.006198
6	Control	0.955823	0.990089	0.619258	0.007736	0.006198	

(g) LC_{50} test of *C. vulgaris* exposed to chloroamphenicol

Log Concentration (X)	Day 10 Control	Day 10 Treatment	Survivability (%)	Mortality (%)	Probit values (Y)
-	9840053	9840053	100	0	-
1.397940009	9840053	9597648	97.53654782	2.463452179	3.033743973
1.698970004	9840053	9244961	93.95234965	6.047650353	3.449214126
2	9840053	7002288	71.16108013	28.83891987	4.441903364
2.301029996	9840053	291399	2.961356001	97.038644	6.886503628
2.602059991	9840053	235732	2.395637503	97.6043625	6.978141464

Appendix 5: Test of means (t-test) results for the specific growth rate of *Chlorella vulgaris* when exposed to chloroamphenicol based on optical density readings and cell count data.

(a) T-Test of *C. vulgaris* when exposed to chloroamphenicol based on optical density readings and cell count data.

(i)

Test of means against reference constant (value)								
	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
Specific Growth Rate (OD_{620})	0.204087	0.043928	3	0.025362	0.00	8.047009	2	0.015094

(ii)

Test of means against reference constant (value)								
	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
Specific Growth Rate (Cell Count)	0.286731	0.027894	3	0.016104	0.00	17.80445	2	0.003140

Appendix 6: Test of means (t-test) results of the purity and yield of DNA extracted from *Chlorella vulgaris*.

(a) T-test results of the purity of DNA

Test of means against reference constant (value)								
	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
Purity	2.007611	0.166200	720	0.006194	0.00	324.1271	719	0.00

(b) T-test results of the total yield of DNA

Test of means against reference constant (value)								
	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
Total DNA yield	54.35231	47.54302	720	1.771824	0.00	30.67591	719	0.00

Appendix 7: Number of transformed *Chlorella vulgaris* clones that gave positive PCR results using four different parameters at each generation.

(a)Positive HBSAg PCR bands obtained from the transformed clones.

Generation	Psi	Distance	Positive PCR HBSAg
22nd	900	6 cm	14
32nd	900	6 cm	0
42nd	900	6 cm	17
53rd	900	6 cm	20
63rd	900	6 cm	20
69th	900	6 cm	20
72nd	900	6 cm	20
82nd	900	6 cm	20
92nd	900	6 cm	20
22nd	900	9 cm	1
32nd	900	9 cm	18
42nd	900	9 cm	20
53rd	900	9 cm	20
63rd	900	9 cm	20
69th	900	9 cm	18
72nd	900	9 cm	20
82nd	900	9 cm	20
92nd	900	9 cm	20
22nd	1100	6 cm	7
32nd	1100	6 cm	3
42nd	1100	6 cm	20
53rd	1100	6 cm	20
63rd	1100	6 cm	20
69th	1100	6 cm	20
72nd	1100	6 cm	20
82nd	1100	6 cm	20
92nd	1100	6 cm	20
22nd	1100	9 cm	1
32nd	1100	9 cm	9
42nd	1100	9 cm	0
53rd	1100	9 cm	20
63rd	1100	9 cm	20
69th	1100	9 cm	17
72nd	1100	9 cm	20
82nd	1100	9 cm	20
92nd	1100	9 cm	20

(b) Transformation efficiency of *C. vulgaris* bombarded using four different parameters

Percentage of growth area	6 cm, 900 psi, R1	6 cm, 900 psi, R2	9 cm, 900 psi, R1	9 cm, 900 psi, R2	6 cm, 1100 psi, R1	6 cm, 1100 psi, R2	9 cm, 1100 psi, R1	9 cm, 1100 psi, R2
P1	1.5713	6.2851	26.7115	14.1414	14.1414	1.5713	61.2794	12.5701
P2	12.5701	0.0000	23.5690	15.7127	7.8563	12.5701	0.0000	6.2851
P3	3.1425	21.9977	0.0000	4.7138	29.8541	4.7138	6.2851	25.1403
P4	21.9977	0.0000	7.8563	3.1425	25.1403	0.0000	0.0000	0.0000
P5	1.5713	1.5713	0.0000	12.5701	26.7115	9.4276	0.0000	0.0000
n=5								
n-1=4								
Sum	40.8529	29.8541	58.1369	50.2805	103.7036	28.2828	67.5645	43.9955
Mean	8.1706	5.9708	11.6274	10.0561	20.7407	5.6566	13.5129	8.7991
Variance	80.7324	86.9046	163.6868	32.8361	86.9046	27.8984	720.4193	110.6058
SD	8.9851	9.3223	12.7940	5.7303	9.3223	5.2819	26.8406	10.5169

(c) Two way ANOVA test of *C. vulgaris* based on the transformation efficiency of bombarded *C. vulgaris* using two different pressures and distances

Univariate Tests of Significance for percentage of cells lawning Log10 (x+1) (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition					
	SS	**Degr. of**	**MS**	**F**	**p**
Intercept	24.21142	1	24.21142	77.99638	0.000000
Distance	0.06410	1	0.06410	0.20650	0.652257
Pressure	0.00922	1	0.00922	0.02970	0.864139
Distance*Pressure	0.85545	1	0.85545	2.75582	0.105588
Error	11.17502	36	0.31042		

Appendix 8: Test of means (t-test) results of the purity and yield of RNA extracted from untransformed and transformed *Chlorella vulgaris*.

(a) T-test for the purity of the total RNA isolated.

Test of means against reference constant								
	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
Purity	1.784625	0.177014	8	0.062584	0.00	28.51571	7	0.000000

(b) T-test for the yield of the total RNA isolated

Test of means against reference constant								
	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
Yield	64.49438	33.24897	8	11.75528	0.00	5.486415	7	0.000920

Appendix 9: Test of means (t-test) results of the concentration of protein extracted from untransformed and transformed *Chlorella vulgaris*.

(a) T-test for the overall concentration of crude protein.

Test of means against reference constant								
	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
Concentrations	239.2387	57.78441	11	17.42266	0.00	13.73147	10	0.000000

(b) T-test for the concentration of crude protein extracted from untransformed C. *vulgaris*.

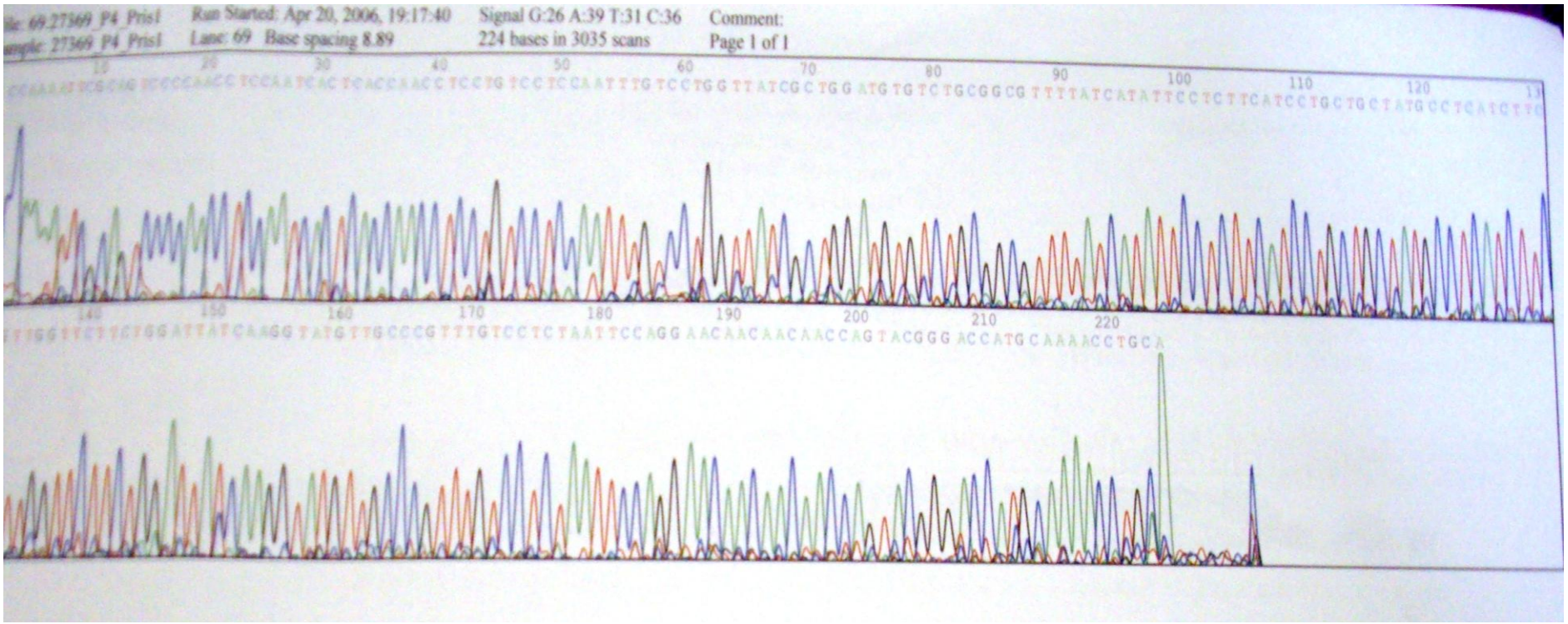
Test of means against reference constant								
	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
Concentrations	228.9065	16.57246	2	11.71850	0.00	19.53377	1	0.032562

(c) T-test for the concentration of crude protein extracted from transformed C. *vulgaris*.

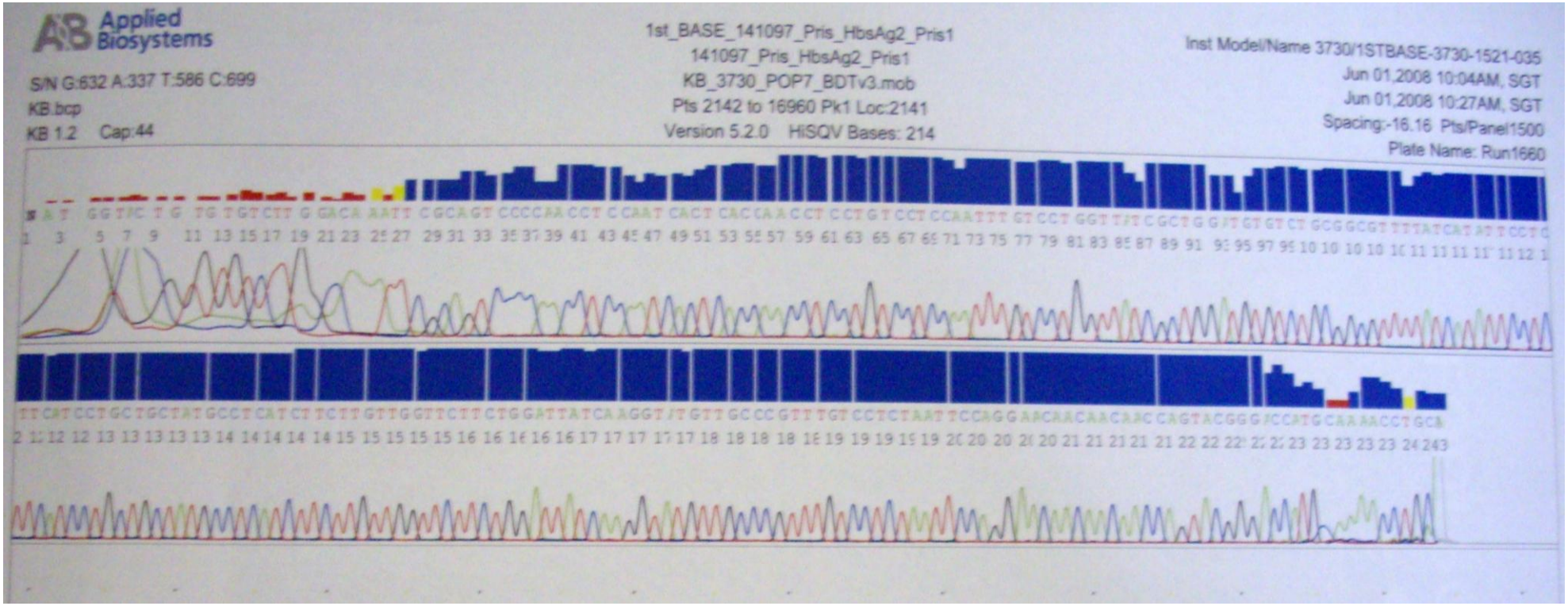
Test of means against reference constant								
	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
Concentrations	258.4375	36.51673	9	12.17224	0.00	21.23171	8	0.000000

Appendix 10: Sequencing results obtained from purified HBSAg PCR product (271p)

(a) Sequencing results of the partially amplified HBSAg PCR product obtained from transformed genomic DNA of *Chlorella vulgaris.*



(b) Sequencing results of the partially amplified HBSAg PCR product obtained from transformed cDNA of *Chlorella vulgaris*.



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