Appendix 1

Plant Tissue Culture Media Formulation

(i) Murashige and Skoog (1962) MS basal nutrients formulation

Components	Concentration in Media (mg/ L)	Stock solution concentration
Macronutrients		
$CaCl_2.2H_2O$	440	10 X
KNO_3	1900	
KH_2PO_4	170	
$MgSO_4.7H_2O$	370	
NH_4NO_3	1650	
Micronutrients		
$CoCl_2.6H_2O$	0.025	100 X
$CuSO_4.5H_2O$	0.025	
H_3BO_4	6.2	
KI	0.83	
$MnSO_4.4H_2O$	22.3	
$Na_2MoO_4.4H_2O$	0.25	
$ZnSO_4.7H_2O$	8.6	
Vitamins		
Glycine	2.0	100 X
Nicotinic acid	0.5	
Pyrodoxine-HCl	0.5	
Thiamine-HCl	0.1	
Myo-inositol	100	1 X
Iron		
$FeSO_4.7H_2O$	27.85	100 X
Na2EDTA	37.25	

The pH of media was adjusted to 5.7 prior to autoclaving.

(ii) Sterilisation methods

a. By steam

All glassware, equipments and media were sterilised in an autoclave with 121 °C, pressure of 1.2 kgf/ cm^2 for 20 minutes.

b. By fitration

Filter sterilisation method was used as alternative for sterilisation of heat-labile, temperature-sensitive components such as certain plant growth regulators and antibiotics. All of the components that would be damaged by steam sterilisation methods are filter sterilised by passing through a 0.22 µm nitrocellulose fliter (Millex ® - GV, Millipore).

(iii) Preparation of plant growth regulators used for plant tissue culture

Plant Growth Regulators	**Solubility**	**Stock Concentration**	**Method of Sterilization**
6 – BA	NaOH	1 mg/ ml	Autoclaving
2, 4 – D	EtOH/ NaOH	1 mg/ ml	Autoclaving
NAA	NaOH	0.1 mg/ ml	Autoclaving
Kinetin	NaOH	0.01 mg/ ml	Filter Sterilise

Appendix 2

Bacterial Cultures Media Preparation

Yeast Extract Broth (YEB) – 1 Litre

Nutrient broth – 14.0 g
Yeast extract – 1.0 g
Sucrose – 5.0 g
Magnesium Sulphate – 10mM

Yeast Extract (YE) Agar Plate – 1 Litre

Nutrient agar – 28.0 g
Yeast extract – 1.0 g
Sucrose – 5.0 g
Magnesium Sulphate – 10mM

The pH of the media was adjusted to 7.5 prior to autoclaving. Media were left to cool to ≈ 50 $^{\circ}$C before adding antibiotics.

Various concentrations of carbenicilin and cefotaxime used in MIC experiments

Combinations	**Cefotaxime**	**Carbenicilin**
1	50 mg/ L	50 mg/ L
2	50 mg/ L	100 mg/ L
3	50 mg/ L	150 mg/ L
4	50 mg/ L	200 mg/ L
5	100 mg/ L	50 mg/ L
6	100 mg/ L	100 mg/ L
7	100 mg/ L	150 mg/ L
8	100 mg/ L	200 mg/ L
9	150 mg/ L	50 mg/ L
10	150 mg/ L	100 mg/ L
11	150 mg/ L	150 mg/ L
12	150 mg/ L	200 mg/ L
13	200 mg/ L	50 mg/ L
14	200 mg/ L	100 mg/ L
15	200 mg/ L	150 mg/ L
16	200 mg/ L	200 mg/ L

Appendix 3

Plasmid Extraction Chemical Solution

(i) Solution I (50 ml)

1.25 ml of 1 M Tris
1.0 ml of 0.5 M EDTA
450 g glucose
Top up to 50 ml with dH_2O

(ii) Solution II (500 µl)

10 µl of 10 N NaOH
50 µl of 10 % (w/v) SDS
440 µl of dH_2O

(iii) Solution III

150 ml of 5 M Potassium acetate
28.75 ml of glacial acetic acid
71.25 ml of dH_2O

Appendix 4

Chemicals for GUS Assessments

Histochemical Staining Reagent

Stock Solution	Working Concentration	Total Volume		
		5 ml	**10 ml**	**25 ml**
0.2 M $NaPO_4$	0.1 M	2.5 ml	5 ml	12.5 ml
0.1 M KFe^{3+}	0.5 mM	25 µl	50 µl	125 µl
0.1 M KFe^{2+}	0.5 mM	25 µl	50 µl	125 µl
0.5 M EDTA	10 mM	100 µl	200 µl	500 µl
0.5 % Triton	0.1 %	1 ml	2 ml	5 ml
Methanol	20 % (v/v)	1 ml	2 ml	5 ml
20 mg/ ml X-Gluc	1.0 mM	250 µl	500 µl	1250 µl
dH_2O	-	100 µl	200 µl	500 µl

FAA Fixing Solution 100 ml

Absolute EtOH – 45 ml
Glacial acetic acid – 5 ml
Formaldehyde – 5 ml
dH_2O – 45 ml

GUS Extraction Buffer (GEB) 100 ml

$NaHPO_4$ (1 M, pH 7.0) – 5.00 ml
2–mercaptoethanol – 0.07 ml
Na_2EDTA (0.5 M, pH 8.0) – 2.00 ml
Sarcosyl (30 % v/v) – 0.33 ml
Triton X-100 (10 % v/v) – 1.00 ml
Distilled water – 91.60 ml

GUS Assay Buffer (GAB) 25 ml

25 mg of 4-methylumbelliferyl β- D- glucuronide in 25 ml of GEB

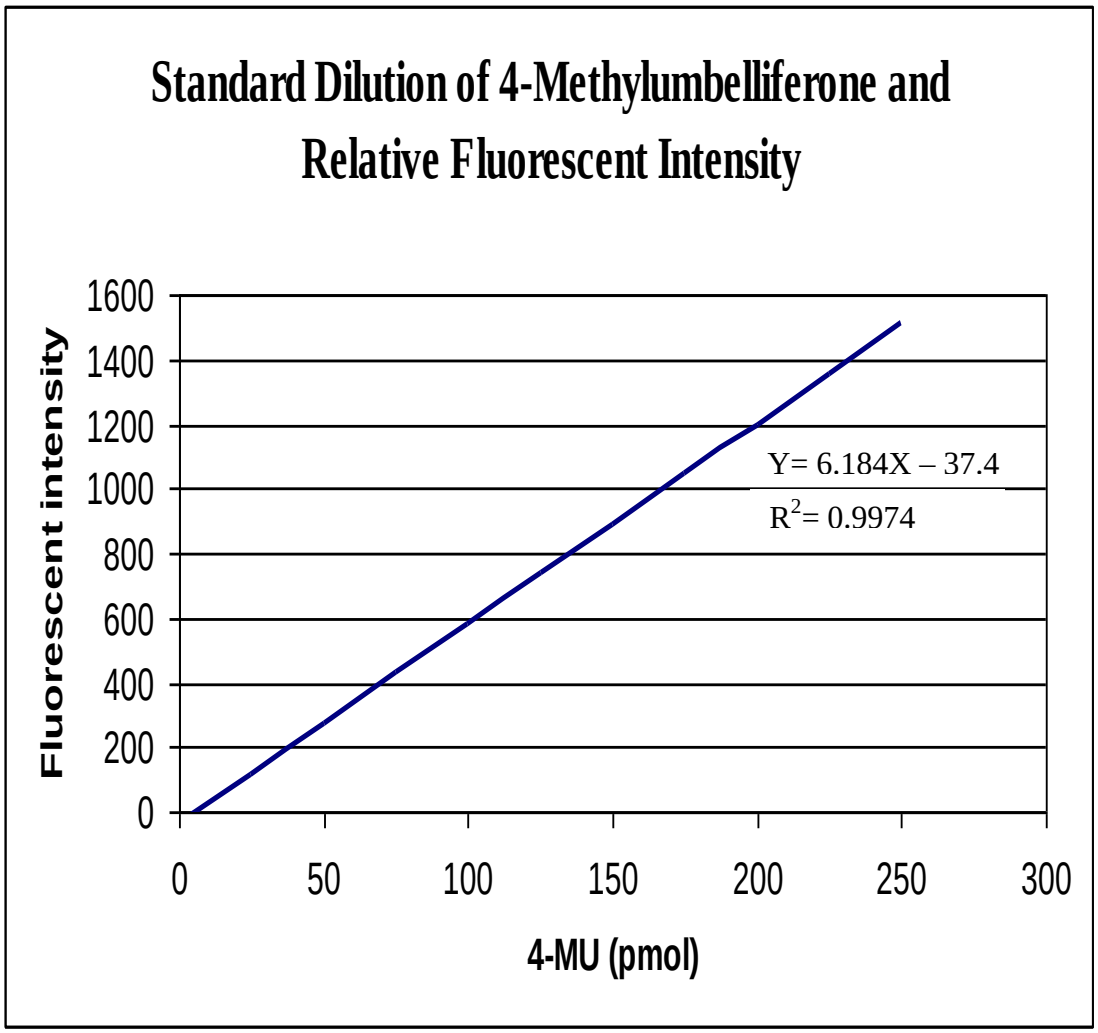
0.2 M Carbonate Stop Buffer (CSB)

Sodium carbonate, anhydrous – 21.2 g
Top up to 1000 ml with ddH_2O

Appendix 5

Standard Dilution of 4-Methylumbelliferone and Relative Fluorescent Intensity

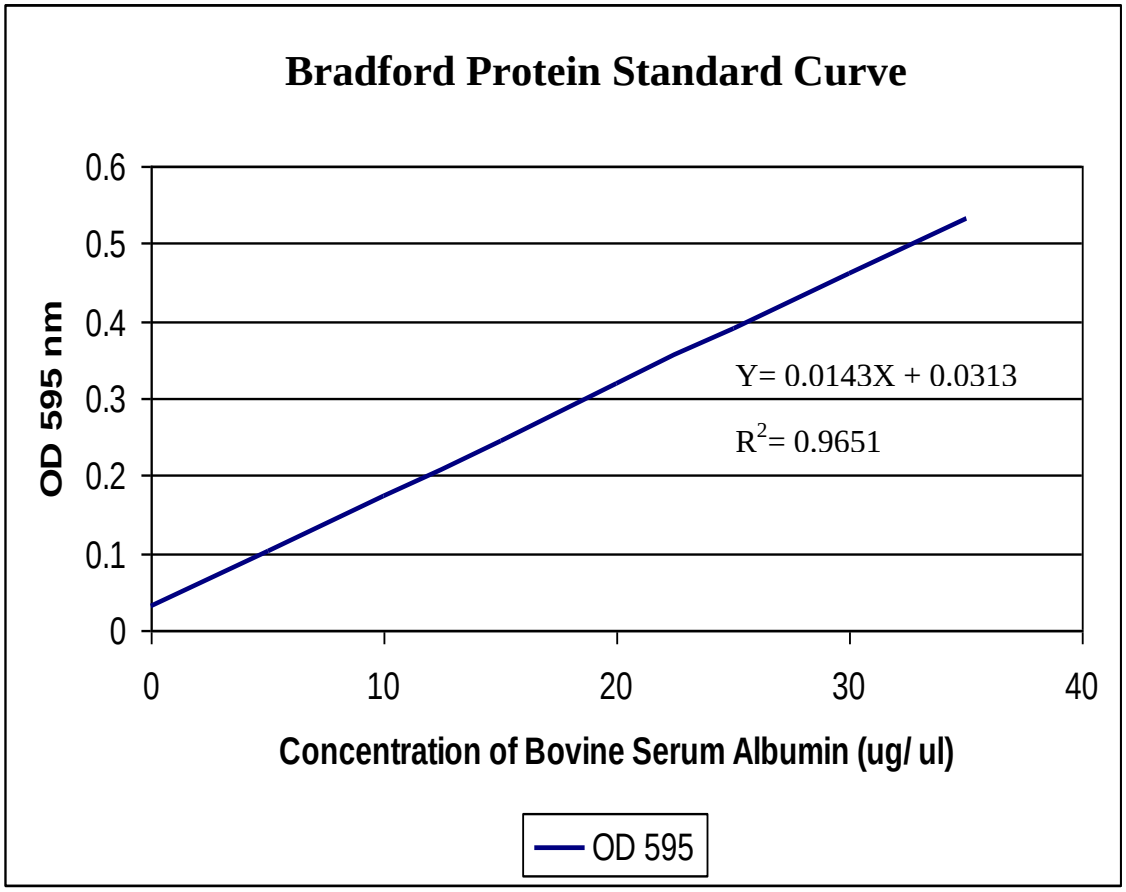
(Wong Wei Chee)



Appendix 6

Bradford Protein Standard Curve with Bovine Serum Albumin as Standard

(Wong Wei Chee)



Appendix 7

Homogenization buffer (1000 ml)

100 mM Tris-HCl	– 15.76 g
20 mM EDTA	– 40 ml of 0.5 M, pH 8.0 EDTA
2% w/v CTAB	– 20 g
1.42 M NaCl	– 81.8 g
2 % w/v PVP- 40	– 20 g
5 mM ascorbic acid	– 0.88 g
4.0 mM DIECA	– 0.96 g

Bring volume to 1000 ml, autoclaved prior to use and kept at room temperature.

TE Buffer

pH 7.4	10 mM Tris-Cl (pH 7.4) 1mM EDTA (pH 8.0)
pH 7.6	10 mM Tris-Cl (pH 7.6) 1mM EDTA (pH 8.0)
pH 8.0	10 mM Tris-Cl (pH 7.8) 1mM EDTA (pH 8.0)

50 X TAE buffer (1000 ml)

Tris base – 121 g
Glacial acetic acid – 28.55 ml
0.5 M, pH 8.0 EDTA – 50 ml

Top up to 500 ml with dH_2O

Diluted into 0.5 X TAE buffer prior to electrophoresis.

(10 ml 50 X TAE buffer in 990 ml dH_2O)

6 X Loading dye

10 mM Tris-HCl (pH 7.6)
0.03% Bromophenol blue
0.03% Xylene cyanol FF
60% Glycerol
60 mM EDTA

Appendix 8

Shoots induction of *Cryptocoryne willisii* cultures

Media supplemented with different concentration of 6 – BA	Numbers of Shoots Formed From A Single Shoot Tip Cultures					Mean Value
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	
MS + 2 mg/ L 6–BA	4	3	3	2	1	2.6
MS + 4 mg/ L 6–BA	1	4	5	1	5	3.2
MS + 6 mg/ L 6–BA	2	7	6	6	4	5
MS + 8 mg/ L 6–BA	3	3	2	5	5	3.6
MS + 10 mg/ L 6–BA	3	3	5	3	3	3.4

Appendix 9

Descriptives

Concentration of 6-BA		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
2		5	2.60	1.140	.510	1.18	4.02	1	4	
4		5	3.20	2.049	.917	.66	5.74	1	5	
6		5	5.00	2.000	.894	2.52	7.48	2	7	
8		5	3.60	1.342	.600	1.93	5.27	2	5	
10		5	3.40	.894	.400	2.29	4.51	3	5	
Total		25	3.56	1.635	.327	2.89	4.23	1	7	
Model	Fixed Effects			1.556	.311	2.91	4.21			
	Random Effects				.397	2.46	4.66			.304

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
2.828	4	20	.052

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.760	4	3.940	1.628	.206
Within Groups	48.400	20	2.420		
Total	64.160	24			

Post Hoc Tests

Homogeneous Subsets

No. of shoots

Duncan

Different concentration of 6-BA	N	Subset for alpha = .05	
		1	2
2	5	2.60	
4	5	3.20	3.20
10	5	3.40	3.40
8	5	3.60	3.60
6	5		5.00
Sig.		.363	.108

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 5.000.

Appendix 11

OD Value obtained for growth of *Agrobacterium tumefaciens* harbouring binary system LBA4404 and pCAMBIA1304 at 550 nm

Time (hours)	OD 550		
	Reading 1	Reading 2	Average
0	0.016	0.016	0.016
1	-0.001	0.032	0.0155
2	0	0	0
3	0.047	0.047	0.013
4	0.026	0.026	0.026
5	-0.006	0.017	0.0055
6	0.011	0.011	0.011
7	-0.04	0.162	0.101
8	0.101	0.069	0.0825
9	0.097	0.13	0.1135
10	0.136	0.168	0.152
11	0.179	0.125	0.152
12	0.151	0.15	0.1505
13	0.219	0.184	0.2015
14	0.175	0.175	0.175
15	0.24	0.244	0.242
16	0.303	0.364	0.3335
17	0.484	0.482	0.483
18	0.584	0.548	0.566
19	0.721	0.657	0.689
20	0.766	0.794	0.78
21	0.896	0.912	0.904
22	1.042	0.951	0.9965
23	1.176	1.17	1.173
24	1.252	1.247	1.2495
25	1.299	1.313	1.306
26	1.359	1.373	1.366
27	1.398	1.388	1.393
28	1.412	1.404	1.408
29	1.412	1.411	1.4115
30	1.412	1.413	1.4125
31	1.41	1.461	1.412
32	1.45	1.45	1.412
33	1.423	1.421	1.412

Appendix 11

Viable cell density of *A. tumefaciens* LBA4404 harbouring pCAMBIA1304. The average numbers of CFU for after incubated at 5, 10, 15 20, and 25 hours obtained *via* dilution spread-plate method.

Incubation time (hours)	**Average number of CFU/ ml**
5	1.08×10^{6}
10	1.30×10^{9}
15	7.00×10^{9}
20	1.53×10^{10}
25	1.39×10^{10}

Appendix 12

	Reading 1	Reading 2	Reading 3	Average	SD
C1 i2	175.3388003	241.3626455	231.054712	215.9187193	35.51916018
C1 i4	154.6759391	214.6539395	171.3035036	180.2111274	30.96529438
C1 i6	520.2162911	678.105879	547.962358	582.0948427	84.29739245
C1 i8	131.4839694	205.3585382	180.4305362	172.4243479	37.58240632
C1 i10	164.695978	221.4121431	180.68528	188.9311337	29.24339963
C2 i2	44.48556313	53.88564096	53.3504133	50.57387246	5.279417588
C2 i4	148.56792	224.8919527	219.456982	197.6389516	42.58355703
C2 i6	57.94601558	76.2380427	75.28966424	69.82457417	10.29805667
C2 i8	143.2026013	197.0193906	172.4307017	170.8842312	26.94170338
C2 i10	103.1367778	157.3459988	142.4186244	134.300467	28.00157622
C3 i2	77.69336185	90.85241981	85.05305041	84.53294403	6.594928702
C3 i4	256.8552931	284.4896682	290.3633347	277.2360987	17.89295714
C3 i6	140.2949182	214.269131	162.0744979	172.212849	38.01493997
C3 i8	188.7807453	339.4886326	330.0660721	286.1118167	84.42274135
C3 i10	0	0	0	0	#DIV/0!
C4 i2	51.08927442	69.85518043	67.43536976	62.79327487	10.20791804
C4 i4	127.308623	176.4347886	152.4923292	152.0785802	24.56569614
C4 i6	99.17381475	173.8921506	122.2050773	131.7570142	38.26404388
C4 i8	50.86273405	72.305509	54.75513381	59.30779229	11.42336088
C4 i10	217.2390619	224.1933214	218.1508085	219.861064	3.77943933

C= Co-cultivation period (days), i = Infection time (mins)

Appendix 13

Statistical analysis of *C. willisii* cocultivated one day with *A. tumefaciens* LBA4404 harbouring pCAMBIA1304

Descriptives

VAR00003

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
2.00	3	215.9190	35.51923	20.50704	127.6843	304.1537	175.34	241.36
4.00	3	180.2113	30.96523	17.87778	103.2894	257.1332	154.68	214.65
6.00	3	582.0947	84.29764	48.66927	372.6877	791.5016	520.22	678.11
8.00	3	172.4247	37.58264	21.69835	79.0642	265.7851	131.48	205.36
10.00	3	188.9310	29.24335	16.88366	116.2865	261.5755	164.70	221.41
Total	15	267.9161	168.30502	43.45617	174.7119	361.1203	131.48	678.11

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
2.712	4	10	.091

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	373383.768	4	93345.942	40.256	.000
Within Groups	23188.365	10	2318.836		
Total	396572.133	14			

Post Hoc Tests
Homogeneous Subsets

	VAR00002	N	Subset for alpha = .05	
			1	2
Duncan(a)	8.00	3	172.4247	
	4.00	3	180.2113	
	10.00	3	188.9310	
	2.00	3	215.9190	
	6.00	3		582.0947
	Sig.		.326	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Appendix 14

Statistical analysis of *C. willisii* cocultivated two day with *A. tumefaciens* LBA4404 harbouring pCAMBIA1304

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
2.00	3	50.5740	5.27917	3.04793	37.4598	63.6882	44.49	53.89
4.00	3	197.6390	42.58353	24.58561	91.8556	303.4224	148.57	224.89
6.00	3	69.8247	10.29814	5.94563	44.2427	95.4067	57.95	76.24
8.00	3	170.8843	26.94132	15.55458	103.9584	237.8103	143.20	197.02
10.00	3	134.3007	28.00151	16.16668	64.7411	203.8603	103.14	157.35
Total	15	124.6445	62.71707	16.19348	89.9130	159.3761	44.49	224.89

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
3.347	4	10	.055

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	48153.642	4	12038.410	17.411	.000
Within Groups	6914.395	10	691.439		
Total	55068.037	14			

Post Hoc Tests
Homogeneous Subsets

	Infection Time	N	Subset for alpha = .05		
			1	2	3
Duncan(a)	2.00	3	50.5740		
	6.00	3	69.8247		
	10.00	3		134.3007	
	8.00	3		170.8843	170.8843
	4.00	3			197.6390
	Sig.		.391	.119	.241

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Appendix 15

ANOVA

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
2.00	3	84.5327	6.59491	3.80757	68.1500	100.9153	77.69	90.85
4.00	3	277.2360	17.89307	10.33057	232.7872	321.6848	256.86	290.36
6.00	3	172.2127	38.01490	21.94791	77.7784	266.6469	140.29	214.27
8.00	3	286.1120	84.42269	48.74146	76.3944	495.8296	188.78	339.49
10.00	3	100.0000	.00000	.00000	100.0000	100.0000	100.00	100.00
Total	15	184.0187	95.05542	24.54320	131.3787	236.6586	77.69	339.49

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
9.049	4	10	.002

Appendix 16

ANOVA

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
2.00	3	62.7930	10.20793	5.89355	37.4351	88.1509	51.09	69.86
4.00	3	152.0787	24.56561	14.18296	91.0543	213.1030	127.31	176.44
6.00	3	131.7570	38.26389	22.09167	36.7042	226.8098	99.17	173.89
8.00	3	59.3080	11.42357	6.59540	30.9303	87.6857	50.86	72.31
10.00	3	219.8610	3.77923	2.18194	210.4729	229.2491	217.24	224.19
Total	15	125.1595	64.65335	16.69342	89.3557	160.9634	50.86	224.19

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
2.866	4	10	.081

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	53887.629	4	13471.907	29.077	.000
Within Groups	4633.154	10	463.315		
Total	58520.783	14			

Post Hoc Tests
Homogeneous Subsets

	Infection Time	N	Subset for alpha = .05		
			1	2	3
Duncan(a)	8.00	3	59.3080		
	2.00	3	62.7930		
	6.00	3		131.7570	
	4.00	3		152.0787	
	10.00	3			219.8610
	Sig.		.847	.274	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.